Difference between dominance and epistasis

Dominance	Epistasis
❑Involves intra-allelic gene interaction.	❑Involves inter-allelic gene interaction.
❑One allele hides the effect of other allele at the same gene pair.	❑One gene hides the effect of other gene at different gene loci.

NELL'EPISTASI UN GENE INFLUENZA L'EFFETTO DI UN ALTRO GENE

L'**epistasi** è un fenomeno per cui un gene influenza l'espressione fenotipica di un altro gene. Per evidenziare un fenomeno epistatico è conveniente studiare la progenie che si forma dall'incrocio di due diibridi. In caso di epistasi, infatti, la comune distribuzione dei fenotipi studiata da Mendel (9:3:3:1) risulta modificata.

Un esempio è costituito dal colore del mantello dei cani di razza Labrador, che dipende da due geni, *B* ed *E*.

- Il gene *B* controlla la produzione del pigmento melanina: l'**allele** dominante *B* produce pigmentazione nera, mentre l'allele recessivo *b* produce pigmentazione marrone.
- Il gene *E* controlla invece la deposizione del pigmento nel mantello: in presenza dell'allele dominante *E* la melanina si deposita normalmente nel pelo; l'allele recessivo *e* invece impedisce la deposizione del pigmento: esso viene prodotto ma non si deposita nella pelliccia. Il risultato è un mantello di colore giallo.

Di conseguenza i cani *BB* o *Bb* sono neri e quelli *bb* sono marroni solo se sono anche *EE* oppure *Ee*; i cani *ee*, invece, sono sempre di colore giallo, indipendentemente dalla presenza degli alleli *B* o *b* (▸figura 14). L'allele recessivo *e* è quindi epistatico sugli alleli *B* e *b*.

Dall'accoppiamento fra due cani *BbEe* si ottiene una cucciolata con 9/16 di cani neri, 3/16 di cani marroni e 4/16 di cani gialli; puoi costruire un quadrato di Punnett per verificare questa previsione.

Figura 14 ↗ **I geni possono interagire reciprocamente tramite epistasi** • L'epistasi si manifesta quando un gene altera l'effetto fenotipico di un altro gene. Nel caso dei cani Labrador retriever il gene *E/e* determina l'espressione del gene *B/b*.

Le parole

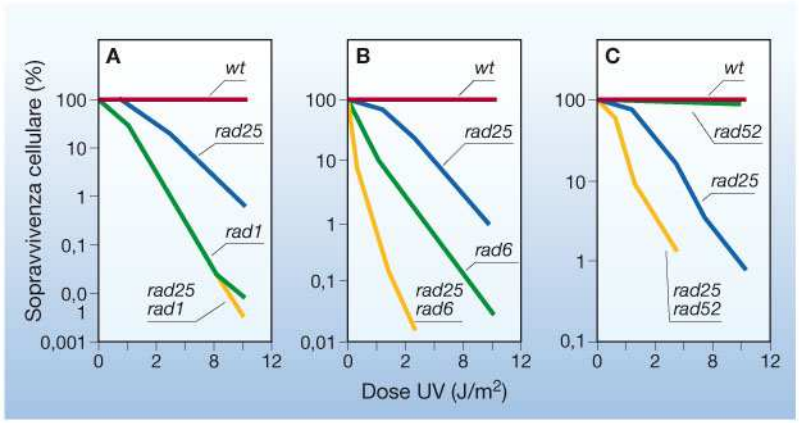
Il termine **epistàsi** deriva dal greco *epí*, «su», e *stásis*, «esser posto», per indicare una relazione concettualmente analoga alla dominanza, ma che andava distinta perché si verificava tra due geni diversi e non tra due alleli dello stesso gene.

Epistatic Gene Interactions

- Gene interactions occur when two or more different genes influence the outcome of a single trait
- Most morphological traits (height, weight, color) are affected by multiple genes
- Epistasis describes situation between various alleles of two genes
- Quantitative loci is a term to describe those loci controlling quantitatively measurable traits
- Pleiotropy describes situations where one gene affects multiple traits

- In **epistasis**, a gene at one locus alters the phenotypic expression of a gene at a second locus.
- *One*, the epistatic gene, determines whether pigment will be present in hair (C)[dominant] or absent (c).
- The *second* determines whether the pigment to be deposited is black (B) [dominant] or brown (b).
  - An individual that is cc has a white (albino) coat regardless of the genotype of the second gene.

  → C/c gene (first) is epistatic to the B/b gene second).



Identification of yeast genes coding for proteins involved in DNA damage repair and definition of epistasis groups.
A): Survival curves of mutants rad25 and rad1 exposed to UV radiation. They have different sensitivity but the double mutant is not additive. Both genes are involved in NER and belong to the same epistasis group.
B) and C); rad6 and rad52 code for two proteins involved in post-replication repair (PPR) and recombination repair (HR), respectively. Rad52 alone does not cause sensitivity to UV (C) but double mutants with rad25 (C) are additive as double mutant of rad6 and rad25 (B) because the genes belong to different epistasis groups.

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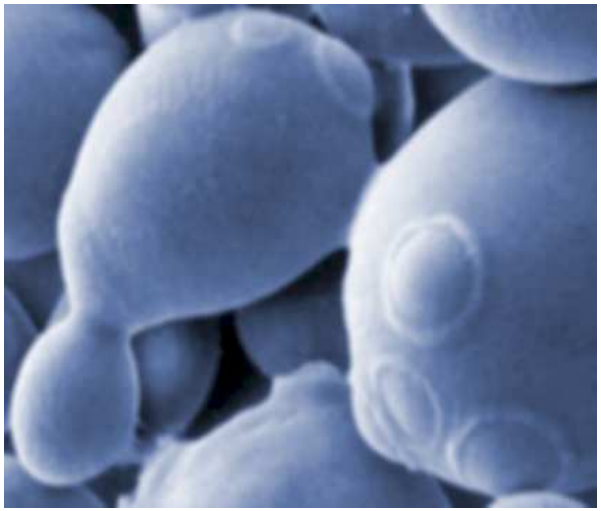
The University of Manchester

Faculty of
Life Sciences

Genomic approaches to fitness in yeasts
Tecnologia di lievito barcoded

Daniela Delneri

La Mitosi: Approcci sperimentali e sistemi modello
Roma, CNR 05/11/2012



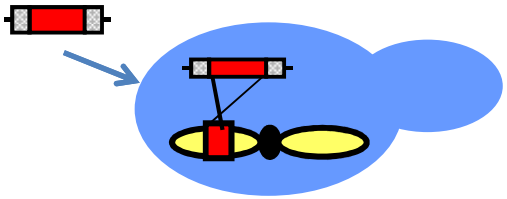
Yeast as model system

Single-celled eukaryote

- Grow in many natural locations, and in animals and insects.
- Rapid growth
- Stable haploid and diploid state
- Non-pathogenic (GRAS) and inexpensive

Simple genetics and physiology

- Efficient transformation system and homologous recombination



Sequence data for *Saccharomycetes*

Several genes involved in human diseases has an homologs in yeast

(Foury F. (1997) Human genetic diseases: a cross-talk between man and yeast, **195**:1-10)

L.H. Hartwell

S. cerevisiae

T. Hunt

Sea urchins

P. Nurse

Sz. pombe

Nobel Price for Medicine and Physiology 2001 for discoveries of proteins that control cell cycle division.

Fitness and haploinsufficiency: the role of the environment

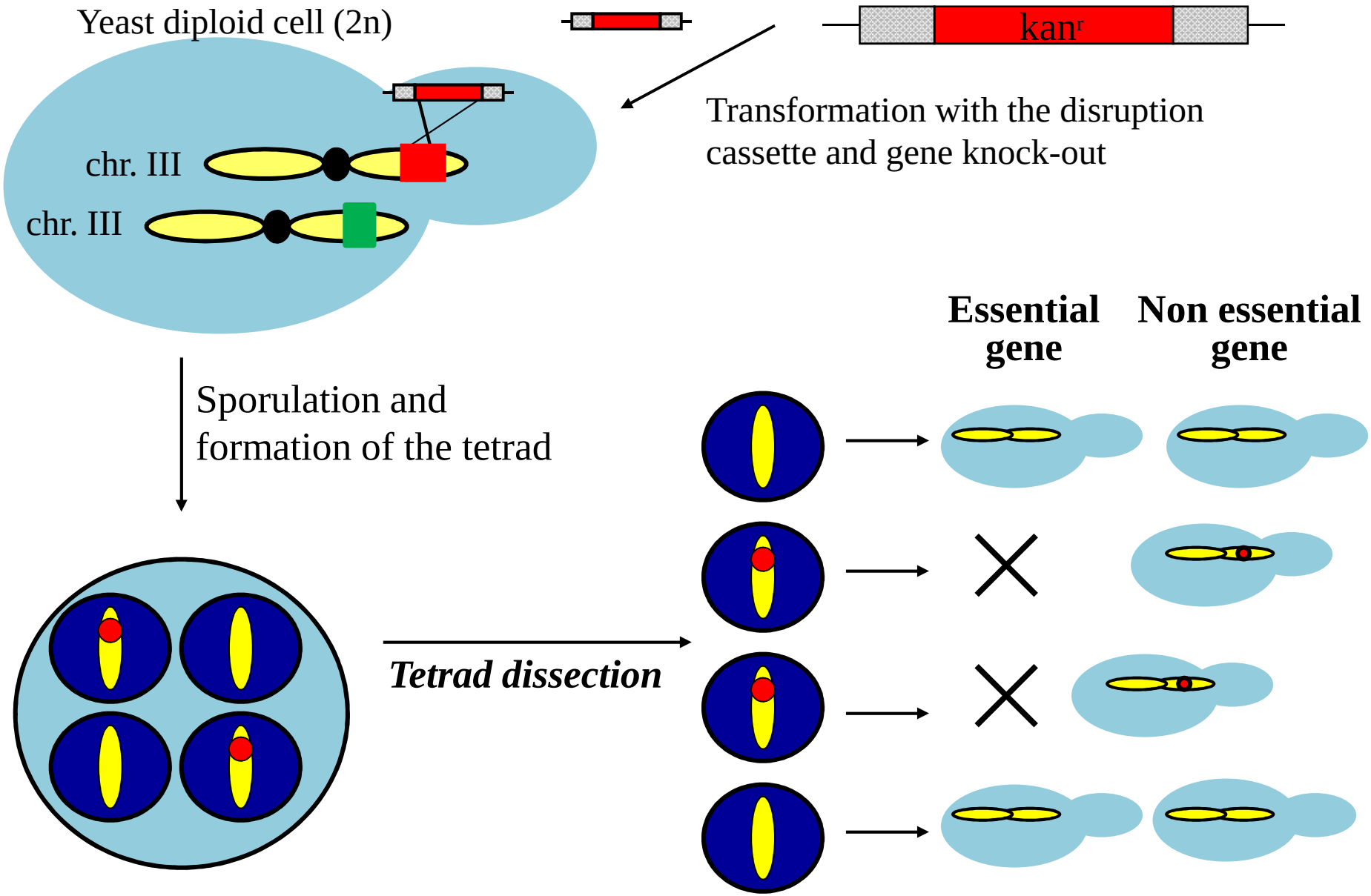
Mutation ⟶ **Phenotype/Fitness**

1. **Is a given gene essential and what is its function ?**
2. **Is the gene function dependent on the environment?**
3. **Is a given gene haplo-insufficient (or haplo-proficient) ?**

↓

Examples with yeast

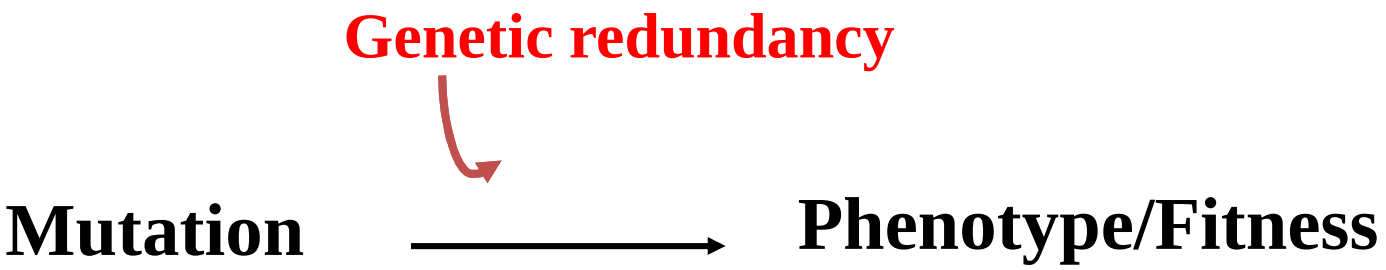
Is a gene essential?



Haploinsufficiency occurs when a diploid organism has only a single functional copy of a gene (with the other copy inactivated by mutation) and the single functional copy does not produce enough of a gene product (typically a protein) to bring about a wild-type condition, leading to an abnormal or diseased state. It is responsible for some but not all autosomal dominant disorders

***Haplo-proficient*, HP are genes** which, when one copy is deleted from a diploid cell, result in an increased rate of proliferation.

Fitness and haploinsufficiency: the role of the environment



1. Is a given gene essential and what is its function ?
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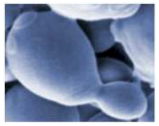
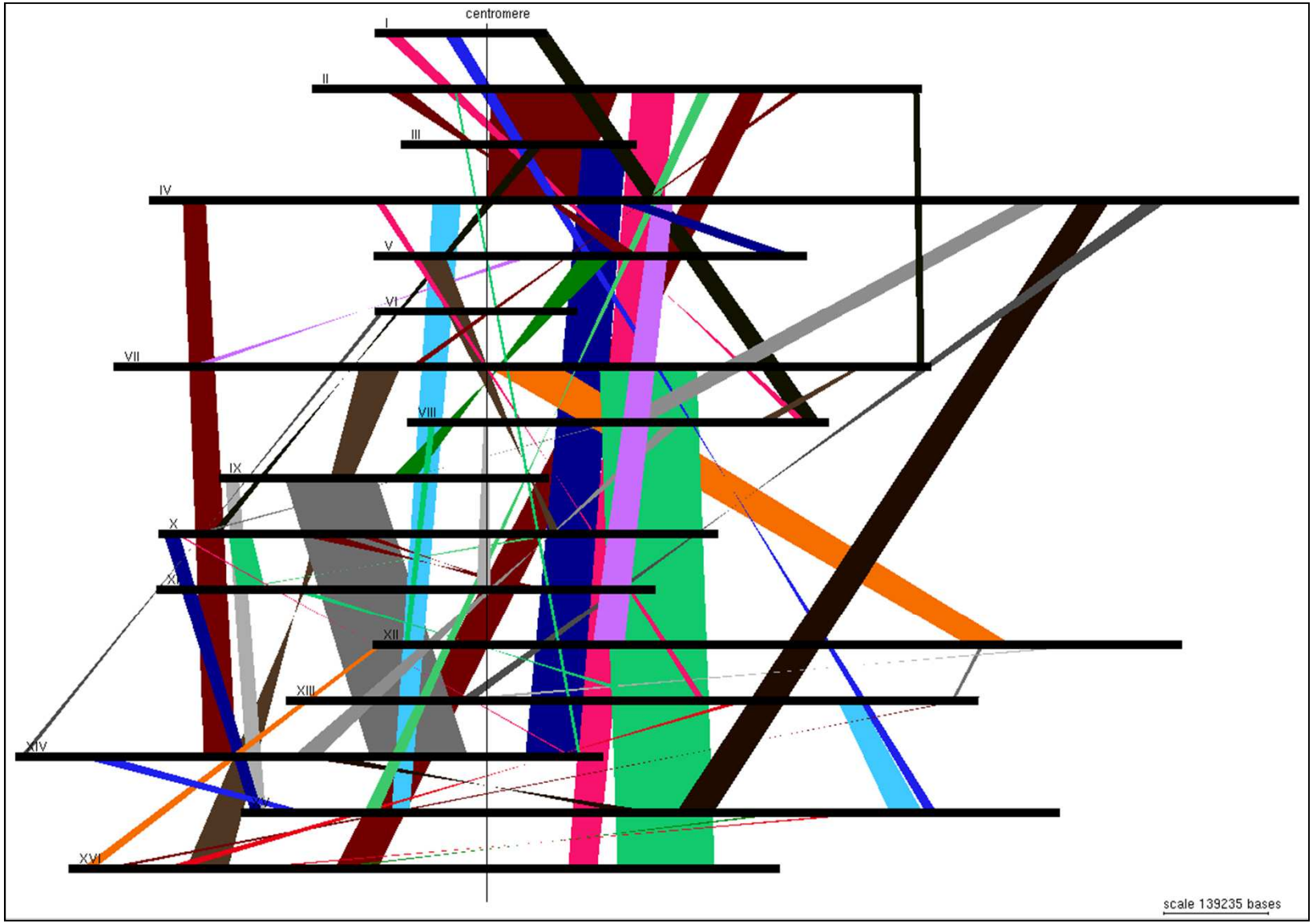
Examples with yeast

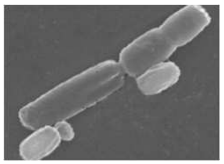
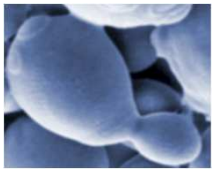
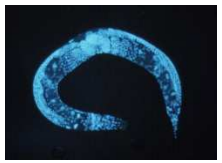


The yeast genome

53 cluster homology regions



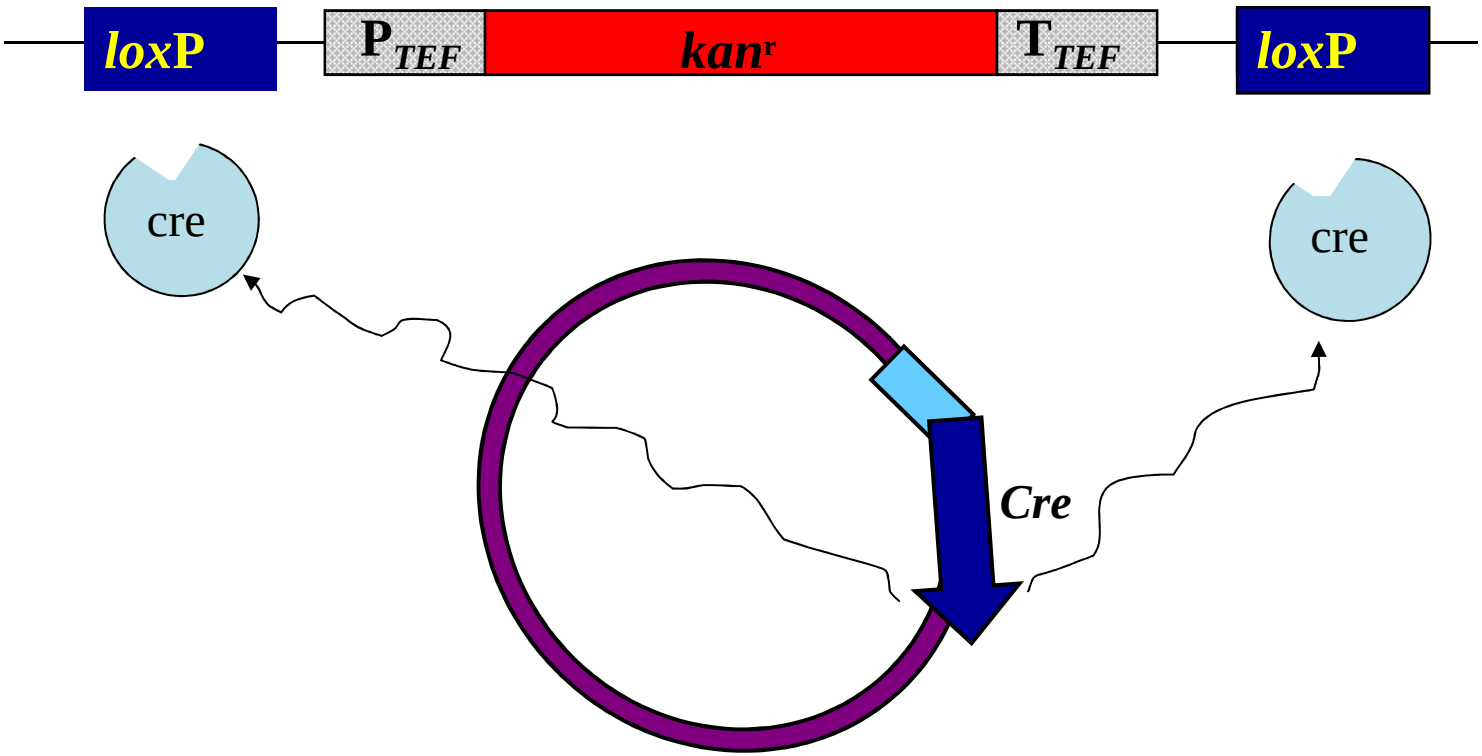
Across many eukaryotes the frequency of dispensable genes is high

