Previous class

- Antibiotics
- Vaccines
- Sequencing microbial genomes
- HMP

Viral Genomes

–Why?

Decipher genes and their products so that agents that block attachment, block replication etc can be made

Examples of medically important viral genomes that have been sequenced

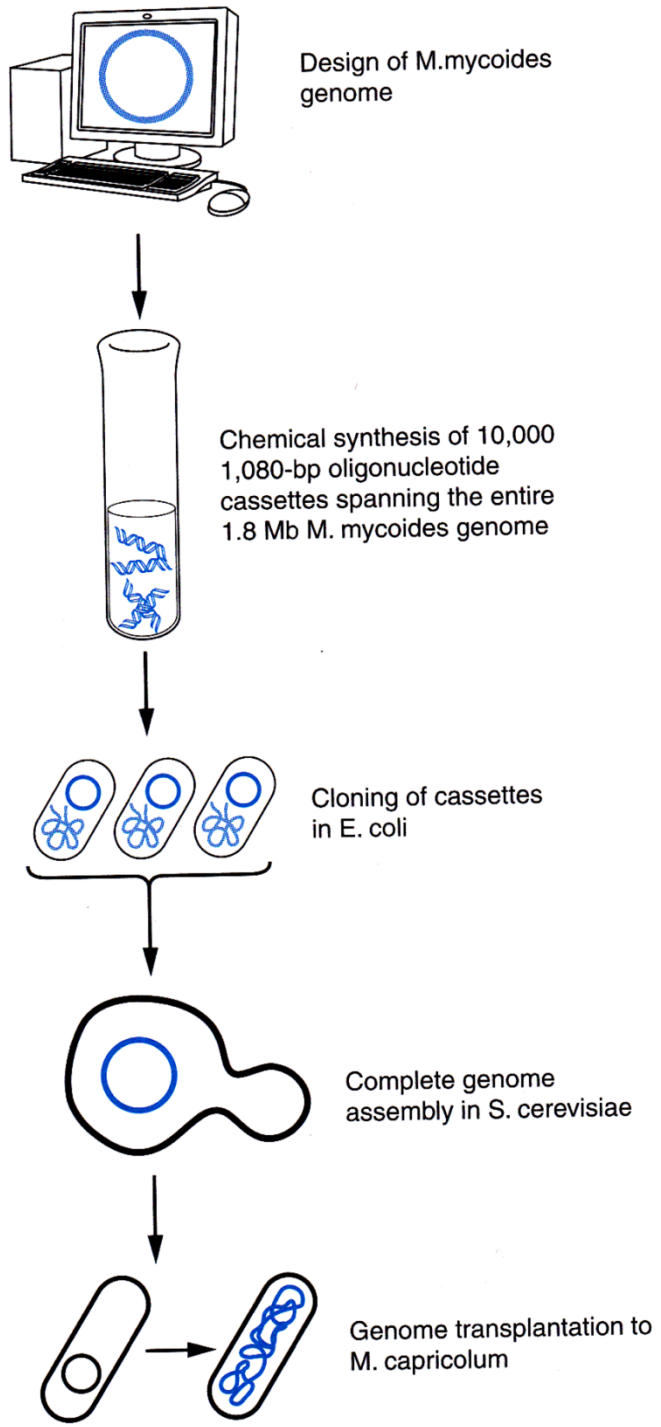
TABLE 5.4 EXAMPLES OF MEDICALLY IMPORTANT VIRAL GENOMES THAT HAVE BEEN SEQUENCED

Virus	Human Disease or Illness	Year Sequenced
Ebola virus	Ebola hemorrhagic fever	1993
Hepatitis A virus	Hepatitis A	1987
Hepatitis B virus	Hepatitis B	1984
Hepatitis C virus	Hepatitis C	1990
Herpes simplex virus, type I	Cold sores	1988
Human immunodeficiency virus (HIV-1)	Acquired immunodeficiency syndrome (AIDS)	1985
Human papillomavirus	Cervical cancer	1985
Human poliovirus	Poliomyelitis	1981
Human rhinovirus	Common cold	1984
Influenza A virus		
• Subtype H5N1 (Avian flu)	Severe flu	2007
• Subtype H5N1 (Swine flu)	Severe flu	2009
Severe acute respiratory coronavirus (SARS-CoV)	Severe acute respiratory syndrome (SARS)	2003
Variola virus	Smallpox	1992

