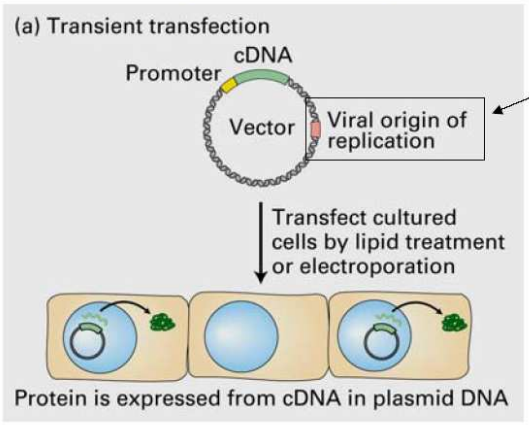
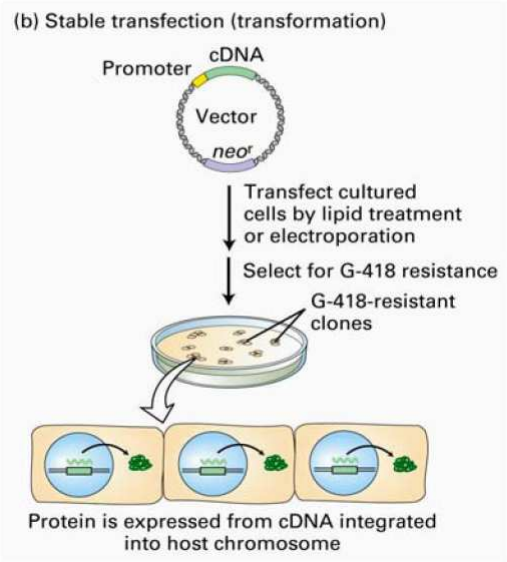
TRANSIENT TRANSFECTION



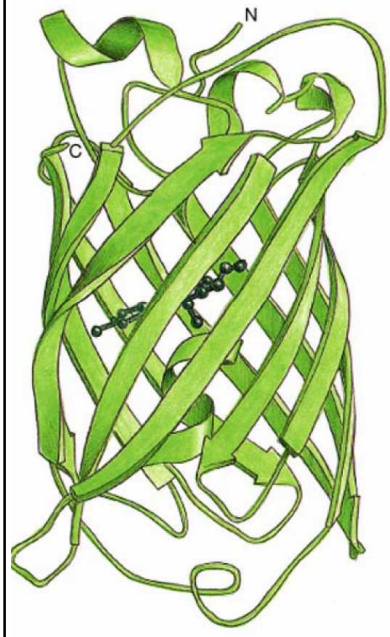
This allows replication if cells are competent for viral replication.
E.g. if SV40 origin, *cos* cells can be used, which express the LT viral antigen, needed for viral replication.

STABLE TRANSFECTION - TRANSFORMATION



For stable transfection, a selection marker is needed (e.g. the *neo* resistance gene or *tk* etc.)

The selection marker gene can be either in the same vector as YFG or in a different vector: animal cells co-integrate exogenous DNA, most commonly at the same locus.



GFP (*green fluorescent protein*)

The most widely used fluorescent fusion tag for studying protein localization and intracellular dynamics, was cloned from *Aequoria victoria* (jellyfish).

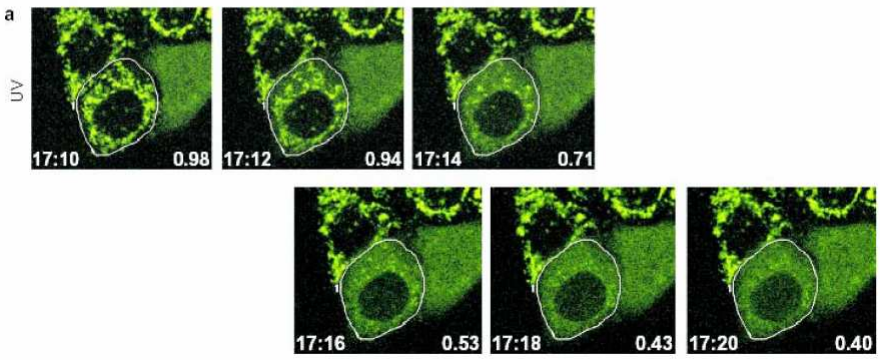
Post-translational modifications of three aminoacid residues give rise to the fluorochrome (dark green).

