Acknowledgement of Country

The University of Queensland (UQ) acknowledges the Traditional Owners and their custodianship of the lands on which we meet.

We pay our respects to their Ancestors and their descendants, who continue cultural and spiritual connections to Country.

We recognise their valuable contributions to Australian and global society.

Image: Digital reproduction of *A guidance through time* by Casey Coolwell and Kyra Mancktelow

Data Use Agreement

- To maximize your learning experience, we will be working with genuine human genetic data.

- Access to this data requires agreement to the following in to comply with human genetic data ethics regulations.

- Please email pctgadmin@imb.uq.edu.au to confirm that you agree with the following: -

  “I agree that access to data is provided for educational purposes only and that I will not make any copy of the data outside the provided computing accounts.”

Aim of the practical: Drug repositioning based on genomic studies.

1. Introduction:

From discovery to develop a drug, it is a long time and expensive process which often fails at number of steps at development stages. Thus, the aim of drug repurposing is to address these issues by finding new indications from existing drugs. This approach can reduce the cost and decrease the time duration for the drug development due to availability of already completed preclinical and safety studies.

2. Objectives

In the current practical, we will identify the drug repurposing candidates by using different genomic resources available at public platform (eg. GWAS datasets).

For this practical we will perform three main steps related to drug repositioning

1: Data selection and expression analysis to identify drug repositioning candidates.

2: Validation of identified signature genes.

3: Mapping list of genes in lLincs to identify drug compounds.

3. Methods used in this study

For the current study we will use MetaXcan software (https://github.com/hakyimlab/MetaXcan) to compute expression analysis of genes using eQTL data from GTEx database which is available at: (https://predictdb.org/post/2021/07/21/gtex-v8-models-on-eqtl-and-sqtl/.)

To perform this study, we will use publicly available GWAS summary statistics of LDL-Cholesterol for disease Hyperlipidemia (Willer et al., 2013; Wu et al., 2022).

4. Data and tool descriptions with path for analysis

All the data and tools are available in folder **"/data/module6/Module_6_Drug_Repurposing/"**

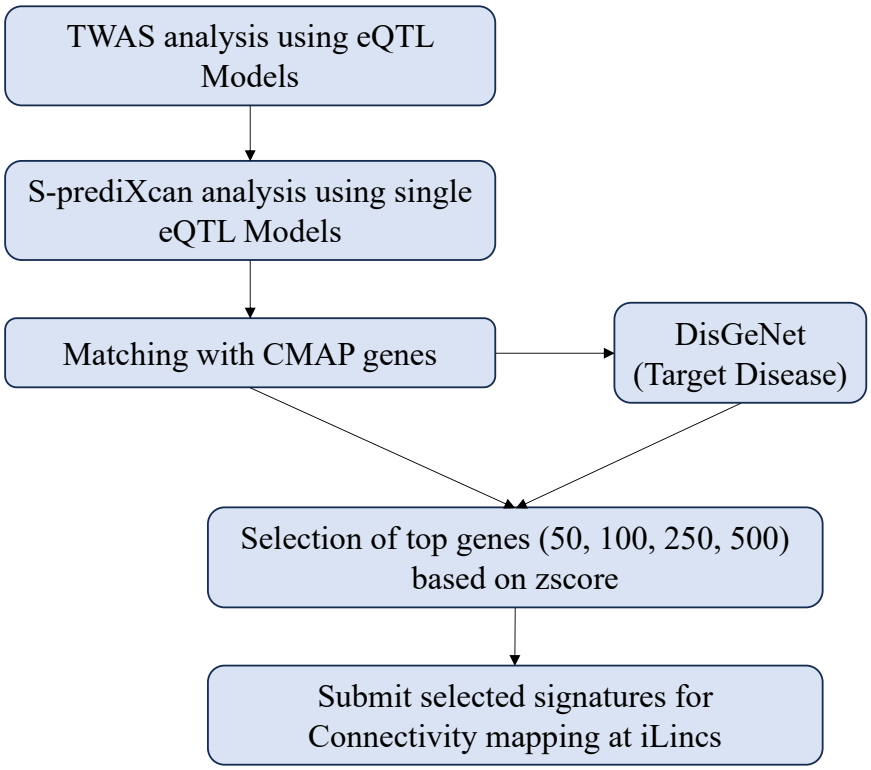
GWAS data was extracted from (https://csg.sph.umich.edu/willer/public/lipids2013/) and stored in the folder:- **"/data/module6/Module_6_Drug_Repurposing/1_GWAS_Hyperlipidimia/** in .txt format.

To compute expression analysis, we have installed "**MetaXcan**" software in Cluster.