Synthetic Genomes

Proposed Applications:

- Synthesize biofuels
- Bioremediation
- Alternative fuels
- Biopharma products
- …



Biofuels

- Use of ethanol as biofuel
- Alcohol-fermenting microbes convert glucose and other sugars in grain to ethanol
- Process is expensive and converting cellulose to glucose is not simple
- Recombinant DNA technology is being used to engineer microbes for this purpose

Microbial Diagnostics

- Microbial Diagnostics – techniques used to detect and track microbes
- Bacterial Detection Strategies
  - RFLP (Restriction fragment length polymorphism) analysis,
  - PCR and
  - DNA sequencing
  - Databases are available for comparison of clinical samples
  - Used to detect and track bacterial contamination of food

Combating Bioterrorism

- **Bioterrorism** – the use of biological materials as weapons to harm humans or the animals and plants we depend on for food
- Only 12 or so organisms could feasibly be cultured, refined, and used in bioterrorism
- Delivered by aerosols, crop duster planes, or water supplies

Table 5.5 POTENTIAL BIOLOGICAL WEAPONS

Agent	Disease Threat and Common Symptoms
Brucella (bacteria)	Different strains of *Brucella* infect livestock such as cattle and goats. Can cause brucellosis in animals and humans. Prolonged fever and lethargy are common symptoms. Can be mild or life threatening.
Bacillus anthracis (bacterium)	Anthrax. Skin form (cutaneous) produces skin surface lesions that are generally treatable. Inhalation anthrax initially produces flulike symptoms leading to pulmonary pneumonia that is usually fatal.
Clostridium botulinum (bacterium)	Botulism. Caused by ingestion of food contaminated with *C. botulinum*or its toxins. Varying degrees of paralysis of the muscular system created by botulinum toxins are typical. Respiratory paralysis and cardiac arrest often cause death.
Ebola virus or Marburg virus	Both are highly virulent viruses that cause hemorrhagic fever. Symptoms include severe fever, muscle/joint pain, and bleeding disorders.
Francisella tularensis (bacterium)	Tularemia. Lung inflammation can cause respiratory failure, shock, and death.
Influenza viruses (a large highly contagious group)	Influenza (flu). Severity and outcome depend largely on the strain of the virus.
Rickettsiae (several bacteria strains)	Different strains cause diseases such as Rocky Mountain spotted fever and typhus.
Variola virus	Smallpox. Chills, high fever, backache, headache, and skin lesions.
Yersinia pestis (bacterium)	"Black" plague. High fever, headache, painful swelling of lymph nodes, shock, circulatory collapse, organ failure, and death within days after infection in a majority of cases.

Think about…

- How can you use biotechnology against bioweapons?

- Should synthetic genomes be made?

Plant Biotechnology

Motivation for genetically engineered crops

- Agriculture is the biggest industrial sector in the world
  - $1.3 trillion of products/year
- Over past 40 years, world population has doubled while agricultural land area has increased by only 10%

Agriculture: The Next Revolution

- **Plant Transgenesis** – transferring genes to plants directly accelerates selective breeding practices used in the past.
  - Cotton fiber strength
    - increased 1.5% per year through conventional breeding
    - Increased 60% by inserting a single gene into the plant
  - Corn and soybean have been targets of much genetic engineering
  - Development of plant vaccines,
  - Development of plants that produce their own pesticides and are resistant to herbicides
- 17 countries are growing more than 200 million acres of crops improved through biotechnology
- Focus of considerable controversy

Methods Used in Plant Transgenesis

- Conventional Selective Breeding and Hybridization
  - Classic breeding (transferring genes by crossing plants) is slow and uncertain
  - Crossing between two different plant lines – repeated backcrossing between hybrid plant's offspring and original parent plant
  - Isolation of desired trait can take years
  - Also plants of different species generally do not hybridize

Methods Used in Plant Transgenesis

- Conventional Selective Breeding and Hybridization
  - **Polyploid plants** – contain multiple chromosome sets greater than normal, usually more than 2N or one set of chromosomes from each parent.
  - Used widely to increase desirable traits
  - By using drug colchicine cells containing double chromosomes are prevented from cell division
  - Hybridization is used to introduce important feature of related species to cultivated new crops
  - Hybrid plants are obtained which contain whole chromosome sets, rather than single genes.

