Previous Class

Animal cloning

Embryo twinning

Cloning from adult cells

Benefits of cloning

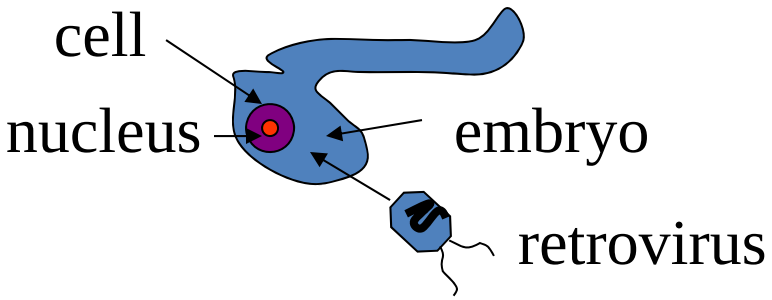
Limitations of cloning

Early experiments on transgenic animals

- A new gene was added to a cell grown in a tissue culture and the effects on that one cell were observed.
- With the introduction of cloning, a gene could be added to many cells, and all the cells could be screened to see which one(s) contained the gene.
  - Each cell that contained the gene could then be used to grow a complete animal using cloning technology

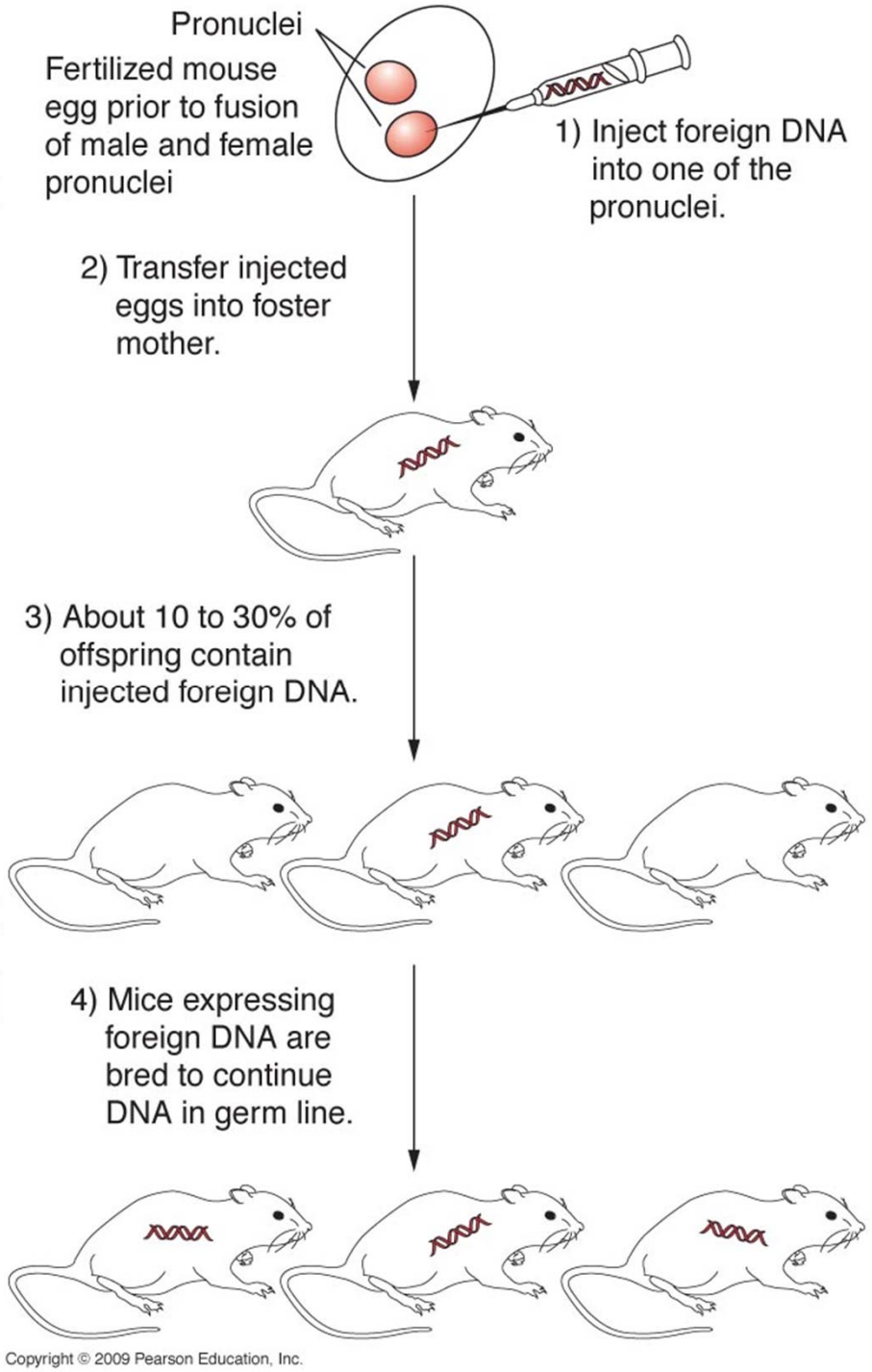
Transgenic techniques

- Retrovirus-mediated transgenesis
  - infect mouse embryo with retroviruses before the embryos are implanted into an animal for gestation.
    - Retrovirus acts as a vector for the new DNA
    - size of new DNA is limited
