

# Yeast Genetics and Molecular Biology

An introductory course

Lecture I – yeast basics and classical yeast genetics

# What is Yeast Genetics?

- **Definition of Genetics in Wikipedia:** “**Genetics** (from Ancient Greek γενετικός *genetikos*, “genitive” and that from γένεσις *genesis*, “origin”), a discipline of biology, is the **science of heredity** and **variation** in living organisms”
- Classical yeast genetics:
  - Desirable traits of naturally occurring yeast strain **variants** were combined by mating of the strains to generate hybrids and **selection** of offspring carrying combinations of these traits
- Modern yeast genetics:
  - the cells are manipulated to generate mutants in pathways and processes of interest (generation of **heritable variation**)
  - Mutants with interesting phenotypes are **selected** or **screened** for and subsequently analyzed with molecular biology and biochemical methods to determine their function in the cell



# YEAST HISTORY

## Chronology

6000-2000 BC

1680

1835

1837

1839

1857

1876

1877

1880

1883

1915

1920

1949

1930-1960

1978

1990-1994

1996

## Milestones

Brewing (Sumeria, Babylonia)

Yeast under the microscope (van Leeuwenhoek)

Alcoholic fermentation associated with yeast

Name (*S.cerevisiae*) created for yeast observed in malt

Sugar as a food source for yeast growth

Fermentation correlated with metabolism (Pasteur)

'Etudes sur la levure de bière' (Pasteur)

Term "enzyme" (in yeast) introduced (Kühne)

Single yeast cells and pure strains for brewing (Hansen)

Alcohol and CO<sub>2</sub> by cell-free extracts (Buchner)

Production of glycerol

Yeast physiology reviewed

First genetic map (Lindegren); mating type system

Yeasts' taxonomy by Kluyver

First transformation of yeast (Hinnen, Hicks & Fink)

First commercial pharmaceutical products from recombinant yeast (Hepatitis B vaccine)

Completion of the yeast genome project

This slide was nicked from internet lecture notes of a course held at the Universität München (Prof. Horst Feldman)

# Pioneers of yeast genetics

- **Øjvind Winge** (1886-1964), Carlsberg laboratory, Copenhagen: <http://www.genetics.org/cgi/content/full/158/1/1>  
  
Discovery of alternation of Haplo – and Diplophase in *Saccharomyces* sp. – “Yeast Sex”; development of mechanical yeast manipulation and dissection methods
- **Carl C. Lindegren** (1896-1987), Washington University, St. Louis; University of Southern Illinois, Carbondale, USA  
Isolation of heterothallic yeast strains (= mutant strains with a stable haploid growth phase)
- **Boris Ephrussi** (1901-1979), Institut Pasteur, Paris; Centre national de la recherche scientifique, Gif-sur-Yvette, France  
  
Cytoplasmic inheritance (= mitochondrial genetics)

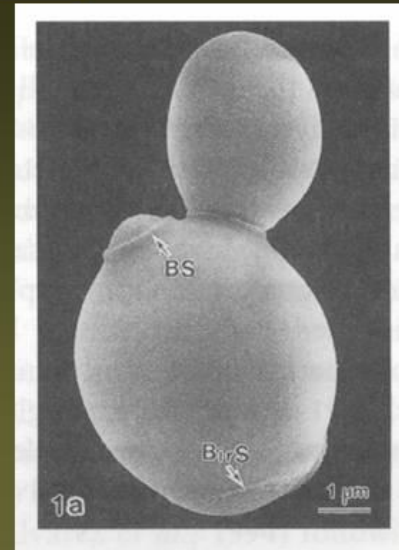
# Baker's Yeast

## *Saccharomyces cerevisiae*:

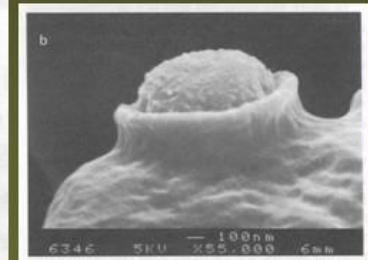
- Also "Budding yeast"
- Ascomycete (ascus as fruiting body)
- Oldest domesticated organism?
- Used in brewing and baking for millennia
- Favorite organism for molecular biologists
- First eukaryotic genome to be sequenced in its entirety (1996)!



### YEAST BUD AND BUD SCAR

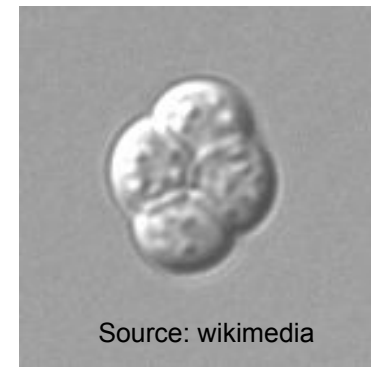


Buds normally develop close to the last bud (bud polarity)



[http://biochemie.web.med.uni-muenchen.de/Yeast\\_Biol/](http://biochemie.web.med.uni-muenchen.de/Yeast_Biol/)

### Yeast ascus with spore tetrad

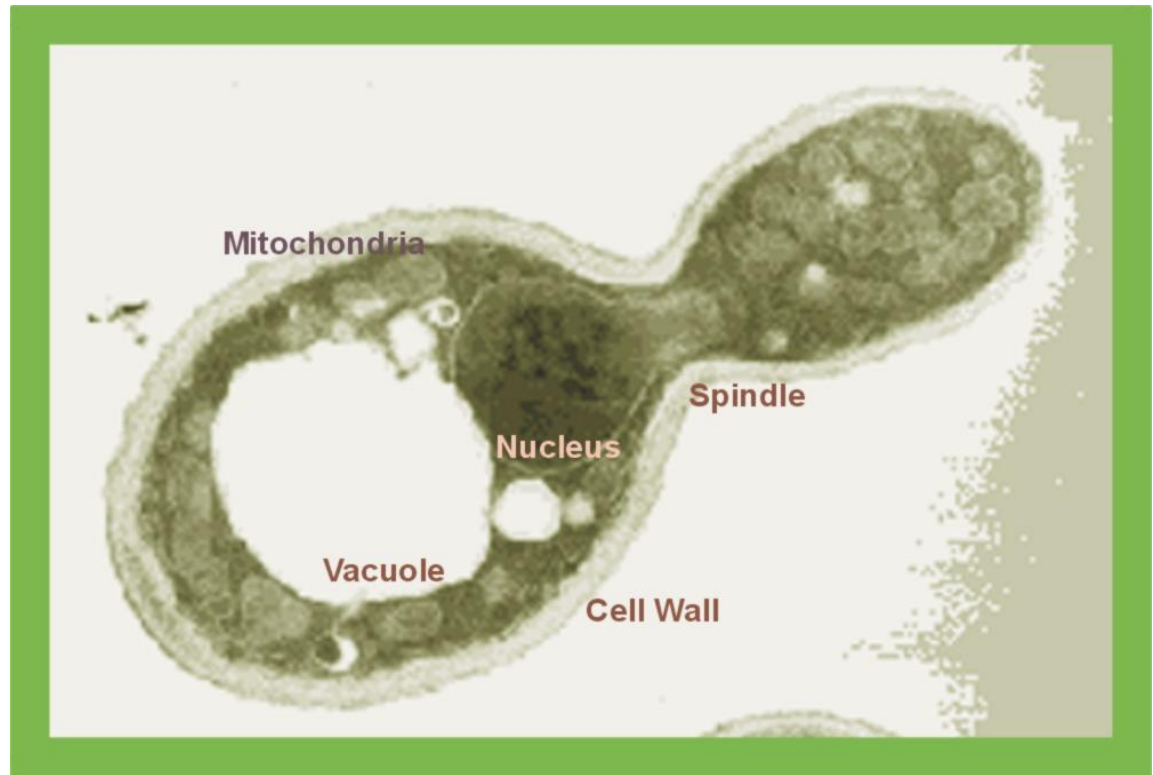


Source: wikimedia

Yeast is a molecular biology **model organism**

# Requirements for Model Organisms:

# Yeast similarity to human cells



## Mammalian homologs (based on *P* value)

<i>P</i> value	Number of ORFs at <i>P</i> value or lower	Percent of total ORFs ( <i>n</i> = 6223)	Percent of ORFs with unknown function
$1 \times 10^{10}$	1914	30.8	34
$1 \times 10^{20}$	1553	25.0	30
$1 \times 10^{40}$	1083	16.8	26
$1 \times 10^{60}$	784	12.6	23
$1 \times 10^{80}$	576	9.3	22
$1 \times 10^{100}$	442	7.1	21
$1 \times 10^{150}$	221	3.6	23
$1 \times 10^{200}$	101	1.6	25

**Yeast as a Model Organism** David **Botstein**, Steven A. **Chervitz**, and J. Michael Cherry *Science* 1997 August 29; 277: 1259-1260. (in Perspectives)



## “Bacterial” aspects of yeast:

- Single cell organism
- Haploid growth phase -> phenotype of recessive mutations shows up in the first mutant generation
- Fast growing (doubling every 1.5 hours on rich media)
- Moderate growth media requirements
- Transformation, gene replacement “easy”

# Processes that can be studied in yeast

- Cell cycle (mitosis, meiosis)
- (Principles of) gene regulation
- Metabolic processes
- Cell-to-cell signaling
- Cell specialization
- Cytoskeletal organization
- Intracellular transport mechanisms
- Compartmentalization
- Mechanisms of retroviral activity

# Growth requirements of Baker's Yeast

- Wild type *S. cerevisiae*: **prototrophic** as long as there is a useable carbon source and nitrogen source as well as trace salts available  
required molecules (amino acids, nucleic acids, polysaccharides, vitamins etc.) can be synthesized by the organism itself (there are, however, mutants available that are **auxotroph** for certain amino acids or nucleic acid precursors)

# Crabtree effect and oxygen requirements of *S. cerevisiae*

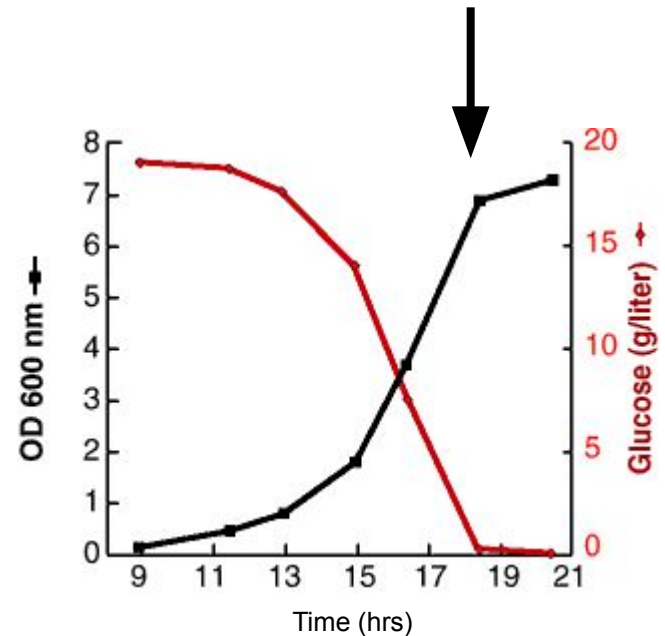
- Preferred carbon source: **glucose**, but many other carbon sources can be used
- If the carbon source allows, *S. cerevisiae* prefers to generate energy mainly by **alcoholic fermentation**
- **When glucose is in abundance, baker's yeast turns off all other pathways utilizing other carbon sources and grows solely by fermenting glucose to ethanol ("Crabtree effect")**
- *S. cerevisiae* is a facultative anaerobe: can grow by fermentation in the complete absence of oxygen, as long as the growth media is substituted with sterols and unsaturated fatty acids
- On non-fermentable carbon sources energy generated solely by respiration, and oxygen in the environment becomes **essential** (required for survival)

# Examples of Fermentable and Non-Fermentable Carbon Sources

<b>Fermentable Carbon Sources</b>	<b>Non-fermentable Carbon Sources</b>
Glucose	Ethanol
Galactose	Acetate
Raffinose	Glycerol
Lactose	Oleate/fatty acids
Sucrose (Saccharose)	Lactate

# Diauxic shift

- Yeast prefers alcoholic fermentation if the carbon source allows for it, until the fermentable carbon source is exhausted
- When there is no more fermentable carbon source in the media, the metabolism switches from fermentative to respiratory
- This process requires the upregulation of genes involved in respiratory breakdown of ethanol, downregulation of genes involved in fermentation
- Growth slows down after the diauxic shift



$OD_{600}$  = optical density at the wavelength of 600 nm;

Not Absorbance!; only linear between 0.3 and 0.7

The corresponding cell count differs from strain to strain (cell size!)

