Hands on with

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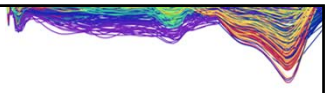
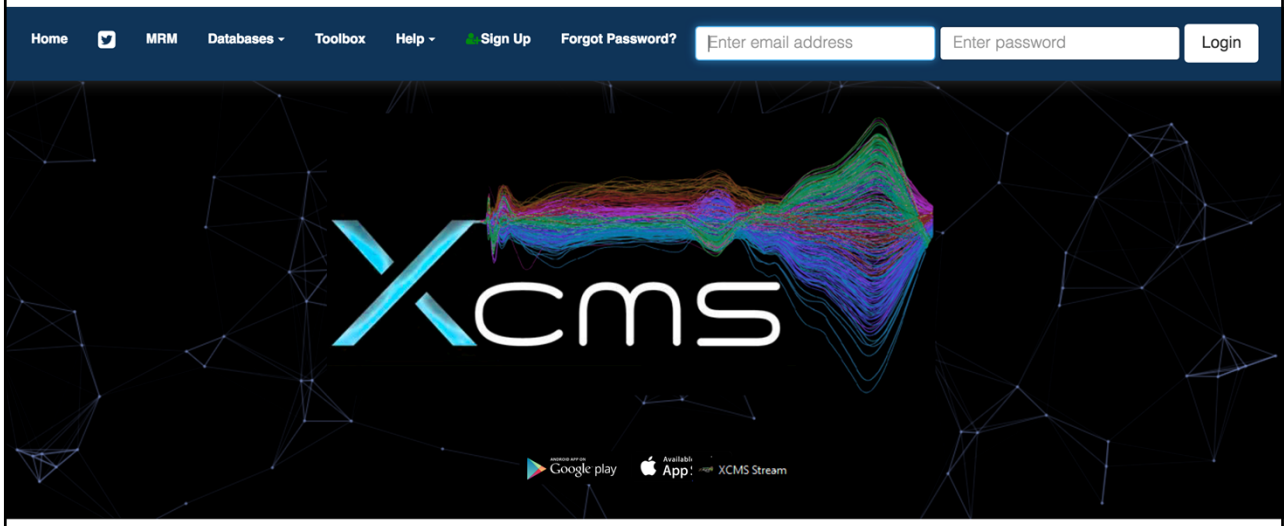
Scripps Research

What we're going to do ….



- It's your choice
  - A – Upload data and run a pairwise/multigroup jobs
  - B – Use a premade cake and find some interesting metabolites
  - C – Run over to xcms-mrm and analyse data there

