Mascot:

Installation and Setup

End User Licence Agreements

MASCOT PROTEIN IDENTIFICATION SYSTEM

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Julian Seward, Cambridge, UK.
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bzip2/libbzip2 version 1.0.3 of 15 February 2005

SWIG

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signature of Ty Coon, 1 April 1990
  Ty Coon, President of Vice

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Regex

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 */
```

PLPA

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```

C Clustering Library

/* The C clustering library.

This library was written at the Laboratory of DNA Information Analysis,

Human Genome Center, Institute of Medical Science, University of Tokyo,

4-6-1 Shirokanedai, Minato-ku, Tokyo 108-8639, Japan.

Contact: mdehoon 'AT' gsc.riken.jp

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*/

Contents

Typographical Conventions

Description	Example
Filenames, pathnames, directory names (folders), programs, and commands are printed in italic fixed pitch font. In Unix, these names are case sensitive.	*nph-mascot.exe* */usr/local/httpd*
The contents of text files are printed in fixed pitch font on a grey background. Where it is necessary to break a long line, this is indicated by an indent and the symbols ↳ ↰. Text omitted from a line is indicated by an ellipsis …, while missing lines are indicated by 3 vertical periods.	`>owl||100K_RAT 100 KD PROTEIN` ↳ ↰`(EC 6.3.2.-).` `>owl||100K_RAT … NORVEGICUS (RAT).`
Text which should be entered literally is shown in bold fixed pitch font on a grey background. Control characters are shown in angle brackets, apart from **<return>** (i.e. carriage return, newline, or enter) which is only shown in ambiguous cases. Bold italic fixed pitch font indicates a variable for which an appropriate value should be entered.	`C:\TEMP> `**`ftp ncbi.nlm.nih.gov`** `% `**`kill `***`PID`*

1
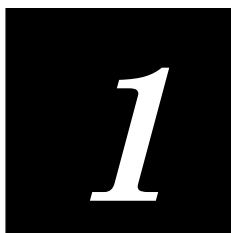
Introduction

Mascot is a software system for protein identification by matching mass spectrometry (MS) data against FASTA format protein or nucleic acid sequence databases. This can be done in three different ways:

1. A Peptide Mass Fingerprint (PMF), in which the MS data are peptide molecular masses from the digestion of a protein by an enzyme.

2. A Sequence Query (SQ), also called a sequence tag, in which MS data are combined with amino acid sequence or composition data.

3. An MS/MS Ions Search (MIS), which uses MS/MS data from one or more peptides.

MS data are submitted to Mascot in the form of peak lists. That is, lists of centroided mass values, possibly with associated intensity values. The result of a search is a ranked list of the most closely matching proteins. Mascot uses a probability based scoring algorithm, so that it is possible to report whether a match is statistically significant. If an exact match is not present in the database, the highest scoring matches will be those entries which exhibit the greatest homology.

Overview

This manual describes how to install, configure and administer Mascot. It is not a User Guide. Mascot includes a linked collection of HTML help pages that provide guidance and application related reference material for end-users.

Mascot conforms to a client / server architecture, and the primary user interface is a JavaScript aware web browser. Searches can be submitted from web browser forms, customised for different types of searches, or from a variety of client software. Mascot Daemon is a client application, bundled with Mascot Server, for batch automation of search submission. Mascot Distiller is a powerful application, licensed separately, that can process a wide range of native file formats into peak lists, submit searches to a Mascot Server, and import the search results for examination or further processing. There are also a number of third party clients, including many mass spectrometry data systems that support search submission to Mascot.

In most cases, the Mascot search engine is executed as a CGI program. On completion of a search, it calls a Perl CGI script that reads the results file and returns an HTML report (or some other machine readable
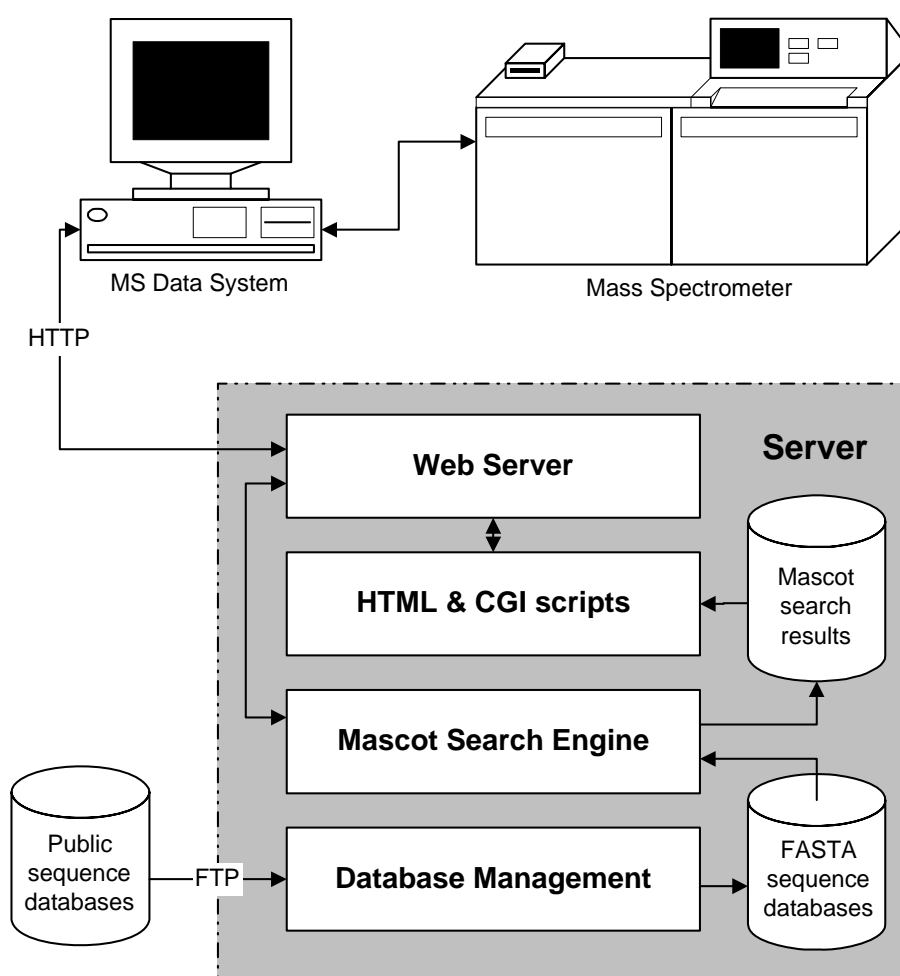
digest of the results) to the client. Links to additional CGI scripts provide more detailed views of the results.

Mascot Components

In this manual, "server" refers to the data system on which the Mascot search engine executes. The term "client" is used very loosely. It may refer to a data system attached to a mass spectrometer, or it may refer to any system at which a user interacts with the Mascot server via a web browser.

In a small laboratory, the server and client may be one and the same computer. This doesn't affect installing or using Mascot, but it does introduce additional considerations, such as the need to adjust system priorities to ensure that the instrument control and data acquisition software is responsive to the real-time needs of both instrument and operator.

Configuration

Mascot configuration files are structured text files. Modifications can be made using a browser-based configuration editor and take effect without a system restart.

Search Engine

The Mascot search engine accepts data and parameters on STDIN in MIME format, executes a search of the specified FASTA format database, and outputs a structured text file containing the search results together with the input data and the complete set of search parameters.

The results file contains everything necessary to repeat the search at a later date, should the need arise. In the default configuration, a new directory is created on the server for each day's results files. If required, the contents of these results files can be parsed into an external database to be queried and analysed.

Monitor

Swapping databases without disrupting ongoing searches is handled by Mascot Monitor. The new database is compressed and tested by running a standard search. If errors are detected in the new database, the database exchange process is abandoned, and searches continue to use the earlier database

Assuming the test is successful, all new searches are performed against the new database, while searches that were in progress against the old database are allowed to continue. Once the final search against the old database is complete, the compressed files are deleted and the FASTA file is moved to an archive directory. If the database being exchanged is memory mapped, the mapping and unmapping are also handled automatically.

Status

The Mascot package includes a CGI application that provides a live status display via a web browser. For each database, the Mascot job queue, the executing jobs, and the completed jobs are listed. The status lines for completed jobs contain hyperlinks to individual results reports.

Review

Review is a CGI application that provides easy access to the flat file database of search result files. Key search parameters, such as time and date, job number, user name, search type, etc. are displayed in a spreadsheet-like table. Columns can be hidden, sorted and filtered to facilitate locating a specific file or group of files. Each row includes hyperlinks, either to generate a Mascot results reports or to display the file contents as raw text.

2

Installation: Linux

Release Notes

Mascot 2.4 is compiled for 32-bit and 64-bit Linux. Refer to the release notes for last-minute additions to documentation and the Matrix Science web site support page for patches and known issues:

> http://www.matrixscience.com/mascot_support.html

Cluster Mode

If you have a licence to run Mascot on multiple processors, and plan to do so on a networked cluster of machines, then please familiarise yourself with the material in Chapter 11, Cluster Mode, before proceeding with the installation.

System Requirements

Web Server

Mascot is compatible with most web servers. Appendix D provides configuration information for Apache.

If a web server is being installed for the first time, in connection with the installation of Mascot, it is essential to verify that it is serving documents correctly before attempting to install Mascot.

Perl

Mascot requires Perl. Perl 5.14 is recommended, Perl 5.8, 5.10, and 5.12 are also supported.

Mascot scripts assume that Perl can be found at */usr/local/bin/ perl*. If Perl is installed in a different path, just add a symbolic link:

```
ln -s /actual/location/of/perl /usr/local/bin/perl
```

If any library modules are missing, this will be identified during the installation procedure. Binary packages of Perl and most Perl modules are available for most Linux distributions. The mechanism for downloading and installing new modules and updates is distribution specific. For example, to install the non-core module Bundle::LWP on some common distributions:

Red Hat/CentOS Linux:

```
yum install perl-libwww-perl
```

Debian/Ubuntu Linux:

```
aptitude install libwww-perl
```

SUSE Linux:

```
yast –i perl-libwww-perl
```

If a module has missing dependencies, you will be prompted to install these. The required non-core modules are:

Module	Debian/Ubuntu	Red Hat/CentOS/SuSE
GD	libgd-gd2-noxpm-perl	perl-GD
Bundle::LWP	libwww-perl	perl-libwww-perl
Algorithm::Diff	libalgorithm-diff-perl	perl-Algorithm-Diff
XML::Simple	libxml-simple-perl	perl-XML-Simple

If other applications require a version of Perl not supported by Mascot or if you have difficulty compiling Perl or one of the required modules, the Mascot DVD includes ActivePerl 5.14 for Linux (32-bit and 64-bit). You can install this for general use or for use by Mascot only. A typical installation might use the following commands:

```
cd /tmp
```

```
gzip -dc /dvdrom/ActivePerl-5.14.2.1402-x86_64-linux-
    glibc-2.3.5-295342.tar.gz | tar xvf -
```

```
cd ActivePerl-5.14.2.1402-x86_64-linux-glibc-2.3.5-
    295342/
```

```
sudo ./install.sh
```

Follow the installation script instructions, and choose to install into an appropriate location. If ActivePerl was being installed for Mascot use only, we might install into /usr/local/ ActivePerl-5.14, and create a symbolic link as follows:

```
ln -s /usr/local/ActivePerl-5.14/bin/perl /usr/local/
    bin/perl
```

Mascot Directory Structure

There are two directory structures to consider. One consists of the "real" paths to files on disk, the other consists of the "virtual" directories which define the web server URL's. The virtual directories are mapped to real directories. For example, the server URL

> http://your.domain/mascot/home.html

might be mapped to the disk file

> */usr/local/mascot/html/home.html*

Any virtual directory that contains CGI executable programs (e.g. *nph-mascot.exe*) or scripts (e.g. *master_results.pl*) must have script execution enabled.

Under normal circumstances, if a directory is mapped to a URL, all of its subdirectories are also accessible as subdirectories of the URL. Figure 2.1 shows the recommended directory structure for Mascot. The root of this structure can be any convenient path.

Some of the directory paths can be changed by using a symbolic link or by modifying the configuration file, *mascot.dat*. For example, it may be desirable to have the sequence or data directories on a separate drive from the rest of the files. Care should be taken with any changes which affect a URL mapped directory or file, because this may require one or more HTML files to be edited to modify links.

In most cases, the contents of the directories can be deduced from their names:

> *bin* contains (non-CGI) executables.

> *cgi* contains CGI executables

> *cluster* contains a sub-directory for platform specific executables, for distribution to the nodes in a cluster

> *config* contains configuration files

> *data* contains Mascot results files. By default, a new sub-directory is created for each day's results files. The name of each sub-directory is that day's date in ISO format, yyyymmdd.

> *html* is the root directory for documents
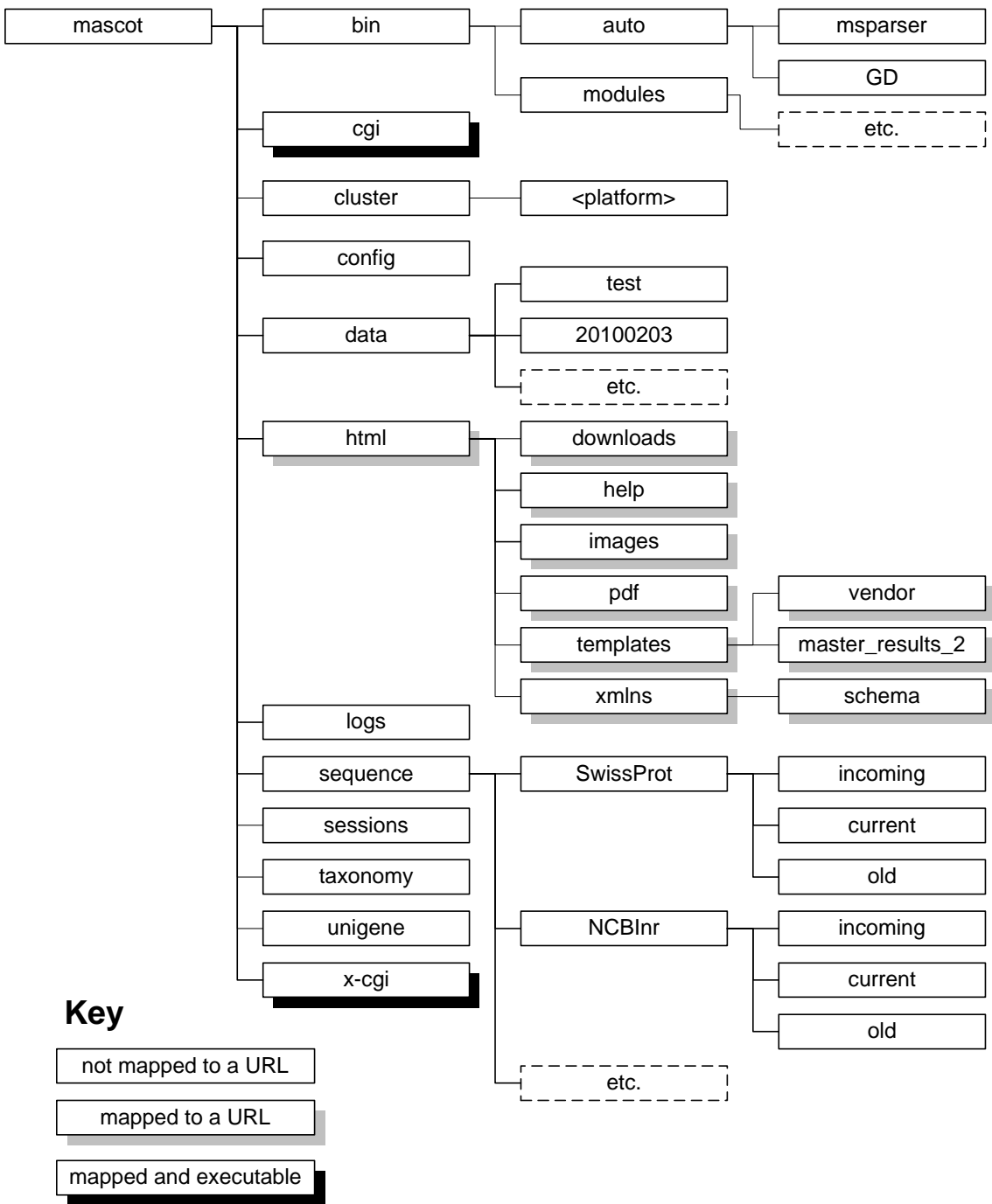
> *logs* contains search and error logs, etc.

Figure 2.1 Mascot Directory Structure

sequence contains a sub-directory for each FASTA database. As illustrated, for each database there are 3 sub-directories to organise the FASTA files into new downloads (*incoming*), active databases (*current*) and the most recently replaced files (*old*).

sessions contains security session files

taxonomy contains taxonomy resources

unigene contains sub-directories for species specific UniGene indexes

x-cgi is a directory for administrative CGI executables, to which access may need to be restricted. This can be achieved using either Mascot security or web server security.

Installation

Clean Installation

Create a directory for the Mascot program files. In documentation, this is assumed to be called *mascot*, but any name can be used. This directory should *not* be in a path mapped to a web server URL.

Version upgrade

If upgrading Perl, do this before upgrading Mascot.

Ensure that no-one will try to use Mascot during the upgrade procedure.

Kill the ms-monitor.exe process.

You might wish to make a backup of the existing files before they are overwritten. All configuration files in the *config* directory apart from *mascot.dat* and the security settings will be overwritten by new files. All results files and sequence databases apart from SwissProt will be retained. The installation script will update *mascot.dat* with by adding any new options, but will retain all existing sequence database configuration settings and other options.

Unpack the Mascot file system

Copy the files *mascot.tar.bz2* and *swissprot.tar.bz2* from the Mascot DVD into the *mascot* directory, and unpack the archives:

```
bzip2 -d mascot.tar.bz2

bzip2 -d swissprot.tar.bz2

tar xvf mascot.tar
```

```
tar xvf swissprot.tar
```

For 32-bit Linux, the 32-bit binaries should be unpacked after *mascot.tar*, so as to over-write the 64-bit files in the *mascot.tar* archive:

bzip2 -d mascot-32.tar.bz2

tar xvf mascot-32.tar

Alternatively, you can combine decompression and tar into a single command, for example:

```
bzip2 -dc /dvdrom/mascot.tar.bz2 | tar xvf -
```

This will create the directory structure illustrated in Figure 2.1. Ensure that the ownership of the files matches the user ID that your web server is configured to use. The *mascot.tar* file has been created using root:root. The required ID when Apache is installed from a RedHat RPM will be apache:apache, and when installed on Ubuntu or Debian, it will be www-data:www-data

```
chown -R apache:apache /usr/local/mascot/*
```

(If this is not acceptable, then the *logs*, *config*, *sessions*, and *data* directories, plus the file *logs/errorlog.txt* must be made writeable by the web server process).

Create URL mappings

If this is a clean installation, add the following mappings to your web server configuration, (substituting your actual disk path to the new mascot directory):

Disk path	URL	Executable
/usr/local/mascot/cgi	/mascot/cgi	Yes
/usr/local/mascot/html	/mascot	No
/usr/local/mascot/x-cgi	/mascot/x-cgi	Yes

You may wish to restrict access to the administrative programs by setting a password or IP address restriction on /mascot/x-cgi.

Notes on web server configuration can be found in Appendix D. Example configuration entries for Apache can be found in the file *config/ apache.conf*.

Installation Script

Step 1: Web Server Operation

Launch a JavaScript aware web browser, and navigate to the URL corresponding to *install.html*, e.g. http://*your.domain*/mascot/ install.html

Follow the instructions on this web page and those that follow to perform some simple system checks and create or update the Mascot configuration file (*mascot.dat*).

Step 2: Perl

If you get an error message, or a "File Save As..." dialog box, after clicking on the "Test Perl" button, then Perl is not functioning correctly. This must be corrected before proceeding. Possible reasons for this problem are listed along with useful links:

- Perl is not installed, or was installed incorrectly.

- Perl, or a soft link to Perl, was not found at */usr/local/bin/ perl*

- The mascot/cgi directory is not configured for CGI execution.

- JavaScript is disabled

If Perl is functioning correctly, the next page displays the Perl version number. If any of the required modules are missing, there will be a warning. Instructions for installing the required modules can be found towards the beginning of this chapter.

Step 3: GD Graphics Library

Assuming Perl and the required modules are present, click on the button to test the GD Graphics library. If GD is installed and working, the next page contains a small graphic to confirm this:
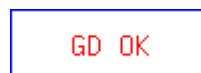
If you do not see this picture, or get an error message, refer to the first part of this chapter for information on installing GD.pm.

Step 4: Configuration
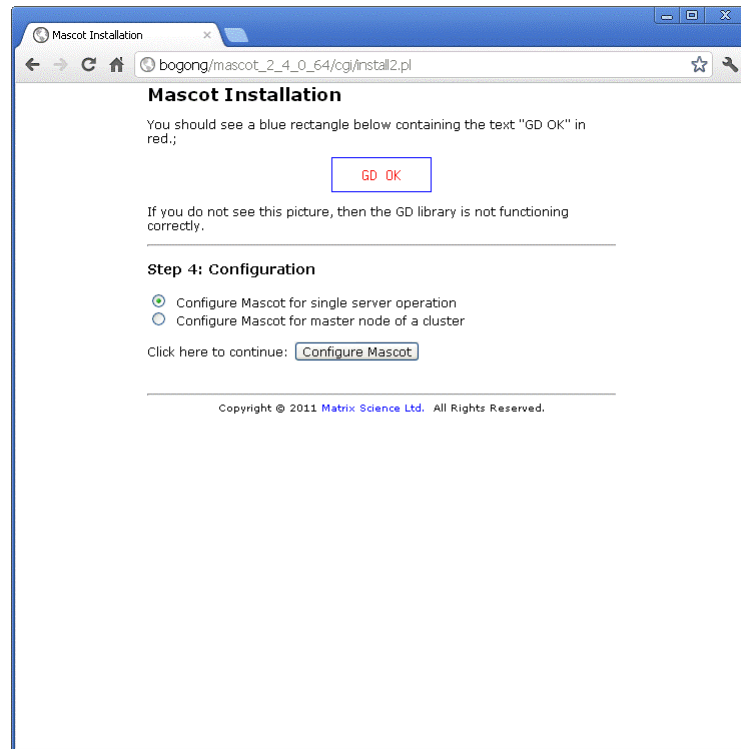
Indicate whether you plan to configure Mascot as a single (SMP) server or a cluster and choose 'Configure Mascot'. If this is a version upgrade, the main configuration file, `mascot.dat`, will be updated. If it is a clean install, a new `mascot.dat` will be created.

Step 5: Start Mascot Monitor
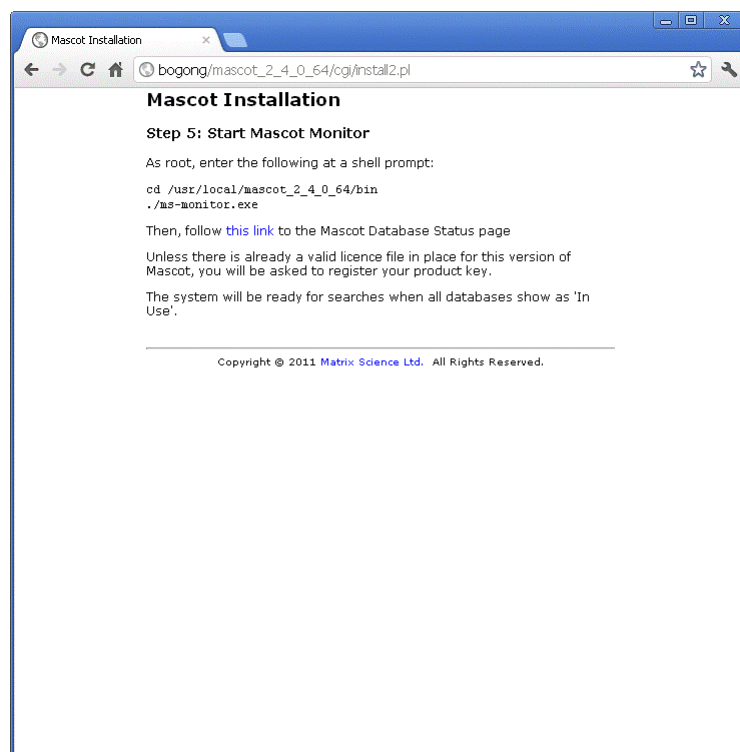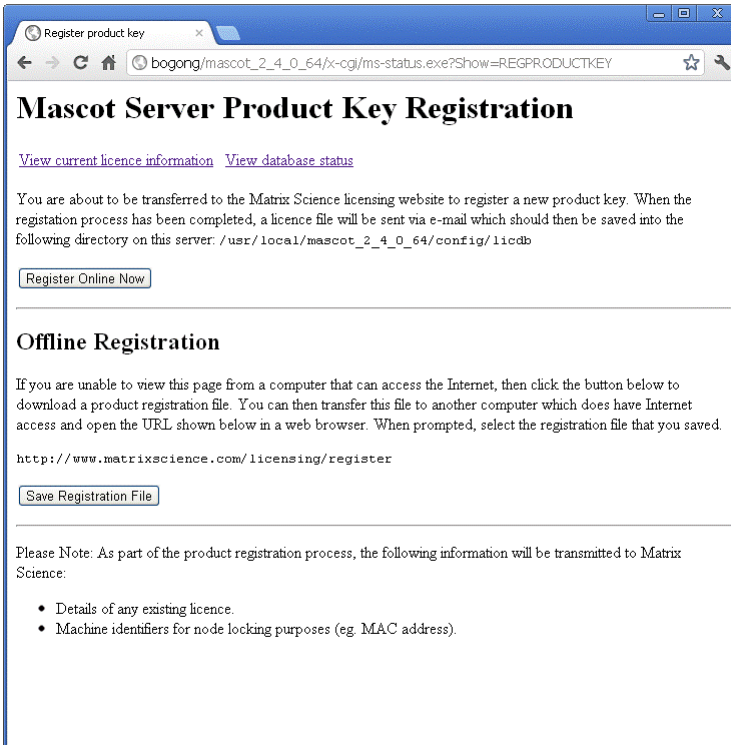
If you chose to configure Mascot as a single (SMP) server, you will see a screen similar to the one above, and can proceed to start Mascot Monitor. If you chose cluster mode, refer to Chapter 11 for further configuration information.

Start Monitor at a shell prompt. (You must have root privileges for this)

```
cd /usr/local/mascot/bin
```

```
./ms-monitor.exe
```

Then follow the hyperlink to the Database Status page to register your product key.

Step 6: Licence Registration

A product key is required and must be registered online. The licence file will be returned by email and must be saved to the specified location on the Mascot server. If the Mascot server cannot connect to the Internet, a file containing registration information can be saved and copied to a system with Internet access for submission.

The registration form allows a second email address to be specified, in case the person installing Mascot is not the end-user. Ensure that the end-user email address is entered into the upper part of the form and the email address to which the licence file should be sent is entered into the CC email field in the lower part of the form.

The licence file must be saved to the `config/licdb` directory as a file with the extension `.lic`.

Verify System Operation

A copy of the SwissProt database is included in the files copied from the DVD. It is recommended that the operation of Mascot is verified and tested using this database before adding further databases or making configuration changes.

Mascot Monitor (*ms-monitor.exe*) is used to manage the swapping and memory mapping of the sequence databases used by Mascot. For Mascot to operate, *ms-monitor.exe* must be running at all times.

Once the new licence file is in place, follow the hyperlink to Database Status. You should see a display similar to the following:

If an error occurs, use the links to the monitor log and the error log to investigate the cause. If all is well, you will see the following messages displayed on the status line for SwissProt:

```
Creating compressed files
Running 1st test
First test just run OK
Trying to memory map files
Just enabled memory mapping
In Use
```

You can begin exploring and using Mascot. However, do not try to run searches or view results reports until the relevant sequence database is 'In Use'.

Usually, you'll want to add *ms-monitor.exe* to the system boot process, so that it is started automatically. An example Linux init script called *mascot* can be found in the Mascot *bin* directory. For RHEL/CentOS , move this to the */etc/init.d* directory with permissions rwxr-xr-x and owner root:root. As root, type:

chkconfig —add mascot

Security

Mascot security is disabled on installation. To enable Mascot security, refer to Chapter 12

Keyword Indexing

Users of Mascot may wish to be able to search the help text by keywords or phrases. The web pages are designed to work with an indexing tool called ht://Dig. This is standard in several Linux distributions. If not installed, we recommend stable release (3.1.6).

Red Hat/CentOS Linux:

yum install htdig

Debian/Ubuntu Linux:

aptitude install htdig

SUSE Linux:

yast -i htdig

A few binary packages are also available at http://www.htdig.org/files/binaries/

Alternatively, if you have a working development system with a C++ compiler, you can download the source code from http://www.htdig.org/

Once installed, you'll need to edit the following values in the ht://Dig configuration file, *htdig.conf*

```
start_url:  http://your_host/mascot/home.html

limit_urls_to:  /mascot/

exclude_urls:  .pl .exe .gif .jpg .pdf .msi .png
```

It is also necessary to add an alias to the Apache configuration. Add the following ScriptAlias entry, immediately before the ScriptAlias for /mascot/cgi:

```
ScriptAlias /mascot/cgi/htsearch /usr/lib/cgi-bin/
htsearch
```

On Red Hat/CentOS, `/usr/lib/cgi-bin` should be replaced with `/var/www/cgi-bin`

You may also need to add the following if you get 403 errors, especially if you have Mascot defined in a separate virtual host:

```
<Directory /usr/lib/cgi-bin>
  Order allow,deny
  Allow from all
</Directory>
```

Finally, build an index of the Mascot web site documents:

```
rundig -v
```

This may need to be run by the web server user or root, depending on how htdig has been installed and configured. Indexing will only take a minute or two. Use of the -v flag causes verbose progress reports to be generated.

Miscellaneous

Hyper-threading

Intel only: Hyper-threading is a technique used by Intel to improve the performance of multi-threaded programs. Hyper-threading does not double performance because pairs of cores share other resources, such as the on-chip cache. On some systems, a BIOS setting can be used to enable and disable hyper-threading.

Hyper-threading is detected automatically. Each CPU in the Mascot licence enables up to 4 cores to be used for searches. Hyper-threading is ignored when counting cores, so that you may see a 1 CPU licence using 8 threads on a system with a quad core processor with hyper-threading enabled.

File System

The file system (NFS or a local file system) needs to support file locking and memory mapping. The following files will be locked/unlocked using the fcntl(,F_SETLKW,) system call: *mascot.job, getseq.job, mascot.control, mascotnode.control.* If Mascot Daemon, Mascot Distiller or any application using the task management functions in *client.pl* is used, then there will be a task_id file in each data/ yyyymmdd directory that will be locked/unlocked. The following files will be memory mapped for r/w: *mascot.control, mascotnode.control.* The location of these files can all be specified in the options section of *mascot.dat* so that if necessary they can be put on a local file system.

Fasta files greater than 2 GB are fully supported on ext2, ext3 and ext4 partitions.

System limits

Memory limits

There are several types of memory limits that can stop Mascot from running:

1. Virtual address space. When files are memory mapped, the address space required can be large – the amount of physical RAM / swap space is not an issue here.

2. The amount of memory that can be locked. On most systems, memory can only be locked by root.

3. Physical memory. It is obviously not possible to lock more memory than is physically available!

4. Data segment size. The amount of memory that an executable or Perl script has access to. The default is sometimes too small to run master_results.pl, and big searches.

5. Swap space. May need to be increased for very large searches.

6. Stack space. Not normally an issue for executables or any of the perl scripts.

7. Thread stack space. Not normally an issue for executables. The perl scripts are not threaded

File size limits.

This is normally unlimited, but a limit may have been configured (e.g. /etc/security/limits.conf).

You should manually verify that your system can successfully FTP a file larger than 2 GB, as FTP doesn't necessarily report an error when it fails.

How the errors are reported

If the Mascot executables report a memory error, the error can be found in the *errorlog.txt* file, including the error code returned by the operating system. For a Perl script running in CGI mode, the web server may just kill the job, and no error will be logged.

Determining what the limits are.

Most systems have two sets of limits – the current limits and the hard limits.

There is no standard Unix command across all platforms, although 'limit' or 'limits' will work on most systems from the 'C' shell

```
$ ulimit -a
cputime      unlimited
filesize     unlimited
datasize     1048576 kbytes
stacksize    65536 kbytes
coredumpsize unlimited
memoryuse    250004 kbytes
descriptors  200
vmemory      1048576 kbytes
threads      1024
```

```
$ ulimit -aH
cputime       unlimited
filesize      unlimited
datasize      1048576 kbytes
stacksize     524288 kbytes
coredumpsize unlimited
memoryuse     524288 kbytes
descriptors   2500
vmemory       1048576 kbytes
threads       1024
```

These values will be different for root and a normal user, and possibly different again for the owner of CGI processes (apache or www-data). Since you may not be able to log in as the CGI user, it can be hard to find out what the real values are. If a script or binary is failing in the web browser, try running from the command line as both root and a normal user.

Changing the default limits

There are different utilities / configuration files on every system. Refer to system documentation.

Detailed Information on each memory limit

This section gives details about how the mascot software reports errors, and tries to increase the limits where appropriate.

Virtual address space

Mascot executables are compiled as both 32-bit and 64-bit programs. If you use the 32-bit executables, the amount of virtual address space is limited to 3 or 4 GB, according to platform, and this limit cannot be exceeded. However, default limit may be set lower than this by the operating system.

If memory cannot be mapped, the error M00048 "Failed to create memory map for [filename]. Error [detailed message]" will be displayed and put into the errorlog.txt file.

The amount of memory that can be locked

As well as the obvious limitation of physical memory, there is generally a limit set on the amount of memory that can be locked. Another frequently used term for locked is 'wired'.

On most systems, memory can only be locked by root. Before a "Failed to lock memory for file xxx" error is given, Mascot Monitor will try and increase the amount of RSS available by calling

```
setrlimit(RLIMIT_RSS, xxx)
```

with the current value plus the size of the file to be locked. Under Solaris, the RLIMIT_AS value is used – (rather confusing use of 'AS' by Sun).

If the resource limit cannot be increased, then error M00114 "Error calling setrlimit(RLIMIT_RSS, [memory requested]) - error [detailed error message]" will be put into errorlog.txt

If the memory cannot be locked, then the error M00073 "Failed to lock memory for file [file name]. Error [detailed text]" will be put into the errorlog.txt file.

If Mascot Monitor, (ms-monitor.exe), is a 32-bit executable, the 3 or 4 GB limit can quickly be reached by having several large databases locked into memory. To work around this limit, a separate ms-lockmem.exe program is provided – this is fork'd / exec'd from ms-monitor.exe when the flag 'SeparateLockMem 1' is added to the options section of mascot.dat.

Physical memory

If the amount of memory locked gets close to the amount of physical memory, the system will grind to a halt! The error M00073 "Failed to lock memory for file [file name]. Error [detailed text]" will also probably be put into the errorlog.txt file.

Data segment size

This amount does not include the space used by memory-mapped files.

Insufficient data segment size will cause a large master_results.pl script to fail and a mascot search to fail with an error M00000 – "Out of memory (malloc) [number of] bytes requested"

Swap space

When all physical memory is exhausted, swap space is used. When all swap space is used, no more memory can be allocated and an error will be reported.

There is a different way of setting up swap space on each system – see system documentation.

Mascot shows free swap space for cluster nodes only.

Stack space

Has not been a problem yet.

Thread stack space

This is not normally an issue, since it is increased by all the binaries at run time to 128k.

3

Installation: Microsoft Windows

Release Notes

Mascot 2.4 is compiled for 32-bit and 64-bit Windows. Refer to the release notes for last-minute additions to documentation and the Matrix Science web site support page for patches and known issues:

http://www.matrixscience.com/mascot_support.html

Cluster Mode

If you have a licence to run Mascot on multiple processors, and plan to do so on a networked cluster of machines, then please familiarise yourself with the material in Chapter 11, Cluster Mode, before proceeding with the installation.

Overview

To install or upgrade Mascot, the following steps need to be performed

1. Verify that the computer has sufficient memory and disk space

2. Verify that the computer has a suitable version of Microsoft Windows installed. Mascot requires Windows XP or later on Intel or AMD.

3. Virus scanning software or Microsoft Outlook should not be running during the installation

4. Install Web server software unless already installed.

5. Install or upgrade Perl, unless a compatible version is already installed.

6. Run setup32.exe (32-bit) or setup64.exe (64-bit) off the Mascot CD

It is essential that steps 4, 5, and 6 are performed in that order

System Requirements

Disk Space

A typical installation of the Microsoft Web server requires about 150 MB. A typical installation of ActiveState Perl requires about 120 MB. A full installation of Mascot requires approx 3.6 GB

The hard disk must be formatted for NTFS. FAT32 has a file size limit of 4GB, which would prevent the use of large sequence databases. It is advisable that NTFS file compression is *not* used for the compressed database files. There are reports that NTFS compression is not fully compatible with memory mapping. NTFS file compression can be used on the FASTA and reference files if you wish.

Memory

To get the best performance from Mascot, the database files need to be memory mapped. It is recommended that you have at least 4 GB of RAM. On a 64-bit system, 12 GB or more will help ensure best performance.

Microsoft Windows versions

XP

Mascot will run under Windows XP Professional; Windows XP Home is not supported.

It is advisable to ensure that the latest service pack has been installed. Check the following URL for current information:

> http://www.microsoft.com/windowsxp/downloads/default.mspx

The Microsoft web server for 32 bit editions of Windows XP is IIS 5.1, which is provided as part of the standard distribution. If IIS is not installed, choose 'Add or Remove Programs' in the Control Panel. Select 'Add/Remove Windows Components', and check the box for Internet Information Services. XP Professional x64 Edition uses IIS 6.0, the same as Server 2003.

Server 2003

Mascot will run under all editions of Windows Server 2003 except those for the Itanium processor.

It is advisable to ensure that the latest service pack has been installed. Check the following URL for current information:

http://technet.microsoft.com/en-us/windowsserver/bb405947

The Microsoft web server for Server 2003 is IIS 6.0. You may need to install IIS by configuring the server as an Application Server. When you start your server, you should see a 'Manage Your Server' wizard. If not, go to Administrative Tools and click on 'Manage Your Server'. When the wizard opens, click on 'Add or Remove a Role', then select Application Server
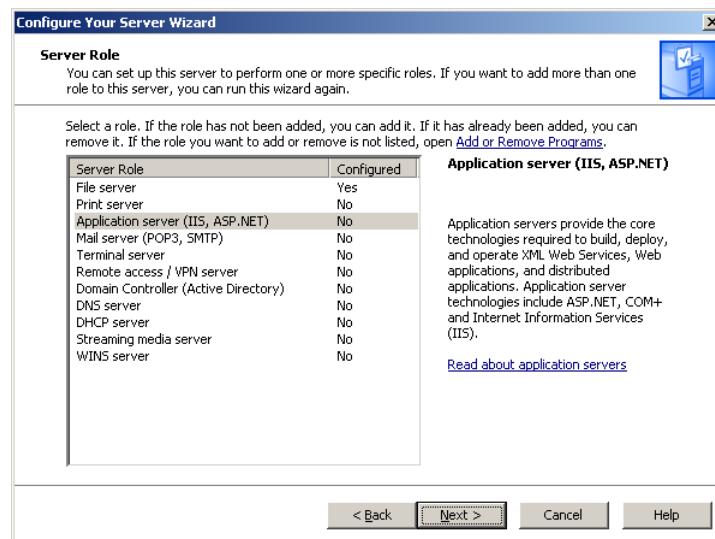
Proceed through the wizard, accepting all the defaults, to install IIS.

IIS 6 does not serve files with unknown MIME types, and its default list of MIME types does not include XML schema documents. See Microsoft Knowledge Base article Q326965 for the procedure to add *.XSD to the IIS 6 list of MIME types

http://support.microsoft.com/default.aspx?scid=kb;en-us;326965

Vista

Mascot will run under all Windows Vista editions except for Starter and Home Basic.

It is advisable to ensure that the latest service pack has been installed. Check the following URL for current information:

http://windows.microsoft.com/en-US/windows/downloads/windows-vista
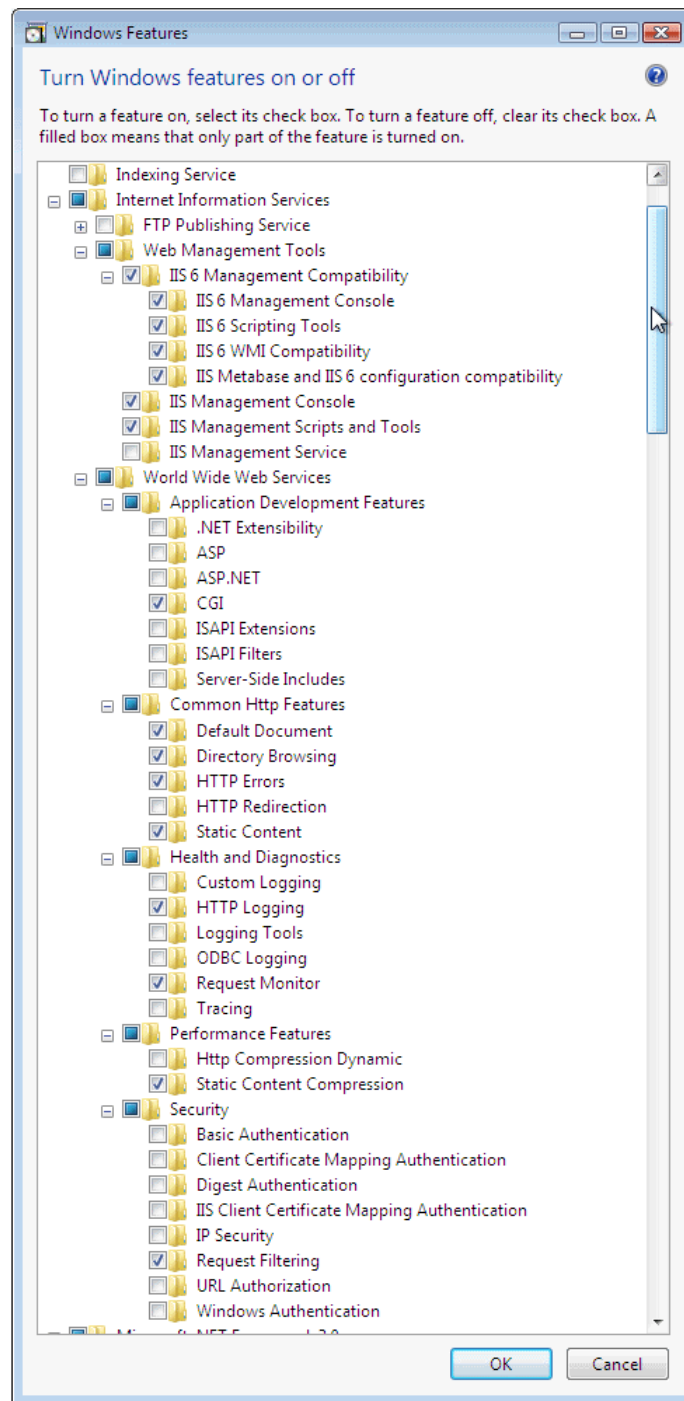
The Microsoft web server for Vista is IIS 7.0, which is provided as part of the standard distribution. A default installation of IIS 7.0 does not support running a CGI application such as Mascot. From the Control Panel, choose 'Programs and Features'. Choose 'Turn Windows features on or off'. Expand the node for Internet Information Services and ensure that all the checkboxes shown below are checked, in addition to any default selections. Then, choose OK.

In Vista Home Premium, the IIS 7 simultaneous request execution limit is 3. In Vista Business, Enterprise, and Ultimate Editions, the limit is 10. This will limit the number of simultaneous searches that can be run from a simple web browser form.

Server 2008 (including R2)

Mascot will run under all Server 2008 editions except for Core.

It is advisable to ensure that the latest service pack has been installed. Check the following URL for current information:

http://msdn.microsoft.com/en-us/windowsserver/bb794698

http://support.microsoft.com/ph/1163#tab1

The Microsoft web server for Server 2008 is IIS 7.0 and 7.5 for Server 2008 R2. From the Control Panel, choose *Turn Windows features on or off* to launch Server Manager. Select *Go to Roles*, scroll down to *Web Server (IIS)*, and choose *Add Role Services*. Then follow the configuration notes under the Windows Vista section, above

Windows 7

Mascot will run under all Windows 7 editions, but note that only Professional and Enterprise support remote desktop

It is advisable to ensure that the latest service pack has been installed. Check the following URL for current information:

http://windows.microsoft.com/en-US/windows/downloads/windows-7

The Microsoft web server for Windows 7 is IIS 7.5. By default, this is not installed. To install IIS, from the Control Panel, choose *Programs and Features*, *Turn Windows features on or off*. Expand the node for Internet Information Services, then follow the configuration notes under the Windows Vista section, above

Web Server

Mascot for Windows is tested with IIS and Apache.

The Mascot installation has been fully automated for Microsoft Internet Information Server 5.0 and later. A good starting point for IIS support information is http://www.iis.net/

> **IMPORTANT:** If you are using IIS 7.x (Vista, Server 2008, Windows 7) you **must** configure it as described in the Windows Vista section, above, **before** proceeding with the installation. Otherwise, the Perl and Mascot installations will fail.

If IIS is configured as a secure server (SSL/TLS), you must change it temporarily to non-secure mode (http: on port 80). Once the installation is complete, you can change back to secure mode.

If you wish to use Apache as your web server, you will need to perform some manual configuration, as described in Appendix D.

IIS 6.0 and later

If a search is submitted from a browser and the connection is broken before the search is complete, the search will be killed. The only known workaround is to use a different web server, e.g. Apache.

Perl

Mascot requires Perl, together with several Perl library modules. ActivePerl 5.14 is recommended, ActivePerl 5.8, 5.10, and 5.12 are also supported.

Active Perl 5.14.2 (build 1402) from ActiveState Corporation is supplied on the Mascot CD. You must install or upgrade Perl *after* installing the Web server, and *before* installing Mascot.

> **IMPORTANT:** You cannot perform single-step upgrades for ActivePerl. You must uninstall the old version before installing the new one. As a precaution, it is also worth deleting the Perl application directory after the uninstall step.

To install ActivePerl from the CD, in Windows Explorer, double click on the appropriate file:

32-bit:

```
ActivePerl-5.14.2.1402-MSWin32-x86-295342.msi
```

64-bit:

```
ActivePerl-5.14.2.1402-MSWin32-x64-295342.msi
```

It is recommended that you accept all the default options for the installation. Full documentation for ActivePerl 5.14 can be found here:

http://docs.activestate.com/activeperl/5.14/full_toc.html

The Mascot installer uses the Windows file association for the *.pl extension to locate Perl. If you have more than one version of Perl installed, ensure that the file association is for the correct version. You can examine the current association by opening a command window and entering 'ftype Perl'

ActiveState Marketing Requirements

The following statements are included to comply with the ActiveState Redistribution Agreement:

Commercial support for ActivePerl is available through ActiveState at:

http://www.activestate.com/enterprise-edition

For peer support resources for ActivePerl issues see:

http://community.activestate.com/forums/activeperl-support

The ActiveState Repository has a large collection of modules and extensions in binary packages that are easy to install and use. To view and install these packages, use the Perl Package Manager (PPM) which is included with ActivePerl.

ActivePerl is the up-to-date, quality-assured ActivePerl binary distribution from ActiveState. Current releases and other professional tools for open source language developers are available at http://www.activestate.com

Mascot Installation

From 'My Computer' or Windows Explorer, select the Mascot CD and double click on *setup32.exe* (for 32-bit) or *setup64.exe* (for 64-bit).

Before the installation of Mascot begins, required Microsoft Visual C++ libraries will be installed.

The following window will be displayed:
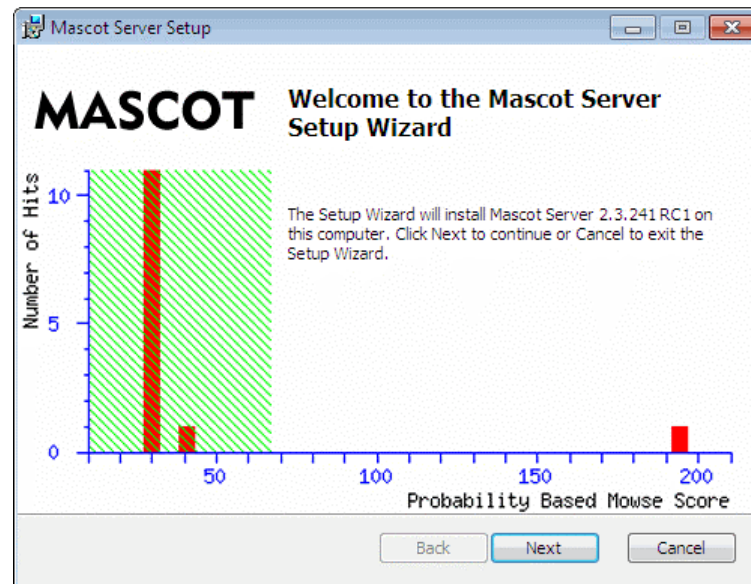
If the installation cannot proceed, a message box will be displayed. Typical problems include:

> **You do not have Administrator privileges:** Log out and log in as a user with local Administrator rights

> **Perl is not installed:** Install Perl as described above

> **Unsupported Windows platform:** Refer to the system requirements at the beginning of this Chapter

Any problem(s) must be fixed before the installer will proceed. Pressing Next displays the Mascot End-User Licence Agreement:

If you do not consent, you cannot proceed with the installation.
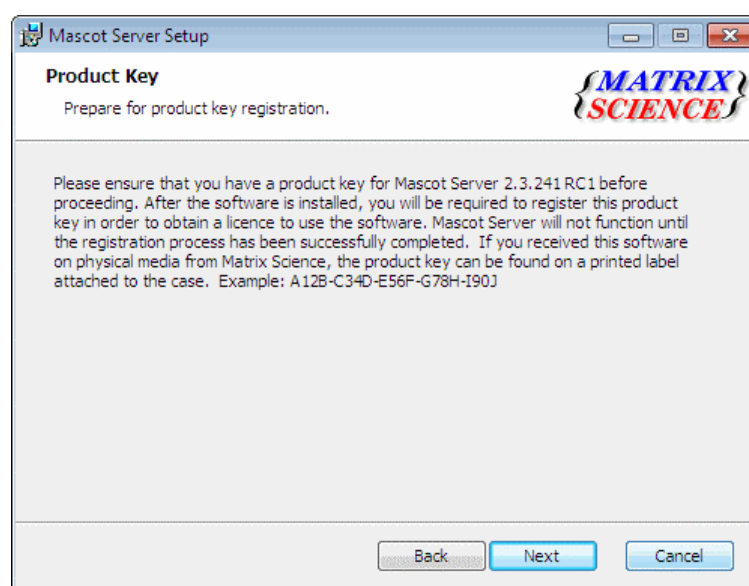
This is a reminder that you will need to register a product key to create a licence file. This product key may be printed on a sticker on the CD case

or it may have been sent by email. If you cannot locate your product key, contact support@matrixscience.com for assistance. The next screen allows you to choose which components will be installed:
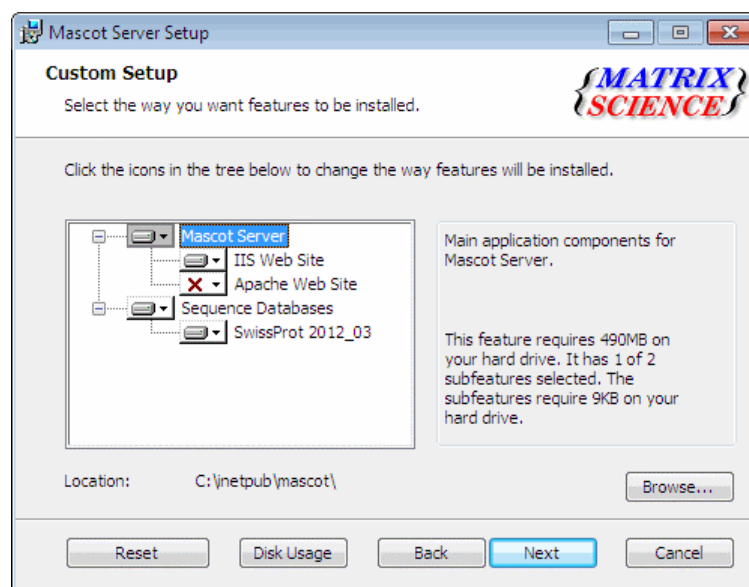
If IIS is installed and functional, the default selections will be as shown above, with IIS being configured automatically. If you don't have IIS installed, the Apache option will be selected instead. A test for whether Apache or some other web server is actually installed comes later.

You can de-select the Swiss-Prot database, but if this is a clean install, you are advised not to do so. It is better to proceed with a full installation, so that correct installation of Mascot can be verified. If you don't want SwissProt to be available, you can easily remove it later

The default location for the installation is $\backslash inetpub\backslash mascot$ on the drive with most free space with the sequence databases in $\backslash inetpub\backslash mascot\backslash sequence$. You can change one or both of these by selecting the component then choosing Browse. If there is insufficient disk space on the selected drive(s), the installation will not be able to continue.

The next step depends on whether IIS or Apache was selected as the web server. For IIS, there will be a drop-down list of all the available web sites. In most cases, you should select 'Default Web Site'. If you select a different web site, refer to the notes on multiple web sites later in this chapter.
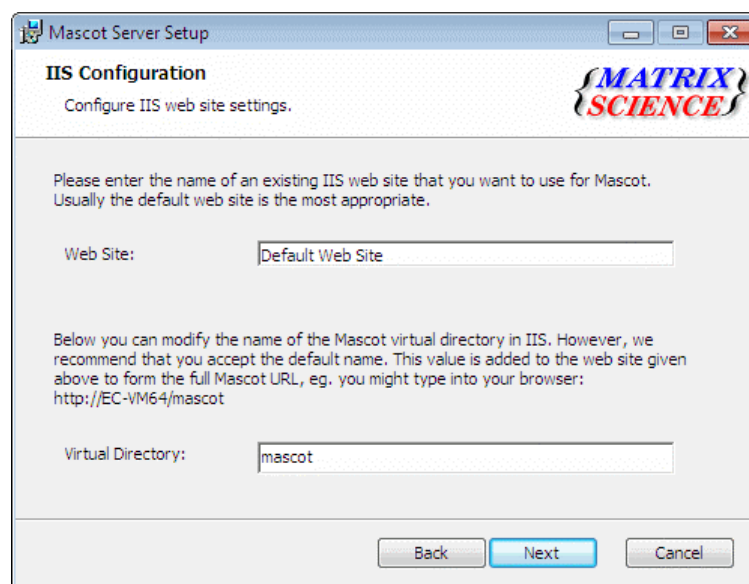
For Apache, or any other web server, you need to confirm the local web server hostname and port. Do not enter localhost as the web site if you wish to access your Mascot server from other computers on your LAN. If there are DNS problems, so that a hostname is not recognised across the LAN, then enter an IP address.

The default ports are 80 for http and 443 for https. The installer will test that the web server responds using the specified hostname and port number. If you have configured your Apache web server as a secure server (https), check the box for 'Use SSL/TLS to access this web site'.
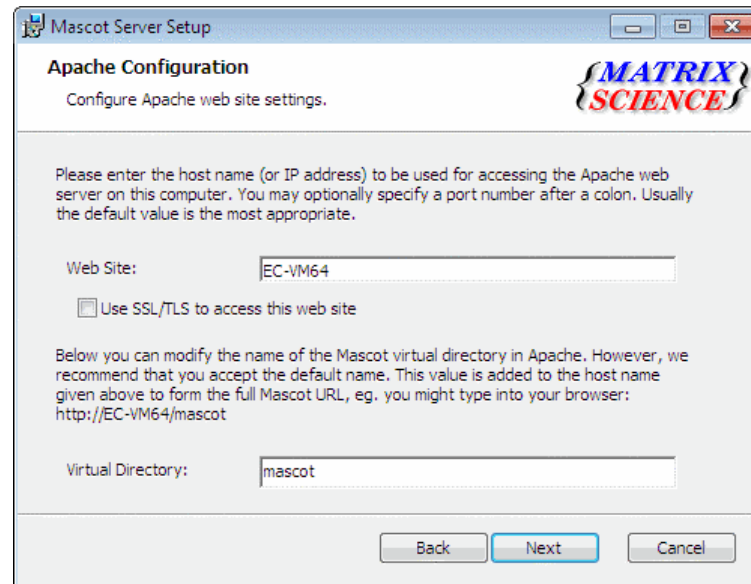
The virtual directory name can be changed, but remember that users are more likely to guess the correct URL if you stick with mascot. Also, some third party software may incorrectly assume the directory name is always mascot.
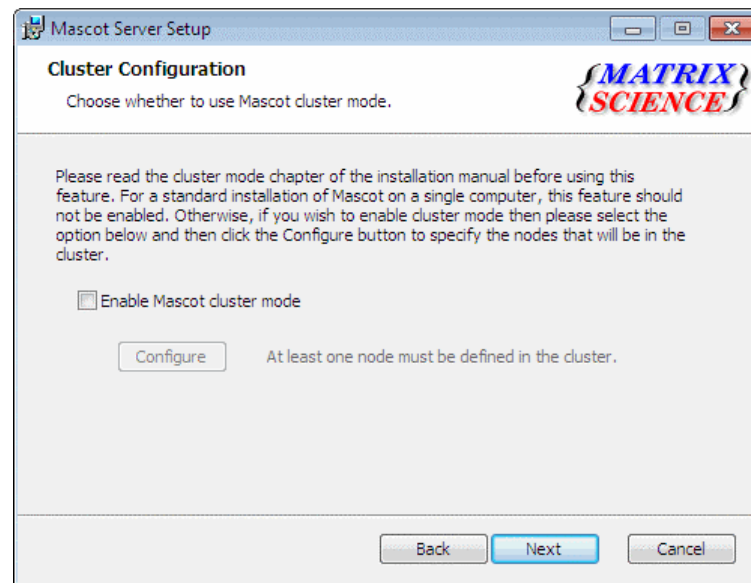
If you have a multi-CPU licence, you can configure Mascot for execution on a networked cluster. If you intend to do this, refer to Chapter 11 for further details before proceeding. If you are installing Mascot on a single multiprocessor server, leave the Enable cluster mode checkbox clear.

The next step is your last opportunity to cancel the installation!
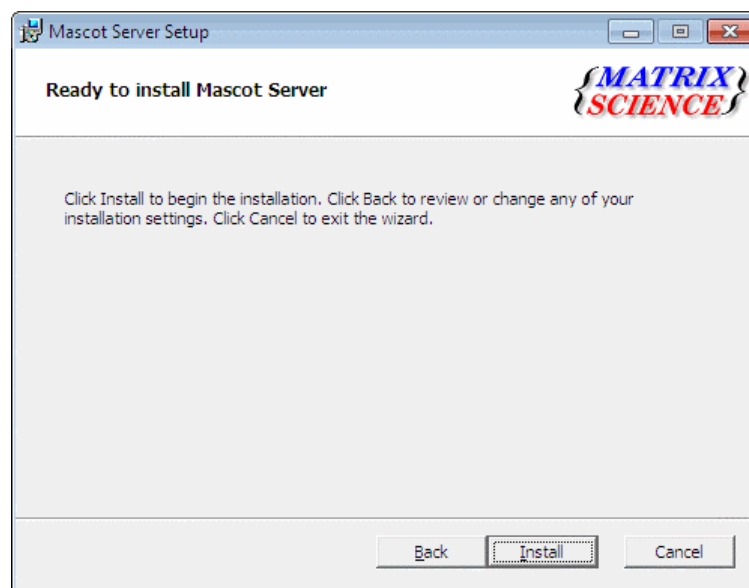
Copying the program files takes only a few minutes
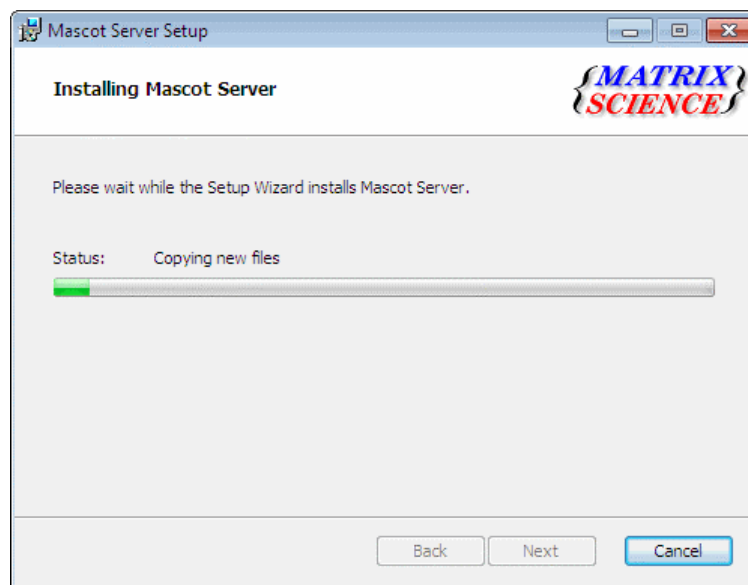
Unpacking the SwissProt files takes longer, and a command window will be displayed at this point. Please be patient and don't try to close the command Window.
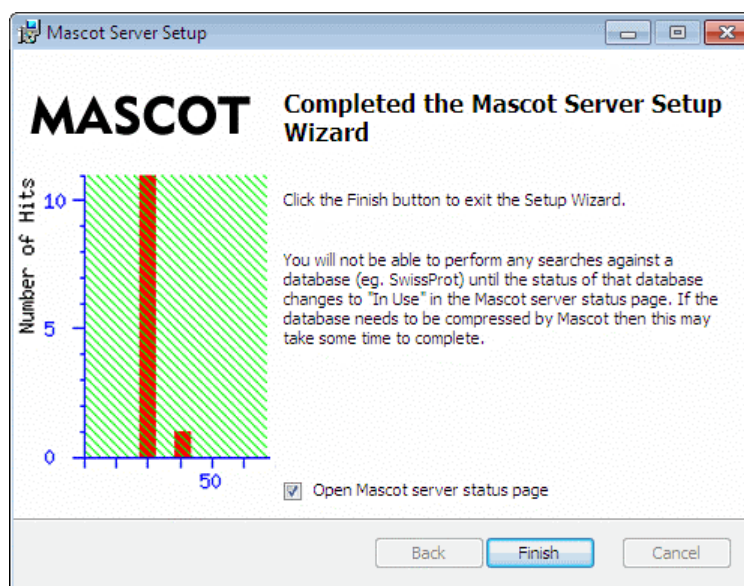
If you are using Apache, model entries for the Apache configuration file can be found in *httpd.conf* in the Mascot *config* directory. Also, ensure that ForkForUnixApache in the Options section of *mascot.dat* is set to 1. Further information on web server configuration can be found in Appendix D.

Installation is finished, but don't clear the checkbox!

Licence Registration

If you cleared the checkbox at the end of the installation wizard, from the Windows Start menu, choose Programs; Mascot; Admin; Database Status. The following screen will be displayed in your default web browser

A product key is required and must be registered online. The licence file will be returned by email and must be saved to the specified location on the Mascot server. If the Mascot server cannot connect to the Internet, a file containing registration information can be saved and copied to a system with Internet access for submission.

The registration form allows a second email address to be specified, in case the person installing Mascot is not the end-user. Ensure that the end-user email address is entered into the upper part of the form and the

email address to which the licence file should be sent is entered into the CC email field in the lower part of the form.

The licence file must be saved to the *config/licdb* directory as a file with the extension *.lic*.

Verify System Operation

A copy of the SwissProt database is included in the files copied from the CD-ROM. It is recommended that the operation of Mascot is verified and tested using this database before adding further databases or making configuration changes.

The Mascot Monitor service is used to manage the swapping and memory mapping of the sequence databases used by Mascot. For Mascot to operate, this service must be running at all times.

Once the new licence file is in place, follow the hyperlink to Database Status. You should see a display similar to the following:

Follow the link at the top centre to view the status of the SwissProt sequence database
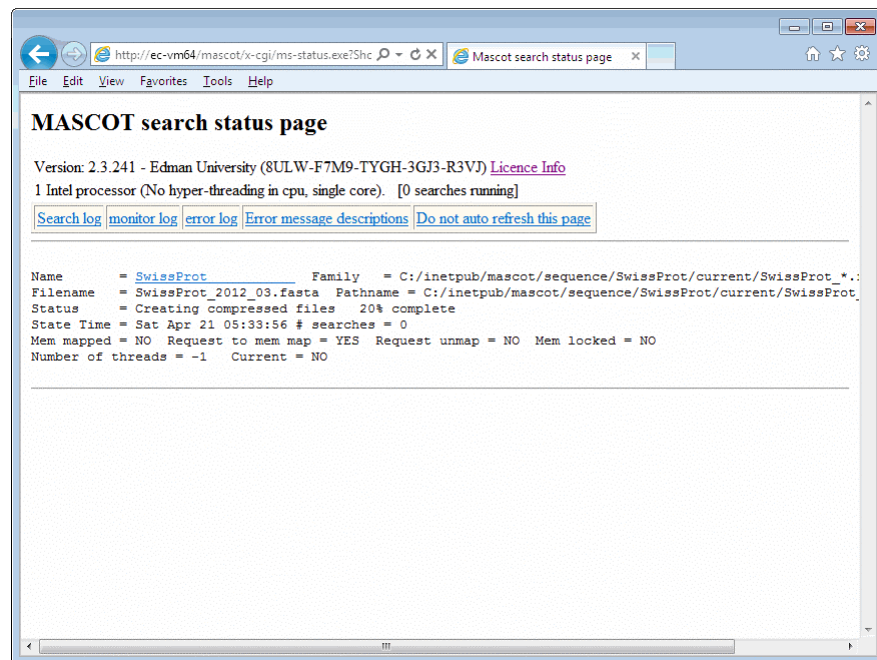
If an error occurs, use the links to the monitor log and the error log to investigate the cause. If all is well, you will see the following messages displayed on the status line for SwissProt:

```
Creating compressed files
Running 1st test
First test just run OK
Trying to memory map files
Just enabled memory mapping
In Use
```

You can begin exploring and using Mascot. However, do not try to run searches or view results reports until the relevant sequence database is 'In Use'.

Windows Firewall

If Windows Firewall is enabled, you may be blocked from accessing the Mascot server from other computers. If so, you need to open up port 80. From the Control Panel, choose Windows Firewall. In the case of Windows XP, on the exceptions tab, check the box for 'World Wide Web Services (HTTP)'.
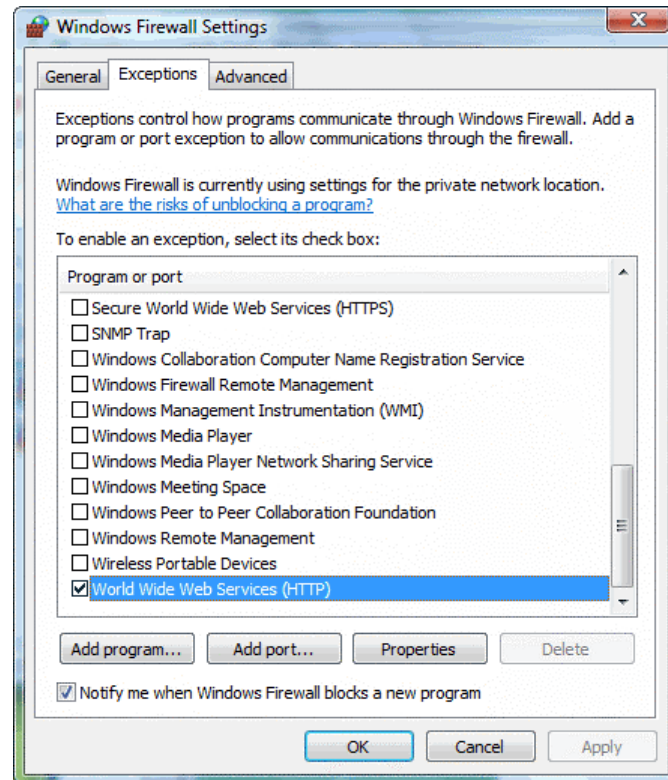
For Windows 7, the appearance is slightly different
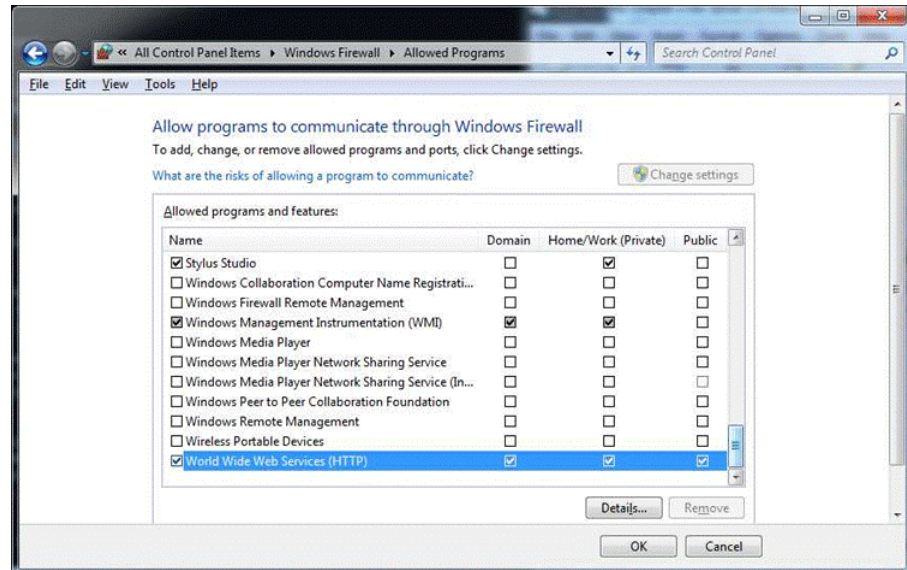
If you installed Apache instead of IIS, there may be no entry for HTTP.
Choose 'Windows firewall with advanced security', then 'Incoming rules'.
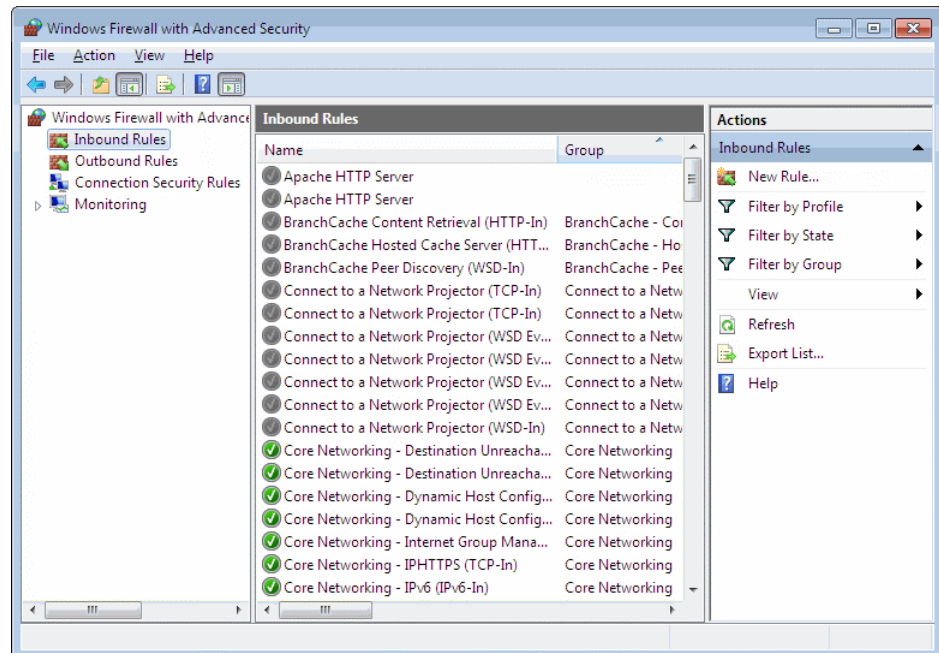
There will be two entries for Apache, one for UDP protocol and one for TCP. Double click the TCP row. On the 'Protocols and ports' tab, configure as shown
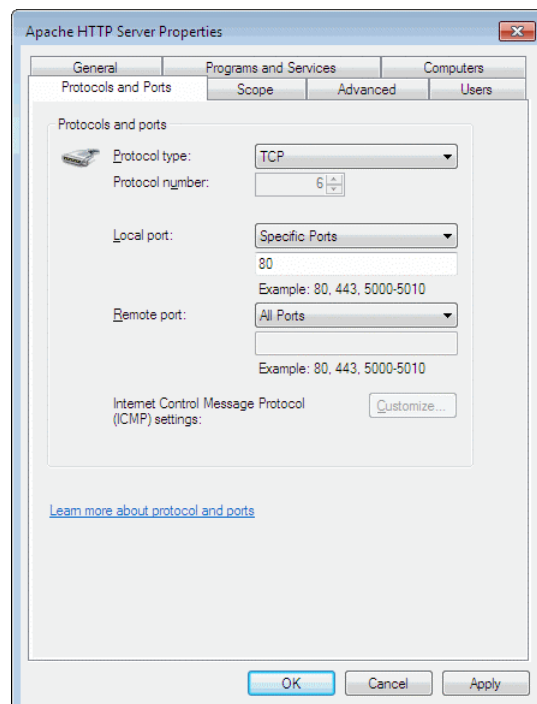
On the Advanced tab, check all three profiles (domain, private, public) and Apply. Back in the top level dialog, with the Apache TCP row selected, choose 'Enable rule'

Security

Mascot security is disabled on installation. To enable Mascot security, refer to Chapter 12

Miscellaneous

LCQ_DTA

This utility, an option on the Mascot search form selection page, makes it possible to a upload a Thermo *.raw file, as opposed to a peak list, when submitting a Mascot search. The HTML form executes a utility from Thermo for generating a peak list from a centroided raw file. For more details, see the Mascot HTML help page

http://www.matrixscience.com/help/instruments_xcalibur.html#EXTRACT.

The script supplied with Mascot expects to find the executable in the path *C:\Program Files\Thermo\ExtractMSn\ExtractMSn.exe*. If the program is in another directory, e.g. on a 64-bit system it might be in *Program Files (x86)*, or if the executable has a different name, open the file *mascot\cgi\lcq_dta_shell.pl* in an editor such as Notepad, and modify the following line:

```
my $lcqExe = "C:\\Program
    Files\\Thermo\\ExtractMSn\\ExtractMSn.exe";
```

Note that the backslashes used as directory delimiters must be entered in pairs, exactly as shown above.

The script needs to create temporary files, and it uses a directory *C:\TEMP*. If this does not exist, you should either create it, or change the following line to point to a suitable temporary directory.

```
my $tempDir = "c:\\temp";
```

To use the lcq_dta form, enter the filename and any other parameters required, and press the "Generate .DTA Files" button. After a few seconds, the Mascot search screen will be displayed. Enter search parameters and proceed as normal.

Multiple web sites

Using IIS on Server versions of Windows, it is possible to create multiple web sites. If multiple web sites exist when Mascot is installed, you will be offered the choice of which web site to install under. However, there

are a number of issues to be aware of if the "Default Web Site" is not used.

Perl installation.

On installation, ActiveState Perl only sets up mappings for the default web site. To add mappings for another web site, right click on the new web site, choose properties, and then click on the "Home Directory" tab. Then, click on the configuration button. If there is no entry in the list for .pl extension, click on "Add" and enter the following information:
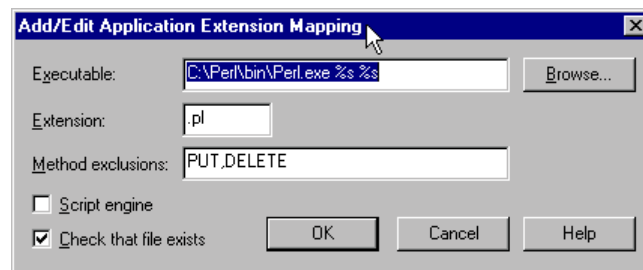
Host name

Mascot will be installed in the correct virtual directory, but the host (base) name may be wrong – the installation program has chosen the computer name.

If you have set up multiple web sites, then it is probable that you have created a DNS entry that is the same as the "Host header name". In this case, replace the computer name with the "Host header name".
Refer to the IIS documentation for details on setting up multiple web sites using the same IP and port address. Briefly, you will need to add a new web site, and then click on the "Web site tab" of the properties box. Next, click on the Advanced button, and enter a host name:
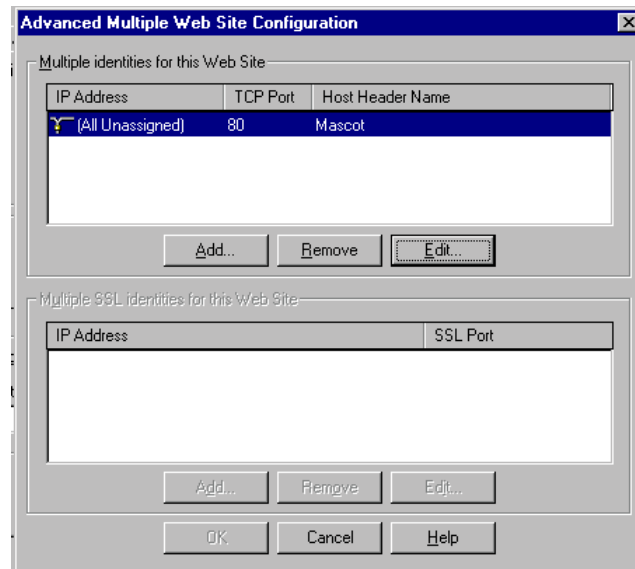
To memory lock databases totalling more than 2 GB

For 32 bit editions of Windows, there is a 2 GB limit on the address space for any single process. Mascot Monitor, (ms-monitor.exe), can easily reach this limit by trying to lock several large databases into memory. To work around the 2GB limit, a separate ms-lockmem.exe program is provided – this is fork/exec'd from ms-monitor.exe when the flag 'SeparateLockMem 1' is added to the options section of mascot.dat. Further details can be found in Chapter 7.

Hyper-threading

Intel only: Hyper-threading is a technique used by Intel to improve the performance of multi-threaded programs. Hyper-threading does not double performance because pairs of cores share other resources, such as the on-chip cache. On some systems, a BIOS setting can be used to enable and disable hyper-threading.

Hyper-threading is detected automatically. Each CPU in the Mascot licence enables up to 4 cores to be used for searches. Hyper-threading is ignored when counting cores, so that you may see a 1 CPU licence using

8 threads on a system with a quad core processor with hyper-threading enabled.

Troubleshooting

Check the Mascot Server Support Page

There may be a fix listed on the Matrix Science Web Site. From the menu, choose Support; Mascot Server and scan down to see if your problem is described.

The installation program doesn't recognise Perl

To test whether Perl is correctly installed, you can open a command window, and type:

```
perl -v
```

The version number should be displayed. If this seems to be functioning correctly, and the Perl version is either 5.8, 5.10, 5.12 or 5.14, re-start the computer and then re-run the Mascot installation program. If it still fails, contact Matrix Science technical support (support@matrixscience.com).

If, when you type **perl -v** you see the text:

```
The name specified is not recognized as an
internal or external command, operable program or
batch file.
```

then Perl is not installed or is not on the path. If you have just installed it, you should try restarting the computer and performing the test again. If that fails, try re-installing Perl, making sure that you choose the option to add it to the path.

The status screen shows an error

If the Mascot Monitor service fails to start, then the following text or something similar will be displayed in the status screen:
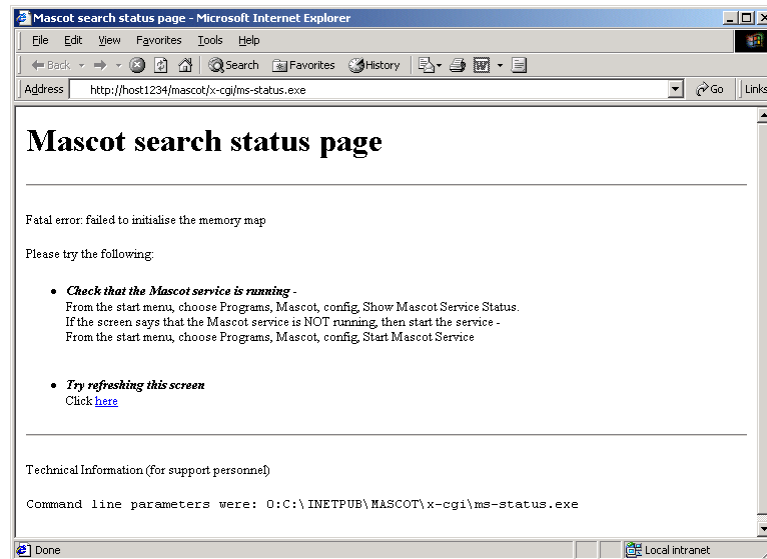
There are several possible causes:

Service not started

Since one of the first things that the Monitor service does is to create the memory mapped file, this could indicate that the service has not started. You can tell whether the service has started by choosing *Start; Programs; mascot; config; Show Mascot ms-monitor service status.*

If the service is not running, check the `monitor.log` and `errorlog.txt` file in the `logs` directory. If there is nothing in those files, then it may be necessary to try and run `ms-monitor.exe` as a command line executable. *You should only do this if the Mascot service is not running.* To do this, open a command prompt window, and change directory to the mascot `bin` directory. If your installation path was the default, you will need to type:

cd \Inetpub\mascot\bin

next start the monitor program:

ms-monitor DEBUG

Any error messages should be displayed on the screen. If possible, correct the faults, and then start the Mascot Service from the start menu. Note that the mascot service should never be running at the same time as `ms-monitor.exe` is being run from the command line.

International Versions Of Windows

If Mascot is installed on a version of Windows that is not in the English language, then when the ms-status screen is displayed, it may have the error 'Failed to initialise memory map"

To correct this fault, the following procedure is required:

1. You will need to find the names of the 'groups' that your version of Windows uses for Administrators and Users. In German, for example, these names are "Administratoren" and "Benutzer" respectively. To see a list of User names, from the start menu, select Programs, Administrative Tools (common), User Manager. The section at the bottom of the screen displays the group names. Make a note of the two names.

2. From the start menu, select
     Programs | Mascot | Config | Stop Mascot Service

3. From the start menu, select
     Programs | Mascot | Config | Mascot Configuration File

4. Scroll down to near the bottom of the file and find the line:
     `NTIUserGroup Users`
   and change this to (for example, for German)
     `NTIUserGroup Benutzer`

5. Find the line
     `NTMonitorGroup Administrators`
   and change this to (for example, for German)
     `NTMonitorGroup Administratoren`

6. Save the mascot.dat file

7. Delete the files:
     `c:\inetpub\mascot\sequence\SwissProt`
                       `\current\SwissProt*.a00`
     `c:\inetpub\mascot\data\mascot.control`
   (Note that these files may be in a different directory if you did not install mascot in the default location)

8. From the start menu, select
     Programs | Mascot | Config | Start Mascot Service

9. Re-load the status page:
     Programs | Mascot | Search Status
   (You may need to re-fresh / re-load the page)

Wait until the files have been compressed and a test search has been done. Mascot is now ready for use.

The site search facility does not work

The local Mascot web pages are indexed using a product called ht://Dig. A log file is made as the indexes are built during the installation. The log file *mascot\htdig\build.log* may contain an error message indicating the nature of the problem.

If the web server was not operational during Mascot installation, it will not have been possible to build the keyword index. To build or rebuild it, open a command window and enter the following commands. If Mascot was installed into a different path, you may have to modify the first two lines

```
C:
cd \inetpub\mascot\htdig
bin\htdig.exe -v
bin\htmerge.exe -v
```

Once the commands have completed, keyword search using the control at the top right of the web pages should be operational

Search status shows a failure to create compressed files

On the search screen, find out what caused the error by clicking on the *Error log* link, fix the fault, (possibly out of disk space), and then click on *retry*.

4

Validation

CGI Operation

To verify that the search engine is functioning correctly when executed as a CGI application, launch a JavaScript aware web browser and load the Mascot home page, (http://your_server/mascot/). Select Mascot from the main menu and then choose the "Peptide Mass Fingerprint" link near the top of the page. This will load the search form for a peptide mass fingerprint.

Enter your name and email address into the fields at the top of the form and type a number, say 1234, into the Query field. Then press the Start Search… button.

The search form will be replaced by the search progress screen. This has a few lines of text at the top, ending in the line "Searching …". Additional lines will appear showing the percentage of the search that has been completed.

Once the search is complete, the Master Results page will appear. Unless you went to the trouble of entering some real mass values, the results will be meaningless!

Monitor Test

When Mascot Monitor is started, it runs a test search against each sequence database. It also runs this same test search against any update to the database as part of the exchange procedure. If the test search fails, an error message will be displayed in the Mascot Status screen and the database will not be available for searching. Error messages from Monitor are logged to *errorlog.txt* in the *mascot/logs* directory. Both this file and *monitor.log* can be viewed using links on the Mascot Status page.

The input file which defines a test search can be found in the *mascot/ data/test* directory. The filename is constructed from the name of the

database together with the extension `.asc`. For example,
SwissProt.asc.

Note: Test files for new databases are generated by modifying
do_not_delete.asc. Never delete this file.

The output of the test search may change slightly with each new update
to a database. Sequences may be corrected or descriptions modified.
Quite often, a new entry appears which is very homologous with one of
the matched proteins so that it appears on the hit list.

Using SwissProt 2012_03, the report from running the standard test
search is shown on the following pages.

{MATRIX}{SCIENCE} Mascot Search Results

