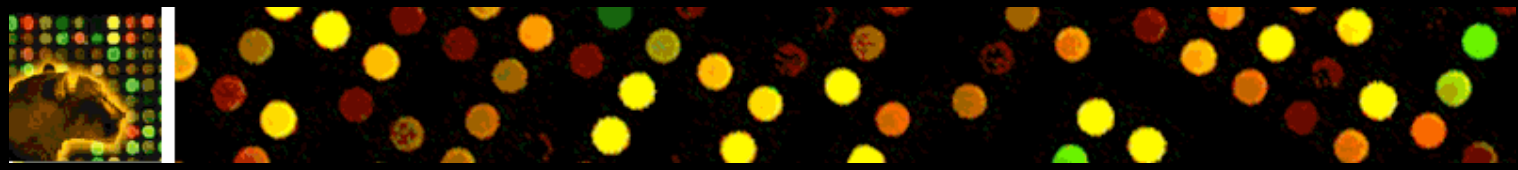
Welcome to PUMAdb



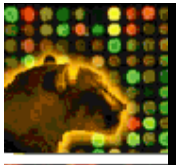
Princeton University MicroArray database

October 17, 2008

John Matese

User Help: Tutorials and Workshops

- Help & FAQ
  - http://puma.princeton.edu/help/
  - http://puma.princeton.edu/help/FAQ.shtml
- Tutorials
  - http://puma.princeton.edu/help/tutorials_subpage.shtml
  - Ideas? Email array@genomics.princeton.edu
- Hybridization & Scanning Individual Instruction
  - Email dstorton@molbio.princeton.edu



Welcome to the database: a tutorial

- What we'll talk about:
  - **User Registration**
  - **Staging Data**
  - **Loading Prerequisites**
  - **Loading Data**
  - **Finding Your Data**
  - **Displaying Your Data**
  - **Data Retrieval and Analysis**
  - **Organizing Data**
  - **Submitting Plate Samples**
- What we will not discuss, or only brush the surface of…
  - **Experimental Design**
  - **Experimental Protocol**
  - **Data Normalization**
  - **Data Quality Assessment**
  - **Data Analysis (clustering)**
  - **External User Tools (XCluster, TreeView, etc.)**

- Please fill out the sign-up sheet and survey form
- Questions? email us at: **array@genomics.princeton.edu**

Welcome to PUMAdb

- User Registration
- Staging Data
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- Loading Data
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User Registration

- PUMAdb is free
- Fill out the registration form
  - http://puma.princeton.edu/cgi-bin/tools/display/registration.pl
- Lab head (PI) should also register
- External collaborators can also be granted accounts (with different levels of access)
- Accounts are occasionally granted to external researchers without an existing on-site collaboration, provided support demands are reasonable.

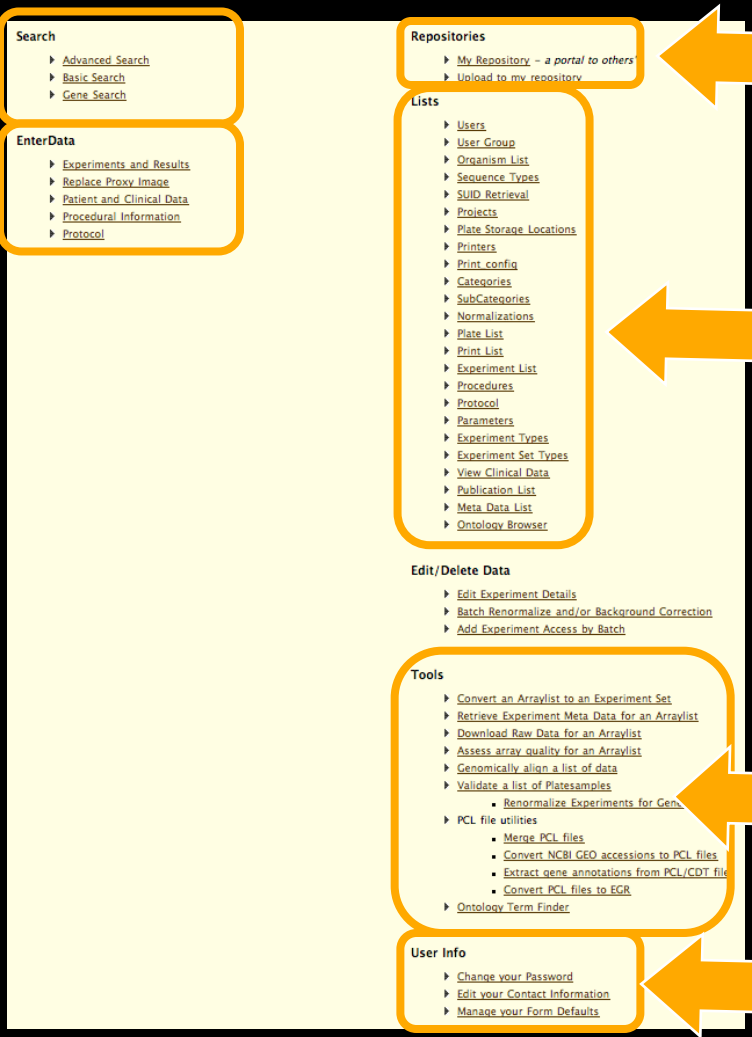
Important Access Policies

- The experimenter for an assay retains all edit and delete privileges, solely
- All users within an access group (i.e. “lab”) can see each others data
- Upon publication, experiments are made public

User Privileges: All Programs

Searches

Data Entry

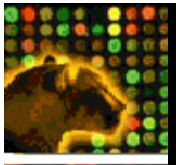


Repository

Lists

Tools

Personal Data



Welcome to PUMAdb

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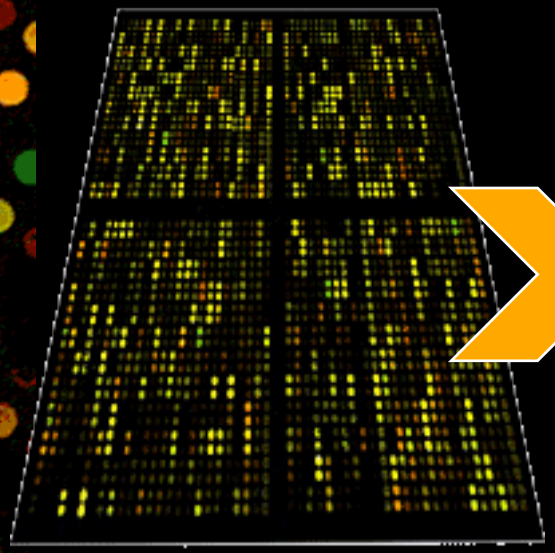
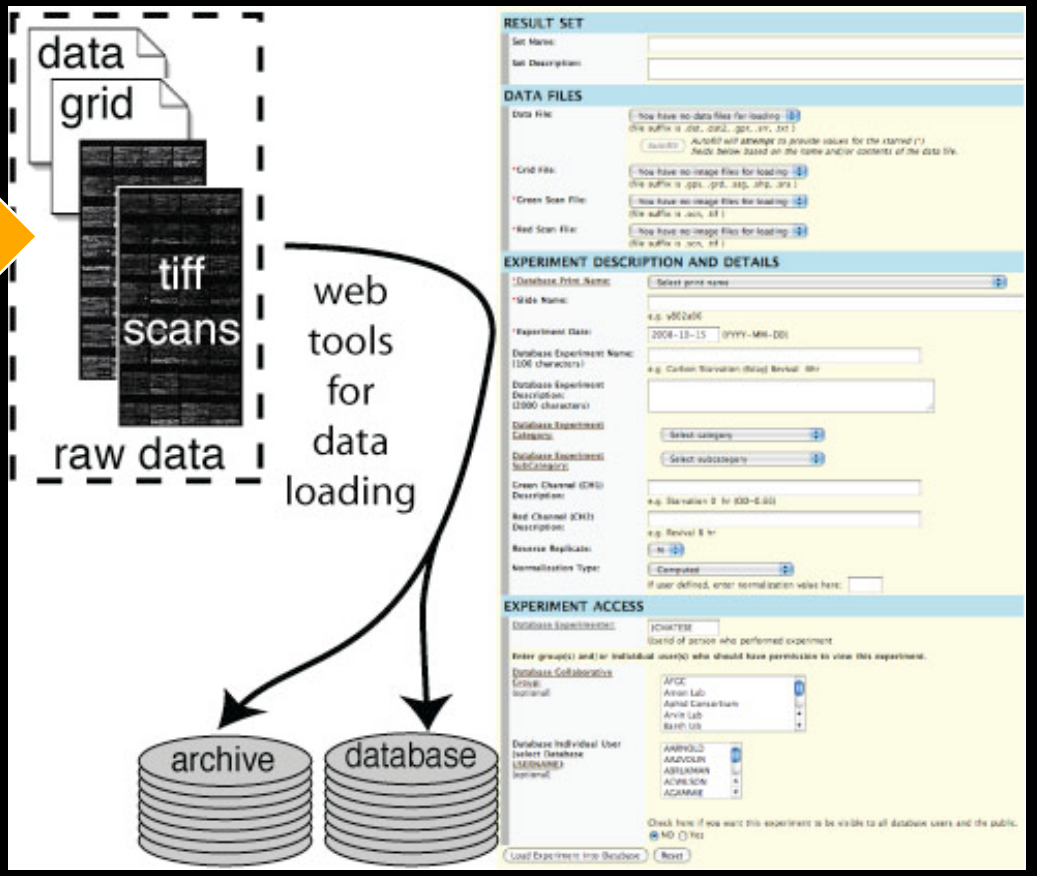
Submitting Data





Array Files vs. Loader

- Array Files, a SMB server
  - for core facility users, only
  - smb://arrayfiles/arraydata
  - Files within personal directory are visible by database automatically
- Loader, a SFTP server
  - for all unrestricted database users
  - With SFTP client, connect to loader.princeton.edu using PUMAdb userid|password and upload your data files to the ‘incoming’ directory

Loader Account: Directories

- incoming

  Stores all files prior to experiment loading. This is temporary storage - eventually, files will be deleted!

- logs

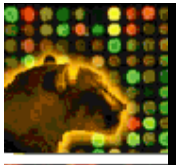
  Feedback files from the database are written to this directory (i.e. experiment loading logs)

- arraylists

  The database will look in this folder to retrieve any arraylists (list of arrays you have grouped together)

- genelists

  The database will look in this folder to retrieve any genelists (a list of genes with possible annotations)



Welcome to PUMAdb

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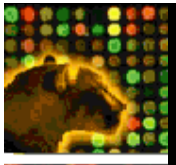

Loading Prerequisites

- Array design
  - Check the existing list of prints, and inform us if not found.
  - If you are using arrays from the core facility, this will be done for you
  - If you are creating your own prints (homemade contact-printed), please stay for the last 15 minutes of the tutorial…
- Experimental category and subcategory
  - Check the existing lists, and inform us if not found.
  - Make sure that your categories and subcategories are meaningful and not cryptic.

If new entries are required, email :

array@genomics.princeton.edu

Loading Prerequisites : Lists

Welcome to PUMAdb

- User Registration
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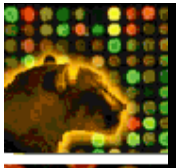


Annotation : Data Standards

- MGED - Micro Array Gene Expression Database Society
- “Minimal Information About a Microarray Experiment” (MIAME)
  - Experimental Design
  - Array Design
  - Biological Samples
  - Hybridizations
  - Measurements
  - Data Normalization and Transformation

Nature Genetics (2001) 29, 365-371.

