Modelling molecular heterogeneity between individuals and single cells

GCC 2015

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Heterogeneity between individuals and single cells

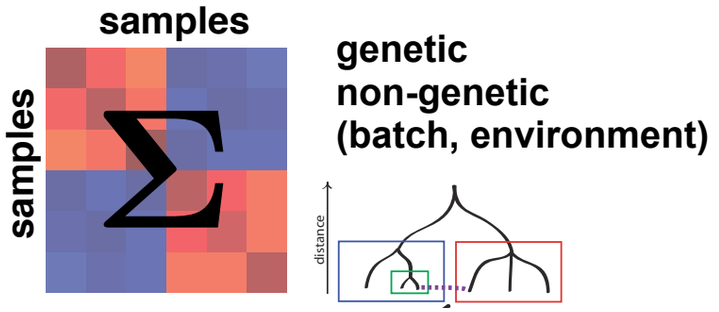
variation of interest

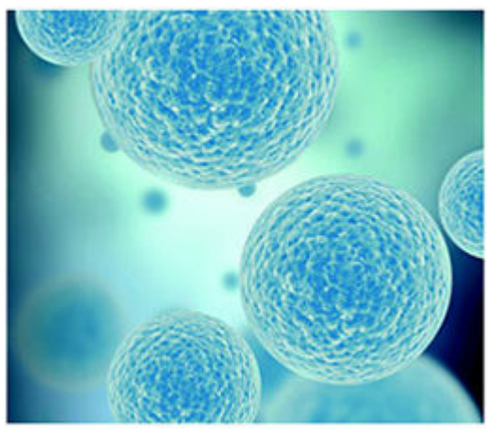
confounding

population variation

genetic associations with phenotype

sample covariance

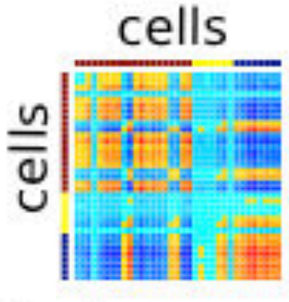




single-cell variation

differentiation processes

cell covariance



cell cycle
stress

UK human iPS cell consortium: genotype to phenotype

iPS genomics

stem cell proteomics

- Discover how
  - genetic variation affects cellular function
  - genetic lesions lead to disease phenotypes

- In so doing, create an open access resource for the wider biomedical community:
  - HapMap-like consent for ~500 healthy normals
  - Controlled access for ~500 disease samples

www.hipsci.org

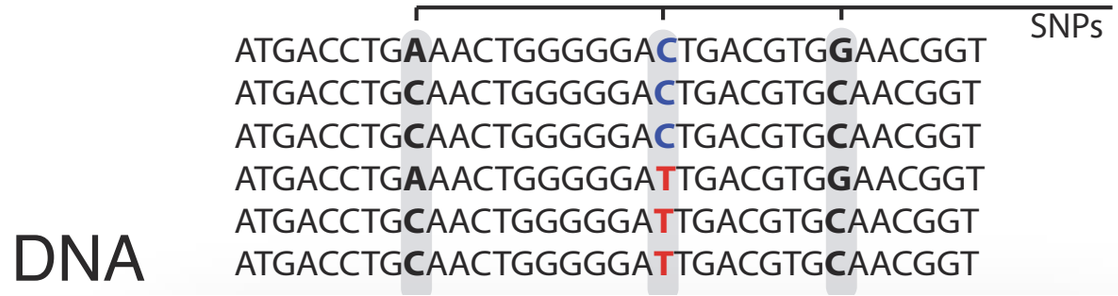
Phil Beales, Ewan Birney,Laura Clarke,
Daniel Gaffney,Angus Lamond, Richard Durbin, Fiona Watt

wellcome trust Strategic Award

MRC Medical Research Council

Multi-omics association genetics

HipSci



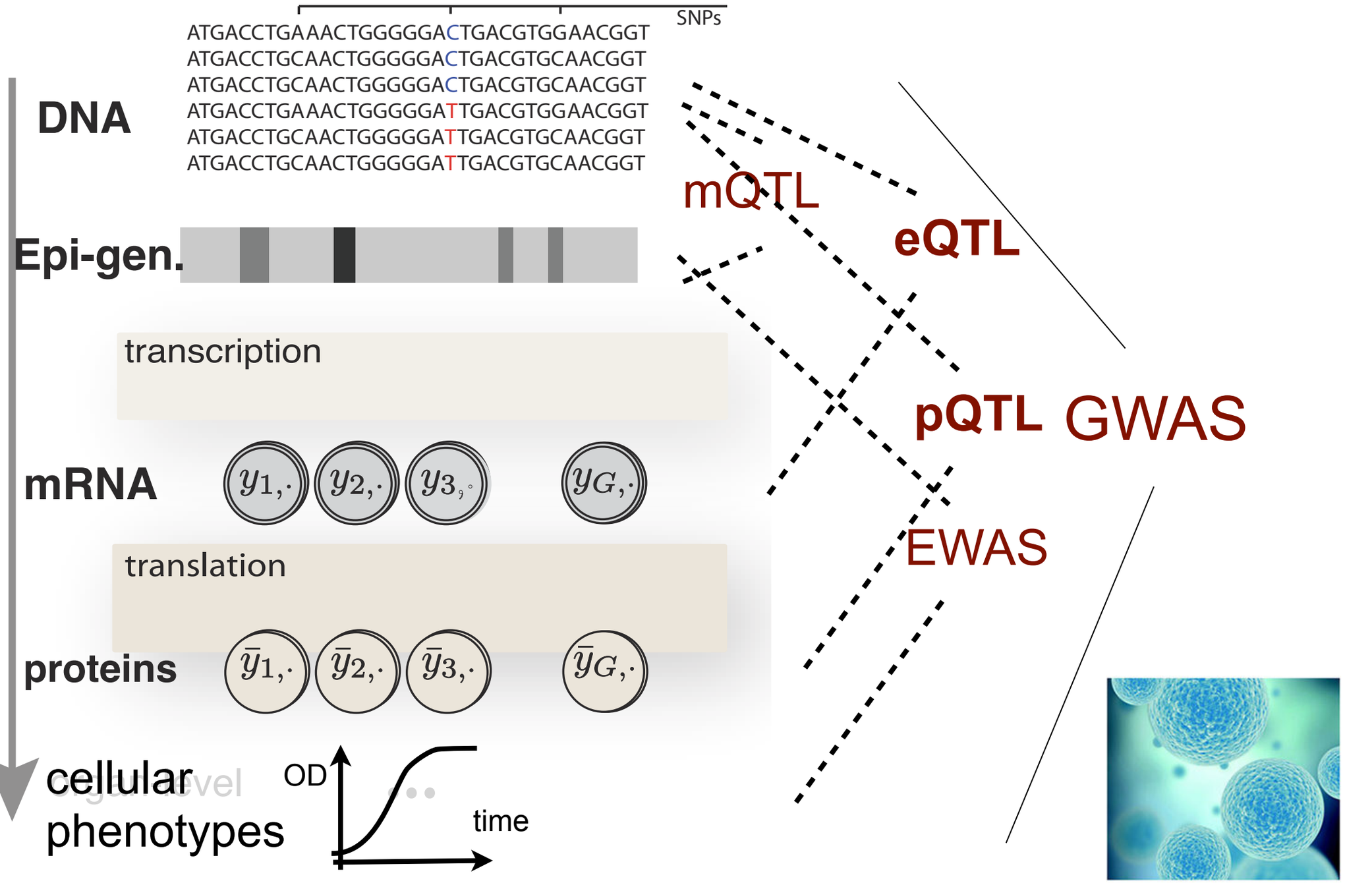
DNA

GWAS

cellular phenotypes

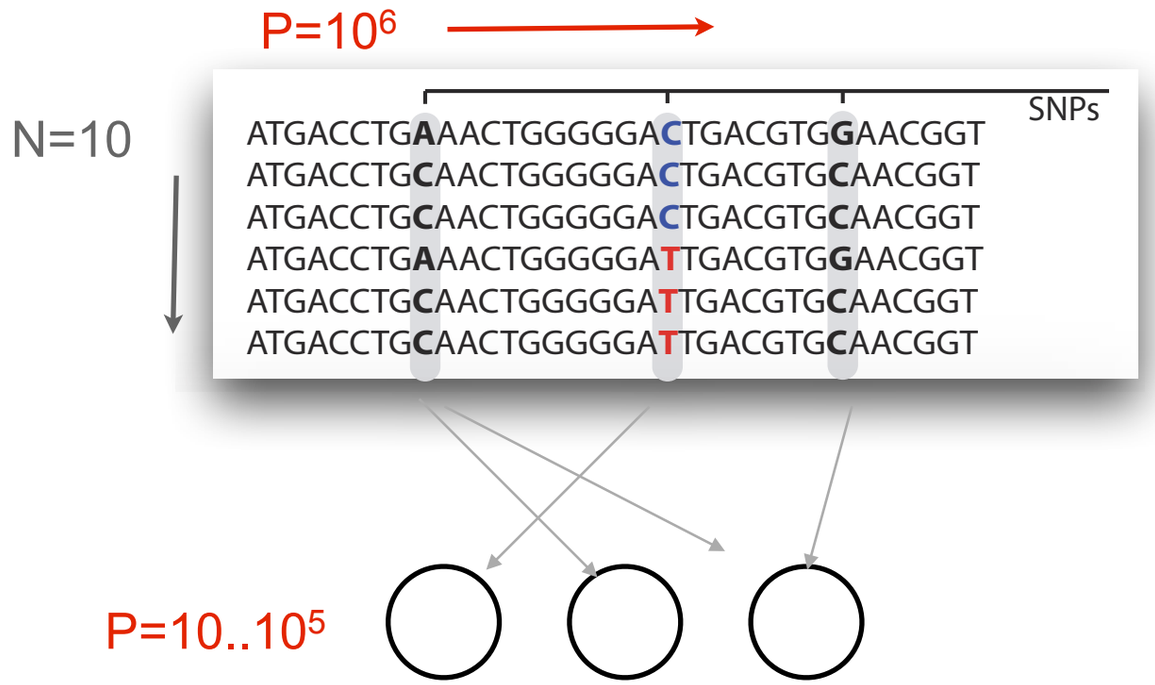
Multi-modal data integration across molecular layers

HipSci

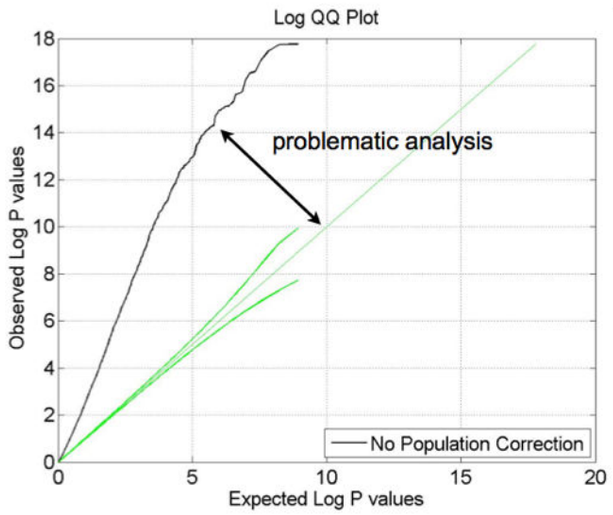


Multi-model association genetics: statistical challenges and opportunities

- **Challenge**: Large-scale multiple testing problem:
  - Need to consider potentially millions of loci and adjust for multiple testing.
  - Account for **confounding**
  - Need appropriate corrections (e.g. False Discovery Rate)
  - Scalability to large cohorts

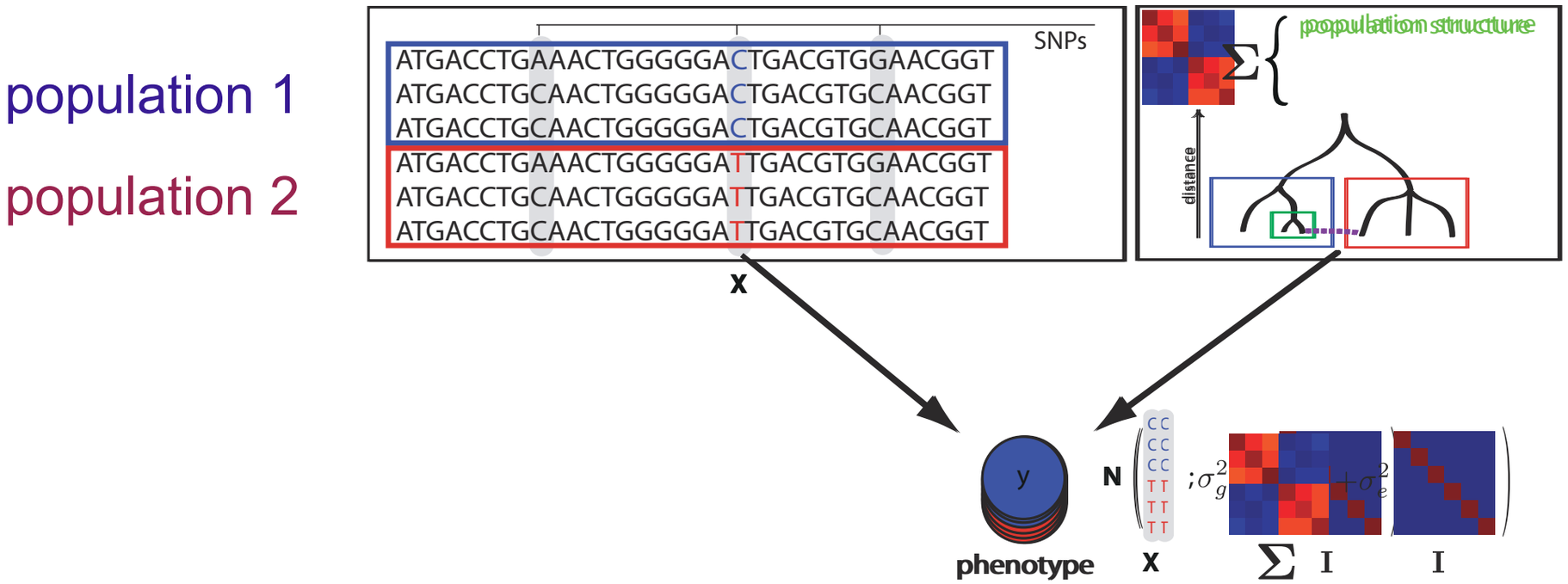


- **Win:** Large dataset allow to test modeling assumptions / fit better models
  - **Inference of confounding structures**
  - Not possible before large-scale hypothesis testing/large datasets
  - More **power due to large datasets**
  - Gain in power by joint analysis of **multiple traits**



Hidden structure: population structure

- Population structure (genetic)



$$\mathbf{y}_g = \underbrace{\mathbf{s}_i \beta_{i,g}}_{\text{genetic}} + \underbrace{\mathbf{u}}_{\text{confounding}} + \underbrace{\boldsymbol{\epsilon}_g}_{\text{noise}} \qquad \mathbf{u} \sim \mathcal{N}(0, \boldsymbol{\Sigma})$$

- Estimate Σ
  - Population structure: genotype data

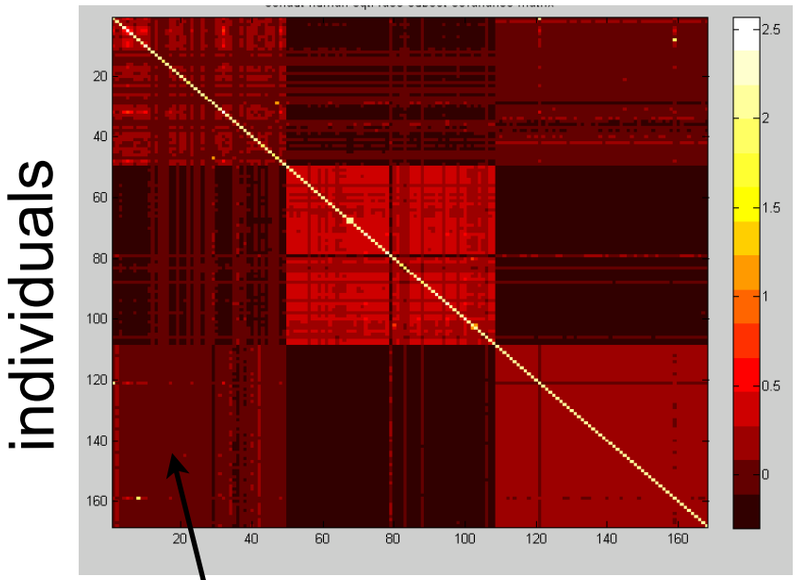
Kang et al., 2008, 2010
Lippert et al. 2011
Zhou et al. 2012

