Genetik, Genomik, Bioinformatik

Teil V Hefegenetik

Behold the awesome power of yeast…

Karsten Weis -IBC

Warum Hefegenetik?

gene -> mRNA -> protein

Verständnis der Physiologie von Zellen & Organismen

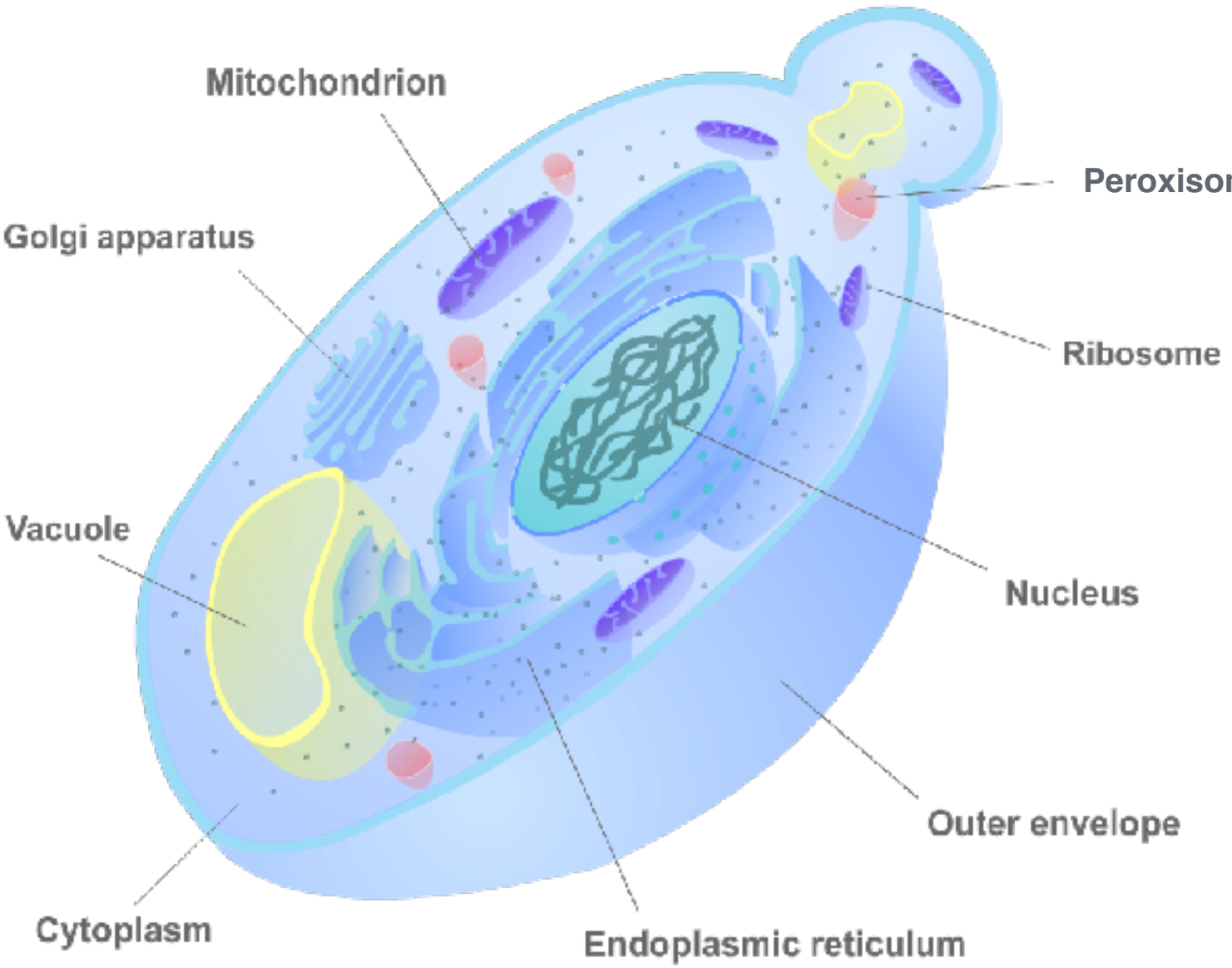
functions of all genes ~> networks
genes never work in isolation, they are always causally induced by preceding genes, factors, feedbacks etc.

in yeast, there are 6k genes, but 15% of all the expressed genes have an unkown cellular function.
when removing these genes, one cannot observe the phenotype which makes it rather hard to define its function

finding homologous genes, one can approximate the function of a gene. of those 15% no homologs have been found yet sadly.

Warum Hefegenetik?

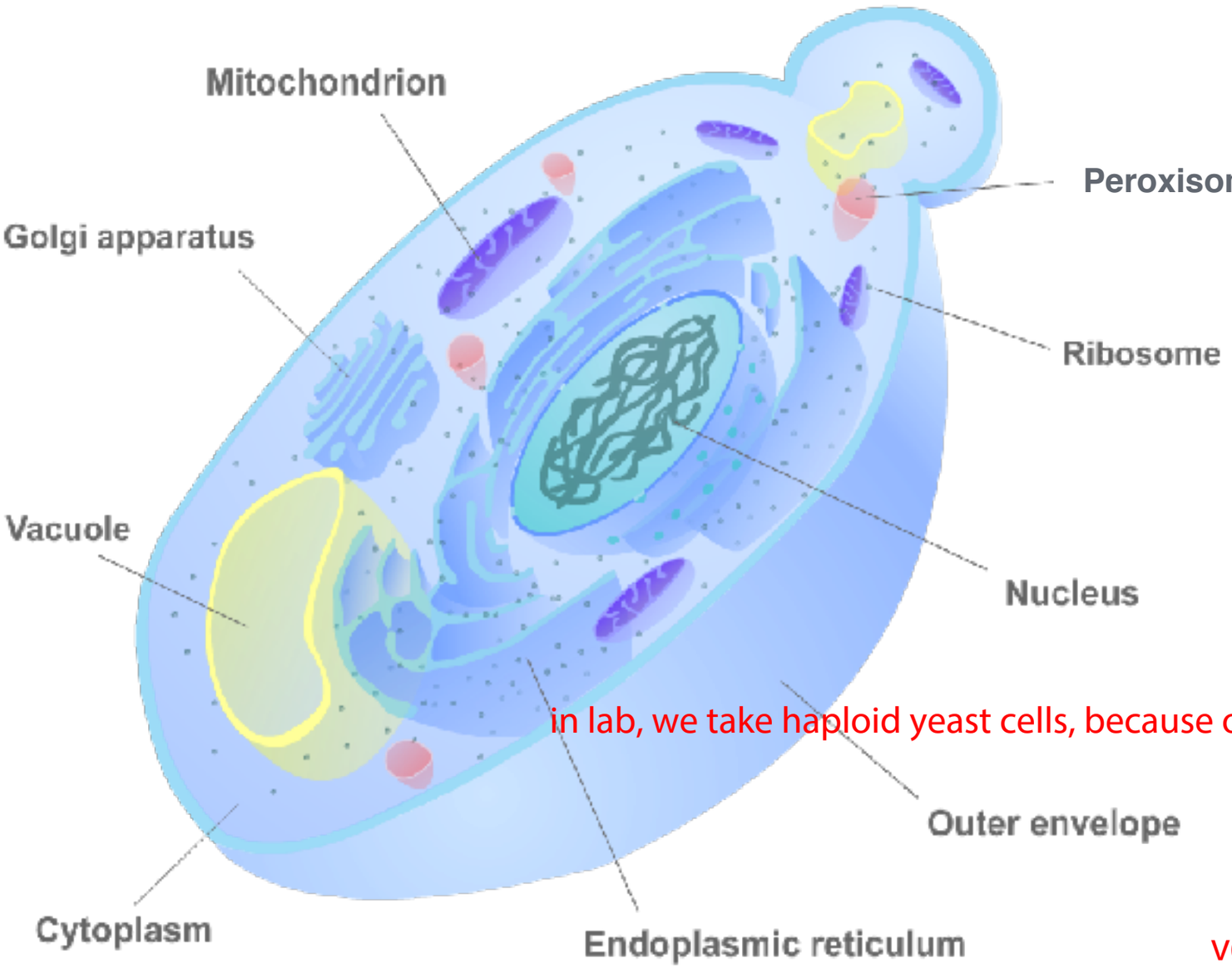
yeast is a good model for eukaryotic lifeforms



- typische eukaryotische Zellstruktur

those dots on the outer wall of the cell nucleous are called nuclear pore complexes (NPC)

Warum Hefegenetik?



advantages of yeast:

- typische eukaryotische Zellstruktur
- kleines, kompaktes Genom
- kurze Generationsdauer
- haploides und diploides Wachstum

in lab, we take haploid yeast cells, because one can observe recessive genes a lot easier

