TempO-SeqR™ Software

User Guide

Version 11 (KT)

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Document 100854

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Scope

TempO-SeqR is software for the analysis of sequencing data obtained by performing the TempO-Seq™ assay. This user guide describes the procedures and equipment necessary for running the TempO-SeqR analysis software.

NOTE: The TempO-SeqR software is not meant for use with data from other assays.

TEMPO-SEQR PIPELINE

TempO-SeqR

Generate Sample Sheet

Any Next-Gen Sequencer

FASTQs

AGGCTAGCTCTGAT
AAGCAGGCTAGCTCTGAT

TACAGATCAGGTAA
TACAGATCAGGTAAACAT
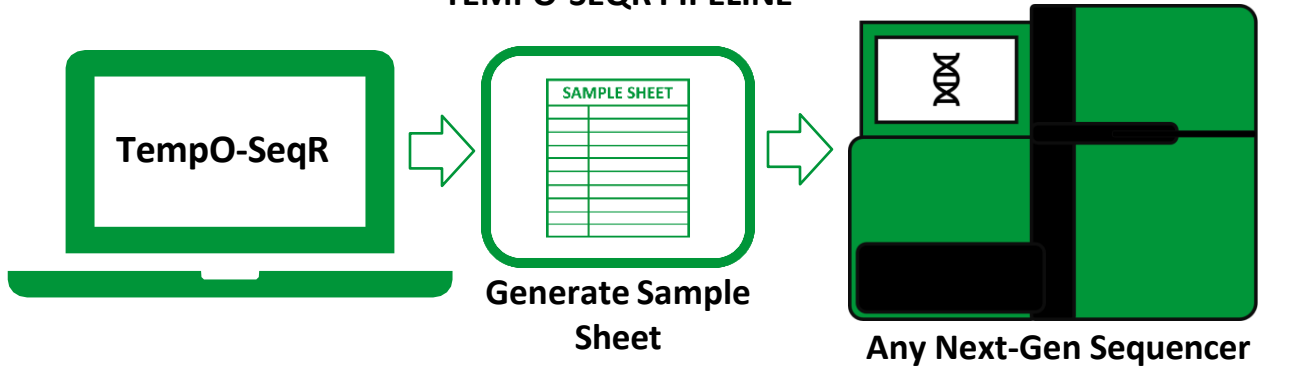
Alignment

GENE	SAMPLE1
NOTCH1	16236
JAG1	2726
GAPDH	52362
ESR1	4130

Counts Table

Quality Control Tools

Barplot   **Statistics**   **Dendrogram**   **Scatterplot**   **PCA**

CTRL1 CTRL2   TX1 TX2

PC2   PC1
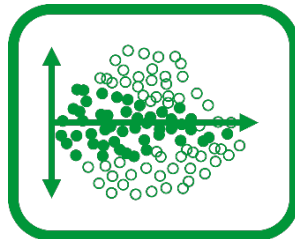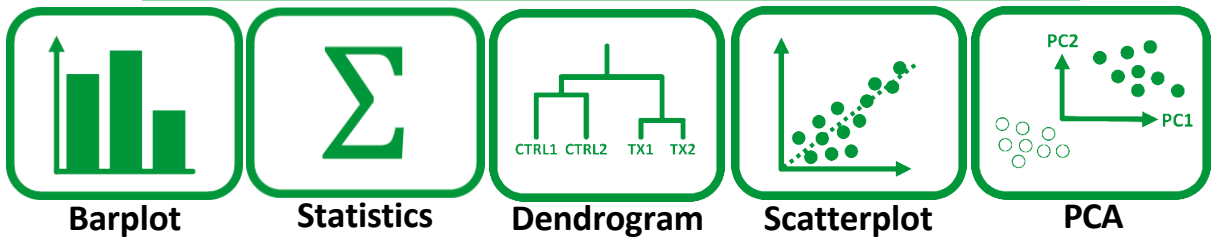
Differential Expression Analysis

Overview of the TempO-SeqR pipeline

The TempO-SeqR program is designed to complement and assist in the execution of the TempO-Seq™ assay.

After the assay has been completed, the user will have a library purified from one or more TempO-Seq™ indexed PCR plates (for 24 or 48-sample TempO-Seq™ kits, please consult the package insert). In order for this library to be sequenced, it is necessary to prepare a Sample Sheet which associates specific index sequences with appropriate samples. The Sample Sheet Generator can be used to easily convert a simple plate map of sample names into a Sample Sheet for any of the supported Next-Generation Sequencing instruments.

The sequencing instrument will use the index information to generate one FASTQ file for every sample denoted in the Sample Sheet. These FASTQ files can then be aligned in the TempO-SeqR program, producing the final output: a count table, showing the signal levels (counts) for each probe in each sample.

Once the count table is available, it can be processed with bioinformatic or statistical tools of user's choice. TempO-SeqR itself can (optionally) be used to check the quality of the data, and to perform the basic differential expression and statistical analyses. It is possible to quickly check correlation of individual samples or controls to each other; to verify grouping of sample types based on similarity; to calculate PCA groupings; or to analyze the basic fold-changes in gene expression between treatment or disease groups.

Terminology

| Term	Definition
TempO-Seq™	Templated Oligo Sequencing Assay.
Alignment	The process of arranging sequences against a set of reference sequences to identify matches.
Alignment ID	A unique identifier for each alignment.
Barplot	A plot showing the total number of mapped reads per sample.
Count Table	Output file containing the number of reads per probe per sample.
Dendrogram	Tree diagram displaying the degree of correlation between samples.
Differential Expression	Calculates quantitative changes in expression levels between groups of samples.
FASTQ Files	Standard output files produced by Next-Generation Sequencing (NGS) instruments containing sequences and their quality scores.
Index	Unique sequence tag used to identify individual samples.
MAplot	Visual representation of differential expression data.
Mapped Reads	Sequences successfully aligned to a reference sequence.
PCA Plot	Principal component analysis; visualizes variance between different samples.
Plate Map	A table that displays the location of each sample in a microtiter plate.
Probe	An oligonucleotide used to detect a target sequence.
Sample Sheet	Standard template file necessary for sorting sequences from multiple samples into FASTQ files (demultiplexing).
Scatterplot	A visualization of correlation between two samples.
Server	Secure online cloud-based system where customer FASTQs and alignment results are stored by BioSpyder.
Reads	The set of genomic sequences obtained from Next-Generation Sequencing.
Reference Table	A table containing probe sequences with their respective gene names and BioSpyder probe IDs. Used for aligning sequencing data.

Accessing TempO-SeqR

TempO-SeqR can be accessed with an internet-connected computer. It is recommended that users interface with TempO-SeqR through the latest build of Mozilla Firefox. Below is a list of the oldest compatible browsers and operating systems supported by the TempO-SeqR software.

Computers with operating systems Windows 7, Mac OSX 10.10, Ubuntu 14.04 (LTS), or newer are supported if users are using any of the following browsers:

Browser	Version (or newer)
Google Chrome	40
Internet Explorer	11
Microsoft Edge	14
Mozilla Firefox	35
Safari	8

Account Information and Login
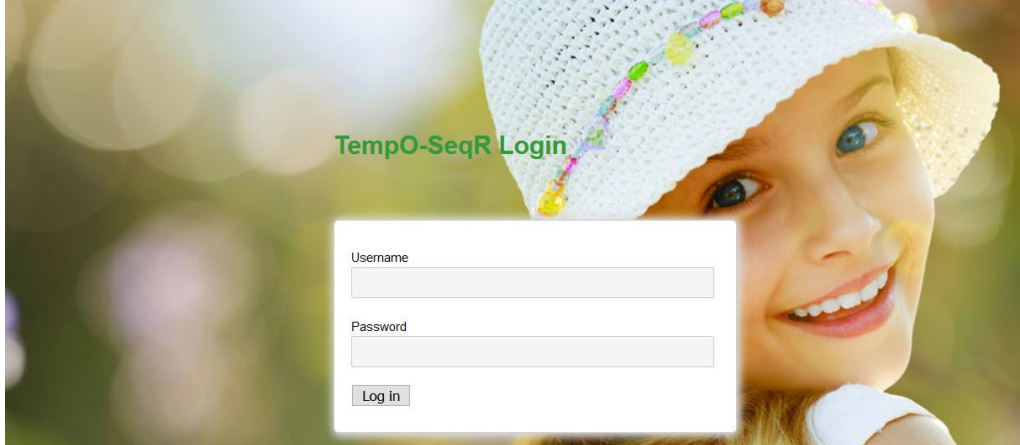
Obtaining username and password

BioSpyder will provide authorized users with a username and password.

Changing your password

Passwords may be changed upon request by contacting TempO-SeqR support (TempO-SeqR@biospyder.com).

Account security

The customer's username links together information about past and current alignments, alignment results, FASTQs, and project and experiment information if provided. These data are not visible or linked to any other account. To maximize security, please keep account usernames and passwords confidential.

Landing Page

Upon logging in, users are greeted with the landing page.

Elements of landing page

BioSpyder Logo

The logo will return the user to the main BioSpyder website.

Menu Bar

The menu bar allows the user to navigate between different modules in TempO-SeqR.

Alignment History

Information regarding the user's current and past alignments will appear in this section of the landing page, including date and time of alignment, alignment ID, and alignment parameters.

The status of the alignment is also shown as one of four possible outcomes:

- New
- Aligning
- Completed
- Failed

Updates
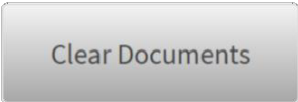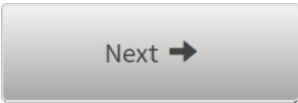
Users will be notified of changes to TempO-SeqR through this section of the landing page.

User Information

The customer's username, company name, and associated email address are displayed next to the green icon.

Common UI Elements

Below is a list of common elements in the interface that the user will encounter as they use TempO-SeqR.

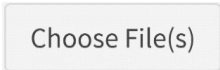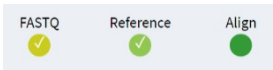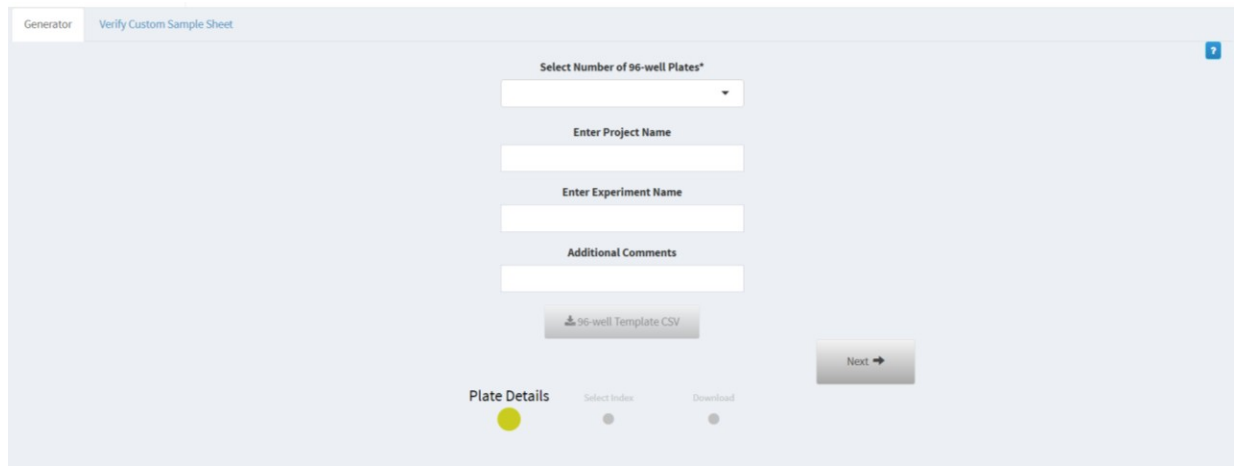| Element Example	Element Description
Main Menu	Returns the user to the landing page.
Logout	Ends the user session and logs the user out of TempO-SeqR.
Reset Alignment	Resets all selections for alignment.
Clear Documents	Clears all currently uploaded documents.
Next ➜	Sends the user to the following step of the process.
?	Indicates a tool tip is available. On click, a brief description on how to use the tool will appear. Tool tips also contain a link to the relevant section of the user guide for more in-depth instruction.
⬇	Indicates a file that can be downloaded, as pictured to the right in context: This applies to graphs, plots, and Sample Sheets. ⬇ 96-well Template CSV
Choose File(s)	Opens a file browser in a new window for the user to select files on the server.
No file selected	Indicates that no file has been selected for upload.
Upload complete	Indicates that a file upload has been completed successfully.
FASTQ ✓ Reference ✓ Align ●	Progress circle indicates current step that user is on, for stepwise processes such as alignment or Sample Sheet generation.

Sample Sheet Generator

The Sample Sheet Generator can be used to make Sample Sheets for use with compatible Next-Generation Sequencing machines.

STEP ONE: Plate Details

From the landing page, select 'Sample Sheet Generator' in the menu bar.

Users may add up to 4 96-well plates for Sample Sheet generation from the drop down menu. After selecting the number of desired plates, click the download button for the 96-well template file.

Note: Selecting the number of plates is all that is required to proceed to the next step. The template file will change based on how many plates have been selected.

Completing the Template

After downloading the provided template file, please fill it out in the spreadsheet program of your choice. The file must be saved in CSV format after editing is complete.

In the example below, we selected 4 plates, and proceeded to download and fill out the template.

Note: See appendix for sample naming tips. Please make sure there are no duplicates, no special characters, and that all empty wells are filled with 'NA'.

Click 'Next' to proceed to the next page.

STEP TWO: Upload Template and Select Index
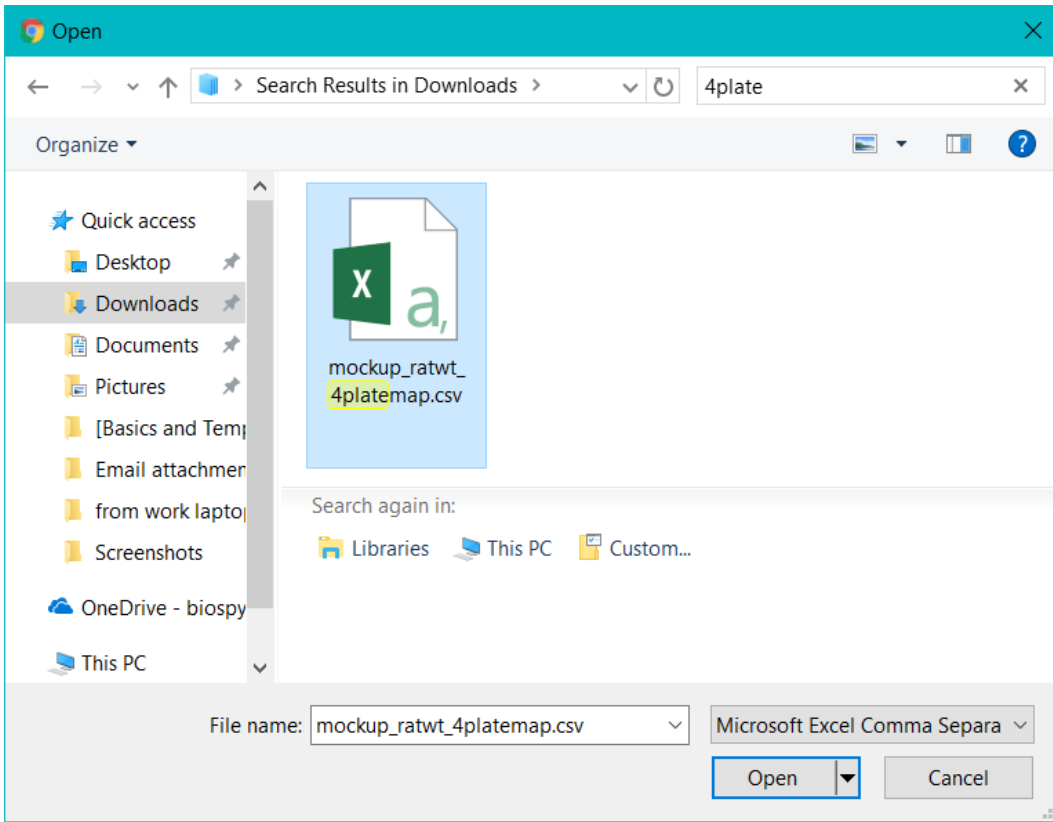
Uploading Completed Template

After saving the template as a CSV file, upload by clicking 'Browse'.
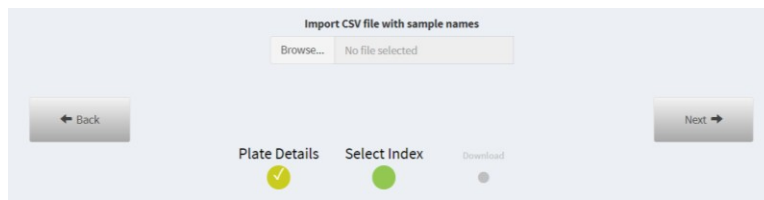
This will bring up the following window to select the completed template from user's computer.

Click 'Open' To upload.

