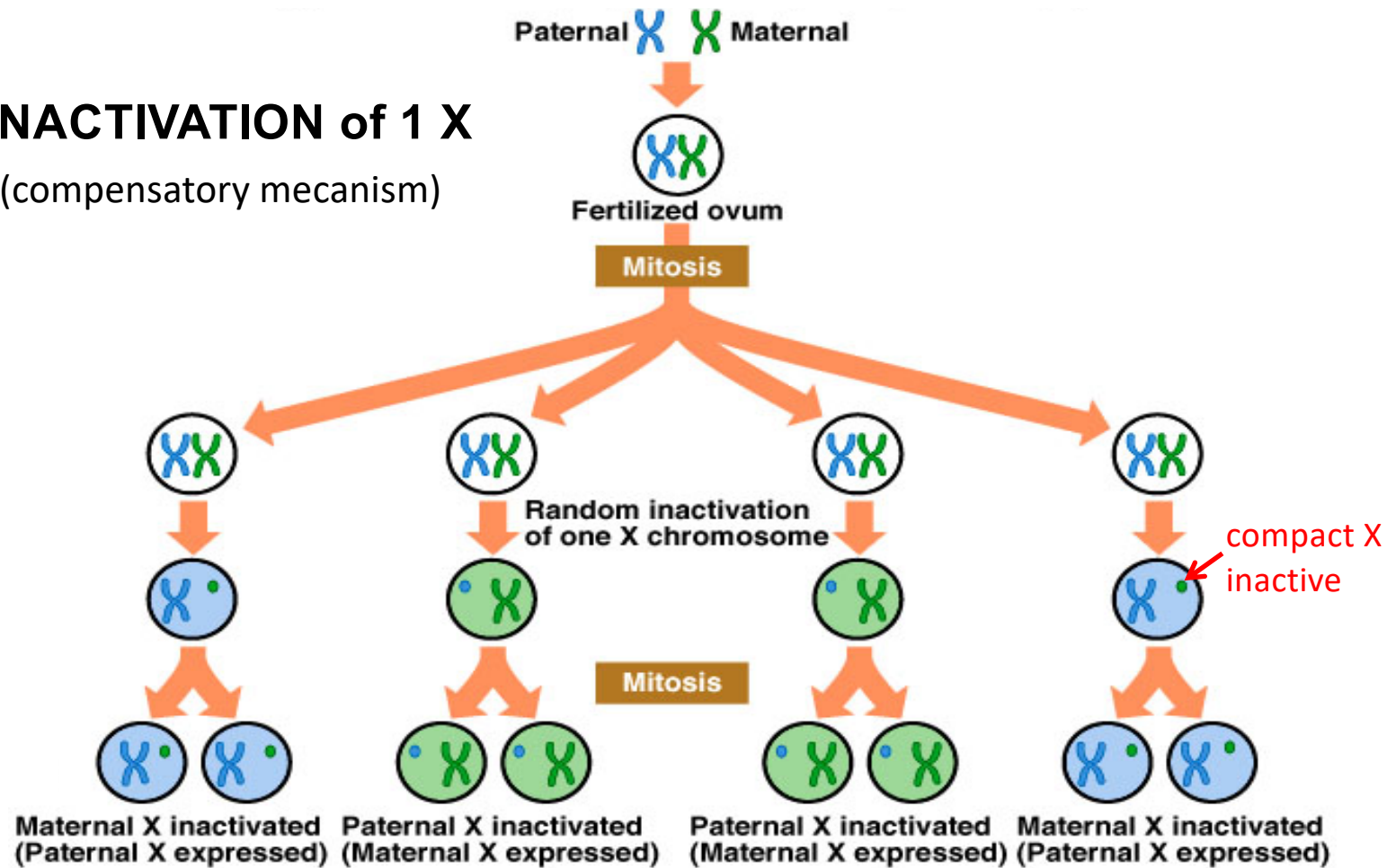


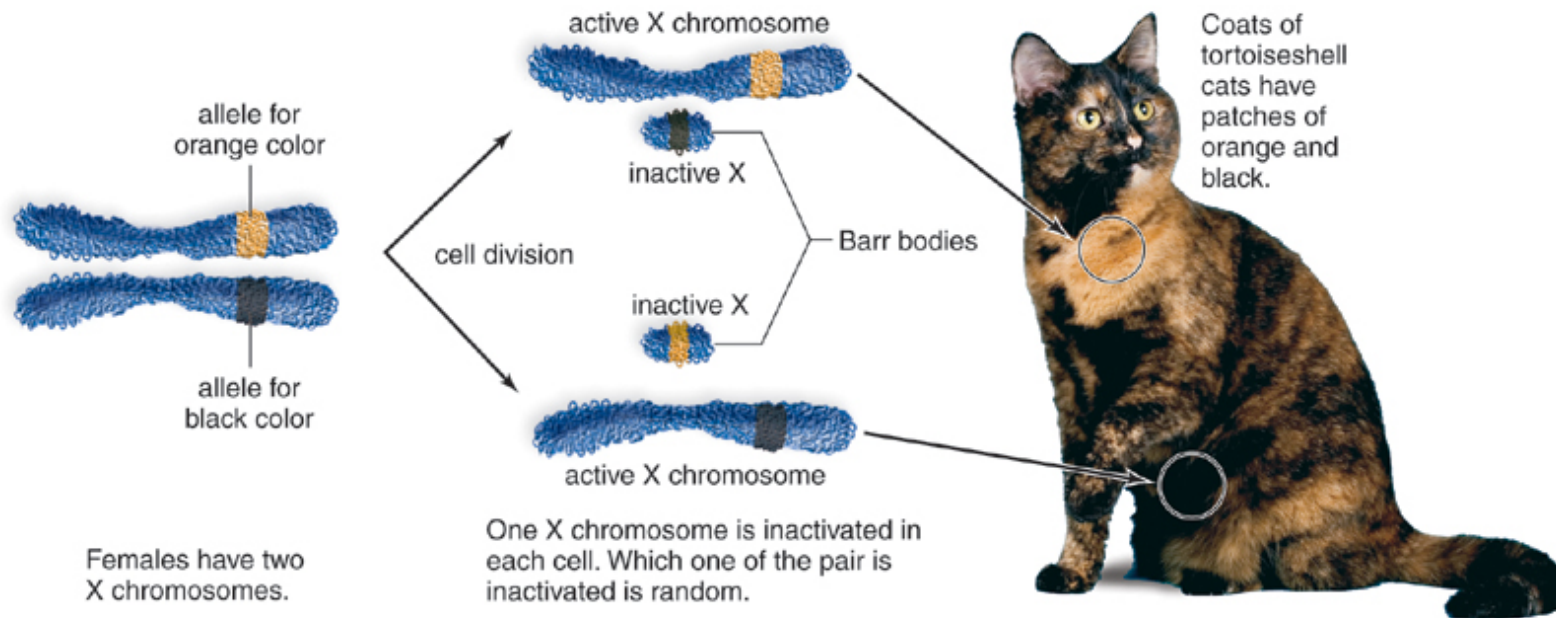
Women and men have the same amount of G6PD
because women and men have only 1 **active** X chromosome.

INACTIVATION of 1 X

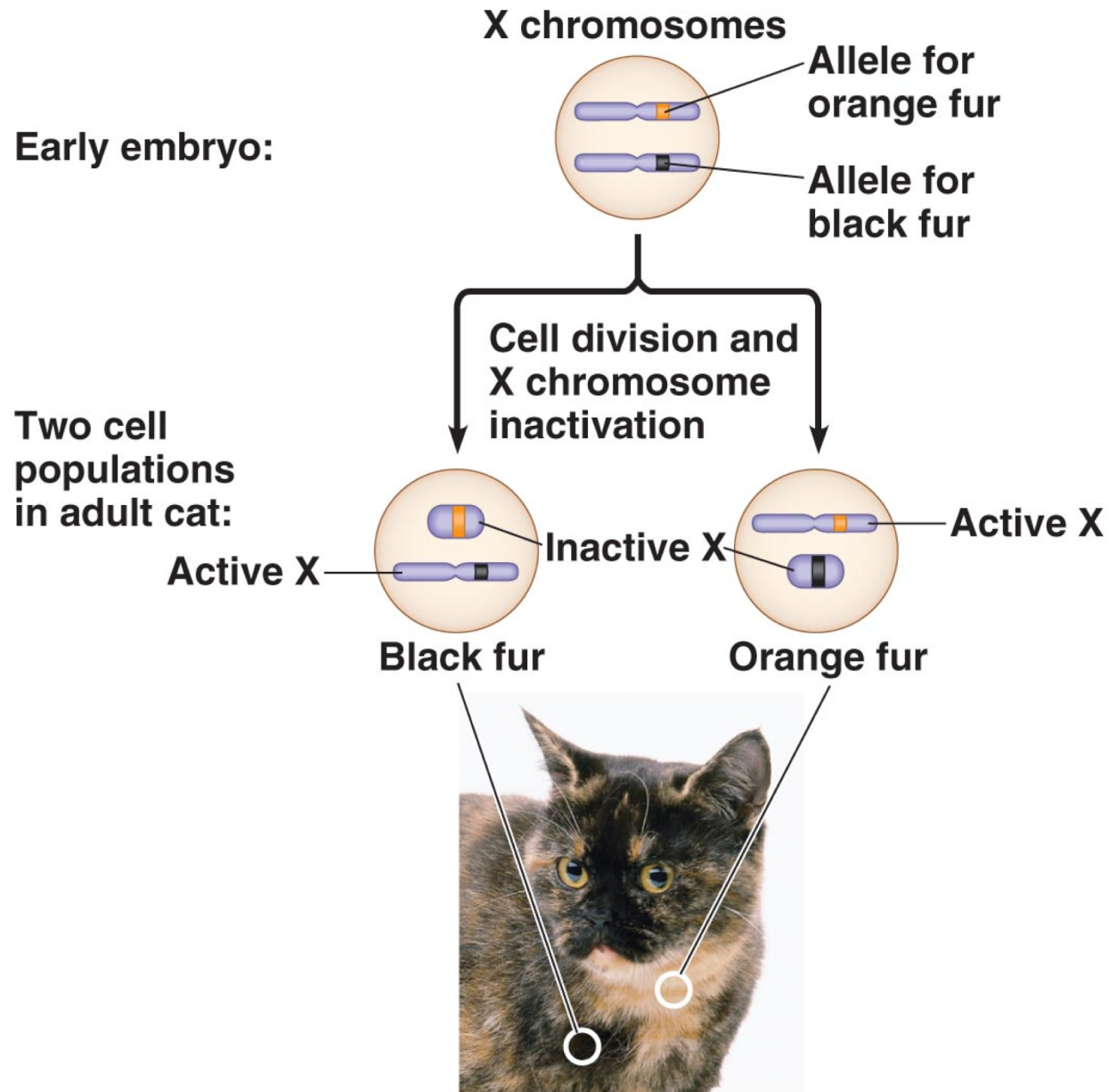
(compensatory mechanism)



X-inactivation



© Chanan Photo 2004



Difference between calico and tortoiseshell

(a)



(b)



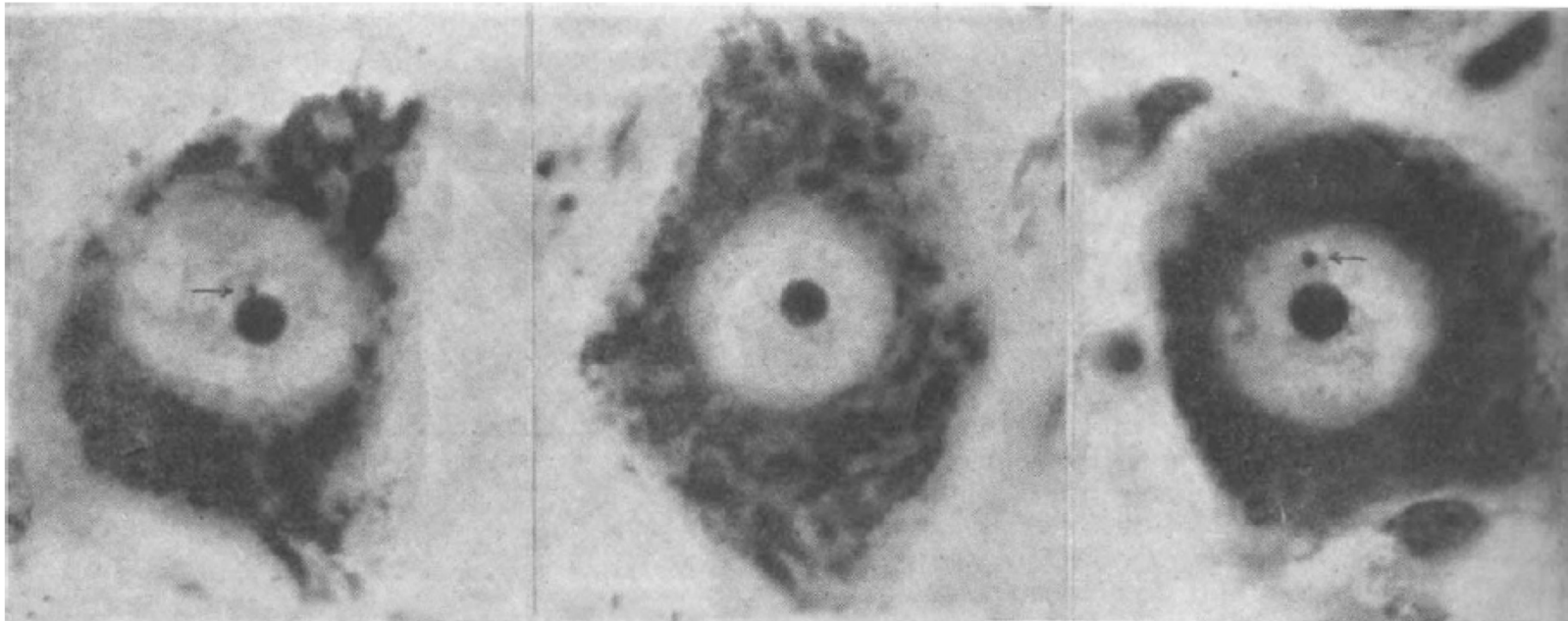
There is no melanocyte



Ewart BERTRAM

**A Morphological Distinction between
Neurones of the Male and Female, and the
Behaviour of the Nucleolar Satellite during
Accelerated Nucleoprotein Synthesis**

