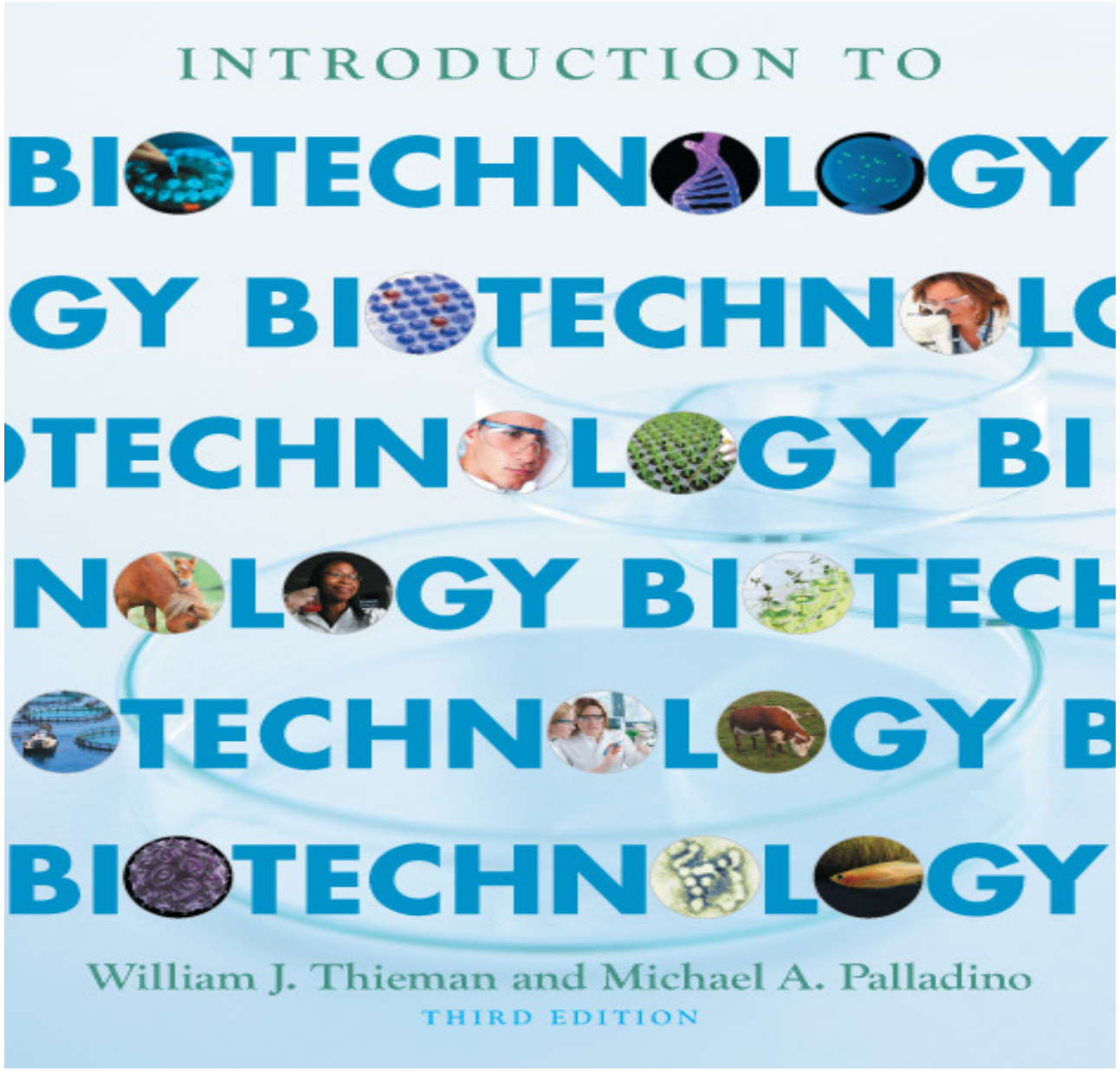


CHAPTER 7

Animal Biotechnology

PowerPoint® Lecture by:
Lisa Werner
Pima Community College

Introduction to Animal Biotechnology

- Genetically engineered animals can be used to
  - Develop new medical treatments
  - Improve our food supply
  - Enhance our understanding of all animals, including humans
- Presents tough scientific and ethical challenges

7.1 Animals in Research

- Animal Models
  - Many genetic and physiological similarities exist between animals and humans
  - Research using animals has been the key to most medical breakthroughs in the past century
    - Polio vaccine
    - Dialysis
    - Cataract surgery
  - Animal health is also directly benefited
    - Biotechnology has developed 111 USDA-approved veterinary biologics and vaccines

7.1 Animals in Research

- Animals most often used are
  - Purebred mice and rats
- Other species used include
  - Zebrafish, fruit flies, nematodes
- Dogs, monkeys, chimpanzees, cats make up less than 1 percent of total number of research animals

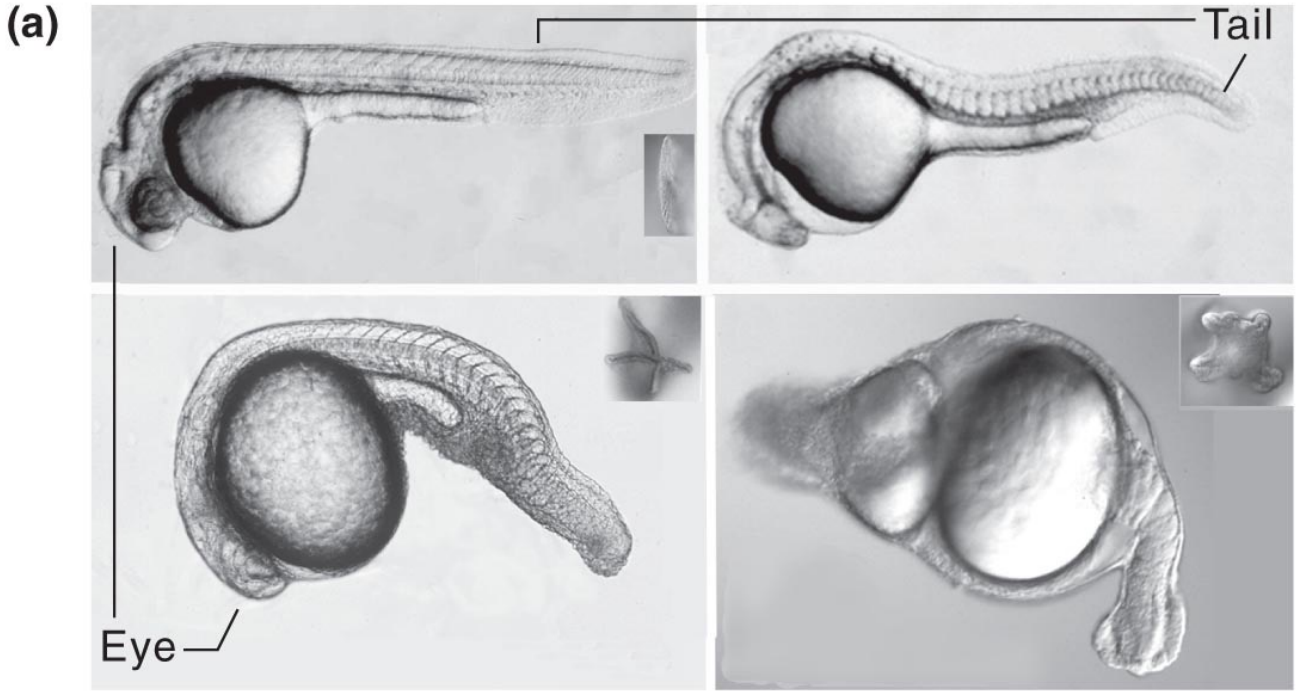
7.1 Animals in Research

- Zebrafish are about 3 cm in length, so can house large numbers in small spaces
- About 3 months between generations
- Average of 200 progeny per female
- Embryogenesis is completed in about 120 hours
  - Gut, liver and kidneys developed in the first 48-72 hours
- Can test drugs for toxicity or adverse effects in 5 days