Selecting Plate Indices

Plate 1 Index	Plate 2 Index	Plate 3 Index	Plate 4 Index
Plate M (900)	Plate N (900)	Plate O (900)	Plate P (900)

Plate 1

	R902	R911	R913	R921	R923	R935	R9
F904	RatWT_NS_01	RatWT_Kidney_01	RatWT_Liver_01	RatWT_Lung_01	RatWT_URR_01	NA	NA
F908	RatWT_NS_02	RatWT_Kidney_02	RatWT_Liver_02	RatWT_Lung_02	RatWT_URR_02	NA	NA
F927	RatWT_NS_03	RatWT_Kidney_03	RatWT_Liver_03	RatWT_Lung_03	RatWT_URR_03	NA	NA
F935	NA	NA	NA	NA	NA	NA	NA
F937	NA	NA	NA	NA	NA	NA	NA
F939	NA	NA	NA	NA	NA	NA	NA
F941	NA	NA	NA	NA	NA	NA	NA
F943	NA	NA	NA	NA	NA	NA	NA

Plate 2

	R908	R912	R920	R922	R924	R936	R938	R943	R946	R959	R986
F904	Exp01test01	Exp01test06	NA	NA	NA	NA	NA	NA	NA	NA	NA
F908	Exp01test02	Exp01test07	NA	NA	NA	NA	NA	NA	NA	NA	NA
F927	Exp01test03	Exp01test08	NA	NA	NA	NA	NA	NA	NA	NA	NA
F935	Exp01test04	Exp01test09	NA	NA	NA	NA	NA	NA	NA	NA	NA
F937	Exp01test05	Exp01test10	NA	NA	NA	NA	NA	NA	NA	NA	NA
F939	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
F941	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
F943	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Plate 3

	R902	R911	R913	R921	R923	R935	R937	R942	R945	R951	R983
F906	Exp02test01	Exp02test06	NA	NA	NA	NA	NA	NA	NA	NA	NA
F916	Exp02test02	Exp02test07	NA	NA	NA	NA	NA	NA	NA	NA	NA
F934	Exp02test03	Exp02test08	NA	NA	NA	NA	NA	NA	NA	NA	NA
F936	Exp02test04	Exp02test09	NA	NA	NA	NA	NA	NA	NA	NA	NA
F938	Exp02test05	Exp02test10	NA	NA	NA	NA	NA	NA	NA	NA	NA
F940	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
F942	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
F944	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Plate 4

	R908	R912	R920	R922	R924	R936	R938	R943	R946	R959	R986
F906	Exp03test01	Exp03test06	NA	NA	NA	NA	NA	NA	NA	NA	NA
F916	Exp03test02	Exp03test07	NA	NA	NA	NA	NA	NA	NA	NA	NA
F934	Exp03test03	Exp03test08	NA	NA	NA	NA	NA	NA	NA	NA	NA
F936	Exp03test04	Exp03test09	NA	NA	NA	NA	NA	NA	NA	NA	NA
F938	Exp03test05	Exp03test10	NA	NA	NA	NA	NA	NA	NA	NA	NA
F940	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
F942	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
F944	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

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Select an index for each plate from the dropdown menus above.

Each index selection must be unique; the same index cannot be used for more than one plate.

Click 'Next' to proceed.

Note: If using 24 or 48 sample kits, please refer to the package insert. The link on the insert will allow the user to download pre-made Sample Sheets.

STEP THREE: Download

Click download for the sequencing machine of choice; upon download, the green circle in the progress bar will be checked off.

The Sample Sheet can be used for demultiplexing sequencing data, as well as uploaded into the quality control tools to map samples to their correct plate location.

	A	B	C	D	E	F	G	H	I	J	K
1	[Header]										
2	IEMFileVe	4									
3	Investigat	BioSpyder									
4	Project Name										
5	Experiment Name										
6	Date	########									
7	Workflow	GenerateF									
8	Applicatic	NextSeq F									
9	Assay	Nextera									
10	Descriptic	NextSeq									
11	Chemistry	Amplicon									
12	[Manifests]										
13											
14	[Reads]										
15	50										
16	[Settings]										
17	CustomIn	C2									
18											
19	[Data]										
20	Sample_II	Sample_N	Sample_P	Sample_W	I7_Index_	index	I5_Index_	index2	Sample_P	Description	
21	1	RatWT_NS_01			R902	TCCTCAGC	F904	GTGCATTAG			
22	2	RatWT_NS_02			R902	TCCTCAGC	F908	AAACACCTT			
23	3	RatWT_NS_03			R902	TCCTCAGC	F927	AGCAAGGTC			
24	4	RatWT_Kidney_01			R911	GGGTTGTT	F904	GTGCATTAG			
25	5	RatWT_Kidney_02			R911	GGGTTGTT	F908	AAACACCTT			
26	6	RatWT_Kidney_03			R911	GGGTTGTT	F927	AGCAAGGTC			
27	7	RatWT_Liver_01			R913	GTTTATAC	F904	GTGCATTAG			
28	8	RatWT_Liver_02			R913	GTTTATAC	F908	AAACACCTT			
29	9	RatWT_Liver_03			R913	GTTTATAC	F927	AGCAAGGTC			
30	10	RatWT_Lung_01			R921	TCAGTACA	F904	GTGCATTAG			
31	11	RatWT_Lung_02			R921	TCAGTACA	F908	AAACACCTT			
32	12	RatWT_Lung_03			R921	TCAGTACA	F927	AGCAAGGTC			
33	13	RatWT_URR_01			R923	TGCATGAC	F904	GTGCATTAG			
34	14	RatWT_URR_02			R923	TGCATGAC	F908	AAACACCTT			
35	15	RatWT_URR_03			R923	TGCATGAC	F927	AGCAAGGTC			
36	16	Exp01test01			R908	AAGACTCT	F904	GTGCATTAG			
37	17	Exp01test02			R908	AAGACTCT	F908	AAACACCTT			
38	18	Exp01test03			R908	AAGACTCT	F927	AGCAAGGTC			
39	19	Exp01test04			R908	AAGACTCT	F935	TAACGCGTC			
40	20	Exp01test05			R908	AAGACTCT	F937	TCTAACCAT			
41	21	Exp01test06			R912	GAACTGTA	F904	GTGCATTAG			
42	22	Exp01test07			R912	GAACTGTA	F908	AAACACCTT			

_PE_NS (2)

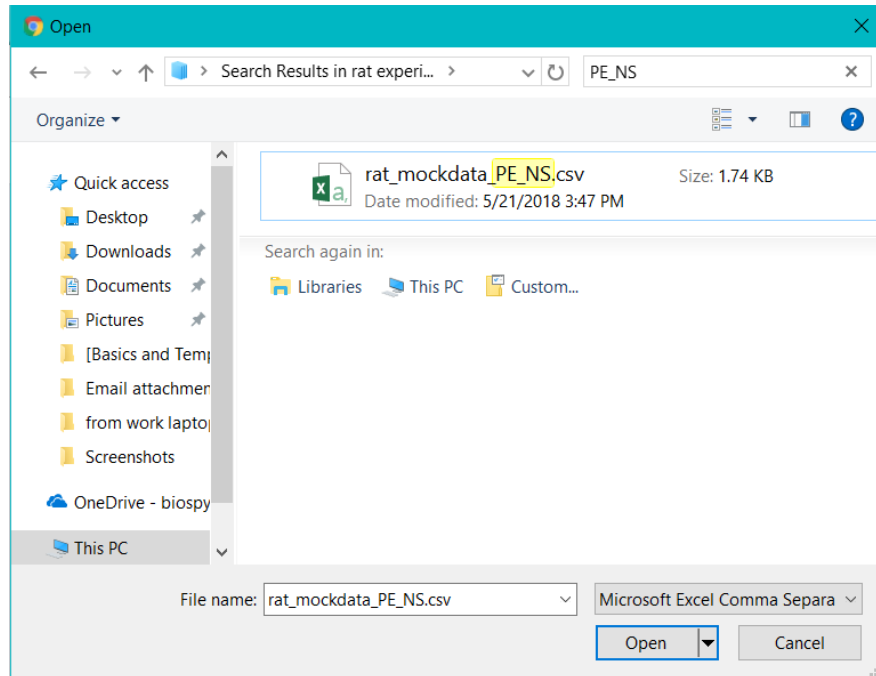**Verify Custom Sample Sheet**

Advanced User Only: BioSpyder recommends generating Sample Sheets from TempO-SeqR. If user makes manual changes to a Sample Sheet, this tool can be used to verify that all sample names and indices remain unique.
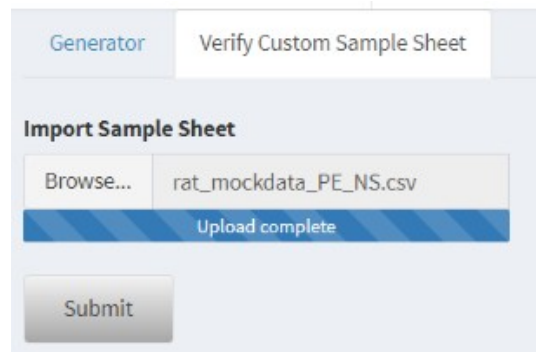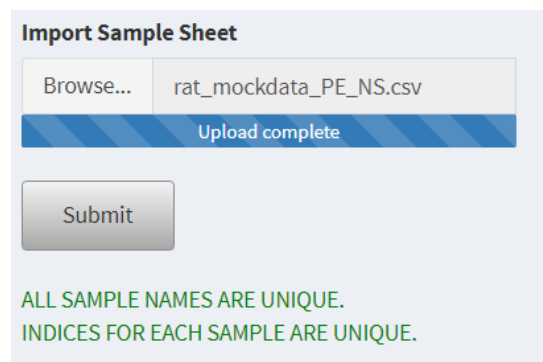

To upload a custom Sample Sheet, click "Browse". This will bring up the system dialogue for file selection.

Select the Sample Sheet of interest and click 'Open'. This will begin upload of the Sample Sheet.

Once uploaded, click 'Submit' to verify that sample names and respective indices are unique.

If sample names and/or indices are not unique, an error message will appear.

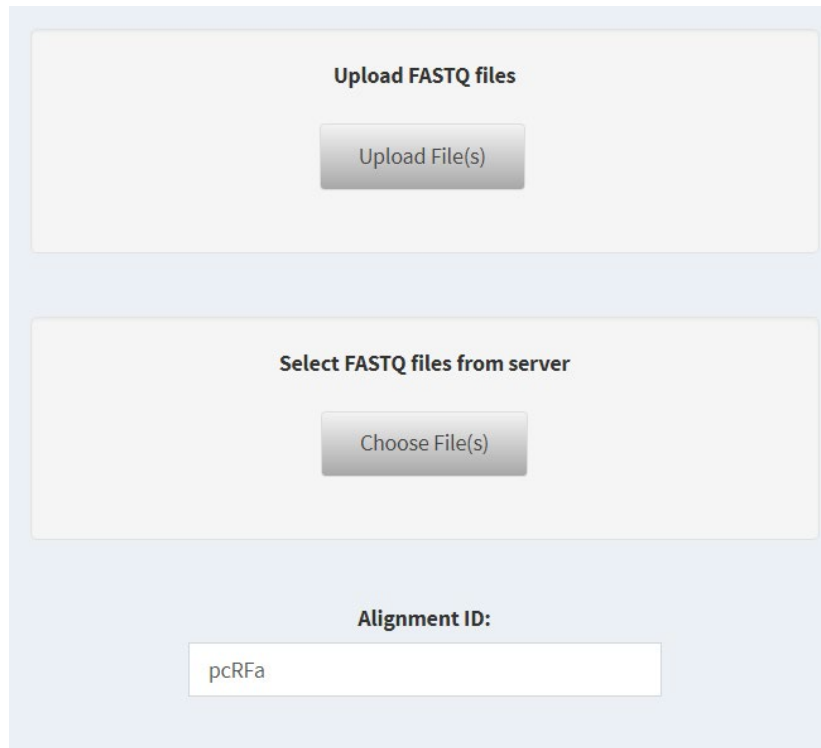ERROR:SAMPLENAMESARENOT UNIQUE. RatWT_Kidney_0I

INDICESFOR EACH SAMPLEAREUNIQUE.

Aligner

The TempO-SeqR aligner takes in FASTQ files generated from the TempO-Seq™ assay and maps them to a user-selected reference table, generating a count table at completion.

Note: If samples are split across lanes have sequencing provider concatenate FASTQ files prior to upload.

STEP ONE: Upload/Select FASTQ Files

After selecting 'Aligner' from the landing page, users will be greeted with the display below.

New FASTQ files must be uploaded from user's local drive for use in TempO-SeqR. FASTQ files will be stored on the BioSpyder server for 48 hours. Click 'Upload File(s)' to proceed.

Uploading files to the BioSpyder server

Note: Please ensure user's computer is not set to 'Sleep' or 'Hibernate' as this may cause interruption in file upload.

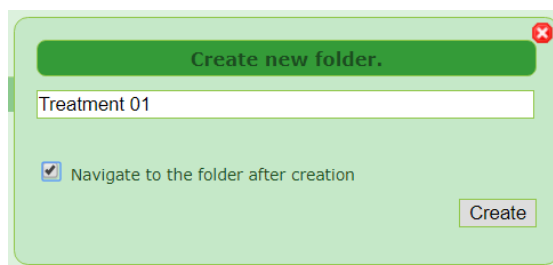Login to the BioSpyder data server using user's provided TempO-SeqR credentials.

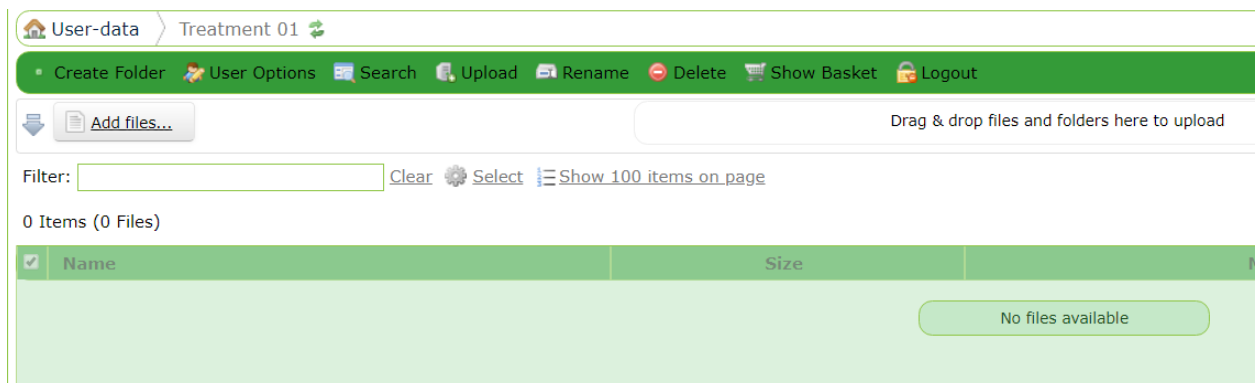Click 'Create Folder' to designate a location for user's FASTQ files.

User will be presented with the dialogue to name folder. Check the 'Navigate to the folder…' to enter the folder you create. Enter a directory name and click 'Create'.
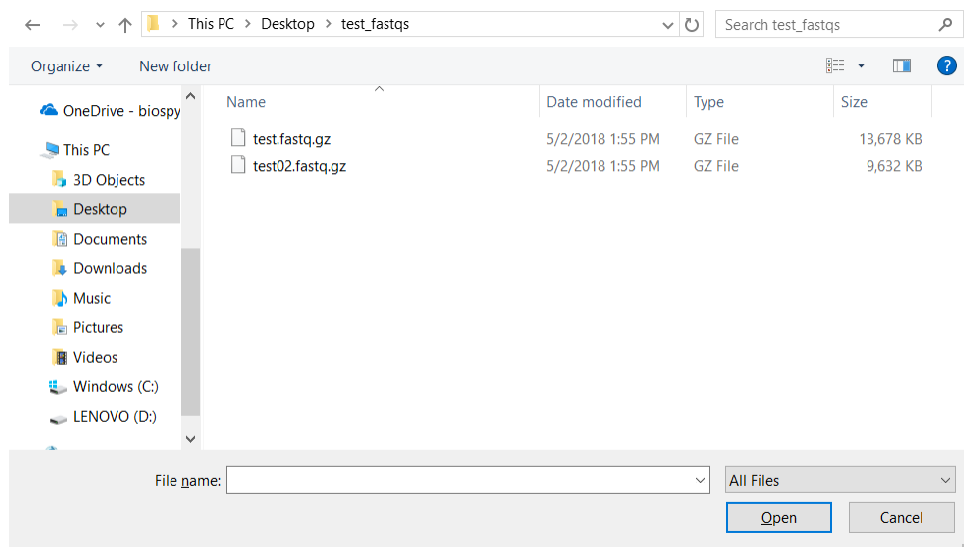

Click 'Add files' or 'Upload' to bring up a file browser.

Select one or more FASTQ files and click 'Open' to start upload of the files.

FASTQ files will upload sequentially. Upload speeds and approximate time remaining will be displayed on screen. File upload times will vary based on file size and network speed.

