READ ME FIRST!

Installing Mascot for the first time?

Please read the notes for specific Windows platforms starting on page 3 before following the installation instructions in Chapter 3 of the Installation and Setup Manual. A complete, up-to-date copy of the Installation and Setup Manual can be found on the Mascot installation CD, (*manual.pdf*).

New: Protein Family Report

The Protein Family Summary displays search results a page at a time, allowing the results of even the largest searches to be browsed without client-side memory or browser limitations. Proteins are grouped into familes using hierarchical clustering, making protein inference more intuitive and more accurate.

New: Percolator Support

Percolator is a utility from the University of Washington that uses machine learning to re-rank the peptide matches. The goal is to maximise sensitivity for a given false discovery rate.

New: Search Multiple Databases

Multi-select more than one database for a search. This is useful when you want to search a single organism database and include the sequences of common contaminants, such as BSA and trypsin.

And lots more

- Export search results as mzIdentML.
- Batch automate quantitation with Mascot Daemon.
- Support for mzML format peak lists.
- 64-bit executables for Windows.

Changes since last release

Details of individual changes since the previous major release of Mascot are listed on page 10 of these notes.

Last minute additions to documentation

See page 2

Last minute additions to documentation

- 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.

- Microsoft Internet Explorer 8 no longer allows text to be pasted into a file upload control. This may break the integration between some older, third party software and the Mascot search form. If the software vendor does not have a fix, you may need to downgrade to Internet Explorer 7 or 6.

- Under Vista and later, you will be unable to change or repair Mascot via the Windows Control Panel, Programs and Features. To change or repair your installation, run the setup program again (setup32.exe or setup64.exe)

- Growth in the size of NCBInr and UniRef100 has meant that there was no room on the Databases DVD for the IPI files referred to in Chapter 5 of the manual

- For a clean install, the Windows installer will default to the drive with the largest amount of free space, not always drive C:

- 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.

- Microsoft Internet Explorer 6 is noticeably slower than other browsers when displaying the new, Protein Family Summary. If possible, use one of the other supported browsers (help/browser_help.html).

Notes for specific Windows Platforms

Windows 2000

Service pack 4 must be installed

A clean installation of Windows 2000 SP4 includes Windows Installer 2. It is very likely that the installer will already have been upgraded to version 3 in the course of installing other software packages. If not, you will see a message when you try to install Mascot stating that the installer must be upgraded. For Windows Installer 3.x download details, go to http://support.microsoft.com/?kbid=893803

Windows XP

The latest service pack should be installed

64 bit support

XP Professional x64 edition uses IIS 6.0. See the notes about this version of IIS under Windows Server 2003, below.

To install Mascot on XP Professional x64 edition, execute setup64.exe instead of setup32.exe

Note that you cannot mix x86 and x64 nodes in a Mascot cluster. All must be 32-bit or all must be 64-bit

Windows firewall

Remember to open port 80 (HTTP) if you want to access the Mascot server from other computers.

The procedure for configuring Windows firewall for a Windows XP search node in a Mascot Cluster is described in Chapter 11 of the Setup & Installation manual.

Windows Server 2003

The latest service pack should be installed

IIS 6.0

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 Apache 2.x as your web server.

IIS 6 does not serve files with unknown MIME types. 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

64 bit support

To install Mascot on a Server 2003 x64 platform, execute setup64.exe instead of setup32.exe.

Note that you cannot mix x86 and x64 nodes in a Mascot cluster. All must be 32-bit or all must be 64-bit

Windows Firewall

Remember to open port 80 (HTTP) if you want to access the Mascot server from other computers.

The procedure for configuring Windows firewall for a Server 2003 search node in a Mascot Cluster is described in Chapter 11 of the Setup & Installation manual.

