Genetic Engineering

Ch 13

13-1 Selective Breeding

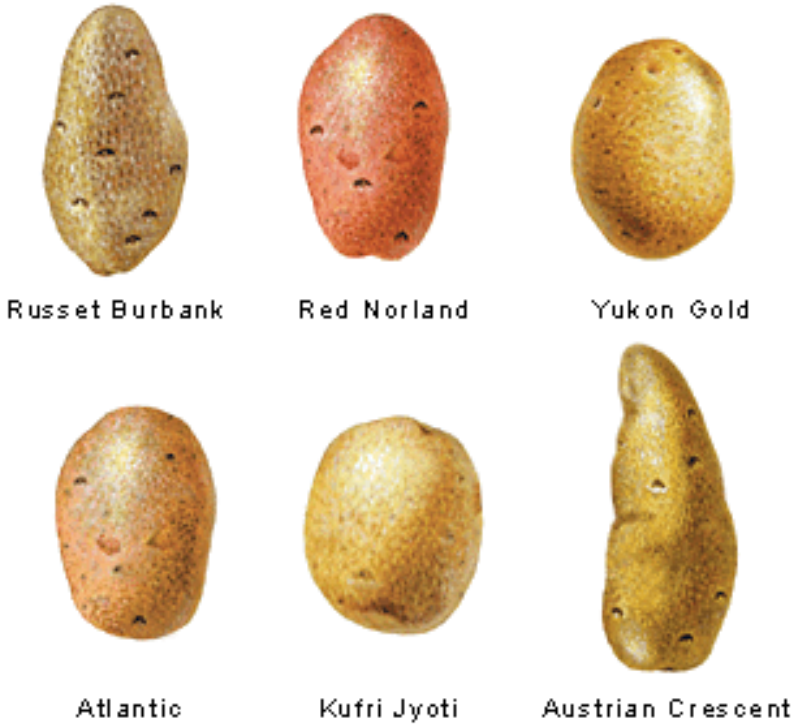
- Used by humans to pass on desired traits

13-1 Selective Breeding

- Luther Burbank (1849-1926)
- Disease resistant potatoes, Gravenstein apples, Shasta daisy

13-1 Hybridization

- Crossing dissimilar individuals to bring together the best of both organisms, like disease resistance and food production in Burbank's potatoes



13-1 Inbreeding

- Continued breeding of individuals with similar characteristics

13-1 Increasing Variation

- Breeders can increase the genetic variation in a population by inducing mutations
- Mutations are the ultimate source of genetic variability
- Mutations occur spontaneously but frequency can be increased by radiation and chemicals
- Many mutations are harmful to the organism but some will produce desired characteristics
- Used to develop useful bacteria strains, like those that can break down oil and plastic

13-1 Polyploidy

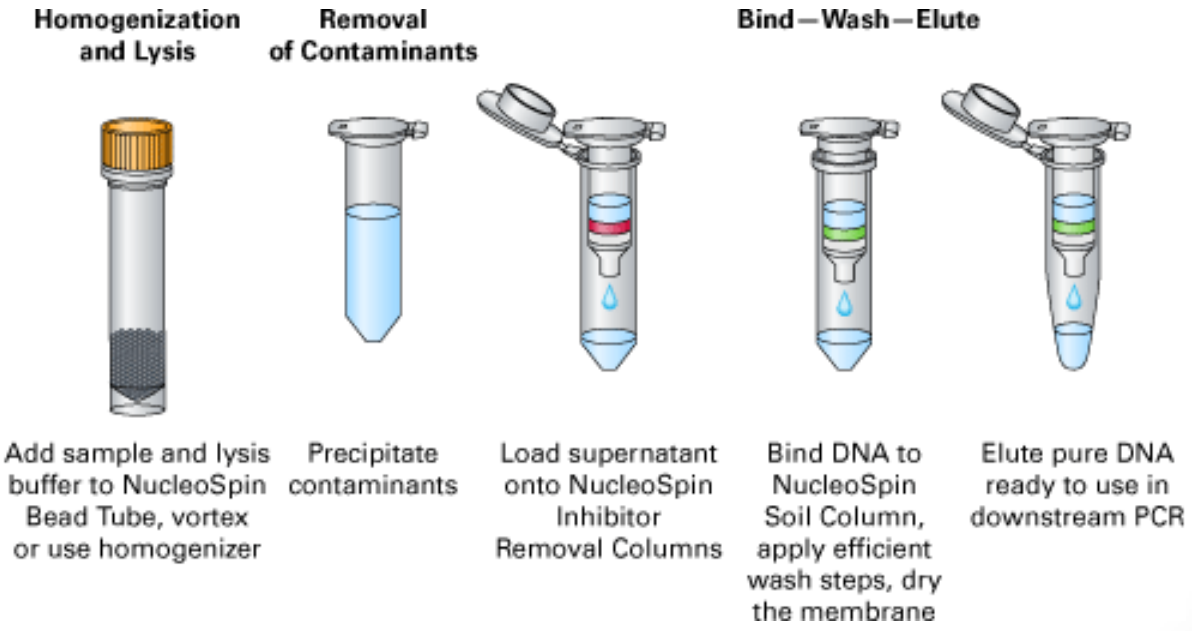
- Some drugs can prevent chromosomal separation in plants and are used to produce seeds that have double or triple the amount of chromosomes
- Polyploidy
- Usually fatal in animals but plants are better at tolerating it
- Makes plant and fruit bigger and stronger

13-2 Manipulating DNA

- DNA can now be extracted from cells, cut into pieces, sequenced, copied, and pasted back together
- Genetic engineering

