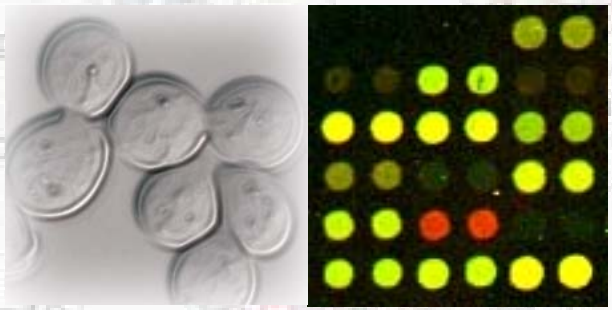
Defining functional modules from genetic interaction profiles obtained with GIM (*Genetic Interactions Mapping*)



Alain Jacquier

Institut Pasteur, Paris, France

Decourty*et al.*, ***PNAS*** 2008

Thank you for inviting me to the UCSB…

CosminSaveanu (with Felix)

Macromolecular Interaction Genetics lab

INSTITUT PASTEUR

Major line of researches:

Study of RNA metabolism in eukaryotes

Use of genomic tools in the yeast *Saccharomycescerevisiae*taken as a model organism

S. cerevisiaegenome sequence

First eukaryotic genome sequenced (1996)

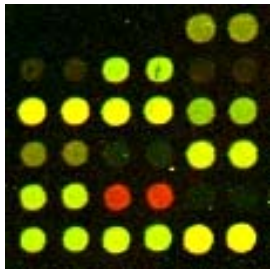
- 6000 genes

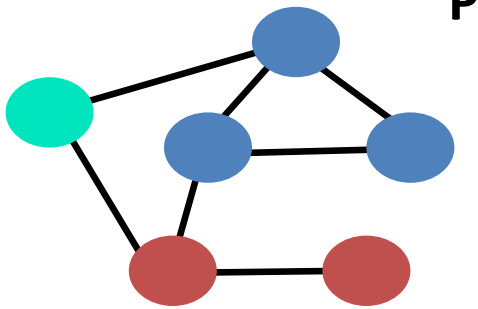
- Only ≈ 2500 - 3500 genes with “some function” assigned

- More than 200 genes coding for proteins with RNA binding motif signatures.

Global yeast genes analyses

Transcriptome analyses
(*PF2*)

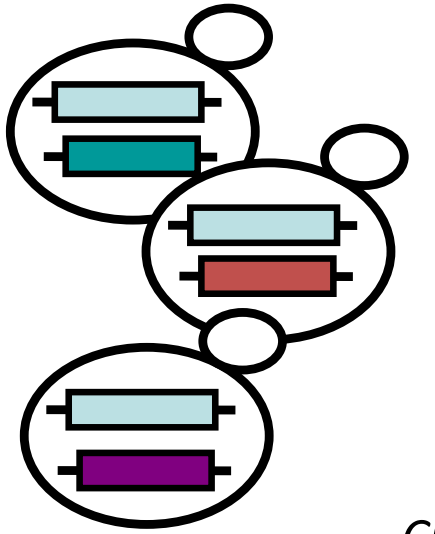




Protein-protein interactions

2-Hybrid
Micheline Fromont
Laurence Decourty

TAP biochemical purifications
Cosmin Saveanu, PF3



Large scale
Genetic Interaction Mapping
(GIM)

Laurence Decourty,
Christophe Malabat, Antonia Doyen, Cosmin Saveanu

Gene deletions or disruption at the genomic scale

- Most of the genes are not essential under laboratory growth conditions:
  - *S. cerevisiae* – Giaever 2002, *Nature*
  - *S. pombe* – Decottigines 2003, *Genome Res*
  - *D. melanogaster* – Boutros, Paddison, 2004 *Science/Nature*
  - *C. elegans* – Kamath 2003, *Nature*

- Macroscopic phenotypes are rarely informative of the gene function

Genetic robustness of cells

In the yeast *S. cerevisiae*, only 19% of the genes are essential.

In addition, only 15% of the non essential genes exhibit a fitness defect when deleted and the cells grown in standard laboratory conditions.

There are potentially several reasons for that:

- Most of the genes might carry functions only required in particular conditions.

    ->Example (auxotrophy in complete medium)

Genetic robustness of cells

-Another, non exclusive, explanation could be a high redundancy of the system.

->*When a pathway is not functional, the cell uses alternative routes.*

One way to address this question is to map genetic interactions to understand the rules that govern these interactions

Understand the rules that govern genetic interactions at the genomic level

- Contribute to the characterization of genes of yet unknown function

- Important in order to understand how a given allele will result in different phenotypes depending on the genetic background

- This has important implications for Genome-Wide Association Studies (GWAS)

What types of genetic interactions?

Synthetic lethality (Synthetic Growth Deffects: aggravating effect)

What types of genetic interactions?

Synthetic lethality (Synthetic Growth Deffects: aggravating effect)

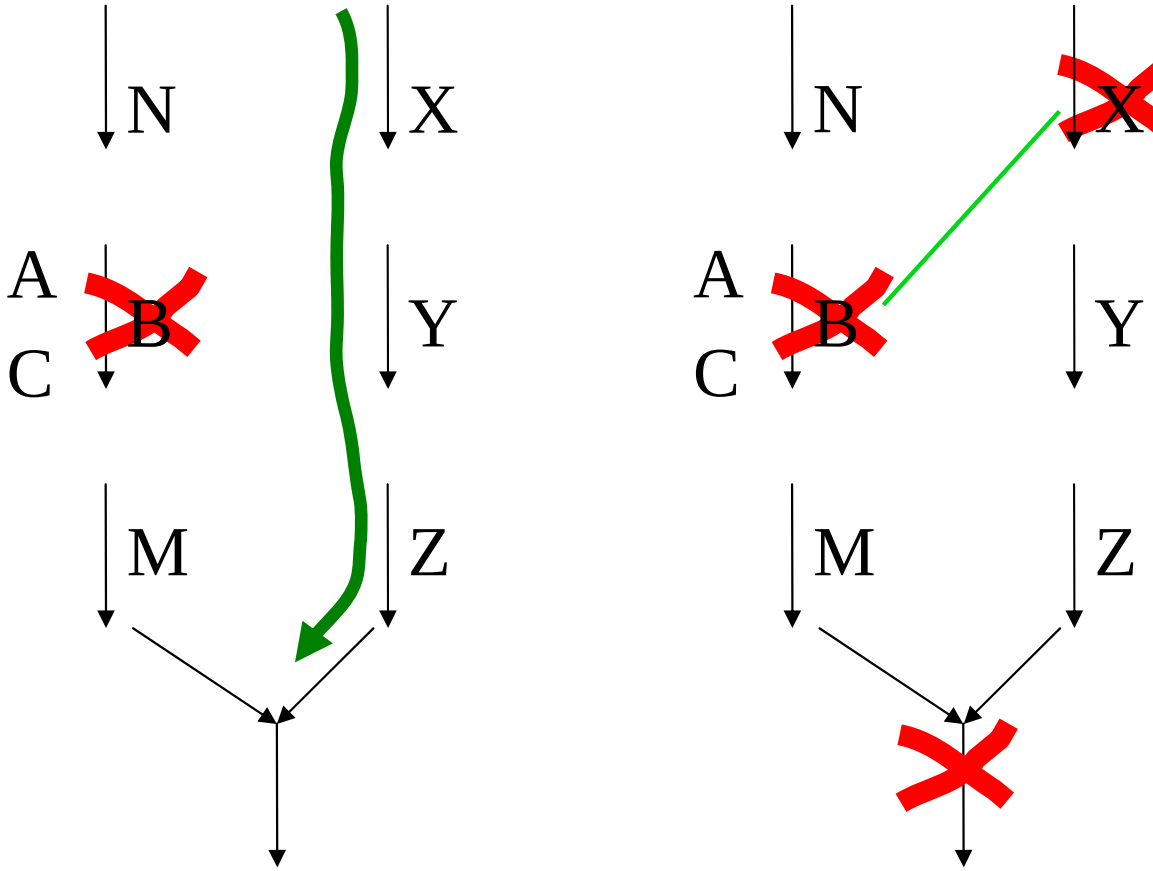
Most simple example: duplicated essential genes.

(Genes coding for Ribosomal Proteins in *S. cerevisiae*for example)

What types of genetic interactions?

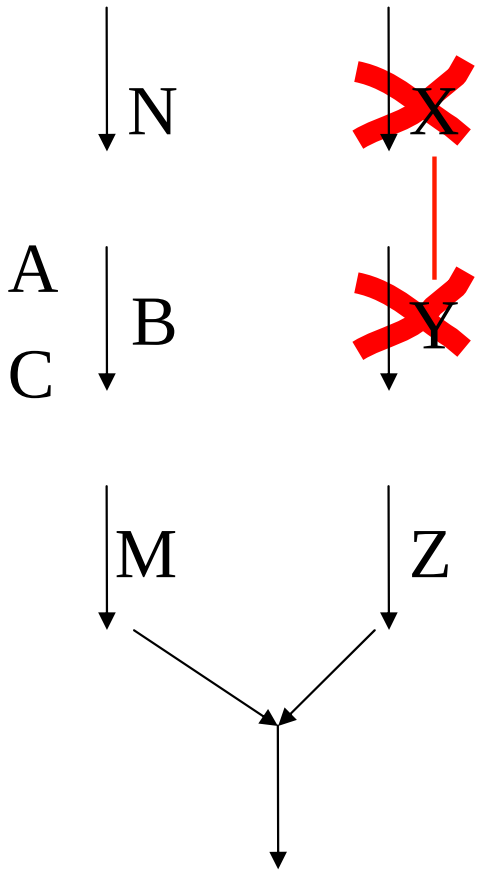
Synthetic lethality (Synthetic Growth Deffects: aggravating effect)

Another simple model



What types of genetic interactions?

Buffering interactions: epistatic interaction

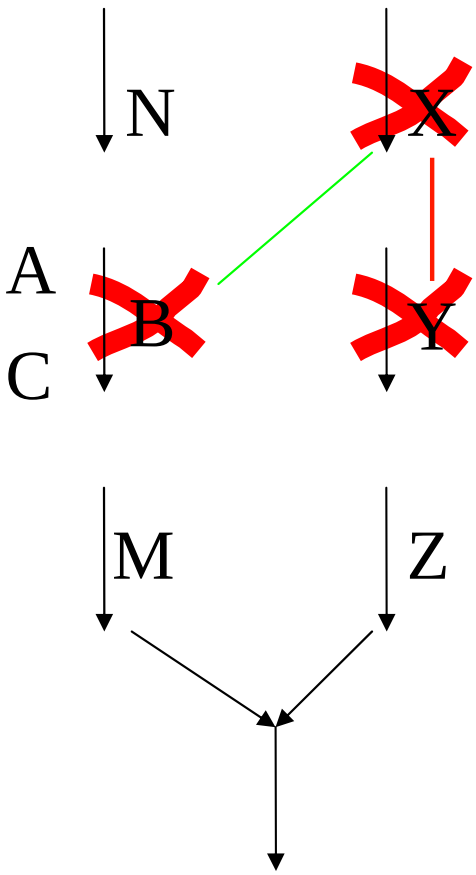


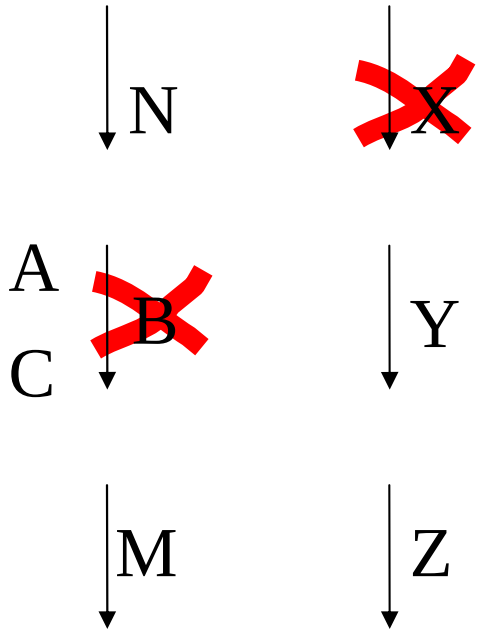
In this simple model, the single mutations *x*and *y*induce the same growth defect as the double mutation *xy*

Suppression

The mutation *y* is a suppressor of the *x* mutation if the double mutant *xy* grows better than the single mutant *x*.

General definition of epistasis





The effect of mutations in different pathways are very rarely perfectly additive !

General definition of epistasis

$$\varepsilon = W_{XY} - W_X W_Y$$

No epistasis	$\varepsilon = 0$
Aggravating[b]	$\varepsilon < 0$
Buffering[c]	$\varepsilon > 0$

W_X and W_Y represent the fitness values of single mutants and W_{XY} represents the fitness value of the corresponding double mutant.

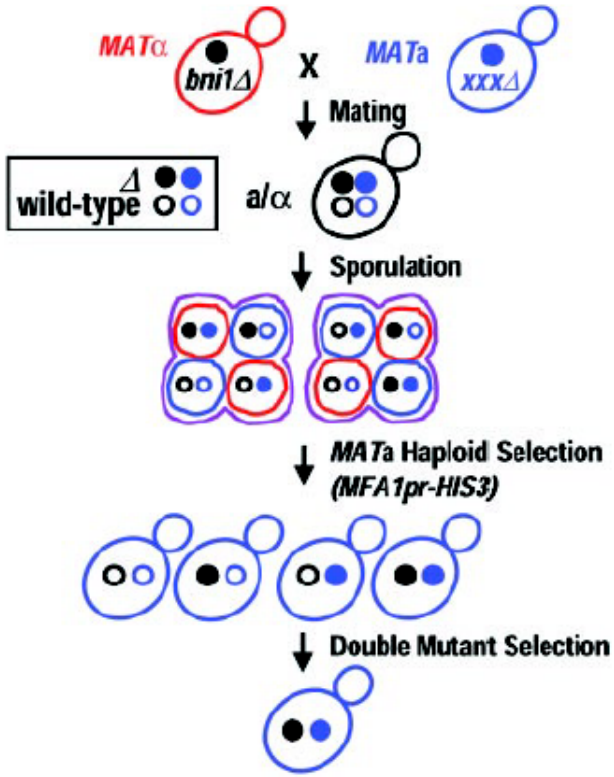
Adaptedfrom: Segré *et al.* (2005) *Nature Genetics*

First large scale synthetic lethality screens

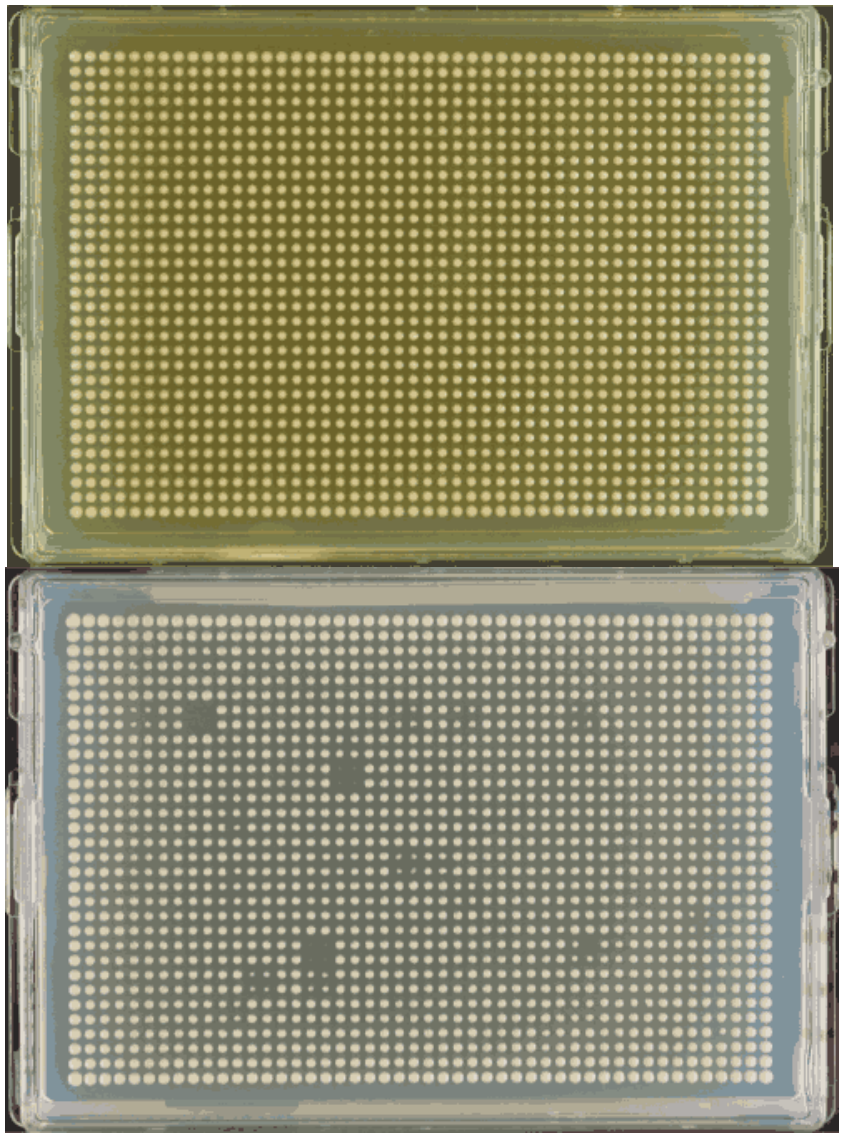
Systematic Genetic Analysis with Ordered Arrays of Yeast Deletion Mutants

Amy Hin Yan Tong,[1,2] Marie Evangelista,[3] Ainslie B. Parsons,[1,2] Hong Xu,[1,2] Gary D. Bader,[4,5] Nicholas Pagé,[6] Mark Robinson,[1] Sasan Raghibizadeh,[7] Christopher W. V. Hogue,[4,5] Howard Bussey,[6] Brenda Andrews,[2*] Mike Tyers,[2,5*] Charles Boone[1,2,3*]

Science, 2001



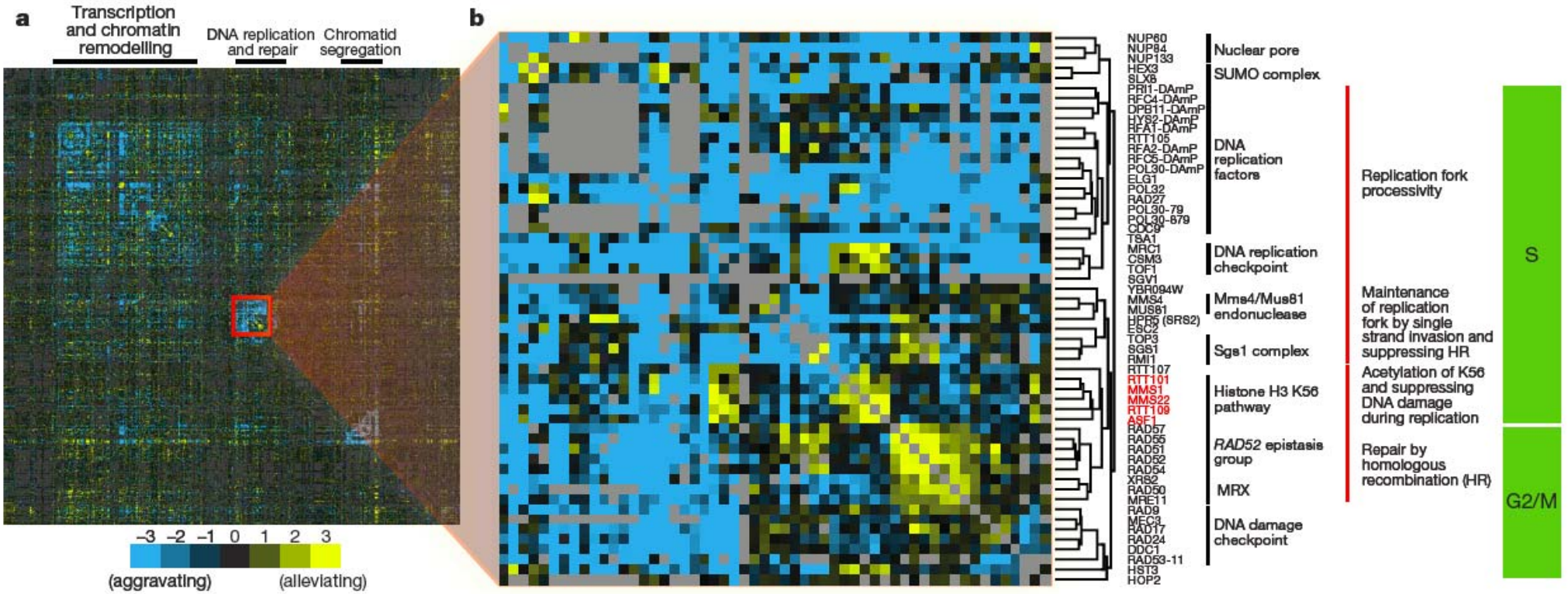
Single mutants from library



double mutants
(with query muation)

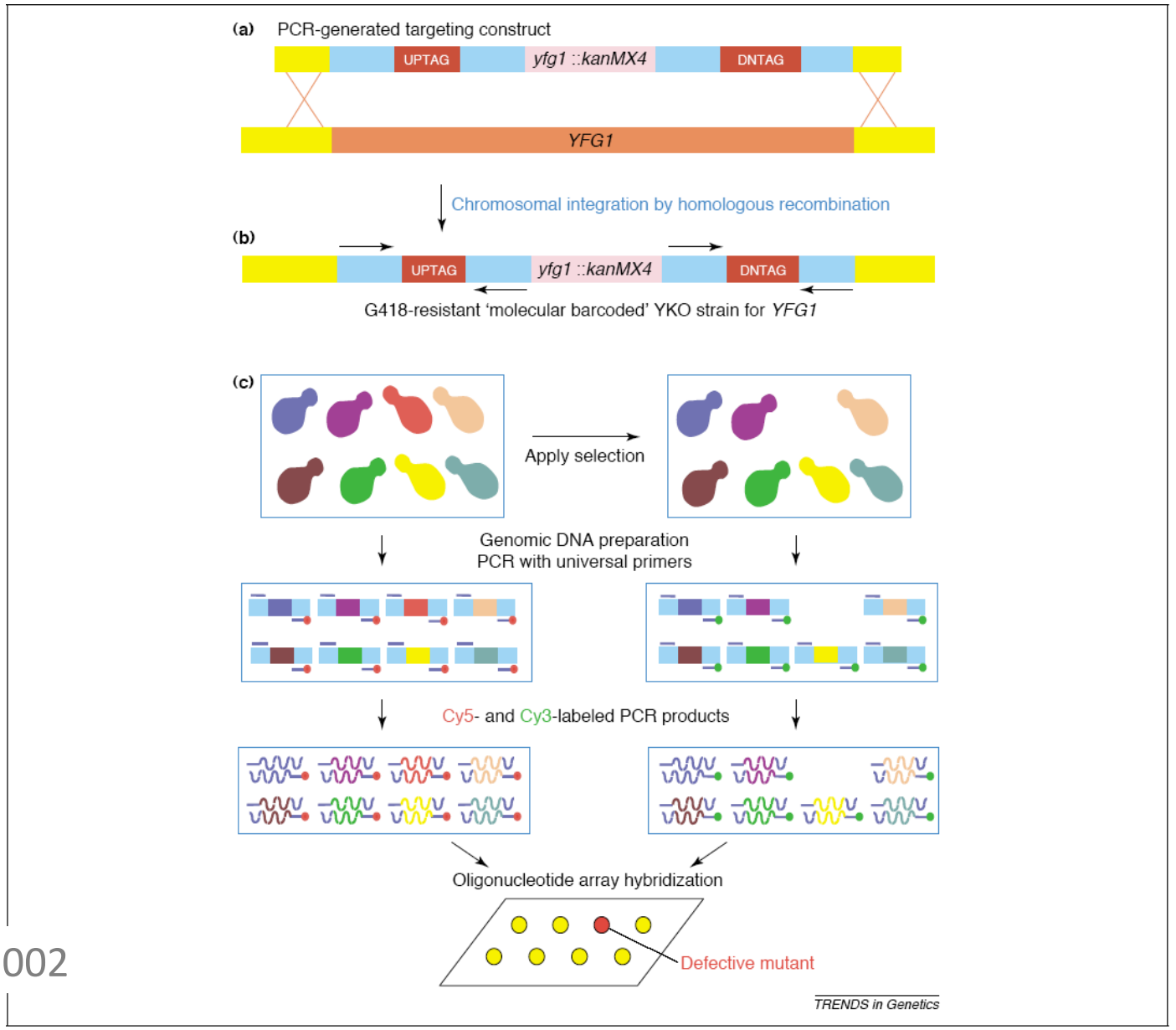
E-maps: quantitative measures and matrices

Krogan'slab: *Cell*2005, *Nature* 2007…



The Genetic Interaction Mapping (GIM) method

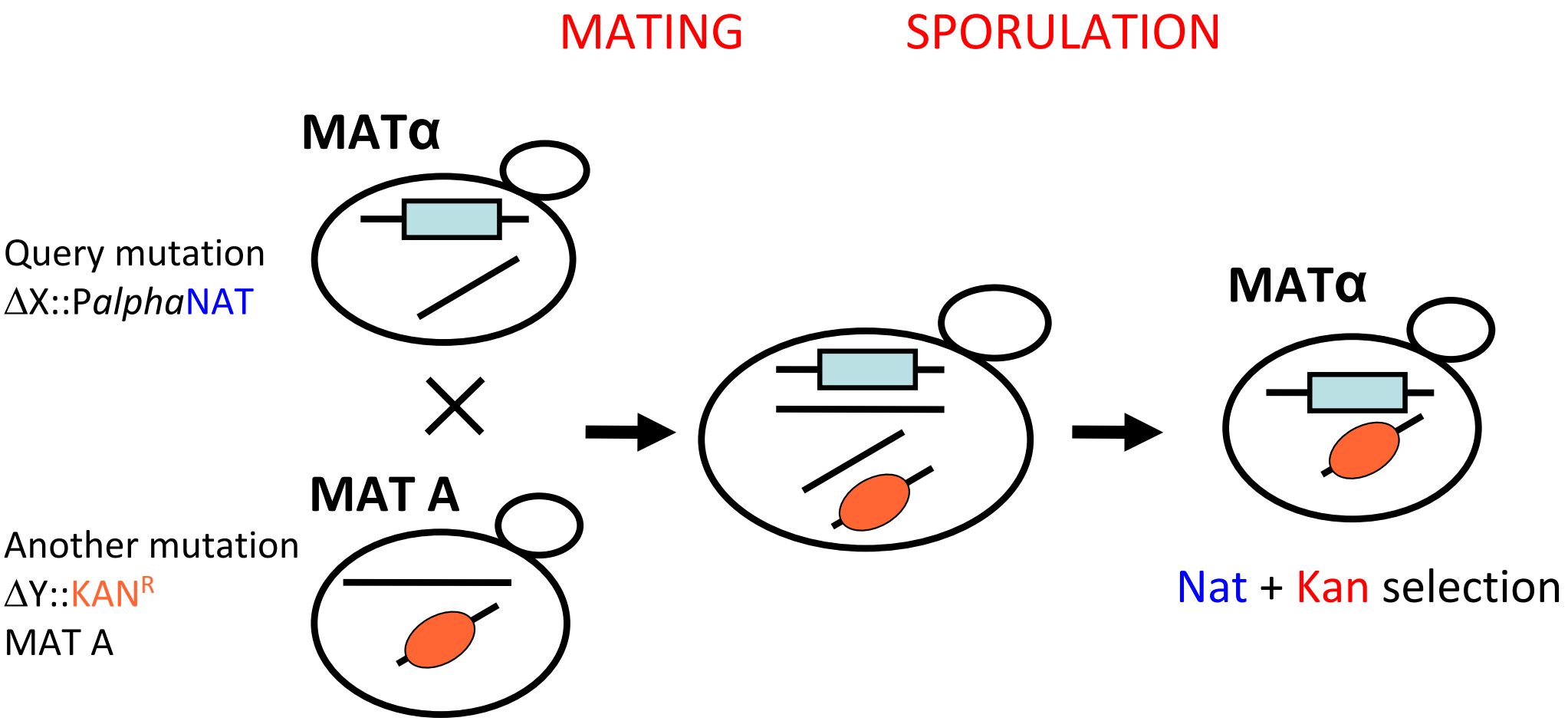
The yeast systematic deletion library is barcoded



