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:
  - Desireable traits of naturally occuring 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	Milestones
6000-2000 BC	Brewing (Sumeria, Babylonia)
1680	Yeast under the microscope (van Leeuwenhoek)
1835	Alcoholic fermentation associated with yeast
1837	Name (S.cerevisiae) created for yeast observed in malt
1839	Sugar as a food source for yeast growth
1857	Fermentation correlated with metabolism (Pasteur)
1876	'Etudes sur la levure de bière' (Pasteur)
1877	Term "enzyme" (in yeast) introduced (Kühne)
1880	Single yeast cells and pure strains for brewing (Hansen)
1883	Alcohol and CO2 by cell-free extracts (Buchner)
1915	Production of glycerol
1920	Yeast physiology reviewed
1949	First genetic map (Lindegren); mating type system
1930-1960	Yeasts' taxonomy by Kluyver
1978	First transformation of yeast (Hinnen, Hicks & Fink)
1990-1994	First commercial pharmaceutical products from recombinant yeast (Hepatitis B vaccine)
1996	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)

http://biochemie.web.med.uni-muenchen.de/Yeast_Biol/

Pioneers of yeast genetics

- **Øjvind Winge** (1886-1964), Carlsberg laboratory, Kopenhagen: http://www.genetics.org/cgi/content/full/158/1/1

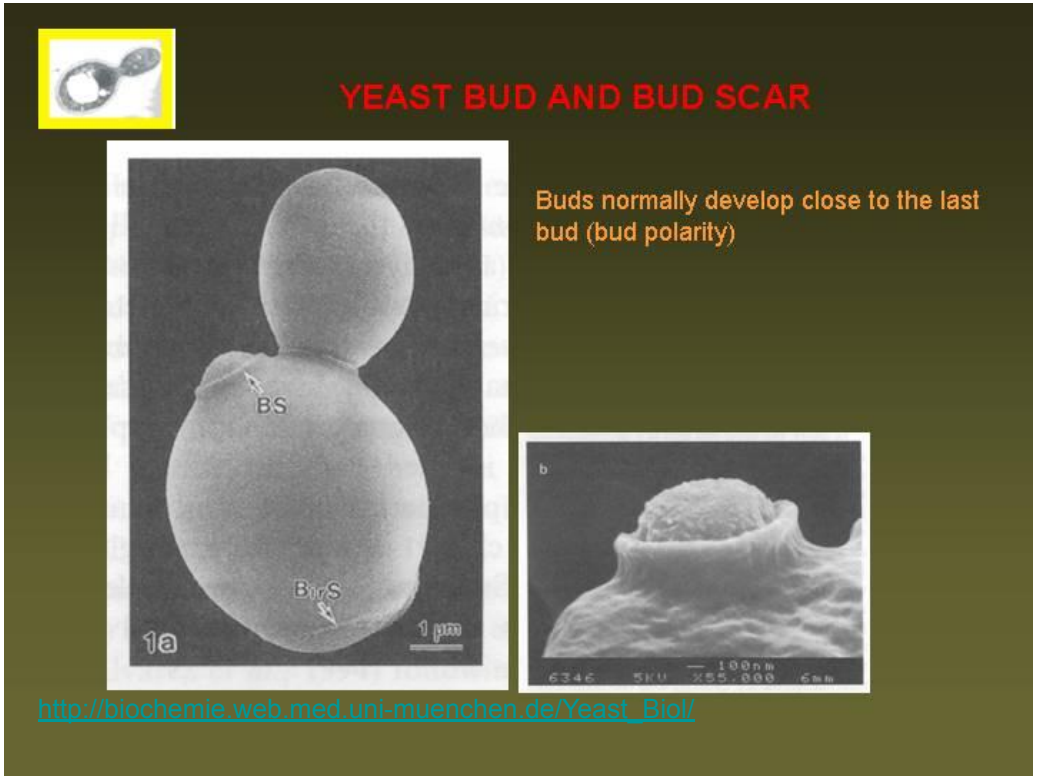
  Discovery of alternation of Haplo – and Diplophase in *Saccharomyces sp.* –"Yeast Sex"; development of mechanical yeast manipulation and dissection methods
- **Carl C. Lindegren** (1896-1987), Washington University, St. Louis; University of Southern Illinois, Carbondale, USA
  Isolation of heterothallic yeast strains (= mutant strains with a stable haploid growth phase)
- **Boris Ephrussi** (1901-1979), Institutes Pasteur, Paris; Centre national de la recherche scientifique, Gif-sur-Yvette, France

  Cytoplasmic inheritance (= mitochondrial genetics)

Baker's Yeast

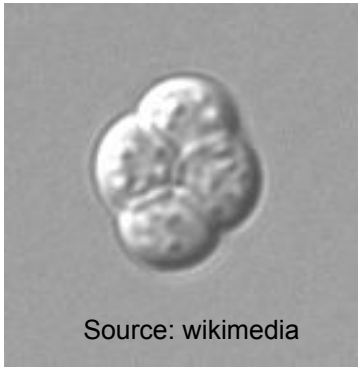
Saccharomyces cerevisiae:

- 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)!

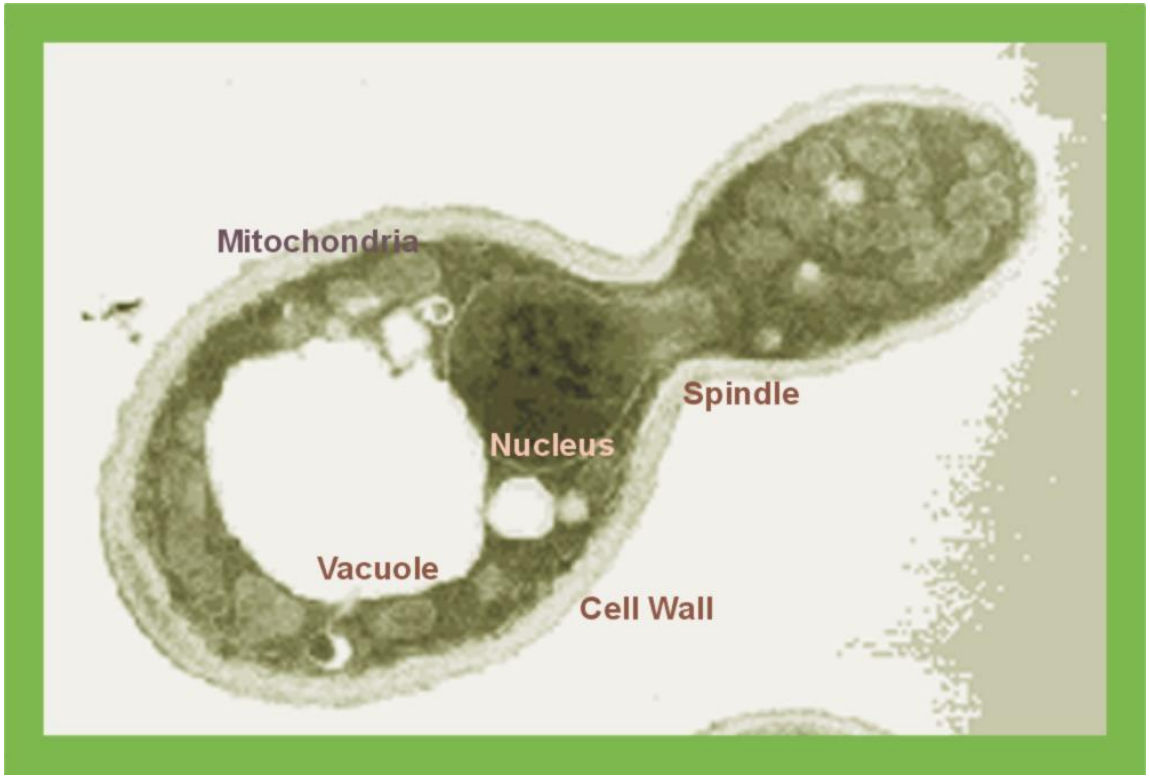
Yeast ascus with spore tertad



Yeast is a molecular biology model organism

Requirements for Model Organisms:

Yeast similarity to human cells



Mammalian homologs (based on *P* value)

P value	Number of ORFs at *P* value or lower	Percent of total ORFs (*n* = 6223)	Percent of ORFs with unknown function
1×10^{10}	1914	30.8	34
1×10^{20}	1553	25.0	30
1×10^{40}	1083	16.8	26
1×10^{60}	784	12.6	23
1×10^{80}	576	9.3	22
1×10^{100}	442	7.1	21
1×10^{150}	221	3.6	23
1×10^{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

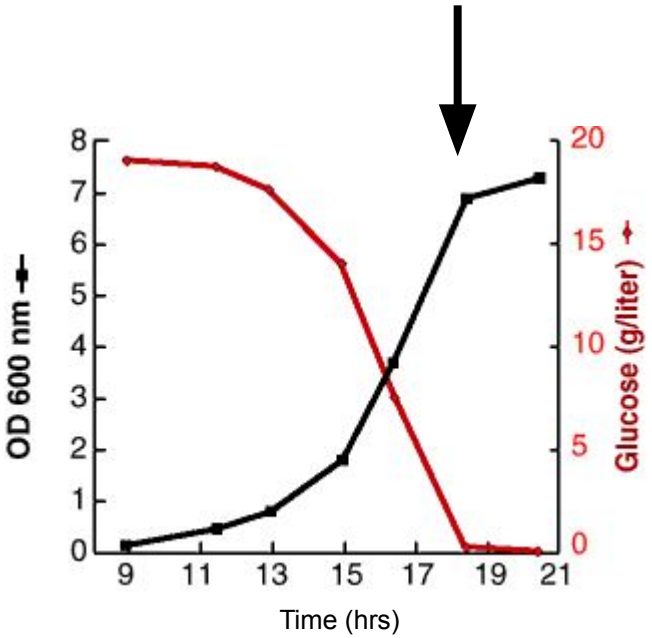
- Preferred carbon source: **glucose**, but many other carbon sources can be used
- If the carbon source allows, *S.cerevisiae* prefers to generate energy mainly by **alcoholic fermentation**
- **When glucose is in abundance, baker's yeast turns off all other pathways utilizing other carbon sources and 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

Fermentable Carbon Sources	Non-fermentable Carbon Sources
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) – containg 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
ARG^{+}	All wild-type alleles controlling arginine requirement
ARG2	A locus or dominant allele
arg2	A locus or recessive allele confering an arginine requirement
arg2^{-}	Any *arg2* 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 protein	The protein encoded by *ARG2*
ARG2 mRNA	The mRNA transcribed from *ARG2*
arg2-Δ*1*	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
arg2::LEU2	Insertion of the functional *LEU2* gene at the *ARG2* locus, and *arg2* is or became nonfunctional
arg2-10::LEU2	Insertion of the functional *LEU2* gene at the *ARG2* locus, and the specified *arg2-10* allele which is nonfunctional
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

