University of Anbar

Department of Biotechnology

College of Science

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Dr. Huda Musleh

Biotechnology 1

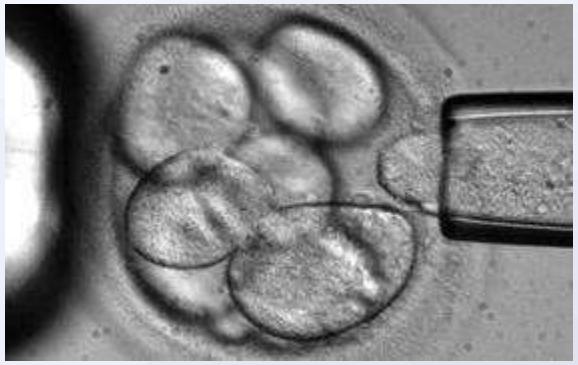
Lecture 1: Biotechnology

In this lecture…

- Agricultural biotechnology
  - Crop engineering
  - Biofuels
- Medical biotechnology
  - Stem cells and animal cell culture
  - Gene therapy
  - Cloning
- Synthetic life

Definition of biotechnology

- "The use of living things and biological processes to produce products"
  - Antibiotics
  - Biofuels
  - Stem cells
  - Beer and cheese

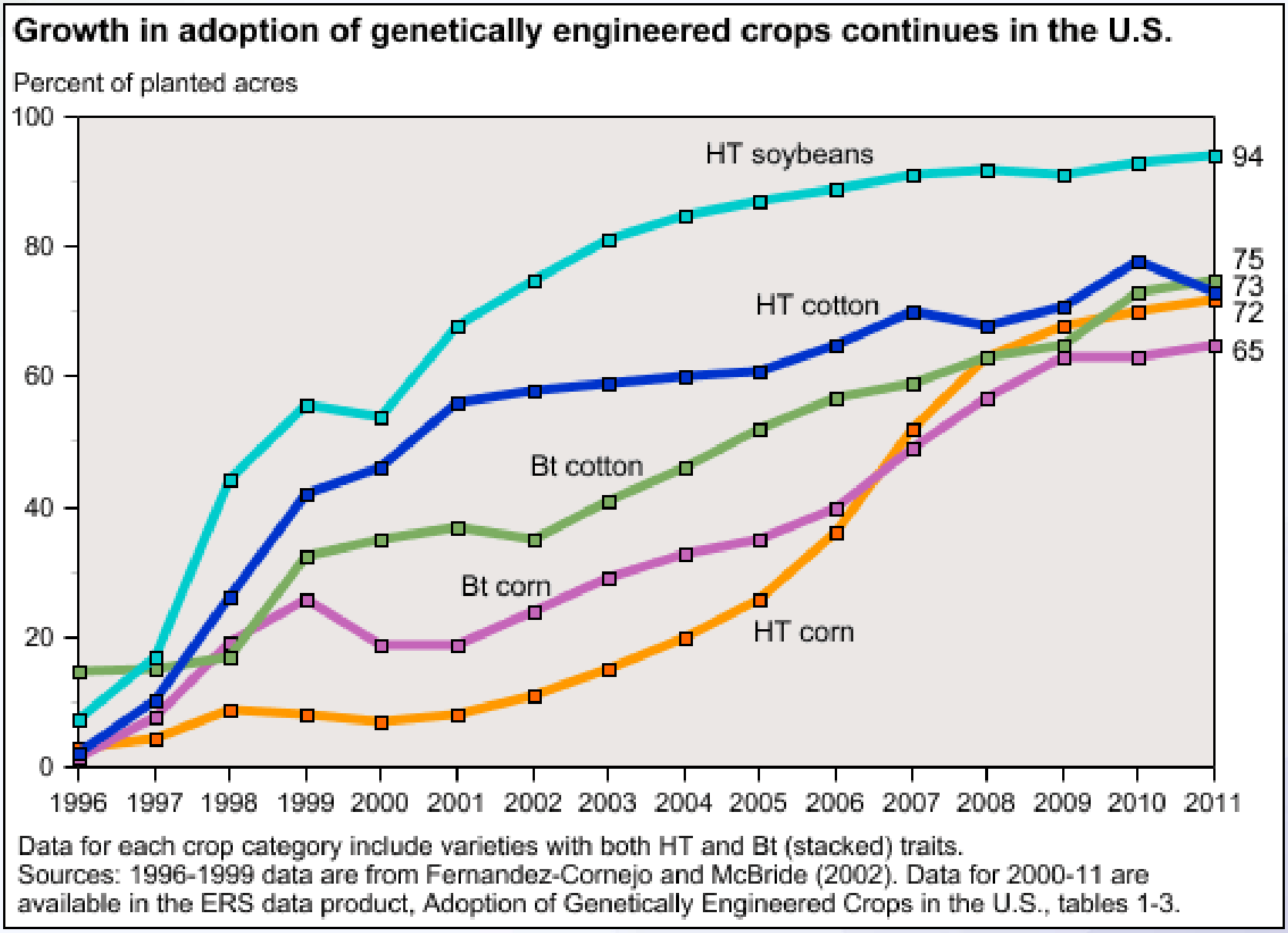


Biotechnology timeline

- 1972 – first transformation of bacteria by Boyer and Cohen
- 1980 – U.S. Supreme Court ruled that genetically modified organisms were patentable in *Diamond v. Chakrabarty*
- 1981 – first genetically engineered plant
- 1981 – mice successfully cloned
- 1982 – insulin produced through bacterial transformation approved for use by the FDA
- 1983 – PCR invented
- 1986 – first field trials of GMOs (tobacco)
- 1986 – first biological drugs approved
- 1990 – first federally approved gene therapy treatment
- 1993 – FDA says GMOs are GRAS
- 1995 – first full genome sequence of a living organism (*Hemophilus influenzae)* finished
- 1997 – Dolly is cloned using DNA from adult sheep cells
- 2001 – human genome sequence finished
- 2010 – first synthetic cell

Agricultural Biotechnology

- Insertion of gene(s) to improve:
  - Taste and nutrition
  - Crop yield
  - Crop hardiness
  - *Reduced dependency on fertilizers, pesticides, etc.
    - Glyphosate, glufosinate, bromoxynil



HT = herbicide tolerance (such as Roundup)

Bt = botulinum toxin

We have been doing artificial selection of crop traits for 10,000 years

Using biotechnology techniques, we can do in months what it took tens or hundreds of years to achieve

Agricultural biotechnology overview

How do you engineer the plants?

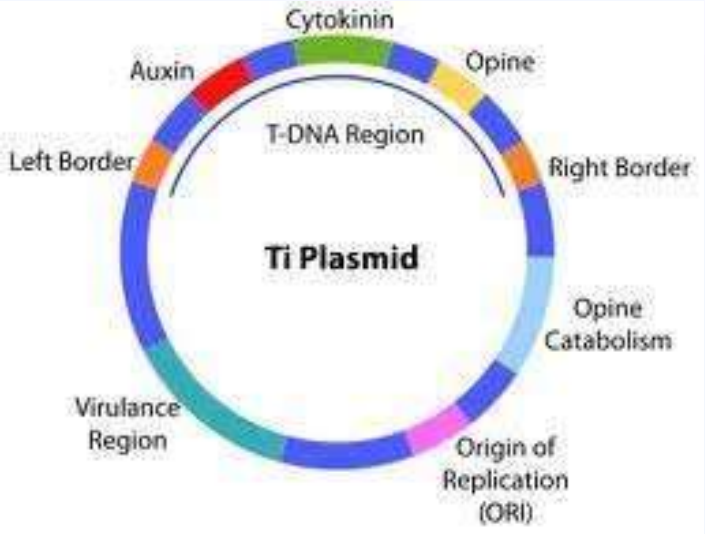
- *Agrobacterium tumefaciens* transformation
- Biolistics

What do you engineer into the plants?

