7.016 Recitation 10 – Fall 2018

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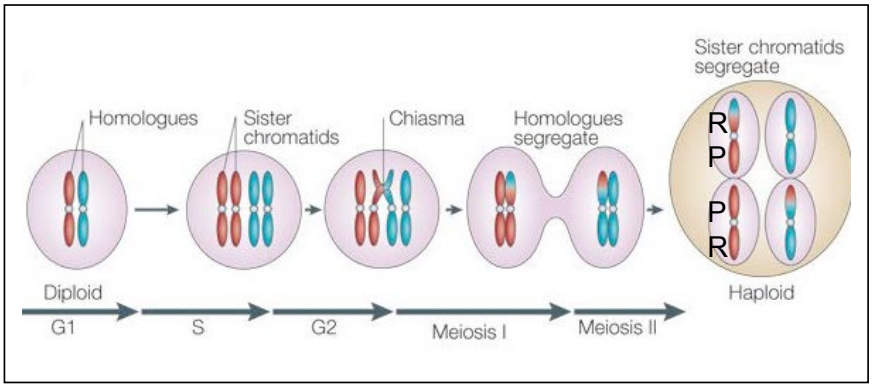
Summary of Lectures 14 (10/12) and 15 (10/15):

Recombination and crossing over: Homologous recombination is the reciprocal exchange of DNA between two homologous chromosomes during metaphase of meiosis I. In this process, two homologs (e.g. chromosome #7 inherited from dad and chromosome #7 inherited from mom) break at the same point and switch ends with each other. This process produces **parental/ non-recombinant (P) and non-parental/ recombinant (R)** gametes as diagrammed below.



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The recombination frequency (RF) is equal to the map distance (represented in cM or map units) between the two genes and is calculated as follows.

RF (%) = map distance (cM) = (number of recombinants in F2) X 100/ Total number of F2

- If RF is 50%: Map distance is 50cM = Parental: recombinant ratio is 1: 1 = Genes are **unlinked**
- If RF is 0%: Map distance is 0cM = Parental: recombinant ratio is 1: 0 = Genes are **completely linked**
- If RF is <50% but >0: = Genes are **incompletely linked**

Tri-hybrid cross: For a tri-hybrid cross you cross two true-breeding organisms that differ by three characteristics, such as P1 plant with genotype: AABBCC (each gene regulating one trait) versus P2 plant that has the genotype aabbcc to produce F1 with genotype AaBbCc and will display the dominant phenotypes. If you cross F1 organisms together, you create the F2 generation.

This cross allows you to detect the order of the genes on a chromosome and the distance between each gene pair. For this to happen:

- The genotypes of the offspring must be able to be observed in order to determine which alleles have been inherited. This is usually done by crossing the F1 (heterozygous at all gene loci) to a triple homozygous.

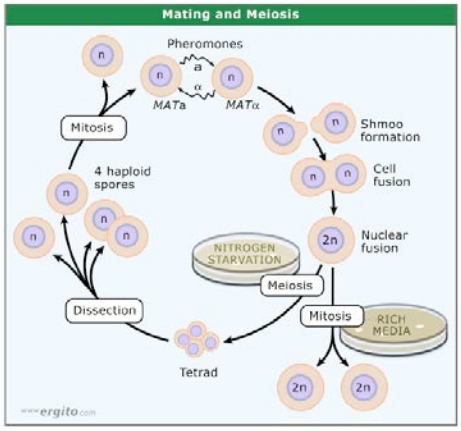
- There must be a sufficient number of offspring produced to give a representative sample that is large enough to get statistically significant results.

It is important to note that in this cross, chromosomes that have undergone no crossing over event, single crossover event (SCO) and double crossover event (DCO) are generated. Because there is a higher probability of having no crossovers, the two genotypes with the highest number of offspring are NCO (no crossover). There are two sets of SCO, and the two with the lowest offspring will be DCO.

You first need to determine which order of alleles on the chromosome will result in the DCO. To determine the distance, add the number of times crossover occurred between the two loci.

SCO+2(DCO)/ Total Offspring * 100= number of map units between genes

Using yeast to connect recombination with chromosomes: One of the main advantages to genetic studies of *S. cerevisiae* is that it can grow mitotically in a stable fashion as either a haploid (with one copy of each chromosome) or a diploid (with two copies of each chromosome).