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 lecure

Classical yeast genetics

Pre-molecular biology

Cloning the Wild-type Gene by Complementation

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

Isolate Ura$^+$ transformants and score for Yfg$^+$

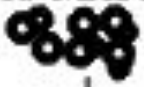
Yfg$^+$

Recover the YCp-*YFG1*$^+$ plasmid in *E. coli*

Analyze the plasmid by digestion with restriction endonucleases and DNA sequencing

Mutant Isolation

Mutagenesis of a haploid *MAT***a** strain



Detection of Yfg$^-$

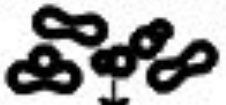
Yfg$^-$

Complementation

Cross the Yfg$^-$ *MAT***a** mutant to *MAT*α tester strains. Isolate diploid strains. Score for Yfg$^+$ and Yfg$^-$

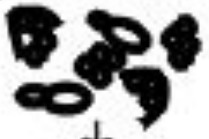
Meiotic Analysis

Cross mutant to *MAT*α *YFG*$^+$



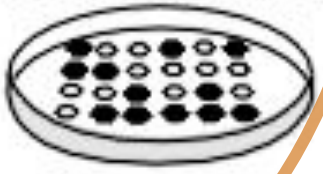
Isolate a diploid strain and Sporulate

Digest ascus walls

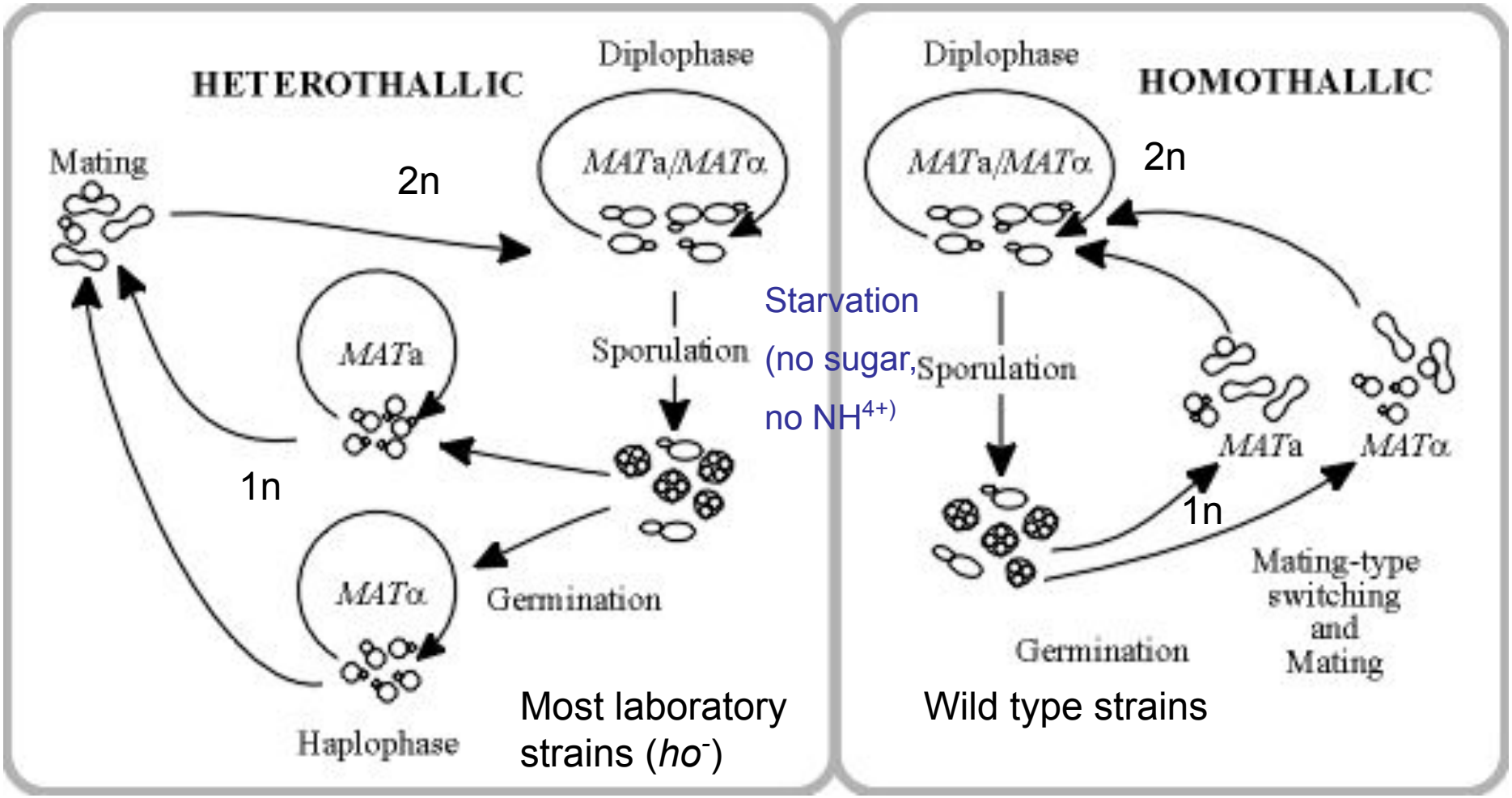


Dissect 4 spores of each tetrad

Score for Yfg$^+$ and Yfg$^-$



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

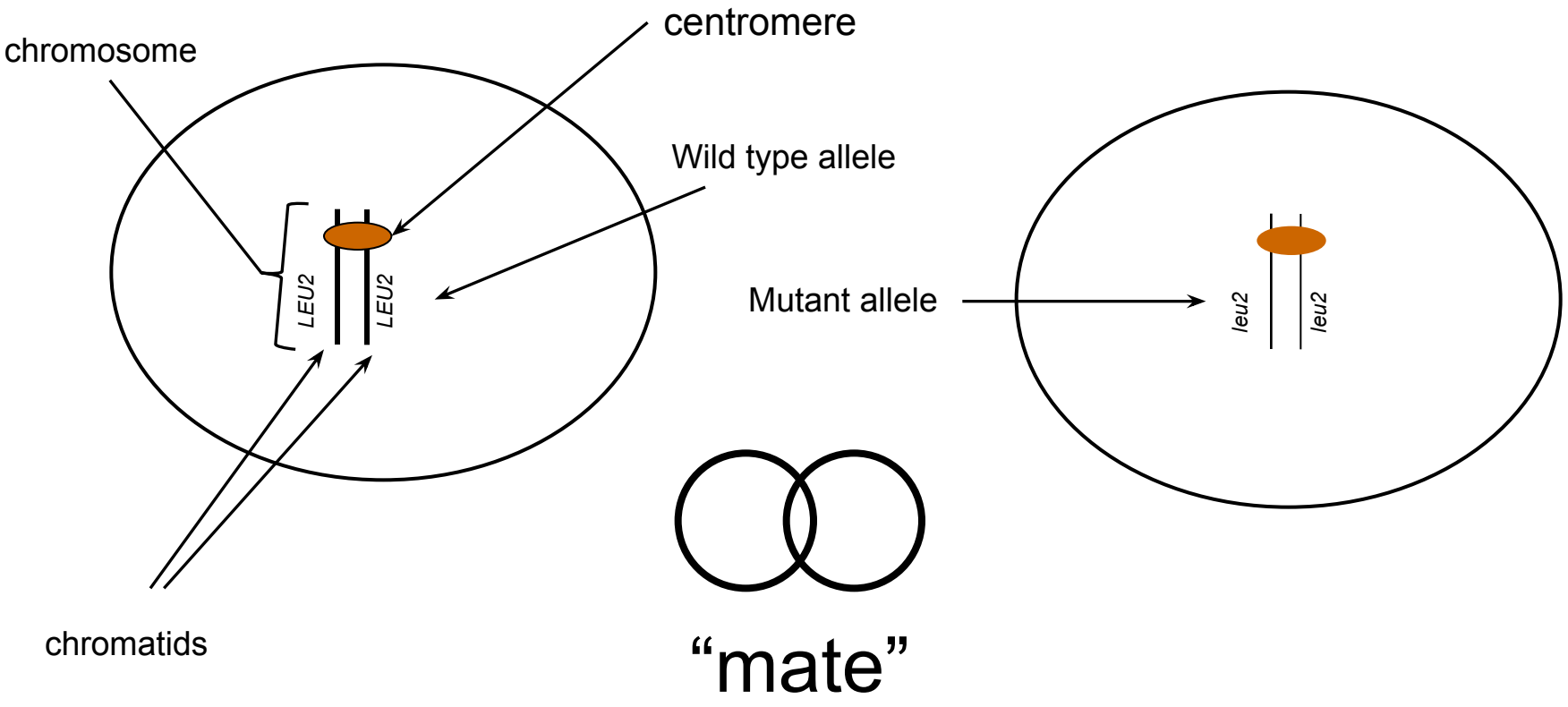
Genetic analysis of a simple mutation

“Wild type” strain (Leu+)

LEU2: functional wild type allele

“mutant” strain (Leu-)

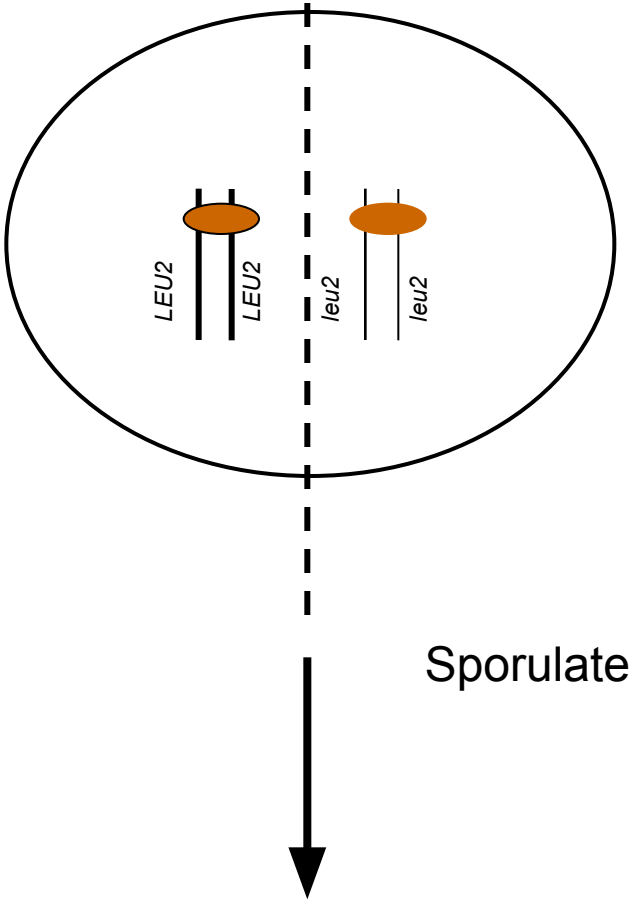
leu2: non-functional, recessive mutant allele