Murray BARR
(1908-1995)



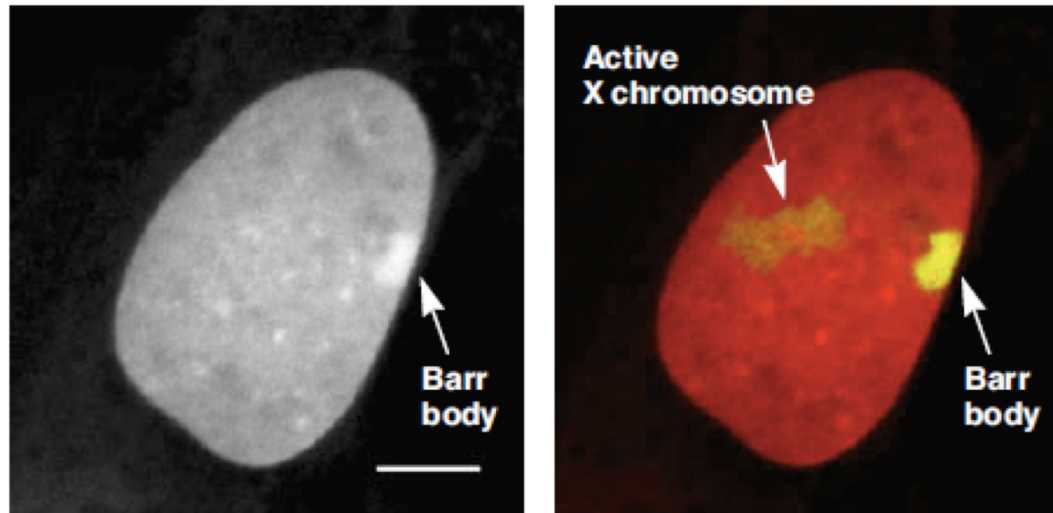
XX Fig. 1

XY Fig. 2

Fig. 3 XX

In 1949, Murray Barr and Ewart Bertram identified a **highly condensed structure** in the interphase nuclei of somatic cells in female cats that was not found in male cats.

This structure became known as the **Barr body**.



(a) Nucleus with a Barr body

In 1960, Susumu Ohno correctly proposed that the Barr body is a highly condensed X chromosome.

Barr body

(inactivated X chromosome)

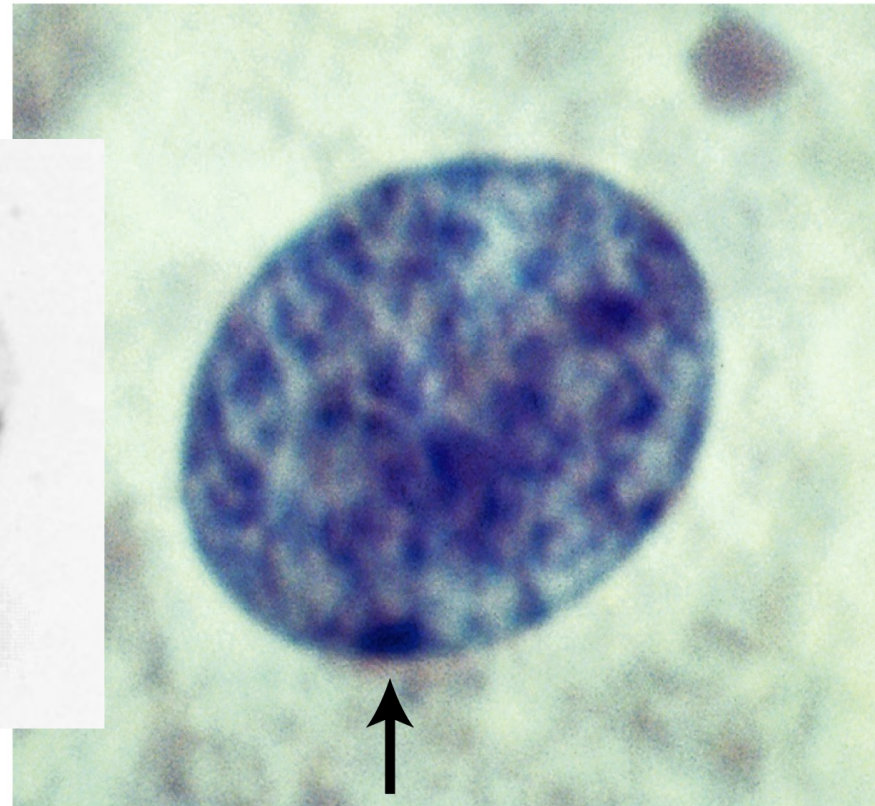
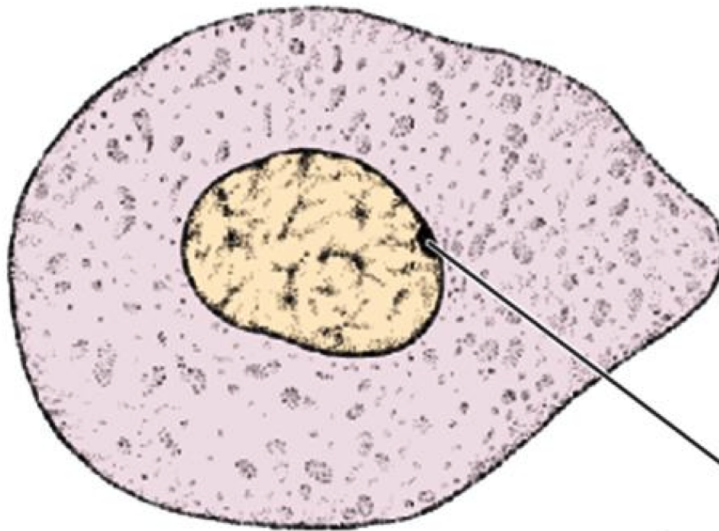


Figure 4-16a
Genetics: A Conceptual Approach, Third Edition
© 2009 W.H. Freeman and Company



Barr body

Buccal epithelium



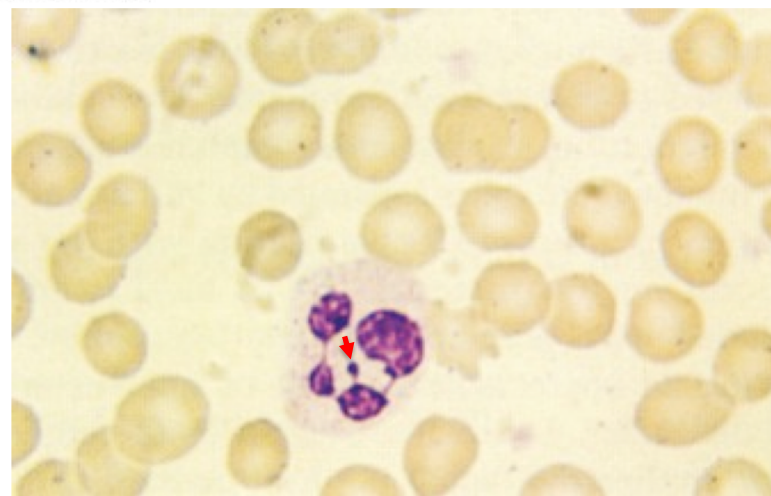
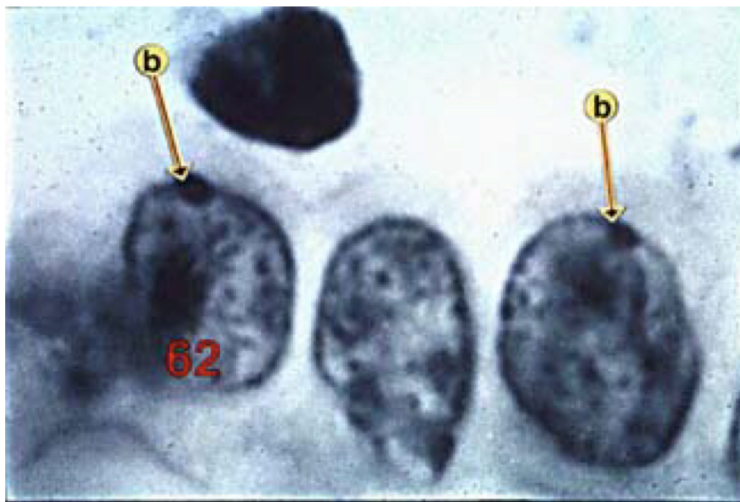
Polymorphonuclear leukocyte



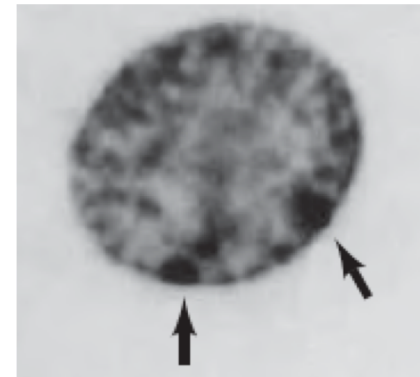
Blood sample

drumstick

Sex chromatin



Triple X (47, XXX)



2 Barr bodies
(2 inactivated X)

One lady with this anomaly was **Ewa Klobukowska**, a 1964 Olympic sprint bronze medallist who was the first woman to fail the sex chromosome test during the 1967 European Cup.

“One chromosome too many to be declared a woman for the purposes of athletic competition” was the statement, and she was obliged to return all her medals. She gave birth to a baby the following year.

➞ 47,XXX women have normal children !!

Feminity test : (competition)

Normal woman :



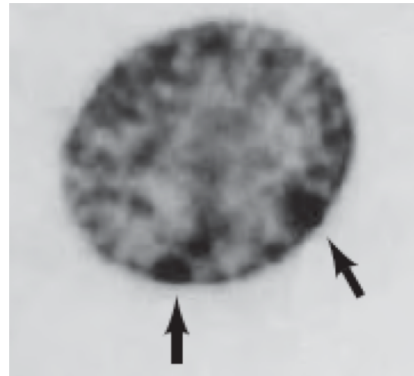
Barr body
= inactivated X chromosome

46, **X** X



47, **X** **X** X

Ewa Klobukowska
Bérénice



Official document attesting gender.

Complete androgen insensitivity

Certificat de féminité pour les sportives :

46,XY female

Miss/Mrs. PATINO CERTIFICATE No. 5341

First name /Prénomial. Maria José Martínez

at/de Spain (Country/Pays)

born/nive (date)

On the occasion of the/A l'occasion des
World Championships
(Games de Championships/
Jeux au Championnat)

held at/à Helsinki 1983

the above mentioned athlete underwent an approved medical test. The result of which was sex chromatin positive. This satisfies the I.A.A.F. requirements for competition in Women's athletic events.

L'athlète mentionnée ci-dessus a subi un examen médical approuvé, et la chromatine sexuelle s'est révélée positive. Ceci répond aux règlements de la F.I.A.A. pour concourir aux preuves féminines.

[Signature]
Athlete's signature/Signature de l'athlète

[Portrait Photo]

I hereby confirm that this Certificate is issued in accordance with the report of this official Medical Panel of the mentioned Games/Championnats.

Je certifie par la présente que ce certificat est délivré conformément au rapport du Comité médical officiel des Jeux/Championnats mentionnés ci-contre.

[Signature]
General Secretary, I.A.A.F.
Secrétaire Général, F.I.A.A.

L'athlète mentionnée ci-dessus
a subi un examen médical approuvé
et la **chromatine sexuelle** s'est
révélée positive.



Maria PATINO

Tested female in 1983 in Helsinki ;
failed the gender test in 1985

Santhi Soundarajan

Doha
2006

46,XY female



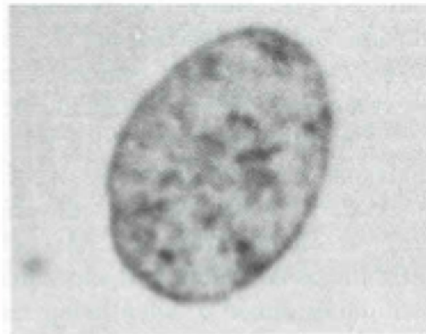
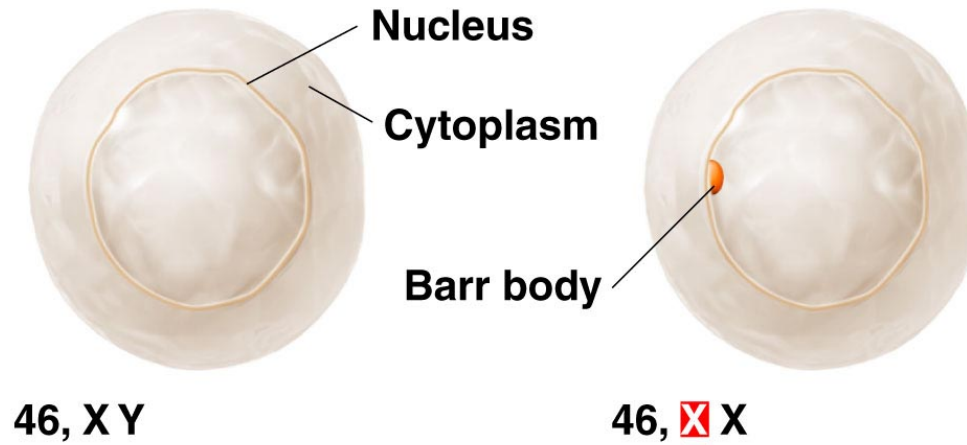
She had to give back her silver medal (800 m).

