PDQuest™ 2-D Analysis Software
Version 6.2 Update Training Package

PDQuest software functionality and tools have been extensively enhanced. Additionally, a new focus on process automation and integration of data from a variety of sources will appeal to the proteome-oriented researcher. Highlights include:

- Greatly enhanced annotation capabilities that allow researchers greater flexibility and speed in annotating databases, as well as allowing spot annotation with URL addresses or document links to server path and file names
- More powerful data export and import capabilities
- More powerful image viewing capabilities and options, including multichannel viewing tools and multi-image reviewing tools
- Volume tools that allow researchers to override Gaussian spot quantitation
- Ability to build "cyber gels" with ReadyStrip™ narrow-range IPG strips
- Full integration with the ProteomeWorks™ system, including control of the Spot-Cutter excision robot as well as sample tracking through data analysis (with the Micromass™ MassPREP™ digestion robot and Micromass M@LDI™ and Q-TOF™ mass spectrometers)

Summary of major changes incorporated into PDQuest, version 6.2

Features/ Interface Design:

- Advanced Annotation Tool
- Improved Spot Data Export/Import
- Image Stack Tool
- Multichannel Image Merging
- Build Cyber Gels from Multiple Gels that Resolve Different pI or Molecular Weight Ranges
- Integrated ProteomeWorks Spot-Cutter Control
- Micromass Mass Spectrometry and Sample Prep Robot Integration
- The Scatter Plot Tool is linked to images, to allow identification of variant proteins
- Volume freehand and auto-contour tools have been added, allowing overwrite of Gaussian quantitation of spots
- Context-sensitive right-mouse-click menus
- Match-offset vectors now indicate outliers in red for better visibility when editing matching of spots

See subsequent pages of this training update for additional detail on the above features

Instrument Integration:

Bio-Rad Devices-

GSD-800 Scanning Densitometer
ProteomeWorks Spot Cutter

Other Devices-

Micromass mass spec data integration (MALDI & Q-TOF)

Advanced Annotation Tool

This tool is easier to work with; it stays resident until closed, and gives feedback on which categories have been annotated on a gel, and which spots within a category have been annotated. Multiple spots to be annotated can be selected from a gel or from an integral spot list and annotated in one operation. Conversion of entire analysis sets into annotations is now possible. You can also display multiple annotation categories simultaneously with unique, user-definable annotation markings. The Annotation Tool allows file path links to documents, including URL links, as annotations, with automatic launching of a web browser to the specified URL. It also dynamically builds HTML pages to view spot annotations.

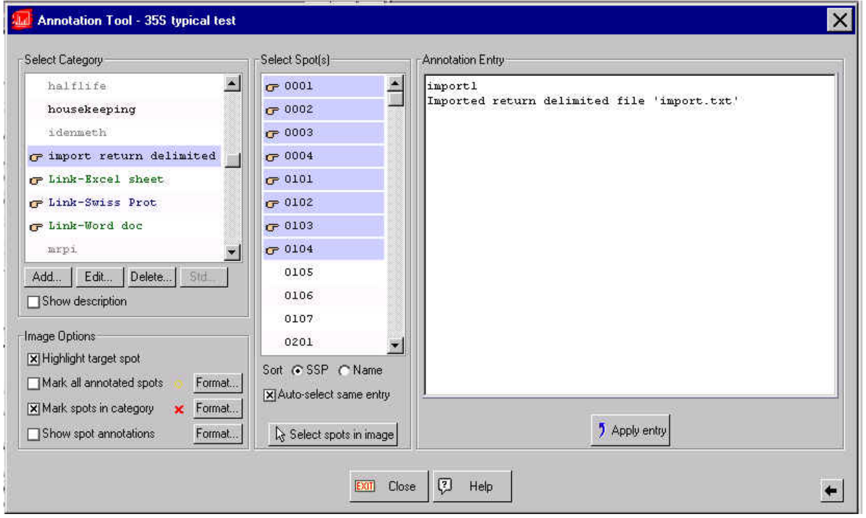

Annotation Tool - 35S typical test

Image Options:
☒ Highlight target spot
☐ Mark all annotated spots
☒ Mark spots in category
☐ Show spot annotations

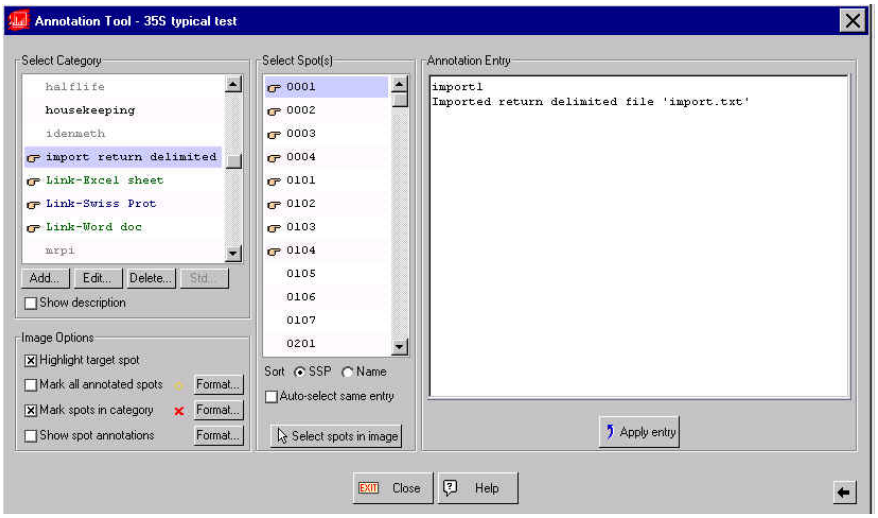
Sort ● SSP ○ Name
☒ Auto-select same entry

☐ Show description

Annotation Entry:
```
import1
Imported return delimited file 'import.txt'
```

annotation 1.

- Positive indication of which categories have annotation entries.
- Positive indication of which spots within a category have annotation entries.
- Color-keyed indication of category format;
  -black for text annotation
  -blue for URL link annotation
  -green for file path name annotation (documents and spreadsheets)
- Positive indication of currently selected category and spot(s)
- Large field allows high annotation entry content.
- Enabling “Auto-select same entry” option allows all spots within a category with the same annotation entry to be selected and/or edited simultaneously in a single operation.



```
import1
Imported return delimited file 'import.txt'
```

annotation 2.

- Disabling "Auto-select same entry" option allows selection or editing of a single spot within a category even if spots with same entry exist

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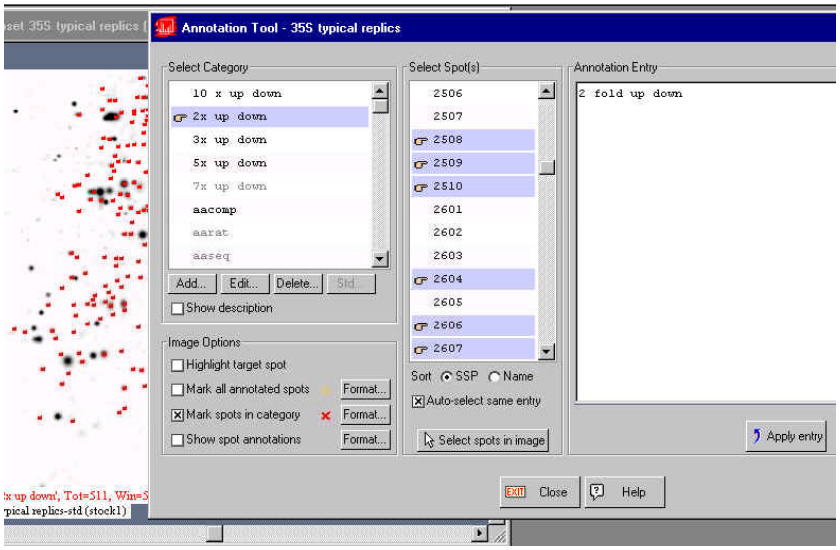

```
2 fold up down
```

annotation 3.

- Can use Shift-click and Ctrl-click conventions to select multiple spots from spot list
- Can use Ctrl-click convention to select multiple spots from gel image

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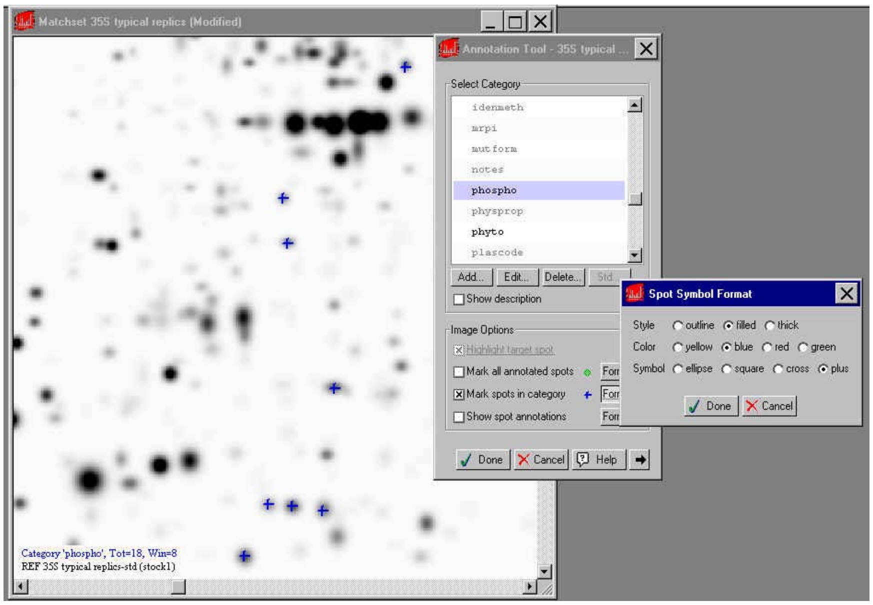


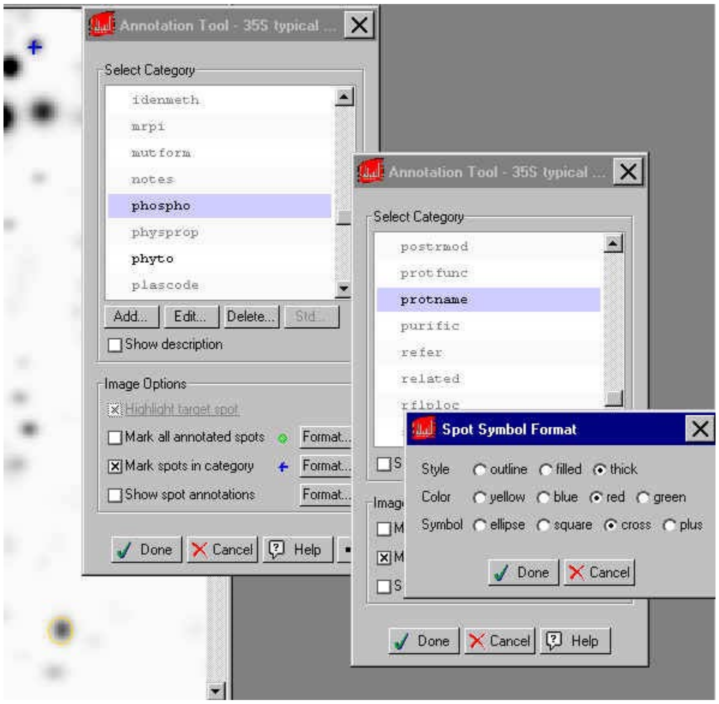
☐ Show description
☒ Highlight target spot
☐ Mark all annotated spots
☒ Mark spots in category
☐ Show spot annotations

Style ○ outline ● filled ○ thick
Color ○ yellow ● blue ○ red ○ green
Symbol ○ ellipse ○ square ○ cross ● plus

Category 'phospho', Tot=18, Win=8
REF 35S typical replics-std (stock1)

annotation 4.

- Gel image symbol overlay display options include;
  -Spots currently targeted (selected) for annotation entry
  -All annotated spots
  -Spots in selected category (*shown in above example*)
  -Annotation Category name
- User can define format of annotation overlay symbol for any specific category
  -style
  -color
  -symbol shape



annotation 5.