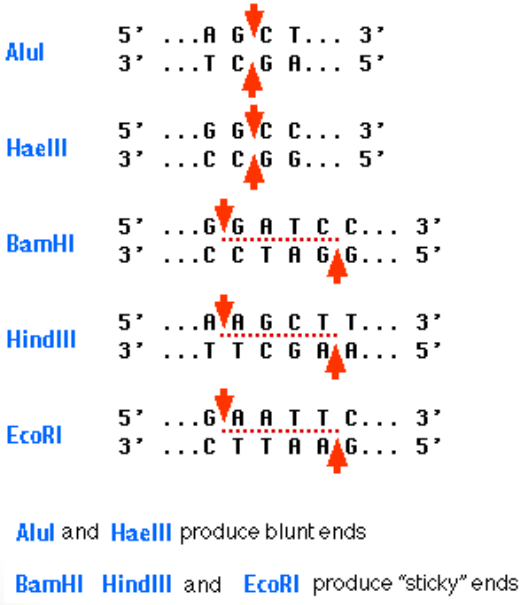
13-2 Manipulating DNA

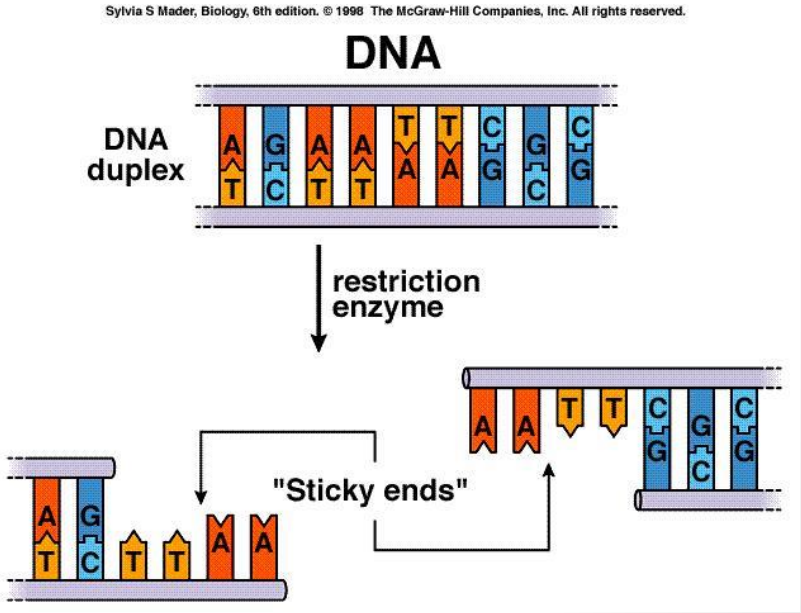
- DNA extraction-cells can be broken open with enzymes and detergents and DNA can be separated from other molecules in cells



13-2 Manipulating DNA

- Cutting DNA-using restriction endonucleases
- Cut at specific sequences
- Hundreds of restriction sites and enzymes
- Enzymes isolated from organisms like bacteria or viruses
- Example BamH1 or EcoR1



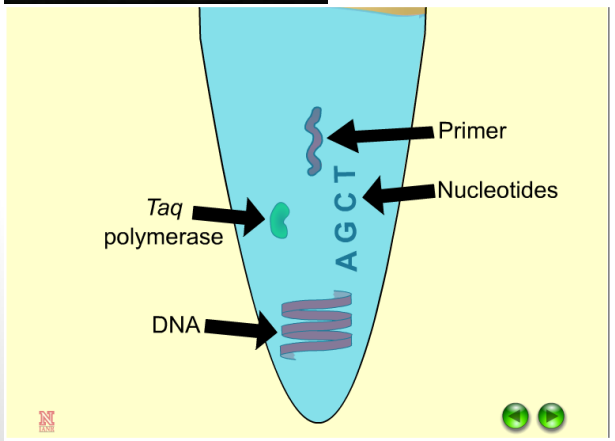


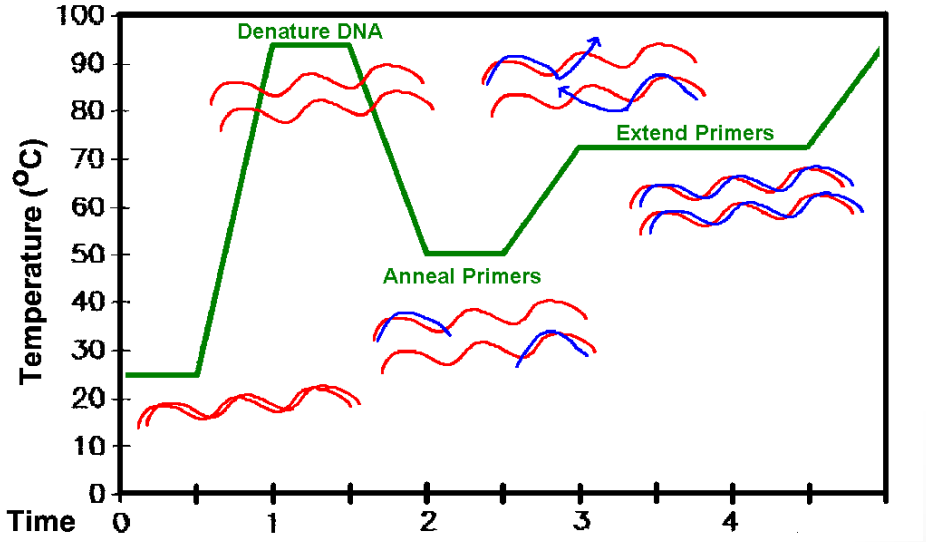
13-2 Manipulating DNA

- Separating DNA and DNA fragments using gel electrophoresis
- Use a polymer gel like agarose or acrylamide-molecular sieve
- Pass electric current through
- DNA is (-) charged and moves in an electric field toward the (+) positive electrode
- Smaller ones move through gel faster, large one slower so can be separated based on size
- Can be used for analysis/diagnostic purposes or fragments can be recovered from gel and used for further studies or manipulation

13-2 Manipulating DNA

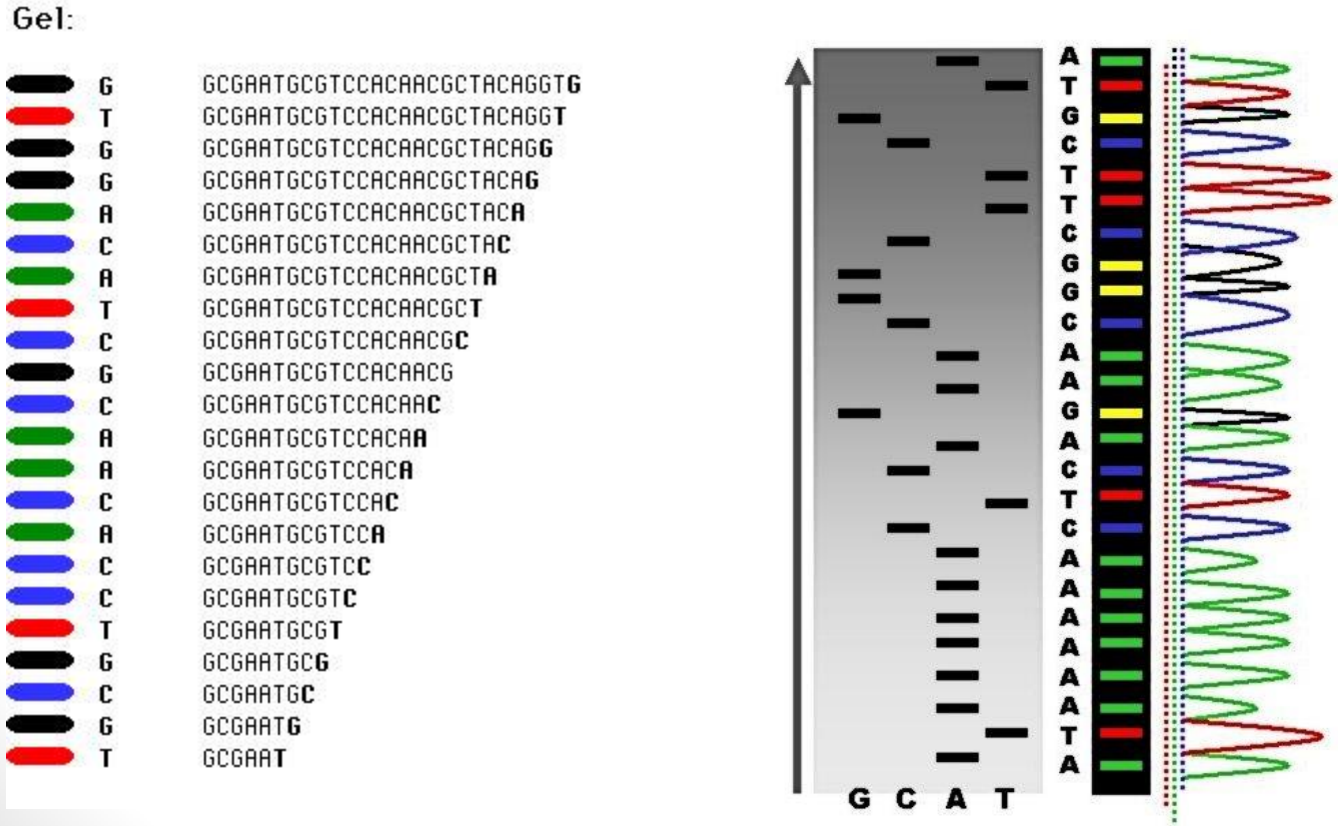
- Making copies of DNA-Polymerase chain reaction-PCR
- Makes millions of copies of DNA by cycling through temperatures that allow DNA to unwind, primers to anneal or stick, and nucleotides to be added or extended