# Growth Media

“Favorite” Media (**RICH** media):

YP (Yeast extract and Peptone=peptic digest of meat) + carbon source

YPD= YP+ dextrose

YPR= YP+ raffinose

YPG= YP+glycerol

YPGal= YP+ galactose

**These are “complex media”** (exact composition not known)

Non-selective! Mutants in amino acid or nucleic acid biosynthetic pathways can grow (unless mutant cannot metabolize carbon source)

# Synthetic complete media

- Contain all the amino acids, some nucleic acid precursors and some vitamins and trace elements
- Nitrogen source: Ammonium sulfate (usually as Yeast Nitrogen Base (YNB) – containing also vitamins and trace salts)
- Carbon source can be varied (SCD, SCR, SCD, SCGal..)
- Non-selective if all amino acids/nucleic acid precursors are included
- Certain amino acids or nucleic acid precursors can be omitted => **selective media**

## **Select against mutations in biosynthetic**

**pathways!** (Select **for** plasmids that carry the wild type copy of a mutated gene □ plasmid marker)



## Minimal media

Carbon source and Nitrogen source (YNB)

Only wild type yeast can grow

# Yeast Gene and Gene Product Nomenclature

- Dominant alleles are written in *italicised* capital letters: *LEU2, ADE3, ARG2*

**Attn:** The number of the gene does not necessarily denote the place of the gene in a metabolic pathway. The numbering is often historical due to the order in which mutant alleles of the gene were obtained

- Recessive alleles are written in *italicised* lower case letters: *leu2, ade3, arg2*

Sometimes mutant allele variants are indicated with a dash and an additional number: *leu2-1, leu2-3....*

- Dominant gene products (=proteins) are written in regular letters, with the first letter capitalized: Leu2, sometimes followed by a lower case p: Leu2p
- Recessive gene products are written in lower case: leu2 (leu2p)

## Genetic nomenclature, using *ARG2* as an example

Gene symbol	Definition
<i>ARG</i> <sup>+</sup>	All wild-type alleles controlling arginine requirement
<i>ARG2</i>	A locus or dominant allele
<i>arg2</i>	A locus or recessive allele conferring an arginine requirement
<i>arg2</i> <sup>-</sup>	Any <i>arg2</i> allele conferring an arginine requirement
<i>ARG2</i> <sup>+</sup>	The wild-type allele
<i>arg2-9</i>	A specific allele or mutation
Arg <sup>+</sup>	A strain not requiring arginine
Arg <sup>-</sup>	A strain requiring arginine
Arg2p	The protein encoded by <i>ARG2</i>
Arg2 protein	The protein encoded by <i>ARG2</i>
<i>ARG2</i> mRNA	The mRNA transcribed from <i>ARG2</i>
<i>arg2-Δ1</i>	A specific complete or partial deletion of <i>ARG2</i>
<i>ARG2::LEU2</i>	Insertion of the functional <i>LEU2</i> gene at the <i>ARG2</i> locus, and <i>ARG2</i> remains functional and dominant
<i>arg2::LEU2</i>	Insertion of the functional <i>LEU2</i> gene at the <i>ARG2</i> locus, and <i>arg2</i> is or became nonfunctional
<i>arg2-10::LEU2</i>	Insertion of the functional <i>LEU2</i> gene at the <i>ARG2</i> locus, and the specified <i>arg2-10</i> allele which is nonfunctional
<i>cyc1-arg2</i>	A fusion between the <i>CYC1</i> and <i>ARG2</i> genes, where both are nonfunctional
<i>PCYCI-ARG2</i>	A fusion between the <i>CYC1</i> promoter and <i>ARG2</i> , where the <i>ARG2</i> gene is functional

**In most cases the wild type allele is denoted in upper case italics: *LEU2*, the mutant allele in lower case italics: *leu2***

**!!!!**

Special nomenclature for mutations involving mitochondrial genes – will not be talked about in this lecture

# Classical yeast genetics

Pre-molecular biology

## Cloning the Wild-type Gene by Complementation

Transform a *MATa yfg1 ura3-52* strain with a YCp50 library.

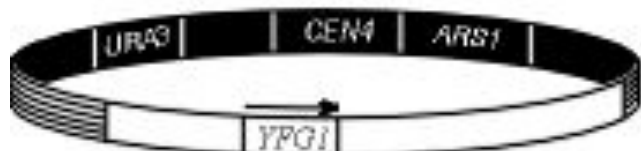
Isolate *Ura*<sup>+</sup> transformants and score for *Yfg*<sup>+</sup>



*Yfg*<sup>+</sup>

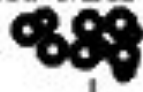
Recover the YCp-*YFGI*<sup>+</sup> plasmid in *E. coli*

Analyze the plasmid by digestion with restriction endonucleases and DNA sequencing



## Mutant Isolation

Mutagenesis of a haploid *MATa* strain



Detection of *Yfg*<sup>-</sup>



*Yfg*<sup>-</sup>

## Complementation

Cross the *Yfg*<sup>-</sup> *MATa* mutant to *MATα* tester strains. Isolate diploid strains. Score for *Yfg*<sup>+</sup> and *Yfg*<sup>-</sup>

*MATa yfg1* X  $\left\{ \begin{array}{l} \textit{MAT}\alpha \textit{ YFG}^+ \\ \textit{MAT}\alpha \textit{ yfg1} \\ \textit{MAT}\alpha \textit{ yfg2} \\ \textit{MAT}\alpha \textit{ yfg3} \\ \textit{etc.} \end{array} \right.$  

## Meiotic Analysis

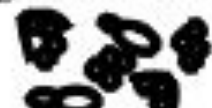
Cross mutant to *MATα YFG*<sup>+</sup>



Isolate a diploid strain and Sporulate



Digest ascus walls



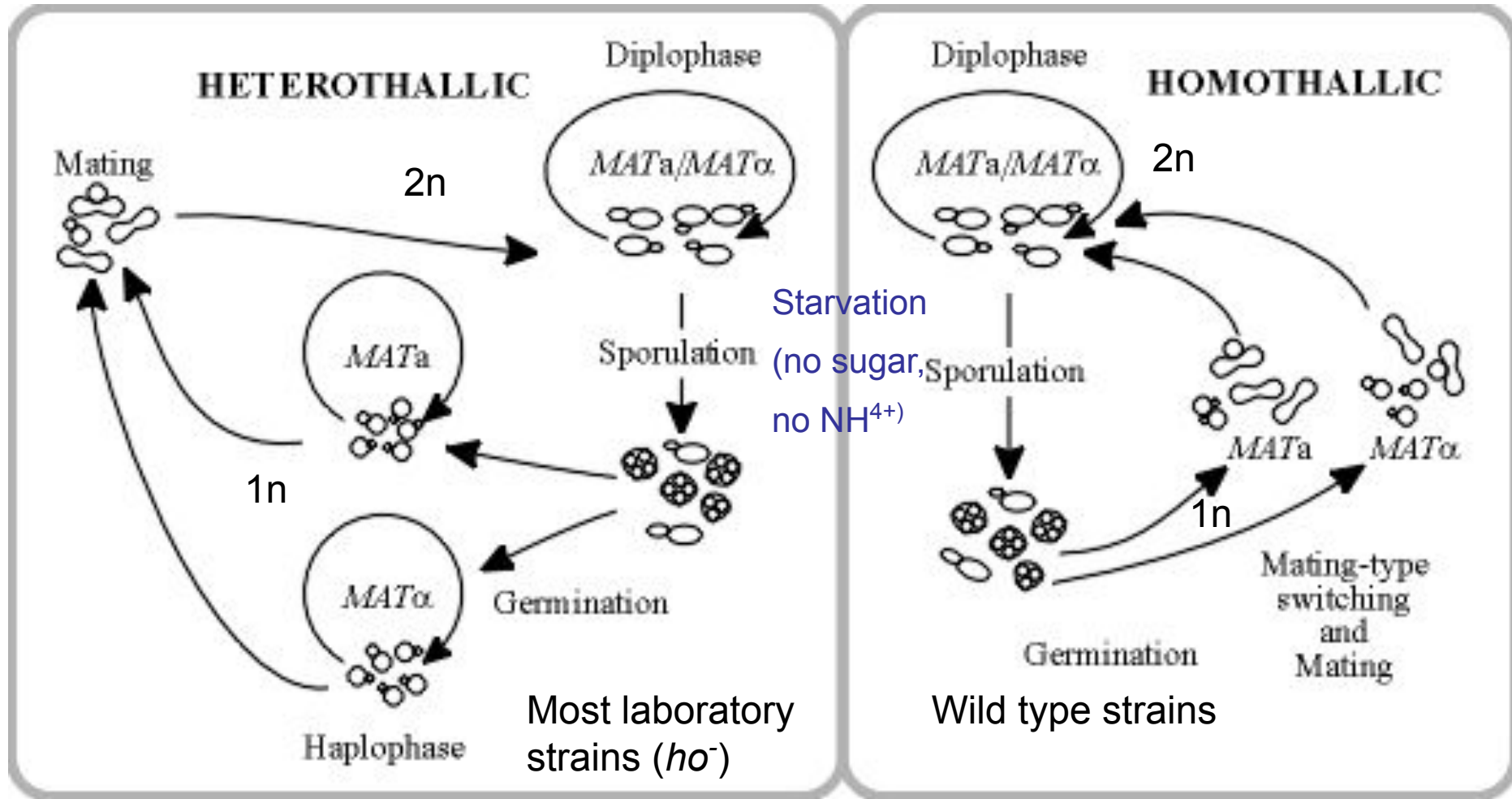
Dissect 4 spores of each tetrad



Score for *Yfg*<sup>+</sup> and *Yfg*<sup>-</sup>



# The Life Cycle of *Saccharomyces cerevisiae*



- Yeast has a haploid growth phase
- Phenotype of mutation apparent immediately
- Every haploid strain is a “pure bred” strain for its genetic traits
- Haploids are “Gametes”
- Sporulation = Meiosis; products of the same meiotic event can be examined!



# Genetic Manipulation

- Ability to mate yeast cells allows combining of mutations
- Meiotic products (spores) are packed in a spore sac (Ascus) and can be physically separated -> dissection of spores allows for dissection of pathways

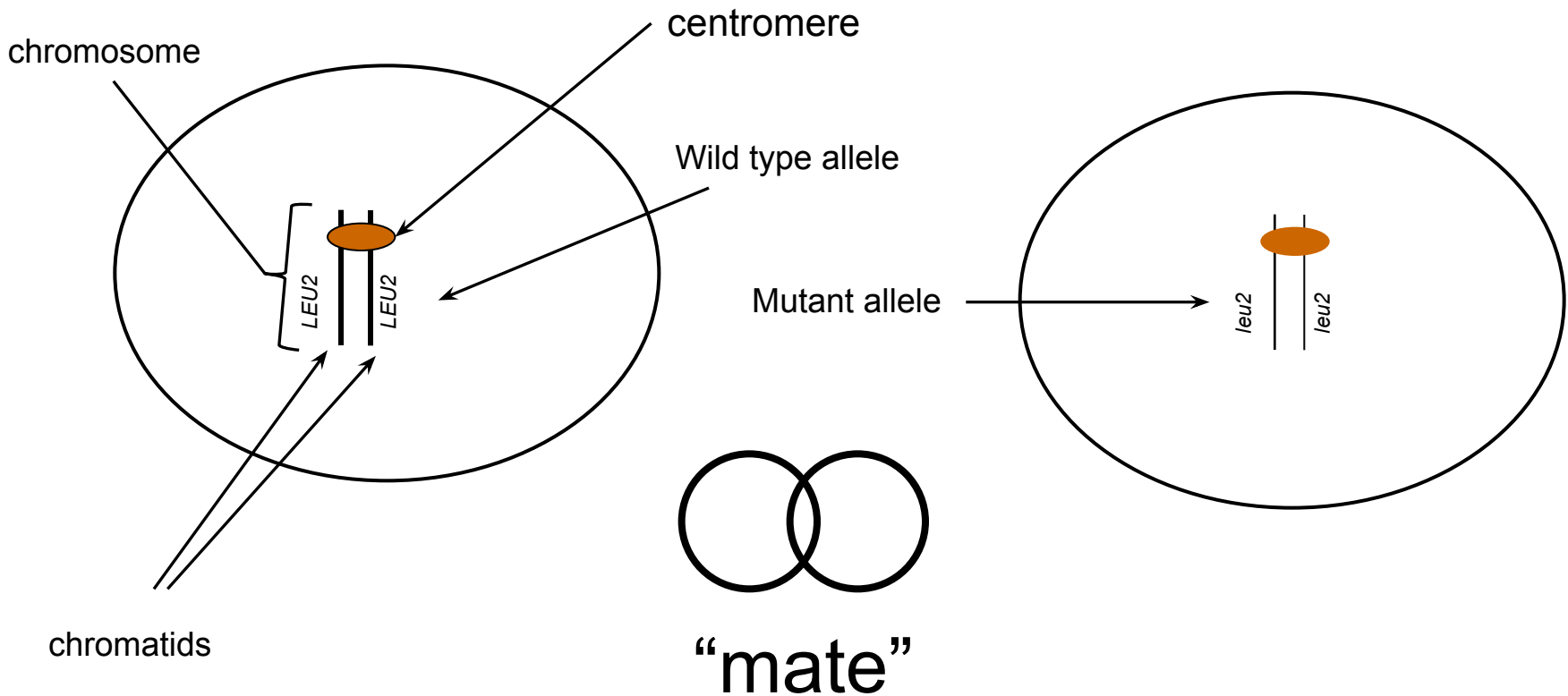
# Genetic analysis of a simple mutation

“Wild type”  
strain (Leu+)