- Tool stays resident until dismissed
- Multiple iterations of tool can be enabled, allowing simultaneous view of multiple categories
  - annotation overlay symbol can be defined independently for multiple categories

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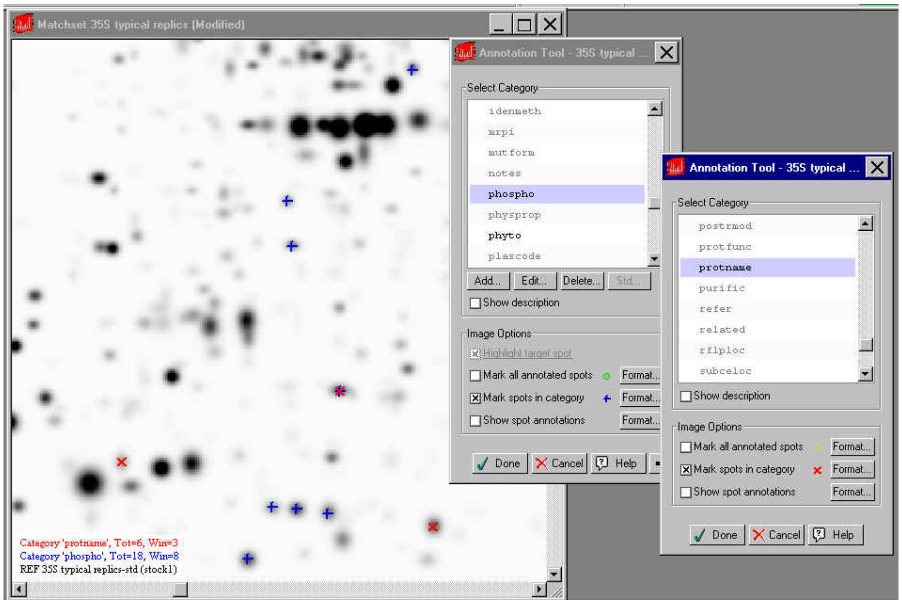
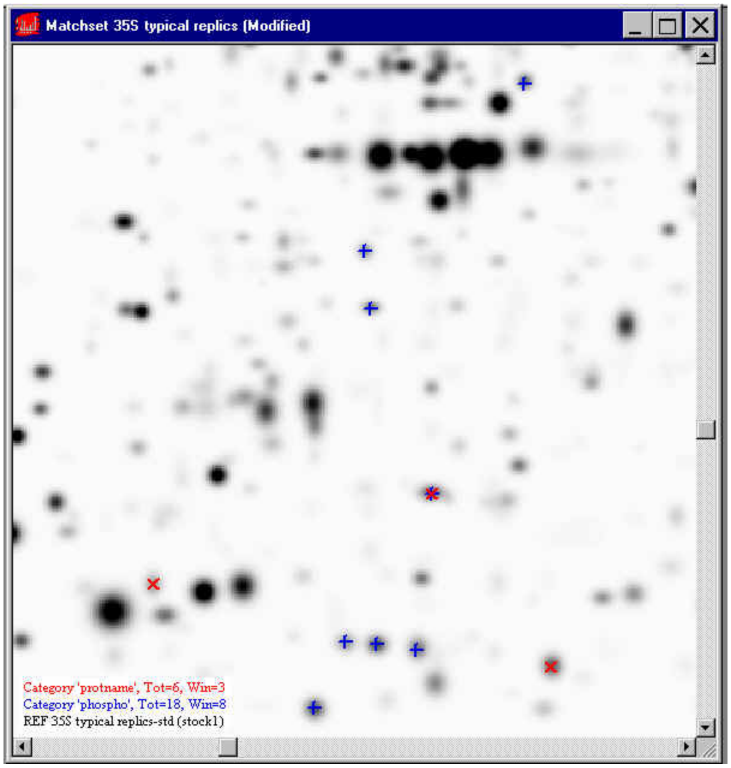


Category 'protname', Tot=6, Win=3
Category 'phospho', Tot=18, Win=8
REF 35S typical replics-std (stock1)

annotation 6.

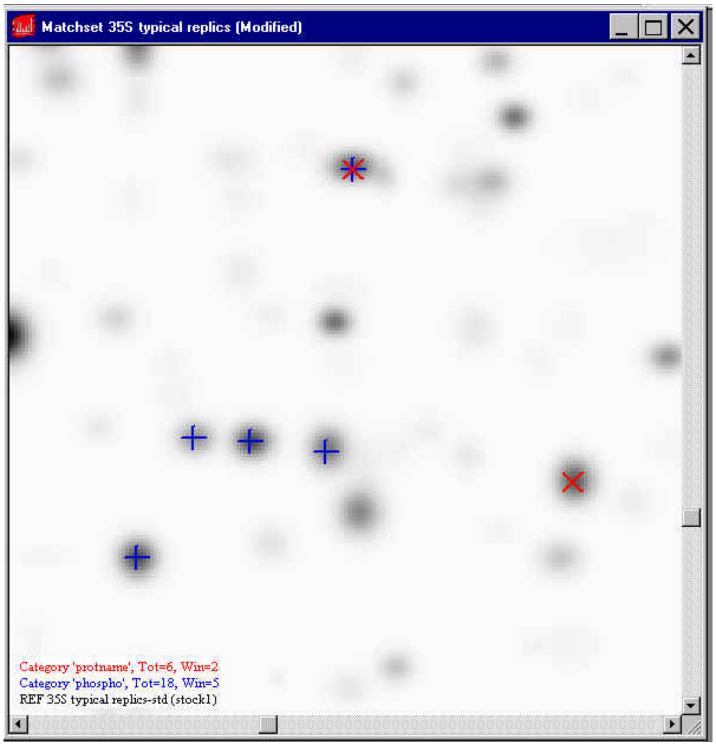
- Simultaneous view of two categories with unique spot symbol overlays
  - phosphorylase proteins (category phospho) as blue pluses
  - proteins with defined protein names (category protname) as red crosses



Category 'protname', Tot=6, Win=3
Category 'phospho', Tot=18, Win=8
REF 35S typical replics-std (stock1)

annotation 7.
CLOSE-UP VIEW

- Simultaneous view of two categories with unique spot symbol overlays
  - phosphorylase proteins (category phospho) as blue pluses
  - proteins with defined protein names (category protname) as red crosses
- Note category legend and spot counts in bottom left corner of window

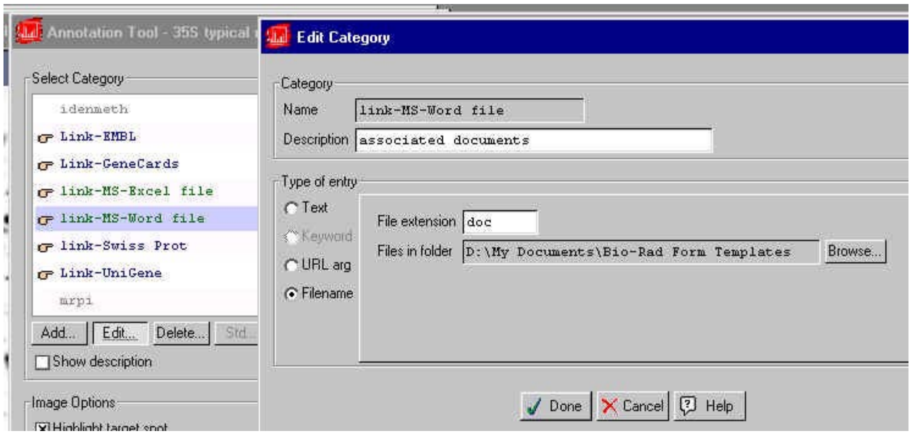


Matchset 35S typical replics (Modified)

Category 'protname', Tot=6, Win=2
Category 'phospho', Tot=18, Win=5
REF 35S typical replics-std (stock1)

annotation 8.
REALLY CLOSE-UP VIEW

- Simultaneous view of two categories with unique spot symbol overlays
  - phosphorylase proteins (category phospho) as blue pluses
  - proteins with defined protein names (category protname) as red crosses
- Note category legend and spot counts in bottom left corner of window
- Note dual-symbol overlay for spot near top of window



Annotation Tool - 35S typical

Select Category
idenmeth
Link-EMBL
Link-GeneCards
link-MS-Excel file
link-MS-Word file
link-Swiss Prot
Link-UniGene
mrpi

☐ Show description

Image Options
☒ Highlight target spot

Edit Category

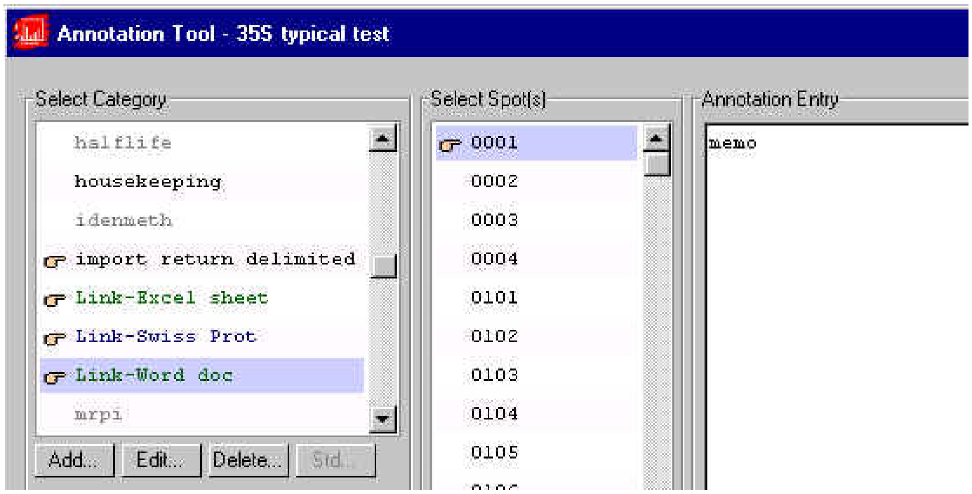
Category
Name: link-MS-Word file
Description: associated documents

Type of entry
○ Text
○ Keyword
○ URL arg
● Filename

File extension: doc
Files in folder: D:\My Documents\Bio-Rad Form Templates

annotation 9.
FORMATTING ANNOTATIONS: File Path Names

- File path links to MS-Word document or MS-Excel documents
- Automatic launch of application to link from right mouse menu
- File path and file name extensions are pre-defined for category



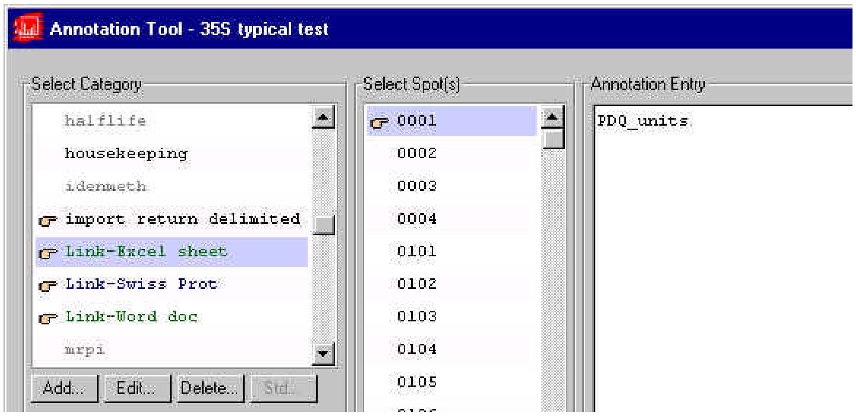
Annotation Entry

memo

annotation 10.

ANNOTATION ENTRIES: File Path Names

- File path link to MS-Word document
- File name extension not required



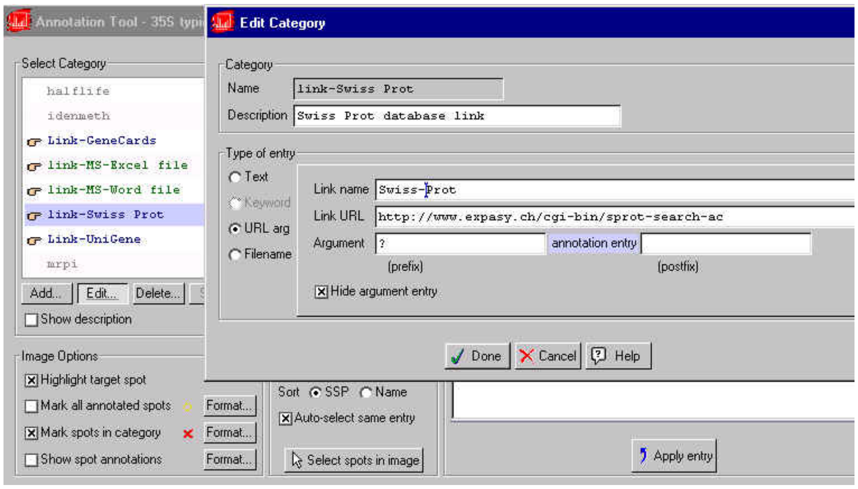
Annotation Entry

PDQ_units

annotation 11.

