Experimental Genetics I

Escherichia coli

Protocol

21st of April 1997
through
26th of April 1997

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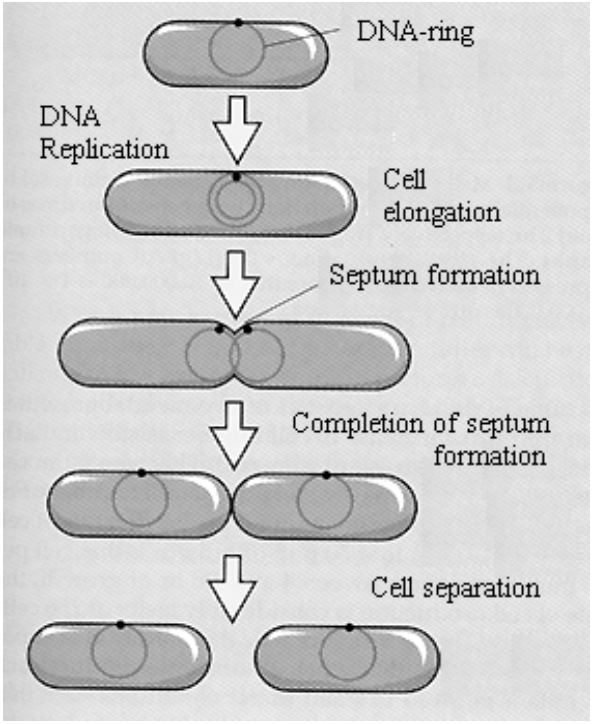
Salzburg, July 4th 1997

A well-known Prokaryote (*Escherichia coli*)

Introduction: *Escherichia coli* is a fast growing (duplicates approx. every 20 minutes), simple to use bacterium, and reveals simple nutritional requirements. Besides that, *E. coli* has something special to offer. It was the first bacterium in which the process of **conjugation** was discovered. Simplified, conjugation can be considered a physical contact, cell to cell or via a pilus, where short segments of DNA, called **plasmids**, can be exchanged from one cell to the other, transmitting special functions the recipient does not yet show; for example, resistance against antibiotics.

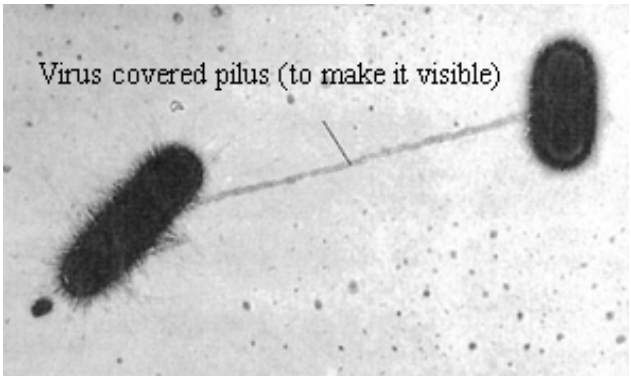
Life Cycle of *E. coli*: In most prokaryotes, growth of an individual cell continues until the cell divides into two new cells, a process called **binary fission**. In a growing culture of the rod shaped *E.coli*, the cells are observed to elongate to approximately twice the length of an average cell and then form a partition that eventually separates the cell into two daughter cells. This partition is referred to as **septum** and is the result of the inward growth of the cytoplasmic membrane and the cell wall from opposing directions until the two daughter cells are pinched off. During the growth cycle all cellular constituents increase in number such that each daughter cell receives a complete chromosome and sufficient copies of all other macromolecules, monomers, and inorganic ions to exist as an independent cell. Partitioning of the replicated DNA molecule between the two daughter cells depends on the DNA remaining attached to membranes during division, with septum formation leading to separation of chromosome copies, one going to each daughter cell (we have to keep in mind that DNA in prokaryotes is found in a circular form).



The time required for a complete growth cycle in *E.coli* is highly variable and is dependent on a number of factors, both nutritional and genetic. Under the best nutritional conditions, it can complete the cycle in about 20 minutes

Mechanism of DNA Transfer (Conjugation): A mechanism of DNA synthesis in certain bacteriophages, called **rolling circle replication**, reveals a simplified model of how this mechanism works. The whole series of events is probably triggered by **cell-to-cell contact**, at which time one strand of the plasmid DNA circle is nicked and one parental strand is transferred. As this transfer occurs, DNA synthesis by the rolling circle mechanism replaces the transferred strand in the donor. A complementary DNA strand is also made in the recipient. The model accounts for the fact that if the DNA of the donor is labeled, some labeled DNA is transferred to the recipient but only a single labeled strand is transferred. Therefore, at the end of the process, both donor and recipient possess completely formed plasmids.

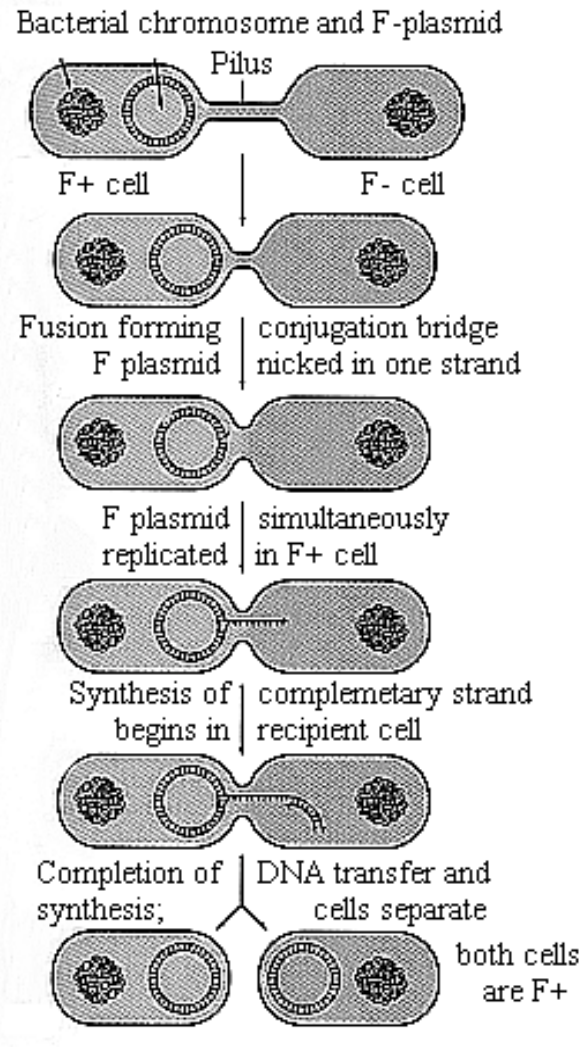
Pilus: Pili are structurally similar to flagella but are not involved in motility. Pili can be visualized under the electron microscope because they serve as specific receptors for certain types of virus particles. Pili are one major utility how bacteria hand-over **F-plasmids** from one organism to the other.



Cell-to-Cell Transfer and Plasmids: Before genetic transfer can be discussed, a very important genetic element has to be dealt with, the plasmid. **Plasmids** are genetic elements that **replicate independently** of the host chromosome. Unlike viruses, plasmids do not have an extra-cellular form and exist inside cells simply as nucleic acid.
Because plasmids lack a distinct extra-cellular form, one can imagine that plasmids are confined almost exclusively to transfer only daughter cells during cell division. Additionally, some prokaryotic cells can take up free DNA from the environment, so it is possible that lysis of the host; however, it may happen, brings the plasmid in contact with a new host. The main mechanism of cell-to-cell transfer is **conjugation**.

F Plasmid: The F-plasmid of *Escherichia coli* is not only conjugative but also has the special property of being able to mobilize the chromosome, so it can be transferred during cell-to-cell contact. The F-plasmid is an **episome**; which means that it can be integrated into the host chromosome. When the F-plasmid is integrated into the chromosome. **Conjugation** can lead to transfer of large blocks of chromosomal genes and genetic recombination between donor and recipient, it can be very extensive. Cells possessing an unintegrated F plasmid are called $\mathbf{F^+}$, and strains that can act as recipients for $\mathbf{F^+}$ (or **HFR**, high frequency of recombination) are called F^-. $\mathbf{F^-}$ lacks the F plasmid; in general, cell that contain a plasmid are very poor recipients for the same or closely related plasmids. Thus, the presence of the F plasmid results in **three** distinct alterations in the properties of a cell:

- ability to synthesize the **F-pilus** (a fimbra-like structure that is present on fertile cells, and is involved in DNA transfer during conjugation).
- mobilization of DNA for transfer to another cell.
- alteration of surface receptors so the cell is no longer able to behave as a recipient in conjugation.

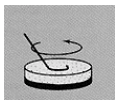


In the experiments listed below the F'-strain was used, which combines both the F^+ and the Hfr properties (see appendix 2)

F Duction or Sex Duction: The transfer of genetic material via a fertility **factor F**. The mating or conjugal system for the transfer of genetic material between strains of *E.coli* is dependent upon a fertility factor (F). Cells containing the F-factor are **donor cells**. Cells lacking F ($\mathbf{F^-}$) behave as **recipient cells**. The donor cells produce specialized **pili** (sex-pili) necessary for the act of **conjugation**. The recipient cells do not produce these pili. Hence, the donor is responsible for the "**one-direction**" transfer of genetic material. Mating of donor and recipient produce **recombinants**. Further, a donor cell when added to a culture of recipient cells soon converts the entire culture of recipient cells to donors, therefore the **F factor** can be considered very infectious. A recipient cell never spontaneously becomes a donor cell, but donors, which loose the F-factor for whatever reason, can become a recipient cell.

