TRANSGENIC ANIMALS AND PLANTS

Purpose

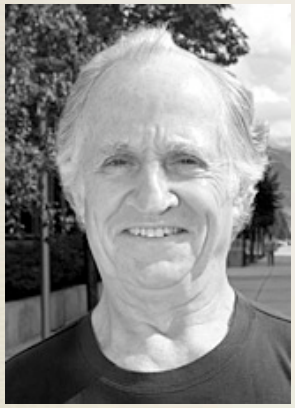
- Study gene function and regulation
- Generate new tools for other fields fields of research
- Cure genetic diseases, test gene therapies
- To create better models of human disease
- New types or sources of bioengineered drugs (plants instead of animals or bacteria)
  Make animal products more suitable for use in humans - humanize blood or organs
- Improve agriculture and raw material production
- Determine how higher order functions are performed.

Transgenic mice

- The organism of choice for mammalian genetic engineers
- small
- hardy
- short life cycle
- genetics are now possible
- interesting stock collections exist

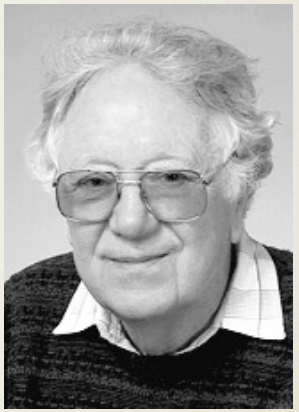
Nobel Prize in Physiology or Medicine 2007

Mario R. Capecchi, **Martin J. Evans** and **Oliver Smithies**
for their discoveries of **"principles for introducing specific gene modifications in mice by the use of embryonic stem cells"**



M. Capecchi
University of Utah

Sir M. Evans
Cardiff University , UK



O. Smithies
University North Carolina, Chapel Hill

The Problem: Heterologous recombination

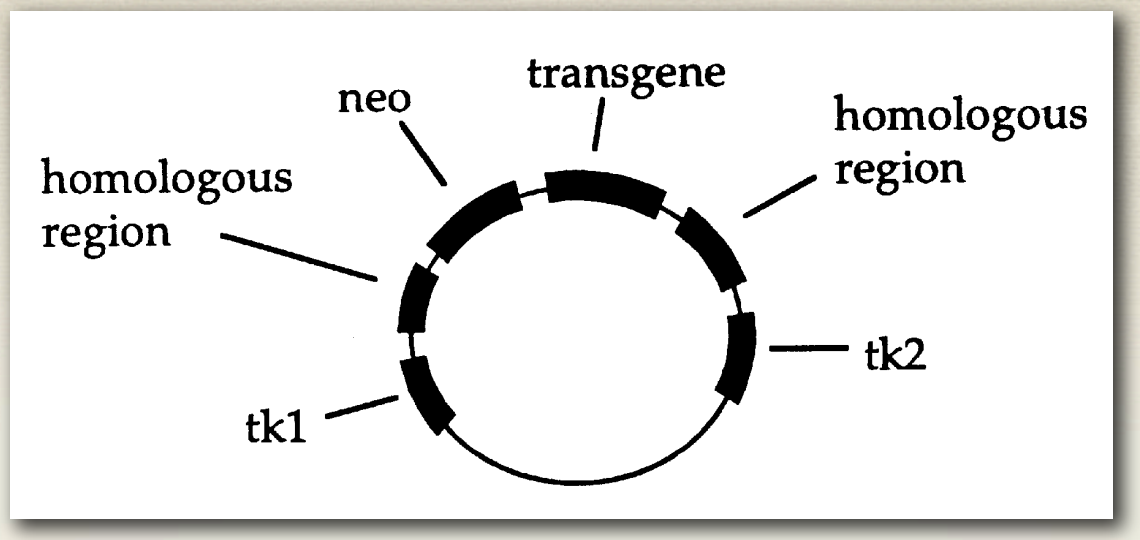
- Sometimes insertion of the transgene at heterologous sites is OK. But it can cause problems. Why?
- DNA integration can occur by homologous or non-homologous recombination.
- Nonhomologous (heterologous) recombination is 1,000 to 10,000 more common than homologous recombination
- To find homologous recombinants you must have a good way to find a needle in a haystack. A selection is best.

Problem is not universal

- Yeast are the opposite.
- In yeast, in a practical sense, in yeast homologous recombination occurs to the exclusion of heterologous recombiantion.

Positive negative selection to identify transfectants

- Positive. Most cells do not pick up DNA. Positive selection identifies those that pick up DNA.
- Negative. The negative selection kills all those that used nonhomologous recombination to pick up the DNA.
- The only cells left are those that acquired it by homologous recombination.



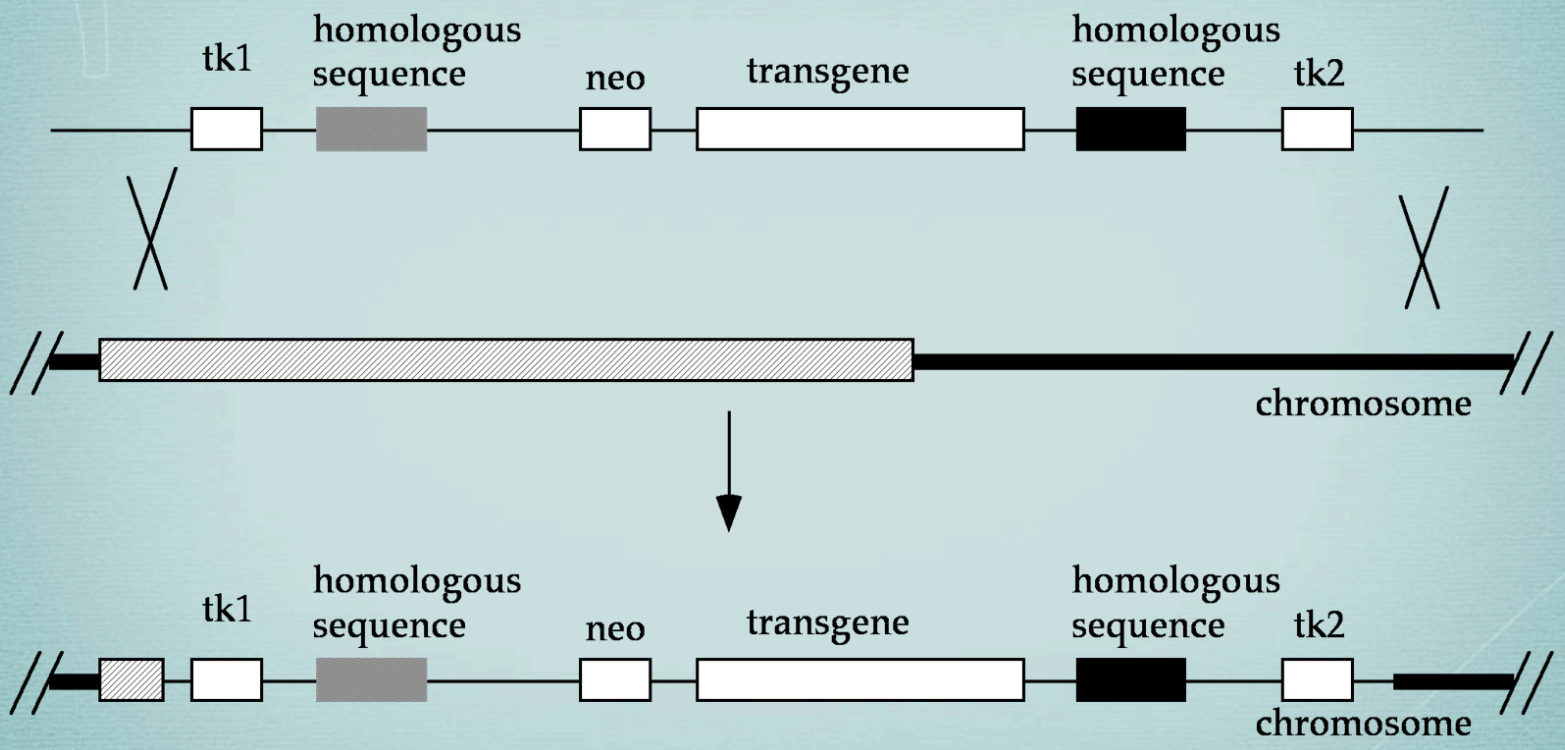
tk1	**HSV type I thymidine kinase gene (HSV=herpes simplex virus)**
homologous regions:	**area homologous to the integration target**
neo	**Neomycin antibiotic resistant gene**
tk2	**HSV type II thymidine kinase gene.**