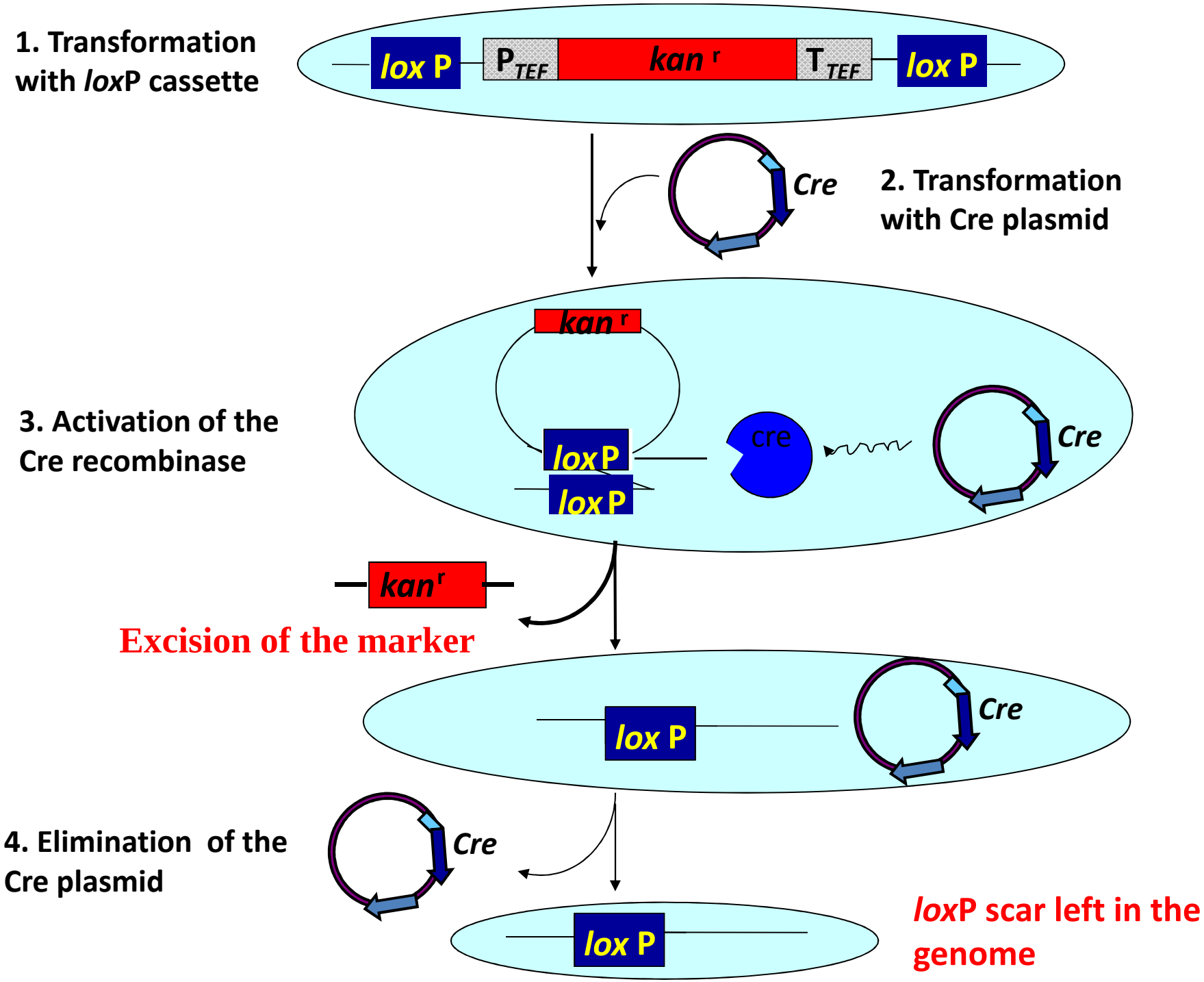
	Organism	Percentage of essential genes
	*B. subtilis*	7.3%
	*S. cerevisiae*	<20%
	*C. elegans*	5.5%
	*M. muscularis*	13.7%-19%

Marker rescue technique: *lox*P-*kan*MX-*lox*P deletion cassette for multiple deletion

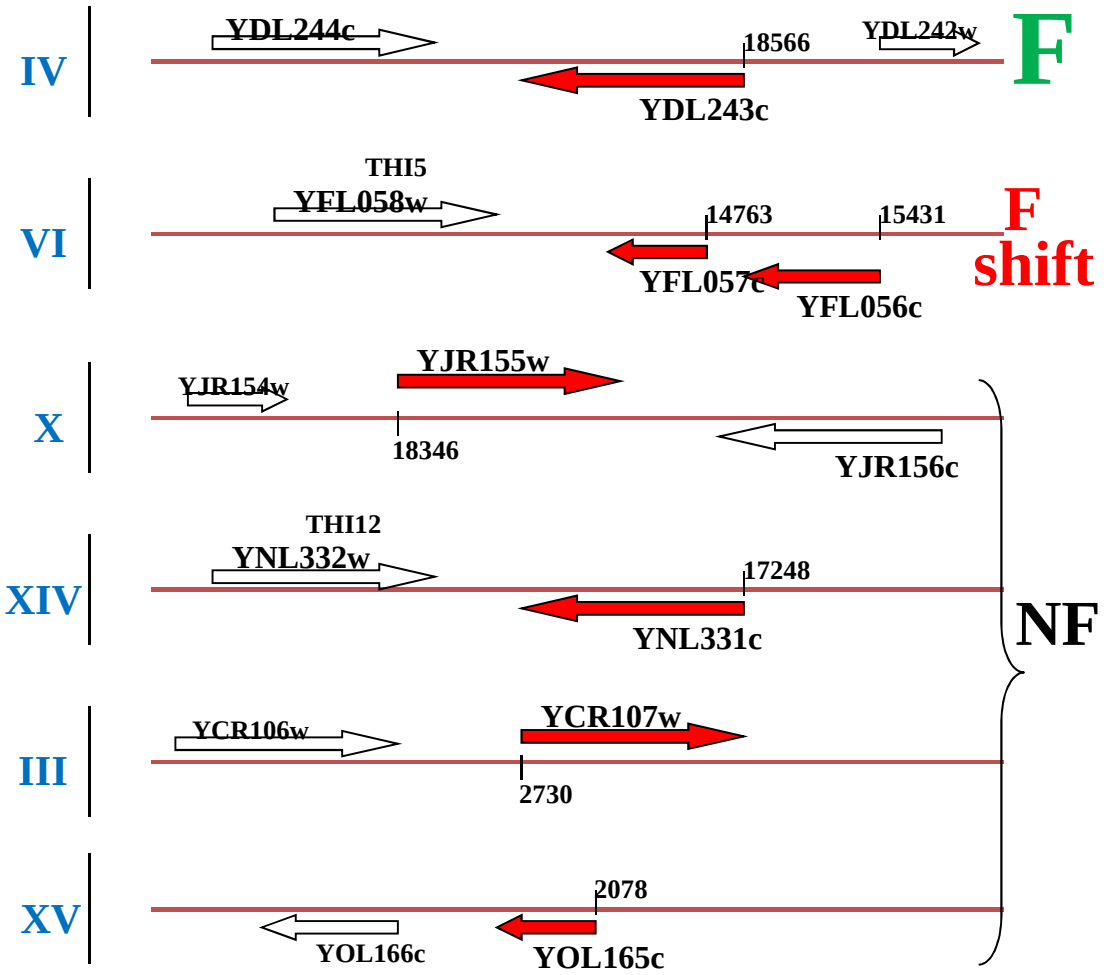
Cre/*lox*P site-specific recombination system from the bacteriophage P1.

This module allow the recycling of the marker: once the *Kan*MX has replaced the gene of interest, it can be excised from the yeast genome via the recombination of the two *loxP* sequences, promoted by the Cre recombinase.



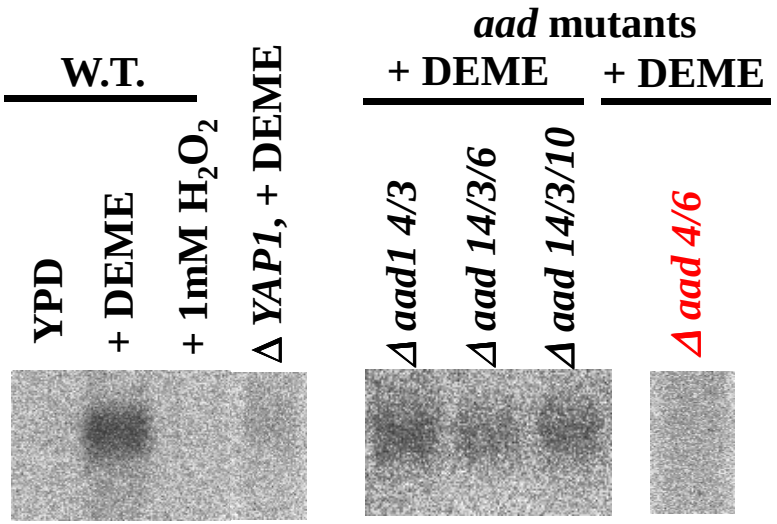

1. Transformation with loxP cassette
lox P
P TEF
kan r
T TEF
lox P
Cre
2. Transformation with Cre plasmid
kan r
3. Activation of the Cre recombinase
lox P
lox P
cre
Cre
kan r
Excision of the marker
lox P
Cre
4. Elimination of the Cre plasmid
Cre
lox P
loxP scar left in the genome

The genetic redundancy is more apparent than real



Locations of the *AAD* (red) homologues in the yeast genome

Northern blot



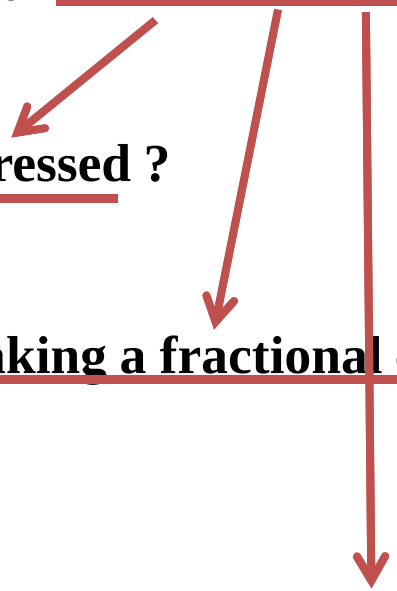
- The *AADs* are expressed under oxidative stress conditions, the expression is *Yap1*p dependent
- Only *AAD4* and *AAD6* are expressed under oxidative stress conditions.

Delneri *et al.*, *Genetics* 153: 1591-1600 (1999)

Genetic redundancy

In the biological and physiological context

- **What is the true meaning of the genetic redundancy within a genome ? Are the members of the gene family truly redundant ?**

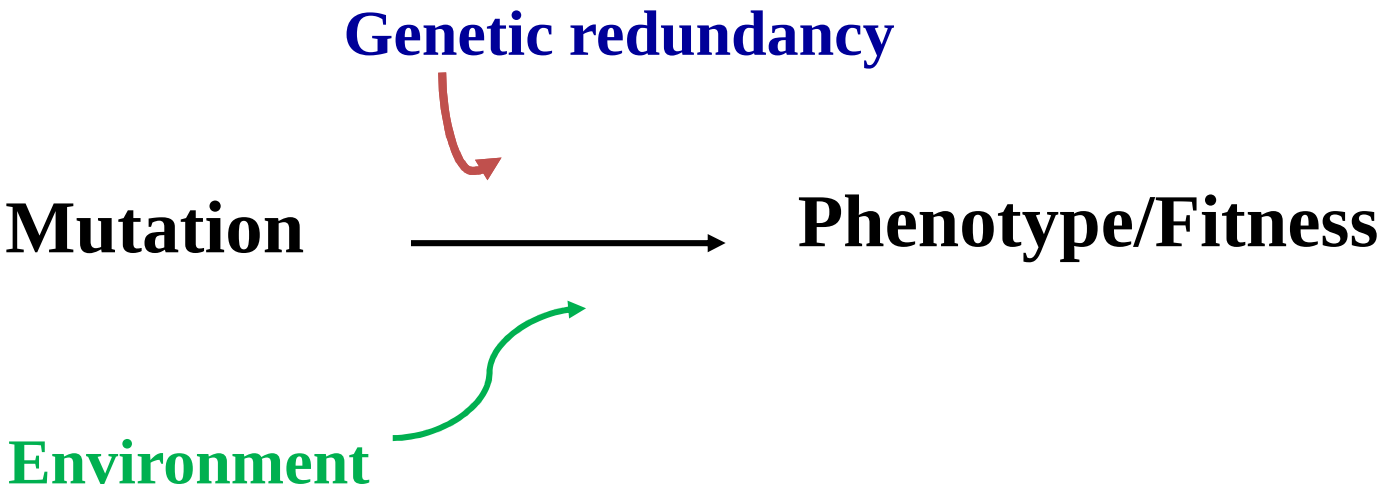


- **Are all the members expressed ?**

- **Is every gene member making a fractional contribution towards the functional activity ?**

- **Are they expressed under different physiological conditions ?**

Fitness and haploinsufficiency: the role of the environment

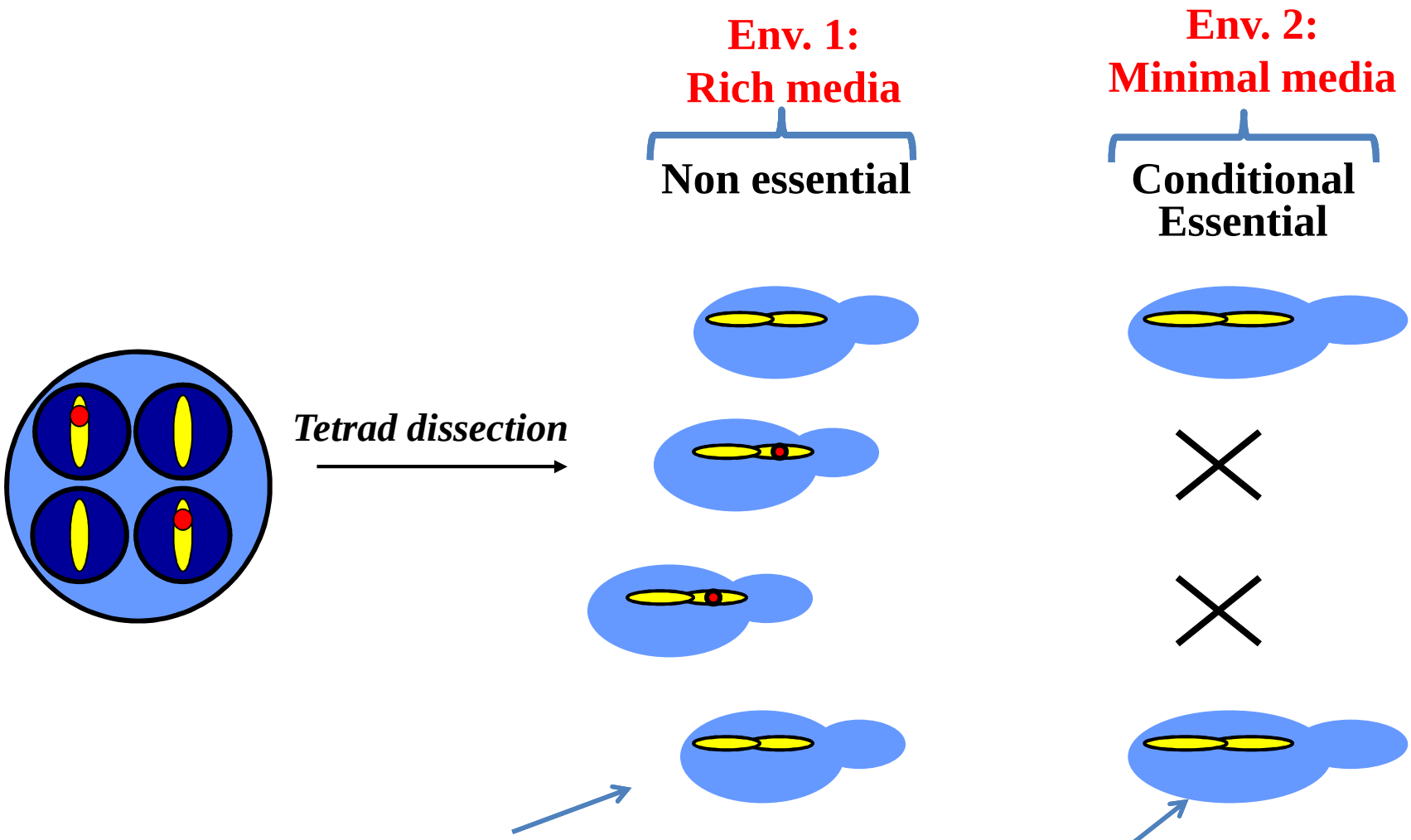


1. Is a given gene essential and what is its function ?
2. Is the gene function dependent on the environment?
3. Is a given gene haplo-insufficient (or haplo-proficient) ?

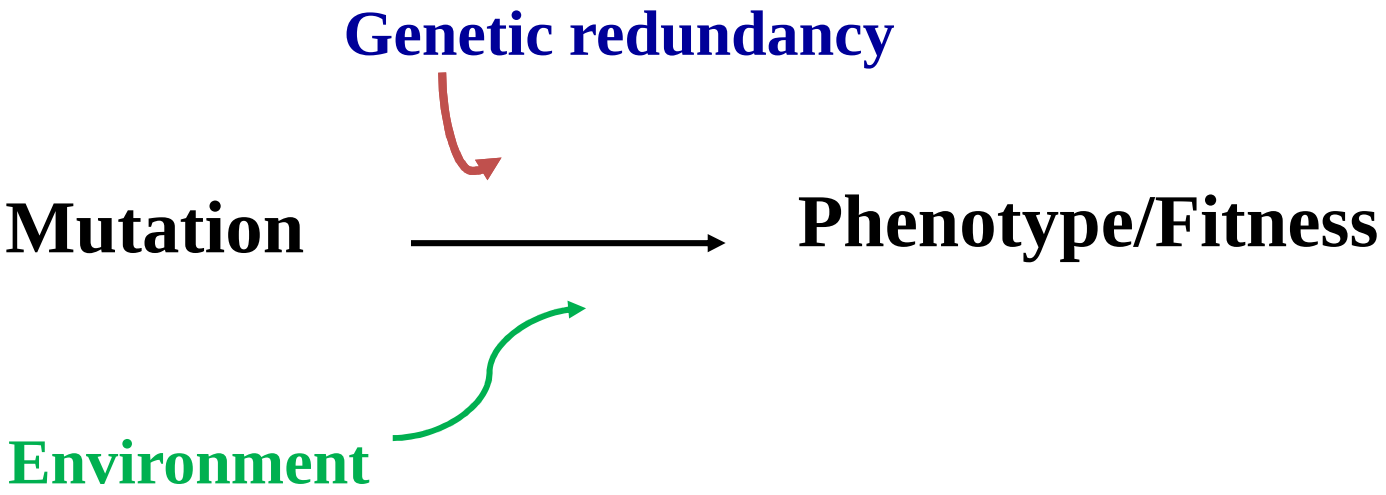
↓

Examples with yeast

Dispensability of a gene depends on the environment



Fitness and haploinsufficiency: the role of the environment



1. Is a given gene essential and what is its function ?
2. Is the gene function dependent on the environment?
3. Is a given gene haplo-insufficient (or haplo-proficient) ?