ANNOTATION ENTRIES: File Path Names

- File path link to MS-Excel document
- File name extension not required



Category
Name: link-Swiss Prot
Description: Swiss Prot database link

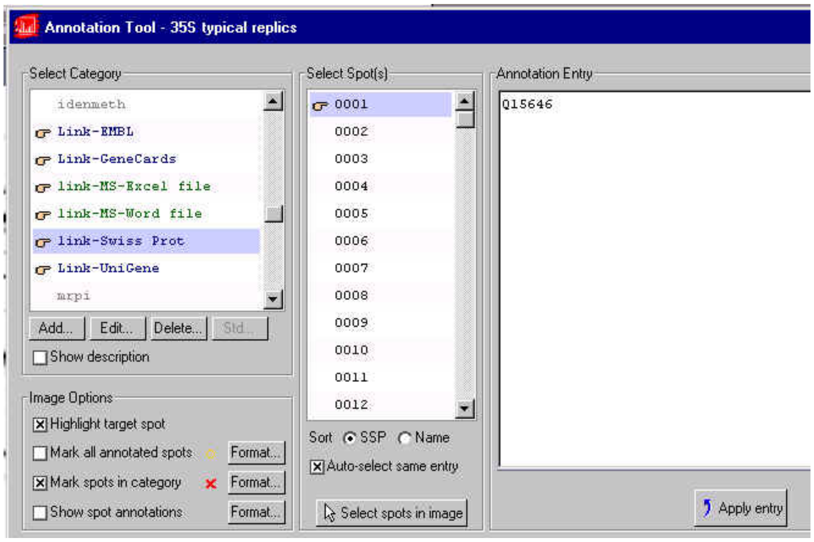
Type of entry
○ Text
○ Keyword
● URL arg
○ Filename

Link name: Swiss-Prot
Link URL: http://www.expasy.ch/cgi-bin/sprot-search-ac
Argument: ? (prefix) annotation entry (postfix)
☒ Hide argument entry

annotation 12.

FORMATTING ANNOTATIONS: URL Links

- Link to URL
- Define URL link address
- Define argument prefix (to annotation entry)
- Define argument postfix (to annotation entry)

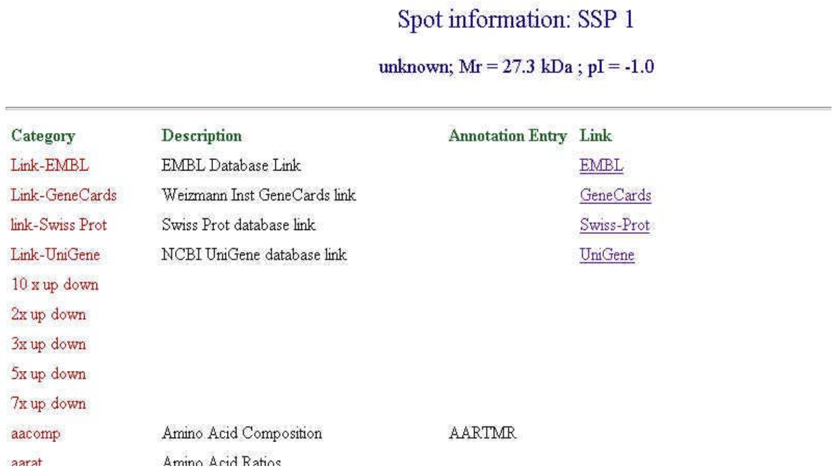


Annotation Entry: Q15646

annotation 13.

ANNOTATION ENTRIES: URL Links

- Link to URL
- Annotation entry is database entry accession number



Spot information: SSP 1

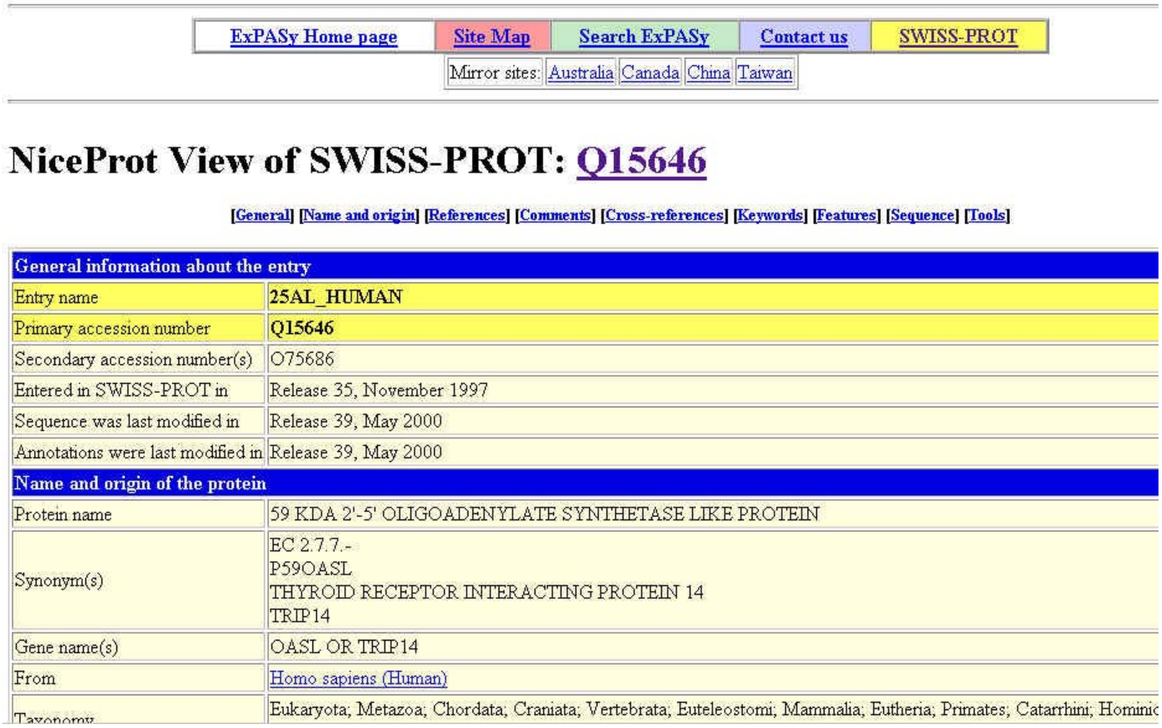
unknown; Mr = 27.3 kDa ; pI = -1.0

Category	Description	Annotation Entry	Link
Link-EMBL	EMBL Database Link		EMBL
Link-GeneCards	Weizmann Inst GeneCards link		GeneCards
link-Swiss Prot	Swiss Prot database link		Swiss-Prot
Link-UniGene	NCBI UniGene database link		UniGene
10 x up down			
2x up down			
3x up down			
5x up down			
7x up down			
aacomp	Amino Acid Composition	AARTMR	
aarat	Amino Acid Ratios		

annotation 14.

BROWSE ANNOTATIONS PAGE:

- HTML page view of spot annotation
- Launched from right-mouse menu from an annotated spot
- Dynamically built in local browser to show all spot annotation updates
- Shows all categories and presence of annotation entries
- Shows File Path Name links
- Shows URL links as hyperlinks

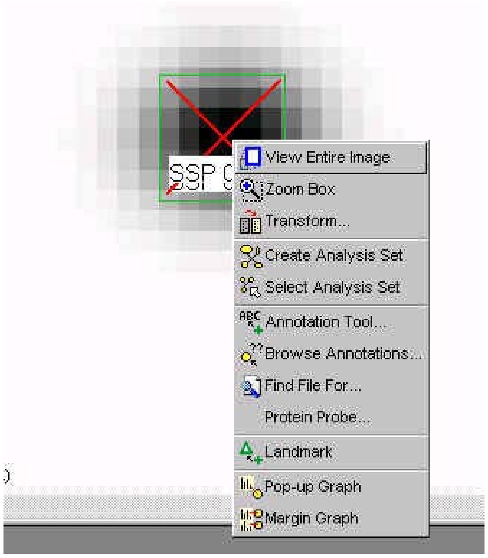


NicePort View of SWISS-PROT: Q15646

General information about the entry	
Entry name	25AL_HUMAN
Primary accession number	Q15646
Secondary accession number(s)	O75686
Entered in SWISS-PROT in	Release 35, November 1997
Sequence was last modified in	Release 39, May 2000
Annotations were last modified in	Release 39, May 2000
Name and origin of the protein	
Protein name	59 KDA 2'-5' OLIGOADENYLATE SYNTHETASE LIKE PROTEIN
Synonym(s)	EC 2.7.7.- P59OASL THYROID RECEPTOR INTERACTING PROTEIN 14 TRIP14
Gene name(s)	OASL OR TRIP14
From	Homo sapiens (Human)
Taxonomy	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Primates; Catarrhini; Hominic

annotation 15.

- Link to URL destination from BROWSE ANNOTATIONS PAGE:
- Automatic launch of local Web Browser

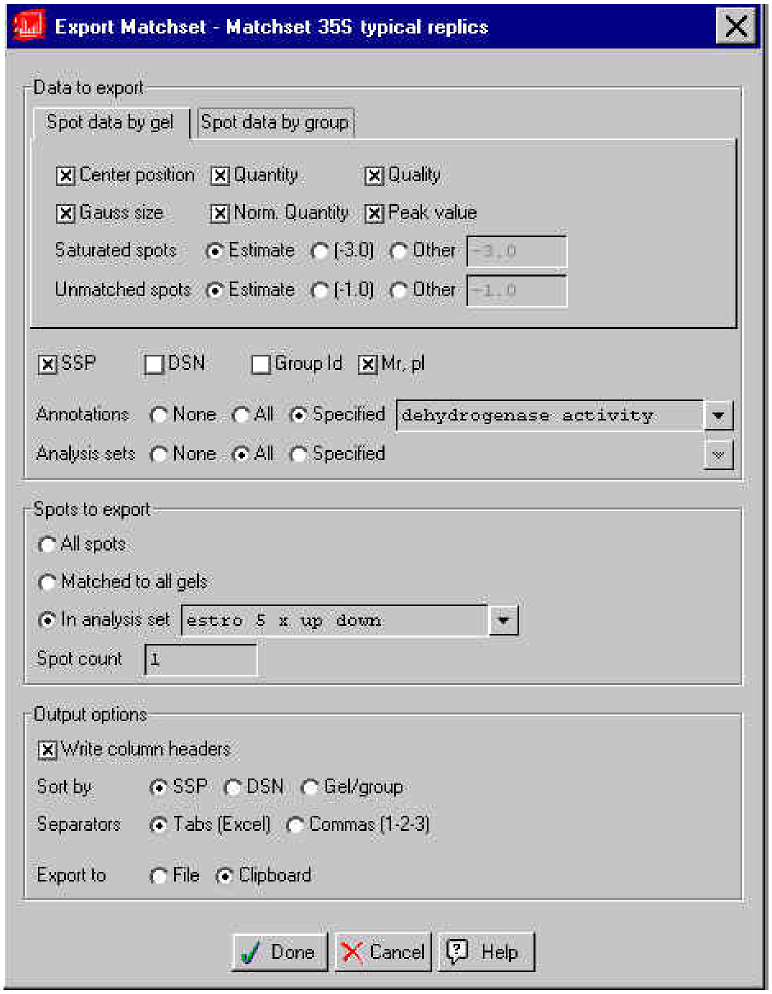


annotation 16.

- Open linked documents through right-mouse menu from spot
- Use “Find File For...” command
- Automatic launch of local application

Improved Spot Data Export/Import

Export of all matchset data is now possible. You can import a list of SSP numbers as a file, and convert it into an analysis set.