All the eQTL models are available at folder: - **"/data/module6/Module_6_Drug_Repurposing/elastic_net_models_v8/".**

In addition, we are providing list of genes available in CMAP as "**cmap_genes.csv**" in folder: - **"/data/module6/Module_6_Drug_Repurposing/CMAP_Genes/cmap_genes.csv".**

5. Steps for data analysis with codes



Step 1:- Open Terminal and activate conda environment to run MetaXcan

Login to cluster with provided details (username, hostname, and password)

```
#Set working environment for MetaXcan by typing:
conda activate imlabtools
```

Inspect the available tissues for expression analysis

Type

```
ls /data/module6/Module_6_Drug_Repurposing/elastic_net_models_v8/
#Select tissue for analysis
```

For this practical, we'll calculate the expression of genes by selecting tissue **"Whole_Blood".**

```
#Blood
SPrediXcan.py \
--model_db_path /data/module6/Module_6_Drug_Repurposing/elastic_net_models_v8/en_Whole_Blood.db \
--covariance /data/module6/Module_6_Drug_Repurposing/elastic_net_models_v8/en_Whole_Blood.txt.gz \
--gwas_folder /data/module6/Module_6_Drug_Repurposing/1_GWAS_Hyperlipidimia \
--gwas_file_pattern ".*txt" \
--snp_column rsid \
--effect_allele_column A1 \
--non_effect_allele_column A2 \
--beta_column beta \
--pvalue_column P-value \
--output_file /scratch/Gagan_DP/NEW_LDL_Blood_V8.csv #create your own output directory at scratch
```

For checking the details of output file copy results from cluster to local system

```
scp user@203.101.229.xxx:/scratch/Gagan_DP/NEW_LDL_Blood_V8.csv Desktop
```

Output file:-

gene	gene_name	zscore	effect_size	pvalue	var_g	pred_perf_r2	pred_perf_pval	pred_perf_qval	n_snps_used	n_snps_in_cov	n_snps_in_model
ENSG00000134222.16	PSRC1	-30.33792101	-0.827687682	3.63E-202	0.012276935	0.081622725	6.40E-12	NA	15	18	18
ENSG00000177051.5	FBXO46	-15.93847812	-1.719510513	3.43E-57	0.000636086	0.011224546	0.01332305	NA	8	13	13
ENSG00000129933.20	MAU2	13.12367542	0.72755404	2.41E-39	0.002642836	0.068922583	3.59E-10	NA	17	20	20
ENSG00000169174.10	PCSK9	10.94466984	0.614274954	7.05E-28	0.004264342	0.083037067	3.24E-12	NA	17	23	23
ENSG00000116641.17	DOCK7	10.85609763	0.211354831	1.87E-27	0.023844024	0.112055681	3.35E-16	NA	71	77	77
ENSG00000175164.13	ABO	-10.80604716	-0.06698474	3.22E-27	0.274637846	0.54305188	1.60E-107	NA	39	46	46
ENSG00000149485.18	FADS1	-10.49934156	-0.358691237	8.70E-26	0.007316963	0.021626361	0.000559655	NA	9	11	11
ENSG00000134825.15	TMEM258	-10.46969085	-0.558357049	1.19E-25	0.002779929	0.036686073	5.80E-06	NA	13	16	16
ENSG00000143126.7	CELSR2	9.018834921	0.379447899	1.90E-19	0.005470723	0.031271326	2.64E-05	NA	18	24	24
ENSG00000162399.6	BSND	8.426269777	0.238444395	3.57E-17	0.020075294	0.119996893	2.56E-17	NA	28	37	37
ENSG00000134824.13	FADS2	-8.003906045	-0.039535701	1.21E-15	0.336541506	0.615839407	3.58E-134	NA	39	58	58
ENSG00000143093.14	STRIP1	7.92860117	0.238526688	2.22E-15	0.006604747	0.047520047	2.17E-07	NA	50	67	67
ENSG00000130202.9	NECTIN2	7.63744237	0.053394303	2.22E-14	0.2275413	0.595386724	1.92E-128	NA	24	37	37
ENSG00000142252.10	GEMIN7	7.608836499	0.770145959	2.77E-14	0.001010828	0.043590775	6.15E-07	NA	11	19	19
ENSG00000186567.12	CEACAM19	7.212386844	0.1684715	5.50E-13	0.015207119	0.047424198	2.25E-07	NA	21	25	25
ENSG00000178719.16	GRINA	-6.809487027	-0.848139956	9.79E-12	0.001182033	0.063173044	1.41E-09	NA	4	6	6
ENSG00000167491.17	GATAD2A	-6.669513287	-0.209158403	2.57E-11	0.008041727	0.14804241	1.11E-21	NA	13	14	14
ENSG00000269976.1	RP11-130L8.2	-6.668255115	-0.296205951	2.59E-11	0.004293929	0.02397146	0.000274764	NA	21	25	25
ENSG00000178209.14	PLEC	-6.337159089	-0.124722662	2.34E-10	0.045592906	0.436165181	1.13E-76	NA	25	33	33
ENSG00000176182.5	MYPOP	-6.300958017	-0.301132644	2.96E-10	0.005583794	0.035576537	8.51E-06	NA	45	54	54
ENSG00000188672.17	RHCE	6.20919277	0.507629711	5.33E-10	0.00188429	0.036390115	6.73E-06	NA	14	17	17
ENSG00000178685.13	PARP10	-5.948695978	-0.299704082	2.70E-09	0.007474583	0.161205386	1.10E-23	NA	28	43	43
ENSG00000130158.13	DOCK6	-5.834446245	-0.131287444	5.40E-09	0.018369535	0.084090576	1.43E-12	NA	23	27	27