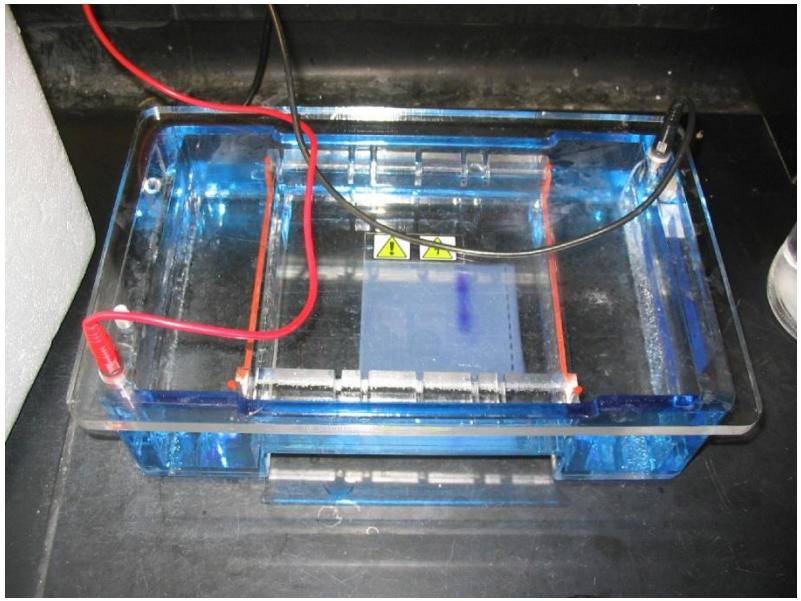
13-2 Manipulating DNA

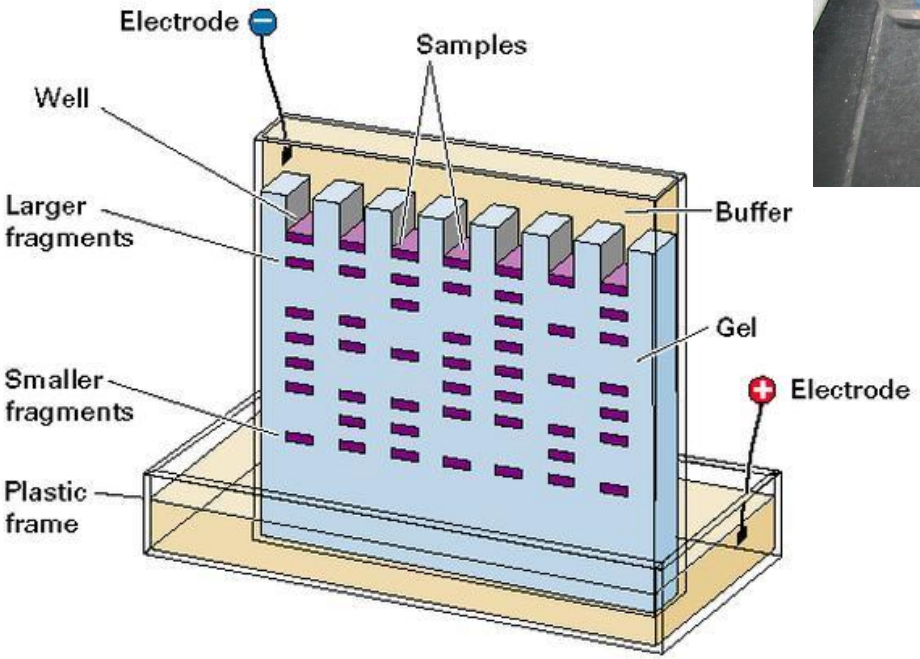
- Can read a DNA sequence by copying the DNA strand, making a complementary copy with fluorescent nucleotides to label the DNA copy then sequencer machine “reads” the sequence