*LEU2*: functional wild type allele

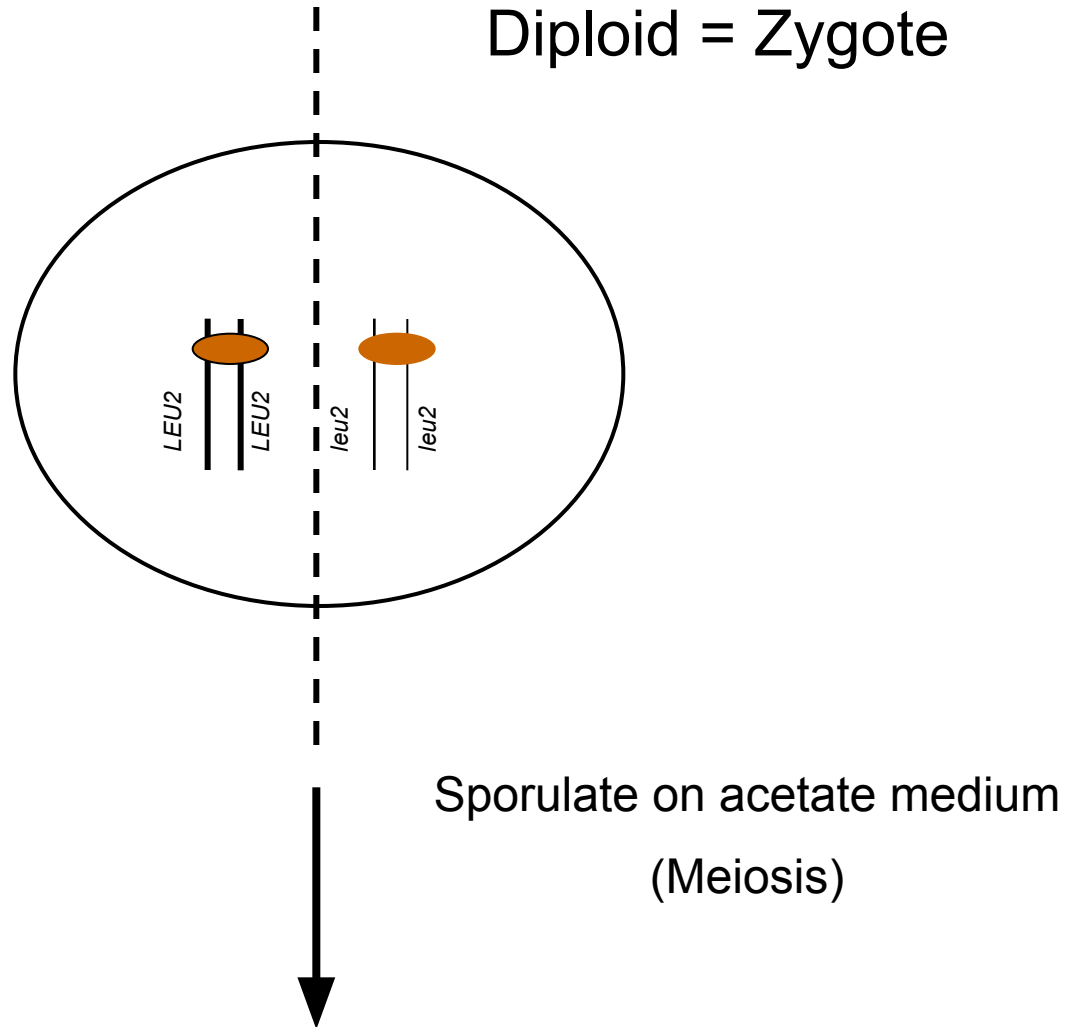
“mutant”  
strain (Leu-)

*leu2*: non-functional, recessive mutant allele

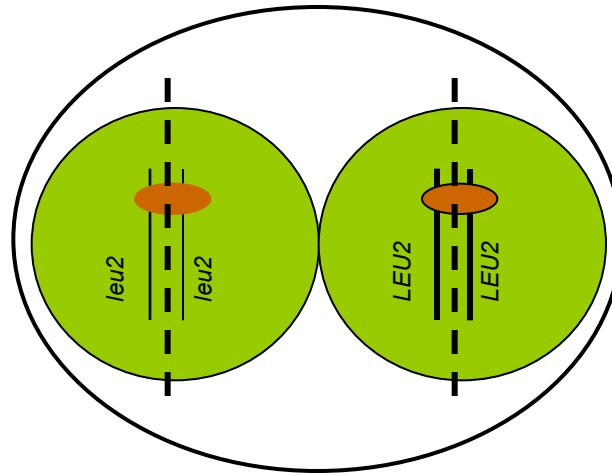


# Segregation of two alleles involved in Leucine biosynthesis

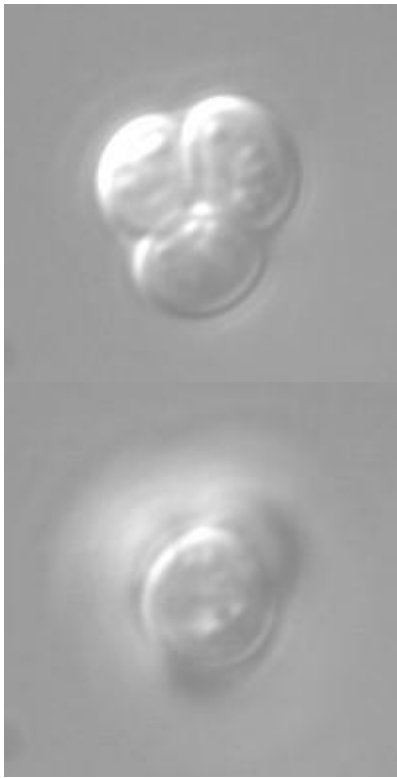
Cells are  $\text{Leu}^+$ , as the functional copy of *LEU2* is sufficient to support growth on media lacking the amino acid Leucine



# Meiosis 1: separation of the homologous chromosomes



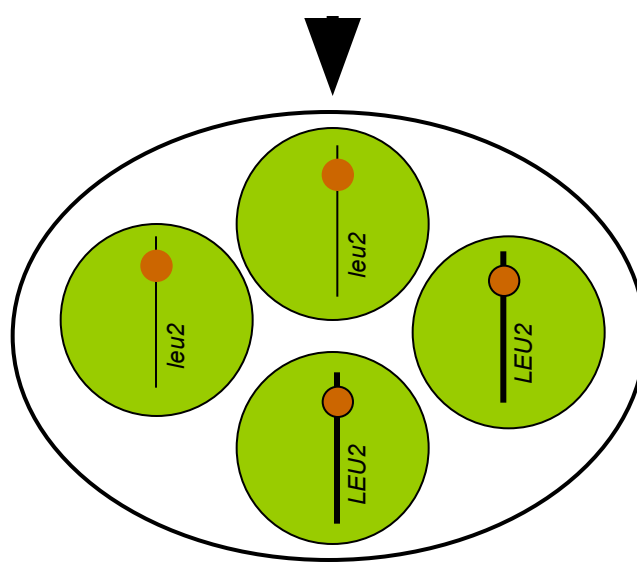
Meiosis 2:  
separation of the  
chromatids



Tetrad with 3 spores visible in one focal plane and 4th spore visible in a second focal plane



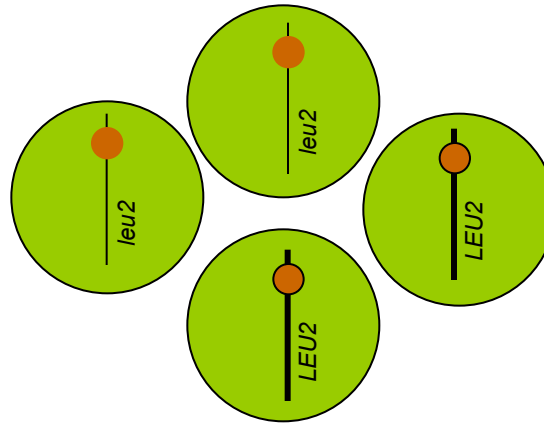
4 spores of a tetrad



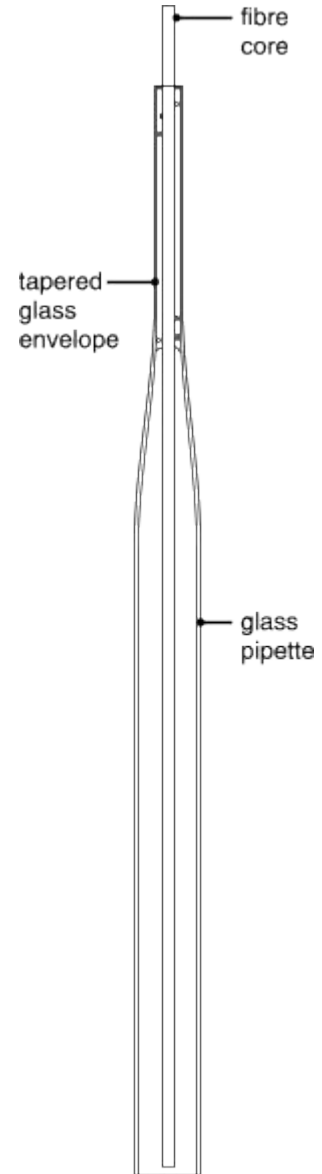
Ascus =  
spore sac

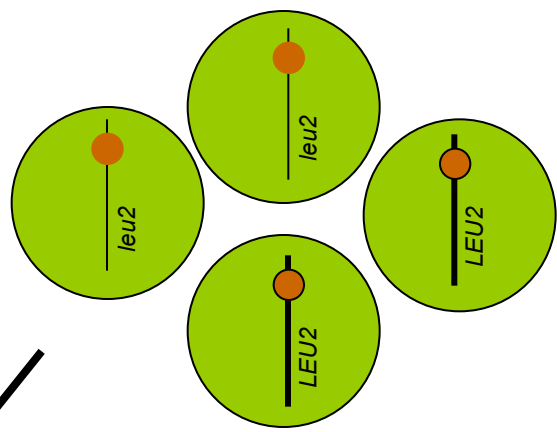
Spores =  
Gametes!

Digest off cell wall



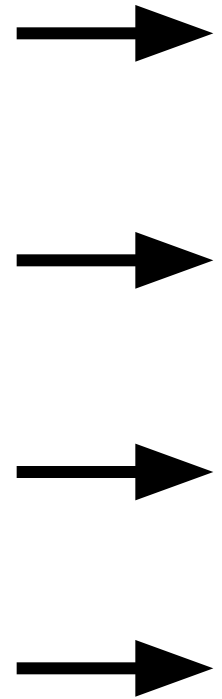
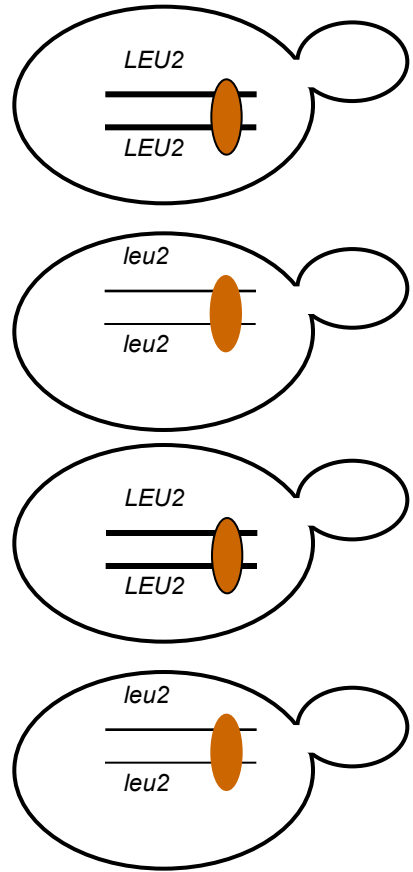
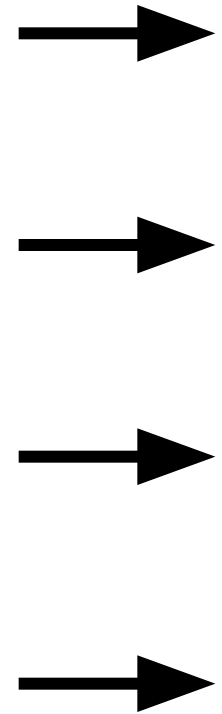
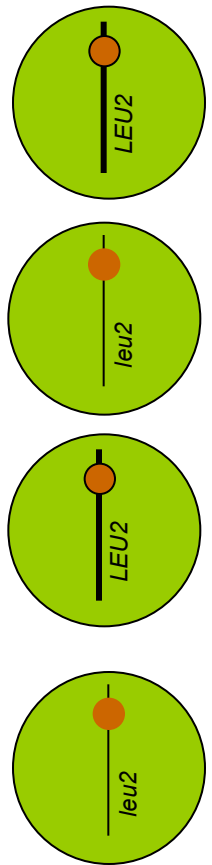
Dissect ascospores!



