

# PLB 161A Molecular Markers: Analysis

## LABORATORY XIV

### XIV a. Location of markers to chromosome

1. Strip the membranes (see protocol laboratory XII)

#### Background information

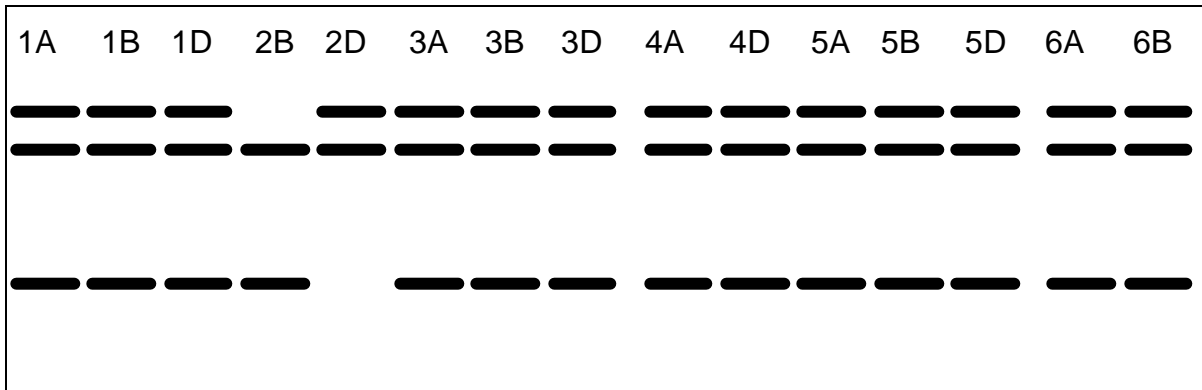
Often it is desirable to locate markers to chromosomes or even parts of a chromosome before genotyping a large set of individuals. This is particularly the case for large genome species such as wheat, when complex segregation patterns are detected for a probe, or when you are only interested in a particular region. To locate a locus to a chromosome or chromosome arm, one uses some type of cytogenetic stock. In maize, both monosomics and B-A translocations are available. Given the difficulty in handling these stocks in maize, most researchers do not take the time to locate new markers to chromosome. However, in wheat cytogenetic stocks are much easier to handle and maintain. Bread wheat (*Triticum aestivum* L.) is an allohexaploid species ( $2n=6x=42$ ) with three separate genomes, A, B, and D. The A genome is thought to come from *T. urartu* and the D genome from *T. tauschii*, while the B-genome has never been identified and may be an extinct species closely related to *T. speltoides*.

Extensive studies showing the close genetic similarities between these genomes began with the late Dr. E. R. Sears, who was able to synthesize numerous aneuploid stocks in the wheat variety Chinese Spring. The triple dose of many genes renders wheat tolerant to the loss of whole chromosomes or chromosome pairs in one genome. The aneuploids are easier to maintain if the missing chromosome pair is compensated by the duplication of one homeologous pair of chromosomes from the other genomes. This condition is called nullitetrasy. The homeologous groups were in fact identified by the ability of some chromosomes to compensate for the absence of others. Other useful cytogenetic stocks are the mono- or ditelosomes for individual chromosomes; in the 1AS ditelosome for example, the 1A chromosome consists of two copies of the short arm and the long arm is absent. These cytogenetic stocks are useful to localize a gene to a specific chromosome arm and even to estimate its distance from the centromere. More recently, B. Gill developed deletion stocks that have homozygous deletions of different length for each chromosome arm.

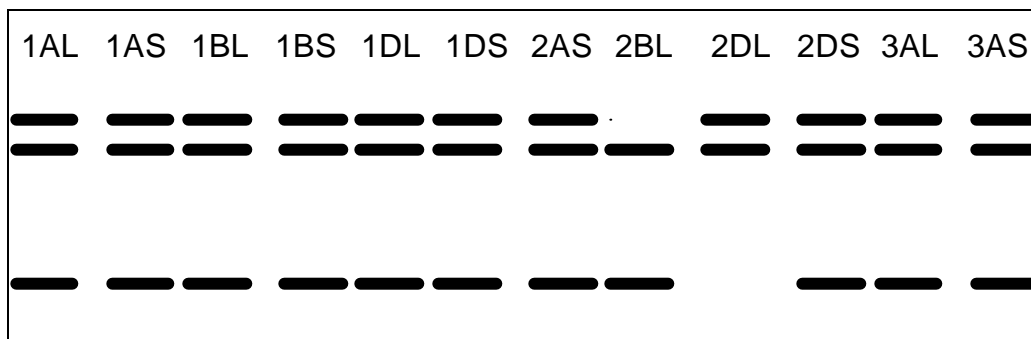
All these stocks have been responsible for almost the whole of the classical wheat map of some 300 morphological, physiological, and biochemical loci. More recently 5,000 ESTs have been map in a set of 101 deletion lines. In barley chromosomal assignments can be made by the analogous use of a series of barley single-chromosome addition lines in Chinese Spring.

## Aneuploid analysis with molecular markers

The nullitetrasomic and ditelosomic series are valuable in molecular mapping as well, by allowing the assignment of individual markers to chromosomes and arms. The figures 1 and 2 below show how this is done.



**Figure 1.** Diagrammatic hybridization pattern of a probe on a Southern blot of Chinese Spring nullitetrasomic stocks. Lanes are labeled with the chromosome missing in each stock.



**Figure 2.** Diagrammatic hybridization pattern of the same probe on a Southern blot of Chinese Spring ditelosomic stock DNAs digested with the same restriction enzyme. Lanes are labeled with the chromosome arm present as a ditelosomic in each stock; its opposite arm is missing.

DNA from each line of the aneuploid series is digested, electrophoresed, and immobilized on a Southern blot for hybridization with a probe of interest. The autoradiogram is inspected for missing bands. In Figure 1 above, the bands missing in 2B and 2D reveal that this probe hybridizes to a sequence on chromosome 2. The 2A-nullisomic stock is not represented because its viability was poor due to the absence of important genes on 2AS. However, the double intensity of one band in N2DT2A (nulli-2D-tetra-2A) identifies the 2A chromosomal band. The three Chromosome 2 loci are certainly orthologous, all

having derived from the same diploid progenitor before the divergence between the A, B, and D genomes. In figure 2, the absence of the bands in the long-arm ditelosomics of chromosome 2 in contrast with their presence in the short-arm ditelosomics shows that this probe hybridizes to the short arm.

## **XIV b. Introduction to Chromosome Mapping**

### **Review of basic genetic concepts:**

Reference: An Introduction to Genetic Analysis. Griffiths et al. 1996.

Mendel established the ground rules for genetic analysis. His work with controlled crosses made it possible to infer the existence and nature of hereditary units and mechanisms. The analysis of frequencies in the progenies of controlled crosses is still an integral part of the experimental approach used in much of modern genetics. Mendel's First Law: The two members of a gene pair segregate from each other into the gametes, so that one-half of the gametes carry one member of the pair and the other one-half of the gametes carry the other member of the pair. Mendel's Second Law: During gamete fecundation the segregation of alleles of one gene is independent of the segregation of alleles of another gene (Griffiths Chapter 2).

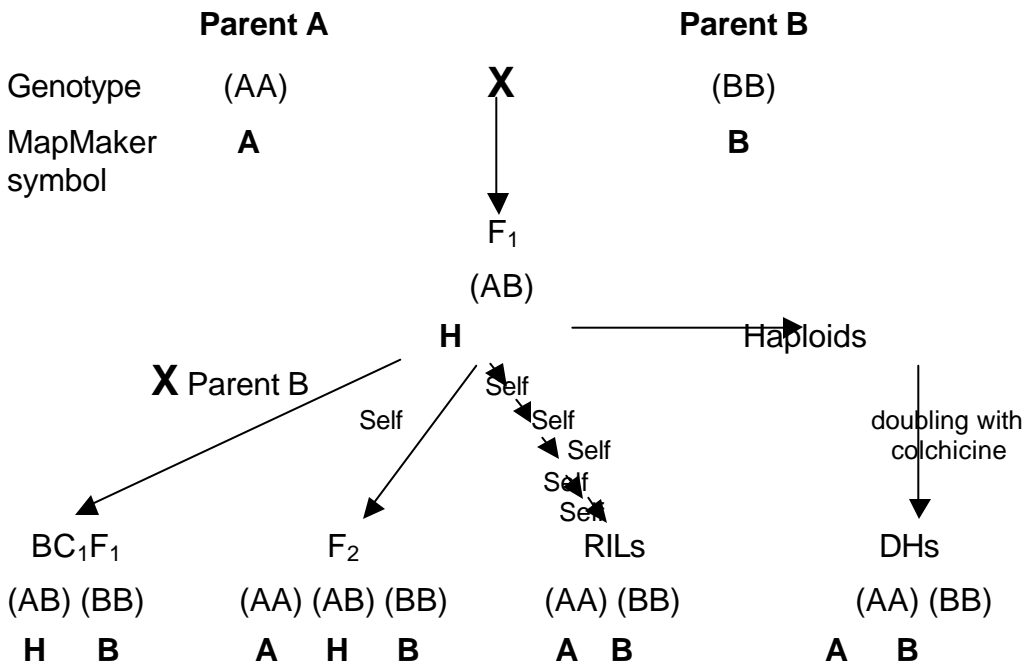
Mendel's laws are only the starting points for understanding heredity. Mendel observed only complete dominance in his experiments. However, incomplete dominance (phenotype of the heterozygote anywhere between the phenotypes of the homozygotes) and codominance (phenotype of both homozygotes in the heterozygote) are also common. Mendel reported genes with two alleles but multiple allelism is also very frequent. To make things more complex, one gene may affect more than one character –that is, it may be pleiotropic, and also several genes may interact to affect a single character (epistatic interactions). In the latter case modified Mendelian ratios will be observed in the  $F_2$ . Finally, the impact of a gene at the phenotype level depends not only on its dominance but also on the modifying effect of the rest of the genome and of the environment. Penetrance and expressivity are useful terms to describe situations where the phenotype ascribed to a gene is known to be dependent on other factors but the precise inheritance of those factors has not been established. Penetrance refers to the percentage of individuals of a specific genotype who express the phenotype associated with that genotype and expressivity refers to the degree to which a particular genotype is expressed as a phenotype (Griffiths Chapter 3).

Sometimes it is difficult to manipulate important agronomic traits because of the complex interactions among genes, phenotype and environment. The use of molecular markers to target the genes of interest can facilitate the manipulation of these genes in breeding or research projects. Molecular markers like RFLP or microsatellites are usually codominant, are not affected by the rest of the genome or the environment, show no interactions with other genes and have no problems of penetrance or expressivity. The molecular markers can be

used as landmarks along the entire length of each chromosome. The strategy of using molecular markers as reference points in the genome has been so successful that complete molecular maps are now available for most of the crops.

### Mapping populations

In deriving a linkage map for a particular species, one must first develop a mapping population that will produce segregation at the desired loci (Fig. 3).



**Figure 3.** Typical mapping populations with genotypes (in parentheses) and Map Maker symbols (in bold)

Many types of populations are possible, and decisions regarding which types are used are based on the desired resolution and ease or possibility of development. F<sub>2</sub> and backcross populations are adequate and easy to produce for self-pollinated species like wheat (Fig. 3). These strategies are fast but produce limited amount of seed of the mapped individuals. If multiple tests in multiple locations are necessary to measure the targeted character, it is necessary to develop immortal mapping populations such as recombinant inbred lines (RILs), recombinant substitution lines (RSLs)<sup>1</sup>, or double-haploids (DHs) (Fig. 3). These three types of populations produce homozygous lines that can be

<sup>1</sup> To produce RSLs a substitution line is crossed with the normal line. The hybrid is like an F<sub>1</sub> but only for the substituted chromosome. The F<sub>1</sub> is crossed as a male with a cytogenetic stock of the same line lacking that chromosome (nullisomic). In the progeny the recombined chromosome will have no companion to pair (monosomic). The monosomic plant is self-pollinated and the recombined chromosome is recovered in double dose.

replicated in the field several times and over several cycles, and are best suited to map quantitative traits (QTLs: Quantitative Trait Loci).

## Linkage

Two genes close together on the same chromosome pair do not assort independently at meiosis. This relationship called linkage explains why the observed parental gene combinations are more frequent in certain experiments than expected by Mendel's second law. The place on the map—and on the chromosome—where a gene is located is called the gene locus (plural, loci).

Linked genes can be separated by a physical exchange of chromosome parts during meiosis. This process, called crossover or intrachromosomal recombination, generates recombinant haploid genotypes that differ from the haploid parental genotypes. There is a rough proportionality between the distance between the linked genes and the chance that nonsister chromatids would cross over in the regions between the genes producing recombinant gametes. Thus, by determining the frequency of recombinants, it is possible to obtain a measure of the map distance between the genes. A recombinant frequency of 0.01 is defined as the distance between genes for which one product of meiosis out of 100 is recombinant (approximately 1 centiMorgan, cM).

Based on the previous concept, it is clear that to estimate the recombination between two loci is necessary to identify recombinant and parental genotypes. All genotypic classes can be identified unequivocally as parental or recombinant in RSL mapping populations. However, some genotypic classes can not be unambiguously identified as parental or recombinant in an  $F_2$  mapping population for a codominant marker such as an RFLP. The double heterozygotes (**HH**) are ambiguous, in that it is not possible to determine whether a parental or recombinant gamete combined to form the individual. The following examples show two different pairs of chromosomes in an  $F_2$  individual, one parental and one recombinant. The crossing over points are indicated by "x".

Example of  $H_1H_2$  genotype derived from parental chromosomes (no recombination between Locus<sub>1</sub> and Locus<sub>2</sub>).

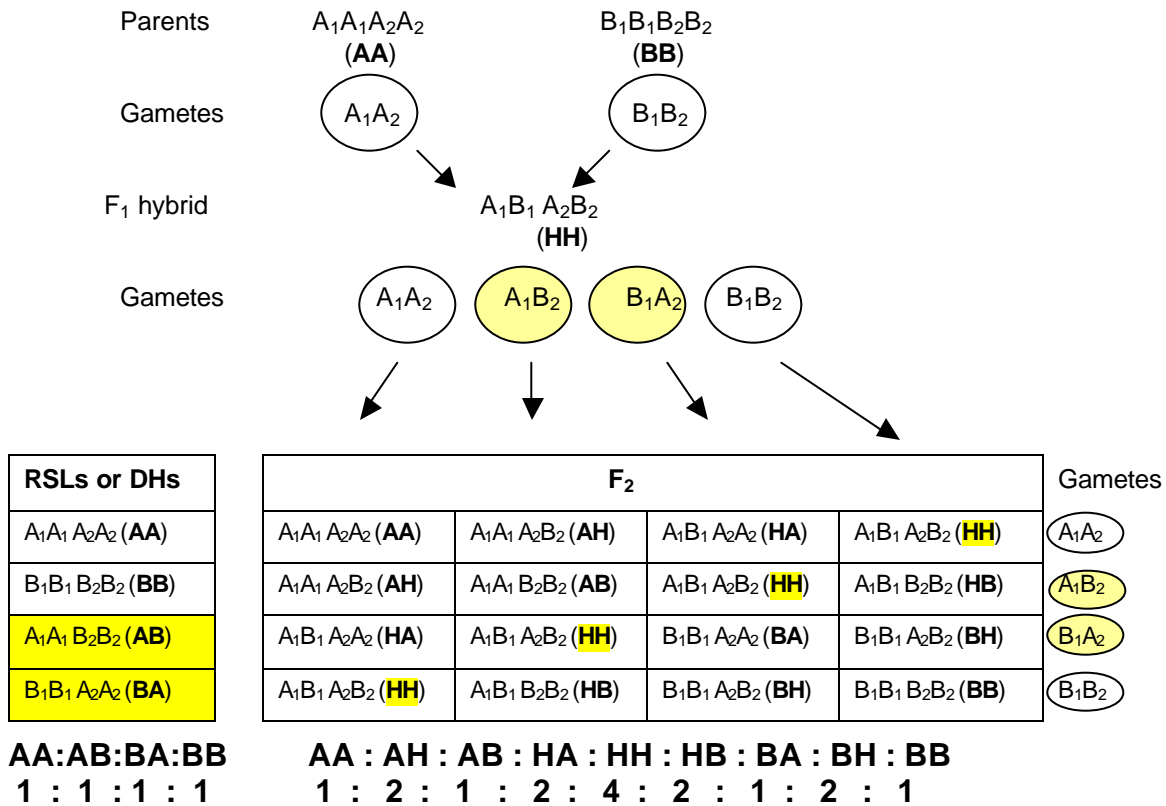
Chromosome 1a:	A	A <sub>1</sub>	A <sub>2</sub>	A x B	B	B
Chromosome 1b:	A x B <sub>1</sub>	B <sub>2</sub>	B	B	B	B
RFLP score:	<b>A</b>	<b>H<sub>1</sub></b>	<b>H<sub>2</sub></b>	<b>H</b>	<b>B</b>	<b>B</b>

Example of  $H_1H_2$  genotype derived from recombinant chromosomes

Chromosome 2a:	A	A <sub>1</sub> x B <sub>2</sub>	B	B	B
Chromosome 2b:	B	B <sub>1</sub> x A <sub>2</sub>	A	A	A
RFLP score:	<b>H</b>	<b>H<sub>1</sub></b>	<b>H<sub>2</sub></b>	<b>H</b>	<b>H</b>

In a double haploid or RSL mapping population the recombination events produced in the  $F_1$  meiosis are immortalized. A recombined chromosome present in the gamete is duplicated and only homozygous plants are produced. Only four genotypic classes are observed in the progeny: two parental classes  $A_1A_1$   $A_2A_2$

and  $B_1B_1 B_2B_2$ , and two recombinant classes  $A_1A_1 B_2B_2$  and  $A_2A_2 B_1B_1$  (Fig. 4). In an  $F_2$  population 9 different genotypic classes will be observed.



**Figure 4.** Expected genotypic classes for a codominant locus in RSLs and  $F_2$  populations.

Once one or more markers have been checked and the genotypes determined the next step would be to determine if any of the markers are linked. Mendel's Second Law concerns the independent assortment of genes. When two homozygous parents are crossed, two classes of individuals will be obtained in the next generation: parental and recombinant. The first step in determining linkage would be to check for independent segregation using the Chi-Square Test. If the proportion of genotypic classes is not significantly different from that expected from independent segregation then the 2 genes are not linked (Tab. 2).

**Table 2.** Example of observed and expected segregation values.

Example of RSL, two locus $L_1 L_2$					$\chi^2$			
Genotype:	AA	:	AB	:	BA	:	BB	
Observed:	6.00	:	5.00	:	7.00	:	7.00	0.44
Expected :	6.25	:	6.25	:	6.25	:	6.25	

**Example of F<sub>2</sub>, two locus L<sub>1</sub> L<sub>2</sub>**

Genotype:	AA	: AH	: AB	: HA	: HH	: HB	: BA	: BH	: BB	
Observed:	5.0	: 2.0	: 0:0	: 0:0	:11.0	: 0:0	: 0:0	: 0:0	: 6.0	38.2
Expected:	1.5	: 3.0	: 1.5	: 3.0	: 6.0	: 3.0	: 1:5	: 3.0	: 1.5	

For the RSLs the calculated Chi-square value is 0.44 (Critical value  $\alpha=0.05, \chi^2_{3df}=7.81$ ). The probability that this value arose by chance is 90-95%, and thus the two markers can be considered unlinked. In the F<sub>2</sub> example the calculated chi-square value is 38.2, that is greater than the critical value at a 99% confidence level (Critical value  $\alpha=0.05, \chi^2_{8df}=15.51$ ) and, thus, the two markers can be considered to be linked.

Table 3 presents additional expected segregation for two dominant and codominant markers in BC<sub>1</sub>F<sub>1</sub>, F<sub>2</sub>, RILs, RSLs and DH populations (Fig. 4).

**Table 3.** Expected segregation for two dominant and codominant markers in BC<sub>1</sub>F<sub>1</sub>, F<sub>2</sub>, RIL RSLs and DH populations (Fig. 4).

Population	Dominant markers	Codominant markers
BC <sub>1</sub> F <sub>2</sub>	1:1:1:1	1:1:1:1
F <sub>2</sub>	9:3:3:1	1:2:1:2:4:2:1:2:1
RIL or RSL	1:1:1:1	1:1:1:1
DH	1:1:1:1	1:1:1:1

**Measure of linkage**

Given two linked markers, the next step would be to calculate how far apart are the two loci. Perhaps the simplest method would be to count the number of crossovers that occurred in the population. The number of crossovers in each individual is indicated below the genotypes of each individual in Table 4.

**Table 4.** Number of crossovers.

RSLs																										
L <sub>1</sub>	A	A	B	B	B	A	B	B	-	A	A	A	A	A	B	A	B	B	B	A	B	A	B	B	B	B
L <sub>2</sub>	A	A	B	B	B	B	A	B	B	-	B	B	B	A	A	A	B	A	A	A	B	A	A	A	A	B
#CO	0	0	0	0	0	1	0	1	-	1	-	1	1	0	1	0	0	1	1	0	0	0	1	1	1	0

RF= 12/25= 0.48

F <sub>2</sub>																										
L <sub>3</sub>	A	H	A	H	B	H	-	H	B	A	H	-	B	H	A	A	H	H	B	B	A	A	H	H	H	B
L <sub>4</sub>	H	H	A	H	B	H	-	H	B	A	H	-	B	H	A	A	H	H	B	B	A	B	H	H	H	B
#CO	1	0	0	0	0	0	-	0	0	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	2	0

To calculate the recombination distance, you simply divide the total number of recombination events detected by the total number of meiotic events. For an F<sub>2</sub>, one can have recombination events in both the male and female

gametes, so the total number of events is twice the number of individuals. Thus, in the  $F_2$  example, the number of crossovers was 3, the total number of individuals was 24 and therefore the total number of meiotic events possible was  $2 \times 24 = 48$ . The calculated recombination frequency is then  $3 / 48 = 0.0625$  (6.25%) and indicates that these two loci were tightly linked. This is an **approximation** since it is not possible to establish if the HH genotypic classes are parental or double recombinants.

While counting recombination events is easy and will result in a good approximation of the frequency, more accurate methods for distance determinations are available. These are based on **maximum likelihood** and allow one to calculate the most probable distance based on the available data. The maximum likelihood method of estimation is a general statistical method to estimate the value of a parameter: the value chosen for the parameter is that which is most probable (or **most likely**) given the occurrence of a certain set of observations.

The advantage of the **maximum likelihood** method is that it is unaffected by segregation distortion or missing data. Both of these problems are often found in typical mapping experiments. The paper by Allard (1956)<sup>2</sup> presents the method to derive the formulas for calculating the distance and standard deviations for several types of populations and loci. Fortunately for us, computer programs have been developed which use these formulas to calculate the distances given large data sets of genotypes. One of the more popular is MapMaker that will be used in this course to construct linkage maps from several sets of genotypes.

To determine whether any two markers are linked, MAPMAKER calculates the maximum-likelihood distance and a parameter called **LOD score** ( $\text{Log}_{10}$  of the **odds ratio**). First the odds ratio is calculated as:

$\frac{\text{Numerator}}{\text{Denominator}} = \frac{\text{Probability of obtaining the observed data under linkage}}{\text{Probability of obtaining the observed data under random assortment}}$
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This quantity is then transformed to  $\log_{10}$ . The higher the value the more likely is the linkage. For example, a  $\text{LOD}=3$  indicates that is 1000 times ( $10^3$ ) more likely to obtain the observed data from linked markers than from unlinked ones.

LOD scores can also be used to determine the most likely order of markers in a map. Mapmaker assigns 0 to the better order and negative LODs to alternative orders. For example, if order  $L_a - L_b - L_c$  has  $\text{LOD}=0$  and order  $L_a - L_c - L_b$  has a LOD score of  $-2$ , that indicates that it is 100 ( $10^2$ ) less likely to obtain the observed data from the second order than from the first order.

Perhaps the most important point to remember is that it is entirely possible to develop a linkage map with bad data. Therefore, extreme care should be

<sup>2</sup> Allard, R. W. (1956). Formulas and tables to facilitate the calculation of recombination values in heredity. *Hilgardia* **24**: 235-278.



taken when entering all genotypic data. If in doubt regarding the genotype of an individual, it is better to enter it as a missing value than to guess. In addition, some form of verification should be used to make sure that the data set is as accurate as possible. This is particularly true if high map resolution (i.e. precise distances) is desired/required.

### Linkage calculations in recombinant inbred populations

There is a strategy called recombinant inbred populations, also known as Single Seed Descent (SSD), which is used to produce permanent mapping populations. Each plant of the F<sub>2</sub> is self-pollinated, and one F<sub>3</sub> seed is harvested from each F<sub>2</sub> plant (without selection). Then each F<sub>3</sub> plant is self-pollinated, and one F<sub>4</sub> seed is harvested from each F<sub>3</sub> plant. The process is repeated until F<sub>6</sub> or F<sub>8</sub>. Remember that at each generation of self-pollination half of the heterozygous loci become homozygous and fixed (proportion of heterozygous = 0.5<sup>n-1</sup>).

SSD lines have fixed genotypes and can be distributed to colleagues who can look at other traits, other environments, etc. It can also be used to analyze the genetics of traits that have a large environmental component and therefore need to be analyzed in replicated trials. Another advantage is that segregation data can be accumulated over labs and years.

Two loci that are linked will be also linked in the SSD. Since the SSD has more generations for recombination, recombination frequencies need to be adjusted. Recombination levels observed in a SSD can be related to recombination in a population derived from a single meiosis using Haldane and Waddington (1931) formulas.

$$R = \frac{2r}{1+2r} \quad \text{or} \quad r = \frac{R}{2-2R}$$

*R* is the proportion of SSD lines showing recombination between two markers and *r* is the RF in a population derived from a single meiosis. When linkages are tight, the recombination frequency among self-pollinated SSDs is twice the conventional F<sub>2</sub> rate. This may represent a disadvantage at the beginning of a mapping endeavor because it will be more difficult to establish linkages. As a map develops, however, it can become an advantage because recombination between closely linked markers will be more readily detected.

Some examples

<i>R</i>	<i>r</i>	<i>R/r</i>
0.01	0.005	1.98
0.1	0.056	1.80
0.2	0.125	1.60
0.3	0.214	1.40
0.4	0.333	1.20
0.5	0.500	1.00

## Class Exercises for linkage and mapping

All these exercises should be included in the lab report

- Exercise 1:

You have assigned four loci to chromosome 1A of wheat using nullitetrasonics. Using fifty double haploid plants developed from a cross between two varieties A and B you recover the proportion of recombinant and parental chromosomes indicated below. The letter indicates the source of the alleles (parent A or B) and the subscript indicates the locus number (AA and BB are the parental combinations):

Locus 1- Locus 2:	A <sub>1</sub> A <sub>2</sub> : 25	A <sub>1</sub> B <sub>2</sub> : 0	B <sub>1</sub> A <sub>2</sub> : 1	B <sub>1</sub> B <sub>2</sub> : 24
Locus 1- Locus 3:	A <sub>1</sub> A <sub>3</sub> : 23	A <sub>1</sub> B <sub>3</sub> : 3	B <sub>1</sub> A <sub>3</sub> : 4	B <sub>1</sub> B <sub>3</sub> : 20
Locus 1- Locus 4:	A <sub>1</sub> A <sub>4</sub> : 25	A <sub>1</sub> B <sub>4</sub> : 1	B <sub>1</sub> A <sub>4</sub> : 1	B <sub>1</sub> B <sub>4</sub> : 23
Locus 2- Locus 3:	A <sub>2</sub> A <sub>3</sub> : 21	A <sub>2</sub> B <sub>3</sub> : 3	B <sub>2</sub> A <sub>3</sub> : 5	B <sub>2</sub> B <sub>3</sub> : 21
Locus 2- Locus 4:	A <sub>2</sub> A <sub>4</sub> : 24	A <sub>2</sub> B <sub>4</sub> : 2	B <sub>2</sub> A <sub>4</sub> : 1	B <sub>2</sub> B <sub>4</sub> : 23
Locus 3- Locus 4:	A <sub>3</sub> A <sub>4</sub> : 23	A <sub>3</sub> B <sub>4</sub> : 2	B <sub>3</sub> A <sub>4</sub> : 3	B <sub>3</sub> B <sub>4</sub> : 22

Construct a map including these four loci. Make distances in the drawing proportional to the genetic distances.

- Exercise 2: Chi Square for Double Haploid, RSL or Backcross

In a similar population of 100 Double Haploid plants you mapped two loci: L1 and L5. You checked parental and recombinant chromosomes and you found:

Locus 1 – Locus 5:	A <sub>1</sub> A <sub>5</sub> :28	A <sub>1</sub> B <sub>5</sub> :22	B <sub>1</sub> A <sub>5</sub> :24	B <sub>1</sub> B <sub>5</sub> :26
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Are these two loci linked?

- Exercise 3: Chi Square for F<sub>2</sub>

Are loci L<sub>1</sub> and L<sub>2</sub> linked?.

Example of F<sub>2</sub>, two locus L<sub>1</sub> L<sub>2</sub>

Genotype:	A <sub>1</sub> A <sub>2</sub> : A <sub>1</sub> H <sub>2</sub> : A <sub>1</sub> B <sub>2</sub> : H <sub>1</sub> A <sub>2</sub> : H <sub>1</sub> H <sub>2</sub> : H <sub>1</sub> B <sub>2</sub> : B <sub>1</sub> A <sub>2</sub> : B <sub>1</sub> H <sub>2</sub> : B <sub>1</sub> B <sub>2</sub>
Observed:	5.0 : 2.0 : 0.0 : 0.0 : 11.0 : 0.0 : 0.0 : 0.0 : 6.0
Expected:	1.5 : 3.0 : 1.5 : 3.0 : 6.0 : 3.0 : 1.5 : 3.0 : 1.5

Indicate the critical 0.05  $\chi^2$  value.

Indicate the procedure to calculate the expected frequencies.

- Exercise 4: Recombination fractions in RI or SSD

The following is the data for MapMaker for a Recombinant Inbred Population of 50 lines and 4 loci (RI or SSD). Calculate the corrected RF 'r' between loci 1-2, 2-3 and 3-4. Remember that in a SSD  $r = R/(2-2R)$  where R is the proportion of recombinants in the RI.

```
data type ri self
50 4 0
*locus1 aaaaa aaaaa bbbbbb bbbbbb aaaaa bbbbbb aaaaa bbbbbb aaaaa bbbbbb
*locus2 aaaaa aaaaa bbbbbb bbbbbb aaaaa bbbbbb aaaaa bbbbbb aaaaa bbbbbb
*locus3 aaaaa aaaaa bbbbbb bbbbbb bbbbbb bbbbbb aaaaa bbbbbb aaaaa bbbbbb
*locus4 aaaaa aaaaa bbbbbb bbbbbb bbbbbb aaaaa bbbbbb bbbbbb aaaaa bbbbbb
```

## Mapping exercises (by groups)

### 1)RSLs

The following matrix represents the map of chromosome 1AL constructed from recombinant substitution lines of the chromosome 1A of bread wheat and chromosome 1A<sup>m</sup> of *Triticum monococcum*<sup>3</sup>. MapMaker does not have an option for RSLs or double haploids but the use of “type f2 backcross” plus the codification of one of the homozygous classes as “h” will produce exactly the same result. In this particular example “h” represents RFLP bands from bread wheat and “a” RFLP bands from *Triticum monococcum*. The markers are organized in the order they are present in the chromosome. As a consequence of this order each column represent a chromosome. The changes between “a” and “h” represent crossing over points.

data type f2 backcross (File: PLB161PH.txt)		
39	18	0
PLANT NUMBER	11111111122222	22222333333333444
	1234568901245678901234	56789012345789023
*Hg	ahaaaahhhahaaaaa-hahhh	hahahaaaahahahhhh
*Xmwg645	-haaaahhaahaahaa-hhhhh	haaahaaaahahahhhh
*Xmwg2048	ahaaaahhaahaahaa-hhhhh	haaahaaaahahhhhhh
*Xmwg2083	ahaahahhaahaahaa-hhhhh	haaaaaaaahahhhhhh
*XChs3	ahaahahhaahaahaa-hhhha	haaaaaaaahahhhhhh
*XksuE18	ahaahaahaahaahah-hhhha	haaaaaaaahahhhhhh
*X5SDna	haaahhahaahaahah-hhhha	haaaaaaaahahhhhhh
*XcsIH69	haaahaahaahaahah-hhhha	haaaaaaaahahhhhhh
*Xabg500	haaahaaaaahaahah-hhhha	haahaaaaahaahhhh
*XTri	haaahaaaaahaahah-hhhha	haahaaaaahaahhhh
*Xesi47	haaahaaaaahahhah-hhhha	haahaaaaahaahhhh
*Xbcd454	haaahaaaaahahhah-hhhha	hhahaaaaahaahhhh
*cdo1396	haaaaaaaahhahhaa-hhhha	hhahhaaaahaahhhh
*XLec	hhaaaahaahhahhaa-hhhha	hhahhahhaaaahhhah
*Xmwg733	hhhhahaahaahhaa-hhhha	hhahhaahhaaahhhah
*Xbcd442	hhhhahaahahahhaahhhha	hhahhaahhaaahahah
*XAdh3	hhhhahaahahahhaahhhha	hhahhaahhaaahahaa
*XksuG34	hhhhahahhahahhaahhha	hhahhaahhahahahah

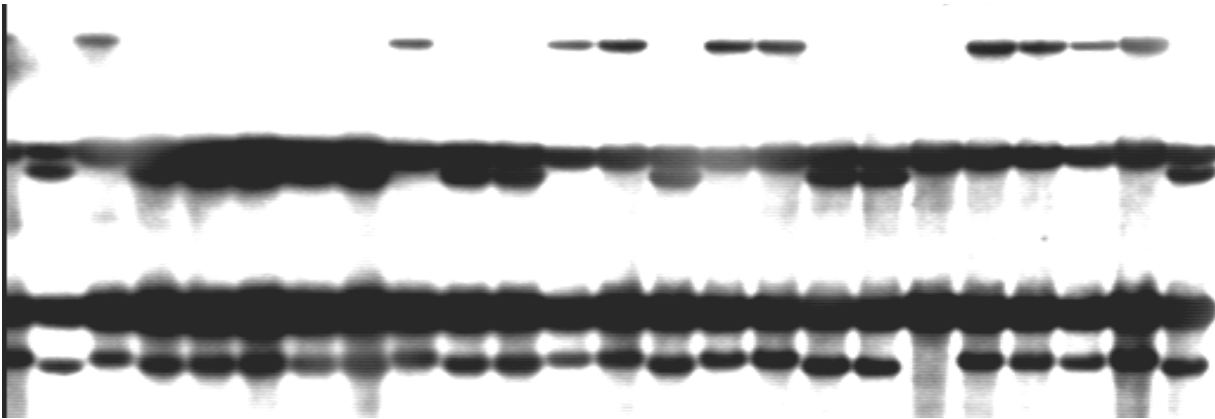
a) Mark with a red dot the crossing over points

Score the High Molecular Weight Glutenin (HMW-G) clone in a small strip of paper. Find the position in the map that minimizes the number of additional crossing over points. This is the most likely chromosome location of the gene in the chromosome.

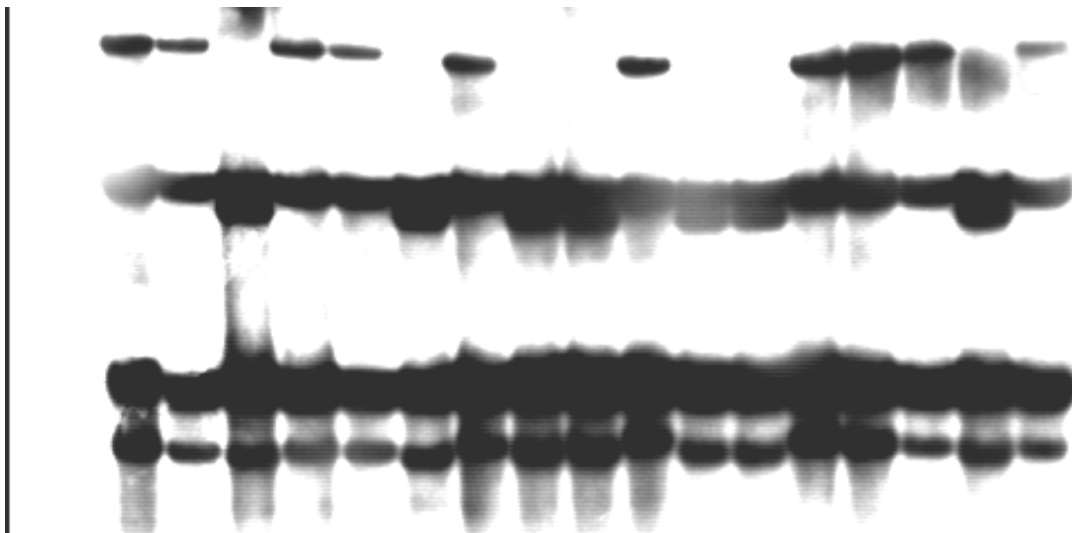
<sup>3</sup> Dubcovsky, J., M.-C. Luo, et al. (1995). “Differentiation between homeologous chromosomes 1A of wheat and 1A<sup>m</sup> of *Triticum monococcum* and its recognition by the wheat *Ph1* locus.” *Proceedings of National Academy of Sciences* **92**: 6645-6649.

# High Molecular Weight Glutenins. RSLs PLB161PH.TXT

CS Tm 1 2 3 4 5 6 8 9 10 11 12 14 15 16 17 18 **19** 20 21 22 23 24



25 26 27 28 29 30 31 32 33 34 35 37 38 39 40 42 43



## 2) Diploid F<sub>2</sub>

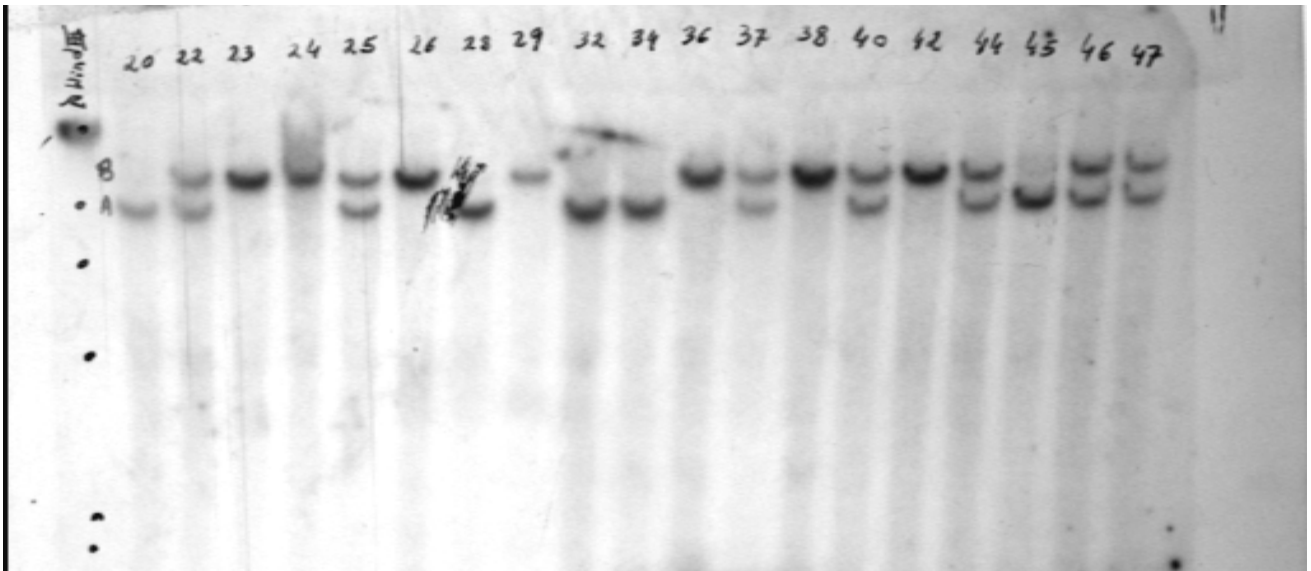
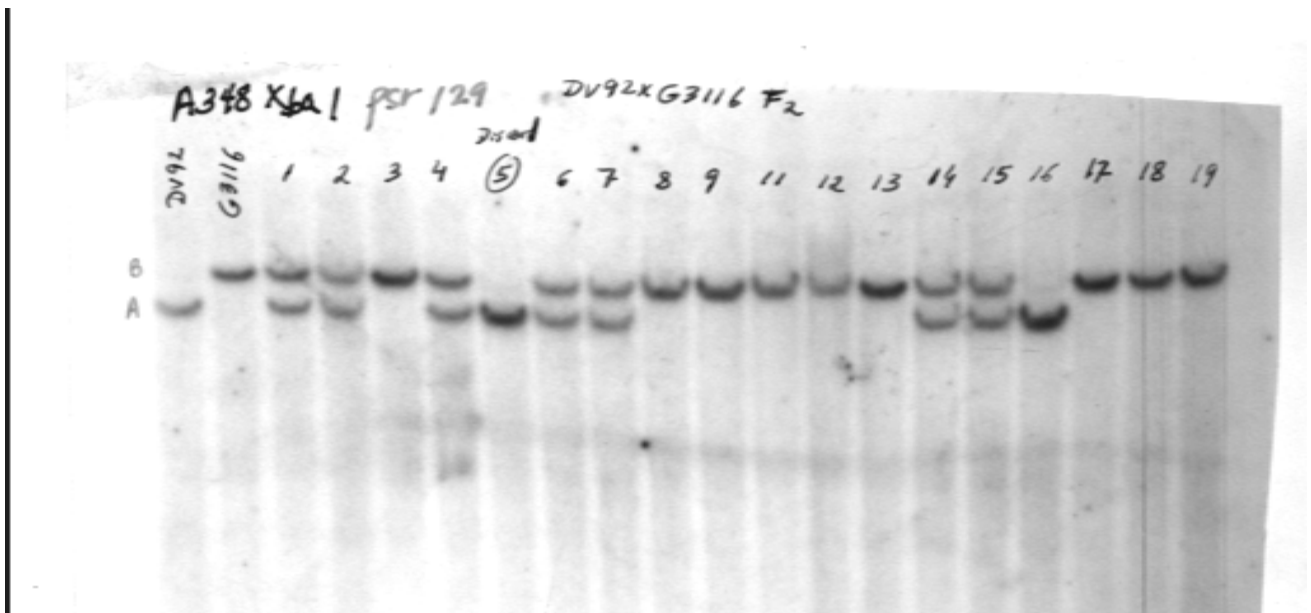
The following matrix represents the map of chromosome 7A<sup>m</sup> constructed from an F<sub>2</sub> mapping population of *Triticum monococcum*<sup>4</sup>. The “A” represents RFLP bands from cultivated *T. monococcum* and the “B” RFLP bands from wild *T. monococcum*. The markers are organized in the order they are present in the chromosome. Since this is an F<sub>2</sub> population each column represent 2 homologous chromosomes coming from two independent meiosis.

data type f2 intercross (File: PLB1617A.txt)				
	36	20	0	
PLANT	111111111 222222223333344444			
NUMBER	123456789123456789 0234568924678024567			
*Xabg704	HABH	AHHHHBAABAHD	HAHBHAC-HAACHHHHAH-	
*Xpsr119	HA-H	AAHHHCAABAHH	HAHBBAHAHAHHHHHAH-	
*Xcdol400	HABH	AABHHBHAVAHHA	HAHBHHHHHA-HHHHAHH	
*Xbcd93	AHBB	AABHH-BABAHD	HHH-BBHHHHAHHB-HHBA	
*Xwg834	AHBB	AABHHHBCBAHHH	AHBHBBHHHHADHBAHB-	
*Xmwg710	AHHB	AHBHHHBHBAHHH	AHBHBBHHHHACHBHAHBA	
*Xabc152	AHHB	AHBHHHBHBAHHH	AHBHBBHHHHHAHHBHAHBH	
*Xglk651	HHHB	AHBHHHBHBAHHH	AHBBBBHCHHAHBAHABC	
*EstA3	HCDB	AHBBBHBHBDDBH-	DCBB-CBAHABDHB-ADHH	
*Xcd057	HHAB	AHBBBBBHBABBH	AHBHBHHAHAB-BBHAAHH	
*Xabc455	AHAB	AHBBBBBHBABBH	AHBHBHHAHABHBBHAAHH	
*Xcd0673	AHHB	AHBBBBBHBABBH	AHBHBHHAHAB-HBBHAAHH	
*XksuH9	DDBD	DDBBBBBDDDBD	DDBDBDBDDB-DBBDDDD	
*Xesi2	HHBH	HHBBBBBHHABBH	AHBHBABAABHHHHBHAAH	
*Xabc305	HBBH	HHBBBBBHHABHB	AHBHBABAABHBBHBAHH	
*XVAtp-B	HBBH	HHBBBBBHHABHB	AHBHBA-AHBHBBHBAH-	
*Xwg380	HBBH	HHBBBBBHHABHB	AABBHBAHAHB-BHBHHHH	
*Xabg461	HBBH	HHBBBHHHACHB	AHBHBAHAHBBBHHHHH	
*Xwg420	HHBH	HHBBBHHHAAHB	AHBHBBCHHHBBBABHHHH	
*Xpsr680	HHHA	HHHHHBBHHAHB	AHBHHBHHHHBBBABHHH-	

- Mark with a red dot the crossing over points
- Score the RFLP marker PSR129 and find the most likely position in the map by minimizing the number of crossing over points.
- Score the autoradiographs hybridized with the Low Molecular Weight Glutenin clone. How many loci are segregating? Find which are on chromosome 7A<sup>m</sup> and determine their position in the map.

<sup>4</sup> Dubcovsky, J., M.-C. Luo, et al. (1996). "Genetic map of diploid wheat, *Triticum monococcum* L., and its comparison with maps of *Hordeum vulgare* L." *Genetics* **143**: 983-999.

RFLP *Xpsr129*. PLB1617A.txt F<sub>2</sub> *Triticum monococcum* 7A<sup>m</sup>



Low Molecular Weight Glutenins **XGlu-A3**  
 PLB1617A.txt F<sub>2</sub> *T. monococcum* 7A<sup>m</sup>