The vector used for transfection is not able to replicate autonomously. It carries your transgene. The only way for it to persist is to integrate into the host cell's genome. Vector enters genome by both heterologous and homologous recombiantion.

neo and TK

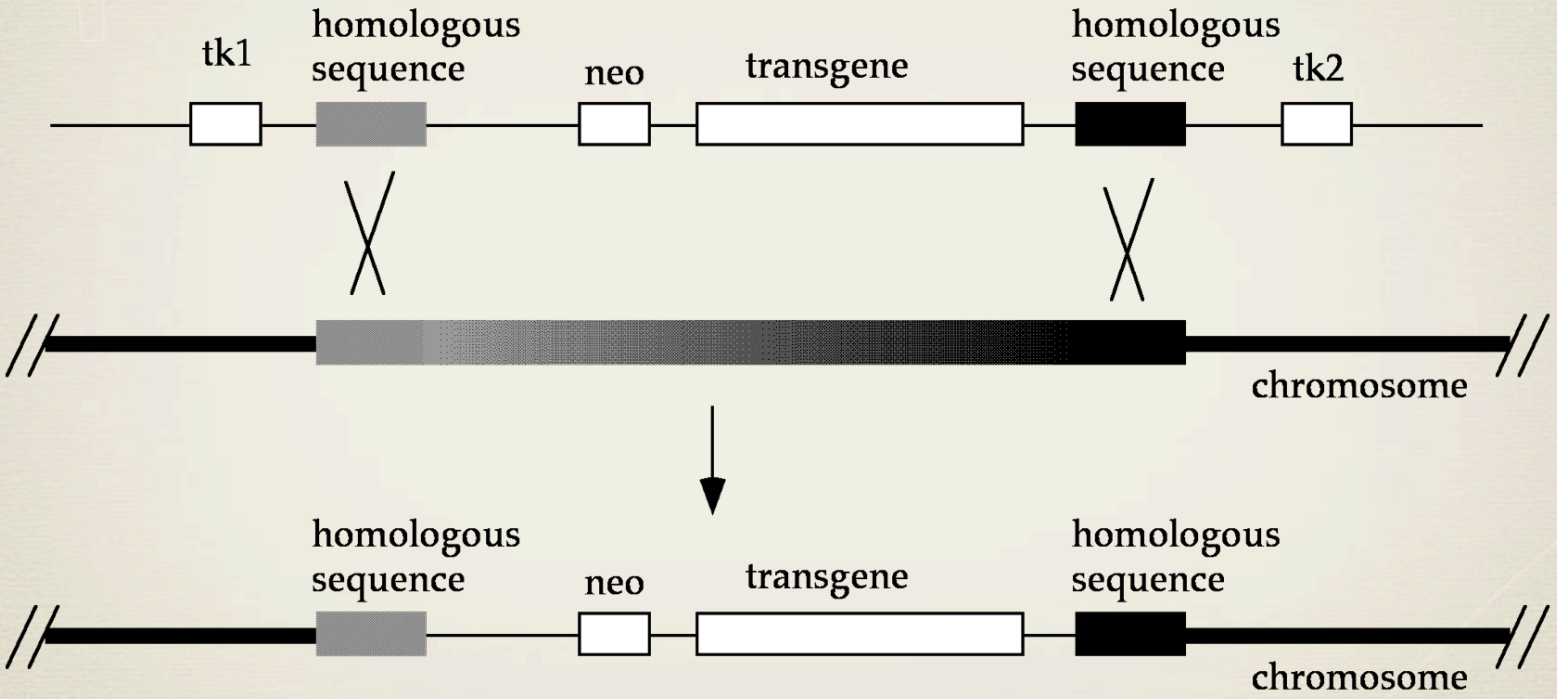
- Cells carrying the neomycin resistance gene are able to grow in the presence of the antibiotic G418 (positive selection).
- Gancyclovir is as nucleotide analog. Viral TK+ converts it to monophosphate which then goes to triphosphate. It interferes with DNA replication. Cells which carry the viral form of TK die. So cells that carry the TK gene from Herpes Simplex Virus (HSV) will die (negative selection).

EXAMPLE OF NON-HOMOLOGOUS RXB



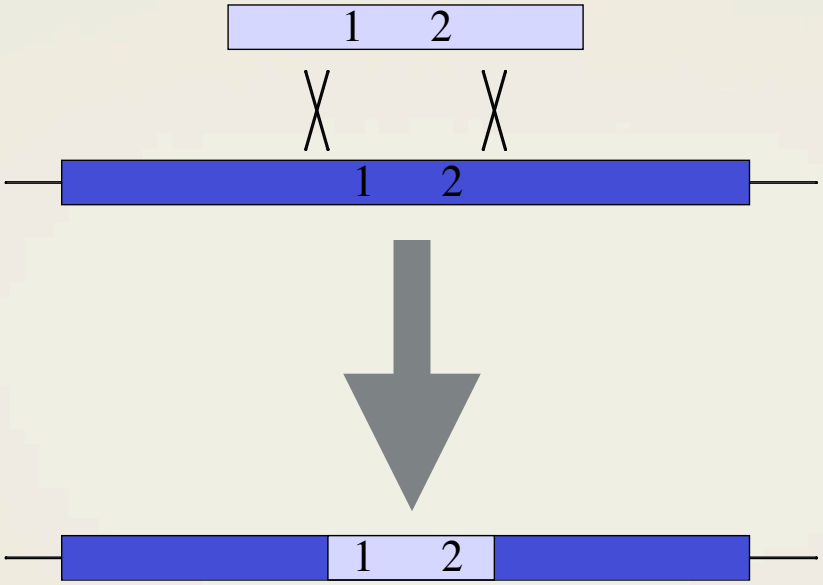
Transformed cells are neo-resistant, but gancyclovir sensitive.