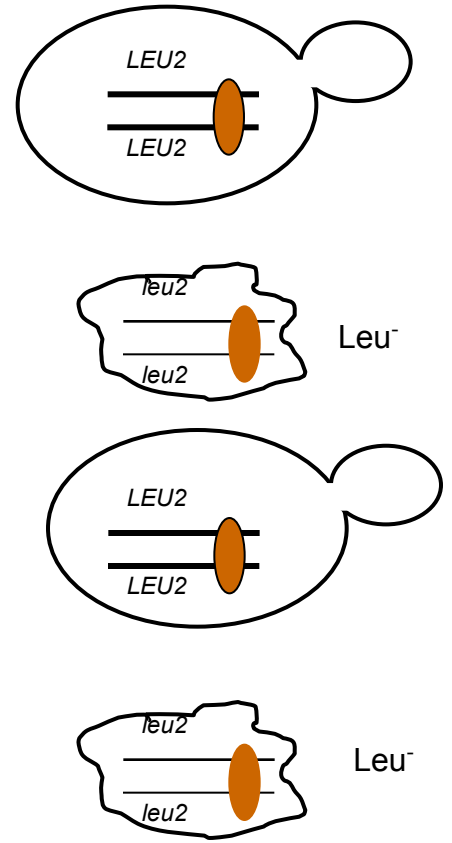


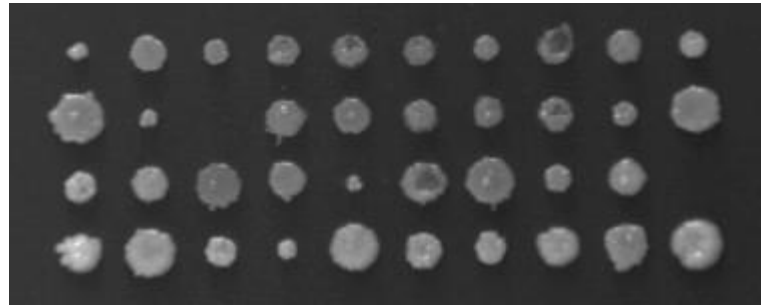
Non-selective media (e.g. YPD)

Line up on grid

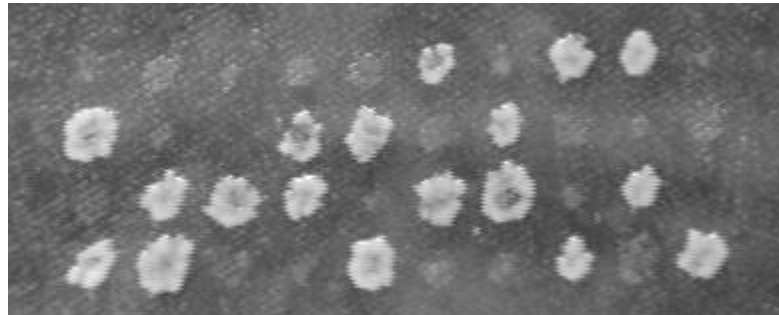


Selective media (SC-Leu)





Original  
Dissection on  
Non-selective  
plate



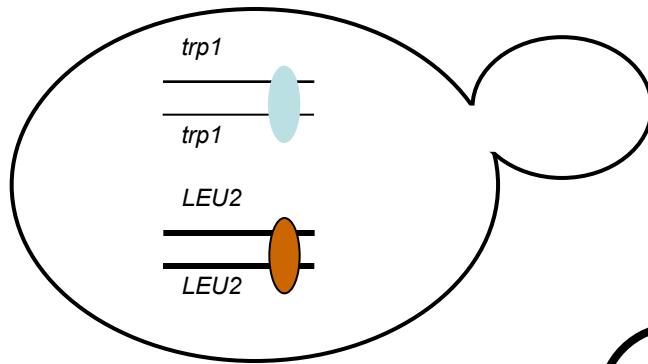
Replica on  
selective plate  
(e.g.  $\text{Leu}^-$  strain  
on SC – Leucine)

2 : 2 segregation ratio  
( $\text{Leu}^+$  vs.  $\text{Leu}^-$  **spores**)

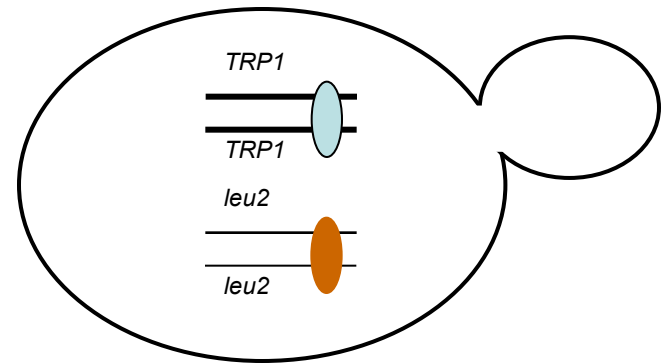


# Segregation of **two** unlinked genes

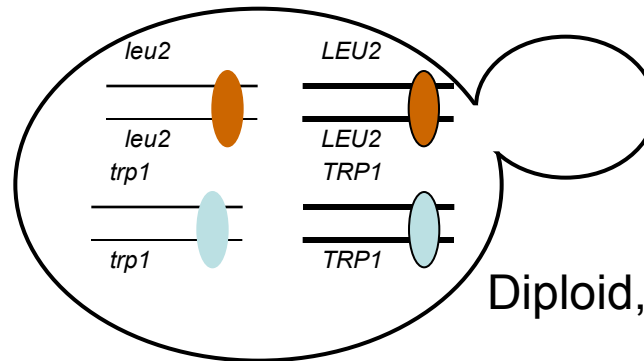
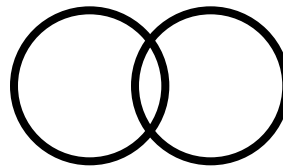
Example: *TRP1*, *LEU2*



Haploid,  
Leu<sup>+</sup>, Trp<sup>-</sup>



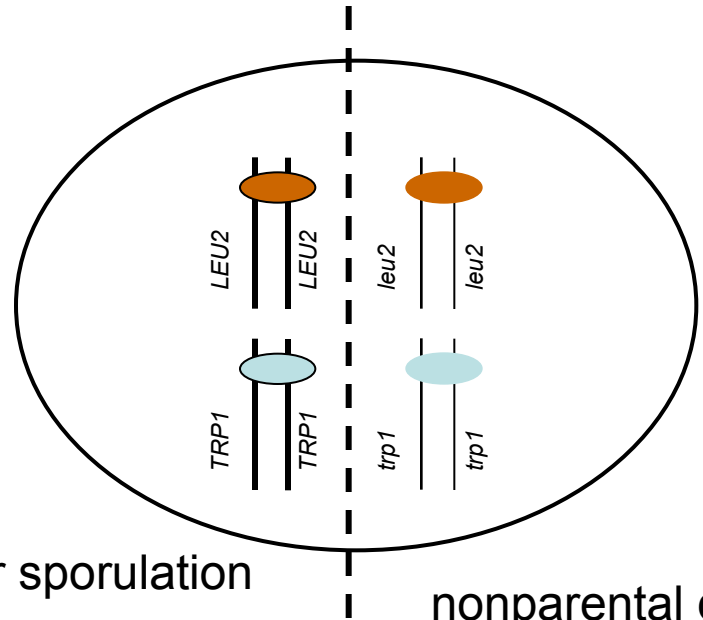
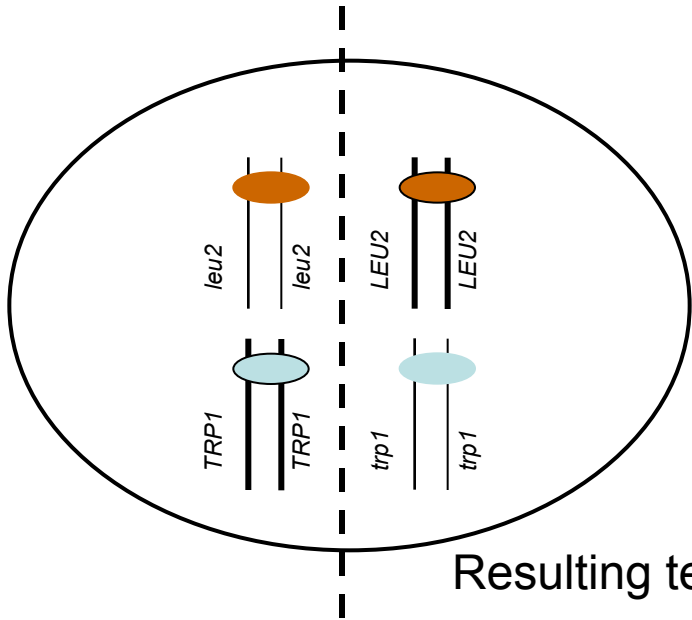
Haploid,  
Leu<sup>-</sup>, Trp<sup>+</sup>



Diploid, Trp<sup>+</sup>, Leu<sup>+</sup>,

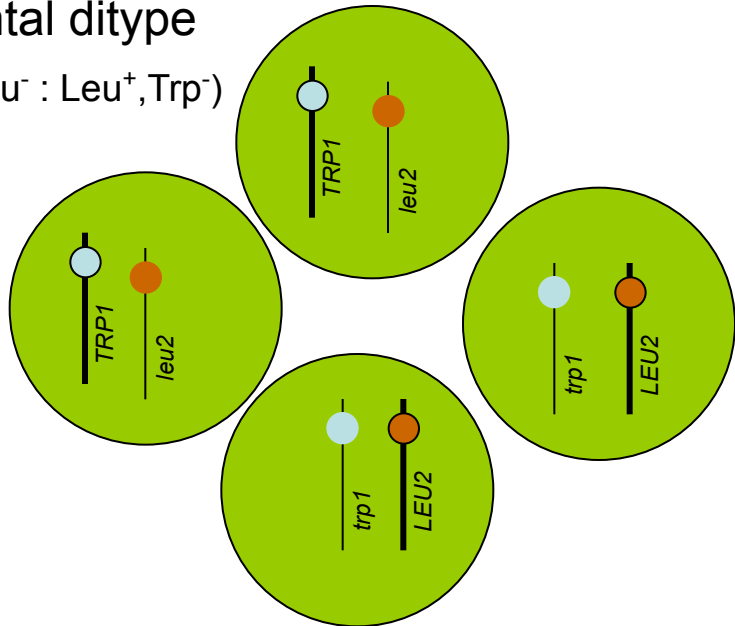


# Possible distribution of chromosomes during meiosis

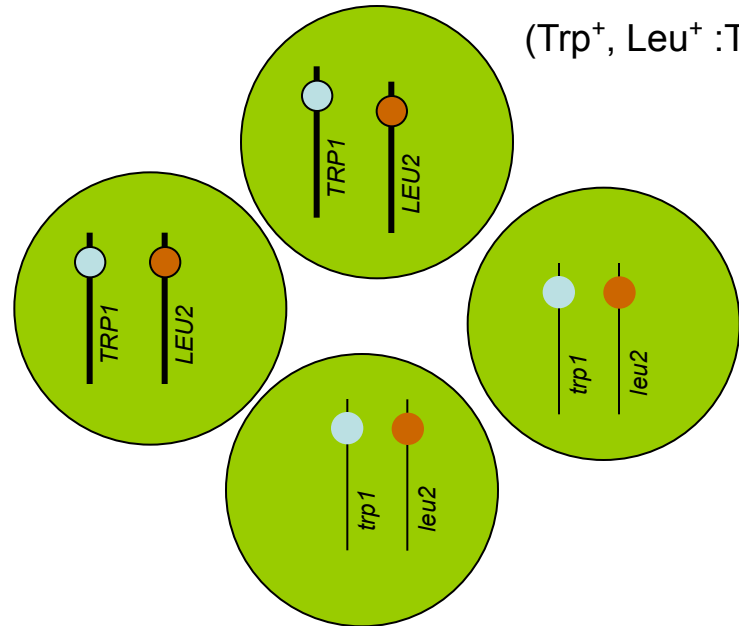


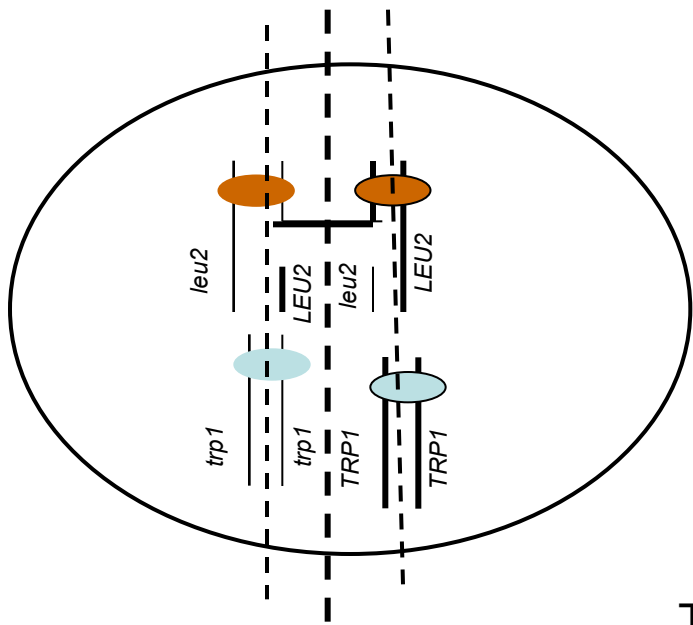
## Resulting tetrads after sporulation

parental ditype  
(Trp<sup>+</sup>, Leu<sup>-</sup> : Leu<sup>+</sup>, Trp<sup>-</sup>)