- einfache Mutagenese

very high recombination rate (especially homologous recombination)

plasmids can be very easily added to yeast

Klassische genetische Methoden

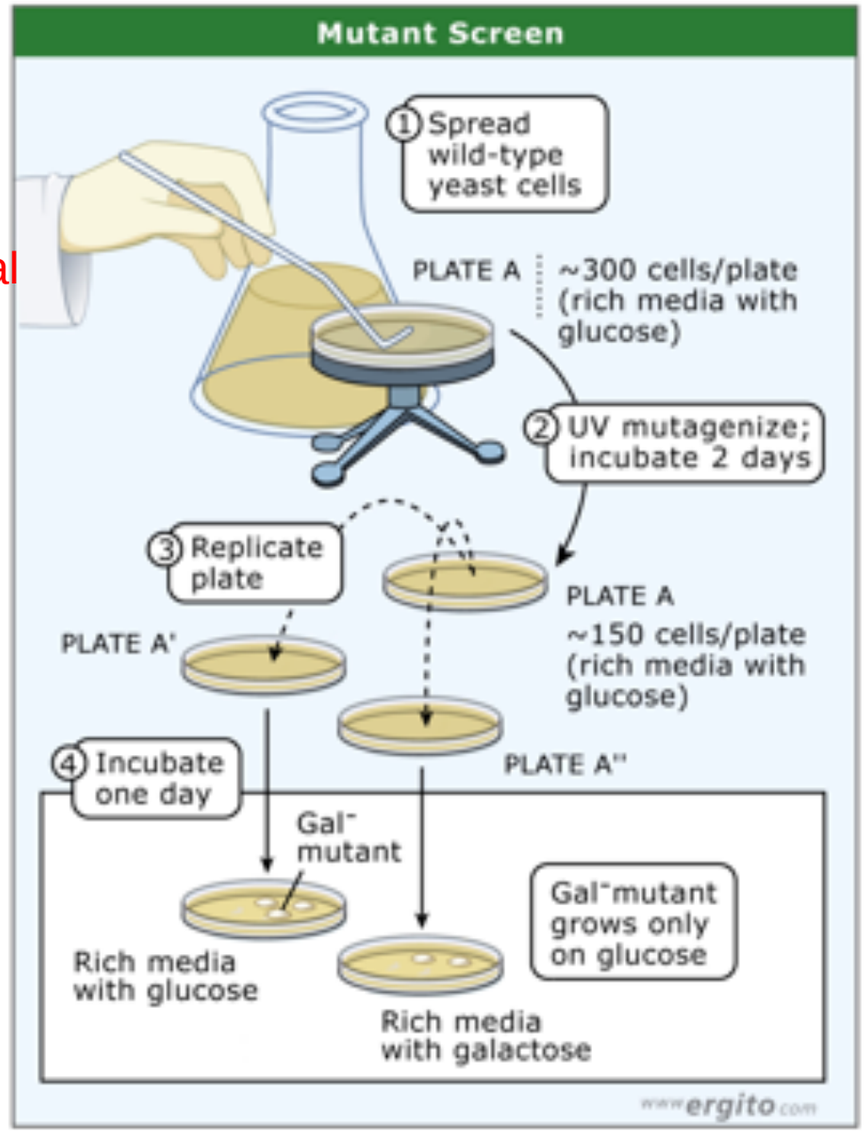
Beispiel - Metabolismus

a clssical genetic study would be, how is glucose fermented into EtOH and CO2 in yeast.
yeast can also metabolize galactose, when the right genes are expressed (genes: GAL2, GAL7, GAL10, GAL4 transcription factor, GAL80 its inhibitor etc.)

Klassische genetische Methoden

15% of the yeast's genes are non-essential.
after mutagenesis, about 15% should die in order to be sure that ca. 1 gene per cell is mutated.

hot to find yeast cells that cannot even grow on glucose?
Use conditional mutants that are normal under certain circumstances and not when they change.



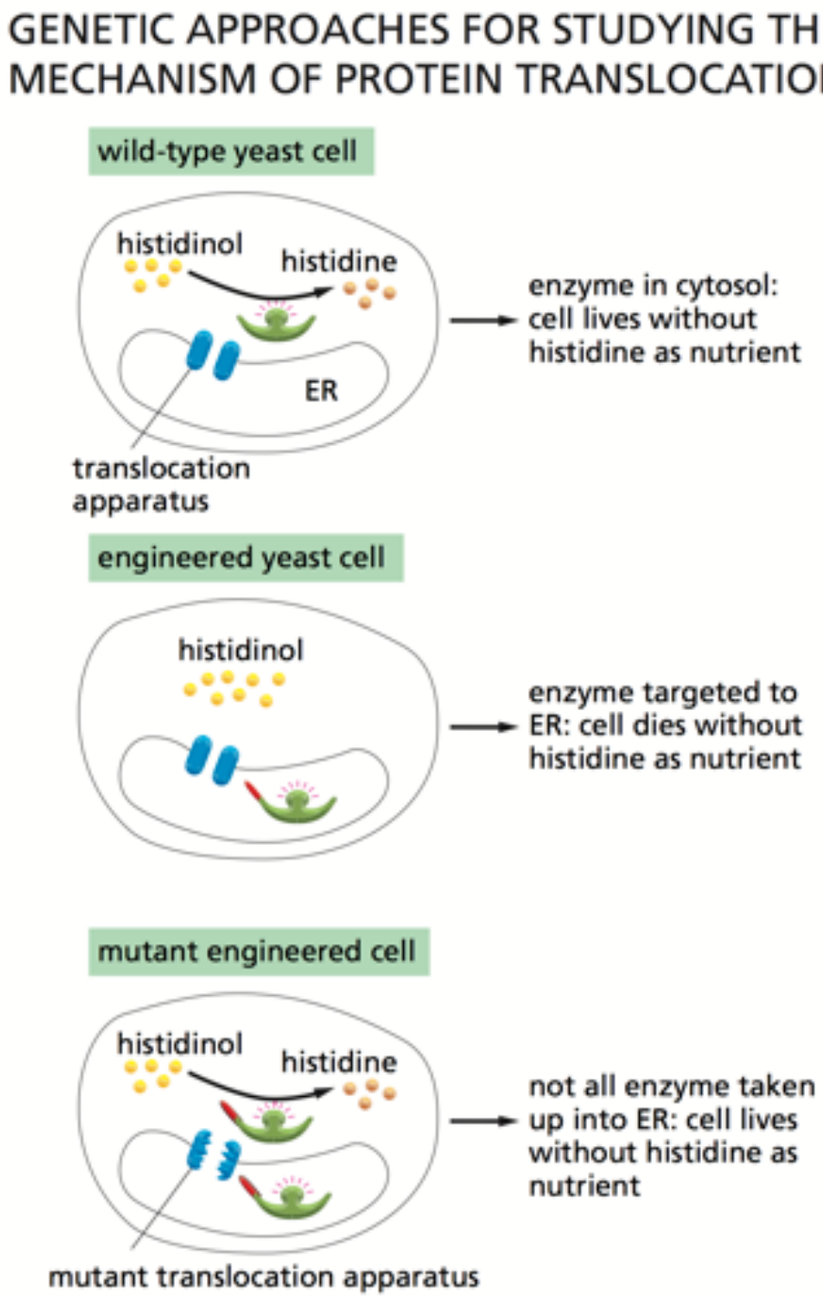
Klassische genetische Methoden

Beispiel - Zellstruktur

proteinbiosynthesis -> ER-translocation - ER -> golgi -> plasma membrane -> secretion

Klassische genetische Methoden

Beispiel - Zellstruktur



(Molecular Biology of Cell, B. Alberts)

In sequencing, one simply sequences the mutant's genome and finds the mutations and compares it to the wild type. easy and cheap.

In complementation, one transforms the plasmids. yeast can very easily take up extra-chromosomal DNA. One checks whether a plasmid can save the yeast cell in its mutation. This complements the mutated phenotype and restores the phenotype. Then, simply sequence the plasmid which is even easier.

Lab of R. Schekman (Nobel 2013)

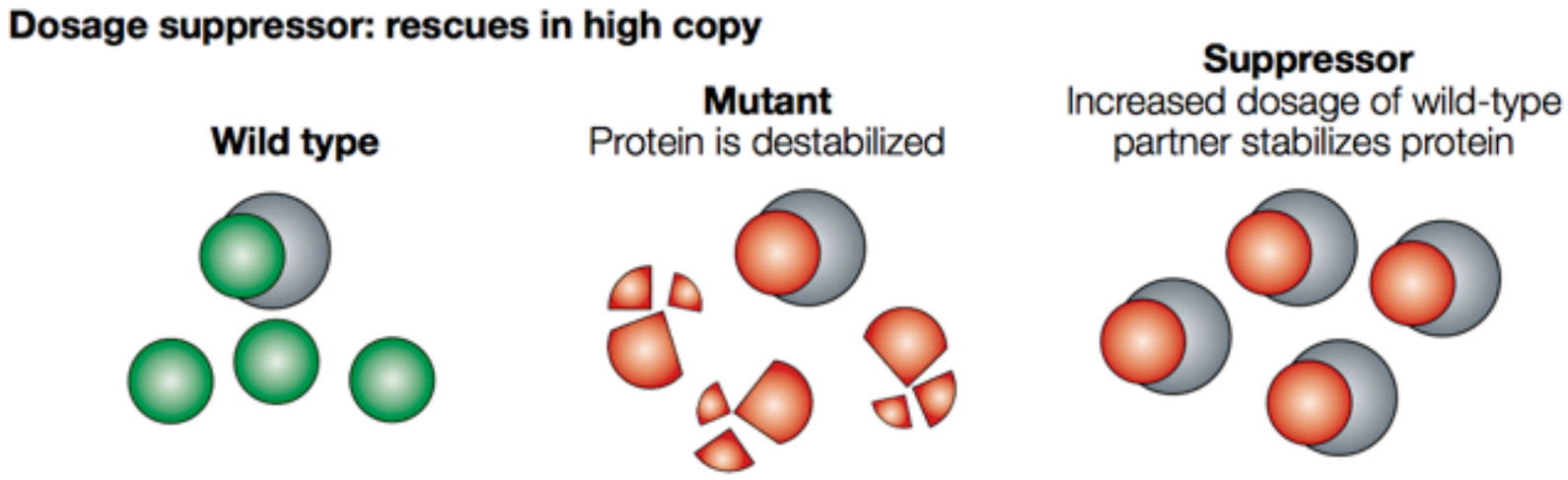
Wo funktioniert klassische Genetik nicht?

