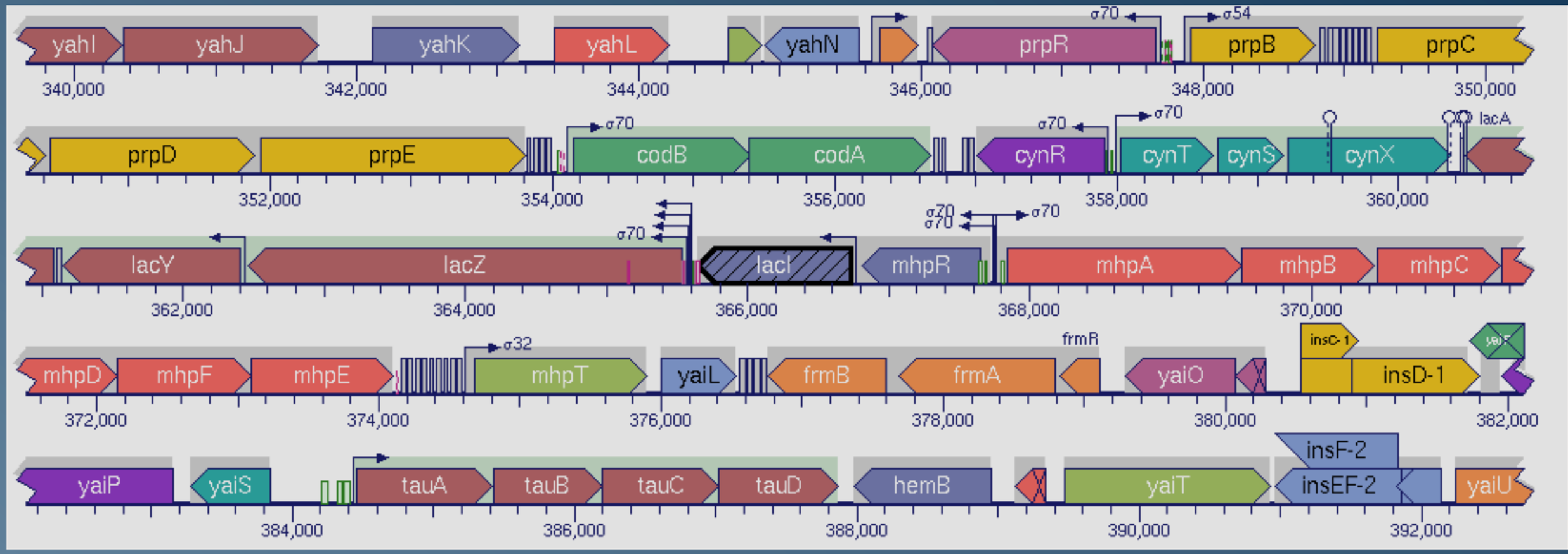
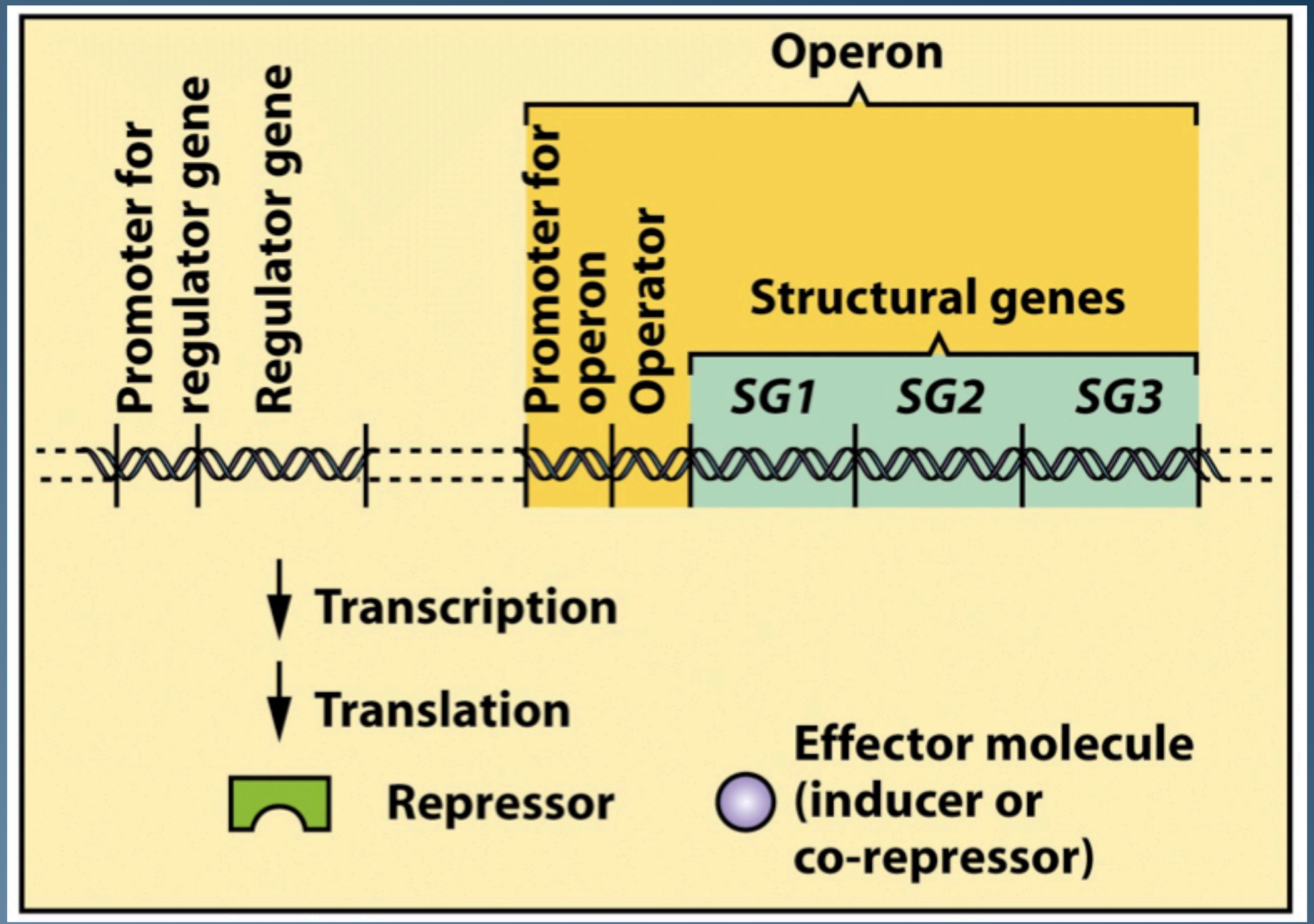
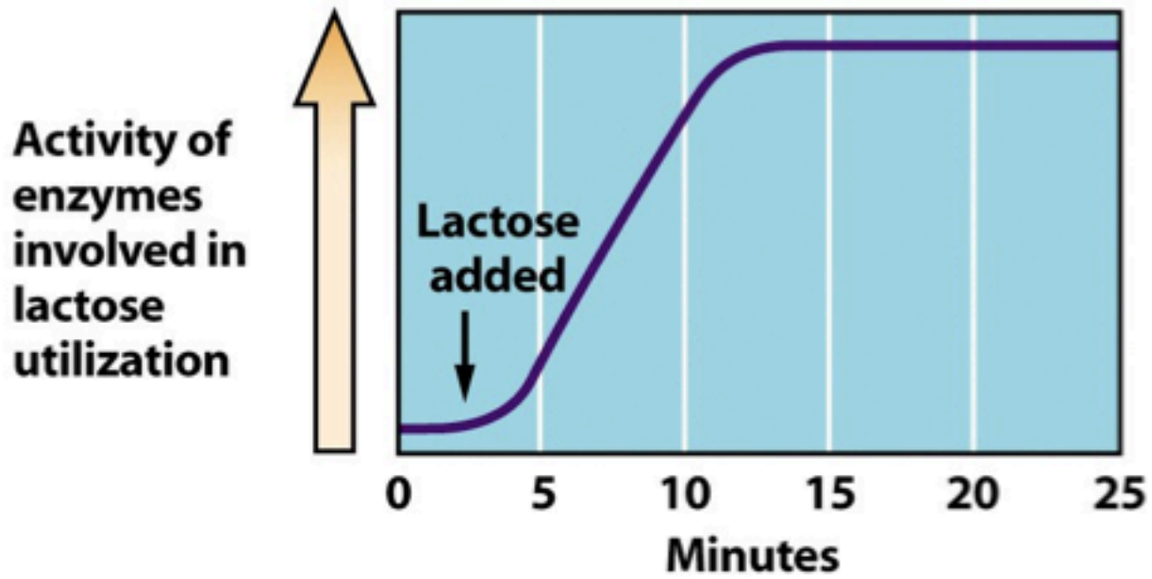
Mapa de una sección del genoma de la cepa K-12 MN1655 de *E. coli*