Resuming File Upload

In the event user's connection drops and file upload is interrupted, user may resume upload by selecting all files and clicking 'Resume All'.
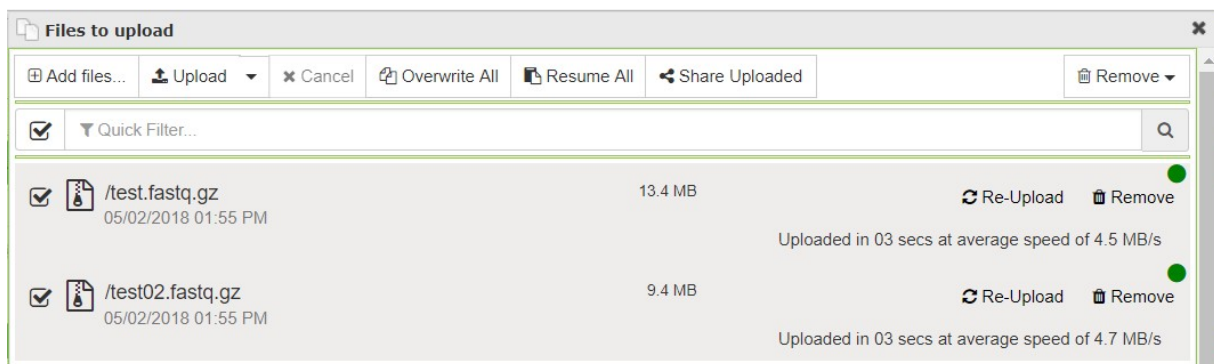
After user navigates away from the upload screen, user's uploaded files will be displayed in their repository.

Note: Files uploaded to the server will not be visible to any other users. BioSpyder uses industry standard security practices to guarantee data privacy.

Toolbar Options

- Search – Tool that allows user to quickly locate files based on file names, file sizes, and keywords.
- Rename – Update file and folder names by checking the file/folder and clicking 'Rename'.
- Delete – To remove files and folders, check the desired file/folder and click 'Delete'.

Selecting Files from Server

Once all FASTQ files are uploaded to the server, return to the TempO-SeqR webpage. Click 'Select File(s)' to choose desired FASTQ files for alignment. This will display the file browser window below.
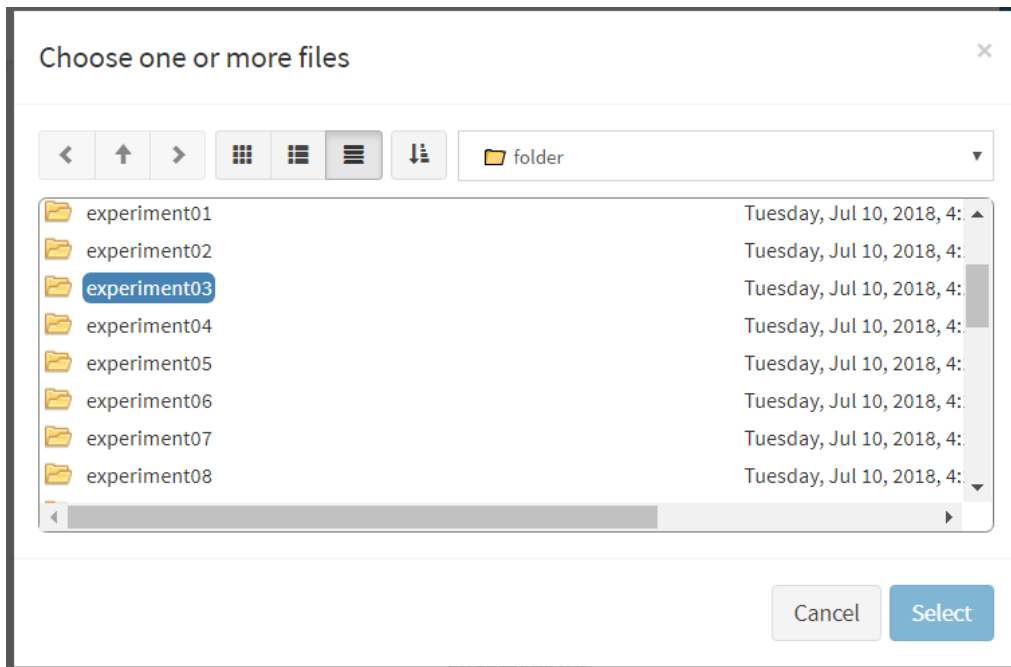

Selecting a folder will open it to show the contents.
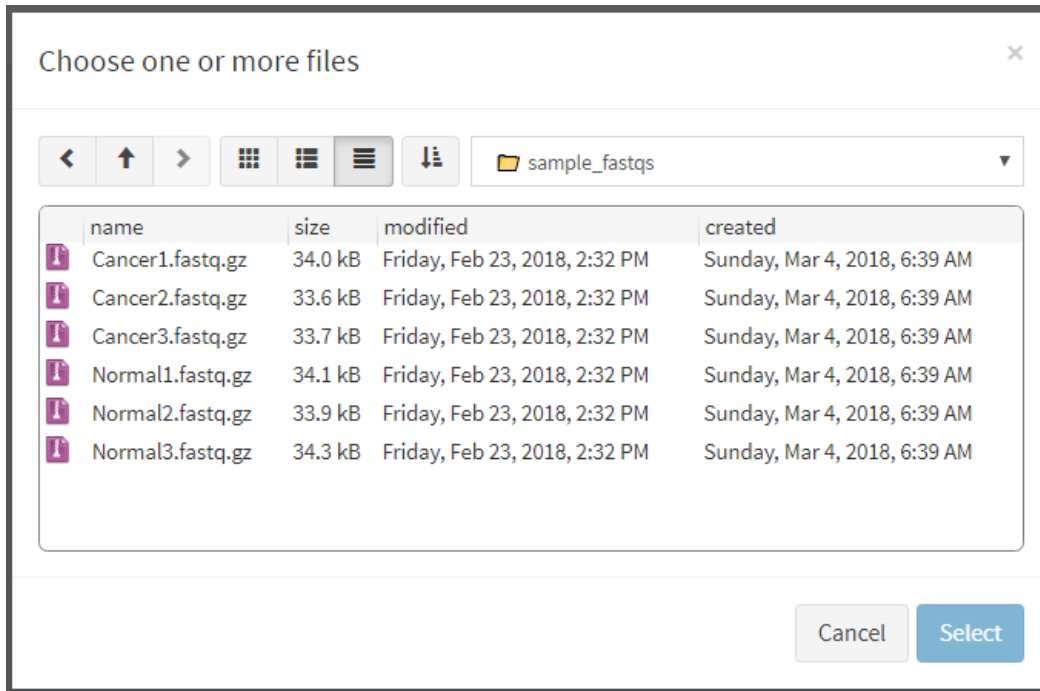
Multiple files may be selected by holding down the Shift key and clicking the first and last FASTQs of the desired grouping. Individual FASTQs may be added or removed from selection by using Ctrl+Click, which will toggle the selected file between inclusion or exclusion.

After selecting your files, click the 'Select' button, which will return you to the alignment screen and display the number of FASTQs that have been selected. If this number is incorrect, click 'Choose File(s)' again to redo your selection.
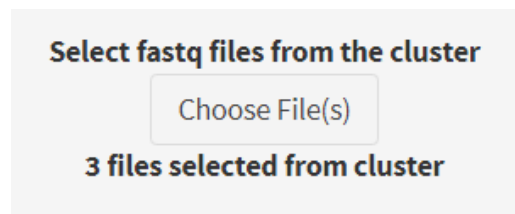
Alignment ID

After your files have been selected and uploaded, enter an appropriate alignment ID. This is the unique name associated with this particular alignment. By default, a random string is generated for the alignment ID. If FASTQs exist in a particular folder, TempO-SeqR will use the folder name as the alignment ID.

Note: Alignment IDs should not contain spaces or special characters. It is recommended to only use alphanumeric characters separated by underscores, e.g. "example_library1234". Alignment IDs are case-sensitive.

Alignment ID:

hz2yB

Click the 'Next' button to proceed to reference table selection.

STEP TWO: Reference Tables and Allowed Mismatches

Choose: Organism

Please select an option below ▼

Choose: Reference table

▼

Choose: Number of allowed mismatches

2 ▼

Select (in the following order) an organism, reference table, and number of allowed mismatches.

Note: The number of allowed mismatches is deducted from a read length of 50 bases. Default value is 2 mismatches. If the user selects 2 mismatches, sequences with 48, 49, or 50 matching bases to a probe sequence in the reference table will all map to that specific probe. Probes with 47 or less matching base pairs will not map to a probe.

Click the 'Next' button to proceed to the next page.

STEP THREE: Review and Submit

Reference Table: TempO-Seq Rat S1500 Surrogate
Mismatches: 2
Number of FASTQs Selected: 3
Alignment ID: testing96
Account Email Address: k▮▮▮▮▮▮▮▮▮▮

Normal1.fastq.gz
Normal2.fastq.gz
Normal3.fastq.gz

Additional email address:

The final page will display the selected reference table, the number of mismatches, number of FASTQs selected, alignment ID, and the email address associated with the user's account. A list of the selected FASTQ file names will also be displayed as an additional checkpoint for the user.

If alignment results need to be sent to other users, please enter their e-mail addresses (separated by commas) in the indicated field. Click 'Run Analysis' to begin Alignment. The user will be required to make an additional confirmation before alignment proceeds.

Upon submitting, the alignment will begin and the user will be returned to be landing page. Alignment status can be tracked from the landing page, while results will be sent by email.

Status on the landing page will display "Complete" upon successful alignment. If an alignment fails, troubleshooting options are explored at the end of this user guide.

Accessing Alignment Data

TempO-SeqR quality control tools can be used on a completed alignment, or a gene count table can be uploaded directly.

Select a Completed Alignment from the Landing Page

Alignment History

Alignment ID	Status	Date/Time Submitted	Reference Table	Reference Organism	Allowed Mismatches
testing96	Complete	2018-06-14 15:25:40	TempO-Seq Rat S1500 Surrogate	Rattus Norvegicus	2

If status is 'Complete', clicking the alignment ID will import the count table and take the user directly to quality control tools.

Uploading Alignment Results

If uploading a count table, click "Upload Documents" from the menu bar (shown below) in the upper right hand corner of the screen.

This will bring up options to upload count table and Sample Sheet. Sample Sheet upload is optional, but recommended for ease of identifying and grouping samples as they were placed in the plate.

Upload a count table by either selecting one that exists on the server through the dropdown menu, or clicking "Browse" under "Upload Count Table from Local". A green checkmark will appear when upload is successful; it will take a few seconds for samples to load.

The user may now optionally upload a Sample Sheet; if upload(s) succeed, samples will be previewed on screen.

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Upload Count Table and Sample Sheet

	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10
F1	CtBrain01	CtBrain02	CtURR01	CtURR02	CtNoSample01	CtNoSample02	Exp01S01	Exp01S02	Exp01S03	Exp0
F2	Exp01S07	Exp01S08	Exp01S09	Exp01S10	Exp01S11	Exp01S12	Exp01S13	Exp01S14	Exp01S15	Exp0
F3	Exp01S19	Exp01S20	Exp01S21	Exp01S22	Exp01S23	Exp01S24	Exp01S25	Exp01S26	Exp01S27	Exp0
F4	Exp01S31	Exp01S32	Exp01S33	Exp01S34	Exp01S35	Exp01S36	Exp01S37	Exp01S38	Exp01S39	Exp0
F5	Exp01S43	Exp01S44	Exp01S45	Exp01S46	Exp01S47	Exp01S48	Exp01S49	Exp01S50	Exp01S51	Exp0
F6	Exp01S55	Exp01S56	Exp01S57	Exp01S58	Exp01S59	Exp01S60	Exp01S61	Exp01S62	Exp01S63	Exp0
F7	Exp01S67	Exp01S68	Exp01S69	Exp01S70	Exp01S71	Exp01S72	Exp01S73	Exp01S74	Exp01S75	Exp0
F8	Exp01S79	Exp01S80	Exp01S81	Exp01S82	Exp01S83	Exp01S84	Exp01S85	Exp01S86	Exp01S87	Exp0

✓

Import Count Table from Server

-- OR --

Upload Count Table from Local

Browse...   mockup_96sample_test_names_05.csv

Upload complete

✓

Import Sample Sheet from Server

-- OR --

Upload Sample Sheet from Local

Browse...   mockup_96sample_SR_HS_test05.csv

Upload complete

Main Menu   Clear Documents   Next →

Click 'Next' to proceed to quality control tools. Click 'Clear Documents' if the incorrect file has been uploaded.

Quality Control Tools

Tools may be accessed from the tool bar after clicking 'Quality Control Tools' from the landing page.

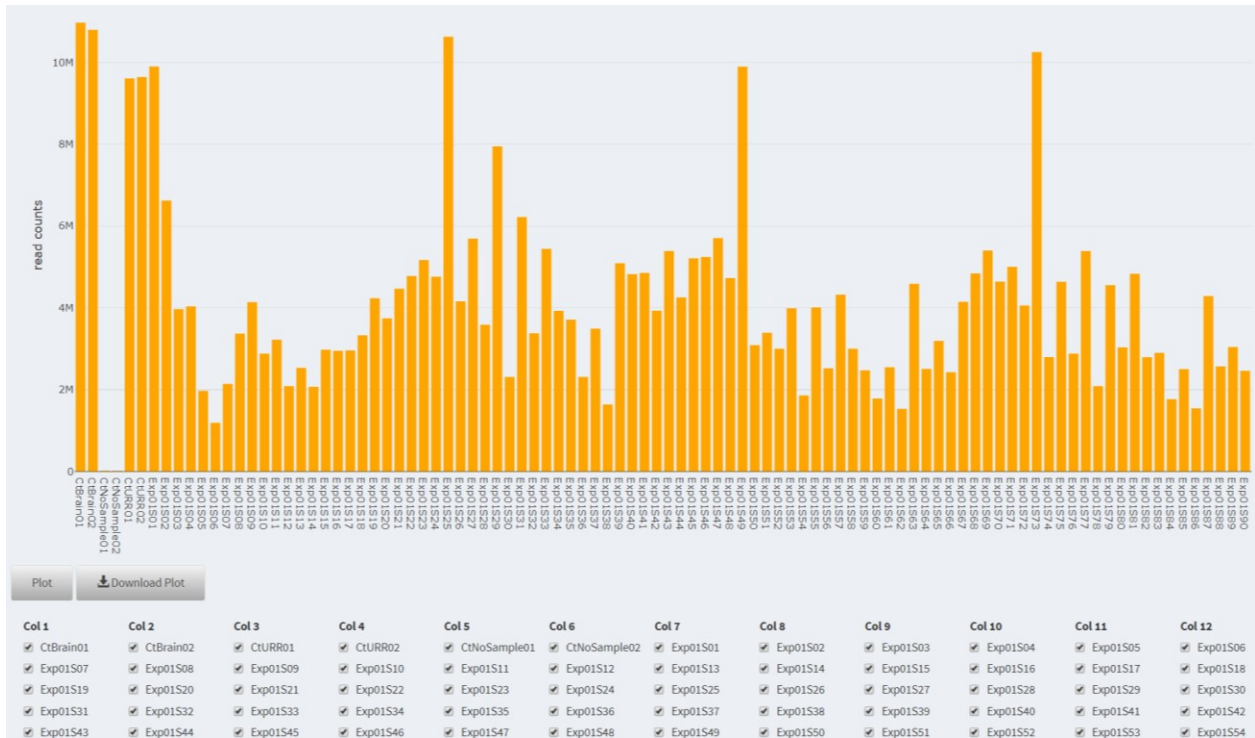| Barplot | Count Table | Statistics | Dendrogram | Scatterplot | PCA |
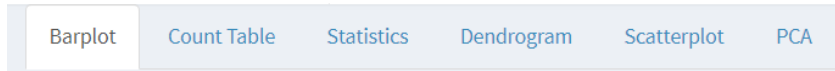
Note: Long and complex sample names may make using these tools difficult. For a better experience, add a short (<15 characters) and unique section separated by an underscore to sample names (i.e. Treatment50uMChemical24hrs → T50uM24_ Treatment50uMChemical24hrs).
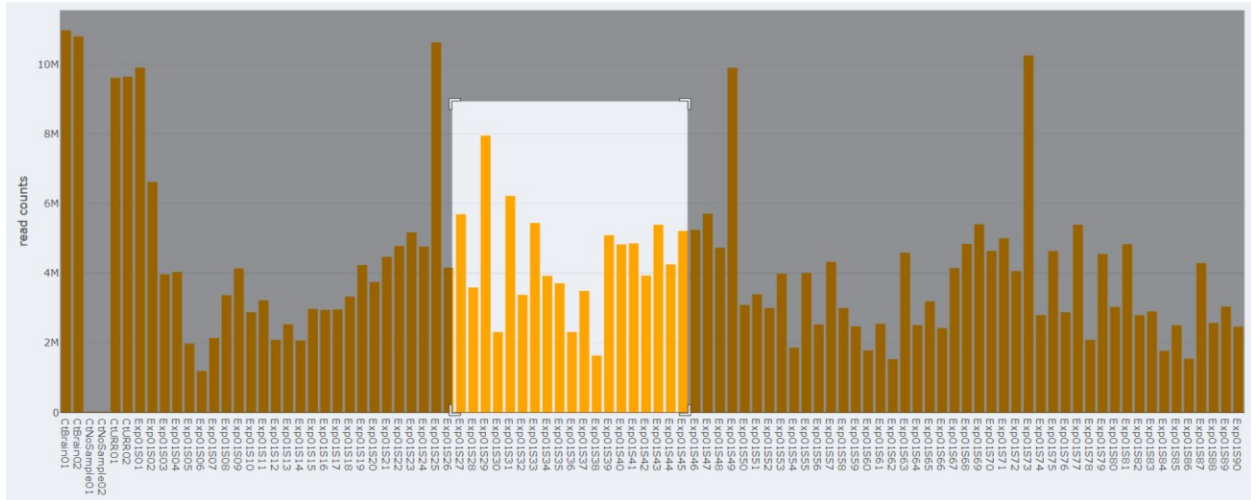
Barplot

The barplot displays the number of reads mapped per sample.