Is it fair ???

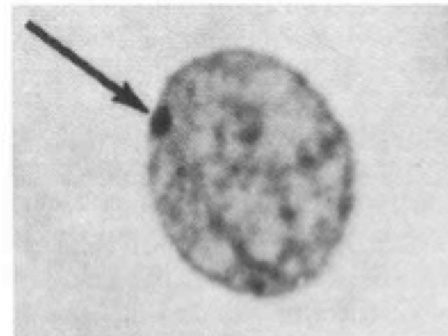


Gender test (competitions)

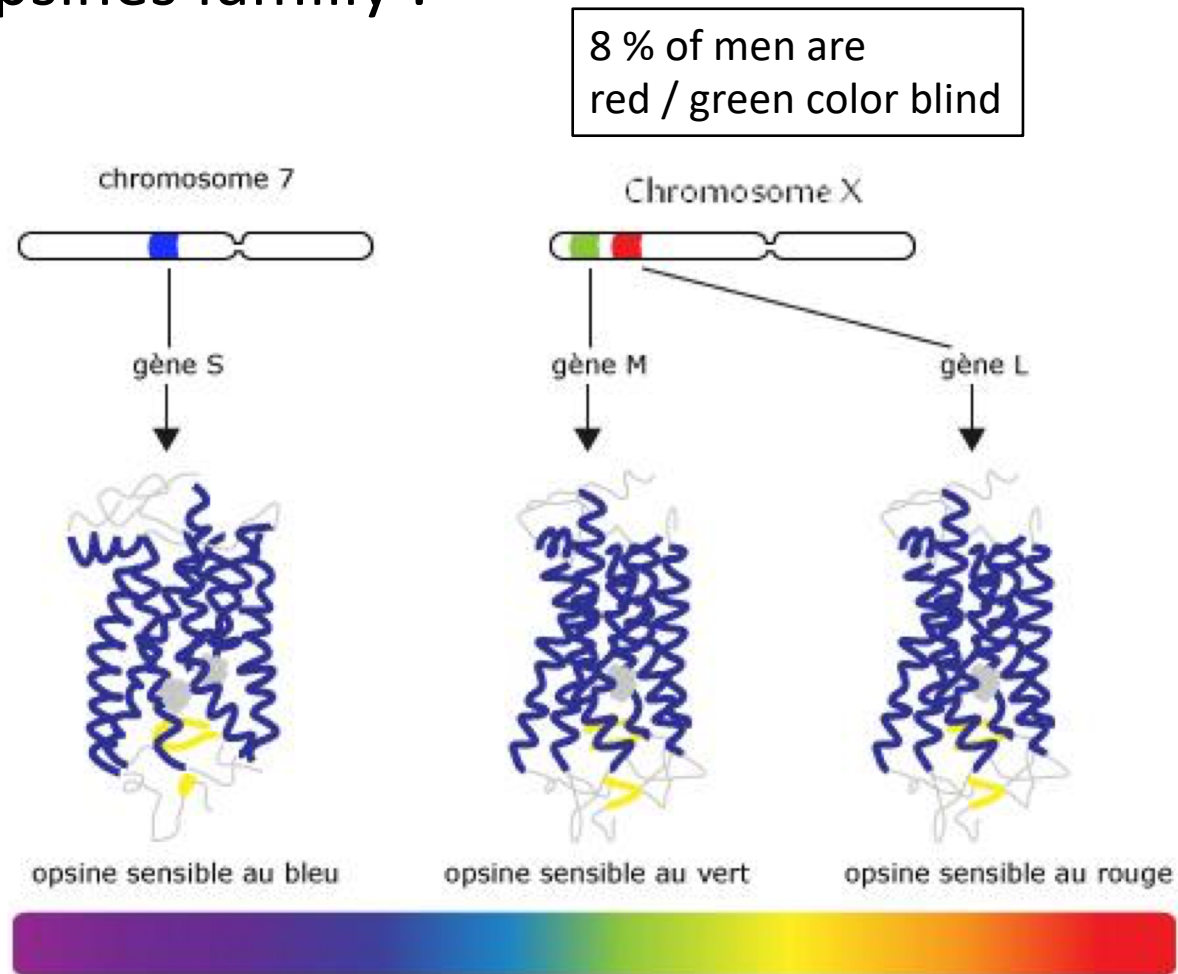
Normal woman :



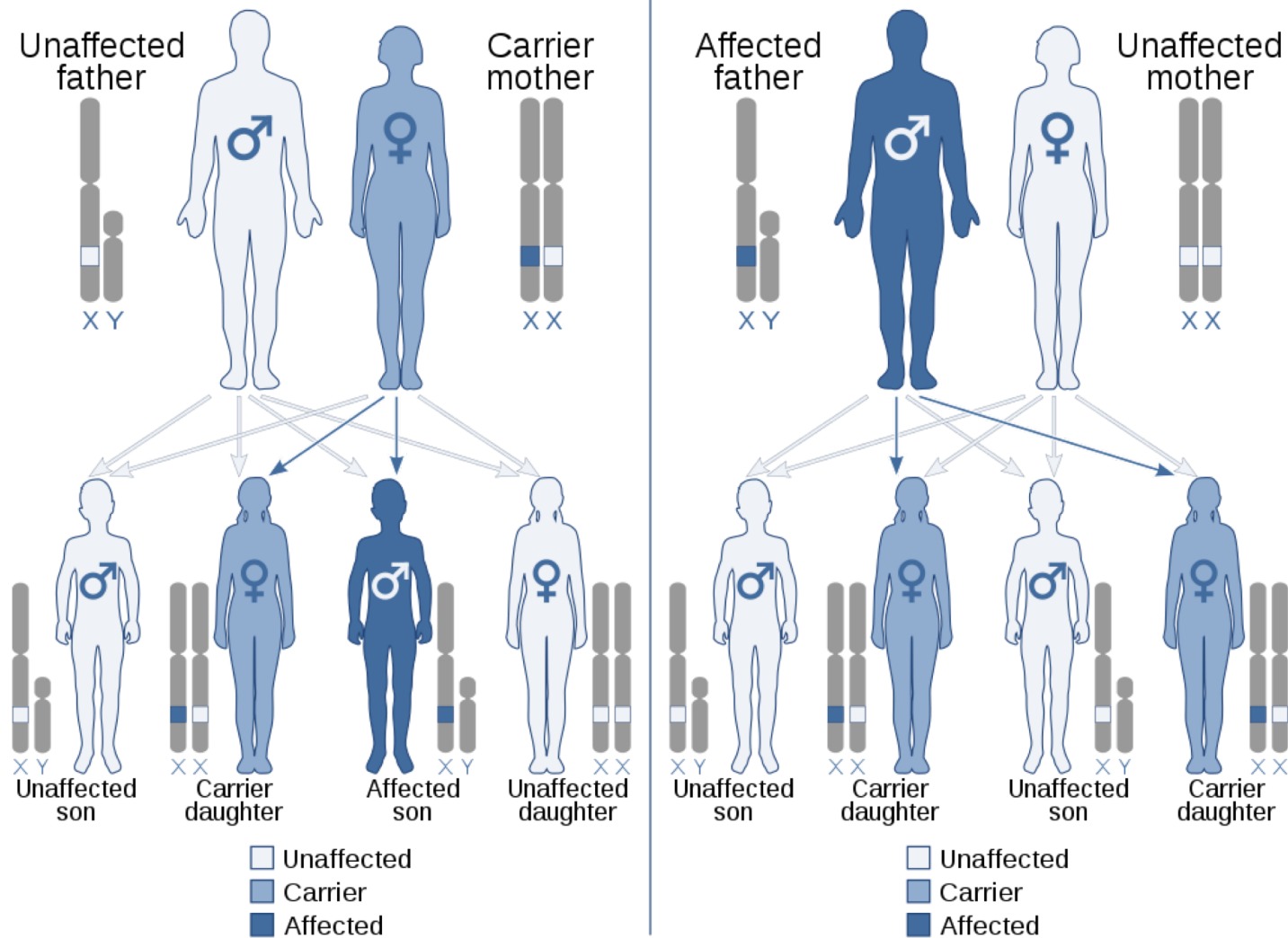
No Barr body



The opsines family :



X-linked recessive

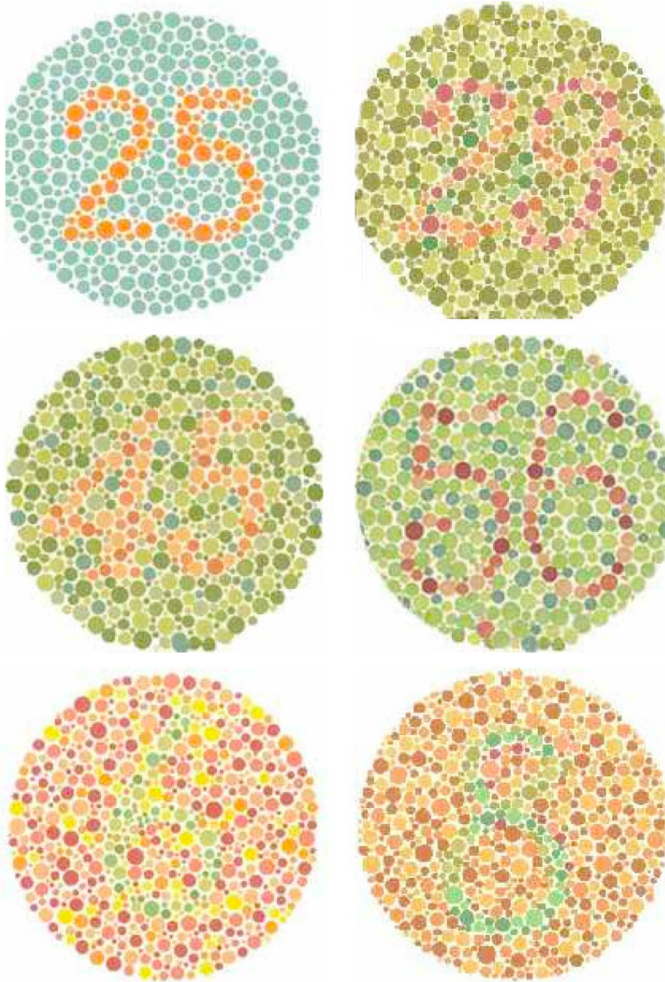


Recessivity is defined only in women.

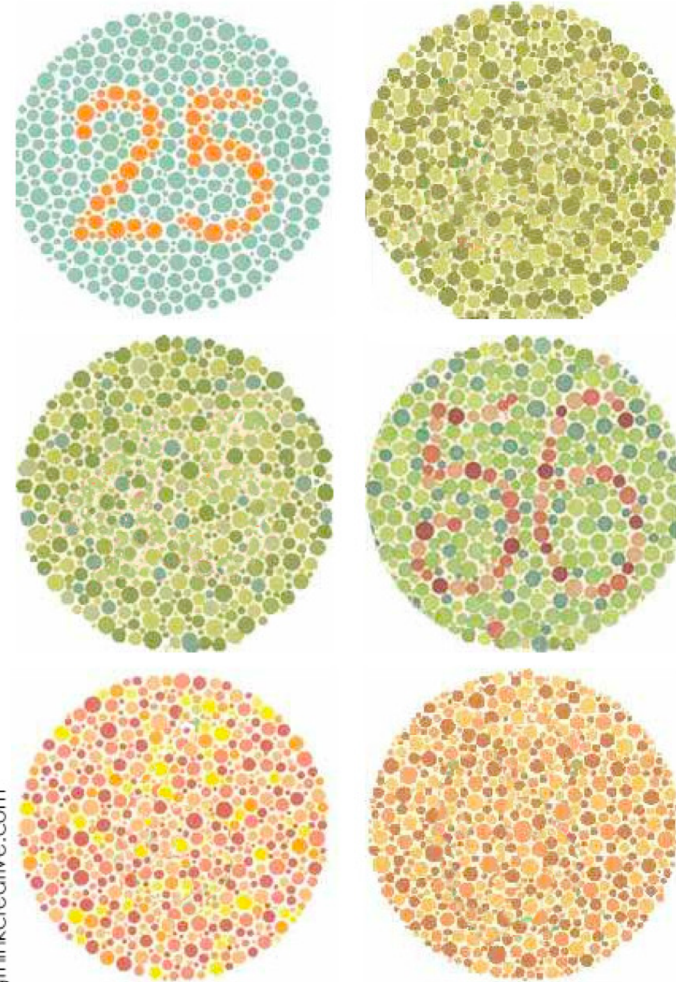
Note: a few carriers may be mildly affected due to skewed X-inactivation.