    - viruses genetic material can interfere with embryo development
    - not very efficient



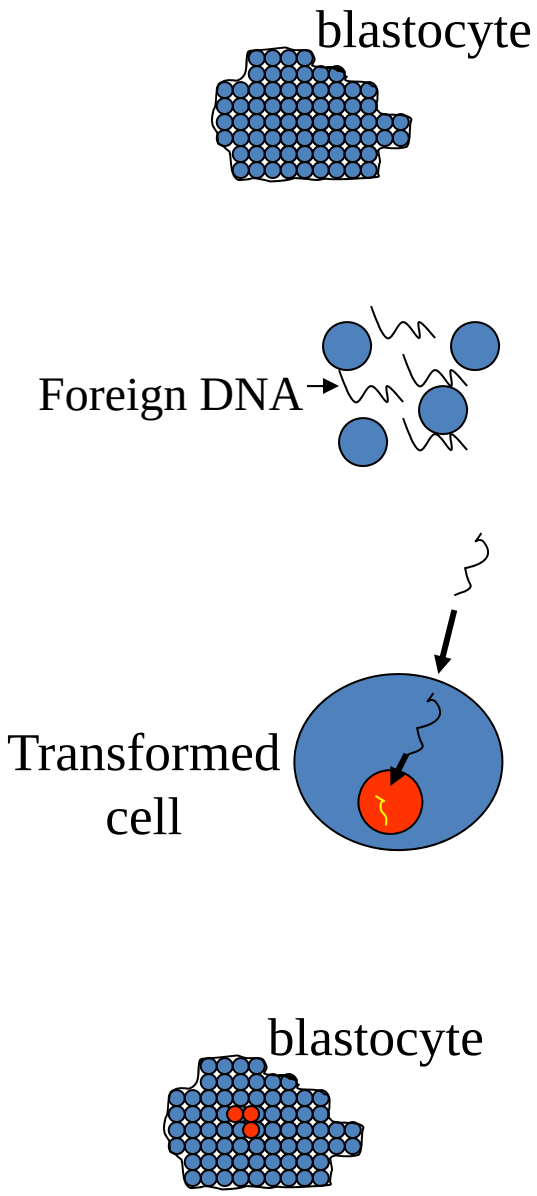
Pronuclear injection

- Introduction of foreign DNA at earliest possible stage of development of the zygote (fertilized egg)
- Just before the egg and sperm cells join, DNA is injected into the nucleus of either cell.
- Since the DNA is injected with a syringe, no vector is required and no vector genetic material is introduced that could complicate outcome



Embryonic stem cell method

- Embryonic stem cells are collected from inner cell mass of blastocytes
- Cells are mixed with foreign DNA
  - some cells take up the foreign DNA and incorporate it into cell's own DNA in the nucleus and are "transformed"
- Transformed cells are injected into the inner cell mass of the host blastocyte for differentiation and development