in redundance, classical genetics does not work

A -> B -> C -> E
-> D

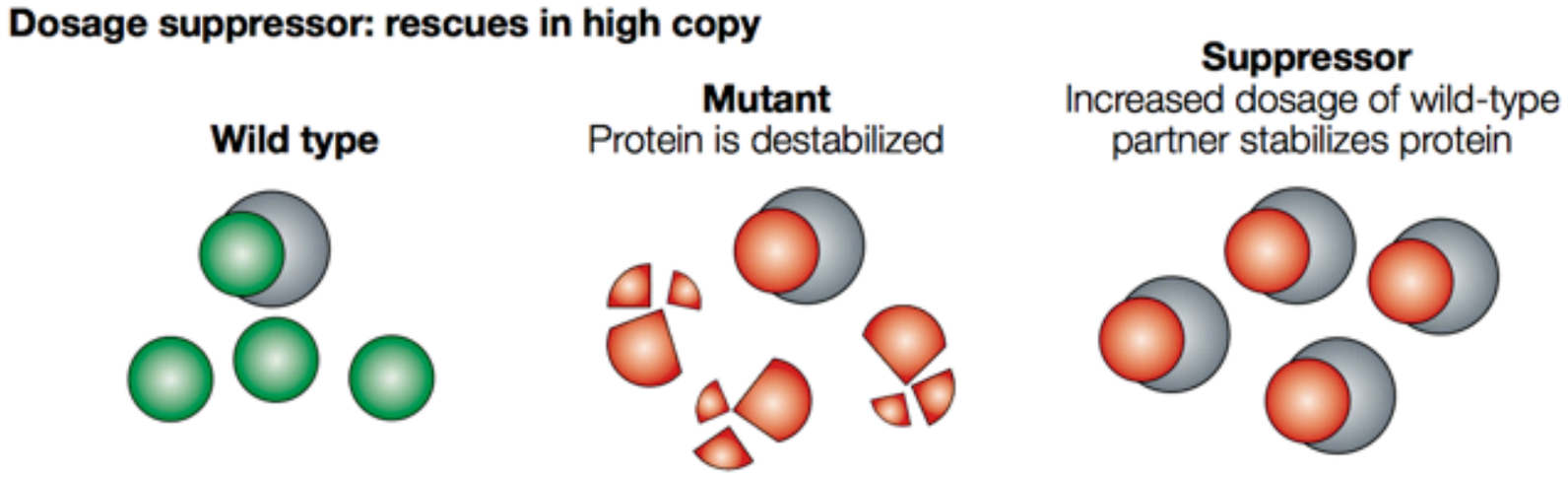
(a pathway, where both ways lead to the same end product E)

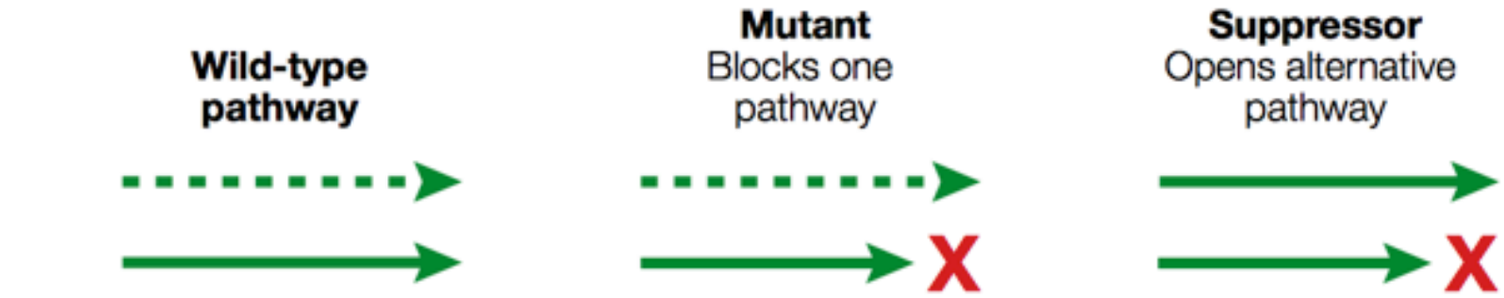
Suppressionsanalyse



plasmids induce high production of the grey balls.
add library in plasmids and look for the right plasmids and sequence it.
needs more than one plasmids per cell to overexpress grey balls

Suppressionsanalyse



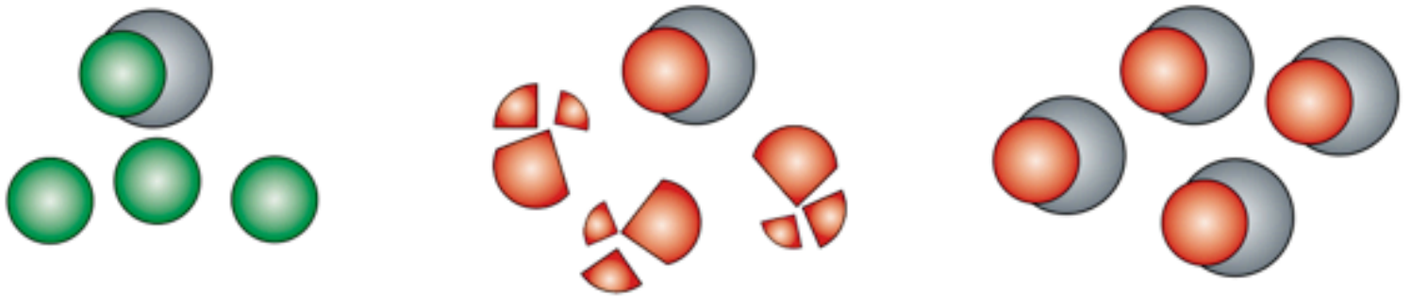


Suppressionsanalyse

type of mutation is important to define discussion

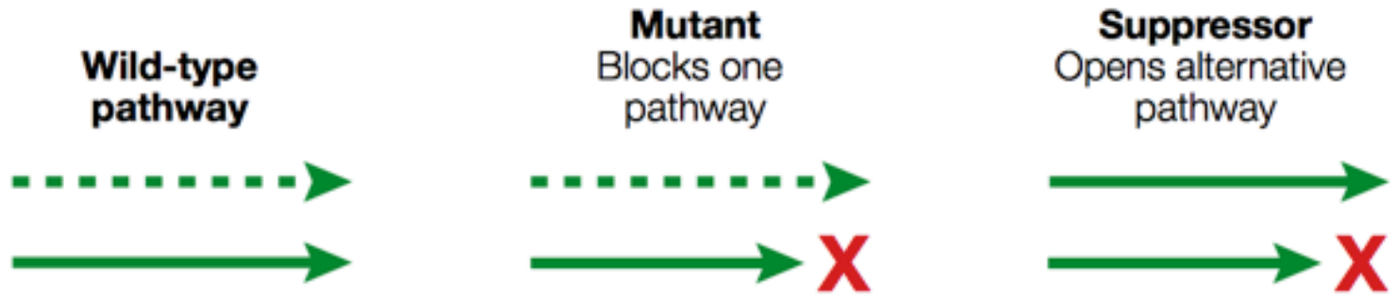
3 types of suppression analysis:

Dosage suppressor: rescues in high copy

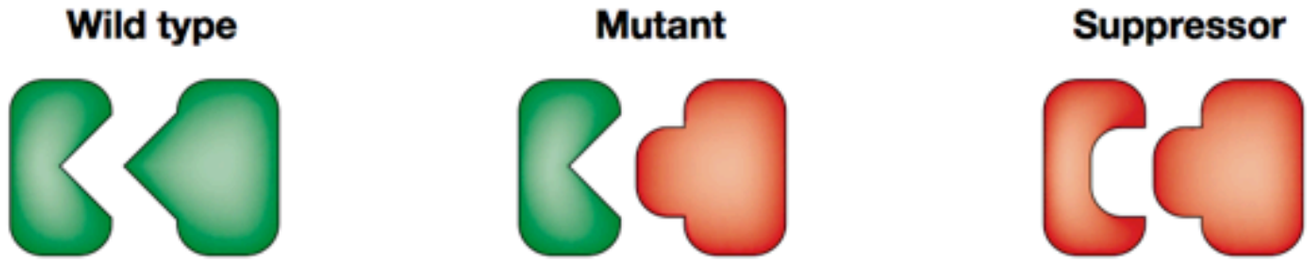


gain of function

Bypass suppressor: pathway specific, rescues null allele

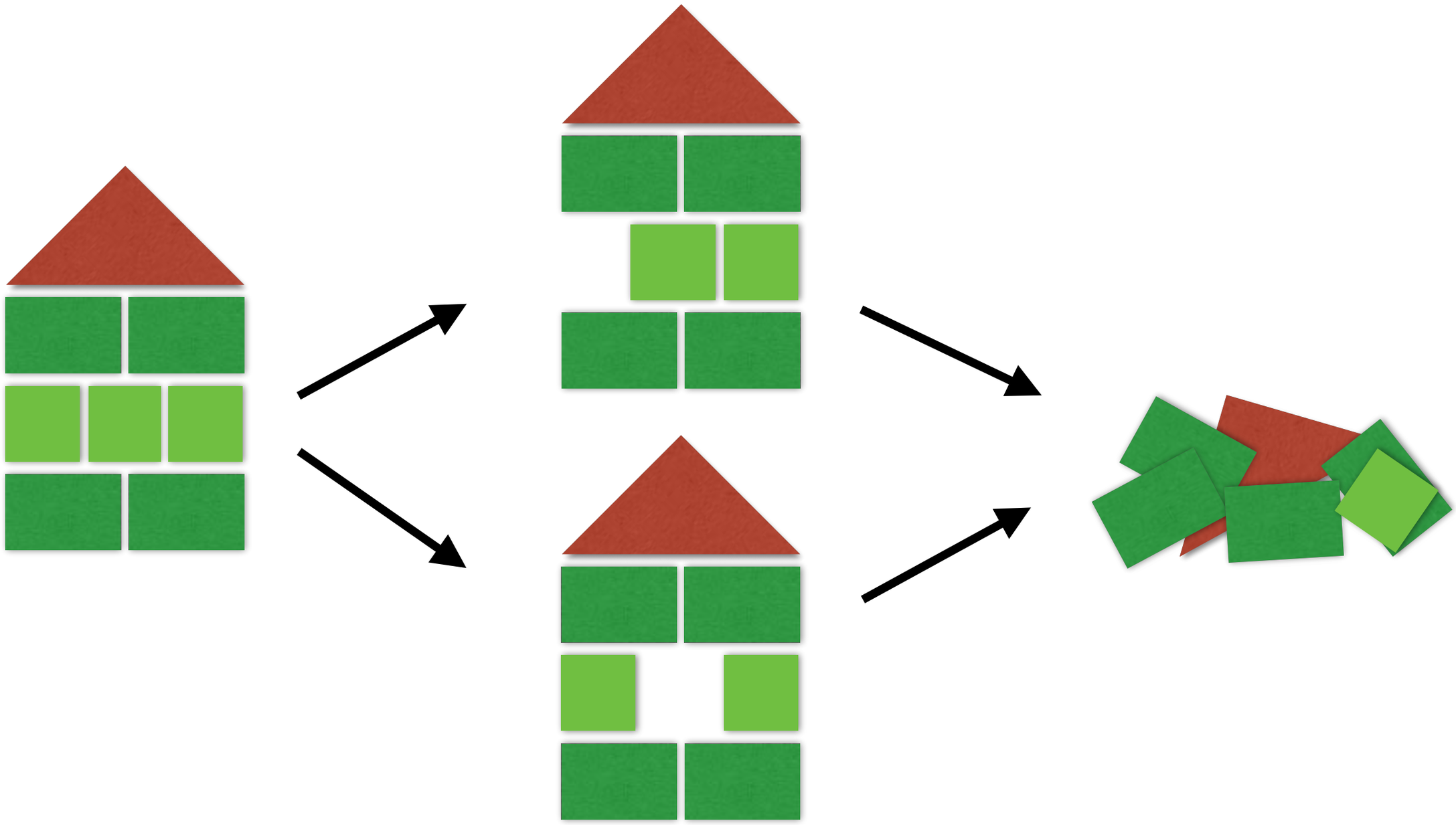


Interaction suppressor: allele specific, gene specific



loss of function

Synthetische Letalität

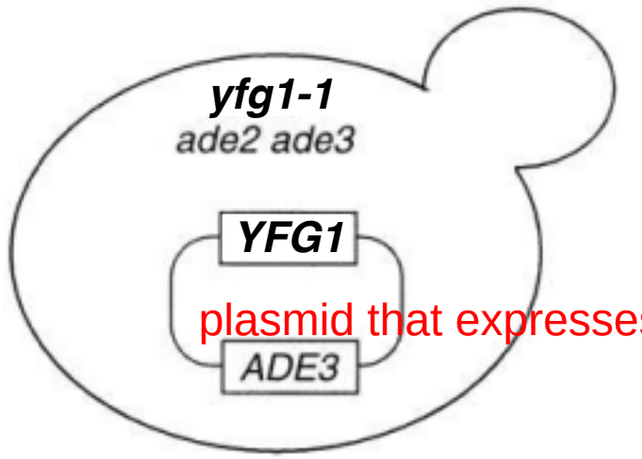


Synthetische Letalität (SL)

ist spezifisch….

Identifizierung von SL Interaktionen

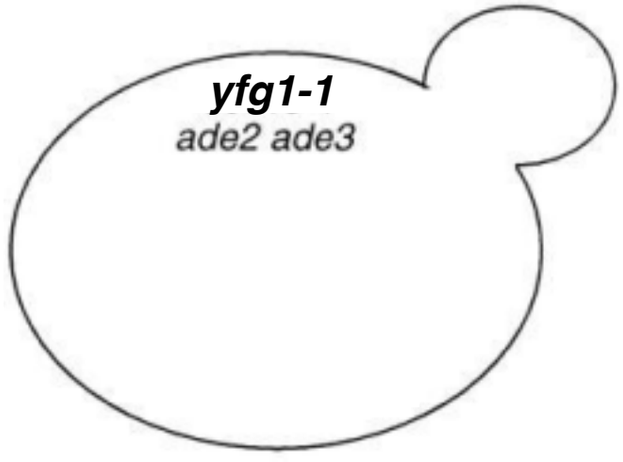
yfg = your favourite gene



Plasmid with wild-type YFG1 with red color marker

plasmid that expresses yfg too and ADE3 is a color molecule (red)

Growth on non-selective medium

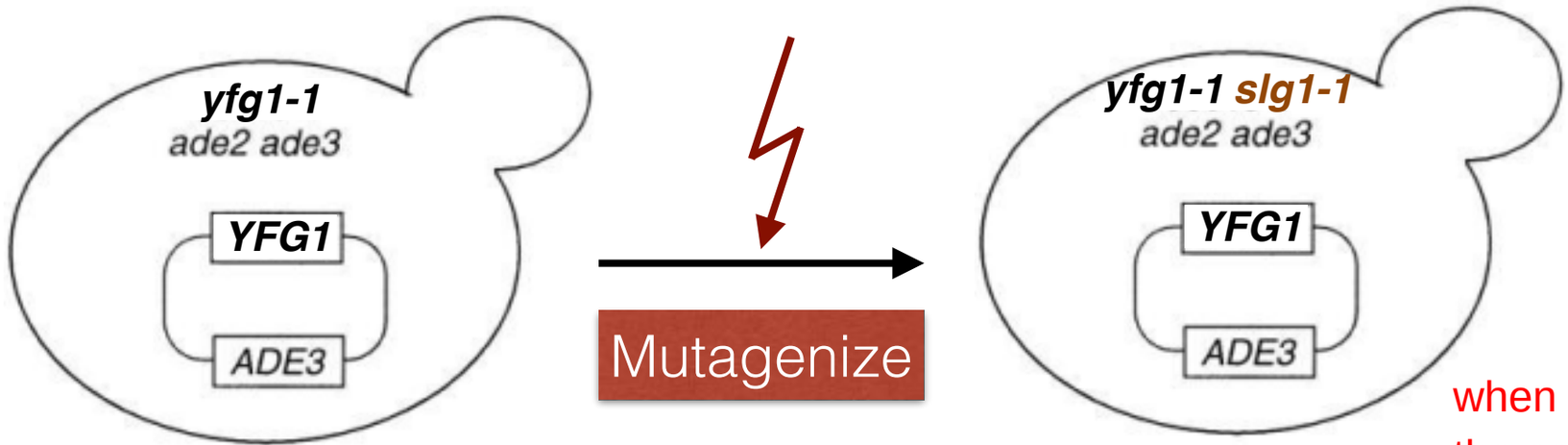


Spontaneous plasmid loss leads to red/white sectoring

the black color is red from ADE3

Identifizierung von SL Interaktionen

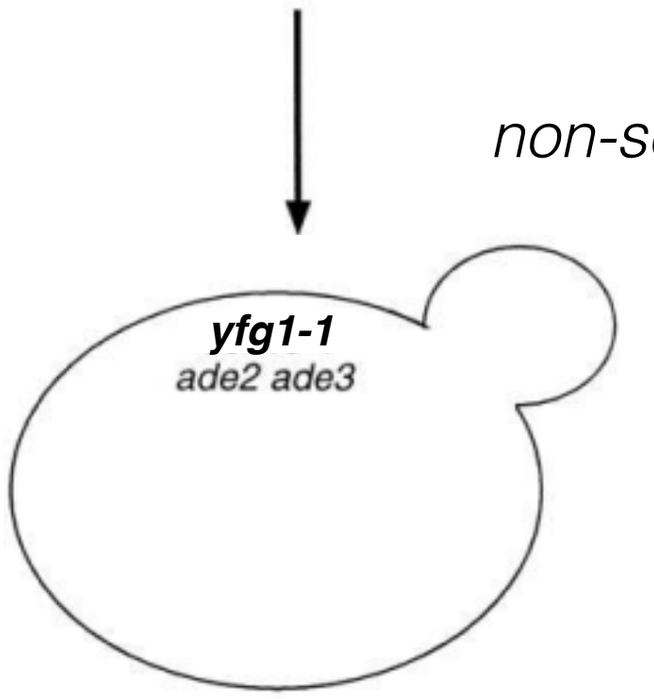
Plasmid with wild-type YFG1 with red color marker



slg is a mutation. both mutations now occur

when plasmid is lost, the cells die. only those who keep the plasmid with the healthy yfg1 survive

Growth on non-selective medium



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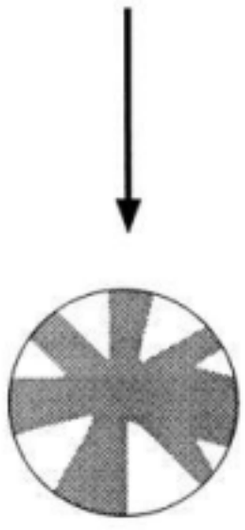