```
User          : Monitor Test DB 0
Email         :
Search title  : MS/MS Test Search
MS data file  : test_search.mgf
Database      : SwissProt 2012_03 (535248 sequences: 189901164 residues)
Timestamp     : 14 Apr 2012 at 18:46:03 GMT
Protein hits  : CH60_HUMAN  60 kDa heat shock protein, mitochondrial OS=Homo sapiens GN=HSPD1 PE=1 SV=2
                CH60_DROME  60 kDa heat shock protein, mitochondrial OS=Drosophila melanogaster GN=Hsp60 PE=1 SV=3
                CH60_CAEEL  Chaperonin homolog Hsp-60, mitochondrial OS=Caenorhabditis elegans GN=hsp-60 PE=1 SV=2
                CH60_STRM5  60 kDa chaperonin OS=Stenotrophomonas maltophilia (strain R551-3) GN=groL PE=3 SV=1
```

Mascot Score Histogram

Ions score is -10*Log(P), where P is the probability that the observed match is a random event.
Individual ions scores > 41 indicate identity or extensive homology (p<0.05).
Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits.
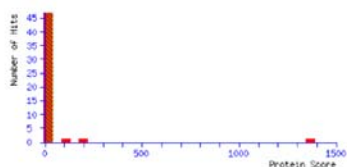
Peptide Summary Report

Format As	Peptide Summary			Help

Significance threshold p< 0.05   Max. number of hits AUTO

Standard scoring ⊙ MudPIT scoring ○   Ions score or expect cut-off 0   Show sub-sets 0

Show pop-ups ⊙ Suppress pop-ups ○   Sort unassigned Decreasing Score   Require bold red □

Select All	Select None	Search Selected	□ Error tolerant	Archive Report

1.   **CH60_HUMAN**   Mass: 61187   Score: 1365   Matches: 33(29)   Sequences: 21(19)
      60 kDa heat shock protein, mitochondrial OS=Homo sapiens GN=HSPD1 PE=1 SV=2
      □ Check to include this hit in error tolerant search or archive report

	Query	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Score	Expect	Rank	Unique	Peptide
☑	11	417.1822	832.3498	832.3828	-39.57	0	45	0.018	1		K.APGFGDNR.K
☑	12	422.7433	843.4720	843.5066	-40.97	0	46	0.037	1	U	K.VGEVIVTK.D
☑	13	430.7328	859.4510	859.4837	-38.02	0	36	0.32	1	U	K.IPAMTIAK.N + Oxidation (M)
☑	15	451.2499	900.4853	900.5280	-47.49	0	56	0.003	1	U	K.LSDGVAVLK.V
☑	16	456.7806	911.5467	911.5804	-37.02	0	59	0.00072	1	U	K.VGLQVVAVK.A
☑	21	480.7447	959.4748	959.5036	-29.99	0	46	0.033	1	U	R.VTDALNATR.A
☑	24	595.7855	1189.5565	1189.6012	-37.62	0	(57)	0.002	1	U	K.EIGNIISDAMK.K
☑	25	603.7720	1205.5294	1205.5962	-55.38	0	60	0.00077	1	U	K.EIGNIISDAMK.K + Oxidation (M)
☑	26	608.3099	1214.6052	1214.6507	-37.42	0	73	4.6e-05	1	U	K.NAGVEGSLIVEK.I
☑	27	617.2857	1232.5569	1232.5885	-25.65	0	81	7.3e-06	1	U	K.VGGTSDVEVNEK.K
☑	31	672.8375	1343.6605	1343.7085	-35.73	0	64	0.00032	1	U	R.TVIIEQSWGSPK.V
☑	34	714.8884	1427.7623	1427.8058	-30.44	0	(65)	0.00027	1	U	R.GVMLAVDAVIAELK.K
☑	35	714.8938	1427.7730	1427.8058	-22.92	0	(73)	4.2e-05	1	U	R.GVMLAVDAVIAELK.K
☑	36	722.8849	1443.7552	1443.8007	-31.49	0	75	2.4e-05	1	U	R.GVMLAVDAVIAELK.K + Oxidation (M)
☑	37	722.8934	1443.7722	1443.8007	-19.74	0	(75)	2.4e-05	1	U	R.GVMLAVDAVIAELK.K + Oxidation (M)
☑	39	752.8643	1503.7141	1503.7490	-23.23	0	(90)	8.5e-07	1	U	K.TLNDELEIIEGMK.F
☑	40	760.8461	1519.6777	1519.7439	-43.58	0	93	3.2e-07	1	U	K.TLNDELEIIEGMK.F + Oxidation (M)
☑	41	886.4059	1770.7972	1770.8458	-27.43	0	46	0.016	1	U	R.CIPALDSLTPANEDQK.I
☑	45	640.3281	1917.9625	1918.0636	-52.67	0	102	4.2e-08	1	U	K.ISSIQSIVPALEIANAHR.K
☑	46	960.0327	1918.0509	1918.0636	-6.62	0	(89)	8e-07	1	U	K.ISSIQSIVPALEIANAHR.K
☑	48	1019.5106	2037.0067	2037.0153	-4.25	0	53	0.0032	1	U	R.IQEIIEQLDVTTSEYEK.E
☑	49	1020.9879	2039.9613	2040.0375	-37.37	0	88	9.8e-07	1	U	K.PVTTPEEIAQVATISANGDK.E
☑	51	1057.0537	2112.0929	2112.1323	-18.66	0	116	1.4e-09	1	U	R.ALMLQGVDLLADAVAVTMGPK.G
☑	52	1065.0399	2128.0653	2128.1272	-29.09	0	(72)	3.6e-05	1	U	R.ALMLQGVDLLADAVAVTMGPK.G + Oxidation (M)
☑	53	1065.0623	2128.1100	2128.1272	-8.10	0	(25)	1.8	1	U	R.ALMLQGVDLLADAVAVTMGPK.G + Oxidation (M)
☑	54	1073.0477	2144.0809	2144.1221	-19.22	0	(92)	3.7e-07	1	U	R.ALMLQGVDLLADAVAVTMGPK.G + 2 Oxidation (M)
☑	58	789.1062	2364.2968	2364.3264	-12.53	1	(56)	0.0012	1	U	R.KPLVIIAEDVDGEALSTLVLNR.L
☑	59	1183.1570	2364.2994	2364.3264	-11.42	1	(64)	0.00018	1	U	R.KPLVIIAEDVDGEALSTLVLNR.L
☑	60	789.1094	2364.3063	2364.3264	-8.50	1	95	1.5e-07	1	U	R.KPLVIIAEDVDGEALSTLVLNR.L
☑	61	828.1238	2481.3495	2481.3942	-18.00	0	(24)	1.7	1	U	R.TALLDAAGVASLLTTAEVVVTEIPK.E
☑	62	828.1322	2481.3748	2481.3942	-7.81	0	45	0.013	1	U	R.TALLDAAGVASLLTTAEVVVTEIPK.E
☑	64	854.0588	2559.1545	2559.2413	-33.90	0	75	1.2e-05	1		K.LVQDVANNTNEEAGDGTTTATVLAR.S

☑ 65 1038.5031 3112.4873 3112.5023 -4.81 0   13   15   1   U   K.DMAIATGGAVFGEEGLTLNLEDVQPHDLGK.V + Oxidation (M

---

2. CH60_DROME   **Mass:** 60885   **Score:** 175   **Matches:** 4(2)   **Sequences:** 4(2)
    60 kDa heat shock protein, mitochondrial OS=Drosophila melanogaster GN=Hsp60 PE=1 SV=3
    ☐ Check to include this hit in error tolerant search or archive report

Query	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Score	Expect	Rank	Unique	Peptide
11	417.1822	832.3498	832.3828	-39.57	0	45	0.018	1		K.APGFGDNR.K
27	617.2857	1232.5569	1232.5885	-25.63	0	42	0.058	2	U	R.VGGSSEVEVNEK.K
59	1183.1570	2364.2994	2364.3264	-11.42	1	12	26	2	U	R.KPLVIIAEDIDGEALSTLVVNR.L
64	854.0588	2559.1545	2559.2413	-33.90	0	75	1.2e-05	1		K.LVQDVANNTNEEAGDGTTTATVLAR.A

---

3. CH60_CAEEL   **Mass:** 60235   **Score:** 139   **Matches:** 3(3)   **Sequences:** 2(2)
    Chaperonin homolog Hsp-60, mitochondrial OS=Caenorhabditis elegans GN=hsp-60 PE=1 SV=2
    ☐ Check to include this hit in error tolerant search or archive report

Query	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Score	Expect	Rank	Unique	Peptide
11	417.1822	832.3498	832.3828	-39.57	0	45	0.018	1		K.APGFGDNR.K
39	752.8643	1503.7141	1503.7490	-23.23	0	(90)	8.5e-07	1	U	K.TLNDELELIEGMK.F
40	760.8461	1519.6777	1519.7439	-43.58	0	93	3.2e-07	1	U	K.TLNDELELIEGMK.F + Oxidation (M)

---

4. CH60_STRM5   **Mass:** 57312   **Score:** 42   **Matches:** 1(1)   **Sequences:** 1(1)
    60 kDa chaperonin OS=Stenotrophomonas maltophilia (strain R551-3) GN=groL PE=3 SV=1
    ☐ Check to include this hit in error tolerant search or archive report

Query	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Score	Expect	Rank	Unique	Peptide
16	456.7806	911.5467	911.6168	-76.92	1	42	0.035	2	U	R.GIVKVVAVK.A

    Proteins matching the same set of peptides:
    CH60_STRMK   **Mass:** 57339   **Score:** 42   **Matches:** 1(1)   **Sequences:** 1(1)
    60 kDa chaperonin OS=Stenotrophomonas maltophilia (strain K279a) GN=groL PE=3 SV=1
    CH60_XANAC   **Mass:** 57131   **Score:** 42   **Matches:** 1(1)   **Sequences:** 1(1)
    60 kDa chaperonin OS=Xanthomonas axonopodis pv. citri GN=groL PE=3 SV=1
    CH60_XANC5   **Mass:** 57163   **Score:** 42   **Matches:** 1(1)   **Sequences:** 1(1)
    60 kDa chaperonin OS=Xanthomonas campestris pv. vesicatoria (strain 85-10) GN=groL PE=3 SV=1
    CH60_XANC8   **Mass:** 57149   **Score:** 42   **Matches:** 1(1)   **Sequences:** 1(1)
    60 kDa chaperonin OS=Xanthomonas campestris pv. campestris (strain 8004) GN=groL PE=3 SV=1
    CH60_XANCB   **Mass:** 57177   **Score:** 42   **Matches:** 1(1)   **Sequences:** 1(1)
    60 kDa chaperonin OS=Xanthomonas campestris pv. campestris (strain B100) GN=groL PE=3 SV=1
    CH60_XANCH   **Mass:** 57190   **Score:** 42   **Matches:** 1(1)   **Sequences:** 1(1)
    60 kDa chaperonin OS=Xanthomonas campestris pv. phaseoli GN=groL PE=3 SV=1
    CH60_XANCP   **Mass:** 57149   **Score:** 42   **Matches:** 1(1)   **Sequences:** 1(1)
    60 kDa chaperonin OS=Xanthomonas campestris pv. campestris GN=groL PE=3 SV=1
    CH60_XANOM   **Mass:** 57121   **Score:** 42   **Matches:** 1(1)   **Sequences:** 1(1)
    60 kDa chaperonin OS=Xanthomonas oryzae pv. oryzae (strain MAFF 311018) GN=groL PE=3 SV=1
    CH60_XANOP   **Mass:** 57121   **Score:** 42   **Matches:** 1(1)   **Sequences:** 1(1)
    60 kDa chaperonin OS=Xanthomonas oryzae pv. oryzae (strain PXO99A) GN=groL PE=3 SV=1
    CH60_XANOR   **Mass:** 57121   **Score:** 42   **Matches:** 1(1)   **Sequences:** 1(1)
    60 kDa chaperonin OS=Xanthomonas oryzae pv. oryzae GN=groL PE=3 SV=1

Peptide matches not assigned to protein hits: (no details means no match)

	Query	Observed	Mr(expt)	Mr(calc)	ppm	Miss	Score	Expect	Rank	Unique	Peptide
☑	33	714.3649	1426.7153	1426.8078	-64.84	1	20	9.2	1		NLRECLLLQLR
☑	9	747.3962	746.3889	746.3381	68.1	0	18	23	1		FAMDAAR + Oxidation (M)
☑	14	442.2283	882.4421	882.5287	-98.15	1	17	15	1		AEPRAVLK
☑	30	663.8379	1325.6612	1325.7667	-79.54	0	15	28	1		VTIIEQALAVNR
☑	4	662.2756	661.2683	661.3217	-80.80	0	14	22	1		QGQMAK
☑	6	673.3495	672.3422	672.3555	-19.74	0	12	82	1		AVNDVR
☑	23	1101.6217	1100.6144	1100.6012	12.0	0	12	60	1		QLLMVAGVDR
☑	8	714.3725	713.3652	713.4072	-58.79	0	11	61	1		LAPAQSK
☑	57	747.0361	2238.0864	2238.1089	-10.08	0	10	52	1		SGESLSLGYPAGMASDEVILVK + Oxidation (M)
☑	38	749.3840	1496.7534	1496.7657	-8.21	0	9	1e+02	1		ELQSLCLDIAAMK
☑	22	1101.5366	1100.5293	1100.5349	-5.09	0	9	1.3e+02	1		ENVIPADSEK
☑	29	642.3536	1282.6926	1282.7357	-33.64	0	8	1.3e+02	1		VVGVAGQGASALVR
☑	2	500.2560	499.2487	499.2278	41.9	0	8	6.4	1		EASPP
☑	19	932.3644	931.3571	931.4433	-92.57	1	6	54	1		KGVESCPVG
☑	56	1119.0452	2236.0758	2236.0947	-8.44	0	6	1.5e+02	1		CQLLFDVNDANILGDNFLR
☑	28	642.3526	1282.6906	1282.6447	35.8	1	4	3.5e+02	1		KMVMGSHIGGIK + 2 Oxidation (M)
☑	44	949.5507	1897.0869	1897.0520	18.4	0	3	2.7e+02	1		ILLVDDLLATGGTAKAGIR
☑	55	1099.0947	2196.1749	2196.1646	4.67	1	3	2.7e+02	1		AGGITGYLNCLIGMKSLVSVK + Oxidation (M)
☑	67	1116.1775	3345.5106	3345.7564	-73.45	1	2	1.4e+02	1		MHGVLVDVYGIGMLITGESGIGKSETALELIK + Oxidation (M)
☑	10	747.4125	746.4052	746.3633	56.2	0	2	1.1e+03	1		MAPSTPK + Oxidation (M)
☑	20	933.4990	932.4917	932.4498	45.0	1	2	8.5e+02	1		SRDPGHVK + Oxidation (M)
☑	7	711.3647	710.3574	710.3711	-19.31	0	1	4.1e+02	1		GGAHEIK
☑	32	711.3707	1420.7269	1420.7054	15.1	0	1	6.3e+02	1		PSVVMMVGLQGSGK + 2 Oxidation (M)
☑	1	498.2729	497.2656								

☑	3	575.5584	574.5511
☑	5	662.4172	661.4099
☑	17	930.6831	929.6758
☑	18	930.7030	929.6957
☑	42	932.4608	1862.9071
☑	43	933.0038	1863.9930
☑	47	665.0096	1992.0069
☑	50	1048.5615	2095.1085
☑	63	832.7986	2495.3739
☑	66	1113.8947	3338.6621

Search Parameters

```
Type of search          : MS/MS Ion Search
Enzyme                  : Trypsin/P
Fixed modifications     : Carbamidomethyl (C)
Variable modifications  : Oxidation (M)
Mass values             : Monoisotopic
Protein Mass            : Unrestricted
Peptide Mass Tolerance  : ± 100 ppm
Fragment Mass Tolerance : ± 0.1 Da
Max Missed Cleavages    : 1
Instrument type         : ESI-QUAD-TOF
Number of queries       : 67
```

Mascot: http://www.matrixscience.com/

5

Sequence Database Setup

S equence database URL's and formats change constantly. Provided your Mascot Server can connect to the Internet, Mascot Database Manager will keep database definitions up-to-date automatically for many popular public databases. For each database, you can configure a file update schedule, so that new releases are downloaded automatically. For more information about Database Manager, refer to the Mascot HTML help pages.

If you want to set up a custom database, such as the proteome or genome of a single organism, download and configuration information can also be found in the Mascot HTML help pages. Note that the HTML help pages for your in-house Server are only updated when you install a new version of Mascot, so for the latest information, go to the help pages on the Matrix Science public web site (http://www.matrixscience.com/help/seq_db_setup.html).

This chapter contains reference material, most of which is only important if you choose not to use Database Manager.

The Fasta Format

Mascot can search any Fasta format sequence database as long as it can parse a unique identifier (accession string) from each entry in a consistent fashion. The accession string can contain any US-ASCII printing characters except comma and double quotes.

The Fasta format is extremely simple. Each entry consists of a one line title followed by one or more lines containing the contiguous sequence string in 1 letter code. Fasta databases can contain either amino acid sequences or nucleic acid sequences, but not both. Nucleic acid databases are translated on the fly by Mascot in all six reading frames.

The Fasta title line begins with a "greater than" character, followed by one or more accession strings, and an optional text string describing the entry. Apart from the use of the "greater than" character, the precise syntax of the title line varies from database to database. The title line is delimited from the sequence that follows by a platform dependent new line character.

The title line is followed by lines of contiguous sequence characters. Line lengths vary between databases; anything from 60 characters to a thousand or more. Mascot can handle lines up to 50,000 characters long. The end of a sequence is indicated when the following line is either a new title line or the end of the file. For example:

```
.
.
.
VYEYVRKYAEHRMLVVAEQPLHAMRKGLLDVLPKNSLEDLTAEDFRLLVNGCGEVNVQML
ISFTSFNDESGENAEKLLQFKRWFWSIVERMSMTERQDLVYFWTSSPSLPASEEGFQPMP
SITIRPPDDQHLPTANTCISRLYVPLYSSKQILKQKLLLAIKTKNFGFV
>104K_THEPA (P15711) 104 KD MICRONEME-RHOPTRY ANTIGEN.
MKFLILLFNILCLFPVLAADNHGVGPQGASGVDPITFDINSNQTGPAFLTAVEMAGVKYL
QVQHGSNVNIHRLVEGNVVIWENASTPLYTGAIVTNNDGPYMAYVEVLGDPNLQFFIKSG
DAWVTLSEHEYLAKLQEIRQAVHIESVFSLNMAFQLENNKYEVETHAKNGANMVTFIPRN
.
.
.
```

Mascot doesn't search the Fasta file directly. When a new database is recognised, Mascot Monitor uses the Fasta file to create a set of compressed files. One reason for doing this to separate the sequence string from the title line, because only the sequence string needs to be memory mapped. In the case of a database with predominantly short sequences, this greatly reduces the amount of memory required. In the case of a nucleic acid database, the limited character set allows Mascot to pack two base codes into each byte of memory. If a taxonomy filter is required, a taxonomy index is built at the same time as the file is compressed.

Naming Conventions and Directory Structure

Although Microsoft Windows permits file and directory names to include spaces, file and directory names to be used by Mascot, or to appear in a URL, cannot include spaces.

By following some simple conventions in database naming, Mascot Monitor enables sequence databases to be automatically updated without any disruption to on-going searches.

The procedure followed by Monitor is that the new database is compressed and tested by running a standard search. If errors are detected in the new database, the database exchange process is abandoned. Assuming the test is successful, all new searches are performed against the new database, while searches that were in progress against the old database are allowed to continue. Once the final search against the old database is complete, the disk file is moved into an archive directory. If the database being exchanged is memory mapped, the mapping and un-mapping are also handled automatically.

Assuming that the new database will be updated periodically, a directory structure similar to the one created for SwissProt during installation is recommended. For example:
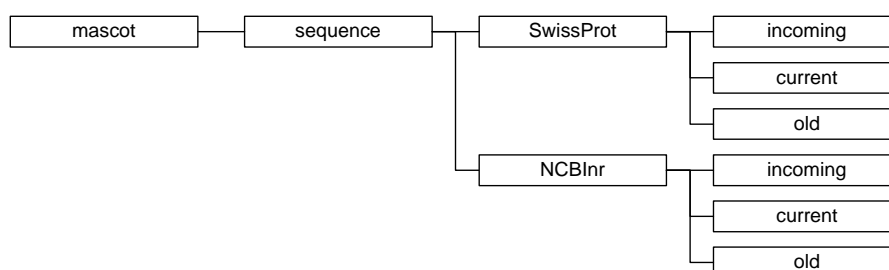
For each database, the *incoming* directory provides a workspace for downloading and expanding a new database file. The *current* directory contains the active database, and this is where Mascot Monitor creates the memory mapped compressed files. The *old* directory is where the immediate past database file is archived … just in case.

In the Mascot configuration, the filename for each database **must** include a wild card. This is to enable the automatic recognition and exchange of an update file. For example, the filename for the SwissProt database might be defined as `SwissProt_*.fasta`. This would match to filenames that included a release number, e.g. *SwissProt_2012_03.fasta,* or a date stamp, e.g. *SwissProt_20120311.fasta*.

Whenever Monitor sees a file in that directory which matches to the database name and is *not* the current database, it will initiate the exchange process. This is why the wild card is important, even though you may not wish to track database dates or revision numbers.

Even if you never intend to swap a database, and have called it (say) *SwissProt.fasta*, you must still define it in the Mascot configuration using a wild card as `SwissProt*.fasta`.

Database File Update Procedure

Mascot Database Manager can update database files automatically to a specified schedule. This section describes how to update the files for a database if your Mascot Server is not connected to the Internet or if you choose not to use Database Manager.

When a new release of a database becomes available, it should be copied or downloaded into the *incoming* directory. In many cases, the downloaded file will have to be de-compressed. The filename may or may not be constant from release to release.

The Fasta database should be renamed to a name that includes a version or date stamp and matches the wild card path for the database, then **moved** to the current directory. Never copy a large file to the current directory under its final name because this will take time and the exchange process may be triggered prematurely.

If you are using a local reference file, rename and move this file first. Otherwise, the exchange process will be triggered by the appearance of the Fasta file, but will immediately fail because the new reference file is not yet available. Note that Fasta and reference files must have identical names apart from the filename extension.

Once Mascot Monitor sees a new Fasta file that matches the wild card path for the database, it will begin the exchange process. Progress can be monitored from the Mascot Database Status page.

Obtaining Fasta files

If your Mascot Server has an Internet connection, and you are able to use Database Manager, ignore the information in this section pertaining to NCBInr. Simply enable the predefined definition for NCBInr in Database Manager and the latest files will be downloaded automatically

If your Mascot Server is not connected to the Internet, download the required files on a PC with Internet access and copy them to your Mascot Server. Download URLs and configuration information for popular databases can be found on the Matrix Science web site at http://www.matrixscience.com/help/seq_db_setup.html

As a convenience for users who have no access to the Internet, a copy of NCBInr, a comprehensive protein sequence database, is included with Mascot on a separate DVD, together with the required taxonomy files. As with the copy of SwissProt, installed with the Mascot program files, these files were current at time of release, but will become increasingly out-of-date.

The procedure to make use of the NCBInr files on the DVD depends on whether you use Database Manager or not:

Using Database Manager

- Choose 'Create New'
- Enter NCBInr as the database name, choose 'Use predefined definition template', and select NCBInr from the list.
- If necessary, modify the location for the sequence database directory, then choose 'Create'
- Unpack the Fasta file into the specified location and unpack the taxonomy files into the Mascot taxonomy directory as described below under Manual Setup step 2
- Choose 'Activate'

Manual Setup

1. Choose a suitable location for the database files

The default location for database directories is under the Mascot sequence directory, but database files can be located on any local drive. If you decide to put the files in a different location, you will need to change the path in step 3.

Create a directory called *NCBInr* and under this, create three directories called *incoming*, *current*, and *old*.

2. Unpack the files from the DVD archive

Linux: Unpack the files using gzip and tar. If the Databases DVD is mounted as /mnt/dvdrom, typical command lines would be

```
cd /usr/local/mascot/sequence
gzip -dc /mnt/dvdrom/NCBInr_20120419.fasta.gz >
      NCBInr/current/NCBInr_20120419.fasta
cd ../taxonomy
gzip -dc /mnt/dvdrom/taxonomy.tar.gz | tar xvf -
```

Windows: Many people will prefer to use a graphical utility, such as WinZip or 7-Zip to unpack these archives. Make sure you use a recent version that can cope with files larger than 4 GB.

Extract *NCBInr_20120419.fasta.gz* into *NCBInr\current* and extract the files in *taxonomy.tar.gz* into the Mascot *taxonomy* directory. (It isn't sufficient just to de-compress taxonomy.tar, make sure you extract the files inside the tar archive.)
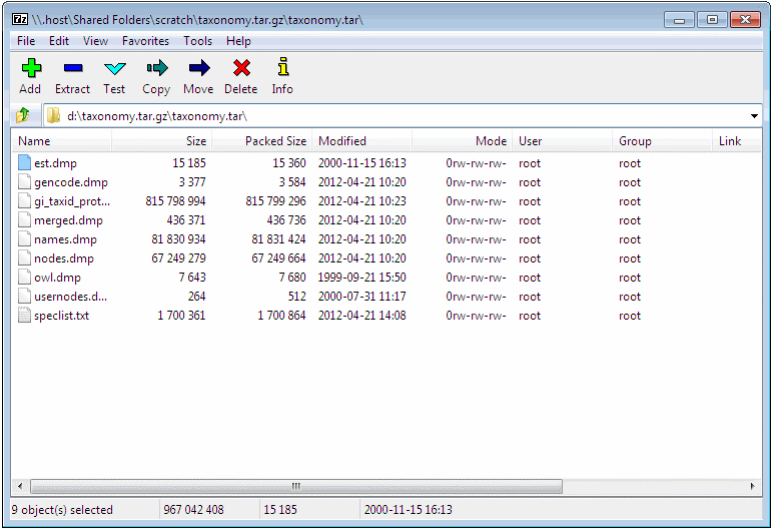
3. Edit mascot.dat

> The first time you use Database Manager, database related configuration information is moved to an XML file, and the database related sections of *mascot.dat* are re-written whenever changes are saved. So, either use Database Manager all the time or edit *mascot.dat* by hand all the time. You cannot swap between the two.

The format of mascot.dat is described in Chapter 6. For NCBInr, configuration information is already present in *mascot.dat*, but commented out to make the database 'inactive'. Once all the files are in place, all you need to do is check the path is correct then remove the comment character '#' and any leading space at the start of the NCBInr line in the Databases section. After saving the changes, monitor progress in the Mascot database status page as the new database is brought on-line.
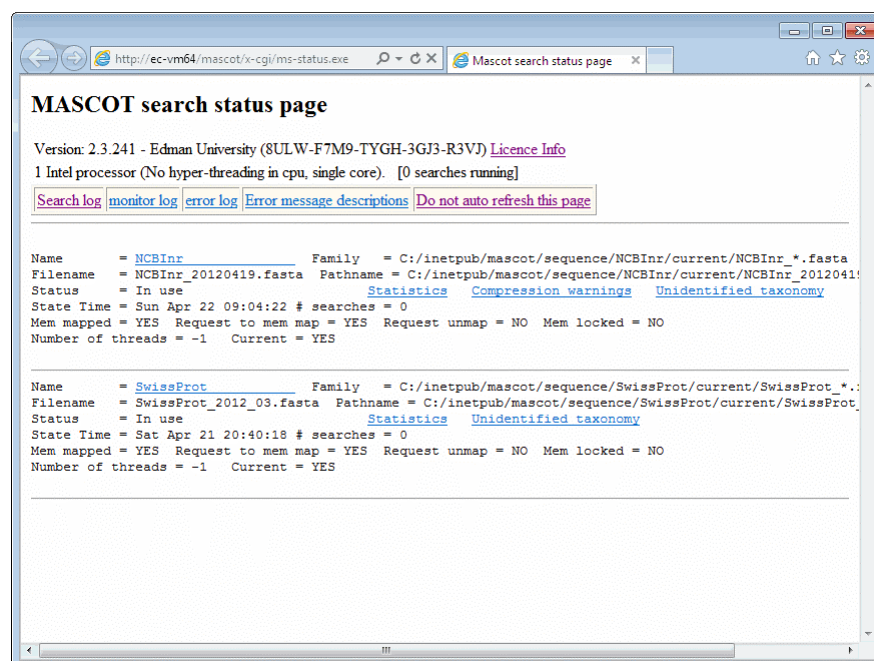
If all is well, the status line for the new database will display the following messages:

```
Creating compressed files
Running 1st test
First test just run OK
Trying to memory map files
Just enabled memory mapping
In Use
```

Troubleshooting

Proxy Server

Several databases do not have a local reference file. The default configuration is to retrieve full annotation text as required from the remote web sites. If there is a proxy server between your Mascot server and the Internet, this may fail unless you define your proxy server in the Options section of *mascot.dat*. The relevant parameters are **proxy_server, proxy_username,** and **proxy_password**. Unless your proxy server uses authentication, you only need the first of these, and a typical entry in *mascot.dat* will look like this

```
proxy_server http://our-cache:3128
```

Permissions / Security

Mascot monitor will need to create the compressed database files in the database *current* directory, and may need to move old database files to the *old* directory. Mascot searches, running as CGI processes with very restricted privileges, need to read the files. Make sure Linux permissions or Windows security settings don't prevent this.

Files not where they are supposed to be

When you enable the database, if nothing happens, double check that the sequence database files are exactly where the Path definition specifies. Note that the taxonomy files are shared, and go into the Mascot *taxonomy* directory, not a sequence database directory. Under Windows, remember that the directory separator in Database Manager and in mascot.dat must be a forward slash, not a back slash.

DVD read errors

File checksums and sizes (as reported by cksum) for the files on the Databases DVD:

3537046107 4218730222 NCBInr_20120419.fasta.gz

657867792 149274785 taxonomy.tar.gz

6

Configuration & Log Files

Configuration Files

Mascot configuration files are located in the *mascot/config* directory:

unimod.xml defines mass values and modifications, including substitutions

enzymes defines enzyme cleavage specificity

fragmentation_rules specifies which fragment ion series correspond to defined instrument types

mascot.dat contains general configuration information. If you use Database Manager, do not modify the sequence database-related sections of mascot.dat because any changes will be lost when Database Manager is next used.

taxonomy specifies the taxonomy filter choices for the search form (described in Chapter 9)

quantitation.xml defines quantitation methods

nodelist.txt configures the systems belonging to a Mascot cluster (described in Chapter 11)

user.xml, group.xml, security_options.xml, and security_tasks.xml are the configuration files for Mascot security, described in Chapter 12

mod_file, masses, and substitutions are obsolete configuration files that are created on the fly from *unimod.xml* to support third party applications that expect to find these files.

Files in *config/dbmanager* are configuration files used by Database Manager. For descriptions, see the Database Manager HTML help page.

A browser-based Configuration Editor is provided to view and edit these files. These files are all text files, so can also be edited in any

text editor. If you choose to edit the files, exercise care and always make a backup first, because seemingly small errors can render Mascot unusable.

Configuration Editor

The local Mascot home page contains a link to the Configuration Editor. The top-level page is a menu. If Mascot security is enabled, there will be an additional menu item for Mascot security administration
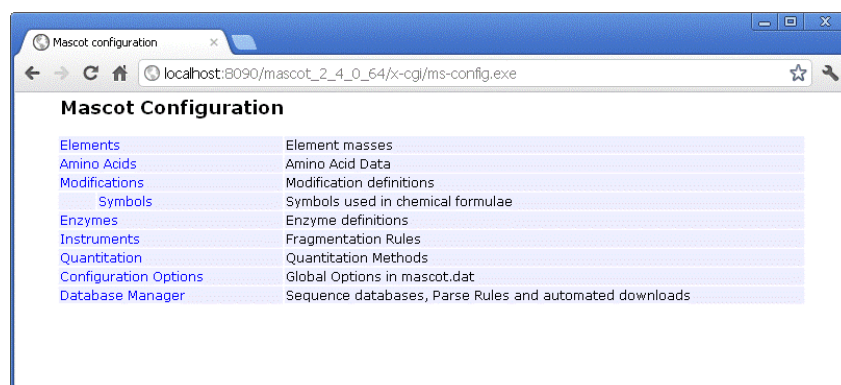
unimod.xml

The first four menu items: Elements, Amino acids, Modifications, and Symbols, are interfaces to different aspects of *unimod.xml*. It should only be necessary to make changes to *unimod.xml* in exceptional circumstances. An example might be if you wanted to add a modification that was confidential or experimental. Otherwise, better to add a new modification to the public Unimod database, www.unimod.org, and later download an updated configuration file from the Unimod help page. By going this route, you share the new modification with others, and benefit in turn from other people's updates.

Most of the pages of the Configuration Editor are self-explanatory. Where necessary, help text is displayed when the mouse rolls over a hyperlink.

For modifications, more detailed help can be found in the Unimod help pages. This is also the place to find details of the file format, which is fully defined by a schema called unimod_1.xsd.

Enzymes
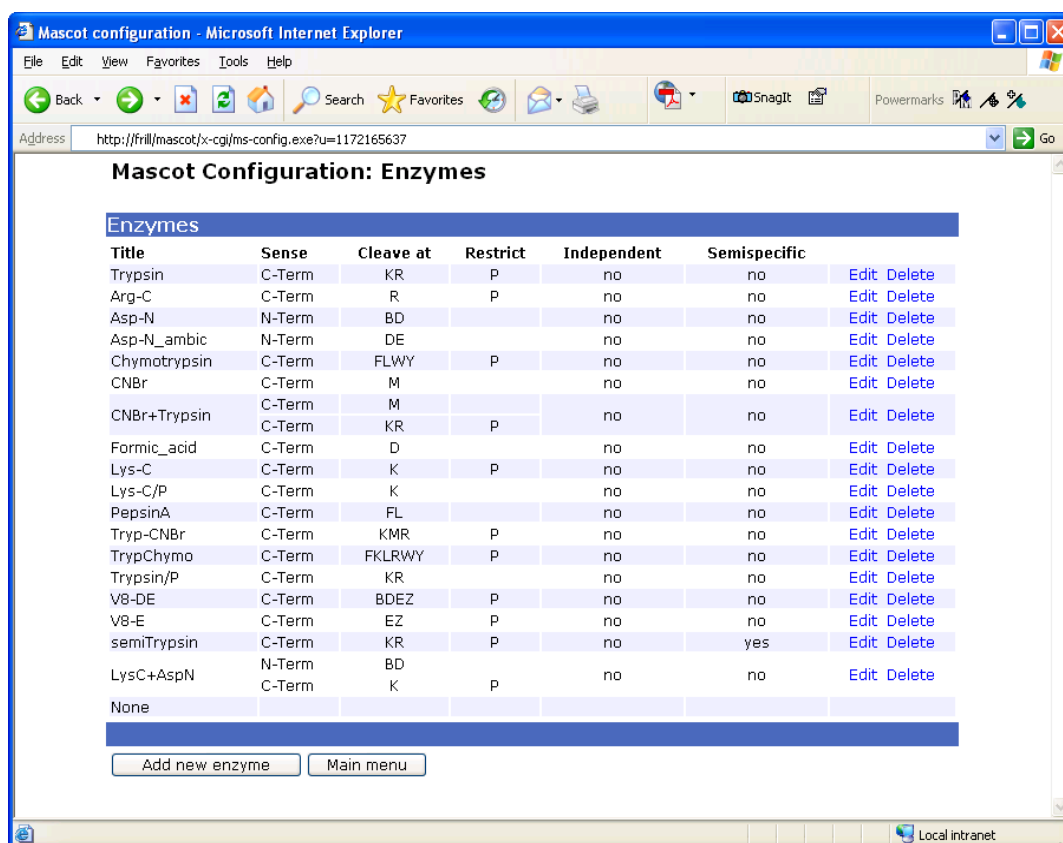
Enzyme 'None' is a special case, which cannot be modified or deleted. All the other enzyme definitions can be edited or deleted, and new ones added.

The edit page allows you to test a new enzyme definition against a protein

File format (enzymes)

Each cleavage agent is defined by a block of lines. Blocks are delimited from one another by a line containing an asterisk. Each line in a block starts with a keyword.

```
Title:Trypsin
Cleavage:KR
Restrict:P
Cterm
*
Title:Asp-N
Cleavage:DB
Nterm
*
```

The first line of each block must start with the `Title:` keyword, followed by a text string that is used to identify the cleavage agent in forms and reports. The definition should be short and self-explanatory. It should only include alphanumeric characters and spaces. Internal spaces are significant.

Each block must also include a line starting with the keyword `Cleavage:` followed by a list of the residues that identify the cleavage site.

Optionally, a block can include a line starting with the keyword `Restrict:` followed by a list of the residues which prevent cleavage if present adjacent to the potential cleavage site.

Finally, the block must include either the keyword `Cterm` or `Nterm` to define whether cleavage occurs on the C terminal or N terminal side of the specified residues.

This syntax can be extended to support multiple cleavage specificities, enabling enzyme mixtures to be modelled, or mixed C-term and N-term cutters. This is achieved by appending zero-based index numbers in square brackets to the keywords `Cleavage`, `Restrict`, `Cterm`, and `Nterm`. For example:

```
Title:CNBr+Trypsin
Cleavage[0]:M
Cterm[0]
Cleavage[1]:KR
Restrict[1]:P
Cterm[1]
Independent:0
*
```

The use of index numbers is optional when only one specificity is defined, but required when there are multiple specificities, as in this example.

For a definition with multiple specificities, if the keyword `Independent` appears and is given a value of 1, this means that the specificities should be treated as if independent digests had been performed on separate sample aliquots and the resulting peptide mixtures combined. Thus, any given peptide will conform to the specificity of one cleavage type only. In the case of CNBr+Trypsin, if `Independent` was set to 1, you would not find any peptides resulting from cleavage after K or R at one end, and

cleavage after M at the other. When `Independent` is omitted or given a value of 0, the specificities are combined, as if the reagents had been applied simultaneously or serially to a single sample aliquot. The keyword `Independent` does not take an index.

```
Title:semiTrypsin
Cleavage[0]:KR
Restrict[0]:P
Cterm[0]
SemiSpecific:1
*
```

If the keyword `SemiSpecific` appears and is given a value of 1, this means that any given peptide need only conform to the cleavage specificity at one end. The other end can result from non-specific cleavage. When `SemiSpecific` is omitted or given a value of 0, peptides are required to conform to the cleavage specificity at both ends. The keyword `SemiSpecific` does not take an index.

Instruments

The INSTRUMENT search parameter is used to select the set of ion series used for scoring MS/MS matches.

File format (fragmentation_rules)

Each instrument is defined by a block of lines. Blocks are delimited from one another by a line containing an asterisk.

The first line of each block must start with the Title: keyword, followed by a text string that is used to identify the instrument in forms and reports. The definition should be short and self-explanatory. It should only include alphanumeric characters and hyphens. The following lines start with an integer, each of which represents an ion series or a rule to be included in the definition. Refer to the file header for a list of available integers. Anything following a hash (#) symbol is treated as a comment.

A block can also specify mass range limits for internal ions. The default range is 0 to 700 Da, and could be changed as in this example:

```
title:MALDI-QIT-TOF
1  # singly charged
4  # immonium
5  # a series
6  # a - NH3 if a significant and fragment includes RKNQ
7  # a - H2O if a significant and fragment includes STED
8  # b series
9  # b - NH3 if b significant and fragment includes RKNQ
10 # b - H2O if b significant and fragment includes STED
13 # y series
14 # y - NH3 if y significant and fragment includes RKNQ
15 # y - H2O if y significant and fragment includes STED
17 # internal yb < 700 Da
18 # internal ya < 700 Da
minInternalMass 200
maxInternalMass 1000
*
```

Quantitation
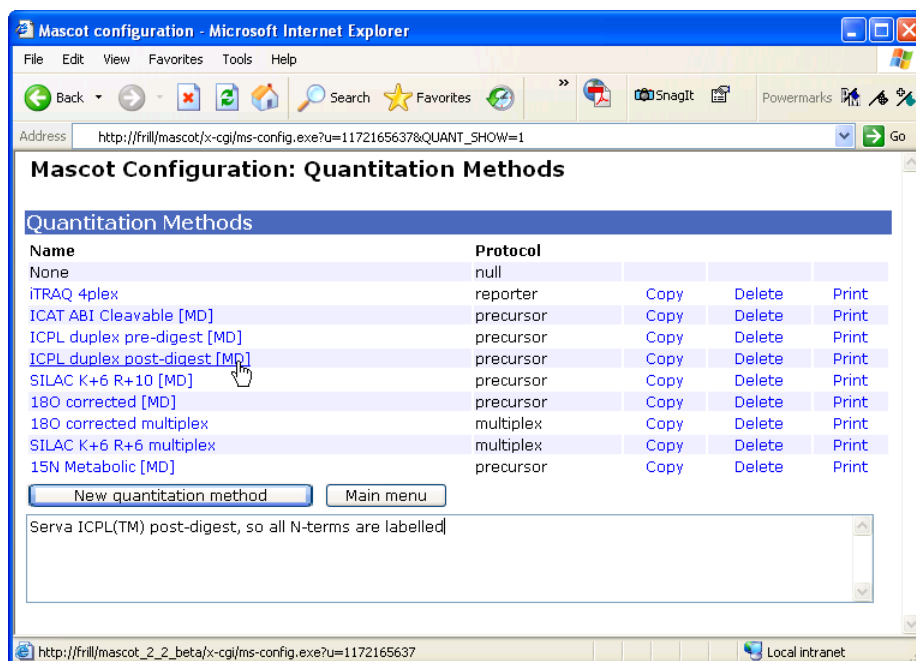
A detailed description of quantitation methods, the relevant Configuration Editor pages, and the underlying file, (`quantitation.xml`), is contained in the HTML help pages. Choose Help from the Mascot main menu bar and then choose Quantitation.

Database Manager

Database Manager is mainly described in the HTML help pages. Choose Help from the Mascot main menu bar and then choose Sequence Database Setup; Database Manager.

Configuration Options

The is a simple interface to the Options section of `mascot.dat`, which contains a variety of global settings. Reference material can be found below.

mascot.dat

Two sections of *mascot.dat*, Processors and Cluster, have no interface in either Database Manager or Configuration Options, and the only way to make changes is to edit *mascot.dat*.

Windows users should note that the path delimiters used in *mascot.dat* must always be forward slashes, never the backward slashes used at the command prompt. If sequence database files are not on a local disk drive, the remote drive must be mapped to a local drive letter. UNC path specifications cannot be used. Finally, spaces are not allowed in file or directory names. Hence:

`C:/InetPub/mascot/config/mascot.dat`   correct ✓

`C:\InetPub\mascot\config\mascot.dat`   wrong ✗

`\\matrix_nt_01\InetPub\mascot\config\mascot.dat`   wrong ✗

`//matrix_nt_01/InetPub/mascot/config/mascot.dat`   wrong ✗

General

mascot.dat is divided into sections. Each section starts with a unique keyword and ends with the keyword 'end'.

Comments and blank lines can be used freely. A line which starts with the # character (pound in the US, hash in Europe) is a comment line.

Databases

Do not modify this section if you ever use Database Manager

```
Databases
.
.
.
# NCBInr c:/inetpub/mascot/sequence/NCBInr/current/
             NCBInr_*.fasta AA 1234 14 1 1 -1 0 0 6 7 0 8
SwissProt c:/inetpub/mascot/sequence/SwissProt/current/
             SwissProt_*.fasta AA 1234 15 1 1 -1 0 1 33 13 15 3
.
.
.
end
```

A line that is commented out with a # character at the start is an *inactive* database definition. Each line defines a database using the following 14 parameters:

1. Name: Each database must have a unique name. Ideally, the name should be short and descriptive. Note that these names are case sensitive, and much confusion can be caused by creating (say) Sprot *and* SPROT. The name does not need to be the same as or even similar to the filename of the actual FASTA file. Allowed characters are alphanumerics and `_ . -$%&()[]`

2. Path: FASTA database files must be available locally. Mascot creates its compressed files in the same directory as the original FASTA file. The location of the FASTA file is defined in the Path field. This must be the fully qualified path to the FASTA file, with a wild card in the filename to allow incoming and outgoing database files with different version or date stamps to be present in the current directory simultaneously. The delimiters between directories must always be forward slashes, even if Mascot is running on a Windows system.

3. AA / NA: AA for an amino acid (protein) database and NA for a nucleic acid (DNA) database.

4. Obsolete: This parameter used to contain the approximate number of entries (sequences) in the database, used for progress reports during a search. The value is now just a place holder.

5. Obsolete: This parameter used to contain a unique identification number. The value is now just a place holder.

6. Mem map: Flag to indicate whether the database file should be memory mapped (1) or not (0). Database files should always be memory mapped. Unlike memory locking, this does not consume physical RAM.

7. Obsolete: This parameter (Blocks) must always be set to 1.

8. Threads: A Mascot search can use multiple threads. If you are running in cluster mode, 'Threads' is ignored. Otherwise, set to –1 to allow the number of threads to be determined automatically. To specify a fixed number of threads in non-cluster mode, set a value of 1 or more.

9. Mem lock: Flag to indicate whether a memory mapped database file should be locked in memory (1) or not (0). This setting is only relevant if column 6 contains a 1.

Memory mapped files can be locked in memory, but only if the computer has sufficient RAM. Having a database locked in memory means that it can never be swapped out to disk, ensuring there will never be a lag if the database files have to be read from disk. Of course, there also needs to be sufficient RAM for the operating system, (Windows consumes approximately 60 MB), anything from tens to hundreds of MB for each Mascot search, and space for any other applications which might be running.

If you try to lock databases into RAM when there isn't room, this will not be a major problem. The locking will fail, generate an error message, and Mascot will carry on regardless. A more serious problem is when there is just sufficient RAM to lock the databases, but none left over for searches or other applications. In this case, the whole system will slow down and the hard disk will be observed to be "thrashing". Eventually, the system is likely to hang or crash.

10. Local ref file: Flag to indicate whether a local reference file is available (1) or not (0). For certain databases, e.g. SwissProt, it is possible to have a local reference file, from which full text information can be taken for a 'Protein View' report.

11. AccessionParseRule: Index of the regular expression in the PARSE section that can be used to parse an accession string from a FASTA file title line.

12. DescriptionParseRule: Index of the regular expression in the PARSE section that can be used to parse a description string from a FASTA file title line.

13. AccessionRefParseRule: Index of the regular expression in the PARSE section that can be used to parse an accession string from a local full text reference file. If there is no local reference file, this value is ignored and can be set to 0.

14. Taxonomy: Index of the taxonomy rule block to be used to parse taxonomy information. If taxonomy information is not available, or is not to be used, this value should be set to 0.

PARSE

Do not modify this section if you ever use Database Manager

The PARSE section contains Basic Regular Expressions used to extract strings from various files.

```
PARSE
.
.
.
# For NCBI accession e.g.
RULE_6                    ">\(gi|[0-9]*\)"
#
# For NCBI description - everything after the first space
RULE_7                    ">[^ ]* \(.*\)"
#
.
.
.
end
```

The syntax of a standard Basic Regular Expression (BRE) is described in Appendix A. Rules defined in this section are referred to by means of their index number in two sections: Databases and WWW.

RULE_6, for example, looks for the ">" at the beginning of the title line. The string to be extracted is in backslashed parentheses: "gi|" then as many digits as possible. The match will stop when a non-digit is encountered, such as a pipe symbol or a space.

If you are not familiar with regular expressions, use the information in Appendix A to understand how the pre-defined rules in *mascot.dat* work.

A mistake in a rule called from the databases section may prevent Mascot from using the database concerned. Always use the Database Manager to configure and test new database definitions before they are brought on-line.

WWW

Do not modify this section if you ever use Database Manager

The WWW section defines where CGI scripts look for the information needed to compile a results report.

At least one line is required for each database, to define the source from which the sequence string of a database entry can be obtained. A second line can optionally define the source from which the full text report of an entry can be obtained. The syntax is very similar in both cases, independent of whether the information originates locally or on a remote system.

Sequence strings can always be retrieved locally, because the FASTA file must be present on a local disk. The Mascot utility *ms-getseq.exe* is normally used to retrieve a sequence string.

If full text for an entry is available locally and the database has been defined as including a ref file, (Column 10 in the Database section of *mascot.dat*), *ms-getseq.exe* can be used to retrieve the full text. Otherwise, a utility or URL must be identified which can accept an accession string and return the report text in a parseable format. An example of a suitable external URL for full annotation text is shown in the example for Trembl, below

Each line in the WWW section contains 5 columns:

```
WWW
.
.
.
Trembl_SEQ "8" "localhost" "80" "c:/inetpub/mascot/x-cgi/ms-getseq.exe
          Trembl #ACCESSION# seq"
Trembl_REP "23" "www.uniprot.org" "80" "/uniprot/#ACCESSION#.txt"
.
.
.
end
```

1. Identifier: An identifier constructed from the name of the database, an underscore character, and either the keyword SEQ or REP. Thus, `Trembl_SEQ` is the source for the sequence string of an entry in the database called Trembl.

2. Parse rule: The index of a rule in the PARSE section that can be used to extract the information required. Note that the rule for parsing a sequence string from `ms-getseq.exe` the same for all databases.

3. Host: The information source. For *ms-getseq.exe* or a similar local executable, this column should contain `localhost`. For a remote source, or a local source that will be queried as a CGI application, enter the hostname. (NB the word `localhost` is used to determine whether the application is a command line executable or a CGI application. If you want to specify a CGI application on the local server, just specify the hostname in some other way, for example `127.0.0.1`).

4. Port: The port number. This should be left at `80` unless another value is required to access a web server operating on a non-default port.

5. Path: A string containing the path to the executable and parameters, some of which are variables.

In the case of a command line executable, the parameters will generally be delimited by spaces. In the case of a CGI application, the parameters may be delimited from the executable by a question mark, and there must be no spaces within the parameter string. In general, spaces in URL's must be replaced by plus symbols, and non-alphanumeric characters should be URL encoded using the %nn notation.

A reminder to Windows users: Do not use backslashes as path delimiters, because these will be interpreted as escape characters.

Most parameters are entered as literal strings, with two exceptions: #ACCESSION# is a place holder that will be replaced by an actual accession string, #FRAME# is a place holder that will be replaced by the number of the reading frame used to translate a nucleic acid sequence. Obviously, this last parameter is only used with NA databases.

The syntax for calling *ms-getseq.exe* is described in Chapter 7. In the examples shown above, the full text report for Trembl is taken from an external URL because the full text file for Trembl is huge (40 GB). The default configuration for SwissProt uses a local full text reference file.

Processors

Mascot licensing is physical CPU or socket-based. For each CPU covered by the licence, Mascot will fully utilise up to 4 logical processors or cores.

If the number of processors available is the same as the number licensed, then it is best not to include a PROCESSORS section. You can include one, if you wish, but this may have a negative impact on system performance.

If the number of processors available is greater than the number licensed, you can use a PROCESSORS section to force specific cores to be used.

Logical processor (core) numbers generally start at 0, but see your computer documentation. The ProcessorSet= line specifies the complete set of logical processors (cores) to be used. Separate processor values with a comma The number in this list must be less than or equal to four times the number of physical CPU licensed, or the system will not run.

Following this, the processors to be used for each database are specified. These numbers must be a subset of the numbers in the ProcessorSet, and there must be the same number of values as the number of threads specified earlier in the database section. For example, if you had a 1 cpu licence and the physical processor had 6 cores, and you wanted to avoid using cores 0 and 1, you could specify this as follows:

```
PROCESSORS
ProcessorSet=2,3,4,5
SwissProt=2,3,4,5
end
```

The PROCESSORS section must be after the Databases section in *mascot.dat*, and ProcessorSet= must come before the other entries in this section.

Taxonomy

Do not modify this section if you ever use Database Manager

The syntax of the taxonomy blocks is fully described in Chapter 9.

Cluster

The syntax of the cluster block is fully described in Chapter 11.

UniGene

Do not modify this section if you ever use Database Manager

UniGene is an index created by automatically partitioning GenBank sequences into a non-redundant set of gene-oriented clusters, (http://www.ncbi.nlm.nih.gov/UniGene/). Each UniGene cluster is a list of the GenBank sequences, including EST's, which represent a unique gene. It is not an attempt to produce a consensus sequence. UniGene can be used to simplify the results of a Mascot search of dbEST.

An index file must be downloaded for each species of interest. For each species, the fully qualified path to the index file is associated with the species name:

```
UniGene
human C:/Inetpub/MASCOT/unigene/human/current/Hs.data
mouse C:/Inetpub/MASCOT/unigene/mouse/current/Mm.data
mosquito C:/Inetpub/MASCOT/unigene/mosquito/current/Aga.data
```

To add a UniGene report option to Mascot for a particular sequence database, add a line containing the name of the database followed by a list of the available species names:

```
EST_human human
EST_mouse mouse
EST_others mosquito
end
```

Options

The Options section is used for miscellaneous parameters, which are listed here in alphabetical order. If a parameter is shown with argument(s), these are the default(s) that apply if the parameter is missing.