Result column discriptions:-

gene: a gene's id: as listed in the Tissue Transcriptome model. Ensemble Id for most gene model releases. Can also be a intron's id for splicing model releases.

gene_name: gene name as listed by the Transcriptome Model.

zscore: S-PrediXcan's association result for the gene, typically HUGO for a gene.

effect_size: S-PrediXcan's association effect size for the gene. Can be computed when beta from the GWAS is used.

pvalue: P-value of the aforementioned statistic.

var_g: variance of the gene expression, calculated as W' * G * W (where W is the vector of SNP weights in a gene's model, W' is its transpose, and G is the covariance matrix)

pred_perf_r2: (cross-validated) R2 of tissue model's correlation to gene's measured transcriptome.

pred_perf_pval: pval of tissue model's correlation to gene's measured transcriptome. (prediction performance.

pred_perf_qval: qval of tissue model's correlation to gene's measured transcriptome. (prediction performance.

n_snps_used: number of snps from GWAS that got used in S-PrediXcan analysis

n_snps_in_cov: number of snps in the covariance matrix

n_snps_in_model: number of snps in the model

Exercise 1:- Compute the expression analysis of genes by selecting

1. Artery_Coronary

2. Artery_Aorta.

Exercise 1 codes:-

```
# Artery_Aorta


SPrediXcan.py \
--model_db_path /data/module6/Module_6_Drug_Repurposing/elastic_net_models_v8/en_Artery_Aorta.db \
--covariance /data/module6/Module_6_Drug_Repurposing/elastic_net_models_v8/en_Artery_Aorta.txt.gz \
--gwas_folder /data/module6/Module_6_Drug_Repurposing/1_GWAS_Hyperlipidimia \
--gwas_file_pattern ".*txt" \
--snp_column rsid \
--effect_allele_column A1 \
--non_effect_allele_column A2 \
--beta_column beta \
--pvalue_column P-value \
--output_file /scratch/Gagan_DP/NEW_LDL_Artery_Aorta_V8.csv



# Artery_Coronary


SPrediXcan.py \
--model_db_path /data/module6/Module_6_Drug_Repurposing/elastic_net_models_v8/en_Artery_Coronary.db \
--covariance /data/module6/Module_6_Drug_Repurposing/elastic_net_models_v8/en_Artery_Coronary.txt.gz \
--gwas_folder /data/module6/Module_6_Drug_Repurposing/1_GWAS_Hyperlipidimia \
--gwas_file_pattern ".*txt" \
--snp_column rsid \
--effect_allele_column A1 \
--non_effect_allele_column A2 \
--beta_column beta \
--pvalue_column P-value \
--output_file /scratch/Gagan_DP/NEW_LDL_Artery_Coronary_V8.csv
```

Identification of drug repurposing candidates:

We will perform this analysis by using R

Type "R"

load required libraries:-

```
library(corrplot)
library(dplyr)
library(reshape2)
library(ggstatsplot)
library(tidyverse)
```

[CMAP: - cmap contains log2 fold changes of 12,437 genes from 1,281 compound treatments for different cell lines corresponding to a total of 3,478 signatures.]

In this step firstly, we will map all the genes obtained after expression analysis with CMAP genes for connectivity mapping.

Path of file with CMAP genes:

"/data/module6/Module_6_Drug_Repurposing/CMAP_Genes/cmap_genes.csv"

Upload CMAP genes:

```
Cmap_genes <- read.csv("/data/module6/Module_6_Drug_Repurposing/CMAP_Genes/cmap_genes.csv")
```

Upload output file obtained from MetaXcan

```
Blood <- read.csv("/scratch/Gagan_DP/NEW_LDL_Blood_V8.csv")
```

Select the list of genes available in CMAP data and arrange according to zscore.

```
Blood_cmap <- inner_join(Blood,Cmap_genes,by = "gene_name") %>% arrange(zscore)
```

Select top 50 expressing genes (upregulated and downregulated) based on zscore

```
Top_up50_B <- tail(Blood_cmap, 50) #Top 50 most upregulated genes
Top_down50_B <- head(Blood_cmap, 50) #Top 50 most downregulated genes
Gene_Signatures_Blood <- bind_rows(Top_up50_B, Top_down50_B) #used for ilincs_input
```

Select identified gene signatures for iLincs_input

We need “gene names”, “zscore” and “pvalue” for this analysis.

```
write_csv(Gene_Signatures_Blood[, c(2,3,5)], "/scratch/Gagan_DP/Gene_signatures_Blood.csv")
```

Copy Gene_signatures file from cluster to local system

```
scp user@203.101.229.XXX:/scratch/Gagan_DP/Gene_signatures_Blood.csv Desktop
```

