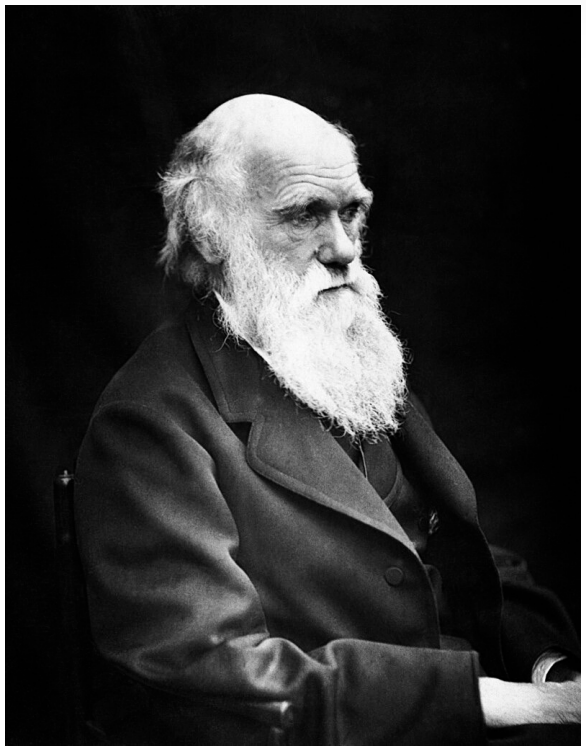
Model systems in biology

Why do we need models in biology?

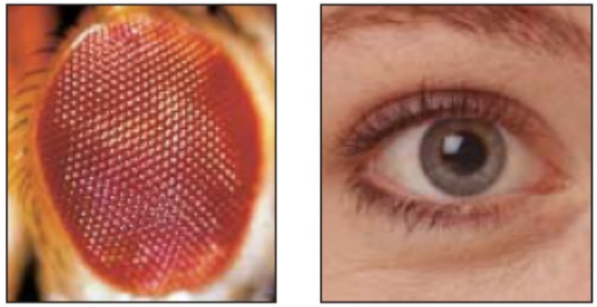
- We can’t perform many experiments in **humans**!
- Also if we could, it would be **too complex**
- We need **NUMBERS** and **REPLICATES**!

The Reductionist approach

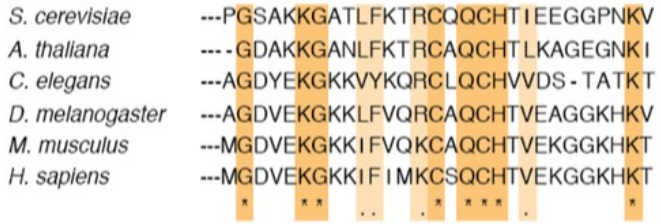
KEY CONCEPT N° 1: EVOLUTION



Charles Darwin



Insect and human eyes have a common origin



An example of protein sequence conservation accross species

- **Basic biological processes** are **shared** accross organisms also if evolutionary really distant
- According to **Charles Darwin's evolution theory**, life has a common origin and many key processes like **metabolism**, **cell cycle** and **embryonic development** are conserved accross species

What is a model system?

Thanks to **evolutionary conservation**, we can study complex human (and non human) processes in 'simpler' organism

Things we can do in model systems:

- **Genetic dissection** (e.g direct and reverse genetics, genetic screenings)
- **Biochemistry** (e.g. interactome studies)
- **Genetic manipulation** (e.g. genome editing)
- **Development** (e.g. in vivo)

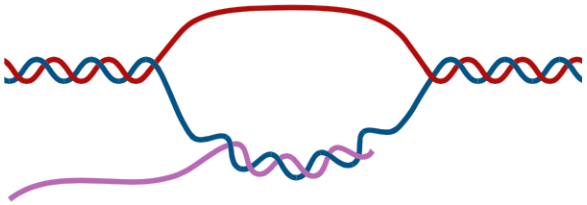
ID card of a model system

a. Cheap
b. Small
c. Easy to handle
d. Safe
e. Fast development and life cycle

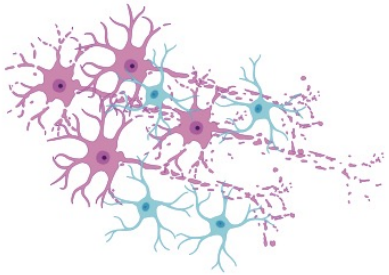
KEY CONCEPT N° 2: BIOLOGICAL QUESTION

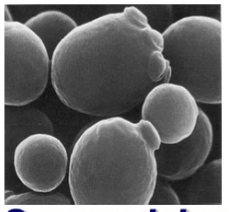
- There is no universal model system
- Each model system is fit to answer a specific BIOLOGICAL QUESTION

Transcription



Neurodegeneration





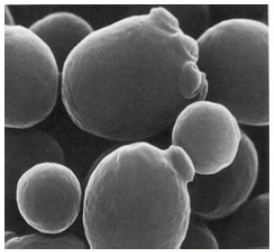
S. cerevisiae

Cell culture

Mus musculus

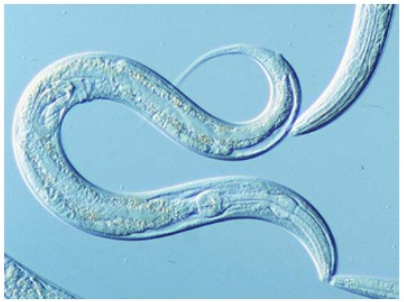
Only ask your model system the answers it is fit to give you!

The most common model systems in biology



S. cerevisiae

D. melanogaster



C. elegans

Cell culture

Zebrafish

Mus musculus

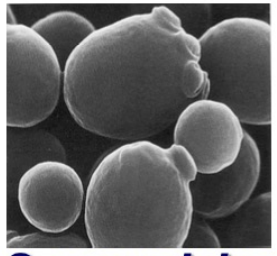
A. thaliana

The most common model systems in biology

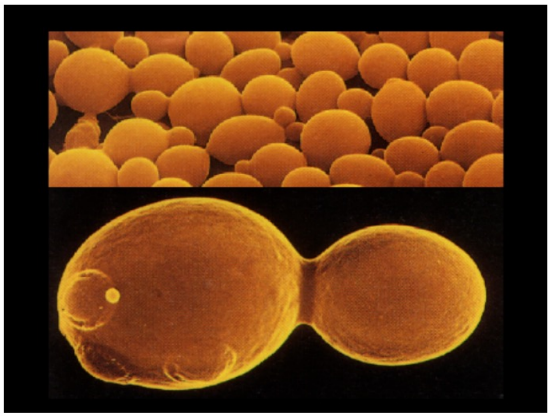
Organism:	Genome	Coding Genes
E. coli	4.6 Mb	4,288
S. cerevisiae	13.5 Mb	6,034
D. melanogaster	165 Mb	12,000
C. elegans	97 Mb	19,099
H. Sapiens	3300 Mb	22,000

“Yeast as a model system for eukaryotic functions”

Yeast



Saccharomyces cerevisiae (Budding yeast)



- Genomic DNA 12.052 kb
- 16 chromosomes
- 6000 genes (3.5x a bacteria), one gene every ~2 kb, ~50-fold higher than the gene density in the human genome
- Most of genes are present as a songle copy
- Only 3.8% contains introns