Transgenic Animals

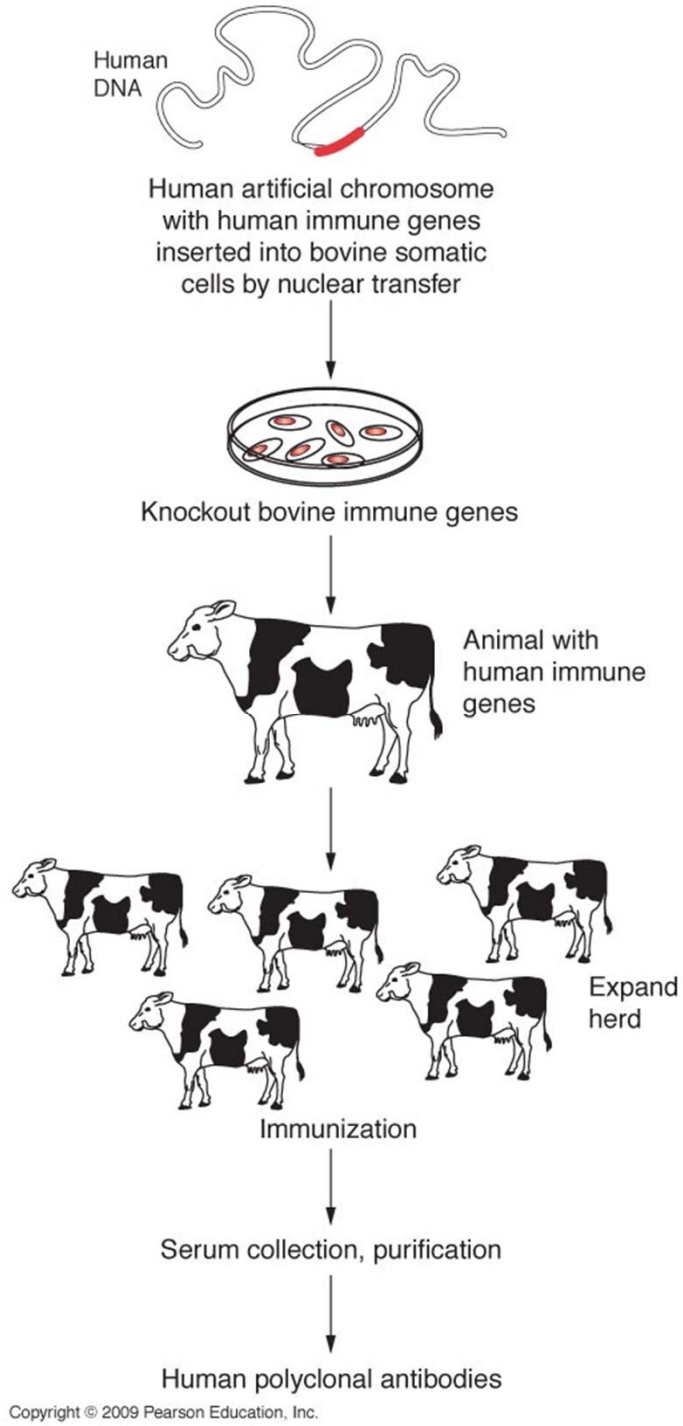
- Introducing New Genetic Material into Animals
  - **Sperm-mediated transfer**
    - Uses “linker proteins” to attach DNA to sperm cells
  - **Gene guns** can also be used on animal cells

Improving Agricultural Products with Transgenics

- Faster growth rates or leaner growth patterns (improve the product), more product
- Lower-cholestrol eggs
- Increase nutritional content-lactoferrin
- Turning the animals into efficient grazers
- Transfer antimicrobial genes to farm animals (for example, trangenic cows contain genes for producing proteins that can kill *S. aureus* which infect cows)
- EnviroPigs – transgenic pigs expressing phytase in their saliva which degrades phosphates in pigs' food thus reducing phosphorus pollution in pig farms

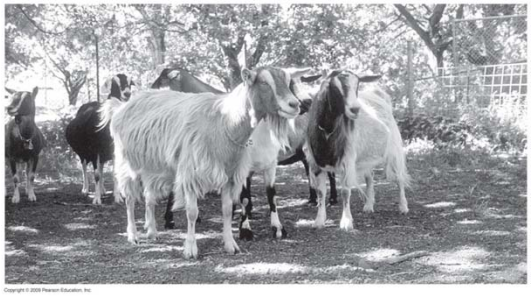
Transgenics to make milk healthier for humans

- Lactoferrin-protein that binds iron needed by human babies for development
  - introduce gene for this protein into cells of cow that are responsible for milk production
- Human immune genes introduced into cows as a factory for **human antibody production**.



Transgenic Animals as Bioreators

- Biosteel otherwise known as spider silk, cloned into goat milk ("silkmilk" goats)
- Pharmaceutical proteins used for treatment of human diseases –
  - eg, *Atryn* gene produce an anticlotting agent needed by patients with hereditary deficiency is produced in goats milk
- Goats reproduce faster than cows and are cheaper than cows
- Hens also make good bioreactors in that they are cheap and a lot of eggs are produced at one time
  - Source of medical products such as lysozyme and attenuated virus vaccines



Transgenics as a means of deleting genes and their functions

- Deleting a gene is a way of determining what its function is in the cell
- Active gene is replaced with a gene that has no functional information
- When the gene is “knocked out” by the useless DNA, the trait controlled by the active gene is eliminated from the animal