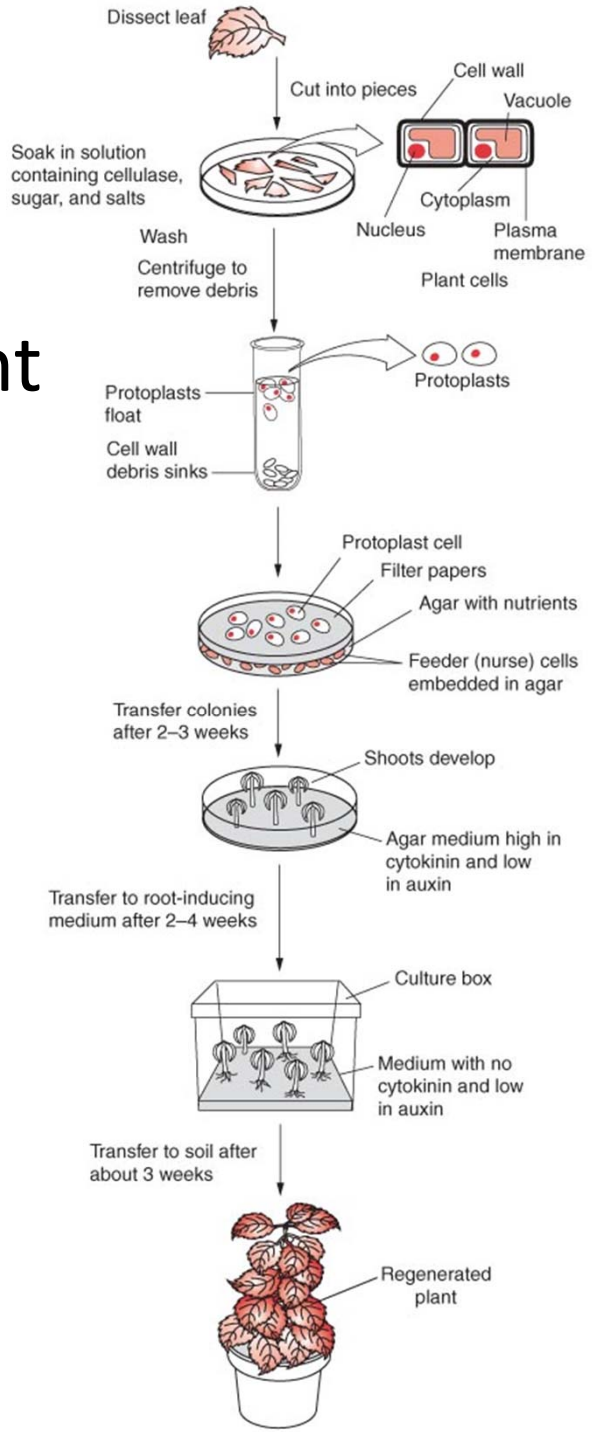
Methods Used in Plant Transgenesis

- Cloning
- Many type of plants can regenerate from single cell – clones
  - Protoplast fusion
  - Leaf fragment technique
  - Gene guns
  - Chloroplast engineering
  - Antisense technology

Methods Used in Plant Transgenesis

- **Cloning –** growing plants from a single cell
  - **Protoplast fusion** is the fusion of two protoplast cells from different species
    - **Protoplast** cell is a callus cell (grow over the site of a wound) whose cell wall has been dissolved by the enzyme cellulase
    - Fusion of the two protoplast cells creates a cell that can grow into a hybrid plant
    - Examples include broccoflower (broccoli and cauliflowed)