Annotation : MIAME Checklist

- In September 2002, MGED sent out a letter to journals and reviews requesting the microarray publications have this “minimal” information/annotation
- Many journals now have policies requiring published data to be well-annotated and deposited in a public repository (i.e. NCBI GEO).

http://www.mged.org/Workgroups/MIAME/miame.html

Loading Data : Required Files

- In order to submit data, you need the following files staged:
  - For Affymetrix Data (dChip/GCOS/MAS5)
    - probeset_data.txt, cell_data.cel, experiment.exp, image.dat
  - For Agilent Data
    - data.txt, shape.shp, channel1.tif, channel2.tif
  - For GenePix Data
    - data.gpr, grid.gps, channel1.tif, channel2.tif
  - For Nimblegen Data
    - genes.txt|genes.calls, cell_data.xys, features.ftr, image.tif
  - For ScanAlyze Data
    - data.dat, grid.sag, channel1.scn, channel2.scn

Loading Data : Problems

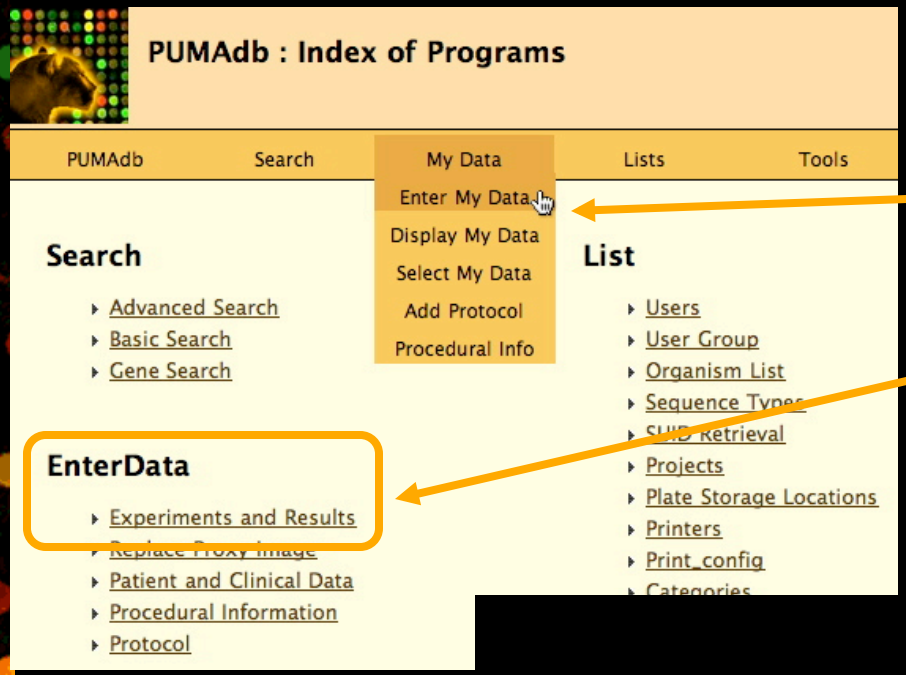
- File names should only include allowed characters:
  - numbers, letters, dots, hyphens, underscores
  - **Spaces** and **slashes** are not allowed
- Images **must** be binary file (transfer issues)
- Only tif files may be compressed at the time of loading

Loading Data to the Database

- The *incoming* directory is emptied automatically every few weeks.
- Although we do archive your data, it does not serve as a raw datafile retrieval service, as of yet.

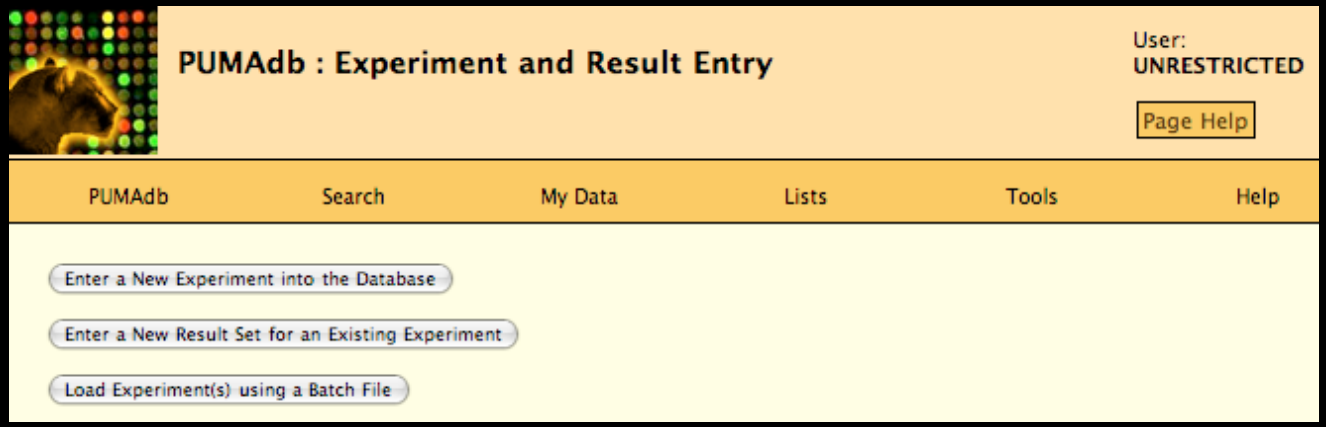
Please Archive Your Data!

Loading Data : Data Entry



- Choose your method
  - Within navigation menu…
  - Experiments and results link…

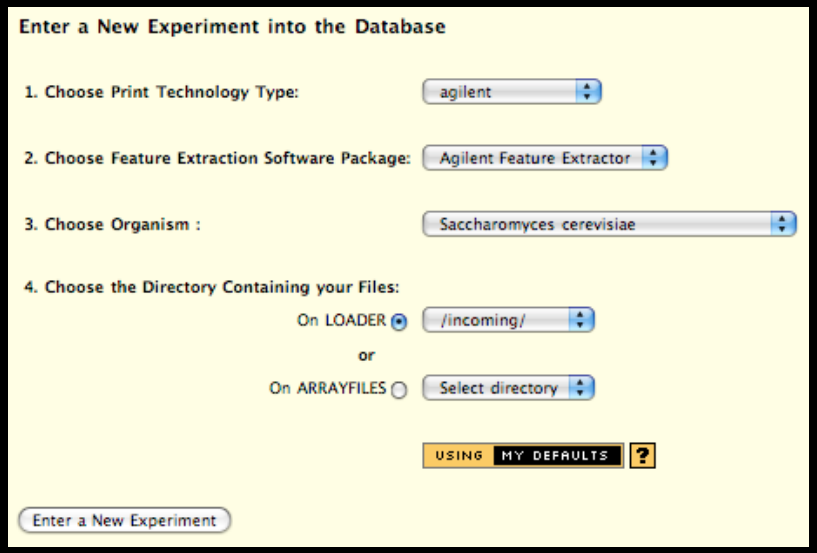
Loading Data: Step 1



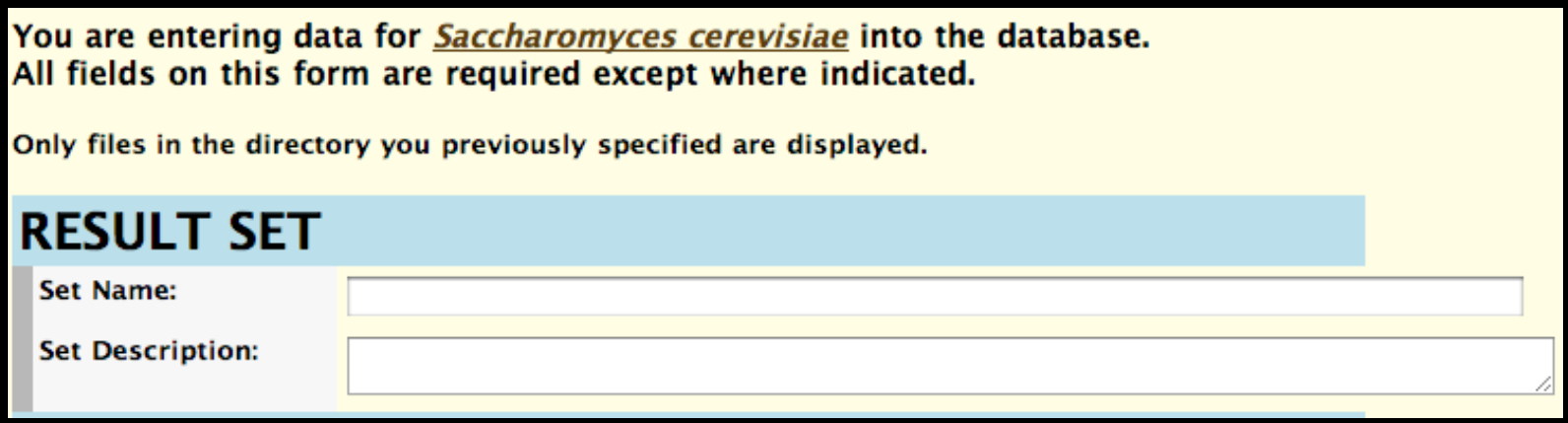
- Decide if you are entering a single experiment or a batch of experiments
- In specialized cases, add additional result sets for existing experiments

Loading Data: Step 2

- Select the print technology (agilent, affymetrix, nimblegen, spotted)
- Select the feature extraction software package was used to generate your data
- Select the organism whose genes are arrayed
- Select the location of staged data

