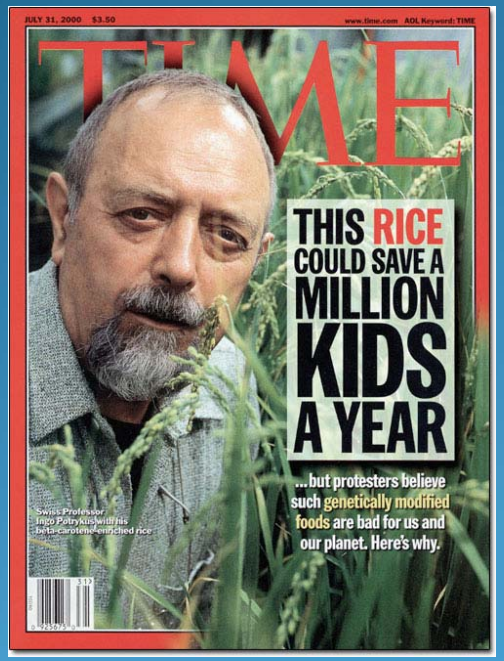
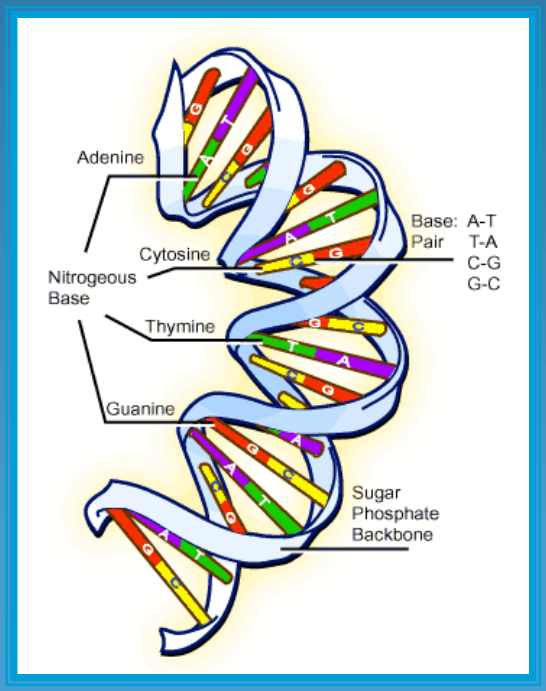
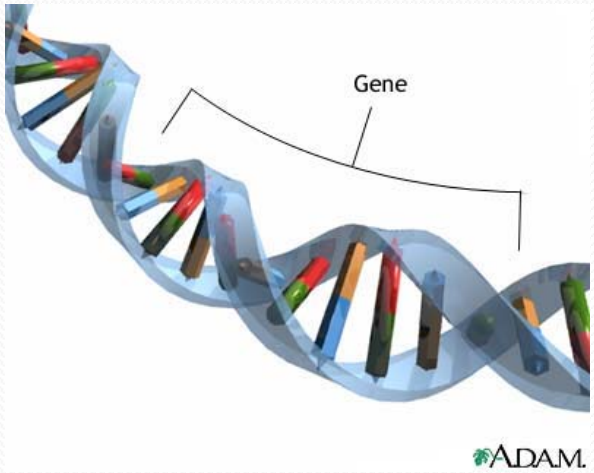
Genetically Modified Plants and Animals





The Manipulation of Genes

- **Gene** – a segment of DNA in a chromosome specifying a particular protein or polypeptide chain, a tRNA or an rRNA
- **Recombinant DNA** – any artificially created DNA molecule which brings together DNA sequences that are not usually found together in nature. (Primrose & Twyman, 2006)



The Manipulation of Genes (cont'd)

- **Gene Manipulation** – a variety of sophisticated techniques for the creation of recombinant DNA, which are then introduced into living cells. (Primrose & Twyman, 2006)
- **Genetic Engineering** – the isolation, manipulation, recombination, and expression of DNA often for the development of genetically modified organisms.