Protoplast Fusion and Regeneration of Hybrid Plant



Cellulase dissolves cell wall

Protoplasts are separated

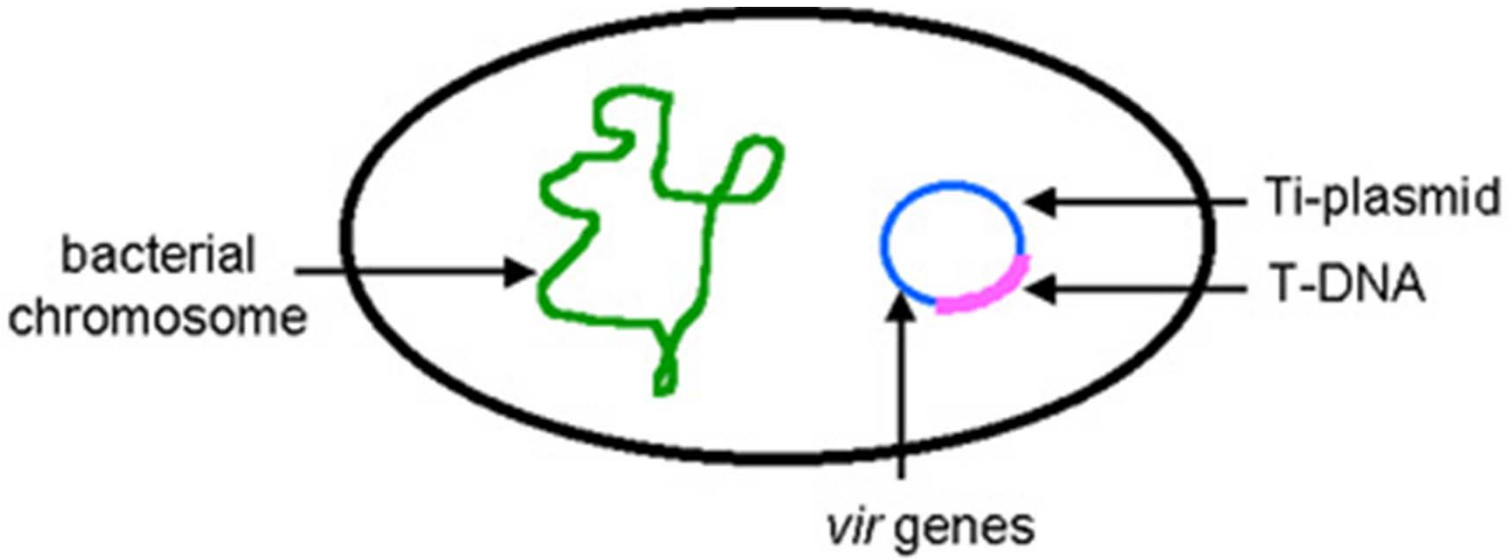
Protoplasts from different plants are fused by culturing them together

