

# Genetic engineering in animals

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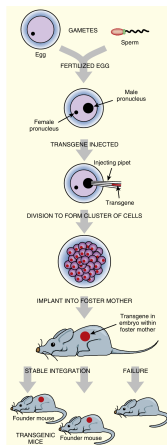
## 1 Transgenic animals

## Transgenic animals

# Background

- Woollier sheep and smarter sheep dogs have both been improved through many generations of selective breeding
- Obviously, the more we know about genetics, the faster and more effectively we can improve our crops and livestock
- Most early experiments in animal transgenics were done with mice
- Now engineered animals:
  - Sheep, goats, cats, dogs and monkeys
- Transgene may be derived from same or from distantly related animals, or even from unrelated organisms.

# Creating transgenic animals: Nuclear microinjection



**Figure 1: Creation of Transgenic Animals by Nuclear Injection**

In vitro fertilization is used to start a transgenic animal. Harvested eggs and sperm are fertilized, and before the pronuclei fuse, the transgene is injected into the male pronucleus. The embryo continues to divide in culture and is then implanted into a mouse. The "foster mother" mouse has been treated with hormones so that she accepts the embryo and carries on with the pregnancy. The offspring are screened for stable integration of the transgene. Founder mice have one copy of the transgene.

## Creating transgenic animals: Nuclear microinjection

- Transgene is injected into fertilized egg cells
- Soon after fertilization, individual nuclei from both sexes are separate as pronuclei.
- Before pronuclei fuse together, DNA is injected into male pronucleus.
- Success rate: 5% - 40%
- After culturing for few days *in-vitro*, embryo is then implanted into womb of a female animal – the **foster mother**.
- Those that received the transgene and maintain it stably are called founder animals.

# Comparison of recombinant protein expression systems

No expression system is perfect, and each recombinant protein must be evaluated for which system will work the best.



Figure 2: Each protein expression system falls on a continuum of worst to best for characteristics such as speed, cost, glycosylation, folding, and government regulations. The other symbols include cultured mammalian cells, insect cells, yeast, and bacteria.

# Comparison of recombinant protein expression system: Insects

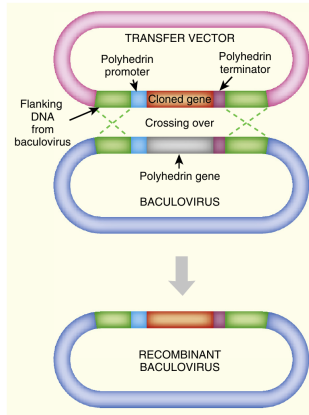


Figure 3: **Baculovirus Expression Vector**

To express a gene in insect cells, the gene must be inserted into a baculovirus genome. First, the gene of interest is cloned into a transfer vector containing the baculovirus polyhedrin gene promoter followed by a multiple cloning site and the polyhedrin terminator. This is done in *E. coli*. The construct is transfected into insect cells along with the normal baculovirus genome. A double crossover between the polyhedrin promoter and terminator replaces the polyhedrin gene with the gene of interest.



## Comparison of recombinant protein expression systems: Insects

- One of most often used is multiple nuclear polyhedrosis virus (MNPV).
- It infects many insects and replicates well in many cultured insect cell lines. A popular cell line used to propagate this baculovirus is from the fall armyworm (*Spodoptera frugiperda*).

## Control of expression

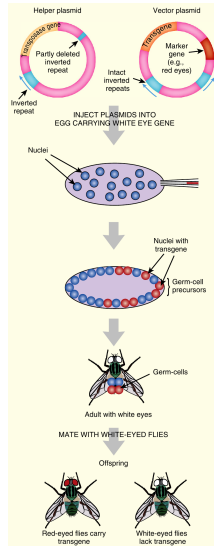
- Transgenes may be artificially regulated by a variety of control systems.
- Engineered versions of the bacterial lac and tet regulators are widely used.
- Transgene expression may be controlled by site-specific recombination. Either the Cre or Flp recombinase can rearrange segments of transgenic DNA to turn transgenes on or off.
- A similar approach uses bacterial recombinases, such as the  $\Phi$  C31 integrase.

## Applications

Marathon Mouse can run about 1800 meters – more than a mile – before exhaustion. This is twice as far as a normal mouse can last. Marathon mouse has enhanced PPAR-delta-a regulator of several genes involved in burning fat and in muscle development.

Mighty Mouse was engineered to lack myostatin, a protein that slows muscle growth. The result is colossal muscle development. There is one known case of a human with a genetic defect leading to lack of myostatin. A German boy, born in Berlin in 2000, has muscles twice the size of other children his age.

# Applications



## Applications: other

- Transgenic animals help fight diseases (Lysozyme fortified livestock milk to prevent diarrhoeal disease)
- Genetically modified mosquitoes
- Growth promoter genes in Atlantic salmon