Identifizierung von SL Interaktionen

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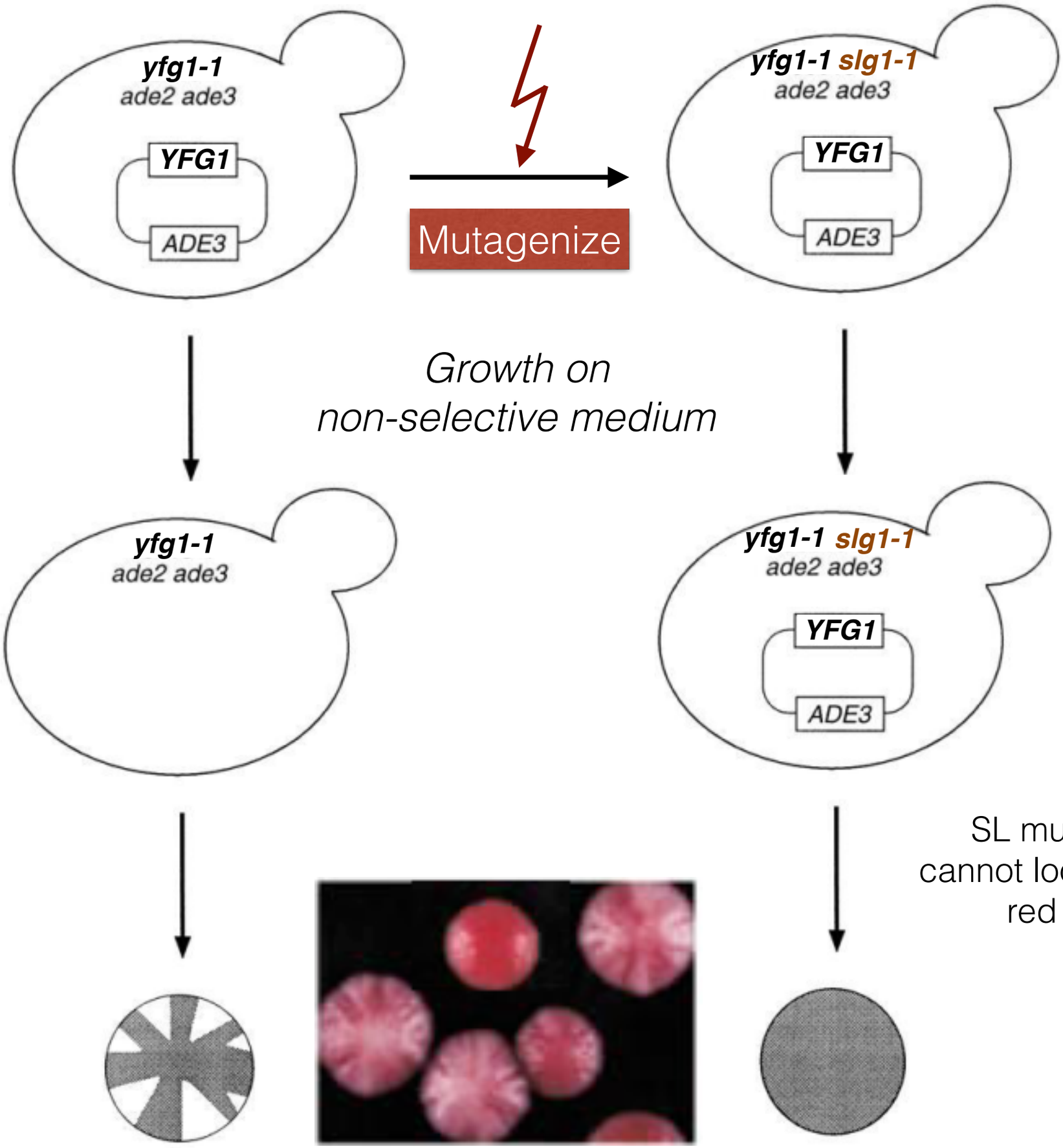
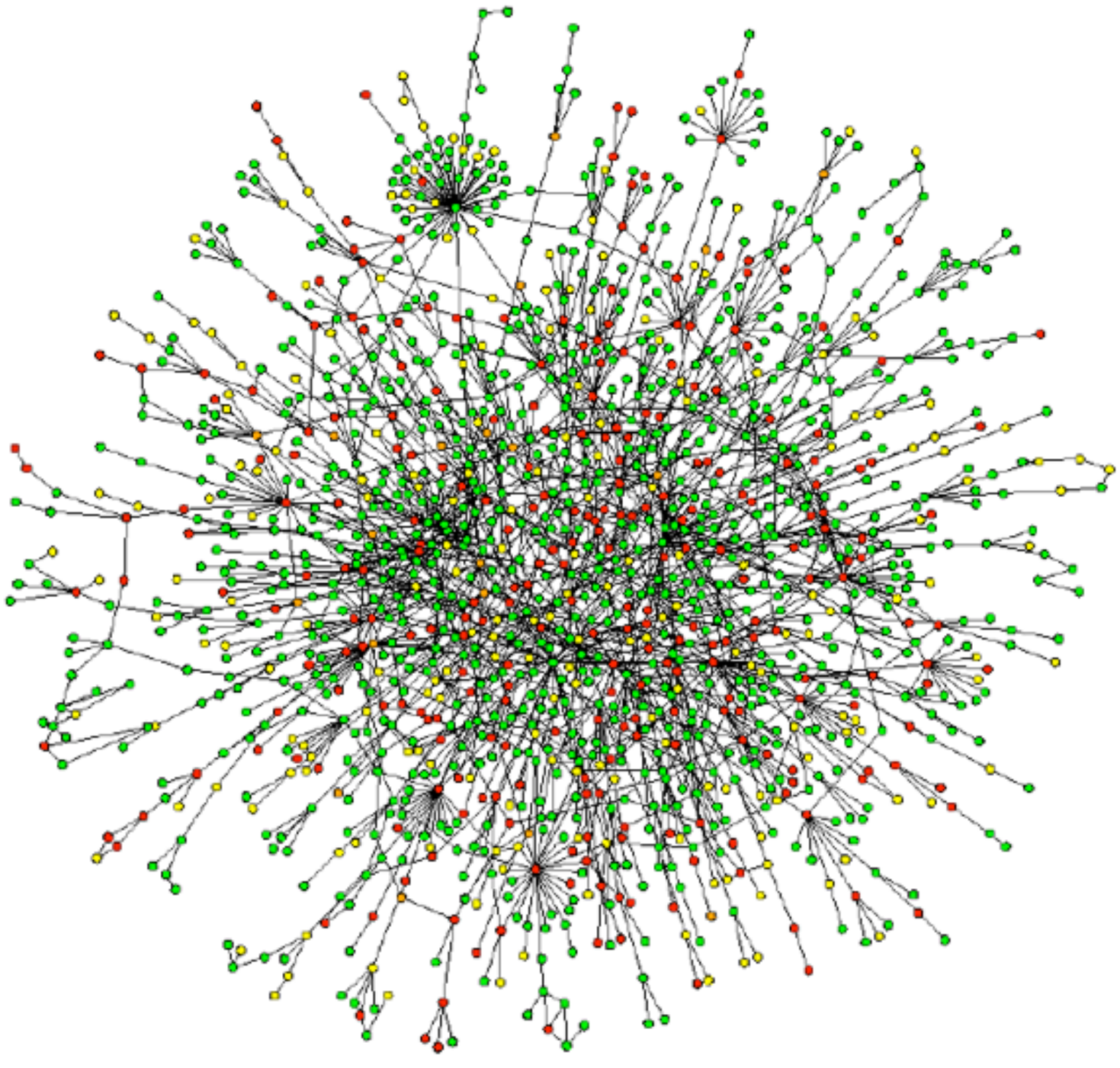


Spontaneous plasmid loss leads to red/white sectoring

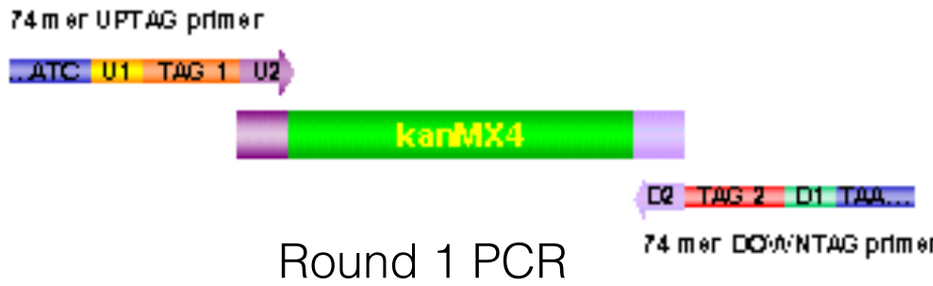
SL mutant cells cannot loose plasmid: red colony