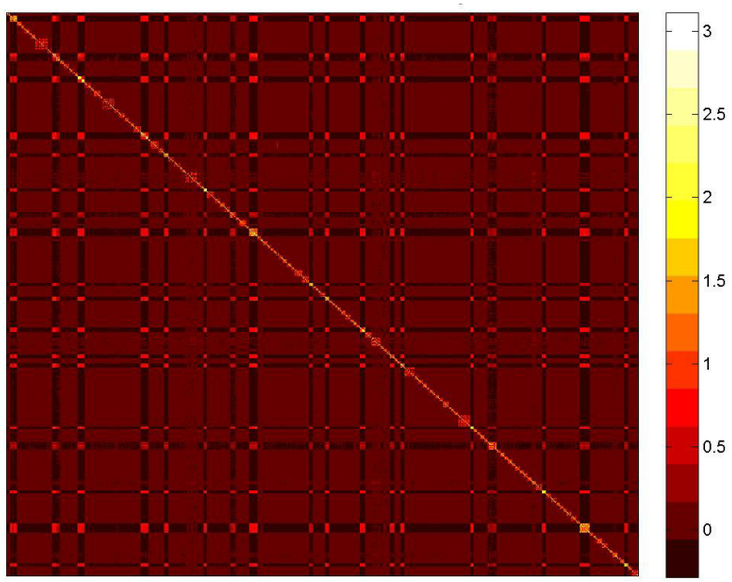
Hidden structure: population structure

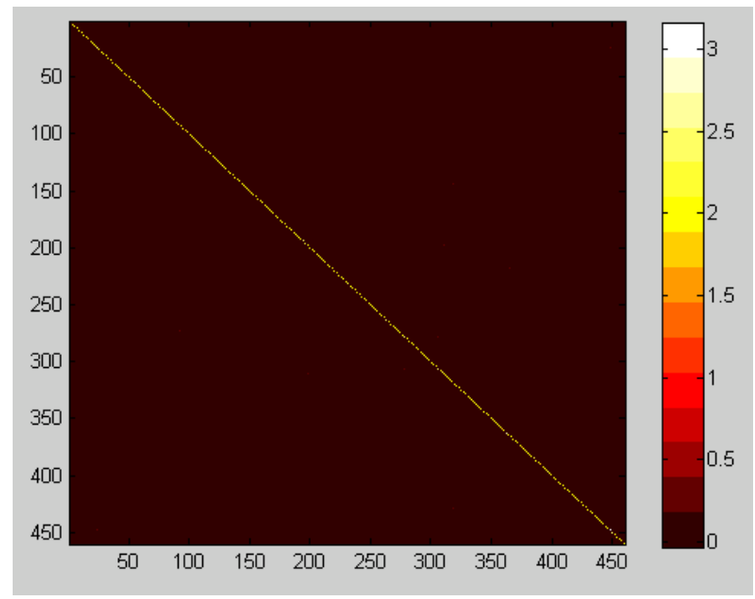
3 populations

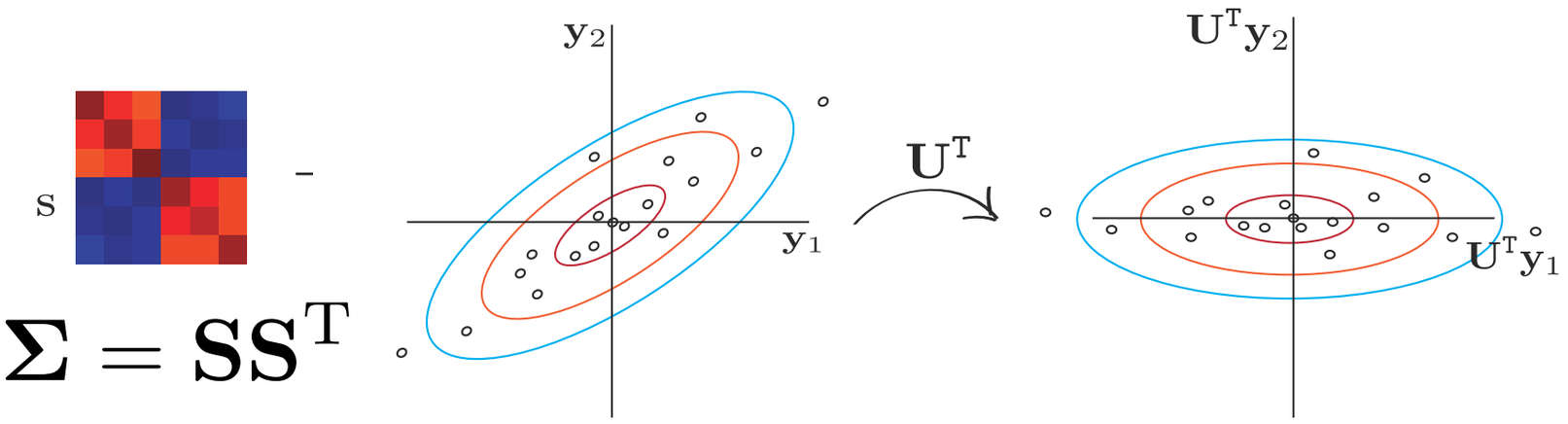
families

no structure









Hidden structure: population structure

LINEAR MODEL

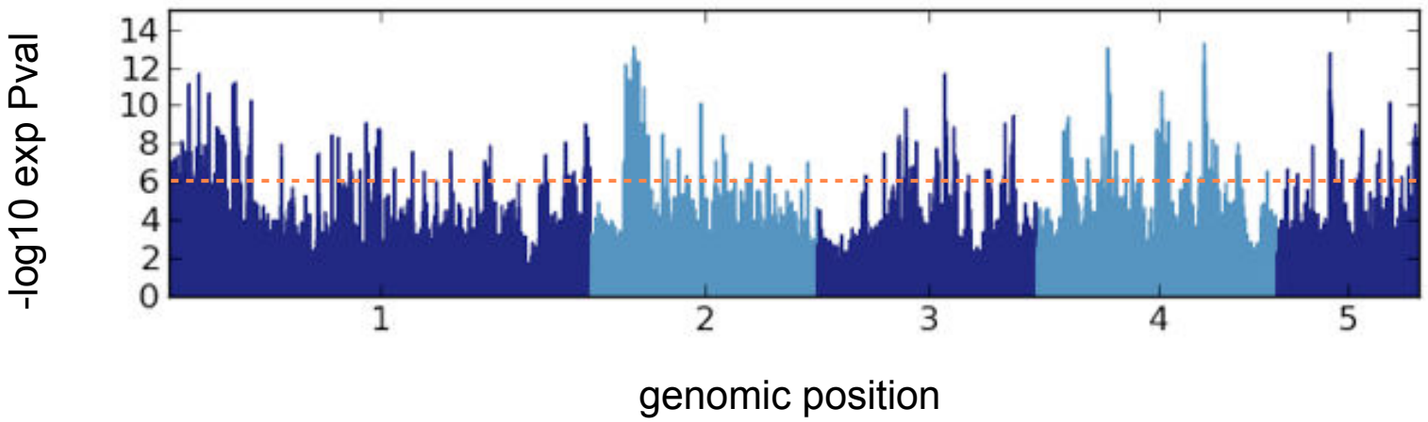
$$\mathbf{y} = \mathbf{X}\beta + \boldsymbol{\psi}$$

pheno | **X SNP** | **noise**

NOISE

$$\boldsymbol{\psi} \sim \mathcal{N}\left(\mathbf{0}, \sigma_e^2 \mathbf{K}\right)$$

N > 1,000



flowering time
A. Thaliana
(Atwell et al. 2010)

Flowering in A. thaliana

Hidden structure: population structure

LINEAR MIXED MODEL

$$\mathbf{y} = \mathbf{X}\beta + \mathbf{u} + \boldsymbol{\psi}$$

pheno | **SNP** | **confounding** | **noise**

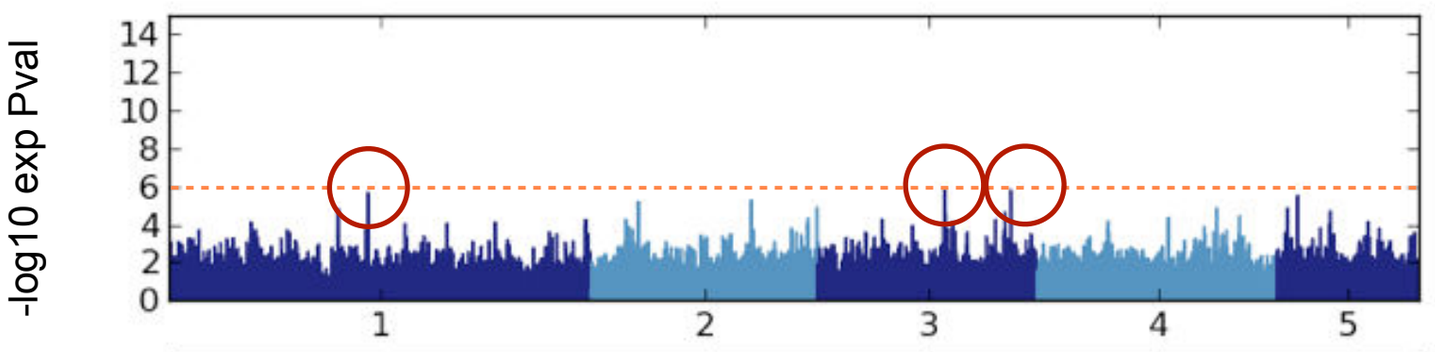
GW GENETIC TERM

$$\mathbf{u} \sim \mathcal{N}\left(\mathbf{0}, \sigma^2_{g\,\mathrm{s}} \right)$$

NOISE

$$\boldsymbol{\psi} \sim \mathcal{N}\left(\mathbf{0}, \sigma^2_e \right)$$

N ~ 5



flowering time
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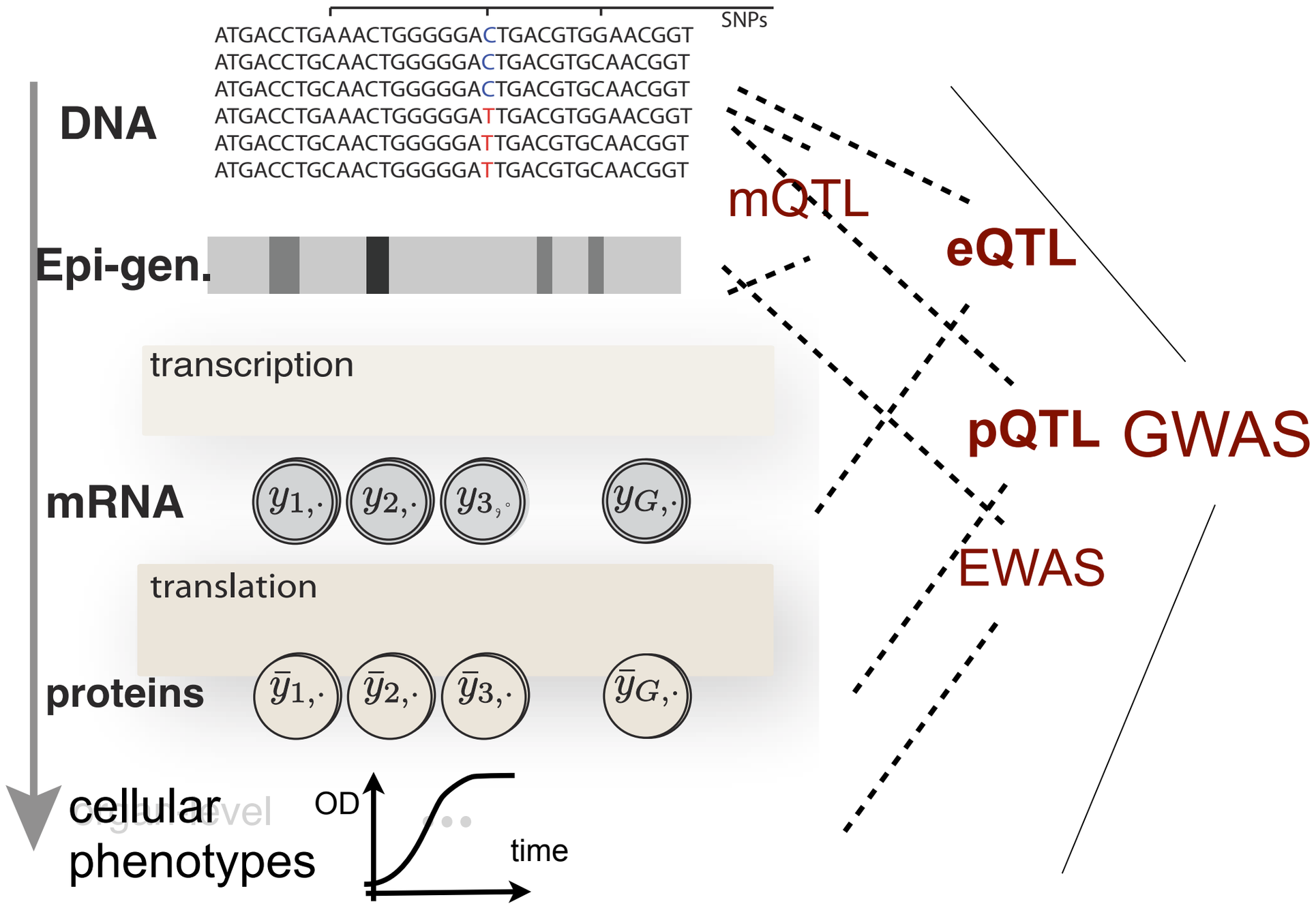
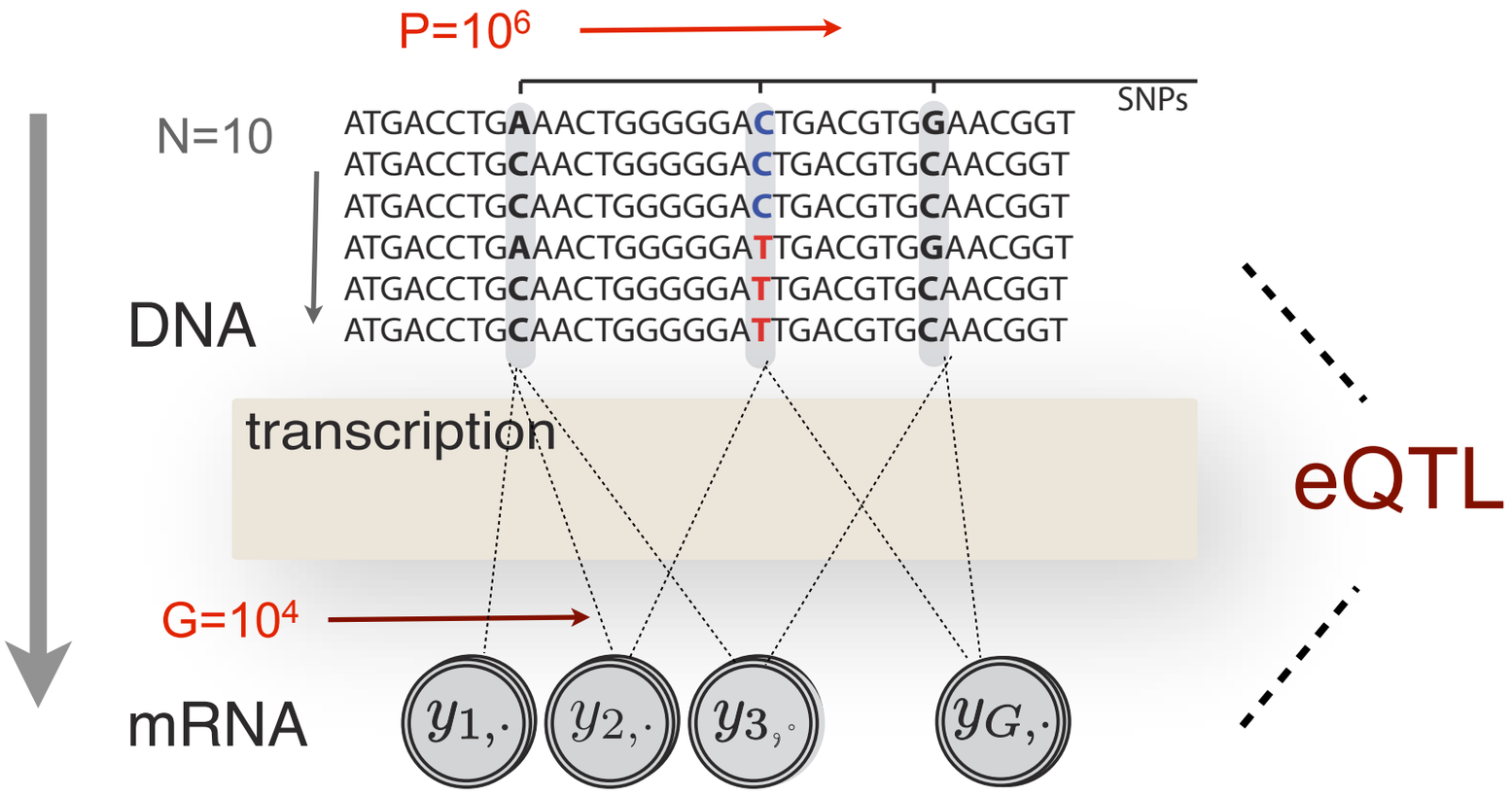
Flowering in A. thaliana

Multi-modal data integration across molecular layers



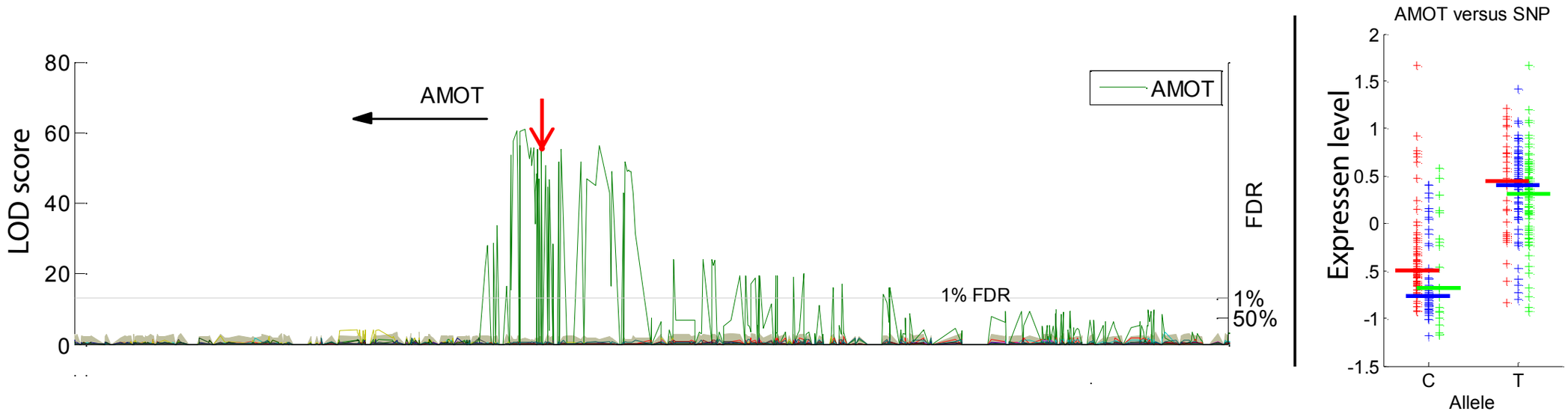
Association genetics with high-dimensional molecular phenotypes



challenge:

- multiple testing
- non-genetic sources of variation

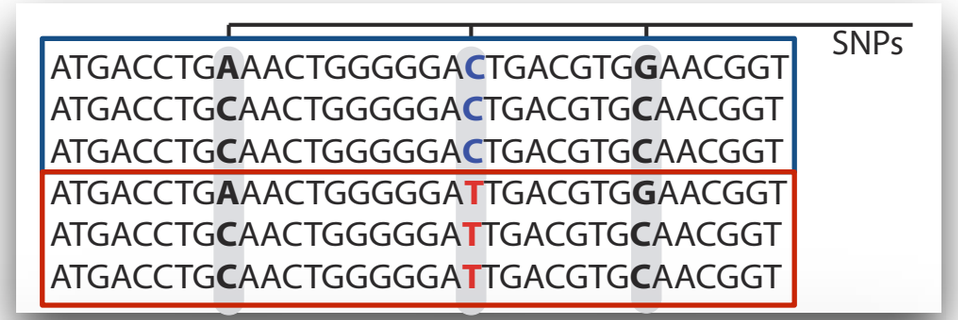
Association genetics with high-dimensional phenotypes



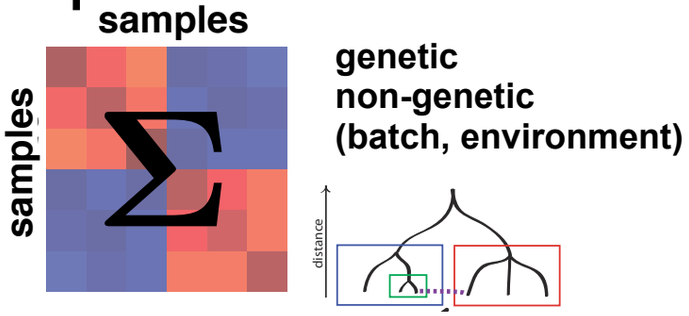
Linear mixed models to account for sample-to-sample covariance

population 1

population 2



sample covariance



transcriptome

- Estimate Σ
  - Population structure: genotype data
  - Environment/batch: gene expression levels

$$\mathbf{y}_g = \underbrace{\mathbf{s}_i \beta_{i,g}}_{\text{genetic}} + \underbrace{\mathbf{u}}_{\text{confounding}} + \underbrace{\boldsymbol{\epsilon}_g}_{\text{noise}}$$

$$\mathbf{u} \sim \mathcal{N}(0, \boldsymbol{\Sigma})$$

Leek & Storey, 2007
Kang et al. 2008
Listgarten et al, 2010
Lipper et al. 2011

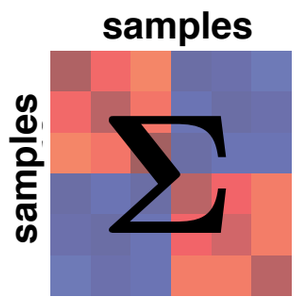
Confounding factors: genetic and non-genetic structure

- genetic

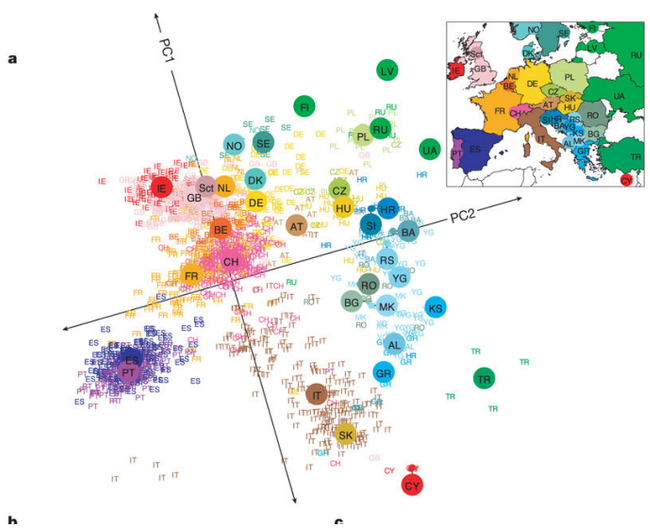
$$\boldsymbol{\Sigma} = \mathbf{S}\mathbf{S}^{\mathrm{T}}$$

- non-genetic

$$\boldsymbol{\Sigma} = \mathbf{Y}\mathbf{Y}^{\mathrm{T}}$$



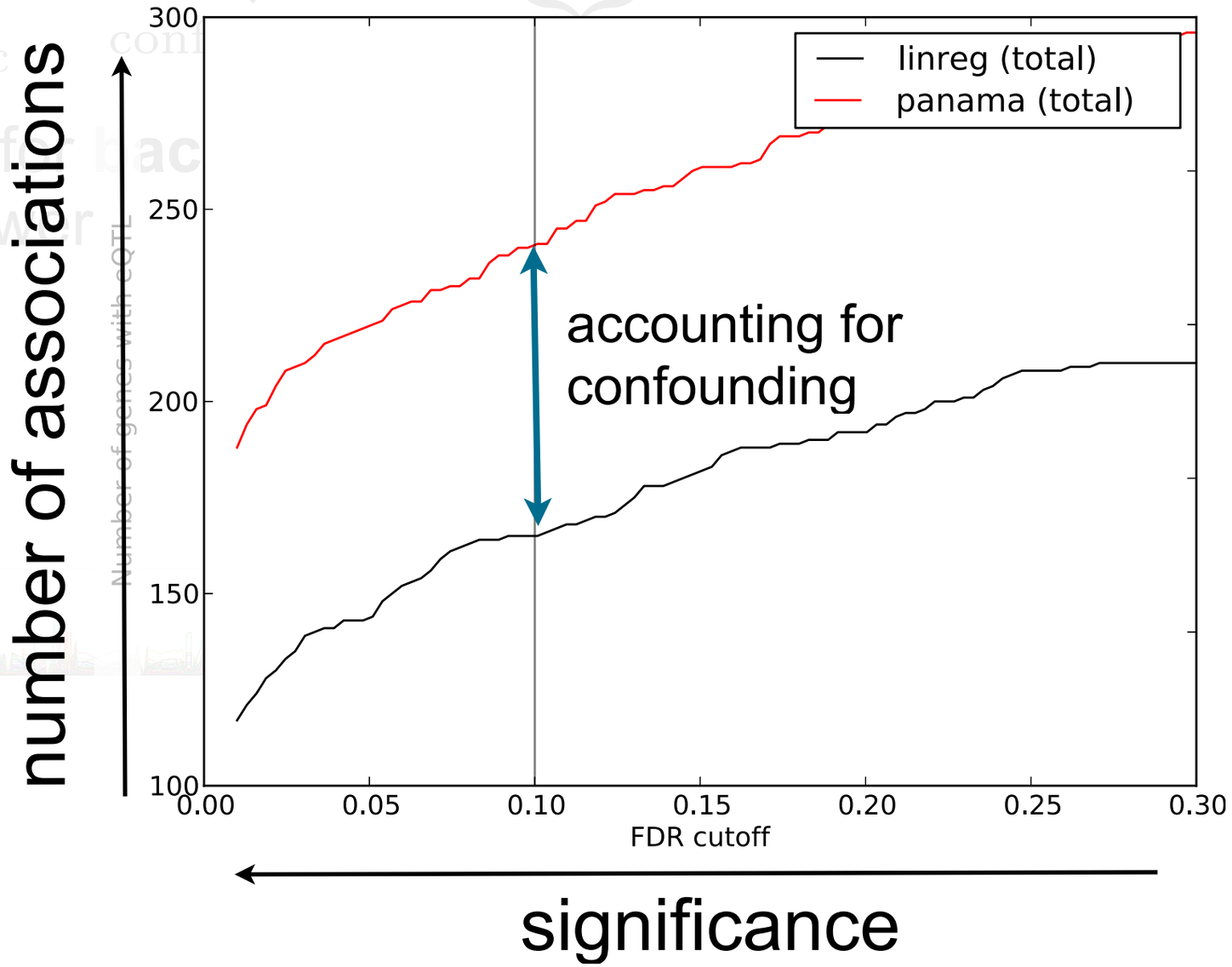
- Empirical gene expression covariance



$$p(\mathbf{Y} \mid \sigma_g^2, \sigma_k^2, \sigma_e^2, \mathbf{S}, \mathbf{X}) = \prod_{g=1}^{G} \mathcal{N}\left(\mathbf{y}_g \mid \mathbf{0}, \sigma_g^2 \mathbf{S}\mathbf{S}^{\mathrm{T}} + \sigma_k^2 \mathbf{X}\mathbf{X}^{\mathrm{T}} + \sigma_e^2 \mathbf{I}\right)$$

genetic non-genetic

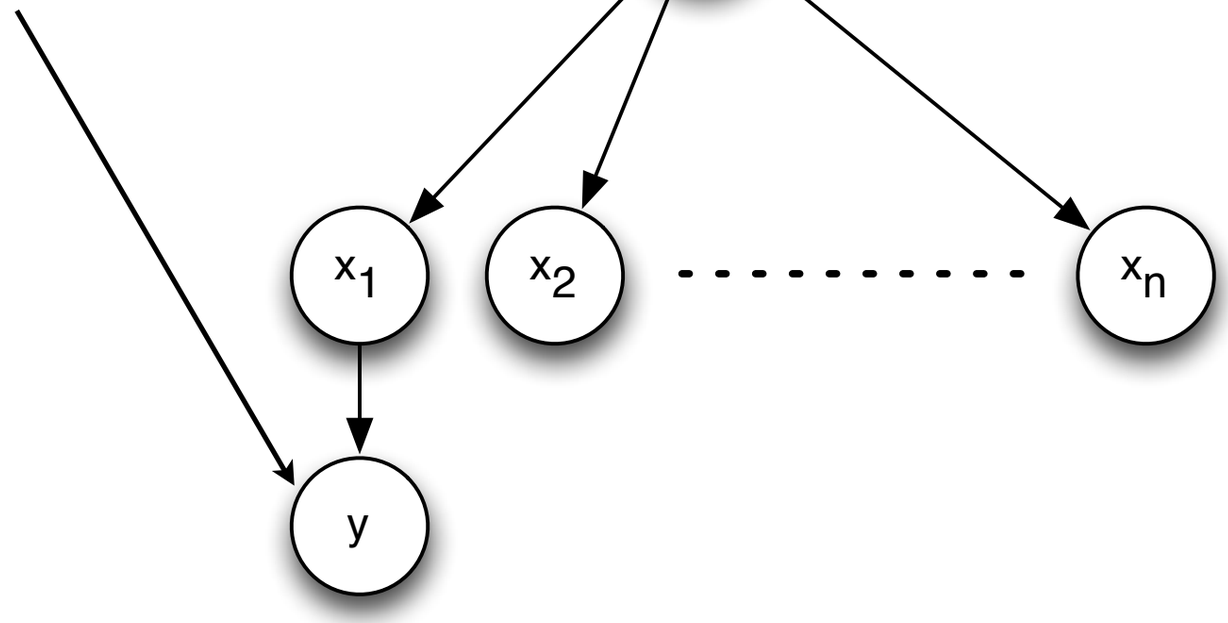
Confounding factors: genetic and non-genetic structure



Nature, Lappalainen et al. 2013

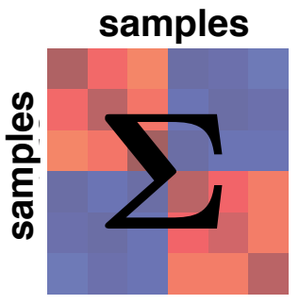
Confounding factors: genetic and non-genetic structure

- non-genetic (batch/env)
- genetic confounding (population structure)



SNPs

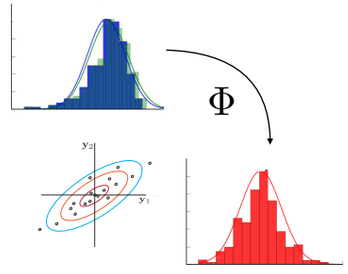
phenotype



Extending linear mixed models: beyond single-SNP single phenotype analyses

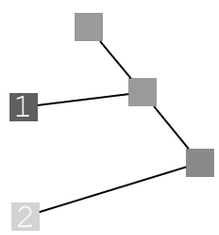
- Statistical challenges in high-dimensional association genetics
  - Normalization and scaling of quantitative traits

    Fusi et al., Nat Comm (2014)



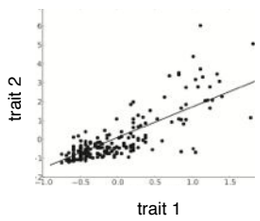
  - Accounting for epistasis and non-linear genetic interactions

    Stephan et al., Nat Comm (2015)

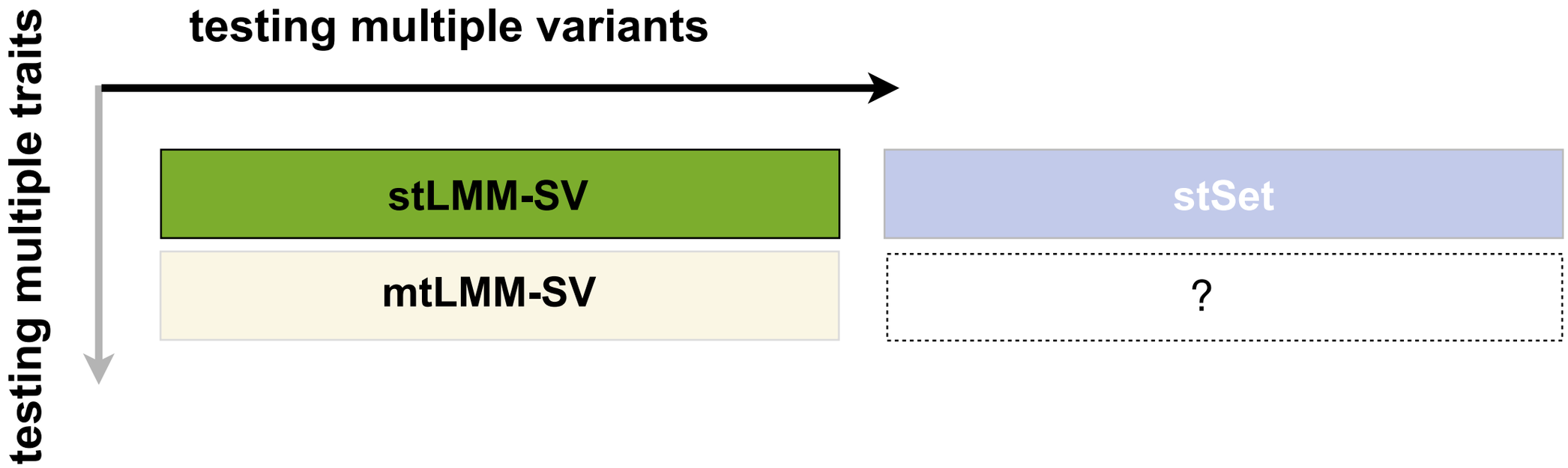


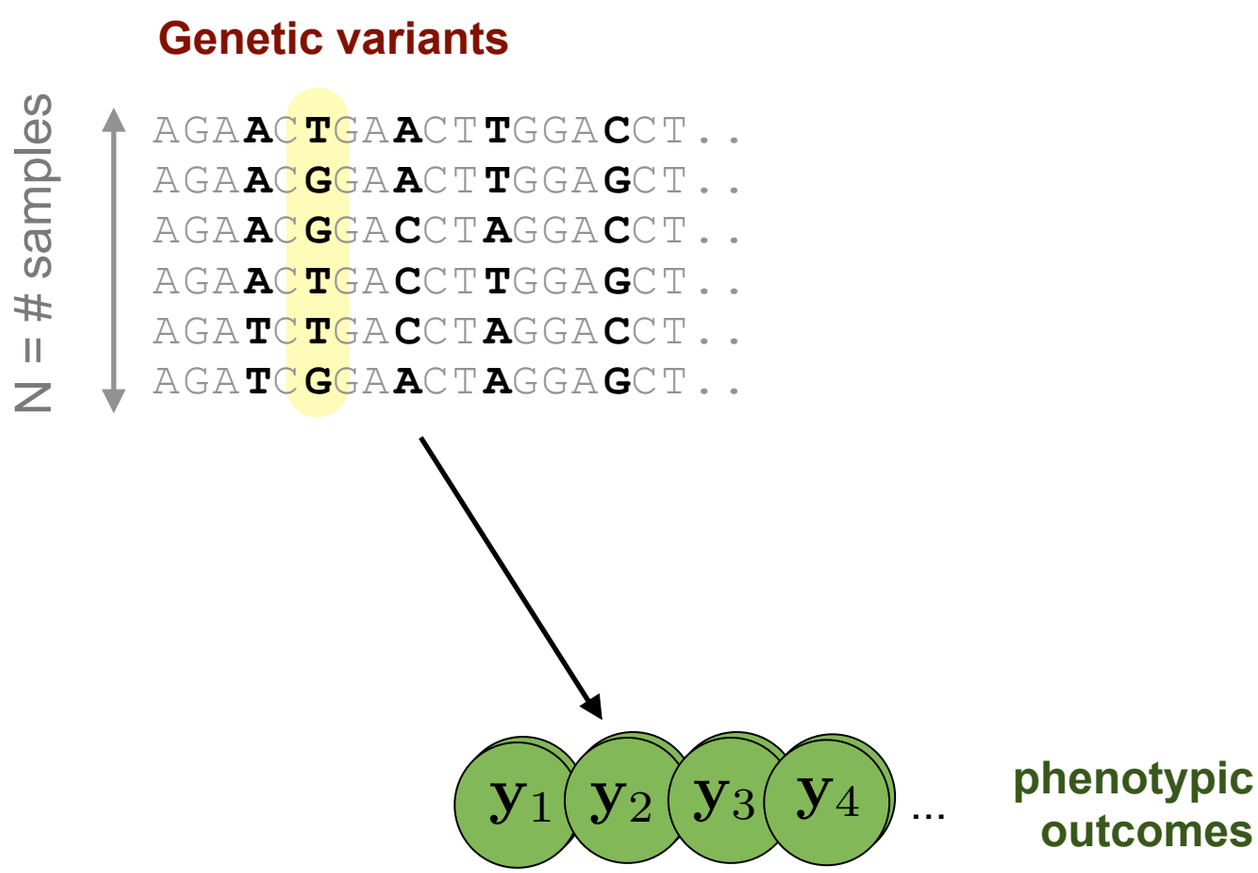
  - **Joint modeling of multiple traits and variants**

