Manual Podbat

Version 0.5

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Introduction

Podbat is a bioinformatics tool to visualize, analyze and store genomic positioning data. Data can be experimentally derived from ChIP-chip, ChIP-seq or expression microarrays.

User interface

Getting started

First, download and install Podbat. Double-click on the `podbat.jar` file Podbat icon (for the Mac bundle) to start Podbat. Alternatively, go to the command line, move to the directory where you saved Podbat and type `java -jar podbat.jar -Xmx1G` (The -Xmx1G part will set the memory allocated to the program to 1GByte). The first time you open Podbat, it will ask you to select which organism you wish work on and the version of its genome annotation. The settings will be saved but you can change or update the annotations at any later time point in Preferences.

Podbat tries to connect to Ensembl to fetch the genome data. If you are not connected to the Internet or your computer fails to connect to the database at Ensembl, you can download some data for some genomes and their annotations from the Podbat download tab or generate you own genome files (see below, section Choose organism). Once Podbat opens, you should see three panels in the window, the 'genome panel', the 'control panel' and the 'selected element panel' (Figure 1).
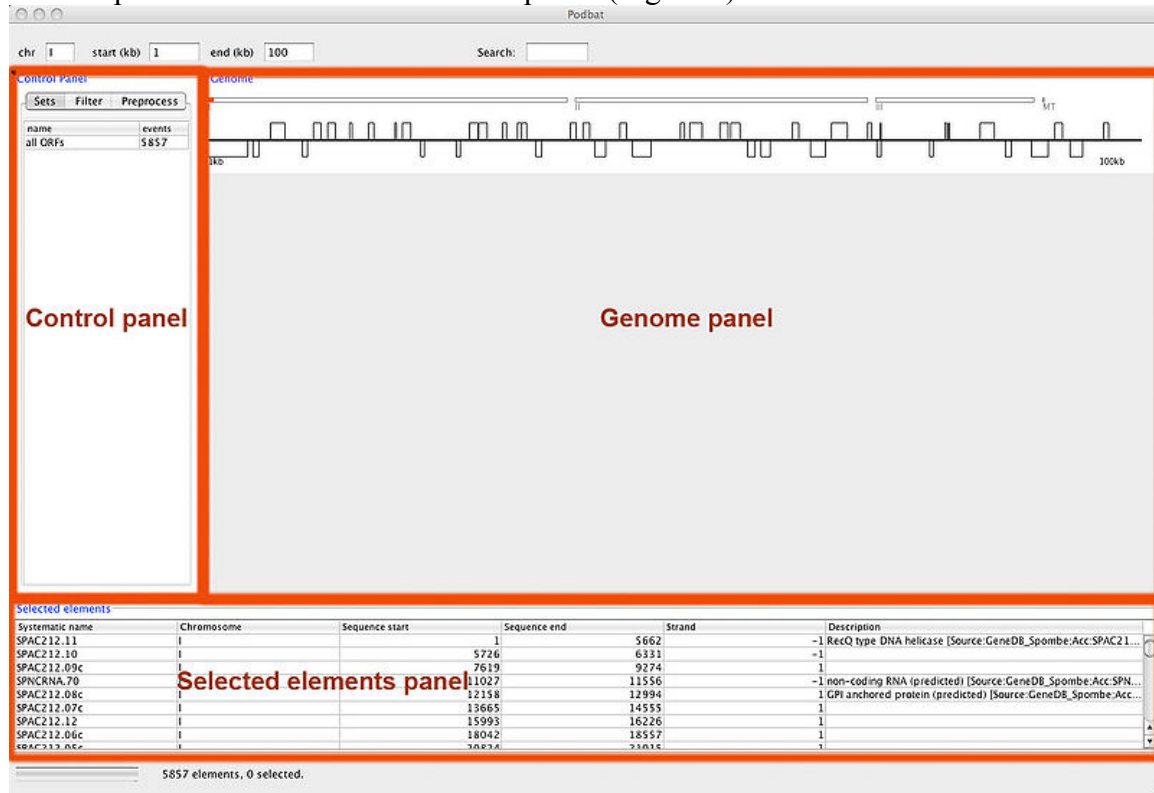

Figure 1. Overview of the Podbat graphical user interface.

The first time you open Podbat, the genome information is missing. You need to set the organism you work on in the Preference panel ().

Preferences

If you go to Preferences (Go to: `File > Preferences`, or for Mac users, `Podbat > Preferences (⌘,)`), you can set some parameters that will be saved between the sessions. You can change the working directory (where files are saved to and retrieved from), select organism and associated annotation (these are saved with the name *"organism_annotation.adata"*, e.g. "schizosaccaromyces_pombe_8_61_1a.adata"). The following row contains information of where to find the links for the web connections for the gene names.

The next sections gives the details for the MySQL database if one is to be used for storage or retrieval. The default is set to the Podbat server at Karolinska Institutet. You can speify the server name, port to be used as well as the name of the database, user and the password. To upload data to the central database you will need a username different from the default.

You can set the coloring scheme for the plots in the lower part of the panel.

Values are saved to the following session. To restore the default values, press the `default` button.

Figure 2. The preference panel.