Example of homologous recombination - knock out.



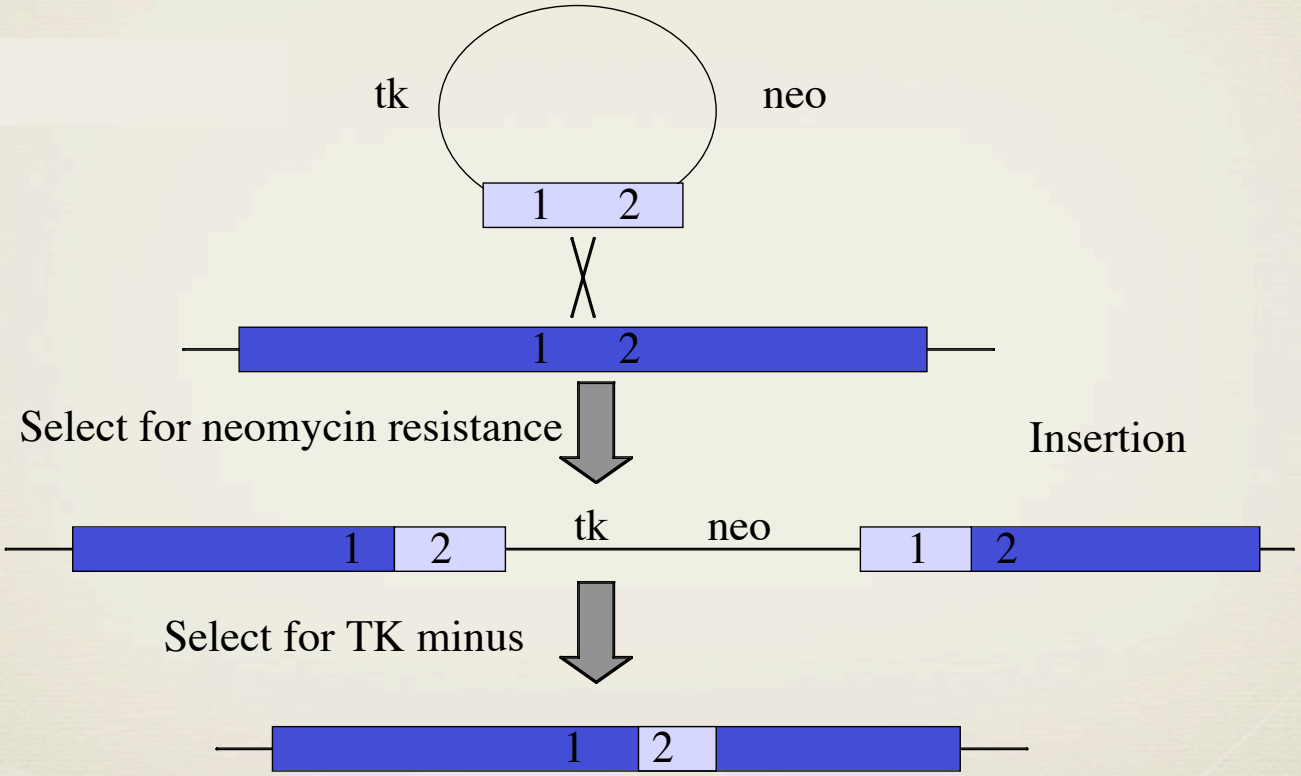
Transformed cells are neo-resistant, but are NOT gancyclovir sensitive.