    Casale et al., Nat Meth (2015)

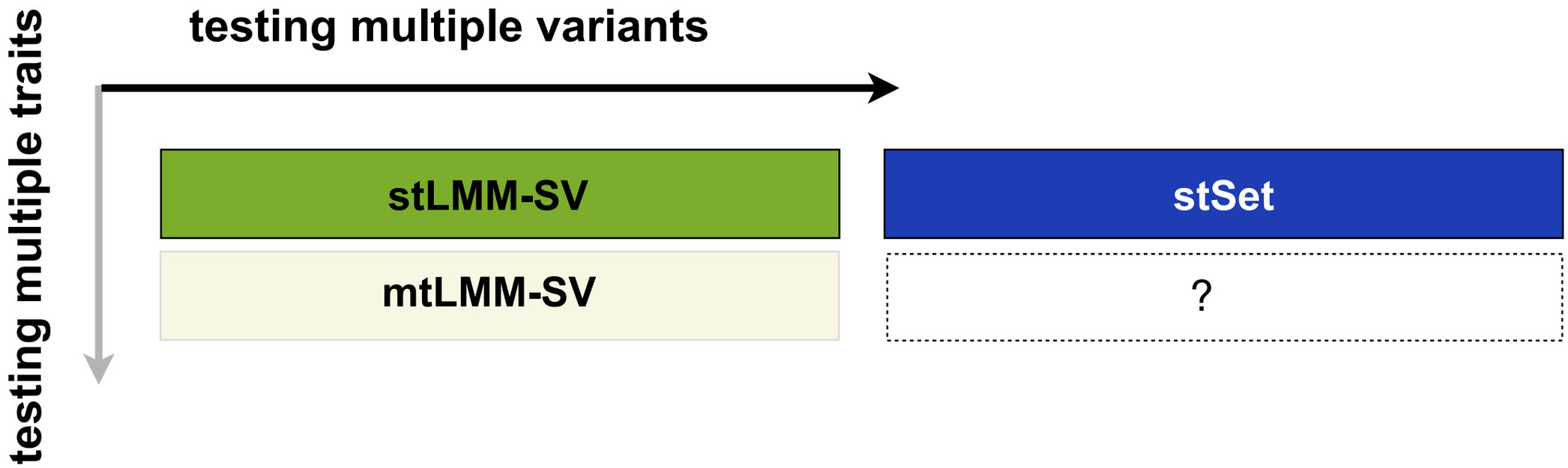


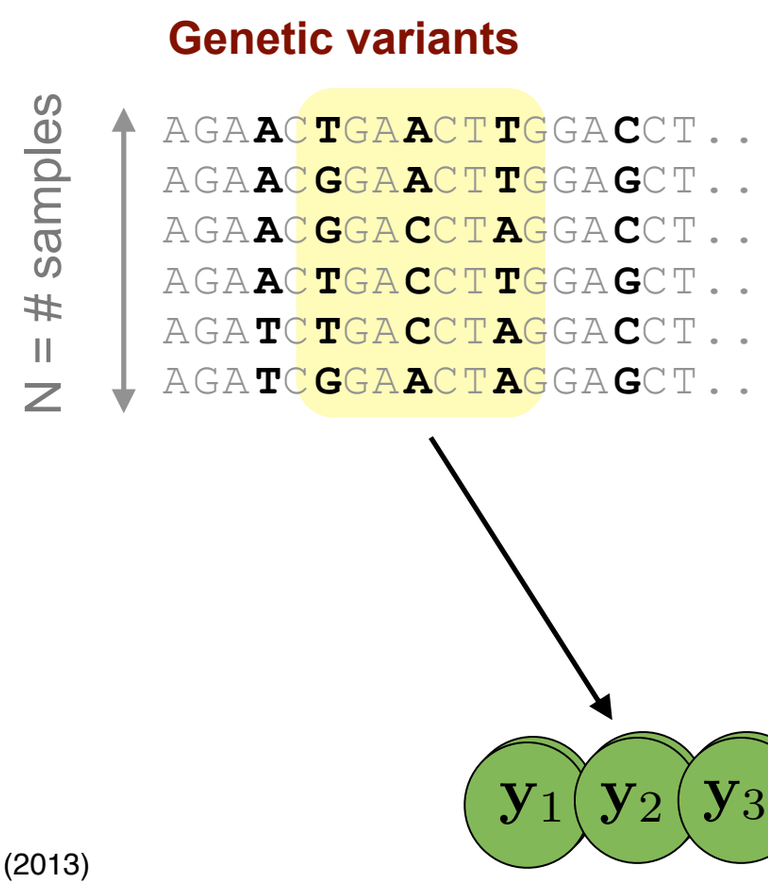
Joint modelling of traits and variants





Joint modelling of traits and variants



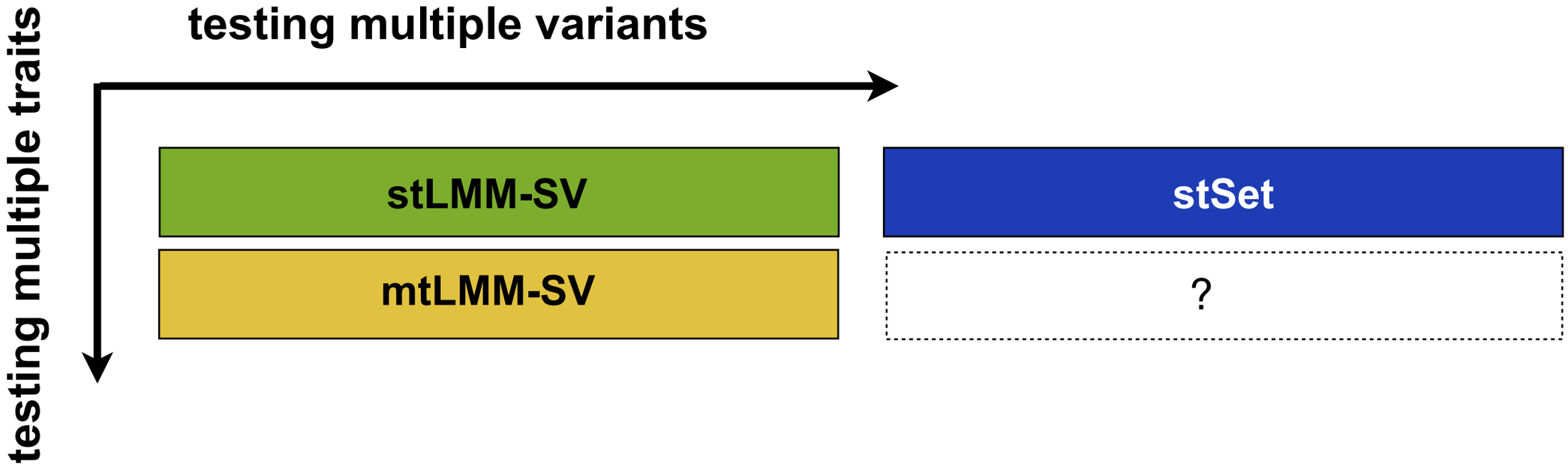


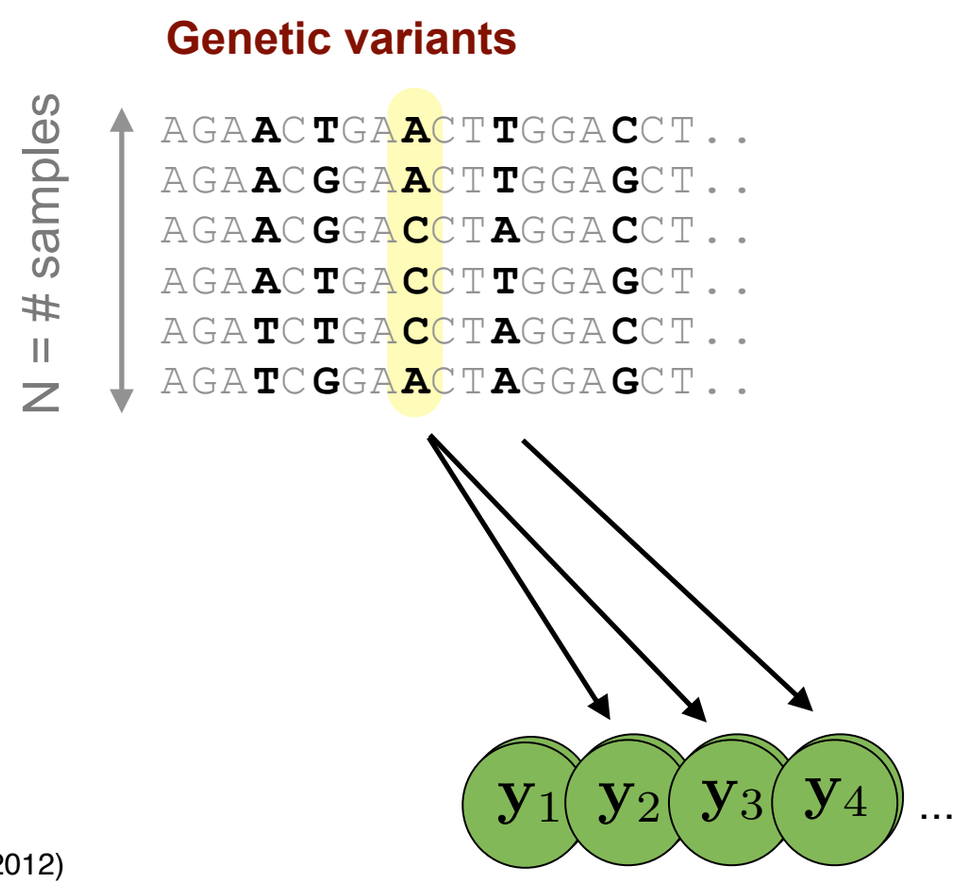
- multiple causal variants
- allelic heterogeneity

phenotypic outcomes

Listgarten et al. Bioinformatics (2013)

Joint modelling of traits and variants

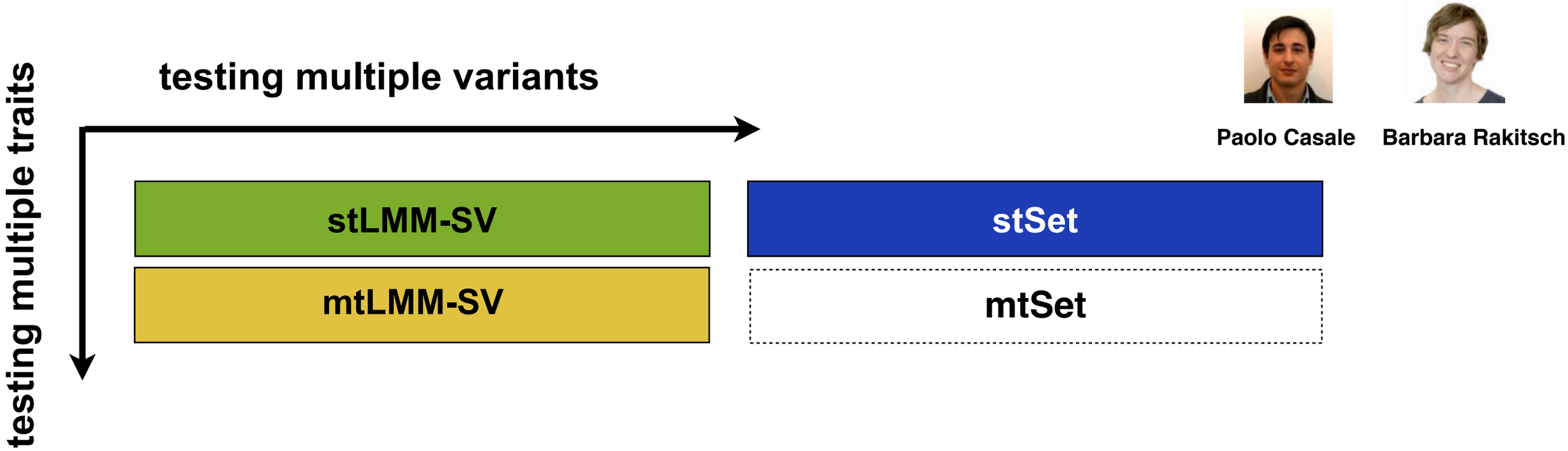




- leverage correlated traits

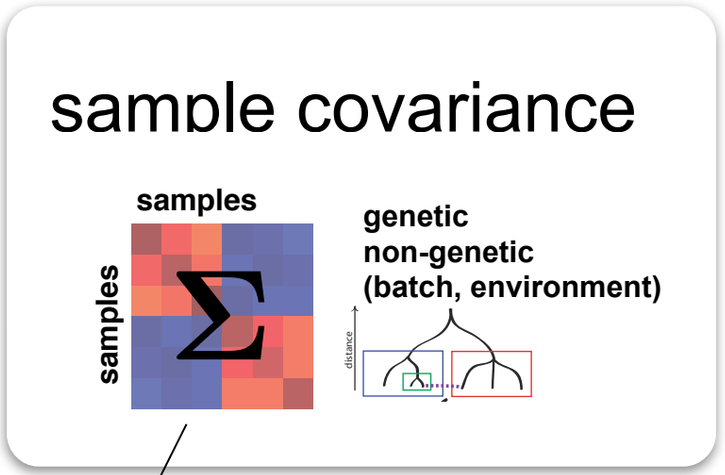
Korte et al., Nature Genetics (2012)

Joint modelling of traits and variants



Casale et al., Nat Meth (2015)

mtSet: aggregation across traits and causal variants



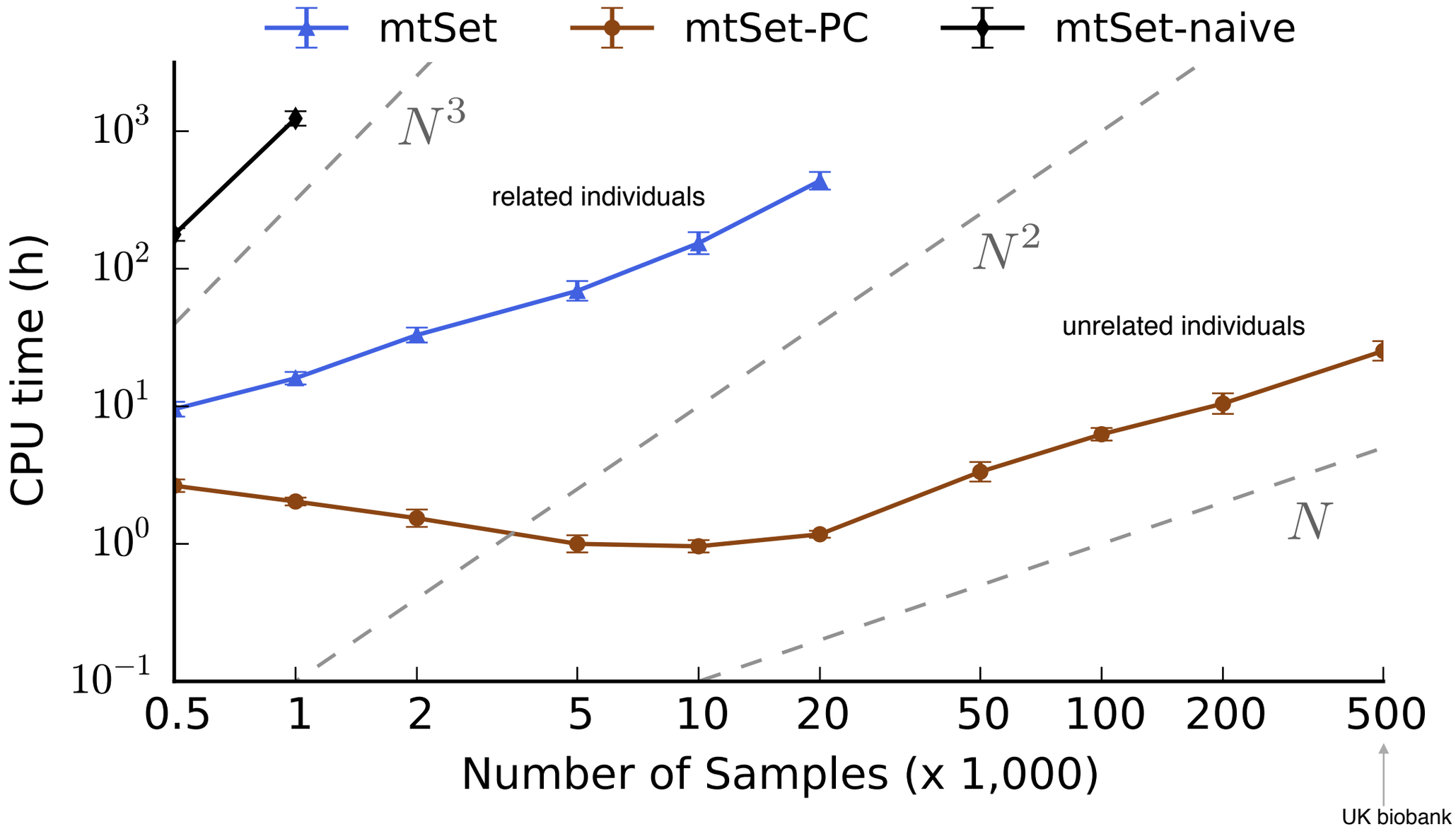
$$\underbrace{\mathbf{Y}}_{\text{phenotypes}} = \underbrace{\mathbf{FW}}_{\text{covariates}} + \underbrace{\mathbf{R}}_{\text{SNPs}} + \underbrace{\mathbf{U}}_{\text{relatedness}} + \underbrace{\mathbf{\Psi}}_{\text{noise}}$$

variance components
(random effects)

$$O(N^3P^3)$$

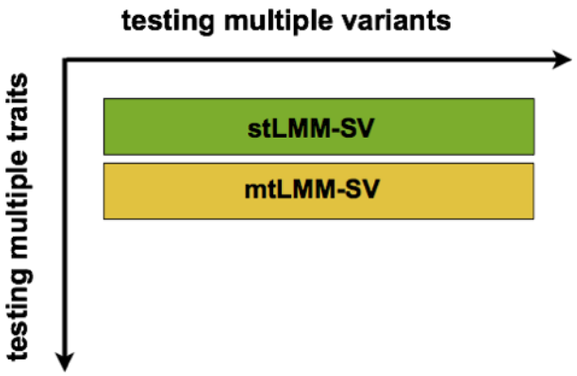
Challenge: Cubical scaling means such an algorithm is impractical for even moderately-size datasets!