Here, you also have the option to provide a file for the DNA sequence in the form:
```
seq_region_id      sequence
2      GATCACGTACATCACCTTGTAAGAATTTATC...
8      AATGTGTAATTTGAATCAATTTCTATCCTTTT...
...
```

Choose organism

Here you also set the organism and the annotation you want to use. If your organism of interest is not in the drop-down menu, you can create a new item. From the annotation drop-down menu you can use the predefined files or you can add a new genome assembly or species. For a certain number of species annotations can be directly downloaded from Ensembl. However, as the format for the genome varies, we cannot guarantee a

successful download. As an alternative, genomes can be imported from text files. Two files required:

> 1) genome organization file with with the length of of the chromosome (or other genetic elements such as plasmids). This file should contain three columns with chromosome name, chromosome id (not used) and chromosome length.

> 2) annotation file with information on the genetic elements (protein coding genes,tRNAs, repeat regions...). This file should contain six columns: unique id, chromosome name (same as in genome organization file), start (actually left) position, end (right) position, strand (+1/-1) and description.

NB When naming the organism, you should use two words separated by "_", e.g. Saccharomyces_cerevisiae.

If the organism has already been loaded, it is possible to only change the annotation file. After an annotation has been loaded once, it should be available at the drop-down menu at the preference panel.

Example genome organization file:
```
1      NC_001133    230208
2      NC_001134    813178
3      NC_001135    316617
4      NC_001136    1531919
5      NC_001137    576869
6      NC_001138    270148
7      NC_001139    1090947
8      NC_001140    562643
9      NC_001141    439885
10     NC_001142    745741
11     NC_001143    666454
12     NC_001144    1078175
13     NC_001145    924429
14     NC_001146    784334
15     NC_001147    1091289
16     NC_001148    948062
17     NC_001224    85779
```
Example annotation file:

```
YBR285W    2     773918     774352     1     Putative    protein    of
unknown function; YBR285W is not an essential gene
YBR286W    2     774696     776309     1     "Vacuolar
aminopeptidase Y, processed to mature form by Prb1p APE3 APY1"
YBR293W    2     787001     788425     1     Permease of basic amino
acids in the vacuolar membrane VBA2
YDL001W    4     447982     449274     1     Cytoplasmic    protein
required for sporulation RMD1
YIR018W    9     384606     385343     1     Basic    leucine    zipper
(bZIP) transcription factor YAP5
YIR035C    9     421023     421787     -1    Putative    cytoplasmic
protein of unknown function
```

Connect to database

Here you also select the database if you want to connect to one. The default option is the central server at Karolinska Institutet, but you can change this to a local database. To create a local database, install MySQL if you do not have it already (download) and create a database with the name of the organism.

Creating session

Once you have selected the organism and annotation, you can load your experimental data. There are several options depending on the data format. You can either 1) load files from your local network or 2) datasets from the database (local or central). Several different formats can be uploaded into Podbat (WIG, BED, GFF, SGR, TXT, TAS). For a description of the formats, go to Appendix A.

Import experimental data from file

File: Click `File > Import > Data from file` A new dialog will open where you are asked to specify the file format and location. If you have one file for each strand, you can upload both in the separate fields, if not, you only need to specify one file. If the data is strand specific, mark this selection. You can also chose to specify the platform that you used for the experiments. Several formats can be uploaded (see Appendix A).

Import experimental data from database

Database: Click `File > Import > Data from database`
A new dialog will open with a spreadsheet with all the datasets available at the server for the organism you are using. You can see a unique identifier as well as some information about each experiment. Select one row and click 'Import' to load the data.

The database settings can be set in the Preference panel. To make use of the central database, make sure your firewall settings are set correctly.

Import annotation data

Data from other sources can be appended to the data table. Text files can be uploaded into Podbat by clicking `File > Import > Annotation (TXT)`. A new window appears where you can specify the file you want to use. Check '`Show Options`' to improve your control of the data (Figure 3). Here you choose which columns will be mapped to each other.
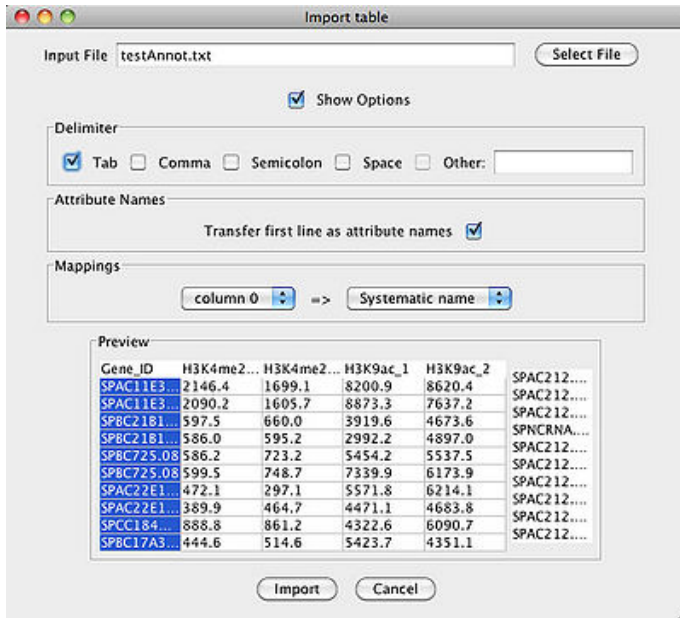
Figure 3. Loading annotation to map to the data

Save/Restore session

You can save the current state of a Podbat session to a named session file. You can use that file to restore the Podbat session yourself or share it with colleagues.

To save a session: Click `File > Save` or `File > Save Session` In the Save window, select a directory and session file name and click `Save`.

To restore a saved session: Click `File > Open.` In the Open session dialog, select a session file and click `OK`. Podbat ends the current session and restores the saved session.