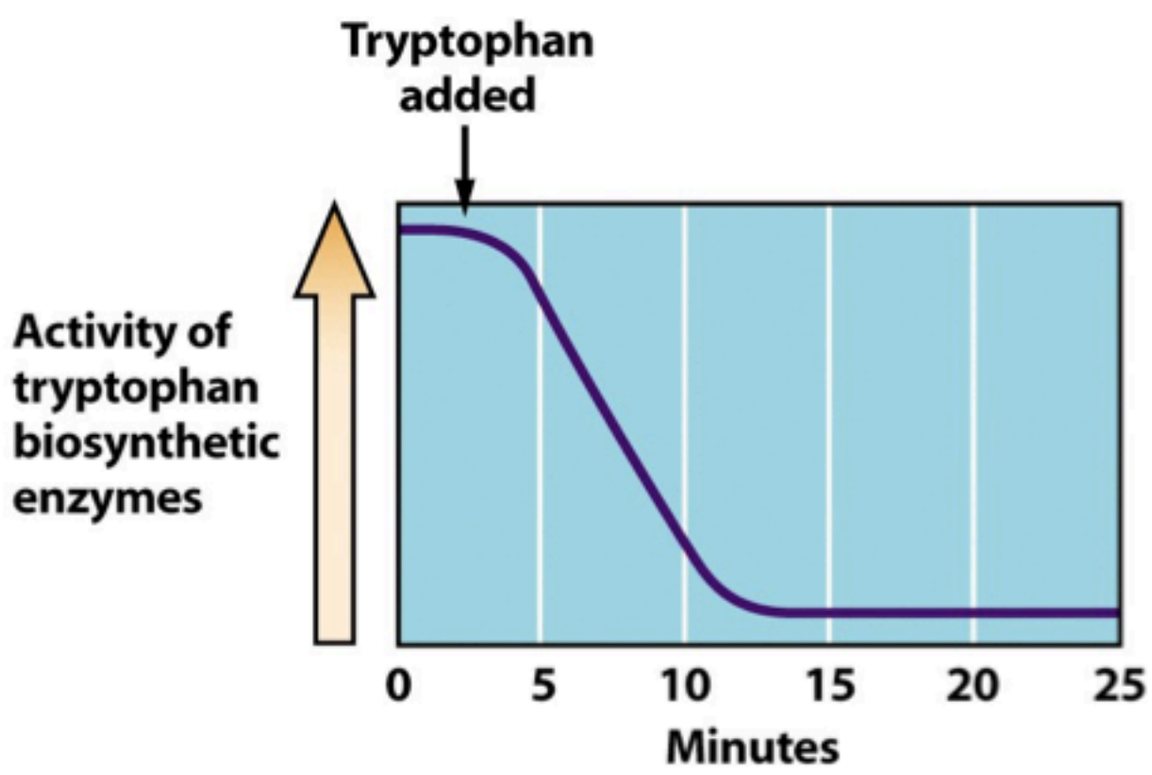


Estructura básica de un operón



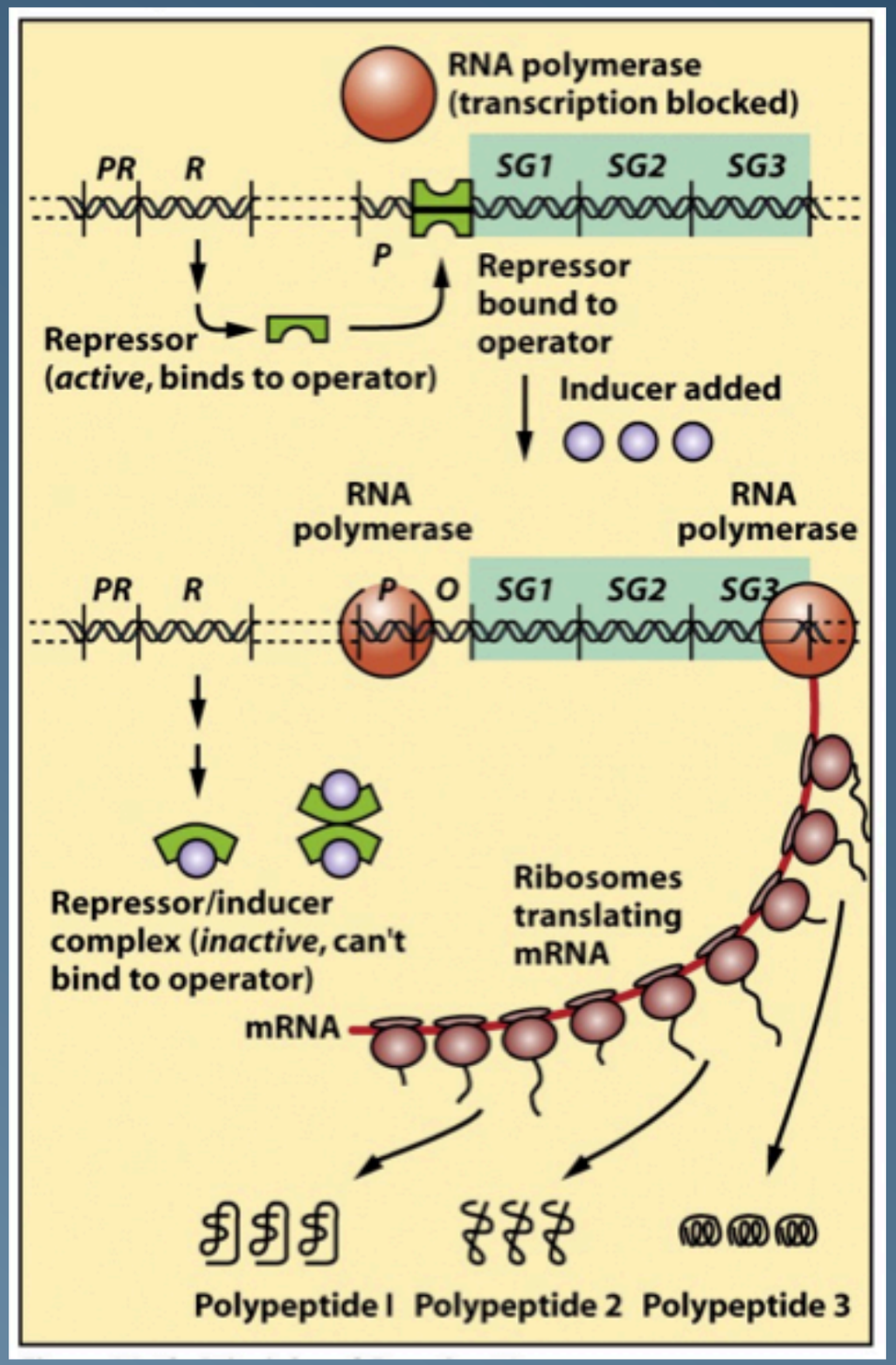


(a) **Induction of enzyme synthesis.**

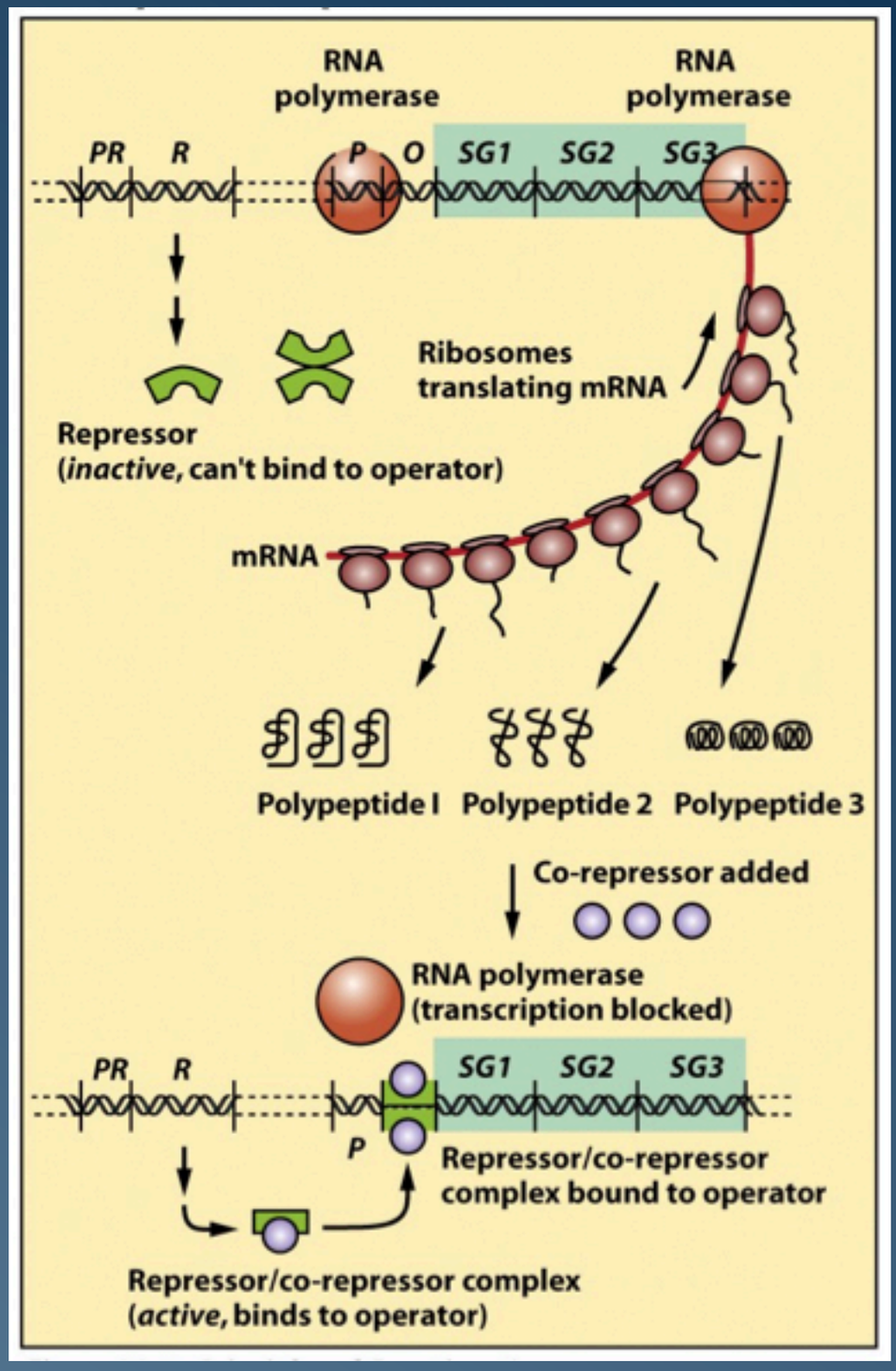


(b) **Repression of enzyme synthesis.**

Operón inducible



Operón reprimible



Sin lactosa

Gen del represor

Operón *lac*

P *lacI*

P *O* *lacZ* *lacY* *lacA*

LacI

El represor se une al operador
e impide la transcripción del operón

Con lactosa

Gen del represor

Operón *lac*

P *lacI*

P *O* *lacZ* *lacY* *lacA*

Alolactosa

LacI

Lactosa

β-galactosidasa

Permeasa

Transacetilasa

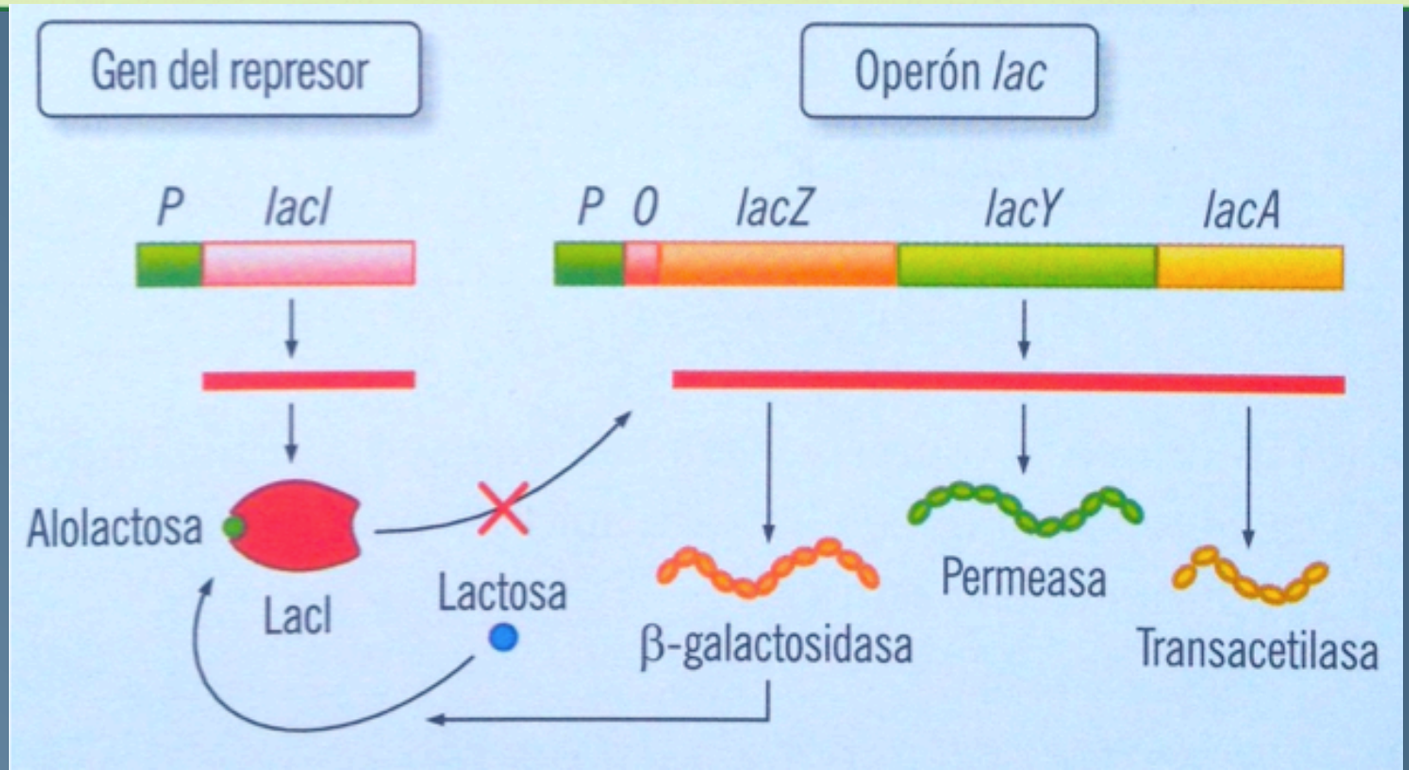
La alolactosa se une al represor, cambia su conformación
e impide su unión al operador. El operón se transcribe

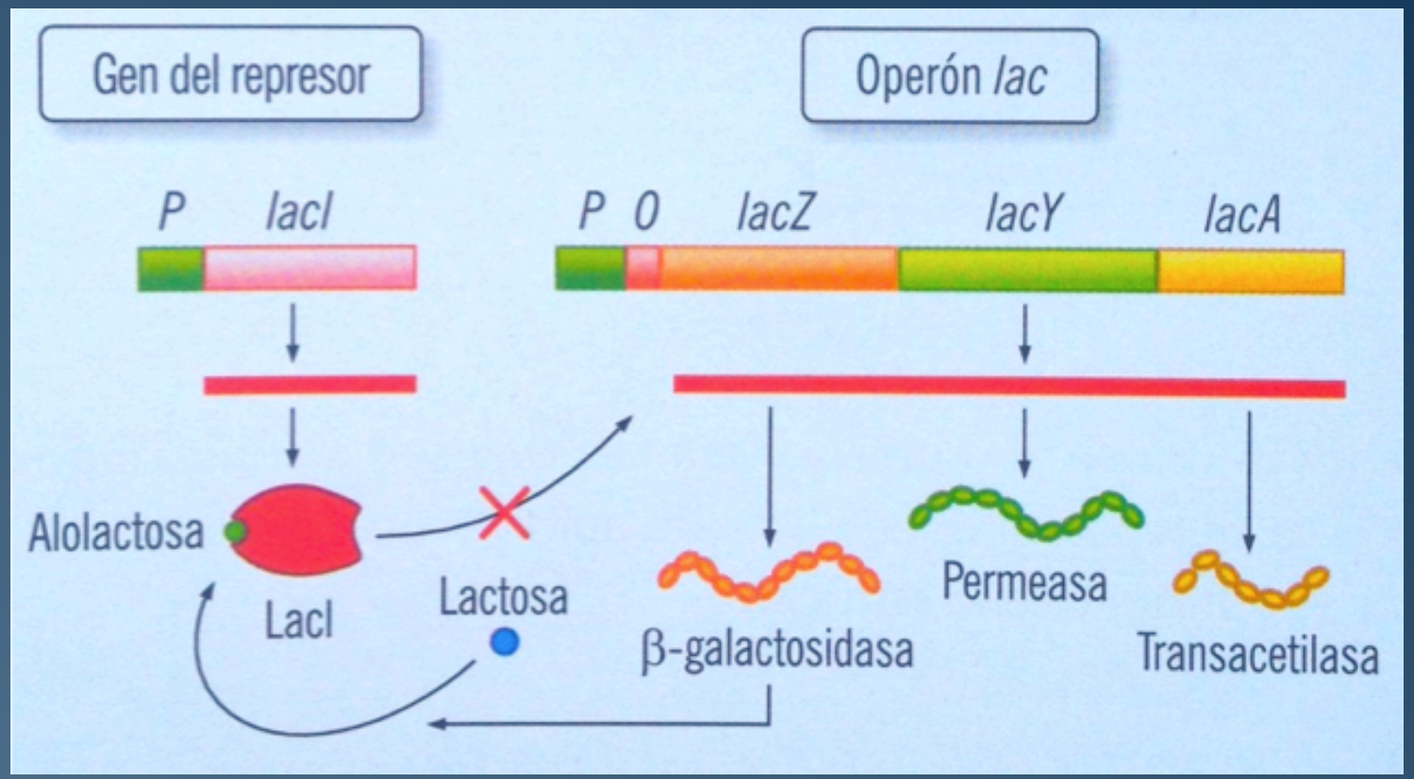
Phenotypic Effects of Mutations in the Repressor Gene (*I*) and the Operator (*O*) Region of the *lac* Operon

Genotype	β-Galactosidase Activity[a]		β-Galactoside Permease Activity[a]		Deduction
	With Lactose	Without Lactose	With Lactose	Without Lactose	
$I^+P^+O^+Z^+Y^+$	100 units	1 unit	100 units	1 unit	Wild-type is inducible
$I^+P^+O^+Z^+Y^+/F'\ I^+P^+O^+Z^-Y^-$	100 units	1 unit	100 units	1 unit	Z^+ is dominant to Z^- Y^+ is dominant to Y^-
