Transgenic Animals

BIT 220
Definitions

Transgenesis
process of introducing foreign or exogenous DNA into an animal’s genome

Transgene
DNA introduced

• Mice
• Cows
• Fish
• Birds
• Sheep
• Goats
Why Transgenesis?

- Improve genetic Features of domesticated Animals
- Provide animal models for study of human diseases
  - Pharming
  - using farm animals for production of human pharmaceuticals
  - mammary glands
- Study the genes regulation, development of animals

How to Get the Transgene Inserted

- Retroviral Vectors
- Microinjection
- Embryonic stem cells
Microinjection

FIGURE 22.13

A. Remove eggs
B. Fertilize \textit{in vitro}
C. DNA is microinjected into male pronucleus (prior to nuclear fusion)
   100-1000 copies of gene
D. Implant eggs into surrogate
Fig 22.13  The production of transgenic animals by microinjection of DNA into fertilized eggs.
Retroviral Vectors

FIGURE 22.8 (19.1)

Infect early stage embryo with replication-defective retrovirus

Limitations
only small DNA inserts
no regulatory sequences
safety
Engineered Embryonic Stem Cells

FIGURE 19.4

- Remove pluripotent ES cells from blastocyst
- Transfect ES
- [Site specific Integration - advantage]
- Selection
- Microinject back into blastocyst
- Implant
Creation of Transgenic Line

Integration of DNA

\( G_0 \) generation - Mosaics

\( G_1 \) progeny

all cells contain transgene

1. Determine whether the progeny are TRANSGENIC
   piece of tail, PCR, Southern Blot

2. Mate this transgenic animal to determine whether the transgene is in GERM line

3. Breed to get homogenous transgenic line
Cloning by Nuclear Transfer

Quiescent [G0] cells Plus EGG

First 3 cell division of Fertilized Egg
• no gene expression
• embryo-initiating protein factors in cytoplasm bind to embryonic development genes

When G0 nucleus is transferred
• cell specific regulatory proteins are stripped
• EIPF bind to EDG
Mice make Human Antibodies

YACs contained many of these heavy and light chain segments

Knock out Mouse Segments, replace with Human segment genes

Fully human antibody made
Transgenic Cattle

Applications

• Increasing casein content of milk increase cheese production
• Lactose free milk (transgene lactase)
• Resistance to bacterial infections
• In vivo immunization
  • transgene is specific Heavy and Light chain genes which
  • create mAn against a specific antigen
Why Express rProtein in Milk

Easy to purify - few other proteins in milk
Doesn’t harm transgenic animal- no change to physiology

rProtein is authentically modified post-translationally

Large quantities

Renewable source

Mammary Gland-specific Promoters
promoter and transcription termination sequences are present
Sheep and Pigs

**PIGS**
- PST porcine somatotropin (growth hormone)
  - adverse effects: kidney, stomach, heart, sterility
- Human Hemoglobin
  - to replace whole blood transfusions

**SHEEP**
- Increase wool production
- Keratin promoter
- Growth factor
Organ Transplant

Problem: Rejection

Antibodies from Host bind to Donor Organ
Elicits Inflammatory Response
Transplanted Organ Lost

Solution:
Transgene in Donor for Complement-Inhibiting Protein
Birds and Fish

Birds

- traditional methods cannot be used because of avian embryogenesis differences
- no ES cells found
- ALV resistant chickens
  - transgene - defective ALV genome
  - makes viral RNA and protein
  - but blocks assembly of wt viral particles

Fish

- aquaculture
- transgene - growth hormone