Connectivity Map (Cmap) processing and querying.

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CMap – One dataset several names

CMap-L1000 version 1:

- L1000-based compendium
- Phase 1
- *A Next Generation Connectivity Map: L1000 platform and the first 1,000,000 profiles (2017)*
- Available at GSE92742

Data:

- 19,811 compounds (drugs and small molecules):
- Different time (6 hours or 24 hours)
- Different concentration (0.04uM to 90uM)
- Different cell lines: (71 different cell lines)
  - **1,319,138 replicates measured.**

The dataset also include genetic perturbation.

CMap – One dataset several names

CMap-L1000 version 2:

- Phase 2
- *No paper published on this dataset (but the data was included in clue.io).*
- Available at GSE70138

Data:

- 1,768 additional compounds:
- Different time (**3hours**, 6 hours or 24 hours)
- Different concentration (0.04uM to **40uM**)
- Different cell lines: (30 different cell lines)
  - **354,123 replicates measured**

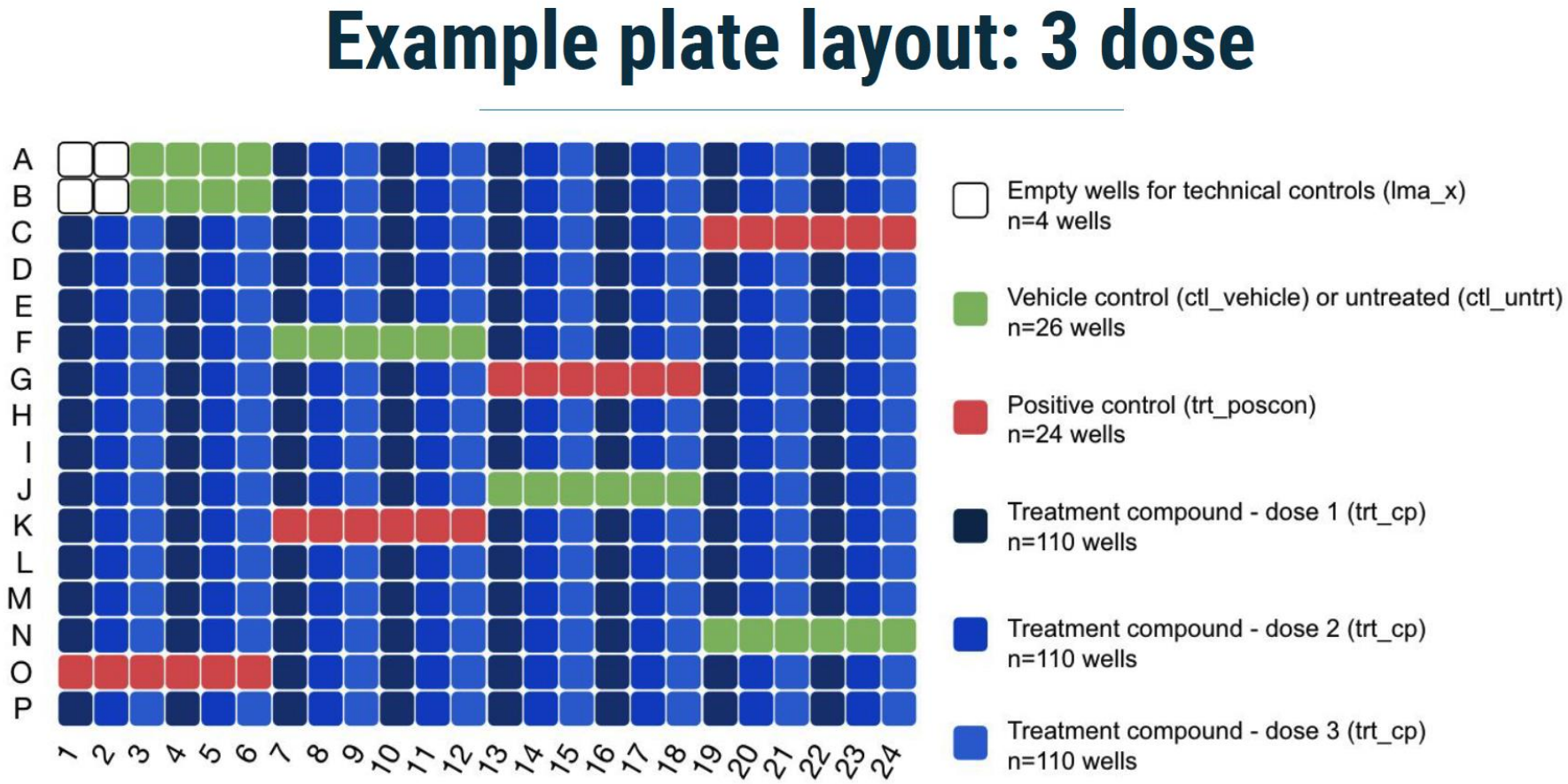
This dataset also include genetic perturbation

CMap – One dataset several names

iLincs:

- Uses both GSE92742 and GSE70138.
- This is a meta-analysis combining phase 1 and phase 2.
- Paper: **Connecting omics signatures and revealing biological mechanisms with iLINCS (2022)**
- Data is accessed through the ilincs website
  - **1,673,261 replicates measured**

CMap – What does it actually look like?



CMap – Microarray.

THE UNIVERSITY OF QUEENSLAND AUSTRALIA

Definition of the landmark genes:

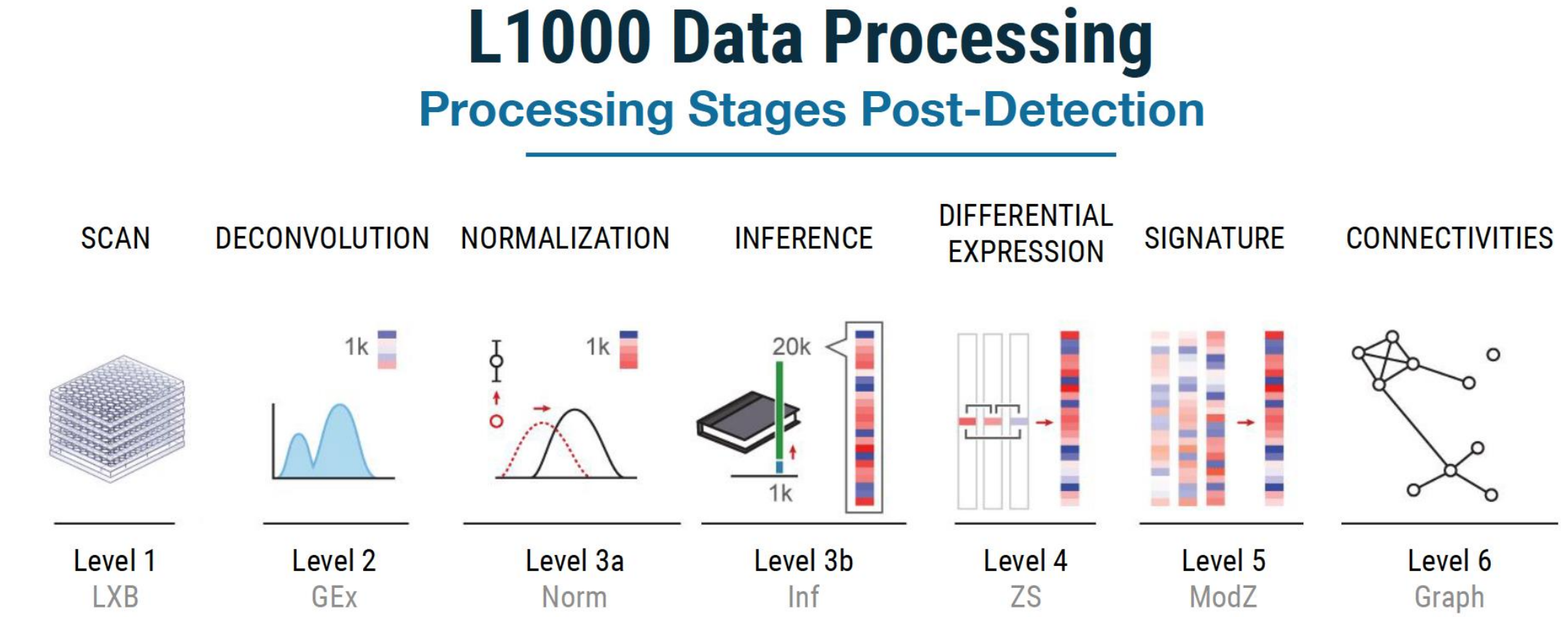
- 12,063 gene expression samples profiles using Affymetrix HG-U133A microarrays from the Gene Expression Omnibus (GEO) called DS_{GEO}.