Output file for selected gene signatures:

gene_name	zscore	pvalue
UEVLD	2.93843678	0.003298719
RTN2	2.988006585	0.002808035
PPP1R13L	3.007875331	0.00263081
TRIM22	3.0290383	0.002453336
WBP2	3.04594798	0.00231948
DDR1	3.100560421	0.001931548
POR	3.113676095	0.001847722
AAR2	3.121383274	0.001800036
DPP3	3.154424466	0.00160815
MPI	3.16475846	0.001552117
CRLF3	3.165228553	0.001549612
HBS1L	3.165548246	0.00154791
TNFAIP8	3.176618838	0.001490027
PRRC2A	3.222361139	0.001271388
CCS	3.227144551	0.001250323

Exercise 2: Identify top 50 upregulated and downregulated genes as drug repurposing candidates in:-

1. Artery_Aorta

2. Artery_Coronary

Exercise 2 codes

Upload CMAP genes:

```
Cmap_genes <- read.csv("/data/module6/Module_6_Drug_Repurposing/CMAP_Genes/cmap_genes.csv")
```

Upload output file obtained from MetaXcan (**Artery_Aorta and Artery_Coronary Tissue**)

```
Artery_Aorta <- read.csv("/scratch/Gagan_DP/NEW_LDL_Artery_Aorta_V8.csv")
Artery_Coronary <- read.csv("/scratch/Gagan_DP/NEW_LDL_Artery_Coronary_V8.csv")
```

Select the list of genes available in CMAP data and arrange according to zscore.

```
Artery_Aorta_cmap <- inner_join(Artery_Aorta,Cmap_genes,by = "gene_name") %>% arrange(zscore)
Artery_Coronary_cmap <- inner_join(Artery_Coronary,Cmap_genes,by = "gene_name") %>% arrange(zscore)
```

Select top 50 expressing genes (upregulated and downregulated) based on zscore

```
Top_up50_AA <- tail(Artery_Aorta_cmap, 50) #Top 50 most upregulated genes
Top_down50_AA <- head(Artery_Aorta_cmap, 50) #Top 50 most downregulated genes
Gene_Signatures_Artery_Aorta <- bind_rows(Top_up50_AA, Top_down50_AA) #used for ilincs_input
Top_up50_AC <- tail(Artery_Coronary_cmap, 50) #Top 50 most upregulated genes
Top_down50_AC <- head(Artery_Coronary_cmap, 50) #Top 50 most downregulated genes
Gene_Signatures_Artery_Coronary <- bind_rows(Top_up50_AC, Top_down50_AC) #used for ilincs_input
```

Select identified gene signatures for iLincs_input

We need “gene names”, “zscore” and “pvalue” for this analysis.

```
write_csv(Gene_Signatures_Artery_Aorta[, c(2,3,5)], "/scratch/Gagan_DP/Gene_signatures_Artery_Aorta.csv")
```

```
write_csv(Gene_Signatures_Artery_Coronary[, c(2,3,5)],
"/scratch/Gagan_DP/Gene_signatures_Artery_Coronary.csv")
```

Copy Gene_signatures file from cluster to local system

```
scp user@203.101.229.XXX:/scratch/Gagan_DP/Gene_Signatures_Artery_Aorta.csv Desktop
scp user@203.101.229.XXX:/scratch/Gagan_DP/Gene_Signatures_Artery_Coronary.csv Desktop
```

2: Validation of identified signature genes

2.1 Based on correlation analysis between related eQTL models.

In this step, we will find the correlation coefficient of genes calculated for expression analysis by selecting two eQTL models related to disease.

As we have already calculated expression analysis for “Artery_Coronary and Artery_Aorta” in Exercise 1.

Thus, for this step we will use the above said eQTL models for correlation analysis.