Export Matchset - Matchset 35S typical replics

Data to export

Spot data by gel | Spot data by group

☒ Center position ☒ Quantity ☒ Quality

☒ Gauss size ☒ Norm. Quantity ☒ Peak value

Saturated spots ● Estimate ○ (-3.0) ○ Other -3.0

Unmatched spots ● Estimate ○ (-1.0) ○ Other -1.0

☒ SSP ☐ DSN ☐ Group Id ☒ Mr, pI

Annotations ○ None ○ All ● Specified dehydrogenase activity

Analysis sets ○ None ● All ○ Specified

Spots to export

○ All spots

○ Matched to all gels

● In analysis set estro 5 x up down

Spot count 1

Output options

☒ Write column headers

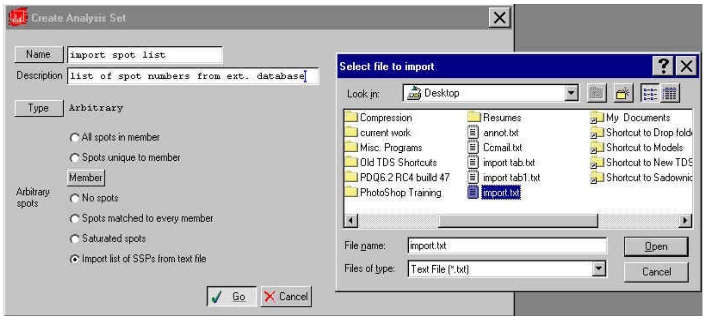
Sort by ● SSP ○ DSN ○ Gel/group

Separators ● Tabs (Excel) ○ Commas (1-2-3)

Export to ○ File ● Clipboard

export 1.

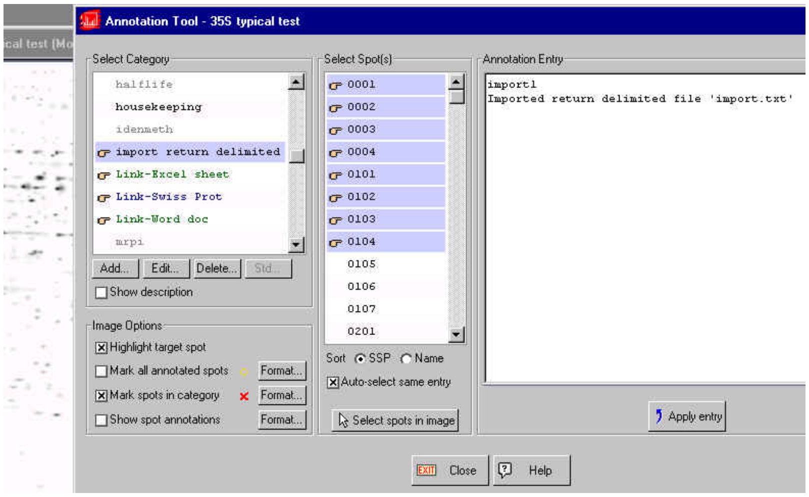
- Check boxes allow tailoring of any or all parameters to be included in export file
- Parameters can include annotation category entries and/or analysis set membership status
  -options include none, all , or specified category or analysis set
- Can restrict selection of spots for export;
  -all spots
  -spots matched to all gels
  -spots in a specific analysis set
- Export to clipboard allows convenient paste into spreadsheet programs for immediate viewing



Name: import spot list
Description: list of spot numbers from ext. database
Type: Arbitrary
Arbitrary spots
○ All spots in member
○ Spots unique to member
Member
○ No spots
○ Spots matched to every member
○ Saturated spots
● Import list of SSPs from text file
File name: import.txt
Files of type: Text File (*.txt)

import 1.

- Create an Arbitrary Analysis Set
- Define Type as “Import List of SSPs from text file”
- Browse to return-delimited text file containing spot list
  -valid spot entries are “101” or “0101”



☐ Show description
Image Options
☒ Highlight target spot
☐ Mark all annotated spots
☒ Mark spots in category
☐ Show spot annotations
Sort ● SSP ○ Name
☒ Auto-select same entry

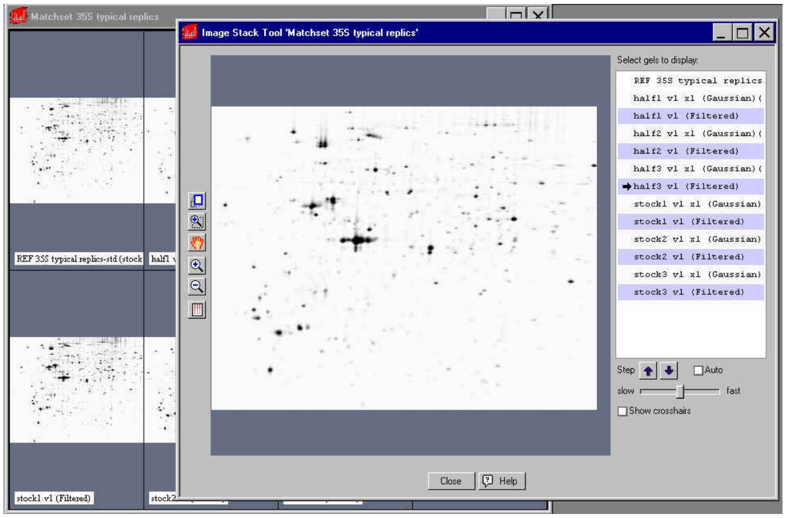
```
import1
Imported return delimited file 'import.txt'
```

import 2.

- Convert Analysis Set into an Annotation Category
  -from menu: **Analysis > More Annot. Tools > Create Annotation from Set**

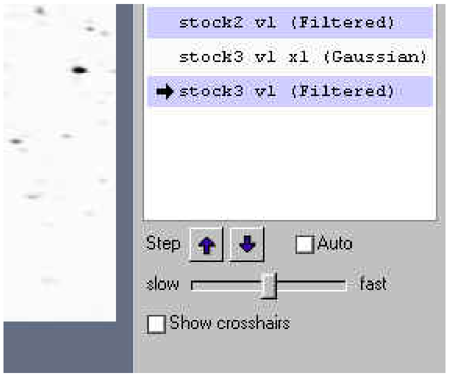
Image Stack Tool

This tool allows aligned-stack viewing of multiple gels, and also offers an auto-flicker option with user-adjustable speed. Aligned stack viewing is only available if gels have been matched. Local alignment will be performed within any window view based on the spot(s) closest to the center of the view.



stack 1.

- All open images in loaded into image list window
- Use Shift-Click and Ctrl-click conventions to choose images for viewing
- If matched

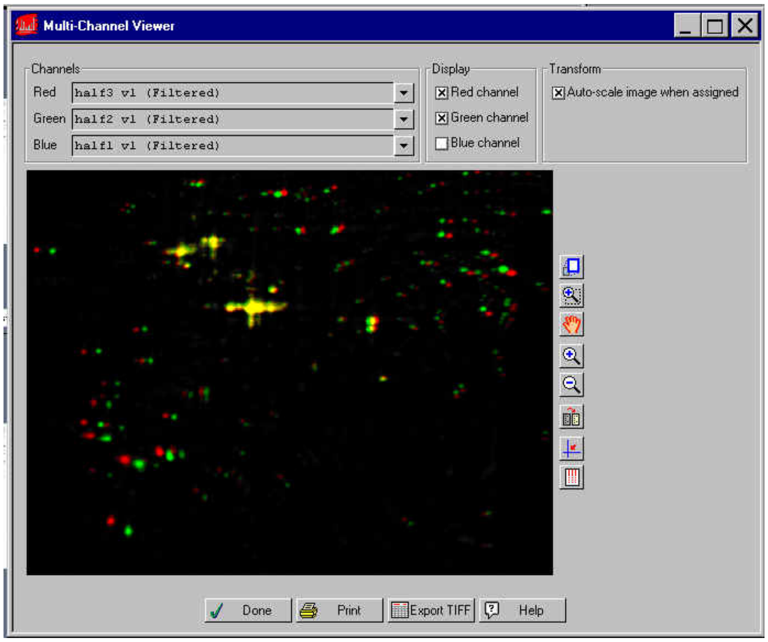


stack 2.

- flicker tools
  - -up/down
- auto-flicker tools
  - -on/off
  - -speed of image flickering

Multichannel Image Merging

Merge up to 3 image channels with independent red, green, or blue color display. Balance the display with independent channel transformation. Zoom in to see fine details like spot overlap.



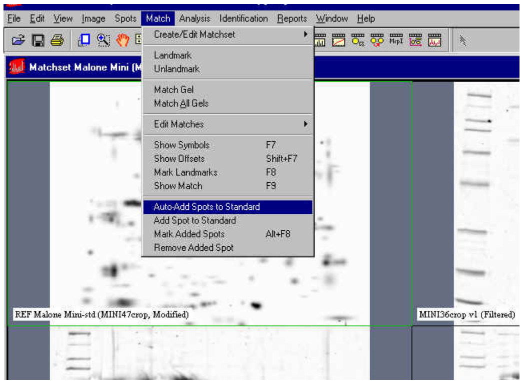
Red half3 v1 (Filtered)
Green half2 v1 (Filtered)
Blue half1 v1 (Filtered)

☒ Red channel
☒ Green channel
☐ Blue channel

☒ Auto-scale image when assigned

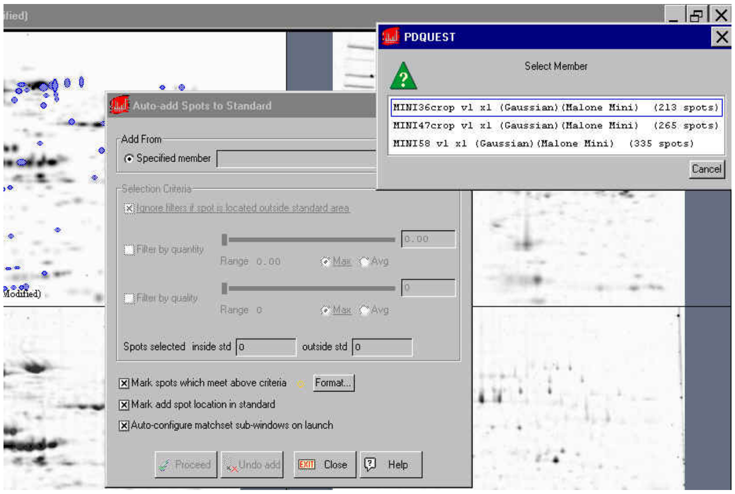
Build Cyber Gels from Multiple Gels that Resolve Different pI or Molecular Weight Ranges

The new Auto-add to Standard function allows addition of all unique spots from a given gel to the standard reference image in one operation. These tools allow expansion of the standard reference image to accommodate a larger area (for example, when combining 2 different IPG strip separations) to build a cyber gel.



Cyber 1.

- Menu : **Match > Auto-add to standard**



Select Member

```
MINI36crop v1 x1 (Gaussian)(Malone Mini)  (213 spots)
MINI47crop v1 x1 (Gaussian)(Malone Mini)  (265 spots)
MINI58 v1 x1 (Gaussian)(Malone Mini)  (335 spots)
```

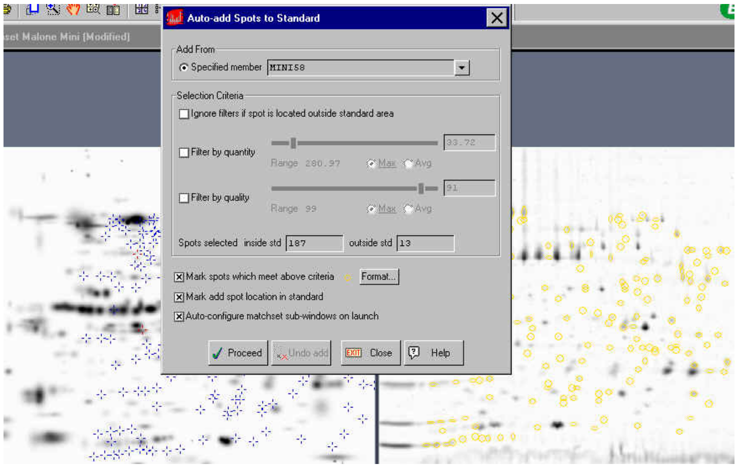
Add From
● Specified member

Selection Criteria
☒ Ignore filters if spot is located outside standard area
☐ Filter by quantity 0.00 Range 0.00 ● Max ○ Avg
☐ Filter by quality 0 Range 0 ● Max ○ Avg
Spots selected inside std 0 outside std 0

☒ Mark spots which meet above criteria
☒ Mark add spot location in standard
☒ Auto-configure matchset sub-windows on launch

Cyber 2.

- Choose gel to add unmatched spots from



Add From
● Specified member MINI58

Selection Criteria
☐ Ignore filters if spot is located outside standard area
☐ Filter by quantity 33.72 Range 280.97 ● Max ○ Avg
☐ Filter by quality 91 Range 99 ● Max ○ Avg
Spots selected inside std 187 outside std 13

☒ Mark spots which meet above criteria
☒ Mark add spot location in standard
☒ Auto-configure matchset sub-windows on launch

Cyber 3.