13-2 Manipulating DNA

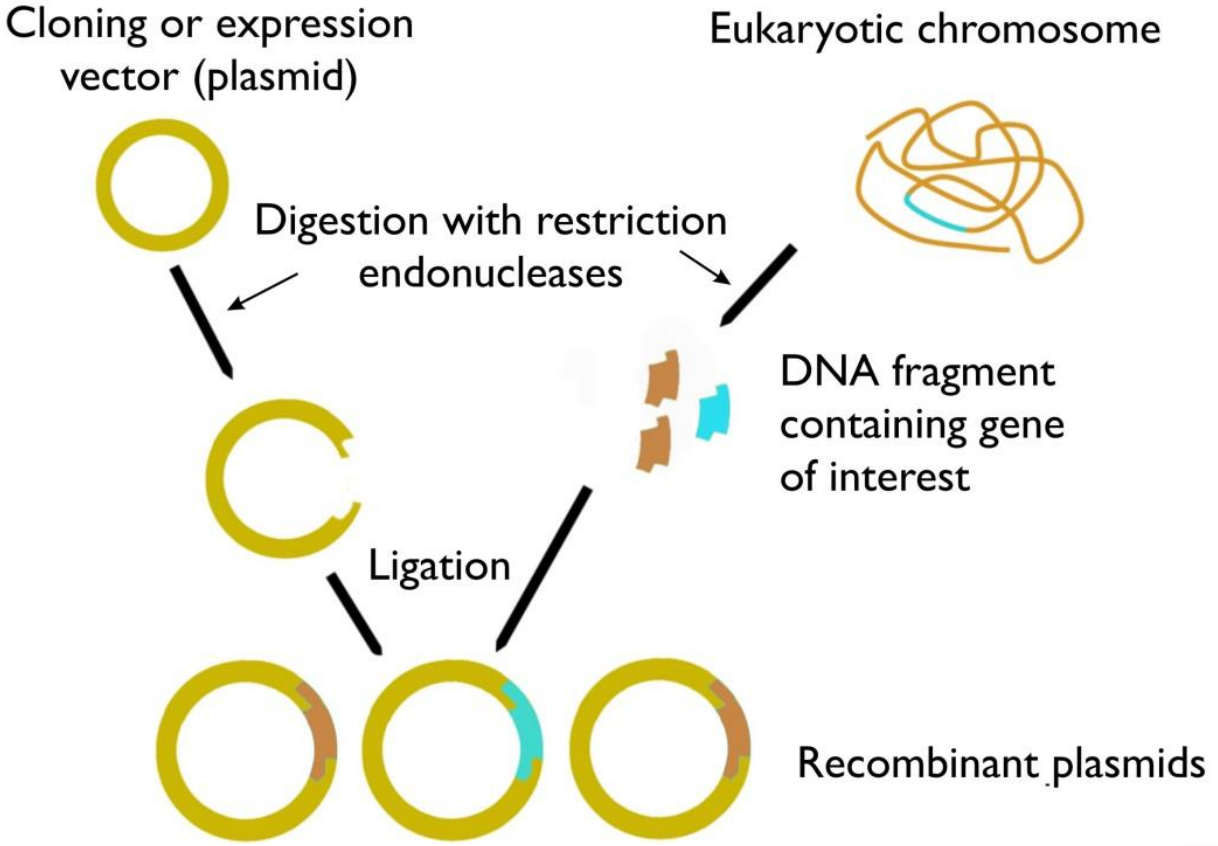
- Gel electrophoresis



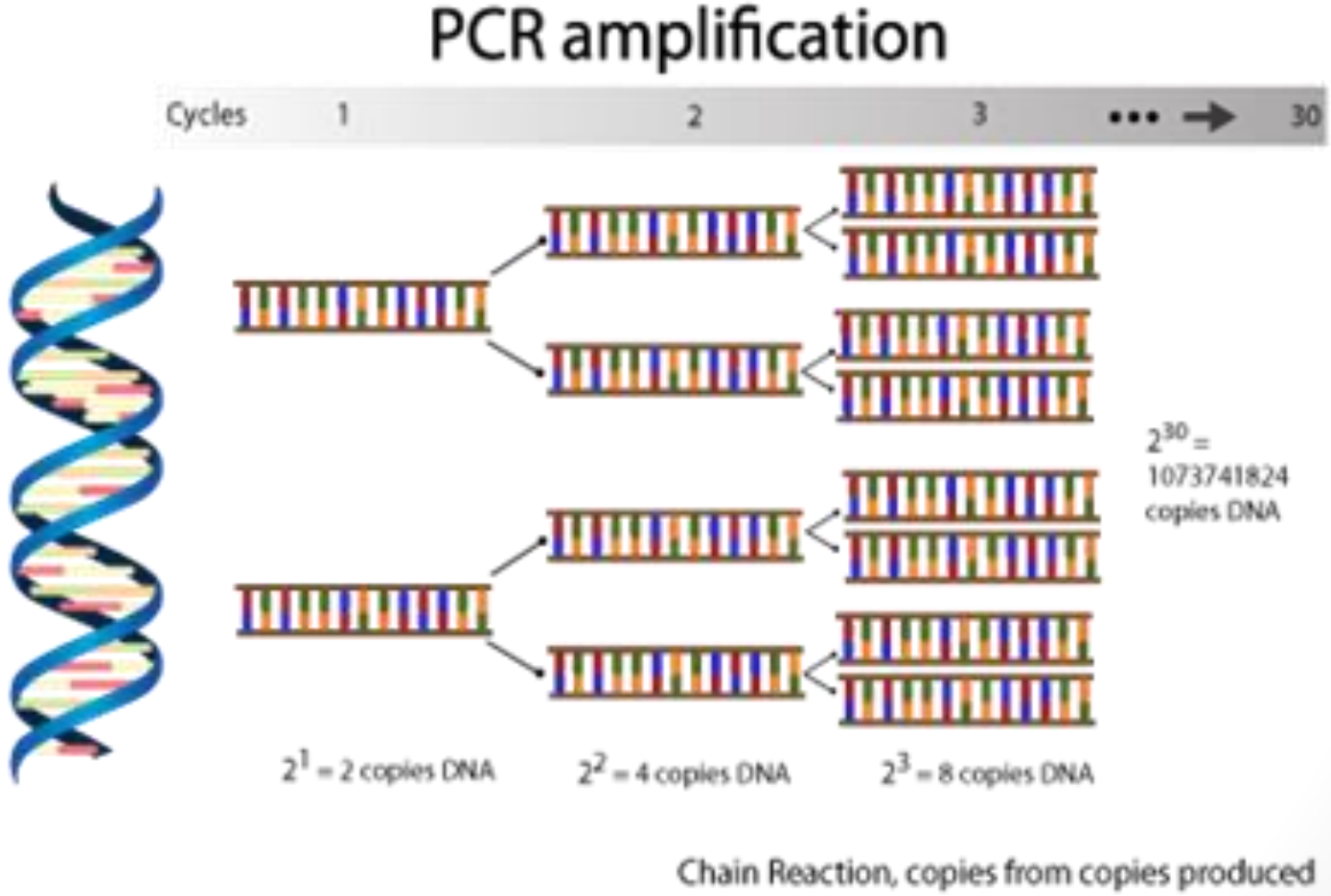


13-2 Manipulating DNA

- Cutting with restriction endonucleases and pasting with ligase to make recombinant DNA