↓

Examples with yeast

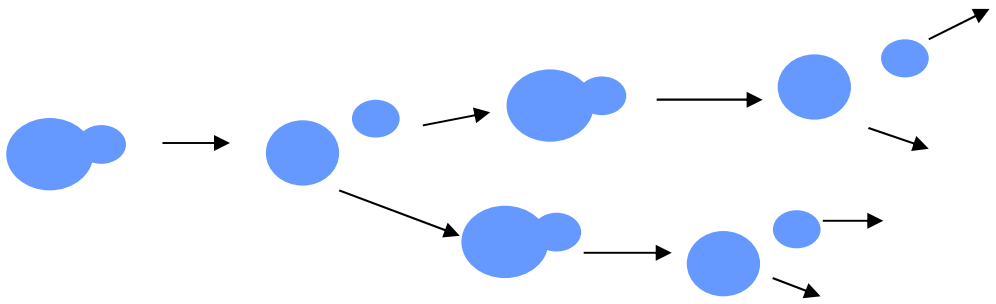
Genome-wide approaches to fitness via competition analysis

- **Measure of fitness and genetic resources**

Fitness

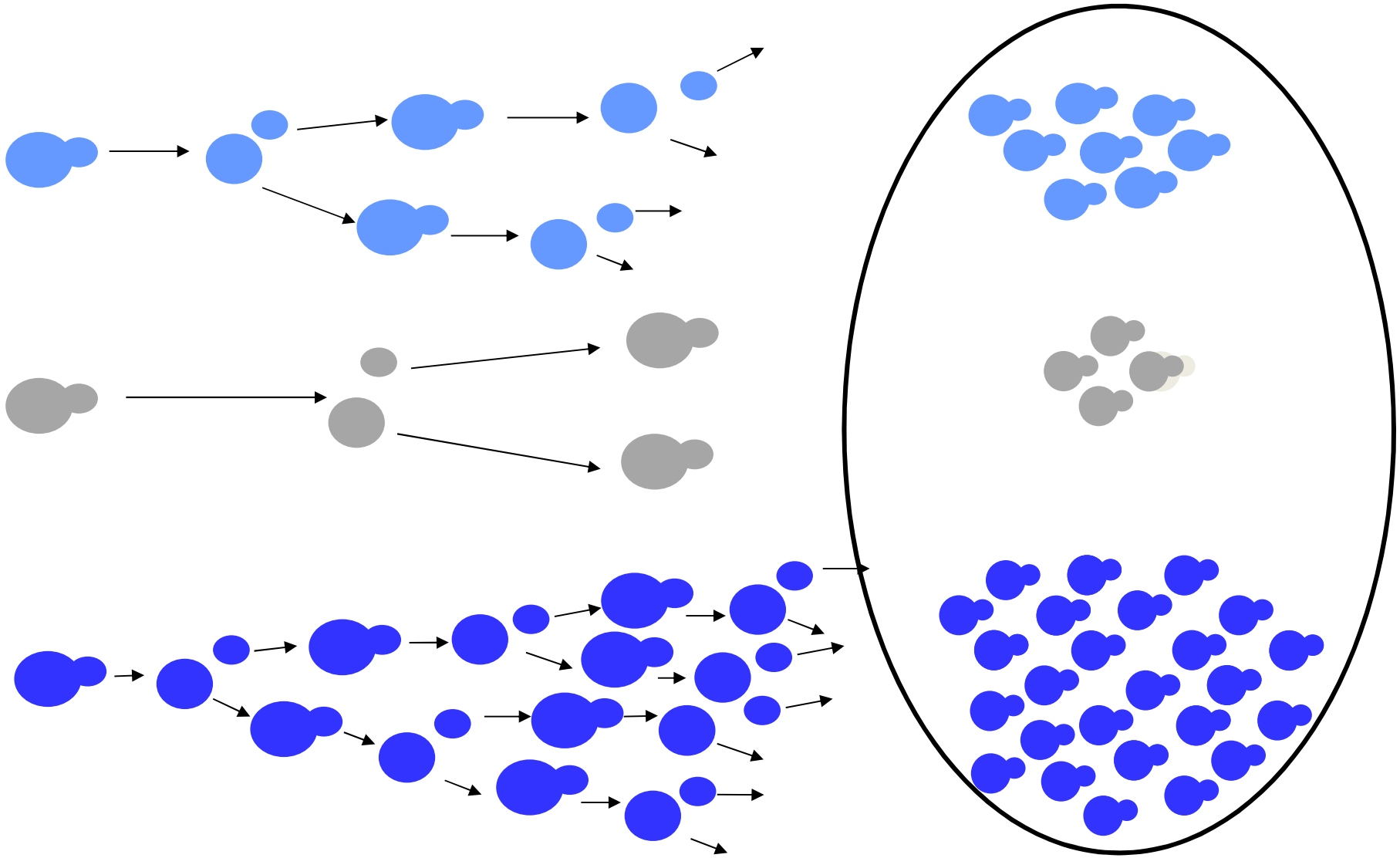
Definition: Probable genetic contribution of an individual to succeeding generations.

In yeast, the *relative fitness* is given by the *growth rate* of different type of cells as they compete for a pool of resources.



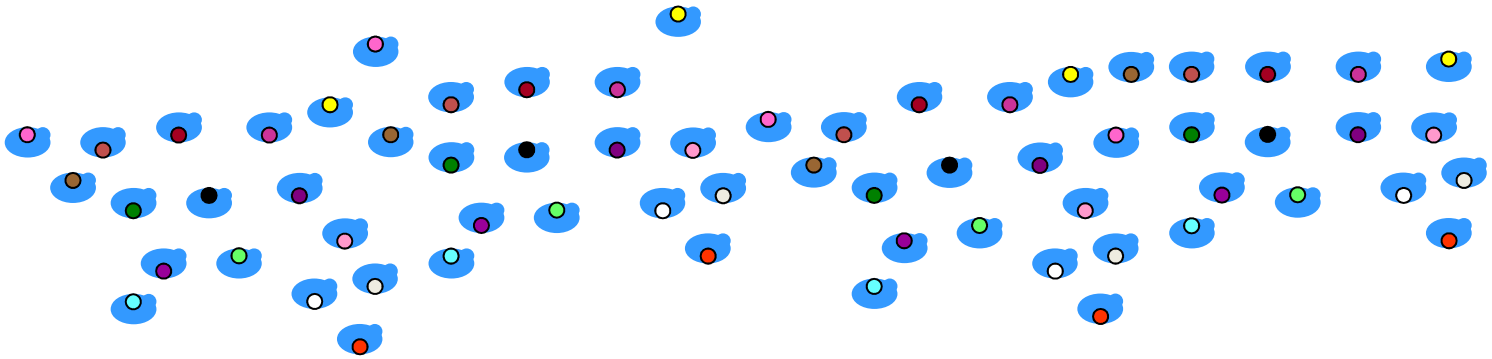
Competition experiments

Competition experiments **provide direct measurement of growth rate, and consequently fitness, in the chosen environment.**



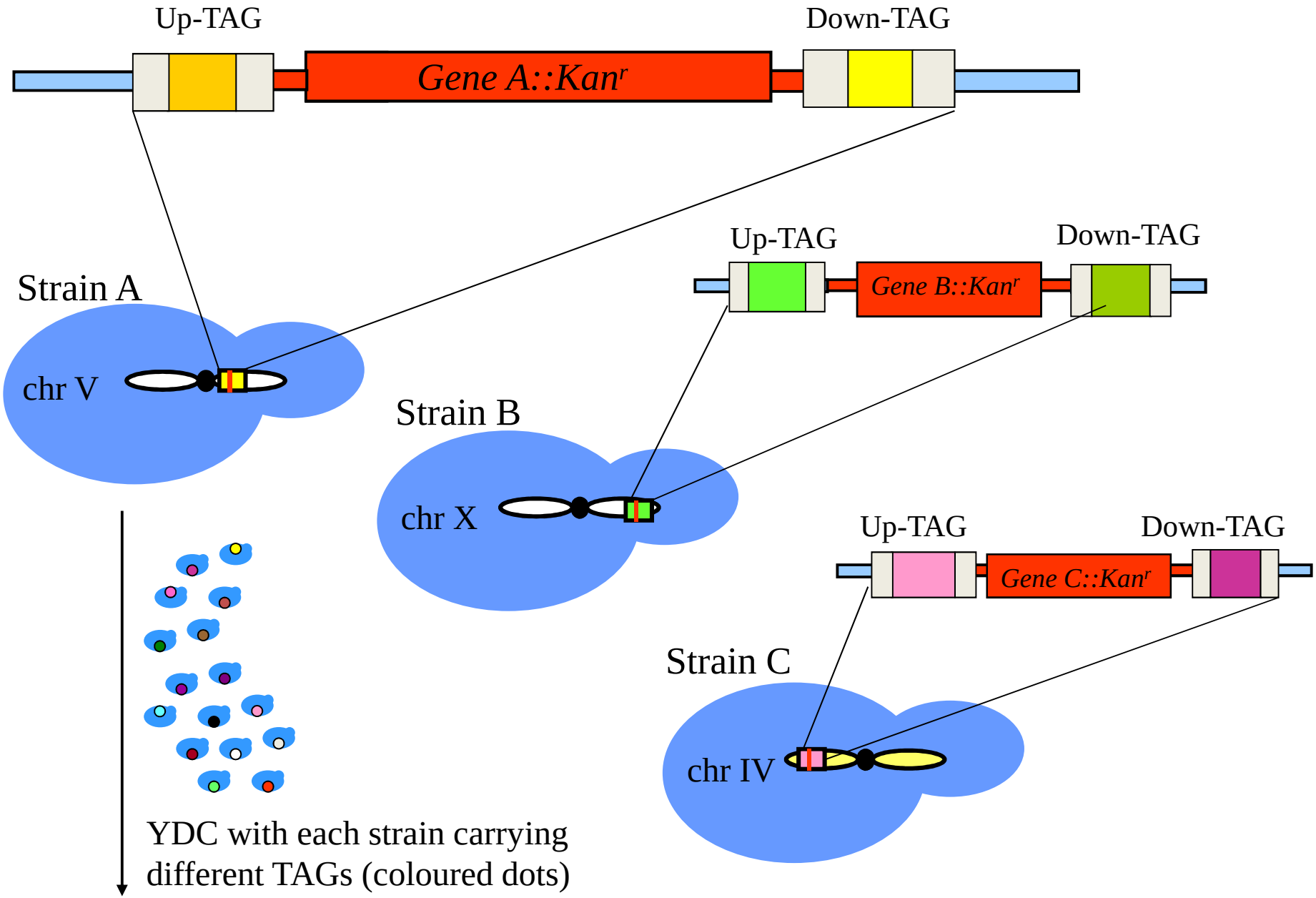
Genome-wide resources

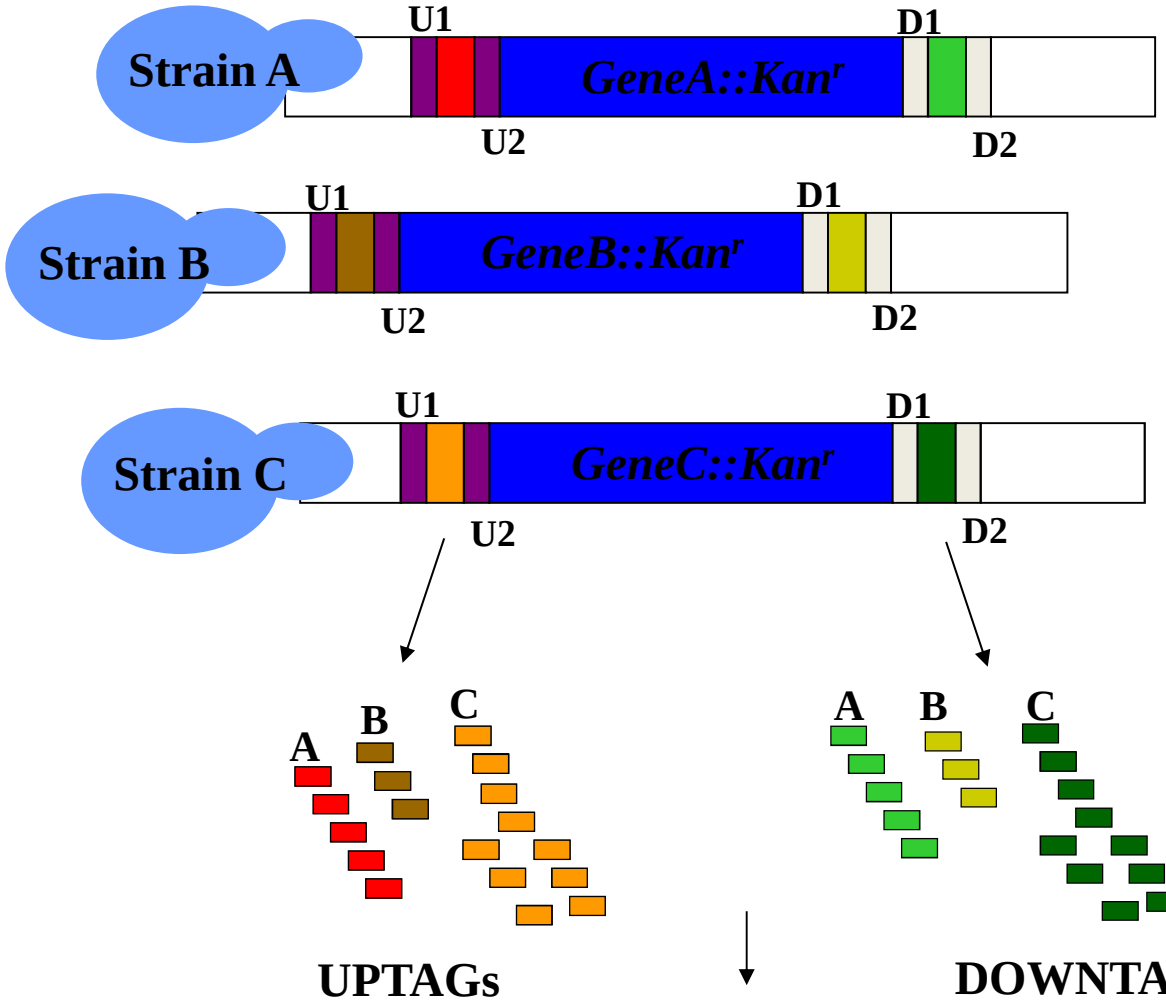
Availability of a *complete set of oligonucleotide-tagged deletion mutants*, produced by the Yeast Genome Deletion Project.



Each deletion is marked by *two unique 20 bp "bar codes"*, so that they can be quantitatively discriminated via *hybridisation array analysis or NGS*.

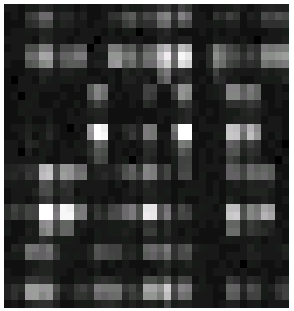
Molecular Barcode Yeast Deletion Collection (YDC)





1. Parallel competition assay of 6,000 yeast barcoded mutant strains.

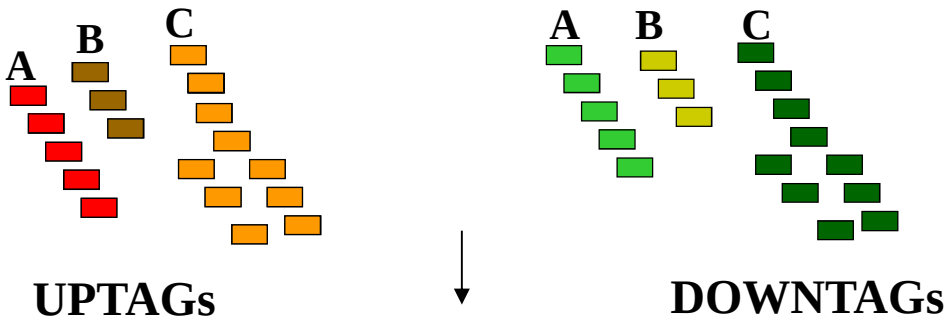
2. Genome extraction and PCR amplification of the TAGs



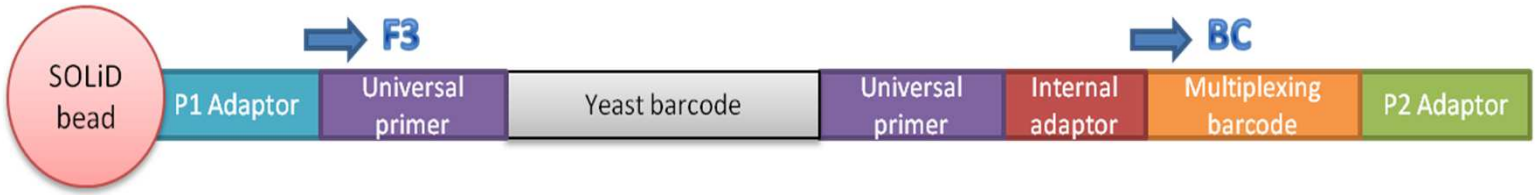
3. Hybridisation of the PCR products to the DNA chip, containing the TAG oligos immobilised on a silicon wafer.

Hybridisation array substituted by NGS

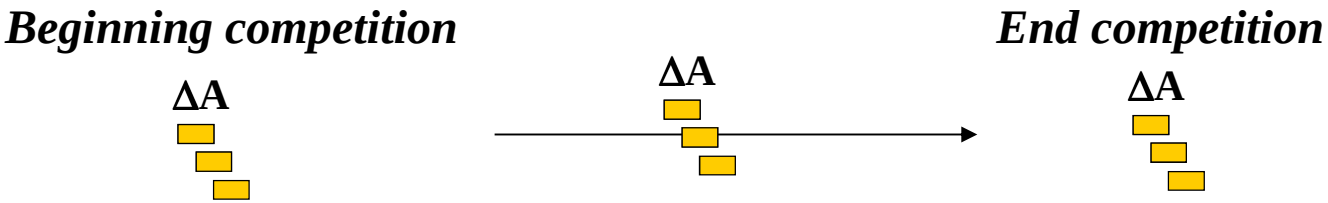
(SOLiD or Illumina technology)



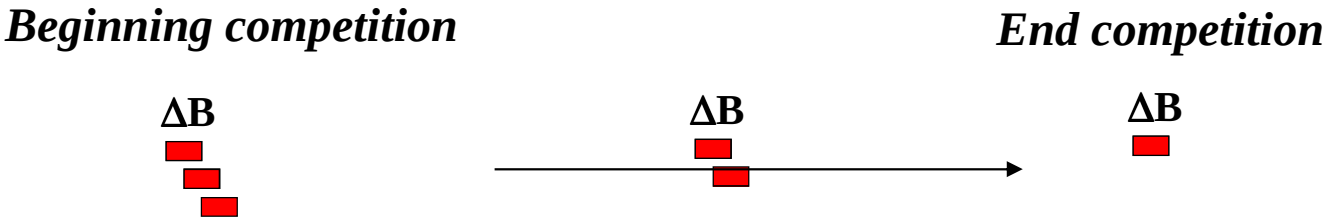
2. Genome extraction and PCR amplification of the TAGs with oligos containing P1 and P2 adaptors and multiplexing barcode)



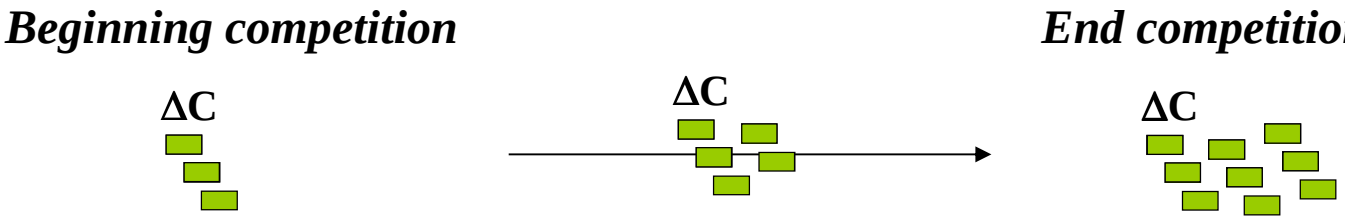
A. **If the deletion of the *Gene A* has no impact on fitness in the growth condition chosen, the intensity of its up- and down-tag will not change**

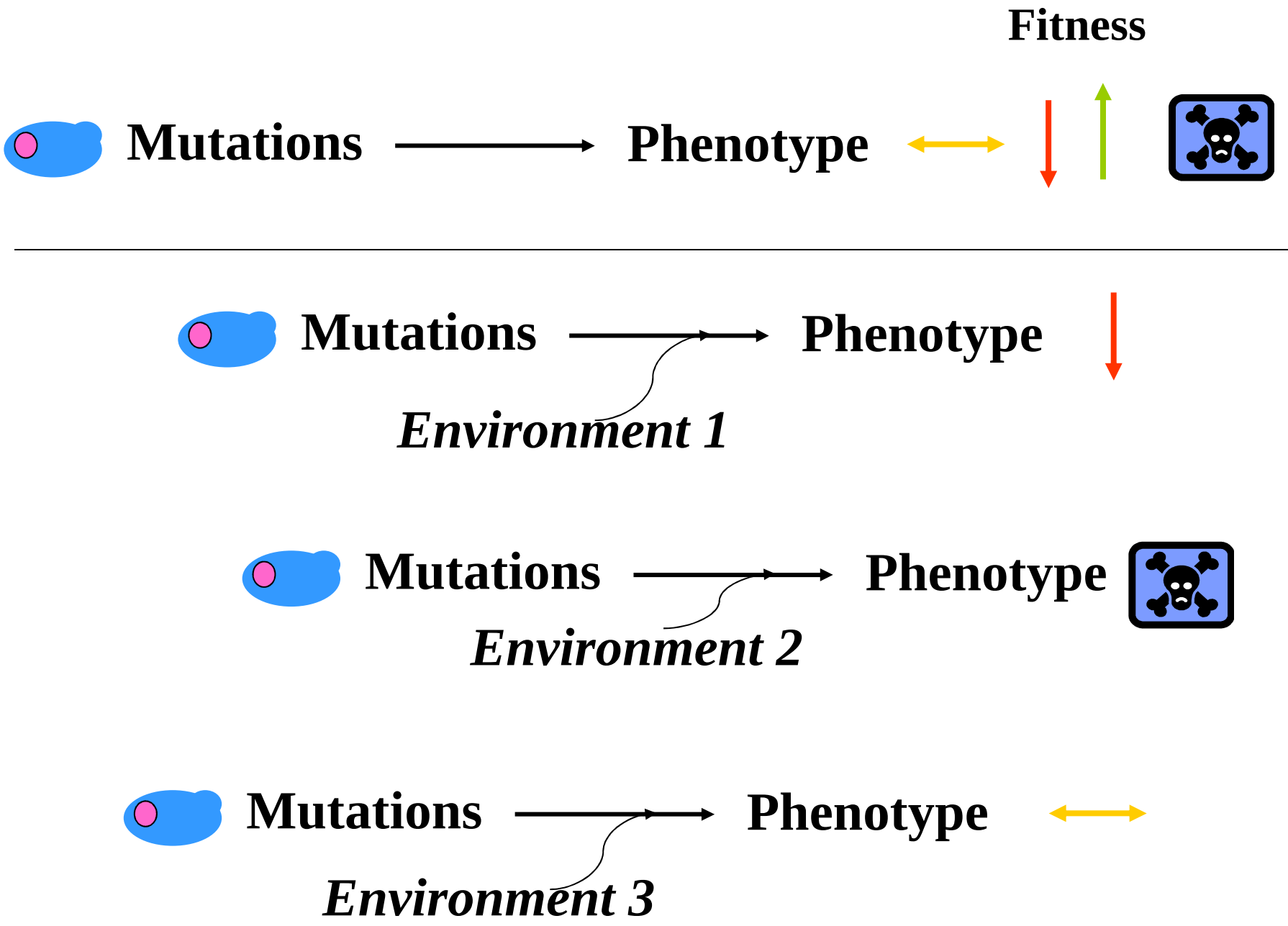


B. **If the deletion of the *Gene B* has a negative impact on fitness in the growth condition chosen, that particular strain will tend to disappear.**