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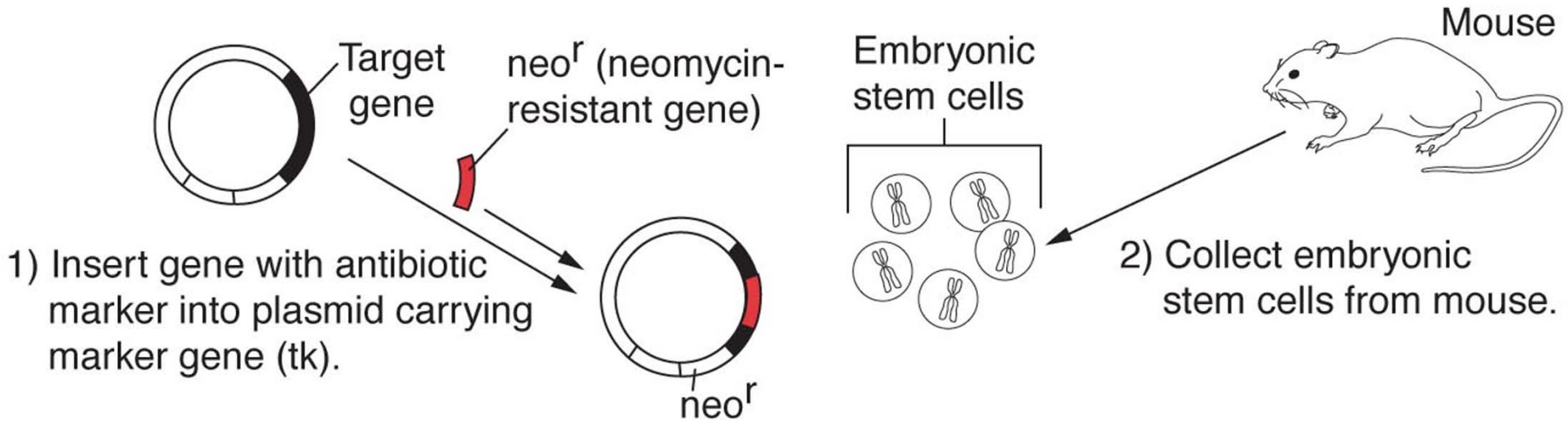
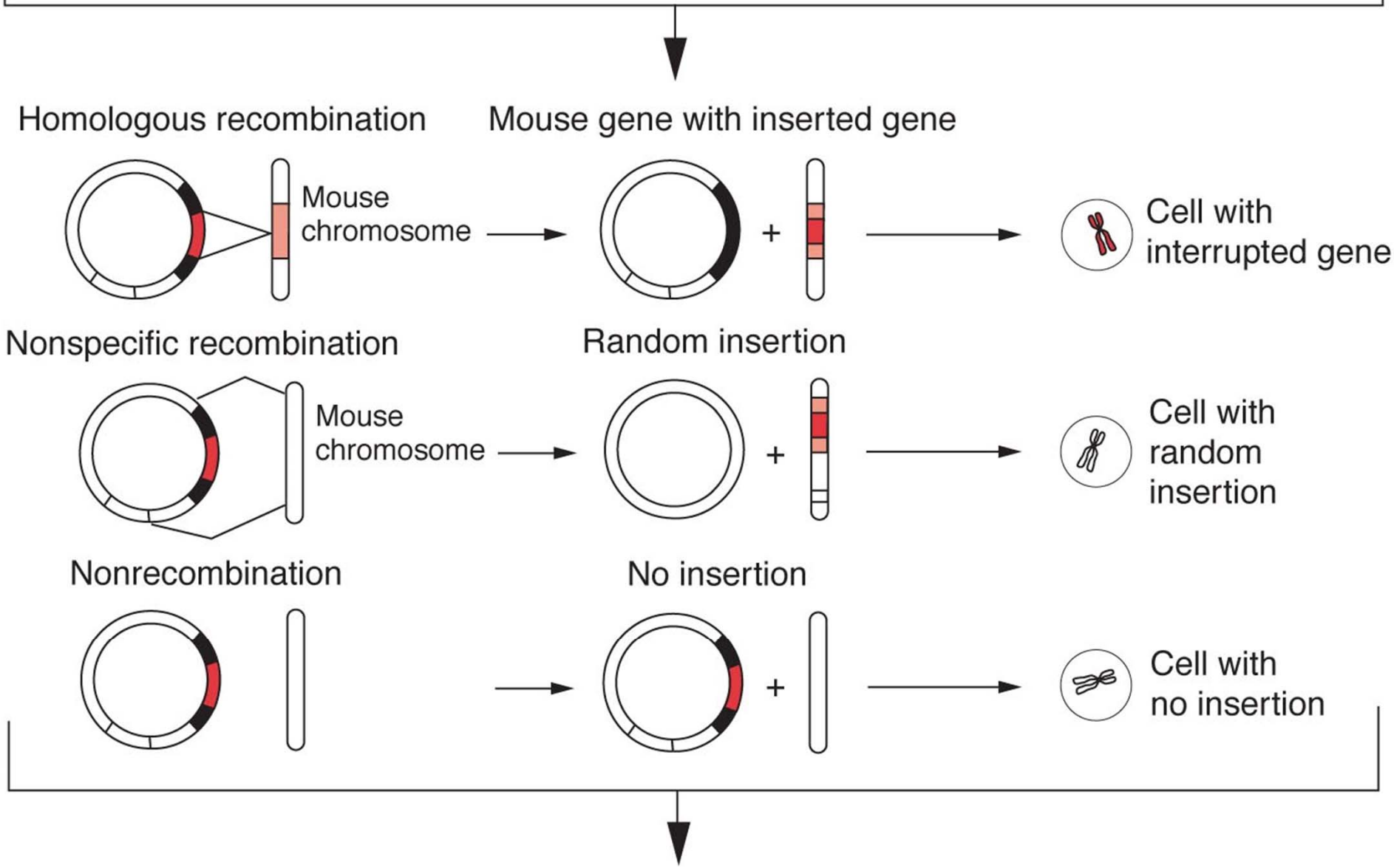
- **Knockouts**: A Special Case of Transgenics
  - Mice that have been genetically engineered so that a specific gene is disrupted
  - DNA is modified and added to the embryonic stem cells, where it recombines with the existing gene on a chromosome
    - Called homologous recombination
  - Modified ES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