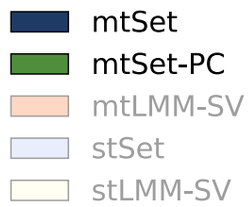
Efficient inference for large-scale GWAS

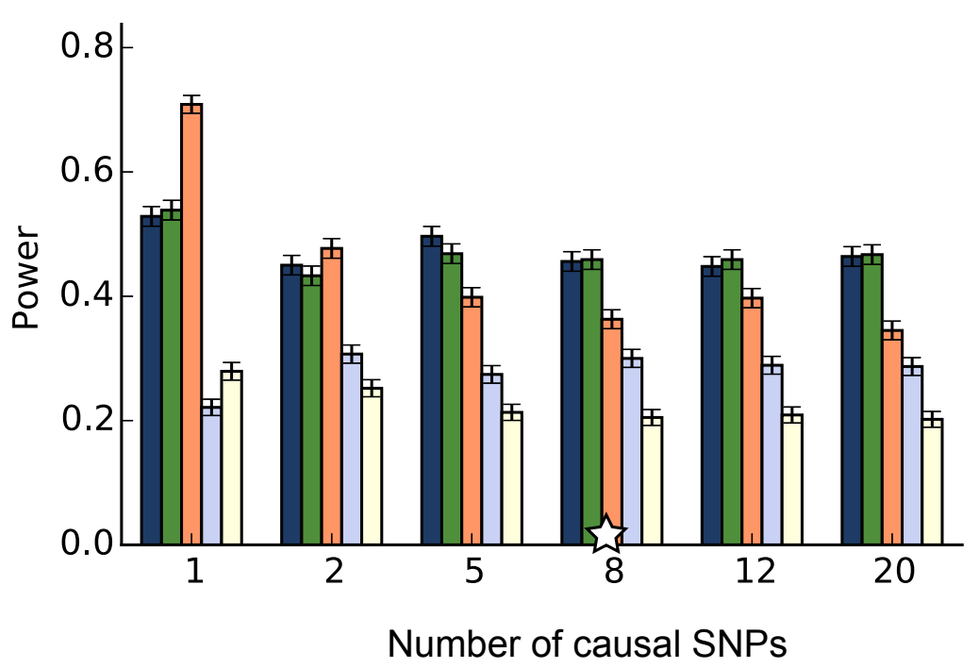


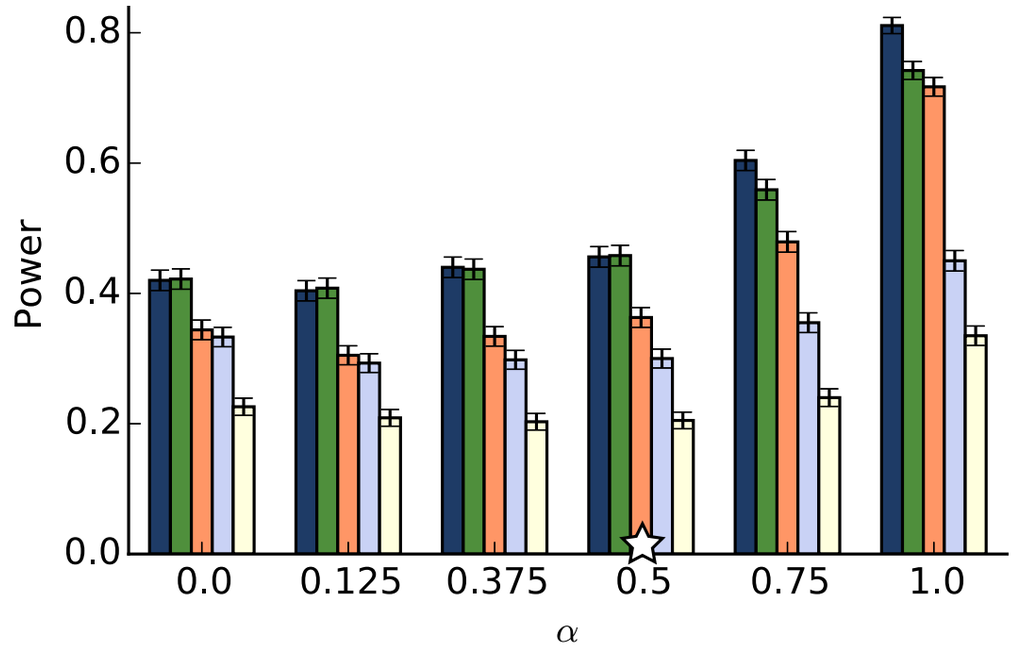
(human chrom20, 3,975 set tests for 4 traits)

Simulation study: aggregating across multiple causal variants and correlated traits









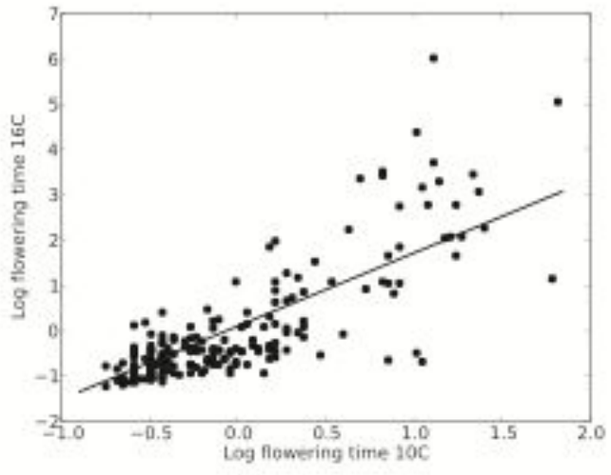
multiple causal variants →

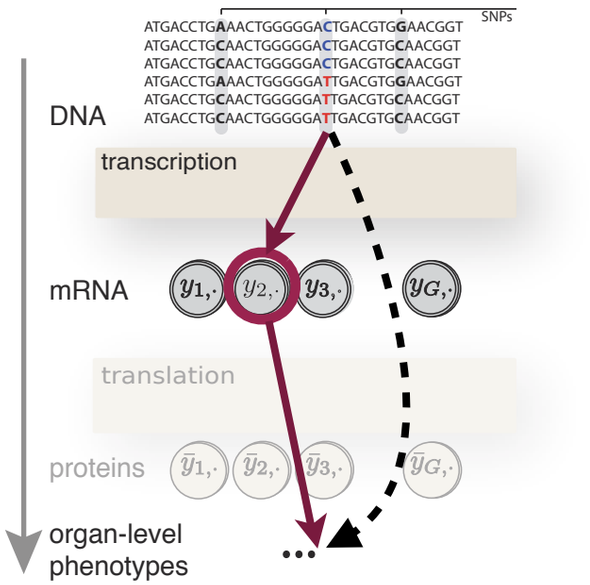
correlation between traits →

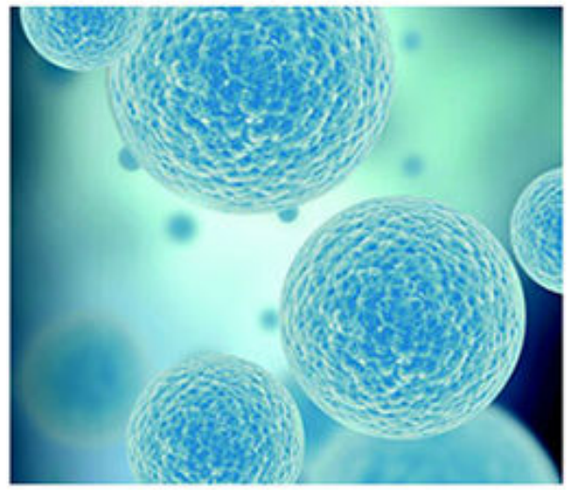
Summary so far

- Linear mixed models enable accounting for sample heterogeneity in genetic analyses
  - genetic (population structure): reduces false positives
  - non-genetic (batch, environment): increases power
- Scalability to large datasets
- Joint modeling of multiple (correlated) traits and multiple causal variants

Accounting for heterogeneity between individuals and single-cells







Statistical genetics

- Software (LIMIX)[1]
- Phenotype normalisation[2]
- Modelling epistatic relationships[3]
- Joint modelling of correlated traits[4]

Molecular heterogeneity

- Genetics of gene expression
- Causality[5]

- Human iPS biology
- Drosophila/yeast/plant genetics
- Cancer
- Drug susceptibility screens

single-cell heterogeneity

- Modelling heterogeneity in scRNA-Seq[6]
- Single cell DNA methylation profiling

1.Lippert et al., BioArxiv
2. Fusi et al., Nat Comm (2014)
3. Stephan et al., Nat Comm (2015)
4.Casale & Rakitsch et al., Nat Meth (2015)
5.Gagneur et al. PLoS Genet (2013)
6.Buettner et al., Nat BioTech (2015)

EMBL-EBI

Single-cell RNA-Seq

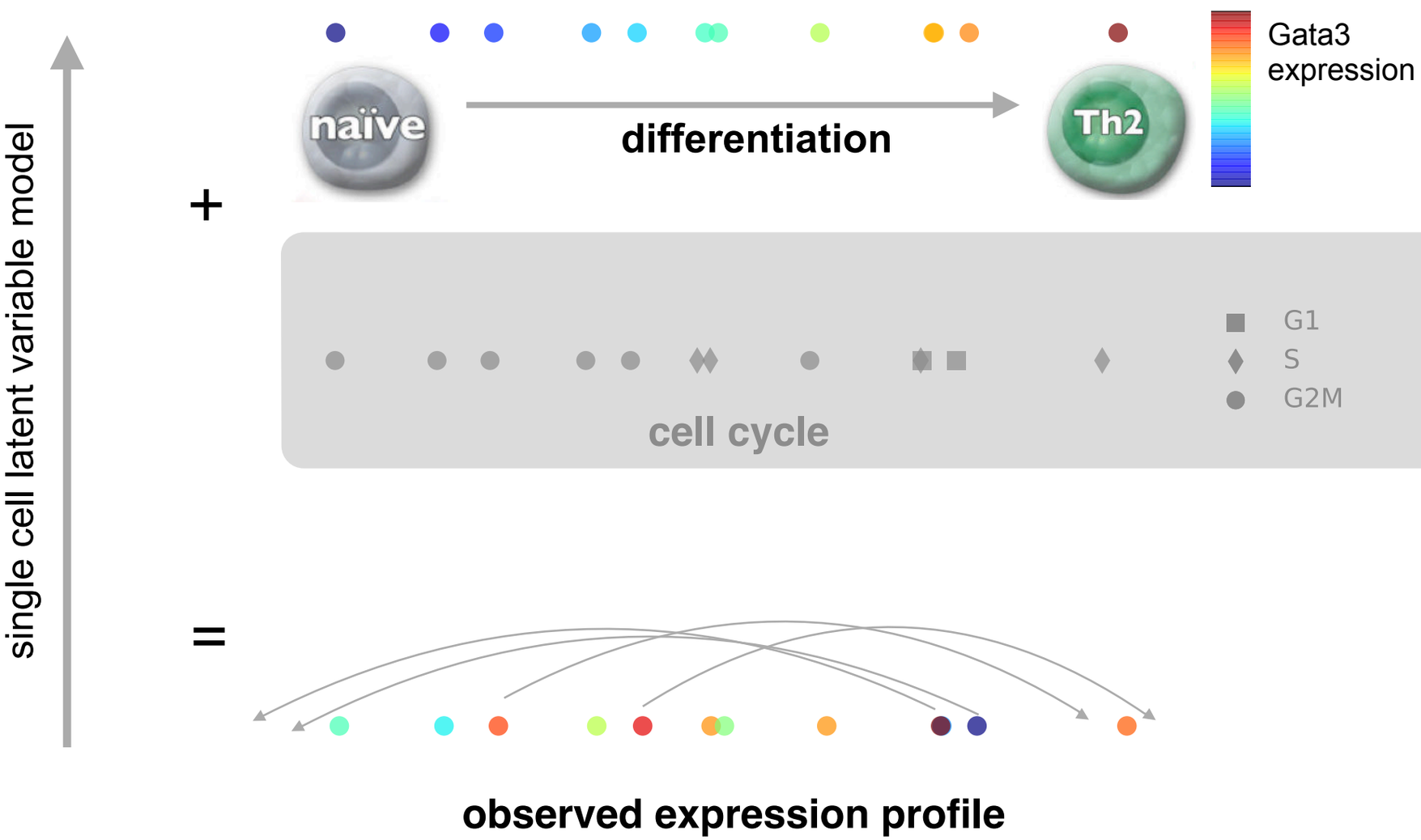
- Conventional RNA-Seq profiles are obtained from a pool of typically ~100,000+ cells.
- Using single-cell RNA-sequencing technologies, we can now assay RNA abundance in single cells.

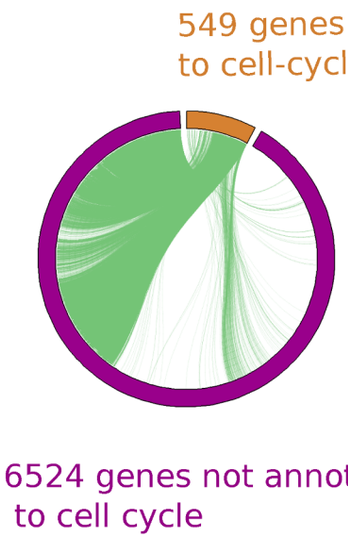
- novel variation between cells:
  cell type composition, **differentiation**

- additional (confounding) expression heterogeneity: **cell cycle**, apoptosis, …

Fluidigm C1®

Cell cycle masks differentiation processes in single-cell RNA-Seq





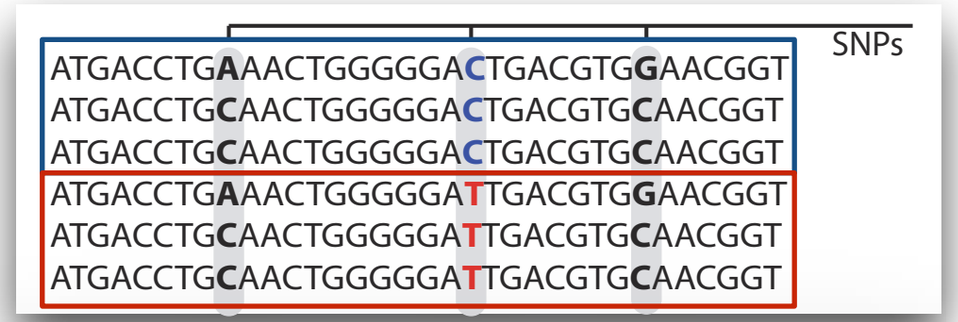
wide-spread correlation between cell cycle genes and non-cycle genes

- Observed expression profiles do not enable recovering of the differentiation process.

Gene expression heterogeneity is not new…

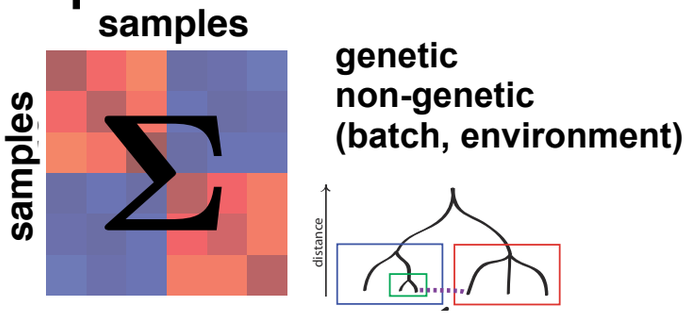
population 1

population 2

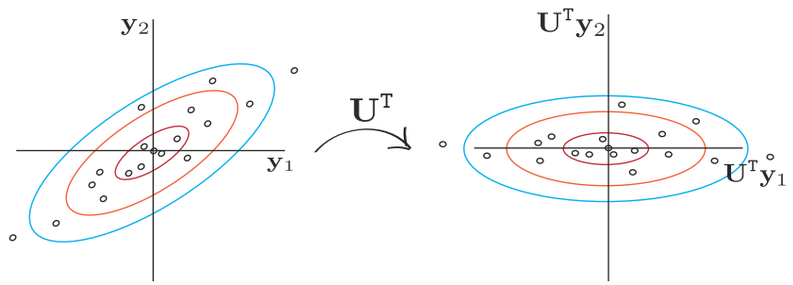


transcriptome

sample covariance



- Estimate Σ
  - Population structure: genotype data
  - Environment/batch: gene expression levels
- Correct for Σ using mixed models
  - "Rotate" phenotypes & genotypes

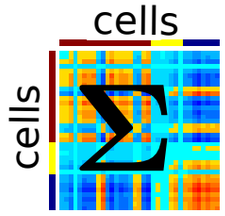


Leek & Storey, 2007
Kang et al. 2008
Listgarten et al, 2010
Lipper et al. 2011
Stegle* & Parts* et al. 2010, 2012
Fusi* & Stegle* et al. 2012

Single-cell latent variable model (scLVM)

Florian Buettner **Kedar Natarajan**

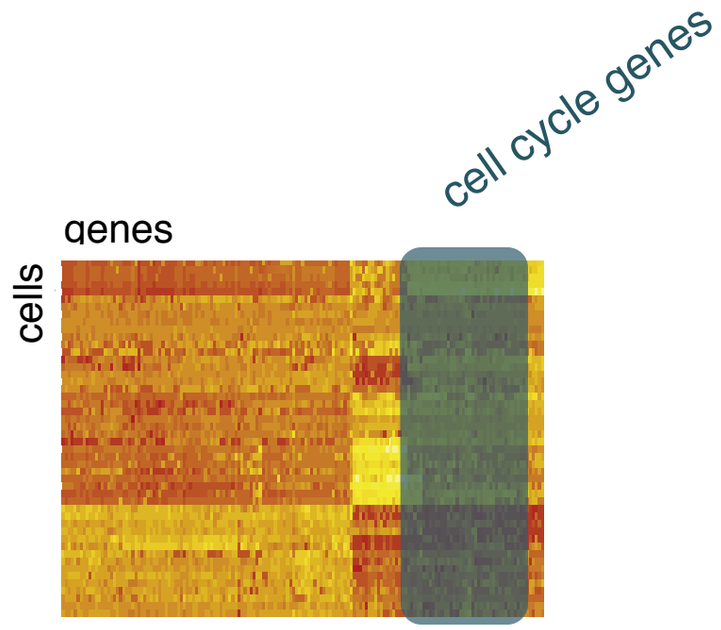
- Random effect model for cell cycle effects. Two-stage approach:
    1. Estimate a cell-cell covariance that captures cell cycle