- Herbicide resistance
- Pesticides
- Increased hardiness
- Taste and nutrition

Bacterial transformation

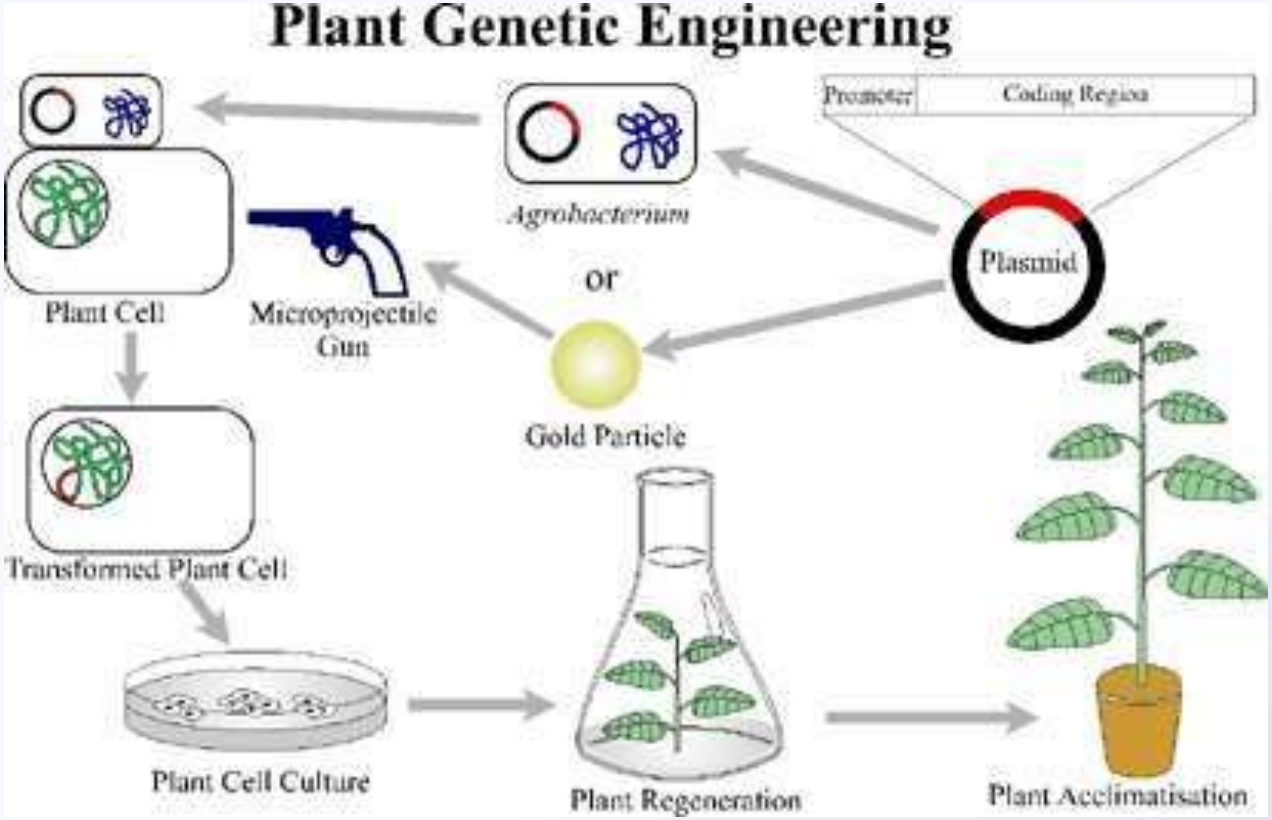
- *Agrobacterium tumefaciens* is a soil-dwelling bacterium that causes crown gall tumors in plants
- *A. tumefaciens* can contain a plasmid called the Ti plasmid
- Tumor formation is caused by the insertion of a plasmid into plant cells from the bacteria



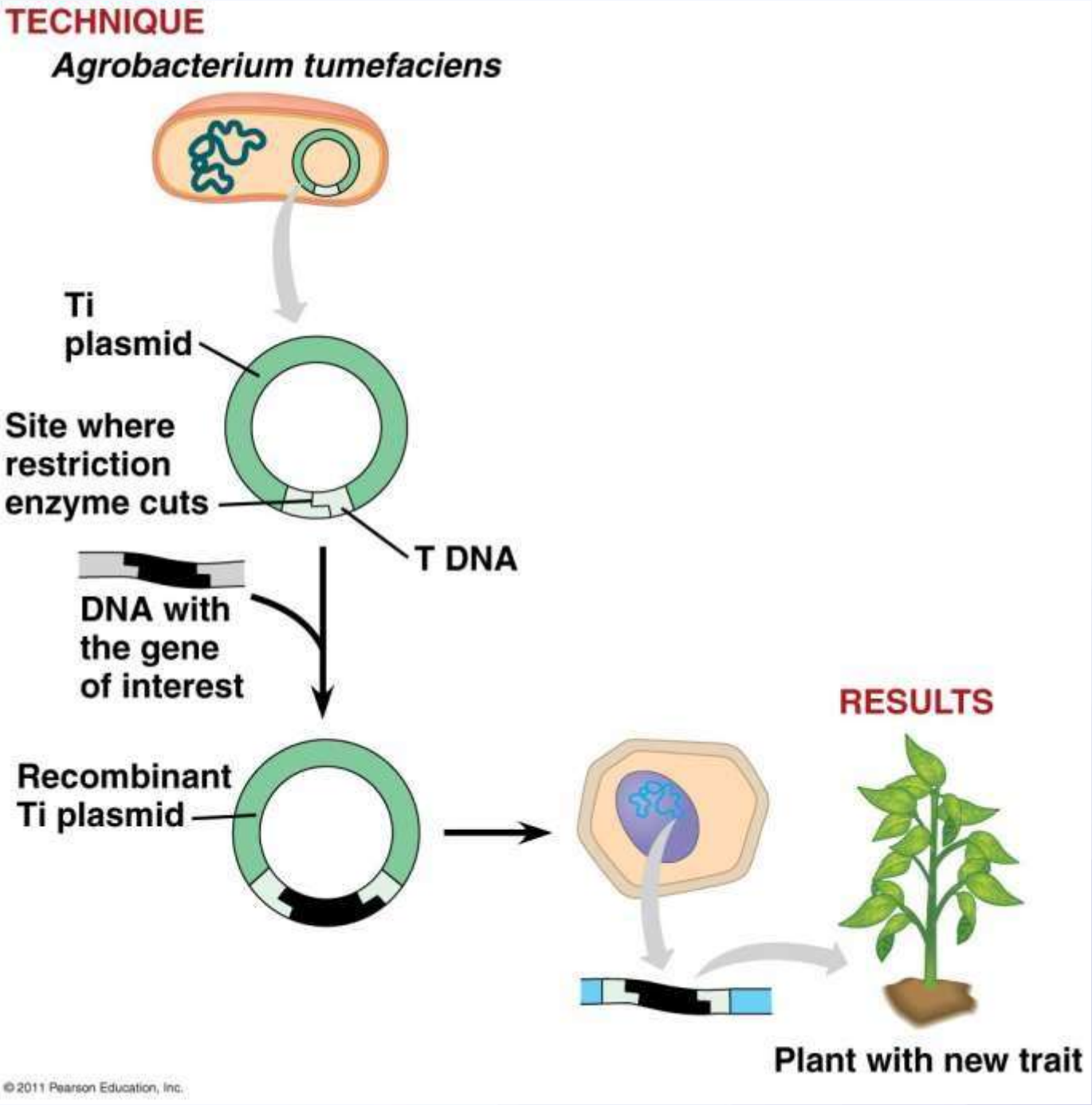
Bacterial transformation

- Isolate your gene of interest (GOI) from the host organism
  - Gene for bt toxin
- Splice together the GOI and the Ti plasmid
  - Considerations: promoter (35S CMV), codon bias, and reporter genes, elimination of virulence region
- Introduce plasmid into *A. tumefaciens*
- Mix transformed *A. tumefaciens* with immature plant cells
- Regenerate/grow plant