Correlation Plot analysis

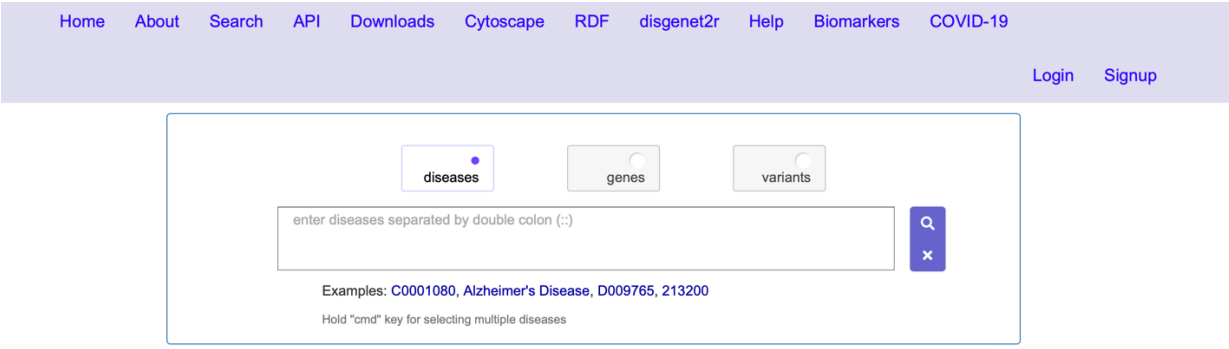
```
#Data_selection
Artery_Aorta <- read.csv("/scratch/Gagan_DP/NEW_LDL_Artery_Aorta_V8.csv")
Artery_Coronary <- read.csv("/scratch/Gagan_DP/NEW_LDL_Artery_Coronary_V8.csv")

#extract gene_Names and Z_Scores
Artery_Aorta_Z <- Artery_Aorta[, c(2,3)]
Artery_Coronary_Z <- Artery_Coronary[, c(2,3)]
#Change Names of respective columns (Z_Scores)
colnames(Artery_Aorta_Z)[2]  <- "Artery_Aorta"
colnames(Artery_Coronary_Z)[2]  <- "Coronary_Artery"
common_genes <- inner_join(Artery_Aorta_Z, Artery_Coronary_Z, by = "gene_name") #ignore Warning

#For_Correlation_Plot
Merged_Z <- subset(common_genes, select = -c(gene_name))

#Pearson_Correlation
CORR_P <- cor(Merged_Z, method = "pearson", use = "pairwise.complete.obs")
corrplot(CORR_P, method="number")
CORR_P
```

2.2. Selecting disease specific genes from DisGeNet:

DisGeNET is a largest publicly available database which consists of genes and variants associated to human diseases from expert curated repositories such as GWAS catalogues, animal models and the scientific literature.

Link to open DisGeNet :- https://www.disgenet.org/search.

Note:- For DisGeNet database there is requirement of login, thus for the current practical, we have downloaded genes related to Hyperlipidemia available in folder:- "/data/module6/Module_6_Drug_Repurposing/DisGeNet_Hyperlipidimia/Hyperlipidimia_genes.csv".




```
#Selection of signature genes from expression analysis
#Upload files in R
Hyperlipidimia_genes <- read.csv("/data/module6/Module_6_Drug_Repurposing/DisGeNet_Hyperlipidimia/Hyperlipidimia_genes.csv")
Blood_cmap <- inner_join(Blood,Cmap_genes,by = "gene_name") %>% arrange(zscore) #already in R
common_genes_Blood <- inner_join(Blood_cmap, Hyperlipidimia_genes, by = "gene_name") %>% arrange(zscore)
Top_up_50_DB <- tail(common_genes_Blood, 50) #Top 50 most upregulated genes
Top_down_50_DB <- head(common_genes_Blood, 50) #Top 50 most downregulated genes
Hyperlipidimia_Signatures_DB <- bind_rows(Top_up_50_DB, Top_down_50_DB)
write.csv(Hyperlipidimia_Signatures_DB[, c(2,3,5)],"/scratch/Gagan_DP/Hyperlipidimia_Signatures_Blood.csv")
scp user@203.101.229.XXX:/scratch/Gagan_DP/Hyperlipidimia_Signatures_Blood.csv Desktop
```

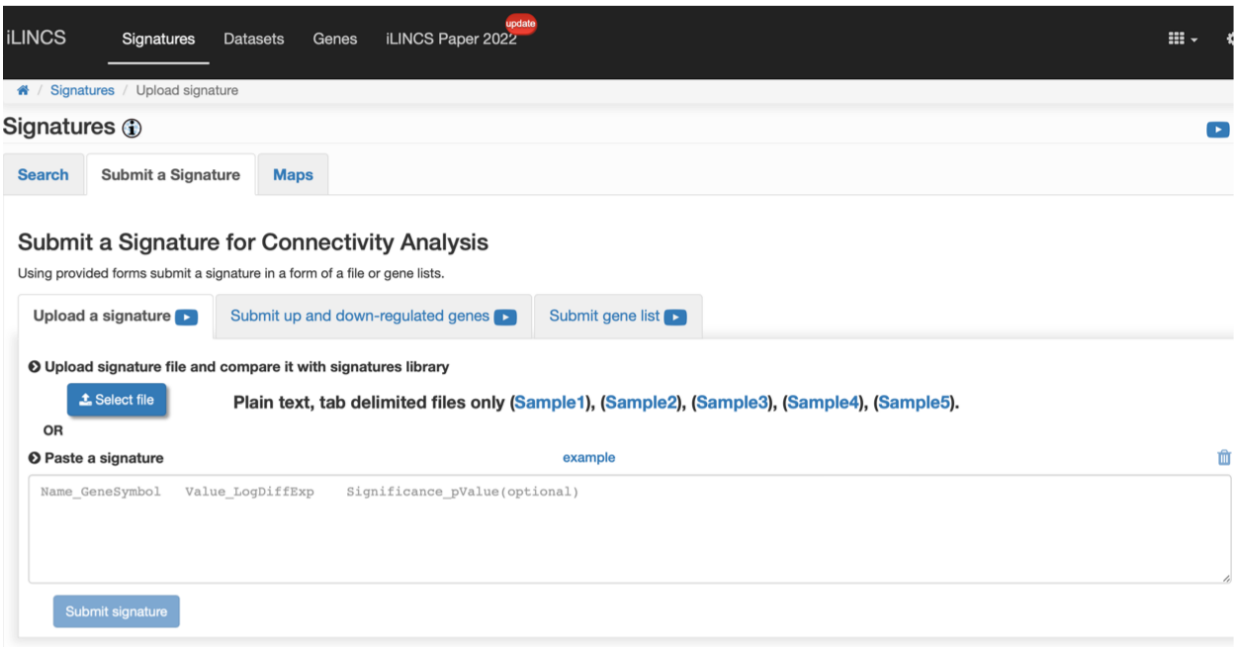
Exercise 3:- Identify disease specific (Hyperlipidemia for current practical) signatures for "Coronary_Artery and Artery_Aorta"