Homologous recombination gene replacement

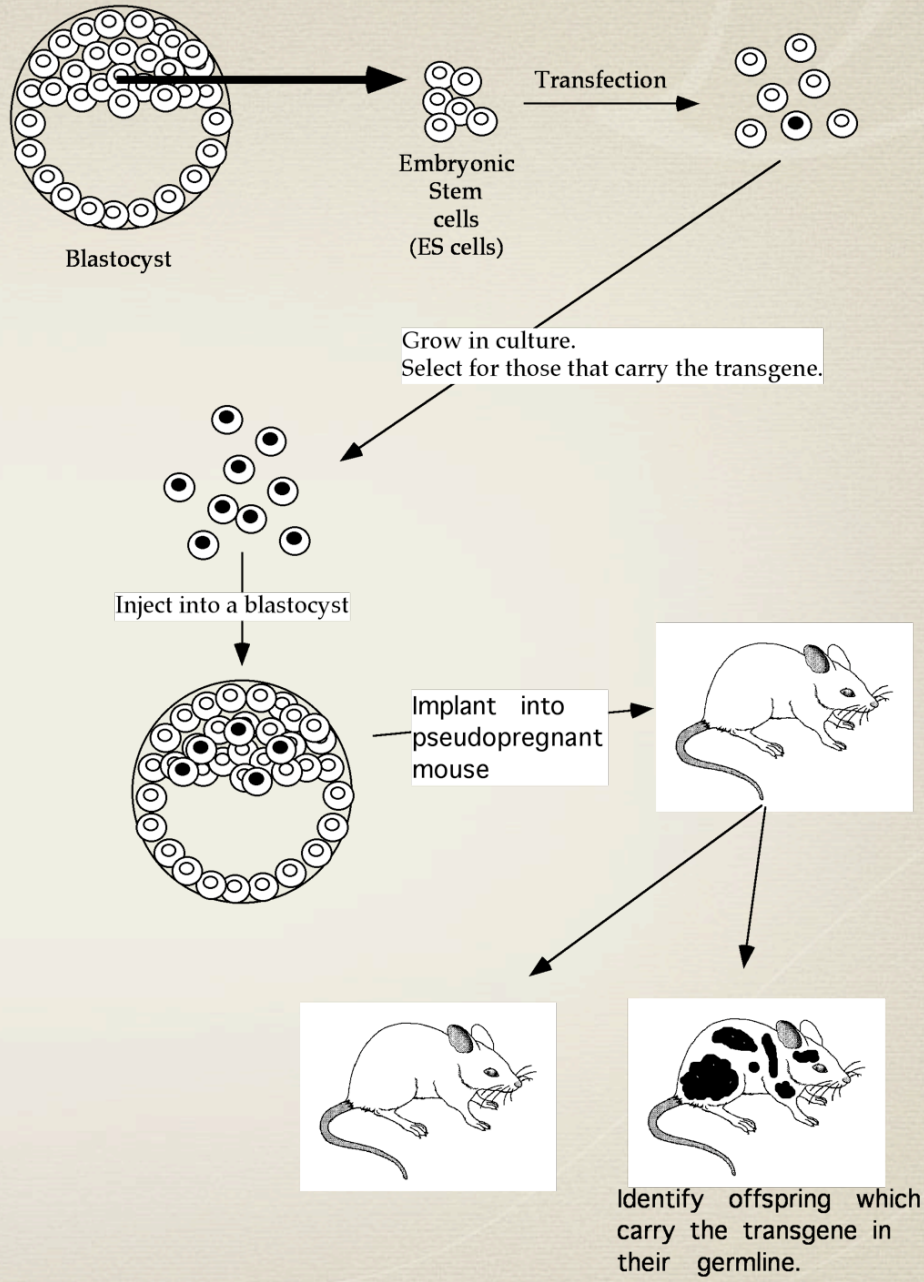


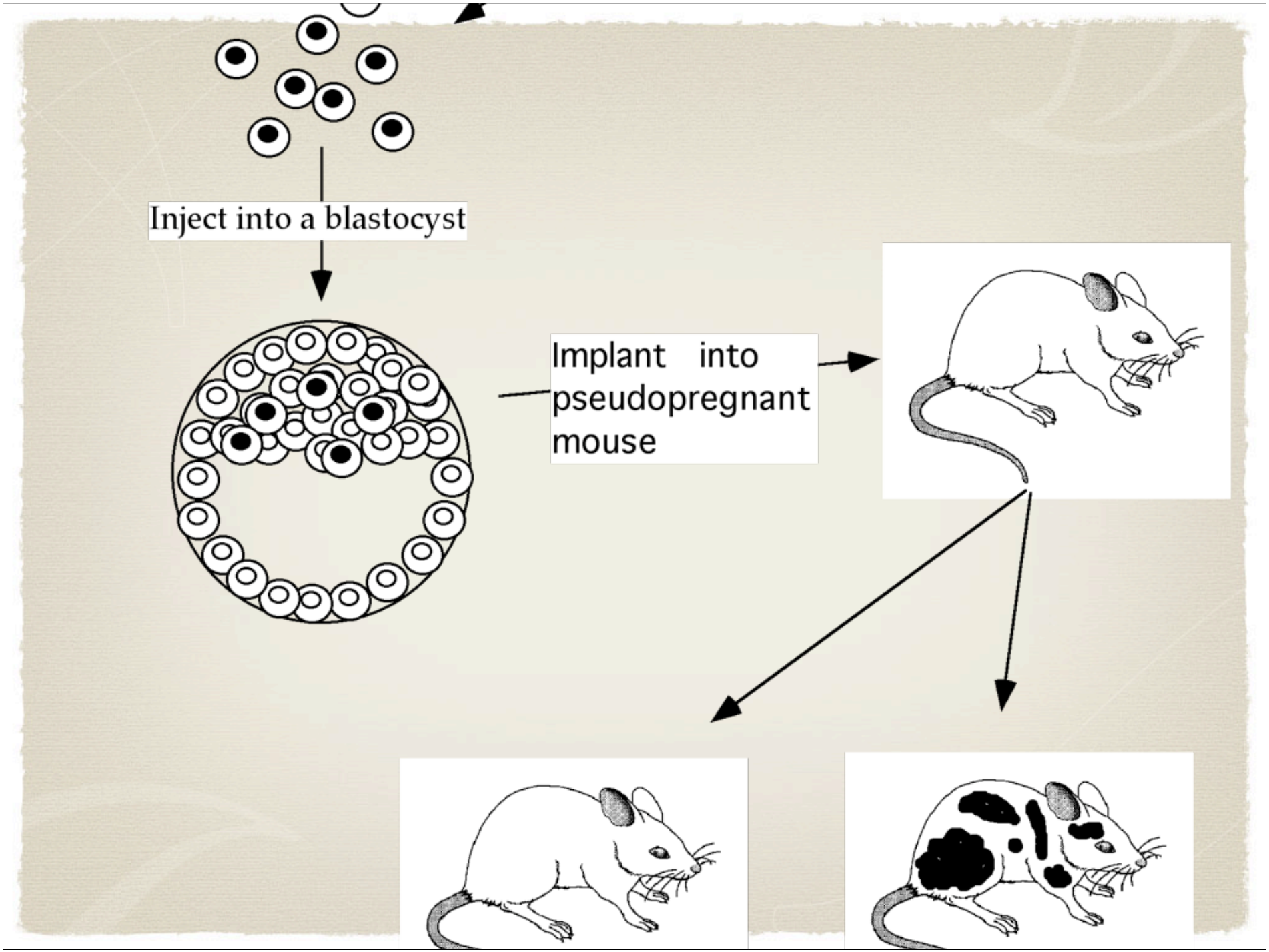
Problem is that this is a very rare event in mammals.

