HEMAP: Online resource for interactive exploration and e-staining of hematopoietic cancer data

Table of Contents

HEMAP Overview 1
- **Home page** 2

Explore: 3
- **e-staining the gene expression state with *FLT3*** 4
- **e-staining Pathway ROSS_AML_WITH_PML_RARA_FUSION** 5
- **e-staining acute precursor B cell vs. chronic lymphocytic leukemia clusters** 7
- **Accessing pairwise analysis results** 8
  - Search: *cancermap cluster CLL and GEXP type* 9
  - Search: *cancermap cluster aml and DrugSigDB type with GSK subfiltering* 10
  - Gene sets of pathway and drug signatures 11

TCGA AML Map 13
- **Additional data types for TCGA AML map: GNAB, CNVR, METH, MIRN and CLIN categories** 14
- **e-staining *mir-125 across METH and MIRNA*** 14
  - Lookup of copy number aberrations by gene name, vice versa 15

Annotation Search, e-staining, and box plotting 16
- **Main Category: *Leukemia* AND Cytogenetics: *crlf2* analysis to IRX3** 16
- **Sample groups from map cluster selection (mouse) and plotting combined with annotation search** 18
- **Annotation categories to gene expression e-staining** 19

Use case examples 24
- **Exploring surface protein expression in lymphomas** 24
- **Finding Myeloma cluster specific genes/pathways** 25
- **Finding subtype specific druggable genes** 28
- **In silico drug repurposing analysis using Hemap and DSigDB** 29
- **In silico drug screening using Hemap** 30

Settings 36

Info 36

HEMAP Overview

Hemap (http://ruoho.uta.fi/hemap) is the interactive online resource component of the project *Hemap: An interactive online resource for characterizing molecular phenotypes across cancers of hematopoietic origin.* Please contact Merja Heinaniemi (merja.heinaniemi@uef.fi) and Matti Nykter (matti.nykter@uta.fi) for credentials. The HTML5 application is designed for interactive exploration of ~10,000 samples across cell types and hematological malignancies. For AML, additional ~100 samples of the TCGA dataset are included. The samples are curated with detailed annotations, including clinical and cytogenetic info. E-staining, dynamic coloring for interactive examination

of sample clustering, is supported across annotation term searches, Pathway/Drug gene set scores and omics profiles. Alternatively, gene expression profiles can be visualized with boxplots of custom sample groups and cluster selections. The main analysis feature of Hemap is the pairwise exploration of cancer cluster, drug, and genomic pathway associations with various feature types to characterize the sample groups. Hemap is optimized for HTML5 compatible browsers and has been extensively tested on recent versions of Chrome, Firefox and IE 11+.

Home page

The resource is organized into visualization, exploration (e-staining and analysis) and annotation tabs, followed by additional tools (boxplots), settings and information tabs. The home page shows the main maps (HEMAP samples, ALL, AML Lymphoma and TCGA). The can be selected and toggle by clicking on the provided links on the upper left corner. Map session element sizes can be changed on the Settings pane. Every map supports zooming and direct viewing of annotations, custom group box plots are enabled using mouse drags. Details are provided below, including e-staining in the Explore panel.

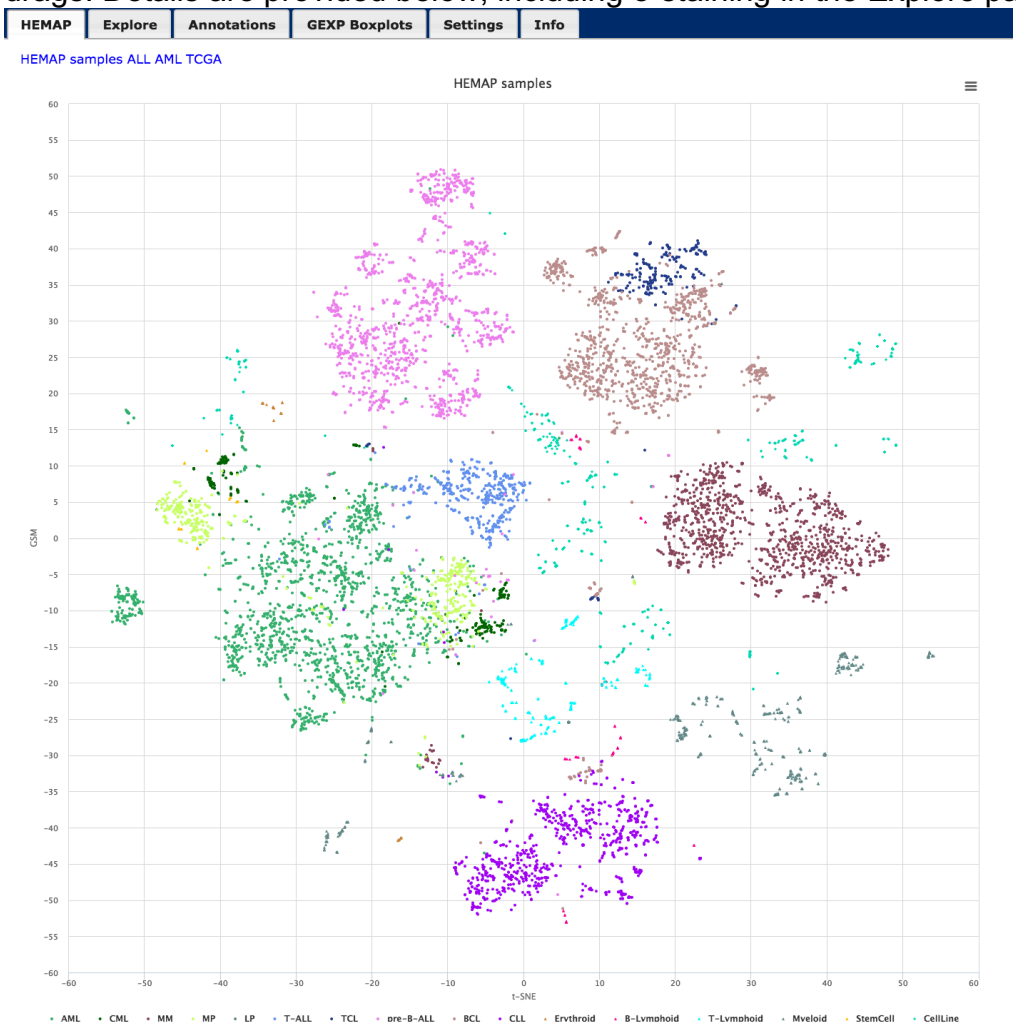
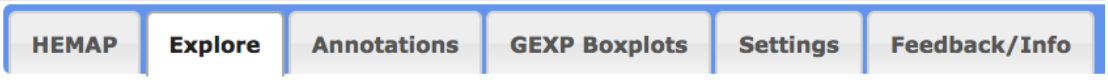


Figure 1. HEMAP home page shows samples (colored data points) on the 2D map obtained by t-SNE. The sample colors are from cell type annotations and disease classifications. The map supports zooming and accessing annotation views from selected samples.

Explore:

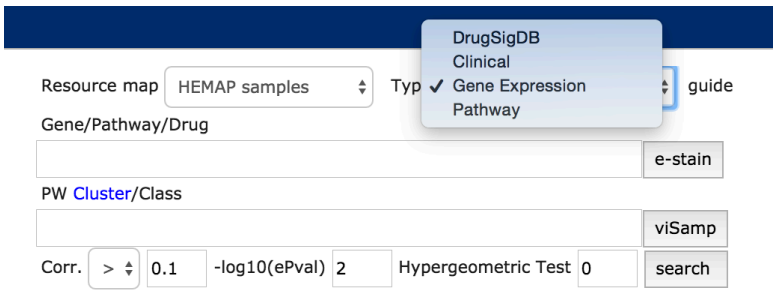


The Explore interface supports e-staining by gene, and pathway and drug marker signatures on maps [HEMAP samples, ALL, AML Lymphoma, and TCGA]. TCGA map allows staining on copy number, methylation and other omic types. In addition, samples annotated by class/clusters can also be selected. Different analysis results for clusters and cluster comparisons can be accessed using inputs provided for searching pairwise/pairwise results. These sortable records can be selected for e-stain for visual inspection directly from the result table. The Settings tab allows customizing map and pairwise search parameters, such as order by, legend placement and visibility, max records and staining category colors. Example usages are listed below.

Interface Overview

The Gene and Pathway/DrugSig form allows users to enter gene/gene set (for pathways and drugs) and cluster/class values for e-staining of selected maps. The Gene/Pathway/Drug Signature input field is implemented with type ahead (auto complete) where the possible choices are determined by **Type** selection. For example, if GEXP is selected, then only Gene names are suggested. Selecting TCGA map with GEXP also dictates the program to have the source type ahead of TCGA genes.

Pairwise results associated with cluster/class values can be explored and downloaded upon searching. The pairwise results are associated with specific Types (DrugSigDB, CLIN, GEXP, GSVA for Leukemia and AML; TCGA those four as well as GNAB, CNVR, METH and MIRN designations). P-Value, Correlation and Hypergeometric Test measures are provided for pairwise filtering, other resultant columns are listed in the pairwise search section below.



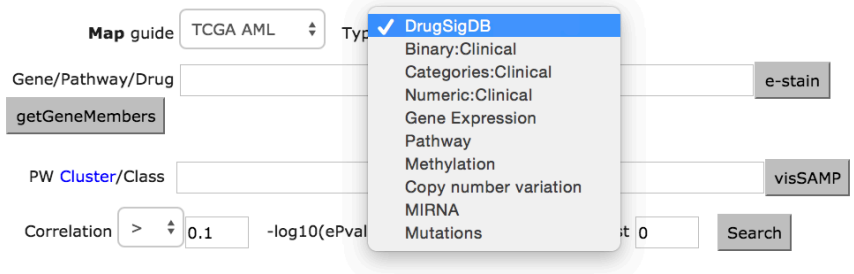
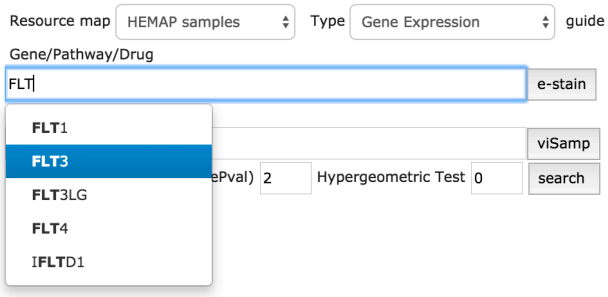


Figure 2. The explore interface provides intuitive search of pairwise cluster to feature associations and then e-staining. The omic types are DrugSigDB, Clinical, GEXP and GSVA. The bottom figure shows additional TCGA cluster/class types, including copy number and methylation.

e-staining the gene expression state with *FLT3*

Auto-suggestions (type aheads) are built into Gene, pathways, and cluster/class fields to ensure valid selections. The example here shows how to visualize *FLT3* e-staining and as well as image export functionality.



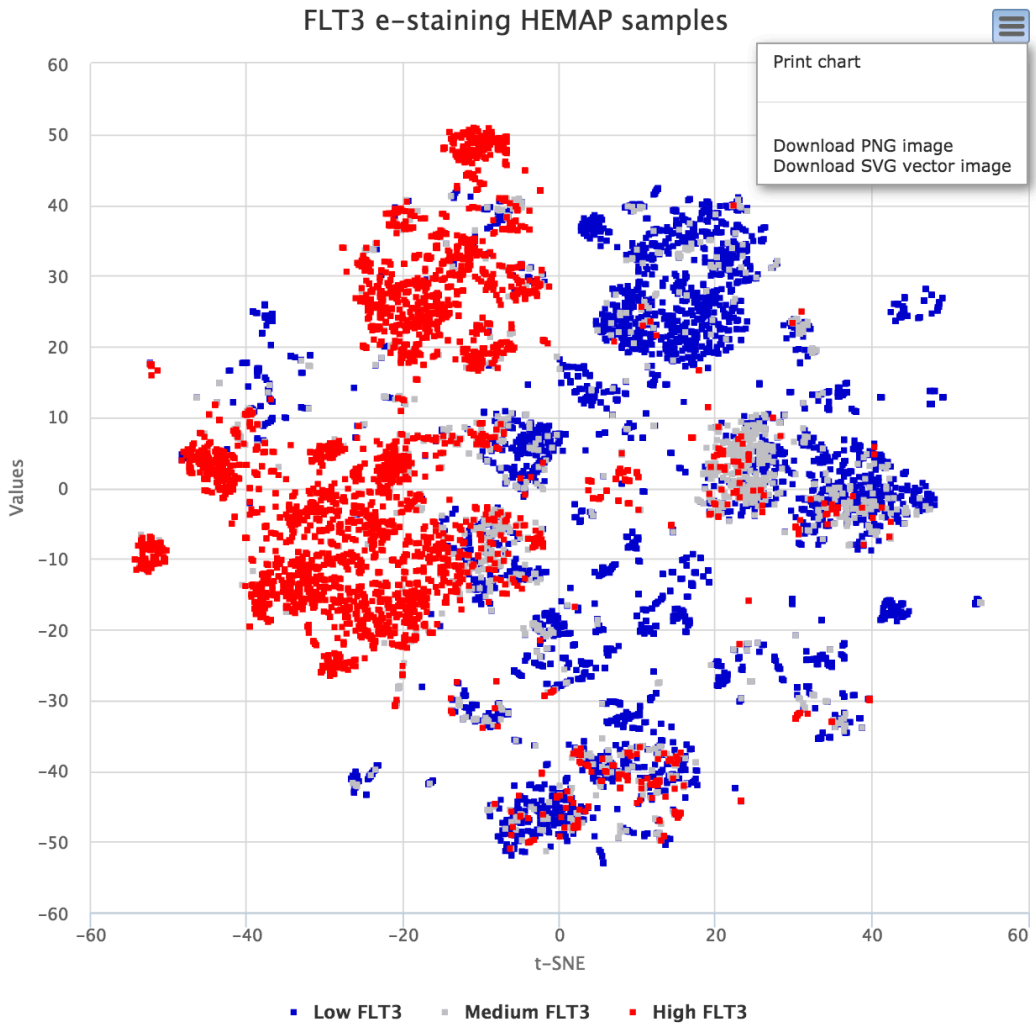
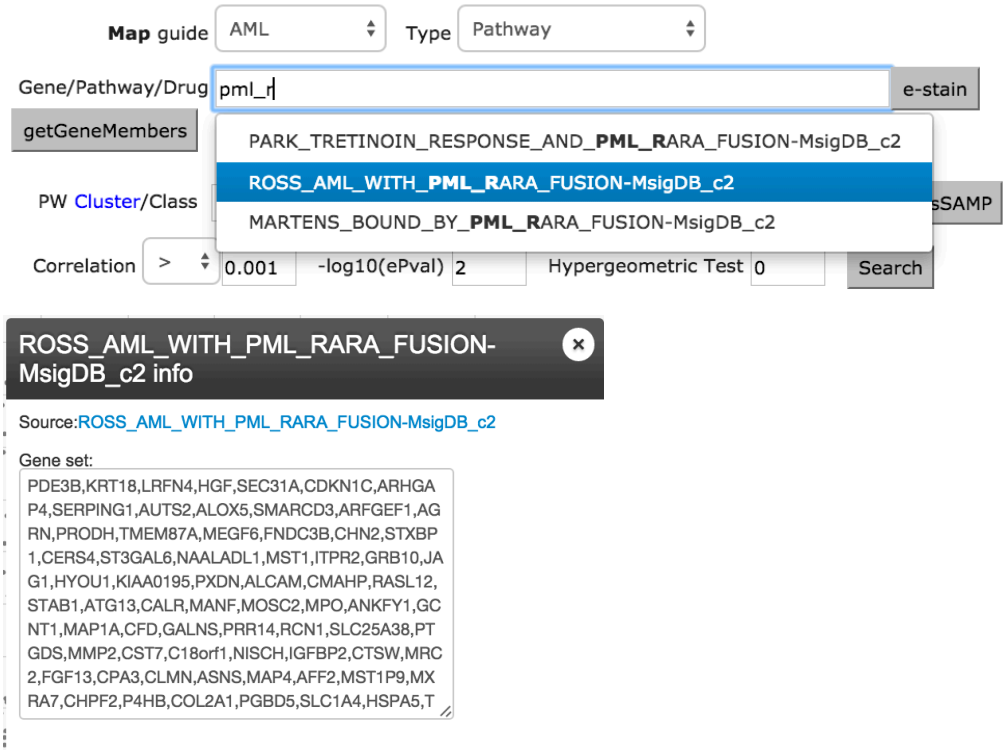


Figure 3. e-staining is performed using FLT3, where low expressing samples are blue, medium grey and high red. Samples in the map are selectable and advanced functions include mouse dragging for summary plotting. Details are provided in the use case section.

e-staining Pathway ROSS_AML_WITH_PML_RARA_FUSION

The example here uses the AML map to locate samples with the PML-RARA fusion gene based on a previously published gene set for this subtype. Shown in figure 4, entry of “PML_RAR” automatically brings up suitable matches and the ROSS_AML_WITH_PML_RARA_FUSION from MsigDB is selected and then e-stained. The graph in the middle lists the gene members of this pathway. To reproduce the result shown below, the FDR cut-off should be set to 0.001 from the Settings tab.