By default, all samples will be plotted automatically when the user views the barplot.

Col 1	Col 2	Col 3	Col 4	Col 5	Col 6	Col 7	Col 8	Col 9	Col 10	Col 11	Col 12
☑ CtBrain01	☑ CtBrain02	☑ CtURR01	☑ CtURR02	☑ CtNoSample01	☑ CtNoSample02	☑ Exp01S01	☑ Exp01S02	☑ Exp01S03	☑ Exp01S04	☑ Exp01S05	☑ Exp01S06
☑ Exp01S07	☑ Exp01S08	☑ Exp01S09	☑ Exp01S10	☑ Exp01S11	☑ Exp01S12	☑ Exp01S13	☑ Exp01S14	☑ Exp01S15	☑ Exp01S16	☑ Exp01S17	☑ Exp01S18
☑ Exp01S19	☑ Exp01S20	☑ Exp01S21	☑ Exp01S22	☑ Exp01S23	☑ Exp01S24	☑ Exp01S25	☑ Exp01S26	☑ Exp01S27	☑ Exp01S28	☑ Exp01S29	☑ Exp01S30
☑ Exp01S31	☑ Exp01S32	☑ Exp01S33	☑ Exp01S34	☑ Exp01S35	☑ Exp01S36	☑ Exp01S37	☑ Exp01S38	☑ Exp01S39	☑ Exp01S40	☑ Exp01S41	☑ Exp01S42
☑ Exp01S43	☑ Exp01S44	☑ Exp01S45	☑ Exp01S46	☑ Exp01S47	☑ Exp01S48	☑ Exp01S49	☑ Exp01S50	☑ Exp01S51	☑ Exp01S52	☑ Exp01S53	☑ Exp01S54

Zoom

To select a smaller subset of the plotted data, click on the plot itself, hold down the mouse button, and drag to select a subset of data.

Releasing the mouse button will change the view to the user's selection.
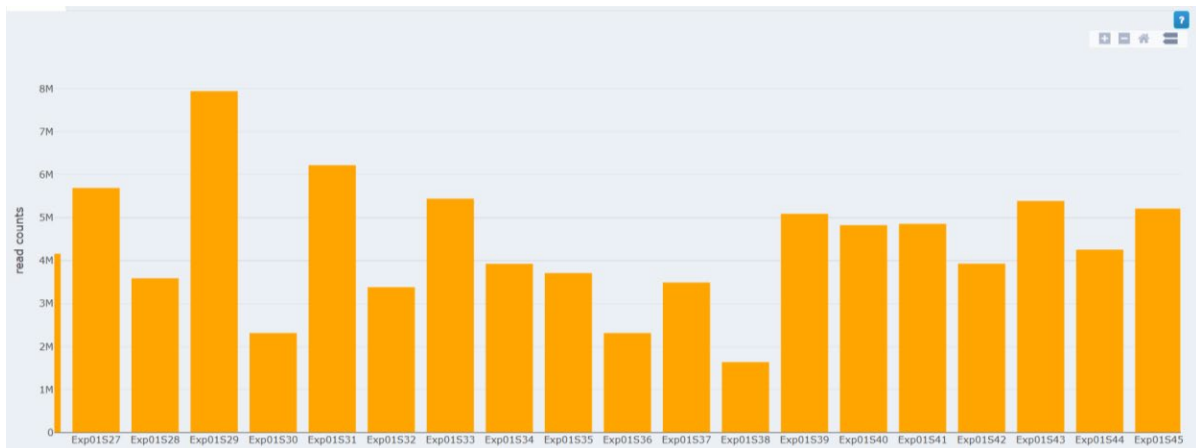
To zoom back out, double click in the area of the plot. The user may also zoom in further by repeating the above steps.

In the upper right hand portion of the plot, the above toolbar will be visible. Clicking the + or - symbols will zoom the user in or out, respectively. The home button will allow the user to return to the default view. The ⬛ symbol shows that on cursor hover, the total mapped read counts for that sample will be displayed at the top of the bar (possible units are M: millions or K: thousands), as shown below.

Sample Selection

Individual samples can be selected by clicking the corresponding checkbox.

To select or deselect multiple samples, click in the area away from the samples, hold down the mouse button, and drag to draw a box with a dashed outline.

The box size can be made as large or as small as the user desires by moving the mouse without releasing. Releasing the mouse will cause all samples in the area of the box to invert their state, so samples that were checked will no longer be checked and vice versa.

Col 5	Col 6	Col 7	Col 8	Col 9	Col 10
☑ CtNoSample01	☑ CtNoSample02	☑ Exp01S01	☑ Exp01S02	☑ Exp01S03	☑ Exp01S04
☐ Exp01S11	☐ Exp01S12	☐ Exp01S13	☐ Exp01S14	☐ Exp01S15	☐ Exp01S16
☐ Exp01S23	☐ Exp01S24	☐ Exp01S25	☑ Exp01S26	☑ Exp01S27	☐ Exp01S28
☐ Exp01S35	☐ Exp01S36	☐ Exp01S37	☑ Exp01S38	☑ Exp01S39	☐ Exp01S40
☐ Exp01S47	☑ Exp01S48	☑ Exp01S49	☐ Exp01S50	☐ Exp01S51	☐ Exp01S52
☐ Exp01S59	☑ Exp01S60	☑ Exp01S61	☐ Exp01S62	☐ Exp01S63	☐ Exp01S64
☐ Exp01S71	☐ Exp01S72	☐ Exp01S73	☐ Exp01S74	☐ Exp01S75	☐ Exp01S76
☐ Exp01S83	☐ Exp01S84	☐ Exp01S85	☐ Exp01S86	☐ Exp01S87	☐ Exp01S88

Download Plot

To download the plot displayed, click 'Download Plot'. Plot images may be downloaded as PNG or SVG files. A description of the differences between these filetypes is listed in the appendix.

Choose Filetype for download

Filetype

◉ PNG ○ SVG

Close   ⬇ Download

Count Table

The count table is the result of alignment of a set of FASTQs to a reference table. This displays the amount of reads mapped to a particular probe, from each individual sample. The number of probes displayed onscreen at any given time can be changed from the dropdown menu in the upper left hand corner of the tool ('Show … entries').

To view a particular sample, click and drag the scroll bar at the bottom of the table to navigate to that sample.

Sorting Data

To sort a column from least counts to greatest, or vice versa, click on the sample name at the top of the column. The ⬍ symbol next to the sample name will toggle to ▲ (sorting least to greatest) or ▼ (sorting greatest to least).

Default View		Sorted Ascending		Sorted Descending	
Genes ⬍	CtBrain01 ⬍	Genes ⬍	CtBrain01 ▲	Genes ⬍	CtBrain01 ▼
NAA50_4434	35	TOP2A_7277	0	MTRNR2L12_33913	365004
P4HB_4890	5	TREH_7332	0	PLP1_16916	182110
PABPN1_4895	5	AURKB_587	0	SLC1A2_24387	69100
TGFB3_7059	9	B4GALT1_603	0	PLP1_5207	67982
TMEM230_7197	740	BZW2_805	0	ATP1B1_527	57087

Page Navigation

To view the read counts for additional probes in the count table, scroll to the bottom of the screen. In the bottom right hand corner will be a listing of the number of pages, where the user can navigate by clicking 'Previous', 'Next', or a particular page number.

AKT1_210	291	240	171	265	1	9	258	511	235	253	84	73	48	154
AKT2_211	41	46	66	67	0	0	80	65	48	4	0	1	14	3
ALDH18A1_218	10	34	65	24	0	0	49	72	18	35	8	9	2	29

Showing 1 to 25 of 20,876 entries   Previous 1 2 3 4 5 … 836 Next

Search Function

To find a particular gene or probe of interest, type in the gene symbol or probe ID in the 'Search' function in the upper right hand corner of the tool. The search will automatically commence, and the word 'Processing' will be displayed on screen while searching occurs.

Show 25 ▼ entries									Search: RACK1
Genes	Hs_Brain_Control_01	Hs_Brain_Control_02	Hs_URR_Control_01	Hs_URR_Control_02	Hs_NS_Control_01	Hs_NS_Control_02	Hs_Treatment_001	Hs_Treatment_002	Hs_Treatme
RACK1_2708	2626	2597	6282	6689	11	5	5948	8740	7552

Showing 1 to 1 of 1 entries (filtered from 20,876 total entries)   Previous 1 Next

Download Data

The user can download the count table by clicking the 'Download Count Table' button in the upper left hand corner of the screen.

Sample Statistics

The sample statistics tab displays the total mapped reads, average reads per probe, and sample type.

The user can denote positive controls and negative controls from the respective dropdown menus.

These statistics can be downloaded as a CSV by the user using the 'Download Sample Statistics' button in the upper left-hand corner.

Dendrogram

Dendrogram shows clustering of samples based on similarity. Samples with low total read counts (<1000 reads) are not shown in the dendrogram. A warning will be shown if a sample with low read counts is selected but not displayed.

By default, all samples will be plotted when the user views the dendrogram.
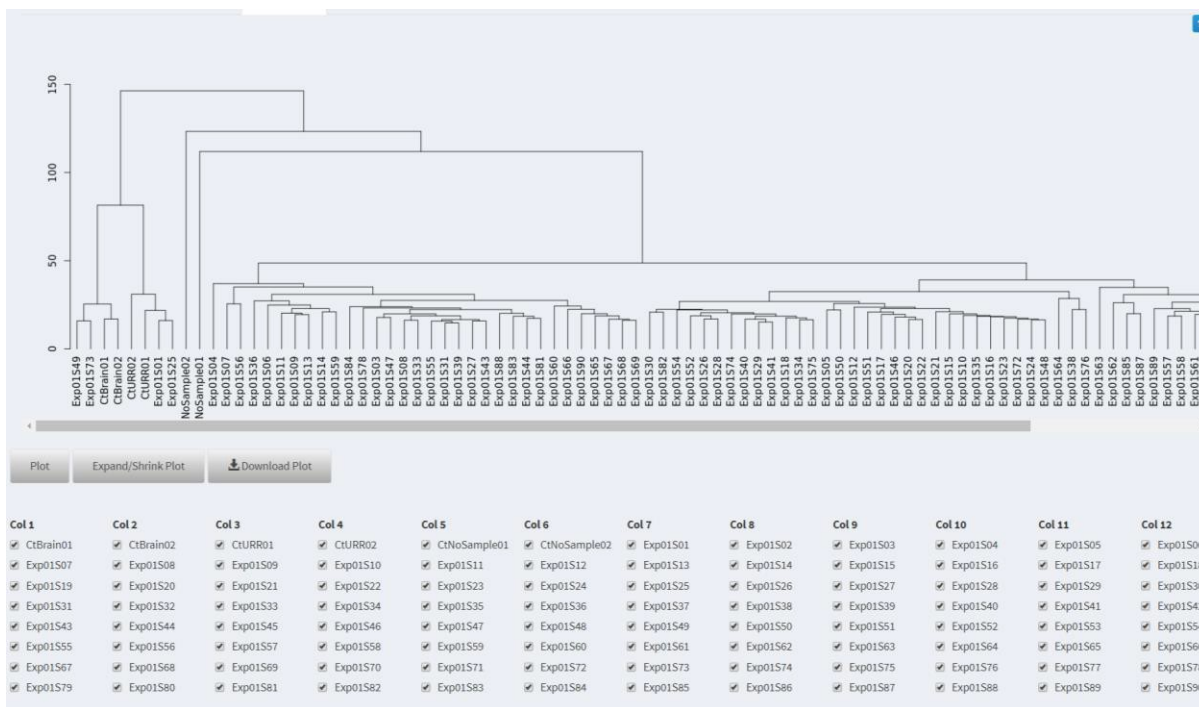
Zoom

To select a smaller subset of the plotted data, click on the plot itself, hold down the left mouse button, and drag to select a subset of data. A blue box will appear. Release the mouse to finalize the selection.
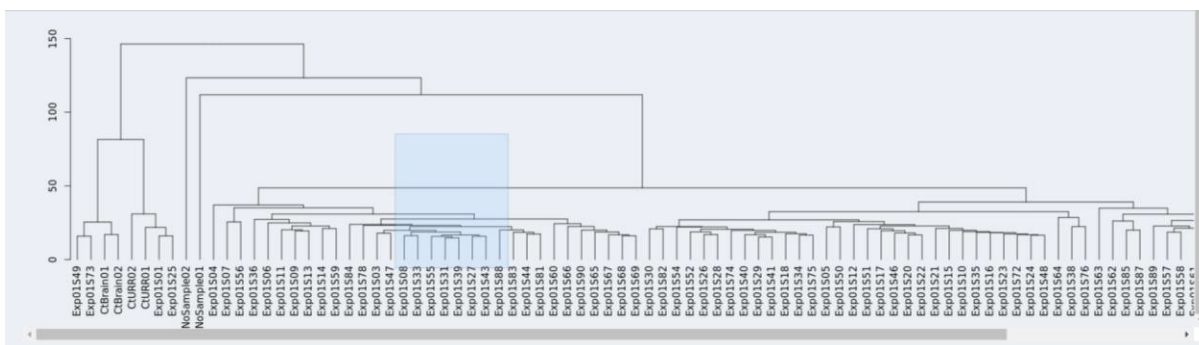
To zoom in on this selection of data, double-click inside the blue box.

If the wrong selection has been made, reset the selection, by clicking outside of the blue box.
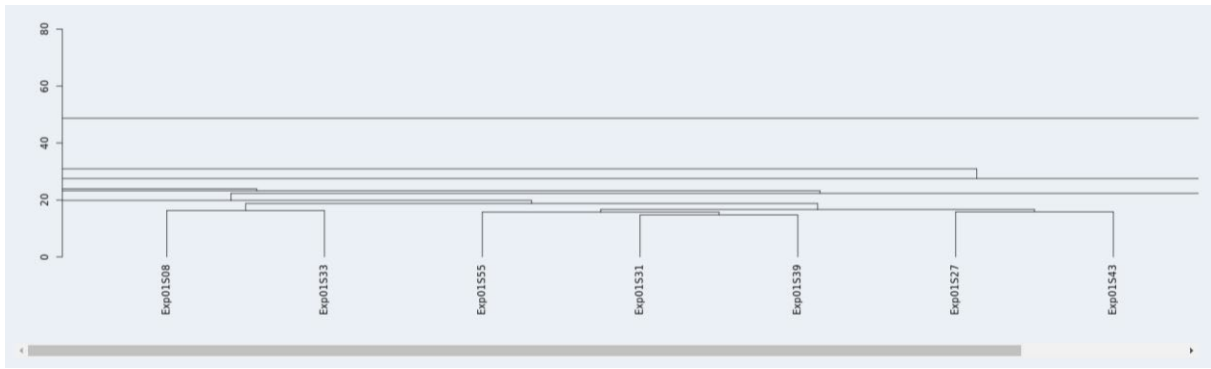
Expand/Shrink Plot