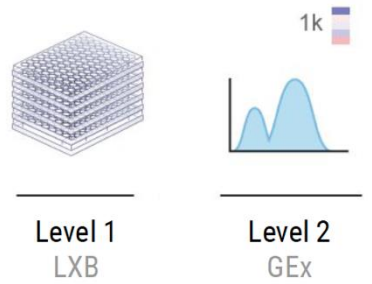
This dataset was used to define two sets of genes:

- Normalising set:
  - 80 genes being invariant within those 12,063 datasets
    - 8 genes, each at 10 levels of low to high expression.
- Landmark set:
  - 978 genes that can be used to impute the rest of the transcriptome.

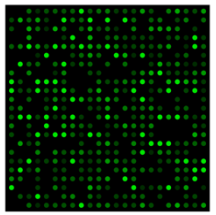
The microarray measures 1,058 transcripts.

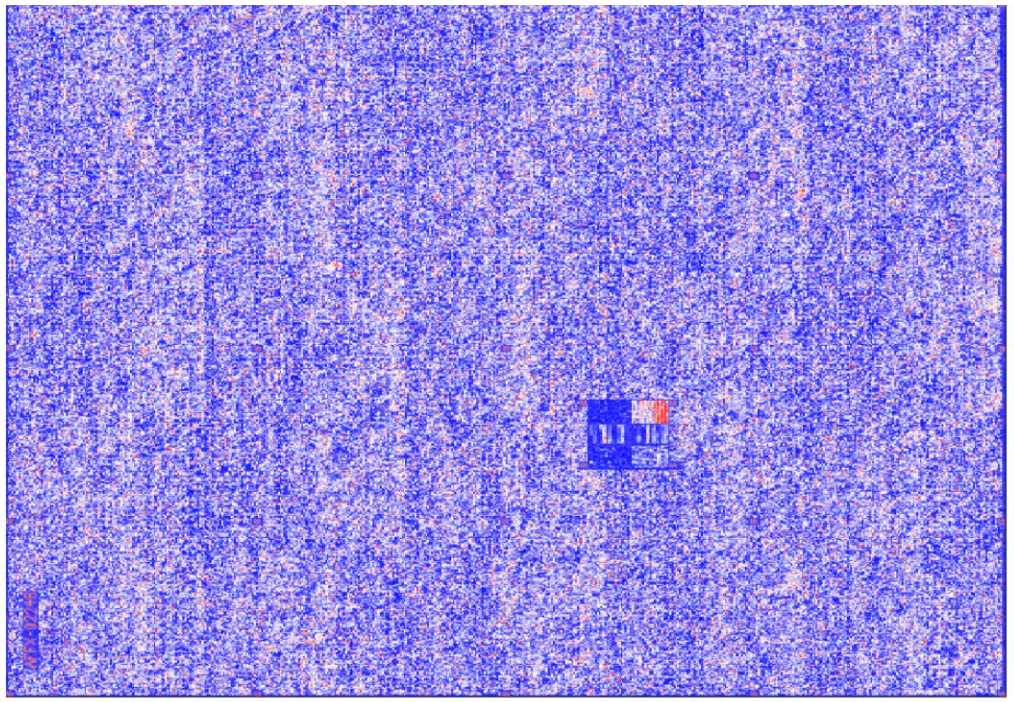
CMap – Data processing:





Microarray processing:





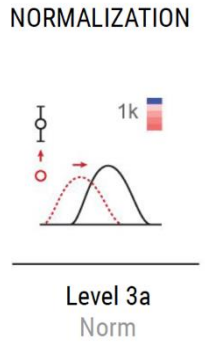
Example Slide Affymetrix microarray (~18,000 genes)

Data generation for each of the replicates:

- Each dot on the slide corresponds to a different gene.
- The intensity and color of the dot represent:
  - **Blue** indicates **low signal intensity** (low expression or hybridization).
  - **Red** indicates **high signal intensity** (high expression or hybridization).
  - **Intermediate shades** (purple, magenta) indicate **medium signal.**

The centre of the image corresponds to control genes of known intensity. This allows to generate a numerical value for each gene measured.

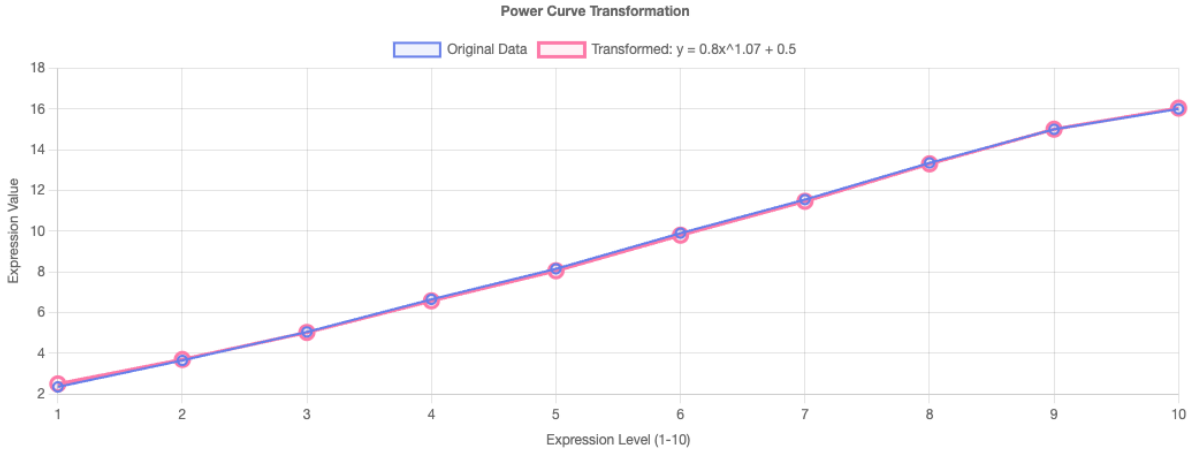
CMap processing - Level 3 normalisation



At this stage, the data contain only 1,058 genes measured for each replicate (1,673,261)

Normalisation is called L1000 Invariant Set Scaling.