Charakterisierung von genetischen Netzwerken

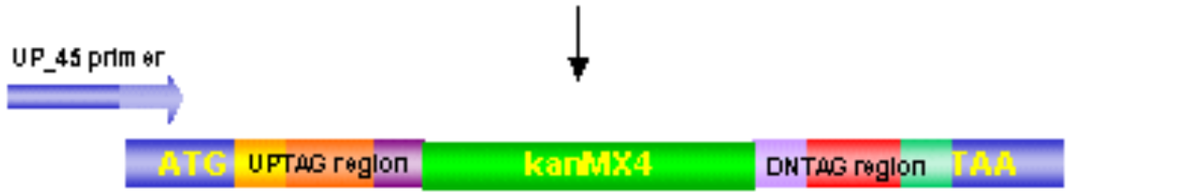


YKO Bibliothek

~4’800 Stämme mit allen nicht essentiellen Deletionen

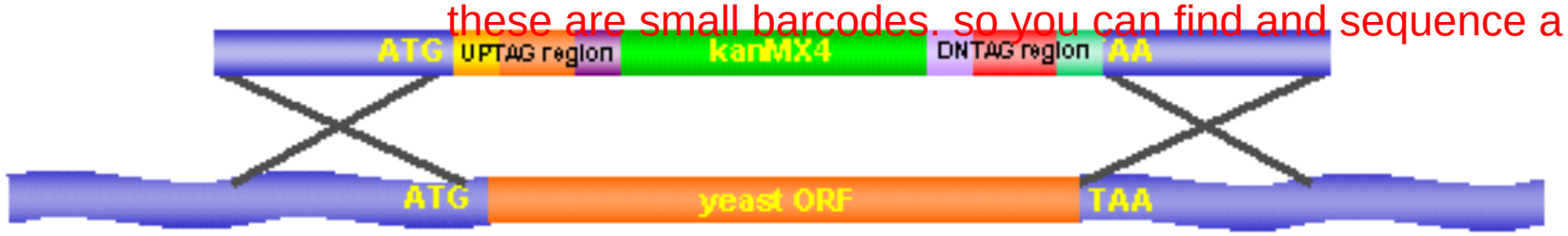


Round 1 PCR



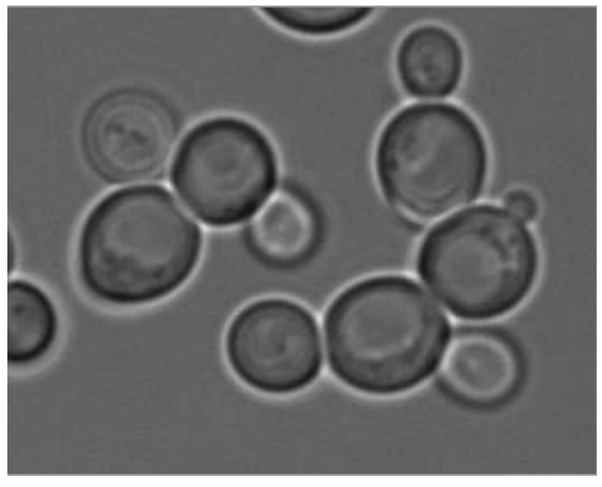
Round 2 PCR

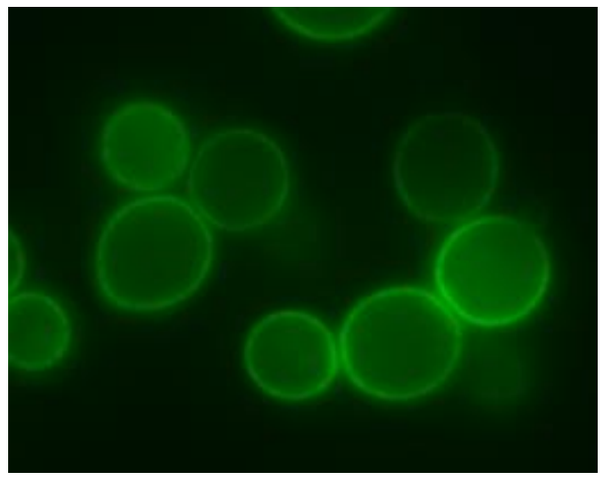
these are small barcodes, so you can find and sequence a gene very very fast



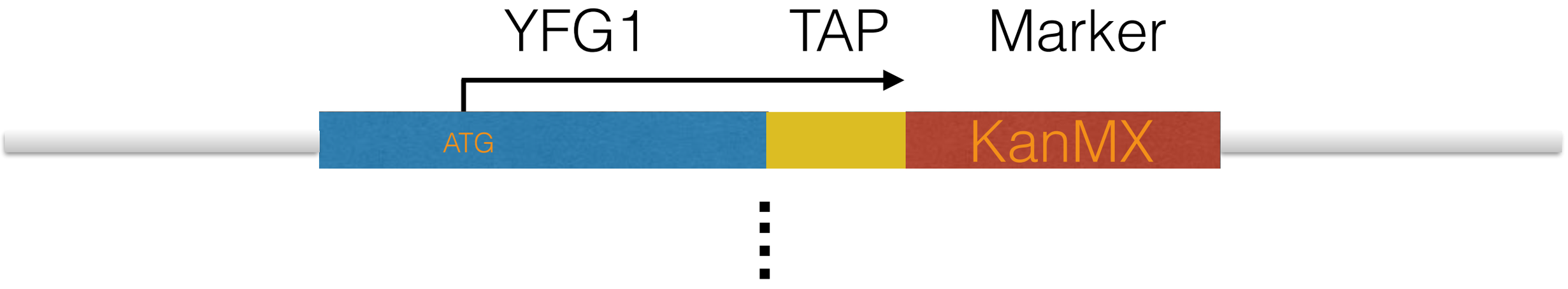
Chromosomal integration by homologous recombination

GFP und Purifikations - Bibliotheken





green fluorescent protein added (GFP). the protein sits on the plasma membrane -> how was it transported there? etc.



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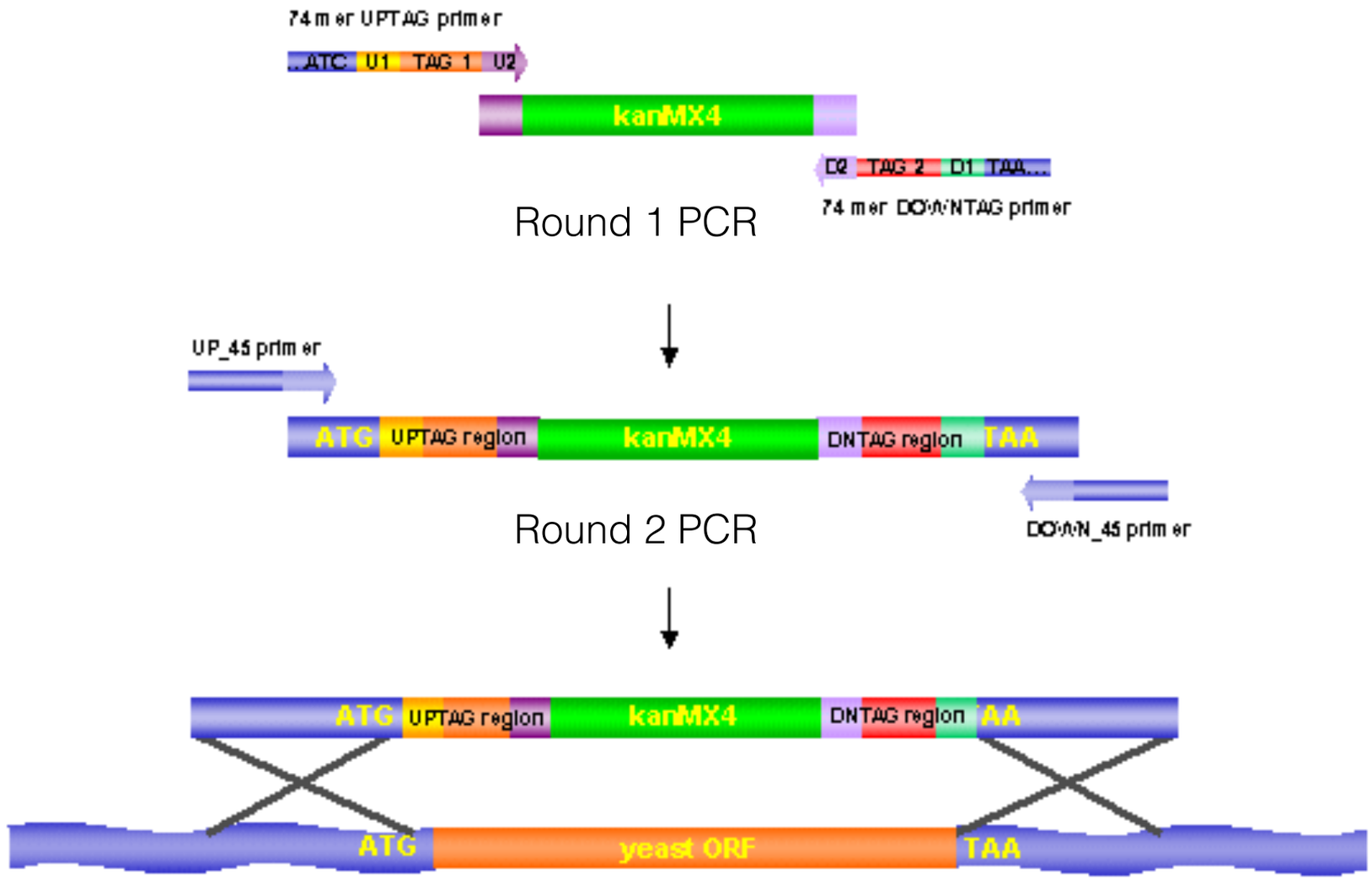