Map guide
AML
Type
Pathway
Gene/Pathway/Drug
pml_r
e-stain
getGeneMembers
PARK_TRETINOIN_RESPONSE_AND_PML_RARA_FUSION-MsigDB_c2
ROSS_AML_WITH_PML_RARA_FUSION-MsigDB_c2
MARTENS_BOUND_BY_PML_RARA_FUSION-MsigDB_c2
PW Cluster/Class
sSAMP
Correlation
>
0.001
-log10(ePval)
2
Hypergeometric Test
0
Search
ROSS_AML_WITH_PML_RARA_FUSION-MsigDB_c2 info
Source:ROSS_AML_WITH_PML_RARA_FUSION-MsigDB_c2
Gene set:
PDE3B,KRT18,LRFN4,HGF,SEC31A,CDKN1C,ARHGAP4,SERPING1,AUTS2,ALOX5,SMARCD3,ARFGEF1,AGRN,PRODH,TMEM87A,MEGF6,FNDC3B,CHN2,STXBP1,CERS4,ST3GAL6,NAALADL1,MST1,ITPR2,GRB10,JAG1,HYOU1,KIAA0195,PXDN,ALCAM,CMAHP,RASL12,STAB1,ATG13,CALR,MANF,MOSC2,MPO,ANKFY1,GCNT1,MAP1A,CFD,GALNS,PRR14,RCN1,SLC25A38,PTGDS,MMP2,CST7,C18orf1,NISCH,IGFBP2,CTSW,MRC2,FGF13,CPA3,CLMN,ASNS,MAP4,AFF2,MST1P9,MXRA7,CHPF2,P4HB,COL2A1,PGBD5,SLC1A4,HSPA5,T

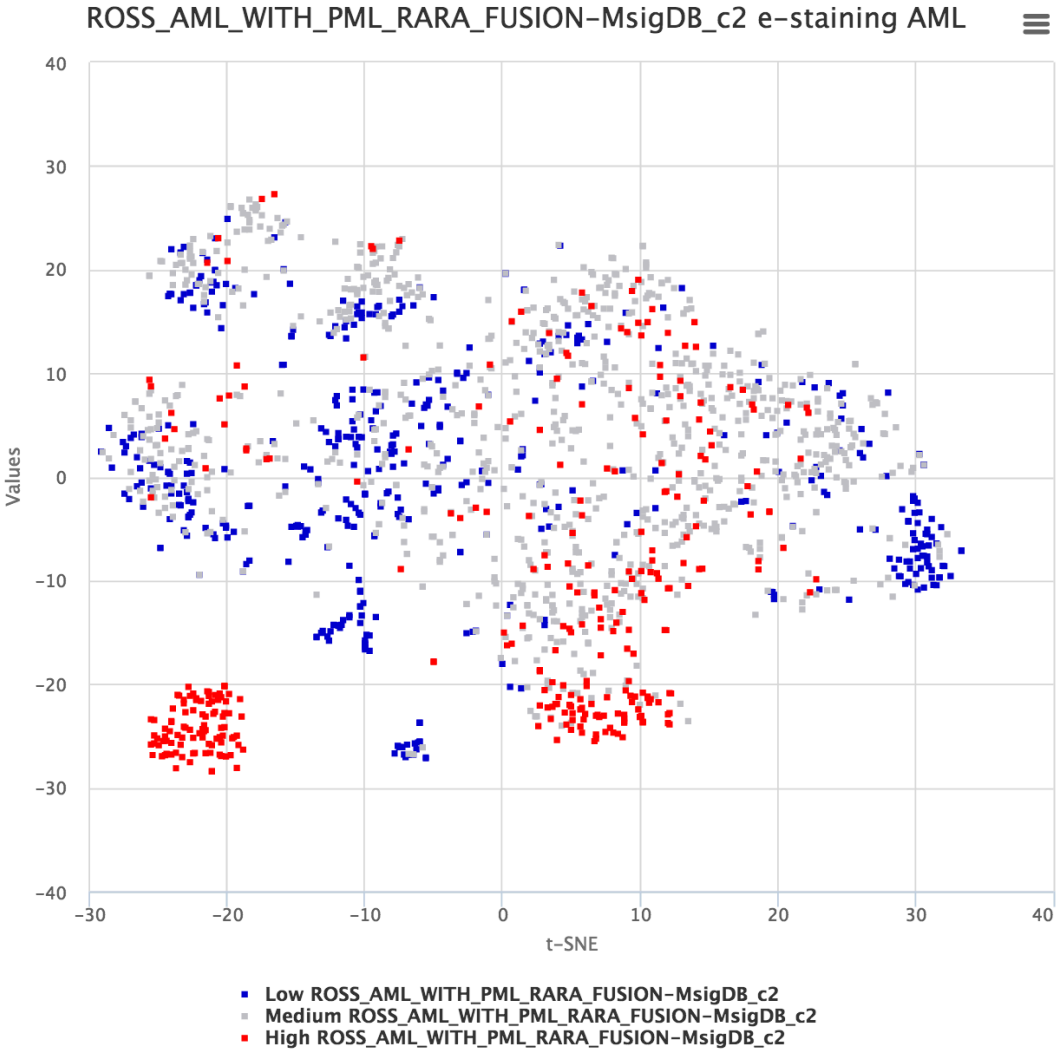


Figure 4. AML map e-stained with ROSS_AML_WITH_PML_RARA fusion event reported in MsigDB. The resource contains type specific autosuggestions and gene membership retrieval functions. The middle figures list gene members of the selected pathway.

e-staining acute precursor B cell vs. chronic lymphocytic leukemia clusters

In order to make the cluster exploration intuitive, it is easy to find out where the samples belonging to a cluster (or clusters being compared) are located on the map as a first step. The scenario below, figure 5, shows the location of acute precursor B cell (pre-B-ALL) vs. chronic lymphocytic leukemia (CLL) using VisSAMP that indicates cluster locations with binary labels vice versa: blue for the 2nd (CLL) category and red for the first (pre-B-ALL), grey nodes are NA. Both diseases have a tight cluster confirming the original map sample placements. VisSAMP stains samples of "_and_" clusters red if both categories applied.

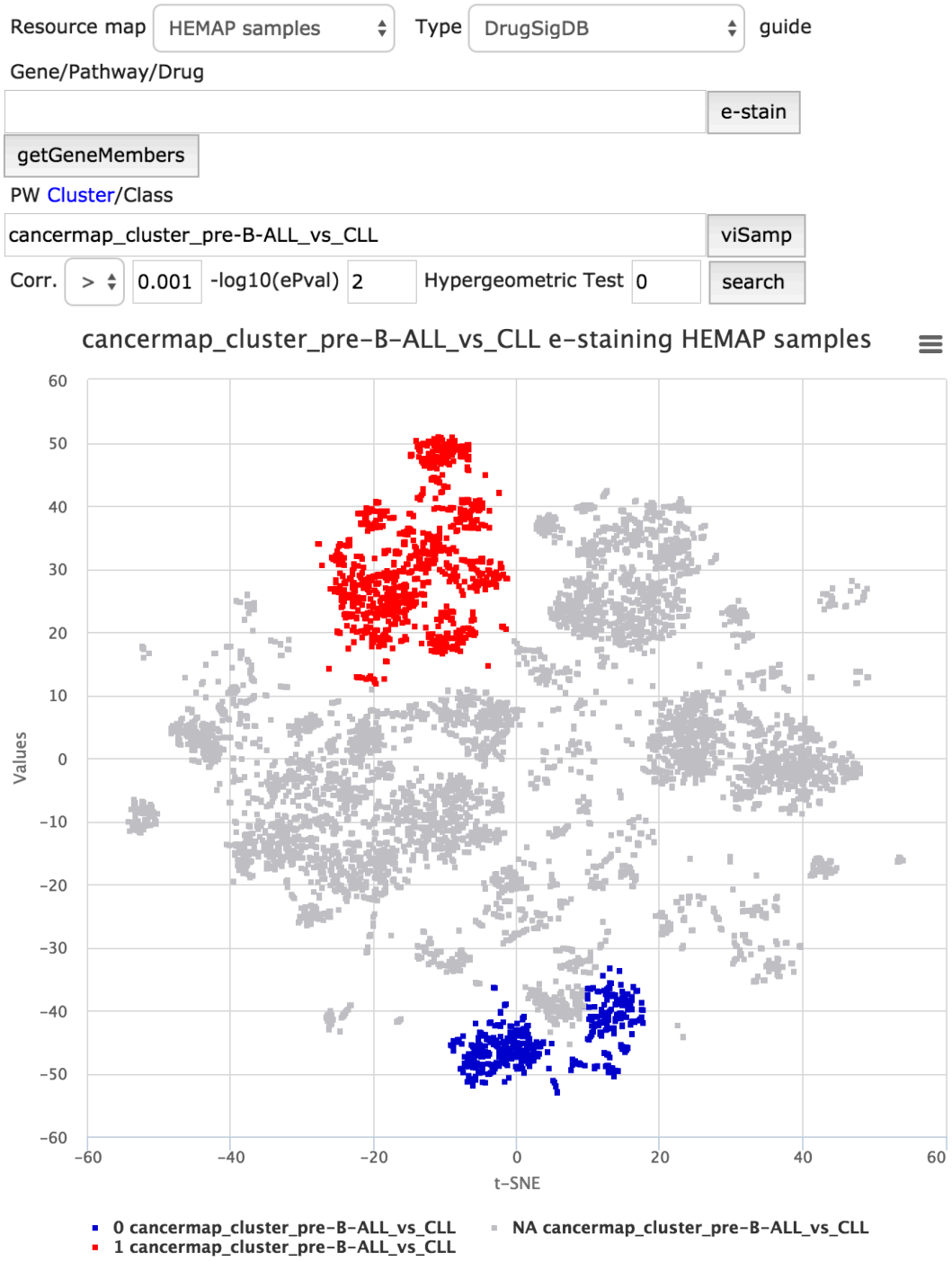


Figure 5. Samples can be directly colored from cluster features. Recalled that pre-B-ALL samples on the original map shared the same location as the red highly expressed samples here. In contrast, the blue samples represent CLL labels.

Accessing pairwise analysis results

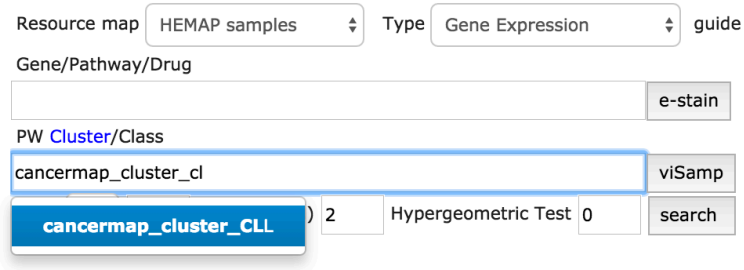
In addition to exploration of selected gene features and gene sets (based on expert knowledge on disease biology), the PW Cluster/Class field enables the user to analyze in an unbiased manner the pairwise comparisons between

cluster assignment and different sample features. The features of interest are selected from the “Type” drop-down menu and the cluster(s) of interest are specified in the PW Cluster/Class field.
The results (default to max 5000, see Settings at the end of the guide for adjustment) can be downloaded as a table and individual features (rows) can be selected directly for e-staining. Each column is sortable and they are:

1. Feature (e.g. a Gene or a pathway gene set)
2. Spearman correlation
3. Adjusted –log 10(P-pvalue) from correlation test
4. Adjusted –log 10(P-pvalue) from cluster enrichment test (hypergeometric test), applicable for GSVA/Drug Signature

Search: *cancermap cluster CLL and GEXP type*

The figure below describes cancer map cluster CLL selection and clicking search where resultant rows (4917) ordered by adjusted P-value are returned and can be downloaded. All columns are sortable. In the example below, the result was sorted by correlation (highest first) (arrows in the column names are clickable to sort in both directions). The map can be directly stained from results by gene name selection on the row; figure 6 bottom panel is map stained by selection of SFMTB1, the 5th record ordered by P-value.



feature	corr.	adjpvalue	BHadj
MATN1-AS1	0.410	292.333	7.667
RNF41	0.410	292.333	7.667
DNMBP	0.410	292.333	7.667
FOXP1	0.410	292.333	7.667
SFMBT1	0.410	292.333	7.667
ADAM28	0.400	292.333	7.667
TNFRSF13C	0.400	292.333	7.667
ISCU	0.400	292.333	7.667
P2RY10	0.400	292.333	7.667
LINC00926	0.400	292.333	7.667
MAP3K1	0.400	292.333	7.667
ZBTB4	0.400	292.333	7.667
ARHGAP24	0.400	292.333	7.667
MAP3K14	0.400	292.333	7.667
CD37	0.400	292.333	7.667