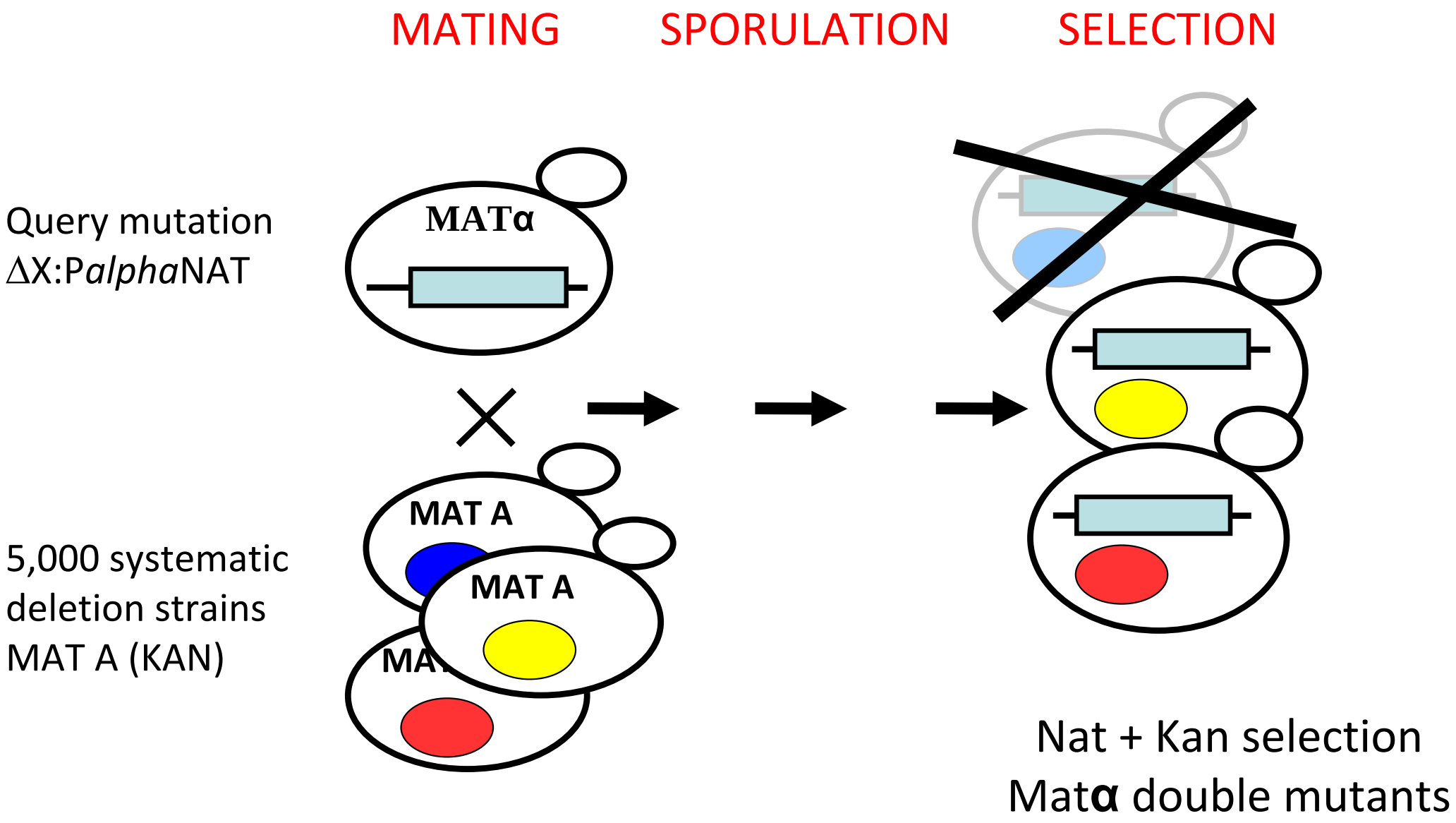
Giaever et al. *Nature* 2002

The Genetic Interaction Mapping (GIM) method

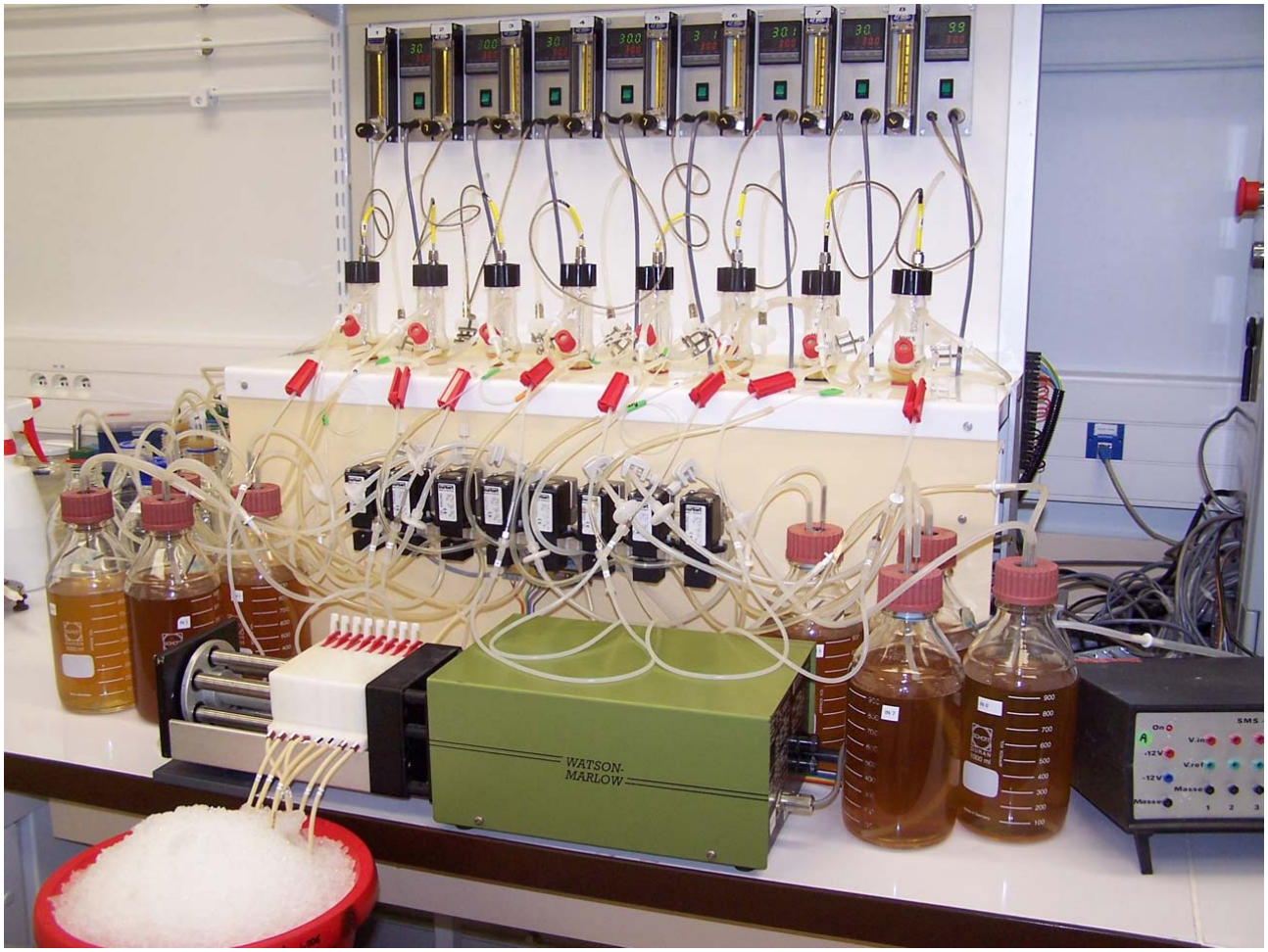
A new marker to easily generate double mutants



The Genetic Interaction Mapping (GIM) method

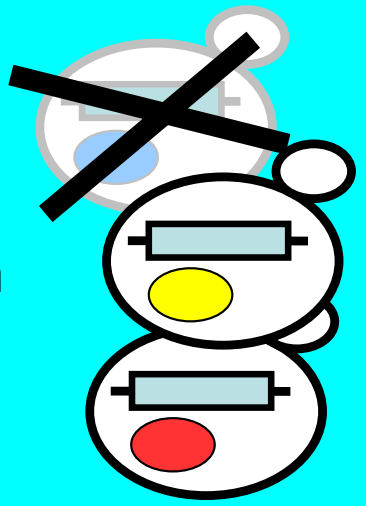


The cultures are performed in a highly controlled manner for a fixed number of generations (18) in a mutiturbidostat (constant OD)

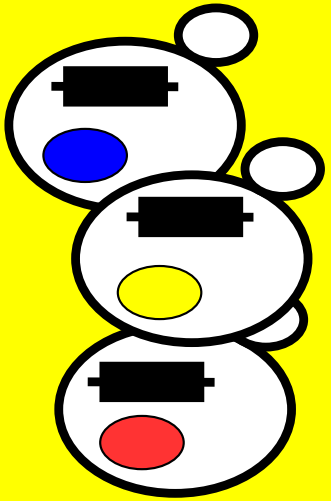


The Genetic Interaction Mapping (GIM) method

Query population

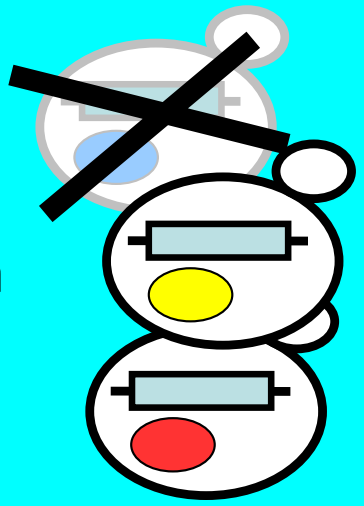


Referenc
e
populatio
n

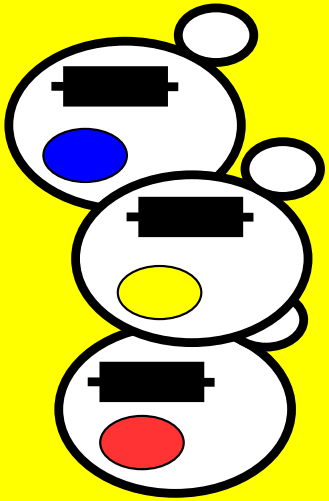


The Genetic Interaction Mapping (GIM) method

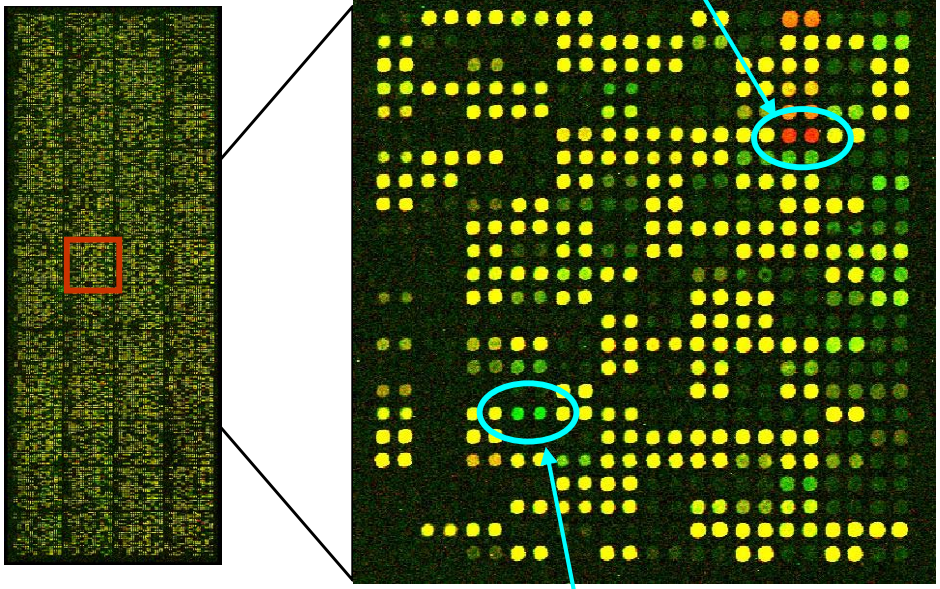
Query population



Reference population



barcodes PCR amplification

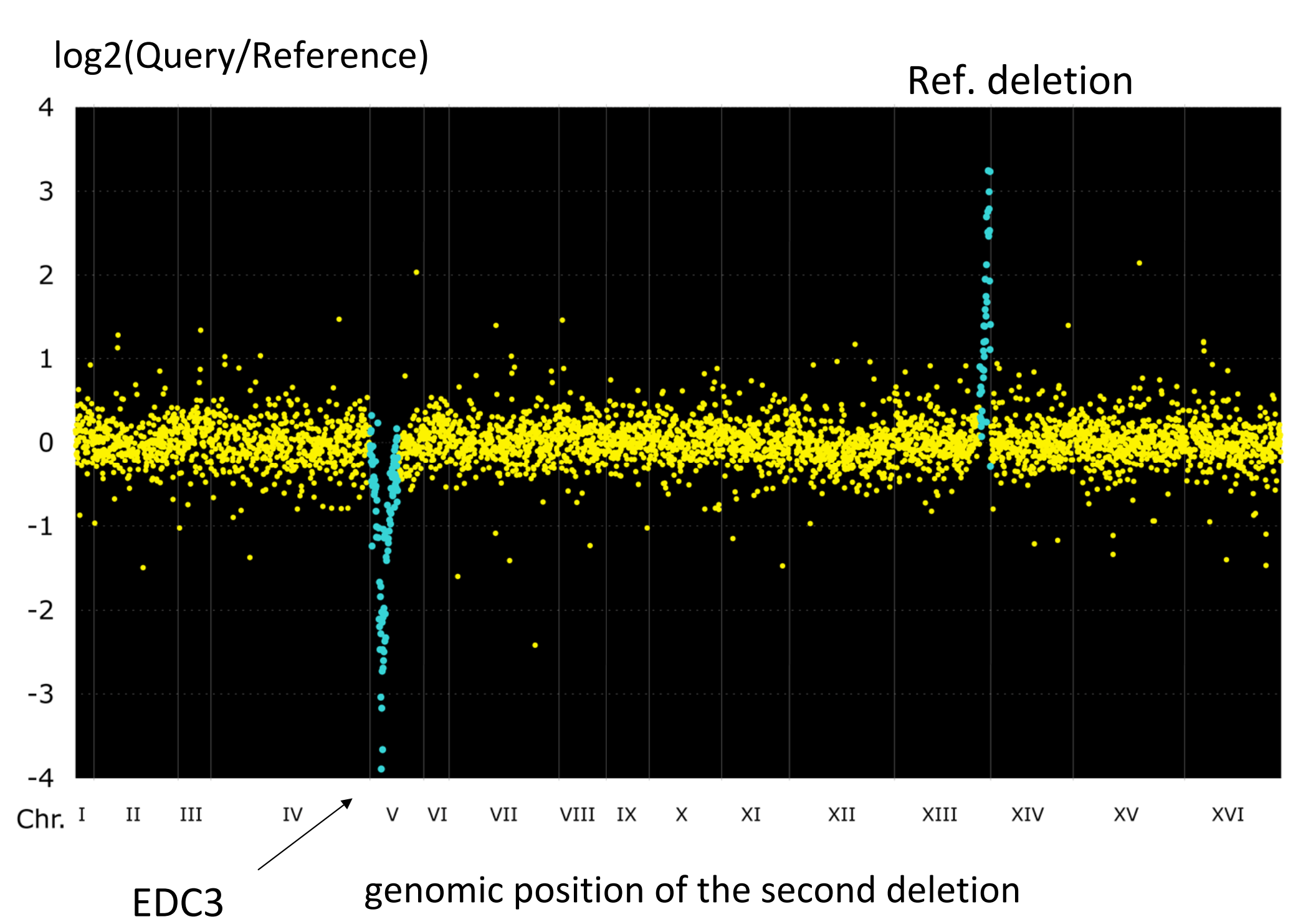


potential alleviating int.

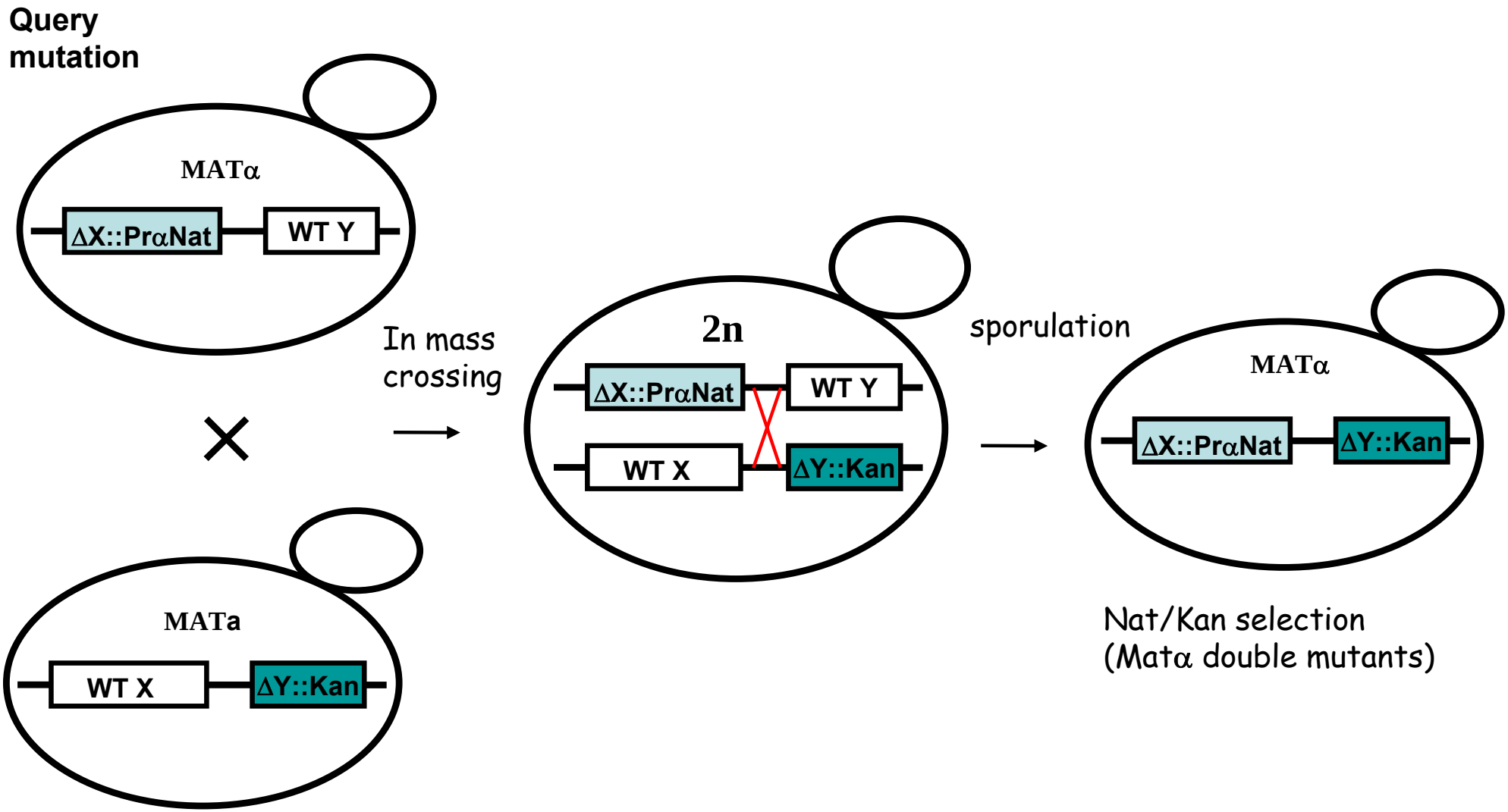
barcodes PCR amplification

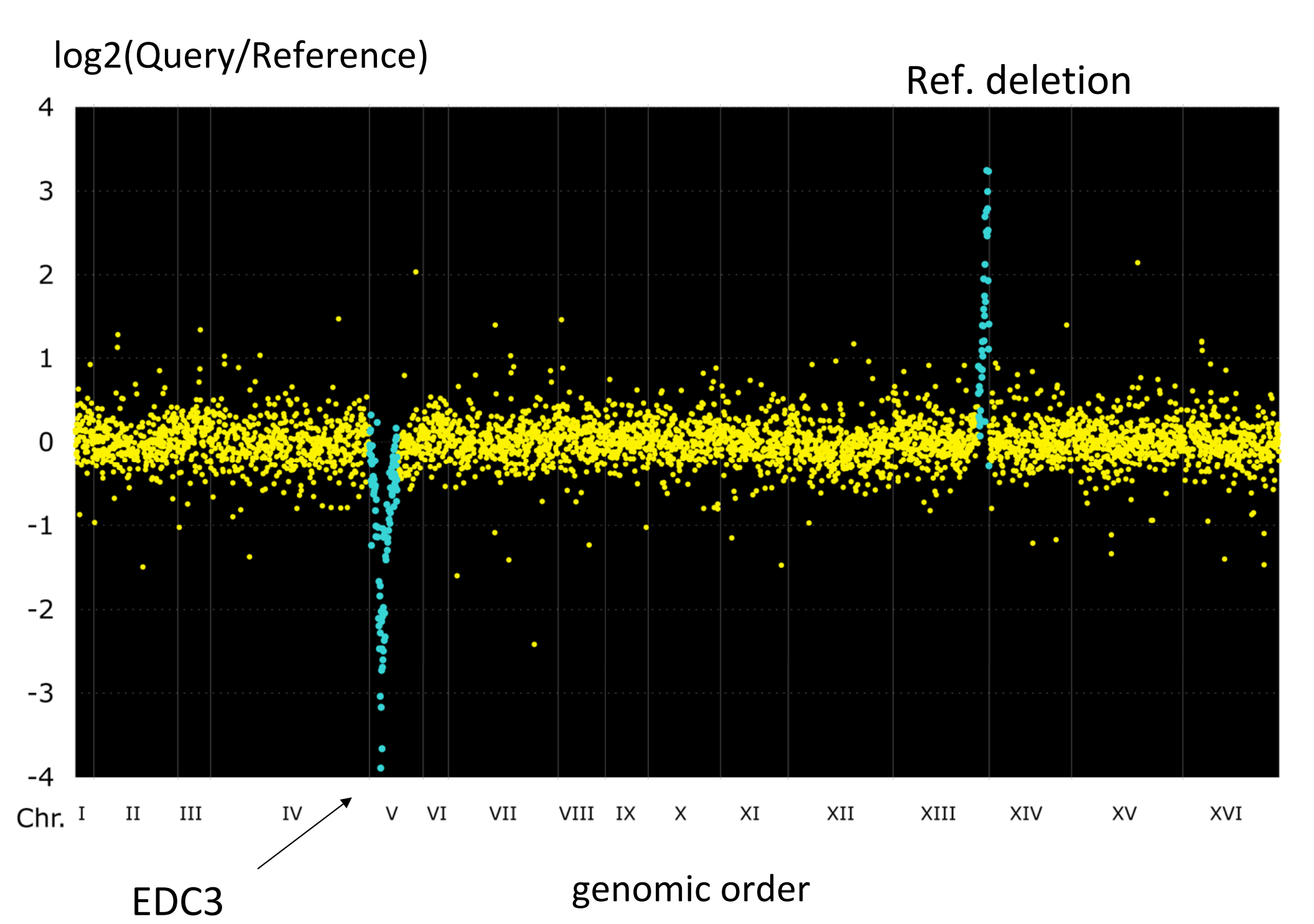
potential aggravating int.

-> log2(Query/Reference) for ~5 000 double mut.


log2(Query/Reference)
Ref. deletion
4
3
2
1
0
-1
-2
-3
-4
Chr.
I
II
III
IV
V
VI
VII
VIII
IX
X
XI
XII
XIII
XIV
XV
XVI
EDC3
genomic position of the second deletion

When the X and Y genes are genetically linked…




log2(Query/Reference)
Ref. deletion
4
3
2
1
0
-1
-2
-3
-4
Chr.
I
II
III
IV
V
VI
VII
VIII
IX
X
XI
XII
XIII
XIV
XV
XVI
EDC3
genomic order

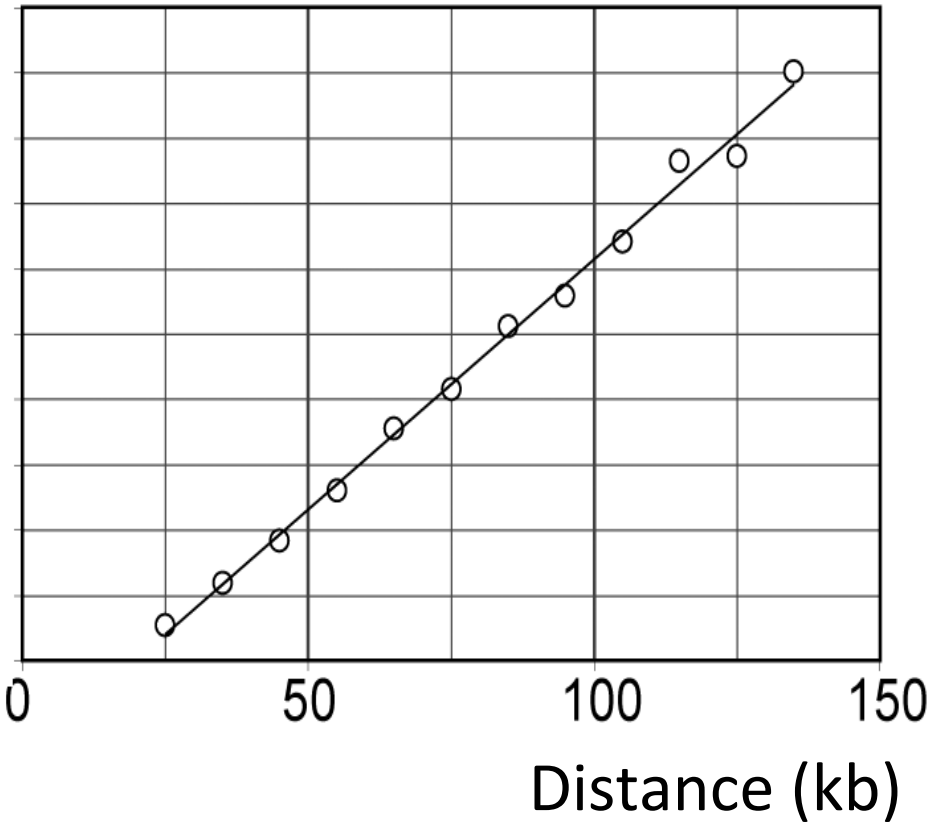
$$r = \frac{1}{2} \cdot (1 - e^{-2 \cdot m})$$