7.1 Animals in Research

- Zebrafish are ideal for studies of development and for genetic research

1. Rapid growth
2. Embryo is visible inside the egg
3. Easy to transfer genes into the egg – do not need donor mother



7.1 Animals in Research

- Rats superior to mice for early drug toxicity tests
  - have more human like responses to drugs
  - larger size facilitates surgical and physiological experimentation
  - more toxicological data has been collected so better understood

7.1 Animals in Research

- Cats, dogs, and primates are used in specific instances when their particular biology is important to research
  - The lung and cardiovascular systems of dogs are similar to those of humans
  - Monkeys and chimpanzees are the only known animals that share humans' vulnerability to HIV

- The numbers of these animals used in experiments has been declining for the past 20 years

TABLE 7.1 FOOD AND DRUG ADMINISTRATION REQUIRED TESTING PHASES FOR DRUG APPROVAL

FDA Phase testing involves the use of animals for pre-clinical testing before allowed in humans. If the new drug candidate has proven to be non-toxic and has benefit, then it can be awarded and Investigational New Drug (IND)status. If it is successful in the three phases of human testing it can receive a New Drug Application (NDA) and likely approval for marketing. The FDA continues evaluating the NDA for another 2.5 years, resulting a total of about 12 years for a successful drug approval.

	Preclinical Testing		Phase I	Phase II	Phase III		FDA		Phase IV
Years	3.5		1	2	3		2.5	12 total	
Tested on	**Animals in the lab**		20–80 healthy volunteers	100–300 patient volunteers	1,000–3,000 patient volunteers				
Purpose	Assess safety and biological activity	File IND at FDA	Determine safety and dosage	Evaluate effectiveness and look for side effects	Verify effectiveness, monitor adverse reactions from long-term use	File NDA at FDA	Review process/ approval		Additional testing after approval required by FDA
Success rate	5,000 compounds evaluated			5 enter trials			1 approved		

Source: www.fda.gov/cder/handbook/develop.htm

7.1 Animals in Research

- Alternatives to Animal Models

1. Cell Culture
   - Preliminary screen to check the toxicity of substances
   - Can answer fundamental questions about biology
   - Cannot provide information about potential impacts on entire living organism
2. Computer Models
   - Simulate specific molecular and chemical structures and their interactions
   - Limited by programming and knowledge of how the physiological system works

7.2 Cloning

- In 1997, the first animal was cloned from the nucleus of another adult animal
  - Dolly the sheep

- Created controversy over the possibility of human cloning

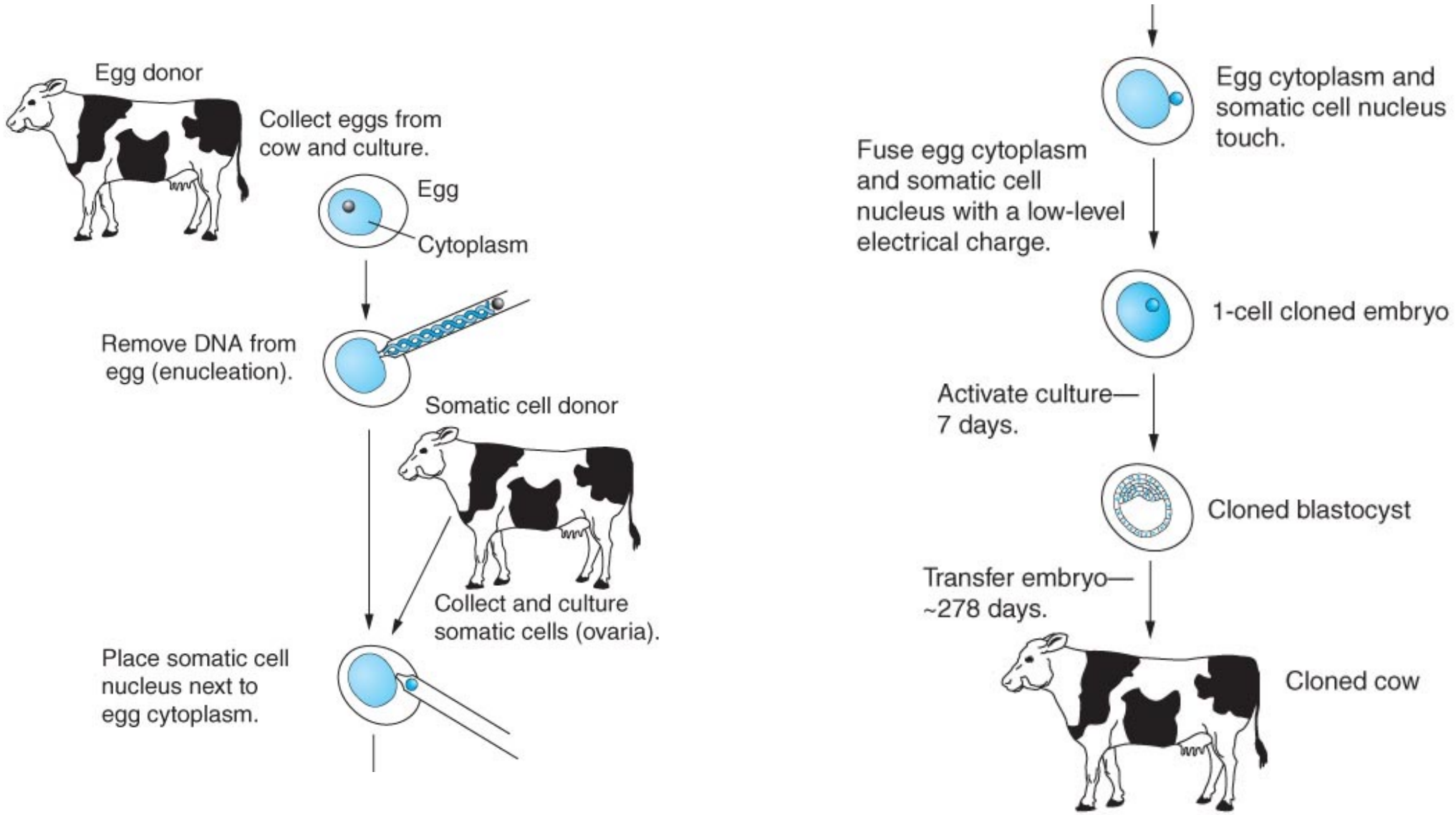
7.2 Cloning

- **Embryo Twinning** – splitting embryos in half
  - First step toward cloning
  - The first successful experiment produced two health calves that were identical twins
  - Procedure is relatively easy, but has limited applications
    - Results in identical twins, but exact nature of those twins is the result of a mix of genetic material from two parents
    - Common practice in the cattle industry today

- Dolly was a breakthrough because she was created from an adult cell
  - Exact duplicate of an adult with known characteristics