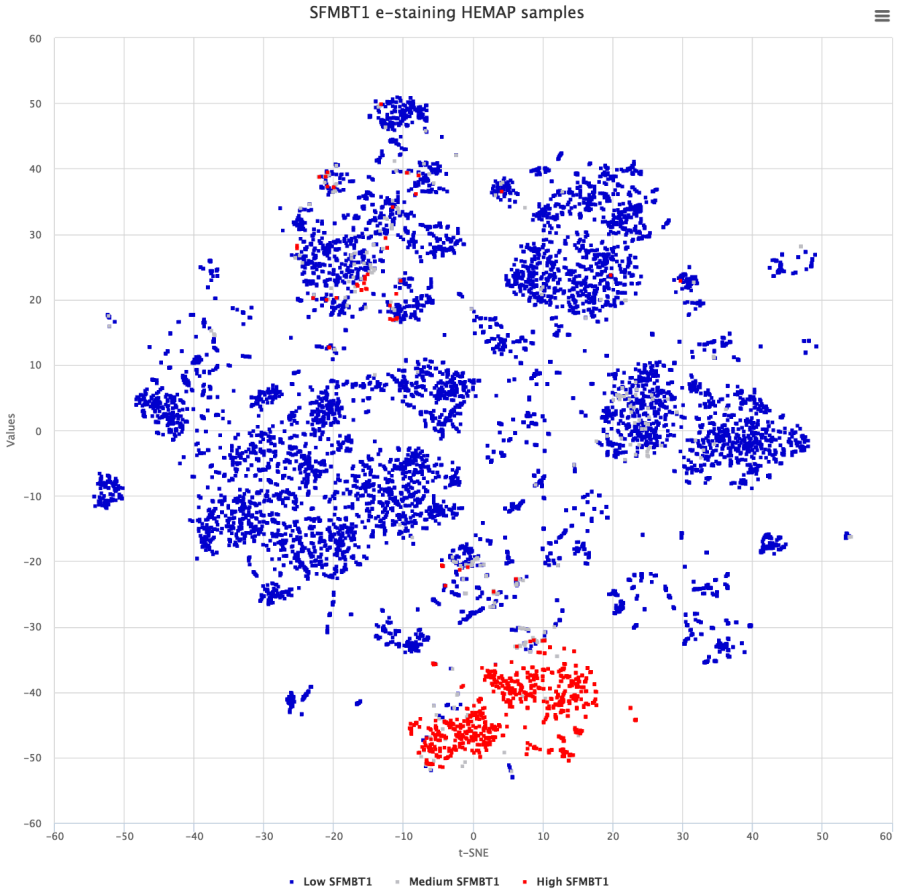


Figure 6. This use case details cancer cluster CLL analysis of finding highly correlated pairwise gene features. The results can be downloaded for advanced statistical studies. Here, SFMBT1 is selected after correlation sorting and then interactively e-stained thus confirming CLL original samples.

Search: *cancermap cluster aml and DrugSigDB type with GSK subfiltering*

DrugSigDB and Pathway features are sets of curated gene lists from known published resources, including Drug Signature DataBase (DSigDB), Wikipathways and PWCommons. The figure below shows the 38 results upon sub-filtering (original with 1990) for pathway features labeled 'GSK' (Glaxo-Smith-Kline) developed drugs. Number of rows shown per page is defaulted to 15 and can be adjusted in Settings.

See http://software.broadinstitute.org/gsea/msigdb/index.jsp for more details on different gene set collections. MsigDB_c2 would filter on MsigDB curated gene sets.

Resource map HEMAP samples Type DrugSigDB guide

Gene/Pathway/Drug

e-stain

getGeneMembers

PW Cluster/Class

annotated_class_AML viSamp

Corr. > 0.1 -log10(ePval) 2 Hypergeometric Test 0 search

Download(1990)

feature	corr.	adjpvalue	BHadj	hypergeom_test	BHadj	nsamp
GW770249X_GSK-DSigDB_D2	0.490	292.194	7.806	307.017	7.806	9544
GW770249A_GSK-DSigDB_D2	0.420	292.194	7.806	202.716	7.806	9544
GW779439X_GSK-DSigDB_D2	0.380	289.994	7.806	128.264	7.806	9544
GW806290X_GSK-DSigDB_D2	0.370	284.494	7.806	121.114	7.806	9544
GW770220A_GSK-DSigDB_D2	0.350	243.294	7.806	38.194	7.806	9544
GW795493X_GSK-DSigDB_D2	0.340	237.694	7.806	65.342	7.806	9544
GW778894X_GSK-DSigDB_D2	0.340	232.494	7.806	57.194	7.806	9544
GW810576X_GSK-DSigDB_D2	0.330	218.994	7.806	132.08	7.806	9544
GW795486X_GSK-DSigDB_D2	0.320	210.694	7.806	80.239	7.806	9544
GSK_3_INHIBITOR_XIII_RBC-DSigDB_D2	0.270	146.194	7.806	63.614	7.806	9544
GSK_3_INHIBITOR_IX_RBC-DSigDB_D2	0.270	143.794	7.806	55.387	7.806	9544
GW830365A_GSK-DSigDB_D2	0.260	137.994	7.806	0	7.806	9544
GW612286X_GSK-DSigDB_D2	0.240	112.794	7.806	9.342	7.806	9544
GSK2110236A_GSK-DSigDB_D2	0.240	111.994	7.806	43.216	7.806	9544
GW784752X_GSK-DSigDB_D2	0.240	111.194	7.806	17.581	7.806	9544

Showing 1 to 15 of 38 entries (filtered from 1,990 total entries) Previous123Next

flip

Filter results

GSK

Figure 7. Sub filtering, using GSK, is demonstrated on AML class features. The number of rows is adjusted to 38 from 1990. Sub-filtering is functional across all columns with character matching.

Gene sets of pathway and drug signatures

Gene members upon pathway/drug gene set selection can be easily looked up. Clicking on the *getGeneMembers* brings up a dialog box showing the source link and gene set.

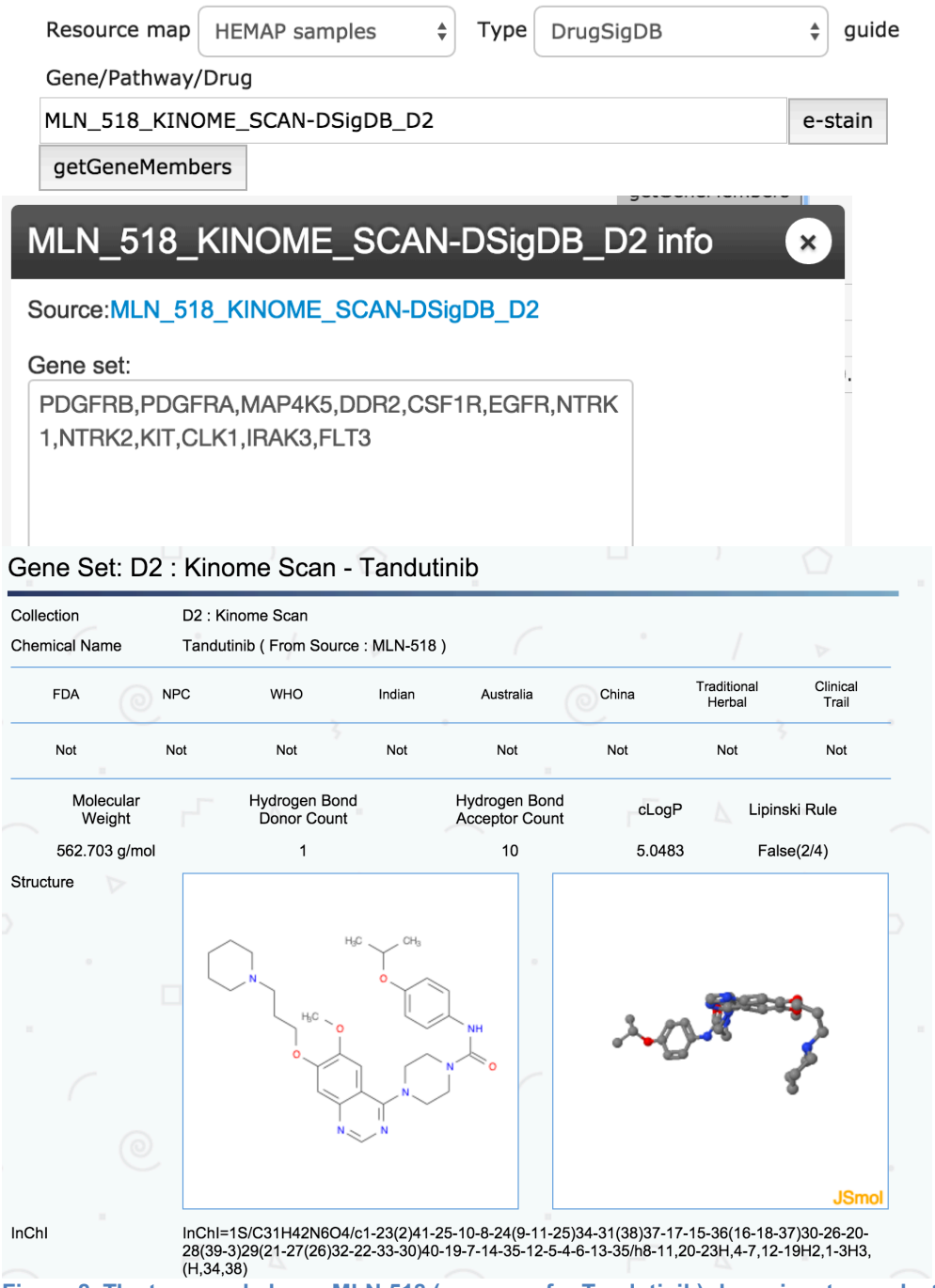


Figure 8. The top panel shows MLN-518 (synonym for Tandutinib) drug signature selection and its gene members are shown. The bottom graph shows results of clicking on the source DSigDB (Tan lab) link.

TCGA AML Map

The default map of TCGA colors the samples by cytogenetic subtypes (fusion genes and mutations) Alternatively, the map can be re-plotted with the t-SNE cluster labels by clicking on Cluster link shown below.

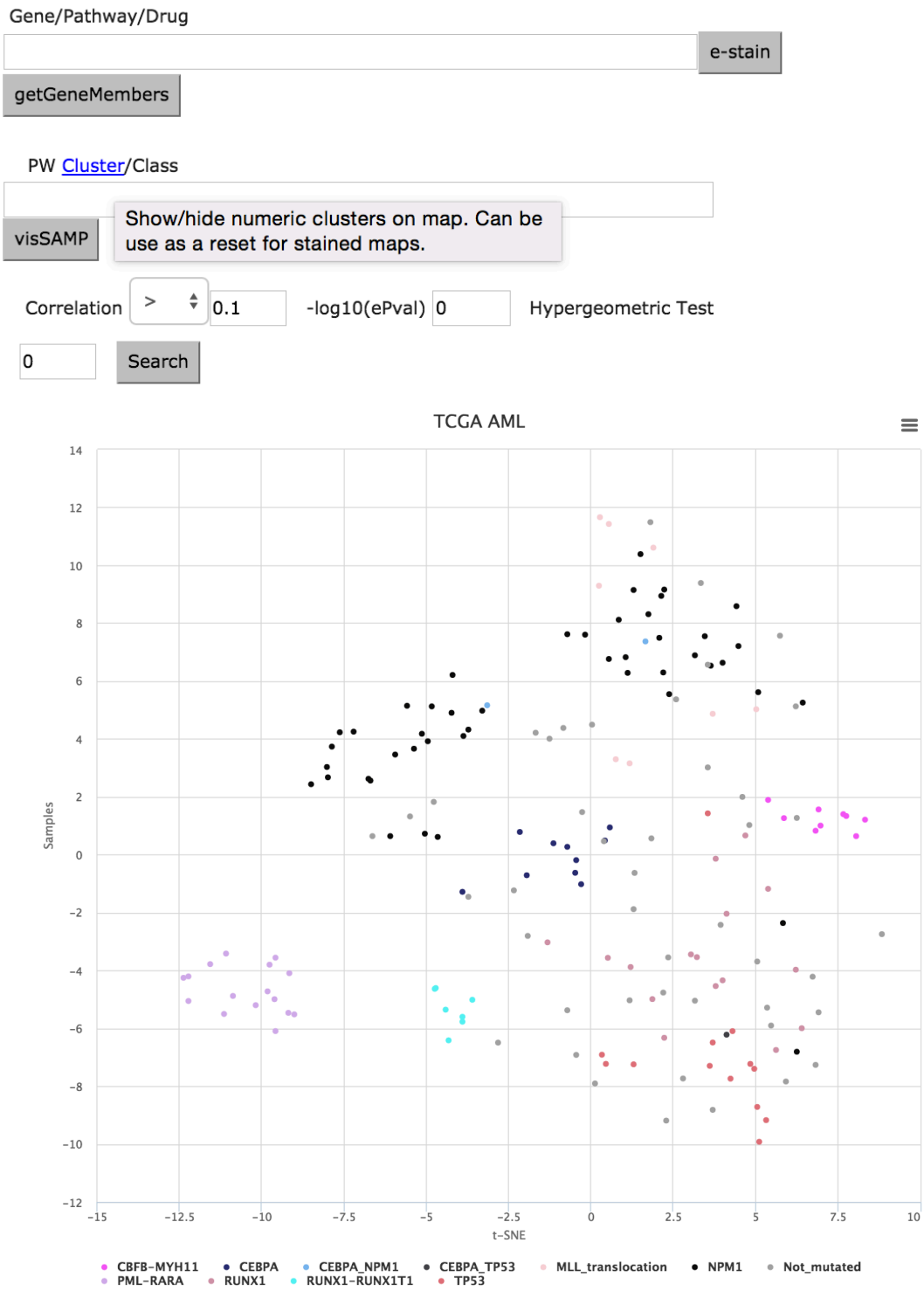


Figure 9. The default view of theTCGA map indicates the location of patients with gene fusion and mutation events in color.

Additional data types for TCGA AML map: GNAB, CNVR, METH, MIRN and CLIN categories

The genome-wide mutation, methylation and copy number profiles are available for the TCGA samples with the feature names GNAB, METH and CNVR. Pairwise exploration of cluster association with these features works the same as described above. Below we will look at a few of the data types and the easy manner they can be explored and e-stained.

Certain TCGA data types are continuous numeric values therefore statistic significance/ranking across each element is rank using z-score, computed as (x – mean)/standard deviation:

$$z = \frac{x - \mu}{\sigma}$$

e-staining *mir-125 across METH and MIRNA*

The scenario below finds mir-125b-1 with high positive correlation (.47 to cancermap cluster 1) after selecting the METH datatype and clicking on cluster 1 (PML-RARA cases) in the map. The quick-selection of interesting clusters for pairwise analysis is also supported for other feature types. As explained earlier, e-staining the methylation data is performed on clicking the feature (gene) name directly on the table (middle figure). It is also possible to check whether the differential methylation status affects the miRNA expression, by choosing Type MIRNA and entering the official identifier to the Gene field (hsa-miR-125b-1) (bottom figure, correlation .48).