$I^+P^+O^+Z^+Y^+/F'\ I^+P^+O^+Z^+Y^+$	200 units	2 units	200 units	2 units	Activity depends on gene dosage
$I^-P^+O^+\ Z^+Y^+$	100 units	100 units	100 units	100 units	*lacI*$^-$ mutants are constitutive
$I^+P^+O^+\ Z^+Y^+/F'\ I^-P^+O^+\ Z^+Y^+$	200 units	2 units	200 units	2 units	I^+ is dominant to I^-
$I^+P^+O^c Z^+Y^+$	100 units	100 units	100 units	100 units	*lacO*^{c} mutants are constitutive
$I^+P^+O^cZ^+Y^-/F'\ I^+P^+O^+Z^-Y^+$	100 units	100 units	100 units	1 unit	O^c and O^+ are *cis*-acting regulators

The *lac* Repressor Gene (I) Acts both *Cis* and *Trans*; the *lac* Operator Acts only in the *Cis* Configuration

Genotype	β-Galactosidase Activity[a]		β-Galactoside Permease Activity[a]		Deduction
	With Lactose	Without Lactose	With Lactose	Without Lactose	
$I^+P^+O^+Z^+Y^+$	100 units	1 unit	100 units	1 unit	Wild-type is inducible
$I^+P^+O^+\ Z^+Y^+/F'\ I^-P^+O^+\ Z^-Y^-$	100 units	1 unit	100 units	1 unit	
$I^-P^+O^+Z^+Y^+/F'\ I^+P^+O^+Z^-Y^-$	100 units	1 unit	100 units	1 unit	I^+ acts both *cis* and *trans*
$I^+P^+O^+Z^+Y^+/F'\ I^+P^+O^cZ^-Y^-$	100 units	1 unit	100 units	1 unit	O^+ acts only in *cis*
$I^+P^+O^+Z^-Y^-/F'\ I^+P^+O^cZ^+Y^+$	100 units	100 units	100 units	100 units	O^c acts only in *cis*