Chromosomal integration by homologous recombination

Charakterisierung von genetischen Netzwerken

Zu untersuchender Stamm

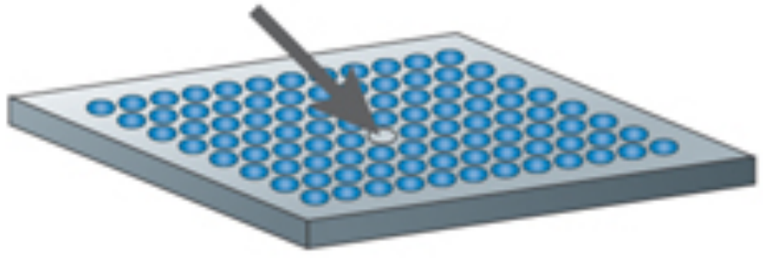
YKO Bibliothek

ausgangsstamm

X

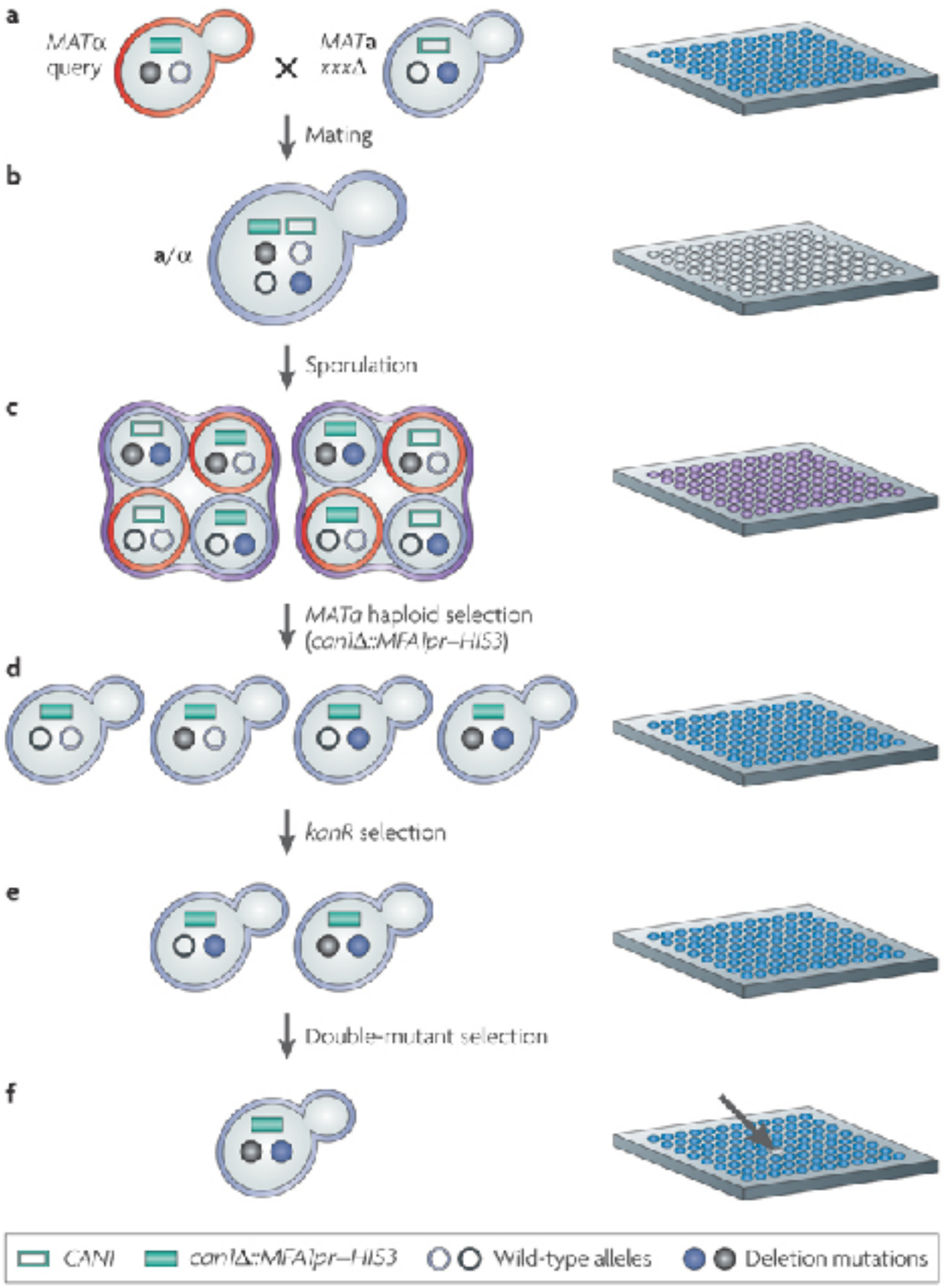
those colors stand for selection markers

Selektion von Doppelmutanten



Charakterisierung von genetischen Netzwerken

not necessary to understand this slide thoroughly



Boone et al, 2007 Nature Reviews | Genetics

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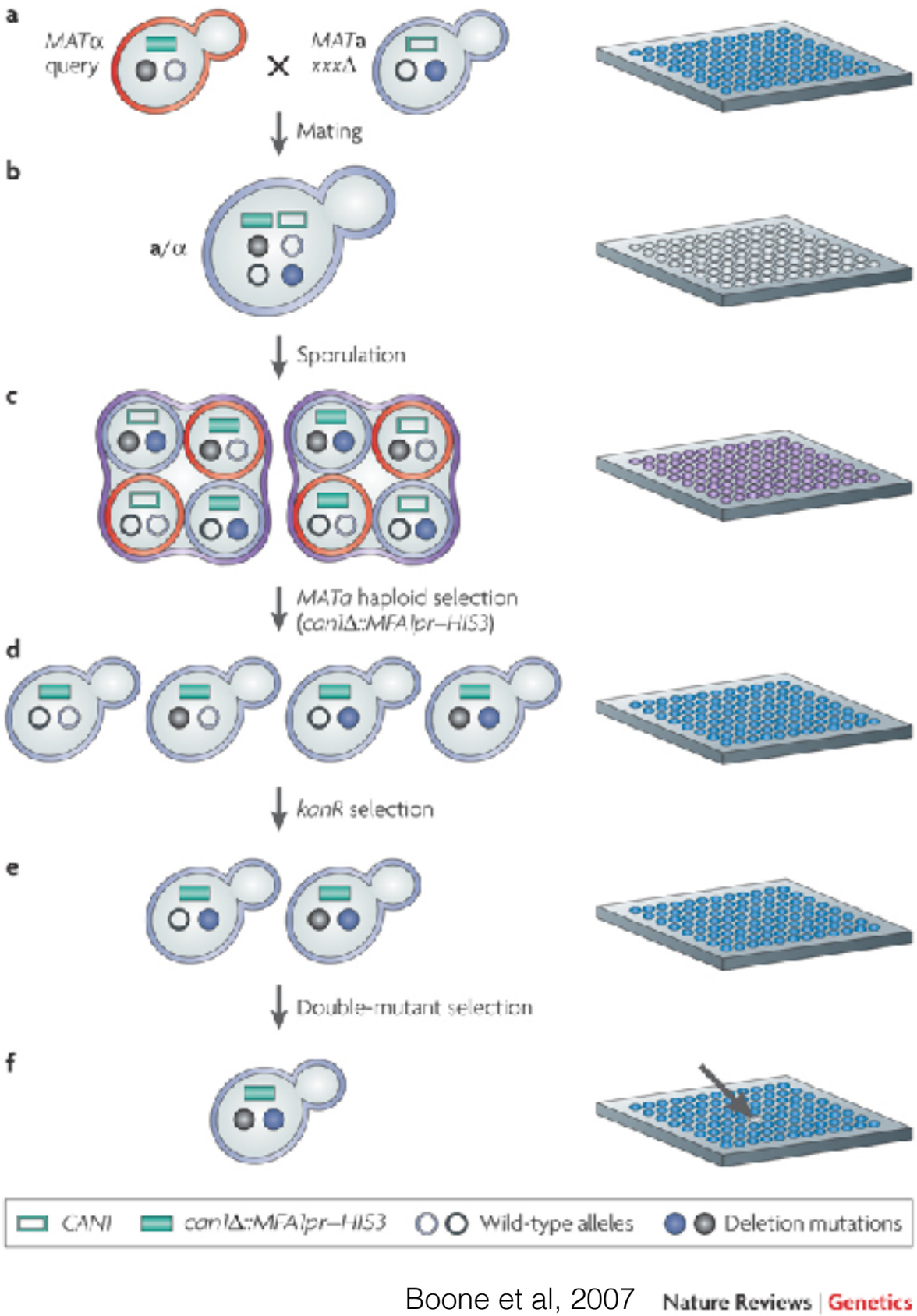