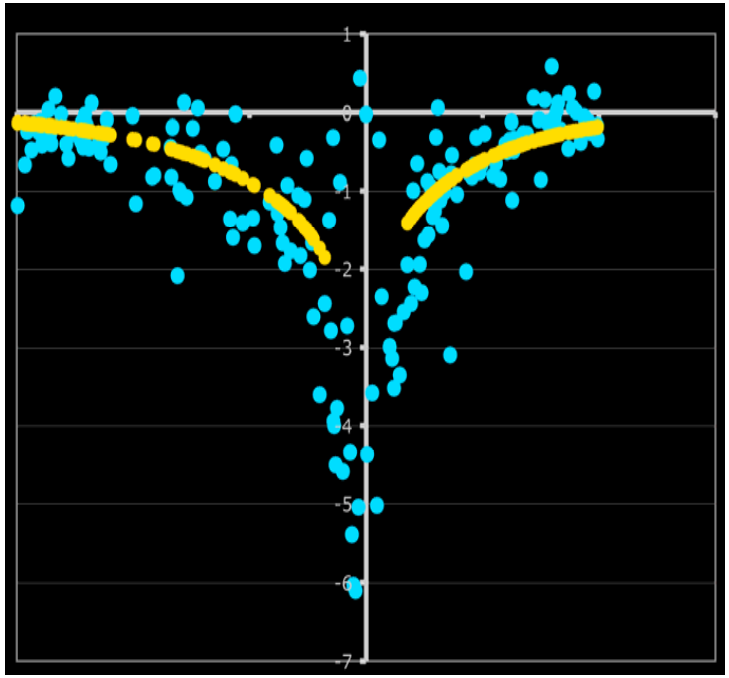
r - *recombination freq.*

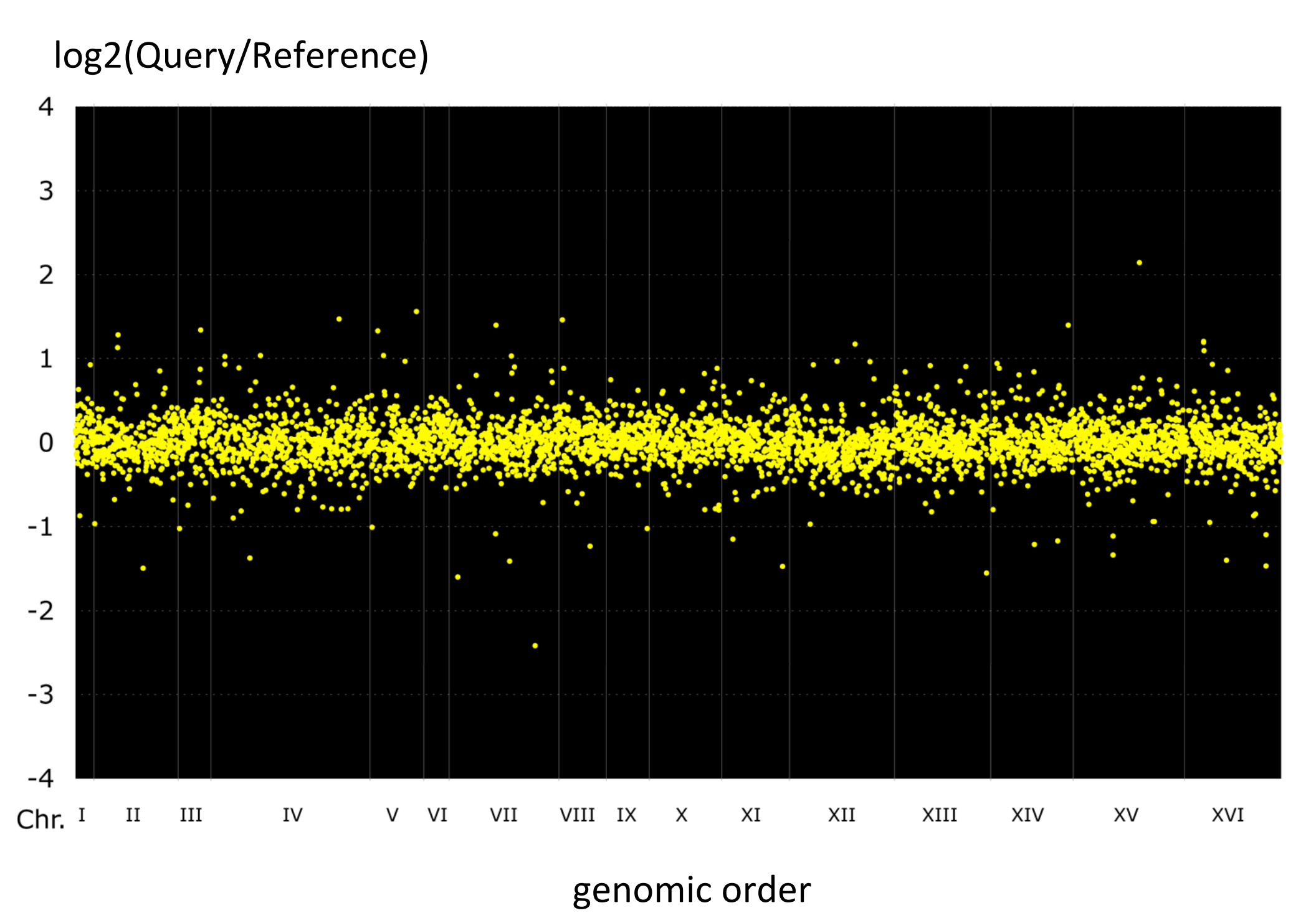
m - *genetic distance*

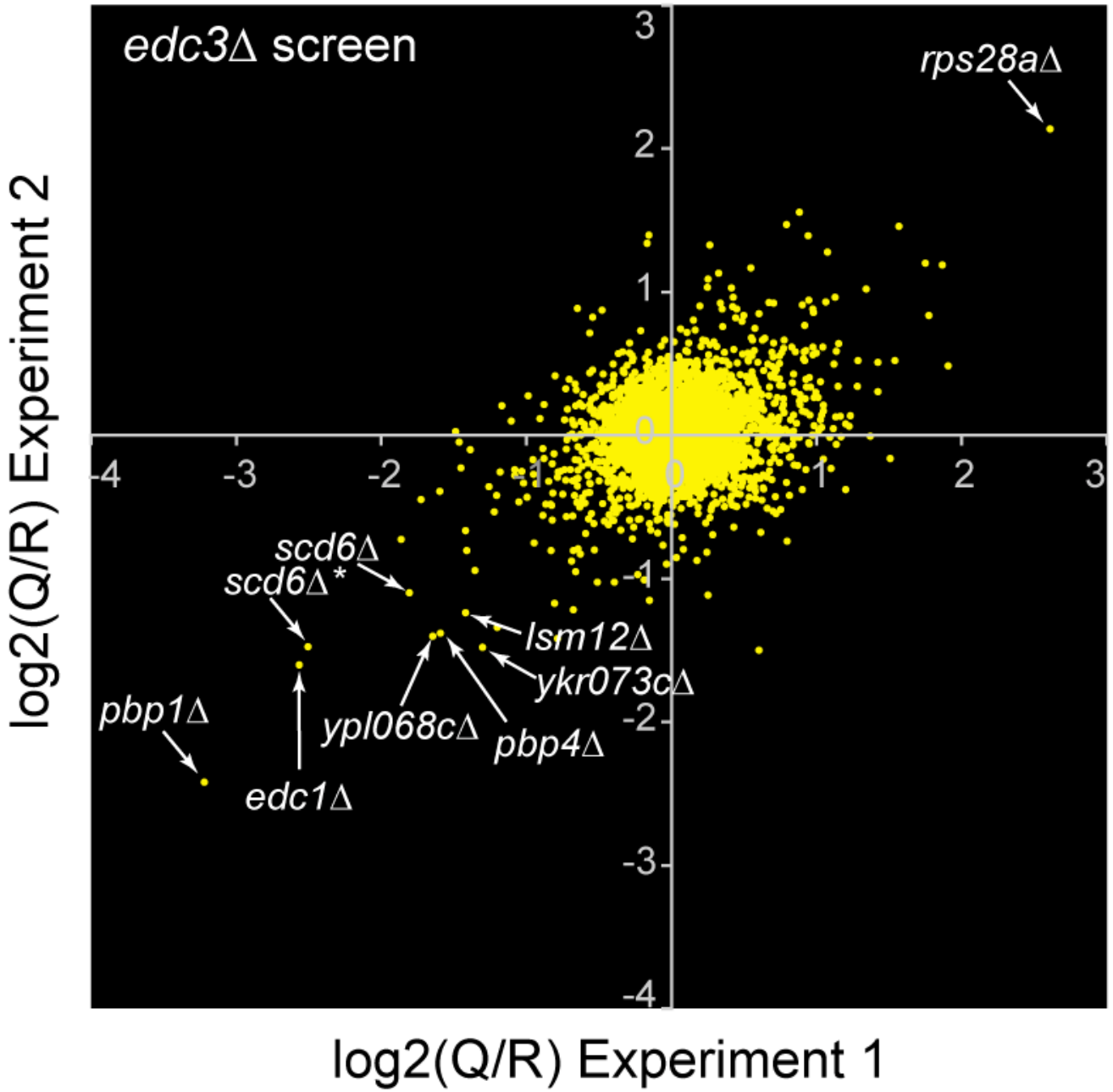
Haldane - 1919

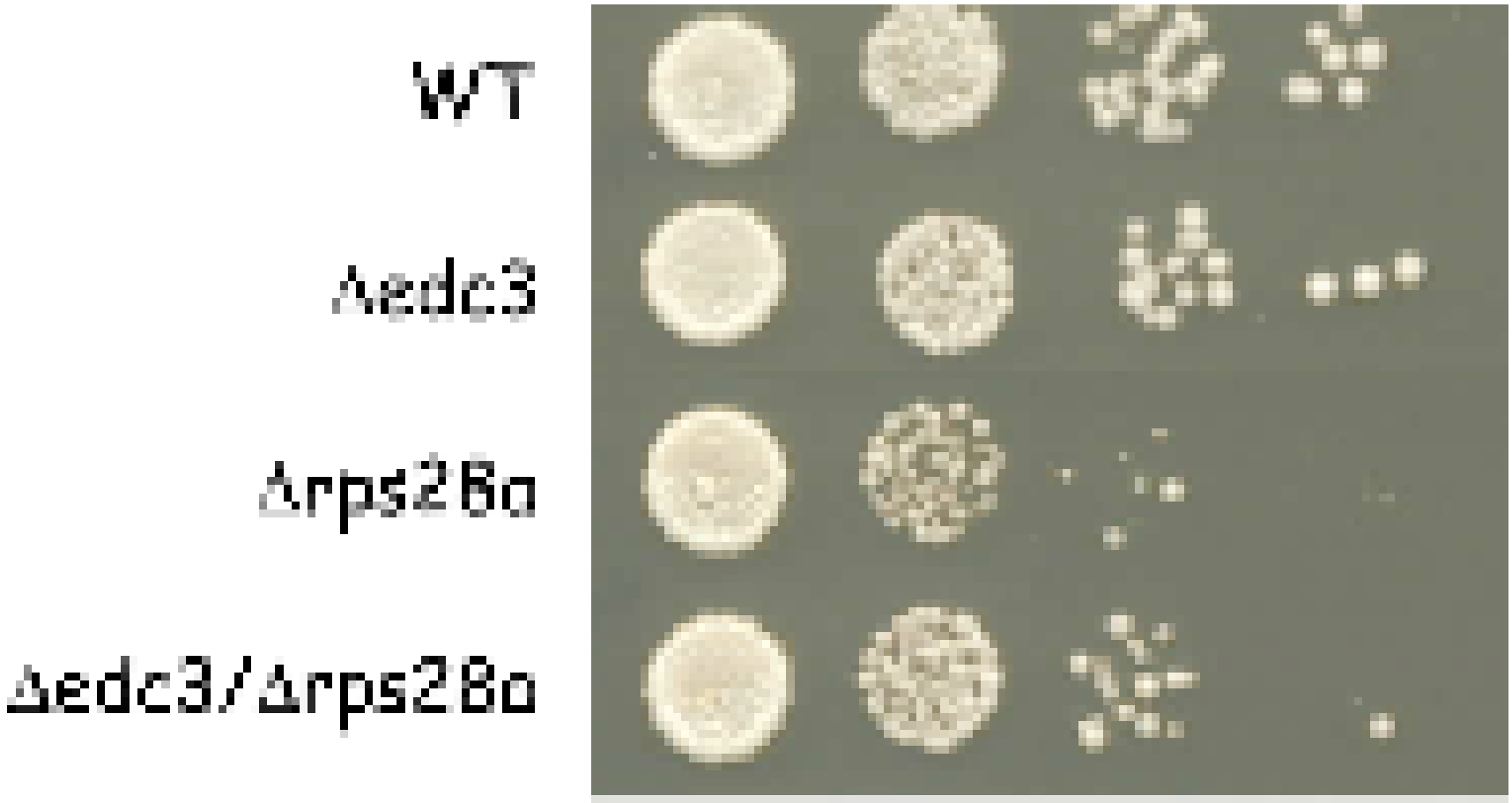
Genetic distance

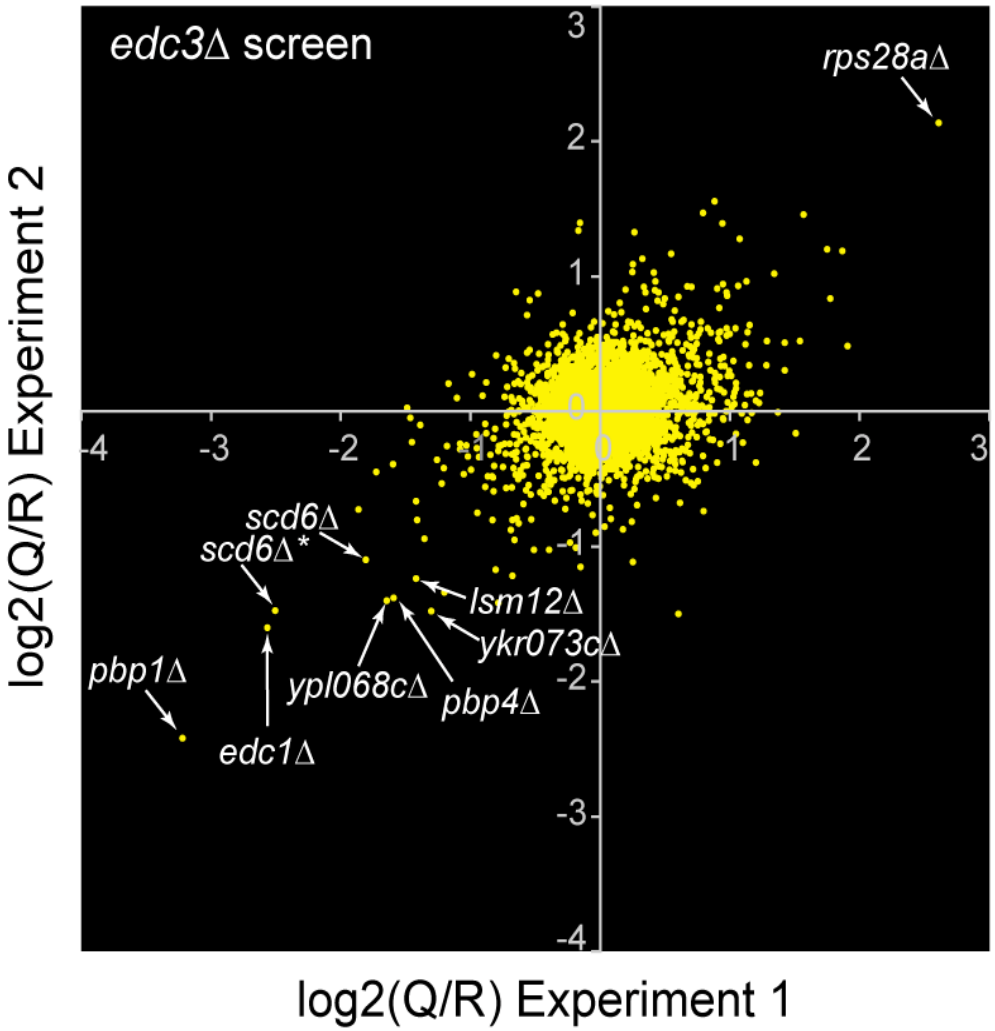


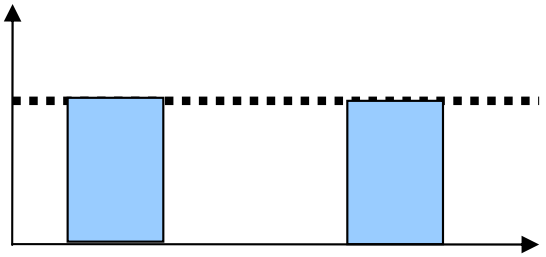



log2(Query/Reference)
4
3
2
1
0
-1
-2
-3
-4
Chr.
I
II
III
IV
V
VI
VII
VIII
IX
X
XI
XII
XIII
XIV
XV
XVI
genomic order

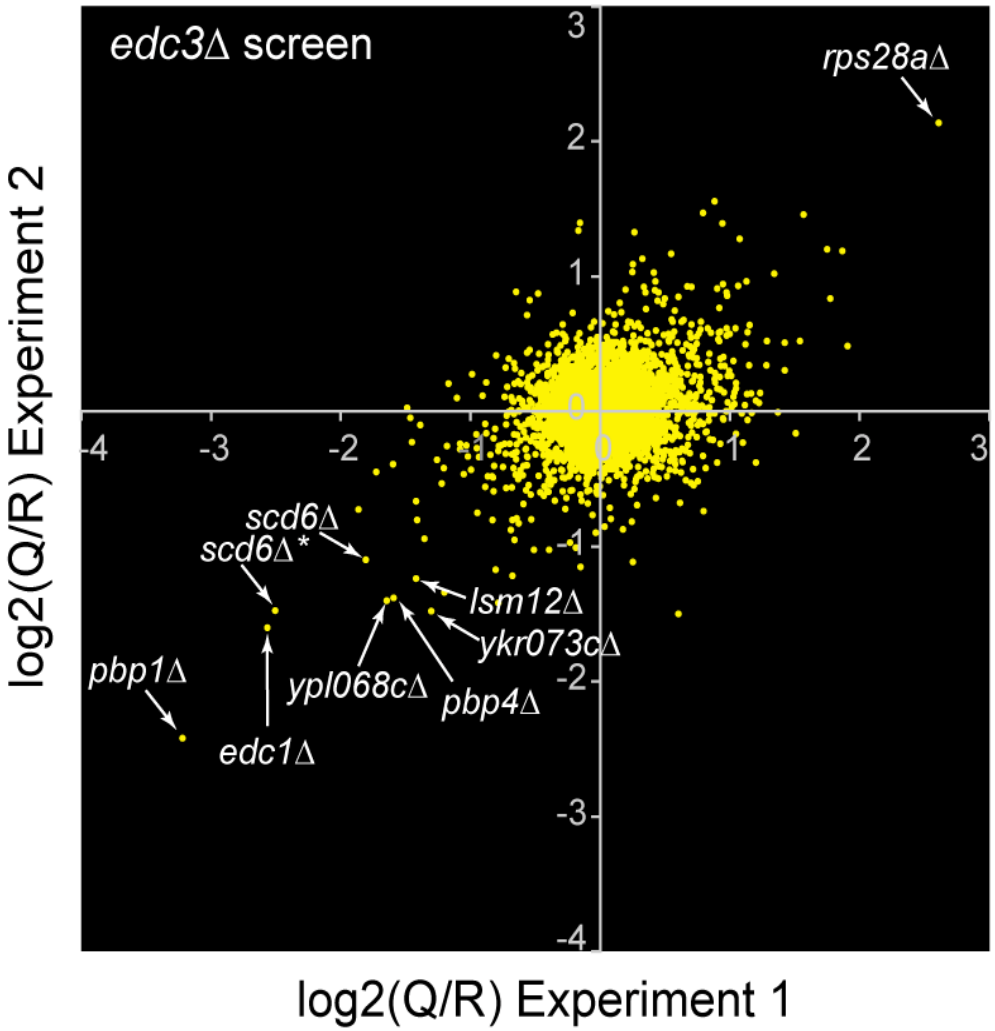

edc3Δ screen
rps28aΔ
scd6Δ
scd6Δ*
lsm12Δ
ykr073cΔ
pbp1Δ
ypl068cΔ
pbp4Δ
edc1Δ
log2(Q/R) Experiment 2
log2(Q/R) Experiment 1
3
2
1
0
-1
-2
-3
-4
-4
-3
-2
-1
0
1
2
3


WT
Δedc3
Δrps28a
Δedc3/Δrps28a





Rps28a Rps28b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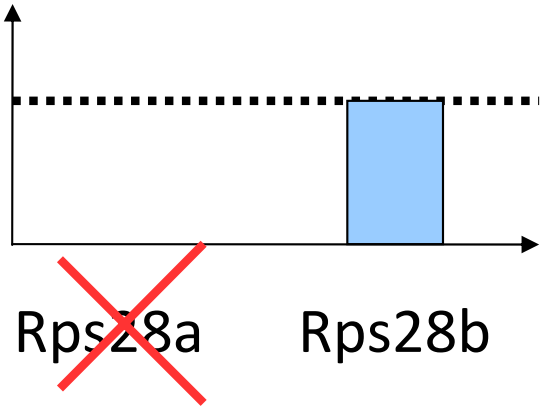
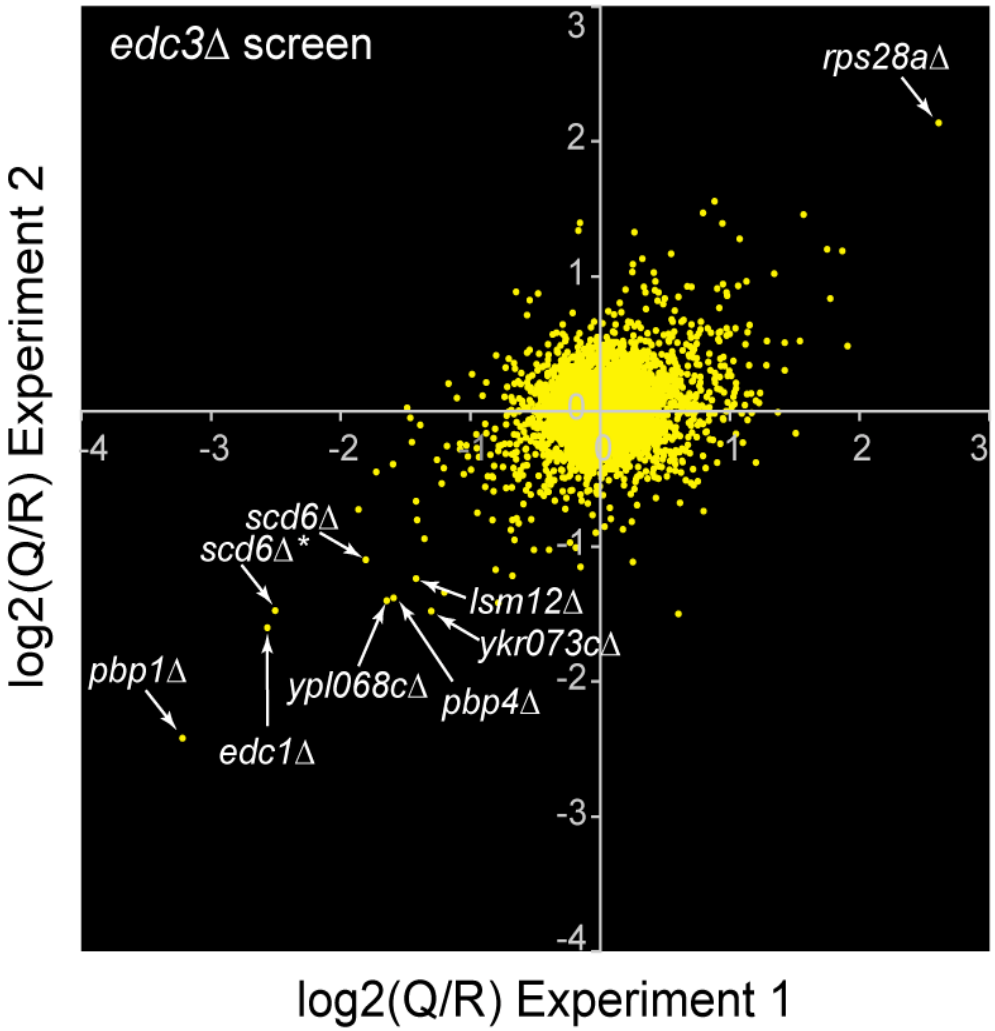
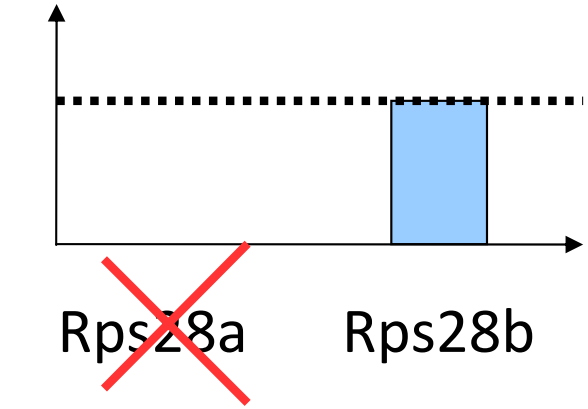
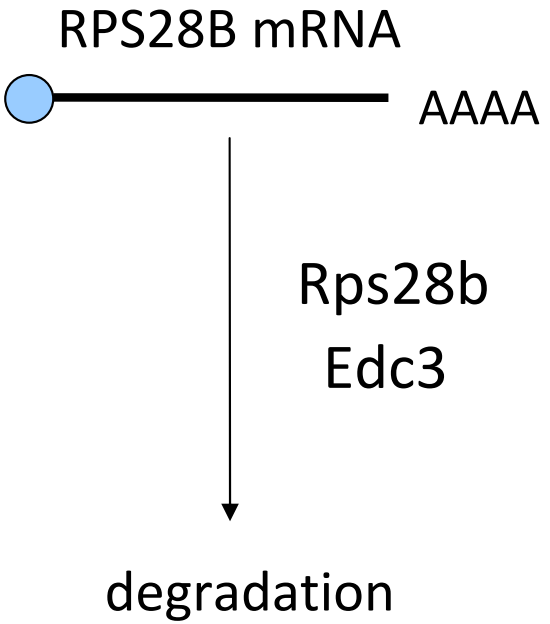
rps28aΔ
scd6Δ
scd6Δ*
lsm12Δ
ykr073cΔ
pbp1Δ
ypl068cΔ
pbp4Δ
edc1Δ
log2(Q/R) Experiment 2
log2(Q/R) Experiment 1

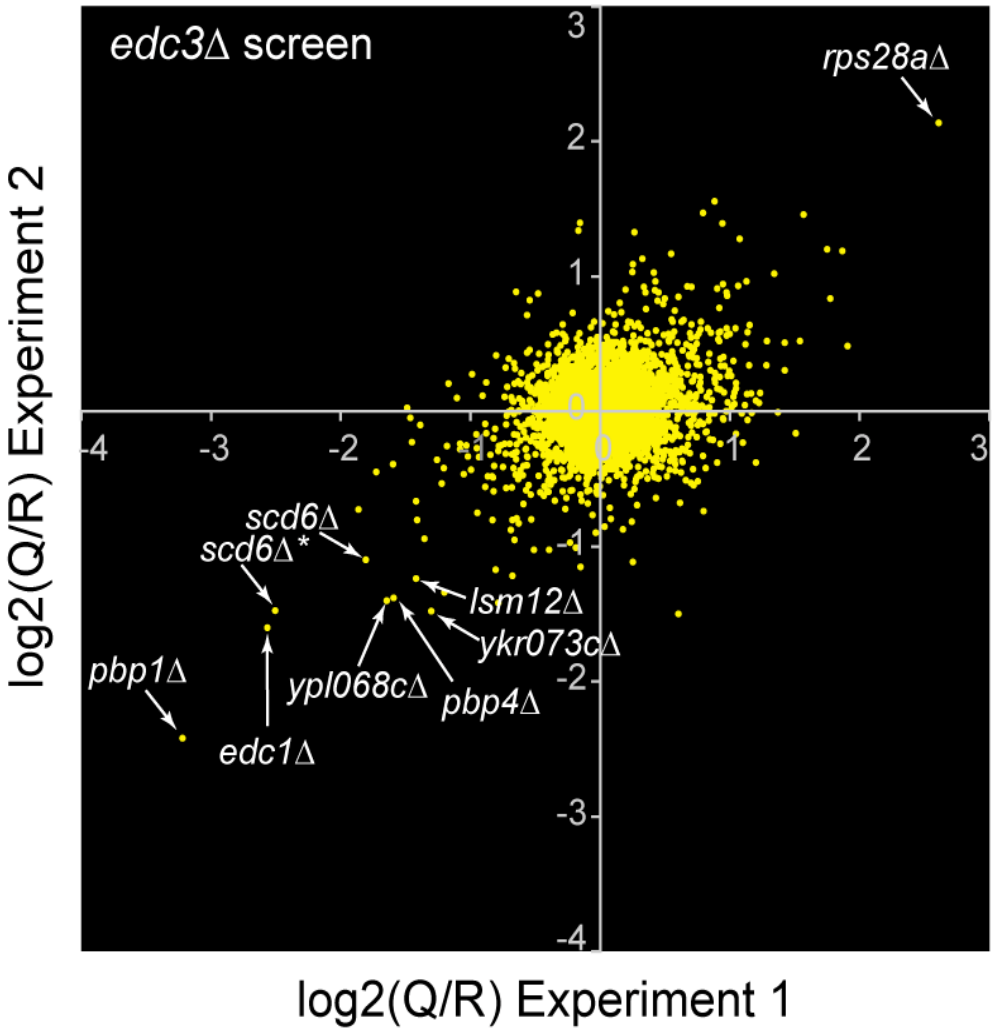

Rps28a
Rps28b

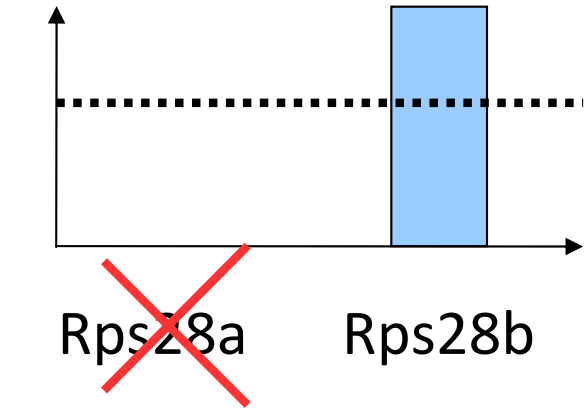


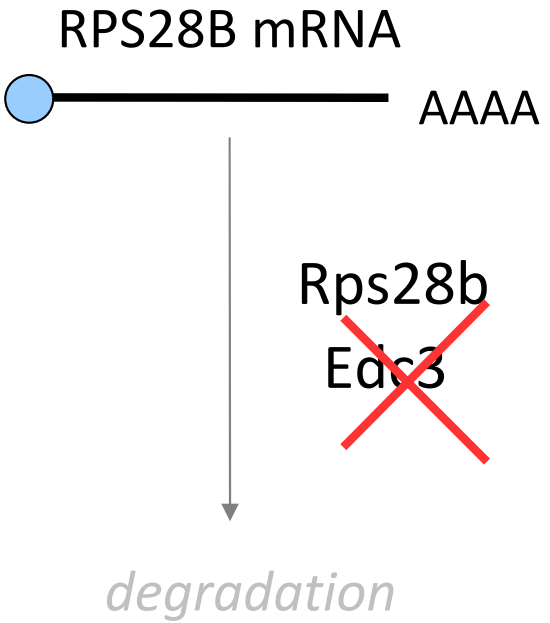


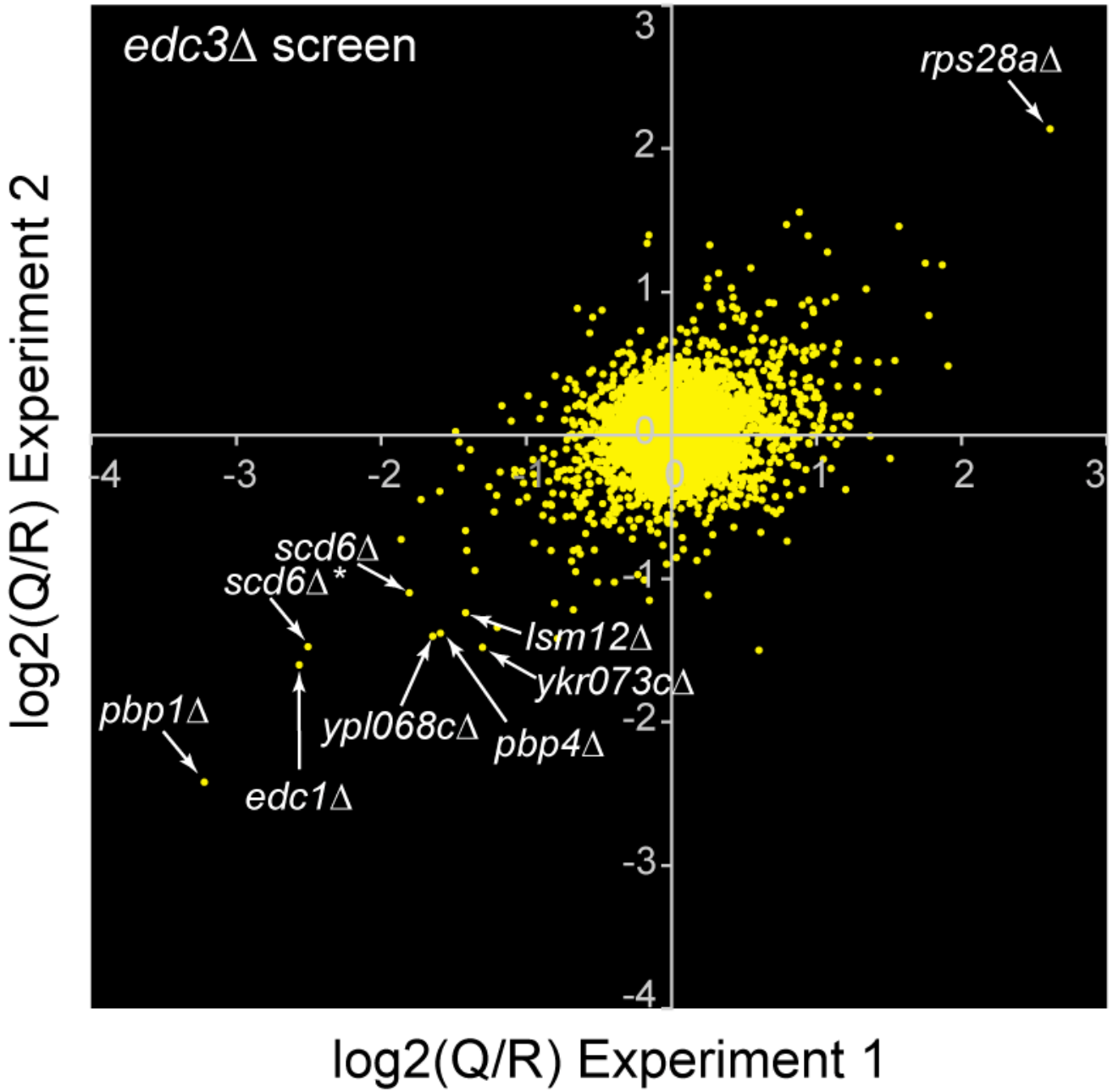


Badis et al., Mol Cell 2004

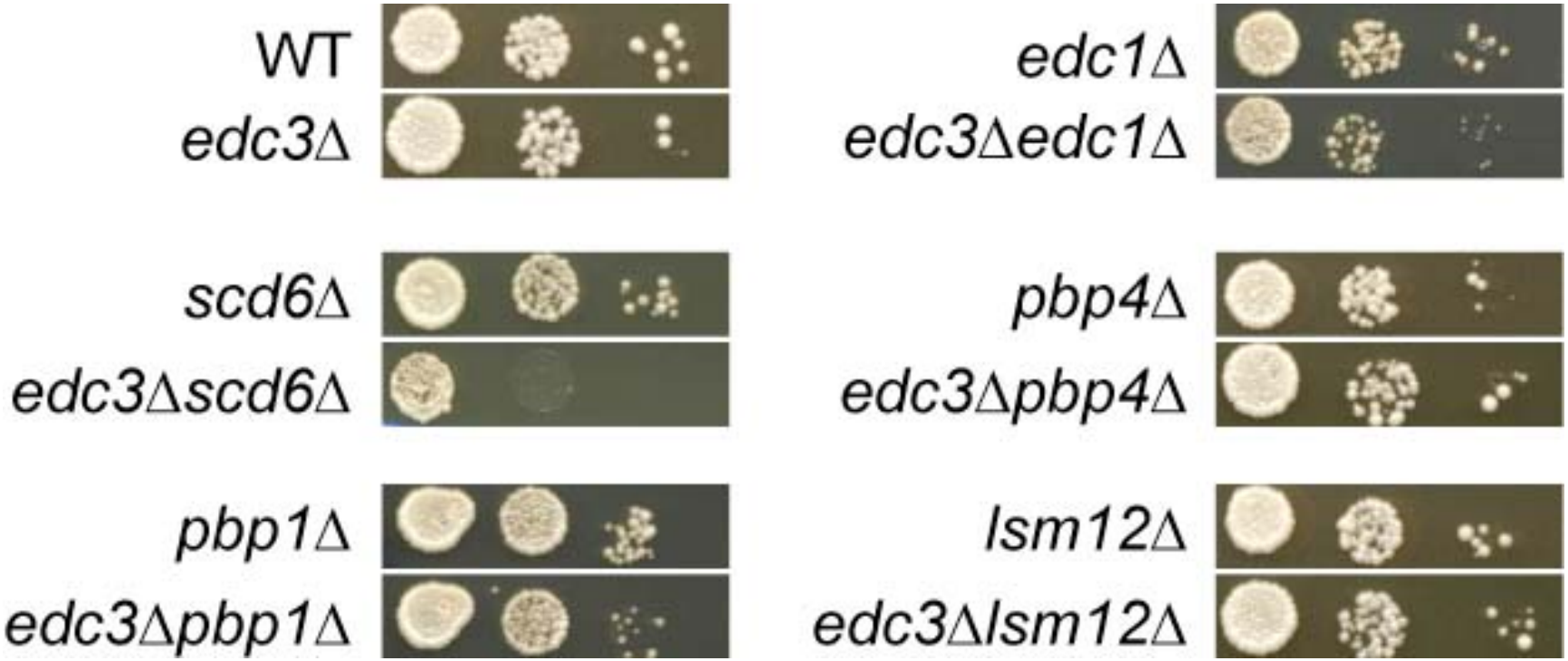

edc3Δ screen
rps28aΔ
scd6Δ
scd6Δ*
lsm12Δ
ykr073cΔ
pbp1Δ
ypl068cΔ
pbp4Δ
edc1Δ
log2(Q/R) Experiment 2
log2(Q/R) Experiment 1