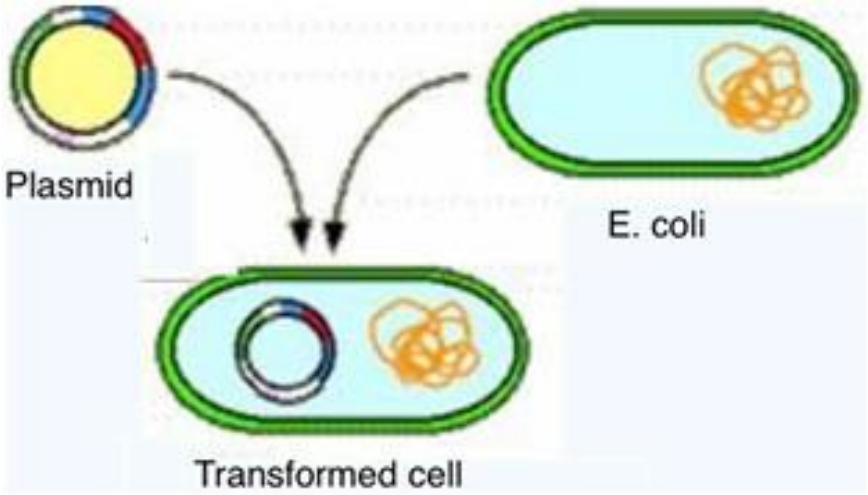


13-2 Manipulating DNA

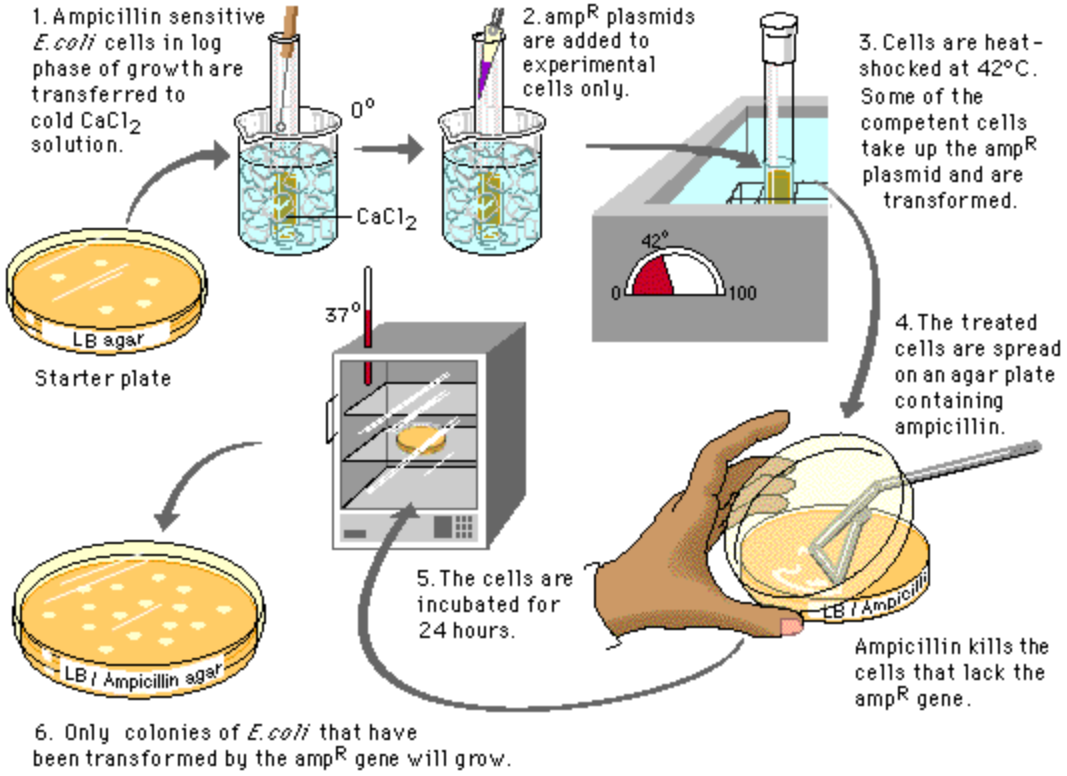


13-3 Cell Transformation

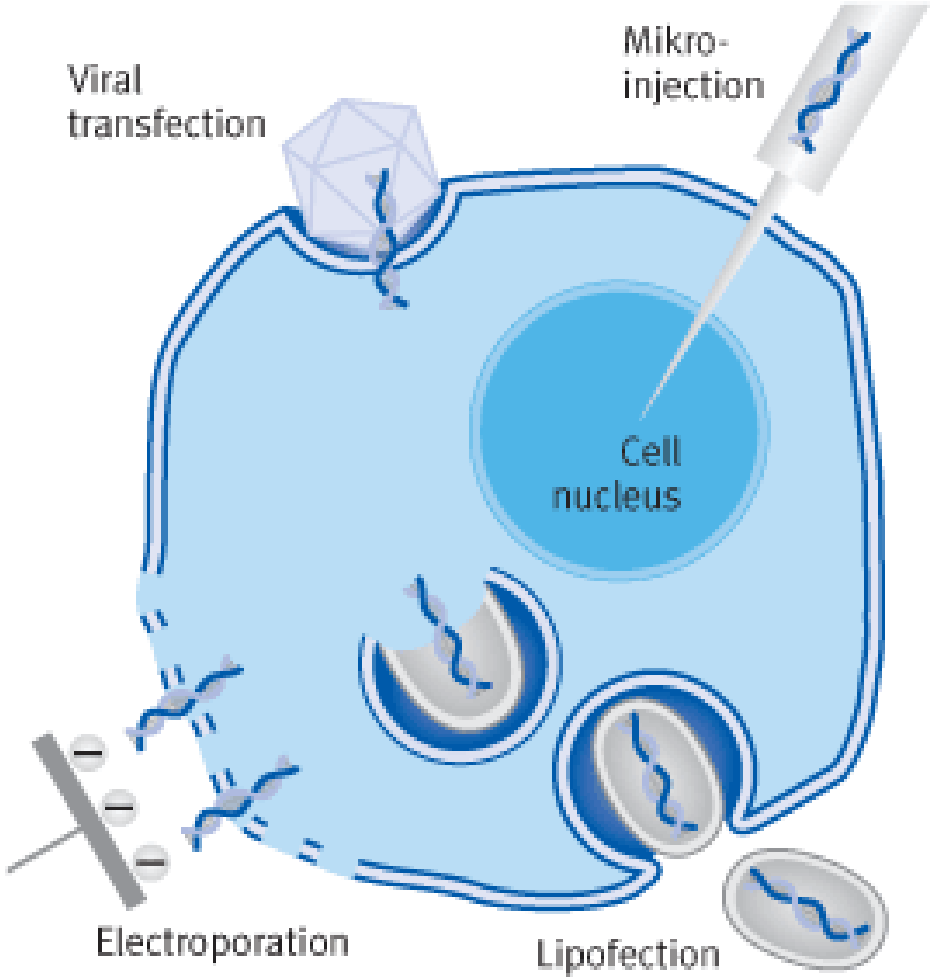
- Cell takes in external DNA in the form of a plasmid, a small circular DNA
- In bacteria, transformation
- In eukaryotic cells, transfection