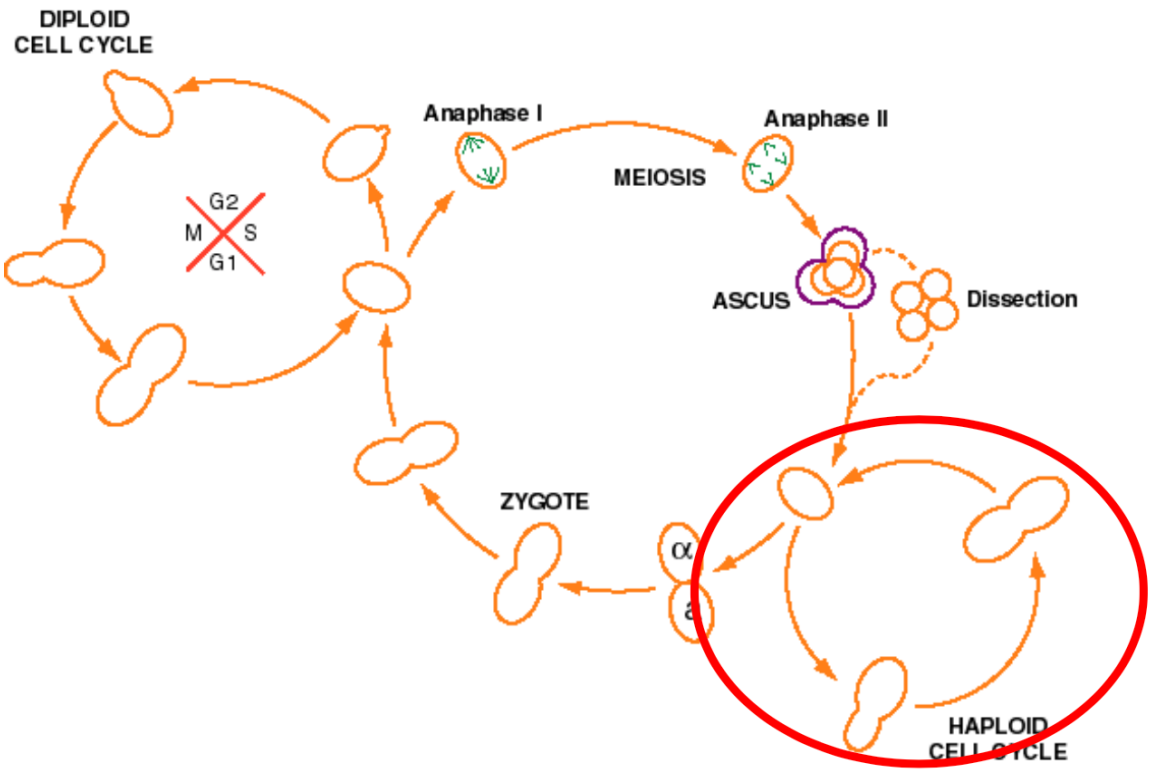
ADVANTAGES

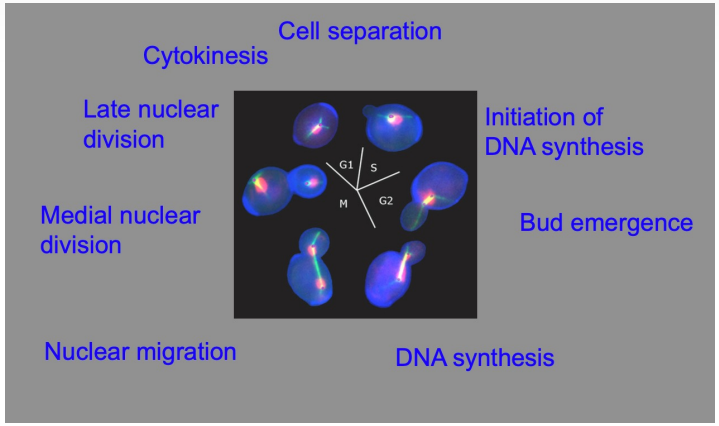
- Rapid growht (90' cell cycle)
- Easy to transform with plasmids
- Both aploid and diploid form
- Genic integration with homologous recombination is efficient
- CHEAP

DISADVANTAGES

- No RNAi machinery
- No splicing

The cell cycle



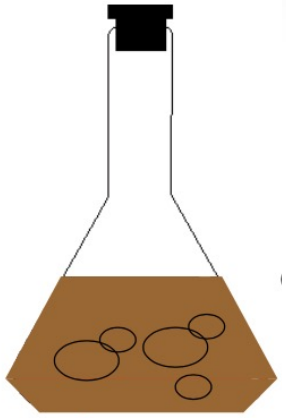


S. cerevisiae cells in nature switch readily between two mating types: haploid **a** cells mate with haploid α cells to form diploids. Under nutrient-poor conditions, diploids can be induced to undergo meiosis and sporulation, forming four haploid spores, two of each mating type

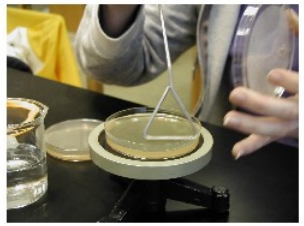
Yeast cells divide as rapidly as once every 90 min under optimal laboratory conditions, through a process of budding in which smaller daughter cells pinch, or bud, off the mother cell. The common name “budding yeast” derives from this notable feature of cell division and distinguishes *S. cerevisiae* from the fission yeast, *Schizosaccharomyces pombe*, also a powerful model organism.

What is it like to work with yeast?

T=30°C



Flask



Agar plates

- Yeast strains are grown in complete medium (YP+sugar) or synthetic minimun medium (SD+sugar), liquid or solid (agar plate)
- Growth is evaluated by OD600 (optical density at 600nm)

Prototrophy v.s. auxotrophy

- An organism that is able to synthesize all the essential molecules for its life is AUTOTROPH
- A wild-type yeast only need sugar and vitamins in the medium. Autotrophy for single molecules is defined **PROTOTROPHY** for that molecule
- A yeast that carries a mutation in an enzyme that produces an essential molecule is an **AUXOTROPH** for that molecule
- An auxotrophic yeast for a certain molecule only grows if it can absord it from the medium
- **Laboratory yeast strains are ALWAYS AUXOTROPHIC FOR ESSENTIAL MOLECULES** (like aa or nucleotides)

In the genotype of a strain you can read the **mating type** (a or alpha) and the **auxotrophies**

ADE2, URA3, trp1, his3 : prototrophic strain for adenine and uracyl and auxotrophic strain for tryptophan and histidine

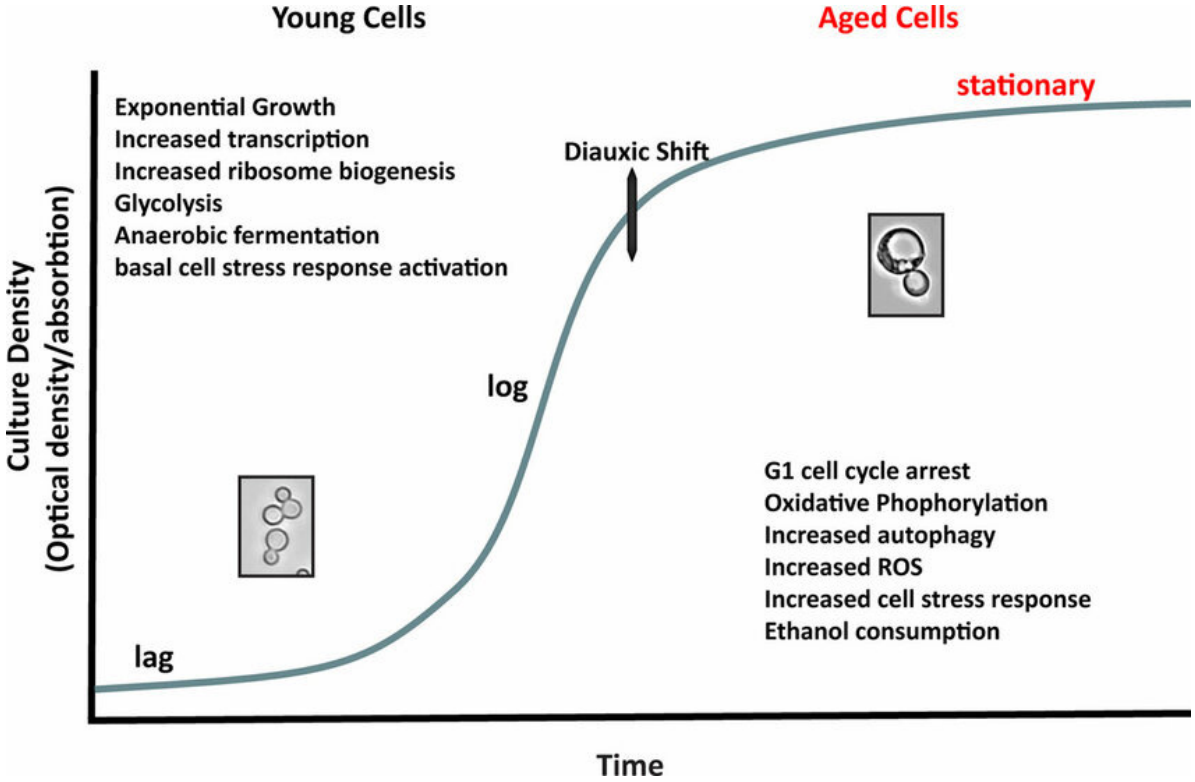
Nomenclature

Ex. Genotype : Mat-a, trp.1.Δ, his3.Δ, ura3.52, lys2.801,ade2.101

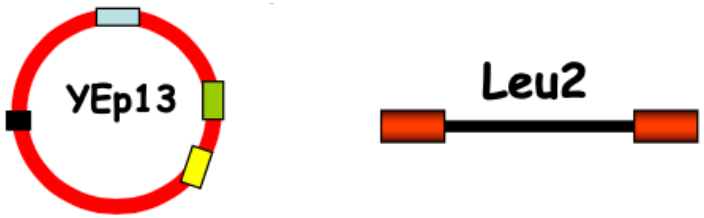
Gene symbol	Definition
ARG2	A locus or dominant allele
arg2	A locus or recessive allele confering an arginine requirement
ARG2+	The wild-type allele
arg2-9	A specific allele or mutation
Arg+	A strain not requiring arginine
Arg-	A strain requiring arginine
Arg2p	The protein encoded by *ARG2*
ARG2 mRNA	The mRNA transcribed from *ARG2*
arg2-Δ1	A specific complete or partial deletion of *ARG2*
ARG2::LEU2	Insertion of the functional *LEU2* gene at the *ARG2* locus, and *ARG2* remains functional and dominant
cyc1-arg2	A fusion between the *CYC1* and *ARG2* genes, where both are nonfunctional
P*CYC1-ARG2*	A fusion between the *CYC1* promoter and *ARG2*, where the *ARG2* gene is functional

Growth in the Laboratory

The **yeast culture** must be maintained in the **exponential phase** (0.1<O.D.600<1.5) to test the effect of the **mutation**or **treatment** on **cell proliferation**.