For each sample the expression of 80 invariable gene is used to generate a “calibration curve”



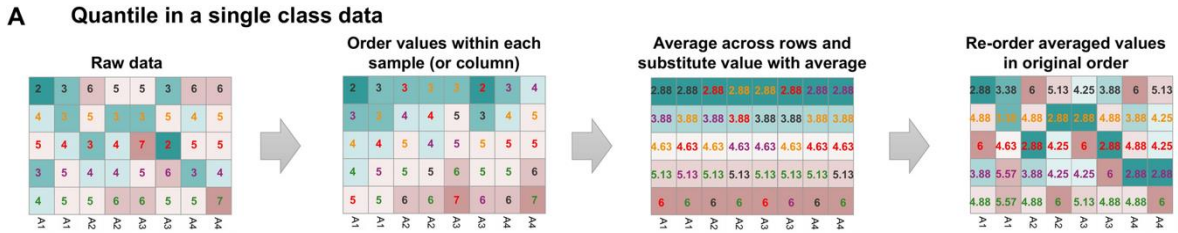
The data is then recalibrated using the following equation:

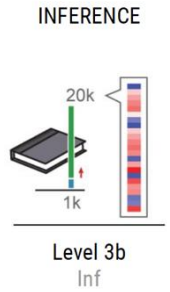
$$y_{scaled} = a y_{raw}^{b} + c$$

Where:

- a,b and c are estimated within each sample using a least square approach.
- y_{raw} is the unscaled data.
- y_{scaled} is the scaled data used for further analysis.

y_{scaled} is the normalized using a quantile normalization.





CMap processing - Level 3 gene inference

To infer the value of the missing genes, they assume that unmeasured genes can be predicted from the measured landmark genes using the following linear combination:

$$x = w_0 + \sum_{i=1}^{978} w_i y_i$$

Where:

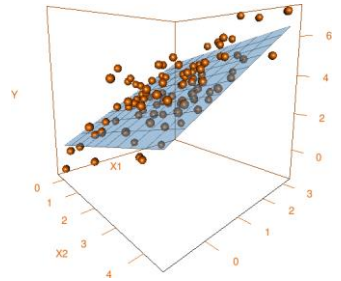
- w_i is the model weights predicted using DS_{GEO}
  - The weights are calculated using an ordinary least square approach within the DS_{GEO} dataset

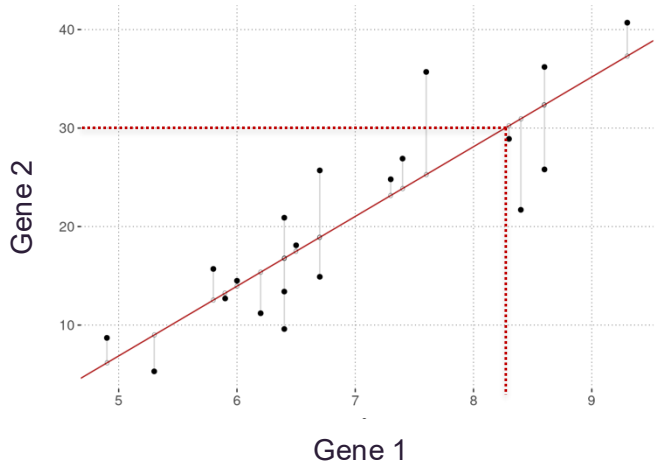
This approach allows the prediction of 12,328 genes.

They benchmark this inference strategy by using the GTEx dataframe and calculate the correlation between the inferred and measure expression.

Gene Symbol	Gene Title	Self-Correlation	Feature_set
ESRRA	estrogen related receptor alpha	0.89	BING
EIF3D	eukaryotic translation initiation factor 3 subunit D	0.90	BING
HAUS2	HAUS augmin like complex subunit 2	-0.38	Inferred

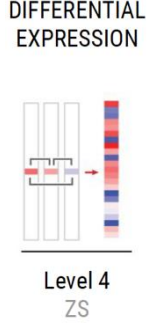
2 response variables:





Example:
Gene 1: Measured value of 8.2
Gene 2: Predicted value of 30

CMap processing - Level 4: Z-score scaling

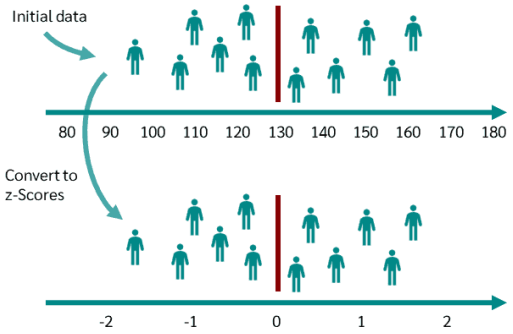


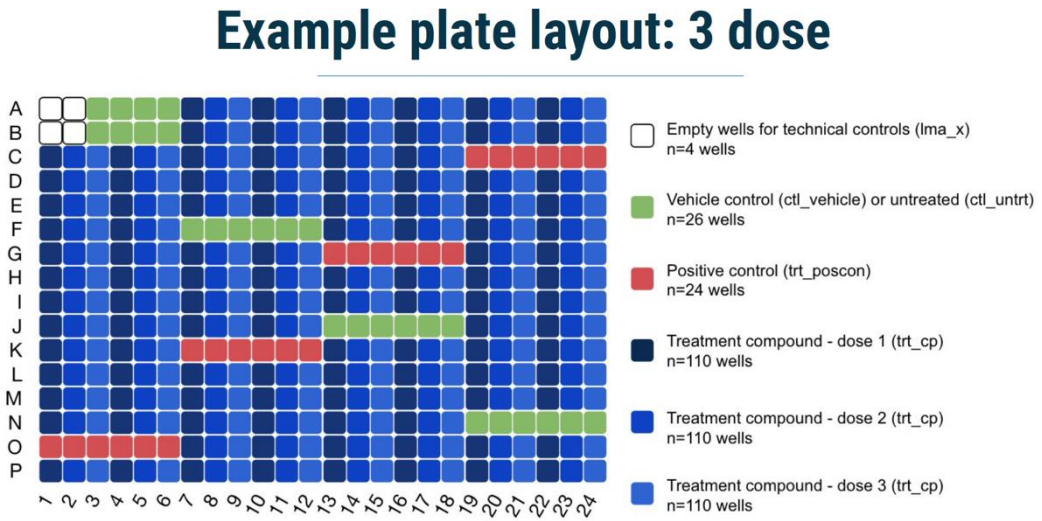
To make genes comparable, they are changed to a z-score scale using the following formula:

$$z_i = \frac{y_{norm} - \mu}{\sigma}$$

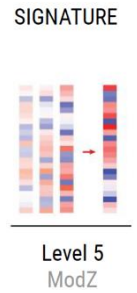
Where:

- z_i is the z-score transformed expression value.
- y_{norm} is the normalized expression (measured or inferred)
- μ is the mean normalized expression *on the plate*
- σ is the standard deviation of the normalized expression *on the plate*





CMap processing – Level 5: consensus signatures



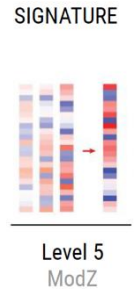
Pairwise correlation is calculated between each replicates of a signature

- Drug
- Time of expose
- Dose
- Cell line

The consensus signature is then calculated as the linear combination of the replicates gene expression.

- The coefficients are the sum of its correlation to the other replicates normalized to sum to 1.