Segregation of two alleles involved in Leucine biosynthesis

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

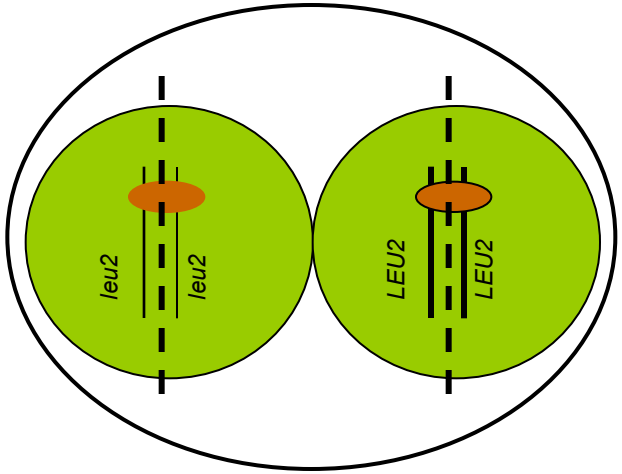
Diploid = Zygote



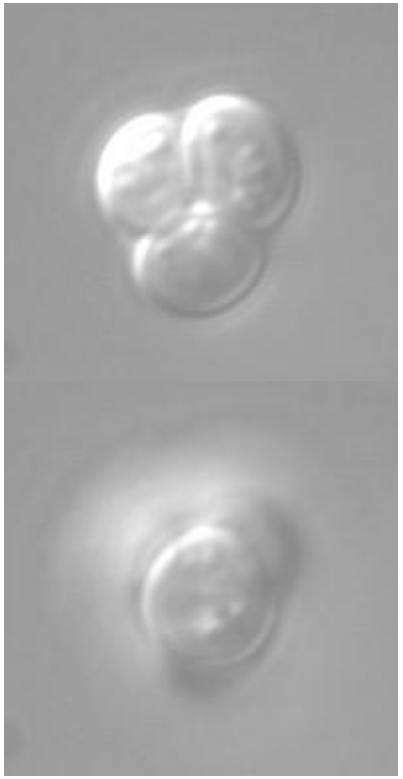
Sporulate on acetate medium

(Meiosis)

Meiosis 1: separation of the homologous chromosomes

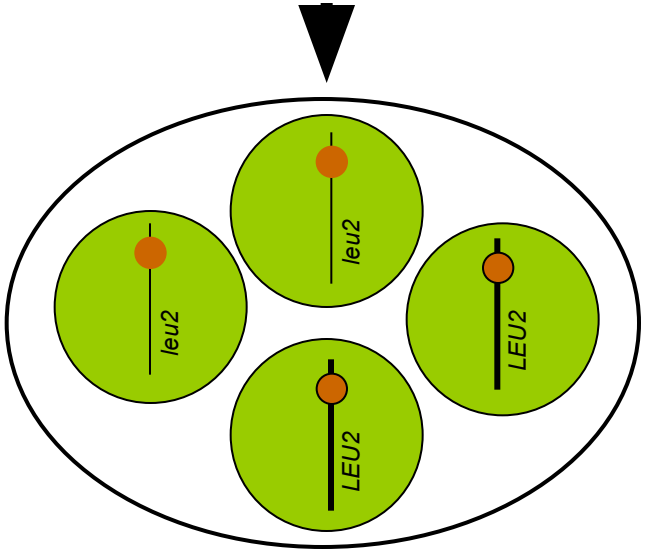


Meiosis 2:
separation of the
chromatids



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

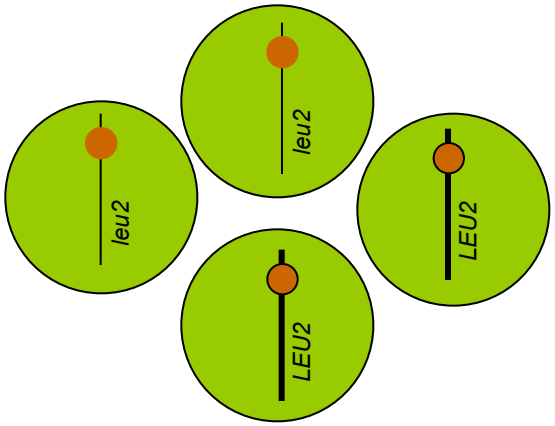
4 spores of a tetrad



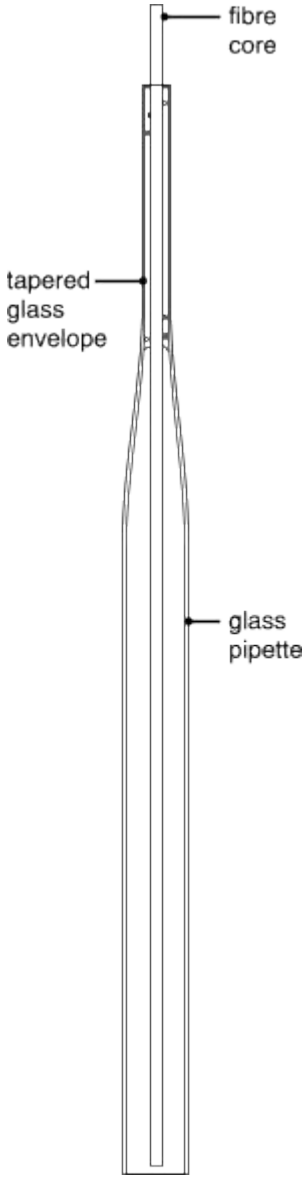
Ascus = spore sac

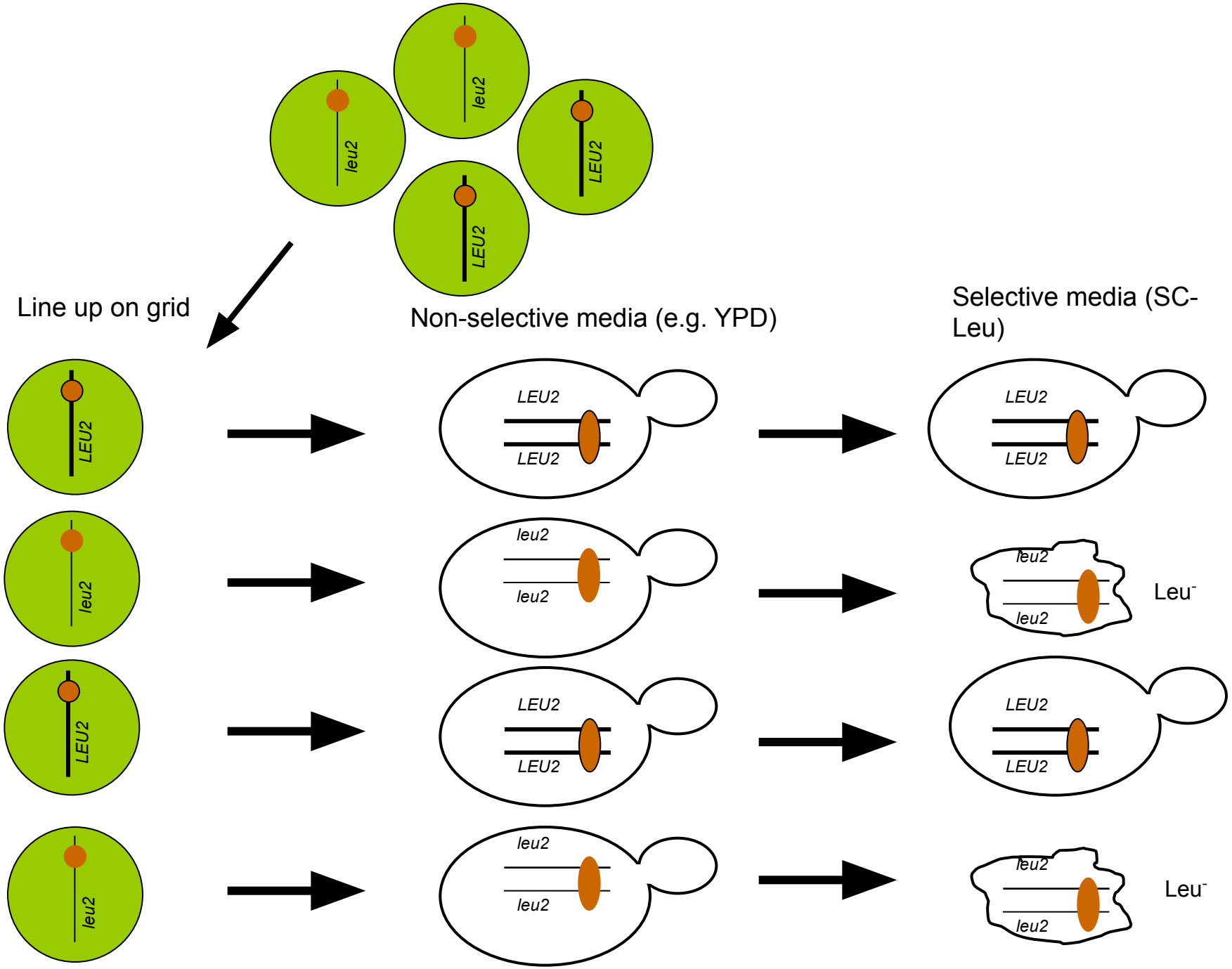
Spores = Gametes!