Visualize

In the main panel, you can select which region to have in view, indicating chromosome number, start and end position (in kb). Press `enter` to execute the view. The selection will be presented in the 'chromosome view' and in the 'genome view' you will see an orange box showing the selection in the genome (Figure 4). The orange box can be dragged by the mouse to pan the genome.

Figure 4. Setting the coordinates for the genome view.

Zoom in/out

You can zoom in to a specific region by clicking and dragging the mouse in the 'chromosome view'. To zoom out, enter a larger interval in the main panel.

Changing representation

By clicking on a sample track, you can change the view of the sample panel or delete a single sample (Figure 5). You are able to set an individual coloring scheme for each sample. To convert the data between linear and logarithmic scale, select option in the drop-down menu. You can also change the scale (this can also be done by clicking on the sample scale top half to increase the scale, bottom half to decrease it) and the offset on the scale here. The easiest way to change the scale is to move the text onto the sample representation and turn the mouse wheel (or slide on the track pad).
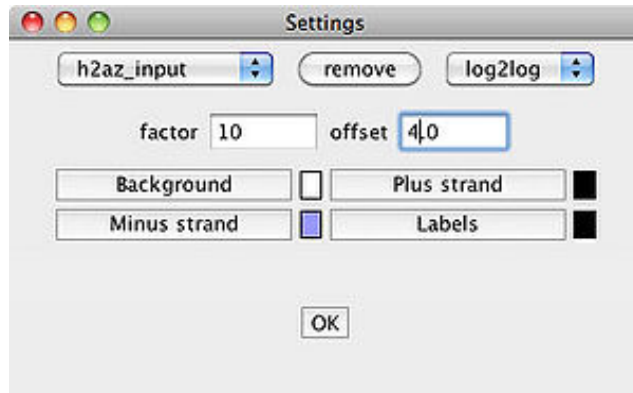

Figure 5. Changing the view for a sample.

Analyze

In the 'Control panel' (Figure 6 and 7) you can organize data in sets, filter data or process the data to determine regions and region averages.
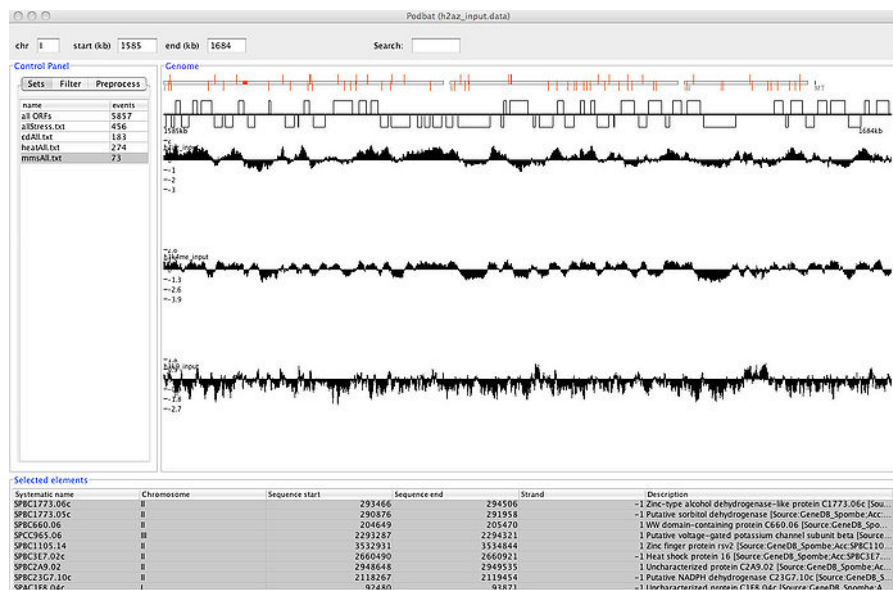

Figure 6. Whole view with selection, control panel in to the left.

Sets of genes or other elements

In this 'sets' view of the Control Panel (Figure 7) you see all the sets that are defined in your session. You can load new sets from a text file with the element identifiers (Click Edit > Load new list) or you can select it from the table (File > New Set from Selection or SHIFT-CTRL-N (SHIFT-⌘-N)).

Example of text file with gene set:
```
SPAC15F9.01c
SPBC713.05
SPBC6B1.12c
SPAC1687.08
I_138872_140253
I_977150_978343
II_430620_432154
III_37173_38830
```


Figure 7. The 'Sets' view of the control panel.

Calculate data-defined regions

In the 'Preprocess' view of the Control Panel you have the choice to determine data-defined regions (e.g. of protein occupancy or RNA expression).

By clicking the 'Determine regions' button, a new panel appears where you select the threshold (above which the average of the probes in the region has to be), minimal length of the region and minimal gap between regions (Figure 3). By checking the 'use HMM' box, you chose to determine the regions using Hidden Markov Models.

Sliding average

Regions are defined by a sliding average. This average is determined over as many probes as specified by the user (in the Length Thres box). When the average is above the intensity threshold (Intensity Thres), a region is defined. Regions closer than a specified threshold (specified in the Gap box) are merged.