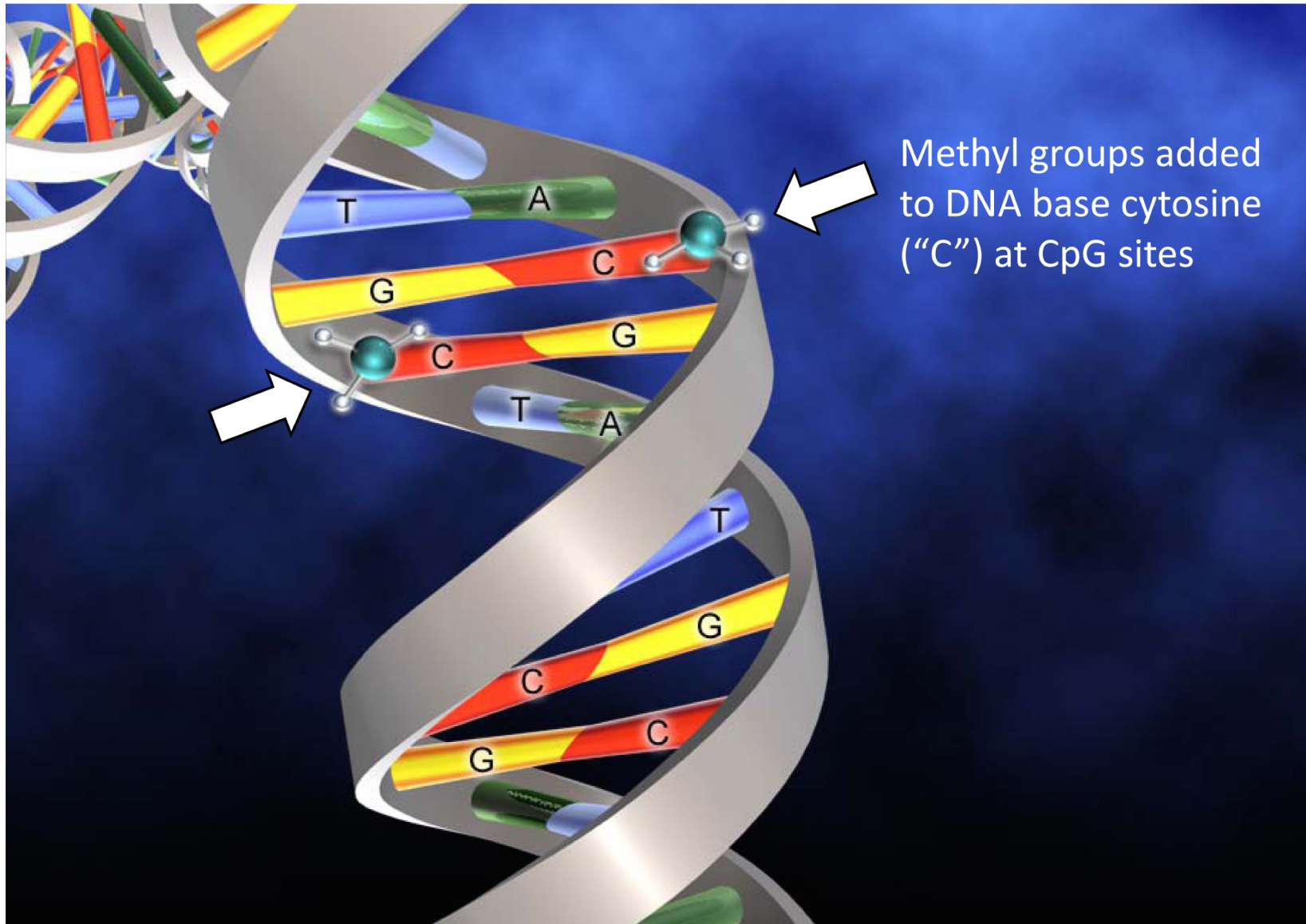
Ishihara Test For Color Blindness

What People With Regular Vision See

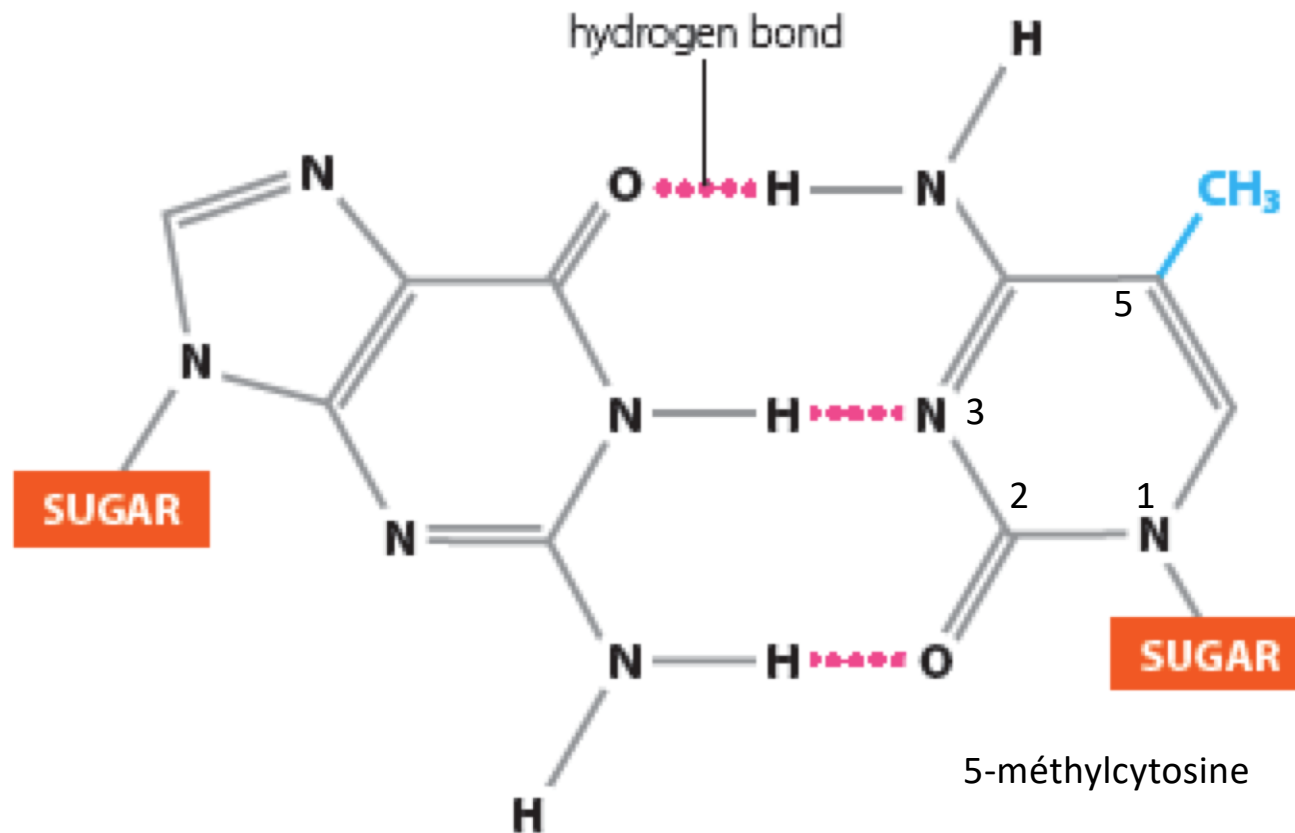


What Red-Green Color Blind People See



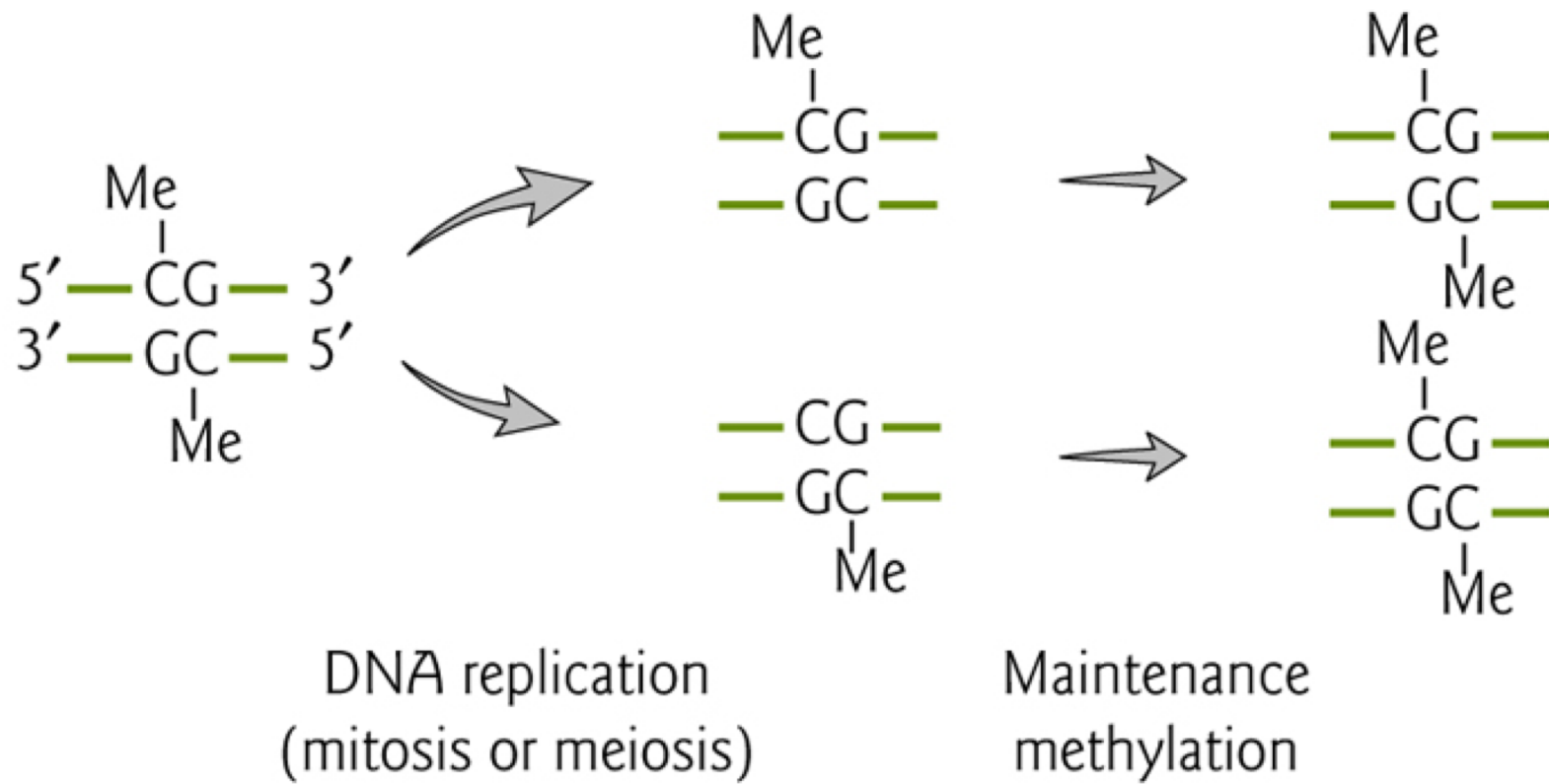


L'appariement n'est pas affecté par la méthylation :

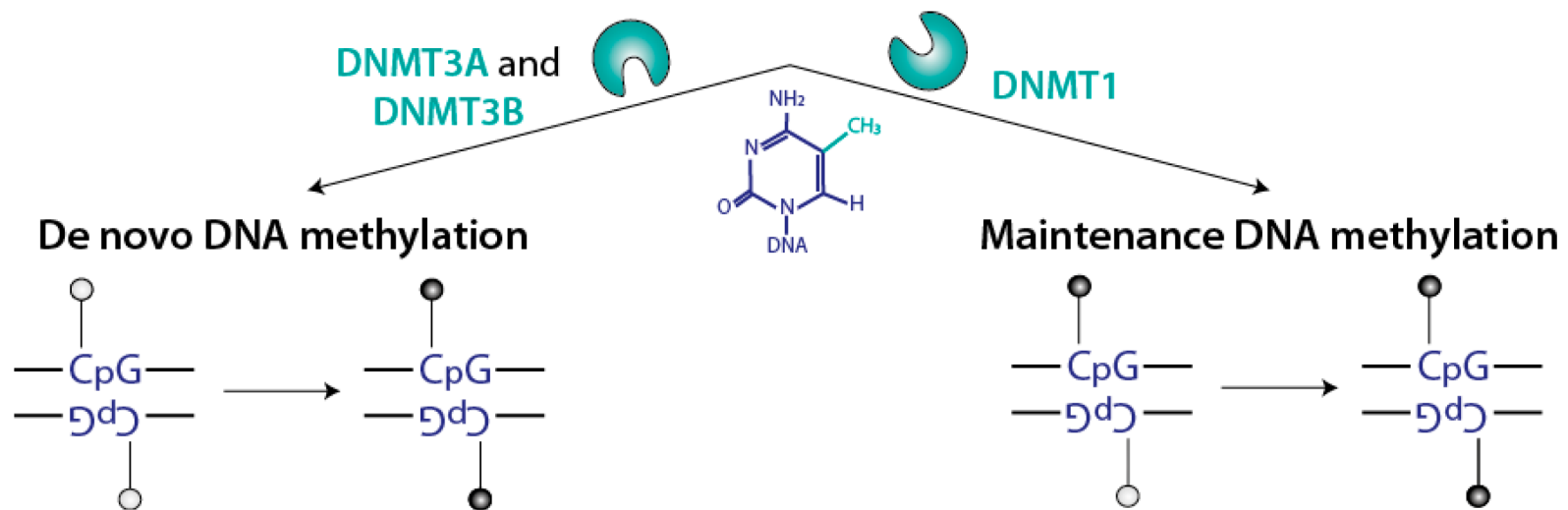


5-methylcytosine base-pairs with guanine in exactly the same way as unmodified cytosine.