Windows Vista

IIS 7.0
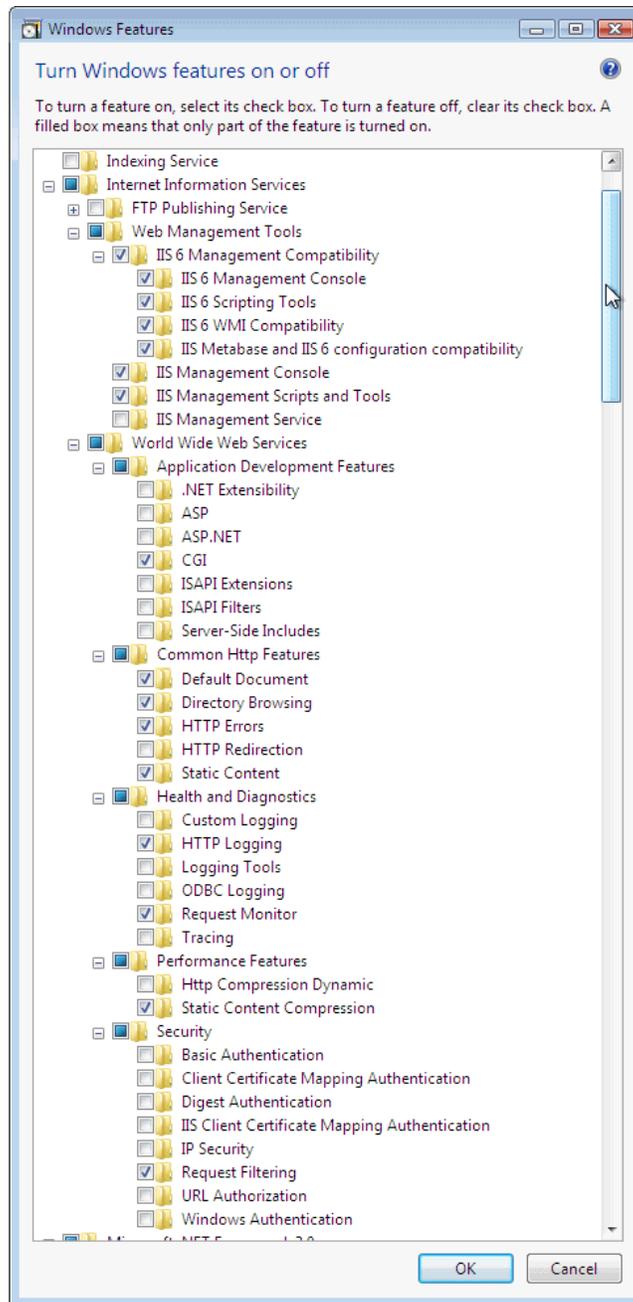
A default installation of IIS 7.0 is not sufficient for running a CGI application such as Mascot. From the Control Panel, choose 'Programs and Features'. Choose 'Turn Windows features on or off'. Expand the node for Internet Information Services and ensure that the checkboxes shown above are checked, in addition to any default selections. Then, choose OK.

Apache

Mascot 2.3 has been tested with Apache 2.2.4 on Vista. It is not known whether earlier releases of Apache are also compatible with Vista. The procedure is as follows:

1. Download the latest Apache MSI installer from http://httpd.apache.org/download.cgi. You must install Apache as Administrator. It is not sufficient to install as a user with administrator privileges. The easiest way to do this is to open a command window by right clicking the start menu item (under accessories) and choosing 'Run as administrator'. Verify that the title bar says 'Administrator: Command prompt'. Execute the installer by typing the filename in this command prompt and pressing return. Accept all defaults.

2. At the end of the installation, you will be prompted by Windows firewall. Choose to unblock port 80 (HTTP). Don't be concerned if you see an empty error message at the end of the installation. This is because the installer is unable to start the Apache service.

3. Install ActiveState Perl from the Mascot installation CD. Accept all defaults

4. Install Mascot

5. Copy the Apache configuration settings from the httpd.conf file created in the Mascot config directory and paste them at the end of the Apache httpd.conf file, (in C:\Program Files\Apache Software Foundation\Apache2.2\conf). Save the changes.

6. In Windows Explorer, go to the Apache bin directory, (C:\Program Files\Apache Software Foundation\Apache2.2\bin). Right click ApacheMonitor.exe and choose properties. On the compatibility tab, check 'Run this program in compatibility mode for' with 'Windows XP SP2' in the drop-down list, then choose OK.

7. Re-start Vista

64 bit support

To install Mascot on a 64-bit Vista platform, execute setup64.exe instead of setup32.exe.

Note that you cannot mix x86 and x64 nodes in a Mascot cluster. All must be 32-bit or all must be 64-bit

Windows Firewall

Remember to open port 80 (HTTP) if you want to access the Mascot server from other computers.