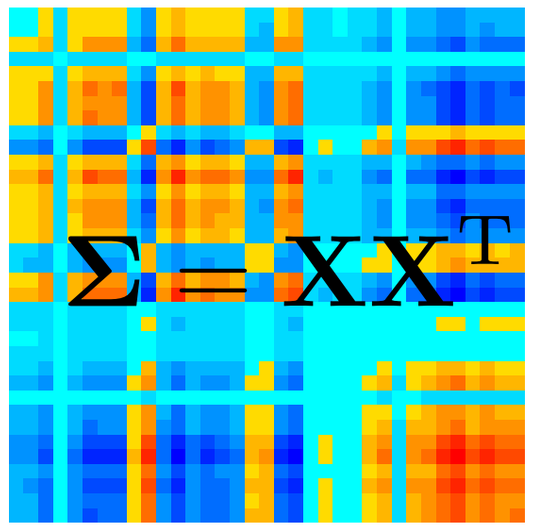
Estimation of cell-cycle induced

Estimating the cell cycle covariance

- Reconstruct cell cycle from the observed expression data
- Use known annotated cell cycle gene set
- Employ latent variable modeling to reconstruct a cell cycle factor (**X**)

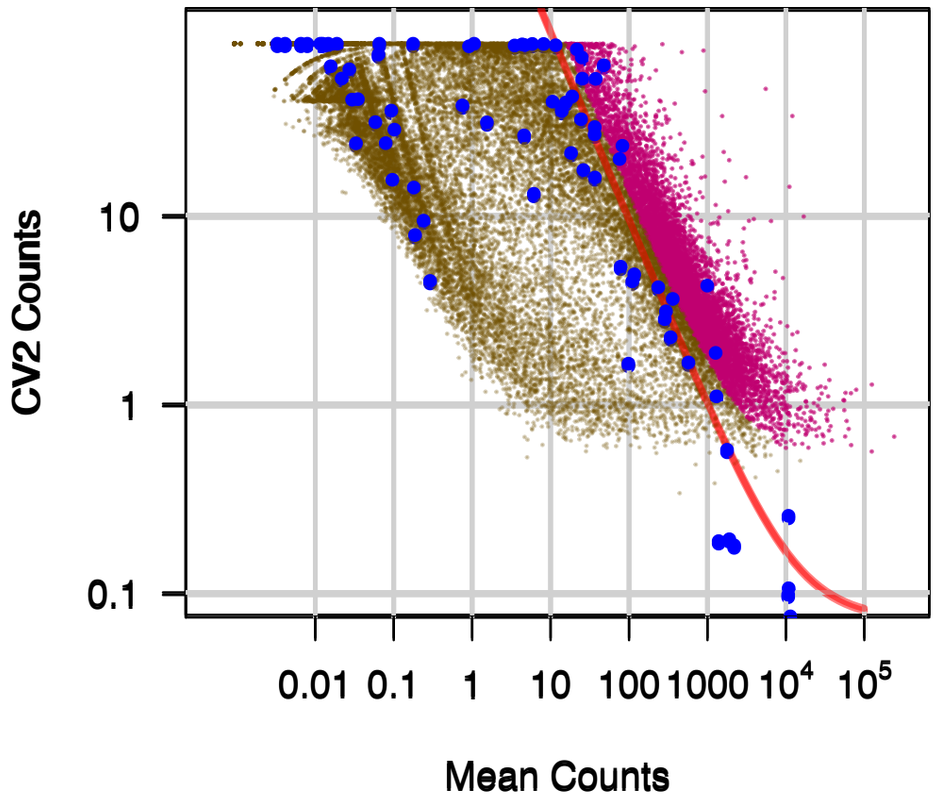


$$\mathbf{Y}_{\mathrm{cc}} \sim \prod_{g} \mathcal{N}(\mathbf{0} \mid \underbrace{\mathbf{X}\mathbf{X}^{\mathrm{T}}}_{\text{cell cycle covariance}} + \underbrace{\delta_b \mathbf{I}}_{\text{residual variance}})$$



Technical noise requires special attention

- Large proportions of technical variability due to low quantities of starting material
- Estimation of technical noise
  - Mean/variance fit from ERCC spike ins
  - Extrapolation to genome-wide genes
  - 7,073 highly variable genes



Brennecke et al. 2013

$$\mathbf{Y}_{cc} \sim \prod_g \mathcal{N}(\mathbf{0} | \underbrace{\mathbf{X}\mathbf{X}^{\mathrm{T}}}_{\text{cell cycle covariance}} + \underbrace{\delta_b \mathbf{I}}_{\text{residual variance}} + \underbrace{\mathrm{diag}(\boldsymbol{\sigma}_g^2)}_{\text{technical variance}})$$

Decomposing sources of gene expression variation

- Variance decomposition of gene expression, considering
  - cell cycle (using estimated covariance)
  - residual biological variability
  - technical noise (estimated via spike-ins)

$$\mathbf{Y}_g = \mu\mathbf{I} + \alpha\mathbf{u}_{\mathrm{cc}} + \delta_b\mathbf{u}_{\mathrm{b}} + \quad \mathbf{u}_{\mathrm{n}}$$

$$N(0,\ \ ) \quad N(0,\ \ ) \quad N(0,\ \ )$$

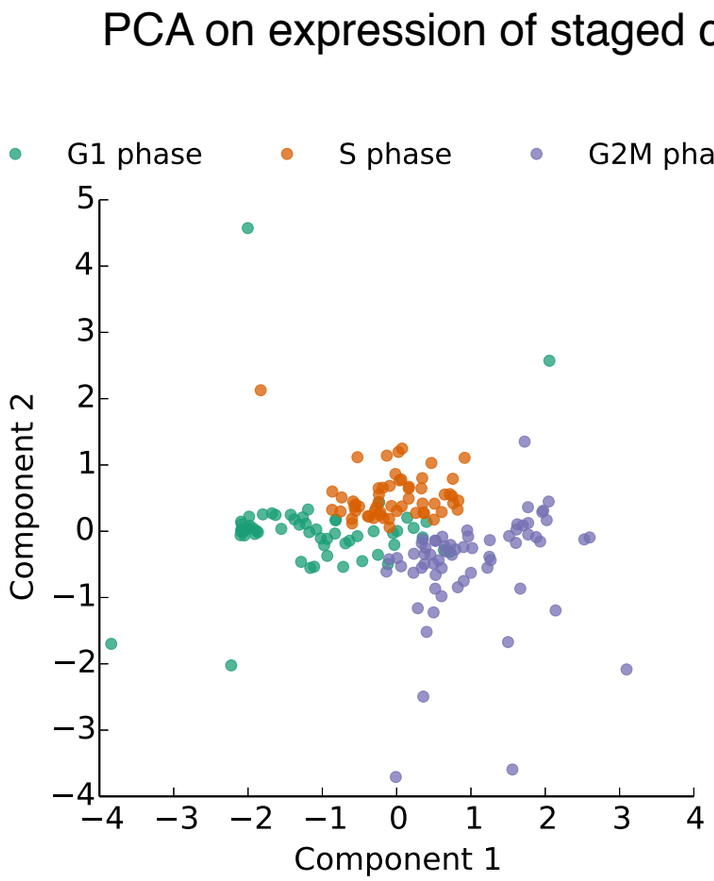
cell cycle

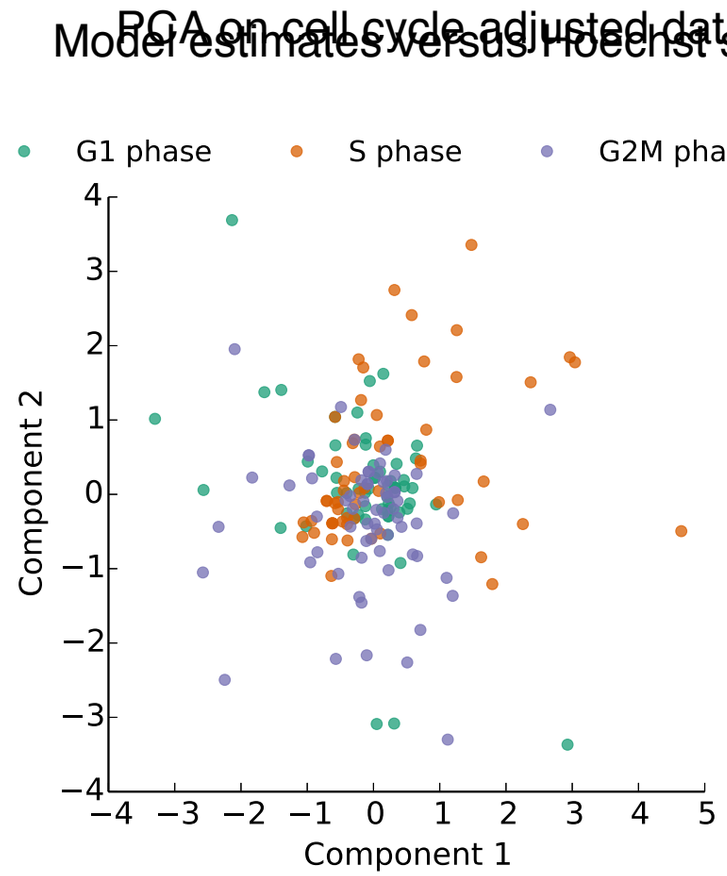
res. biological variability

technical noise

Model validation on mouse ESCs

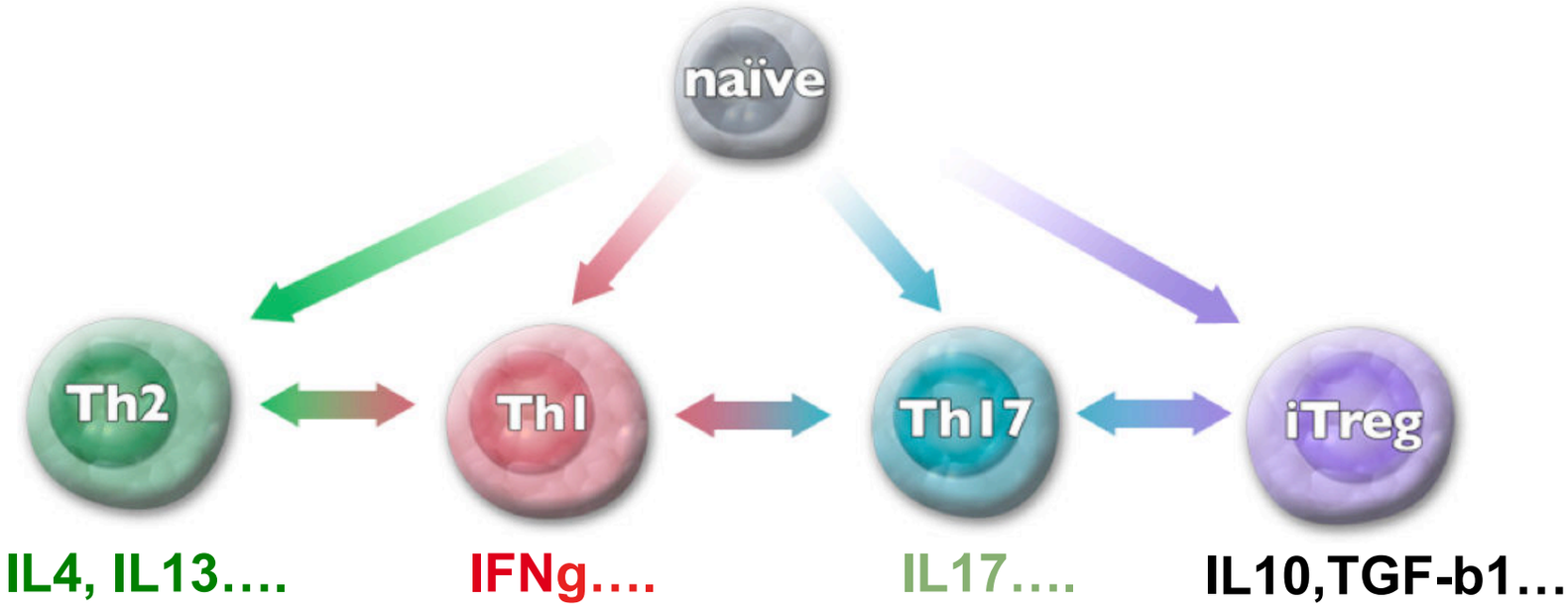
- To test our model, we used single-cell RNA-Seq data generated from ~300 ES cells collected at different stages of the cell cycle





- scLVM accurately estimates variability due to the cell cycle.
- Cell cycle effects are not visible on the model residuals.

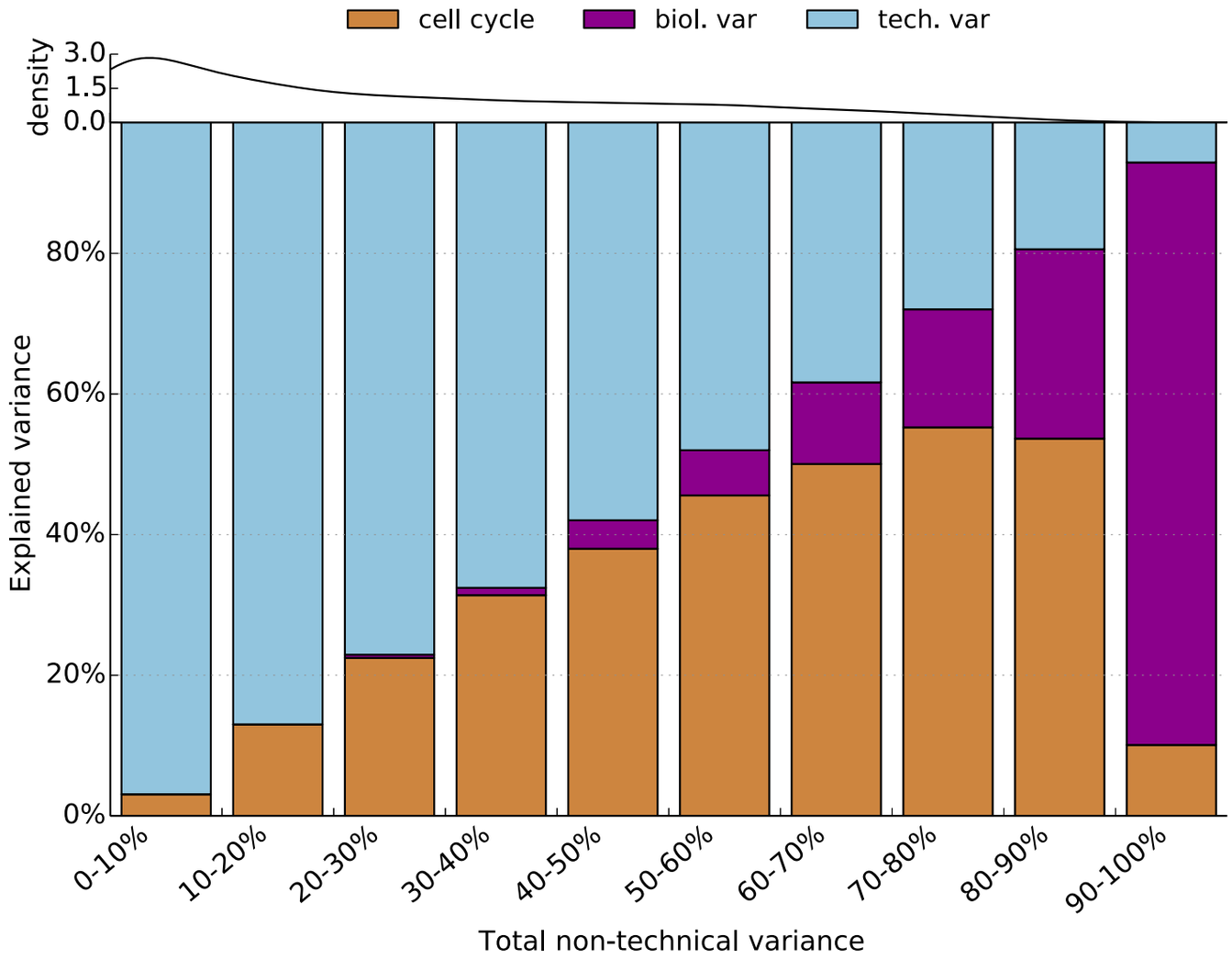
Application to T-cell differentiation



- Focus on cells being differentiated in vitro from the naïve state towards the Th2 cell type
- 96 cells transcription profiled using the Fluidigm C1 system

Dissecting the sources of transcriptional variation

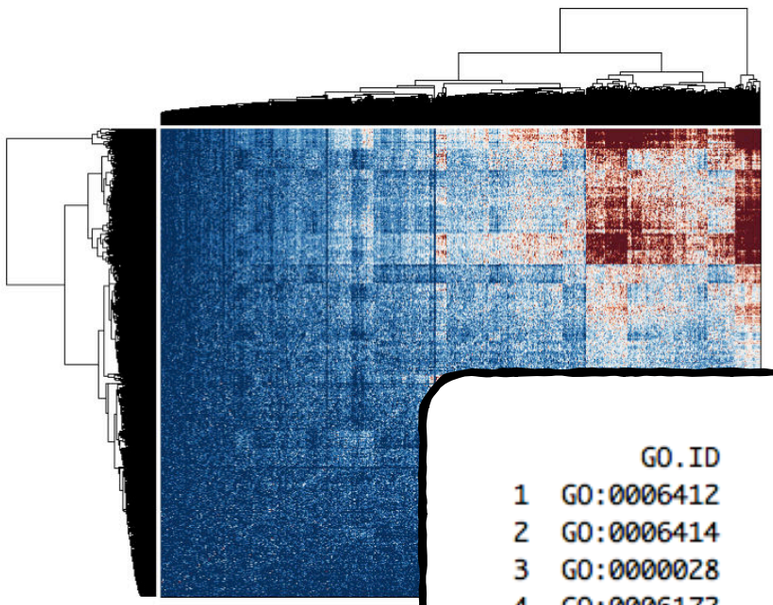
- **Technical noise**
  For 27% of the genes, variation of expression can be entirely explained by the (technical) null variability.
- **Cell-cycle**
  For 42% of the genes, >30% of the observed variance is explained by the cell cycle state.