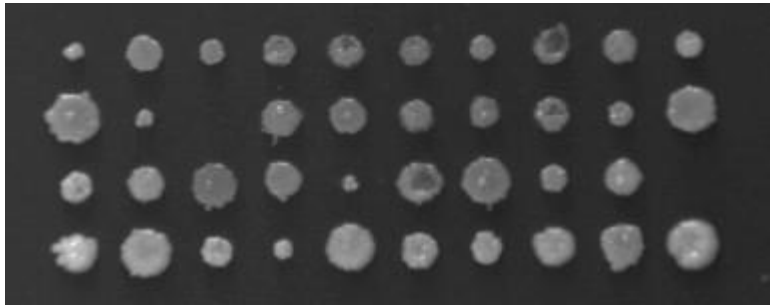
Digest off cell wall



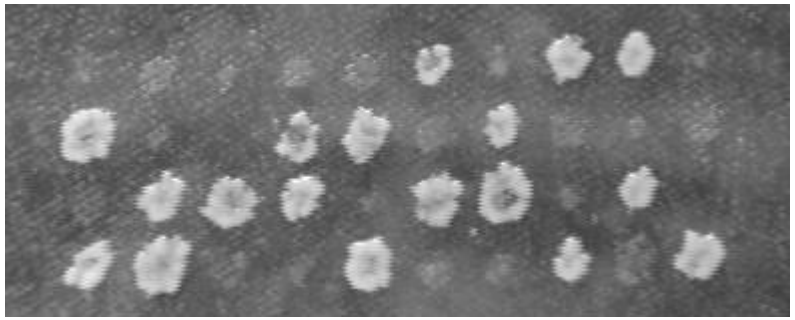
Dissect ascospores!


fibre
core
tapered
glass
envelope
glass
pipette


leu2
leu2
LEU2
LEU2
Line up on grid
Non-selective media (e.g. YPD)
Selective media (SC-Leu)
LEU2
LEU2
LEU2
LEU2
LEU2
LEU2
leu2
leu2
leu2
leu2
Leu-
LEU2
LEU2
LEU2
LEU2
LEU2
LEU2
leu2
leu2
leu2
leu2
Leu-



Original Dissection on Non-selective plate



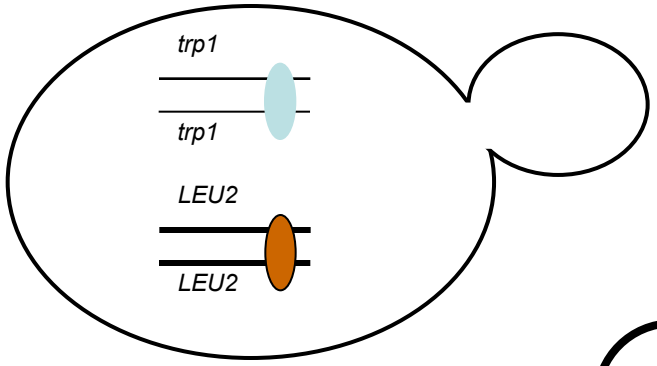
Replica on selective plate (e.g. Leu^- strain on SC – Leucine)

2 : 2 segregation ratio

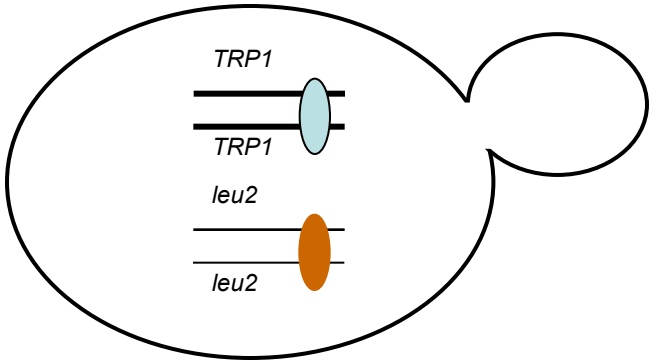
(Leu^+ vs. Leu^- **spores**)

Segregation of **two** unlinked genes

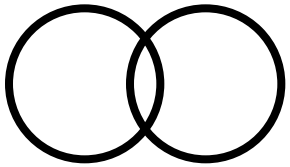
Example: *TRP1*, *LEU2*

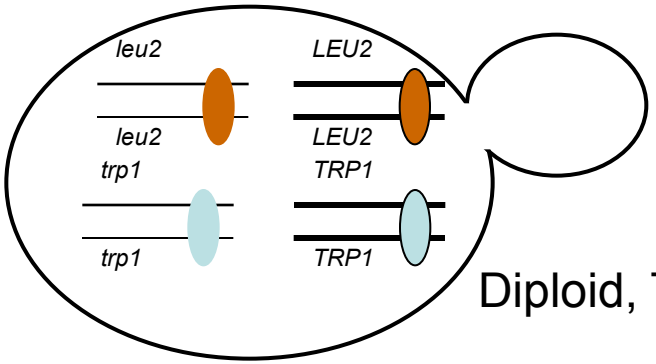


Haploid,
Leu$^+$, Trp$^-$

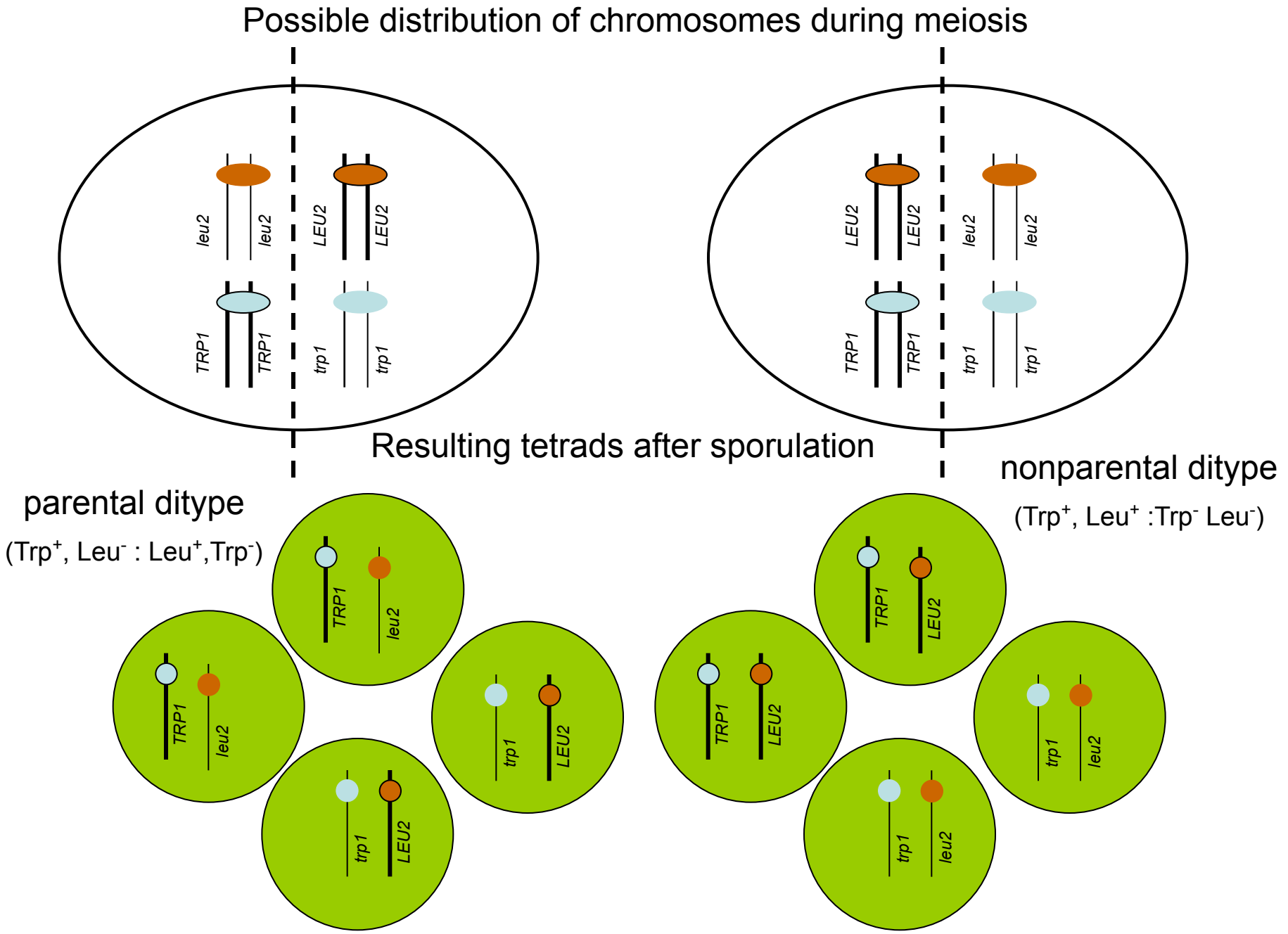


Haploid,
Leu$^-$, Trp$^+$



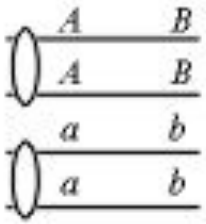
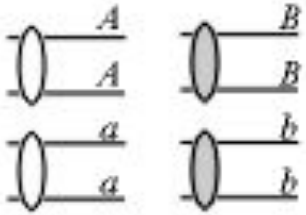
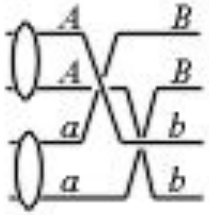
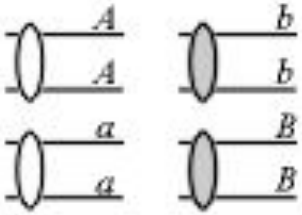
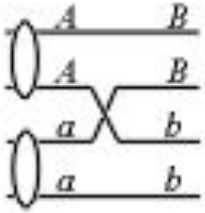
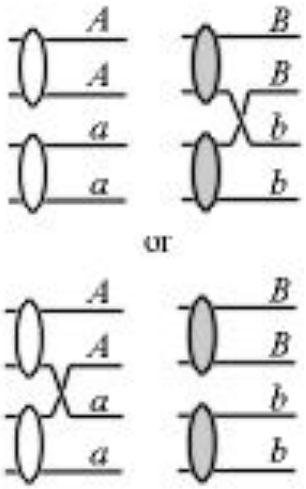


Diploid, Trp$^+$, Leu$^+$,


Possible distribution of chromosomes during meiosis
leu2
leu2
LEU2
LEU2
TRP1
TRP1
trp1
trp1
LEU2
LEU2
leu2
leu2
TRP1
TRP1
trp1
trp1
Resulting tetrads after sporulation
parental ditype
(Trp+, Leu- : Leu+,Trp-)
nonparental ditype
(Trp+, Leu+ :Trp- Leu-)
TRP1
leu2
TRP1
leu2
trp1
LEU2
trp1
LEU2
TRP1
LEU2
TRP1
LEU2
trp1
leu2
trp1
leu2

or

Tetratype

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

	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:

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

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)