7.2 Cloning

- **Creating a Clone from an Adult**
  - DNA from donor cell must be inserted into an egg
  - Egg is prepared by enucleation
    - Pipette suctions out the nucleus
  - DNA from donor cell put into egg cell
  - Embryo is transferred to a surrogate mother for gestation
  - Sheep, pigs, goats, cattle, and a gaur have been cloned



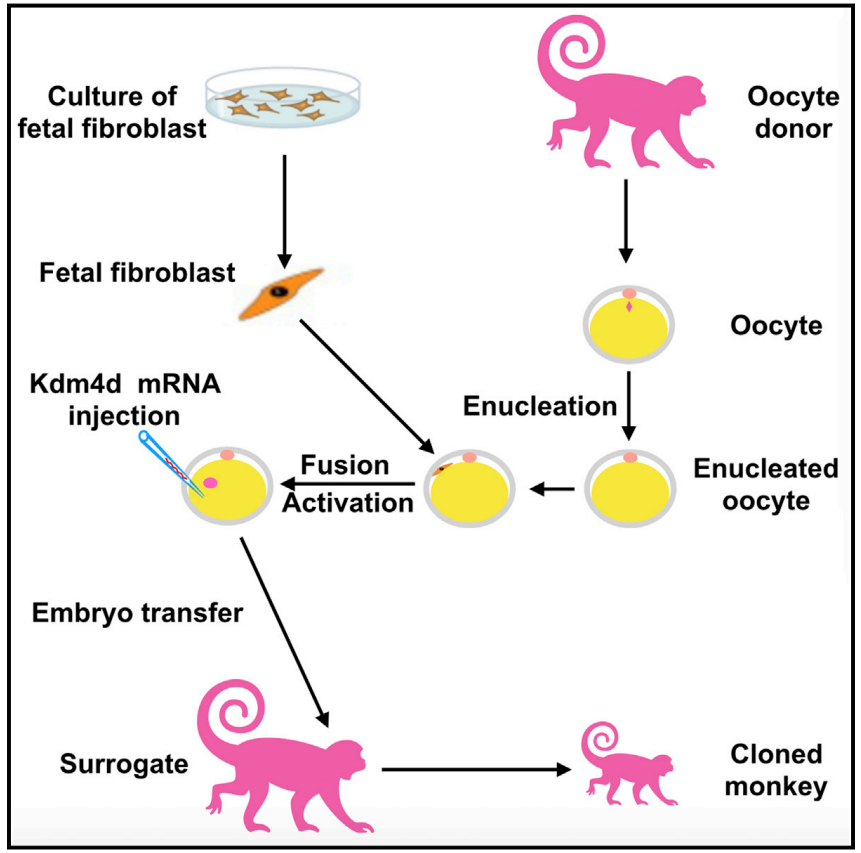
7.2 Cloning

- The Limits to Cloning
  - Donor cell must come from a living organism
  - Clones are not exactly identical
    - Shaped by experiences and environments
  - Present success rate is quite low
    - Dolly was result of 277 efforts
    - Carbon Copy (Cc) was only success out of 87 implanted clone embryos
  - Clones may be old before their time
    - Shortened telomeres

Cell

Cloning of Macaque Monkeys by Somatic Cell Nuclear Transfer

Graphical Abstract



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In Brief

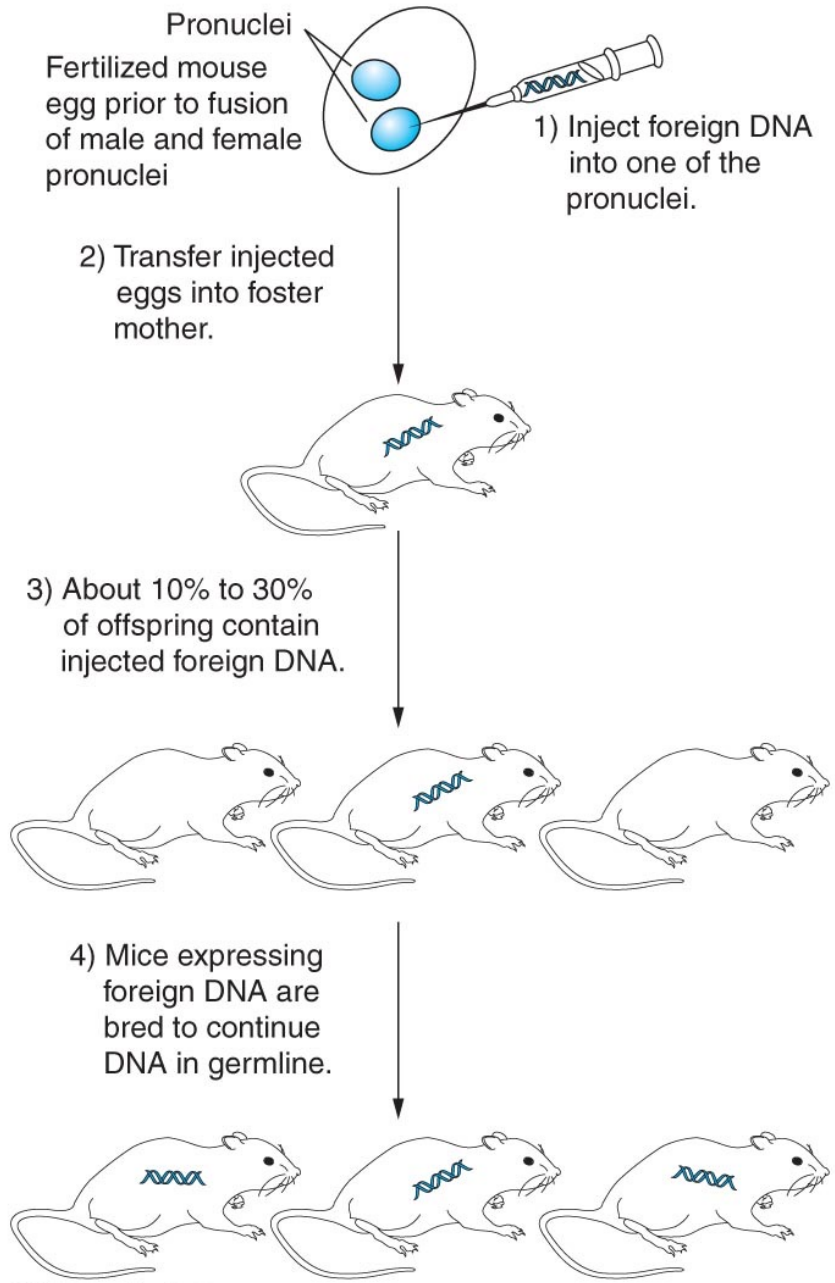
Generation of cloned cynomolgus monkeys by somatic cell nuclear transfer using fetal monkey fibroblasts.

Highlights

- Somatic cell nuclear transfer (SCNT) using fetal fibroblasts yielded two live monkeys
- Epigenetic modulators promoted development and pregnancy rate of SCNT embryos
- SCNT using adult cumulus cells yielded live births of monkeys that were short-lived
- Genetic analysis confirmed the clonal origin of the SCNT monkey offspring

7.3 Transgenic Animals

- Introducing New Genetic Material into Animals
  - **Retrovirus-mediated transgenics**
    - Infecting mouse embryos with retroviruses before the embryos are implanted
    - Size of transgene (transferred genetic material) is limited
  - **Pronuclear microinjection**
    - Introduces the transgene DNA at the earliest possible stage of development of the zygote
    - DNA is injected directly into nucleus of egg or sperm


Pronuclei
Fertilized mouse egg prior to fusion of male and female pronuclei
1) Inject foreign DNA into one of the pronuclei.
2) Transfer injected eggs into foster mother.
3) About 10% to 30% of offspring contain injected foreign DNA.
4) Mice expressing foreign DNA are bred to continue DNA in germline.

7.3 Transgenic Animals

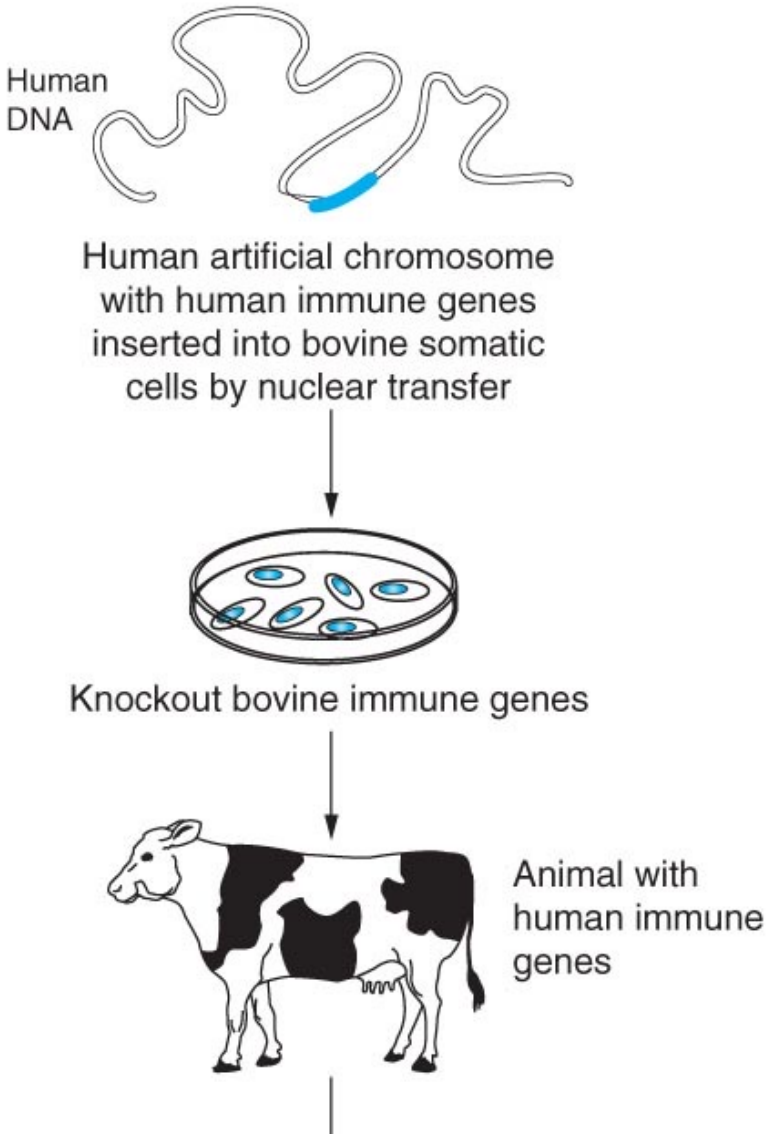
- Introducing New Genetic Material into Animals
  - **Embryonic stem cell method**
    - Embryonic stem cells are mixed with DNA and will absorb the DNA
  - **Sperm-mediated transfer**
    - Uses "linker proteins" to attach DNA to sperm cells
  - **Gene guns** can also be used on animal cells

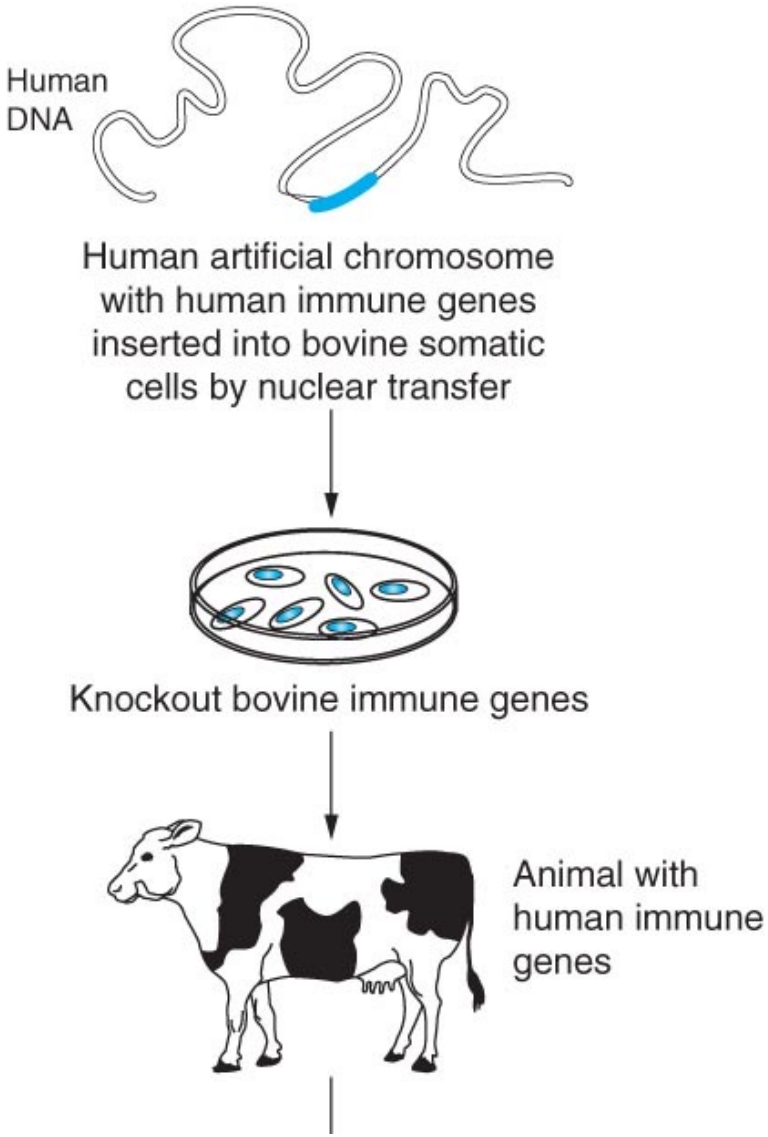
7.3 Transgenic Animals

- Can use gene transfer to improve the productivity of livestock
  - Introduce genes for faster growth rates or leaner growth patterns = quicker to market and less costly

7.3 Transgenic Animals

- Gene transfer can produce healthier foods
  - Tweaking the genes responsible for cholesterol production could result in healthier, lower-cholesterol eggs
  - Herman, a bull produced by transgenics nuclear transfer carries the human gene for lactoferrin which increases the availability of iron in milk





7.3 Transgenic Animals

- Animals can be engineered with genes that make them resistant to diseases

- The U.S. Department of Agriculture in Maryland created transgenic dairy cows that could resist mastitis
  - Highly contagious condition caused by the bacterium *S. aureus*

- The transgenic cattle have a gene that produces lysostaphin which kills *S. aureus*
  - Preliminary data show some resistance, though may not be fully protected

7.3 Transgenic Animals

- Improvements in food safety
  - Transfer of antimicrobial genes to farm animals could reduce the thousands of food poisoning deaths in the US each year
  - Also reduce the use of antibiotics in agriculture

7.3 Transgenic Animals

- Transgenic Animals as Bioreactors
  - Whole animals can serve as bioreactors to produce proteins
  - Gene for a desired protein is introduced via transgenics to the target cell
  - By using cloning techniques, cell is raised to become an adult animal
  - Produce milk or eggs that are rich in the desired protein
  - Goats are used to produce milk, rather than cattle, because they reproduce more quickly and are cheaper to raise

7.3 Transgenic Animals

- **Transgenic Animals as Bioreactors**
  - Biosteel may be used to strengthen bulletproof vests and suture silk
    - Spiderweb protein, one of the strongest givers on earth has been transferred to goats where it is expressed in their milk
  - A human gene, *Atryn*, is needed by patients with a hereditary clotting deficiency
    - Transgenic goats developed by the company GTC have *Atryn* under control of a mammary specific promoter so can produce the anticlotting protein faster, more reliably and more cheaply than traditional pharmaceutical methods

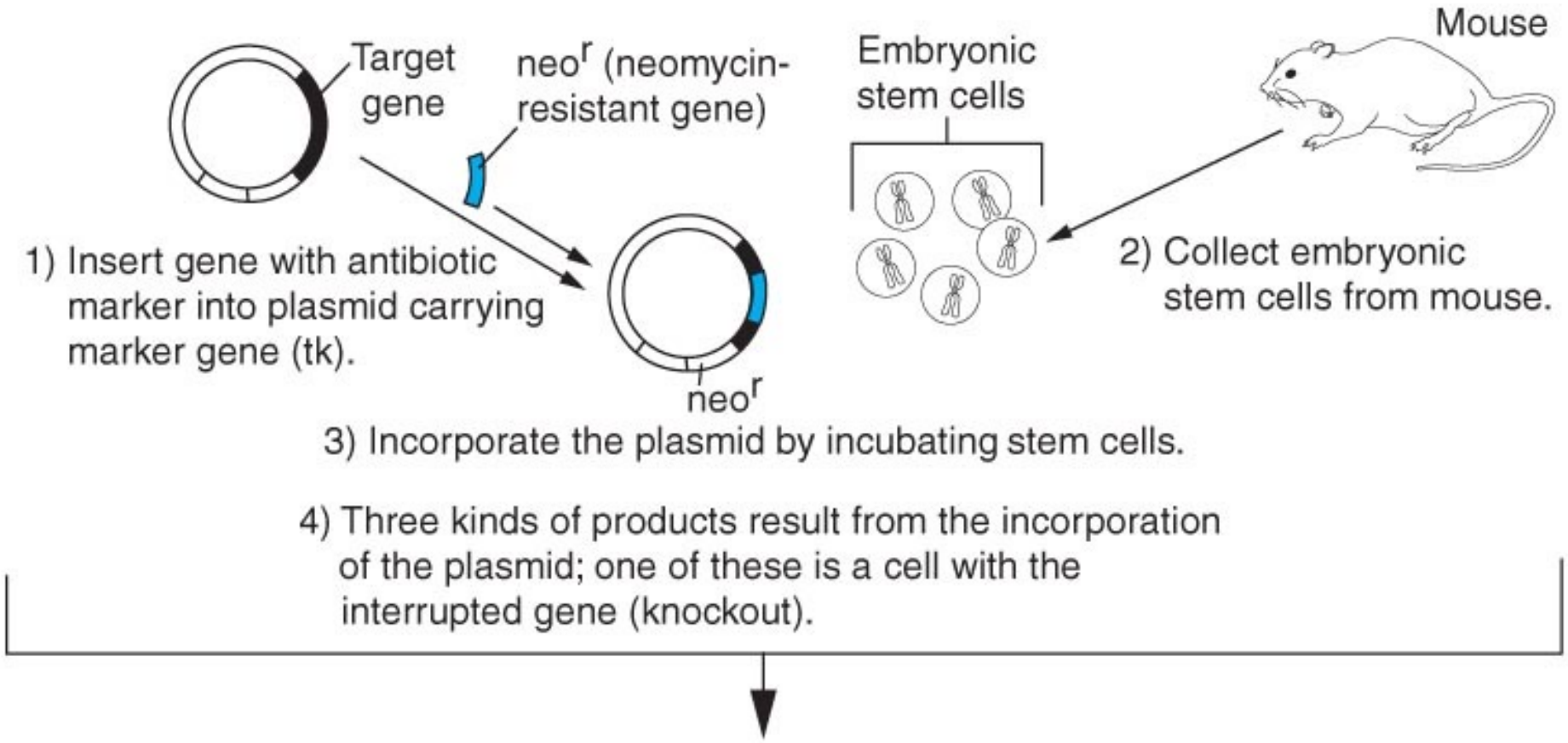
7.3 Transgenic Animals

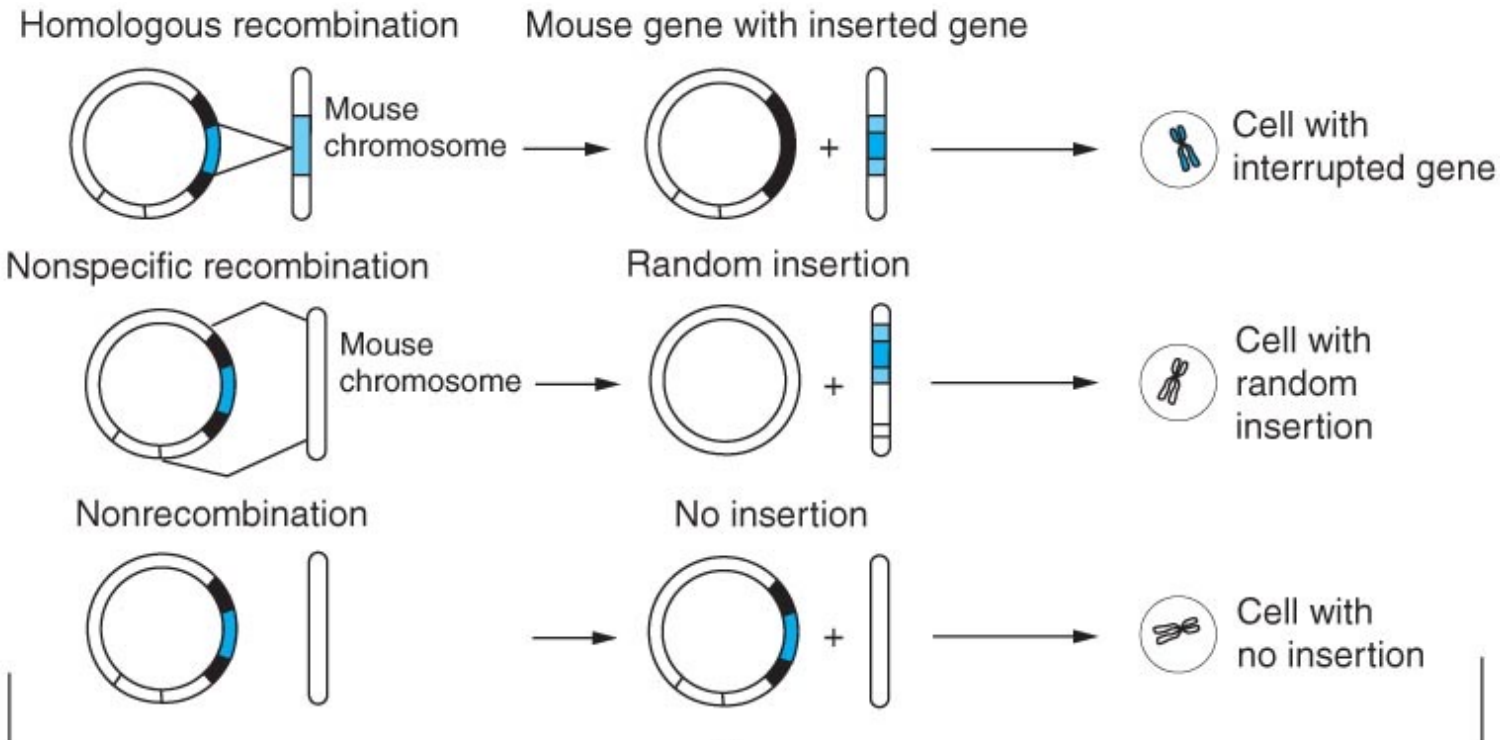
- **Knockouts:** A Special Case of Transgenics
  - Mice that have been genetically engineered so that a specific gene is disrupted
  - DNA is modified and added to the embryonic stem cells, where it recombines with the existing gene on a chromosome
    - Called homologous recombination
  - Modified ES cells are introduced into normal embryo and embryo is implanted into a mother

7.3 Transgenic Animals

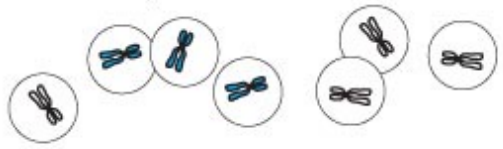
- Knockouts: A Special Case of Transgenics
  - The mouse pup is a **chimera** – some cells are normal and some are knockouts
  - Two generations of breeding are required to produce complete knockouts

- Knock-in animals – have a human gene inserted to replace their own counterpart





5) Collect cells.



6) Select with neomycin and tk substrate. Only the cells that have the interrupted gene will grow in the medium and resist the drug that kills cells with functional tk gene.

7) The cells that survive are microinjected into mouse embryo.

7.3 Transgenic Animals

- Being able to study knock out animals will provide new insights into human diseases
  - diabetes, cystic fibrosis, muscular dystrophy

- The breast cancer mouse, patented in 1988 by Harvard University scientist, has been used extensively to test new breast cancer drugs and therapies

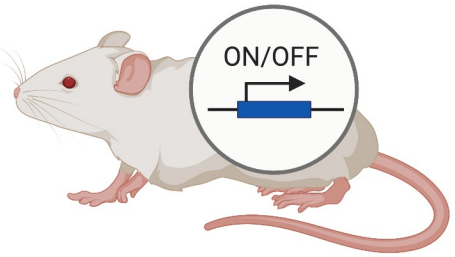
Genetic and Genomic Variations (Mouse Model)

Human genetic material

Mouse genetic material

Inducible and conditional

Temporal or spatial control of gene expression



Knockout

Functionally delete a gene

Knockin

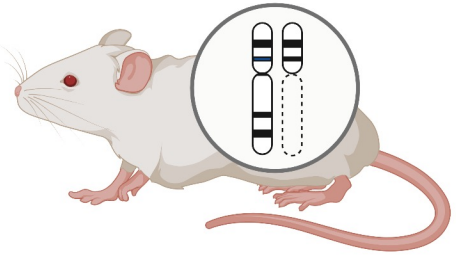
Replace mouse sequence with another sequence expressed physiologically

Genomically humanized

Replace mouse sequence with orthologous human genomic region

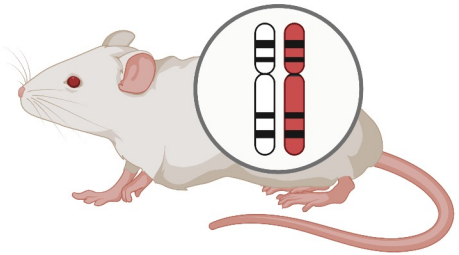
Chromosome engineered

A chromosome region is duplicated or deleted



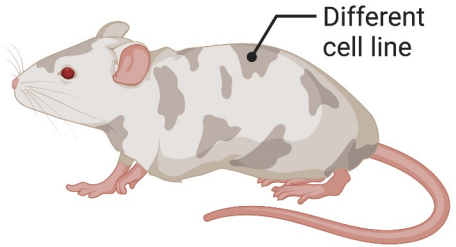
Transchromosomal model

Human chromosome added to the mouse genome



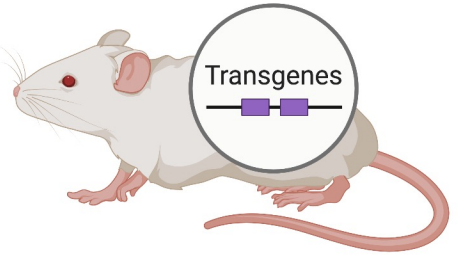
Chimeras

Mice consisting of two different cell lines, e.g., mouse-mouse or mouse-human



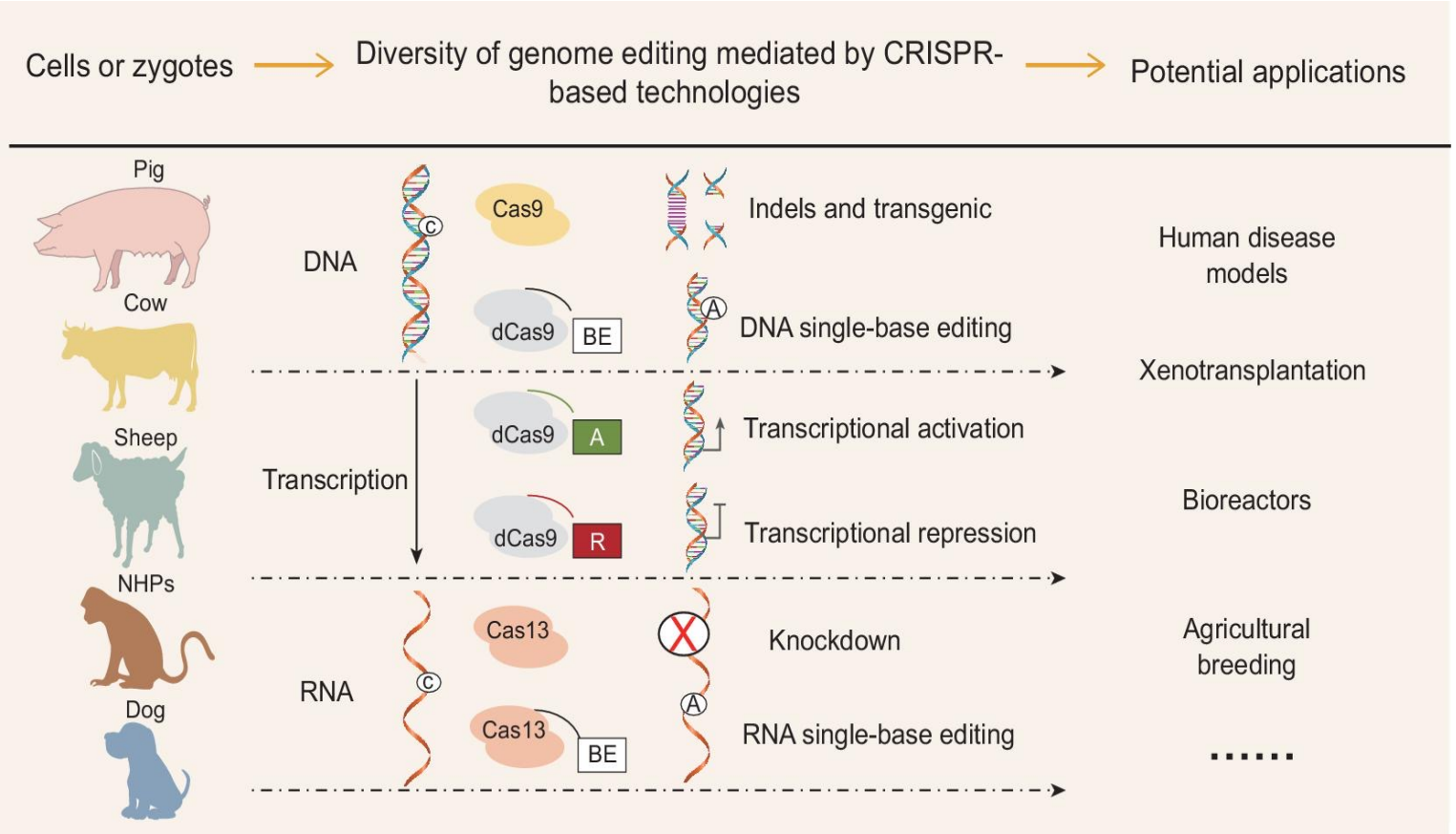
Transgenic

Multiple copies of exogenous DNA



7.3 CRISPR/Cas technology in animals

- Major strategies to recruit DNA- and RNA-targeting and modifying enzymes via the CRISPR/Cas systems



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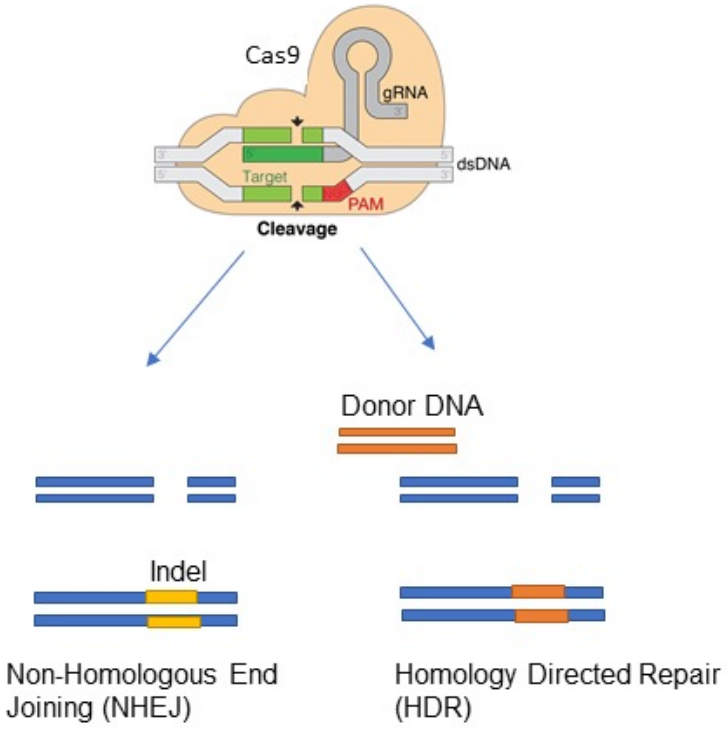
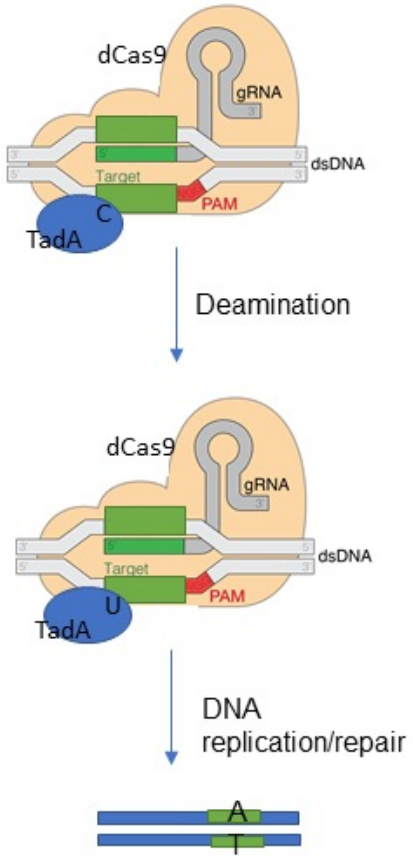
7.3 CRISPR/Cas technology in animals

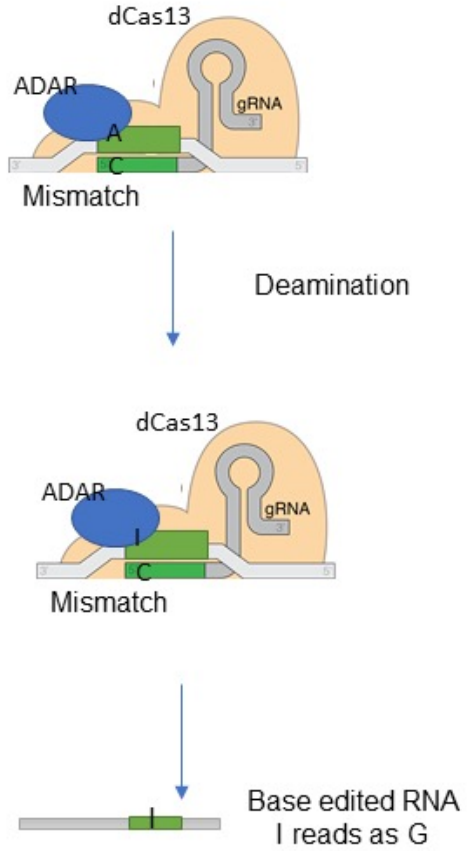
- Base-editing by CRISPR/Cas systems



GENE editing with CRISPR



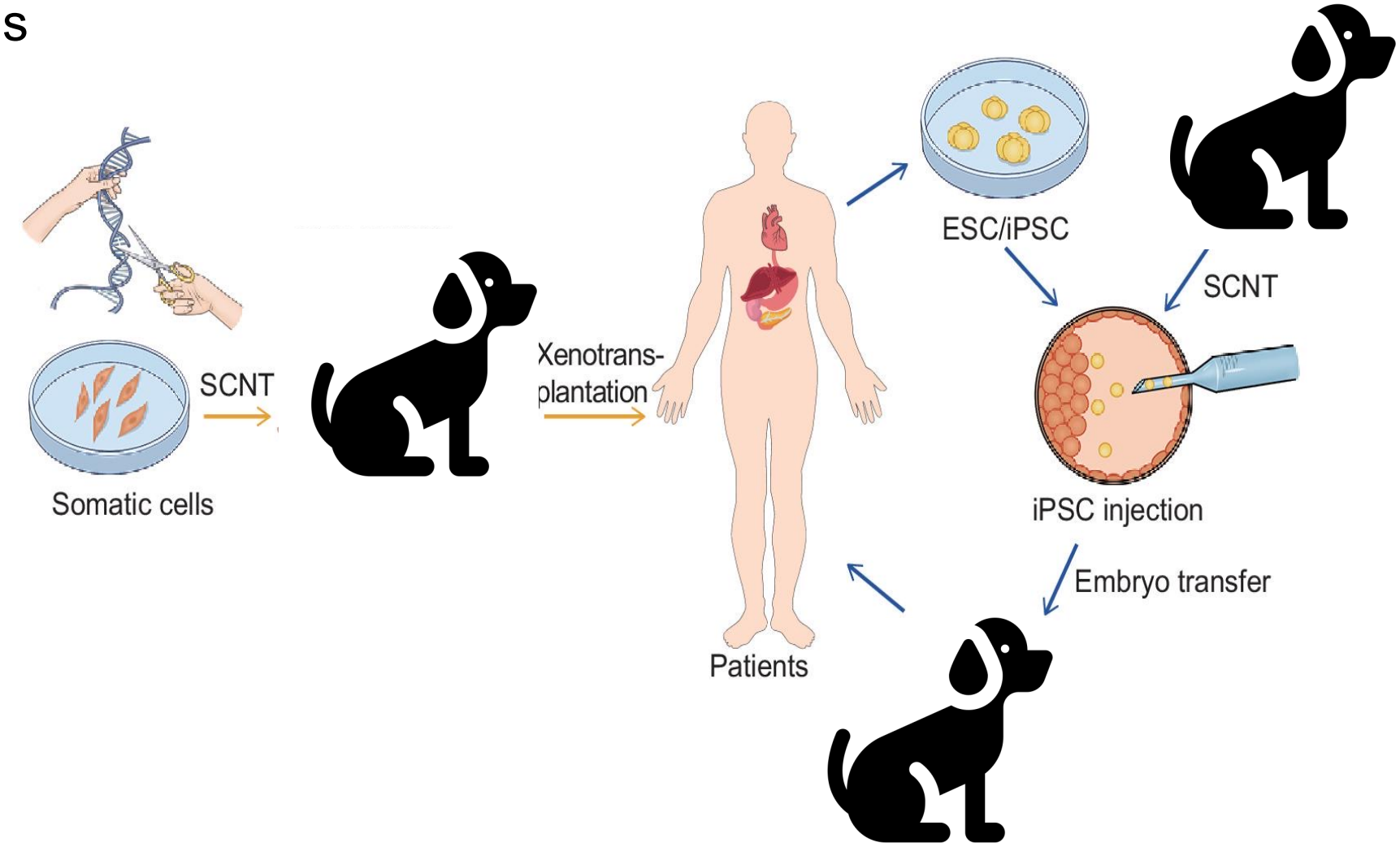
DNA base editing



RNA base editing

7.3 CRISPR/Cas technology in animals

- Strategies to manipulating animal organs to make them compatible to humans



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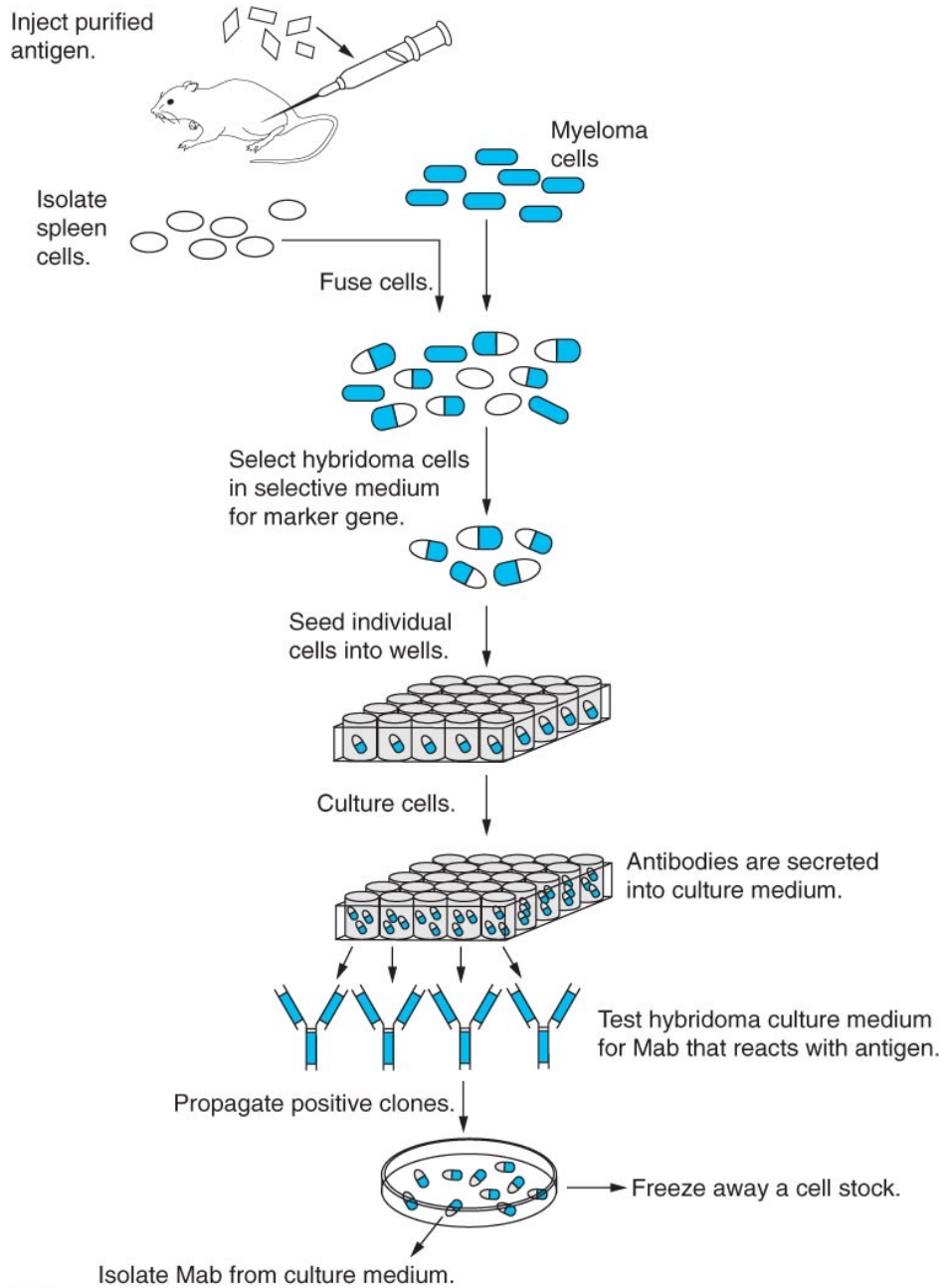
7.4 Producing Human Antibodies in Animals

- Production of Monoclonal Antibodies (Mabs)
  - Mouse or rat inoculated with the antigen (Ag) to which an antibody is desired
  - Spleen harvested after an immune response is produced
  - Spleen cells are fused with a specialized myeloma cell line that no longer produces an antibody of its own
    - Myeloma is an antibody-secreting tumor

7.4 Producing Human Antibodies in Animals

- Production of Monoclonal Antibodies (Mabs)
  - The resulting hybridoma (fused cells) grows continuously and rapidly like the tumor and produces the antibody specified by the spleen cells

- Mabs used to treat
  - Cancer
  - Heart disease
  - Transplant rejection


Inject purified antigen.
Myeloma cells
Isolate spleen cells.
Fuse cells.
Select hybridoma cells in selective medium for marker gene.
Seed individual cells into wells.
Culture cells.
Antibodies are secreted into culture medium.
Test hybridoma culture medium for Mab that reacts with antigen.
Propagate positive clones.
Freeze away a cell stock.
Isolate Mab from culture medium.