Identifying drug-targetable key drivers of disease

Expression data

Public data

Phenotypes

UMCG

Genetics Department

‘To capture something small
you need something big’

CERN

© Ruben van Leer

DNA

AC

GT

‘To capture something small you need something big’

DNA Sequencers

© Sanger Institute

‘To capture something small you needed something big’

DNA Sequencer

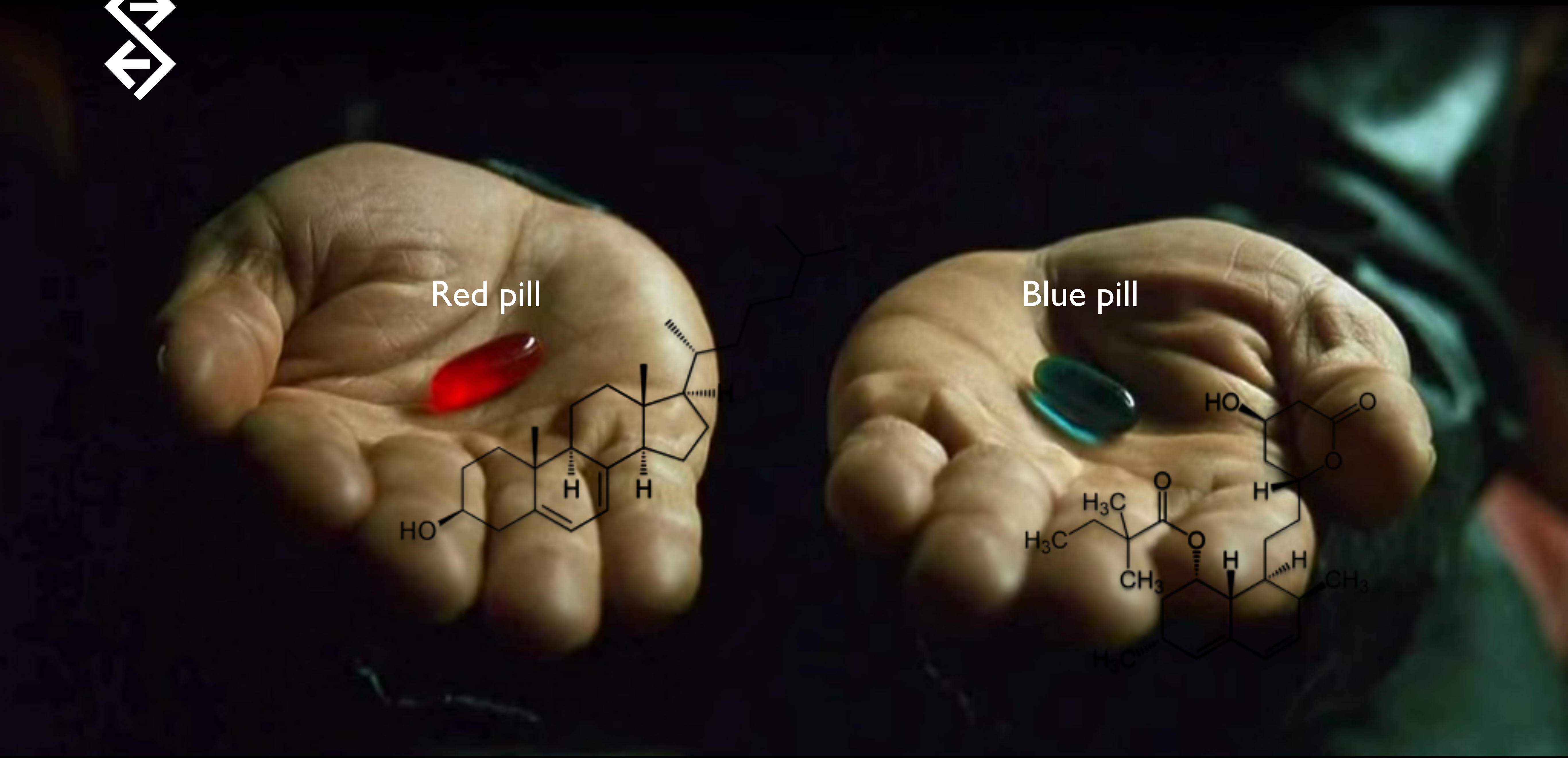
Minion

© Oxford Nanopore

more data now available

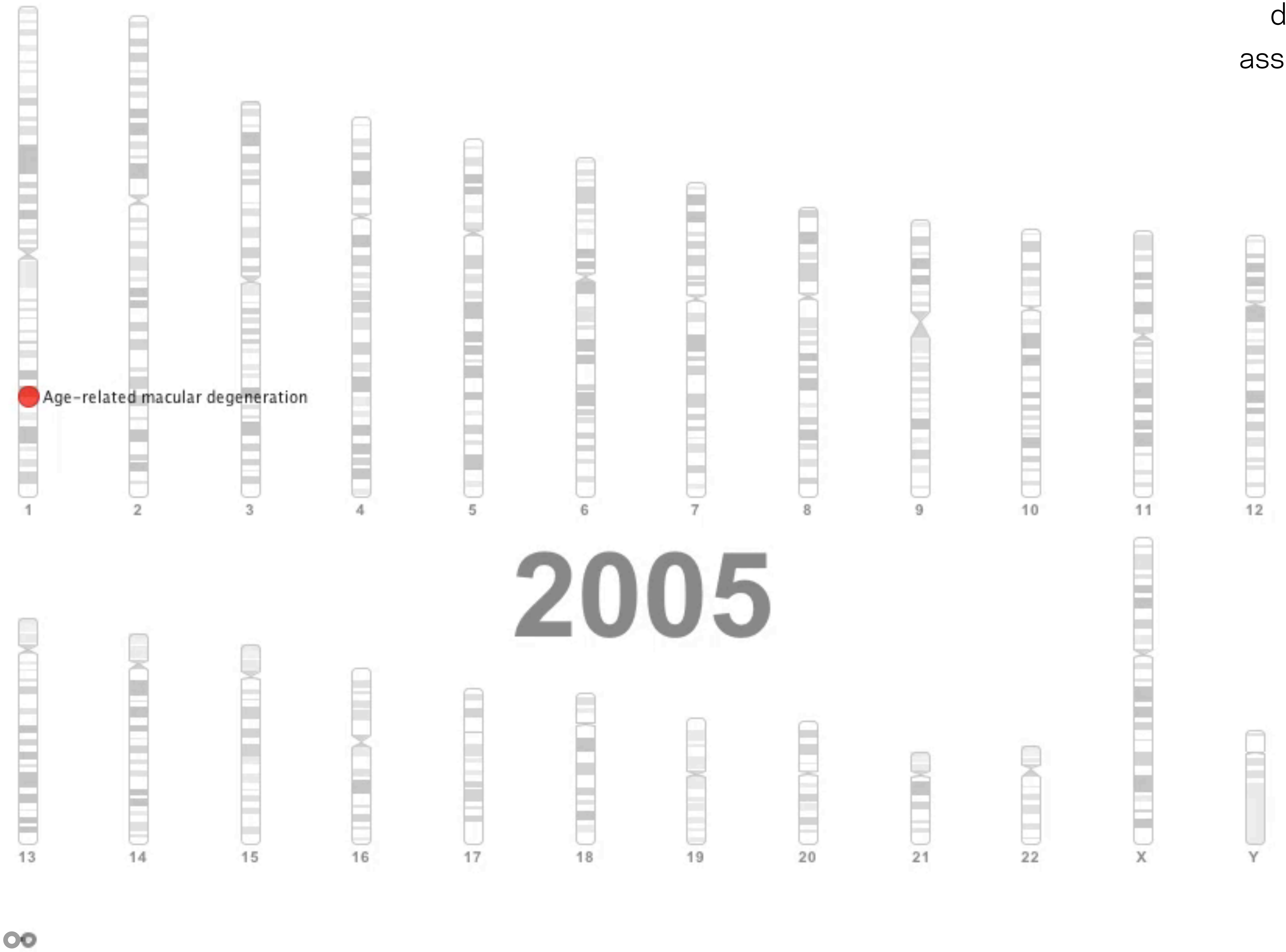
large amounts of data now available

Goal: better diagnose and treat patients



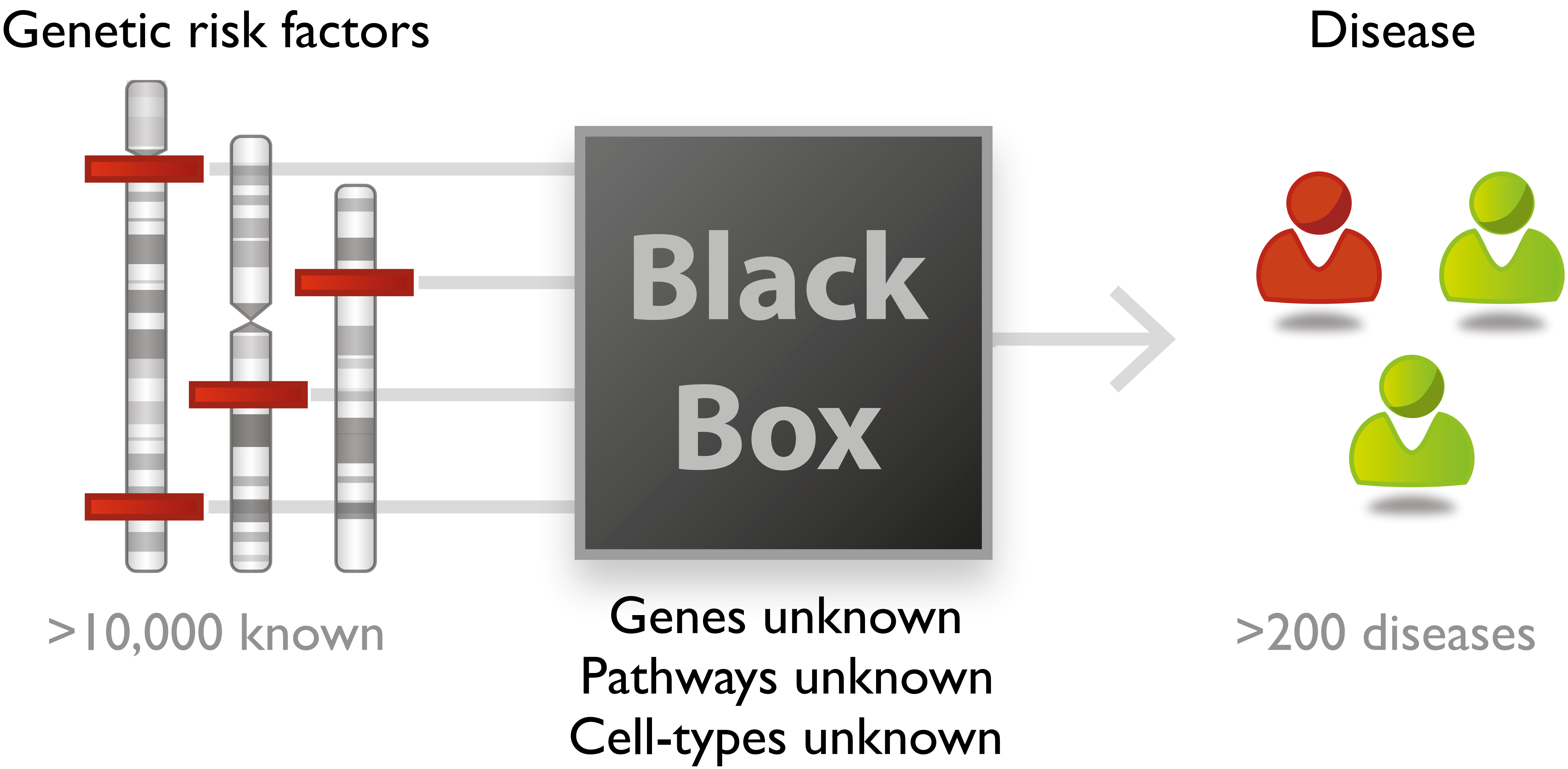
Seven years of GWAS studies

6,054
disease
associations



Teri Manolio *et al*: A catalog of published genome-wide association studies

Problem of life science community



Genetic risk factors

Disease

Black Box

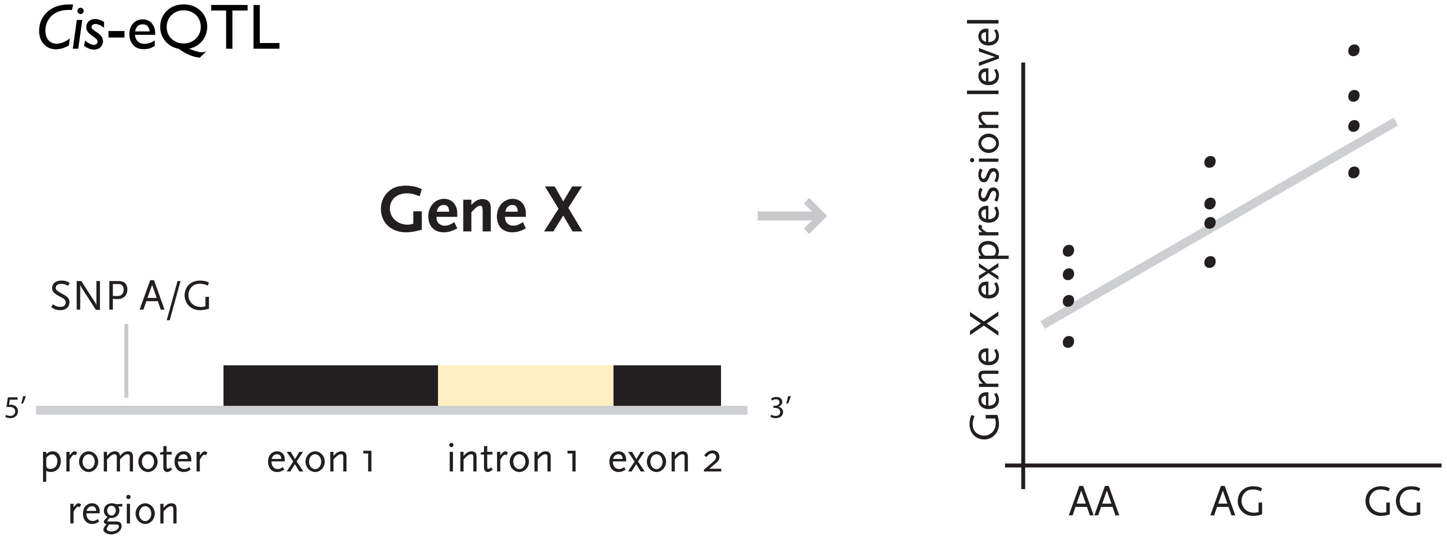
>10,000 known

Genes unknown
Pathways unknown
Cell-types unknown

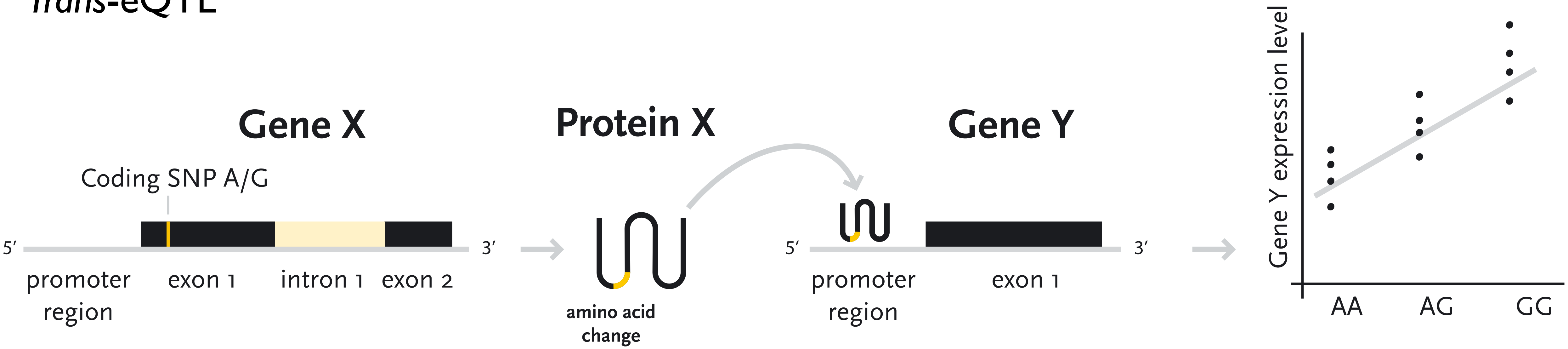
>200 diseases

Expression quantitative trait locus (eQTL)

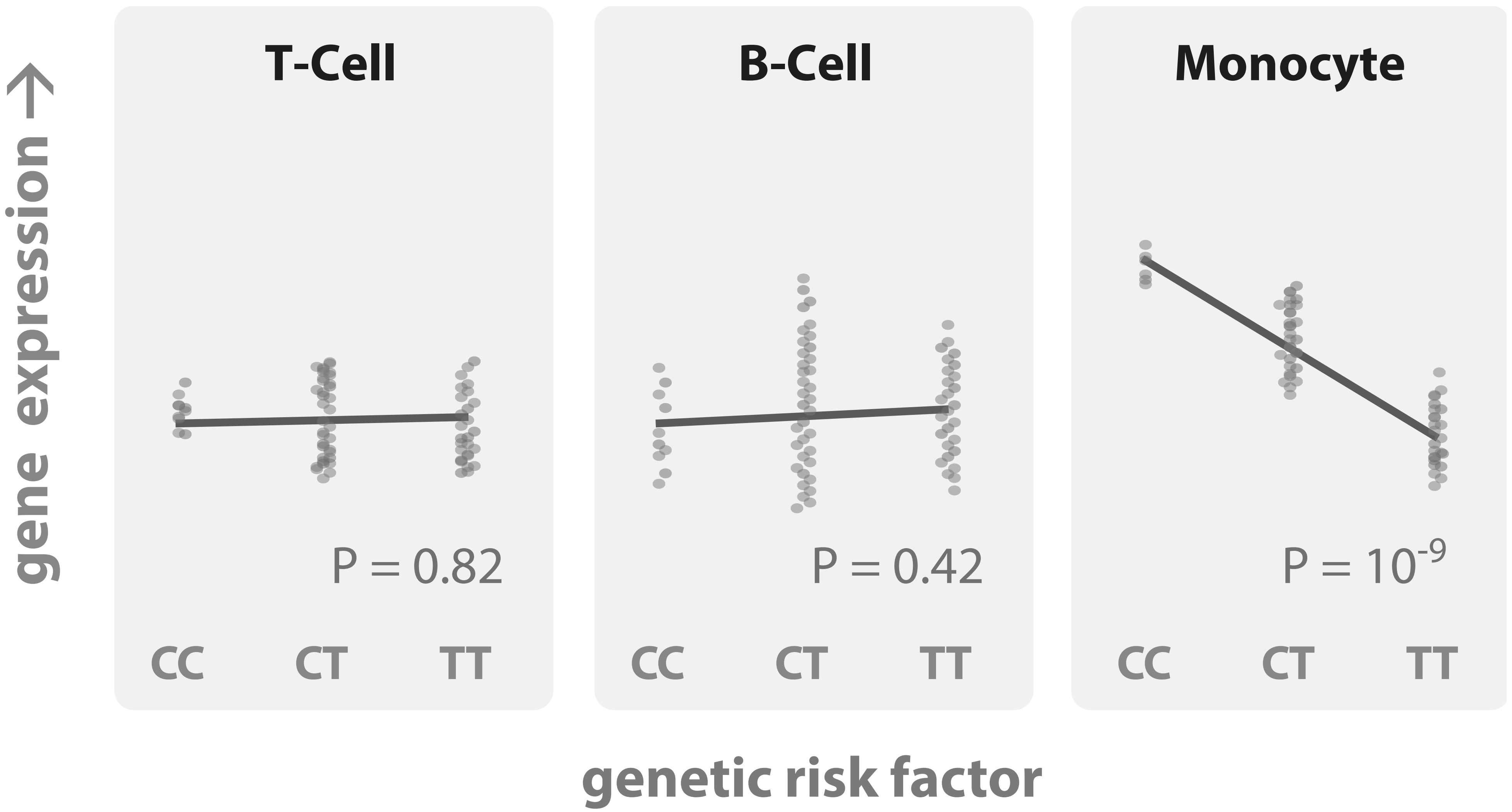
Cis-eQTL



Trans-eQTL



Far majority of genetic risk factors affect gene expression



Dubois *et al*, Nature Genetics 2010
Fehrmann *et al*, PLoS Genetics 2011
Fu *et al*, PLoS Genetics 2012
Westra *et al*, Nature Genetics 2013

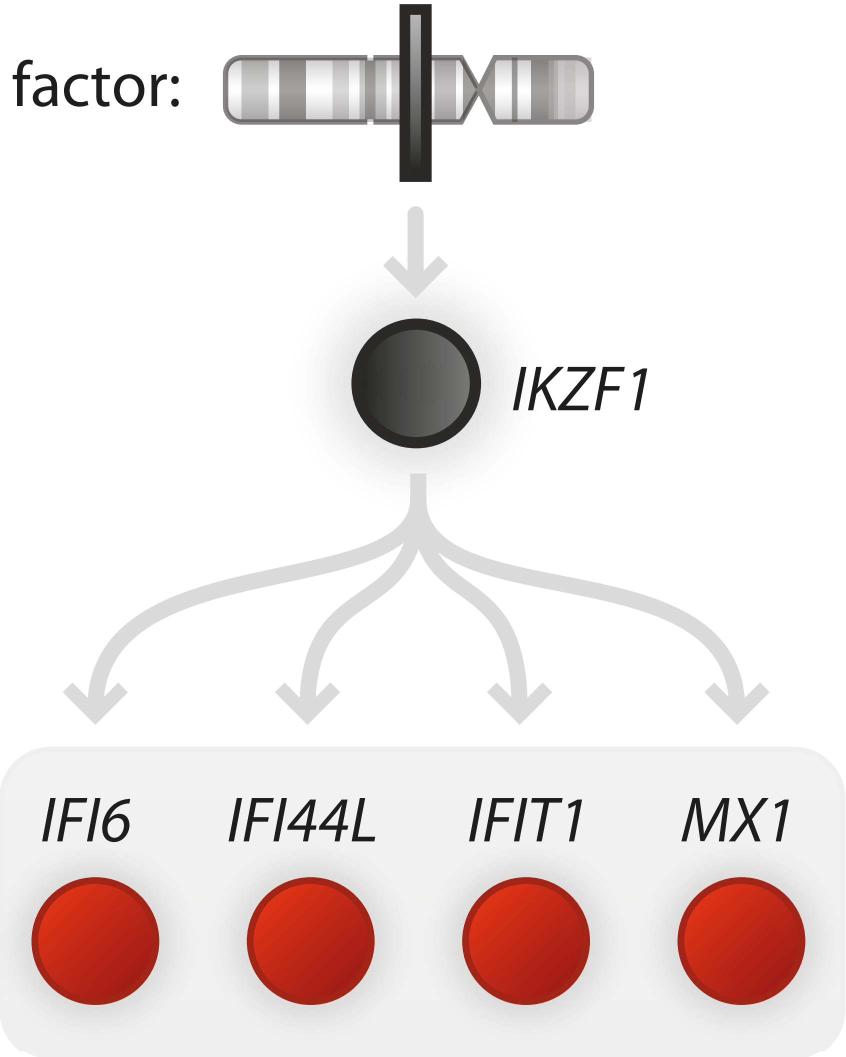
Get larger sample-sizes: meta-analysis in 5,311 samples

Systemic lupus erythematosis risk factor:

Local expression effect:

Type 1 interferon response:

(in Monocytes)