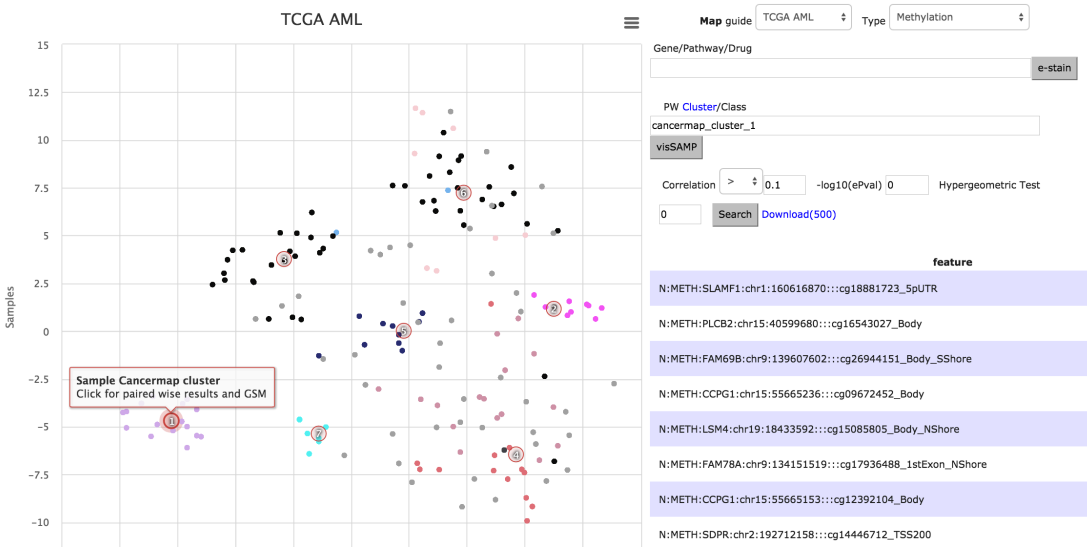


Figure 10. Data analysis is performed visually for mir-125 via e-staining across methylation and miRNA. Pairwise results are interactively retrieved with cluster label mouse click on map.

Lookup of copy number aberrations by gene name, vice versa

Copy number aberrations often include large chromosomal region gains or losses that houses multiple genes. As such, it is useful to search by gene name and then look up the corresponding copy number labels, often in cytoband terms. The figure below illustrates the situation of looking up *TP53*, *20q13.2* is displayed upon clicking *Gene2Cyto*, *Cyto2Genes* click returns gene members.

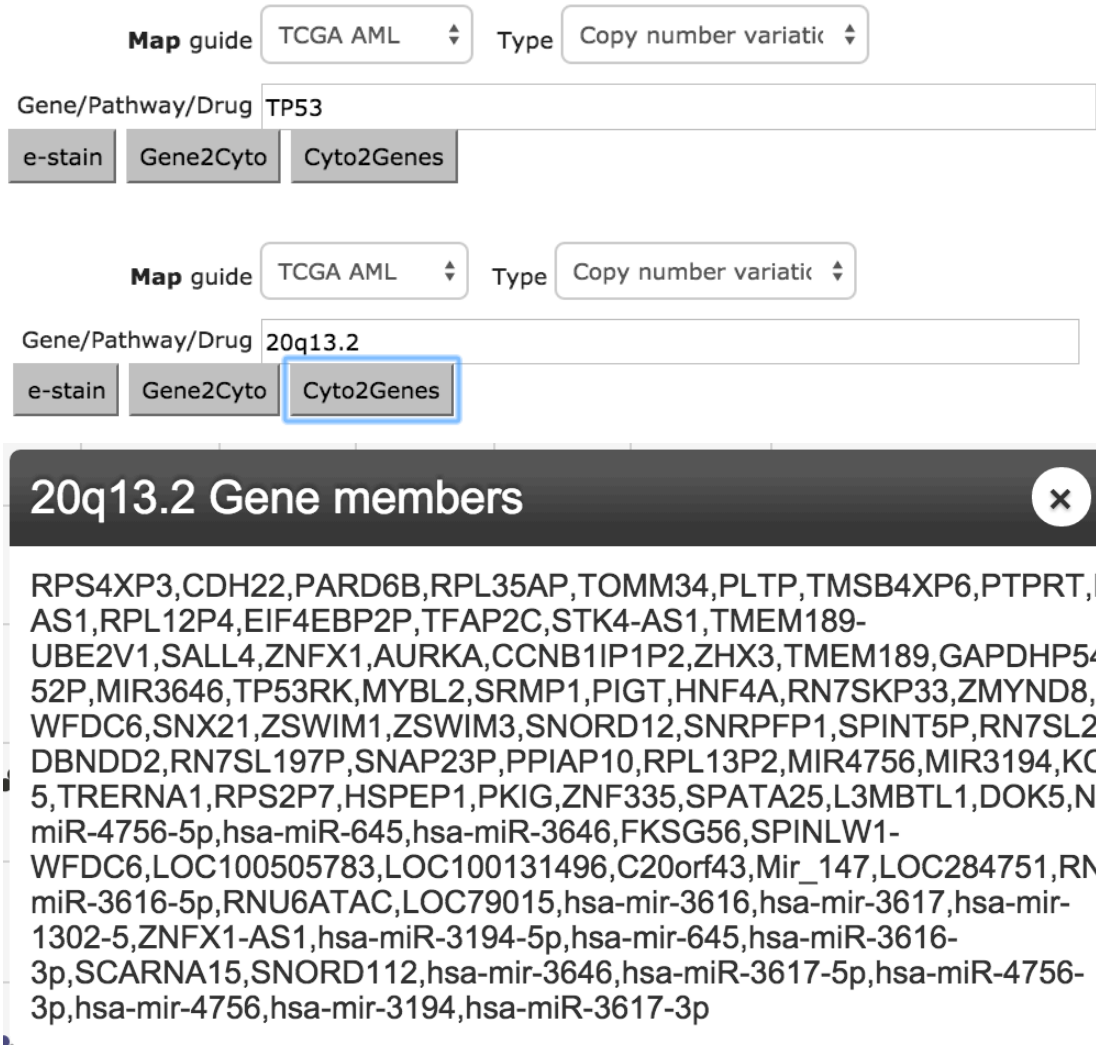


Figure 11. Copy number features are often named as cytobands inclusive of many genes. Hemap allows for lookups in both directions. Example depicts looking up TP53 (top) for 20q13.2(middle) and then all members within the cytoband.

Annotation Search, e-staining, and box plotting

Main Category: *Leukemia* AND Cytogenetics: *crlf2* analysis to IRX3

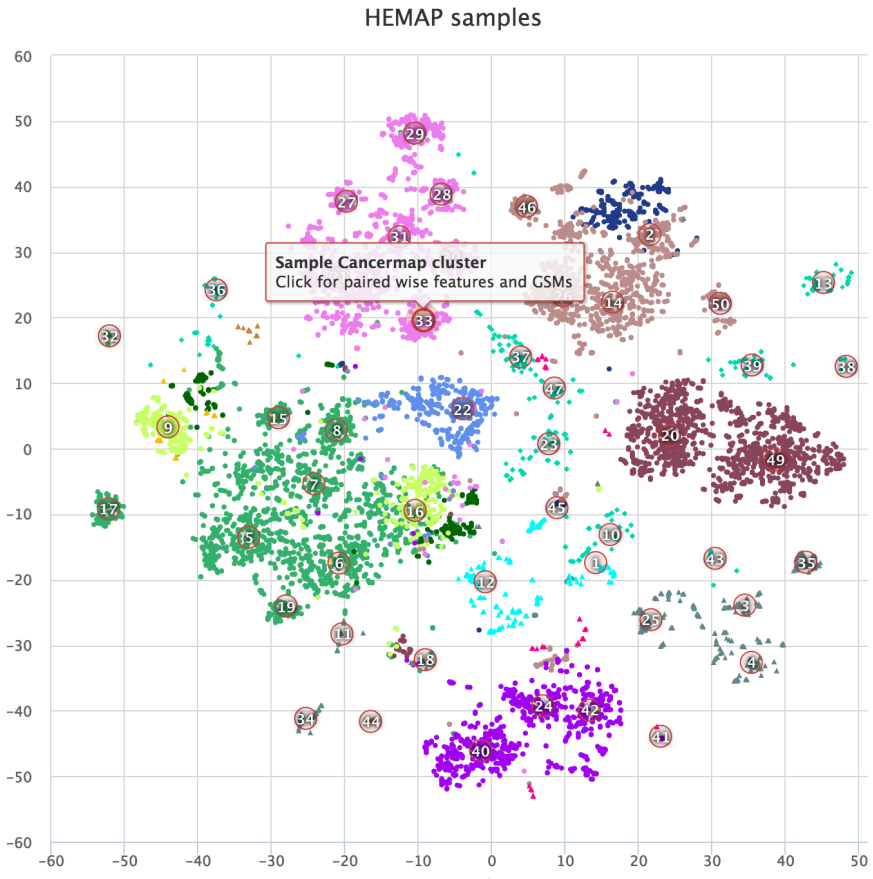
The web application allows for flexible searches across all annotations supporting type ahead and wild card search. For example and detailed in figure 12, updating the column dropdown to *Main Category* and typing in Leukemia (or use type ahead) returns 4778 records (not shown). The advanced interface is turned on (or hide) by clicking on Advanced link and setting the 2nd column dropdown to *Cytogenetics* with *crlf2* entry with AND clause, 26 records are returned as depicted in figure 11 (top with partial number of rows). Using the button "See Results on Map" on upper right corner, the middle figure shows the 26 samples (including all crlf2 cytogenetic deletions and fish) in blue (color picker available as shown). From the annotation results next to the Search button is AddResults2BoxPlot. This adds the sample group to Hemap GEXP

box plots; more custom groups can be added across user selected gene(s); in this case *IRX3*.

Figure 12. Annotation search allows for intuitive basic search but also provides powerful advanced functions. Almost 5000 records are labeled Leukemia and shown here is Leukemia samples annotated also with Cytogenetics *crlf2*. The 26 results are then visualized on the map and then gene FLT3's expression summary statistics are plotted and contrasted, revealing substantially higher mean expression.

Sample groups from map cluster selection (mouse) and plotting combined with annotation search

From the main map, the 9,544 samples have been classified into 50 clusters. Prior, we described toggling the map labels using Explore interface 'Clusters' link for the main map and for pairwise TCGA searches. Cluster selection on the main map can also add the clustered samples for box plotting. Figure 13 shows selection of Cluster 33 (169 samples) and then the box plot result with IRX3 expression values. User needs to select the cluster label (red enlarged circles), not sample nodes (selection on sample nodes opens up GEO), the 2nd panel shows a zoomed in area of cluster 33 for easier selection. Upon clicking, the AddCluster33_2Boxplot button needs to be clicked, also in the Explore interface, above the tabular results and shown in same panel. The IRX1 box plot for the 169 samples (from prompt after button) is shown in 3rd panel of figure 13. Hemap is integrated across maps, annotations and plotting. The bottom panel includes cluster 33 IRX3 together with annotation search of subtype pre-B-ALL. Other annotation searches or cluster selections are also compatible.



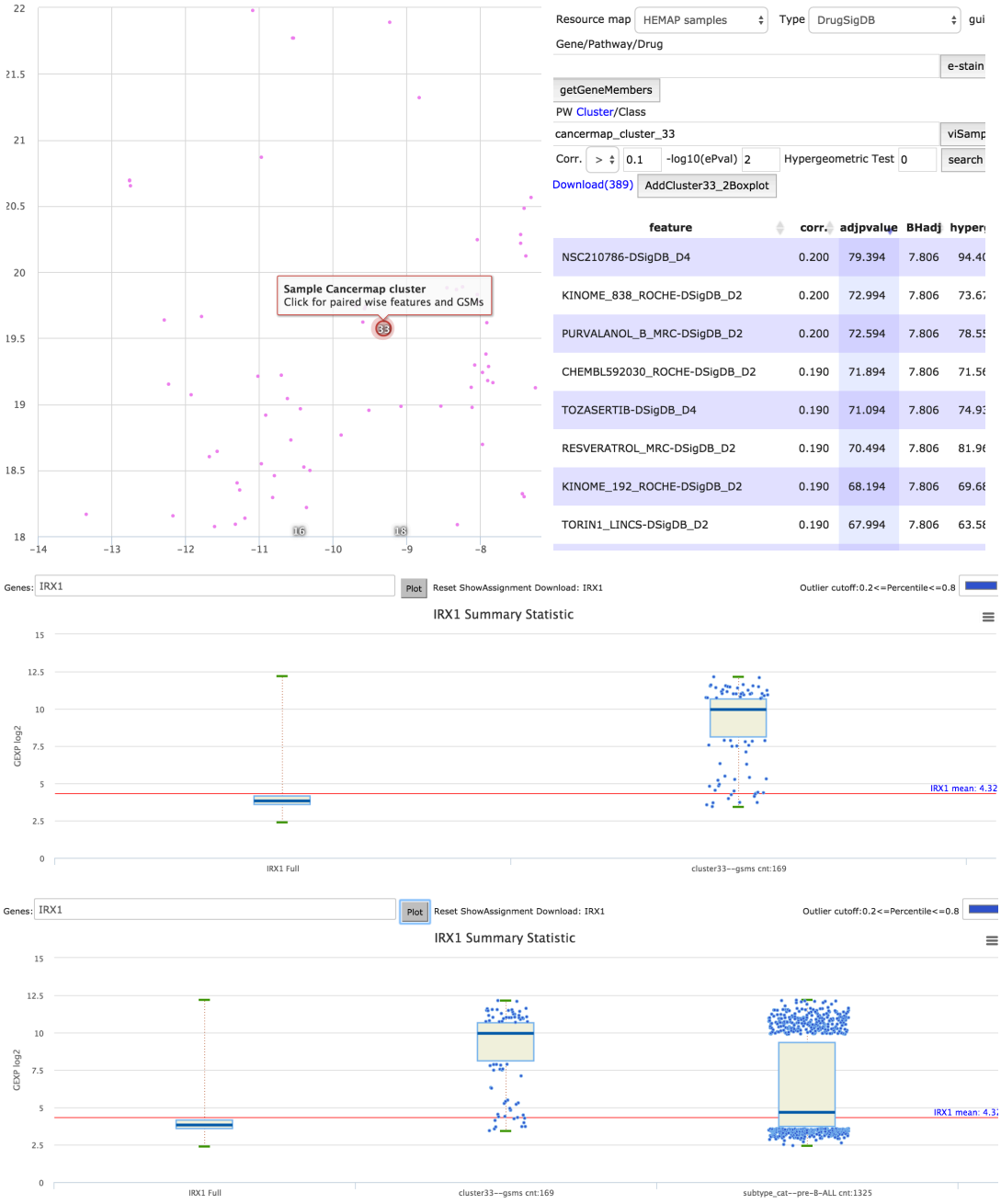
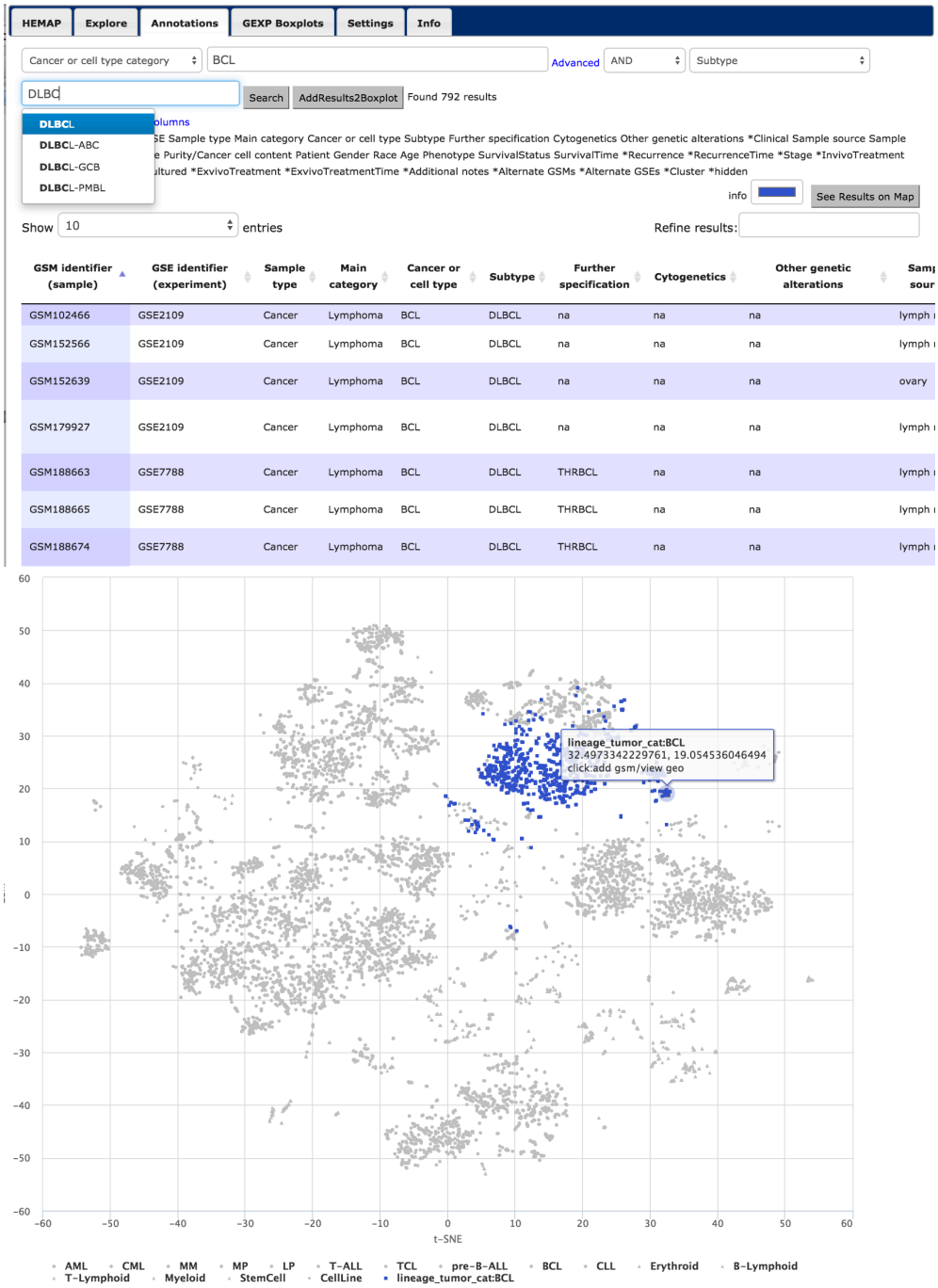