```
AutoSelectCharge 1
```

Controls how MS/MS queries are treated when the CHARGE parameter specifies more than one charge state (e.g. 1+. 2+, and 3+). This is usually because no charge information was available for a query, so the search form defaults applied.

If set to 0, a query is generated for each charge state and these queries are searched and reported independently. This is the default setting because this was the behaviour in earlier versions of Mascot.

If set to 1, each charge state will be searched, but only the charge state that gets the highest scoring match is saved to the result file and reported. This is the recommended setting

Note that this switch only applies to MS/MS queries, (including tags). Independent queries are always generated if multiple charge states are specified for molecular mass queries.

```
CacheDirectory ../data/cache/%Y/%m
```

Cache files are created and to improve performance when viewing large search results. This option specifies the relative path from the cgi directory to the location for saving report cache files. The actual directory will be, for example, ../data/cache/2010/02/uwcuxlsxx3s524f4vnnz3btmni/ where the lowest level directory is an md5sum of the .dat filename, the size and last-modified date of the .dat file. The tokens are % followed by any of the conversion specifiers supported by the strftime function (http://www.cplusplus.com/reference/clibrary/ctime/strftime/). For example, %Y gets converted to the year as a decimal number including the century, %m to the month as a decimal number (range 01 to 12) and %d to the day of the month as a decimal number (range 01 to 31). The date used will be the last modified date of the .dat file (rather than the time that the search started). See also ResfileCache and ResultsCache

```
CentroidWidth 0.25
CentroidWidthCount 1000
```

CentroidWidth is the width in Daltons of the sliding window used for re-centroiding profile data. Must be a floating point number between 0 and 10. Re-centroiding is applied whenever the number of peaks in a single scan exceeds CentroidWidthCount

```
DecoyTypeNoEnzyme 3
DecoyTypeSpecific 1
```

These parameters determine how decoy sequences are created for Mascot Auto-decoy searches. DecoyTypeSpecific applies to MS/MS searches using fully specific or semi-specific enzymes. DecoyTypeNoEnzyme applies to MS/MS searches with no enzyme. For PMF, random protein sequences are used, whatever the settings. For NA databases, the sequences are randomized before translation. Classifications are based on G. Wang, et al. (2009), "Decoy Methods for Assessing False Positives and False Discovery Rates in Shotgun Proteomics", Anal Chem. 81(1):146-159. Values supported in Mascot 2.4 are:

1 Reverse the sequence of each protein entry.

3 For each protein entry, generate a random sequence of the same length, with the composition based on the average composition of the whole database. This is the default in Mascot 2.3 and earlier.

4 Digest each protein sequence into peptides, then generate a random sequence for each peptide, but keep the same terminal residues and don't introduce new cutting sites.

```
EmailErrorsEnabled 0
EmailFromTextName
EmailFromUser
EmailPassword
EmailProfile
EmailService
EmailTimeOutPeriod 120
EmailUsersEnabled 0
ErrMessageEmailTo
MailTempFile C:/TEMP/MXXXXXX
MailTransport 2
MonitorEmailCheckFreq 300
SendmailPath /usr/lib/sendmail
```

Mascot can be configured to use email for two purposes:

1. When the search engine executes as a CGI application, email can be used to send the results of a search to a user who accidentally or deliberately disconnected before the search was complete. This facility can be enabled by setting `EmailUsersEnabled` to 1 or disabled by setting it to 0.

2. Serious error messages can be emailed to an administrator. This facility can be enabled by setting `EmailErrorsEnabled` to 1 or disabled by setting it to 0. Error messages that are considered serious are identified in the file *errors.html*. This file can be found in the root directory of the installation CD-ROM, and is displayed by clicking on the link 'Error message descriptions' at the top of the database status page.

A number of parameters are used to define how email should be sent:

`MailTransport` should be set to one of the following values:

    0 for CMC
    1 for MAPI
    2 for sendmail
    3 for Blat

`EmailService` is the service name (CMC only)

`EmailPassword` is the password (if any) required to log onto MAPI or CMC

`EmailProfile` is the MAPI profile name

`sendmailPath` is the path to sendmail, or an equivalent.

`EmailFromUser` is the name which will appear in the 'From' field of the email message.

`EmailFromTextName` will appear in the 'Title' field of the message.

If `EmailUsersEnabled` is set to 1, search results will be emailed to a user if their web browser does not respond within the number of seconds specified in `EmailTimeOutPeriod` following the completion of a search.

Email messages can be sent in batches at intervals specified by `MonitorEmailCheckFreq` (in seconds). `MailTempFile` is the name of the temporary file used to store email messages until they can be sent.

If `EmailErrorsEnabled` is set to 1, serious error messages will be emailed to `ErrMessageEmailTo`.

MAPI Configuration (Windows Only)

Set `MailTransport` to 1.

Set the `EmailPassword` to the password (if any) that is required to log onto MAPI.

Set the `EmailProfile` to the profile name used by MAPI. This can be found by opening the Windows Control Panel and clicking on Mail. (Depending on whether you have an 'internet mail only' or a 'corporate or workgroup' installation of MS-Outlook, you will have a list of either account names or profile names to choose from).

Sendmail Configuration (Linux Only)

Set `MailTransport` to 2.

Set the `EmailFromUser` parameter to the name that is required in the 'From' field of the email messages.

Set `EmailFromTextName` as the name of the server that is running mascot. For example setting `EmailFromUser` to www and `EmailFromTextName` to Mascot Server will result in emails from www (Mascot Server). The From field of the email will be www@www.your_domain.com.

Set `sendmailPath` as the path for the sendmail program, e.g. /usr/lib/sendmail

Set `MailTempFile` as the name of the file used to store email messages until they can be sent (must be the path followed by a filename in the form: MXXXXXX). This will create temporary files that begin with M

followed by a unique number. Typically this parameter will be /var/tmp/ MXXXXXX.

Blat Configuration (Windows only)

Blat is a free, easily installed mail program for Windows. For more information, visit:

http://www.blat.net/

Set `MailTransport` to 3.

Set the `EmailUserFrom` parameter to the name that is required in the 'From' field of the email messages.

Set `EmailFromTextName` as the name of the server that is running mascot. For example setting `EmailUserFrom` to www and `EmailFromTextName` to Mascot Server will result in emails from www (Mascot Server). The From field of the email will be www@www.your_domain.com.

Set `sendmailPath` as the fully qualified path (including drive letter) for the Blat program.

Set `MailTempFile` as the name of the file used to store email messages until they can be sent (must be in the form path/MXXXXXX). This will create a new temp file where the first letter will be an M and the next 6 characters will make up a unique number. Typically this parameter will be c:/temp/MXXXXXX

```
ErrorLogFile ../logs/errorlog.txt
GetSeqJobIdFile ../data/getseq.job
InterFileBasePath c:/inetpub/mascot/data (Windows)
              /usr/local/mascot/data (Linux)
InterFileRelPath ../data
MascotCmdLine ../cgi/nph-mascot.exe
MascotControlFile ../data/mascot.control
MascotJobIdFile ../data/mascot.job
MascotNodeControlFile ../data/mascotnode.control
MonitorLogFile ../logs/monitor.log
SearchLogFile ../logs/searches.log
TestDirectory ../data/test
UniqueJobStartNumber 001234
```

These entries determine local paths (not URL's). `ErrorLogFile`, `MascotCmdLine`, `MonitorLogFile`, `SearchLogFile`, and `TestDirectory` are self-explanatory.

`GetSeqJobIdFile` contains the next available job number for the `ms-getseq.exe` utility. These numbers wrap around at 999 and do not

appear in the search logs. If this file is deleted, the next job number will be reset to 1 and a new `jobId` file created automatically

Mascot output files are written to a path given by:

`InterFileBasePath/InterFileRelPath/`*yyyymmdd*`/F`*nnnnnn*`.dat`

Where *yyyymmdd* is the current ISO date, and *nnnnnn* is a sequential job number with a minimum of 6 digits. The path is split into a base path and a relative path as seen by the CGI scripts so that the search engine can pass a file path to (say) *master_results.pl* as:

`InterFileRelPath/`*yyyymmdd*`/F`*nnnnnn*`.dat`

`TestDirectory` contains the input files used by Monitor to test new sequence databases.

`MascotControlFile` contains critical internal parameters. This file must be memory mapped and locked to provide interprocess communication between different Mascot components. `MascotNodeControlFile` is a similar, additional file used in cluster mode

`MascotJobIdFile` contains the next available job number. If this file is deleted, the next job number will be initialised to the value given by `UniqueJobStartNumber`, and a new `jobId` file created automatically. NB `UniqueJobStartNumber` must never be set lower than 1000.

`ErrTolMaxAccessions 0`

The maximum number of database entries allowed for a manual error tolerant search. Default is 0, meaning no limit.

`ExecAfterSearch_n flag:num[flag:num], title string, command string`

Defines a command to be run after a search is complete. N is one or two digits in the range 1 to 10. The Mascot installer creates the following two entries which provide Percolator integration:

`ExecAfterSearch_1 waitfor:0;logging:0, Creating percolator input, ../bin/ms-createpip.exe -i %resultfilepath -o %percolator_pip`

`ExecAfterSearch_2 waitfor:1; logging:1, Percolating, ../bin/percolator.exe $PercolatorExeFlags`

The following flags may be specified:

flag	num	description
waitfor	0..10	The command should wait for completion of the command specified by num. A value of 0 means don't wait, equivalent to omitting the flag
logging	0..3	0 – no logging 1 – log successful commands (return code 0) 2 – log unsuccessful commands (return code not 0) 3 – log successful and unsuccessful commands
percolator	0..1	0 – no dependency on Percolator 1 – command should only be run if search fulfills criteria for running Percolator

The title string will be displayed in the search progress while the process is running. This string must not contain a comma

The command string can include literals and also the following tags, which will be substituted at run time:

Tag	Replaced with
%resultfilepath	Relative path from the cgi directory to the results file
%resultfilename	File name part of *%resultfilepath*
%percolator_pip	Relative path from the cgi directory to the Percolator input file
%percolator_decoy_pop	Relative path from the cgi directory to the Percolator output file for the decoy matches
%percolator_target_pop	Relative path from the cgi directory to the Percolator output file for the target matches. %session_id is the session identifier of the logged in user when Mascot Security is enabled. %task_id is the task identifier assigned using client.pl when called from client applications.
$PercolatorExeFlags	The value of PercolatorExeFlags

Paths to executables and any paths included as arguments should use forward slashes and should not include spaces

```
FeatureTableLength 30000
```

If a nucleic acid sequence is longer than 30000 bases, the protein view report will automatically switch to feature table mode and output the matches as a GenBank feature table. The threshold for switching to feature table mode can be altered using the parameter FeatureTableLength in the Options section of mascot.dat or by append-

ing _featuretablelength=X to the protein view URL, where X is the length in bases.

`FeatureTableMinScore`

By default, only matches with significant scores ($p < 0.05$) are output. A different score threshold can be specified using the parameter FeatureTableMinScore in the Options section of mascot.dat or by appending _featuretableminscore=X to the protein view URL, where X is the score threshold.

`ForkForUnixApache 0`

If a user presses 'Stop' or goes to another page in their browser when a search is running, then the intended behaviour is that the search should continue, and the user be emailed with their results. However, when running some versions of Apache, the search is terminated by Apache when the connection to the browser is lost. To stop this from happening, set this value to 1. Setting this parameter to 1 with other servers can cause problems, so only use this setting if necessary. (When set to 1, the result is that *nph-mascot.exe* ignores PIPE signals, does a fork, the parent exits and the child then ignores HUP signals).

`FormVersion 1.01`

Mascot users may save search forms off-line, or submit searches using scripts or private forms. When the search engine is upgraded, there is the possibility that old scripts or forms may contain invalid or obsolete parameters. If a search is submitted to Mascot without a version number, or if the version number is lower than that specified by `FormVersion`, a warning will be included in the results file and in the master results report.

`GetSeqJobIdFile see ErrorLogFile`

`ICATQuantitationMethod ICAT`

For backward compatibility, if a search is submitted from an old client with ICAT=ON, then the specified quantitation method will be used.

`IgnoreDupeAccessions EST_others`

A comma separated list of database names. For any database in this list, don't check for duplicate accession numbers when creating the compressed files. A database should only be added to this list if it has a very

> large number of sequence which may causes the system to run out of memory when creating the compressed files.

```
IgnoreIonsScoreBelow 0.0
```

> When a report is generated, any ions score lower than this value will be set to zero and ignored. The parameter is a floating point number, default 0.0. Values greater than 0 and less than 1 act as an expect value threshold, and the scores for any peptide matches with higher expect values are set to 0. This global default can be over-ridden on an individual report URL by appending &_ignoreionsscorebelow=X, where X is the cut-off value.

```
IntensitySigFigs 2
```

> The precision of intensity values written to the result file.

```
InterFileBasePath see ErrorLogFile

InterFileRelPath see ErrorLogFile

IonsDecimalPlaces 2
```

> Mascot calculates all masses to an accuracy of 1/65535 Daltons. The number of decimal places used to display fragment ion masses in reports can be altered by changing this value.

```
IteratePMFIntensities 1
```

> Set this option to 0 to prevent selection of PMF values on the basis of their intensity.

```
LabelAll 0
```

> Set this option to 1 to make the initial display in Peptide View one in which all peaks that match a calculated mass value are labelled.

```
LastQueryAscFile ../logs/lastquery.asc
SaveEveryLastQueryAsc 1
SaveLastQueryAsc 0
```

> SaveLastQueryAsc is a flag which controls whether the most recent input file to Mascot (i.e. the MIME format file containing MS data and search parameters) should be saved to disk (1) or not (0). This can be a

useful debugging tool when writing scripts or forms to submit searches to Mascot. If `SaveLastQueryAsc` is set to 1, the name of the file is determined by `LastQueryAscFile`. Each new search over-writes this file. NB `LastQueryAscFile` is a disk path, not a URL.

An additional debugging tool is provided by `SaveEveryLastQueryAsc`. If set to 1, the Mascot input file will be saved for any search that fails to complete because it generates a fatal error. The name of the output file follows the same naming convention as a normal Mascot result file, except for the additional suffix `.inp`. If a search goes to completion, this file is deleted as soon as the normal output file has been written to disk.

`LogoImageFile ../images/88x31_logo_white.gif`

This is the URL of the Matrix Science logo, used at the top of a search progress report. You can customise this by substituting the URL of your own logo. For optimum appearance, the image should be 88 pixels wide and 31 pixels high.

`MailTempFile see EmailErrorsEnabled`

`MailTransport see EmailErrorsEnabled`

`MascotCmdLine see ErrorLogFile`

`MascotControlFile see ErrorLogFile`

`MascotJobIdFile see ErrorLogFile`

`MascotMessage`

A text string to be displayed ahead of the progress reports when a search is run

`MassDecimalPlaces 2`

Mascot calculates all masses to an accuracy of 1/65535 Daltons. The number of decimal places used to display peptide mass values in reports can be altered by changing this value.

`MaxAccessionLen`

Obsolete

`MaxConcurrentSearches 10`

This parameter limits the maximum number of concurrent searches so as to avoid overloading the Mascot server. Default is 10

MaxDatabases 64

The maximum number of concurrently active sequence databases. Increasing this value uses more RAM, so don't set unnecessarily high. There is no upper limit to this value. You need to restart the Mascot service after changing this value.

MaxDescriptionLen 100

Description text parsed from the FASTA title line will be truncated at this number of characters. (Note: There is no need to recompress a database if this parameter is changed).

MaxEtagMassDelta 1770
MinEtagMassDelta -130

In an error tolerant tag search with a fully specific enzyme, these values set the limits on the amount the mass is allowed to increase (MaxEtagMassDelta) or decrease (MinEtagMassDelta) in order to reach the first available cleavage point.

MaxEtVarMods 2

The maximum number of variable mods allowed in the first pass of an automated error tolerant search (global default, can be over-ridden for a group in security)

MaxNumPeptides

The maximum number of peptides that can be expected from the enzymatic digest of a single entry. The default is `MaxSequenceLen / 4`

MaxPepNumVarMods 5

The maximum number of variable mods allowed for a PMF

MaxQueries 10000

The maximum number of MS/MS spectra allowed in a single search. Note that the maximum number of mass values in a PMF is hard-coded to 1000

MaxSearchesPerUser 0

>   Sets the maximum number of concurrent searches from a single IP
>   address. A value of 0 means no limit. (global default, can be over-ridden
>   for a group in security)

MaxSequenceLen 50000

>   The maximum length of a database entry in characters, (bases for NA or
>   residues for AA). The default is 50,000. The length of the longest se-
>   quence in a database can be found in the *.stats file, created by Mascot
>   Monitor when the database is compressed. The larger the value of
>   MaxSequenceLen, the more memory mascot uses. So, if you need to
>   increase it, make it just a little greater than the length of the longest
>   sequence. On a 32 bit system, try not to exceed 3 million, because
>   searches may run slower than normal. If you are trying to search an
>   assembled genome, you might want to consider searching shorter se-
>   quences instead, such as a database of the contigs.

MaxVarMods 9

>   The maximum number of variable mods allowed for an MIS search
>   (global default, can be over-ridden for a group in security). Value is an
>   integer in the range 0 to 32

MinPeaksForHomology 6

>   For an MS/MS search, a homology threshold will not be reported if the
>   number of peaks in a spectrum is less than this value

MinPepLenInPepSummary 7

>   In a Peptide Summary report, two proteins are reported as distinct
>   matches if the peptide matches to one protein are not identical to or a
>   sub-set of the peptide matches to the other protein. Since matches to
>   very short peptides are usually random, peptides shorter than
>   MinPepLenInPepSummary are not considered in this comparison.

MinPepLenInSearch 7

>   Peptides shorter than MinPepLenInSearch are rejected during the
>   search. Matches to very short peptides are meaningless because a 2-mer
>   or 3-mer can occur in almost every entry in a database. If such matches

are allowed in the peptides section, it can cause serious bloating of the result file.

`MonitorEmailCheckFreq` see `EmailErrorsEnabled`

`MonitorLogFile` see `ErrorLogFile`

`MonitorPidFile monitor.pid`

The name for the file that holds the process ID number for *ms-monitor.exe*. Default is `monitor.pid`.

`MonitorTestTimeout 1200`

A time-out can be applied to the test searches used to validate a new database. If the test search on a new database does not produce a valid result within the number of seconds specified by `MonitorTestTimeout`, the problem is assumed to be with the new database, and the exchange process is halted.

`MoveOldDbToOldDir 1`

After a successful database swap, the old Fasta file and old reference file (if any) are moved to the `../old` directory unless this parameter is present and set to 0. Note that, if set to 0, the old files are not deleted. Some other application must take care of this or there will be problems next time Monitor starts up.

`Mudpit 1000`

Obsolete, see MudpitSwitch

`MudpitSwitch 0.001`

Mascot has two ways to calculate protein scores in a Peptide or Select summary report. Standard scoring is used when the ratio between the number of queries and the number of database entries, (after any taxonomy filter), is small. The standard score is the sum of the ion scores after excluding duplicate matches and applying a small correction. Protein score calculation switches to large search mode when the ratio between the number of queries and the number of database entries, (after any taxonomy filter), exceeds the value specified by MudpitSwitch. Only those ions scores that exceed one or both significance thresholds contribute to the score, so that low scoring, random matches have no effect. The global default can also be over-ridden on an individual report

URL by appending &_server_mudpit_switch=X, where X is the ratio between the number of queries and the number of database entries, (after any taxonomy filter).

```
NoResultsScript ../cgi/master_results.pl
ProteinFamilySwitch 300
ResultsFullURL ###URL###/cgi/master_results.pl
ResultsFullURL_2 ###URL###/cgi/master_results_2.pl
ResultsPerlScript ../cgi/master_results.pl
ResultsPerlScript_2 ../cgi/master_results_2.pl
```

These are URL's (not disk paths) for the scripts to be called by the search engine at the completion of a search. A successful search calls ResultsPerlScript if the number of queries is less than ProteinFamilySwitch otherwise ResultsPerlScript_2. A search that didn't find any hits calls NoResultsScript.

The ResultsFullURL and ResultsFullURL_2 are used when a link to the search results is emailed to a user. Since the email will probably be received on another system, the link needs to have the full URL including the Web server hostname. ###URL### is replaced by the server URL during installation

```
NTIUserGroup Users
NTMonitorGroup Administrators
```

Under Windows, the Mascot service is generally run using the 'Local System' account. It has to create, write and read the memory mapped files. The CGI scripts (such as *nph-mascot.exe*) are run by the Web server, and will be run using a different user name with different permissions from the service. These programs also need to be able to read and write to these files. For example, with the Microsoft Web server (IIS), a new user with the name IUSR_<name_of_pc> is created when the server is installed, and the scripts are run using this user name. The installation program sets these values appropriately. Other Web servers may use different user names, with different permissions.

NTIUserGroup is the name of a group that the user name of the process to run CGI scripts belongs to. NTMonitorGroup is the name of the local Administrators group.

If not using IIS, check the documentation that comes with the server to find out which user name is used for running scripts, then from the start menu, choose, Programs, administrative tools (common), and User Manager. Double click on the user name, and press the groups button to find out which groups this user name belongs to. This is the name to put in *mascot.dat* for NTIUserGroup.

Failure to put the correct group name will generally result in one of two error messages:

```
Failed to open memory mapped file <filename>.  ⤶
        ⤷ Error: access denied
```

or

```
Failed to create memory map for <filename>. ⤶
        ⤷ Error Access denied
```

After changing either of these entries, the Mascot service will need to be stopped, (from the start menu, choose *Programs; Mascot; config; Stop Mascot service)*. All compressed database files must be deleted. Then the Mascot service can be re-started (*Programs; Mascot; config; Start Mascot service*).

```
Percolator 0
PercolatorFeatures mScore, lgDScore, mrCalc, charge, dM, dMppm, absDM,
            absDMppm, isoDM, isoDMppm, mc, varmods, totInt,
            intMatchedTot, relIntMatchedTot
PercolatorMinQueries 100
PercolatorMinSequences 100
PercolatorUseProteins 0
PercolatorUseRT 0
PercolatorExeFlags -i 10 -D 14 -v 0
```

Set `Percolator` to 1 if percolated results should be opened by default, 0 otherwise. `PercolatorFeatures` specifies the list of features used by Percolator. To see the list of available features, run *ms-createpip.exe –help*. Percolator will only be run if the number of queries in the search is at least `PercolatorMinQueries` and the number of entries in the sequence database is at least `PercolatorMinSequences`. Percolator will use the assignment of proteins to peptides as a feature if `PercolatorUseProteins` is set to 1. This can have undesirable results and should be used with great care. This flag is not supported in the current release. Percolator will use the retention times of peptides as a feature if `PercolatorUseRT` is set to 1. `PercolatorExeFlags` is used to specify the Percolator command line arguments with the exception of the file path arguments –j –B –r. If the string includes the argument –D num, this will be removed unless `PercolatorUseRT` is set to 1

```
PrecursorCutOut –1,-1
```

The precursor peak can often have very high intensity relative to the fragment peaks, which may give rise to spurious fragment ion matches. It is usually best if the precursor is removed before the search.

With the default arguments of −1,−1, a smart filter is created. This removes peaks within the fragment ion tolerance window about each of the precursor isotope peaks. The number of isotopes is assumed to be as follows:

```
            Mr        Number
        < 1000           3
  1000 − 1999            4
  2000 − 2999            5
  3000 − 3999            6
  4000 − 4999            7
  5000 − 5999            8
  6000 − 6999            9
        > 7000          10
```

So, if the precursor m/z was 800, the charge was 2, and fragment ion tolerance was +/− 0.1 Da, the filter would remove 4 notches of width

```
m/z 800.0 +/- 0.1
m/z 800.5 +/- 0.1
m/z 801.0 +/- 0.1
m/z 801.5 +/- 0.1
```

At first sight, this may seem a strange mix of m/z and Da. The reason is that we need to avoid matches from 1+ fragment ions, whatever the charge on the precursor.

If the arguments are anything other than −1,−1, a single notch is used where the first argument is the mass offset of the beginning of the notch and the second value is the mass offset of the end of the notch. For the precursor in the last example, if the arguments were −1,4 then the notch would run from m/z 799.5 to m/z 802.0. However, if the precursor charge was 1, then the notch would be from m/z 799 to m/z 804.

The mascot.dat setting can be over-ridden in a search by using the search parameter CUTOUT. Note that the peaks removed by this filter are not recorded in the result file, so cannot be recovered by changing this parameter in a repeat search.

```
ProteinFamilySwitch see NoResultsScript
```

```
ProteinsInResultsFile 2
```

Determines the number of protein title lines saved to each results file.

1   As in Mascot 1.7 and earlier, only proteins that appear in the Summary section will appear in the Proteins section

2   Include proteins with at least one top ranking peptide match to a peptide of length greater than `MinPepLengthInPepSummary`

3   Include all proteins

```
proxy_password
proxy_server
proxy_username
```

These entries support a proxy server between the Mascot server and the outside world. A typical entry might be

```
proxy_server http://our-cache:3128
```

If there is no `proxy_server` entry, scripts will look for proxy information in the server environment. The `proxy_username` and `proxy_password` parameters are only required if the proxy server requires authentication. Remote host authentication should be included directly in the URLs specified in mascot.dat. e.g. `http://username:password@hostname/`

```
RemoveOldIndexFiles 1
```

After a successful database swap, the compressed files in the current directory are deleted unless this parameter is present and set to 0

```
ReportBuilderColumnArrangement
```

Set the column arrangement at the given index. Column arrangements are used by Report Builder (introduced in Mascot 2.4) to provide a default list of columns to show. These can be selected from a dropdown list in the report. Each column arrangement is of the form "Name:[columns]" where Name is the column arrangement name (e.g. Standard) and [columns] is a comma-separated list of column names, as used by Report Builder. The following is the standard list of column names, available in every report:

family

> member
> db
> acc
> score
> mass
> matches
> matches-sig
> sequences
> sequences-sig
> empai
> frame
> desc

Frame will not be shown in the report if the search is against a proteindatabase. Quantitation methods add additional column names, but these are generated from the quantitation ratio names. The easiest way to create a column arrangement is to arrange the columns in Report Builder, then "export" the arrangement as a string.

```
ReportNumberChoices 5,10,20,30,40,50
```

If present, this list will define the choices provided in the search form 'Report top' drop down list.

```
RequireBoldRed 0
```

If this flag is set to 1, only protein matches which have one or more 'bold red' peptide matches will be listed in a peptide summary report. That is, proteins that include at least one top ranking peptide match that has not already appeared in the report. This global default can be overridden on an individual report URL by appending &_requireboldred=X, where X is 0 or 1.

```
ResfileCache master_results.pl, master_results_2.pl, peptide_view.pl,
             protein_view.pl, export_dat.pl, export_dat_2.pl, ms-
             createpip.exe, MSAnatomiser.class, mi_getpeaklist.pl,
             msms_gif.pl, nph-mascot.exe, ms-searchcontrol.exe
```

```
ResultsCache master_results.pl, master_results_2.pl, protein_view.pl,
             export_dat.pl, export_dat_2.pl, ms-createpip.exe,
             MSAnatomiser.class, mi_getpeaklist.pl, nph-mascot.exe,
             ms-searchcontrol.exe
```

Comma, space or tab delimited string of scripts and applications that will use cache files to speed up access to the results files. To prevent the use of the cache for a particular script, remove it from this list. There are

two sets of cache files, one for the results file, independent of any particular report format, controlled by ResfileCache, and one for each combination of summary report format settings, controlled by ResultsCache. See also CacheDirectory.

`ResultsFileFormatVersion`

If present, and the argument is 2.1, the result file format will be "2.1 compatible". That is, no xml sections. No other arguments are supported at this time.

`ResultsFullURL see NoResultsScript`

`ResultsFullURL_2 see NoResultsScript`

`ResultsPerlScript see NoResultsScript`

`ResultsPerlScript_2 see NoResultsScript`

`ReviewColWidths 7,8,8,27,30,100,32,25,6,13,2,4,6,16,7`

This sets the widths of the columns in ms-review.exe.

`SaveEveryLastQueryAsc see LastQueryAscFile`

`SaveLastQueryAsc see LastQueryAscFile`

`ScoreThresholdForAuto`

Deprecated, use `SigThreshold`.

`SearchControlLifetime 7200`
`SearchControlSaveE 0`

Obsolete.

`SearchLogFile see ErrorLogFile`

`SendmailPath see EmailErrorsEnabled`

`SelectSwitch 1000`

If the number of queries in an MS/MS search is less than or equal to this number, the default report is the Peptide Summary. If it is greater than this number, the default report is the Select Summary.

`SeparateLockMem 0`

> Only required for 32-bit versions if the total amount of memory to be locked is greater than 2Gb (or lower if some system limit is set). Setting this value to 1 indicates that *ms-monitor.exe* will run a separate program (*ms-lockmem.exe*) that will lock the memory blocks. A value greater than 1 specifies the block size in Mb. For example, if there is a 1.5 Gb *.s00 file, and this parameter is set to 750, then two instances of *ms-lockmem.exe* will be run.

`ShowAllFromErrorTolerant 0`

> Standard behaviour for the result report of a manual error tolerant search is to show only those matches that satisfy two criteria: (i) the score must be at least as high as the match for the same query in the original 'parent' search, (ii) the score equals or exceeds the identity threshold for the same query in the original 'parent' search. Setting `ShowAllFromErrorTolerant` to 1 causes all matches to be displayed. This global default can be overridden on an individual report URL by appending &_showallfromerrortolerant=X, where X is 0 or 1.

`ShowSubSets 0`

> If this is set to 1, under each protein match in a peptide summary report, matches to proteins that contain a sub-set of the same peptides will also be listed. This was the default behaviour in version 1.6 and earlier. If this flag is set to 0, which is now the default, the sub-set matches will not be shown. Values between 0 and 1 represent the fraction of the protein score of the primary hit that a subset hit can lose and still be listed. For example, if ShowSubSets is 0.2, and the primary hit has a protein score of 200, sub-set hits with scores of 160 or more will be listed.

> If multiple entries contain the full set of peptides, they are all displayed, whatever the setting of this parameter. This global default can be overridden on an individual report URL by appending &_showsubsets=X, where X is 0 or 1.

`SigThreshold 0.05`

> Significance threshold used in result reports, default 0.05. Valid range is 1 to 1E-18. This global default can be overridden on an individual report URL by appending &_sigthreshold=X, where X is the significance threshold.

```
SiteAnalysisMD10Prob 0.1
```

> Used to calculate relative probabilities of modification assignments in Peptide View. It defines the factor in probability that a peptide score difference of 10 corresponds to. The default is 0.1, which means a score difference of 10 corresponds to a factor of 10 in probability. Similarly, 0.05 corresponds to a factor of 20.

```
SortUnassigned scoredown
```

> In a peptide summary report, peptide matches that are not assigned to protein hits are initially sorted by descending score (scoredown). Alternatives for `SortUnassigned` are ascending query order (queryup) and descending intensity order (intdown). This global default can be overridden on an individual report URL by appending &_sortunassigned=X, where X is scoredown, queryup, or intdown.

```
SplitDataFileSize 10000000
```

> Large searches are divided into 'chunks', and no single chunk can exceed this number of bytes – default 10 Mb. When a search is divided into chunks, protein and peptide match data are no longer written to the summary section of the result file. This means that a Protein summary report cannot be generated.

```
SplitNumberOfQueries 1000
```

> Large searches are divided into 'chunks', and no single chunk can exceed this number of queries – default 1000. When a search is divided into chunks, protein and peptide match data are no longer written to the summary section of the result file. This means that a Protein summary report cannot be generated.

```
StoreModPermutations 1
```

> If set to 0, only the highest scoring permutation of variable modifications for each unique peptide sequence is retained in the list of the top 10 ions scores. If set to 1, then different permutations of variable modifications are treated as independent matches, creating the possibility that all 10 top ions scores correspond to the same primary sequence. Default is 1.

```
TargetFDRPercentages 0.1, 0.2, 0.5, 1+, 2, 5
```

Choices available for the FDR drop down list in the Protein Family Summary report of an auto-decoy search. Each item in the list is a percentage. The + symbol specifies the default setting of the control, 1% in this case.

```
TaxBrowserURL
```

(No default). The URL used in reports to retrieve taxonomy information for a Protein View report. By default, this points to the NCBI. If you don't want to send such queries out to the internet, the URL can be replaced by a call to the ms-gettaxonomy.exe utility:

```
TaxBrowserUrl ../x-cgi/ms-
      gettaxonomy.exe?4+#DATABASE#+#ACCESSION#
```

```
TestDirectory see ErrorLogFile
```

```
UniqueJobStartNumber see ErrorLogFile
```

```
UnixDirPerm 777
```

Specify the Linux permissions for the 'daily' result file directories. For example, 775 makes each directory world readable but not writeable. This option provides more fine grained control than UnixWebUserGroup

```
UnixWebUserGroup
```

This entry, if present, will restrict access to the files created by *ms-monitor.exe*, and hence improve system security. The UnixWebUserGroup is the <u>number</u> of the group used by the web server to run CGI programs. With Apache, the group name will generally be nobody, and you will need to ascertain the group number from the group file. For other Web servers, check the documentation that comes with the server to find out which user name is used for running CGI programs.

A value of -2 can be used if the same user name is used to run Web server scripts as runs *ms-monitor.exe*. (This is generally only possible under Irix, using capabilities). In this case, The files created by *ms-monitor.exe* will not be world accessible, and 'chown' is not used on the files to change ownership.

Failure to put the correct group name will generally result in one of two error messages:

```
        Failed to open memory mapped file <filename>. ↳
            ↳ Error: access denied
```

or

```
        Failed to create memory map for <filename>. ↳
            ↳ Error Access denied
```

Vmemory -1

Obsolete.

Cron

Do not modify this section if you ever use Database Manager

Database Manager uses the information in this section to schedule database updates.

```
Cron
CronEnable 1
Logfile ../logs/cron.log
Logging 3
0-59 * 1-31 * * /usr/local/mascot/bin/dbman_process_tasks.pl
end
```

`CronEnable` is set to 1 to enable cron functionality, 0 to disable.

`Logfile` specifies the path to the log for recording cron events, `Logging` controls the verbosity:

> 0 - No logging
> 1 - Log successful commands (return code 0)
> 2 - Log unsuccessful commands (return code not 0)
> 3 - Log successful and unsuccessful commands

The remaining lines in this section simulate a crontab file. Each line contains six fields, separated by spaces or tabs. The first five are integer patterns that specify the following: minute (0-59), hour (0-23), day of the month (1-31), month of the year (1-12), day of the week (0-6 with 0=Sunday). Each of these patterns may be an asterisk (meaning all legal values), a range of integers or a list of comma separated integers.

An element is either a number or two numbers separated by a minus sign (meaning an inclusive range). Note that days may be specified in two different ways (day of the month and day of the week). If both are specified as a list of elements, both are adhered to. For example,

```
0 0 1,15 * 1
```

would run a command on the first and fifteenth of each month, as well as on every Monday. To specify days by only one field, the other field should be set to * (for example, 0 0 * * 1 would run a command only on Mondays).

The sixth field is a string that is executed by the shell (command prompt) at the specified times. The string must be on a single line. The entire string, up to the end of the line, is passed to the command prompt for execution. The part of the string up to the first space must be the fully qualified path to an executable. The remainder of the line will be passed to the command as parameters.

Log files

Mascot maintains several log files, which are described below. When trouble-shooting, it can be useful to inspect the web server log files, also. Errors in Perl scripts, for example, will be appear in the web server error log, not the Mascot error log.

Error Log

All errors are logged to *logs/errorlog.txt*. This is may be the only place to find a fatal error message resulting from a major configuration problem.

Examples of typical error messages are shown below. A comprehensive list of all Mascot error messages can be found in the file *errors.html*, in the root directory of the Mascot CD-ROM.

```
Error [M00088 - Job 2636 - X00123:file-upload] - Thu Mar 11 10:59:30 2009
   - Invalid command/mass at line 1 of your query.
 Line is where am I?

Error [M00034 - Job 2638 - X00251:modifications] - Thu Mar 11 10:59:59 2009
   - Modification conflict: Both Carbamidomethyl (C) and ↘
                 ↳ Carboxymethyl (C) modify the same residue

Error [M00133 - Job 2639 - X00938:www] - Thu Mar 11 11:00:21 2009
   - Peptide mass of -1234 is too small. The minimum mass allowed is 30
```

Searches Log

Every Mascot search is listed in *logs/searches.log*. The Mascot Review utility provides a web browser interface to this file, displaying filtered and sorted listings of searches. Mascot Review is described in Chapter 7.

Alternatively, the file can be opened in a spreadsheet program. The file consists of 14 columns, delimited by tabs. Row 1 contains column titles. An example of a single entry is shown below:

```
2633 \t 185 \t NCBInr \t JSC \t JSC@gmail.com \t  \t ↳
      ↳ ../data/20090311/F002633.dat \t Thu Mar 11 09:10:36 2009 ↳
      ↳ \t 17 \t User read res \t 1 \t PMF \t Yes \t 192.168.42.4
```

(Tabs indicated by \t for clarity). The individual columns contain the following information:

Column 1: Mascot job number. Job numbers are allocated sequentially, but will appear in the log in the order in which searches are completed. If the submitted search contained an error which prevented the search starting, there will be no entry in searches.log, but there should be an entry in errorlog.txt.

Column 2: Process ID

Column 3: Sequence Database searched

Column 4: User name. User names are required by the (JavaScript) search forms, but not by the search engine, so this field may be empty. If an entry logs utility program activity, rather than a search, this field contains the name of the utility, e.g. TESTPARSE or GETSEQ.

Column 5: User email address. User email addresses are required by the (JavaScript) search forms, but not by the search engine, so this field may be empty.

Column 6: Search title. Empty if none supplied.

Column 7: Relative path to Mascot search results file

Column 8: Start time in the format illustrated in the example above.

Column 9: Duration in seconds

Column 10: Completion Status, normally "User read res". If EmailUsersEnabled is set to 1, and the user disconnected before the search was complete, this entry would read "user emailed".

Column 11: Job Priority. Not currently implemented

Column 12: Type of search: PMF, SQ, or MIS

Column 13: Enzyme: Either yes (if user selected an enzyme) or no (if user selected enzyme type None).

Column 14: User IP address

Monitor Log

Mascot Monitor activity, such as sequence database exchange, is logged to *logs/monitor.log*. The following extract shows a typical example of the contents:

```
Fri Apr 20 17:21:28 2012 - ───────────────── ms-monitor 2.4.0 started
Fri Apr 20 17:21:28 2012 - Locked memory for file ../data/mascot.control
Fri Apr 20 17:21:28 2012 - Waiting for valid licence
Fri Apr 20 17:30:28 2012 - Licensed to: Edman University  (XQ5P-TFRR-3APW-FB33-7H6X)
Fri Apr 20 17:30:28 2012 - Starting up                     to Checking that Mascot
                           Nodes exist
Fri Apr 20 17:30:28 2012 - Checking that Mascot Nodes exist     to Loading DB informa-
                           tion
Fri Apr 20 17:30:28 2012 - Loading DB information           to Started up success-
                           fully
Fri Apr 20 17:30:29 2012 - SwissProt0 Not in use                to Preparing to run
                           1st test
Fri Apr 20 17:30:29 2012 - SwissProt0 Preparing to run 1st test   to Waiting
Fri Apr 20 17:30:30 2012 - SwissProt0 Waiting                 to About to compress
                           files
Fri Apr 20 17:30:30 2012 - SwissProt0 About to compress files     to Creating compressed
                           files
Fri Apr 20 17:30:33 2012 - Creating compressed files from /usr/local/mascot/sequence/
                           SwissProt/current/SwissProt_2012_03.fasta
Fri Apr 20 17:30:33 2012 - Creating compress file /usr/local/mascot/sequence/SwissProt/
                           current/SwissProt_2012_03.i00
Fri Apr 20 17:30:33 2012 - Creating compress file /usr/local/mascot/sequence/SwissProt/
                           current/SwissProt_2012_03.s00
Fri Apr 20 17:30:33 2012 - Creating compress file /usr/local/mascot/sequence/SwissProt/
                           current/SwissProt_2012_03.a00
Fri Apr 20 17:30:33 2012 - Creating compress file /usr/local/mascot/sequence/SwissProt/
                           current/SwissProt_2012_03.t00
Fri Apr 20 17:30:33 2012 - Creating compress file /usr/local/mascot/sequence/SwissProt/
                           current/SwissProt_2012_03.stats
Fri Apr 20 17:32:26 2012 - SwissProt0 Creating compressed files   to Finished compress-
                           ing files
Fri Apr 20 17:32:26 2012 - SwissProt0 Finished compressing files  to Running 1st test
Fri Apr 20 17:32:33 2012 - SwissProt0 Running 1st test            to First test just run
                           OK
Fri Apr 20 17:32:33 2012 - SwissProt0 First test just run OK      to Waiting for other
                           DB to end
Fri Apr 20 17:32:33 2012 - SwissProt0 Waiting for other DB to end to Trying to memory
                           map files
Fri Apr 20 17:32:33 2012 - SwissProt0 Trying to memory map files  to Just enabled memory
                           mapping
Fri Apr 20 17:32:33 2012 - SwissProt0 Just enabled memory mapping to In use
.
.
.
```

IPC Log

In cluster mode (only) an interprocess communication log can be enabled by setting IPCLogging (in the cluster section of *mascot.dat*) to 1 or 2. This log can be used to investigate communications errors at the socket level.

7

Program Reference

M ascot implements a client-server architecture using the HTTP protocol, (web server / web browser). In this mode, the search engine is run by the web server as a CGI application.

It is also possible to execute the search engine as a 'console' or 'command line' application. This Chapter provides the information that is required to write scripts or applications which interface to the Mascot search engine and associated programs.

Mascot Search Engine

The Mascot search engine, `cgi/nph-mascot.exe`, accepts command line arguments and a MIME format ASCII text file on standard input (STDIN) containing search data and parameters.