Bacterial transformation

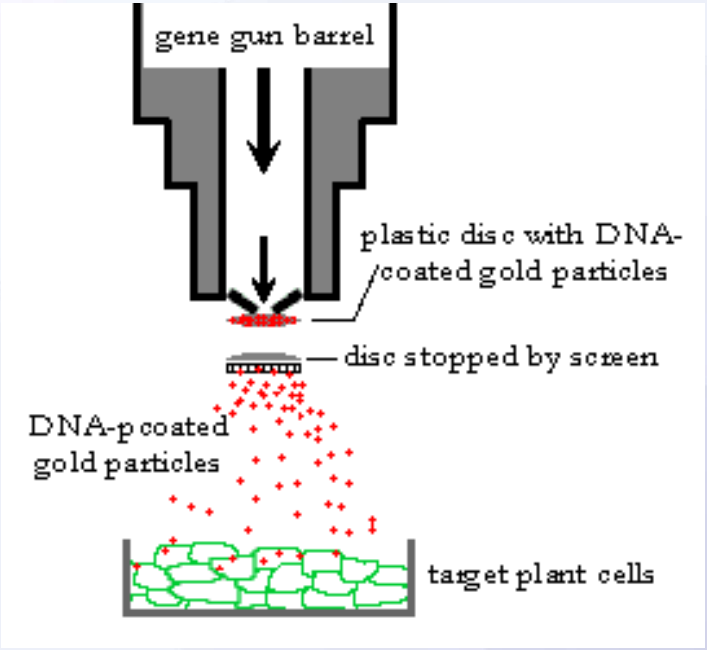


Transformation using *A. tumefaciens* is the most common plant engineering method


TECHNIQUE
Agrobacterium tumefaciens
Ti plasmid
Site where restriction enzyme cuts
T DNA
DNA with the gene of interest
Recombinant Ti plasmid
RESULTS
Plant with new trait
© 2011 Pearson Education, Inc.

Biolistics

- Gold particles coated in plasmid DNA are ‘fired’ into plant cells using a gene gun
- Gets past cell wall and hopefully hits nucleus

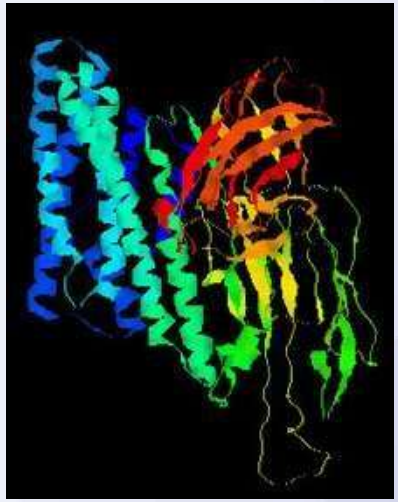


The bt toxin as a pesticide

- *Bacillus thuringiensis* produces a crystal-like (*cry)* toxin deadly to insects but safe for mammals
  - Different bt toxins will affect different insects
- The toxin binds to proteins in insect guts and punches holes through the gut
- Organic – no long-term environmental contamination

A 12-day-old cotton bollworm larva raised on a diet with no Bt. Source: USDA

A 12-day-old cotton bollworm larva raised on a diet containing Bt proteins. Source: USDA



Use of Bt toxin in agriculture

- Traditionally the toxin is mass-produced in the bacteria then used as a spray on crops
- Almost all corn and soybeans now contain a gene for production of bt within the plant
- Reduced need for spraying of insecticides
- Acceptable in organic agriculture since it is biological in origin

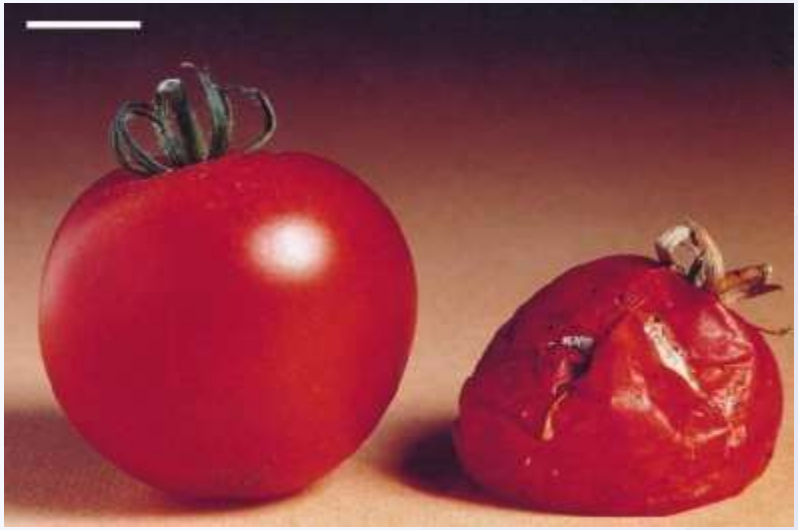
Roundup-ready

- Roundup (glyphosate) is an herbicide used to kill plants, primarily weeds
- Glyphosate competitively inhibits an enzyme involved in amino acid synthesis
- Crop plants like corn, soybeans, and have been engineered using an enzyme that allows them to break down glyphosate
- They are now resistant to glyphosate so farmers don't risk crop damage when spraying
- Reduces amount of glyphosate necessary for spraying

93% of soybeans in the US are Roundup-ready

Flavr Savr Tomato

- First commercially grown genetically engineered food approved for human consumption in 1992 through Calgene
- Normal tomatoes are picked unripe so they are firm and easy to handle, then artificially ripened using ethylene gas
- If allowed to ripen on the vine, the enzyme polygalacturonase would kick in and begin to degrade pectin in the cell walls, turning the tomato soft and easy to rot

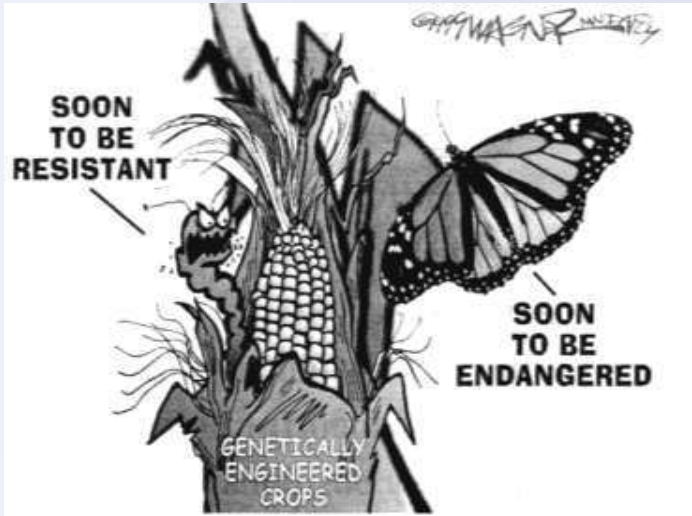


Flavr Savr Tomato

- Calgene hoped to slow softening while still maintaining tomato nutrition and taste
- Inserted an antisense gene that would interfere with production of polygalacturonase, and allow tomatoes to ripen on the vine yet remain firm
- Flavr Savr tomato discontinued in 1997 due to poor business practices by Calgene and shifting public perception of GMO food
- FDA did not require labeling of GMO food because it deemed the tomato to be identical in terms of nutritional content and safety to regular tomatoes