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Haploids exist in either one of two mating types, called a and α. Mating type is determined at a genetic locus named MAT that can exist in either of two states, MATa or MATα. Haploids of opposite mating type can mate to form diploids. Mating occurs when cells of the opposite mating type become physically close to each other. Each haploid cell type secretes a specific mating pheromone that arrests the growth of cells of the opposite mating type at START of the cell cycle. The cells then fuse, an event followed by nuclear fusion, resulting in an a/α diploid.

Diploids are mitotically stable (that is, they will not undergo meiosis) in most growth conditions. However, when a/α diploids are starved for nitrogen, carbon, or sulfur, they arrest mitotic growth at G1 and undergo meiosis. Each meiotic diploid gives rise to four haploid progeny (spores) in a tetrad as illustrated in Figure above. Because the progeny are haploid, the phenotype is directly related to the genotype and therefore no test cross is needed to observe the recessive trait.

Chromosomes segregation in *S. cerevisiae,* proceeds similarly to that of other eukaryotes, with meiosis-I and II. During meiosis, the cells undergo dramatic morphogenetic changes. Each diploid cell that undergoes meiosis produces four haploid progeny as spores within an ascus/ tetrad. The physical association of all of the products of a single meiosis within a tetrad allows powerful genetic analyses of meiotic progeny in yeast to be performed. The genetic analysis of the meiotic products within a tetrad requires a method known as tetrad dissection, which is the act of separating the four spores of a tetrad on a solid growth medium.

While tetrad dissection is the act of separating spores from within tetrads, tetrad analysis involves determining the segregation of genetic markers in the tetrads and interpreting these segregation patterns. In addition to using tetrad analysis to determine single-gene segregation, we can also use it to measure the genetic map position of a gene, two genes, determined if they are linked or lot. This can be either relative to another gene (two-gene segregation), or relative to its centromere (gene-centromere segregation). This is because the centromeres of non-sister chromatids always segregate away from each other in meiosis I, and the centromeres of sister chromatids always segregate away from each other in meiosis II.

- A **parental ditype (PD)** is a tetrad in which two genetic markers are segregating, and two spores have one parental genotype and two spores have the other parental genotype.
- A **nonparental ditype (NPD)** is a tetrad in which two genetic markers are segregating, and two spores have one nonparental genotype and two spores have the other nonparental genotype.
- A **tetratype (TT)** is a tetrad in which two genetic markers are segregating. Two of the four spores have parental genotypes and two have recombinant genotypes.

If PD: ND: TT is 1:1: 4 then it means that the two markers are unlinked and segregate independently of each other. If PD: ND is 1:1 then the two genes are unlinked but they are linked at the centromere. If PD>> ND+TT then the two genes are linked.

Genetic screens: Genetic screening methods are used in model organisms (like flies and worms) to identify genes that are involved in multiple aspects of cellular and organismal physiology such as apoptosis and circadian rhythm and eye development. One such screening experiment in fruit flies is described below.

Here the male flies are fed ethyl methane sulphonate (EMS) to induce random mutations at a high rate in gametes. They are then crossed with normal females. The individual F1 fly is crossed with a wild-type fly to generate multiple F2 progeny (males and females) that are the heterozygous for the mutation. These F2 siblings are then crossed to each other to get F3, 25% of which will be homozygous for the recessive allele of mutated gene. These F3 flies will exhibit a phenotype corresponding to the absence of that gene.

Questions

1. Tomato plants can be **tall** or **short** and have **notched** or **smooth** leaves. You cross a tall, smooth leafed plant with a short, notched leafed plant. All of the progeny are **tall**, and **notched** leafed.

a) Which traits are **dominant**?

b) What are the genotypes of the two **true-breeding** parents? ***Note:*** *Use the letters H or h to represent the alleles of the height gene and the letters S or s to represent the alleles of the leaf gene. In each case, use the uppercase letter for the allele associated with the dominant phenotype and the lower case letter for the allele associated with the recessive phenotype.*

Tall & smooth: _____ Short and notched; _____

c) Two tall, notched F1 plants were crossed (self cross) to get 1600 F2 plants

i. What ratio of phenotype do you expect in the F2 generation?

ii. If you get 400 plants in F2, how many of these will be homozygous recessive for both traits?

d) You cross an F1 Plant with a double homozygous recessive plant (genotype: hhss) and get 1600 plants in F2 generation. Give the four classes of the F2 plants that you will see. Give the genotype, phenotype and the number of each of F2 classes generated.

e) If you assume that the two genes recombine at a frequency of 10%, what is the map distance between them?