CpG Methylation

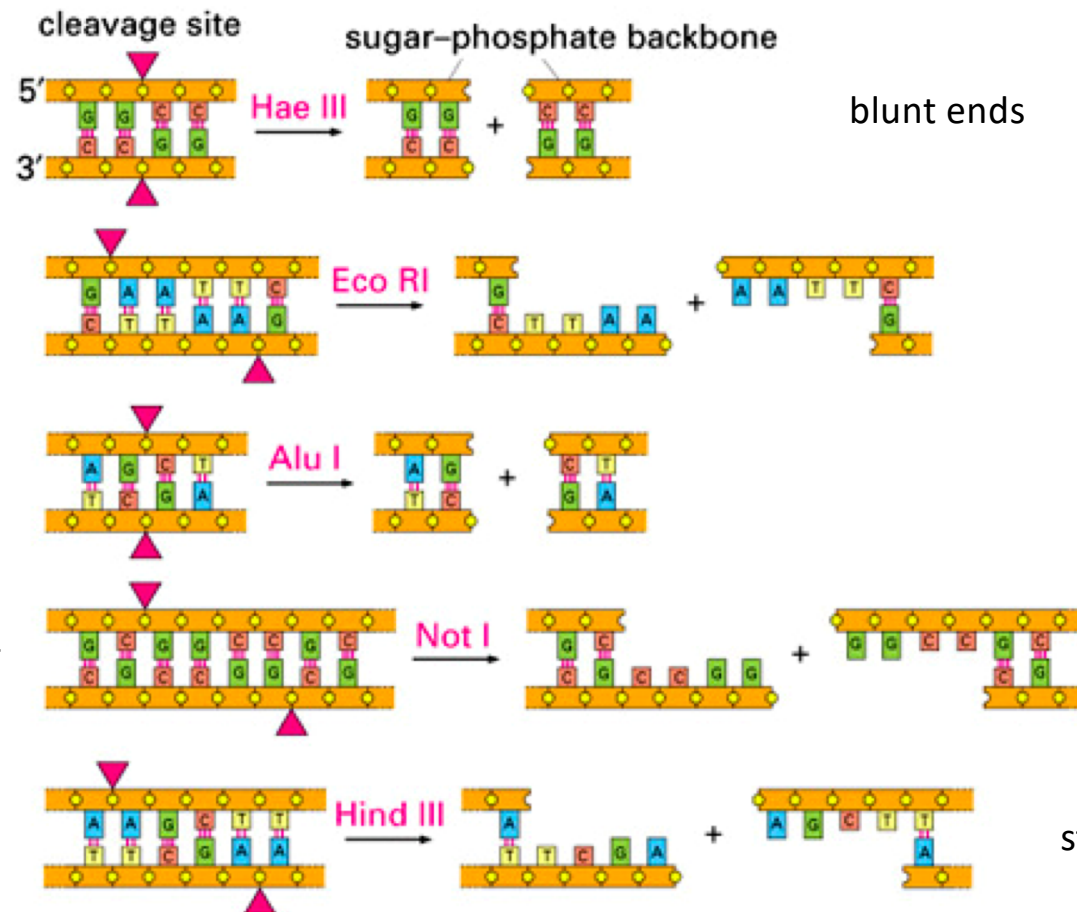


CpG Methylation



DNMT = **D**Na **M**ethyl **T**ransferase

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**

Isoschizomeres

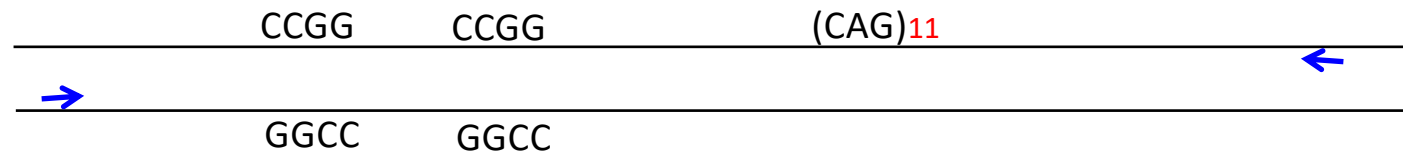
	cut	no cut
Hpa II recognizes	5'- C C G G - 3' 3'- G G C C - 5'	5'- C ^m C G G - 3' 3'- G G ^m C C - 5'
Msp I recognizes	5'- C C G G - 3' 3'- G G C C - 5'	5'- C ^m C G G - 3' 3'- G G ^m C C - 5'
	cut	cut

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

However there is one important difference :

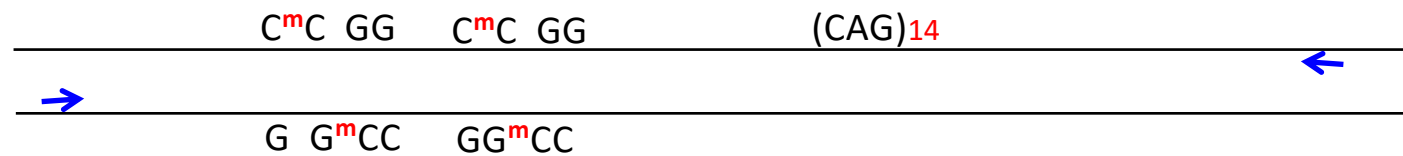
Hpa II is **sensitive** to DNA methylation

Msp I is **insensitive** to DNA methylation

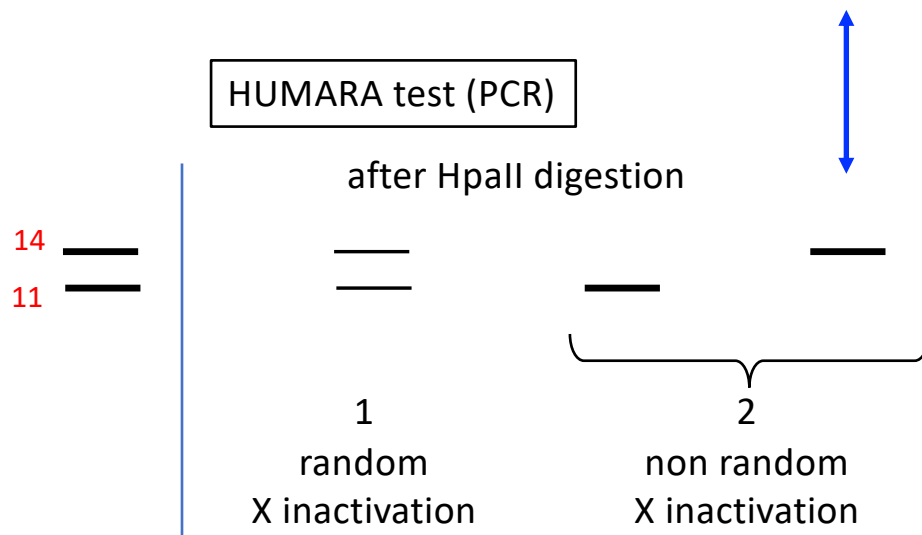


The active X
is not methylated

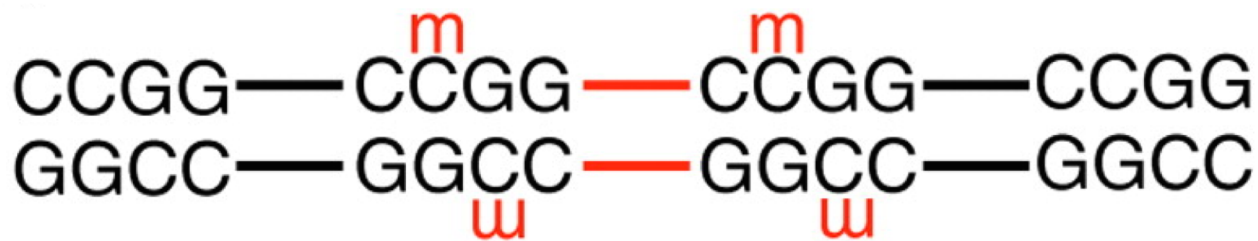
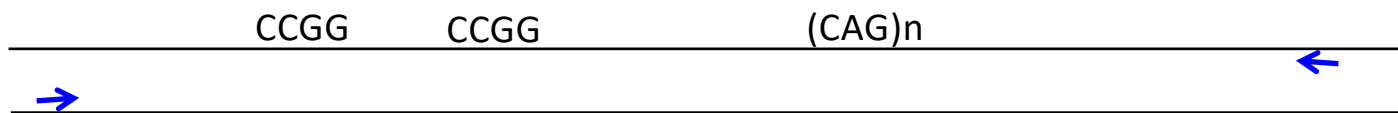
microsatellite



The inactive X
is methylated



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



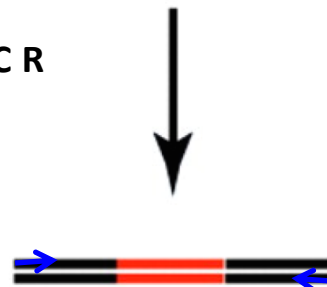
Digestion with *Hpa*II
(blocked by CG methylation)

Digestion with *Msp*I
(not blocked by CG methylation)