Health and environmental concerns of GMOs

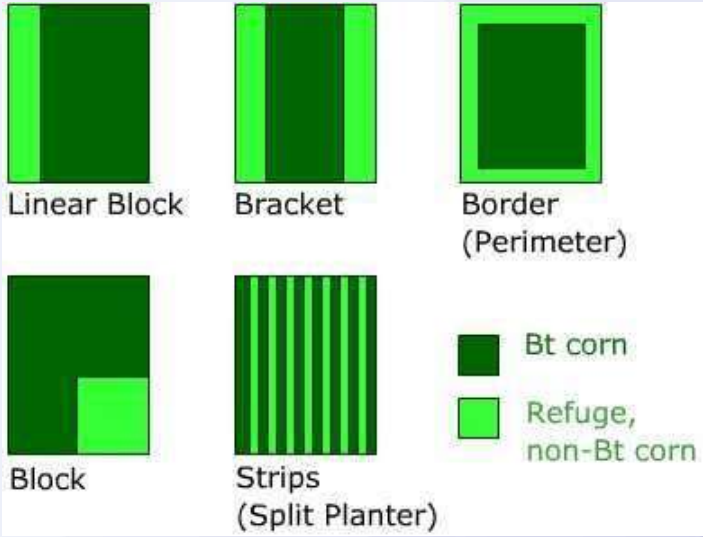
- Insect resistance management
  - Alternation of synthetic pesticides and bt, crop rotation
- Gene flow and outcrossing
- Preserving genetic diversity in crop plants
- Economic impacts (patents)
- No human health concerns



Bt refuge areas

- Development of resistance to bt by insects is a huge worry
- EPA requires farmers must plant 80% bt cotton and 20% non-bt (wildtype) cotton
- Insects from refuge areas will breed with those in transgenic areas to hopefully produce nonresistant progeny

A field of Bt cotton with a border of non Bt cotton. Source: California Agriculture



Biofuels

- Fuels made from biological processes

1st generation biofuel: made from sugar, starch, or oil, and other nonsustainable feedstock

2nd generation biofuel: made from plant portions that are nonedible (cellulose)

3rd generation: made from non-food plants (algae, switchgrass)

Ethanol Biofuels

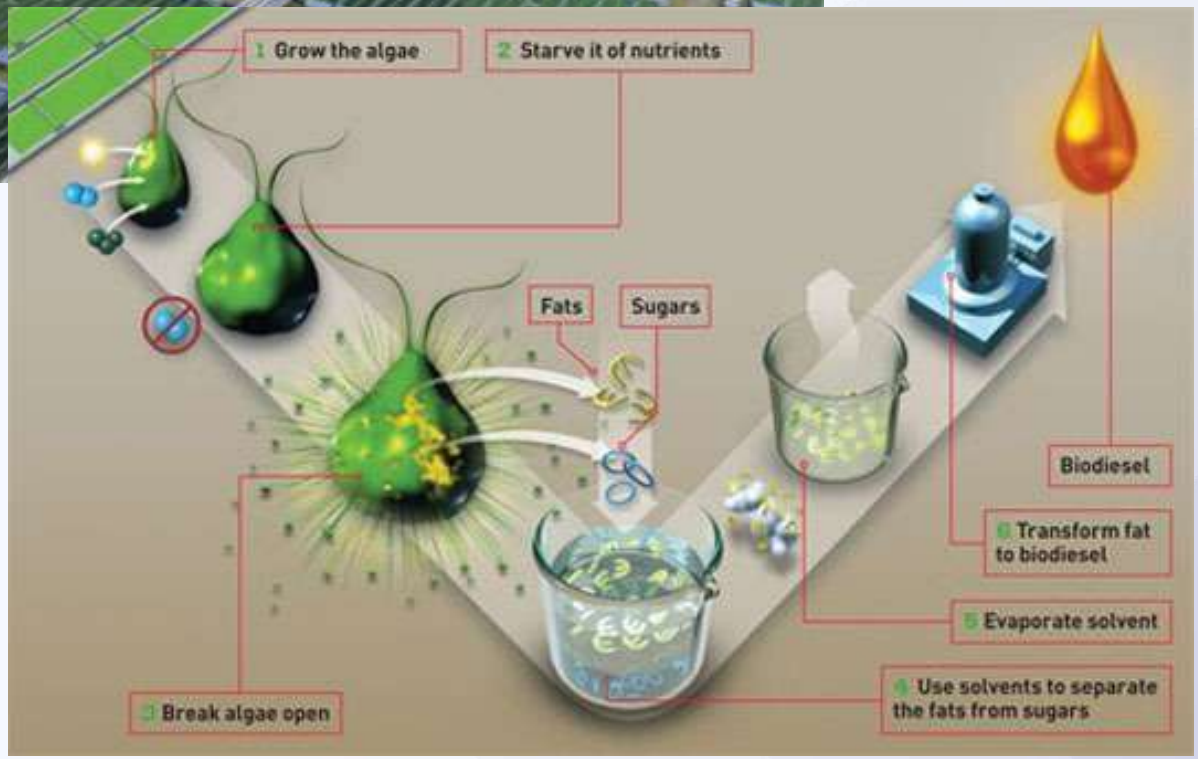
- 55% of the energy of gasoline - $3.45 per gallon tradeoff
- 1st generation produced through fermentation of starches and sugars
  - Corn is the major source of starch and sugar in the U.S.
  - Other sources include sugar cane and vegetable oil
  - Net energy gain over gasoline is very small due to lack of infrastructure and
- 2nd generation produced from cellulose
  - Problem: hydrolyzing cellulose into glucose so bacteria can ferment it
  - Problem: lignin is very hard to break down

Biodiesels

- 90% of the energy of regular diesel
- Produced by reacting animal and plant fats with alcohol (esterification)
  - Soybean oil, vegetable oil, waste oil, frying oil
- Biodiesel can be blended with conventional diesel – up to 20% with no modifications on vehicles required

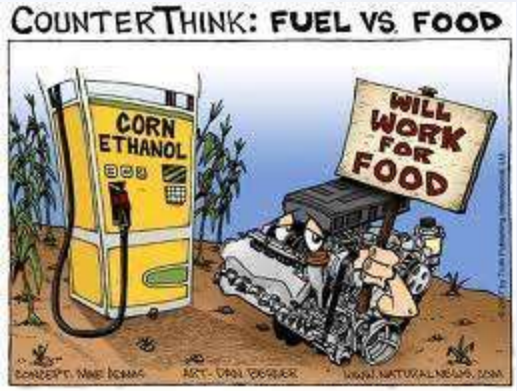
3rd generation biofuels

- Almost exclusively algae, which produce oil in their cell walls
- The algal oil is refined into usable fuel using esterification
- Algae can be grown almost exclusively indoors, and takes up far less room than corn and other biofuel crops
- Problem: difficult to grow correctly, has not yet been tested thoroughly in cars


1 Grow the algae
2 Starve it of nutrients
Fats
Sugars
Biodiesel
4 Transform fat to biodiesel
5 Evaporate solvent
3 Break algae open
6 Use solvents to separate the fats from sugars