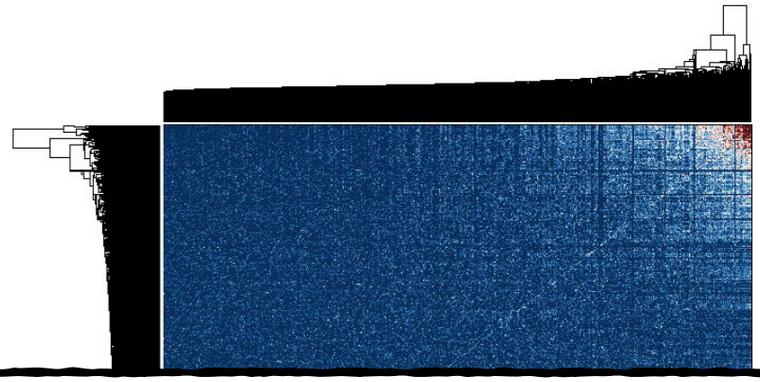


The impact of cell cycle on gene-gene correlations

Gene-gene correlations (unadjusted)

Gene-gene correlations (adjusted for cell cycle)

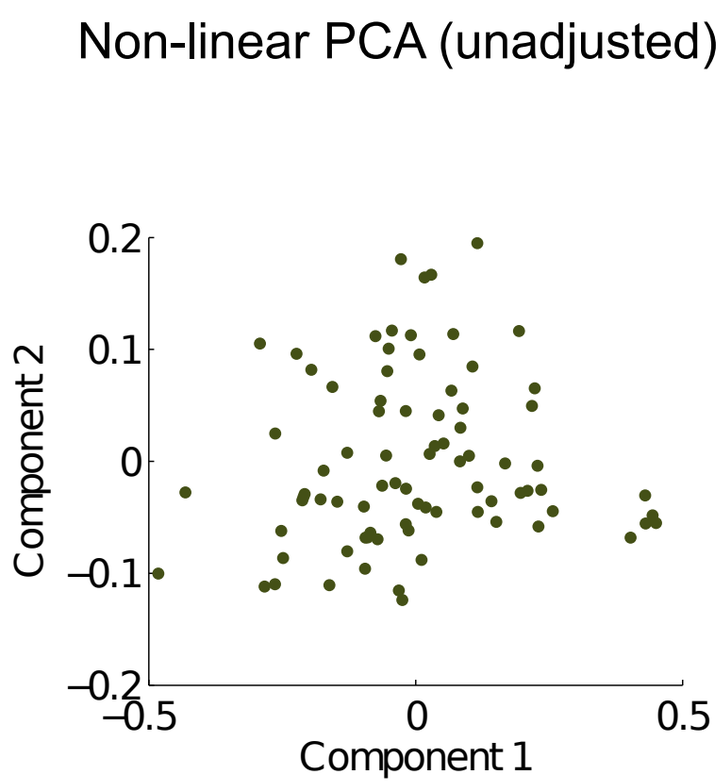


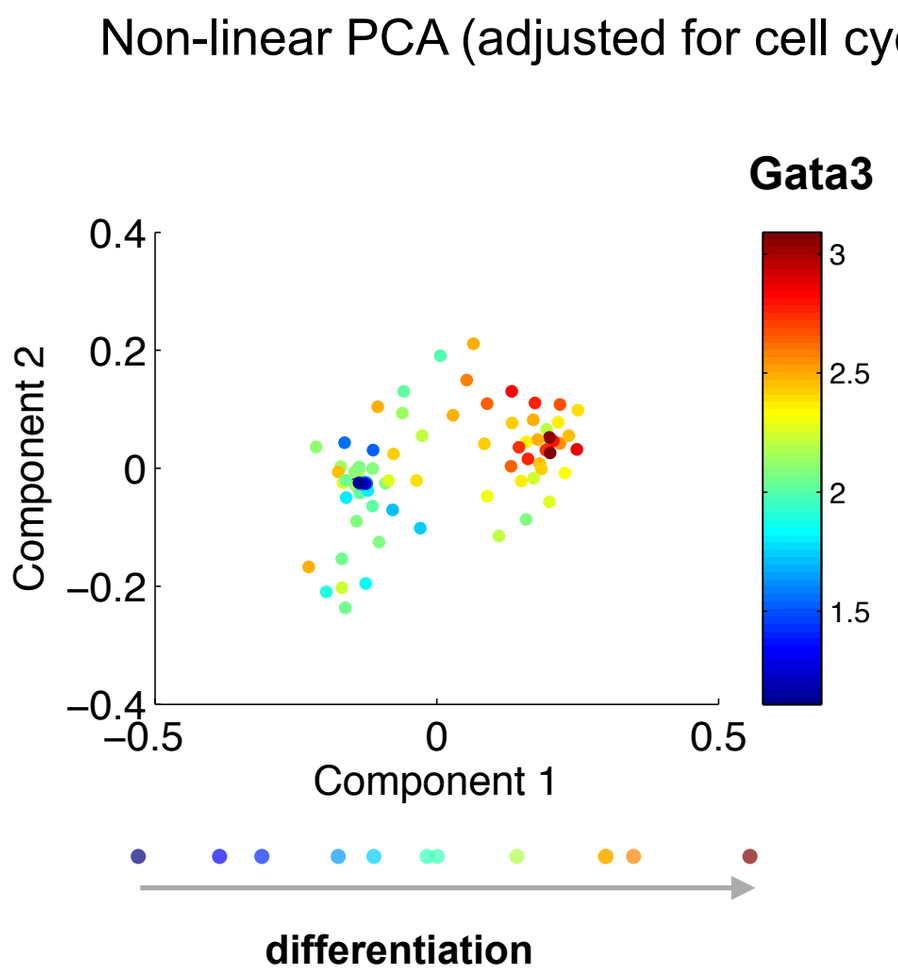


> 500,

	GO.ID	Term	Annotated	Significant	Expected	result1
1	GO:0006412	translation	416	55	6.49	8.0e-17
2	GO:0006414	translational elongation	45	13	0.70	1.2e-13
3	GO:0000028	ribosomal small subunit assembly	10	6	0.16	2.8e-09
4	GO:0006172	ADP biosynthetic process	8	5	0.12	4.8e-08
5	GO:0015986	ATP synthesis coupled proton transport	17	6	0.27	1.5e-07
6	GO:0006096	glycolysis	59	8	0.92	3.6e-06
7	GO:0006413	translational initiation	92	12	1.44	9.5e-06
8	GO:0001916	positive regulation of T cell mediated c...	21	5	0.33	1.5e-05
9	GO:0071353	cellular response to interleukin-4	22	5	0.34	1.9e-05
10	GO:0008284	positive regulation of cell proliferatio...	642	28	10.02	2.6e-05
11	GO:0000462	maturation of SSU-rRNA from tricistronic...	5	3	0.08	3.7e-05
12	GO:0015991	ATP hydrolysis coupled proton transport	25	5	0.39	3.7e-05
13	GO:0006662	glycerol ether metabolic process	13	4	0.20	3.7e-05
14	GO:0002474	antigen processing and presentation of p...	19	6	0.30	5.0e-05
15	GO:0042273	ribosomal large subunit biogenesis	14	4	0.22	5.2e-05

Discrimination between differentiated and undifferentiated cells

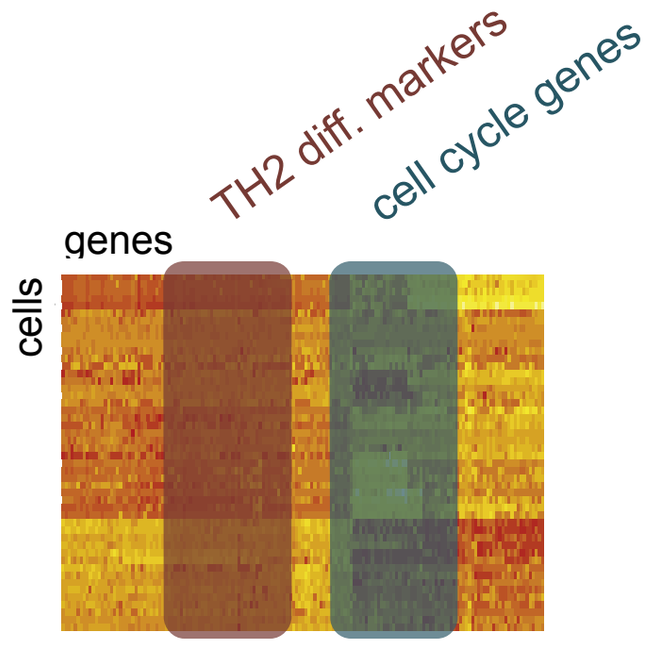




- After correction for cell heterogeneity, cells appear to separate better into two groups than without correction.

Can we better tease apart the effect of cell cycle and differentiation ?

- scLVM also enables learning multiple latent factors
  - Genes annotated for cell cycle
  - Th2 differentiation marker genes



- Extended variance component analysis

$$\mathbf{Y}_g = \mu\mathbf{I} + \alpha\mathbf{u}_{\mathrm{cc}} + \beta\mathbf{u}_{\mathrm{th2}} + \delta_b\mathbf{u}_{\mathrm{b}} + \quad \mathbf{u}_{\mathrm{n}}$$

$N(0,$) $N(0,$) $N(0,$) $N(0,$)

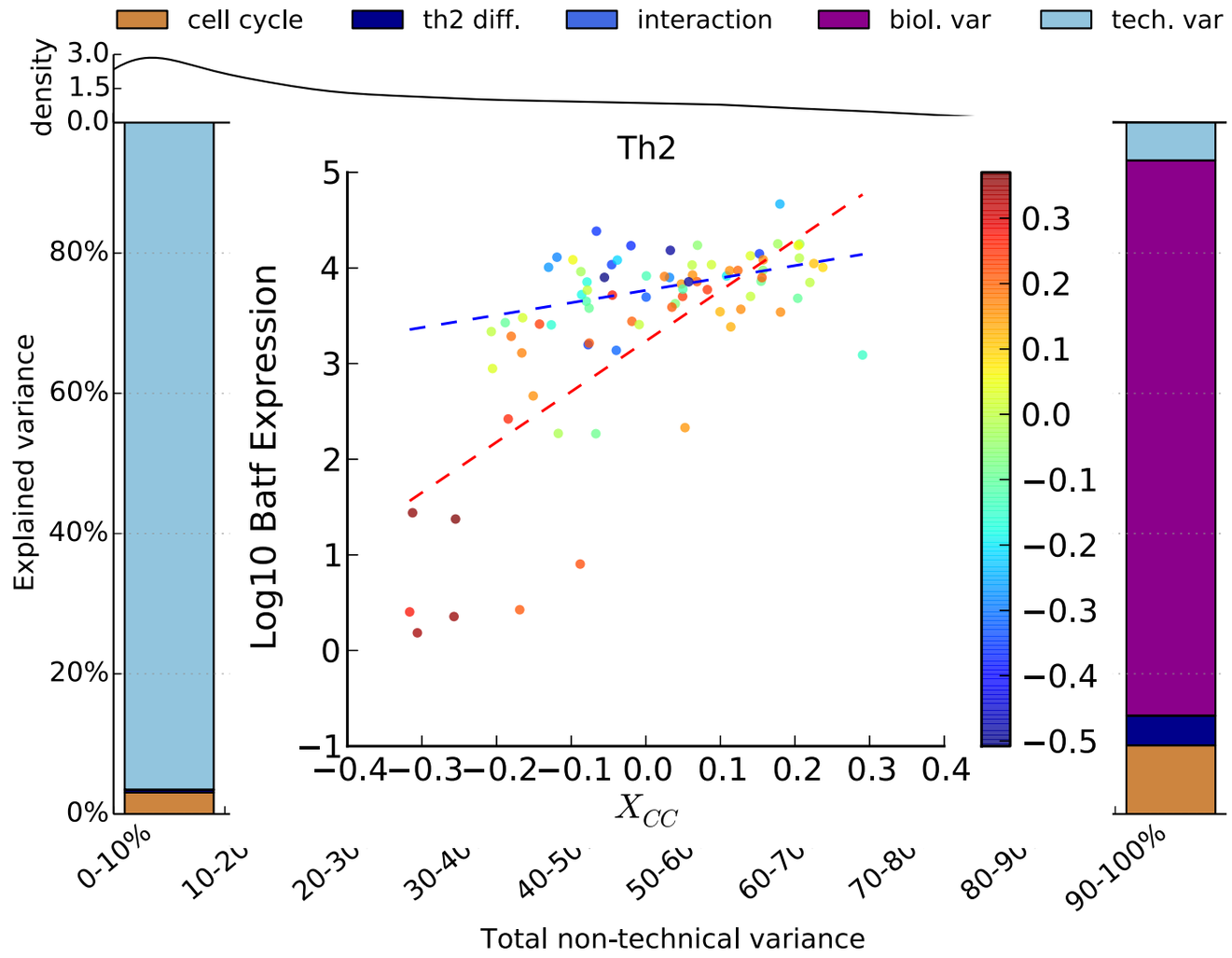
cell cycle
th2 differentiation
res. biological variability
technical noise

Can we better tease apart the effect of cell cycle and differentiation ?

- **Th2 differentiation**
  928 genes with affected by the Th2 differentiation factor

- **Th2/cell-cycle interaction**
  200 genes with interaction effects

- Enriched for
  positive cell proliferation
  negative regulation of apoptosis



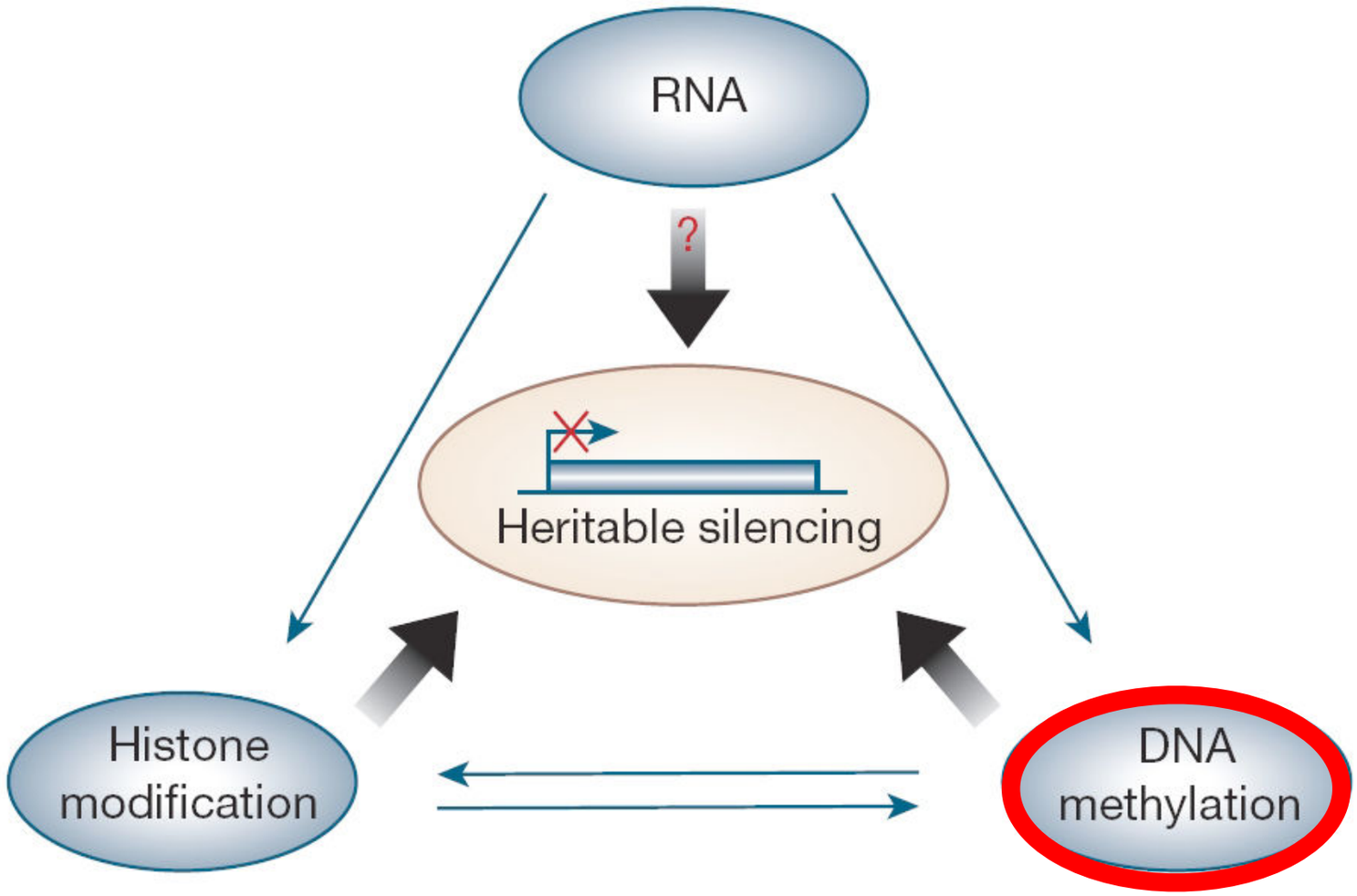
The origin of transcriptome diversity?