.- El mapa del operón ***Lac*** de *E. coli* es: ***I P O Z Y***

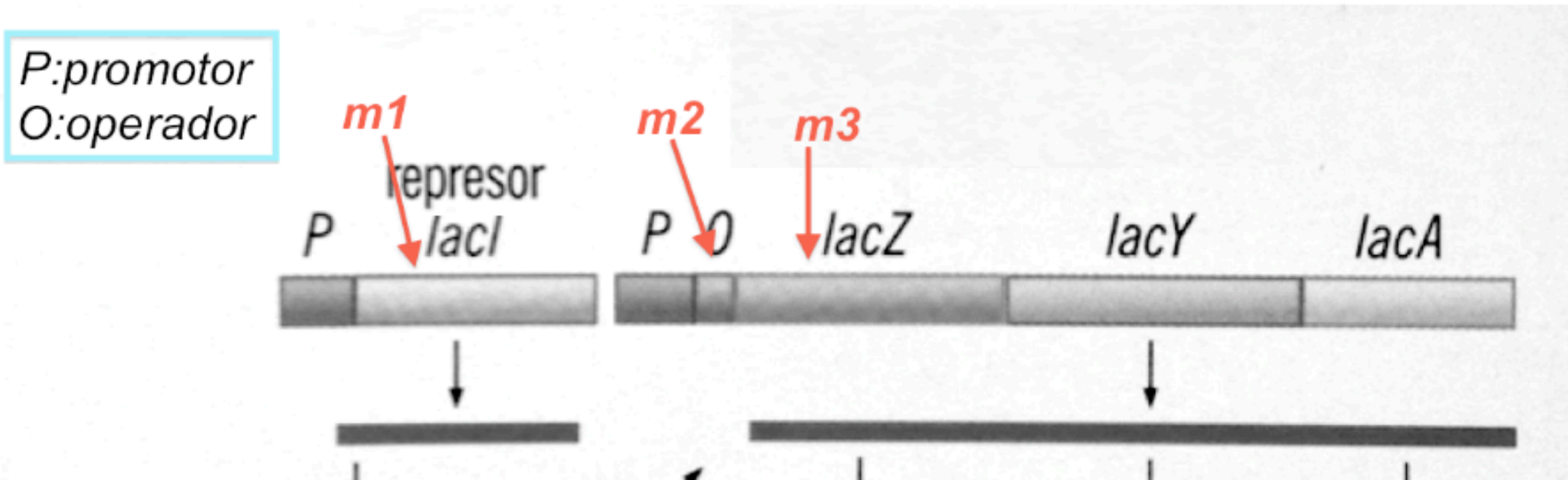
Utilice sus conocimientos sobre el operón lactosa para completar la tabla siguiente. Escriba + donde se produzca enzima y – en el caso contrario.

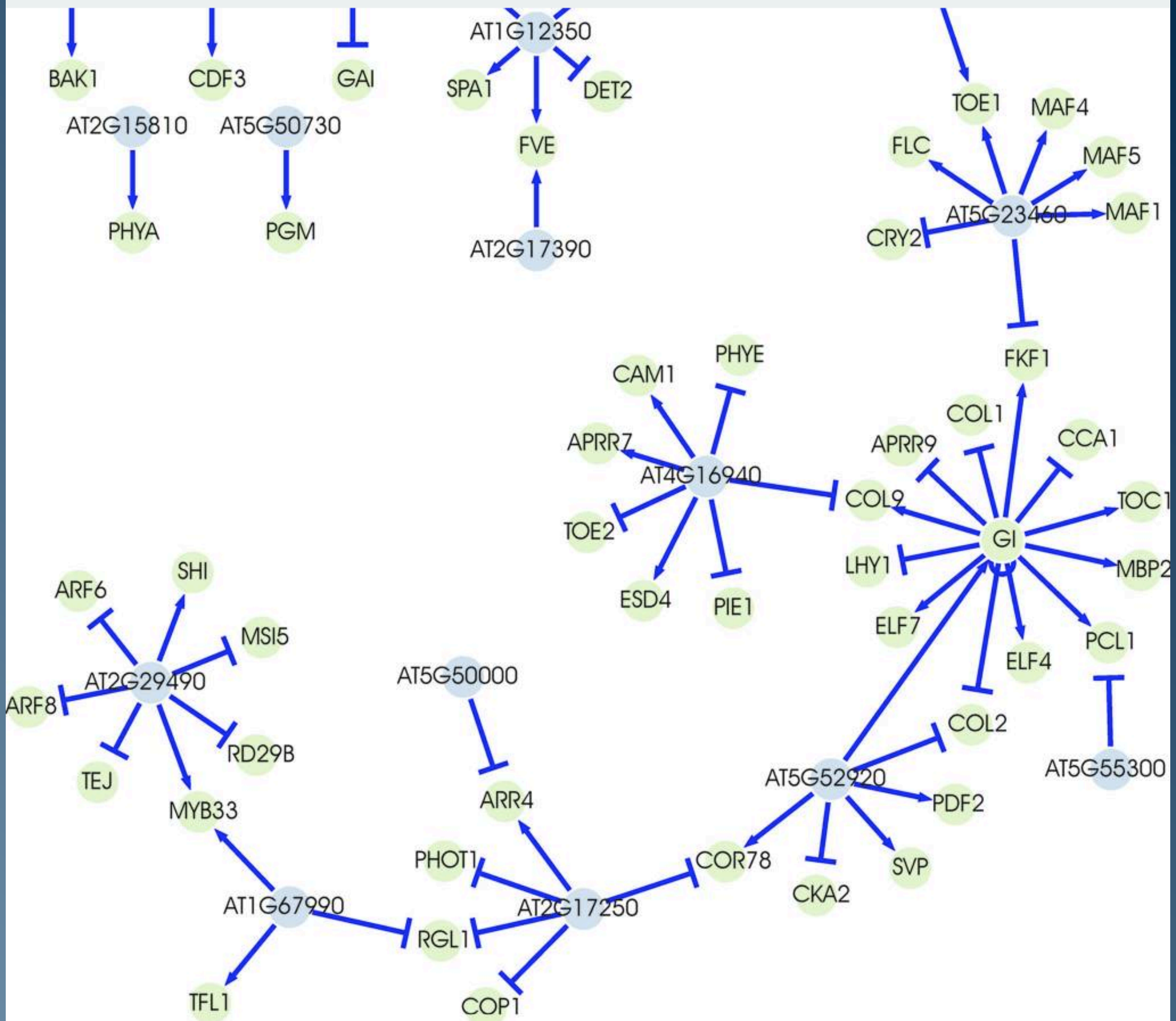
genotipo	β galactosidasa		permeasa	
	sin lactosa	con lactosa	sin lactosa	con lactosa
$I^+P^+O^+Z^+Y^+$ / F' $I^+P^+O^+Z^+Y^+$	-	+	-	+
$IP^+O^cZ^+Y^-$ / F' $I^+P^+O^+Z^-Y^+$				
$I^+P^-O^cZ^-Y^+$ / F' $IP^+O^cZ^+Y^-$				
$I^sP^+O^+Z^+Y^-$ / F' $I^+P^+O^+Z^-Y^+$				
$I^sP^+O^+Z^+Y^+$ / F' $IP^+O^+Z^+Y^+$				
$IP^+O^cZ^+Y^-$ / F' $IP^+O^+Z^-Y^+$				
$IP^-O^+Z^+Y^+$ / F' $IP^+O^cZ^+Y^-$				
$I^+P^+O^+Z^-Y^+$ / F' $IP^+O^+Z^+Y^-$				

2/3- Se dispone de tres cepas de *E. coli* (*m1, m2 y m3*); cada una de las cuales lleva una sola mutación puntual que afecta a un único gen o región reguladora del operón lactosa. Las cepas *m1* y *m2* son mutantes constitutivos (han perdido la capacidad de regular la expresión del gen de la β-galactosidasa) mientras que la cepa *m3* no tiene actividad β-galactosidasa. A partir de estas cepas se han construido otras cepas diploides parciales que poseen dos copias del operón lactosa: una en el cromosoma bacteriano y otra en un factor *F'*. La siguiente tabla da los fenotipos en presencia y ausencia de lactosa en el medio (+ significa actividad del enzima β-galactosidasa) para las distintas cepas, así como las procedencias de las dos copias en los diploides parciales:

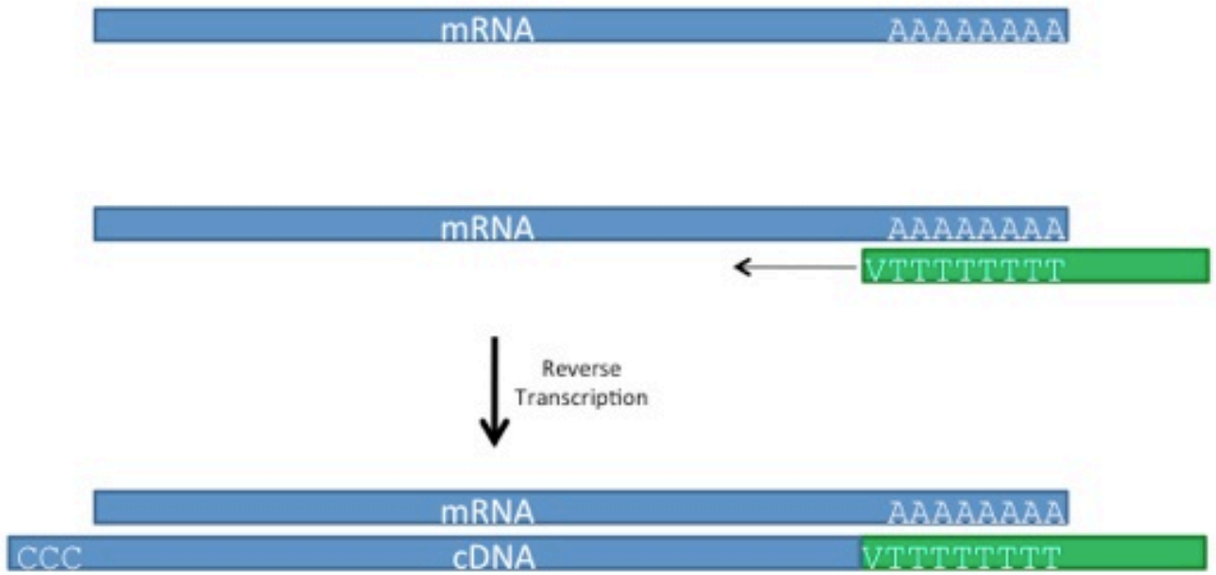
	Actividad β-galactosidasa	
Cepa:	con lactosa	sin lactosa
m1	+	+
m2	+	+
m3	-	-
m1/F'm2	+	+
m1/F'm3	+	-
m2/F'm3	+	+