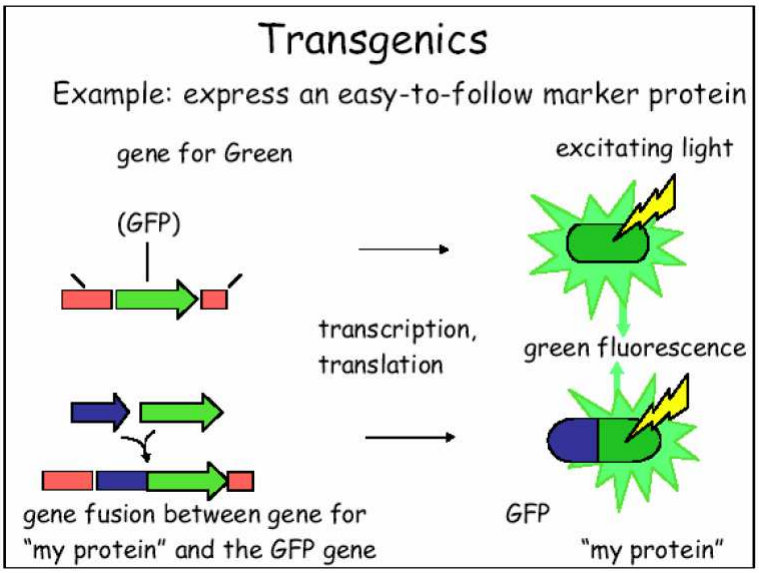
Extensive mutational analysis has produced several variants of the original GFP, giving a range of variously colored proteins for molecular studies (CFP, YFP, etc.).

questa proteina ha la proprietà di catalizzare essa stessa la modificazione di alcuni residui aminoacidici per dare il fluorocromo

se la sequenza codificante la GFP viene fusa al cDNA di YFG, la nostra YFP sarà attaccata a GFP, cioè "tagged" (etichettata)

in questo modo, possiamo "vedere" il destino cellulare della nostra YFP





Knock-out animals and Transgenic animals

TRANSGENIC MICE

Introduction of foreign genes or altered forms of an endogenous gene into an organism (mice)

The introduced genes are called TRANSGENES

The organisms carrying this gene are called TRANSGENICS

TRANSGENIC MICE

Useful to study:

- Normal mammalian biology
- Disease processes (i.e. oncogenes)
- Development

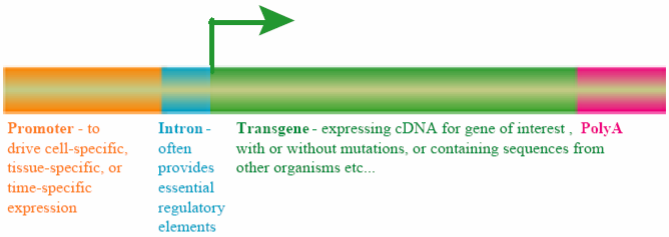
TRANSGENIC MICE

To produce a transgenic mouse you have to:

1) prepare a construct carrying the desired transgene

2) insert it in the animal

Basic Transgenic Construct

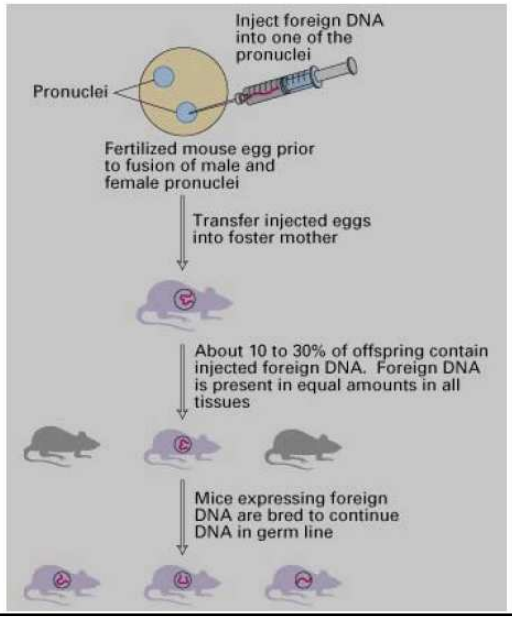


Useful for:

- Studying gene expression profiles
- Overexpression
- Dominant negative, insertional/point mutants
- Complementation

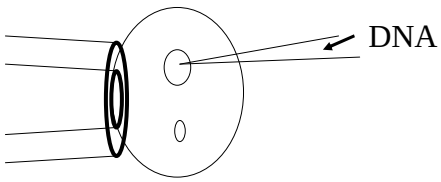
Dove inietto questo costrutto?

Production of transgenic mice



-Dove si integra il DNA?
-In quale cromosoma?
-Quali cellule o tessuti esprimono la proteina d'interesse?
-Da cosa dipende l'eventuale specificità di espressione?