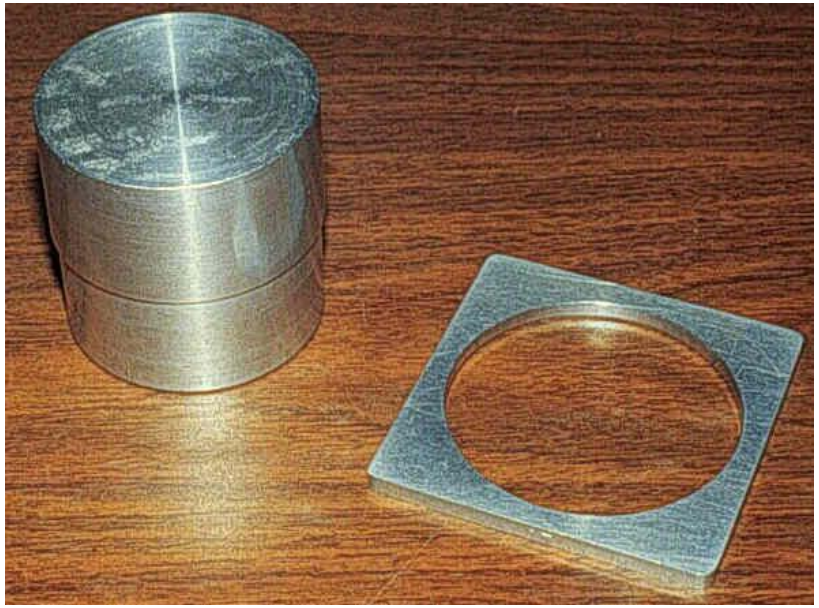
Dissecting Metabolic Pathways in Yeast

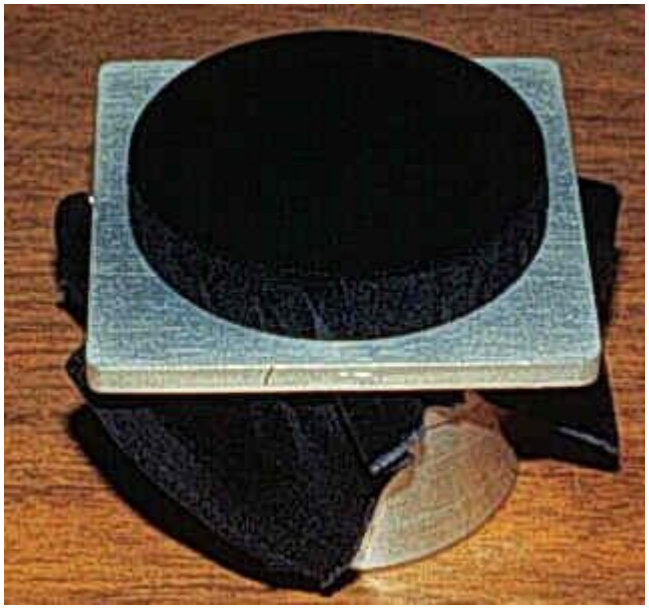
- Question: What enzymes are involved in the Biosynthesis of Uracil?

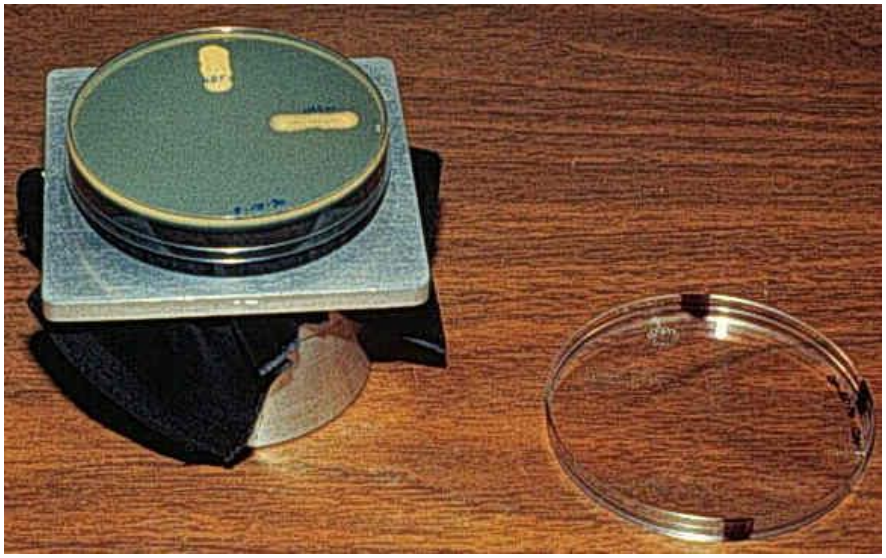
Approach: Screening for mutants dependent on uracil in the growth media

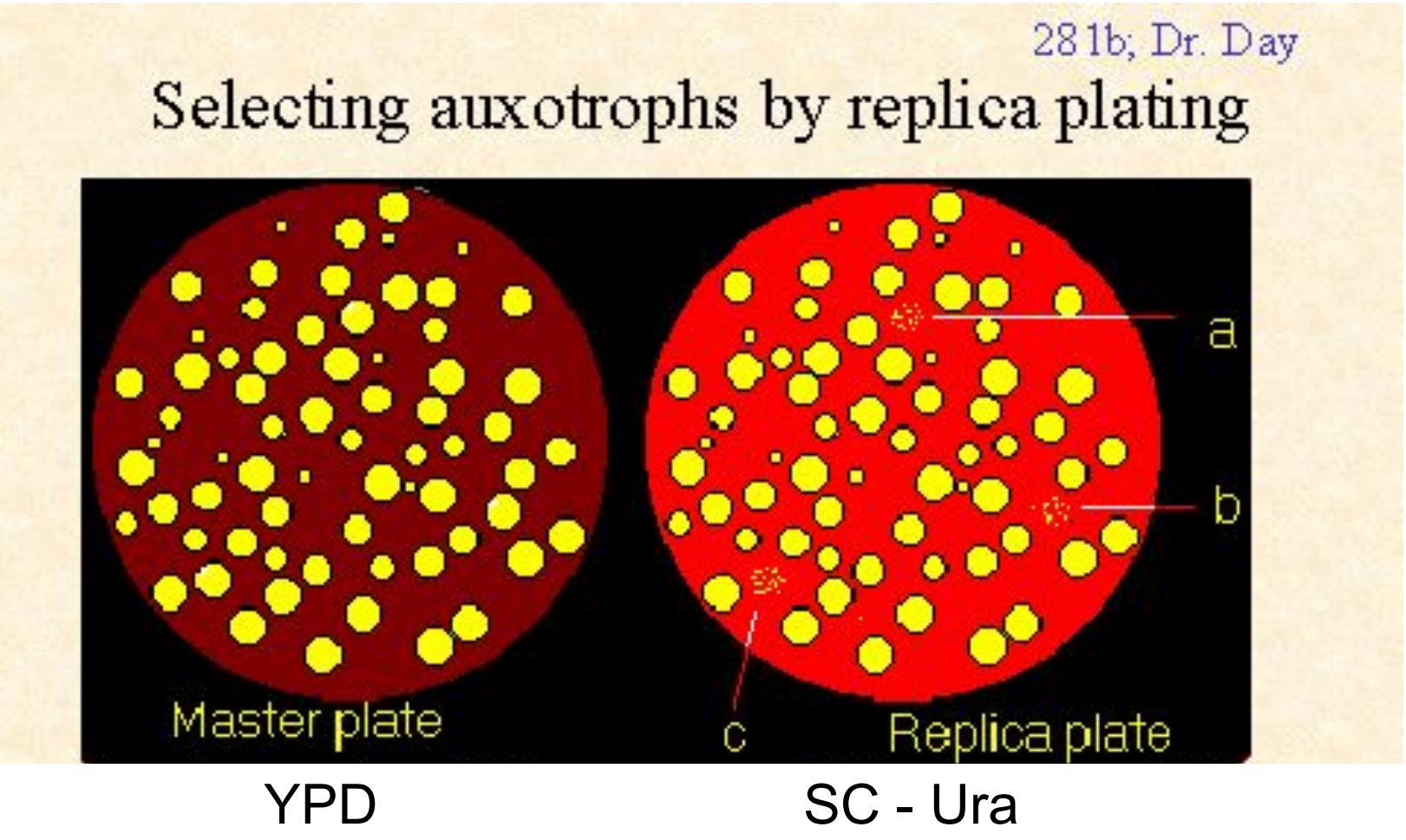
- Mutagenize a healthy yeast strain (UV light, alkylating agents)
- Plate mutagenized cells on non-selective media
- Replica plate onto synthetic media lacking uracil (SC – Ura)

Replica plating:









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

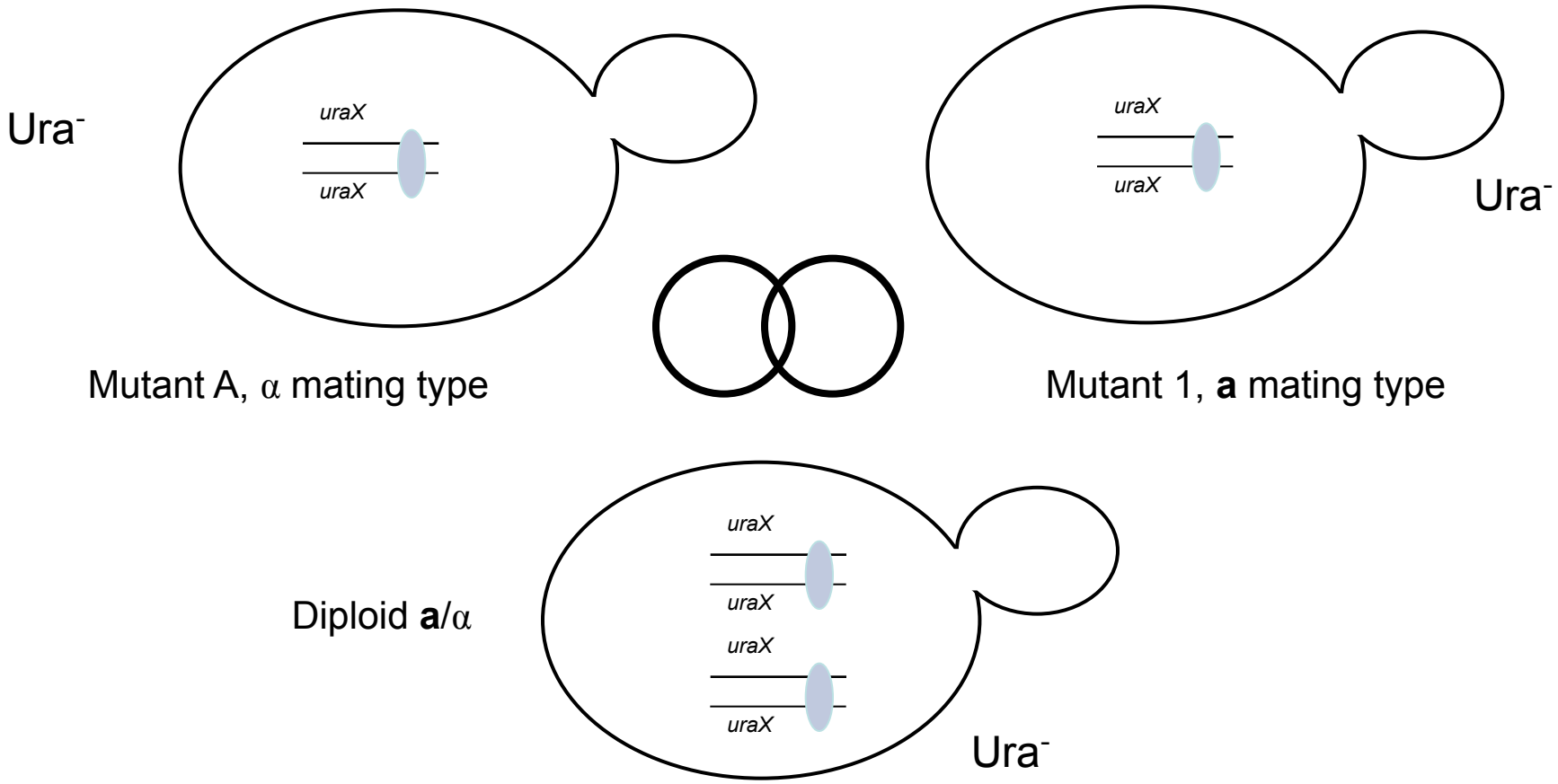
- In our hypothetical screen, we have identified several haploid mutants in the uracil biosynthesis pathway in both mating types
- To test if the mutations are in the same pathway, we carry out **Complementation analysis**