Yeast transformation

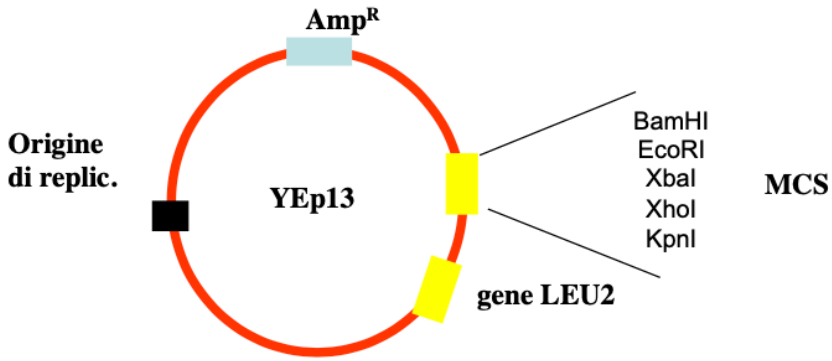


Yeast can be easily transformed with DNA (plasmids and PCR fragments).

- Yeast vectors usually contain regions that allow their replication and genes for the production of aa or nucleotides tha complement the auxotrophies of the laboratory strains
- **These genes function as selectable markers for transformants**

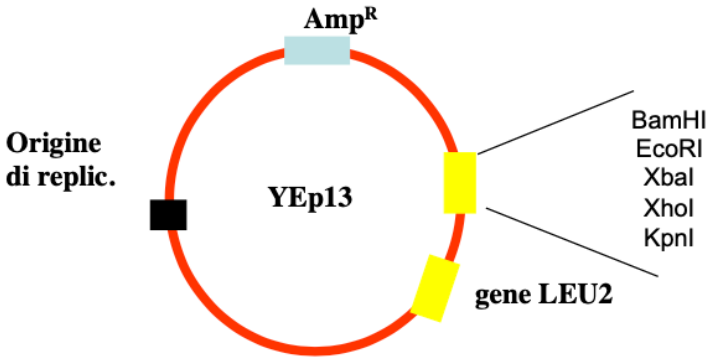
Yeast Vectors

They are defined as **"shuttle vectors"** because they are able to **replicate in both *E. coli* and yeast.**
Yeast vectors always contain:



- Origin of replication
- **Drug resistance gene** for growth in bacteria
- **Polylinker** for cloning
- genes for the production of aa or nucleotides tha complement the auxotrophies of the laboratory strains.**These genes function as selectable markers for transformants**

Yeast Vectors



The selection is based on an enzyme involved in leucine synthesis. LEU2 codes for β-isopropylmalate dehydrogenase, one of the enzymes required for the conversion of pyruvic acid to leucine.

- **To use a plasmid that contains LEU2 you need a leu2 auxotrophic strain!**

Yeast Vectors

The **marker gene** codes for an enzyme involved in the synthesis of an amino acid.

Gene	Enzyme	Selection Compound
HIS3	Imidazole glycerol-phosphate dehydrogenase	Histidine
LEU2	β-Isopropylmalate dehydrogenase	Leucine
LYS2	α-Aminoadipate reductase	Lysine
TRP1	N-(5'-phosphoribosyl)-anthranilate isomerase	Tryptophan
URA3	Orotidine-5'-phosphate decarboxylase	Uracil (a nucleotide base)

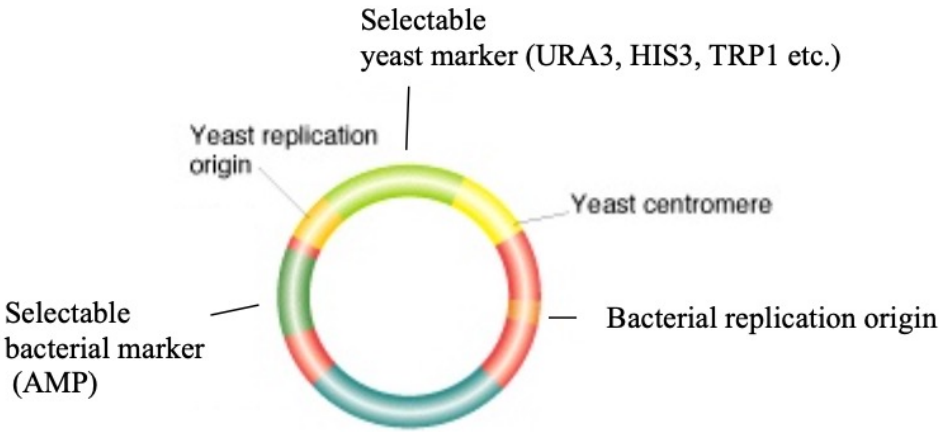
•**The yeast strains used possess one or more mutated marker genes:**

Mat a, his3−Δ, leu2−101, lys2−801, trp.1−Δ, ura3−52

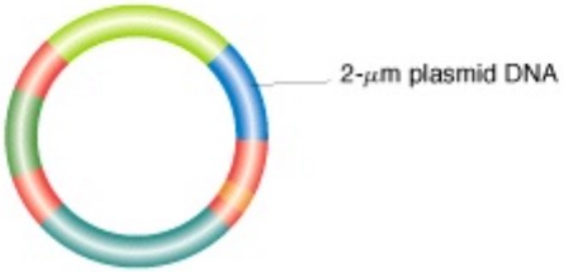
•**It will therefore be possible to select the transformed cells using a minimal growth medium that does not contain the amino acid used as a marker gene:**

- •Yeast Nitrogen Base (YNB) without amino acids
- •2% sugar
- •Amino acids for the auxotrophies, **except for the one corresponding to the marker gene**.

Yeast vectors



CENTROMERIC VECTOR
Centromere CEN and ARS sequence, behave like stable minichromosomes ½ copies per cell



2uM VECTOR
1-10 copies per cell

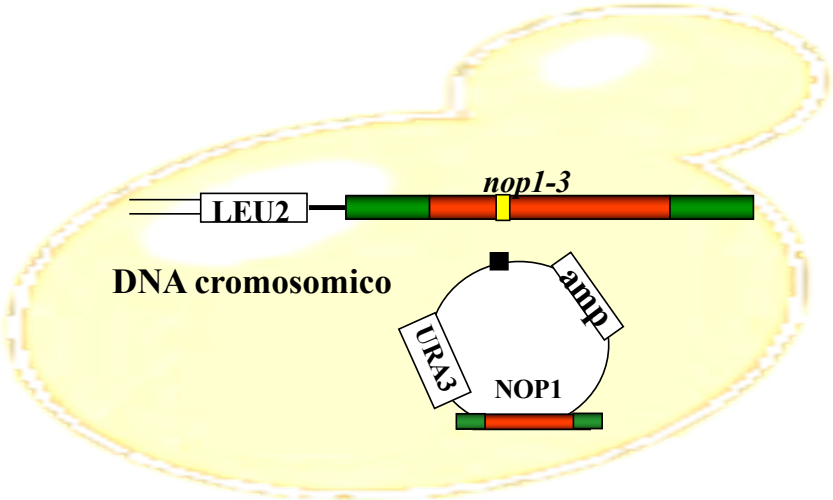
Marker genes for genetic studies

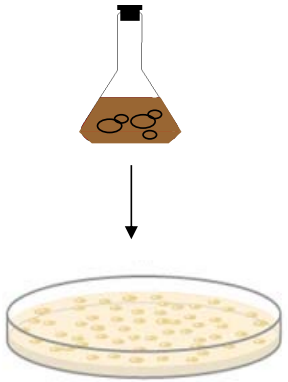
URA3

It allows for obtaining both a positive selection and a negative selection of the vector that contains it:

•Positive selection is obtained by complementing the auxotrophy of the strain used.

•Negative selection (favoring the cells that lose the vector) is obtained by adding 5-fluoro-orotic acid (5-FOA) to the culture medium.





Terreno minimo: YNB,zucchero, DO-Leu, FOA

How can we study gene function in yeast?

Forward genetics (classic)

Creating random mutations in an organism's DNA which result in visible changes in the phenotype. The mutation and its associated phenotype are identified, and gene locus on the chromosome is mapped.

Reverse genetics (modern)

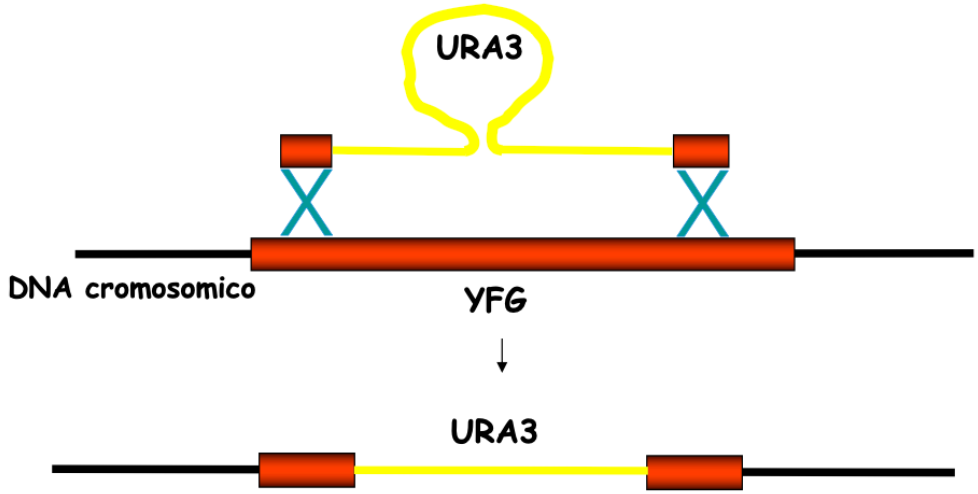
Disruption of known genes, followed by screening for mutant phenotypes resulting from these manipulations. Requires the sequence of the genome

In 1996, the *S. cerevisiae* genome became the first fully sequenced eukaryotic genome

YFG= your favourite gene

Integration of a linear fragment

- Integration occurs via homologous recombination.
- Integration causes the disruption of the target gene.
- The recombined loci are called "disrupted" (or disrupted loci).
- The integrations are stably propagated; "pop-out" never occurs.