Microinjection in fertilized eggs



The transgene is injected into the male pronucleus of a fertilized egg

The DNA is inserted in the genome RANDOMLY by non-homologous recombination

G0 offsprings from surrogate mothers contain transgene in ALL cells

G0 crossed with non-transgenics. Offsprings called FOUNDERS

KNOCK-OUT MICE

Selective inactivation of a gene by replacing it with a mutant allele in an otherwise normal organism (mice)

KNOCK-OUT MICE

To produce a knock-out mouse you have to:

1) prepare a construct carrying the desired mutant allele

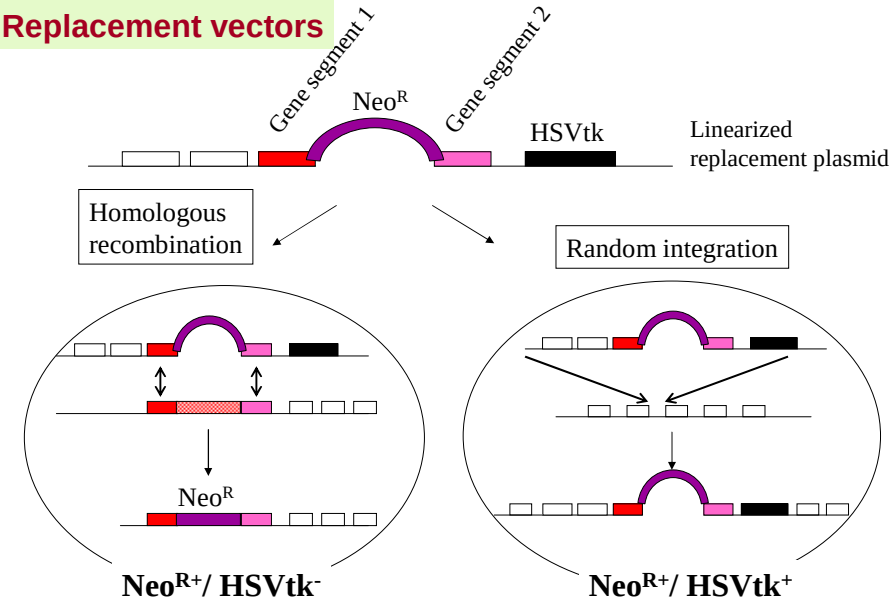
2) insert it in the animal

KNOCK-OUT MICE

Useful to study:

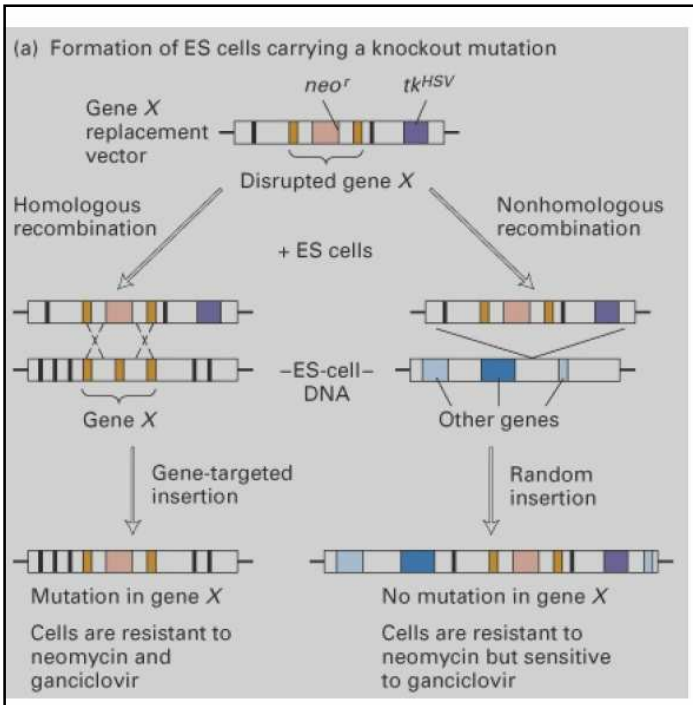
- Development
- Behavior
- Physiology
- Human genetic diseases