Señale en el esquema una posible localización de cada una de las tres mutaciones. **(2,5 puntos)**

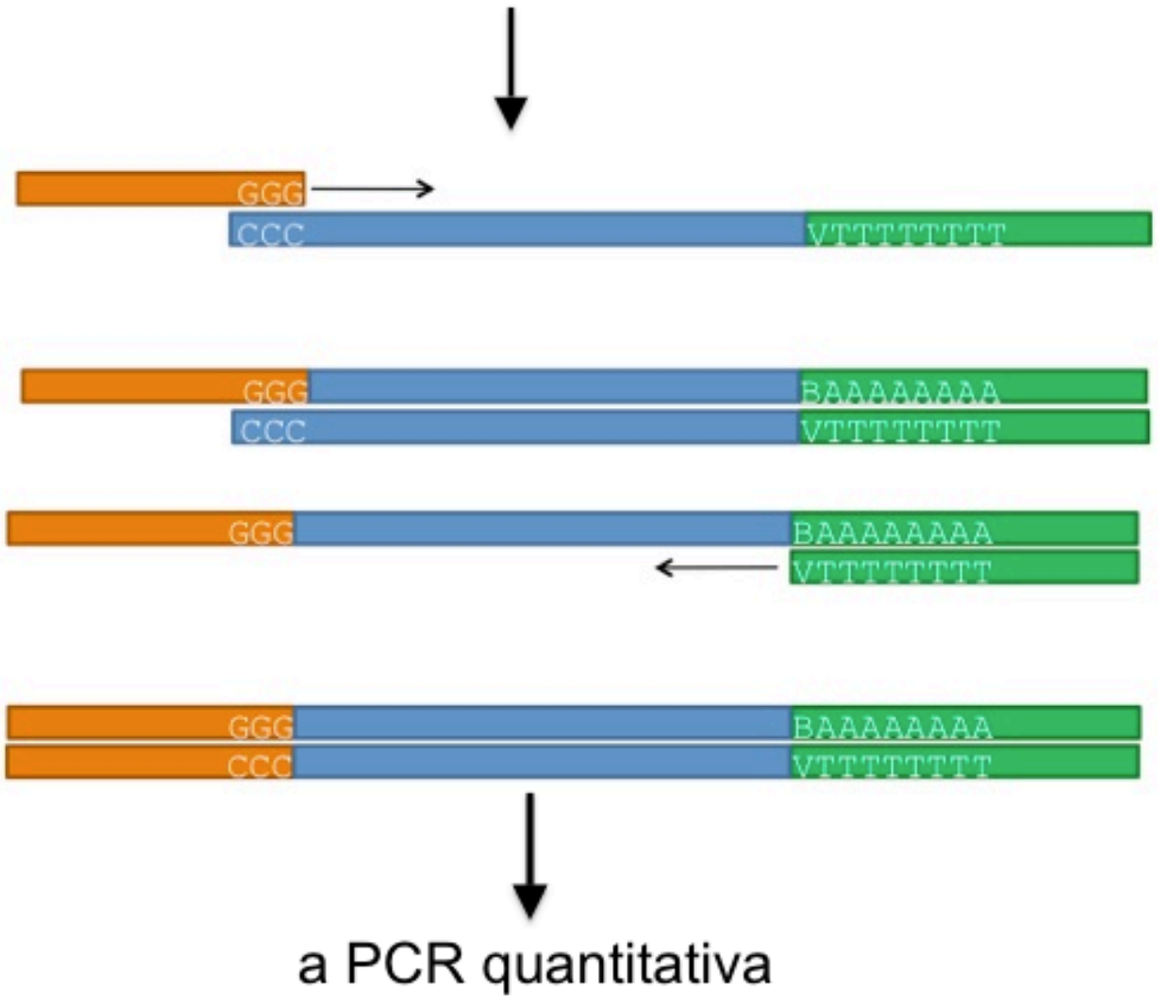



BAK1
CDF3
GAI
AT2G15810
AT5G50730
PHYA
PGM
AT1G12350
SPA1
DET2
FVE
AT2G17390
TOE1
MAF4
FLC
MAF5
AT5G23460
MAF1
CRY2
FKF1
PHYE
CAM1
APRR7
AT4G16940
COL9
TOE2
ESD4
PIE1
COL1
APRR9
CCA1
TOC1
GI
LHY1
MBP2
ELF7
ELF4
PCL1
AT5G55300
COL2
AT5G52920
PDF2
SVP
CKA2
COR78
SHI
ARF6
MSI5
AT2G29490
ARF8
TEJ
RD29B
MYB33
TFL1
AT5G50000
ARR4
PHOT1
AT2G17250
RGL1
COP1
AT1G67990

First strand synthesis



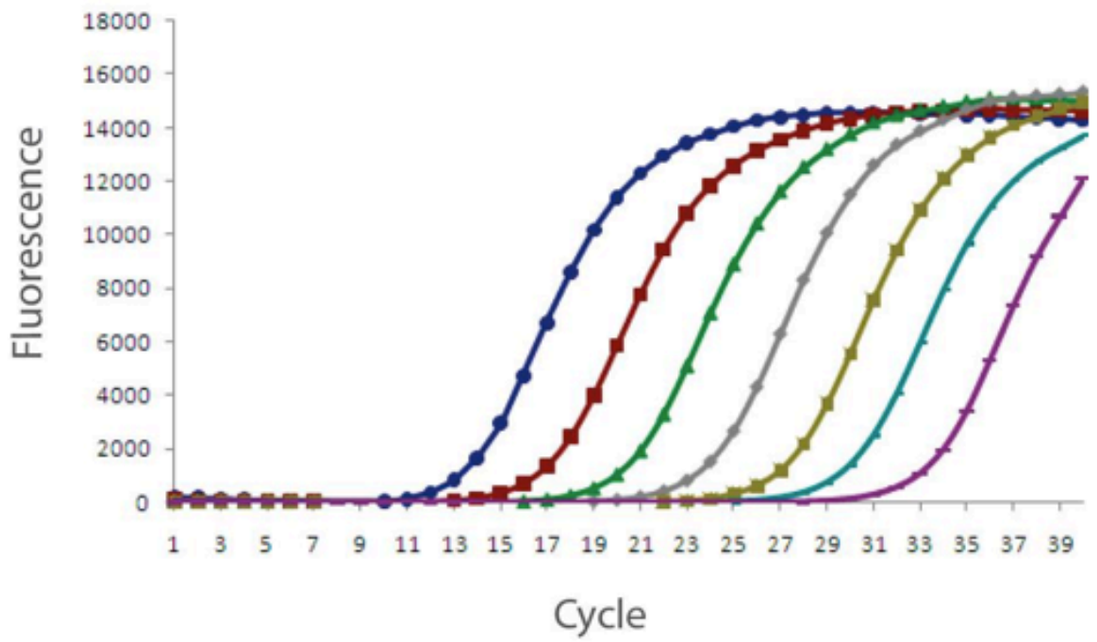
Reverse Transcriptase adds cytosines when it reaches the 5' end of the template RNA