Figure 13. Labeled cluster 33 selection triggers pairwise selection and the samples classified to cluster 33 can also be added to box plot. This sample groups is plotted to IRX1 and the bottom most figure demonstrates combining annotation search on subtype pre-B-ALL, contrasting with cluster selection and also revealing higher IRX1 expression on pre-B-ALL samples.

Annotation categories to gene expression e-staining

This example illustrates the usage of combining filtering values to locate samples from three cancer types. The first filter is BCL cancer/cell type and next we select across subtypes DLBCL (792 samples stained blue (Fig. 14 rows 1 and 2)) or CHL (139 samples stained purple, see row 3 usage of color picker

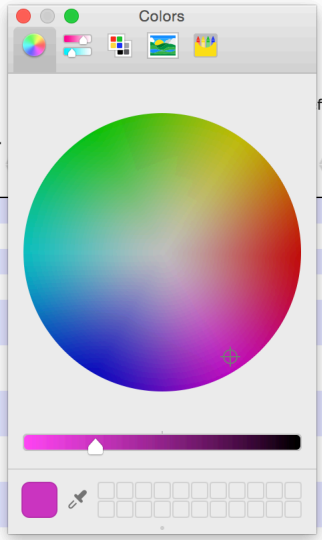
(Fig. 14 rows 3 and 4)). In a similar manner, more BCL types can be stained on the map (not shown). Without the advanced AND subtype filtering, there are 1280 BCL labeled samples. As third example, the subtype selection of pre-B-ALL with MLL cytogenetic labeled samples (238) are stained pink in the same figure.



Cancer or cell type category | BCL | Advanced | AND | Subtype

CHL | Search | AddResults2Boxplot | Found 139 results

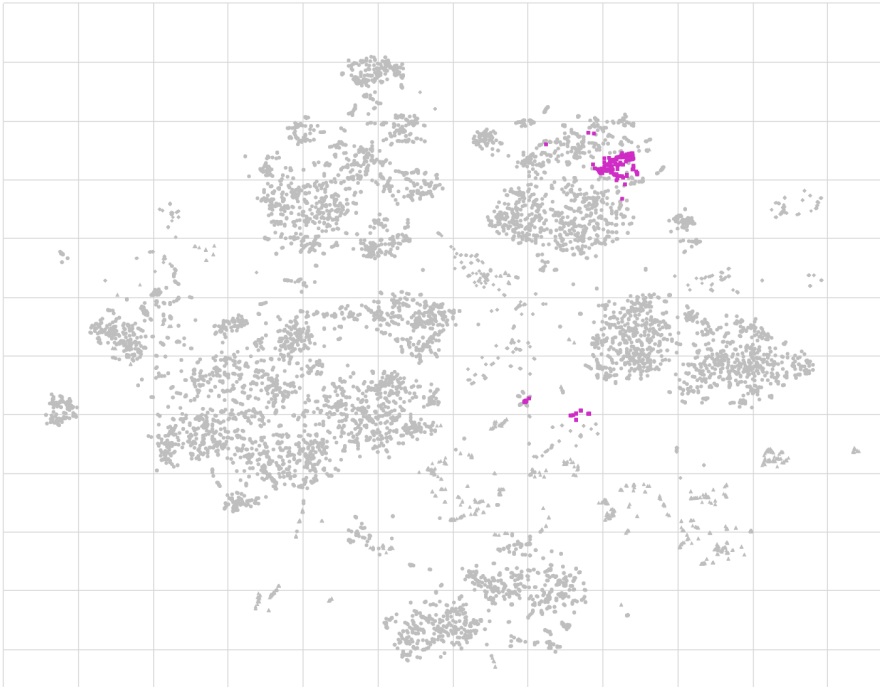
Manage Annotation Columns



info | See Results on Map

Show 10 entries | fine results:

GSM identifier (sample)	GSE identifier (experiment)	Sample type	Main category	Cancer or cell type				Other genetic alterations	Clinical
GSM311194	GSE12427	CellLine	Lymphoma	BCL				na	na
GSM311195	GSE12427	CellLine	Lymphoma	BCL				na	na
GSM311200	GSE12427	CellLine	Lymphoma	BCL				na	na
GSM311203	GSE12427	CellLine	Lymphoma	BCL				na	na
GSM312814	GSE12453	Cancer	Lymphoma	BCL				na	na
GSM312816	GSE12453	Cancer	Lymphoma	BCL				na	na
GSM312817	GSE12453	Cancer	Lymphoma	BCL				na	na
GSM312818	GSE12453	Cancer	Lymphoma	BCL				na	na
GSM312819	GSE12453	Cancer	Lymphoma	BCL				na	na
GSM312820	GSE12453	Cancer	Lymphoma	BCL	CHL	na	na	na	na
GSM312821	GSE12453	Cancer	Lymphoma	BCL	CHL	na	na	na	na



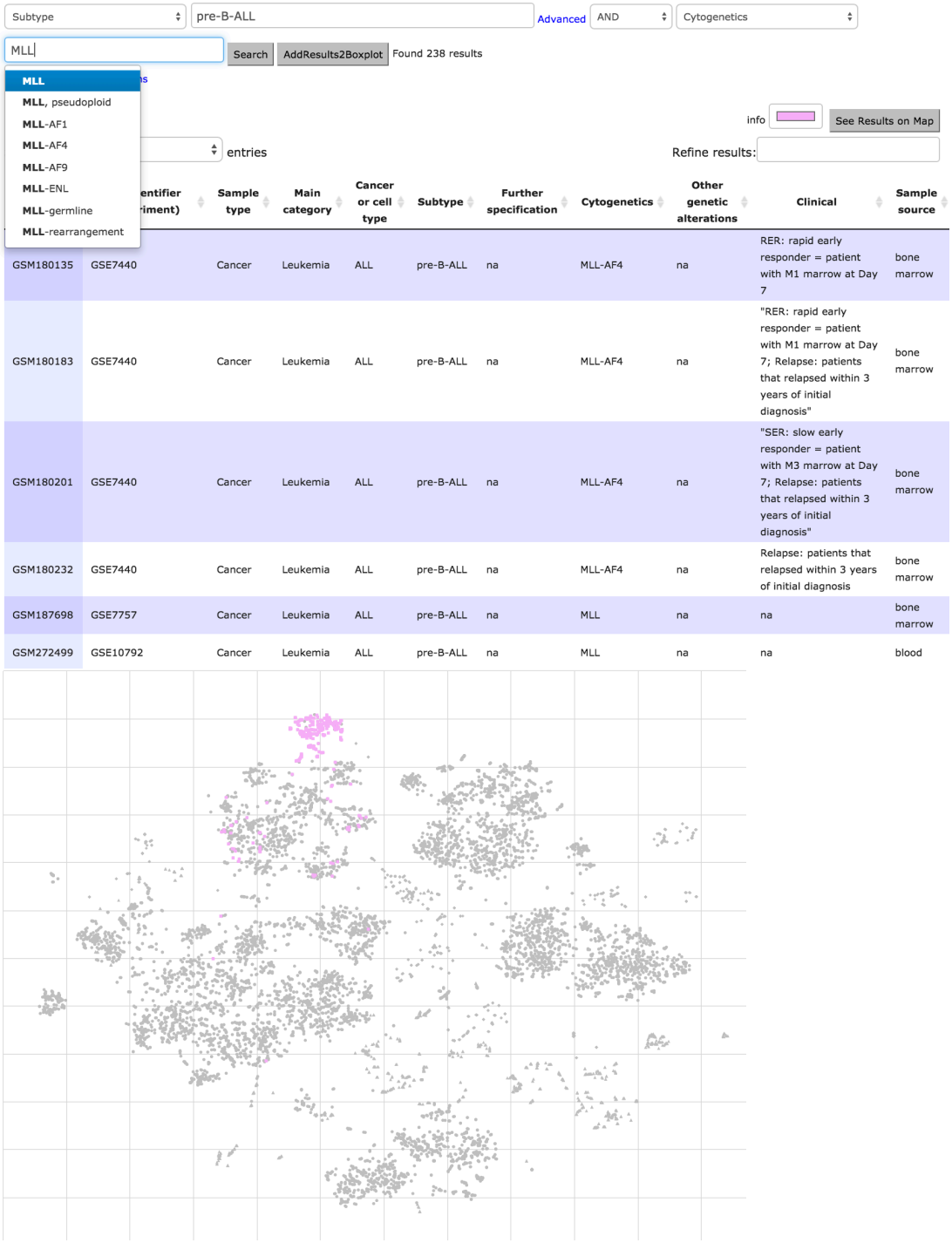


Figure 14. HEMAP samples are further classified and e-stained based on cancer type BCL subtypes DLBCL (1st 2nd rows) and CHL (middle 3rd,4th rows). The results are obtained via combining cancer/cell type with subtype. Filtering and its combinations are highly flexible, as demonstrated in rows 5 and 6 of 238 samples via using subtype pre-B-ALL and MLL cytogenetic event.

The Explore tab allows for easy gene and pathway e-staining as well as pairwise statistical data analysis, highlighted in the next Advanced use case section. Here in Fig. 15, the cell surface genes *CD19* and *MME* (aka *CD10*) e-staining maps are plotted. The gene input, as well as pathway and drug signature fields, provides type ahead.

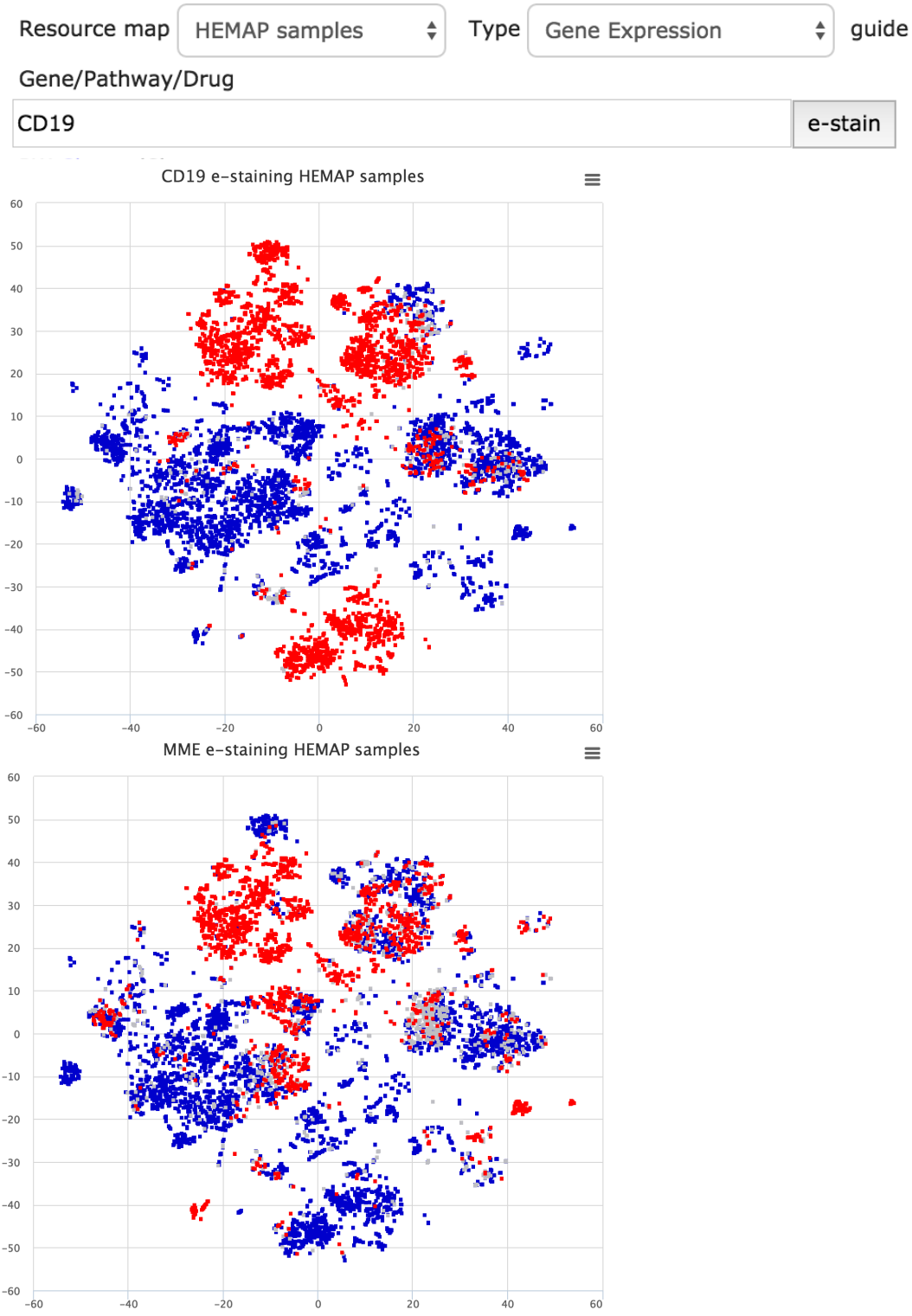


Figure 15. Gene based e-staining is accomplished for cell surface protein coding *CD19* and *MME* (aka *CD10*) genes. The maps are exportable. Low, medium and high colors can be customized using the settings, described in the last section of this guide.