Chr. 7

Chr. 7

Downstream *trans*-eQTL effects

Downstream effects identified for >200 genetic risk factors

New meta-analysis ongoing in 25,000 blood samples

Westra *et al*, Nature Genetics 2013

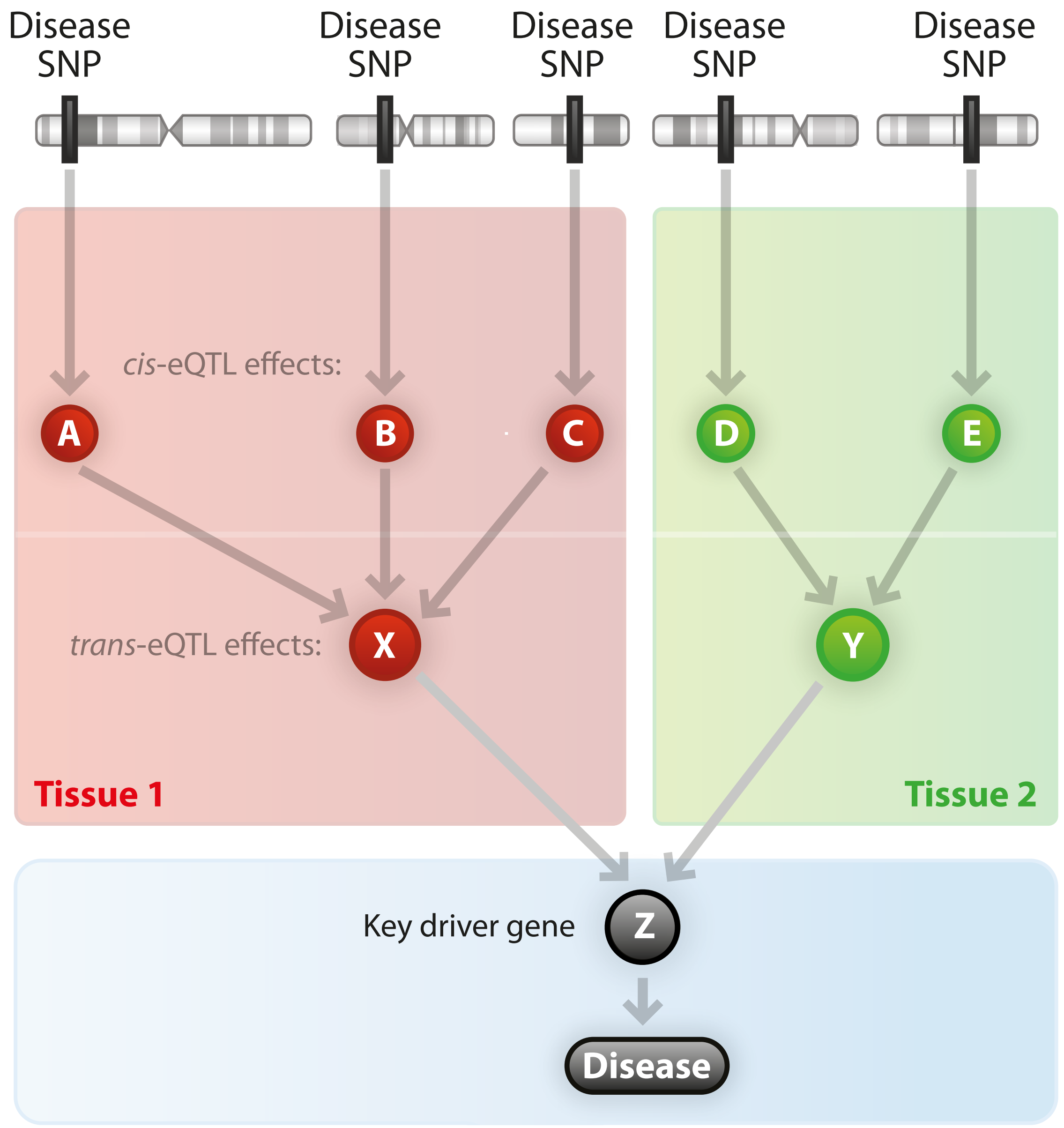
Goal

Genome-wide association studies

cis-eQTL mapping

trans-eQTL mapping

Key driver gene identification

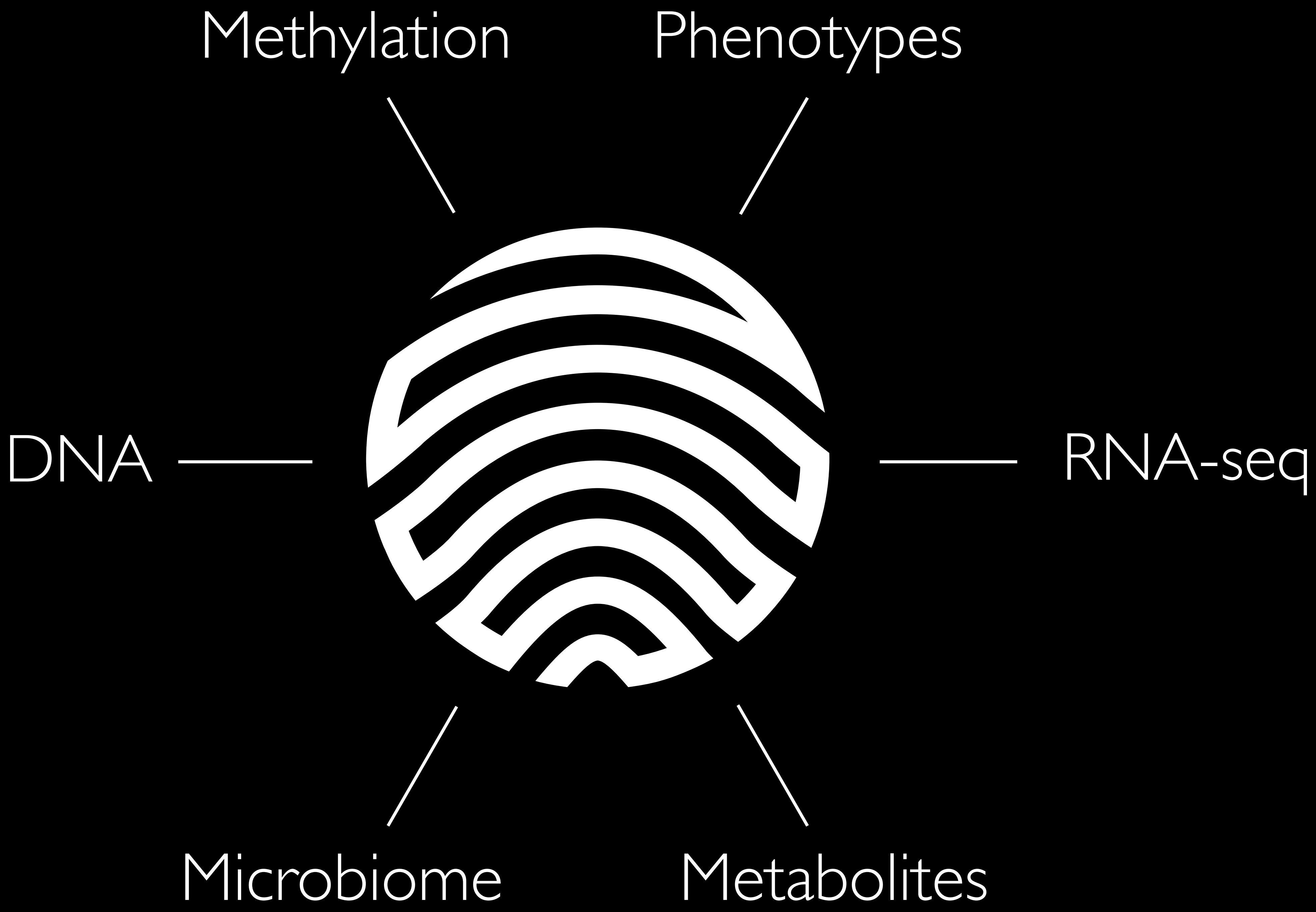


Possible to identify all these downstream effects?

~~Im~~possible

This is not going to be possible!

- Massive sample-sizes required
- Many cell-types required
- Genotype and gene expression data required from the same samples



Methylation

Phenotypes

DNA

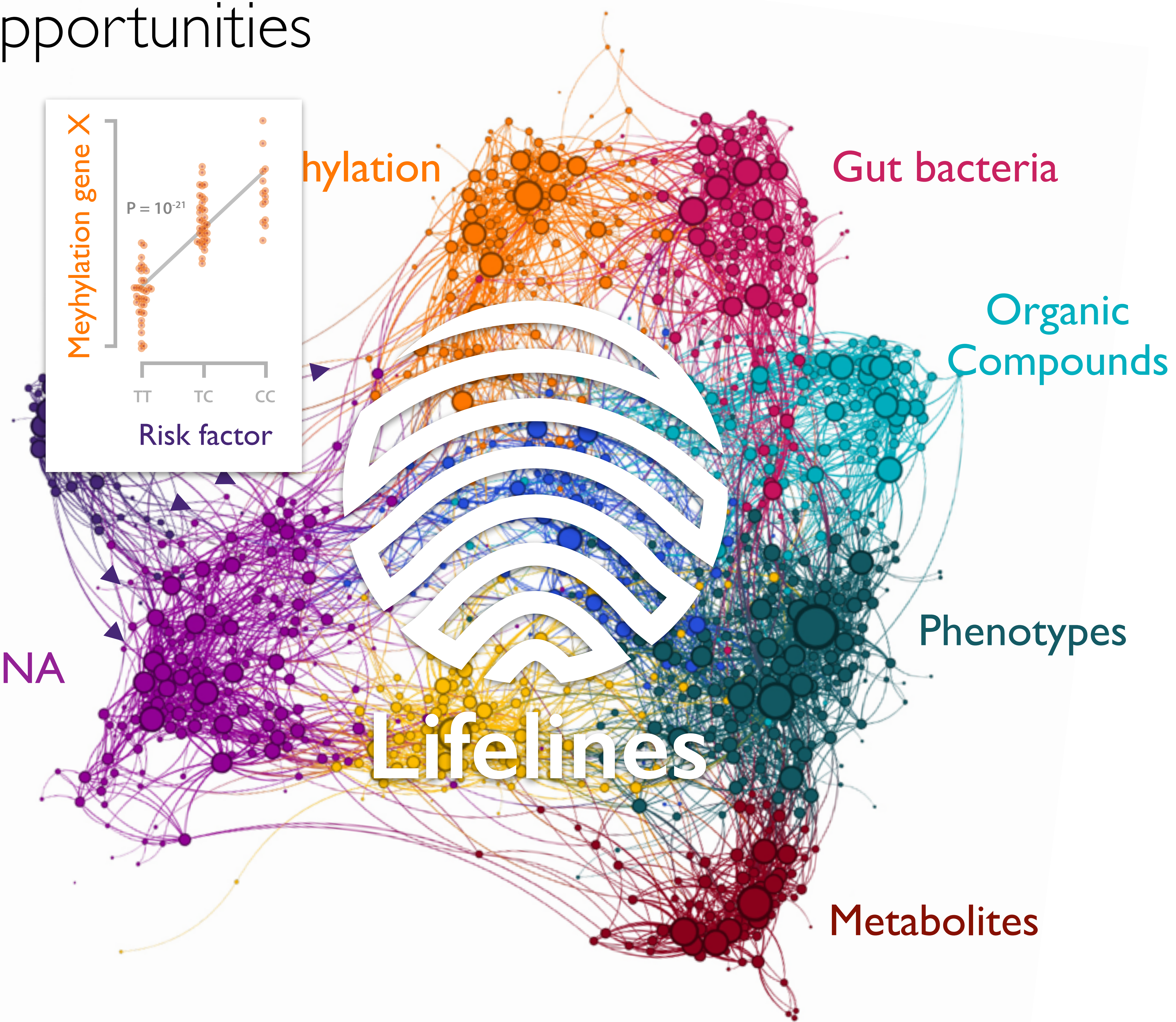
RNA-seq

Microbiome

Metabolites

Lifelines Deep (1500 samples)

The opportunities



Trans-meQTL meta-analysis in 3,840 samples

- 34.4% of 405,709 tested CpG sites are *cis*-meQTL (FDR < 0.05)

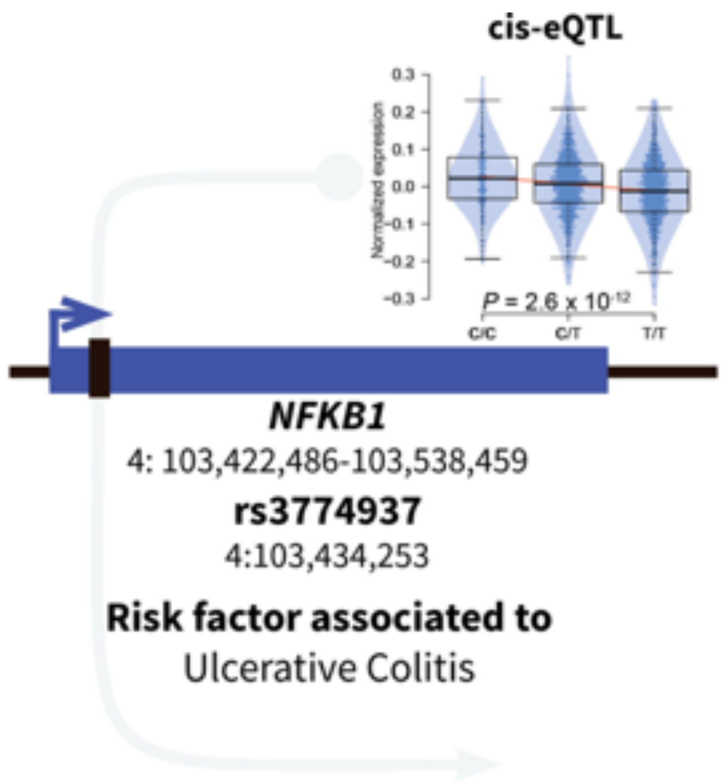
- 31.2% of established GWAS risk factors give *trans*-meQTL effect (FDR < 0.05). 1,907 SNPs affecting 10,141 unique CpG sites in *trans*

- *Trans*-meQTL replicate in monocytes: 95% identical allelic direction

- *Trans*-SNPs affect expression of nearby TFs, subsequent methylation of downstream targets of these TF

Bonder *et al*, BiorXiv preprint

Trans-meQTL meta-analysis in 3,840 samples



NFKB1

4: 103,422,486-103,538,459

rs3774937

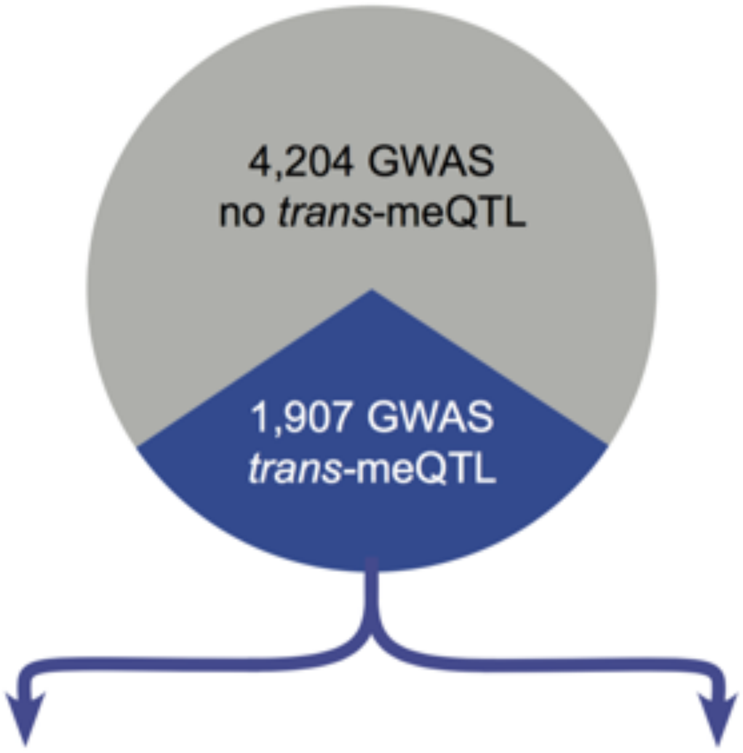
4:103,434,253

Risk factor associated to

Ulcerative Colitis

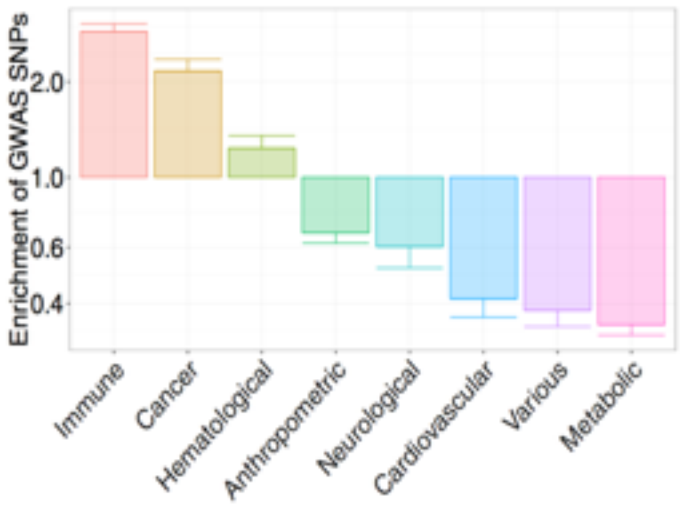
Bonder *et al*, BiorXiv preprint

Trans-meQTL meta-analysis in 3,840 samples



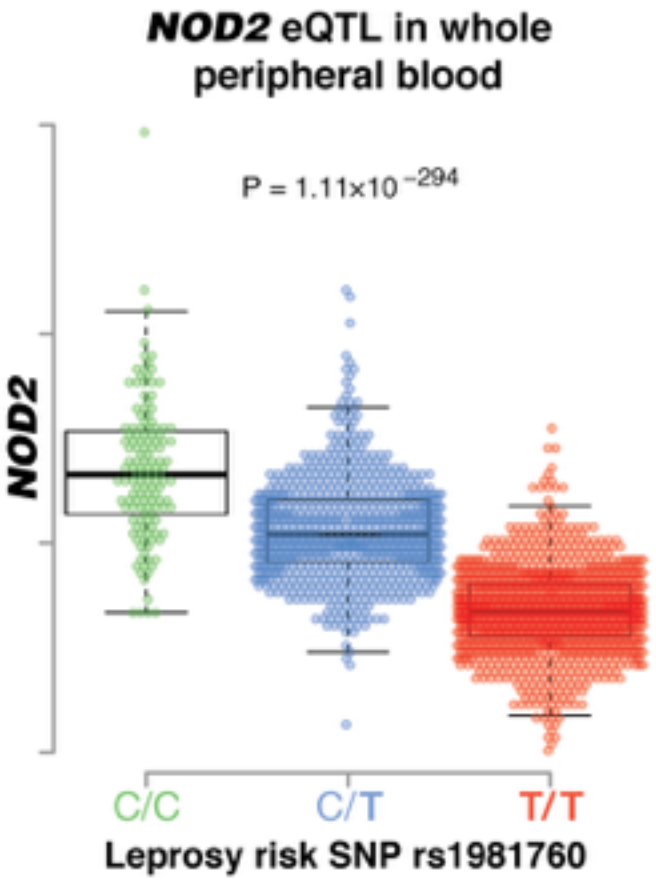
4,204 GWAS
no *trans*-meQTL

1,907 GWAS
trans-meQTL

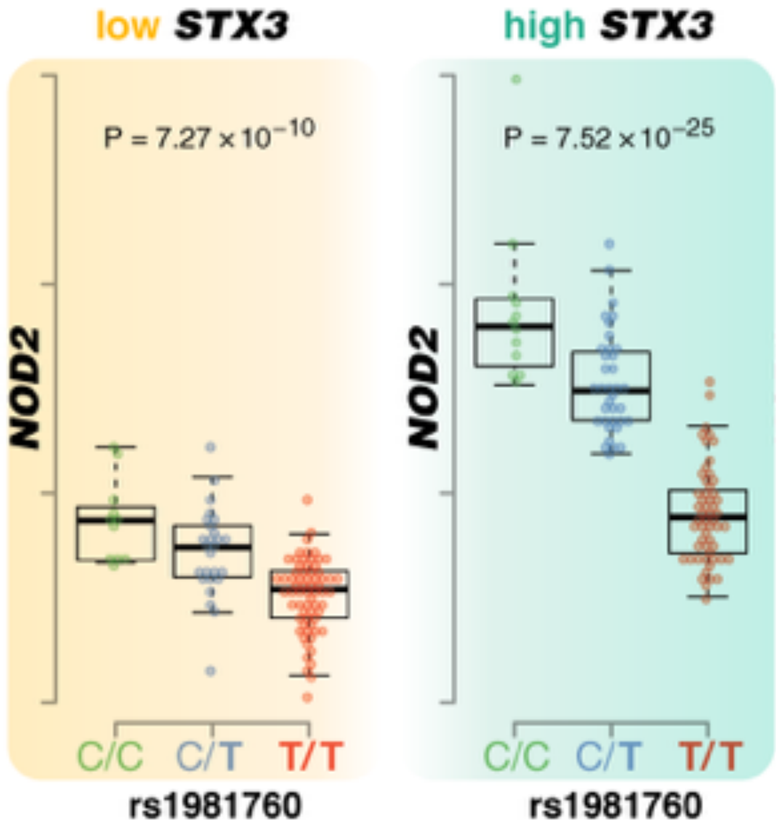


Bonder *et al*, BiorXiv preprint

Detecting cell-type dependent eQTLs in whole blood

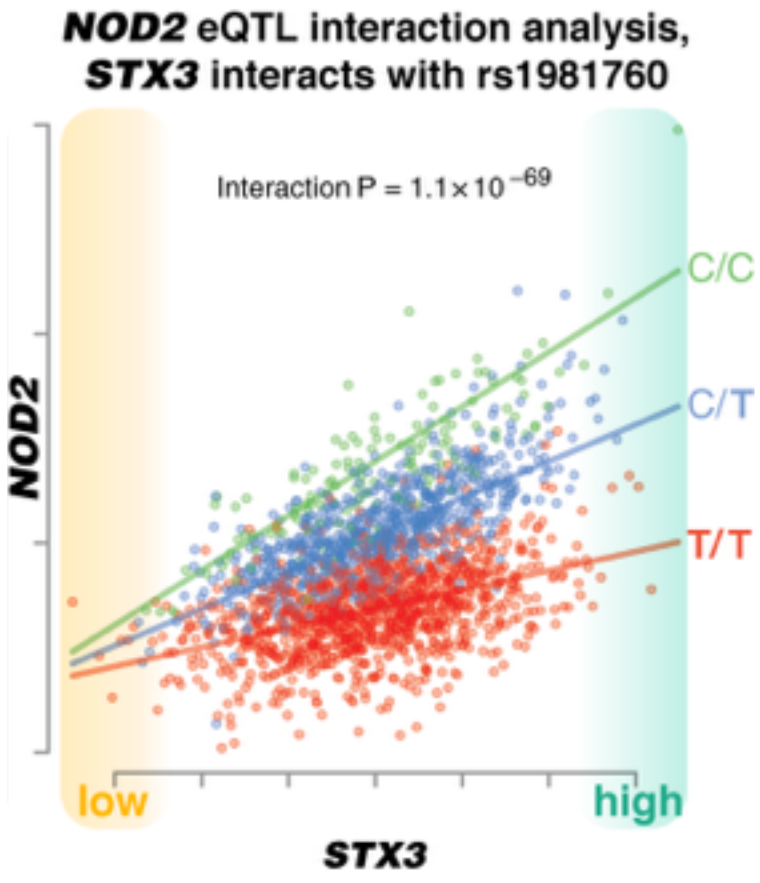


NOD2 **eQTL in whole peripheral blood**

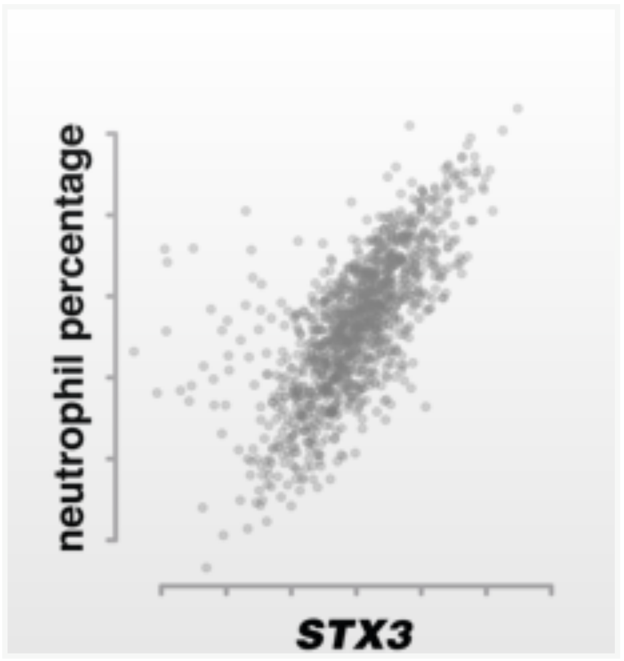


low ***STX3***

high ***STX3***

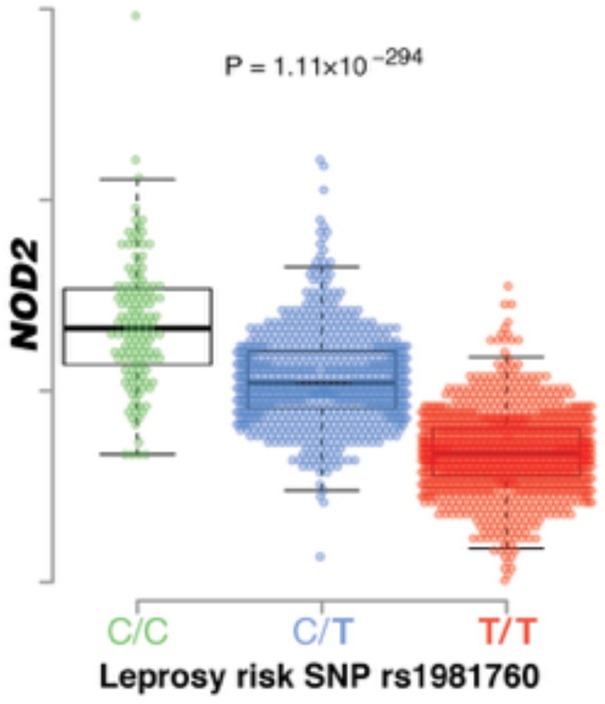


NOD2 **eQTL interaction analysis,** ***STX3*** **interacts with rs1981760**

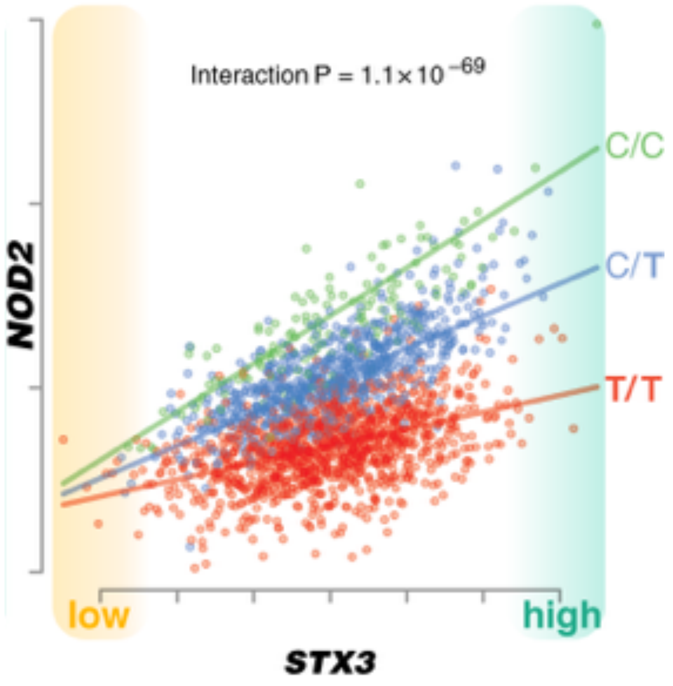


Context specific *cis*-eQTL analysis in 2,116 samples

NOD2 **eQTL in whole peripheral blood**



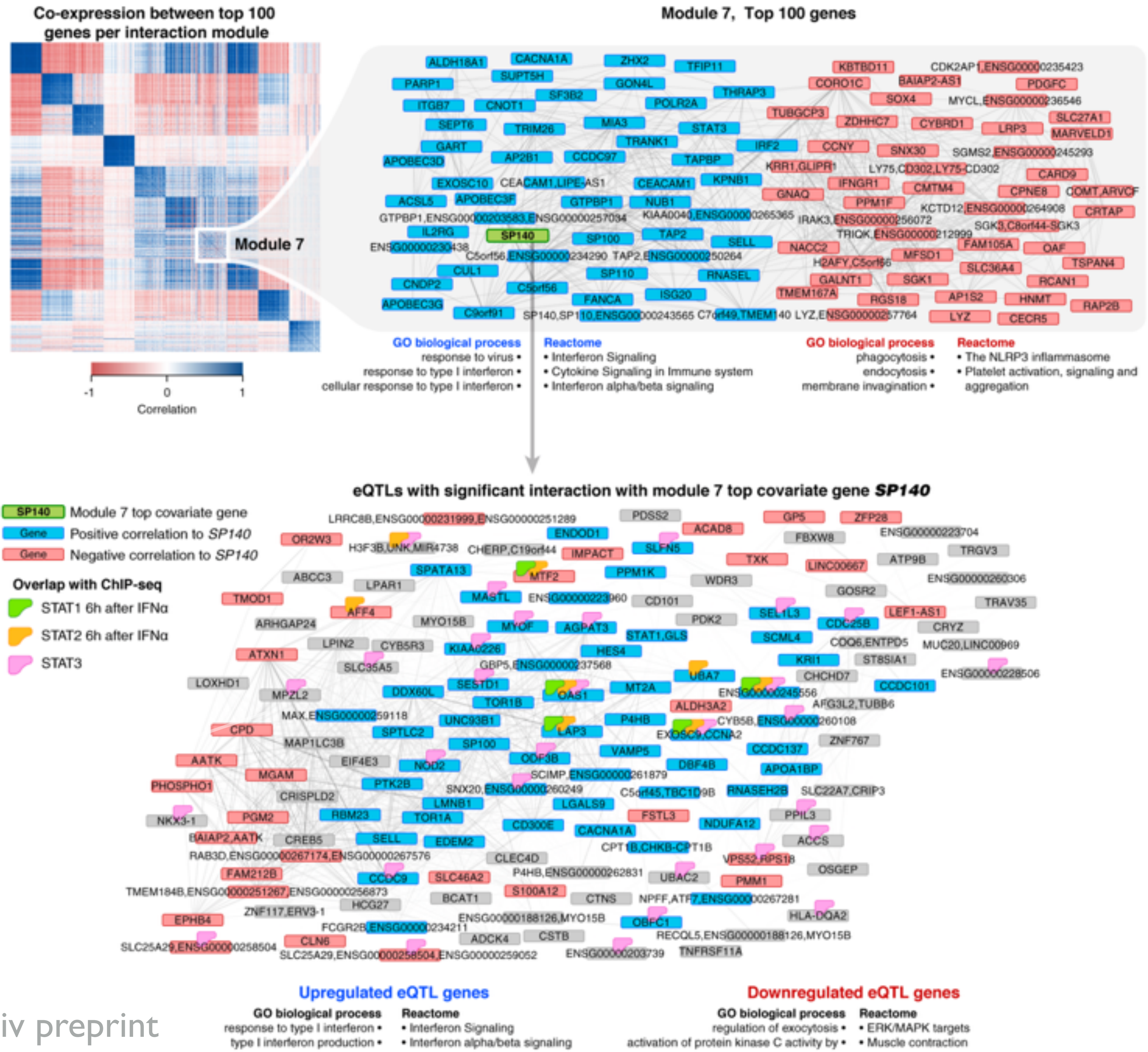
NOD2 **eQTL interaction analysis,**
STX3 **interacts with rs1981760**