First lets check your User



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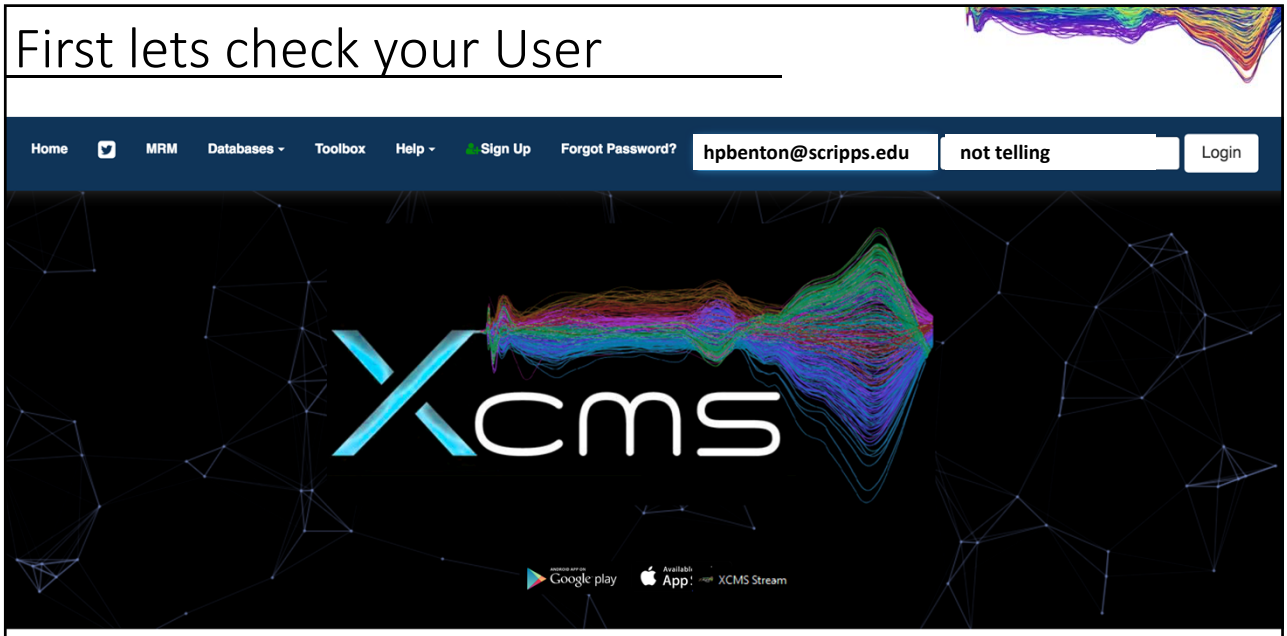
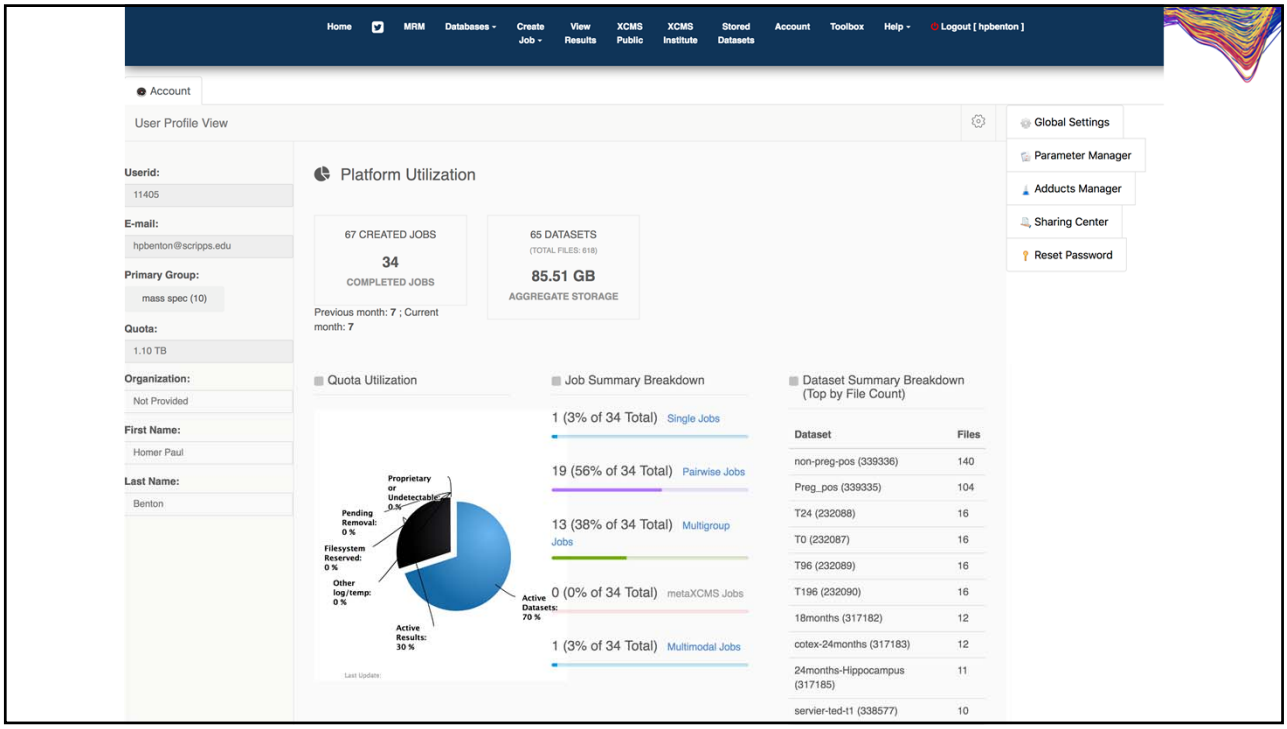


What we're going to do ….

- Can everyone login ?
  - If you cannot please let me know!
- Otherwise –
  - Have a look at our help section. Please have a scan over this -
    - We have an FAQ page that we constantly update with questions that have been posted
    - We have a help page that we have need to get back to
    - Contact us page - E-mail us here- Not instantaneous!
  - Lets check out the account tab



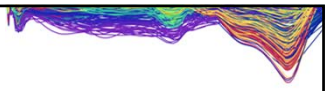
What we're going to do ….

- Datasets to use –
  1. Chronic Pain – SHAM rats vs (TNT) tibial nerve transected rats
  2. *Halobacterium salinarum* KO models – VNG111
  3. Coke Vs Pepsi – A dataset to find the secret ingredients
  4. MRM Plasma with 10 Standards

A bit about the datasets

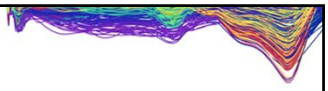
- Chronic Pain – SHAM rats vs (TNT) tibial nerve transected rats
  - **Alterations in Spinal Cord Metabolism during Treatment of Neuropathic Pain** https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4548716
  - **Metabolomics implicates altered sphingolipids in chronic pain of neuropathic origin https://www.nature.com/articles/nchembio.767**
  - Main metabolites were identified
    - *N,N*-dimethylsphingosine (DMS) 328.321
    - Due to the Sphingomyeline-ceramide pathway having a non-reversible pathway from sphingosine to DMS
- https://www.dropbox.com/sh/wk4qggnzugphqkt/AACSln5rmIUVMFIQ_oxhn4fEa?dl=0

