Lanceotron Documentation

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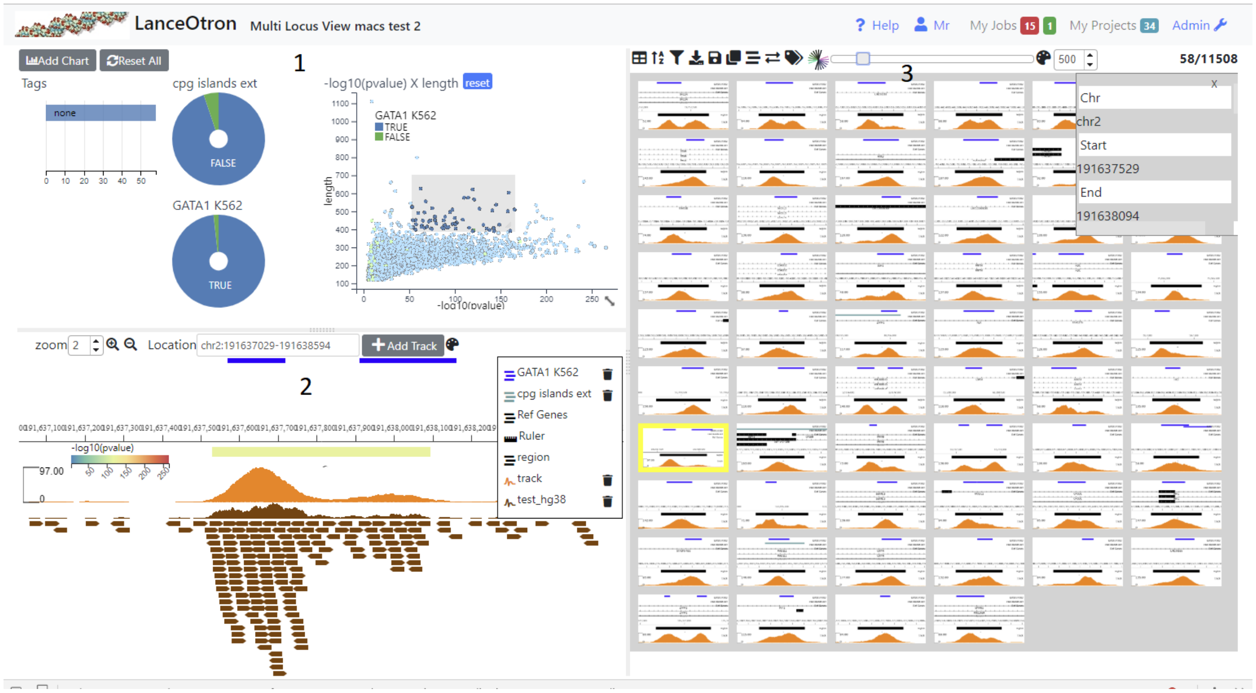
CHAPTER 1

Multi Locus View

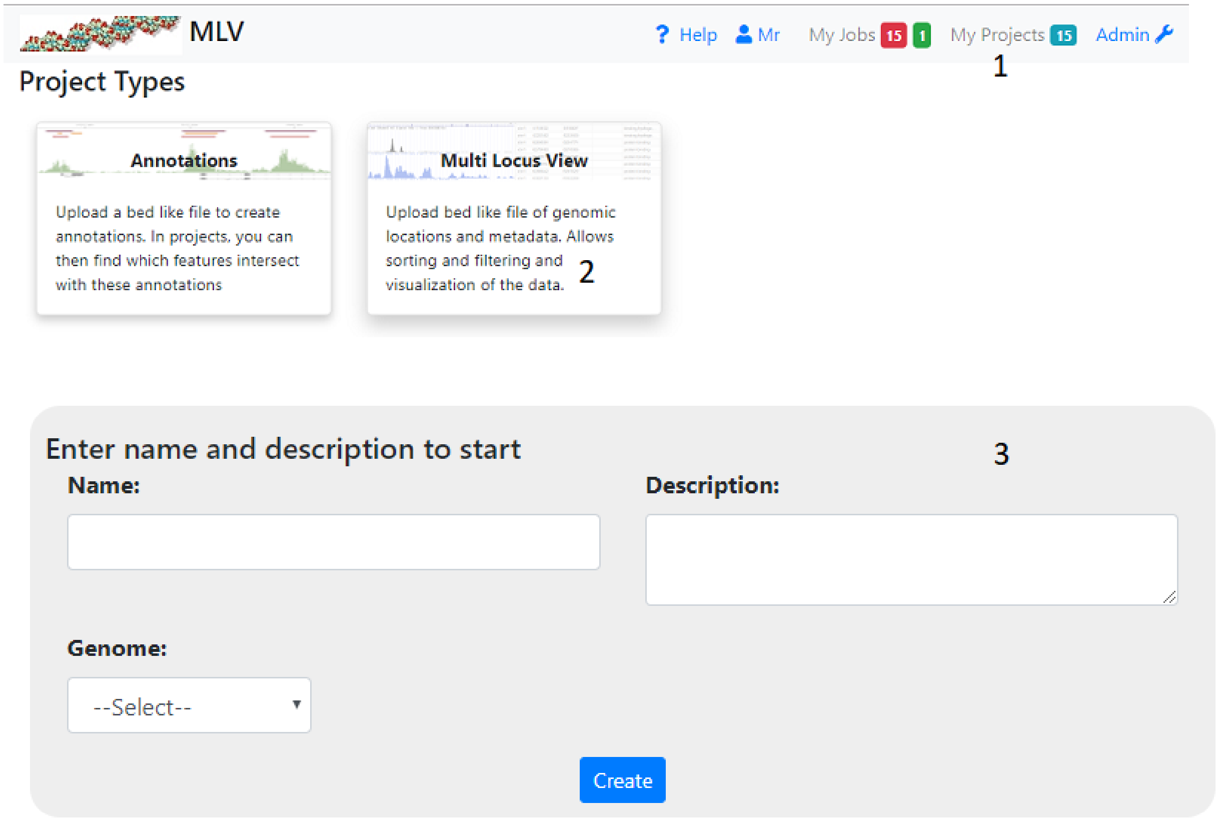
1.1 Summary

Multi Locus View (MLV) enables the user to visually inspect the underlying data from NGS experiments e.g. RNA-seq, ChIP-seq. Initially, the user uploads a list of genomic locations and associated data (*Uploading a File*), which can then intuitively filtered, sorted and viewed to drill down to regions of interest.



The view is divided into three sections. The top left shows charts (1), the bottom left a simple genome browser (2) and the right section shows a table containing all the genomic locations (3). Initially no charts are present and the browser only shows a track of the uploaded genomic regions (and possibly a RefGene track if a genome was selected). In order to make it easier to view and filter your data you can add charts (*Adding Graphs/Charts*) and browser tracks (*Adding Tracks*) . In addition analysis jobs such as finding intersections, claculating signal at each location from a bigWig track and dimension reduction can be carried out (*Running Analysis Jobs*). Locations can be annotated (*Tagging Locations*) by the user and downloaded or exported to the data visualisation tool Zegami

1.2 Creating a Project



Upload a bed like file to create annotations. In projects, you can then find which features intersect with these annotations

Upload bed like file of genomic locations and metadata. Allows sorting and filtering and visualization of the data.

To create a project click on 'My Projects' (1) on the top navigation bar and then the Multi Locus View panel (2). This will take you to a page where you have to fill in the name and description of the project (3). You also have to select the gemome required. If the genome you want is not available select 'other'. In this case gene information and other annotations will not be available. When you press 'Create' you wll be taken to a page where you can upload your file (see below)

1.2.1 Uploading Data

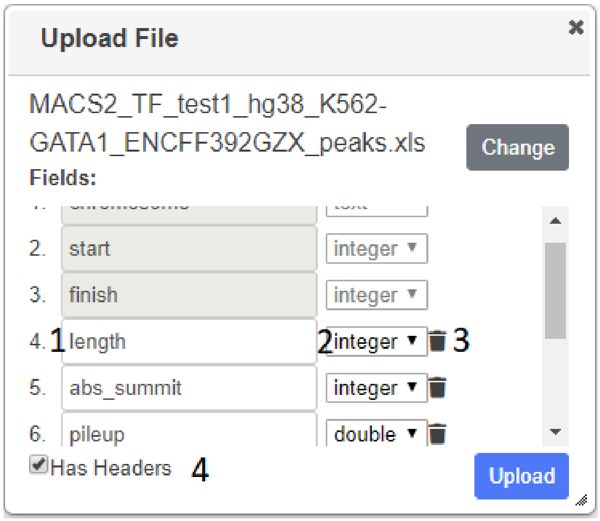
Input File Required

The file format is quite flexible and can be either tab(.tsv) or comma(.csv) delmited and can also be gzipped (.gz). The only requirement is that the first three columns (in order) specify the genomic location i.e. chromosome, start and finish. Normal bed files fulfill these criteria as well as excel data that has been saved as a .csv or .tsv file.

Other file types apart from bed-like files are not supported in the initial upload but can be added later to the browser (see *Adding Tracks*). In addition, in subsequent analysis bigWig files can be uplaoded in order to calculate peak stats at each genomic location (see *Calculate Peak Stats*)

Chromosome names can either be the UCSC e.g. chr1, chr2, chrX etc or Ensembl style 1,2,X. However, if subsequent files are added to the anaylis they have to be of the same format. This is not the case for calculating intesections, where you can mix and match between Ensembl and UCSC style chromosome names.

Uploading a File



Upload File

MACS2_TF_test1_hg38_K562-GATA1_ENCFF392GZX_peaks.xls

Fields:

2. start integer
3. finish integer
4. length integer
5. abs_summit integer
6. pileup double

☑ Has Headers

Press Choose in the displayed dialog and select your file containing genomic locations. The file will be parsed and the column (feild) types will be ascertained and displayed. Ensure the correct datatype has been deduced and change if necessary using the dropdowns (2). The header names are taken from the file (1), but you can change these and you can also delete any columns that you do not want (3). If no headers were detected in the file, enter the name of the column - the value of the first row is given to help you (1). If the file contains a header, but it was not detected, check the Has Headers box (4) at the the bottom of the dialog.

Once you are satisfied, press the upload button. There will be a delay as the data is uploaded and processed and if there are no problems you will be presented with an initial view

1.2.2 Saving A Project

When tracks, graphs or columns are added and you have edit permissions (see *Permissions*), they are permanantly added to the project. Also, when an analysis job has completed, any graphs,tracks or columns produced will be permanant. However, when you alter a graph or tracks's settings or reize/move them, these are not saved. Similary any changes you make to the table (column resizing/ordering, sorting etc.) will not automatically be saved. In order to save these changes you need to save the layout > Save Layout.

If you wish to make changes to public project then you will have to clone the project > Save As. The project will be copied into your name and you can them make any changes you wish.

1.3 Adding Graphs/Charts

Charts help you get a picture of the data as a whole and also help you filter the data. By selecting regions (dragging with th mouse) on scatter plots and histograms or clicking on sections in pie charts, row charts and box plots, the data can be intutitively filtered. With each filtering step, all charts will update (as well as the table and browser) to reflect the filtered data. Filters on individual charts can be removed by clicking the reset button which appears on the chart's title bar when a filter is applied or filters on all charts can be removed with the 'Reset All' button.

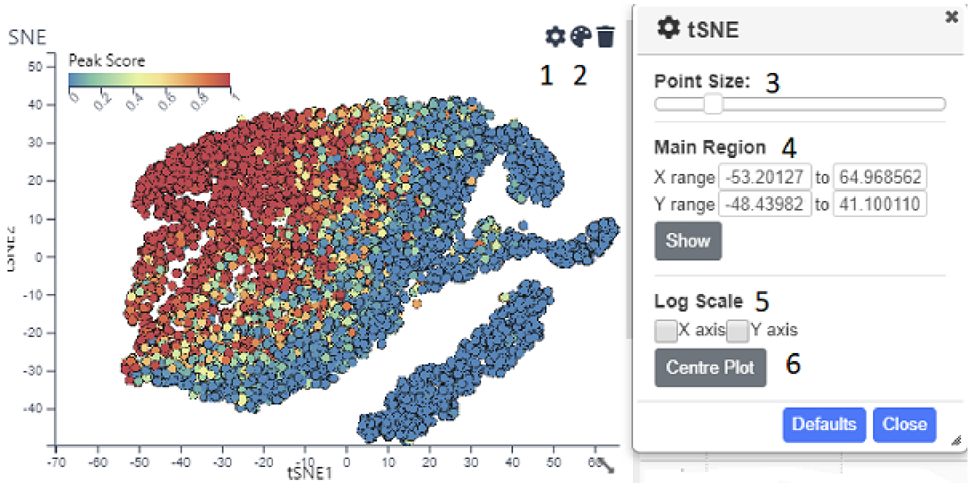
Charts can be moved by dragging on the title bar, and resized by dragging on the resize icon, which appears in the bottom right hand corner of chart when you hover over a chart.

Initially the only chart visible will be a row chart showing Tags (see *Tagging Locations*) so you need to add other charts to get a better insight into your data (see below)

1.3.1 Adding a Chart

Clicking on the 'Add Chart' button will show a dialog where you have to select the type of chart, the fields to use in the chart and its name. Once created you can change the chart's settings (icon), which differ according to the chart's type and with some charts color it (icon). Charts can moved by dragging them via the title bar and resized by the resize icon which appears in bottom left hand corner when the mouse is over the chart. The chart can be removed by clicking the trash icon, which appears when you hover over the graph's title. Once charts have been added and the appropriate settings/colors added, they can be saved using the icon above the table. The following chart types are available

Scatter Plot



Point Size:

Main Region

X range -53.20127 to 64.968562

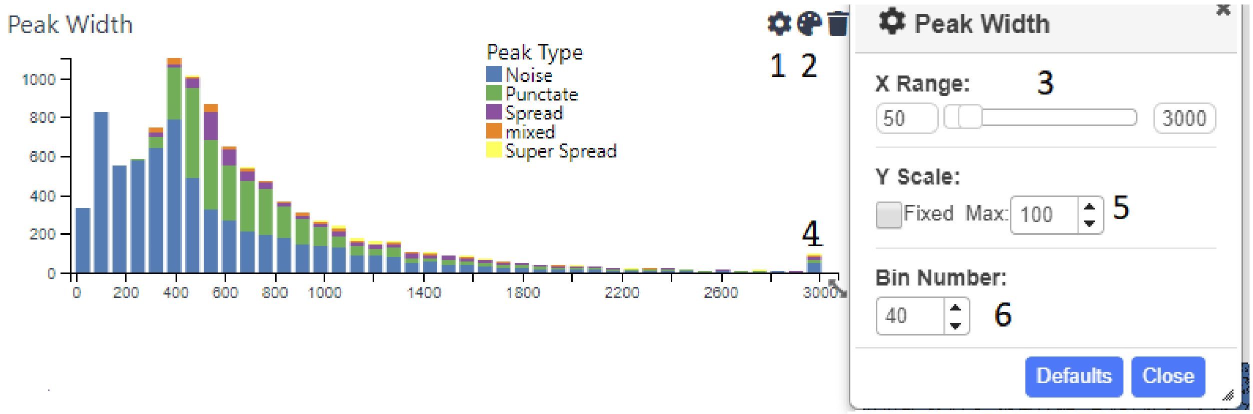
Y range -48.43982 to 41.100110

Log Scale

☐ X axis ☐ Y axis

A standard scatter plot requiring numerical fields for the x and y axis. Once created, the points can be coloured (on the title bar). Also by opening up the settings dialog (icon) you can alter the point size (3). By default the graph will show all the points, but you can zoom in and out using the mouse wheel and pan by pressing shift and dragging with the mouse. However if you want the default view to be a particular region, you can set this using the inputs in the Main Region section (4)and pressing show. The x and/or y axis can also be set to a log scale (5). After zooming and panning, the Centre Plot button (6) will restore the plot to show all the points or the region specified in (4). Normal mouse dragging (without shift) will cause pruduce a brush that filters the data, once created the brus can be dragged to different regions of the plot.