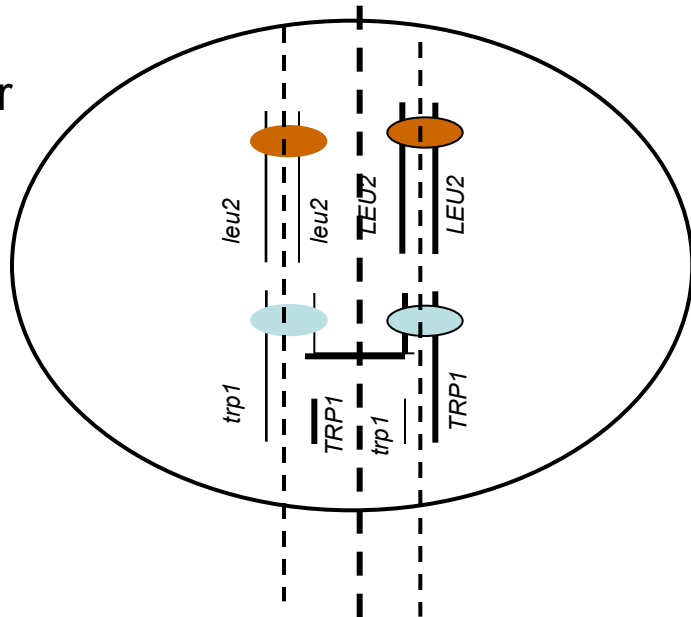


nonparental ditype  
(Trp<sup>+</sup>, Leu<sup>+</sup> : Trp<sup>-</sup>, Leu<sup>-</sup>)

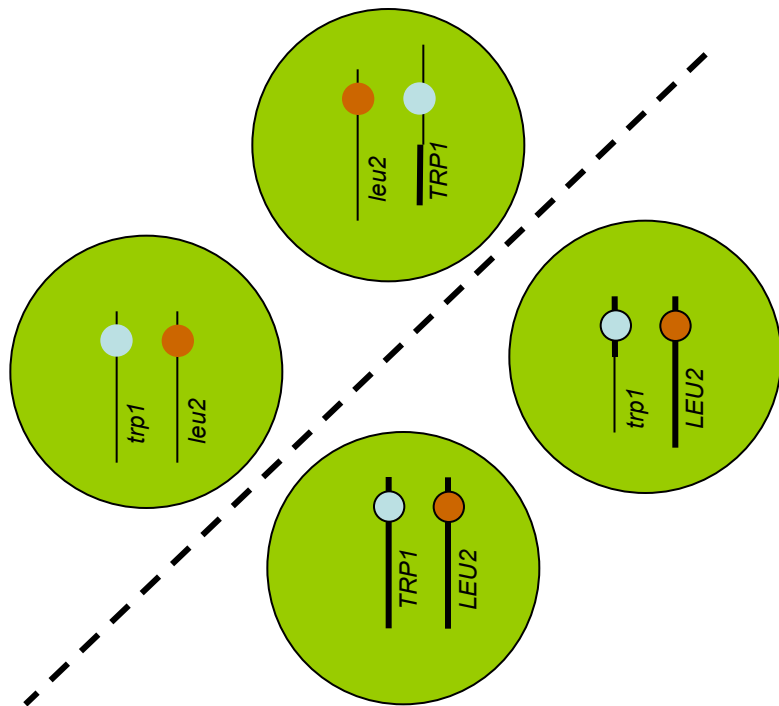
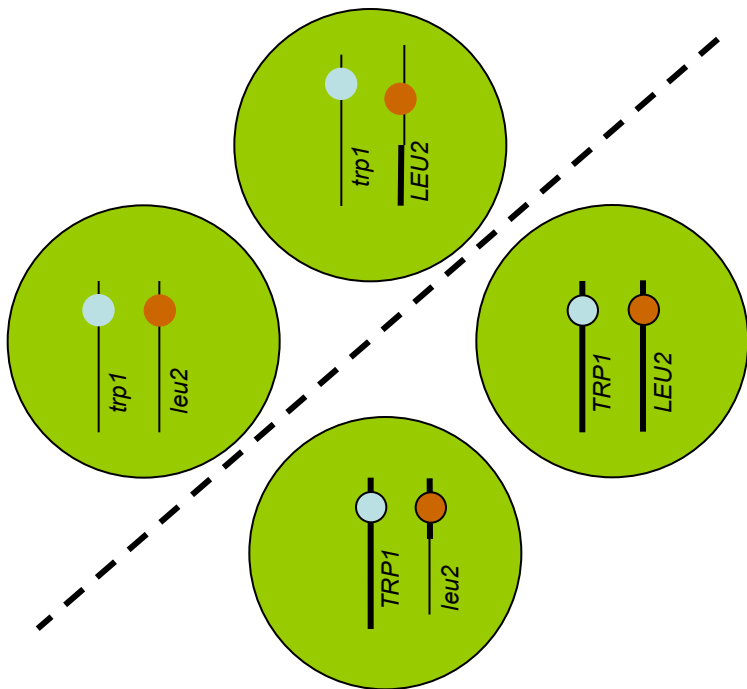


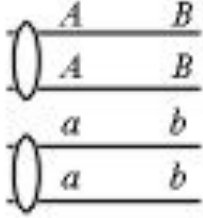
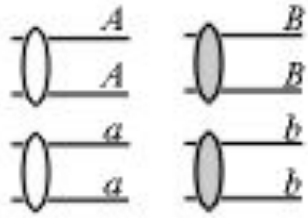
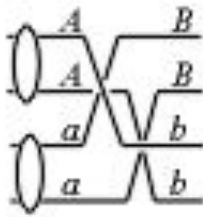
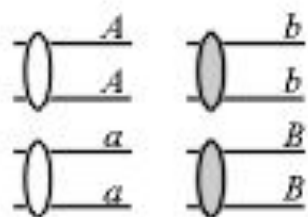
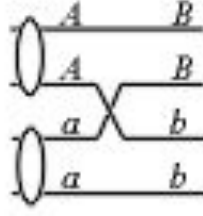
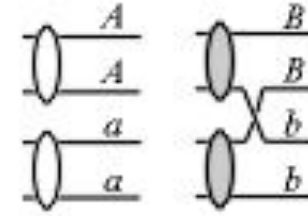
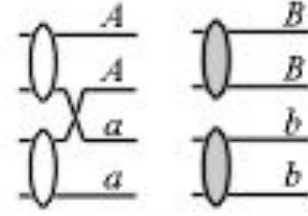


or



Tetratype



Tetrad type	Genes on homologous chromosomes	Genes on nonhomologous chromosomes
Parental ditype (PD) <i>A B</i> <i>A B</i> <i>a b</i> <i>a b</i>	No crossover 	No crossover 
Non-parental ditype (NPD) <i>A b</i> <i>A b</i> <i>a B</i> <i>a B</i>	Double crossover 	No crossover 
Tetratype (T) <i>A B</i> <i>A b</i> <i>a B</i> <i>a b</i>	Single crossover 	Single crossovers  or 

	PD	NPD	T
Spore 1	AB	aB	AB
Spore 2	AB	aB	Ab
Spore 3	ab	Ab	ab
Spore 4	ab	Ab	aB
Random assortment	1 :	1 :	4
Linkage	>1 :	<1	?
Centromere linkage	1 :	1 :	<4


 Ratios of different types of **tetrads!**  
**(NOT spores)**

Distances between linked genes can be calculated by counting the different tetrad types;

Formula:

$$\frac{\frac{1}{2} T + \text{NPD (recombinants)}}{\text{Total tetrads}} \times 100$$

Distance is expressed as recombination frequency in %

1% recombination = 1cM (centimorgan, after the famous fruit fly geneticist **Thomas Hunt Morgan**)

Recombination frequencies can never be > 50%

(= random assortment; genes behave unlinked)

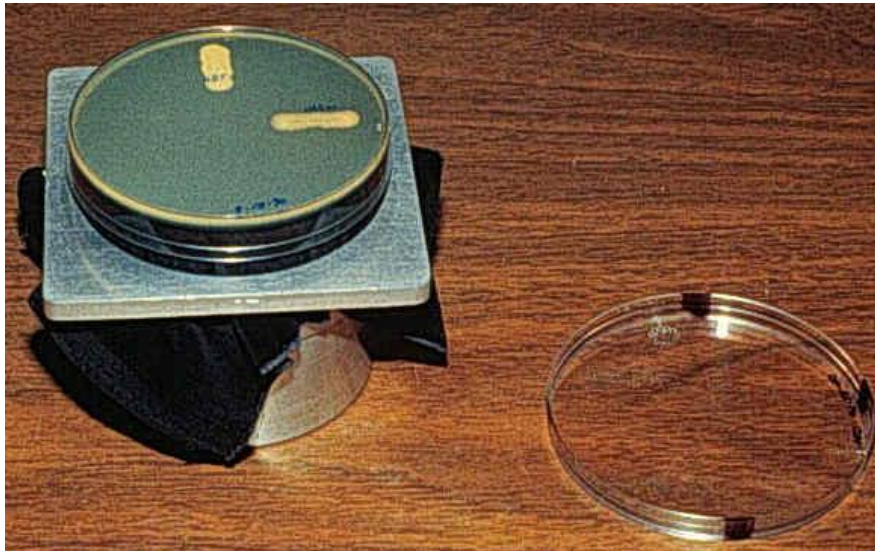
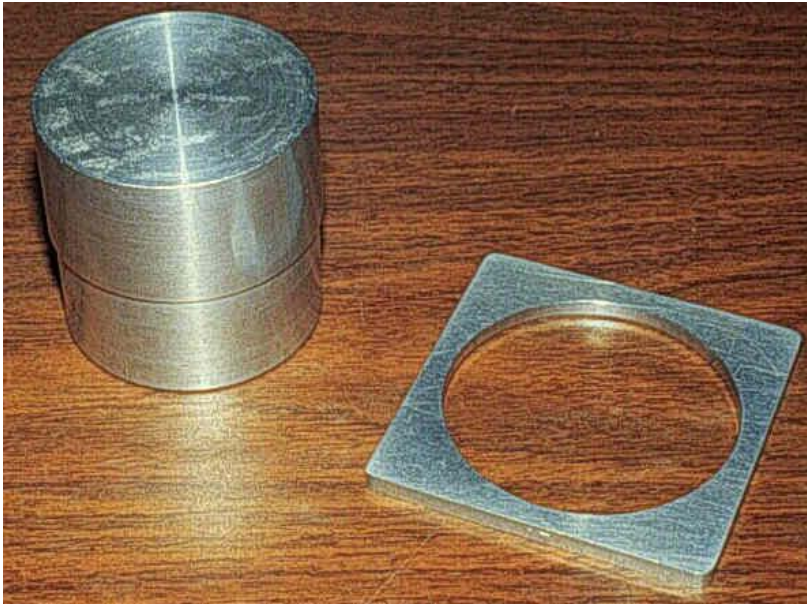
# Dissecting Metabolic Pathways in Yeast

- Question: What enzymes are involved in the Biosynthesis of Uracil?