- Gel image overlay with targeted spots in member and targeted position to add in Standard Reference

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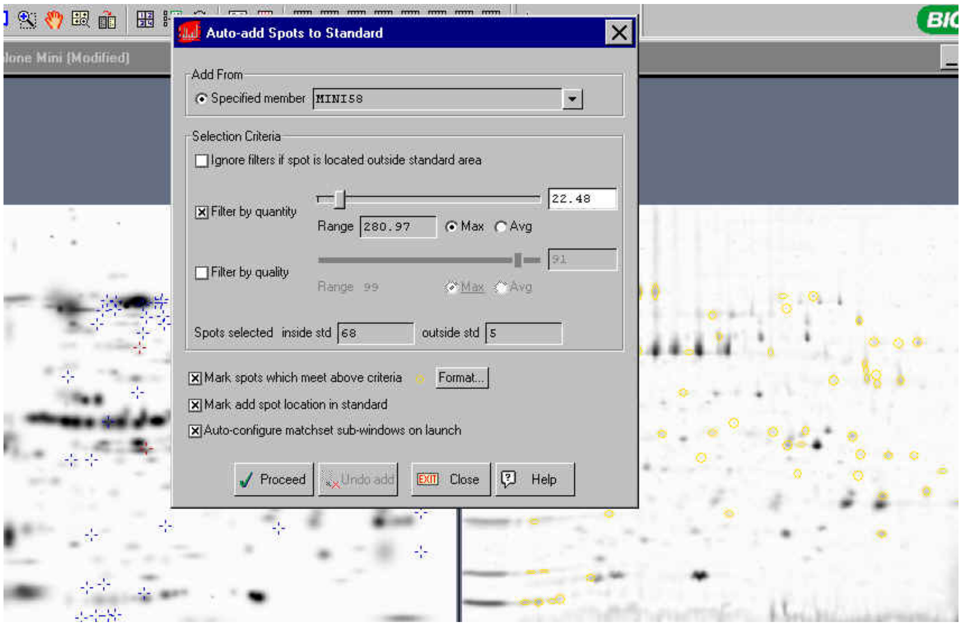

Auto-add Spots to Standard

Add From

● Specified member MINI58

Selection Criteria

☐ Ignore filters if spot is located outside standard area

☒ Filter by quantity 22.48 Range 280.97 ● Max ○ Avg

☐ Filter by quality 91 Range 99 ● Max ○ Avg

Spots selected inside std 68 outside std 5

☒ Mark spots which meet above criteria

☒ Mark add spot location in standard

☒ Auto-configure matchset sub-windows on launch

Cyber 4.

- Filtering of spot selection by both **quantity** and **quality**
- Instant feedback in gel image and spot selection number fieel

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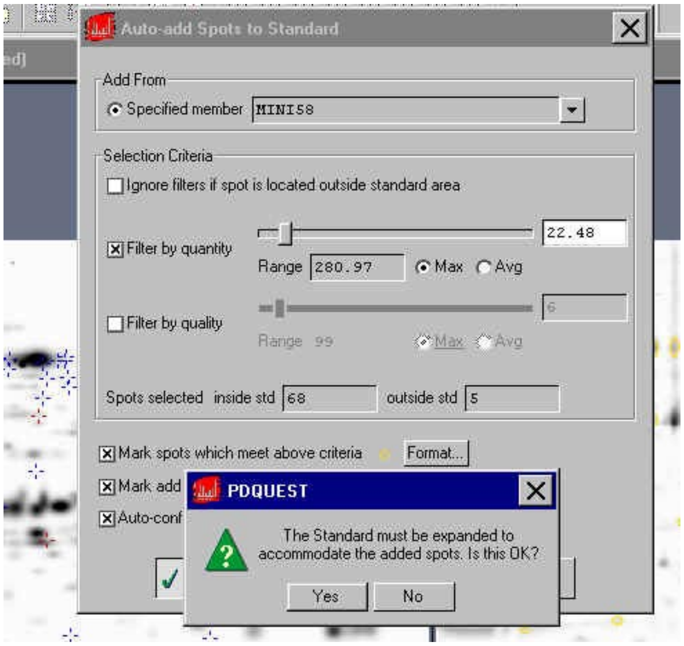

Auto-add Spots to Standard

Add From

● Specified member MINI58

Selection Criteria

☐ Ignore filters if spot is located outside standard area

☒ Filter by quantity 22.48 Range 280.97 ● Max ○ Avg

☐ Filter by quality 6 Range 99 ● Max ○ Avg

Spots selected inside std 68 outside std 5

☒ Mark spots which meet above criteria

☒ Mark add

☒ Auto-conf

PDQUEST

The Standard must be expanded to accommodate the added spots. Is this OK?

Cyber 5.

- Ability to expand Standard Reference to accommodate a wider pH or MW range
- User can select to ignore filters if targeted spot is outside the Standard Reference area

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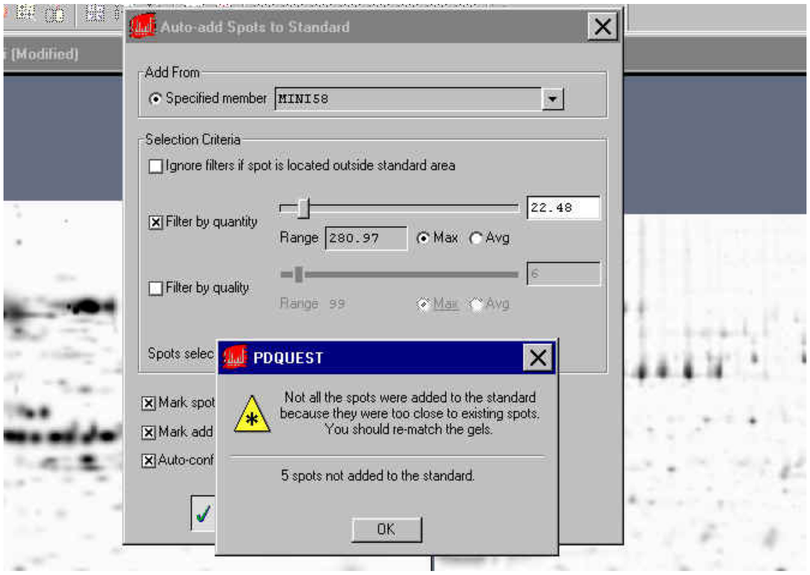


Add From
● Specified member MINI58

Selection Criteria
☐ Ignore filters if spot is located outside standard area
☒ Filter by quantity 22.48
Range 280.97 ● Max ○ Avg
☐ Filter by quality 6
Range 99 ● Max ○ Avg

Not all the spots were added to the standard because they were too close to existing spots. You should re-match the gels.

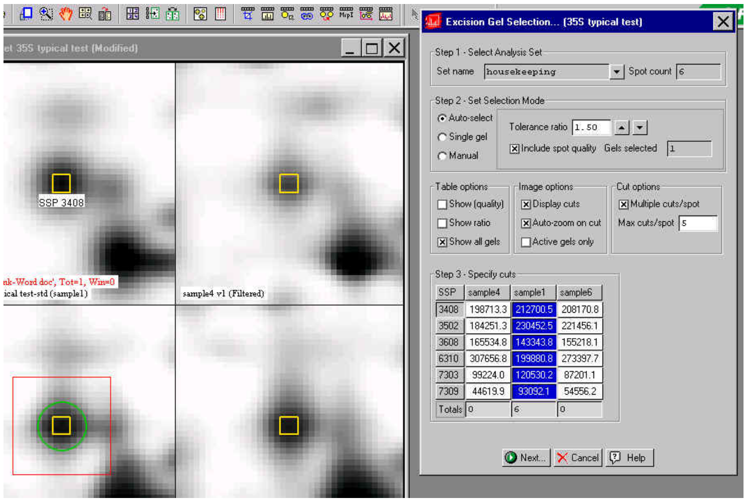
5 spots not added to the standard.

Cyber 6.

- Proximity over-ride and warning

Integrated ProteomeWorks Spot-Cutter Control

The software will drive the spot cutter to cut out any analysis set defined in PDQuest. in PDQuest software, with automatic targeting of spots to the correct location on the gel. A Basic Excision (point-and-click) cutting mode is also available. Basic Excision will also run in PDQuest Viewer mode.



Step 1 - Select Analysis Set
Set name: housekeeping
Spot count: 6

Step 2 - Set Selection Mode
● Auto-select
○ Single gel
○ Manual
Tolerance ratio: 1.50
☒ Include spot quality
Gels selected: 1

Table options
☐ Show (quality)
☐ Show ratio
☒ Show all gels

Image options
☒ Display cuts
☒ Auto-zoom on cut
☐ Active gels only

Cut options
☒ Multiple cuts/spot
Max cuts/spot: 5

Step 3 - Specify cuts

SSP	sample4	sample1	sample6
3408	198713.3	212700.5	208170.8
3502	184251.3	230452.5	221456.1
3608	165534.8	143343.8	155218.1
6310	307656.8	199880.8	273397.7
7303	99224.0	120530.2	87201.1
7309	44619.9	93092.1	54556.2
Totals	0	6	0

Spot Cutter 1.
GEL SELECTION TOOL

- Spots are pre-selected from PDQuest Analysis Sets
- Spot Cutter software can automatically target the gel with the highest expression of any given spot (to maximize excision yield)
  -automatically tracks and prompts for correct gel during excision
  -user can over-ride or supplement automatic selection
- Targeted spots in gel images presented for easy verification

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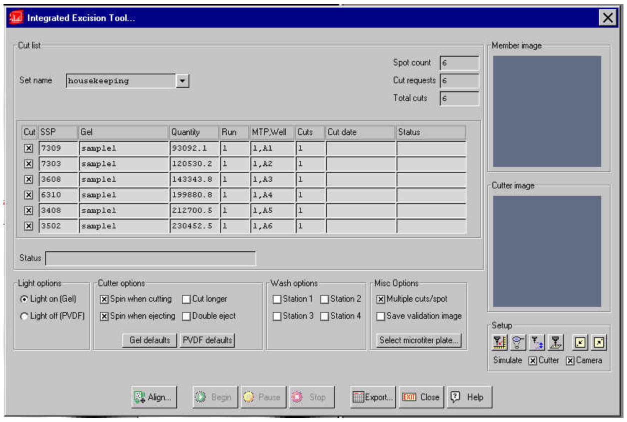


Cut list
Set name: housekeeping
Spot count: 6
Cut requests: 6
Total cuts: 6

Cut	SSP	Gel	Quantity	Run	MTP,Well	Cuts	Cut date	Status
☒	7309	sample1	93092.1	1	1,A1	1		
☒	7303	sample1	120530.2	1	1,A2	1		
☒	3608	sample1	143343.8	1	1,A3	1		
☒	6310	sample1	199880.8	1	1,A4	1		
☒	3408	sample1	212700.5	1	1,A5	1		
☒	3502	sample1	230452.5	1	1,A6	1		

Status:

Light options
● Light on (Gel)
○ Light off (PVDF)

Cutter options
☒ Spin when cutting
☐ Cut longer
☒ Spin when ejecting
☐ Double eject

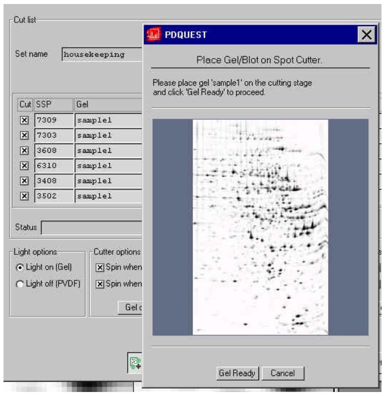
Wash options
☐ Station 1
☐ Station 2
☐ Station 3
☐ Station 4

Misc Options
☒ Multiple cuts/spot
☐ Save validation image

☒ Cutter
☒ Camera

Spot Cutter 2.
INTEGRATED EXCISION TOOL

- Controls alignment and cutting/re-cutting operations



Set name: housekeeping

Cut	SSP	Gel
☒	7309	sample1
☒	7303	sample1
☒	3608	sample1
☒	6310	sample1
☒	3408	sample1
☒	3502	sample1

Status

● Light on (Gel)
○ Light off (PVDF)
☒ Spin when
☒ Spin when

Place Gel/Blot on Spot Cutter.

Please place gel 'sample1' on the cutting stage and click 'Gel Ready' to proceed.

Spot Cutter 3.