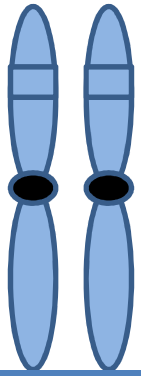


C. **If the deletion of *Gene C* has an advantage in the media of choice, the strain carrying that deletion will become over-represented in the population over the time.**



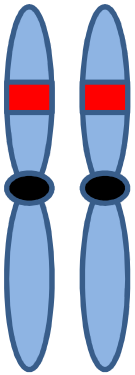

Fitness
Mutations
Phenotype
Mutations
Phenotype
Environment 1
Mutations
Phenotype
Environment 2
Mutations
Phenotype
Environment 3

Diploid organism (WT)



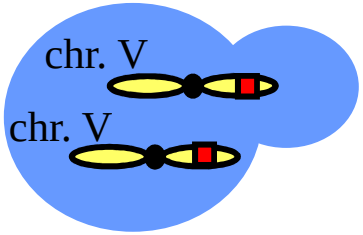
A/A

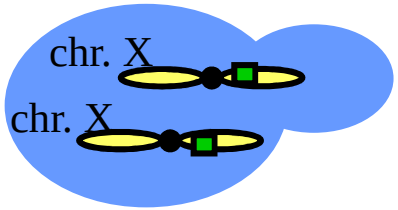
Homozygote deletant

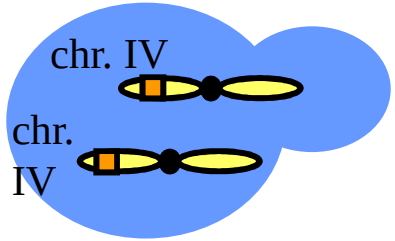


ΔA/ΔA

Studies on gene function







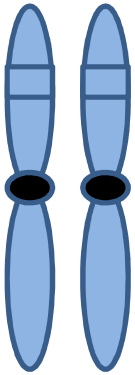
Collection of homozygous diploid

(ca. 4,500 strains out 6,000 ORFs)

Discovered genes that are necessary for optimal growth under six well-studied conditions: high salt, sorbitol, galactose, pH 8, minimal medium and nystatin treatment (Giaever et al., *Nature*, 2002).

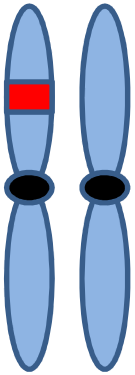
Discovered genes involved in the mitochondrial function and biogenesis. (Steinmetz et al., *Nature Genetics*, 2002).

Diploid organism (WT)



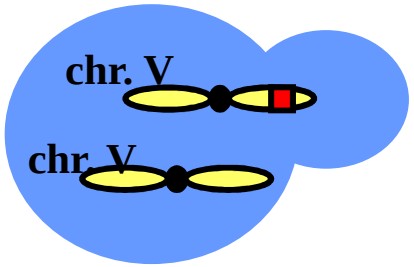
A/A

Heterozygote deletant



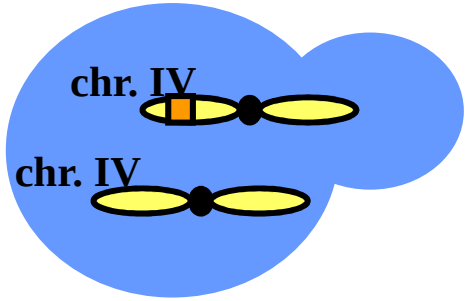
ΔA/A

Studies on gene dosage



Collection of heterozygous diploids

(ca. 6,000 strains)



- Heterozygous strains will allow the study of essential genes
- Genes with a haplo-insufficiency phenotype can be isolated: study gene dosage

↓

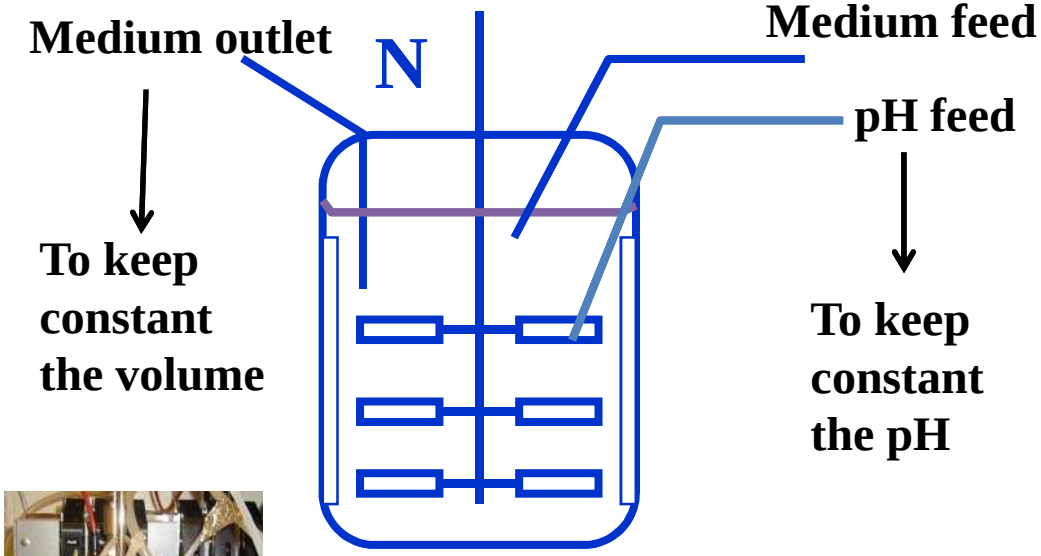
Problem: how do we obtain a fair detection of fitness changes since one copy of the gene is still there?

Competition between a population of deletant strains.

Batch: closed system in which cells are grown in a fixed volume where the nutrients and pH change during growth.

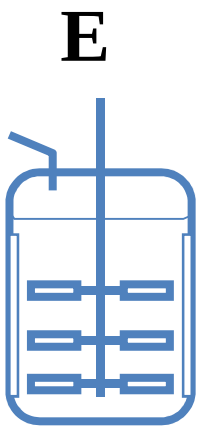
the culture environment continuously changes

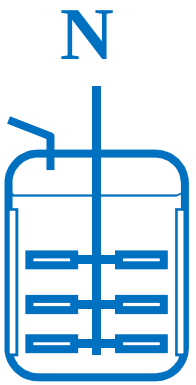
Chemostat: open system where nutrients, pH and culture volume are kept constant.

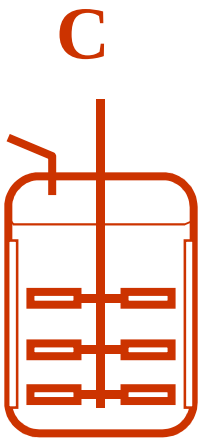


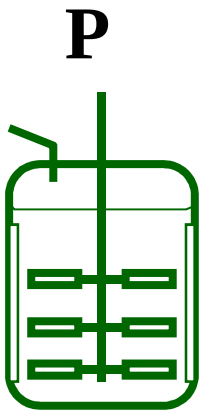
physiological steady state with constant growth rate

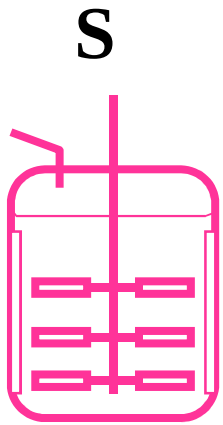
Cultures in chemostat under different nutrient limitations











Genome profiling in different environments

Small-scale parallel fermentation modular system

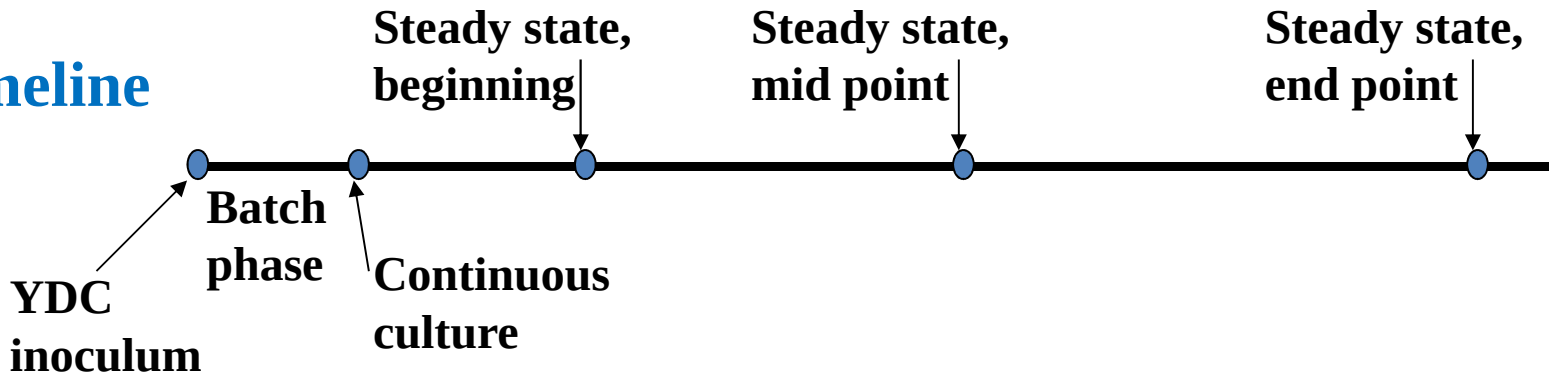
Fitness profile in:

- **C-limited**
- **N-limited**
- **P-limited**

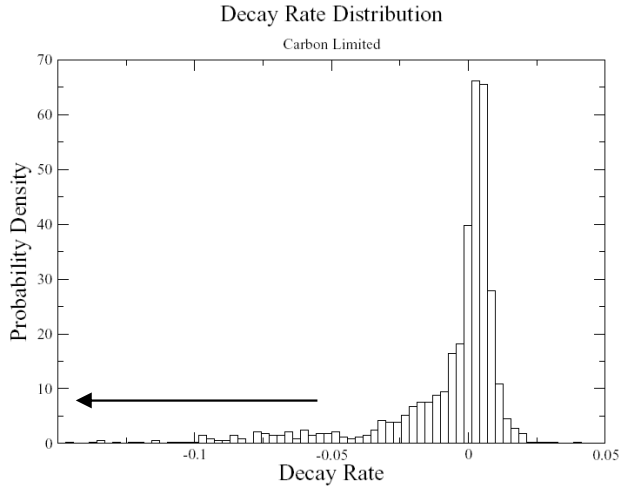
Chemically defined media

- **Grape juice** **(natural environment)**

Timeline



Genes with an haplo-*insufficient* phenotype

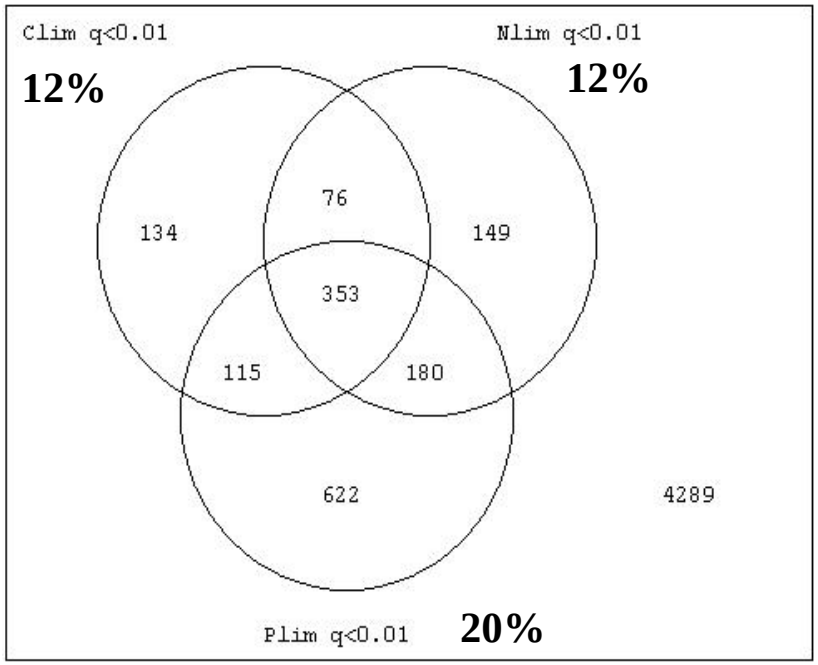


YPD: 3%

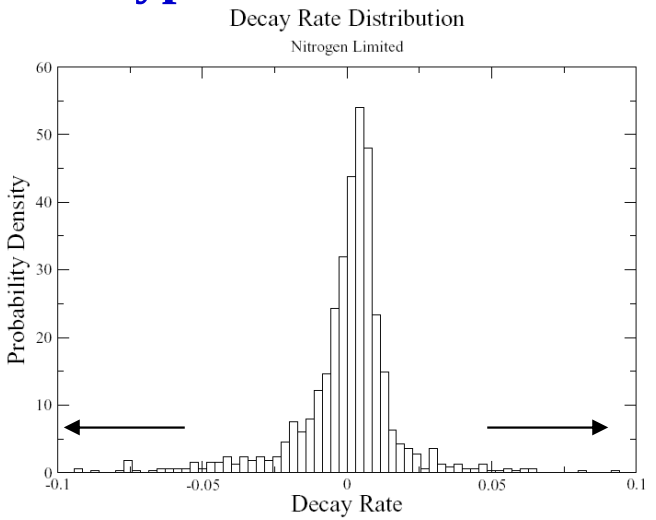
(Giaever *et al.*, 2004)

GJ: 3.8%

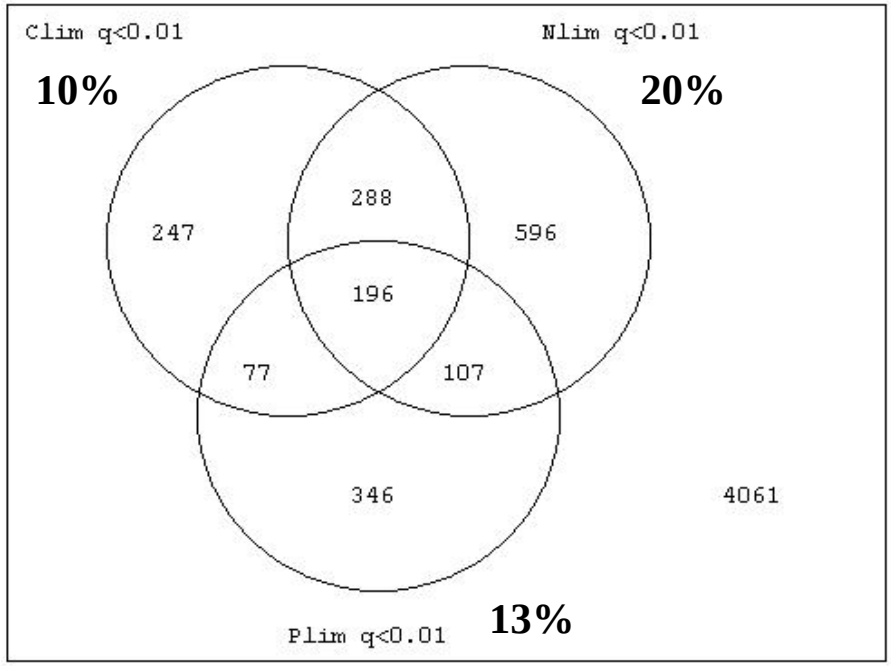
Daniela -ve decay rate



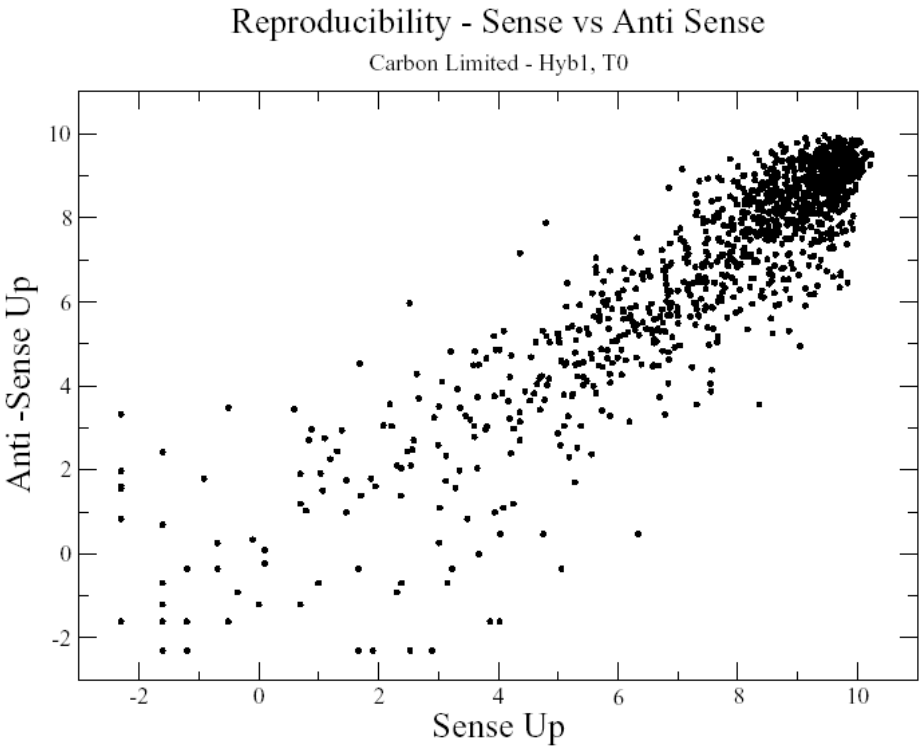
Genes with an haplo-*proficient* phenotype