2. The following are the results of a tri-hybrid cross. ***Note:*** *In this question "v", "cv" and "ct" are three genes. An allele with "+" represents an allele that confers the wild-type phenotype and "m" represent the mutated allele.*

$v^m cv^+ ct^+$	580
$v^+ cv^m ct^m$	592
$v^m cv^m ct^+$	42
$v^+ cv^+ ct^m$	43
$v^m cv^m ct^m$	91
$v^+ cv^+ ct^+$	92
$v^m cv^+ ct^m$	4
$v^+ cv^m ct^+$	4

Draw the arrangement of the three genes on the chromosome and specify the distance between them.

3. To understand how tetrad data can be used to measure gene-centromere linkage, you do the following cross using yeast.

(Trp1new1) ***X*** ***(trp1New1)***

You obtain the following numbers of tetrads: 40 PD, 40 NPD and 20 TT.

Are the two genes **linked or unlinked**?

Solutions to questions

1. Tomato plants can be **tall** or **short** and have **notched** or **smooth** leaves. You cross a tall, smooth leafed plant with a short, notched leafed plant. All of the progeny are **tall**, and **notched** leafed.

a) Which traits are **dominant**? *Tall & notched*

b) What are the genotypes of the two **true-breeding** parents? *Use the letters H or h to represent the alleles of the height gene and the letters S or s to represent the alleles of the leaf gene. In each case, use the uppercase letter for the allele associated with the dominant phenotype and the lower case letter for the allele associated with the recessive phenotype.*

Tall & smooth: HHss *Short and notched; hhSS*

c) Two tall, notched F1 plants were crossed (self cross) to get 1600 F2 plants

i. What ratio of phenotype do you expect in the F2 generation?
Tall and notched: Tall and smooth: short & notched: short & smooth = 9: 3: 3: 1

ii. If you get 400 plants in F2, how many of these will be homozygous recessive for both traits?
25

d) You cross an F1 Plant with a double homozygous recessive plant (genotype: hhss) and get 1600 plants in F2 generation. Give the four classes of the F2 plants that you will see. Give the genotype, phenotype and the number of each of F2 classes generated.

Tall & smooth (400): Short & notched (400): Tall and notched (400): short and smooth (400)

e) If you assume that the two genes recombine at a frequency of 10%, what is the map distance between them?
10cM or 10 map units.

2. The following are the results of a tri-hybrid cross. ***Note:*** *In this question "v", "cv" and "ct" are three genes. An allele with "+" represents an allele that confers the wild-type phenotype and "m" represent the mutated allele.*

$v^m cv^+ ct^+$	580
$v^+ cv^m ct^m$	592
$v^m cv^m ct^+$	42
$v^+ cv^+ ct^m$	43
$v^m cv^m ct^m$	91
$v^+ cv^+ ct^+$	92
$v^m cv^+ ct^m$	4
$v^+ cv^m ct^+$	4

Draw the arrangement of the three genes on the chromosome and specify the distance between them.

v-ct = 91+92+3+5/ 1448=13.2map units

$ct^+ cv$ = 4+4+42+43/ 1448= 6.4map units

*Chromosomal map: **v** (13.2cM) **ct** (6.4cM) **cv***

3. To understand how tetrad data can be used to measure gene-centromere linkage, you do the following cross using yeast.

(Trp1new1) ***X*** ***(trp1New1)***

You obtain the following numbers of tetrads: 40 PD; 40 NPD and 20 TT.

Are the two genes **linked or unlinked**?
Because PD = NPD, we know that trp1 and new1 are unlinked to each other but linked at the centromere.

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7.016 Introductory Biology
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