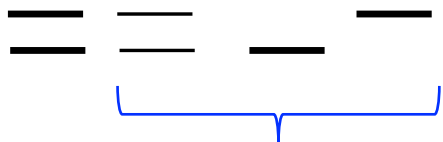


No amplification product

P C R



HUMARA test

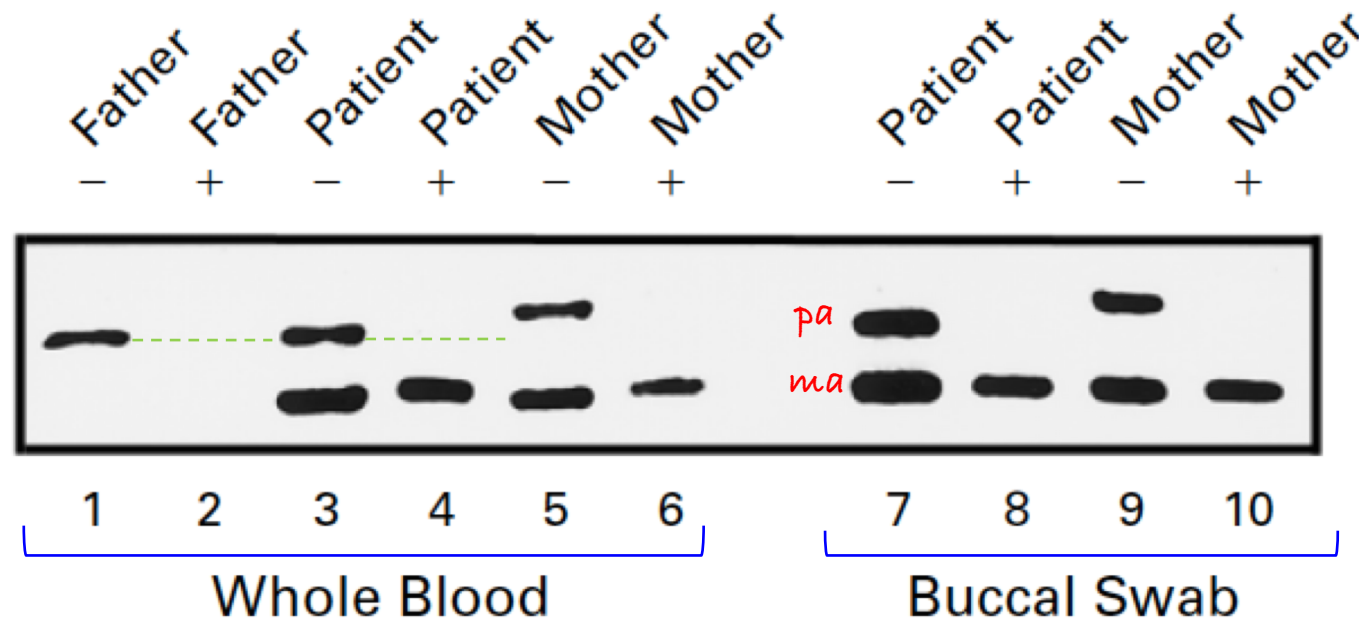


after *Hpa*II digestion

Example of testing

Mother shows non random X inactivation

Patient shows non random X inactivation



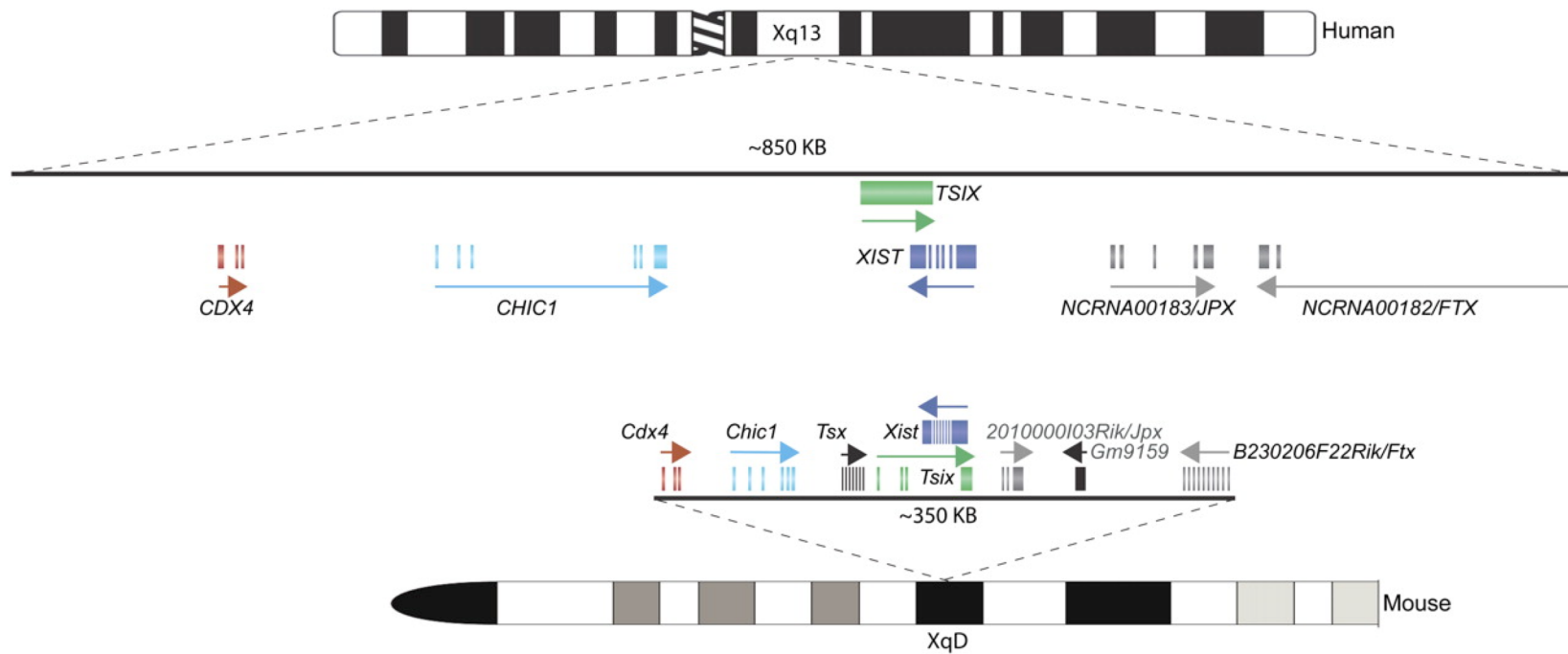
Whole blood and buccal swab give identical results

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.

XIC = X Inactivation Center

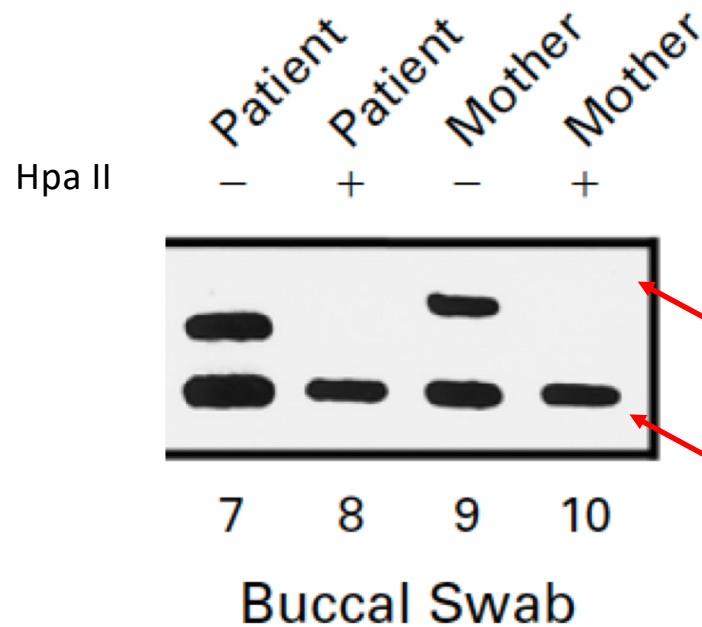
TSIX and XIST are non coding RNA



Mutations of XIC can be

- loss-of-function
- gain-of-function

Hpa II digested *before* PCR



Mother shows
non random
X inactivation

Why ? XIC is mutated

never inactivated

always inactivated

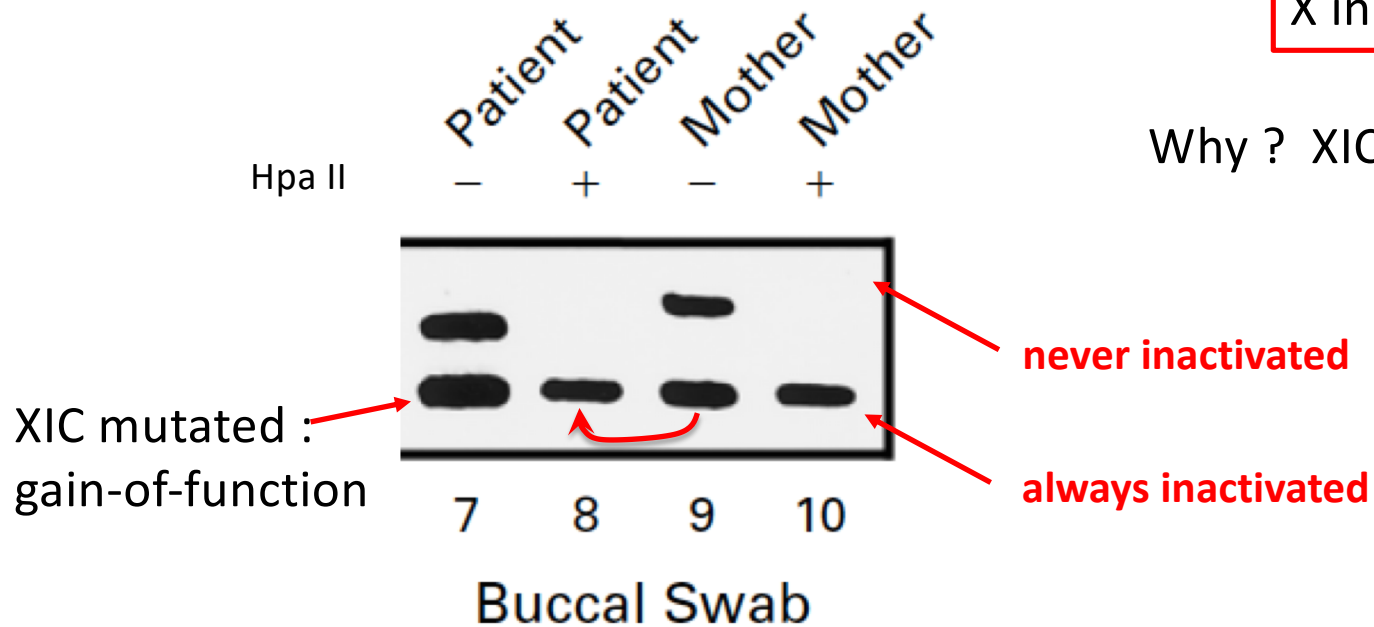
A priori 2 possibilities :

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

Hpa II digeston *before* PCR

Patient shows
non random
X inactivation

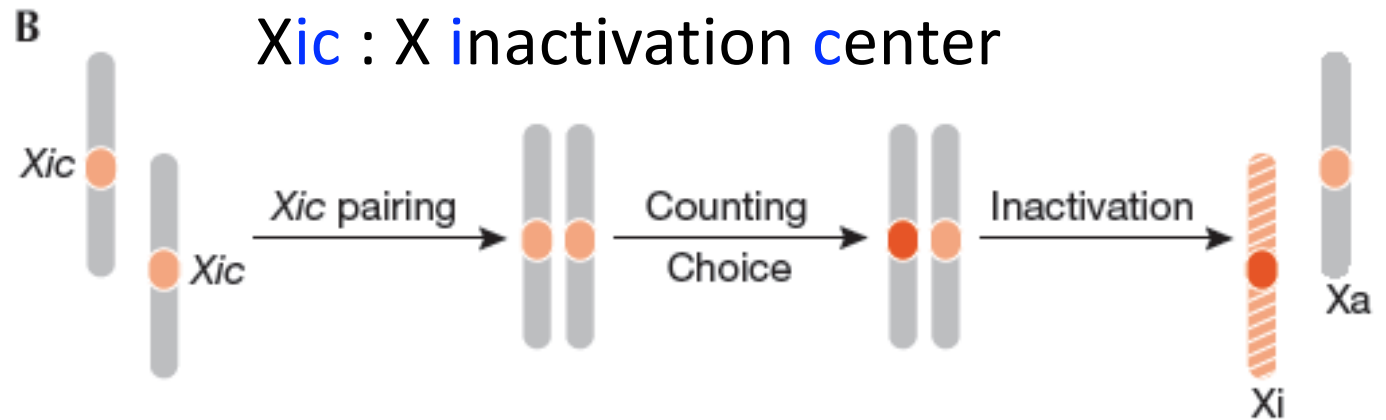
Why ? XIC is mutated



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

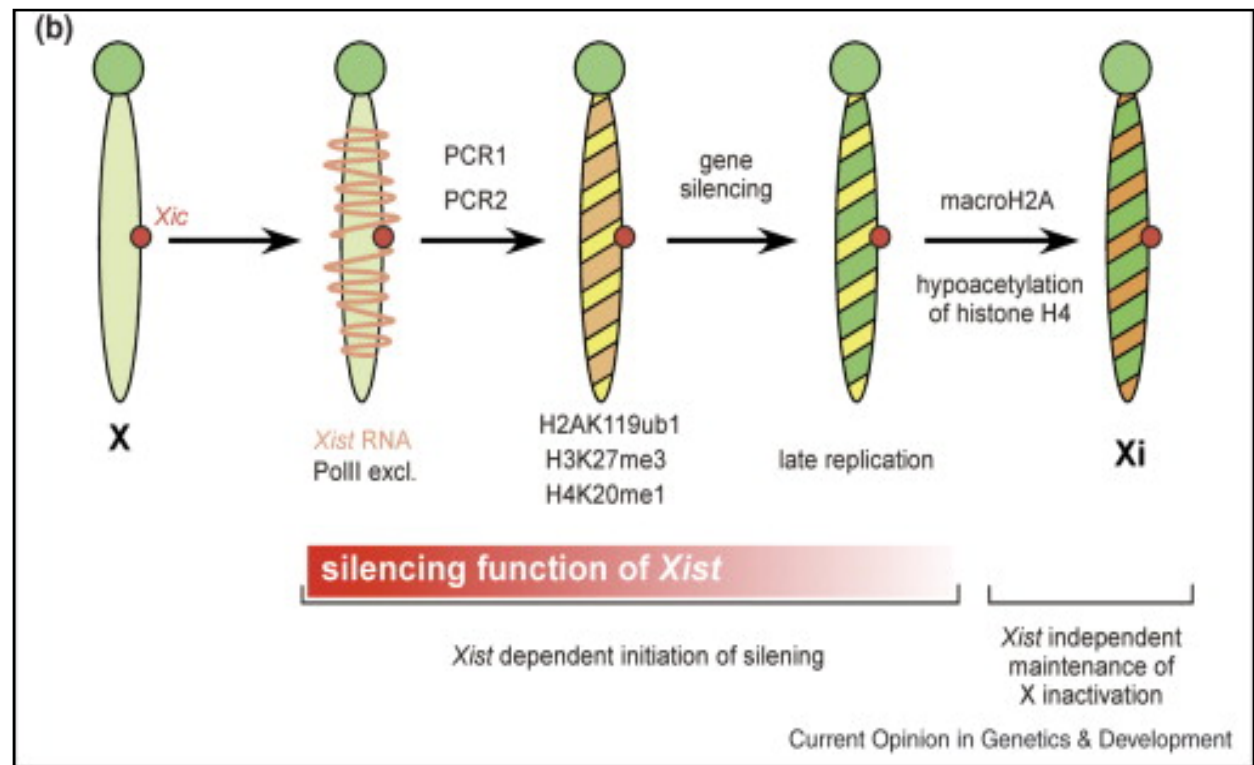


Xic is required for X inactivation.
Loss of function of Xic makes the X chromosome unable to inactivate.