Use case examples

Exploring surface protein expression in lymphomas

In the following analysis, a new surface protein candidate for immunotherapy is compared to CD19 that is currently used in B-lymphoid cancers. High expression of CADM1 has been previously linked with T-cell lymphoma. The relevant reference for CADM1 is here https://www.ncbi.nlm.nih.gov/pubmed/22706526

Figure 16: CADM1 shows a consistent RNA expression within the lymphoma disease context and low levels in normal blood cell types. Its expression in lymphoma patient material can be further confirmed using Surface Protein Atlas.

Gene expression box plots across main categories for CD19 and CADM1.

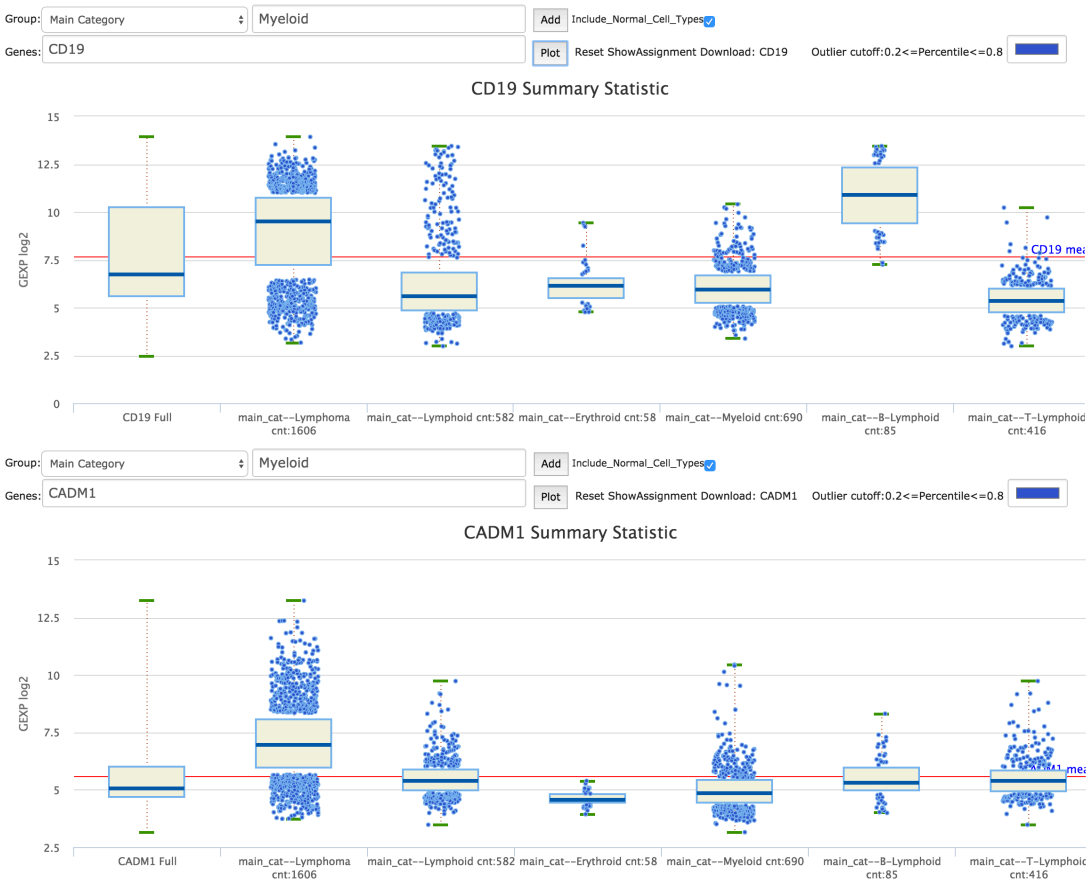


Figure 17. Comparing CD19 and CADM1 gene expressions. From the GEXP Boxplot interface, categories Lymphoma, Lymphoid, T-Lymphoid, B-Lymphoid, Erythroid, and Myeloid are entered one at a time and then the genes CD19 and CADM1 are plotted. It is visually evident the difference in their expressions across the different lymphoma categories.

Finding Myeloma cluster specific genes/pathways

In this example Hemap resource is used for characterizing Myeloma clusters. First, genes that best distinguish two myeloma clusters are extracted from the pairwise results. Similar analysis is also used for finding pathways that can distinguish the clusters.

Resource map HEMAP samples Type Gene Expression guide

Gene/Pathway/Drug e-stain Download raw LOC284889

PW Cluster/Class cancermap_cluster_20_vs_49 viSamp Corr. < 0.1 -

log10(adjPval) 10 -log10(hypergeom_test_adjPval) 0 search Download(4486)

feature	correlation	adj_pvalue (-log10)	hypergeom_test_pvalue (-log10)
LOC284889	-0.830	156.333	na
IL1RN	-0.810	151.033	na
TOP3B	-0.810	150.133	na
PDLIM4	-0.810	148.833	na
NKX2-5	-0.810	148.733	na
TGM2	-0.810	148.633	na
DRD2	-0.800	146.233	na
KCNK16	-0.800	145.833	na
P2RX2	-0.800	145.533	na
SOX18	-0.800	144.633	na
ZDHHC3	-0.800	144.333	na
MSC	-0.790	143.833	na
FCAR	-0.790	143.633	na
PTRH1	-0.790	143.333	na
ZNF503	-0.790	141.833	na

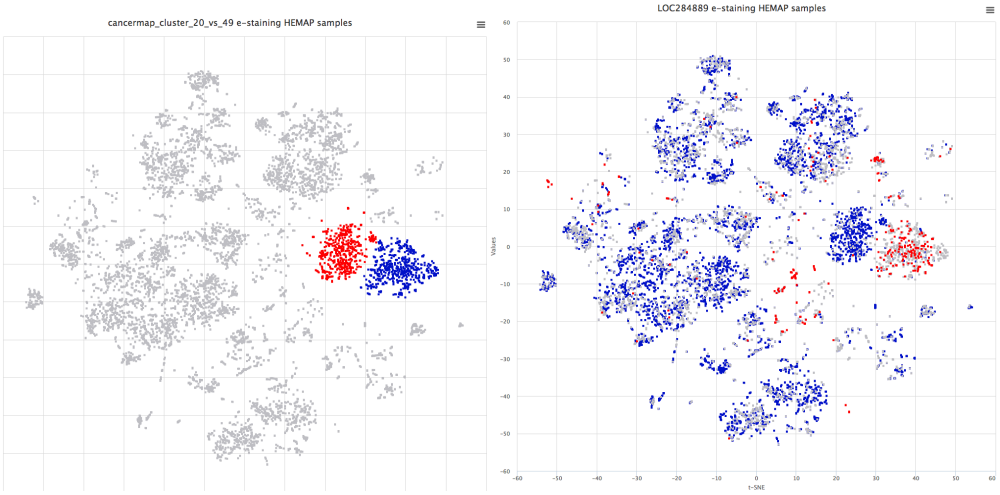


Figure 18. Genes distinguishing cluster 49 from cluster 20 are explored using pairwise results. Top result LOC284889 (MIF-AS) is plotted to cancermap using e-staining.

Resource map HEMAP samples Type Pathway guideGSVA/FDR +/-:0.11

Gene/Pathway/Drug KRAS_SIGNALING_DN-MsigDB_HALLMARKS e-stain getGeneMembers

PW Cluster/Class cancermap_cluster_20_vs_49 viSamp Corr. ab 0.5 -

log10(adjPval) 50 -log10(hypergeom_test_adjPval) 50 search Download(435)

feature	correlation	adj_pvalue (-log10)	hypergeom_test_pvalue (-log10)
XENOBIOTIC_METABOLISM-MsigDB_HALLMARKS	-0.750	127.694	70.54
CHOLESTEROL_HOMEOSTASIS-MsigDB_HALLMARKS	-0.730	121.394	55.415
HYPOXIA-MsigDB_HALLMARKS	-0.670	101.594	93.832
MTORC1_SIGNALING-MsigDB_HALLMARKS	-0.640	91.894	64.591
HEME_METABOLISM-MsigDB_HALLMARKS	-0.610	81.794	53.08
KRAS_SIGNALING_DN-MsigDB_HALLMARKS	-0.570	69.594	68.423
REACTIVE_OXIGEN_SPECIES_PATHWAY-MsigDB_HALLMARKS	-0.550	65.694	54.637
MYOGENESIS-MsigDB_HALLMARKS	-0.540	63.594	54.675

Showing 1 to 8 of 8 entries (filtered from 435 total entries) Previous1Next

Filter results MsigDB_HALLMARKS

flip

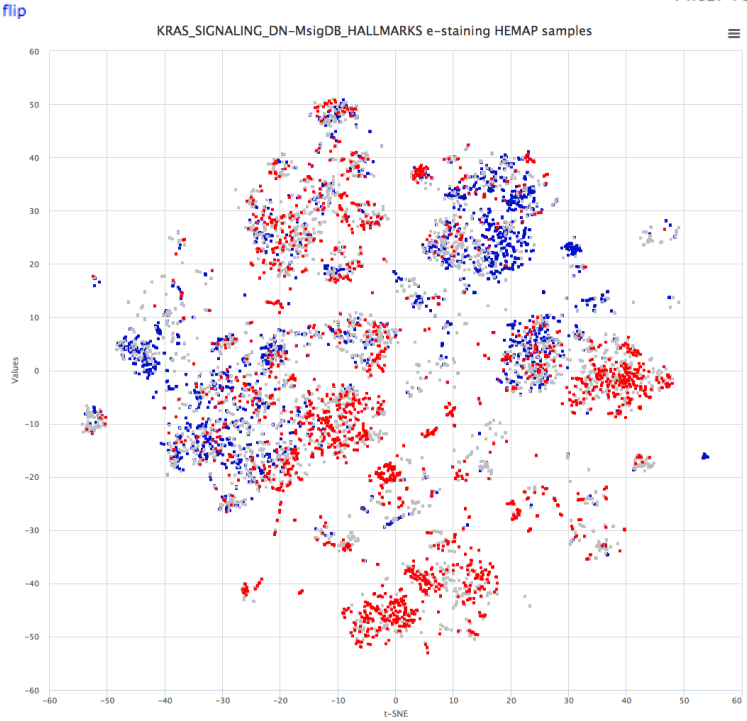
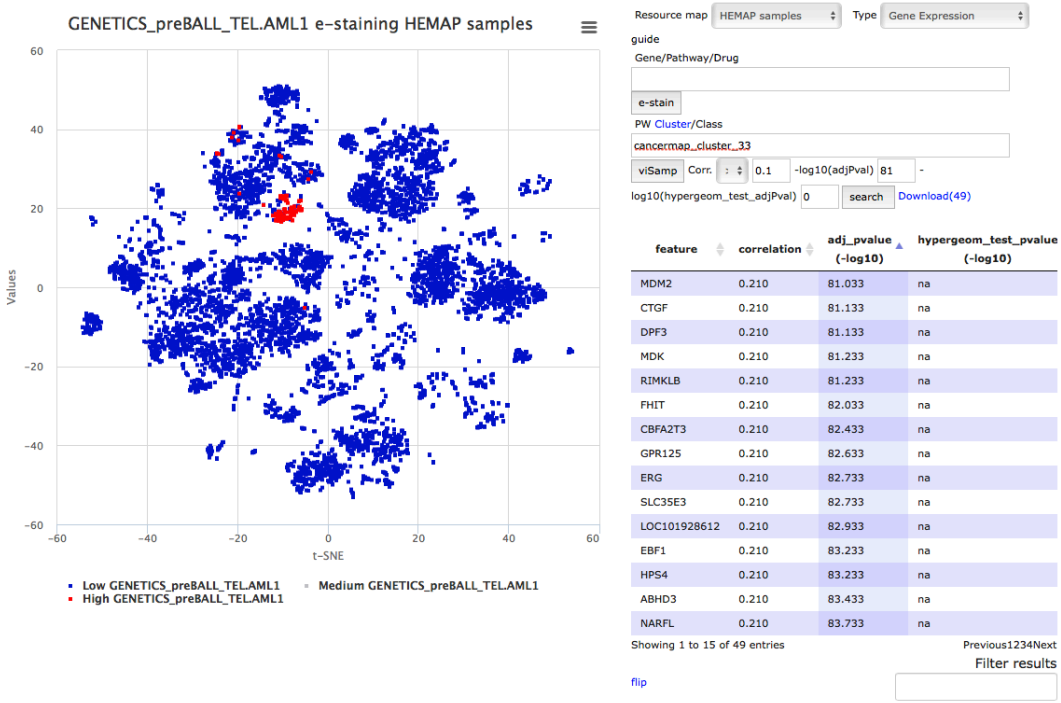


Figure 19. Pathways distinguishing cluster 49 from cluster 20 are explored using pairwise results. Results are filtered using correlation and hypergeometric test adjusted P-values and correlation coefficient. Pathway source (MsigDB_HALLMARKS) is filtered using "Filter results" field. KRAS signature separates clusters 49 and 20.