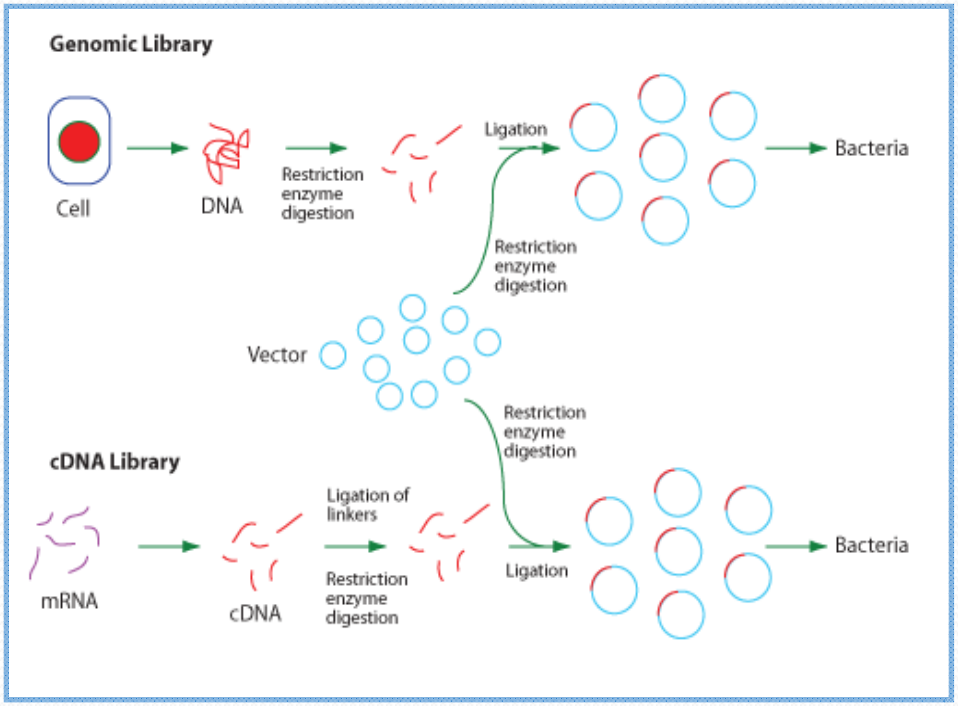
Finding the Right Gene

- All genetic engineering starts by identifying and isolating the correct clone containing the gene
- This can be done by:
  - Making *gene libraries* from total genomic DNA
  - Or, if the gene is identified, cloning the DNA fragment by PCR (polymerase chain reaction)