Knockout Mice

Knockout mice begin as embryonic stem cells with specifically modified DNA that has been prepared by recombinant techniques. The modification results in a nonsense mutation in the normal gene of the animal.



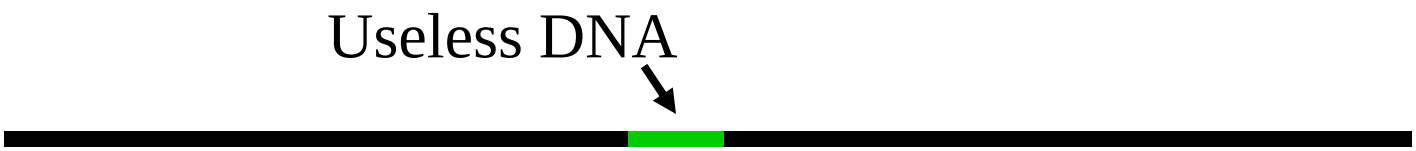
4) Three kinds of products result from the incorporation of the plasmid, and one of these is a cell with the interrupted gene (knockout).



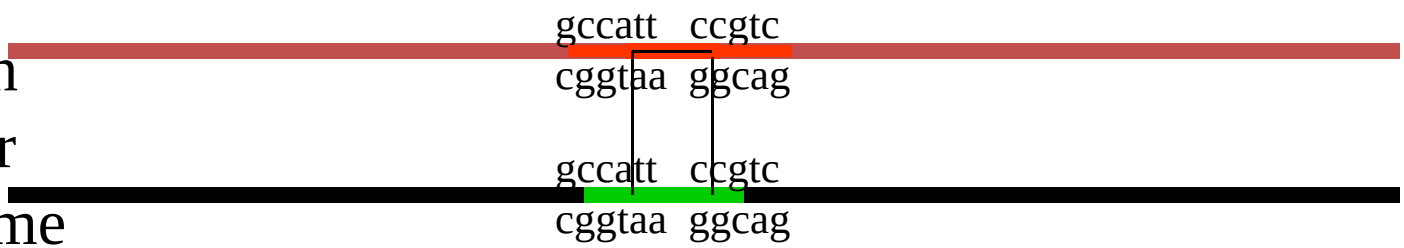
Homologous recombination within target gene

Chromosome with normal gene

Plasmid with useless DNA



Recombination between vector and chromosome



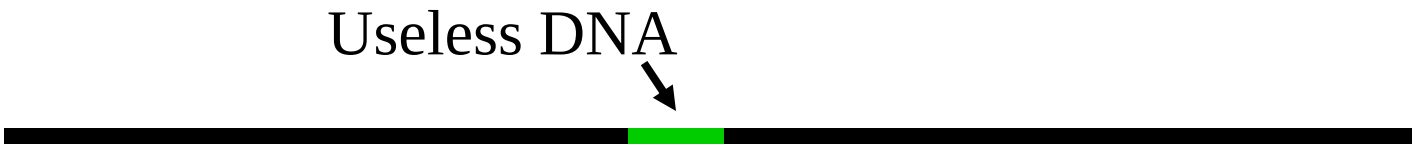
insert section of DNA of gene on vector into a section of DNA containing good gene on chromosome of stem cells.