‘Food vs. Fuel’ Debate

- Corn, sugar cane, etc. can be used as both food and fuel
  - 25% of corn in the US goes to ethanol production
  - Does this impact the volatility of food prices?
- 2nd and 3rd generation biofuels use the nonuseful parts of a food plant, or a plant that is not used as food in the first place

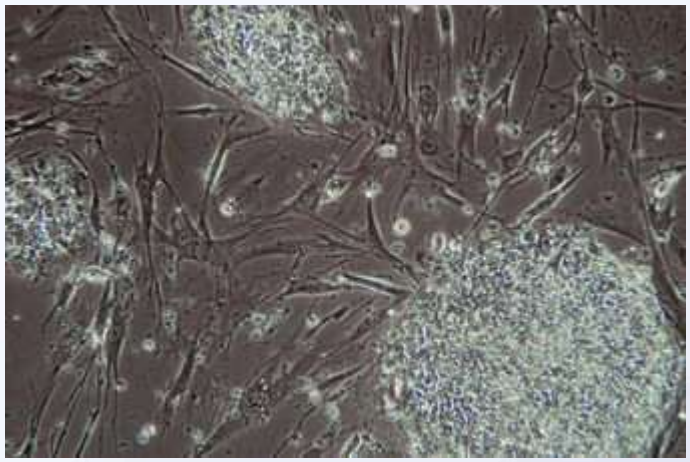


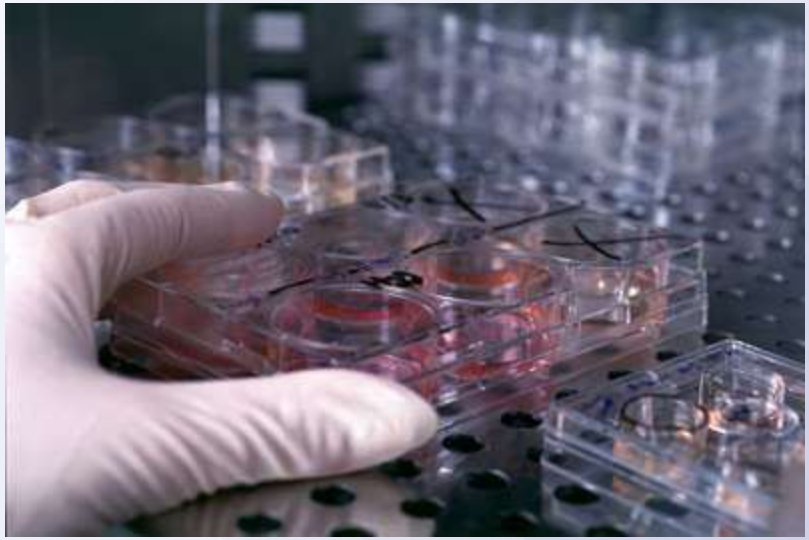
Medical Biotechnology

- Pharmaceuticals
- Stem cells
- Gene therapy
- Gene chips

Stem cell treatments – a biological drug

- What are stem cells?
- Adult stem cells
- Embryonic stem cells
- iPS cells



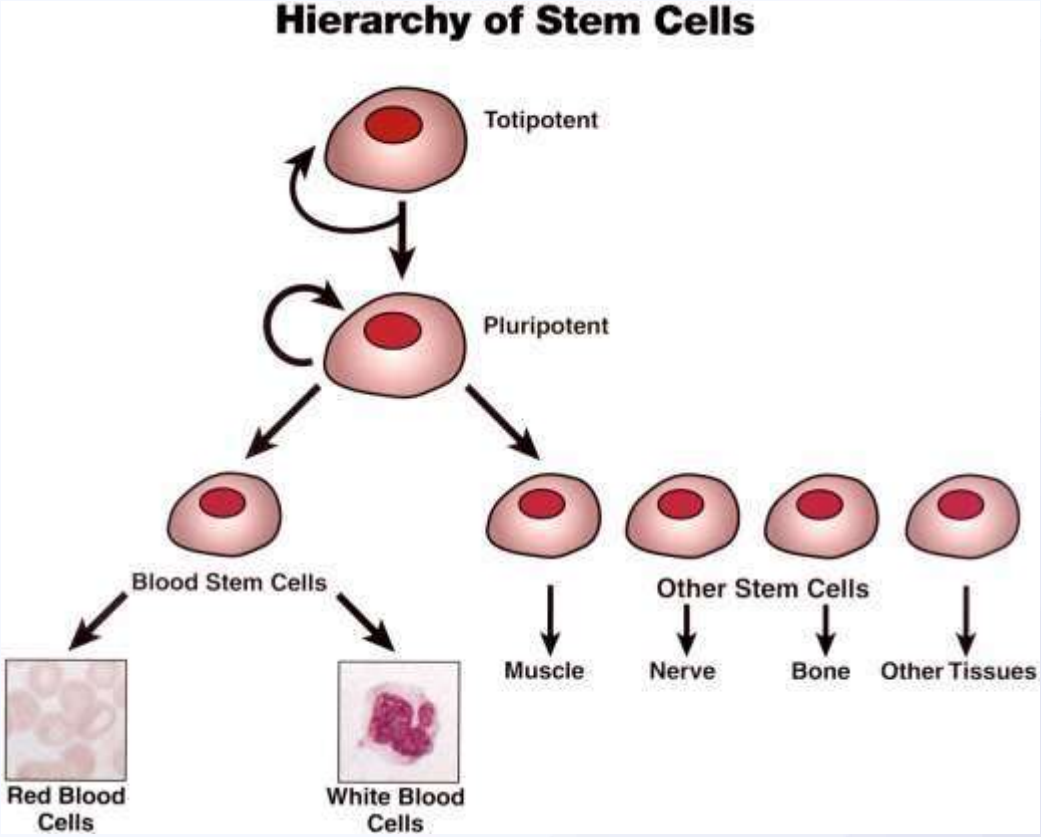


What are stem cells?

- Normal body cells can only produce more of themselves
  - Muscle cells → muscle cells, skin cells → skin cells
  - Limited number of divisions (partly because of telomere shortening)
- **Stem cells** are undifferentiated cells that can develop into any other cell type in the body
  - Unlimited division – one cell can become millions
  - **Totipotent** – can give rise to an entire organism and any cell in the body
  - **Pluripotent** – stem cells that can become any other cell type in the body, but can't form a full organism by themselves
  - **Multipotent** –can only become a limited number of other types of cells

What are stem cells?

- Stem cells were first derived from mouse embryos in 1981
- Human embryonic stem cells were derived and grown *in vitro* in 1998