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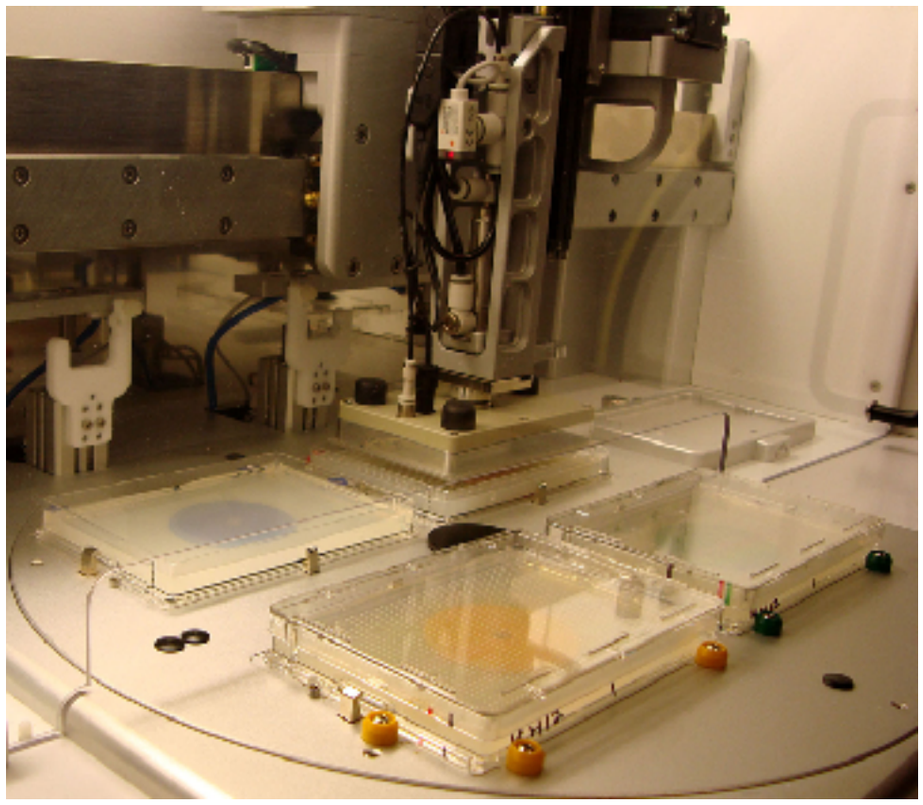
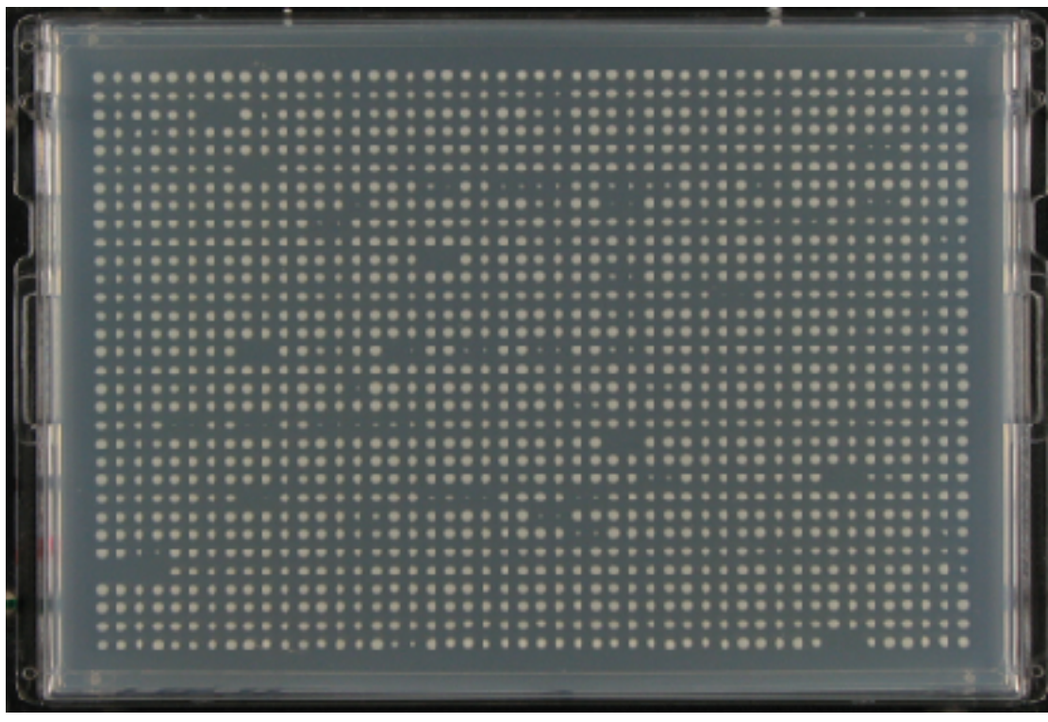
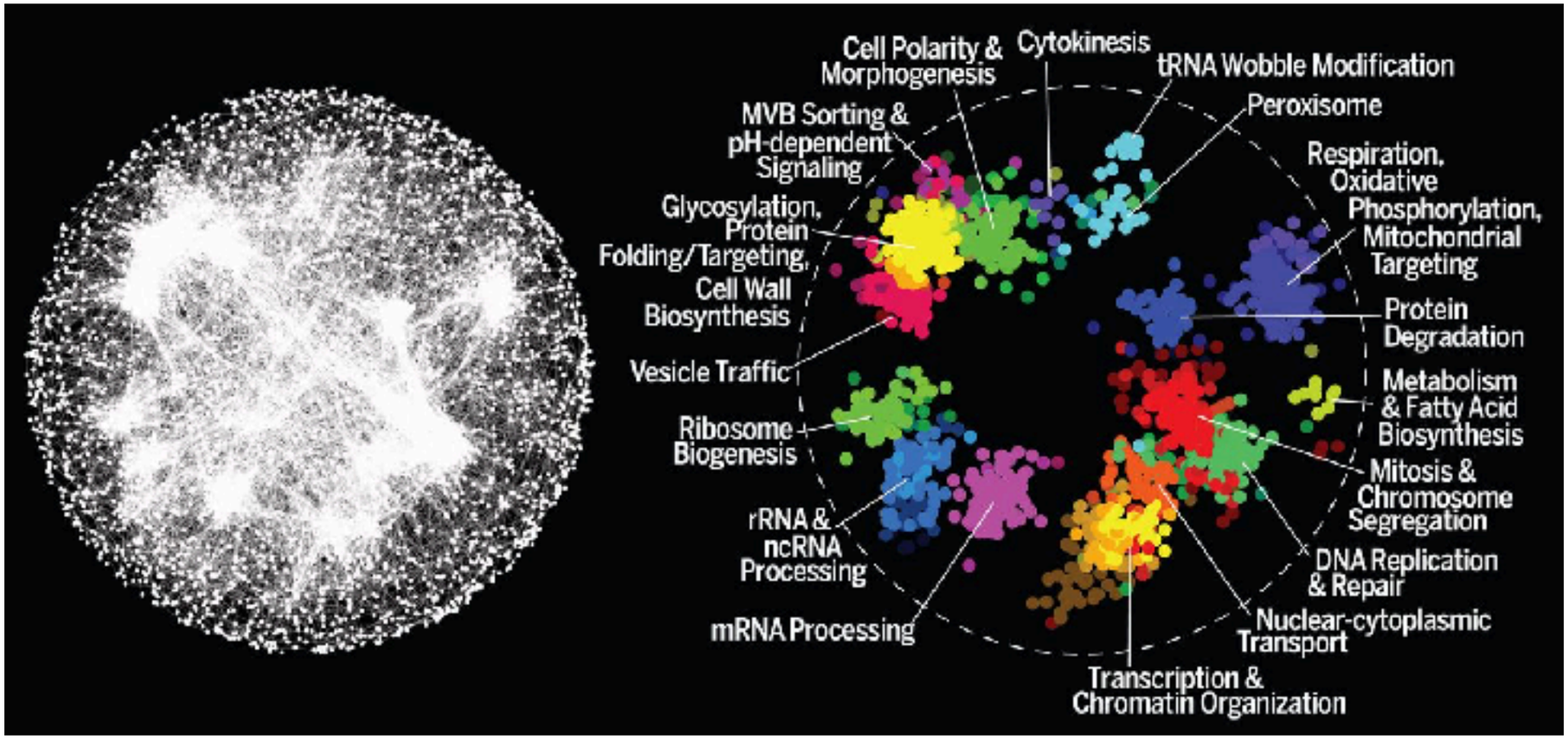




Systematische Analyse von allen Doppelmutanten in *S. cerevisiae*

[~4800 x ~4800 = ~23’000’000]



Charlie Boone’s group Costanzo et al. Science 2016

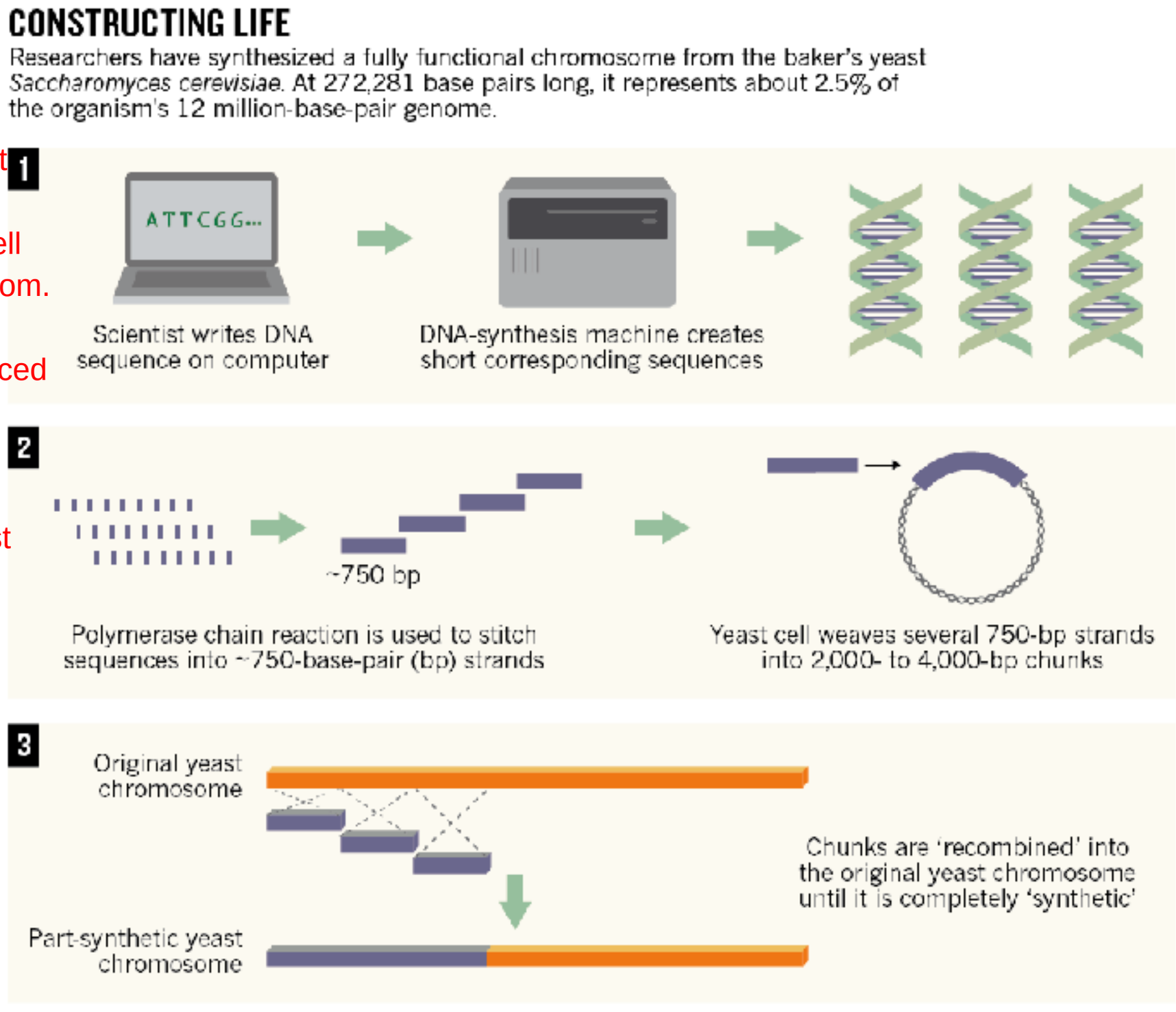
550’000 negative & 350’000 positive Interaktionen

these died (synthetic lethality) two sick cells combined give a healthy well-growing one

In Richtung eines synthetischen Genoms in *S. cerevisiae*

scientist were able to synthesize the smallest chromosome in yeast and add it to a yeast cell in vitro. the original chrom. was removed and the synth. one was introduced to yeast cell.

yeast cell genome was manipulated for the first time.



J. Boeke et al - Synthetisches Chromosome III (Annalura et al., Science 2014)

In Richtung eines synthetischen Genoms in *S. cerevisiae*

- synIII ist etwa 15% kleiner als Chr III
- hat TAG/TAA stop-codon Austausch
- Deletion der subtelomeren Regionen, Introne, transfer RNAs, Transposons
- Einführung von Rekombinations-Sequenzen (loxPsym) erlaubt Neuorganisation des Genoms.

J. Boeke et al - Synthetisches Chromosome III (Annalura et al., Science 2014)