The procedure for configuring Windows firewall for a Vista search node in a Mascot Cluster differs from that shown in Chapter 11 of the Setup & Installation manual as follows:

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*.
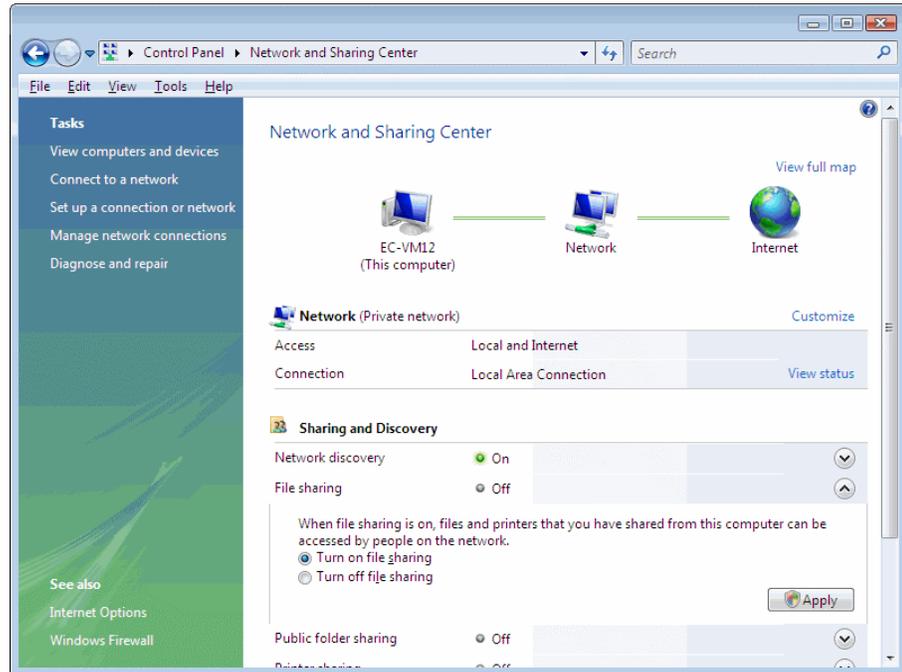
Select *Administrative Tools* and launch *Windows Firewall with Advanced Security*. Select *Inbound Rules* in the left hand panel and *New Rule* in the action panel.
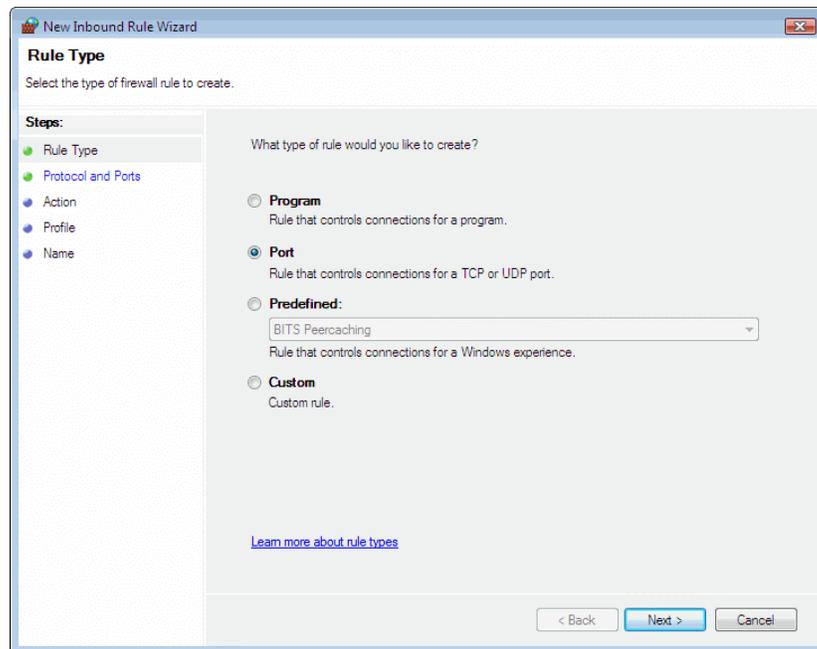
In the wizard, choose Port, Next, TCP, Specific Local Ports, 5001, Next, Allow Connection, Next, Clear the checkbox for Domain and Public, Next, Enter the name as MascotNodePort5001, Finish. The new rule will be added to the list of Inbound Rules.