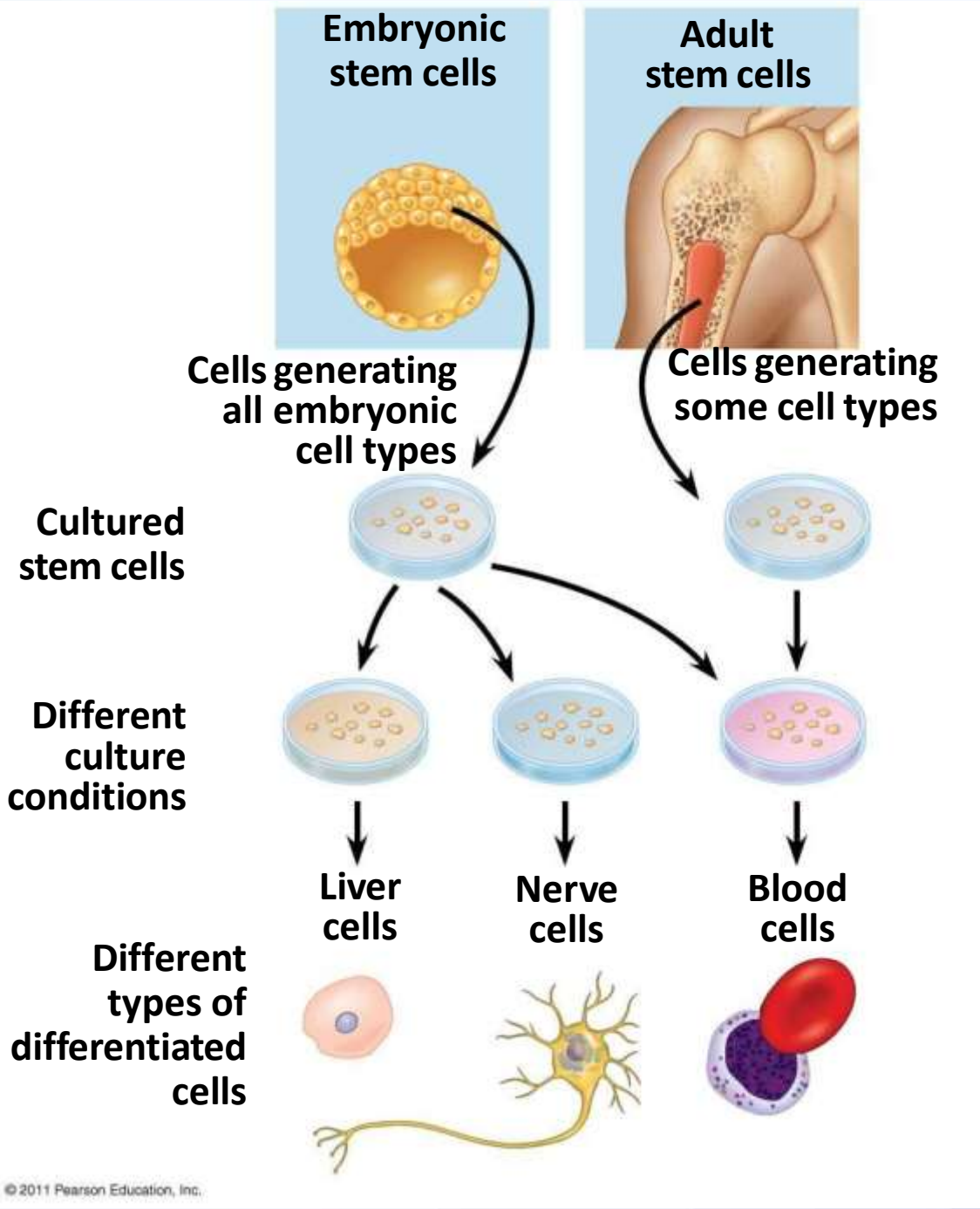


Types of stem cells

- Three types of stem cells:
  - **Embryonic stem cells**
  - **Adult stem cells**
  - **iPS cells**

As stem cells develop and differentiate, their gene expression patterns change – different genes expressed at different times. Methylation probably plays a big role!

Figure20.21


Embryonic stem cells
Adult stem cells
Cells generating all embryonic cell types
Cells generating some cell types
Cultured stem cells
Different culture conditions
Liver cells
Nerve cells
Blood cells
Different types of differentiated cells
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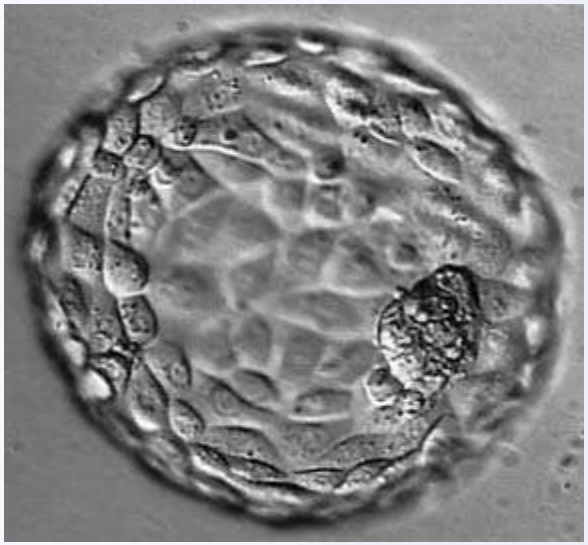
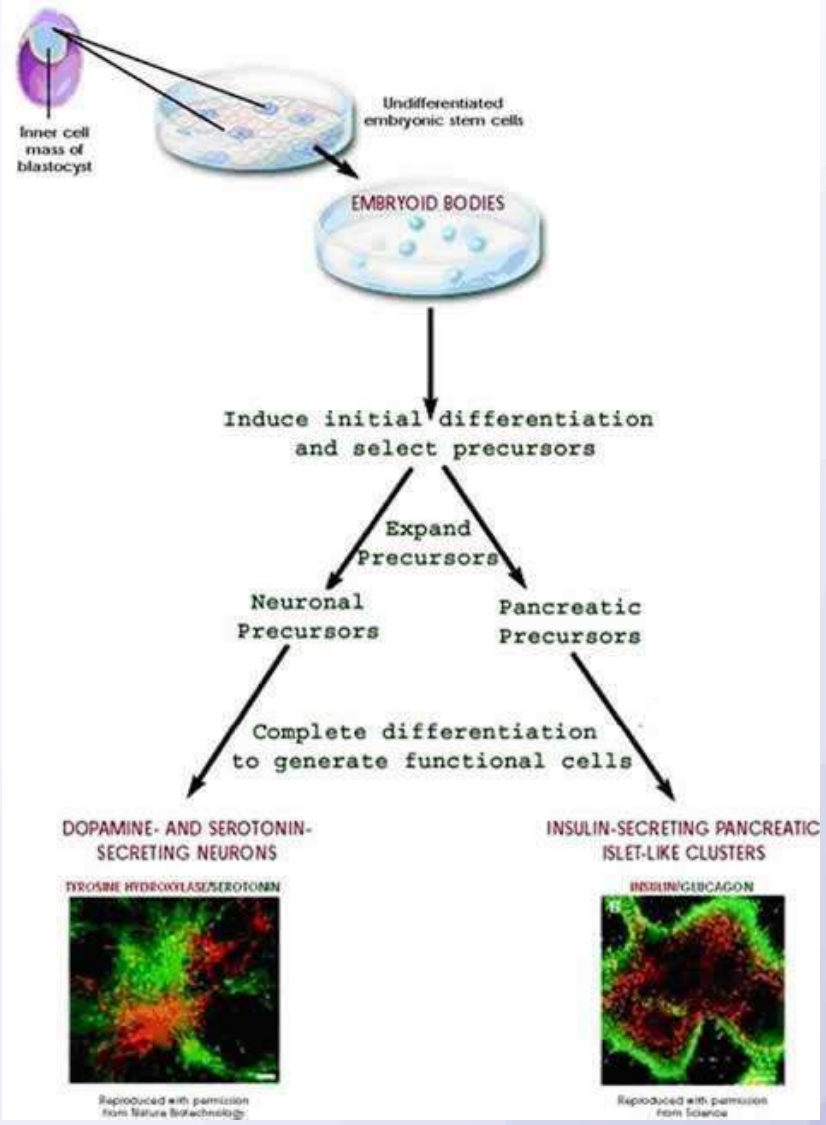
Where do embryonic stem cells come from?