```
nph-mascot.exe 1 [-commandline] [-f path]
           [--taskID number] [--sessionID string] < in.asc
```

The first argument is required, and is a digit, between 1 and 4, which determines the mode of operation:

> 1: Normal search; MS/MS data, if any, form part of the MIME format input file
>
> 2: Monitor test mode 0
>
> 3: Monitor test mode 1
>
> 4: Repeat search; the MIME format input file contains a reference to a Mascot results file which may contain MS/MS data

Optional argument –commandline is a flag. If present, HTML formatted output is not written to STDOUT.

Optional argument –f allows a result file path to be specified. In the absence of this argument, the result file will be written to a daily sub-directory of `mascot/data` and have the filename `F123456.dat`, where 123456 is an auto-incremented job number.

Optional argument --taskID is used to specify a unique numeric identifier. This identifier should be obtained from the SearchControl utility, described later in this chapter. By specifying an identifier, progress reports and search results can be obtained asynchronously from SearchControl.

Optional argument --sessionID is used to specify a Mascot security session identifier, (see Chapter 12).

The file piped to STDIN must be a MIME format file containing the search parameters and mass spectrometry data.

Monitor test mode has a different syntax:

nph-mascot.exe 2|3 *path [number]* **< in.asc**

Required argument *path* is the path to a flag file, e.g. *../data/test/ SwissProt_2011_06.fasta.bu253neb5rcnpqtv2jiiannc2y.testedOk* and optional argument *number* is the cluster number. The input file, e.g. *../data/test/SwissProt.asc*, is created automatically from the *do_not_delete.asc* template.

The Monitor application must be running before search engine can be invoked. During search execution, warnings, errors, progress reports, etc. are written to standard output (STDOUT). This output is formatted as HTML text for viewing on a web browser. If the search engine is not being executed as a CGI application, the calling application may need to parse the output to remove the HTML tags.

When a search is complete, an HTML string is written to STDOUT, which causes the client browser to invoke the script defined in *mascot.dat* for displaying a results report, (*master_results.pl or master_results_2.pl*). If the search engine is not being executed as a CGI application, the name of the results file can be parsed directly from this string. The output to STDOUT from a successful search will closely resemble the following:

```
(null) 200 OK
Server: (null)
Content-type: text/html
Pragma: no-cache

<HTML>
<HEAD><TITLE>Mascot searching...</TITLE>
<META HTTP-EQUIV="Expires" CONTENT="0">
<META HTTP-EQUIV="Pragma" CONTENT="no-cache">
</HEAD><BODY BGCOLOR='#FFFFFF'>
<!-- comment here -->
<!-- comment here -->
<!-- comment here -->
<!-- comment here -->
<!-- comment here -->
```

```
<!-- comment here -->
<!-- comment here -->
<!-- comment here -->
<!-- comment here -->
<!-- comment here -->
<H1><IMG SRC="../images/88x31_logo_white.gif" WIDTH="88" HEIGHT= ↵
↳ "31" ALIGN="TOP" BORDER="0" NATURALSIZEFLAG="3"> Mascot Search</H1>
Licensed to: Matrix Science In-house test system.<BR>Not a real form
Finished uploading search details...<BR>
<B>IMPORTANT:</B> If you get disconnected or choose not to wait ↵
↳ for your search results<BR>DO NOT RESUBMIT THE SEARCH. Your ↵
↳ results will be sent by email when the search is complete<BR>


Searching....<BR>
.10% complete<BR>
..20% complete<BR>
...30% complete<BR>
....50% complete<BR>
.....60% complete<BR>
......70% complete<BR>
.......90% complete<BR>
271397 sequences and 86500527 residues checked.<BR>
<SCRIPT LANGUAGE="JavaScript">
<!-- Begin hiding Javascript from old browsers.
if(window.navigator.userAgent.indexOf("MSIE") != -1){
  window.location.replace("../cgi/master_results.pl?file= ↵
↳ ../data/20090312/F002642.dat");
} else if (window.location.replace == null){
  window.location.assign("../cgi/master_results.pl?file= ↵
↳ ../data/20090312/F002642.dat");
} else {
  window.location.replace("../cgi/master_results.pl?file= ↵
↳ ../data/20090312/F002642.dat");
}


// End hiding Javascript from old browsers. -->
</SCRIPT>
<NOSCRIPT>
<A HREF="../cgi/master_results.pl?file=../data/20090312/F002642.dat"> ↵
↳ Click here to see Search Report</A>
</NOSCRIPT>
</BODY></HTML>
```

The executable called *nph-mascot1.exe* is for Mascot TD ("BIG" Mascot), where the precursor mass limit of 16 kDa has been removed. It will only be used for searches if enabled in the licence.

Monitor

The primary function of Mascot Monitor, *bin/ms-monitor.exe*, is to manage the sequence databases. Monitor must be running in order for the search engine to execute. Under Linux this runs as a daemon, and under Windows this runs as a service.

Monitor does the following:

1. Creates compressed files from the databases, checking that the FASTA database files are valid; minor errors in the files are reported as warnings, more serious errors stop the databases from being used

2. These files can then be mapped into memory to improve search times

3. Allows swapping and updating of databases without interruption to executing searches. This means that Mascot can be available for running searches 24/7.

4. Deletes old copies of the FASTA databases to stop the disk becoming full; only the most recent copy is kept.

5. Optionally email a system administrator with serious errors requiring immediate attention. Configuration of email settings in the options section of *mascot.dat* is described in Chapter 6.

6. Optionally email users with their results if they didn't wait for them. Configuration of email settings in the options section of *mascot.dat* is described in Chapter 6.

Sequence Of Events When A New Database Is Added

When a new or updated database is added to a directory, the following sequence of events takes place:

1. If the entry in the *mascot.dat* file indicates that there should also be a reference file containing full text entries, Monitor looks for a file with the same name as the new file but with a *.ref* or *.dat* extension instead of *.fasta*. If there is no such file, the swap to the new database stops.

2. Compressed index files are made from the .fasta and reference files. For example, the following files would be created for the database SwissProt_2012_03:

```
SwissProt_2012_03.a00
SwissProt_2012_03.i00
SwissProt_2012_03.s00
SwissProt_2012_03.stats
```

```
SwissProt_2012_03.NoTaxonomyMatch.txt
SwissProt_2012_03.t00
```

The final two files are only created if taxonomy is specified in the database configuration. Compressed files are a proprietary format, which is unlikely be useful for other applications.

3. If a serious error occurs while creating these files, then the conversion to the new database stops, an error is put into the error log and (optionally) the error message is emailed to the administrator. Also, if the status screen is shown, the existence of the error is shown on that screen. Searches on the existing database will continue until the problem is resolved.

4. A test search is performed on the new database. The test uses the appropriate file in the `../data/test` directory. If the test is successful, then a file with the name <database_name>.<unique hash key>.fasta.testedOk is put into the `../data/test` directory. If the test fails, then an error is put into the error log and (optionally) the error message is emailed to the administrator. Also, if the status screen is shown, the existence of the error is shown on that screen. Searches on the existing database will continue until the problem is resolved.

5. Any new searches submitted by users will now use the new database.

6. When there are no more searches running that use the old database, the files for the old database will be unmapped from memory, and the new files are then mapped into memory.

7. Any files in the `old` directory for the database, which have the same base name as the current files, are deleted.

8. The `.fasta` and `.ref` files for the outgoing database are moved to the `old` directory

9. The compressed index files are for the outgoing database are deleted.

Why Memory Map and Lock the FASTA Files?

To speed up the processing of the FASTA files, they should be mapped into memory. Databases can be configured in three operational modes:

1. Without memory mapping. Do not choose this option, it will make searches very slow.

2. Memory mapping the database files, but not locking the memory. This gives the best performance in most cases. When the system gets low on memory, the files are swapped out of memory to disk.

On most platforms, this will give better performance than simply relying on the system file cache.

3. Memory mapping the files, and locking the memory. This gives the best possible performance, but does require sufficient RAM for the databases, the operating system, searches, and any other applications that are to run concurrently with Mascot.

In order to reduce the amount of memory required, and to prevent memory fragmentation, the sequence strings from the FASTA database are saved separately in a number of files that are then memory mapped. The description line(s) are not memory mapped, only an index to the description in the original database. Compared with mapping the original FASTA database, this can reduce memory requirements by more than 30%. Furthermore, the savings for a nucleic acid database are even greater because the files are compressed with a 2:1 ratio.

For trouble-shooting purposes, Monitor can be started from a command or shell prompt with the argument DEBUG. Under Windows, *ms-monitor.exe* must not be started from the command line if it is already running as a service.

Status and error messages from Monitor can be viewed from a web browser using the Mascot Status application, described below.

GetSeq

GetSeq is a utility for retrieving the sequence, title, or full text of an entry in a database configured for use by Mascot. The utility can be used to retrieve information for a single entry, or in batch mode

Single entry mode

The executable, *x-cgi/ms-getseq.exe*, accepts the following command line parameters:

1. The name of the database, e.g. NCBInr This argument is required.

2. An accession string, e.g. 100K_RAT. This argument is required.

3. One of five keywords: seq, all, len, title or pI. This argument is required, and is explained further below.

4. (Nucleic acid databases only) Frame number between 1 and 6 to retrieve a sequence translated into protein or 0 for the original nucleic acid sequence.

5. (Optionally, if Mascot sercurity enabled) —sessionID followed by a space and then the security session identifier

If the keyword `seq` is supplied, the output from GetSeq has the following format:

```
Content-type: text/plain

*MMSARGDFLNYALSLMRSHNDEHSDVLPRLY ... PLYSSKQILKQKLLLAIKTKNFGFV
>100K_RAT 100 KD PROTEIN (EC 6.3.2.-). - RATTUS NORVEGICUS (RAT).
```

The keyword, all, is only applicable if a local, full text database is available and configured in *mascot.dat*. In which case, the returned text has a format similar to the following:

```
Content-type: text/plain

*MMSARGDFLNYALSLMRSHNDEHSDVLPRLY ... PLYSSKQILKQKLLLAIKTKNFGFV
>100K_RAT 100 KD PROTEIN (EC 6.3.2.-). - RATTUS NORVEGICUS (RAT).
>P1;100K_RAT
100 KD PROTEIN (EC 6.3.2.-). - RATTUS NORVEGICUS (RAT).
.
.
.
C;DOMAIN 827 847 PRO-RICH.
C;BINDING 858 858 UBIQUITIN (BY SIMILARITY).
C;Keywords: UBIQUITIN CONJUGATION; LIGASE.
```

In all cases, the first line is a content-type specifier, followed by a blank line.

For `seq` and `all` there is then an asterisk followed by the unformatted sequence in one letter code. The next line is identical to the FASTA title line, beginning with a right angle bracket.

In the case of a full text report, this is followed by the raw text entry, as retrieved from the sequence database full text file.

If the keyword len is supplied, then the length of the sequence is returned as ascii text. If the database is a nucleic acid database, then the length returned will depend on the translation frame number specified.

If the keyword title is supplied, the FASTA title line is returned, beginning with a right angle bracket.

If the keyword pI is supplied, the calculated iso-electric point is returned.

Batch mode

Request format

GET-request always means single entry mode. POST-request automatically means batch mode. A batch mode request should use UTF-8 encoding and be of "multipart/form-data"-enctype, for example:

```
————————————41184676334
Content-Disposition: form-data; name="db"

SwissProt
————————————41184676334
Content-Disposition: form-data; name="accession"

"RL19_YEAST"
"G3P2_YEAST", "ERROR_YEAST"
————————————41184676334
Content-Disposition: form-data; name="accession"

"TRY1_BOVIN"
————————————41184676334
Content-Disposition: form-data; name="showpi"

on
————————————41184676334
Content-Disposition: form-data; name="showtitle"

on
————————————41184676334
Content-Disposition: form-data; name="showlen"

on
————————————41184676334
Content-Disposition: form-data; name="showsequence"

on
————————————41184676334
Content-Disposition: form-data; name="showreference"

off
————————————41184676334
Content-Disposition: form-data; name="sessionID"

123456
————————————41184676334—
```

Maximum number of accession strings submitted at once shouldn't be more than 100 000 and the total size of request shouldn't be more than 10 Mb.

All request parameter names are case-insensitive. Any parameter value can be optionally quoted.

DB – mandatory parameter and can only appear once. If several databases are searched than ms-getseq must be called separately for each database.

ACCESSION – must appear at least once and consist of entries in the format "accession_string"[:frameNo]

Quotes around accession strings are mandatory, Frame number can be integer from 0 to 6 and can only be specified for NA-databases. Otherwise, an error will be reported. Accessions can be delimited with commas, spaces, tabs or new-line characters. Several ACCESSION fields will be merged by ms-getseq.exe into one internally.

SHOWPI – can appear only once and if set to TRUE pi-values will have to be calculated for each sequence and output.

SHOWTITLE – can appear only once and if set to TRUE a description for each db-entry has to be output.

SHOWLEN – can appear only once and if set to TRUE a length of sequence string is output for each db-entry.

SHOWSEQUENCE – can appear only once and if set to TRUE a sequence string should be output for every db-entry.

SHOWREFERENCE – can appear only once and if set to TRUE reference lines should be output for each db-entry.

SESSIONID – an optional parameter and can appear at most once. If no session ID is supplied then ms-getseq can either process the request when security is disabled or try to retrieve the ID from cookies.

Boolean values can be coded in different ways:

> true = TRUE = True = on = any number except 0 = any string except an empty string

> false = FALSE = False = 0 = ""

All missing parameters are defaulted to "false" value. Missing frame-parameter by default is equal to 0.

Output format

In response to any POST-request, XML format output is returned. Encoding UTF-8 is to be used for output. XML output is schema-vali-

dated and schema-versioned. All XML output must be XML escaped using the following substitutions:

>   >

<   <

&   &

'   '

"   "

Proteins are returned in the order requested. A <msgs:frame> element will only be output for an NA database.

The example input file would produce output similar to this (edited for brevity):

```
<?xml version="1.0" encoding="UTF-8" standalone="no" ?>
<msgs:ms_getseq_out xmlns:msgs="http://www.matrixscience.com/xmlns/
schema/msgetseq_1"
                    majorVersion="1" minorVersion="0"
                    xmlns:xsi="http://www.w3.org/2001/XMLSchema-in-
stance"
                    xsi:schemaLocation="http://www.matrixscience.com/
xmlns/schema/msgetseq_1 msgetseq_1.xsd">
  <msgs:all_errors>
    <msgs:error code="461">
      <msgs:err_description>Sequence not found</msgs:err_description>
      <msgs:err_param name="accession">ERROR_YEAST</msgs:err_param>
    </msgs:error>
  </msgs:all_errors>
  <msgs:all_proteins jobid="873">
    <msgs:protein>
      <msgs:accession>RL19_YEAST</msgs:accession>
      <msgs:db>SwissProt</msgs:db>
      <msgs:prot_title>&gt;sp|P05735|RL19_YEAST 60S ribosomal protein
L19 OS=Saccharomyces cerevisiae GN=RPL19A PE=1 SV=
5</msgs:prot_title>
      <msgs:prot_len>189</msgs:prot_len>
      <msgs:prot_pi>11.35</msgs:prot_pi>
      <msgs:prot_sequence>MANLRT ... ALLKEDA</msgs:prot_sequence>
    </msgs:protein>
    <msgs:protein>
      <msgs:accession>G3P2_YEAST</msgs:accession>
      .
      .
      .
    </msgs:protein>
```

```
    <msgs:protein>
      <msgs:accession>TRY1_BOVIN</msgs:accession>
      .
      .
      .
    </msgs:protein>
  </msgs:all_proteins>
</msgs:ms_getseq_out>
```

Error messages

All errors have unique codes and are logged to both the XML output and the Mascot error log, (but only the first 10 instances of any particular error number). The XML output contains a full set of error messages in a structured format that can be processed automatically.

Fatal Errors (no database entry is going be retrieved)

403   "Error while reading mascot.dat"

        Parameters:

errstring – error message as generated by ms-parser

463   "'db' parameter is missing"

464   "'accession' parameter is missing"

440   "Invalid session or session ID"

        Parameters:

   errstring – error message as returned by security objects

443   "Not allowed to search the database"

        Parameters:

   db – database name that was requested

462   "One or more errors happened while loading taxonomy nodes"

        Parameters:

messages – more detailed error information

460   "Failed to register job. Please inspect mascot error log."

270   "A POST-request is submitted with zero content length"

55   "Cannot find boundary string"

56   "First line was not a boundary"

259   "Corrupted input - possibly a binary file is submitted"

72   "Corrupted input or incompatible browser"

458   "Invalid accession format for ms-getseq.exe"

459   "Too large POST-request"

54   "Standard input stream error"

               Parameters:

                    bytesread – number of bytes already read

                    lengthofdata – total size of input data in the stream

Non-fatal Errors:

461   "Sequence not found"

               Parameters:

          accession – accession string

               frame – frame number (0 if not supplied in the input or
missing if AA-database)

        Warnings that are only reported in the end of the XML document:

400   "Missing or invalid gencode id. Table 1 is used for transla-
tion"

               Parameters:

          accession – accession string

               frame – frame number (0 if not supplied in the input or
missing if AA-database)

470   "Cannot find taxonomy id"

               Parameters:

          accession – accession string

               frame – frame number (0 if not supplied in the input or
missing if AA-database)

104   "Sequence is too long for translation"

               Parameters:

          accession – accession string

               frame – frame number (0 if not supplied in the input or
missing if AA-database)

Status

The Database Status utility, `x-cgi/ms-status.exe`, provides an overview of the active and recent searches on all of the configured databases. The top level display will resemble this:

By clicking on a database hypertext link, a page is displayed showing the activity on that particular database:

From which, links allow details of any specific search to be displayed:

Status can also be used to print Mascot configuration and result files to STDOUT. This provides a method to display these files in a browser. For example:

http://your_server/mascot/x-cgi/ms-status.exe?Show=MS_ENZYMES

where the argument to Show determines the file to be displayed:

MS_ENZYMES	enzymes
MS_FRAGMENTATION_RULES	fragmentation_rules
MS_MASCOT_DAT	mascot.dat
MS_MASSES	masses
MS_MOD_FILE	mod_file
MS_QUANTITATIONXML	quantitation.xml
MS_SUBSTITUTIONS	substitutions
MS_TAXONOMY	taxonomy
MS_UNIMODXML	unimod.xml
MS_USERS	users.xml

The above files are all displayed as plain text, without any formatting. If Show=RESULTFILE, then a results file from any directory under mascot/data can be returned, with HTML formatting. For example:

http://your_server/mascot/x-cgi/ms-status.exe?
    Show=RESULTFILE&DateDir=20031231&ResJob=F006983.dat

For security reasons, the following characters are not allowed in the DateDir or ResJob: ~ / \ :

The argument MS_USERS returns a list of users that can be spoofed by the user whose session ID was supplied. This may be an empty list. Output format is: "username","user id","user type","full name","email address". E.g.:

```
"guest","1","1","Guest user","guest@localhost"
"admin","2","1","Administrator","admin@localhost"
"daemon","4","1","Mascot Daemon","daemon@localhost"
"(system)","6","2","Mascot Integra system account","integra@localhost"
```

MS_STATUSXML returns an XML formatted document equivalent to the main status page. The schema is html\xmlns\schema\msstatus_1\msstatus_1.xsd

Review

Mascot Review, *x-cgi/ms-review.exe*, provides similar functionality to Status, but takes its input from *searches.log*. The tabular display can be filtered and sorted to locate specific searches by title, user name, or any one of the following log fields:

1. Mascot job number.
2. Process ID
3. Sequence Database
4. User name
5. User email address
6. Search title
7. Results file path
8. Start time and date
9. Duration in seconds
10. Completion Status
11. Job priority
12. Type of search: PMF, SQ, or MIS
13. Enzyme: Either yes (if user selected an enzyme) or no (if user selected enzyme type None).
14. User IP address

At the top of each column is a checkbox and a radio button. Select the radio button to sort the display on that column. Uncheck the checkbox to hide that column.

Along the top of the screen are a series of controls:

The Sort/filter button updates the display to reflect changes in parameters.

If you have multiple log files, a specific file can be displayed by entering its path into the Log File text field.

Start can be used to page through a long listing in blocks of entries specified by the number in the following field. Setting start to -1 displays the list starting from the last entry in the log file rather than the first

Finally, there is a field to specify a path to the data files. The log file only contains a relative path. If the data files have been moved, possibly to an archive directory or CD-ROM, the path to the new location can be specified here so as to restore the validity of the relative path.

An example of the Status display, filtered to show MS/MS searches of NCBInr, is shown below:

GetTaxonomy

GetTaxonomy is a utility for retrieving taxonomy details for an entry in a database configured for use by Mascot. The utility can be used to retrieve information for a single entry, ot in batch mode

Single entry mode

The executable, *x-cgi/ms-gettaxonomy.exe*, can be called from the command line, or via a URL as a CGI application.

When calling as a CGI application, with arguments appended to the URL, the parameter list must be URL escaped. (Spaces replaced by '+' and characters other than letters or numbers replaced by a '%xx' where xx is the ASCII code for the character as a hexadecimal number).

When running from a command line, the accession string should be enclosed in single or double quotes. This is essential for accession strings beginning gi| , because the pipe character has special meaning in Linux and Windows.

In the table below, the first argument supplied to ms-gettaxonomy.exe is an integer to specify the mode. The remaining arguments are selected from:

database	Mascot database name, e.g. NCBInr
accession	accession string, e.g. gi|7633482

tax_ID	taxonomy ID number, e.g. 9606
species	name of species, e.g. "homo sapiens"
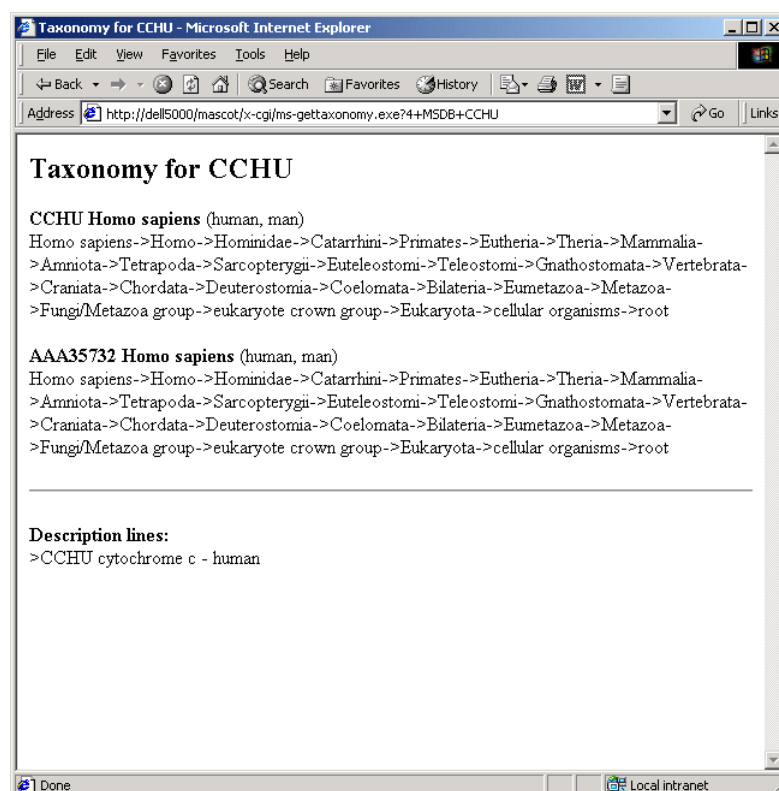
Parameters	Returns
1 database accession	Space separated list of accession string, tax_ID number, and scientific species name. Where a database entry represents multiple accessions, this information is repeated for each accession. Plain formatted.

2 database accession	Space separated pair of accession string and scientific species name. Where a database entry represents multiple accessions, this information is repeated for each accession. Followed by the FASTA title line for the accession supplied as an argument. Pretty formatted.
3 database accession	Same as mode 2, plus a list of common species names in parentheses.
4 database accession	Same as mode 3, plus complete taxonomy tree
5 database tax_ID	The scientific species name as a string. Pretty formatted.
6 database tax_ID	Same as mode 5, plus a list of common species names in parentheses.
7 database tax_ID	Same as mode 6, plus complete taxonomy tree
8 database species	verbose tax_ID information
9 database accession	genetic code number

Batch mode

Request format

GET-request always means single entry mode. POST-request automatically means batch mode. A batch mode request should use UTF-8 encoding and be of "multipart/form-data"-enctype, for example:

```
————————————41184676334
Content-Disposition: form-data; name="db"

SwissProt
————————————41184676334
Content-Disposition: form-data; name="accession"

"RL19_YEAST"
————————————41184676334
Content-Disposition: form-data; name="taxID"

1061
————————————41184676334
Content-Disposition: form-data; name="showtitle"
```

```
on
─────────────────41184676334
Content-Disposition: form-data; name="showSynonyms"

on
─────────────────41184676334
Content-Disposition: form-data; name="showTaxTree"

on
─────────────────41184676334
Content-Disposition: form-data; name="sessionID"

123456
─────────────────41184676334─
```

The batch format aggregatesboth "find taxonomy from accession" and "find taxonomy from id" requests.

Maximum number of accessions / taxIDs submitted at once must not exceed 100000 and the total size of request should be no more than 10 MB.

All request parameter names are case-insensitive. Any parameter value can be in quotes.

DB – mandatory parameter and can only appear once. If several databases are searched than ms-getseq must be called separately for each database.

ACCESSION – can appear any number of times. Quotes are mandatory. Can have a list of accessions delimited by commas, spaces, tabs or new line characters. All ACCESSION-fields are merged into one list of accession strings internally.

TAXID – can appear any number of times and contains a list of taxonomy ids delimited by commas, spaces or new line characters. All such fields are merged into one list internally.

SHOWTITLE – can appear only once and if set to TRUE a description for each db-entry has to be output.

SHOWSYNONYMS – can appear only once and if set to TRUE a list of common names should be output for each taxonomy.

SHOWTAXTREE – can appear only once and if set to TRUE taxonomy tree should be output for each taxonomy.

SESSIONID – an optional parameter and can appear at most once. If no session ID is supplied then ms-gettaxonomy can either process the request when security is disabled or try to retrieve the ID from cookies.

Boolean values can be coded in different ways:

true = TRUE = True = on = any number except 0 = any string except an empty string

false = FALSE = False = 0 = ""= off

All missing parameters are defaulted to "false" value.

Translation table number is always output as well as taxonomy Id and scientific name.

Output format

In response to any POST-request, XML format output is returned. Encoding UTF-8 is to be used for output. XML output is schema-validated and schema-versioned. All XML output must be XML escaped using the following substitutions:

> >

< <

& &

' '

" "

Taxonomy information is returned in the order requested. A <msgs:frame> element will only be output for an NA database.

The example input file would produce output similar to this (edited for brevity):

```
<?xml version="1.0" encoding="UTF-8" standalone="no" ?>
<msgt:ms_gettaxonomy_out xmlns:msgt="http://www.matrixscience.com/
xmlns/schema/msgettaxonomy_1"
                majorVersion="1" minorVersion="0"
                xmlns:xsi="http://www.w3.org/2001/XMLSchema-in-
stance"
                xsi:schemaLocation="http://www.matrixscience.com/
xmlns/schema/msgettaxonomy_1 msgettaxonomy_1.xsd">
  <msgt:results jobid="874">
    <msgt:db_entry>
      <msgt:db>SwissProt</msgt:db>
      <msgt:accession_str>RL19_YEAST</msgt:accession_str>
      <msgt:title>&gt;sp|P05735|RL19_YEAST 60S ribosomal protein L19
OS=Saccharomyces cerevisiae GN=RPL19A PE=1 SV=5</ms
gt:title>
      <msgt:all_accessions>
        <msgt:accession>
```

```
            <msgt:accession_str>RL19_YEAST</msgt:accession_str>
            <msgt:taxonomy>
              <msgt:db>SwissProt</msgt:db>
              <msgt:taxonomy_id>4932</msgt:taxonomy_id>
              <msgt:scientific_name>Saccharomyces cerevisiae</
msgt:scientific_name>
              <msgt:translation_table_id>1</msgt:translation_table_id>
              <msgt:common_names>
                <msgt:synonym>Candida robusta</msgt:synonym>
                <msgt:synonym>Saccaromyces cerevisiae</msgt:synonym>
                <msgt:synonym>Saccharomyces capensis</msgt:synonym>
                <msgt:synonym>Saccharomyces italicus</msgt:synonym>
                <msgt:synonym>Saccharomyces oviformis</msgt:synonym>
                <msgt:synonym>Saccharomyces uvarum var. melibiosus</
msgt:synonym>
                <msgt:synonym>Saccharomyes cerevisiae</msgt:synonym>
                <msgt:synonym>Sccharomyces cerevisiae</msgt:synonym>
                <msgt:synonym>YEAST</msgt:synonym>
                <msgt:synonym>baker&apos;s yeast</msgt:synonym>
                <msgt:synonym>brewer&apos;s yeast</msgt:synonym>
                <msgt:synonym>lager beer yeast</msgt:synonym>
                <msgt:synonym>yeast</msgt:synonym>
              </msgt:common_names>
              <msgt:tree>
                <msgt:node level="12">Saccharomyces cerevisiae</
msgt:node>
                <msgt:node level="11">Saccharomyces</msgt:node>
                <msgt:node level="10">Saccharomycetaceae</msgt:node>
                <msgt:node level="9">Saccharomycetales</msgt:node>
                <msgt:node level="8">Saccharomycetes</msgt:node>
                <msgt:node level="7">Saccharomycotina</msgt:node>
                <msgt:node level="6">Ascomycota</msgt:node>
                <msgt:node level="5">Dikarya</msgt:node>
                <msgt:node level="4">Fungi</msgt:node>
                <msgt:node level="3">Fungi/Metazoa group</msgt:node>
                <msgt:node level="2">Eukaryota</msgt:node>
                <msgt:node level="1">cellular organisms</msgt:node>
              </msgt:tree>
            </msgt:taxonomy>
          </msgt:accession>
        </msgt:all_accessions>
      </msgt:db_entry>
      <msgt:tax_from_id>
        <msgt:taxonomy>
          <msgt:db>SwissProt</msgt:db>
          <msgt:taxonomy_id>1061</msgt:taxonomy_id>
          <msgt:scientific_name>Rhodobacter capsulatus</
msgt:scientific_name>
```

```
        <msgt:translation_table_id>11</msgt:translation_table_id>
        .
        .
        .
      </msgt:taxonomy>
    </msgt:tax_from_id>
  </msgt:results>
</msgt:ms_gettaxonomy_out>
```

The way information is represented in the XML output will be clearer if a few rules are kept in mind:

- msgt:title element will only appear in the output if showTitle=true,

- msgt:common_names element will only appear in the output if showSynonyms=true,

- msgt:tree element will only appear in the output is showTaxTree=true,

- order of elements within msgt:tree is essential,

- in msgt:tree "root" element is not listed but always assumed,

- msgt:translation_table_id element may not be available,

- Any of the elements msgt:db_entry, msgt:tax_from_id can be missing or repeated several times depending on request.

Error messages

All errors have unique codes and are logged to both the XML output and the Mascot error log, (but only the first 10 instances of any particular error number). The XML output contains a full set of error messages in a structured format that can be processed automatically.

Fatal Errors (no database entry is going be retrieved)

403   "Error while reading mascot.dat"

Parameters:

errstring – error message as generated by ms-parser

463   "'db' parameter is missing"

465   "POST-request to ms-gettaxonomy is empty"

440   "Invalid session or session ID"

Parameters:

errstring – error message as returned by security objects

443   "Not allowed to search the database"

Parameters:

db – database name that was requested

27   "Database is not available or not active"

Parameters:

db – database name that was requested

251   "No taxonomy indexes for this database"

Parameters:

db – database name that was requested

469   "Failed to load species file"

Parameters:

messages – more detailed error message

462   "One or more errors happened while loading taxonomy nodes"

Parameters:

messages – more detailed error information

460   "Failed to register job. Please inspect mascot error log."

270   "A POST-request is submitted with zero content length"

55   "Cannot find boundary string"

56   "First line was not a boundary"

259   "Corrupted input - possibly a binary file is submitted"

72   "Corrupted input or incompatible browser"

466   "Invalid accession format for ms-gettaxonomy.exe"

468   "Too large POST-request"

467   "Invalid taxID format for ms-gettaxonomy.exe"

54   "Standard input stream error"

Parameters:

bytesread – number of bytes already read

lengthofdata – total size of input data in the stream

Non-fatal errors:

461   "Sequence not found"

Parameters:

accession – accession string

470   "Cannot find taxonomy id"

Parameters:

accession – accession string (empty if non-fatal error, can be non-empty only in warning-section for accession-requests)

taxid – taxonomy id

Warnings that are only reported in the end of XML document:

400   "Missing or invalid gencode id. Table 1 is used for translation"

Parameters:

accession – accession string (empty if non-fatal error, can be non-empty only in warning-section for accession-requests)

taxid – taxonomy id

470   "Cannot find taxonomy id"

Parameters:

accession – accession string (empty if non-fatal error, can be non-empty only in warning-section for accession-requests)

taxid – taxonomy id

SearchControl

Any helper application can call *bin/ms-searchcontrol.exe* to implement asynchronous automation of search submission. Available commands are:

```
--status
--result_file_name
--result_file_mime
--result_file_ini
--results
--xmlresults
--create_task_id
```

```
--mascot_job_number
--kill_job
--pause_job
--resume_job
--nice_job
--set_to_queued
--version
```

ms-searchcontrol.exe --status --taskID <number> [--sessionID <string>]

The 'status' command will return one of the following:

unknown_id (referring to task ID)

id_assigned (referring to task ID)

error=nnnn

running=yy%

complete

queued

searchcontrol-error=nnn

where error indicates an error in the search, and will be the Mascot error number or one of:

```
TASK_ERROR_NO_ERROR             =   0
TASK_ERROR_JOB_CRASHED          = -1
TASK_ERROR_JOB_KILLED           = -2
```

And searchcontrol-error indicates a problem with the ms-searchcontrol.exe program. Values will be one of:

```
ERR_TASKID_NOERROR          = 0
ERR_TASKID_FAILOPEN             = 1
ERR_TASKID_FAILCREATE           = 2
ERR_TASKID_FAILREAD             = 3
ERR_TASKID_FAILWRITE            = 4
ERR_TASKID_FAILCLOSE            = 5
ERR_TASKID_CHANGEDRECORD        = 6
ERR_TASKID_INVALIDMASCOTDAT     = 7
ERR_TASKID_MISSINGRESULTSFILE       = 8
```

```
ERR_TASKID_FILENAMETOOLONG        = 9
ERR_TASKID_SESSIONTIMEDOUT        = 10
ERR_TASKID_PERMISSIONDENIED       = 11
```

ms-searchcontrol.exe --result_file_name --taskID <number> [--sessionID <string>]

This will return either the results file name:

filename=<filename>

or

searchcontrol-error=nnn

with values of 'nnn' as for --status.

Note that <filename> may be empty for some states – this is not an error.

This may then be used from the command line for other applications to provide functionality that is not in ms-searchcontrol.exe For example, a client application needs the USER name from a search. In this case, a perl script 'getusername.pl' could be written that takes the passed unique task ID, finds the results file name using:

```
ms-searchcontrol.exe --result_file_name
```

and then looks for the user name in the results file.

ms-searchcontrol.exe --result_file_mime --taskID <number> [--sessionID <string>]

This will return the results file as a mime format file or

searchcontrol-error=nnn

with values of 'nnn' as for --status.

ms-searchcontrol.exe --result_file_ini --taskID <number> [--sessionID <string>]

This will return the results file as a windows '.ini' format file or

searchcontrol-error=nnn

with values of 'nnn' as for --status.

ms-searchcontrol,exe --results --taskID <number> [--sessionID <string>]

If the job is complete, then this will return the search results in a format recognised by Mascot Daemon:

For a peptide mass fingerprint, the output is of the form:

```
###daemon###file=..\data\F981122.dat
###daemon###release=MSDB_20020121.fasta
###daemon###queries=8
###daemon###num_hits=6
###daemon###h1=1A6K,103,1.00,17004.1
###daemon###h1_text=myoglobin - sperm whale
###daemon###reptype=concise
###daemon###sigscoreprot=72
###daemon###ionquery1=734.992175 from(736.000000,1+)
###daemon###ionquery2=746.992175 from(748.000000,1+)
###daemon###ionquery3=939.992175 from(941.000000,1+)
###daemon###ionquery4=1515.992175 from(1517.000000,1+)
###daemon###ionquery5=1591.992175 from(1593.000000,1+)
###daemon###ionquery6=1853.992175 from(1855.000000,1+)
###daemon###ionquery7=1980.992175 from(1982.000000,1+)
###daemon###ionquery8=2111.992175 from(2113.000000,1+)
###daemon###selectpeptides=0
```

For an MS/MS ions search, the output is of the form:

```
###daemon###file=..\data\F981123.dat
###daemon###release=MSDB_20020121.fasta
###daemon###queries=4
###daemon###num_hits=1
###daemon###h1=Q9XZJ2,286.477,1.00,79480.1
###daemon###h1_text=HEAT SHOCK PROTEIN 70.- Crassostrea gigas (Pacific
oyster).
###daemon###reptype=peptide
###daemon###sigscoreprot=72
###daemon###ionquery1=1341.784350 from(671.900000,2+) query(1)
###daemon###score1=95.12
###daemon###sigscore1=49
###daemon###ionquery2=1614.584350 from(808.300000,2+) query(2)
###daemon###score2=74.55
###daemon###sigscore2=48
###daemon###ionquery3=1945.784350 from(973.900000,2+) query(3)
###daemon###score3=89.84
###daemon###sigscore3=47
###daemon###ionquery4=2167.784350 from(1084.900000,2+) query(4)
###daemon###score4=39.11
```

```
###daemon###sigscore4=47
###daemon###selectpeptides=1
```

>   If the job is incomplete, or has failed, then an error will be returned:
>
>   `unknown_id`
>
>   `searchcontrol-error=nnn`
>
>   with values of 'nnn' as for --status.

ms-searchcontrol,exe --xmlresults --taskID <number> --reporttop [FILE|AUTO|num_hits] [--sessionID <string>]

>   If the job is complete, then this will return the results formatted as an XML instance document that conforms to the schema

mascot/html/xmlns/schema/DistillerMascotSearch_1/DistillerMascotSearch_1.xsd

>   If the job is incomplete, or has failed, then an error will be returned:
>
>   `unknown_id`
>
>   `searchcontrol-error=nnn`
>
>   with values of 'nnn' as for --status.

ms-searchcontrol.exe --create_task_id [--sessionID <string>]

>   On failure, this will return
>
>   `searchcontrol-error=nnn`
>
>   with values of 'nnn' as for --status.
>
>   And on success it will return:
>
>   `taskID=nnn`

ms-searchcontrol.exe --mascot_job_number --taskID <number> [--sessionID <string>]

>   This will return either the job number:
>
>   `mascotjobnumber=nnnn`
>
>   or
>
>   `searchcontrol-error=nnn`
>
>   with values of 'nnn' as for --status.

ms-searchcontrol.exe --kill_job --taskID <number> [--sessionID <string>]

If the task is successful, this will return the text:

job_killed

If there is an error, one of the following will be returned:

unknown_id

job_not_running

searchcontrol-error=nnn

with values of 'nnn' as for --status.

The 'kill' is implemented by setting a flag in the mascot.control memory mapped file. The nph-mascot.exe task is responsible for 'killing' itself.

ms-searchcontrol.exe --pause_job --taskID <number> [--sessionID <string>]

If the task is successful, this will return the text:

job_paused

If there is an error, one of the following will be returned:

unknown_id

job_not_running

job_already_paused

searchcontrol-error=nnn

with values of 'nnn' as for --status.

The 'pause' is implemented by setting a flag in the mascot.control memory mapped file. The nph-mascot.exe task is responsible for 'pausing' itself.

ms-searchcontrol.exe --resume_job --taskID <number> [--sessionID <string>]

If the task is successful, this will return the text:

job_resumed

If there is an error, one of the following will be returned:

unknown_id

```
job_not_running
```

```
job_not_paused
```

```
searchcontrol-error=nnn
```

with values of 'nnn' as for --status.

The 'resume' is implemented by setting a flag in the mascot.control memory mapped file. The nph-mascot.exe task is responsible for 'resuming' itself.

ms-searchcontrol.exe --nice_job --taskID <number> [--nice <integer>] [--sessionID <string>]

The task ID need to be supplied. and an optional nice value

If a valid new nice value is supplied, this will return the text:

```
job_niced
```

If a nice value is not supplied, the program will return the current nice value:

```
nice=xxx
```

If there is an error, one of the following will be returned:

```
unknown_id
```

```
job_not_running
```

```
searchcontrol-error=nnn
```

with values of 'nnn' as for --status.

The 'nice' is implemented by setting a flag in the mascot.control memory mapped file. The nph-mascot.exe task is responsible for 'resuming' itself. Nice values range from –20 to +20. A value of +20 will set the task to a very low priority. The Mascot status screen shows the 'nice' value as a priority, which is simply –1 * the nice value. Microsoft Windows does not allow such a fine grained control of priorities, so, for example +20 and +19 will map to the same priority.

ms-searchcontrol.exe --set_to_queued --taskID <number> [--sessionID <string>]

If the task is successful, this will return the text:

```
queued
```

If there is an error, one of the following will be returned:

```
unknown_id
```

```
already_running

already_complete

searchcontrol-error=nnn
```

with values of 'nnn' as for --status.

A batch processing client can make queued jobs visible to the Mascot system by getting a taskID and using this call to set the status to 'queued'. When the search is eventually submitted, nph-mascot.exe will set the status 'running'. A queued job will return 'queued' when ms-searchcontrol.exe is called with the —status argument.

ms-searchcontrol.exe --version [--sessionID <string>]

If the task is successful, this will return the version number

CreatePIP

Usage: ms-createpip.exe [OPTION] -i filename

Options:

-h, —help	display this help page and exit
-f, —features	display list of features defined in mascot.dat and exit
—sessionID <id>	not normally used because this is run from command line
-o output_file	default is filename.pip
-q <#queries>	override minimum number of queries set in mascot.dat
-s <#sequences>	override minimum number of sequences set in mascot.dat
-a <feature>	add a feature to the list specified in mascot.dat
-r <feature>	remove a feature to the list specified in mascot.dat
-c	use cached results
-p <interval>	progress reports of Process:X% every <interval> seconds
—nocache	do not use cached results
—version	display version number and exit

Features:

retentionTime	Retention time in seconds if available
dM	Calculated minus observed peptide mass in Da
mScore	Mascot score (always on)
lgDScore	Mascot score minus Mascot score of next best non-isobaric peptide hit
mrCalc	Calculated Mr
charge	Charge
dMppm	Calculated minus observed peptide mass in ppm
absDM	Absolute value of calculated minus observed peptide mass in Da
absDMppm	Absolute value of calculated minus observed peptide mass in ppm
isoDM	Calculated minus observed peptide mass, after eliminating possible isotope errors up to 2 Da, in Da
isoDMppm	Calculated minus observed peptide mass, after eliminating possible isotope errors up to 2 Da, in ppm
mc	Number of missed cleavages (always 0 if no enzyme)
varmods	Number of modified sites divided by number of modifiable sites
varmodsCount	The number of variable mods used in the peptide. That is, if there are 10 Met and 5 of these are oxidised, this counts as 1. A peptide with Met-OX, phosphoS, deamidation, and acetylation, would count as 5.
modifiable	Total number of modifiable sites
modified	Total number of modified residues and terminii
totInt	Log total ion intensity. The 20 most intense peaks in each 100 Da bin are used for all features, and totInt reports this value
intMatchedTot	Log total matched ion intensity
relIntMatchedTot	Total matched ion intensity divided by total ion intensity as a percentage (no logs involved)
fragDeltaMed	Median value of all matched fragment errors in Da
fragDeltaIqr	Interquartile range value of all matched fragment errors in Da
fragDeltaMedPPM	Median value of all matched fragment errors in ppm
fragDeltaIqrPPM	Interquartile range value of all matched fragment errors in ppm
fragDeltaPolyFit	2nd order polynomial fit to m/z vs delta. Result is RSquared multiplied by the number of points divided by 100
longest	Longest sequence matched ions, reported separately for each ion series (backbone only), as with fracIonsMatched

```
fracIonsMatched     Fraction of calculated ions matched, reported sepa-
                    rately for each ion series, with NLs lumped to-
                    gether (e.g. fracIonsMatchedB1,
                    fracIonsMatchedB1deriv, fracIonsMatchedB2,
                    fracIonsMatchedB2deriv)
matchedIntensity    Matched ion intensity, reported separately for each
                    ion series, as with fracIonsMatched
   numUniqPeps *    The excess of the number of unique peptide matches
                    (unique primary sequence) over the number of
                    matches expected by chance
        qmatch      The number of peptide matches for which an ms-ms
                    match was attempted
       peptide      The peptide string that was matched
      proteins      A tab separated list of accessions of proteins that
                    contain this peptide. Must be last
```

'*' indicates that this feature is not implemented

Error codes

Return Description

-1   Invalid parameters. Use —help for help

-2   Missing or invalid mascot.dat. Error:

-3   No MS-MS spectra in results file

-4   Automatic decoy search not enabled

-5   Insufficient number of queries.

-6   Insufficient number of sequences searched.

-7   Cannot read the results file. Error:

-8   Failed to create output file:

-9   Invalid feature in mascot.dat options:

-10   Invalid feature for -a option:

-11   Invalid feature for -r option:

Miscellaneous Utilities

Service

Supplied for Windows only. This application shows the status of the
Matrix Science Mascot Service, and allows it to be stopped and started.
It is normally accessed from the start menu -

Programs; Mascot; config; Show Mascot Service Status

Programs; Mascot; config; Start Mascot Service

Programs; Mascot; config; Stop Mascot Service

These options run the program *x-cgi/ms-service.exe* with the first parameter set to the service name (MatrixScienceMascotService) and the second parameter being 0, 1, or 2 respectively.

It is also possible to run this program as a CGI script by entering the following URL in the browser:

http://your.host/mascot/x-cgi/ ↰
↳ *ms-service.exe?MatrixScienceMascotService+0*

Where *your.host* is replaced by the host name of the Mascot server. This CGI script can be run from any computer on the network. However, it is not usually possible to start and stop the service from another computer using the default access rights.

There is a final option, which will allow removal of the service. This may be required for a manual de-installation and will not normally be required. If this option is used, Mascot will not run again without re-running the installation program. The command to enter is:

ms-service MatrixScienceMascotService remove

Compress

Compress is a utility for compressing FASTA files independently of Mascot monitor.

The executable, *bin/ms-compress.exe* is executed from a shell or command prompt.

ms-compress.exe db_name fasta

where

db_name is the database family name from mascot.dat - e.g. MSDB

fasta is the fully qualified path to the FASTA file

ms-compress.exe compresses the fasta file using the rules specifed in mascot.dat and must be run so that it's current working directory is *mascot/bin*.

Return value of 0 for success, > 0 for failure

MakeSearchLog

The bin/ms-makesearchlog.exe utility is used to rebuild the search log by scanning all the result files located in sub-directories of *mascot/ data*. This can be useful if the original search log has been damaged or if result files have been pruned after archiving. There are no arguments.

LockMem

On 32 bit platforms, the 2GB address space limit can quickly be reached by having several large databases locked into memory. To work around this limit, the *bin/ms-lockmem.exe* utility is provided.

LockMem is enabled by adding the parameter 'SeparateLockMem 1' to the options section of mascot.dat. Specifying a value greater than 1 specifies the block size in MB. For example, if there is a 1.5 GB *.s00 file, and this parameter is set to 750, two instances of *ms-lockmem.exe* will be run.

GetError

The utility *cgi/ms-geterror.exe* takes an error number as an argument and returns the corresponding text string. For example:

```
C:\Inetpub\MASCOT\cgi>ms-geterror.exe 23
You specified enzyme %s which is not available. Choose another.
```

8
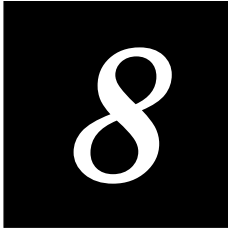
I/O File Formats

Both Mascot search input files and results output files are in MIME format. This is a text file which can be viewed easily for inspection or debugging purposes.

The MIME format is defined in various "request for comment" documents. The following are the most relevant:

ftp://ftp.isi.edu/in-notes/rfc2045.txt

ftp://ftp.isi.edu/in-notes/rfc2046.txt

ftp://ftp.isi.edu/in-notes/rfc2388.txt

Very briefly, a unique boundary string is used to divide the file into sections, each of which contains data in a format defined by a Content-type.

Each section begins with two hyphens followed by the boundary string. The next line contains the content definition and name, followed by a blank line. Then data, until the beginning of the next section For example:

```
MIME-Version: 1.0 (Generated by Mascot version 1.0)
Content-Type: multipart/mixed; boundary=gc0p4Jq0M2Yt08jU534c0p

--gc0p4Jq0M2Yt08jU534c0p
Content-Type: application/x-Mascot; name="first_name"

first_value
--gc0p4Jq0M2Yt08jU534c0p
Content-Type: application/x-Mascot; name="another_name"

another_value
--gc0p4Jq0M2Yt08jU534c0p
.
.
.
--gc0p4Jq0M2Yt08jU534c0p
```

```
Content-Type: application/x-Mascot; name="final_name"

final_value
--gc0p4Jq0M2Yt08jU534c0p--
```

Search Input File

The search input file is normally generated by the web browser. If another application is used to generate an input file, simply ensure that it conforms to the MIME format standard.

The Mascot Monitor test searches use "captured" input files. Hence, an example of a file can be seen by opening `mascot/data/test/SwissProt.asc` in any text editor.