As the branches of the dendrogram may be closely packed and indistinct, the user can click 'Expand Plot' to enlarge the spacing between samples.
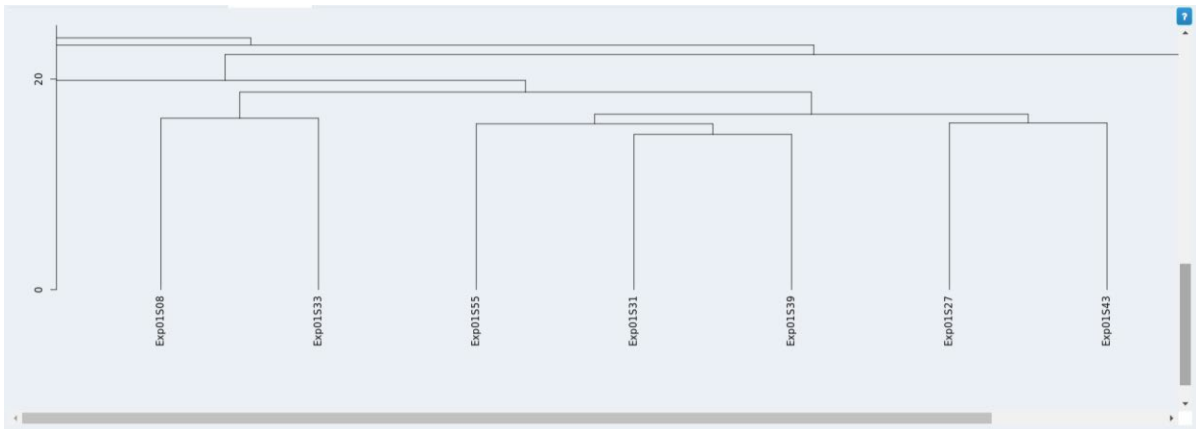
To select fewer samples for display, the user can individually click in the checkbox next to each sample to select and deselect the sample. If the checkbox is marked, the sample will be included in the plot. Replot by clicking the 'Plot' button after changing the selection of samples.
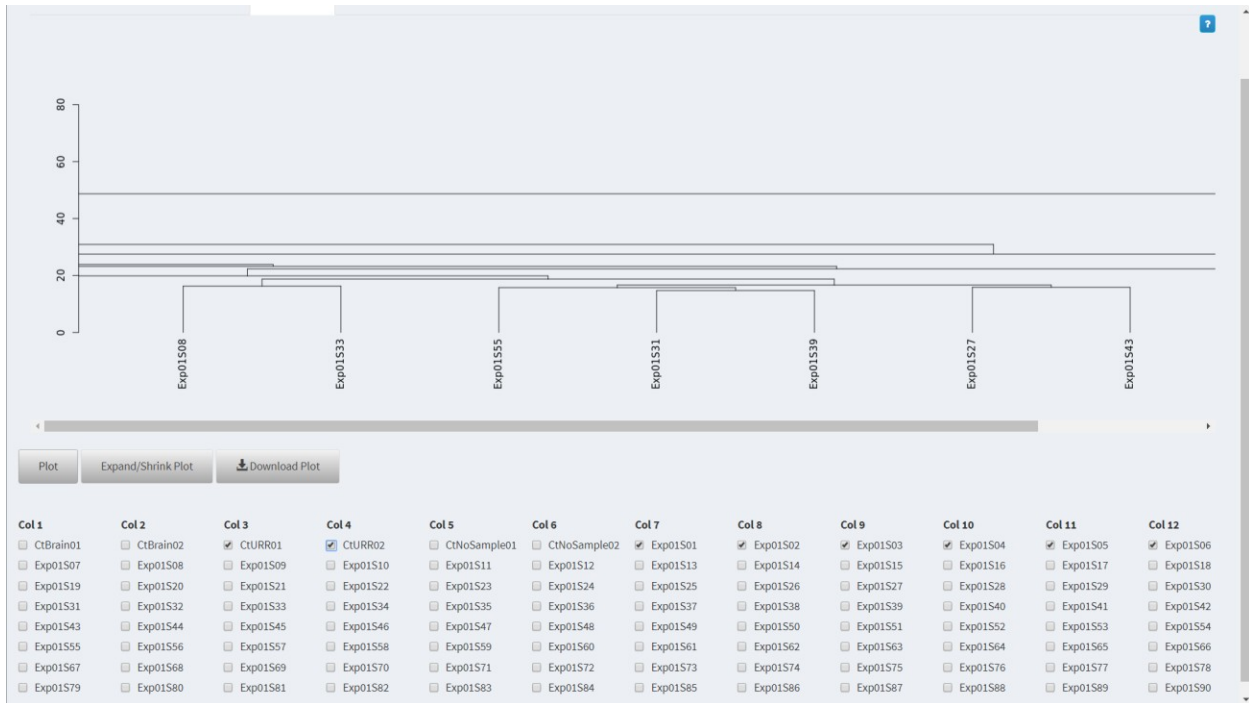
Next, click the 'Plot' button to plot the new selection. The new plot will now be displayed in the plot area.

Note: The 'Plot' button should be used every time the user is changing their sample selection, or else no change will appear on screen.

Sample Selection

Individual samples can be selected by clicking the corresponding checkbox.

To select or deselect multiple samples, click in the area away from the samples, hold down the mouse button, and drag to draw a box with a dashed outline.

The box size can be made as large or as small as the user desires by moving the mouse without releasing. Releasing the mouse will cause all samples in the area of the box to invert their state, so samples that were checked will no longer be checked and vice versa.

Col 5	Col 6	Col 7	Col 8	Col 9	Col 10
☑ CtNoSample01	☑ CtNoSample02	☑ Exp01S01	☑ Exp01S02	☑ Exp01S03	☑ Exp01S04
☐ Exp01S11	☐ Exp01S12	☐ Exp01S13	☐ Exp01S14	☐ Exp01S15	☐ Exp01S16
☐ Exp01S23	☐ Exp01S24	☐ Exp01S25	☑ Exp01S26	☑ Exp01S27	☐ Exp01S28
☐ Exp01S35	☐ Exp01S36	☐ Exp01S37	☑ Exp01S38	☑ Exp01S39	☐ Exp01S40
☐ Exp01S47	☑ Exp01S48	☑ Exp01S49	☐ Exp01S50	☐ Exp01S51	☐ Exp01S52
☐ Exp01S59	☑ Exp01S60	☑ Exp01S61	☐ Exp01S62	☐ Exp01S63	☐ Exp01S64
☐ Exp01S71	☐ Exp01S72	☐ Exp01S73	☐ Exp01S74	☐ Exp01S75	☐ Exp01S76
☐ Exp01S83	☐ Exp01S84	☐ Exp01S85	☐ Exp01S86	☐ Exp01S87	☐ Exp01S88

Download Plot

To download the plot displayed, click 'Download Plot'. Plot images may be downloaded as PNG or SVG files. A description of the differences between these filetypes is listed in the appendix.

Choose Filetype for download

Filetype
◉ PNG   ◯ SVG

Close   ⬇ Download

Scatterplot

The scatterplot shows the degree of correlation between two samples.
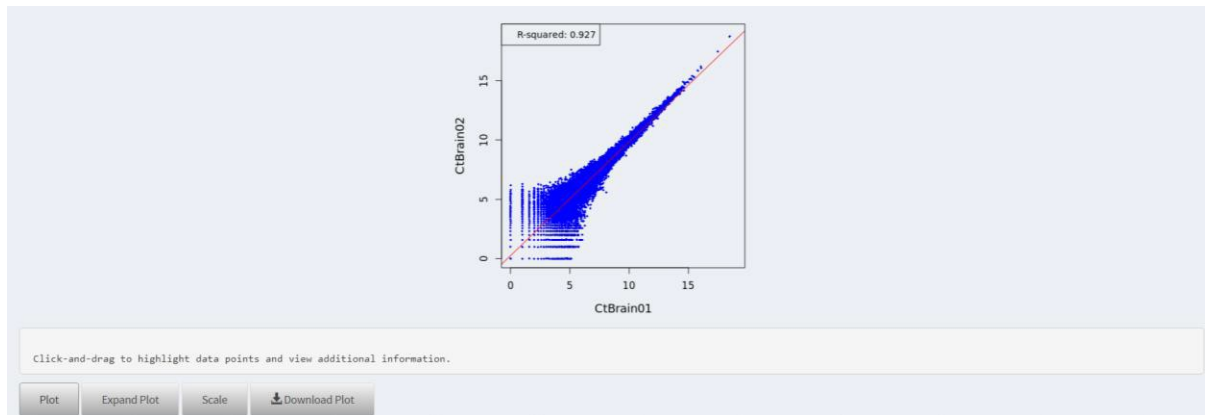
Plotting Samples

By default, no samples are selected.

Users may check the boxes next to two samples. If the checkbox is marked, the sample will be included in the plot. Re-plot by clicking the 'Plot' button after changing the selection of samples.

View Data

To select a smaller subset of the plotted data, click on the plot itself, hold down the mouse button, and drag to select a subset of data. A blue box will appear. Release the mouse to finalize the selection.

The gene names and values for the selected data will be displayed in the box below.
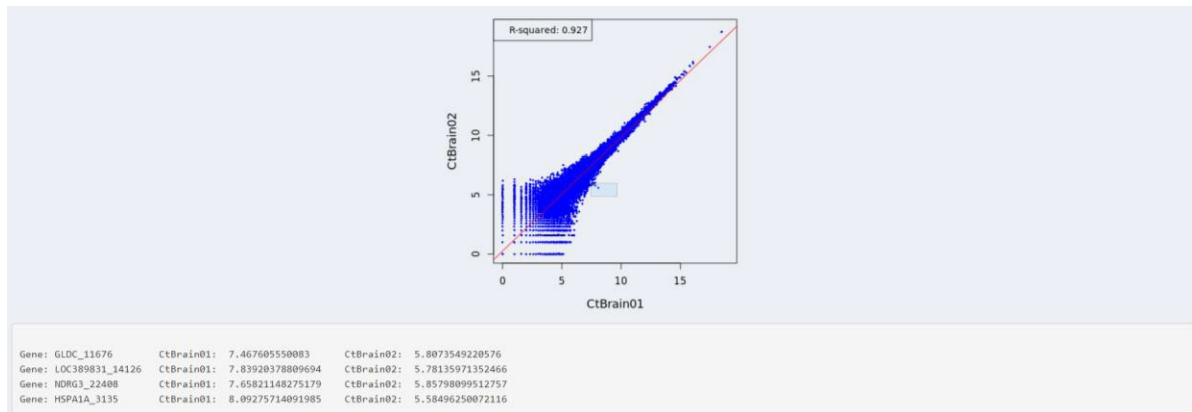
If the wrong selection has been made, reset the selection by clicking outside of the blue box.

Changing Plot Scale
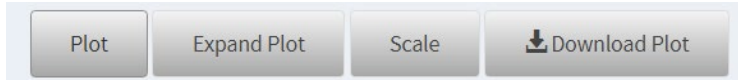
To plot samples using a different axis scale, click 'Scale'. This will allow the user to pick between log2, log10, or linear scales.
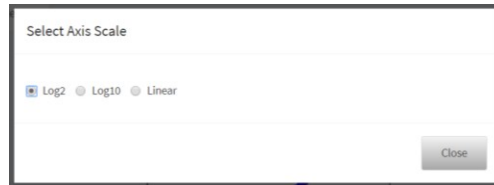
After making a selection, click 'Close' and then 'Plot'. Here, 'Linear' is selected and replotted for the same two samples shown above.
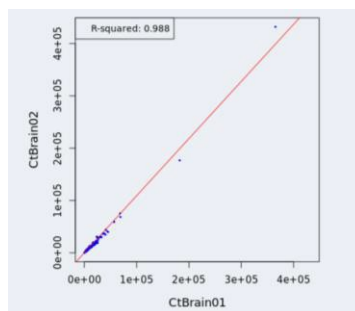
Expand Plot

To view the data at a larger view, click 'Expand Plot' in order to enlarge the plot to the size of the browser window.
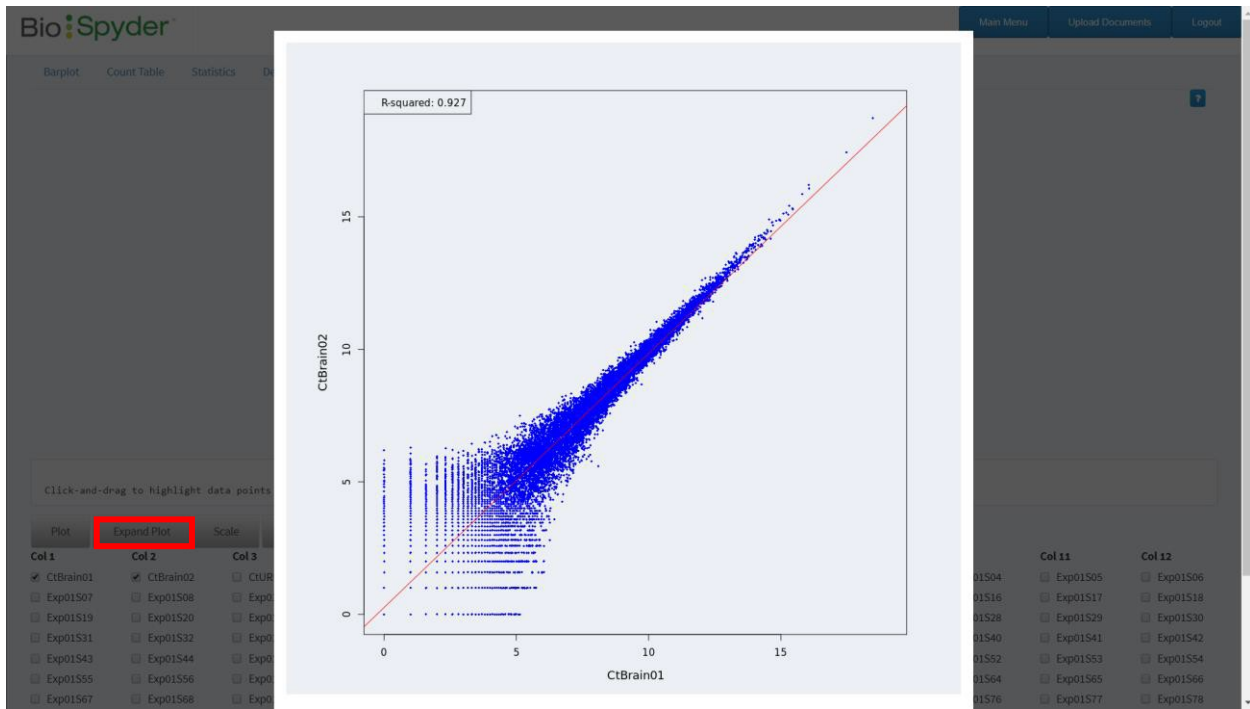
As with the smaller plot, selecting samples by clicking-and-dragging will display the values for the sub-selection of data, which can also be downloaded as a CSV file.

Sample Selection

Individual samples can be selected by clicking the corresponding checkbox.

To select or deselect multiple samples, click in the area away from the samples, hold down the mouse button, and drag to draw a box with a dashed outline.

Col 5	Col 6	Col 7	Col 8	Col 9	Col 10
✔ CtNoSample01	✔ CtNoSample02	✔ Exp01S01	✔ Exp01S02	✔ Exp01S03	✔ Exp01S04
☐ Exp01S11	☐ Exp01S12	☐ Exp01S13	☐ Exp01S14	☐ Exp01S15	☐ Exp01S16
☐ Exp01S23	✔ Exp01S24	✔ Exp01S25	☐ Exp01S26	☐ Exp01S27	☐ Exp01S28
☐ Exp01S35	✔ Exp01S36	✔ Exp01S37	☐ Exp01S38	☐ Exp01S39	☐ Exp01S40
☐ Exp01S47	☐ Exp01S48	☐ Exp01S49	✔ Exp01S50	✔ Exp01S51	☐ Exp01S52
☐ Exp01S59	☐ Exp01S60	☐ Exp01S61	✔ Exp01S62	✔ Exp01S63	☐ Exp01S64
☐ Exp01S71	☐ Exp01S72	☐ Exp01S73	☐ Exp01S74	☐ Exp01S75	☐ Exp01S76
☐ Exp01S83	☐ Exp01S84	☐ Exp01S85	☐ Exp01S86	☐ Exp01S87	☐ Exp01S88

The box size can be made as large or as small as the user desires by moving the mouse without releasing. Releasing the mouse will cause all samples in the area of the box to invert their state, so samples that were checked will no longer be checked and vice versa.

Col 5	Col 6	Col 7	Col 8	Col 9	Col 10
✔ CtNoSample01	✔ CtNoSample02	✔ Exp01S01	✔ Exp01S02	✔ Exp01S03	✔ Exp01S04
☐ Exp01S11	☐ Exp01S12	☐ Exp01S13	☐ Exp01S14	☐ Exp01S15	☐ Exp01S16
☐ Exp01S23	☐ Exp01S24	☐ Exp01S25	✔ Exp01S26	✔ Exp01S27	☐ Exp01S28
☐ Exp01S35	☐ Exp01S36	☐ Exp01S37	✔ Exp01S38	✔ Exp01S39	☐ Exp01S40
☐ Exp01S47	✔ Exp01S48	✔ Exp01S49	☐ Exp01S50	☐ Exp01S51	☐ Exp01S52
☐ Exp01S59	✔ Exp01S60	✔ Exp01S61	☐ Exp01S62	☐ Exp01S63	☐ Exp01S64
☐ Exp01S71	☐ Exp01S72	☐ Exp01S73	☐ Exp01S74	☐ Exp01S75	☐ Exp01S76
☐ Exp01S83	☐ Exp01S84	☐ Exp01S85	☐ Exp01S86	☐ Exp01S87	☐ Exp01S88

Click plot to display the scatterplot. By default, the correlation between the log2 of both samples will be plotted.

Download Plot

To download the plot displayed, click 'Download Plot' and select a filetype, as shown below. A description of the differences between these filetypes is listed in the appendix.

Choose Filetype for download

Filetype
◉ PNG ◯ SVG

Close   ⬇ Download

Click 'Close' to return to the normal plot.

PCA Plot

PCA shows clustering of samples based on similarity.