YFG= your favourite gene

Structural Inactivation of Genes or Gene disruption in yeast

Study of gene function through inactivation by homologous recombination (gene disruption).

The integration is by **homologous recombination**

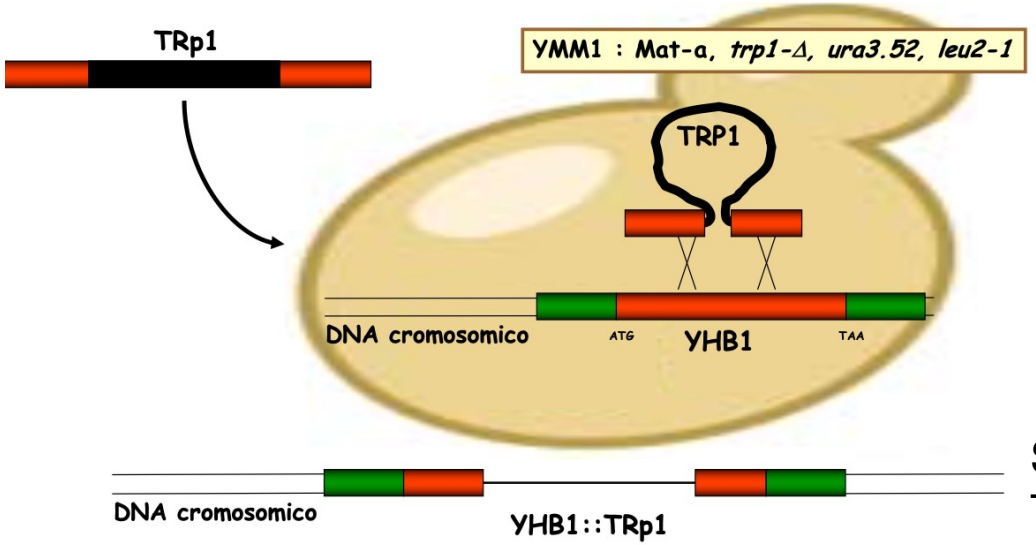
- The integration produces the disruption of the target gene
- The integration is stable, ‘pop-out’ never happen

PCR fragment that contains

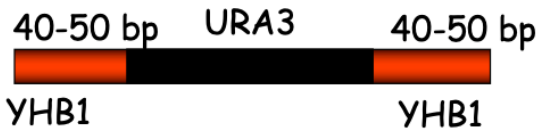
- Marker gene
- Homology arms for the yeast genome

Gene disruption in yeast

EX.: We want to disrupt the non-essential gene YHB1

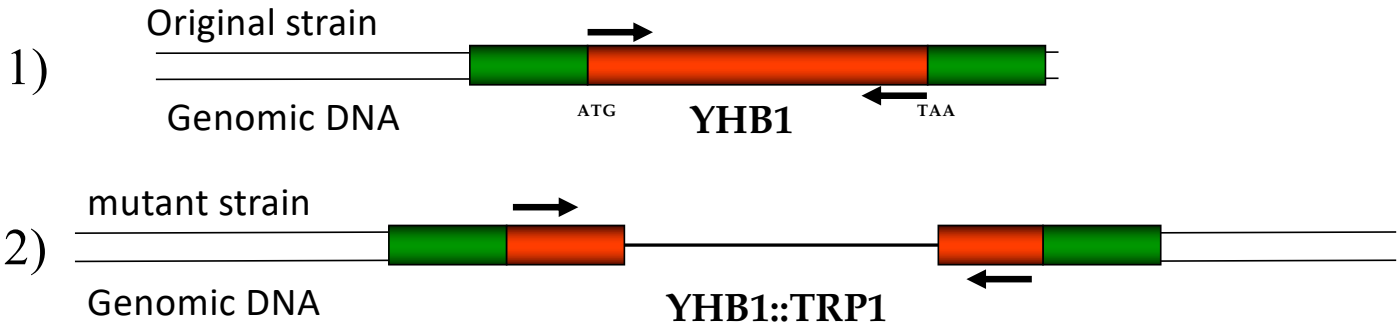


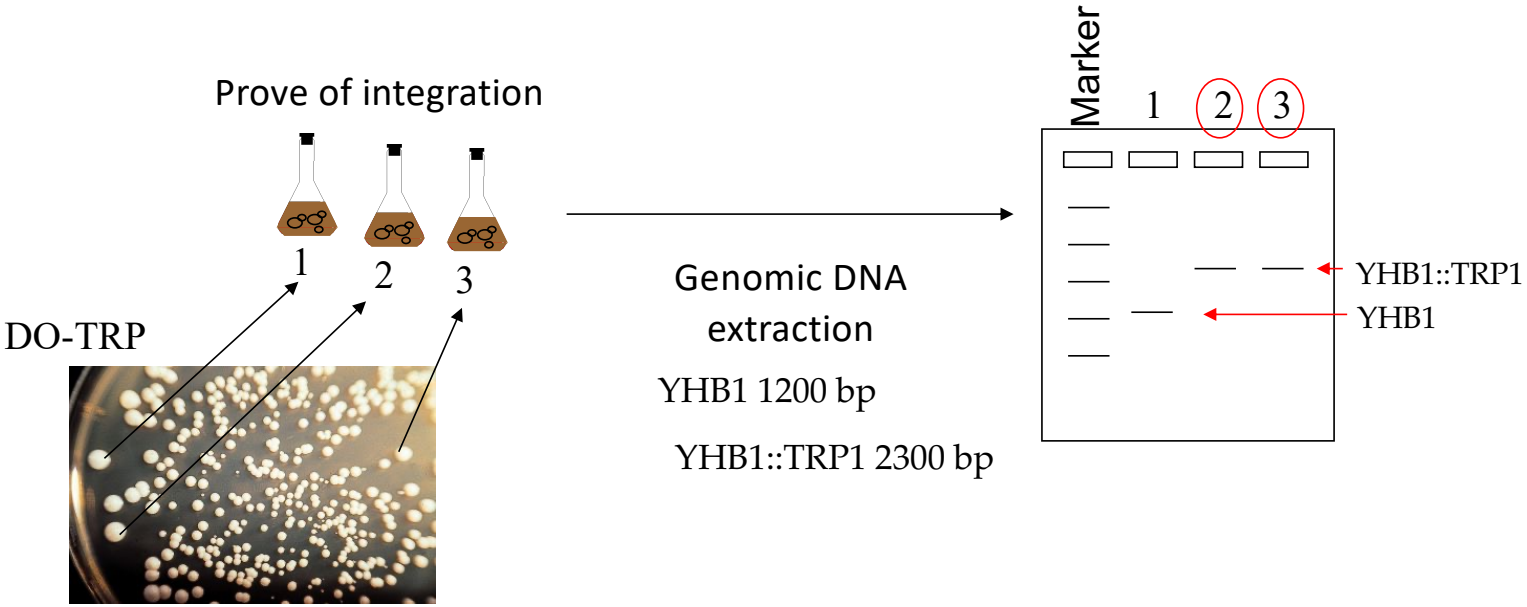
Selection of Transformants: Minimal medium with DO-TRP



We have constructed a new yeast strain whose genotype will be:

YBM2 : Mat-a, *trp1-Δ, ura3.52, leu2-1, YHB1::TRP1*


1)
Original strain
Genomic DNA
ATG
YHB1
TAA
2)
mutant strain
Genomic DNA
YHB1::TRP1


Prove of integration
1
2
3
DO-TRP
Genomic DNA
extraction
YHB1 1200 bp
YHB1::TRP1 2300 bp
Marker
1
2
3
YHB1::TRP1
YHB1