Replacement vectors



HSVtk will convert gancyclovir into a toxic drug and kill HSVtk+ cells

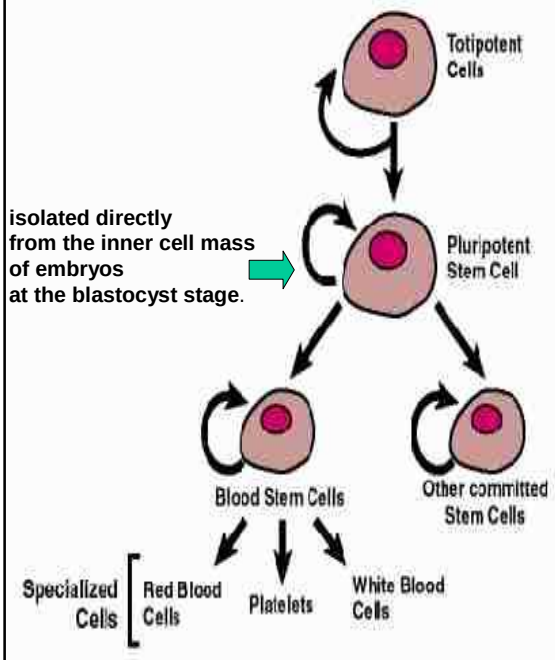
- Dove inietto questo costrutto?



Embryonic stem (ES) cells

Pluripotent stem cells derived from the inner cell mass of the blastocyst

Can be cultured, manipulated and then reinjected into blastocysts, where they can go on to contribute to all parts of embryo.

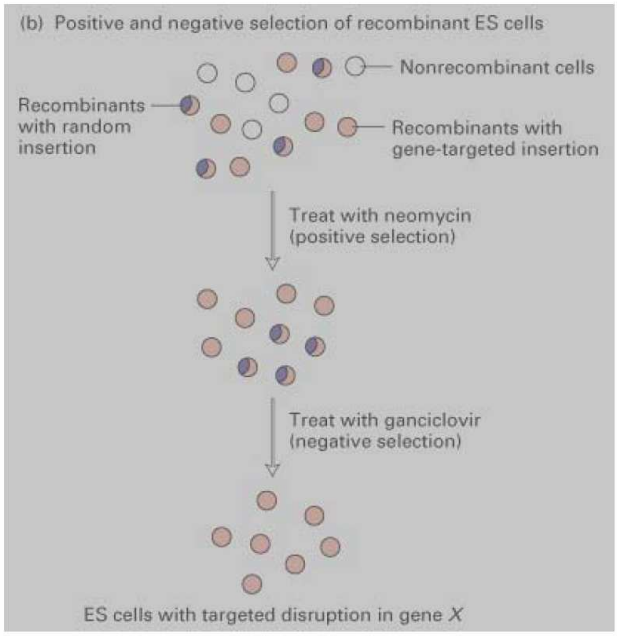


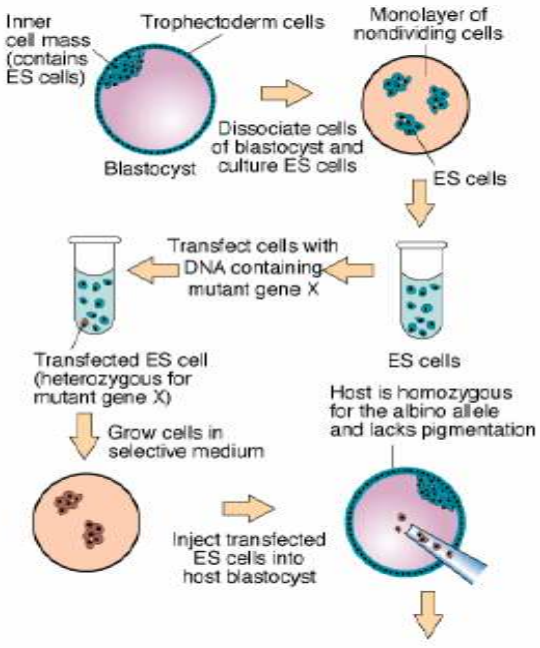
Totipotent =
meaning that
its potential is total.

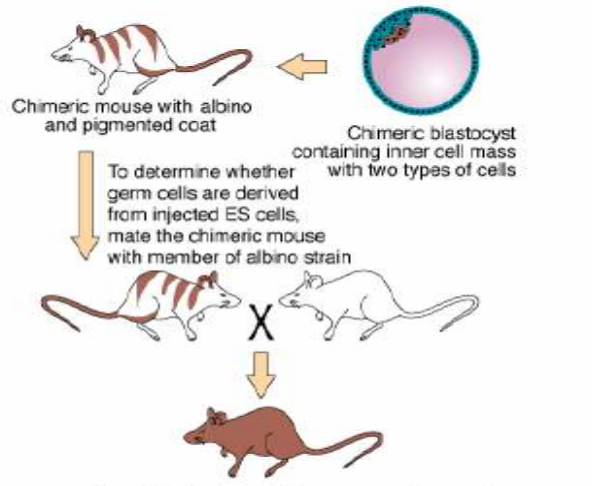
Pluripotent =
they can give rise
to many types of cells
but not all types of cells
(no fetus developed).