Rps28a
Rps28b

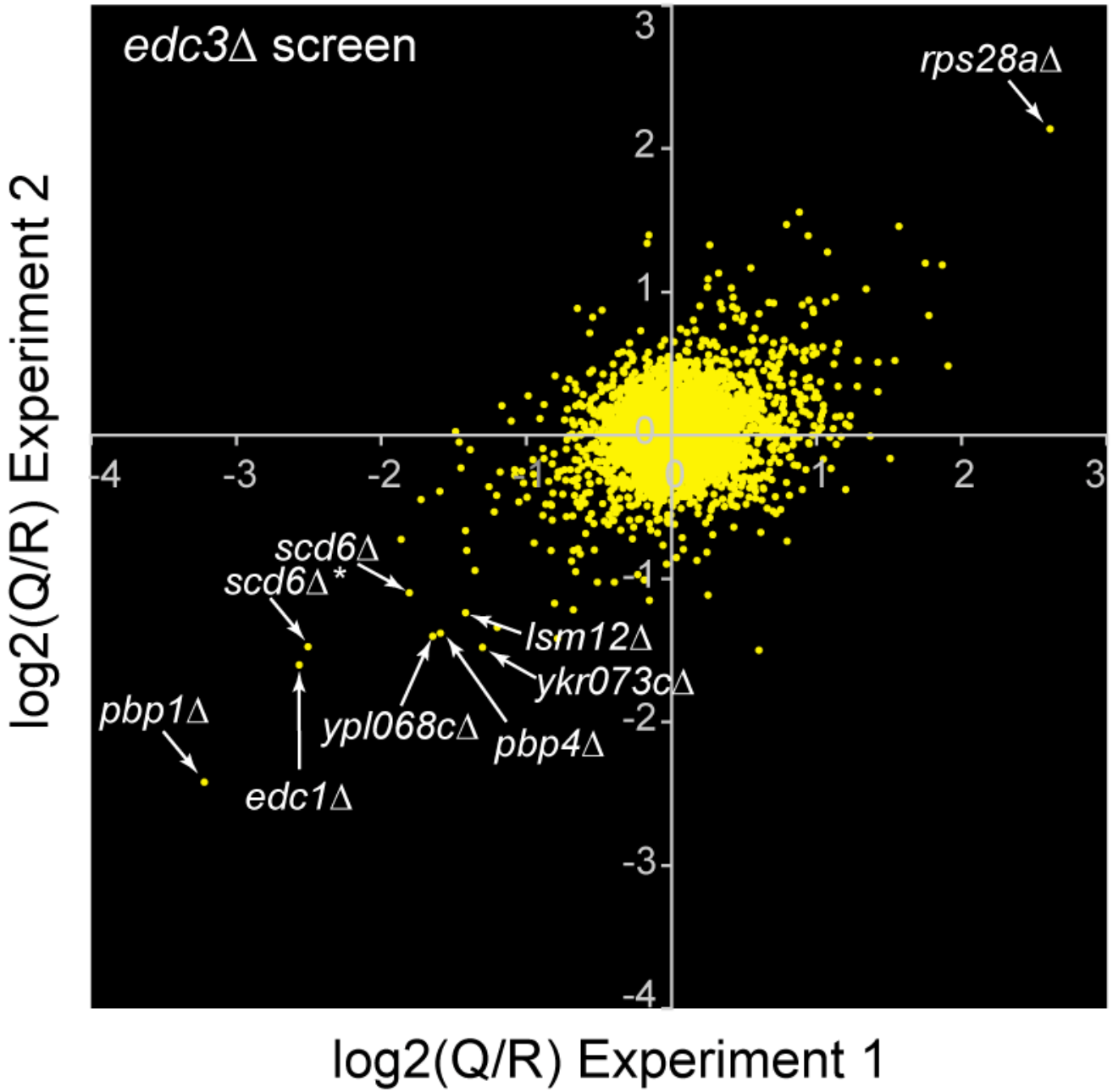

RPS28B mRNA
AAAA
Rps28b
Edc3
degradation


edc3Δ screen
rps28aΔ
scd6Δ
scd6Δ*
lsm12Δ
ykr073cΔ
pbp1Δ
ypl068cΔ
pbp4Δ
edc1Δ
log2(Q/R) Experiment 2
log2(Q/R) Experiment 1
3
2
1
0
-1
-2
-3
-4
-4
-3
-2
-1
0
1
2
3

Validation: 2x2 plate assay



YPD 30°C


edc3Δ screen
rps28aΔ
scd6Δ
scd6Δ*
lsm12Δ
ykr073cΔ
pbp1Δ
ypl068cΔ
pbp4Δ
edc1Δ
log2(Q/R) Experiment 2
log2(Q/R) Experiment 1
3
2
1
0
-1
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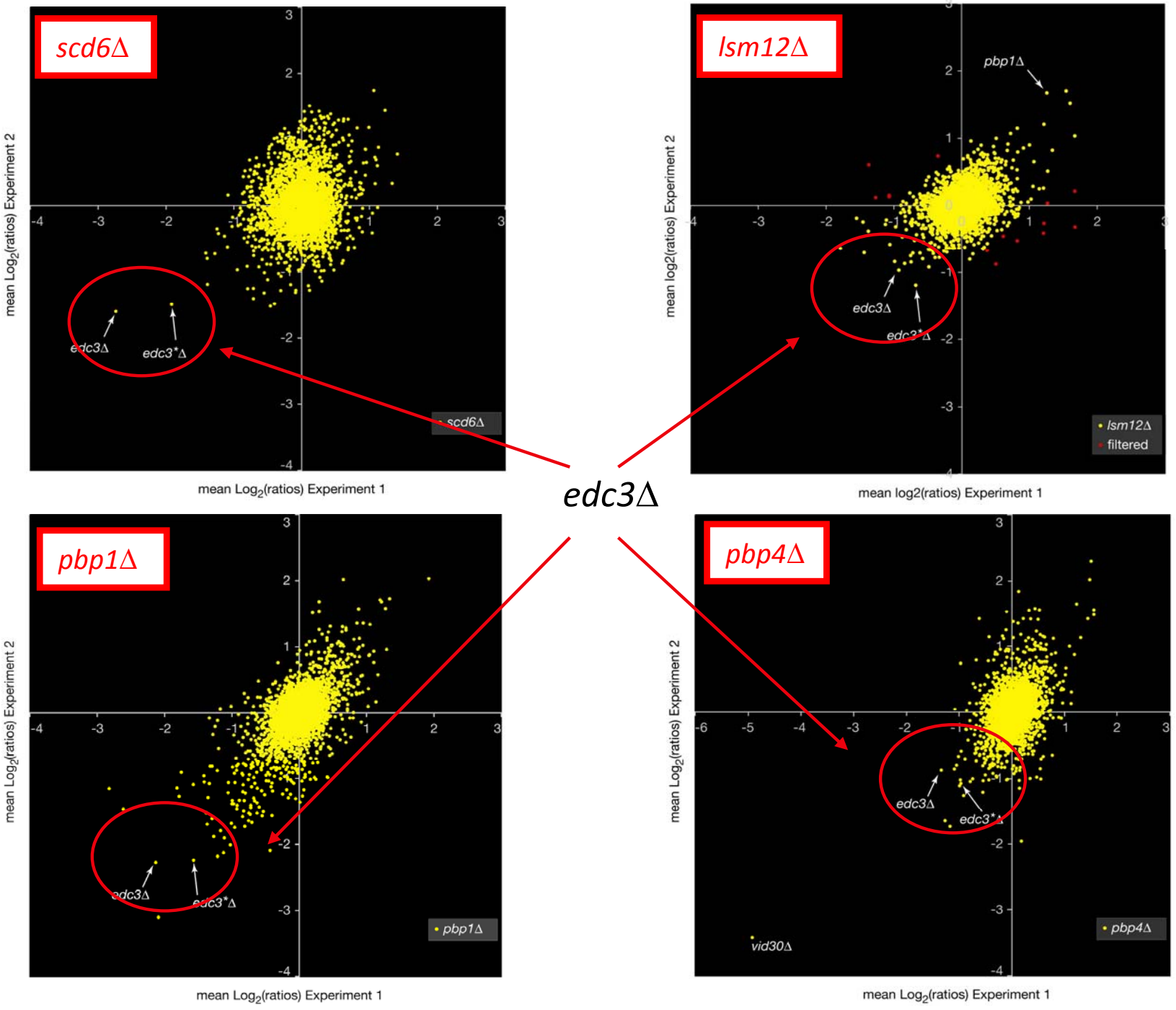
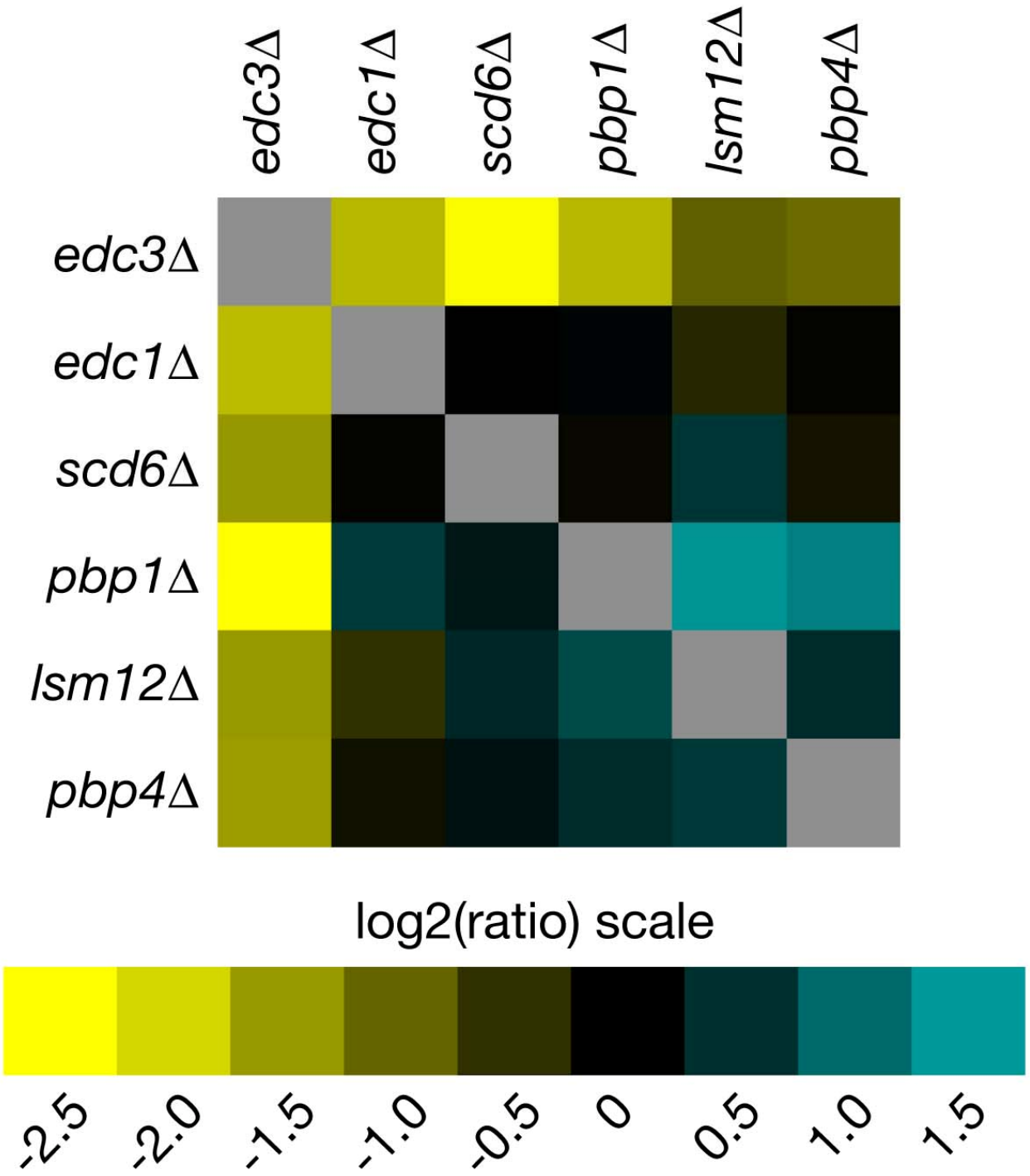
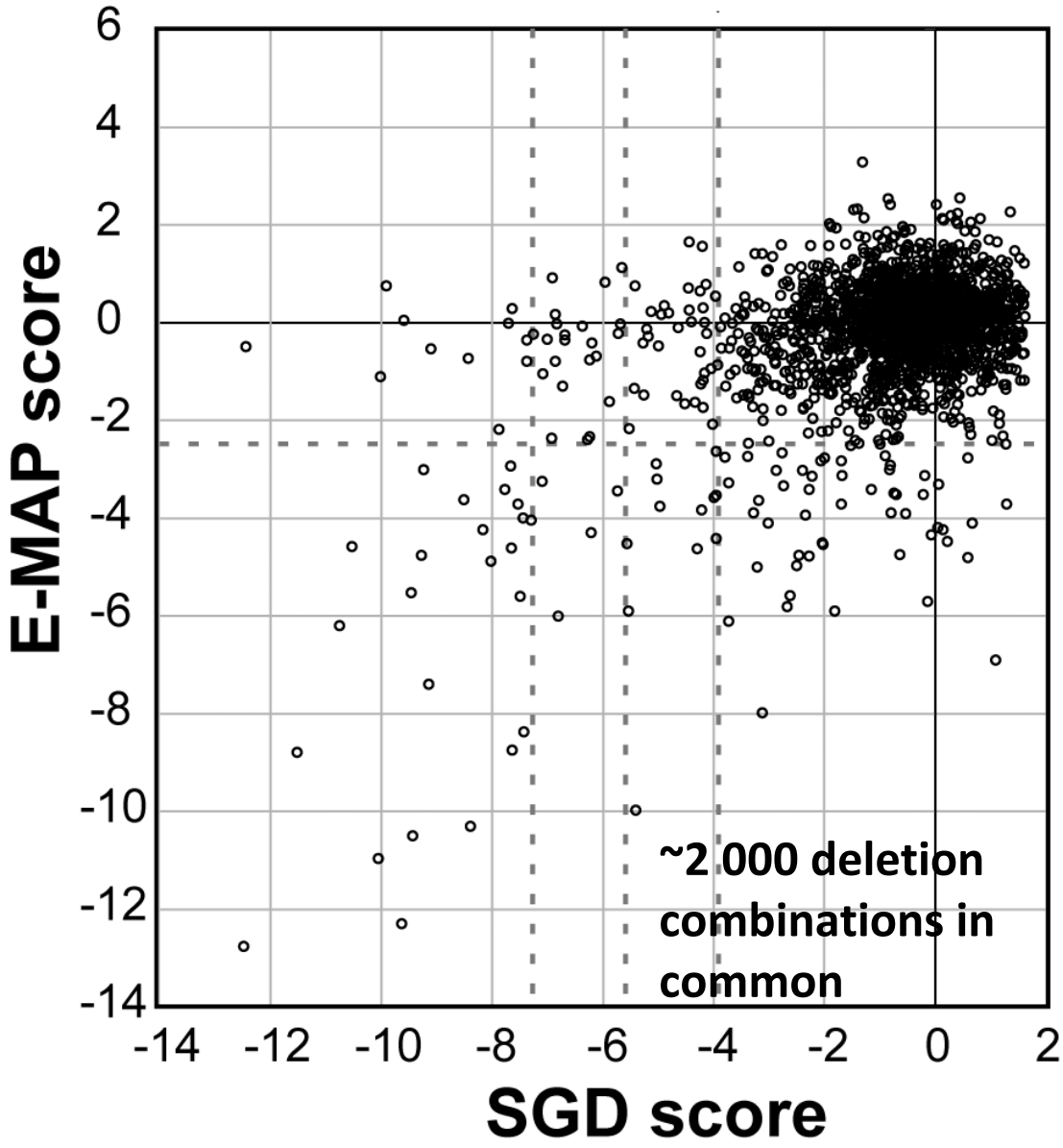
-3
-4
-4
-3
-2
-1
0
1
2
3

Even weak interactions are reproducible and reciprocal




edc3Δ
edc1Δ
scd6Δ
pbp1Δ
lsm12Δ
pbp4Δ
edc3Δ
edc1Δ
scd6Δ
pbp1Δ
lsm12Δ
pbp4Δ
log2(ratio) scale
-2.5
-2.0
-1.5
-1.0
-0.5
0
0.5
1.0
1.5

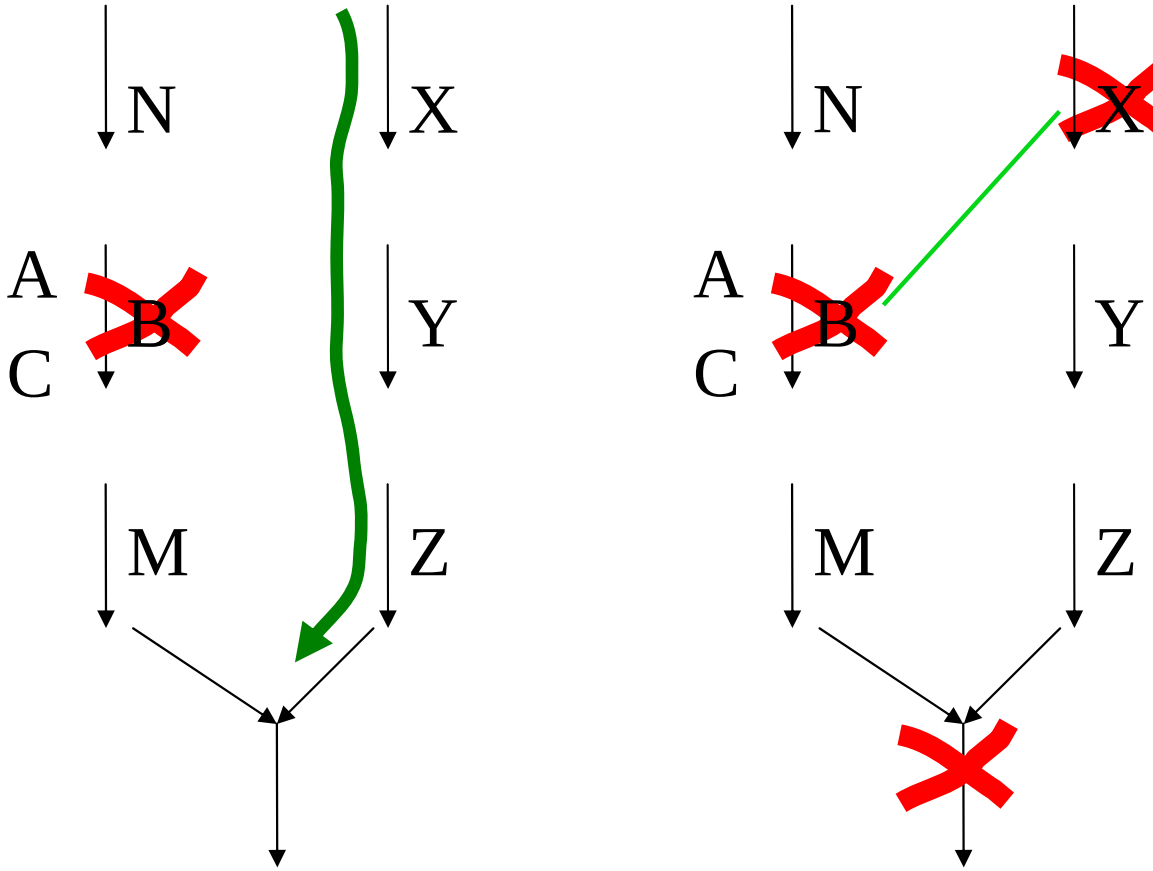
E-MAP data, Collins et al. *Nature*, 2007



GIM direct interactions, Decourty et al. *PNAS*, 2008

Inferring functional relationships from genetic interactions

Are direct genetic interactions strongly predictive of functional similarities?



BMC Genomics

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Research article

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Finding function: evaluation methods for functional genomic data

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Email: Chad L Myers - clmyers@princeton.edu; Daniel R Barrett - drbarret@princeton.edu; Matthew A Hibbs - mhibbs@cs.princeton.edu; Curtis Huttenhower - chuttenh@cs.princeton.edu; Olga G Troyanskaya* - ogt@cs.princeton.edu

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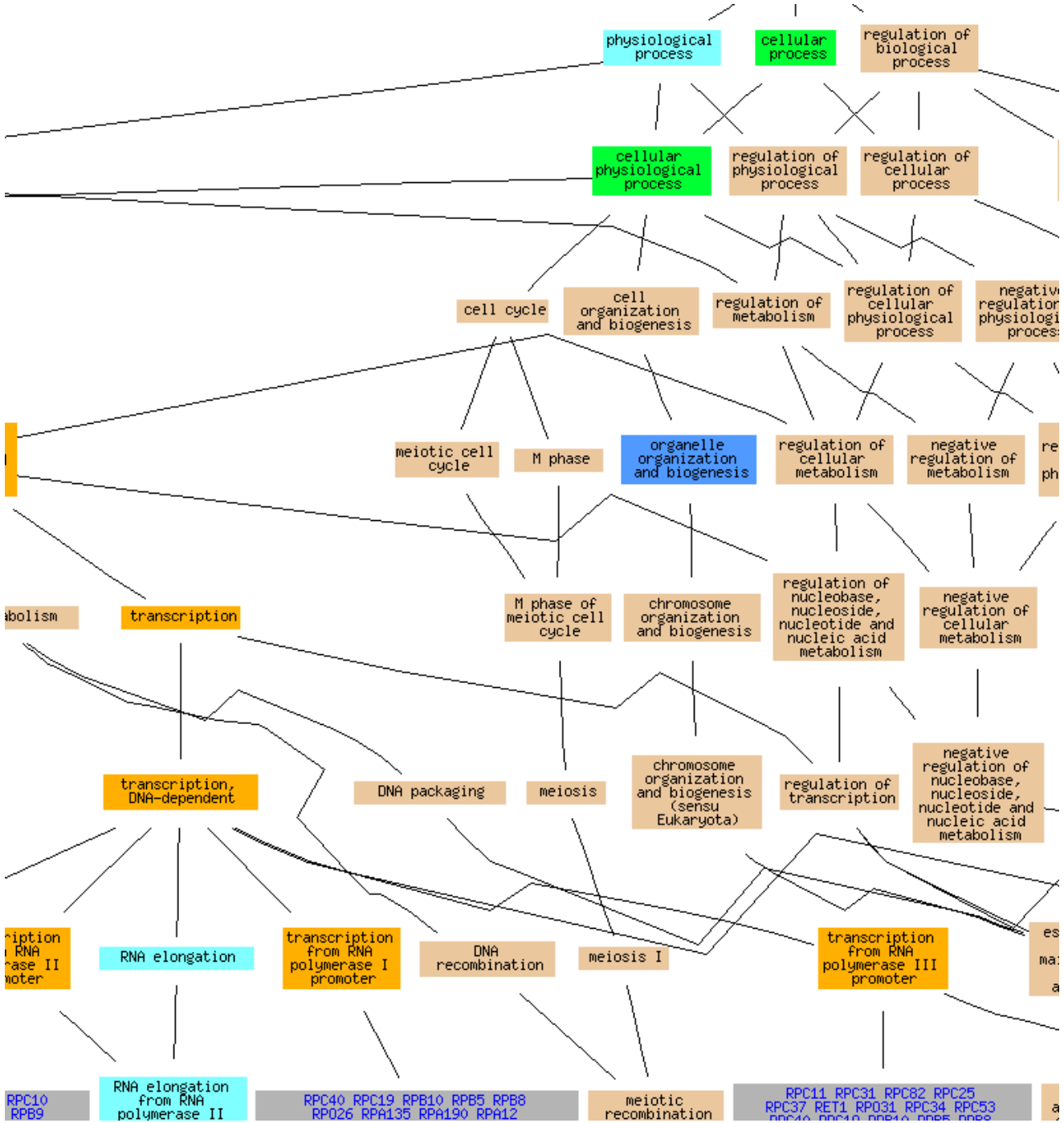
Accepted: 25 July 2006

GO tree

with

GO Term Finder



Estimation of the functional information content of a data set

Myers et al. *BMC Genomics* 2006

Gene Ontology gold standard

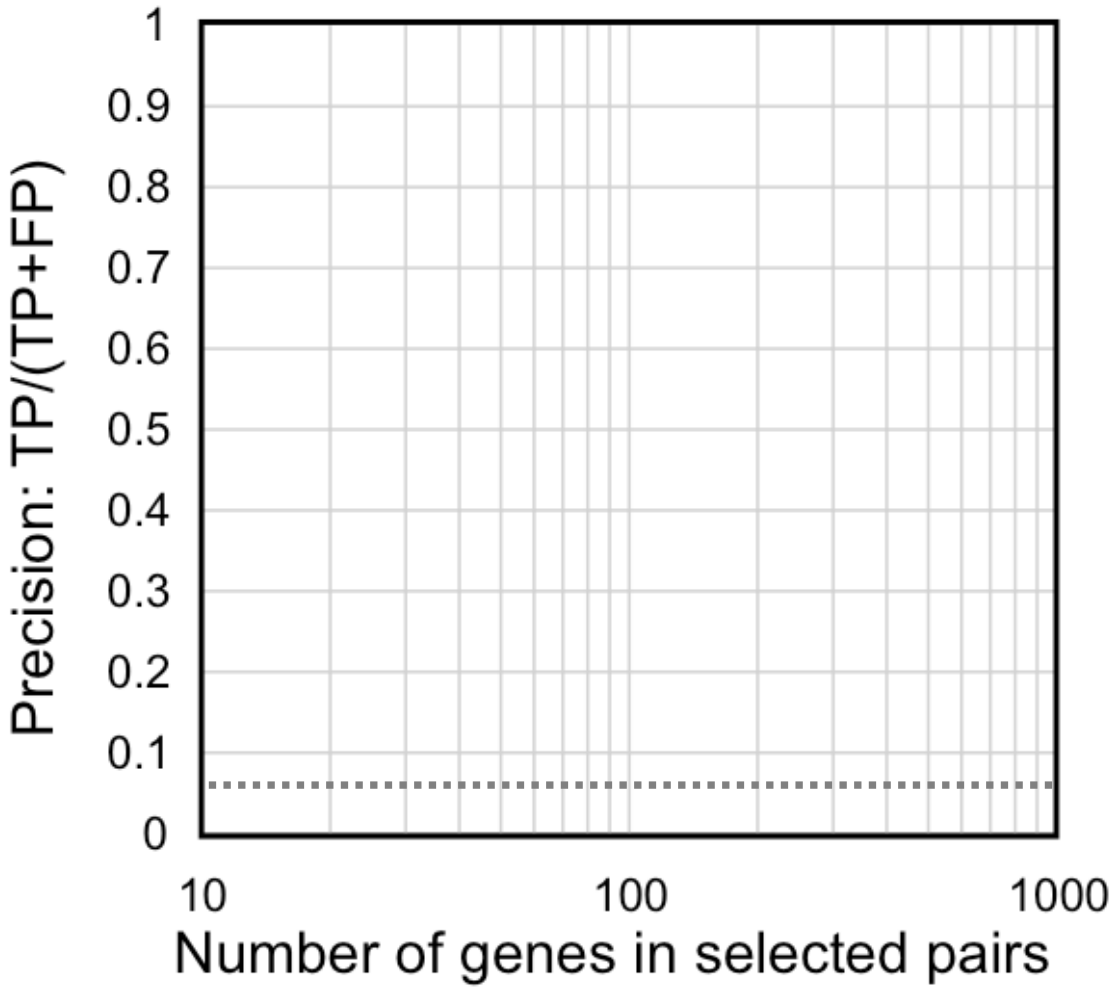
Positive set: ~ 5 10^5 True Positive gene pairs (TPs)