Module number	Module description	Number of affected eQTLs	# eQTLs in strong LD with known GWAS hits	GO biological process top enriched pathway
1	Neutrophils 1	917	75	Detection of bacterium
2	CD4+ T-cells	337	25	T cell selection
3	NK cells / CD8+ T-cells	226	19	Cellular defense response
4	Erytrocytes	188	8	Hemoglobin metabolic process
5	Monocytes / Macrophages	181	11	Defense response to virus
6	Growth factor	156	10	Nerve growth factor receptor signaling pathway
7	Type 1 interferon	145	11	Regulation of defense response
8	Neutrophils 2	121	3	Detection of bacterium
9	B-cells	123	11	B cell receptor signaling pathway
10	Eosinophil	120	7	Regulation of myeloid leukocyte mediated immunity

Zhernakova *et al*, BiorXiv preprint

Context specific *cis*-eQTL analysis in 2,116 samples



GO biological process
- response to virus
- response to type I interferon
- cellular response to type I interferon

Reactome
- Interferon Signaling
- Cytokine Signaling in Immune system
- Interferon alpha/beta signaling

GO biological process
- phagocytosis
- endocytosis
- membrane invagination

Reactome
- The NLRP3 inflammasome
- Platelet activation, signaling and aggregation

Upregulated eQTL genes

GO biological process
- response to type I interferon
- type I interferon production

Reactome
- Interferon Signaling
- Interferon alpha/beta signaling

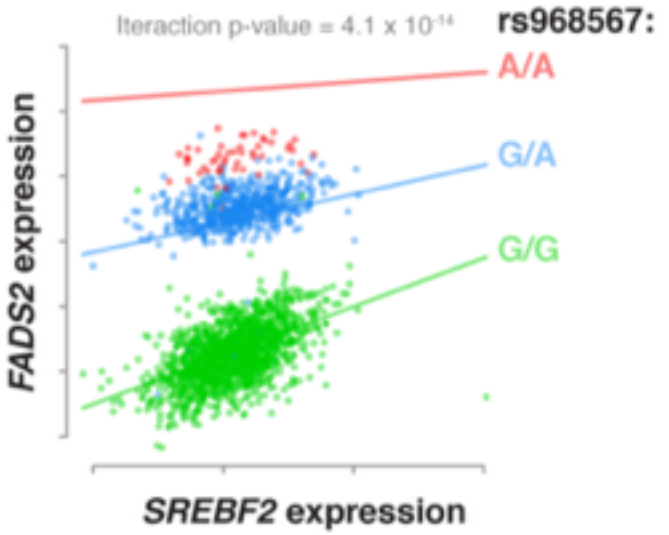
Downregulated eQTL genes

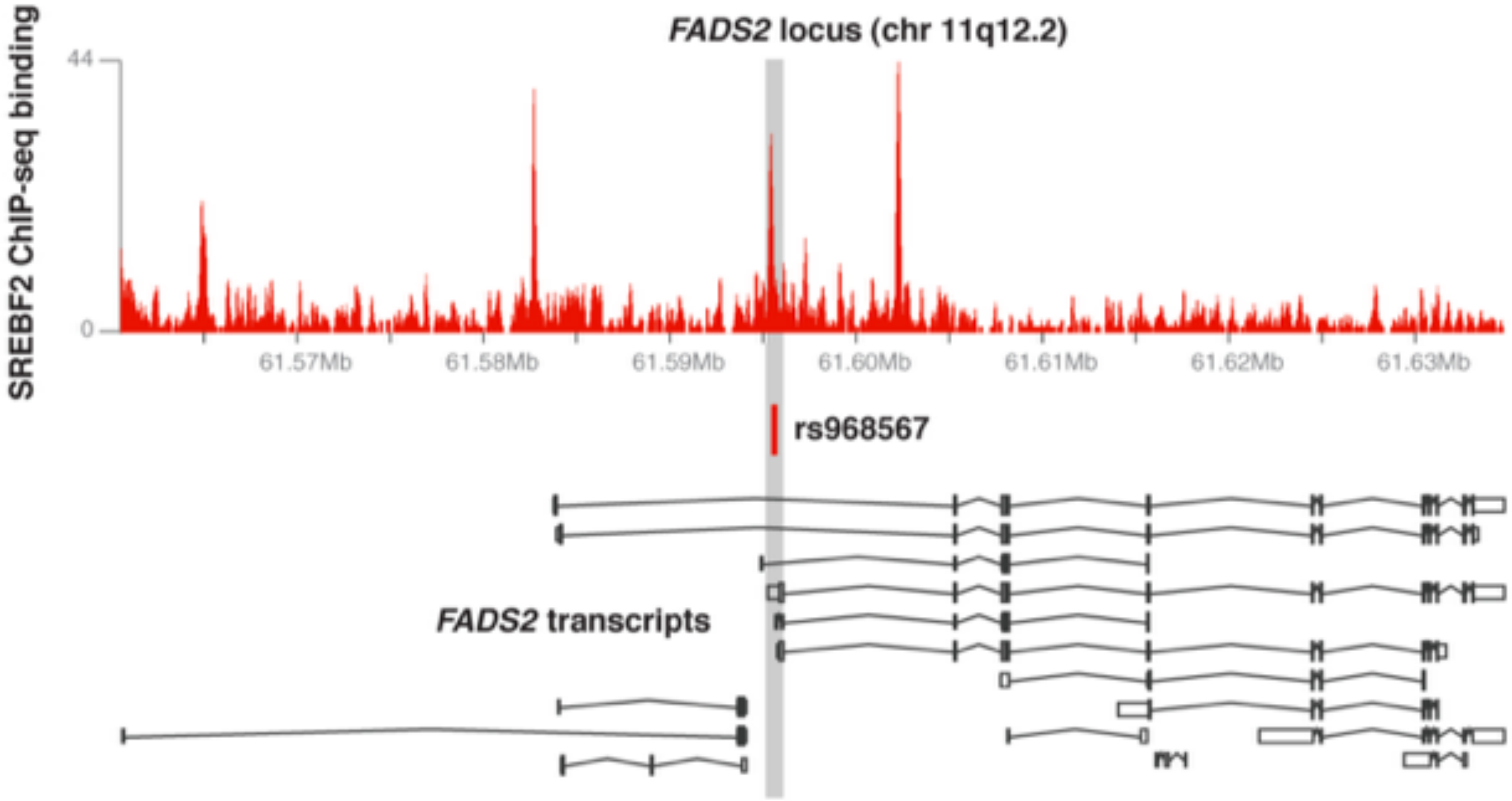
GO biological process
- regulation of exocytosis
- activation of protein kinase C activity by

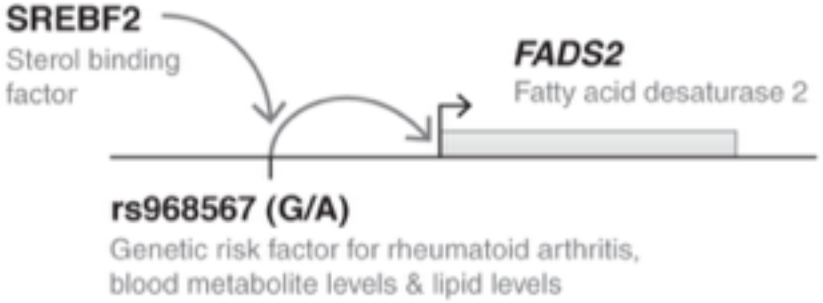
Reactome
- ERK/MAPK targets
- Muscle contraction

Zhernakova *et al*, BiorXiv preprint

Regulatory network reconstruction in 2,116 samples







SREBF2
Sterol binding factor
FADS2
Fatty acid desaturase 2
rs968567 (G/A)
Genetic risk factor for rheumatoid arthritis, blood metabolite levels & lipid levels

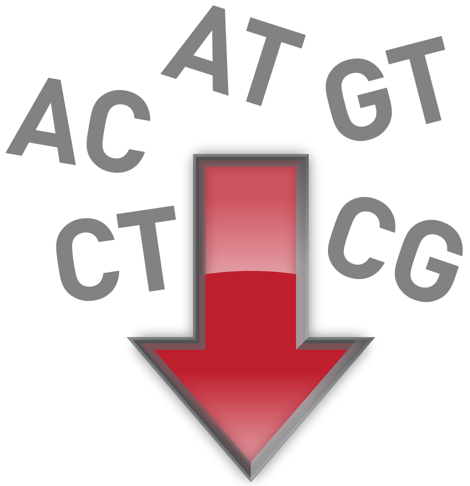
Zhernakova *et al*, BiorXiv preprint

but is this relevant for my patients?

But what about patients we see?

Patient with a severe disease.
You suspect a genetic cause.
What do you do?

- Targeted gene panel?
- Whole exome sequencing?
- Whole genome sequencing?



Problem:
Many (rare) variants
of unknown significance

Smart ways to filter?

gene expression?

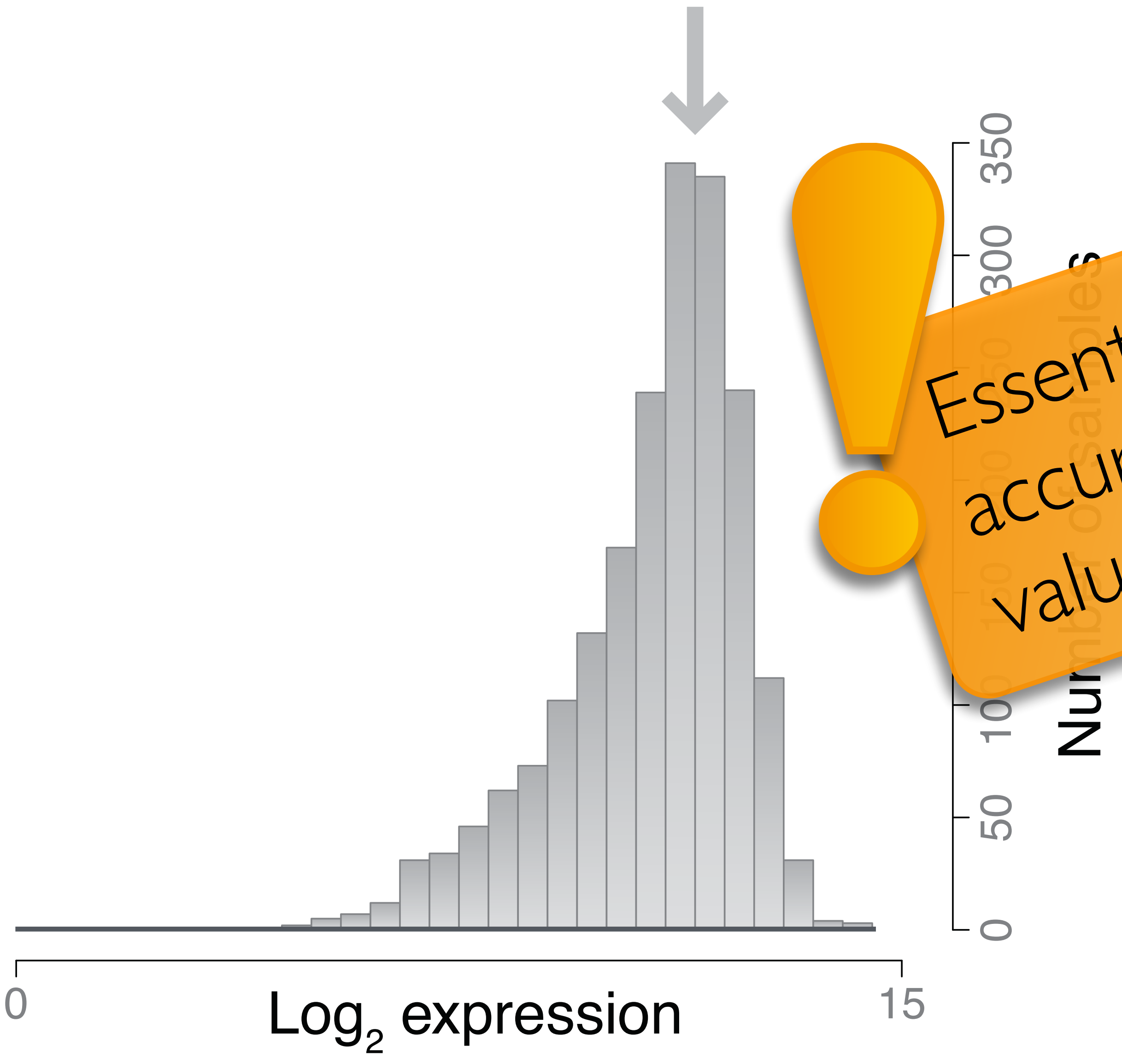
Transcriptome of the Netherlands

- Rare genetic variants also have effects on gene expression
- Rationale BBMRI-NL BIOS Consortium to establish ‘Transcriptome of the Netherlands’ in 5,000 population based samples
- Generate RNA-seq data on patients. Contrast these expression values to the Transcriptome of the Netherlands.

BBMRI • NL

Transcriptome of the Netherlands

***TRIM51BP* gene expression distribution in the Dutch population**



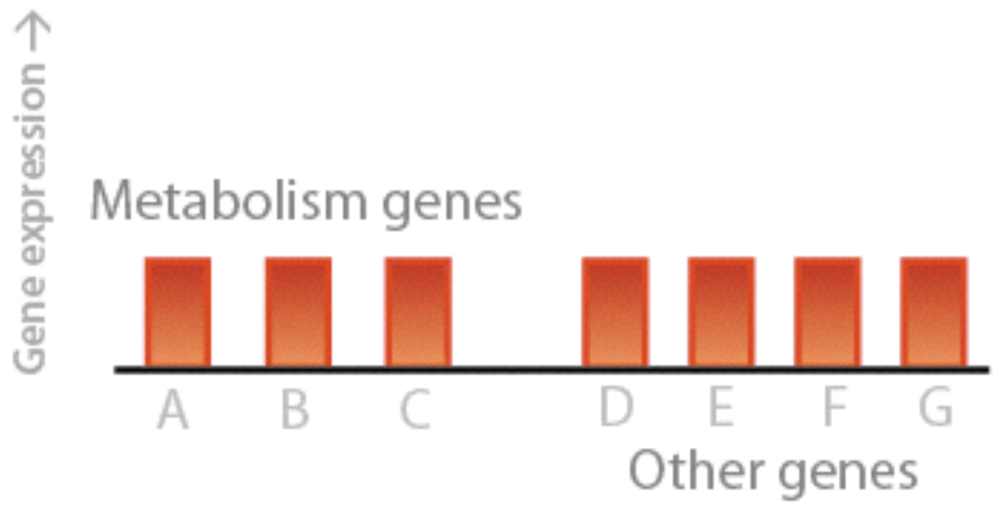
Essential to get very accurate reference values for each gene

Remove non-genetic expression variation

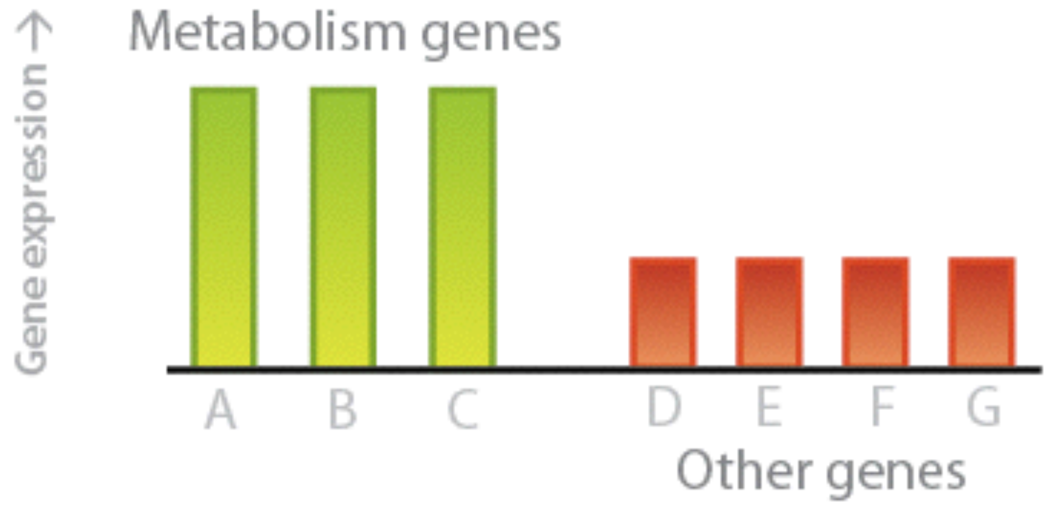
Most expression variation due to:

- Physiological state
- Metabolic state
- Environmental state

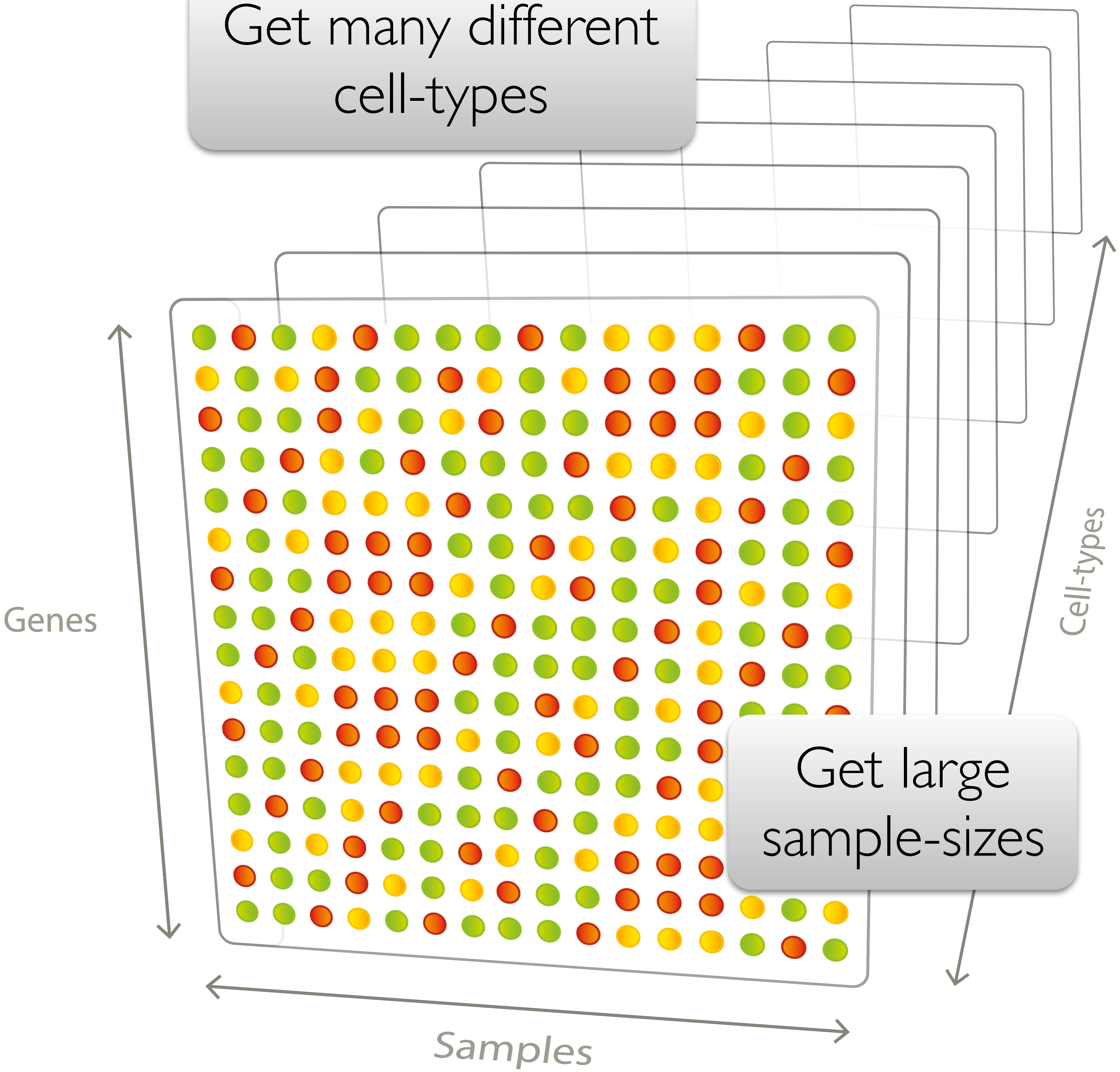
RNA blood expression
when you wake up



RNA blood expression
after nice diner



Strategies

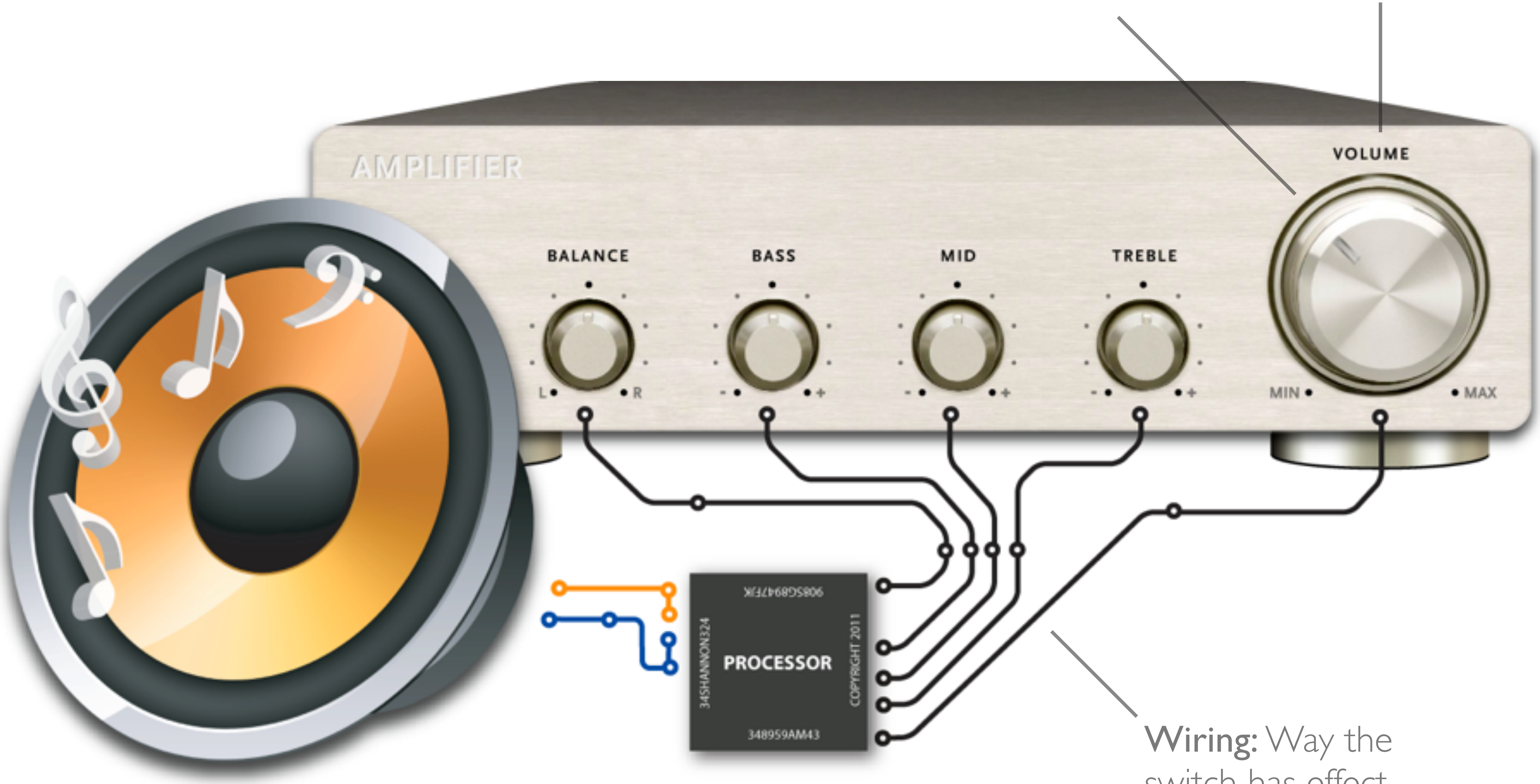


Get many different cell-types

Get large sample-sizes

Recycle big data

Amplifier can change many aspects of music

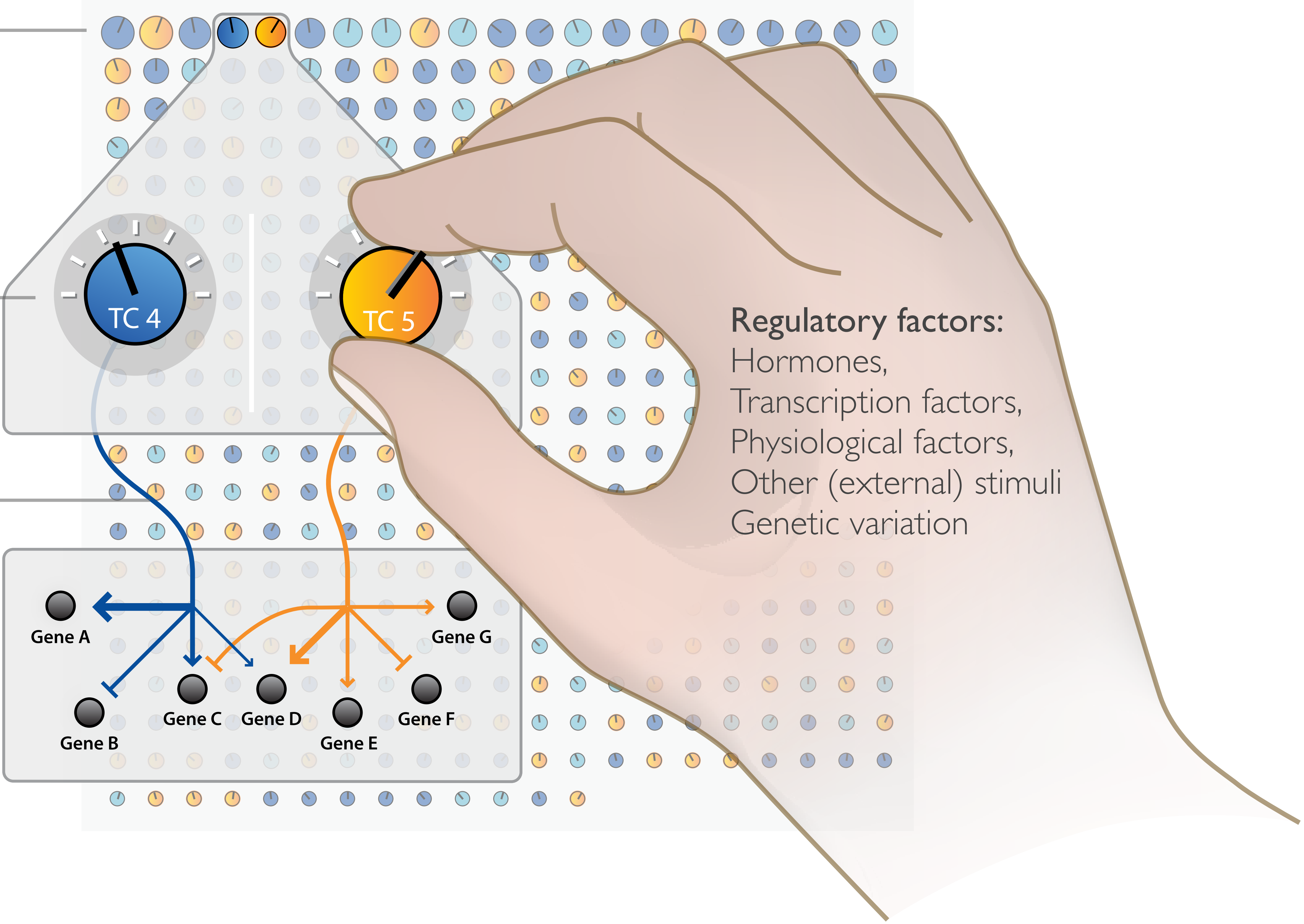


Setting: Activity of switch

Size of switch: Importance of switch

Wiring: Way the switch has effect

A control panel that determines gene expression?



Size of switch:
Importance

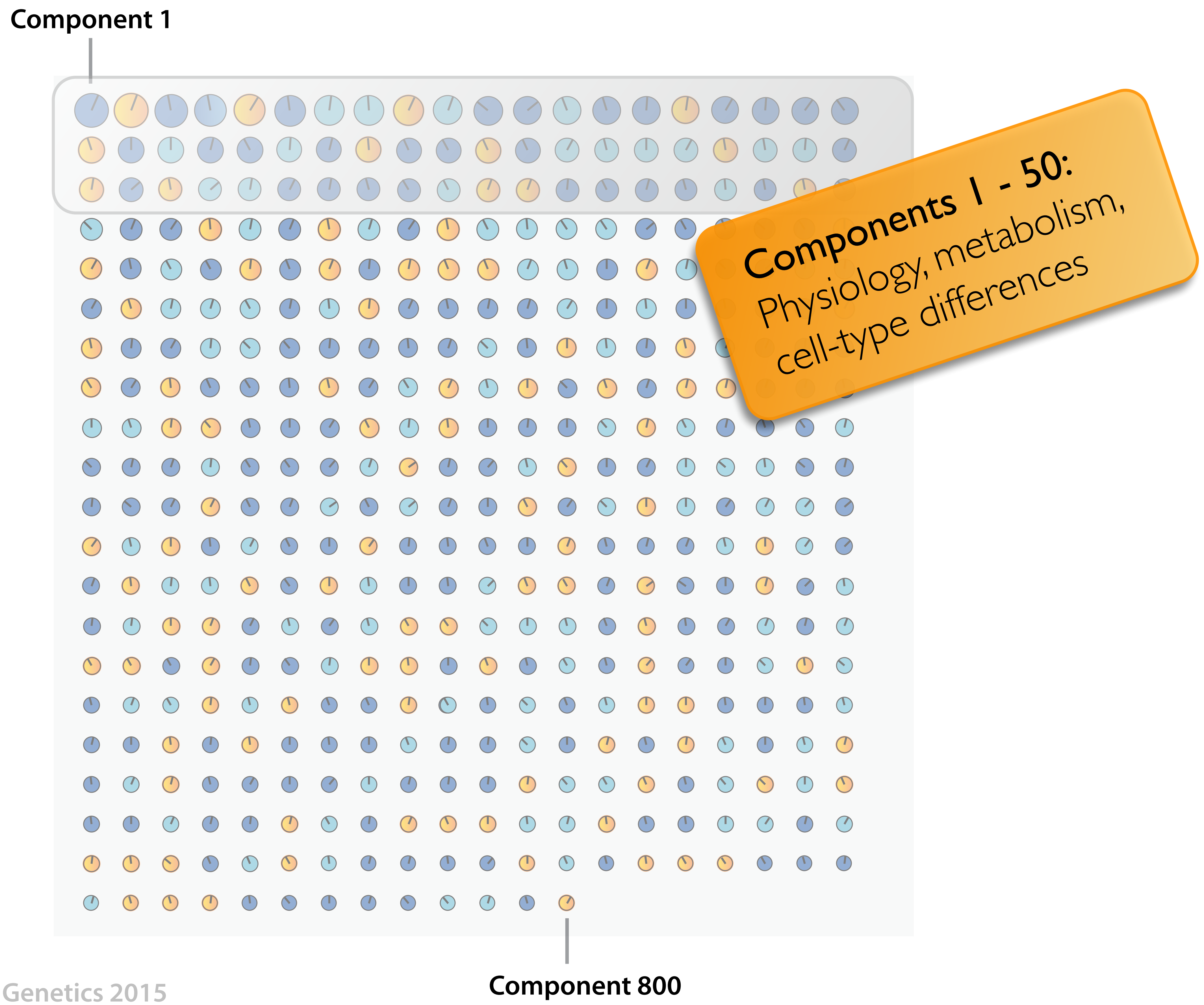
Setting: State of
a certain sample

Wiring: Effect on
individual genes

Regulatory factors:
Hormones,
Transcription factors,
Physiological factors,
Other (external) stimuli
Genetic variation

Fehrmann *et al*, Nature Genetics 2015

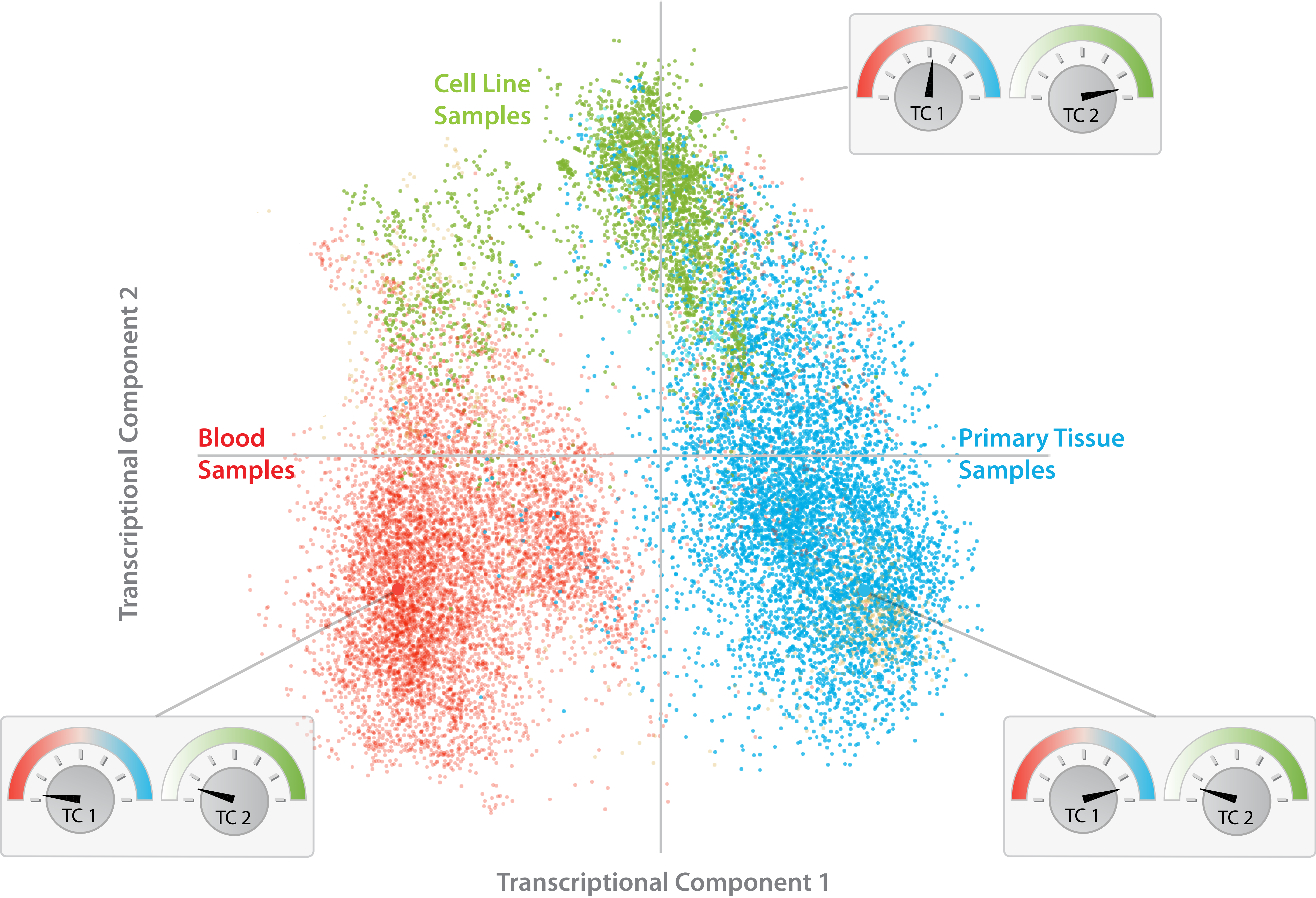
800 ‘transcriptional components’: Component 1 - 50



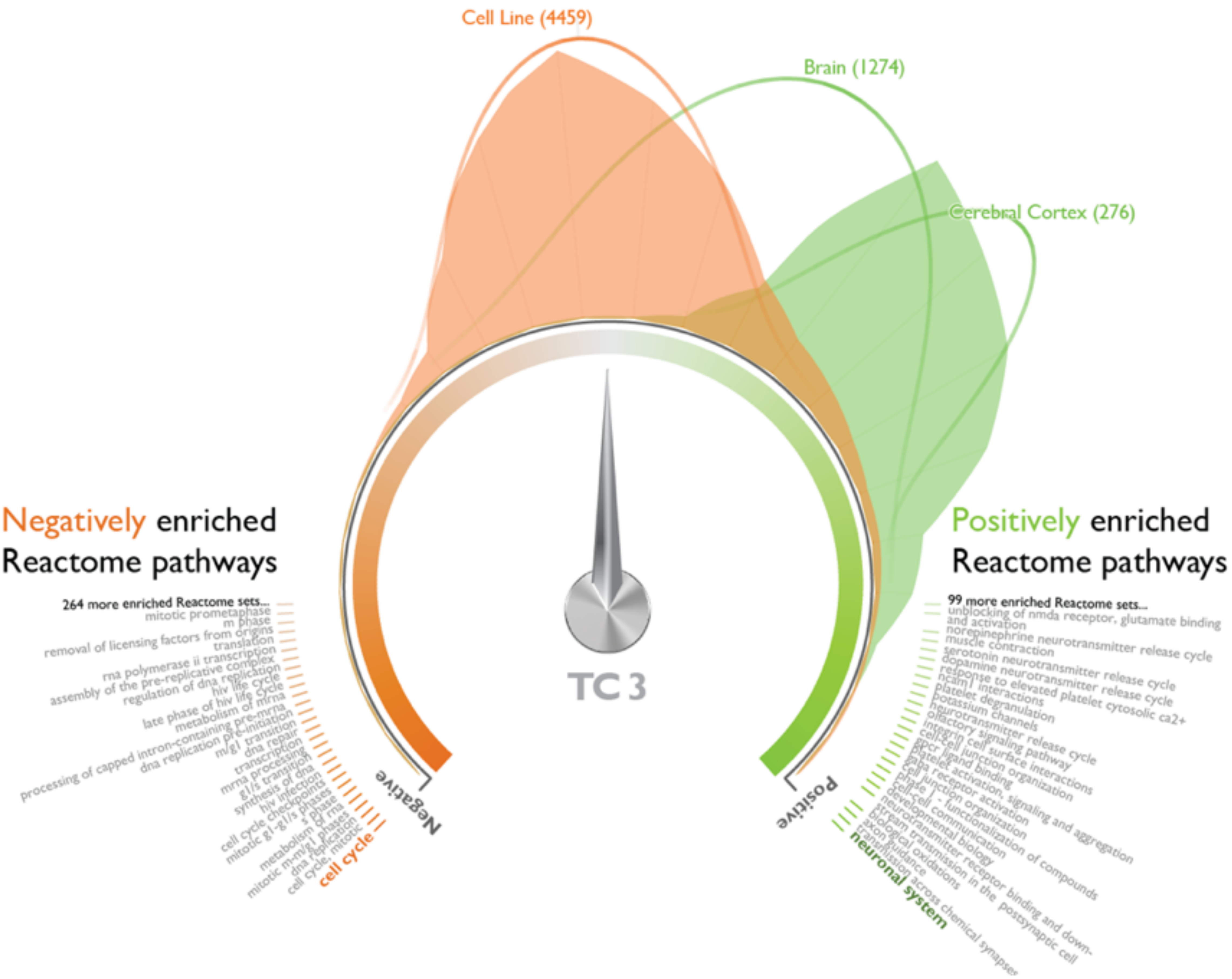
Components 1 - 50:
Physiology, metabolism, cell-type differences

Fehrmann *et al*, Nature Genetics 2015

Component 1 and 2



Transcriptional component 3



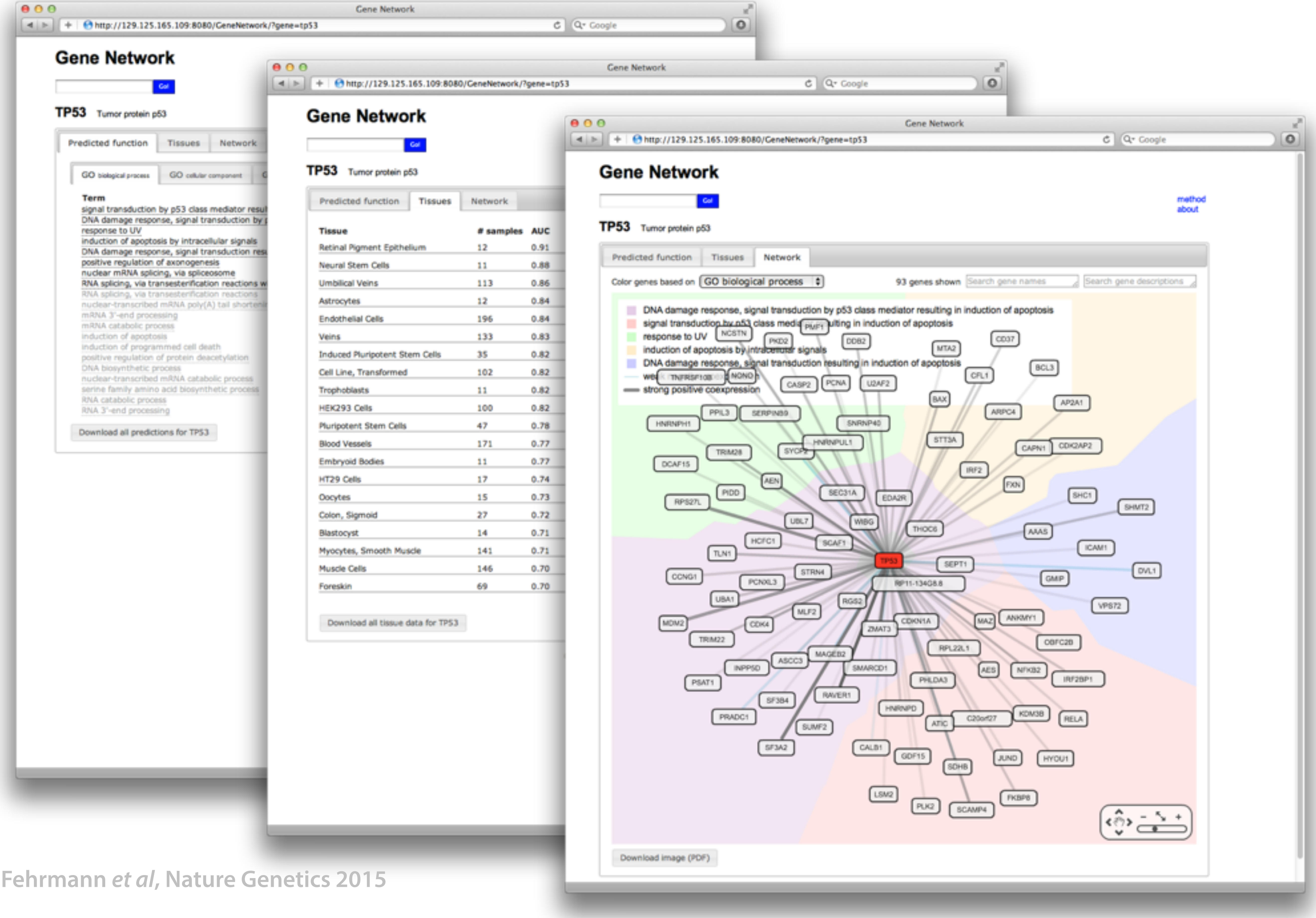
Negatively enriched Reactome pathways

264 more enriched Reactome sets...
mitotic prometaphase
m phase
removal of licensing factors from origins
translation
rna polymerase ii transcription
assembly of the pre-replicative complex
regulation of dna replication
hiv life cycle
late phase of hiv life cycle
metabolism of mrna
processing of capped intron-containing pre-mrna
dna replication pre-initiation
m/g1 transition
dna repair
transcription
mrna processing
g1/s transition
synthesis of dna
hiv infection
cell cycle checkpoints
mitotic g1-g1/s phases
s phase
metabolism of rna
mitotic m-m/g1 phases
dna replication
cell cycle, mitotic
cell cycle

Positively enriched Reactome pathways

99 more enriched Reactome sets...
unblocking of nmda receptor, glutamate binding and activation
norepinephrine neurotransmitter release cycle
muscle contraction
serotonin neurotransmitter release cycle
dopamine neurotransmitter release cycle
response to elevated platelet cytosolic ca2+
ncam1 interactions
platelet degranulation
potassium channels
neurotransmitter release cycle
olfactory signaling pathway
integrin cell surface interactions
cell-cell junction organization
gpcr ligand binding
platelet activation, signaling and aggregation
gaba receptor activation
cell junction organization
phase 1 - functionalization of compounds
cell-cell communication
developmental biology
neurotransmitter receptor binding and down-stream transmission in the postsynaptic cell
biological oxidations
axon guidance
transmission across chemical synapses
neuronal system

Predicted gene functions: www.genenetwork.nl



Gene Network

http://129.125.165.109:8080/GeneNetwork/?gene=tp53

Gene Network

Go!

TP53 Tumor protein p53

Predicted function | Tissues | Network

GO biological process | GO cellular component

Term
- signal transduction by p53 class mediator resul
- DNA damage response, signal transduction by p
- response to UV
- induction of apoptosis by intracellular signals
- DNA damage response, signal transduction res
- positive regulation of axonogenesis
- nuclear mRNA splicing, via spliceosome
- RNA splicing, via transesterification reactions w
- RNA splicing, via transesterification reactions
- nuclear-transcribed mRNA poly(A) tail shorteni
- mRNA 3'-end processing
- mRNA catabolic process
- induction of apoptosis
- induction of programmed cell death
- positive regulation of protein deacetylation
- DNA biosynthetic process
- nuclear-transcribed mRNA catabolic process
- serine family amino acid biosynthetic process
- RNA catabolic process
- RNA 3'-end processing

Download all predictions for TP53

Gene Network

http://129.125.165.109:8080/GeneNetwork/?gene=tp53

Gene Network

Go!