⇒ Mutants are mated against each other

⇒ If the mutants are in the same gene, they will not complement each other an 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

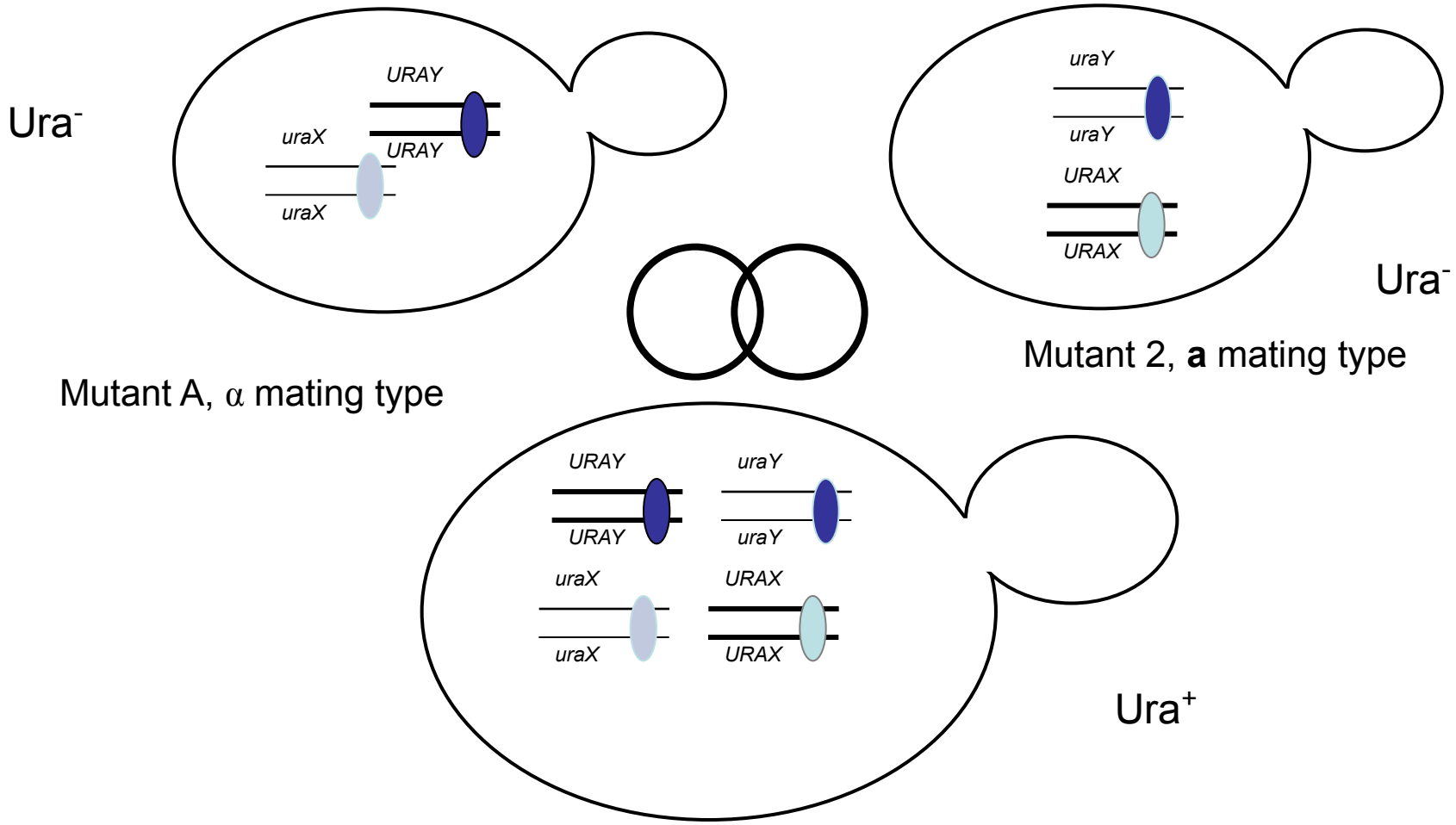
Complementation analysis Scenario 1: mutations are in the same gene



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

Conclusion: Mutant A (α) and mutant 1 (**a**) are in the same gene *ura*X; as there is no functional copy of *uraX* in the cells, they are uable to synthesize uracil;

Complementation analysis Scenario 2: mutations are in different genes



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

Conclusion: Mutant A (α) and mutant 2 (**a**) are in different genes *ura*X and *ura*Y; 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

α^{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

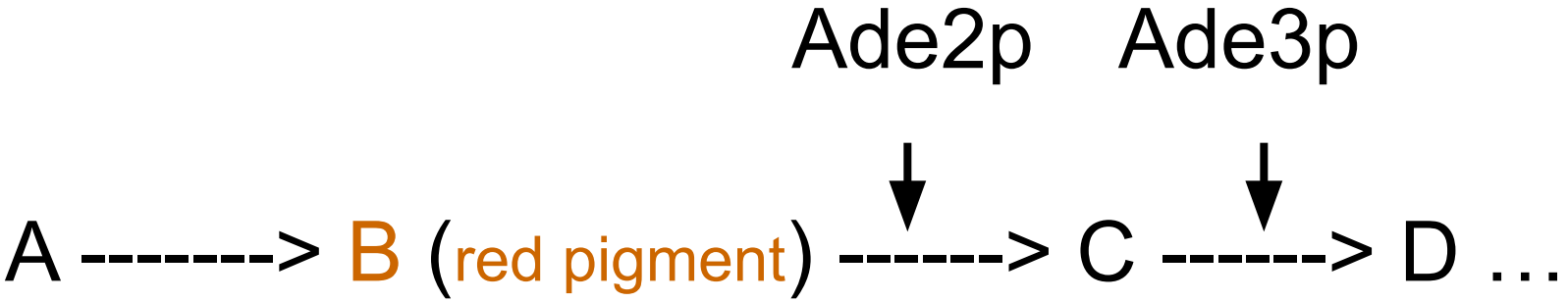
Epistasis - the interaction between two or more genes to control a single phenotype

Epistatic Analysis: determine the order and/or relation ship of genes in a pathway

Example of Epistatic analysis

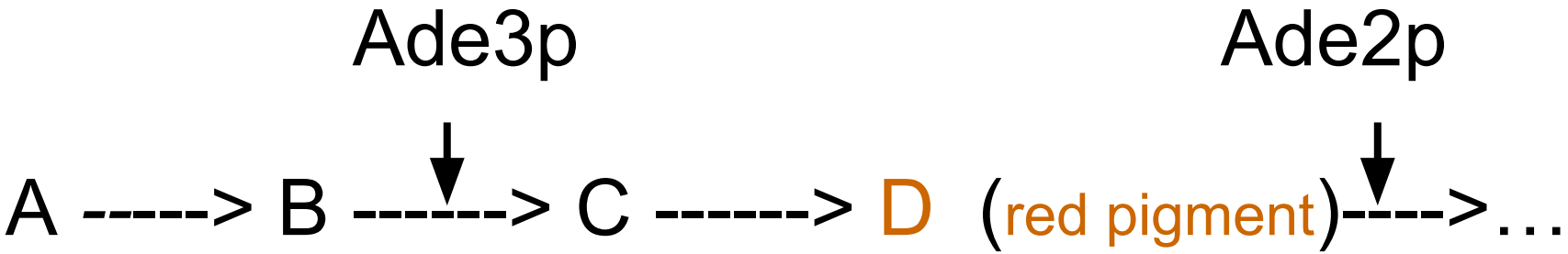
- Example: Adenine biosynthesis mutants *ade2* and *ade3* (unlinked genes):
- *ade2* mutants are Ade-, make red colonies
- *ade3* mutants are Ade-, make white colonies
- Double mutant will reveal position of genes/gene products in the adenine biosynthesis pathway relative to each other

Two possibilities of order of action of Ade2p and Ade3p in this pathway:

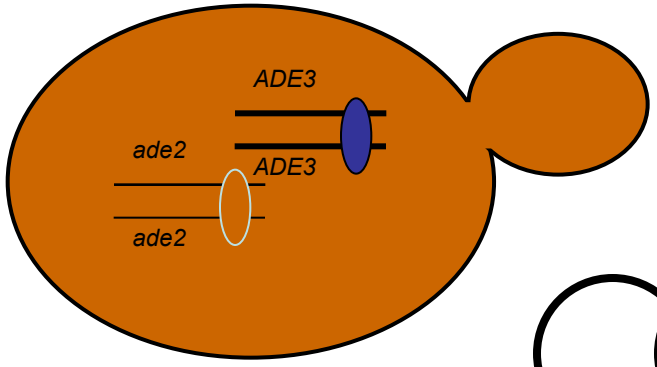


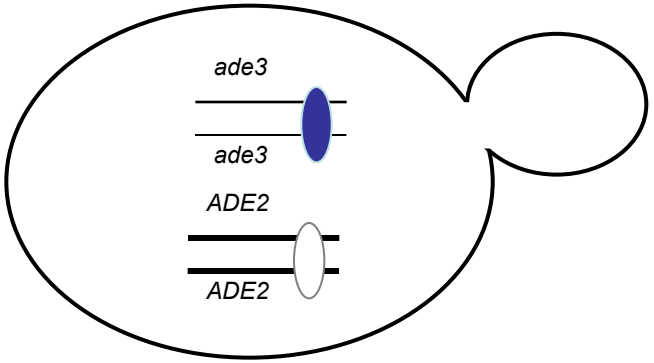
Scenario I

or



Scenario II



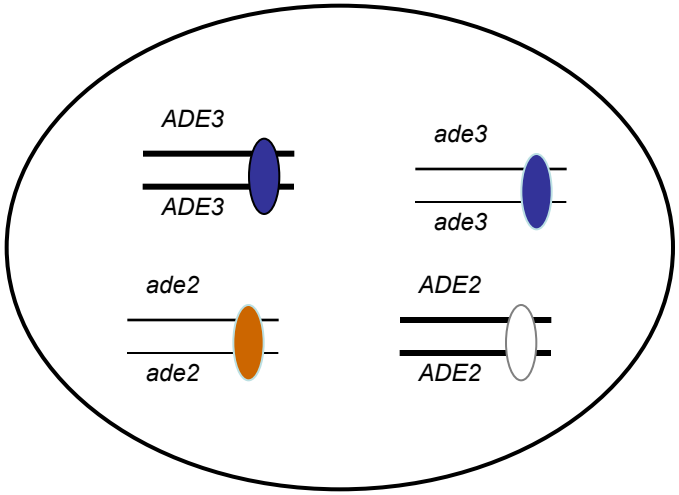


ade2 mutant, α mating type

Ade $^{-}$, RED

ade3 mutant, **a** mating type

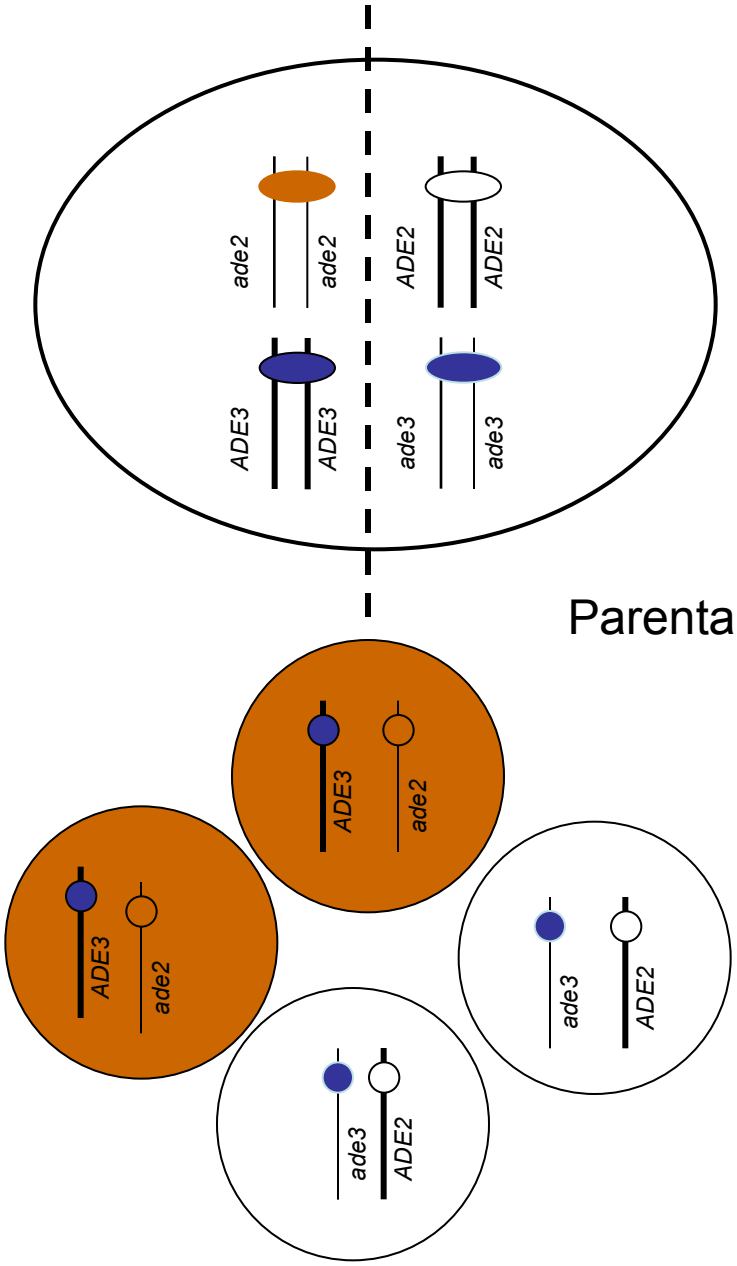
Ade^{-}, creamy white



Diploid is white, Ade^{+}

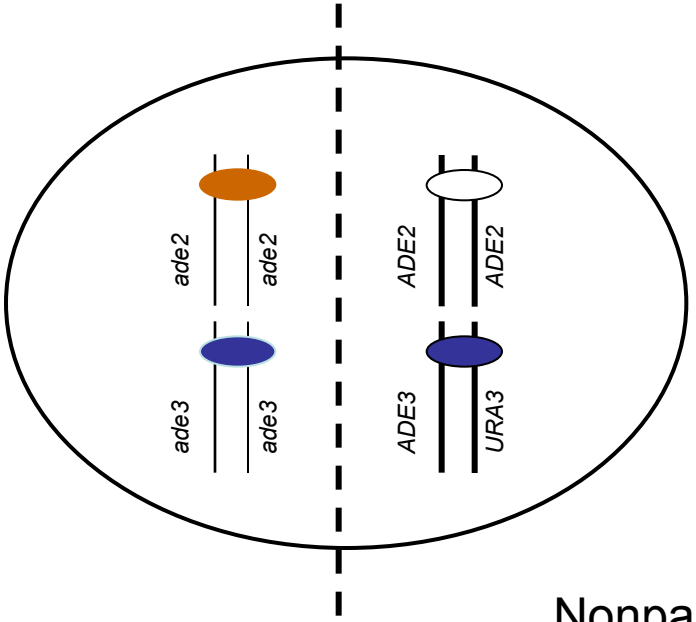
sporulate

Possible distribution of chromosomes during meiosis

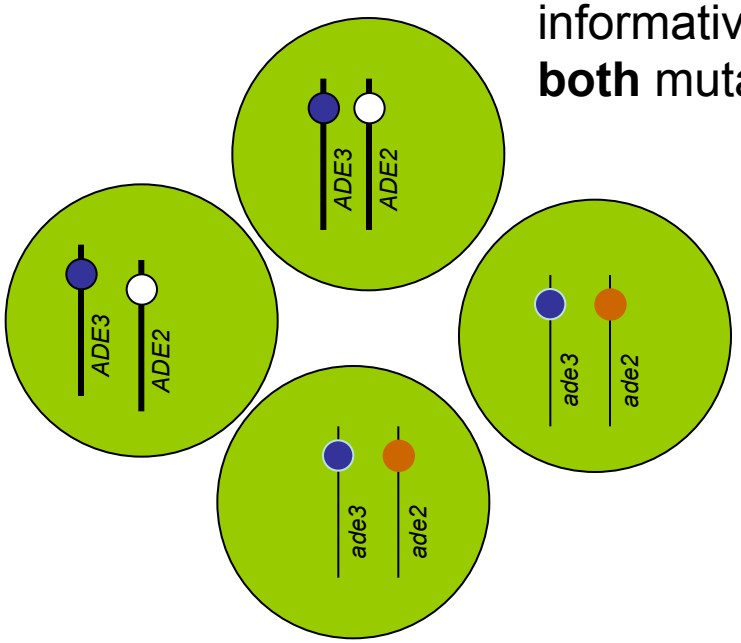


Parental Ditype - uninformative

All Ade^{-}

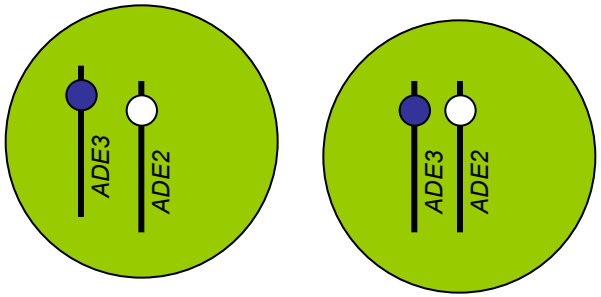


Nonparental Ditype – informative (two spores carry **both** mutations)