Plotting Samples

By default, no data is plotted until the user manually selects the sample data to plot.

To select samples for display, the user can individually click in the checkbox next to each sample. If the checkbox is marked, the sample will be included in the plot. Replot by clicking the 'Plot' button after changing the selection of samples.
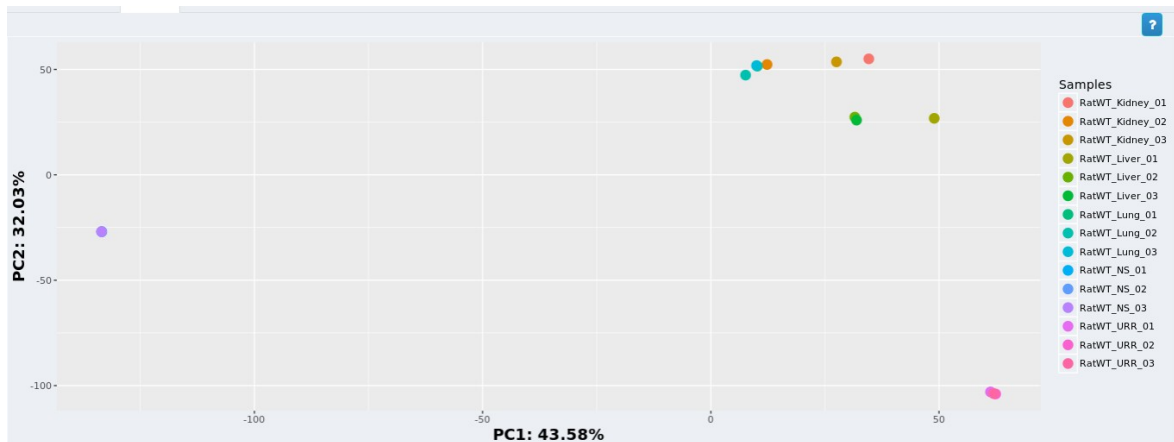
The user can change which principle components are displayed by using the selection on the left hand side.

Features
- ☑ PC1: 60.23%
- ☑ PC2: 18.27%
- ☐ PC3: 7.98%
- ☐ PC4: 6.32%
- ☐ PC5: 4.79%
- ☐ PC6: 0.88%

<u>View Data</u>

To select a smaller subset of the plotted data, click on the plot itself, hold down the mouse button, and drag to select a subset of data. A blue box will appear. Release the mouse to finalize the selection.

PCA values for the selected data will be displayed in the light grey box below.

Note: This function is not available for 3D PCA.
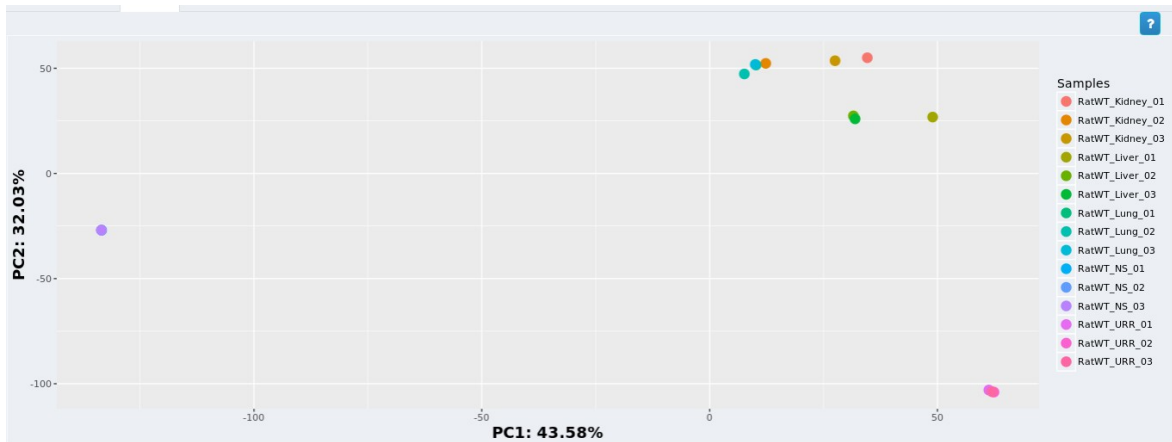
If the wrong selection has been made, reset the selection by clicking outside of the blue box.

Grouping Replicates for PCA

As shown below, all samples with the same labels are displayed with the same color. If it is desired that samples of the same type have the same color in the PCA plot, the Sample Sheet should be modified to designate sample groupings.
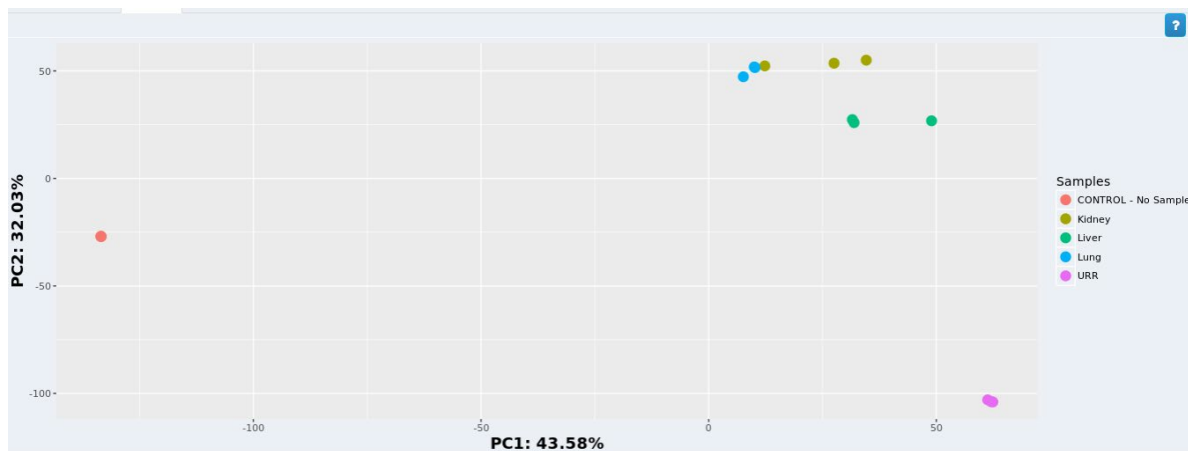
Sample Sheet Formatting

To assign samples to a group, enter group descriptors into the column titled 'Description' within the Sample Sheet. Group samples together by using the same descriptor, as shown below (all no sample controls are labeled 'CONTROL – No Sample').

	A	B	C	D	E	F	G	H	I	J	K	L
1	[Header]											
2	IEMFileVe	4										
3	Investigat	BioSpyder										
4	Project Na	rat_mockd										
5	Experimer	rat_mockd										
6	Date	########										
7	Workflow	Generatel										
8	Applicatic	NextSeq F										
9	Assay	Nextera										
10	Descriptic	NextSeq										
11	Chemistry	Amplicon										
12	[Manifests]											
13												
14	[Reads]											
15	50											
16	[Settings]											
17	CustomIn	C2										
18												
19	[Data]											
20	Sample_II	Sample_N	Sample_P	Sample_V	I7_Index_	index	I5_Index_	index2	Sample_P	Description		
21	1	RatWT_NS_01			R927	GTGATCTC	F933	ACGACTTGG		CONTROL - No Sample		
22	2	RatWT_NS_02			R927	GTGATCTC	F935	TAACGCGTC		CONTROL - No Sample		
23	3	RatWT_NS_03			R927	GTGATCTC	F937	TCTAACCAT		CONTROL - No Sample		
24	4	RatWT_Kidney_01			R929	GGAATGC	F933	ACGACTTGG		Kidney		
25	5	RatWT_Kidney_02			R929	GGAATGC	F935	TAACGCGTC		Kidney		
26	6	RatWT_Kidney_03			R929	GGAATGC	F937	TCTAACCAT		Kidney		
27	7	RatWT_Liver_01			R931	GCATGGA	F933	ACGACTTGG		Liver		
28	8	RatWT_Liver_02			R931	GCATGGA	F935	TAACGCGTC		Liver		
29	9	RatWT_Liver_03			R931	GCATGGA	F937	TCTAACCAT		Liver		
30	10	RatWT_Lung_01			R933	TCCTTCGT	F933	ACGACTTGG		Lung		
31	11	RatWT_Lung_02			R933	TCCTTCGT	F935	TAACGCGTC		Lung		
32	12	RatWT_Lung_03			R933	TCCTTCGT	F937	TCTAACCAT		Lung		
33	13	RatWT_URR_01			R935	TAGCTCTT	F933	ACGACTTGG		URR		
34	14	RatWT_URR_02			R935	TAGCTCTT	F935	TAACGCGTC		URR		
35	15	RatWT_URR_03			R935	TAGCTCTT	F937	TCTAACCAT		URR		
36												
37												

rat_mockdata_PE_NS_labeled

3D PCA

Users also have the option to plot PCA in 3D, by selecting an additional feature and clicking 'Plot'.

Features

- ☑ PC1: 60.23%
- ☑ PC2: 18.27%
- ☑ PC3: 7.98%
- ☐ PC4: 6.32%
- ☐ PC5: 4.79%
- ☐ PC6: 0.88%

Note: Selecting more than 3 features will not result in a plot.
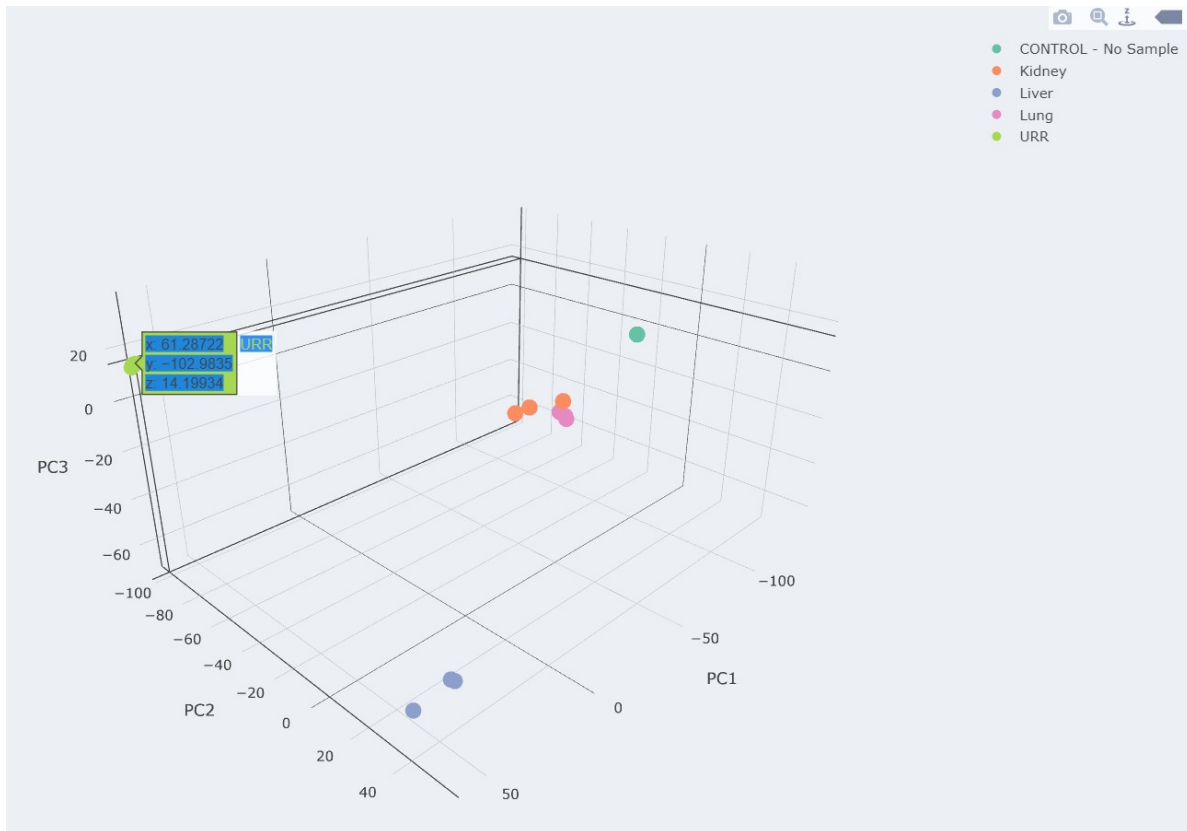
Hovering over individual points will give the 3D coordinates.

Users may then navigate using the cursor and following toolbar, in the upper right hand corner of the plot area.

   Saves the picture as a PNG.

   Zooms in on the plot.

   User can rotate the graph around a vertical axis.

   Raw value on hover is turned on.

Sample Selection

Individual samples can be selected by clicking the corresponding checkbox.

To select or deselect multiple samples, click in the area away from the samples, hold down the mouse button, and drag to draw a box with a dashed outline.

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Col 5	Col 6	Col 7	Col 8	Col 9	Col 10
☑ CtNoSample01	☑ CtNoSample02	☑ Exp01S01	☑ Exp01S02	☑ Exp01S03	☑ Exp01S04
☐ Exp01S11	☐ Exp01S12	☐ Exp01S13	☐ Exp01S14	☐ Exp01S15	☐ Exp01S16
☐ Exp01S23	☑ Exp01S24	☑ Exp01S25	☐ Exp01S26	☐ Exp01S27	☐ Exp01S28
☐ Exp01S35	☑ Exp01S36	☑ Exp01S37	☐ Exp01S38	☐ Exp01S39	☐ Exp01S40
☐ Exp01S47	☐ Exp01S48	☐ Exp01S49	☑ Exp01S50	☑ Exp01S51	☐ Exp01S52
☐ Exp01S59	☐ Exp01S60	☐ Exp01S61	☑ Exp01S62	☑ Exp01S63	☐ Exp01S64
☐ Exp01S71	☐ Exp01S72	☐ Exp01S73	☐ Exp01S74	☐ Exp01S75	☐ Exp01S76
☐ Exp01S83	☐ Exp01S84	☐ Exp01S85	☐ Exp01S86	☐ Exp01S87	☐ Exp01S88

The box size can be made as large or as small as the user desires by moving the mouse without releasing. Releasing the mouse will cause all samples in the area of the box to invert their state, so samples that were checked will no longer be checked and vice versa.

Col 5	Col 6	Col 7	Col 8	Col 9	Col 10
☑ CtNoSample01	☑ CtNoSample02	☑ Exp01S01	☑ Exp01S02	☑ Exp01S03	☑ Exp01S04
☐ Exp01S11	☐ Exp01S12	☐ Exp01S13	☐ Exp01S14	☐ Exp01S15	☐ Exp01S16
☐ Exp01S23	☐ Exp01S24	☐ Exp01S25	☑ Exp01S26	☑ Exp01S27	☐ Exp01S28
☐ Exp01S35	☐ Exp01S36	☐ Exp01S37	☑ Exp01S38	☑ Exp01S39	☐ Exp01S40
☐ Exp01S47	☑ Exp01S48	☑ Exp01S49	☐ Exp01S50	☐ Exp01S51	☐ Exp01S52
☐ Exp01S59	☑ Exp01S60	☑ Exp01S61	☐ Exp01S62	☐ Exp01S63	☐ Exp01S64
☐ Exp01S71	☐ Exp01S72	☐ Exp01S73	☐ Exp01S74	☐ Exp01S75	☐ Exp01S76
☐ Exp01S83	☐ Exp01S84	☐ Exp01S85	☐ Exp01S86	☐ Exp01S87	☐ Exp01S88

Downloading Plot

Both the plot as well as the sub-selected data may be downloaded by clicking on their respective download buttons. The sub-selection of data will be downloaded as a comma separated value. For the plot, user will be prompted to select a PNG or SVG image for download.

Choose Filetype for download

Filetype
◉ PNG   ◯ SVG

Close   ⬇ Download

Differential Expression Analysis

Selecting 'Differential Expression' from the landing page will take you to both the MAplot and DESeq tools, which can be selected from the toolbar.

MAPlot	DESeq Table

MAPlot

Visual representation of differential expression data.
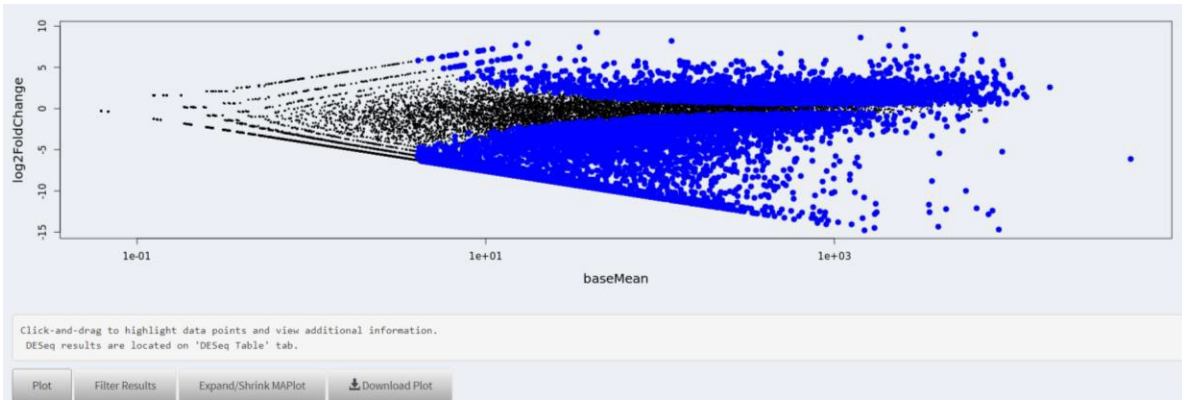
By default, no samples are plotted. The user must select at least one Control sample and one Experimental sample. Samples are deemed 'differentially expressed' and displayed in blue if they have a p-value < 0.05.

