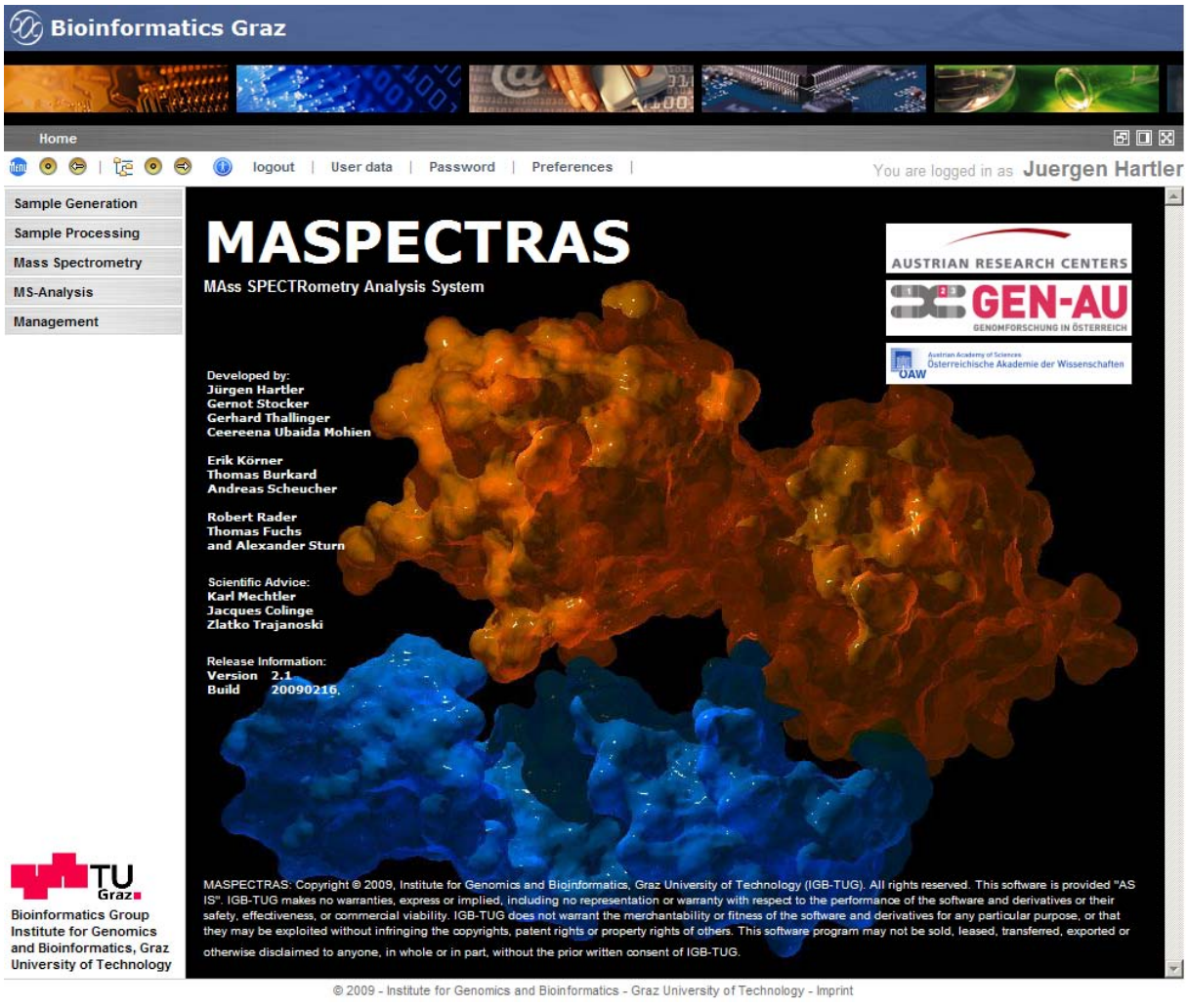
MASPECTRAS

Users Guide

In this user guide every page and functionality is described in detail. To work with MASPECTRAS it is not necessary to read the whole document, because many things work similar to other sections. To work with MASPECTRAS without neglecting any advantages it should be sufficient to read the chapters 1, 2, 6 and 7. The rest should serve as look-up for clarifying ambiguities.

1. General Information:



This will be your first impression of MASPECTRAS.
The main view is divided into 3 sections:

1. The header section consists of some images on the top, of one bar concerning the display and one bar concerning the AAS(Authentication and Authorization System)
2. The left side bar contains the menu
3. The centre frame contains the displayable information

1.1 The header section:

1.1.1 The display bar:

The “Home”-link leads you back to the start page.
At the right side there are 3 icons where you can change the spatial usage of the browser window:

 : brings the window back to the normal size (default setting)

 : with this link you can use the full width of your screen for displaying the information section

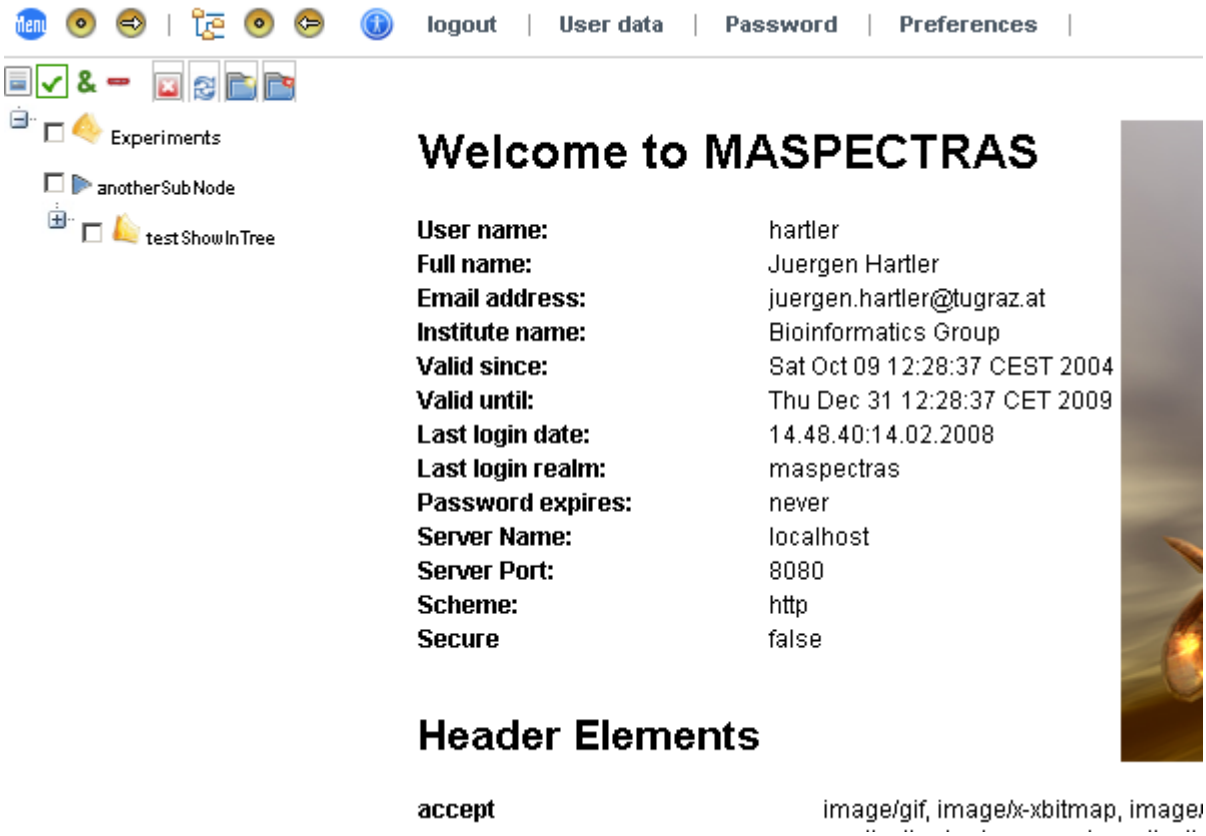
 : uses the full width of the window and the images at the header section disappear, only the display bar and the AAS bar will stay.

1.1.2 The menu administration bar:

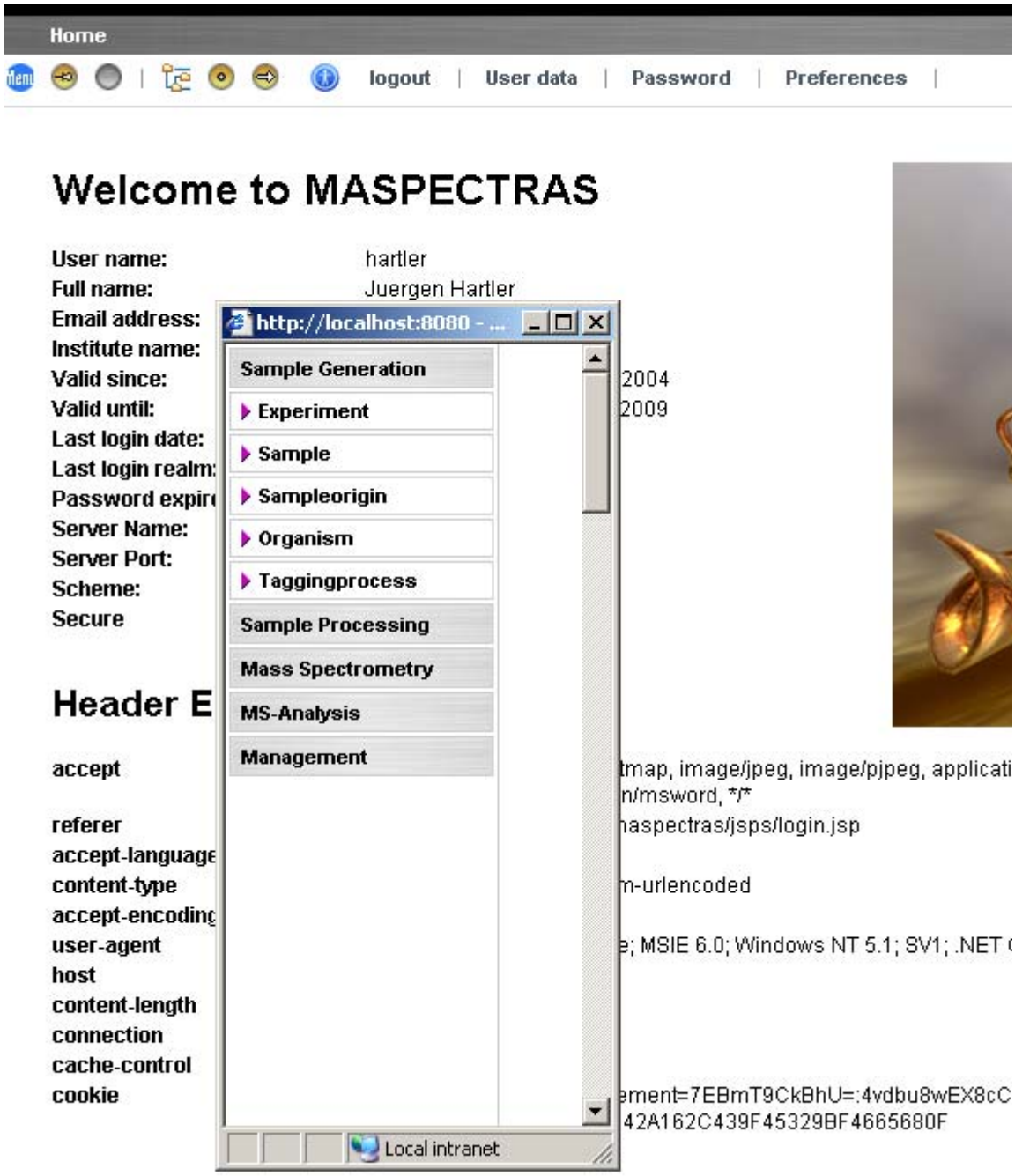
This bar allows the customization of the menu bars. The icons which come after  change the settings of the normal menu while the icons which come after  change the settings of the tree menu. The meaning of the symbols is the same:

- : this removes the menu bar on the left side of the screen so that all of the space is available for the main information page:

- : this shows a menu bar again on the left side (here the tree menu is shown):



: this shows a menu bar in a new window:



: this removes the opened window.

1.1.3 The user (login) bar:

If you are not logged in:

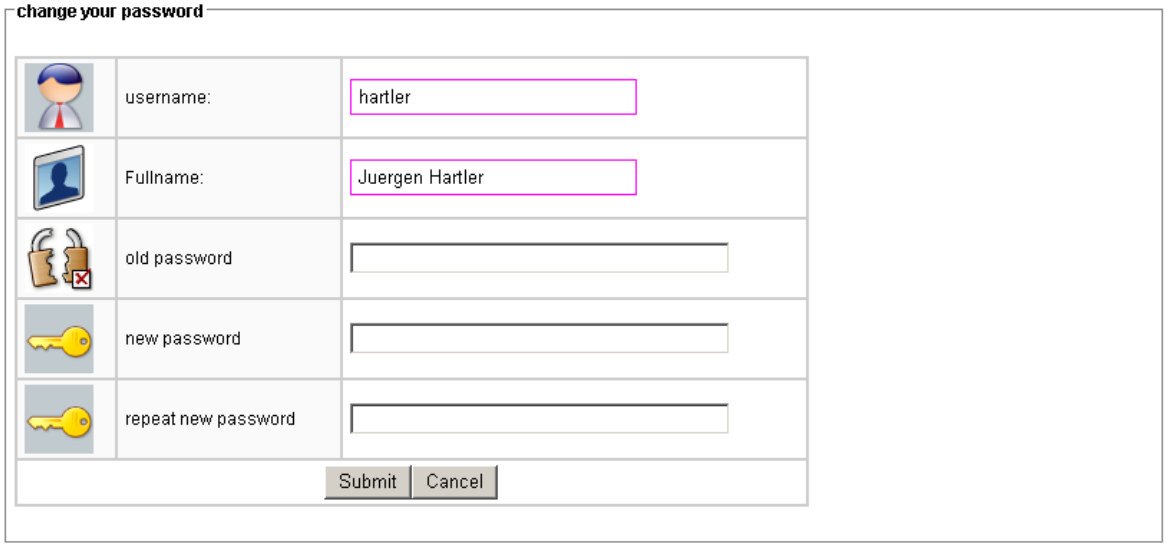
gives the possibility to log in

If you are logged in:

gives the possibility to:

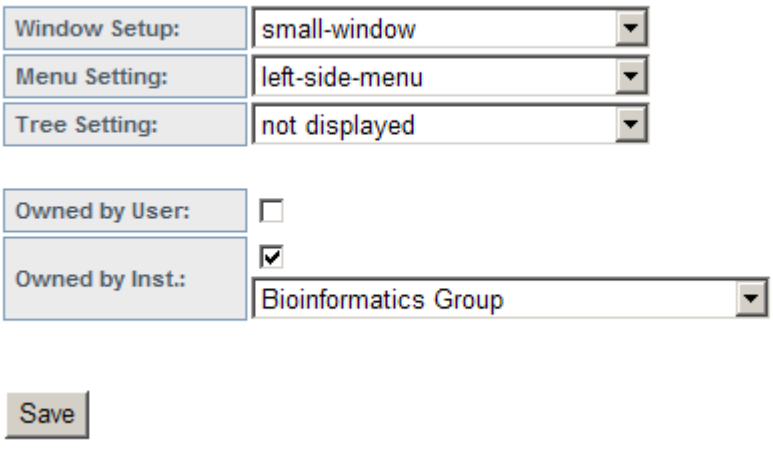
- Log out
- Show detailed information about your user account
- Change your password
- Displays your user
- Sets the preferences for the display

1.1.3.1 Change Password:



You must enter your old password and repeat the new one two times. The password must have at least 8 characters. One character must be a number and one character must be special character (!"@=?...).

1.1.3.2 Change Preferences:



This allows customization of the menu structure for each user. Whenever the user logs in the preferences are loaded and the menu display changes correspondingly.

Window-setup:

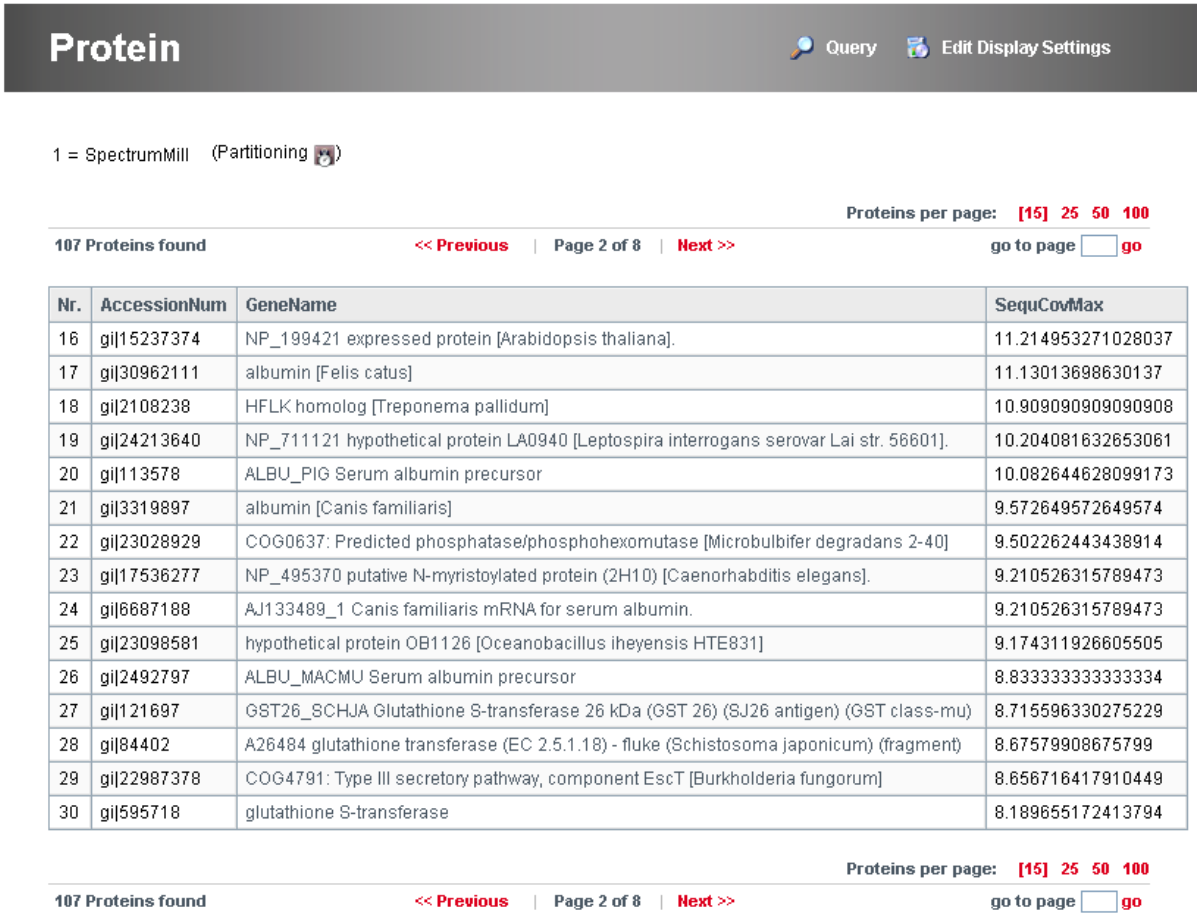
- small-window: same meaning like  in 1.1.1
- stretched-window: same meaning like  in 1.1.1
- fullscreen-window: same meaning like  in 1.1.1

The “Menu Setting” and the “Tree Setting” have the same options. The option “left-side-menu” is just possible for one of them, since otherwise to much space for the display of information is lost:

- left-side-window: the menu is displayed at the left side of the information screen (default setting for the “Menu Setting”)
- new-window-menu: the menu is displayed in a new window; for this option the pop-ups for the Maspectras application must not be blocked.
- not-displayed: the menu is displayed not at all.

The “Owned by” fields allow the specification of the default displayed ownership of entered or edited data. This is just for the default view, while in every page the change of the ownership is possible.

1.2 The information section:



Nr.	AccessionNum	GeneName	SequCovMax
16	gi|15237374	NP_199421 expressed protein [Arabidopsis thaliana].	11.214953271028037
17	gi|30962111	albumin [Felis catus]	11.13013698630137
18	gi|2108238	HFLK homolog [Treponema pallidum]	10.909090909090908
19	gi|24213640	NP_711121 hypothetical protein LA0940 [Leptospira interrogans serovar Lai str. 56601].	10.204081632653061
20	gi|113578	ALBU_PIG Serum albumin precursor	10.082644628099173
21	gi|3319897	albumin [Canis familiaris]	9.572649572649574
22	gi|23028929	COG0637: Predicted phosphatase/phosphohexomutase [Microbulbifer degradans 2-40]	9.502262443438914
23	gi|17536277	NP_495370 putative N-myristoylated protein (2H10) [Caenorhabditis elegans].	9.210526315789473
24	gi|6687188	AJ133489_1 Canis familiaris mRNA for serum albumin.	9.210526315789473
25	gi|23098581	hypothetical protein OB1126 [Oceanobacillus iheyensis HTE831]	9.174311926605505
26	gi|2492797	ALBU_MACMU Serum albumin precursor	8.833333333333334
27	gi|121697	GST26_SCHJA Glutathione S-transferase 26 kDa (GST 26) (SJ26 antigen) (GST class-mu)	8.715596330275229
28	gi|84402	A26484 glutathione transferase (EC 2.5.1.18) - fluke (Schistosoma japonicum) (fragment)	8.67579908675799
29	gi|22987378	COG4791: Type III secretory pathway, component EscT [Burkholderia fungorum]	8.656716417910449
30	gi|595718	glutathione S-transferase	8.189655172413794

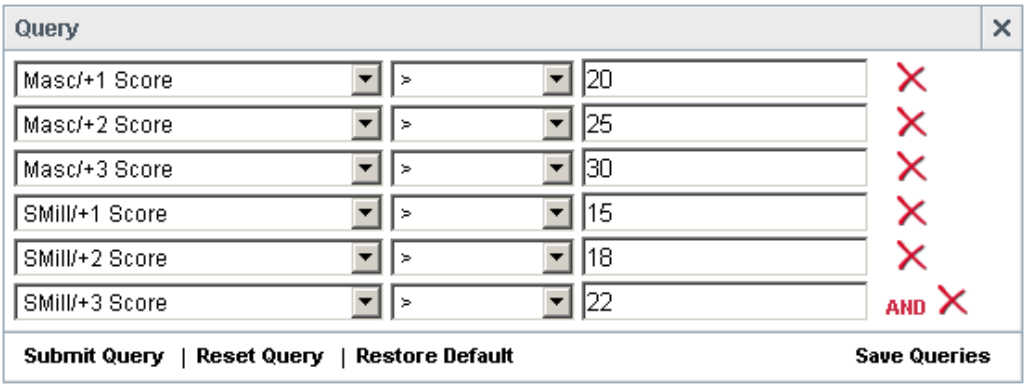
The general presentation of the data in MASPECTRAS looks like the figure above.
In the header section there are 2 links:

- Customizable queries
- Customizable display

The table with the data is always enclosed by the bars for the scrolling and almost every column in the table is sortable.

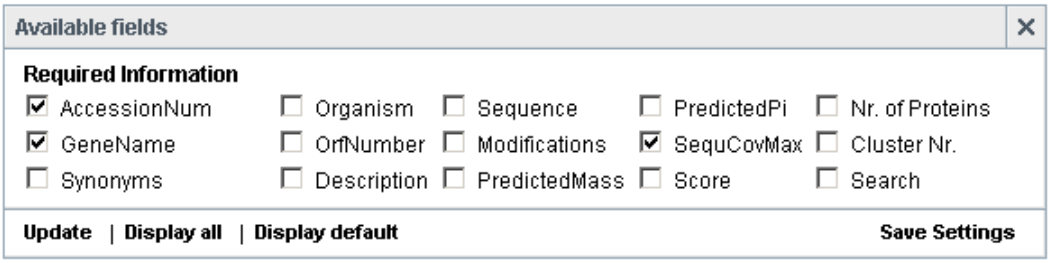
If you come from another page, at the bottom of the page, there is a return button, which brings you to page you have visited before.

1.2.1 Customizable queries:



The query box enables the combination of as many queries as you like. The queries can be added or removed. The operators “LIKE” and “NOT LIKE” need a preceding or trailing asterisk
The button “Submit Query” submits the entered query and changes the view on the data correspondingly.
“Reset Query” removes all entered queries and submits a query without any user-defined filters.
Restore Default” restores the default set of queries and submits them.
“Save Queries” saves the actually entered set of queries as default to the database and submits them. Unless you change the queries your data on that page will always be filtered with this default set of queries.

1.2.2 Customizable display:



The information that will be displayed on the screen is customizable to the needs of the end-user. The user can select the information by clicking on the checkboxes and update the view on the data by pressing the button “Update”.
“Save Settings” allows the user to store his own display settings. Whenever the user enters the same page his settings will be displayed by default.

1.2.3 Scrolling bar:

On the left the scrolling bar shows the number of elements that have been found (depending on the query the user submitted). In the centre section the total number of pages with the actual page is displayed, plus the two arrows to go to next or the previous page. In the centre section the actual page is displayed and it is possible to switch to the previous and the next page. On the right you can choose how many proteins you prefer to be shown on one page. At the right side you can define the number of items per page and you jump to any page by entering the page number and pushing the “go” button.

Nr.	ID	Upload Name	Category	Added Date				
1	2650	casein_NL_MS3	xcalibur	2005-06-29				
2	2700	Task1ms22400-3601	sequest	2005-07-06				
3	2600	testBigMascot	mascot	2005-06-21				
4	2850	newMascot	mascot	2005-08-04				
5	2001	karlDB	synthDatabase	2005-06-07				
6	2002	kPEP_phospho_BSA	synthDatabase	2005-06-07				
7	2003	myTestDB	synthDatabase	2005-06-07				
8	2004	SynthDB	synthDatabase	2005-06-07				
9	2005	SynthPep	synthDatabase	2005-06-07				
10	2006	SpectrumMill	spectrummill	2005-06-07				
11	2007	Task1ms22400-3600	sequest	2005-06-07				
12	2009	Task2synthDBAll	sequest	2005-06-07				
13	2010	Task2testKarlDB2	sequest	2005-06-07				
14	2011	Task2CompToMasc	sequest	2005-06-07				
15	2012	MSDB	mascot	2005-06-07				

The table view consists by default of the following parts:

- The header: if you hover your mouse over a column-name the colour changes to blue and you can sort by this column

- The number in the first column indicates the hit number of the entry corresponding to the order you sorted your data
- Links to data connected to the entries are normally located on entries in the list
- : Indicates that you can edit your data here.
- : Indicates if there is some information downloadable
- : Indicates if you can delete this data entry here.
- : Indicates that there is additional information available
- : Indicates that you can share your data to other users of the system

When you click on the share icon you move to a page where you can select other users or institutes and make the data available for them:

Sharing

You are about to share item: quantTestJune2006

	Name	E-Mail	
☐	Institute for Genomics and Bioinformatics	zlatko.trajanoski@tugraz.at	
☐	Institue of Pathology, University of Graz	karin.wagner@klinikum-graz.at	
☐	Inserm U255	jerome@irgendwas.fr	
☐	Visitors	none	
☐	Ludwig Boltzmann Institut	gudrun.gann@klinikum-graz.at	
☐	ARC Seibersdorf	dieter.kopecky@arcsmed.at	
☐	Sandoz GmbH	thomas.specht@sandoz.com	
☐	I.M.P.	Karl.Mechtler@imp.univie.ac.at	
☐	Institute of Molecular Biotechnology	Helmut.Schwab@tugraz.at	
☐	Institut fuer Chemie	Christoph.Kratky@uni-graz.at	
☐	Aging Research	guenter.lepperdinger@oeaw.ac.at	
☐	Information Design Department, FH JOANNEUM	informations-design@fh-joanneum.at	
☐	Dept. Immunology, School of Pathology	none	
☐	Biocenter, Innsbruck	Zellbiologie@i-med.ac.at	
☐	Department for Specialized Gynaecology	teresa.wagner@akh-wien.ac.at	
☐	Oridis BioMed	info@oridis-biomed.com	

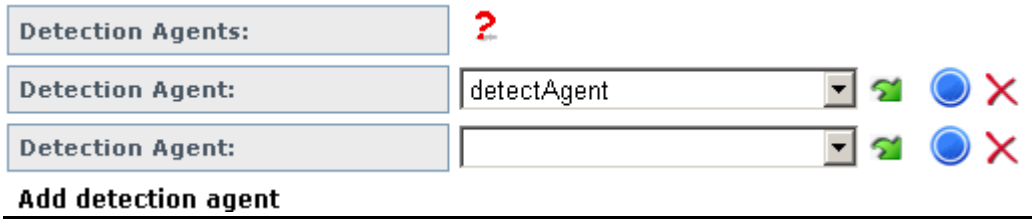
	Name	Full Name	E-Mail	
	hartler	Juergen Hartler	juergen.hartler@tugraz.at	
☐	testmaspectras	Test Maspectras	juergen.hartler@tugraz.at	
☐	stocker	Gernot Stocker	gernot.stocker@tugraz.at	
☐	mechtler	Karl Mechtler	Karl.Mechtler@imp.univie.ac.at	

When you select a user or an institute the checkboxes at and are enabled and you can additionally specify if the user has edit or delete rights on your data.

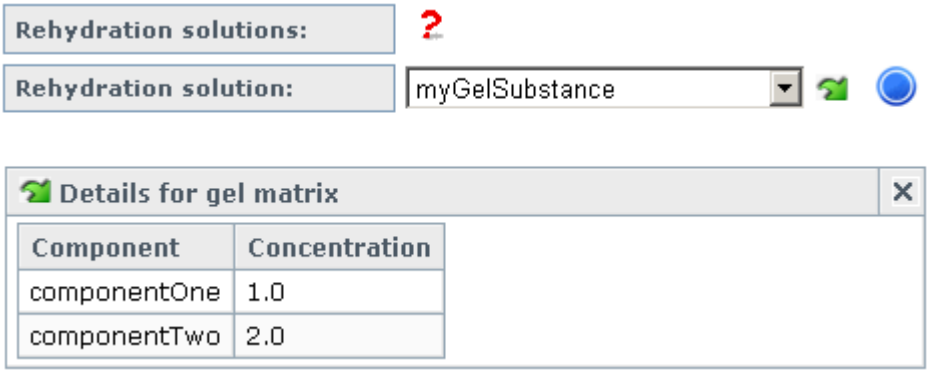
1.2.4 Select input Fields:

When you have an input field like the one above and your element of choice is not in the drop down menu, you can push the blue button and enter your element. The button can lead either to an input page of an element or to add dictionary elements. Dictionary elements are unified text elements. The main purpose is to overcome words with different spellings (or different level of detail in description) but the same meaning. For more detailed information about Dictionaries, see chapter 2.5 “Dictionary”.

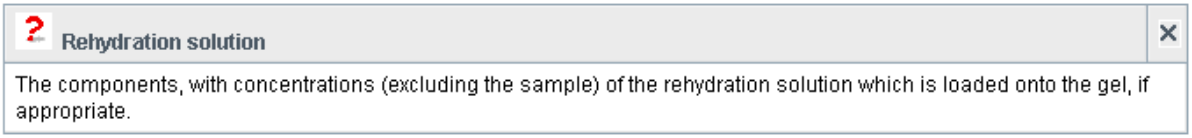
1.2.5 Multiple input Fields and other buttons:



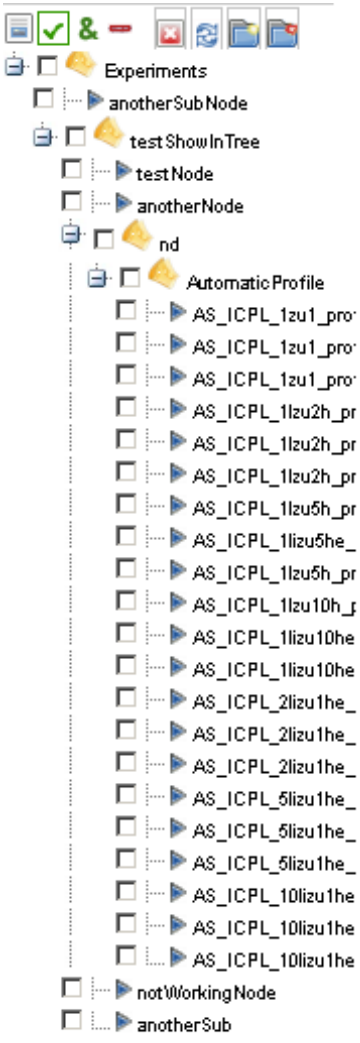
In MASPECTRAS there are very often multiple select or other input fields provided. With the “Add …” you can add additional input fields to your input mask, or with the you can remove them again. On **important** thing is, that when you add an object, or you any other changes, the changes will be stored in the database when you press the Update Button, while when you press the Button the data object will be deleted in the database, immediately.
When you press the Button you retrieve additional information about the selected object.



In this example the solution consists of two components and they are shown below the select field. When such a details field is open and you change the selected selection this field is updated automatically. Such fields can be closed again with the . When the button is next to an image the image is displayed at the bottom of the page.
The provides you a help, so that it is clear what has to be entered at this input field. The information appears at the top of the page.



1.3 The tree menu:



The tree menu has at the top the command line and below it is showing a tree existing of experiments and sub-experiments (see 3.1). Their child nodes can be samples (3.2) and the child nodes of the samples can be massspecexperiments (see 6.1).

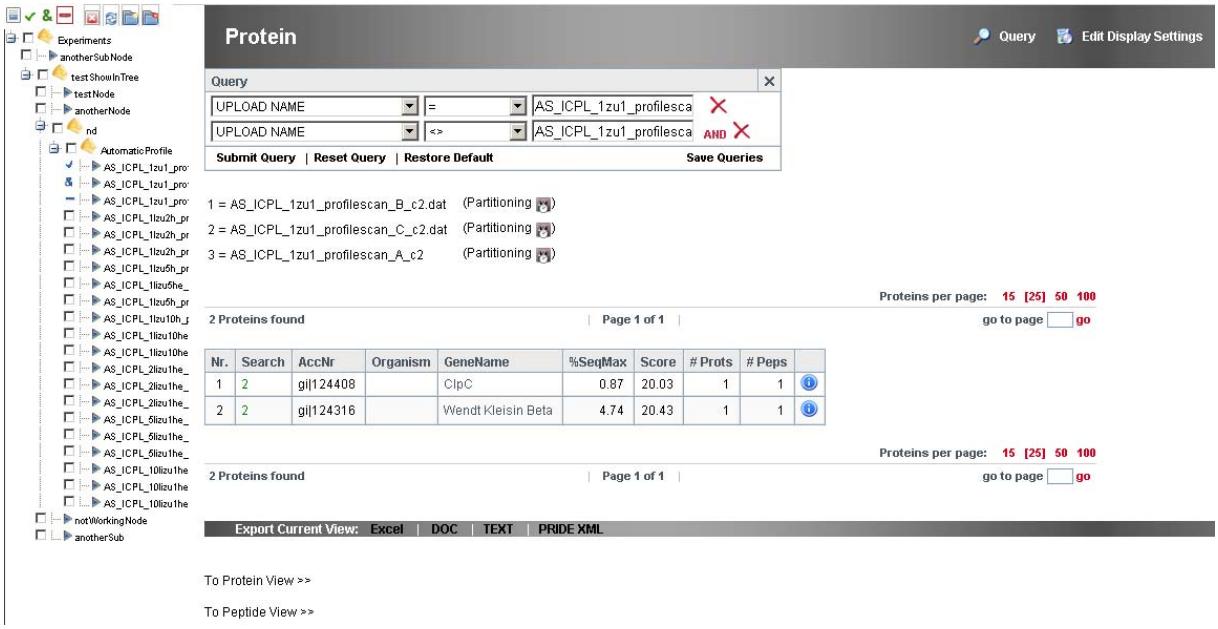
In the top menu you have 3 different selection possibilities:

: shows all of the elements of this search and does not affect any of the other searches.

: shows all of the elements of this search and from the other elements just the ones which are in this search (just the common ones of several searches are shown)

: elements marked with this icon are subtracted from the rest of the selection.

For the selection of elements first the corresponding element has to be selected from the command line at the top, and then you have to click on the checkbox which you want to select.
To accept the selection click on .
In the next picture you can see a selection. You can see that for the “&” and the “-“ selection automatically queries has been generated. For the “&” the operator is “=” and for the “-“ the operator is “<>”.



When a folder is selected the same operator is applied to all massspecexperiments which are in this folder. When a name of a folder or massspecexperiment is clicked for all of the massspecexperiments associated the ☑ operation is applied.

Other menu items:

: unselects all of the selections

: refreshes the tree

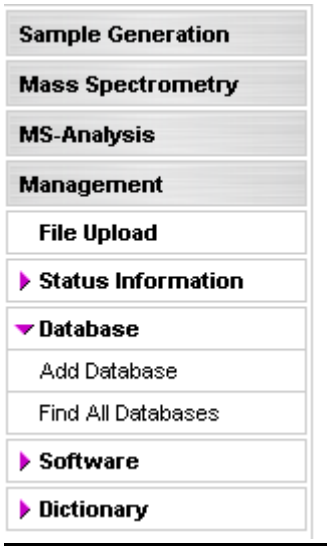
: adds a new subexperiment to an experiment (will not work for samples or msexperiments)

: moves one node to another one; the node to move must be marked with & and the receiving node with ☑

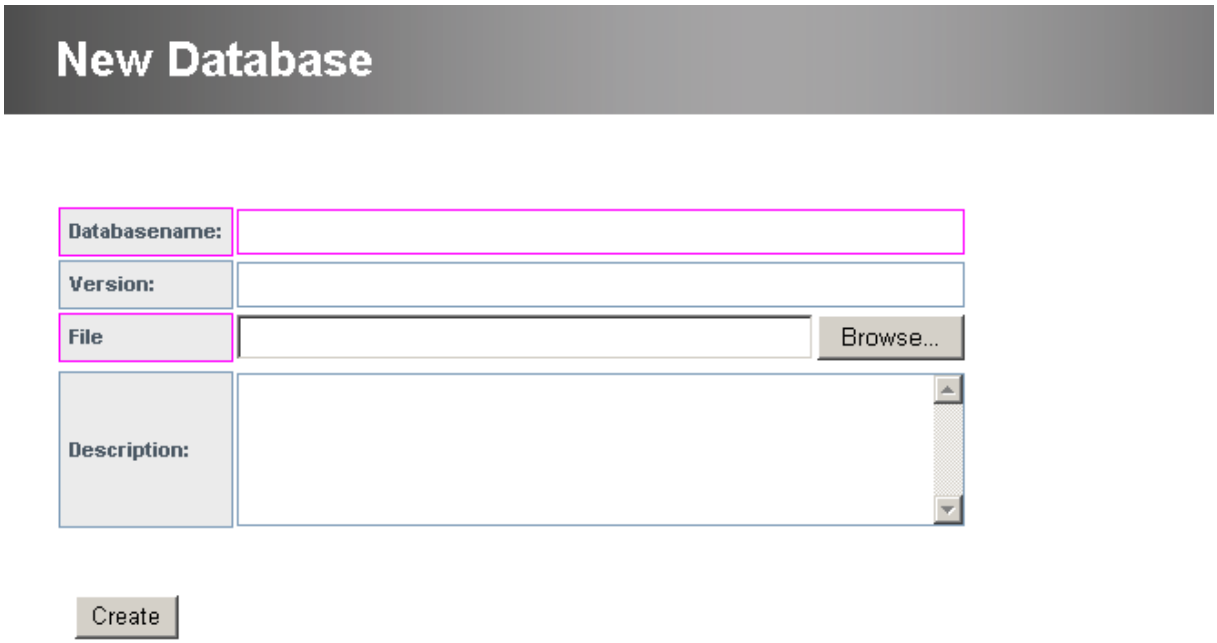
2. Management Section:

2.1 Database:

By clicking Management-> Database in the menu-bar you reach the general Database Section. MASPECTRAS needs the original sequence databases to find out the corresponding protein sequence.



With the “Add Database” you can add a new database. The second possibility to upload a database is the MultipleFiluploadApplet (see 2.2). This applet can upload databases bigger than 2GB.

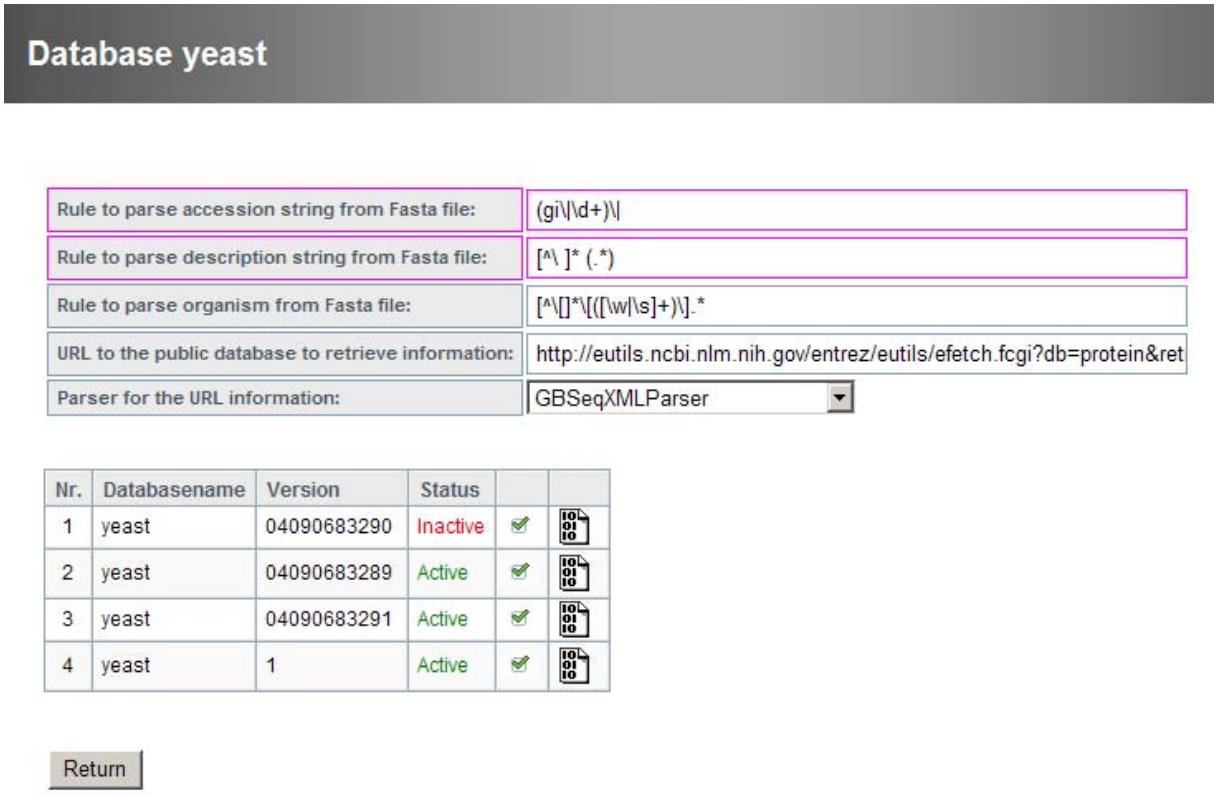


When you select a file, the fields databasename and version are filled out automatically. When you enter no version the version is set to 1 automatically.

With the “Find All Databases” you get an overview of all your databases.

Nr.	Databasename	
1	testdb	
2	nr	
3	yeast	
4	bovine	
5	karl	
6	mouse	
7	MSDB	

When you have created a database or pushed the button you get to the detailed view of your database:



At the top you can define your parsing rules for the accession string, the description string and the organism string. Examples for parsing rules you will find in your installation package at /doc/parsingRules. The meaning for the regexs you can find at http://java.sun.com/j2se/1.5.0/docs/api/java/util/regex/Pattern.html. Accession rule and the description rule are mandatory. In the next line you can specify the URL to the external information fetch page (this URL has to end with ‘id=’ or whatever your external information page requires, so that the program can add the accession numbers it has to look for) and below the parser for the information page. By default parsers are provided for IPI and GenBank

(although the GenBank parser delivers not so much information as the IPI parser since not so much is available directly). If you want to enhance the existing parsers or write your own parser please visit 2.2.1. .
With the green checkbox you can test your parsing rules and you get the output of the first 10 entries at the bottom of this page and the links to the external sources at the top:

```
External data link:http://eutils.ncbi.nlm.nih.gov/entrez/eutils/efetch.fcgi?db=protein&retmode=xml&id=gi|19114688,gi|1723488,gi|7490714,gi|1213267
External html link: http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=protein&id=gi|19114688,gi|1723488,gi|7490714,gi|1213267,
================================================================
Complete Entry:
>gi|19114688|ref|NP_593776.1| hypothetical homeobox domain protein [Schizosaccharomyces pombe]□gi|172
MRSYSNPENGGQINDNINYSEKRPTMLPENLSLSNYDMDSFLGQFPSDNNMQLPHSTYEQHLQGEQQNPTNPNYFPPEFD
ENKVDWKQEKPKPDAPSFADNNSFDNVNSSKLTNPSPVQPNIVKSESEPANSKQNEVVEATSVEKAKENVAHESGTPESG
GSTSAPKSKKQRLTADQLAYLLREFSKDTNPPPAIREKIGRELNIPERSVTIWFQNRRAKSKLISRRQEEERQRILREQR
ELDSLNQKVSQAFAHEVLSTSPTSPYVGGIAANRQYANTLLPKPTRKTGNFYMKSGPMQSSMEPCIAESDIPIRQSLSST
YYNSLSPNAVPVSSQRKYSASSYSAIPNAMSVSNQAFDVESPPSSYATPLTGIRMPQPESDLYSYPREVSPSSGGYRMFG
HSKPSSYKASGPVRPPNMATGHMRTSSEPTSYDSEFYYFSCTLLVIGLWKRLRASPQDLMCFYSPPKKLFAYLIQFQGIQ
YRIEYSFFVIESIHVFRVEEPLLNELSATASSRDKPAPNEYWLQMDIQLSVPPVFHMITSEGQGNCTDFTEGNQASEVLL
HSLMGRATSMFQMLDRVRRASPELGSVIRLQKGLNPHQFLDPQWANQLPRQPDSSVFDHQGRNPPIQGLSHDTSSEYGNK
SQFKRLRSTSTPARQDLAQHLLPPKTNTEGLMHAQSVSPITQAMKSANVLEGSSTRLNSYEPSVSSAYPHHNLALNLDNT
QFGELGTSNISYPLSAPSDVGSLPRASNSPSRPVMHPNTQGINTEIKDMAAQFPNSQTGGLTPNSWSMNTNVSVPFTTQN
REFGGIGSSSISTTMNAPSQQLSQVPFGDVSLATENSVPSYGFEVPSEESVYAQARTNSSVSAGVAPRLFIQTPSIPLAS
SAGQDSNLIEKSSSGGVYASQPGASGYLSHDQSGSPFEDVYSPSAGIDFQKLRGQQFSPDMQ

Rule accession_rule: gi|19114688,gi|1723488,gi|7490714,gi|1213267,
Rule description_rule: hypothetical homeobox domain protein [Schizosaccharomyces pombe],Hypothetical
Rule organism_rule: Schizosaccharomyces pombe,null,null,Schizosaccharomyces pombe,
================================================================
Complete Entry:
>gi|496693|emb|CAA56020.1| B-127 protein [Saccharomyces cerevisiae]
MPFSFLAQPFPPCKISSTHSLGVNSPGRGSHGNLNVFWYKLSISGLIEEDIVVDSPGFVVISLLLWLVEVGDLILVLFPV
AFVPGFATVVPIPLKLENVFLGDIWFVVDVGLDSSDVLSSIVFIPGL

Rule accession_rule: gi|496693
Rule description_rule: B-127 protein [Saccharomyces cerevisiae]
Rule organism_rule: Saccharomyces cerevisiae
================================================================
Complete Entry:
>gi|6323056|ref|NP_013128.1| AICAR transformylase/IMP cyclohydrolase; Ade16p [Saccharomyces cerevisia
MGKYTKTAILSVYDKTGLLDLAKGLVENNVRILASGGTANMVREAGFPVDDVSSITHAPEMLGGRVKTLHPAVHAGILAR
NLEGDEKDLKEQHIDKVDFVVCNLYPFKETVAKIGVTVQEAVEEIDIGGVTLLRAAAKNHSRVTILSDPNDYSIFLQDLS
KDGEISQDLRNRFALKAFEHTADYDAAISDFFRKQYSEGKAQLPLRYGCNPHQRPAQAYITQQEELPFKVLCGTPGYINL
LDALNSWPLVKELSASLNLPAAASFKHVSPAGAAVGLPLSDVERQVYFVNDMEDLSPLACAYARARGADRMSSFGDFIAL
SNIVDVATAKIISKEVSDGVIAPGYEPEALNILSKKKNGKYCILQIDPNYVPGQMESREVFGVTLQQKRNDAIINQSTFK
EIVSKNKALTEQAVIDLTVATLVLKYTQSNSVCYAKNGMVVGLGAGQQSRIHCTRLAGDKTDNWWLRQHPKVLNMKWAKG
IKRADKSNAIDLFVTGQRIEGPEKVDYESKFEEVPEPFTKEERLEWLSKLNNVSLSSDAFFPFPDNVYRAVQSGVKFITA
PSGSVMDKVVFQAADSFDIVYVENPIRLFHH

Rule accession_rule: gi|6323056,gi|1709914,gi|7433574,gi|1480728,gi|2204263,
Rule description_rule: AICAR transformylase/IMP cyclohydrolase; Ade16p [Saccharomyces cerevisiae],Bif
Rule organism_rule: Saccharomyces cerevisiae,null,null,null,Saccharomyces cerevisiae,
================================================================
```

First you get the complete database entry. At “Rule accession_rule:” you get your returned accession strings. If there are multiple ones for one entry they are always separated by “,”. It is mandatory that the accession string that you see here is the same like in your result files because this one is used for the indexing. At “Rule description_rule” you get the description of your protein. At “Rule organism_rule:” you get the result of your organism rule. If there is a “null” within the string, than this rule didn’t return anything (happens sometimes, when there are no organisms declared). The first line (this will be just there URL and the parser is chosen) is the external link how the data is returned for the parser, the second line is the link which will be put on the accession number in the protein overview (see 7.1)

If you are content with your result push the button to index your database.
The database can have the following stati:

Active : The database is active and can be used for file parsing.