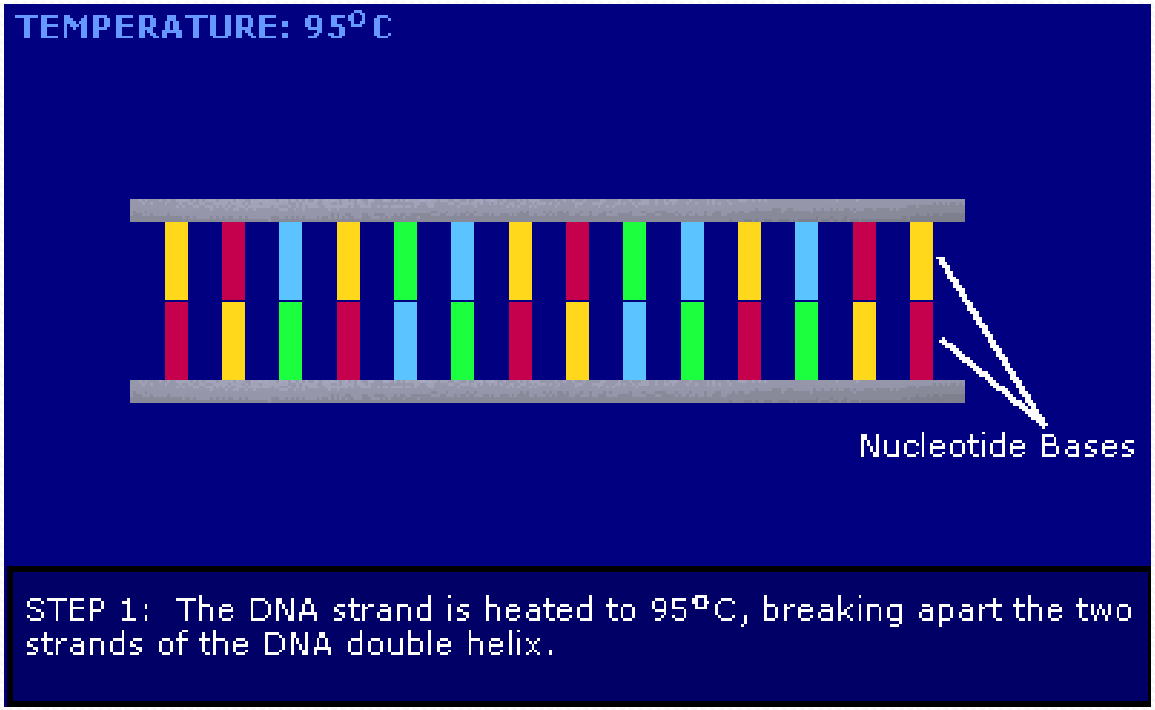
Gene Libraries

- **cDNA Libraries** - These libraries will only contain DNA from transcribed genes.

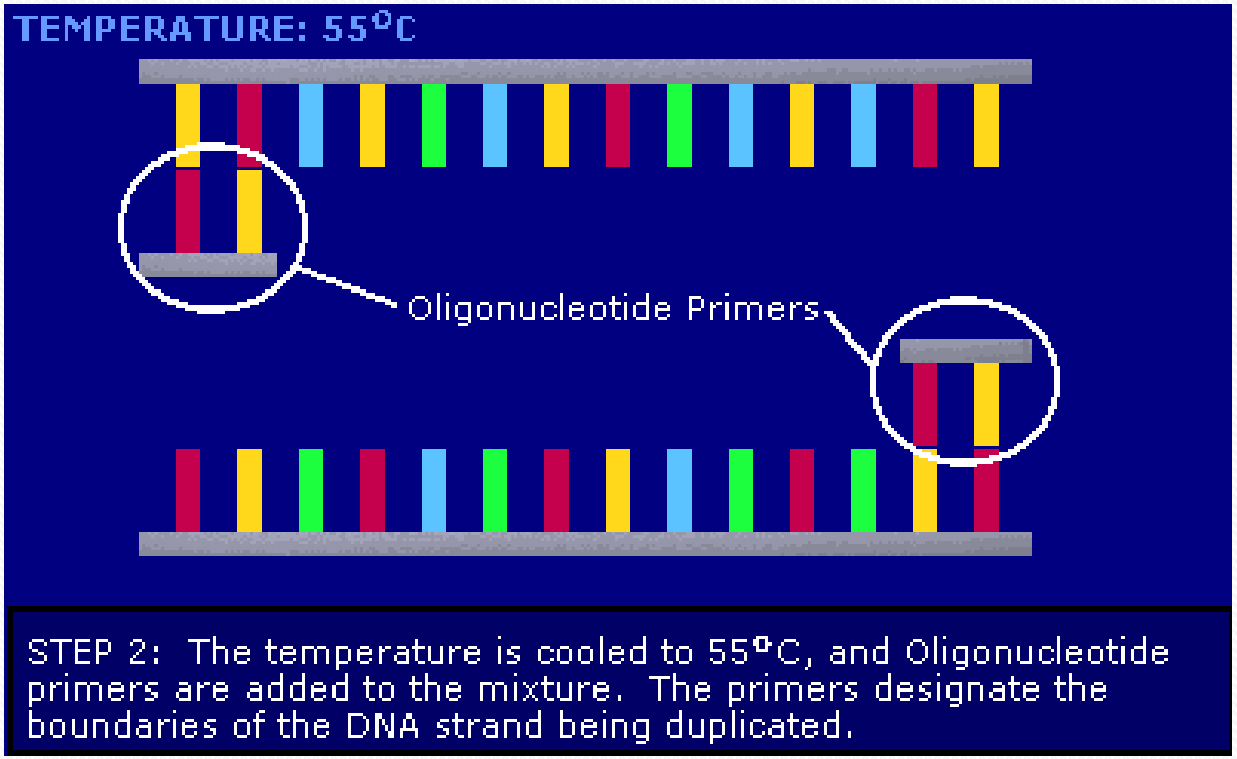
- **Genomic DNA Libraries** - These libraries will contain all DNA sequences