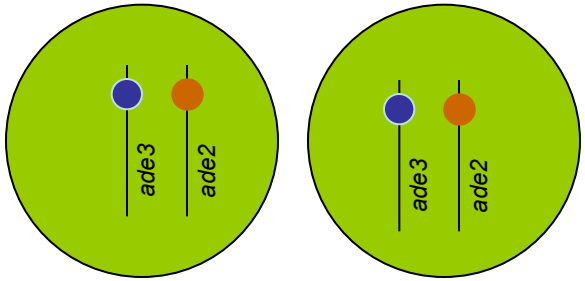


Two Scenarios

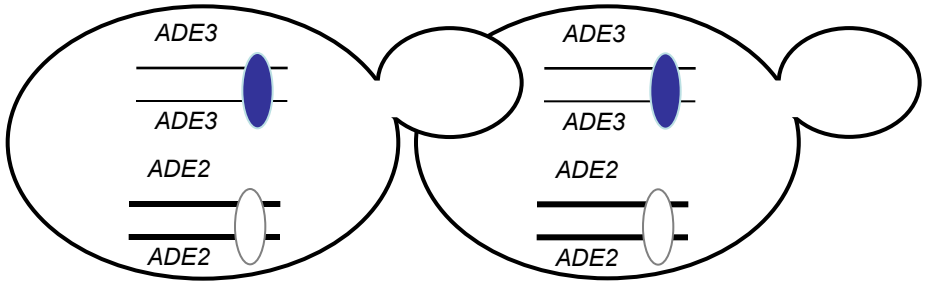
Scenario 1

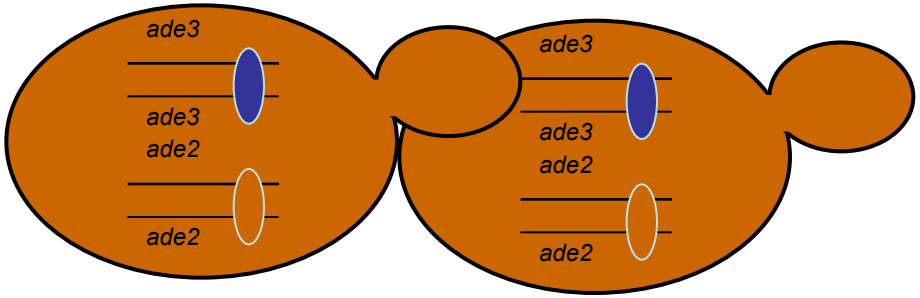


2 x Ade$^+$, white



Double mutant, Ade-, RED



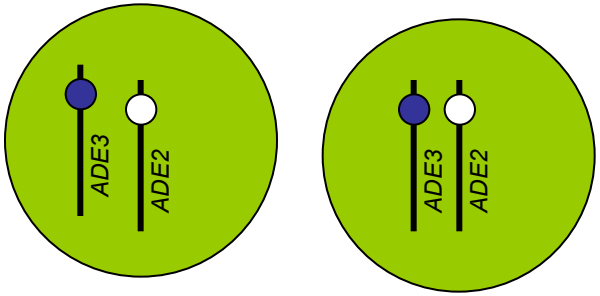


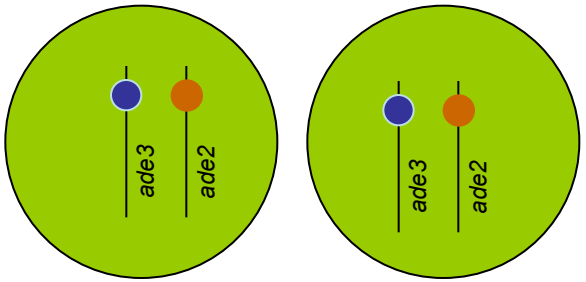
Ade2p Ade3p

A -------> B (red pigment) ------> C ------> D …

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

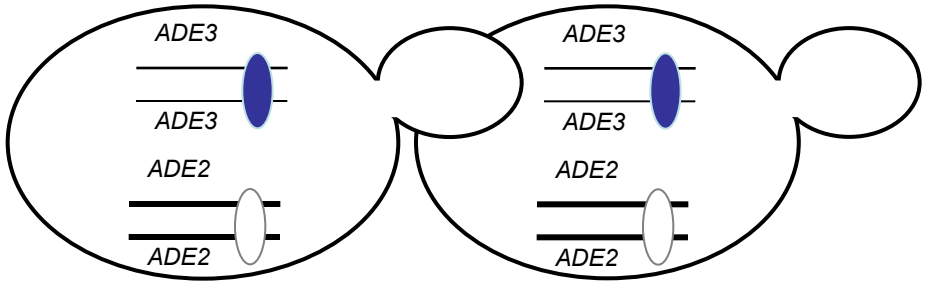
Scenario 2

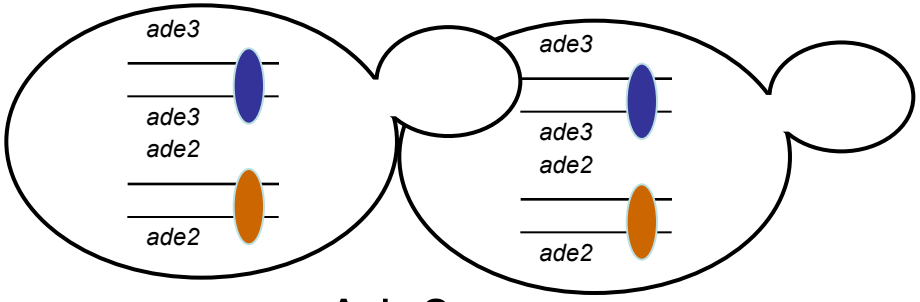




2 x Ade^{+}, white

Double mutant 2x Ade-, WHITE



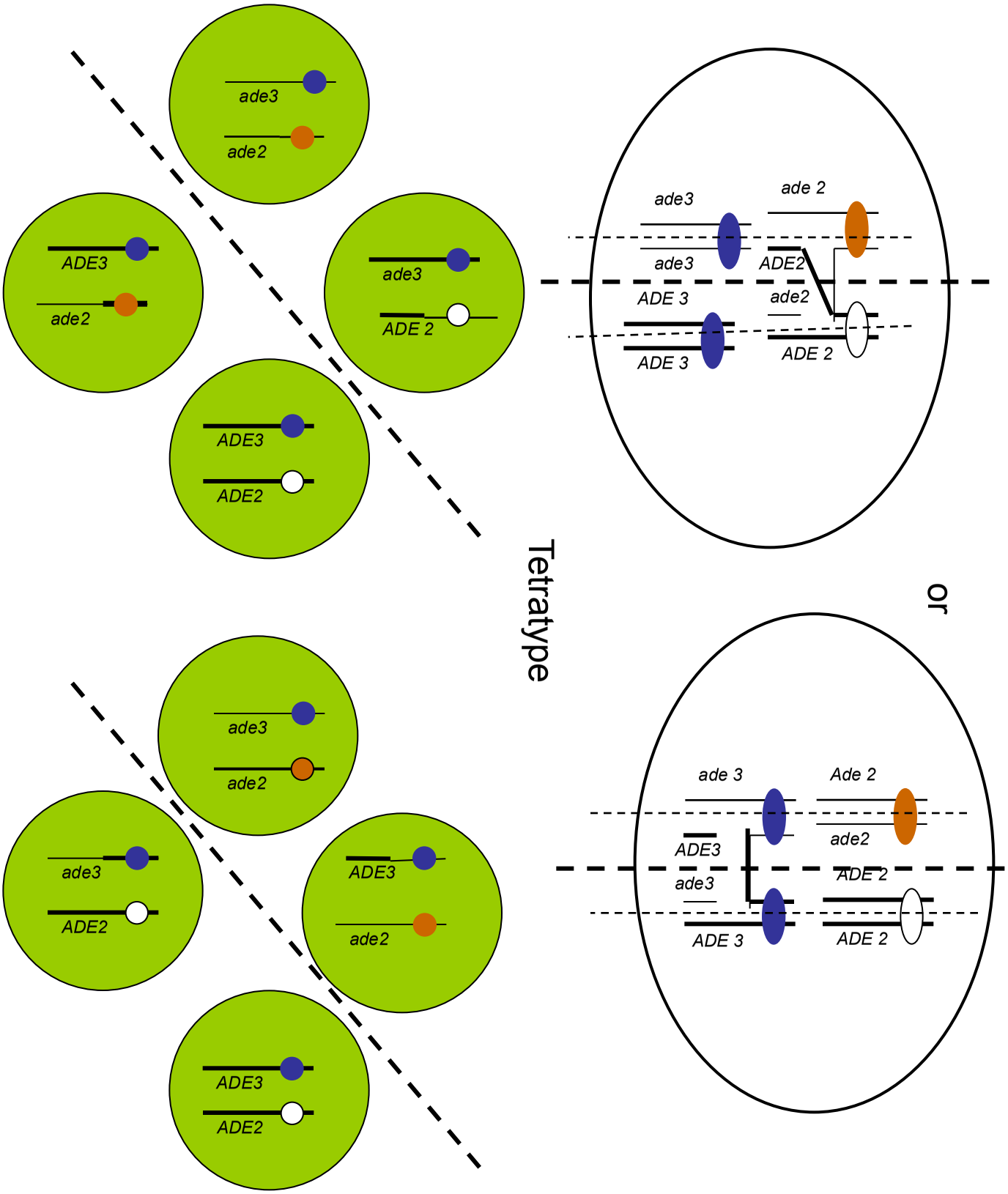


Ade3p

Ade2p

A -----> B ------> C ------> D (red pigment)

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.


ade3
ade2
ADE3
ade2
ade3
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ADE3
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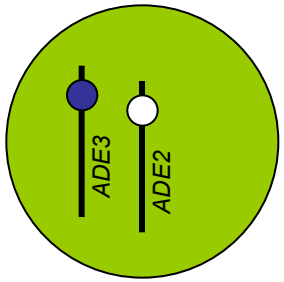
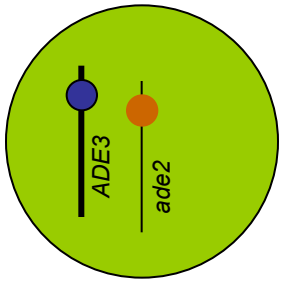
ade3
ade2
ade3
ade 2
ade3
ADE2
ADE 3
ade2
ADE 3
ADE 2
Tetratype
or
ade3
ade2
ade3
ADE2
ADE3
ade2
ADE3
ADE2
ade 3
Ade 2
ADE3
ade2
ade3
ADE 2
ADE 3
ADE 2

Scenario1

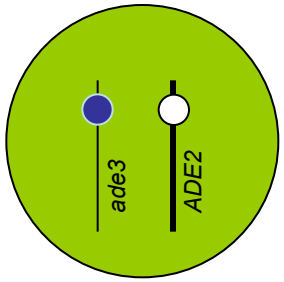
Tetratype



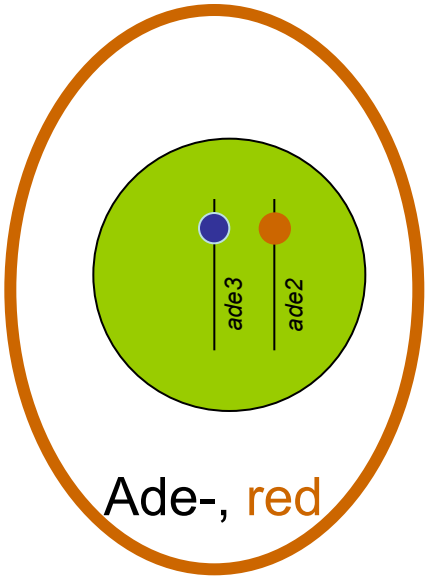
Ade+, white



Ade-, red



Ade-, white

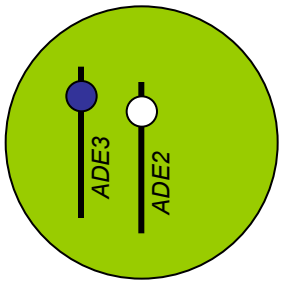


Ade-, red

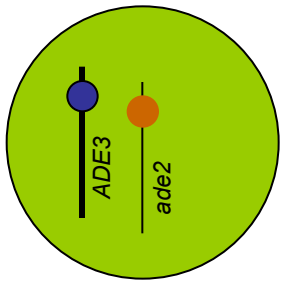
Ade2p Ade3p

A -------> B (red pigment) ---> C ---> D …

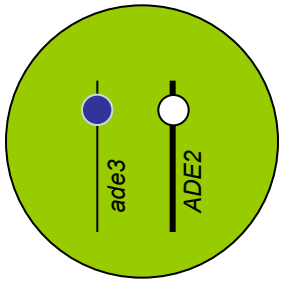
Scenario2



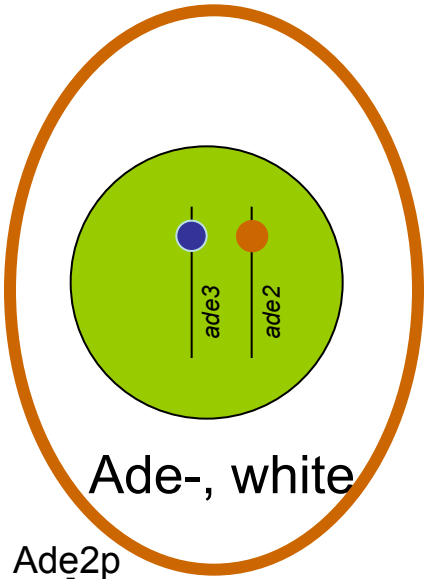
Ade+, white



Ade-, red



Ade-, white



Ade-, white

Ade3p

A ---> B ------> C ------> D (red

Ade2p

The Adenine Biosynthesis pathway