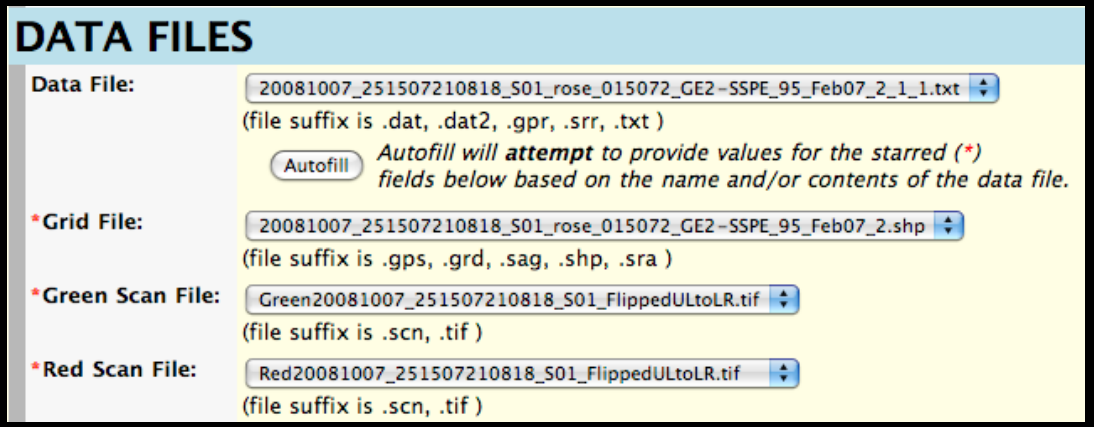


Loading Data: Result set



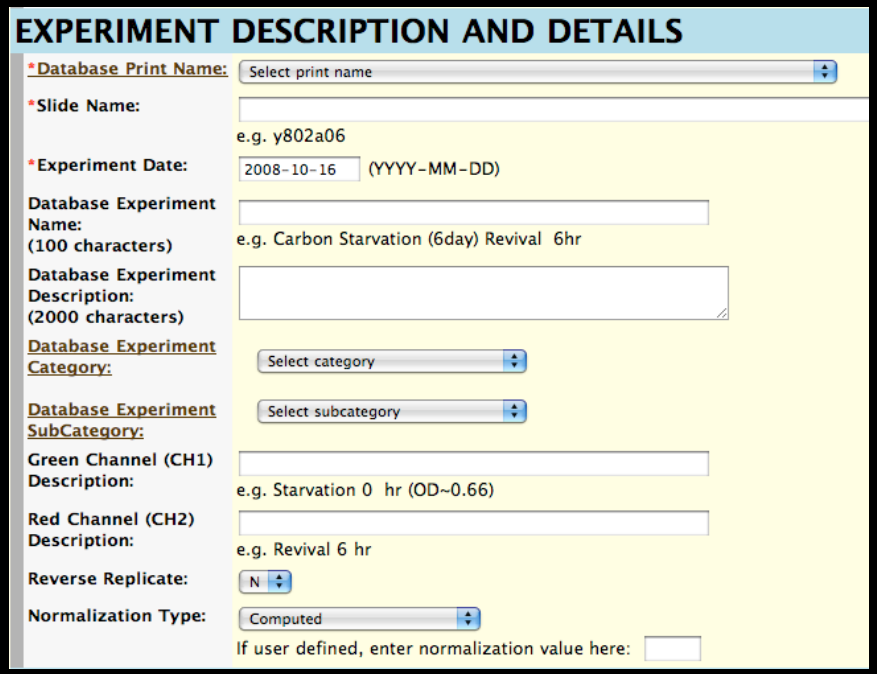
- For Affymetrix, Agilent, and Nimblegen results
- Provide a Result Set Name and Description
  - As for any single experiment there may be *n* result sets derived from raw data. You must create a name for each of these sets so that each result set may be identified and retrieved unambiguously from the database

Loading Data : Data File Locations



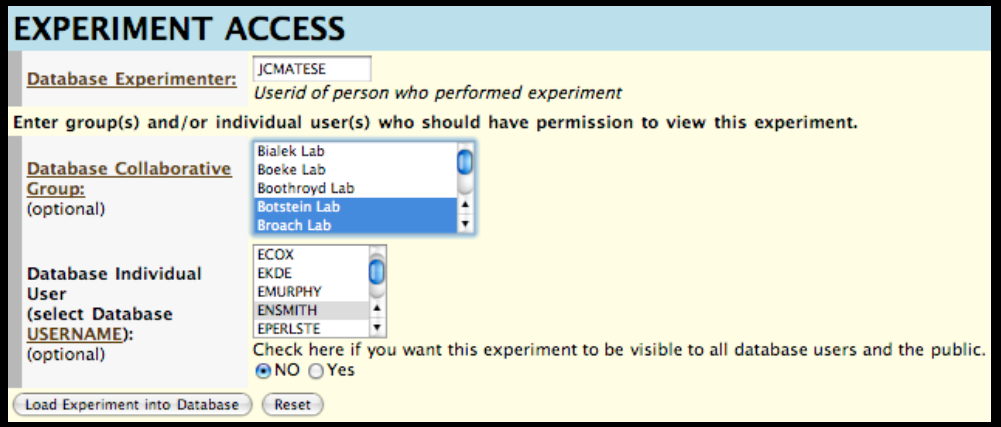
- Choose the data, grid, green scan and red scan files to be loaded from your staging directory
- Each pull down menu should be populated with the appropriate file
- Autofill button may help select the correct file, given a data file to start…

Loading Data : Platform & Experiment Details



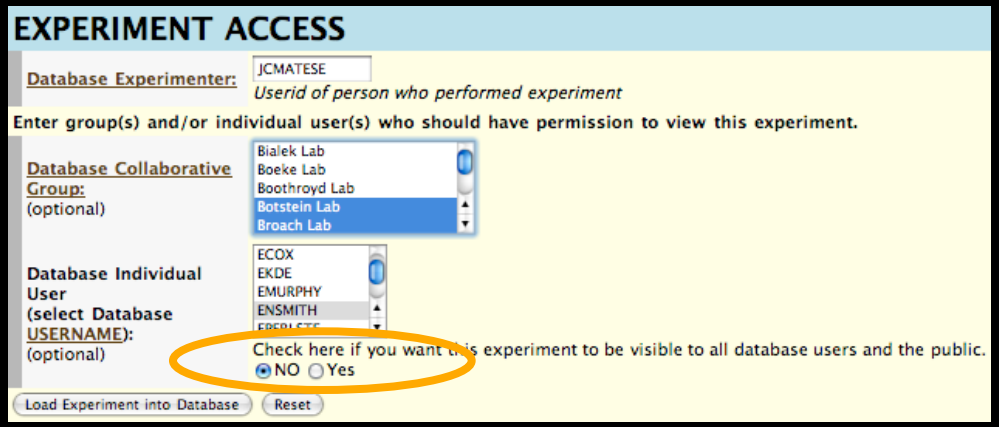
- Print Name
- Slide Name
  - Unique, descriptive
- Experiment Date
- Experiment Name
  - Unique, descriptive
- Loading Prerequisites
- Channel Descriptions
- Reverse Replicate?
- Normalization Type
  - (describe later)

Loading Data : Experiment Access



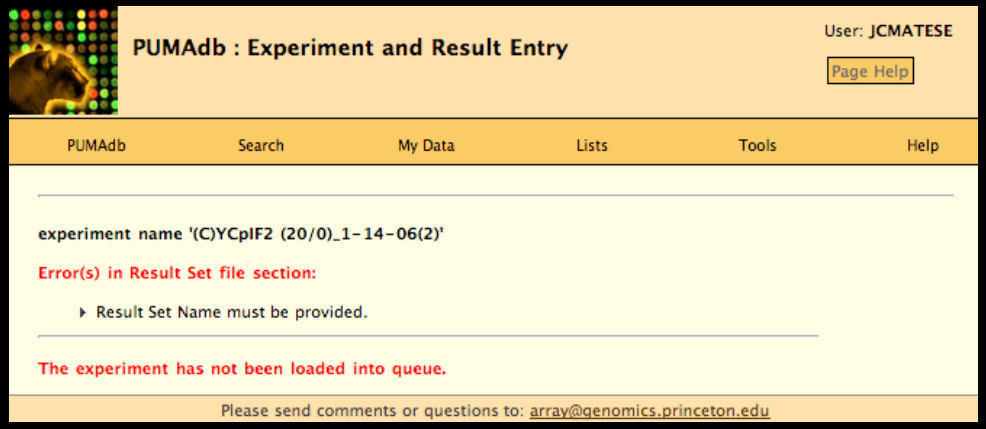
- Experimenter (i.e. 'Owner')
  - Person who will have edit/delete/access privileges
- Collaborative Groups
  - By default, your lab group will be able to see all your experiments
  - If you wish for another entire group to view your experiments, you select the group name here
- Individual Users
  - Give an individual user the ability to view your experiment

Loading Data : Experiment Access



- World Access
  - Selecting ‘Yes’ here makes your data viewable by the WORLD
  - usually only done for open collaborations

Loading Data : Errors



- Loading software checks for common errors
- Experiments will not be loaded if there are errors. You must go back, correct your error(s) and resubmit your data

Loading Data : Queue

- After passing the checks, your data goes into a loading *queue*
- The queue holds all experiments being loaded and processes them in an ordered fashion
- You can monitor the progress of your experiment entry
- You will also be sent an email with the hyperlink and Batch_No to check the loading process

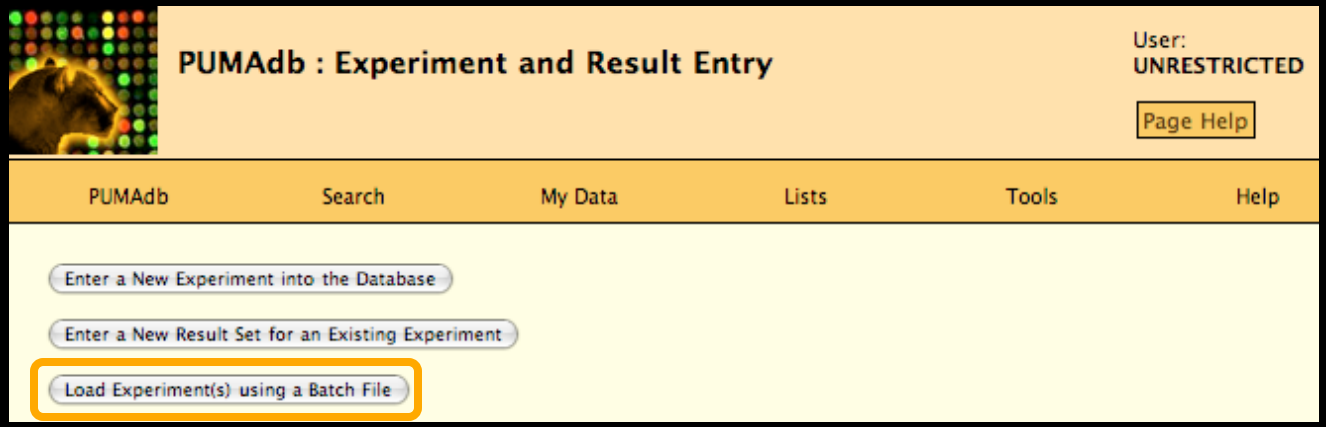
Loading Data : Successful Experiment Entry

- Once your experiment has been loaded into the database, there are 2 methods to get the details of the experiment loading process
  - From the queue page
  - A file will be created on your loader account in the *logs* directory
    - batch_no.log

Loading Data : Experiment Entry Log File

- The log file will give you the following information:
  - ExptID (experiment ID)
  - Information on experiment access
  - Information on normalization value
    - Number of spots that pass criteria
    - Spots used to calculate normalization
    - Percentage of spots that passed criteria
    - Normalization Value

Loading Data : Batch Loading

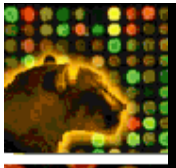


- Instead of loading experiments one by one, you can choose to load a batch of experiments
- All experiments need to be listed in a tab-delimited file (a batch file) in your *incoming* directory
- There are sample batch files located on the batch entry help page

Loading Data : Assembling a Batch File

- (Result Set Name)
- Print Name
- Experiment Category
- Experiment SubCategory
- Slide Name
- Data File Location
- Grid File Location
- Green Scan File Location
- Red Scan File Location
- Experiment Date
- Experiment Name
- Green Channel (CH1) Description
- Red Channel (CH2) Description
- Normalization Type
- Norm Value
- Experimenter
- Experiment Description
- Collaborative Group
- Individual User

All underlined column headers are required data



Loading Data via Batch File

	A	B	C	D
1	Print Name	Experiment Category	Experiment SubCategory	Slide Name
2	yeast6000-1	cell growth	glucose signaling	Gbc-051
3	yeast6000-1	cell growth	glucose signaling	Gbc-052

Print, Categorization, and Slide

E	F	G	H
Data File Location	Grid File Location	Green Scan File Location	Red Scan File Location
yw106_0-20_-03-15-02.gpr	proxy.gps	yw106_0-20_-03-15-02-W_532.tif	yw106_0-20_-03-15-02-W_635.tif
yw106_0-20_-10-8-02.gpr	proxy.gps	yw106_0-20_-10-8-02-W_532.tif	yw106_0-20_-10-8-02-W_635.tif