TP53 Tumor protein p53

Predicted function | Tissues | Network

Tissue	# samples	AUC
Retinal Pigment Epithelium	12	0.91
Neural Stem Cells	11	0.88
Umbilical Veins	113	0.86
Astrocytes	12	0.84
Endothelial Cells	196	0.84
Veins	133	0.83
Induced Pluripotent Stem Cells	35	0.82
Cell Line, Transformed	102	0.82
Trophoblasts	11	0.82
HEK293 Cells	100	0.82
Pluripotent Stem Cells	47	0.78
Blood Vessels	171	0.77
Embryoid Bodies	11	0.77
HT29 Cells	17	0.74
Oocytes	15	0.73
Colon, Sigmoid	27	0.72
Blastocyst	14	0.71
Myocytes, Smooth Muscle	141	0.71
Muscle Cells	146	0.70
Foreskin	69	0.70

Download all tissue data for TP53

Gene Network

http://129.125.165.109:8080/GeneNetwork/?gene=tp53

Gene Network

Go!

method
about

TP53 Tumor protein p53

Predicted function | Tissues | Network

Color genes based on: GO biological process

93 genes shown

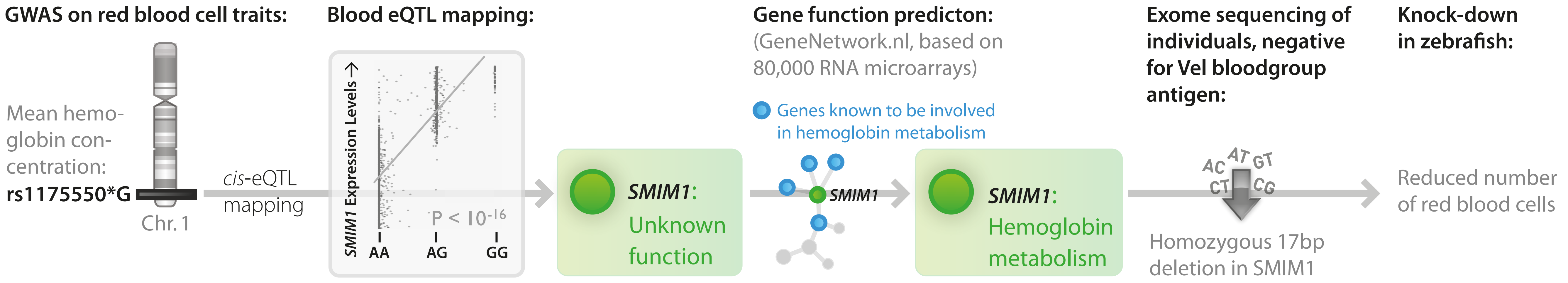
Search gene names | Search gene descriptions

- 🟪 DNA damage response, signal transduction by p53 class mediator resulting in induction of apoptosis
- 🟥 signal transduction by p53 class mediator resulting in induction of apoptosis
- 🟩 response to UV
- 🟧 induction of apoptosis by intracellular signals
- 🟦 DNA damage response, signal transduction resulting in induction of apoptosis
- — weak positive coexpression
- — strong positive coexpression

Download image (PDF)

Fehrmann *et al*, Nature Genetics 2015

GeneNetwork gene function predictions



GWAS on red blood cell traits:

Mean hemoglobin concentration: **rs1175550*G**

cis-eQTL mapping

Blood eQTL mapping:

SMIM1: Unknown function

Gene function predicton: (GeneNetwork.nl, based on 80,000 RNA microarrays)

● Genes known to be involved in hemoglobin metabolism

SMIM1: Hemoglobin metabolism

Exome sequencing of individuals, negative for Vel bloodgroup antigen:

Homozygous 17bp deletion in SMIM1

Knock-down in zebrafish:

Reduced number of red blood cells

Van der Harst *et al*, Nature 2012

Cvejic *et al*, Nature Genetics 2013

Amounts of data integrated:

GWAS in 135,000 samples

eQTL mapping in 1,500 samples

Transcriptomics in 80,000 samples

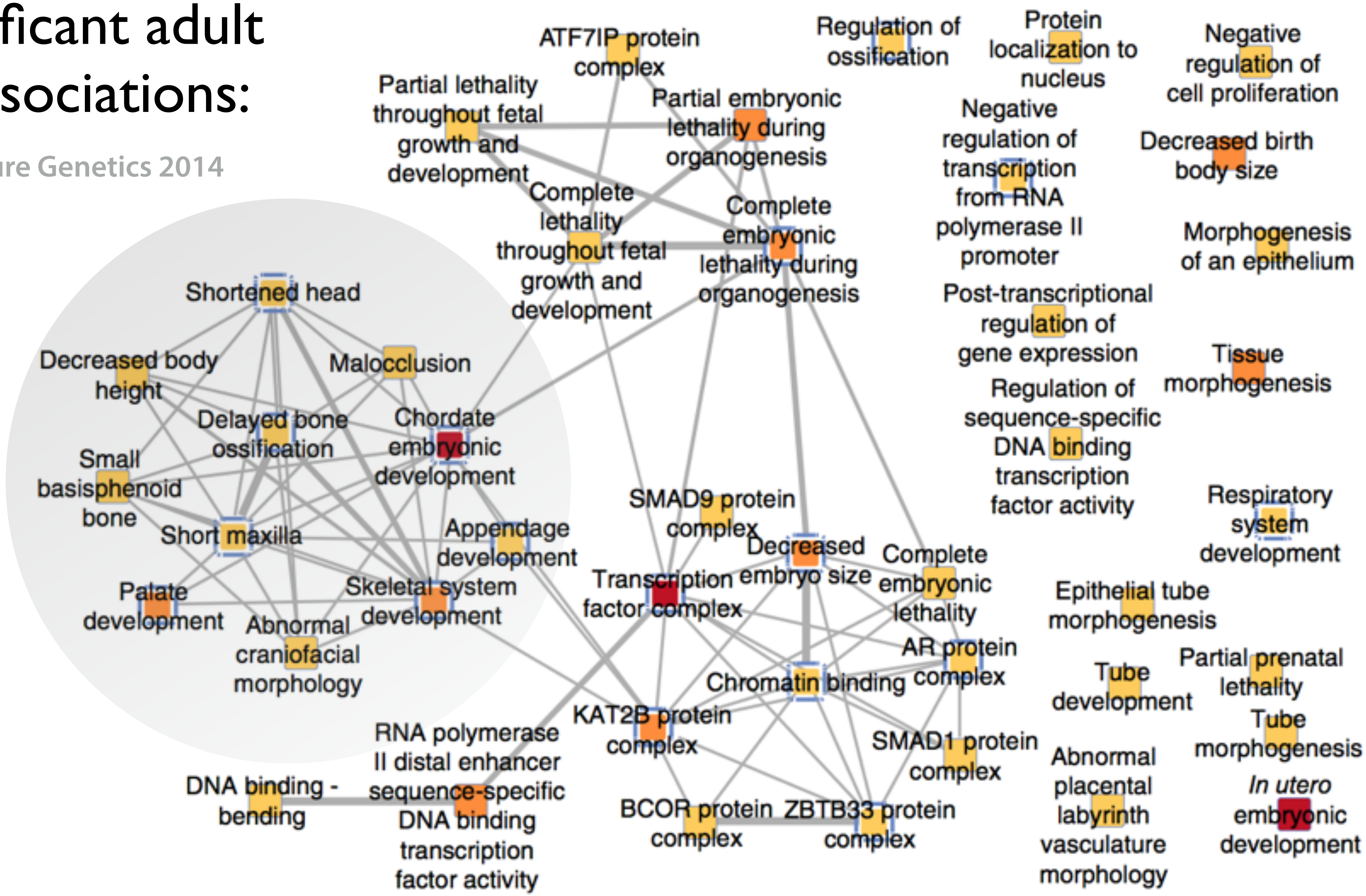
Exome sequencing

Wet lab proof

DEPICT: New prioritisation algorithm for GWAS

697 significant adult height associations:

Wood *et al*, Nature Genetics 2014



ATF7IP protein complex
Regulation of ossification
Protein localization to nucleus
Negative regulation of cell proliferation
Partial lethality throughout fetal growth and development
Partial embryonic lethality during organogenesis
Negative regulation of transcription from RNA polymerase II promoter
Decreased birth body size
Complete lethality throughout fetal growth and development
Complete embryonic lethality during organogenesis
Morphogenesis of an epithelium
Post-transcriptional regulation of gene expression
Shortened head
Decreased body height
Malocclusion
Tissue morphogenesis
Regulation of sequence-specific DNA binding transcription factor activity
Delayed bone ossification
Chordate embryonic development
Small basisphenoid bone
Short maxilla
SMAD9 protein complex
Respiratory system development
Appendage development
Decreased embryo size
Complete embryonic lethality
Palate development
Skeletal system development
Transcription factor complex
Epithelial tube morphogenesis
Abnormal craniofacial morphology
AR protein complex
Partial prenatal lethality
Tube development
Chromatin binding complex
Tube morphogenesis
KAT2B protein complex
RNA polymerase II distal enhancer sequence-specific DNA binding transcription factor activity
SMAD1 protein complex
Abnormal placental labyrinth vasculature morphology
In utero embryonic development
DNA binding - bending
BCOR protein complex
ZBTB33 protein complex

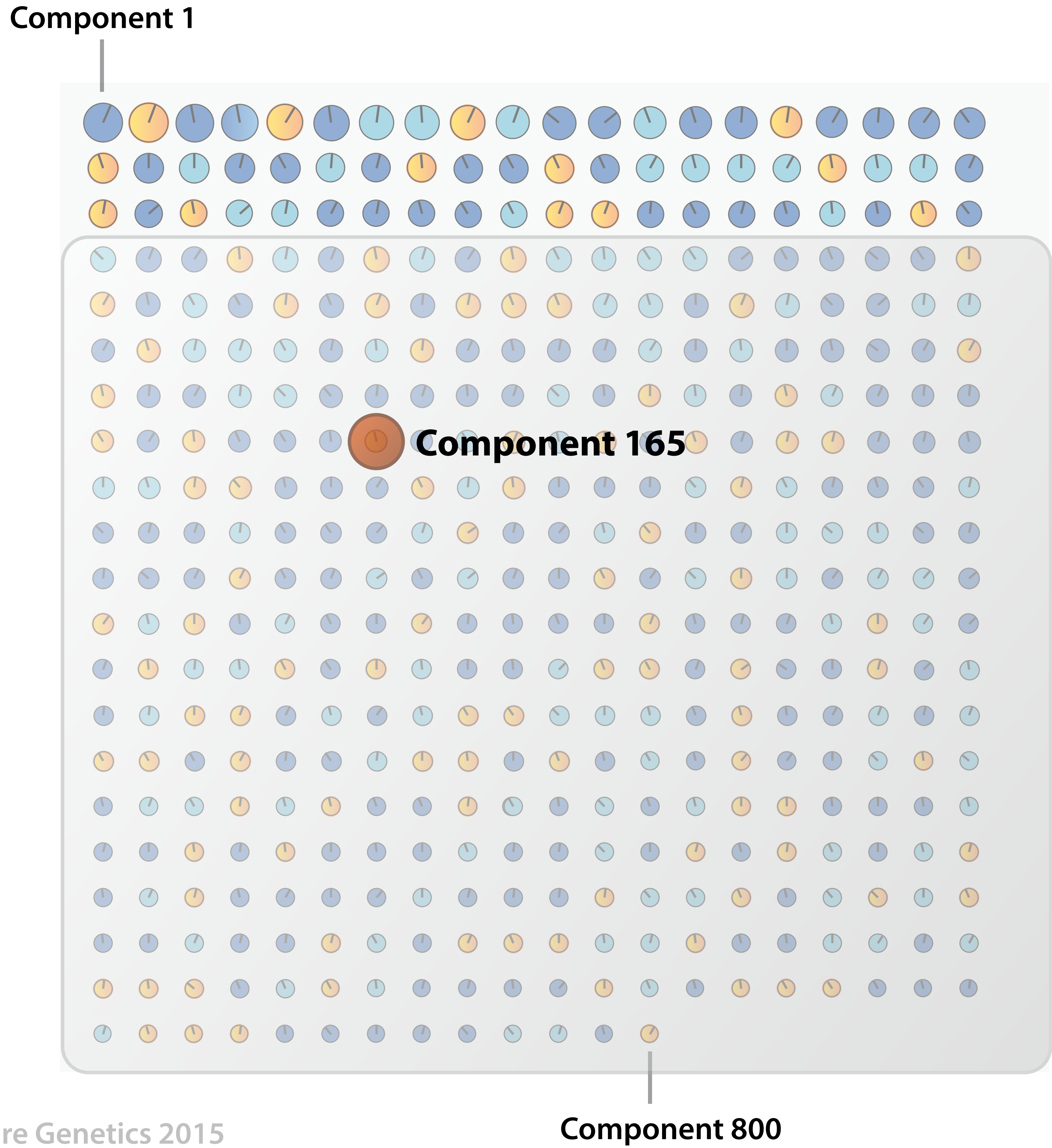
DEPICT Method:

Pers *et al*, Nature Communications 2015

DEPICT used for:

Body mass index (Locke *et al*, Nature 2015)
Waist hip ratio (Shungin *et al*, Nature 2015)
Hypospadias (Geller *et al*, Nature Genetics 2014)
Lipid Levels (Surakka, Nature Genetics 2015)

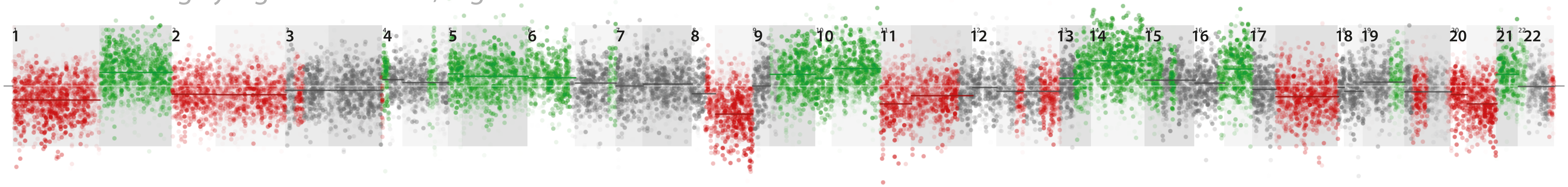
Components 51 - 800

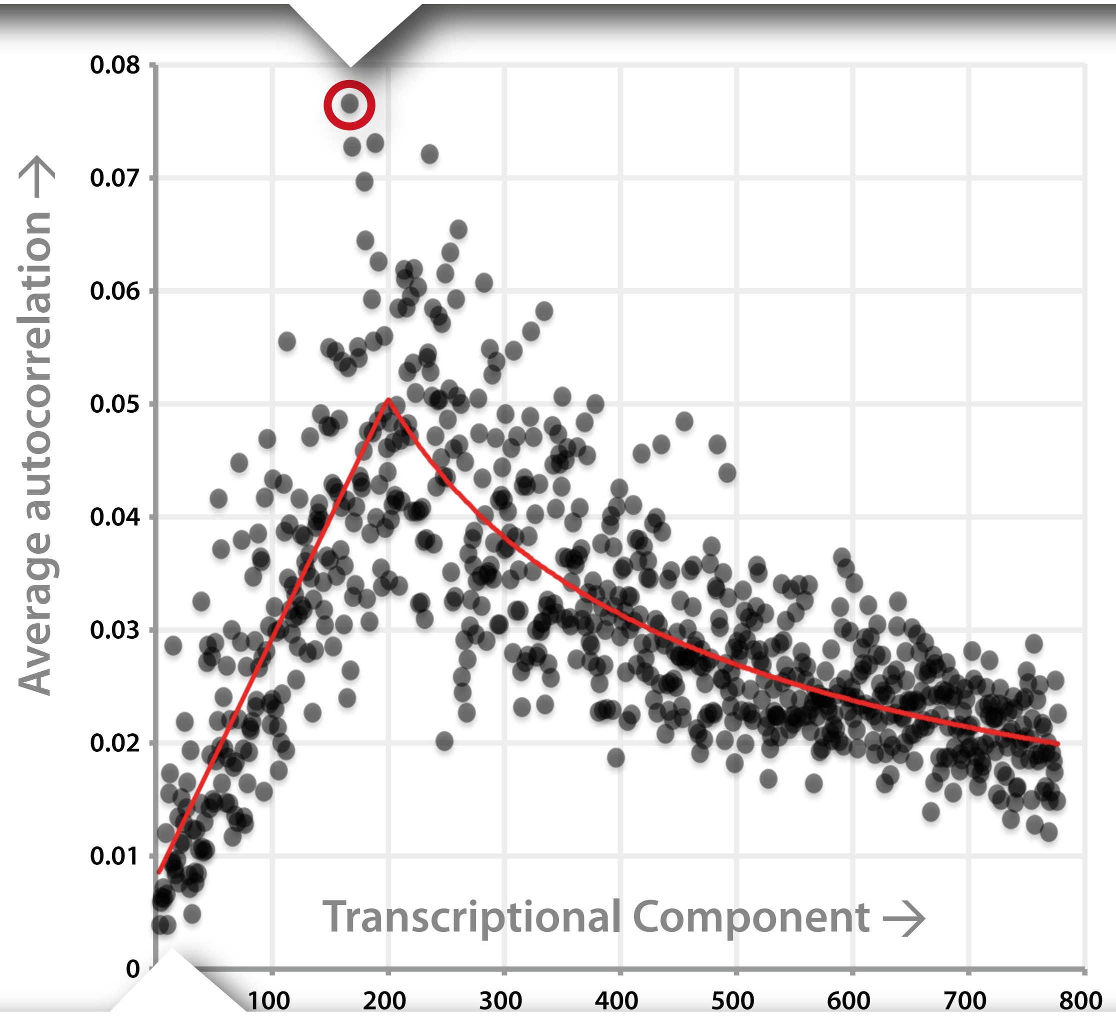


Fehrmann *et al*, Nature Genetics 2015

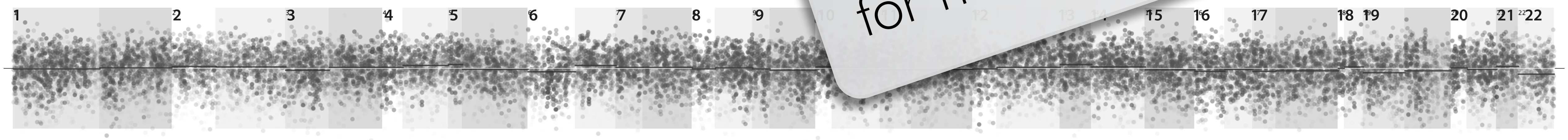
Some component show weird behaviour

TC 165: Strong cytogenetic effects, high autocorrelation





TC 1: No cytogenetic effect, zero autocorrelation

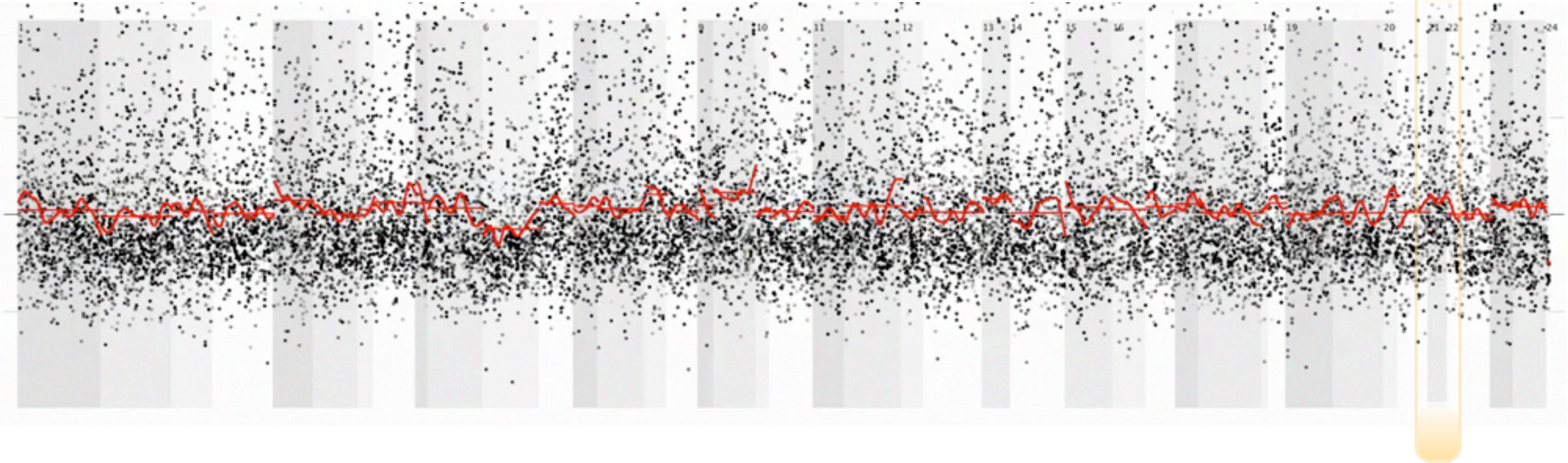


Redo analysis in healthy samples, correct cancer data for healthy components

Detection cytogenetic aberration in expression data

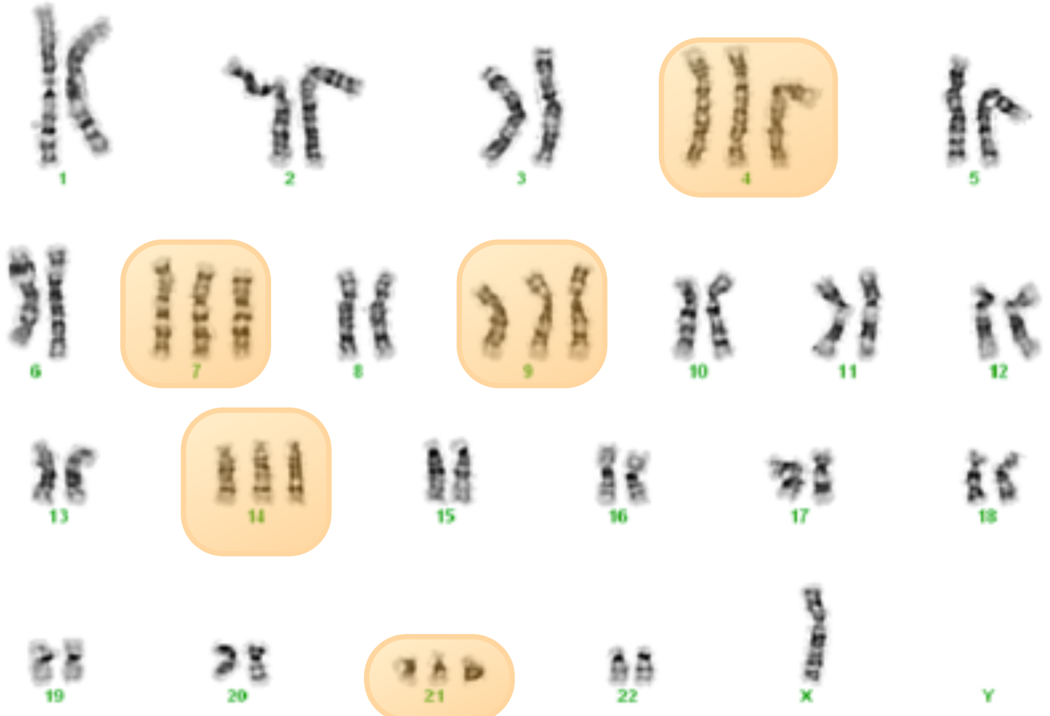
Chromosome

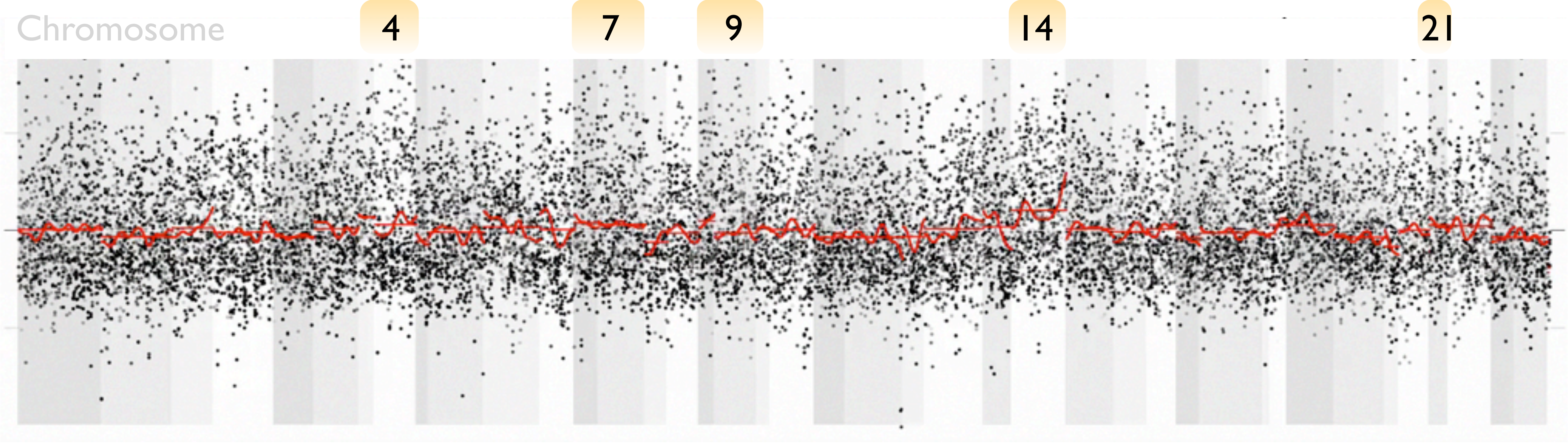
Down Syndrome patient: dup 21



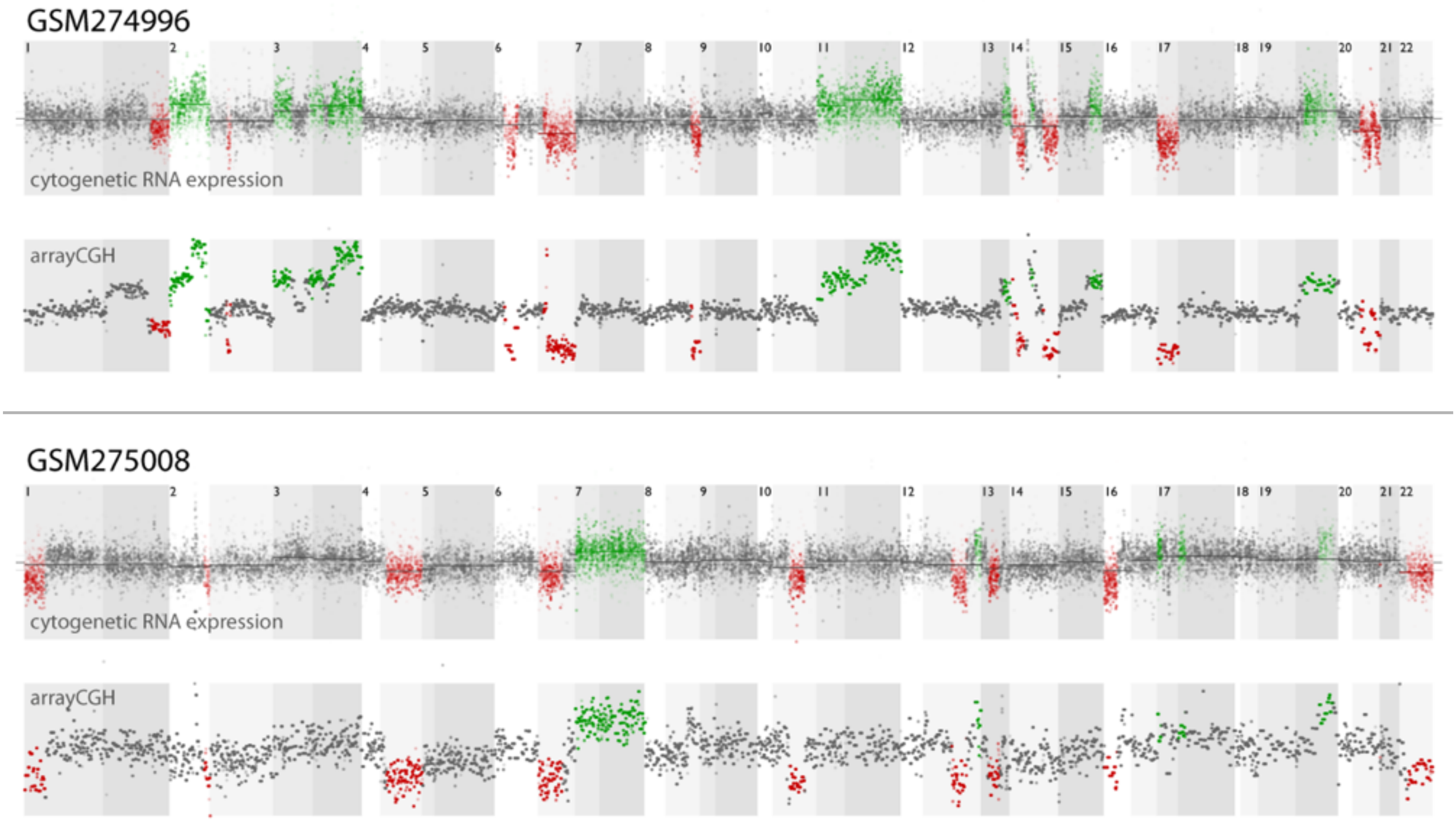
Identifying five chromosome duplications