Histogram



Peak Width

X Range: 50 3000

Y Scale: ☐ Fixed Max: 100

Bin Number: 40

Shows the distribution of a numerical field in the data. The x range is automatically set to include the largest and smallest values. However, this will often lead to the chart looking skewed due to low numbers of extreme outliers. Therefore, you can use the cog icon (1) to open up the settings dialog, where an upper and/or lower limit can be set (3). Values higher or lower than these limits will be lumped together in the outermost bin (4). The y axis can also be capped (5) in order to get a better handle on bins conatining fewer counts. The number of bins can be adjusted using the appropriate spinner(6). Each bar can be coloured by categorical data use the icon (2).

Pie Chart

Shows categorical data. By default the maximium number of categories shown are the 8 largest ones, any reamining categories are lumped into ‘Others’. This can be changed by opening up the settings dialog (). Clicking on a segment (category) will select that category and clicking on further segements will add these to the filter. To filter again with a single category, use the reset button.

Row Chart

A chart showing catgories on the y axis and usually the number of records belonging to this category on the y axis. You can also choose a numerical field for the x axis, in which case the values of this field will be summed for each category. However a boxplot is usually more informative for this kind of information as the average and quartile ranges of the values are shown instead of the sum. As with the pie chart, the maximium number of categories shown are the 8 the largest ones, but this can be changed by opening up the settings dialog ()

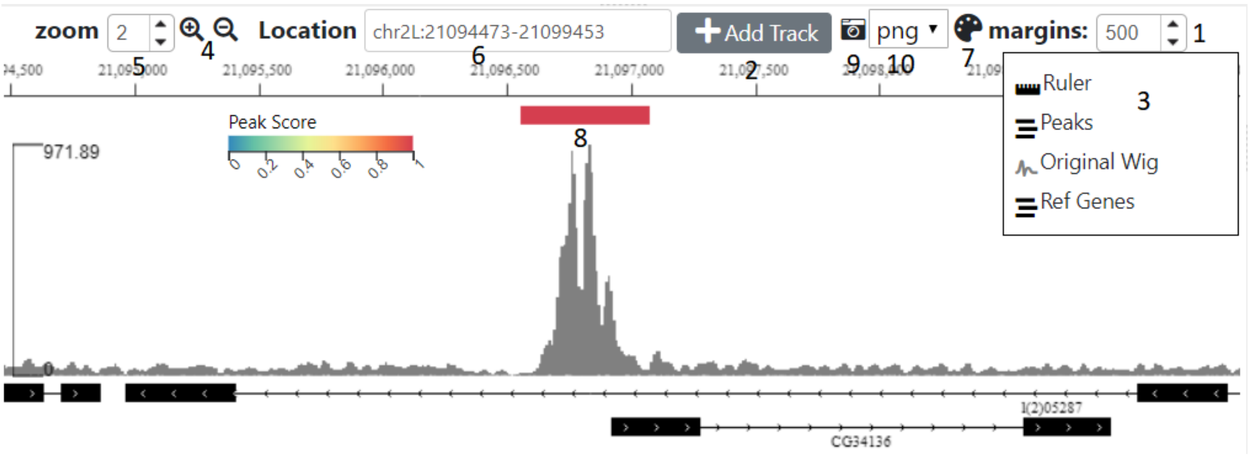
BoxPlot

A chart showing categeories and average/quartile ranges of the values of another field for that category. Box plots work best for fields that contain only a small number of categories. They are scaled to include all the datapoints, so if there are extreme outliers, the boxes will appear squashed.

Bar Chart

A Bar Chart showing the column average of any number of supplied fields. Because fields may differ in scale,to ensure differing values can fit on the same scale, the average is scaled between the median +/- 1.5*IQR (the same as the whiskers on a boxplot). The graph changes to only include those datapints in the current filter. No Selection is possible with this chart, as it would make no sense to filter on a column

1.4 The Genome Browser



zoom 2

Location chr2L:21094473-21099453

png

margins: 500

The browser shows the genomic location of the currently selected table row (or image). The distance either side of the region to also show can be controlld using the margin spinner (1) above the browser

1.4.1 Adding Tracks

Initially only two tracks will be displayed, the genomic locations you uploaded and if you didn't select 'other' for the genome, a track is displaying the genes. Other tracks can be added with the 'Add Tracks' button (2), which shows a dialog where you need to enter the url of a publically accessible track. The hosting server of the track should allow Cross Origin Resource Sharing (CORS). The type of track will try and be ascertained based on the url, although you can manually overide this by clicking on one of the radio buttons

Tracks that can be added are:-

- bed(tabix) - A bed file that has been gzipped and indexed with tabix
- BigBed
- BigWig
- Bam - A bam index is also required
- UCSC session - either cut and paste the url from the UCSC browser or use a session url. The latter will be more stable as the former uses a temporary id, which is only valid for a short period.

1.4.2 Altering Track Appearance

Clicking on the track label in the legend (3) will open a dialog for that track. The contents of the dialog will vary according to the type of track. The track height can be altered from this dialog

1.4.3 Zooming/Panning

There are five ways you can navigate using the browser:-

- You can zoom in and out using the mouse wheel and scroll left and right by deagging the mouse
- Use shift and drag to highlight and zoom into a region on the browser
- Use the magnifying glass icons (4), the zoom amount can be controlled by the adjacent spinner (5)

- Type the location co-ordinates (chr:<start>-<stop>) in the location text box (6)
- Click on a row or image in the right hand table to go to that feature. The margin spinner (1) shows how many bp either side of the feature will be displayed.

1.4.4 Feature track

This shows the uploaded regions(features) displayed in the right hand table. Clicking on the settings icon (7) will bring up a dialog where the following can be adjsuted:-

- Set the field you wish to a label the features with
- Set the field to color the feature by
- Set the field with which to position the feature on the y axis. By default the feature layout depends on the layout (Collapsed, expanded or squished) but can be a numeric field
- Choose the margins (distance either side of the feature) that will be displayed when you click on an image or a row in thr table

1.4.5 Saving the Browser Layout

Use the disk icon above the the table to save all settings including the current layout of the browser (tracks and track settings)

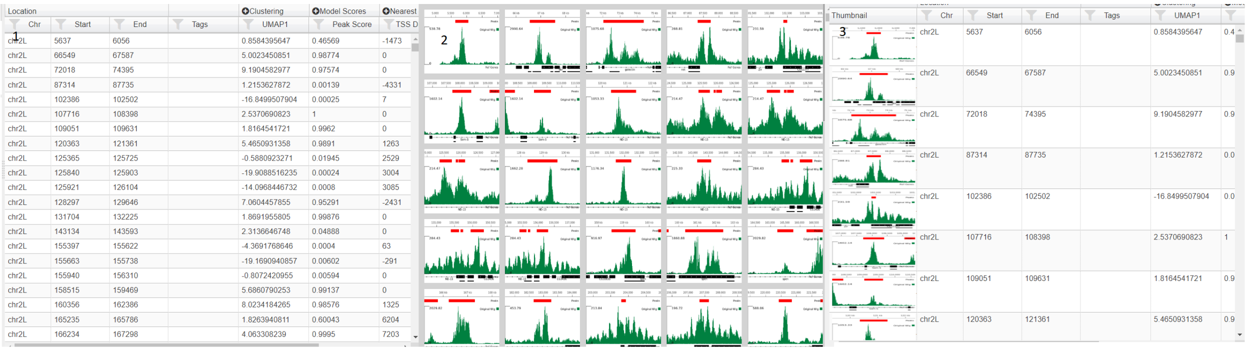
1.4.6 Capturing An Image

Use the icon (9) to download an image of the current browser view. The image format (png, pdf or svg) can altered using the adjacent dropdown (10)

1.5 The Table/Images

The default table behaves as a typical spread sheet, you can alter the column width by dragging the header's left and right borders and move columns by dragging the column's header. Clicking on the header will sort by that column. Clicking on that row will select it and update the browser.

1.5.1 Table Mode



Location				Clustering	Model Scores	Nearest
Chr	Start	End	Tags	UMAP1	Peak Score	TSS D
chr2L	5637	6056		0.8584395647	0.46569	-1473
chr2L	66549	67587		5.0023450851	0.98774	0
chr2L	72018	74395		9.1904582977	0.97574	0
chr2L	87314	87735		1.2153627872	0.00139	-4331
chr2L	102386	102502		-16.8499507904	0.00025	7
chr2L	107716	108398		2.5370690823	1	0
chr2L	109051	109631		1.8164541721	0.9962	0
chr2L	120363	121361		5.4650931358	0.9891	1263
chr2L	125365	125725		-0.5880923271	0.01945	2529
chr2L	125840	125903		-19.9088516235	0.00024	3004
chr2L	125921	126104		-14.0968446732	0.0008	3085
chr2L	128297	129646		7.0604457855	0.95291	-2431
chr2L	131704	132225		1.8691955805	0.99876	0
chr2L	143134	143593		2.3136646748	0.04888	0
chr2L	155397	155622		-4.3691768646	0.0004	63
chr2L	155663	155738		-19.1690940857	0.00602	-291
chr2L	155940	156310		-0.8072420955	0.00594	0
chr2L	158515	159469		5.6860790253	0.99137	0
chr2L	160356	162386		8.0234184265	0.98576	1325
chr2L	165235	165786		1.8263940811	0.60043	6204
chr2L	166234	167298		4.063308239	0.9995	7203

Thumbnail	Chr	Start	End	Tags	UMAP1	
	chr2L	5637	6056		0.8584395647	0.4
	chr2L	66549	67587		5.0023450851	0.9
	chr2L	72018	74395		9.1904582977	0.9
	chr2L	87314	87735		1.2153627872	0.0
	chr2L	102386	102502		-16.8499507904	0.0
	chr2L	107716	108398		2.5370690823	1
	chr2L	109051	109631		1.8164541721	0.9
	chr2L	120363	121361		5.4650931358	0.9

If your project contains images (see *Adding Images*) then then you change how the table is displayed using the table icon (). Three choices are table (1), images (2) and table with thumbnials (3).In image mode, the genomic location can be selected by clicking on the image and using the arrow keys to select the next/previous image. In this mode, the data can be sorted and filtered using the icons ( ) in the menu above the table. Also in image mode you can alter image size using the slider in the table menu and also color the border around the image by a field (). This opens up a dialog where you can choose the field and the color scale to use

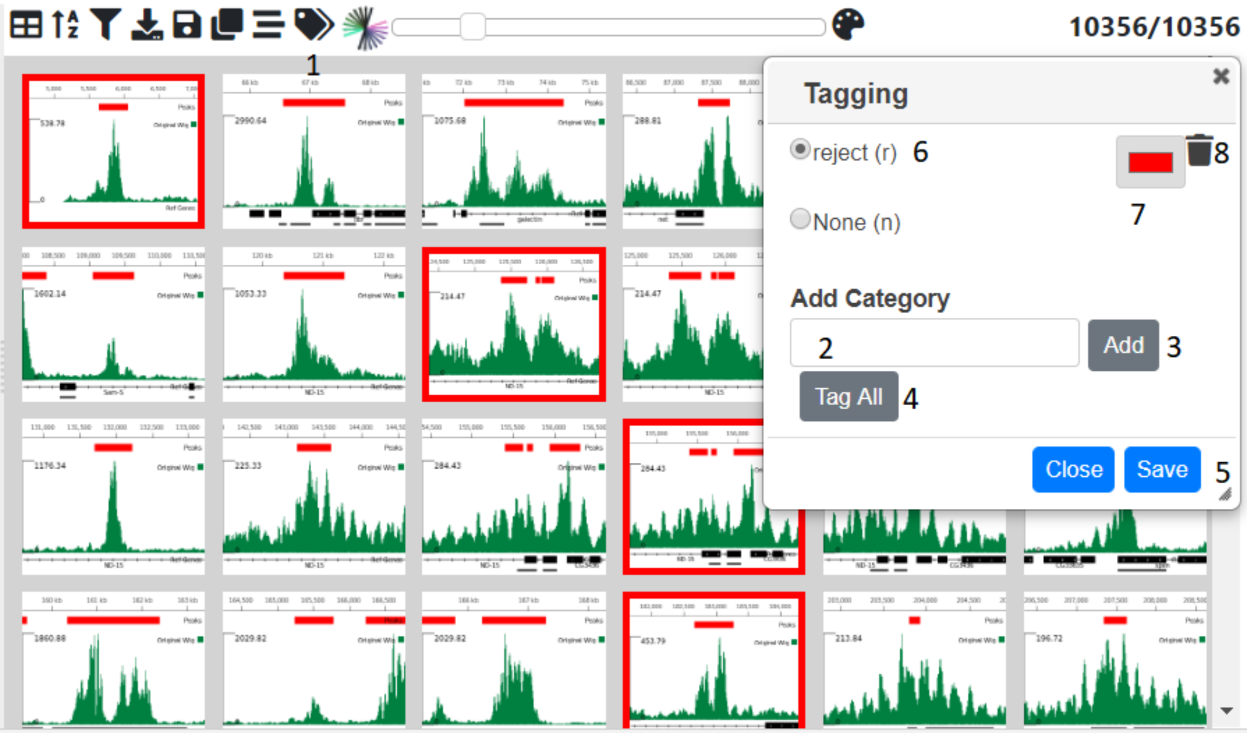
1.5.2 Filtering Data

It is often more intuitive to filter using graphs (see *Adding Graphs/Charts*), however data can be filtered by clicking on filter icon in each column header. To filter on multiple columns or when the table is only showing images,press the filter icon on the top table menu. This will bring up a dialog showing filtering options for all fields in the data. Whenever any filters are added or changed, any charts will update accordingly,but the filters are not added to the charts or existing filters on the charts updated as they are completely independant.

1.5.3 Sorting Data

The data can be sorted on columns by clicking the column header (shift click to sort on multiple columns). The data can be also be filtered by clcking the sort icon in the table menu. In the sort dialog,the columns to be sorted on are added usng the plus icon and then either Ascending (ASC) or descending (DESC) can be chosen . The sort order can be changed by dragging the labels or columns removed from the sort by clicking on the trash icon

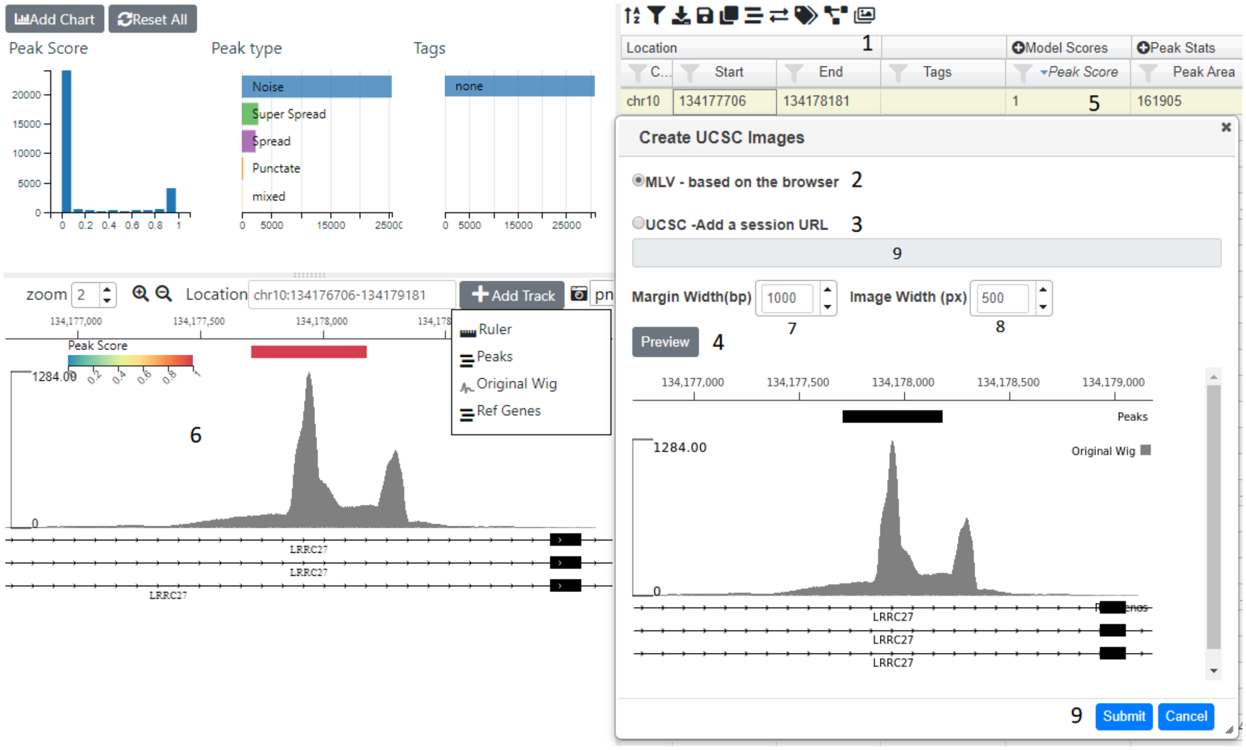
1.6 Tagging Locations



Sometimes it may be useful to catgeorise or tag the genomic location based on a trend theat that you have discovered. This can be done by opening up the tagging dialog with tag icon (1) in the menu above the table. Initially only the none category is present. To add other ones type a name in the text box (2) and press the add button (3) . The category will then be added to the list at the top of the dialog. By selecting the radio button next to it, then clicking on an image or a cell in the tagging column in the table will tag that genomic location. Multiple locations can be tagged by clicking and image/cell and the shift clicking another one and all the images/rows in between will be tagged. The 'Tag All' will tag all the currently filtered locations with the currently selected catogry. Another way to tag is to use the arrow keys to go to the next previous image/row and then press the shortcut key shown in brackets next to the category to tag the currently selected items with that category. The category color can be changed by clicking on the appropriate color chooser (7). The category can be removed (which will remove all tags of this category from the data using the trash icon next to the category (8)

N.B. To permanantly save the tags press the Save button (5) which will commit the changes to the database.