Negative set: ~ 9 10^6 False Positive gene pairs (FPs)

Precision Score: TPs/(TPs+FPs)

Random sampling: 5 10^5 /(5 10^5 + 9 10^6) ≈ 0.05

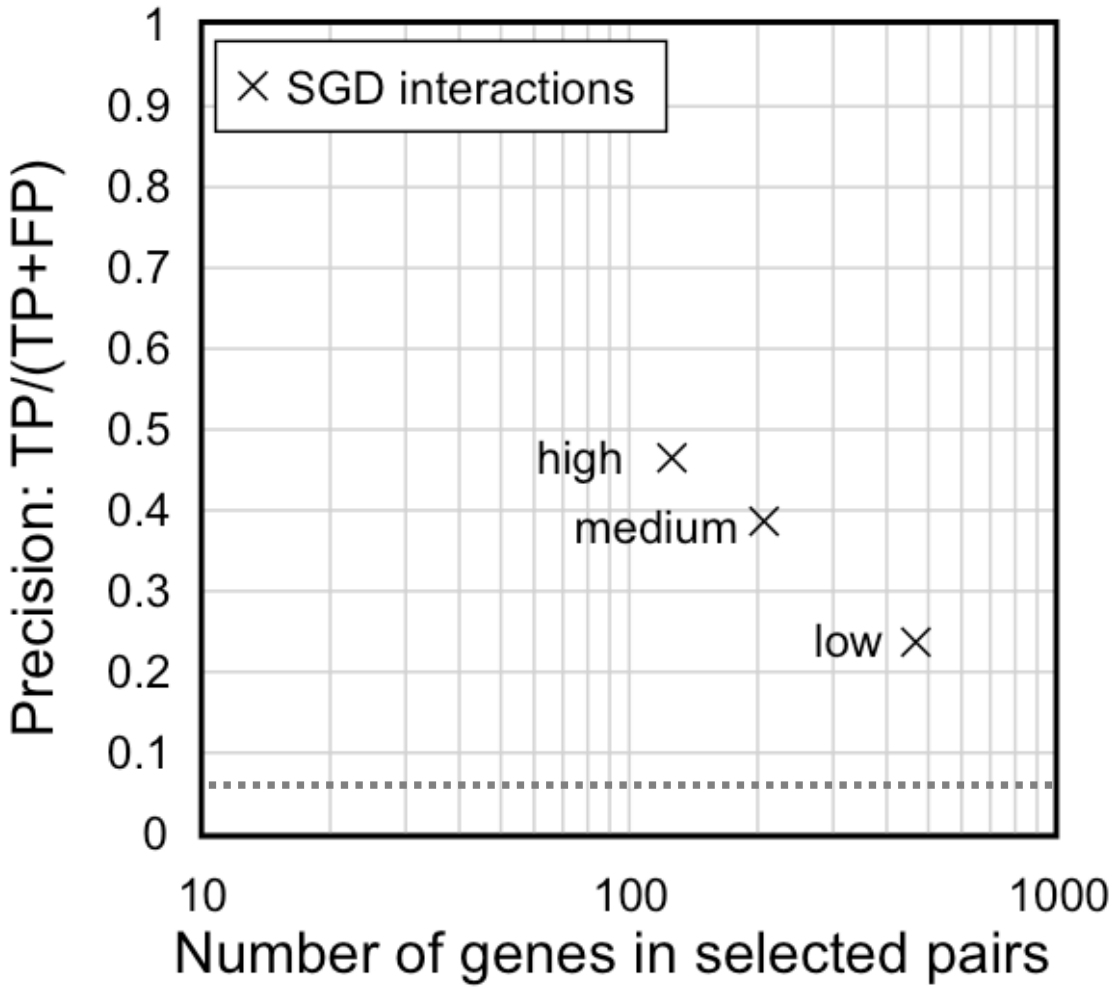
Precision vs sensitivity



Synthetic Growth Defect (SGD) interactions

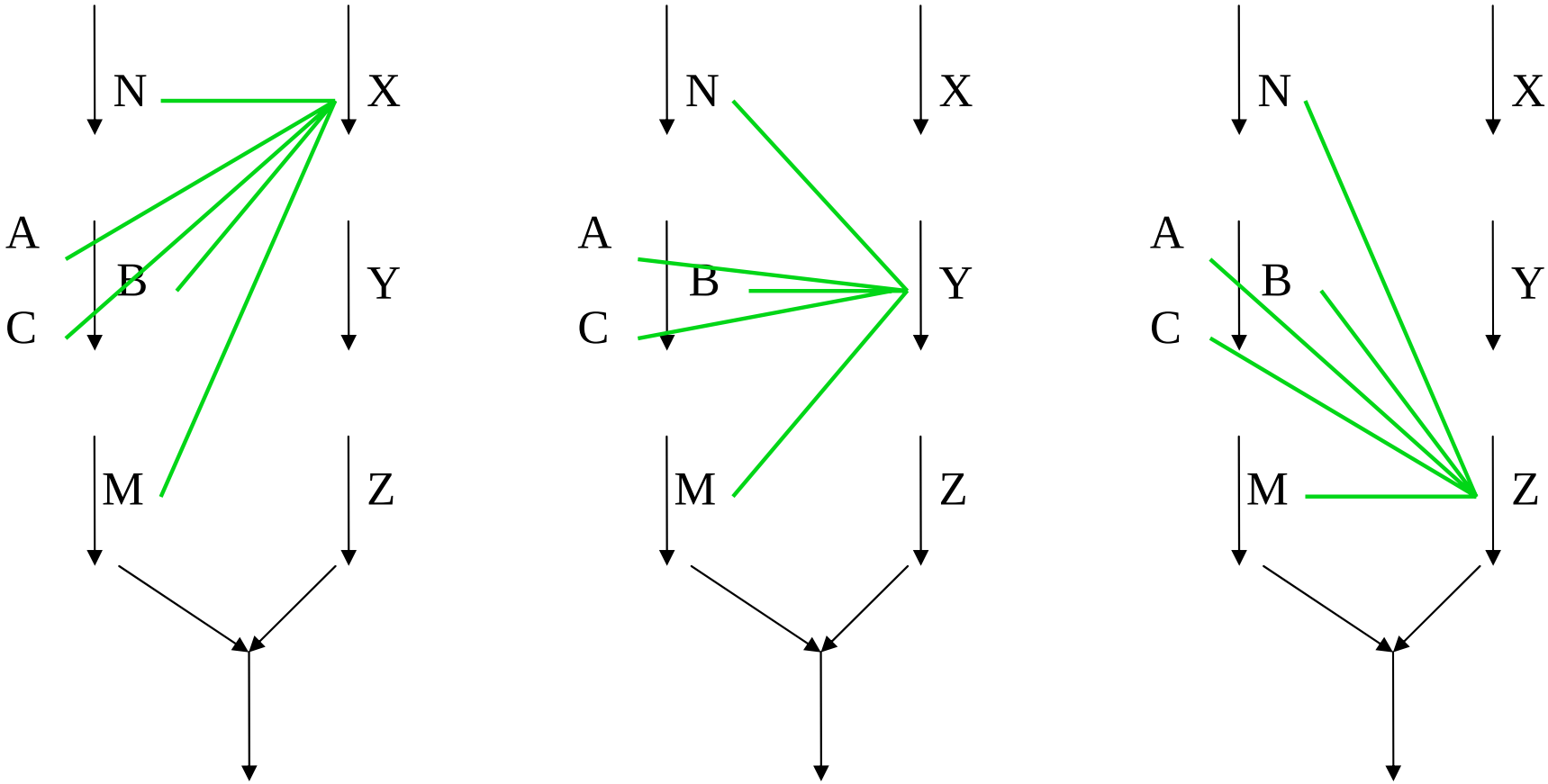
(direct genetic interactions)

41 screens

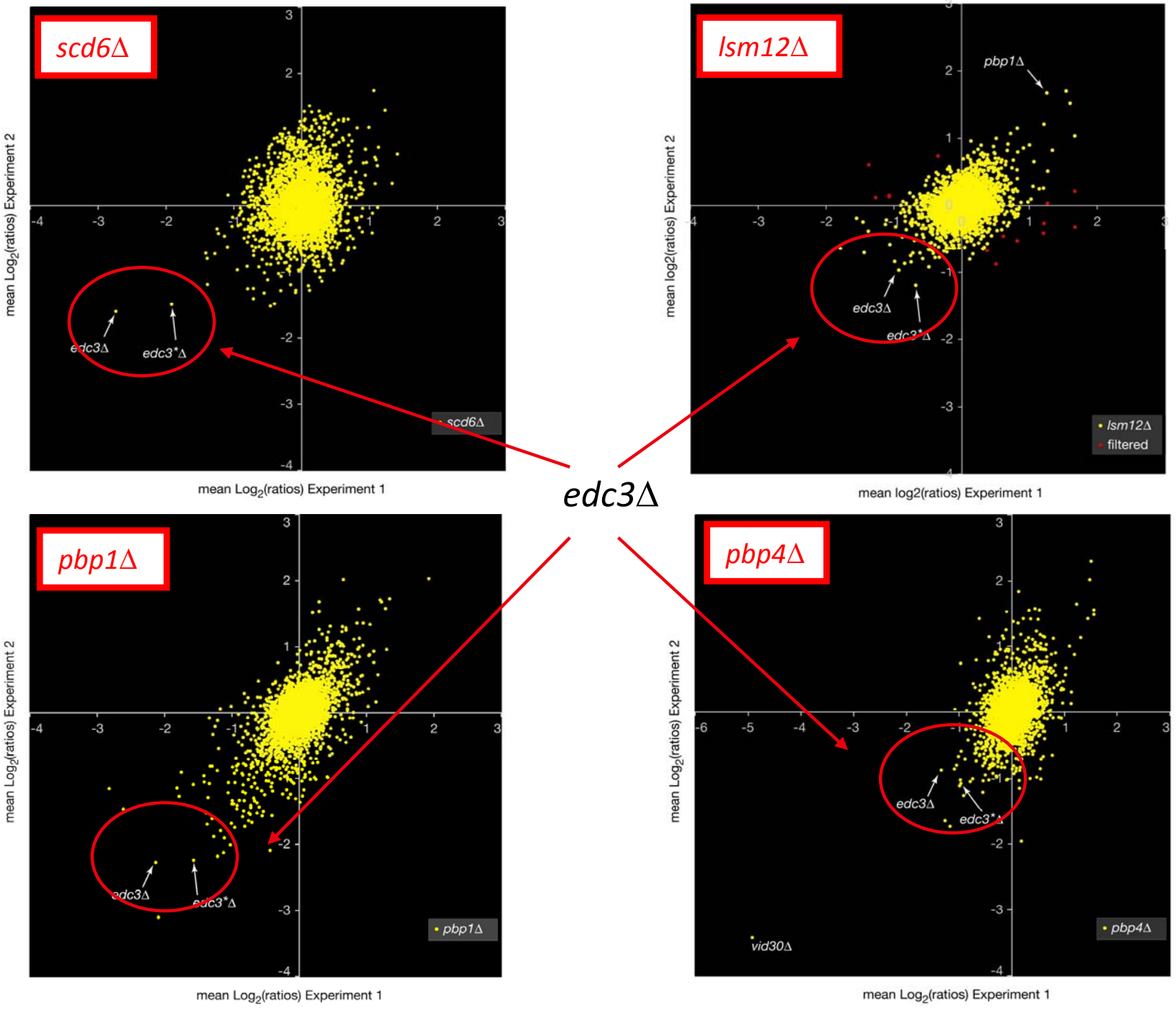


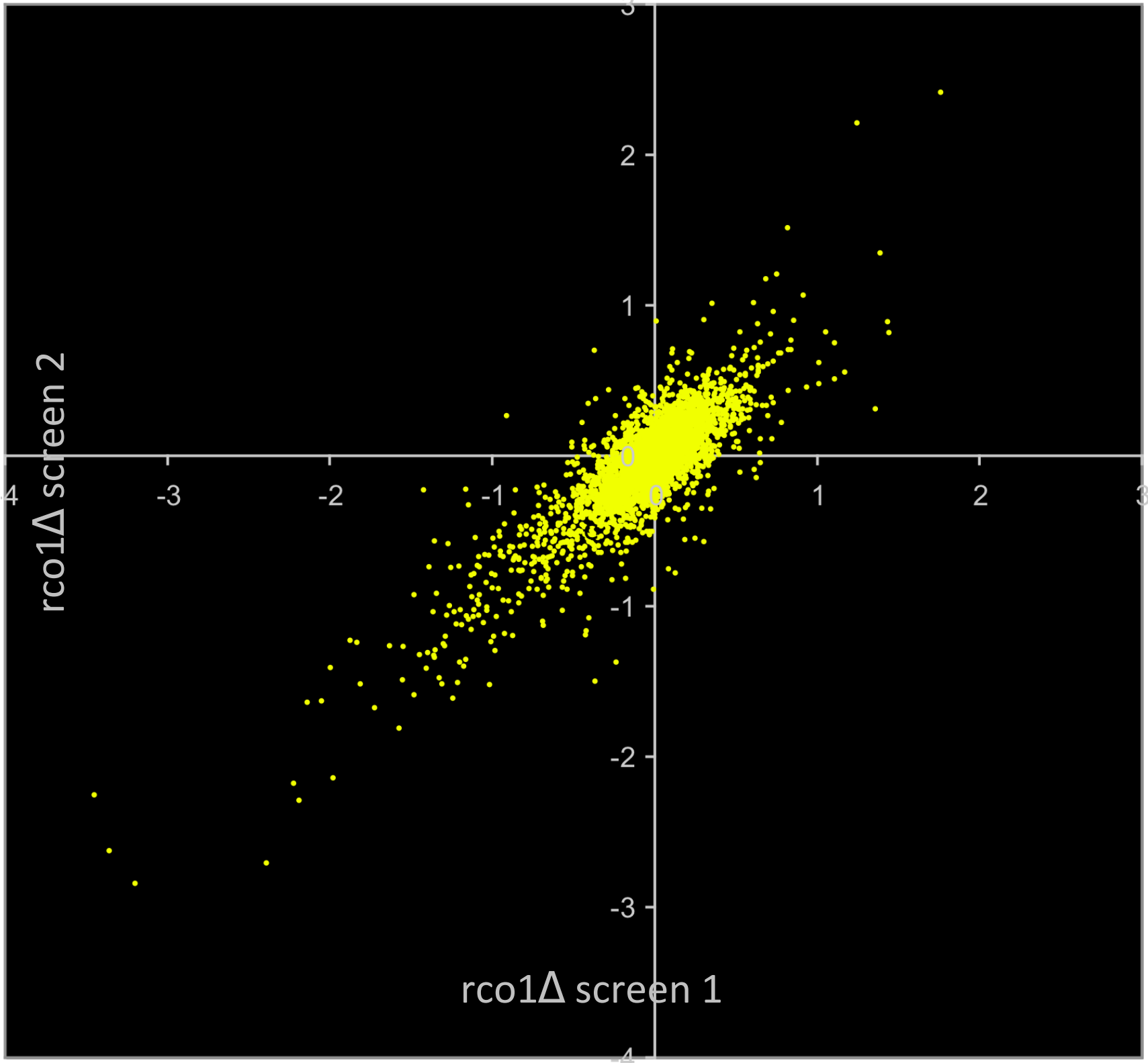
Principle of Synthetic Growth Defects

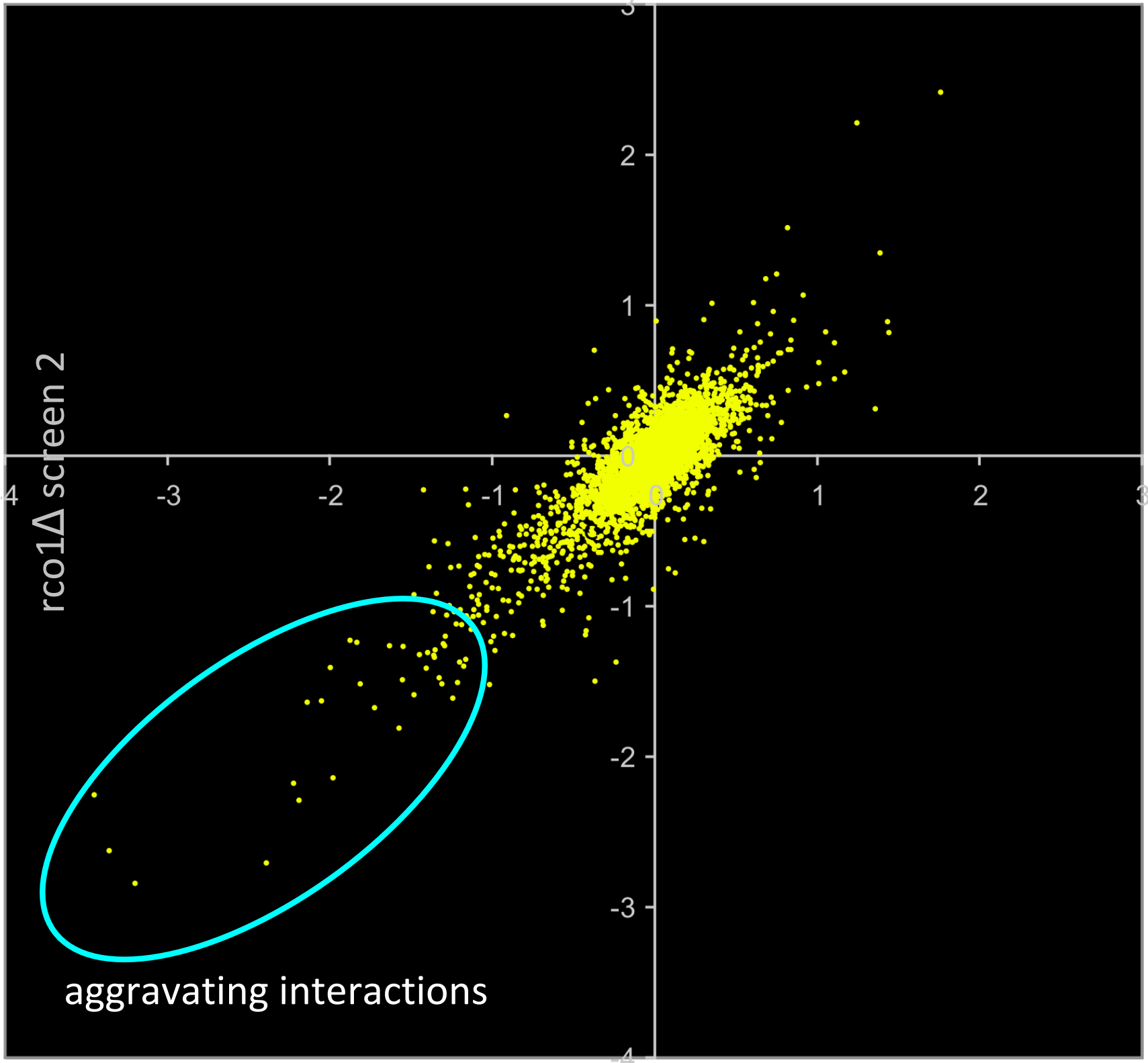
Same Genetic Interaction Profiles : **congruency**

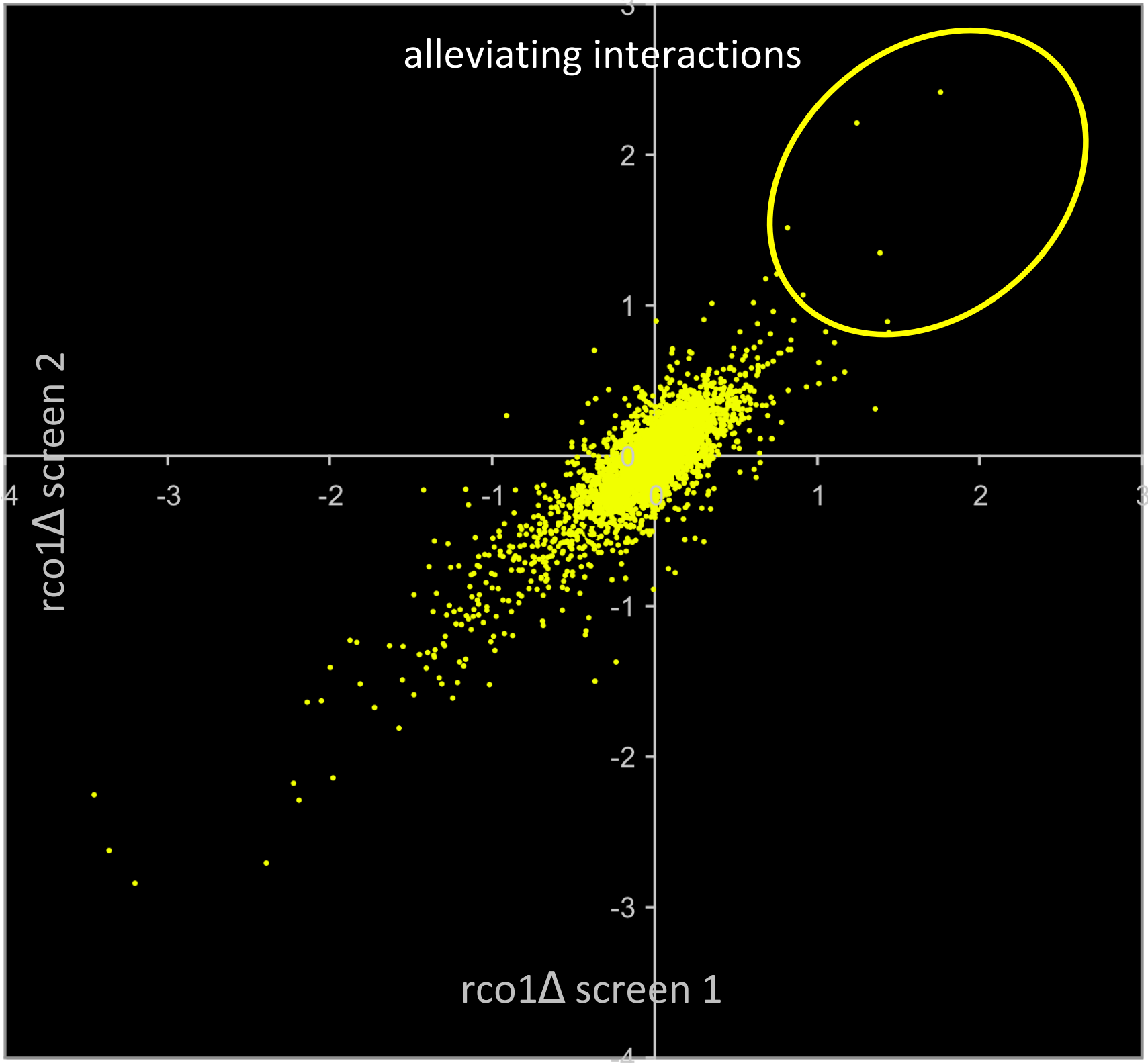


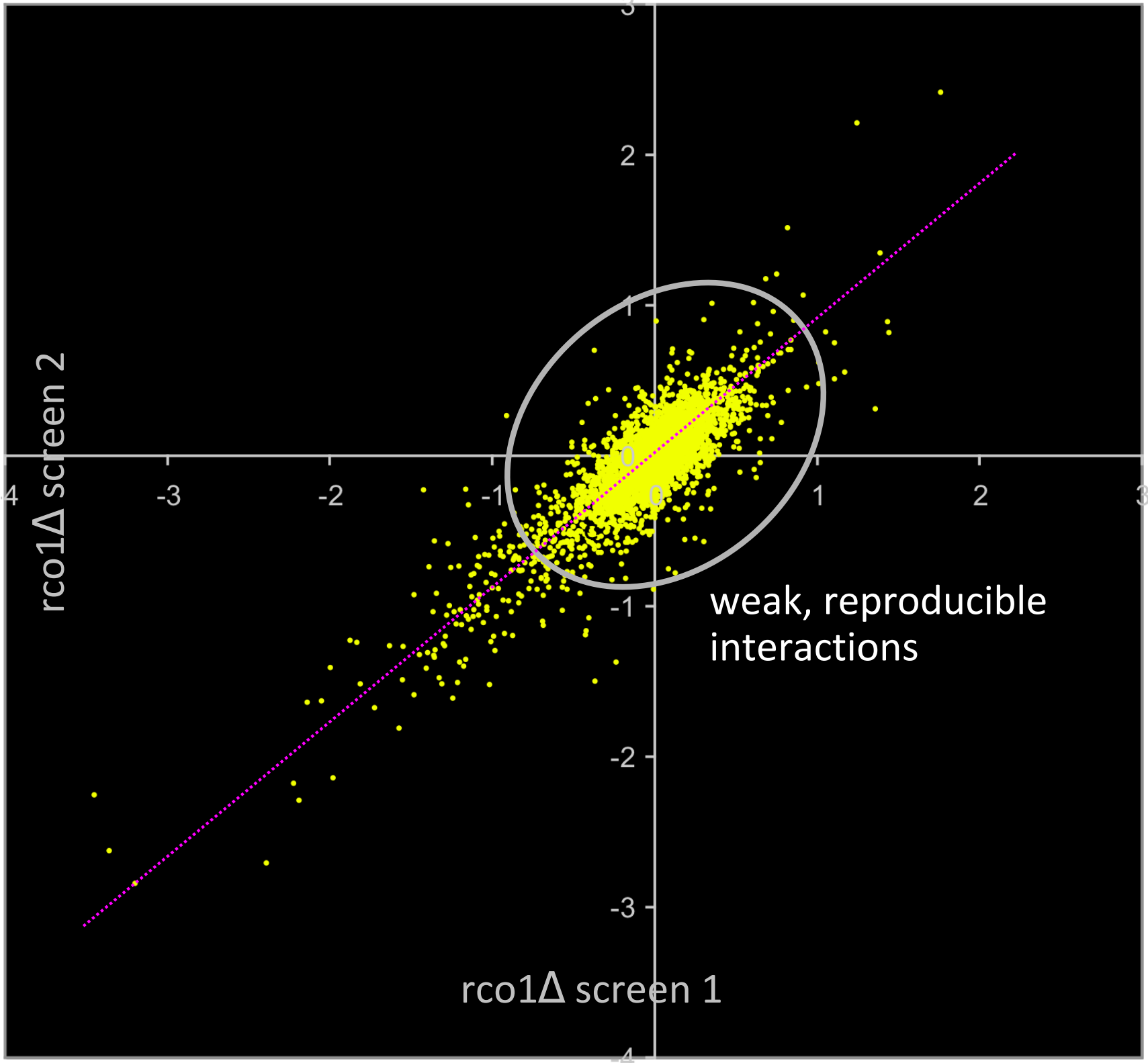
Even weak interactions are reproducible and reciprocal