A bit about the datasets



- *Halobacterium salinarum* KO models – VNG111
  - **Meta-analysis of untargeted metabolomic data from multiple profiling experiments** https://www.nature.com/articles/nprot.2011.454
  - Knock out models of *Halobacterium Salinarum* at VNG1816G, VNG1179c & VNG2094G vs the wild type URA3
  - Glutamic acid was seen to be important in some of the mutants but not all mutants. Copper trafficking was shown to be responsible for this change. (overlap analysis was able to find these results)
- https://www.dropbox.com/sh/vkhja1ss21pear5/AADA1gqF2HNPcZ4yJIGWEIKYa?dl=0

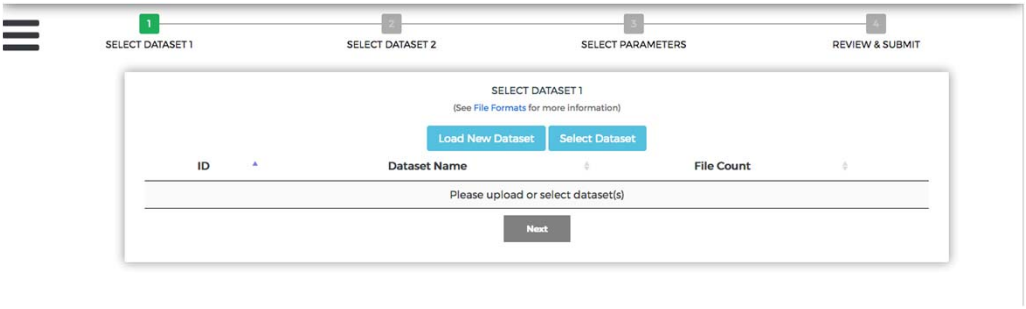
A bit about the datasets



- Coke Vs Pepsi – A dataset to find the secret ingredients
  - A highly complex study hidden in trade and lab secrets
  - Coke and pepsi was extracted using sea water type extraction
  - 10ml was injected for each
- *https://www.dropbox.com/sh/xviewq92n9mpsck/AADgfIpNK9bGvs6NTUZqTTeKa?dl=0*

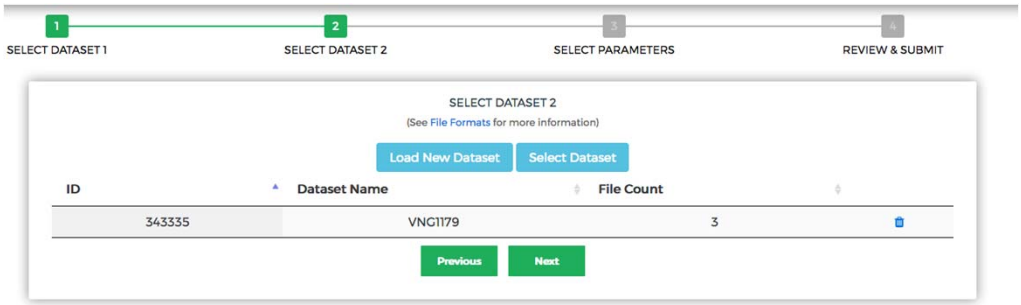
The lab was never legally pursued by either company as long as the ingredients were never declared.

A bit about the datasets



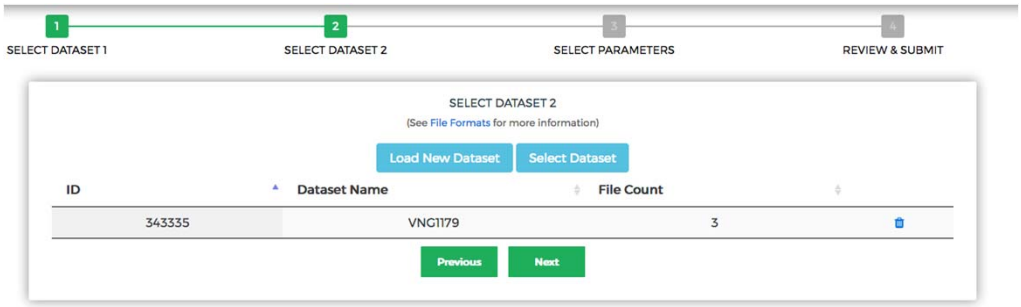
- Here you can upload the data and you'll be greeted by a upload box.
- Within the upload window you can drag and drop the files dierectly onto the uploader or select and find the files
- Wait till all the data is uploaded. This means a green check mark next to each dataset.
- Name the dataset something useful that you'll remember for later.
- Hit 'Next'

A bit about the datasets



- Do the same again for the 2nd dataset you want to compare to.
- Once uploaded you will see the dataset with the saved name and the number of files that were uploaded.
- Again lets hit next to proceed.