Homologous recombination gene replacement

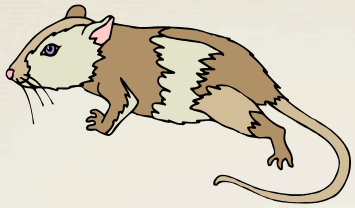


How to make a transgenic mouse



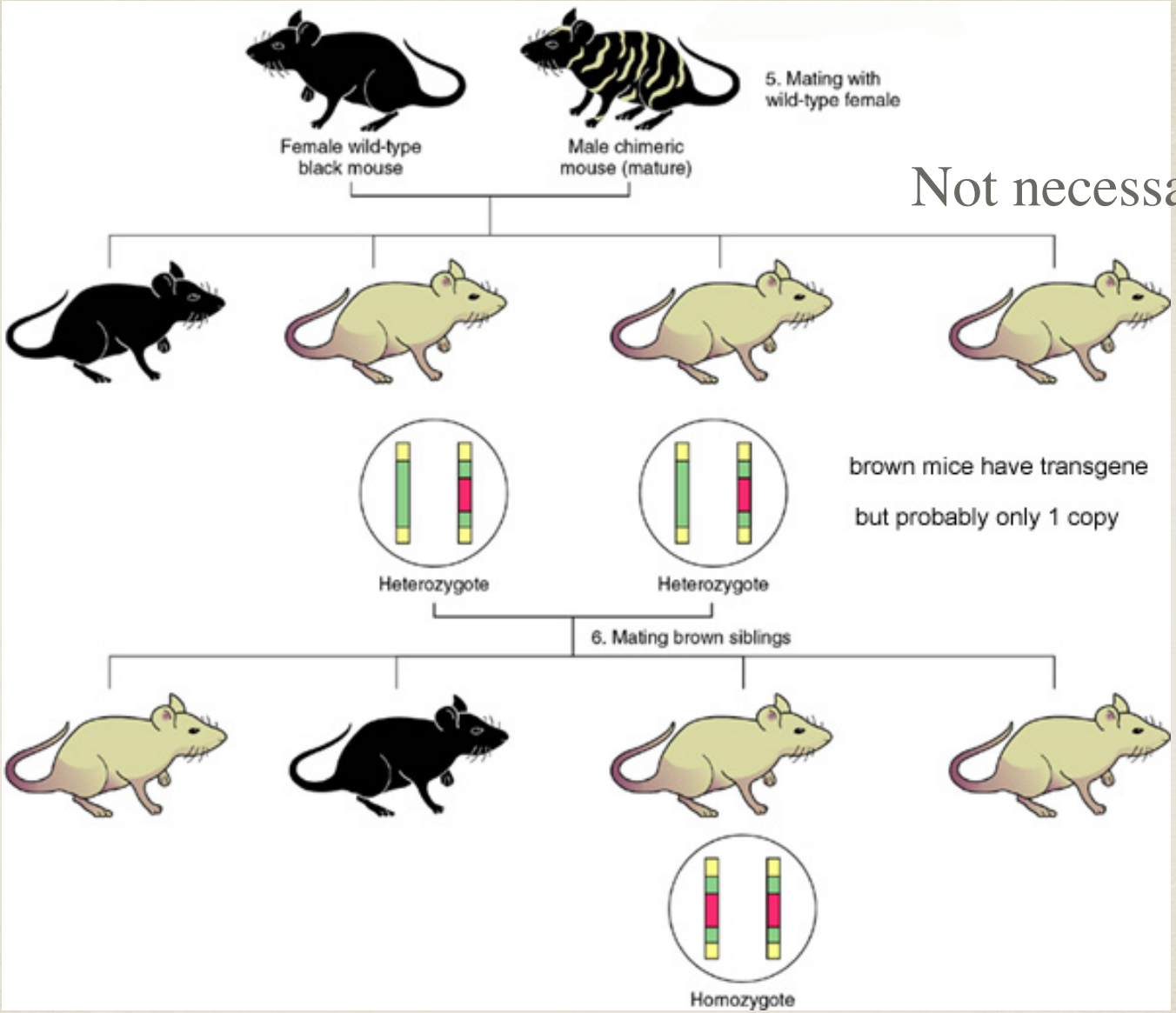

Inject into a blastocyst
Implant into pseudopregnant mouse

(a) If the recipient stem cells are from a brown mouse, and the transgenic cells are injected into a black (female) mouse, chimeras are easily identified by their Brown/Black phenotype.



(b) To get a completely transgenic KO mouse (where all cells have KO gene), mate the chimera with a black mouse. Some of the progeny will be brown (its dominant), because some of the germ line cells will be from the KO cells. ½ the brown mice will have the transgene KO, because the paternal germ-line cell was probably heterozygous.

(c) To get a homozygous KO mouse (both chromosomes have the KO transgene), cross two brown transgenic heterozygotes. ~1/4 will be homozygous at the transgene locus.



Not necessarily 3:1

brown mice have transgene

but probably only 1 copy

Similar to
Fig. 5.41

Conditional mutants

- CRE-LOX system: derived from bacteriophage P2. A way to cause the conditional loss of exons or genes. Exons are said to be FLOXed.
- Tet-on, Tet-off systems

CRE LOX EXAMPLE

Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 5547–5551, June 1992
Cell Biology

Tight control of gene expression in mammalian cells by tetracycline-responsive promoters

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Communicated by W. J. Gehring, March 3, 1992 (received for review January 7, 1992)

ABSTRACT **Control elements of the tetracycline-resistance operon encoded in Tn*10* of *Escherichia coli* have been utilized to establish a highly efficient regulatory system in mammalian cells. By fusing the *tet* repressor with the activating domain of virion protein 16 of herpes simplex virus, a tetracycline-controlled transactivator (tTA) was generated that is constitutively expressed in HeLa cells. This transactivator stimulates transcription from a minimal promoter sequence derived from the human cytomegalovirus promoter IE combined with *tet* operator sequences. Upon integration of a luciferase gene controlled by a tTA-dependent promoter into a tTA-producing HeLa cell line, high levels of luciferase expression were monitored. These activities are sensitive to tetracycline. Depending on the concentration of the antibiotic in the culture medium (0–1 μg/ml), the luciferase activity can be regulated over up to five orders of magnitude. Thus, the system not only allows differential control of the activity of an individual gene in mammalian cells but also is suitable for creation of "on/off" situations for such genes in a reversible way.**

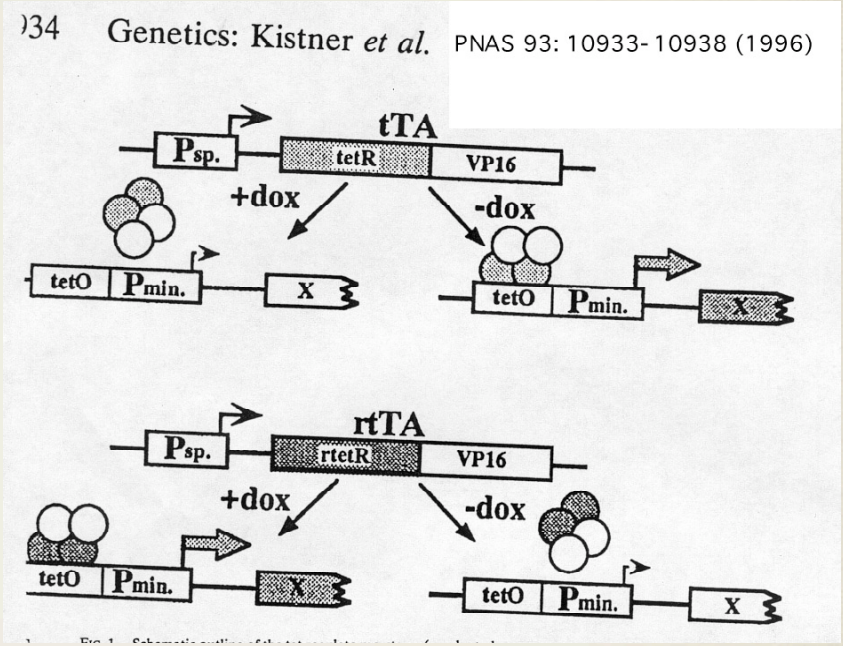
The study of gene function in complex genetic environments such as mammalian cells would greatly profit from systems that would allow stringent control of the expression of individual genes. Ideally, such systems would not only mediate an "on/off" situation of gene activity but also would IPTG. The temperature dependence and the inherent IPTG-related problems, however, may once again limit this approach.

Here we describe a control system that in HeLa cells allows regulation of expression of an individual gene over up to five orders of magnitude. This system is based on regulatory elements of the Tn*10*-specified tetracycline-resistance operon of *E. coli* (19), in which transcription of resistance-mediating genes is negatively regulated by the tetracycline repressor (*tet*R). In the presence of the antibiotic tetracycline *tet*R does not bind to its operators located within the promoter region of the operon and allows transcription. By combining *tet*R with the C-terminal domain of VP16 from HSV, known to be essential for the transcription of the immediate early viral genes (20), a hybrid transactivator was generated that stimulates minimal promoters fused to tetracycline operator (*tet*O) sequences. These promoters are virtually silent in the presence of low concentrations of tetracycline, which prevents the tetracycline-controlled transactivator (tTA) from binding to *tet*O sequences.