CMap processing – Level 5: consensus signatures



Example 3 genes, 3 replicates:

	Rep1	Rep2	Rep3
Gene 1	10	13	9
Gene 2	8	6	0
Gene 3	2	2	2

Step 1: correlation matrix:

$$\begin{bmatrix} 1 & 0.9 & 0.5 \\ 0.9 & 1 & 0.82 \\ 0.5 & 0.82 & 1 \end{bmatrix}$$

Step 2: Set self-correlation to 0:

$$\begin{bmatrix} 0 & 0.9 & 0.5 \\ 0.9 & 0 & 0.82 \\ 0.5 & 0.82 & 0 \end{bmatrix}$$

Step 3: Raw weights:

$$\begin{bmatrix} 1.4 & 1.72 & 1.32 \end{bmatrix}$$

Normalizing factor: 4.44

Step 4: Normalized weights:

$$\begin{bmatrix} 0.31 & 0.39 & 0.30 \end{bmatrix}$$

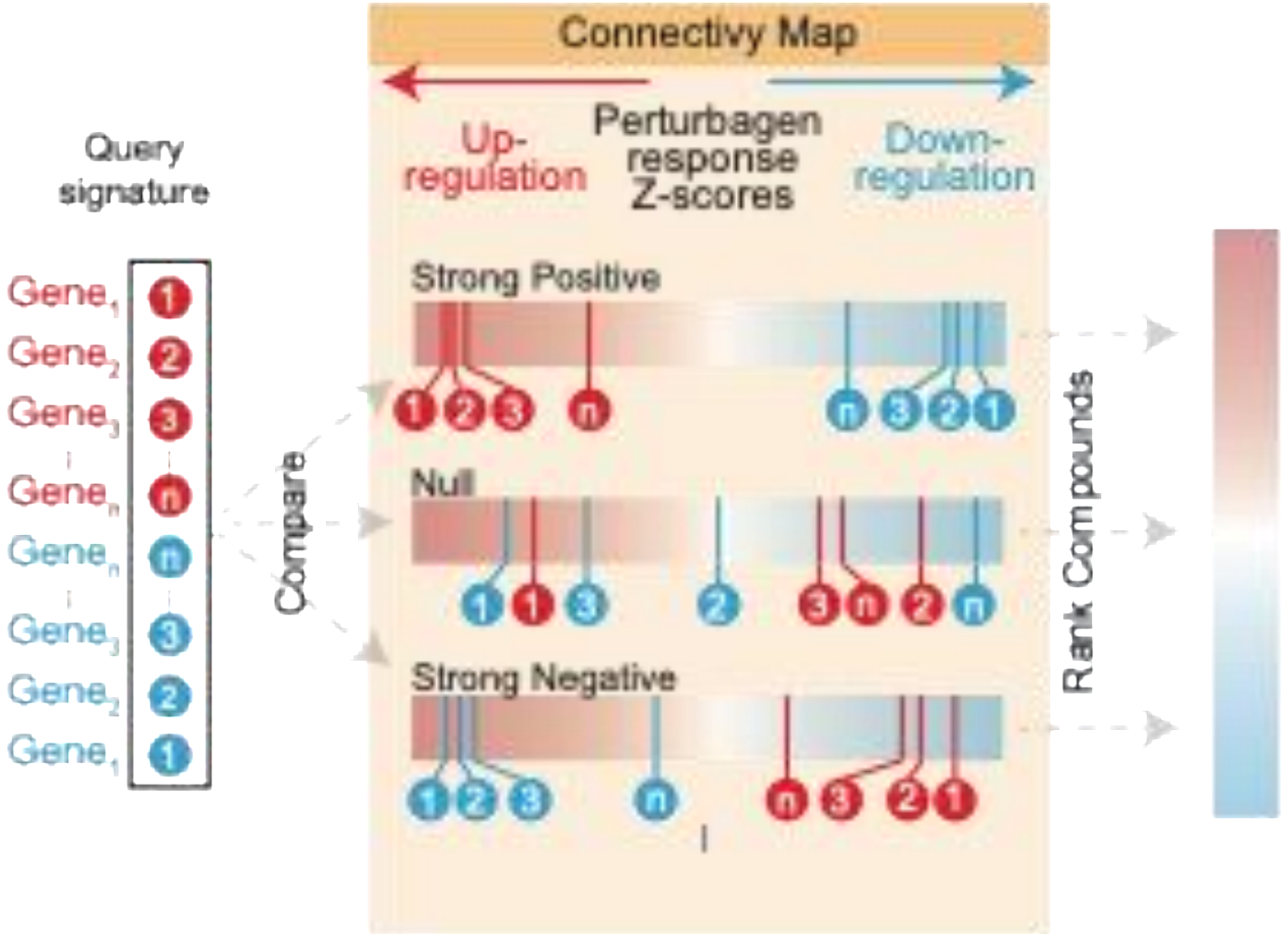
Step 5: Linear combination

$$\begin{bmatrix} 0.31 * 10 + 0.39 * 13 + 0.30 * 9 \\ 0.31 * 8 + 0.39 * 6 + 0.30 * 0 \\ 0.31 * 2 + 0.39 * 2 + 0.30 * 2 \end{bmatrix} = \begin{bmatrix} 10.87 \\ 4.82 \\ 2 \end{bmatrix}$$

→ Consensus Signature

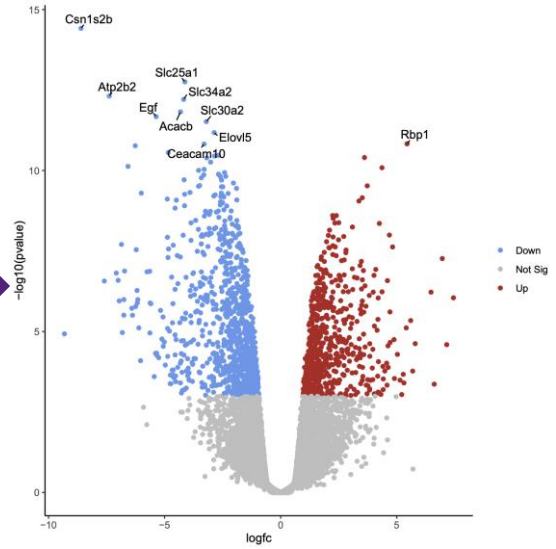
Questions?

How to query CMap:



Disease signature

Data Generation Process
e.g.
- **mRNA**
- Protein
- DNA



Gene	LogFC	P-value
Csn1s2b	-8.02	5.29E-16
atp2b2	-6.52	3.52E-11
slc25A	-4.53	2.95E-06
Egf	-5.11	1.61E-07
slc34a2	-8.08	5.04E-07
Slc30a2	-3.98	3.45E-05
Rbp1	5.61	1.01E-08
Elovl5	-3.28	5.19E-04
Ceacam	-3.68	1.17E-04

What data type should be used?

Disease signature

RNA sequencing:
Pro:
- Easy to generate
- Easy to analyze

Cons:
- **Function:** Transcriptomic changes do not necessarily have an impact on protein level
- **Causality:** RNA can be caused by the disease instead of causing the disease.
- **Sampling:** Obtaining the tissue of interest can be hard or impossible.