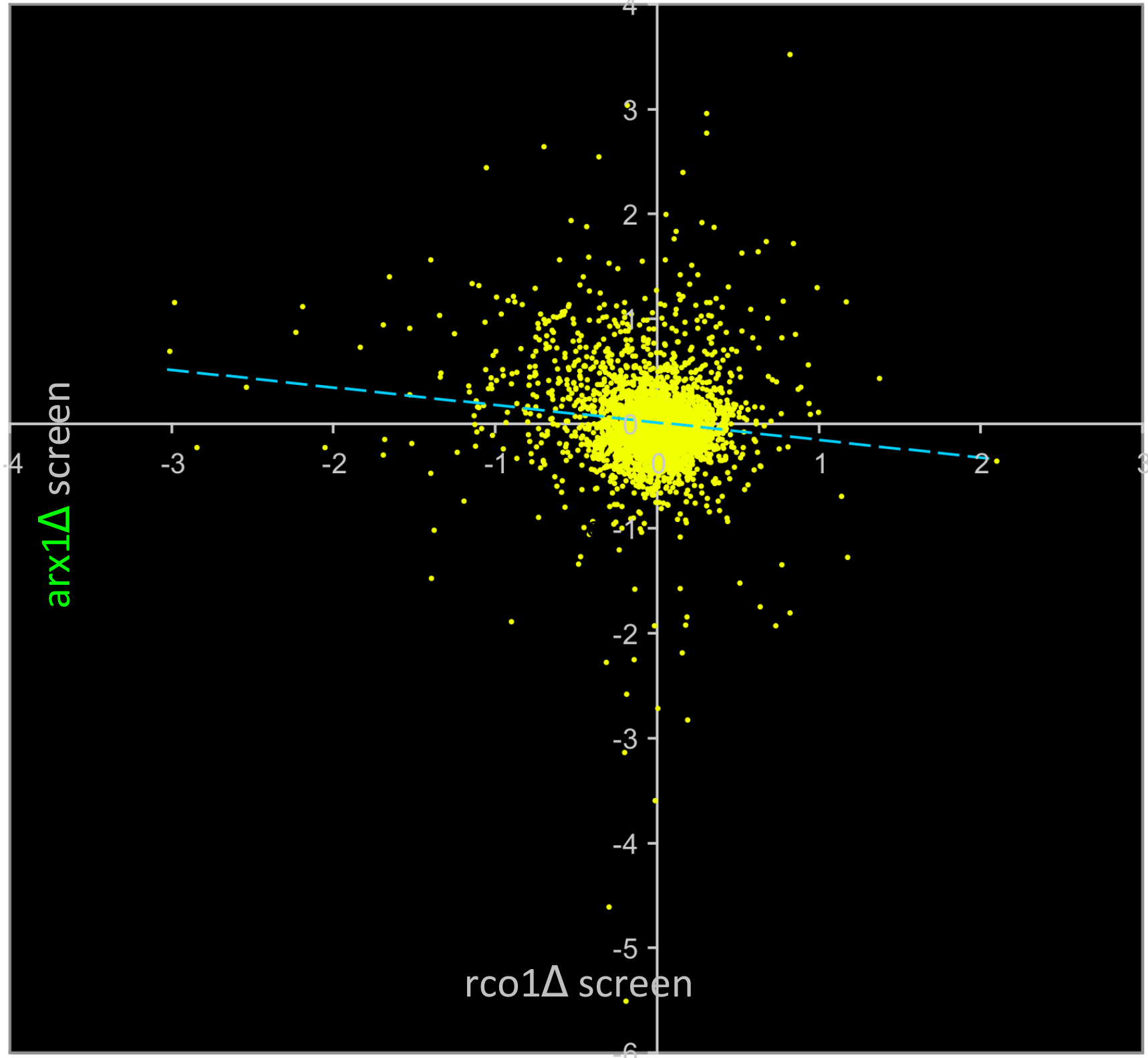


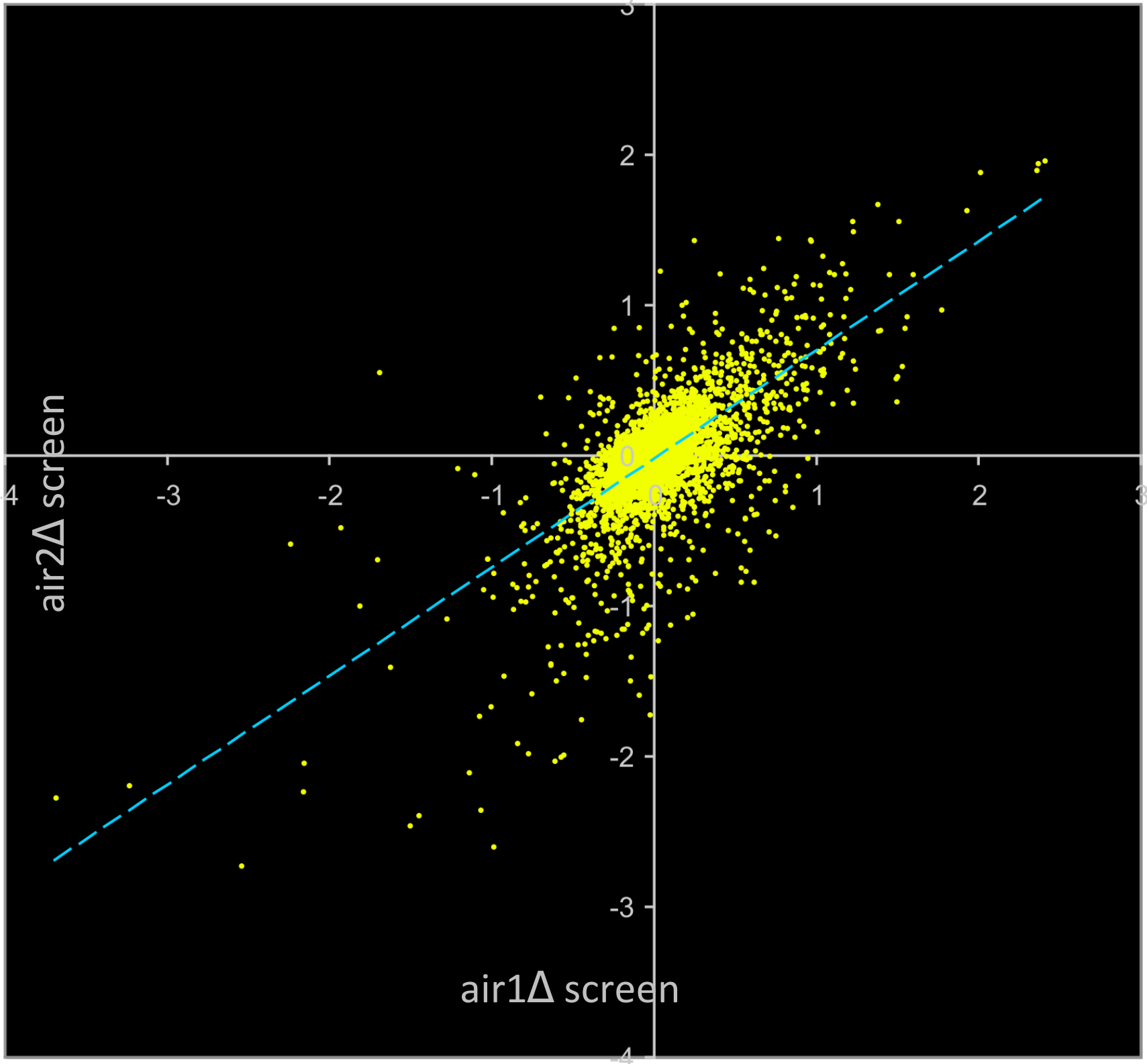

rco1Δ screen 2
rco1Δ screen 1
3
2
1
0
-1
-2
-3
-4
-3
-2
-1
0
1
2


rco1Δ screen 2
aggravating interactions
2
1
0
-1
-2
-3
-4
-3
-2
-1
0
1
2


alleviating interactions
rco1Δ screen 2
rco1Δ screen 1
3
2
1
0
-1
-2
-3
-4
-3
-2
-1
0
1
2

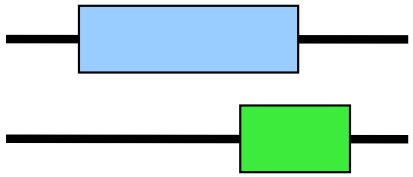

rco1Δ screen 2
rco1Δ screen 1
weak, reproducible
interactions
3
2
1
0
-1
-2
-3
-4
-3
-2
-1
0
1
2
3

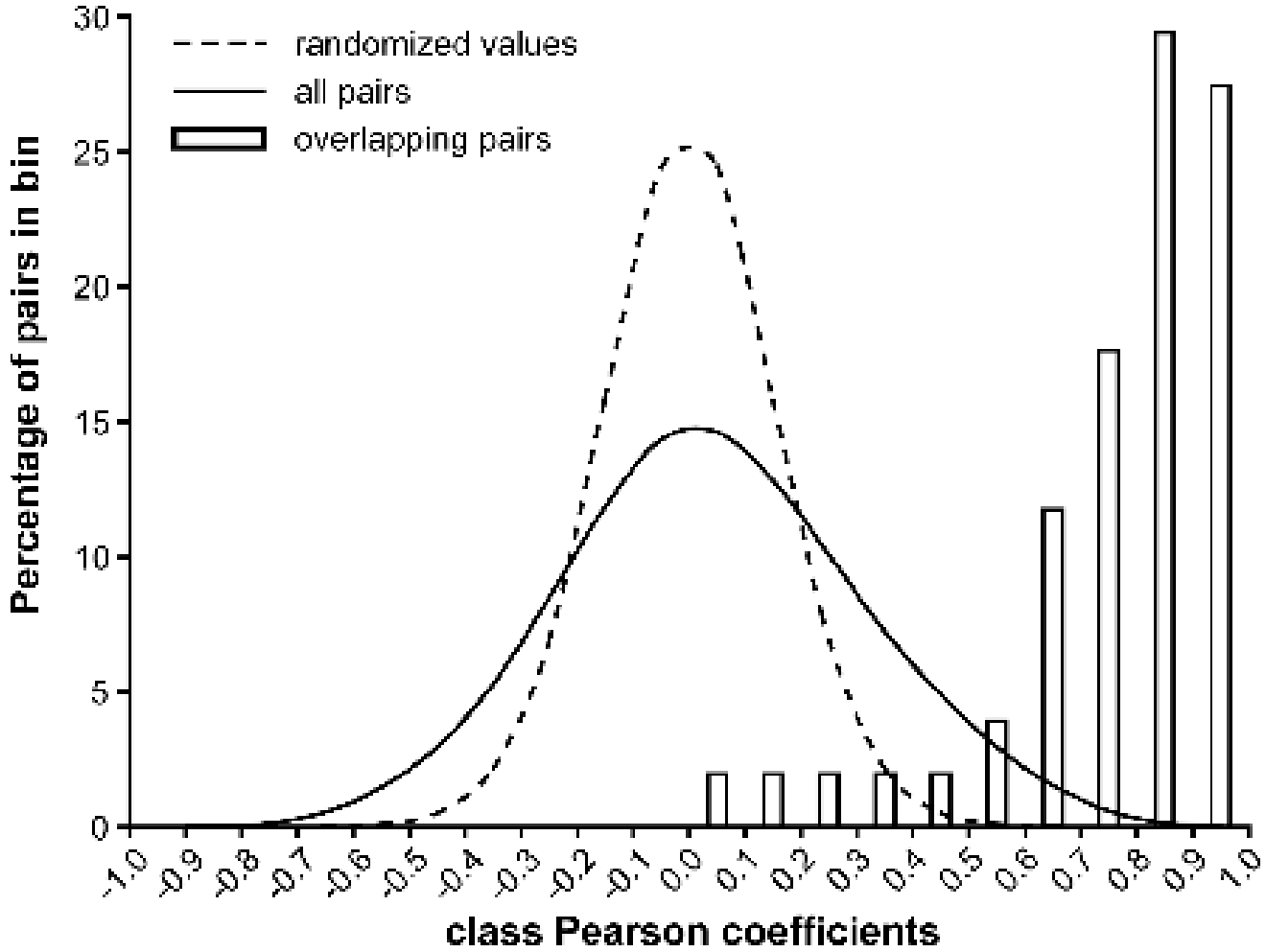

arx1Δ screen
rco1Δ screen
4
3
2
1
0
-1
-2
-3
-4
-5
-6
-4
-3
-2
-1
0
1
2
3


air2Δ screen
air1Δ screen
-4
-3
-2
-1
0
1
2
3

Correlations between Genetic Interaction Profiles (GIPs)

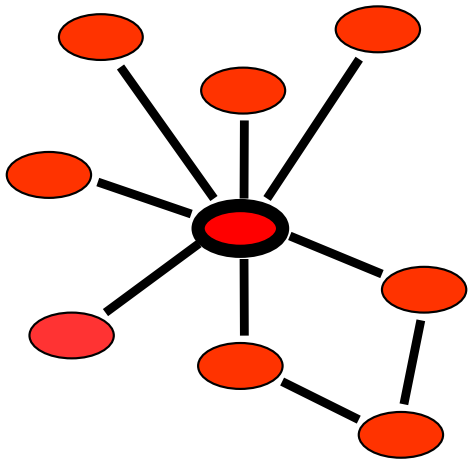
41 screens in duplicates

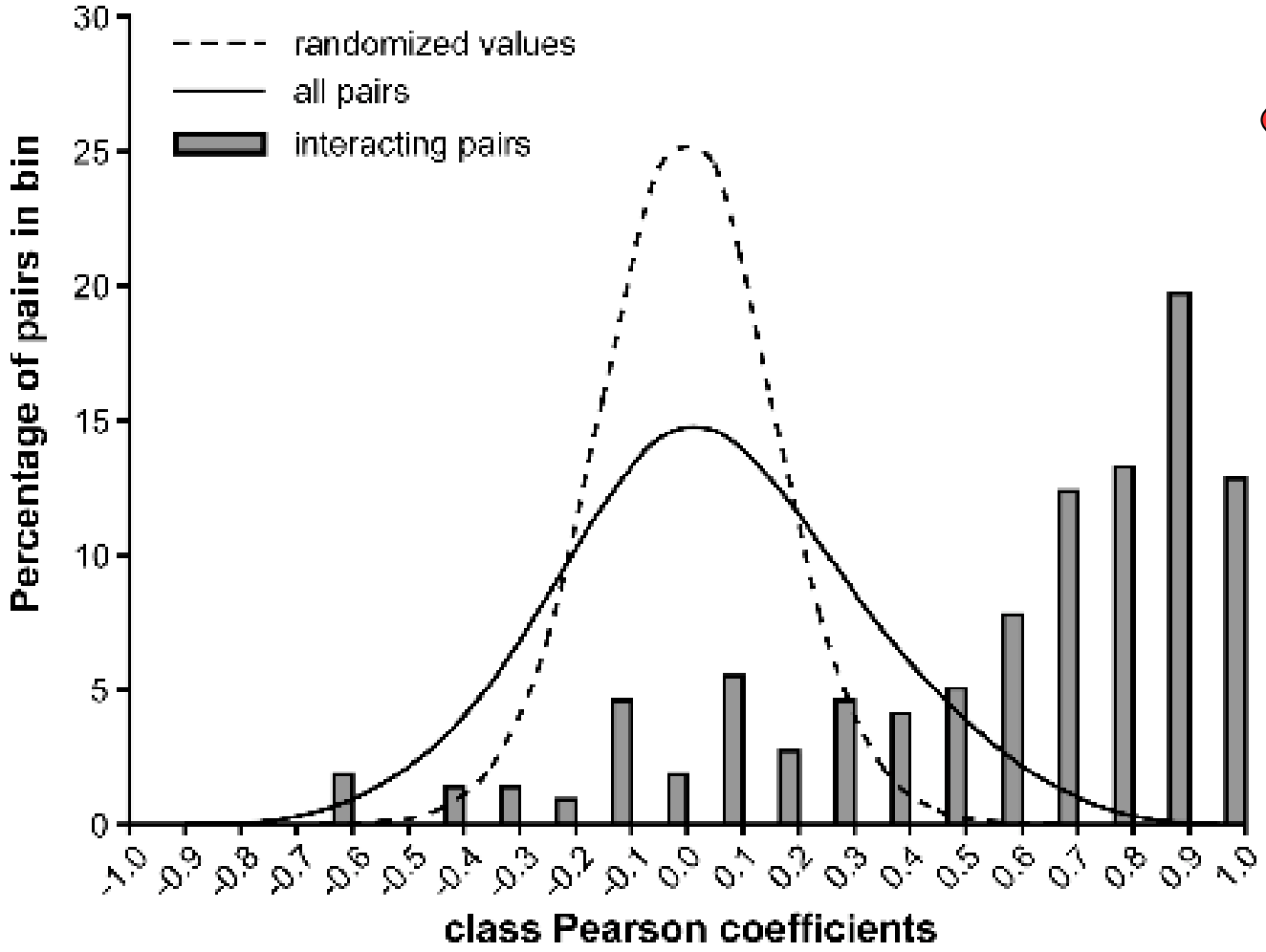




Correlations between Genetic Interaction Profiles (GIPs)

41 screens in duplicates



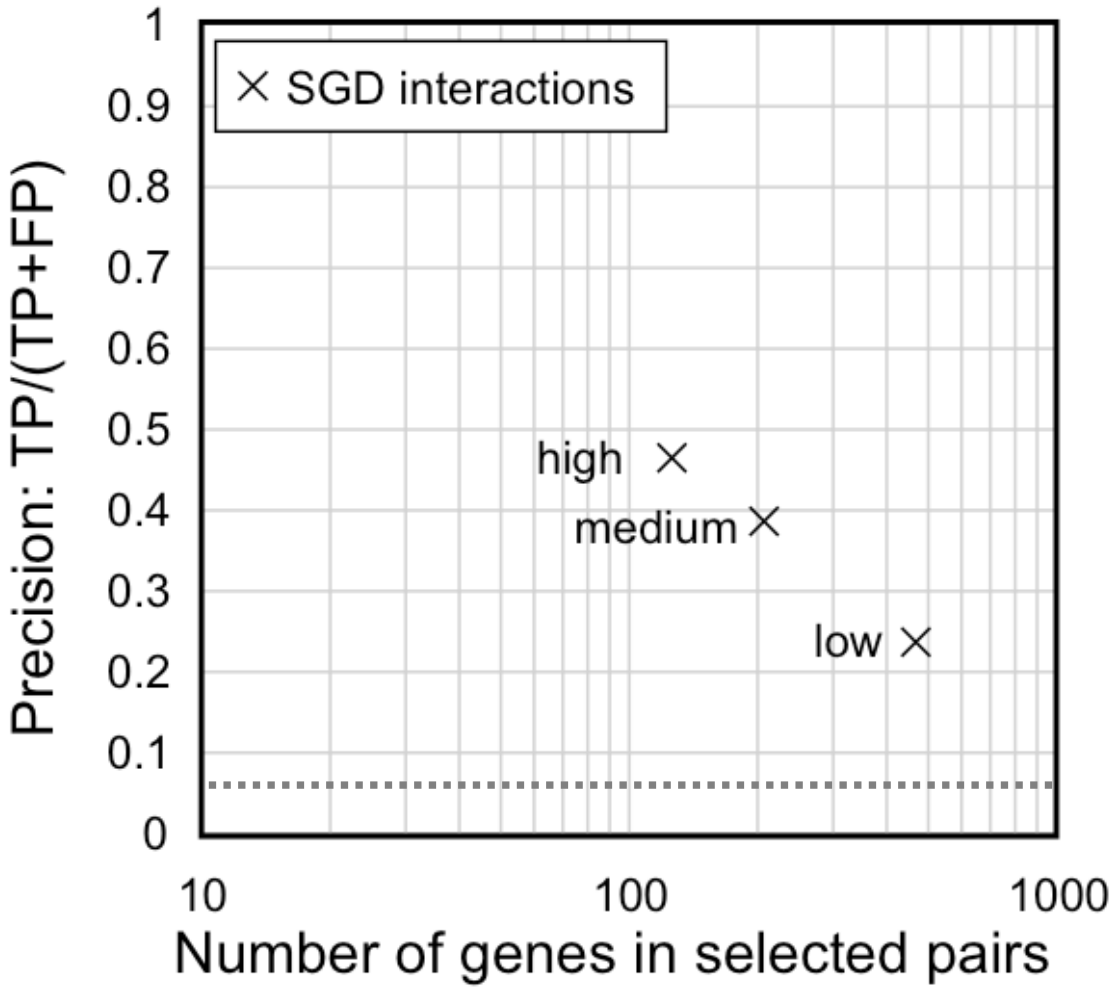


Collins et al.
MCP 2007

Synthetic Growth Defect (SGD) interactions

(direct genetic interactions)

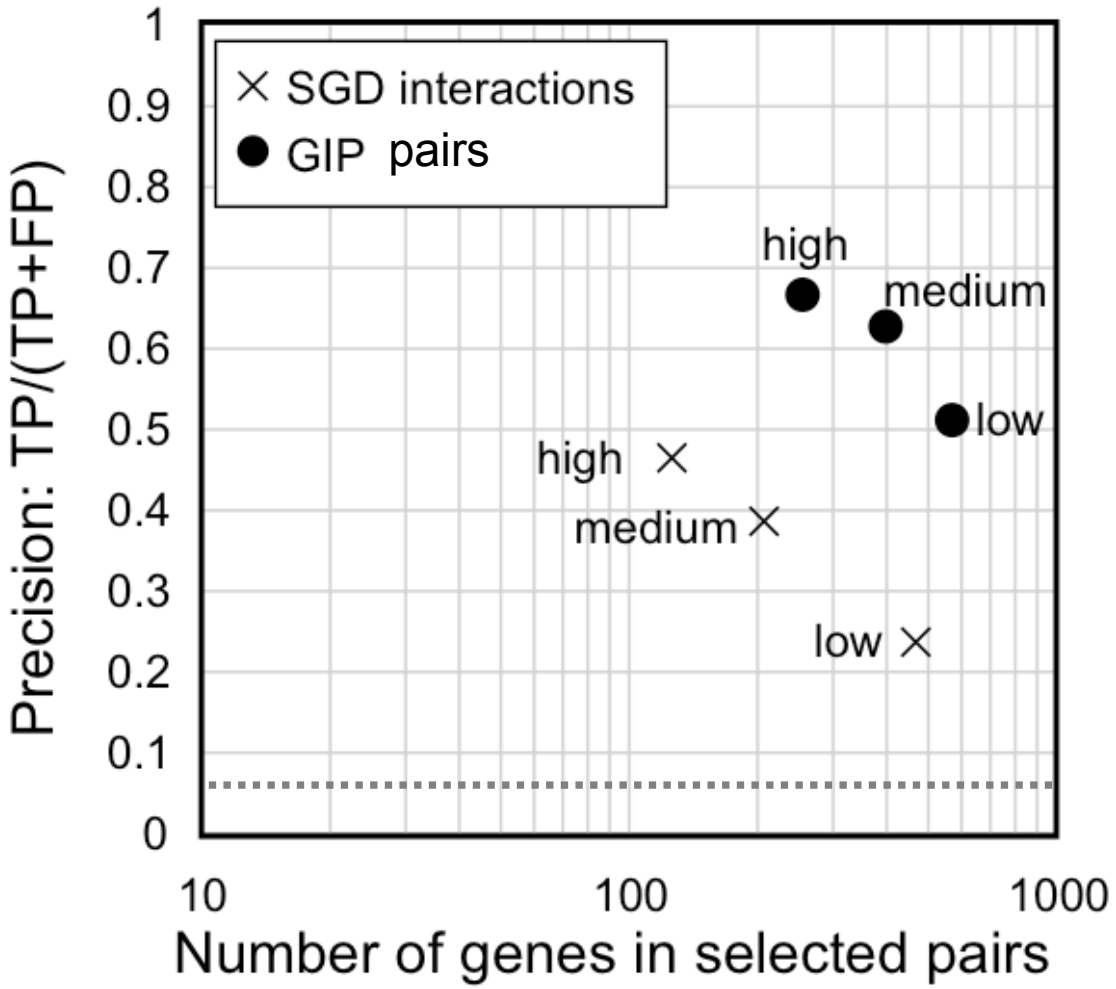
41 screens in duplicates

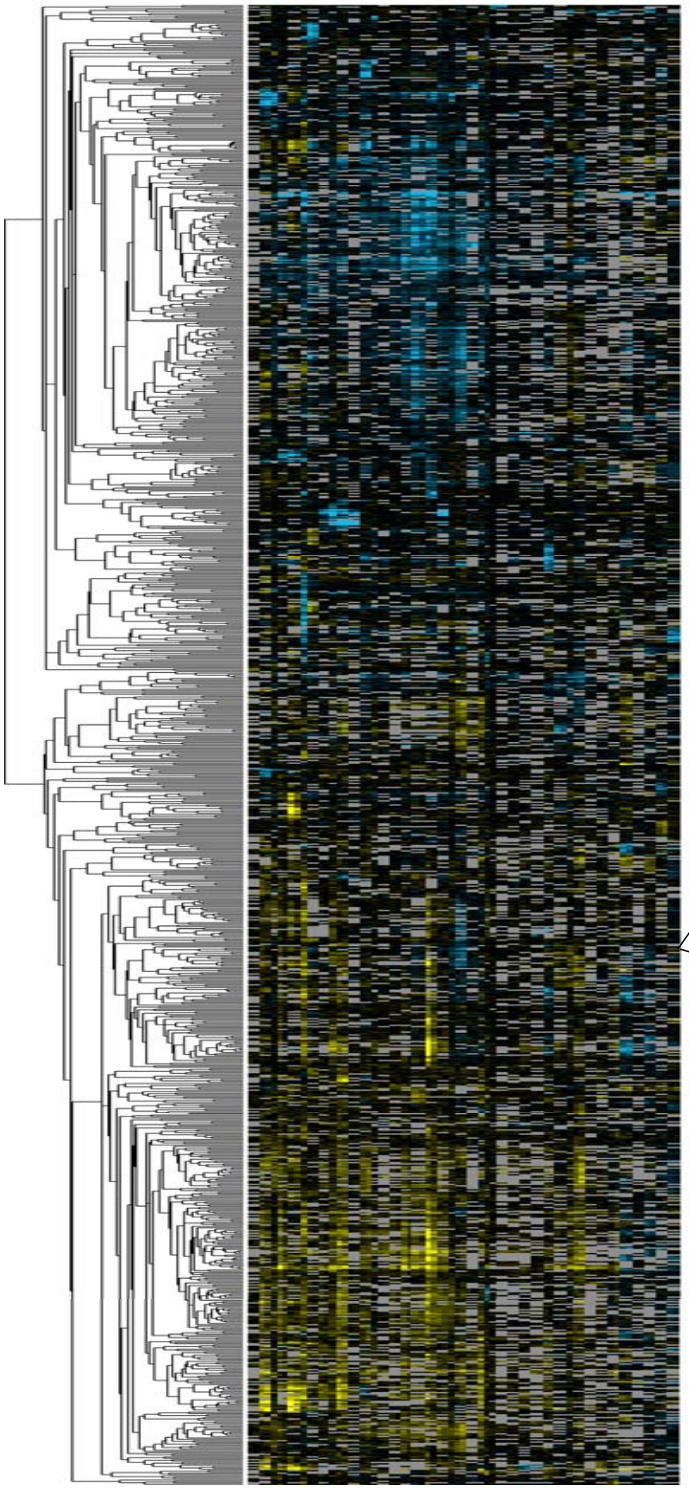


Genetic Interaction Profile (GIP) pairs

(Pearson correlation coefficient & specificity)

41 screens in duplicates





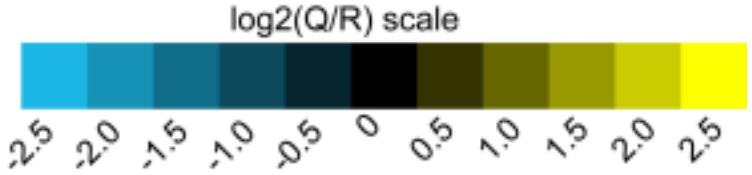
41 screens (duplicated)