Karyogram
HapMap LCL





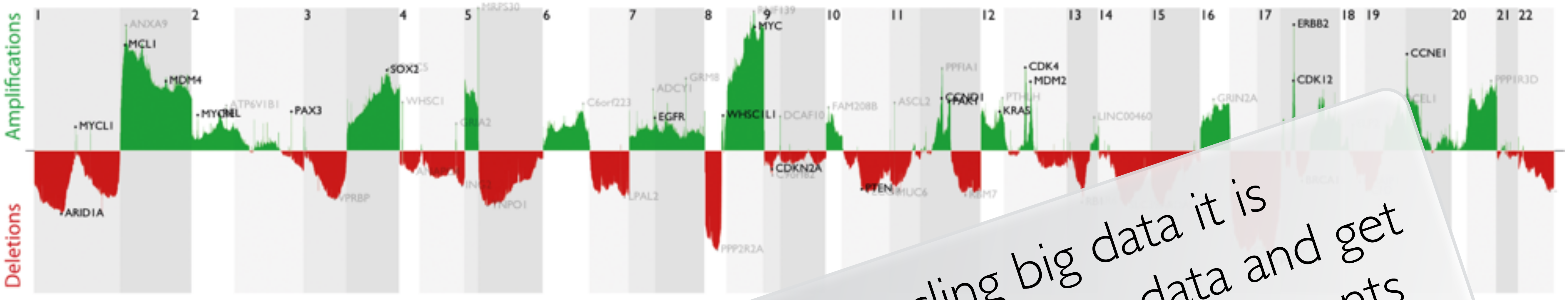
Comparison of arrayCGH and cytogenetic RNA profiles



Fehrmann *et al*, Nature Genetics 2015

Known driver genes in amplification and deletion peaks

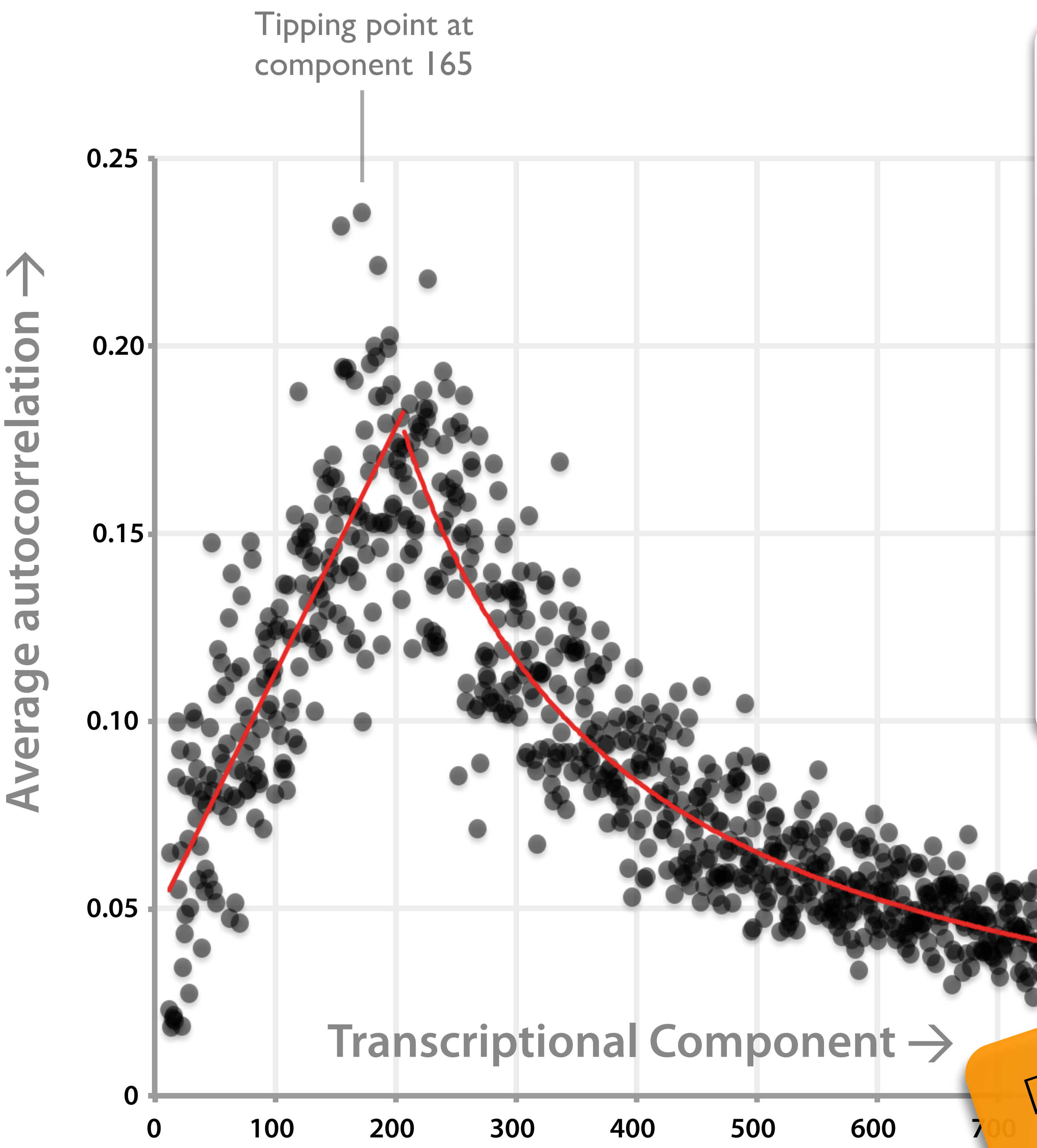
Average somatic copy number aberration profile of 16,172 primary tumor samples (GPL570 + GPL96 platforms)



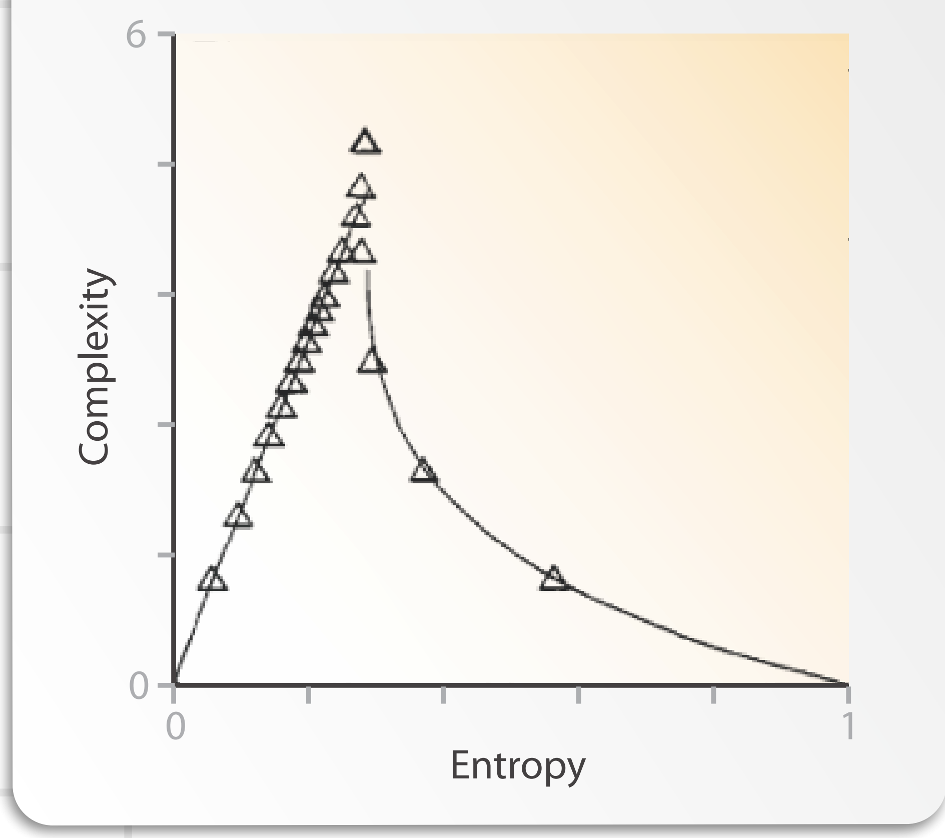
By recycling big data it is possible to clean data and get very accurate measurements

Fehrmann *et al*, Nature Genetics 2015

Amount of cytogenetic aberrations



Transition to chaos in the logistic map
Crutchfield *et al*, 1990

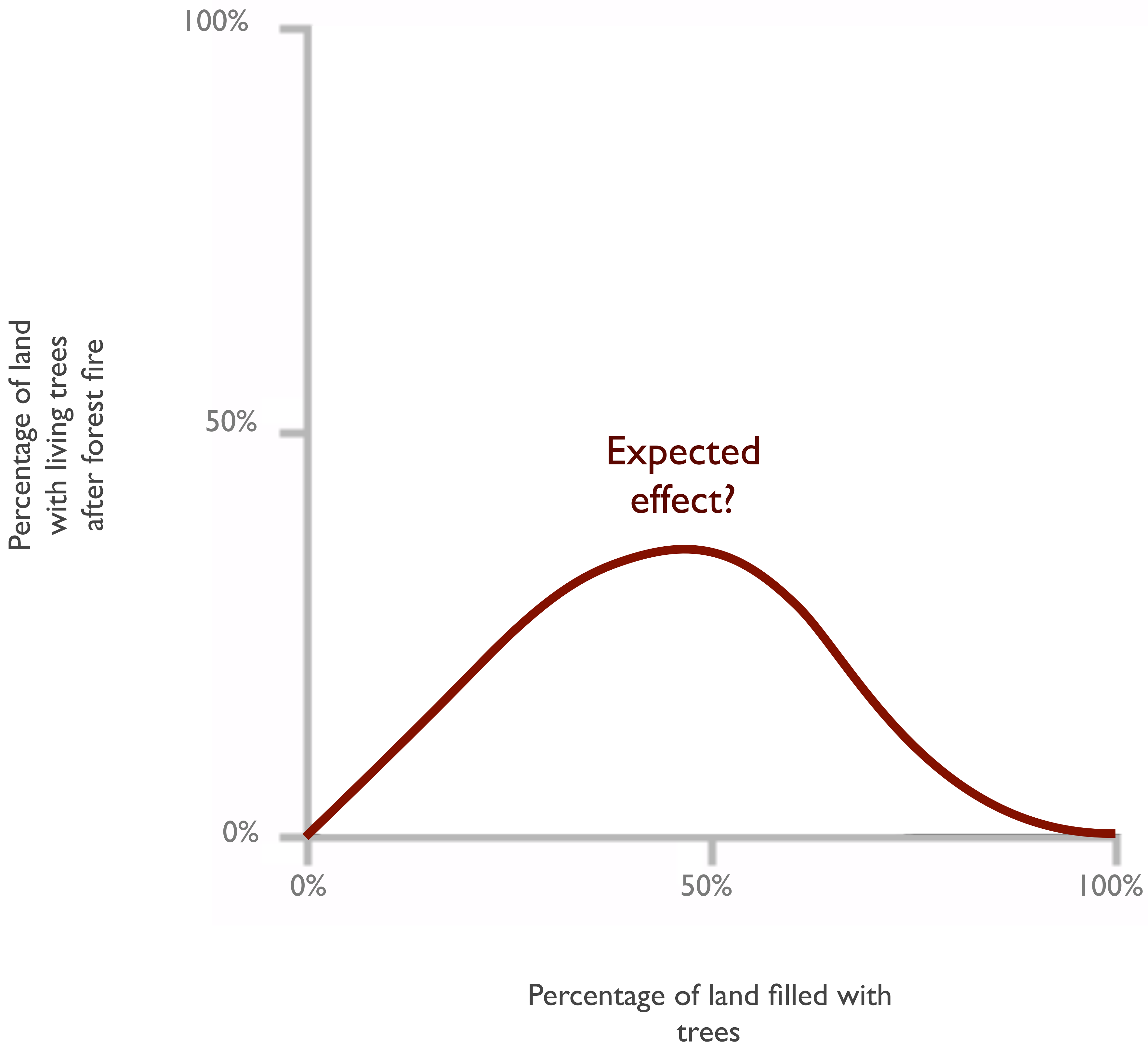


Distribution identical to simulations in complexity theory

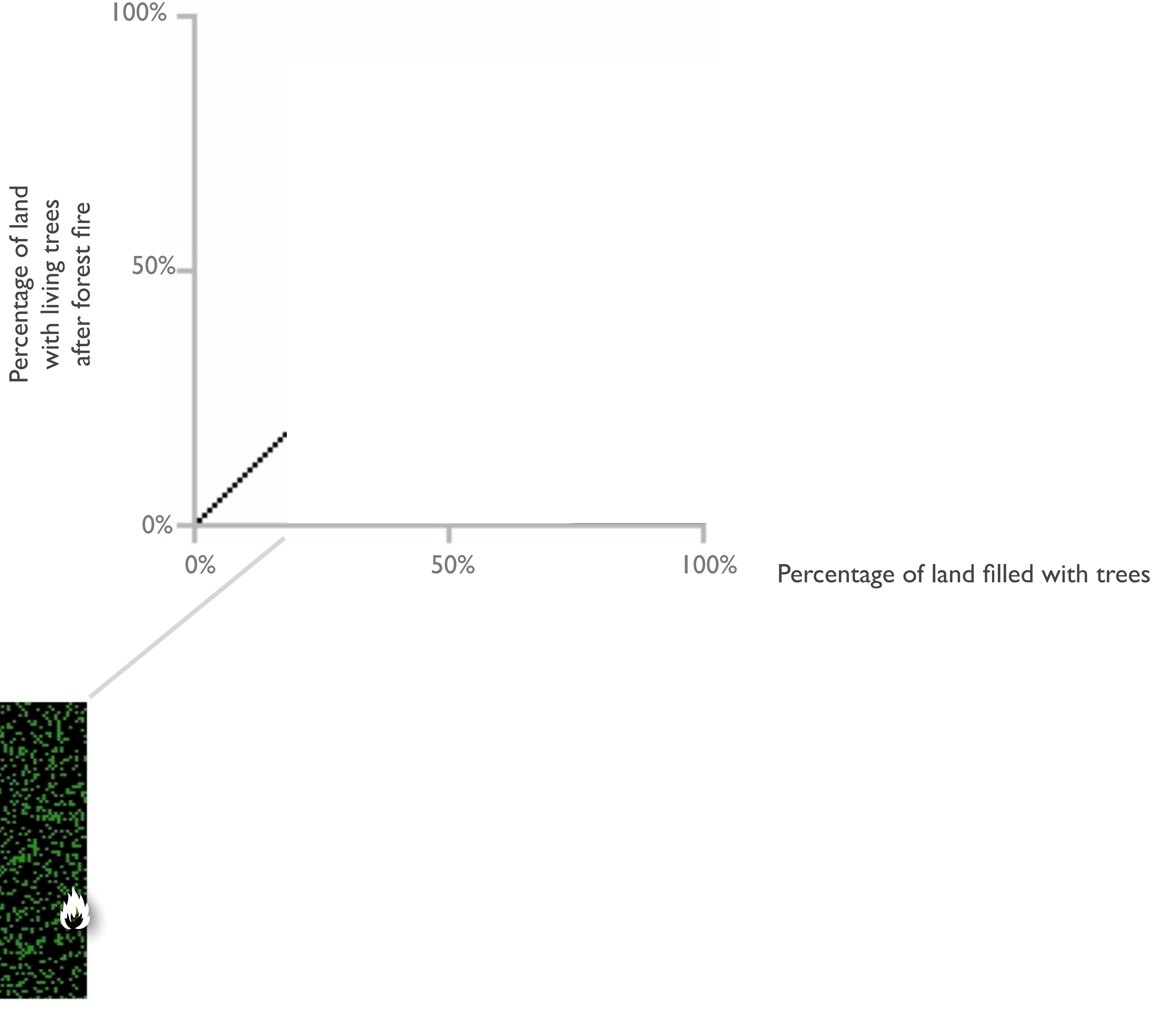
Forest fire: when will a forest burn down entirely?

How many trees can you plant without the risk that everything burns down?