The specificity of the *tet*R for its operator sequence (19) as well as the high affinity of tetracycline for *tet*R (21) and the well-studied chemical and physiological properties of tetracyclines constitute a basis for an inducible expression system in mammalian cells far superior to the *lac*R/O/IPTG system.

Tet-off

- This system is derived from the transposon Tn10's tetracycline resistance gene.
- Doxycycline is a tetracycline type antibiotic.



PLANTS

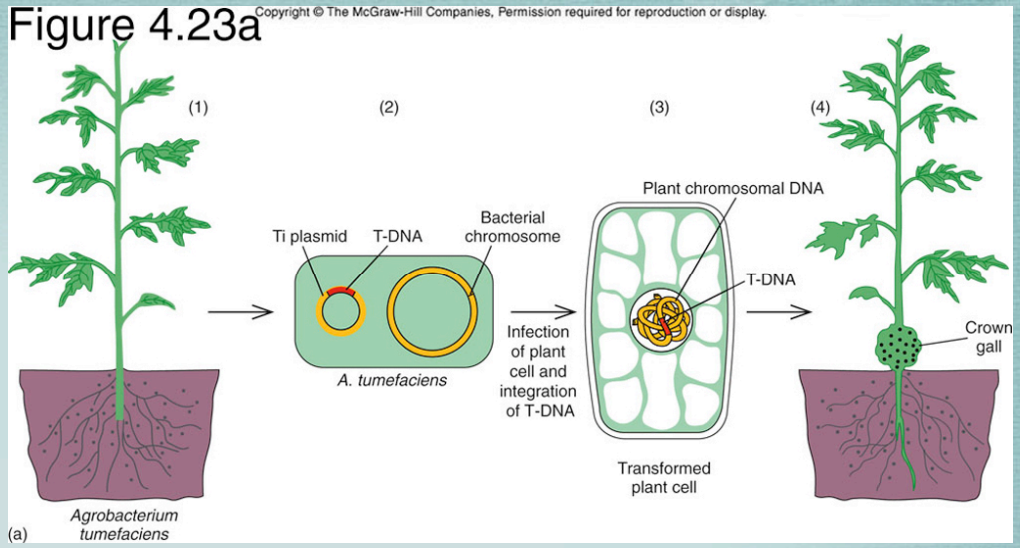
Purpose

- Pest resistant plants
- Herbicide resistant plants
- Vaccine production C.J. Arntzen et al. (2005) Plant-derived Vaccines and Antibodies: Potential and Limitations. Vaccine 23, 1753-1756.
- Production of other therapeutic proteins

Agrobacteria

Agrobacterium tumefaciens caues crown gall for dicots such as corn

- Ti plasmid is large (>200 kb)
- Conjugative. Portion of plasmid (T-DNA) integrates into plant genome
- Engineers plant cell to produce strange amino acids (opines) that only a bacterial cell carrying the Ti plasmid can eat.

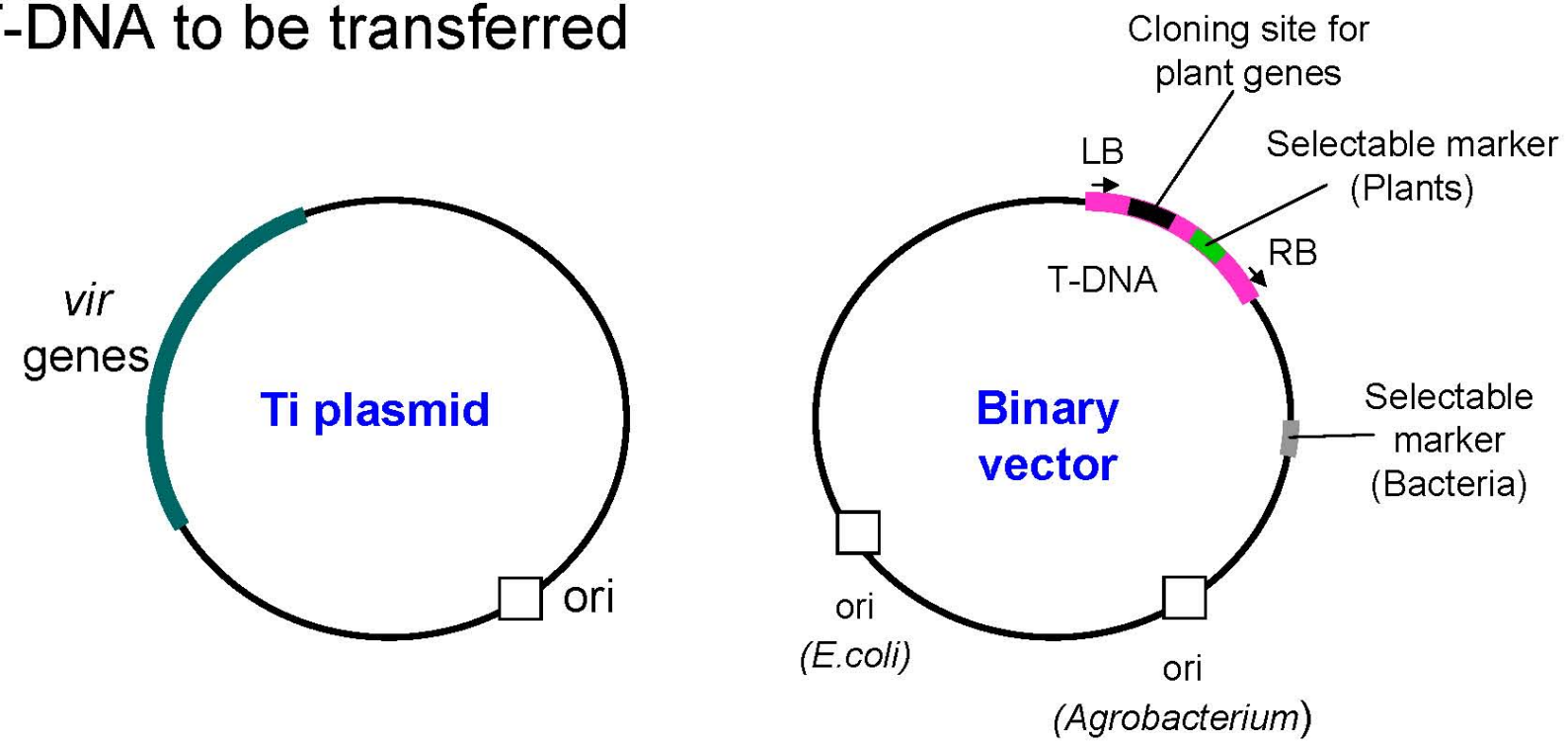


Ti plasmid

- T-DNA genes have typeically EUKARYOTIC EXPRESSION SIGNALS

Binary vector system
Agrobacterium tumefaciens as a tool for genetic engineering

- *vir* genes and T-DNA can be on separate plasmids
- only left and right borders (LB & RB) are required for T-DNA to be transferred