To select samples as controls, the user must click the 'Control' button, which will turn it yellow.

To select samples for display, the user can individually click in the checkbox next to each sample to select and deselect the sample. If the checkbox is marked, the sample will be included in the plot. Re-plot by clicking the 'Plot' button after changing the selection of samples.

Sample Selection

Individual samples can be selected by clicking the corresponding checkbox.

To select or deselect multiple samples, click in the area away from the samples, hold down the mouse button, and drag to draw a box with a dashed outline.

Col 5	Col 6	Col 7	Col 8	Col 9	Col 10
☑ CtNoSample01	☑ CtNoSample02	☑ Exp01S01	☑ Exp01S02	☑ Exp01S03	☑ Exp01S04
☐ Exp01S11	☐ Exp01S12	☐ Exp01S13	☐ Exp01S14	☐ Exp01S15	☐ Exp01S16
☐ Exp01S23	☑ Exp01S24	☑ Exp01S25	☐ Exp01S26	☐ Exp01S27	☐ Exp01S28
☐ Exp01S35	☑ Exp01S36	☑ Exp01S37	☐ Exp01S38	☐ Exp01S39	☐ Exp01S40
☐ Exp01S47	☐ Exp01S48	☐ Exp01S49	☑ Exp01S50	☑ Exp01S51	☐ Exp01S52
☐ Exp01S59	☐ Exp01S60	☐ Exp01S61	☑ Exp01S62	☑ Exp01S63	☐ Exp01S64
☐ Exp01S71	☐ Exp01S72	☐ Exp01S73	☐ Exp01S74	☐ Exp01S75	☐ Exp01S76
☐ Exp01S83	☐ Exp01S84	☐ Exp01S85	☐ Exp01S86	☐ Exp01S87	☐ Exp01S88

The box size can be made as large or as small as the user desires by moving the mouse without releasing. Releasing the mouse will cause all samples in the area of the box to invert their state, so samples that were checked will no longer be checked and vice versa.

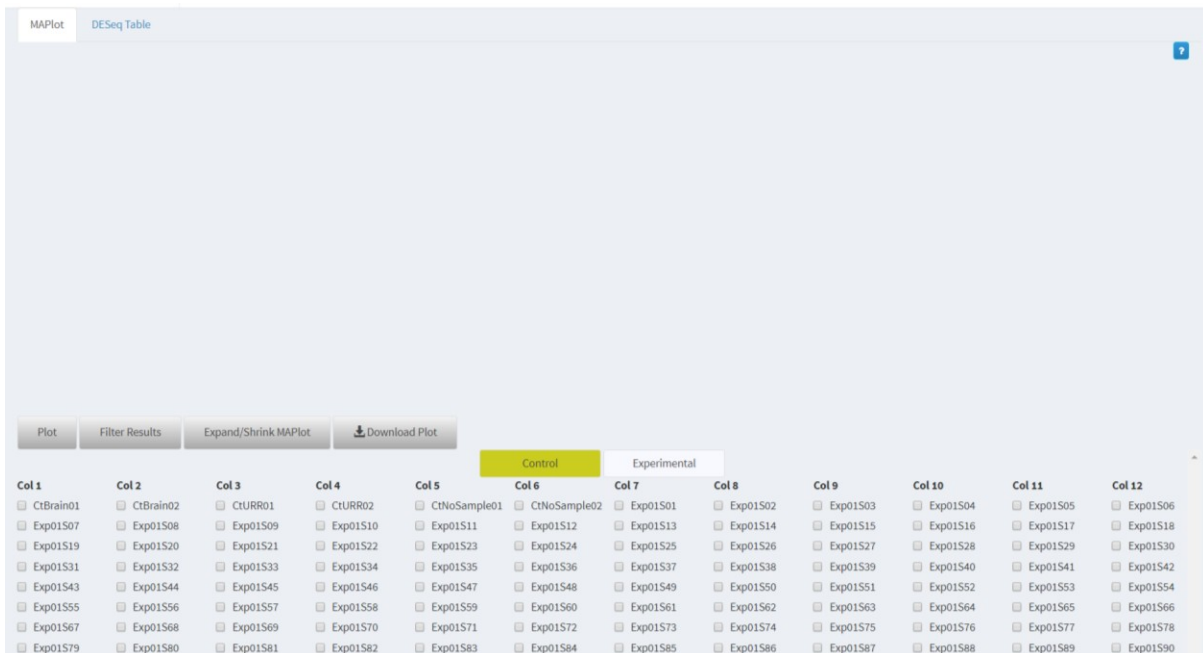| Col 5	Col 6	Col 7	Col 8	Col 9	Col 10
☑ CtNoSample01	☑ CtNoSample02	☑ Exp01S01	☑ Exp01S02	☑ Exp01S03	☑ Exp01S04
☐ Exp01S11	☐ Exp01S12	☐ Exp01S13	☐ Exp01S14	☐ Exp01S15	☐ Exp01S16
☐ Exp01S23	☐ Exp01S24	☐ Exp01S25	☑ Exp01S26	☑ Exp01S27	☐ Exp01S28
☐ Exp01S35	☐ Exp01S36	☐ Exp01S37	☑ Exp01S38	☑ Exp01S39	☐ Exp01S40
☐ Exp01S47	☑ Exp01S48	☑ Exp01S49	☐ Exp01S50	☐ Exp01S51	☐ Exp01S52
☐ Exp01S59	☑ Exp01S60	☑ Exp01S61	☐ Exp01S62	☐ Exp01S63	☐ Exp01S64
☐ Exp01S71	☐ Exp01S72	☐ Exp01S73	☐ Exp01S74	☐ Exp01S75	☐ Exp01S76
☐ Exp01S83	☐ Exp01S84	☐ Exp01S85	☐ Exp01S86	☐ Exp01S87	☐ Exp01S88

Click 'Plot' to display.

Expanding Plot

To change the size of the plot, click 'Expand/Shrink MAPlot'.
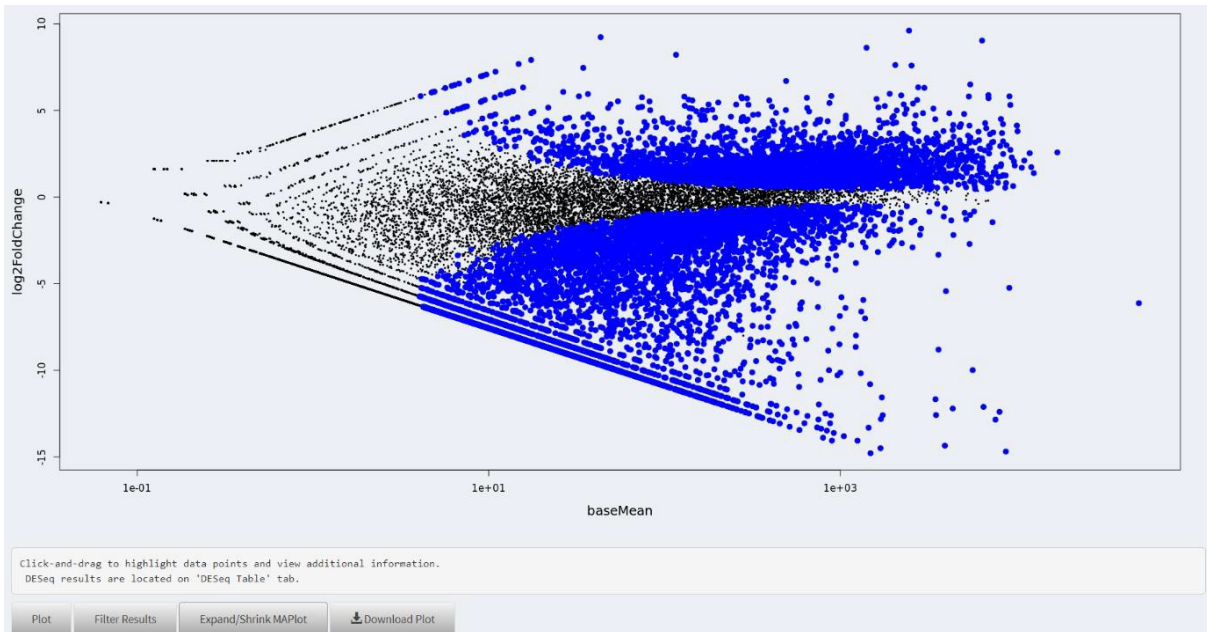
Filtering Results

To filter results by p-value or BaseMean, click 'Filter Results', which will bring up the following dialogue.

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Subselected p-value range of 0.8-1.0.
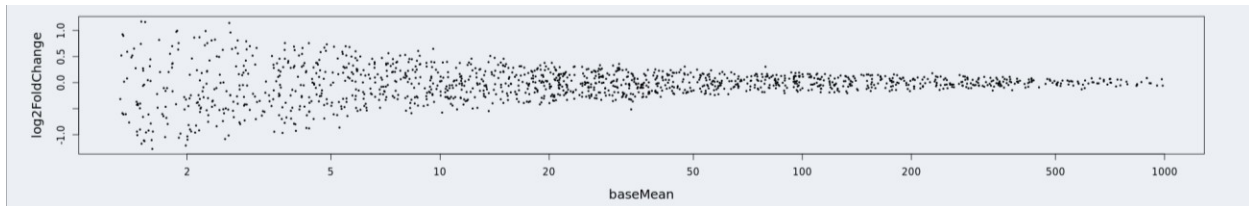
Subselected p-value range of 0-0.2.
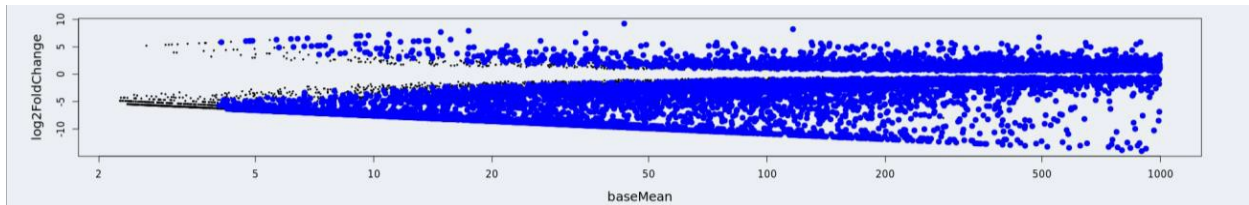
Plot of 0-0.2 p-value range, expanded.

Click-and-drag to highlight data points and view additional information.
DESeq results are located on 'DESeq Table' tab.

| Plot | Filter Results | Expand/Shrink MAPlot | ⬇ Download Plot |
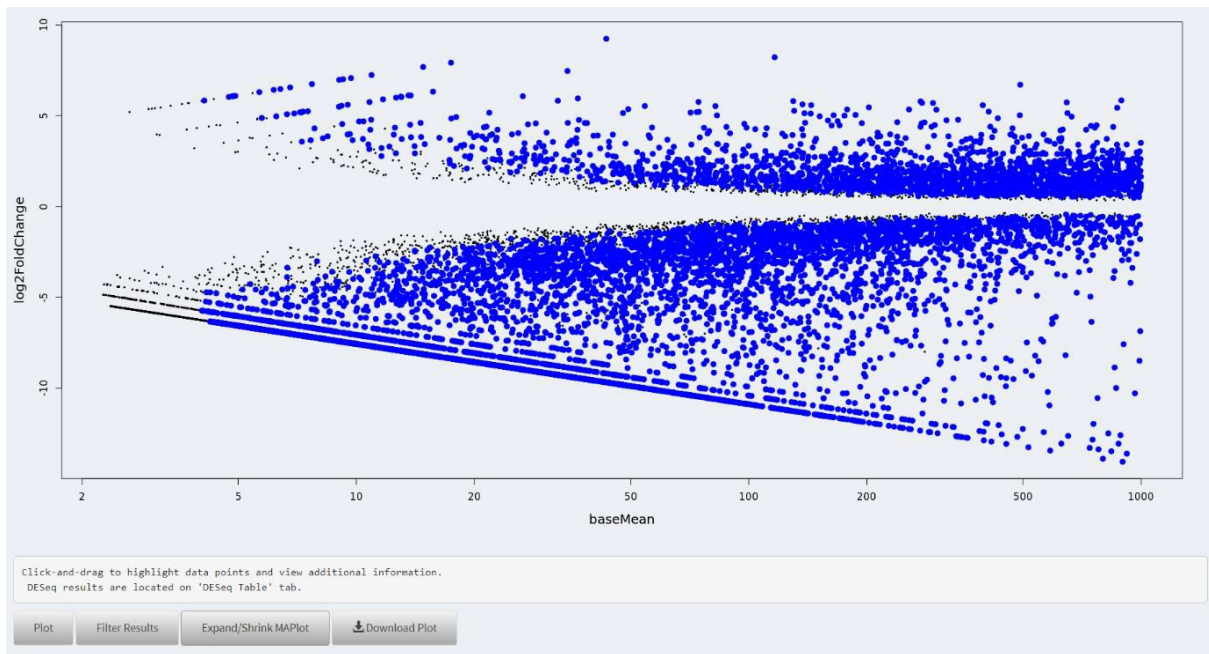
View Data

To view baseMean, log2 fold change, and p-value for a subset of data, click on the plot itself, hold down the mouse button, and drag to select a subset of data. A blue box will appear. Release the mouse to

finalize the selection. Information about the data points selected will appear in the light grey box below the plot and can be downloaded as a CSV.


```
Gene: TFF1_7046      baseMean: 116    log2FoldChange: 8.22   p-value: 1.62e-12
Gene: KIF4A_28251    baseMean: 43.3   log2FoldChange: 9.24   p-value: 1.82e-08
Gene: IL20_16401     baseMean: 34.5   log2FoldChange: 7.46   p-value: 9.22e-06
```

| Plot | Filter Results | Expand/Shrink MAPlot | ⤓ Download Plot | ⤓ Donwload Sub-Selected Data |
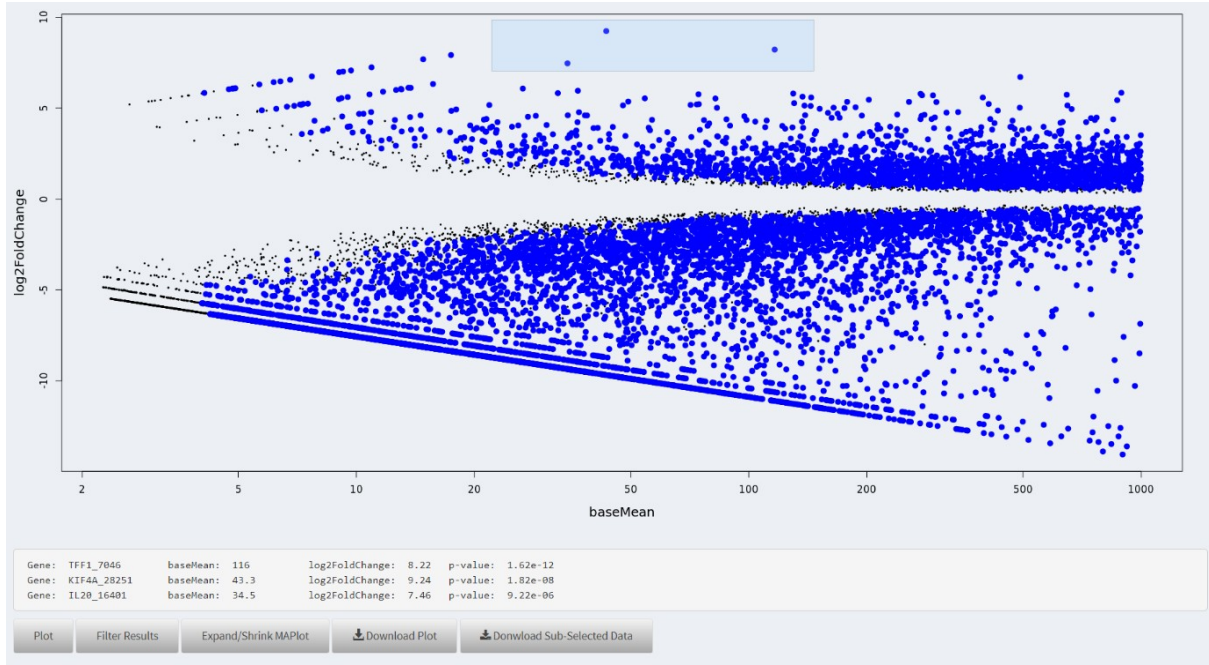
DESeq

Table showing the raw data generated by differential expression analysis.