Indexing : This database is indexing. (This page is not refreshed automatically at the moment)

Inactive : The database has not been indexed or something at the indexing has gone wrong

It is not mandatory to keep all the versions of your databases. Once a search result file has been parsed into MASPECTRAS it stays conserved and does not need the old database again. The database section should be reserved to an administrator of MASPECTRAS, because when the definition string is changed in a running instance, you have to be aware that there may be pending data uploads which need information with the old settings. Once the data is uploaded into MASPECTRAS there is no need to keep the old database, the whole sequence is stored within MASPECTRAS.

2.2.1 Enhanced or self-written parsers plug-in:

MASPECTRAS features a plug-in mechanism to extend the external DB-information fetch. A self-written parser (in a JAR-file) has just to be put in the $JBOSS_HOME$/server/maspectras/sequenceParsers folder to be detected at the next restart of MASPECTRAS. The current implementation provides parsers for IPI and GenBank (here not all of the information available) and therefore there are the IPIParser.jar and the GBSeqXMLParser.jar. The IPIParser can be regarded as show-case of how to implement a self-written parser and therefore it is provided including the source code (IPIParser.java) (in the GBSeqXMLParser the source code is provided as well but rather for self-written enhancements).
The JAR-file must contain the parser class (in binary format), all classes which are required for your parser and the following entry in the META-INF/MANIFEST.MF:
`Parser-Class: at.tugraz.genome.maspectras.parser.sequenceInformation.IPIParser`.
This is the fully qualified name of your parser class, so that MASPECTRAS knows which Class to instantiate for the parsing. The self-written parsing class has to implement at.tugraz.genome.maspectras.parser.interfaces.SequenceInformationParser interface to be accepted, which can be found in the maspectras.jar. The parsers have to return a Vector<SequenceResultVO>, which contain the identifier of the external plus a description (not mandatory can be ‘null’ as well). The current implementation allows the fetch of information about:

- Entrez Gene
- Ensembl;
- Ensembl Havanna
- Gen3D
- GO
- NCBI Taxonomy
- InterPro
- PathoSign
- Pfam
- PROSITE
- RefSeq
- SMART
- UniProt-SwissProt
- UniProt-TrEMBL
- Vega
- PubMed
- iHOP
- KEGG

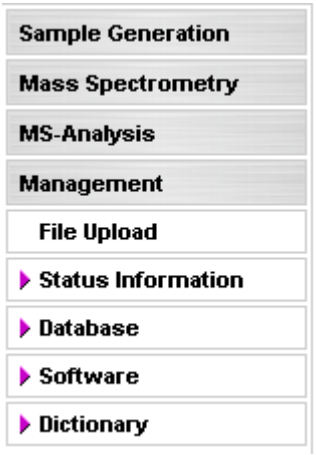
- Gene symbol
- Synonym

If your required web-page does not deliver some of the required information, just return ‘new Vector<SequenceResultVO>;’ or null. If further information entries are desired please contact the developers at maspectras@genome.tugraz.at . It is **important** that the parser can handle the fetch of several entries at once identified by an accession number, since MASPECTRAS fetches always a bundle of 50 entries, since one by one would take too much time. For a detailed documentation of a parser please take a look at the IPIParser.java.
The external fetch strategy itself works like the following:

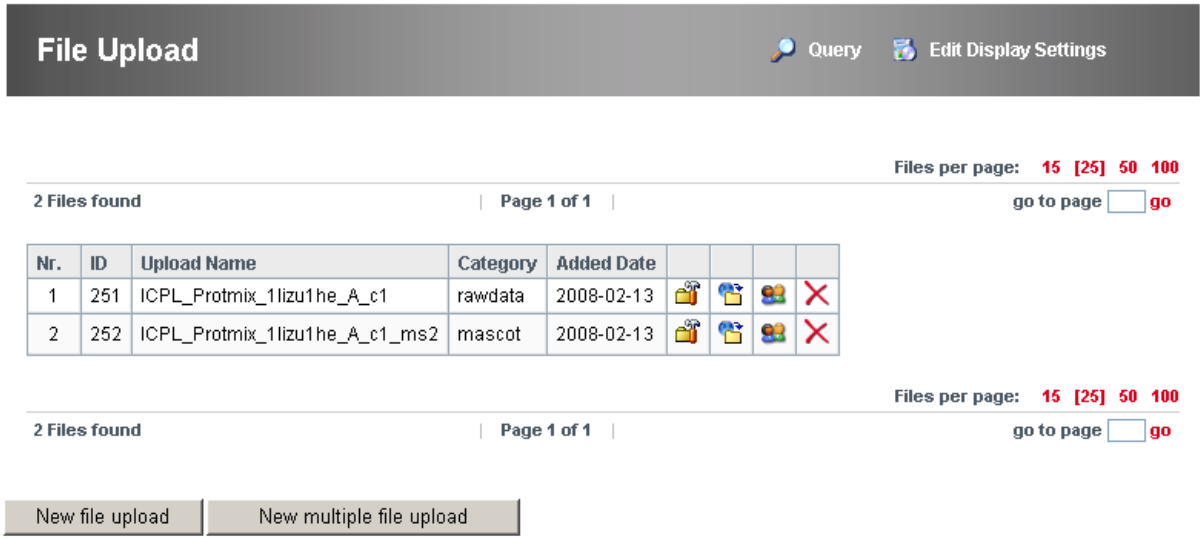
- HTTP connection to the external data resource you specified in 2.1
- Parsing with a standard parser or your self-written parser
- If a Uniprot-Swissprot entry exists the system makes a connection to Uniprot as well to receive further information (e.g. Pubmed identifiers)
- Parsing of Uniprot files by existing MASPECTRAS parsers

Therefore it is possible that further information is fetched via Uniprot if your parser returns Uniprot identifiers, which is not in the original data file.

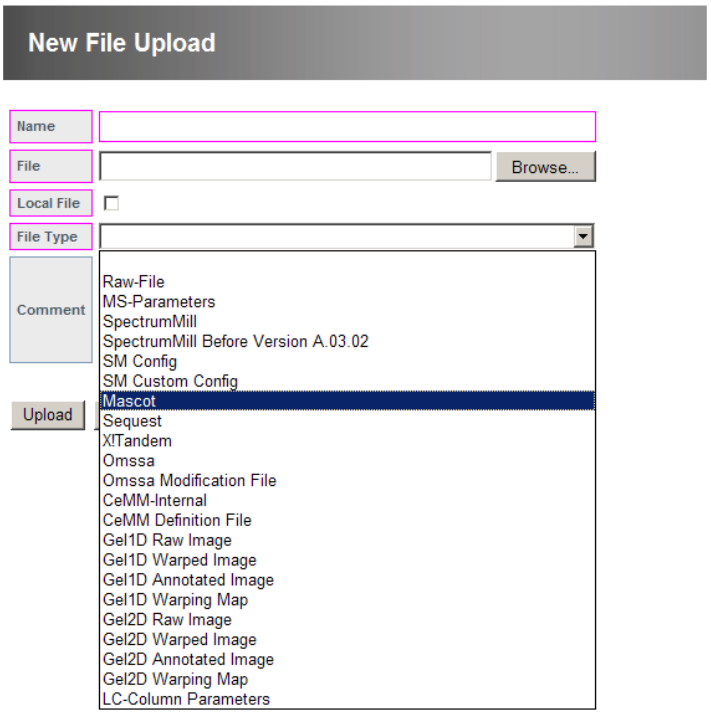
2.2 File Upload:



By clicking Management->FileUpload in the menu-bar you reach the general Upload Section, where all your already uploaded files are listed:



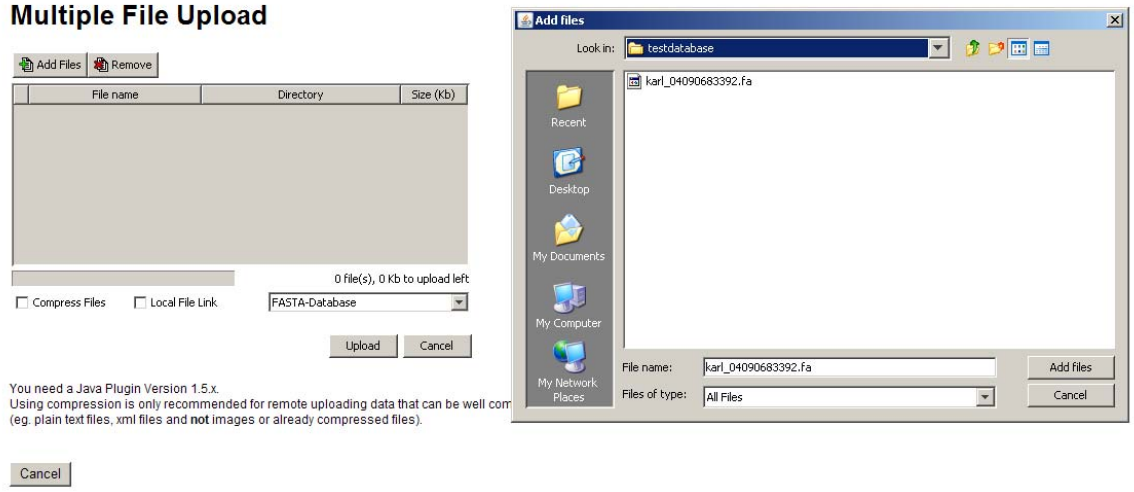
With “New file upload” you come to the upload page:



The important thing is that you have to add your file to the corresponding category.
The Sequest-Files and SpectrumMill-Files must be uploaded in a *.zip directory. Spectrum Mill is differentiated in “Spectrum Mill” (new version) and “Spectrum Mill Before Version A.03.02” (old version). For the new version a SM Config File (your smconfig.xml file) is necessary. The SM Custom Config (your smconfig.custom.xml) is not mandatory, but needed when you searched with modifications and elements which you created by yourself. For OMSSA searches the Omssa Modification File (mods.xml) is needed. As “Raw-File” mzXML, mzData and XCalibur Version 1.3 RAW are accepted. After the raw file is uploaded an automatic conversion in a more convenient format for the calculation is started (you can see the progress in the upload status page see 2.3). automatically. If this is not desired, there is the option to work directly on one of the formats, but then the real 3D view does not work, just the quasi-3D view (see 7.7). If you want to turn off this feature contact the administrator

of your system. In the file $DATAROOTDIRECTORY$/analyses/partitioning/cluster.properties there is an attribute translateChromatograms=true. Set this attribute to translateChromatograms=false. The “Local File” check box is just applicable if the file is on the same system (or mounted on the system) where MASPECTRAS is located. Here no upload is made just a link to the file is stored. Through this file duplication can be avoided and disk space saved.

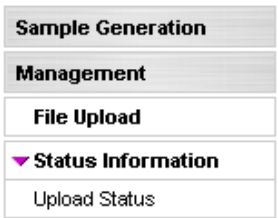
When you click “New multiple file upload” you come to the multiple file upload applet:



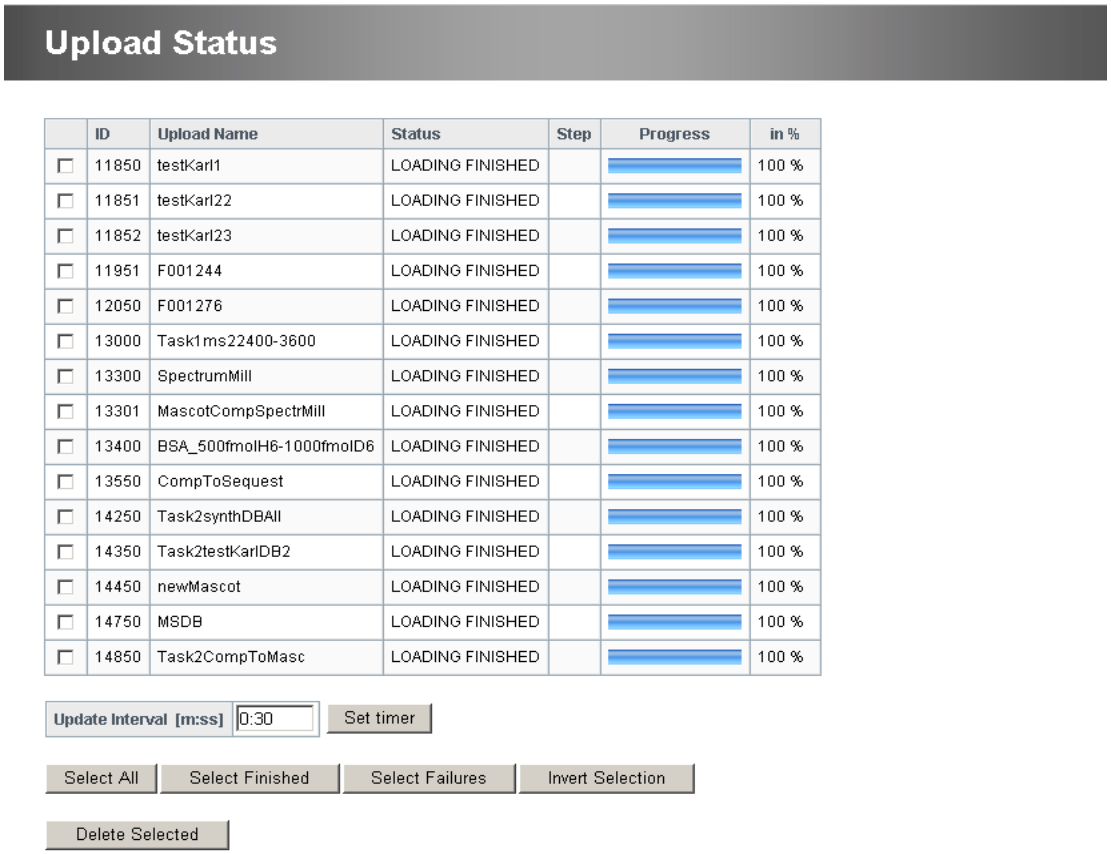
With the “Add Files” a new window opens where you can choose your files and they are displayed in the list below. In the list you can select the files and with “Remove” you can remove them from the list. The “Compress File” option can be used to reduce the transfer over the network but it takes some time to compress and decompress the file again. This option should not be used for already zipped files. Then there is a select box with the categories. There are the same ones like in the normal “New file upload”, plus the category “FASTA-Database”. With this option you can upload databases bigger than 2GB into the system. The database is not displayed in the normal “FileUpload” list but moves directly to the databases (see 2.1). The “Upload” button starts the upload of the selected files. When the upload was successfully finished a green check icon appears in front of the name. When there was an error a red “cross-out” icon appears.

2.3 Upload Status:

By clicking Management->Status Information->Upload Status in the menu-bar you reach the general Upload Status Section.

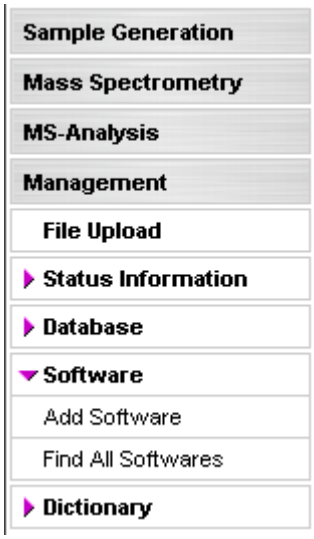


This page gives information about the progress of tasks, which are processed asynchronously because of their time consume.



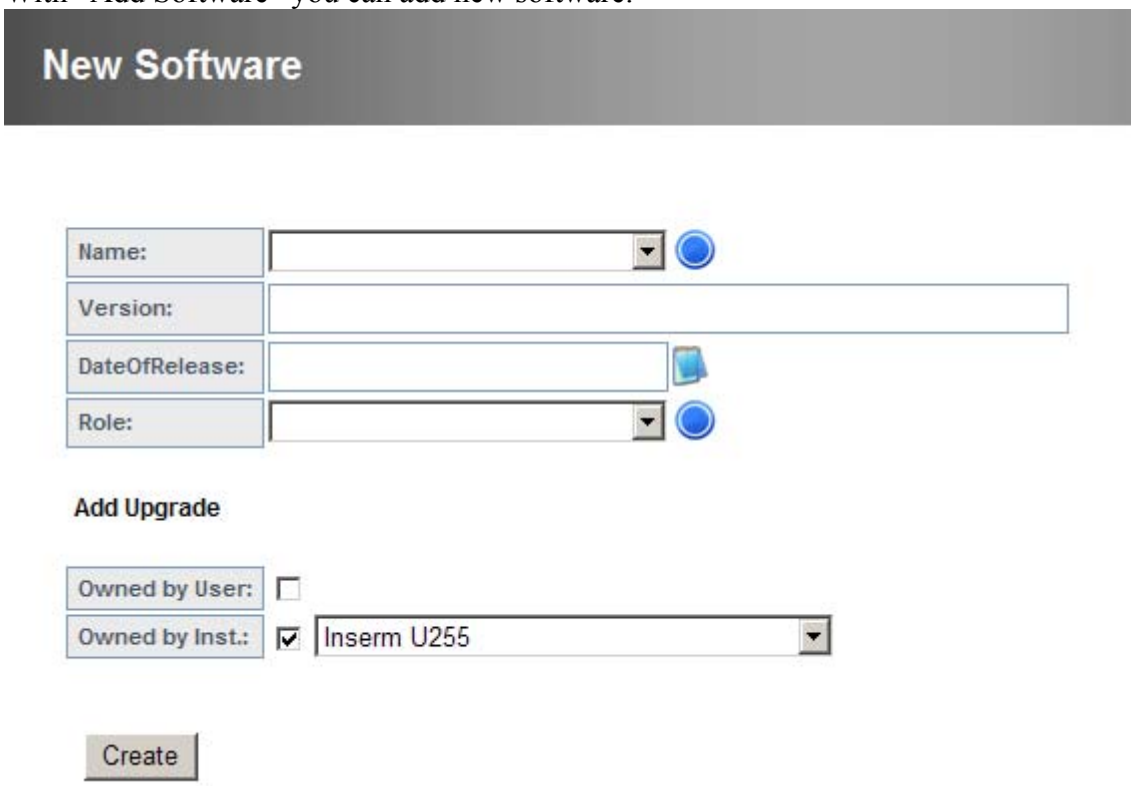
2.4 Software:

By clicking Management->Software in the menu-bar you reach the general Software Section.



The general software section is used to document all the software used in MASPECTRAS. Here you can get an overview about the software and edit them. When the software is needed in a select box in another table you can add new software from there directly (e.g. see chapter 5.5 “Controlsoftware”).

With “Add Software” you can add new software.

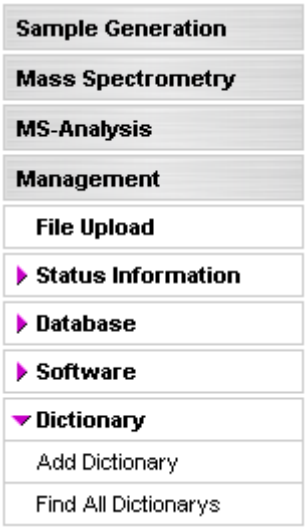


With the link “Add Upgrade” you can enter software upgrades.

With “Find All Softwares” you get an overview of all your general software.

2.5 Dictionary:

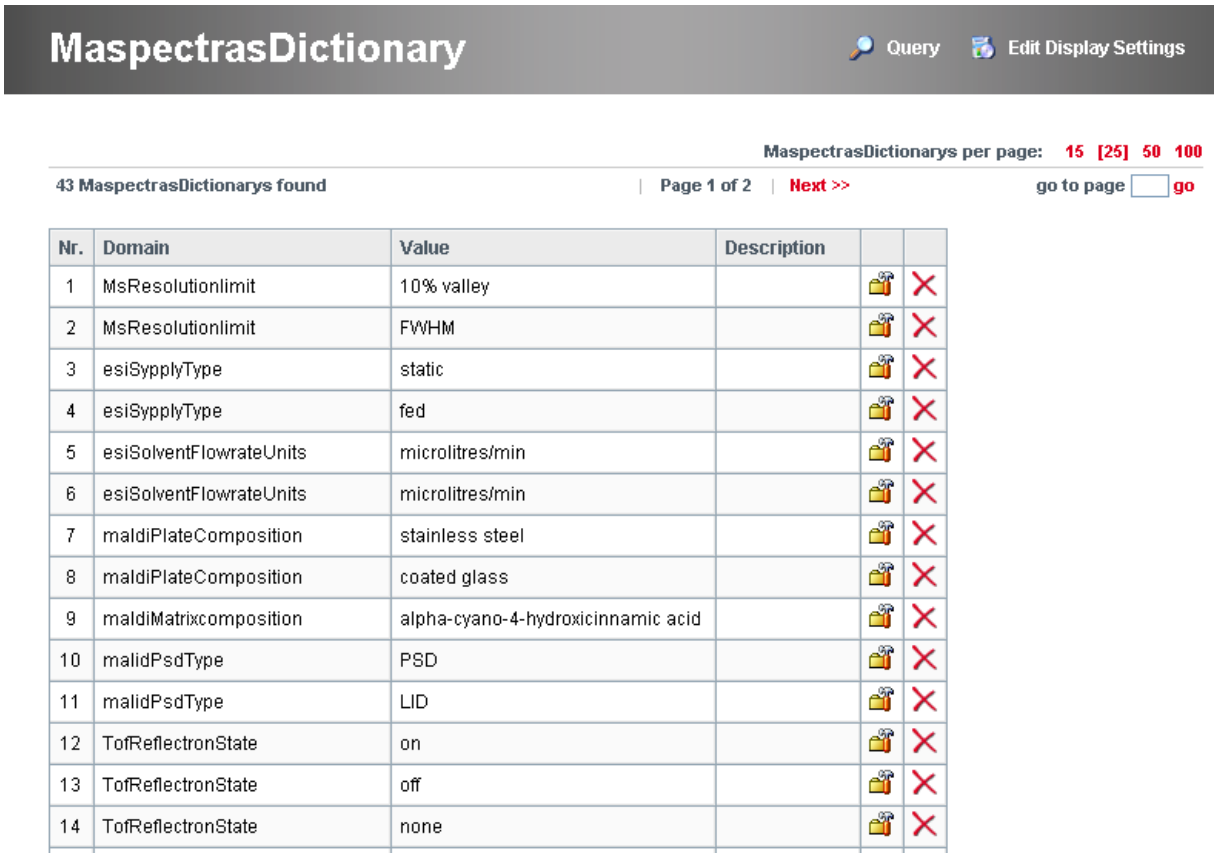
By clicking Management->Dictionary in the menu-bar you reach the general dictionary section.



The dictionary section stores commonly used values for certain input fields. Here are you can add, edit and change dictionary values from all domains, while when you are in another table you can only select an existing dictionary field and add values for this certain domain.

With the “Add Dictionary” you can add a new dictionary entry.

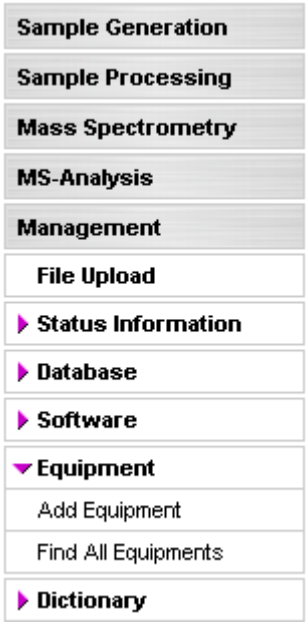
By clicking the “Find All Dictionarys” you will get an overview of all your dictionaries.



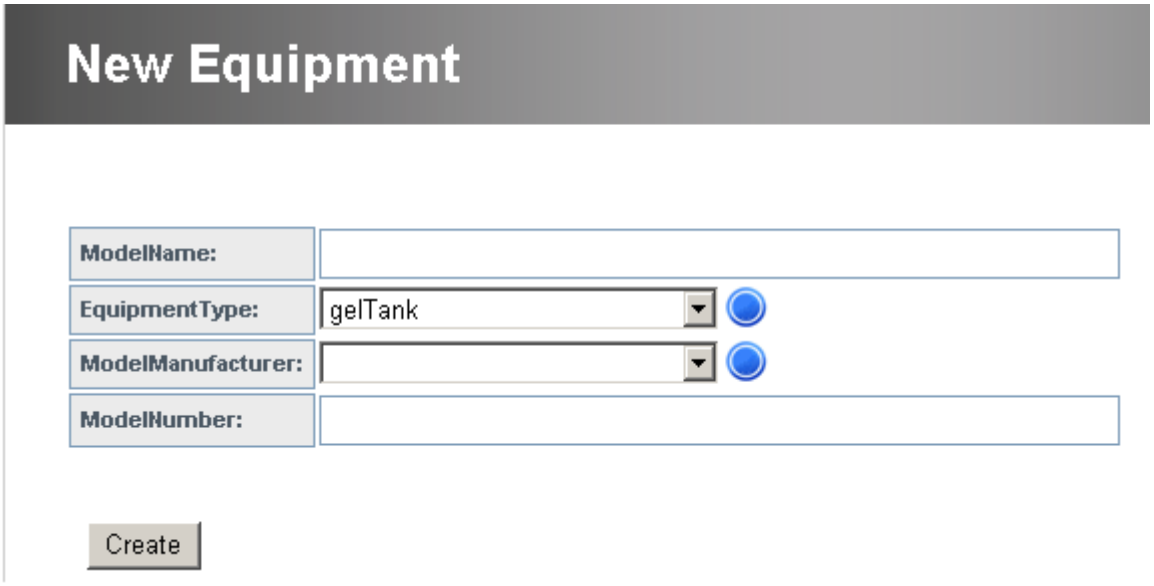
Nr.	Domain	Value	Description		
1	MsResolutionlimit	10% valley			
2	MsResolutionlimit	FWHM			
3	esiSypplyType	static			
4	esiSypplyType	fed			
5	esiSolventFlowrateUnits	microlitres/min			
6	esiSolventFlowrateUnits	microlitres/min			
7	maldiPlateComposition	stainless steel			
8	maldiPlateComposition	coated glass			
9	maldiMatrixcomposition	alpha-cyano-4-hydroxicinnamic acid			
10	malidPsdType	PSD			
11	malidPsdType	LID			
12	TofReflectronState	on			
13	TofReflectronState	off			
14	TofReflectronState	none			

2.6 Equipment:

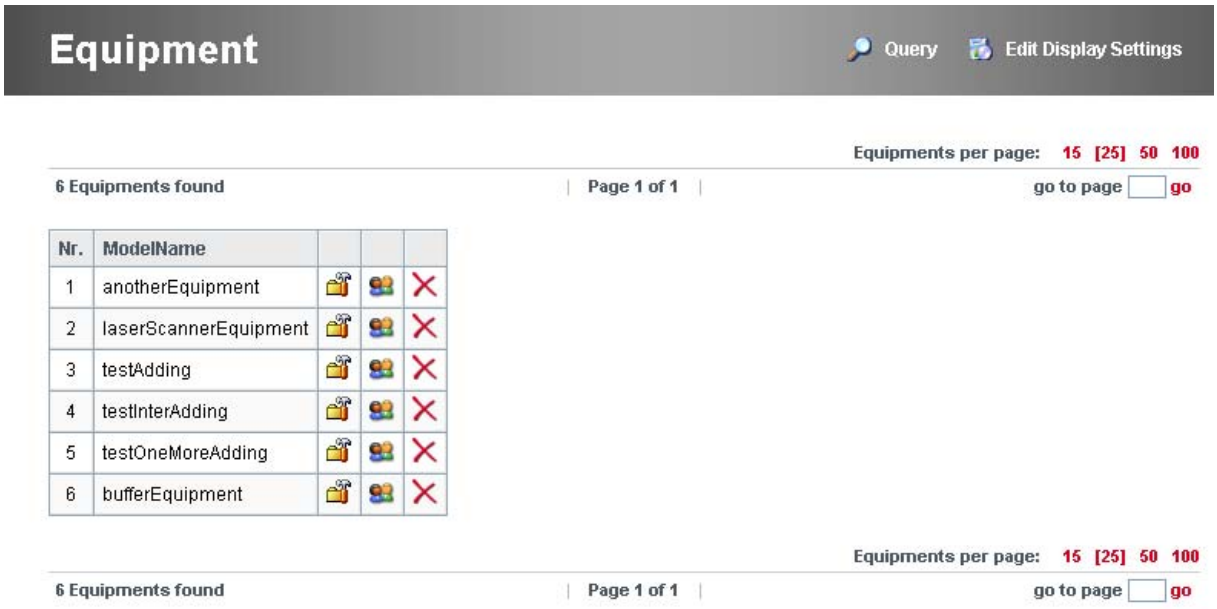
By clicking Management->Equipment in the menu-bar you reach the general equipment section.



The equipment section stores all kinds of equipment needed (for 1D Gels, 2D Gels, …). With the “Add Equipment” you can add a new equipment entry.



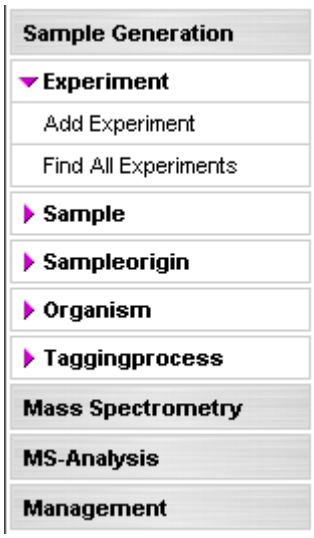
Because of the fact that the equipment section is general, it is necessary to enter the type of the equipment. Then it is easier to find the wanted one.
By clicking the “Find All Equipments” you will get an overview of all your dictionaries.



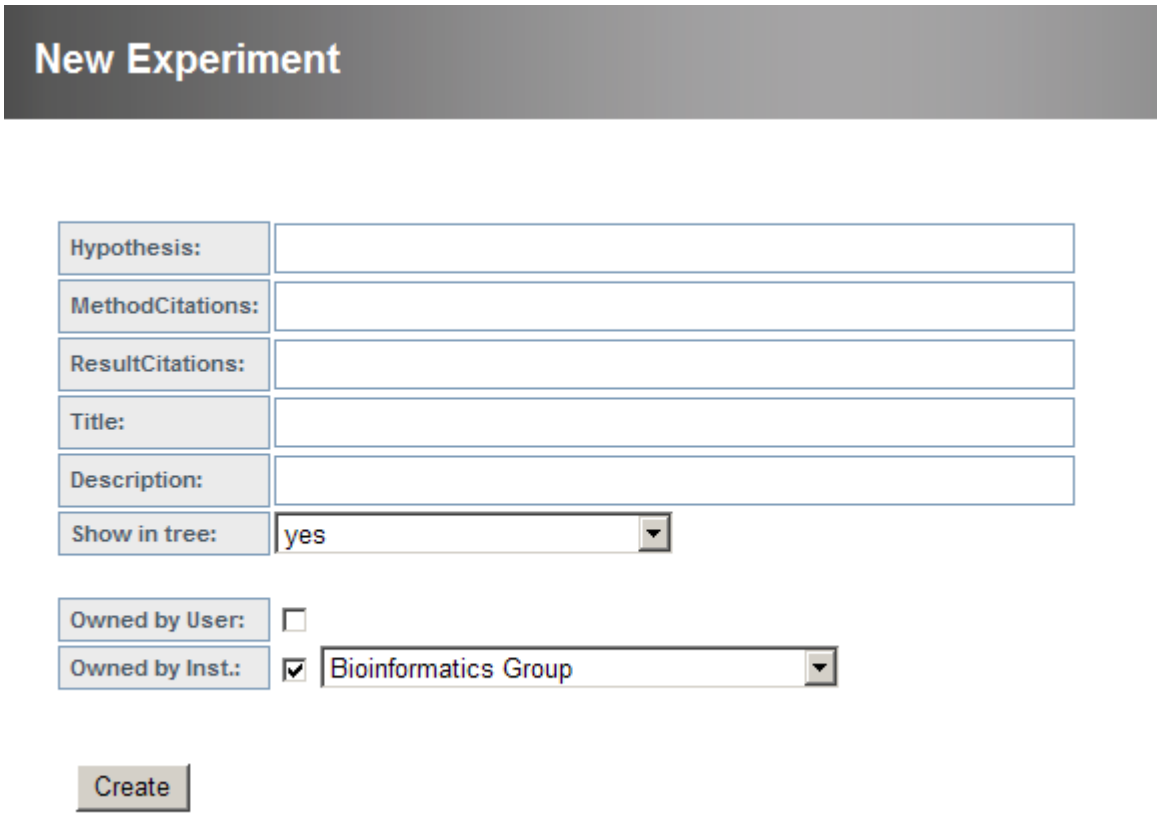
3. Sample Description:

3.1 Experiment:

By clicking Sample Generation->Experiment you reach the experiment section.

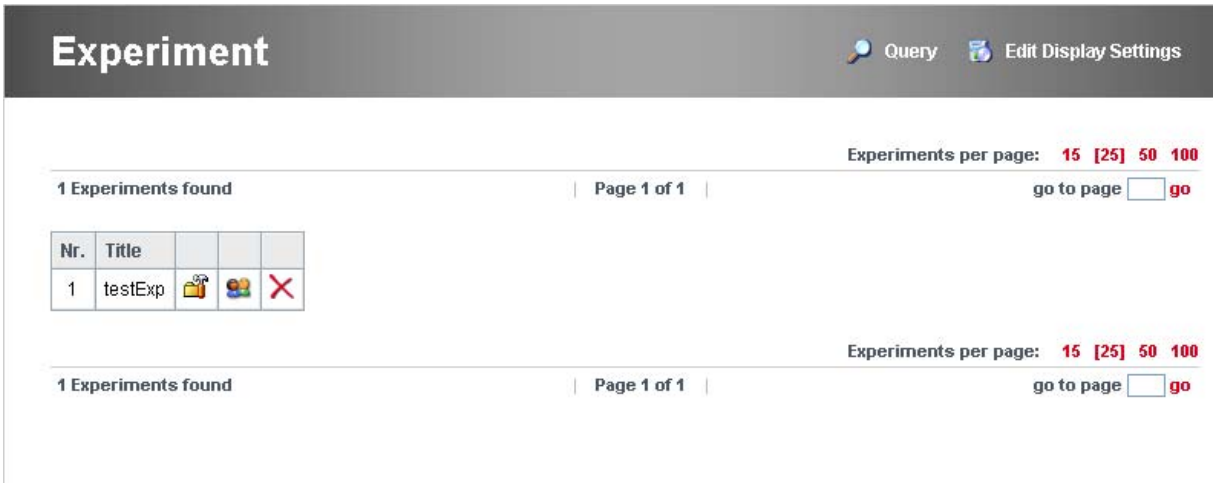


With the “Add Experiment” you can add new experiments.



With the “Show in tree” option you can specify if this experiment should be shown in the tree.

With the “Find All Experiments” you get an overview of all your experiments.

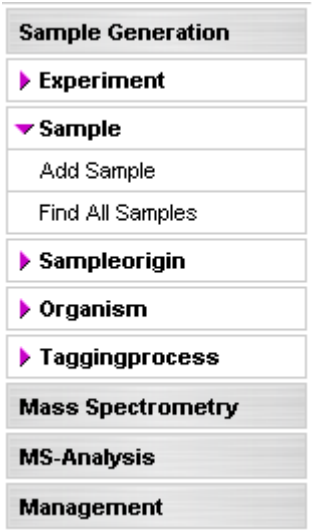


3.2 Sample:

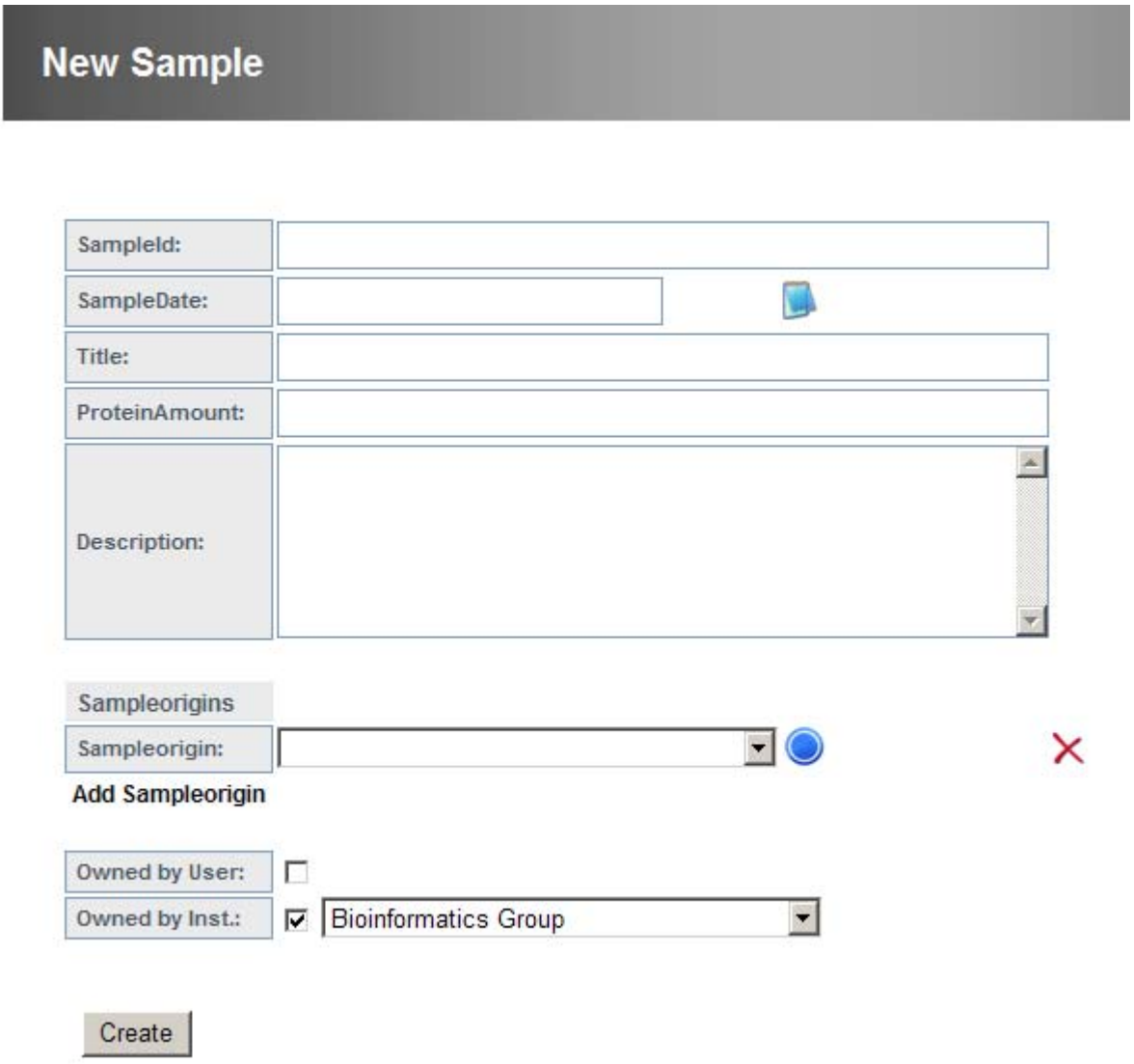
There are 2 ways to generate your sample:

3.2.1 Sample directly:

Here it works in the same way like in the experiment.
By clicking Sample Generation->Sample you reach the sample section.

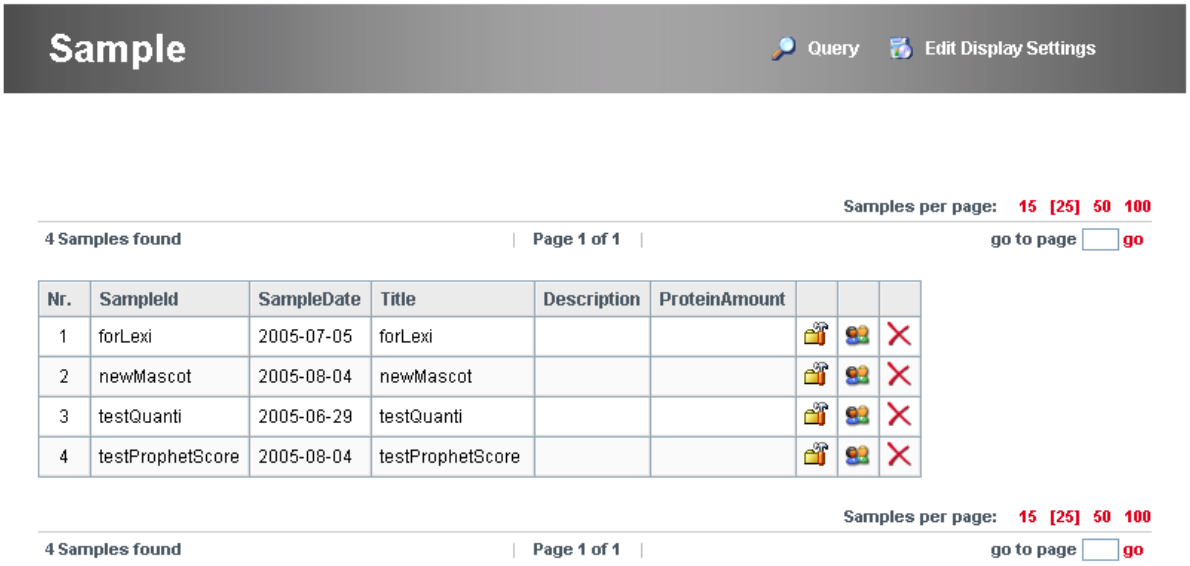


With the “Add Sample” you can add a new sample:



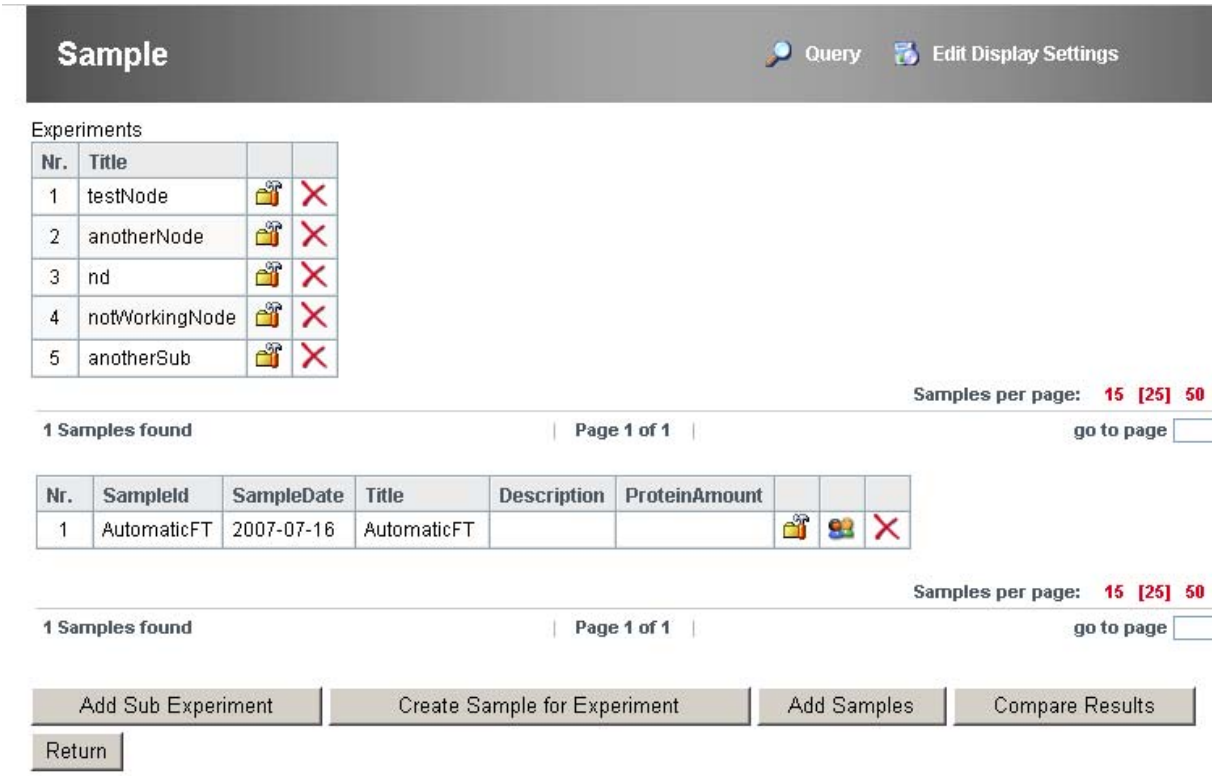
With the link on “Add Sampleorigin” you can add additional origins to the sample. If your desired sample origin is not in the list you can add it directly with the blue button on the right side of the select field. Read more about sample origins in chapter 3.3 “Sampleorgin”.

With a click on the button “Find All Samples” you get an overview of all your samples:



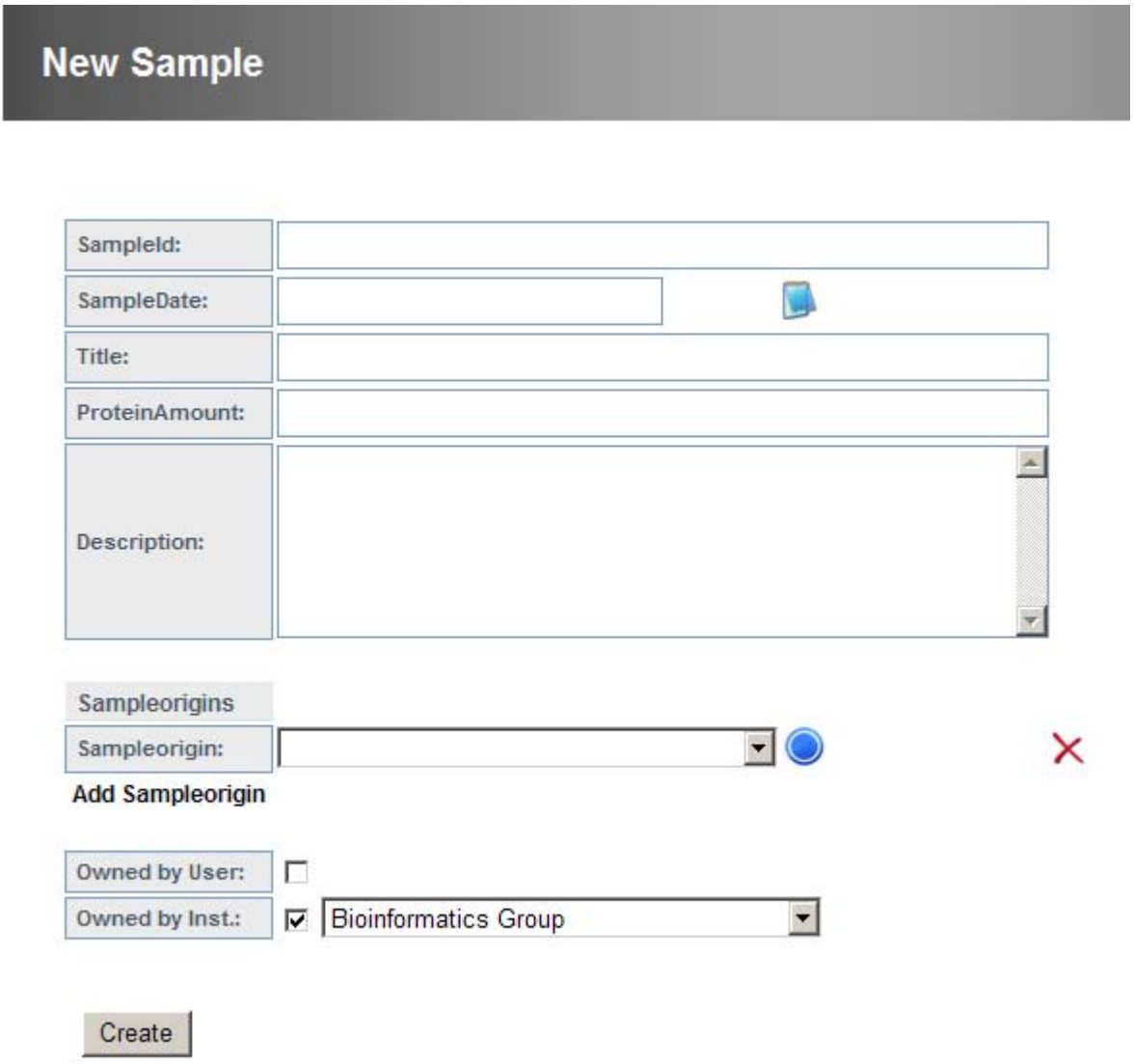
3.2.2 Sample over experiment:

When you click on the title of your experiment where you are interested in then you get an overview of all your samples which has been added to this experiment.

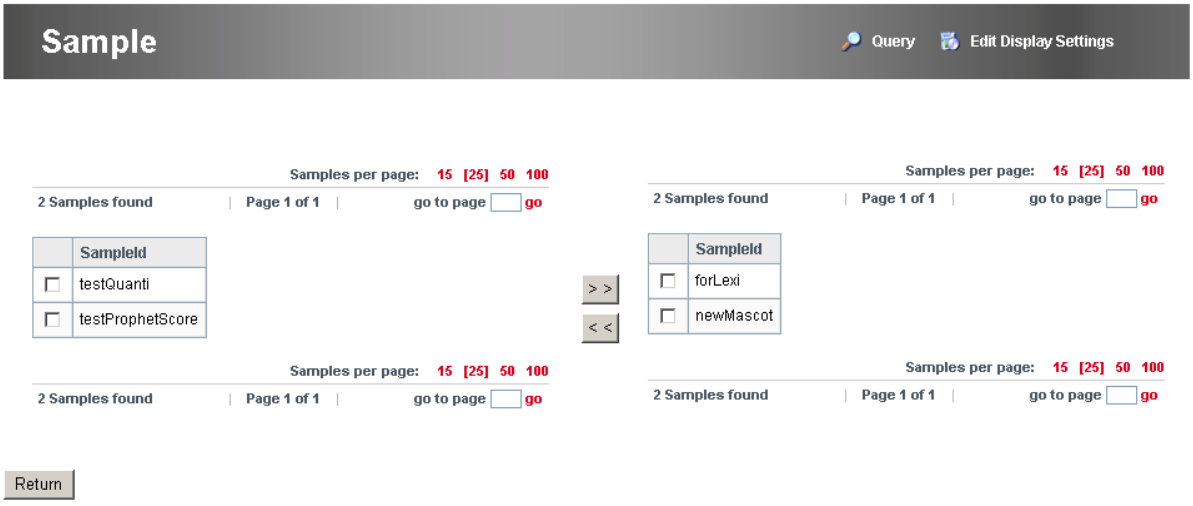


With the “Add Sub Experiment” you can create a sub-experiment.

When you push the “Create Sample for Experiment” button you can generate a new sample and it will be added directly to the experiment.



When you use the “Add Samples” button you can add or remove existing samples to or from your experiment.