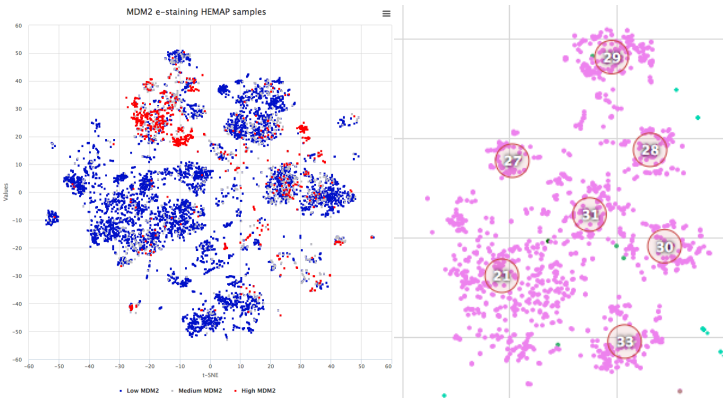
Finding subtype specific druggable genes

In this example, genes with high expression are obtained for the ETV6-RUNX1 (also known as TEL-AML1) pre-B-ALL subtype, here visualized based on e-staining the clinical feature (GENETICS_preBALL_TEL.AML1). One of these genes is MDM2 that represents a recently characterized vulnerability in pre-B-ALL and particularly TEL-AML1 (PMIDs: 26459177, 24240203). MDM2 is also a top gene for cluster 33 when analyzing existing drug targets. Expression of MDM2 is also compared to other leukemia subtypes as boxplots to confirm the association.



feature	correlation	adj_pvalue (-log10)	hypergeom_test_pvalue (-log10)
MDM2	0.210	81.033	na
CTGF	0.210	81.133	na
DPF3	0.210	81.133	na
MDK	0.210	81.233	na
RIMKLB	0.210	81.233	na
FHIT	0.210	82.033	na
CBFA2T3	0.210	82.433	na
GPR125	0.210	82.633	na
ERG	0.210	82.733	na
SLC35E3	0.210	82.733	na
LOC101928612	0.210	82.933	na
EBF1	0.210	83.233	na
HPS4	0.210	83.233	na
ABHD3	0.210	83.433	na
NARFL	0.210	83.733	na

Figure20. Finding TEL-AML1 (cluster 33) specific genes.



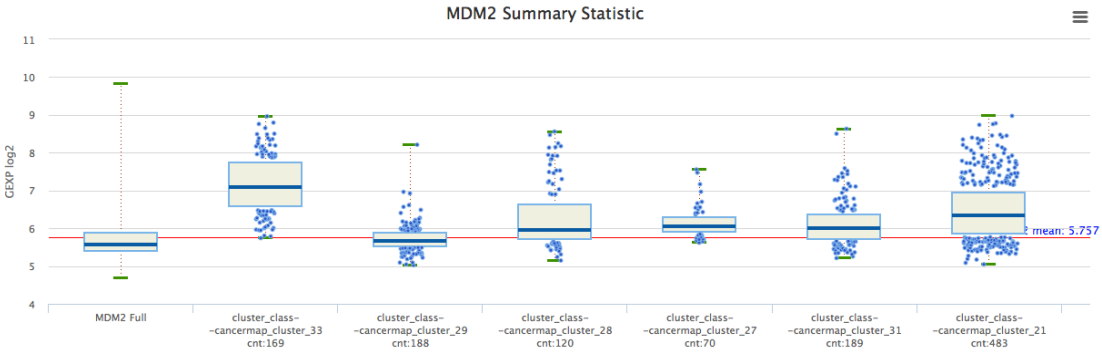


Figure 21. Comparing MDM2 expression to other pre-B-ALL clusters to verify high subtype specificity. Clusters were added to boxplot from the GEXP Boxplot interface.

In silico drug repurposing analysis using Hemap and DSigDB

This example illustrates how the Hemap resource can be used for *in silico* drug repurposing analysis (following the target example from PMID:28885610). Dasatinib is currently in use to treat pre-B-ALL. Several Dasatinib targets were expressed also in lymphoma and in T-ALL, motivating exploring drug repurposing in those diseases. LCK was identified as one of the top Dasatinib targets for T-ALL. Drugs targeting LCK were retrieved assessed from chemical screening database to evaluate their specificity for LCK.

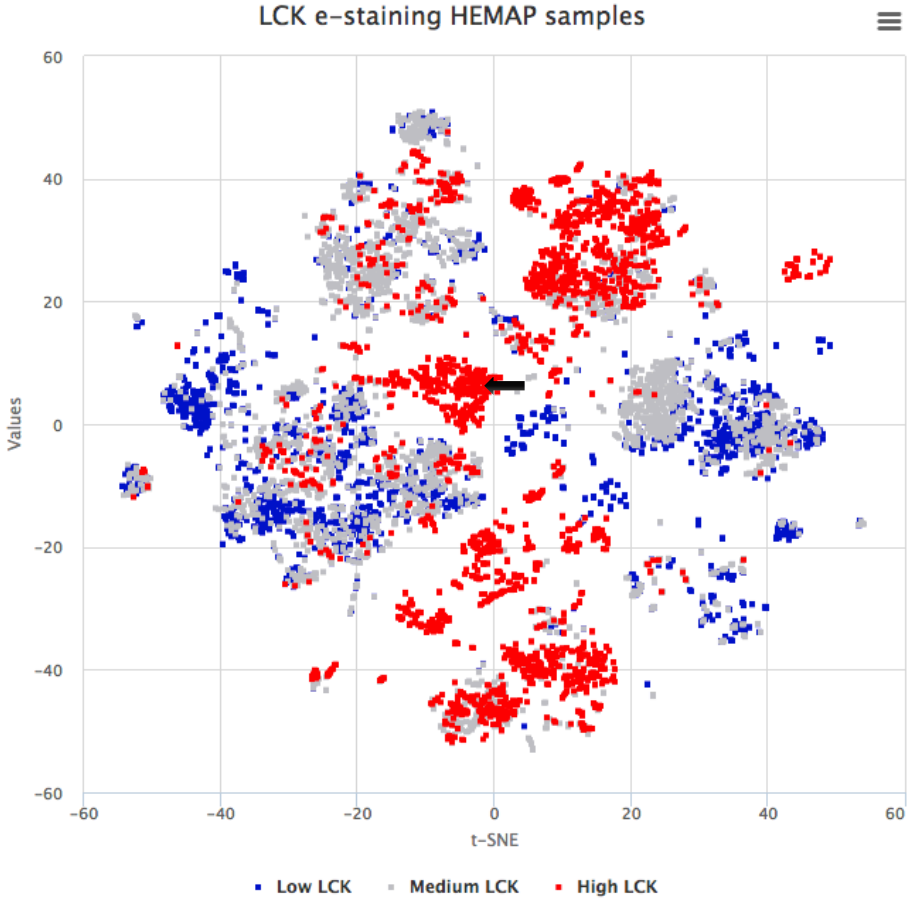


Figure 22. E-staining of LCK

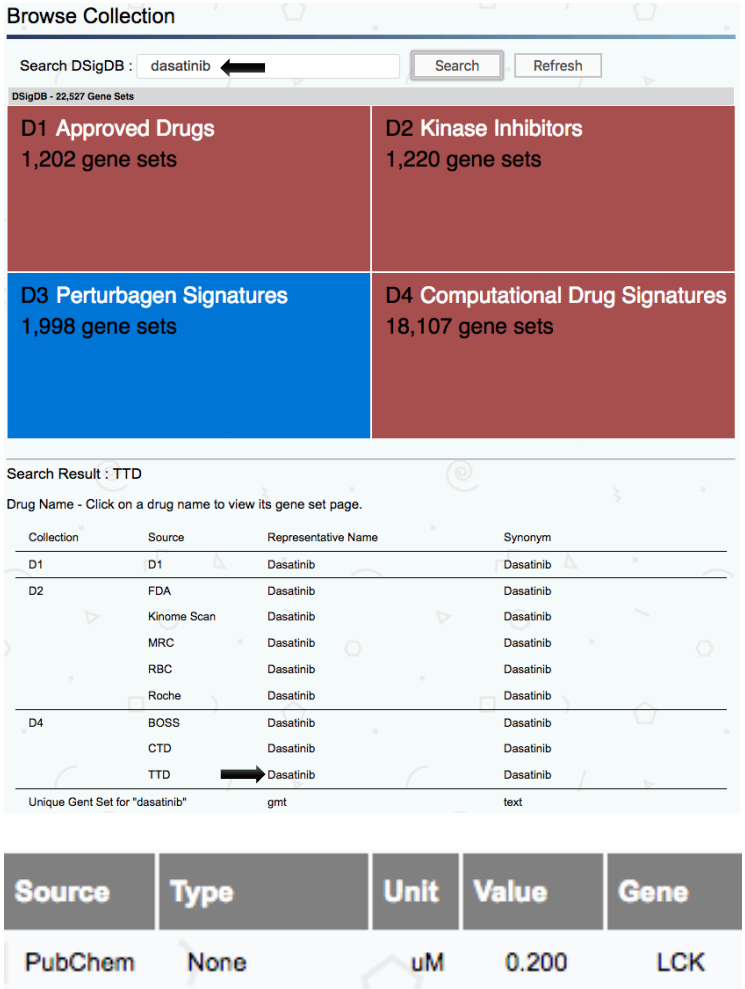


Figure 23. Checking Dasatinib target specificity from DSigDB. Several sources contain chemical screening data. Clicking TTD same targets as in tableS6 for Dasatinib are shown with chemical screening results from PubChem.

In silico drug screening using Hemap

This example illustrates how the Hemap resource can be used for *in silico* drug screening. First, the pairwise results are used to find signaling pathways that could be targeted in pre-B-ALL and as a next step to find candidate drugs that specifically target this signaling pathway. Furthermore, as a third step, drug gene set details of drug chemical screening are used to evaluate drug target specificity. As a final step, gene e-staining and boxplot functions are used to compare gene expression in disease and normal cells to assess drug safety and potential side effects.

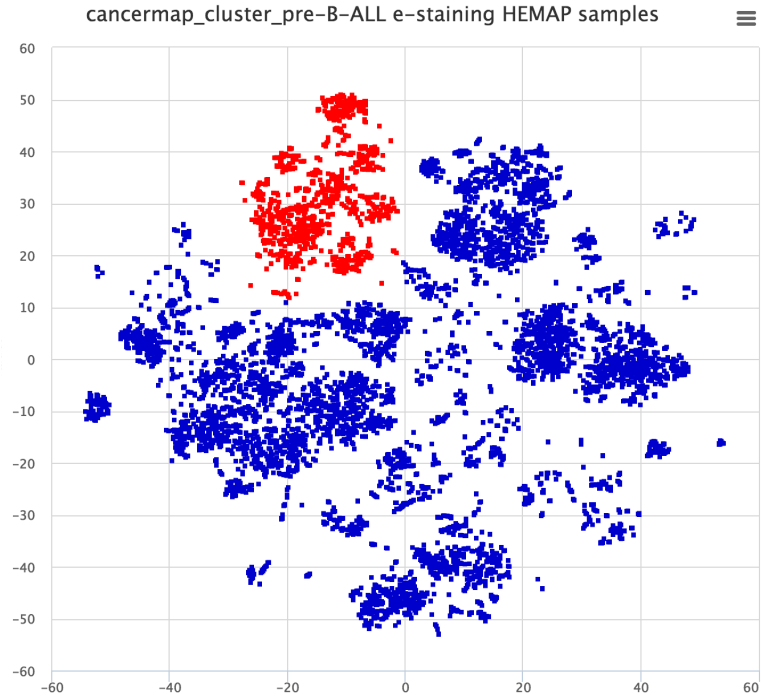


Figure 24. pre-B-ALL clusters stained using visSAMP.

Step 1: Identification of candidate pathways

Resource map HEMAP samples Type Pathway guide

Gene/Pathway/Drug e-stain getGeneMembers

PW Cluster/Class cancermap_duster_pre-B-ALL viSamp Corr. 0.1

-log10(ePval) 2 Hypergeometric Test 0 search Download(3238)

view/hide: nsamples ndif1 ndif2 nna1 nna2

feature	corr.	adjpvalue	BHadj
INSULIN_IGF_PATHWAY_PROTEIN_KINASE_B_SIGNALING_CASCADE-PWCOMMONS	0.410	292.194	7.806
WNT_SIGNALING_PATHWAY-PWCOMMONS	0.430	292.194	7.806
RNF_MUTANTS_SHOW_ENHANCED_WNT_SIGNALING_AND_PROLIFERATION-PWCOMMONS	0.400	292.194	7.806
MISSPLICED_LRP5_MUTANTS_HAVE_ENHANCED_BETA_CATENIN_DEPENDENT_SIGNALING-PWCOMMONS	0.400	292.194	7.806
TCF_DEPENDENT_SIGNALING_IN_RESPONSE_TO_WNT-PWCOMMONS	0.400	292.194	7.806
SIGNALING_BY_WNT-PWCOMMONS	0.380	292.194	7.806
WNT_SIGNALING_PATHWAY_NETPATH_HOMO_SAPIENS-WIKIPW	0.370	272.394	7.806
WNT_SIGNALING_PATHWAY-KEGG_MsigDB_c2	0.350	252.794	7.806
PIP3_SIGNALING_IN_CARDIAC_MYOCTES-SIG_MsigDB_c2	0.350	250.294	7.806
ANDROGEN_RECEPTOR_SIGNALING_PATHWAY_HOMO_SAPIENS-WIKIPW	0.350	246.594	7.806
PI3K_EVENTS_IN_ERBB2_SIGNALING-REACTOME_MsigDB_c2	0.340	240.094	7.806
SIGNALING_EVENTS_MEDIATED_BY_THE_HEDGEHOG_FAMILY-PWCOMMONS	0.340	239.494	7.806
REGULATION_OF_NUCLEAR_SMAD2_3_SIGNALING-NCI_NATURE_V4_PID	0.340	239.394	7.806
REGULATION_OF_NUCLEAR_SMAD2_3_SIGNALING-PWCOMMONS	0.340	239.394	7.806
PI3K_EVENTS_IN_ERBB4_SIGNALING-REACTOME_MsigDB_c2	0.340	234.594	7.806

Showing 1 to 15 of 251 entries (filtered from 3,238 total entries) Previous12345...

flip Filter results signaling

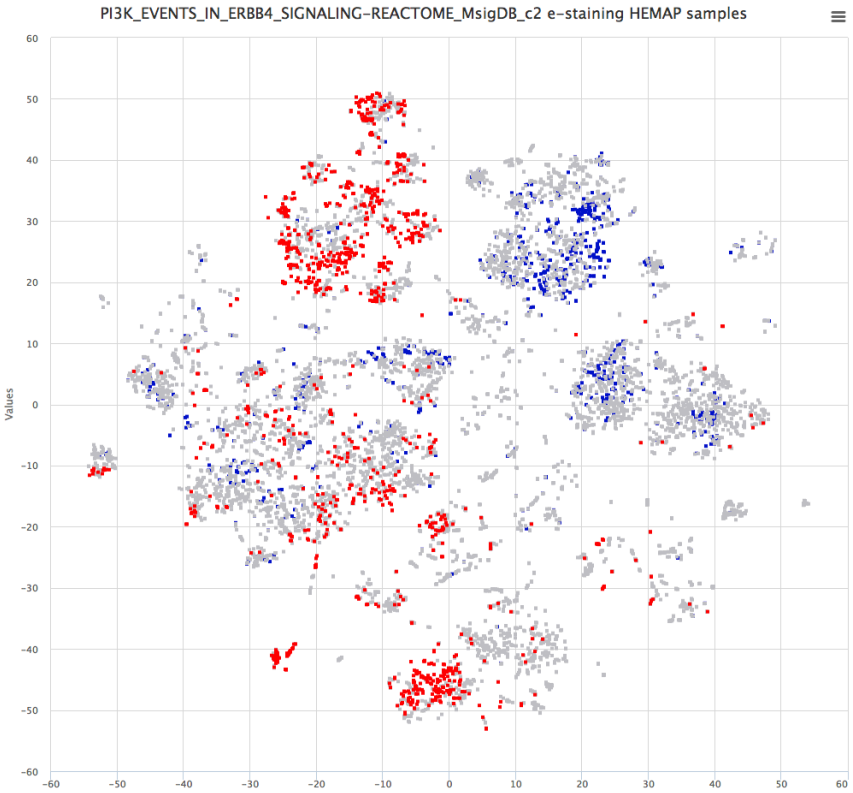


Figure 25. Searching for pre-B-ALL cluster correlated gene sets in pairwise results and filtering using term signaling. PI3K pathway is enriched in pre-B-ALL samples as shown in e-staining.