```
——————————243501029130836
Content-Disposition: form-data; name="INTERMEDIATE"


——————————243501029130836
Content-Disposition: form-data; name="FORMVER"

1.01
——————————243501029130836
Content-Disposition: form-data; name="SEARCH"

MIS
——————————243501029130836
Content-Disposition: form-data; name="PEAK"

AUTO
——————————243501029130836
Content-Disposition: form-data; name="REPTYPE"

peptide
——————————243501029130836
Content-Disposition: form-data; name="ErrTolRepeat"

0
——————————243501029130836
Content-Disposition: form-data; name="SHOWALLMODS"


——————————243501029130836
Content-Disposition: form-data; name="USERNAME"

Monitor Program Test
```

```
————————————243501029130836
Content-Disposition: form-data; name="COM"

MS/MS Test Search
————————————243501029130836
Content-Disposition: form-data; name="DB"

SwissProt
————————————243501029130836
Content-Disposition: form-data; name="CLE"

Trypsin/P
————————————243501029130836
Content-Disposition: form-data; name="PFA"

1
————————————243501029130836
Content-Disposition: form-data; name="QUANTITATION"

None
————————————243501029130836
Content-Disposition: form-data; name="TAXONOMY"

All entries
————————————243501029130836
Content-Disposition: form-data; name="MODS"

Carbamidomethyl (C)
————————————243501029130836
Content-Disposition: form-data; name="IT_MODS"

Oxidation (M)
————————————243501029130836
Content-Disposition: form-data; name="TOL"

100
————————————243501029130836
Content-Disposition: form-data; name="TOLU"

ppm
————————————243501029130836
Content-Disposition: form-data; name="PEP_ISOTOPE_ERROR"

0
————————————243501029130836
Content-Disposition: form-data; name="ITOL"

0.1
————————————243501029130836
```

```
Content-Disposition: form-data; name="ITOLU"

Da
————————————243501029130836
Content-Disposition: form-data; name="CHARGE"

1+
————————————243501029130836
Content-Disposition: form-data; name="MASS"

Monoisotopic
————————————243501029130836
Content-Disposition: form-data; name="FILE";
filename="test_search.mgf"
Content-Type: application/octet-stream

BEGIN IONS
PEPMASS=498.272888
CHARGE=1+
157.096962 23.72
185.160000 26.69
286.134951 80.7
385.210000 13.49
.
.
.
2000.120000 3.142
2000.568167 4.108
2001.020697 2.098
2001.820000 1.103
END IONS


————————————243501029130836
Content-Disposition: form-data; name="FORMAT"

Mascot generic
————————————243501029130836
Content-Disposition: form-data; name="PRECURSOR"


————————————243501029130836
Content-Disposition: form-data; name="INSTRUMENT"

ESI-QUAD-TOF
————————————243501029130836
Content-Disposition: form-data; name="REPORT"

AUTO
————————————243501029130836—
```

Results File

The results file contains the search results together with the search input parameters and peak list data. This means that a results file contains everything necessary to generate a report, repeat the search at a later date, or act as the self-contained input file to a project database or LIMS.

Mascot Parser provides an object-oriented Application Programmer Interface (API) to Mascot result files and configuration files, making it easy for programs written in C++, Java, Perl or Python to access Mascot results.

We strongly recommend that anyone writing software to process Mascot results uses Mascot Parser, just like all of the Mascot result report scripts:

- It makes application development much faster

- It makes your code simpler and easier to debug

- You don't have to worry about updating your code every time a new version of Mascot is released

The Mascot Parser package, which includes object libraries, header files, binary executables, extensive documentation, and example code for many functions, is available as a free download. For more information, go to http://www.matrixscience.com/msparser.html

For reference, the result file contents are divided into logical sections:

1. Search parameters
2. Mass values
3. Quantitation method (if used)
4. Unimod extract
5. Enzyme definition
6. Taxonomy (if a taxonomy filter was used)
7. Misc. header information
8. Summary results (for Protein Summary)
9. Mixtures (if PMF)
10. Summary of decoy results (if automatic decoy)
11. Summary of error tolerant results (if automatic ET)
12. Mixtures in decoy results (if automatic decoy PMF)
13. Peptides (if SQ or MIS)
14. Decoy peptides (if SQ or MIS and automatic ET)
15. Error tolerant peptides (if SQ or MIS and automatic ET)
16. Proteins (if SQ or MIS)
17. Query data, one block for each query
18. Index

General Notes

1. Values are shown in italics
2. Label case doesn't matter.
3. Labels are used to assist readability, but kept short to minimise file size
4. Parameters are grouped logically
5. Order of blocks is not important except that the index block must be the last block. Presence of blank lines within the index block may cause a problem.
6. Because the MIME type is defined as an unknown application, if this file passes through a mail agent, it will be treated as an "octet stream" and encoded "base64" for transmission.

Search parameters

```
--gc0p4Jq0M2Yt08jU534c0p
Content-Type: application/x-Mascot; name="parameters"

USERNAME=user name in plain text
USEREMAIL=email address in plain text
SEARCH=PMF
COM=search title text
DB=SwissProt
CLE=Trypsin
MASS=Monoisotopic
MODS=Mod 1,Mod 2
.
.
.
RULES=1,2,5,6,8,9,13,14
--gc0p4Jq0M2Yt08jU534c0p
```

The Parameters section contains the complete set of parameter values from the search form apart from the contents of the uploaded data file or the query window. Labels must be unique, independent of case. Where a parameter can be multivalued (e.g. mods) the values are listed on one line separated by commas.

RULES contains a list of the rule numbers that define the instrument type in the configuration file `fragmentation_rules`. The rule numbers are listed explicitly because the contents of the configuration file may have changed since the search was run.

Masses

```
--gc0p4Jq0M2Yt08jU534c0p
```

```
Content-Type: application/x-Mascot; name="masses"

A=71.037110
B=114.534940
C=160.030649
D=115.026940
E=129.042590
F=147.068410
G=57.021460
H=137.058910
I=113.084060
J=0.000000
K=128.094960
L=113.084060
M=131.040480
N=114.042930
O=0.000000
P=97.052760
Q=128.058580
R=156.101110
S=87.032030
T=101.047680
U=150.953630
V=99.068410
W=186.079310
X=111.000000
Y=163.063330
Z=128.550590
Hydrogen=1.007825
Carbon=12.000000
Nitrogen=14.003074
Oxygen=15.994915
Electron=0.000549
C_term=17.002740
N_term=1.007825
delta1=15.994919,Oxidation (M)
NeutralLoss1=0.000000
FixedMod1=57.021469, Carbamidomethyl (C)
FixedModResidues1=C
--gc0p4Jq0M2Yt08jU534c0p
```

This block contains "actual" mass values. That is, average or monisotopic residue masses, including any fixed modifications; C and N terminus groups also include any fixed modifications.

FixedMod1, FixedMod2, etc., records the delta mass and name for each fixed modification as comma separated values. FixedModResidues1 gives the site specificity. If multiple residues are affected, they are listed as a

string, e.g. STY. If there was a neutral loss, the delta mass is given by the value of FixedModNeutralLoss1.

```
FixedModn=delta, Name
FixedModResiduesn=[A-Z]|C_term|N_term
FixedModNeutralLossn=mass
```

Fixed modifications cannot have peptide neutral losses, multiple neutral losses and cannot be protein-terminal or residue-terminal. In all these cases, fixed modifications are automatically converted into variable ones.

Variable modifications are reported in delta1, delta2, etc. Each entry defines the difference in mass introduced by the modification together with the name of the modification, separated by a comma. If a variable modification suffers a neutral loss on fragmentation, the delta is specified by a NeutralLoss*n* entry. By definition, this is always a master neutral loss. If there are multiple neutral losses, then two more lines appear:

```
NeutralLossn_master=mass[[,mass] ...]
NeutralLossn_slave=mass[[,mass] ...]
```

The first neutral loss (defined by NeutralLoss*n*) has an implicit index number of 1. Any additional neutral losses (defined by NeutralLoss*n*_master or followed by NeutralLoss*n*_slave) have implicit index numbers of 2 and up.

If a modification includes a required or optional neutral loss from the precursor, this is recorded as follows:

```
ReqPepNeutralLossn=mass[[,mass] ...]
PepNeutralLossn=mass[[,mass] ...]
```

Error-tolerant modifications are not listed in masses section.

Quantitation

```
--gc0p4Jq0M2Yt08jU534c0p
Content-Type: application/x-Mascot; name="quantitation"

<?xml version="1.0" encoding="UTF-8" standalone="no" ?>
<quantitation majorVersion="1" minorVersion="0" xmlns="http://
www.matrixscience.com/xmlns/schema/quantitation_1" xmlns:xsi=
"http://www.w3.org/2001/XMLSchema-instance" xsi:schemaLocation="http:/
/www.matrixscience.com/xmlns/schema/quantitation_1 qu
antitation_1.xsd">
```

```
    <method constrain_search="false" description="15N metabolic label-
ling" min_num_peptides="2" name="15N Metabolic [MD]" pro
t_score_type="mudpit" protein_ratio_type="weighted"
report_detail="true" require_bold_red="true" show_sub_sets="0.5"
sig_th
reshold_value="0.05">
    <component name="light">
      <isotope/>
  </component>
    <component name="heavy">
      <isotope>
        <old>N</old>
        <new>15N</new>
      </isotope>
```

> This section is an extract from quantitation.xml containing the
> quantitation method specified for the search. For more details and a link
> to the schema, refer to the Mascot HTML help pages for quantitation.

Unimod

```
—gc0p4Jq0M2Yt08jU534c0p
Content-Type: application/x-Mascot; name="unimod"


<?xml version="1.0" encoding="UTF-8" standalone="no" ?>
<umod:unimod xmlns:umod="http://www.unimod.org/xmlns/schema/unimod_2"
majorVersion="2" minorVersion="0" xmlns:xsi="http://w
ww.w3.org/2001/XMLSchema-instance" xsi:schemaLocation="http://
www.unimod.org/xmlns/schema/unimod_2 unimod_2.xsd">
  <umod:elements>
    <umod:elem avge_mass="1.00794" full_name="Hydrogen"
mono_mass="1.007825035" title="H"/>
    <umod:elem avge_mass="2.014101779" full_name="Deuterium"
mono_mass="2.014101779" title="2H"/>
    <umod:elem avge_mass="6.941" full_name="Lithium"
mono_mass="7.016003" title="Li"/>
    <umod:elem avge_mass="12.0107" full_name="Carbon" mono_mass="12"
title="C"/>
```

> This section is an extract from unimod.xml containing data for the
> elements, amino_acids, and any modifications specified in the search
> form. For more details and a link to the schema, refer to the help pages
> at www.unimod.org

Enzyme

```
—gc0p4Jq0M2Yt08jU534c0p
Content-Type: application/x-Mascot; name="enzyme"

Title:Trypsin
Cleavage:KR
Restrict:P
Cterm
*
```

This section is simply an extract from the enzyme file. Syntax details can be found in Chapter 6

Taxonomy

```
—gc0p4Jq0M2Yt08jU534c0p
Content-Type: application/x-Mascot; name="taxonomy"

Title:. . . . . . . . . . . . . . . . . Homo sapiens (human)
Include: 9606
Exclude:
*
```

This section is simply an extract from the taxonomy file. Syntax details can be found in Chapter 9

Header

```
--gc0p4Jq0M2Yt08jU534c0p
Content-Type: application/x-Mascot; name="header"

sequences=number of sequences in DB
sequences_after_tax=number of sequences after taxonomy filter
residues=number of residues in DB
distribution=see below
distribution_decoy=see below
decoy_type=n (type of decoy, 1..8)
exec_time=search time in seconds
date=timestamp (seconds since Jan 1st 1970)
time=time in hh:mm:ss
queries=number of queries, (>= 1)
max_hits=maximum number of hits to be listed
version=version information
fastafile=full path to database fasta file
```

```
release=filename of actual database used - e.g. Owl_31.fasta
taskid=unique task identifier for searches submitted asynchronously
pmf_num_queries_used=number of mass values selected for PMF match
pmf_queries_used=comma separated list of selected query numbers
Warn0=
Warn1=
Warn2=
--gc0p4Jq0M2Yt08jU534c0p
```

The Header section contains general values, used in the master results page header paragraph.

`Distribution` is a comma separated list of values that represent a histogram of the complete protein score distribution. The first value is the number of entries with score 0, the second is the number of entries with score 1, and so on, up to the maximum score for the search. Scores are converted to integers by truncation. This distribution is only meaningful for a peptide mass fingerprint search.

If intensity values are supplied for a peptide mass fingerprint, Mascot iterates the experimental peaks to find the set that gives the best score. The number of values selected is reported in `pmf_num_queries_used` and the selected queries listed in `pmf_queries_used`.

Summary results

```
--gc0p4Jq0M2Yt08jU534c0p
Content-Type: application/x-Mascot; name="summary"

qmass1=Mr
qexp1=m/z for query 1,
      charge
qintensity1=intensity value for query1 (if available)
qmatch1=Total number of peptide mass matches for query1 in database
qplughole1=Threshold score for homologous peptide match (MIS only)
qmass2=…
qexp2=…
qintensity1=
qmatch2=…
qplughole2=…
.
.
.
qmassn=…
qexpn=…
qintensityn=
qmatchn=…
qplugholen=…
num_hits=number of hits in the summary block (<= max_hits)
```

```
h1=accession string,
      total protein score,
      obsolete,
      intact protein mass
h1_text=title text
h1_frame=frame_number (between 1 and 6, for nucleic acid only)
h1_q1=missed cleavages, (-1 indicates no match)
      peptide Mr,
      delta,
      start,
      end,
      number of ions matched,
      peptide string,
      peaks used from Ions1,
      variable modifications string,
      ions score,
      multiplicity,
      ion series found,
      peaks used from Ions2,
      peaks used from Ions3,
      total area of matched peaks
h1_q1_et_mods=modification mass,
            neutral loss mass,
            modification description
h1_q1_et_mods_master=neutral loss mass[[,neutral loss mass] … ]
h1_q1_et_mods_slave=neutral loss mass[[,neutral loss mass] … ]
h1_q1_primary_nl=neutral loss string
h1_q1_na_diff=original NA sequence,
            modified NA sequence
h1_q1_tag=tagNum:startPos:endPos:seriesID,…
h1_q1_drange=startPos:endPos
h1_q1_terms=residue,residue
h1_q1_subst=pos1,ambig1,matched1 … ,posn,ambign,matchedn
h1_p1_summed_mods=variable modifications string
h1_q2=…
.
.
.
h1_qm=…
h2=…
.
.
.
hn_qm=…
--gc0p4Jq0M2Yt08jU534c0p
```

Where a parameter has multiple values, these are shown on separate lines for clarity. In the actual result file, all values for a parameter are on a single line and there are no spaces or tabs between values.

Variable modifications is a string of digits, one digit for the N terminus, one for each residue and one for the C terminus. Each digit specifies the modification used to obtain the match: 0 indicates no modification, 1 indicates delta1, 2 indicates delta2 etc., in the masses section. If the number of modifications exceeds 9, the letters A to W are used to represent modifications 10 to 32. X is used to indicate a modification found in error tolerant mode.

`neutral loss string` is the same concept as the variable mod string, except each character represents the index of the primary neutral loss (one of the master NL). Any position that is not modified, or where the mod has no neutral loss, is set to 0. hn_qm_primary_nl will only be output if the string contains at least one non-zero character.

If a new modification is found in an error tolerant search, its position is marked by X, and details are recorded in an additional entry, hn_qm_et_mods. If the error tolerant search is of a nucleic acid database, and the modification is a single base change in the primary sequence, the two mass fields will be set to zero, and one of the keywords `NA_INSERTION`, `NA_DELETION`, or `NA_SUBSTITUTION` will appear in the description field. The additional parameter hn_qm_na_diff is then used to record the 'before' and 'after' nucleic acid sequences.

If the search includes a quantitation method and the search parameter MULTI_SITE_MODS is set to 1 then a single site can carry two modifications. When this occurs, a second modifications string, e.g. h1_p1_summed_mods, is used to record the additional modification(s).

Ion series is a string of 19 digits representing the ion series:

> a
> place holder
> a++
> b
> place holder
> b++
> y
> place holder
> y++
> c
> c++
> x
> x++
> z
> z++
> z+H
> z+H++

```
        z+2H
        z+2H++
```

A digit is set to 1 if the corresponding series contains more than just random matches and 2 if the series contributes to the score.

Multiplicity means number of peptide mass matches for a query in a protein

For each sequence tag, four colon separated values are output: 1-based tag number, 1-based residue position where tag starts, 1-based residue position where tag ends, ion series into which the tag was matched:

```
-1 means no matches for the tag
 0 "a" series (single charge)
 1 "a-NH3" series (single charge)
 2 "a" series (double charge)
 3 "b" series (single charge)
 4 "b-NH3" series (single charge)
 5 "b" series (double charge)
 6 "y" series (single charge)
 7 "y-NH3" series (single charge)
 8 "y" series (double charge)
 9 "c" series (single charge)
10 "c" series (double charge)
11 "x" series (single charge)
12 "x" series (double charge)
13 "z" series (single charge)
14 "z" series (double charge)
15 "a-H2O" series (single charge)
16 "a-H2O" series (double charge)
17 "b-H2O" series (single charge)
18 "b-H2O" series (double charge)
19 "y-H2O" series (single charge)
20 "y-H2O" series (double charge)
21 "a-NH3" series (double charge)
22 "b-NH3" series (double charge)
23 "y-NH3" series (double charge)
25 "internal yb" series (single charge)
26 "internal ya" series (single charge)
27 "z+H" series (single charge)
28 "z+H" series (double charge)
29 high-energy "d" and "d'" series (single charge)
31 high-energy "v" series (single charge)
32 high-energy "w" and "w'" series (single charge)
33 "z+2H" series (single charge)
34 "z+2H" series (double charge)
```

If there are multiple tags for a query, comma separated groups of these numbers are output for each tag.

h*n*_q*m*_drange is output for a query that includes an error tolerant sequence tag. It defines the range of positions within which an unsuspected modification has been located. For a peptide of 10 residues, position 0 would indicate the amino terminus and position 11 would indicate the carboxy terminus. If there is no location information, the range is output as 0,256

h*n*_q*m*_terms shows the residues the bracket the peptide in the protein. If the peptide forms the terminus of the protein, then a hyphen is used instead.

h*n*_q*m*_subst is output when the matched peptide contained an ambiguous residue, (B, X, or Z). The argument is one or more triplets of comma separated values. For each triplet, the first value is the residue position, the second is the ambiguous residue, and the third is the residue that has been substituted to obtain the reported match.

For a large MS/MS search, `num_hits` is set to zero, and the summary block only contains entries for `qmass`*n*, `qexp`*n*, `qmatch`*n*, `qplughole`*n*. The threshold for switching to this mode is specified using two parameters in the Options section of `mascot.dat`. SplitDataFileSize is the size of the search process in bytes, (default 10000000), and SplitNumberOfQueries is the size of the search in queries, (default 1000).

If this is a two-pass search, either an automatic decoy database search or an automatic error tolerant search, a second summary block appears, containing the second set of results. The section name is either et_summary or decoy_summary. The syntax of the contents is identical

Mixture

```
--gc0p4Jq0M2Yt08jU534c0p
Content-Type: application/x-Mascot; name="mixture"

num_hits=number of mixtures found
h1_score=total score for mixture 1
h1_numprot=number of proteins in mixture 1
h1_nummatch=number of queries matched
h1_m1=accession string for protein component 1
h1_m2=accession string for protein component 2
.
.
.
```

```
h1_mm=accession string for protein component m
h2_score=
.
.
.
hn_mm=
--gc0p4Jq0M2Yt08jU534c0p
```

The Mixture section is only output for a peptide mass fingerprint. If any statistically significant protein mixtures are found, the mixture components are summarised. For details of individual components, use the accession strings to refer back to the Summary section.

If this is an automatic decoy database search, a second mixture block appears, containing the second set of results. The section name is decoy_mixture. The syntax of the contents is identical

Peptides

```
--gc0p4Jq0M2Yt08jU534c0p
Content-Type: application/x-Mascot; name="peptides"

q1_p1=missed cleavages, (-1 indicates no match)
      peptide Mr,
      delta,
      number of ions matched,
      peptide string,
      peaks used from Ions1,
      variable modifications string,
      ions score,
      ion series found,
      peaks used from Ions2,
      peaks used from Ions3;
      "accession string": data for first protein
            frame number:
            start:
            end:
            multiplicity,
      "accession string": data for second protein
            frame number:
            start:
            end:
            multiplicity,
      etc.
q1_p1_et_mods=modification mass,
            neutral loss mass,
            modification description
q1_p1_et_mods_master=neutral loss mass[[,neutral loss mass] … ]
q1_p1_et_mods_slave=neutral loss mass[[,neutral loss mass] … ]
```

```
q1_p1_primary_nl=neutral loss string
q1_p1_na_diff=original NA sequence,
            modified NA sequence
q1_p1_tag=tagNum:startPos:endPos:seriesID,…
q1_p1_drange=startPos:endPos
q1_p1_terms=residue,residue[[:residue,residue] … ]
q1_p1_subst=pos1,ambig1,matched1 … ,posn,ambign,matchedn
q1_p1_comp=quantitation component name
q1_p1_summed_mods=variable modifications string
q1_p2=…
.
.
.
qn_pm=…
--gc0p4Jq0M2Yt08jU534c0p
```

Each line contains the data for a peptide match followed by data for at least one protein in which the peptide was found.

If there multiple entries in the database containing the matched peptide, there will be a corresponding number of pairs of bracketing residues listed in q*n*_p*m*_terms.

Otherwise, individual field descriptions are identical to those for the Summary section

If this is a two-pass search, either an automatic decoy database search or an automatic error tolerant search, a second peptides block appears, containing the second set of results. The section name is either et_peptides or decoy_peptides. The syntax of the contents is identical

Proteins

```
--gc0p4Jq0M2Yt08jU534c0p
Content-Type: application/x-Mascot; name="proteins"

"accession string"=protein mass,
            "title text"
.
.
.
"accession string"=protein mass,
            "title text"
--gc0p4Jq0M2Yt08jU534c0p
```

This block contains reference data for the proteins listed in the peptides block.

Input data for query n

```
--gc0p4Jq0M2Yt08jU534c0p
Content-Type: application/x-Mascot; name="queryn"

title=query title
index=query index
seq1=sequence qualifier (e.g. N-ABCDEF)
seq2=…
.
.
.
seqn=
comp1=composition qualifier (e.g. 0[P]2[W])
comp2=…
.
.
.
compn=…
PepTol=peptide tolerance qualifier (e.g. 2.000000,Da)
IT_MODS=Mod 1[,Mod 2[,…]]
INSTRUMENT=instrument identifier, (e.g. ESI-TRAP)
RULES=1,2,5,6,8,9,13,14
INTERNALS=min mass,max mass
CHARGE=charge state (e.g. 2+)
RTINSECONDS=a[[-b][,c[-d]]]
SCANS=a[[-b][,c[-d]]]
tag1=sequence tag (e.g. t,889.4,[QK]S,1104.54)
.
.
.
tagn=…
mass_min=lowest mass
mass_max=highest mass
int_min=lowest intensity
int_max=highest intensity
num_vals=number of mass values
num_used1=-1 (obsolete)
ions1=1344.65:34.3,1365.41:13.2
ions2=y-1344.65:34.3,1365.41:13.2
ions3=b-1344.65:34.3,1365.41:13.2

--gc0p4Jq0M2Yt08jU534c0p
```

Value "query*n*" runs from "query1" (no leading zeros). ions*n* values are sorted in the order that they were selected for scoring.

Most searches will only require a few of these fields. For example, a peptide mass fingerprint would only include the charge field.

The index is a 0 based record of the original query order before sorting by Mr

ions2 and ions3 are only required when fragment ions are specified in a sequence query as being N-terminal or C-terminal series.

The first field in a tagn value is t for a standard sequence tag and e for an error tolerant sequence tag

Some search parameters can be define in the local scope of a query. These are CHARGE, COMP, INSTRUMENT, IT_MODS, TOL, TOLU. Any that are used are listed here. If the MGF file contained scan range information in terms of seconds or scans, this is written to RTINSECONDS and/or SCANS.

Index

```
—gc0p4Jq0M2Yt08jU534c0p
Content-Type: application/x-Mascot; name="index"

parameters=4
masses=78
unimod=116
enzyme=322
taxonomy=329
header=336
summary=351
et_summary=6059
peptides=6473
et_peptides=7143
proteins=7292
query1=7362
query2=7374.
.
.
.
query81=8322
query82=8334
—gc0p4Jq0M2Yt08jU534c0p—
```

Values in index are the line number offsets of the section "Content-Type:" lines (starting from 0 for the first line of the file).

9

Taxonomy

Mascot supports the use of a taxonomy filter to limit the database entries to be searched. This is useful because it speeds up the search, and can reduce the proteins in the results list to those expected in the sample being analysed.

Some databases record taxonomy in a manner that makes it difficult to extract the information reliably. The major problems are:

1. The location of the text containing the species identifier is mostly not defined, and can even vary within one database

2. There are often many names for one particular species - e.g. homo sapiens, human, man.

3. Names are sometimes misspelled - e.g. homo sapeins.

4. Continual re-classification of species is taking place

5. Some non-redundant databases only reliably give one species when several submissions from different species have identical sequences.

6. There are differences of opinion regarding the taxonomy 'tree' structure.

This section describes how the Mascot taxonomy filter works and how to configure it. Most of the configuration that will be required should be simple to change - for example the list of species displayed in the search form can be modified easily, and it is fairly simple to download updated taxonomy lists from the vendors of public web sites. However, to modify the configuration to use a new format and a different numbering system is a more complex task that may take some time.

The NCBI keeps a list of taxonomy ID's up to date, and guarantees that the ID for a given species will not change (although some of the names used for that ID may change). Mascot configurations all use the NCBI IDs, but it would be possible to configure mascot to use a different system.

Modifying the list in the search form window

The list in the search form is taken from the *taxonomy* file in the mascot *config* directory.
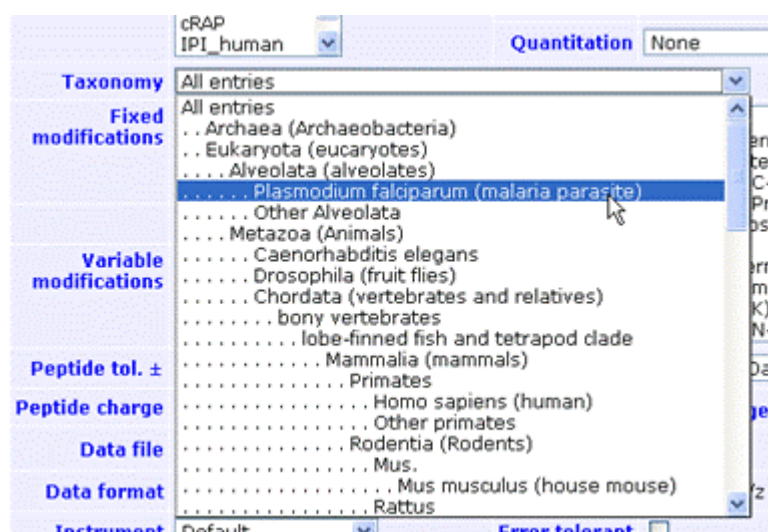
This file can be edited using any text editor. (Under Windows, from the start menu, choose Programs, Mascot, Config, Mascot taxonomy file).

The following is an extract from the supplied file:

```
Title:All entries
Include: 1
Exclude: 0
*
Title:. . Archaea (Archaeobacteria)
Include: 2157
Exclude:
*
Title:. . Eukaryota (eucaryotes)
Include: 2759
Exclude:
*
Title:. . . . Alveolata (alveolates)
Include: 33630
Exclude:
*
Title:. . . . . . . . . . . . . Primates
```

```
Include: 9443
Exclude:
*
Title:. . . . . . . . . . . . . . . Homo sapiens (human)
Include: 9606
Exclude:
*
Title:. . . . . . . . . . . . . . Other primates
Include: 9443
Exclude: 9606
*
```

The first line of each block must start with the `Title:` keyword, followed by a text string that is used to identify the species in forms and reports. The definition should be short and self-explanatory. To show the tree structure, indentation can be used. Unfortunately, it is not possible to use tabs or multiple spaces for indentation in an html form, so a full stop (period) and a space are used to indent the list. Internal spaces are significant, and there should never be two or more spaces together.

This should be followed with a definition line starting with the `Include:` keyword, followed by one or more NCBI taxonomy IDs separated with commas.

This should be followed with a definition line starting with the `Exclude:` keyword, followed by one or more NCBI taxonomy IDs separated with commas. Any sequence with a taxonomy ID that passes the 'include' test, may then be rejected by any entry in the exclude list.

Finally, each entry must end with a *

There are two ways of finding the NCBI taxonomy ID for a given species. The first is to open the file *names.dmp* in the mascot taxonomy directory (Under Windows, from the start menu, choose Programs, Mascot, Config, NCBI taxonomy names.dmp file), and search for the species name. The ID is the number on the left. For example, the ID for Filicophyta is 3263:

```
3263          | Filicophyta   |          |    scientific name      |
3263          | ferns      |      |    preferred common name  |
```

Alternatively, the NCBI taxonomy browser can be used:

http://www.ncbi.nlm.nih.gov/Taxonomy/

For example, to add a choice of Ferns or human, add the following to the taxonomy file:

```
Title:. Ferns or human
Include: 9606, 3263
Exclude:
*
```

And to add the choice of Not human or mice add the following to the taxonomy file:

```
Title:. Not human or mice
Include: 1
Exclude: 9606, 10088
*
```

Note that 'all species', or root has the ID '1'.

It is, of course, possible to accidentally specify a selection that will result in no species matching - for example include humans, and exclude animals.

If you wish to include species in the taxonomy file without having them appear on the search form, the keyword 'Hidden' should appear on the line following the title line.

Location and format of species lists

NCBI Files

The NCBI provide two files that list all the species for which they have one or more sequences. These files are called *names.dmp* and *nodes.dmp*. As shown above, *names.dmp* is a list of scientific names, synonyms and misspellings for the species. From this list, you can easily find the ID for the given species. For example:

```
3701 | Arabidopsis          | | scientific name       |
3701 | Cardaminopsis        | | synonym               |
3702 | Arabidopsis thaliana | | scientific name       |
3702 | Arbisopsis thaliana  | | misspelling           |
3702 | thale cress          | | preferred common name |
3702 | thale-cress          | | common name           |
3702 | mouse-ear cress      | | common name           |
```

The file *nodes.dmp* specifies the tree structure. The first column is a taxonomy ID and the second column is the parent taxonomy ID. Note that the 'parent' of Arabidopsis thaliana (3702) is Arabidopsis (3701).

```
3700 | 3699 | family  |    | 4 | 1 | 1 | 1 | 1 | 1 | 0 | 0 |   |
3701 | 3700 | genus   |    | 4 | 1 | 1 | 1 | 1 | 1 | 0 | 0 |   |
3702 | 3701 | species | AT | 4 | 1 | 1 | 1 | 1 | 1 | 0 | 0 |
```

Both files can be obtained from the NCBI ftp site:

ftp://ftp.ncbi.nih.gov/pub/taxonomy/taxdump.tar.gz

For NCBInr, you will also need *gi_taxid_prot.dmp.gz.* For NCBI EST databases, you will need *gi_taxid_nucl.dmp.gz.*

You should not modify the *names.dmp* and *nodes.dmp* file in the taxonomy directory. If you wish to add more entries, a new file should be made with just the new entries. Mascot will load multiple files as specified below. Most Mascot updates will contain the updated *names.dmp* and *nodes.dmp* files.

PDBeast File

This file contains a list of entries that are derived from the Brookhaven Protein databank (PDB). The file is available at:

ftp://ftp.ncbi.nih.gov/mmdb/pdbeast/table

SwissProt File

SwissProt also supplies a file, *speclist.txt* that is similar to the NCBI *names.dmp* file, except that it gives the NCBI taxonomy ID for the SwissProt Code. A regular expression is used to extract the "Code" and "Taxon Node" from the file. The regular expression should be defined in any Taxonomy_ x section that uses speclist.txt and is defined as:

SWISSPROTRegex   "^\([A-Z0-9]*\) *[ABEV] *\([0-9]*\):"

```
Code    Taxon   N=Official name
        Node    C=Common name
                S=Synonym
_____ _ _____ _____
AAV2  V 010804: N=Adeno-associated virus 2
                C=AAV2
ABDS2 B 056673: N=Antarctic bacterium DS2-3R
ABIAL E 045372: N=Abies alba
                C=Edeltanne
```

This file is available at:

ftp://ftp.ebi.ac.uk/pub/databases/uniprot/   ↳

↳   knowledgebase/docs/speclist.txt

If you wish to add more entries, a new file should be made with just the new entries- Mascot will load multiple files as specified below. Most Mascot updates will contain the updated *speclist.txt* file.

Genetic code selection

During a search of a nucleic acid database, Mascot also uses the taxonomy of each entry to choose the correct genetic code for translation. The genetic codes are defined in the NCBI file *gencode.dmp*, which is included in the archive *taxdump.tar*, mentioned above.

Nodes.dmp is used as a lookup table to obtain a genetic code number from a TaxID.

For many species, the genetic code is different for mitochondrial and nuclear proteins. Although Mascot could try to determine whether a database entry is mitochondrial by performing a keyword search of the FASTA description, this is unreliable. In any case, mitochondrial proteins will usually represent only a very small fraction of the entries in any comprehensive database. The most important requirement is to use the correct code for a database that is specifically mitochondrial proteins. The solution is to include a flag in each *mascot.dat* taxonomy definition to specify, at a database level, which code is to be used.

For further information on genetic codes, see:

http://www.ncbi.nlm.nih.gov/Taxonomy/Utils/wprintgc.cgi?mode=c

Modifying the "Taxonomy lineage' link

In the protein view, a link to taxonomy lineage is shown:
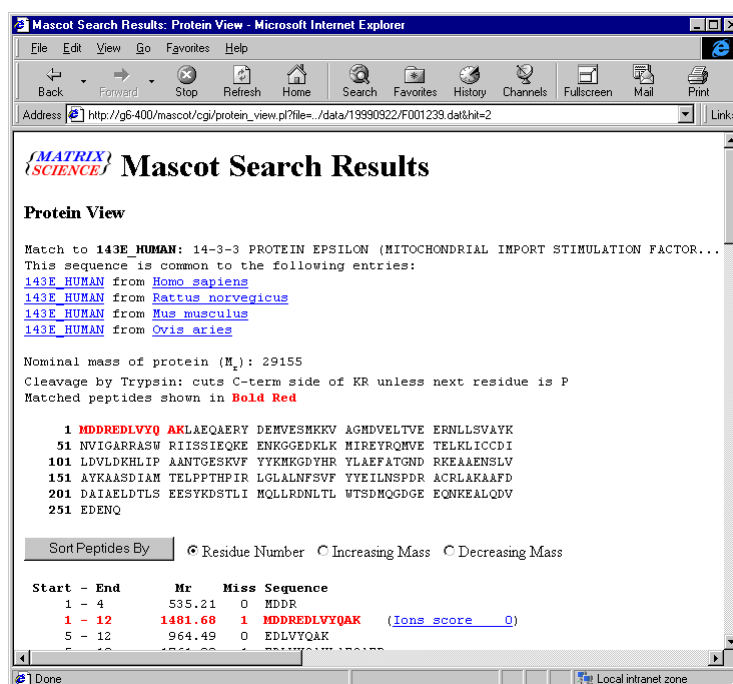
The default behaviour is for this to link to the NCBI taxonomy browser. For non-redundant databases with more than one species source per sequence, there will be a list of the species, each with a link. For the NCBInr database, a separate 'gi' number will be shown for each database entry, with a link to Entrez and the NCBI Taxonomy browser for each entry.

If security and confidentiality protocols may make this unacceptable for your installation, then change the entry in the Options section of the *mascot.dat* file from:

```
TaxBrowserUrl http://www.ncbi.nlm.nih.gov/htbin-
     post/Taxonomy/wgetorg?lvl=0&lin=f&id=#TAXID#
```

to

```
TaxBrowserUrl ../x-cgi/ms-gettaxonomy.exe?4+#DATABASE#+#ACCESSION#
```

In this case, the link will display the information in the following format

Taxonomy for gi|4501885

gi|4501885 Unknown species

gi|113270 Homo sapiens (human, man, HUMAN, 103D, 10GS, 11GS, 121P, 12CA, 12GS, 133L)
Homo sapiens->Homo->Hominidae->Catarrhini->Primates->Eutheria->Theria->Mammalia-> Amniota->Tetrapoda->lobe-finned fish and tetrapod clade->bony vertebrates->Gnathostomata-> Vertebrata->Craniata->Chordata->Deuterostomia->Coelomata->Bilateria->Eumetazoa->Metazoa-> Fungi/Metazoa group->eukaryote crown group->Eukaryota->root
.
.
.
gi|3320892 Trichosurus vulpecula (Tricosurus vulpecular, Trichosurus vulpecular, common brush-tailed possum, TRIVU)
Trichosurus vulpecula->Trichosurus->Phalangeridae->Diprotodontia->Metatheria->Theria-> Mammalia->Amniota->Tetrapoda->lobe-finned fish and tetrapod clade->bony vertebrates-> Gnathostomata->Vertebrata->Craniata->Chordata->Deuterostomia->Coelomata->Bilateria-> Eumetazoa->Metazoa->Fungi/Metazoa group->eukaryote crown group->Eukaryota->root

Description lines:
>gi|4501885|ref|NP_001092.1|pACTB| beta actin
gi|113270|sp|P02570|ACTB_HUMAN ACTIN, CYTOPLASMIC 1 (BETA-ACTIN)
gi|71618|pir||ATHUB actin beta - human
gi|71619|pir||ATMSB actin beta - mouse
gi|279669|pir||ATCHB actin beta - chicken
gi|28252|emb|CAA25099| (X00351) beta-actin [Homo sapiens]
gi|49866|emb|CAA27307| (X03672) beta-actin (aa 1-375) [Mus musculus]
gi|55575|emb|CAA24528| (V01217) beta-actin [Rattus norvegicus]
gi|177968 (M10277) cytoplasmic beta actin [Homo sapiens]
gi|211237 (L08165) beta-actin [Gallus gallus]
gi|2116655|dbj|BAA20266| (AB004047) beta-actin [Homo sapiens]
gi|2182269 (U39357) beta actin [Ovis aries]
gi|2661136 (AF035774) beta actin [Equus caballus]
gi|3320892 (AF076190) beta-actin [Trichosurus vulpecula]

Other parameters are possible for ms-gettaxonomy.exe - see the reference section 'ms-gettaxonomy' in Chapter 7.

Common Questions

Why do I sometimes get results for a species I didn't specify?

Sometime, when specifying for example 'Human' species, the results may appear at first sight to be from for example a Mouse sample. The most common reason for this is that, for a non-redundant database, exactly the same sequence has been found in many species. To check this, look at the protein view, where you should see at least one entry for the species you selected.

What is the "unclassified" and "other" species?

The NCBI cannot always classify every sequence - either because no species information was supplied with the data or because it currently doesn't fit into any currently classification. There are about 1500 such sequences in the NCBI nr database.

"Other" species include plasmids, and artificial sequences.

How do I see which sequences Mascot couldn't assign a taxonomy ID?

In the status screen, click on the "Unidentified taxonomy" link. This will show sequences where one or more of the species names were not identified by Mascot.

Why do I get the message "Taxonomy 'xxx' ignored. No taxonomy indexes for this database"

Check the following:

In the mascot.dat file, is parameter 14 for the problem database a valid number and is there a taxonomy section in mascot.dat for that number?

When the compressed files are built, the taxonomy index has the name 'database_name.t00' If this file doesn't exist for the database, it may be necessary to stop Mascot Monitor, delete the *.stats file for the database, and restart Monitor.

How Mascot gets a species ID for sequences

This section contains complex configuration information. It is normally only necessary to read and understand this section when adding a new database of a different type.

When ms-monitor creates the compressed files, it also makes a file containing the taxonomy ID(s) for each sequence. To do this it needs to follow certain rules. These rules are defined in mascot.dat. The rule number for each database is specified as the 14th parameter in the databases section of the mascot.dat file. To help explain these rules, the following sections describe these rules for NCBInr, SwissProt, and EST_others.

All text searches and comparisons are case insensitive, except where stated. Taxonomy definition keywords in the mascot.dat file are also case insensitive.

Several taxonomy definition blocks are obsolete, and retained only for backwards compatibility. Only the current definitions are described below.

NCBInr

This non-identical protein database from the NCBI may contain multiple title lines for each sequence. The titles are separated by a control 'A' (character code 01). The definition block for NCBI is number 8, and this contains the following:

```
# TAXONOMY FOR NCBInr using GI2TAXID
Taxonomy_8
Enabled             1    # 0 to disable it
FromRefFile         0
ErrorLevel          0
DescriptionLineSep  1    # ctrl a - hex code '1'. For multiple descrip-
        tions per entry
SpeciesFiles        GI2TAXID:gi_taxid_prot.dmp, NCBI:names.dmp
NodesFiles          NCBI:nodes.dmp, NCBI:merged.dmp
DefaultRule         GI2TAXID,      CHOP: "gi|\([0-9]*\)"    #The gi
        number
Identifier          NCBI protein FASTA using GI2TAXID
AccFromSpeciesLine  "\(gi|[0-9]*\)"
End
```

To turn off taxonomy for NCBInr, set the enabled flag to 0

FromRefFile is set to 0 indicates that the taxonomy should be found in the .fasta file rather than in a reference file.

ErrorLevel is set to zero, to indicate the type of warnings or errors that are found when creating the taxonomy information. If this is set to 0, then an entry is put into the '*NoTaxonomyMatch.txt*' file for every sequence where **no** taxonomy information is found. If it is set to 1, then an entry is put into the file '*NoTaxonomyMatch.txt*' for every sequence that had **any** gi number in a sequence without a match. Since some sequences will have up to 200 gi numbers (sources), there is a reasonable chance that some of these entries will not have species information, and this would cause the errors files to become very large.

As mentioned earlier, each entry in the description line is separated by a CTRL-A, so *DescriptionLineSep* is set to 1, the ASCII character code for CTRL-A.

There are two SpeciesFiles - gi_taxid_prot.dmp and names.dmp, and two NodesFiles - nodes.dmp and merged.dmp. All four files must be present and up-to-date

The method of finding the species is particularly simple for NCBI databases. The default rule is applied to the FASTA title line to extract the 'gi' number from the accession string. The species file gi_taxid_prot.dmp is a look-up table that returns an NCBI taxonomy ID number.

For example, the FASTA title line

```
>gi|2147497|pir||S73153 hypothetical protein 10 - red ↳
      ↳ alga (Porphyra purpurea) chloroplast
```

returns a gi number 2147497. Looking this number up in gi_taxid_prot.dmp returns a taxonomy ID of 2787. Looking this number up in names.dmp returns the string

```
2787 |     Porphyra purpurea  |          | ↳
      ↳ scientific name     |
```

SwissProt

The rules for SwissProt are fairly simple. Block 3 should always used for SwissProt and Trembl, even if you have a local full text (dat) file.