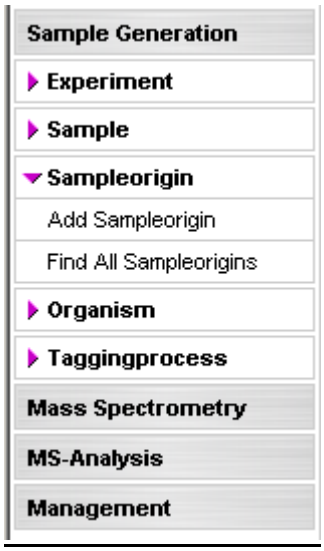


On the left side the addable samples are listed and on the right side the already added samples are listed. The left side is completely queryable. When you want to add samples you simply check the desired checkboxes of the samples on the left side and push the “>>” button. When

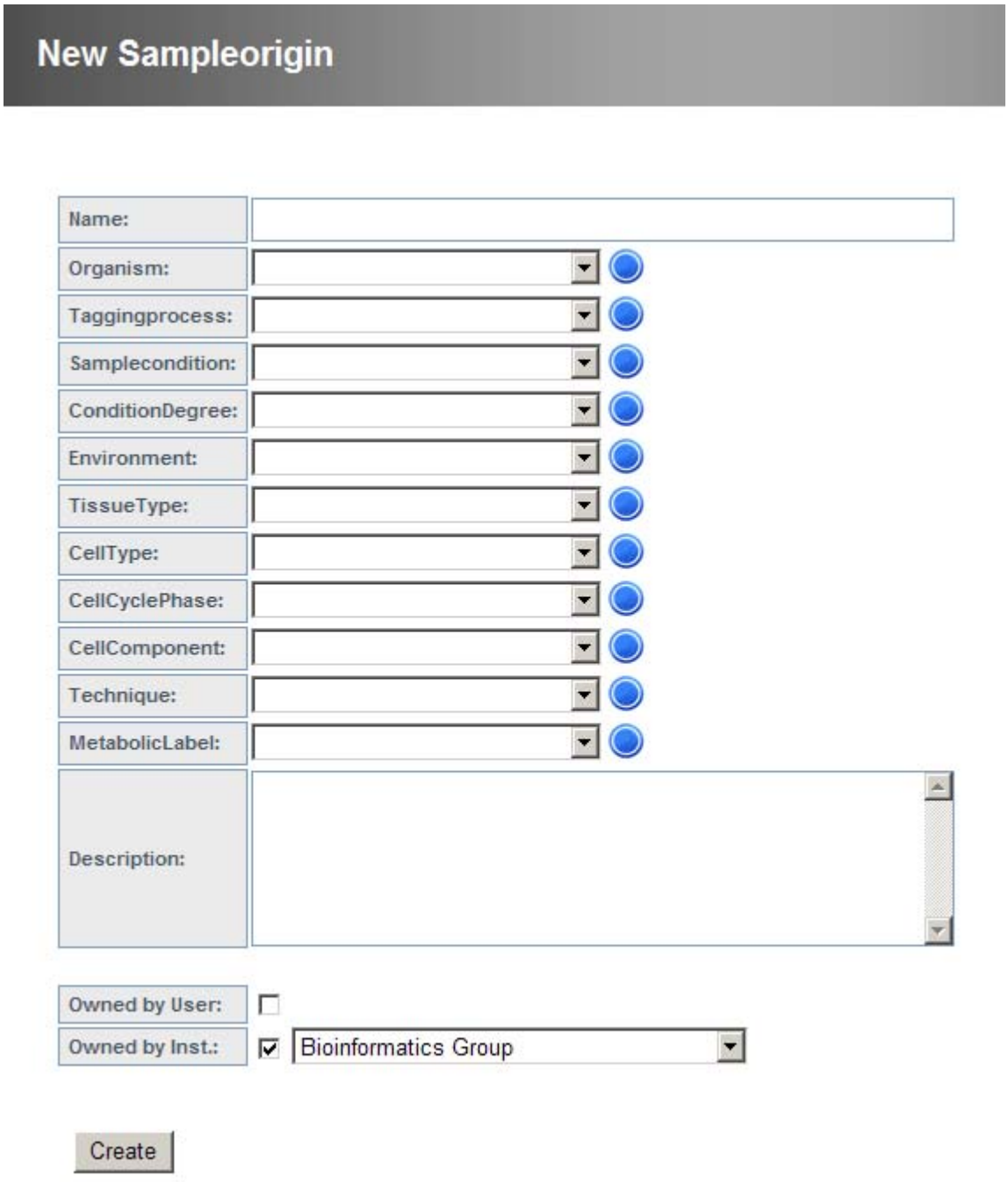
you want to remove samples you simply check the desired checkboxes of the samples on the right side and push the "<<" button.
The meaning of the "Compare Results" button will be explained in the Analysis section (4).

3.3 Sampleorigin:

By clicking Sample Generation->Sampleorgin you reach the sample origin section.



With the "Add Samplorigin" you can add new sample origins.

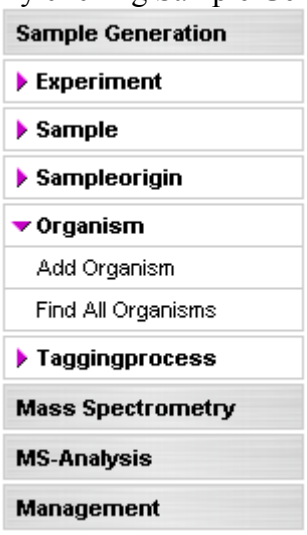


If your desired organism or tagging process is not in the list you can add it directly with the blue button on the right side of the select field. Read more about organisms in chapter 3.4 “Organism” and about tagging processes in chapter 3.5 “Taggingprocess”.

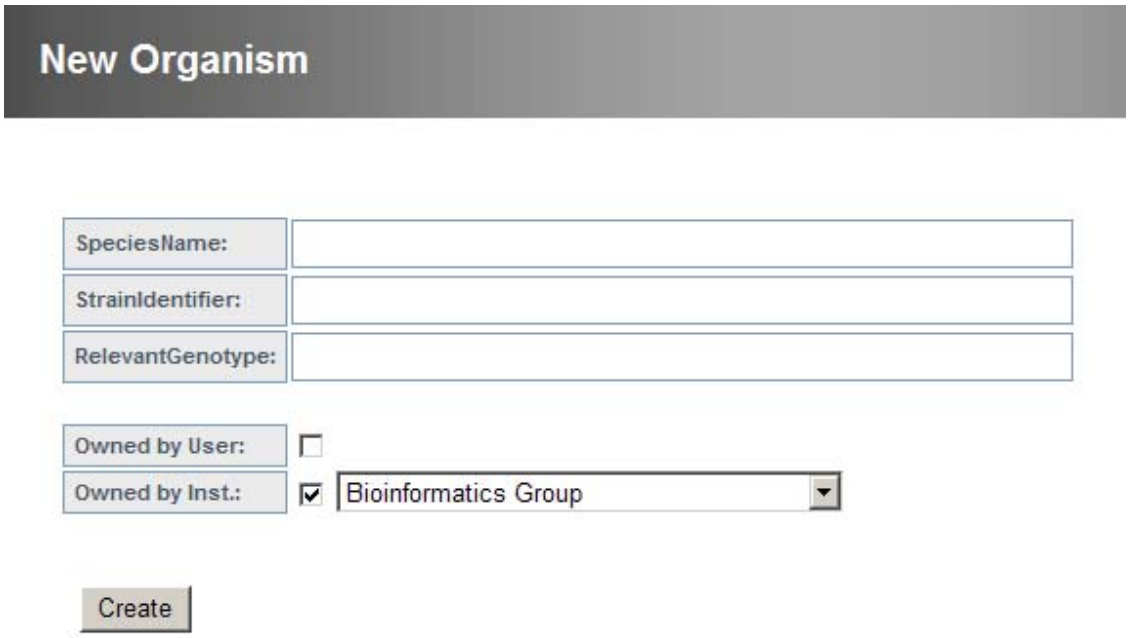
With a click on the button “Find All Sampleorigins” you get an overview of all your sample origins:

3.4 Organism:

By clicking Sample Generation->Organism you reach organism section.



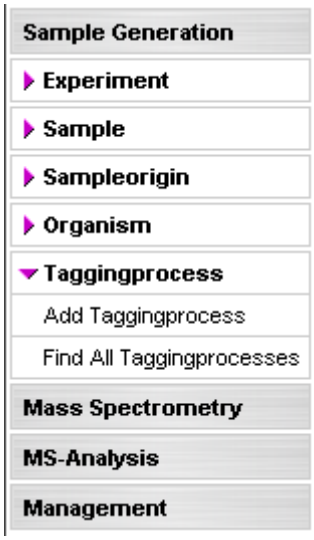
With the “Add Organism” you can add new organisms.



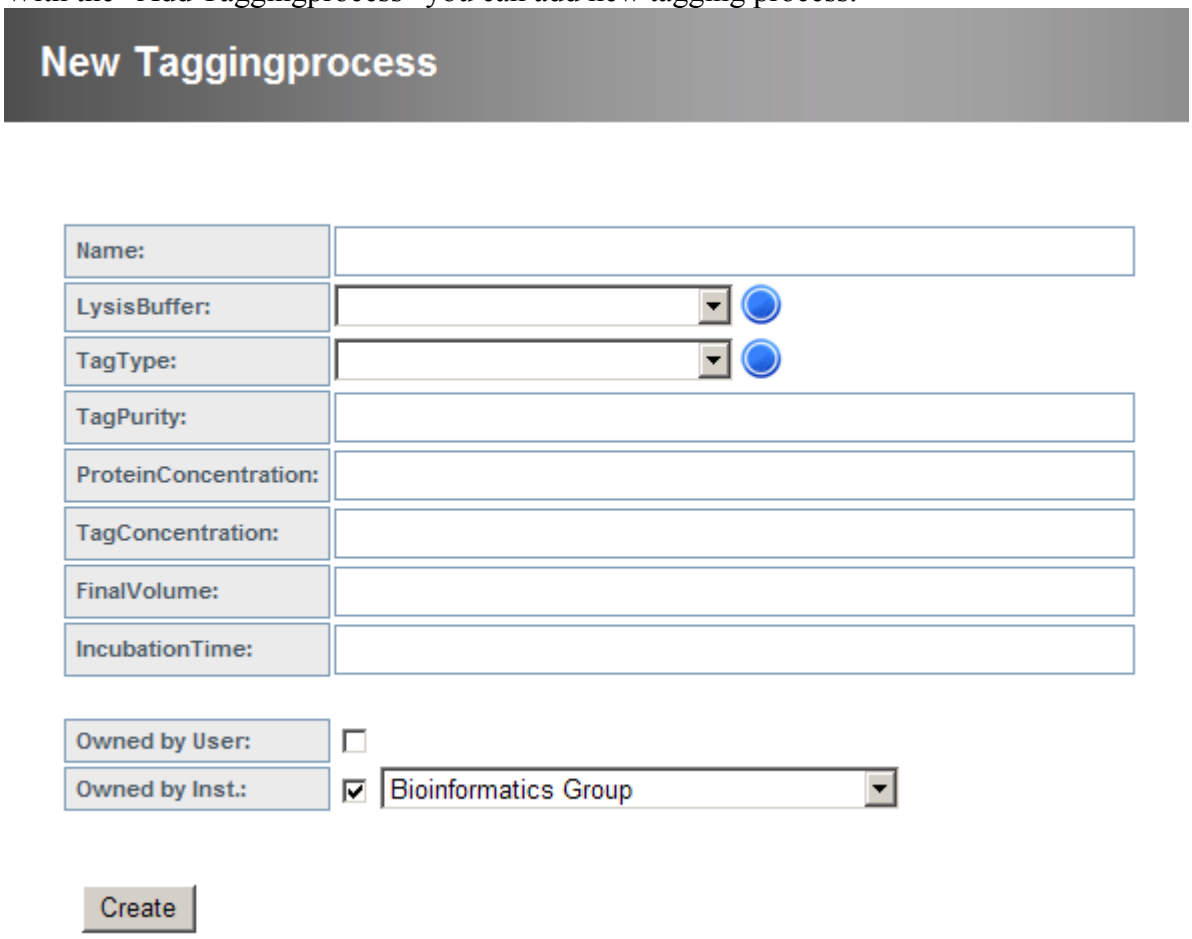
With a click on the button “Find All Organisms” you get an overview of all your organisms:

3.5 Taggingprocess:

By clicking Sample Generation->Taggingprocess you reach tagging process section.



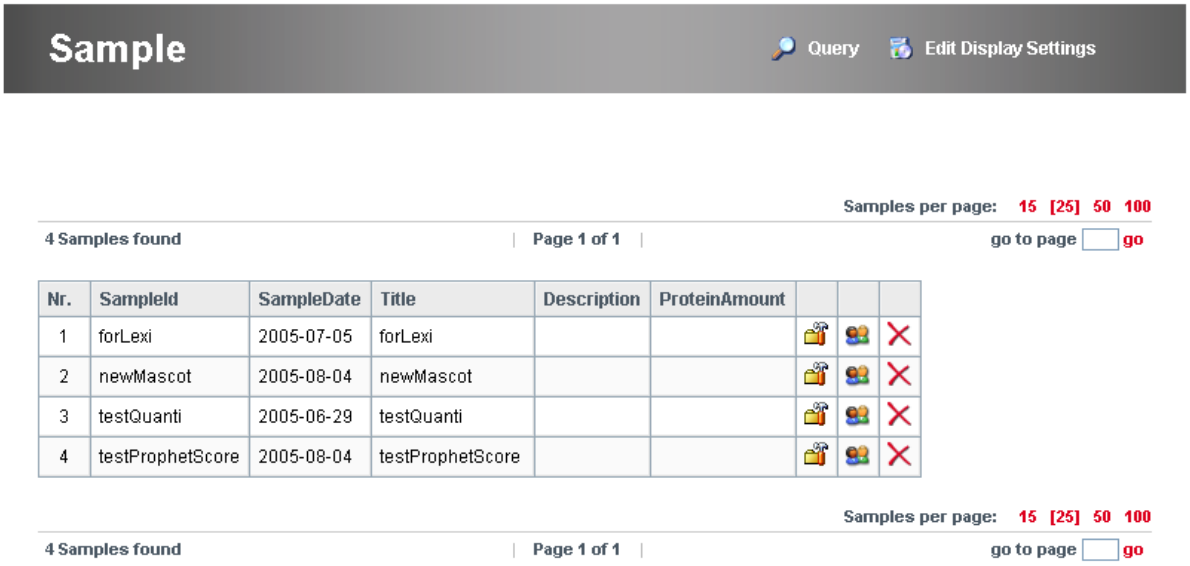
With the “Add Taggingprocess” you can add new tagging process.



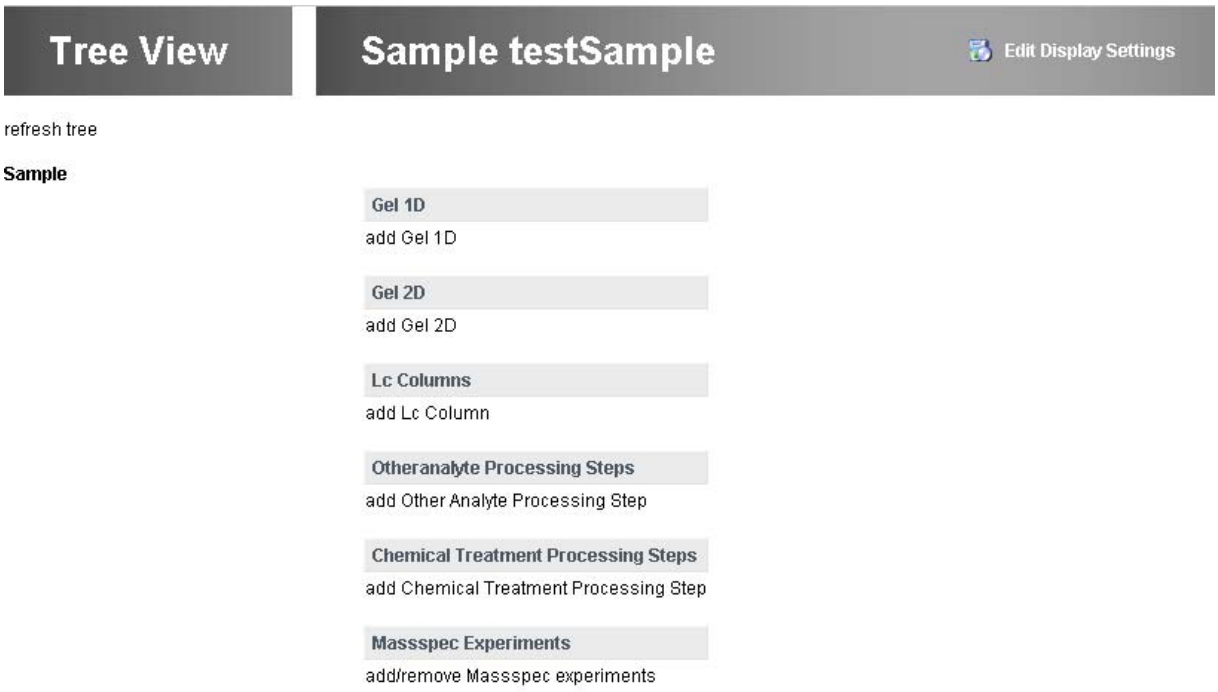
With a click on the button “Find All Taggingprocesses“ you get an overview of all your tagging processes:

4. Sample Preprocessing:

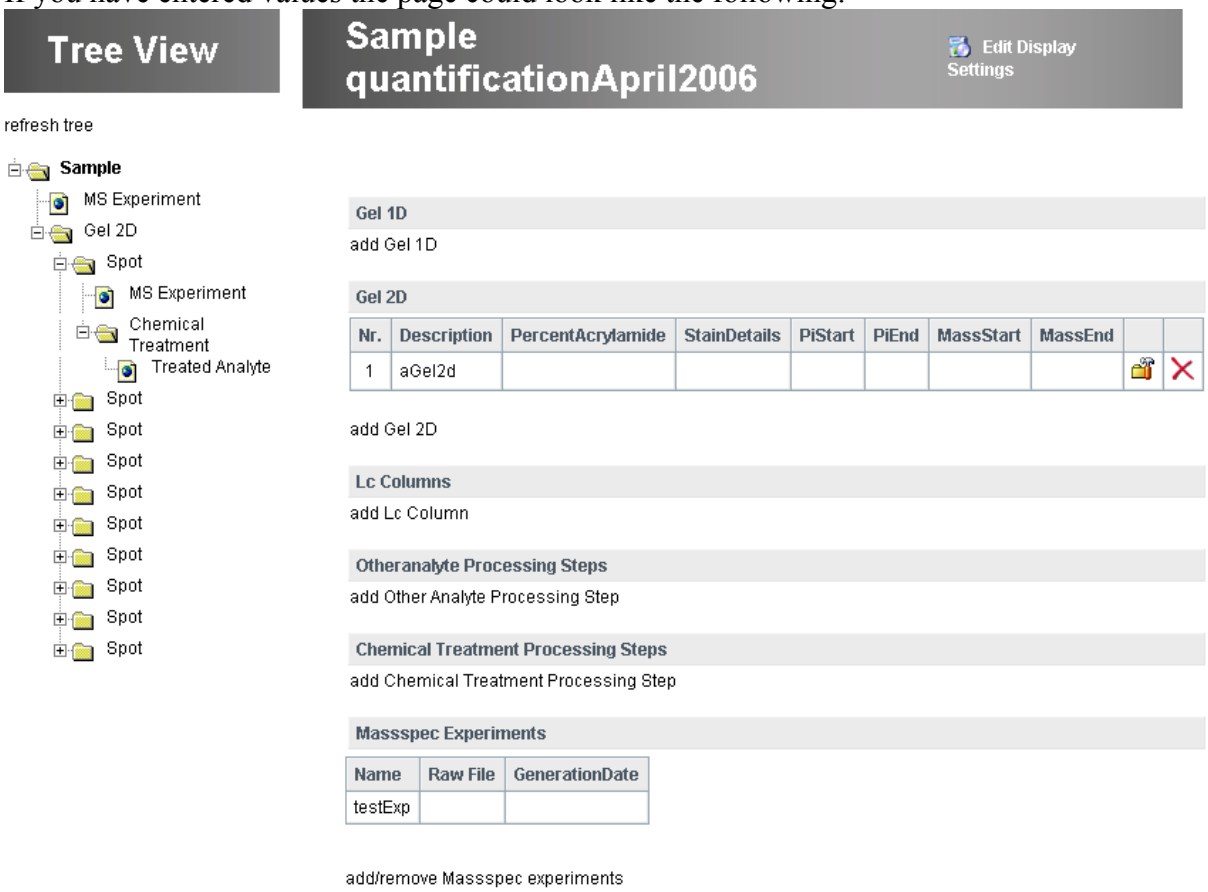
Here, information about the preparation steps of a sample can be entered.
First, you have to click on “Sample Generation->Sample->Find All Samples” and you get an overview of all your samples:



When you want to get more information on a sample, you click on the name for “sampleId” in the corresponding column to you reach the sample processing part. When you have a sample with no entries you will get the following page:

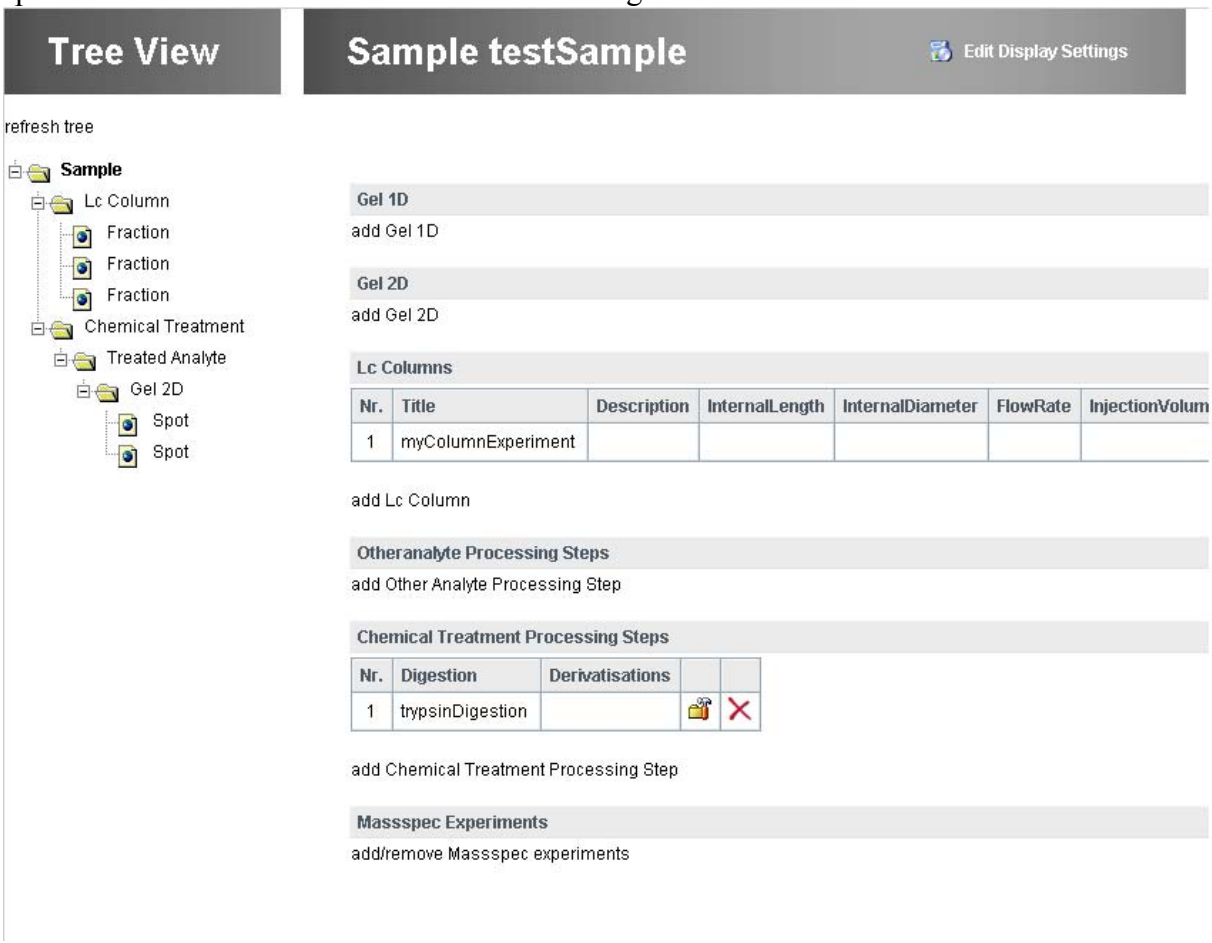


On the left side there should be a tree but now only the root element is there (the sample). If you have entered values the page could look like the following:

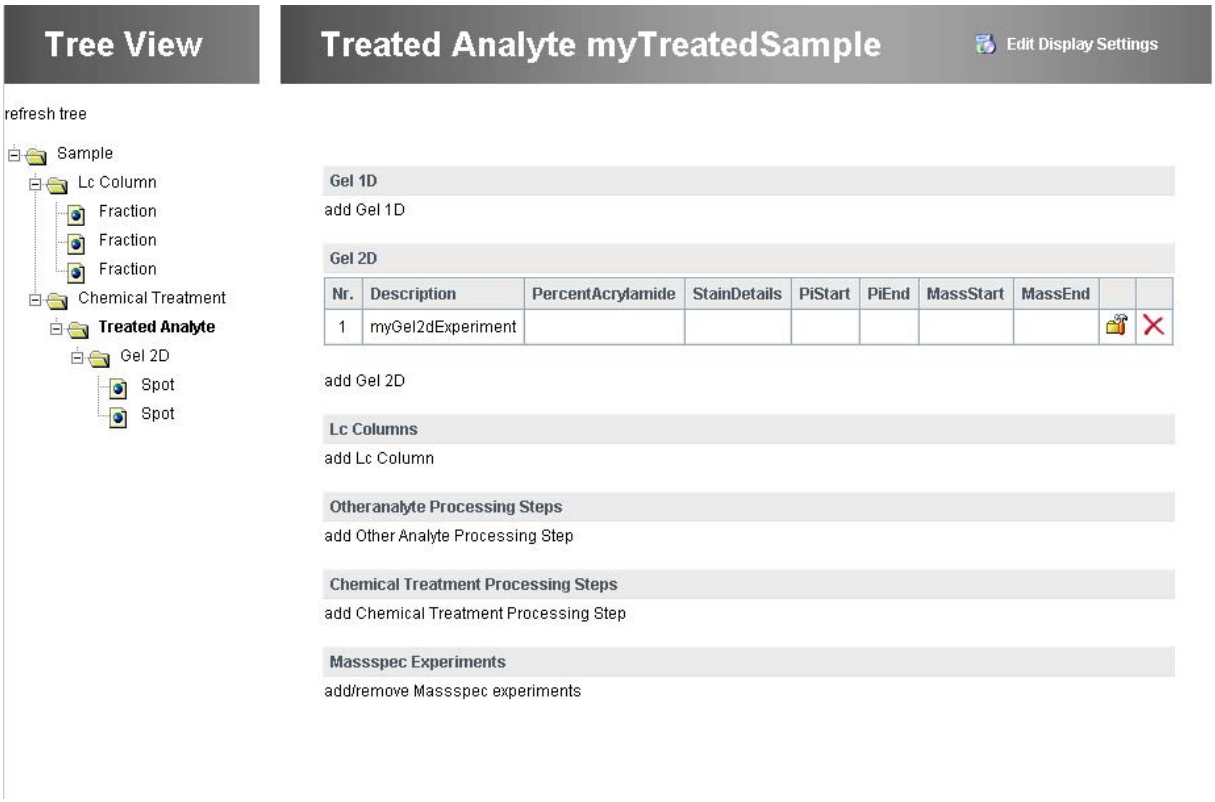


The page splits into two parts, the tree view (see chapter 4.1 “Tree View”) and the information view where you can display and edit your data. You can arbitrarily manage your

preparation steps here. E.g. you have a sample. With one half you ran it over an LC-Column, and got 3 Fractions which are interesting. The other half was first digested with trypsin and you got one treated analyte. With this one you made a 2D-Gel where you got a 2 interesting spots. Then the tree would look like the following:



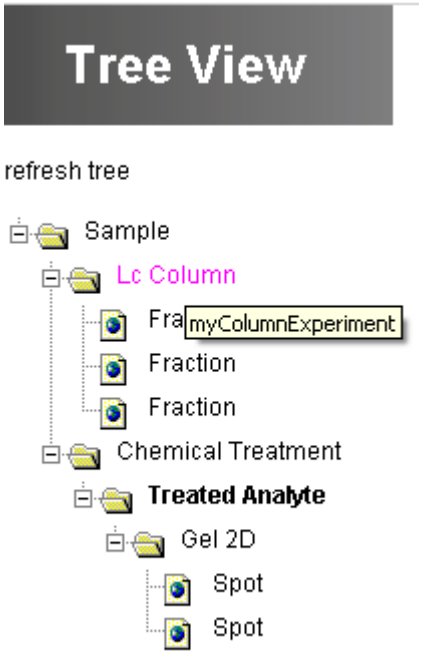
That means you can illustrate any splitting and any consecutive treatment. Regardless of the separation method you choose the organization is always the same. First you have a page where you can enter information about the separation method itself. After you have entered it once you can add with the edit option an arbitrary number of analytes. When you click on one of these analytes you will get again to a page where you can choose again between different analyte processing methods:



There are 5 different processing methods. A Gel1D leads to bands (for detailed information see chapter 4.2 Gel1D), a Gel2D leads to spots (for detailed information see chapter 4.3 Gel2D), a LC-Column leads to Fractions (for detailed information see chapter 4.4 LC-Column), a Chemical Treatment leads to Treated Analytes (for detailed information see chapter 4.5 Chemical Treatment), and Other Analyte Processing Steps (for detailed information see chapter 4.6 Other Analyte Processing Step) leads to Other Analytes. For all of the analytes Massspec experiments can be added (for detailed information how to add them see chapter 4.7 “Adding of Massspec experiments”). How you generate a Massspec Experiment see chapter 6.1 “Mass spectrometry experiment”.

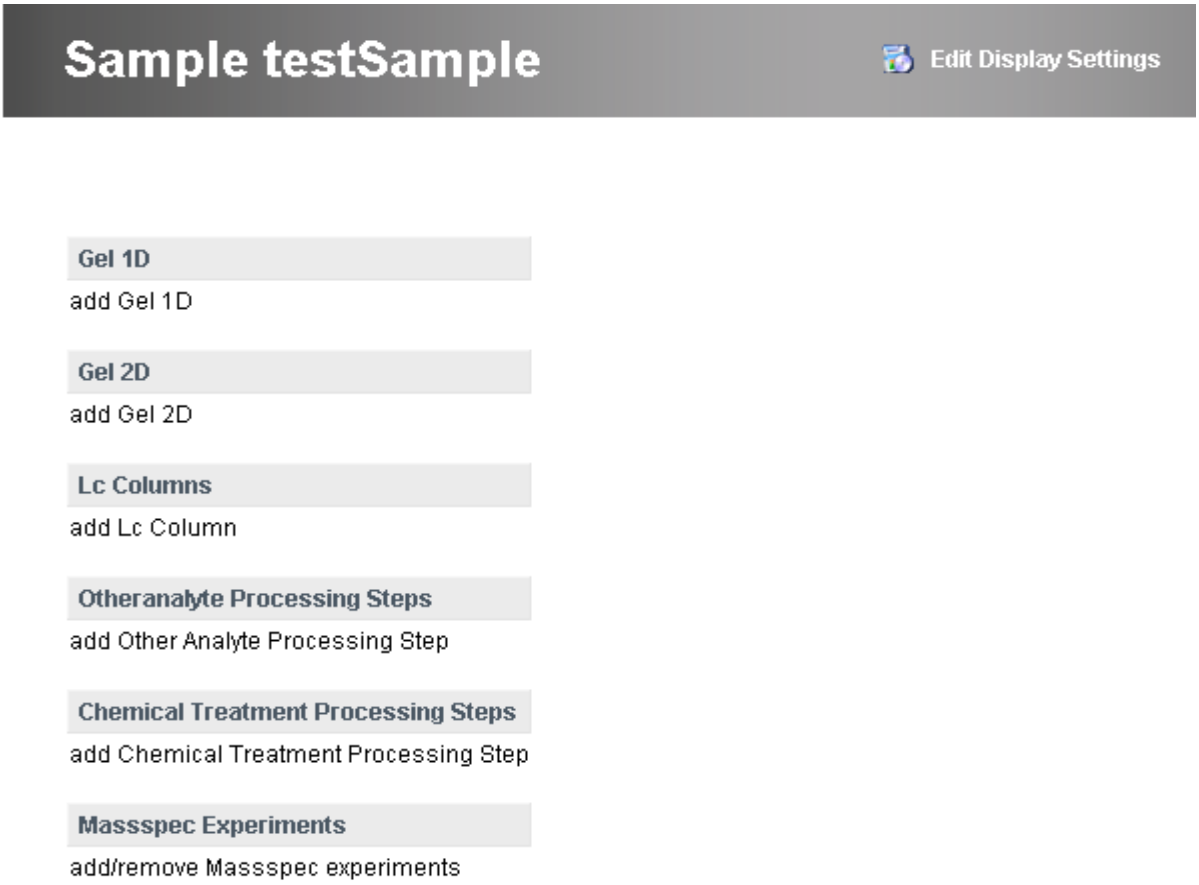
4.1 Tree view:

In the tree view the cross linking of the data is displayed graphically. In the tree the types of the analyte processing steps and the analytes are displayed. If you want to know the name of an element, you have to move your mouse over the element and a tool tip with the name will appear.

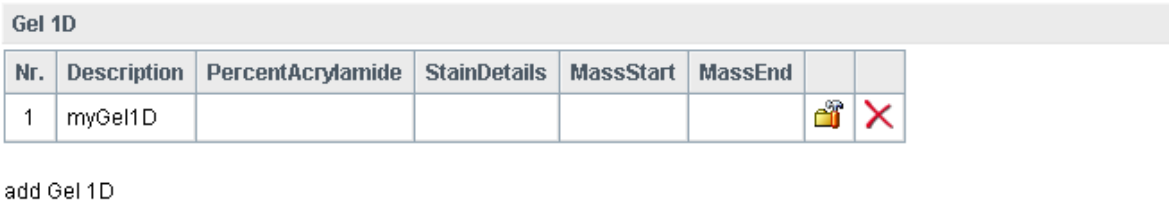


When you click on an element, information about this element will be displayed.
When you enter information on the right side the tree won't be updated automatically.
Press "refresh tree" to update it.

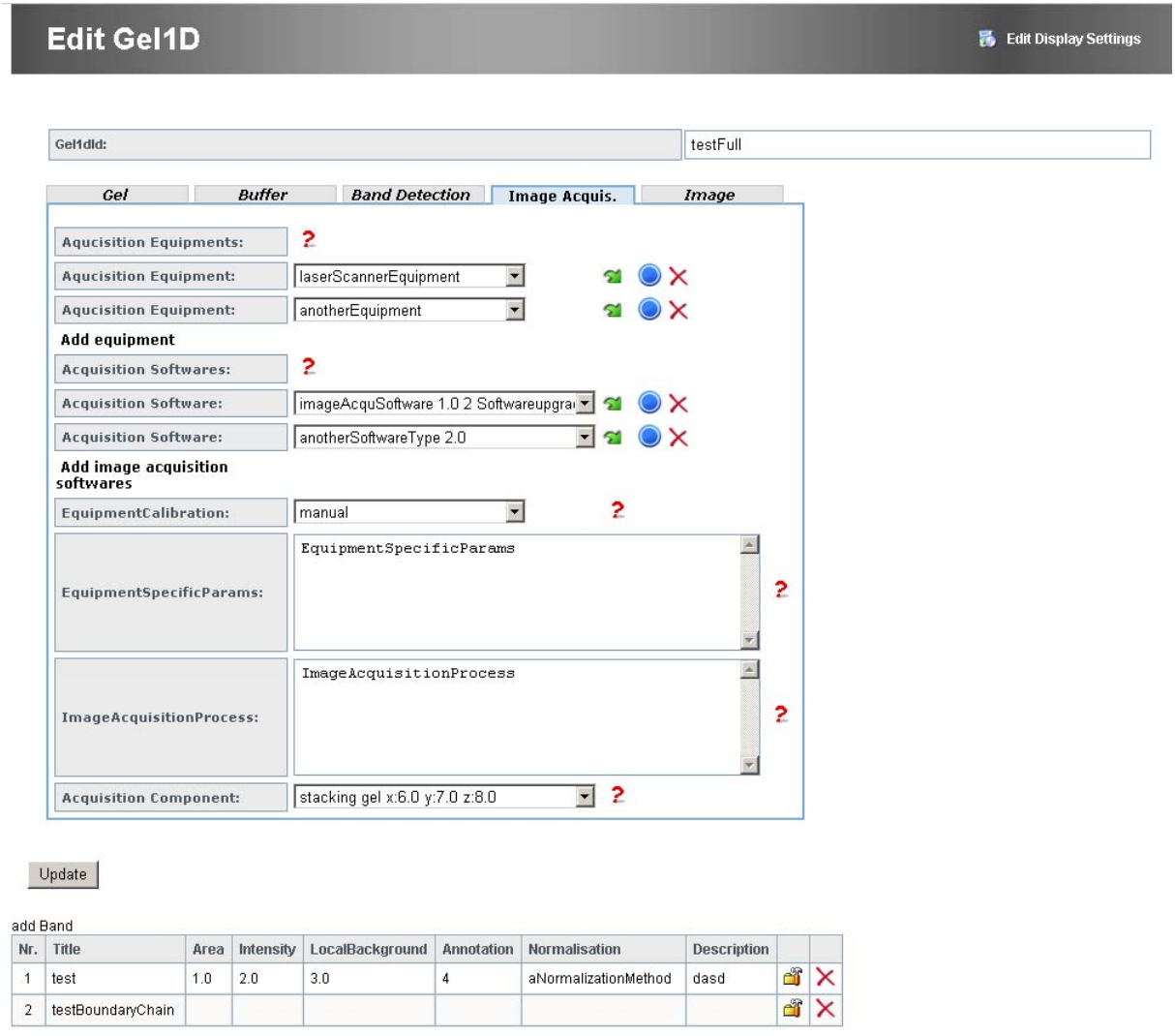
4.2 Gel1D:



When you are on the page of a sample or an analyte you can add a Gel1D with the link “add Gel 1D”. When you have added a Gel1D you will be redirected to the previous page displaying the added Gel1D.

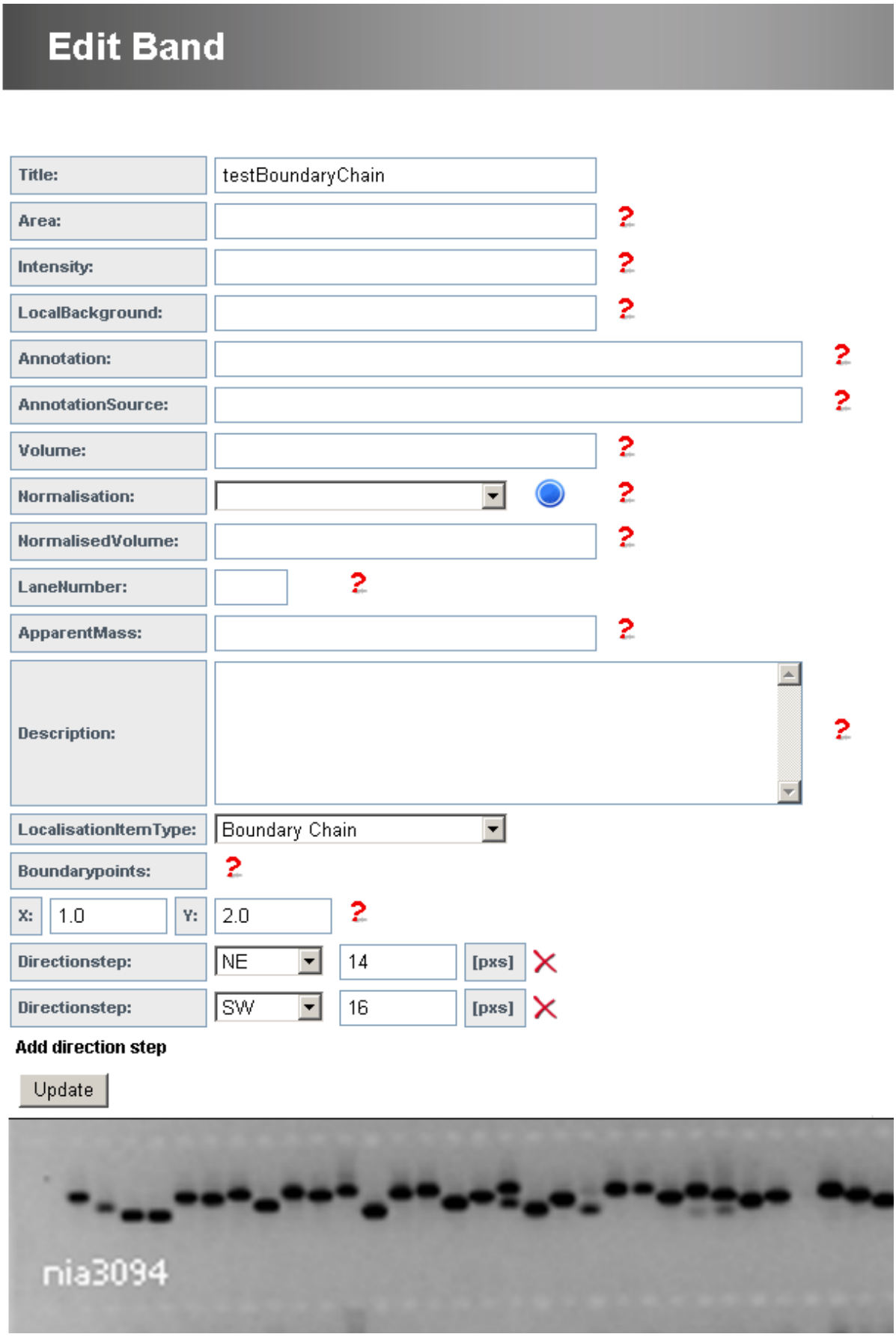


When you click on the description name or on the edit button you can edit it again.



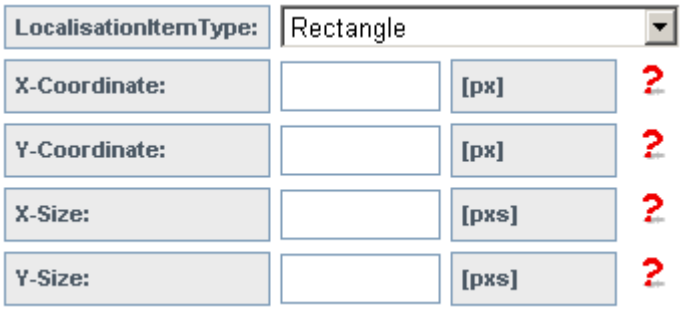
For the “Acquisition Component” you can select the whole selected “Gelmatrix” named with “Main” (you will find this select field, when you click on the “Gel” tab) or one of the components of the “Gelmatrix”. The references to the files (mostly images) which you can select in the “Image” tab, must be uploaded with the following upload types: Gel1D Raw Image for the Raw Image; Gel1D Warped Image for the Warped Image; Gel1D Warping Map for the Warping Map; Gel1D Annotated Image for the Annotated Image. All of the images can be displayed on this page as well.

Additionally to the create page link the “add Band” link for adding bands and a list with added bands will be displayed (here the edit page is shown).

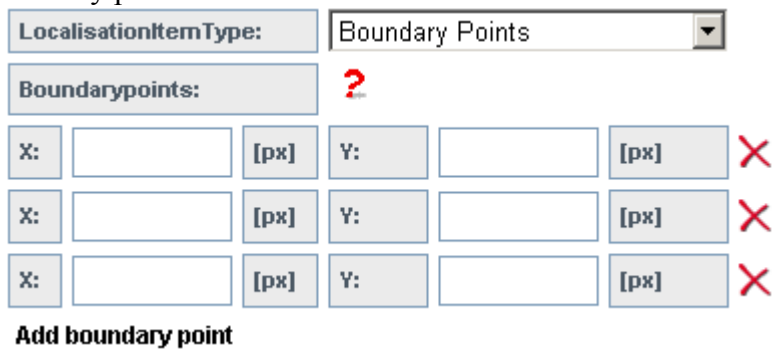


When you click on the edit or delete button of a band you reach this “create/edit” page again and you can make your changes, but by clicking on the title of the band (in this case “myBand”) you reach the page where you can add additional preparation steps or mass spectrometry experiments to the band.

At the bottom of the page the annotated image is shown (for demonstration purposes only an arbitrary image is shown). The localization of the band can be described in three different ways. The boundary chain is depicted in the image above.
Rectangle:

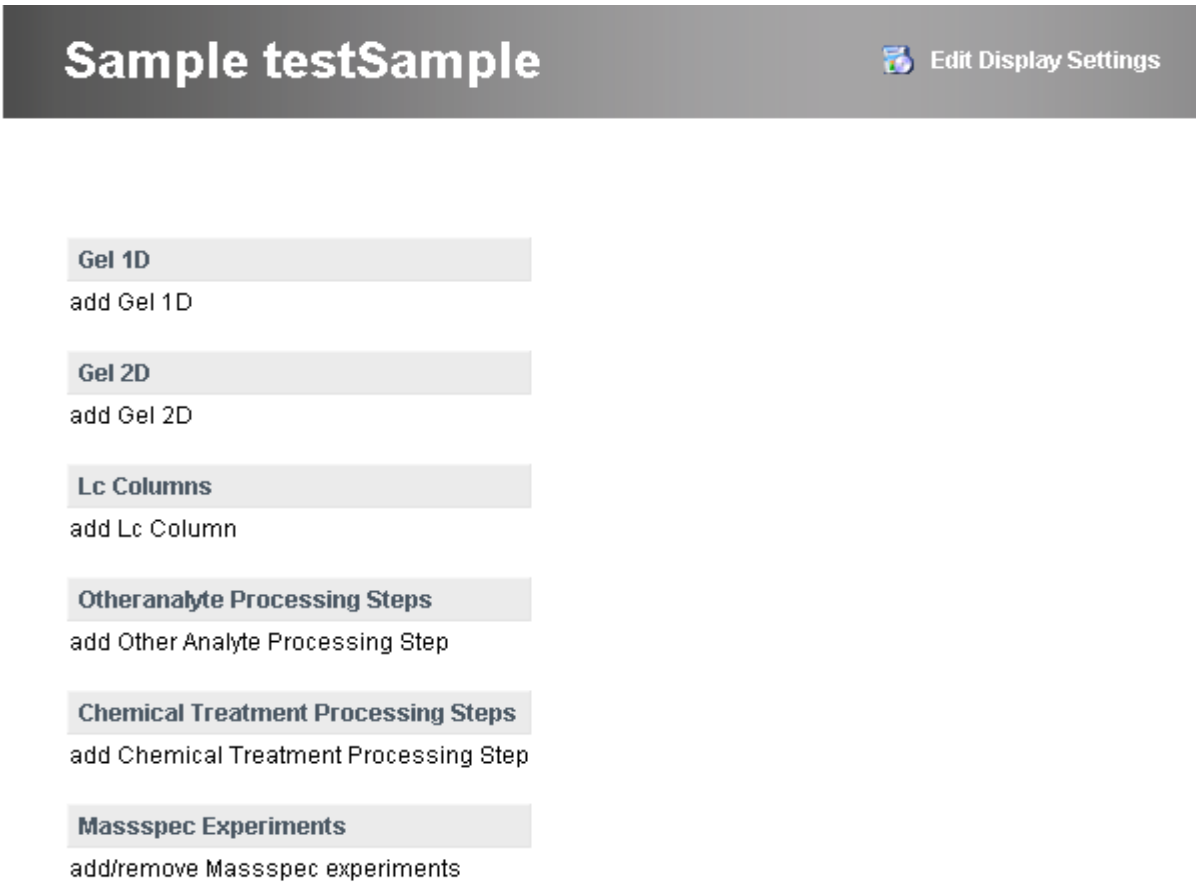


Boundary points:



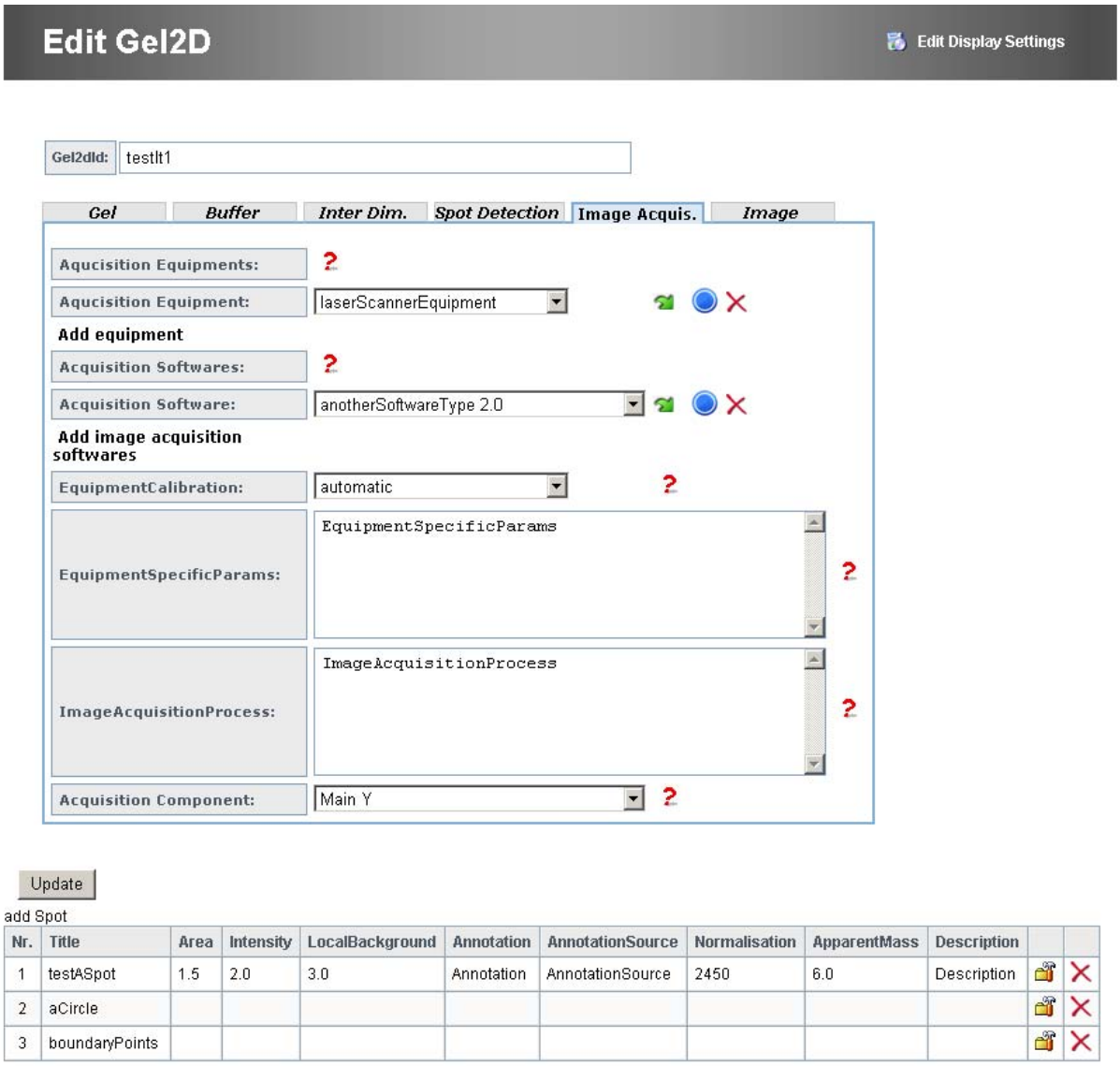
For the boundary chain and the boundary points the sequence of the entered values is important.