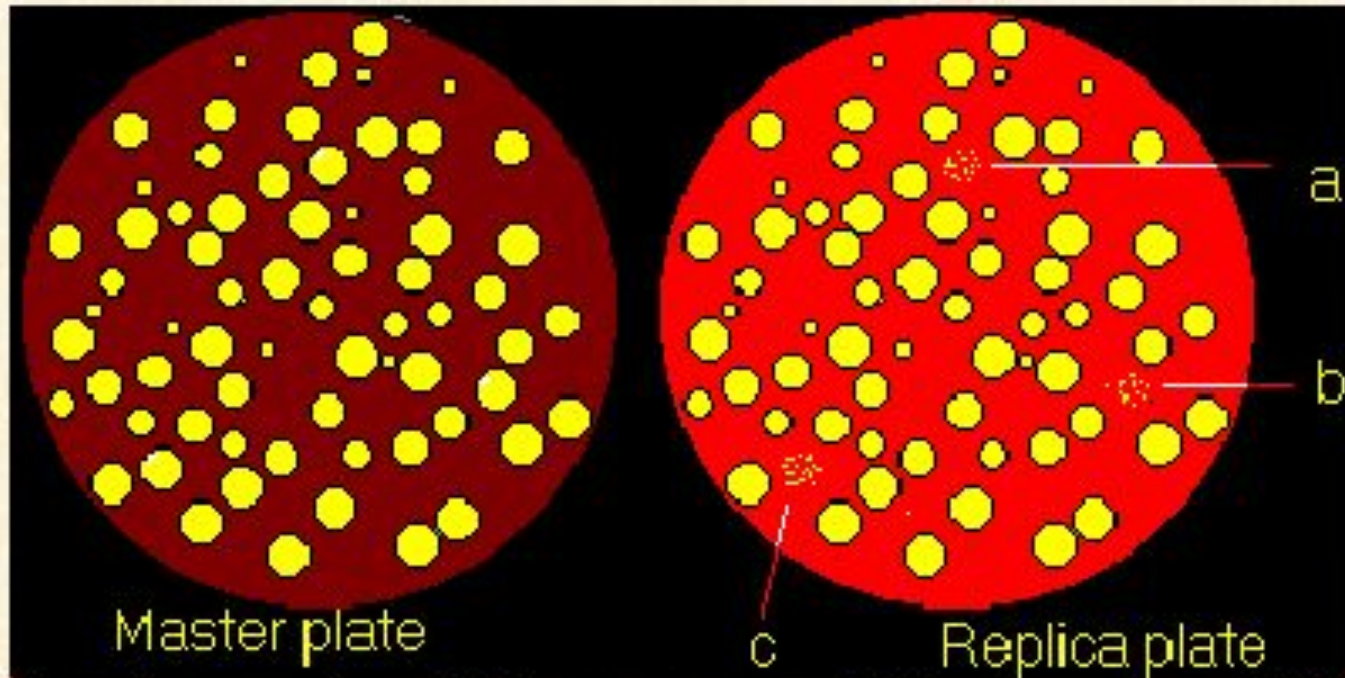
## **Approach: Screening for mutants dependent on uracil in the growth media**

- Mutagenize a healthy yeast strain (UV light, alkylating agents)
- Plate mutagenized cells on non-selective media
- Replica plate onto synthetic media lacking uracil (SC – Ura)

# Replica plating:



## Selecting auxotrophs by replica plating



YPD

SC - Ura

Most colonies still wild type – can grow on synthetic media lacking uracil, but a, b and v are uracil **auxotrophs** – they have a new growth requirement (presence of uracil in the media) – and can't grow on synthetic media lacking uracil

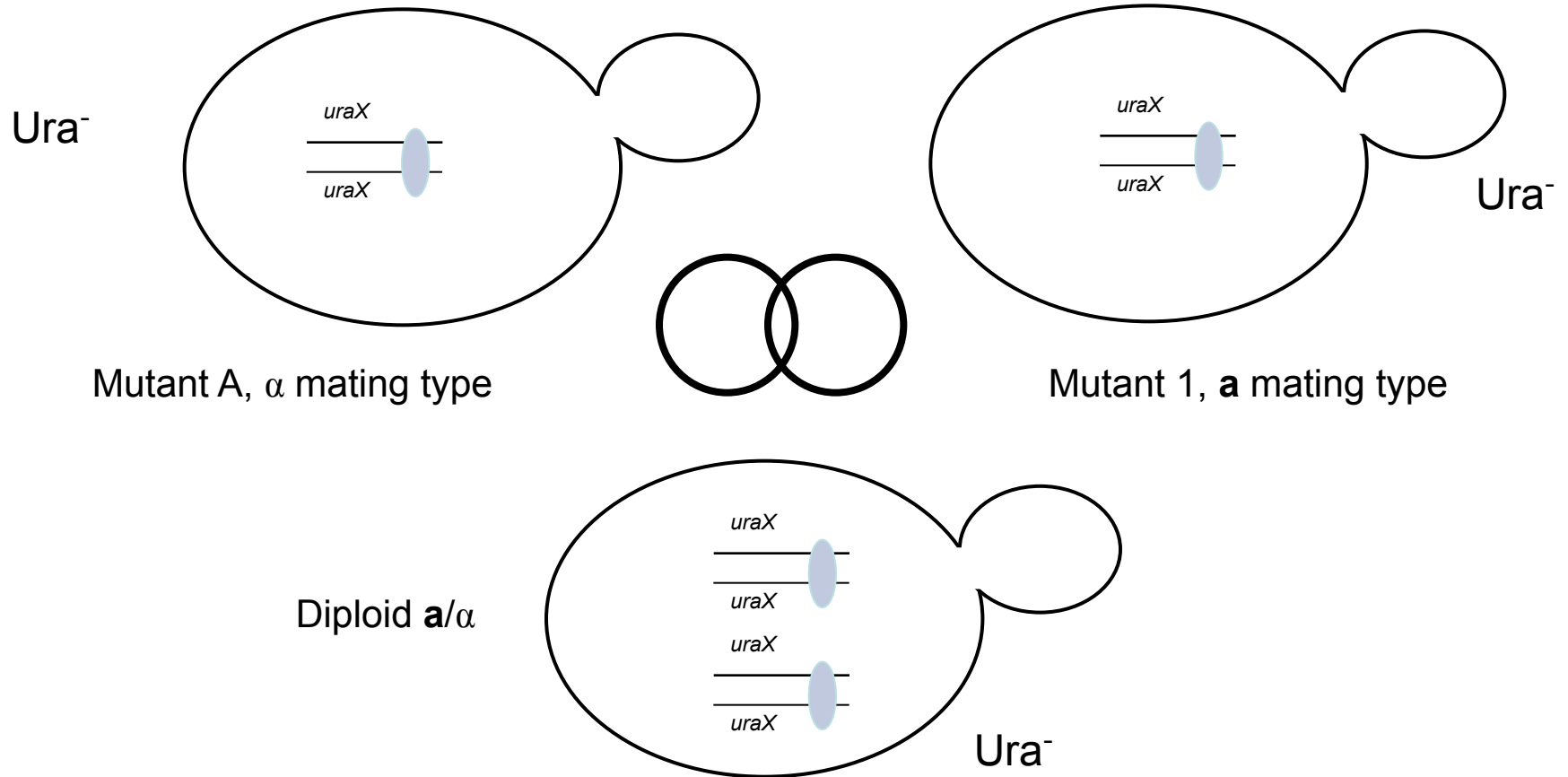


# Sorting of mutations

- In our hypothetical screen, we have identified several haploid mutants in the uracil biosynthesis pathway in both mating types
  - To test if the mutations are in the same pathway, we carry out **Complementation analysis**
- ⇒ Mutants are mated against each other
- ⇒ If the mutants are in the same gene, they will not complement each other and the diploid will be a uracil auxotroph
- ⇒ If the mutants are in different genes, they will complement each other, and the diploids will be able to grow on media lacking uracil

# Complementation analysis

Scenario 1: mutations are in the same gene

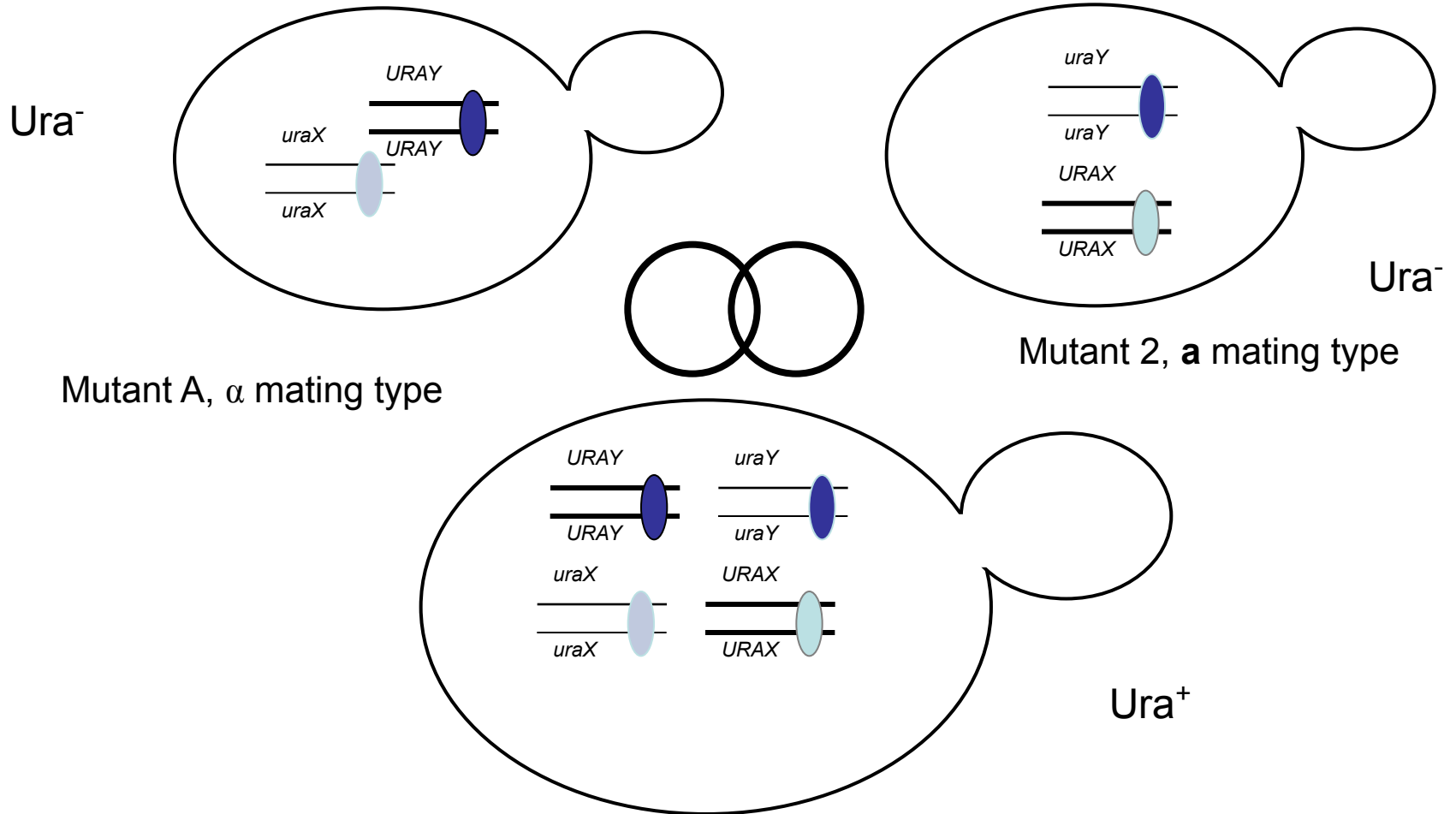


Diploid cannot grow on SC – *ura* => Mutant A ( $\alpha$ ) and mutant 1 (**a**) **cannot** complement each other and are therefore in the **same** complementation group

Conclusion: Mutant A ( $\alpha$ ) and mutant 1 (**a**) are in the same gene *uraX*; as there is no functional copy of *uraX* in the cells, they are unable to synthesize uracil;

# Complementation analysis

Scenario 2: mutations are in different genes



The diploid is able to grow on SC – *ura* => Mutant A ( $\alpha$ ) and mutant 1 (**a**) are able to complement each other and are in **different** complementation groups

Conclusion: Mutant A ( $\alpha$ ) and mutant 2 (**a**) are in different genes *uraX* and *uraY*; as there is **one** functional copy of each *URAX* and *URAY* in the cells, they are able to synthesize uracil;

## Complementation of mutants in the uracil biosynthesis pathway

(+) = mutants complement each other ; (-) = mutants do not complement each other

$\alpha$ a	1	2	3	4	5	6
A	-	+	-	-	+	+
B	+	+	+	+	-	-
C	+	-	+	+	+	+
D	-	+	-	-	+	+
E	+	-	+	+	+	+

Complementation groups: **1.** A,D, 1, 3, 4      **2.** B, 5, 6      **3.** C,E, 2

Mutants in the same complementation groups have mutations in the same gene

# Epistatic Analysis

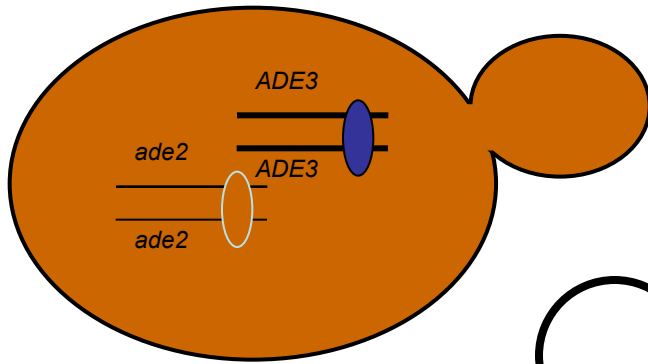
**Epistasis** - the interaction between two or more genes to control a single phenotype