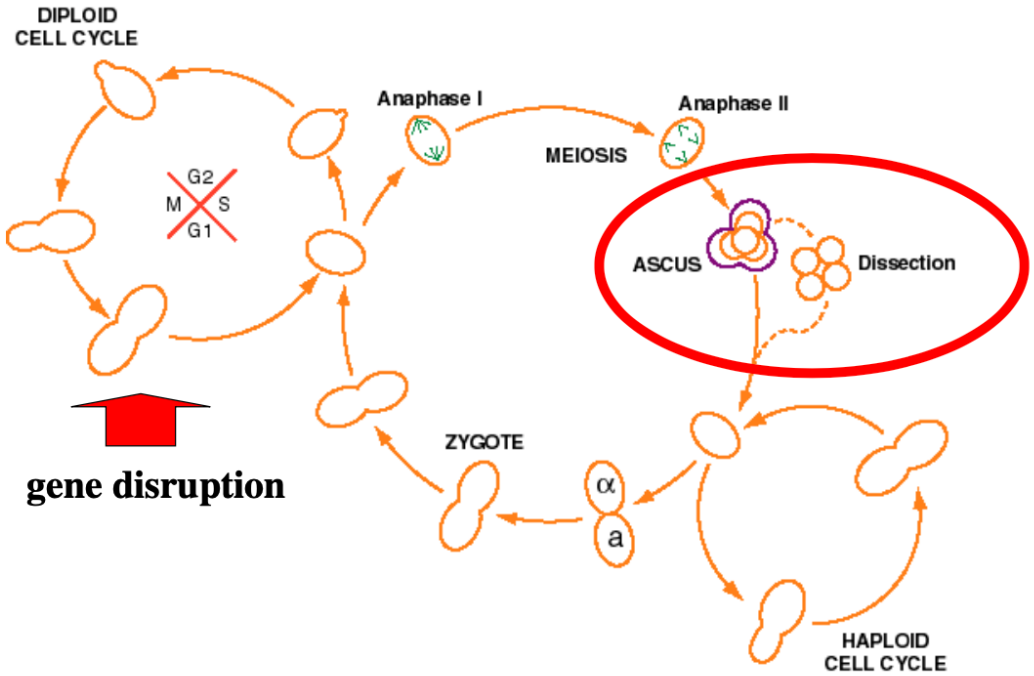
Gene disruption in yeast

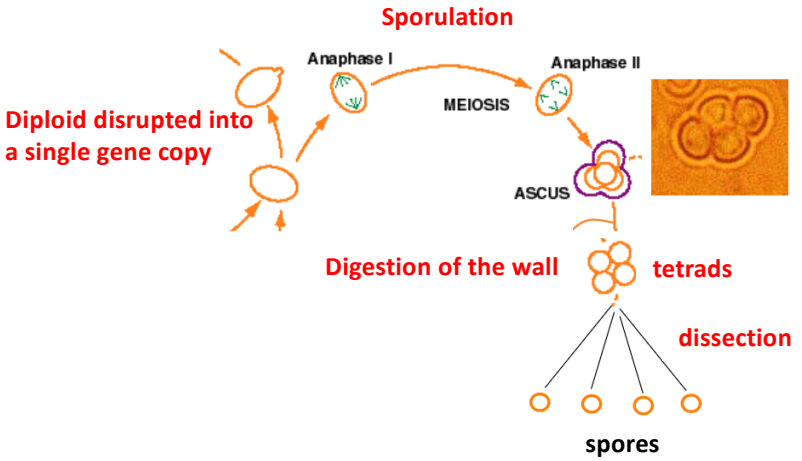
All the *S.cerevisiae* ORFs were disrupted to determine its essentiality for cell survival
(www.yeastgenome.org)

- 6183 ORFs, of which 5700 encode proteins
- 1100 genes are essential for cell growth

How can we handle essential genes?
(they cause lethality)

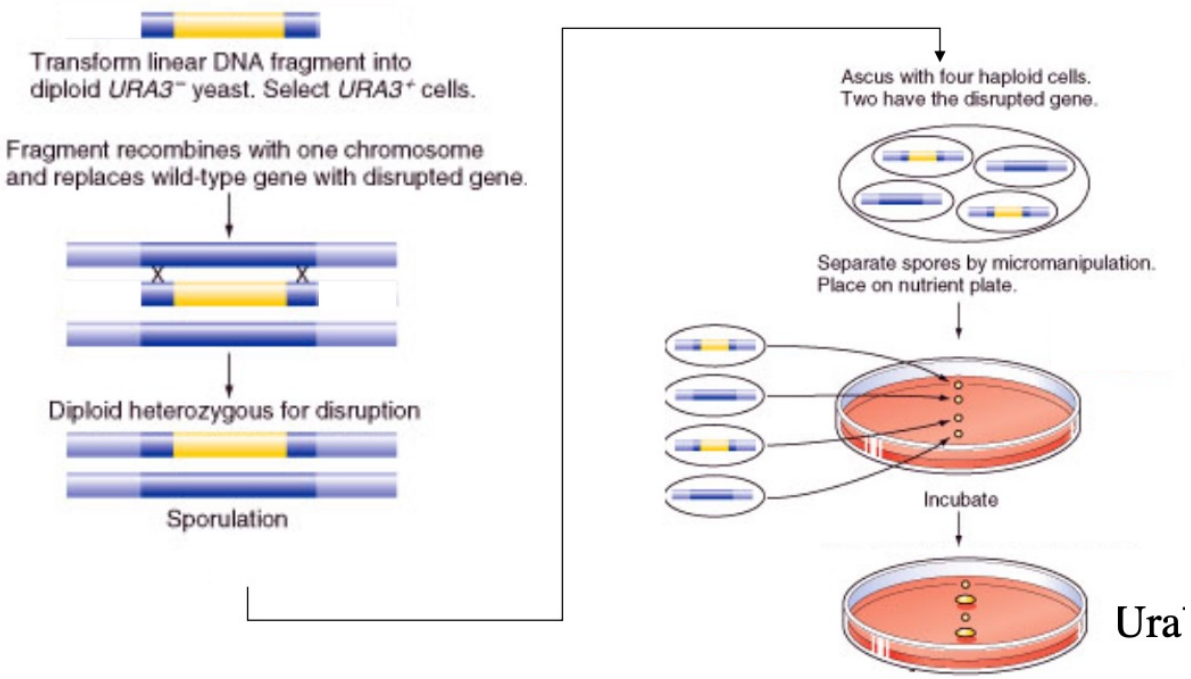
Tetrad analysis





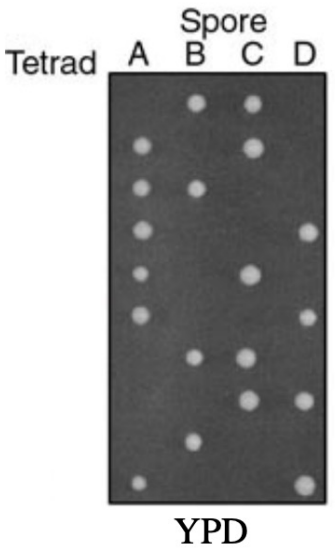
We need **DIPLOID strains** that undergo sporulation (**meiosis**) and produce 4 aploid spores

Is a gene essential?

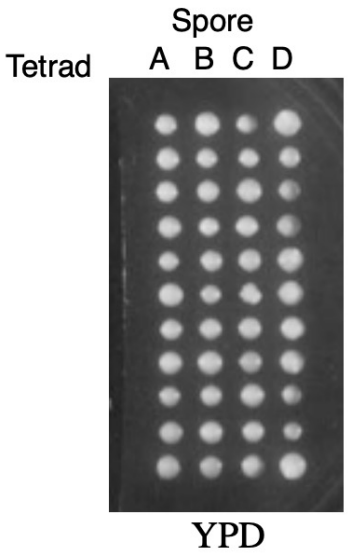


Non essential gene

Essential gene



Non essential gene



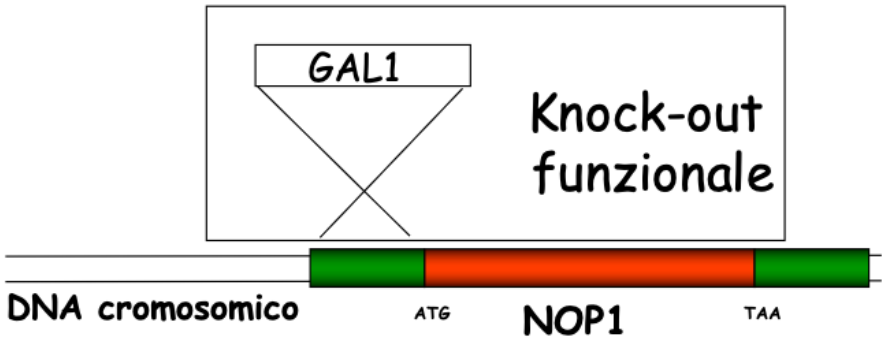
Functional inactivation of genes

If a gene is essential we need to produce a **conditional mutant**:

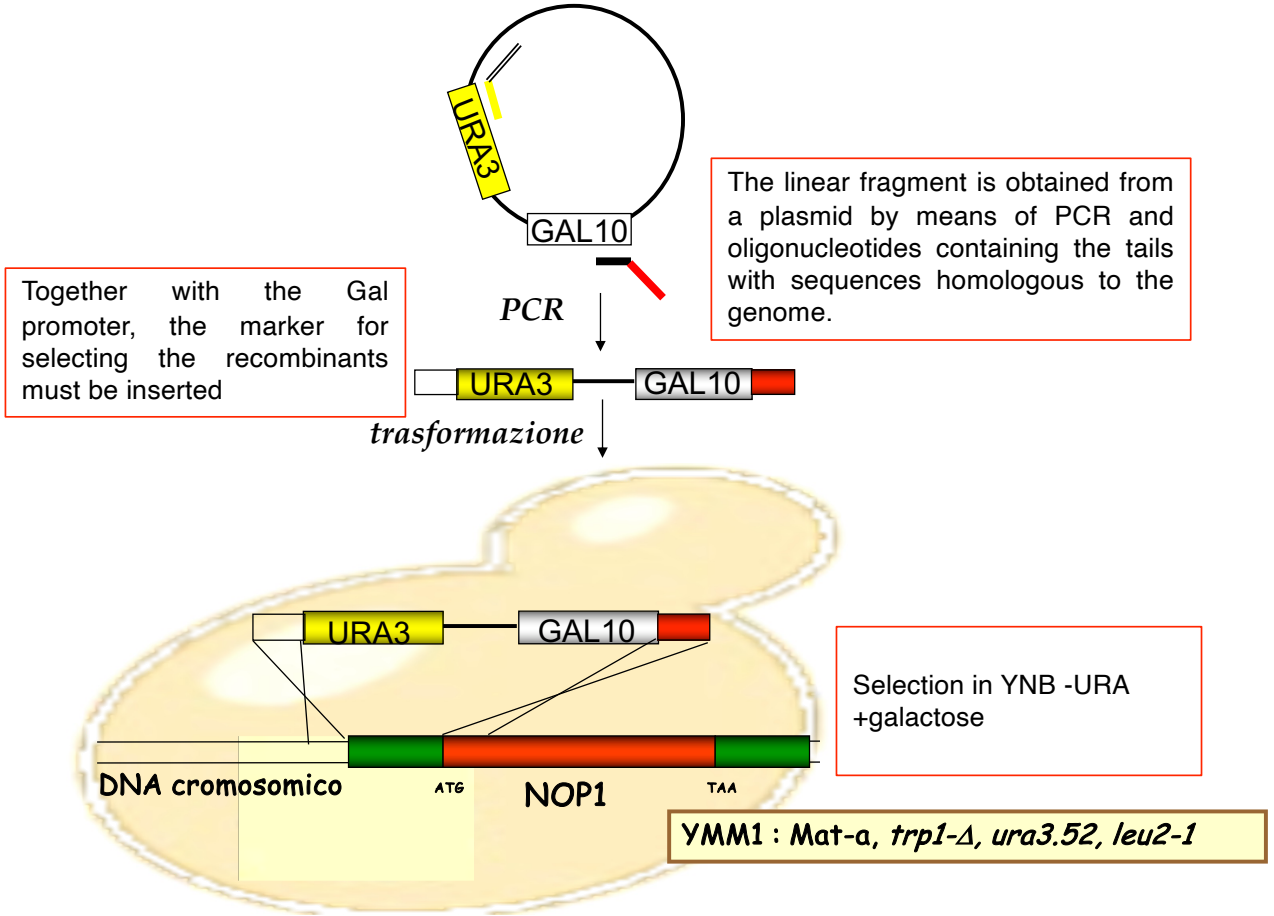
- Thermosensitive mutant
- Genes under control of inducible promoters

Functional inactivation of genes

GAL1 inducible promoter



Functional inactivation of genes



The linear fragment is obtained from a plasmid by means of PCR and oligonucleotides containing the tails with sequences homologous to the genome.

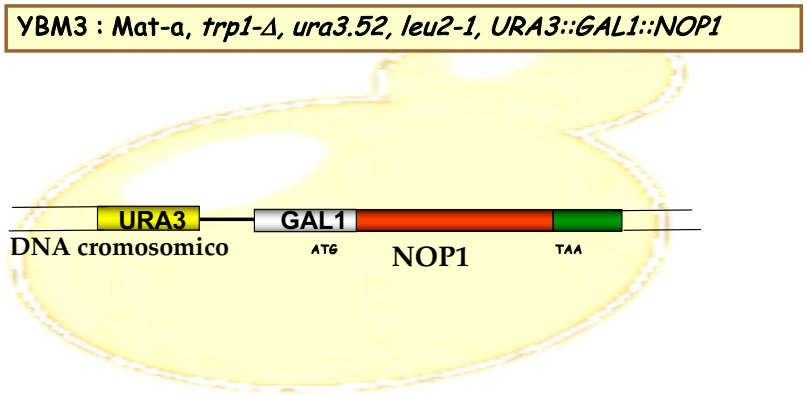
Together with the Gal promoter, the marker for selecting the recombinants must be inserted

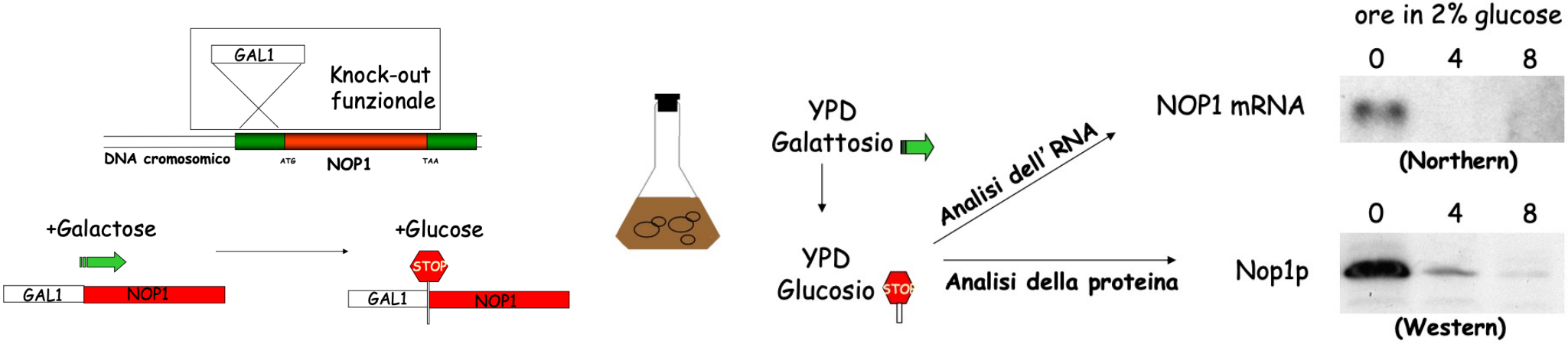
Selection in YNB -URA +galactose

YMM1 : Mat-a, *trp1-Δ, ura3.52, leu2-1*

Functional inactivation of genes

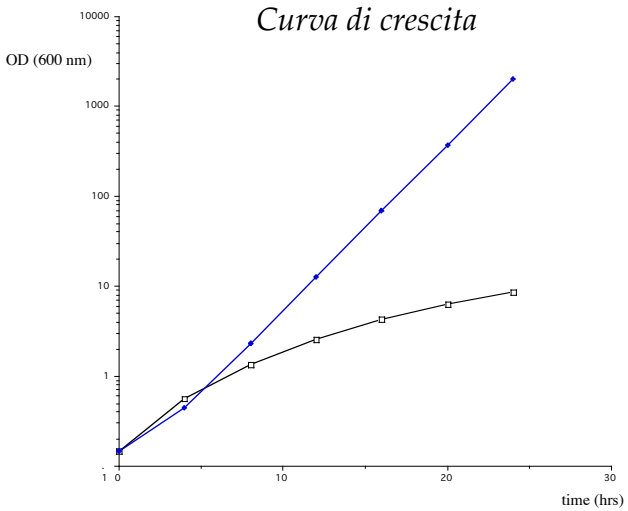
We have raised a new yeast strain with the genotype:

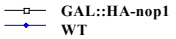


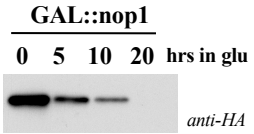


GAL::Nop1 allele

The insertion of HA TAG will facilitate the detection by WB



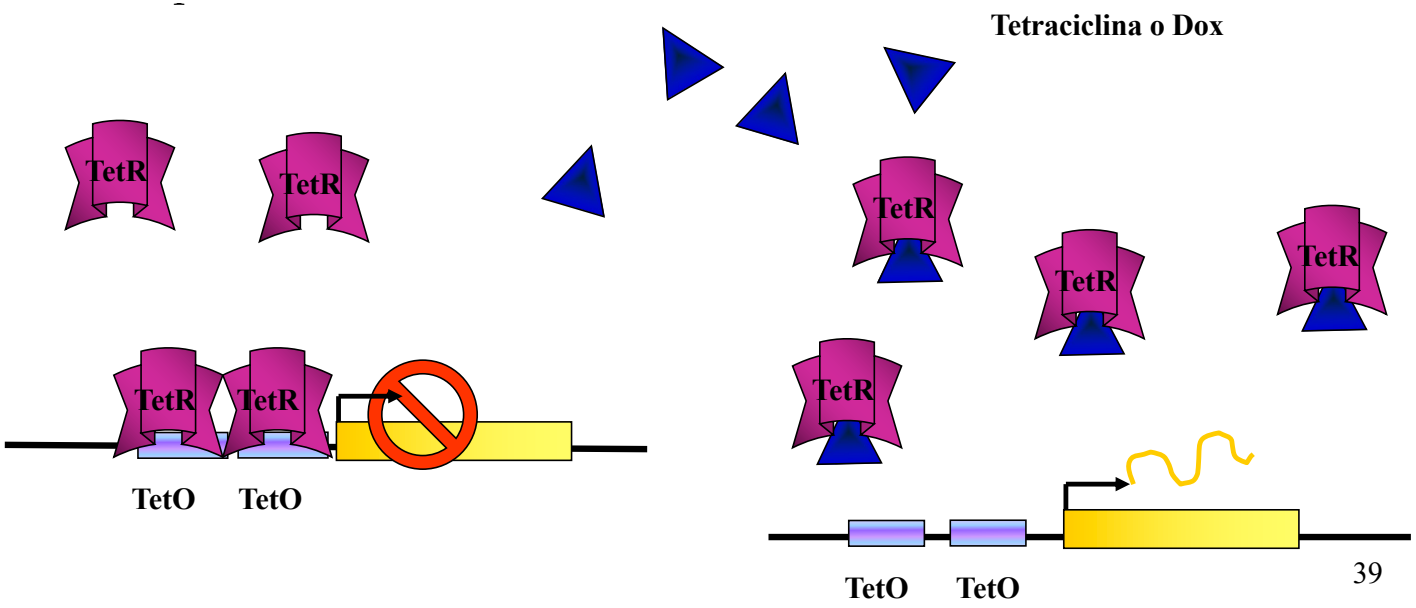




The growth curve is used to understand the timing for molecular analyses

TET ON/OFF system

- This system uses the tetracyclin resistence operon of Tn10 transposon of E.coli
- In E.coli the protein Tet is a repressor (TetR) that in absence of tetracyclin binds to TetO sites and inhibits transcription of the operon