4.3 Gel2D:

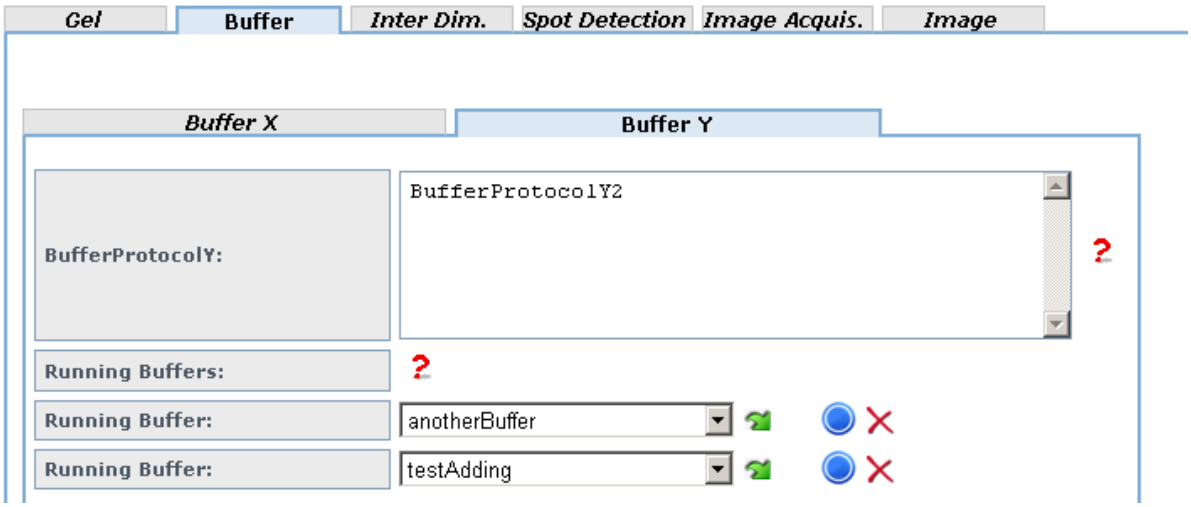


When you are on the page of a sample or an analyte you can add a Gel2D with the link “add Gel 2D”. When you have added a Gel2D you will be redirected to the previous page containing the added Gel2D.

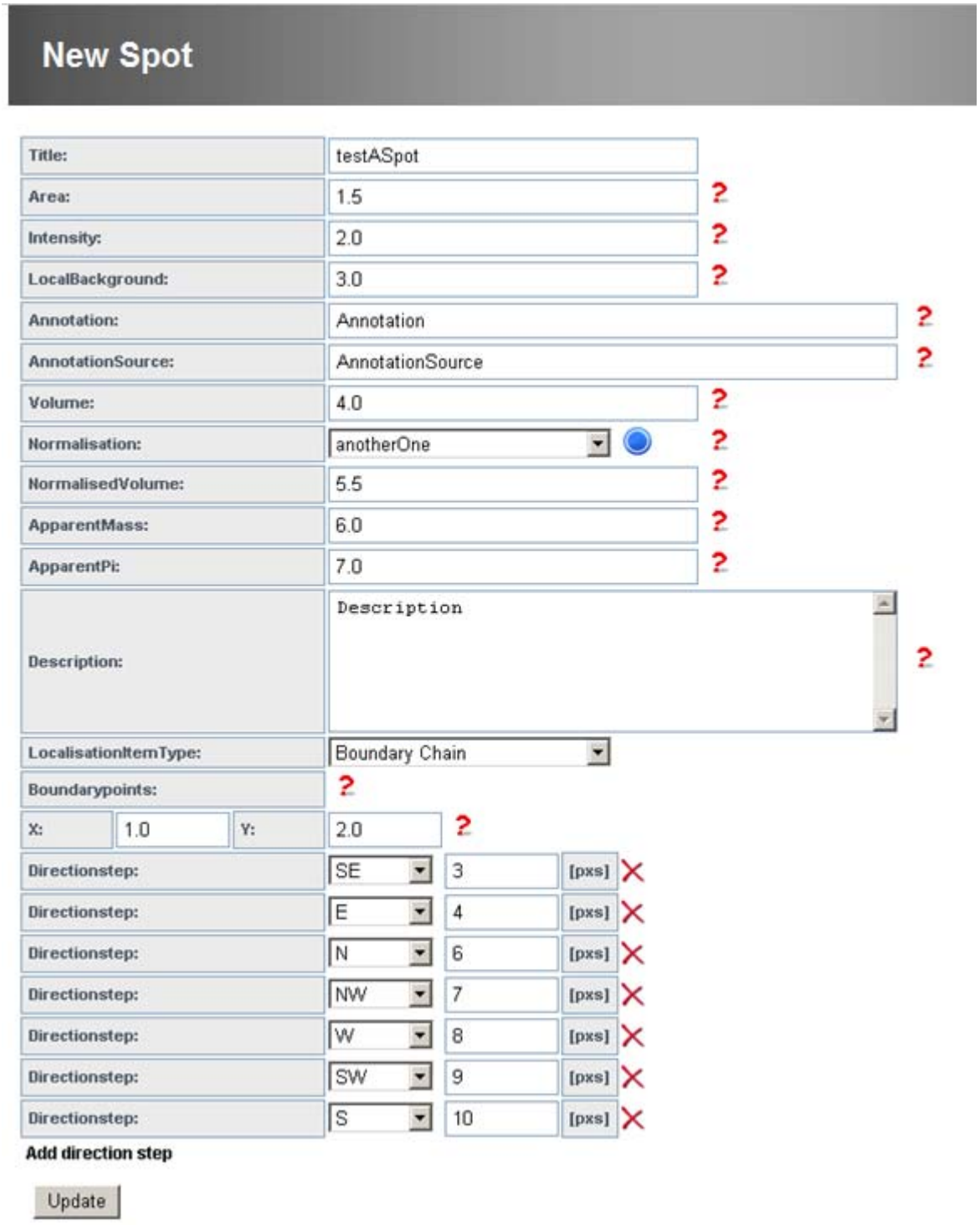
When you click on the description name or on the edit button you will be directed to the “create/edit” page again.



For the “Acquisition Component” you can select the whole selected “Gelmatrix” for X and Y named with “Main X” and “Main Y” (you will find this select field, when you click on the “Gel” tab) or one of the components of the “Gelmatrix X” “Gelmatrix Y”. The references to the files (mostly images) which you can select in the “Image” tab, must be uploaded with the following upload types: Gel2D Raw Image for the Raw Image; Gel2D Warped Image for the Warped Image; Gel2D Warping Map for the Warping Map; Gel2D Annotated Image for the Annotated Image. All of the images can be displayed on this page as well. In contrast to the Gel1D the input mask is quite often divided by additional tabs in information concerning the X and concerning the Y section.



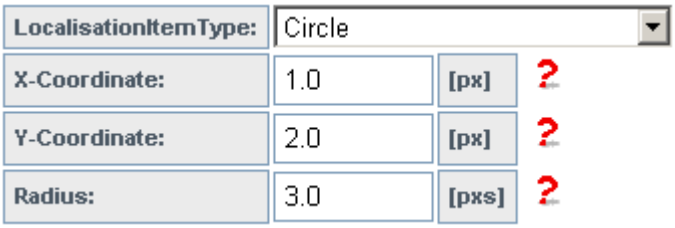
Additionally to the create page link the “add Spot” link for adding spots and a list with added spots will be displayed.



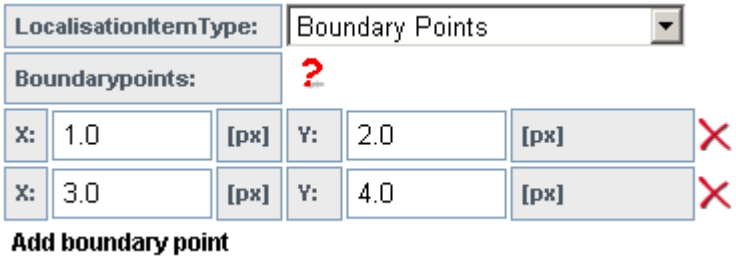
When you click on the edit or delete button of a spot you reach this “create/edit” page and you can make your changes, but by clicking on the title of the band (in this case “mySpot”) you reach the page where you can add additional preparation steps or mass spectrometry experiments to the spot.

At the bottom of the page the annotated image is shown (for demonstration purposes only an arbitrary image is shown). The localization of the spot can be described in three different ways. The boundary chain is depicted in the image above.

Circle:

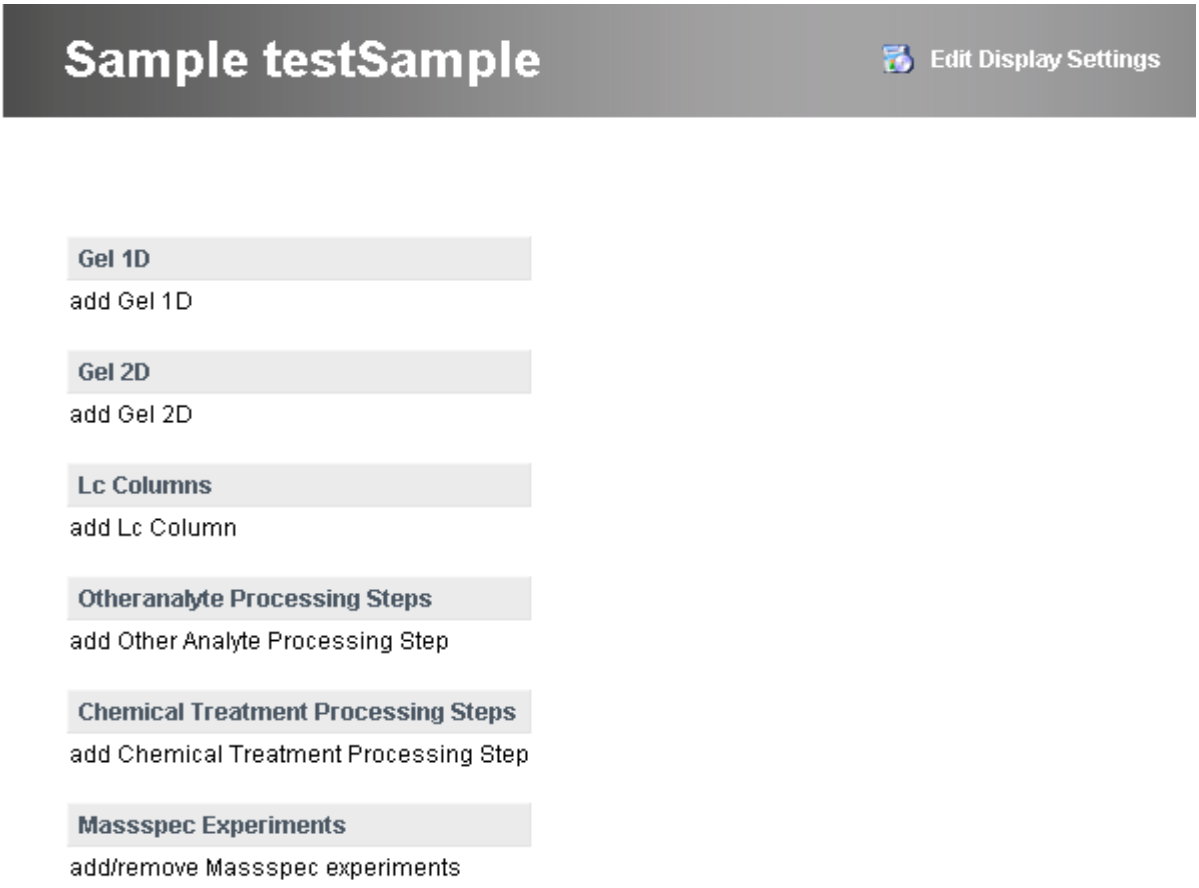


Boundary points:



For the boundary chain and the boundary points the sequence of the entered values is important.

4.4 LC-Column:



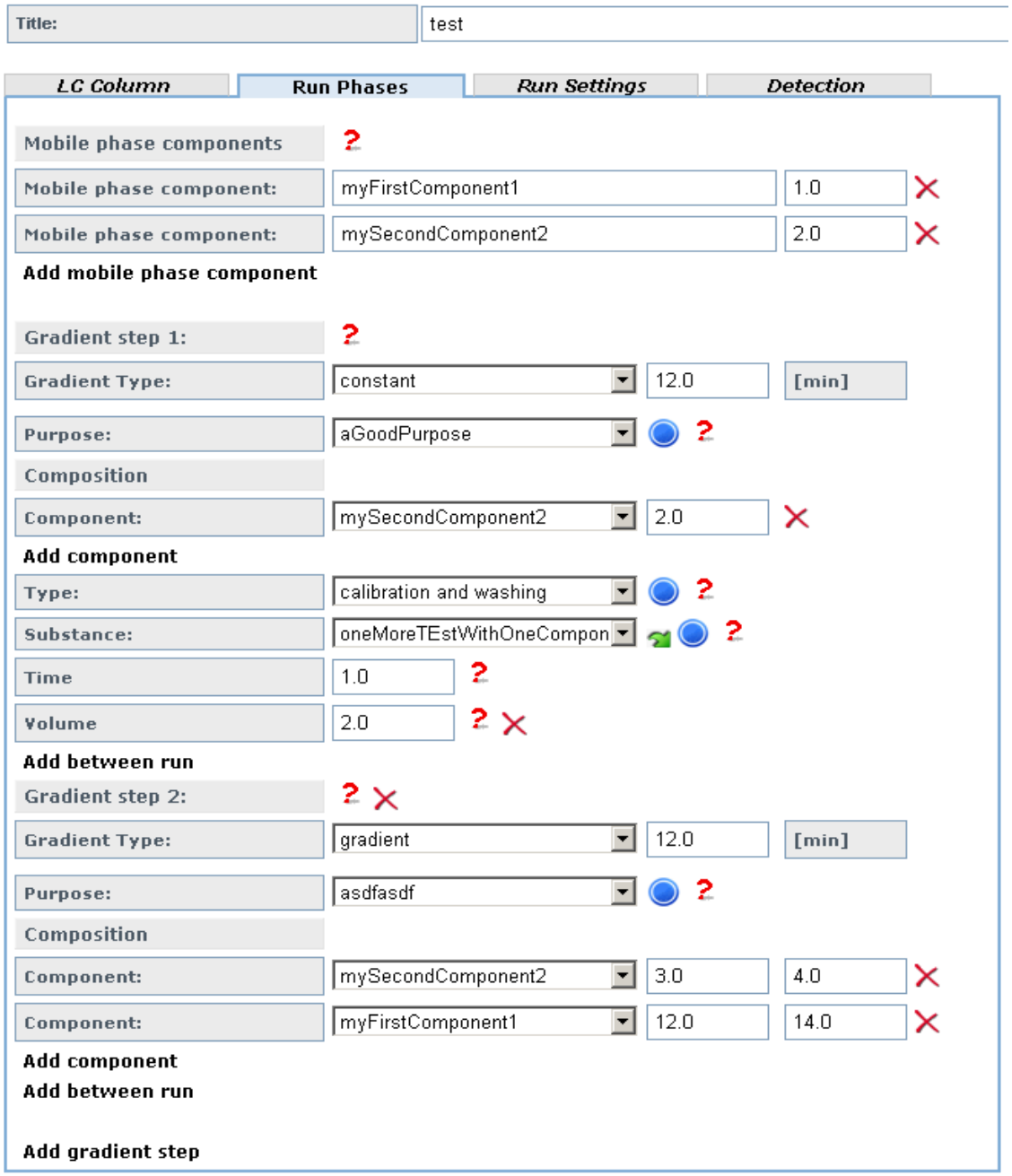
When you are on the page of a sample or an analyte you can add a LC-Column with the link “add Lc Column“. When you have added an LC-Column you will be redirected to the previous page containing the added LC-Column.

Lc Columns

Nr.	Title	Description	InternalLength	InternalDiameter	FlowRate	InjectionVolume		
1	myColumnExperiment							

add Lc Column

When you click on the title name or on the edit button you will be directed to “create/edit” page again.



Title: test

LC Column | Run Phases | Run Settings | Detection

Mobile phase components

Mobile phase component: myFirstComponent1 1.0

Mobile phase component: mySecondComponent2 2.0

Add mobile phase component

Gradient step 1:

Gradient Type: constant 12.0 [min]

Purpose: aGoodPurpose

Composition

Component: mySecondComponent2 2.0

Add component

Type: calibration and washing

Substance: oneMoreTEstWithOneCompon

Time 1.0

Volume 2.0

Add between run

Gradient step 2:

Gradient Type: gradient 12.0 [min]

Purpose: asdfasdf

Composition

Component: mySecondComponent2 3.0 4.0

Component: myFirstComponent1 12.0 14.0

Add component

Add between run

Add gradient step

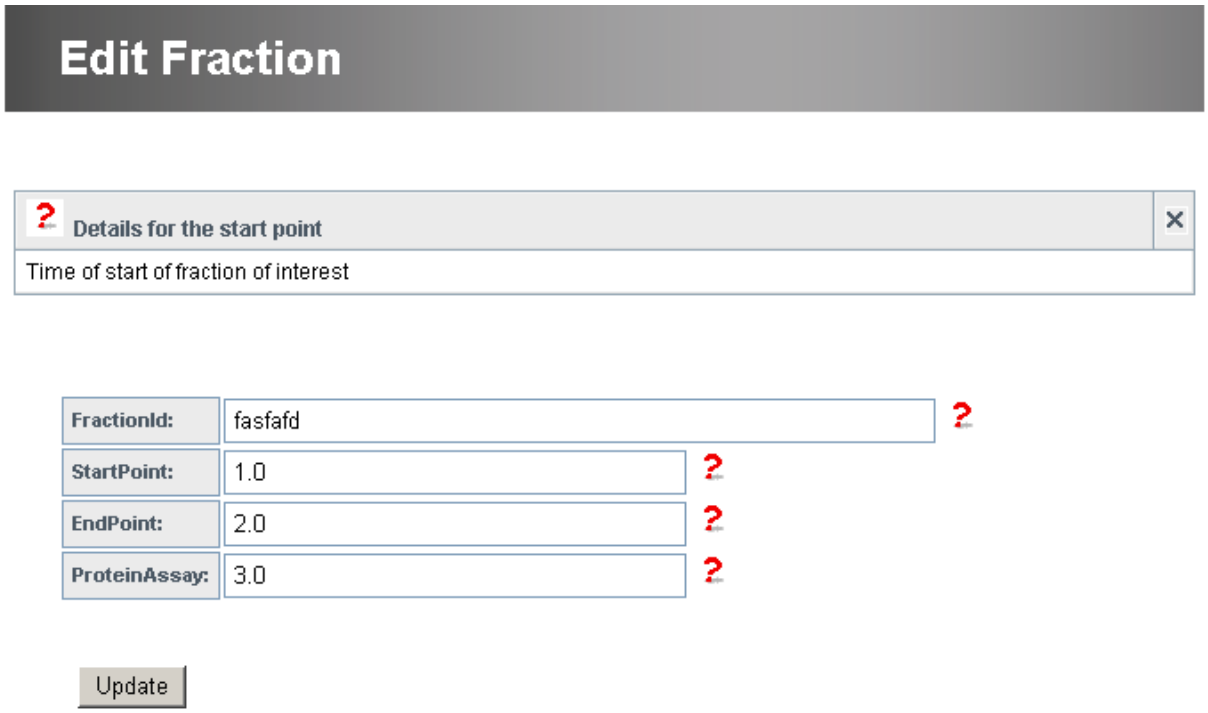
Update

add Fraction

Nr.	FractionId	StartPoint	EndPoint	ProteinAssay		
1	fasfafd	1.0	2.0	3.0		

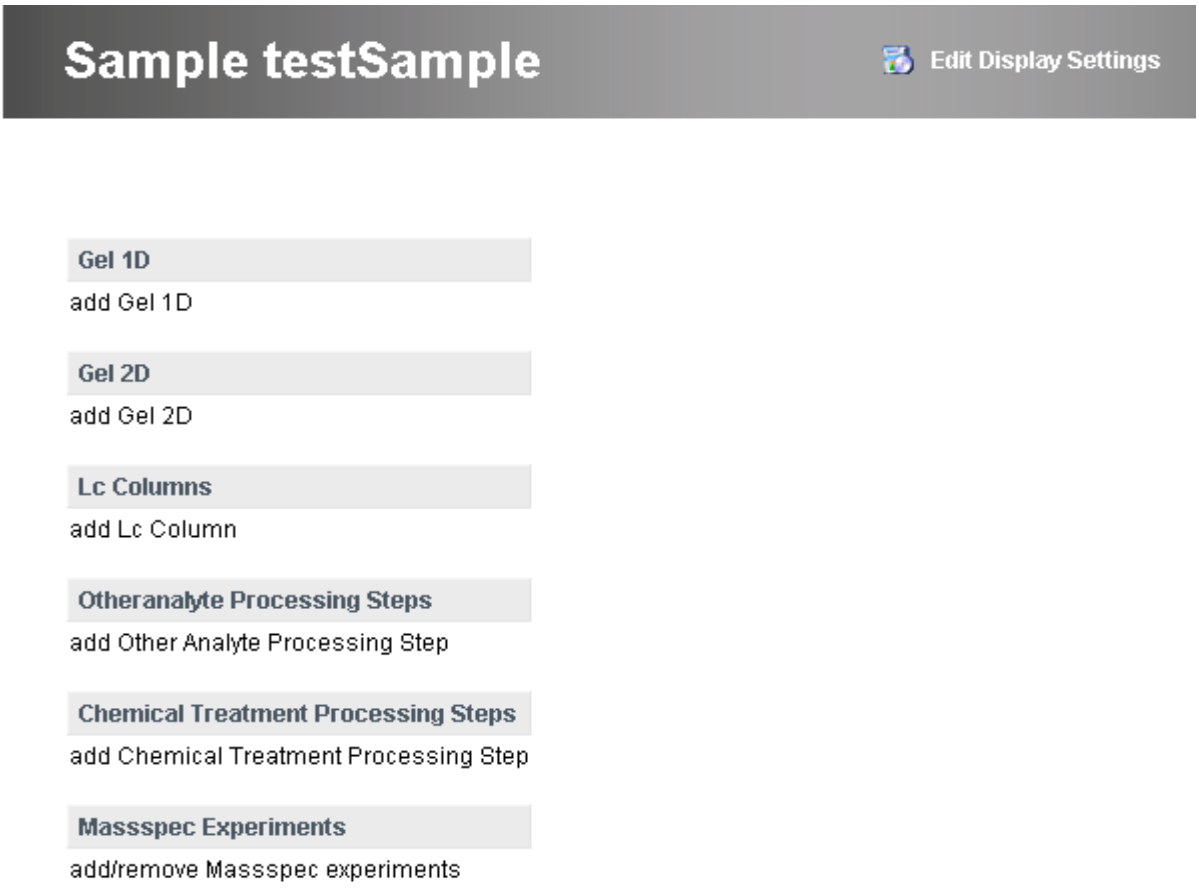
In this page you can enter different mobile phase components, which you can select (after entering) in the component select field.

Additionally to the create page link the “add Fraction” link for adding fractions and a list with added fractions will be displayed.

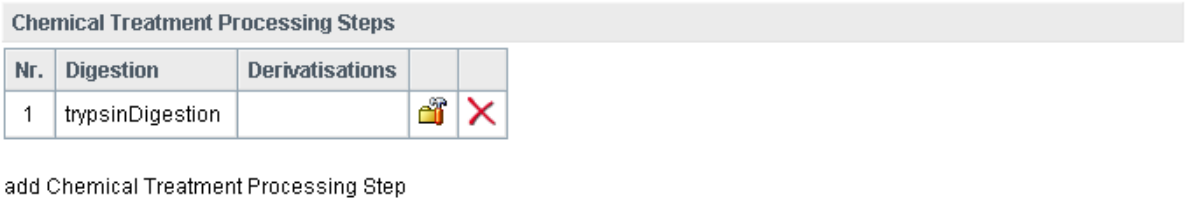


When you click on the edit or delete button of a Fraction you reach this “create/edit” page and you can make your changes, but by clicking on the title of the fractionId (in this case “firstFraction”) you reach the page where you can add additional preparation steps or mass spectrometry experiments to the fraction.

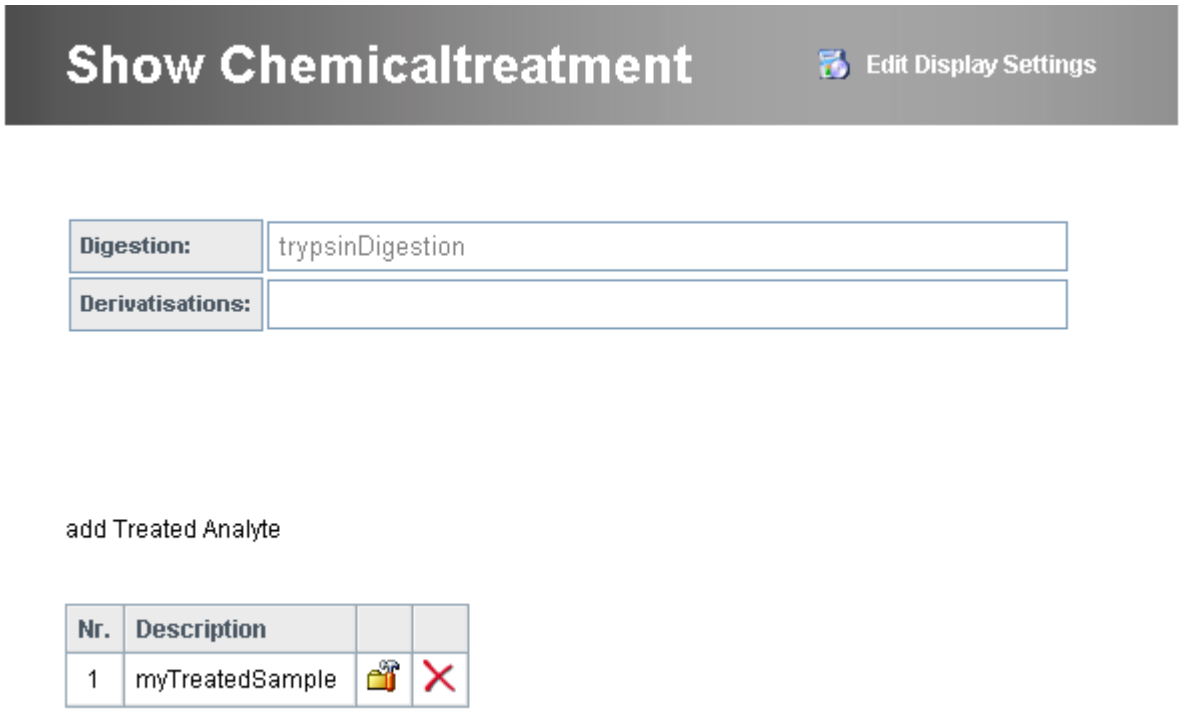
4.5 Chemical Treatment:



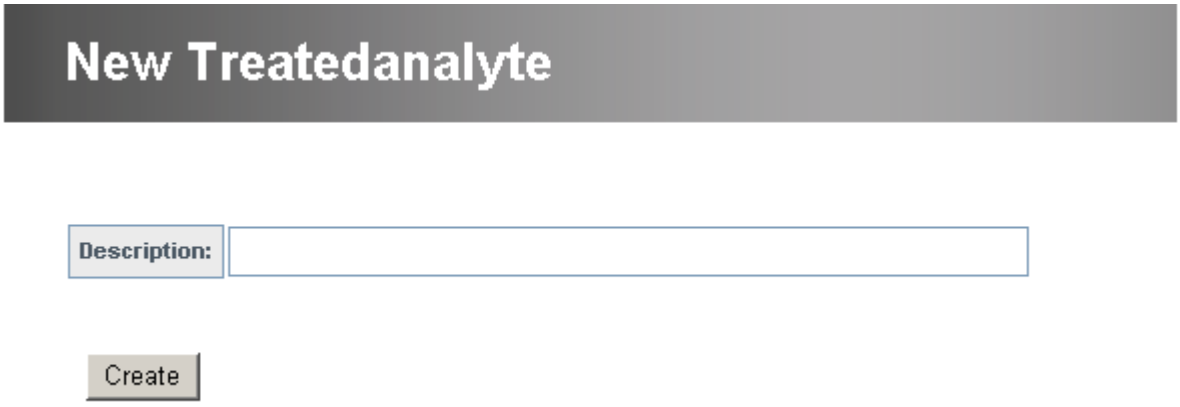
When you are on the page of a sample or an analyte you can add chemical treatments with the "add Chemical Treatment Processing Step"link. When you have added a chemical treatment you will be redirected to the previous page containing the added chemical treatment.



When you click on the digestion name or on the edit button you will be directed to the same "create/edit" page again.

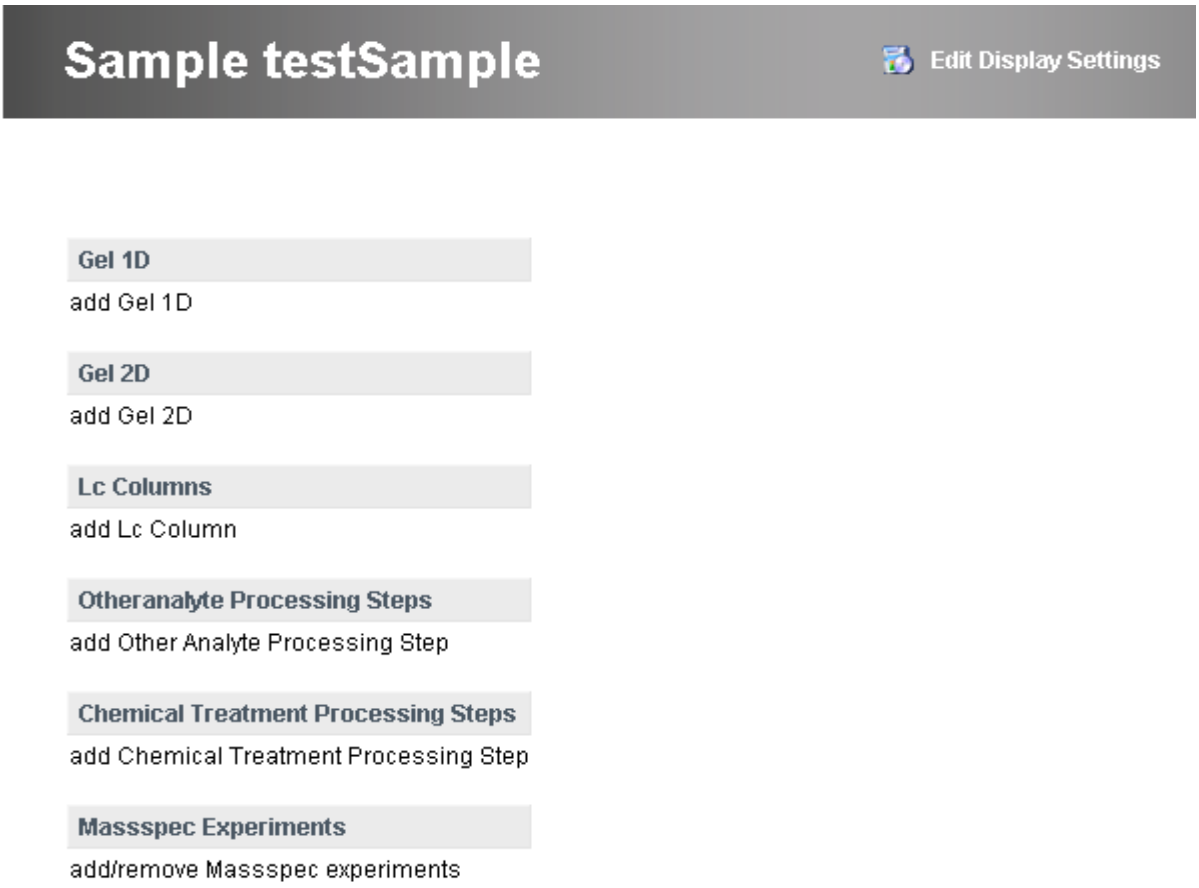


Additionally to the create page link the “add Treated Analyte” link for adding treated analytes and a list with added treated analytes will be displayed.

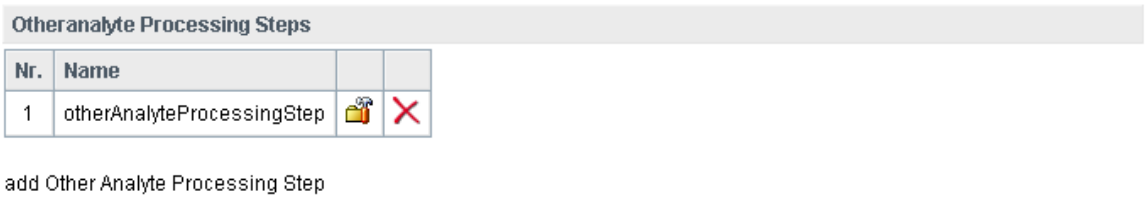


When you click on the edit or delete button of an treated analyte you reach this “create/edit” page again and you can make your changes, but by clicking on the name of the description (in this case “myTreatedSample”) you reach the page where you can add additional preparation steps or mass spectrometry experiments to the treated analyte.

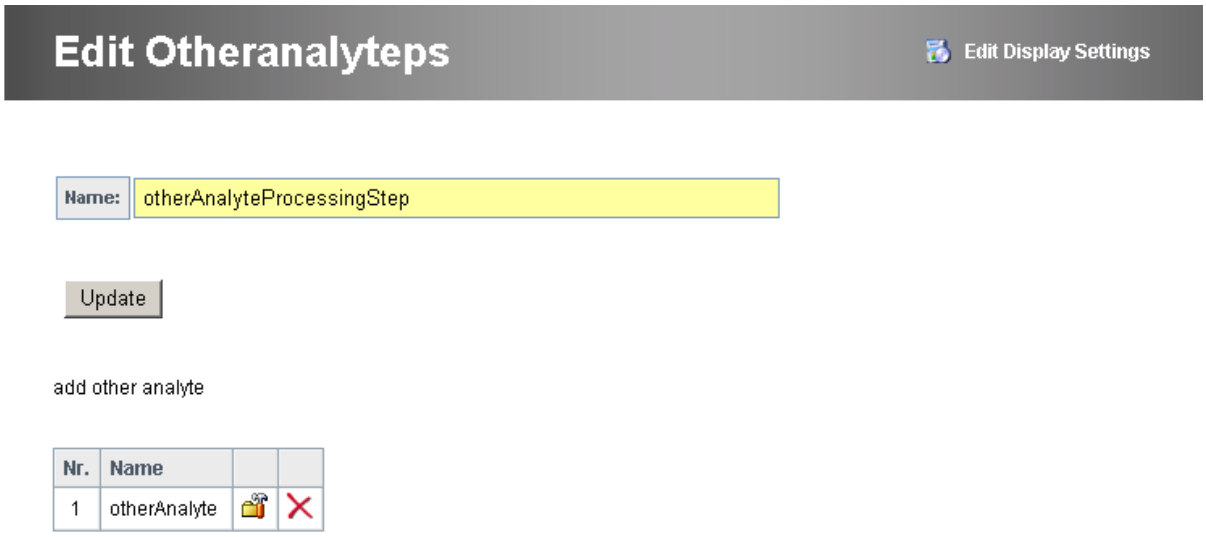
4.6 Other Analyte Processing Step:



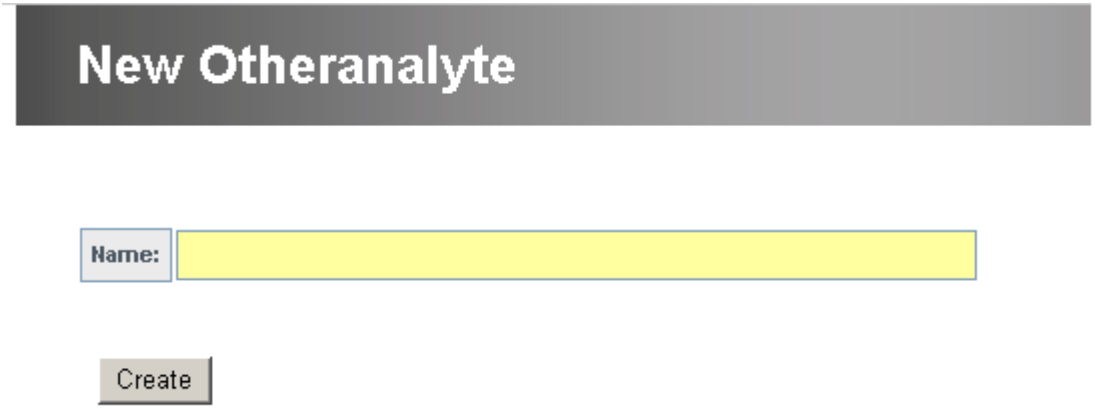
When you are on the page of a sample or an analyte you can add other analyte processing steps with the link “add Other Analyte Processing Step“. When you have added an other analyte processing step you will be redirected to the previous page containing the added other analyte processing step.



When you click on the name or on the edit button you will be directed to the same “create/edit” page again.

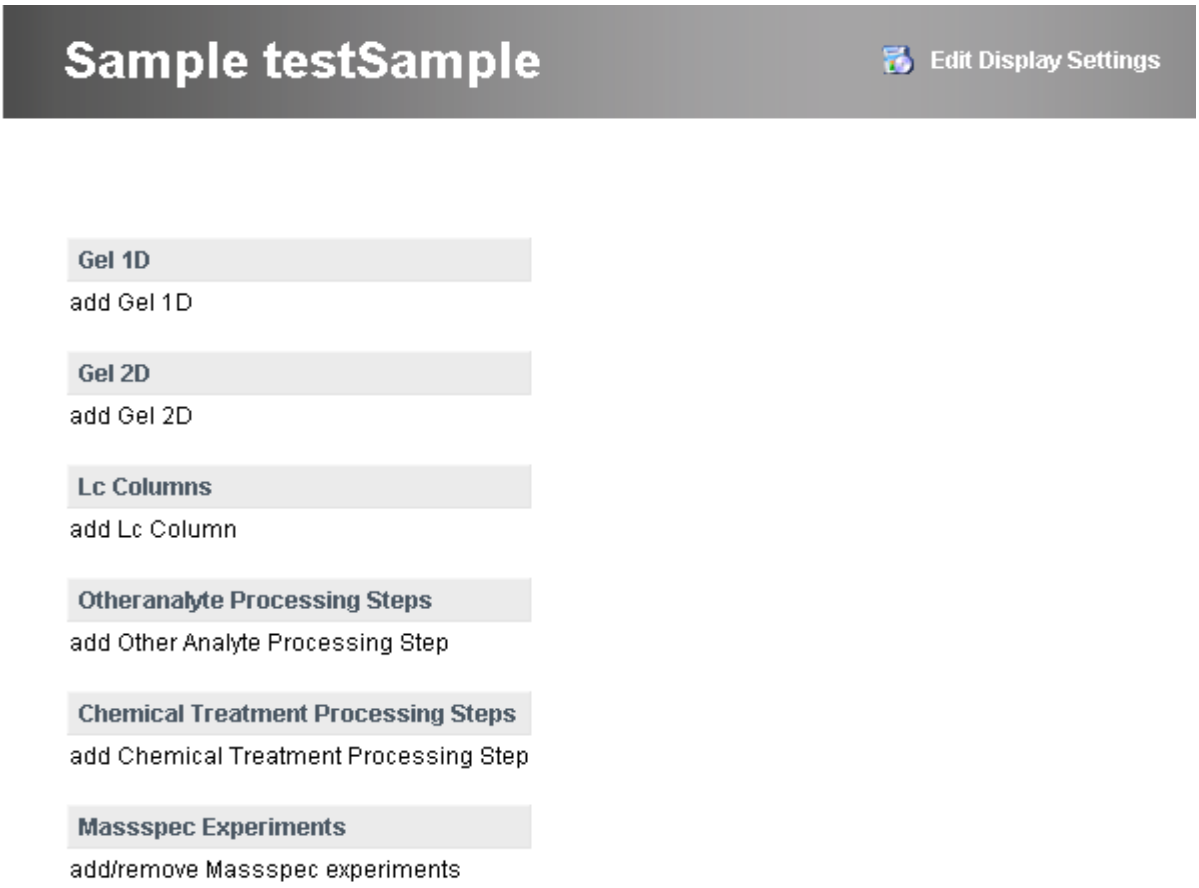


Additionally to the create page link the “add other analyte” link for adding other analytes and a list with added other analytes will be displayed.

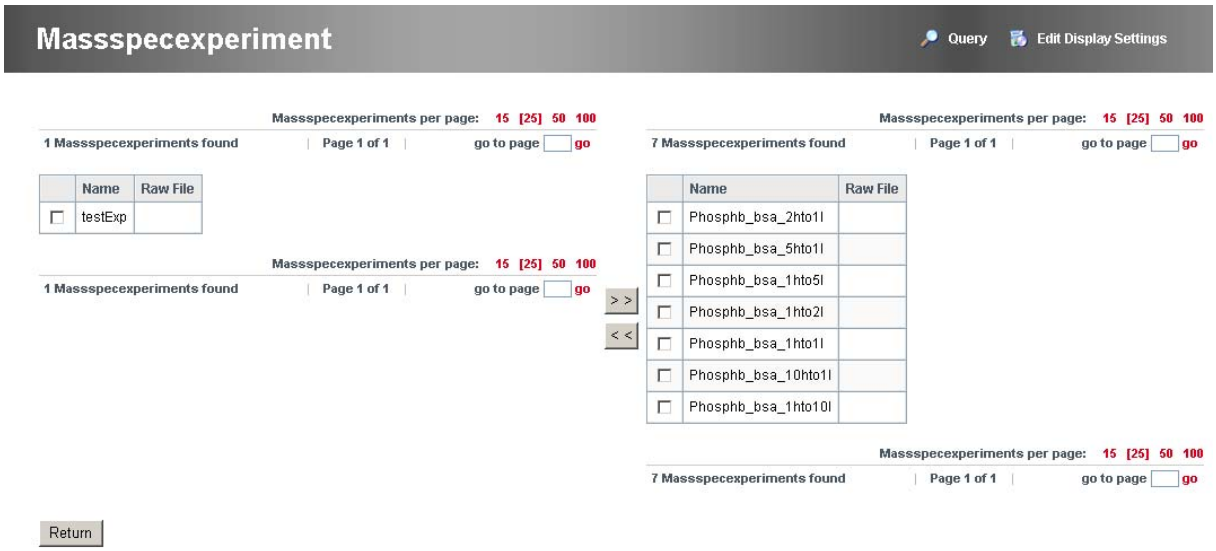


When you click on the edit or delete button of an analyte you reach this “create” page and you can make your changes, but by clicking on the name (in this case “otherAnalyte”) you reach the page where you can add additional preparation steps or mass spectrometry experiments to the otherAnalyte.

4.7 Adding of Massspec experiments



When you have added some mass spectrometry experiments, there is a direct link on the title of the mass spectrometry experiment to the mass spectrometry experiment.
When you are on the page of a sample or an analyte you can add other mass spectrometry experiments using the "add/remove Massspec experiments"link.



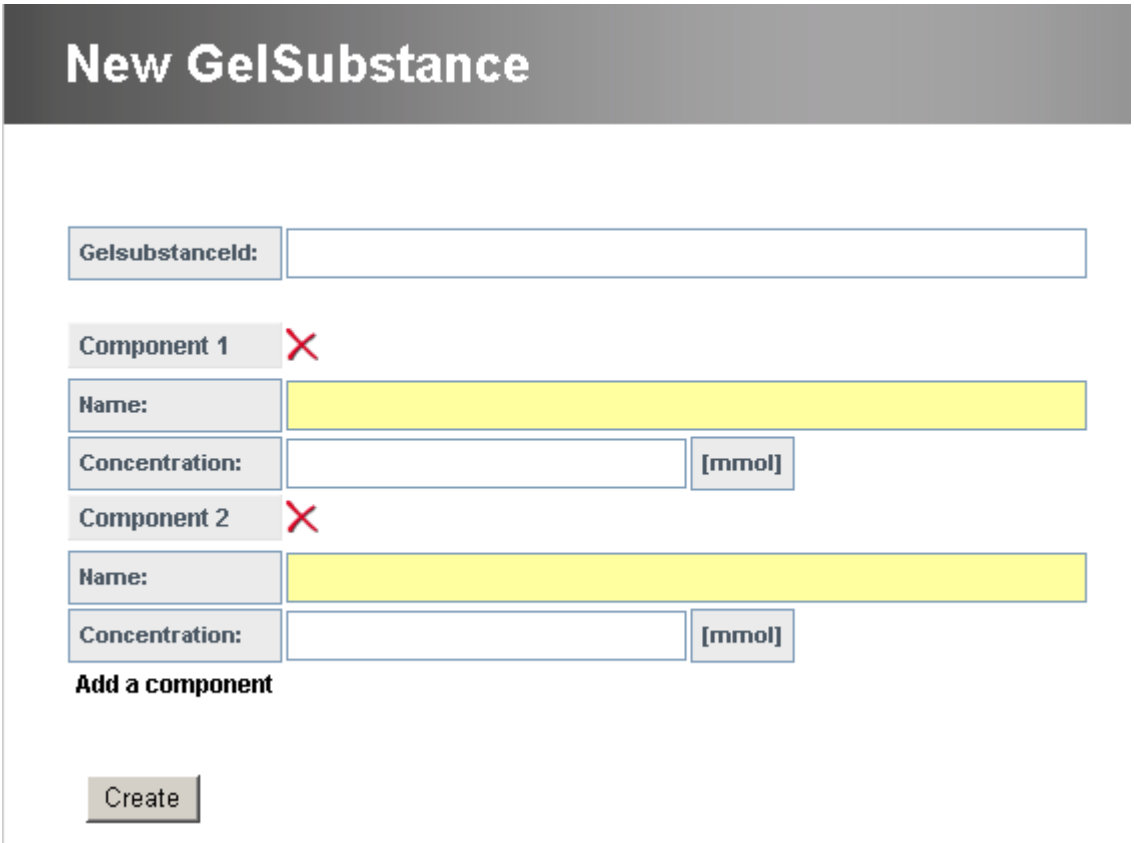
Adding massspec experiments to an analyte works the same way as adding samples to experiments (see chapter 3.2.2). The only difference is that only those mass spectrometry experiments are displayed on the left side, which are not already added to an analyte, while

the sample can be added to several experiments. For detailed information how to create mass spectrometry experiments see 6.1 “Mass spectrometry experiment”.

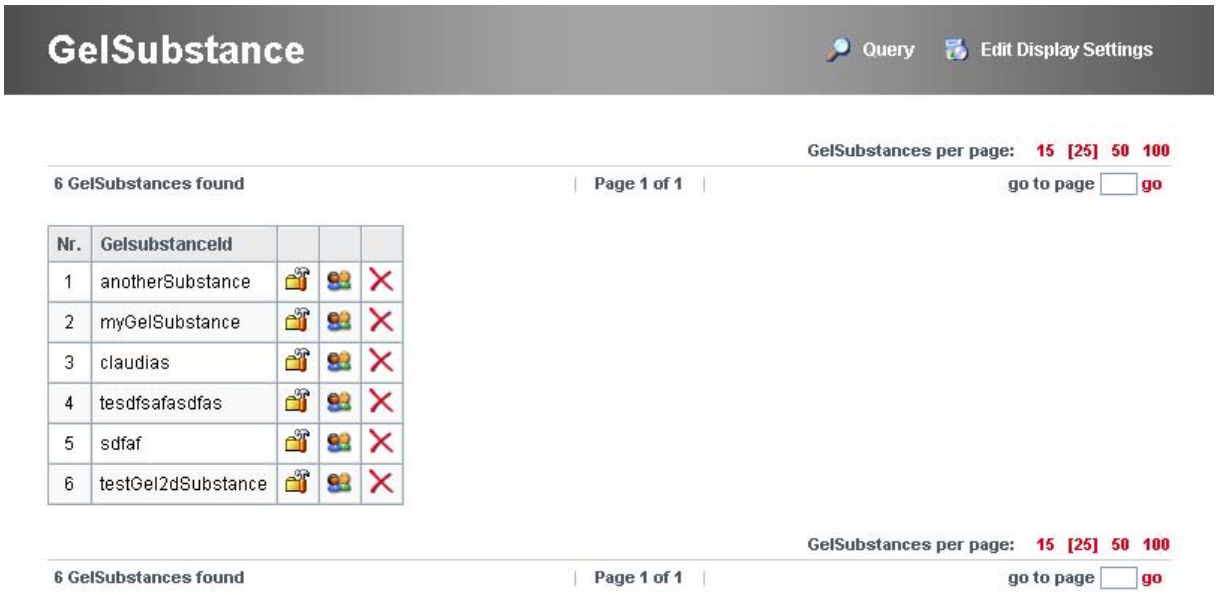
4.8 Gel Substance

By clicking Sample Processing->GelSubstance you reach gel substance section.

With the “Add GelSubstance” you can add gel substances.