```
# TAXONOMY FOR SwissProt or Trembl from the fasta file
Taxonomy_3
Identifier          SwissProt FASTA
Enabled             1          # 0 to disable it
FromRefFile         0
DescriptionLineSep  0          # ctrl a - hex code '1'. For multiple
      descriptions per entry
SpeciesFiles            NCBI:names.dmp, SWISSPROT:speclist.txt
NodesFiles              NCBI:nodes.dmp, NCBI:merged.dmp
DefaultRule             SWISSPROT, CHOP: ">[^_]*_\([^ ]*\) "    # Anything
      after _ before space
SWISSPROTRegex          "^\([A-Z0-9]*\) *[ABEV] *\([0-9]*\):"
End
```

There is just a single species per sequence, so the DescriptionLineSep is set to 0.

The SpeciesFiles are from NCBI and SwissProt, and the NodesFiles are taken from NCBI as before.

There is only one database source, so the DefaultRule can be used. This takes everything after the first underscore to the next space. For example:

```
>104K_THEPA (P15711) 104 KD MICRONEME-RHOPTRY ANTIGEN.
```

Would find the text THEPA, which it would look up in speclist.txt.

NCBI EST

Definition 9 for EST_others is very similar to that for NCBInr:

```
# TAXONOMY FOR dbEST using GI2TAXID
Taxonomy_9
Enabled              1   # 0 to disable it
FromRefFile          0
ErrorLevel           0
DescriptionLineSep   1   # ctrl a - hex code '1'. For multiple descrip-
       tions per entry
SpeciesFiles         GI2TAXID:gi_taxid_nucl.dmp, NCBI:names.dmp
NodesFiles           NCBI:nodes.dmp, NCBI:merged.dmp
DefaultRule          GI2TAXID,       CHOP: "gi|\([0-9]*\)"   #The gi
       number
Identifier           NCBI nucleotide FASTA using GI2TAXID
GencodeFiles         NCBI:gencode.dmp
AccFromSpeciesLine   "\(gi|[0-9]*\)"
MitochondrialTranslation 0
End
```

A different species file, gi_taxid_nucl.dmp, is used for nucleic acid se-
quences.

The file containing genetic code data is specified as an argument to
GencodeFiles, while *MitochondrialTranslation* is set to 0, specify-
ing that all entries should be translated using the genetic code for nu-
clear proteins.

EMBL EST

```
# TAXONOMY FOR EMBL EST
Taxonomy_13
Identifier           EMBL EST Fasta
Enabled              1   # 0 to disable it
FromRefFile          0
ErrorLevel           0
SpeciesFiles         ACC2TAXID:acc_to_taxid.mapping.txt, NCBI:names.dmp
NodesFiles           NCBI:nodes.dmp, NCBI:merged.dmp
DefaultRule          ACC2TAXID,      CHOP: ">EM_EST:\([A-Z0-9]*\)"
GencodeFiles         NCBI:gencode.dmp
MitochondrialTranslation 0
end
```

The ACC2TAXID identifier is used to identify a file that contains a
simple mapping of accession to taxonomy ID. It has two values per line:

Accession taxonomyID

For example:
A00001 10641
A00002 9913

where A00001 and A00002 are accessions and 10641 is the NCBI taxonomy id for "Cauliflower mosaic virus" and 9913 is the NCBI taxonomy id for "Bos taurus"

The accession and ID can be separated using any white space. The acc_to_taxid.mapping.txt file from the EMBL contains entries for all the EMBL EST databases so is very large (approximately 3Gb). The file is created at the start of each EMBL release (every 3 months), and so does not include the latest entries in the 'cumulative' Fasta files.

Performance when creating the compressed files is faster if the order of entries in the taxonomy file is the same as the order of sequences in the fasta file. When the ACC2TAXID file is first used or is updated, lookup files (.cdb) are created in the taxonomy directory. These files are only used when compressing the database and are not required when running a search.

Species specific nucleic acid databases

Even a species specific database, such as EST_human, requires taxonomy to be defined at the database level, so that the correct genetic code can be chosen.

For EST_human, the default taxonomy block in mascot.dat is:

```
# TAXONOMY FOR EST_human with TaxID
Taxonomy_10
Enabled              1   # 0 to disable it
SpeciesFiles         NCBI:names.dmp
NodesFiles           NCBI:nodes.dmp, NCBI:merged.dmp
Identifier           All human with TaxID 9606
GencodeFiles         NCBI:gencode.dmp
MitochondrialTranslation 0
TaxID        9606
End
```

MitochondrialTranslation is set to 0, (off), and *TaxID* is set to 9606, specifying that all database entries are homo sapiens. So, genetic code 1, (standard), will be selected for all entries.

HUPO PSI PEFF Format

The HUPO Proteomics Standards Initiative PEFF Fasta format is described here http:// www.psidev.info/index.php?q=node/363

```
# TAXONOMY FOR PEFF
Taxonomy_14
Identifier          HUPO PSI PEFF Format
Enabled             1   # 0 to disable it
FromRefFile         0
ErrorLevel          0
SpeciesFiles        NCBI:names.dmp
NodesFiles          NCBI:nodes.dmp, NCBI:merged.dmp
DefaultRule         EXPLICIT, CHOP: "\\NcbiTaxId=\([0-9]*\)"
end
```

The NCBI taxonomy ID can be parsed directly from the title line.

10

Mascot Daemon

Overview

Mascot Daemon is a client application that automates the submission of searches to a Mascot Server. Functionality includes:

1. Batch mode, in which an arbitrary group of files can be defined for searching, either immediately or at some pre-set time.

2. Real-time monitor mode, in which new files on a pre-defined path are searched as they are created.

3. Data dependent follow-up tasks. For example, automatically repeating an unsuccessful search at a later date or against a different sequence database.

Tasks

The functional unit of Mascot Daemon is a task. A task can be created or modified in the Task Editor. A task is defined by:

1. The data source (e.g. a file list or a file path)

2. How the data are to be searched (an associated set of search parameters)

3. When the searches are to take place

4. Any follow-up activities, such as conditional repeat searches.

Tasks can be in one of four states: running, paused, completed, or cancelled. A paused task can be resumed. A paused or completed task can be cancelled or deleted.

Data Files

Data files can be any of the peak list formats supported by Mascot. Other types of file, such as binary data, can be specified if an appropriate data import filter is available:

1. A wide range of native file formats can be processed using the Mascot Distiller library, (requires an additional licence).

2. If AB SCIEX Analyst is installed on the same system as Mascot Daemon, Analyst WIFF data files can be processed using the mascot.dll "script".

3. If AB SCIEX Data Explorer is installed on the same system as Mascot Daemon, Voyager DAT files can be processed.

4. If a copy of ExtractMsn.exe or similar is installed on the same system as Mascot Daemon, Thermo Xcalibur RAW files can be imported.

5. A utility called TS2Mascot can be used to import peak lists from an AB SCIEX 4000 / 5000 series database

Flexibility

Several Mascot Daemon clients can submit searches to a single Mascot Server, and can even share a common task database. If you have several mass spectrometers, you can choose whether to install separate copies of Daemon on each instrument data system or whether to have a single copy of Daemon somewhere on the LAN, marshalling searches for all instruments.

User Help

Mascot Daemon includes comprehensive, context sensitive on-line help. Press F1 at any time to jump to the relevant topic.

Installation

After Mascot Server has been installed, go to your local home page for links to a help page that describes how to install, upgrade or troubleshoot Mascot Daemon. All the required installation files are hyperlinked from this page.

11

Cluster Mode

Introduction

Mascot has been designed and implemented to work efficiently on a cluster of computers. A cluster of single or dual processor boxes provides a highly cost effective solution for high throughput protein identification. Mascot can be run in cluster mode on all supported hardware platforms and operating systems.

Mascot Cluster Topology
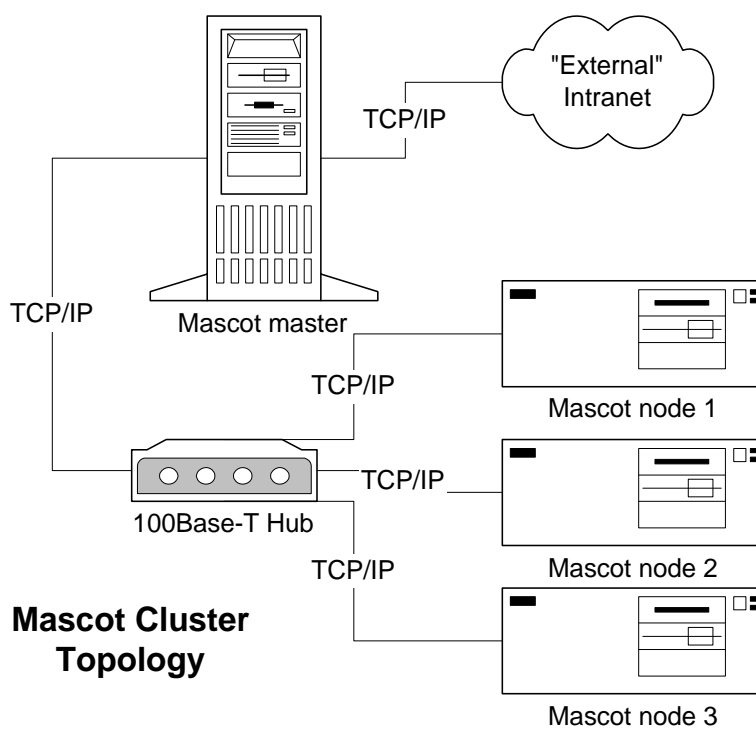
Hardware Requirements

All machines in a cluster should have processors of the same speed. Otherwise, the box with the slower processor(s) will become a bottleneck.

Two network ports are required on the master server; one for external access and one for communication on the private local area network (LAN) that connects the master to the nodes. The LAN for the cluster should run at least at 100Mb/s.

The total amount of RAM required in a cluster is a function of how many sequence databases need to be held in memory simultaneously. Mascot supports an unlimited number of databases, but only those that are searched frequently justify being locked in memory. The others can be allowed to swap in and out of memory as needed. For example, a 5 node, 8 processor cluster (non-searching head node) might have 12 GB on the master, and 6 GB on each of the search nodes. Assuming memory requirements for the OS are negligible, this gives nearly 24 GB in total for searches and databases. Even though 5 or 10 searches might be running, this should be sufficient to allow the more popular databases to remain resident in memory.

Each search node requires sufficient free disk space for the Mascot application software and the compressed FASTA databases. The master also requires sufficient disk space for the original FASTA databases and the accumulating search result files. The amount of space required for the results files depends on how heavily the system is used and how often the files are backed-up and deleted.

For best performance, it is advisable for the nodes to have local hard disks. If you prefer to use shared storage, then each node must have its own dedicated directory structure.

Mascot nodes may have any number of processors, but the number of cores in each node should be a multiple of 4 to make maximum use of the number of CPUs in the licence.

A search node does not require a keyboard, monitor or mouse. If you are running Windows on the nodes, and want to be able to "see" the individual desktops, you might consider using a KVM switch so that a single keyboard, monitor and mouse can be shared between all the nodes. Alternatively, Windows Remote Desktop or VNC can be used.

http://www.realvnc.com/

Operating System Requirements

For nodes running Windows, it is not necessary to use a 'Server' version of Windows on the search nodes.

For Linux clusters, it must be possible for the master to communicate with the search nodes using ssh or rsh without quoting a password or passphrase.

Search nodes do not require Perl or web server software.

You cannot mix x86 and x64 nodes in a Mascot cluster. All must be 32-bit or all must be 64-bit.

The master detects that search nodes are responding by issuing an echo command to TCP on port 7 under Linux and ICMP echo under Windows. Hence, these services must not be disabled or blocked by firewalls

Overview of Implementation

Each search is distributed to all the cluster nodes, but each node searches just an allocated portion of the sequence database. Search results are returned to the master, which merges them, writes the result file to disk, and optionally generates HTML reports to be returned to a client web browser.

All master - node communication is via TCP/IP.

Configuration and program files are distributed and updated automatically from the Master node.

Mascot administration tools provide web browser based system status reports. These are continuously updated and show at a glance important parameters such as processor usage and free disk space for each of the nodes. As an option, critical alerts can also be sent to the system administrator by email.

In cluster mode, Mascot is intended to run as a dedicated system. Trying to run other applications on the cluster simultaneously may have unpredictable effects on search speed.

Installation of Mascot

It is only necessary to install or upgrade Mascot on the master system. In fact, no files are copied to the Mascot nodes during installation. The distribution of files and executables is all handled when Mascot Monitor starts.

Windows

During Mascot installation on a Windows system, the following dialog will be displayed:
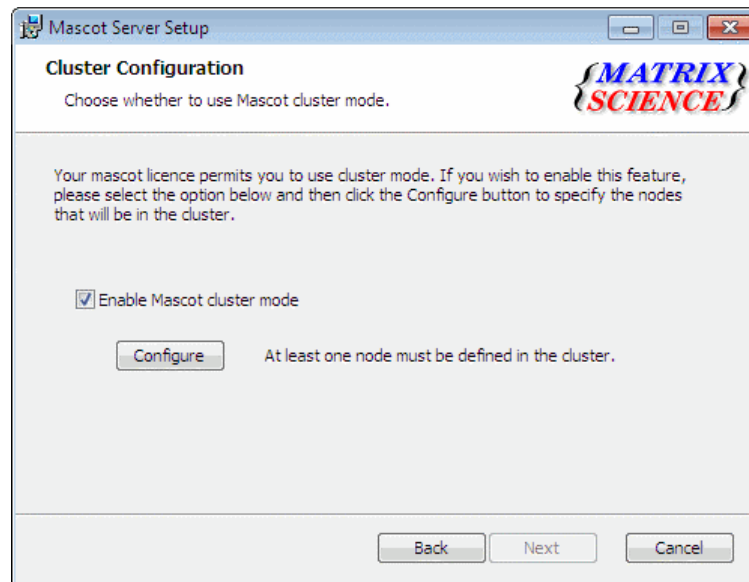
If you enable cluster mode, the configure button invokes the following dialog
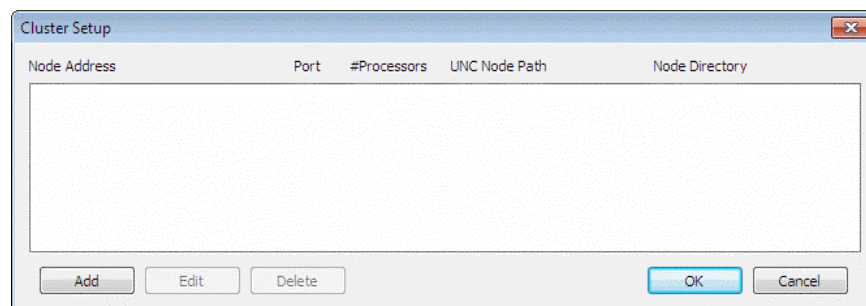
Choose Add to configure each cluster node

Use the Browse button to ensure that the UNC path to the node is correct. If the machines are in a Windows domain, and the remote drive is not explicitly shared, you can enter C$ for drive C, etc., to use the administrative shares. If the base directory does not exist, create it using the 'Make New Folder' button. The recommended base folder name is MascotNode.

Ensure that the local path to the MascotNode directory matches the UNC path. This must be a local or mapped drive on the node so that the path can be specified using a drive letter. The dialog will try to guess the local path from the UNC path, but it may get it wrong. Ensure that this path is correct before pressing OK.

It is not necessary to fill in the Host name and IP Address fields unless the node is a multi-homed system and it is necessary to define which network interface will be used for communication with the Mascot master.

The default port number for cluster communication is 5001. If there are conflicts, this can be changed

The number of processors must be specified. The total number of processors specified for all nodes can be greater than the number of processors in the Mascot licence. The surplus processors will then behave as 'hot-

spares', to be swapped into the cluster as required if there is a hardware problem on another node.

NOTE: If the master is also a search node, and will execute Mascot searches in addition to running Mascot Monitor and the web server, it must be added as a search node using this dialog.

Use the Add, Edit, and Delete buttons to specify the complete cluster.
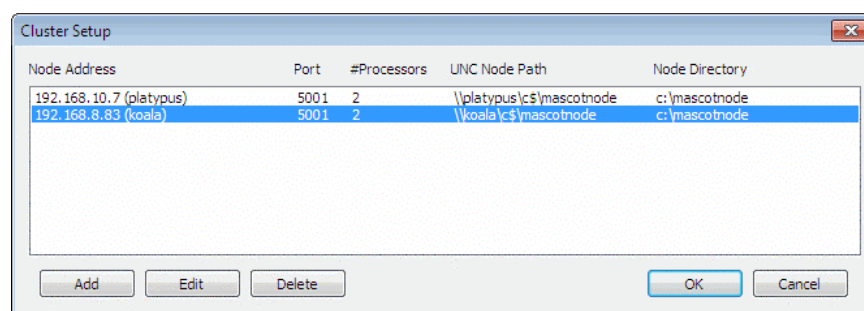
Press OK to return to the installation wizard, and file installation will begin. Copying the files and configuring the system may take some time.

Once complete, you will be presented with a message requesting that you configure and start the Mascot Monitor service. This has to be done manually. The Monitor service on the master needs to be run under an account that has local Administrator rights on each node because it needs to write to the registry, install, start and stop services on each node. (If you later change the password for this account, remember to change it in the Logon tab of the Matrix Science Mascot Service properties, also).

Very large clusters

Defining a very large cluster using the Add node... dialog can be tedious. It is usually faster to define a small cluster, let the installation program run to completion, then edit the configuration files using a text editor.

From the Program menu, stop the Mascot service, and edit the cluster and sub-cluster configuration details into `mascot.dat` and `nodelist.txt`. A full description of these files can be found below in the 'Reference' section. Then, start the Mascot service.

Windows Firewall on Search Nodes

Windows XP and later includes a software firewall called Windows Firewall. You can avoid the configuration steps in this section by turning off Windows Firewall on the search nodes. If the search nodes are on a separate subnet, that can only connect to the master node, having a firewall enabled on a search node is of little use. It is redundant until the

master node has been compromised, by which time it is too late. If the search nodes are not on a separate subnet, or if you simply want to enable Windows Firewall because the operating system keeps nagging you to do so, it is necessary to run through the following steps on each search node.

Windows firewall configuration varies across the different editions of Windows and also according to whether it was part of the original installation or added in a service pack.
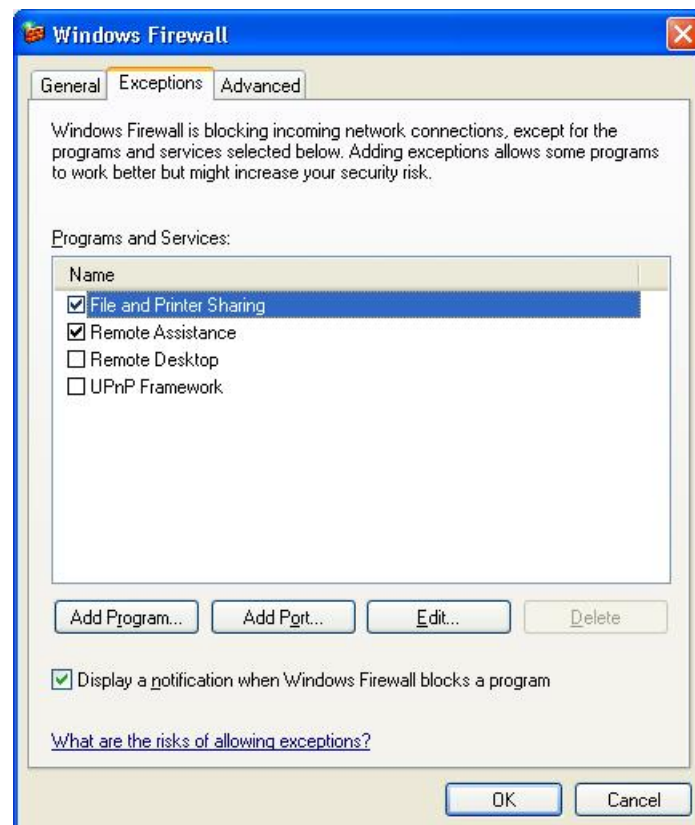
Windows XP and Server 2003:

On each search node, log in as a user with local administrator rights. Go to Control Panel and launch Windows Firewall. On the Advanced tab, make sure the network connection to the master is checked.
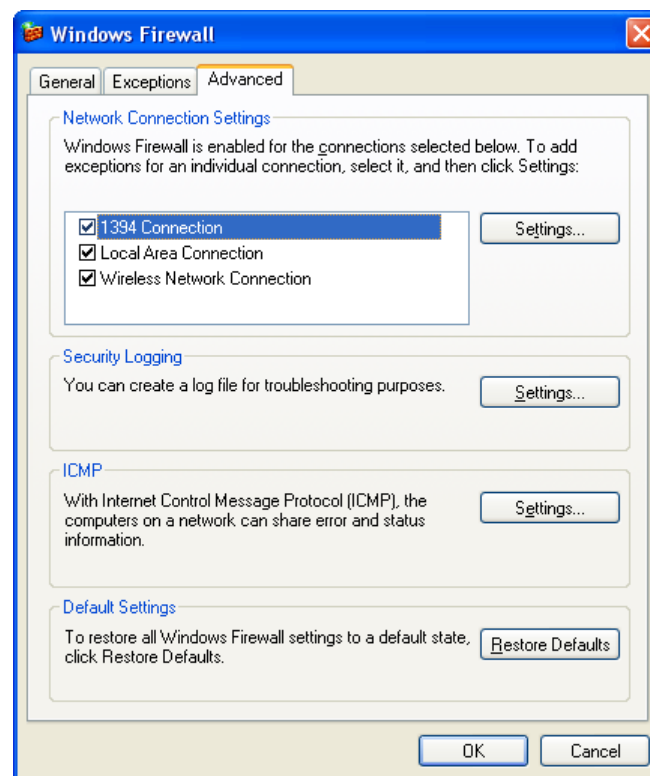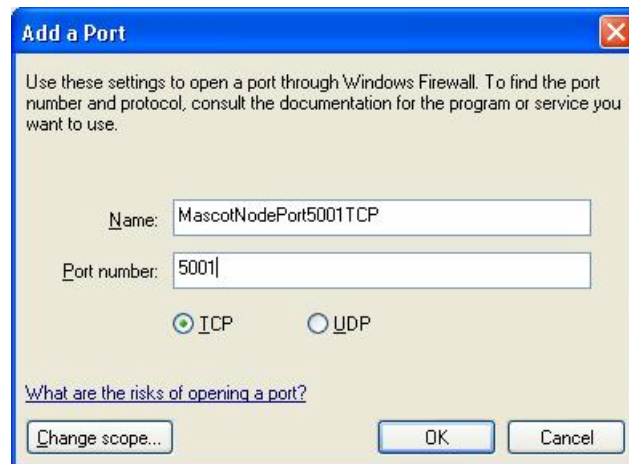

Choose ICMP Settings, check *Allow incoming echo request*, and choose OK.
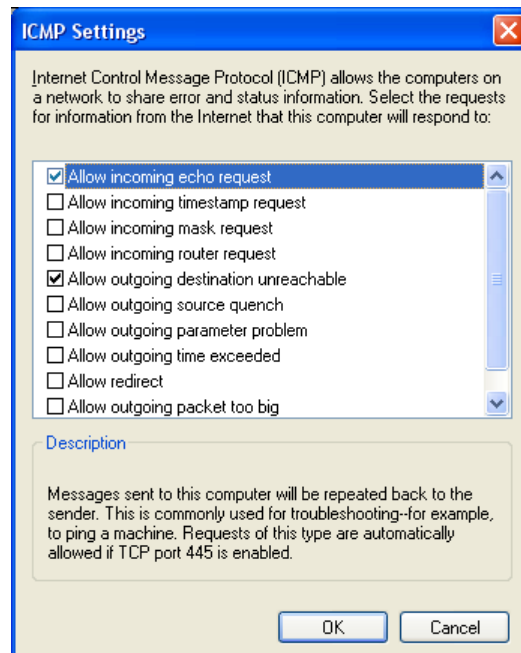
Now, go to the Exceptions tab and ensure that *File and Printer Sharing* is checked. If you plan to use Remote Desktop from the master, you might want to check this at the same time.
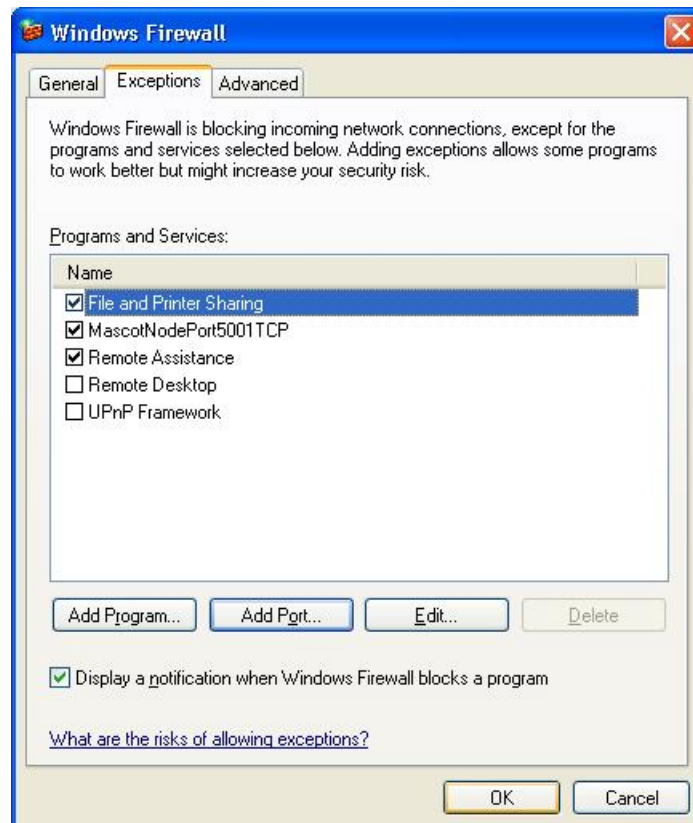
Choose *Add Port* and enter the following data. **Don't** press OK yet.

Choose *Change scope* and select the second option: *My network (subnet) only*.
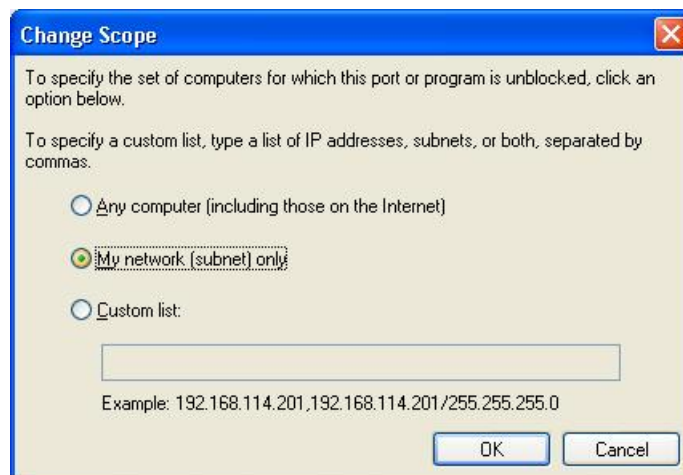
Now press OK buttons in this and in the previous window in order to return to the Windows Firewall dialog, which should now look like this
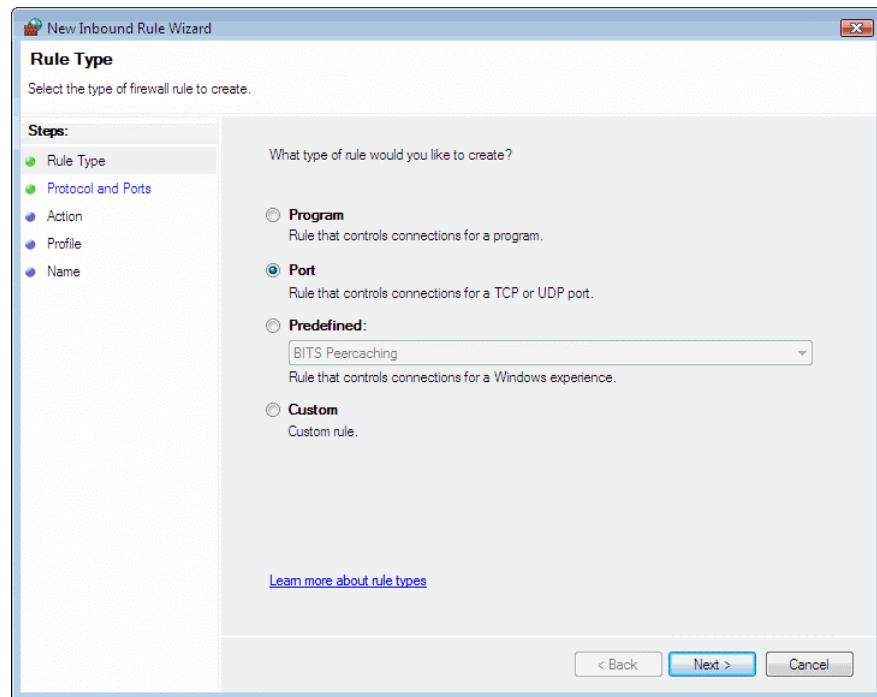
Press OK. Repeat this entire procedure on every search node.

Vista and Server 2008:

On each search node, log in as a user with local administrator rights. Go to Control Panel, Network Status and ensure the network connection to the master node is described as Private. If it shows as Public, choose customise to change it. Under *Sharing and Discovery*, Enable *File Sharing*.
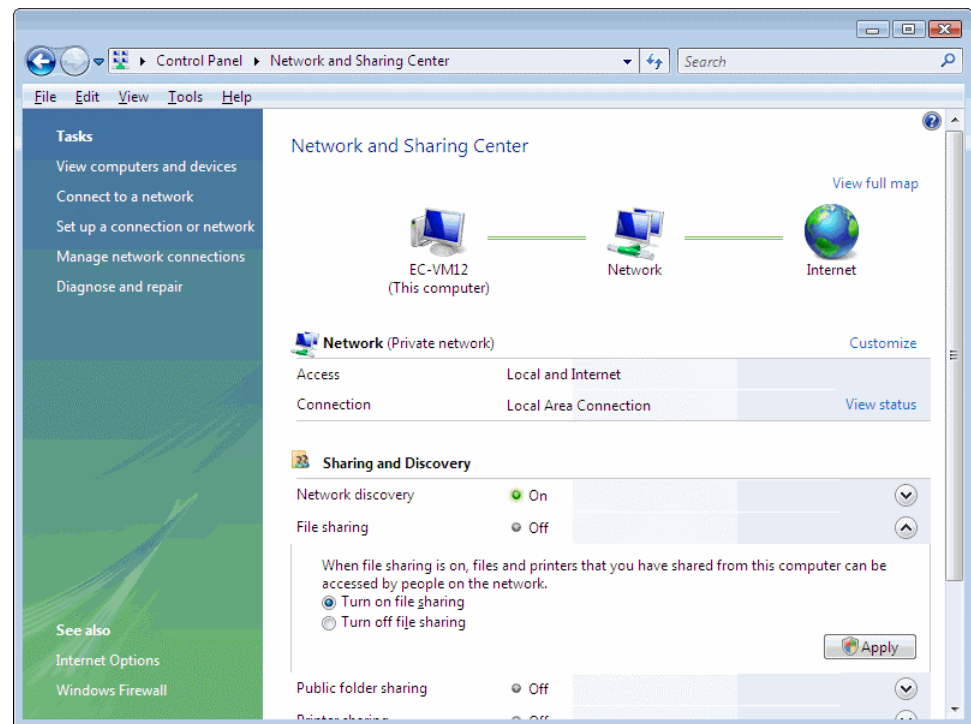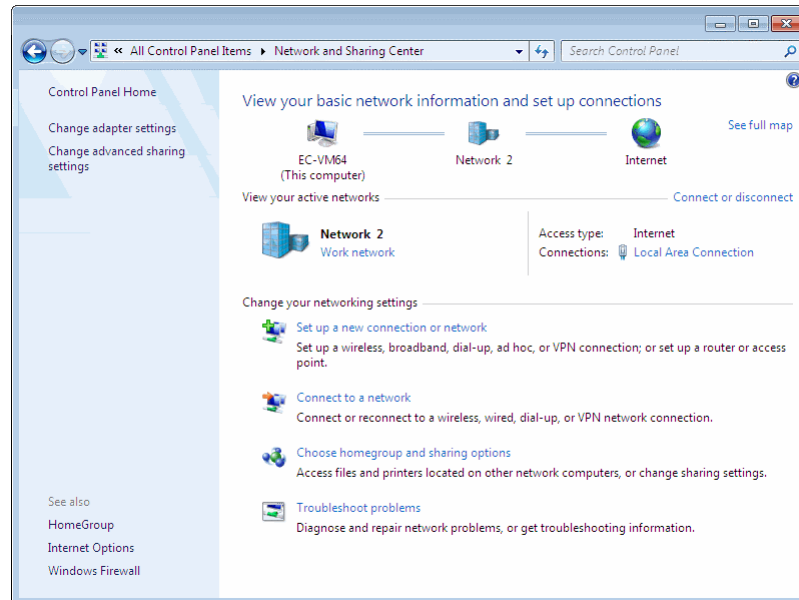
Select *Administrative Tools* and launch *Windows Firewall with Advanced Security*. Select *Inbound Rules* in the left hand panel and *New Rule* in the action panel.

In the wizard, choose Port, Next, TCP, Specific Local Ports, 5001, Next, Allow Connection, Next, Clear the checkbox for Domain and Public, Next, Enter the name as MascotNodePort5001, Finish. The new rule will be added to the list of Inbound Rules.

Windows 7:

On each search node, log in as a user with local administrator rights. Go to Control Panel, Network & Sharing Center and ensure the network connection to the master node is described as Work. If it shows as Public, click on the hyperlink to change it. Choose *Change Advanced sharing settings* and ensure *File and Printer Sharing* is enabled.

Choose Windows Firewall then Advanced Settings. Select Inbound Rules in the left hand panel and New Rule in the action panel.

In the wizard, choose Port, Next, TCP, Specific Local Ports, 5001, Next, Allow Connection, Next, Clear the checkbox for Domain and Public, Next, Enter the name as MascotNodePort5001, Finish. The new rule will be added to the list of Inbound Rules.

Nodes belonging to a Workgroup

The steps in this section are **<u>not</u>** required if all the nodes belong to a Windows domain.

For XP and Server 2003, from the Control Panel, select Administrative tools. Choose Local Security Policy item and double-click on it. Go down the following path: Security settings > Local Policies > Security Options. On the right-side panel select *Network access: Sharing and security model for local accounts*
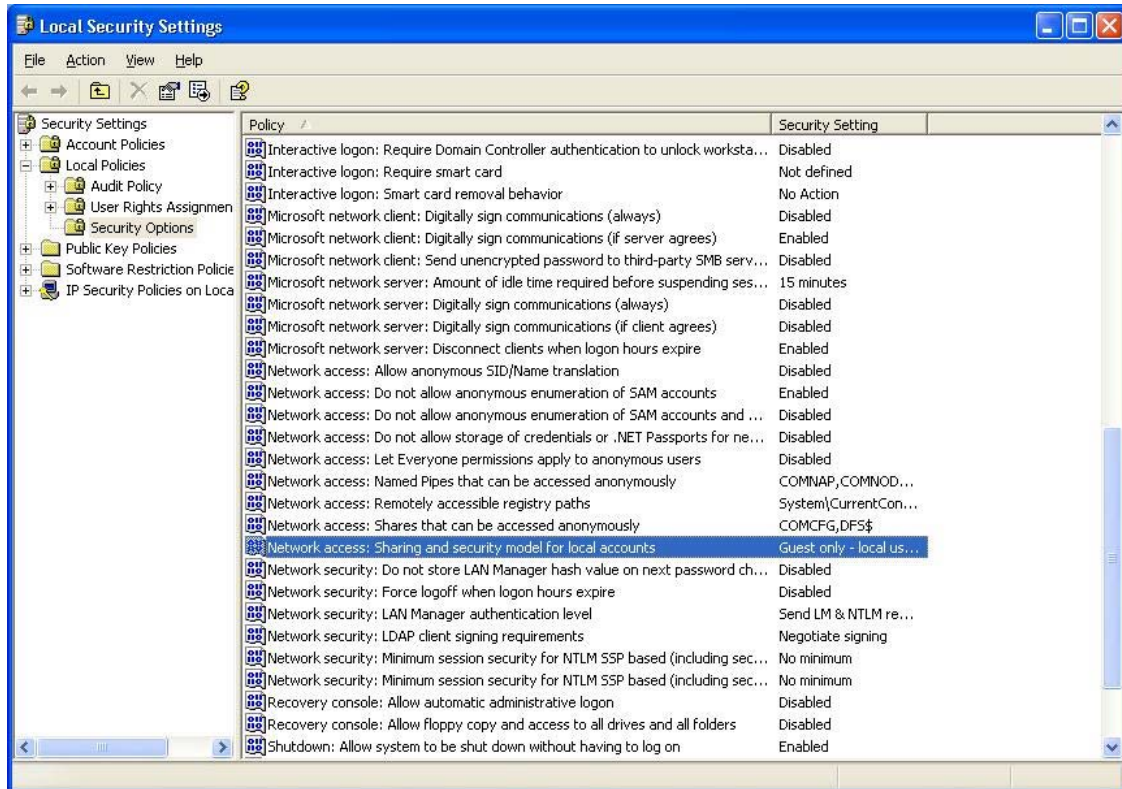
If the current setting is *Guest only*, double-click on the item to change the setting.

Select *Classic – local users authenticate as themselves* and press OK. Close the Local Security Settings window.

For Vista, Server 2008, and Windows 7, a registry change is required to allow administrator rights when logging in using a local (SAM) account. This procedure is taken from Microsoft KB article 951016

> 1. Click Start, click Run, type regedit, and then press ENTER. If the start menu does not have a Run… option, then open a Command Prompt window from the Accessories program folder and use this instead.
>
> 2. Locate and then click the following registry subkey: HKEY_LOCAL_MACHINE\SOFTWARE\Microsoft\Windows\CurrentVersion\Policies\System
>
> 3. If the LocalAccountTokenFilterPolicy registry entry does not exist, follow these steps:
>
>> A. On the Edit menu, point to New, and then click DWORD Value.
>>
>> B. Type LocalAccountTokenFilterPolicy, and then press EN-TER.
>
> 4. Right-click LocalAccountTokenFilterPolicy, and then click Modify.
>
> 5. In the Value data box, type 1, and then click OK.
>
> 6. Exit Registry Editor.

Repeat this entire procedure on every search node.

Vista, Server 2008, and Windows 7

On each search node, from the Control panel, Administrative Tools, open the Services dialog and select *Remote Registry*. Unless already set to Automatic, right click and choose Properties. On the General tab, set *Startup type* to Automatic and also start the service. Choose OK.

Starting the Mascot service for the first time

On the master node, from the Control panel, Administrative Tools, open the Services dialog and select Matrix Science Mascot Service. Right click and choose Properties. Go to the *Log On* tab and choose *This account*. Enter the user name and password for a domain account with local Administrator rights on each search node. (*not* the local administrator account on the master). You could use a domain administrator account, but this might be considered risky.

If the nodes do not belong to a domain, all nodes (including the master) must have a user defined with administrator rights and the *same user name and password*. The service must be set to log in as this user.
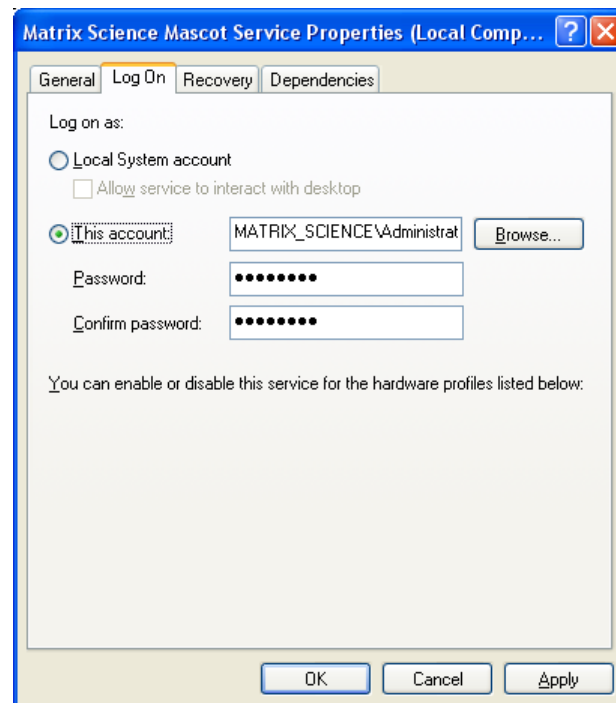
Press OK, and you will be returned to the Services dialog:
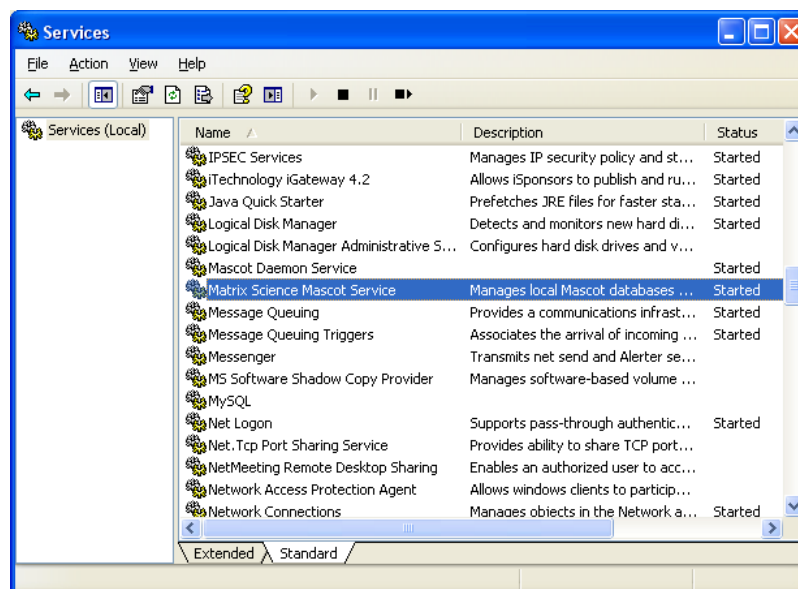
Highlight the entry for Matrix Science Monitor Service and press Start. If the service fails to start, the cause must be investigated and the problem fixed before proceeding.

Monitor progress using the Database Status page on the master. Choose *Monitor log* and watch for error messages as the program files and database files are copied to the search nodes.

Completion

The installation is now complete. There will be a lot of disk activity while the Mascot service compresses the SwissProt sequence database. Searches on the database cannot be performed until the files have been compressed. You should open up the status screen in a web browser (Start menu; Programs; Mascot; Search Status) and verify the cluster status.

If this is a clean installation or a version update, you will need to follow the links to register a product key as described in Chapter 2 (Linux installation) or Chapter 3 (Windows installation). Once the licence file has been saved to `config/licdb` on the master node, you will be able to proceed to Database Status.

If all is well, you will see rows of happy faces and the status line will display the following messages:

```
Creating compressed files
Running 1st test
First test just run OK
Trying to memory map files
Just enabled memory mapping
In Use
```

Once the database is "In use", you can begin exploring and using Mascot. Clicking on the links in the cluster node table will display more detailed status information for individual nodes.

Linux

Communication

Under Linux, the master node communicates with the search nodes using either ssh (preferred), or rsh. If communication can be established using ssh, then scp is used for file copying. If rsh is used for communication, then rcp is used for file copying.

Whether ssh or rsh is used, it is essential that communication can be established without requiring passwords or passphrases. In the case of ssh, key based authentication is the preferred mechanism. A less secure alternative for rsh is provided by file based authentication using .rhosts or hosts.equiv.

A detailed description of the many ways to configure ssh or rsh is outside the scope of this manual. For key based authentication, read the man pages for: ssh, sshd, ssh-keygen, ssh-add, ssh-agent. For file based authentication, read the man pages for: rsh, rshd, rlogin, hosts.equiv.

The minimum procedure to set up key based authentication for ssh on a clean Linux system, where there are no pre-existing keys, is as follows:

1.   Login to the master node as the user who will own the ms-monitor.exe process, (generally root), and generate a version 2 RSA key pair by executing:

ssh-keygen –t rsa

2.   When asked for a passphrase, press return to indicate no passphrase is required. Accept the default location for saving the key files, ($HOME/.ssh)

3.   The contents of the public key, $HOME/.ssh/id_rsa.pub, must be added to a file called $HOME/.ssh/authorized_keys on each of the search nodes.

4.   Test communication by logging in to each search node from the master node using ssh. The first time a connection is made, confirm that the new host should be added to the list of known hosts, $HOME/.ssh/known_hosts

Installation

Perform a standard installation of Mascot onto the master system according to the procedure in Chapter 2. Verify correct system operation as a single server by performing searches of SwissProt and familiarise yourself with administrative tools such as ms-review.exe and ms-status.exe (Chapter 7). Any problems need to be resolved before reconfiguring for cluster operation.

Cluster Configuration Procedure

1. Kill *ms-monitor.exe*

2. Open *mascot/config/mascot.dat* in a text editor. Move down to the "Cluster" section and enter configuration information for the cluster. The parameters are fully described below in the Reference section. In the databases section, verify that the threads and blocks parameters are set to 1 for all databases. If this is not the case, make the necessary changes, then save *mascot.dat*. For a 5 node, 10 cpu cluster, typical entries might be:

```
Cluster
#
# Enable (1) or disable (0) cluster mode
Enabled 1
#
# MasterComputerName must be the hostname
MasterComputerName zx80
#
# Node defaults
DefaultNodeOS Linux
DefaultNodeHomeDir /usr/local/mascotnode
#
# Following line must be commented out WHEN this is a homogeneous
MascotNodeScript       /usr/local/mascot/bin/load_node.pl
#
# Sub-cluster definition
# Syntax is SubClusterSet X Y where X is the sub-cluster number
# and Y is the maximum number of CPUs to use within the given sub-
#
SubClusterSet 0 10
#
# Time outs, log files
IPCTimeout    5    # seconds with no response before timeout
IPCLogging    0    # no logging = 0, minimal = 1, verbose = 2
IPCLogfile    ../logs/ipc.log    # relative path
CheckNodesAliveFreq    30   # seconds between node health checks
SecsToWaitForNodeAtStartup  20   # seconds to wait for node to
#
end
```

3. Open *mascot/config/not.nodelist.txt* in a text editor. Enter configuration information for the cluster. The parameters are fully described below in the Reference section. Save as *nodelist.txt*. For a 5 node, 10 cpu cluster, typical entries might be:

```
# Cluster node definitions
#
# Each line begins with the word Node, followed by a space and
# then a comma delimited list of configuration parameters:
#      ip address:port
#      computer (host) name
#      maximum number of node CPU's to be used
#      operating system
#      local path to home directory
#      home directory as seen from master (specify for NT master only)
#
Node 10.0.0.1:5001, search01, 2, Linux, /usr/local/mascotnode
Node 10.0.0.2:5001, search02, 2, Linux, /usr/local/mascotnode
Node 10.0.0.3:5001, search03, 2, Linux, /usr/local/mascotnode
Node 10.0.0.4:5001, search04, 2, Linux, /usr/local/mascotnode
Node 10.0.0.5:5001, search05, 2, Linux, /usr/local/mascotnode
```

4. Re-start *ms-monitor.exe*. Note that you must change directory to *mascot/bin* and have super user privileges to execute *ms-monitor.exe*.

Note: (Linux only) Under Redhat Linux 8.0, if *ms-monitor.exe* terminates immediately after launch, without any error messages, the problem may relate to a bug in gethostbyname_r(). In the cluster section of *mascot.dat*, try using the IP address for the master node, rather than the hostname, as the argument to MasterComputerName.

5. In a web browser, navigate to *ms-status.exe* and verify that the system starts up correctly.

Reference

The Cluster section in mascot.dat

```
Cluster
#
# Enable (1) or disable (0) cluster mode
Enabled 1
#
# MasterComputerName must be the hostname
MasterComputerName mascot-master
#
```

```
# Node defaults
DefaultNodeOS Windows_NT
DefaultNodeHomeDir c:/mascotnode
#
# Following line must be commented out UNLESS this is a
DefaultNodeHomeDirFromMaster \\<host_name>\c$\mascotnode
#
# Following line must be commented out WHEN this is a
# MascotNodeScript        ###ROOT###/bin/load_node.pl
#
# Sub-cluster definition
# Syntax is SubClusterSet X Y where X is the sub-cluster number
# and Y is the maximum number of processors in the sub-cluster
SubClusterSet 0 10
#
# Time outs, log files
IPCTimeout              5                    # seconds with no
IPCLogging              0                    # no logging = 0,
IPCLogfile            ../logs/ipc.log        # relative path
CheckNodesAliveFreq     30                   # seconds between node
SecsToWaitForNodeAtStartup  20               # seconds to wait for
#
end
```

Enabled

1 to enable cluster mode, 0 to enable single server mode

MasterComputerName

Enter the host name for the master computer and, optionally, the IP address separated by a comma. The IP address may need to be specified for a multi-homed master where it is necessary to define which network card is on the LAN and which is the gateway to the outside world.

DefaultNodeOS

If no OS is defined for a particular node, then this OS is assumed. Must be one of:

- Windows_NT
- Linux

Note that these names are case sensitive.

DefaultNodeHomeDir

If no specific home directory is specified for a particular node, then this default is used.

On a Linux system, this will typically be */usr/local/mascotnode*. It is best not to use */usr/local/mascot* as this is the directory mostly used for the master.

On a Windows system, this will typically be *C:/MascotNode* or *D:/MascotNode*.

To override this setting for a particular node, enter the directory on the node line

DefaultNodeHomeDirFromMaster

This is the directory on the node as seen from the master. For a Windows cluster, this must be present and specified as a UNC name.

The text <host_name> will be replaced by the host name as specified in the Node line.

For a Linux cluster, this parameter must be commented out.

MascotNodeScript

This script is run for each node with the following parameters:

-i   ip address of node - required

-t   The task to be performed - required, either

'StopNode' – the script will try and stop the Mascot Node daemon or service on the specified node.

or

'StartNode' – the script will unconditionally update *ms-mascotnode.exe*, *mascot.license*, and *mascot.dat* on the specified node, then start the Mascot Node daemon or service.

-f   Full path to the node's home directory - required

-r   Port number of node - required

-o   The operating system running on the node – required

For a Linux cluster, the master and search nodes must be able to communicate using either ssh (preferred), or rsh without requiring passwords or passphrases. In the case of ssh, key based authentication is the preferred mechanism. A less secure alternative for rsh is provided by file based authentication using .rhosts or hosts.equiv.

As shipped, load_node.pl executes *ms-mascotnode.exe* as root on each search node. If this is not acceptable, the script can be edited.

SubClusterSet X Y

Large clusters can be divided into sub-clusters. X is a unique integer value (0 based) used to identify the sub-cluster. Y is the maximum number of processors in the sub-cluster. A single cluster must have a single entry with X set to 0.

IPCTimeout

The timeout in seconds for inter-process communication

IPCLogging

0 for no logging of inter-process communication
1 for minimal logging
2 for verbose logging

IPCLogfile

The relative path to the inter-process communication log file

CheckNodesAliveFreq

The interval in seconds between 'health checks' on the nodes

SecsToWaitForNodeAtStartup

At startup, if a node is not available within this time, the system will continue to startup without that node. If the value is set to 0, then the system will wait indefinitely. Default is 60 (seconds).

This timeout is also used if a node fails while the system is running. The system will wait for this number of seconds before re-initialising ms-monitor.exe. This means that a short-lived interruption in network communication doesn't create a major service interruption.

MascotNodeRebootScript

Path to an optional CGI script to re-boot a cluster node. If this parameter is defined, there will be a link at the bottom of each Mascot Cluster Node status page. Clicking on this link will execute the specified CGI script with the host name of the specified node as an argument.

DefaultPort

Sets the default port number to be used when this parameter is missing from *nodelist.txt*. Recommended default is 5001

UseCompleteDatabase

Not used. If specified, must be set to 1.

nodelist.txt

This file is used to define the nodes that belong to the cluster. For a very large cluster, it is advisable to define a few percent of additional nodes as 'spares'. For example, if 51 nodes with 102 processors were available, and Mascot was configured to use 2 sub-clusters, each of 50 processors, the node with the 2 spare processors could be used to replace a failed node automatically.