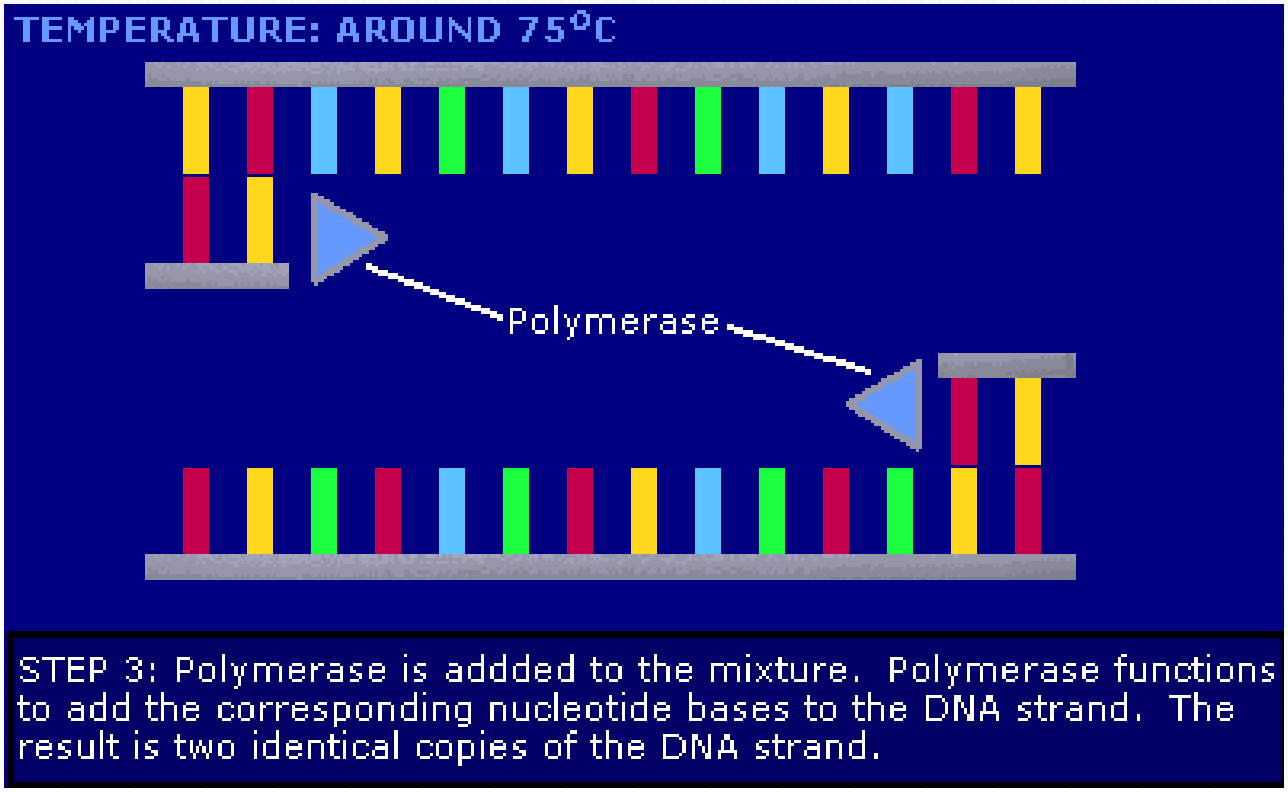
Polymerase Chain Reaction (PCR) Step 1



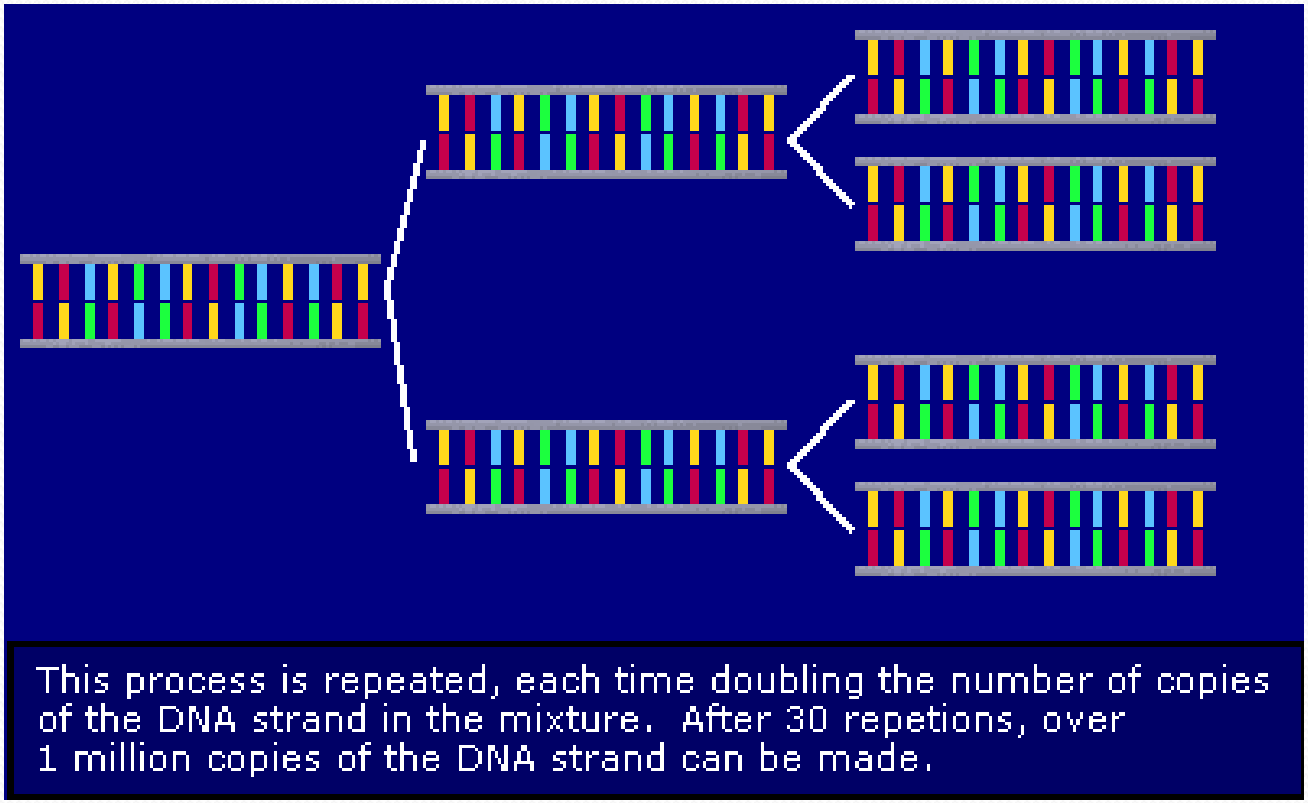
PCR – Step 2


TEMPERATURE: 55°C
Oligonucleotide Primers
STEP 2: The temperature is cooled to 55°C, and Oligonucleotide primers are added to the mixture. The primers designate the boundaries of the DNA strand being duplicated.

PCR – Step 3


TEMPERATURE: AROUND 75°C
Polymerase
STEP 3: Polymerase is addded to the mixture. Polymerase functions to add the corresponding nucleotide bases to the DNA strand. The result is two identical copies of the DNA strand.

PCR – Step 4



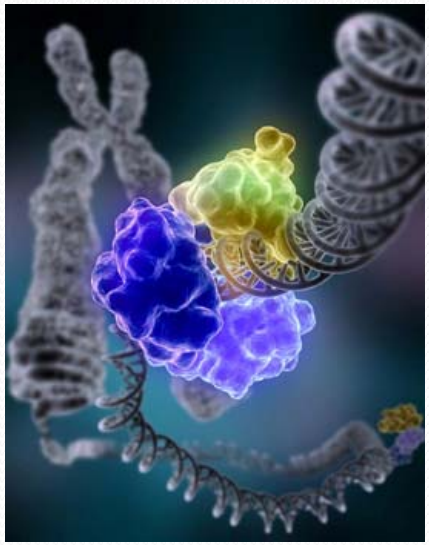
This process is repeated, each time doubling the number of copies of the DNA strand in the mixture. After 30 repetions, over 1 million copies of the DNA strand can be made.

“Cutting the DNA”

- Once identified, the next step is to remove the gene you are interested in from its host organism.
- To “cut” the DNA, substances called restriction enzymes are used. Restriction enzymes cut at specific locations as determined by the DNA sequence.

"Cutting the DNA" (cont'd)

- When using bacteria, the "cut" segment is then inserted into a small, circular piece of bacterial DNA, called a plasmid.
- The enzyme DNA ligase seals the bond between the transferred gene and the plasmid DNA



DNA ligase repairing chromosomal damage. (Image courtesy of the National Institutes of Health.)

“Growing the Gene”

- The plasmid is then mixed with the bacteria and spread onto a growth medium in a Petri dish.