Gene	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj	CtURR01	CtURR02	Exp01S21	Exp01S33	Exp01S45	Exp01S22	Exp01S34	Exp01S46
TRIM37_14843	6974.03	5.888918e+00	0.1699302	34.64	5.881722e-263	1.042123e-258	1.430823e+02	1.691661e+02	9048.330289	8.203844e+03	8617.004621	9915.048738	10411.625478	9284.128944
ACTA2_14836	1384.90	-7.000574e+00	0.2146265	-32.62	2.319266e-233	2.054637e-229	5.542320e+03	5.282347e+03	51.040037	3.773709e+01	39.255131	40.118012	44.457354	41.896911
SPTSSB_25421	6419.40	9.038674e+00	0.2803118	32.25	4.123986e-228	2.435626e-224	1.146652e+01	2.128218e+01	10108.301291	7.524577e+03	6936.079762	9553.986627	7890.463275	9309.040620
LMNB1_27441	6988.12	5.711407e+00	0.1780918	32.07	1.154832e-225	5.115328e-222	2.078931e+02	1.451554e+02	8394.305628	8.868216e+03	9766.475393	9231.862588	9591.315591	9699.701004
TFAP2C_21692	1608.86	5.668388e+00	0.1841048	30.79	3.687613e-208	1.306743e-204	4.337337e+01	4.038158e+01	2104.511295	2.198682e+03	2165.071480	2116.815118	2076.588663	2125.418957
ARHGAP11A_18300	2335.90	4.814721e+00	0.1757328	27.40	2.899617e-165	8.562569e-162	1.106769e+02	1.080480e+02	2929.460731	3.136151e+03	3463.510443	3052.508757	3200.929486	2685.931681
FOXA1_22204	5497.85	6.502753e+00	0.2396649	27.13	4.053433e-162	1.025982e-158	9.322783e+01	6.766643e+01	6345.582281	5.810518e+03	6751.882607	7365.195076	9797.827171	7750.928482
SLC38A1_14804	8222.47	4.509621e+00	0.1664763	27.09	1.338991e-161	2.965531e-158	4.701275e+02	4.785763e+02	9246.555549	1.082657e+04	10841.462069	9792.334818	12240.113423	11884.002106
CDT1_21099	1649.58	5.182112e+00	0.1929783	26.85	7.710077e-159	1.517857e-155	5.683408e+01	6.330085e+01	2053.471258	2.072561e+03	2357.320970	2025.959619	2466.666091	2100.507280
APPBP2_370	8444.33	4.217783e+00	0.1581151	26.68	9.081706e-157	1.609097e-153	5.653495e+02	6.237317e+02	10185.454836	1.177199e+04	12287.862681	11610.624727	9737.594627	10772.035448
SYTL2_21368	2097.04	5.002116e+00	0.1888288	26.49	1.256314e-154	2.023580e-151	8.275839e+01	9.004000e+01	2846.372299	2.899797e+03	3072.972212	2920.355305	2435.115711	2428.888472
SPC24_26328	1912.87	5.968526e+00	0.2269338	26.30	1.880922e-152	2.777181e-149	4.486901e+01	3.601600e+01	2530.636256	2.181800e+03	2193.254651	2492.036527	2984.379149	2839.931137
TPD52L1_23148	6682.70	4.270051e+00	0.1634258	26.13	1.735672e-150	2.365587e-147	4.447017e+02	4.632968e+02	9133.792677	8.598098e+03	7364.866582	9616.523529	8293.447677	9546.833897
HIST1H3F_17317	9184.59	5.820299e+00	0.2239166	25.99	5.917930e-150	7.489563e-147	1.909426e+02	2.401067e+02	10998.534496	1.026945e+04	9766.475393	13802.956103	12416.508731	15791.738291

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The number of genes displayed onscreen at any given time can be changed from the dropdown menu in the upper left hand corner of the tool, next to 'Show … entries'.

Sorting Data

To sort a column from least counts to greatest, or vice versa, click on the sample name at the top of the column. The ⬍ symbol next to the sample name will toggle to ▲ (sorting least to greatest) or ▼ (sorting greatest to least).

Search Function

To find a particular gene or probe of interest, type in the gene symbol or probe ID in the 'Search' function in the upper right hand corner of the tool. The search will automatically commence, and the word 'Processing' will be displayed on screen while searching occurs.

Show 25 ▾ entries   Search: NOTCH

Gene	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj	CtURR01	CtURR02	Exp01S21	Exp01S33	Exp01S45	Exp01S22	Exp01S34	Exp01S46
NOTCH3_20764	660.73	1.353808e+00	0.3405380	3.98	7.023199e-05	2.541087e-04	3.315321e+02	2.783055e+02	487.847796	7.060808e+02	1181.680110	527.433867	935.038542	837.938214
NOTCH4_27592	60.33	-2.763632e+00	0.9824329	-2.81	4.907410e-03	1.207967e-02	1.949309e+02	1.396984e+02	1.186978	4.965406e+00	43.281299	9.439532	41.589138	47.558655
NOTCH2NL_27591	97.83	-9.140595e-01	0.5864321	-1.56	1.190723e-01	1.889756e-01	1.899455e+02	1.124136e+02	45.105149	1.062597e+02	115.752311	43.657837	41.589138	127.955430
NOTCH2_18421	168.17	-1.805017e-01	0.3997952	-0.45	6.516398e-01	7.300247e-01	1.989193e+02	1.697118e+02	225.525745	1.559138e+02	84.549514	194.690354	162.054226	153.999456
NOTCH1_4642	94.70	6.972406e-02	0.7149886	0.10	9.223153e-01	9.455839e-01	1.191521e+02	6.330085e+01	15.430709	5.958488e+01	140.915856	95.575265	182.131740	81.529124

Showing 1 to 5 of 5 entries (filtered from 21,111 total entries)   Previous **1** Next

Download Data

Users can download the raw data by clicking the 'Download' button in the upper left hand corner of the screen.

FAQ

In the event that the user's question is not addressed, please contact the TempO-SeqR support at TempO-SeqR@biospyder.com.

Q: Why do we need to upload a count table and sample sheet?

A count table is required to use any of the quality control and analysis tools, while a sample sheet is not required.

However, selecting a sample sheet in tandem with a count table will format the sample names in the same order they appear on the micro-titer plate.

Additionally, the 'Description' column on the Sample sheet can be used to designate replicates for use in PCA (see User Guide section 'PCA: Grouping Replicates for PCA').

Q: Why are my files taking so long to upload?

File upload speeds are dependent on the size of the file(s) and speed of the internet connection.

Q: How do I designate my replicates for PCA?

Fill out the 'Description' column in the Sample Sheet to designate sample replicates. Label each replicate within a group with the same alphanumeric characters (e.g. Liver, Kidney, Group1) and upload the Sample Sheet (see User Guide section 'PCA: Grouping Replicates for PCA).

Q: I don't have my results. How long is an alignment expected to take?

Alignment times vary based on the number and size of the FASTQ files. Typically, alignments take 1 minute per 5 million reads.

However, when under heavy user load the BioSpyder High Performance Computing Cluster may take longer to return results. View the 'Alignment History' section on the landing page to see the status of your alignment.

Q: My alignment failed. What happened and what do I do?

Alignments can fail at two separate points, during FASTQ validation and while mapping sequences to the reference genome.

If the alignment email contains the message, "FASTQ validation failed. Alignment process could not complete for user." there is an issue with the format of one or more FASTQ files.

Receiving the message, "Aligning process did not complete." indicates the alignment failed while mapping sequences to the reference genome.

Upon a failed alignment, a BioSpyder representative will reach out by email within 48 hours to help troubleshoot any issues.

Appendix A

Common Inputs and Outputs

Inputs

- FASTQs
  - These are text files containing quality scores. Please refer to Illumina documentation for the standard layout of a FASTQ file.
- Template file for Sample Sheet Generator
  - CSV (comma-separated value) format, which can be opened in common spreadsheet editors such as Microsoft Excel, LibreOffice Calc, or OpenOffice Calc.
- Count table
  - Count tables are generated in CSV (comma-separated value) format, which can be opened in common spreadsheet editors such as Microsoft Excel, LibreOffice Calc, or OpenOffice Calc.

Outputs

- Count table – DESeq, Statistics, Count table
  - Count tables are generated in CSV (comma-separated value) format, which can be opened in common spreadsheet editors such as Microsoft Excel, LibreOffice Calc, or OpenOffice Calc.
- Sample Sheet
  - Sample Sheets are generated in CSV (comma-separated value) format, which can be opened in common spreadsheet editors such as Microsoft Excel, LibreOffice Calc, or OpenOffice Calc.
- Plots - PNG, SVG
  - PNG – Portable Network Graphic; images saved as PNG are compressed losslessly and provide high resolution images at relatively small file sizes.
  - SVG – Scalable Vector Graphic; these are images that remain high quality even when zoomed in, with the tradeoff of a much larger file size.
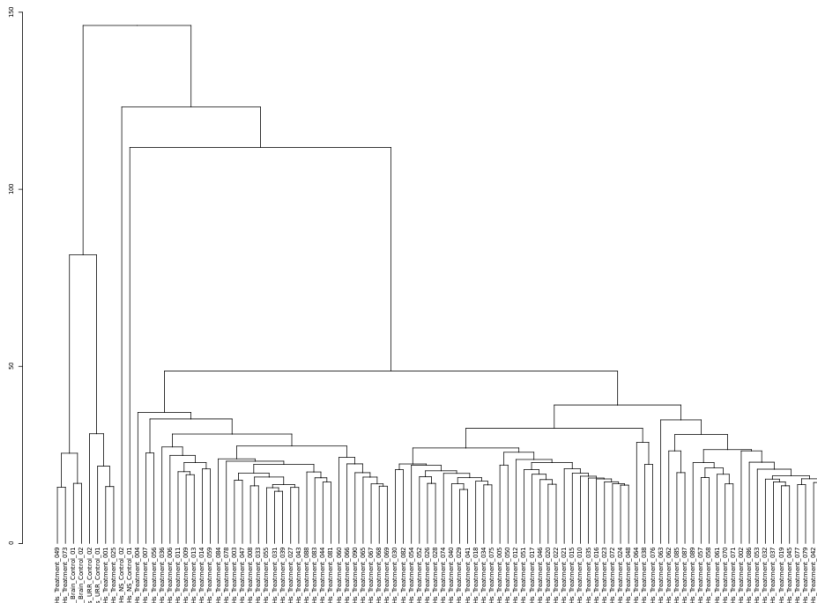
PNG image (above)
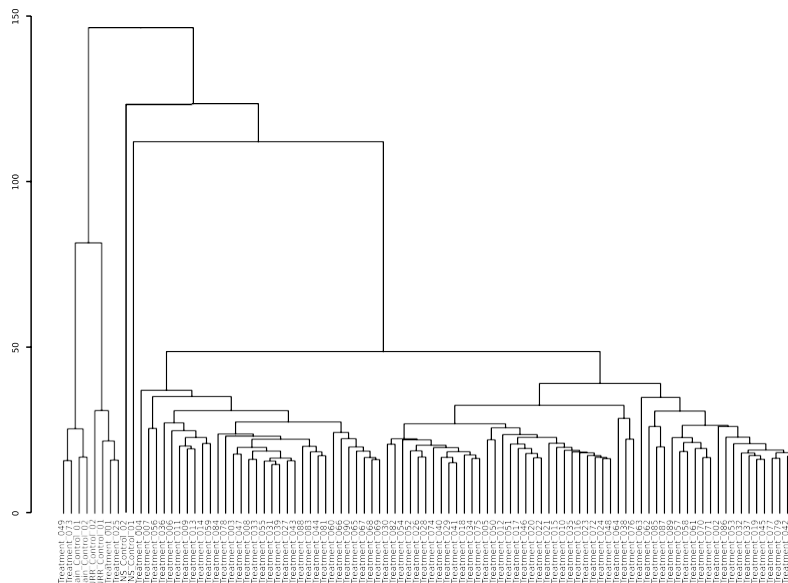
SVG image (above)

PNG vs. SVG (500% zoom)

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xtment_065
xtment_067
xtment_068
xtment_069
xtment_030
xtment_082
xtment_054
xtment_052
xtment_026
xtment_028
xtment_074
xtment_040
xtment_029

LITIII:::IIL_UUU
tmenC0ll
tment-009
tment-013
tment-014
tmenC059
tment-084
tment-078
tment-003
tment-047
tment-008
tment-033
tment-055
tmenC031
tment-039
tment-02_7_

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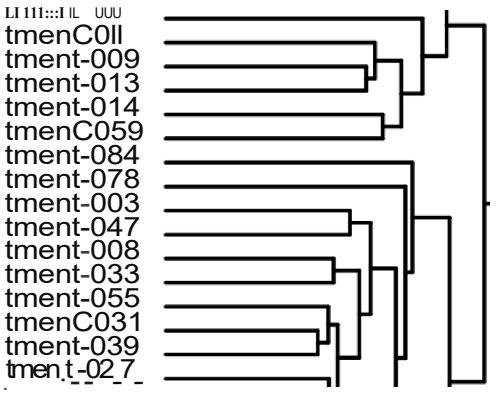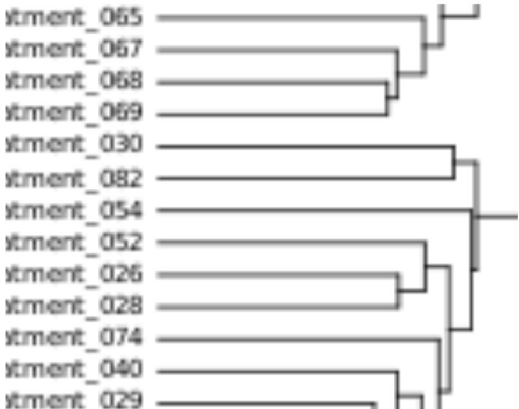
Appendix B: Possible Error Messages

Error Message	Likely Cause	Solution
FASTQ validation failed and alignment could not be attempted.	One or more FASTQ files are corrupt or missing information.	Check FASTQ files for error(s) and correct them.
Aligning process did not complete.	FASTQ validation was successful, but an error arose during alignment.	Contact BioSpyder.
Duplicate sample names found.	Two or more samples have identical names.	Replace with unique names and re-upload the file.
Count table contains a value that is NOT an integer.	Count table contains a value that is not an integer.	Correct and re-upload the table.
Count table contains too many samples. A maximum of 384 samples is allowed.	More than 384 samples are present in the count table.	Remove superfluous sample columns from the count table.
Error detected in Sample Sheet. Please check format and re-upload.	Sample Sheet could not be correctly parsed due to errors in the format.	Correct Sample Sheet and re-upload.
Sample Sheet contains too many samples. A maximum of 384 samples is allowed.	More than 384 samples are present in the Sample Sheet.	Remove superfluous samples from the Sample Sheet.
Duplicate index pairs detected.	The same indices are selected for more than one plate.	Choose a new plate index.
Indices are not unique for each sample.	Two or more samples have identical indices.	Samples must have unique indices.
Sample names are not unique.	Two or more samples have identical names.	Replace with unique names and re-upload the file.

Appendix C: Naming of Samples and Files

Naming files and samples correctly is vital to streamlining the process of data analysis using the TempO-SeqR Software.

FASTQ naming

There should be no spaces or special characters (?/\=+ ()<> [].:;"',*^|&) in the FASTQ file name.
- Underscores or hyphens should be used to separate fields in the name, rather than spaces.
- FASTQ extensions should be .fastq, .fastq.gz, or .fq.

Sample naming

- There should be no spaces or special characters (?/\=+ ()<> [].:;"',*^|&) in the sample name.
- Underscores or hyphens should be used to separate fields in the name, rather than spaces.
- Unique sample names
  - Samples may have long, complex names. The TempO-SeqR software will split sample names on underscores before determining which fields are unique to the sample.
  - Sample names will then be kept from the beginning up to the field which is unique to each sample.
  - For instance, as seen below, the first field is unique to all samples, so the sample name is truncated to the first field only. This allows clear labeling in TempO-SeqR analysis tools.

B1	fx	CtBrain01_S1_L001_R1_001		
	A	B	C	D
1		CtBrain01_S1_L001_R1_001	CtBrain02_S2_L001_R1_001	CtURR01_S3_L001_R1_001
2	NAA50_44	35	15	12
3	P4HB_489	5	1	1
4	PABPN1_4	5	26	24
5	TGFB3_70	9	9	46
6	TMEM230	740	710	371
7	TOP2A_72	0	1	260
8	TPRKB_72	8	6	41
9	TREH_733	0	1	65
10	TSC22D3_	873	995	970
11	TSPAN4_7	1	0	6
12	ADCK3_11	502	419	425
13	ADCY8_12	125	80	28
14	ADO_144	1366	1205	1196
15	ADPRHL2_	3	4	30
16	GRK2_152	196	167	145
17	ADSL_153	12	1	13
18	AFMID_15	380	411	367
19	AGPAT2_1	39	20	78
20	AGRN_165	1074	944	1379
21	AHCY_174	151	92	294
22	AIFM1_18	192	152	594
23	AKAP8L_1	65	62	43
24	AKT1_210	291	240	171
25	AKT2_211	41	46	66