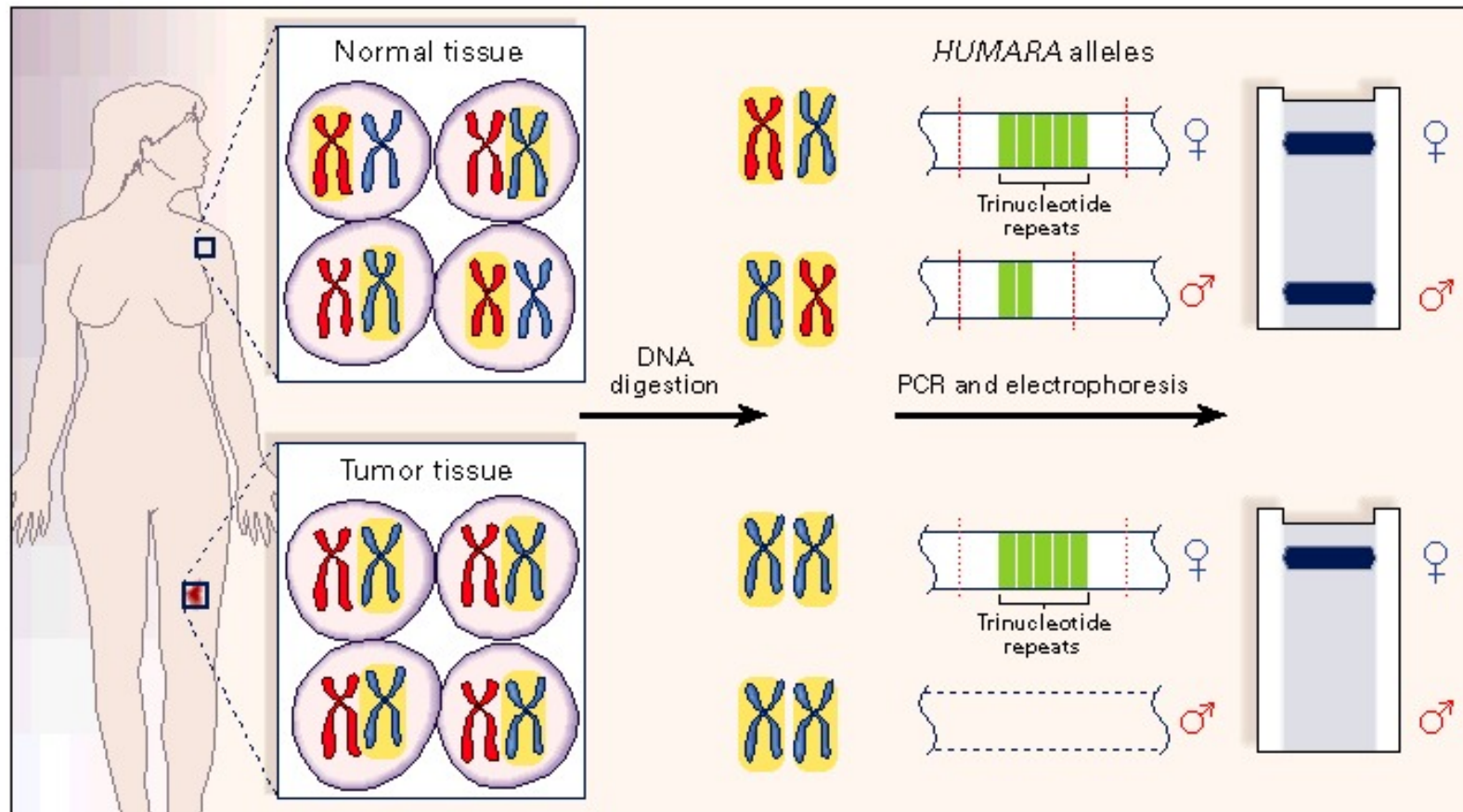
Gain of function of Xic makes the X chromosome always inactive.

Consequence :

The X inactivation is ***not random***.



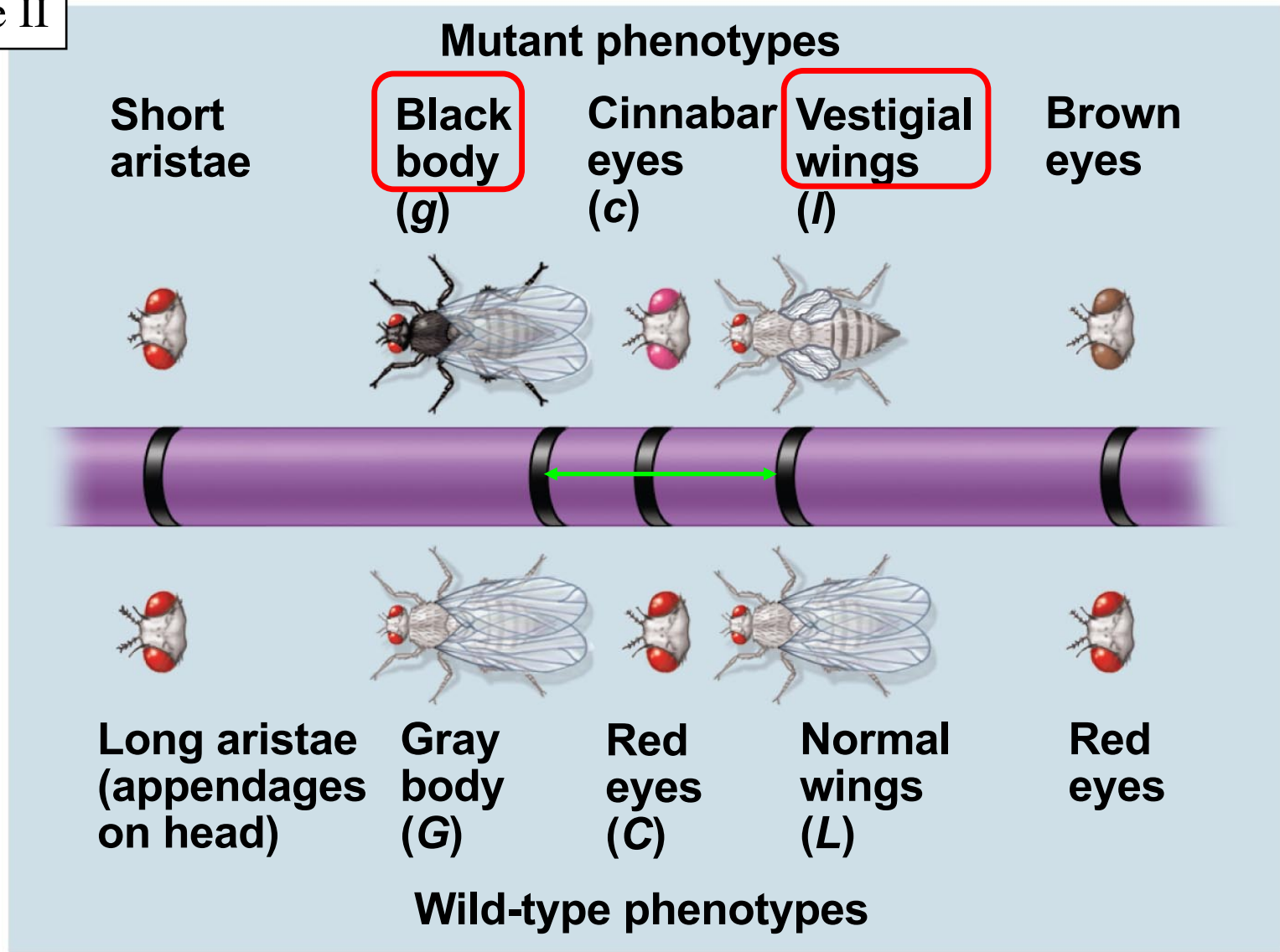
HUMARA testing in cancer patients



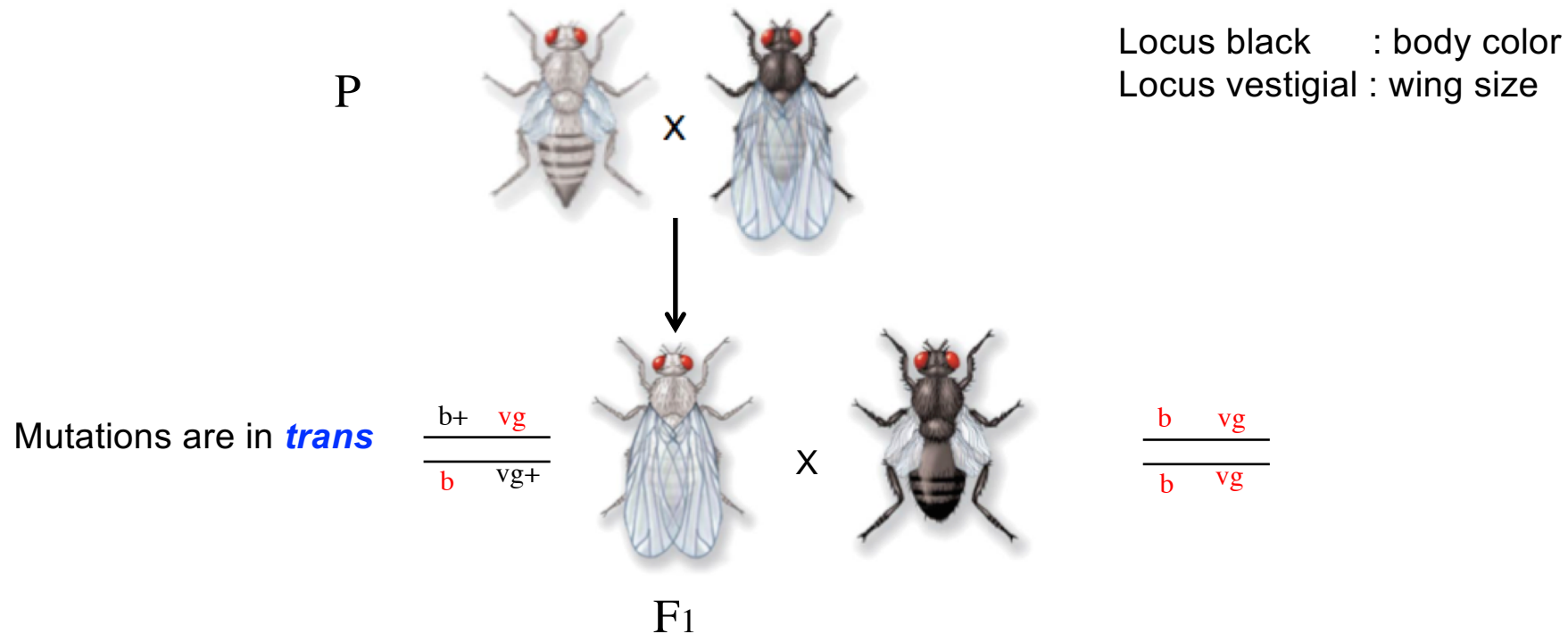
Evidence for monoclonal origin of cancer tumor.

Chromosome II

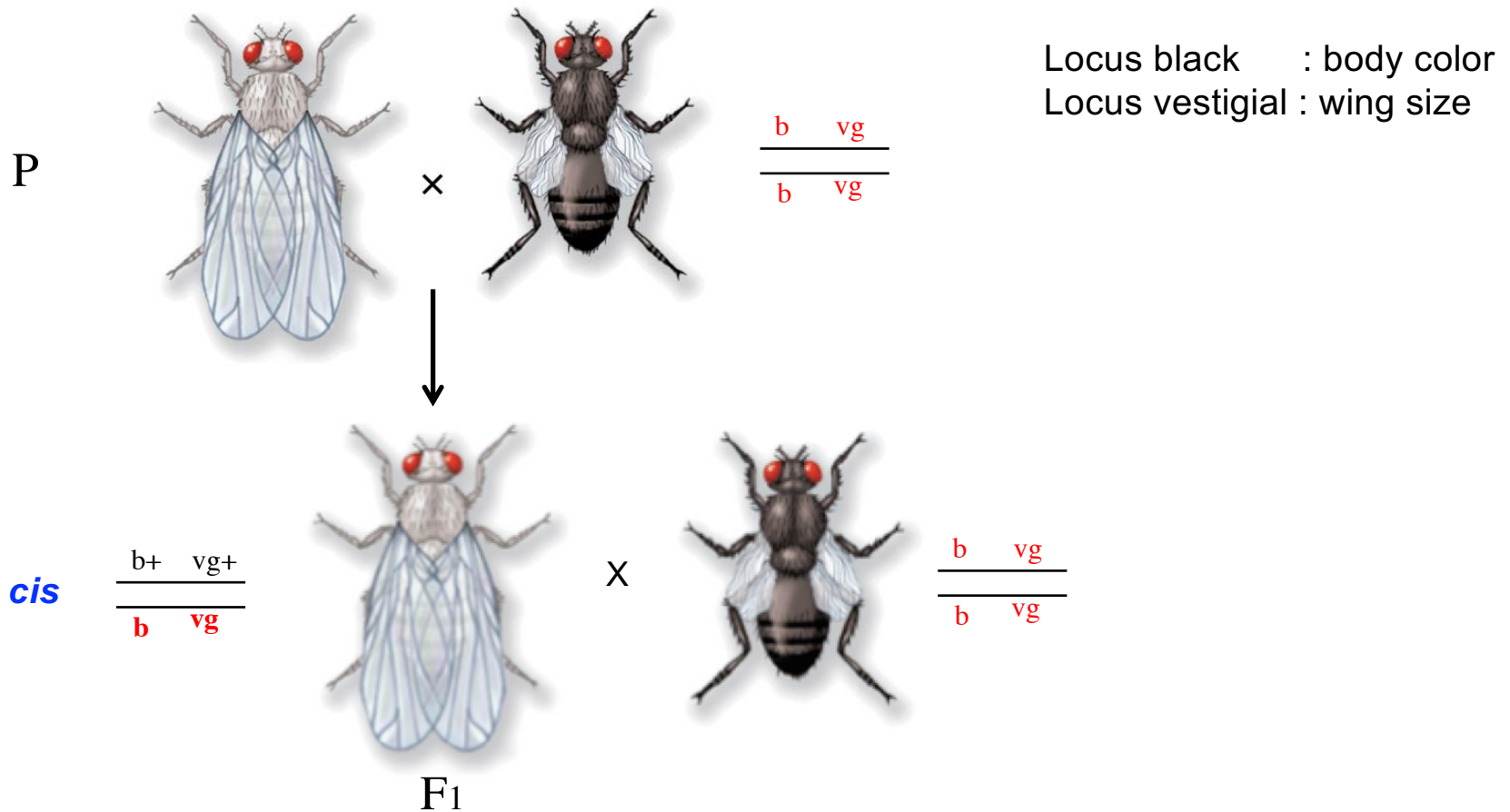
Question :
What is the **genetic distance** between the **black locus** and the **vestigial locus** ?