A bit about the datasets



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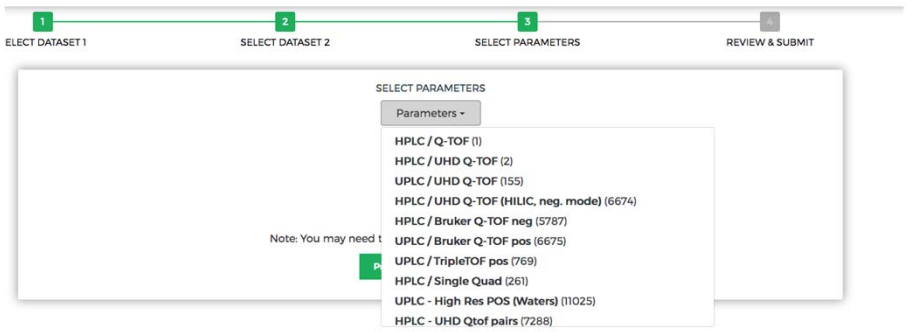
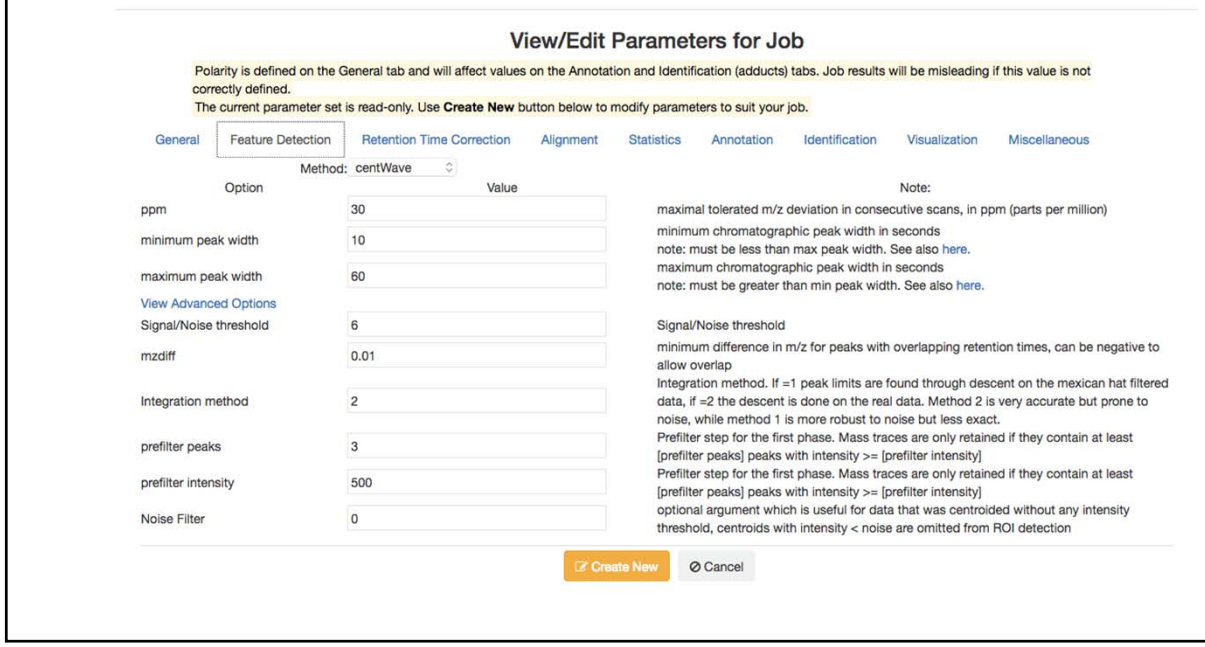
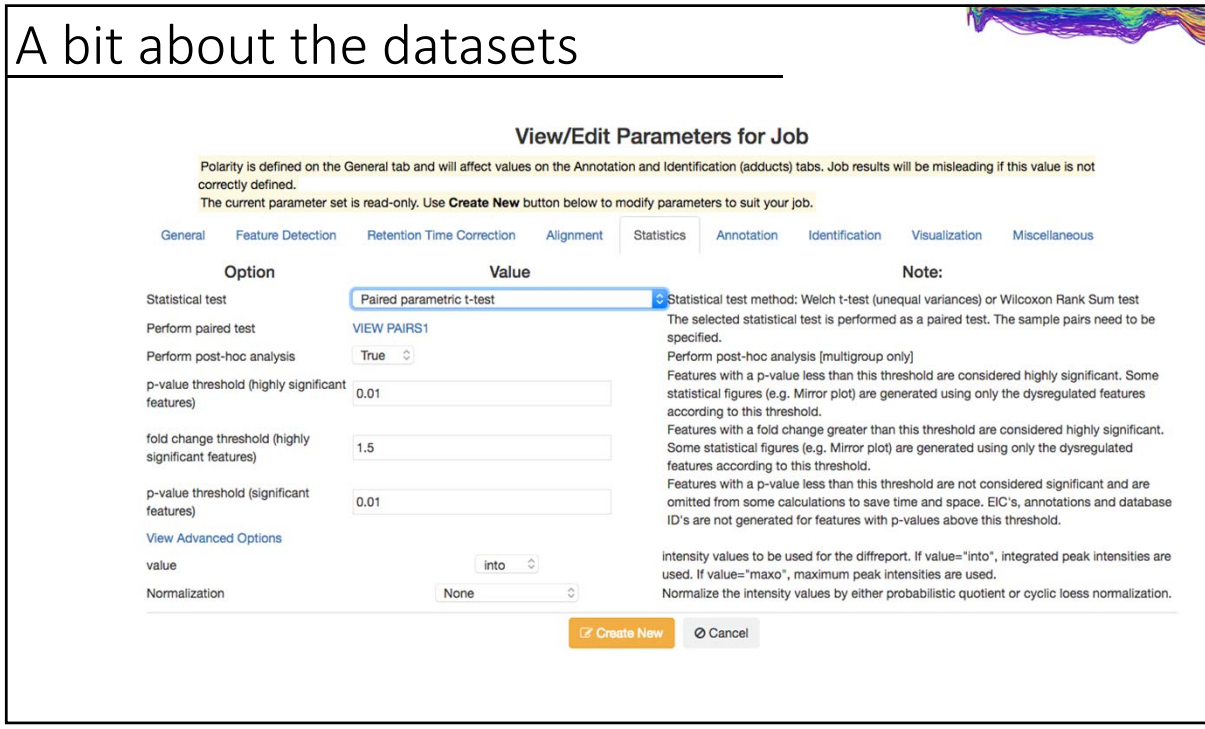