Filenames

I	J	K	L	M	N	O	Q	R
Experiment Name	Green Channel (CH1) Description	Red Channel (CH2) Description	Normalization Type	Norm Value	Experimenter	Experiment Date	Collaborative Group	Individual User
yw106_0-20_-03-15-02	Y2872 0min mRNA level	Y2872 20min mRNA level	user defined	0.841665	XIUYINGZ	11/28/01		
yw106_0-20_-10-08-02	Y2872 0min mRNA level	Y2872 20min mRNA level	user defined	0.860216	XIUYINGZ	8/16/02		

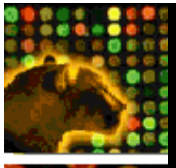
Experiment/Hybridization name, Experimenter description, Normalization Type

Access

P
Experiment Description
Y2872 was grown in SC + 3% glycerol and shaken. When OD600 reached 0.64, cells were harvested as 0min, kept added 2% galactose to rest and kept growing for 20, 40, 60, 80 minutes. Lisa did all cell growth for this data batch.
Y2872 was grown in SC + 3% glycerol and shaken. When budding index reached 19%, cells were harvested as 0min, kept added 2% galactose to rest and kept growing for 20, 40, 60, 80 minutes

Loading Data : Batch Loading

- After you select your organism, select your batch file
- First, check your batch file (catch common errors)…
- Next, queue/load your batch file…
- Load proceeds as for single experiment entry



Loading Data : Example queue logfile

```
===== Loading Expt Batch NO : 3279 =====
Experiment Name: blah blah
Thu Dec 13 15:54:01 2001

Processing Data File : /loader/ftphome/youruserid/incoming/slidename.gpr

 ===== Inserting experiment info into experiment table... =====
exptID = 28765
The experiment data has been successfully inserted into experiment table!

 ===== Updating Experiment Access Control Table ... =====
Updating expt_access for experimenter YOURUSERID (#) ...  OK
Updating expt_access for Brown/Botstein labs (#) ...  OK

===== Calculate norm value... =====
Reading all data from datafile and doing all calculation now...
PassCriteria = 16005
Using 36490 spots for normalization  43.8% passed criteria of a good spot  with 0.65

===== Updating exptNorm table... =====
NormType = Computed  NormValue = 0.96
===== Updating Result table... =====
=====  Total Record : 43200 =====
===== Updating Result table... =====
===== Expected = 43200, actual is 43200 =====
1000 . . .
```

Replace a Proxy Image

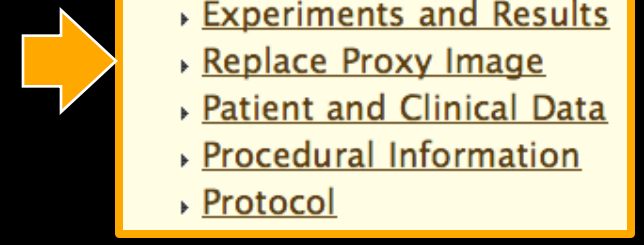
When:

- The image (.png) created by the default process is not acceptable
- After renormalization

How:

- Use your copy of tif files
- Make composite and save as .png
- Upload on loader into *incoming*
- Replace the copy
- Use the Replace Proxy Image link

Data Entry

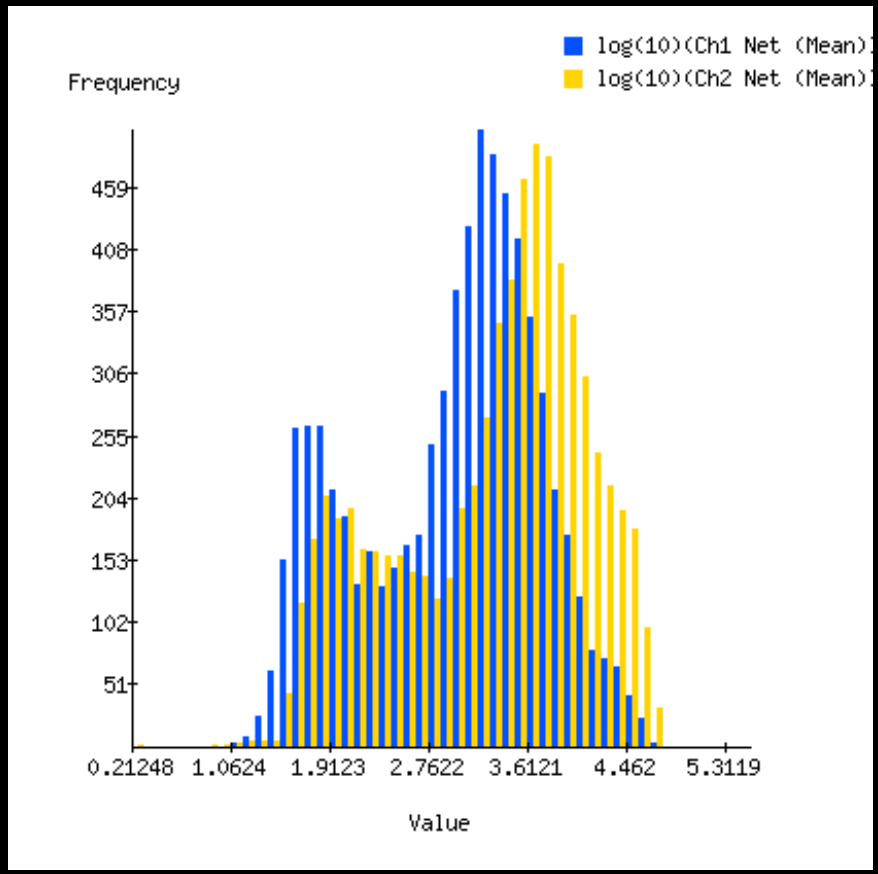


Normalization: Why normalize data?

- Normalization reduces the effects of labeling bias
- Normalization allows you to recognize the biological information in your data
- Normalization allows you to compare data from one array to another

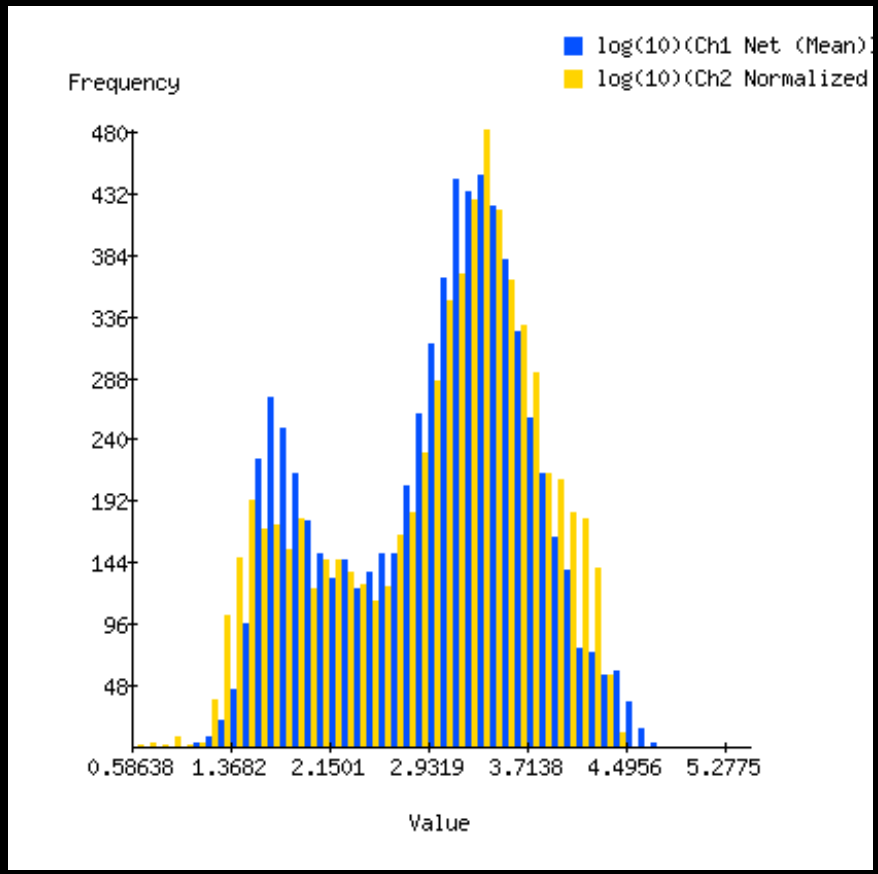
Normalization: Channel biases

Before Normalization…



Normalization: Channel biases

After Normalization…



Normalization Steps

1. Assume that for the vast majority of spots on the array, the ratio should be 1 (i.e. no difference between samples/channels)
2. Choose those spots with “well-measured” data
3. Calculate a factor based on the initial assumption for these spots
4. Apply this factor to the second channel’s data for all spots

Normalization: Choosing Spots

- The database offers two options for selecting "well-measured"spots for normalization:
  - "**Regression correlation**": only non-flagged spots are used, with regression correlation greater than 0.6
  - "**Computed**": based on the percentage of pixels in each unflagged spot whose intensity is at least one standard deviation greater than background (for Scanalyze spots, it is the fraction of pixels 1.5 fold greater than the background)

Normalization: “computed” method

- “well-measured” spots are those with at least 65% of pixels significantly above background.
- If less than 10% of spots on the array meet the threshold, the 65% threshold is reduced stepwise until either 10% of spots pass or the threshold reaches 55% of pixels above background (whichever comes first)

Normalization: Calculating Factor

- Default normalization factor is the geometric mean of the red/green ratio of the selected “well-measured” spots
- Alternatively, a user can specify a normalization factor
- These methods can be applied for a genelist (in batch too)

Normalization: Applying the factor

- To apply the normalization factor, both the intensity and background of channel 2 (red) for all spots are divided by the normalization factor
- Other normalized values are calculated from these
- NOTE: Agilent data are not normalized in the database

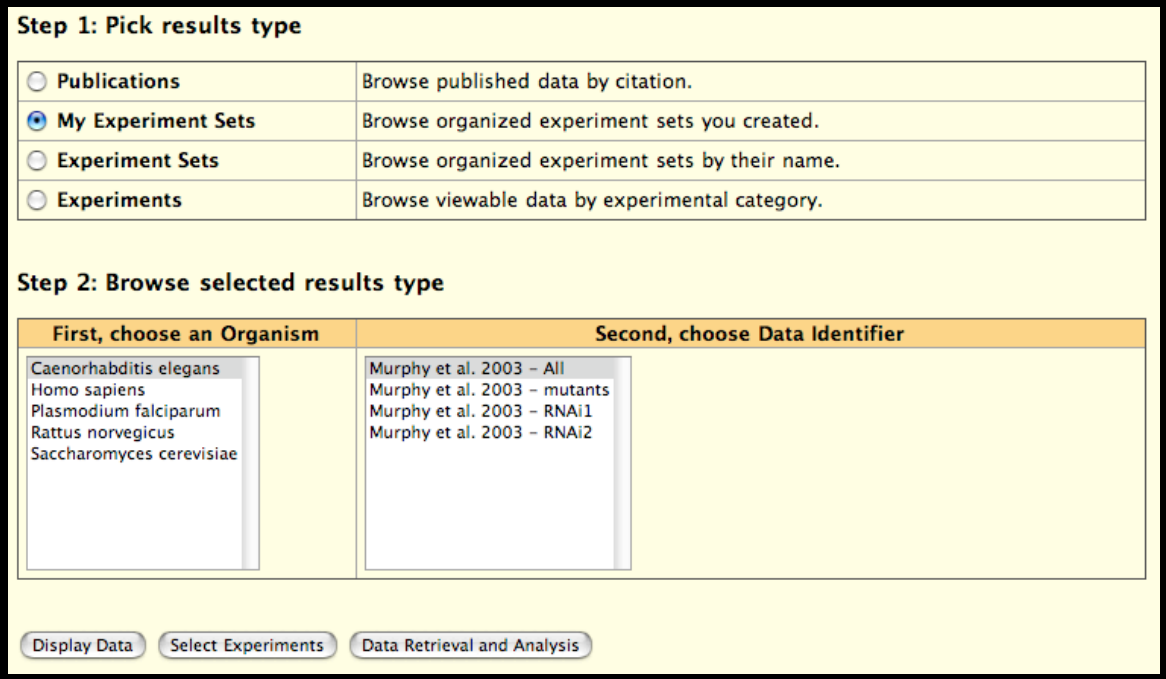
Welcome to PUMAdb

- User Registration
- Staging Data
- Loading Prerequisites
- Loading Data
- Finding Your Data
- Displaying Your Data
- Data Retrieval and Analysis
- Organizing Data
- Submitting a Printlist