1. Diluting *E.coli* Suspension and Determining Rate of Growth (Experiment 2)

1.1 Colony Count - Day 1: 21st of April 1997



....see appendix 1 for technical procedures and handling

material: marking pen
6 100ul plastic bullets
1 1ml pipette plus blue tips
1 200µl pipette plus yellow tips
2 LB dishes
aqua destillata
1 glass spreader
1 beaker filled with ethanol
1 pocket lighter
colony: plastic-bullet of 100µl
E.coli K12 F' lac^+ rec^- Str^S

Purpose: This technique is used to count only living colonies, which means, cells are able to divide and form offspring within a colony. To obtain a viable count it is important that the number of colonies on the plate not to be too large because on crowded plates some cells may not form colonies and some colonies may fuse, leading to erroneous counts. It is also essential that the number of colonies not be too small, or the statistical significance of the calculated count will be too low. To obtain the appropriate colony number, the sample to be counted **must** always be diluted. Furthermore, it is important to determine the incubation condition such as medium, temperature, and time that will give the maximum number of colonies of a given organism and then use these conditions throughout the experiment.

Procedure: A way to use the plate counting is to use both the **serial dilution row** and the **spread plate method** (see appendix 1).
To increase credibility of the data, prepare two counting plates, with the first one going as far down as 10^{-6} and the second to 10^{-7}. Use the proper pipette size for water (max. capacity of 1ml - with blue tips) and culture suspension (max. capacity of 0.2ml - with yellow tips).
10^{-6} extraction on petri dish: Extract 0.1ml of the 10^{-5} bullet and drip it onto one LB petri dish.
Repeat the same procedure as above for the 10^{-7} extraction on petri dish by extraction 0.1ml from the 10^{-6} bullet.
Once both extractions are done, don't forget to mark them with name, date, strain, and dilute concentration. Place the plates into an incubator and leave them there for two days at 28°C.

Note: Dilution row: Every time a dilution step is made **discard the mouth pieces** of the pipette each and every time to avoid falsifying the dilutive concentration.
Spread plate method: Place the beaker of ethanol is in a considerable distance away from the "burning" spreader.

Results and Evaluation: Day 3: 23rd of April 1997

2. F-Duction of *E.coli* (Experiment 4)

This experiment is preceeded by two experimental tests. These experimental tests are needed (2.1 and 2.2) to confirm the characteristics of the strains involved.

2.1 Streptomycin Test - Day 2: 22nd of April 1997

material: marking pen
1 LB-Str dish
2 sterile toothpicks
donor: plastic-bullet of undiluted 1ml
E.coli K12 F' lac^+ rec^- Str^S
recipient: plastic-bullet of undiluted 1ml
E.coli K12 F^- lac^- rec^- Str^R

Purpose: Control experiment of streptomycin resistance by using two different strains of *E.coli* - Streptomycin sensitive (**Str^S**) and streptomycin resistant (**Str^R**) strands which are tested on the **LB-Str**, a streptomycin containing petri dish.

Procedure: Take one culture at a time, starting with the donor, using a sterile toothpick, place a streak in a zigzag manner of this strain directly down one half of the LB-Str dish and discard the pick. Repeat same procedure with the recipient strain, by spreading it on the remaining half of the dish; cover and mark the plate. Incubate for two days at 28°C.

Note: Use a light touch, try not to dig into the agar.

Results and Evaluation: Day 4: 24th of April 1997

2.2 Lactose Test - Day 2: 22nd of April 1997

material: marker pen
2 EMB-Lac dishes with eosin and methylblue as dyes
6 sterile toothpicks
donor: plastic-bullet of undiluted 1ml
E.coli K12 F' lac^+ rec^- Str^S
recipient: plastic-bullet of undiluted 1ml
E.coli K12 F^- lac^- rec^- Str^R

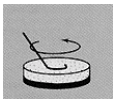
Purpose: Control experiment of the two different strains of *E.coli* - **lactose**$^-$ and **lactose**$^+$ on two **EMB-lac** petri dishes lactose enriched indicator plates plus two colors (eosin and methylblue) which will reveal a dark stained colonial growth if the strain is capable of fermenting lactose (**lac**$^+$), or light-colored if not able to ferment lactose (**lac**$^-$) by using the Cross-streaking technique to dilute the incident stroke which makes the last one very effective i.e.: a single colony can be detected.

Procedure: Take one culture at a time, starting with the donor, using a sterile toothpick, place a single streak of this strain directly down on the top quarter of the EMB-lac dish and discard the pick. Grab an other sterile pick and cross the first stroke into the second quarter of the dish. Again discard the pick. Finally cross the second stroke once moving down to the remaining half of the plate in a zigzag manner, discard this pick as well, cover and mark the plate (name, date, strain, etc.)

Note: Don't cross the previous strokes made on the dish, otherwise a cross-infection will occur and enrich the diluted strokes.
Mark the plate with your name, date, and strain.
Repeat same procedure with the recipient strain on the second plate, mark as well and incubate both dishes for two days at 28°C.

Results and Evaluation: Day 4: 24th of April 1997

2.3 F-Duction - Day 2: 22nd of April 1997



....see appendix 1 for technical procedures and handling

material: marker pen
1 250ml sterile flask
12 100ul plastic bullets
1 1ml pipette plus blue tips
1 200µl pipette plus yellow tips
6 EMB-lac dishes with eosin and methylblue as dyes
aqua destillata
1 glass spreader
1 beaker filled with ethanol
1 pocket lighter
donor: plastic-bullet of undiluted 1ml *E.coli* K12 F' lac^+ rec^- Str^S
recipient: plastic-bullet of undiluted 1ml *E.coli* K12 F^- lac^- rec^- Str^R

Purpose: To proof the transfer of genetic material via a **fertility factor F** from cells containing it (donor cells) via specialized pili (sex-pili) necessary for the act of conjugation to the recipient cells lacking F(F^-). A donor cell when added to a culture of recipient cells soon converts the entire culture of recipient cells to donors; the F factor can be considered very infectious.
The number of genes transferred being dependent on the allowed mating time and temperature.

Procedure: Pipette both the donor and the recipient strain into one flask, mix and shake it once and keep it at 37°C (bagno maria) for the time the experiment is running.

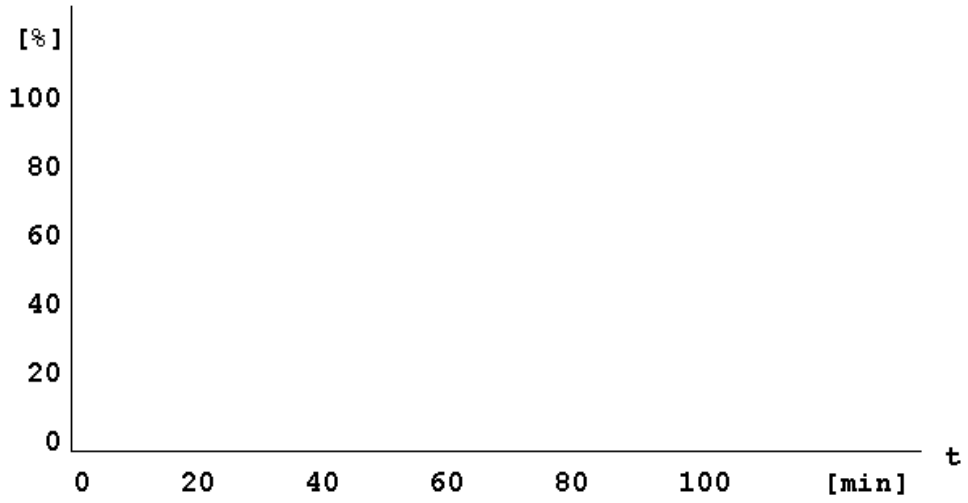
Note: Keep track of running time and **do not** shake the flask anymore (formed pili might break).
Go ahead with the first round of **serial dilution** (as shown in appendix 1)

- At **0 minutes** after mixing, use the first four bullets until the concentration of 10^{-3} and 10^{-4} is reached;
  Avoid falsification by **discarding the mouth piece** of the pipette every time a probe is taken.
  A way to use the plate counting method is to use the **spread plate method** (see appendix 1);
  10^{-4} extraction on petri dish: Extract 0.1ml of the 10^{-3} bullet and drip it and spread it onto a EMB-Lac petri dish.
  Note: When using the spread plate method make sure that the beaker of ethanol is in a considerable distance **away** from the "burning" spreader.
  10^{-5} extraction on petri dish: Repeat the same procedure as above with the 10^{-4} bullet.
- After **40 minutes**, go ahead with the second round of serial dilution, using another four bullets and repeat the spread plate method mentioned above with two fresh EMB-Lac dishes.
  Remember: Keep track of the running time and **do not** shake the mixture in the flask.
- Finally, after **60 minutes** execute the third round with the remaining bullets and dishes.
  Once all six dishes have been plated, place them in an incubator at 28°C until the colonies appear (usually after one or two days).

Results and Evaluation: Day 4: 24th of April 1997

	0 [min]	40 [min]	60 [min]
10^{-4} white 10^{-5}			
10^{-4} blue 10^{-5}			
10^{-4} averaged # 10^{-5}			
blue [%]			

Sigmoidal growth of the Hfr strain:



Experimental Genetics I

Saccharomyces cerevisiae

Protocol

21st of April 1997
through
26th of April 1997

Headed by: **Prof. Dr. Michael Breitenbach**

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Handed in by:

Pierre Madl (Mat-#: 9521584)
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Salzburg, July 4th 1997

A common eukaryota, YEAST (*Saccharomyces cerevisiae*)

Introduction: The yeast *Saccharomyces cerevisiae* is an ideal eukaryotic microorganism for biological studies:

- It grows rapidly, doubling in about two hours, forming thousands of cloned yeast colonies which can be cultured on petri-dishes in two days.
- Genome of yeast is very small, about $1.4 \cdot 10^7$ bp with a total number of 16 chromosomes only, which greatly simplifies both genetic and molecular analysis.
- Yeast cells can be maintained either as haploids or diploids; therefore, genetically recessive mutations can be easily obtained by working with haploid cells, and genetic complementation.