- So now we have to look at parameters !!!
- We can either choose a general selection of parameters that will most likely work pretty well but for fine tuning select a close set of parameters and then click the 'View/edit'
- Before proceeding please note that statistics and model type are saved with the job and not with the parameter set.
- If you save the parameters and then go back to view it within the job the parameters will reset to default settings for the statistics and model type

A bit about the datasets



A bit about the datasets



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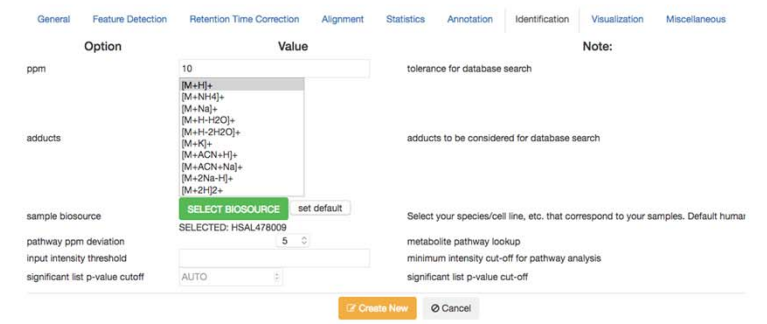

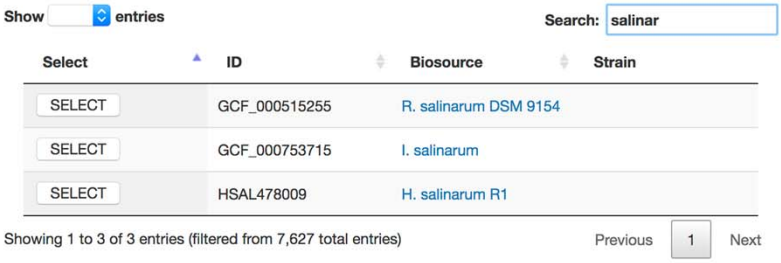

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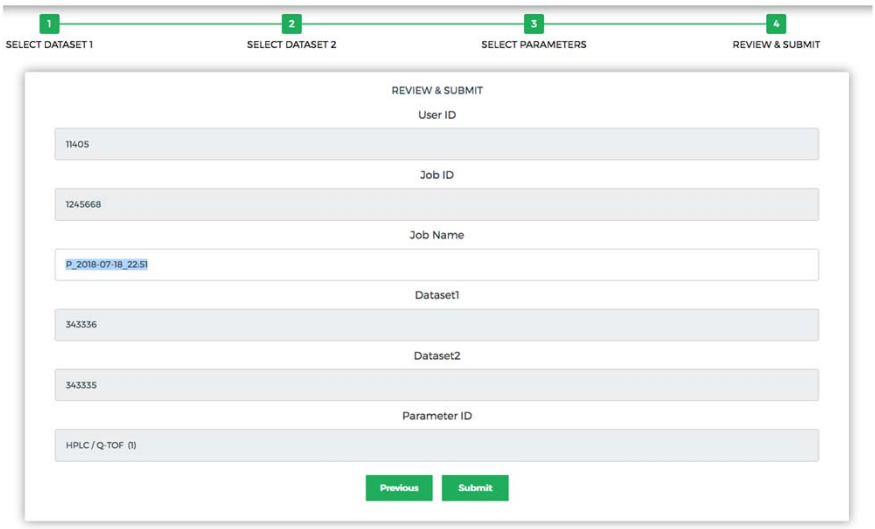