1095 mutants (~25% of the mutant library) with a detectable genetic interaction

Pearson's correlation coeff.: 0.92

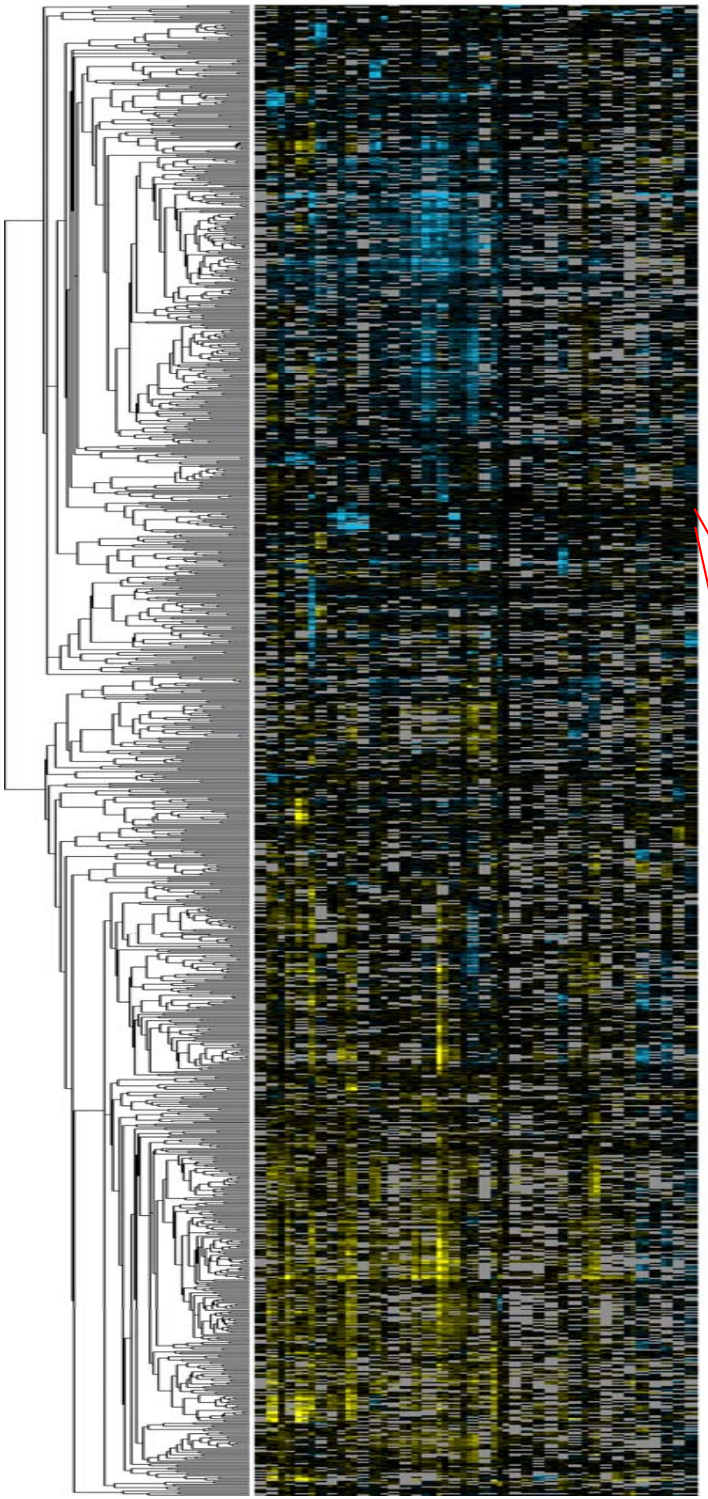
Synthetic growth defect

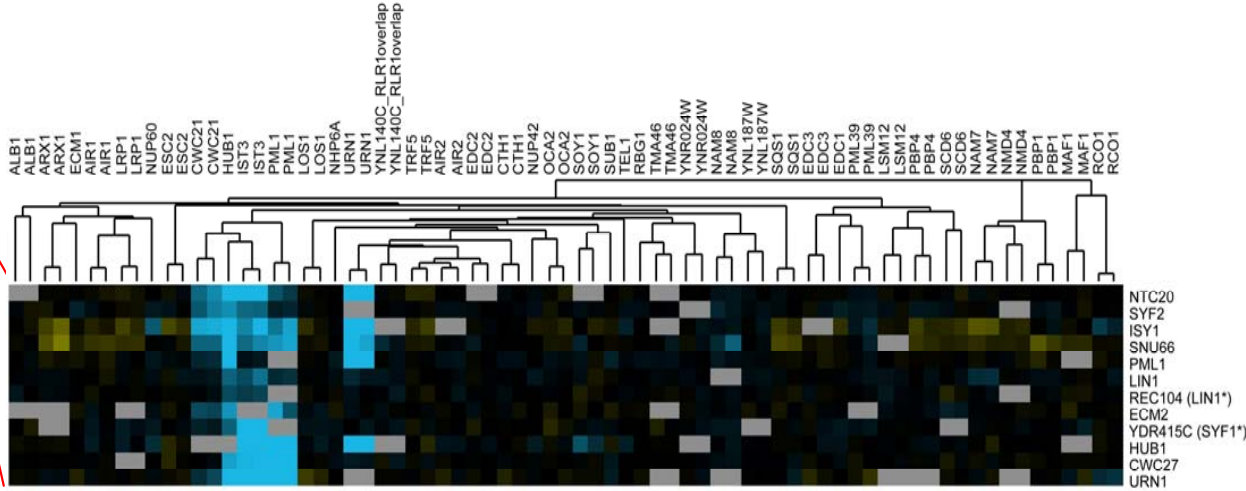
Epistasis/Suppresion/Buffering



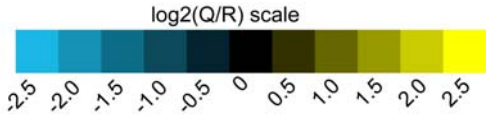
41 screens in duplicate

1095 mutants (~25% of the mutant library) with a detectable genetic interaction

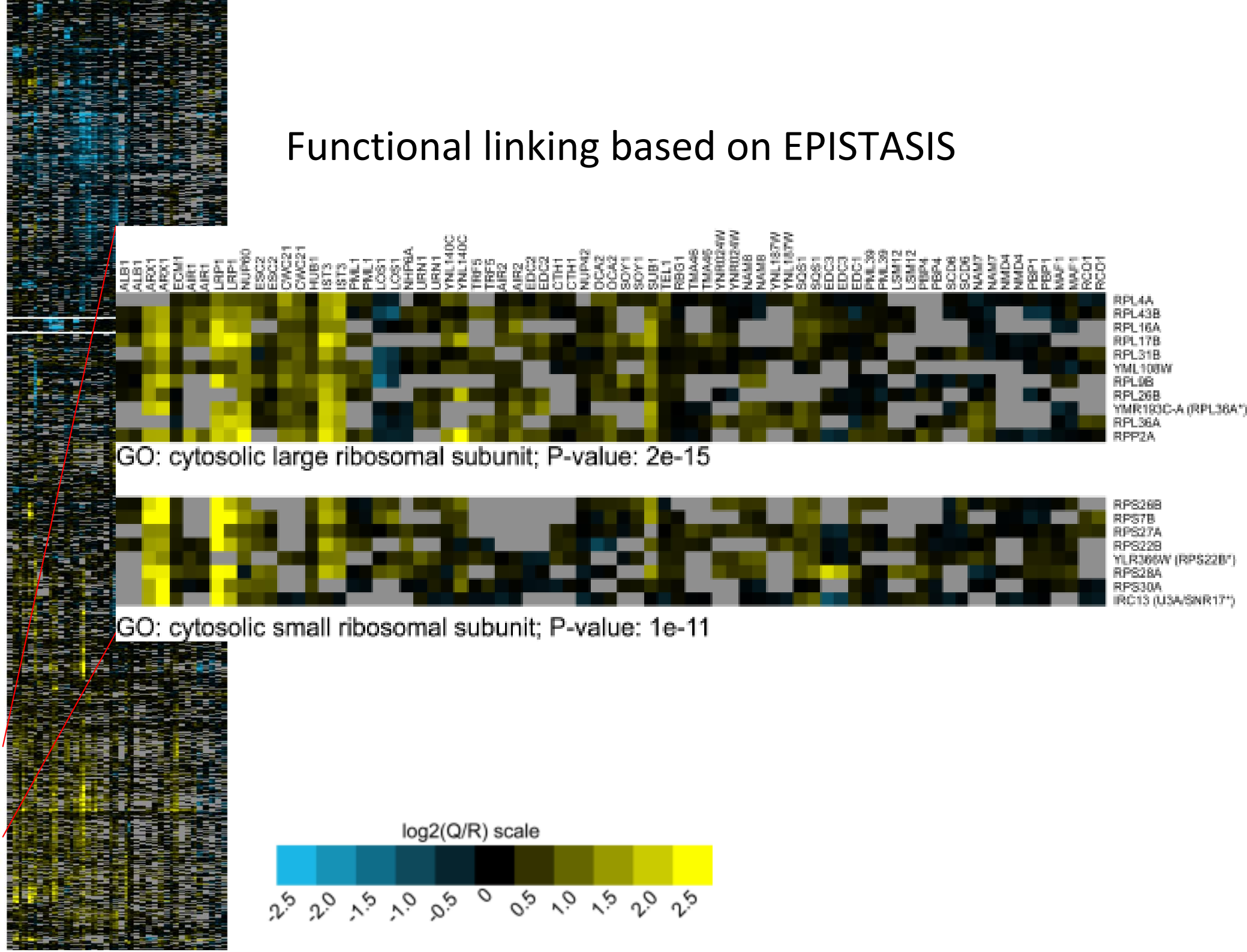




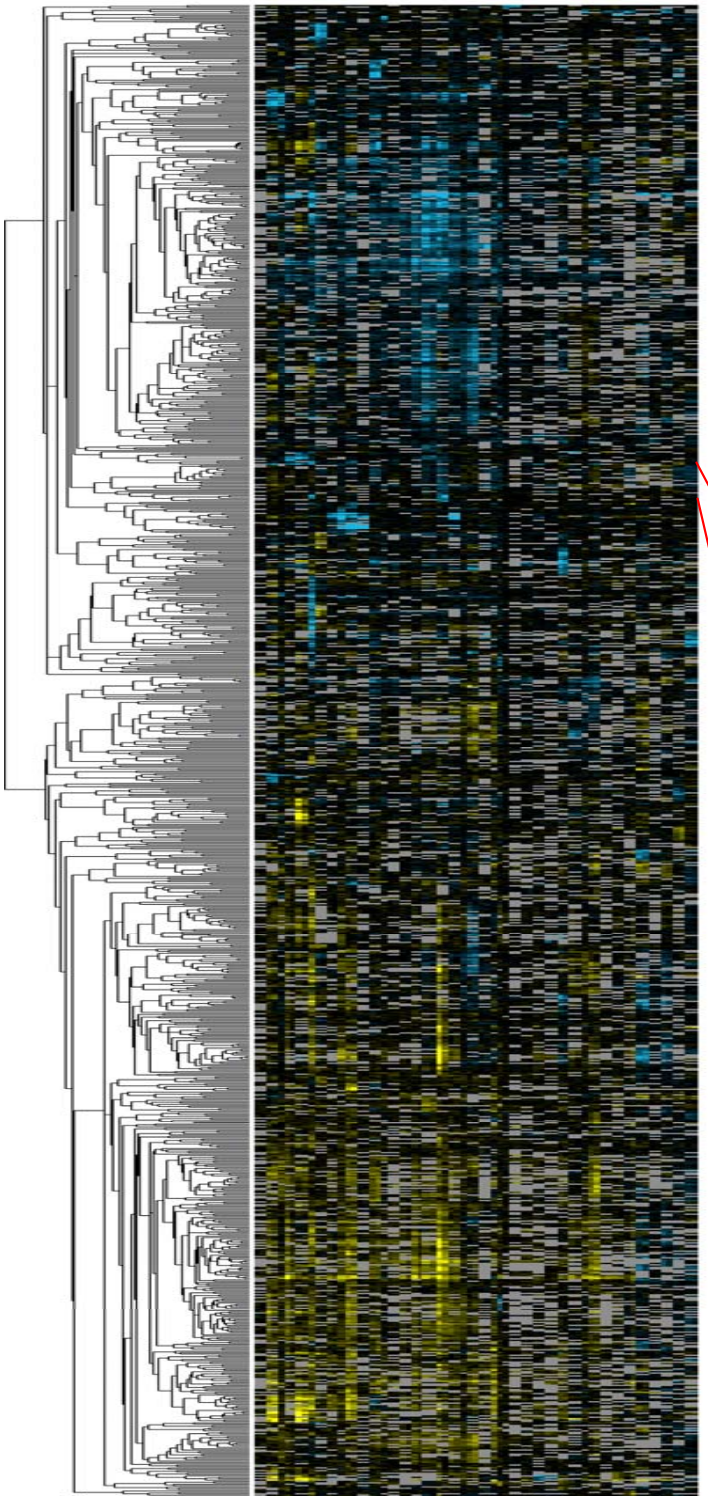
GO: nuclear mRNA splicing, via spliceosome; P-value: 4.5e-09

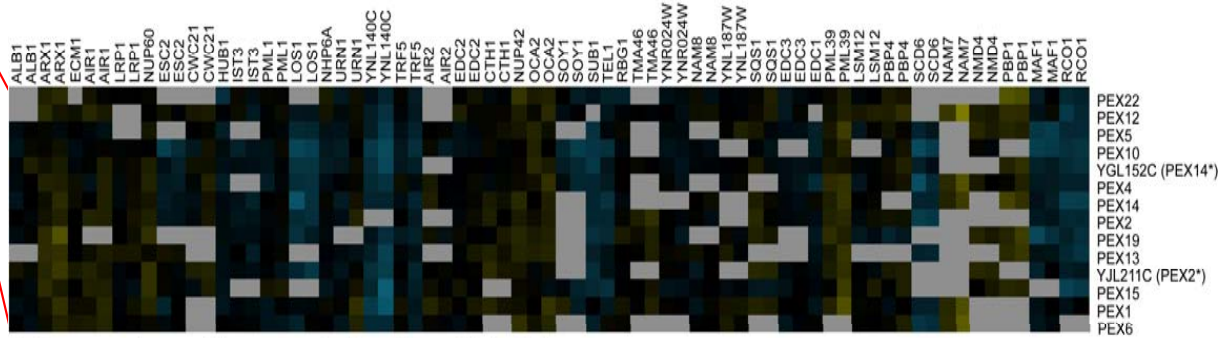


Functional linking based on EPISTASIS

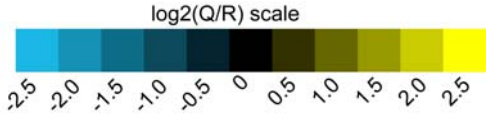


Very weak interactions can globally define high GIP scores

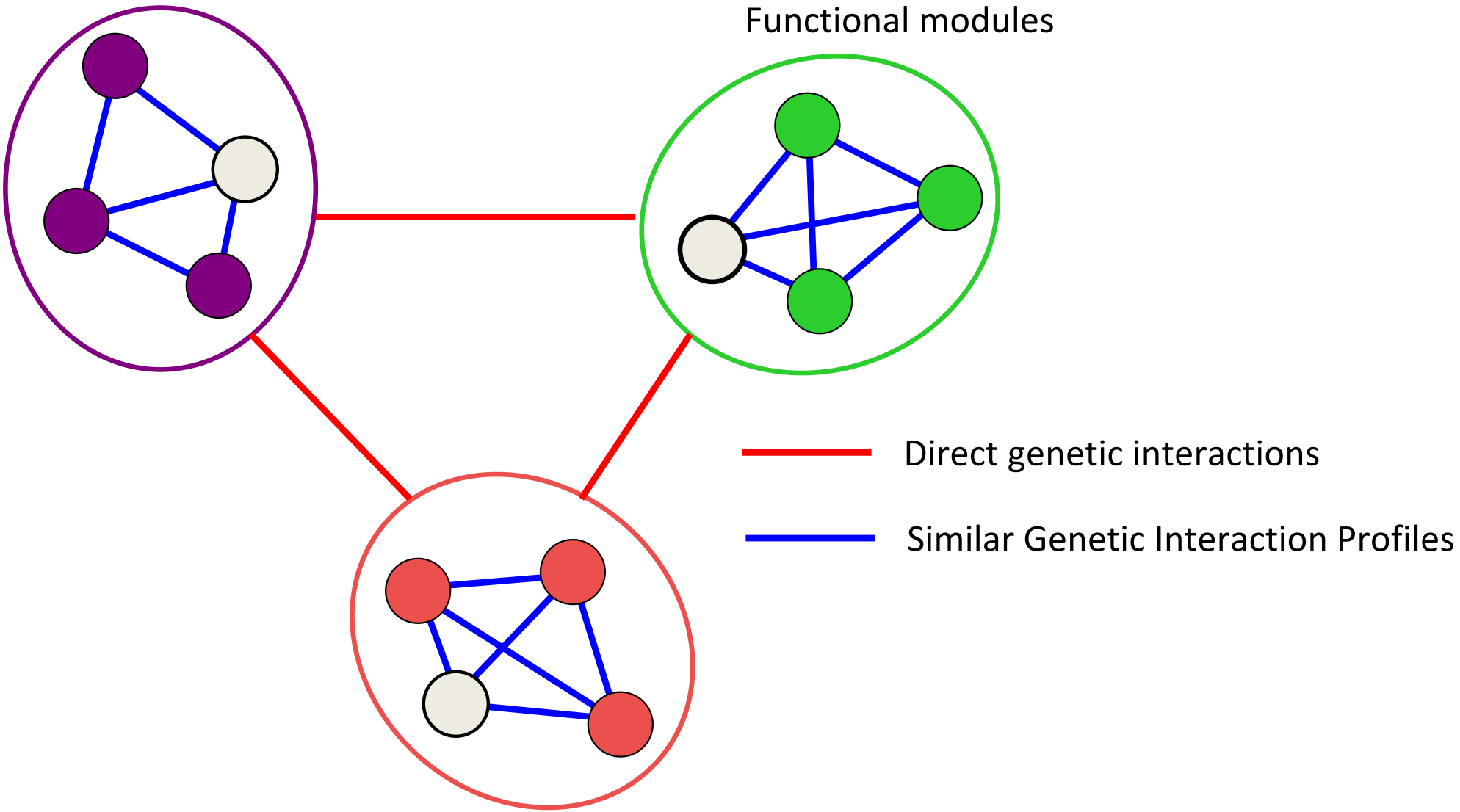


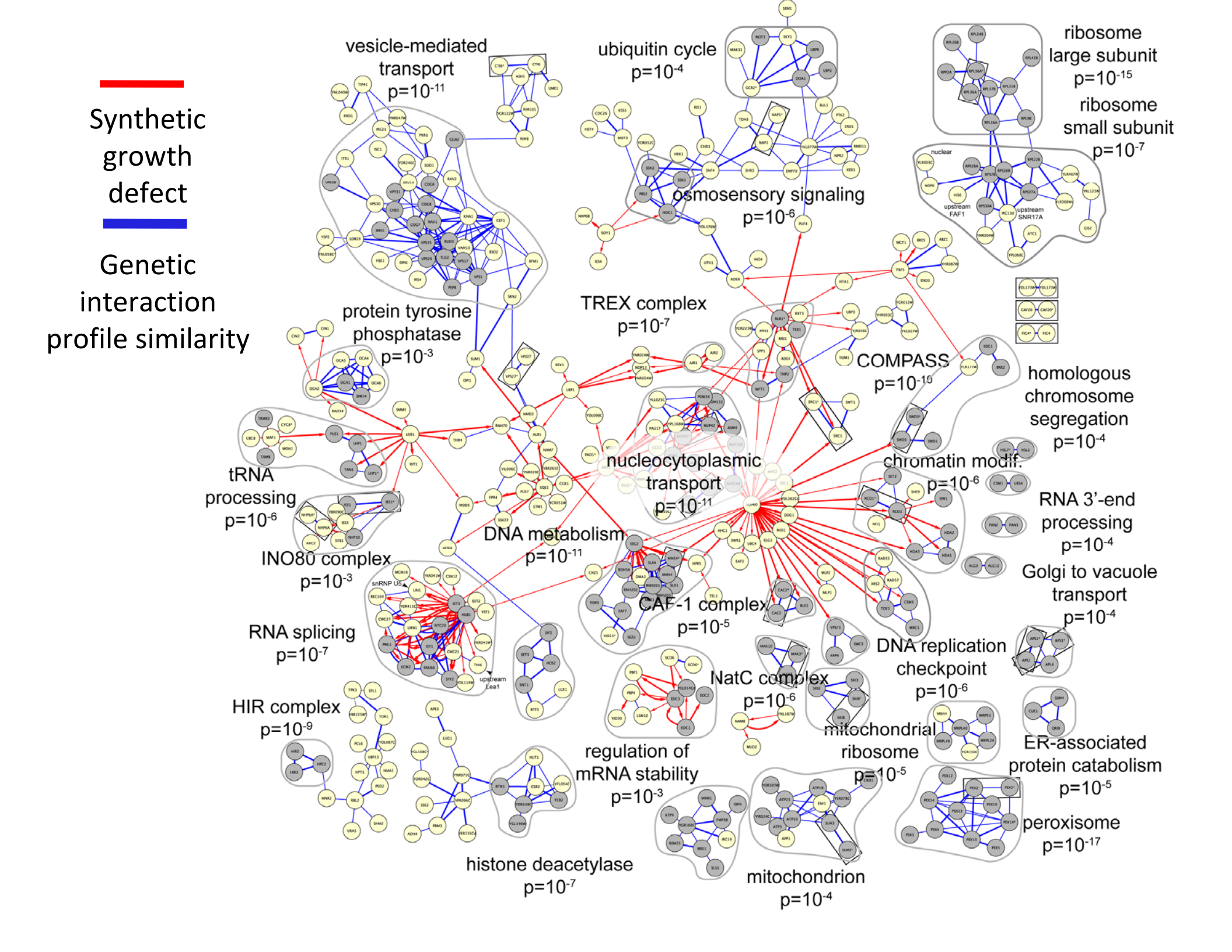


GO: peroxisomal transport; P-value: 1.5e-31



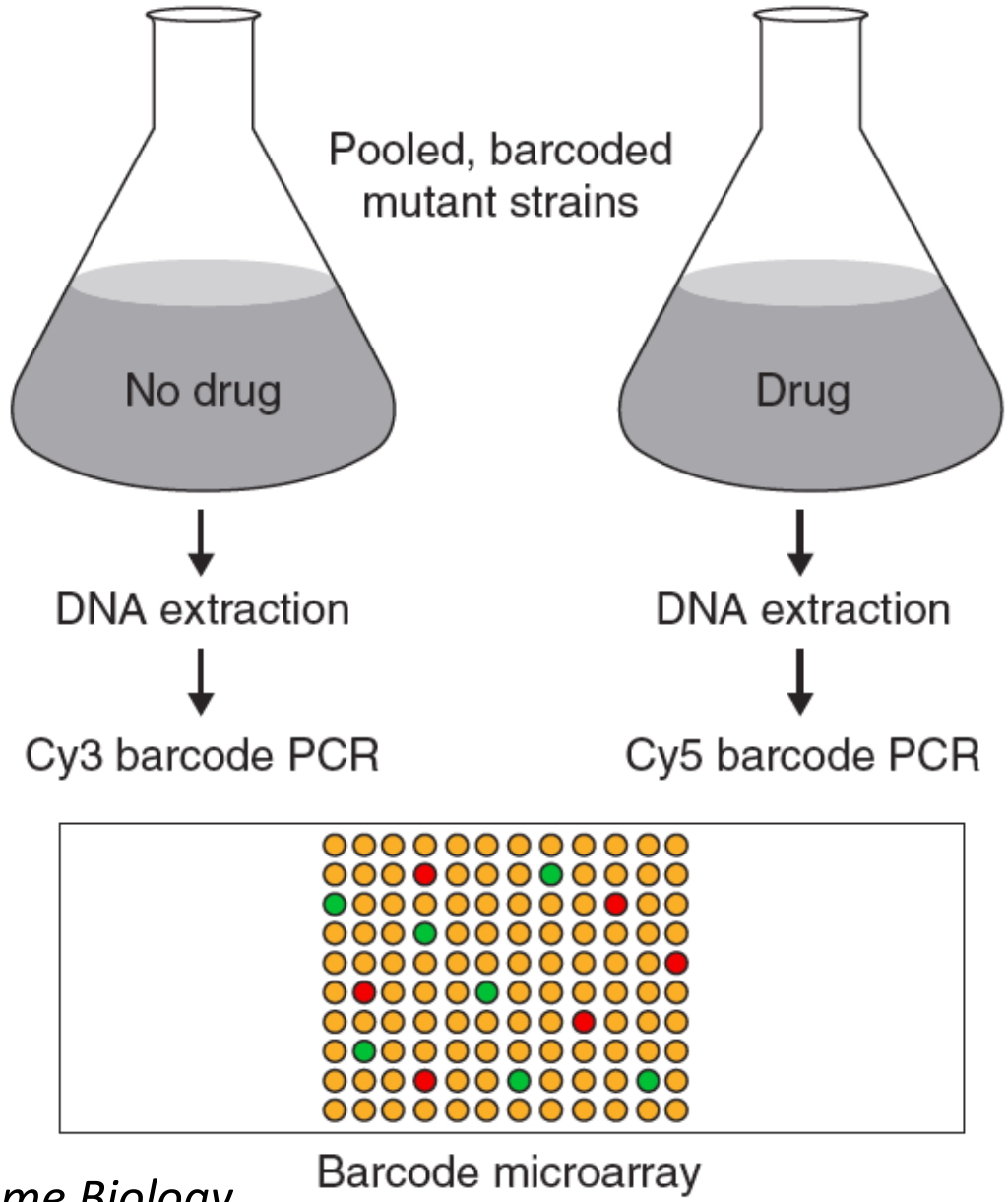
Schematic Genetic Network




Synthetic growth defect
Genetic interaction profile similarity
vesicle-mediated transport p=10-11
ubiquitin cycle p=10-4
ribosome large subunit p=10-15
ribosome small subunit p=10-7
nuclear
upstream FAF1
upstream SNR17A
osmosensory signaling p=10-6
protein tyrosine phosphatase p=10-3
TREX complex p=10-7
COMPASS p=10-10
homologous chromosome segregation p=10-4
chromatin modif. p=10-6
RNA 3'-end processing p=10-4
Golgi to vacuole transport p=10-4
tRNA processing p=10-6
nucleocytoplasmic transport p=10-11
DNA metabolism p=10-11
INO80 complex p=10-3
snRNP U5
upstream Lea1
RNA splicing p=10-7
CAF-1 complex p=10-5
DNA replication checkpoint p=10-6
NatC complex p=10-6
HIR complex p=10-9
regulation of mRNA stability p=10-3
mitochondrial ribosome p=10-5
ER-associated protein catabolism p=10-5
peroxisome p=10-17
histone deacetylase p=10-7
mitochondrion p=10-4

Variations on the theme

Chemogenomics



Taken from: Brenner (2004) *Genome Biology*

Chemogenomics

Parson,… & Boon (2004) *Cell*

Hoon,… &Nislow (2008) *Nature Chemical Biology*

Hillenmeyer, … &Giaver (2008) *Science*

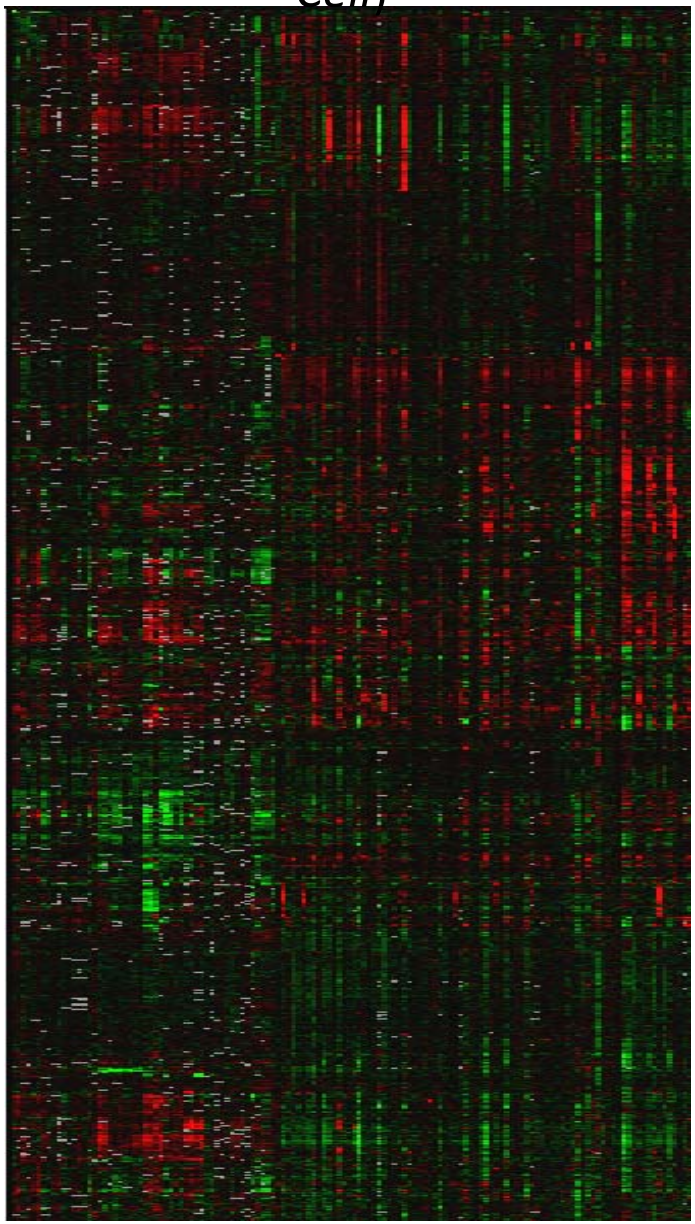
etc…

Chemogenomics

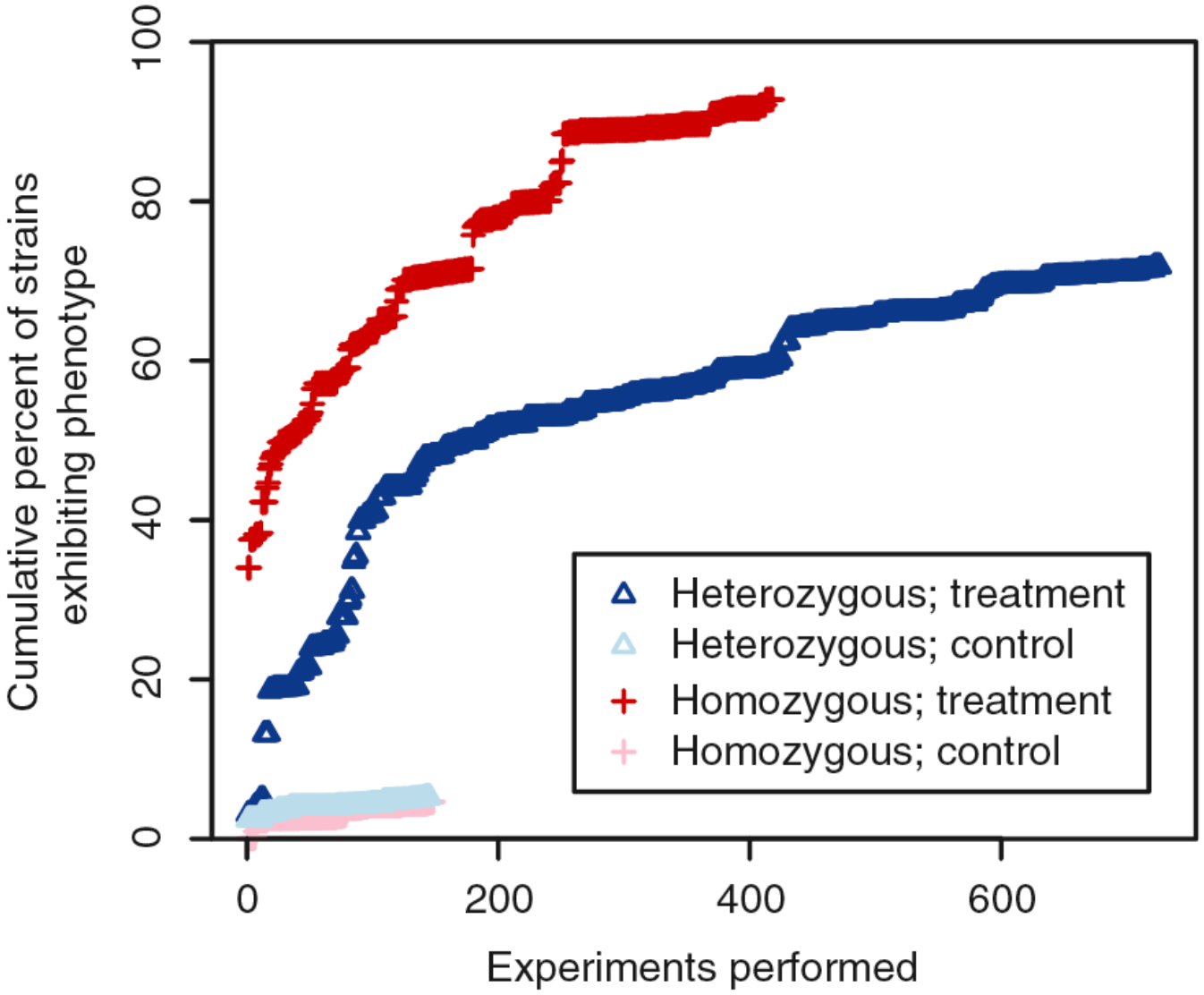
GIM *vs*Chemical Profiling

GIM

Chemical profiling (Parson & Boon (2004) *Cell*)



A phenotype for everyone



(Hillenmeyer et al., *Science*, **2008**)

A phenotype can be found for every gene deletion in yeast, under particular drug conditions

CosminSaveanu

Laurence Decourty

Collaborators:

Christophe Malabat

DomenicoLibri (CNRS, Gif-sur-Yvette)
David Tollervey (Edinburgh, UK)
(Milligan et al., MCB, 2008)
Bertrand Seraphin (CNRS, Gif-sur-Yvette)
Olivier Gadal (CNRS, Toulouse)
(Berger et al., MCB, 2007)
Franck Feuerbach-Fournier (IP)
Olivier Lefebvre (CEA, Saclay)
Denis Lafontaine (ULB, Bruxelles)