Chromosome is modified with a useless form of the gene. Look for a trait that has changed

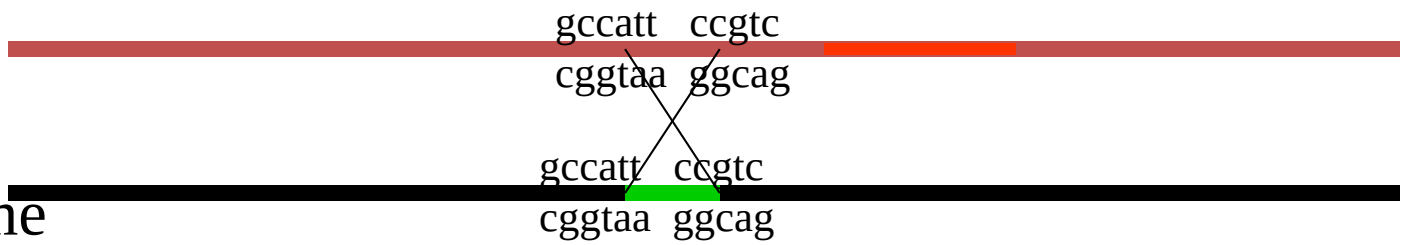
Random insertion of useless gene at a location other than the target gene

Chromosome with normal gene

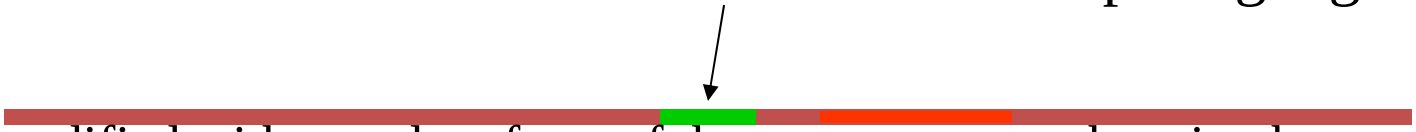
Vector with useless DNA



Recombination between vector and chromosome

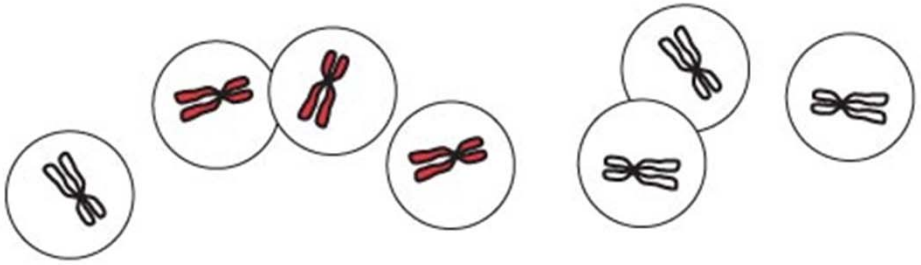


Insert section of DNA of useless gene on vector into a section of chromosome that does not disrupt target gene.



Chromosome is modified with a useless form of the gene at some other site than target gene

5) Collect cells.