Protein sequencing:
Pro:
- Direct link to the biology
- (fairly) easy to analyze

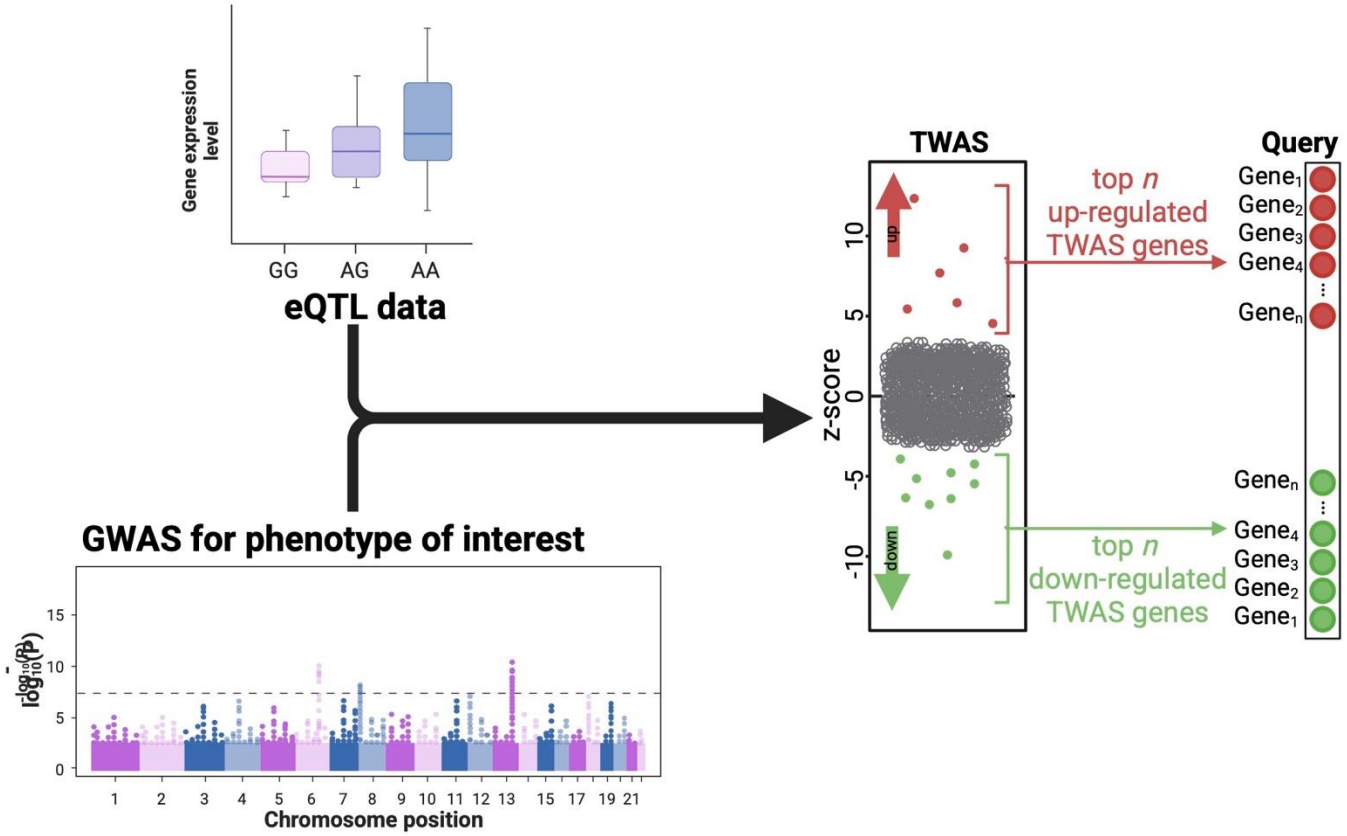
Cons:
- **Causality:** Protein abundance changed can be caused by the disease instead of causing the disease.
- **Sampling:** Obtaining the tissue of interest can be hard or impossible.
- **Function:** Protein abundance do not necessarily translate to protein function

DNA sequencing:
Pro:
- Massive biobank available.
- **Causality:** Disease does not influence DNA.

Cons:
- **Function:** Not linked to expression changes
- **Specificity:** Not tissue specific

Using DNA for a disease signature

Transcriptome wide association study:



Disease signature

Gene	Z-score	P-value
Csn1s2b	-8.02	5.29E-16
atp2b2	6.52	3.52E-11
slc25A	-4.53	2.95E-06
Egf	5.11	1.61E-07
slc34a2	-8.08	5.04E-07
Slc30a2	-3.98	3.45E-05
Rbp1	5.61	1.01E-08
Elovl5	-3.28	5.19E-04
Ceacam	-3.68	1.17E-04

How to define a disease signature?

1. What threshold do we want to use?
    1. P-value or Z-score - is there a difference between the two?

2. How many genes do we want to include in the signature?
    1. All significant genes?
    2. Highly significant genes?
        1. How do you define high here?

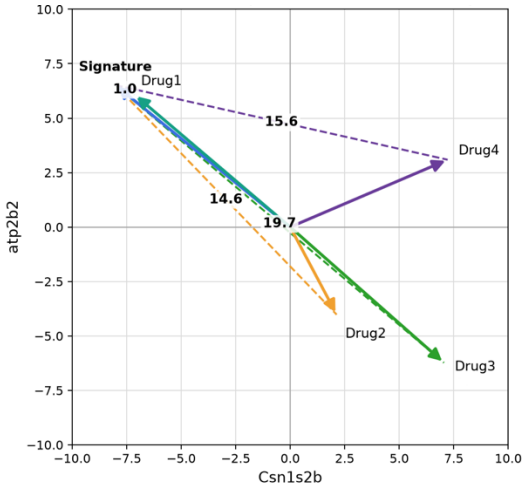
Don’t use a single signature.

Selection bias and winner’s curse can lead to inflated signal.

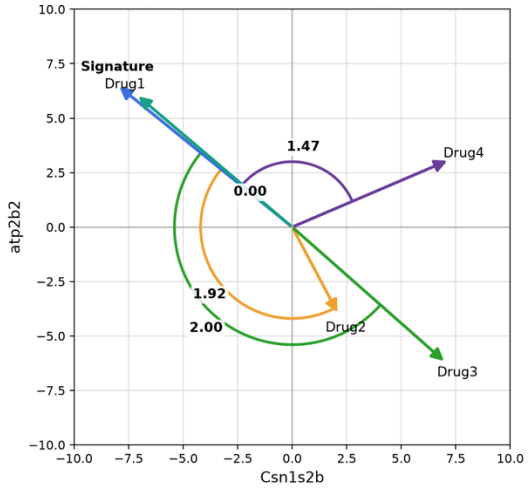
How to think about similarity?

Gene	Disease Signature	Drug1	Drug2	Drug3	Drug4
Csn1s2b	-8.02	-7.14	2.13	7.05	7.22
atp2b2	6.52	6.08	-4.02	-6.23	3.09
slc25A	-4.53	-4.27	7.31	4.18	-2.14
Egf	5.11	5.36	3.45	-5.42	8.03
slc34a2	-8.02	-6.91	-8.16	6.88	4.95