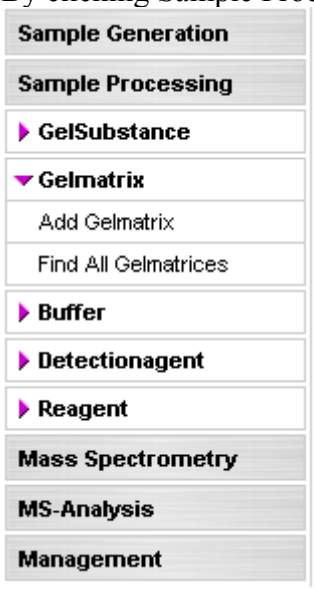


With a click on the button “Find All GelSubstances” you get an overview of all your gel substances:

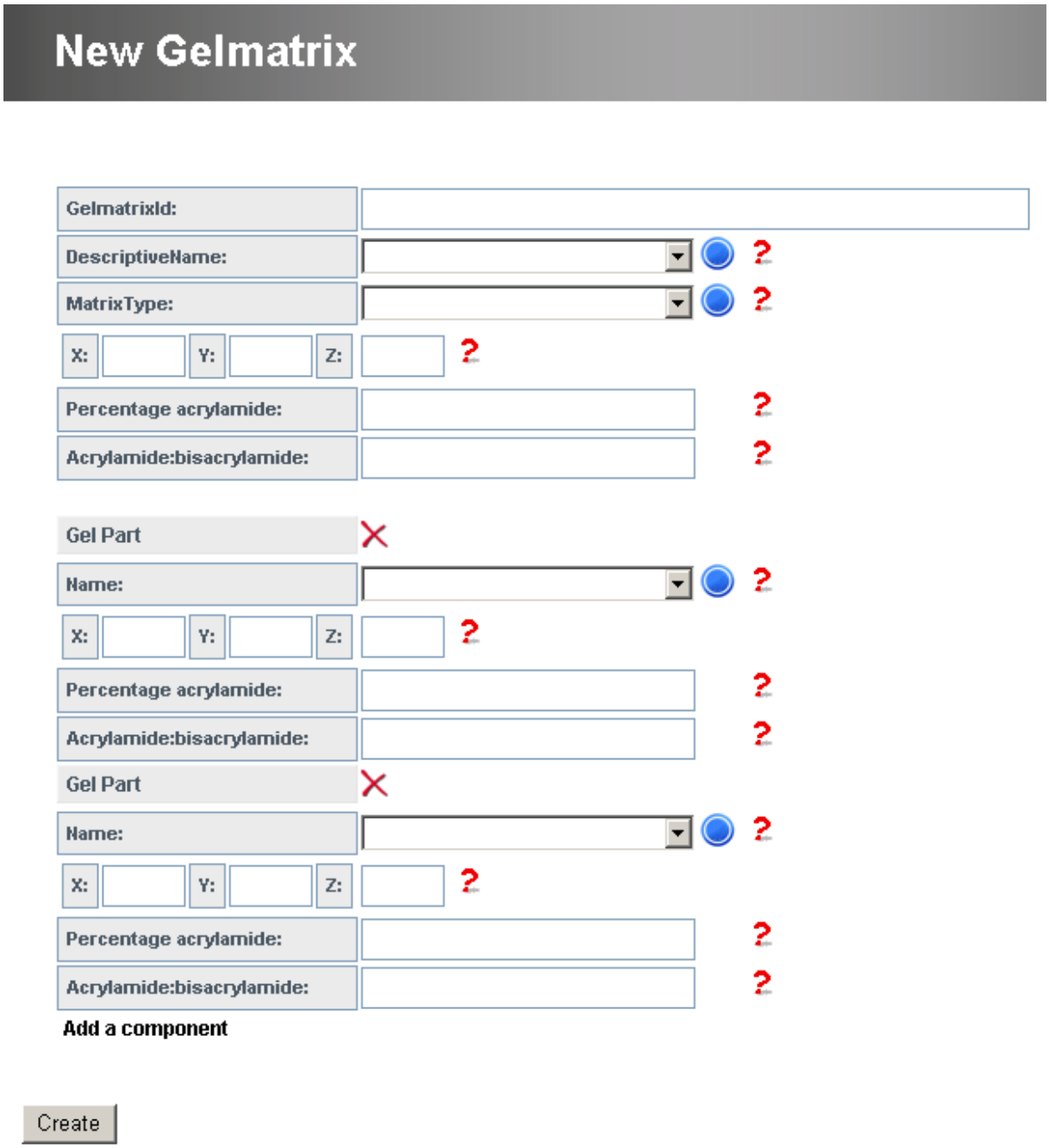


4.9 Gel Matrix

By clicking Sample Processing->Gelmatrix you reach gel matrix section.



With the “Add Gelmatrix” you can add gel matrices.

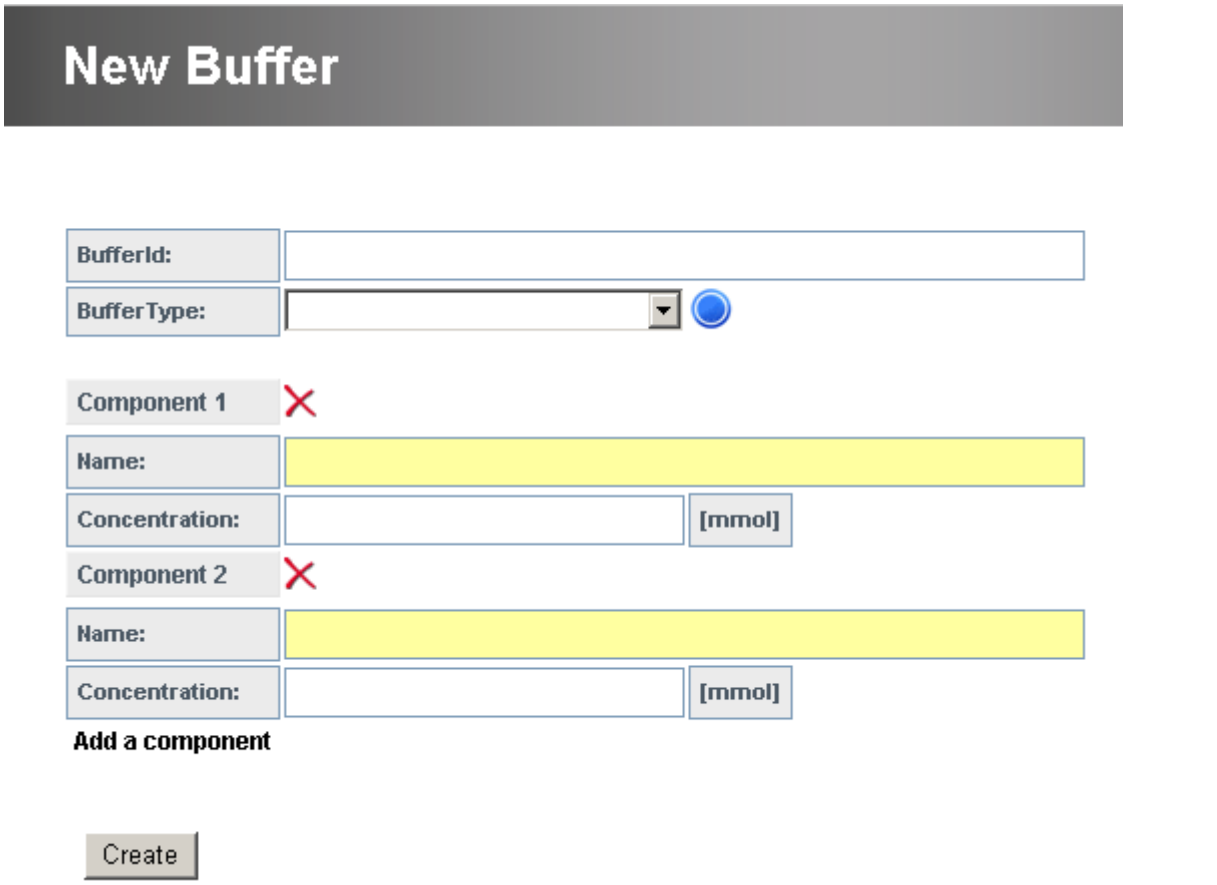


With a click on the button “Find All Gelmatrices” you get an overview of all your gel matrices:

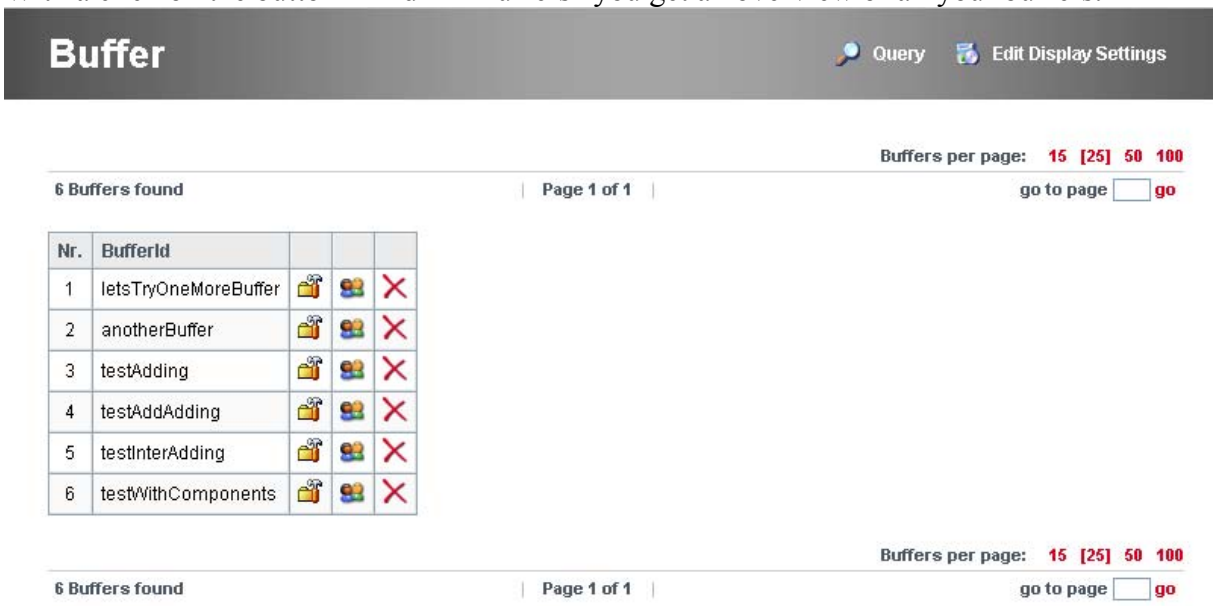
4.10 Buffer

By clicking Sample Processing->Buffer you reach buffer section.

With the “Add Buffer” you can add buffers.

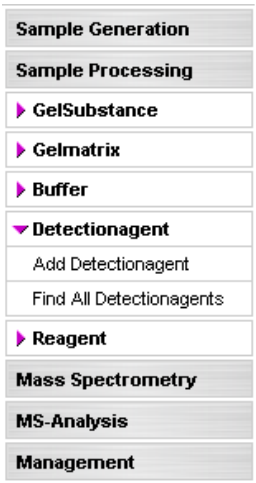


With a click on the button “Find All Buffers” you get an overview of all your buffers:

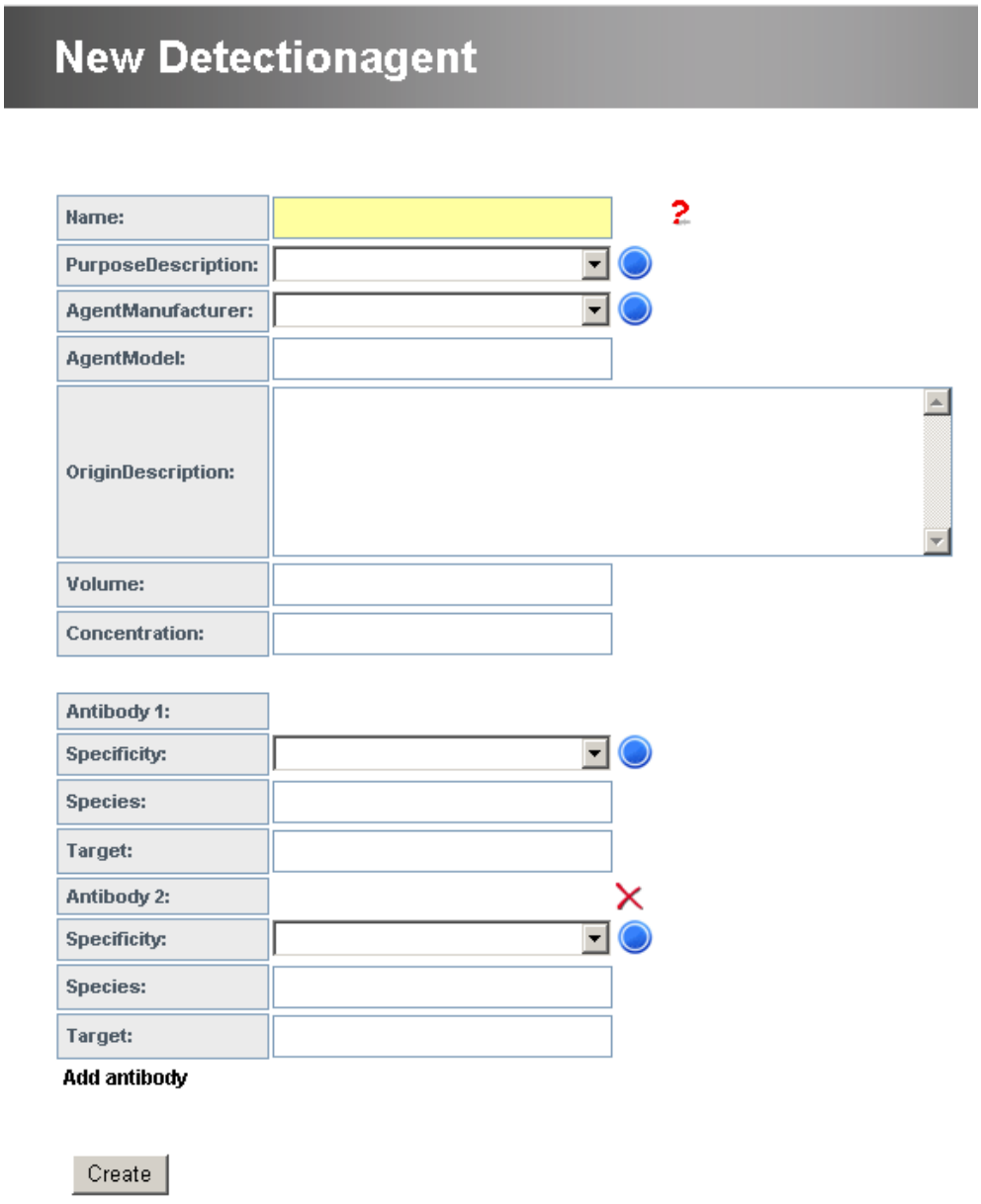


4.11 Detection agent

By clicking Sample Processing->Detectionagent you reach detection agent section.



With the “Add Detectionagent” you can add detection agents.

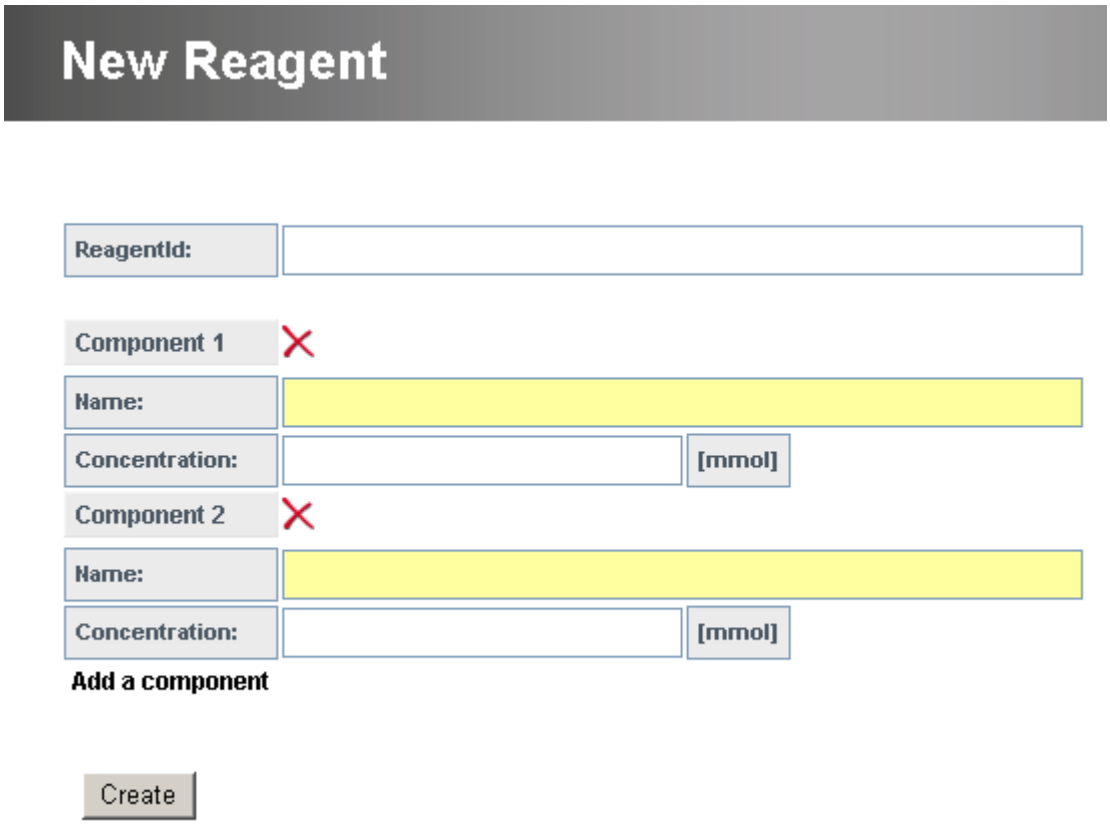


With a click on the button “Find All Detectionagents” you get an overview of all your detection agents:

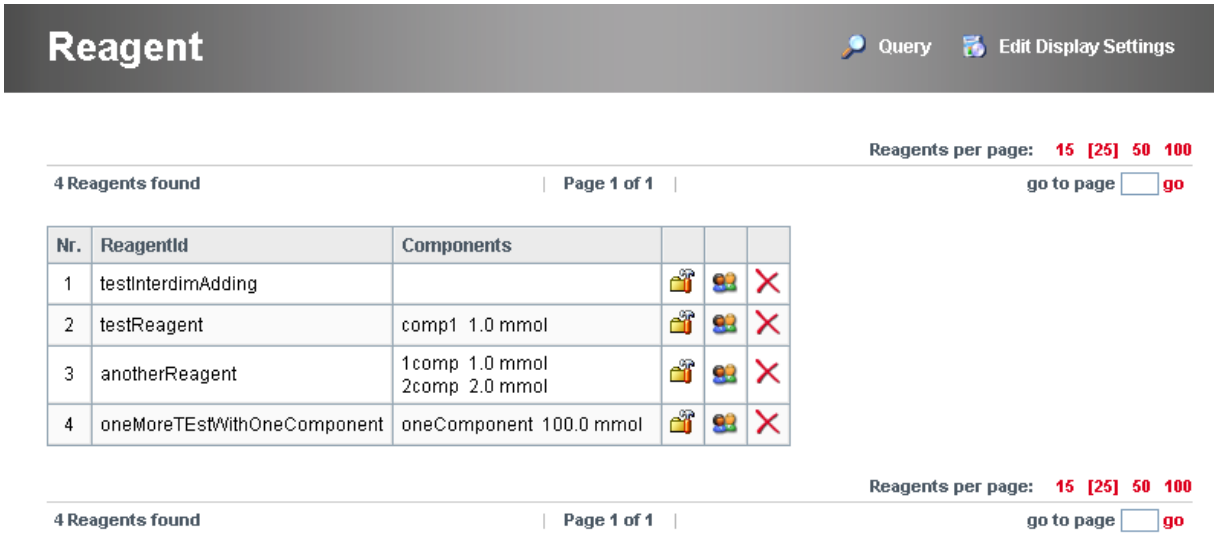
4.12 Reagent

By clicking Sample Processing->Reagent you reach reagent section.

With the “Add Reagent” you can add reagents.



With a click on the button “Find All Reagents” you get an overview of all your reagents:



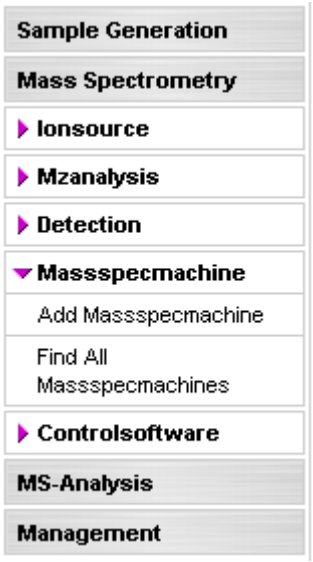
5. Mass Spectrometry:

This section describes machine and software settings for the mass spectrometry experiment.

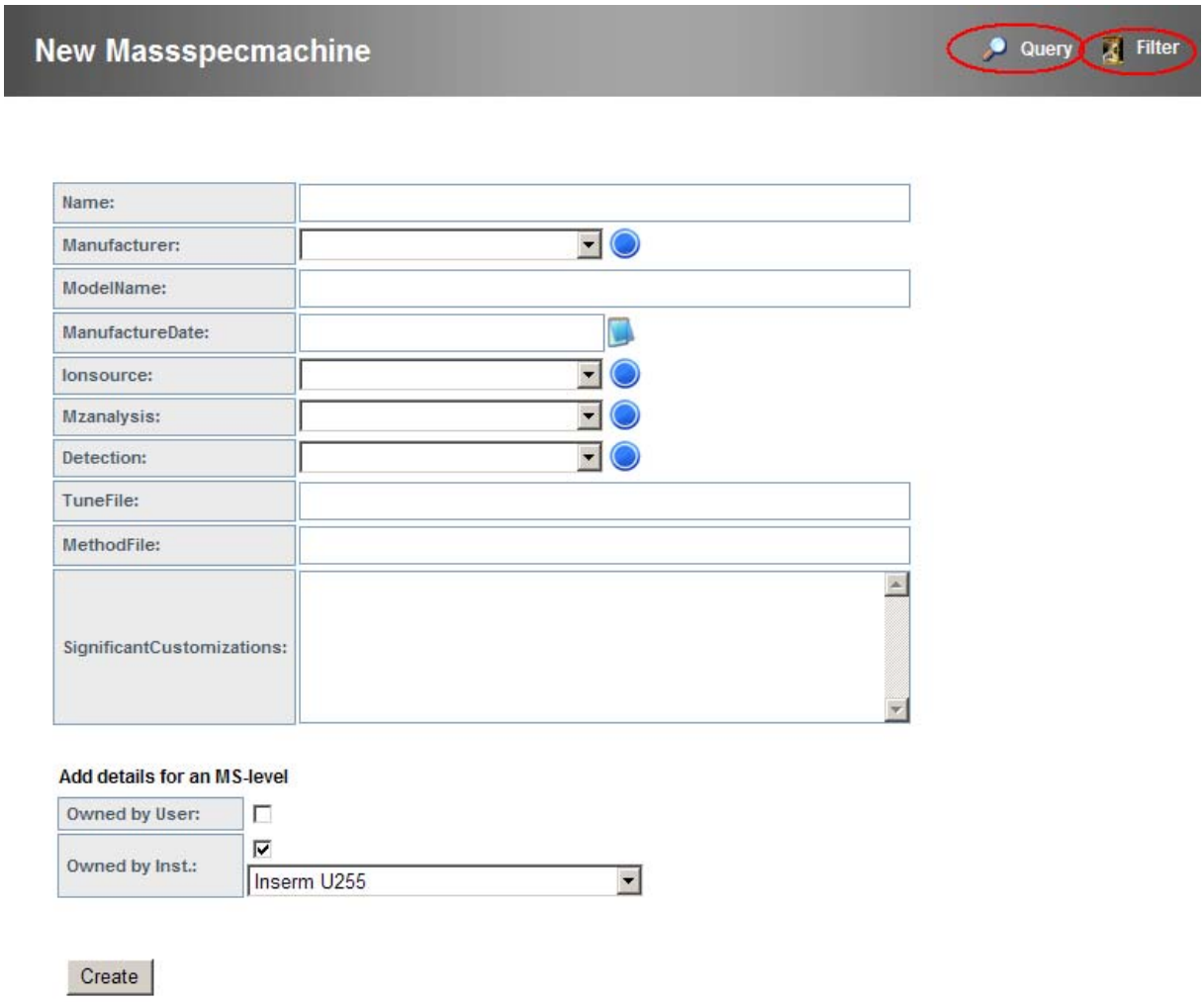
5.1 Mass Spectrometry Machine:

The main part of this section is the mass spectrometry and the other parts (except “Controlsoftware” see chapter 5.5 “Control Software”) are linked to this part. There are two

ways how to reach this part. The first one is by the link in the create/edit page of the mass spectrometry experiment (see chapter 6.1 “Mass spectrometry experiment”), the second one is by clicking on Mass Spectrometry->Massspecmachine.



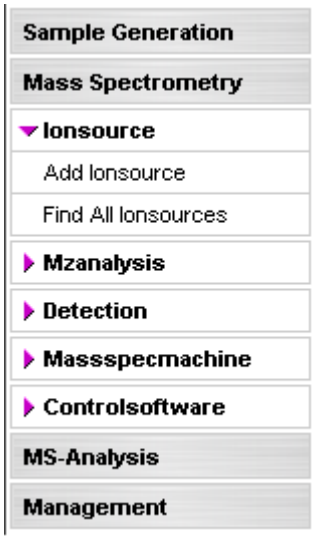
With the “Add Massspecmachine” you can add new mass spectrometry machines.



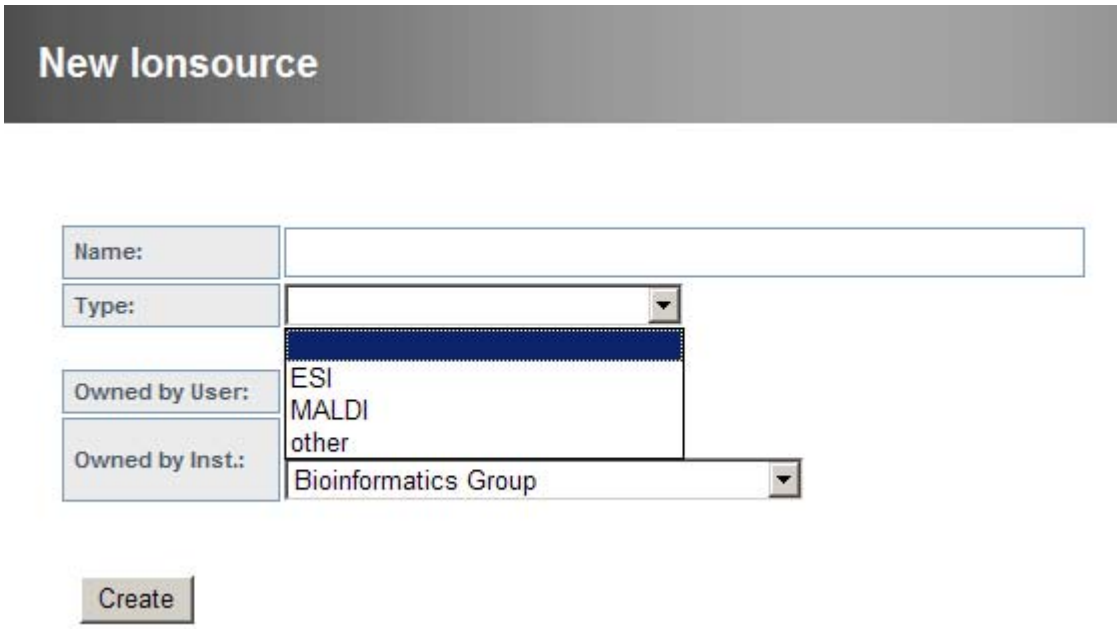
If your desired ionsource,mz analysis or detection is not in the list you can add it directly with the blue button on the right side of the select field. Read more about organisms in chapter 5.2 “Ionsource”, about mz analysis in chapter 5.3 “Mzanalysis” and about detection in chapter 5.4 “Detection”. The links “Add details for an MS-level” add details for each MS-level. You should enter details for all used MS-levels. With the links at the top for “Filter” and “Query” you can specify machine-specific filters for the upload (see 6.2) or machine specific default filters for the protein/clustered view (see 7.1).
With a click on the “Find All Massspecmachines” button you get an overview of all your mass spectrometry machines:

5.2 Ionsource:

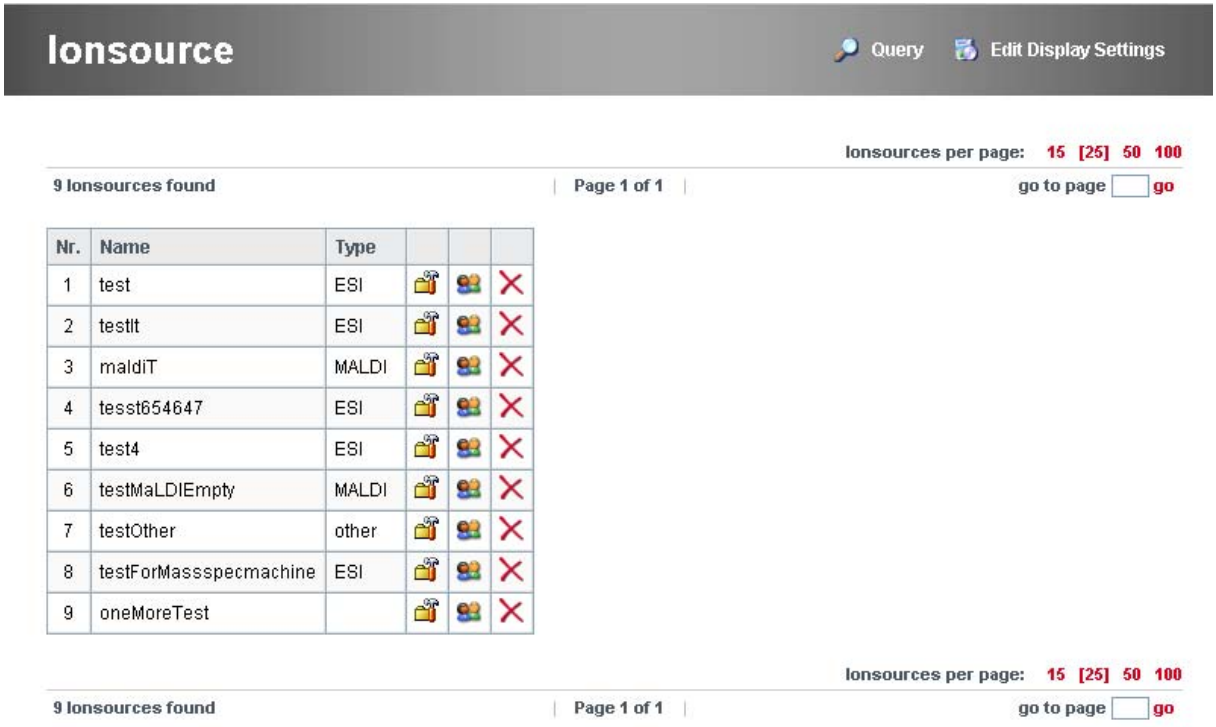
By clicking Mass Spectrometry->Ionsource you reach the ionsource section.



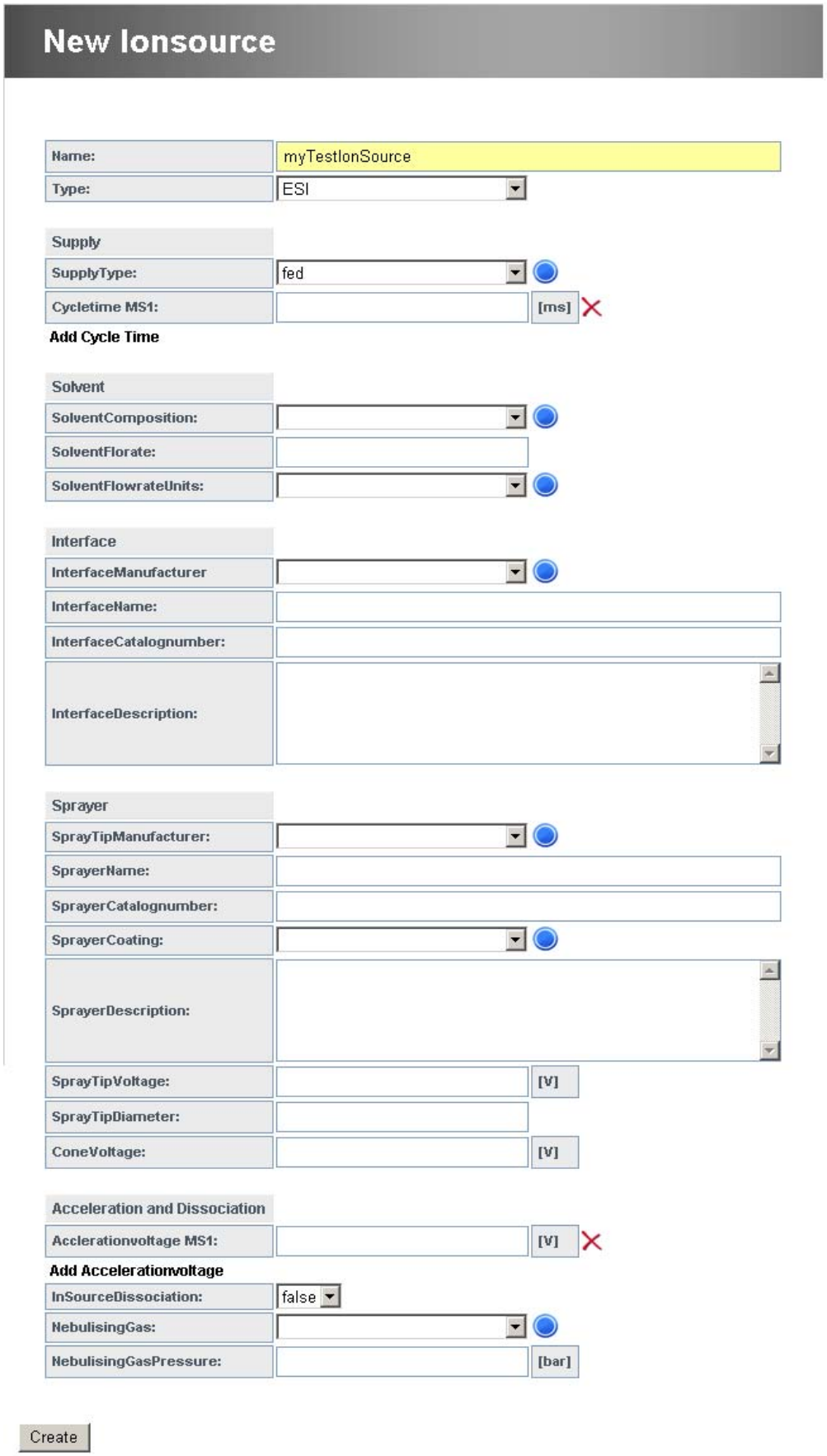
With the “Add Ionsource” you can add new ionsources.



There are 3 types of ionsources (Electrospray chapter 5.2.1, MALDI 5.2.2 and other 5.2.3) available and the input page changes correspondingly.
With a click on the button “Find All Ionsources” you get an overview of all your ionsources:

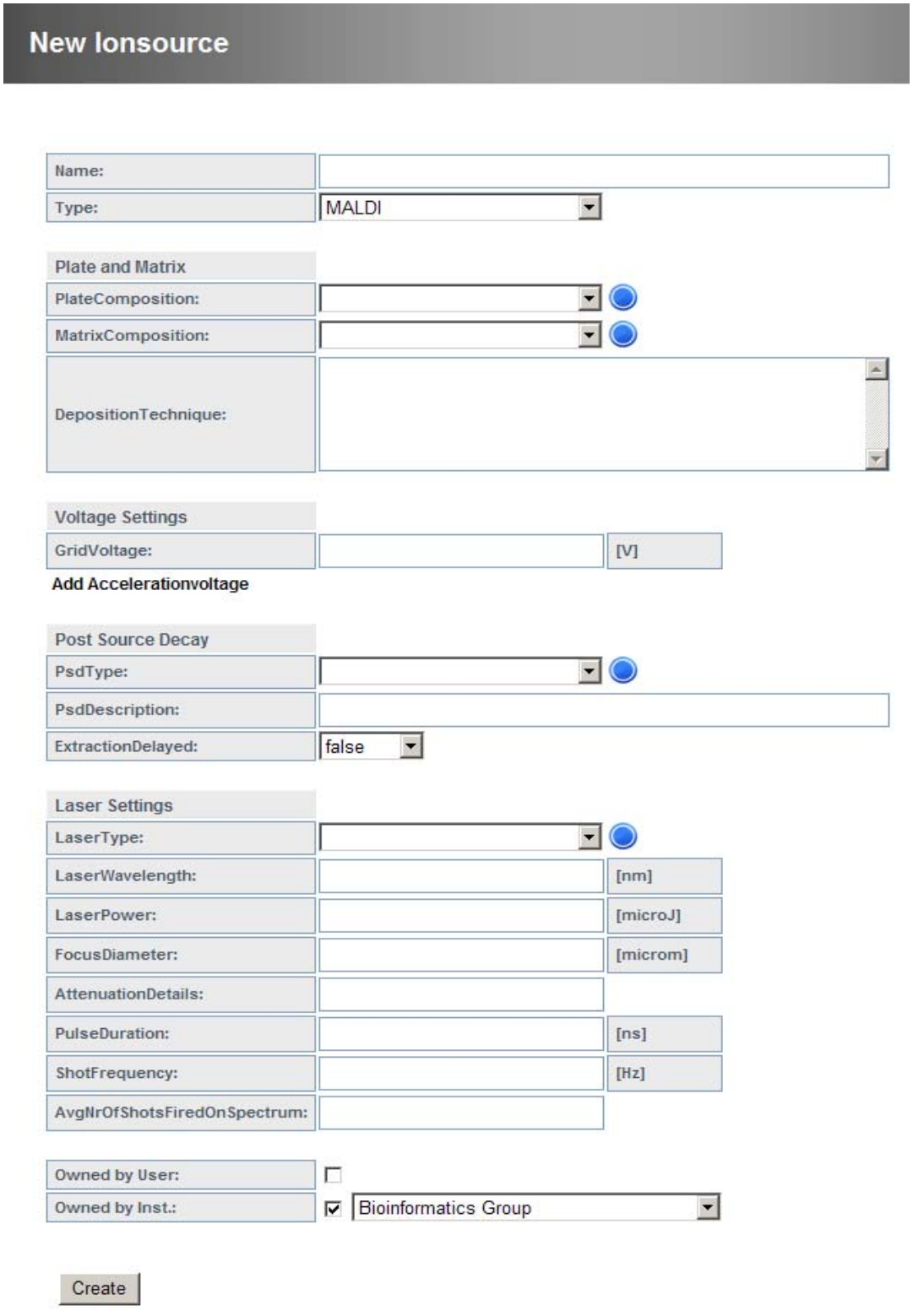


5.2.1 Electrospray:



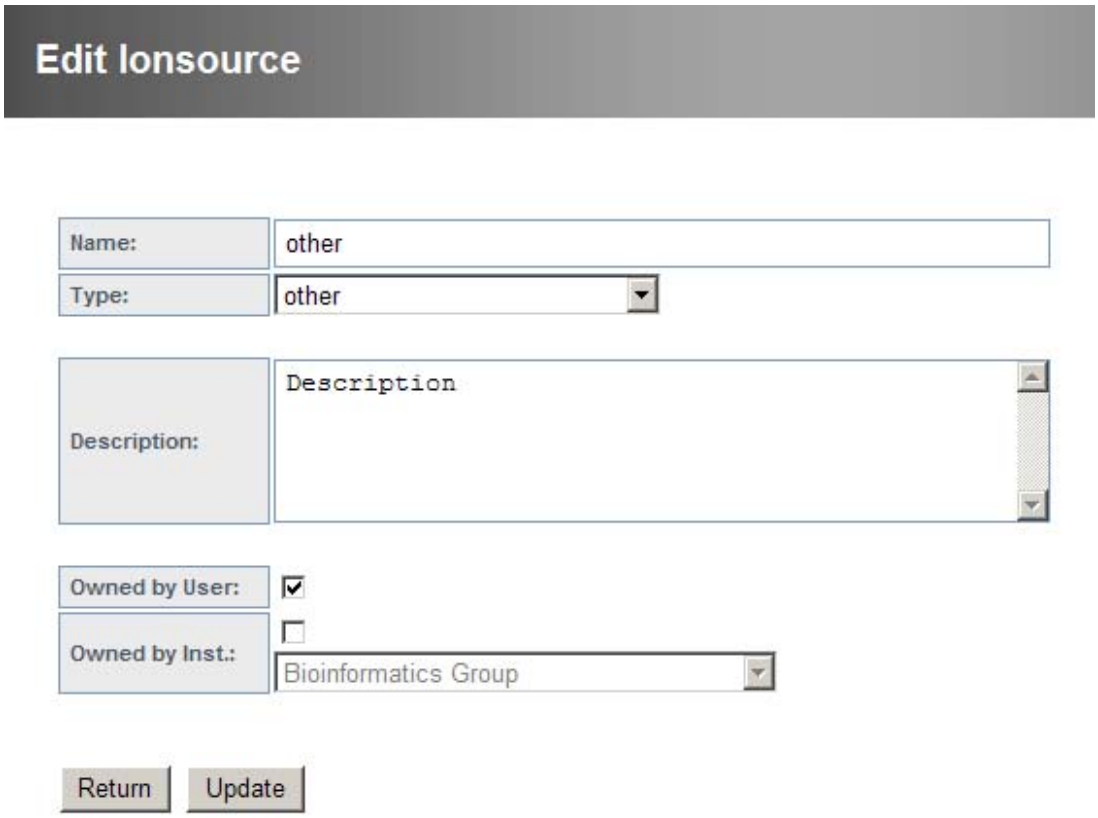
When you change the type to “ESI”, you get the electro spray input form. When you change the “SupplyType” to “fed” then the link “Add Cycle Time” appears and you can enter cycle times for all your MS-levels. In the section “Aceleration and Dissociation” there exists a second link “Add Accelerationvoltage”, where you can enter the acceleration voltages for each MS-level.

5.2.2 MALDI:



When you change the type to “MALDI” then you get the MALDI input page. Use the link “Add Accelerationvoltage” to enter the acceleration voltages for each MS-level.

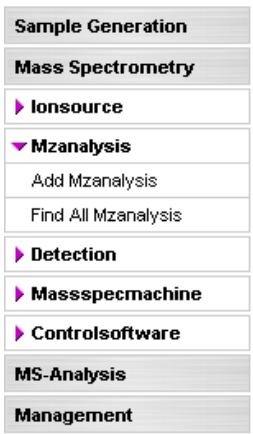
5.2.3 other:



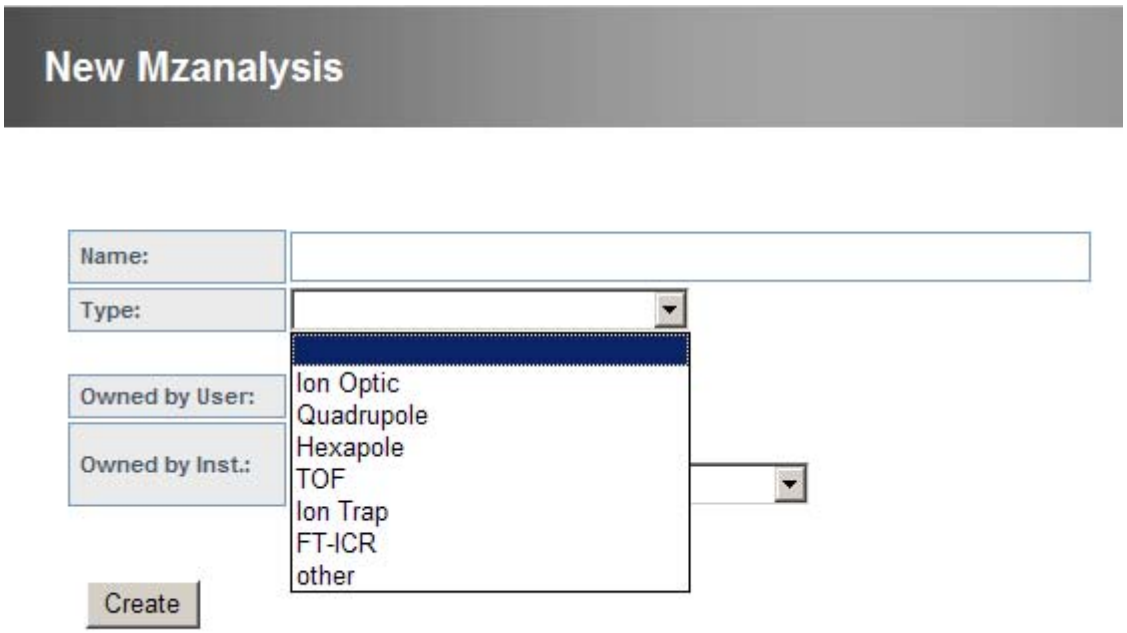
When you change the type to “other” then you get the other ionization input page. There is only an input field for the description of other ionization techniques.

5.3 Mzanalysis:

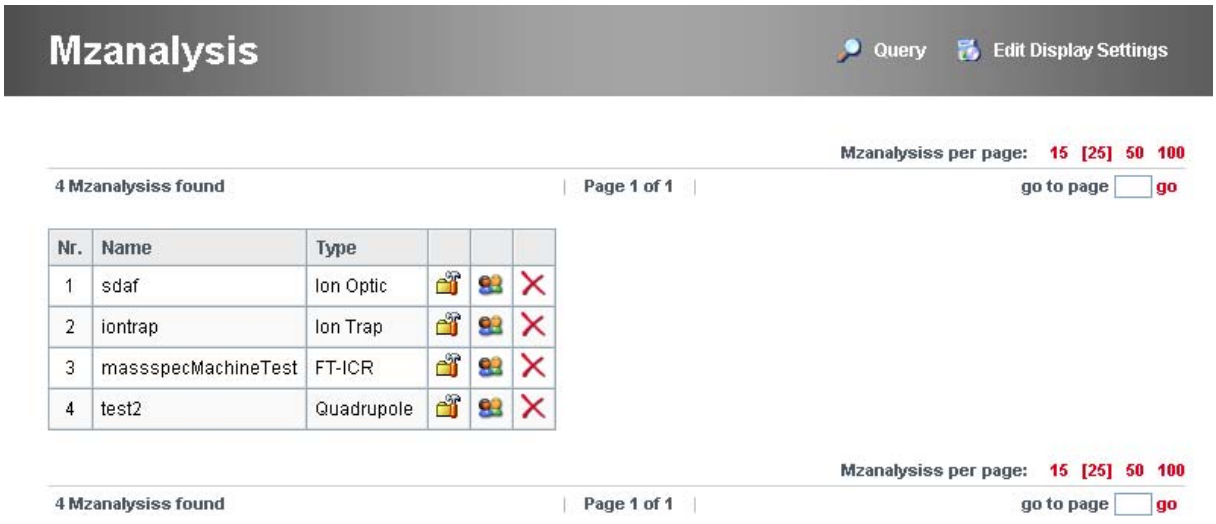
By clicking Mass Spectrometry->Mzanalyisis you reach the mzanalysis section.



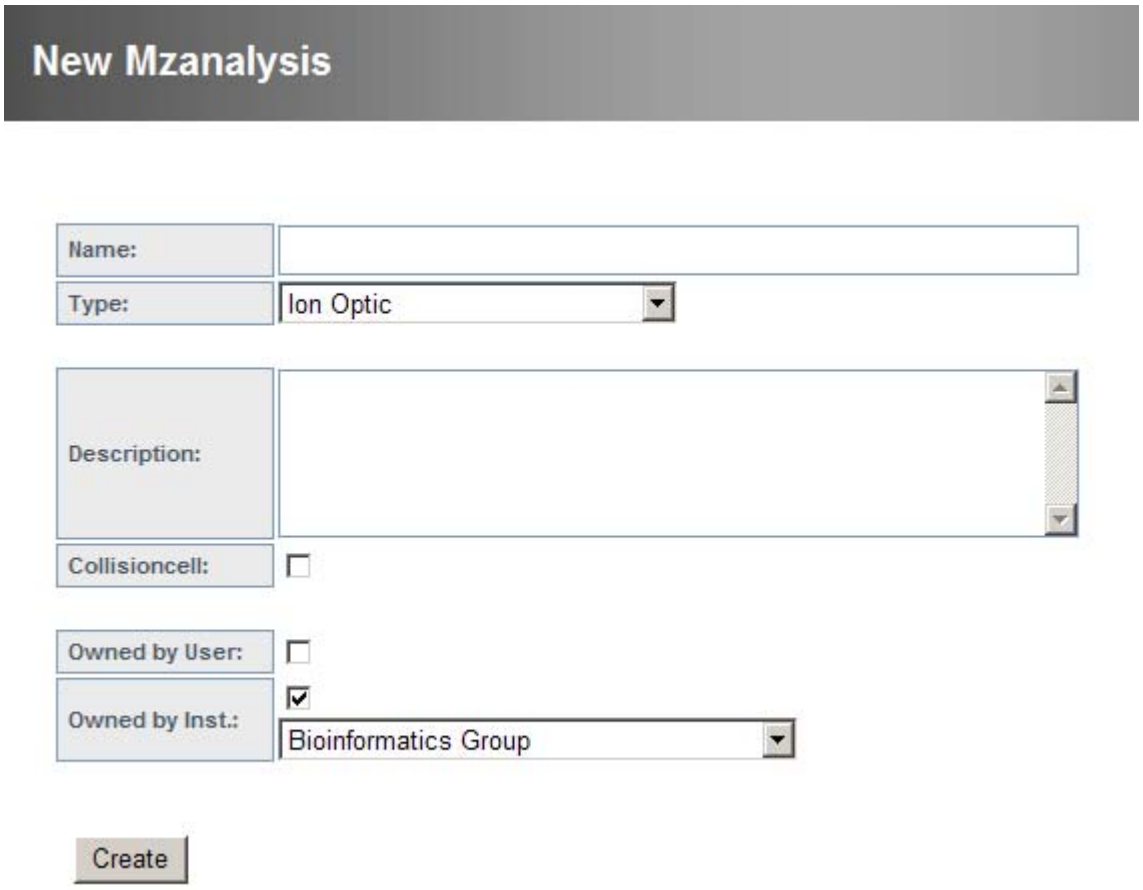
With the “Add Mzanalysis” you can add new mz analysis apparatus.



There are 7 types of mz analysis apparati (Ion optic chapter 5.3.1, Quadrupole chapter 5.3.2, Hexapole chapter 5.3.3, TOF chapter 5.3.4, Ion Trap chapter 5.3.5, FT-ICR chapter 5.3.6 and other 5.3.7) available and the input page changes correspondingly.
With a click on the button "Find All Mzanalysis" you get an overview of all your mz analysis apparati:



5.3.1 Ion optic:



For the ion optic only a description field is necessary. All of the mzanalysis types have a check box where you can enter details about the collision cell (see chapter Collision Cell 5.3.8).