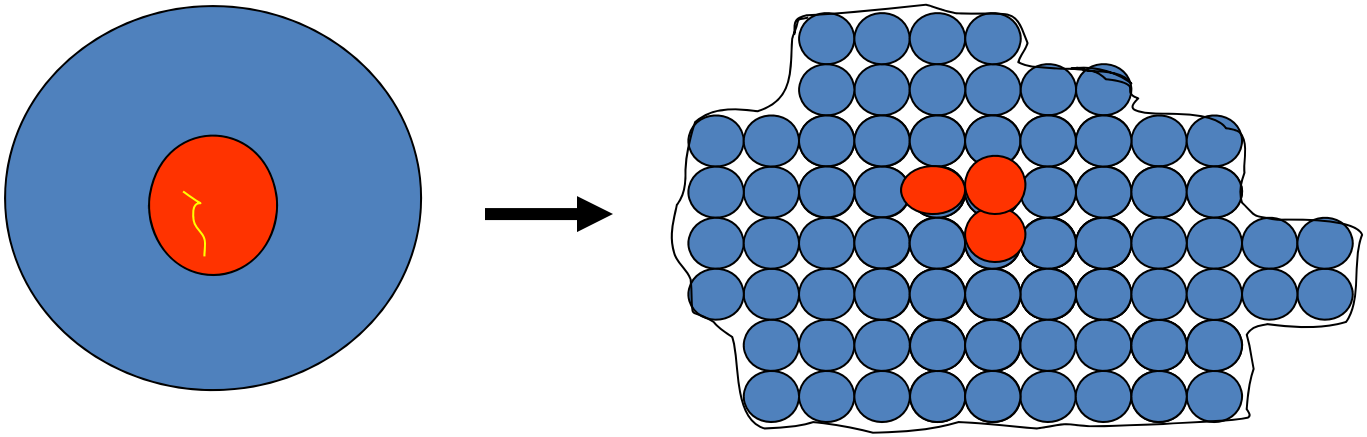


6) Select with neomycin and tk substrate. Only the cells that have the interrupted gene will grow in the medium and resist the drug that kills cells with functional tk gene.

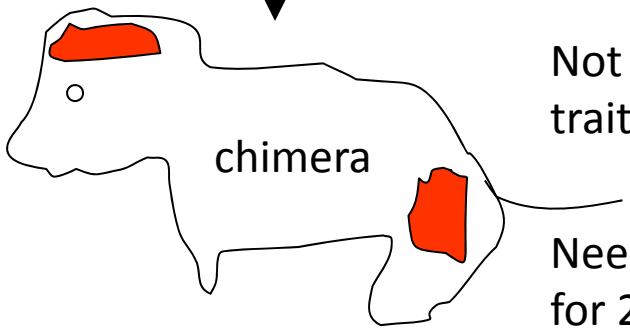
7) The cells that survive are microinjected into mouse embryo.

Transformed stem cell

Blastocyte



Knockout mouse with nonfunctional gene in all its differentiated somatic cells



Not all cells had the trait changed

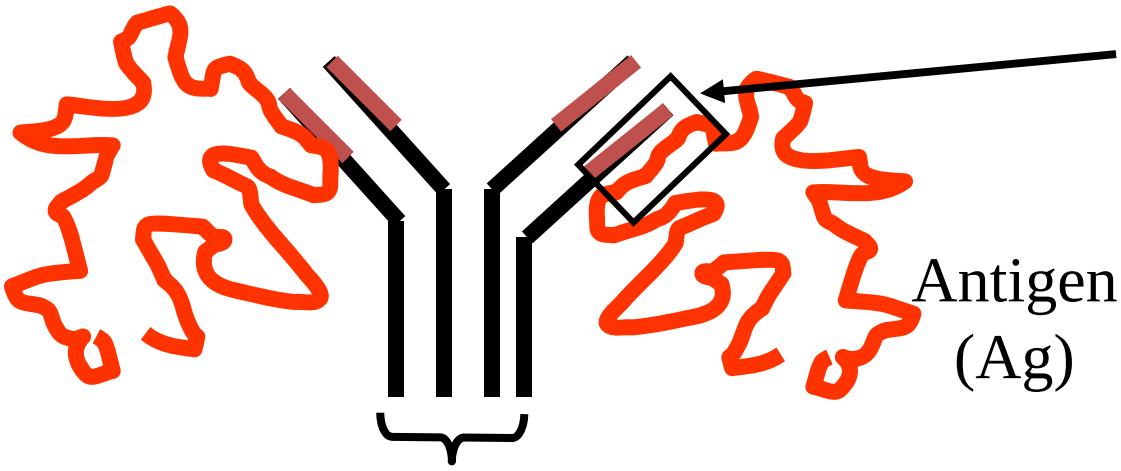
Need to crossbreed for 2 generations to get all cells to lose trait.

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

Antibodies

- One limitation in the therapeutic use of antibodies is the production of a specific antibody in large quantities
- Antibodies are proteins whose structure gives it the ability to bind very specifically to other proteins



Antibodies

- Antibodies could be designed that target and inactivate cancer cells in our bodies.

- **Myelomas**: antibody-secreting tumors

- Monoclonal Abs (mAb) are produced from myeloma cells that produce an Ab that reacts with only one region of an antigenic protein

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

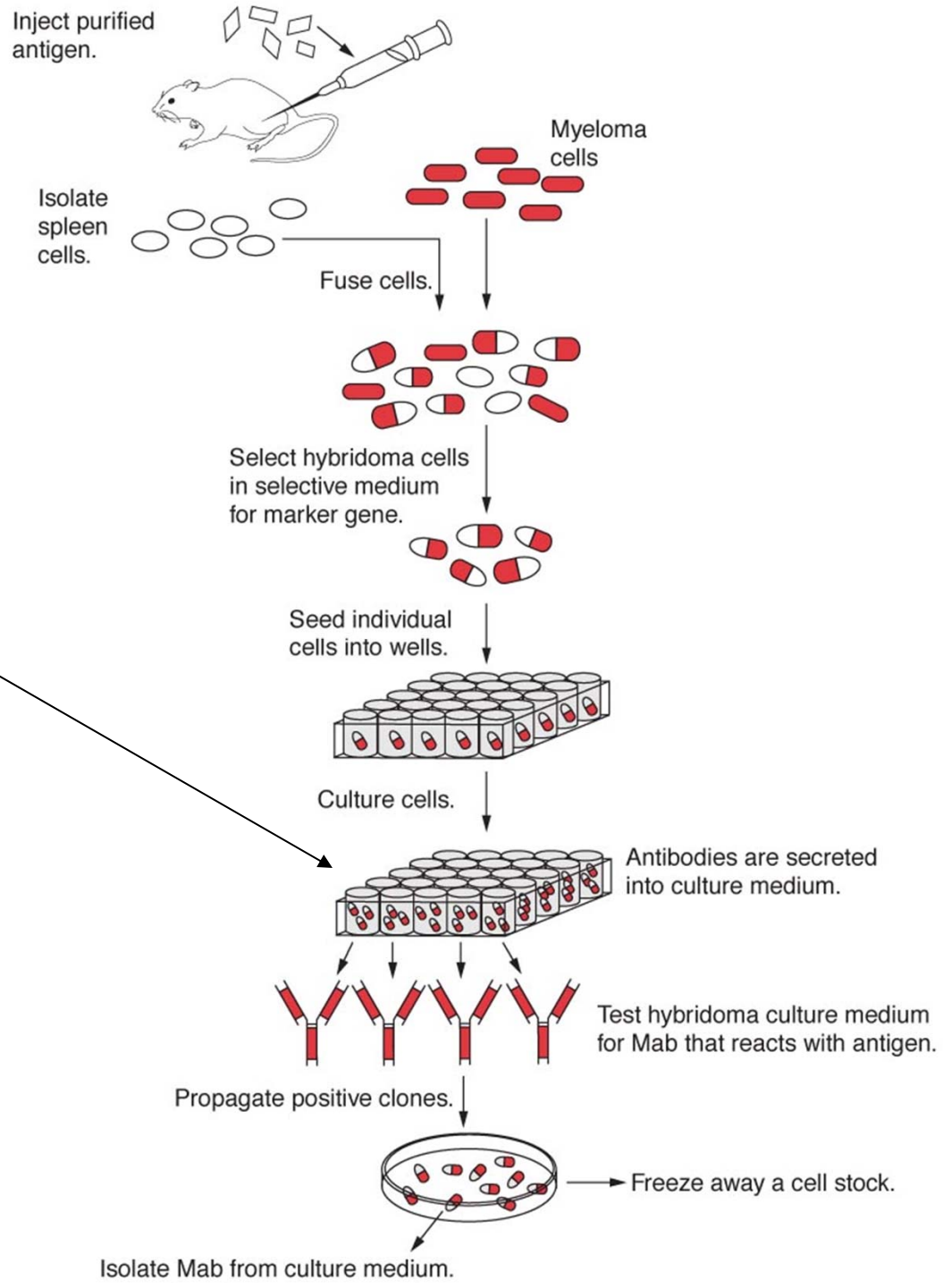
Producing Human Antibodies in Animals

- 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

Making cells that produce monoclonal antibodies

The specific antibody is released into the culture medium and recovered

Once a cell line is identified that produces an antibody against a specific antigen, it can be replicated and the cells frozen until needed to make the specific antibody



Producing Human Antibodies in Animals

- Abs derived from mouse hybridoma cells provoked an immune response in human patients.
- Mouse hybridomas cause the HAMA response
  - Human antimouse antibody response
- Working to solve this problem by creating antibodies in different organisms

Producing Human Antibodies in Animals

- Panitumab – human Ab used against epidermal growth factor receptor as a treatment for colorectal cancer pateints
- Does not produce HAMA response
- Ab produced from transgenic mice
- But mouse antibody machinery is inactivated by removing genes responsible for it and human equivalent genes were added by homologous recombination