1.7 Adding Images



Location				Model Scores	Peak Stats
C...	Start	End	Tags	Peak Score	Peak Area
chr10	134177706	134178181		1	161905

Create UCSC Images

● MLV - based on the browser

○ UCSC -Add a session URL

Margin Width(bp) 1000

Image Width (px) 500

zoom 2

Location chr10:134176706-134179181

Images for every genomic location can be added to the project and then displayed in the table. The icon (1) opens up a dialog where you can choose to either have images created based on the internal browser (2) or by the UCSC browser (3). with the USCS option, you can have more detailed images, but is image generation is much slower and you are limited in the number of images you can create. One option is to create a smaller subset (see *Creating Subsets* and then produce images from this.

1.7.1 MLV Images

Clicking on the Preview button (4) will show a preview of the image for the currently selected row (5). The image is based on the tracks and settings in the browser (6) see *The Genome Browser* on how to add tracks and alter their appearance. You can adjust the image width and the width of margins shown either side of the genomic location by using the apprpriate spinners (7 and 8). Once you are happy with image you can press submit button (9) and images for all genomic locations will be created. This will take a few minutes (approx 800 images/min).

1.7.2 UCSC Images

Clicking on the UCSC radio (3) will enable the the URL input (9) where you can paste a UCSC browser URL or session. Pressing preview will check the url is valid and produce an image based ob the margin width (7), image width(8) and selected gemomic location (5). If a preview was sucessfuly produced then you can press the submit button (9) to generate images for all genomic locations. This will take quite a while.

An email will be sent when all images have been generated. You can then view the table in image or thumbnail mode (see *Table Mode*) and upload the project to Zegami

1.8 Running Analysis Jobs

Analysis jobs are run in the background on the server and the results, in the form of tracks, graphs and extra columns in the table are added to the project once the job is complete. The following types of analysis are possible:-

- **Annotation Intersection** - calculates whether each location overlaps a set of annotations or locations from another project.
- **Find TSS Distances** - calculates whether each location overlaps a Transcription Start Site (TSS) and if not, the distance to nearest site, either upstream (+) or downstream (-).
- **Calculate Peak Stats** - calculates the area and max height of the signal from a bigWig file at at each location in the project.
- **Cluster on Columns** - carries out dimension reduction (UMAP,tSNE) on any number of given columns in the project.

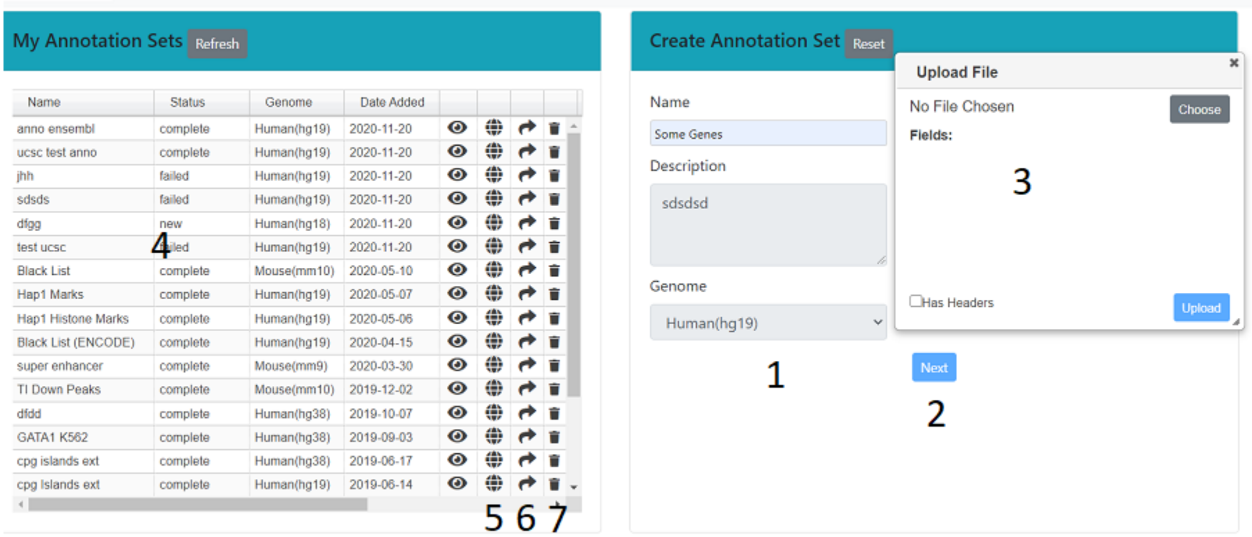
Jobs are run in the background and can be viewed on thr 'My Jobs' page (link in the top navigation bar) or in the history dialog (), which will automatically open when you send a job. You do not need to stay on the page whilst jobs are running, although if you do when the job is finished, you will be notified and the appropriate results loaded in.

1.8.1 Annotation Intersection

Intersections can be carried out between an Annotation Set, which us basically just a list of genomic locations, or another project. In the simplest case, a single column will be added, with TRUE/FALSE values, depending on whether a region in your project overlaps with a region in the query set.

Annotation Sets

Annotation sets are just lists of genome locations and can be created by clicking on 'Annotations' in the upper panel of my projects page, which will open up the following page:-



Name	Status	Genome	Date Added				
anno ensembl	complete	Human(hg19)	2020-11-20	👁	🌐	➦	🗑
ucsc test anno	complete	Human(hg19)	2020-11-20	👁	🌐	➦	🗑
jhh	failed	Human(hg19)	2020-11-20	👁	🌐	➦	🗑
sdsds	failed	Human(hg19)	2020-11-20	👁	🌐	➦	🗑
dfgg	new	Human(hg18)	2020-11-20	👁	🌐	➦	🗑
test ucsc	failed	Human(hg19)	2020-11-20	👁	🌐	➦	🗑
Black List	complete	Mouse(mm10)	2020-05-10	👁	🌐	➦	🗑
Hap1 Marks	complete	Human(hg19)	2020-05-07	👁	🌐	➦	🗑
Hap1 Histone Marks	complete	Human(hg19)	2020-05-06	👁	🌐	➦	🗑
Black List (ENCODE)	complete	Human(hg19)	2020-04-15	👁	🌐	➦	🗑
super enhancer	complete	Mouse(mm9)	2020-03-30	👁	🌐	➦	🗑
TI Down Peaks	complete	Mouse(mm10)	2019-12-02	👁	🌐	➦	🗑
dfdd	complete	Human(hg38)	2019-10-07	👁	🌐	➦	🗑
GATA1 K562	complete	Human(hg38)	2019-09-03	👁	🌐	➦	🗑
cpg islands ext	complete	Human(hg38)	2019-06-17	👁	🌐	➦	🗑
cpg Islands ext	complete	Human(hg19)	2019-06-14	👁	🌐	➦	🗑

Name: Some Genes

Description: sdsdsd

Genome: Human(hg19)

No File Chosen

Fields:

☐ Has Headers

Fill in the name, description and genome in the right hand panel (1), then press next (2). A dialog will open (3), which allows you to upload a bed like file (see *Uploading a File*). In the left hand panel (4) is a list of all the annotation sets that you own. You can make these public (5), share (6) or delete them (7).

Another way to create an annotation set is within a project, > create annotation set. **N.B.** The set will be created from the currently selected (filtered locations), shown the top right hand corner. Again, the dialog allows you to fill in the name and description of the set and you can also check any columns that you want included in the set.

Intersections

> 'Annotation Intersection' will being up a table with all the Annotation Sets and projects that you are able to inteserct with. You can select single or multiple (ctrl or shift) sets/projects. If you select a single project/set then a dialog will ask you whether you want just a TRUE/FALSE column or whether you want extra columns, with information from the intersecting set.

Once an annotation set has run, columns will be added to the table, either a single TRUE/FALSE column for each intersecting set or the data columns you selected in the previous step. In addition, pie charts showing this TRUE/FALSE distribution will be added, along with a track for each intesecting set.

1.8.2 Find TSS Distances

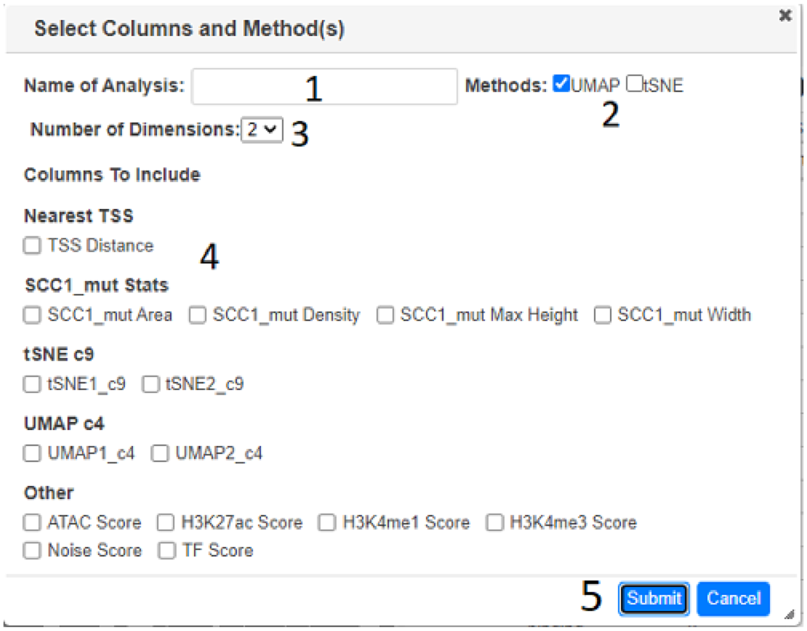
will bring up a simple dialog, with the only choice being whether you want to include Gene Ontolgy annotations. These are taken from the go-basic.obo file (http://geneontology.org/) and collpsed, such there is only one term (the most frequent) at each hierarchical level. If you include GO annotations, you can choose upto which hierarchical level you want.

Once the job has run four columns will be added to the table

- TSS distance - the distance to the nearset TSS, + being upstream and - downstream
- Gene ID - the Genbank gene id of the nearest gene
- Gene Name - the common name of the nearest gene
- Ovelaps TSS - either TRUE or FALSE depending on whether the region overlaps the TSS

1.8.3 Cluster on Columns

In order to get a better handle on the data it may be useful to collapse some of the fields into two (or more) dimensions, such that they can be visually clustered in a 2D scatter plot. To do this click on the cluster icon () above the table, which brings up the dialog below.



Select Columns and Method(s)

Name of Analysis: ______ Methods: ☑ UMAP ☐ tSNE

Number of Dimensions: 2

Columns To Include

Nearest TSS
☐ TSS Distance

SCC1_mut Stats
☐ SCC1_mut Area ☐ SCC1_mut Density ☐ SCC1_mut Max Height ☐ SCC1_mut Width

tSNE c9
☐ tSNE1_c9 ☐ tSNE2_c9

UMAP c4
☐ UMAP1_c4 ☐ UMAP2_c4

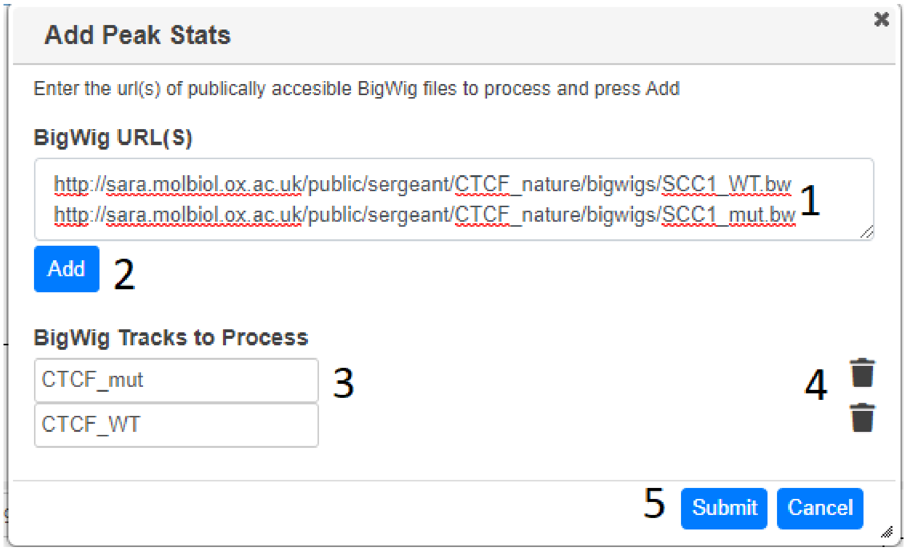
Other
☐ ATAC Score ☐ H3K27ac Score ☐ H3K4me1 Score ☐ H3K4me3 Score
☐ Noise Score ☐ TF Score

Type a name in the text box (1) and select the dimension reduction methods required (2). The number of dimensions can also be increased (default 2) using the dropdown (3). All numerical fields in the analysis are displayed (4), check all the ones to be used in the analysis and then press submit (5).

The outputs are columns for each dimension for each method named '<method><number>_<anlysis_name>' e.g tSNE1_anal1, tSNE2_anal1 etc. For each method, a scatterplot of the first two dimensions will be also be added. You can change these scatter plot e.g color by a specific field (see *Scatter Plot*) or use the dimensions to add another graph (see *Adding a Chart*)

1.8.4 Calculate Peak Stats

If you have bigWig files and you want to find out the peak area/height in each of the genomic locations in your project, use the icon which brings up the dialog below.



Add Peak Stats

Enter the url(s) of publically accesible BigWig files to process and press Add

BigWig URL(S)

http://sara.molbiol.ox.ac.uk/public/sergeant/CTCF_nature/bigwigs/SCC1_WT.bw
http://sara.molbiol.ox.ac.uk/public/sergeant/CTCF_nature/bigwigs/SCC1_mut.bw

BigWig Tracks to Process

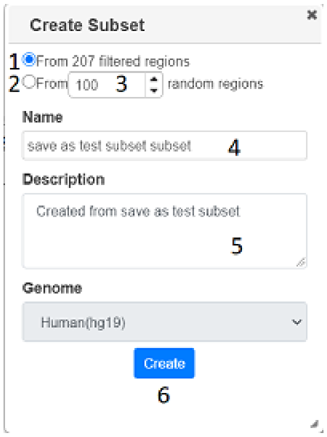
CTCF_mut
CTCF_WT

Paste the url (or a list of urls) corresposnding to bigwig files you want to analayse in the text box (1) and press Add (2). If the bigWig files can be located and they are the correct format, they will be added to the “bigWig Tracks to Process” section (3)in the dialog. The name is taken from the file name, but this can be changed. When you have specified all the bigWig files required, press submit The area, max height, width and density (area/width) in each location will be calulated. You do not have to stay on the page whilst the stats are being calculated.

When complete, columns will be added to the table with the relevent information and each bigWig track will be added to the Browser. The bigWig tracks are added with default settings, so you may need to change them to suit your needs (see *Altering Track Appearance_*). Note it just calculates the amount of signal in each region and reports the width of the region, it does not try and call peaks and work out the the width of the peak.

1.9 Creating Subsets

Clicking on brings up a dialog, which allows you to create a subset of the currently selected locations



Create Subset

1 ● From 207 filtered regions
2 ○ From 100 3 random regions

Name: save as test subset subset

Description: Created from save as test subset

Genome: Human(hg19)

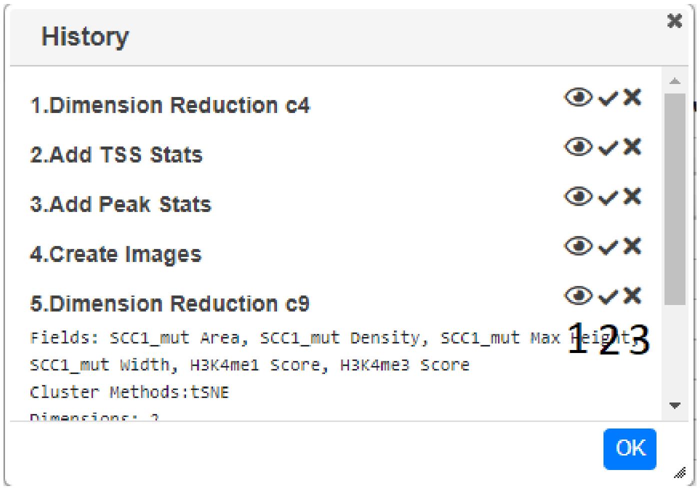
You can choose to create the subset either from the currently filtered locations (1) or from a random subset (2) with a specified number (3). After filling in a name and description (4) and (5), press ‘Create’ and the subset will be created. Once this has happened, you will get a link to the subset. You can create a susbset of any project you have viewing rights too, including public projects, and you will be the owner of the subset. All graphs/tracks/columns are copied, although the graphs may look different as there will be fewer locations in the new project.

1.10 Exporting Data

Click on the download icon to download the currently filtered locatons. The data is just downloaded as a text file, although you get a choice to download in either tsv or csv format. Only the currently displayed columns will be downloaded, so expand any column groups by clicking the plus icon if you want these in your file. If you have images in your project, you can export the data to Zegami. Click on the Zegami icon and a dialog will appear, wher you have to fill in your zegami username, project id and password. Once the project is created, you will emailed a link to the project

1.11 Project History

Every action such as adding a graph/track/column is recorded and be viewed by opening the history dialog ()



1.Dimension Reduction c4

2.Add TSS Stats

3.Add Peak Stats

4.Create Images

5.Dimension Reduction c9

Fields: SCC1_mut Area, SCC1_mut Density, SCC1_mut Max Height, SCC1_mut Width, H3K4me1 Score, H3K4me3 Score
Cluster Methods:tSNE
Dimensions: 2

Clicking on the eye icon (1) will toggle information about the action. The second icon (2) shows the status of the action, a tick means it is complete, a spinning circle shows that it is still processing an an exclamation mark showd there was an error whilts trying to perform the action. If you have edit permissions you are able to undo the action (N.B. there is no redo action). This will remove the action from the dialog and remove any tracks,columns or graphs that the action generated. If columns are removed, than any graphs which use these columns will also be removed, even if they were not added by the action.

1.12 Permissions

There are two types of permission for a project, view and edit.

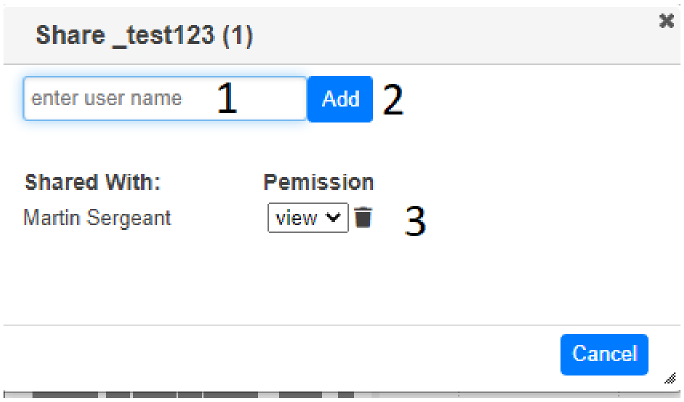
If you have a view permission for a project (anyone has view permission for a public project), you can open the project and add tracks and charts, as well as edit existing charts and tracks. However, you cannot save any updates or run any jobs such as finding TSS's or creating images. If you want to do this, you will have to copy the project (you need to be logged in) - click the disk icon () and the select 'save as'. This will clone the project in your name and then you can make any changes you wish.

If you have editing rights to a project you can make any changes you want , run any jobs and save the layout . You automatically have editing rights to a project you own it or if you are an adminisrator. You can also be assigened editing rights to a project (see below)

The icon on the menu allows you to share the project with another user and assign them view or editing rights

1.12.1 Sharing a Project

Click on share icon () above the table and select 'share project'



Share _test123 (1)

Shared With: Martin Sergeant

Pemission: view

Start typing the name of user you want to share the project with in the text box (1) and then select the name from the drop down. When you press 'Add' (2), the project will be shared and the name of the user will be added to the dialog (3). You can change the editing rights of the user to view or edit using the dropdown (4) or stop sharing by pressing the trash icon (5)

1.12.2 Making a Project Public

Click on share icon () above the table and select 'share project', you will be prompted to see whether you really want the project to become public. If you click OK then then anyone (including non users) will be able to view the project. You can share the project by sharing the link in the browser's address bar.

1.13 Submitting an Issue

An Issue or question, can be asked within MLV (if you are logged in) using the help link in the top navigation bar > 'Send Question'. You can also submit an issue to he GitHub page

1.14 Frequently Asked Questions

1.14.1 Can MLV be viewed on a mobile/small screen device?

No. The whole idea is to see how each component ie. the graphs,tracks and images change as you filter the data, which would not be possible, if only one component was displayed at once. All panels and individual tracks/graphs/table columns/images can be resized, to get the exact layout that the user requires, rather than relying on adapative screen size techniques which limit viewing to a single compnent/panel on small screen sizes

1.14.2 Can I upload a bigWig file?

Not initially. The only files that can be initially uplaoded are bed or bed like files with genomic locations. However, bigWig files can be added to the browser and uplaoded for processing later (see *Calculate Peak Stats*). Another application, Lanceotron does take bigWig files and identifies peaks based on machine learning.

CHAPTER 2

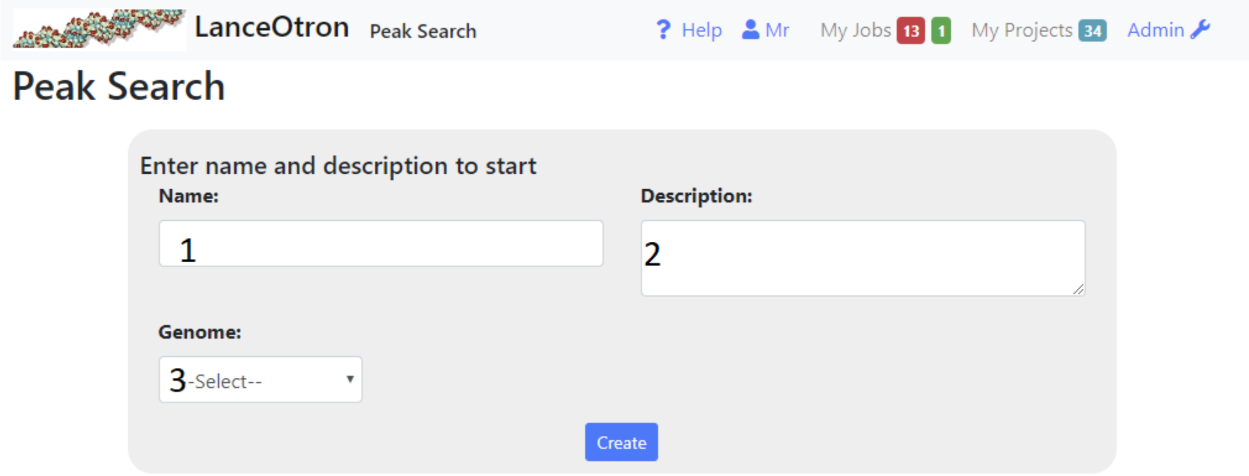
Lanceotron

2.1 Peak Search

2.1.1 Summary

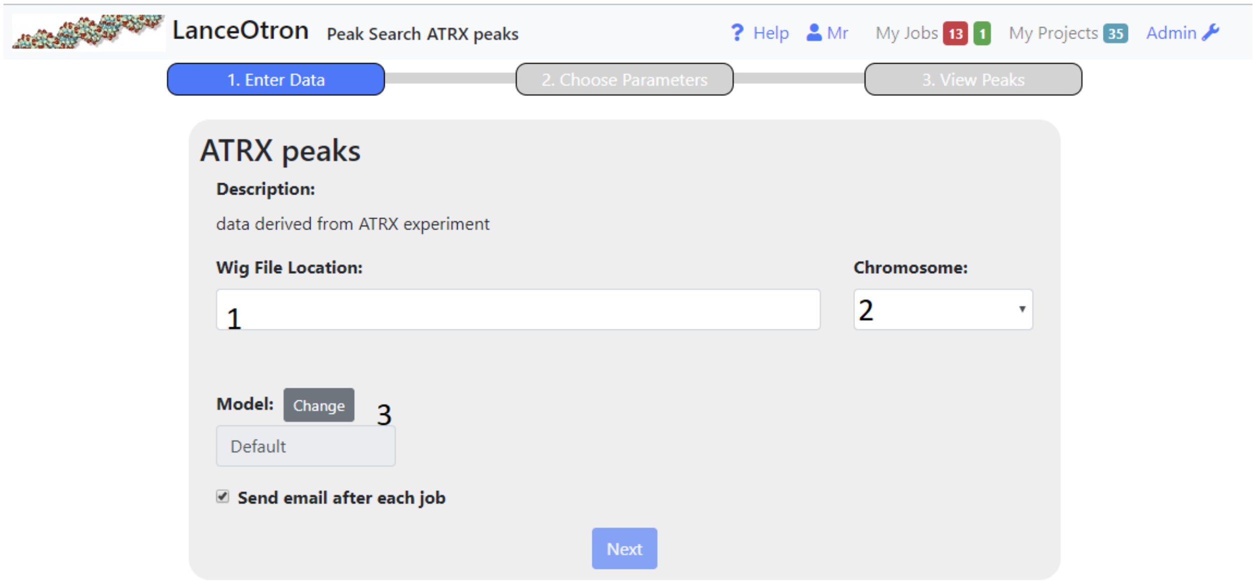
Peak Search allows you to call peaks from a wig file and classify them based on shape using a machine learning approach. Initially peaks are only called on a single chromosome with no classification. From this, the correct parameters can be chosen, which are used to call and classify peaks on the entire genome. The peaks can then be analysed using various tools, filtered and downloaded as a bed file.

2.1.2 Create the Project



1. Type the name and description of the project into the inputs (1 and 2 above)
2. Select the correct genome from the dropdown box(3). If the required genome is not available, select 'other'. In this case, certain features will not be avaialable, such as finding the distance to TSSs and annotation intersections.

2.1.3 1.Enter Details



ATRX peaks

Description:

data derived from ATRX experiment

Wig File Location:

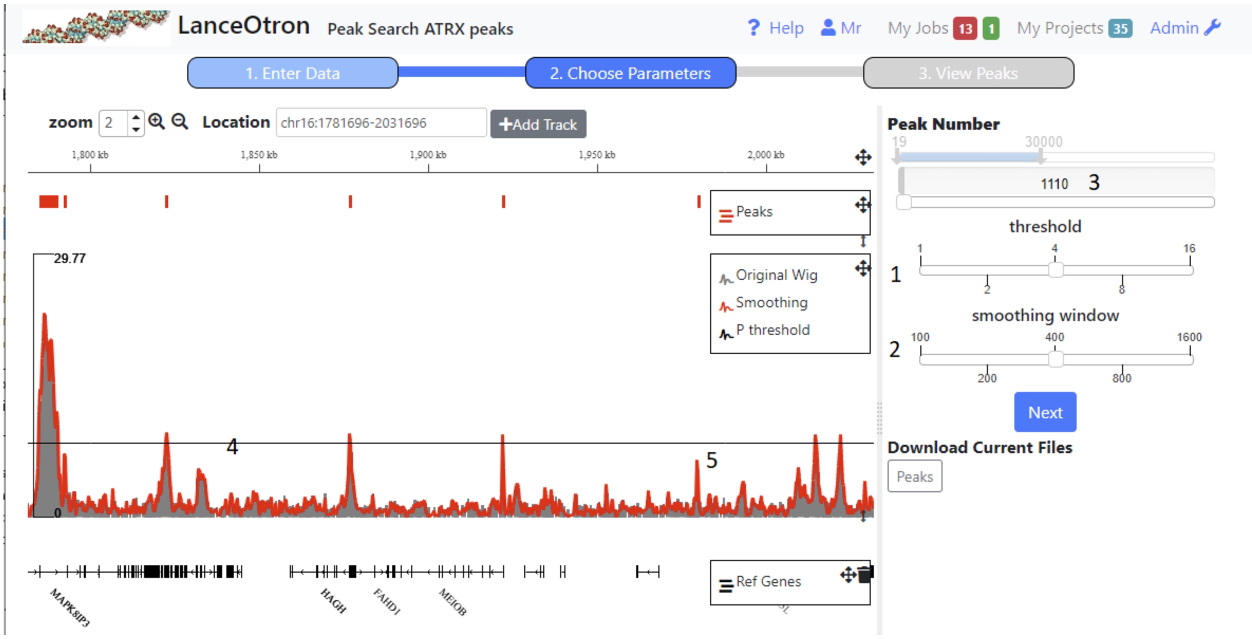
Chromosome:

Model: Default

☑ **Send email after each job**

1. Enter the http address of a publically accessible bigwig file into the text box(1). Once you have pressed enter or lost focus, the path entered will be checked to see if it is indeed a bigwig file and then the chromosomes displayed in the right hand drop down(2) for you to choose from.
2. Choose the chromosome from the dropdown(2) for the initial peak calling. Preferably the one you are most familiar with, as this will aid when choosing parmameters in the next step
3. Choose the model that will be used to assign peak scores, in most cases the default model will suffice.
4. Once you have entered the correct information, the next button should become enabled and you can proceed with the next step. The web page need not be kept open (although it will update once the processing is complete) . If you checked the appropraite box an email will be sent to you when the processing is complete.

2.1.4 2.Choose Parameters



zoom 2

Location chr16:1781696-2031696

Peak Number 1110

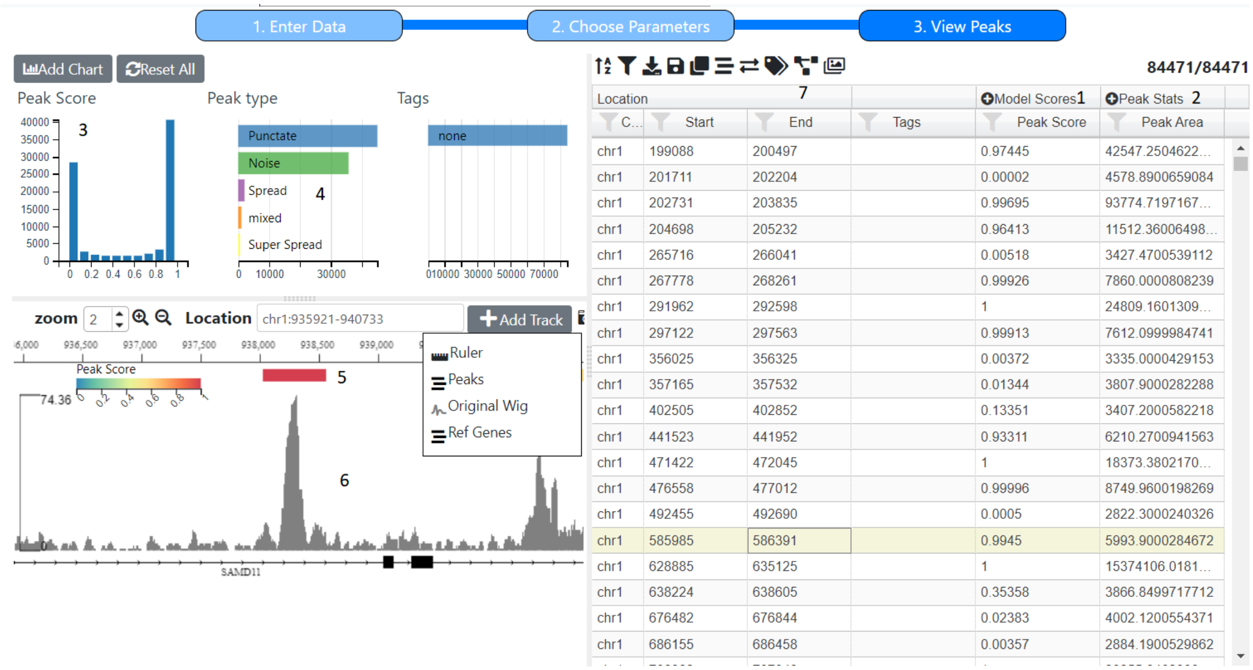
Once peaks have been generated for each combination of parameters (threshold and smoothing window) for the chromosome chosen, you can choose which ones to use to call and classify peaks genome wide.

1. Using the sliders on the right (1,2) will update the browser showing the peaks called using the selected parameters.Navigate to an area you are familiar with and choose the parameters, which result in sane peak calls. You can only continue when the total peak number is less than 30000.

   - **smoothing window** This is represented by the red line in the browser. The greater the smoothing, the flatter the line becomes, resulting in fewer broader peaks.
   - **threshold** The black horizontal line(4) in the browser represents the threshold. Peaks are called where the smoothing line bisects the threshold. Thus a higher threshold will result in only the taller peaks being called.N.B. When zoomed out in the browser, due ti the nature of bigwig files, peak height is an average of the region and hence where the smoothing line crosses the threshold does not always tally with peaks called (see 5 above) .You will need to zoom in to get a more accurate representation.
   - **Peak Number** This shows the number of peaks that have been called on the chromosome with the parameters selected. You can also use the peak number slider to see the effect this has on the parameters and peak calling (shown in the browser)

2. Once you are satisified with the parameters, pressing next will trigger peak calling/classification genome wide. Again, you can navigate away from the page whilst this is ocurring and you will be sent an email when the the process is complete

2.1.5 3. View Peaks



Location 7				Model Scores 1	Peak Stats 2
C...	Start	End	Tags	Peak Score	Peak Area
chr1	199088	200497		0.97445	42547.2504622...
chr1	201711	202204		0.00002	4578.8900659084
chr1	202731	203835		0.99695	93774.7197167...
chr1	204698	205232		0.96413	11512.36006498...
chr1	265716	266041		0.00518	3427.4700539112
chr1	267778	268261		0.99926	7860.0000808239
chr1	291962	292598		1	24809.1601309...
chr1	297122	297563		0.99913	7612.0999984741
chr1	356025	356325		0.00372	3335.0000429153
chr1	357165	357532		0.01344	3807.9000282288
chr1	402505	402852		0.13351	3407.2000582218
chr1	441523	441952		0.93311	6210.2700941563
chr1	471422	472045		1	18373.3802170...
chr1	476558	477012		0.99996	8749.9600198269
chr1	492455	492690		0.0005	2822.3000240326
chr1	585985	586391		0.9945	5993.9000284672
chr1	628885	635125		1	15374106.0181...
chr1	638224	638605		0.35358	3866.8499717712
chr1	676482	676844		0.02383	4002.1200554371
chr1	686155	686458		0.00357	2884.1900529862

The final screen shows a summary of the peaks that were called and the scores that were assigned to them with the model used. The screen is based charts, a table and browser, see *Adding Graphs/Charts*

Model Scores

These are the scores assigned by the machine learning model. The Peak Score, shown in the table (1) and histogram (3) is just the sum of all the scores for 'peaks', which in the case of the default model is all scores except Noise. Individual peak scores (H3K4me1,TF etc) and peak types can be displayed in the table by clicking on the icon in the Model Scores column (1) which will expand the column.. The Peak Type is calculated using simple rules based on the scores, for example the default uses the following rules:-

- Super Spread if H3K4me1 > 0.5
- Spread if the sum of H3K4me1, H3K4me3 and H3K27ac > 0.5
- Punctate if TF and ATAC > 0.5
- Noise if Noise > 0.5
- Mixed if none of the above apply

Peak Stats

For each predicted peak, the width, max height, area and density (area/width) are calculated. To see them all click on the icon in the Peak Width column (2).

Tracks

Initially there will be two tracks are the original wig file (6) that was uploaded and the peaks identified (5). Other tracks can be added (see *Adding Tracks*). The peaks tracks is coloured by the peak score but can this can be altered using the icon in the browser tool bar

Charts

Initialy a hostogram of peak socre (3) and row chart of peak types (4) and current Tags are shown, however other charts can be addeed (see *Adding a Chart*). For example you could create a scatter plot of peak height X peak width.

Clustering

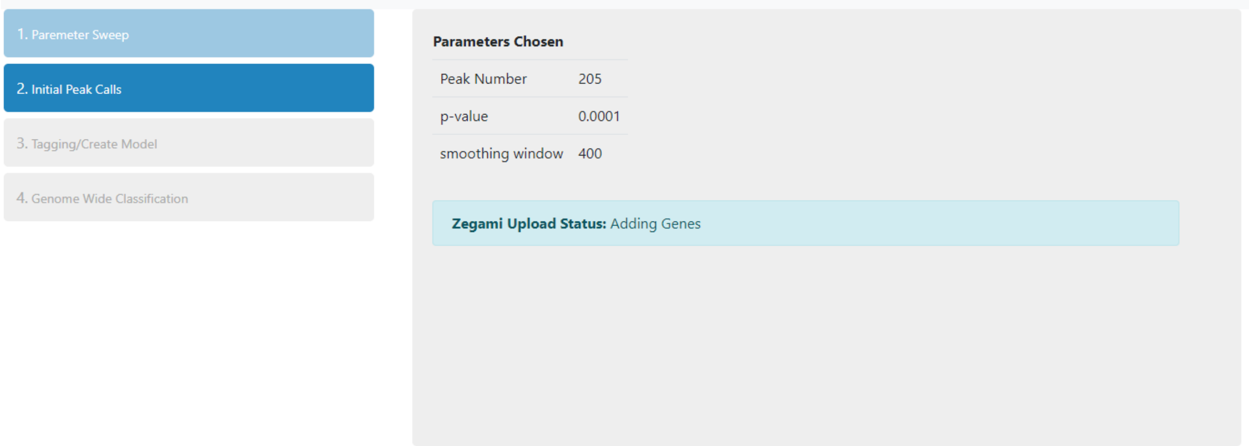
In order the peaks will be clustered based on their shapes . The brings up a dialog . You can choose UMAP tSNE or PCA You don't have to remain on the web page. Once completed, graphs see (mlv- see *Scatter Plot*) will be added to the as well as columns with the clustering values.Clusters can then be selected by dragging a region

Other

You may want peaks you thing are good or bad (see *Tagging Locations*) These tags can then be used to create a model This can be aided by creating images for all the candidate peaks

Choosing Parameters

Processing Peaks



Parameters Chosen

Peak Number	205
p-value	0.0001
smoothing window	400

Zegami Upload Status: Adding Genes

Once the parameters have been chosen and submitted, the following steps will be carried out on all the peaks identified on the selected chromosome:-

- Peak statistics (width, height, area) are calculated
- Overlap with any genes, annotations are calculated
- Thumbnail created for each peak
- tSNE and PCA carried out bases on peak shape
- Peaks are classified using the default model and assigned a Peak and Noise score
- Images are uplaoded to Zegami and a collection is created

You will be informed about which stage is currently running. You do not have to remain on the page, but can return to it later to see if all the processing has been completed.

Also, whilst the above is being carried out, peaks for the whole genome (not just the specified chromosome) will be called and classified according to the parameters initially chosen and the default model.

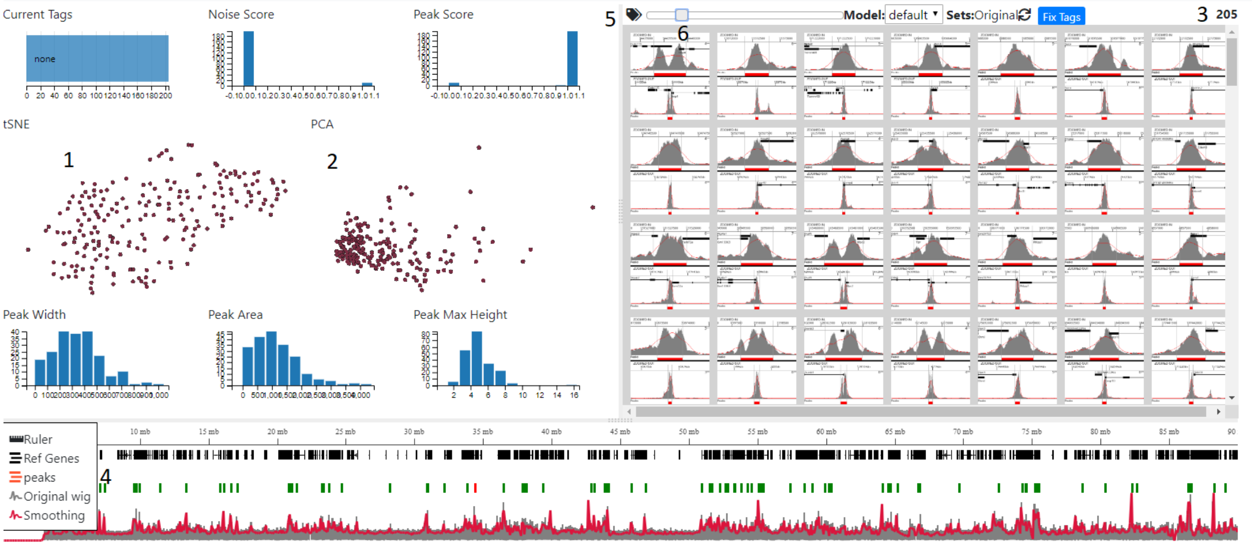
Once processing is complete, you will have access to the Tagging/Create Model and Whole Genome Classification tabs.

2.1.6 TaggingCreating Model

Tagging Using MLV

General Navigation

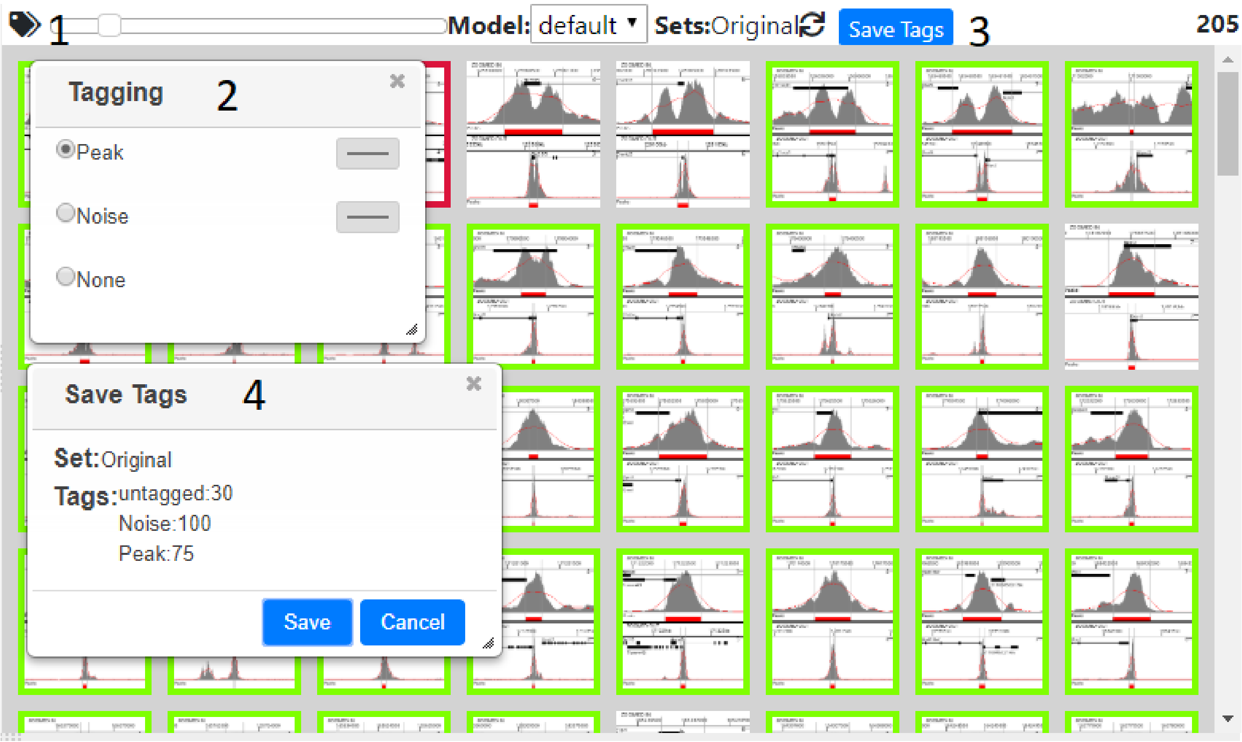
Press the 'Tag With MLV' button ob the Tagging/Create Model panel and you will be taken to the MLV page



Initially all the peaks will be shown in the right-hand tab and the the total number on the far right (3) The graphs on the left show peak statistics, clustering (tSNE/PCA) and also scores given to the peaks using the default model. In addition, overlap with any annotations will also be shown in pie charts, if this was specified.