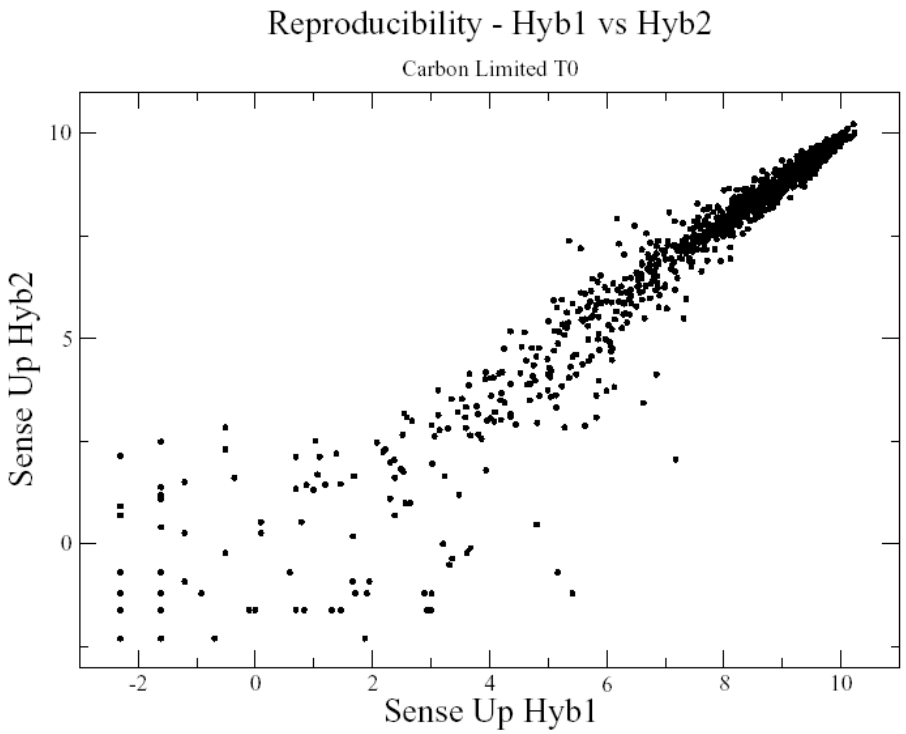


Daniela +ve decay rate

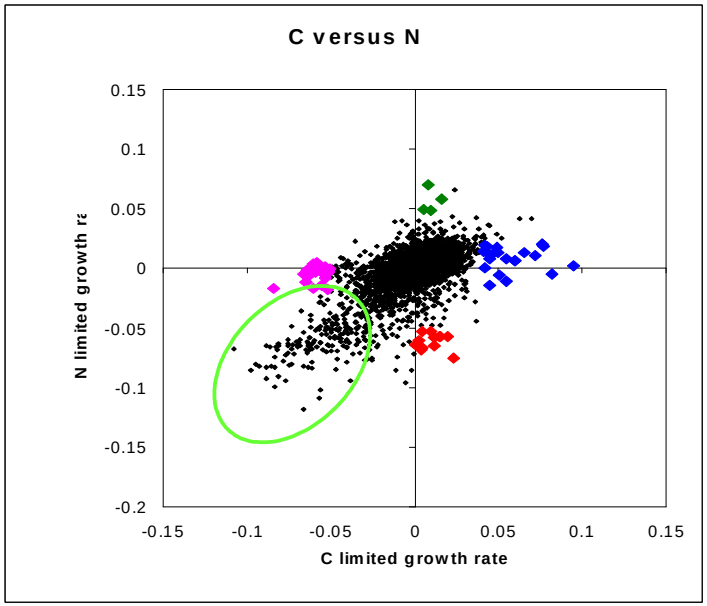


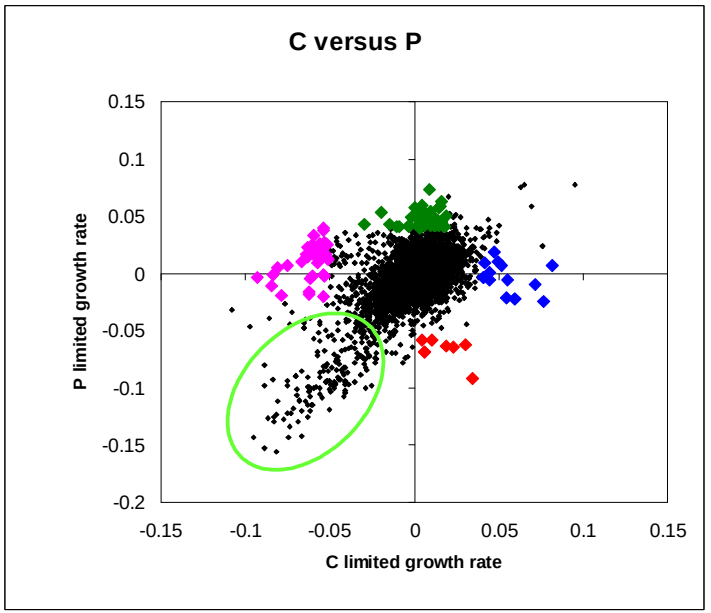
Reproducibility of the data

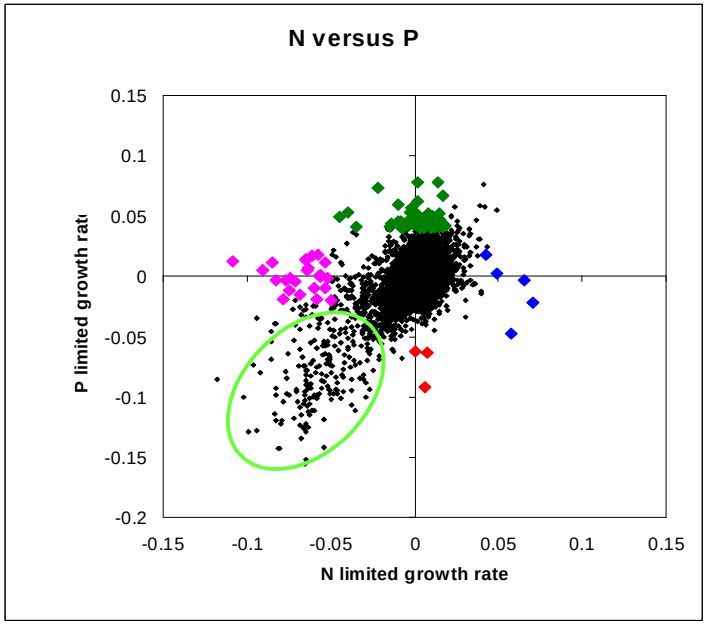


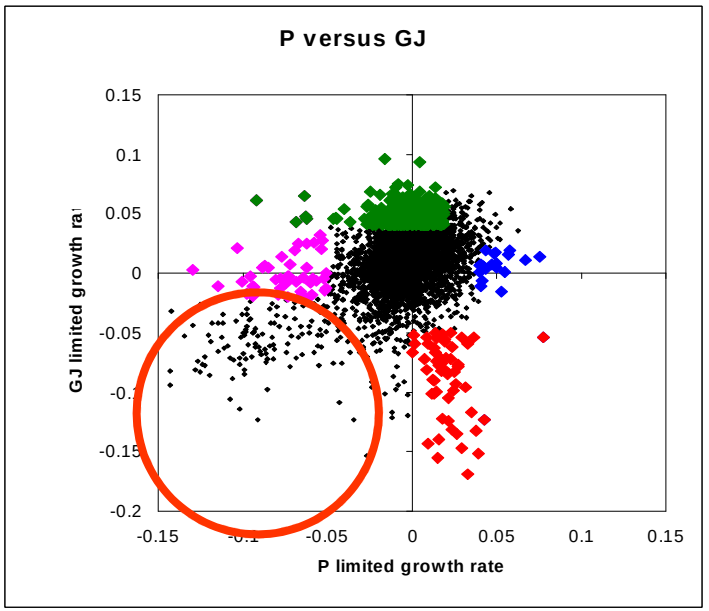


Growth rate comparisons









Haplo-insufficiency profile mainly common to all environments

C-limited

1. COPI vesicle coat
2. Ornithine biosynthesis
3. Response to pH
4. Poly(A)tail shortening
5. CCR4-NOT core complex
6. Nucleosome remodeling complex
7. SWI/SNF complex
8. Hydrogen-transporting ATPase domain V0 domain
9. Mediator complex
10. Regulation of transcription from Pol I promoter
11. Protein kinase CK2 activity
12. Protein kinase CK2 complex

….

21. Negative regulation of meiosis

N-limited

1. Nucleosome remodelling complex
2. COPI vesicle coat
3. RNA polymerase II transcription mediator activity
4. Mediator complex
5. Poly(A)tail shortening
6. CCR4-NOT complex
7. Ornithine biosynthesis
8. Sin3 complex
9. Negative regulation of meiosis
10. SWI/SNF complex
11. Nuclear nucleosome
12. DNA-directed RNA polymerase II\, core complex
13. NAD-independent histone deacetylase activity
14. Regulation of transcription from Pol I promoter
15. Protein kinase CK2 activity
16. Protein kinase CK2 complex

…

25. Nitrogen utilisation

P-limited

1. Negative regulation of meiosis
2. NAD-independent histone deacetylase activity
3. Glycolysis
4. Mitotic chromosome condensation
5. COPI vesicle coat
6. Microtubule binding
7. NAD-dependent histone deacetylase activity
8. Metallopeptidase activity
9. Protein kinase CK2 activity
10. Protein kinase CK2 complex

…

26. poly(A)tail shortening

Protein trafficking is the cell category most affected by changes in gene dosage

Haplo-insufficient phenotype: **COPI vesicles coat components**

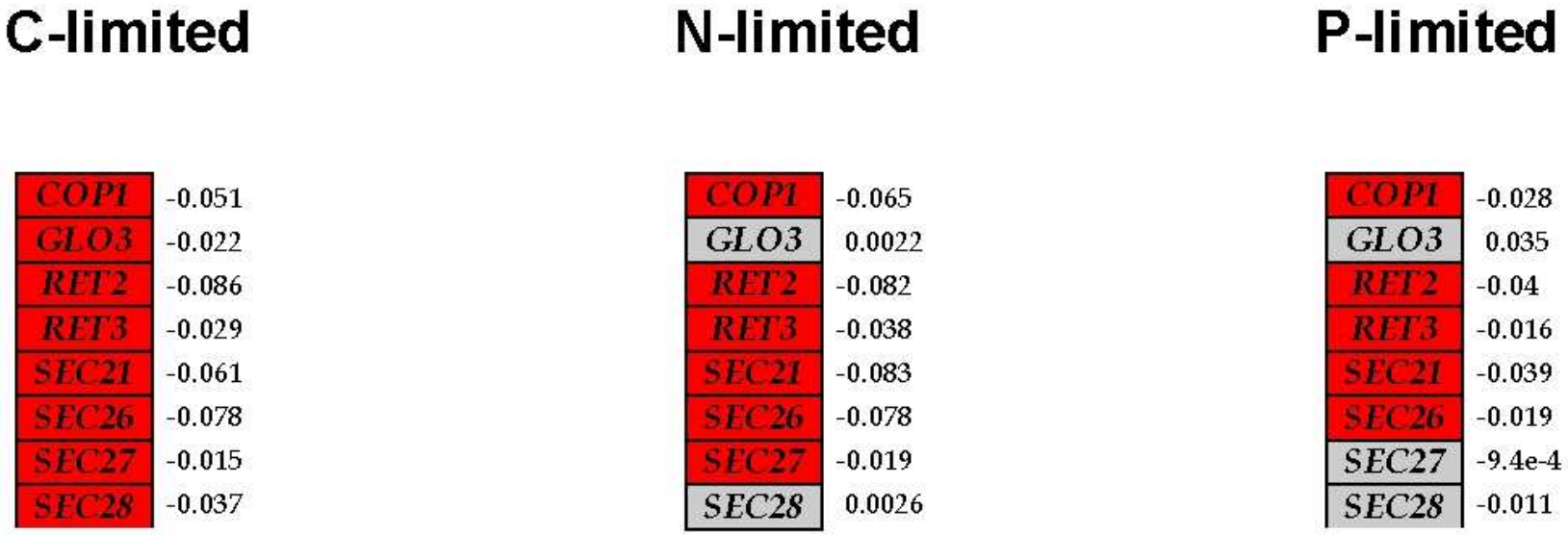


Figure S1: **COPI vesicle coat components**

Gene Ontology analysis results associated with the GO term 'COPI vesicle coat', using haplo-insufficiency data from CL, NL and PL cultures, respectively. Red boxes indicate haploinsufficiency, while grey shading is used when the specified criteria are not met (negative growth rate effect and/or q-value < 0.01). The numbers next to each gene name represent the growth rate differences (relative to the imposed dilution rate of 0.1 h^{-1}).

Haplo-proficiency data: Gene ontology analysis

C-limited

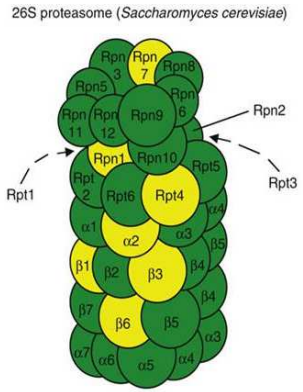
1. Cytosolic large ribosomal subunit
2. Commitment complex
3. Chromatin accessibility complex
4. Arp2/3 protein complex
5. snRNA cap binding complex
6. snRNP U1
7. Actin cytoskeleton organization and_biogenesis
8. Actin filament organization
9. Bud site selection
10. Pyruvate dehydrogenase complex
11. Chromatin silencing at telomere
12. Spliceosome assembly
13. Telomeric DNA binding
14. Transcription coactivator activity
15. Telomere maintenance

N-limited

1. Endopeptidase activity
2. Ubiquitin-dependent protein catabolism
3. Actin cytoskeleton organization and biogenesis
4. Proteasome regulatory particle \, lid subcomplex
5. Proteasome core complex \, alpha-subunit complex
6. Actin filament organization
7. NADH oxidation
8. Receptor activity
9. Proteasome core complex \, beta-subunit complex
10. Protein phosphatase regulator activity
11. Microsome
12. Ubiquitin conjugating enzyme activity
13. Bud tip
14. Histone binding
15. MAP kinase kinase activity
16. Proteasome regulatory particle \, base subcomplex

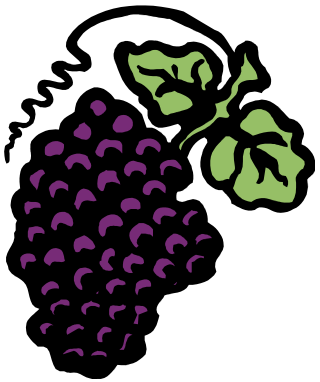
P-limited

1. Transmembrane receptor activity
2. Mitochondrial inner membrane protein insertion complex
3. Osmosensory signalling pathway
4. Nucleotide-excision repair \, DNA damage recognition
5. Sphingolipid biosynthesis
6. snRNP U1
7. DNA-dependent DNA replication
8. Cytochrome c oxidase biogenesis



Delneri D, *et al.* (2008) *Nat Genet*. **40**: 113-117

Genes showing haploinsufficient phenotype in grape juice



FUR4 is synthetically lethal with *URA3*

COPI vesicle coat (p=0.020)

Molecular function

Category	p-value	In Category from Cluster	k	f
nucleobase, nucleoside, nucleotide and nucleic acid transporter [GO:0015932]	0.0017	FUR4 FCY2 ANT1	3	8
solute\:cation symporter [GO:0015294]	0.0025	FUR4 MAL31 FCY2	3	9
ATP binding [GO:0005524]	0.0078	ATP1 PCA1 RBK1 RAD18 RVB1 PMA1 PPA1 RRP3 ATP2 DBP7 MSH2 RVB2	12	165
electrochemical potential -driven transporter [GO:0015290]	0.0088	FUR4 CTP1 MAL31 PHO87 FCY2 DIP5	6	55

Biological Process

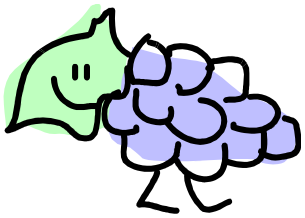
pyrimidine transport [GO:0015855]	0.0010	FUR4 FCY2	2	2
fatty acid oxidation [GO:0019395]	0.0025	TES1 ECI1 ANT1	3	9
regulation of transcription by glucose [GO:0046015] /carbon catabolites [GO:0045990]	0.0031	VPS36 SNF8	2	3
regulation of translational initiation [GO:0006446]	0.0100	PAT1 TIF2	2	5
regulation of nitrogen utilization [GO:0006808]	0.0100	GAT 1 URE2	2	5

Cellular component

CCR4 -NOT complex [GO:0030014]	0.0004	CDC39 CAF130 CAF4 POP2	4	12
hydrogen -transporting ATP synthase, catalytic core (sensu Eukarya) [GO:0005754]	0.0010	ATP1 ATP2	2	2

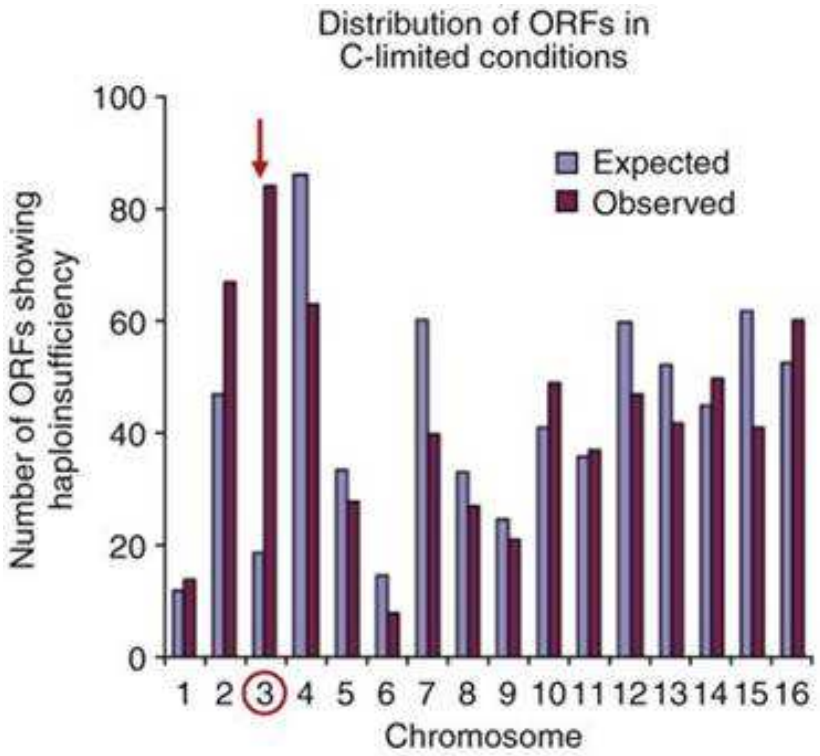
Genes showing haploproficient phenotype in grape juice

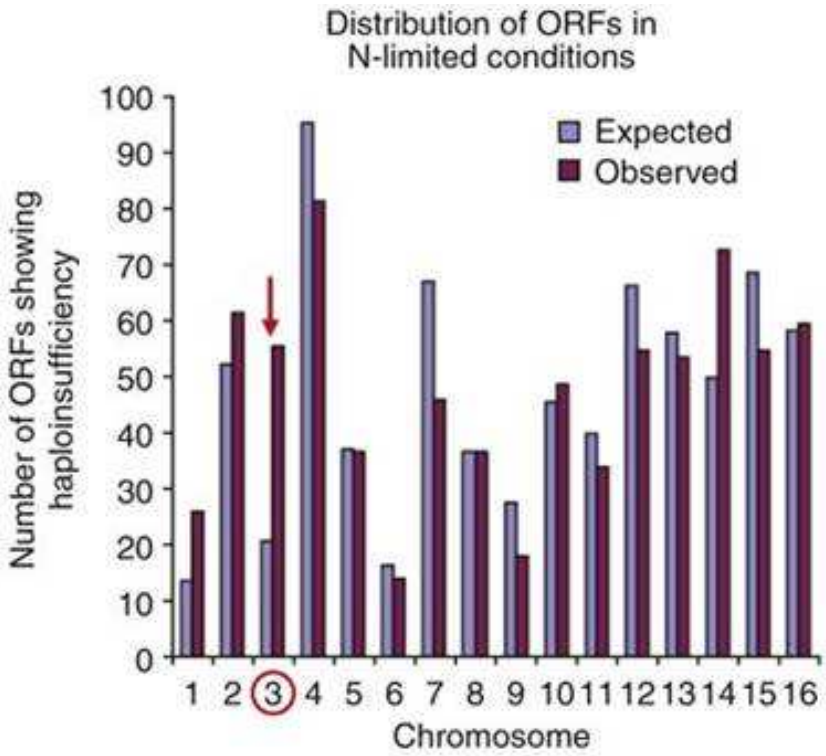
	Category	p-value	In Category from Cluster	k	f
Molecular function	cytoskeletal adaptor [GO:0008093]	0.005	ENT1 YAP1802 ENT3 ENT2	4	8
	NADH dehydrogenase [GO:0003954]	0.010	NDE2 NDE1	2	2
	RAN small monomeric GTPase [GO:0003929]	0.010	GSP1 GSP2	2	2
Biological Process	endocytosis [GO:0006897]	0.002	ENT1 YDR036C UBC5 CUP5 YAP1802 YSC84 ENT3 YPK1 YPT52 ENT2 YPT53 SLA2	12	48
	vacuole inheritance [GO:0000011]	0.007	SEC18 VAC8 TRX2 VPS34 VAC14	5	13
	ethanol fermentation [GO:0019655]	0.009	NDE2 PDC1 NDE1	3	5
Cellular component	cytosolic large ribosomal subunit (sensu Eukarya) [GO:0005842]	0.003	RPL21A RPL41B RPL35B RPL35A RPL12B RPL7A RPL1B RPL27A RPL2B RPL37A RPP0 RPL26A RPL6A RPL13B RPL36A RPL20A RPL18B	17	83
	19S proteasome regulatory particle, base subcomplex [GO:0008540]	0.003	RPT3 RPT6 RPT5	3	4



Grape juice is low in nitrogen

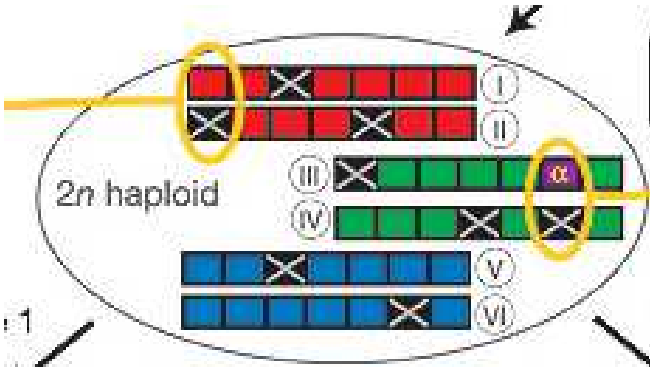
Haploinsufficiency genes accumulate on chromosome III





Chromosome III harbour the mating type locus

Accumulation of haplo-insuffcient genes on chr. III may be a mechanisms to prevent its loss.



Deletion of one MAT locus or loss of the chromosome III in a diploid cell, result in the cell perceiving itself as a haploid.