Hidden Markov Models

To stratify the data, a Hidden Markov Trace can be determined (Figure 8). For the novice to Hidden Markov Models (HMM), here is an excellent primer on HMM. The user may use *a priori* estimated values for the emission and transmission probabilities as well as the initial population. In this implementation , there are two states (0 - background, 1 - above background). The distance between the states is determined by the user defined HMM threshold (HMM Thres) in the preprocess panel. The probabilities can be estimated by the Baum-Welsh algorithm. To save computational power, by default the estimation is done using 500 probes (from 10,000 to 10499). These numbers can be changed at the advanced frame ('first probe' and '# probe' after clicking the 'Adv' button). Podbat uses the Java implementation of HMM related algoritms provided in Jahmm.
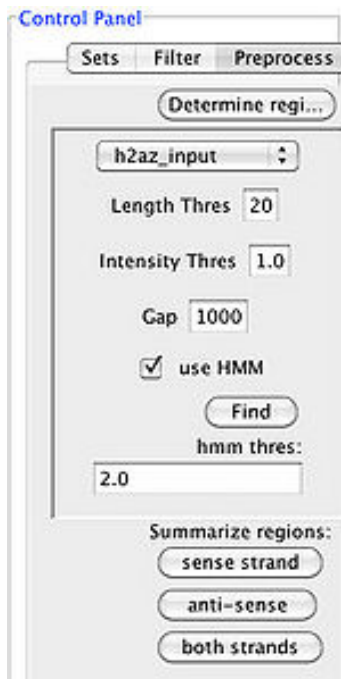

Figure 8. Determine regions within the data.

Once regions have been determined, they will be given identifiers as of their position (chromosome, start and end). The directionality of the region is given by the order of start and end position in case of strand-specific data. Example: `II_593002_590222` is positioned on chromosome II, resides between bases 590222 and 593002 on the negative (Crick) strand.The regions are visualized in the view as empty orange squares. Two sets are created, one of the actual regions (in the description you find the genes that are overlapping with the region on the same strand or opposite strand (nt)), and one of the genes overlapping with the region (Figure 9).
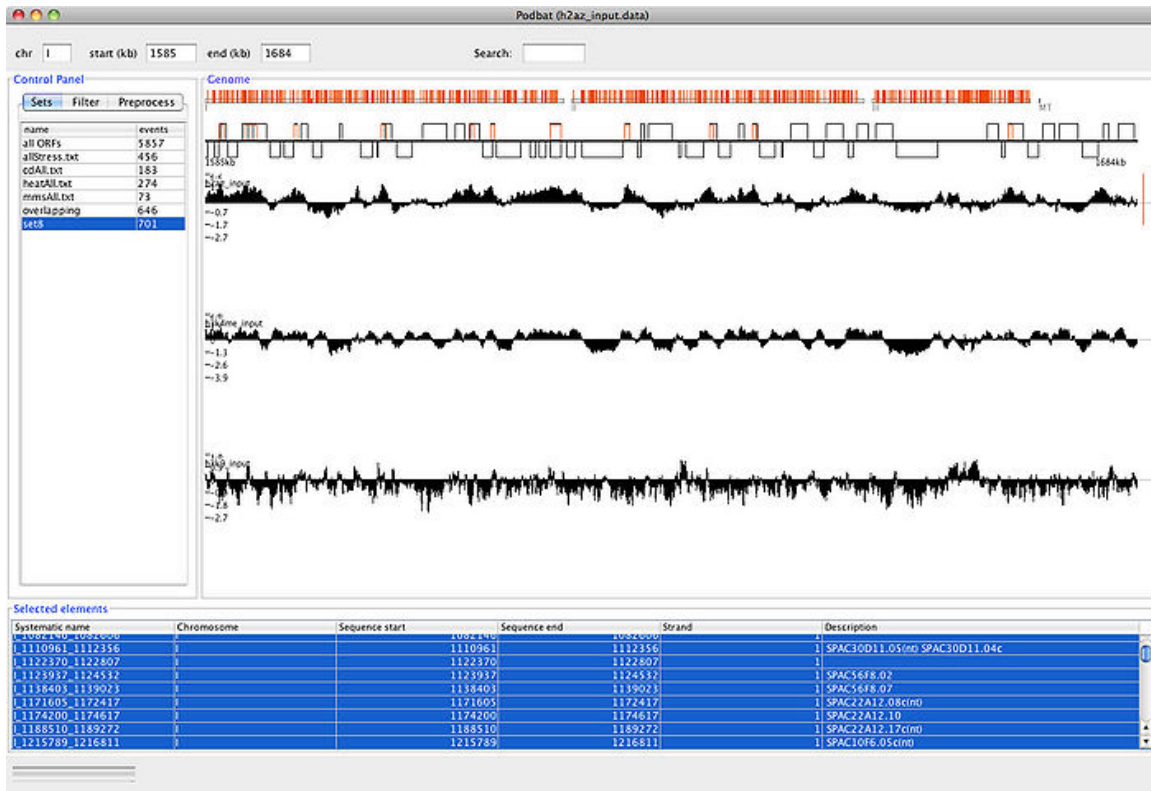

Figure 9. Results after determining regions in the data.

Summarize intensities over regions

Data within regions (either predefined in annotations or data-defined) can be summarized into a single value. Currently, this is done by taking the mean of the values within the region. This can be done for the data irrespective of strand (for protein occupancy data, `both strands` button), or for only the sense strand (for RNA expression data, `sense strand` button). A third alternative, is to look at the anti-sense or the regions (used to look for aberrant or read-through transcription, or just anti-sense transcription, `anti sense` button) (Figure 10). After the calculations, new columns will appear in the `Selected elements panel`.

Figure 10. Buttons used to calculate the mean intensity value within a region.

Sort, filter data

Data in the spreadsheet can be sorted by clicking on the header. A filtering function is present in the 'Control panel' (Filter), where you can combine several attributes. You can always make a new gene set by going to File > New set from selection.