Peaks can be filtered by selecting areas on the graphs in the left hand panels. Areas in the scatter plots can be selected by pressing shift and dragging the mouse. Normal dragging will cause panning and zooming can be achieved using the mouse wheel. Filtered peaks are also reflected in the bottom browser window and are coloured by Peak score from green (score=1) to red (score =0).Image size can be altered using the slider (6)

Tagging Images

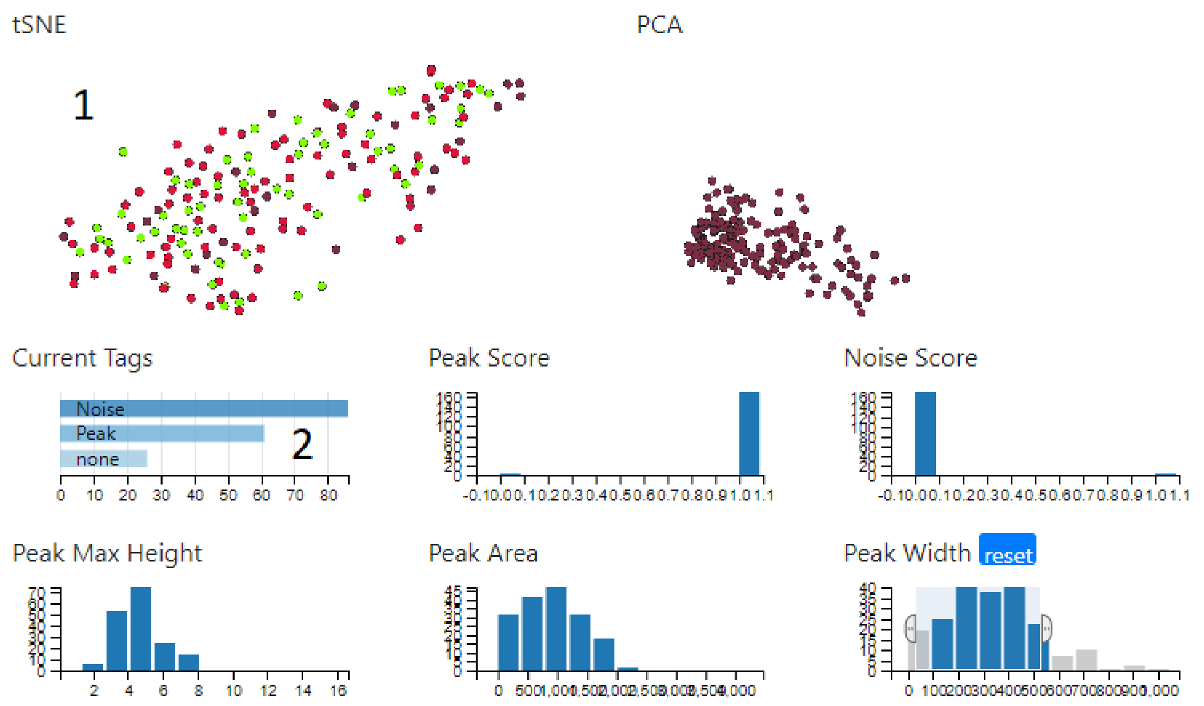


Set:Original

Tags:untagged:30
Noise:100
Peak:75

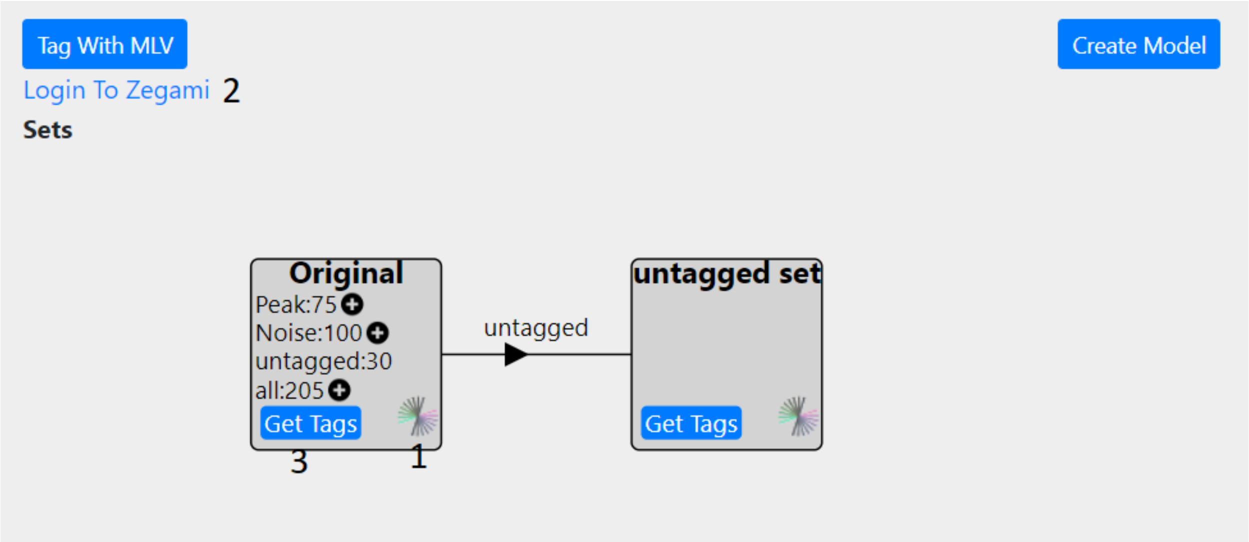
Pressing the tag icon (1) will open the tagging dialog (2), now clicking on an image will then tag that image with the tag selected in the dialog's radio buttons, or remove the tag, if none was selected. Multiple images can be tagged at once, by pressing shift and clicking, which tags all the images between the last and currently tagged item. Closing the tagging dialog will remove the coloured border from the image, but not remove the tags, and the images will become highlighted again once the dialog is reopened.

Tags can be saved at any time using the 'Save Tags' button (3). This opens a dialog (4) that shows the current set, which initially will be 'Original' and the number and type of tags. Pressing the save button will save/update the tags in the database and if enough tags are present allows a model to be created see *Classifying/Generating a Model*



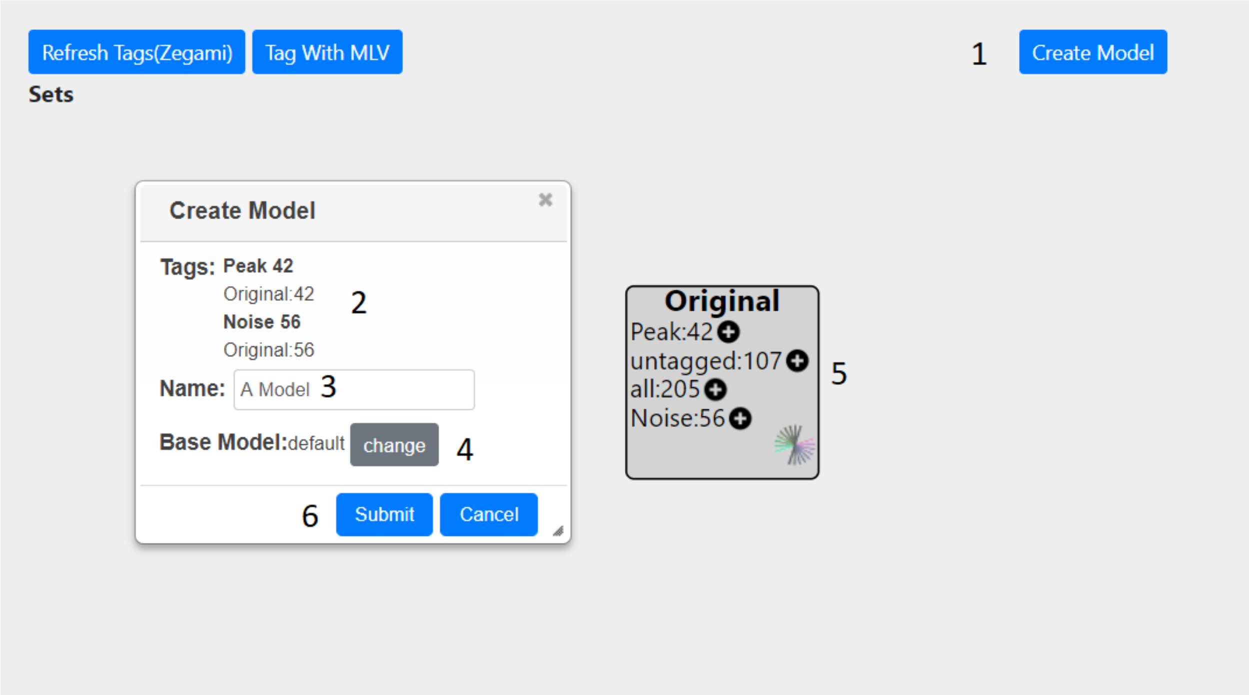
When the tagging dialog is open, the tSNE plot (1) will be coloured according to the type of tag. In addition, the 'Current Tags' bar chart (2) will show the number and type of tags in the current set. Clicking on a bar will select just those tags.

Tagging Using Zegami



On the main project page in the 'Tagging/Create Model' tabe, click the Zegami icon to go to the Zegami set (1). You need to login to Zegami first using the link on the page (2) with the correct password/username, otherwise a request error will be shown on the Zegami page. Then tag the sets in Zegami- see the Zegami web site for instructions on how to tag images. When you have finished tagging, you will need to return to the main project page to proceed and press the 'Get Tags' button (3). This will update the database with all the tags from Zegami and overwrite any existing onces. Once enough tags have been generated, a model can be created, see *Classifying/Generating a Model*

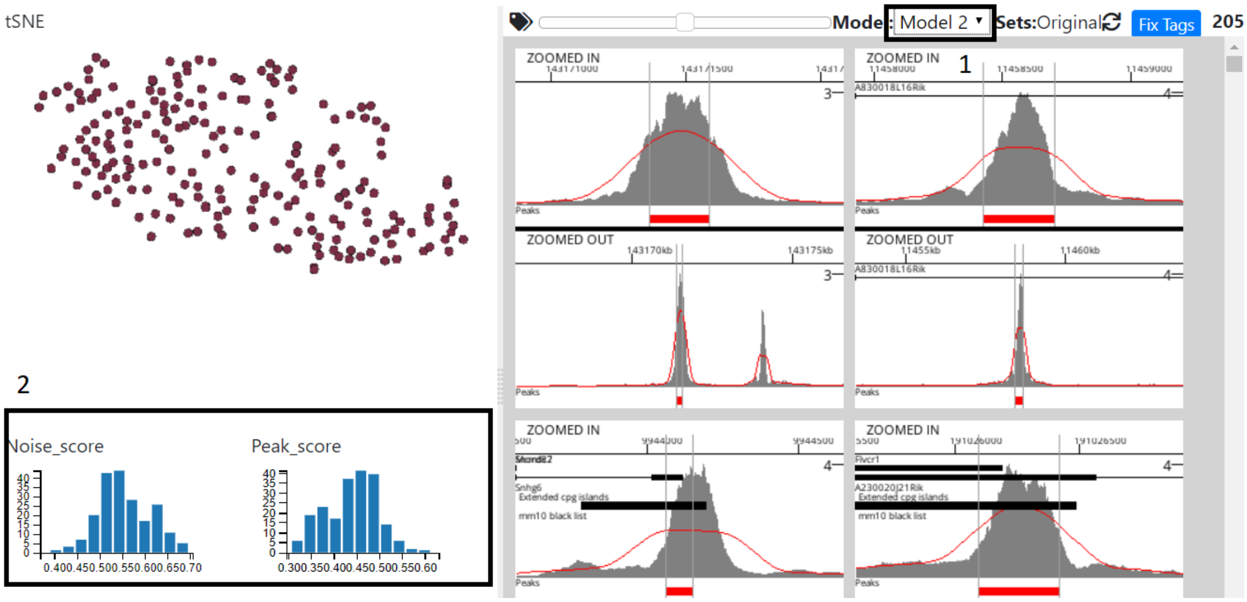
Classifying/Generating a Model



Pressing the ‘Create Model’ button (1) will bring up a dialog, showing the number and type of peaks which have been tagged (2) The source of these tags is also shown. In this example, only the original set is present (5) hence all tags are taken from this. If other sets are present, for each peak, the tag will be taken from the last created set, if there is not one present, then the next set will be examined for tag and so on until the original set is reached.

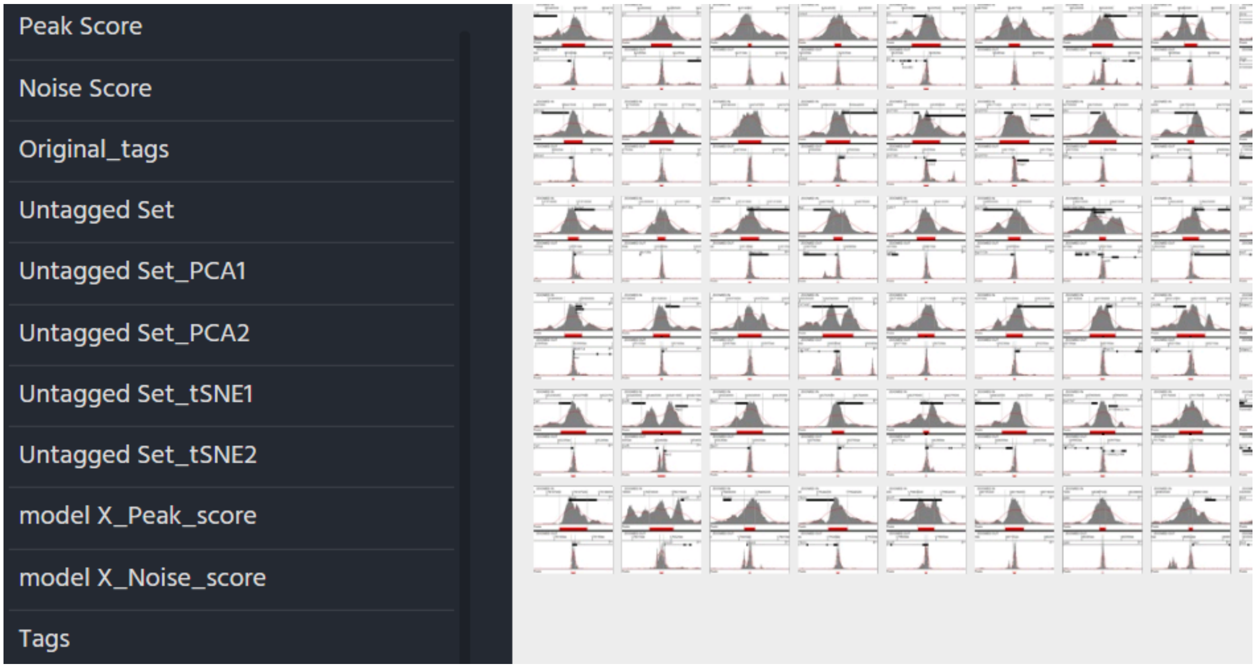
An appropriate name should be given to the model (3). The base model can also be changed from default to a previously created one (4). Finally, the model can be submitted(6) At the top of the screen, a message will show that the model is being created and a dialog will appear to inform you when the job is finished. Once the model has been created you can view it either in Zegami or MLV. The model can then be used to call peaks genome wide.