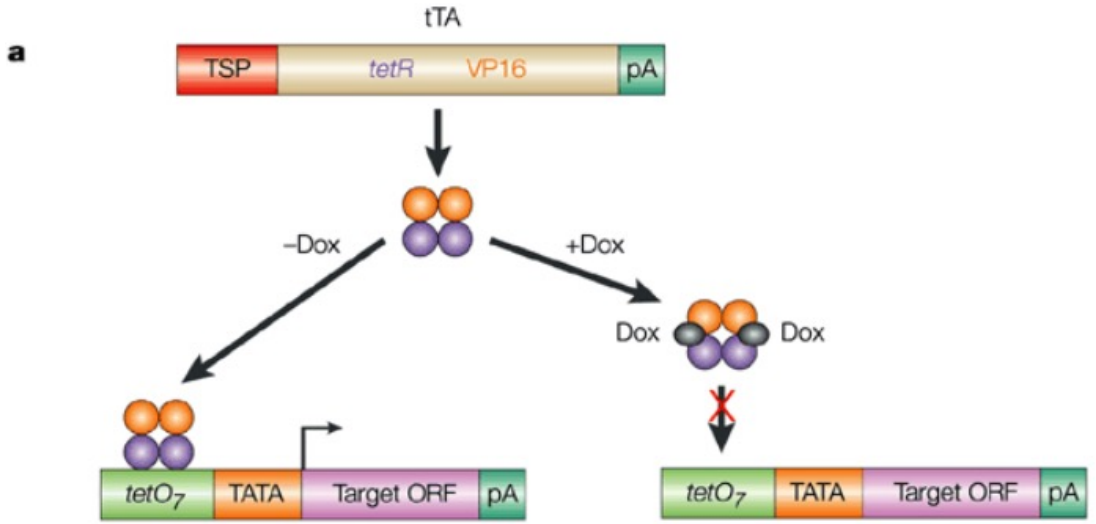


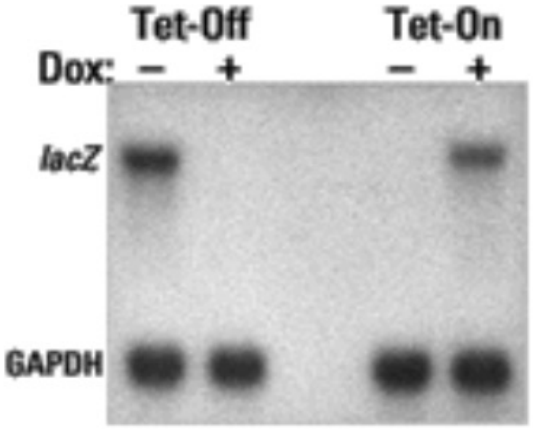
TET ON/OFF system

- The target gene in under control of a minimal promoter (no enhacer) but carrying tetO sittes

- Tet-OFF: TetR is fused to HSV VP16 activation domain, and becomes tTA. tTA is a transcriptional activator that in absence of Dox activates transcription

TET-OFF



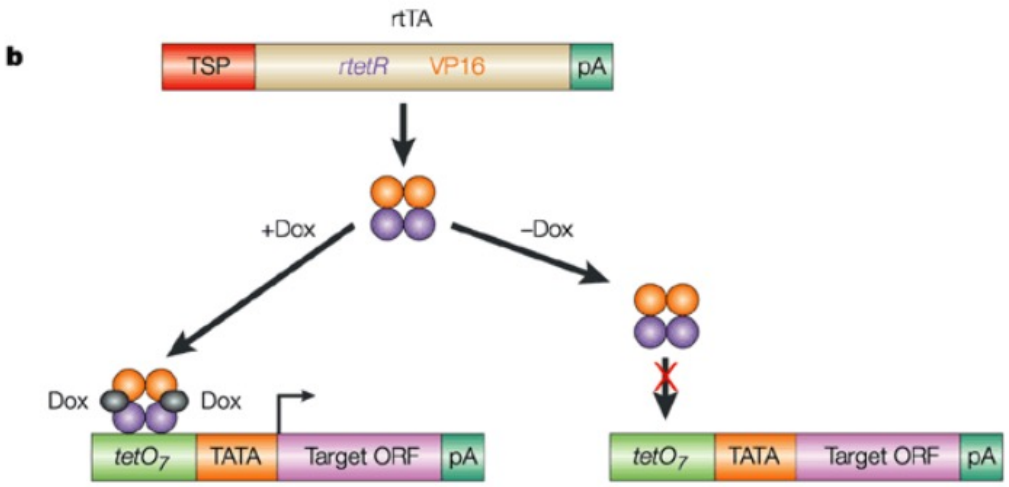


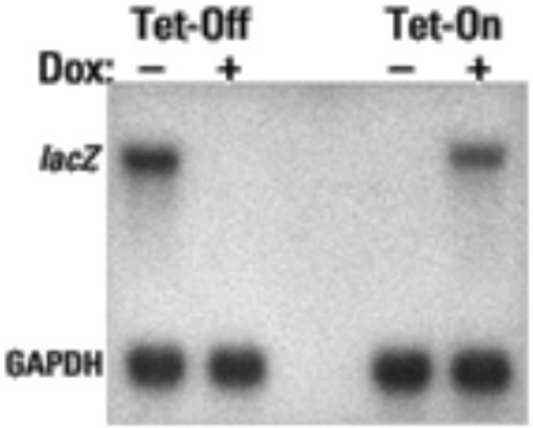
TET ON/OFF system

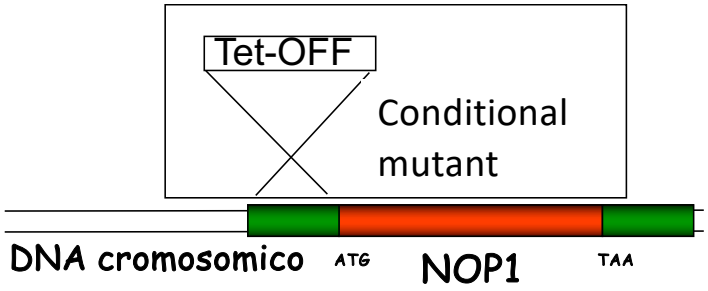
- The target gene in under control of a minimal promoter (no enhacer) but carrying tetO sittes

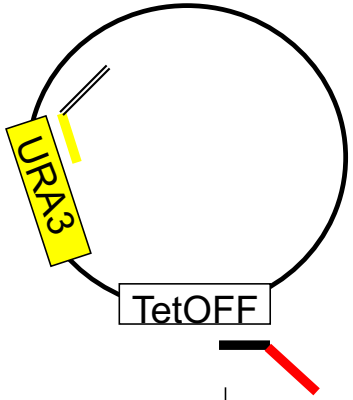
- Tet-ON: four aa substitutions in TetR influece its binding to DNA and produce ‘reverse tTA’ or rtTA. rtTA only activates transcription in the presence of DOX