a PCR quantitativa

Medida del nivel de expresión de un gen

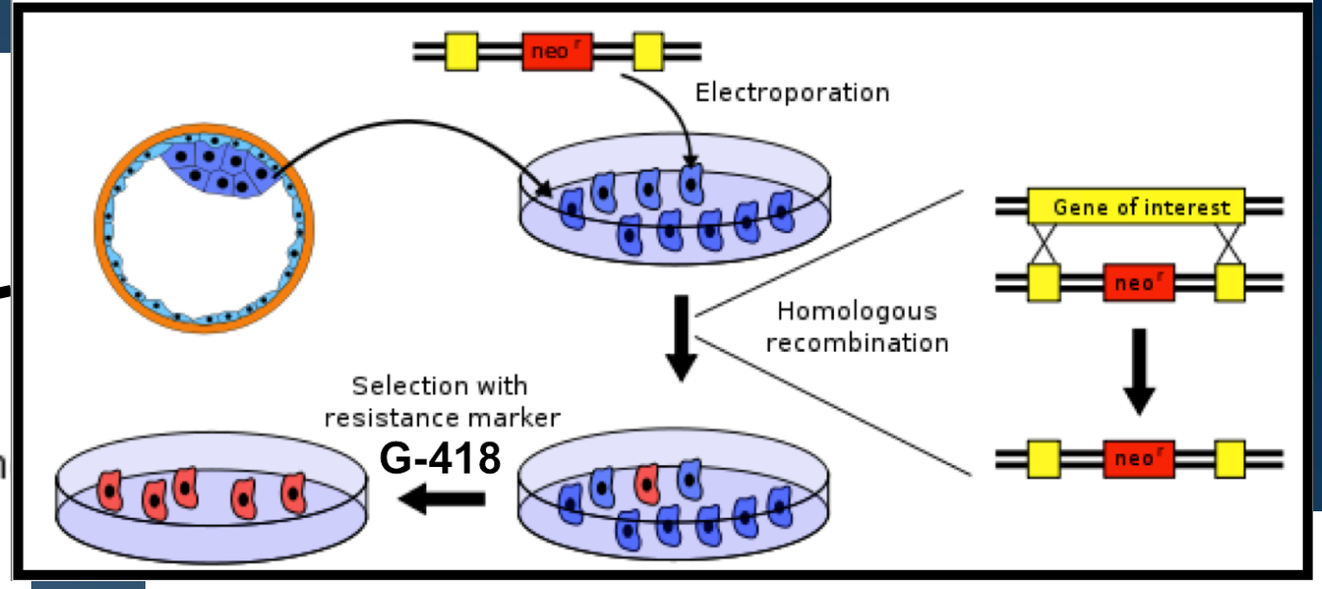
PCR cuantitativa

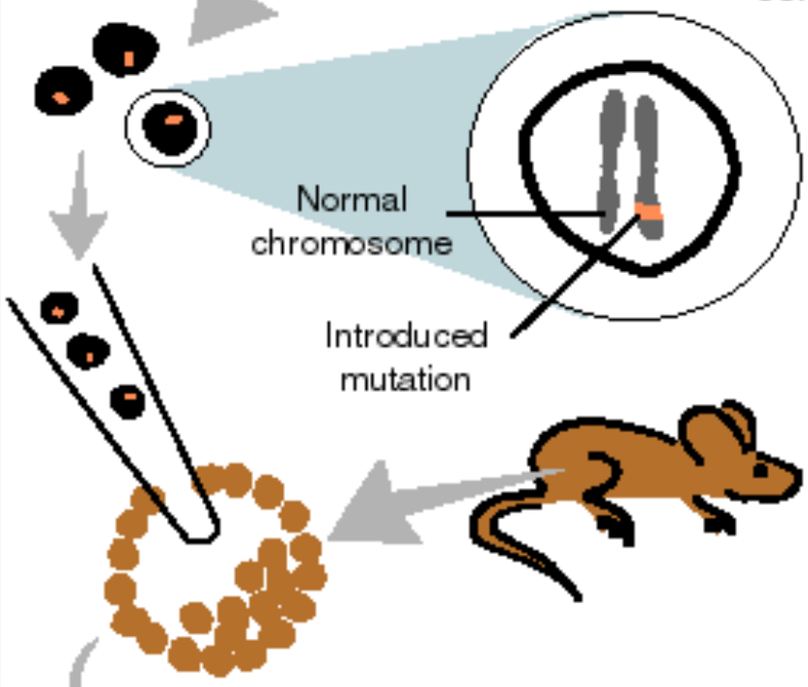


Engineering a Knock-out Mouse

Embryonic Stem (ES) cells from black mouse

Introduce DNA containing mutant gen

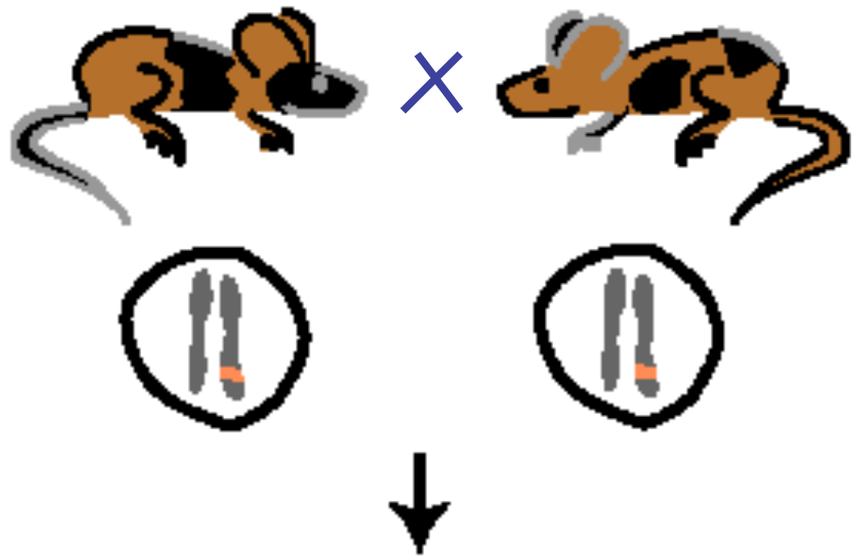




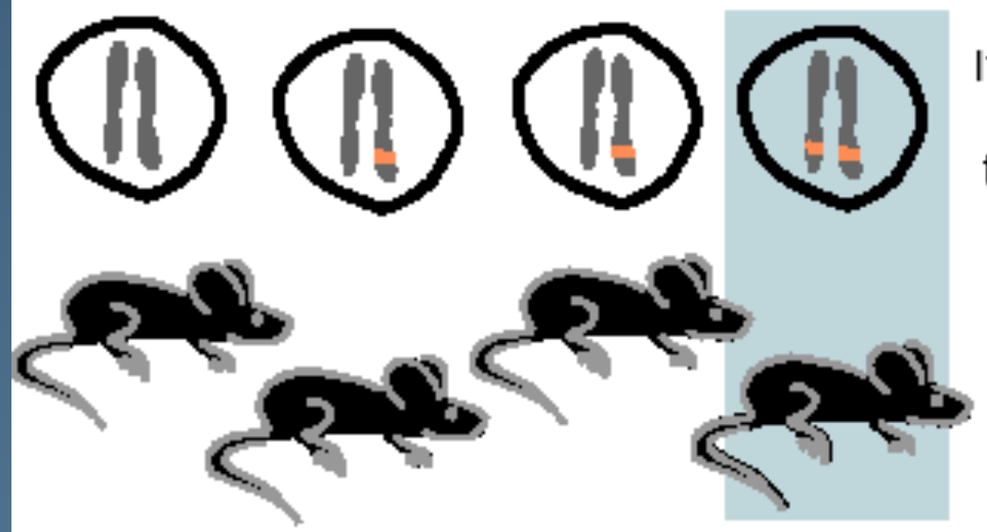
Inject altered ES cells into blastocyst stage of embryo from brown mouse

Implant embryo into surrogate mother mouse

Surrogate mother gives birth to chimeric pup; this pup has cells from the black strain and cells from the brown strain



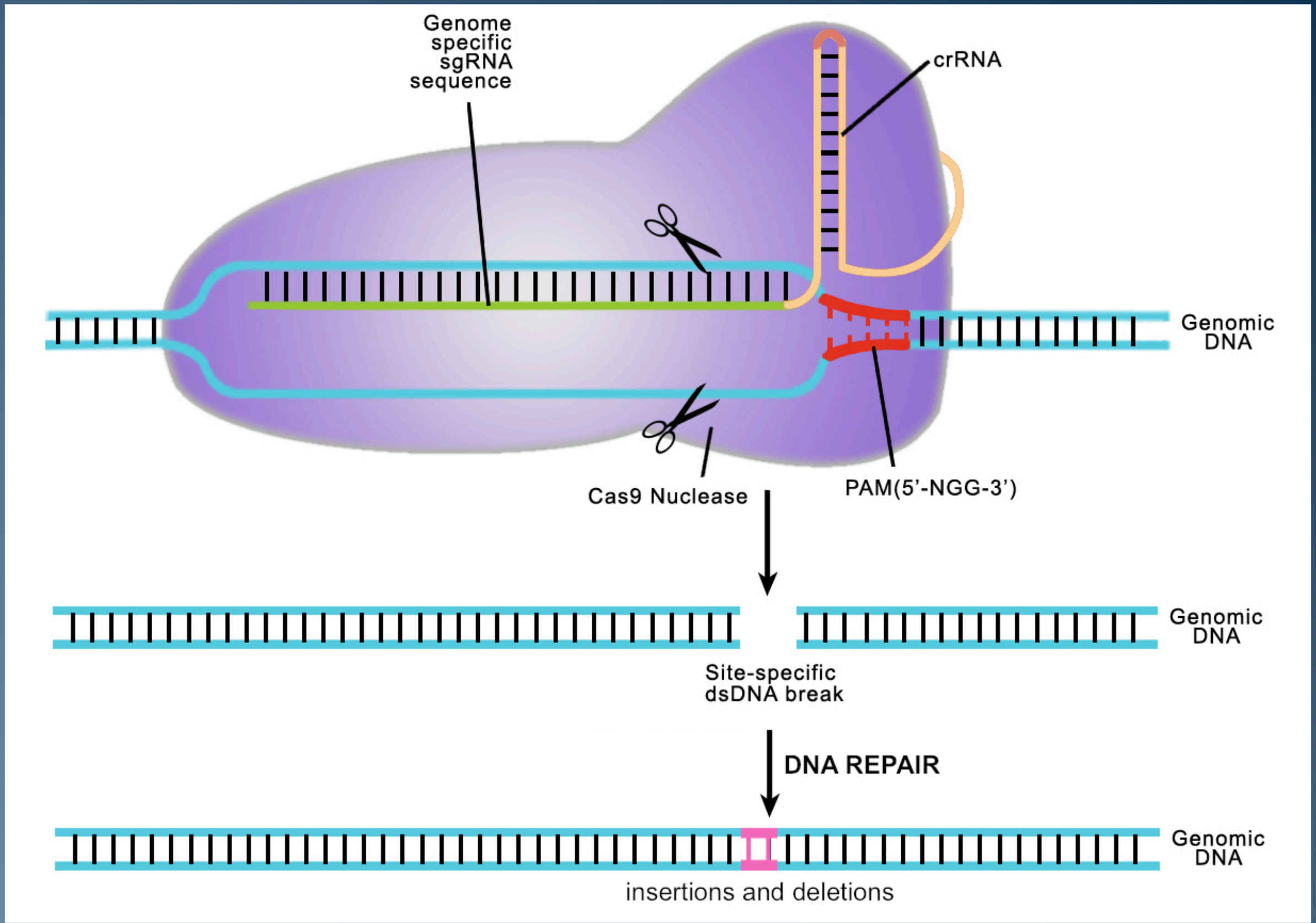
Mate male and female chimeras, each having one mutant gene in black cells



If each parent's gonad carries the introduced mutation, these are the possible offspring

Knock-out mouse (both copies of gene are mutant; no good copy of gene is present)