Eukaryotes can mate during sexual reproduction, and therefore DNA transfer and recombination differ in many ways from that in prokaryotes. The complex nuclear organization of eukaryotes and the existence in each nucleus of a number of linear chromosomes rather than circular lead to more regular mechanism of gene assortment and segregation.

Typically, eukaryotic cells like yeast, can be of two types, **haploid** or **diploid** (see meiosis), depending on the chromosome number. In the haploid phase the number of chromosomes per cell is **1n**, and in the diploid phase **2n**. Thus in the case of yeast, a haploid cell contains 16 chromosomes and the diploid 32. In diploid cells, two copies of each gene are present, one on each of the two **homologous** chromosomes.

The term **allele** is used to refer to the two alternate forms of the same gene present on the two homologous chromosomes. If the allele on one of the chromosomes has a mutation preventing the normal product from being expressed, the allele on the other chromosome can continue to be expressed, and so the effect of the mutation may not be evident. Thus, the expression of one allele may be **masked** by the other. The gene that is expressed is said to be **dominant** to the other allele, which is said to be **recessive**. Diploidity represents difficulties in genetic research because isolation of mutants is much easier in a haploid cell, where only one form of the gene is present; therefore, the effect of a mutation can be directly determined.

Mating Types: As mentioned before, the products of meiosis are four haploid gametes. By definition, eggs are formed by females and sperm are formed by males, but in many microorganisms there is no clear sexual distinction, only different mating types.

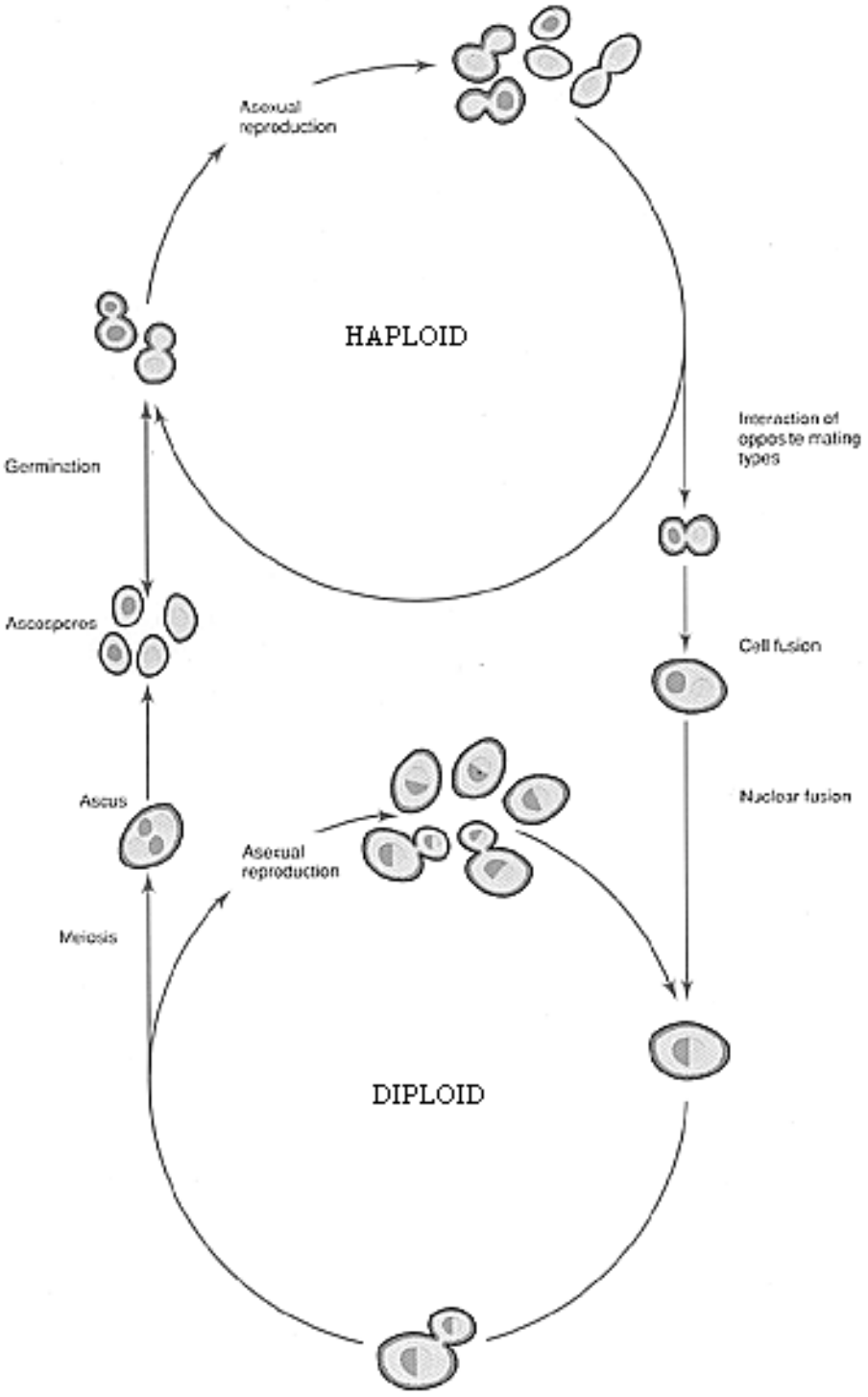
The two **mating types** of *Saccharomyces cerevisiae* are designated $\boldsymbol{\alpha}$ and ***a***. Cells of type α mate only with cells of type *a*, and whether a cell is *alpha* or *a* is itself determined genetically. However, although a yeast cell line generally remains either α or *a*, haploid yeast cells are periodically able to **switch their mating type** from one to the other (one consequence of this switching is that a pure culture of a single mating type can ultimately form diploids). There is a single active genetic locus, called the **MAT** (mating type) locus, at which either gene α or gene *a* can be inserted. At this active locus, the MAT promoter controls the transcription of whichever mating type gene is present. Thus, if gene α is at that locus, then the cell is mating type α, whereas if gene *a* is at that locus, the cell is mating type *a*. Somewhere else on the yeast genome are copies of both genes, α and *a*, which are not expressed. These **silent copies** serve as the source of the gene that is inserted when the switch occurs, the appropriate gene, α or *a*, is copied from its silent site and then inserted into the MAT location, replacing the gene already present. Thus the old gene is excised out of the locus and discarded and the new gene is inserted.

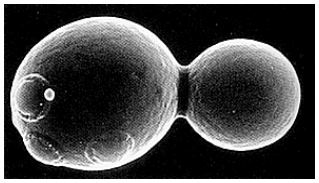
Beyond determining the mating types, α and *a* also sign for signaling each other the proper "sex" by excreting **peptide hormones** called α- and *a*-factors. These **hormones** bind to cells of opposite mating type and bring about changes in the cell surface of these cells. It seems that α cells have receptors on their surfaces only for *a*-factor, and *a* cells receptors for α-factors.

Life Cycle of Yeast:

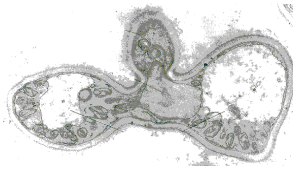
Once two cells of opposite mating type have associated, the two cells fuse, giving rise to a **zygote**.

The diploid cell formed can then become the forerunner of a new population of genetically identical diploid organisms or, as is the case with most eukaryotic microorganisms, meiosis can generate haploid cells that can divide asexually.





Bud scars of asexually reproducing *Saccharomyces cerevisiae*



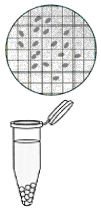
Bud of sexually reproducing *Saccharomyces cerevisiae*

1. Diluting yeast suspension and determining rate of growth (Experiment 3)

The following two experiments (1.1 and 1.2) describe two basic tools of counting colonies

1.1 Direct Microscope Count Day 1: 21st of April 1997

....see appendix 1 for technical procedures and handling



material: 2 100µl plastic bullets
1 1ml pipette plus blue tips
1 200ul pipette plus yellow tips
Petroff-Hausser counting chamber
1 glass covering slip
1 microscope
colony: 100ul plastic-bullet of
Saccharomyces c. W303e D

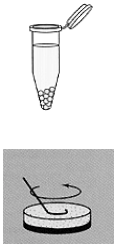
Purpose: This procedure is a quick way of estimating microbial cell number of the original suspension (either dead or alive). The number of cells per unit area of the very precisely cut **Petroff-Hausser counting chamber** (see appendix 1) can be counted under the microscope. Converting the counted value by multiplying with a conversion factor based on the volume of the chamber, gives an estimate of the number of cells per milliliter of suspension.

Procedure: The pure suspension has to be diluted via a **serial dilution row** to a 10^{-1} or 10^{-2} concentration. Extract a 5µl sample from the diluted plastic bullet and drip it carefully at the edge of the **Petroff-Hausser counting chamber** with the covering slip already in place (see appendix 1 for both procedures). Make sure that the liquid is completely withdrawn into the chamber, without leaving room for air. Finally count the cells per unit square of the 16-square field. And extrapolate it to obtain the suspension-density per milliliter of the original solution, as described in the appendix.

Note: Every time a dilution step is made **discard the mouth pieces** of the pipette each and every time to avoid falsifying the dilutive concentration.

Results and Evaluation: same day, just some hours later

1.2 Plate Counting or colony count - Day 1: 21st of April 1997



....see appendix 1 for technical procedures and handling

material: marker pen
6 100ul plastic bullets
1 1ml pipette plus blue tips
1 200ul pipette plus yellow tips
2 YPD dishes
aqua distilata
1 glass spreader
1 beaker filled with ethanol
1 pocket lighter
colony: 100ul plastic-bullet of *Saccharomyces c.* W303e D

Purpose: This technique is used to count only living cells, which means, they are able to divide and form offspring within a colony. To obtain a viable count it is important to determine the incubation condition such as medium, temperature, and time that will give the maximum number of colonies of a given organism and then use this conditions throughout the experiment.