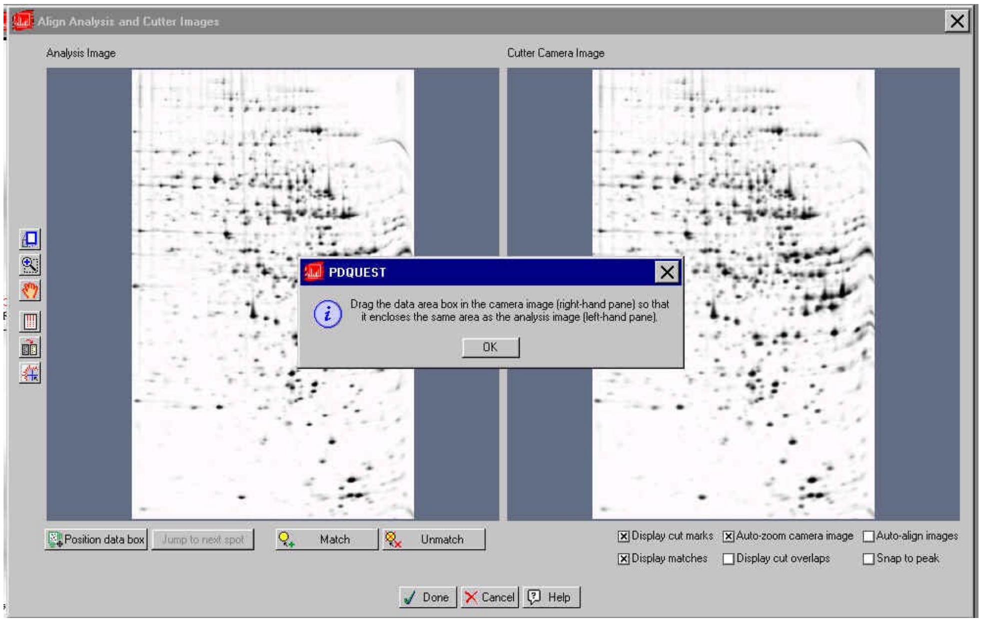
- Prompt for appropriate gel

Cutter not responding.

Would you like the simulation?

Spot Cutter 4.

- Spot Cutter Simulation mode for demos/training

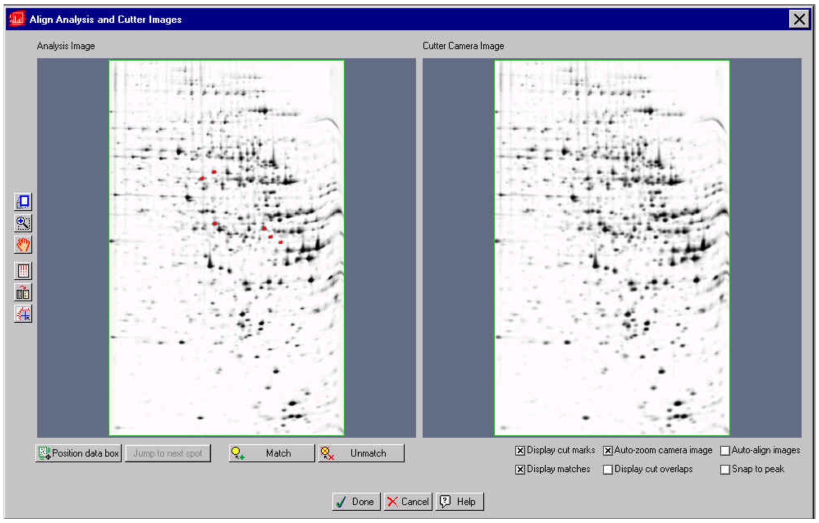


Drag the data area box in the camera image (right-hand pane) so that it encloses the same area as the analysis image (left-hand pane).

☒ Display cut marks
☒ Auto-zoom camera image
☐ Auto-align images
☒ Display matches
☐ Display cut overlaps
☐ Snap to peak

Spot Cutter 5.

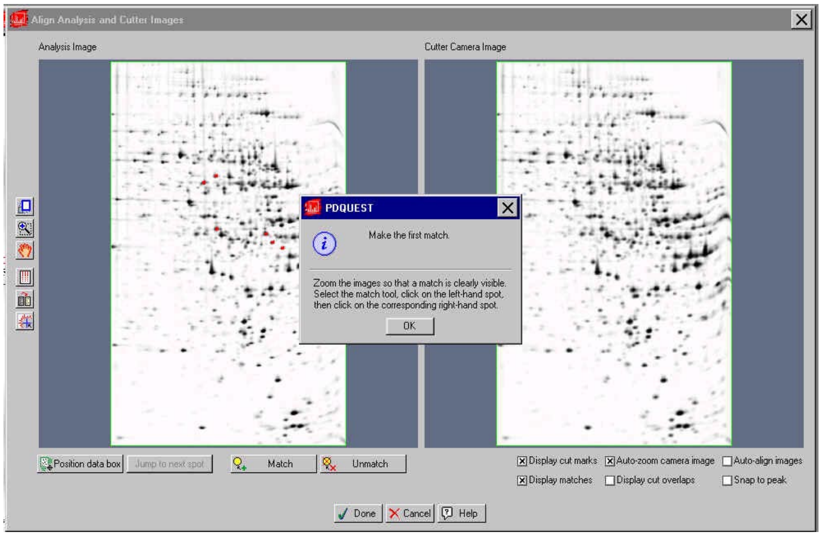
- Prompts for initial alignment; gel area



☒ Display cut marks
☒ Auto-zoom camera image
☐ Auto-align images
☒ Display matches
☐ Display cut overlaps
☐ Snap to peak

Spot Cutter 6.

- gel area aligned



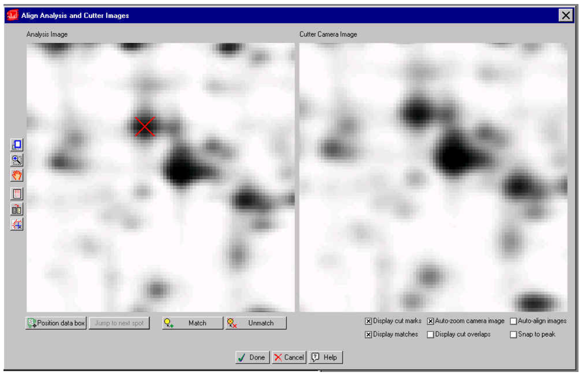
Make the first match.

Zoom the images so that a match is clearly visible. Select the match tool, click on the left-hand spot, then click on the corresponding right-hand spot.

☒ Display cut marks
☒ Auto-zoom camera image
☐ Auto-align images
☒ Display matches
☐ Display cut overlaps
☐ Snap to peak

Spot Cutter 7.

- Prompts for gel alignment; targeted spot in analysed image versus gel on Spot Cutter table

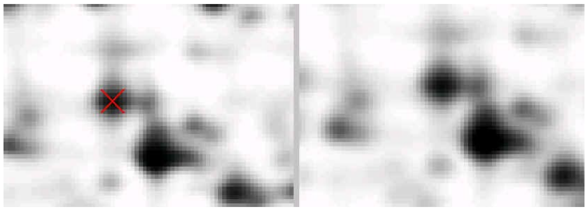


☒ Display cut marks ☒ Auto-zoom camera image ☐ Auto-align images
☒ Display matches ☐ Display cut overlaps ☐ Snap to peak

Spot Cutter 8.

- First targeted cut
- Analysis image on left, Spot Cutter target on image on right

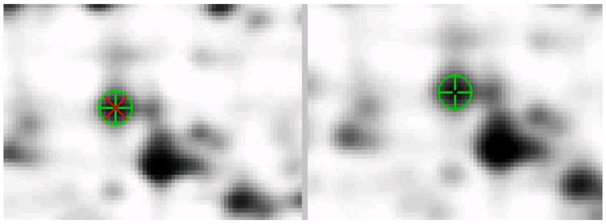
---



Spot Cutter 9.
CLOSE UP

- First targeted cut
- User clicks on matching spot in right side image to confirm cut location

---

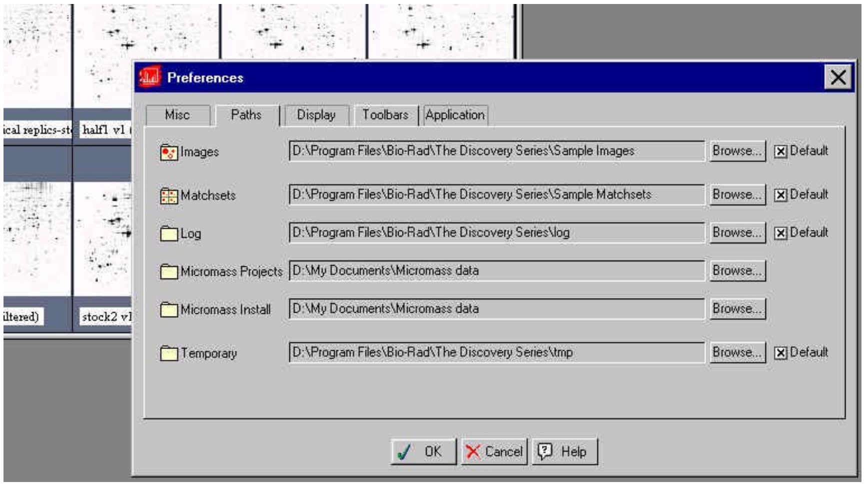


Spot Cutter 10.
CLOSE UP

- First targeted cut after alignment
- Target confirmation can be dragged to adjust accuracy of cutter tip placement
- Green circle is actual size of cutting tip
- After alignment, Shift-Click to jump to next targeted cut

Micromass Mass Spectrometry and Sample Prep Robot Integration

You can track the spot (after excision with the spot cutter) through the trypsin digestion robot and to the data file generated for that spot. Automatically update all spot annotations in a matchset with protein identification annotations from the resulting Micromass data files. Launch Micromass software via the ProteinProbe(tm) browser to see the associated mass spectra for a spot.

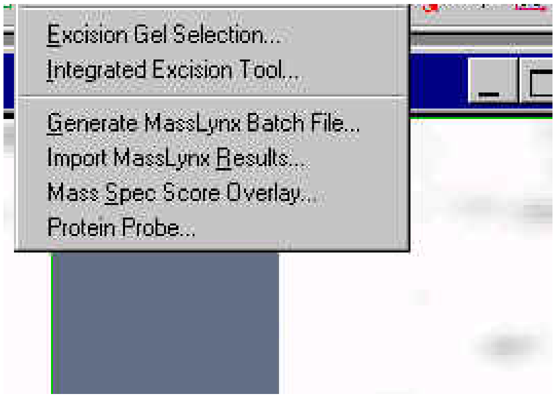


Images: D:\Program Files\Bio-Rad\The Discovery Series\Sample Images ☒ Default
Matchsets: D:\Program Files\Bio-Rad\The Discovery Series\Sample Matchsets ☒ Default
Log: D:\Program Files\Bio-Rad\The Discovery Series\log ☒ Default
Micromass Projects: D:\My Documents\Micromass data
Micromass Install: D:\My Documents\Micromass data
Temporary: D:\Program Files\Bio-Rad\The Discovery Series\tmp ☒ Default

Micromass 1.

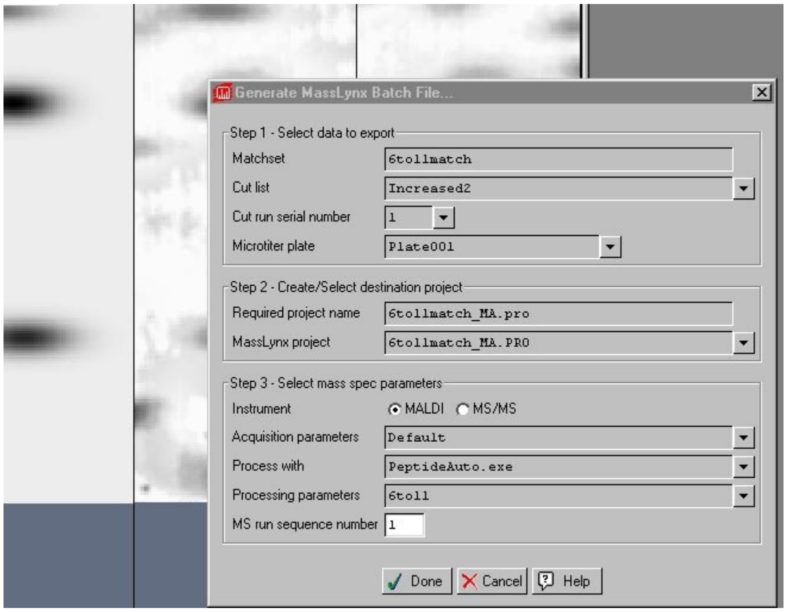
- Define pathways for Micromass data exchange
    - -where to export data file to establish a MassLynx project file
    - -where to find resulting protein identification results file

---



Micromass 2.