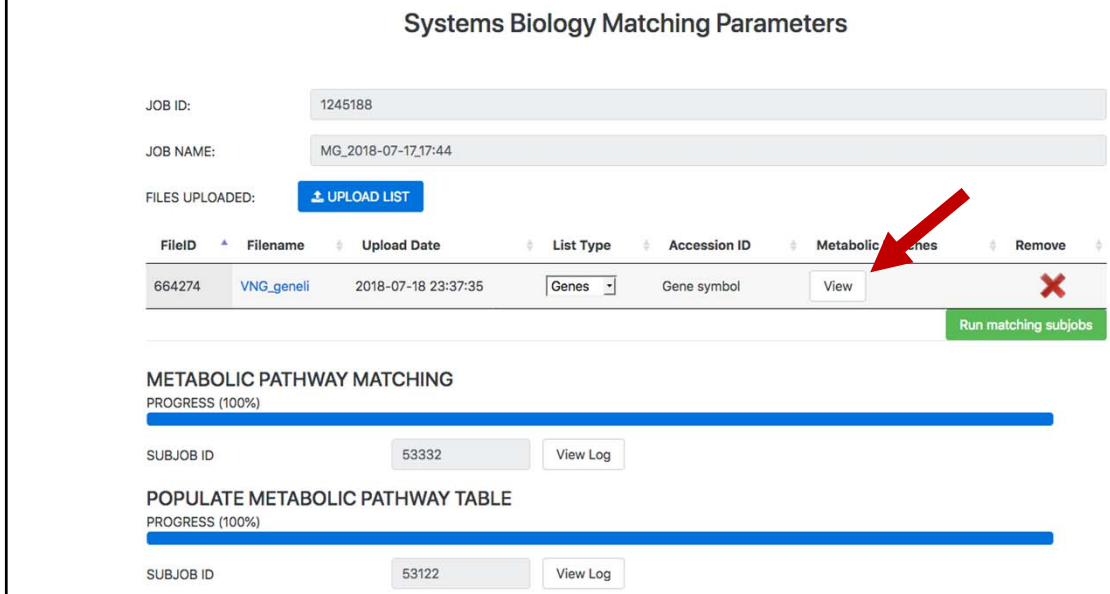
So now what ….

- Normally at this point you would have to wait until your job was finished.
- For today I have given everyone multi-submit access and setup our own processing server so things should be fast for us.
- If you were following the above you should see something like the below image -