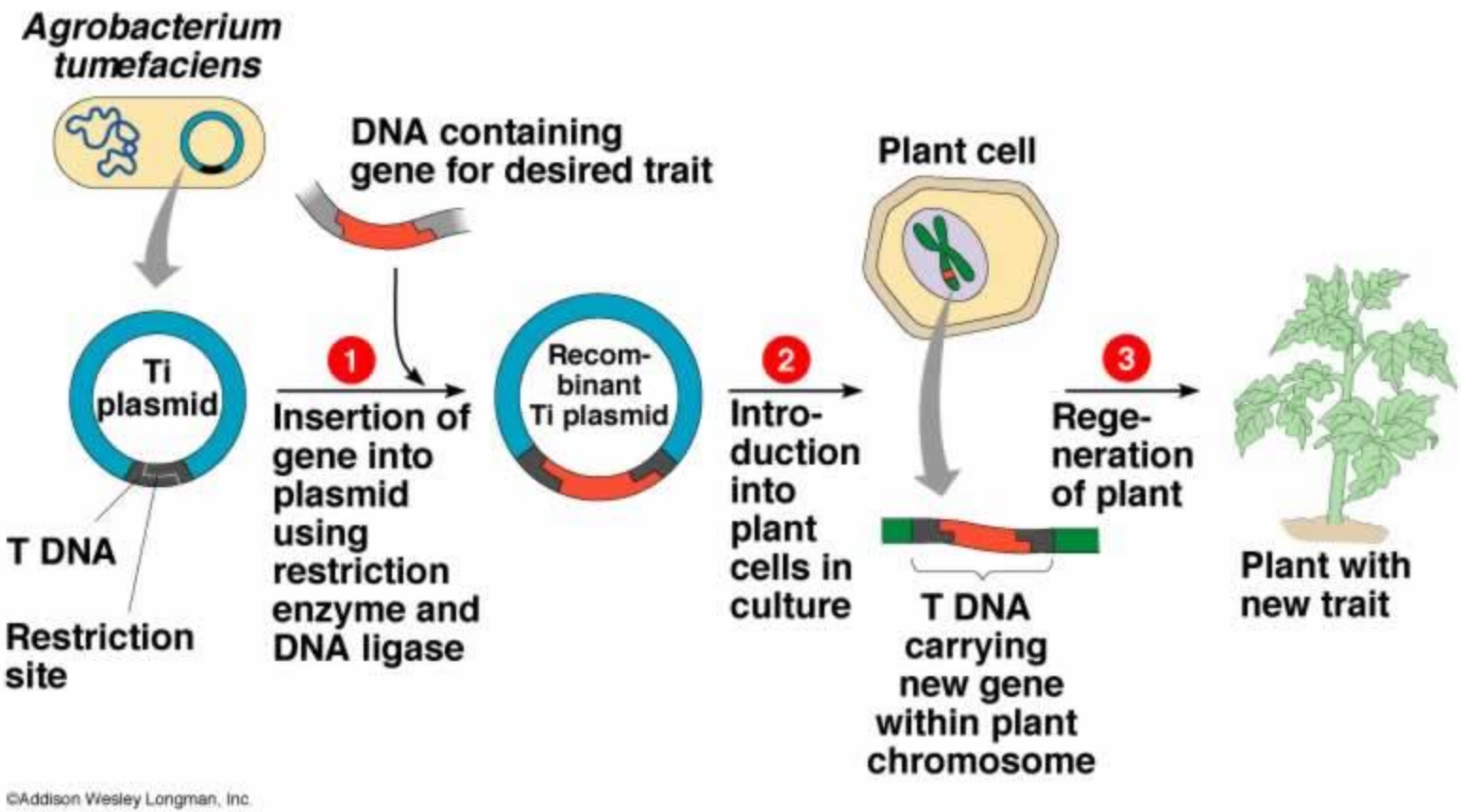
13-3 Cell Transformation



13-3 Transfection

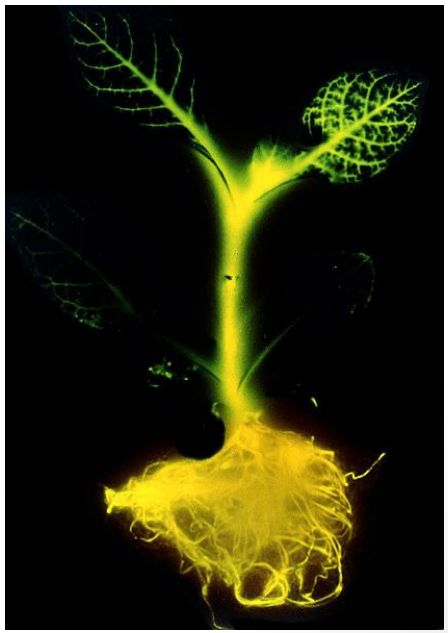


13-3 Plant cell transformation

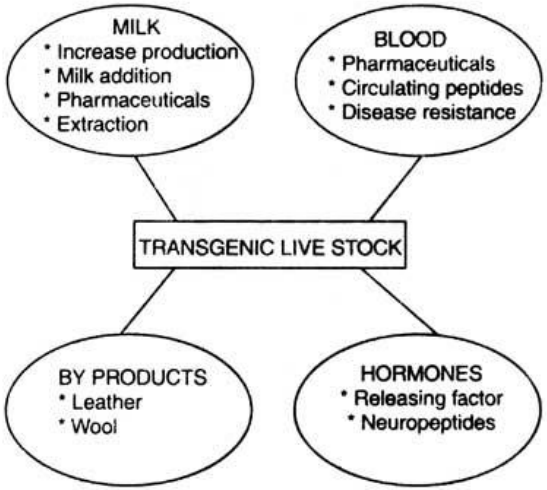


13-4 Applications of Genetic Engineering

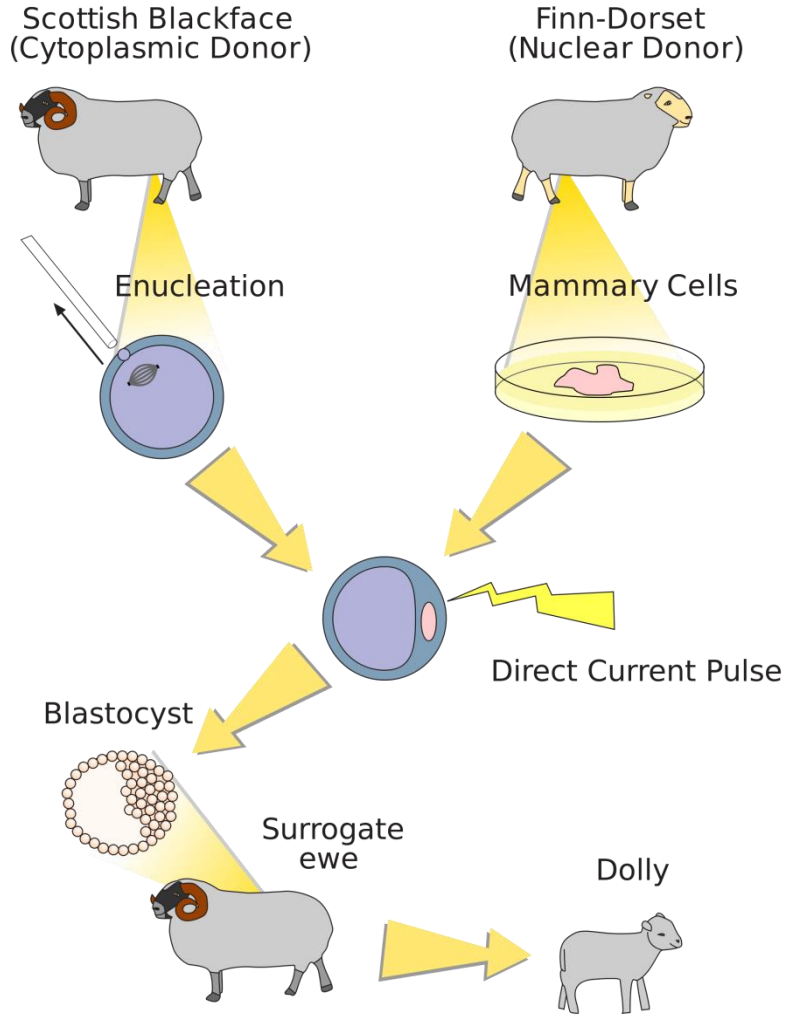
- Transgenic organisms contain genes from other organisms
- Bacteria and yeast are used to make proteins cheaply and in great abundance
- Insulin, growth hormones, clotting factors
- Works because mechanism of gene expression (DNA-RNA-Protein) works the same in all organisms