Hybrid plants grow

Leaf fragment technique

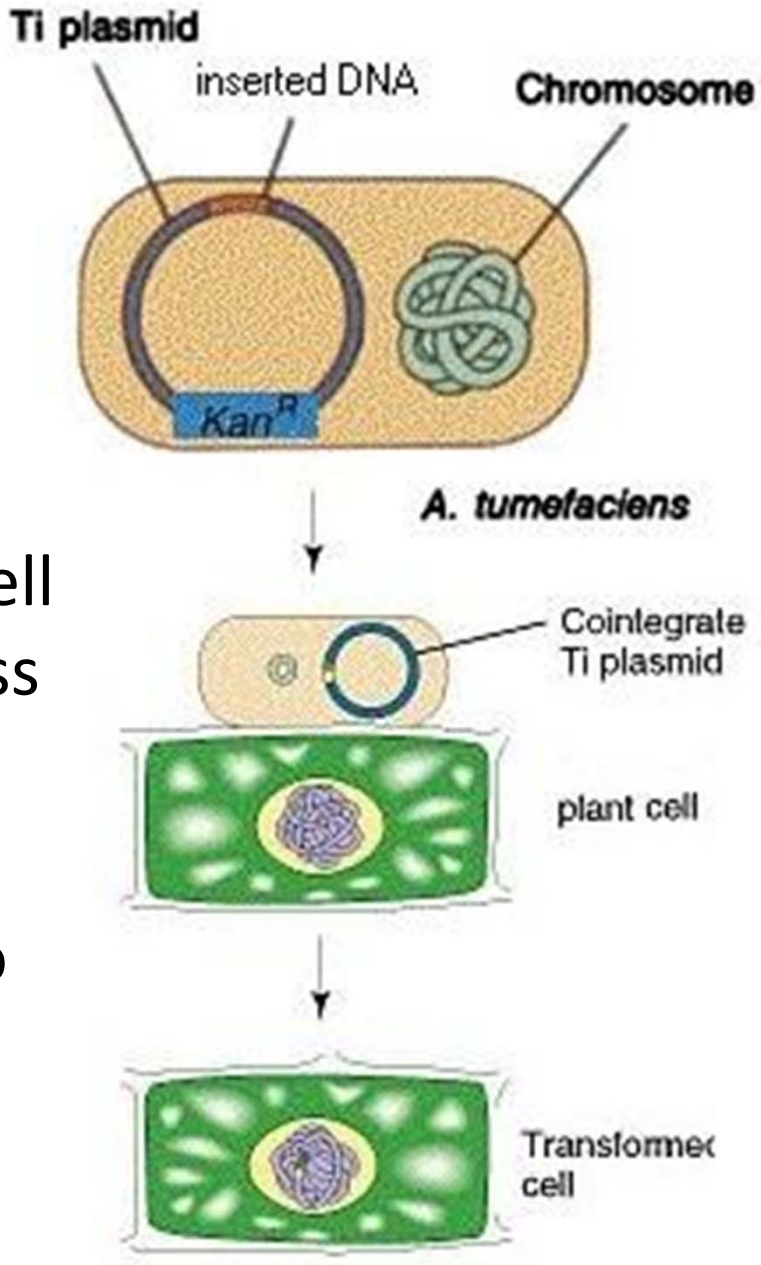
Agrobacterium tumefaciens as a vector for transferring foreign genes into plant chromosome

- This bacterium naturally infects plant cells causing cancerous growths - crown gall disease
- Infection (*vir*) genes carried on Ti plasmid



Infection Process

- Vir genes copy T-DNA
- Open channel in bacterial cell membrane for T-DNA to pass through
- T-DNA enters plant through wound, integrates itself into plant chromosome



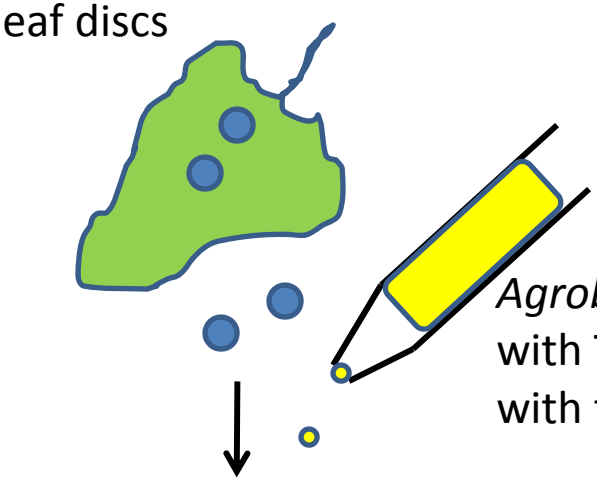
http://www.bio.davidson.edu/people/kabernd/seminar/2002/method/dsmeth/ds.htm

Leaf fragment technique used to introduce foreign genes into plant

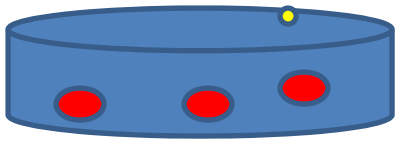
- Small discs are cut from plant leaf
- Discs are cultured to start a new plant
- Early in the regeneration process, the bacterium *Agrobacterium tumefaciens* carrying a Ti plasmid is introduced into the culture
- The plasmid DNA combines with the plant chromosome
- Discs are treated with hormones to encourage shoot and root development and then the new plant is planted in the soil