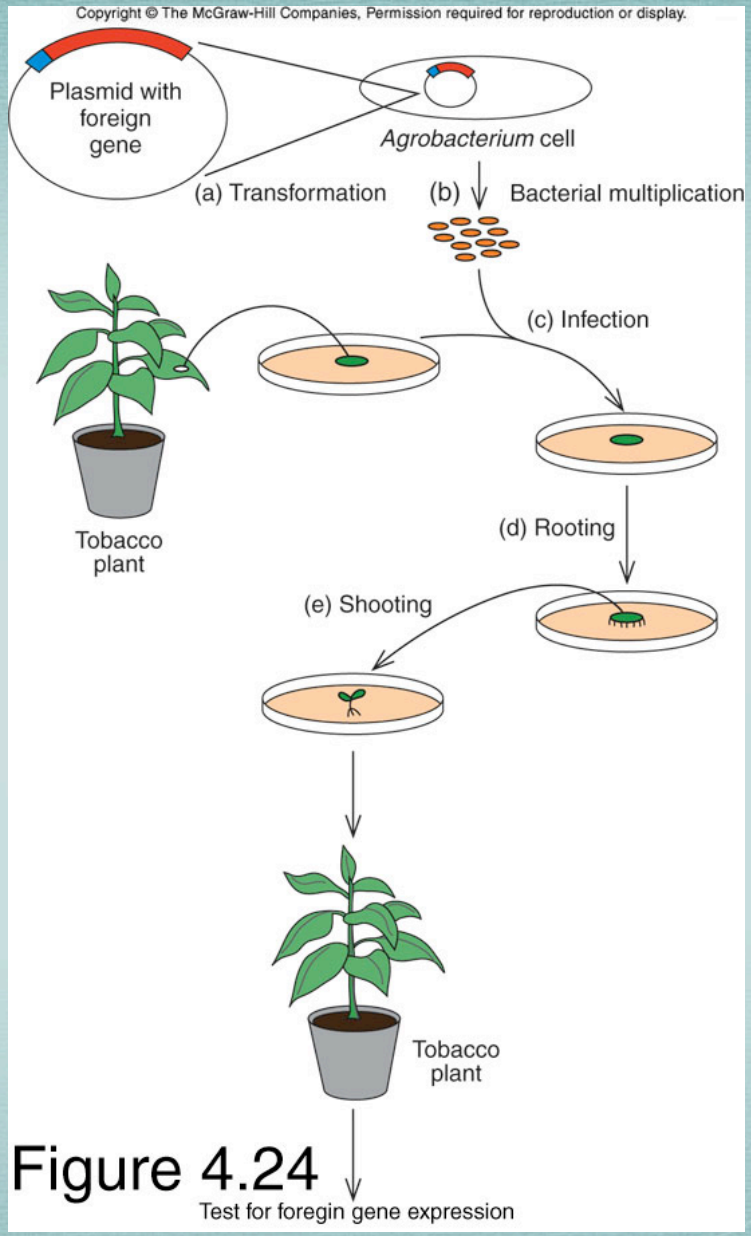


Figure 4.24

Other Transformation Protocols

1. Leaf-disc transformation - after selection and regeneration with tissue culture, get plants with the introduced gene in every cell

2. Floral Dip – does not require tissue culture. Reproductive tissue is transformed and the resulting seeds are screened for drug-resistant growth. (Clough and Bent (1998) Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. Plant Journal 16, 735–743)

Concerns that have been raised about cultivating/consuming GM crops (or GMOs)

1. They may be toxic or allergenic.
2. They may become established in the wild and outcompete other plants.
3. They may negatively affect insects or other organisms that use crops.
4. They may outcross to a nearby wild relative spreading the *trans*gene into a wild population.

References on regulation and eco-risk assessment vis-à-vis the cultivation of GM crops

- Nap *et al.* (2003) *Plant Journal* 33, 1-18
  - –Focuses on current status and regulations
- Conner *et al.* (2003) *Plant Journal* 33, 19-46
  - –Focuses on ecological risk assessment

Fin

Probably out of date.

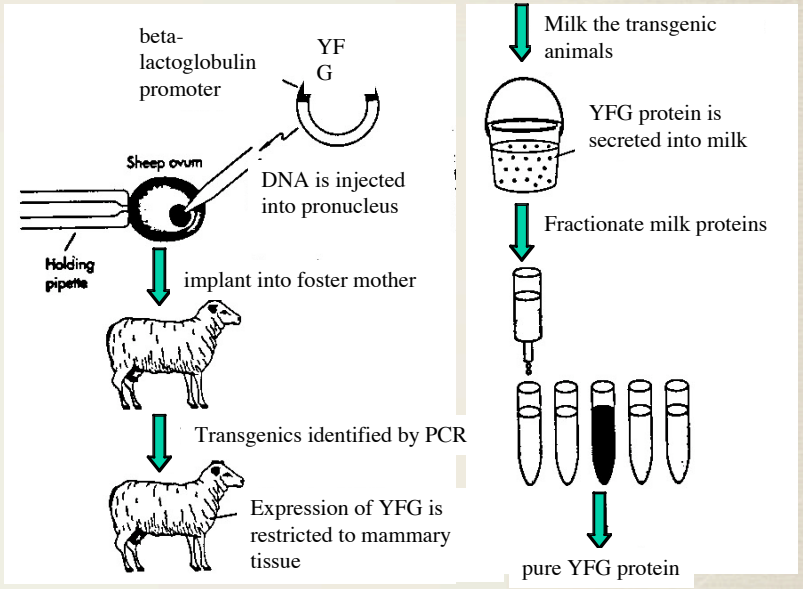
Transgenic animals are expensive to make. It would be nice to be able to harvest the protien product without killing the animal. Expression in mammary glands provide a way to do this.

Milk contains a relatively small number of proteins. Therefore, a protein expressed in milk is already partially purified.