“Growing the Gene” (cont’d)

- Many of the bacteria will pick up the plasmid.
- To determine which bacteria possess the new gene, specific markers such as antibiotic resistance are inserted along with the gene.
- The growth media contains the target antibiotic; therefore organisms which grow on the medium must contain the new gene

Using the New Gene

- The main reason for creating a new gene is to produce the protein.
  - Some proteins are used to make plants that are resistant to insects or insecticides

  - Some proteins are used to enhance the characteristics of the product

Flavr Savr Tomato

  - Some proteins are used to make pharmaceuticals

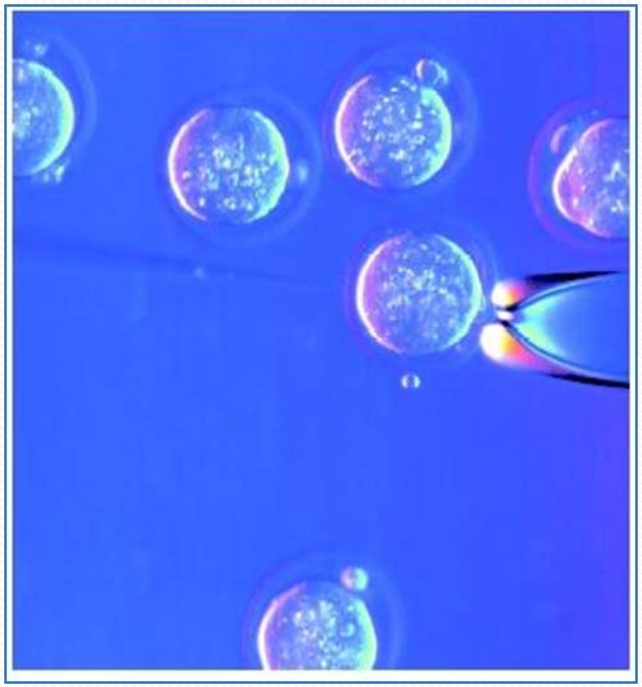
Insulin

Genetically Modified Animals (Transgenic Animals)

- The FDA Center for Veterinary Medicine(CVM) regulates genetically altered animal products
- Currently no transgenic animals have been approved for human consumption
- Transgenic animals have been approved for use as *biopharm animals* (for producing drugs and hormones) and they produce such products as milk and wool

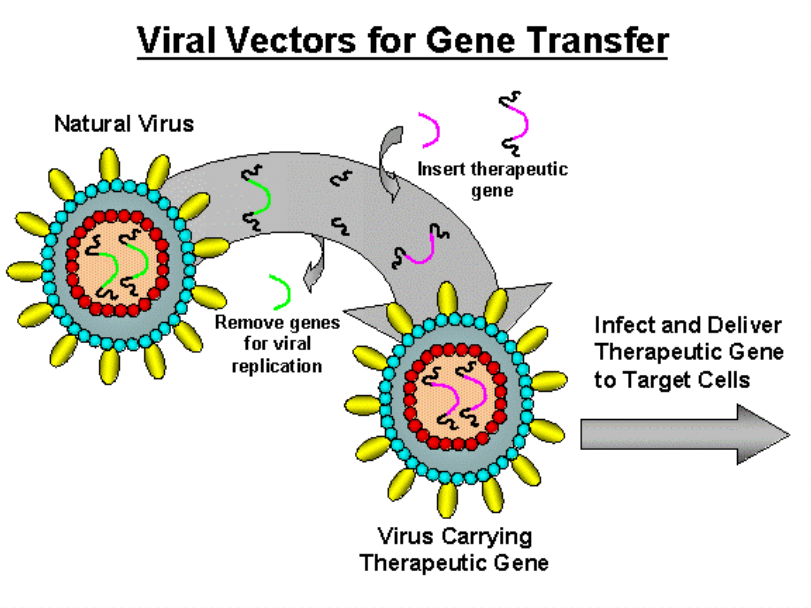
Making Transgenic Animals

- Making a genetically modified animal can be done by:
  - DNA microinjection- The new gene is inserted directly into the fertilized ovum