13-4 Transgenic Bacteria and Animals



13-4 Transgenic Animals-Cloning



8.1 Overview of genetic engineering

- Pharmaceuticals
  - Protropin HGH, Actimmune interferon, Herceptin anticancer antibody
- Industrial enzymes
  - Indiage cellulase, Purafect protease
- Agricultural products
  - Round up ready soybeans, New leaf potatoes pest resistant

8.1 Overview of genetic engineering

- General steps
- 1. The coding region for a desired characteristic or protein is identified and isolated from a donor cell, confirmed by restriction digest and sequencing and pasted into a vector to form rDNA

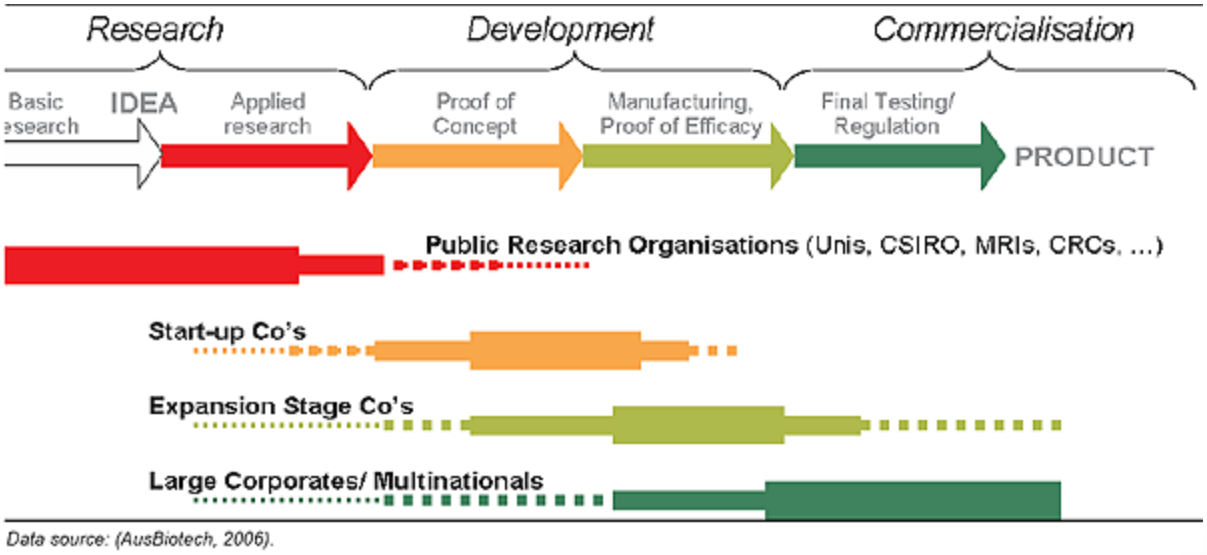
8.1 Overview of genetic engineering

- 2. Recombinant cells are transformed/transfected with the rDNA and cells are assayed to confirm presence of the rDNA and expression of the protein

8.1 Overview of genetic engineering

- 3. The recombinant cells are grown in culture at a small (fermentation) then a large (manufacturing) scale

- 4. Recombinant protein is isolated and purified from cell cultures, analyzed for purity and activity, then goes to market



8.1 Overview of genetic engineering

- A product must be better produced by genetic engineering than by conventional methods in order to justify the investment in R&D
  - Scale
  - Safety
  - Ease of isolation/production
  - Chymosin/Renin
    - Renin isolated from calf gut
    - Chymosin produced in yeast cells (Chymax by Genencor)

8.3 After Transformation

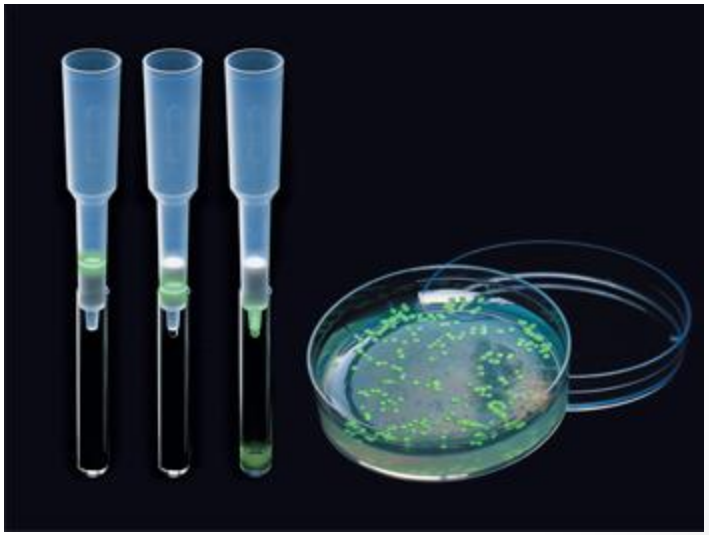
- Scale up-need more than a few colonies to purify enough protein to use as a product
- 1-2L spinner flasks are appropriate size for R&D
- For production, fermentation tanks 10K liters
- Require sterile conditions and strict protocols for cleaning and sterilizing equipment

8.3 After Transformation

- Using assays during scale up
- Test at every step of scale up and production for
  - Presence
  - Activity
  - Concentration
- Conducted by Quality Control (QC) Department

8.4 Fermentation, Manufacturing and GMP

- Fermentation in biotechnology is growing cells under optimal conditions for maximum cell division and product production
- Highly controlled large scale growth
- A seed colony is a colony growing on a petrie dish
- Because of exponential growth (every 20 minutes the number of cells in the culture double) large cultures can quickly be reached as long as optimal conditions are maintained
- Then product is isolated