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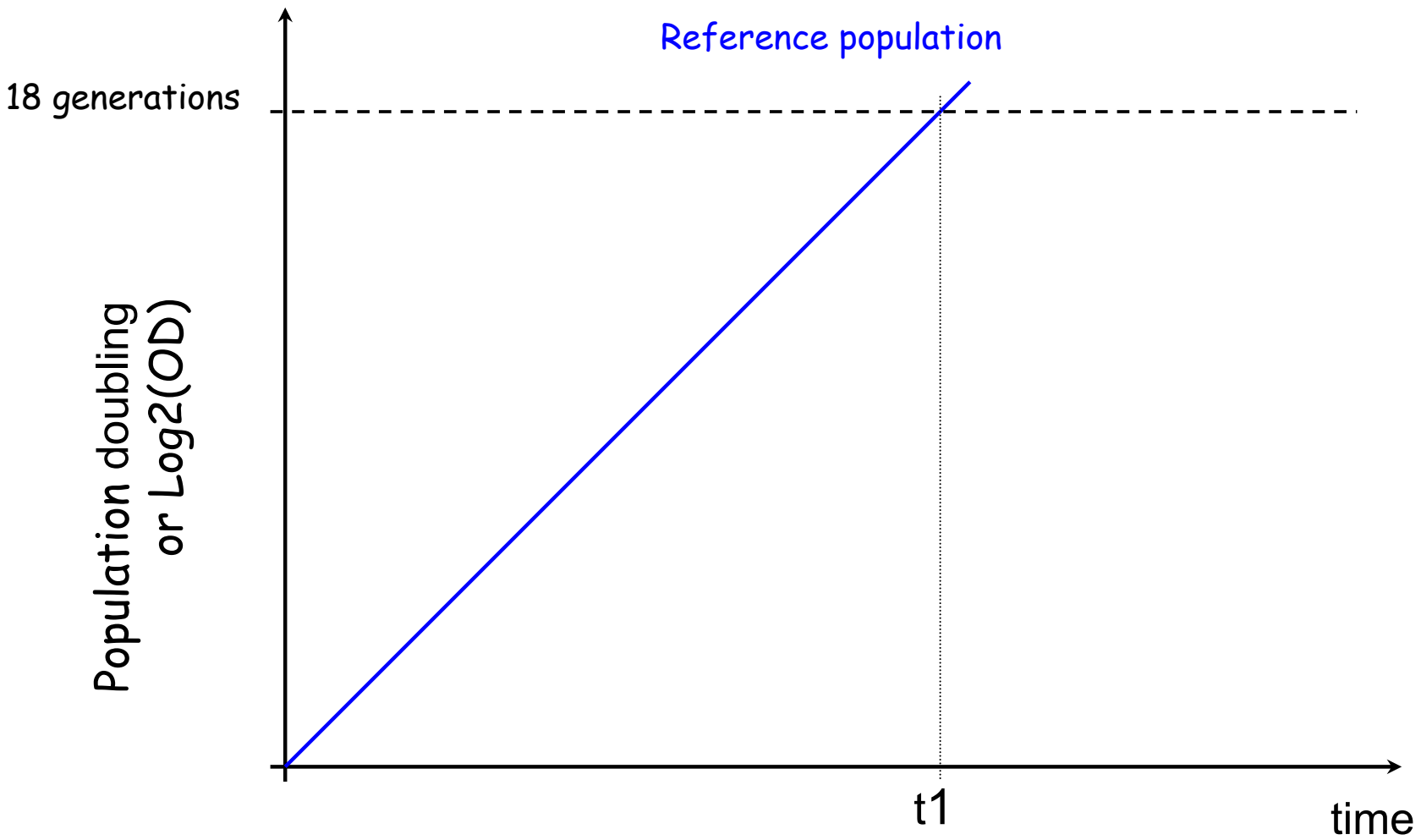
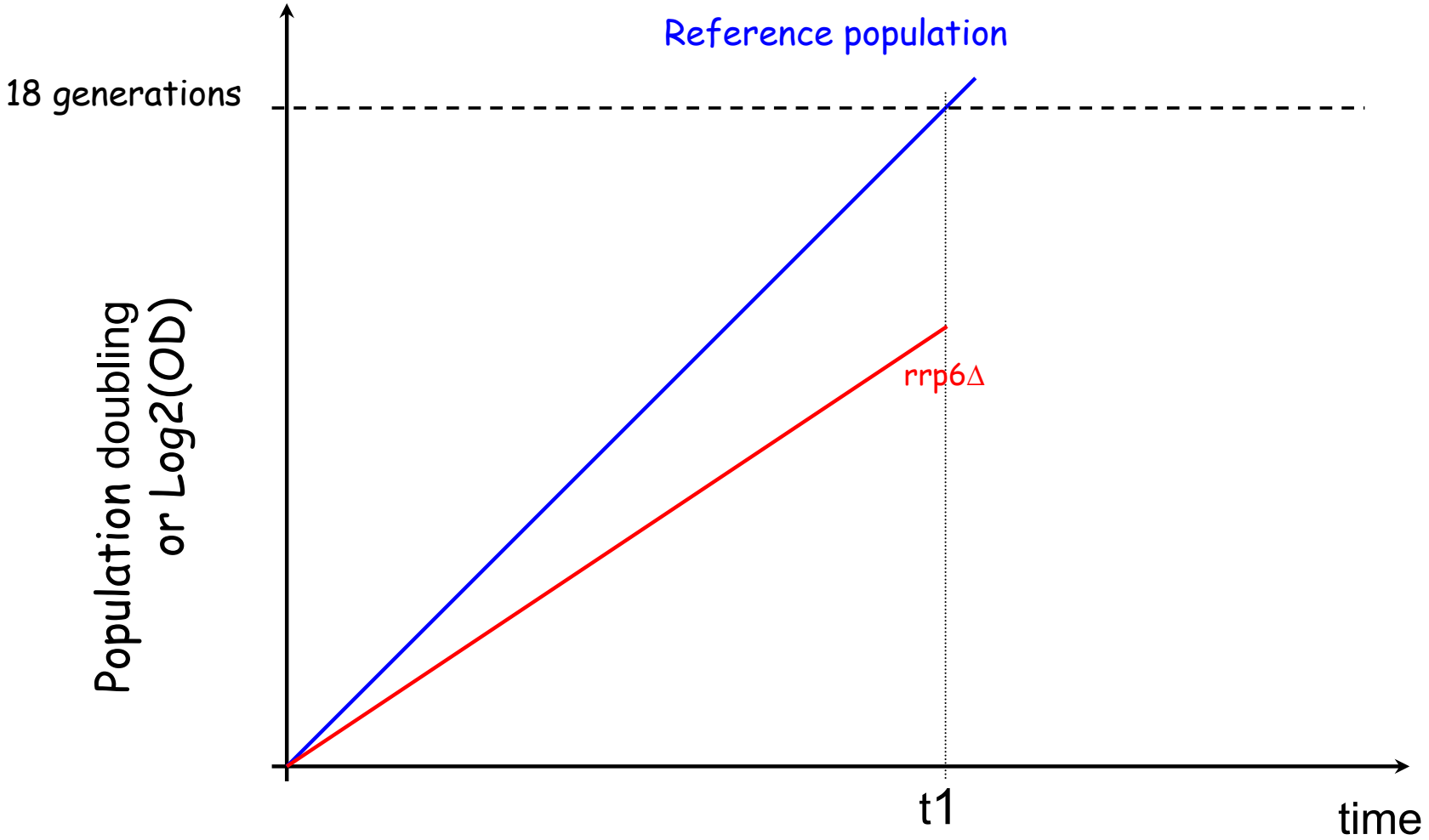
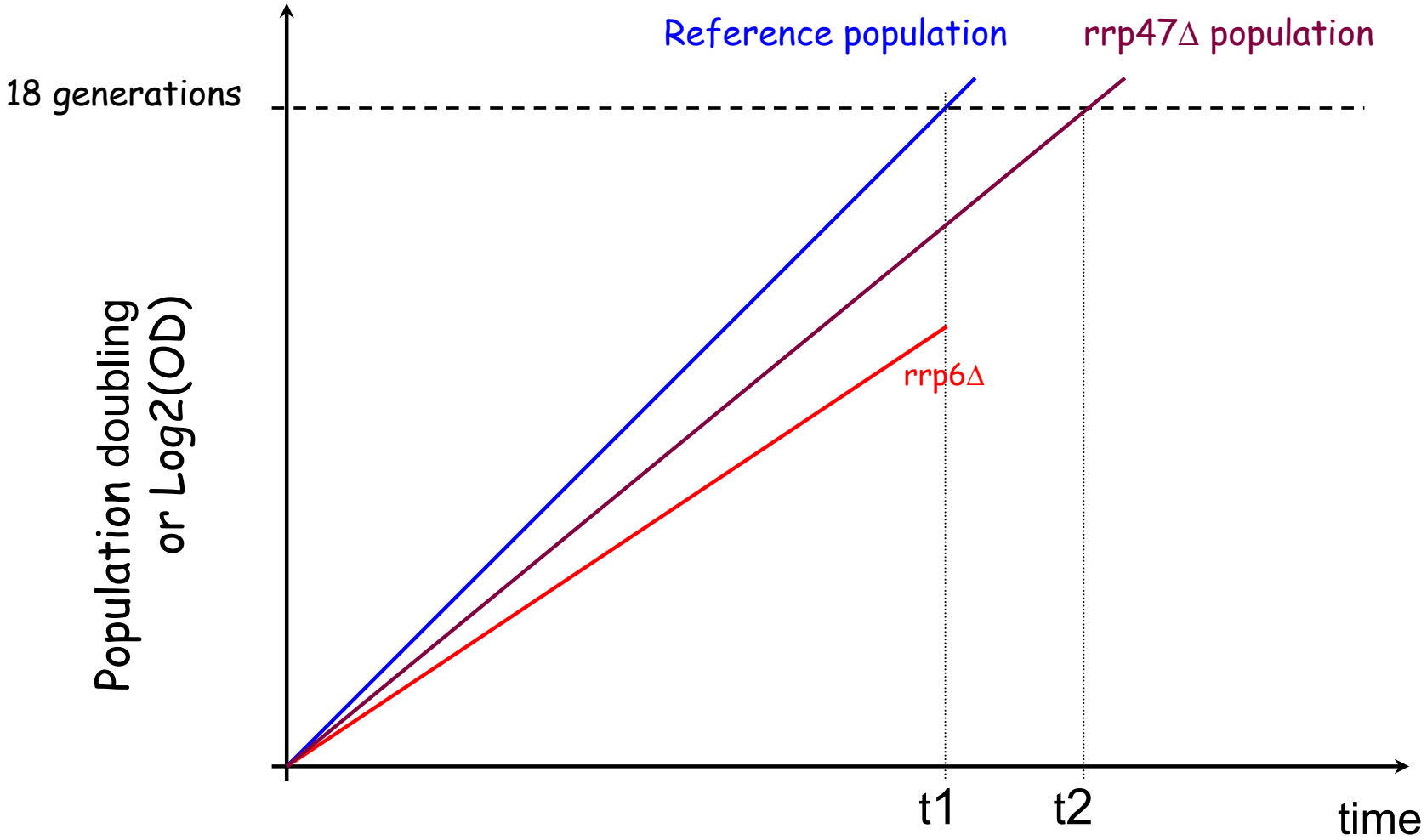
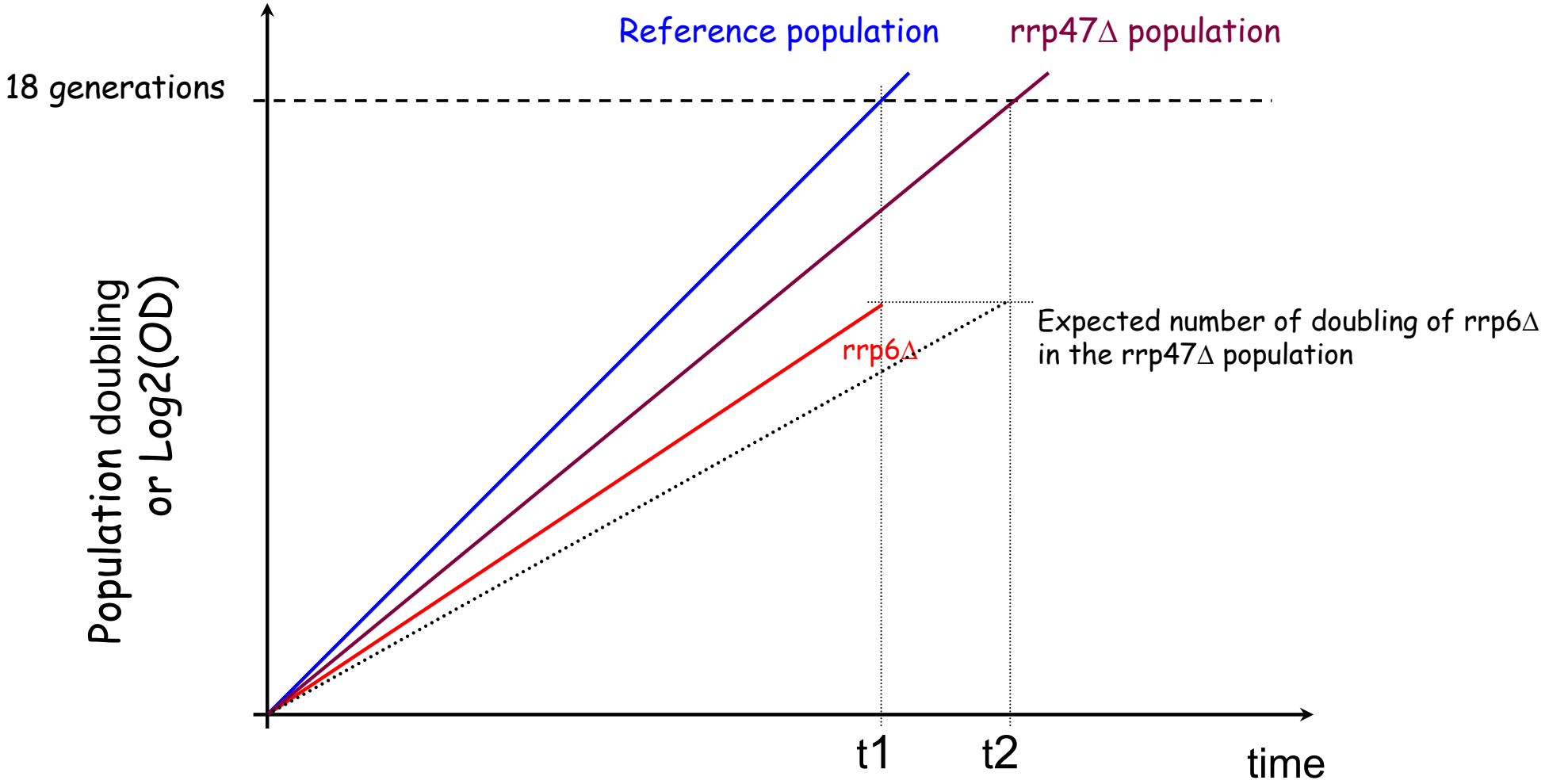
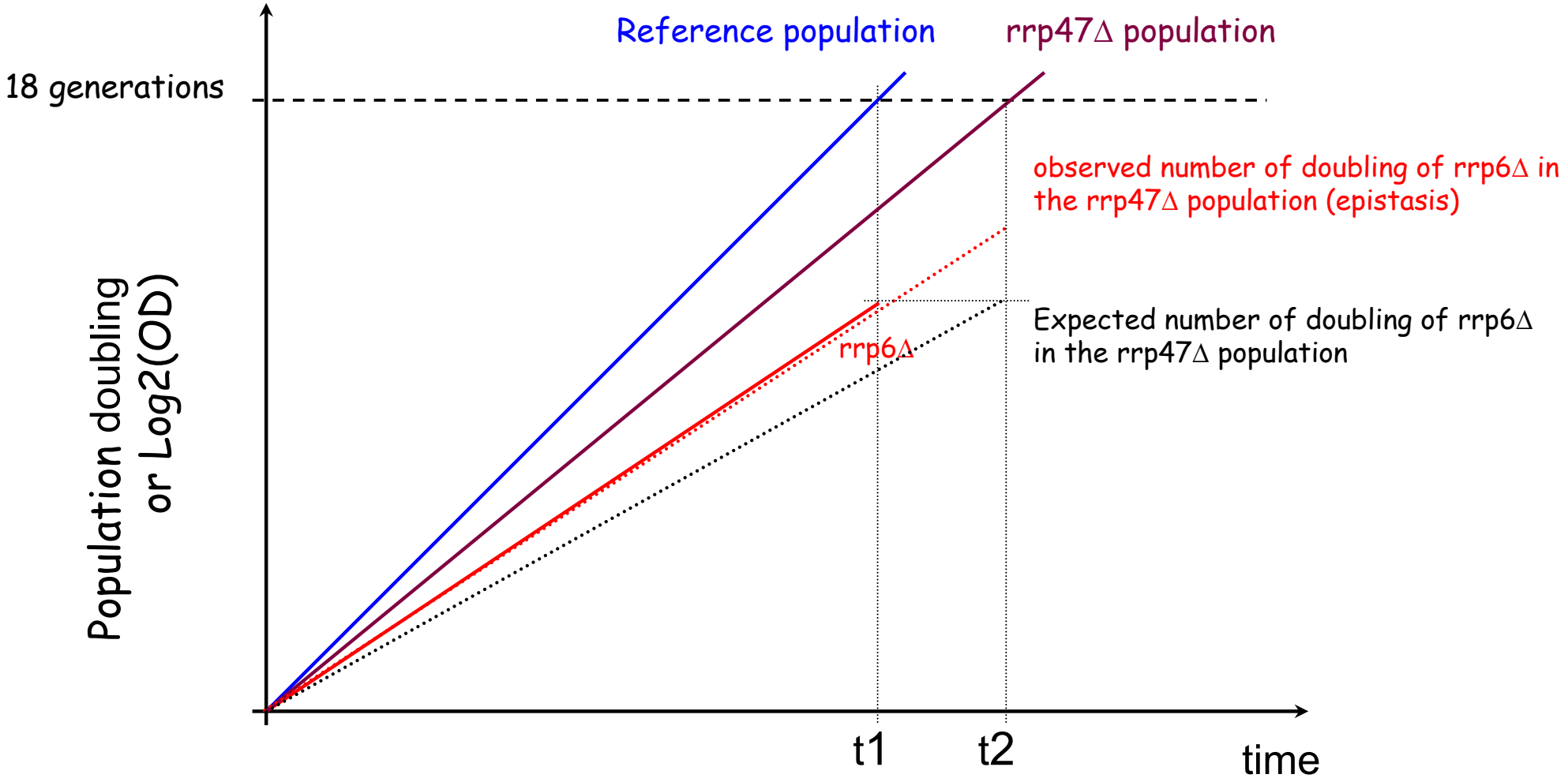
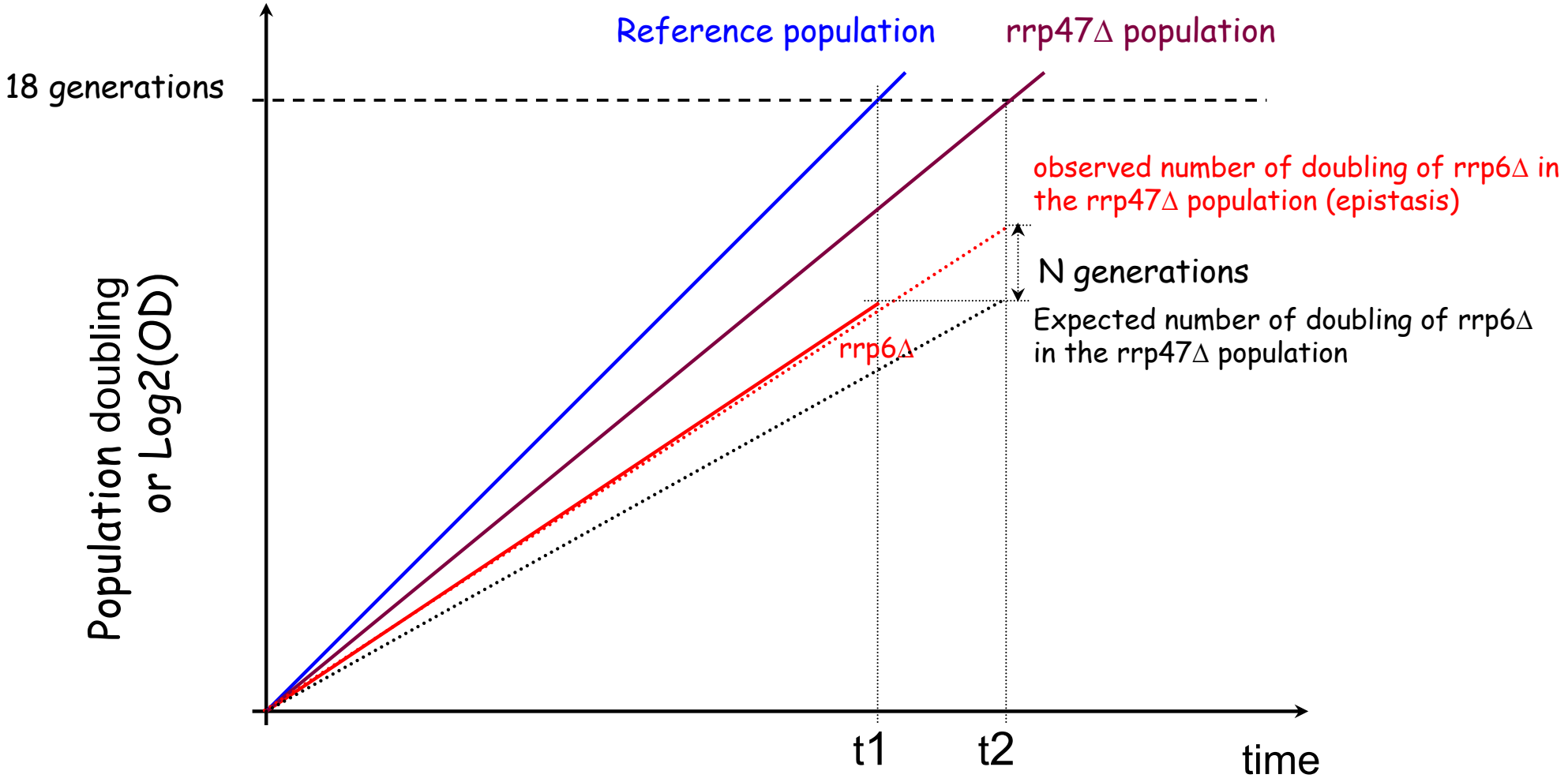

Reference population
18 generations
Population doubling
or Log2(OD)
t1
time

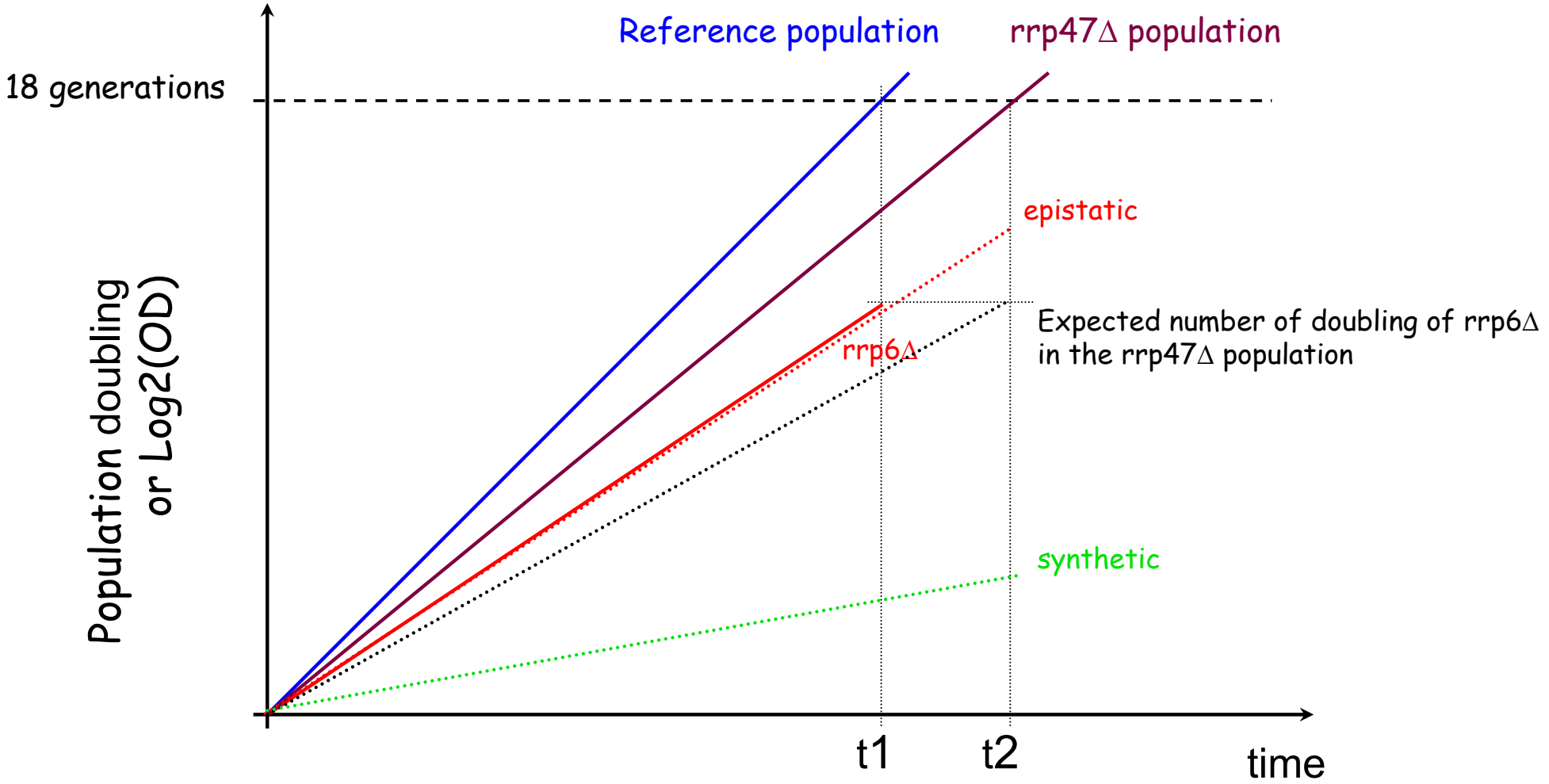

Reference population
18 generations
rrp6Δ
Population doubling
or Log2(OD)
t1
time

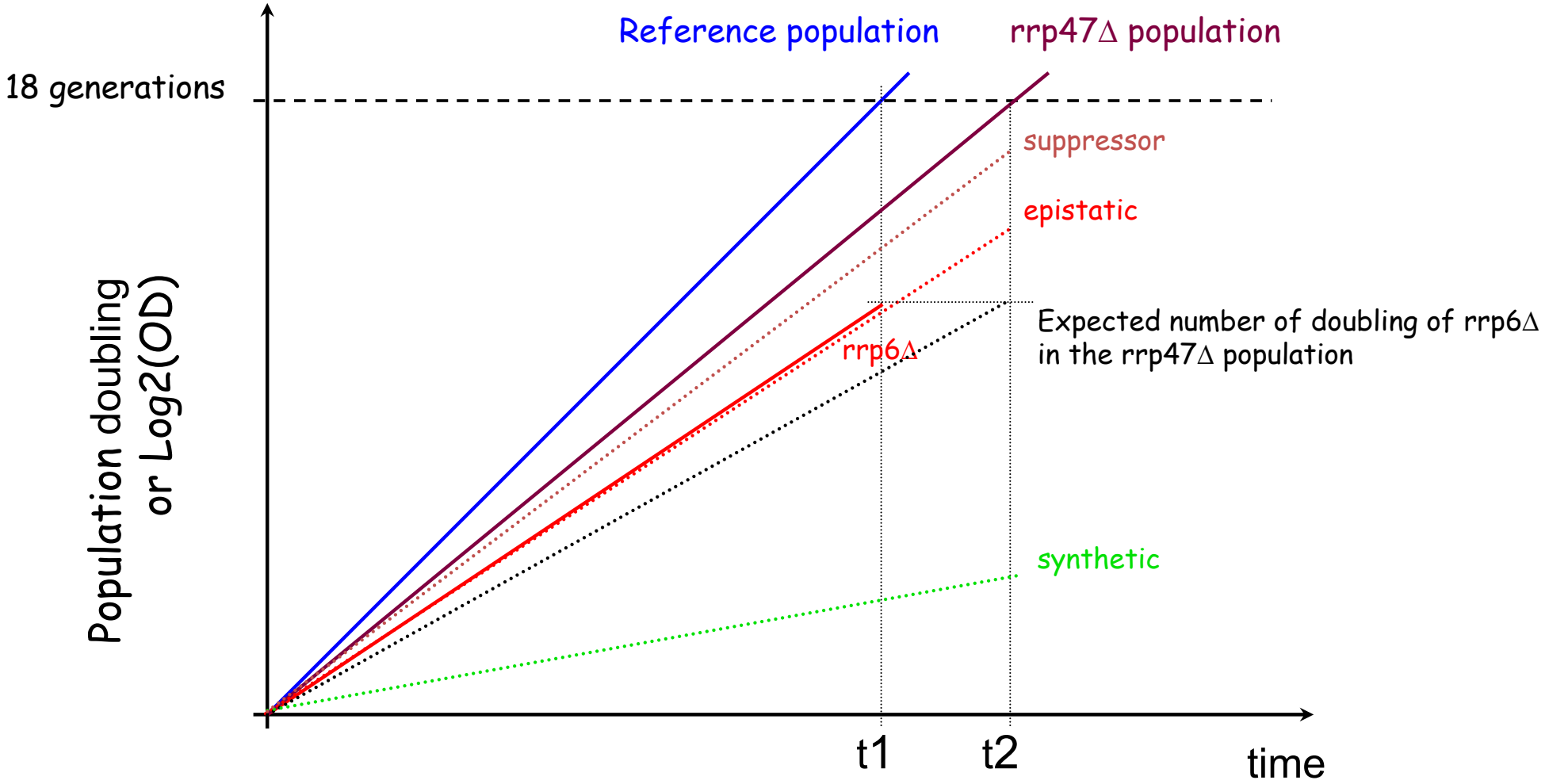

Reference population
rrp47Δ population
18 generations
rrp6Δ
Population doubling
or Log2(OD)
t1
t2
time


Reference population
rrp47Δ population
18 generations
Population doubling
or Log2(OD)
rrp6Δ
Expected number of doubling of rrp6Δ
in the rrp47Δ population
t1
t2
time


Reference population
rrp47Δ population
18 generations
observed number of doubling of rrp6Δ in the rrp47Δ population (epistasis)
Expected number of doubling of rrp6Δ in the rrp47Δ population
rrp6Δ
Population doubling or Log2(OD)
t1
t2
time


Reference population
rrp47Δ population
18 generations
observed number of doubling of rrp6Δ in the rrp47Δ population (epistasis)
N generations
Expected number of doubling of rrp6Δ in the rrp47Δ population
rrp6Δ
Population doubling or Log2(OD)
t1
t2
time


Reference population
rrp47Δ population
18 generations
epistatic
Expected number of doubling of rrp6Δ
in the rrp47Δ population
rrp6Δ
synthetic
Population doubling
or Log2(OD)
t1
t2
time


Reference population
rrp47Δ population
18 generations
suppressor
epistatic
Expected number of doubling of rrp6Δ
in the rrp47Δ population
rrp6Δ
synthetic
Population doubling
or Log2(OD)
t1
t2
time