Exercise 3 codes

```
#Upload Hyperlipidemia genes obtained from DisGeNet database.

Hyperlipidimia_genes <-
read.csv("/data/module6/Module_6_Drug_Repurposing/DisGeNet_Hyperlipidimia/Hyperlipidimia_genes.csv")


#Selection of signature genes from expression analysis


#Hyperlipidemia Signatures for Artery Aorta

#Upload common genes (CMAP and Artery Aorta) and arrange based on z-score

Artery_Aorta_cmap <- inner_join(Artery_Aorta,Cmap_genes,by = "gene_name") %>% arrange(zscore)

common_genes_Artery_Aorta <- inner_join(Artery_Aorta_cmap, Hyperlipidimia_genes, by = "gene_name") %>%
arrange(zscore)

Top_up_50_AA <- tail(common_genes_Artery_Aorta, 50) #Top 50 most upregulated genes

Top_down_50_AA <- head(common_genes_Artery_Aorta, 50) #Top 50 most downregulated genes

Hyperlipidimia_Signatures_AA <- bind_rows(Top_up_50_AA, Top_down_50_AA)


write.csv(Hyperlipidimia_Signatures_AA[,
c(2,3,5)],"/scratch/Gagan_DP/Hyperlipidimia_Signatures_Artery_Aorta.csv")

#Save file on local system

scp user@203.101.229.XXX:/scratch/Gagan_DP/Hyperlipidimia_Signatures_Artery_Aorta.csv Desktop


#Hyperlipidemia Signatures for Artery Coronary

#Upload common genes (CMAP and Artery Coronary) and arrange based on z-score

Artery_Coronary_cmap <- inner_join(Artery_Coronary,Cmap_genes,by = "gene_name") %>% arrange(zscore)

common_genes_Artery_Coronary <- inner_join(Artery_Coronary_cmap, Hyperlipidimia_genes, by = "gene_name")
%>% arrange(zscore)

Top_up_50_AC <- tail(common_genes_Artery_Coronary, 50) #Top 50 most upregulated genes

Top_down_50_AC <- head(common_genes_Artery_Coronary, 50) #Top 50 most downregulated genes

Hyperlipidimia_Signatures_AC <- bind_rows(Top_up_50_AC, Top_down_50_AC)

write.csv(Hyperlipidimia_Signatures_AC[,
c(2,3,5)],"/scratch/Gagan_DP/Hyperlipidimia_Signatures_Artery_Coronary.csv")


#Save file on local system

scp user@203.101.229.XXX:/scratch/Gagan_DP/Hyperlipidimia_Signatures_Artery_Coronary.csv Desktop
```