```
# Cluster node definitions
#
# Each line begins with the word Node, followed by a space and
# then a comma delimited list of configuration parameters:
#      ip address:port
#      computer (host) name
#      maximum number of node CPU's to be used
#      operating system
#      local path to home directory
#      home directory as seen from master (specify for NT master only)
#
Node 10.0.0.1:5001, search01, 2, Windows_NT, c:/MascotNode, ↳
     ↳ \\search01\c$\MascotNode
Node 10.0.0.2:5001, search02, 2, Windows_NT, c:/MascotNode, ↳
     ↳ \\search02\c$\MascotNode
Node 10.0.0.3:5001, search03, 2, Windows_NT, c:/MascotNode, ↳
     ↳ \\search03\c$\MascotNode
Node 10.0.0.4:5001, search04, 2, Windows_NT, c:/MascotNode, ↳
     ↳ \\search04\c$\MascotNode
Node 10.0.0.5:5001, search05, 2, Windows_NT, c:/MascotNode, ↳
     ↳ \\search05\c$\MascotNode
```

Important: Because Mascot frequently writes status information to *nodelist.txt*, you should open the file in a text editor that puts a lock on the file (e.g. vi or wordpad). This will prevent Mascot from modifying the file while it is being edited. *nodelist.txt* can be viewed using Mascot Status.

Node

There must be one or more node entries. Items in square brackets are optional – but the commas must always be supplied.

IP address:Port, Host name, Number of processors, [OS], [Home dir], [Home dir from master]

IP address, port, and host name must always be specified.

The number of processors to be used on the node can be less than the number of processors available. If the total number of processors speci-

fied in all the nodes exceeds the number of licenses available, only the licensed number will be used at any one time.

If the OS is not specified, then the DefaultNodeOS is used. Must be one of the choices shown under DefaultNodeOS.

The home directory is the local path on the node to the root of the Mascot directory structure. If this is not specified, then DefaultNodeHomeDir is used.

Home directory from master is the home directory on the node as seen from the master. This parameter is only applicable to a Windows cluster and must be omitted for a Linux cluster.

Once a cluster has been started, an additional four status values will be written periodically to *nodelist.txt*. If you edit this file while Mascot is *not* running, these values can be deleted.

> subcluster ID number (0 based)
>
> node within subcluster (0 based)
>
> status: 0 unknown status
>
> > 1 attempting to bring into use
> >
> > 2 no response to ping
> >
> > 3 failed to start service
> >
> > 4 in use
>
> number of CPU's actually being used

File Replication

The configuration files, such as *mascot.dat*, that are on the Mascot master are automatically replicated to the nodes. So, it is only necessary to update a file on the master. The *ms-monitor.exe* program (run as the Matrix Science Mascot Service under Windows), continually looks to see if a file has been updated, and will distribute new versions to the nodes as required. The dates, times and lengths of the distributed files should be identical on all systems.

The same process is used for updates to executable programs, except that these updates will only be made when the *ms-monitor.exe* service first starts.

The Status screen will indicate if any executable files need updating.

Files required on each Mascot Node

Target File name and directory relative to node home directory	Notes
./bin/ms-mascotnode.exe	Updated at start-up
./bin/nph-mascot.exe	
./config/enzymes	
./config/mascot.dat	Updated at start-up
./config/unimod.xml	
./config/mascot.license	Updated at start-up
./config/taxonomy	
./config/fragmentation_rules	
./config/quantitation.xml	
./taxonomy/nodes.dmp	
./taxonomy/usernodes.dmp names.dmp is not required on the Mascot Nodes.	Not required by most users. Note that

Start-up of ms-monitor.exe

The following sequence occurs (for each node) when *ms-monitor.exe* starts for the first time on the master system. (items marked * are for Windows clusters only).

- See if the computer is available by opening a socket to the ping port (port 7)

- If there is an entry 'StopMascotNodeCmd' in the *mascot.dat* file, then run that command to stop the Mascot node daemon - or
  * See if there is a MascotNodeService installed on the computer - if there is, then stop that service

- If there is no *ms-mascotnode.exe* or if it is out of date on the Mascot node, then copy/update the file from the cluster/<OS> directory on the Mascot Master system to the specified directory on the Mascot Node.

- * If the service is not installed, then install the service, and add a registry entry for the directory to be changed to at start up

- Make a *logs* and *config* directory and copy *mascot.dat* and *mascot.license*

- * Start the MascotNodeService on the Mascot Node computer. (With a Linux based system, the *ms-mascotnode.exe* daemon will be started).

- Check that the service / daemon now communicates through TCP/IP sockets – if it fails, then a message indicating which Mascot node it is waiting for is displayed in the ms-status screen.

- Initialise the MascotNodeService / daemon by sending the appropriate commands

- See if any files are missing or out of date (see above), and if necessary, update them. This is done though the TCP/IP socket, so no directory mapping / NFS mounts are required.

Once all the Mascot nodes have been successfully initialised, then Mascot Monitor starts as normal.

Licensing

The number of processors that the search is permitted to run on is restricted by the number of mascot licenses. The Mascot master node is not included in this list, since it merely distributes the search and collates the results. The number of processors to be used for Mascot will never exceed the number specified in the licence.

Error messages and emails

In the single server version of Mascot, selected warning messages can optionally be emailed to the system administrator when something critical, such as a database update, fails on the server. The following additional messages, specific to a cluster, can also be emailed:

M00323 One or more cluster nodes has stopped responding

M00316 Dr. Watson log updated (indicating a software crash) on one of the cluster nodes.

Who Am I?

If the Mascot master is also being used as a node, when nph-mascot.exe is run, it needs to know whether it is running as a node task or as master task. Since the different *mascot.dat* files are identical, it determines this from a file *mascot/config/iam.dat* that is created by the Mascot node service when it starts up. Do not copy or replace this file.

Windows Manual Configuration

The following configuration steps on each search node are performed automatically as part of the Windows installation
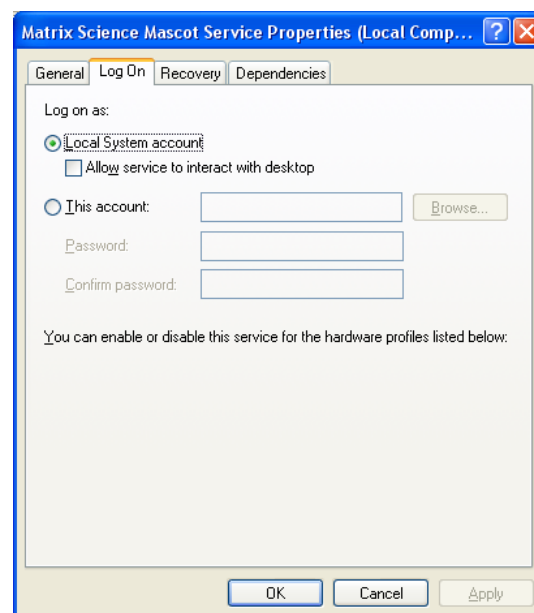
MascotNodeService

Under Windows, *ms-mascotnode.exe* is configured to run as a service. This should be taken care of automatically. If there are any problems, service creation or deletion requires the Microsoft utility *sc.exe*, which can be found in the *mascot/cluster/Windows_NT* directory.

The command to create the service is:

```
sc create MascotNodeService ⤳
  type= own ⤳
  binpath= c:\mascotnode\bin\ms-mascotnode.exe ⤳
  start= auto
```

You may need to change the path to the executable, and note that the spaces after the equals signs are significant.

To verify that the service has been created successfully, from the Control panel, open the Services control panel and choose MascotNodeService. Select Startup… and the following dialog should be displayed:

To delete the service, first stop it, close the services control panel, then enter:

```
sc delete MascotNodeService
```

Dr. Watson

To prevent (invisible) dialog boxes from being displayed if a fatal error occurs, edit the registry key

```
HKEY_LOCAL_MACHINE\Software\Microsoft\DrWatson
```

Set the value of `VisualNotification` to 0. When the Mascot node service starts on a Windows system, it sets a Dr. Watson registry entry to ensure that Dr. Watson log files are written to the node *logs* directory.

Registry Settings

Two registry entries are used on each search node to record the root directory of the mascot file structure and the port number used for communication. For example:

```
[HKEY_LOCAL_MACHINE\SOFTWARE\MatrixScience\Mascot\1.00]
"MascotNodeFolder"="C:/mascotnode/bin"
"MascotNodePort"="5001"
```

Very large Mascot clusters

Very large clusters (> 30 nodes) pose certain special problems:

- Even with reliable hardware, node failures can be expected relatively frequently

- LAN communication can become a bottleneck

- Need to avoid mixing processors with different speeds, because the slower processors become a bottleneck

Mascot allows large clusters to be divided into sub-clusters. Each sub-cluster uses identical databases and configuration files, but operates independently of the other sub-clusters. An incoming search can be directed to a specific sub-cluster or the first available sub-cluster.

Should a node go down, only the sub-cluster is affected. Ideally, there will be one or more "spare" nodes defined. Mascot will reconfigure the sub-cluster using a spare node and re-start. If there are no spare nodes, Mascot will reconfigure the sub-cluster to exclude the faulty node and re-start.

Configuration

mascot.dat

SubClusterSet X Y

Large clusters can be divided into sub-clusters. X is a unique integer value (0 based) used to identify the sub-cluster. Y is the maximum number of licensed processors assigned to the sub-cluster. Since a licensed processor is good for up to 4 cores, it may be clearer to think of Y as cores/4. A single cluster must have a single entry with X set to 0.

nodelist.txt

This file is used to define the nodes that belong to the cluster. For a very large cluster, it is advisable to define a few percent of additional nodes as "spares". For example, if 51 nodes with 102 processors were available, and Mascot was configured to use 2 sub-clusters, each of 50 processors, the node with the 2 spare processors could be used to replace a failed node automatically. At start-up, ms-monitor starts each sub-cluster in turn, taking the required number of nodes from nodelist.txt in the order specified in the file. If you wish to override this behaviour, specify a sub-cluster number in nodelist.txt:

```
# Each line begins with the word Node, followed by a
space and
# then a comma delimited list of configuration param-
eters:
#      ip address:port
#      computer (host) name
#      maximum number of node CPU's to be used
#      operating system
#      local path to home directory
#      status: 0 = available
#      sub-cluster
```

Mascot system limits

The following are relevant to very large clusters. Other system limits are listed in Appendix C of manual

- Maximum number of processors per machine 64

- Maximum number of sub-clusters in a cluster 50

- Maximum number of machines in a sub-cluster 1024

- Maximum number of processors in a sub-cluster 65536

- Maximum number of nodes in nodelist.txt 4096

Directing jobs to a sub-cluster

The SUBCLUSTER search parameter is used to direct jobs to a sub-cluster. This can be added to the web-browser search form as a hidden field by editing the Perl script.

To use the next free sub-cluster:

SUBCLUSTER=-1

If all of the sub-clusters have searches running, and the search has been submitted from a browser, then the following will be displayed in the browser until a sub-cluster becomes free:

Waiting for sub cluster to become available......

To use a specific sub-cluster, e.g sub-cluster 2

SUBCLUSTER=2

The default value is 0 so, if this parameter is not specified, a search will go to the first sub-cluster.

Specifying which sub-cluster a particular job goes to usually implies some third party job queuing system is being used. For example

- Job gets submitted to PBS and PBS decides which sub-cluster to run the search on

- PBS adds a SUBCLUSTER=x to the search parameters

- PBS creates a task_id using ms-searchcontrol.exe — create_task_id

- PBS submits the search, passing the returned task_id

- You can monitor / control the running search using ms-searchcontrol.exe

A simpler, "static" system could be implemented by adding a SUBCLUSTER command to a Daemon parameter set. SwissProt-SC1.par might contain SUBCLUSTER=1, so selecting this for a task would direct searches to sub-cluster 1, etc.

Database Status

If multiple sub-clusters are defined, the database status screen (ms-status.exe) only shows one sub cluster at a time. An additional summary table is shown at the bottom of the page, with links for the other sub-clusters.

12

Security

Overview

The security model allows a Mascot administrator to:

- Prevent un-authorised changes of Mascot server configuration files using, for example, the database maintenance utility

- Restrict access to results files and sequence databases based on group and user definitions

- Provide standard 'session' support (with time-outs) so users do not need to continually re-enter passwords

- Restrict access to Mascot server based utilities that allow deletion of searches and other job control functions

- Provide read-only access to configuration files for third party applications without requiring login

- Optionally allow submission of searches etc. for 3rd party applications without a login

- Switch OS platform painlessly if Mascot or Mascot Integra authentication is used

- Easily set-up Mascot Daemon to run searches as the 'customer' in a service or core lab environment

Some third party applications require helper scripts to be installed on the Mascot web server. If Mascot security is enabled, you should be aware that such scripts may create security holes.

Enabling security

When Mascot is first installed, the security system is disabled. To enable security, open a command prompt or shell on the Mascot server and change to the mascot/bin directory. Enter the command:

```
perl enable_security.pl
```

The Mascot service (ms-monitor.exe) must then be stopped and re-started.

Disabling security

To disable security, open a command prompt or shell on the Mascot server and change to the mascot/bin directory. Enter the command:

```
perl disable_security.pl
```

The Mascot service (ms-monitor.exe) must then be stopped and re-started.

Authentication

There are two different ways in which users can be authenticated:

1. Mascot authentication. The passwords are stored and maintained by the Mascot security libraries and/or by Mascot Integra.

2. Web server authentication. Available with any web server that supports authentication. Refer to your web server documentation for details on how to set up authentication

The type of authentication is set up at the user level, and not as a global setting. Even if the server has web authentication switched on, it may be useful to set some users to be authenticated using the Mascot authentication. A typical case for this might be for a service lab manager running Windows and IIS with integrated authentication. This user would not typically want to create a separate Windows login account for the administrator, but would choose to login explicitly as administrator to update configuration files etc. For an Apache server, with authentication switched on, most users would want to be set to use the authenticated login

Users

New users are added using the Mascot security administration utility. There are 6 special "system" user accounts:

guest

The guest user is not enabled by default. If this account is enabled, then any user is automatically logged in as guest, and needs to explicitly login as another user to gain further access rights. The guest account cannot be deleted, but the account can be disabled. The userid is 1.

admin

This account should be used to perform administration on the Mascot server. It is recommended that you always log in as administrator to perform security and other administration rather than assign administrator rights to another user. The administrator account cannot be deleted or disabled and the admin user cannot be removed from the administrators group. By default, the administrator can access all the administrator screens, but cannot submit searches. The userid is 2. The initial password for admin is admin, but this must be changed on first login.

command line

This pseudo user is always used when running programs from the command line, and can perform any task without restriction. This 'user' doesn't appear in the security administration utility and hence the account cannot be deleted or disabled. The userid is 3.

daemon

This user should be used to run searches in Mascot Daemon. See the Mascot Daemon help for details. The user account is disabled by default, so it will need to be enabled and before use. The userid is 4.

public_searches

This is a pseudo user that is used for the example searches. This 'user' doesn't appear in the security administration utility and hence the account cannot be deleted or disabled. It isn't possible to login as this user. The userid is 5.

(system)

The Mascot Integra system account is used to query data on the Mascot server. Do not change the name of this account or the type of the account. There is no password associated with this account since it can only be called from the secure Mascot Integra server. The userid is 6.

Types of user

Six 'types' of user are available, and the appropriate type should be selected using a the drop down list in the administration screen:

Standard Mascot User

The user name and password are stored by Mascot

Mascot Integra User

The password, password expiry and timeouts for these users are set in the Mascot Integra administration screens.

The standard Mascot login screen will be displayed, but authentication is performed using Mascot Integra

The Mascot Integra server details must be specified in the options section of the security administration utility.

IP address

This 'user' should only used for third party legacy applications, that do not support Mascot security. Instead of a user name, enter the static IP address of the computer that will access the Mascot server. Do not enter a password.

Computer name

Same as the IP address, but the computer name is used instead. A computer name is more practical where dynamic IP addresses are being used.

Agent string

Should only be used as a last resort for third party applications that haven't implemented Mascot security and where the computer name / IP address is not reliable. A case sensitive substring comparison will be made with the HTTP_USER_AGENT environment variable.

Use built in web server authentication

See description of 'authentication' above.

Mascot will never prompt these users for username and password, and hence passwords and password expiry will be ignored.

Mascot security session time-outs do not apply.

In Microsoft Internet Information Services, (IIS), if anonymous access and integrated authentication are both enabled, then users will generally be 'logged in' as anonymous until they try to access a file where permission is denied. This almost certainly means that anonymous login must be disabled to use this option.

IIS user names generally include the Domain name: e.g. matrix_science/charles. The comparison will be with everything after the last forward or back slash. So, in this case, you would enter 'charles' as the user name.

Groups

Access rights can are assigned to groups, not users. Therefore, a user has no effective rights unless they belong to one or more groups. If a user belongs to more than one group, then their rights are the combination of the rights in both groups.

There are 5 special built in groups:

Guests

By default, the guest user is the only member of this group and the guest group can only submit PMF searches against any database. This can easily be changed using the security administration utility.

Administrators

The admin user always belongs to this group. Members of the group can perform any administration task, but cannot submit searches.

PowerUsers

Members of this group can submit all types of searches and perform some administration. They cannot access the security administration utility.

Daemons

The daemon user belongs to this group by default.

MascotIntegraSystem

The (system) user is the only member of this group.

Using the security administration utility

When the security administration utility is started for the first time, you will need to login as admin/admin. You are then forced to change the password.

The main page lists the current users and groups and has buttons for deleting adding and editing users and groups. The global security options can also be modified from this page. On all the pages, there is a help window that gives details about specific options – just position the mouse over the relevant hyperlink to see the help.
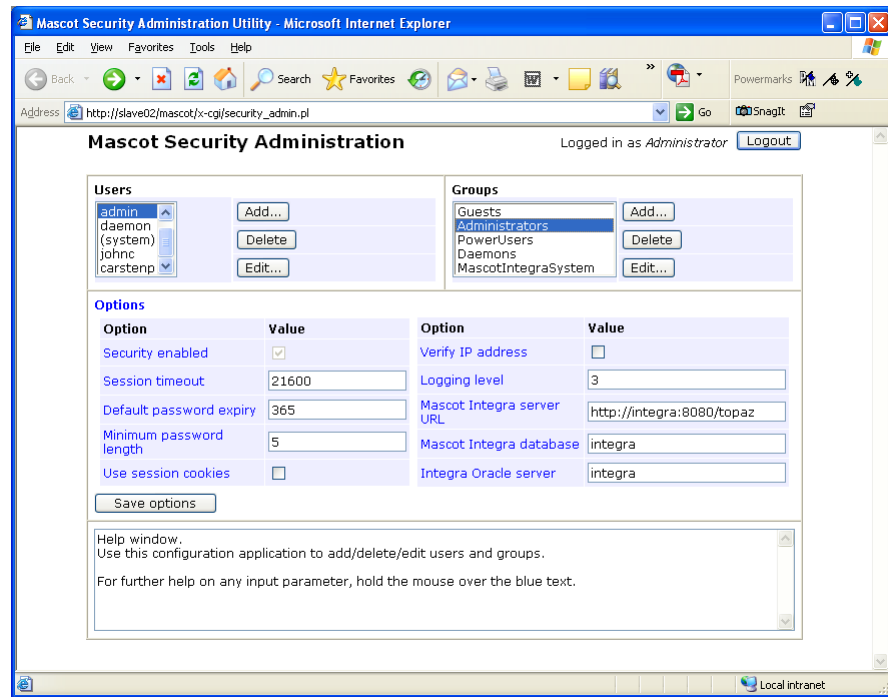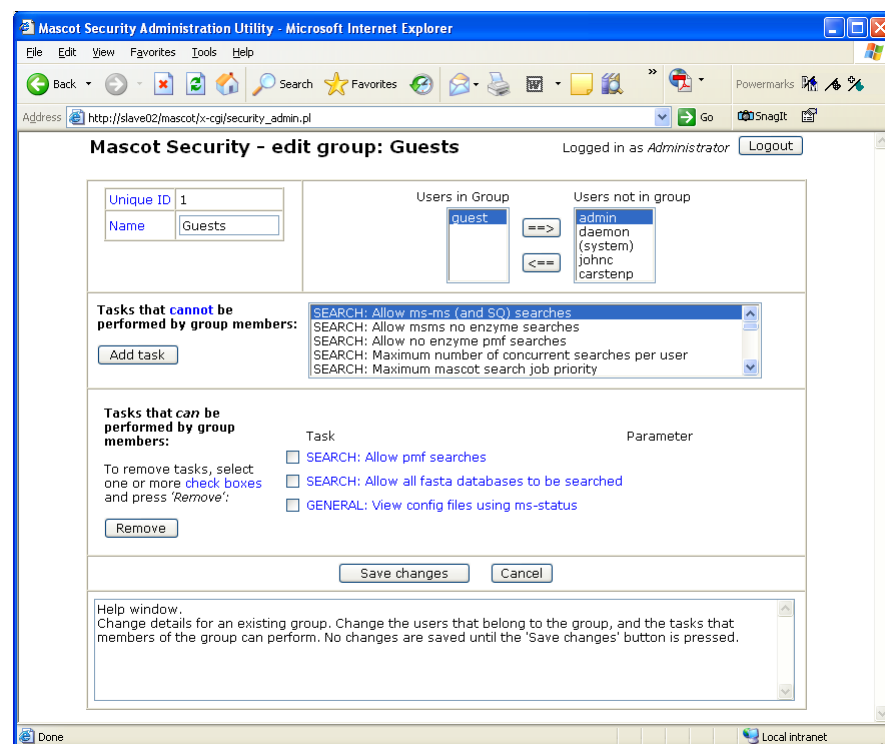
To add a new user, click on the Add... button:
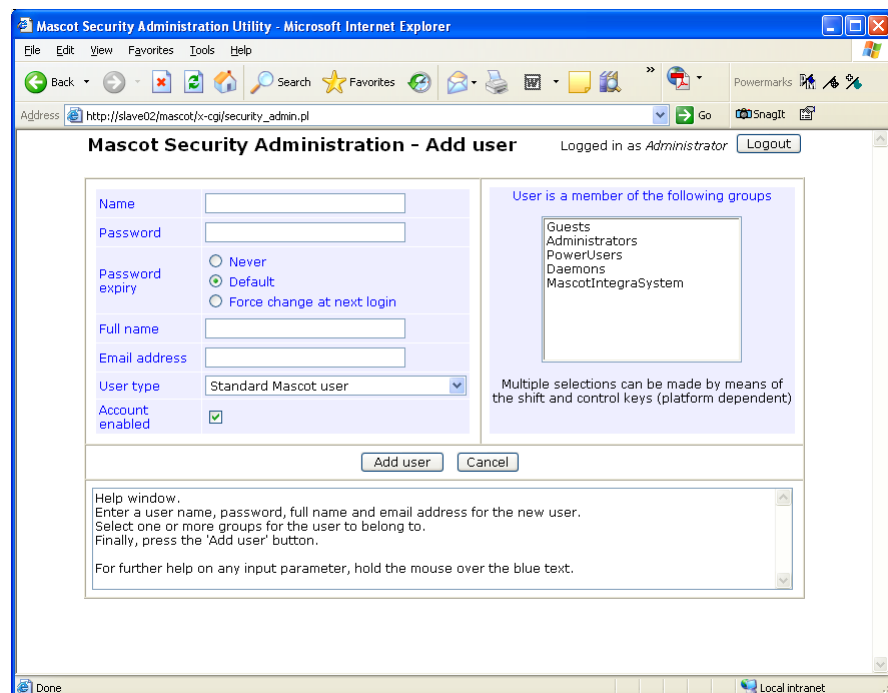
The new user must be given a name, password, full name and email address. There is a description of the different user types of user earlier in this chapter. You should also select one or more groups that the user should belong to before pressing the 'Add user' button.

New groups may be added or edited:

Each group has a unique ID that cannot be changed.

Users can be added to or removed from groups either on this screen or from the edit/add user screens.

Mascot security is fine grained. There is a list of about 20 tasks that members of a group can (or cannot perform). The tasks that are not permitted are in the top list. To allow group members to perform one of these tasks, click on the task in the list, and then 'Add task'. This task will then appear in the lower list. Similarly, to remove a task, click on the check box in the lower list, and click on the 'Remove' button. To get further information about any task, hold the mouse over the task in the lower window and further details will appear in the help box.

No changes to a group are saved until the 'Save changes' button is pressed.

Session files

Session files are created in the mascot/sessions directory. Sessions that have expired will be deleted automatically by ms-monitor.

Log file

The log file 'security.log', in the mascot/logs directory contains information about all security changes. The file is not available from any web based application for security reasons. The level of logging can be controlled from the security administration utility.

Configuration Files

Security information is saved in three configuration files in the mascot/config directory:

```
security_options.xml

security_tasks.xml

group.xml

user.xml
```

The schema for these files is mascot_security_1_0.xsd.

Use the security administration utility or Mascot Parser rather than editing these files manually.

Automating addition of new users

Mascot Parser users have access to all of the documentation for the lower level functions to administer Mascot security programmatically. The security administration utility uses some of these functions.

To simply to add a large number of users, then the add_user.pl script in the mascot/bin directory can be used:

```
Usage: add_user.pl -u username
                   -p password
                   -x password_expiry
                   -f fullname
                   -e email_address
                   -g group to which user should belong
```

The password expiry should be 0 for never expires or 1 to force the user to change the password when they first log in.

Resetting the administrator password

If the admin user password is lost, the easiest way to reset it is to re-run 'enable_security.pl' from the command line as described above. This will not affect any existing groups or users, but will just reset the password.

User ID

The user ID for each search is saved in the results file. If security is disabled, then the search ID will be set to zero. Special user IDs are listed above. Other users will have an automatically assigned IDs starting at 1000.

A

Basic Regular Expressions

Sequence database parsing in Mascot is defined using rules which conform to Basic Regular Expression (BRE) notation as defined in standard ISO/IEC 9945-2: 1993. BRE notation is widely used in Unix, e.g. in the grep command, but it may be less familiar to those from a DOS or Windows background. Man pages containing a rigorous definition of BRE notation can be found on most Unix systems.

The following description is much simplified, and is intended to provide just enough information to understand the existing rules in mascot.dat, and to enable someone without prior knowledge of regular expressions to write simple rules for new databases. Only the most basic aspects of BRE notation are touched on.

In mascot.dat, the PARSE section contains a number of rules. For each rule, the pattern in double quotes is a BRE which is used to identify a string so that it can be parsed from the surrounding text. For example:

```
#Report text from NCBI excluding sequence (used for AA entries)
RULE_10 "\(LOCUS .*\)ORIGIN "
```

The part of the BRE between the backslashed parentheses \(and \) is the string which we are trying to locate and extract. This rule looks for the word LOCUS followed by a space. It will extract all the text, including the word LOCUS, up to but excluding the word ORIGIN followed by a space.

BRE Rules

The rules for performing this match are as follows:

The BRE always looks for the longest, leftmost matching string.

Matching is case sensitive.

Newline characters (LF in Unix or CR+LF in Windows) are treated like any other character

The sub-expression to be extracted from the surrounding text is defined using backslashed parentheses \(\). The parentheses are ignored for matching purposes.

Some characters are "Special":

. [\   The period, left-bracket and backslash are special except when used in a bracket expression.

*   The asterisk is special except when used in a bracket expression, as the first character of an entire BRE (after an initial ^, if any), as the first character of a subexpression (after an initial ^, if any).

^   The circumflex is special when used as an anchor, or as the first character of a bracket expression.

$ The dollar sign is special when used as an anchor.

Matching Single Characters

Any character that is not a special character is an ordinary character. An ordinary character, or a special character preceded by a backslash, matches to itself.

A period, used outside a bracket expression, matches to any single character, including a newline character.

A bracket expression (a list of characters enclosed in square brackets, [ ]) matches any single character from the enclosed list. The following rules and definitions apply to bracket expressions:

A bracket expression is either a matching list expression or a non-matching list expression. The right-bracket] loses its special meaning and represents itself in a bracket expression if it occurs first in the list (after an initial circumflex ^, if any). Otherwise, it terminates the bracket expression. The special characters: . * [\ (period, asterisk, left-bracket and backslash, respectively) lose their special meaning within a bracket expression.

A matching list expression matches any one of the characters in the list. The first character in the list must not be the circumflex. For example, [abc] matches any one of the characters a, b or c.

A non-matching list expression begins with a circumflex ^ and specifies a list that matches any character except for the characters in the list after the leading circumflex. For example, [^abc] matches any one character except the characters a, b or c. The circumflex will have this special meaning only when it occurs first in the list, immediately following the left-bracket.

A range expression represents the inclusive set of characters between two characters in the ASCII character set. The starting and ending characters are separated by a hyphen. For example, `[A-Z]` will match to any single upper case letter, while `[0-9_A-Za-z]` matches any single alphanumeric character.

Matching Multiple Characters

When a BRE matching a single character or a subexpression is followed by the special character asterisk `*`, together with that asterisk it matches what zero or more consecutive occurrences of the character. For example, `[ab]*` and `[ab][ab]` are equivalent when matching the string ab. The expression `ab*c` will match to `ac` or `abc` or `abbbbbbc`.

When a BRE matching a single character or a subexpression is followed by an interval expression of the format `\{m\}`, `\{m,\}` or `\{m,n\}`, together with that interval expression it matches what repeated consecutive occurrences of the BRE would match. The values of m and n will be decimal integers in the range `0 <= m <= n <= 255`, where m specifies the exact or minimum number of occurrences and n specifies the maximum number of occurrences. The expression `\{m\}` matches exactly m occurrences of the preceding BRE, `\{m,\}` matches at least m occurrences and `\{m,n\}` matches any number of occurrences between m and n, inclusive.

For example, in the string `abababcccccccd` the BRE `c\{3\}` is matched by characters seven to nine, the BRE `\(ab\)\{4,\}` is not matched at all and the BRE `c\{1,3\}d` is matched by characters ten to thirteen.

The behaviour of multiple adjacent duplication symbols produces undefined results.

Expression Anchoring

A BRE can be limited to matching strings that begin or end a line; this is called anchoring. The circumflex and dollar sign special characters will be considered BRE anchors in the following contexts:

A circumflex `^` is an anchor when used as the first character of an entire BRE. The circumflex will anchor the expression to the beginning of a string; only sequences starting at the first character of a string will be matched by the BRE. For example, the BRE `^ab` matches `ab` in the string abcdef, but fails to match in the string cdefab.

A dollar sign `$` is an anchor when used as the last character of an entire BRE. The dollar sign will anchor the expression to the end of the string being matched (not including a final newline character, if present).

A BRE anchored by both `^` and `$` matches only an entire string. For example, the BRE `^abcdef$` matches strings consisting only of abcdef.
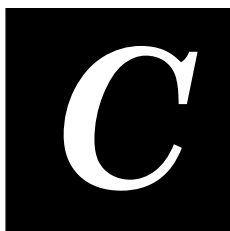
B
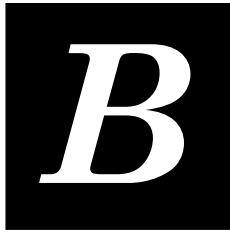
Error Messages

A complete listing of Mascot error codes, messages, and explanations can be found at the URL mascot/cgi/ms-geterror.exe?ALL.
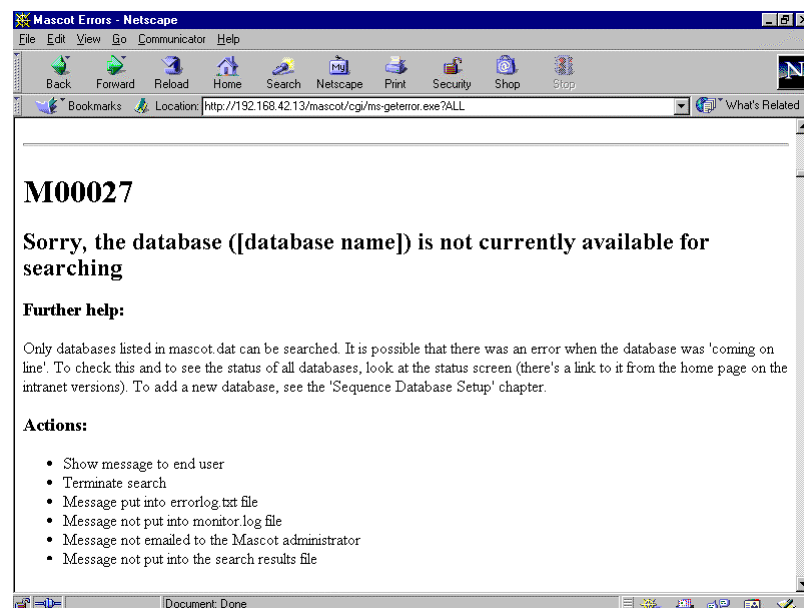
The same text can also be found in the file `errors.html` in the root directory of the Mascot CD-ROM.

C

System Limits

Number of different modifications in unimod.xml	unlimited
Number of enzymatic peptides per sequence	user definable (MaxNumPeptides)
Length of a sequence (number of residues)	user definable (MaxSequenceLen)
Number of seq(), comp(), and ions() type qualifiers per query	20
Maximum number of tags and etags in a search	100
Number of peptide masses (MS/MS search)	unlimited
Number of peptide masses (PMF search)	1000
Number of enzymes in the enzymes file	100
Number of protein hits saved in the results file summary section (PMF)	50
Number of peaks per MS/MS spectrum	10,000
Number of lines with name= in MIME format file	1,000,000
Maximum mass of any peptide in standard Mascot (Daltons)	16,000
Minimum mass of any peptide (Daltons)	100
Maximum mass of an unmodified amino acid residue	300
Length of any peptide in residues in standard Mascot	254
Length of name (TITLE=) for any query when 'escaped'	30,000
Length of database name	19
Length of enzyme name	50
Length of modification name	50
Simultaneous variable modifications	9
Number of missed cleavage sites in a peptide	9
Maximum number of cleavage rules per enzyme	20
Number of active sequence databases	user definable (MaxDatabases)

Number of threads per search	1024
Number of concurrent jobs per database	100
Number of parse rules	256
Length of parse rule	128
Maximum length of an accession string	200
Maximum number of processors per server	64
Maximum number of sub-clusters in a cluster	50
Maximum number of machines in a sub-cluster	1024
Maximum number of processors in a sub-cluster	65536
Maximum number of nodes in nodelist.txt	4096

D
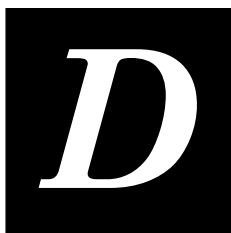
Web Server Configuration

Mascot Directory Structure

The Mascot directory structure is described in Chapter 2, Installation: Linux

Microsoft Internet Information Services

The Mascot installation program automatically configures Microsoft IIS 5.0 or later.

CGI Timeout

The CGI timeout is set to 1 day, and any searches running longer than this will be terminated. If you wish, you can increase this timeout.

As of Mascot 2.2, the CGI timeout value is set only on the parent node /w3svc/1/root/mascot so that it is inherited by both the cgi and x-cgi nodes. If a value is also set on /w3svc/1/root/mascot/cgi, e.g. from a previous Mascot installation or set by an administrator, then it will override any inherited value.

IIS 5.x and 6.0 (2000, XP, 2003 Server)

At the command prompt, go to *c:/Inetpub/AdminScripts* directory

To get the value of the current Mascot cgi timeout:

```
cscript adsutil.vbs get /w3svc/1/root/mascot/cgi/cgitimeout
```

If you get an error message saying "not set at this node", go up one level to the mascot node:

```
cscript adsutil.vbs get /w3svc/1/root/mascot/cgitimeout
```

If you still get an error message saying "not set at this node", then you should set a value at this node. If cgitimeout was already set at this node

or at the cgi node, you can change the value. The default value as set by Mascot will be 86400 seconds (= 1 day)

To change the timeout value at the cgi node:

```
cscript adsutil.vbs set /w3svc/1/root/mascot/cgi/cgitimeout x
```

To set a new timeout value at the mascot node, or change the existing value:

```
cscript adsutil.vbs set /w3svc/1/root/mascot/cgitimeout x
```

where x is the value in seconds that you want to set

You will then need to re-start IIS (from the IIS Management console)

IIS 7.x (Vista, Server 2008, 7)

In the Windows Start menu, go to Control panel, Administrative Tools, Internet Information Services (IIS) Manager. On the connections tree, expand Sites and Default web site and select mascot. In the central pane, double click the CGI properties icon. The CGI time-out will be displayed and can be edited. If you make changes, choose Apply in the Action pane.
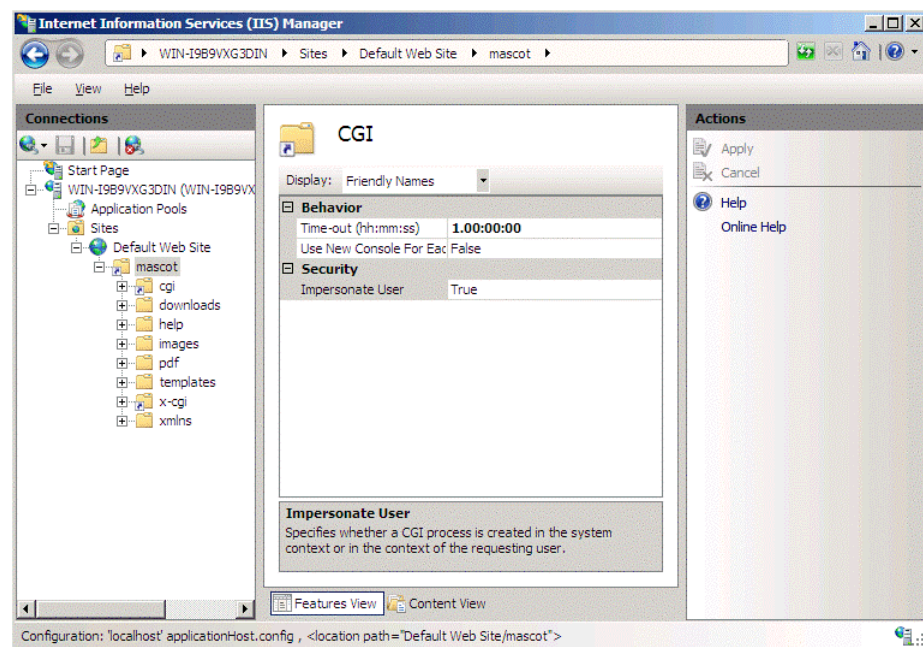
If you have configured IIS 7 with multiple web sites, and the Mascot server is not installed in the default web site, you will need to browse to

the appropriate location. You can also inspect the CGI timeout at other connection nodes, in case a different timeout has been set manually at the cgi node or even at the level of individual files (inadvisable).

Apache

Apache is a very rugged and popular server for Unix platforms. It is a less obvious choice for Windows, since the Mascot installation program will configure Microsoft IIS automatically.

Important: When using Apache, in the Options section of *mascot.dat*, ensure that ForkForUnixApache is set to 1

If the URL /mascot is mapped to disk path *mascot/html*, then URL / mascot/images will correspond to disk path *mascot/html/images*. So, it is important that the entries for the *cgi* and *x-cgi* directories come before that for the *html* directory. Otherwise, the server will report that it cannot find the *cgi* and *x-cgi* paths, because it has assumed from the URL that they are sub-directories of *mascot/html*.

If the web browser connection breaks when submitting a large search or viewing a large result report, add or increase the Timeout directive in the configuration file. Remember to restart Apache after saving the change. The argument is in seconds

```
Timeout 3600
```

Linux configuration

The following lines illustrate typical mappings and permissions for the Mascot directories:

```
ScriptAlias /mascot/cgi/htsearch /usr/lib/cgi-bin/
        htsearch

<Directory /usr/local/mascot/cgi>
  AllowOverride None
  Options None
  Order allow,deny
  Allow from all
</Directory>
ScriptAlias /mascot/cgi /usr/local/mascot/cgi

<Directory /usr/local/mascot/x-cgi>
  AllowOverride None
  Options None
```

```
  Order allow,deny
  Allow from all
</Directory>
ScriptAlias /mascot/x-cgi /usr/local/mascot/x-cgi

<Directory /usr/local/mascot/html>
  AllowOverride None
  Options None
  Order allow,deny
  Allow from all
</Directory>
Alias /mascot /usr/local/mascot/html
```

Windows Installation

If you choose to use Apache under Windows, a good starting point for support information is:

> http://httpd.apache.org/docs/2.4/platform/windows.html

Important: If IIS is installed, stop the IIS service before installing Apache, Perl and Mascot.

Mascot 2.4 has been tested with Apache 2.2.22 on Windows 7.

After Mascot has been installed, from the Windows Start menu, choose Programs; Apache HttpServer 2.2; Configure Apache Server. Copy the customised Apache configuration settings from the *httpd.conf* file in the Mascot *config* directory and paste them at the end of the Apache *httpd.conf* file. Save the changes. From the Windows start menu, choose Programs; Apache HttpServer 2.2; Control Apache Server; Re-start

You should now be able to view Mascot pages in a web browser and proceed with licence registration.

If Windows Firewall is enabled, you will probably have to open up port 80 as described in Chapter 3, before the Mascot Server can be accessed from other computers.

Keyword Indexing

The keyword index required for site search will not have been built during Mascot installation because the web server mappings were not in place.

To build the keyword index, open a command window and enter the following commands. If Mascot was installed into a different path, you may have to modify the first two lines

```
C:
cd \inetpub\mascot\htdig
bin\htdig.exe –v
bin\htmerge.exe –v
```

Once the commands have completed, keyword search using the control at the top right of the web pages should be operational

Using shebang under Windows

The configuration file created by the installer includes this directive:

```
ScriptInterpreterSource Registry
```

This enables Windows-style registry file associations. Assuming Perl has been installed correctly, the extension *pl* will be associated with *perl.exe*.

Without this directive, Apache uses the shebang line at the top of each Perl script to associate the script with the Perl interpreter. The default shebang line is:

```
#!/usr/local/bin/perl
```

If you want to use this on a Windows system, you will need to change the shebang lines of all scripts to something similar to:

```
#!c:/perl/bin/perl.exe
```

User authentication.

Apache provides several ways to restrict access to directories or files. One method is to limit access to clients from a range of IP addresses or a particular domain. Another method is to require a username and password, which may be a convenient way for a system administrator to limit access to the *x-cgi* directory.

Setting up user authentication takes two steps: firstly, creating a file containing the usernames and passwords. Secondly, telling the server what resources are to be protected and which users are allowed (after entering a valid password) to access them.

Creating a User Database

A list of users and passwords needs to be created in a file. For security reasons, this file should not be under the document root. This example assumes the file is called */usr/local/mascot/config/.passwd*.

The file will consist of a list of usernames and a password for each. The format is similar to the standard Unix password file, with the username and password being separated by a colon. However you cannot just type in the usernames and passwords because the passwords are stored in an encrypted format.

The program *htpasswd* is used to add create a user file and to add or modify users. This can be found in the *bin* directory of the Apache distribution. To create a new user file and add the username "mickey", the command would be:

> *./htpasswd -c /usr/local/mascot/config/.passwd mickey*

The *-c* argument tells *htpasswd* to create new users file. You will be prompted to enter a password for mickey, and confirm it by entering it again. Other users can be added to the existing file in the same way, except that the *-c* argument is not needed. The same command can also be used to modify the password of an existing user.

Specifying the password protected resources

Having created a password file, the next step is to modify the configuration mapping for the *x-cgi* directory. Instead of the mapping shown earlier, you would use a directive like this:

```
<Directory /usr/local/mascot/x-cgi>
  AllowOverride None
  Options None
  AuthType Basic
  AuthName Restricted
  AuthUserFile /usr/local/mascot/config/.passwd
  require valid-user
</Directory>
ScriptAlias /mascot/x-cgi /usr/local/mascot/x-cgi/
```

You will need to stop and restart Apache, or send a *kill -HUP* to the parent process, to activate the new configuration. For further information on restricting access to the server, see the "Authentication and Access Restrictions" section of the Apache FAQ documentation.