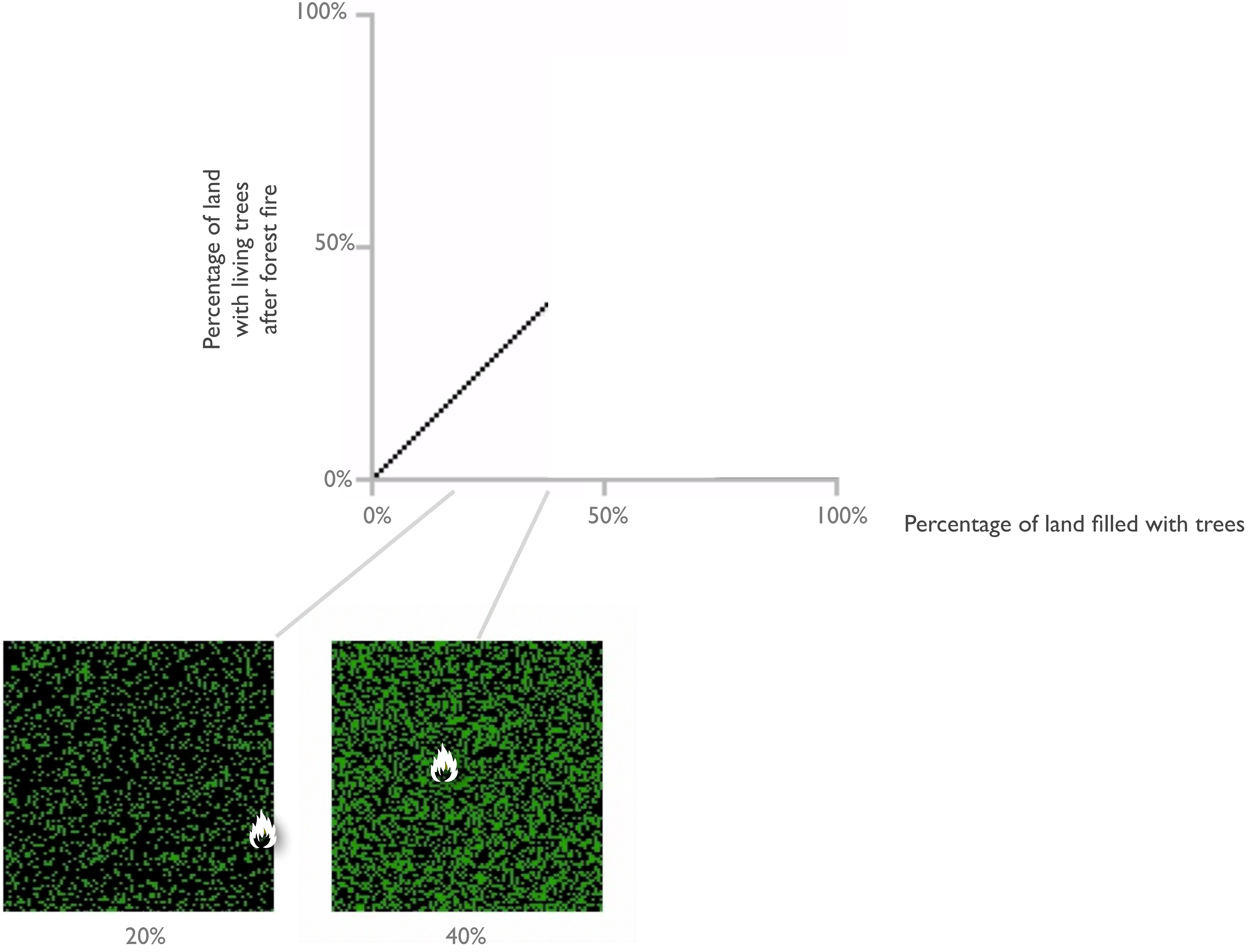
Complexity: Forest fire



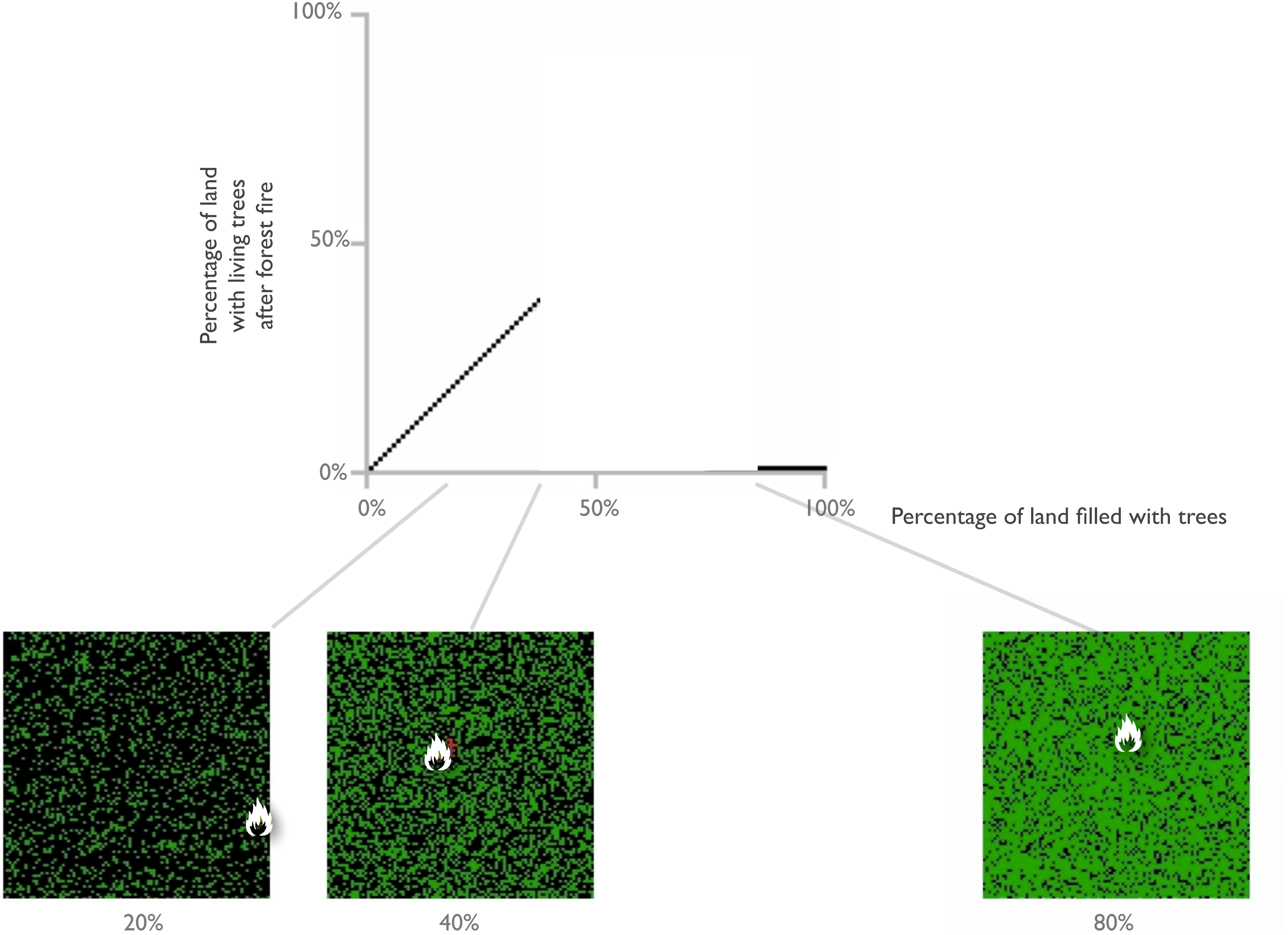
Complexity: Forest fire



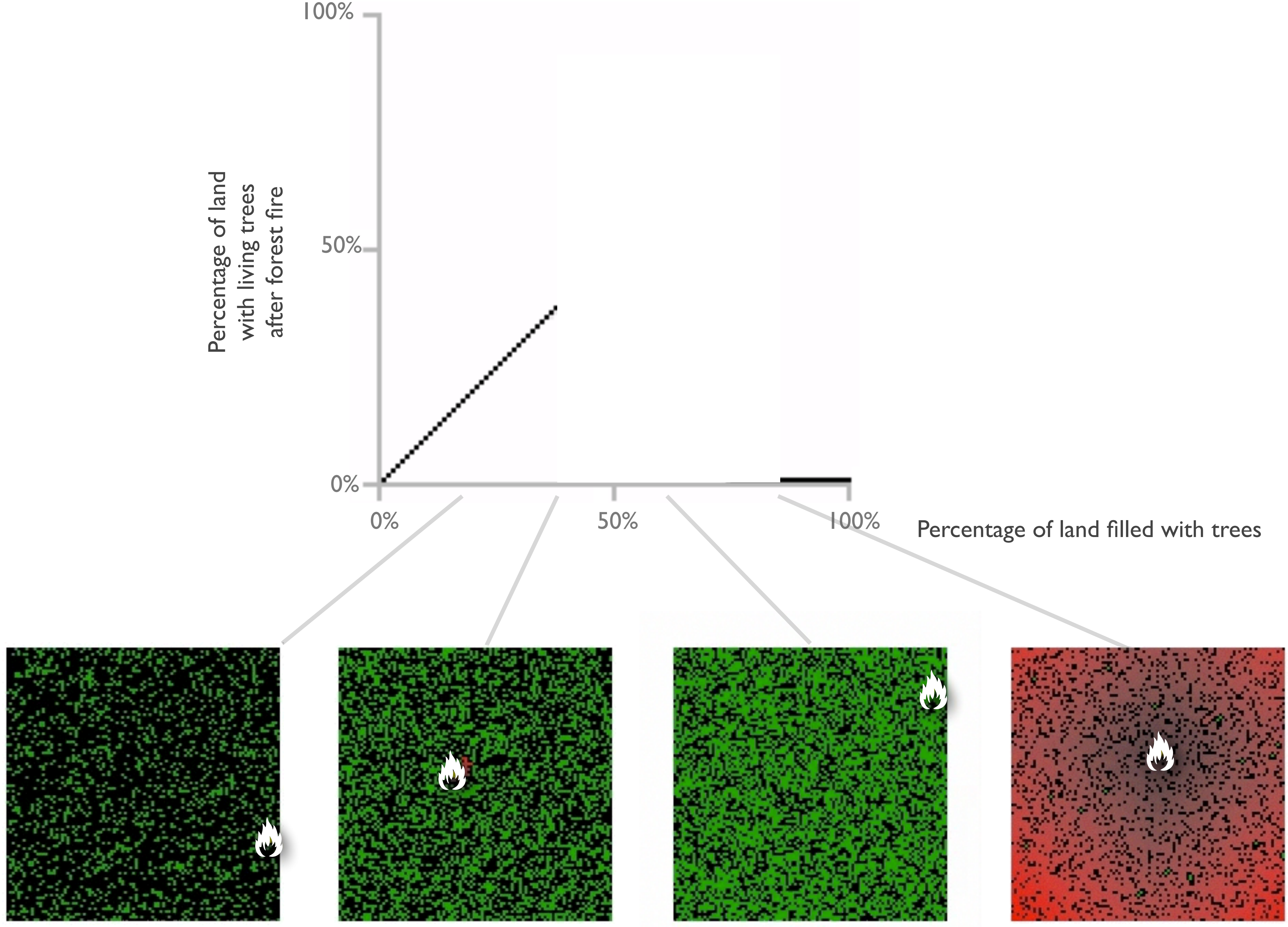
Complexity: Forest fire



Complexity: Forest fire



Complexity: Forest fire



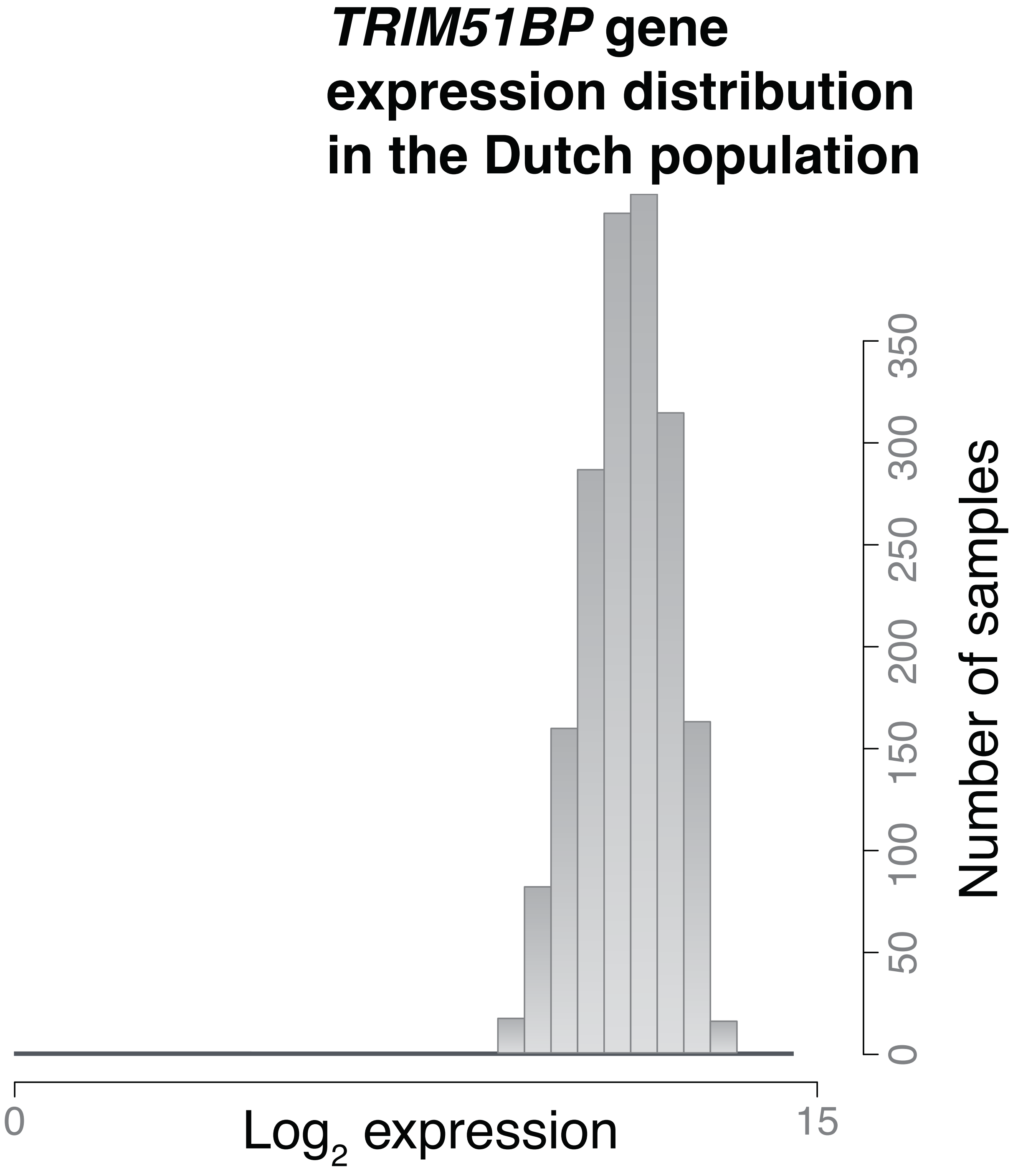
20%

40%

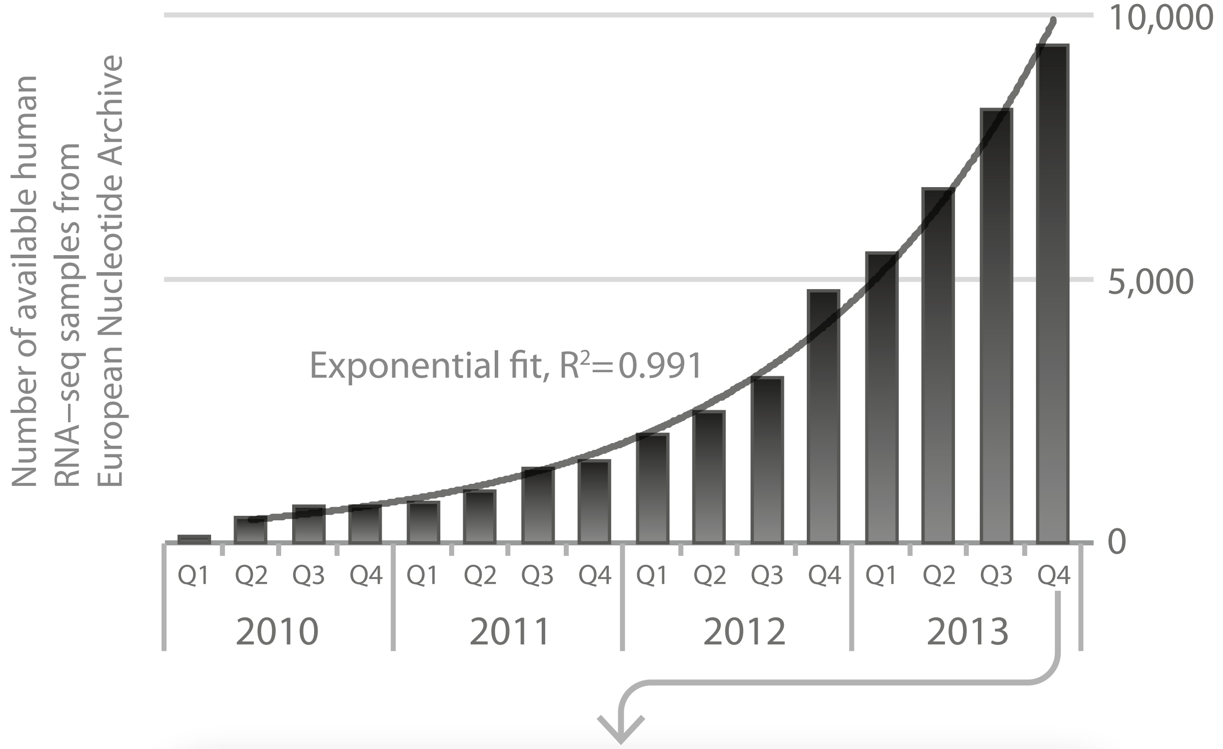
60%

80%

More accurate reference values for genes



Explosion of publicly available RNA-seq data

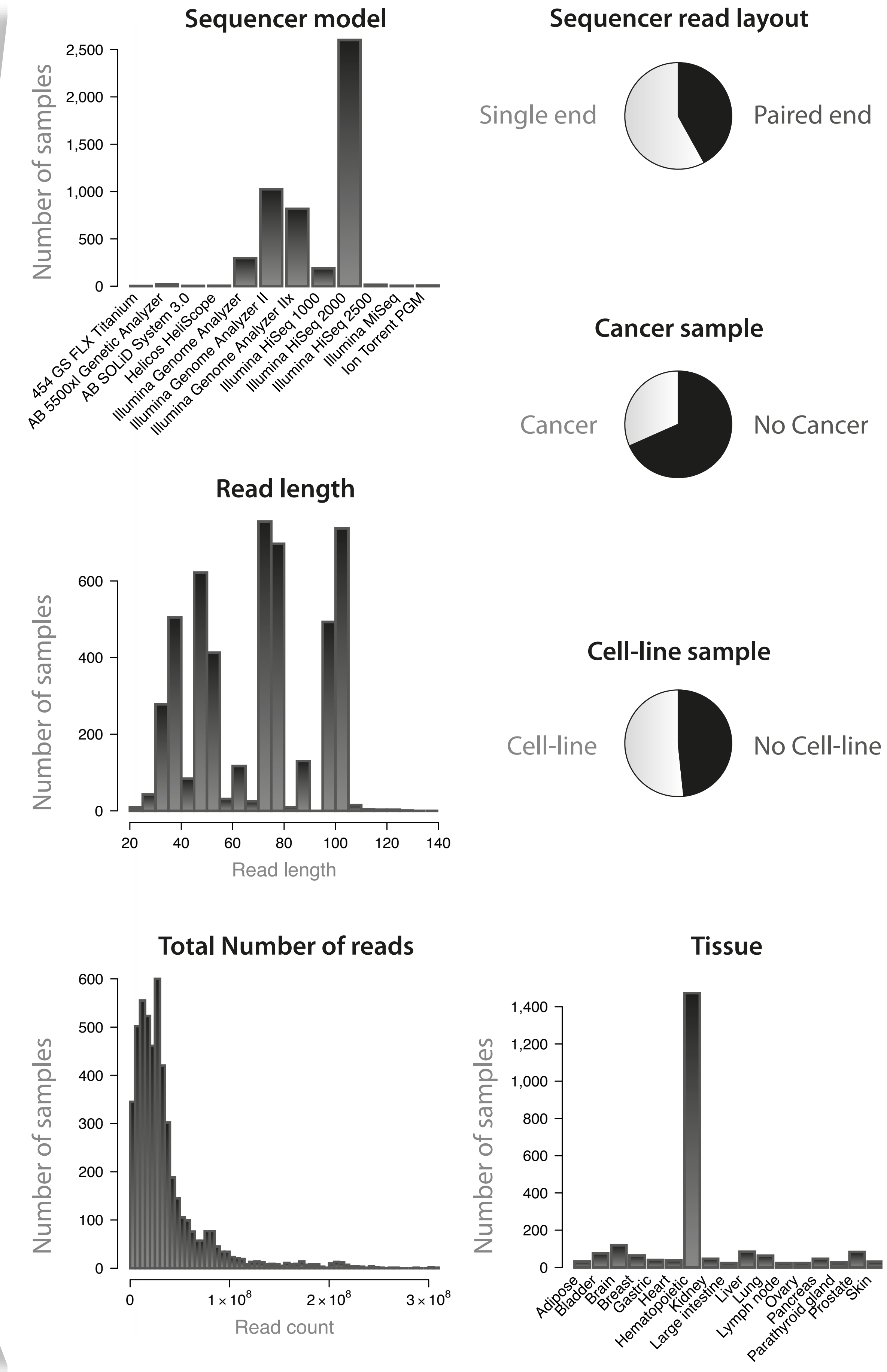


9,527 public human RNA-seq runs from ENA

Read alignment, epression quantification, normalization and PCA:

- 4,028 runs with low mapping statistics removed
- 521 expression outliers removed

4,978 samples (used for expression clustering)



Sequencer model

Sequencer read layout

Cancer sample

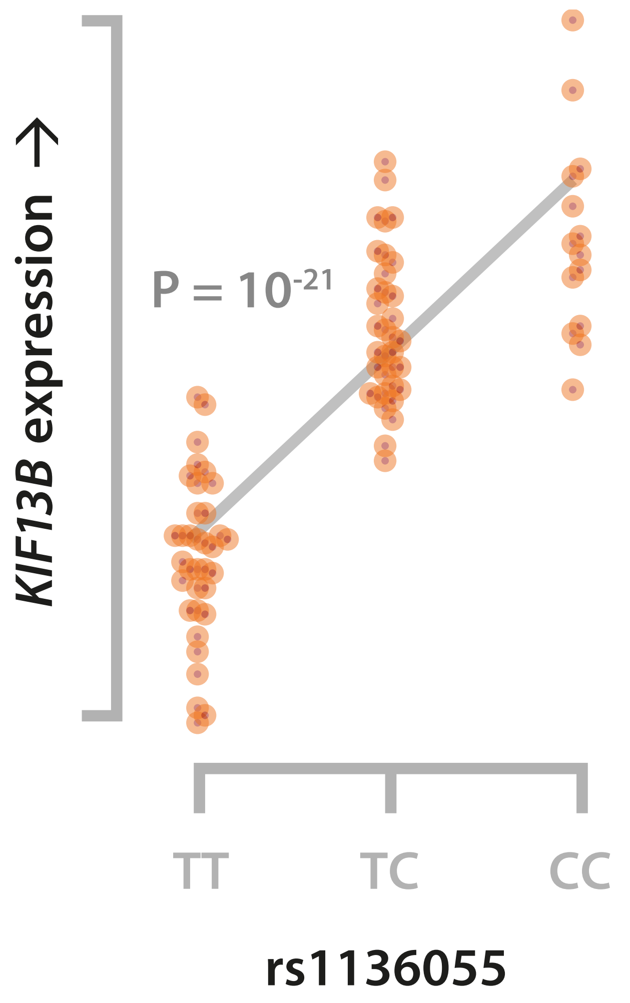
Read length

Cell-line sample

Total Number of reads

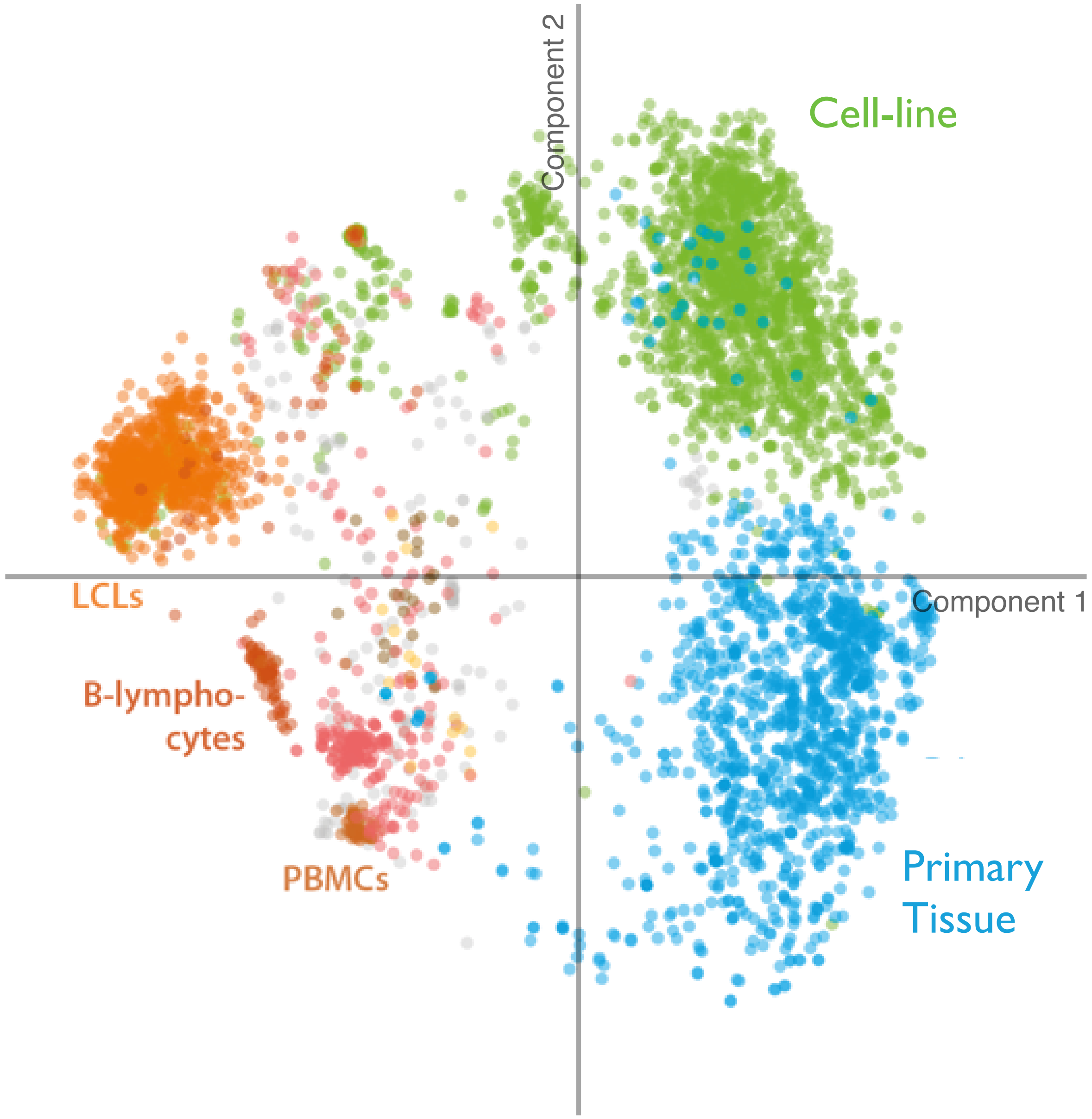
Tissue

Derive SNP genotypes from RNA-seq data



Infer genotypes

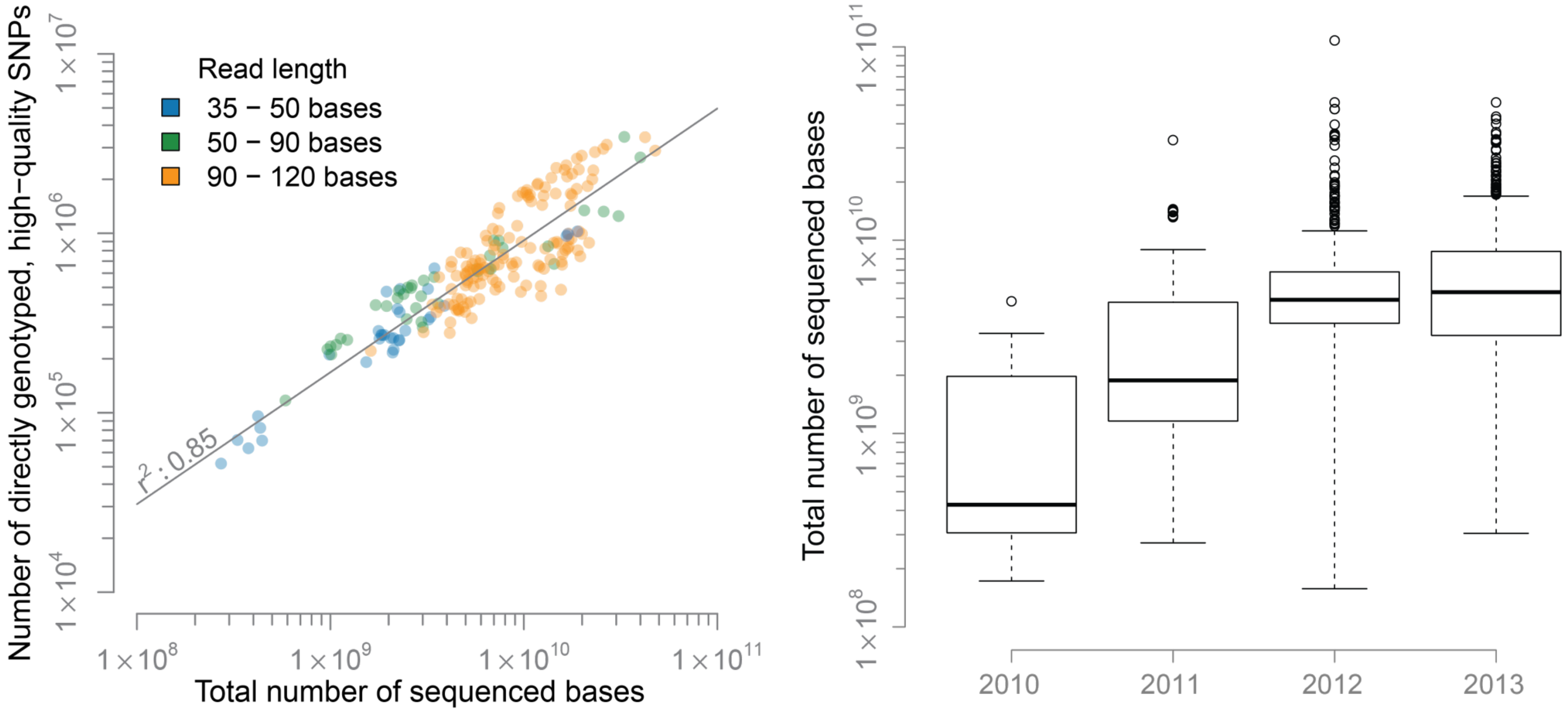
Public RNA-seq data (5,000 samples)



Deelen *et al*, Genome Medicine 2015

Calling genotypes in RNA-seq data

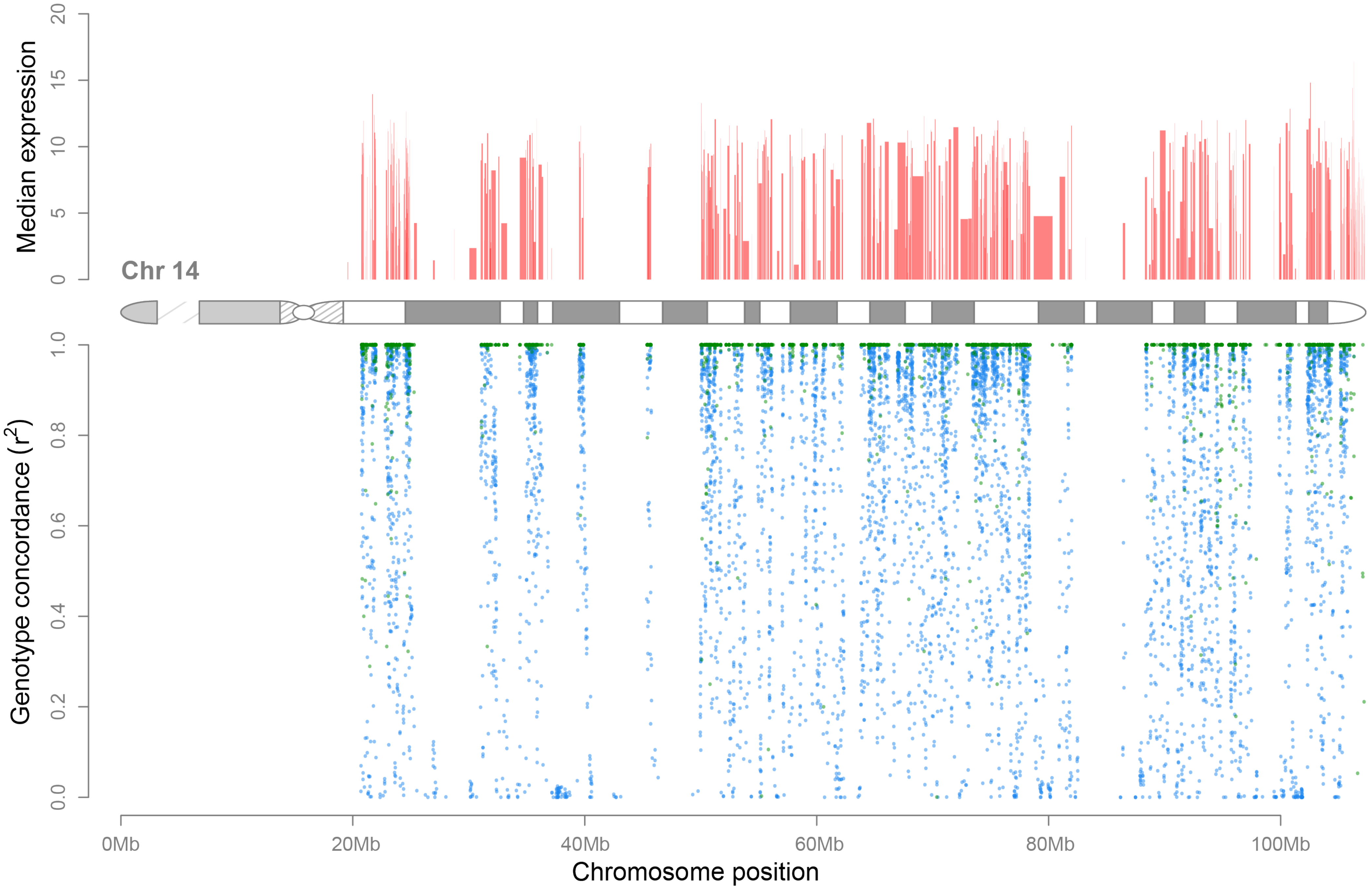
GATK to call genotypes and output genotype likelihoods, BEAGLE used for imputation towards Genome of the Netherlands