Finding Your Data

- There are several entry points for queries:
  - Advanced Search
  - Basic Search
  - Experiment List
  - Gene Search
  - Navigation Menu

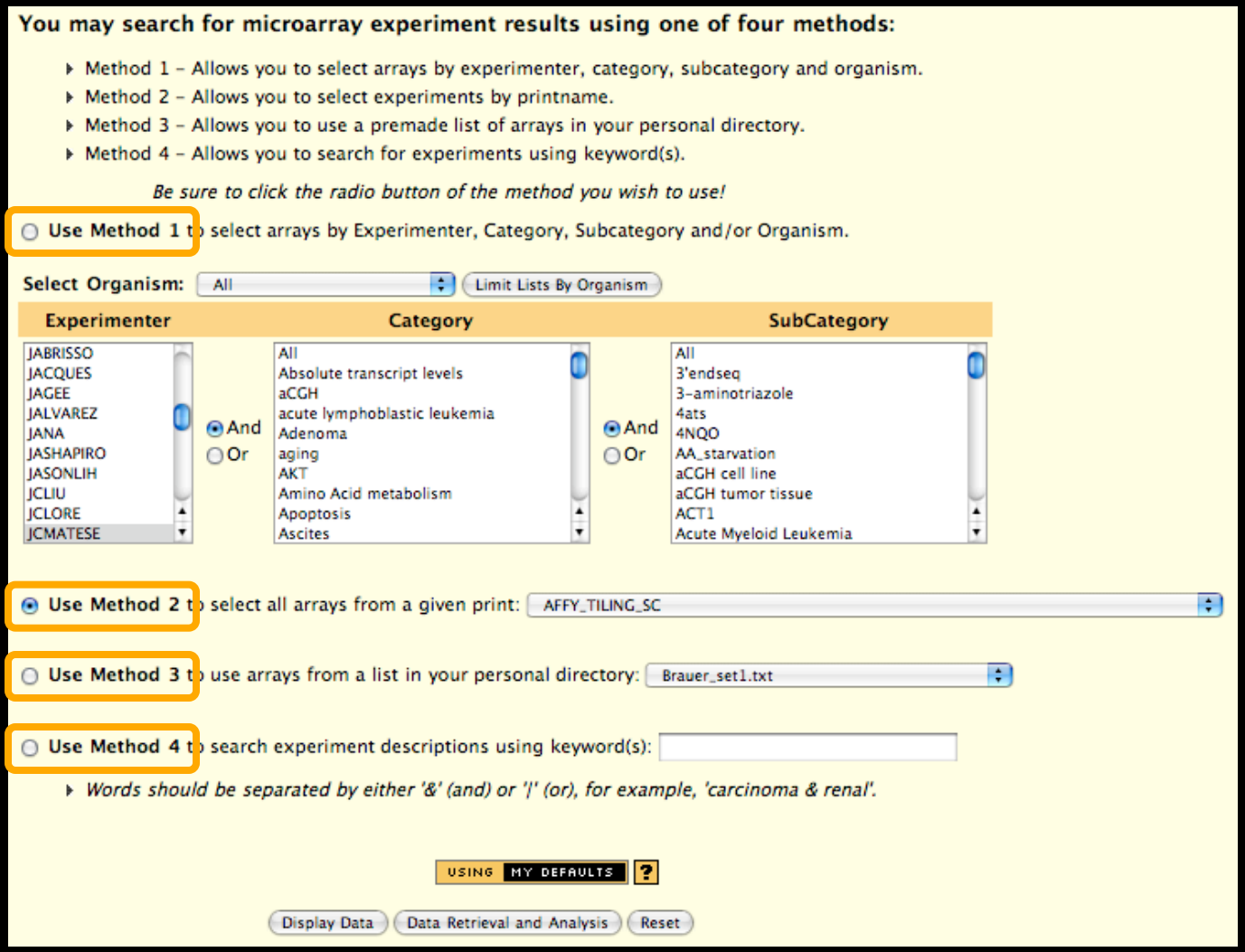
Finding Your Data : Basic Search



There are three ways to find your data via Basic Search:

- Publications include all published data in the database
- Experiment sets allow you to search data on pre-defined experiment groups. (This will be described later)
- Retrieve data by experiment category

Finding Your Data : Advanced Search Results



Assay retrieval : Search software

Use 'Basic Search' to browse/retrieve:

- a single Publication
- a single Experiment set
  * your personal sets
  * others', if viewable
- a single Experimental category

Use 'Advanced Search' to perform:

- A boolean search
  * by Experimenter
  * by Category
  * by Subcategory
- A search by Print
- A search by arraylist
- A text search

Welcome to PUMAdb

- User Registration
- Staging Data
- Loading Prerequisites
- Loading Data
- Finding Your Data
- Displaying Your Data
- Data Retrieval and Analysis
- Organizing Data
- Submitting a Printlist

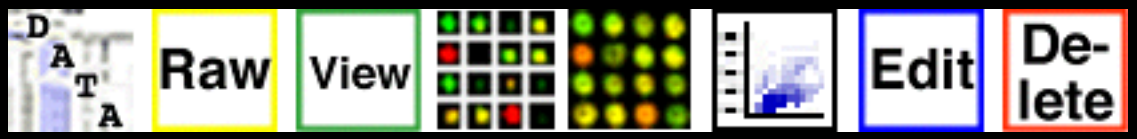
Display Data from Searches

- Click on a Category/Subcategory to retrieve a list of all arrays using that category/subcategory
- Click on an experimenter to view their details
- DATA = View and Sort Array Data
- Raw Data = Download Raw Data
- View = View Array Details
- = View Array Image and Grids
- = Clickable Image
- = Plot Array Data
- = Align Data to Chromosomes

Your query returned 4 result sets.
Re-sort by: ExptName Re-sort

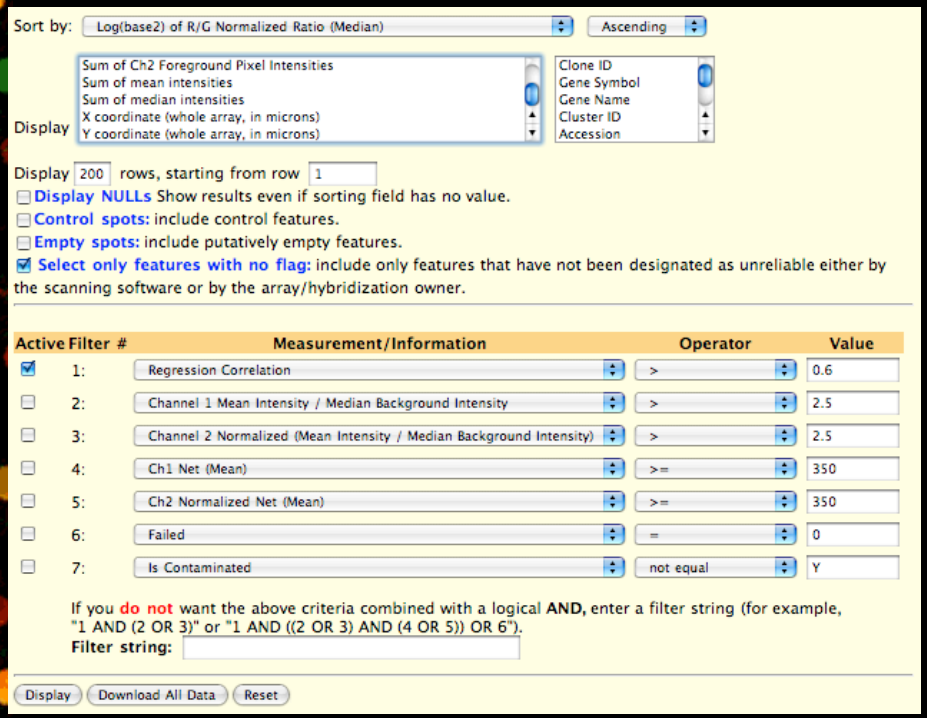
ExptID	Experiment	Category	Subcategory	SlideName	Result Set	Options	Experimenter	ExptDate
101259	Y2864 2%glu (0-20)_6_15_05	Yeast expression	glucose signaling	251144713617_A01	RGT2-1	DATA Raw Data View Edit De-lete	SZAMAN	2005-06-15
101260	Y2864 2%glu (0-40)_6_16_05	Yeast expression	glucose signaling	251144713523_A01	RGT2-1	DATA Raw Data View Edit De-lete	SZAMAN	2005-06-16
101261	Y2864 2%glu (0-60)_6_15_05	Yeast expression	glucose signaling	251144713618_A01	RGT2-1	DATA Raw Data View Edit De-lete	SZAMAN	2005-06-15
101262	Y2864 2%glu (0-80)_6_15_05	Yeast expression	glucose signaling	251144713618_A02	RGT2-1	DATA Raw Data View Edit De-lete	SZAMAN	2005-06-15

Display Data



- = View and Sort Array Data
- = Download Raw Data
- = View Array Details
- = View Array Image and Grids
- = Clickable Image
- = Plot Array Data
- = Edit Array Details
- = Delete Array

Display Options: View Data



- Select Data to use for sorting
- Select Columns to be displayed
  - Spot metrics
  - Biological Annotation
- Select how many rows to be displayed per page
- Include Controls/Nulls?
- Make downloadable file?
- Select filtering criteria