Procedure: A way to use the plate counting is to use the **spread plane method**;
10^{-4} extraction on petri dish: Extract 0.1ml of the 10^{-3} bullet and drip it onto a YPD petri dish. Follow the indications listed in appendix 1.

Note: Make sure that the beaker of ethanol is in a considerable distance away from the "burning" spreader.
Repeat the same procedure as above for the 10^{-3} extraction on petri dish by extraction 0.1ml from the 10^{-4} bullet.
Don't forget to mark the plate with your name, date, dilute concentration and place both plates in the incubator for two days at 28°C.

Results and Evaluation: Day 3: 23rd of April 1997

2. Life Cycle - Mating (Experiment 1)

The procedures listed under this chapter (2.1 til 2.5) describe the basic experimental tools needed to manipulate *Sacchromyces cerevisiae* throughout its (haploid and diploid) life cycle.

2.1 Preparation of haploid strains of different mating type

material: marker pen
4 toothpicks
1 YPD dish
colony: 100ul plastic-bullet of
7456-ID as mating type *a*
100ul plastic-bullet of
XD16-3D as mating type α

Purpose: Two haploid strains of different mating type are brought together into a full medium petri dish and incubated to allow asexual reproduction for two days at 28°C.

Procedure: Take one culture at a time, starting with strain *a*, using a sterile toothpick, place a streak of this strain on a small area directly down one half of the YPD dish and discard the pick. Repeat same procedure with the strain α, by spreading it on the remaining half of the dish; cover and mark the plate. Incubate for two days at 28°C.

Note: Use a light touch, try not to dig into the agar. Use sterile toothpicks only once.

Results and Evaluation: samples already given

2.2 Mating and Formation of Zygotes - Day 1: 21st of April 1997

material: marker
4 toothpicks
microscope
colony: 1 YPD dish from exp.2.1

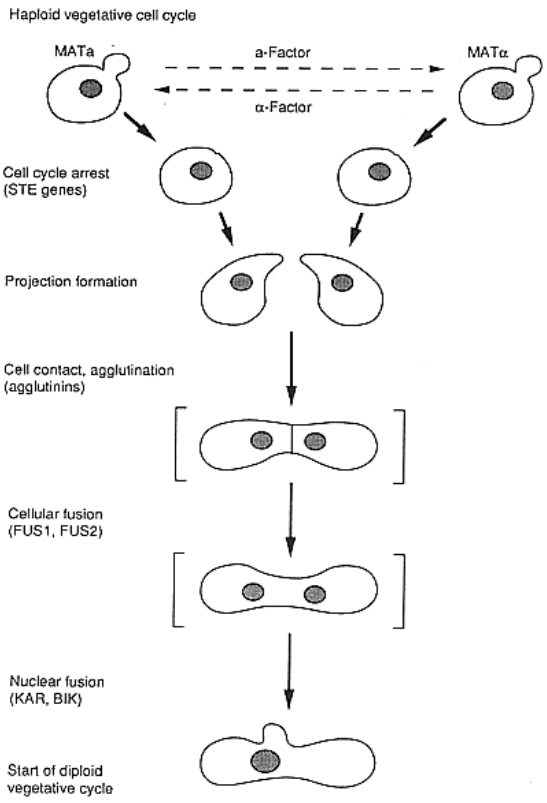
Purpose: The incubated strains *a* and *α* have been given time to reproduce asexually; enough of the haploid material has accumulated, the crossing of those haploid strains can be now executed. Several hours after mating, the characteristic diploid zygotes can be seen under the microscope.

MAT ***α***		MAT ***a***
XD16-3D	X	7456-ID

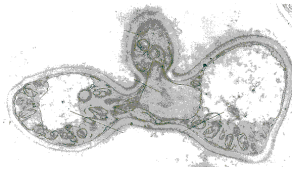
Procedure: Make a crossing by picking first a tiny probe from strain *a* with a sterile toothpick and place it gently on the lower central part of the same dish. Repeat the same procedure with the strain *α*, by spreading it together with the previous one; cover and mark the plate, and incubate for one day at 28°C.

Note: Use a light touch, try not to dig into the agar. Use sterile toothpicks only once.

Results and Evaluation: same days some hours later (probes taken from the early morning class)



Electromicroscopic view of the former haploid cells (outer left and right) and the diploid bud (center - top)



2.3 Sporulation - Day 2: 22nd of April 1997

....see appendix 1 for technical procedures and handling

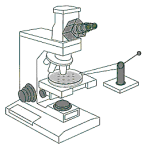
material: marker pen
1 sporulation medium dish
aluminum cylinder
plastic hoop
sterilized velveteen
colony: YPD-plate from previous experiment 2.2

Purpose: The diploid zygote were allowed to grow on a YPD medium for one day. This colony will be replicated on a sporolation medium (which lacks the nitrogen source) by using the **replica plating** method (see appendix 1).
Sporulation results in the creation of four haploid spores (formation of tetrad) tightly encased in a structure called an ascus.

Procedure: Once the tools for the replica plating procedure are ready, proceed to transfer the culture from the YPD dish onto the sporulation medium; cover and mark it with your name and date. Place the freshly made dish into the incubator for three days at 28°C.

Results and Evaluation: Day 5: 25th of April 1997

2.4 Micromanipulation - Day 5: 25th of April 1997



....see appendix 1 for technical procedures and handling

material: marker pen
micromanipilator
centrifuge
1 YPD dish
aqua distilata
1 test tube of 0.5ml aqua distilata
1 plastic bullet of 10µl glasulase
colony: 1 sporulation medium dish from previous experiment 2.3

Purpose: With the help of the **micromanipulator** (see appendix 1 for details) the four spores from the ascus can be isolated and placed on a new culture medium. The tetrads will reproduce asexually and will be ready for further manipulation .

Procedure: The asci are stripped off from the sporulation medium and suspended into the flask of sterile water. To get rid of the ascus wall an enzyme (glasulase) is added to the suspension. Shake the tube vigorously in a vortex manner and let it incubate at room temperature for up to 10 minutes. Place the glass tube for two minutes into the centrifuge at 2000rpm's. Rinse the centrifugated material twice with distilled water.

Note: Don't shake the tube after centrifugation, the tetrad might get damaged.
Now carefully place the tetrads onto one half of the YPD plate. Through the use of a joystick-operated device called a micromanipulator (see appendix 1), each tetrad is dissected into four individual spores. Every separated haploid spore of the tetrads is brought onto the other half of the YPD medium dish. The spores are each allowed to germinate and grow into a colony by incubating them over night at 28°C.

Results and Evaluation: tetrad 33 and 34 given by the tutors.

2.5 Vegetative Reproduction - samples given

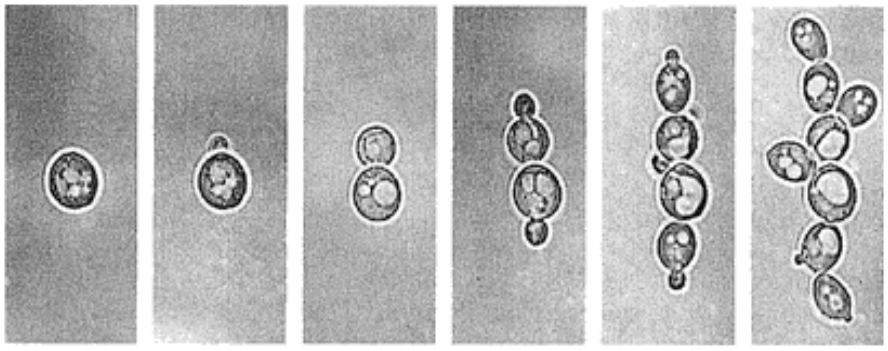
material: marker peb
8 toothpicks
1 YPD dish
colony: tetrade medium dish from previous experiment 2.4

Purpose: Two individual tetrad colonies have to be transferred to YPD medium dishes to be available for meotic recombination.

Procedure: Take one culture at a time, starting with the first strain of tetrad one, using a sterile toothpick, place a streak of this strain on a small area directly down one half of the YPD dish and place it horizontally along one line; discard the pick after completing the first streak. Repeat same procedure with the other three strains.

Note: Use a light touch, try not to dig into the agar. Use sterile toothpicks only once.
Repeat the entire procedure with the second tetrad by placing it on the remaining half of the dish; cover and mark the plate. Incubate for two days at 28°C.

Results and Evaluation: tetrad 33 and 34 already prepared and given by tutors



3. Meiotic Recombination (Experiment 5)

The experiments preceeding the final one, are needed to prepare and verify the charactristics of the strains involved. The results of experiment 3.2 will be of essential importance to locate the position of the markers used on the respective chromosomes of *Saccharomyces cerevisiae*.

3.1 Preparation of Test Strain - samples given

material: marker pen
2 toothpicks
1 YPD dish
colony 1: YPD-dish with test strain *a*
JJ-1A; Mat a, arg-1, thr-1
colony 2: YPD-dish with test strain *a*
JJ-1C; Mat α, arg-1,thr-1

Purpose: A test strain master plate has to be prepared to be able to determine the mating type of the tetrad colony for the experiment 3.2. The known test strains with different mating type are placed onto YPD-medium to allow to grow asexually.

Procedure: Take one culture at a time, starting with the first strain *a*, using a sterile toothpick, place a streak of this strain on a small area directly down one half of the YPD dish and place it horizontally along on line; discard the pick after completing the first streak.

Note: Use a light touch, try not to dig into the agar. Use sterile toothpicks only once.
Repeat same procedure with the other test strains α.; cover and mark the plate. Incubate for two days at 28°C.