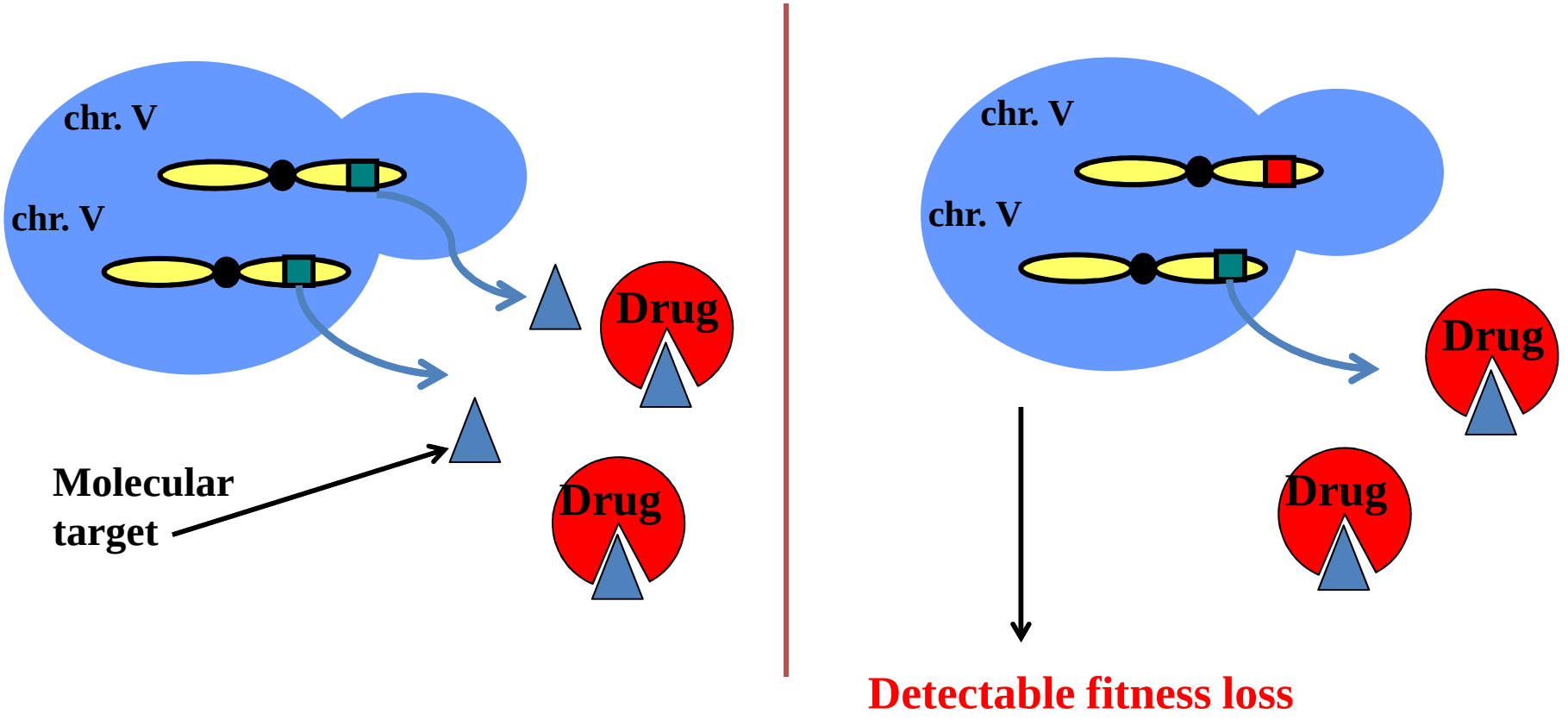
List of genes showing haploinsufficiency in C- and N-limitation

C-limited: decreased fitness		N-limited: decreased fitness	
YER038c	*KRE29* (unknown)	YNL287w	*SEC21* (ER-Golgi transportation)
YNL156c	*YIG2* (unknown)	YCRO52w	*RSC6 (chromatin modelling)*
YHR166c	*CDC23* (cyclin catabolism)	YML085C	*TUB1* (cytoskelton, chromosomes segregation)
YJL091c	*GWT1* (unknown)	YDL196w	unknown
YNL245c	*CWC25* (unknown)	YGR115C	unknown
YDL196w	unknown	YNL245C	*CWC25* (unknown)
YDR361c	*BCP1* (unknown)	YGL150C	*INO80* (DNA repair)
YPL146c	unknown	YGL093w	*SPC105* (microtuble)
YNL137c	*NAM9, MNA6* (protein biosynthesis)	YBR202w	*CDC47* (DNA replication initiation)
YGL122c	*NAB2* (mRNA polyadenilation)	YFL035c-a	*MOB2* (cell polarity)

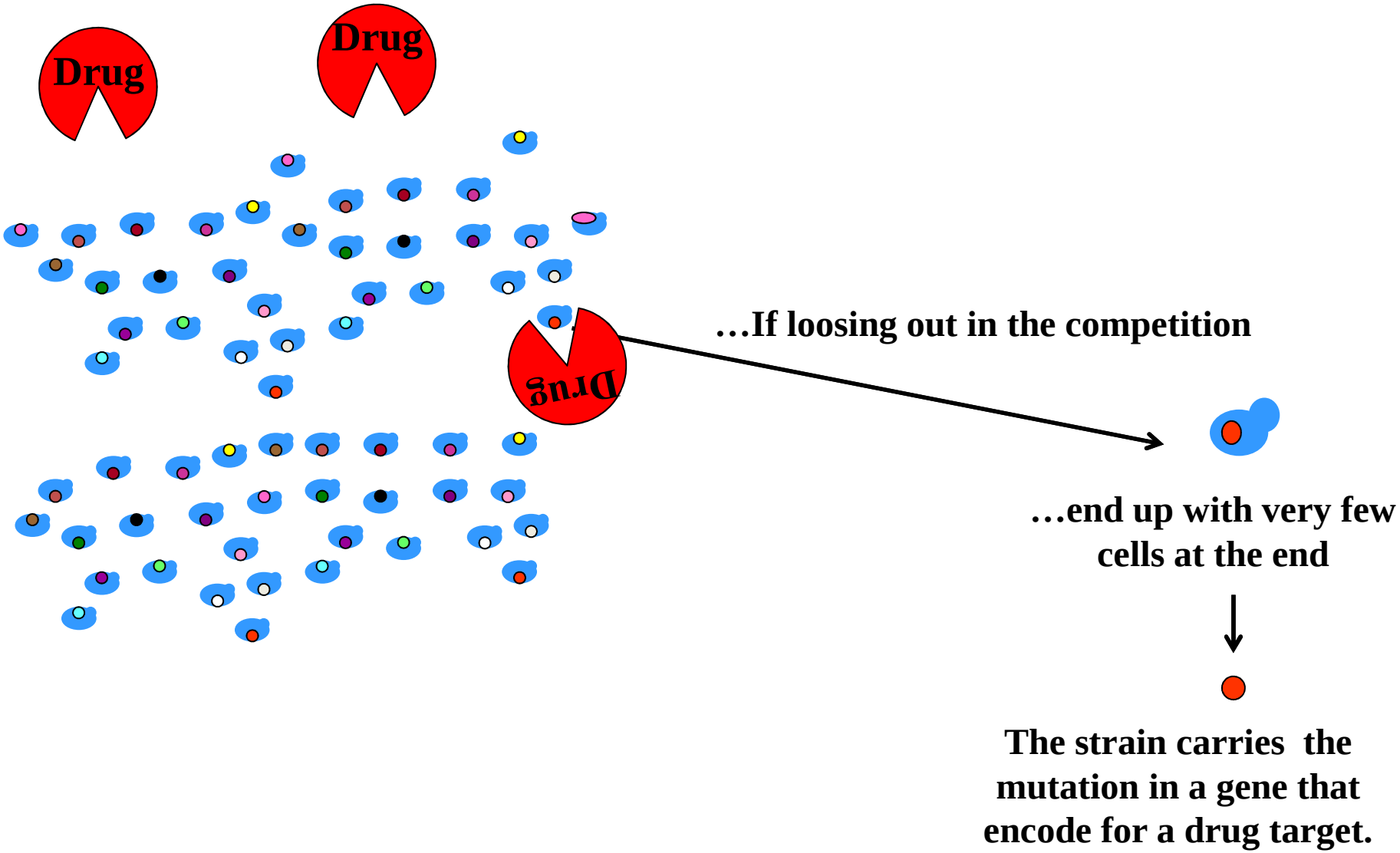
Application to Pharmacogenomics

Principle: Reducing the gene copy number of drug targets result in sensitisation to the drug of interest.

Discovery of new molecular drug targets

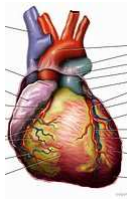


YDC in presence of the drug



Screening of medically relevant drugs using yeast genome profiling

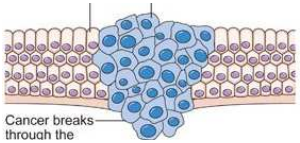
Antianginal drug (molsidomine) →



Genes in sterol biosynthesis results haploinsufficient.

Explanation why patients treated with this drug showed lower cholesterol

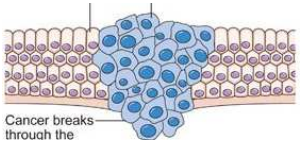
Anticancer drug 5-FOA →



rRNA processing exosome results haplo insufficient

5-FOA inhibit cell growth by inducing RNA damage.

Anticancer drug methotrexate →

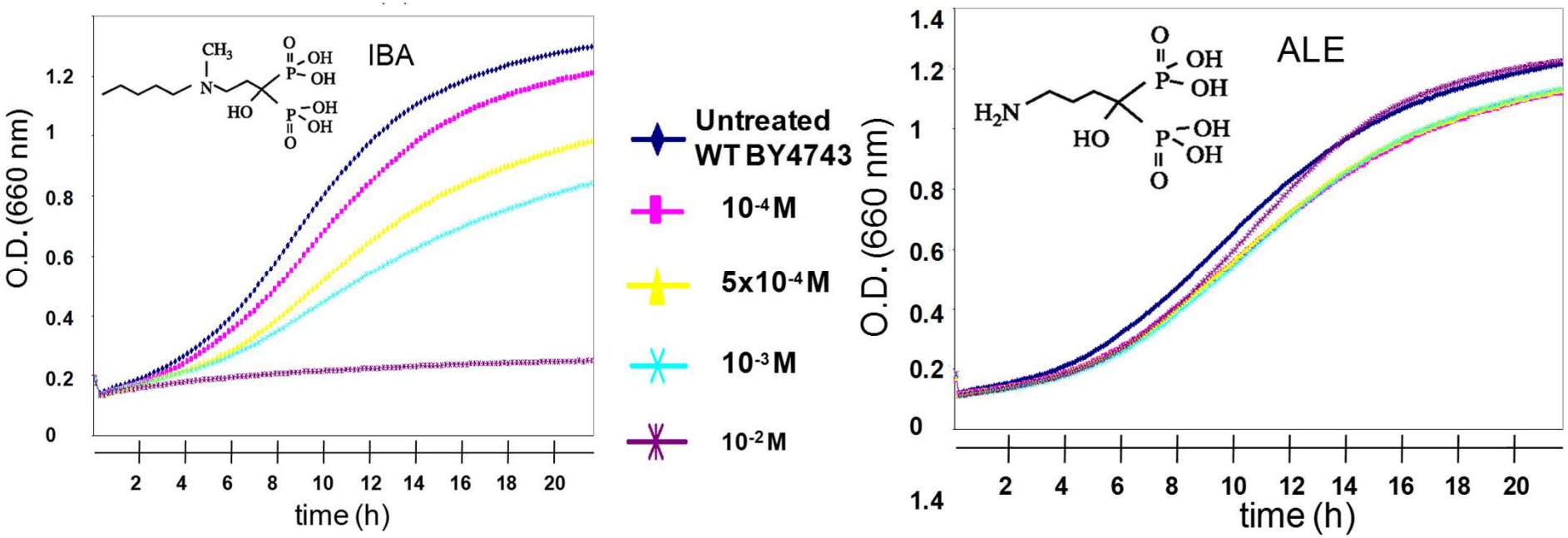


Identified new target *FOL1* and *FOL2* involved in folate biosynthesis.

***FOL1* has a homolog in *C. albicans*, but no human homolog.**

***FOL2* has a human homolog**

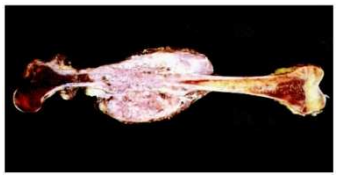
Screening for secondary targets of N-bisphosphonates (anti-cancer agents)



Screening for secondary targets of N-bisphosphonates

Haplo-insufficient

N-bisphosphonates (anti-cancer agents) → **Identified secondary targets: i.e. genes involved in organisation of cytoskelton.**



Haplo-proficient

Fitness advantage of *RAV1* mutant responsible for acidification of the endocytic vesicles.

↓

N-BP needs correct acidification to be released in cytosol → ***RAV1* hemizygote mutants have impaired acidification and therefore N-BP cannot release in the cytosol and cell develop resistance to this drug.**

Summary of chemogenomic screening with different drugs

Drugs	Class	Genes identified	References
benomyl	Fungicide drug	*TUB1* and *TUB2*	Giaever *et al.*, 1999
tunicamycin	Antibiotic drug	*ALG7*	Giaever *et al.*, 1999
fluconazole	Antifungal drug	*ERG11*	Giaever *et al.*, 1999
molsidomine	Antianginal drug	*ERG7*	Lum *et al.*, 2004
5-fluorouracil	Antiproliferative drug	*CDC21*, *RRP6*, *RRP41*, *RRP44*, *RRP46*, *NOP4*, *MAK21*, *SSF1* and *YPR143W*	Lum *et al.*, 2004
methotrexate	Anticancer drugs	*FOL1* and *FOL2*	Giaever *et al.*, 2004
atorvastatin and lovastatin	Anticholesterol drugs	*HMG1*, *PDR5* and *ERG13*	Giaever *et al.*, 2004
N-bisphosphonates	Osteoporosis and anticancer drugs	*ERG20*, *MRH1*, *ATG4*, *ALF1*, *TUB3*, *DBF4* and *RAV1*	Bivi *et al.*, 2009
chitosan	Antimicrobial compound	*SLN1*	Zakarewska *et al.*, 2007
rapamycin	Antifungal agents and immunosuppressant drug	*SER1*, *SER2*, *HOM2* and *HOM3*	Chan *et al.*, 2000
wortamannin	Anticancer drug	*SML1*, *POL1*, *POL2*, *RAD7*, *MSH1*, *MSH2*, and *MLH3*	Zewail *et al.*, 2003

Conclusions on studies of gene dosage effect on fitness and environmental dependency of gene function

• Yeast Deletion Collection (YDC) used as model to study gene function and gene dispensability

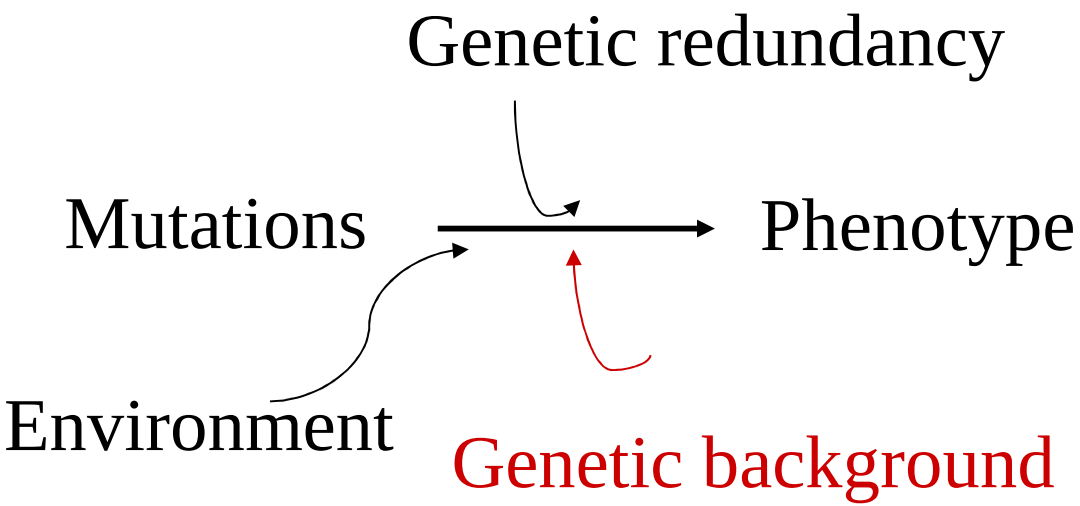
•YDC Homozygote collections to study the biological function of non essential genes in different environments

• YDC Heterozygote collection to study the gene dosage effects on fitness in different environments (haploinsuffcient phenotypes)

•YDC Heterozygote collection employed in pharmacogenomics screening to discover molecular drug targets.

Interaction of genetic background & environment

Investigating the impact that intra-specific and inter-specific variation have on the phenotypes and fitness



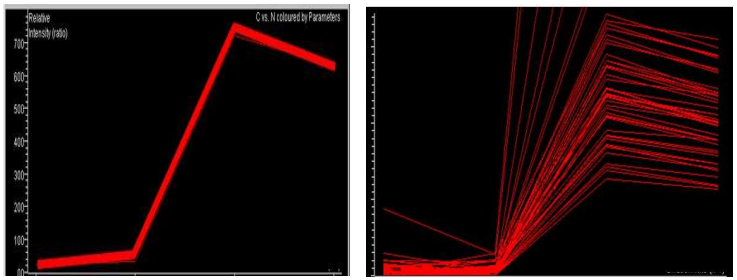
- Cross all Yeast Deletion Collection with different yeast strain and species.
- Study of the haplo*insufficiency* profile in different environments

Dowell R.D. Ryan O., *et al*.,…& Boone C. (2010), *Science* **328**: 469

Functional analysis from single gene to gene network

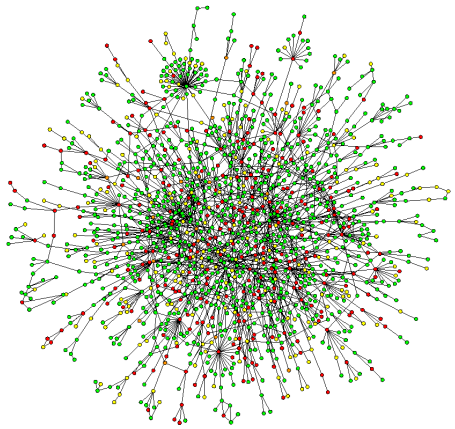
Genomic level → Single/multiple gene knockout → Phenotypic assays → Env1, Env2, Env3, Env4

Transcriptome → Study of the expression profile of the cell under different physiological conditions. → Cluster analysis



Proteome

Study of the proteins produced by a cell under different physiological conditions. Protein-protein interaction.



Abstract

A genetic interaction occurs when the combination of two mutations leads to an unexpected phenotype. Screens for synthetic genetic interactions have been used extensively to identify genes whose products are functionally related. In particular, synthetic lethal genetic interactions often identify genes that buffer one another or impinge on the same essential pathway. For the yeast *Saccharomyces cerevisiae*, we developed a method termed synthetic genetic array (SGA) analysis, which offers an efficient approach for the systematic construction of double mutants and enables a global analysis of synthetic genetic interactions. In a typical SGA screen, a query mutation is crossed to an ordered array of ~5000 viable gene deletion mutants (representing ~80% of all yeast genes) such that meiotic progeny harboring both mutations can be scored for fitness defects. This approach can be extended to all ~6000 genes through the use of yeast arrays containing mutants carrying conditional or hypomorphic alleles of essential genes. Estimating the fitness for the two single mutants and their corresponding double mutant enables a quantitative measurement of genetic interactions, distinguishing negative (synthetic lethal) and positive (within pathway and suppression) interactions. The profile of genetic interactions represents a rich phenotypic signature for each gene and clustering genetic interaction profiles group genes into functionally relevant pathways and complexes. This array-based approach automates yeast genetic analysis in general and can be easily adapted for a number of different genetic screens or combined with high-content screening systems to quantify the activity of specific reporters in genome-wide sets of single or more complex multiple mutant backgrounds. Comparison of genetic and chemical-genetic interaction profiles offers the potential to link bioactive compounds to their targets. Finally, we also developed an SGA system for the fission yeast *Schizosaccharomyces pombe*, providing another model system for comparative analysis of genetic networks and testing the conservation of genetic networks over millions of years of evolution.

