Chapter 19-Transgenic Animals: Methodology and Applications

- Transgenic mice: methodology (Retrovirus vector, DNA microinjection, Engineered embryonic stem cell, Cre-loxP recombination system, High capacity vectors)
- Transgenic mice: applications (Alzheimer disease, test systems, conditional regulation, control of cell death)
- Cloning livestock by nuclear transfer
- Transgenic cattle, sheep, goats and pigs
- Transgenic birds
- Transgenic fish

Fig. 19.1 Establishing transgenic mice with retroviral vectors (rarely used)

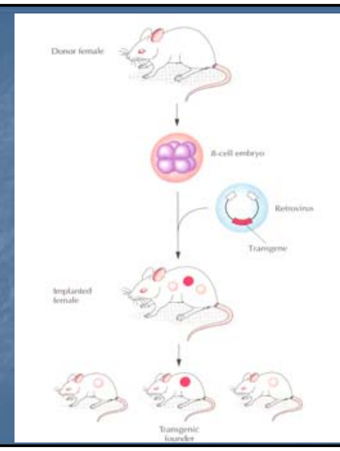
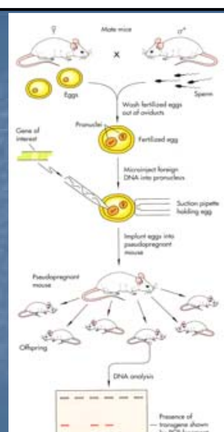


Fig. 19.2 Establishing transgenic mice by DNA microinjection

- Most commonly used method
- Only 5% or less of the treated eggs become transgenic progeny
- Need to check mouse pups for DNA (by PCR or Southerns), RNA (by northerns or RT-PCR), and protein (by western or by some specific assay method)
- Expression will vary in transgenic offspring: due to position effect and copy number

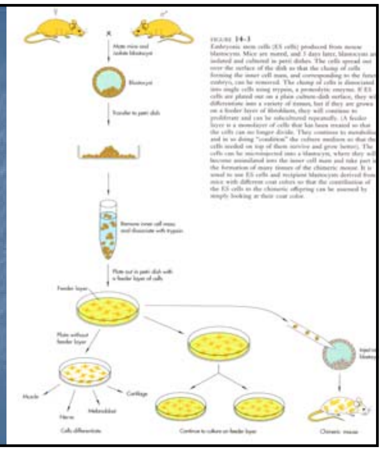


Creating a transgenic mouse using the DNA microinjection method

Creating a Transgenic.mov

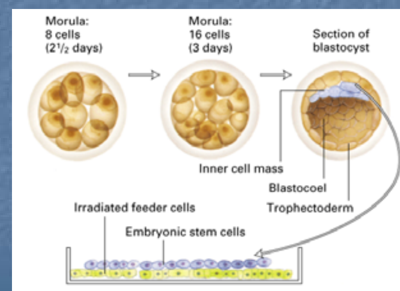
Establishing transgenic animals using engineered embryonic stem (ES) cells

But what are ES cells?

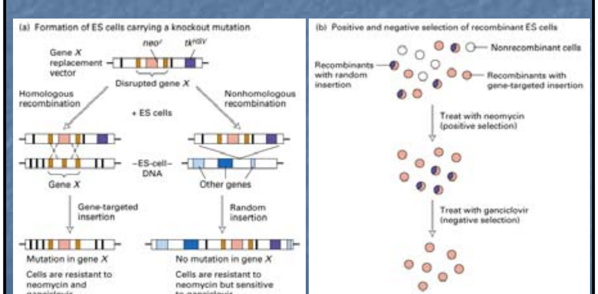


Transgenic animals-Engineered embyronic stem cell method (used for gene knockouts)

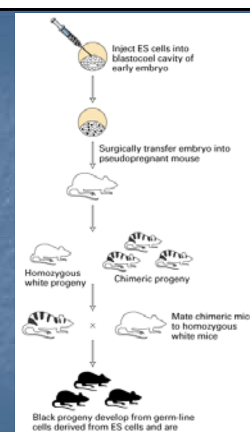
Step 1: Get the ES cells (Fig. 19.4)