**Epistatic Analysis:** determine the order and/or relationship of genes in a pathway

# Example of Epistatic analysis

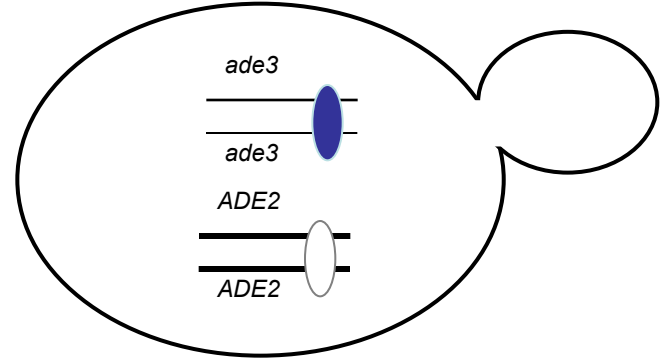
- Example: Adenine biosynthesis mutants *ade2* and *ade3* (unlinked genes):
- *ade2* mutants are Ade-, make **red** colonies
- *ade3* mutants are Ade-, make white colonies
- Double mutant will reveal position of genes/gene products in the adenine biosynthesis pathway relative to each other





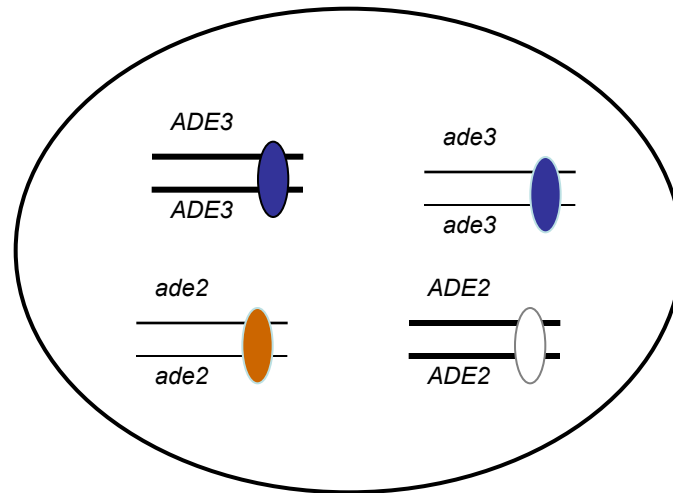
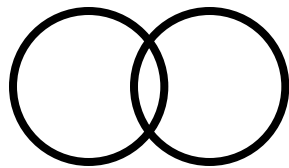
*ade2* mutant,  $\alpha$  mating type

Ade<sup>-</sup>, RED



*ade3* mutant, **a** mating type

Ade<sup>-</sup>, creamy white



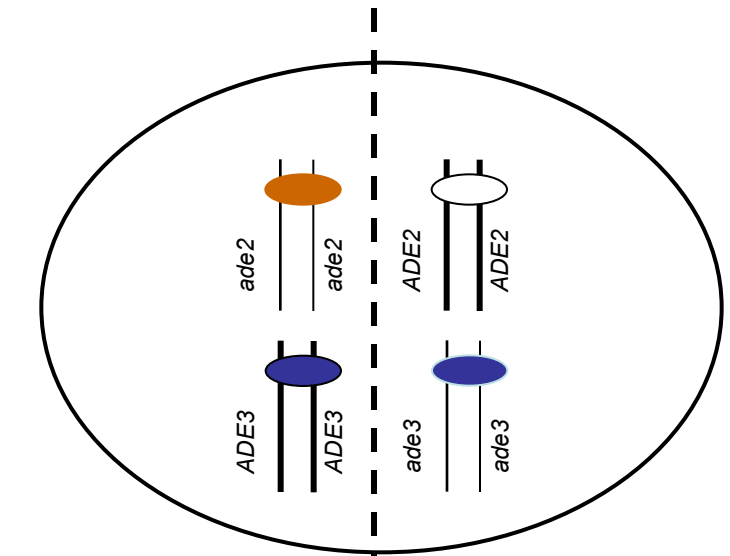
Diploid is white, Ade<sup>+</sup>



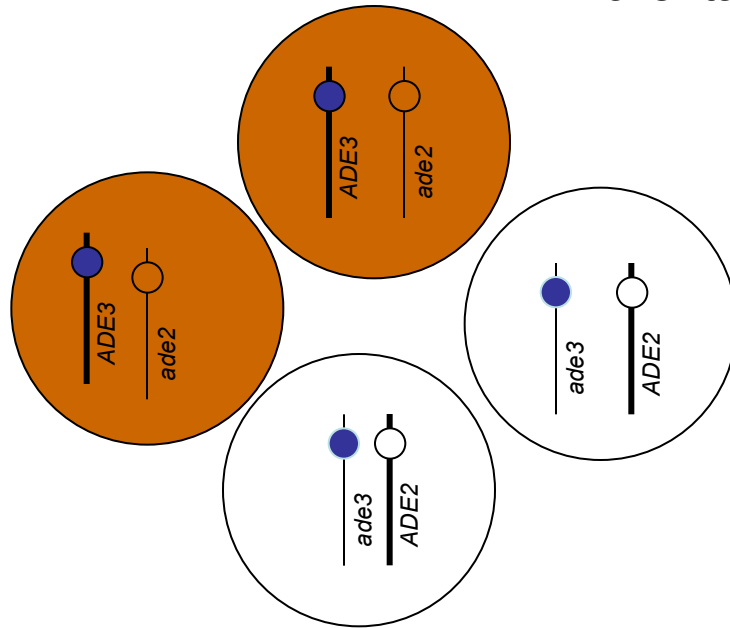
sporulate



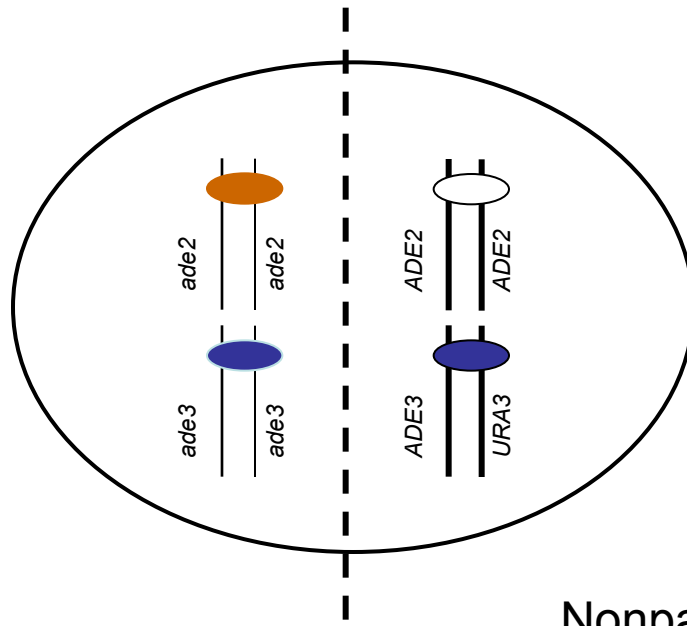
# Possible distribution of chromosomes during meiosis



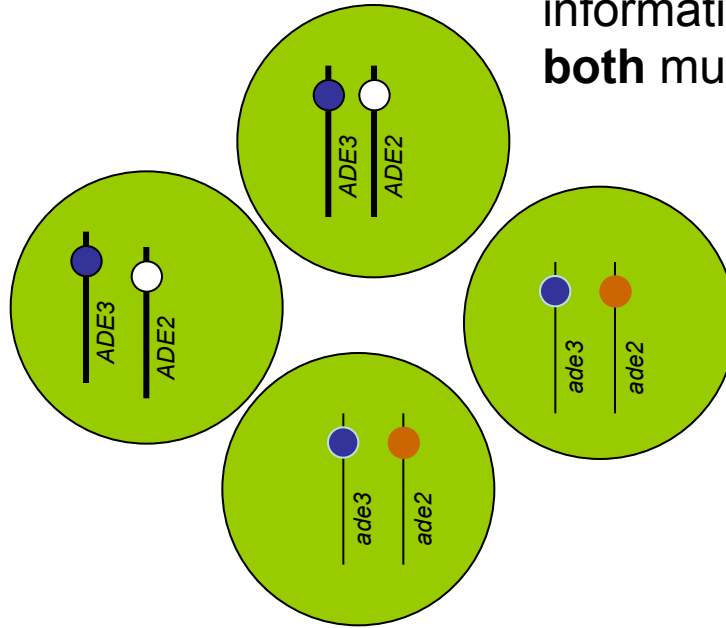
Parental Ditype - uninformative



All Ade<sup>-</sup>

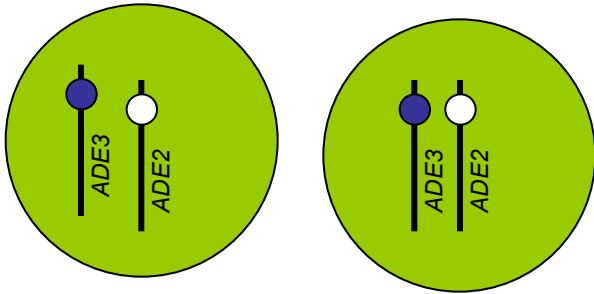


Nonparental Ditype –  
informative (two spores carry  
**both** mutations)

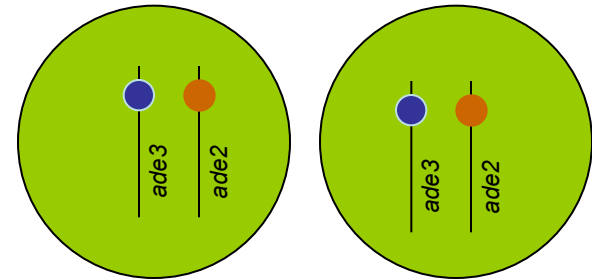


Two Scenarios

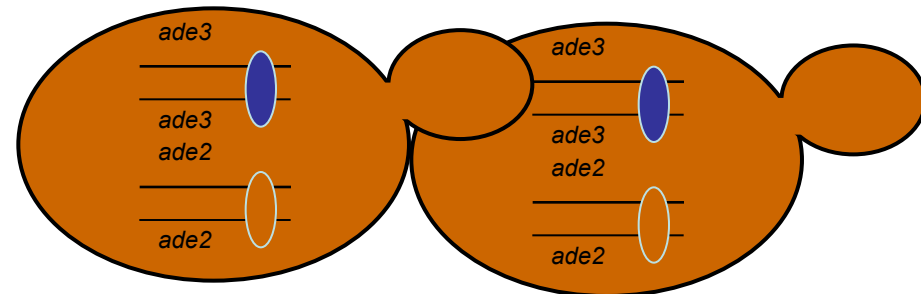
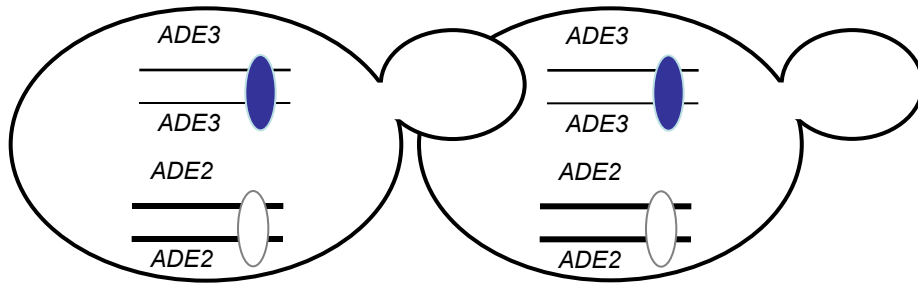
# Scenario 1



2 x Ade<sup>+</sup>,  
white

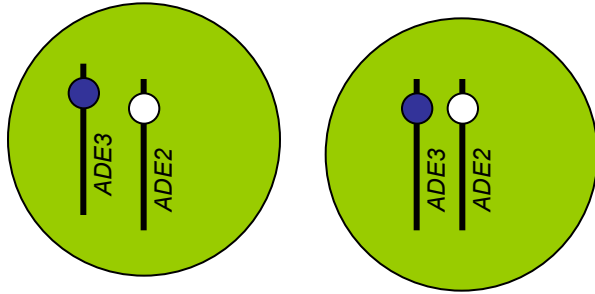


Double mutant, Ade<sup>-</sup>,  
**RED**

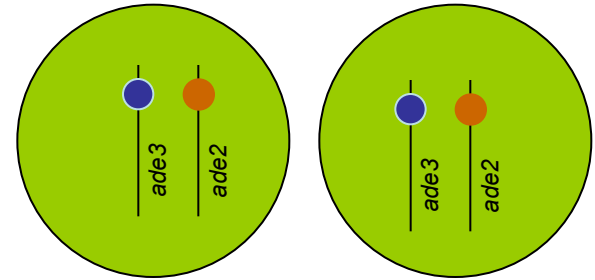


The *ADE2* gene product catalyzes a reaction upstream of the *ADE3* gene product. A mutation of *ade2* blocks adenine synthesis at a point where the intermediate is a red pigment