Windows Server 2008

The latest service pack should be installed

IIS 7.0, 7.5

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

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 Apache 2.x as your web server.

64 bit support

To install Mascot on a Server 2008 x64 platform, execute setup64.exe instead of setup32.exe.

Note that you cannot mix x86 and x64 nodes in a Mascot cluster. All must be 32-bit or all must be 64-bit

Windows Firewall

Remember to open port 80 (HTTP) if you want to access the Mascot server from other computers.

The procedure for configuring Windows firewall for a Server 2008 search node in a Mascot Cluster differs from that shown in Chapter 11. Instead, follow the procedure described above under Windows Vista

Windows 7

IIS 7.5

From the Control Panel, choose *Turn Windows features on or off*. Expand the node for Internet Information Services, then follow the configuration notes under the Windows Vista section, above

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 Apache 2.x as your web server.

64 bit support

To install Mascot on a Windows 7 x64 platform, execute setup64.exe instead of setup32.exe.

Note that you cannot mix x86 and x64 nodes in a Mascot cluster. All must be 32-bit or all must be 64-bit

Windows Firewall

Remember to open port 80 (HTTP) if you want to access the Mascot server from other computers.

The procedure for configuring Windows firewall for a Windows 7 search node in a Mascot Cluster differs from that shown in Chapter 11 of the Setup & Installation manual as follows:

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.
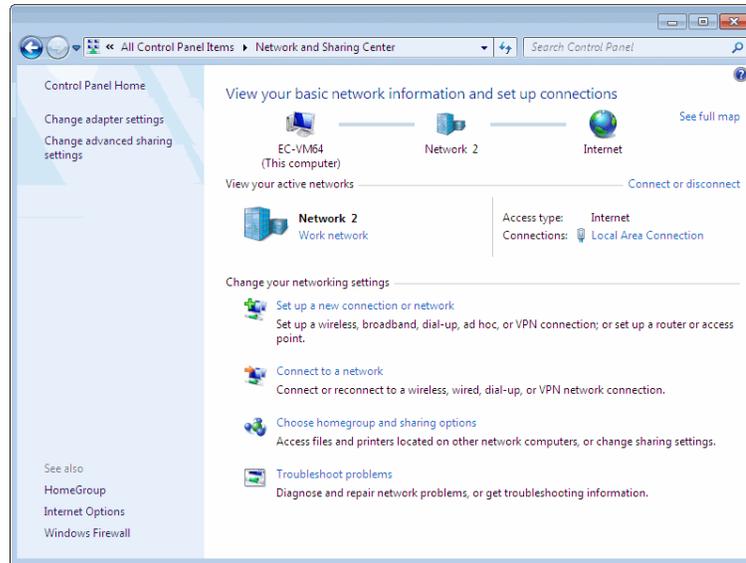
Choose Windows Firewall then Advanced Settings. Select Inbound Rules in the left hand panel and New Rule in the action panel.

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.

Changes since Version 2.2.02

ID	Title
203	Support multiple Fasta databases
599	Installation - service name missing under Windows 2000
781	Delete a database from mascot.dat, still shows up in Database Status
1108	ignore_ions not working properly
1754	Non-linear progress reports in error tolerant search of large data set
1765	New installation program required
1792	Spurious M00251 messages. Warning : Taxonomy 'Viruses' ignored.
1834	Some URL's in Entrez reports in Protein View are relative
1883	Daemon check for Analyst 1.x is not correct
2062	Master results needs to display flag for unique vs shared
2136	Select summary (unassigned) has check boxes but no repeat search button.
2191	Add /LARGEADDRESSAWARE flag for Windows
2196	Crash when .dat or taxonomy file being updated on server when search starts
2209	New functions for client.pl
2233	Installer fails to set permissions adequately
2236	mzData ignores spectra with precursorList count > 1
2246	ms-getseq.exe and ms-gettaxonomy.exe not working for guest user
2338	Accession numbers should not be displayed in the status bar.
2341	Modifications on search form should use single master list
2378	Reference file entry not completely retrieved for first seq in SwissProt using AC rather than ID
2382	Changes to not.mascot.dat for Windows installer
2393	Support for QUERYLIST to enable repeat search from Select summary
2433	ET search or search with accessions on NA db is wrong
2434	peptide match parameter discrepancies for multiple NLs
2438	Reproducible crash in CMassComponent::makeQueryArray
2441	RDBI Daemon parameter fails to clear decoy/error tolerant
2442	DNA modifications are not output in Peptide Summary
2443	progress report is slightly wrong for NA searches with FRAMES
2445	nph-daemon_helper.pl missing from shipper (required by GPS Explorer)
2447	Confusing error message from Daemon with security and Integra user
2448	get_params.pl fails with Integra system user when security is enabled on Linux
2449	Modifications from hidden list not escaped in search form