Step 2: Identification of candidate drugs

Resource map HEMAP samples Type DrugSigDB guideGSVA/FDR

+/-:0.49

Gene/Pathway/Drug BEZ235_LINCS-DSigDB_D2

e-stain getGeneMembers

PW Cluster/Class cancermap_cluster_pre-B-ALL

viSamp Corr. > 0.1 -log10(ePval) 2 Hypergeometric Test 0 search

Download(1192)

feature	corr.	adjpvalue	BHadj	hypergeom_test	BHadj	nsam
BEZ235_LINCS-DSigDB_D2	0.470	292.194	7.806	316	7.806	954
GALLAMINE_TRIETHIODIDE-DSigDB_D4	0.450	292.194	7.806	316	7.806	954
HC_TOXIN_ALL_DOWN-DSigDB_D3	0.400	292.194	7.806	306.259	7.806	954
REPAGLINIDE-DSigDB_D4	0.400	292.194	7.806	269.54	7.806	954
KINOME_192_ROCHE-DSigDB_D2	0.440	292.194	7.806	263.938	7.806	954

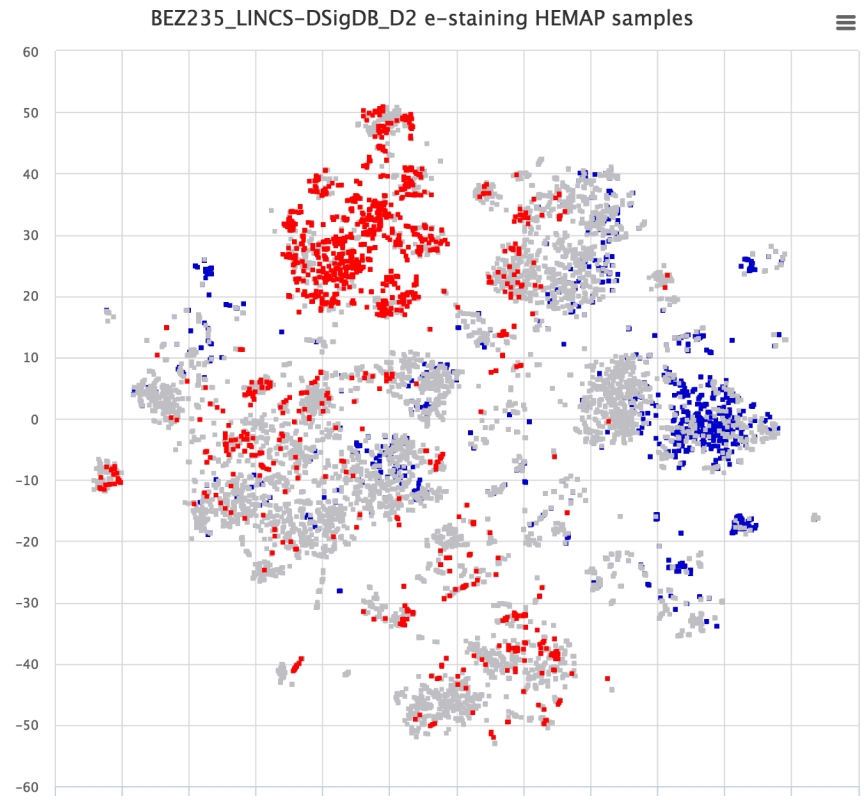


Figure 26. Searching for pre-B-ALL cluster correlated drug gene sets in pairwise results. Search is filtered to contain only LINCS chemical screen drugs. Two PI3K inhibitors, BEZ235 and AZD_6482, are among top correlated drug gene sets for pre-B-ALL. BEZ235 e-staining reveals high specificity for pre-B-ALL

Step 3: Examining the drug gene set and accessing drug target information

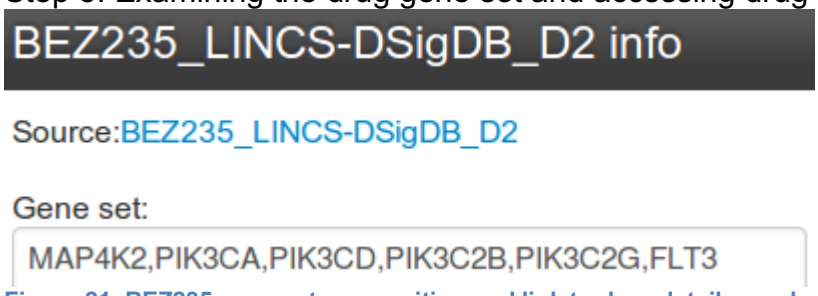


Figure 21. BEZ235 gene set composition and link to drug details can be accessed by clicking GetGeneMembers

Gene Set: D2 : HMS LINCS - Nvp-Bez235

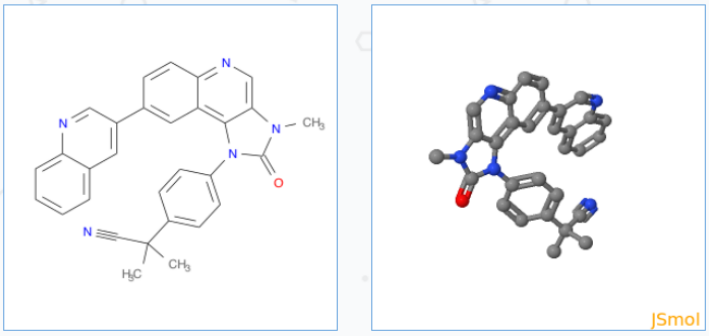
Collection D2 : HMS LINCS

Chemical Name Nvp-Bez235 (From Source : BEZ235)

FDA	NPC	WHO	Indian	Australia	China	Traditional Herbal	Clinical Trail
Not	Not	Not	Not	Not	Not	Not	Not

Molecular Weight	Hydrogen Bond Donor Count	Hydrogen Bond Acceptor Count	cLogP	Lipinski Rule
469.537 g/mol	0	6	5.89378	False(3/4)

Structure



InChI InChI=1S/C30H23N5O/c1-30(2,18-31)22-9-11-23(12-10-22)35-28-24-15-19(21-14-20-6-4-5-7-25(20)32-16-21)8-13-26(24)33-17-27(28)34(3)29(35)36/h4-17H,1-3H3

InChIKey JOGKUKXHTYWRGZ-UHFFFAOYSA-N

Links

CAS Num : 915019-65-7

CAS Num : 1146702-52-4

Gene (14 / 14)

⊖ Less

Value Type	Value↑	Concentration	Gene	PMID / Source
POC	2.800	1uM	PIK3C2B	HMS LINCS
POC	2.800	1uM	PIK3CA	HMS LINCS
POC	2.800	1uM	PIK3CA(E542K)	HMS LINCS
POC	3.000	1uM	PIK3CA(E545K)	HMS LINCS
POC	3.800	1uM	PIK3CA(Q546K)	HMS LINCS
POC	4.400	1uM	PIK3C2G	HMS LINCS
POC	4.600	1uM	PIK3CA(C420R)	HMS LINCS
POC	4.800	1uM	PIK3CA(H1047L)	HMS LINCS
POC	5.100	1uM	PIK3CA(E545A)	HMS LINCS
POC	6.200	1uM	PIK3CA(H1047Y)	HMS LINCS
POC	7.200	1uM	PIK3CD	HMS LINCS
POC	11.000	1uM	MAP4K2	HMS LINCS
POC	11.000	1uM	PIK3CA(M1043I)	HMS LINCS
POC	12.000	1uM	FLT3(D835Y)	HMS LINCS

Download gmt, text, Detailed text gene sets

Figure 27. Drug target details for BEZ235 reveal that PIK3C2B and PIK3CA have the highest specificity for BEZ235.

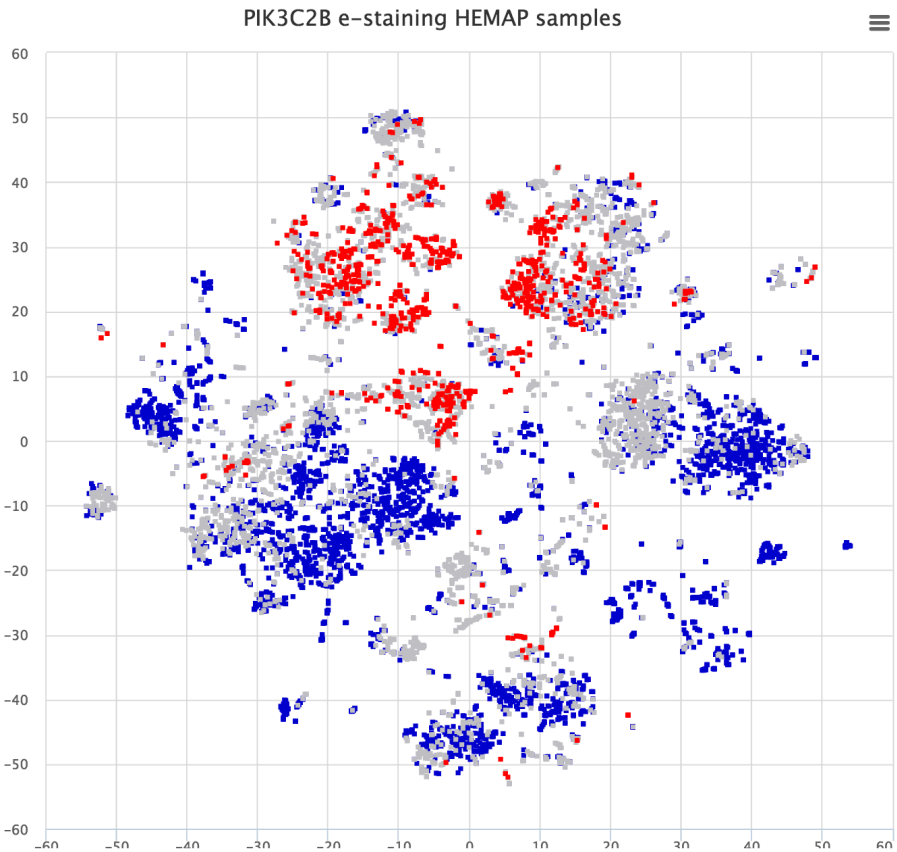


Figure 28. e-staining PIK3C2B shows that high expression of this drug target is prevalent in pre-B-ALL.

Final step: Comparing the expression in cancer to normal cell types using boxplots

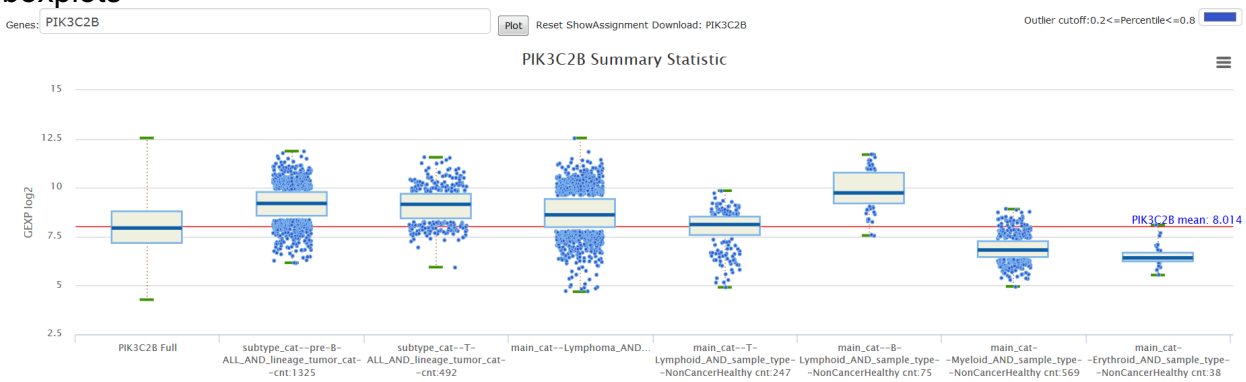


Figure 29. Sample sets of interest (pre-B-ALL, T-ALL and lymphoma vs T-Lymphoid, B-lymphoid, Erythroid and Myeloid) were defined using the Annotation table and selected for box plotting. The result shows that PIK3C2B is highly expressed in pre-B-ALL and T-ALL but is also highly expressed in normal B-lymphoid cells, which could indicate potential side effects.

Settings

Users can specify custom plotting and database search settings. It should be noted that plotting and search peformance have large dependencies on network and browser RAM memory capacity. Brief descriptions are listed below.

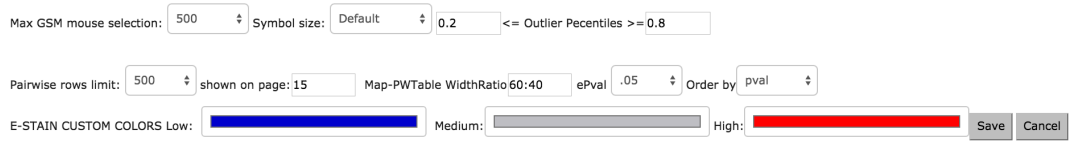


Figure 30. Hemap allows for session customization of max number of selections, rows returned and colors. These settings are stored at the session and will revert to defaults on closing of the browse.

The default settings can be individually updated using the Settings interface.
Max mouse sample map selections – default 500
Map Symbol size, can increase or decrease using drop down.
Outlier Percentiles for Boxplots <20% and >80%
Max number of pairwise rows – default 5000, values 500 to 50,000
Rows shown per page – default 15
Map:Table Explore Screen Ratio – default 60:40 (flip function in Explore interface)
ePval default .05, empirical Pvalue used for pairwise cutoff – drop down
Custom e-staining colors

Info

This section provides contact information and project issue tracker. Hemap is open sourced and free for non-profit usage. The software is use as it is and does not offer any warranty or guarantee. Please contact matti.nykter@uta.fi and merja.heinaniemi@uef.fi for commercial usage permissions.