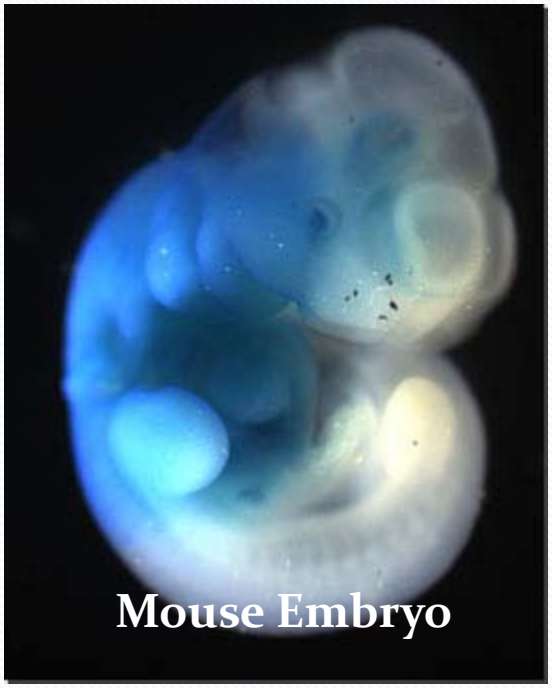
Making Transgenic Animals (cont'd)

- Retrovirus-mediated gene transfer – RNA viruses are used to transfer the gene into the cell

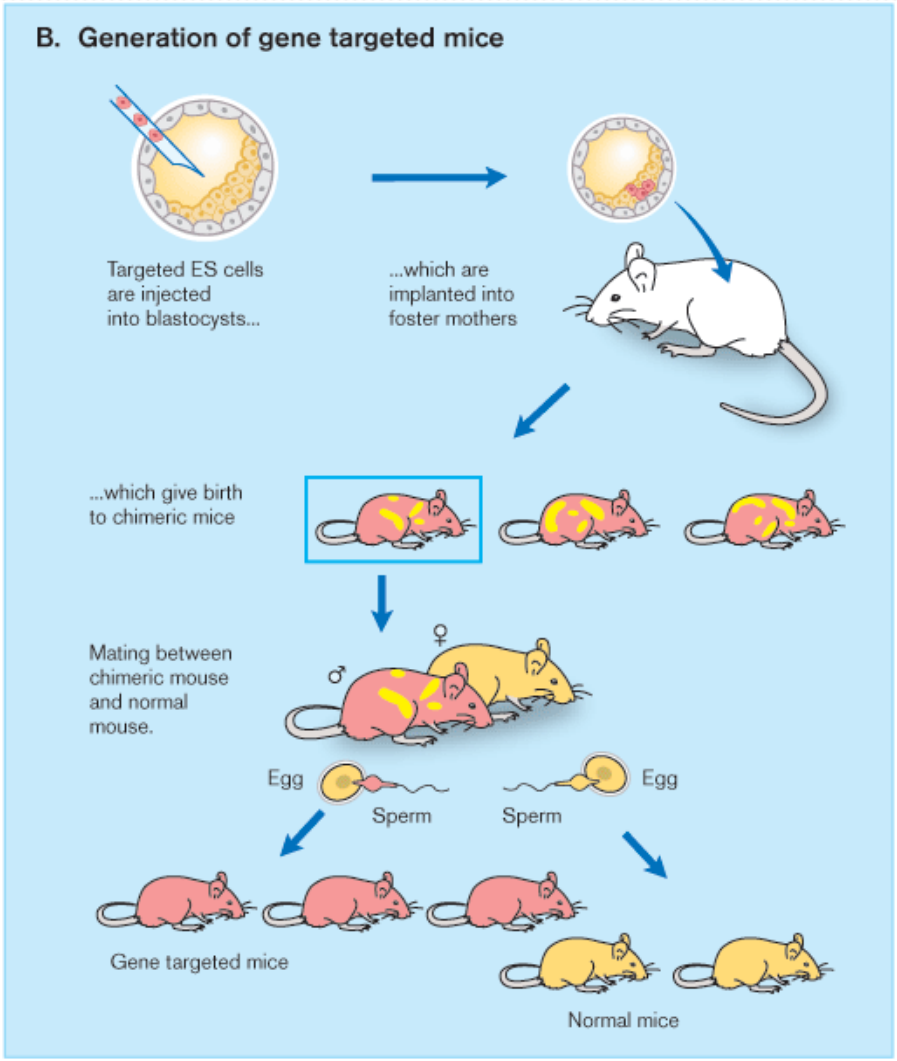


Making Transgenic Animals (cont'd)

- Embryonic Stem Cell-Mediated Gene Transfer – the gene is inserted into embryonic stem cells soon after fertilization and then implanted into surrogate mothers