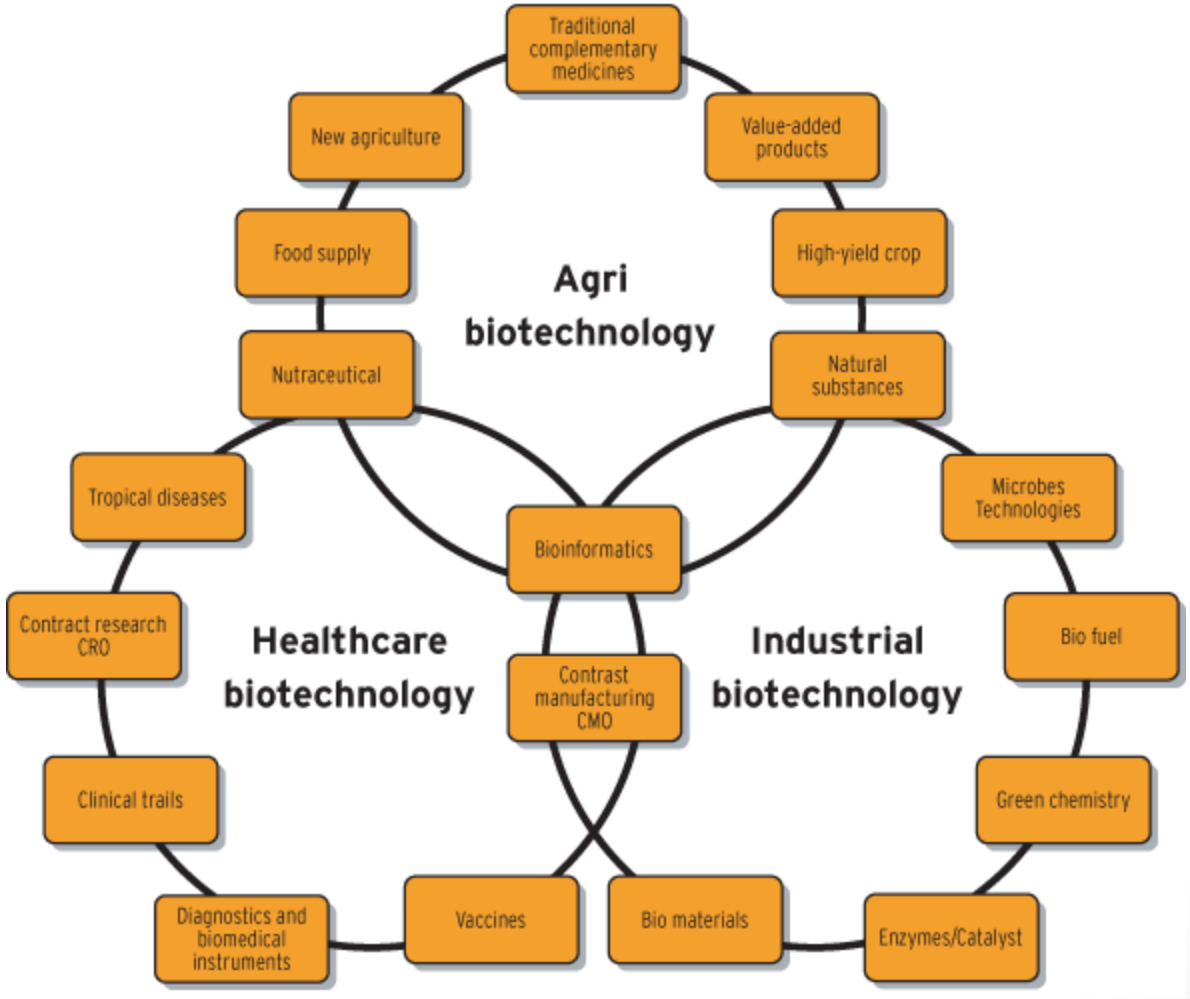
8.4 Fermentation, Manufacturing and GMP

- After product isolation it must be formulated
- Prepared for delivery and storage
  - Route of drug administration must be determined-oral, IV, etc
  - Testing and market analysis involved
- Often an entire department is devoted to this

8.4 Fermentation, Manufacturing and GMP

- During manufacture, current good manufacturing practices (cGMP) must be followed

What is Biotechnology



How Companies Select Products to Manufacture

- Biotechnology Products
- More nutritious foods
- Better medicine
- Improved living conditions
- Cleaner environment
- Commonality is that the potential product must make it through the product pipeline and generate sales in a reasonable amount of time and at a reasonable cost

How Companies Select Products to Manufacture

- Product pipeline-what is that?

CANDIDATE	RESEARCH	PRECLINICAL	PHASE 1	PHASE 2	PHASE 3	NEW DRUG APPLICATION
Ambrisentan	Pulmonary Arterial Hypertension					
Aztreonam Lysine for Inhalation	Cystic Fibrosis					
Tenofovir Disoproxil Fumarate	Chronic Hepatitis B					
Darusentan	Resistant Hypertension					
GS 9137 integrase inhibitor	HIV/AIDS					
GS 9190 polymerase inhibitor	Hepatitis C					
Small Molecule Therapeutics	Viral Infections					
Small Molecule Therapeutics	Cardiopulmonary					
Inhaled Therapeutics	Respiratory Infections					

How Companies Select Products to Manufacture

- Product development plan

- Does the product meet a critical need?
- Who will use it? Is the market large enough? How many customers?
- Do preliminary data support that the product will work? Will the product do what the company claims?
- Can patent protection be secured? Can the company prevent another company from making it?
- Can the company make a profit? How much will it cost to produce and how much can it be sold for?

How Companies Select Products to Manufacture

- Product development is based on criteria set by the
  - Food and Drug Administration (FDA)
  - Environmental Protection Agency (EPA)
  - United States Department of Agriculture (USDA)

How Companies Select Products to Manufacture

- Clinical Trials
- Test potential therapeutics in human patients
- Three phases
- I. Test on terminally ill patients with no other treatment options
  - Is it safe? Does it work?
  - How should it be given/ What is the dose?
  - Small sample size (a dozen patients)
- II. Further safety tests
  - How well does it work?
  - Tested with a specific type of cancer
- III. Test compared to other drugs used for the same condition
  - Does it work better?
- Overseen by the FDA

Careers in the Biotechnology Industry

- Funding is necessary for research and development (R&D) and manufacturing
- Raw materials, utilities and building maintenance
- Majority of budget spent on employee salaries and benefits
- Once a product starts making money, the profits repay the cost, and additional profit is reinvested

Careers in the Biotechnology Industry

- Scientific and non-scientific jobs
- Scientific-R&D, manufacturing and production, clinical research, quality control
- Non-Scientific-information systems, marketing and sales, regulatory affairs, administration/legal affairs

Careers in the Biotechnology Industry

- HS Diploma-Lab assistant
- Certificate (1-2 years community or career college)-Biotechnician
- Bachelor's Degree (4 yrs college)-Research Associate
- Masters degree (1-3 yrs graduate school)- Research Associate
- Doctorate (4-6 yrs after bachelor's degree)-Scientist
- Postdoctorate (1 or more years after doctorate)-Scientist