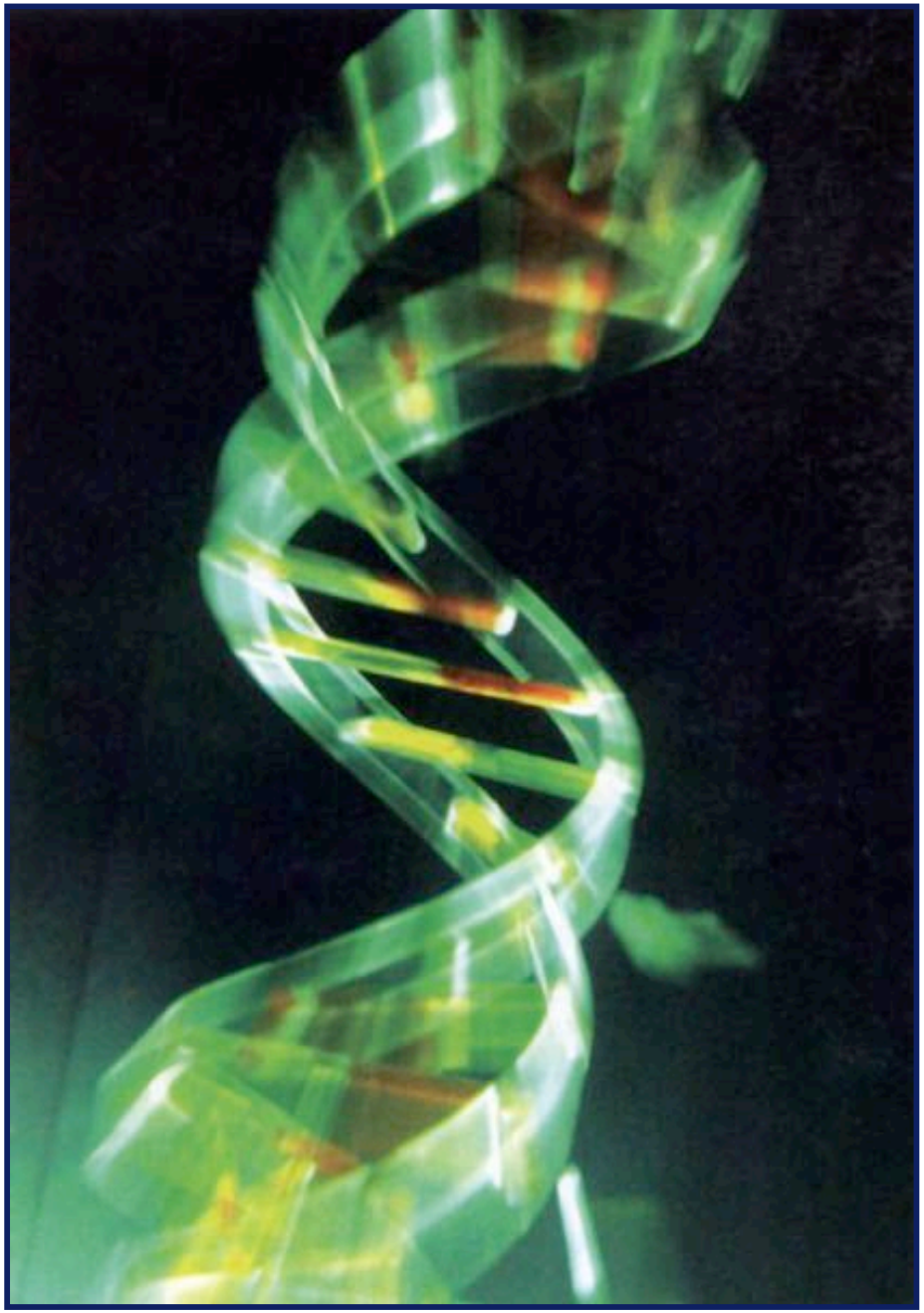
Chapter 6

Linkage Analysis and Mapping

Three point crosses

- mapping
- strategy
- examples

➢ Mapping human genes



Three point crosses

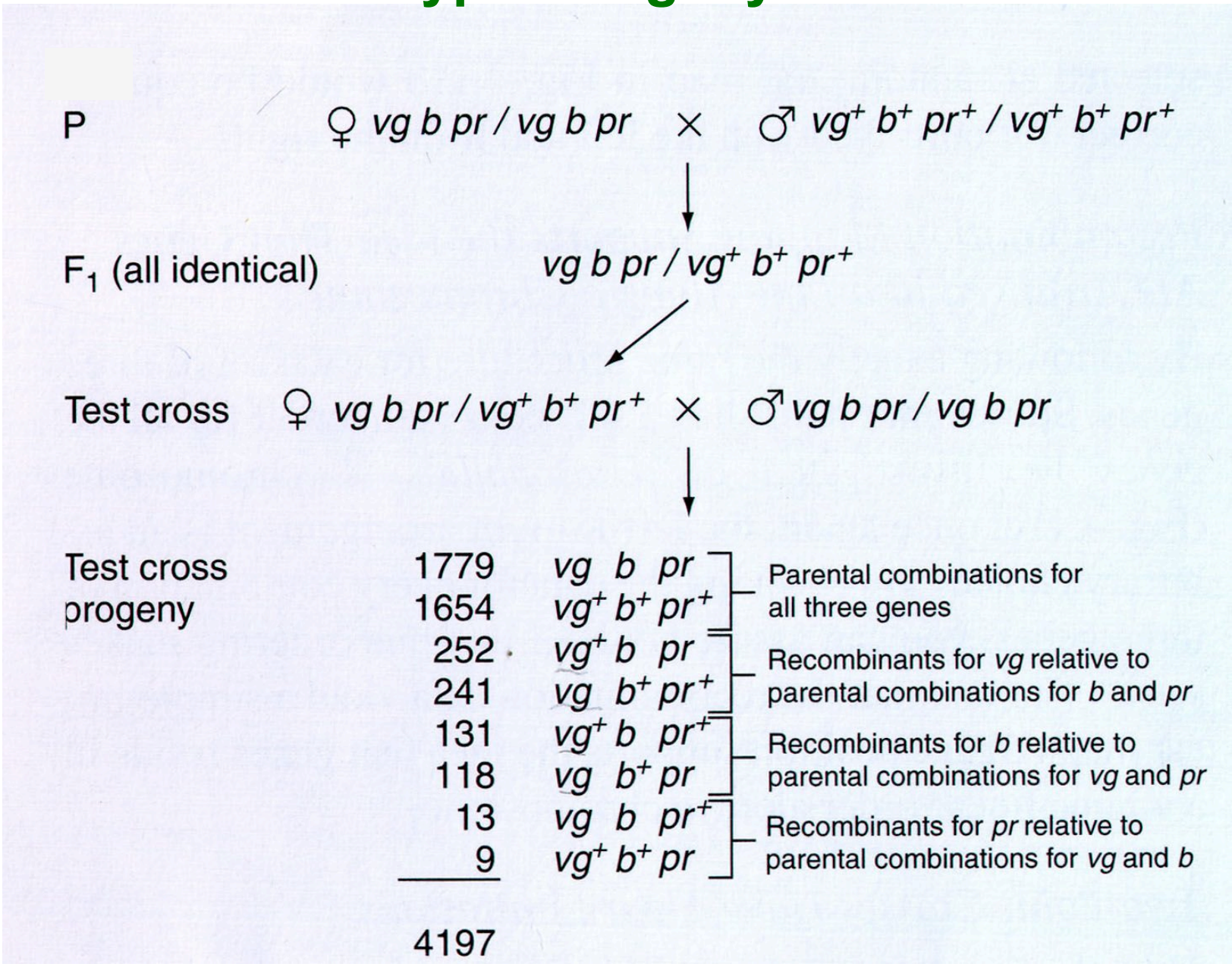
- Faster and more accurate way to map genes
- Simultaneous analysis of three markers

- Information on the position of three genes relative to each other can be obtained from one mating rather than two independent matings.

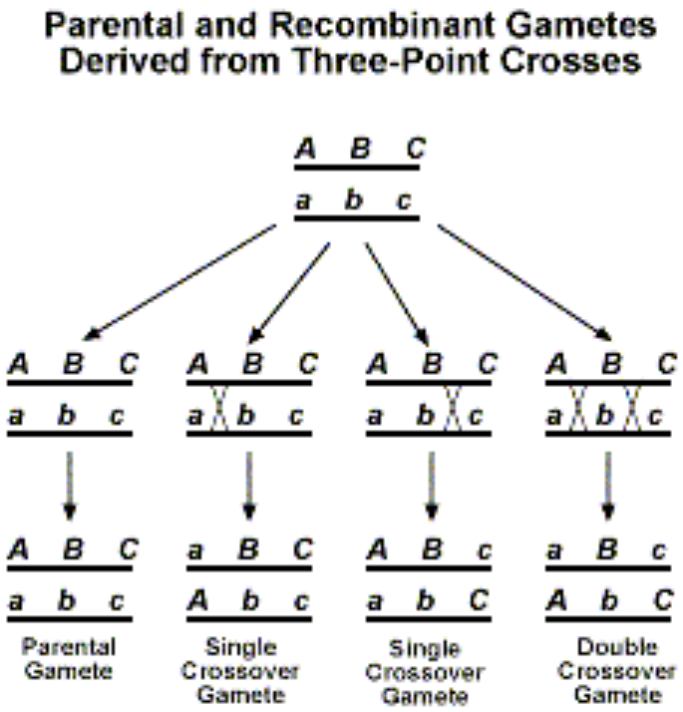
  - Example: *Drosophila* autosomal genes:
    - ***vg*** = vestigial wings; ***vg+*** = normal
    - ***b*** = black body; ***b+*** = normal body
    - ***pr*** = purple eyes; ***pr+*** = normal eyes

- Cross of pure breeding vestigial winged, black bodied, purple eyed female to a pure breeding wild type male:
  - ***vgvg bb prpr × vg+vg+ b+b+ pr+pr+***

Three Factor Testcrosses Result in Eight Phenotypic Progeny Classes



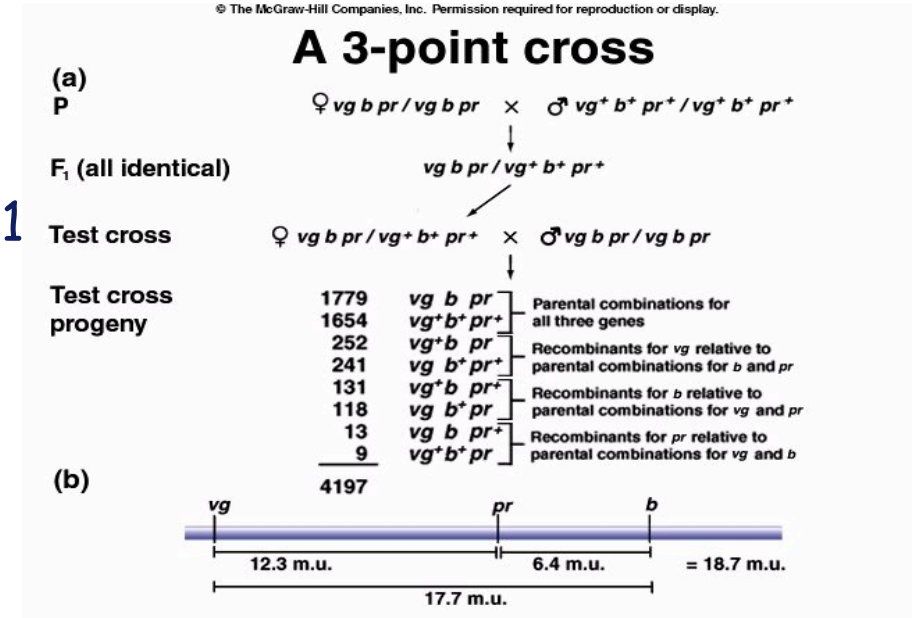
Types of Gametes



- Parental Types
- Single Crossover between A & B/a & b
- Single Crossover between B & C/b & C
- Double Crossover

Data Analysis

- There are eight gametes from the F1
  - largest number is parental
  - smallest number is double crossover
- Identify the parental and recombinants
  - Two genes at a time.
  - Compare recombinant to parental
  - Double crossover change (oddball) is guy in the middle

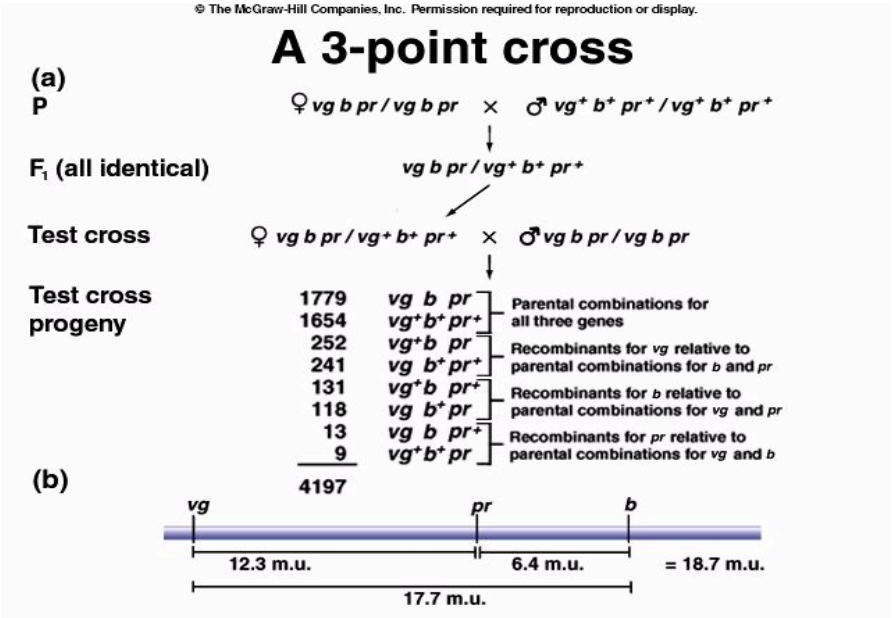


- Write order of genes
- The orientation from left to right is purely arbitrary.
- What the hell are you talking about??????????????????????????

Data Analysis

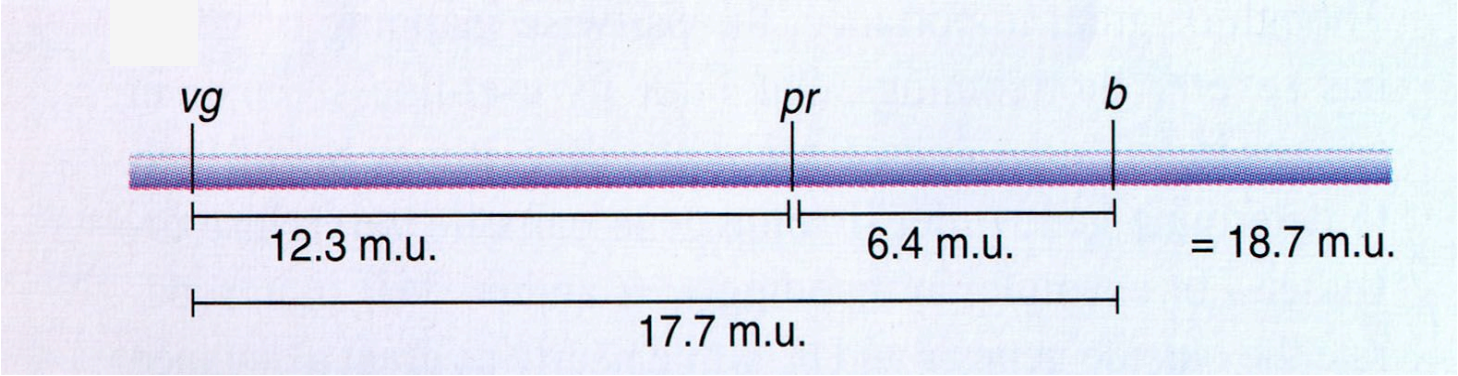
- There are eight gametes from the F1
  - largest number is parental (P)
  - smallest number is double crossover (DCO)

- Identify the parental and recombinants
  - Two genes at a time.
  - Compare recombinant to parental
  - Double crossover change (oddball) is guy in the middle
  - Compare DCO to P
  - 2 genes should be the same,
  - one should be the opposite.
  - Check both to check yourself
  - Write order of genes
- vg - pr - b
  - The orientation from left to right is purely arbitrary.

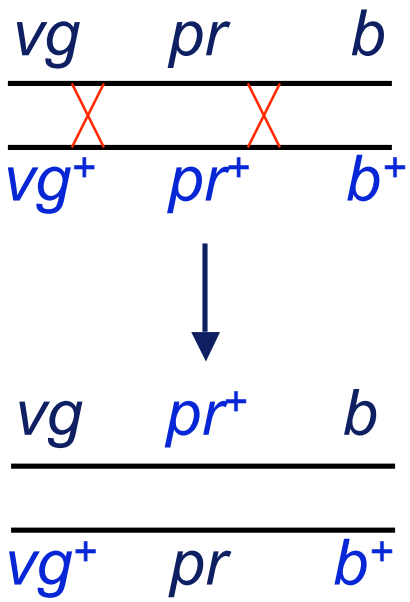


- What the hell are you talking about??????????????????????????????

Find the **double recombinant** class (the class with the least number of progeny) -- the gene that is different from the parental chromosome in this class is the middle gene.



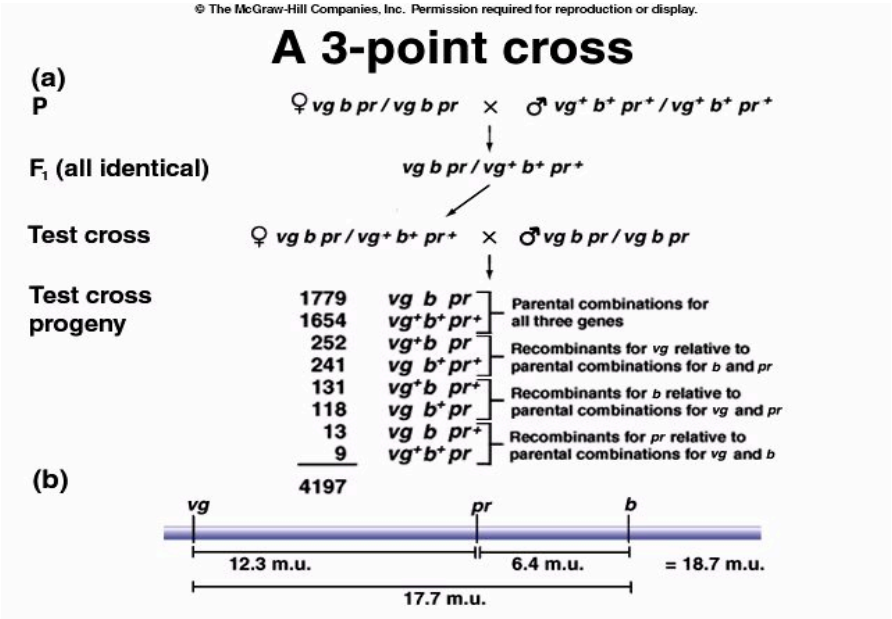
1779	*vg b pr*	P
1654	*vg*$^+$ *b*$^+$ *pr*$^+$	P
252	*vg*$^+$ *b pr*	
241	*vg b*$^+$ *pr*$^+$	
131	*vg*$^+$ *b pr*$^+$	
118	*vg b*$^+$ *pr*	
13	*vg b pr*$^+$	DCO
9	*vg*$^+$ *b*$^+$ *pr*	DCO



double cross over chromosomes

Data Analysis

- There are eight gametes from the F1
  - largest number is parental (P)
  - smallest number is double crossover (DCO)
- Identify the parental and recombinants
  - Two genes at a time.
  - Compare recombinant to parental
  - Double crossover change (oddball) is guy in the middle
  - Compare DCO to P
  - 2 genes should be the same,
  - one should be the opposite. (the oddball)
  - Check both to check yourself
  - Write order of genes

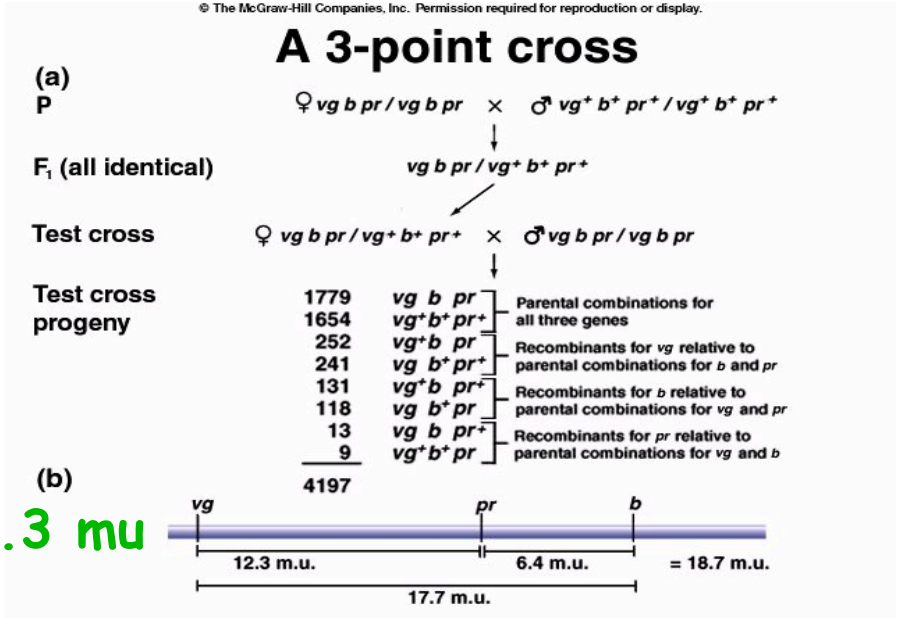


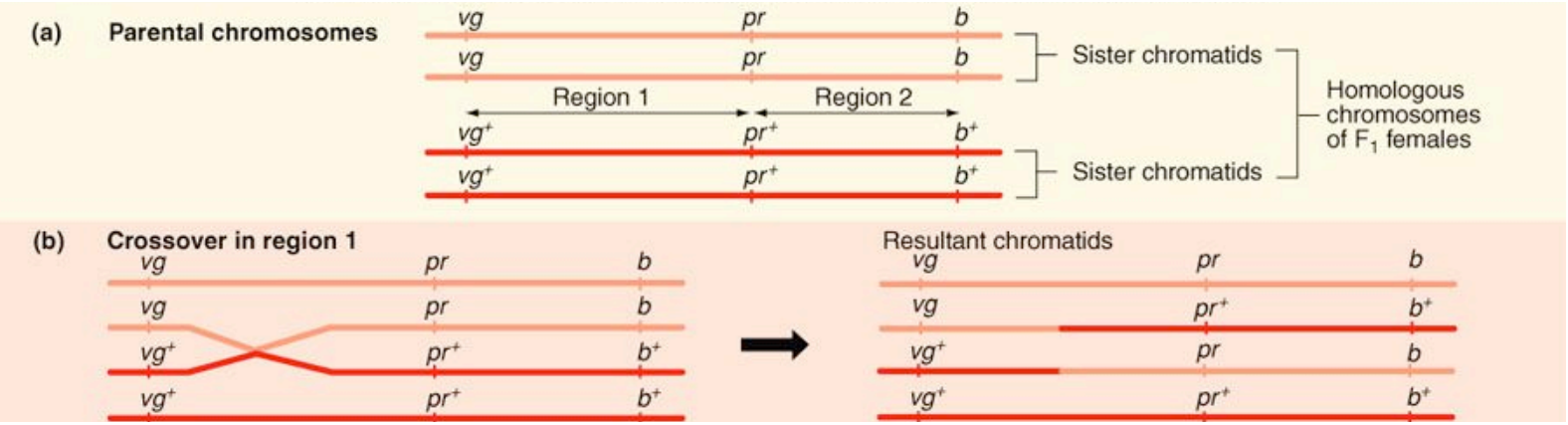
- vg - pr - b
  - The orientation from left to right is purely arbitrary.
- Now determine which numbers go to which genes.
  - Find the numbers where the "vg" is the oddball and others are same
  - These are numbers for vg to pr region. (252 and 241)
- What the hell are you talking about??????????????????????????

Analyzing the results of a three point cross

- Look at two genes at a time and compare to parental
  - vg and pr
- vg-pr parentals are:
  - vg - pr - b
  - vg+ - pr+ - b+
- vg-pr recombinants are:
  - vg+ - pr - b
  - vg - pr+ - b
- Numbers that correspond are

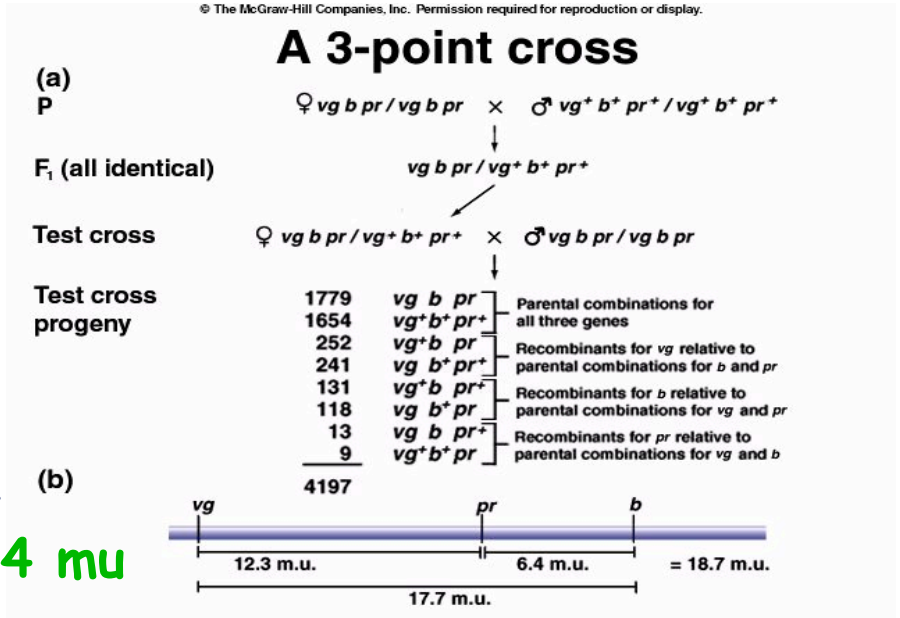
$$\frac{252+241+13+9}{4197} = 0.123 \times 100 = 12.3 \text{ mu}$$

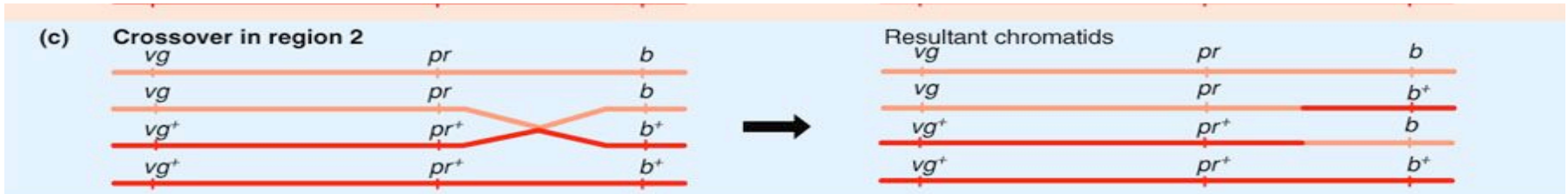




Analyzing the results of a three point cross

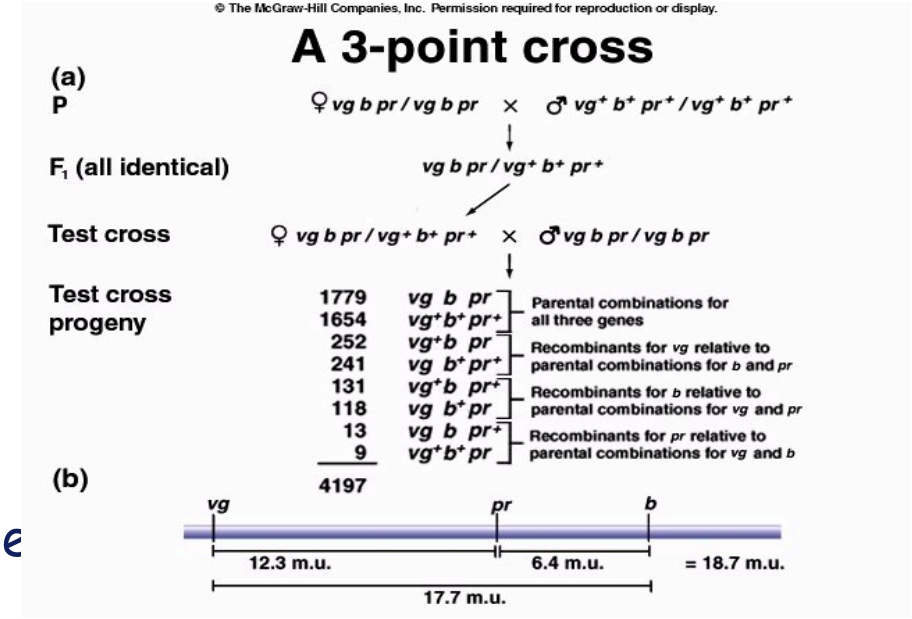
- Look at two genes at a time
- and compare to parental
  - Vg - pr - b
  - Vg+ - pr+ - b+
- b-pr recombinants are:
  - Vg+ - b - pr+
  - Vg - b+- pr
- Numbers that correspond are
  - $\frac{131+118+13+9}{4197} = 0.064 \times 100 = 6.4$ mu





Analyzing the results of a three point cross

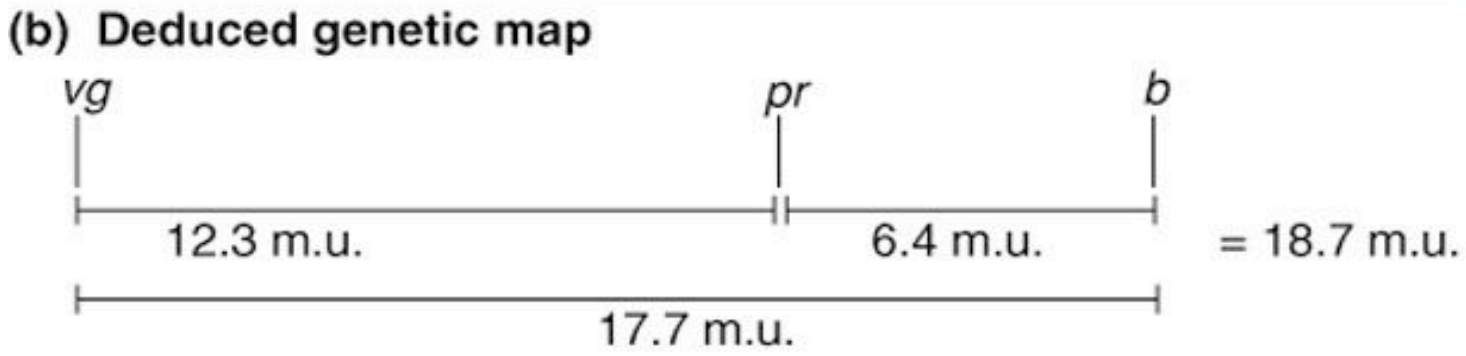
- Look at two genes at a time and compare to parental
  - vg and b



- vg-b recombinants are:
  - vg-b$^+$
  - vg$^+$-b
  - This checks to be sure you have
    - the correct middle gene also.
    - Should be the largest distance
- Numbers that correspond are

$$\frac{252 + 241 + 131 + 118 + 13 + 13 + 9 + 9}{4197} \text{ X } 100 = 18.7 \text{ m.u.}$$

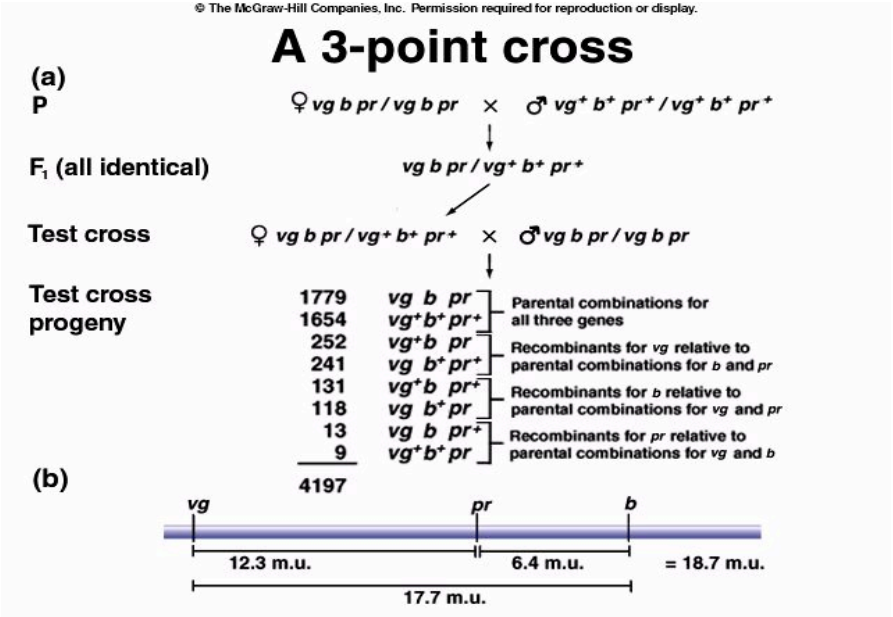
- Why count dco twice?



WRONG Without dco ⟶ ***vg – b* dist** $\frac{252 + 241 + 131 + 118}{4197}$ **X 100 = 17.7 m.u.**

***vg – pr* dist** $\frac{252 + 241 + 13 + 9}{4197}$ **X 100 = 12.3 m.u.**

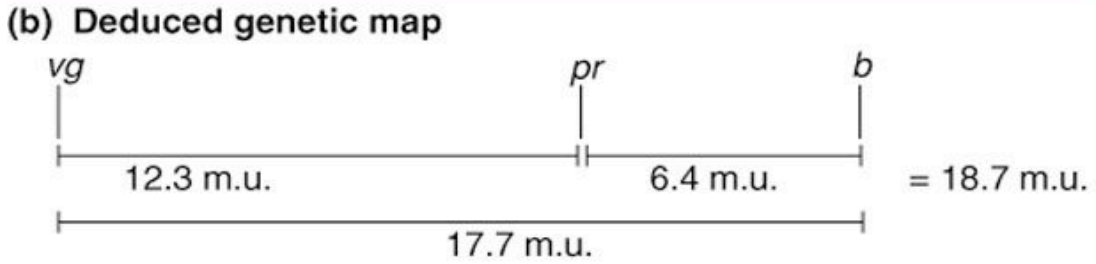
***b – pr* dist** $\frac{131 + 118 + 13 + 9}{4197}$ **X 100 = 6.4 m.u.**

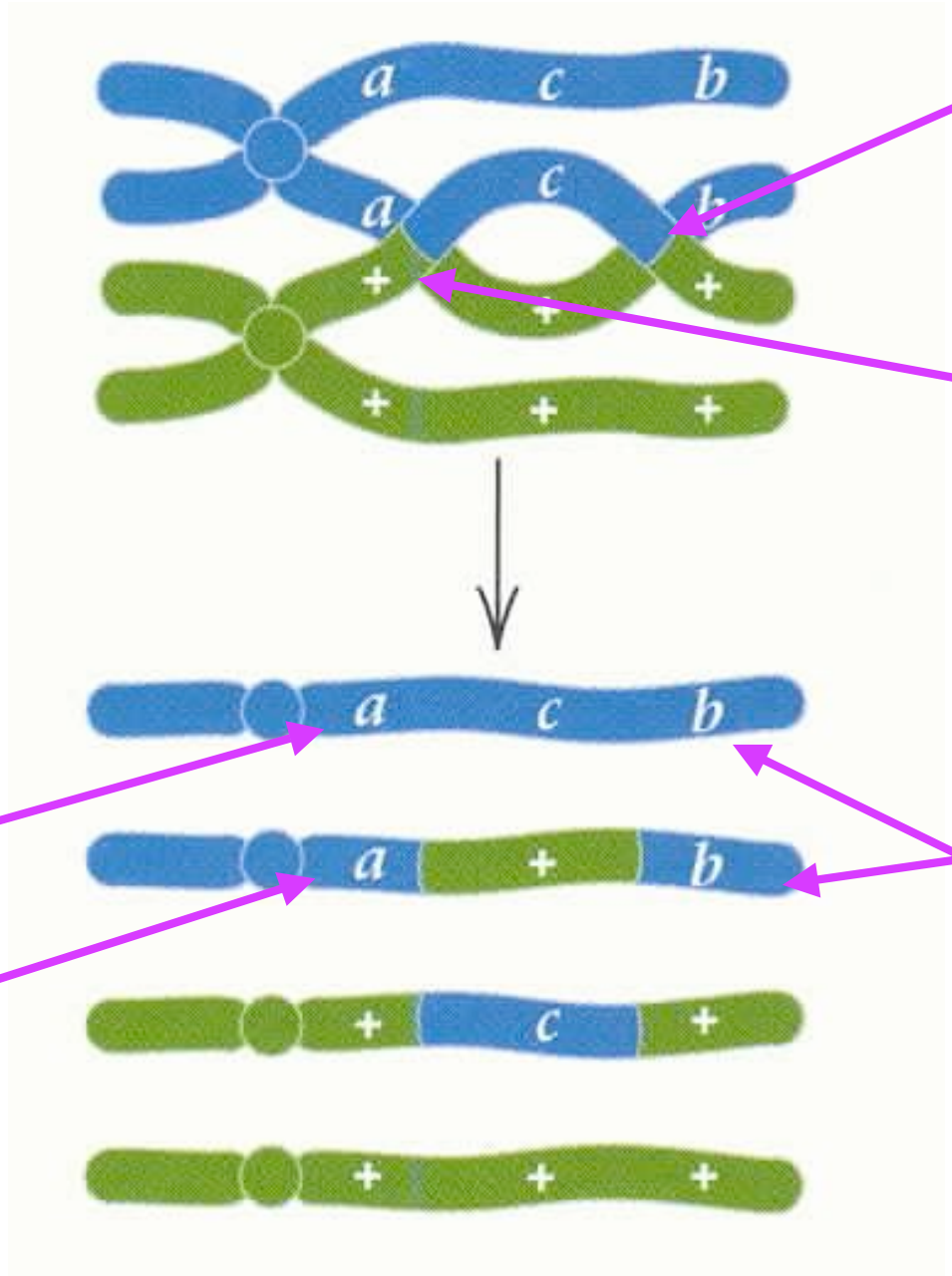


17.7 m.u. is not 18.7 m. u. !!!!!!

Double Crossovers

- Recombination is caused by formation of chiasmata along the chromosome at multiple points.
- If the distance between two genes is large enough, there can potentially be multiple chiasmata formation between them;
  - so there could be multiple crossovers.
- What would happen if there were two crossovers between the two outside genes (in this case *vg* and *b*)?
  - ***Answer***: there would appear to be fewer recombinants between the two genes:
  - it would appear as if the genes are closer;
  - the calculated map distance between these genes will be less than actual.





Two recombination events occurred in the interval between a and b.

- We must count them for each of the single crossover events.
- They are crossing over events that occur on both sides.
- Must be used twice in calculations

➢We need to add the number of double recombinants **TWICE** to our total for the outside markers:

1779	*vg b pr*	P
1654	*vg*$^+$ *b*$^+$ *pr*$^+$	P
252	*vg*$^+$ *b pr*	
241	*vg b*$^+$ *pr*$^+$	
131	*vg*$^+$ *b pr*$^+$	
118	*vg b*$^+$ *pr*	
13	*vg b pr*$^+$	DCO
9	*vg*$^+$ *b*$^+$ *pr*	DCO

vg-b distance…

$$\frac{252 + 241 + 131 + 118 + \mathbf{13 + 9 + 13 + 9}}{4197} \times 100 = \mathbf{18.7}\ \text{m.u.}$$

Question

- Which type of class would you expect to account for the lowest frequency?

1) Parental
2) Single Recombinants
3) Double Recombinants
4) Middle class

Do Genetic and Physical maps correspond?

- Order of genes in correctly predicted by physical maps
- Distance between genes is not always similar to physical maps
  - Double, triple, and more crossovers
  - Only 50% recombination frequency observable in a cross
  - Variation across chromosome in rate of recombination
- Mapping functions compensate for inaccuracies, but are often imprecise.
- In addition, a process called Interference may occur.

Interference: The number of double crossovers may be less than expected

- Sometimes the number of observable double crossovers is less than expected if the two exchanges are not independent
  - Occurrence of one crossover reduces likelihood that another crossover will occur in adjacent parts of the chromosome
  - Chromosomal interference –
    - crossovers do not occur independently
  - Interference is not uniform among chromosomes or even within a chromosome

Interference

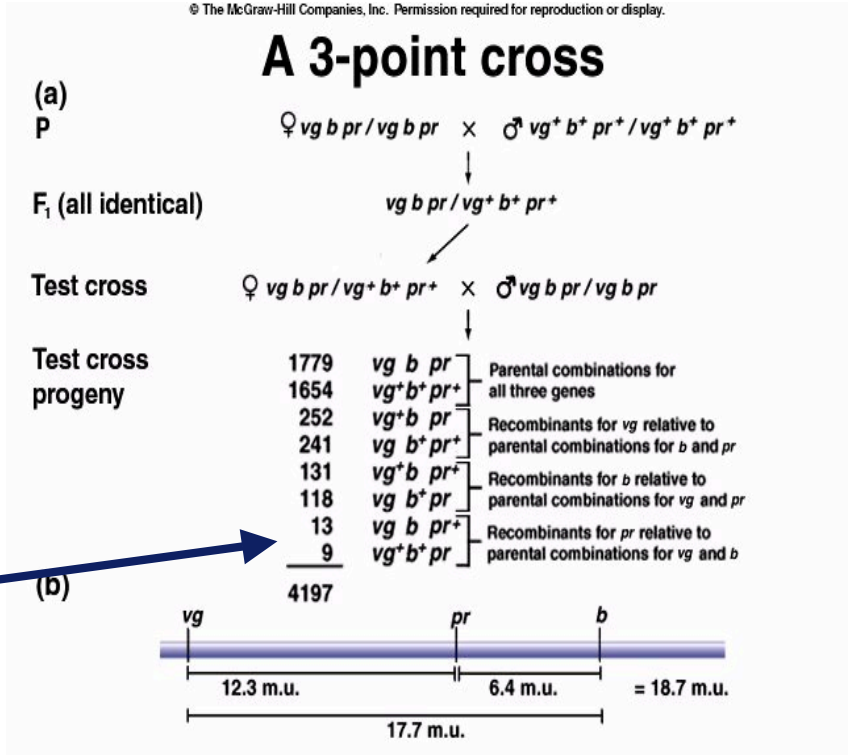
- The product rule allows us to predict the likelihood of a double crossover from the individual probabilities of each single crossover

Expected		Probability		Probability
(double crossover)	=	(single crossover)	X	(single crossover)
0.00787	=	0.064	X	0.123

- If we analyzed a total of
- 4197 fly offspring
  - The expected number
  - of double crossover offspring is

= 4197 X 0.00787 = 33

- Observed number is 22



Interference

- Therefore, we would expect 33 offspring to be produced as a result of a double crossover
  - However, the observed number was only 22!
- This lower-than-expected value is due to a common genetic phenomenon, termed interference
  - The first crossover decreases the probability that a second crossover will occur nearby

Measuring interference

- Coefficient of coincidence =
  - ratio between actual or observed dco and expected dco
- coefficient of coincidence :
  = observed dco / expected dco
- Interference = 1 – coefficient of coincidence

p(crossover in region 1) X p(crossover in region 2) =
.123 X .064 = .0078

from a total of 4197 progeny, we should have seen (**expect**)
.0078 x 4197 = 32.7 or about 33 double recombinants

1779	*vg*	*b*	*pr*
1654	*vg+*	*b+*	*pr+*
252	*vg+*	*b*	*pr*
241	*vg*	*b+*	*pr+*
131	*vg+*	*b*	*pr+*
118	*vg*	*b+*	*pr*
13	*vg*	*b*	*pr+*
9	*vg+*	*b+*	*pr*
4197			

$$I = 1 - \text{C of C}$$

$$I = 1 - \frac{\text{Observed \# DCO}}{\text{Expected \# DCO}}$$

$$I = 1 - \frac{13 + 9}{33} = 0.333 \text{ or } 33.3\%$$

- This suggests that a cross over in one gene interval physically inhibited a crossover in adjacent regions by 33.3%.