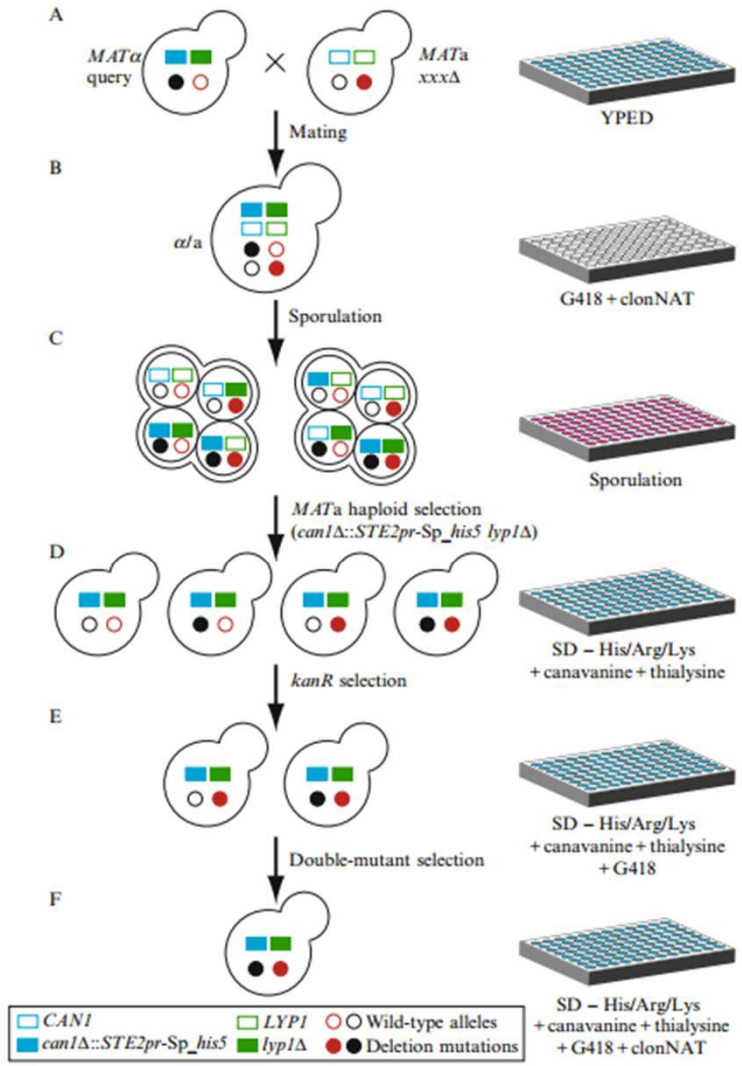


Figure 7.1 Synthetic genetic array (SGA) methodology. (A) A *MAT*α strain carries a query mutation linked to a dominant selectable marker (filled black circle), such as the nourseothricin-resistance marker, *natMX4*, and the SGA reporter, *can1Δ::STE2pr-Sp_his5* (in which *STE2pr-Sp_his5* is integrated into the genome such that it deletes the open reading frame (ORF) of the *CAN1* gene, which normally confers sensitivity

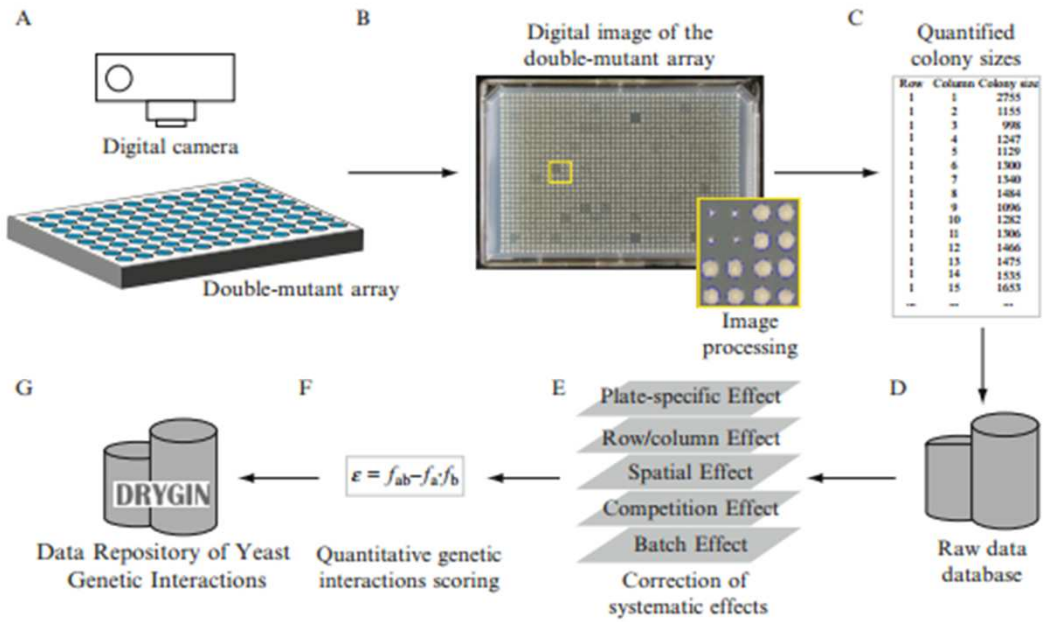
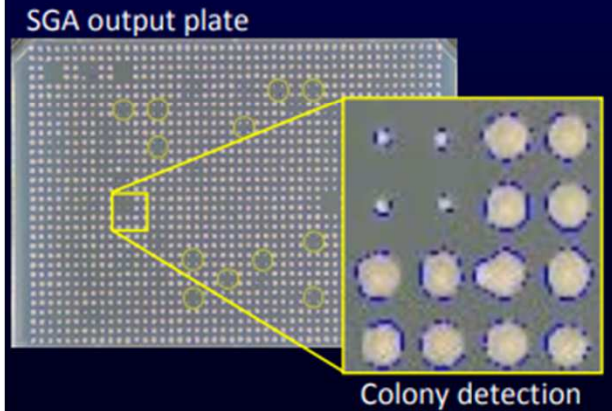


Figure 7.2 Computational pipeline for processing SGA data. (A) Double mutant array plates are photographed by a high-resolution digital camera. (B, C) Digital images of the double mutant array plates are processed by a custom-developed image processing software that identifies the colonies and measures their areas in terms of pixels. (D) Quantified double mutant colony sizes are stored in the database for further manipulation and analysis. (E) To identify quantitative genetic interactions, the yeast colony data is retrieved from the database and a series of normalizations are applied to correct for numerous systematic experimental effects. (F) Genetic interactions are measured by combining the corrected double mutant fitness and the fitnesses of the two single mutants. (G) Genetic interaction data is made available via the DRYGIN web database system.

Generating epistasis data: metabolic SGA miniarray

Quantitative epistasis data generated by Charles Boone's lab in *S. cerevisiae*:

- ~ 378 000 gene pairs tested (~1000 genes), 1246 negative and 322 positive interactions identified
- Genes include enzymes, transporters and regulators



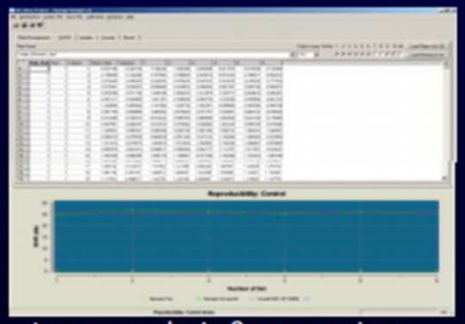


Image analysis & processing

Data Correction:

1) Systematic effects (plate/position)

2) Competition effects

3) Measure DM fitness
   Estimate SM fitness

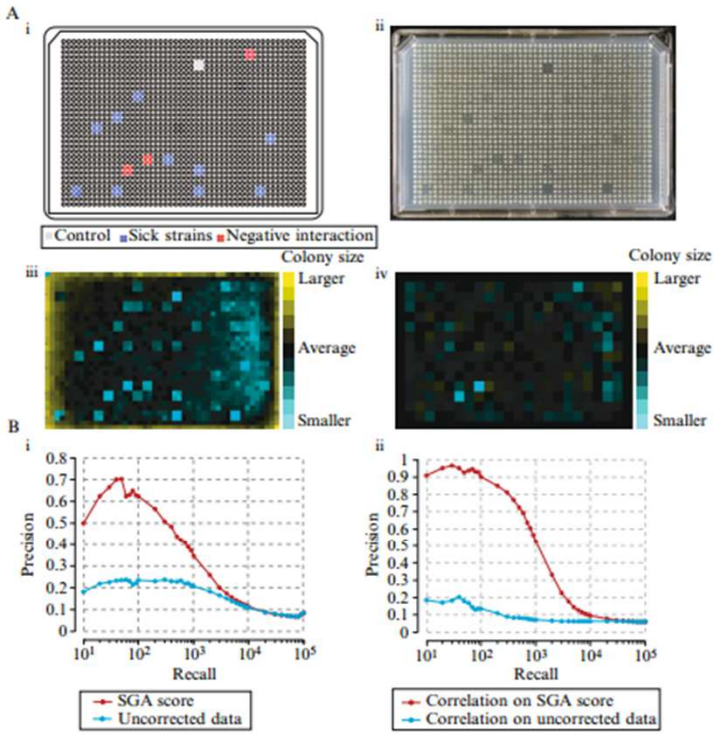


Figure 7.3 Systematic effect correction. (A) A schematic of a typical double mutant array plate shows the systematic biases affecting colony sizes. (i, ii) A typical double mutant array plate contains control spots (gray circles), strains with low fitness (blue circles), negative interactions (red circles), and positive interactions (not shown). On visual inspection, all three cases appear as small colonies or empty spots. (iii) Quantification of colony areas shows a distinctive spatial pattern affecting opposite sides of the plate (bigger colonies on the right, smaller colonies on the left) that was not obvious on visual inspection. Failure to correct for this spatial pattern will result in false-positive interactions. (iv) Corrects spatial patterns, eliminates false positives, and highlights true genetic interactions. (B) Precision–recall curves on genetic interaction scores (i) and genetic profile similarity (ii) show the increased functional prediction capacity of genetic data after correcting for systematic biases. A set of 1712 genome-wide SGA screens (Costanzo *et al.*, in press) were processed using the SGA score (Baryshnikova *et al.*, manuscript in preparation) and a version of the SGA score without systematic effect correction. Both direct genetic interactions and genetic profile similarities, as measured by Pearson correlations, were assessed for function by calculating precision and recall of functionally related gene pairs as described in the study of Myers *et al.* (2006). As a measure of functional relatedness, we used coannotation to the same Gene Ontology term.

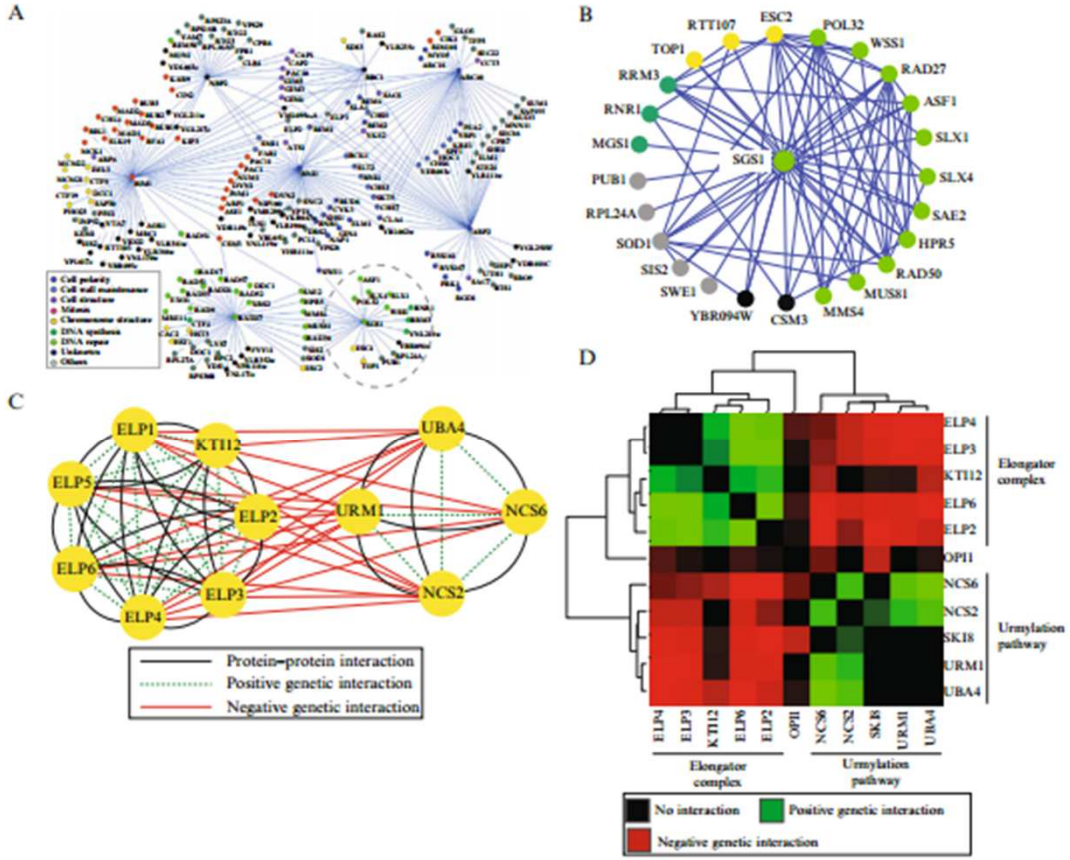


Figure 7.4 (*Continued*)

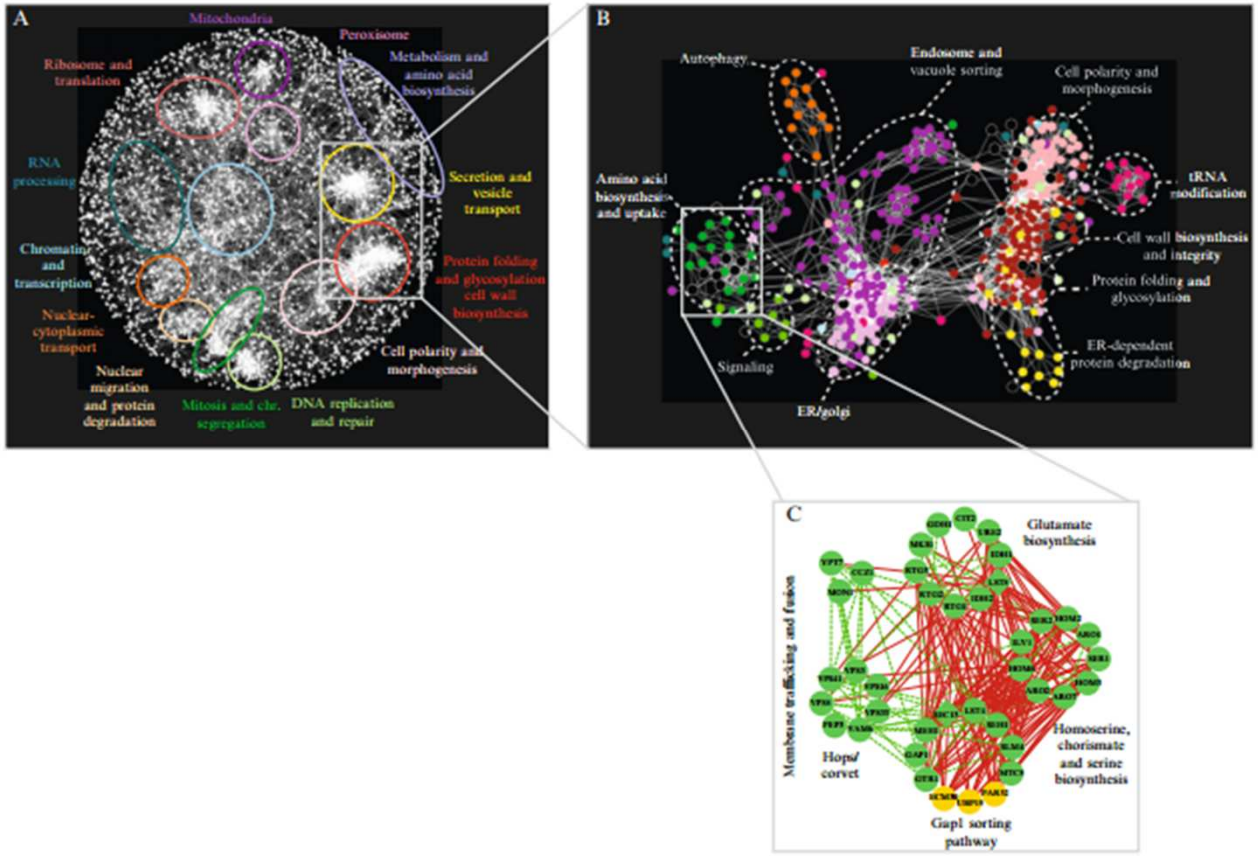
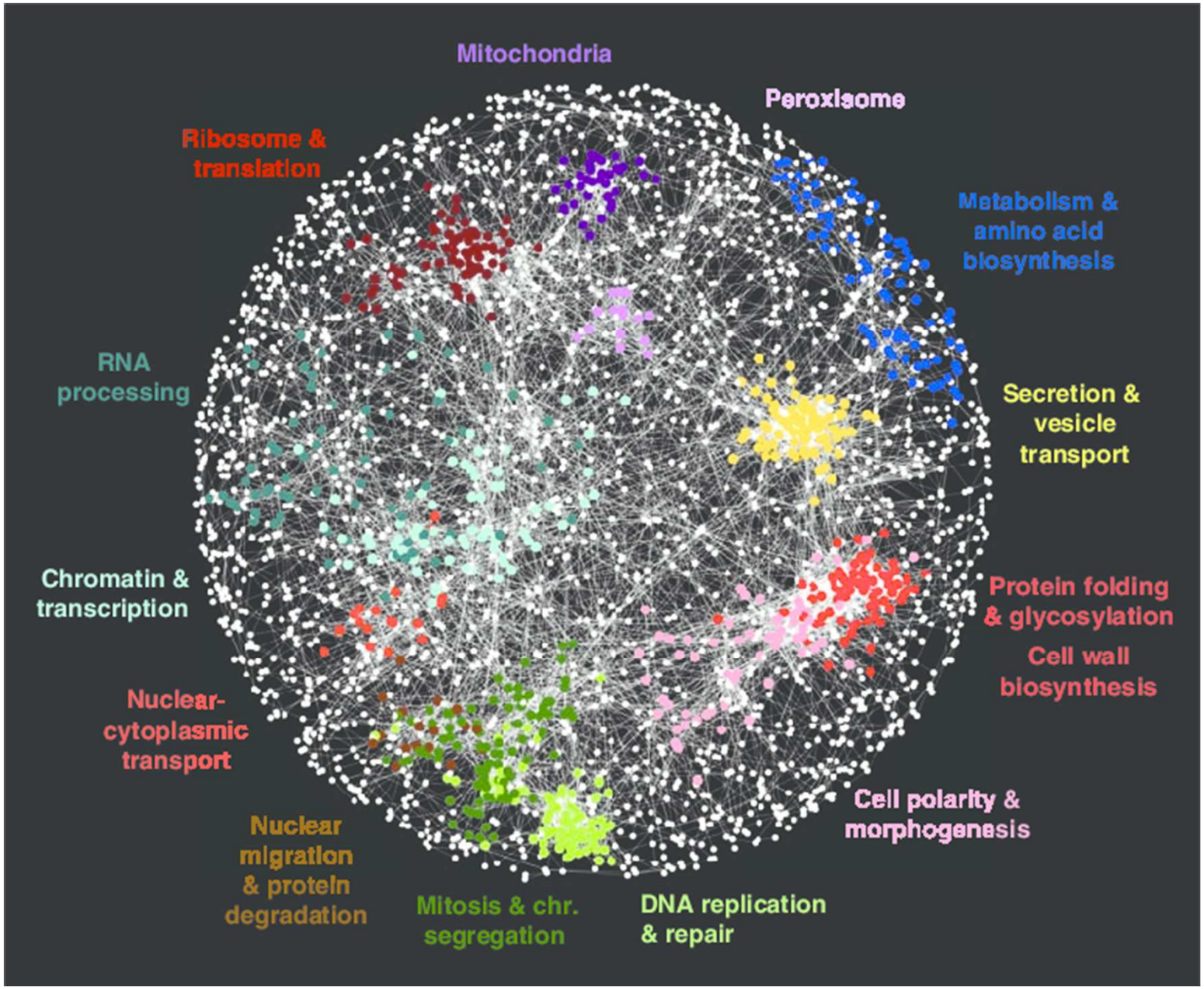


Figure 7.5 (*Continued*)


Mitochondria
Peroxisome
Ribosome & translation
Metabolism & amino acid biosynthesis
RNA processing
Secretion & vesicle transport
Chromatin & transcription
Protein folding & glycosylation
Cell wall biosynthesis
Nuclear-cytoplasmic transport
Cell polarity & morphogenesis
Nuclear migration & protein degradation
Mitosis & chr. segregation
DNA replication & repair

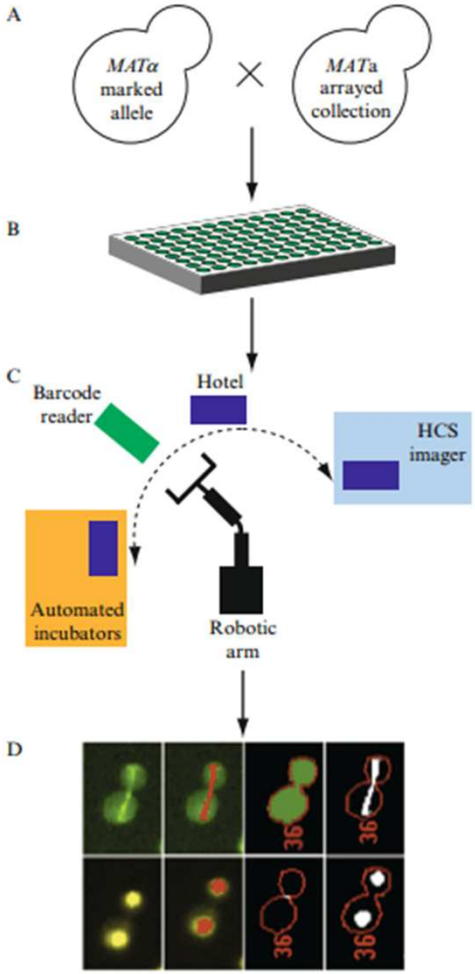


Figure 7.7 SGA-HCS pipeline for evaluation of cell biological phenotypes. (A) Using the SGA methodology, a fluorescent marker can be introduced into the arrayed deletion collection . (B) Deletion mutant colonies expressing a fluorescent marker are transferred to optical plates containing liquid selection media for imaging. (C) A robotic arm is used to move optical plates between incubators and the HCS imaging system. (D) Automated image analysis software, such as MetaXpress, is used to detect fluorescent signal and measure morphological features.