Christof Angermueller

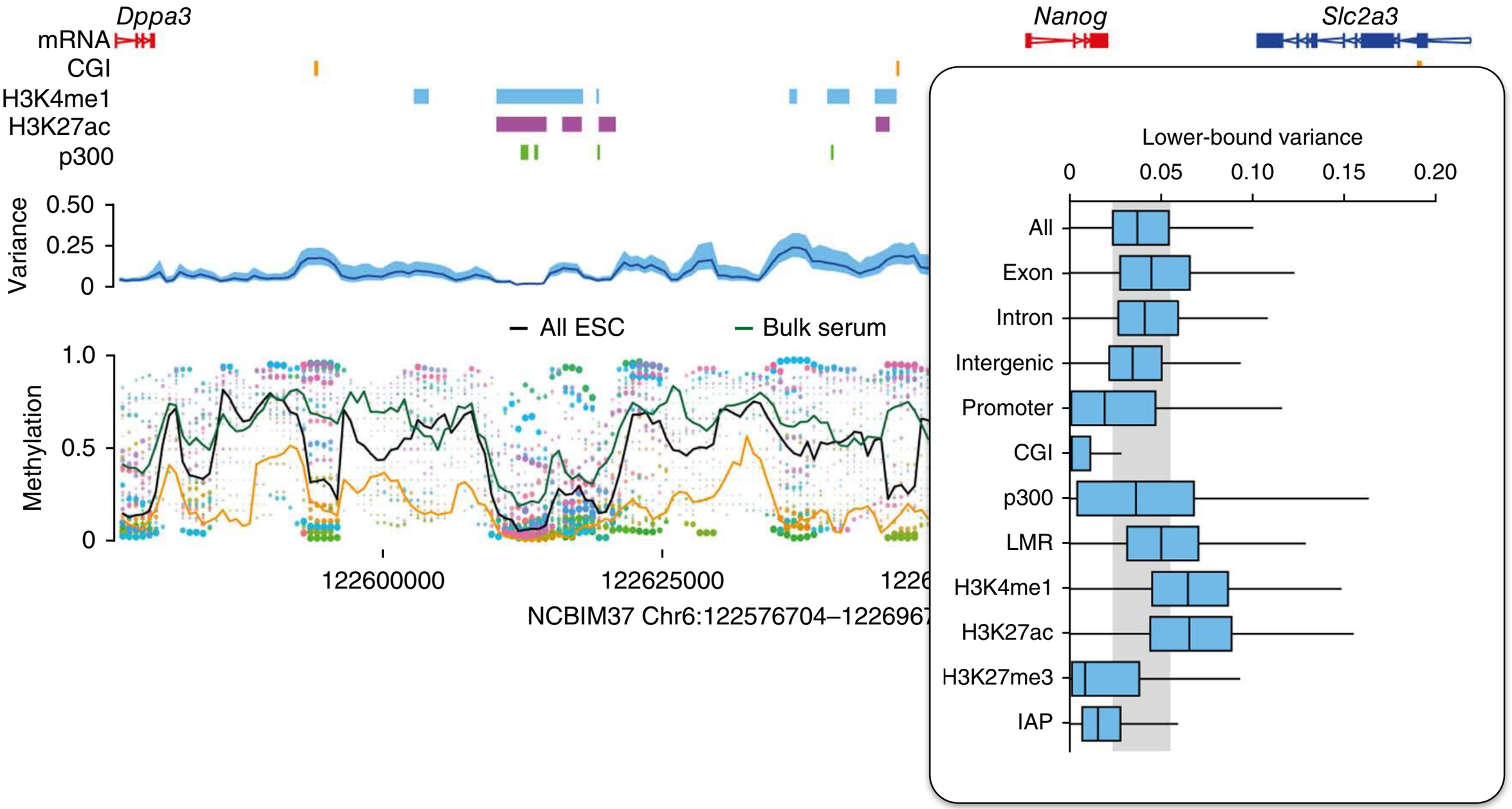
Heather Lee

Wolf Reik

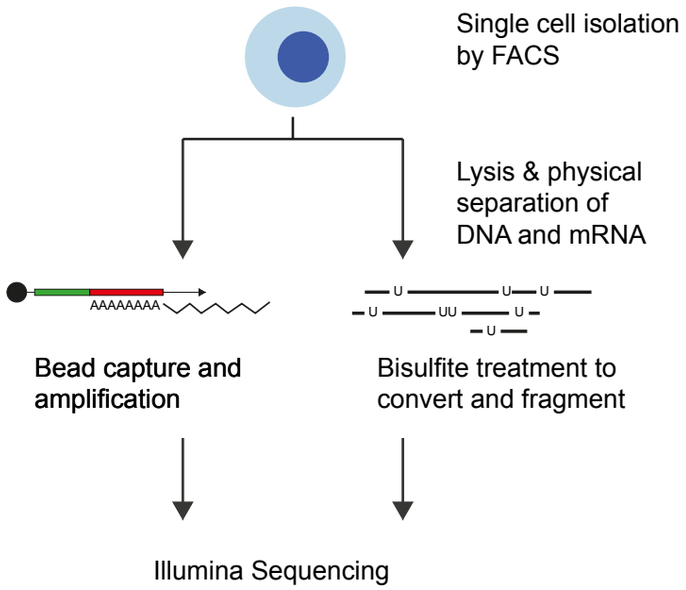
Thierry Voet



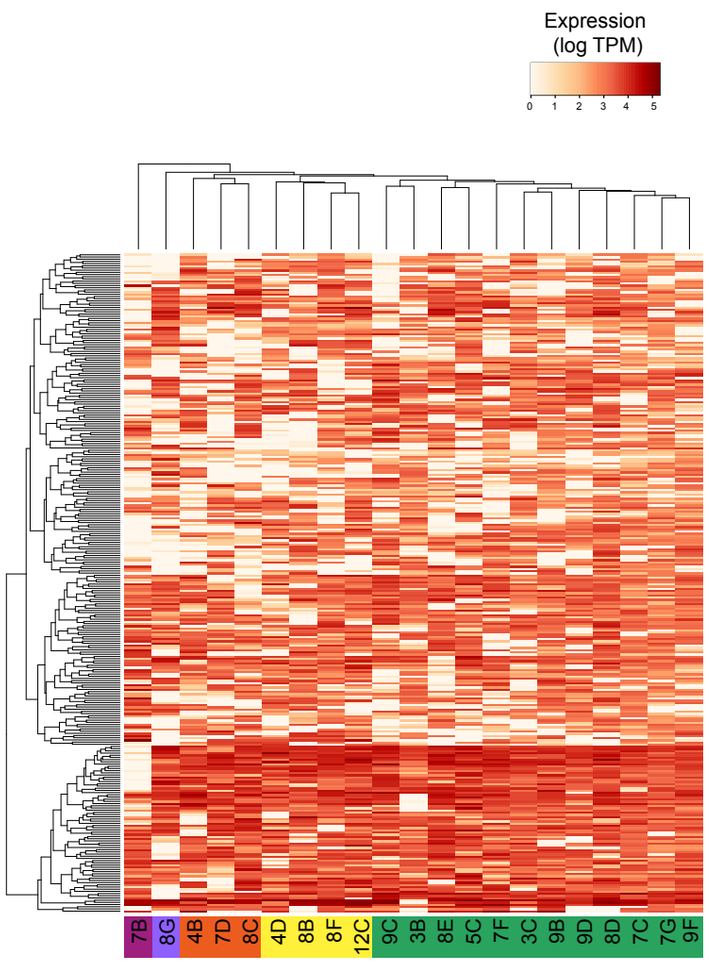
Single-cell bisulfite sequencing (20 ESC cells)



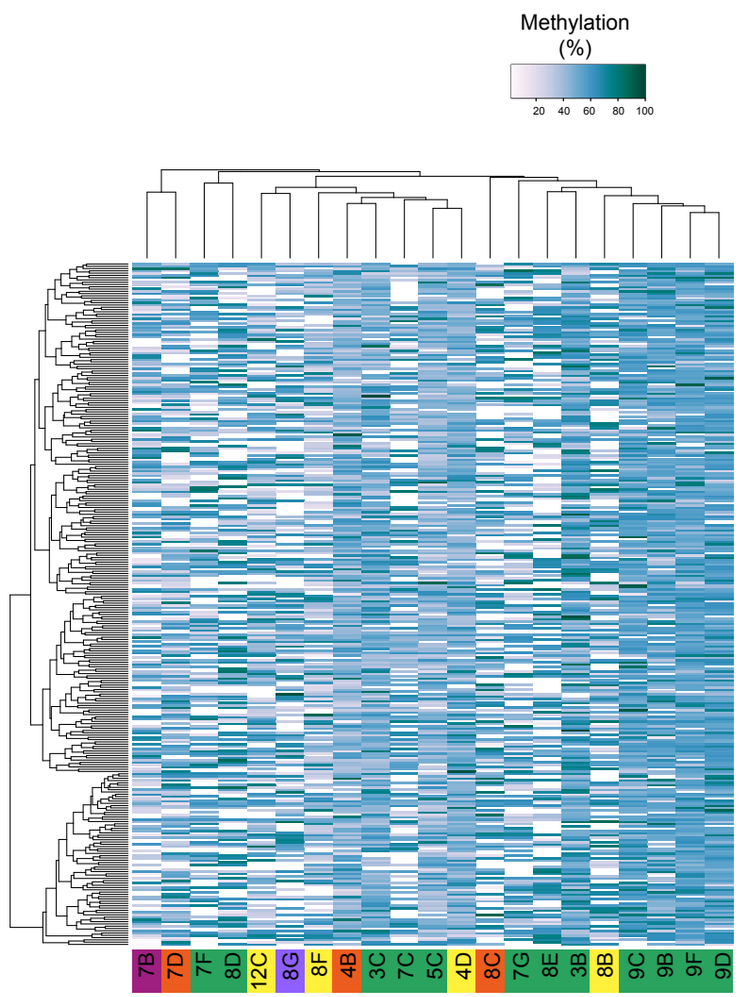
Parallel bs-seq & RNA-seq profiling in 21 serum ES cells



Gene expression

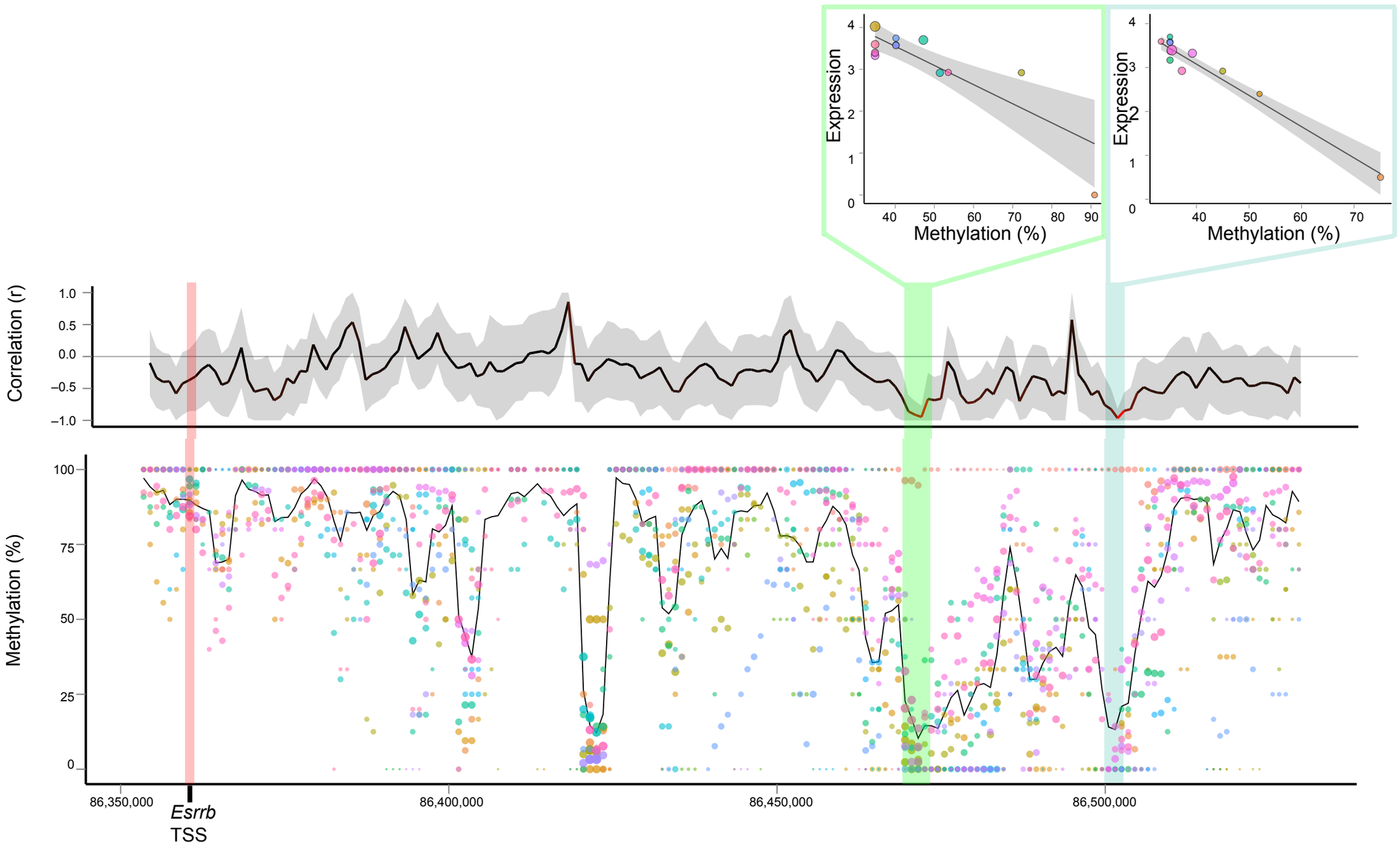


DNA methyatlion



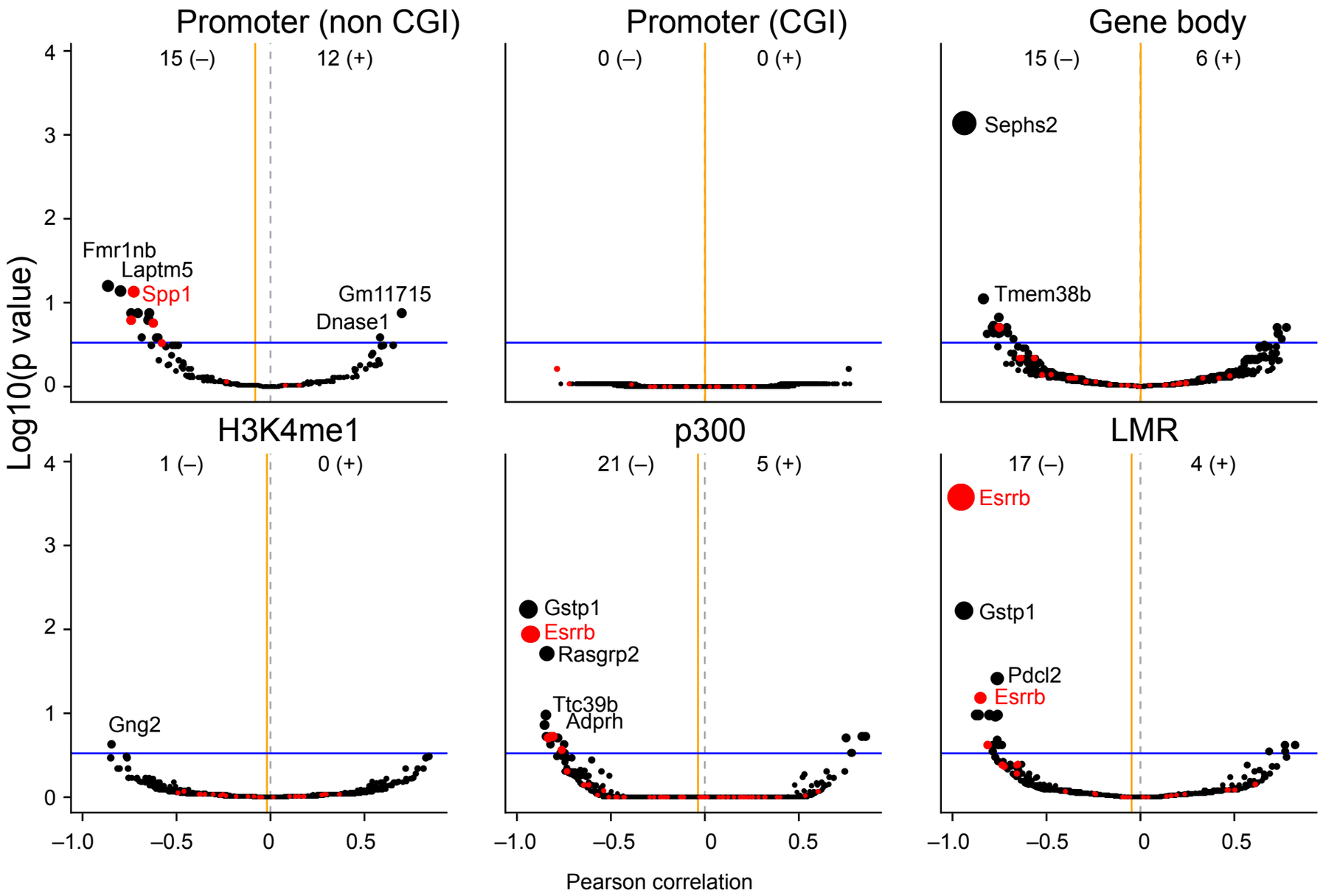
EMBL

Parallel bs-seq & RNA-seq profiling in 21 serum ES cells



EMBL

Parallel bs-seq & RNA-seq profiling in 21 serum ES cells



Conclusions

- Latent variable models can effectively account for gene expression heterogeneity & confounding
- (e)QTL analysis
  - population structure & env. /technical confounding to improve power
- Single-cell RNA-seq analysis
  - a small number of genes with known cell cycle annotation is sufficient to estimate a cell covariance due to cell cycle
  - more compact gene-gene correlations
  - detection of genes with interactions involving multiple biological processes
  - Parallel bs-seq/RNA-seq profiling in the same cells reveals associations between methylation and transcriptome variation

Acknowledgments

group
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Iain Macaulay

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Heather Lee
Stephen Clark

Helmholtz Munich
Fabian Theis

Microsoft Research
Nicolo Fusi
Christoph Lippert

University of Sheffield
Neil Lawrence

Mixed model software: https://github.com/PMBio/limix

scLVM: https://github.com/PMBio/scLVM

Postdoc opportunities:
Single-cell genomics
Statistical genetics