Embryonic Stem Cell-Mediated Gene Transfer



This method works very well in mice – producing the "knock-out" mice used for laboratory research

Benefits of Transgenic Animals

- Production of animals with specific traits much quicker than with traditional breeding methods
  - Results in
    - Better quality and increased milk production
    - Better quality and increased wool production
    - Increased growth rates

Benefits of Transgenic Animals (cont'd)

- Efficient production of pharmaceuticals, nutritional supplements, and hormones
  - Most pharmaceuticals are produced from the milk of goats, cows and sheep
    - Included are such drugs as
      - Insulin
      - Growth hormone

Benefits of Transgenic Animals (cont'd)

Biopharm Animals Reduce Production Costs

- Experts estimate that producing therapeutic protein using traditional methods cost approximately $300 - $3,000 per gram.
- In contrast, using a transgenic goat to produce the protein in milk costs approximately $20 - $105 per gram
- Transgenic hen eggs are even cheaper, costing approximately $.10 - $.25 per gram of protein

Genetically Modified Plants

- Plants are genetically modified to be:
  - Herbicide resistant
  - Pesticide resistant
  - Insect resistant
  - Drought tolerant
  - Extreme temperature tolerant
  - Have added nutrients, such as vitamins and minerals

"Roundup ready" crops

- Roundup is a common herbicide manufactured by Monsanto that is harmful to weeds and plants alike
- For this reason, Monsanto developed a line of "Roundup ready" crops that are resistant to the herbicide
- By inserting gene 5'-enolpyruvylshikimate-3'-phosphate (EPSP) from the bacteria *Agrobacterium*, plants such as corn, soybeans, cotton, and alfalfa could be made herbicide resistant

Insect resistance

- Corn, cotton, and several other plants have been genetically modified to be insect resistant.
- Insect resistance in crops is accomplished by identifying and isolating a gene from the soil bacterium *Bacillus thuringiensis* that produces a toxin called *Cry* that is toxic to plant insects.
- By cutting and inserting the gene of interest from *B. thuringiensis* into plant DNA, a new genetically modified plant is created that is resistant to insects.

Benefits of insect resistance

- GM cotton plants that are insect resistant are protected from tobacco budworm, bollworm, and pink bollworm caterpillars
- GM corn plants that are insect resistant are protected from European corn borers and corn rootworms
- These insects cause severe damage to the plants and ultimately prove costly to the farmer.
  - GM plants are beneficial in that they increase crop yield and save farmers time and money by having to use less insecticides

GM plants with added nutrients - Rice

- Rice, a staple food in many countries, has been genetically modified to be an improved source of vitamin A
- This GM rice is able to biosynthesize β-carotene, which leads to production of vitamin A in the human body
- Biosynthesis of beta-carotene in GM rice was accomplished by inserting phytoene synthase (*psy)* gene from daffodils and phytoene desaturase (*ctrI*) gene from the bacteria *Erwinia uredovora* into rice DNA

“Golden rice”

- The additional beta-carotene produced by the endosperm (rice grain that is eaten by the humans) gives it a characteristic yellow or golden hue
- Because of this the vitamin-enriched GM rice is also known as “golden rice”

White, non-GM rice

Golden, GM rice

GM plants with added nutrients - Strawberries

- Strawberries, which are a good source of vitamin C, have been genetically modified to provide 3 times as much vitamin C
- A gene in the strawberry plant called GalUR gene codes for an enzyme that converts a protein in the plant to vitamin C
- A similar gene is found in the thale cress *Arabidopsis thaliana*.
- Researchers created a DNA plasmid using the *A. thaliana* gene and the bacteria *Agrobacterium* and inserted into the strawberry plant to over-express GalUR gene and produce 3 times as much vitamin C

More GM crops

- Currently, researchers around the world are working at creating and perfecting:
  - Drought resistant wheat, corn, and rice
  - Salt tolerant tomatoes
  - Frost resistant strawberries
  - Heat tolerant beans such as kidney, red, black, and pinto beans
  - Carrots that produce a vaccine against hepatitis B

Benefits of GM plants

- GM plants could:
  - Provide additional nutrients
  - Resist insects, herbicides, and diseases
  - Tolerate environmental stresses to provide an increased crop yield
  - Provide enough food for the growing population
  - Be a source of vaccines and drugs for infectious diseases

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