Question

A plant heterozygous for three dominant traits N, T, and U is test crossed, the resulting progeny are as follows:

N	U	T	2
n	u	T	70
N	u	T	21
n	u	t	4
N	U	t	82
n	U	t	21
n	U	T	13
N	u	t	17

What is the linkage arrangement of the N, U and T alleles in the parental?

What is the linkage arrangement of the N, U and T alleles in the dco?

Which gene is in the middle?

1) N
2) U
3) T

Three Point Cross -- Example where order is not known: start with 2 pure breeding strains, F1 test crossed.

Testcross offspring

progeny phenotype	number
+++	6
r++	359
rs+	98
rsw	4
r+w	47
+s+	43
+sw	351
++w	92

Testcross offspring

phenotype	number
+++	6
r++	359
rs+	98
rsw	4
r+w	47
+s+	43
+sw	351
++w	92

largest class: r++ 359, +sw 351

smallest class: +++ 6, rsw 4

Order: determine an order where it takes two cross-overs to go from parentals to DCO.

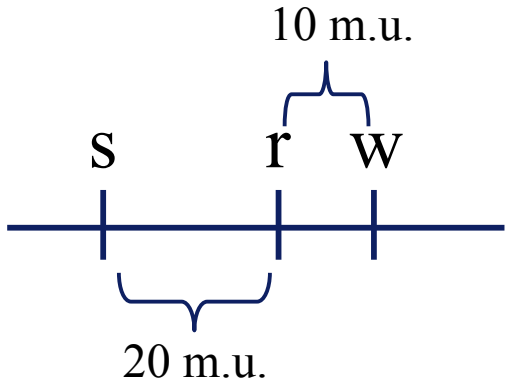
s r w

w r s

The order of three markers is: s-r-w or w-r-s

- Next, sort according to reciprocal products and determine where crossovers occur:

F2 products	#	class
+ r +	359	parental
s + w	351	parental
s r +	98	SCO(s-r)
+ + w	92	SCO(s-r)
+ r w	47	SCO(r-w)
s + +	43	SCO(r-w)
+ + +	6	DCO
s r w	4	DCO

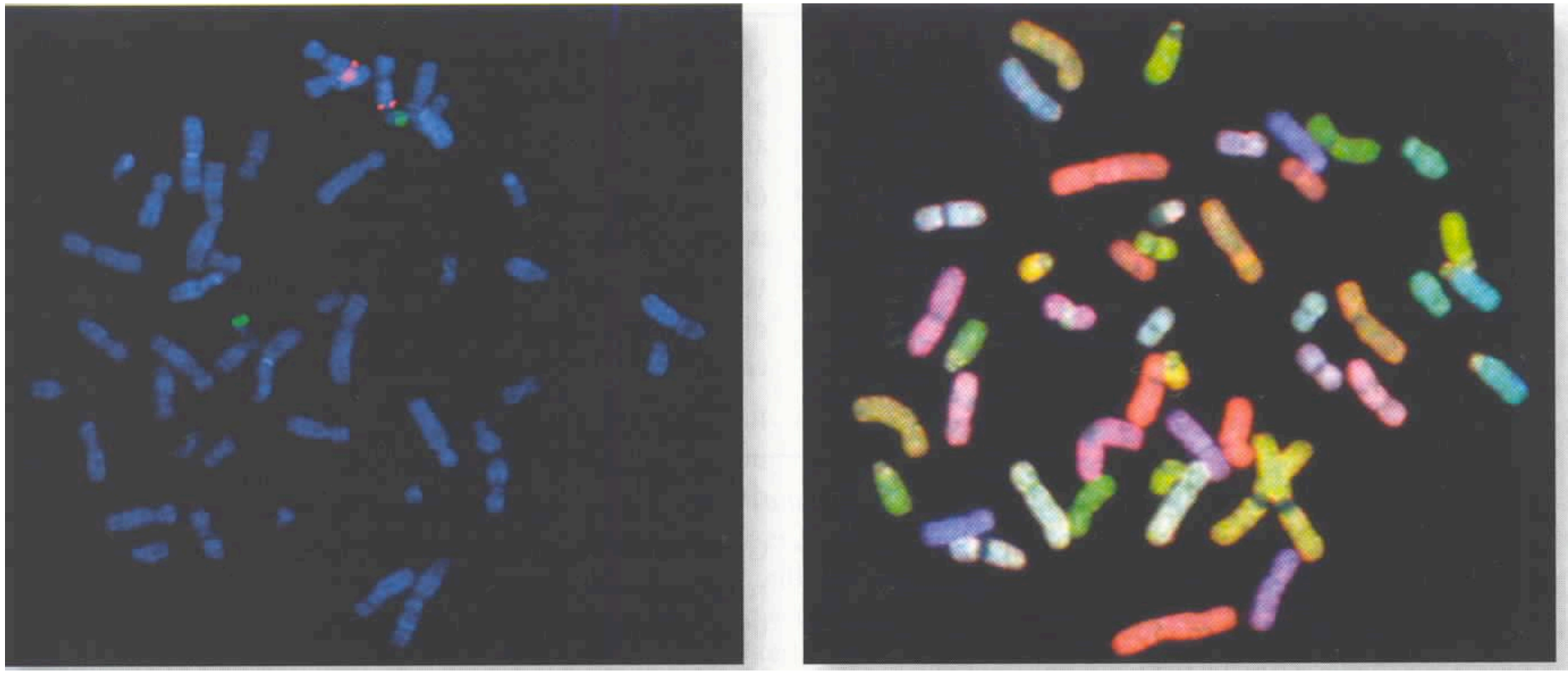


$$\text{map distance s-r} = \frac{98+92+6+4}{1000} = 0.20 = 20 \text{ m.u.}$$

$$\text{map distance r-w} = \frac{47+43+6+4}{1000} = 0.10 = 10 \text{ m.u.}$$

Recent Advances in the Field

Fluorescent *in situ* hybridization can also be used to localize cloned genes to a particular chromosome.



Probe for a specific gene

Visualized with chromosome specific probes

Homework Problems

Chapter 6

19, 20, 21, 22, 23, 24, 27

- DON'T forget to take the online QUIZ!!
- DON'T forget to submit the online iActivity
  - "Tomato"