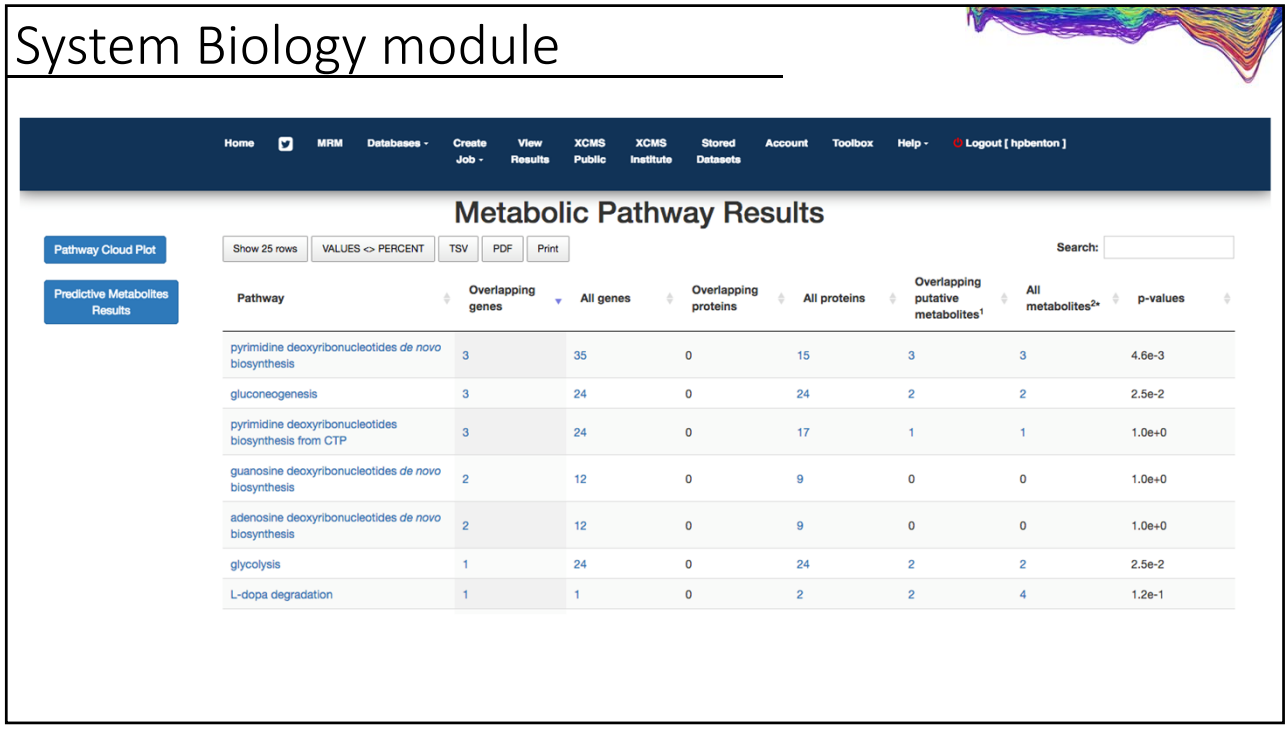
Running the other job types …

- I recommend to run the VNG-URA datasets as both a pairwise setup
  - VNG1816G VS URA (WT)
  - VNG1179c VS URA (WT)
  - VNG2094G VS URA (WT)
- Then again as a multigroup and finally
- The above pairwise as a meta-XCMS analysis to see the differences between the multigroup and meta analysis

System Biology module



System Biology module

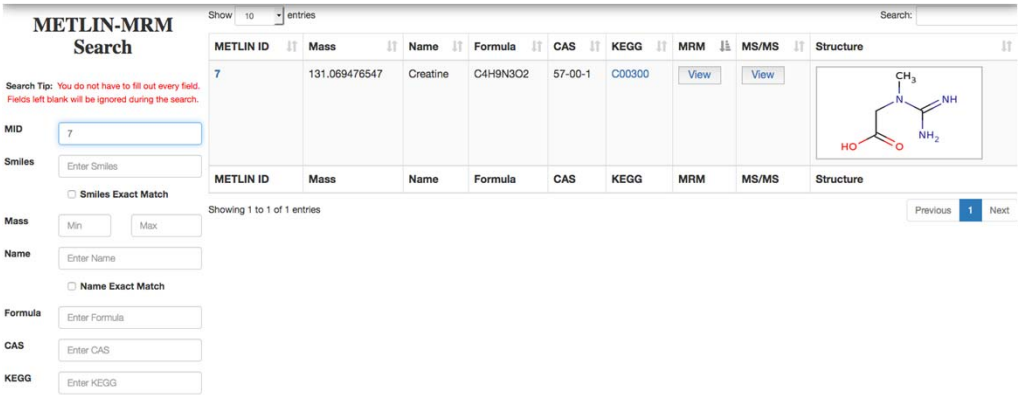


XCMS-mrm & METLIN-MRM

- First you want to get your transitions

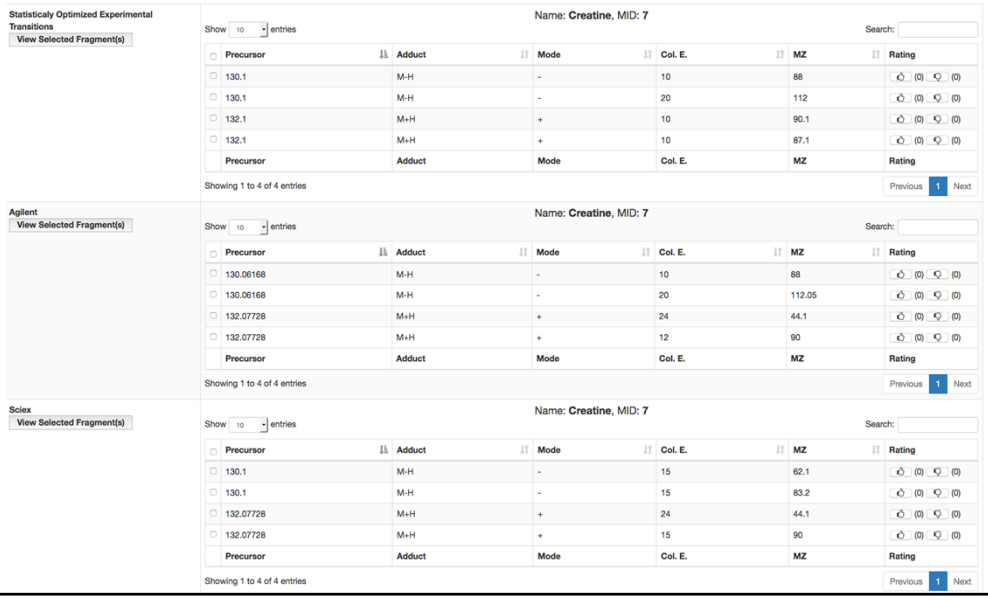
XCMS-mrm & METLIN-MRM

- Search for a metabolite that you want the transition for



XCMS-mrm & METLIN-MRM

- Decided which is the best transition for you…



Statisticaly Optimized Experimental Transitions
View Selected Fragment(s)

Name: **Creatine**, MID: **7**

Show 10 entries — Search:

Precursor	Adduct	Mode	Col. E.	MZ	Rating
130.1	M-H	-	10	88	(0) (0)
130.1	M-H	-	20	112	(0) (0)
132.1	M+H	+	10	90.1	(0) (0)
132.1	M+H	+	10	87.1	(0) (0)