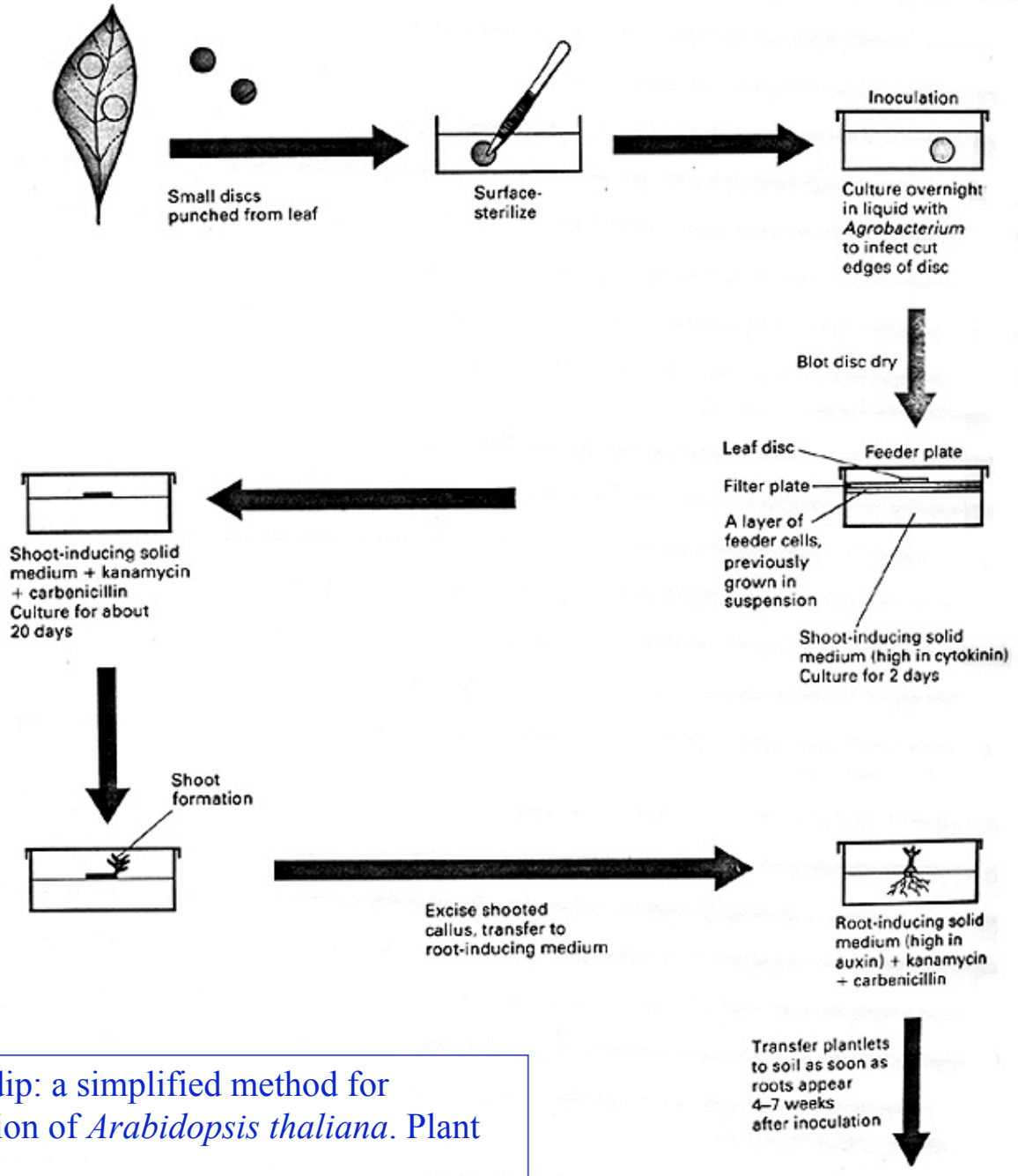
Use the beta-casein transcriptional control region. Make a fusion so that protein is shed into the milk.

B-lactoglobin whey acidic protein can be used.

CFTR, interleukin 2, tPA proteins are early examples.

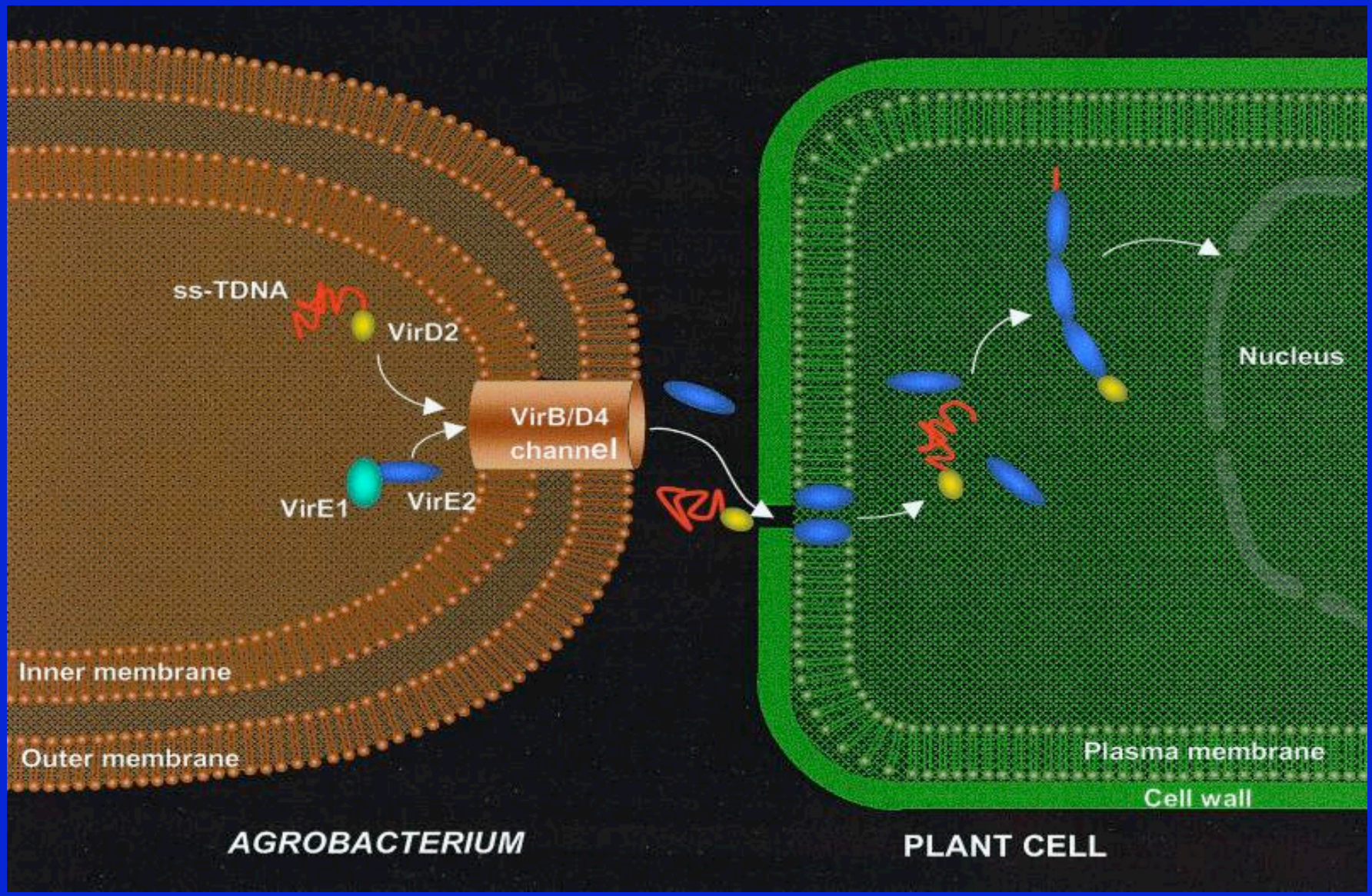


Making a transgenic plant by leaf disc transformation with *Agrobacterium.*



S.J. Clough, A.F. Bent (1998) Floral dip: a simplified method for Agrobacterium-mediated transformation of *Arabidopsis thaliana*. Plant Journal 16, 735–743.

VirE2 may get DNA-protein complex across host PM



Dumas et al., (2001), Proc. Natl. Acad. Sci. USA, 98:485