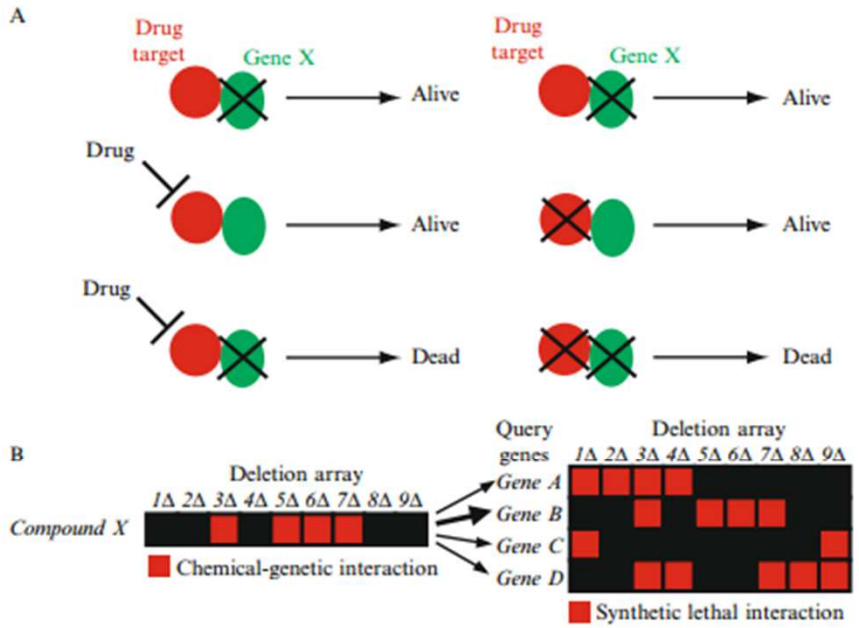
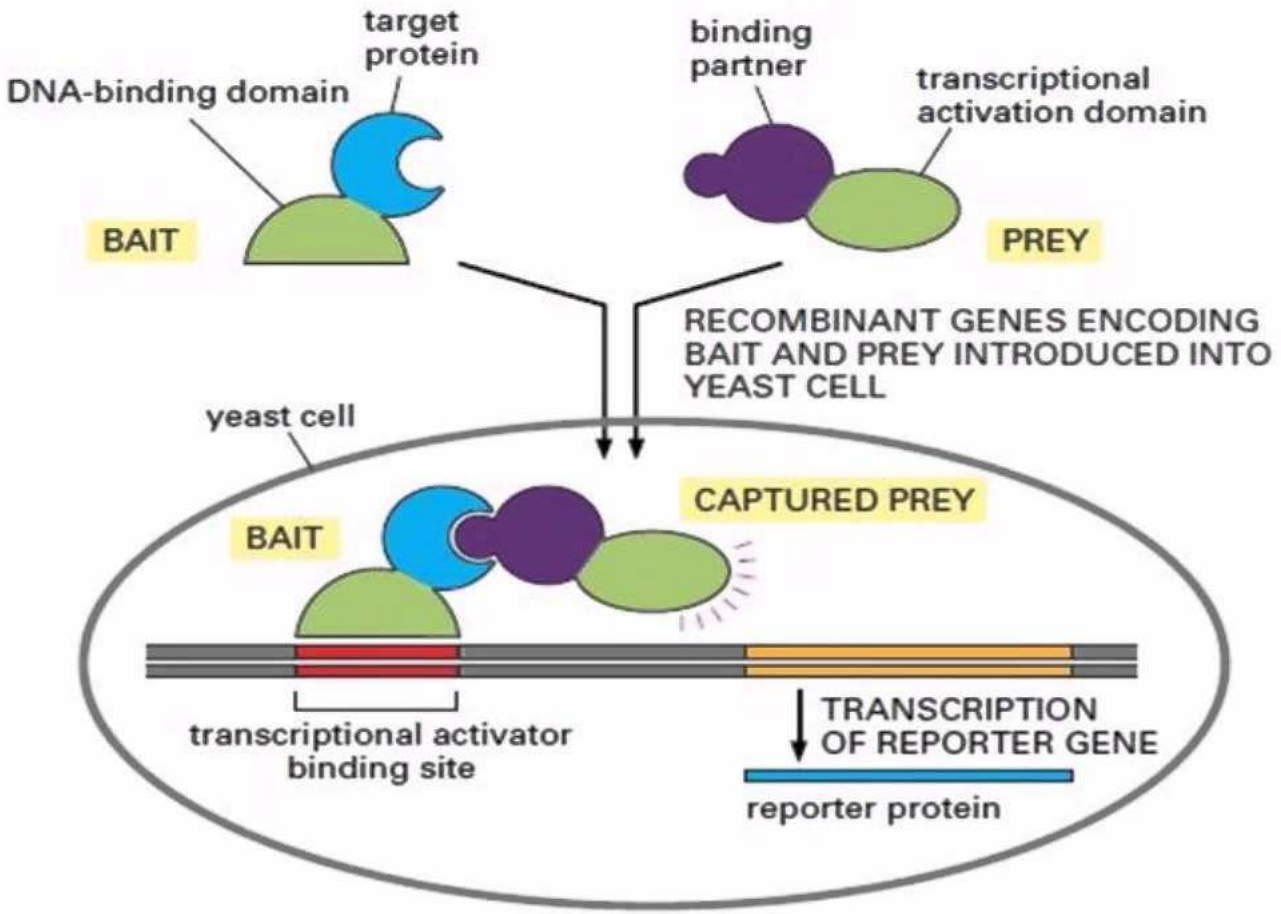


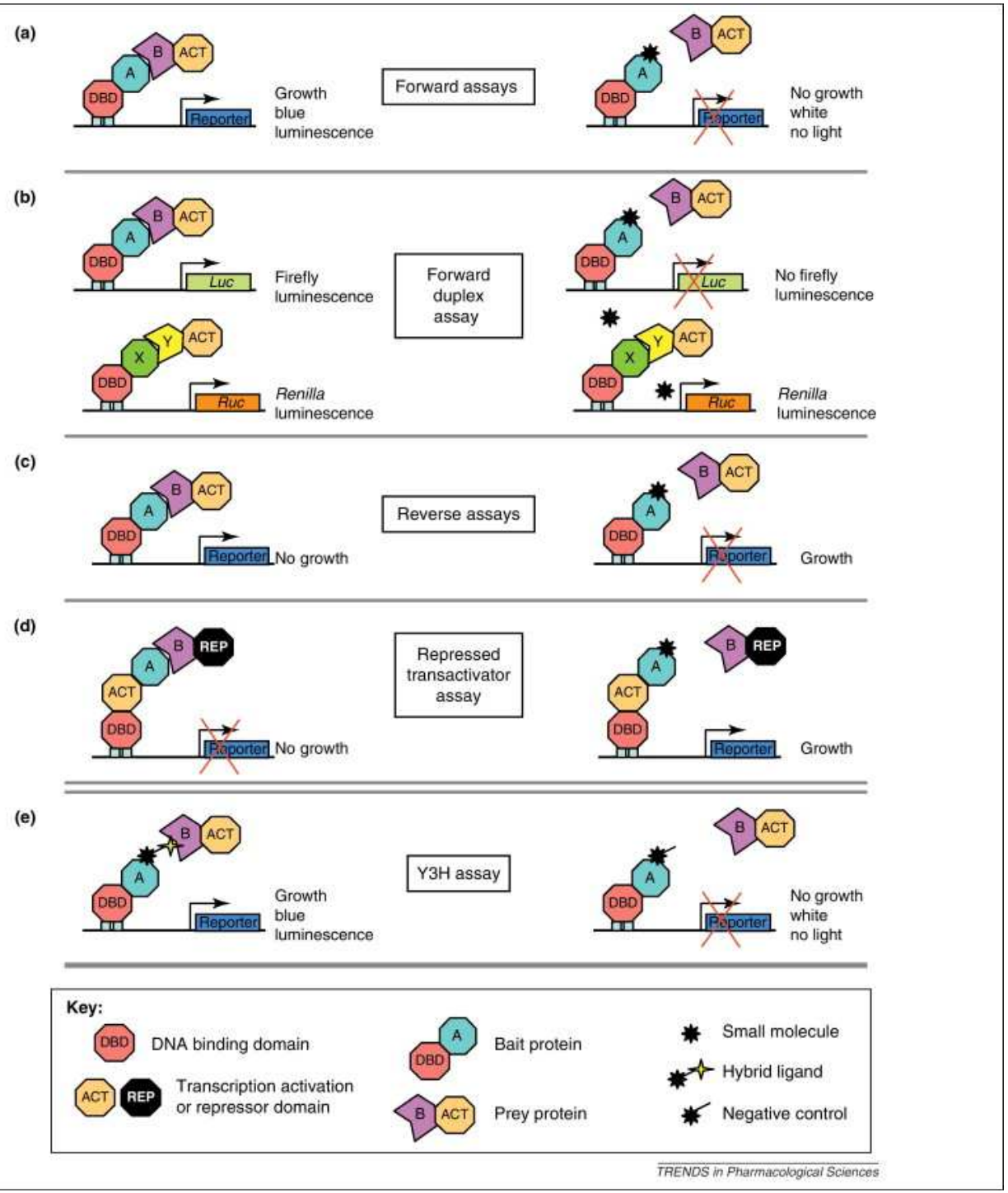
Figure 7.8 Chemical-genetic interactions can be modeled by synthetic genetic interactions. (A) In a chemical-genetic interaction (at left), a deletion mutant, lacking the product of the deleted gene (represented by a black X), is hypersensitive to a normally sublethal concentration of a growth-inhibitory compound. In a synthetic lethal genetic interaction (right), two single deletions lead to viable mutants but are inviable in a double-mutant combination. Gene deletion alleles that show chemical-genetic interactions with a particular compound should also be synthetically lethal or sick with a mutation in the compound target gene. (B) Comparison of a chemical-genetic profile to a compendium of genetic interaction (synthetic lethal) profiles should identify the pathways and targets inhibited by drug treatment. In this hypothetical figure, chemical-genetic and genetic interactions are both designated by red squares. For example, deletion mutants 3, 5, 6, and 7 are hypersensitive to compound X and a mutation in query gene A leads to a fitness defect when combined with deletion alleles 1, 2, 3, and 4. Here, the chemical-genetic profile of compound X resembles the genetic profile of gene B, thereby identifying the product of gene B as a putative target of compound X.

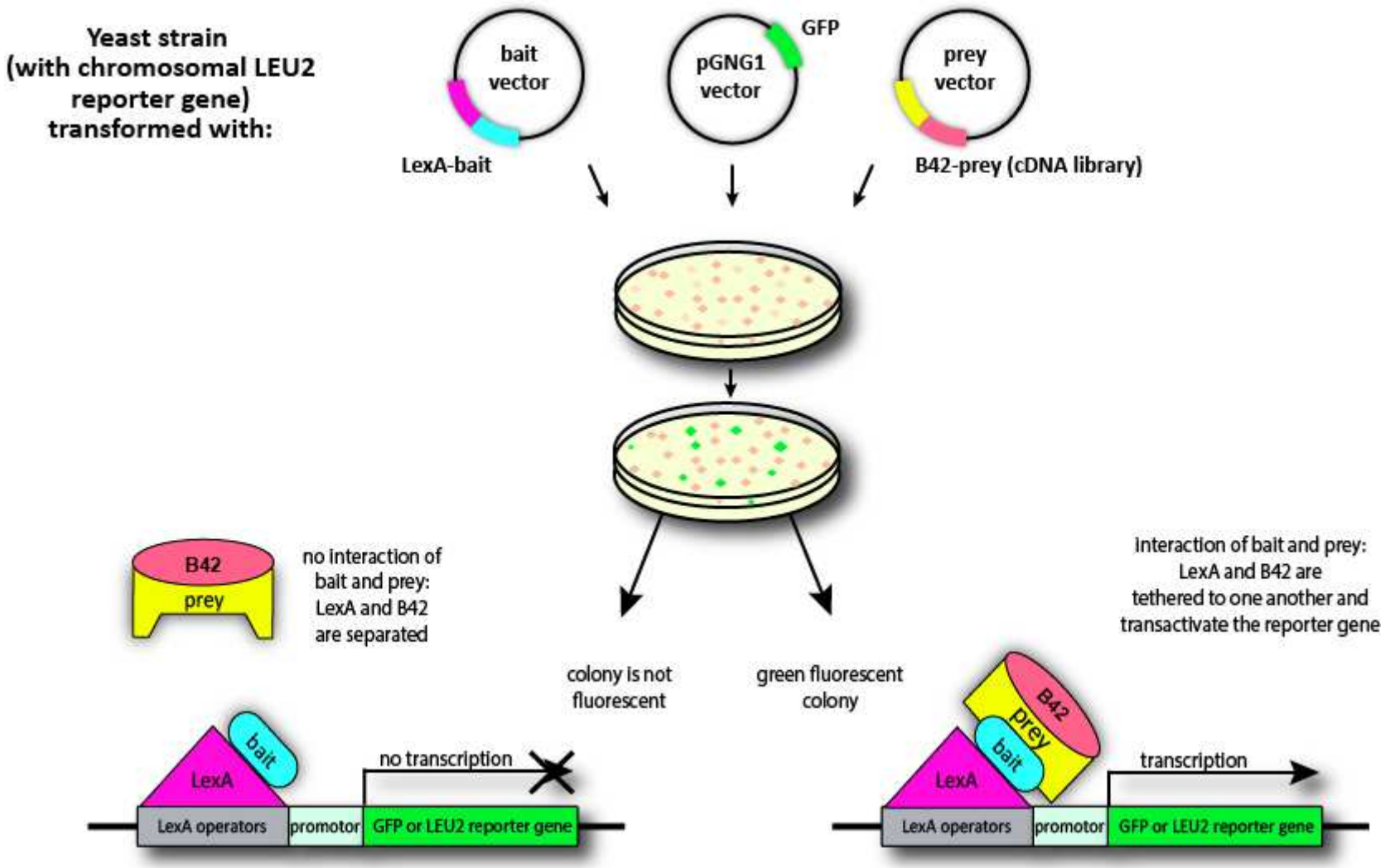
Yeast Two Hybrid System

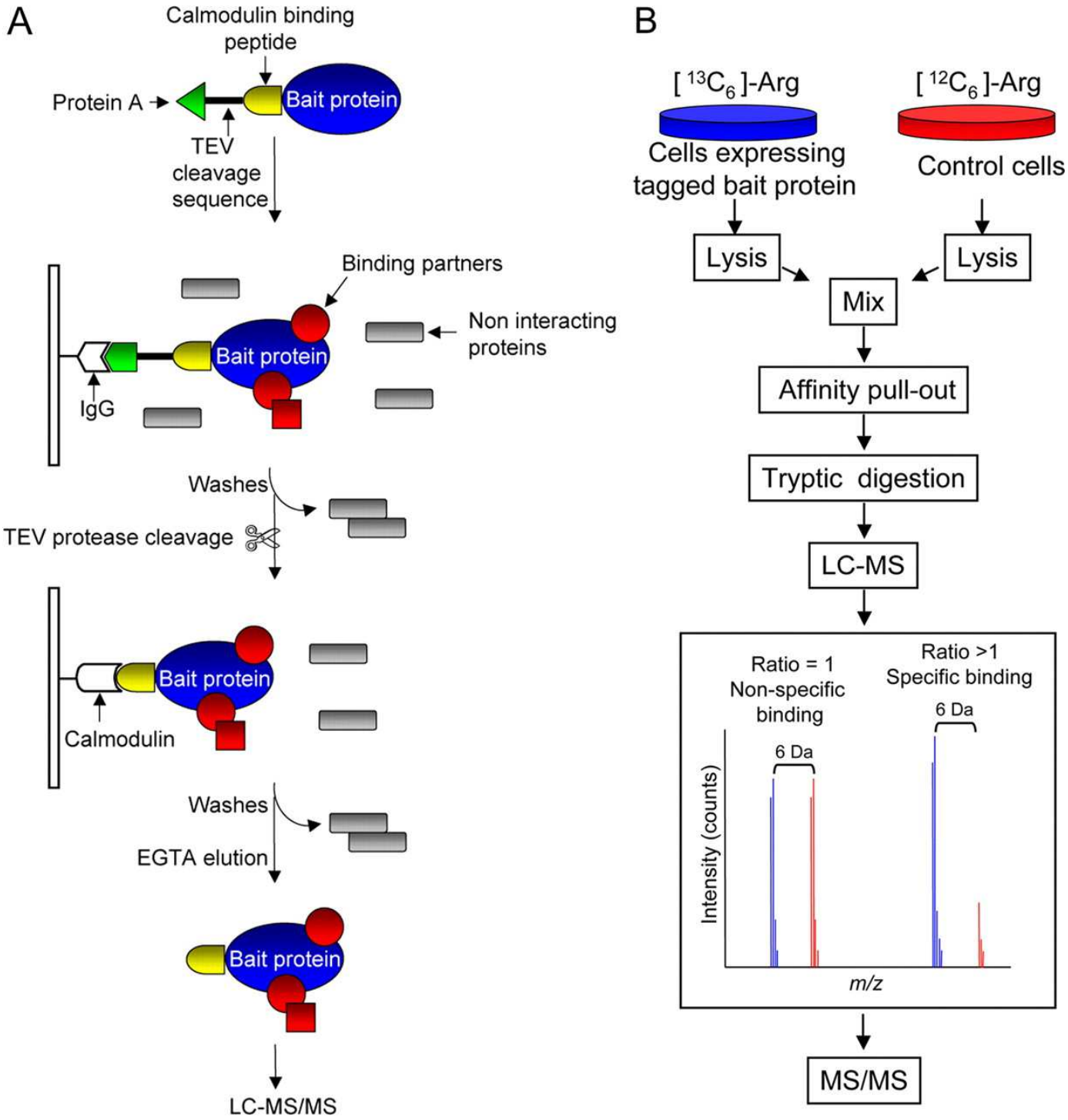


Yeast Two Hybrid System

www.technologyinscience.blogspot.com


(a)
DBD
A
B
ACT
Reporter
Growth
blue
luminescence
Forward assays
No growth
white
no light
(b)
Luc
Firefly
luminescence
Forward
duplex
assay
No firefly
luminescence
X
Y
Ruc
Renilla
luminescence
(c)
No growth
Reverse assays
Growth
(d)
REP
Repressed
transactivator
assay
(e)
Y3H assay
Key:
DNA binding domain
Transcription activation
or repressor domain
Bait protein
Prey protein
Small molecule
Hybrid ligand
Negative control
TRENDS in Pharmacological Sciences


Yeast strain
(with chromosomal LEU2
reporter gene)
transformed with:
bait
vector
LexA-bait
pGNG1
vector
GFP
prey
vector
B42-prey (cDNA library)
B42
prey
no interaction of
bait and prey:
LexA and B42
are separated
colony is not
fluorescent
green fluorescent
colony
Interaction of bait and prey:
LexA and B42 are
tethered to one another and
transactivate the reporter gene
bait
LexA
no transcription
LexA operators
promotor
GFP or LEU2 reporter gene
B42
prey
bait
LexA
transcription
LexA operators
promotor
GFP or LEU2 reporter gene


A
Calmodulin binding peptide
Protein A
Bait protein
TEV cleavage sequence
Binding partners
Non interacting proteins
Bait protein
IgG
Washes
TEV protease cleavage
Bait protein
Calmodulin
Washes
EGTA elution
Bait protein
LC-MS/MS
B
[13C6]-Arg
[12C6]-Arg
Cells expressing tagged bait protein
Control cells
Lysis
Lysis
Mix
Affinity pull-out
Tryptic digestion
LC-MS
Ratio = 1
Non-specific binding
Ratio >1
Specific binding
6 Da
6 Da
Intensity (counts)
m/z
MS/MS

Genetic interaction (epistasis) refers to the non-independence of mutational effects

Epistasis between two gene deletions: