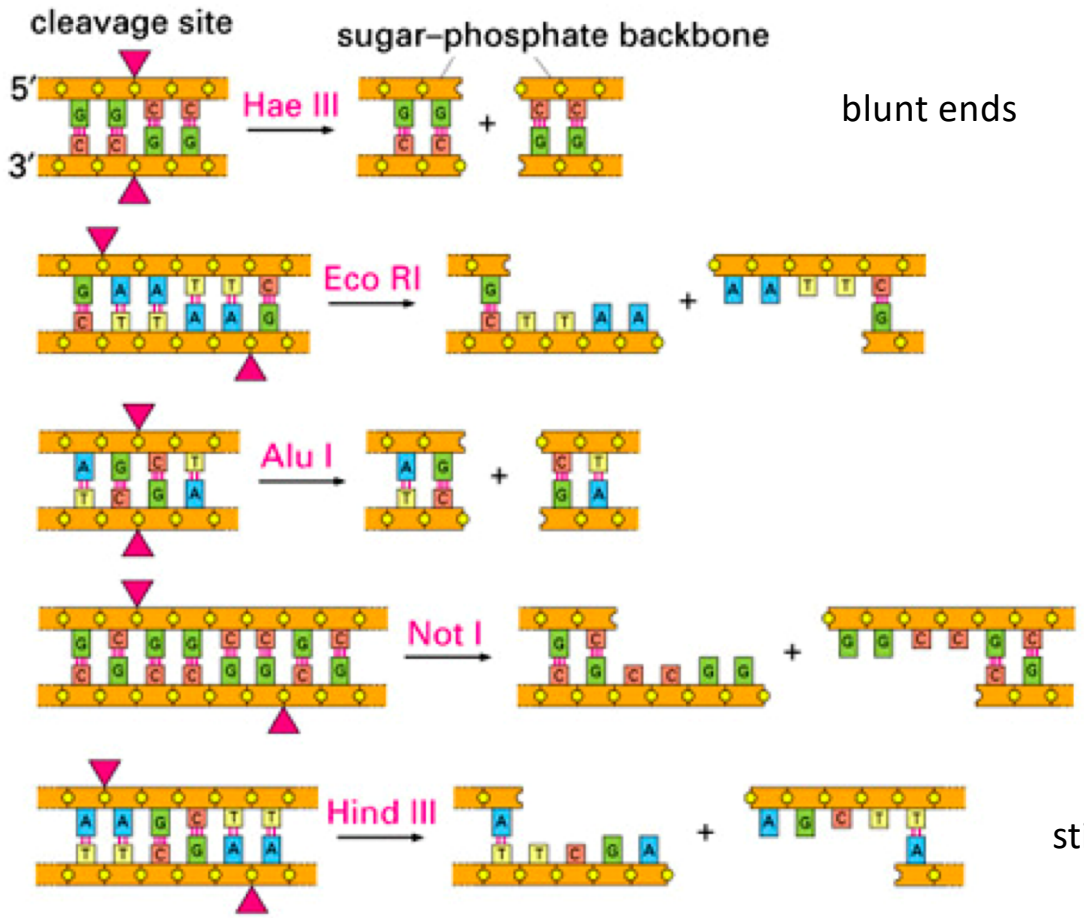
DNA can be cut by restriction nucleases



- cut double-stranded DNA
- at particular sites: recognition sites
- highly specific sequences of
  4 nucleotides: frequent cutters
  6 nucleotides: medium cutters
  8 nucleotides: rare cutters
- from bacteria; defense mechanism against phages

Figure 10-4 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Isoschizomeres

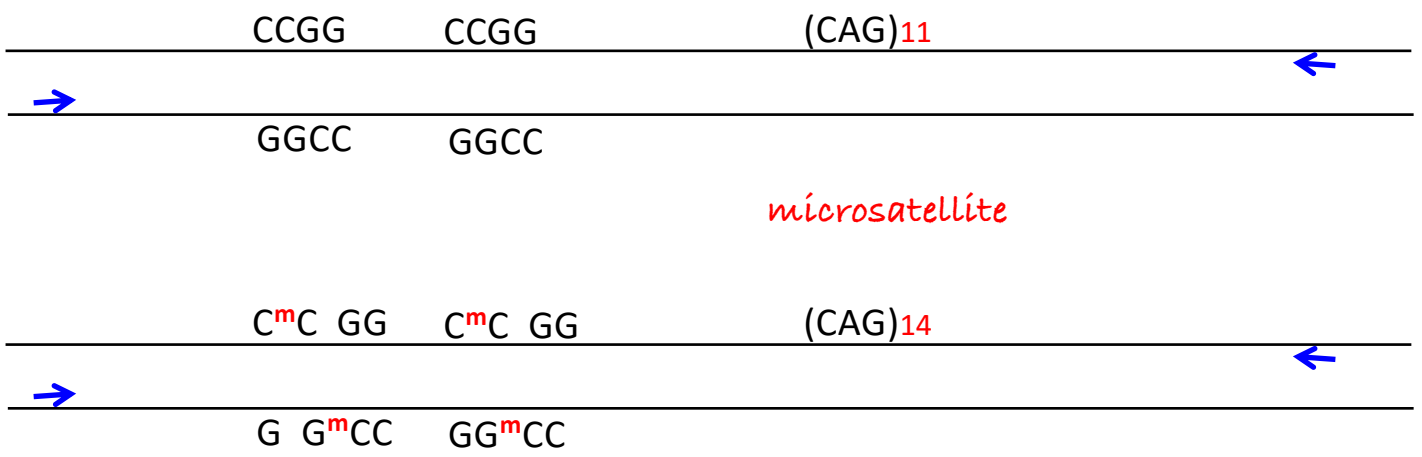
	cut	no cut
Hpa II recognizes	5’- C C G G – 3’ 3’- G G C C – 5’	5’- C ^{m}C G G – 3’ 3’- G G ^{m}C C – 5’
Msp I recognizes	5’- C C G G – 3’ 3’- G G C C – 5’	5’- C ^{m}C G G – 3’ 3’- G G ^{m}C C – 5’
	cut	cut

Hpa II and Msp I recognize the same restriction site : they are isoschizomeres.

However there is one important difference :

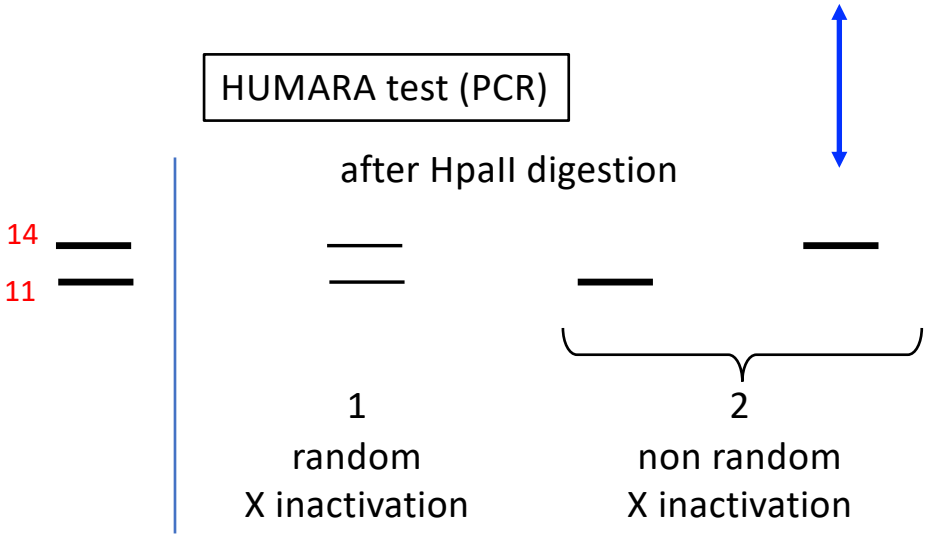
Hpa II is **sensitive** to DNA methylation

Msp I is **insensitive** to DNA methylation

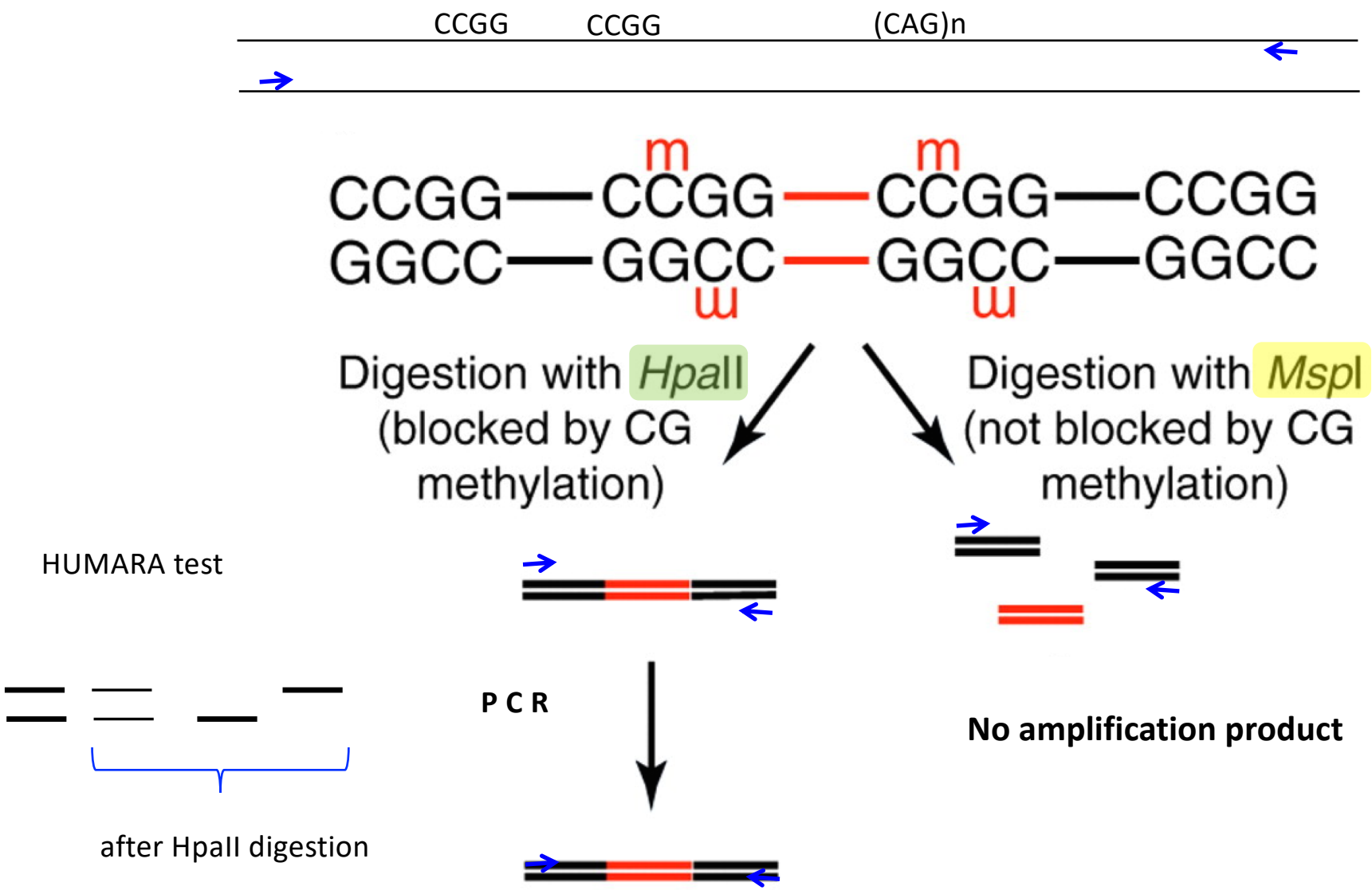


The active X is not methylated

The inactive X is methylated



Only the DNA from the **inactive X** can be amplified by PRC.


CCGG
CCGG
(CAG)n
m
m
CCGG—CCGG—CCGG—CCGG
GGCC—GGCC—GGCC—GGCC
Digestion with HpaII (blocked by CG methylation)
Digestion with MspI (not blocked by CG methylation)
HUMARA test
P C R
No amplification product
after HpaII digestion

Example of testing

Mother shows non random X inactivation

Patient shows non random X inactivation

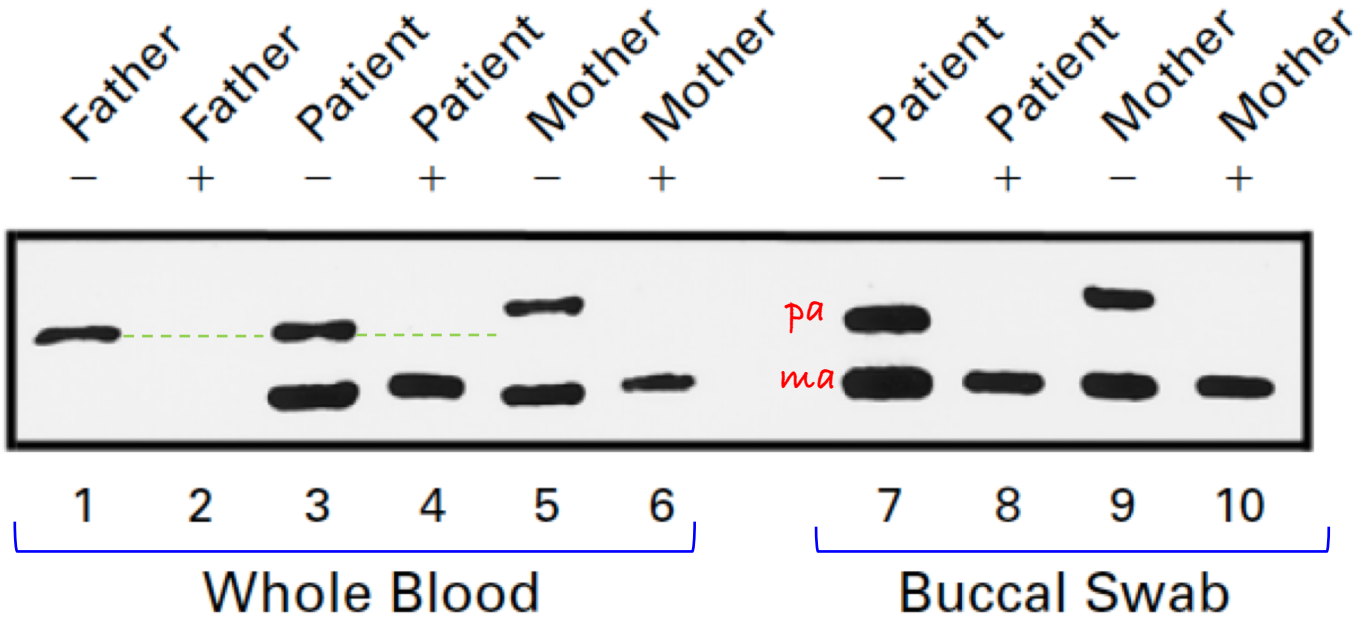


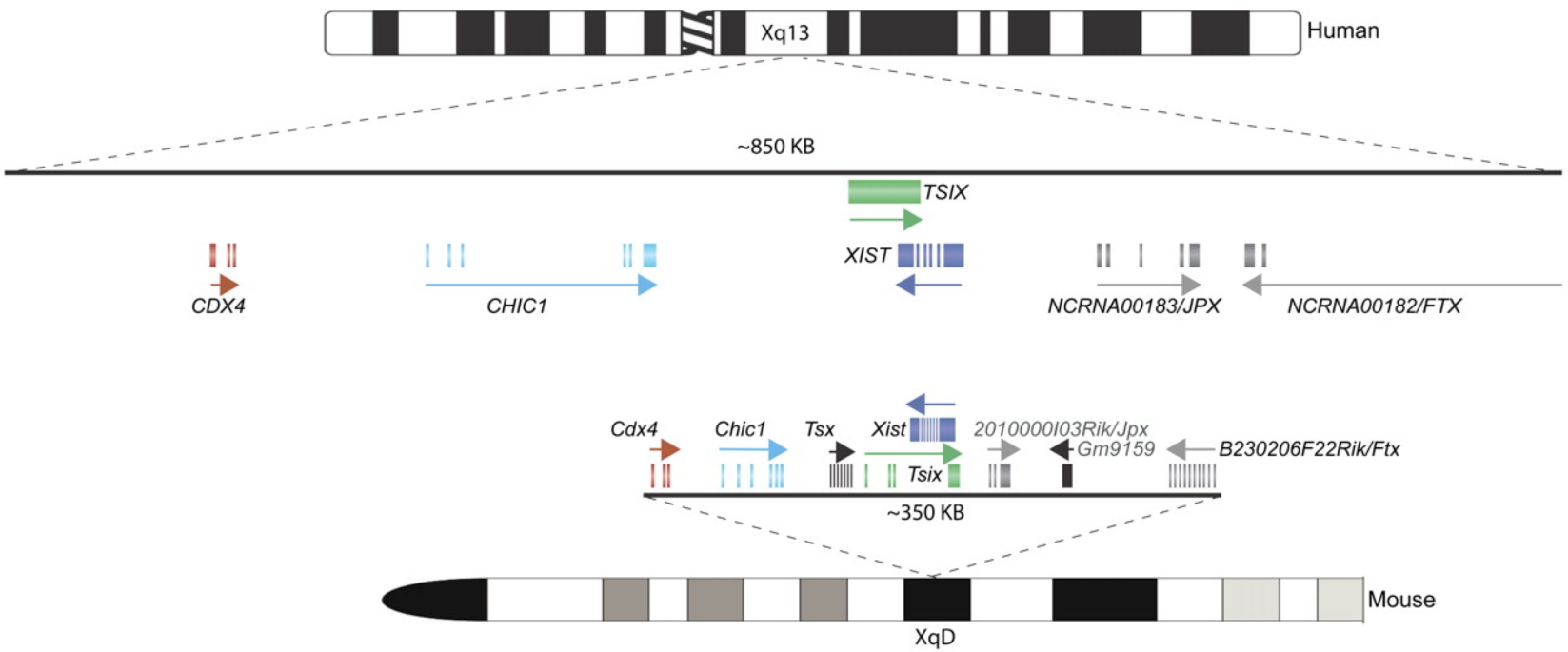
Figure 1. Analysis of the Pattern of X-Chromosome Inactivation in the Patient and Her Parents.

DNA was extracted from whole blood or oral mucosal cells from the patient and her parents and amplified by PCR with specific primers that flank the *HUMARA* locus (lanes 1, 3, 5, 7, and 9; labeled with a minus sign). In addition, the DNA was digested with the methylation-sensitive enzyme *Hpa*II before PCR amplification (lanes 2, 4, 6, 8, and 10; labeled with a plus sign). The samples were analyzed on 3 percent agarose gel and stained with ethidium bromide.

Whole blood and buccal swab give identical results

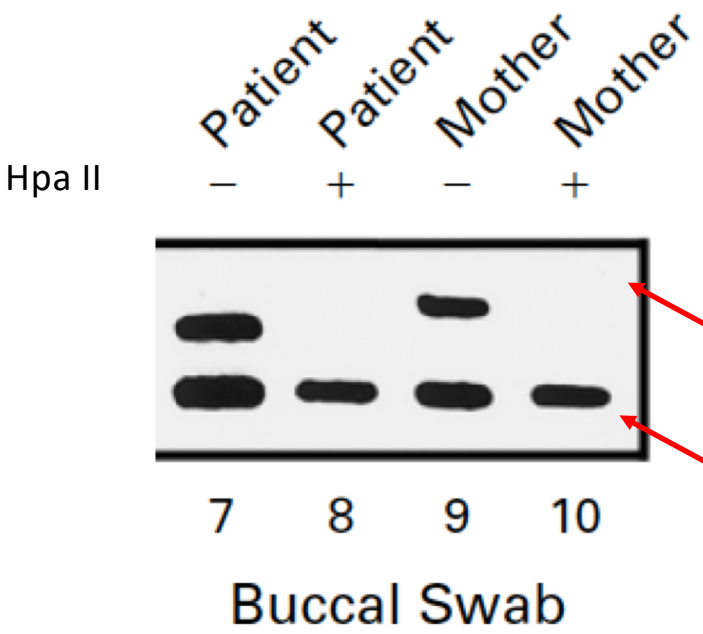
XIC = X Inactivation Center

TSIX and XIST are non coding RNA



Mutations of XIC can be
- loss-of-function
- gain-of-function

Hpa II digeston ***before*** PCR



Mother shows
non random
X inactivation

Why ? XIC is mutated

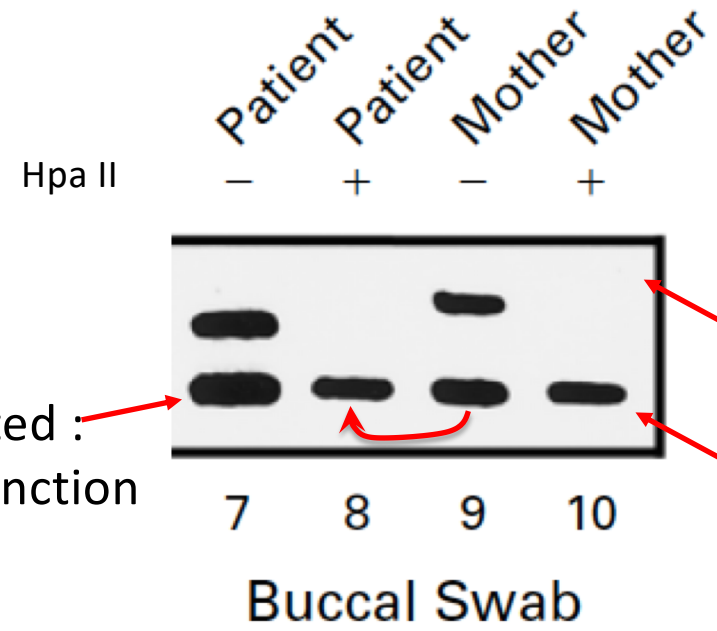
never inactivated

always inactivated

A priori 2 possibilities :

1. Top allele has a **loss-of-function** mutation
2. Bottom allele has a **gain-of-function** mutation

Hpa II digeston ***before*** PCR



XIC mutated :
gain-of-function

never inactivated

always inactivated

Patient shows
non random
X inactivation

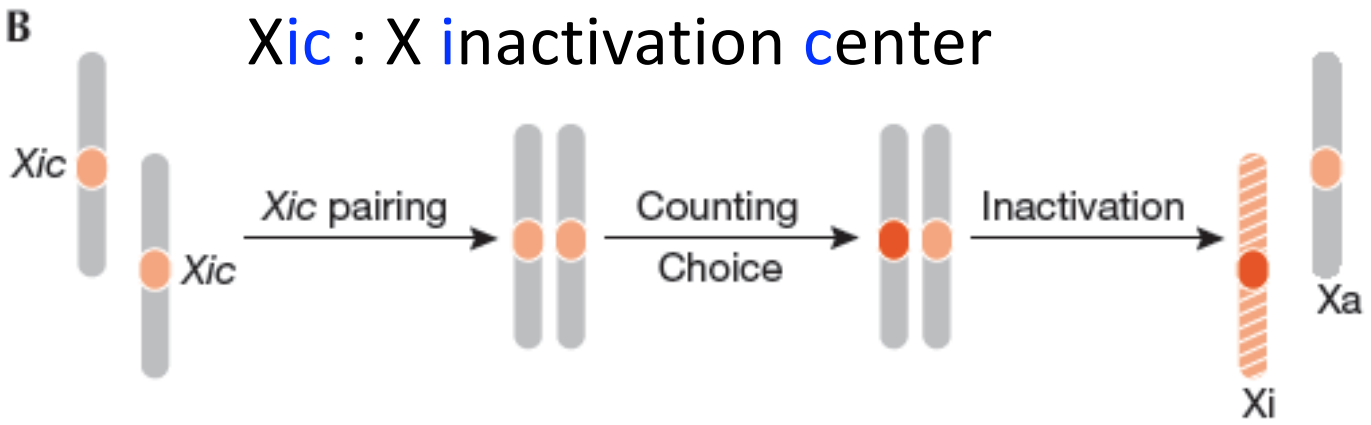
Why ? XIC is mutated

Observation in the patient shows that the mother has a gain-of-function mutation

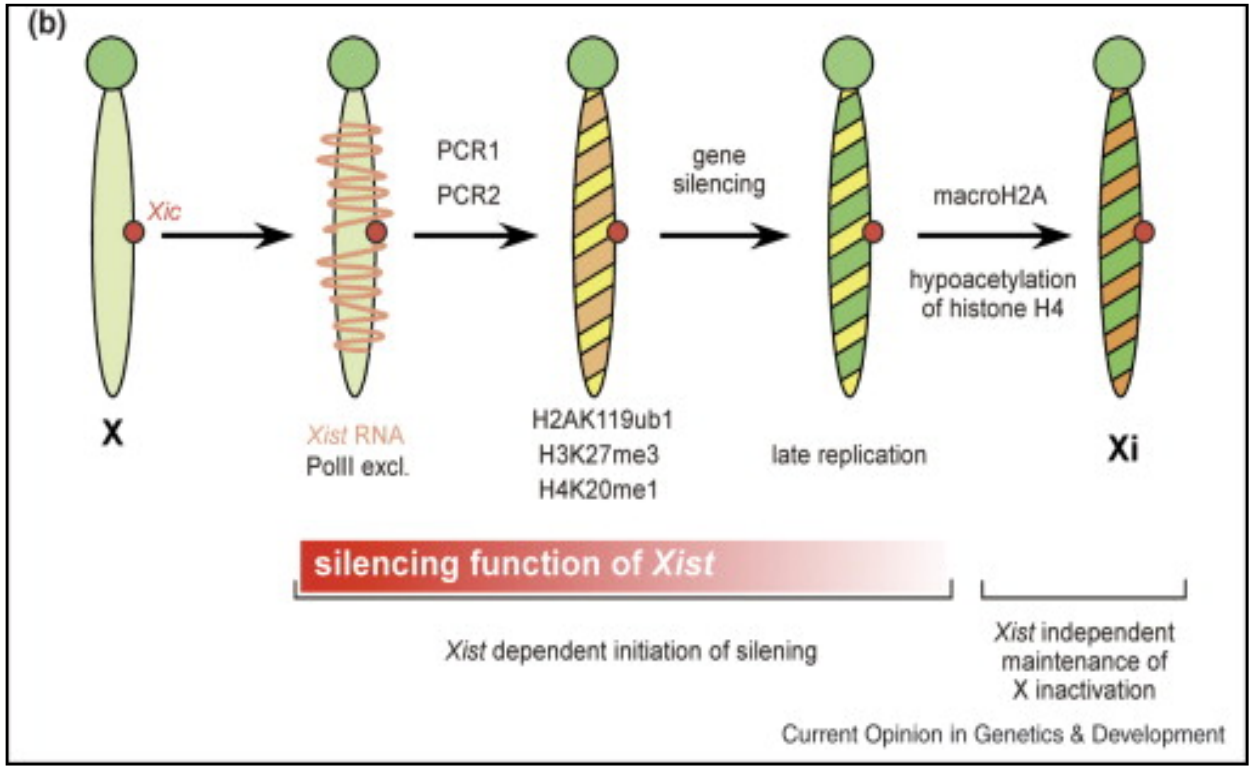
2 possibilities :

1. Top allele has a **loss-of-function** mutation
2. Bottom allele has a **gain-of-function** mutation

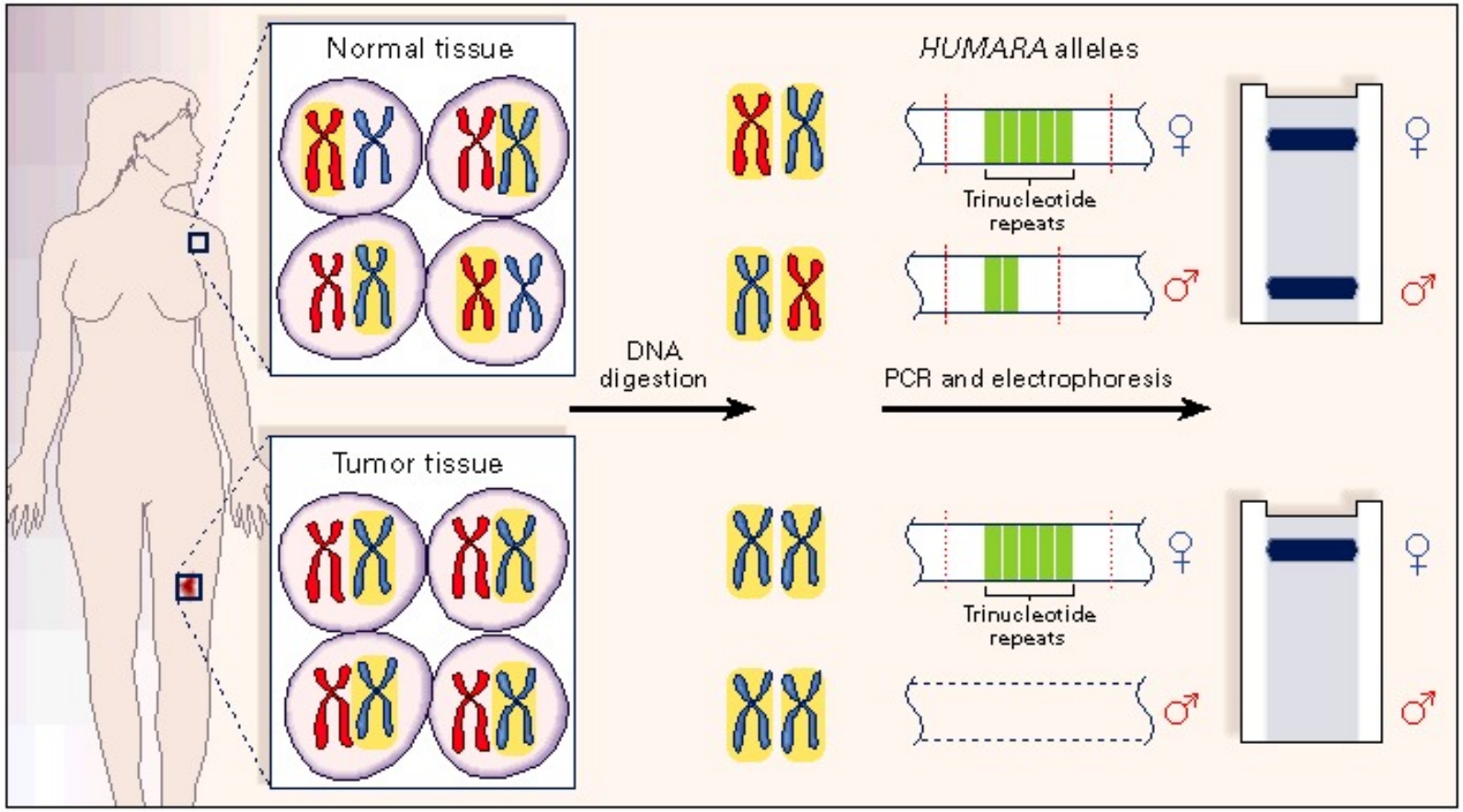
Xic : X inactivation center



Xic is required for X inactivation.
Loss of function of Xic makes the X chromosome unable to inactivate.
Gain of function of Xic makes the X chromosome always inactive.
Consequence :
The X inactivation is ***not random***.