Showing 1 to 4 of 4 entries — Previous 1 Next

Agilent
View Selected Fragment(s)

Name: **Creatine**, MID: **7**

Show 10 entries — Search:

Precursor	Adduct	Mode	Col. E.	MZ	Rating
130.06168	M-H	-	10	88	(0) (0)
130.06168	M-H	-	20	112.05	(0) (0)
132.07728	M+H	+	24	44.1	(0) (0)
132.07728	M+H	+	12	90	(0) (0)

Showing 1 to 4 of 4 entries — Previous 1 Next

Sciex
View Selected Fragment(s)

Name: **Creatine**, MID: **7**

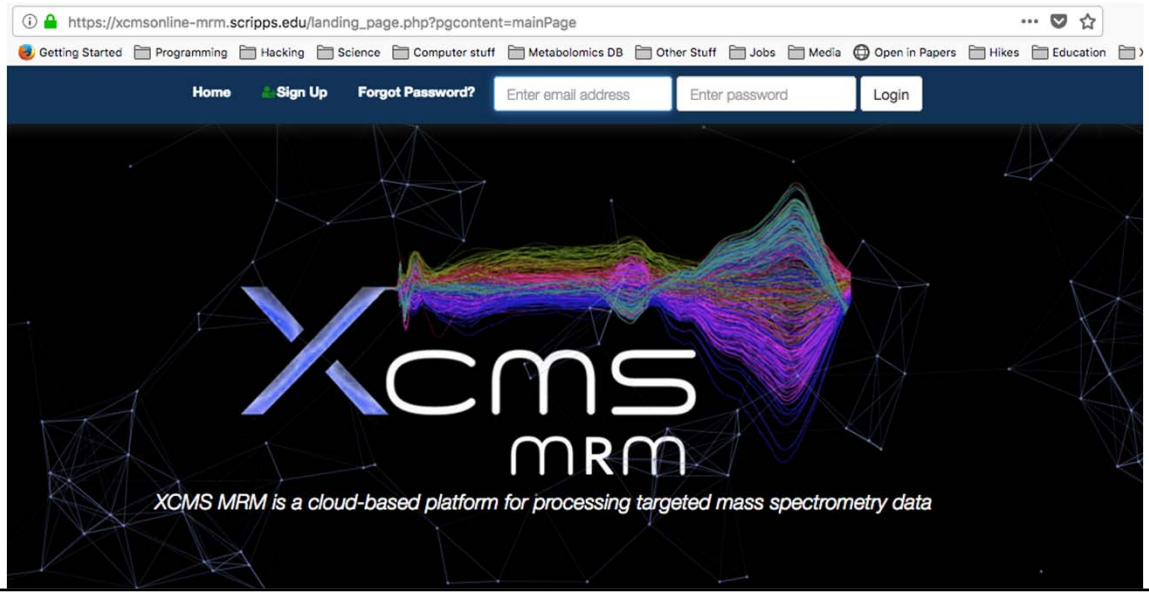
Show 10 entries — Search:

Precursor	Adduct	Mode	Col. E.	MZ	Rating
130.1	M-H	-	15	62.1	(0) (0)
130.1	M-H	-	15	83.2	(0) (0)
132.07728	M+H	+	24	44.1	(0) (0)
132.07728	M+H	+	15	90	(0) (0)

Showing 1 to 4 of 4 entries — Previous 1 Next

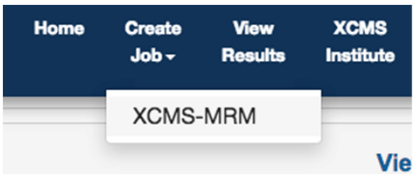
XCMS-mrm & METLIN-MRM

- Decided which is the best transition for you…



XCMS-mrm & METLIN-MRM

- Run a job the same way we did before



- After uploading your files you'll need a selection of transitions that you have used –

Name	Precursor	Product	RT.min	RT.max	Prec.Labeled	Prod.Labeled
Leucine	132.1	43.096	0.709	1.7		
Leucine	132.1	44.096	0.707	1.7		
Isoleucine	132.1	44.096	0.708	1.705		
Isoleucine	132.1	69.066	0.706	1.703		
Leucine	132.1	86.086	0.704	1.7		
Isoleucine	132.1	86.086	0.705	1.701		
Phenylalanine	166.08	103.096	2.012	3.004		
Phenylalanine	166.08	120.076	2.01	3.003		
Phenylalanine	166.08	130.996	2.007	3.003		
Tyrosine	182.08	136.096	0.607	1.599		
Tyrosine	182.08	146.996	0.604	1.598		
Tyrosine	182.08	165.096	0.601	1.597		
Caffeine	195.08	83.056	2.863	3.867		
Caffeine	195.08	110.066	2.862	3.864		
Caffeine	195.08	138.056	2.861	3.862		