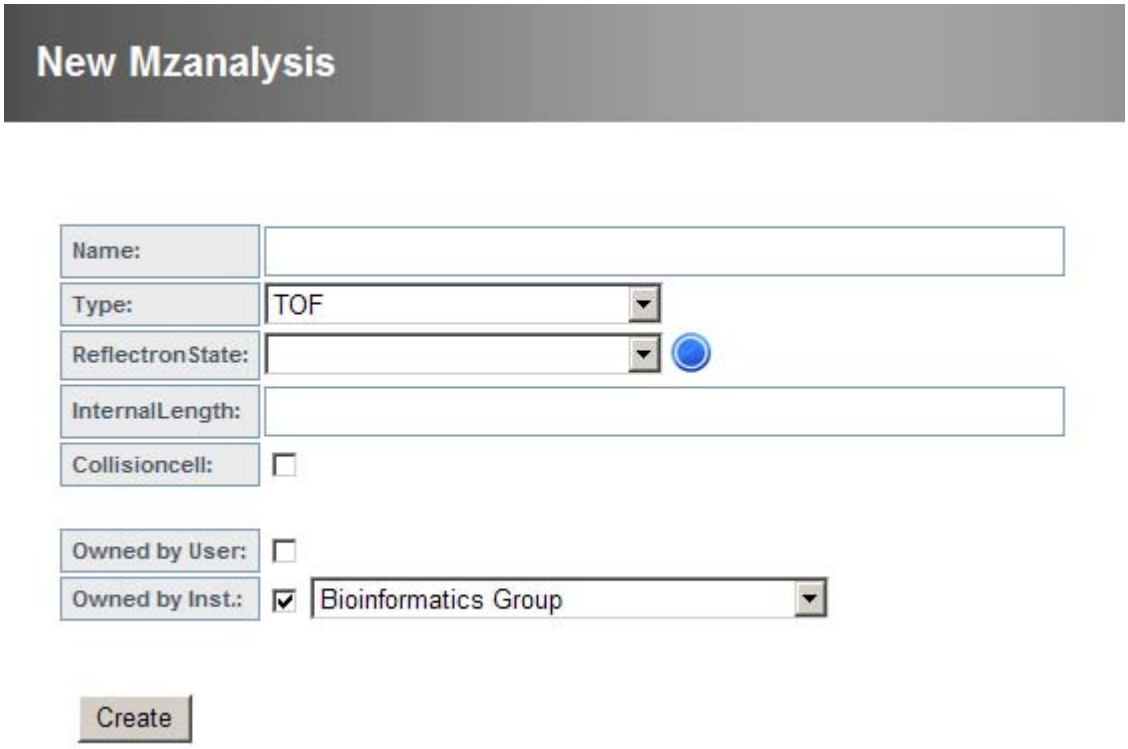
5.3.2 Quadrupole:

Same input page like ion optic see 5.3.1.

5.3.3 Hexapole:

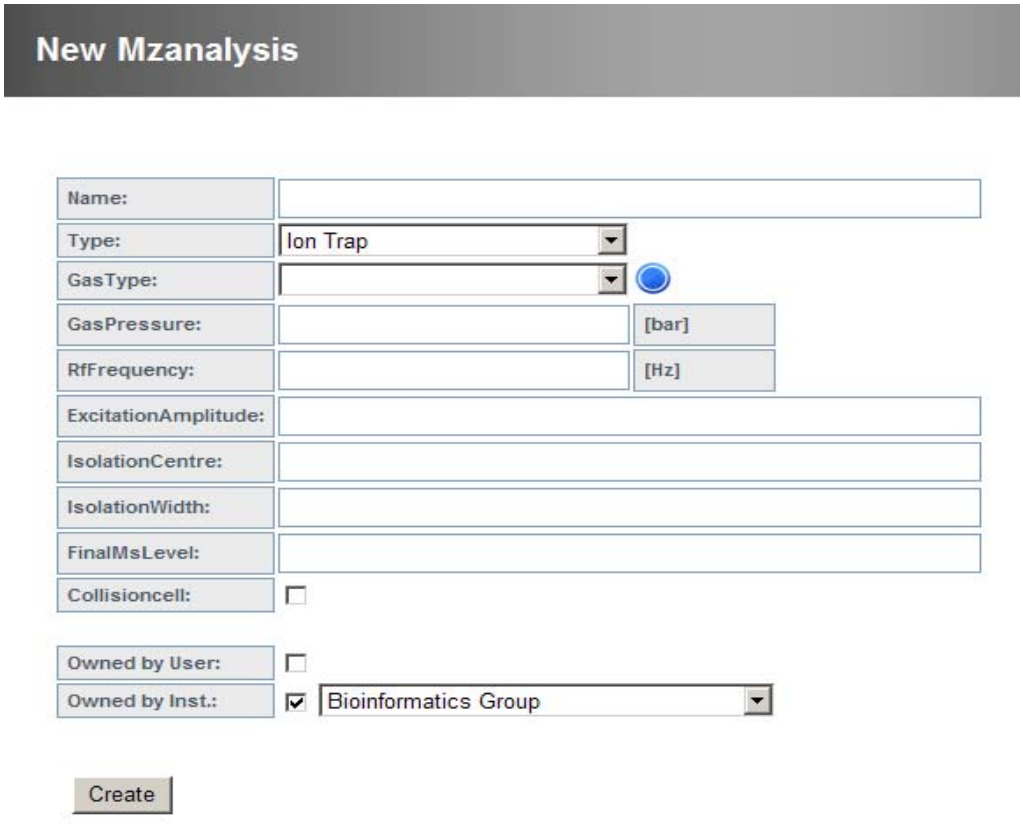
Same input page like ion optic see 5.3.1.

5.3.4 TOF:



All of the mzanalysis types have a check box where you can enter details about the collision cell (see chapter Collision Cell 5.3.8).

5.3.5 Ion Trap:



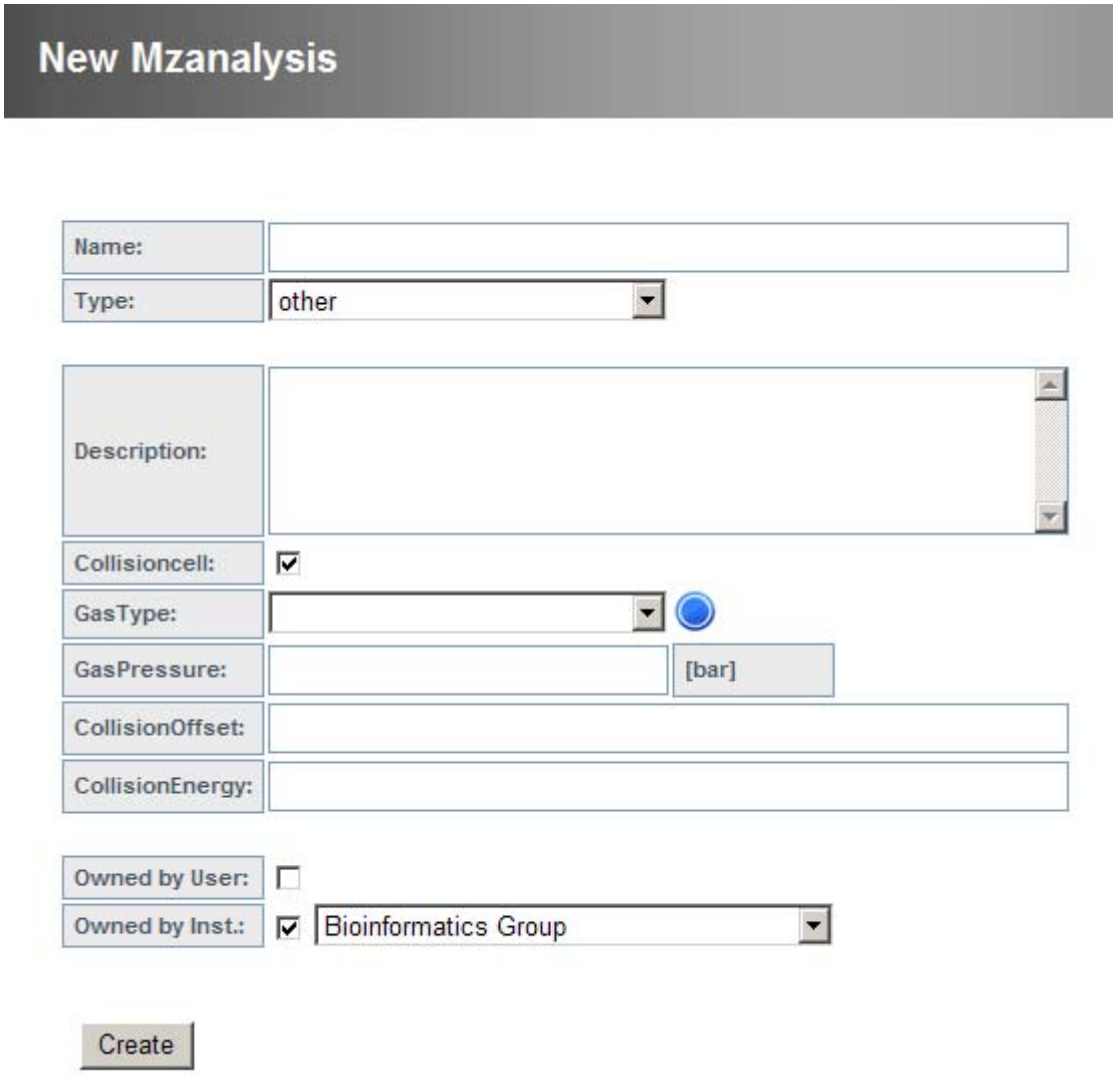
5.3.6 FT-ICR:

Same input page like ion trap see 5.3.5.

5.3.7 Other:

Same input page like ion optic see 5.3.1.

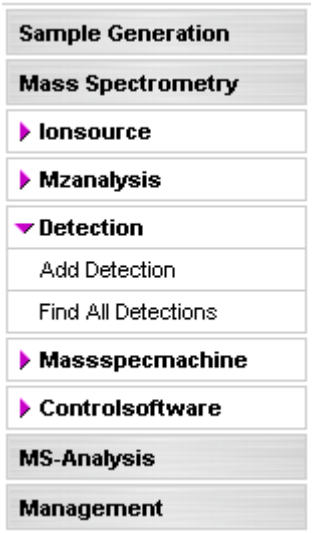
5.3.8 Collision Cell:



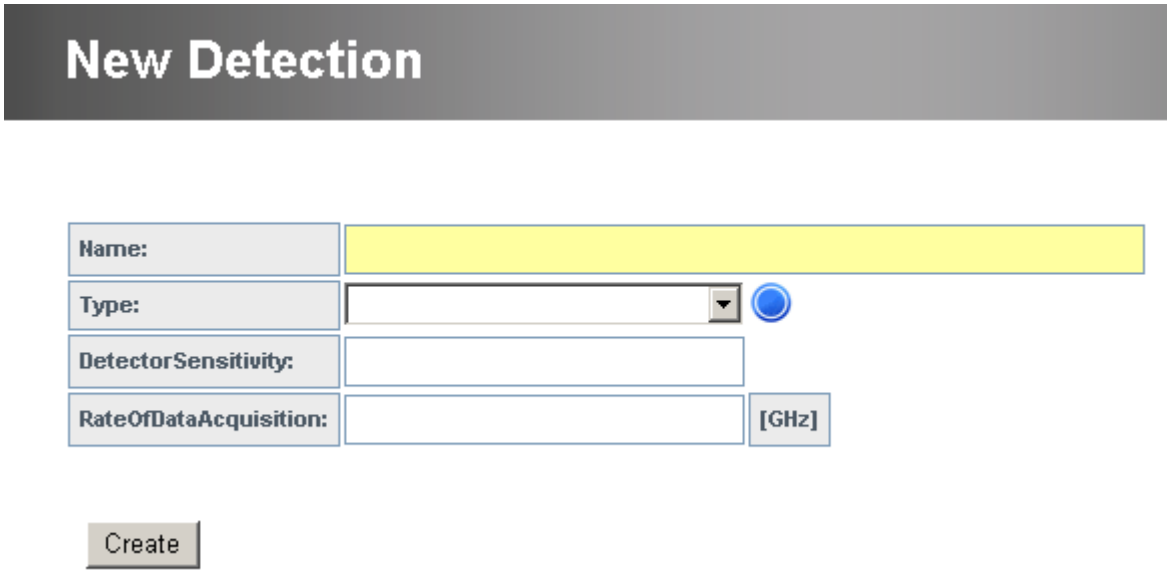
When you check the “Collision cell” check box you can enter information about the collision cell.

5.4 Detection:

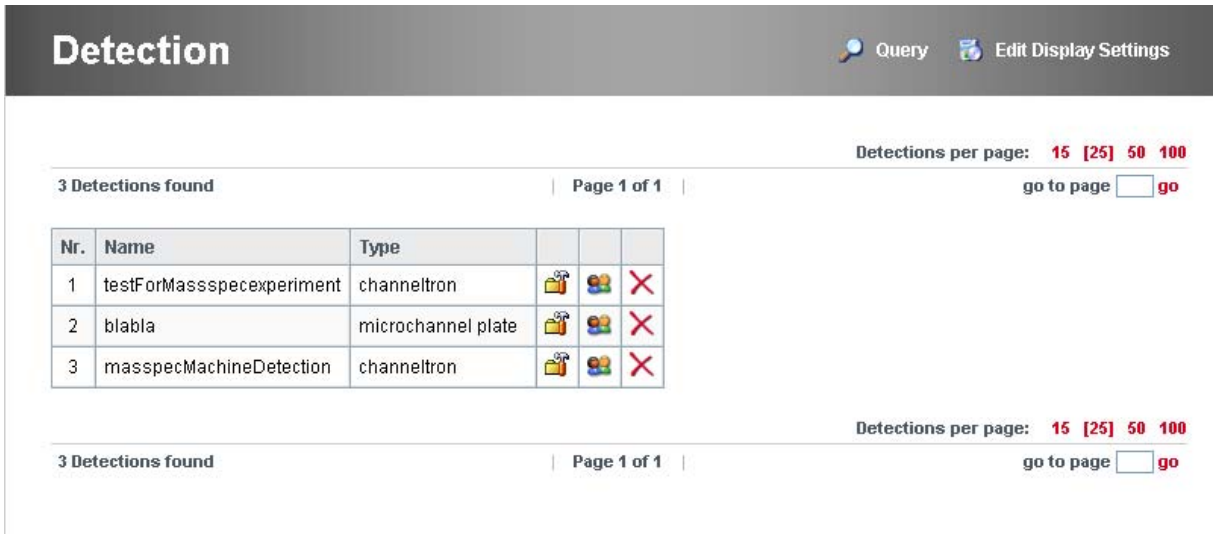
By clicking Mass Spectrometry->Detection you reach the detection section.



With the “Add Detection” you can add a new detector.

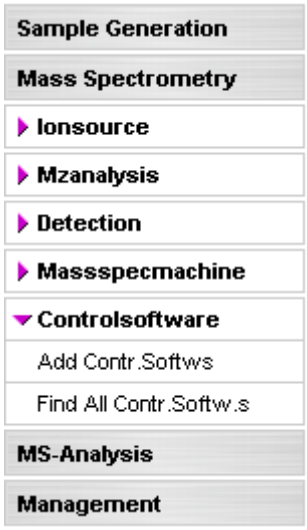


With a click on the button “Find All Detection” you get an overview of all your detectors:

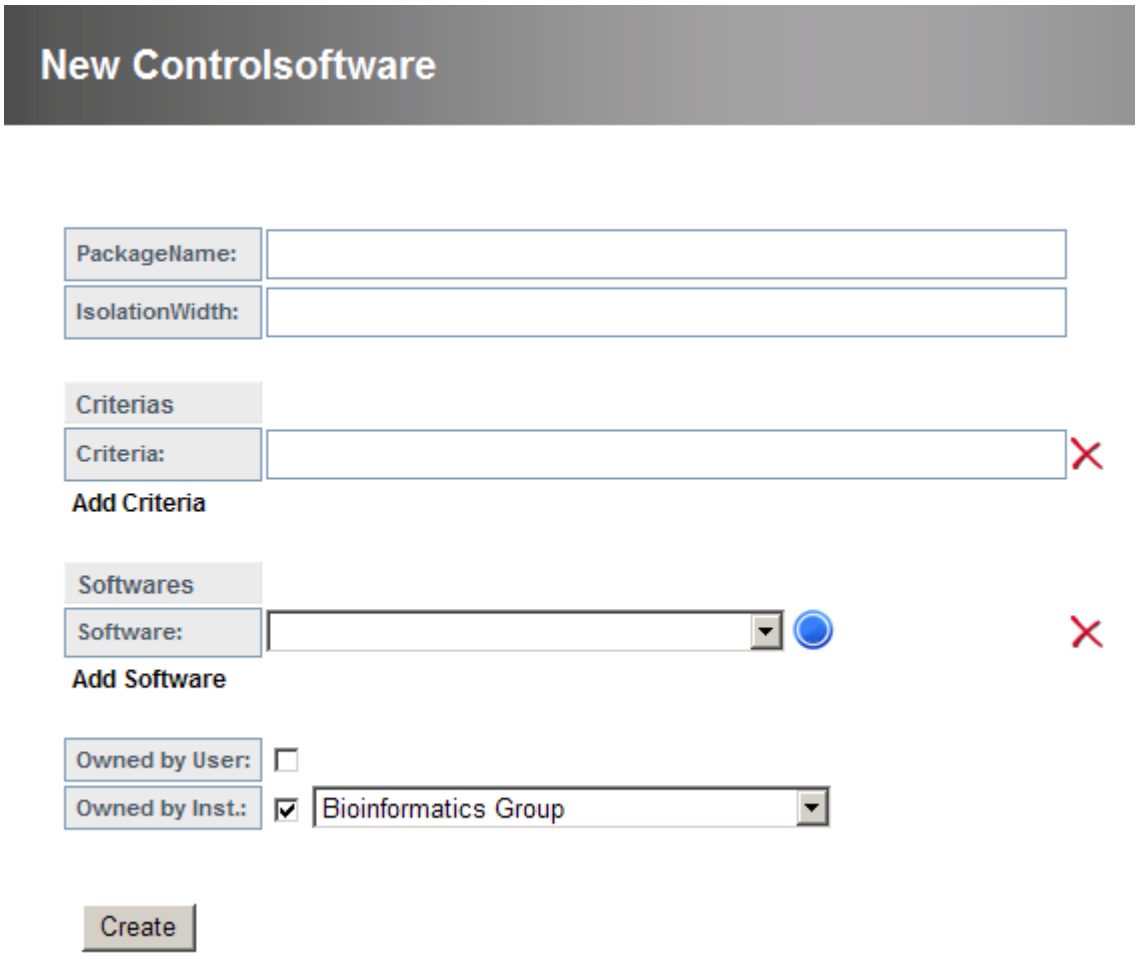


5.5 Control Software:

The control software is needed for mass spectrometry experiments (see chapter 6.1 “Mass spectrometry experiment”). By clicking Mass Spectrometry->Controlsoftware you reach the control software section.

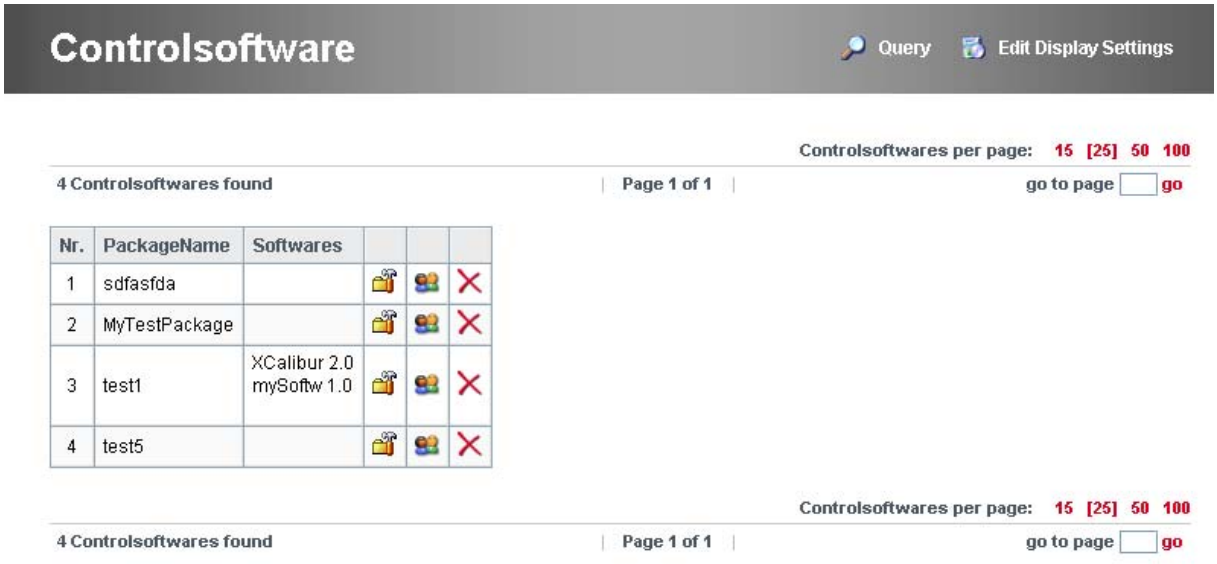


With the “Add Contr.Softws” you can add new control software.



With the link “Add Criteria” you can add switching criteria. With the link “Add Software” you can add software, which the control software consists of. If your software is not in the selection list you can add it with the blue button and you come to the create software page (see chapter 2.4 “Software”).

With a click on the button “Find All Contr.Softw.s” you get an overview of all your control software:

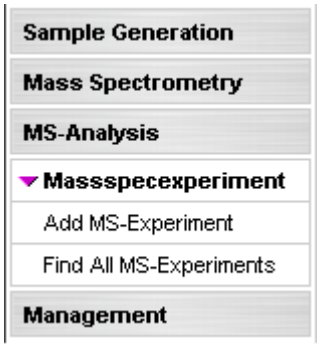


6. Mass Spec Experiment and File Uploading:

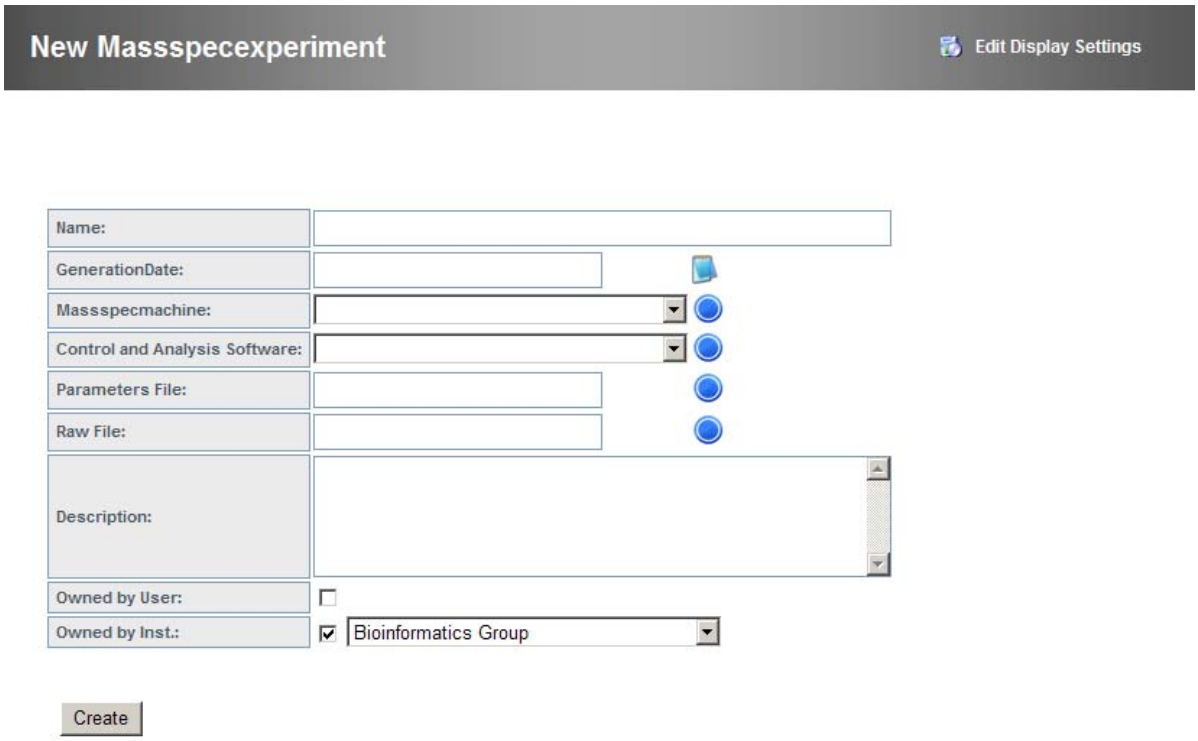
This section describes the generation of mass spectrometry experiments and how you can add searches from different search engines to them.

6.1 Mass spectrometry experiment:

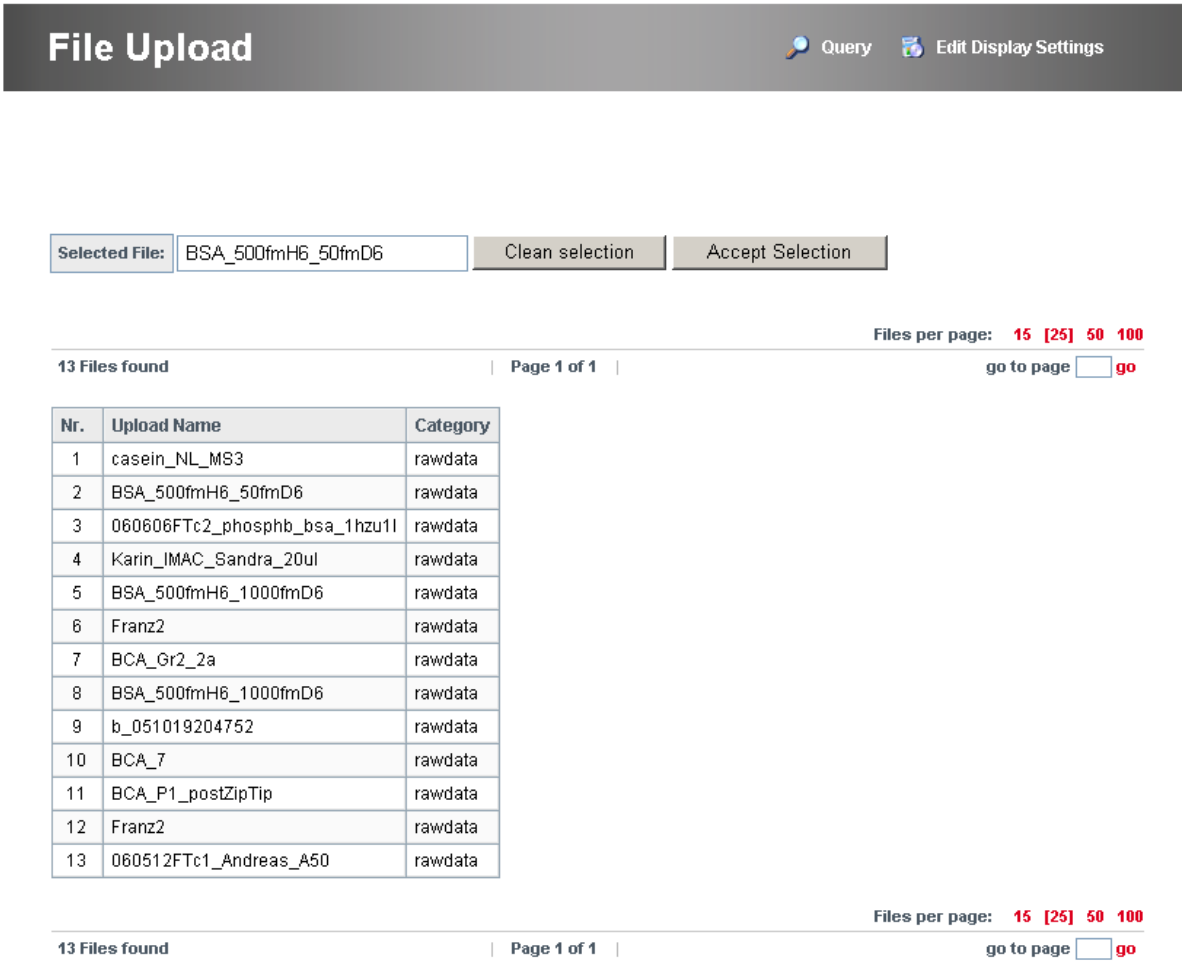
By clicking MS-Analysis->Massspecexperiment you reach the mass spectrometry experiment section. This is a central point, where all the information is linked to one another.



With the “Add MS-Experiment” you can add new mass spectrometry experiment.

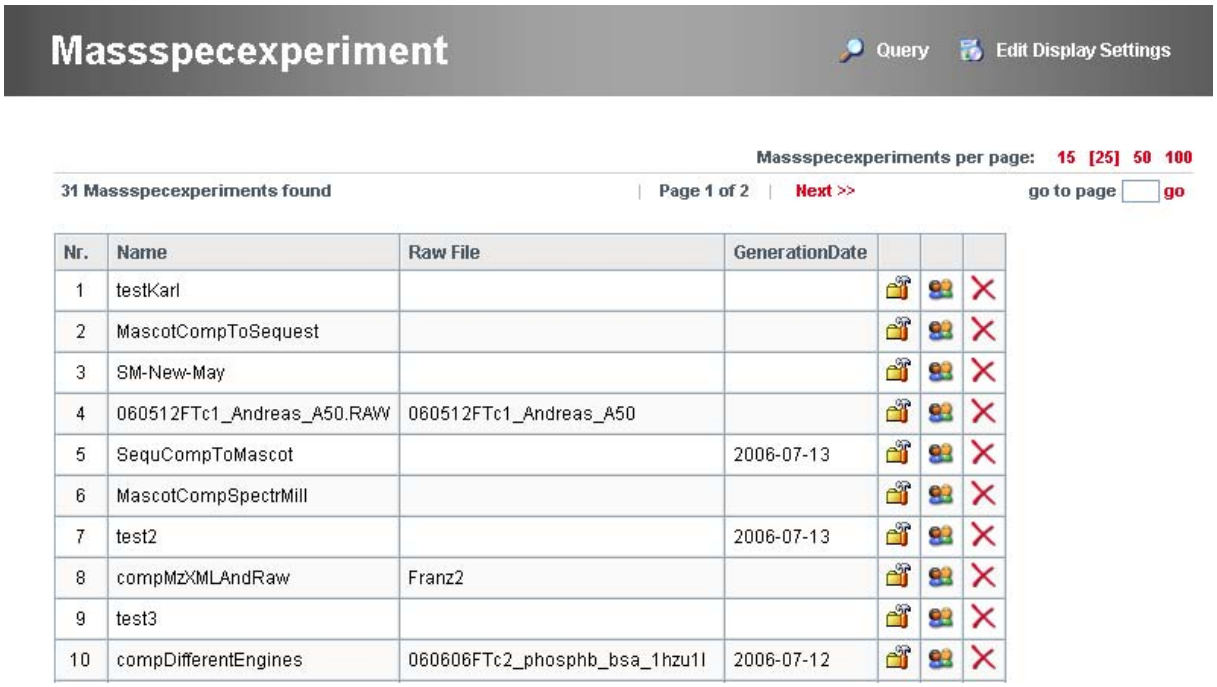


If the desired mass spectrometry machine is not in the select box you can click the blue button on the right side of the select box and you will reach the create page of the mass spectrometry machine (see chapter 5.1 “Mass Spectrometry Machine”). If the desired control and analysis software is not in the select box, click the blue button on the right side of the select box and you will reach the create page of the control software (see chapter 5.5 “Control Software”). To select a raw File click the blue button next to the “Raw File” input field. The following page will appear:



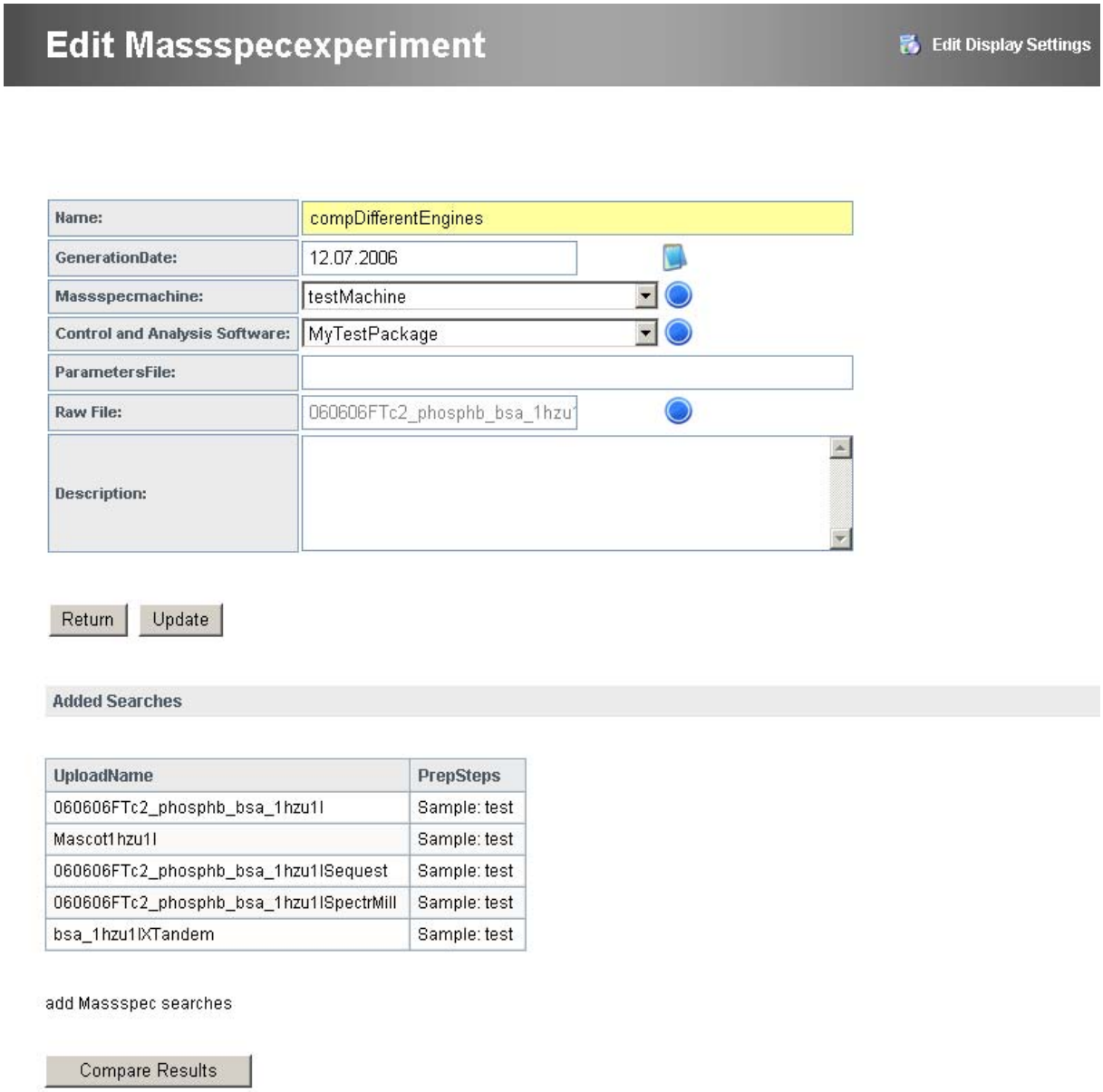
A list of all the raw files uploaded appears. When you click any of the “Upload Names” in the list the name will appear in “Selected File” field. With “Clean selection” you can clean the entry again. With “Accept Selection” this raw file is accepted for that mass spectrometry experiment and will be used for quantitative evaluations, and you return to the create page of the mass spectrometry experiment. If you want to add your mass spectrometry experiment to an analyte, see chapter 4.7 “Adding of Massspec experiments”.

With a click on the button “Find All MS-Experiments” you get an overview of all your mass spectrometry experiments:



6.2 File parsing into MASPECTRAS:

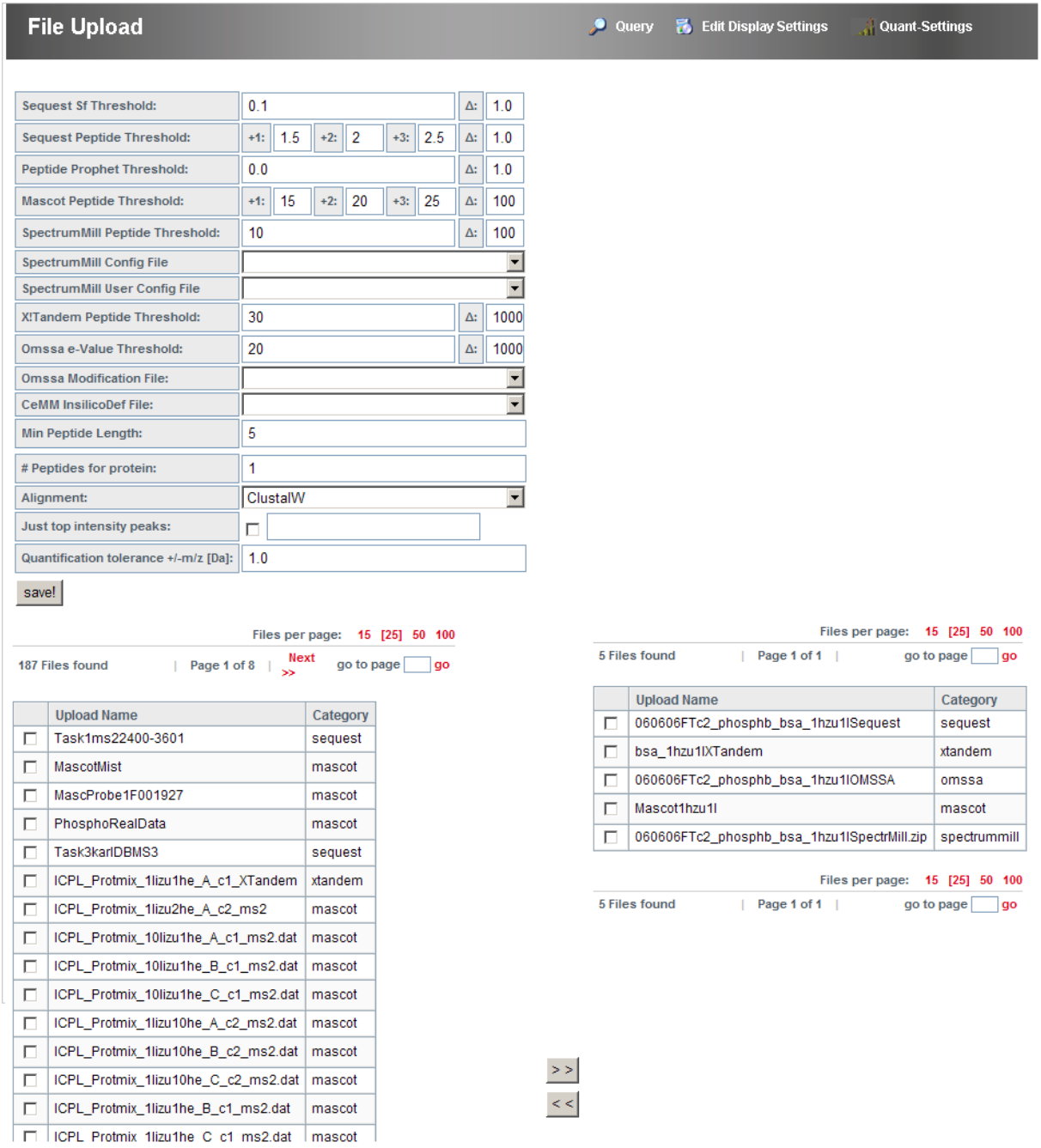
When you click on the name of the mass spectrometry experiment or the edit button, you will get the following view of your mass spectrometry experiment:



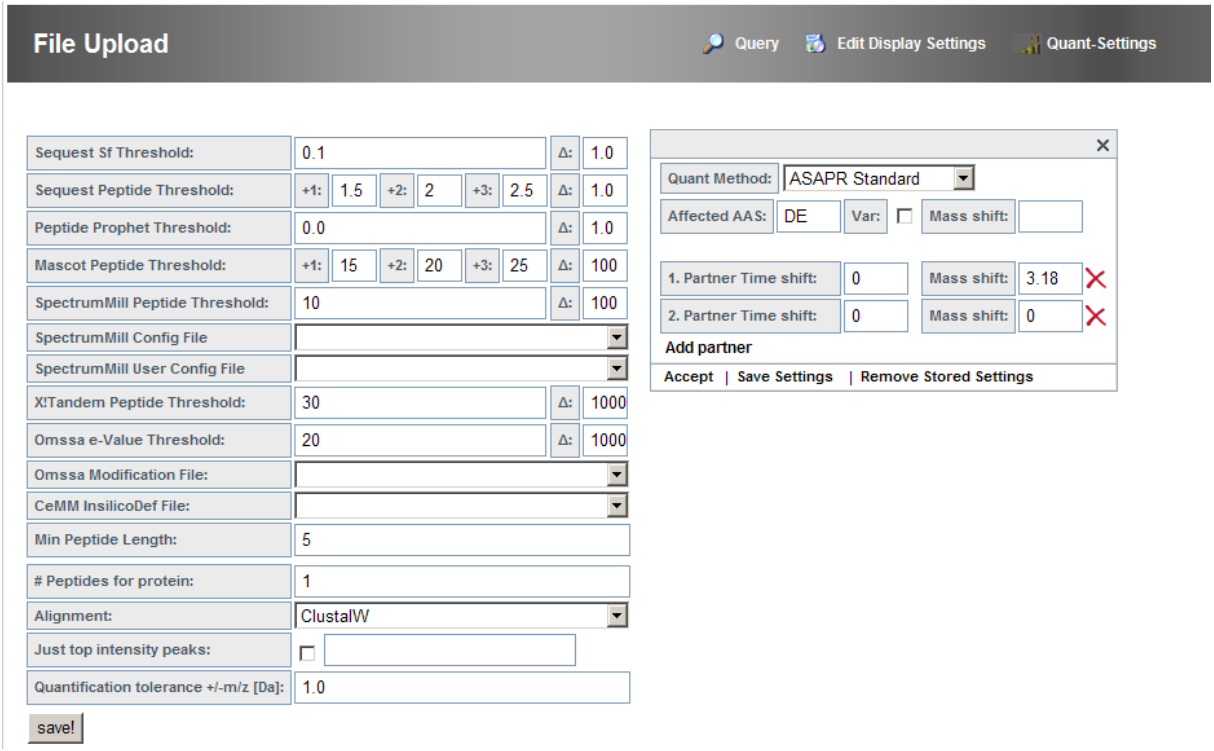
When you follow the link “add Massspec searches”, you will get a page where you can upload you search results from Sequest, Mascot, Spectrum Mill, X! Tandem, or OMSSA.

The thresholds are necessary to remove the most unlikely data. The peptide prophet threshold affects Sequest and Mascot only. For SpectrumMill (new version) you have to specify your Spectrum Mill Config File (smconfig.xml) and if you have added modificitations also the Spectrum Mill User Config File (smconfig.custom.xml). For OMSSA you have to specify the Omssa Modifications File (mods.xml). The Δ means the allowed threshold difference between the first and the rest of the found hits for one search. The “Min Peptide Length” specifies how many amino acids a peptide must have at least and the “# Peptides for protein” specifies how many peptides have to be found to accept a protein. With the “Alignment” option you can specify if an alignment of the sequence based clustering has to be done by ClustalW. The reason why this option has been made was that it was a time consuming step and that there was much more disk space consumption with the alignment. If you select “NONE” the only thing not possible is the clustered view described at 7.2. The “Just top intensity peaks” option allows the save of disk space. It is specifiable how many (the ones with the best intensity)

should be store (e.g. if you enter 100 the 100 peaks with the highest intensity will be stored in the database while the rest will be discarded).



The  icon opens a new box where you can specify quantification options (if needed):



These settings are for the detection of partners which are not identified by MS/MS. With “Quant Method” you can specify the used quantification method. “ASAPR Standard” is the standard ASAPRatio peak detection (works better with Ion Trap data) and “ASAPR Enh. Valley” is the new version (works better with FT and Orbitrap data). In general, the more accurate the mass detection of the mass spectrometer is the more feasible the “ASAPR Enh. Valley”. Then you can specify which amino acids carry the modification (for C-terminus and N-terminus write: C-term or N-term). When you just searched with a fixed modification and you have not found partners than uncheck “Var:” (the meaning is the first partner for the comparison a variable modification). Then in the next line you have to specify the expected mass and time shift to the first partner/fixed modification. It is possibly to specify as many partner modifications as you like.

Adding and removing of searches to a spot (or band) works the same way like adding of samples to experiments works (see section 3.2.2).

After the files have been selected the following processes are started (you will see the same steps in the Upload Status Section):

“Step 1/5 (Parsing)”: Reads the necessary file (or files), filters the data and builds the corresponding value objects
“Step 2/5 (Transferring hits)”: Stores the found proteins into the database
“Step 3/5 (Storing peaklists)”: Stores the peaklists and the connected peptidehits and links them to the corresponding proteins
“Step 4/5 (Calculating)”: Retrieves the protein sequences from the database (if not already stored), calculates the proteinhit score and the sequence coverage of the hit
“Step 5/5 (Protein Grouping)”: Clusters similar proteins together in protein groups.

After these five steps an automatic calculation of a relative quantity for each peptide is started, when a raw file for the mass spectrometry experiment is selected (see chapter 6.1 “Mass Spectrometry Experiment”). The progress bar for the calculation starts again at 0%. You can meanwhile validate your data. The view on the data is the same, the only difference is that in the peak-area file you will find no value until the calculation has finished.

7. Analysis:

There are two ways to analyse (compare) your data:

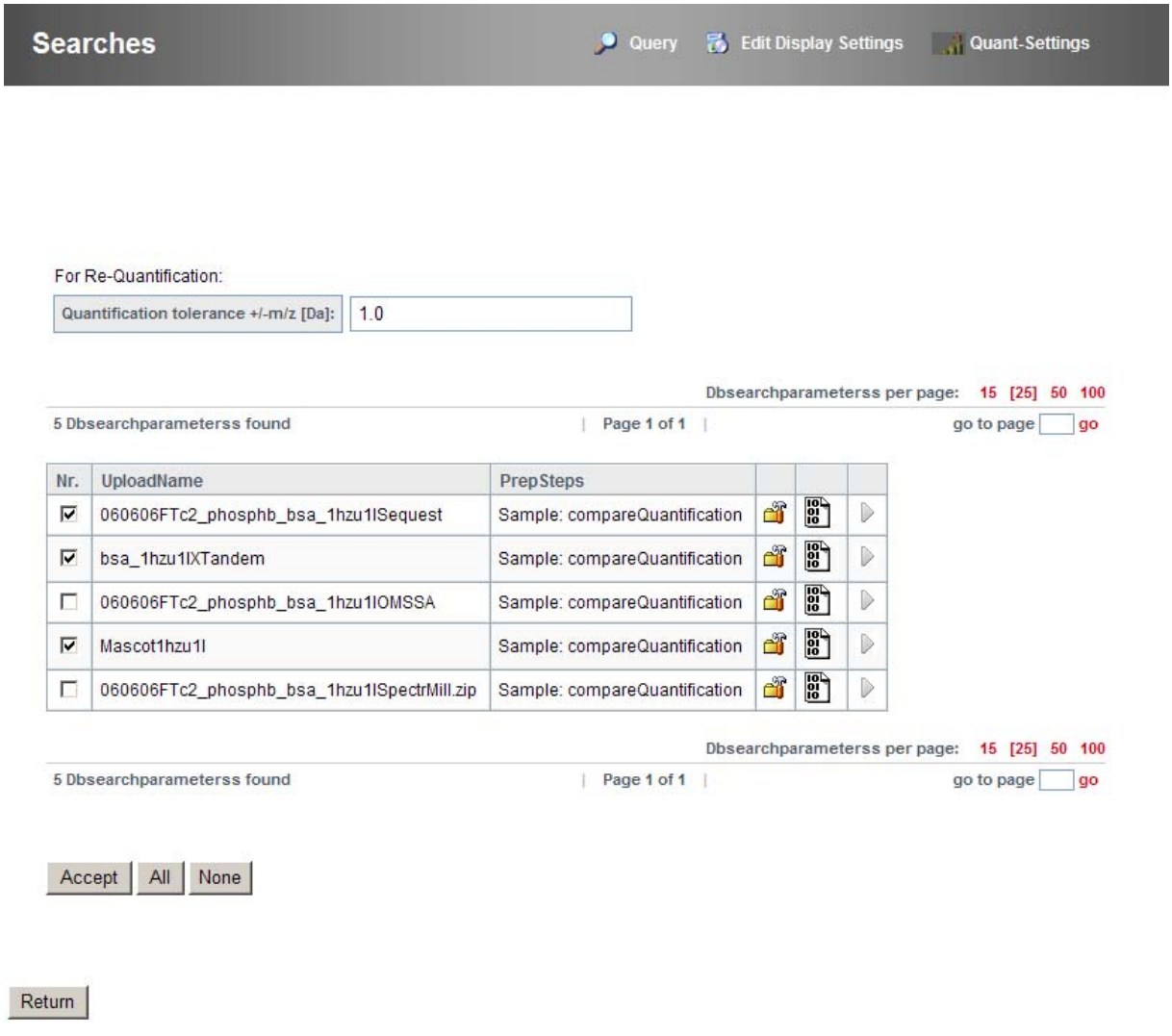
1. To click directly on the upload name table below the mass spectrometry experiment (see first picture section 6.2 “File parsing into MASPECTRAS”)
2. To use the

   Compare Results

   button.

You will find this button when you list your samples from one experiment (then you can compare all searches that are in this experiment) or in a list of the “Uploaded Searches” in the mass spectrometry experiment (see first picture section 6.2 “File parsing into MASPECTRAS). Further buttons of that type are planned at every analyte and at every sample processing step.

When you push this button you can select which of the uploaded searches you want to compare. All uploaded searches below this data point are displayed. Also the preparation steps that have been used are shown.



When you click the , you can edit the mass values of your uploaded modifications. This could be useful for the comparison, because the system could only group together peptides with the same mass shift.