Analyze gene sets

Compare gene sets

You can compare you different gene sets (Edit > Compare Lists). By selecting two sets and clicking the 'compare' button, Podbat will calculate the overlap and the p-value given the hypogeometrical distribution between the two sets. Note that for very small p-values, the results will show as 0. You can also make new gene sets from the union, intersection or the unique elements (Figure 11).
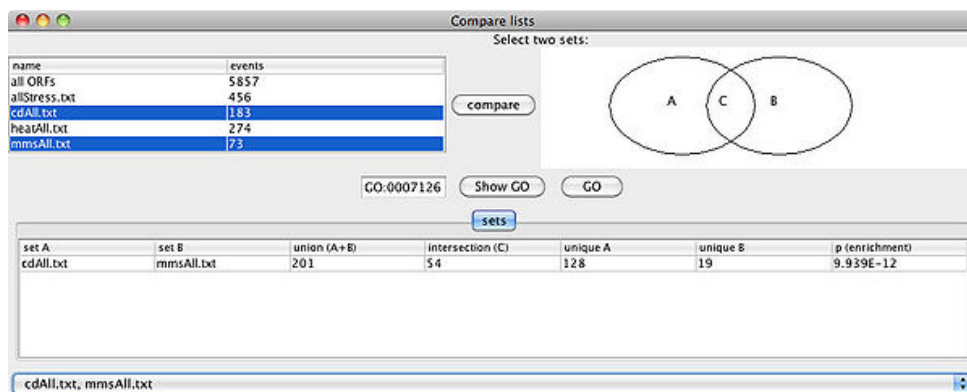

Figure 11. Comparison between gene sets.

Enrichment of gene sets

To compare gene sets to previously determined sets, e.g. the sets defined by the Gene Ontology consortium, you need first to fetch the database (Edit > Compare lists, then GeneSets > Fetch GO. For this, you will need the NCBI organism number (e.g. 4896 for *Schizosaccharomyces pombe*). Fetching the GO database is a very time consuming task so for budding and fission yeast, the data is available at the Podbat download site.

Once you have the file on the working directory on your computer, you just need to load and activate GO (`GeneSets > Activate GO`). You can also create a gene set data file yourself with the other gene sets (e.g. determined in previous experiments). The tab-delimited text file has one line for each gene set. For each line, the first field: unique identifier, second field: description, third field: number of genes in gene set, subsequent fields: the gene identifiers of the genes in the gene set (see example file below).

Example of gene set data file:
```
schizosaccharomyces_pombe
GO:0000001  mitochondrion inheritance      7      SPAC1782.06c
      SPBC28F2.06c       SPBC27B12.01c      SPAC17H9.17c
      SPAC30C2.06c       SPBC1215.02c       SPBC12C2.08
GO:0000009  alpha-1,6-mannosyltransferase activity     3
      SPAC1006.05c       SPBC1734.12c       SPBC11B10.01
GO:0000011  vacuole inheritance       3      SPBP8B7.13  SPBC25H2.03
      SPBC354.14c
GO:0000012  single strand break repair      1      SPCP31B10.05
GO:0000014  single-stranded DNA specific endodeoxyribonuclease activity
      3      SPBC4F6.15c SPAC13C5.07 SPCC970.01
GO:0000015  phosphopyruvate hydratase complex    2      SPBPB21E7.01c
      SPBC1815.01
```

Now you can select a single gene set from your list and click the '`calc enrichment`' button. Podbat will calculate and, in a new tab with the name of the gene set, display the ratio within your selection and the overall ratio for each GO term, as well as the number of genes, an enrichment ratio and a p-value (hypogeometric distribution (Figure 12). You can sort the data by clicking on the header, or you can copy paste the data into another program (such as Excel) for further processing.

By checking the '`Show settings`' box, you can change the default values of the background set, which gene identifier to use, as well as thresholds for the number of genes that should be in the selection, the enrichment factor and the significance level. Click '`show set`' to make a new gene set of the ID selected or written in the text box adjacent to the button.

Figure 12. Enrichment of GO terms.

Aligning genes by genomic positions

Gene average plots

Podbat can also align the data for the selected elements after the start or end position. Click `Edit > Average` gene plot and a new window will open (Figure 13). It is the gene set selected in the main control panel that will be aligned.
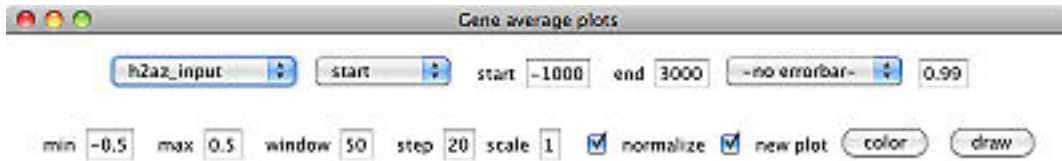
Figure 13. Aligning data of the regions.

You can specify several parameters: the size of the alignment region, if you want error bars (CI -confidence interval, then specify the level in box adjacent, SD - standard deviation, SDx2 - twice the standard deviation, SEM - standard error of the mean), the scale on the Y-axis (min and max), the window and step size to smoothen the curve, normalize will set the genome average to 0. You can also choose to stay in the same canvas or make a new plot, and which color it will have. By clicking 'draw' the data will be processed (Figure 14). **Note:** For large gene sets and big regions, this process will take long time and the program might not be able to finish it. To resolve it, try reducing the size (either by breaking the gene set up in smaller sets, or dividing the region in smaller regions) or starting up Podbat with more memory.

Figure 14. Data alignment.

Add statistics

You can calculate if there is a significant difference between two gene sets. For each bin (determined by the window and step size), a t-test will be performed under the null hypothesis that there is no difference between the two sets. To comp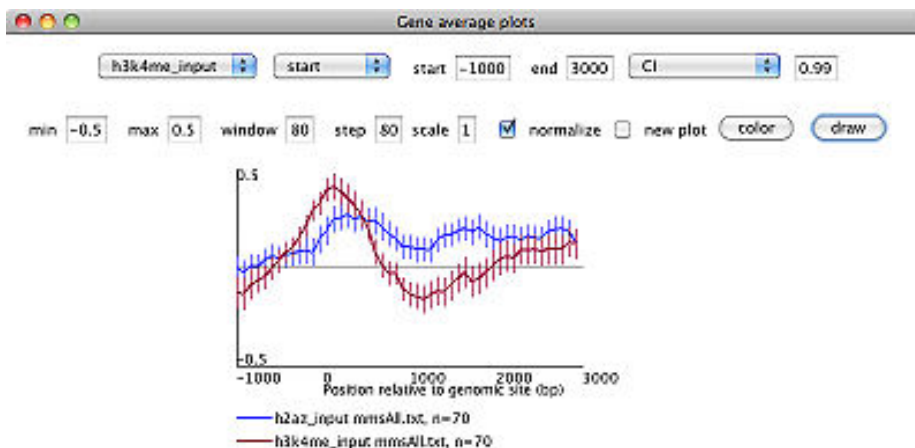are to sets, you go to the menu `Edit > Add statistics`. Select the two sets you want to compare in the pull down menus (both have to be from the latest plot). There are two options for showing the regions which are significantly different: 1) by setting the p-value threshold (enter the -log10 value, i.e. `3` for p<0.001) or 2) by checking the continuous option (Figure 15).
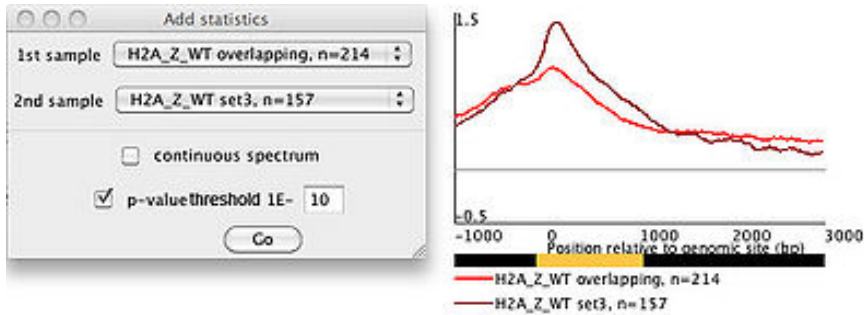
Figure 15. Adding statistics to the alignment analysis, fixed threshold at 10^{-10}

By checking the continuous option, the bar will show the p-values on a continuous scale, from black (p=1) via red to yellow (p=0) (Figure 16).
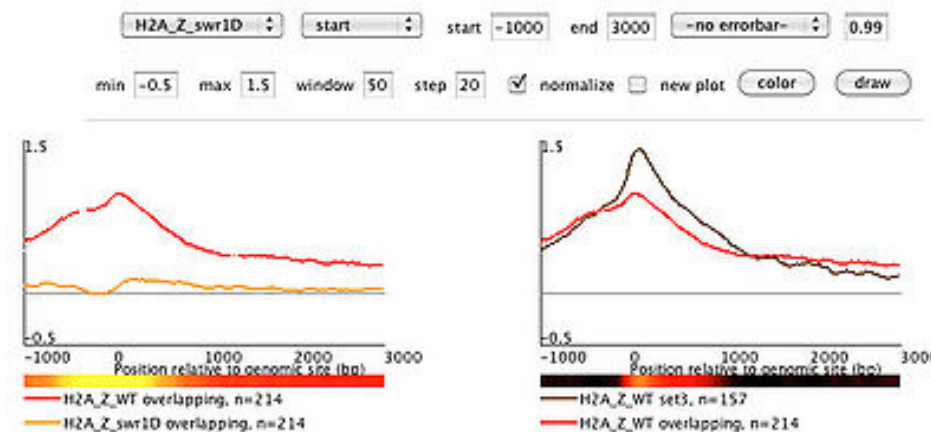
Figure 16. Adding statistics to the alignment analysis, showing p-values over the entire region with the color corresponding to the significance.

Export plot

The lastly made figure can be exported as EPS by going to the menu `Edit > Export as EPS`.

Interact With Database

You can access the central database and view data. To upload data, get in contact with the Podbat development team and you will be sent a password that you can use.

Alternatively, you can create a local database. Installing a local MySQL database, and change the settings in Podbat Preferences so that Podbat can locate it and communicate with the local database.

Export

Data in tables can be exported by selecting the cells of interest and the copy (`CTRL-C` or `⌘-C`) and paste (`CTRL-V` or `⌘-V`) into any text editor or Excel.

Export to database

To export to a local database, go to menu `File > Export > Data to Database`You will need to adjust the default settings in the Preference window and make sure you have writer privileges to the database you are trying to connect with. To upload data to the central Podbat database at Karolinska Institutet, please contact the Podbat development team and you will be issued a password so that you can upload your data.