TET-ON



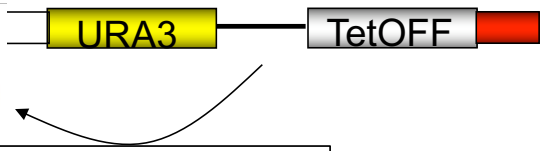




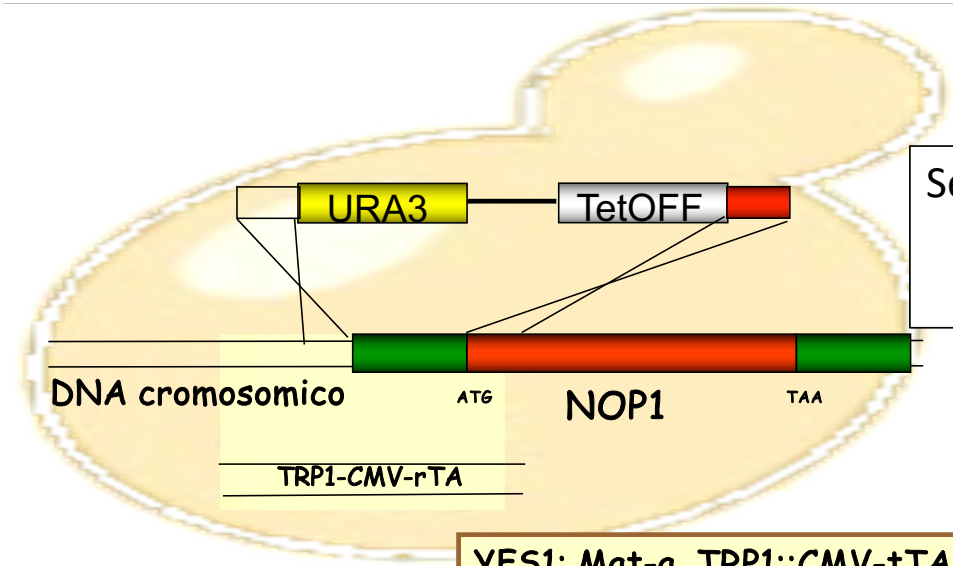


Use a strain expressing tTA

PCR



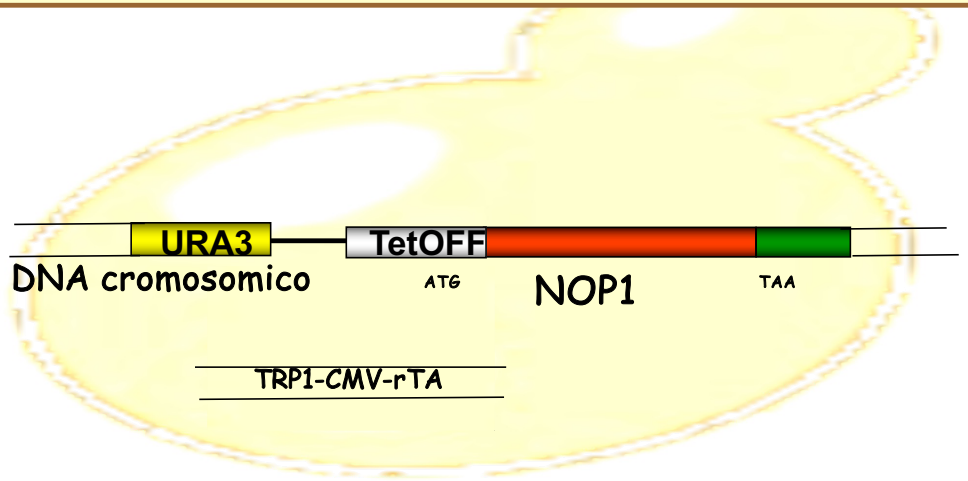
Selection: YNB
DO-URA
Glucose

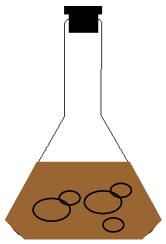


YFS1: Mat-a, TRP1::CMV-tTA,, *trp1-Δ, ura3.52, leu2-1*

We have raised a new yeast strain with the genotype:

YREGE2 : Mat-a,TRP1::CMV-tTA, *trp1-Δ, ura3.52, leu2-1, URA3::TETOFF::NOP1*



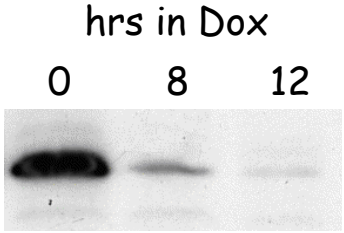


YNB
DO-URA
Glucose

YNB
DO-URA
Glucose
+DOX

Analisi della proteina

Nop1p

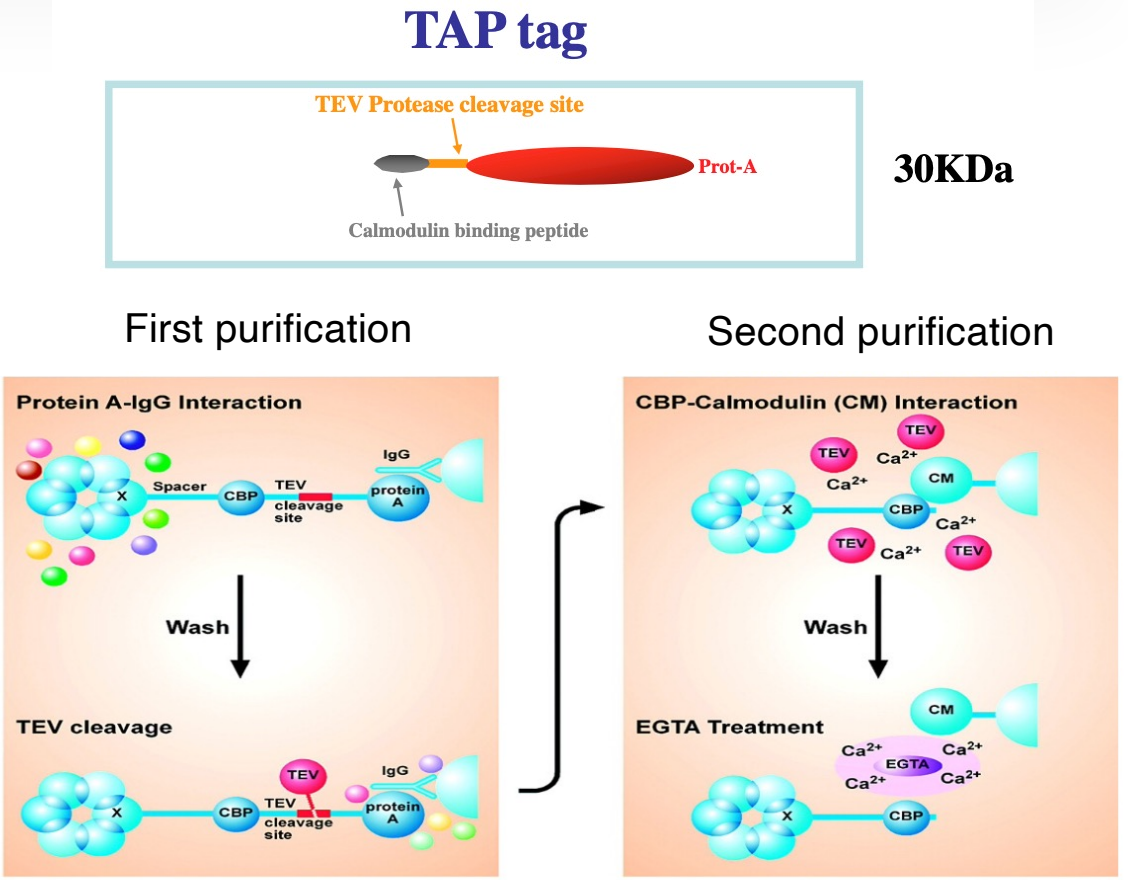


TAP ‘tagging’

It’s a system to isolate multiprotein complexes with a two-stage affinity purification

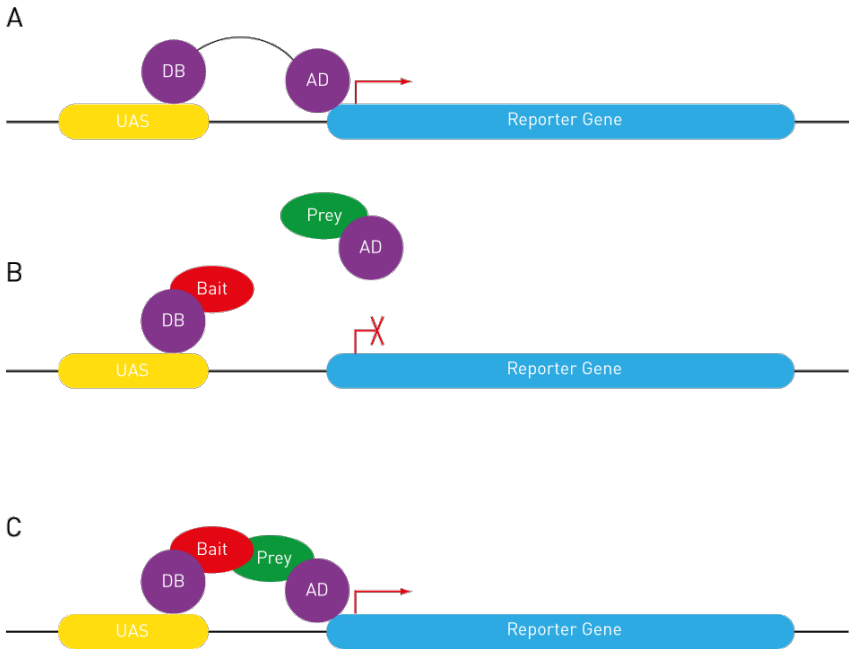
Advantages
- Tagging can be performed on the genomic copy
- Purification of ‘native’ multiprotein complexes
- Elimination of aspecific background
- Easy sequencing of new components by mass spec

Disadvantages
- It’s huge! Almost 30kDa



Two-hybrid system in yeast

The yeast two hybrid system is a method to detect the **interaction between two proteins (**from any organism) in yeast



In this method, the yeast transcription factor Gal4 is split into a DNA-binding domain (**DBD**) and an activation domain (AD). Protein pairs are fused to the DBD and AD, and the resulting DBD-X and AD-Y fusions are generally referred to as bait and prey.

The third component is a reporter cassette, which consists of the DNA-binding site for the first hybrid protein in the context of a minimal promoter, upstream of the coding sequence for an easily scored reporter gene

Only when bait and target interact, a reporter gene will be activated

Two-hybrid system in yeast