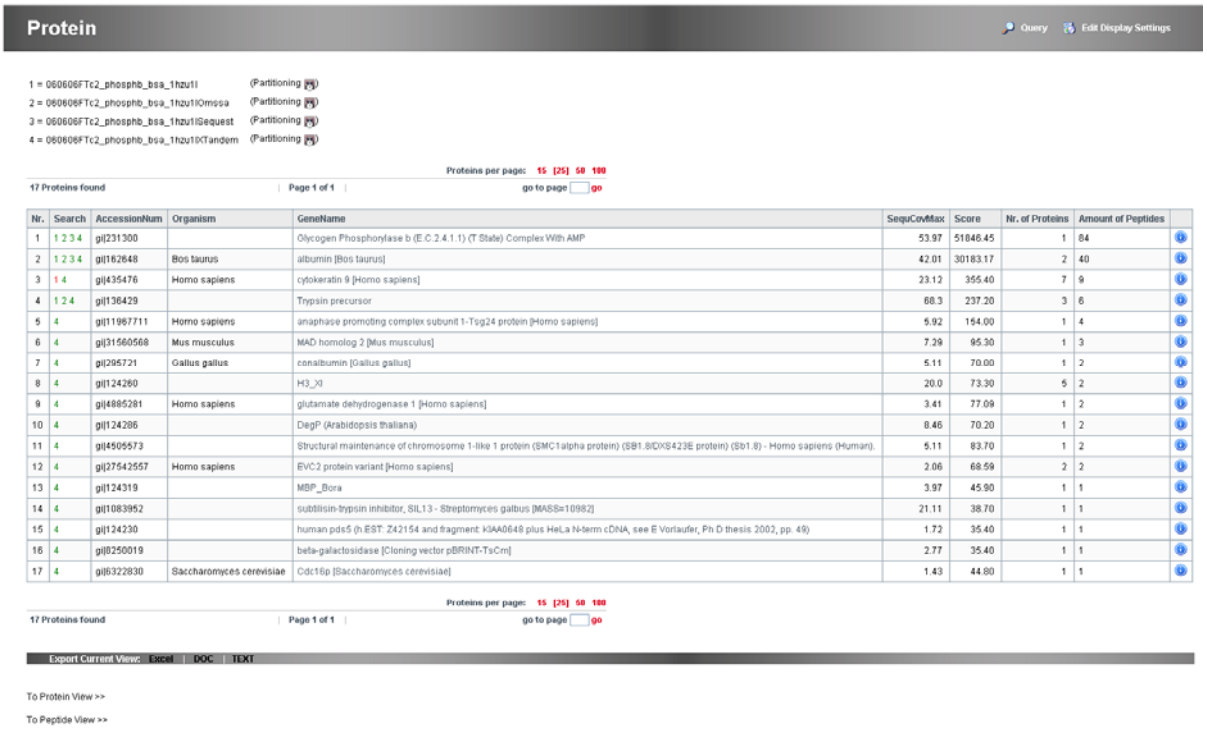
Hydrogen	1.007825
Carbon	12.0
Nitrogen	14.00307
Oxygen	15.99491
Electron	5.49E-4
C_term	17.002735
N_term	1.007825
Oxidation (M)	15.994904
NeutralLoss1	0.0
Phospho (ST)	79.966324
NeutralLoss2	97.976896
Phospho (Y)	79.966324
NeutralLoss3	0.0

Return Update

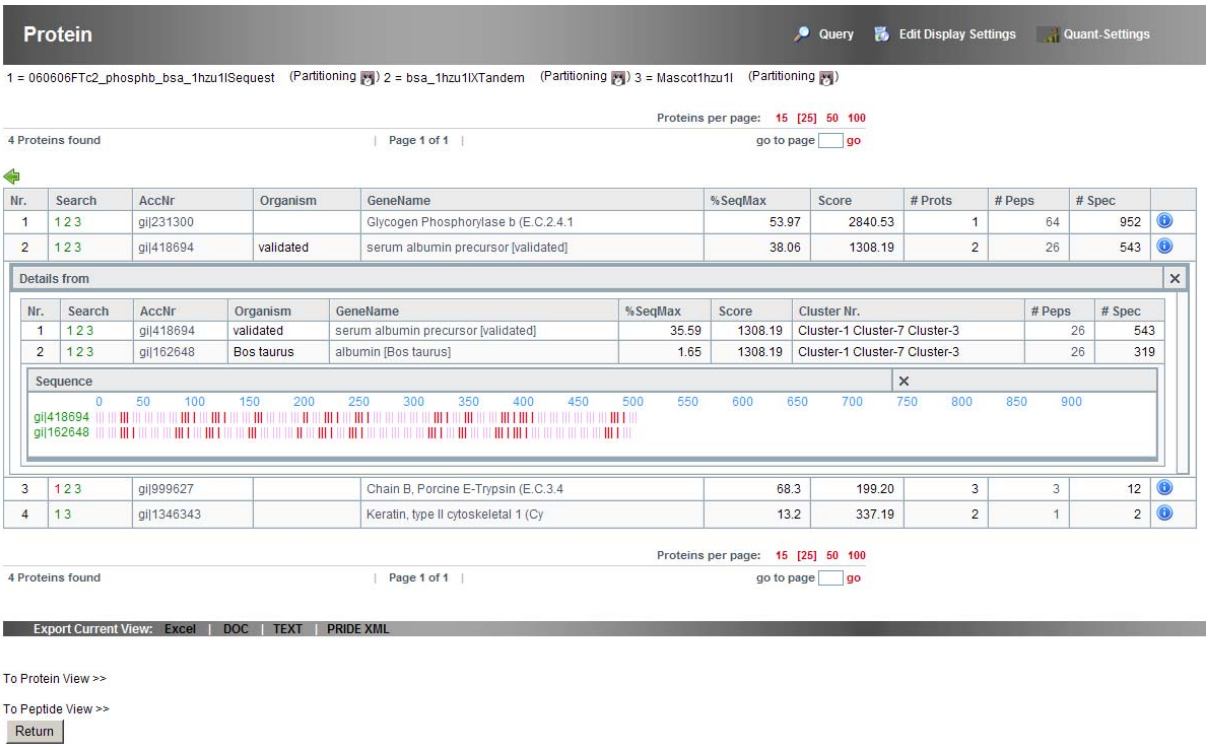
The icon restarts the quantification of an uploaded file. After clicking you have to watch the upload status page to see the progress (see 2.3). If there is no raw file specified the icon is greyed out.

The icon fetches or re-fetches the external information. The icon is greyed out if the corresponding information about the external sources is not given (see 2.1). If you want to know how to display the external information see chapter 7.4.

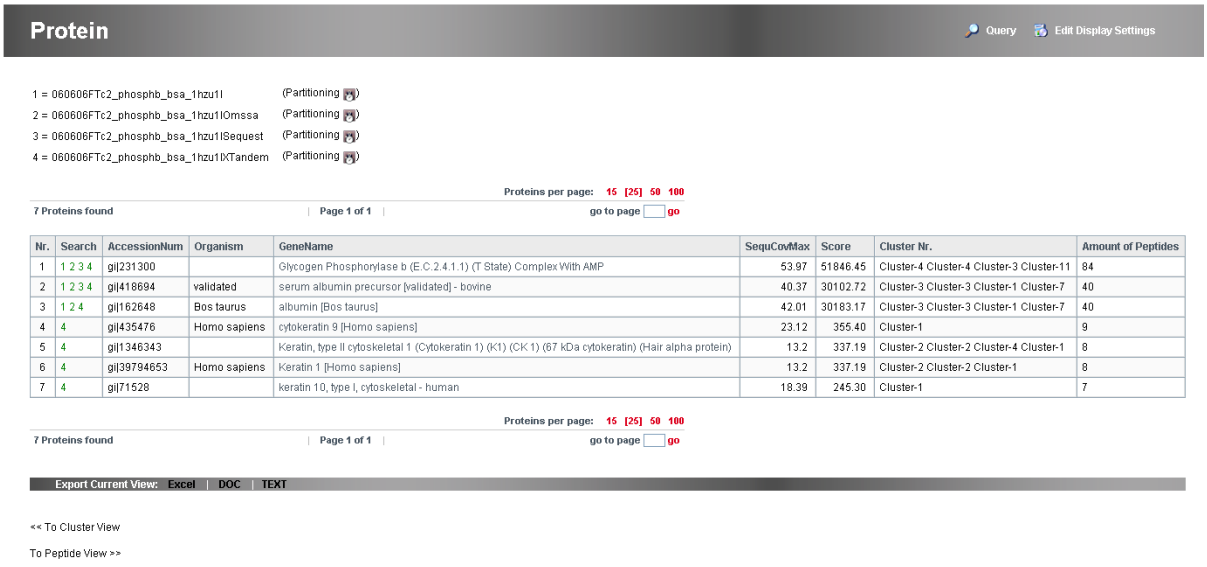
7.1 Protein comparison:



Below the header the searches that you have selected are listed by their names and numbers are assigned to find them in the table below. Next to the names there are links in brackets called “Partitioning”. With these links you reach a page with a more detailed description of the cluster (7.2).
The table below lists the found proteins. When you reach the page the proteins are clustered together. The proteins are sorted by their sequence coverage. The protein with the best sequence coverage is getting displayed as substitute for all the proteins in the cluster. In the “Search” column the numbers indicate the searches, by which a protein has been found. You can reach the combined peptide view of the protein when you click on the “GeneName” of the protein (7.5). If you want to see the peptide view of only one search there is a link on the number if the number is green. A red number indicates that this substitute protein was not found with this search but another protein in the cluster has been found with this search.
The “Nr. of Proteins” column shows you how many proteins have been put together in one cluster. When you push the blue button you get all proteins of that cluster listed.



The “Cluster Nr.” indicates the cluster where the protein is located. The order is the same as in the “Search” column.
If you don’t want to see the clustered view at all you can click on the “To Protein View>>” at the bottom of the page to get all proteins displayed.



The “<< To Cluster View” brings you back the cluster view.

The export bar lets you export the table with the selected columns in different file formats.

The “PRIDE XML” link generates a XML File in the PRIDE 2.0 XML Format, which is needed to export your Experiment to the PRoteomics IDEntifications database, a centralized, standards compliant, public data repository for proteomics data (http://www.ebi.ac.uk/pride/).

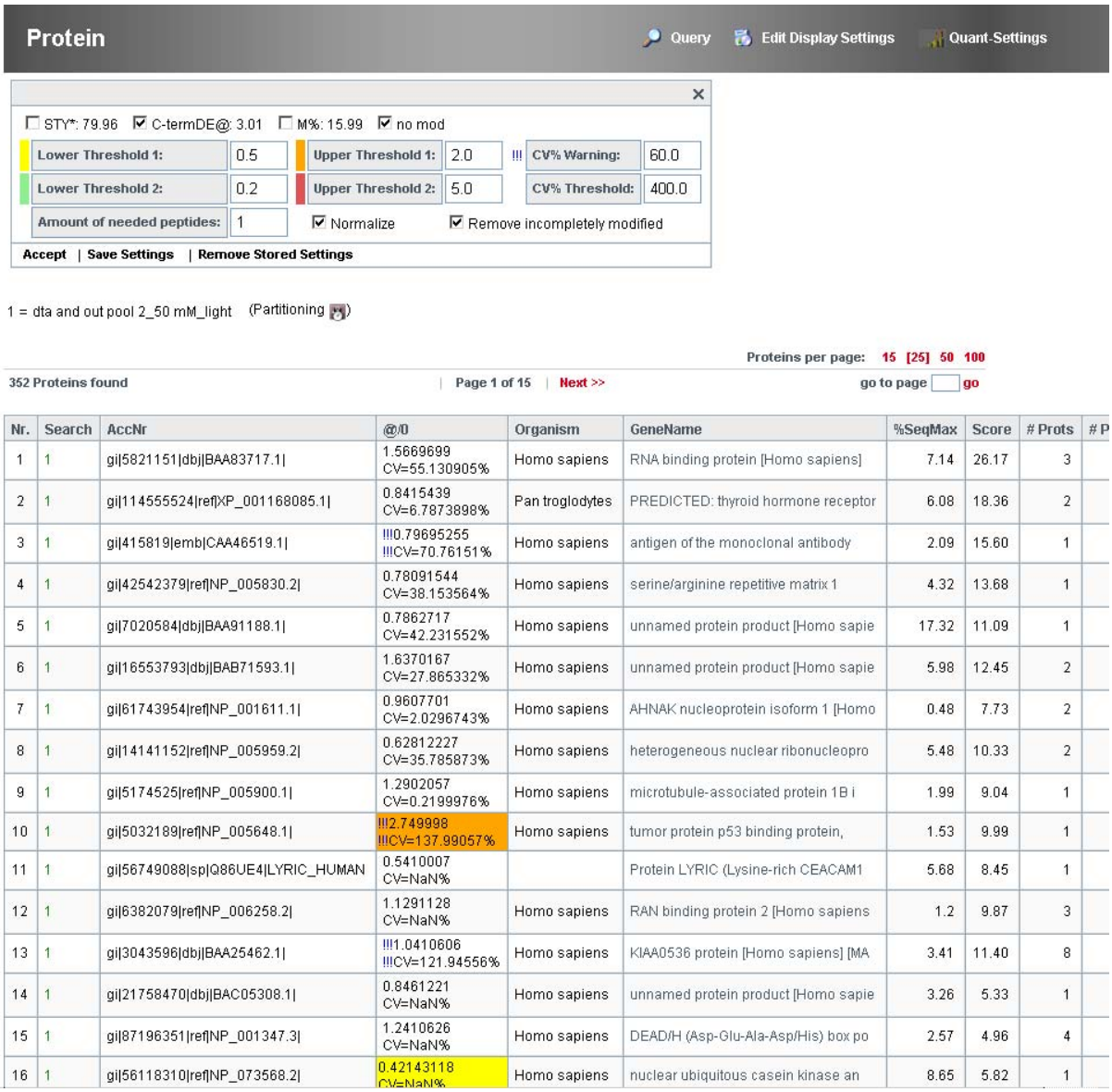
To get a valuable XML file be sure that you have entered detailed information about the sample and the massspecmachine including for example the sample origin the masspecmachine analyzers and detectors and the controlsoftware.

The ">> To Peptide View" brings you to the peptide view, where all the peptides of your searches are displayed. It is the same like in 7.5 but the protein sequence is not colored.

Concerning the querying:
The meaning of most of the query fields is clear by the name they carry. And most of the query fields are executed as directly on the database which is quite fast. The queries that are described here are post-database filters, that means that elements that do not meet the criteria are removed later, which takes a little bit longer:

- NrOfDifferentPepSequences: a specific amount of peptide sequences (irrespective if they are carrying different modifications) must be found for one protein in one search
- NrOfSpectraForPepSequence: a specific amount of spectra must be found for one peptide sequence in one search (irrespective if they are carrying different modifications)
- NrOfFhSpectraForPepSequence: a specific amount of first hit spectra must be found for one peptide sequence in one search (irrespective if they are carrying different modifications)
- NrOfDifferentPepSeqAndModi: a specific amount of peptide sequences (each modified peptide is count as a separate peptide sequence) must be found for one protein in one search
- NrOfSpectraForPepSequAndModi: a specific amount of spectra must be found for one peptide sequence in one search (each modified peptide is count as a separate peptide sequence)
- NrOfFhSpectraForPepSequAndModi: a specific amount of first hit spectra must be found for one peptide sequence in one search (each modified peptide is count as a separate peptide sequence)
- TotalSpectraForPepOfSearches: a specific amount of spectra must be found for one peptide of a protein over several searches
- TotalFhSpectraForPepOfSearches: a specific amount of first hit spectra must be found for one peptide of a protein over several searches
- SpectraForOneProteinFromMultiSearches: a specific amount of spectra must be found for one protein over several searches
- FhpectraForOneProteinFromMultiSearches: : a specific amount of first hit spectra must be found for one protein over several searches

In the protein list the quantification of the proteins can be displayed, when you click on the  button.

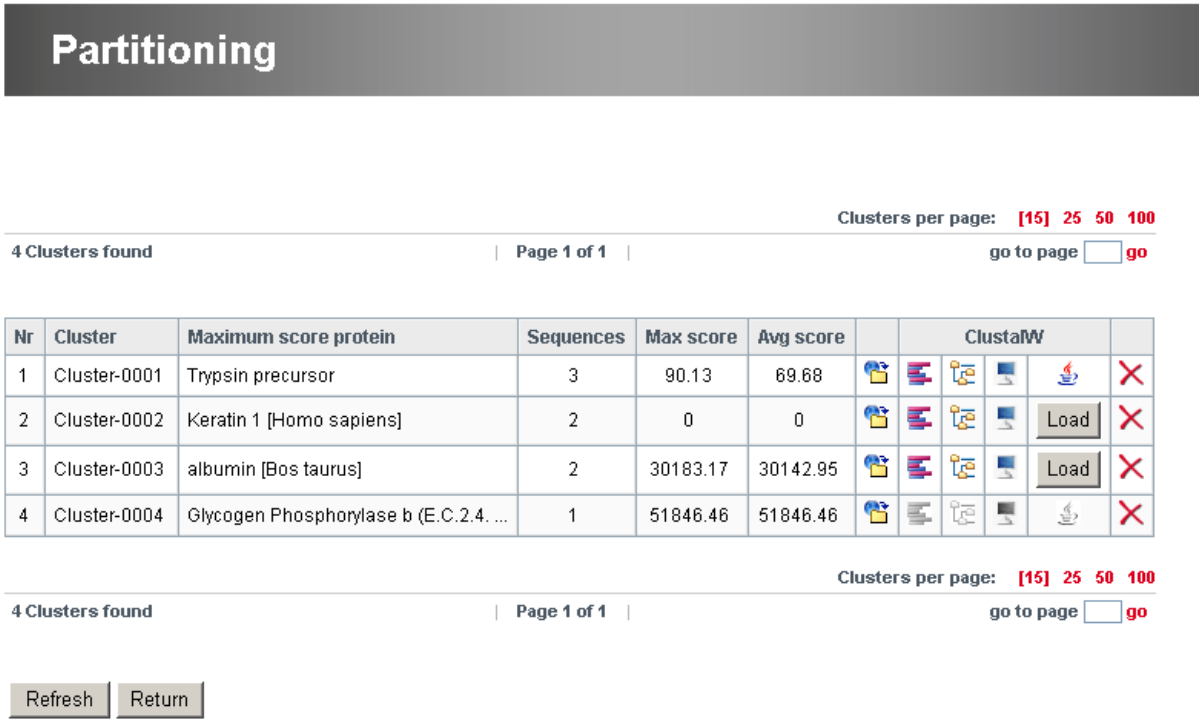


Nr.	Search	AccNr	@/0	Organism	GeneName	%SeqMax	Score	# Prots	# P
1	1	gi|5821151|dbj|BAA83717.1|	1.5669699 CV=55.130905%	Homo sapiens	RNA binding protein [Homo sapiens]	7.14	26.17	3	
2	1	gi|114555524|ref|XP_001168085.1|	0.8415439 CV=6.7873898%	Pan troglodytes	PREDICTED: thyroid hormone receptor	6.08	18.36	2	
3	1	gi|415819|emb|CAA46519.1|	!!!0.79695255 !!!CV=70.76151%	Homo sapiens	antigen of the monoclonal antibody	2.09	15.60	1	
4	1	gi|42542379|ref|NP_005830.2|	0.78091544 CV=38.153564%	Homo sapiens	serine/arginine repetitive matrix 1	4.32	13.68	1	
5	1	gi|7020584|dbj|BAA91188.1|	0.7862717 CV=42.231552%	Homo sapiens	unnamed protein product [Homo sapie	17.32	11.09	1	
6	1	gi|16553793|dbj|BAB71593.1|	1.6370167 CV=27.865332%	Homo sapiens	unnamed protein product [Homo sapie	5.98	12.45	2	
7	1	gi|61743954|ref|NP_001611.1|	0.9607701 CV=2.0296743%	Homo sapiens	AHNAK nucleoprotein isoform 1 [Homo	0.48	7.73	2	
8	1	gi|14141152|ref|NP_005959.2|	0.62812227 CV=35.785873%	Homo sapiens	heterogeneous nuclear ribonucleopro	5.48	10.33	2	
9	1	gi|5174525|ref|NP_005900.1|	1.2902057 CV=0.2199976%	Homo sapiens	microtubule-associated protein 1B i	1.99	9.04	1	
10	1	gi|5032189|ref|NP_005648.1|	!!!2.749998 !!!CV=137.99057%	Homo sapiens	tumor protein p53 binding protein,	1.53	9.99	1	
11	1	gi|56749088|sp|Q86UE4|LYRIC_HUMAN	0.5410007 CV=NaN%		Protein LYRIC (Lysine-rich CEACAM1	5.68	8.45	1	
12	1	gi|6382079|ref|NP_006258.2|	1.1291128 CV=NaN%	Homo sapiens	RAN binding protein 2 [Homo sapiens	1.2	9.87	3	
13	1	gi|3043596|dbj|BAA25462.1|	!!!1.0410606 !!!CV=121.94556%	Homo sapiens	KIAA0536 protein [Homo sapiens] [MA	3.41	11.40	8	
14	1	gi|21758470|dbj|BAC05308.1|	0.8461221 CV=NaN%	Homo sapiens	unnamed protein product [Homo sapie	3.26	5.33	1	
15	1	gi|87196351|ref|NP_001347.3|	1.2410626 CV=NaN%	Homo sapiens	DEAD/H (Asp-Glu-Ala-Asp/His) box po	2.57	4.96	4	
16	1	gi|56118310|ref|NP_073568.2|	0.42143118 CV=NaN%	Homo sapiens	nuclear ubiquitous casein kinase an	8.65	5.82	1	

In the first line of the quantification box you have to specify the modifications with have to be compared (in this example a fixed modification is selected, therefore “no mod”, against a modification at C-termDE with the value of 3.01). Then you can specify two thresholds to display deviations from the 1:1 ratio in colour. “Normalize” means that a total ratio over all of the peptides is calculated to see if there are any differences in the labelling efficiency and the other values are corrected automatically with this value. This option takes a little bit longer since a lot of peptides have to be fetched from the database. The option “Remove incompletely modified” removes all peptides which do not carry a modification on all of the possible positions. If you want to measure incompletely modified peptides do not use this option.

There is a link on the ratio of the comparison which leads directly to the quantitative peptide overview for this protein (see 7.8).

7.2 Cluster (Partitioning):



The detailed view of the clusters is reachable by the protein comparisons (7.1). The proteins are sorted by the size of the cluster.

- : Download of the involved proteins in FASTA format

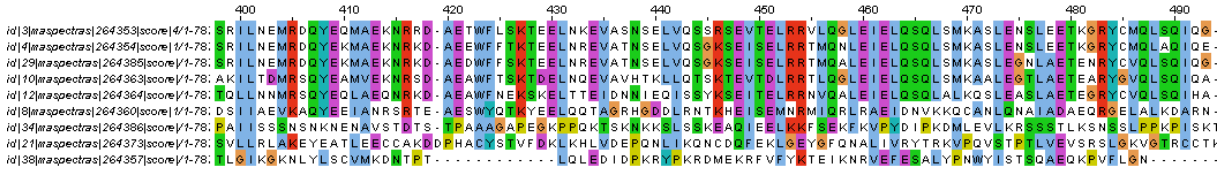
: Download of the alignment of the proteins

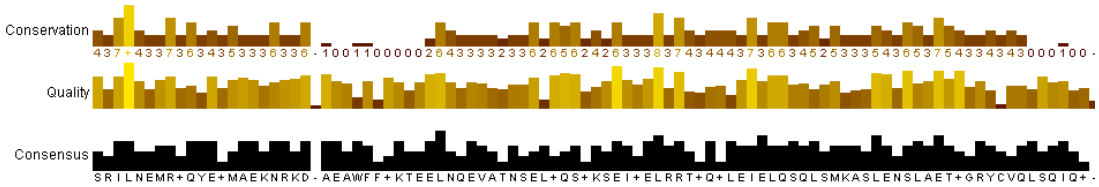
: Download the storage of the tree that you can see in Jalview at the end of this section

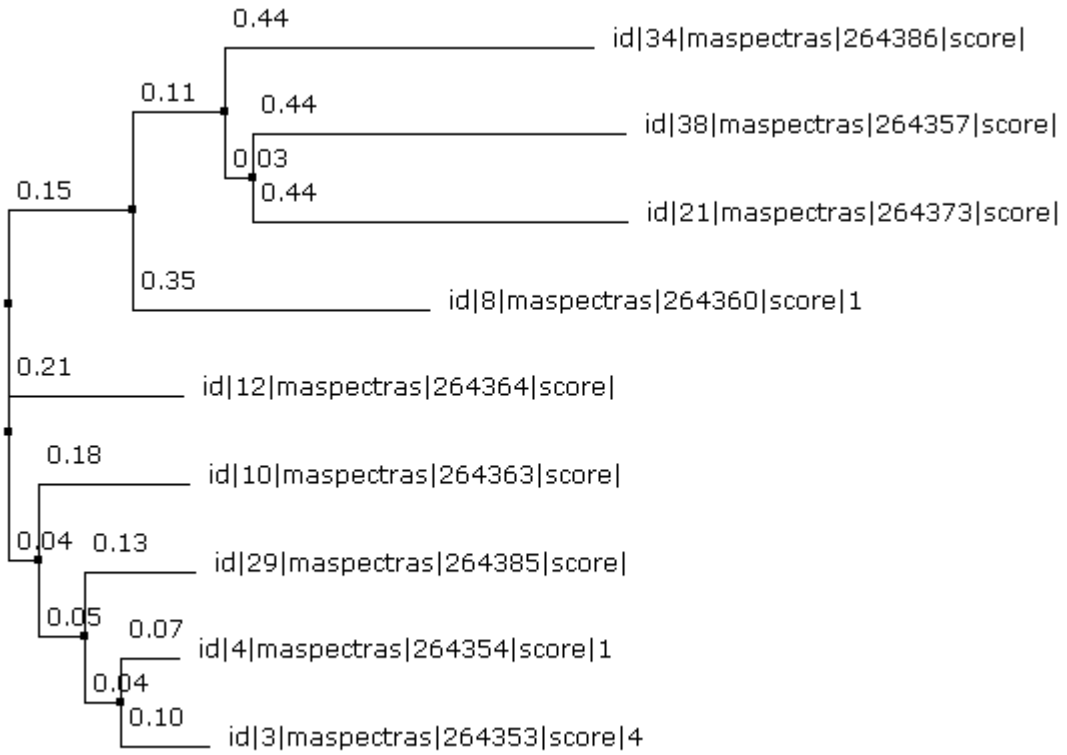
: The log-file of the alignment

Load : The buttons are Java applets itself and when you want to display a big list all of the buttons all the applets would have to be loaded. As this takes to much time, the Load" button has to be presses to get a corresponding applet.

: Starts Jalview applet to see the alignment

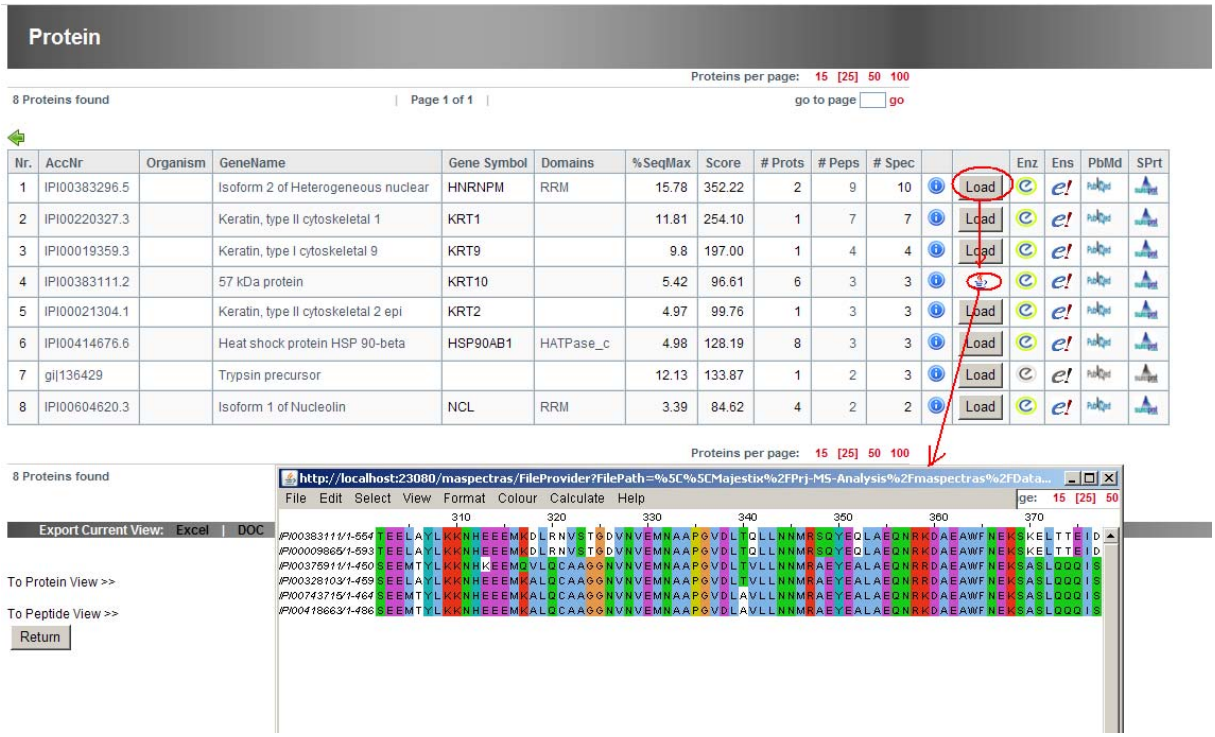

400
410
420
430
440
450
460
470
480
490
id|3|maspectras|264353|score|4/1-78: SRILNEMRDQYEQMAEKNRRD-AETWFLSKTEELNKEVASNSELVQSSRSEVTELRRVLQGLEIELQSQLSMKASLENSLEETKGRYCMQLSQIQG-
id|4|maspectras|264354|score|1/1-78: SRILNEMRDQYEKMAEKNRKD-AEEWFFTKTEELNREVATNSELVQSGKSEISELRRTMQNLEIELQSQLSMKASLENSLEETKGRYCMQLAQIQE-
id|29|maspectras|264385|score|1/1-78: SRILNEMRDQYEKMAEKNRKD-AEDWFFSKTEELNREVATNSELVQSGKSEISELRRTMQALEIELQSQLSMKASLEGNLAETENRYCVQLSQIQG-
id|10|maspectras|264363|score|1/1-78: AKILTDMRSQYEAMVEKNRSD-AEAWFTSKTDELNQEVAVHTKLLQTSKTEVTDLRRTLQGLEIELQSQLSMKAALEGTLAETEARYGVQLSQIQA-
id|12|maspectras|264364|score|1/1-78: TQLLNNMRSQYEQLAEQNRKD-AEAWFNEKSKELTTEIDNNIEQISSYKSEITELRRNVQALEIELQSQLALKQSLEASLAETEGRYCVQLSQIHA-
id|8|maspectras|264360|score|1/1-78: DSIIAEVKAQYEEIANRSRTE-AESWYQTKYEELQQTAGRHGDDLRNTKHEISEMNRMIQRLRAEIDNVKKQCANLQNAIADAEQRGELALKDARN-
id|34|maspectras|264386|score|1/1-78: PAIISSSNSNKNENAVSTDTS-TPAAAGAPEGKPPQKTSKNKKSLSSKEAQIEELKKFSEKFKVPYDIPKDMLEVLKRSSSTLKSNSSLPPKPISKT
id|21|maspectras|264373|score|1/1-78: SVLLRLAKEYEATLEECCAKDDPHACYSTVFDKLKHLVDEPQNLIKQNCDQFEKLGEYGFQNALIVRYTRKVPQVSTPTLVEVSRSLGKVGTRCCTK
id|38|maspectras|264357|score|1/1-78: TLGIKGKNLYLSCVMKDNTPT-----------LQLEDIDPKRYPKRDMEKRFVFYKTEIKNRVEFESALYPNWYISTSQAEQKPVFLGN------


Conservation
437+433736343533336336-1001100000264333323362656242633383743444373663452533354365375433434300010 0-
Quality
Consensus
SRILNEMR+QYE+MAEKNRKD-AEAWFF+KTEELNQEVATNSEL+QS+KSEI+ELRRT+Q+LEIELQSQLSMKASLENSLAET+GRYCVQLSQIQ+-


0.44
id|34|maspectras|264386|score|
0.11
0.44
id|38|maspectras|264357|score|
0.03
0.44
id|21|maspectras|264373|score|
0.15
0.35
id|8|maspectras|264360|score|1
0.21
id|12|maspectras|264364|score|
0.18
id|10|maspectras|264363|score|
0.04
0.13
id|29|maspectras|264385|score|
0.05
0.07
id|4|maspectras|264354|score|1
0.04
0.10
id|3|maspectras|264353|score|4

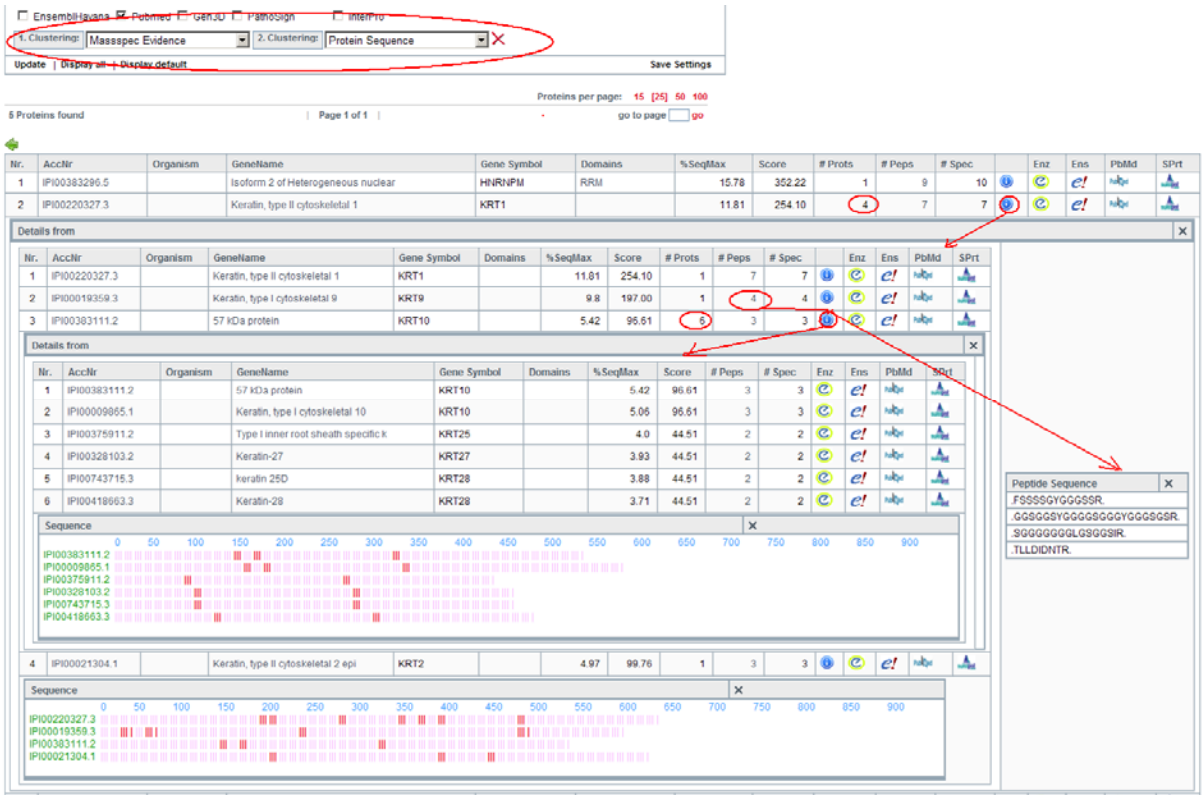
7.3 Clustering possibilities in MASPECTRAS:

The clustering settings in the clustered protein view can be made in the “Edit Display Settings”. Generally it is possible to select clustering based on the protein sequence (the possibility to show the alignment of the cluster is described at 7.2) or based on mass spectrometry evidence. Since the mass spectrometry evidence based calculated on the fly, no pre-calculation of the alignment is possible. Therefore the quick alignment algorithm KAlign has been included.



The procedure to display the alignment is similar to 7.2. First the “Load” button has to be pressed to start the alignment. When the Java icon appears you can click on it and the Jalview Alignment editor will be started to show the results.

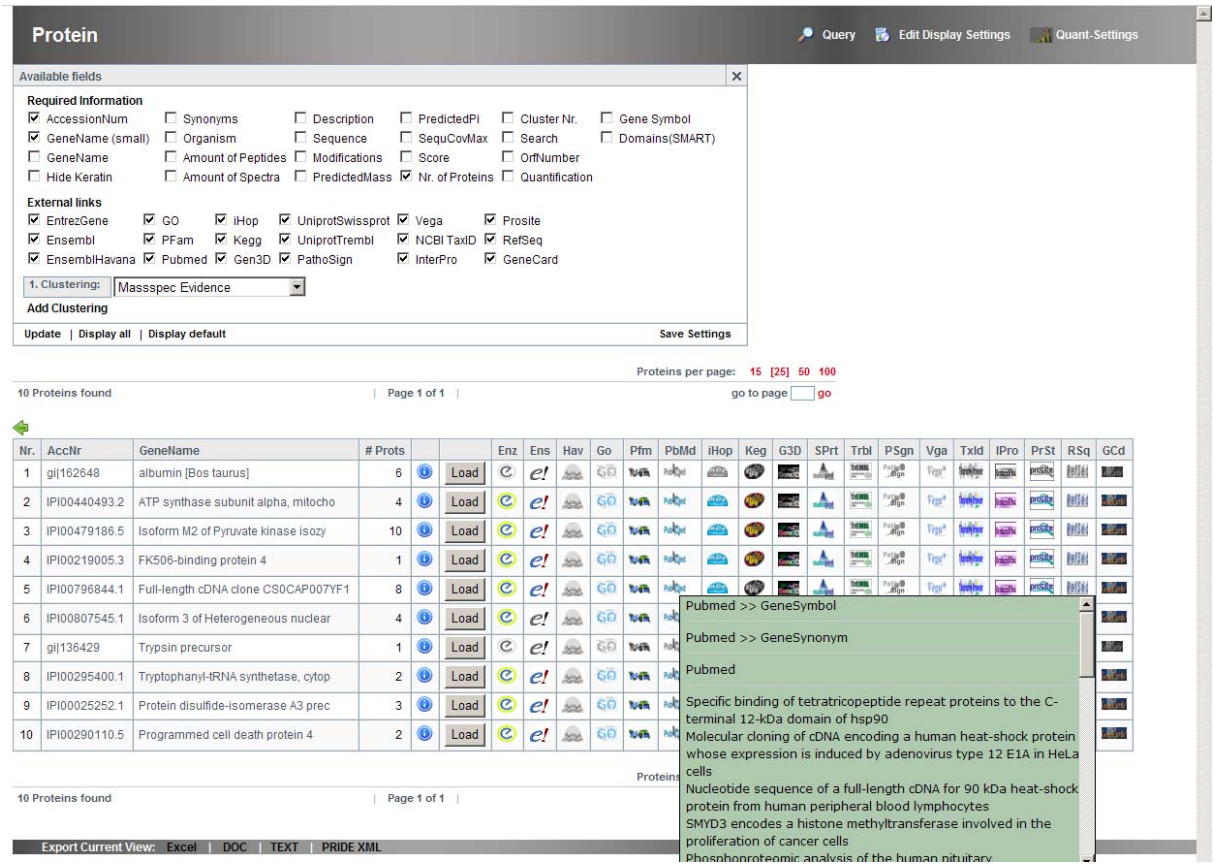
The system has further the ability to invoke more than one clustering sequentially (e.g. first the proteins are clustered by MS evidence and then by the sequence). Further clustering methods are planned in future.



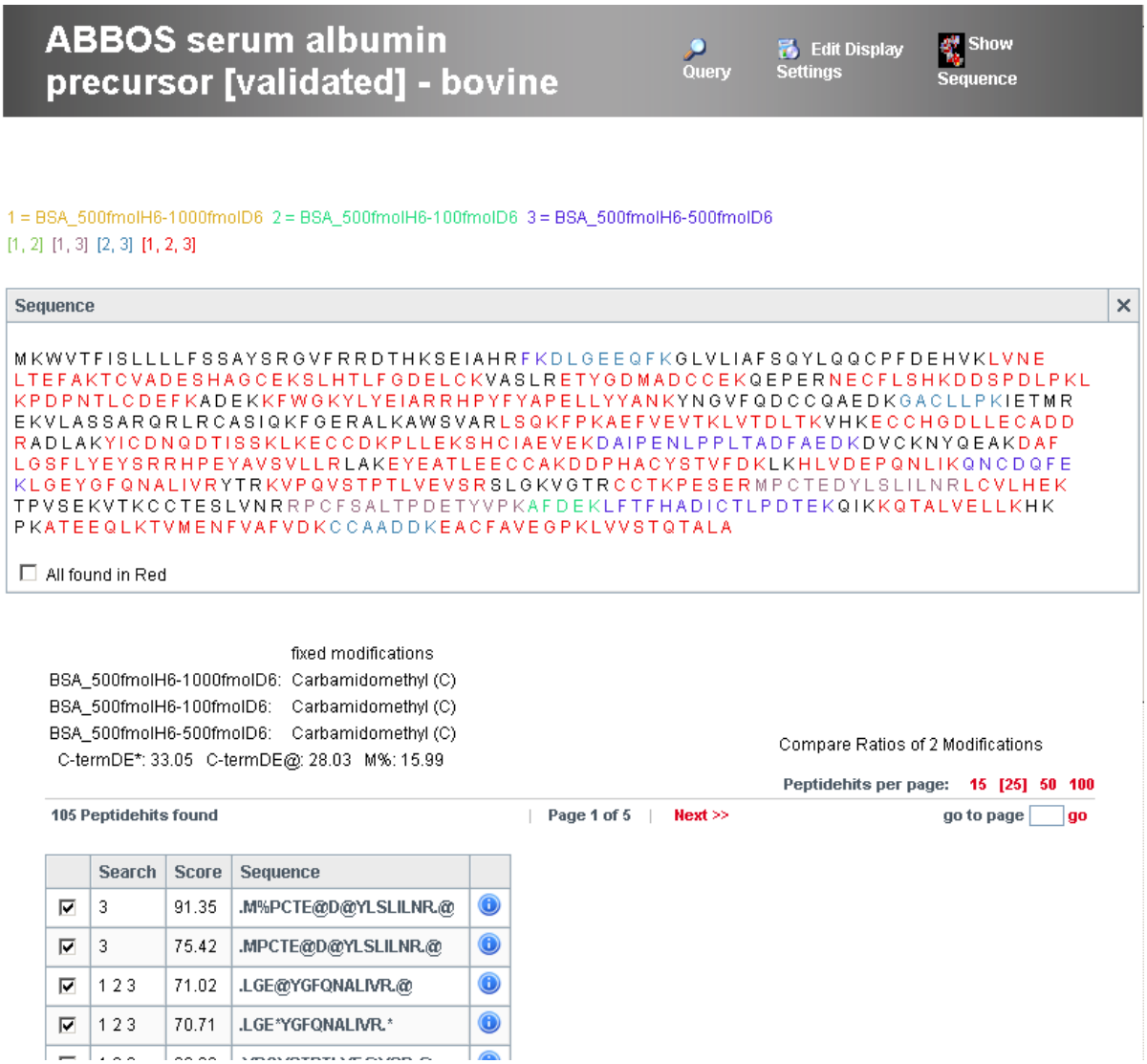
This example shows a protein list which is first clustered by the mass spectrometry evidence and then by the protein sequence. The second row has been expanded with the blue button und the content of the cluster (4 proteins) is shown (clustering by protein sequence). The protein at row three of the cluster is again the representative of a cluster (clustered by MS evidence) which is again expanded by the blue button. The fourth element of the first extended cluster will be continued after the extension of the cluster with second cluster with six elements. Additionally this view has the ability to show the peptides of a specific protein at the right side if you click on the number in the column "# Peps".

7.4 External information:

The system provides the possibility to directly link the found proteins to many external sources. If you want to know how to configure the system to fetch the necessary external information please visit section 2.1 and how you restart just the fetch (and not to do a removal of the uploaded data and start the upload again) please visit section 7 at the beginning.
The fields that should be shown can be set in the "Edit Display Settings" of the page. If you hover the mouse over an external link a popup will open, where there are links to external information. Normally the link is displayed by its ID while in some cases like PubMed the description of link (in this case the title) is getting displayed. On the accession number of the protein is a link on the primary fetch page for the external links (in the example below IPI).


Protein
Query
Edit Display Settings
Quant-Settings
Available fields
Required Information
AccessionNum
Synonyms
Description
PredictedPI
Cluster Nr.
Gene Symbol
GeneName (small)
Organism
Sequence
SequCovMax
Search
Domains(SMART)
GeneName
Amount of Peptides
Modifications
Score
OrfNumber
Hide Keratin
Amount of Spectra
PredictedMass
Nr. of Proteins
Quantification
External links
EntrezGene
GO
iHop
UniprotSwissprot
Vega
Prosite
Ensembl
PFam
Kegg
UniprotTrembl
NCBI TaxID
RefSeq
EnsemblHavana
Pubmed
Gen3D
PathoSign
InterPro
GeneCard
1. Clustering:
Massspec Evidence
Add Clustering
Update | Display all | Display default
Save Settings
Proteins per page: 15 [25] 50 100
10 Proteins found
Page 1 of 1
go to page go
Nr. AccNr GeneName # Prots Enz Ens Hav Go Pfm PbMd iHop Keg G3D SPrt Trbl PSgn Vga Txld IPro PrSt RSq GCd
1 gi|162648 albumin [Bos taurus] 6 Load
2 IPI00440493.2 ATP synthase subunit alpha, mitocho 4 Load
3 IPI00479186.5 Isoform M2 of Pyruvate kinase isozy 10 Load
4 IPI00219005.3 FK506-binding protein 4 1 Load
5 IPI00796844.1 Full-length cDNA clone CS0CAP007YF1 8 Load
6 IPI00807545.1 Isoform 3 of Heterogeneous nuclear 4 Load
7 gi|136429 Trypsin precursor 1 Load
8 IPI00295400.1 Tryptophanyl-tRNA synthetase, cytop 2 Load
9 IPI00025252.1 Protein disulfide-isomerase A3 prec 3 Load
10 IPI00290110.5 Programmed cell death protein 4 2 Load
Pubmed >> GeneSymbol
Pubmed >> GeneSynonym
Pubmed
Specific binding of tetratricopeptide repeat proteins to the C-terminal 12-kDa domain of hsp90
Molecular cloning of cDNA encoding a human heat-shock protein whose expression is induced by adenovirus type 12 E1A in HeLa cells
Nucleotide sequence of a full-length cDNA for 90 kDa heat-shock protein from human peripheral blood lymphocytes
SMYD3 encodes a histone methyltransferase involved in the proliferation of cancer cells
Phosphoproteomic analysis of the human pituitary
10 Proteins found
Page 1 of 1
Export Current View: Excel | DOC | TEXT | PRIDE XML

7.5 Peptide comparison:



The gene-name is displayed at the page head. The  button opens the box with the protein sequence again, if you have closed it. Below the page head the searches are listed again. This time dyed in order to recognize them in the protein sequence. Underneath the possible combinations of the searches are colour-encoded as well.
The “Sequence” box has a little checkbox “All found in Red”, which shows all found parts of the sequence in red, if one colour is not easily visible.
Then the searches are listed again and the fixed modifications are given. At the end of the searches the variable modifications are indicated in one row. The affected amino acids are shown followed by the substitute for the modification in the peptide list and the mass shift after the colon.
Below the searches the found peptides are listed, sorted by the score. To indicate by which search the peptide has been found the numbers in the search column are denoted (the same way like in 7.1). If this sequence is a first hit, the sequence is in bold letters. When you uncheck the checkbox in front of a peptide, this peptide will be removed as found in the “Sequence” box. At the upper right part of the peptides listed there is the link “Compare Ratios of 2 Modifications”. Here you can compare the quantitative ratios of differentially

labelled proteins (e.g ICPL-light to ICPL-heavy), or all found peptides which carry a modification versus ones that do not carry the modification (see 7.8).
When you push the blue button you get detailed information about a peptide. That means you are on the level of the single searches. Here you get more detailed information about the peptides. On that level the quantitative comparison is possible as well (the "Peak Area" column).

	Search	Score	Sequence	PeakArea	
☑	1	45.59	.TVM%E*NFVAFVD*K.*		
☑	1	63.65	.TVM%E@NFVAFVD@K.@		
☑	1	34.19	.VPQVSTPTLVE@VSR.@		
☑	1	49.59	.VPQVSTPTLVE*VSR.*		
☑	1	24.34	.YICD@NQD@TISSK.@		
☑	1	45.04	.YICD*NQD*TISSK.*		
☑	1	26.62	.YICDNQD@TISSK.@		
☑	1	34.54	.YLYE*IAR.*		
☑	1	26.44	.YLYE@IAR.@		

Peptidehits per page: [15] 25 50 100

69 Peptidehits found << Previous | Page 5 of 5 | go to page go

Details from YLYEIAR

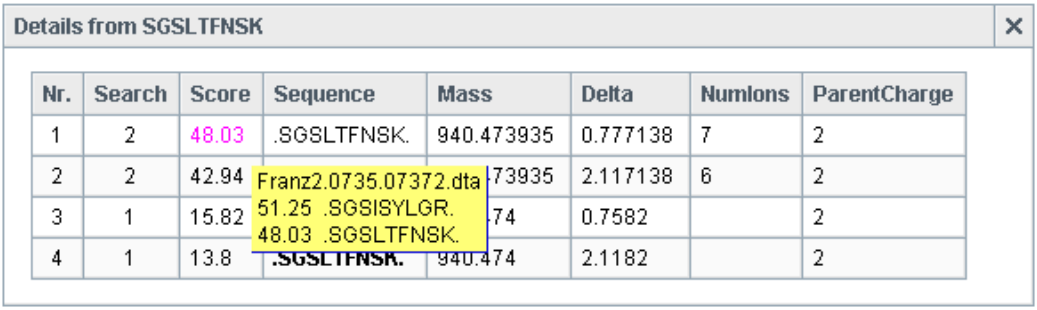
Nr.	Search	Score	Sequence	Mass	Delta	NumIons	ParentCharge	PeakArea
1	1	35.05	.YLYE*IAR.*	993.613782	0.537291	6	2	8.7043128E7
2	1	34.54	.YLYE*IAR.*	993.613782	0.197291	6	2	8.7043128E7
3	1	33.37	.YLYE*IAR.*	993.613782	-0.022709	6	2	8.7043128E7
4	1	28.85	.YLYE*IAR.*	993.613782	2.497291	5	2	8.7043128E7

Details from YLYEIAR

Nr.	Search	Score	Sequence	Mass	Delta	NumIons	ParentCharge	PeakArea
1	1	32.99	.YLYE@IAR.@	983.556501	0.304572	6	2	4.1418732E7
2	1	32.88	.YLYE@IAR.@	983.556501	0.564572	6	2	4.1418732E7
3	1	26.44	.YLYE@IAR.@	983.556501	0.674572	5	2	4.1418732E7

Return

When you move your mouse over one entry of the column "Search", "Sequence" or "Score" a tooltip with the hits will be displayed.