Export sets to text file

The element (gene) sets that has been made in a session and are visualized in the 'Sets' tab of the 'Control Panel' can be exported to a text file. Go to menu `File > Export > Sets to File`. The format is the same as is imported in the Gene Set Comparison. The first set containing the entire genomic annotation will not be included. To export this you can copy and paste it from the 'Selected elements panel.

Export graphics

The genome view can be exported to EPS files for publication grade figures. Go to menu `File > Export > Graphics as EPS`

Export sequence information

Also the sequences of the selected genetic elements can be retrieved and send to a text file in FASTA format. Go to menu `File > Export >Element Sequences`This can be useful to design PCR primers for verification studies, or to find protein binding motifs using e.g. REDUCE.

Example FASTA format:
```
>SPCC16C4.22
TGGAGAAAACATATGGGAAAACTGTATTGCCATTGAGCAGAGTAAAGAGGATAATAAAGCAAGAT
GAAGACGTACACTACTGTTCTAATGCATCTGCTTTGTTAATTTCAGTAGCTACTGTATGTGTGTTG
TTGATAAATCAGCTATCTGGGCGACTTAGCACCATTTTGATTGCATAAAAGAAAAAGAAAAATAAA
AGCTTACCTATTTTATGATTTAGGAATTATTTGTTGAAAAGTTAGCAACAGAGGCTTATCAACTTG
CCAAATTACAAAAACGTAAAGGAATACGATACAGGGACGTTGAGGACGTTGTACGTAAGGACGAT
CAATTTGAATTTTTATCAGATTTGTTTTCTATATAA

>SPCC16C4.21
TGCAGCTCTATCCGGCAAATGTTAAGGGATTGAGATATAGTGAATGTTGCATCTTAACCGCTAAC
CCATCGGTTCAACATTTTGTCATGCATGATGATGCTGGTGTTCCTCTAACGTACAGCGTTTCCAC
ACTGCACGCCGAACTTACCGACCACCTATACCTCCCAACCACCAGGAAAACAGTCGCAGTAGGC
AAGAAAACGCTTTTTGTAGTGCAAAAAAAAAGATTTAAGCCGGAAACATAG
```

Plug-ins

The functionality of Podbat can be extended by plugins. A selection of plugins are available at the plug-ins tab of the Podbat web page.
Instructions on how to write plugins can be found in Appendix B.

Quickstart

To test the functionality of Podbat, first download the Java package here.
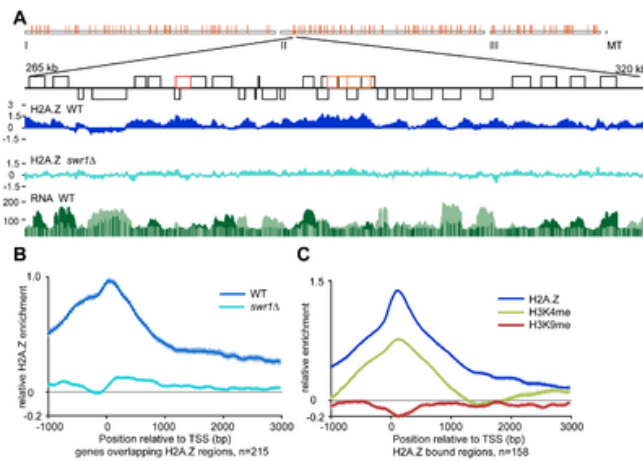Follow the instruction available in this pdf-document (Figure 17).

Figure 17. Example of Podbat analysis.

Podbat is developed and maintained at Karolinska Institutet, Stockholm, Sweden.

Appendix A. File formats

Before loading data files into Podbat, some preprocessing of the data need to be performed, such as aligning the sequences/probes onto the genome and quantifying the data at each location of the genome. For more information, we refer to others. There are many options for this depending on the platform, e.g. for Affymetrix, TAS can be used. There are also R packages to do this. Data files can be uploaded in many different formats. If the data files are compressed (zip, gz, ...), you need to decompress them before they will be recognized and imported. If you have data in other formats than the ones mentioned here, you can convert the data to fit into Podbat. Examples of these conversions are FindPeaks - used to generate WIG files from ELAND alignment files (Solexa/Illumina), Eland2Wig - used to generate WIG files from ELAND alignment files, STAR - used to generate WIG files from BED files.

Spreadsheet data (TXT)

To load spreadsheet data, two files are needed, 1) a description file giving the genome position information of all data points and 2) a data file with the numerical values for each sample and data point. Comments can be added by starting a line with '#' or '!'. Most datasets in the ArrayExpress or Geo database are available in this format.

Example description file:
```
ID      chr     strand          start
3       II      -1      1318254
4       II      -1      2614339
5       I       -1      2718766
6       II      -1      4462259
8       I       -1      4461193
9       III     1       2233423
10      III     1       1397485
12      II      -1      1005011
```
Example data file:
```
        sample1         sample2         sample3         sample4
3       -1.34 -0.05 -0.73 -0.06
4       0.06  -0.08 -0.15 -0.09
5       -0.1  -0.14 -0.19 -0.12
6       -0.15 -0.09 -0.24 -0.41
8       -0.22 0.15  0.12  0.53
9       -1.42 -0.05 -0.84 -0.06
10      2.62  1.91  3.01  2.3
12      3.58  3.26  2.66  3.37
```

Affymetrix data (TAS)

When using Affymetrix GeneChip data, these are ofter processed in (TAS). When using TAS, after defining the analysis group (TAG file), calculate signal and p-values (BAR file). Choose the option to export the BAR file as text (the BAR file cannot be read by Podbat) and the signal.txt file can be uploaded in Podbat. For data with the two strands analyzed separately , you will upload two separate files: one for the + strand and another for the - strand. For analysis where the data shuld be kept strand specific (transcriptional data but usually not protein binding data), check the box for "strand specific".