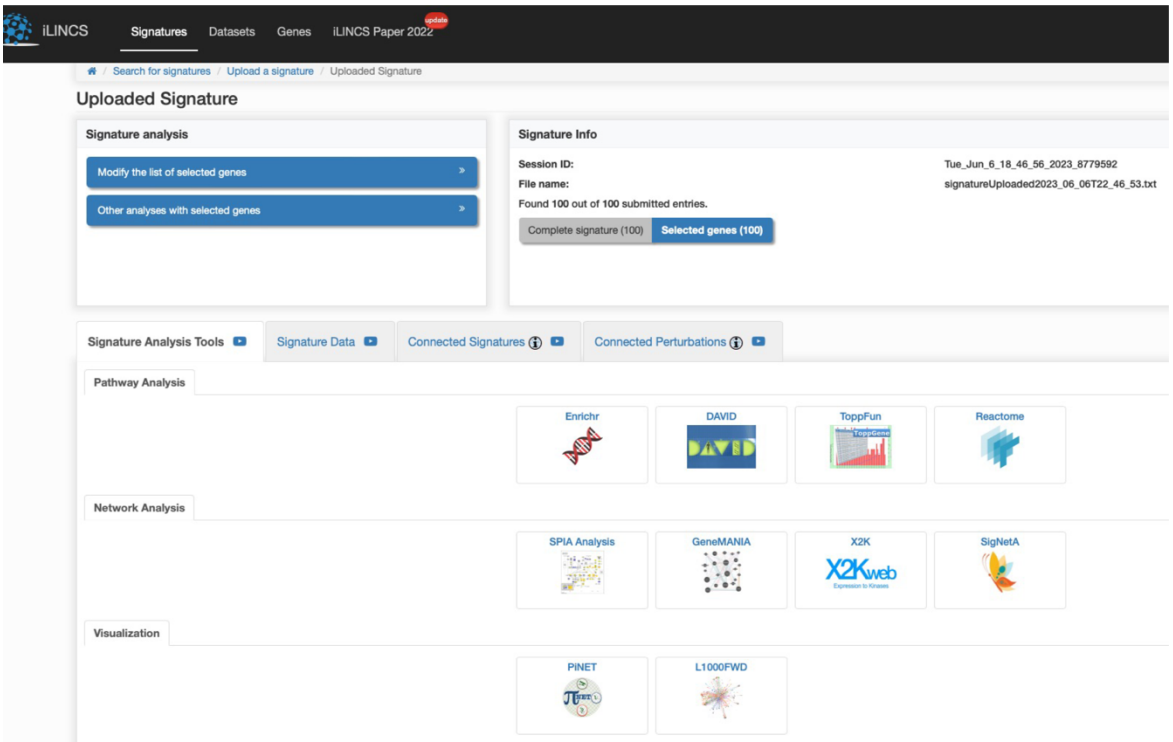
Step 3: Mapping signature genes in iLincs database.

ILincs database is available at http://www.ilincs.org/ilincs/signatures/main

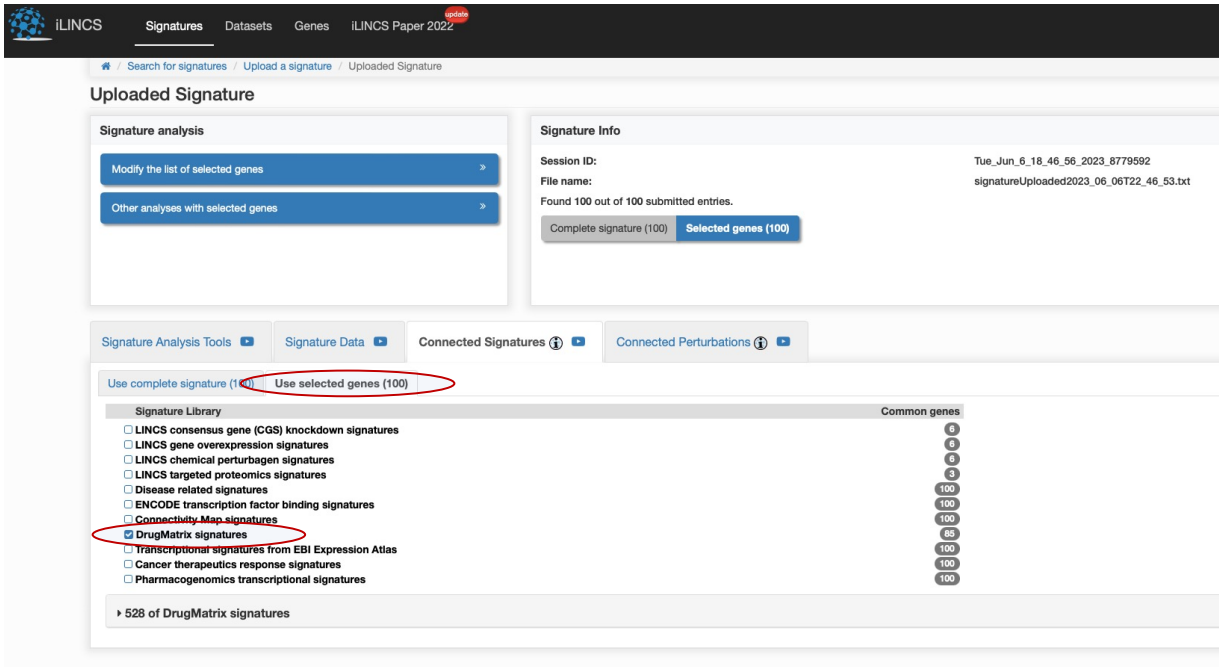


Go to Tab and select obtained list of genes with “gene_name” “zscore” and “pvalue” and submit signatures.