Sistema CRISPR-Cas9

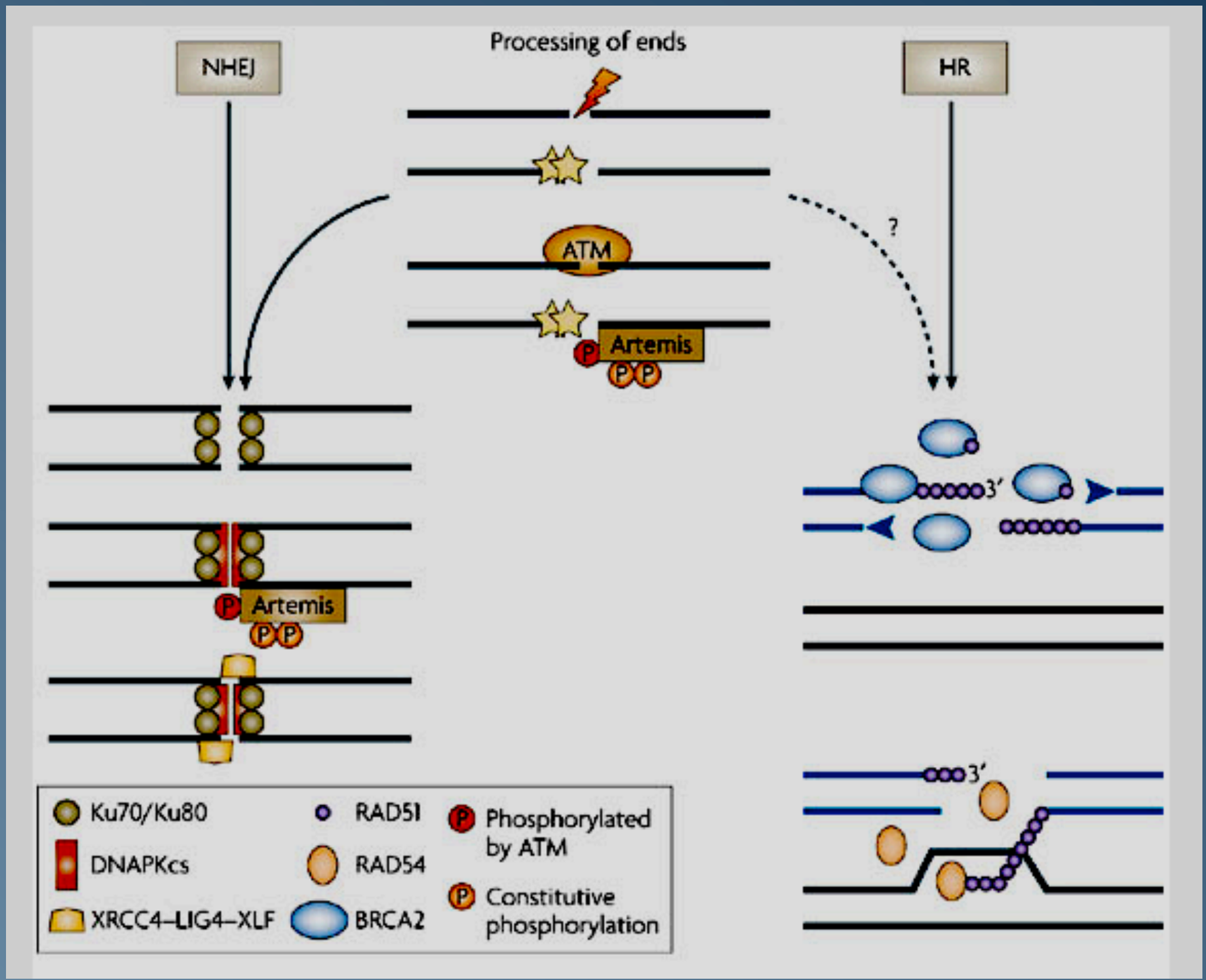


Reparación de roturas de doble cadena

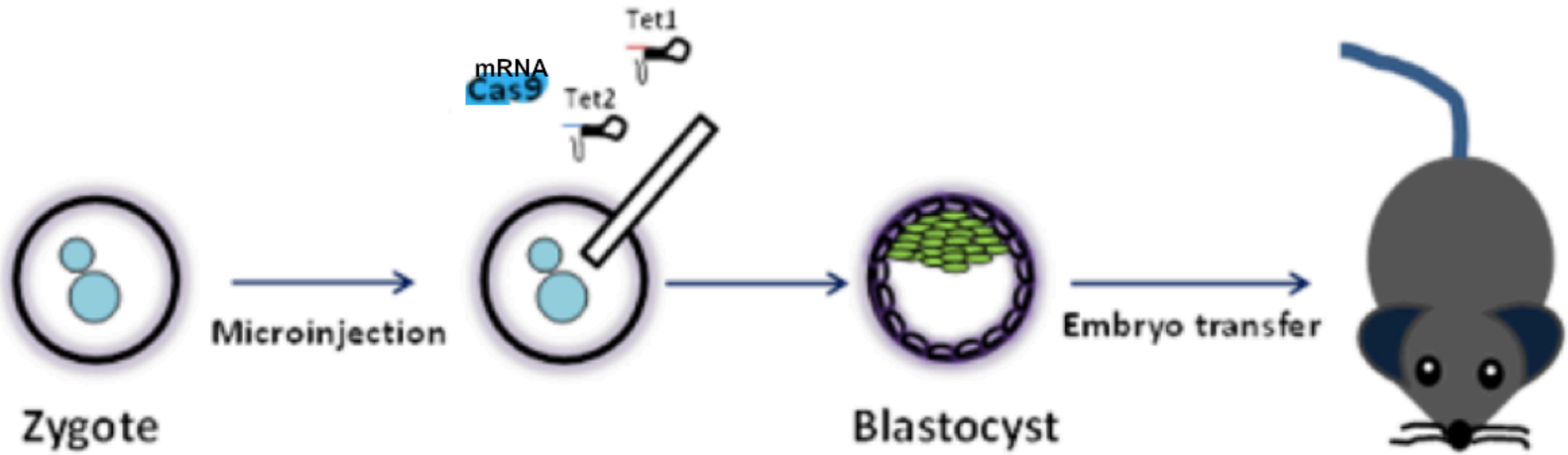
Unión de sitios no homólogos.