Results and Evaluation: JJ-1A and JJ-1C already prepared and given by tutors

3.2 Tetrad Testing and Gene Mapping - Day 2: 22nd of April 1997

....see appendix 1 for technical procedures and handling

material: marker pen
1 SC-leu petri dish
1 SC-ura petri dish
1 SC-ade petri dish
1 YPG petri dish
1 YPD petri dish (control medium)
aluminum cylinder
plastic hoop
sterilized velveteen

parental strains: master tetrad dish from experiment 2.5
XD16-3D: MAT α dit101::LEU2, his4, leu2-3 112, ura3-52,ade2-101
7456-1D: MAT a his4-917, leu2-1, pet9

Purpose: First to test the progeny, various kinds of gelled selective media are used to test the strains for their auxotrophic character (aux^- or aux^+). Nutritional mutants can be detected by the **replica plating method** (see apendix 1). The colonies of the parental type will grow normally, whereas those of the mutant will not. The inability of a colony to grow on a replica plate is a signal that it is a mutant.
The media are complete media (SC) with either adenine, uracil, or leucine absent in the dish. The YPG medium contains glycerin instead of glucose to determine the capability of the parental strain to see whether they are able to proliferate on glycerin rather than on a glucose medium. Second to determine whether there was a crossover during meiotic division.

Procedure: Place the master parental plate onto the velvet of the replica plate and press it gently, making sure that enough of the parental strain are passed on on the velveteen. Then proceed imprinting it with the four complete media (SC-leu, SC-ura, SC-ade, YPG) and finally replicate it also onto the remaining YPD dish to use as control if enough of the original strains are left over to grow. For orientation, it is helpful to mark each of the replicated tetrads with the first four letters of the alphabet. Put your name, date, and place them into the incubator for three days at 28°C.

Results and Evaluation: Day 4: 24th of April 1997

3.3 Determining the Mating Type - Day 2: 22nd of April 1997

....see appendix 1 for technical procedures and handling

material: marker pen
1 SD dish (synthetic deficient medium)
aluminum cylinder
plastic hoop
sterilized velveteen
parental strains: master tetrad dish from experiment 3.2
XD16-3D: MAT α dit101::LEU2, his4, leu2-3 112, ura3-52,ade2-101
7456-1D: MAT *a* his4-917, leu2-1, pet9
test strains: master plate from experiment 3.1
JJ-1A; MAT a, arg-1, thr-1
JJ-1C; MAT α, arg-1,thr-1

Purpose: To determine the mating type of the parental dish, the two tetrads are crossed with two known test strains and replicated onto a deficient medium. This is used, to make sure that only diploid cells proliferate on this medium, which makes evaluation a lot easier.
Diploid cell growth will only occur on those cross-points where strains of alternating mating type come together, thus allowing determination of parental mating type.

Procedure: Use the master tetrad dish from the previous experiment to make the cross with the master plate containing the test strains using the **replica plating** technique (see appendix1).
Place the master tetrad dish from the previous experiment onto the replication device, then use the test strain dish and place it perpendicularly onto it.
Finally press the deficient medium onto the criss-cross of the velveteen cloth.
For orientation mark the new dish with *a* and α of the test strains and a, b, c, d for both parental tetrads, add name and date and incubate for two days at 28°C.

Results and Evaluation: Day 4: 24th of April 1997

Tetrad	JJ-1A a	JJ-1C α
33 A		
33 B		
33 C		
33 D		
34 A		
34 B		
34 C		
34 D		

4. Gene Mapping (Experiment 5)

Introduction: Meiotic analysis is the traditional method for genetically determining order and distances. Yeast is especially suited for meiotic mapping because the four spores in an ascus are the products of a single meiotic event (**haploid**), and the genetic analysis of this tetrad provides a sensitive means for determining linkage relationships of genes presented in the **heterozygous** condition. There are two basic advantages in the study of fungi when it is in the haploid state:

1. There is only one chromosome set per spore, dominance and recessiveness normally do not obscure gene expression; the phenotype is a direct reflection of the genotype.
2. Random meiotic analysis is easier.

Tetrad analysis is useful for linkage studies and for constructing strains necessary in genetic and biochemical experiments. Prerequisites are a **dihybrid** cross, which involves two different markers of **heterozygous** origin.
Keeping this in mind, it is possible to map a gene relative to its centromere, if known **centromere-linked** genes are present in the cross. Although the isolation of the four spores from an ascus is one of the more difficult techniques in yeast genetics, requiring a micro-manipulator and considerable practice (as described in experiment 2.4).

- The closer a gene is to its centromere, the fewer times a crossover will occur between that gene and its centromere.
- However the farther apart the gene and its centromere, the more frequently crossing over will occur. Thus, the distance separating a gene from its centromere is reflected by the frequency of recombinants found in their progeny.

It is important to remember that markers (genes) are placed on a genetic map relative to each other, the order being determined by the recombination between them. The unit of recombination is the centiMorgan (**cM**). Two markers are one cM apart (recombinant frequency, of 0.01; RF = 1%), if they recombine in meiosis once in every 100 opportunities. The cM is a genetic measure, not a physical one, but a useful rule of thumb is that one cM is equivalent to approx. 10^3 basepairs (bp).

$$cM = 50 \cdot \frac{TT + 6 \cdot NPD}{PD + NPD + TT}$$

$$cM' = 50 \cdot \frac{TT}{PD + NPD + TT}$$

The equation for deducing map distances in cM is accurate for distances up to approx. 35 cM.
For larger distances up to approx. 75 cM, the value should be corrected (not exemplified in this text).
Similarly, the distances between a marker and its centromere can be approximated from the percentage of TT tetrads with a tightly linked centromere marker, such as *ura3*; the cM' formula should be used instead).

Tetrad types (by using the leu and ade marker):

Spores of one tetrad	Marker (leu / ade)	Marker (leu / ade)	Marker (leu / ade)
A	+ +	+ +	+ -
B	+ +	+ -	+ -
C	- -	- -	- +
D	- -	- -	- +
	PD	**TT**	**NPD**
Random ass.	1	4	1
Linkage	>1	(0 - <4)*	<1
CEN-linked	1	0 - <4	1

The term marker refers to an easily testable gene (its function) to keep track of individual genes during the experimental cycle.

*) irrelevant

PD (parental ditype): there is **no crossover**, hence progeny reflects parental genotype; RF = 0; (valid for both homologue and non-homologue chromosomes).

TT (tetratype): **One crossover** between any non-sister parental pair or non-homologues (4 types of segregant - all possible types); RF = 50%

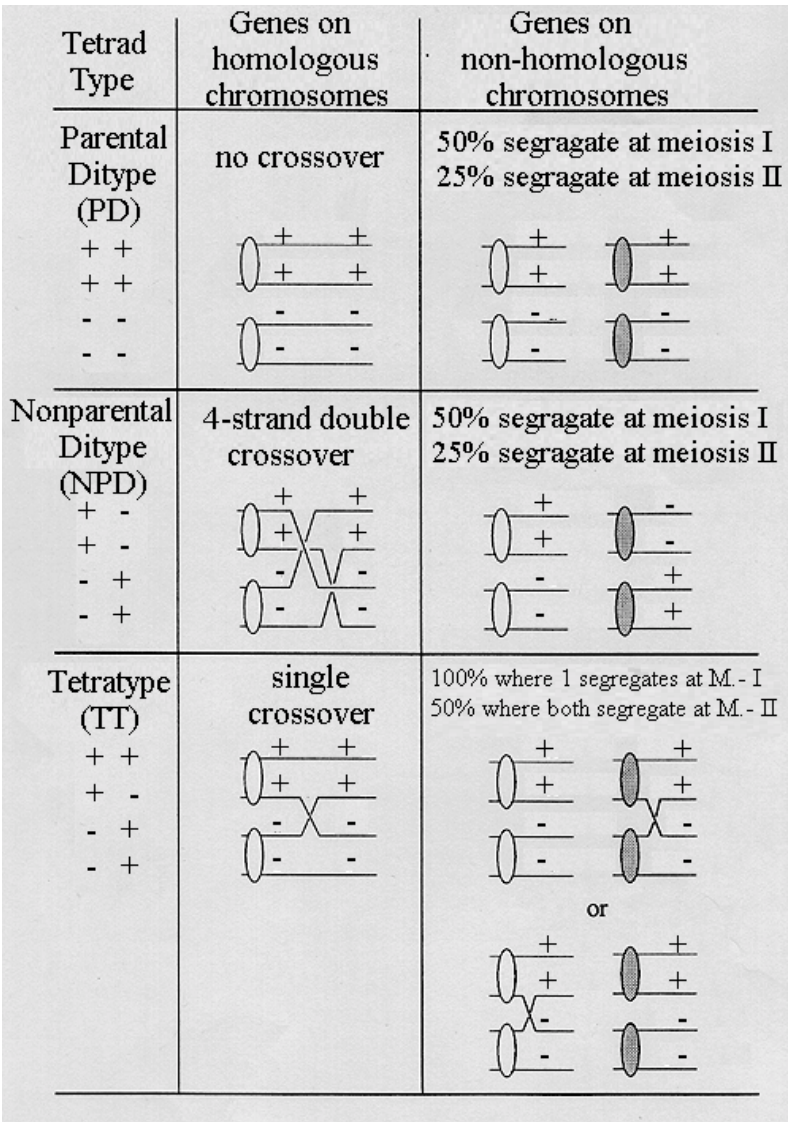
NPD (nonparental ditype): **Two crossovers** between non-sister parental (2 types of segregants - both non parental) or a single exchange of non-homologues chromosomes (w/o crossing over) RF = 0-100%.

When pairs of homologues come together during meiosis, they exchange segments (recombination occurs).

Random assortment: No linkage between genes (markers) since they are either located on different chromosomes or widely separated on the same chromosome. Tetrads of PD and NPD occur in equal numbers (unlinked genes); the ratio of ditypes (PD + NPD) to TT is 1:3, neither shows centromere linkage.

Linkage: Genes located close to each other on the same chromosome that tend to be transmitted as a single unit. PD outnumbers NPD since NPD requires a double crossover; (PD > NPD).