www.laskerfoundation.org/ news/weis/estemcell.html

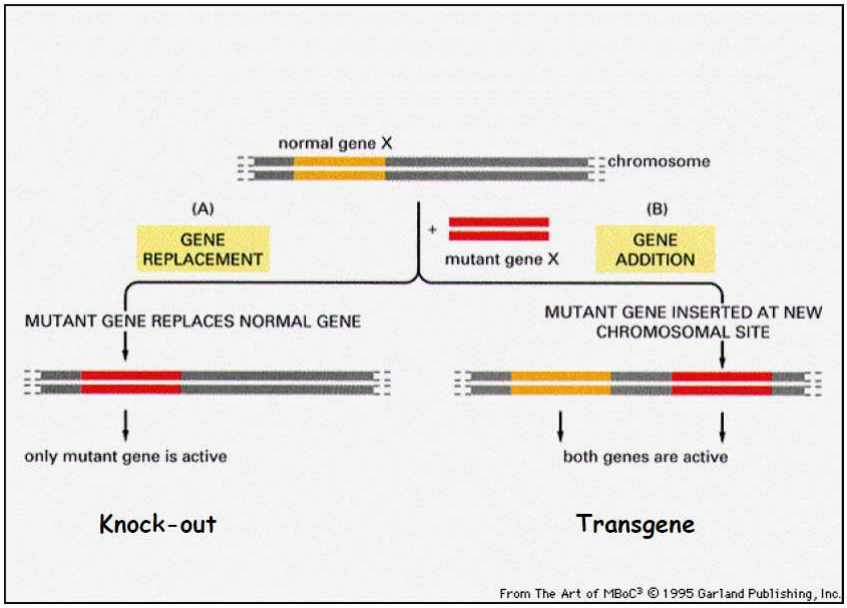
ES cells selection



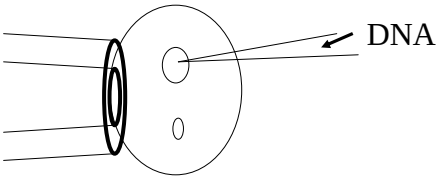




Transgene vs Knock-out



Microinjection in fertilized eggs



The transgene is injected into the male pronucleus of a fertilized egg

The DNA is inserted in the genome RANDOMLY by non-homologous recombination

Transformation of ES cells

ES cells are selected by Neomycin (Neo accompany Your Gene)

Transformed ES cells are injected into 3 day embryo (blastula)

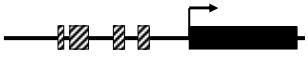
Chimerae etc as for knocks

G0 offsprings from surrogate mothers contain transgene in ALL cells

G0 crossed with non-transgenics. Offsprings called FOUNDERS

Conditional knock-outs

inactivate a gene only in specific tissues and at certain times during development and life.

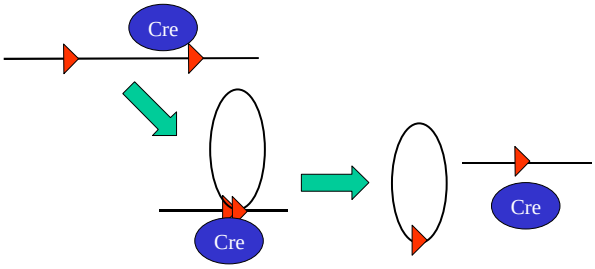


Promoter element **Cre**

Cre-lox technology

Cre – a site-specific recombinase enzyme from the P1 phage.

Recognises a 34bp DNA sequence *loxP* =



Your gene of interest is **flanked by 34 bp *loxP* sites (floxed).**

Where CRE recombinase is expressed at least once

Gene between loxP sites is removed

Quale tipo di approccio devo utilizzare per ottenere il topo “loxato”?

Quale tipo di approccio devo utilizzare per ottenere un topo con una cre ricombinasi tessuto specifica?

Che tipo di costrutto dovrò preparare per il topo lox?

Che tipo di costrutto dovrò preparare per il topo cre?

Utilizzo cellule ES? Inietto il DNA nel pronucleo maschile dell’uovo fertilizzato o nella blastocisti?

Cosa succede quando incrociate un topo “CRE” con un topo “loxato”?

se incrocio un topo con la cre-ricombinasi regolata dal promotore della nestina con un topo che presenta ErbB4 “loxato”,

Quali cellule esprimeranno ErbB4?

Quali non lo esprimeranno?

Perché?

L’effetto è reversibile?