ID	Title
2454	mod_file should be re-written if a user edits it
2455	Changes requested to modifications configuration editor
2457	Installer fails if IIS web site is bound to a specific interface or URL contains an IP address
2458	Installer needs to set permissions on unimod.xml and quantitation.xml
2464	Add the fixed mod delta to the XML export
2465	Mascot Daemon 2.2 no longer works with IIS integrated authentication
2466	Need to leak bytes in additional place for quant reports
2467	ms-monitor fails when shutting down under Windows with ms-lockmem enabled
2470	Support higher charge states (current limit 8+)
2471	PMF performance improvements
2472	Multiple instances of too many ions error in mzData causes nph-mascot.exe to crash
2473	Cannot have space in lcq_dta.exe path name in Daemon
2475	Quant. Want option to require modified residue to be present.
2476	Mascot is ignoring mzData retention time information
2477	Local modification being used in the wrong spectra?
2478	Perl scripts ouputting http headers with charset=ISO-8859-1
2479	Invalid etags can get matches
2481	Error tolerant search: discrepancies between Peptide Summary and Peptide View
2482	performance improvements for etag searches
2483	In reports, modifications could be hyperlinked to the Unimod entry
2484	Export help not clear
2485	getseq.pl takes a sessionID parameter, but doesn't pass it on to ms-getseq.exe
2486	Kludge modification names for GPS
2487	Add proper support for Vista
2488	Installer needs to configure IIS 6 to allow cgi and x-cgi
2489	The Mascot paths in the registry now have a trailing slash.
2490	Installer needs to install new config file called mod_aliases
2491	Configuration Editor bugs roll-up
2492	Mascot Security issue with ms-getseq.exe. Change to getseq.pl
2494	Daemon fails to report all Distiller errors
2496	SwissProt taxonomy is partly broken...
2497	NA searches sometimes crash while writing results file
2498	Missing graphs - improve error reporting in scripts
2502	nph-mascot.exe opens all compressed files on node. Should only open required ones?

ID	Title
2503	emPAI documentation updates
2505	Add button to display all matches in Peptide View
2506	URL arguments for quant formatting not being passed to export script
2507	Daemon drag and drop to repeat a search problems
2510	Search crash after failing to lock with ms-lockmem
2511	MS/MS search speed improvements
2512	primary_nl string confused if two matches have identical score
2515	Cannot remove multiple privileges with single click in security admin
2516	Add raw result file to export list
2518	Daemon should not halt a task if Distiller produces multiple peak lists
2519	State of hide error tolerant checkbox not passed to protein view
2520	Protein view and enzymes created by config editor are incompatible
2522	Increase range of charge states available in lcq_dta_shell
2524	Intermittent crash with integrated ET mode
2529	Table of matches at bottom of peptide view doesn't always agree with master results
2530	Scripts and parser had different upper limit on significance threshold
2533	ms-makesearchlog.exe crashes when corrupt result files encountered
2534	Create windows installer patch package
2535	Daemon external process parameter "<resultfilepath>" doesn't return a full path like the help says.
2536	automatic BIG mascot mode
2538	Intermediate file name is missing or empty. [M00398] - misleading error message.
2539	Command string for Daemon external processes is truncated unnecessarily at 255 characters
2540	If ITOL not set Mascot will search anyway
2541	If heavy or light isotope not found in Unimod, error messages are unclear
2542	Master results slow after fix for 2529
2543	Legacy upgrade doesn't remove old ARP entry
2544	High PMF score for single large protein with no enzyme (crazy search)
2550	Isotope correction for multiplex quantitation not implemented correctly
2559	Decoy stats shouldn't be displayed in protein summary for mixed ms and ms-ms
2561	Configuration editor limits number of residues in cleave and restrict fields to 10. Limit should be 20
2562	Protein View fails for hit in peptide summary with a protein score below threshold
2563	Spurious error message: Error [M00267 - Job -1 - X00875:ms_fileutil] - Failed to lock file
2564	Zero length fasta file causes ms-monitor.exe to crash