Viewing in MLV



Select the model in model dropdown (1) and the Noise,Peak Score graphs will update to reflect the new model

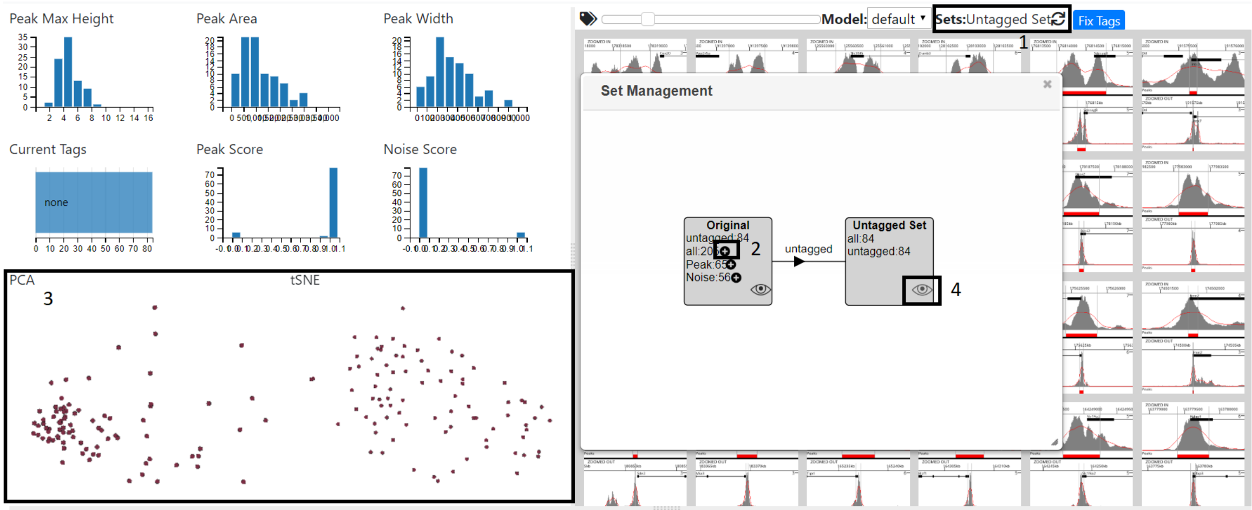
Viewing in Zegami



For each model created An extra field will be added to the original zegami collection with the title model_name tag score (see above)

Working with Sets

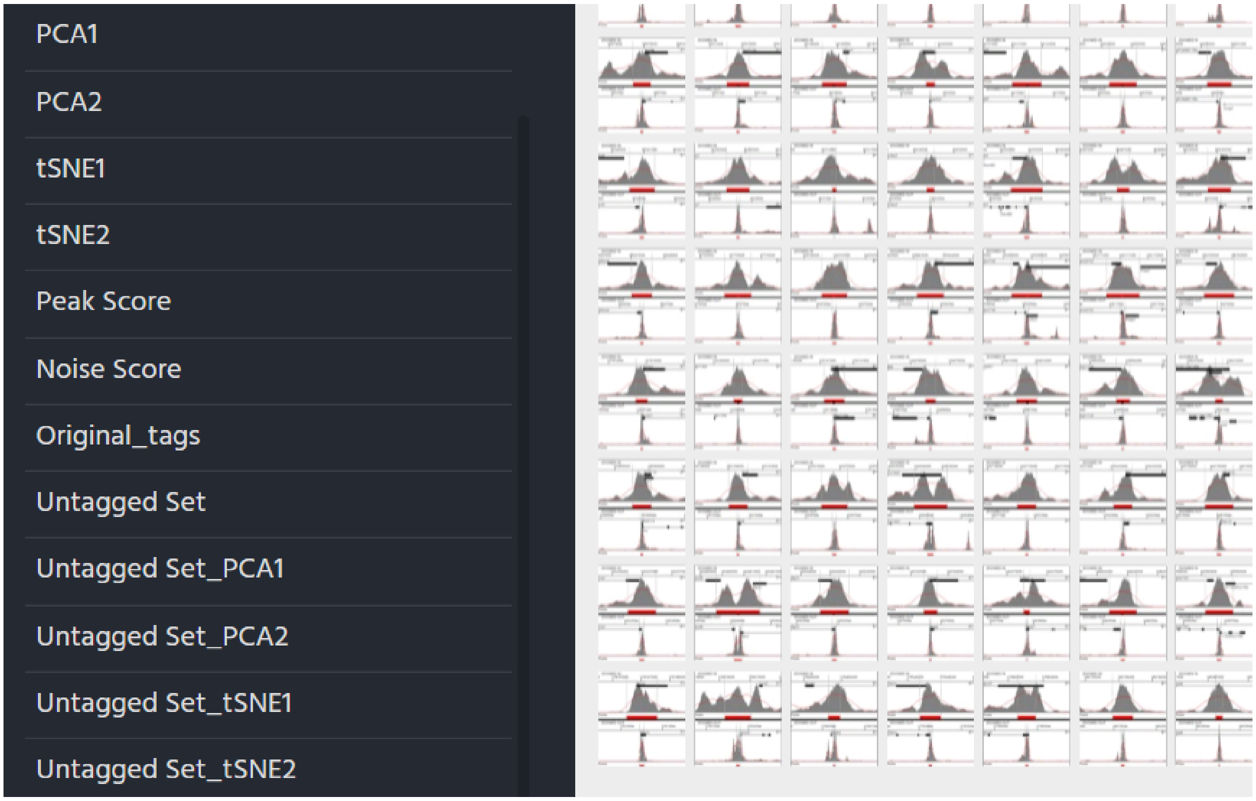
In MLV



The set dialog can be opened by clicking on the sets label (1). Once tags have been fixed, pressing the plus sign next to a tag (2) will create a new set. This may take a while as peaks are re-clustered. Once this is complete the view will automatically change to the reflect the set, with the charts showing the new clustering (3). You can than tag this set and fix the tags in the normal way - see *Tagging Using MLV* To switch between sets press the eye icon and the graphs and images will update accordingly. As the number of setsexpands, you can zoom (mouse wheel) and pan (mouse drag)in the diagram.

In Zegami

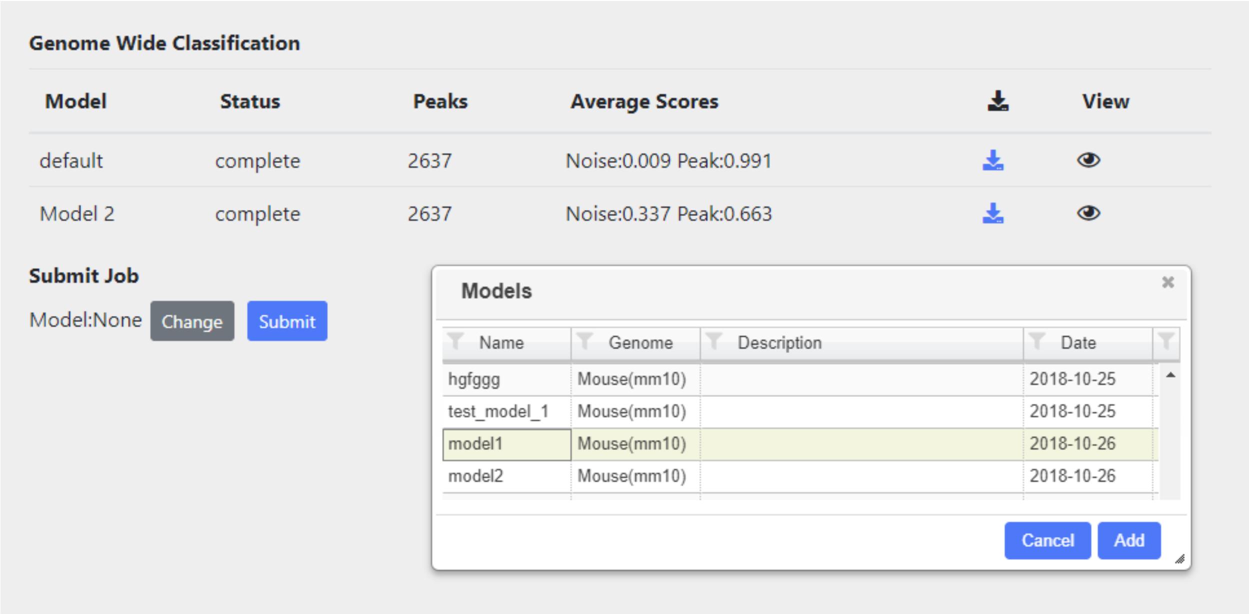
Sets can be created in the Tagging/Create Model tab of the main project page. Once tags have been fixed in a set, pressing the plus icon next to a tag will create a set from those tags (see above). Once the set has been created, it can be viewed in Zegami (using the zegami icon in the set), although you may have to wait a while for the set to be processed.



The new zegami collection will consist only of items in the new set and contain extra fields for PCA/tSNE1, which are prefixed with the set name.The set can be tagged and fixed in the normal way *Tagging Using Zegami*

For each set created, all the fields relevent to the sets (tSNE/PCA, tags) will also be added to the original Zegami collection (prefixed with the set name) allowing comparison between sets

2.1.7 Genome Wide Classification



Genome Wide Classification

Model	Status	Peaks	Average Scores	⤓	View
default	complete	2637	Noise:0.009 Peak:0.991	⤓	👁
Model 2	complete	2637	Noise:0.337 Peak:0.663	⤓	👁

Submit Job

Model:None

Models

Name	Genome	Description	Date
hgfggg	Mouse(mm10)		2018-10-25
test_model_1	Mouse(mm10)		2018-10-25
model1	Mouse(mm10)		2018-10-26
model2	Mouse(mm10)		2018-10-26

Peaks in the whole genome are tagged and classified according to the model chosen. Classification automatically runs for the default model. Other models can also be rub and these can be selected by using the 'change' button and then selecting the appropriate model from the table.

All classifications, either running or complete are shown in the table. Once complete the Average scores of each tag submiited will be displayed, as well as icon which allows downloading of a bigbed file containing all the peak calls and associated scores.

You can aslo view the results of the classification in a viewer by presssing the eye icon.

CHAPTER 3

CaptureSee

3.1 Summary

CaptureSee enables the user to browse highly multiplexed CaptureSee experiments Initially, the user uploads a list of genomic locations and associated data, which can then filtered, sorted and viewed. Operations such as finding the nearest TSS, intersections with other annotations/views can also be carried out. In addition charts can be created based on any the the parameters (or combinations of) and used to filter the data. Extra genomic tracks can be added and images generated for each location, so that trends in the data can be more easily recognised. Locations can be annotated (tagged) by the user and downloaded or exported to the data visualisation tool Zegami for further analysis.

3.2 Creating a Project

To create a project click on 'My Projects' (1) on the top navigation bar and then the Multi Locus View panel (2). This will take you to a page where you have to fill in the name and description of the project (3). You also have to select the gemome required. If the genome you want is not available select 'other'. In this case gene information and other annotations will not be available. When you press 'Create' you wll be taken to a page where you can upload your file (see below)

3.2.1 Uploading Data

Press Choose in the displayed dailog and select your file containing genomic locations. The file format is quite flexible and can be either tab(.tsv) or comma(.csv) delmited and also can also be gzipped (.gz) The only requirement is that the first three columns (in order) specify the genomic location i.e. chromosome, start and finish. Normal bed files fulfill these criteria as well as excel data that has been saved as a .csv or .tsv file. The file will be parsed and the column (feild) types will be ascertained.

CHAPTER 4

MLV Developer Documentation

4.1 Installing the Application

4.1.1 Clone from Git

```
git clone https://github.com/Hughes-Genome-Group/mlv.git
```

4.1.2 Setting up a Python Environment

MLV requires python 3.6 or later. One way to manage the python environment is to use use virtualenv and virtualenvwrapper.

```
pip install -U virtualenv
pip install -U virtualenvwrapper
```

Then add the following to your .bashrc (paths may vary)

```
export WORKON_HOME=$HOME/envs
export VIRTUALENVWRAPPER_PYHTON=/usr/bin/python3.6
. /usr/local/bin/virtualenvwrapper.sh
```

Next create a virtual environment and install all the required python modules:-

```
mkvirtualenv mlv
workon mlv
pip install -r requirements.txt
```

4.1.3 Installing Dependencies

The following programs need to installed and avialable in the path:-

- tabix and bgzip (https://github.com/samtools/htslib)
- rabbitmq-server (https://www.rabbitmq.com/download.html)
- bedtools (https://github.com/arq5x/bedtools2/releases)
- bedToBigBed,bigBedToBed and bigWigInfo (http://hgdownload.cse.ucsc.edu/admin/exe/)
- nodejs (https://nodejs.org/en/download/)
- nodejs modules najax, jquery,xhr2,extend and canvas
- NGINX (https://www.nginx.com/) or another http server (for production)
- PostgreSQL 9.5 or above (https://www.postgresql.org/)

4.1.4 Creating the Database

MLV requires PostgreSQL 9.5 or above, which can be runnning on the same or a separate server. The first thing to do is to create the system database and associated tables by running *create_system_db.sql* in *app/dyatabases/*. To do this via the the psql cosole, log in as a user with the correct permissions and run the following:-

```
createdb mlv_user;
\c mlv_user;
\i /path/to/app/databases/create_system_db.sql;
```

If your PostgreSQL instance does not have a suitable user, you need to create one and grant access to tables generated in the previous step.

```
CREATE USER mlv WITH PASSWORD 'pword';
GRANT SELECT, INSERT, UPDATE, DELETE ON ALL  TABLES IN SCHEMA public TO mlv;
GRANT SELECT UPDATE, DELETE ON ALL SEQUENCES IN SCHEMA public TO mlv;
```

You also need to change the DB parameters in settings.py (or a custom config)

```
DB_USER="mlv"
SYSTEM_DATABASE="mlv_user"
DB_HOST="localhost" #or host name
```

Make sure you allow the user to connect to the database in PostgreSQL's *hb_config*:-

```
#allow connection from another host
host   mlv_user,generic_genome   mlv    x.x.x.x/32 md5
#allow local connection
local  mlv_user,generic_genom mlv   md5
```

If the database is hosted on a different server, you may have to update the firewall settings on this server to allow the mlv server to connect to it on port 5432

Creating The First Users

To create a user you will need to run the appropriate script (see *Executing Scripts*) In order to do this, the app needs to know the location of the scripts module and also the password to the database. These can be stored in a secure file and added to the environment variables:-

```
export FLASK_APP=/path/to/install/app/commands/cli_commands.py
export DB_PASS=pword
```

Then you can add the guest user and an admin:-

```
flask find_or_create_user --firstname John --last_name Doe --email guest@somewhere.com
flask find_or_create_user --first_name Mr --last_name admin --email me@gmailcom \
                          --password password --admin True
```

Adding Genomes

Genomes are housed in separate databases to the system database. A single databases can hold many genomes or separate databases can be created for each genome (not recommended). A fallback genome called ‘other’ initially needs to be added. The following code creates a database ‘generic_genome’ and then adds the fallback ‘other’ and the C. Elegains(cel1) genome:-

```
flask create_new_genome_database -db_name generic_genome
flask add_new_genome --name other --label Other --database generic_genome
flask add_new_genome --name ce11 --label C. Elegans(ce11) --database generic_genome
```

4.1.5 Running and Serving the Application

For testing porposes the application can be run using the *run_app* script.

```
flask run_app --port 5000
```

This will run the application locally on port 5000. For production purposes it should be run through a web server gateway interface such a Gunicorn. For example, the following code will run the app locally on port 5000 using 3 threads.

```
/path/to/virtualenv/gunicorn "app:create_app('lanceotron_config')"\
-b 127.0.0.1:5000  \
--workers 3 \
--error-logfile /path/to/gunicorn.log
```

MLV should also be run through a webserver such as Nginx or Apache. Although static files such as images and tracks can be served through the app via flask, this is not recommended in a production setting. It is more efficient to serve such files from the webserver. An Nginx config to allow this is given below:-

```
#redirect to the flask app
location / {
    proxy_pass http://localhost:5000;
    proxy_read_timeout 180s;
    proxy_set_header   Host                 $host;
    proxy_set_header   X-Real-IP            $remote_addr;
    proxy_set_header   X-Forwarded-For      $proxy_add_x_forwarded_for;
    proxy_set_header   X-Forwarded-Proto    $scheme;


}
#directly serve any static files
location /static/ {
       alias /home/sergeant/mlv_dev/app/static/;
}
#temporary images
location /data/temp/ {
        alias /data/mlv/temp/;
```

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```
}
#static files belonging to a module
location ~ /(.*)/static/(.*) {
             alias /home/sergeant/mlv_dev/app/modules/$1/static/$2;
}
#genome tracks
location /tracks/ {
    alias /path/to/tracks;
}
#any images belonging to projects
location ~ /data/(.+\.(?:jpg|jpeg|gif|png))$  {
    alias /data/mlv/$1;
}
```

4.1.6 Running the Message Queue

MLV uses celery and rabbit-mq to queue time consuming tasks and run simple pipelines. Two queues need to initialised, a default queue for jobs whuch should return quickly and are required for the user to continue and a ‘slow queue’ for longer tasks and pipelines. To start the queues use the following commands:-

```
/path/to/venv/bin/flask runcelery
/path/to/venv/bin/flask runcelery --queue slow_queue
```

The default number of threads is 3, although this can be changed using the *–threads parameter* . For debugging purposes celery can be disabled by changing the config setting *USE_CELERY=False*, which will cause jobs to run directly in the flask thread and hence would be impractible for a production environment.

4.1.7 Using Supervisord

Supervisor (http://supervisord.org/) can be used to populate environment variables, and run the server and job queues as daemon threads. An example of a suitable config would be:-

```
[unix_http_server]
file=/path/to/supervisor.sock   ; (the path to the socket file)

[supervisord]
logfile=/path/to/supervisord.log  ; (main log file;default $CWD/supervisord.log)
user=username                     ; (default is current user, required if root)
directory=/path/to/root_dir       ; (default is not to cd during start)
environment=FLASK_APP="/path/to/root_dir/app/commands/cli_commands.py",\
        FLASK_CONFIG="my_config",\
        DATABASE_PASS="pword"

[supervisorctl]
serverurl=unix:///var/run/supervisor/supervisor.sock ; use a unix:// URL  for a unix␣
↪socket

[program:mlv]
command=/path/to/venv/bin/gunicorn "app:create_app('lanceotron_config')"\
                                    -b 127.0.0.1:5000 \
                                    --workers 3 \
                                    --error-logfile /path/to/gunicorn.log
autostart=true
```

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```
autorestart=true

[program:celery]
command=/path/to/venv/bin/flask runcelery
autostart=true
autorestart=true

[program:celery_slow]
command=/path/to/venv/bin/flask runcelery --queue slow_queue
autostart=true
autorestart=true
```

4.2 Config Settings

The main config is *settings.py* in the main app directory. Another config, which can add or override variables in *settings.py* can be specified either in the *create_app* method or in the environment variable FLASK_CONFIG. The following shows the config variables which may need to be changed.