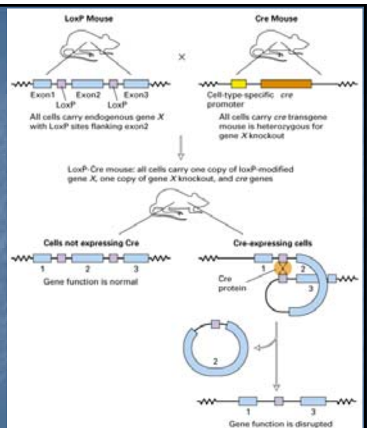
Step 2: Genetically engineer the ES cells (Figs. 19.5 and 19.6)



Step 3: Place engineered ES cells into an early embryo (Fig. 19.4)



Transgenic animals-Using Cre-*loxP* for tissue or time-specific gene knockouts



Transgenic mice can be produced with high capacity vectors

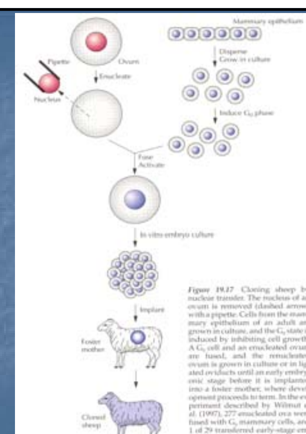
- Generally done by microinjection of numerous genes contained in a YAC
- Production of mice that can produce human antibodies is one notable example

Transgenic mice: applications

- Transgenic models for Alzheimer disease, amyotrophic lateral sclerosis, Huntington disease, arthritis, muscular dystrophy, tumorigenesis, hypertension, neurodegenerative disorders, endocrinological dysfunction, coronary disease, etc.
- Using transgenic mice as test systems (e.g., protein [CFTR] secretion into milk, protection against mastitis caused by *Staphylococcus aureus* using a modified lysostaphin gene)
- Conditional regulation of gene expression (tetracycline-inducible system in Fig. 19.15)
- Conditional control of cell death (used to model and study organ failure; involves the organ-specific engineering of a toxin receptor into the mice and then addition of the toxin to kill that organ)

Fig. 19.7 Cloning livestock by nuclear transfer (e.g., sheep)

"Hello Dolly"



Transgenic cattle, sheep, goats, and pigs

- Using the mammary gland as a bioreactor (see adjacent figure)
- Increase casein content in milk
- Express lactase in milk (to remove lactose)
- Resistance to bacterial, viral, and parasitic diseases
- Reduce phosphorous excretion

Table 19.3 Some exogenous proteins that have been expressed in the mammary glands of transgenic animals

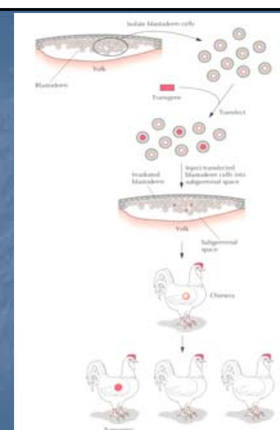
- Erythropoietin
- Factor IX
- Factor VIII
- Fibrinogen
- Growth hormone
- Hemoglobin
- Insulin
- Monoclonal antibodies
- Tissue plasminogen activator (TPA)
- α1-antitrypsin

"Enviro-pigs"

- Transgenic pigs expressing the phytase gene in their salivary glands
- The phytase gene was introduced via DNA microinjection and used the parotid secretory protein promoter to specifically drive expression in the salivary glands
- Phytate is the predominant storage form of phosphorus in plant-based animal feeds (e.g., soybean meal)
- Pigs and poultry cannot digest phytate and consequently excrete large amounts of phosphorus
- "Enviro-pigs" excrete 75% less phosphorus

Fig. 19.20 Establishing transgenic chickens by transfection of isolated blastoderm cells

- Resistance to viral, bacterial, and coccidial diseases
- Better feed efficiency
- Lower fat and cholesterol levels in eggs
- Better meat quality
- Eggs with pharmaceutical proteins in them



Transgenic fish

- Genes are introduced into fertilized eggs by DNA microinjection or electroporation
- No need to implant the embryo; development is external
- Genetically engineered for more rapid growth using the growth hormone gene (salmon, trout, catfish, tuna, etc.)
- Genetically engineered for greater disease resistance
- Genetically engineered to serve as a biosensor for water pollution