- Derived from the inner cell mass of a fertilized embryo
  - Most are derived from leftover cells from *in vitro* fertilization clinics with donor consent
  - Usually kills the blastocyst
- Chemical 'cocktails' can be added to keep them pluripotent, or to force them to **differentiate** into other cell types



Uses of ESCs

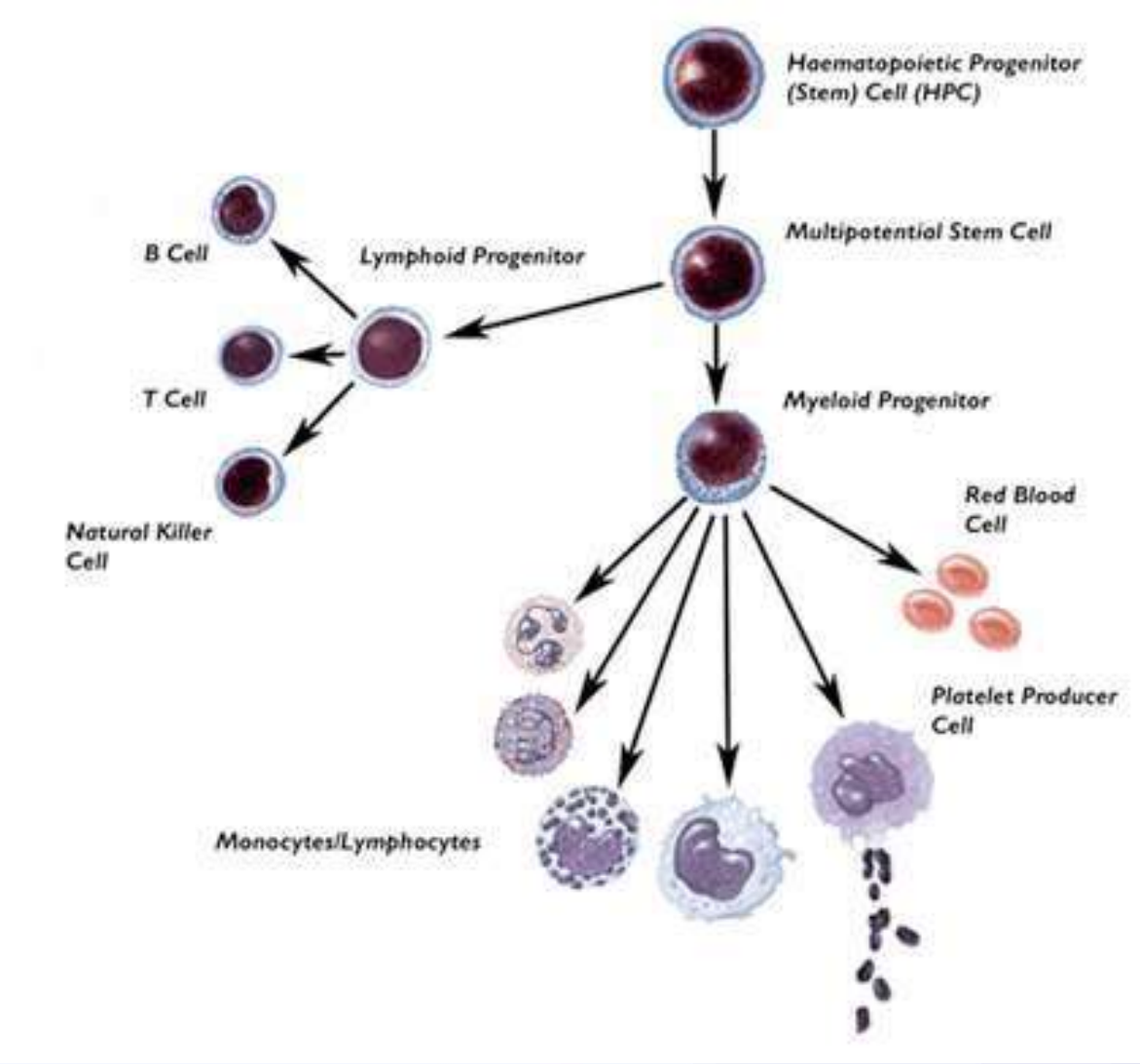
- Pluripotent
- Capable of producing large amounts of cells
  - These cells can then be forcibly differentiated
  - If we can find the triggers that reliably differentiate them…
- Also used to study basic developmental biology
  - What cell signaling pathways contribute to development?



Adult stem cells

- Multipotent
- Within adult bodies, we have populations of partially differentiated stem cells called **adult stem cells**
- These cells can become only a few designated special types of cells
  - Adult blood cells can become red blood cells, white blood cells, etc.
  - Adult neuronal cells can become astrocytes, oligodendrocytes, etc.
  - Some evidence that adult stem cells can act as ESCs
- In some adult tissues, such as bone marrow, muscle, and brain, adult stem cells generate replacements for cells that are lost through normal wear and tear, injury, or disease

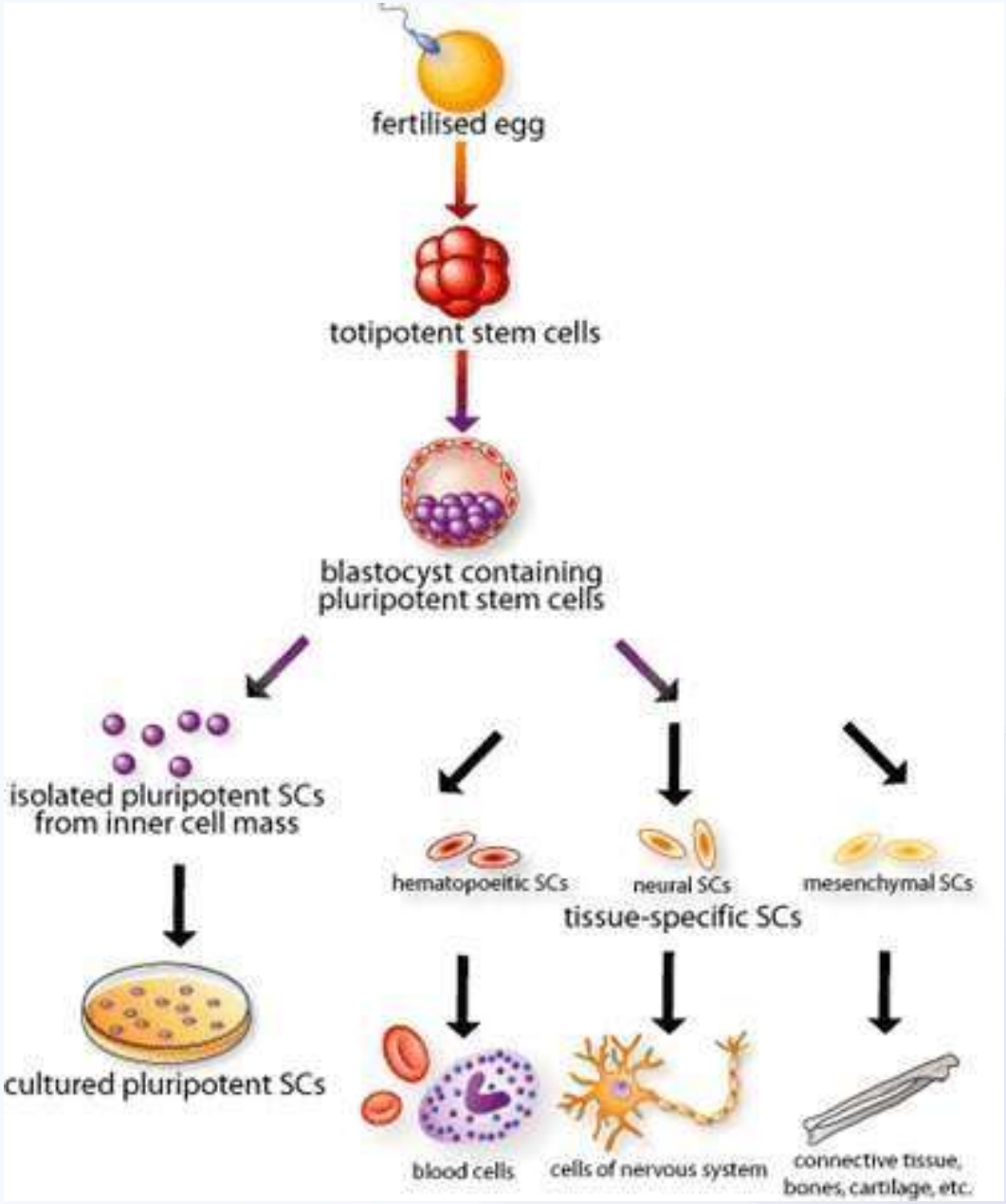
Adult stem cells



Treatments with adult stem cells

- Regenerating spinal cords using hNSCs
- Replacing dead pancreatic beta cells killed in diabetes
- Regrowing teeth
- Regrowing corneas
- Skin grafts for burn victims
- Transdifferentiation?