CEN linked: In close association with the centromere, hence hardly any crossover will ever occur. The frequencies of PD, NPD, will predominate (in equal frequencies) over TT tetrads.



Results and Evaluation: Day 5: 26th of April 1997

material: replicated petri dishes from experiment 3.2
1 SC-leu petri dish
1 SC-ura petri dish
1 SC-ade petri dish
1 YPG petri dish

Procedure:

1. Determine the auxotrophic / prototrophic pattern of the two tetrads on each of each SC petri dish and write the results in the evaluation sheet.
2. Classification of tetrad types (PD, TT, NPD).
3. Evaluation of tetrad types by calculating the map distances (cM).

parental strains: Master tetrad dish from experiment 2.5 used as reference
XD16-3D: MAT α dit101::LEU2, his4, leu2-3 112, ura3-52,ade2-101
7456-1D: MAT *a* his4-917, leu2-1, pet9

Tetrad	leu	ade	YPG	ura	leu / ade		YPG / leu		YPG / ura		leu / ura	
XD16-3D	+	-	+	-	+ / -		+ / +		+ / -		+ / -	
7456-1D	-	+	-	-	- / +		- / -		- / -		- / -	
33 A					/		/		/		/	
33 B					/		/		/		/	
33 C					/		/		/		/	
33 D					/		/		/		/	
34 A					/		/		/		/	
34 B					/		/		/		/	
34 C					/		/		/		/	
34 D					/		/		/		/	

	leu / ade	YPG / leu	YPG / ura	leu / ura
	PD : TT : NPD	PD : TT : NPD	PD : TT : NPD	PD : TT : NPD
Tetrad 1 - 30*	: :	: :	: :	: :
Tetrad 31 - 54*	: :	: :	: :	: :
Tetrad 55 - 60*	: :	: :	: :	: :
Σ	: :	: :	: :	: :
Ratio	: :	: :	: :	: :

Type of linkage				
Formula to use				

*) tetrads 1-30 obtained from morning class; tetrads 31-54: afternoon class, tetrads 55-60: tutors

Calculation of cM:

leu / ade ----------o----------

YPG / leu ----------o----------

YPG / ura ----------o----------

leu / ura ----------o----------

Technical Procedures and Handling

1. Serial Dilution:

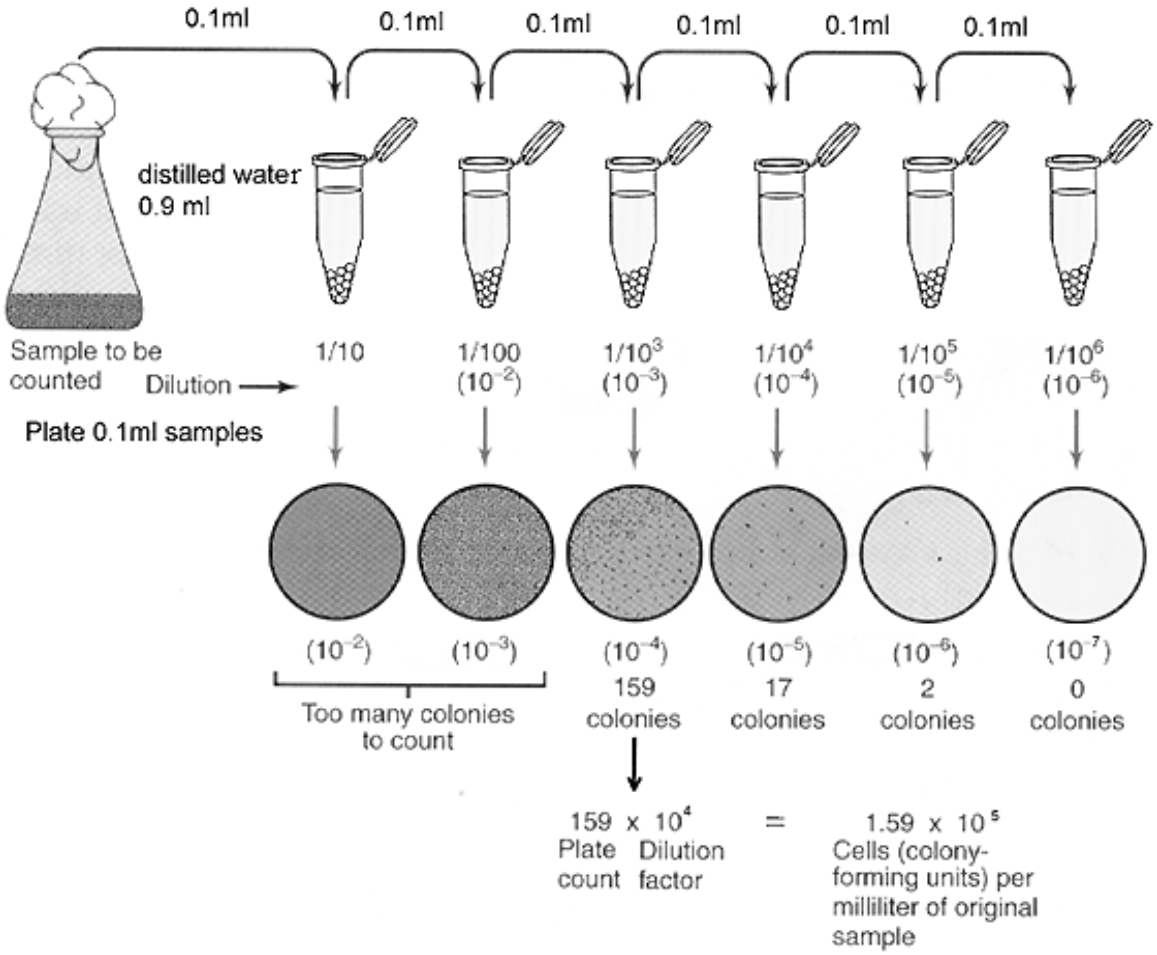
Purpose: A serial dilution procedure is to obtain a significant colony number.

It is important that the number of colonies on the plate not to be too large because on crowded plates some cells may not form colonies and some colonies may fuse, leading to erroneous measurements. It is also essential that the number of colonies not be too small, or the statistical significance of the calculated count will be low.

To obtain the appropriate colony number, the sample to be counted **must** always be diluted.

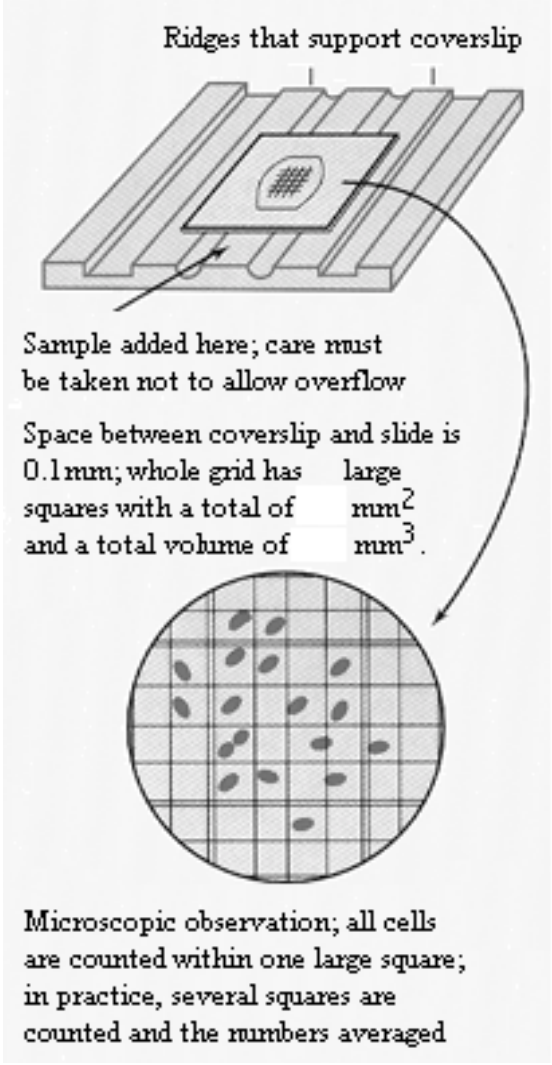
Procedure: Mix 0.1ml of undiluted suspension with 0.9ml of distilled water in a plastic bullet. Shake it vigorously in a vortex manner. Then, extract 0.1ml from it and pipette it together with another 0.9ml of distilled water in an other plastic bullet. Keep doing so by pouring, diluting and shaking the plastic bullets, until the desired dilution is reached, i.e.: 10^{-3} and 10^{-4}. Every dilutive step equals a 10^{-1} -fold decrease in concentration of the original suspension liquid.

Note: Every time a dilution step is made **discard the mouth pieces** of the pipette each and every time to avoid falsifying the dilutive concentration. Use the proper pipette size for water (max. capacity of 1ml - with blue tips) and culture suspension (max. capacity of 0.2ml - with yellow tips).



2. Direct Microscope Count

Purpose: This procedure is a quick way of estimating microbial cell number of the original suspension (either dead or alive) if the organism is not too small.
Therefore only applicable in microorganisms from a certain size onwards i.e.: yeast.
The **Petroff-Hausser** counting chamber is a grid marked on the surface of the glass slide, with squares of tiny known areas. Over each square of the grid is a volume of known size which is very small and precisely measured. The number of cells per unit area of grid can be counted under the microscope, giving a measure of the number of cells per small chamber volume. Converting this value to the number of cells per milliliter of suspension is easily done by multiplying it with a conversion factor based on the volume of the chamber sample.



Procedure: Extract a 5µl sample of from a serial dilution row and drip it carefully at the edge of the counting chamber with the covering plate already in place.
Make sure that the liquid is completely withdrawn into the chamber, without leaving room for air. Finally count the cells per unit square of the 16-square field. To obtain an approximate estimate of the total number of cells, count all the cells of each of the 16 fields and divide it by 4. The average resulting number is then multiplied by a conversion factor of 10^6 to obtain the approximate total number of cells per milliliter.