4.2.1 Database Settings

- **DB_HOST** The database host, either a server name or an IP address. By default, the environment variable DB_HOST will be used or localhost if it is not set.
- **DB_USER** The name of the database user, mlv by default.
- **SYSTEM_DATABASE** The name of the system/user database, mlv_user by default.
- **DB_PASS** The database password, set to the environment variable DATABASE_PASS by default (passwords shouldn't be stored in the config)

4.2.2 Folder Locations

- **DATA_FOLDER** The location to store all the data, can be a mapped drive
- **TEMP_FOLDER** The location to store temporary data. Data here can be deleted
- **TRACKS_FOLDER** The location to store and serve genome tracks (BigWigs,BigBed files etc)

4.2.3 Message Queue (Celery) Settings

- **BROKER_URL** The message queue url. By default is the localhost but potentially it could be a different server
- **USE_CELERY** The default is True, only set to False when debugging

4.2.4 App Settings

- **HOME_PAGE** the url of the home page of the application
- **HOST_NAME** the name of the machine hosting the app
- **APPLICATION_NAME** The name of the application

- **APPLICATION_LOGO** The url of the application logo
- **MODULES** A list of modules to load. [“multi_locus_view”] by default

4.2.5 Email Settings

- **MAIL_SERVER** The email server, smtp.gmail.com by default
- **MAIL_PORT** The email server port, 587 by default
- **MAIL_USE_SSL** False by default
- **MAIL_USE_TLS** True by default
- **MAIL_USERNAME** The mail username . The dummy entry mlv@gmail.com is the default.
- **MAIL_PASSWORD** password by default
- **HELP_EMAIL_RECIPIENTS** A list of email addresees to which user questions are sent (an empty list bt default)
- **MAIL_DEFAULT_SENDER** The name to attach to emails that are sent out. ‘The MLV Team’ by default.

4.2.6 Misc. Setting

- **SECRET_KEY** Enables secure password hashing, should be sent to large random string
- **JS_VERSION** Should be changed each time a new version is rolled out as this will cause existing js and css caches on the user’s computers to be refreshed.

4.3 Executing Scripts

To run any script requires the environment variable FLASK_APP to point to the module *cli_commands.py*. Other environment variables that may be required include FLASK_CONFIG, which points to a custom config and DATABASE_PASS, which holds the database password.

```
export FLASK_APP=/path/to/install/app/commands/cli_commands.py
export FLASK_CONFIG=linux_test_config
export DATABASE_PASS="pword"
```

4.3.1 Writing scripts

To write a script, simply import app from *cli_commands.py* and wrap your code in the app context.

```
from app.commands.cli_commands import app
from app.jobs.jobs import get_job
from appp.ngs.project import get_project

with app.app_context():
    j=get_job(1234)
    j.process()
    p=get_project(4321)
    p.delete(True)
```

4.3.2 Built in Scripts

There are a number of utility scripts in *cli_commands.py* that can be run with

```
flask name_of_script --param value
```

create_new_genome_database

creates a new empty genome database.

- - -*db_name* - The name of the database

add_new_genome

Adds a genome to the specified database. If the name matches a public genome in the UCSC genome browser, the RefSeq genes and chromosome file will automatically be added. Oterwise, the chromosome file (tab delimited chromosome to length) can be added manually to *data_root/<genome_name>/<genome_name>/chrom.sizes*.

- - -*name* - The name of the database (required)
- - -*label* - The label e.g. Human(hg19) (required)
- - -*icon* - The url of an icon to represent the database (24px x 24px). If not supplied a default icon will be used
- - -*database* - The name of the database to store the genome in (required)
- - -*connections* - The numner of connections optional - default 5

run_app

Runs the app on the local host.

- - -*port* - The port

runcelery

Runs the message queue

- - -*queue* - The name of the queue . the default is celery, the other oprion is slow_queue
- - -*threads* - The number of threads to give the queue - optional (default is 3)

remove_deleted_projects

Removes all projects (and associated jobs) that are tagged as deleted. All data associated with the project is permanantly deleted.

check_all_jobs

Calls *check_status* on all running jobs. This is not required for jobs in the local queue, only those running on remote servers that have their *check_process* method overwritten. In which case this script should be run at frequent time intervals e.g. in crontab

find_or_create_user

Manually adds a user to the database

- - -*first_name* - The user's first name (required)
- - -*last_name* - The user's last name(required)
- - -*email* - The user's email(required)
- - -*password* - The user's password (required)
- - -*admin* - If True,true or TRUE,the user will habe admin rights (default False)

4.4 Modules

Modules are a way of creating independent applications with discrete templates (html), static files (js,css and images) and python modules. A module can be added to a system by simply adding the module folder to the *app/modules* directory and then adding the name of the folder(module) to the MODULES list in the app's config.

4.4.1 Folder Structure

```
app
|--modules
   |--module_name
      |--jobs
      |--projects
      |--static
      |--templates
      |--__init__.py
      |--config.json
```

4.4.2 Templates

The templates folder should contain subfolders named after each project in the module. Each subfolder should contain *home.html* (see *Project Home Page*) as well as any other templates required by the project. Templates are referenced in the normal way. e.g the file *template.html* in the subfolder *project1* of the *templates* directory

```
/app/modules/<module_name>/templates/project1/template.html
```

would be referenced in the view method as:-

```
get_template(self,args):
    return "project1/template.html"
```

4.4.3 Static Files

Any static files (js,css,images) go in the static subfolder of the module and can be referenced from template files by prefixing the module name before static e.g. the js file

```
/app/modules/static/myjsfile.js
```

would be accessed by:-

```
<script src="/<module_name>/static/myjsfile.js?version={{config['JS_VERSION']}}"></
↪script>
```

4.4.4 Projects

Any projects need to be specified in a dictionary in the projects list of the module's config see *App Settings*. This will ensure the project is imported and registered when the app is initialised. The actual project code needs to be in a python file named after the project in the module's project folder - see *Project Class*.

4.4.5 Jobs

Jobs need to be specified in the 'jobs' list of the module's config. The job's code should then be in a module named after the job in the module's jobs folder - see *Jobs*.

4.4.6 Config

The config should have three keys:- jobs, projects and config. The jobs and projects specify the jobs and projects that the module contains and the config will update the app's config with any extra variables required. For example:-

```
"jobs":[
    {"name":"peak_search_job"}
],
"projects":[
    {
        "name":"peak_search",
        "label":"Peak Search",
        "large_icon":"/lanceotron/static/img/peak_search.png",
        "can_create":true,
        "is_public":true,
        "main_project":true,
     }
],
"config":{

"NEW_APP_PARAMETER":"value"
}
```

4.5 Projects

A Project represents an analysis or pipeline and is represented by a JSON config, a python class, html (Jinja) templates and JavaScript files. The metadata for each project is kept in the 'projects' table of the system database.

4.5.1 Config

Each project needs to described by an entry in the project's list of a module's config. The config should contain the following:-

- **name** - The name of the project type (that will be stored in the database as type)
- **label** - The name shown to user.

- **large_icon** - The url of the icon which is displayed in the panels on the main page.
- **can_create** If True then this type of project can be directly created from the home page.
- **description** A short description which is displayed in the create panel on the main page.
- **is_public** If False, then the user must have the permission 'view_project_type' with the value of the project's type.
- **main_project** If True, then individual projects of this type will be accessible to view from the home page.
- **enter_genome** (optional) - If True then the genome can entered during the initial creation page, usually only name and description can be entered.
- **anonymous_create** (optional) If True, then projects of this type can be created by an anonymous user that is not logged in.

Users have to be given a specific permission to create a project If the project type is public, new users will automatically get permission to create that project type.

4.5.2 HTML Templates

Project Home Page

Projects that can be created directly will have the following url:

```
http://<server_name>/projects/<project_type>/home
```

This page allows the user to enter a name, description and genome and then creates an empty project. The actual template file should be located at

```
app/modules/<module_name>/templates/<project_name>/home.html
```

and contain the following html :-

```
{% extends "projects/home_base.html" %}
{% block project_explanation %}
   Information about the project here
{% endblock %}
```

Individual Project Page

A second url points to a specific instance of the project:-

```
http://<server_name>/projects/<project_type>/<project_id>
```

The flask view behind this url checks the user has permission to view/edit the project and calls the project's get_template method. The template is then rendered with the following kwargs (plus any extra returned by the get_template method)

- project_id
- project_type
- project_name
- project_description

The project's *get_template* method receives the args from the request and should return the location of the template and a dictionary containing any key word arguments (in addition to the above) required by the template. Different templates can be returned depending on the state of the project.

```
def get_template(self,args):
    kwgs={}
    template="<project_name>/<template_name>.html"
    #alter template and kwgs based on args and the project's current state
    return template,kwgs
```

The html templates for each project need to be located in the directory

```
app/modules/<module_name>/<project_name>/
```

with the following template:-

```
{% extends "common/page_base.html" %}

{% block stylesheets %}
    {{super() }}
    <!-- extra styles -->
{% endblock %}

{% block outercontent %}
    <!-- main content -->
{% endblock %}

{% block scripts %}
    {{ super() }}
    <!-- scripts -->
{% endblock %}
```

4.5.3 Project Class

Projects should inherit from the GenericObject in *app.ngs.project*, which supplies methods amongst others for sharing, making public, deleting projects. In addidion the *projects* member of *app.ngs.project* should be updated with the class name

To expose a project's method to an HTML Client use the static member 'methods', which is a dictionary with the exposed method as the key and a dictionary containing the following parameters:-

- **permission required, either view or edit**
    - view - allow all users with view permission to access the method. This will include all users if the object is public
    - edit - allow only users with edit permission to access the method
- **async optional, either True or False (False by default)**
    - False - The method will be run in the browser thread
    - True - The method will be processed asynchronously
- **running_flag** optional, The projects's data will have this parameter set to the supplied value immediately before the method is sent to any queue. Should be a list containing the parameter and value to set

As an example a project *new_project* with a single method *get_data* that can be accessed by all users (including anonymous ones) would be written as follows:-

```
from app.ngs.project import GenericObject,projects

class CaptureCompareProject(GenericObject):
    def get_template(self,args):
        return "new_project/temp.html",kwgs
    def get_data(self,param1="default",param2="default):
             #get the data using params
        return data

projects["new_project"]= NewProject

NewProjects.methods=
    {
        "filter_peaks":
            {
               "permission":"view",
               "running_flag":["filter_status","filtering"]
             }
    }
```

The method can then be called from JavaScript using:-

```
$.ajax({
    url:"/meths/execute_project_action/<project_id>",
    type:"POST",
    dataType:"json",
    contentType:"application/json",
    data:JSON.stringify({
        method:"get_data",
        args:{
            param1:"value1",
            param2:"value2"
        }
    })
})
```

The ‘args’ parameter contains the key word arguments sent to the project’s method. However, if ‘project_data’ is present in the args then this will be used to update the project’s data immediately and not passed to the method. This is useful if the method is beng run asynchronously with the async flag.

4.6 Jobs

Jobs run tasks asynchronously, either locally through celery or remotely on another server. Local jobs should extend *LocalJob* from *app.jobs.jobs*, whilst remote jobs should extend *BaseJob*

4.6.1 Constructor

To construct a job, user_id, inputs (a dictionary of key/value pairs) and genome should be specified although default values of 0, an empty dictionary and ‘other’ will be used. A type, however must be specified. Inputs should contain all the information required to send or resend the job. Once a job is constructed in this way, it will be added to the database and can be retrieved in the future with *app.jobs.jobs.get_job(<job_id>)*.

4.6.2 Registering a job

In a module, each job should be in separate python file in the jobs folder of the module with the name of the job type e.g. new_job.py and registered in the config:-

```
{  .....,
   "jobs":[
      {"name":"new_job"}
   ],
   .....
}
```

The job's type needs to be linked to its class using *job_types* from *app.jobs.jobs*. For example new_job.py could contain:-

```
from app.jobs.jobs import BaseJob,job_types
import traceback

class NewJob(LocalJob):
    def __init__(self,job=None,inputs={},user_id=0,genome="other"):
        if (job):
            super().__init__(job=job)
        else:
            super().__init__(inputs=data,genome=genome,type="new_job",user_id=user_id)

    def process(self):
            try:
         #run the job
       except:
         self.failed(traceback.format_exc())


job_types["type"] = JobClass
```

4.6.3 Methods to Override

send(data)

For local jobs this simply calls *process* asynchronously in the celery thread. For remote jobs, this method needs to be over-ridden in the subclass to call a pipeline on a remote server add it to a remote queue etc. Usually no parameters need to be passed as they should all be stored in the database when the job is created. However, data can be passed for example a password, which you would not want to store.

resend()

The default implementation is just to call *send*. However it should be over-ridden to clean up any mess that was created when the job was first sent.

check_status()

The default is to return the job's status (the field in the underlying database). This is sufficient for local jobs, as the status is updated by the celery thread. For Remote jobs this method should just return the status if it is 'complete', 'new', 'processing' or 'failed'. Otherwise (i.e. the job is still running) it should actually check on the status of the remote script/pipeline (query the remote server, check the queue etc.) and if the job has failed or is complete, call *failed* or *process* respectively.

process()

For local jobs this should be the meat of the job and do all the heavy lifting ,storing the results to the database. For remote jobs this should take the results from the remote pipeline and process/store them in the database. Exceptions should be caught and if catastrophic, call *failed* passing the description of the exception.

delete()

This method just removes the job from the database. Sub-classes should over-ride this method and delete any files or other resources before removing the database entry.

failed(msg)

sets the status and time finished, writing the error message to the database. If there is more cleaning up required then this method should be over-ridden with the relevent code.

kill()

The default implementation of this method is simply to set the job's status as failed and add a message to the outputs. For remote jobs it should send a message to the server/queue to kill the job. For local jobs - if possible the job'status should be checked periodically and processing stopped if the status is 'failed'.

4.6.4 Utility Methods

Job objects have the instance variable 'job', which is just an SQLAlchemy object referring to the database entry. This can be manipulated directly or there are the following convenience methods:-

- **complete** Sets date finished and status fields in the database
- **has_permission(user)** Returns whether the user has permission for this job
- **set_input_parameter(param,value)** Sets the input parameter
- **set_output_parameter(param,value)** Sets the output parameter
- **get_input_parameter(param,value)** Gets the input parameter
- **get_output_parameter(param,value)** Gets the output parameter
- **get_user_name()** Gets the full name of the job's owner
- **set_status(self,value)** Sets the status in the database
- **get_info()** Returns a dictionary containing the job's inputs and outputs

CHAPTER 5

Indices and tables

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- modindex
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