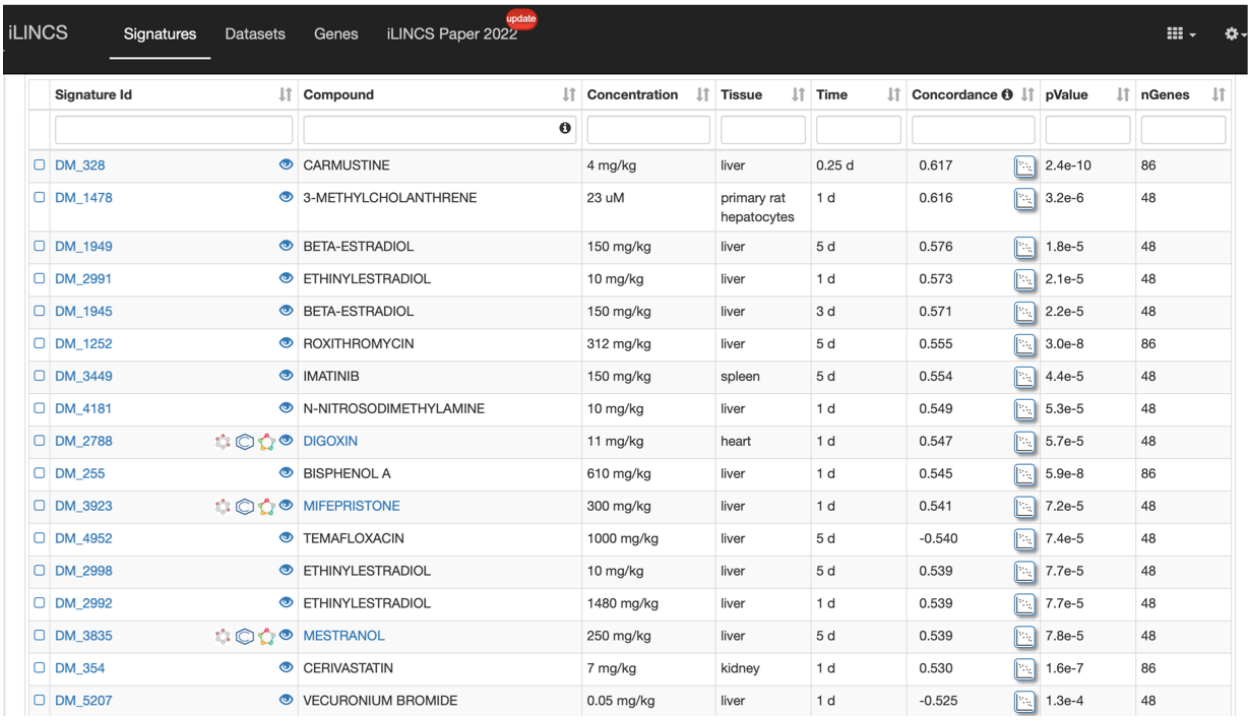
After completion of the analysis, click on connected signatures.



For identifying of list of drugs select tab "use selected genes" and "DrugMatrix signatures"



List of Drugs with targeting tissue will come as a output (Shown in result folder)



Signature Id	Compound	Concentration	Tissue	Time	Concordance	pValue	nGenes
DM_328	CARMUSTINE	4 mg/kg	liver	0.25 d	0.617	2.4e-10	86
DM_1478	3-METHYLCHOLANTHRENE	23 uM	primary rat hepatocytes	1 d	0.616	3.2e-6	48
DM_1949	BETA-ESTRADIOL	150 mg/kg	liver	5 d	0.576	1.8e-5	48
DM_2991	ETHINYLESTRADIOL	10 mg/kg	liver	1 d	0.573	2.1e-5	48
DM_1945	BETA-ESTRADIOL	150 mg/kg	liver	3 d	0.571	2.2e-5	48
DM_1252	ROXITHROMYCIN	312 mg/kg	liver	5 d	0.555	3.0e-8	86
DM_3449	IMATINIB	150 mg/kg	spleen	5 d	0.554	4.4e-5	48
DM_4181	N-NITROSODIMETHYLAMINE	10 mg/kg	liver	1 d	0.549	5.3e-5	48
DM_2788	DIGOXIN	11 mg/kg	heart	1 d	0.547	5.7e-5	48
DM_255	BISPHENOL A	610 mg/kg	liver	1 d	0.545	5.9e-8	86
DM_3923	MIFEPRISTONE	300 mg/kg	liver	1 d	0.541	7.2e-5	48
DM_4952	TEMAFLOXACIN	1000 mg/kg	liver	5 d	-0.540	7.4e-5	48
DM_2998	ETHINYLESTRADIOL	10 mg/kg	liver	5 d	0.539	7.7e-5	48
DM_2992	ETHINYLESTRADIOL	1480 mg/kg	liver	1 d	0.539	7.7e-5	48
DM_3835	MESTRANOL	250 mg/kg	liver	5 d	0.539	7.8e-5	48
DM_354	CERIVASTATIN	7 mg/kg	kidney	1 d	0.530	1.6e-7	86
DM_5207	VECURONIUM BROMIDE	0.05 mg/kg	liver	1 d	-0.525	1.3e-4	48

Exercise 4: - Mapping signatures for "Coronary_Artery and Artery_Aorta" in iLincs.

Exercise 5:- Mapping disease specific signatures (hyperlipidemia) for above said tissues.

Extension Questions: -

1. How to select signature genes when disease is associated with multiple tissues?

2. How to select signature for specific Drugs?

3. How to identify signatures from RNASeq data with control and disease conditions?

References

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