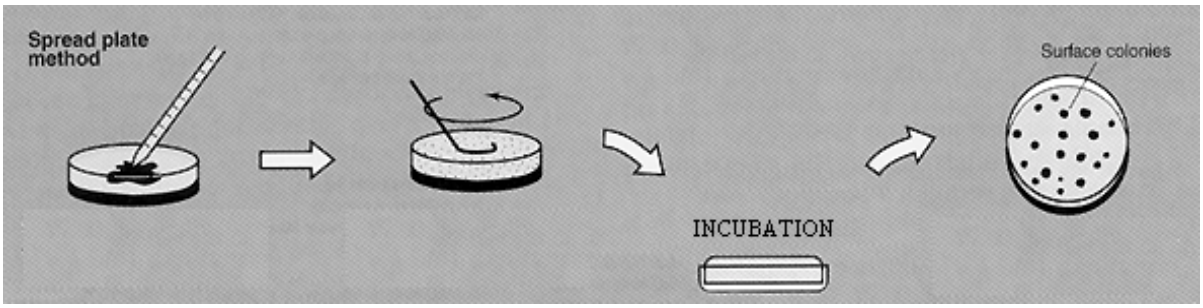
3. Spread Plate method

Purpose: This procedure is an easy way to spread a diluted sample (usually from a serial dilution row) onto a medium dish to obtain statistical results concerning the mother-plate or flask of original suspension once the culture has been incubated for a certain period.

Procedure: Extract 0.1ml sample from a serial dilution row and drip it onto the petri dish.

Use a glass spreader to distribute the solute evenly over the agar. To make the spreader sterile, dip it into a beaker of ethanol, remove it carefully and disinfect it by igniting it. Place the spreader on the edge of the agar plate until it cooled off before distributing the dilution.

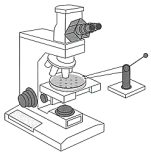
Note: Make sure that the beaker of ethanol is in a considerable distance away from the "burning" spreader.



4. Micromanipulation

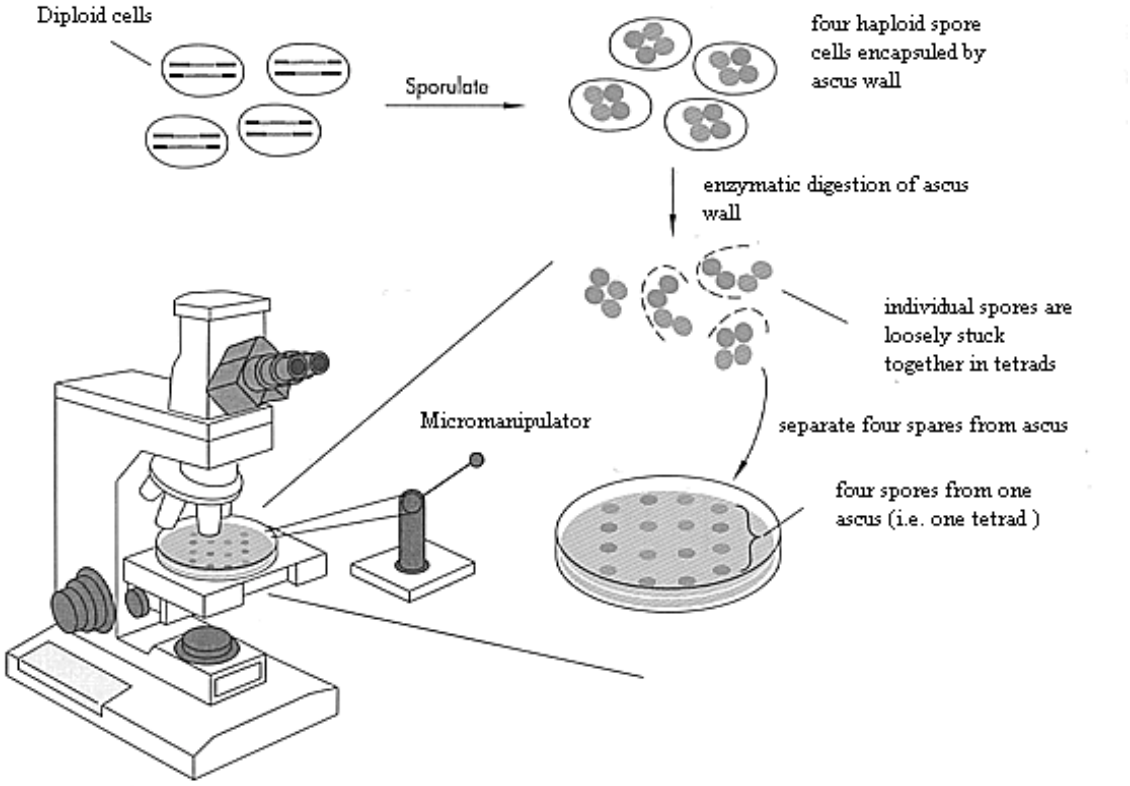
Purpose: Micromanipulators are used for yeast studies; they operate with control levers or joysticks that can translate hand movement into synchronously reduced movements and microtools. Most instruments were designed so that movement of the tool in the horizontal (X,Y) plane is directly related to the movement to the control handle; whereas, the vertical (Z) direction tool movement is controlled by rotating a knob, located either on or near the horizontal control handle.

Procedure: The ascus wall is stripped off with an enzyme (glasulase), and the spore clusters (tetrads) are placed on a plate.



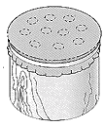
Through the use of a joystick-operated device called a micromanipulator, each tetrad is dissected into four individual spores. Every separated haploid spore of the tetrads is brought onto a YPD medium dish. The spores are each allowed to germinate and grow into a colony by incubating them over night at 28°C. Finally the colonies of two individual tetrads asexually reproduced haploid cells are transferred with a toot-pick onto YPD medium (two tetrads on one dish, with every spore-colony spread over in a linear manner).

Note: Do not attempt to move the pincer of the micromanipulator horizontally once it touches the plate; it can break.



5. Replica Plating

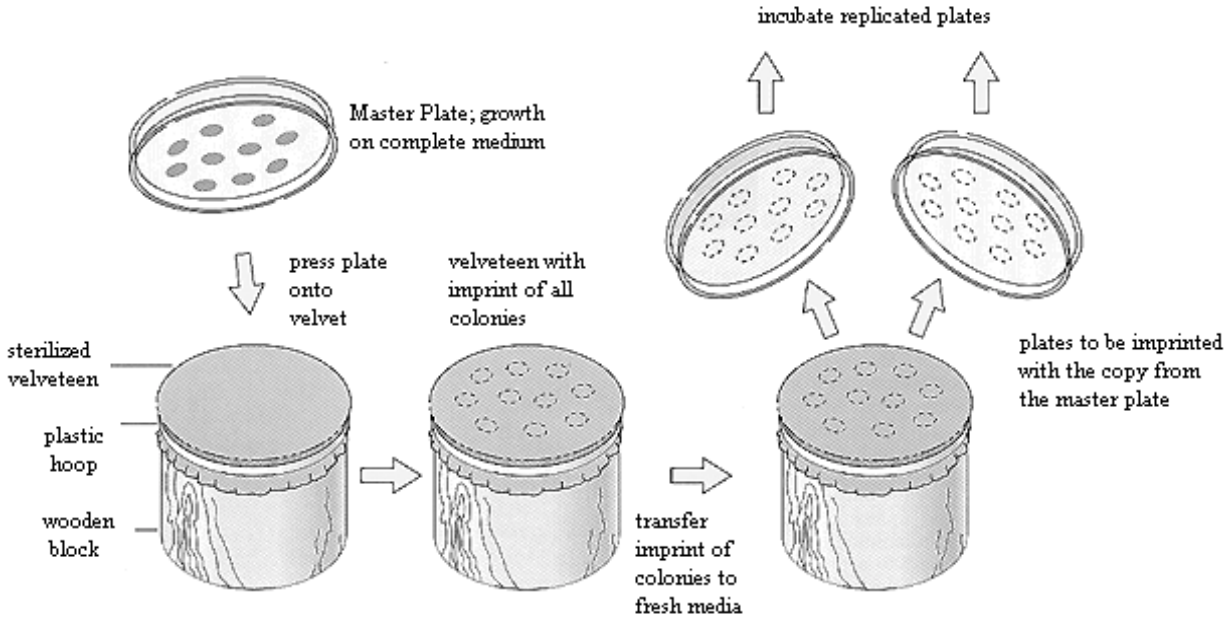
Purpose: Replica plating allows the experimenter to transfer microbial colonies quickly from one plate to another.



Colonies are grown on a mother plate and pressed onto a sterile piece of velvet. The microbes adhere to the velvet, yielding a print of all the colonies. A fresh plate (SC,YPD, etc.) is placed on the velvet and the transfer is reversed generating a replica of the colonies from the master plate onto the new plate. Several new plates can be imprinted from a single velvet (up to 20). Replica plating can be used to screen colonies, selected for one marker, for presence of another etc.

Procedure: Place the sterile velveteen onto the aluminum cylinder with the soft part on top. Place the plastic hoop onto the block to tighten the cloth firmly. Pop the mother plate upside down (with the marking matching that one on the hoop) onto the velvet and make sure that the pressure is evenly distributed over the sample. Replace the mother plate with the new media dishes where the microorganism have to be replicated onto (again match the marking).

Note: Use a sterile piece of velvet - to be handled at the edges only and make sure to avoid contamination with body fluids and by other objects.