ID	Title
2566	Specific case where sequence tag fails
2567	ms-searchcontrol.exe should not output warnings if they will confuse client
2568	quantitation normalisation not consistent with help
2569	Quantitation not reporting protein ratio when there is a single peptide ratio
2571	Daemon should automatically remove MGF header lines when merging files
2572	Database maintenance corrupts extended characters which can lead to SetEntriesInAcl errors
2573	Swapping database with SeparateLockMem=1 and /3GB /PAE
2574	Update Daemon help re connection strings for SQL Server
2575	Add security to client.pl
2578	ms-gettaxonomy.exe fails if you request a tax id of -5 or lower
2580	Small memory leaks
2581	minor ms/ms scoring bugs (no visible symtoms)
2582	Need paged report for very large result files
2584	Decoy search on cluster only performs decoy part on first node
2589	ms-review - outputs invalid html if there is no searches.log file
2595	User parameters not HTML escaped during report generation
2597	No reason why tolerance needs to be set wide for multiplex quantitation
2598	Changing the case of a database name causes problems in Windows
2601	Supply a (sample) contaminants database
2602	Increase limit for number of databases to 256
2605	Improved reporting of protein hit
2609	PMF Mixture false positive
2612	Crash with large mod and very large peptide
2613	negative modification deltas with BIG mascot are output to result file as huge numbers
2616	Over-merging peptide matches when delta is the same
2618	Charge state in result different from input file
2619	Incorrect delta mass written to result file when fixed and variable apply to terminus
2620	Should probably include merged.dmp in mascot.dat (and supply it) to reduce M00384 warnings.
2622	Order of etag-results and of pmf queries is variable in result file
2623	Results slow to load in a browser because javascript var lines too long / large
2626	Protein view gives: "Fatal error no source defined" when database name contains parentheses
2629	Rare crash on Windows cluster. No message in error log files
2631	Progress reports for ET search inaccurate

ID	Title
2632	Upgrade projects to VS2005
2633	No point having sort unassigned control in a (protein) select summary
2635	Uniref100 help page needs updating
2637	Daemon failing to post correctly to https (ssl enabled) Mascot server
2639	Add support and doco for new MGF field "RAWSCANS".
2644	ProteinsInResultsFile set to 2 doesn't include rank 2 peptides with score same as rank 1
2646	Protein view: Fatal Error protein sequence has changed since the search was performed when there are base substitution in an error tolerant search
2647	Add support for UltraSPARC T1 and T2, and IV+ processors
2648	Minor CSV format changes for export script
2650	Mascot Daemon crash if number of formats supported by Distiller is reduced (e.g. uninstall Agilent MassHunter support)
2659	Don't use peptide length limit in error tolerant search
2662	Configuration Editor should use the system locale
2663	Crash with multiple subclusters
2664	PKL files with charge as float breaks Daemon merge
2666	Quantitation method can not be found [M00473] when there are strings containing multiple consecutive spaces in name.
2668	protein_view.pl should take enzyme definition from the results file
2669	BIG mascot mode has a few problems
2670	Error M00248 - Invalid taxonomy name class (authority |) and (unpublished name |)
2671	new version Uniprot SwissProt 56 has changed format for fasta
2673	Spurious 'Too many modifications' message for quantitation method
2674	Need to change certain text labels in ms_config Instruments section
2675	small databases with mutliple threads are not iterated properly
2676	Daemon follow up loses track of Distiller project after first follow up
2678	Illegal division by zero at ./quant_subs.pl line 3653
2679	Cannot have same modification appearing in more than one component in quantitation method
2680	ms-config crashes on empty environment
2681	Crash when writing to ipc.log, or rarely other log files. The ctime function is not thread safe
2683	Inconsistent report of quant method mods at the bottom of peptide summary.
2685	Mascot Integra Daemon not starting tasks with a long parameterset name
2686	If two local definitions in a quantitation method, second is ignored
2688	Change minimum ms-ms fragment tolerance in search form so that users don"t have to switch units to mmu