Example TAS file:

```
# Number Sequences        1
# Data        signal
# Scale       Log2
# Genomic Map        sp20r_M_v04_new.bpmap
# AlgName    TAS
# AlgVersion        1.1.02
# probe_coordinate_convention 0_indexed_center

# Sequence   10
# Name        chr3
# Number of Hits   17

59987 -0.31479
60016 -0.38421
60041 -0.36757
60061 -0.34329
60082 -0.30381
60104 -0.42574
60125 -0.49113
60145 -0.54901
60174 -0.54096
60195 -0.53333
60215 -0.51947
60236 -0.52353
60257 -0.59274
60280 -0.67085
60301 -0.57876
60327 -0.77498
60351 -0.86848
```

WIG

The wiggle (WIG) file can contain several samples. The file also contains the sample name and extras such as colors and the genome position where the browser will open. More information on the WIG format.

Example WIG file:

```
browser position chrI:4200-1340700
#      first track with name "VariableStep"
#      150 base wide bar graph at arbitrarily spaced positions,
track type=wiggle_0 name="variableStep" color=50,150,255
variableStep chrom=chrI span=150
4701  10.0
4901  12.5
5401  15.0
5601  17.5
5901  20.0
6081  17.5
#      second track with name "fixedStep"
#      200 base wide points graph at every 300 bases
#      lines and background are in different colors
track        type=wiggle_0        name="fixedStep"        color=0,200,100
altcolor=255,29,100
fixedStep chrom=chrI start=7401 step=300 span=200
10.3
```

```
4.9
-0.8
2.7
6.6
```

BED

The BED file consist of three required columns: chromosome, start and end. Nine additional columns are optional, of which three are used by Podbat: name (not implemented), score and strand (+/-). More information of the BED format.

Example BED file:
```
track name="bed_format"
chrI 1000 5000 cloneA -5.6 +
chrI 2000 6000 cloneB 12.4 -
```

BedGraph

More information of the BedGraph format.

Example BedGraph file:
```
browser position chrI:5302001-5354701
#     300 base wide bar graph
track    type=bedGraph    name="BedGraph_Format"          color=200,100,0
altColor=0,100,200
chrI 5302000 5302300 -1.0
chrI 5302300 5302600 -0.75
chrI 5302600 5302900 -0.50
chrI 5302900 5303200 -0.25
chrI 5303200 5303500 0.0
chrI 5303500 5303800 0.25
chrI 5303800 5304100 0.50
chrI 5304100 5304400 0.75
chrI 5304400 5304700 1.00
```

GFF/GTF

The GFF file contains 9 columns: seqname, source, feature, start, end, score, strand, frame and group. The columns must be separated by tab. Only four of these are used in Podbat (start, end, score and strand). Since the GFF and GTF formats only differ at the nineth, unused column, GTF data can be uploaded using this option as well. More information of the GFF format. Note, for files to be used in Podbat, the "raw" GFF file with each sequence cannot be used. The GFF files to be imported need to have a numeric score field and non-overlapping sequences. To use Solexa/Illumina data, convert the ELAND alignment files to e.g. WIG format (FindPeaks,Eland2Wig).

Example GFF file:
```
track name="GFF_example"
chrI  SEQ1  enhancer  100000  101000  4.5 +  .  TG1
chrI  SEQ1  promoter  110000  110100  9.3 +  .  TG1
```

```
chrI  SEQ1  promoter  120000  125000  -6.4      -  .  TG2
```

SGR

A simplified version of the GFF file, containing only three columns: chromosome name, position and score.

Example SGR file:
```
chr1  5722  1.02
chr1  6312  0.80
chr1  6639  1.45
chr1  6892  4.35
chr1  6898  9.68
chr1  7694  0.34
chr1  7695  0.30
chr2  10989 0.28
chr2  10993 0.14
chr2  11325 0.73
chr2  11338 0.55
chr2  11343 0.83
chr2  12104 1.17
chr2  12104 0.52
chr2  14847 0.56
chr3  24584 0.73
chr3  24587 0.65
chr3  24629 0.80
chr3  24629 0.60
```

Appendix B. Develop Plugins

Podbat allows programmers to write plugins that access the core data structures and windows of Podbat to allow a wide variety of operations. Here we explain how to write a plugin and how to get Podbat to load and use your plugin.

The PodbatPlug interface

The PodbatPlug interface is very simple. Your Podbat plugin class needs to implement this interface to be recognized. It also needs to contain the method run().

```
package plugin.YourPlugIn;

public class YourPlugIn implements PodbatPlug {
public void run() {}
}
```

For plugin *xxx*, the name of the package needs to be plugin.*xxx* and saved in a file named plugin.*xxx*.jar. The class name needs to be *xxx*.

To access the Podbat data, you need to import the `Podbat.jar` file (import Podbat.*;)

Example: HelloWorld plugin

```
package plugin.HelloWorld;

import Podbat.PodbatPlug;
import javax.swing.*;

public class HelloWorld implements PodbatPlug {
public void run() {
JFrame helloFrame= new JFrame();
JPanel helloPane = new JPanel();
helloPane.add(new JLabel("Hello world"));
helloFrame.add(helloPane);
helloFrame.setSize(50, 50);
helloFrame.setVisible(true);
}
}
```