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Abbreviations used in experiments of *E.coli* -

donor: *E.coli* K12 F` lac^+ rec^- Str^S
recipient: *E.coli* K12 F` lac^- rec^- Str^R
K12.... Name of colony
F.... the **F'** is called the **F-prime** and it shows characteristics of both $\mathbf{F^+}$ and **Hfr** strains, that the F prime is the factor associated or attached to various-sized segments of the host chromosomes.
$\mathbf{F^+}$ is the donor cell carring the F factor in the non integrated state (that is, with its cytoplasma and not associated with the host chromosomes).
Certain cells of an F^+ culture were able to transfer the chromosomes efficiently therefore termed Hfr (high frequency transfer cells)
lac.... lactose; + able to ferment lactose; - not able to ferment lactose;
rec.... recessive;
Str....Streptomycin; **S** sensitive to streptomycin; **R** resistant to streptomycin;

Media used in *E.coli*

LB-Str	2% agar 1% trypton 0.5% yeast extract 0.5% NaCl 1l H20 dest. 4mg streptomycin
EMB-lac	3.6% EMB-agar (indicator plate) lactose eosin (colorant) methylblue (colorant)
EMB-lac-Str	as EMB-lac 4mg streptomycin

Abbreviations used in experiments of *Saccharomyces cerevisiae*

test strain:	genotype:
JJ-1A	MATa, arg-1, thr-1
JJ-1C	MATα, g-1, thr-1

mapping parrent strain:	genotype:
XD16-3D	MATα, dit101::LEU2, his4, leu2-3 112, ura3-52, ade2-101
7456-1D	MAT*a*, his4-917, leu2-1, pet9
W303e D	

legend:		
	dit 101::LEU2	LEU2 is in place of dit101
	his4	histidine requiring, auxotropic
	leu	leucine requiring, auxotrophic
	ura	uracil requiring, auxotrophic
	ade	adenine requiring, auxotrophic
	pet	unable to grow on nonfermentable carbon sources (glycerin)
	thr	threonine requiring, auxotrhophic
	arg	argenine requiring
	ura **3-52**	third enzyme of the uracil pathway defect; specific 52nd allele / amino acid
	MAT*a*	mating phenotype *a*
	MATα	mating phenotype α

Media used for *S.cerevisciae*

YPD	yeast peptone diglucose, is a complex medium for routine growth of yeast	2% agar 2% glucose 2% peptone 1% yeast extract 1l H20 destil.
YPG	yeast peptone glycerin, is a complex medium containing a non-fermentable carbon source (glycerol) that does not support the growth of e- or petit mutants.	2% agar 2% glycerin 2% peptone 1% yeast extract 1l H20 destil.
Sporulation M.	medium forcing the colony to withdraw into spore forming status containing only a very limited amount of essential substances	1% K-acetat 0.1% yeast extract 0.5% glucose 2% agar 1l H20 destil.
SD Medium	synthethic minimal medium (or synthetic deficient)	0.67% bacto yeast nitrogen based w/o amino acid 2% agar 2% glucose 0.5% ammunium sulfat 1l H20 destil.

SC Medium	synthethic complete medium in which an essential amino acid is missing.
SC-leu	leucine absent - testing of the leu marker on a synthetic complete medium
SC-ura	uracil absent - testing of the ura marker
SC-ade	adenine absent - testing of the ade marker

Glossary of *Escherichia coli* and *Saccharomyces cerevisiae*

Agar: A gelatinous substance derived from certain red algae; used as a solidifying agent in the preparation if nutrient media for growing microorganisms

Auxotroph: A mutant that has a growth factor requirement (compare prototroph).

Auxotroph: (Gr. auxo, self; trophos, feeder) A strain of microorganism that will proliferate only when the medium is supplemented with some specific substance (growth factor) not required by the wild type; humans are auxotroph for certain amino acids (see mutation, compare prototroph).

Cell Cycle: Set of events that occur during the division of mitotic cells - periodically cycling between mitosis (M phase) and interphase. **Interphase** can be subdivided in order into G1, S, and G2 phase. DNA synthesis occurs during S-Phase. The length of the cell cycle is regulated through a special option

G1-phase: in which G1 cells can enter a resting phase also called G0; preceding S-phase (haploid).

S-phase: the phase in which DNA synthesis occurs (doubling of DNA).

G2-phase: preceding M-phase (diploid).

M-phase: the mitotic phase of the cell.

cM - **CentiMorgan**: (T.H. Morgan) The distance between two linked gene pairs where one percent of the products of meiosis are recombinant; a unit of distance in a linkage map; also known as unit map.

Centromer: A kinetochore; the point or constricted region (CEN) on the chromosome where the spindle (microtubuli) attaches and also where the two chromatids are joined during cell division (see also mitosis and meiosis).

Chiasma: (Gk. chiasma, cross)A cross-shaped structure commonly observed between non-sister chromatids during meiosis; the site of crossing over.

Complementation: The production of a wildtype-phenotype when two different mutations are combined, mixed in a diploid or heterokaryon (compare recombination).

Conjugation: Transfer of genes from one prokaryotic cell to another by mechanism involving cell-to-cell contact and a plasma.

Crossing Over: The exchange of corresponding chromosome parts between homologs (synapsis) by breakage and reunion (see also chiasma and meiosis).

Genetic Code: The system of nucleotide triplets (codons) in DNA and RNA that dictates the aminoacid sequence in polypeptide chains (proteins) except for the start (AUG) and stop (UAA,UGA,UAG) signals

Gene Map: A linear designation of mutant site within a gene, based upon the various frequencies of interallelic (intragenic) recombination.

Genetic Mapping: The process of locating the position of genes on chromosomes (see locus).

Genome: The entire complement of genetic material in a chromosome set (see gene dose, mutation).

Genotype: The specific allelic composition of a cell - either of the entire or, more commonly, for a certain gene or set of genes; genetic characteristics (makeup) that determine the structure and function of an organism (see also phenotype).

Homozygot: (Gk. homo, same) Has two identical alleles of a gene either *AA* (dominant) or *aa* (recessive).

Independent Assortment Structure: see Mendels 2nd Law.

Kilobase (kb): A 1000 base fragment of nucleic acid. A kilobase pair is a fragment containing 1000 base pairs.

Linkage: The tendency of genes located on the same chromosome to be inherited together.

L. **Group**: Closely located genes on the same chromosome that tend to be transmitted as a single unit.

Locus: The particular physical location on the chromosome of which the gene for a given trait occurs.

Marker: Alleles used as experimental probes to keep track of an individual, a tissue, a cell, a nucleus, a chromosome, or a gene; usually those genes and their mutants which can easily be tracked.

Mating Type: Is the equivalent in lower organisms of the sexes in higher organisms; the mating types typically defer only physiologically and not in physical forms.

M.T. Test: Are best carried out with MAT-*a* and MAT-α testing strains, each containing markers not in the strains to be tested.

Meiosis: (Gk. replication) Two successive nuclear divisions (with corresponding cell division) that produce gametes (in animals) or sexual spores (in plants or fungi) have one-half of the genetic material of the original cell (1n).
Prior to **meiosis-I**, each chromosome is duplicated in the pre-meiotic S-phase to form a tetrad (synaptonemal complex) resulting in tetraploidity (4n); During prophase-I (synapsis) chiasma/ta are formed between non-sister-chromosomes resulting in crossing over; In metaphase-I the sister chromosomes are separated (2n - centromer still in tact); Ana-, and telophase-I similar to mitosis. **Meiosis-II** follows (no interphase in-between) producing haploid cells (see mitosis (1n)).

Mendels laws: **1st**: Law of equal segregation; The two members of a genome-pair segregate from each other during anaphase of both meiosis-I and -II; each gamete has an equal probability of obtaining either member of the gene pair (2:2) .
2nd: Law of independent assortment; unlinked or distantly linked segregating gene-pairs assort independently in the rpophase of meiosis-I (recombination).

Mitosis: (Gk. mitos, thread) A type of nuclear division (occurring at cell division) that produces two daughter nuclei identical to the parent nucleus; (di-, polyploid).
Prophase: (Gk. Pro, early; phasis, form) Early stage of nuclear division; nucleus disappears, mitotic spindle forms, chromosome condenses and becomes visible.
Metaphase: (L. meta, half) Intermediate stage o.n.d.; chromosomes align along the equatorial plane.
Anaphase: (Gk. ana, away) Spindle separates centromere, pulling chromatids apart to the opposed poles of the cell.
Telophhase: (Gk,. Telo, late) Late stage o.n.d.; spindle dissolves, nuclear envelope reappears daughter nuclei re-form (segregation and cytokinesis).

Mutant: An organism or cell carrying a mutation.

Mutation: (L. mutare, to change) A permanent change in chemical structure, organization, or amount of DNA; produces a gene or a chromosome set differing from the wild type, resulting in a faulty protein (loss or gain of function; gains and selection are the tools of evolution).

Plasmid: An autonomously replicating circular, extra-chromosomal DNA molecule in prokaryota, bearing often genes of antibiotic resistance.

Prototroph: A strain of organism that will proliferate on minimal medium (compare auxotroph).

Recombination: The formation of offspring by combination of genes that are present in either chromatid, resulting from the assortment of chromosomes and their genes during the production of gametes (meiosis) and their subsequent fertilization (ovum and testis) from different individuals. (the reshuffling of maternal and paternal chromosomes during meiosis, resulting in new genetic recombinations (compare complementation).

Replica Plating: A velvet covered piston which allows the experimental transfer of microrganic colonies from one petri-plate to another for genetic analysis.

Sporolation: A diploid cell undergoing meiosis, leading to the generation of four haploid spores (tetrad).

Tetrad: (Gk. Tetra , four) Products of a single meiosis remain together as a group of four; in the case of *Saccharomyces cerevisiae* it produces four spores..

Wild Type: The genotype or phenotype that is found in nature or in the standard laboratory stock for a given organism (see also mutant).

Zygote: (Gr. zygotos, paired together) the diploid cell that results from the fusion of an egg and a sperm cell.
Heterozyg.: An individual having heterozygous gene pair (a gene pair having different alleles in the two chromosome sets of the diploid individual).
Homozyg.: An organism carrying identical alleles at the corresponding sites and homologous chromosomes.