ID	Title
2689	Parser functions requiring a string must not be passed a perl number
2692	implement mzML input data format
2693	More problems with large variable mods
2694	Can't lock both SwissProt and NCBInr on Server 2003 (x64) cluster
2696	Selecting Merge MS/MS into single search and Distiller peak list format mzData gives error
2697	Crash with average quantitation protocol and no variable mods.
2699	pass spectrumID as SCANS in mzData
2701	Masses in help page are out of date
2702	Daemon fatal error with myODBC 5.1.5
2705	Need documentation for different neutral loss types somewhere
2706	index.html on databases DVD needs updating for 64 bit windows
2707	lcq_dta_shell.pl still using 1.008 for proton mass
2709	Change Daemon to have default Min. Scans / Group of 1 for lcq_dta.exe
2711	Crash on public web site with multiple very large modifications (glycosylations)
2717	Export with show_queries=1 or query_master=1 will run out of memory on large result file
2719	Some very short peptides with very low scores missed in multi-threaded search
2720	taxonomy ignored warning is reported twice
2721	Support IPv6 in the search log
2722	Search of Trembl crashes before it can even get started when "constrain search" is true.
2723	Crash with ETD-CID Instrument
2725	Configuration Editor allows duplicate values for report_ratio name
2726	Select summary should not collapse matches for different charge states
2733	Database maintenance doesn't set number of threads correctly in some cases
2735	ms-ms mzData file fails with out of memory if first spectrum is ms with 8000 peaks
2738	Add support for Distiller quantitation into Daemon so as to enable batch automation
2740	Add charge states "2+, 3+, and 4+" to search form for ETD
2741	In Daemon, support MDRO IPeakListFormatOptions4 functions
2742	mascot.dll memory leak has become unacceptable. Add work-around to Daemon
2743	Add a security task that set a separate query limit for no enzyme searches
2744	Test search fails when there are multiple sub-clusters
2745	Daemon should provide proper UI for web authentication username and password
2746	semi-specific enzyme or ET search on NA databases doesn't properly count number of Xs

ID	Title
2748	Failure to read .stats file doesn't report an error
2751	Error M00251 - taxonomy indexes ignored when searching SwissProt with large number of threads.
2752	homology thresholds can differ in cluster and standalone mode or with different chunks
2753	Mass of electron not considered when reading from dta files.
2754	discrepancies in mod deltas as output to dat-file
2757	Update Configuration Editor to support quantitation_2.xsd
2760	quant_subs.pl crashes when normalisation either average or median and some peptide matches have no ratios
2761	Integrate Mascot Percolator
2763	Add support for analysisXML (now called mzIdentML)
2764	Export script fails to include error tolerant matches in pepXML
2766	Update IPI help pages
2767	Allowed p value for significance threshold should be 1E-18 < p < 1
2768	iTRAQ reporter ion masses don't allow for loss of electron
2769	Mascot Daemon Parameter editor needs to allow multiple fasta
2770	ms-monitor checks far too frequently for fasta file changes
2771	If a set of values is identical, should report SD(geo) as 1.0, not NN
2778	Add MALDI-ISD option to Instruments file
2780	Daemon needs heartbeat function for Integra
2784	Add copy and paste for command line to export script
2785	If file path and name are too long Daemon can not open the data file
2786	Mascot Daemon hyperlink to Protein Family Summary for large MS/MS
2790	Peptide view not listing fixed mods specified in quant method
2791	Config editor hangs when deleting quant ratios
2793	Save an index into the mgf/pkl/dta file for mzIdentML export
2797	Update test search and change template name to do_not_delete.asc
2799	Duplicate fasta filenames in different paths cause confusion
2804	Update multiplex to handle isobaric peptides (IPTL)
2805	Taxonomy entry with apostrophe breaks search form cookies
2814	Speed up for mzIdentML exporter when creating fragment ions
2815	Fragment masses wrong when ET mod applies to fixed mod site
2823	If IgnoreIonsScoreBelow is not zero in mascot.dat, cannot set to zero in form
2824	ms-monitor.exe crash when multiple threads write to monitor.log at same time