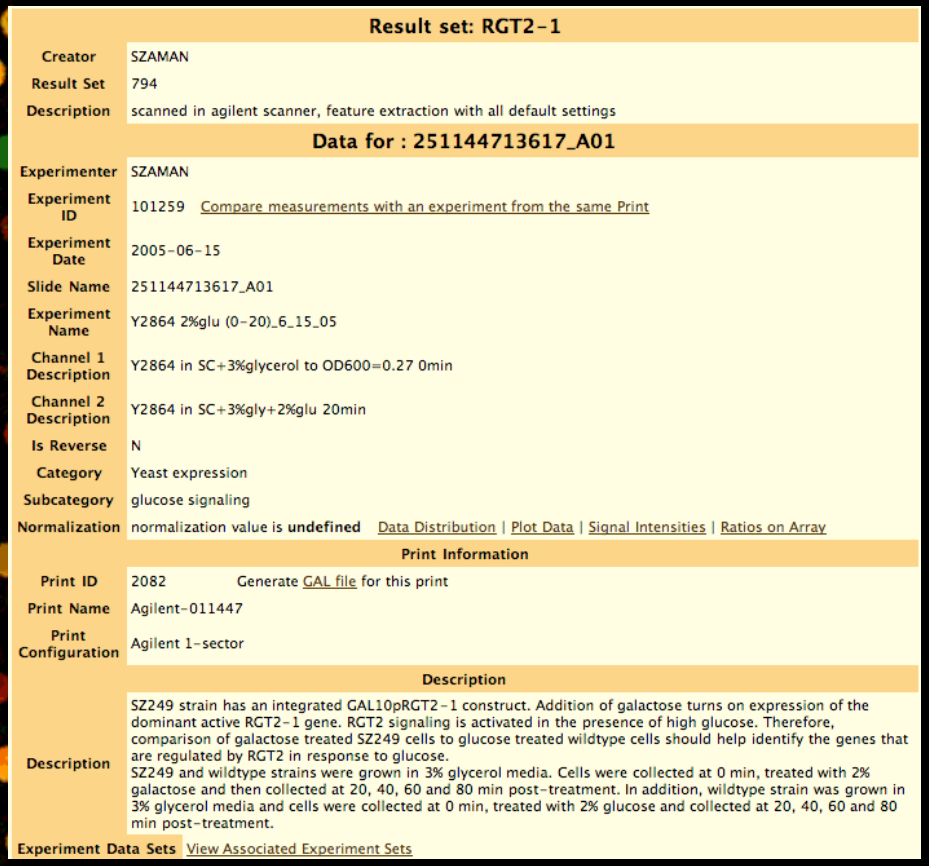
Display Options: Raw Data

Raw

- This will save a file on your computer of all the columns of raw data
- The file is named *exptid*.xls
- The file is actually a tab-delimited file that can be opened in any program

View

Display Options: View Details



Result set: RGT2-1

Creator	SZAMAN
Result Set	794
Description	scanned in agilent scanner, feature extraction with all default settings

Data for : 251144713617_A01

Experimenter	SZAMAN
Experiment ID	101259 Compare measurements with an experiment from the same Print
Experiment Date	2005-06-15
Slide Name	251144713617_A01
Experiment Name	Y2864 2%glu (0-20)_6_15_05
Channel 1 Description	Y2864 in SC+3%glycerol to OD600=0.27 0min
Channel 2 Description	Y2864 in SC+3%gly+2%glu 20min
Is Reverse	N
Category	Yeast expression
Subcategory	glucose signaling
Normalization	normalization value is **undefined** Data Distribution | Plot Data | Signal Intensities | Ratios on Array

Print Information

Print ID	2082 Generate GAL file for this print
Print Name	Agilent-011447
Print Configuration	Agilent 1-sector

Description

Description	SZ249 strain has an integrated GAL10pRGT2-1 construct. Addition of galactose turns on expression of the dominant active RGT2-1 gene. RGT2 signaling is activated in the presence of high glucose. Therefore, comparison of galactose treated SZ249 cells to glucose treated wildtype cells should help identify the genes that are regulated by RGT2 in response to glucose. SZ249 and wildtype strains were grown in 3% glycerol media. Cells were collected at 0 min, treated with 2% galactose and then collected at 20, 40, 60 and 80 min post-treatment. In addition, wildtype strain was grown in 3% glycerol media and cells were collected at 0 min, treated with 2% glucose and collected at 20, 40, 60 and 80 min post-treatment.

Experiment Data Sets View Associated Experiment Sets

- Gives you all the experimental annotation
- Allows you to compare measurements with another experiment from same print.
- Gives you the normalization method and value (if applicable)
- Gives you several options to access the quality of data

View

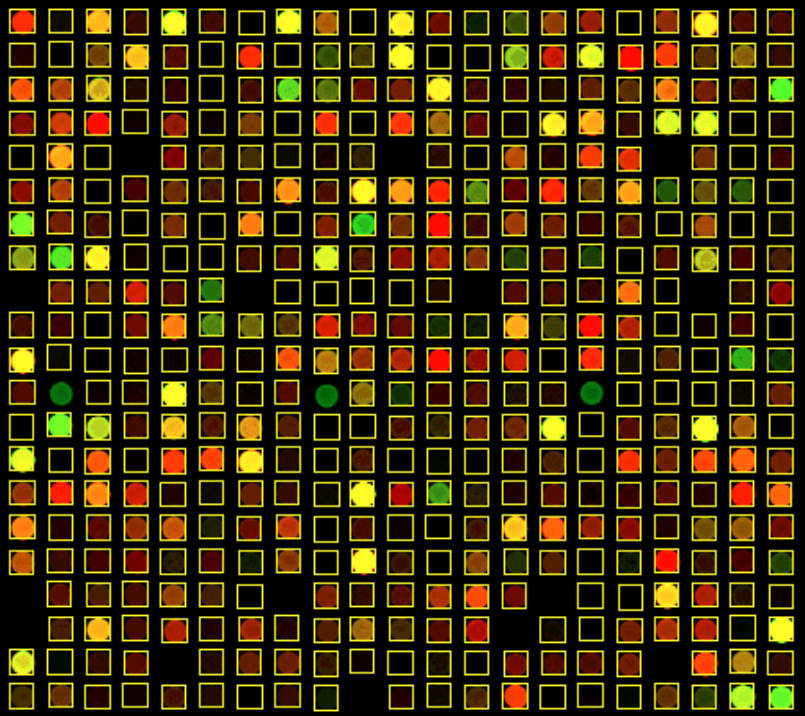
Display Options: View Details

Normalization	**Computed** normalization value is **2.06**	Data Distribution | Plot Data | Signal Intensities | Ratios on Array

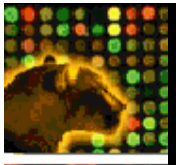
- Data Distribution
- Plot Data
- Signal Intensities
- Ratios on Array

These graphs are covered in the data analysis tutorial.

Display Data: View images with grids



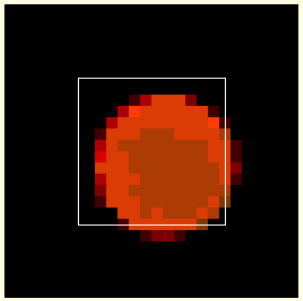
- Select data matching criteria
- Grid for array
- If you see a spot of interest, clicking on the spot yields…



Display Data: Spot Image

Individual Spot Data ((Agilent_batch_1))

1 spot(s) found matching criteria.

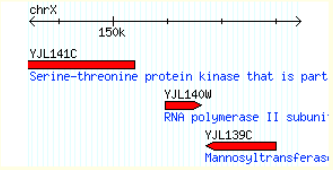


Click spot to see in array context

Biological Information

Sequence Name	A_06_P4161
GENE NAME	RPB4
CHROMOSOME	10
STRAND	W
Beginning Coordinate	150958
Ending Coordinate	151623
BIOLOGICAL_PROCESS	transcription*
MOLECULAR_FUNCTION	transferase activity*
ORF Name	YJL140W
Sequence Description	YJL140W|ebi.ac.uk:Database:sgd:A_06_P4161||www.chem.agilent.com:Database:agp

Relative Genome Map



View expression history of this entity

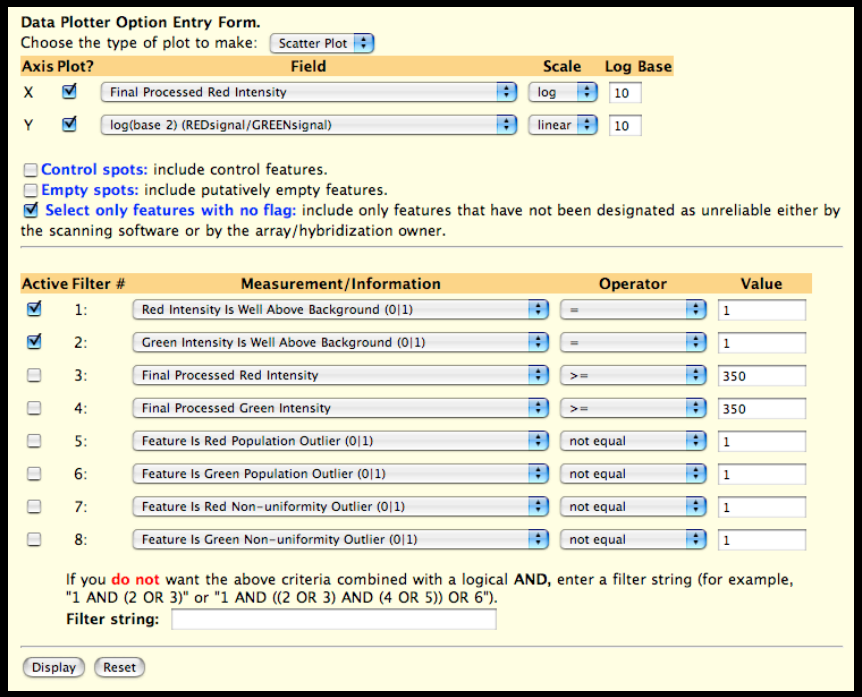
Flag Spot

Feature	5384
X coordinate	746.908
Y coordinate	1003.88
Green Intensity (mean)	323.8644
Green Intensity (median)	325
Red Intensity (mean)	976.4407
Red Intensity (median)	970
Green Background (mean)	23.89744
Green Background (median)	24
Red Background (mean)	29.79121
Red Background (median)	30
Number of Green Inlier Pixels	59
Number of Red Inlier Pixels	59
Standard Deviation of Green Inlier Pixels	21.01515
Standard Deviation of Red Inlier Pixels	57.41509
Number of Green Background Pixels	273
Number of Red Background Pixels	273
Standard Deviation of Green Background Inlier Pixels	2.82916
Standard Deviation of Red Background Inlier Pixels	4.219658
Box Top	997
Box Bottom	1010
Box Left	740
Box Right	753
Feature Is Used for Global Background (0|1)	
log(base 10) (REDsignal/GREENsignal)	.1442506872
Final Processed Green Intensity	4231.357
Final Processed Red Intensity	5898.348
GHighEndCorrDNSig	
RHighEndCorrDNSig	
Green Normalized Net Intensity	4231.36
Red Normalized Net Intensity	5898.35
Green Net Intensity	298.156
Red Net Intensity	946.158
Green Background Intensity Used	25.708
Red Background Intensity Used	30.2825
GProcessedSigError	38.82774
RProcessedSigError	46.59793
Error of Log Ratio	.05029484891
log(base 10) (P-value of Log Ratio)	-2.38410865315161
log(base 10) (P-value of Green Difference from Background)	-68.4480830206348