Leaf fragment technique

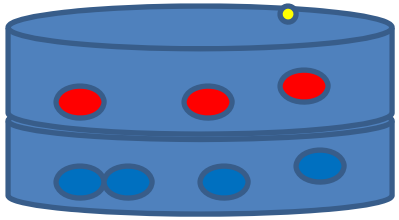
Make leaf discs



Agrobacterium with Ti plasmid with foreign gene



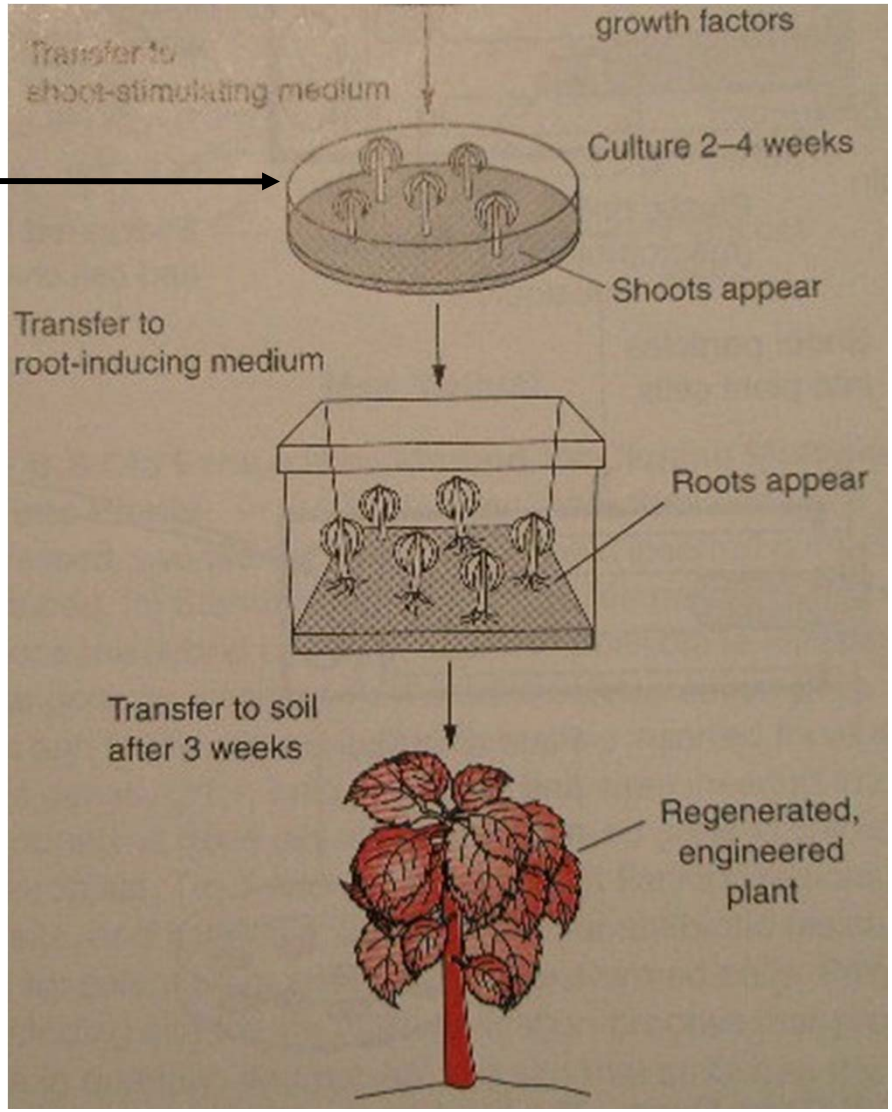
Briefly culture discs with genetically modified *Agrobacterium*



Transfer to filter paper over nurse cells

Culture 2-3 days

Transfer to shoot stimulating medium



Gene Guns

- Used to blast tiny metal beads coated with DNA into an embryonic plant cell
- Hit or miss
- Aimed at the nucleus or the chloroplast
- Use marker genes to distinguish genetically transformed cells
  - Antibiotic resistance
- Technique is useful in plants that are resistant to *Agrobacter*