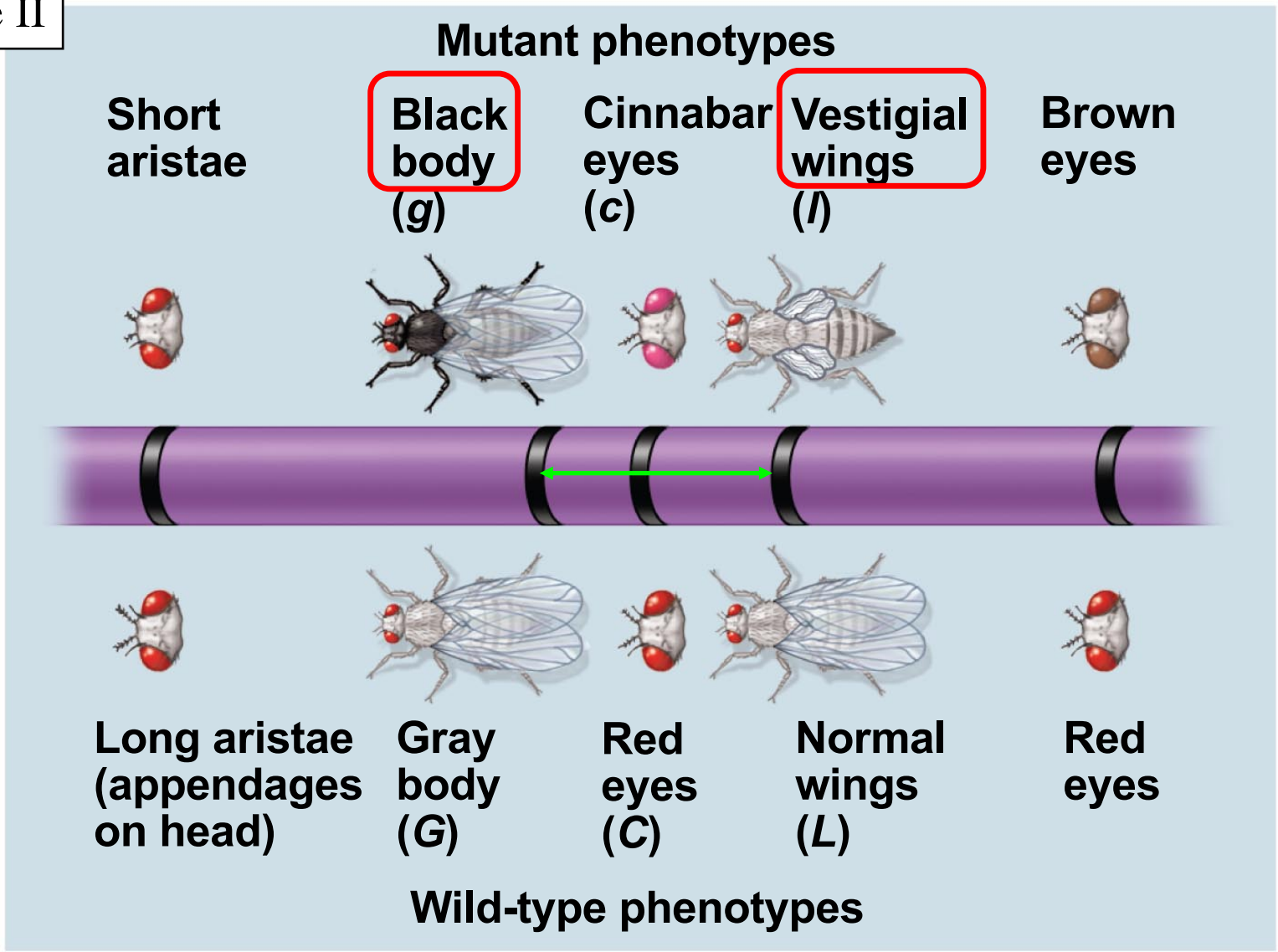
HUMARA testing in cancer patients



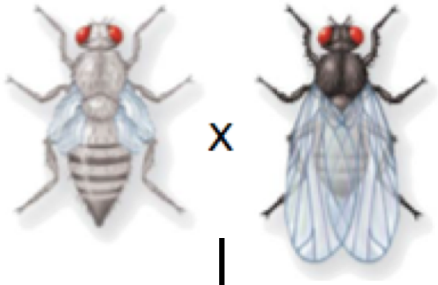
Evidence for monoclonal origine of cancer tumor.

Chromosome II

Question :
What is the **genetic distance** between the **black locus** and the **vestigial locus** ?



Measuring the genetic distance between 2 loci:

P  x

Locus black : body color
Locus vestigial : wing size

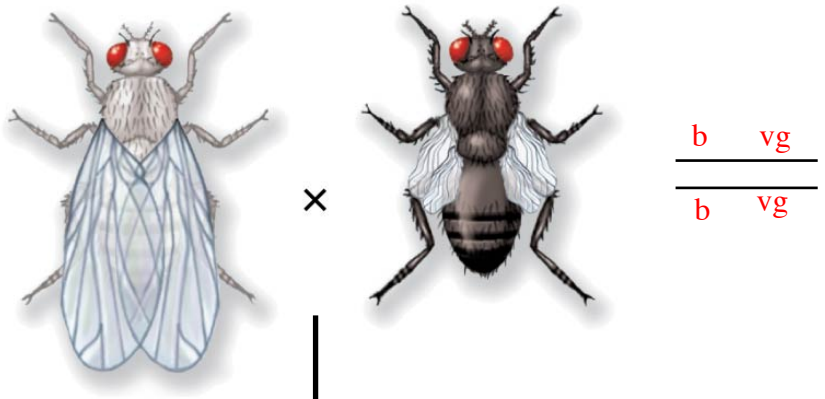
Mutations are in ***trans***

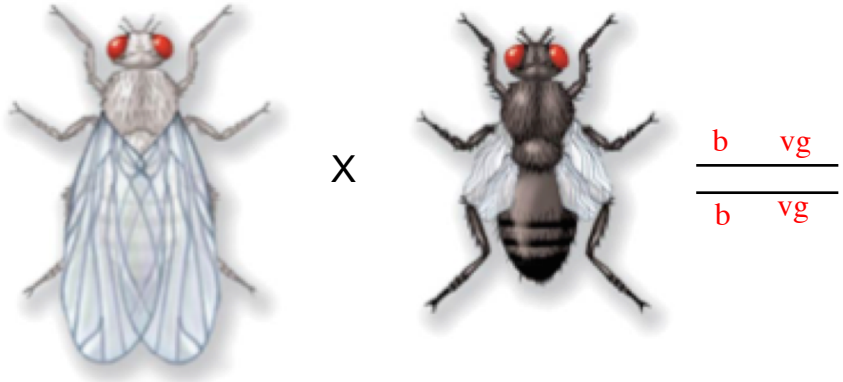
$$\frac{b+ \quad vg}{b \quad vg+}$$  x  $$\frac{b \quad vg}{b \quad vg}$$

F_1

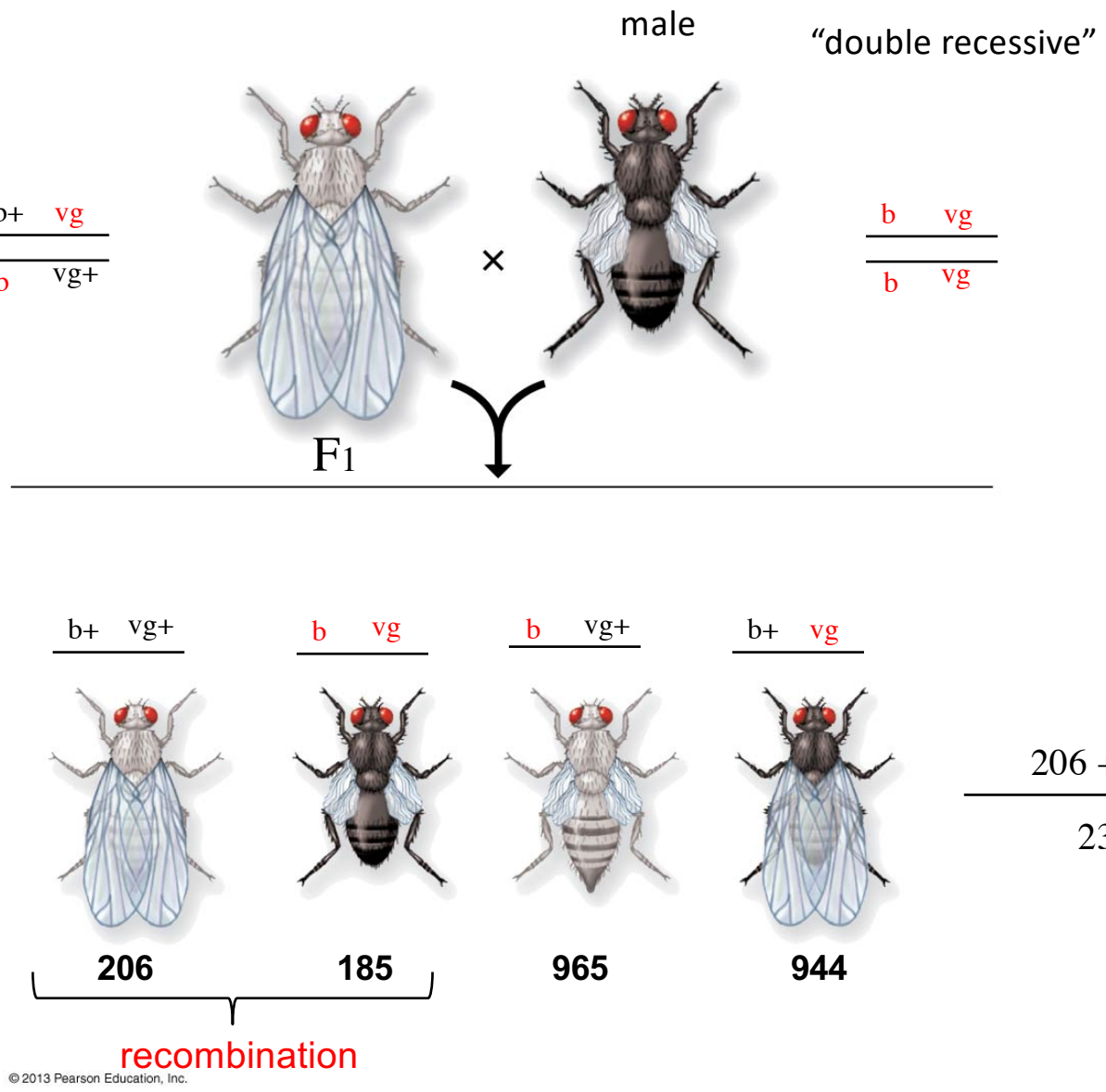
Measuring the genetic distance between 2 loci:

Locus black : body color
Locus vestigial : wing size

P  $\frac{b \quad vg}{b \quad vg}$

Mutations are in ***cis*** $\frac{b+ \quad vg+}{b \quad vg}$  F1 X $\frac{b \quad vg}{b \quad vg}$

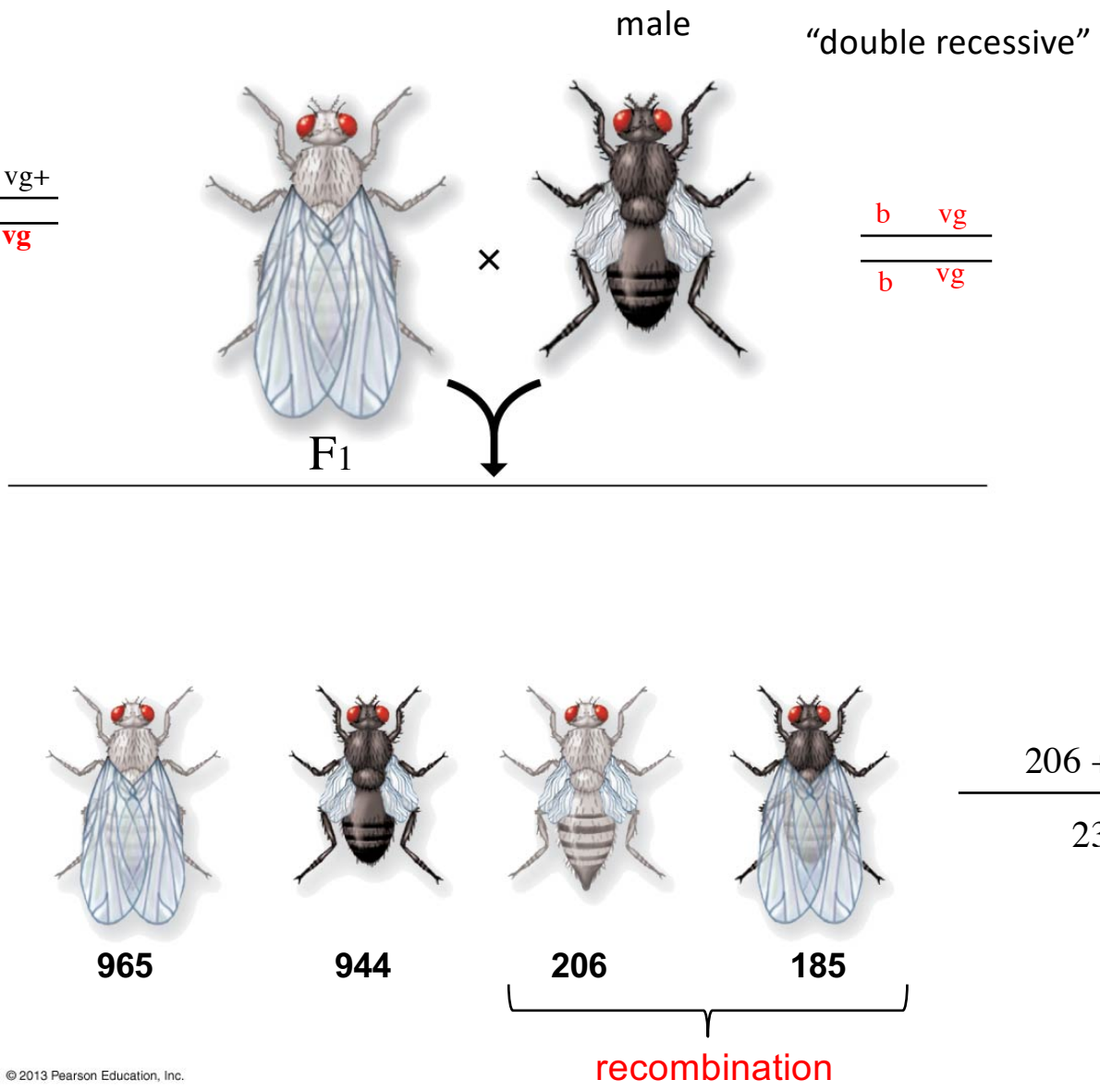
Mutations are in *trans*



$$\frac{206 + 185}{2300} = 0.17$$

17 centiMorgan

Mutations are in *cis*

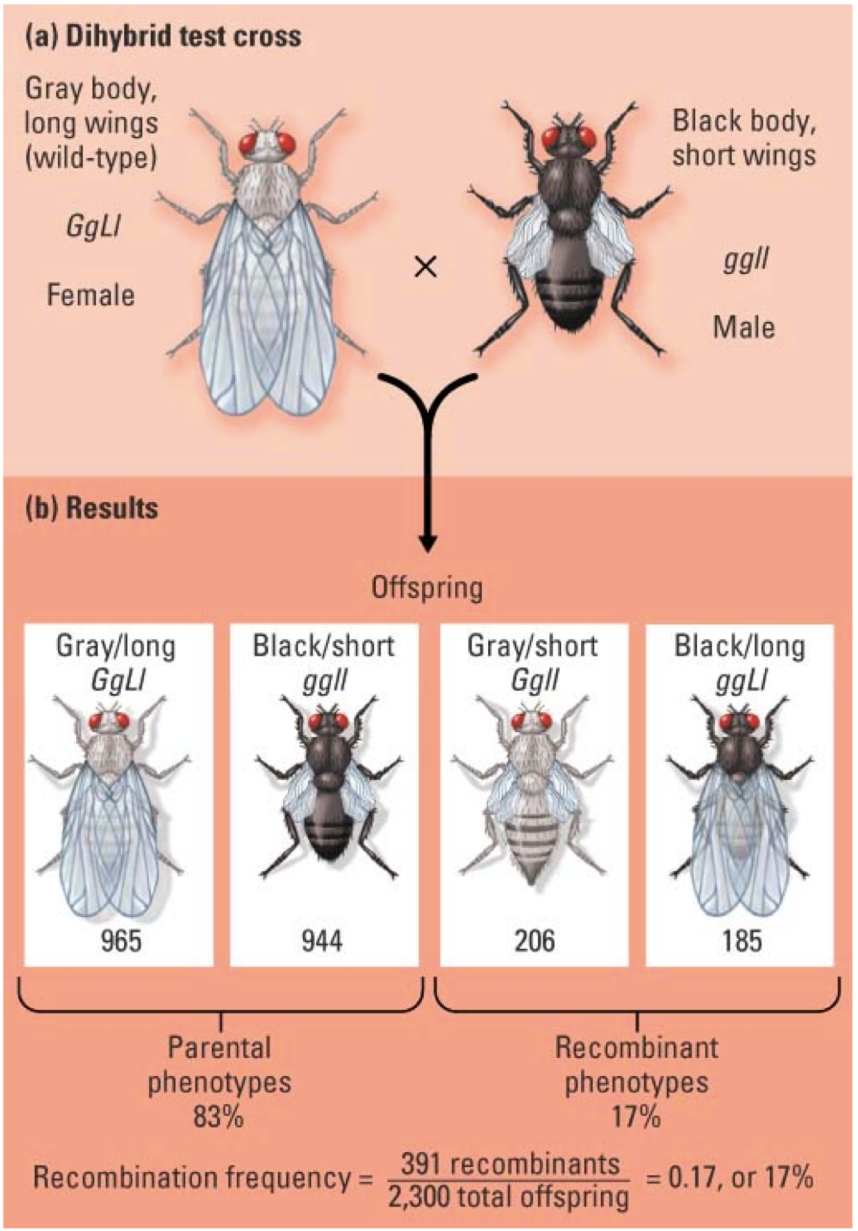


$$\frac{206 + 185}{2300} = 0.17$$

17 centiMorgan

In textbooks, the mutations are in ***cis***.

The case with mutations in ***trans*** is omitted.

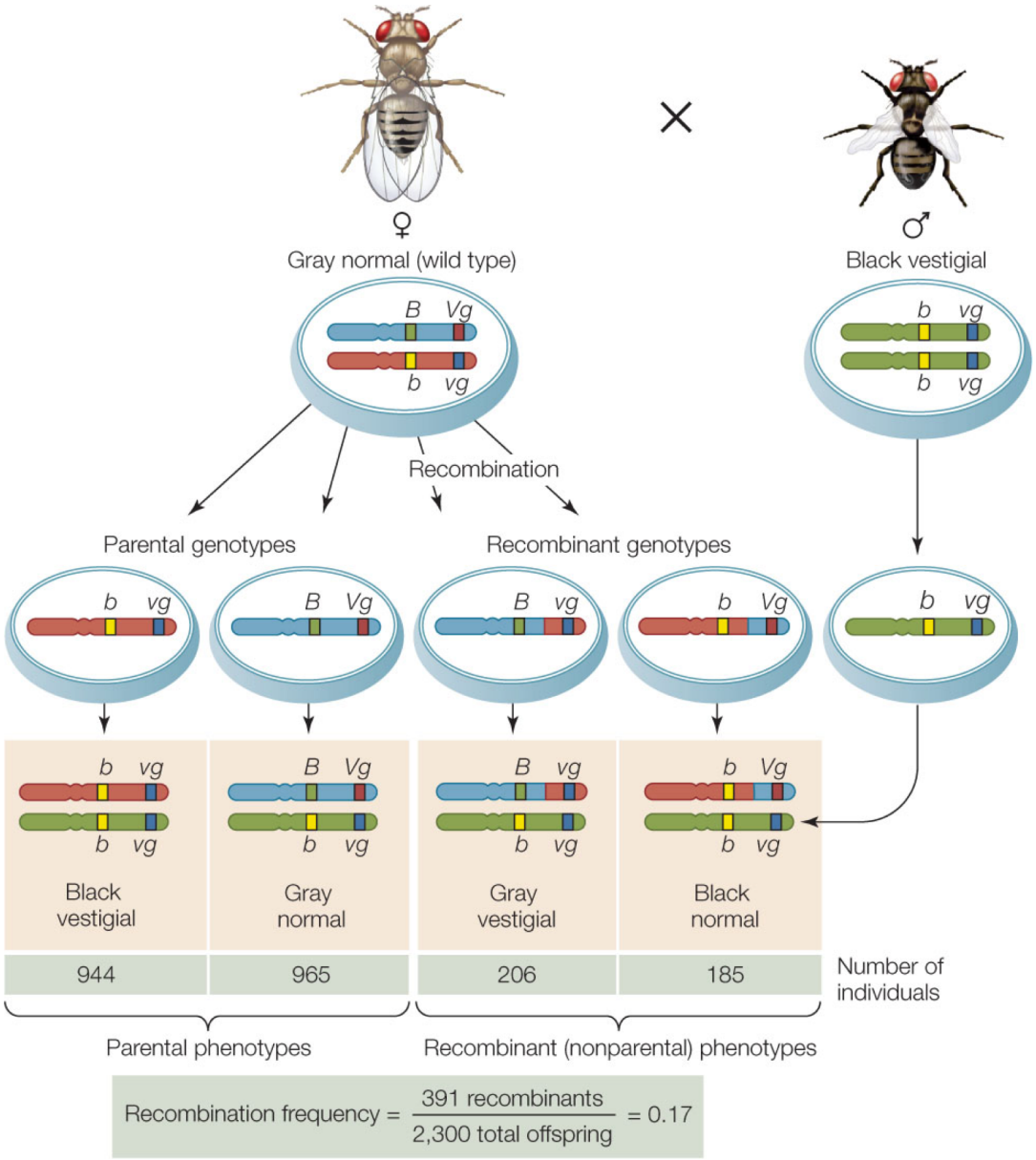


Essential Biology
Fig 9.24

Hillis, chapter 8

We observe crossovers during meiosis in the female. The female produces 4 types of gametes.

The phenotype of the offspring reflects the genetic information transmitted by the mother.



The male produces only 1 type of gametes

What is expected from you :

1. Given the genetic distance between the 2 loci and the number of offspring you should be able to calculate the expected numbers for all 4 phenotypes.

2. Given the numbers for all 4 phenotypes among the offspring you should be able to calculate the genetic distance between the 2 loci.

Remarkable cases :

d < 50cM Detection of linkage

d = 50 cM $\frac{25 + 25}{25 + 25 + 25 + 25} = 0.5$ The loci behave as if they were not linked

d > 50 cM The genetic distance cannot be deduced from one single cross; several crosses are needed to measure the genetic distance.