Display Data: Clickable Image

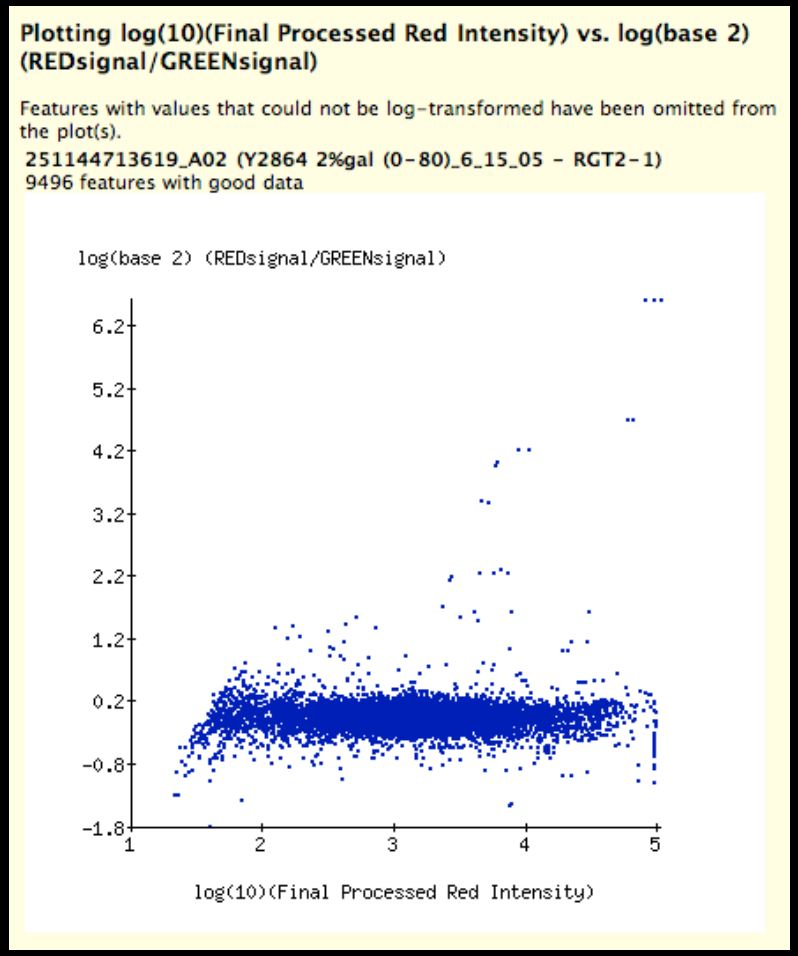
- Gives you the array image without the grid
- Does not give you the filtering option
- If you click on a spot, you get the same spot detail as the previous option

Display Data: Plot Array Data



Evaluate data quality by plotting values for any array, using any measurement you wish to.

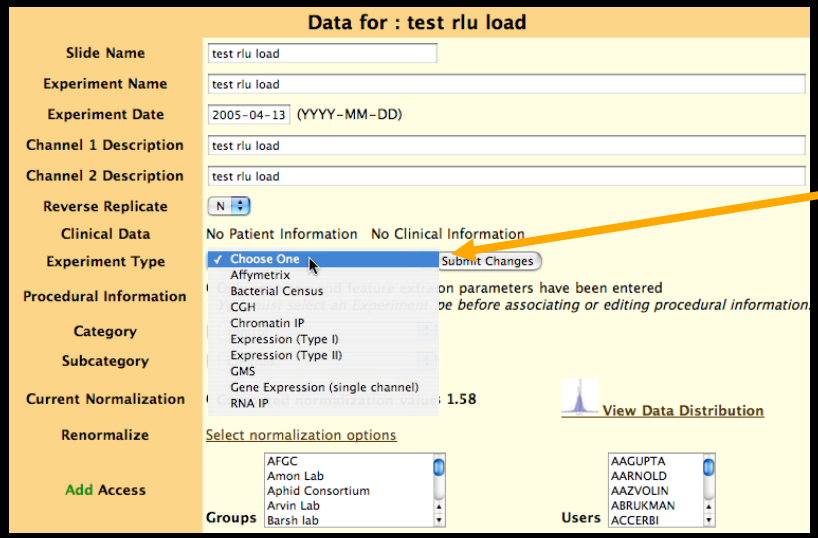
Display Data: Plot Array Data



Evaluate data quality by plotting values for any array, using any measurement you wish to.

Edit

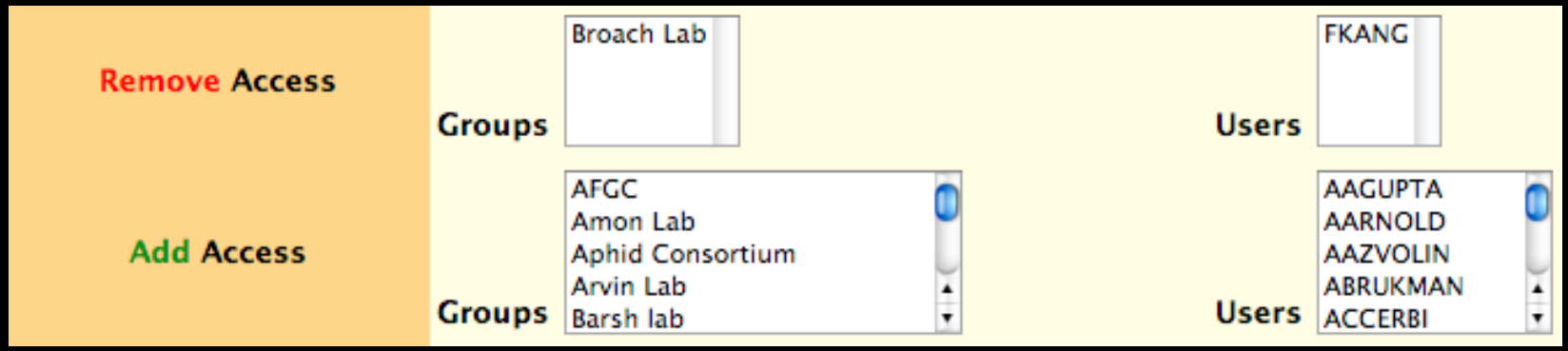
Display Data: Edit Experiment Details



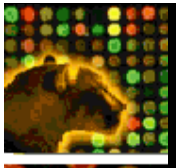
- Edit all names and descriptions
- Experiment Type
- Associate procedural information
- View Data Distribution
- Re-normalize data

Edit

Display Data : Editing Access



- Under Edit Experiment Details, you can add or remove experiment access
- You can give access to an entire group or an individual user
- To give access to collaborators:
  - Register your collaborator
  - In Experiment Details, Click on collaborator's name to grant access to view experiment



De-
lete

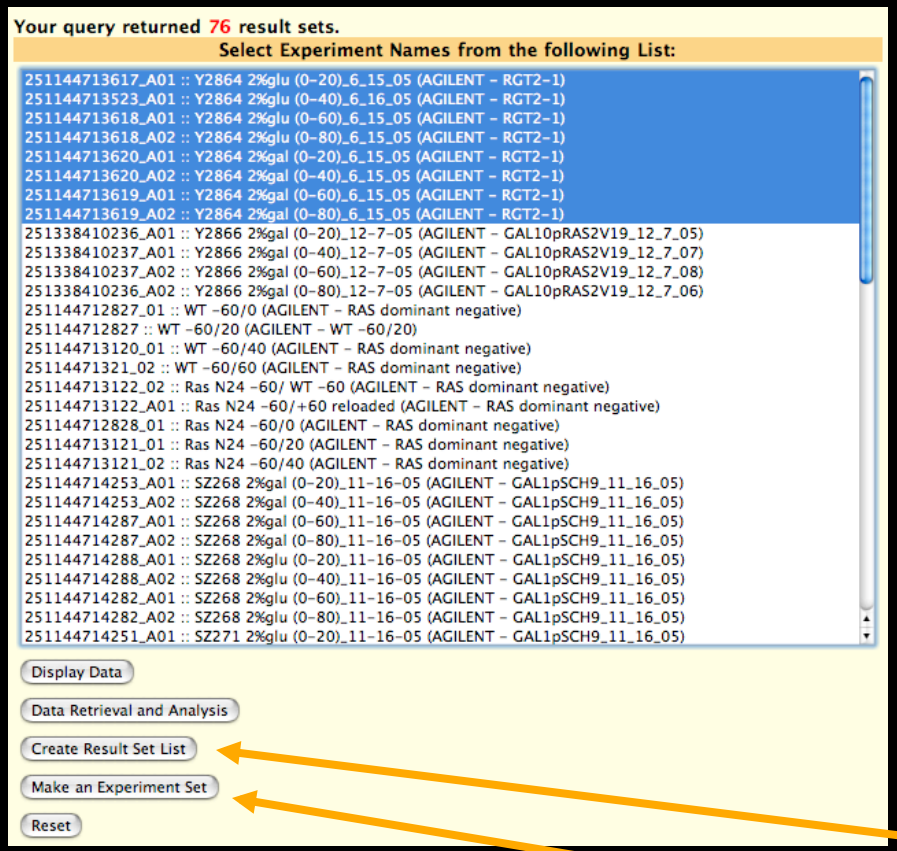
Display Data: Delete an Experiment

- Only the owner of an experiment can delete it
- Once an experiment is deleted from the database, it can not be recovered easily
- Once an experimenter leaves the lab, the lab head should consider what to do with his/her experiments, i.e. should the user still have the ability to delete all their experiments?

Welcome to PUMAdb

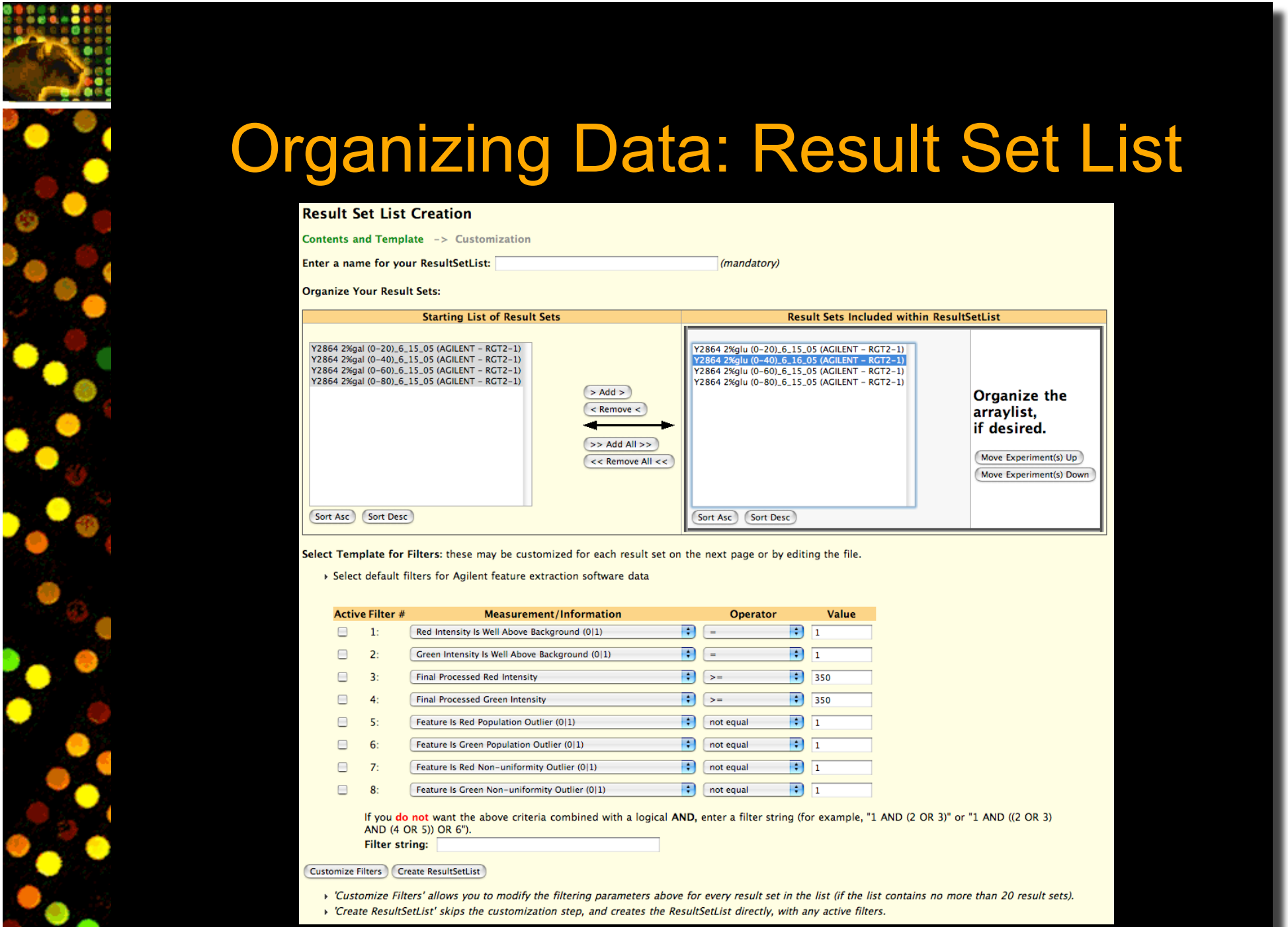
- User Registration
- Staging Data
- Loading Prerequisites
- Loading Data
- Finding Your Data
- Displaying Your Data
- Organizing Data
- Submitting a Printlist