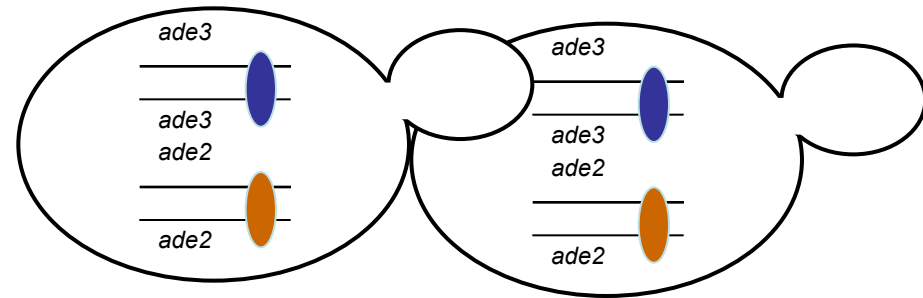
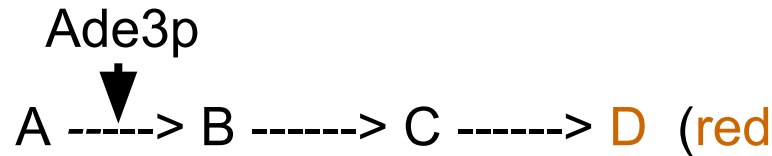
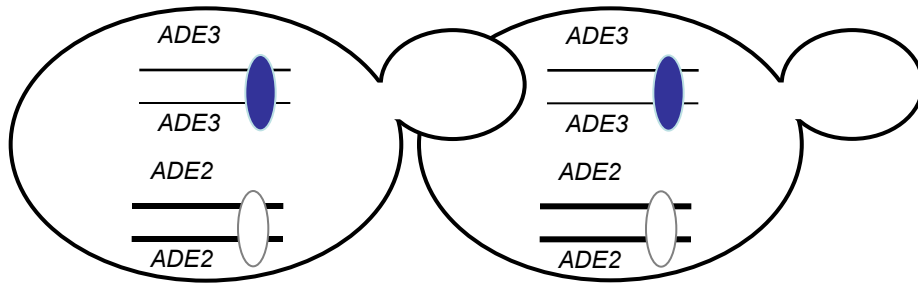
## Scenario 2



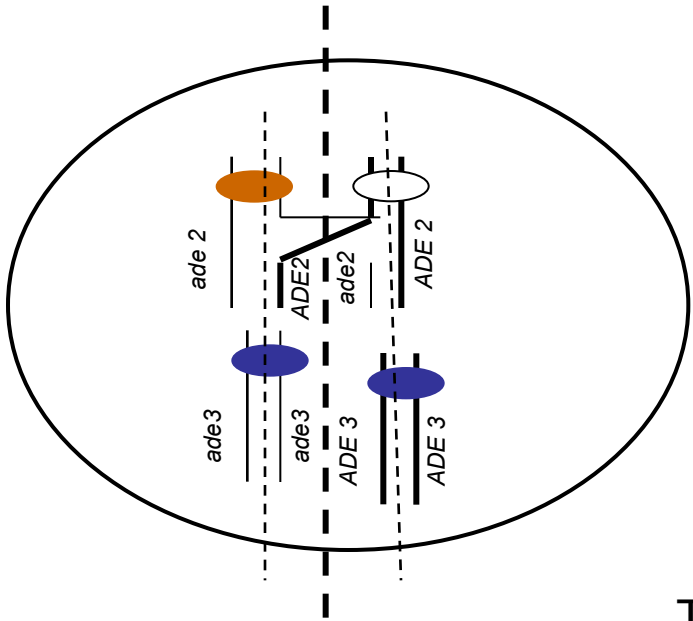
2 x Ade<sup>+</sup>,  
white



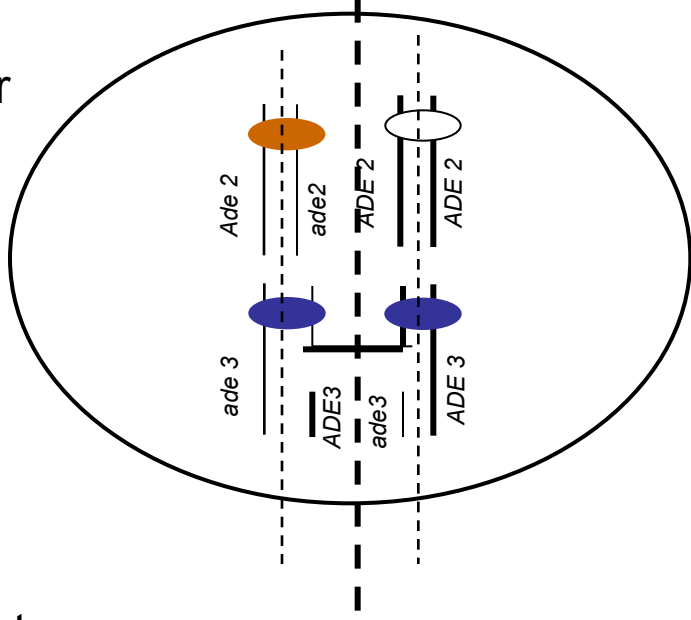
Double mutant  
2x Ade<sup>-</sup>, WHITE



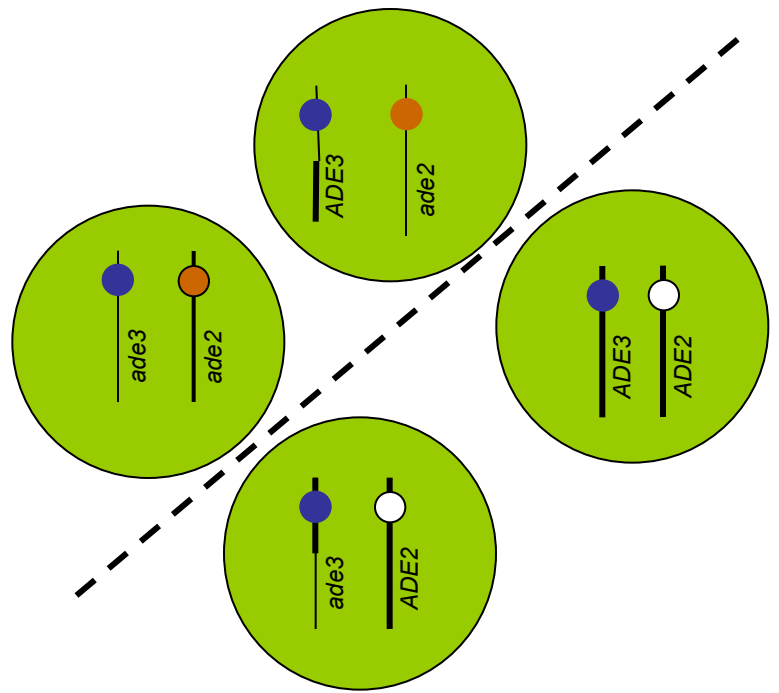
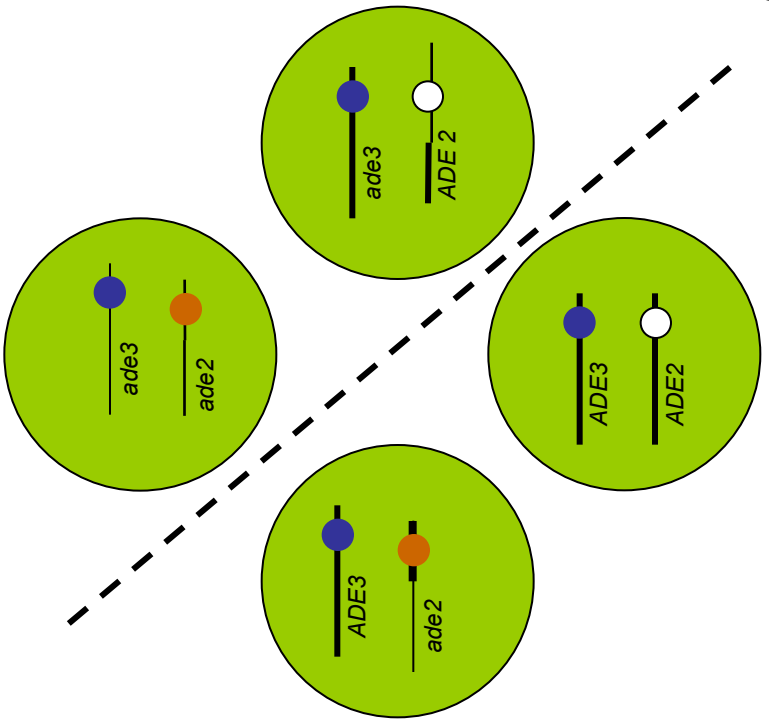
The *ADE3* gene product catalyzes a reaction upstream of the *ADE2* gene product. A mutation of *ade3* blocks adenine synthesis at a point upstream of the formation of the red pigment. The cells are white.



or

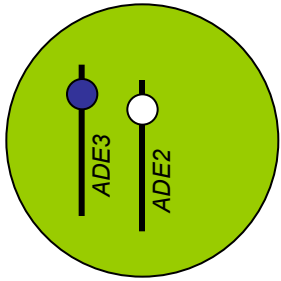


Tetratype

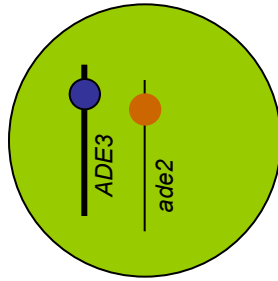


# Scenario1

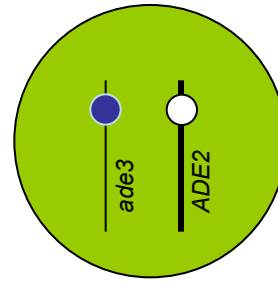
# Tetratype



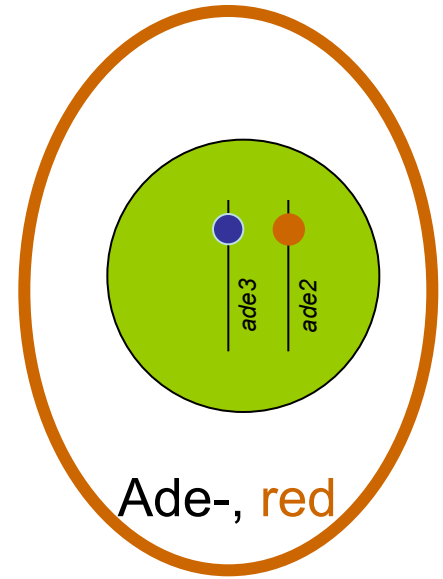
Ade+, white



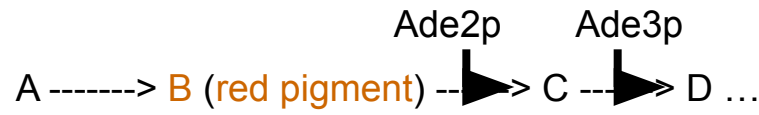
Ade-, red



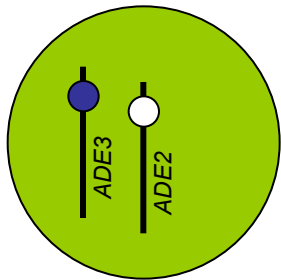
Ade-, white



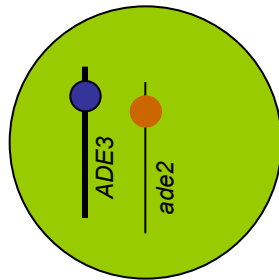
Ade-, red



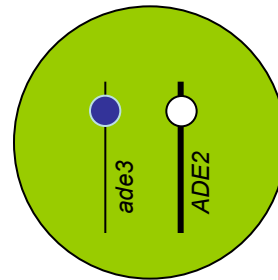
# Scenario2



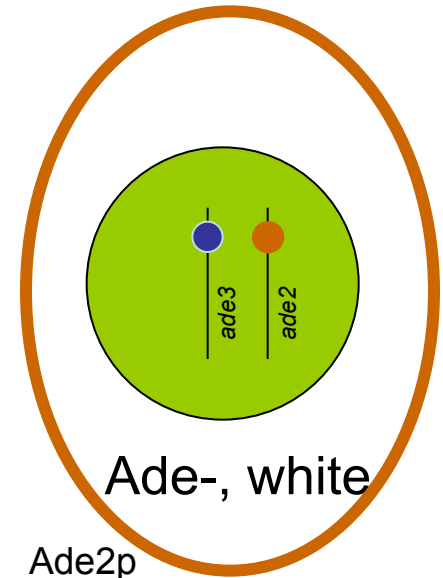
Ade+, white



Ade-, red



Ade-, white



Ade-, white



# The Adenine Biosynthesis pathway