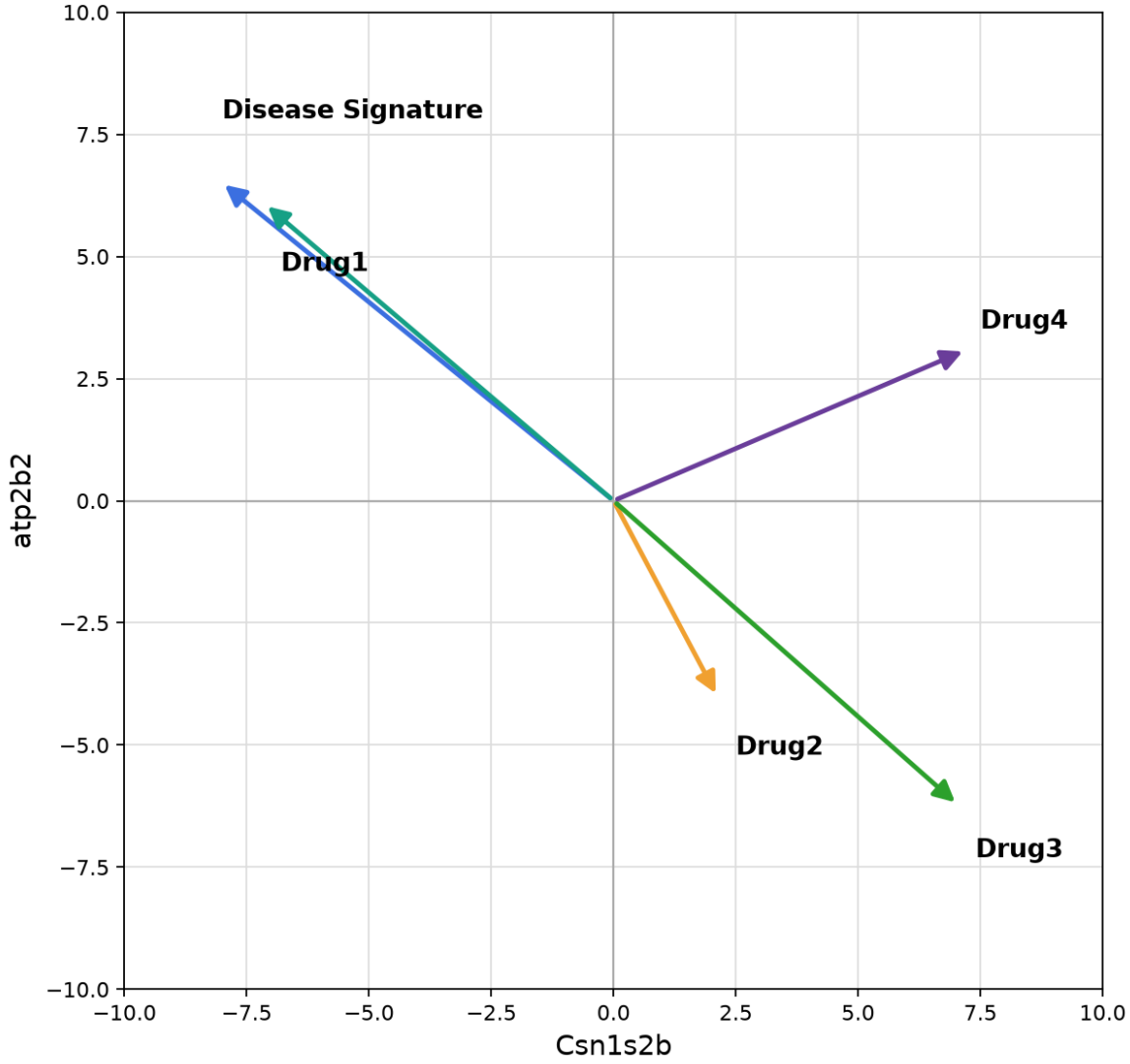
Euclidean distance



Cosine distance

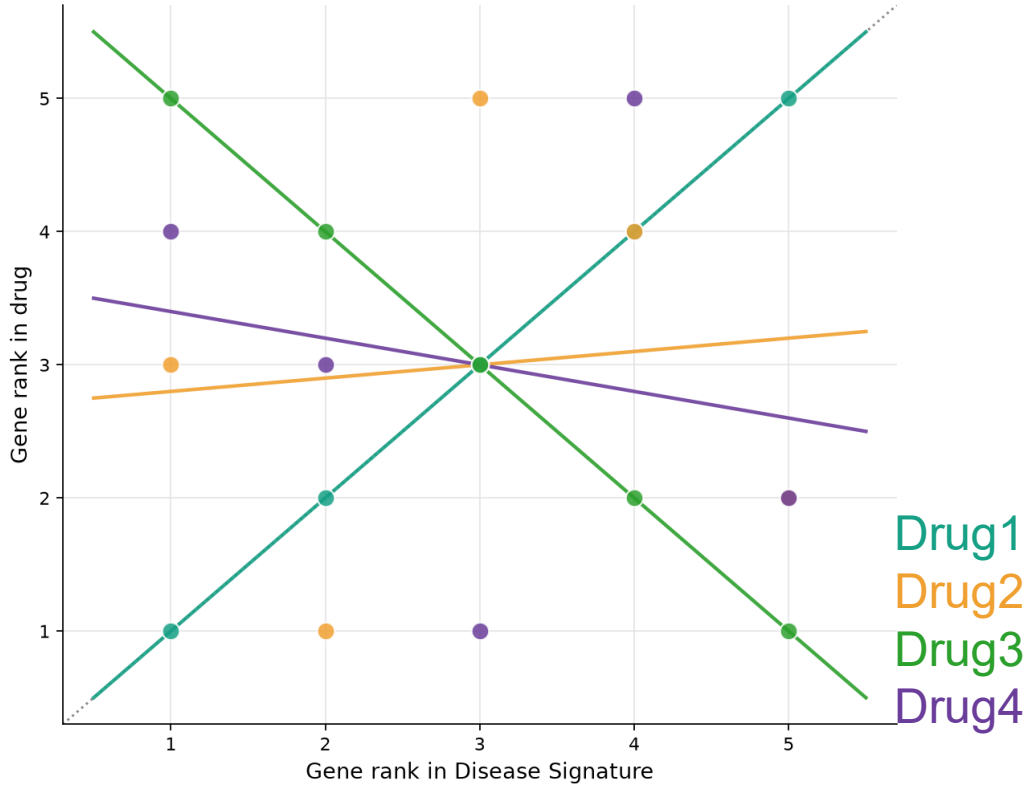


Visual representation of the perturbations in 2D



How to think about similarity?

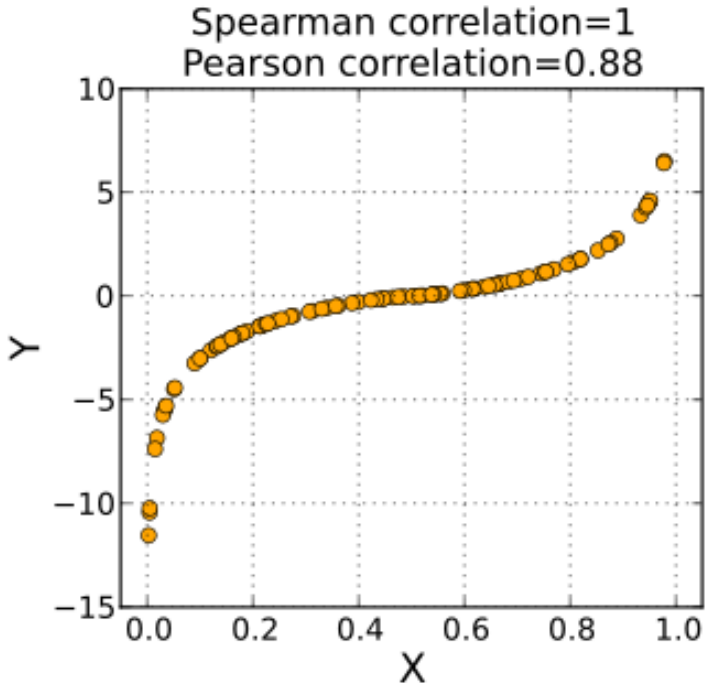
Similarity based on rank



There are no obvious similarity metric being ‘better’ than another.

But… some metric have a natural advantage:

- Spearman correlation is resistant to scale effect.



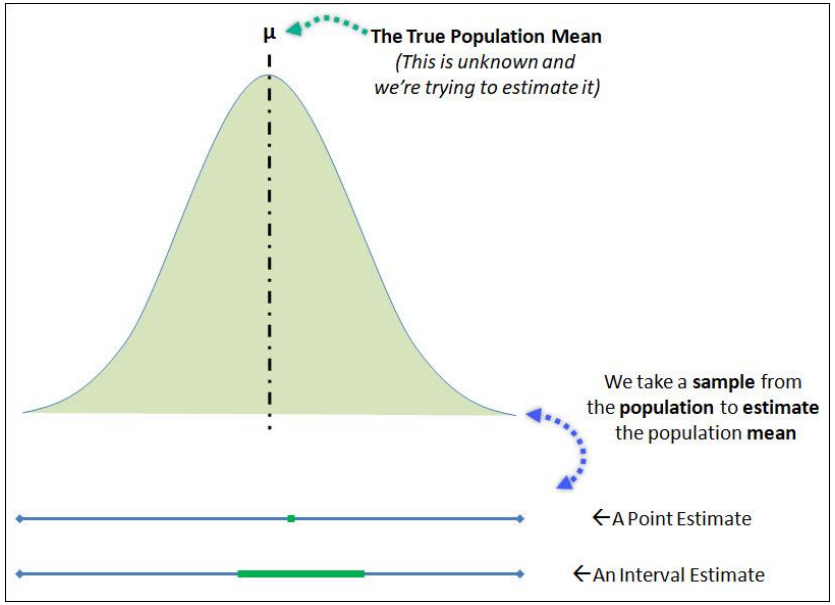
Drug recommendations:

We rank drugs based on their similarity:

1. Drug1
2. Drug2
3. Drug4
4. Drug3

Based on our hypothesis Drug3 is therefore the most likely to be a candidate to help patients with the disease

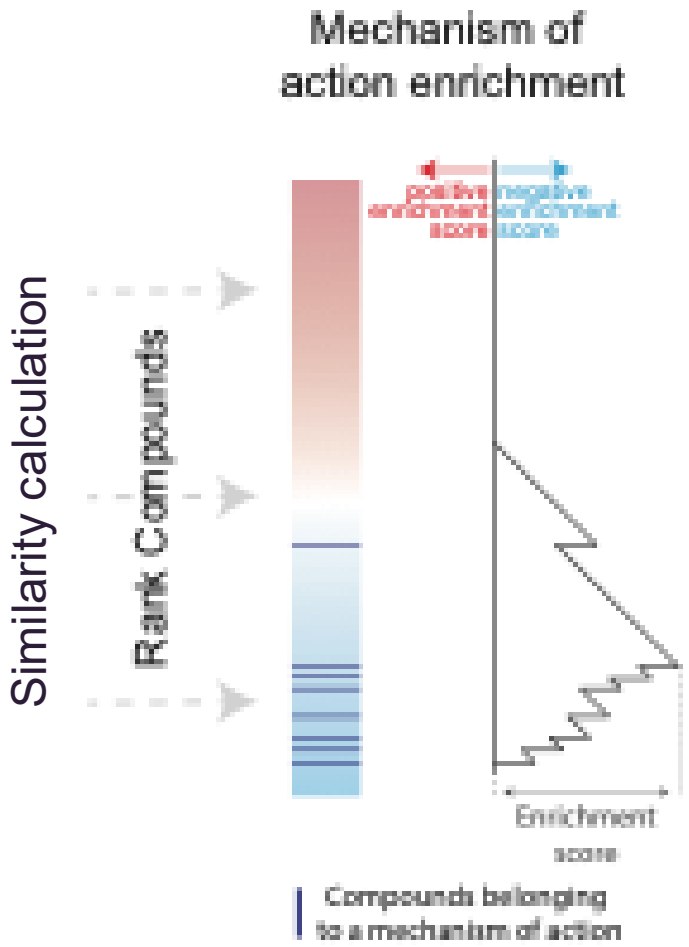
How likely is it to be true?



We have two possible ways of improving our drug prediction:

1. Increase the sample size of our perturbagen dataset (more accurate measurement of every gene)
2. Group similar compounds together.

Enrichment:



Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles

Aravind Subramanian [1], Pablo Tamayo, Vamsi K Mootha, Sayan Mukherjee, Benjamin L Ebert, Michael A Gillette, Amanda Paulovich, Scott L Pomeroy, Todd R Golub, Eric S Lander, Jill P Mesirov

Affiliations + expand

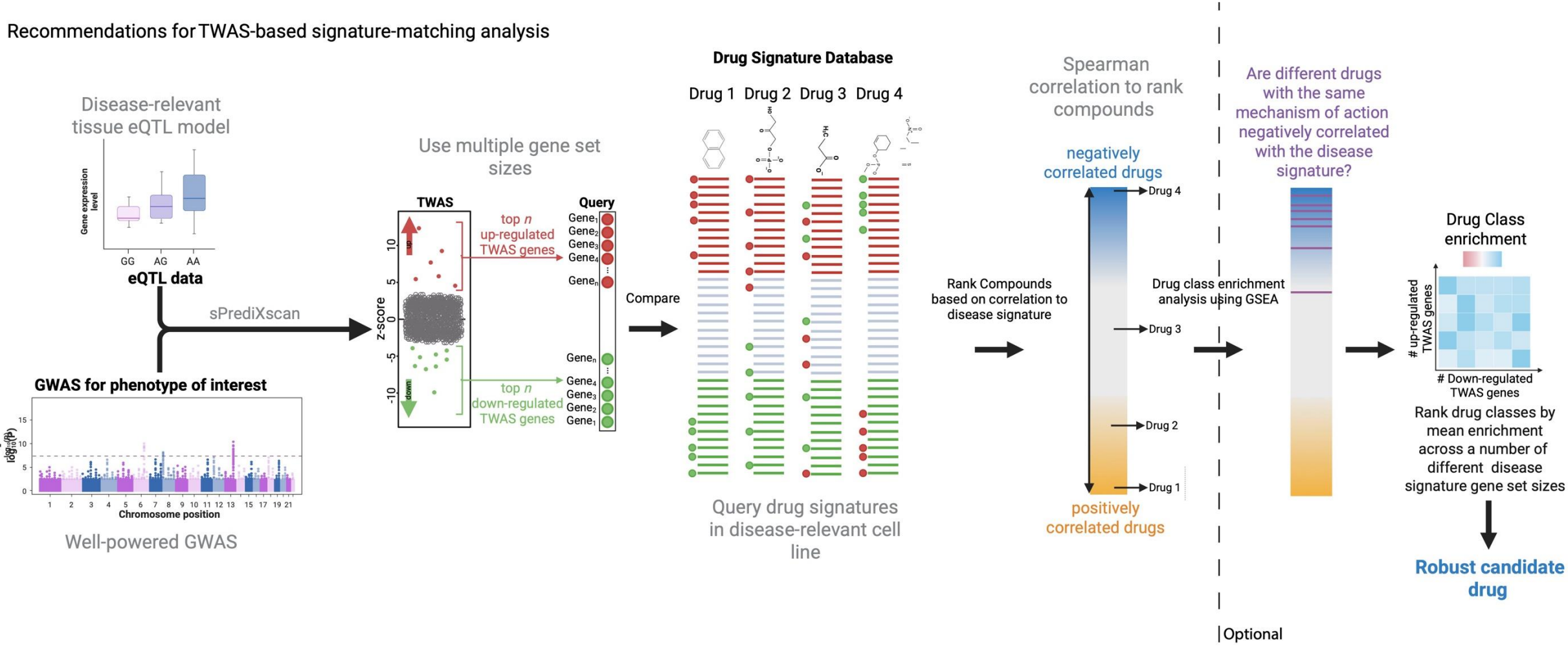
PMID: 16199517 PMCID: PMC1239896 DOI: 10.1073/pnas.0506580102

Grouping compounds based:

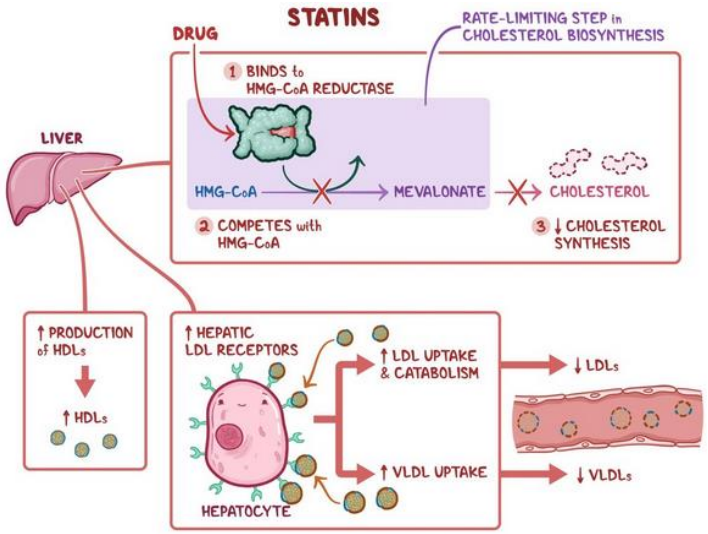
- Known mechanism of action
- Indications
- …
- Hypothesis based group
- Chemical structure

This allows us to increase the amount of evidence underlying the proposed grouping.