When you click on one of the entries with the tooltip a window pops up with the corresponding spectrum, so that manual validation is possible (see 7.6).

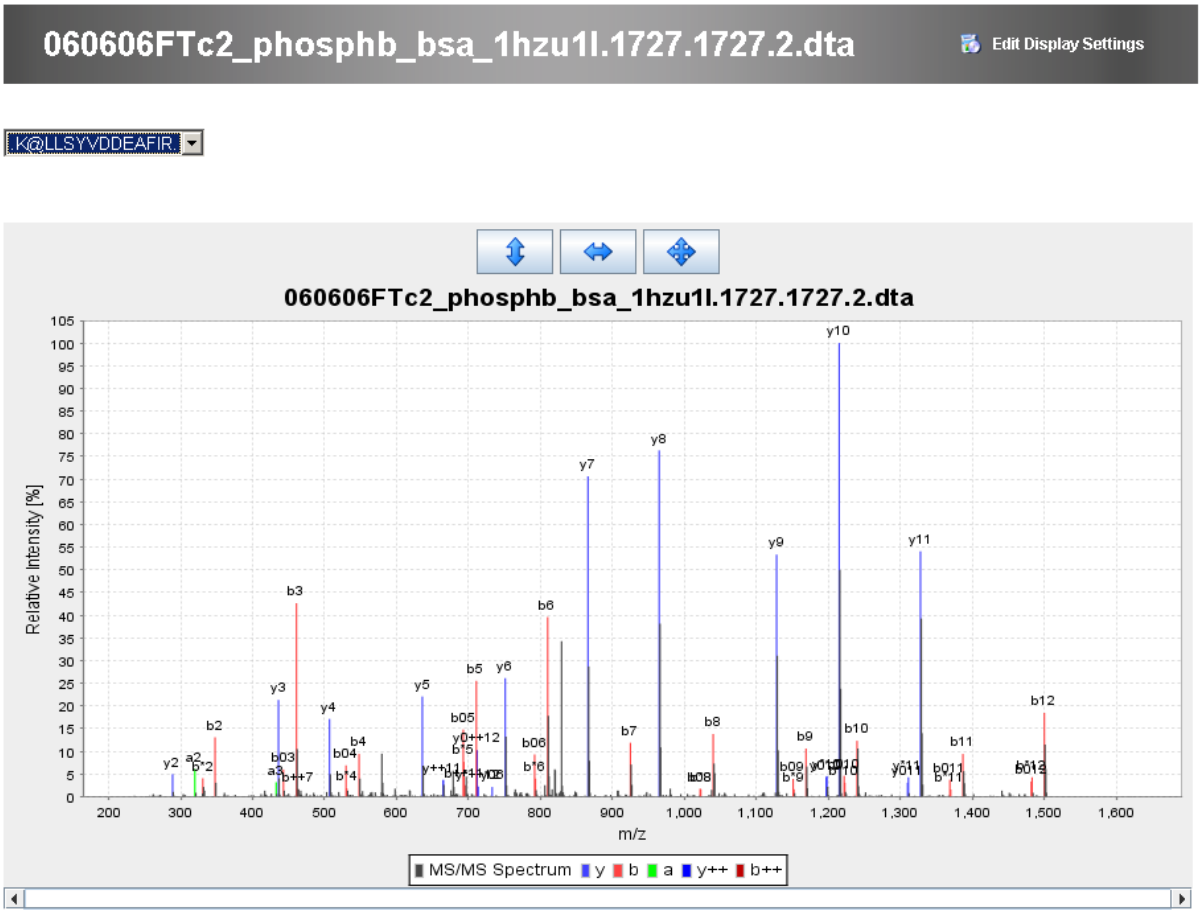
When you click on the link on the peak area entries you receive a chromatogram viewer for the manual inspection and correction of the automatically calculated peak areas (see 7.7.)

Concerning the querying:

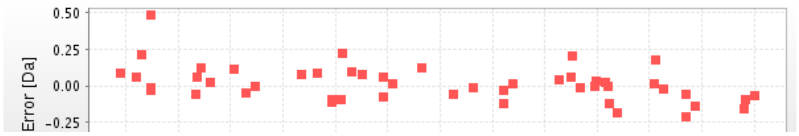
The meaning of most of the query fields is clear by the name they carry. And most of the query fields are executed as directly on the database which is quite fast. The queries that are described here are post-database filters, that means that elements that do not meet the criteria are removed later, which takes a little bit longer:

- NrOfPassingSpectra: a specific amount of spectra must be found for one peptide hit in one search
- NrOfPassingFirstHitSpectra: a specific amount of spectra must be found for one peptide hit in one search
- NrOfTotalPassingSpectra: a specific amount of spectra must be found for one peptide hit in several searches
- NrOfTotalPassingFirstHitSpectra: a specific amount of spectra must be found for one peptide hit in several searches

7.6 Spectrum View:

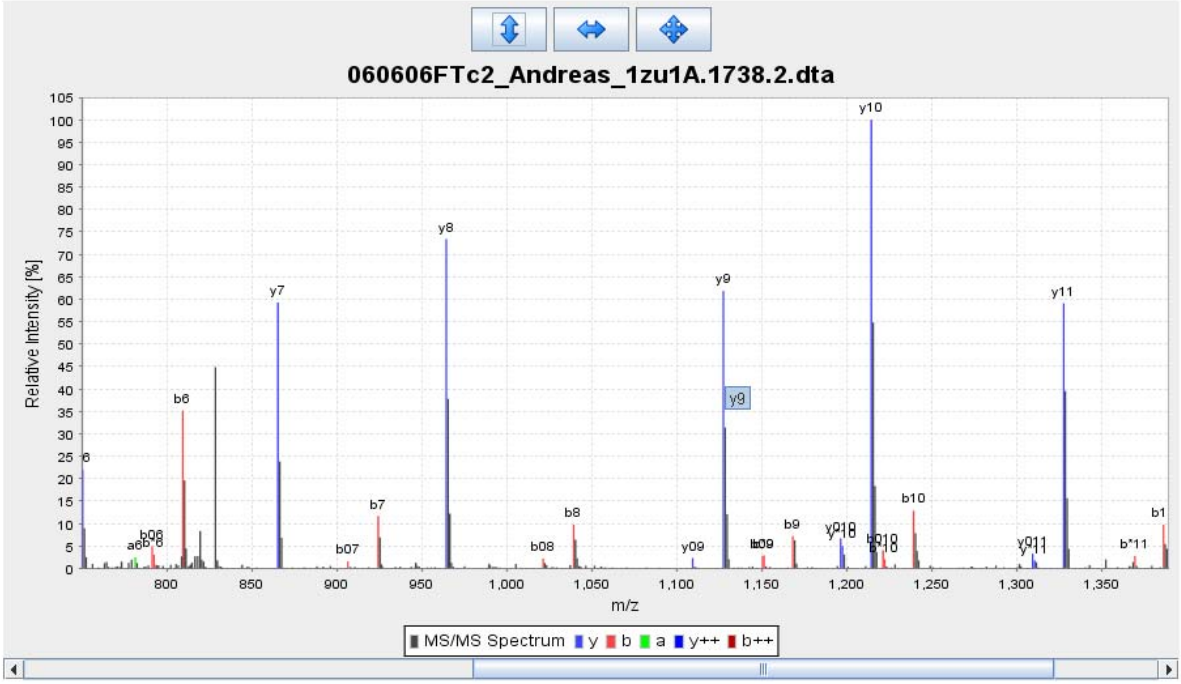


	a	b	b*	b0	b++	b*++	b0++		y	y*	y0	y++	y*++	y0++	
1	206.12	234.12	217.09	216.11	117.56	109.05	108.56	K							13
2	319.21	347.2	330.18	329.19	174.1	165.59	165.1	L	1440.73	1423.71	1422.72	720.87	712.35	711.86	12
3	432.29	460.29	443.26	442.28	230.64	222.13	221.64	L	1327.65	1310.62	1309.64	664.33	655.81	655.32	11
4	519.32	547.32	530.29	529.31	274.16	265.65	265.16	S	1214.56	1197.54	1196.55	607.78	599.27	598.78	10
5	682.39	710.38	693.35	692.37	355.69	347.18	346.69	Y	1127.53	1110.51	1109.52	564.27	555.75	555.26	9
6	781.45	809.45	792.42	791.44	405.23	396.71	396.22	V	964.47	947.44	946.46	482.74	474.22	473.73	8
7	896.48	924.48	907.45	906.47	462.74	454.23	453.73	D	865.4	848.37	847.39	433.2	424.69	424.2	7
8	1011.51	1039.5	1022.48	1021.49	520.25	511.74	511.25	D	750.37	733.35	732.36	375.69	367.17	366.68	6
9	1140.55	1168.55	1151.52	1150.54	584.77	576.26	575.77	E	635.35	618.32	617.34	318.17	309.66	309.17	5
10	1211.59	1239.58	1222.56	1221.57	620.29	611.78	611.29	A	506.3	489.28	488.29	253.65	245.14	244.65	4
11	1358.66	1386.65	1369.63	1368.64	693.83	685.31	684.82	F	435.27	418.24	417.26	218.13	209.62	209.13	3
12	1471.74	1499.74	1482.71	1481.73	750.37	741.86	741.36	I	288.2	271.17	270.19	144.6	136.09	135.6	2
13								R	175.11	158.09	157.1	88.06	79.55	79.05	1



With “Edit Display Settings” you can select the series you want to be displayed. You can save your own display settings like in all the other pages.
With the select box below the “Edit Display Settings” box you can switch between the found hits.
Then there is a Java Applet with the spectrum (see 7.6.1) and after the spectrum view a box with calculated masses of the fragments is added. At the bottom of the page the mass error of the single hits of the different series is displayed.

7.6.1 The spectrum viewer:



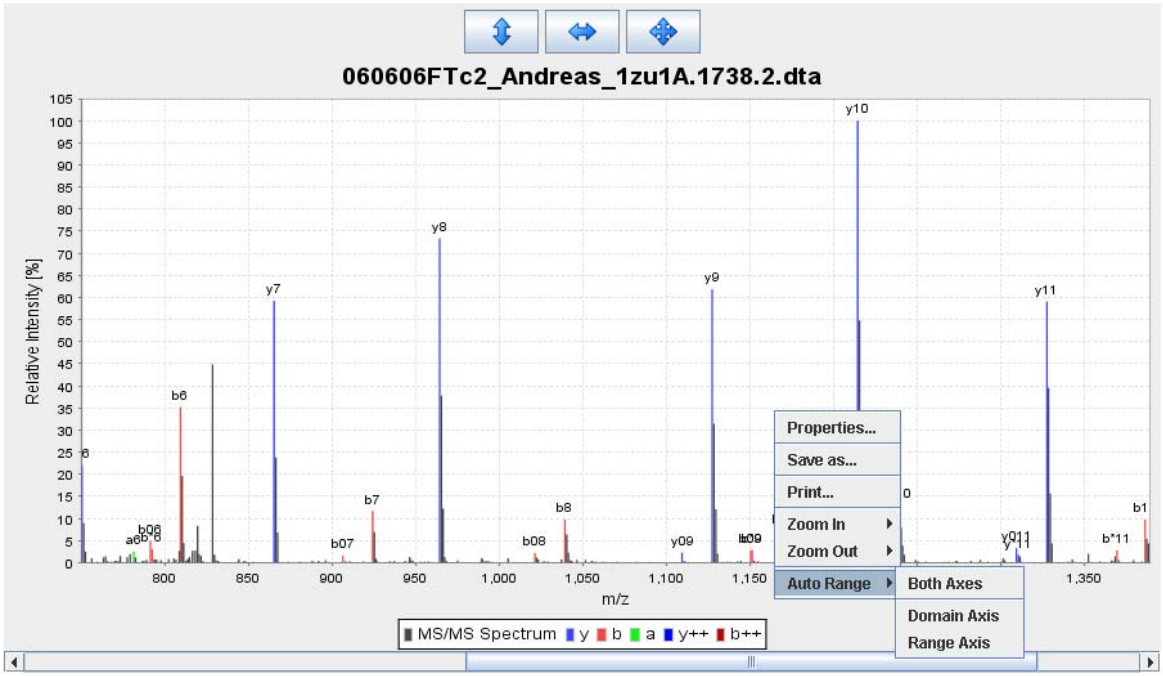
The not assigned peaks are displayed in red. The assigned fragment name is written on the top of the peak. If you hover your mouse over one peak the name will be displayed in a tooltip as well. You can zoom into your spectrum and scroll the x-axis with the bar at the bottom.

 : zooms out the y-axis

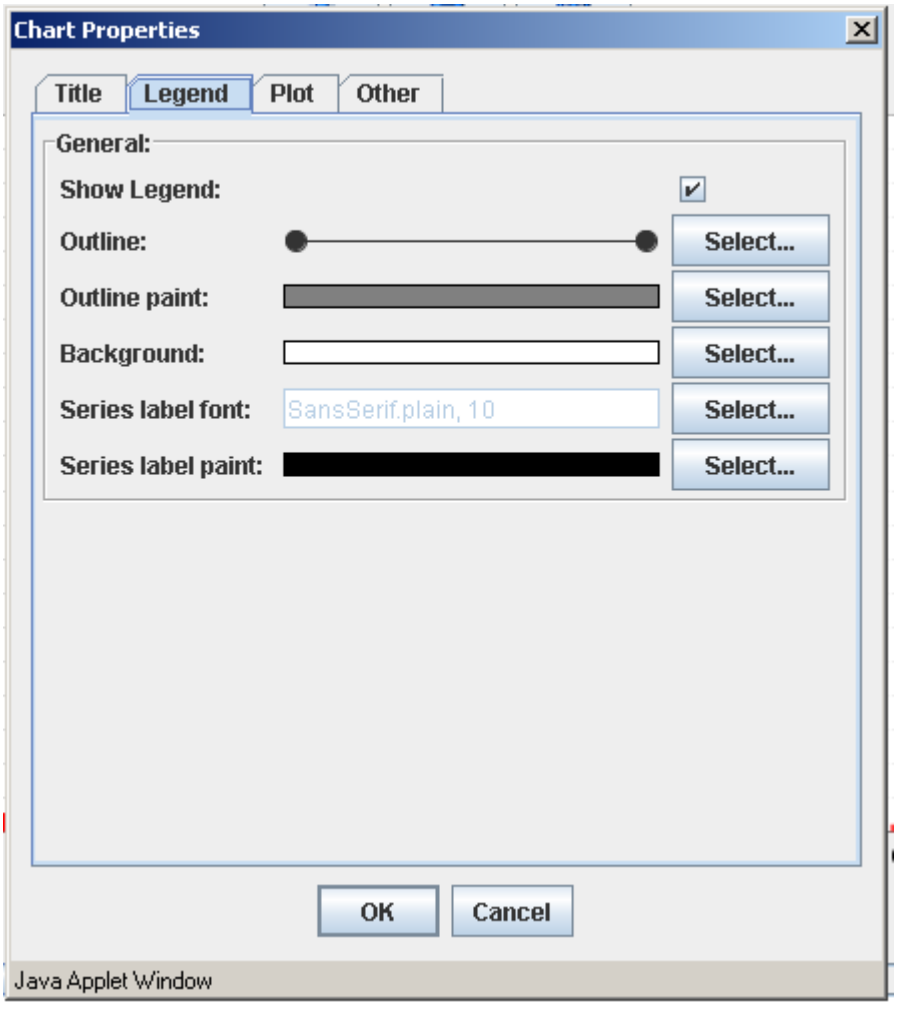
 : zooms out the x-axis

 : zooms out both axes

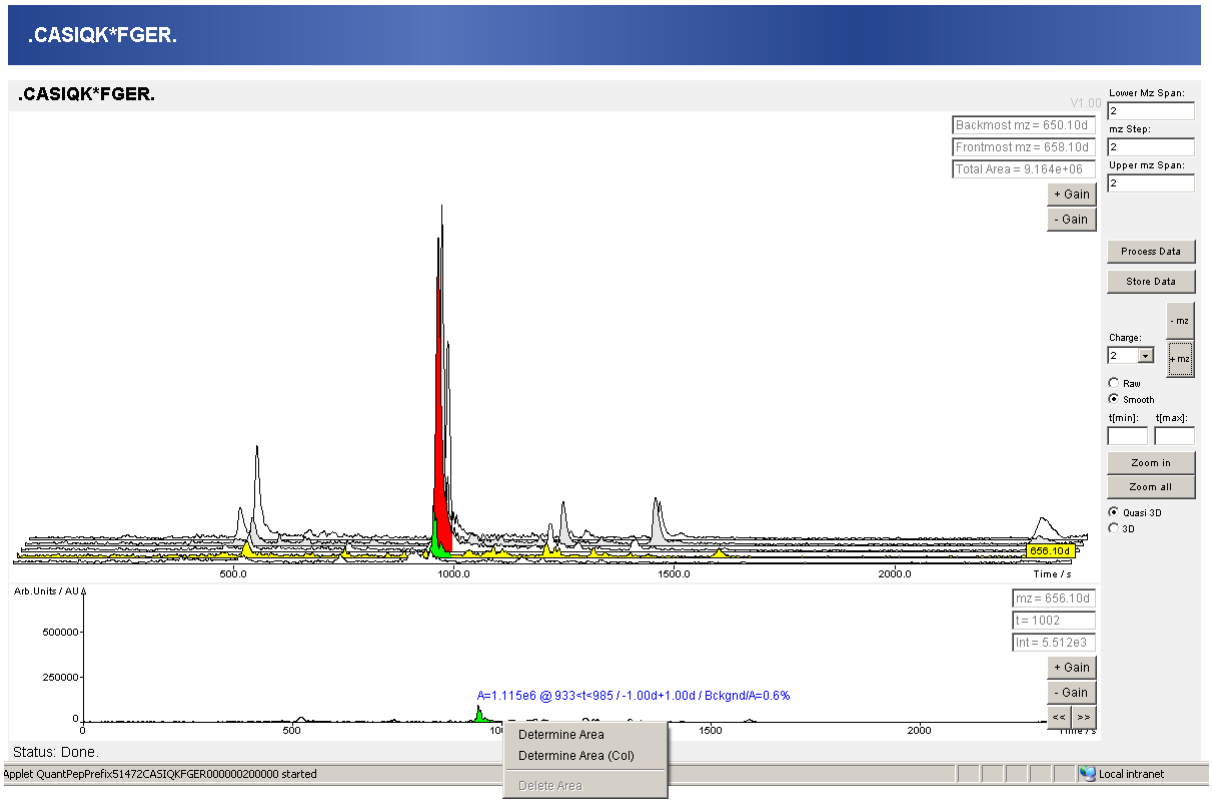
When you first click on the spectrum and then click with the right mouse button you will get a popup window where you have additional features:



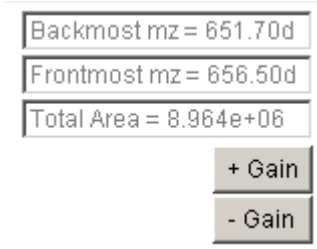
You can print your actual zoom scan. In the “Properties…” you can customize your font and other settings.



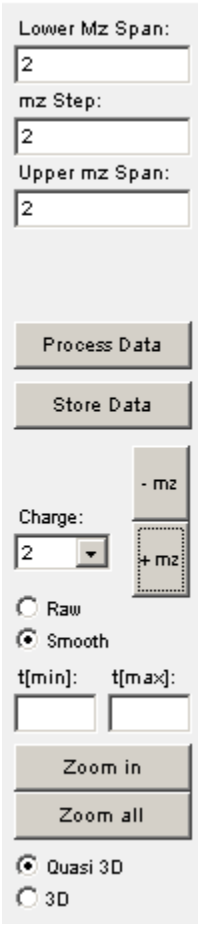
7.7 Chromatogram viewer



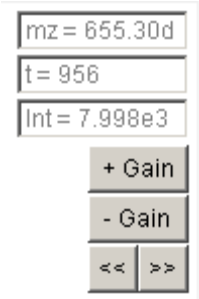
At the top the name of the peptide is written. The upper view shows the chromatogram plus the chromatograms in the neighbourhood. The red peak is the quantified one the green peak indicates one that has been selected manually. The second view shows one of the upper chromatograms in a 2 dimensional view. The one chromatogram which has been selected is shown in yellow in the upper view. The mass to charge ratio of the selected chromatogram is shown in the yellow box on the right side of the upper view.



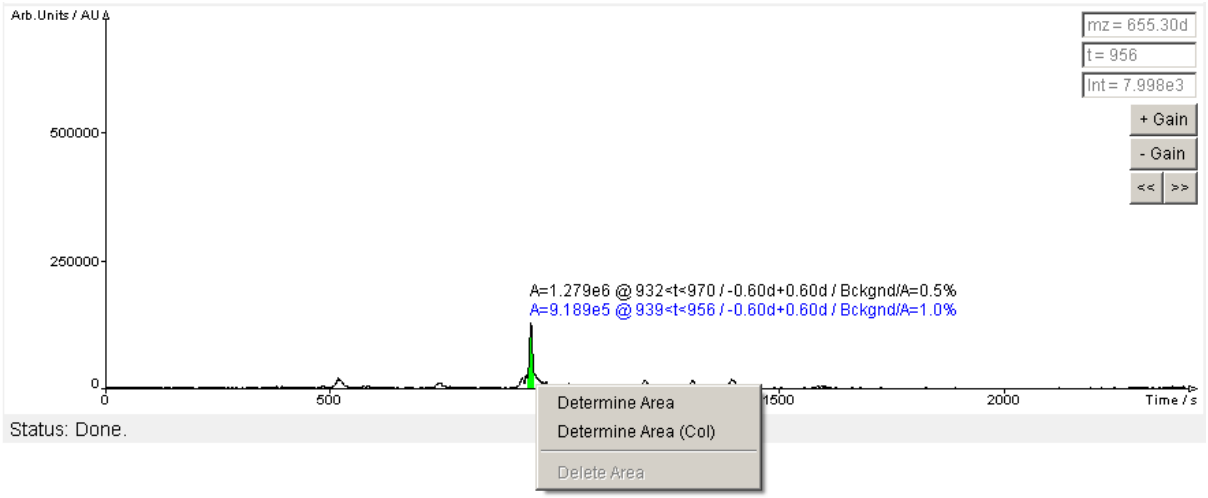
The box at the upper right part of the upper view shows the m/z borders where the chromatograms are depicted and the total area calculated. With “+Gain” and “-Gain” you can zoom in and out the amplitude.



In the menu on the right side you can determine how many chromatograms you want to see in positive/negative m/z direction with “Upper mz Span”/”Lower Mz Span”. With the mz Step you can select the distance between two chromatograms. Peaks within one half of the distance in positive direction and one half of the distance in negative direction are taken for the calculation of the chromatograms.
Once you changed something there you must press the “Process Data” button to retrieve the chromatograms from the server. The “Store Data” button stores manually changes (additional or removed peaks). With the “Charge” you can switch between the charge states of the peptide (only found charge states are calculated). With the “-mz” and the “+mz” you can select the chromatogram for the 2D view. With “Raw” and “Smooth” you can see the smoothed chromatogram and the raw chromatogram. In the “t[min]” and “t[max]” you can fill in the time borders and with “Zoom in” you can zoom to this borders for the 2D view. Use “Zoom all” to go back. The last check-box changes the quasi-3D view to real 3D viewer. The problem is that the real 3D viewer needs Java3D installed on the client machine and needs much more main memory on the machine (see 7.7.1)



The box at the upper right part of the lower view shows the current m/z value, the time where the curser is actually and the amplitude where the cursor is actually. With “+Gain” and “-Gain” you can zoom in and out. With “<<” “>>” you can move in the zoomed view left and right.

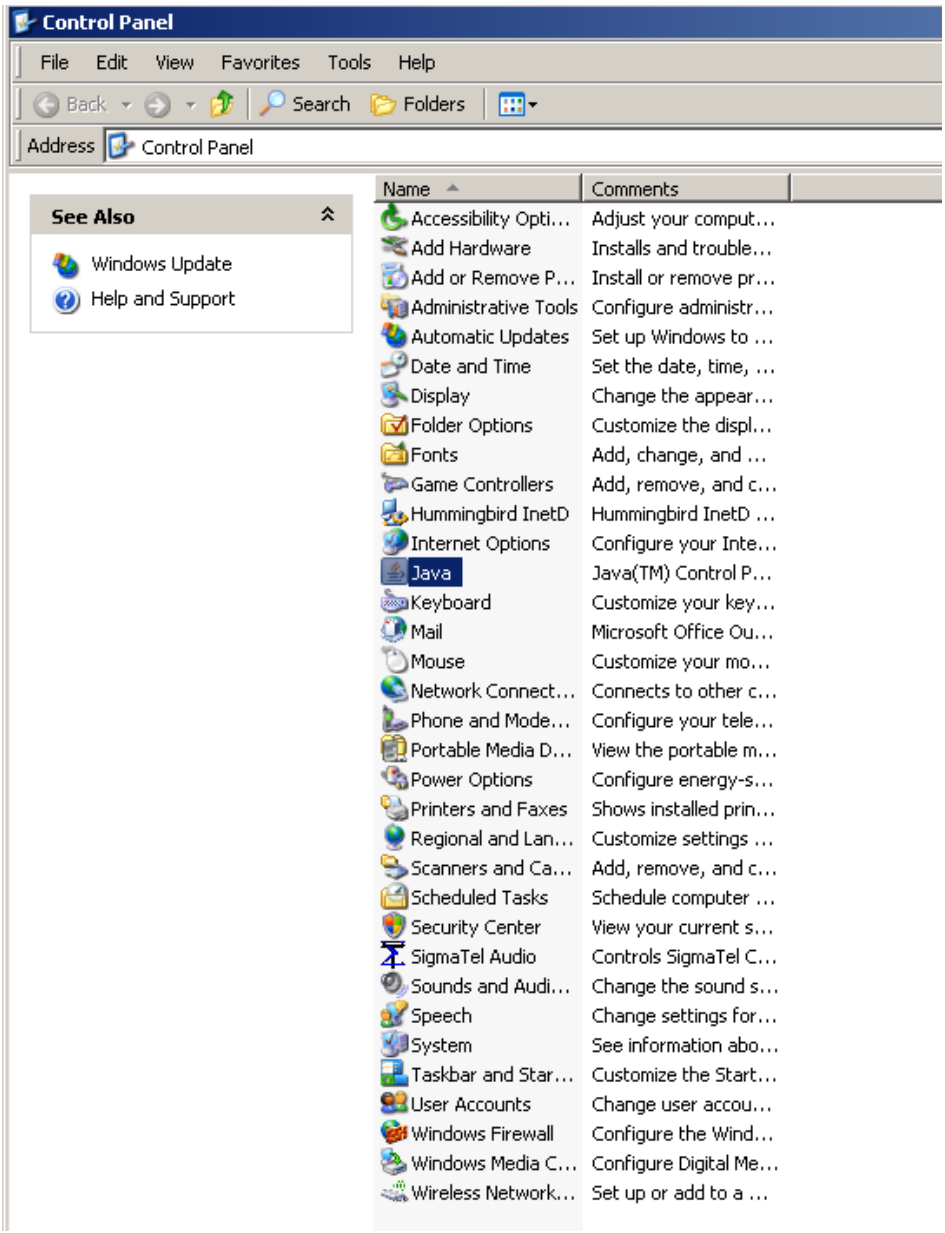


In the lower view you can select and deselect peak areas when you move the cursor inside the peak area you want to select and click the right mouse button the popup will appear. With the "Determine Area" you select a peak like it is chosen in ASAPRatio. With "Determine Area (Col)" the new peak finding algorithm is taken which quantifies peaks with saddle-points and foothills. The black "A=…" shows the stored area in the database the blue "A=…" shows the actually selected area for that peak. If a peak with the same boundaries is stored in the database the peak appears in red otherwise it is shown in green. At the bottom there is a progress bar. When there "Done" appears you can work on the chromatograms, when "Processing Data … " the applet is fetching data from the server and there is no use to work on the data now because the data will be overwritten when it is finished.

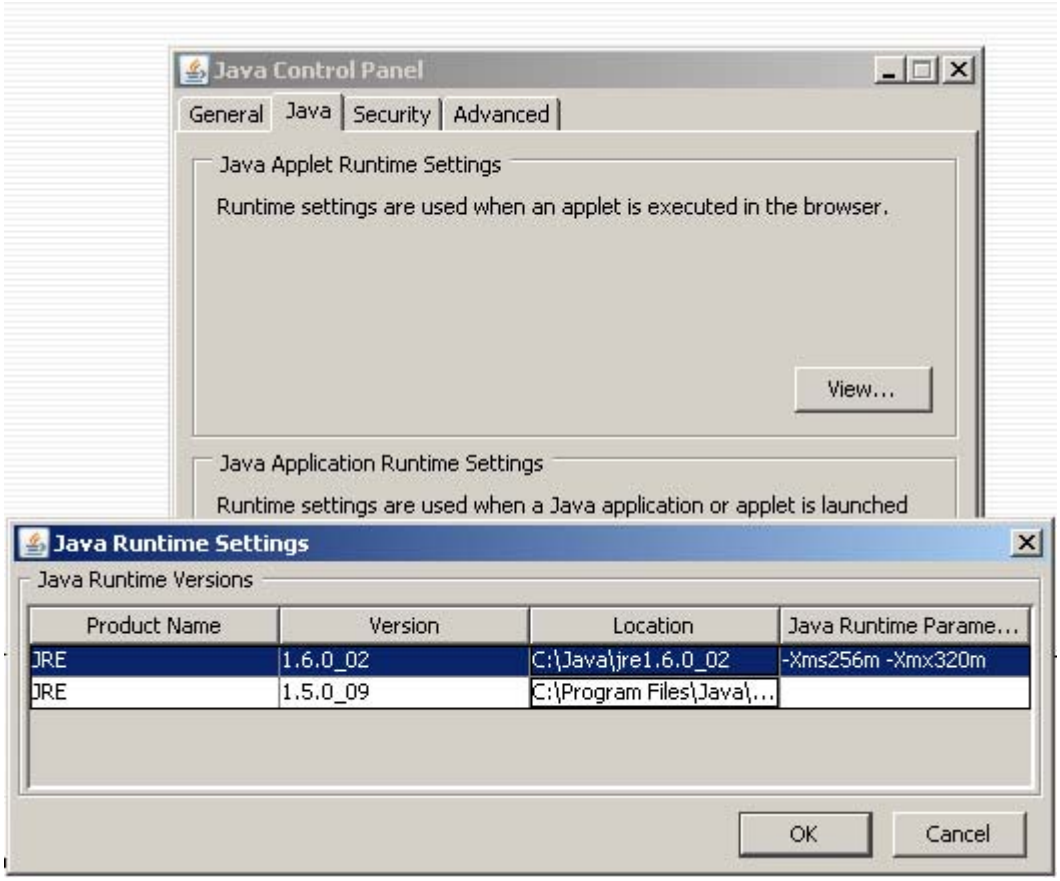
7.7.1 Chromatogram 3D viewer:

To run the 3D-viewer Java3D must be installed. You can download this from:
http://java.sun.com/products/java-media/3D/download.html

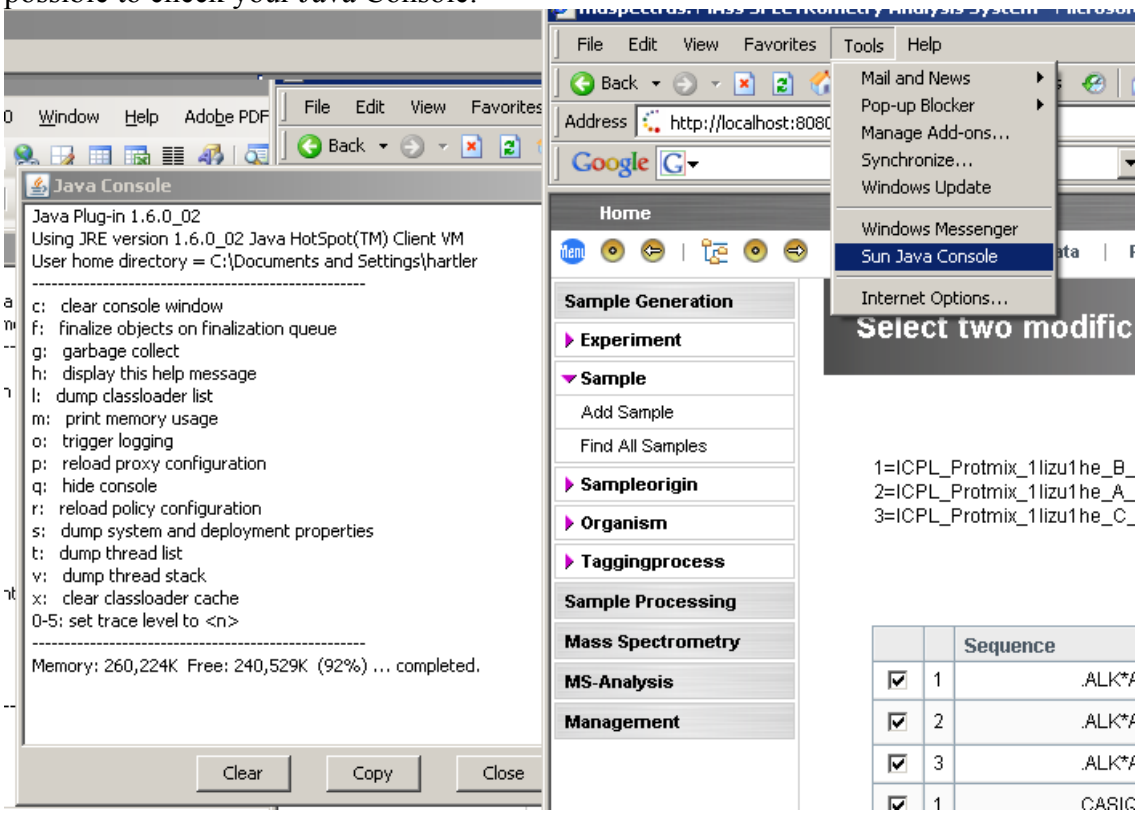
When you installed Java3D you have to reserve more memory for the applet. In Windows you have to go to "Control Panel" and then double-click on Java.



Then the Java Control-Panel opens:

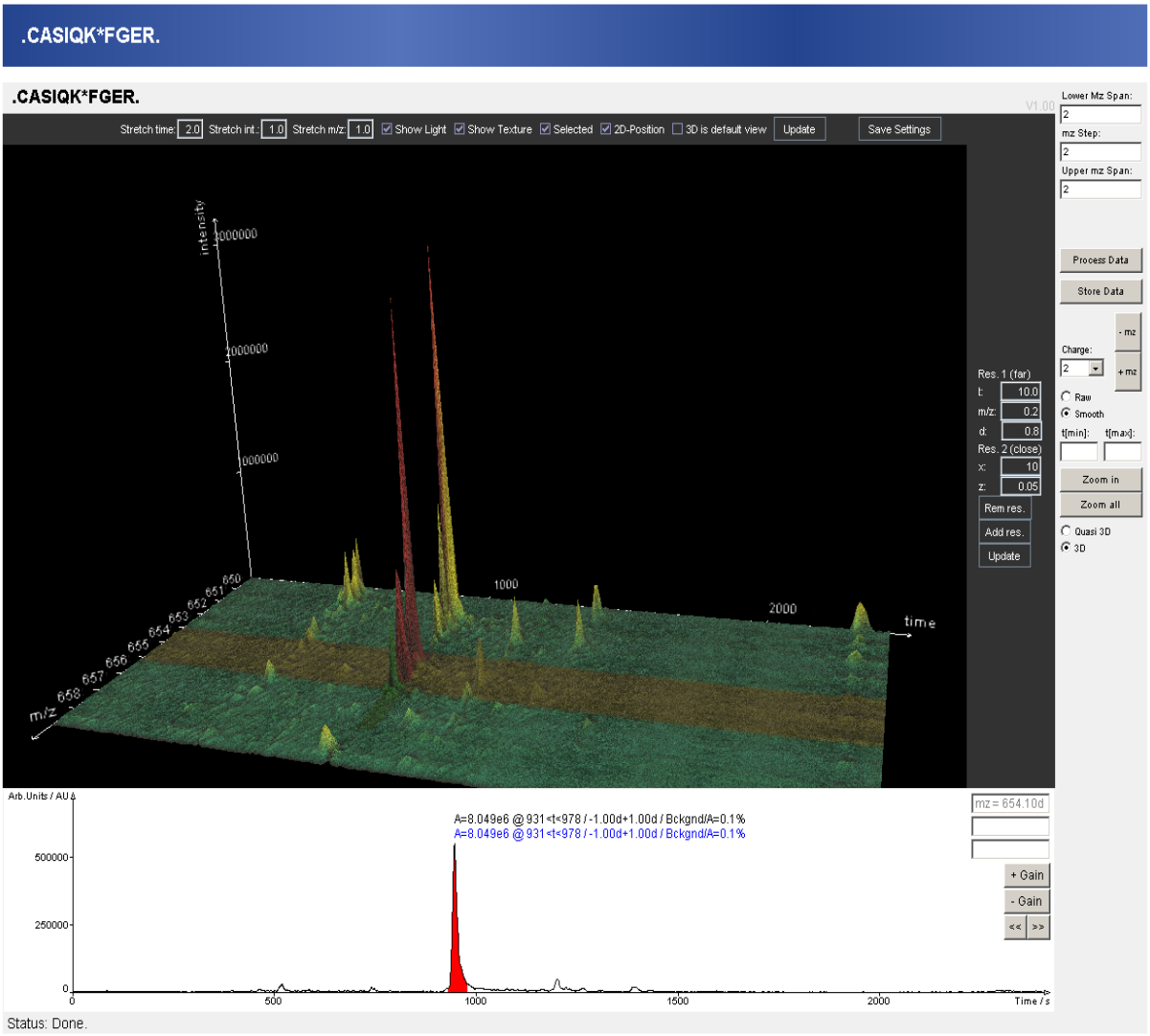


Click on the Java tab and then in the Java Applet Runtime Settings on the "View" button. You should enter approximately the values I entered here (at least –Xmx320m should be used) and click on "OK". Then it is necessary to restart your browser. Then in your browser it is possible to check your Java Console:



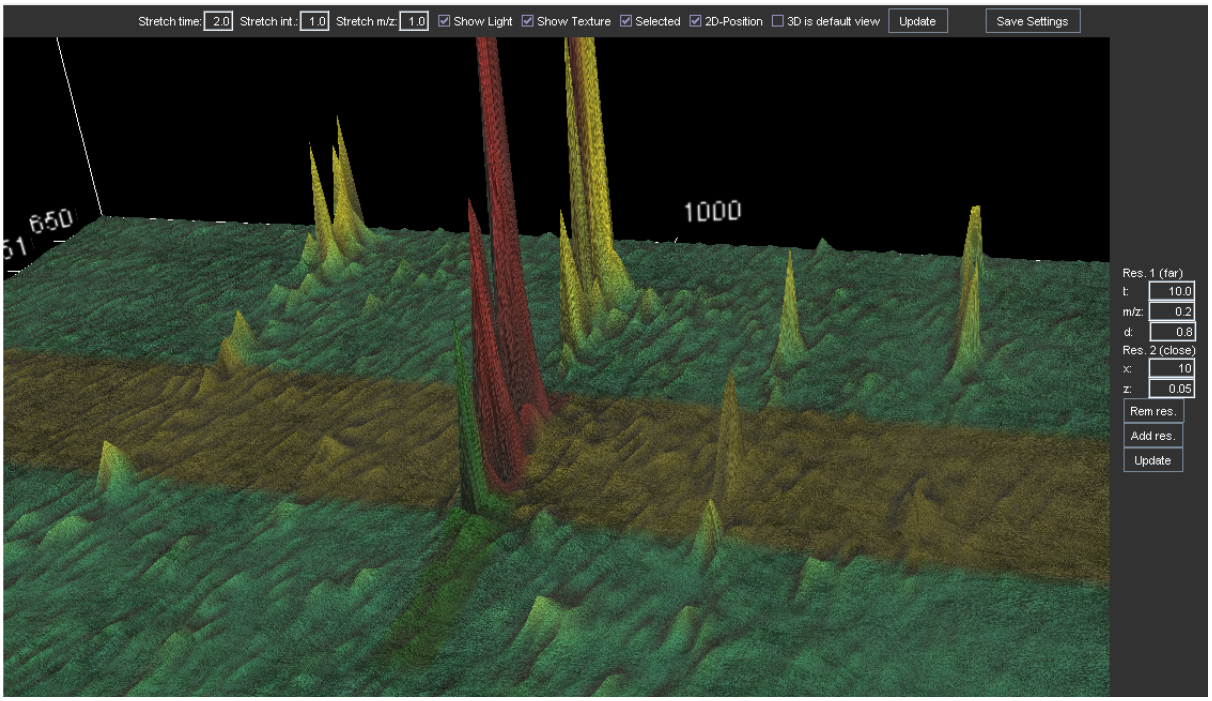
When you click in the Java Console on “m” you can check the memory used. The option –Xms is the permanently reserved memory and the –Xmx is the maximum memory that could be used if needed. When you start the Java3D viewer (clicking on the radio button Java 3D) and you get java.lang.OutOfMemory you have to less memory to run the 3D applet.

Now to the 3D viewer:

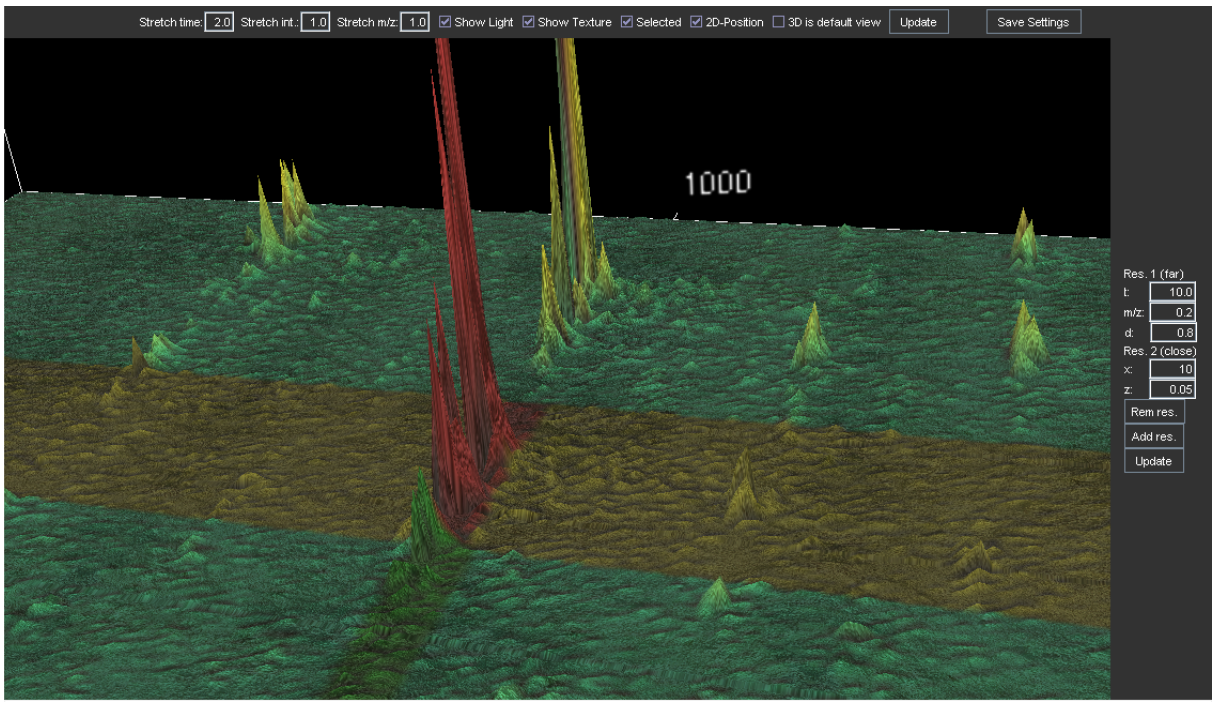


The memory used for this applet is mainly dependant on the resolution used and on effects like “Show Light” or “Show Texture”.
With the “Update” buttons you execute your changed settings.
With “Stretch time” you can stretch or tighten the time coordinate. With “Stretch int.” you can stretch or tighten the intensity coordinate. With “Stretch m/z” you can stretch the m/z axis. With show lights you can have light effects and with “Show Texture” the surface is covered with a texture. The light and texture option makes it easier to realize bumpiness on the surface. The “Selected” option shows the selected peaks in red and green. The “2D-Position” option shows the position of the 2D chromatogram displayed below in gold in the 3D chromatogram. On the right menu you can specify how the time and the m/z axis should be resolved. Here in this example I used two resolutions depending on the distance to the object. When you come nearer it will automatically switch to a higher resolution.

Far resolution:



Close resolution:



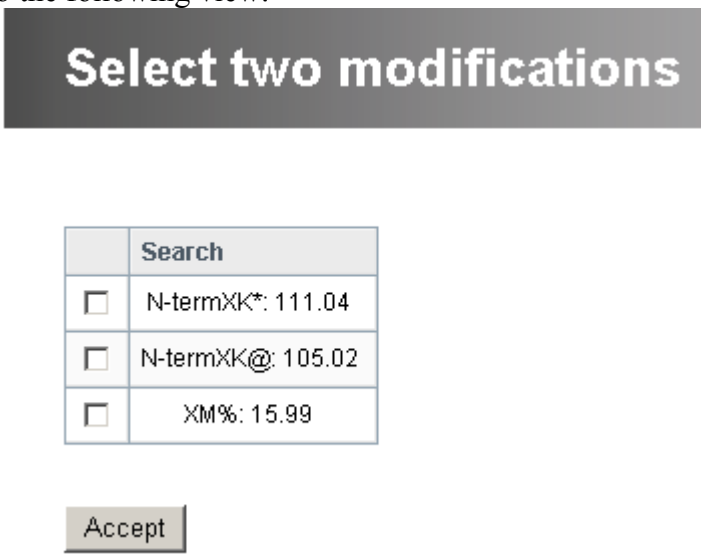
t: time in seconds which is used for one data point
m/z: m/z distance which is used for one data point
d: distance to the chromatogram object to switch to a higher resolution (has nothing to do with m/z or t).

The user can store for himself his own resolution settings and the rest of the settings described here with “Save Settings”. The “3D is default view” option has just an effect when you click afterwards on “Save settings”. When you checked this option, the next time you open a chromatogram viewer applet the 3D view will be used automatically.

Be careful with the resolution settings because they are causing the memory consumption of the applet. When you first visit the 3D view an automatic setting is calculated, which is adjusted to your data. When you once stored your own settings, these automatic settings will never be called again. So be careful when you first took a look at high resolution data (in a smaller range) and switch then to low resolution data (in a broader range). The viewer will still use the high resolution settings (if you did not save different ones) and run out of memory. Once the Java Console runs out of Memory all the browser windows have to be closed and the browser must be restarted. Very often the Java Console is still causing problems. The best is, once a browser window is opened to open the Java Console before any page is visited (then I have never problems). For this applet I had the experience that the Firefox browser is better, since for the Firefox more memory can be allocated for applets than the IE.

7.8 Evaluation of quantitative ratios

When you click on the link “Compare Ratios of 2 modifications” in the peptide list you come to the following view:



Her all the possible variable modifications are listed. You can select one ore two between them you want to calculate ratios and click the “Accept” button.

(See next figure) At the upper left the selected searches are listed and a number for them. Then you have a list of your comparable peptides. In the first column you can select and deselect the peptide, the second columns indicates the search which is compared, the third column is the peptide sequence which is compared, the fourth column shows the charge states which are comparable, the fifth column shows the area for one modification, the sixth for the other one, the seventh and the eighth column shows the ratios of the areas to one another. At the end of the column the mean and the standard deviation of the selected values is calculated. The whole list can be exported to Excel, Doc and txt. At the bottom of this is a link called “Refresh areas” which refreshes the list when you changed quantified areas manually. The picture depicts the found ratios graphically and calculates a regression line for the values. On the one axis the area for one modification and on the other to area for the other modification is depicted. The picture can be copied directly out of the browser.

1=ICPL_Protmix_1lizu1he_A_c1_ms2
2=ICPL_Protmix_1lizu1he_B_c1_ms2
3=ICPL_Protmix_1lizu1he_C_c1_ms2

		Sequence	Z	N-termXK*: 111.04	N-termXK@: 105.02	Ratio 1/2	Ratio 2/1
☑	3	.ALK*AWSVAR.	2	8856610.6171875	8761861.0	1.0108138690156692	0.9893018197047497
☑	2	.ALK*AWSVAR.	2	9542612.75	9225358.75	1.0343893401435473	0.9667539689274303
☑	1	.ALK*AWSVAR.	2	1.0170150546875E7	9550851.375	1.0648423001844691	0.9391061942475086
☑	2	.CASIQK*FGER.	3	876386.25	958544.125	0.9142888961945284	1.0937461935305353
☑	1	.CASIQK*FGER.	2	9323722.59375	9311852.25	1.0012747564535294	0.9987268664816393
☐	3	.CLK*DGAGDVAFVK.	2	128293.22	661789.1	0.19385816417949464	5.15841055357407
☐	2	.CLK*DGAGDVAFVK.	2	195198.17	634894.9	0.30744957945007906	3.2525658411654166
☐	3	.CLVEK*GDVAFVK.		0.0	0.0	NaN	NaN
☑	2	.CLVEK*GDVAFVK.	2	1307267.75	1664997.8046875	0.7851468310166081	1.2736471198708146
☑	1	.CLVEK*GDVAFVK.	2	1319871.453125	1459752.2	0.9041750052680174	1.1059805835968424
☑	3	.DDTVCLAK*LHDR.	2	2808382.15625	2191846.390625	1.2812860281915999	0.7804658585182535
☑	3	.DHMK*SVIPSDGPSVACVK.	2	164365.38	166958.88	0.9844662350394301	1.0157788702219408
☑	1	.YLGEEYVK*AVGNLR.	3	506337.73046875	416672.78125	1.2151927201718316	0.8229147388725283
		Mean:				0.9666037807938672	1.0660752058942036
		Standard Dev.:				0.16904034	0.18760616

Export Current View: Excel | DOC | TEXT

fresh Areas