Organizing Data:Data Retrieval and Analysis

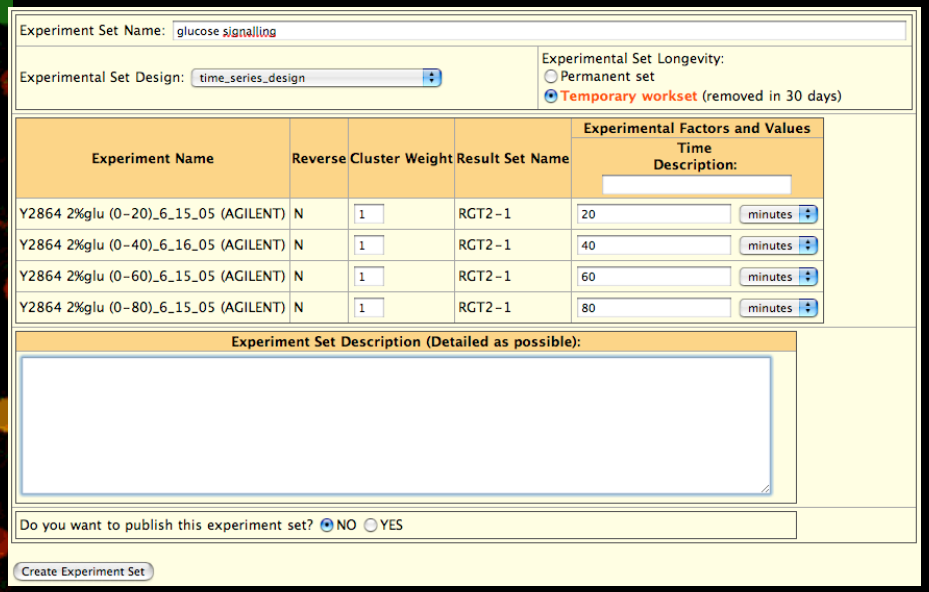


- Once you have selected a group of experiments, you need to select the experiments you wish to work with
- You have several different options:
  - *Display Data*
  - *Data Retrieval and Analysis* (clustering)
  - *Create Result Set List*
  - *Create Experiment Set*

Organizing Data: Result Set List



Organizing Data: Experiment Sets



- Order your experiments
- Select experimental factors (optional)
- Next provide more details
  - Name, Experiment set design, Longevity
  - Weights for clustering
  - Set description
    - For publications, this would be the abstract or figure legend
  - Publication Radio Buttons
    - All experiments must be world viewable in order to publish the set

Organizing Data: Result Set List vs Experiment Set

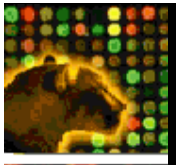
- Result Set (Arraylist)
  - Text file that exists in your loader account arraylists directory
  - Only visible to you
  - Contains no annotation
  - Customized filtering
  - Accessed through Advanced Search
- Experiment Set
  - Exists in the database therefore dynamic (edit, delete, or annotate through a web interface)
  - Visible to users & collaborators
  - Can be well annotated
  - Required for publication within the database
  - Accessed through Basic Search

Organizing Data: Genelists

- What is a genelist?
  - A file containing a list of genes that exists in your loader account in the directory *genelists*
- What is the purpose of a genelist?
  - Cluster and analyze only a set of genes
  - When retrieving your data, you may choose to retain the annotation from your genelist instead of using the database annotation
- There are several shared standard files of genelists that are available for many organisms.
- You may create your own precompiled list of genes.
- Normalization values can be calculated based on a genelist.

Organizing Data: Creating your own genelist

- Create a tab-delimited text file
- The first line of the file must have the appropriate label for the data contained within it
  - NAME (YPR119W, IMAGE:1542757, or HPY1808)
  - SUID
  - LUID
  - SPOT
- Your file may contain one additional column with any type of annotation data you desire for each gene
- This information can be extracted during data analysis and carried all the way over through clustering



Questions?

Send e-mail:
array@genomics.princeton.edu

Office: CIL 135
Phone: 258 - 8309

Online help: http://puma.princeton.edu/help/

Welcome to PUMAdb

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- Staging Data
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- Loading Data
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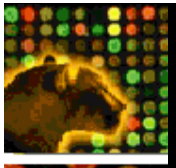
Submitting Plate Samples and ArrayDesigns

- The creation of a print within the database is a complex process.
- If you receive your arrays from the core facility, this is done for you
- Plate samples are conveyed as a tab-delimited list (well address + contents)
- There is a program to assist you in platesample submission:
  - Located under "*Tools*" on the "**Index of Programs**" page
  - Printlist must be in your incoming directory on loader

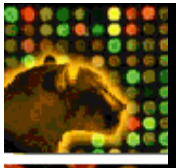
Submitting Plate Samples : Is a new list required?

- **Yes**, if the plates used have not been previously entered into the database
- **Yes**, if the plate was entered in the past, but their contents have changed over time (well contamination, well emptied)
- **No**, if your lab makes 3 different prints using the exact same plates in the same or different order
  - Just need to tell a curator the a list of database plateIDs and plateNames from the first print in their new order.



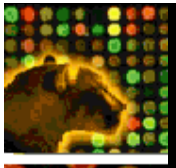
Submitting Plate Samples: Column Headers

- **PLAT**: The plate number; eg 1, 2, 3, etc. *INTEGER*
- **PROW**: The plate row; eg A, B, C, etc. *CHARACTER*
- **PCOL**: The plate column; eg 1, 2, 3, etc. *INTEGER*
- **NAME**: The sequence name
  - usually a systematic name or clone identifier (I.e. YBL016 or IMAGE:753234)
  - This is the only name used for samples of TYPE other than CDNA.
- **TYPE**: The sequence type
  - Usually ORF, CDNA, CONTROL, or EMPTY.
  - List of types can be seen from the SMD homepage under *List Data: Sequence Type*
- **FAIL**: Whether the PCR failed
  - 0 : one distinct band - success
  - 1 : no signal - fail
  - 2 : multiple distinct bands
  - 3 : signal, but not a distinct band (smear)
  - 4 : multiple smears
  - 5 : unknown
  - 101 : worst cases of peeled away or haloed spots(assigned on a 96 well plate basis)
  - 102 : less bad cases of peeled away or haloed spots(assigned on a 96 well plate basis)
  - Null is assumed to be 0 (success)



Submitting Plate Samples: Additional Columns for cDNA data

- **CLONEID**:
- **ACC**: Required if CLONEID is absent/null.
  - This is the GenBank accession, usually acquired from dbEST.
- **IS_CONT**: Whether the sample is known to be contaminated. A blank entry will default to unknown (U)
- **IS_VER**: Whether the DNA in a well has been verified. A blank entry will default to unverified (U).
- **SOURCE**: A string describing the source of the clone or DNA. This has typically been used to indicate the original plate source, and the 96 and 384 well plate locations that a clone has been in
  - GF200:96(1A1):384(1A1).
  - GF200 refers to a set of resgen plates



Submitting Plate Samples: Optional Columns

- **DESC**: A description of the molecular entity. This description is associated with the SUID itself (not a clone or platesample description)
- **LUID**: Laboratory Unique ID: For those samples that have identical NAME and TYPE, but require distinction within the laboratory for experimental reasons (different sources, new PCR,new plate). If you wish to enter LUIDs for your lab's platesamples, please contact the curators: array@genomics.princeton.edu
- **GENE_NAME**: Sometimes clones will stop being included in UniGene for spurious reasons, but users have a 'Preferred Name' for those clones.
- **ORIGIN**: For CDNA clones, this can indicate whether this is a public or private clone.
- **SAMPLE_DESC**: A description, if any, about that particular sample. This description is specific to the plate sample.
- **ORGANISM**: If submitting a print containing samples from multiple organisms (i.e. human, yeast). For those few rows where the sample is derived from an organism *other* than the default (user-defined), the organism code must be specified.

Submitting Plate Samples: Creating New SUIDs

- New samples in your plates (i.e. those not currently in the database) will need to have a unique sequence identifier assigned to them (SUID)
- A SUID is meant to represent a unique molecular entity within the database. It is relatively meaningless outside the context of the database.
- The combination NAME::TYPE::ORGANISM uniquely identify an SUID
  - YBL001C::ORF::SC → SUID:3429
  - IMAGE:486544::CDNA::HS →SUID:28546
- SUIDs allow comparison of the same samples across different prints.
  - It is extremely important that erroneous SUIDs are not created.
    - This will prevent comparisons between prints/experiments

Submitting Plate Samples: Avoiding Common Name Errors

- Erroneous SUIDs are usually created by a bad NAME
  - misspelled, non-standard, or non-systematic
    - ACT1:ORF:SC or Actin:ORF:SC ➔ YFL039C:ORF:SC
    - 3X SSC:CONTROL:SC ➔ 3xssc:CONTROL:SC
  - Every new sample must be verified by the user before it is assigned a new SUID and before the printlist can be entered.
  - Please be a conscientious user and verify that any new SUIDs you approve are valid.

- Empty wells must be specified as such
  - All empty wells must be designated NAME=>EMPTY and TYPE=>EMPTY.
  - Do not use "blank“ or "control" to describe empty wells.

Submitting a Printlist: Avoiding Common Errors

- Headers misspelled or absent
- Required data missing
  - except FAIL, CLONEID, but column header must still be present
- Correct Plate ordering
- No wells may be skipped (with the exception of the last plate in the print run).
- Useful check: number of plate samples = number of printed spots

  #samples = (#printlist rows-1) <= #tips * #rows per sector * #columns per sector = #spots

Submitting Plate Samples: Validation Program

- The printlist must be placed in your *incoming* directory on your loader account
- This program will assist you in printlist submission
  - It follows the rules stipulated above.
- The program will send all feedback to your *logs* directory
  - Filename.new
  - Filename.errors

Submitting a Printlist: Notify Curators

- Additional information needed:
  - Number of sector rows/columns
  - Distance of rows/columns in sector
  - Printing algorithm: http://puma.princeton.edu/help/createPrint.shtml
  - Number of slides printed
  - Plate location
  - Printer used for printing
- When your printlist is correct - send email with info above to ***array@genomics.princeton.edu***