- Export a batch file which contains the neccessary info to establish links to the MassLynx project name for the mass spec results and instrument / processing software control parameters
- Import the resulting data file with automatic annotation category creation and data entry for identified spots



Step 1 - Select data to export

Matchset: 6to11match
Cut list: Increased2
Cut run serial number: 1
Microtiter plate: Plate001

Step 2 - Create/Select destination project

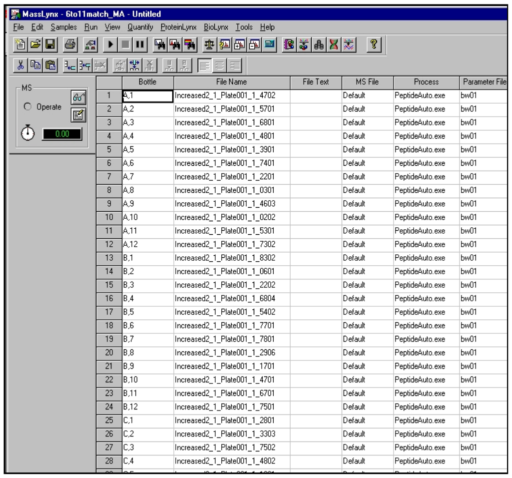
Required project name: 6to11match_MA.pro
MassLynx project: 6to11match_MA.PRO

Step 3 - Select mass spec parameters

Instrument: ● MALDI ○ MS/MS
Acquisition parameters: Default
Process with: PeptideAuto.exe
Processing parameters: 6to11
MS run sequence number: 1

Micromass 3.

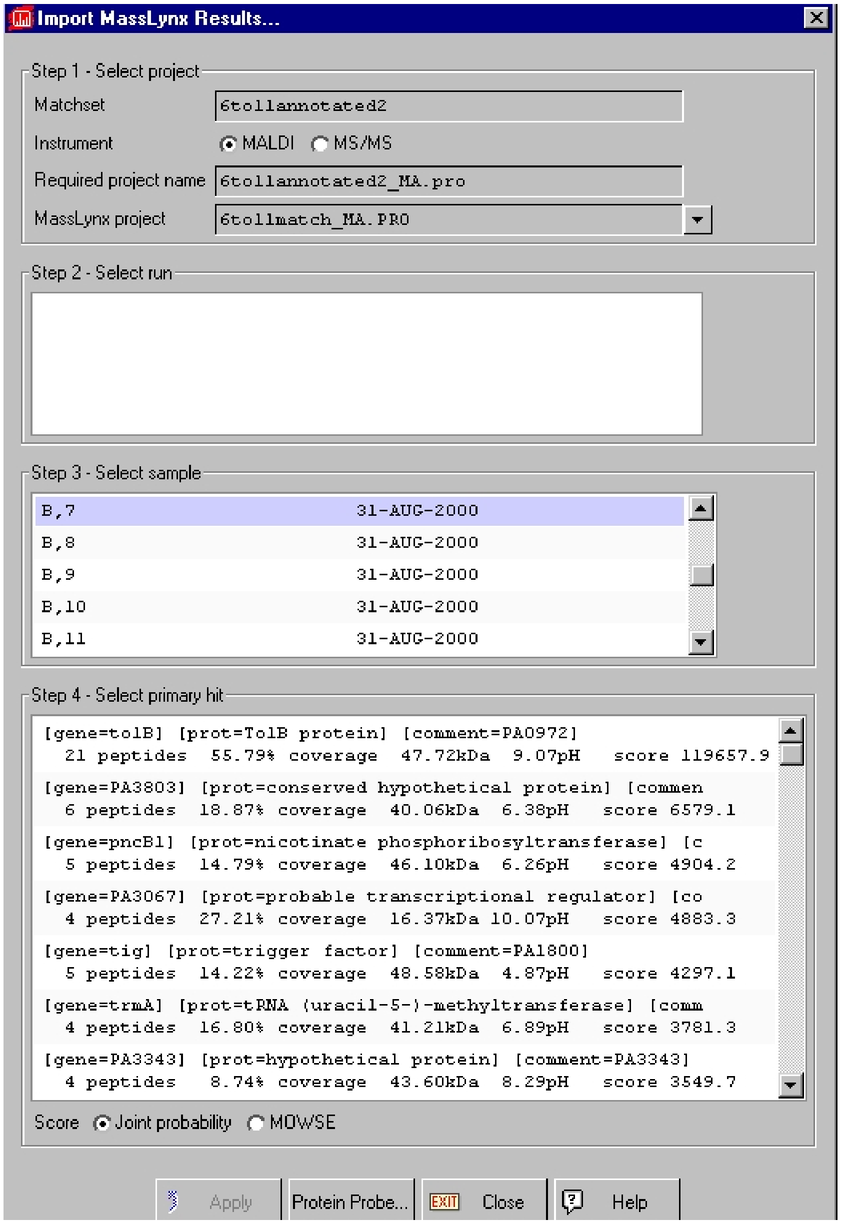
- Export file defines MassLynx project name and sets Mass Spec parameters (for either MALDI or Q-TOF instruments)
  - -Mass spec acquisition parameters
  - -spectra processing parameters
  - -database search/comparison parameters
- MassLynx project name must match PDQuest required name and must use default file name extensions



	Bottle	File Name	File Text	MS File	Process	Parameter File
1	A,1	Increased2_1_Plate001_1_4702		Default	PeptideAuto.exe	bw01
2	A,2	Increased2_1_Plate001_1_5701		Default	PeptideAuto.exe	bw01
3	A,3	Increased2_1_Plate001_1_6801		Default	PeptideAuto.exe	bw01
4	A,4	Increased2_1_Plate001_1_4801		Default	PeptideAuto.exe	bw01
5	A,5	Increased2_1_Plate001_1_3901		Default	PeptideAuto.exe	bw01
6	A,6	Increased2_1_Plate001_1_7401		Default	PeptideAuto.exe	bw01
7	A,7	Increased2_1_Plate001_1_2201		Default	PeptideAuto.exe	bw01
8	A,8	Increased2_1_Plate001_1_0301		Default	PeptideAuto.exe	bw01
9	A,9	Increased2_1_Plate001_1_4603		Default	PeptideAuto.exe	bw01
10	A,10	Increased2_1_Plate001_1_0202		Default	PeptideAuto.exe	bw01
11	A,11	Increased2_1_Plate001_1_5301		Default	PeptideAuto.exe	bw01
12	A,12	Increased2_1_Plate001_1_7302		Default	PeptideAuto.exe	bw01
13	B,1	Increased2_1_Plate001_1_8302		Default	PeptideAuto.exe	bw01
14	B,2	Increased2_1_Plate001_1_0601		Default	PeptideAuto.exe	bw01
15	B,3	Increased2_1_Plate001_1_2202		Default	PeptideAuto.exe	bw01
16	B,4	Increased2_1_Plate001_1_6804		Default	PeptideAuto.exe	bw01
17	B,5	Increased2_1_Plate001_1_5402		Default	PeptideAuto.exe	bw01
18	B,6	Increased2_1_Plate001_1_7701		Default	PeptideAuto.exe	bw01
19	B,7	Increased2_1_Plate001_1_7801		Default	PeptideAuto.exe	bw01
20	B,8	Increased2_1_Plate001_1_2906		Default	PeptideAuto.exe	bw01
21	B,9	Increased2_1_Plate001_1_1701		Default	PeptideAuto.exe	bw01
22	B,10	Increased2_1_Plate001_1_4701		Default	PeptideAuto.exe	bw01
23	B,11	Increased2_1_Plate001_1_6701		Default	PeptideAuto.exe	bw01
24	B,12	Increased2_1_Plate001_1_7501		Default	PeptideAuto.exe	bw01
25	C,1	Increased2_1_Plate001_1_2801		Default	PeptideAuto.exe	bw01
26	C,2	Increased2_1_Plate001_1_3303		Default	PeptideAuto.exe	bw01
27	C,3	Increased2_1_Plate001_1_7502		Default	PeptideAuto.exe	bw01
28	C,4	Increased2_1_Plate001_1_4802		Default	PeptideAuto.exe	bw01

Micromass 4.

- Project file is set up within MassLynx software by mass spec operator



Matchset: 6tollannotated2

Instrument: ● MALDI ○ MS/MS

Required project name: 6tollannotated2_MA.pro

MassLynx project: 6tollmatch_MA.PRO

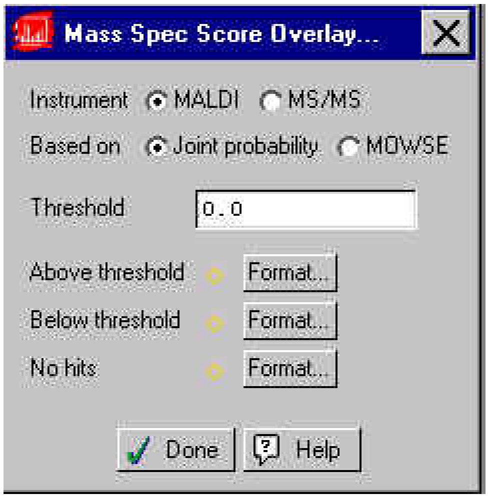
```
B,7                       31-AUG-2000
B,8                       31-AUG-2000
B,9                       31-AUG-2000
B,10                      31-AUG-2000
B,11                      31-AUG-2000
```

```
[gene=tolB] [prot=TolB protein] [comment=PA0972]
  21 peptides  55.79% coverage  47.72kDa  9.07pH   score 119657.9
[gene=PA3803] [prot=conserved hypothetical protein] [commen
  6 peptides  18.87% coverage  40.06kDa  6.38pH   score 6579.1
[gene=pncB1] [prot=nicotinate phosphoribosyltransferase] [c
  5 peptides  14.79% coverage  46.10kDa  6.26pH   score 4904.2
[gene=PA3067] [prot=probable transcriptional regulator] [co
  4 peptides  27.21% coverage  16.37kDa 10.07pH   score 4883.3
[gene=tig] [prot=trigger factor] [comment=PA1800]
  5 peptides  14.22% coverage  48.58kDa  4.87pH   score 4297.1
[gene=trmA] [prot=tRNA (uracil-5-)-methyltransferase] [comm
  4 peptides  16.80% coverage  41.21kDa  6.89pH   score 3781.3
[gene=PA3343] [prot=hypothetical protein] [comment=PA3343]
  4 peptides   8.74% coverage  43.60kDa  8.29pH   score 3549.7
```

Score ● Joint probability ○ MOWSE

Micromass 5.

- File import after MS run complete includes “most probable hit selection” dialogue
- Hit data summary for probable hits includes
  -number of matched peptides in spectra
  -percentage of the targeted protein covered by the identified peptides
  -molecular weight and isoelectric point of the probable match
  -match probability score (user selectable for Joint Probability or MOWSE algorithms)



Mass Spec Score Overlay...

Instrument ● MALDI ○ MS/MS

Based on ● Joint probability ○ MOWSE

Threshold 0.0

Above threshold ○ Format...

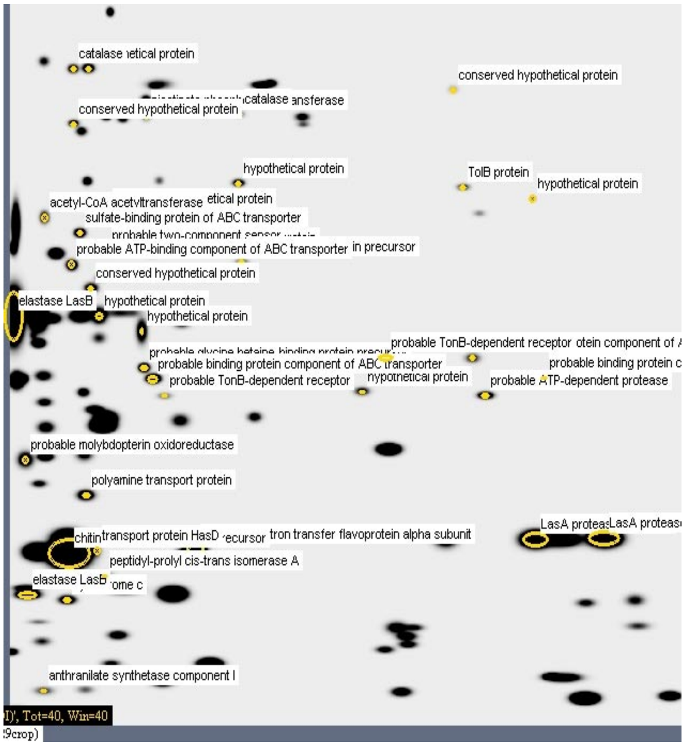
Below threshold ○ Format...