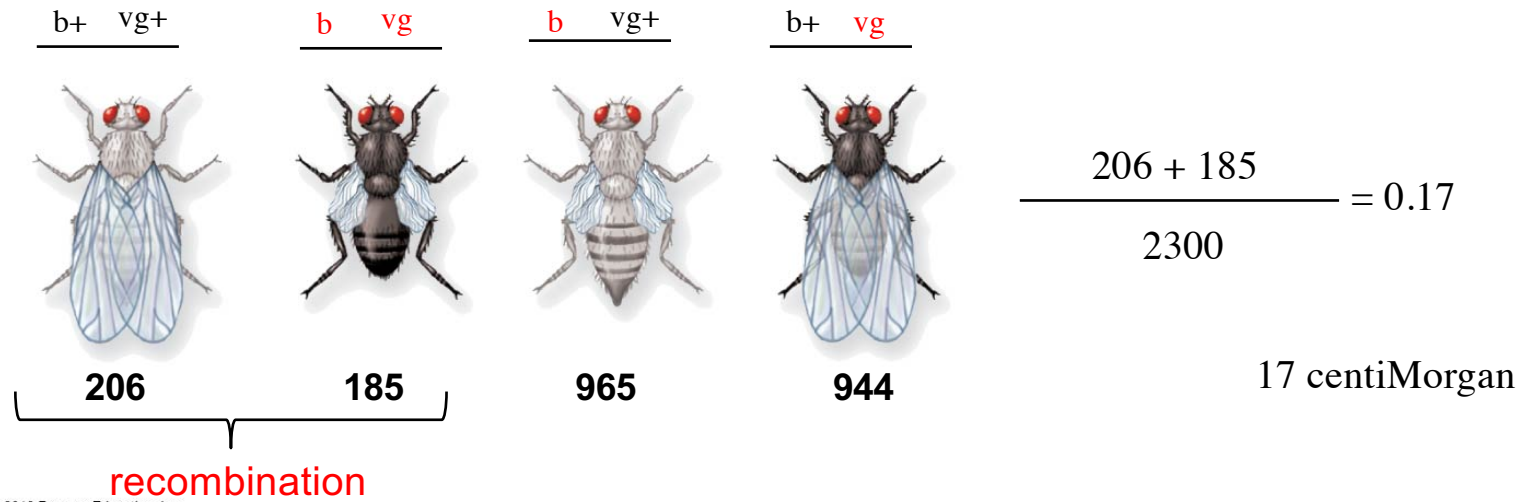
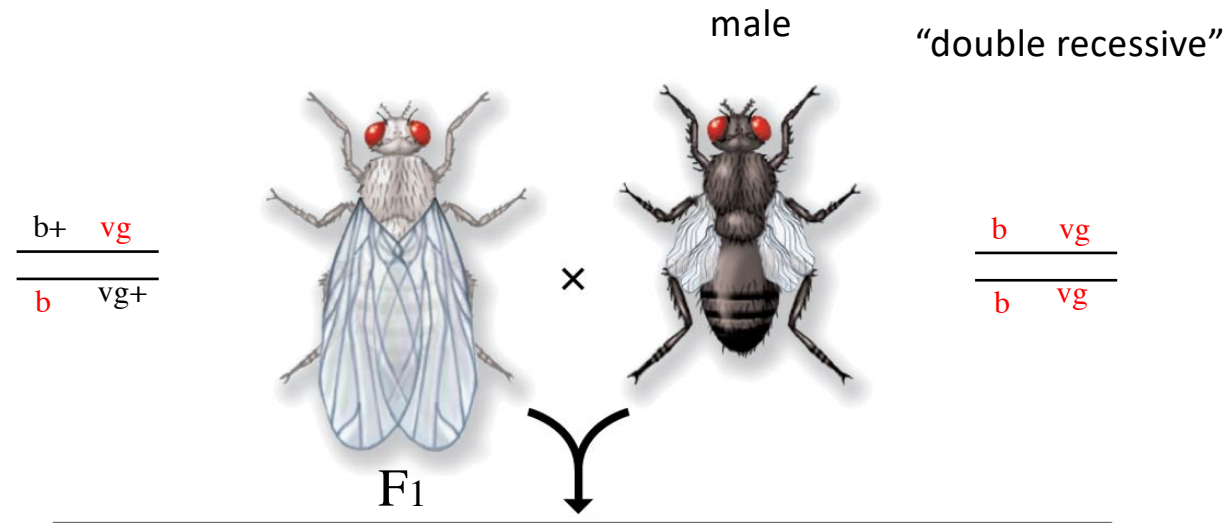
Measuring the genetic distance between 2 loci:



Measuring the genetic distance between 2 loci:

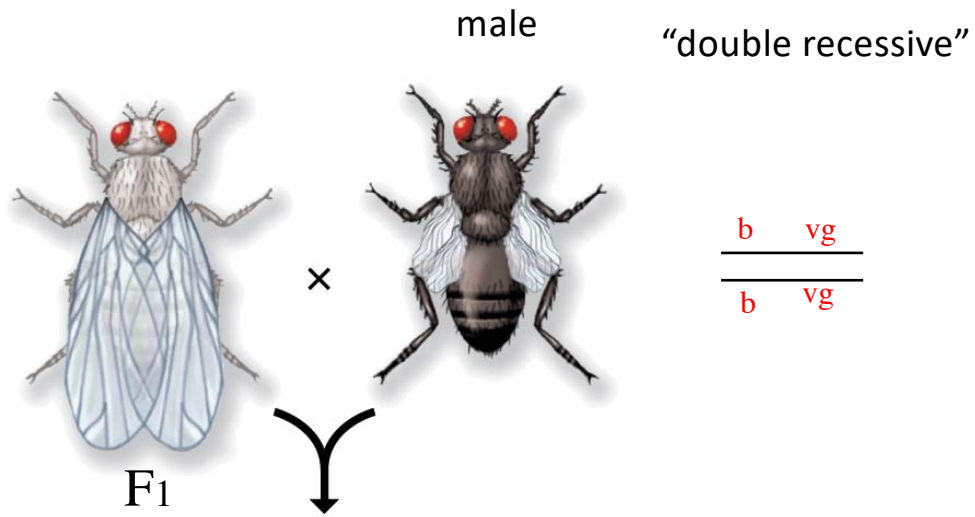


Mutations are in *trans*



Mutations are in *cis*

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



965



944



206



185

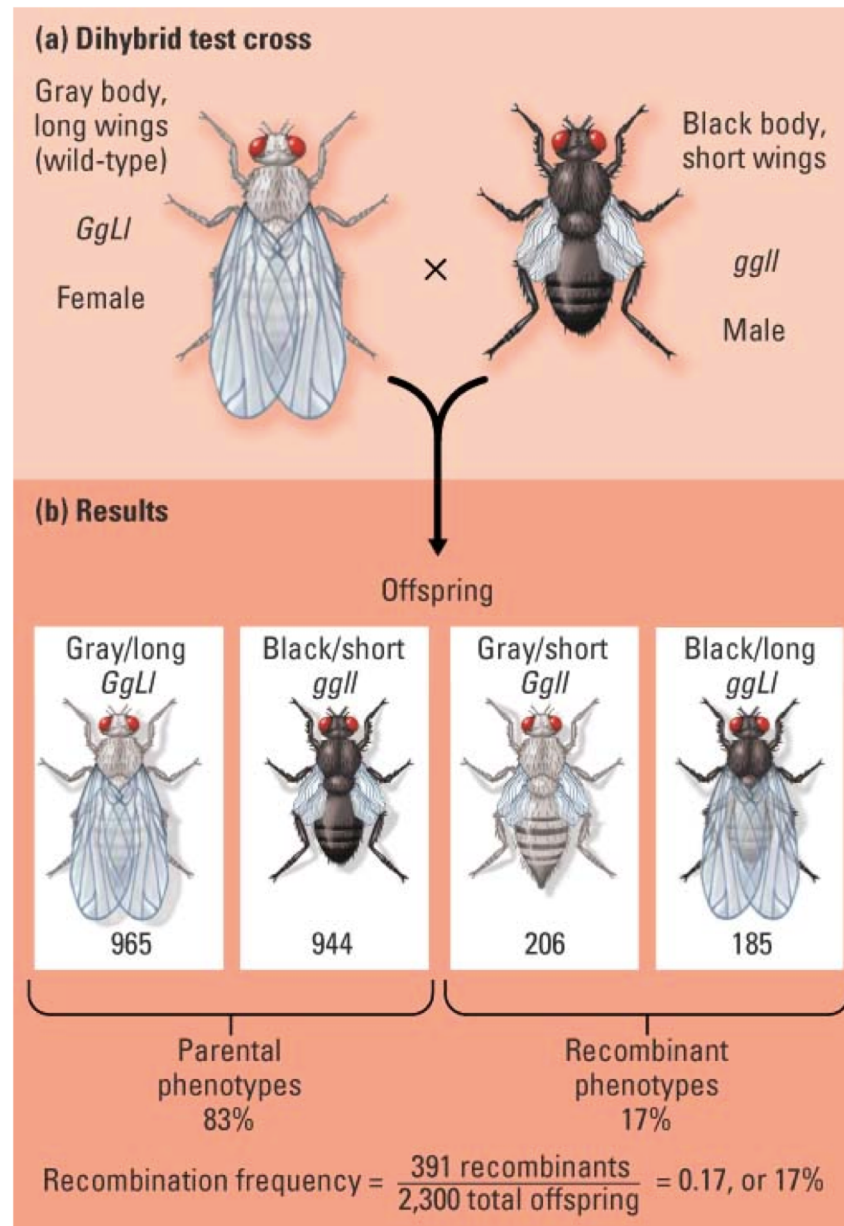
$$\frac{206 + 185}{2300} = 0.17$$

17 centiMorgan

recombination

In textbooks, the mutations are in *cis*.

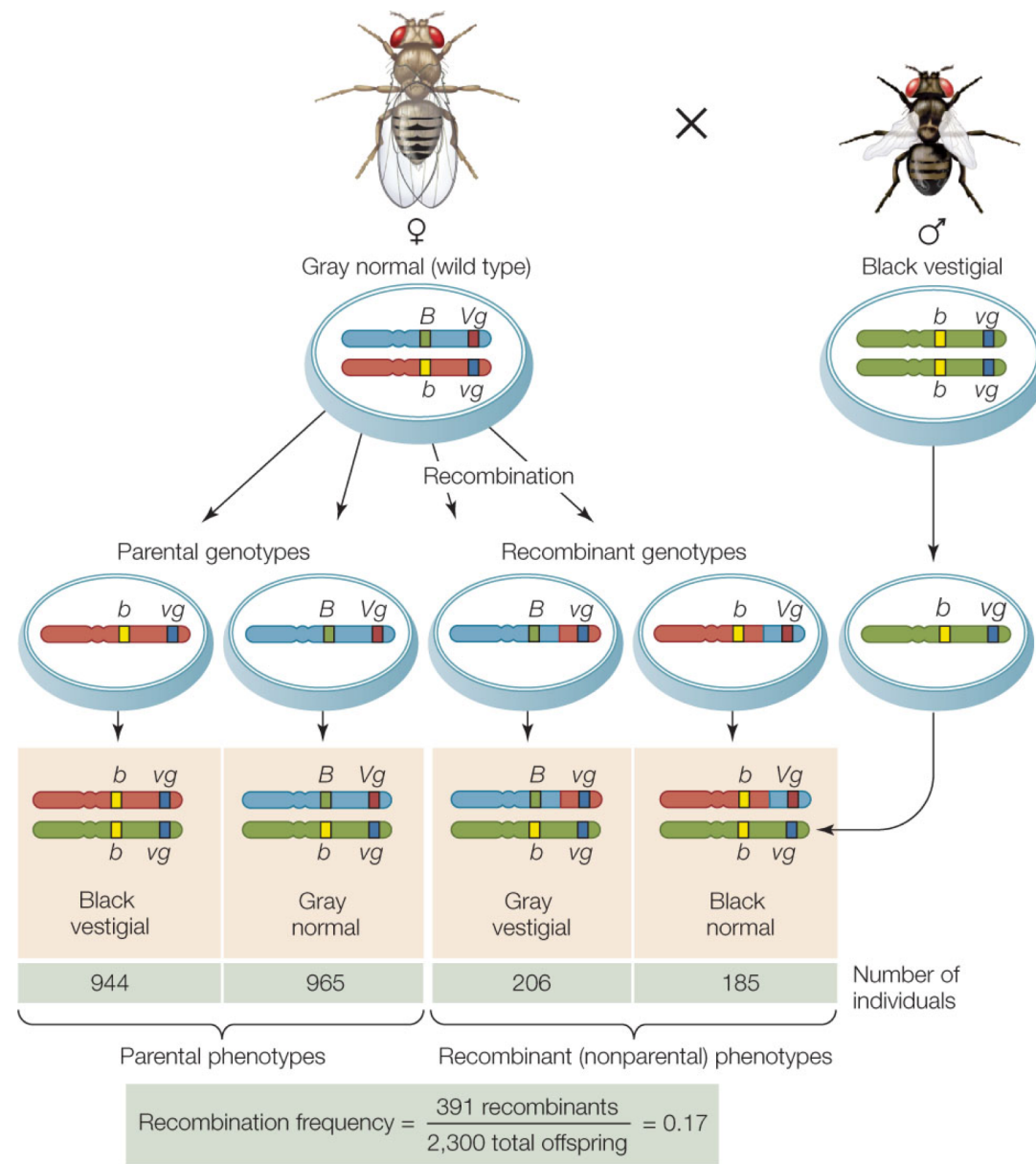
The case with mutations in *trans* is omitted.



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 :

$d < 50\text{cM}$ Detection of linkage

$d = 50\text{ cM}$ $\frac{25 + 25}{25 + 25 + 25 + 25} = 0.5$

The loci behave as if they were not linked

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