Deelen *et al*, Genome Medicine 2015

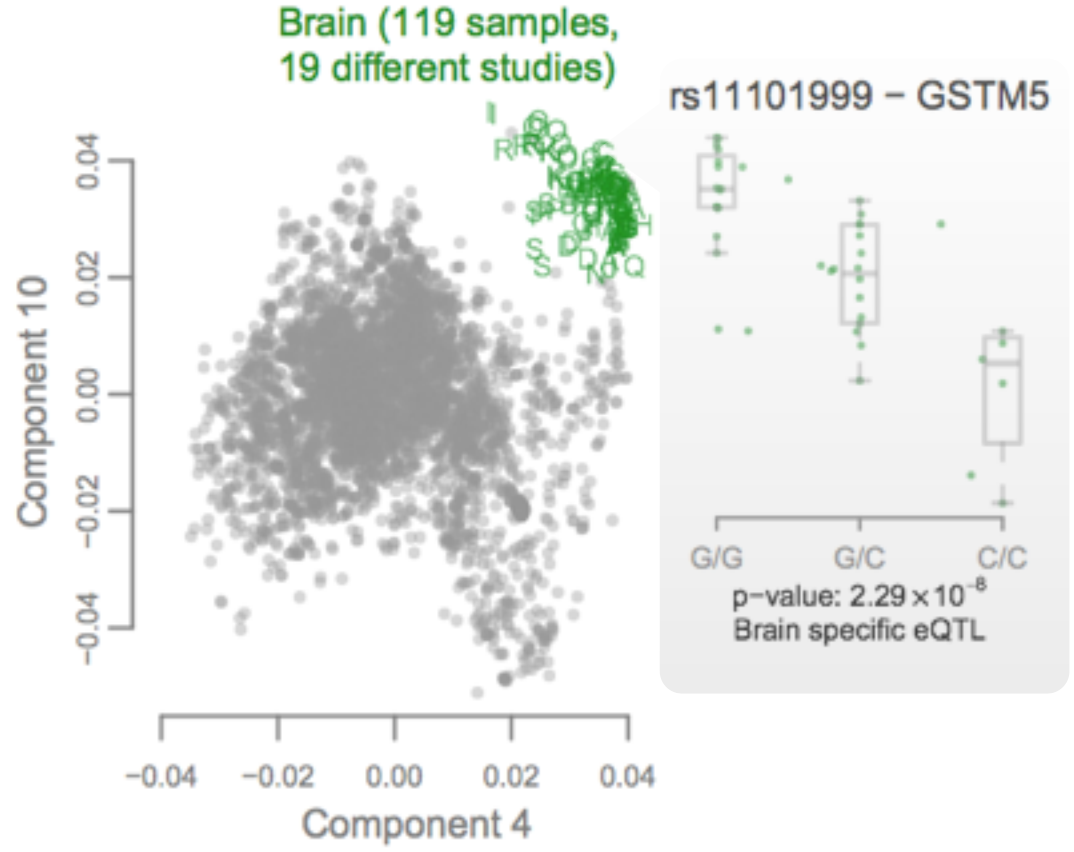
Calling genotypes in RNA-seq data

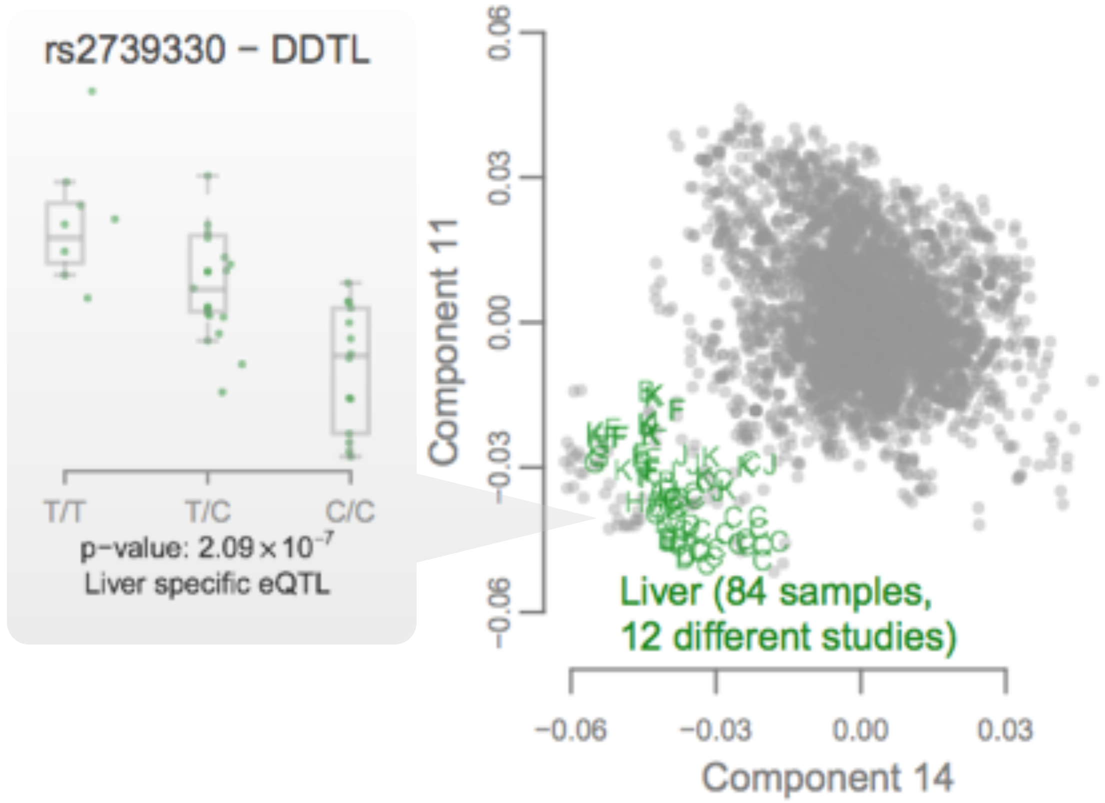
Ability to call SNP is largely dependent on expressed transcripts

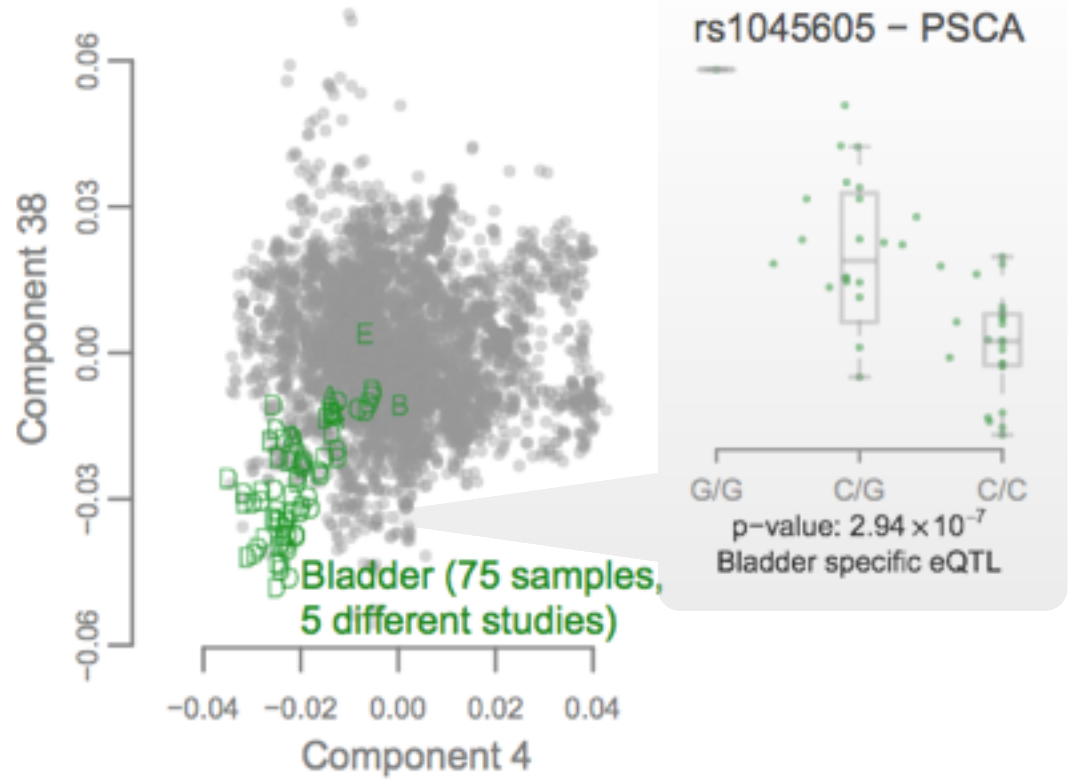


Deelen *et al*, Genome Medicine 2015

Tissue-specific eQTL mapping for free

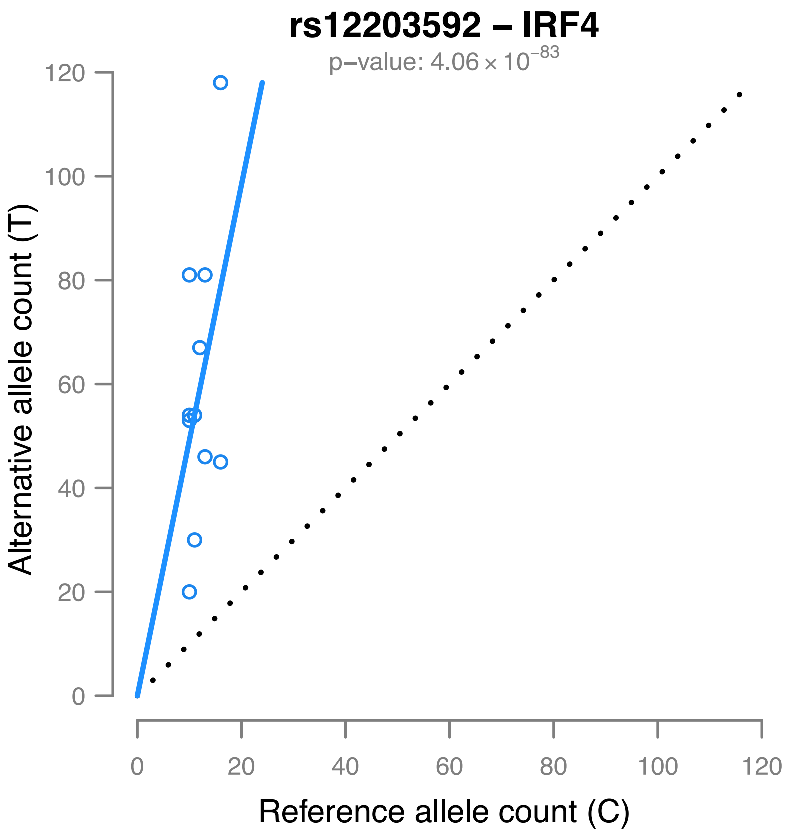


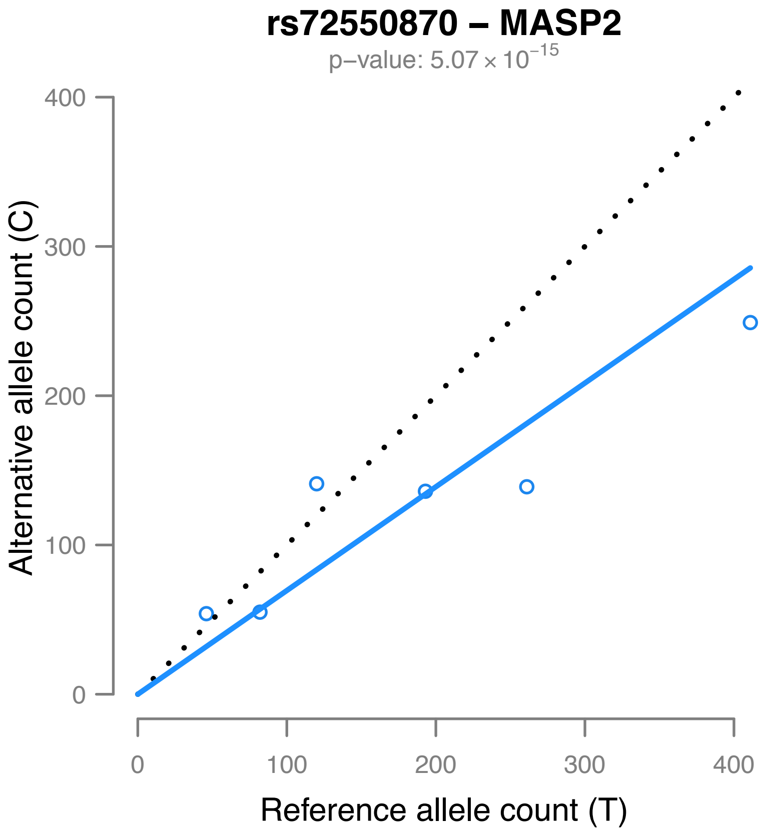


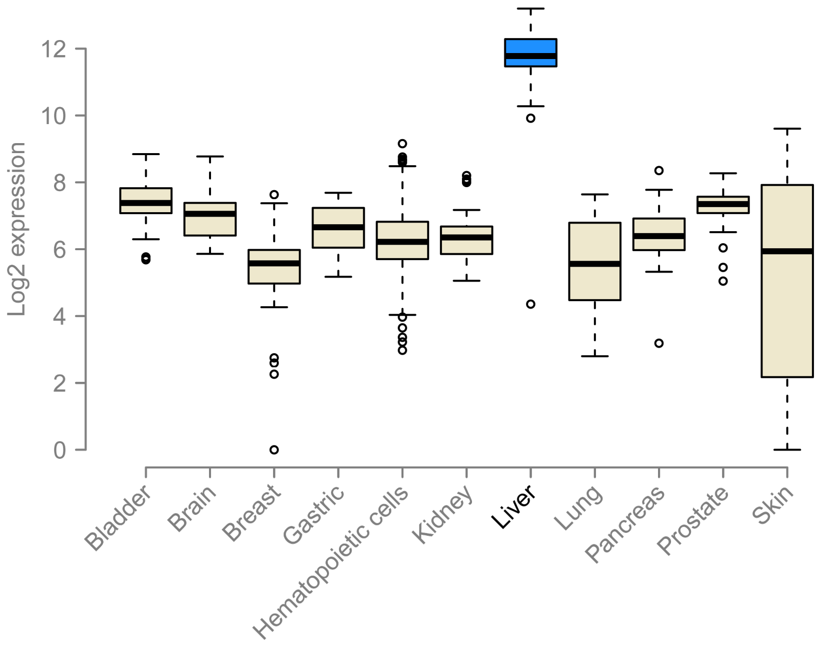


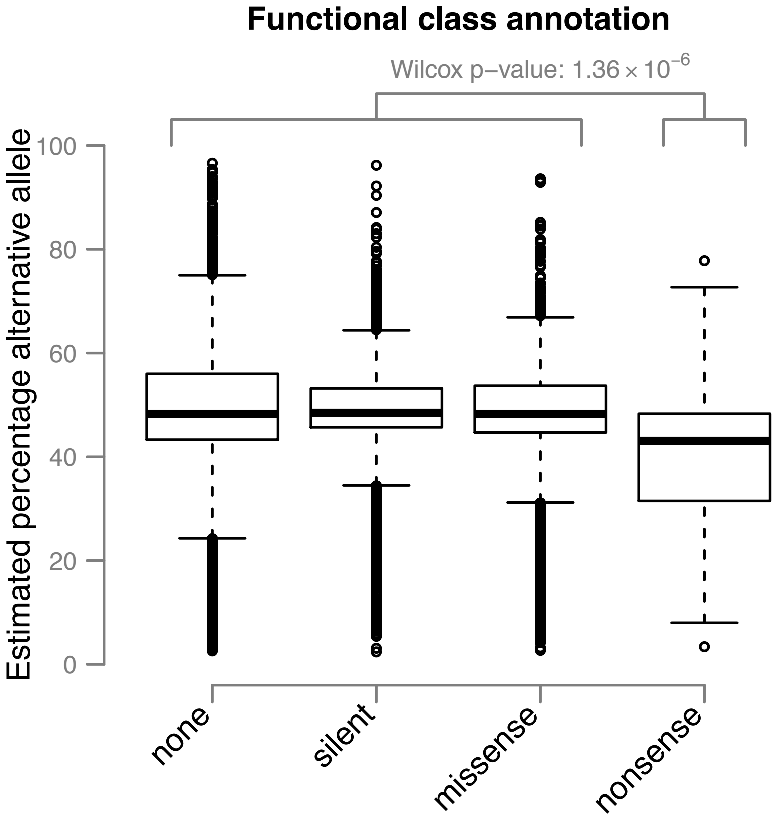
Deelen *et al*, Genome Medicine 2015

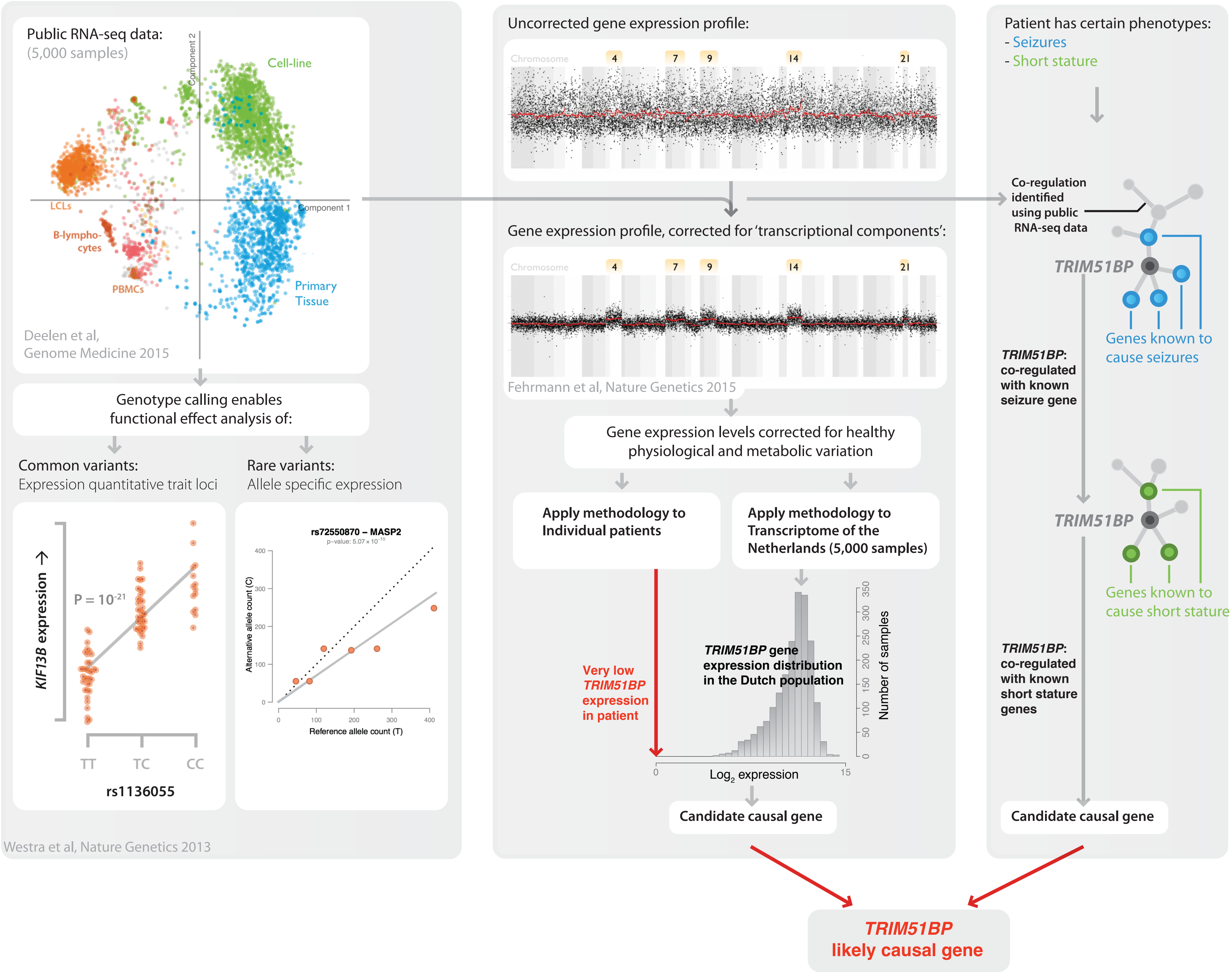
Allele specific effects for rare variants











Public RNA-seq data:
(5,000 samples)

Deelen et al,
Genome Medicine 2015

Genotype calling enables
functional effect analysis of:

Common variants:
Expression quantitative trait loci

Rare variants:
Allele specific expression

KIF13B expression →
P = 10^{-21}
TT
TC
CC
rs1136055
rs72550870 – MASP2
p-value: 5.07×10^{-15}
Alternative allele count (C)
Reference allele count (T)
0
100
200
300
400

Westra et al, Nature Genetics 2013

Uncorrected gene expression profile:

Gene expression profile, corrected for ‘transcriptional components’:

Fehrmann et al, Nature Genetics 2015

Gene expression levels corrected for healthy
physiological and metabolic variation

Apply methodology to
Individual patients

Apply methodology to
Transcriptome of the
Netherlands (5,000 samples)

Very low
TRIM51BP
expression
in patient

TRIM51BP gene
expression distribution
in the Dutch population

Candidate causal gene

Patient has certain phenotypes:
- Seizures
- Short stature

Co-regulation
identified
using public
RNA-seq data

Genes known to
cause seizures

TRIM51BP:
co-regulated
with known
seizure gene

Genes known to
cause short stature

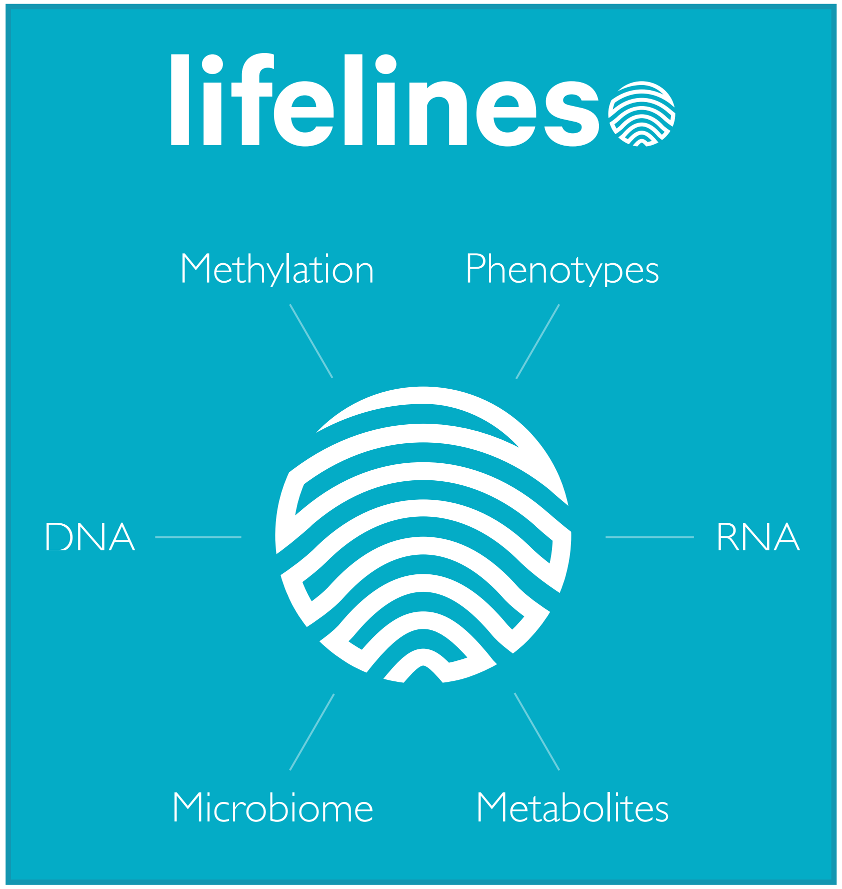
TRIM51BP:
co-regulated
with known
short stature
genes

Candidate causal gene

TRIM51BP
likely causal gene

Integration of different datasets

Lifelines Deep



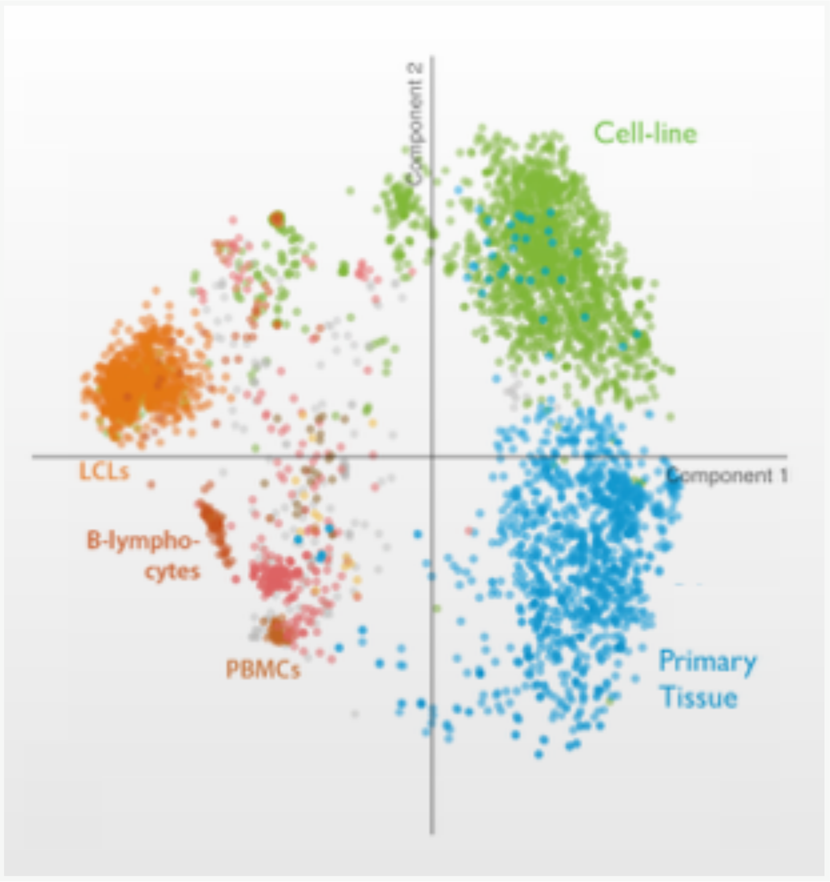
- 1,500 samples
- Many omics levels
- Genotype data
- Extensive phenotyping

Transcriptome of the Netherlands

BBMRI•NL

- 5,000 samples
- RNA-seq data
- Genotype data
- Methylation 450k data

Public RNA-seq data



- 25,000 samples
- RNA-seq data
- Genotype data

Conclusions

- Enormous opportunities exist when recycling ‘big data’, permits gaining insight into downstream consequences of (rare) genetic variants

- Workshop: how to conduct these analyses yourself:
  - Pointers to the software that is available
  - Identifying sample mix-ups
  - Correcting for unknown confounders
  - Multiple testing correction
  - Allele specific expression

Acknowledgements >

UMC Groningen	Juha Karjalainen Dasha Zhernakova Patrick Deelen Marc Jan Bonder Sipko van Dam Morris Swertz	Freerk van Dijk Niek de Klein Urmo Vosa Annique Claringbould Rudolf Fehrmann Cisca Wijmenga
BBMRI-NL BIOS Consortium	Peter-Bram ’t Hoen	Bas Heijmans
eQTLGen Consortium	Tonu Esko	Tune Pers
Target Project CIT RUG	Haije Wind	

Funding >

erc

SEVENTH FRAMEWORK PROGRAMME

NWO

BBMRI-NL