No hits ○ Format...

Micromass 6.

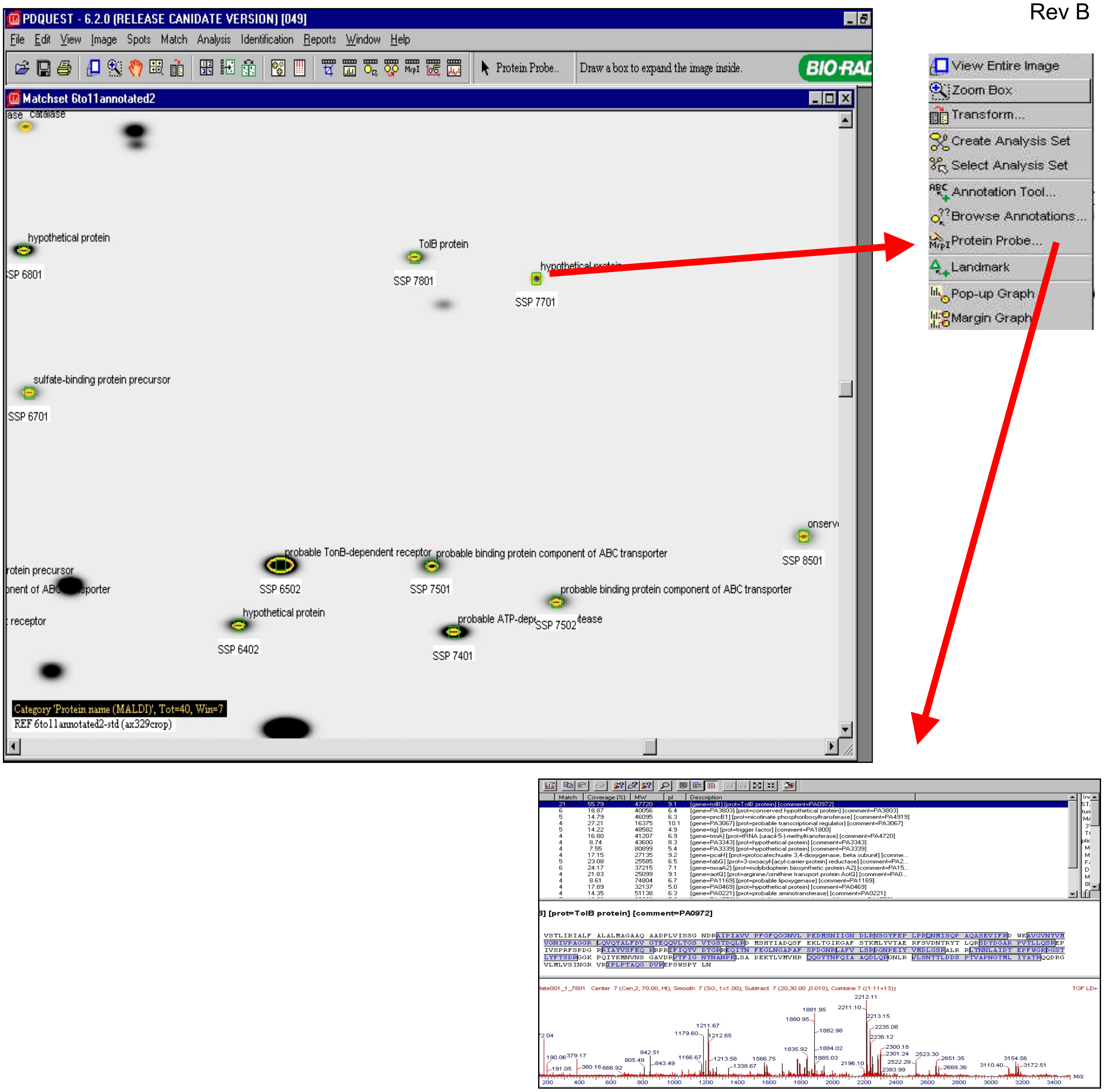
- Identified proteins in the gel image can be highlighted with hit indicator overlays based on the Match Probability Score
- Overlays can be color coded for user-defined confidence thresholding

---



Micromass 7.

- Import MassLynx data files to automatic create Annotation Categories and/or update Annotation Category entries with protein identification info



Micromass 8.

- Launch ProteinProbe software to view actual mass spectra and structure information associated with protein identification
- Advantages of integrated 2D Analysis software / Spot Cutter control / Mass Spec data

  -“Eliminates errors”

  -“Cuts time by 80%”

  -“Allows gel map to be used as point of reference for other data - visual image”

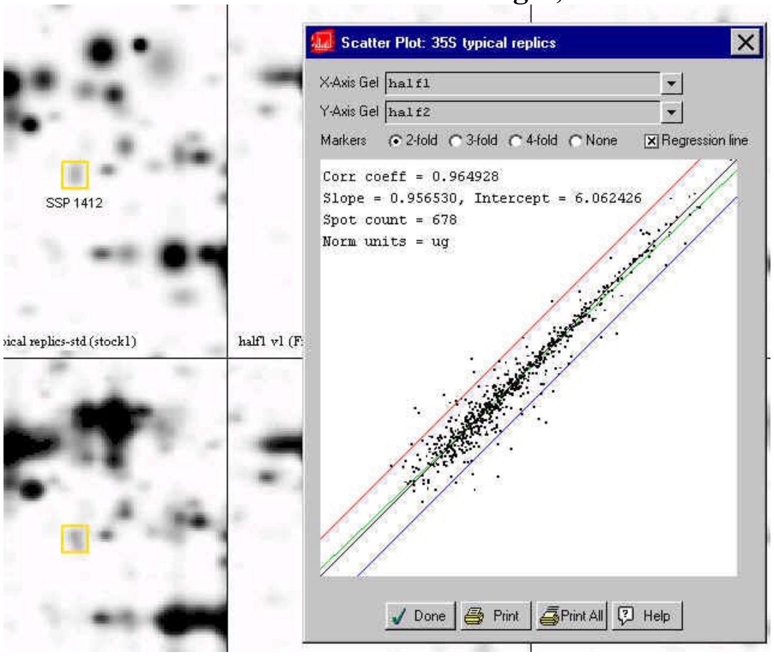
  -“Facilitates user compliance”

  -“Allows study of more complex proteomes”

  ProteomeWorks system evaluation by Dr. Brad Walsh, APAF

  September 2000, Sienna Proteome Conference

The Scatter Plot Tool is linked to images, to allow identification of variant proteins



Scatter Plot: 35S typical replics

X-Axis Gel half1
Y-Axis Gel half2
Markers ● 2-fold ○ 3-fold ○ 4-fold ○ None ☒ Regression line

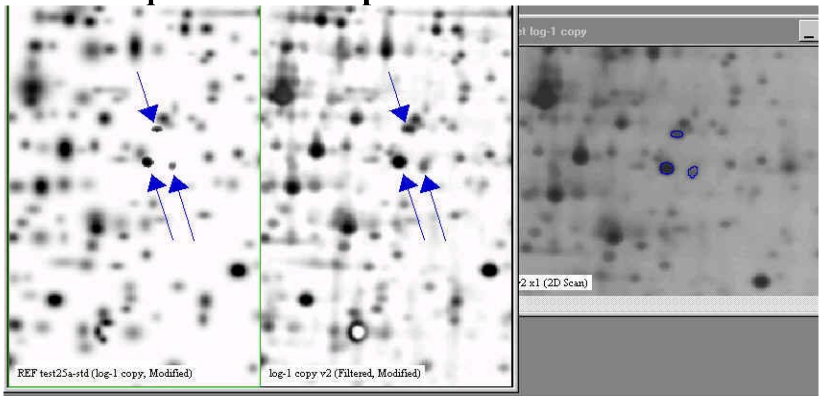
```
Corr coeff = 0.964928
Slope = 0.956530, Intercept = 6.062426
Spot count = 678
Norm units = ug
```

scatter plot 1.

- Clicking on any data point in the plot will target the correlating protein on the gel
- Spots falling outside the standard deviation can now be easily identified on the gel image

  -only matched spots are plotted in the tool

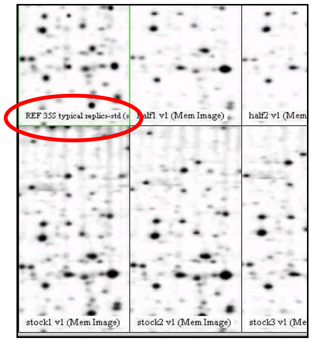
Volume freehand and auto-contour tools have been added, allowing overwrite of Gaussian quantitation of spots

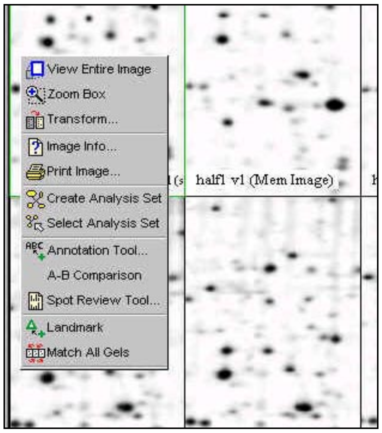


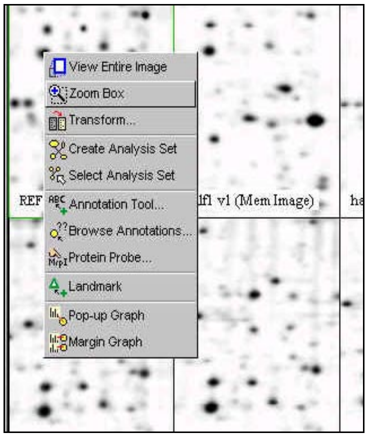
contour 1.

- Can apply a contour to scanset or matchset images

Context-sensitive right-mouse-click menus





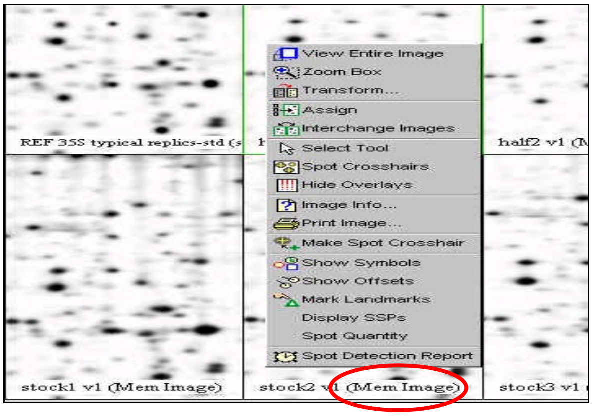


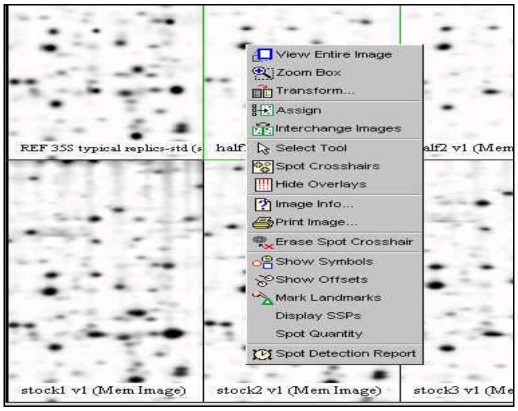
menu 1. Spot menu background menu

Menus available from the **Standard Reference image**

-context referenced to cursor location on spots or gel background

---





menu 2. Spot menu background menu

Menus available from the **member images**

-context referenced to cursor location on spots or gel background

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Match-offset vectors now indicate outliers in red for better visibility when editing matching of spots

offset 1.

- Offsets are compared to local neighbors for direction and length of distortion
- outliers are indicated in red

Ordering Information

Catalog #	Description
170-8603	PDQuest 2-D Analysis Software, PC
170-8611	PDQuest 2-D Analysis Software, Mac
170-8626	PDQuest Software Upgrade, PC/Mac