Suele llevar a deleciones e inserciones

Recombinación homóloga

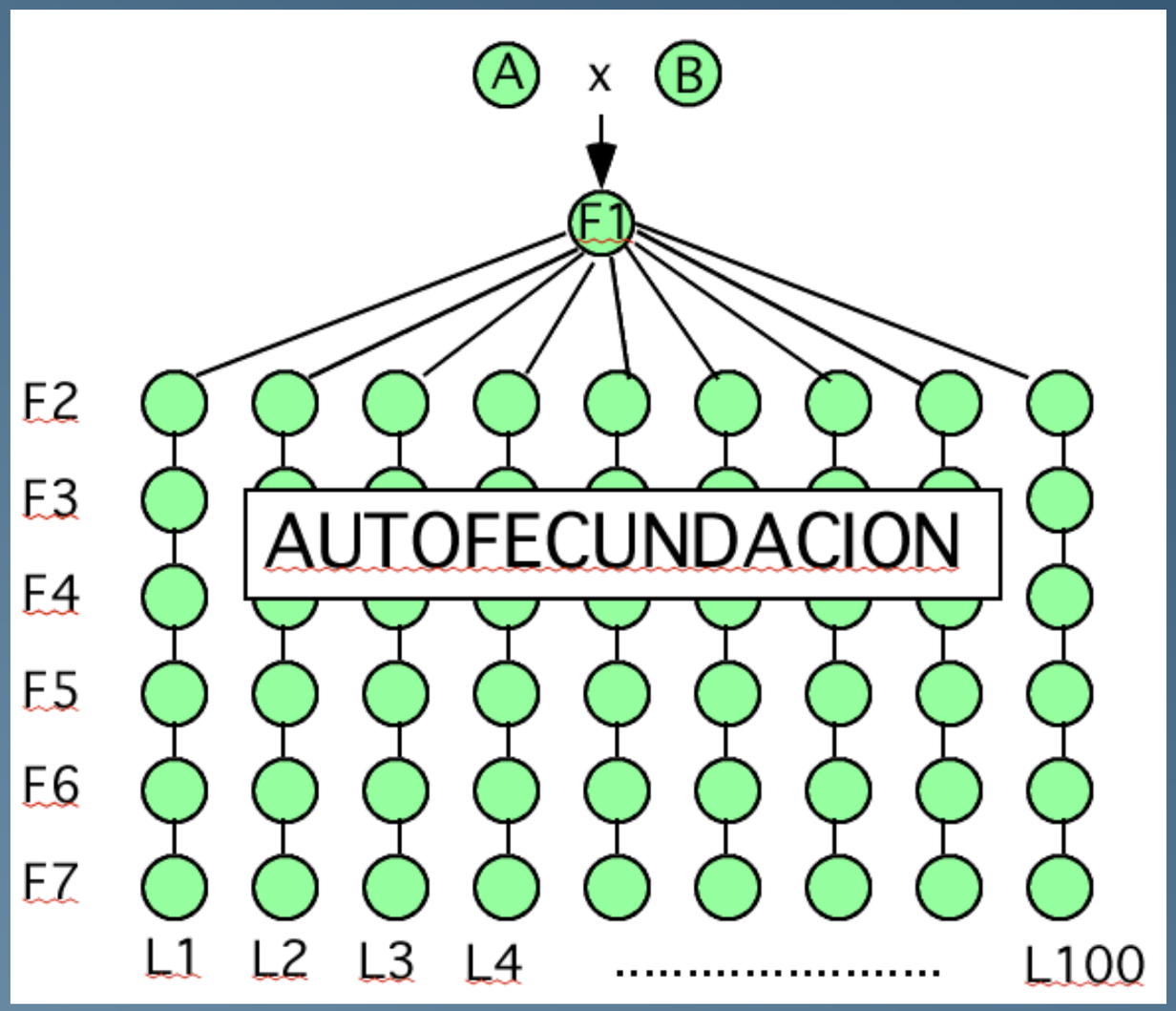


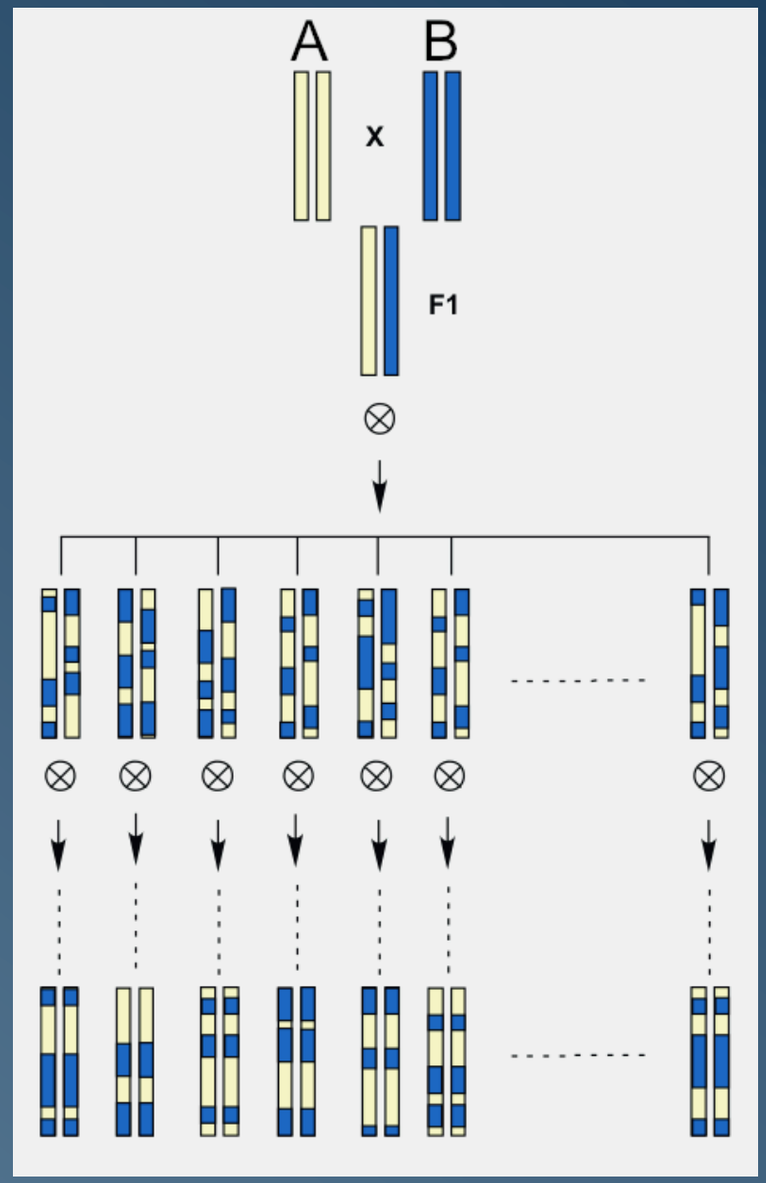
Producción de knockouts mediante CRISPR-Cas9



Obtención de líneas (individuos) para análisis de QTLs (Quantitative Trait Locus)

- Las líneas pueden ser líneas endogámicas (preferentemente en vegetales). Un método de obtención son las RILs (Recombinant Inbred Lines)



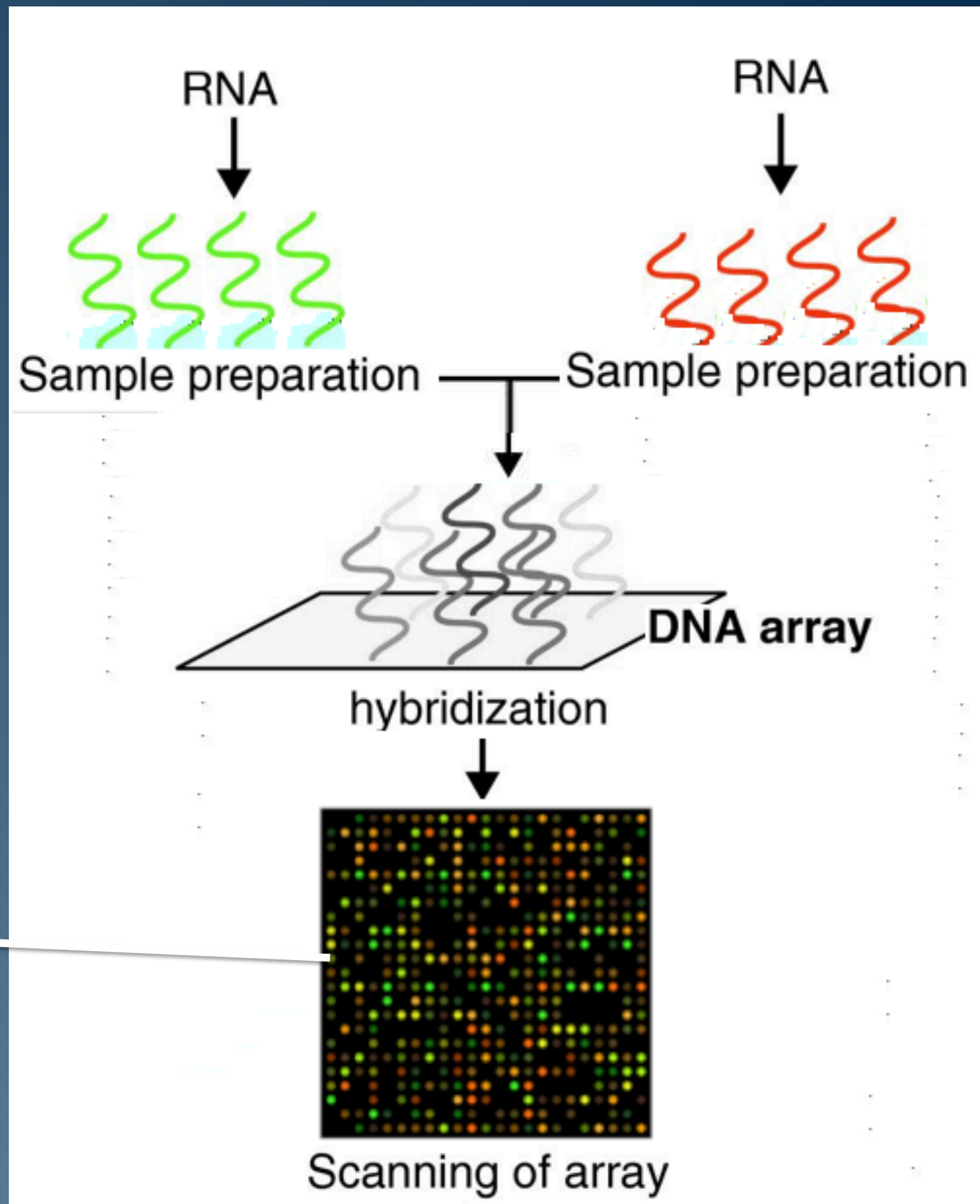


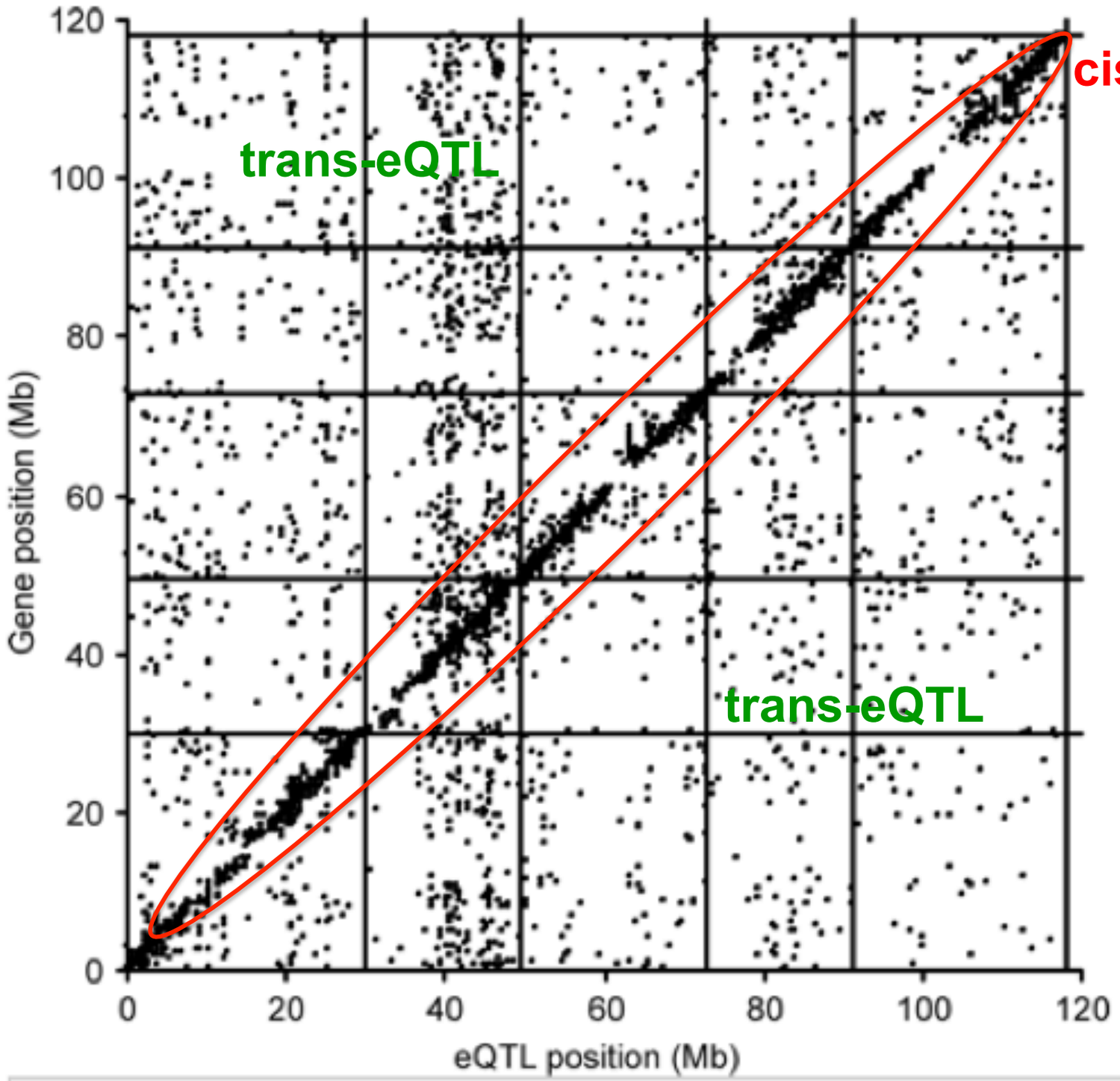
eQTLs:

Caracteres:

Marcadores SNps

QTL Analysis





Distribution of mapped genes versus the position of their accompanying eQTL. Positions of detected eQTL are plotted against the position of the gene for which that eQTL was found. Chromosomal borders are depicted as horizontal and vertical lines. Mb, megabase.

Identificación de sitios de unión de proteínas reguladoras (footprint)