Putting it all together and more questions:



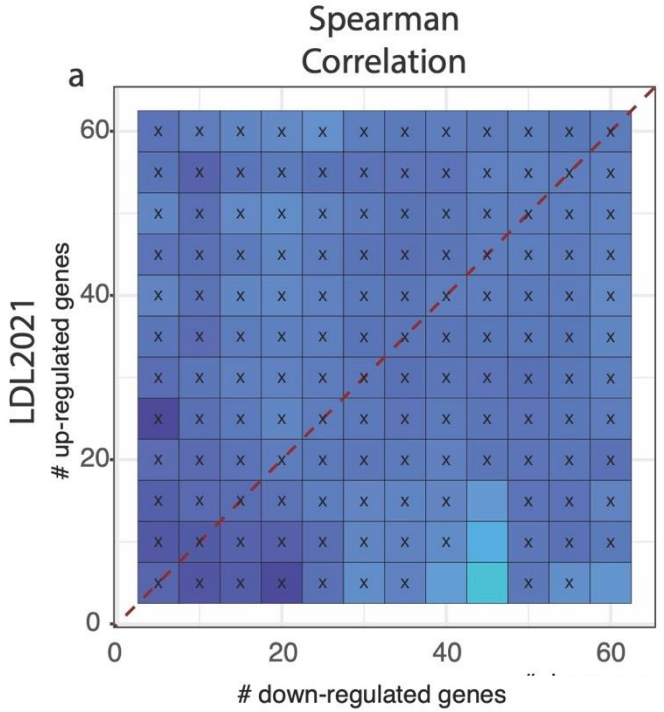
GWAS and true positive signal



GWAS:

Phenotype	n	Ancestry	PMID
LDL2021	1,320,016	European	34887591
LDL2013	188,578	European	24097068

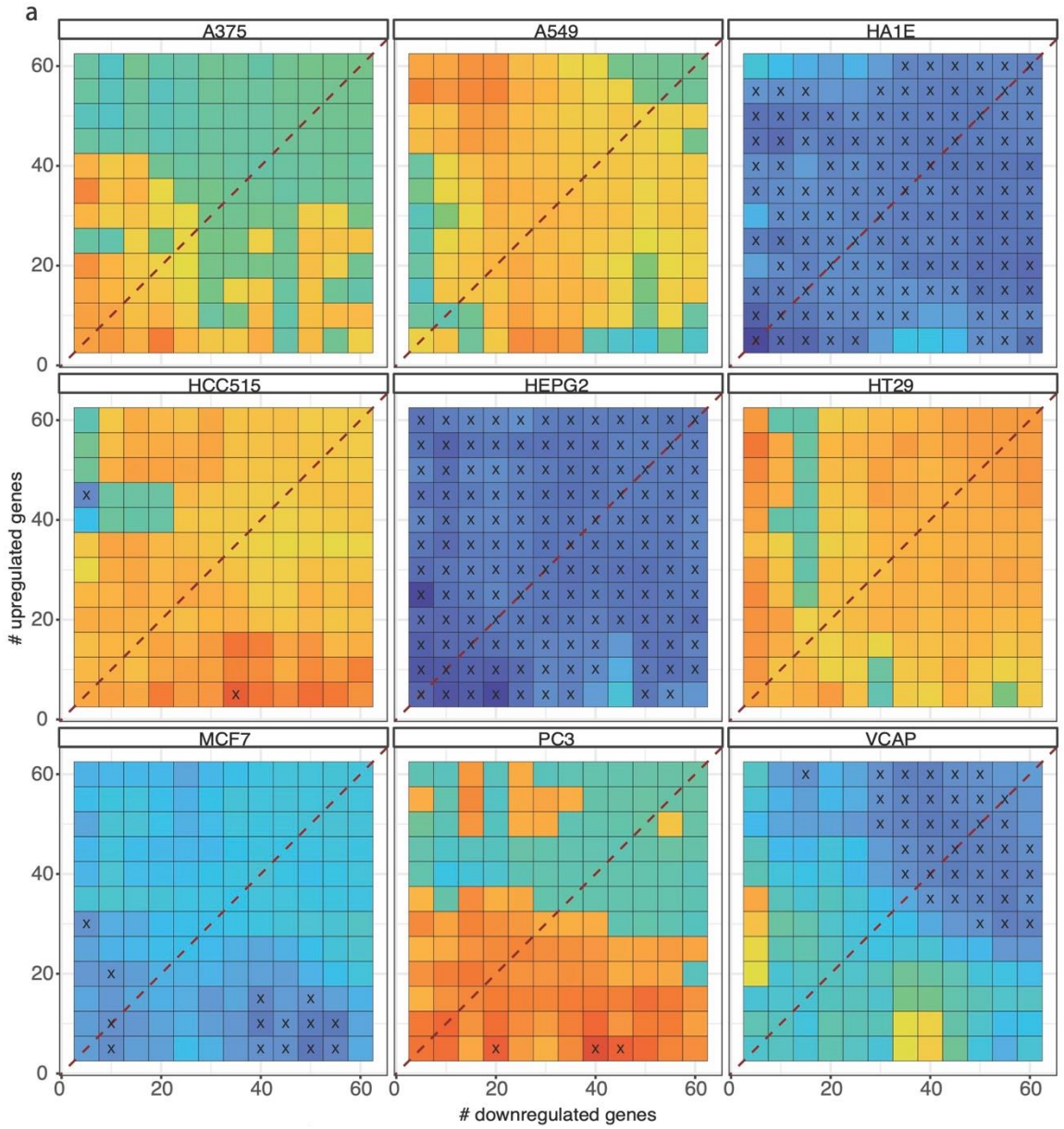
eQTL:
GTEx version 8: Liver tissue

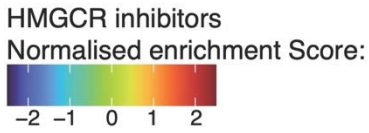


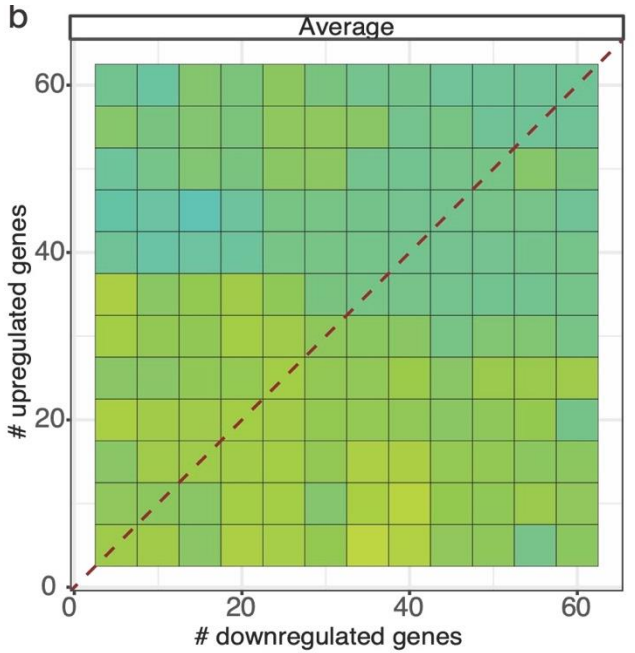
Enrichment of HMGCR inhibitors for LDL-C:

- Spearman correlation:
  - -1.76±0.15, p= 0.014

Analytic considerations:







TWAS: sPrediXcan
eQTL: Liver
GWAS: LDL-C
Cell Line: Changing