Neural stem cells are the only cells that are not **immunogenic**


fertilised egg
totipotent stem cells
blastocyst containing
pluripotent stem cells
isolated pluripotent SCs
from inner cell mass
cultured pluripotent SCs
hematopoeitic SCs
neural SCs
mesenchymal SCs
tissue-specific SCs
blood cells
cells of nervous system
connective tissue,
bones, cartilage, etc.

ESCs vs. Adult stem cells

- Advantages:
  - No ethical or moral barriers to use
  - Are already partially differentiated
- Disadvantages
  - Can only divide a limited number of times
  - Difficult to isolate from adult tissues
  - May be more immunogenic than ESCs

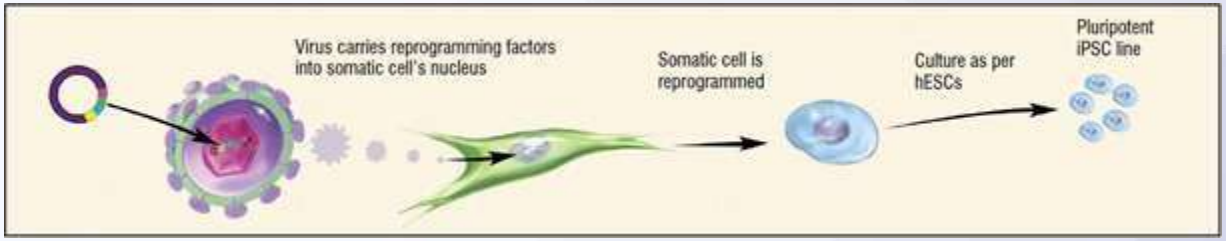
iPS Cells

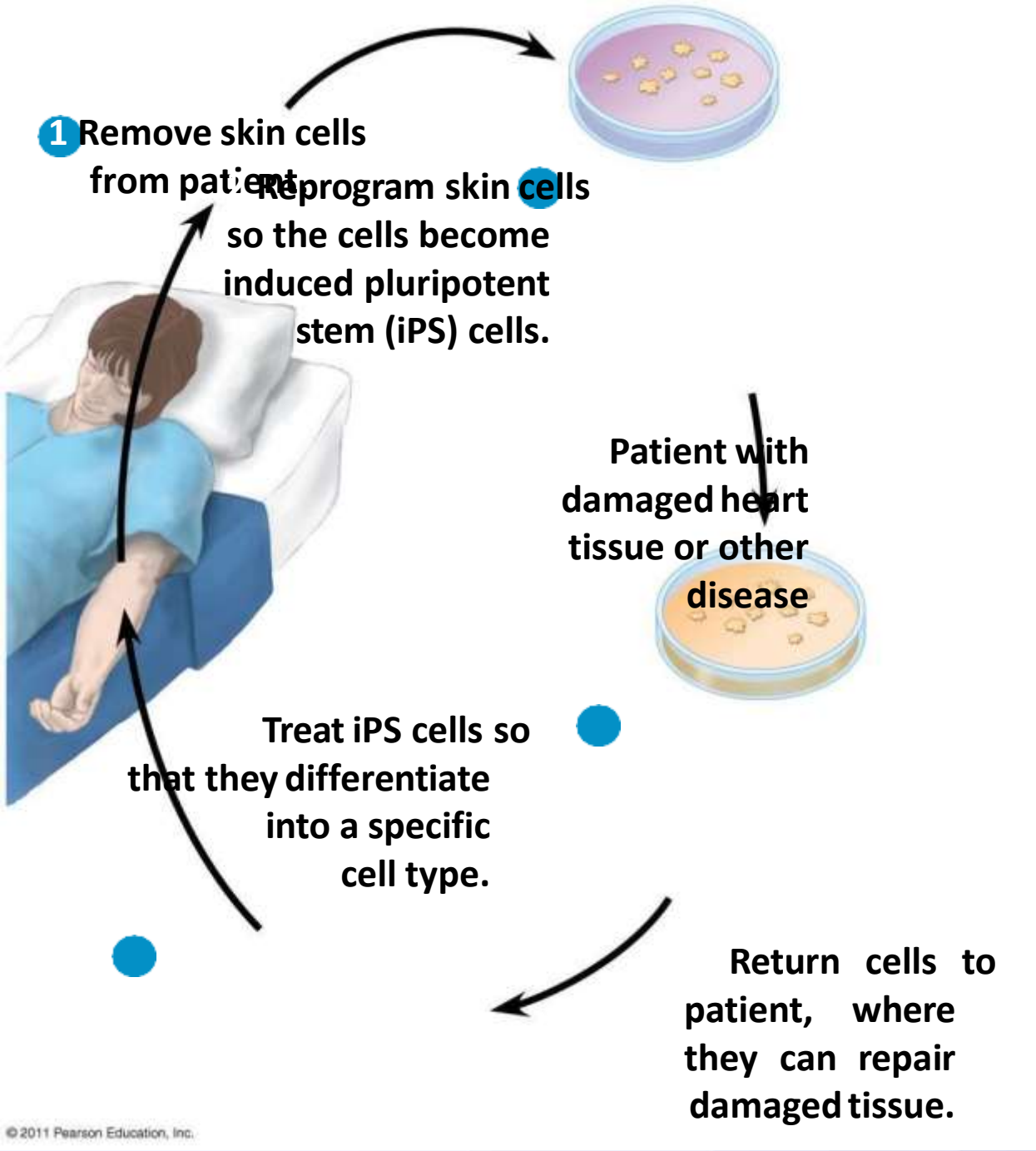
- Induced pluripotent stem cells, method created in 2006
- Adult cells genetically engineered to become stem cells
- Cells can be taken from a donor, reprogrammed to become iPSCs, then put back into the donor
  - No immunogenicity?
- Not known if they actually have the same properties of normal pluripotent stem cells
- Useful for *in vitro* drug development and disease modeling

http://www.youtube.com/watch?v=cgw19KMcWw4&feature=related

How IPSCs are created

- Four genes that encoded for transcription factors (Oct4, Sox2, Klf4, and c-Myc) were forcibly expressed using retroviruses
  - Leftover retroviral elements left over in the genome after insertion
- These genes help to express *other* genes that are important in creating and maintaining and ESC-like state
- <1% of cells in a tissue sample will be reprogrammed




1 Remove skin cells from patient.
2 Reprogram skin cells so the cells become induced pluripotent stem (iPS) cells.
Patient with damaged heart tissue or other disease
3 Treat iPS cells so that they differentiate into a specific cell type.
4 Return cells to patient, where they can repair damaged tissue.
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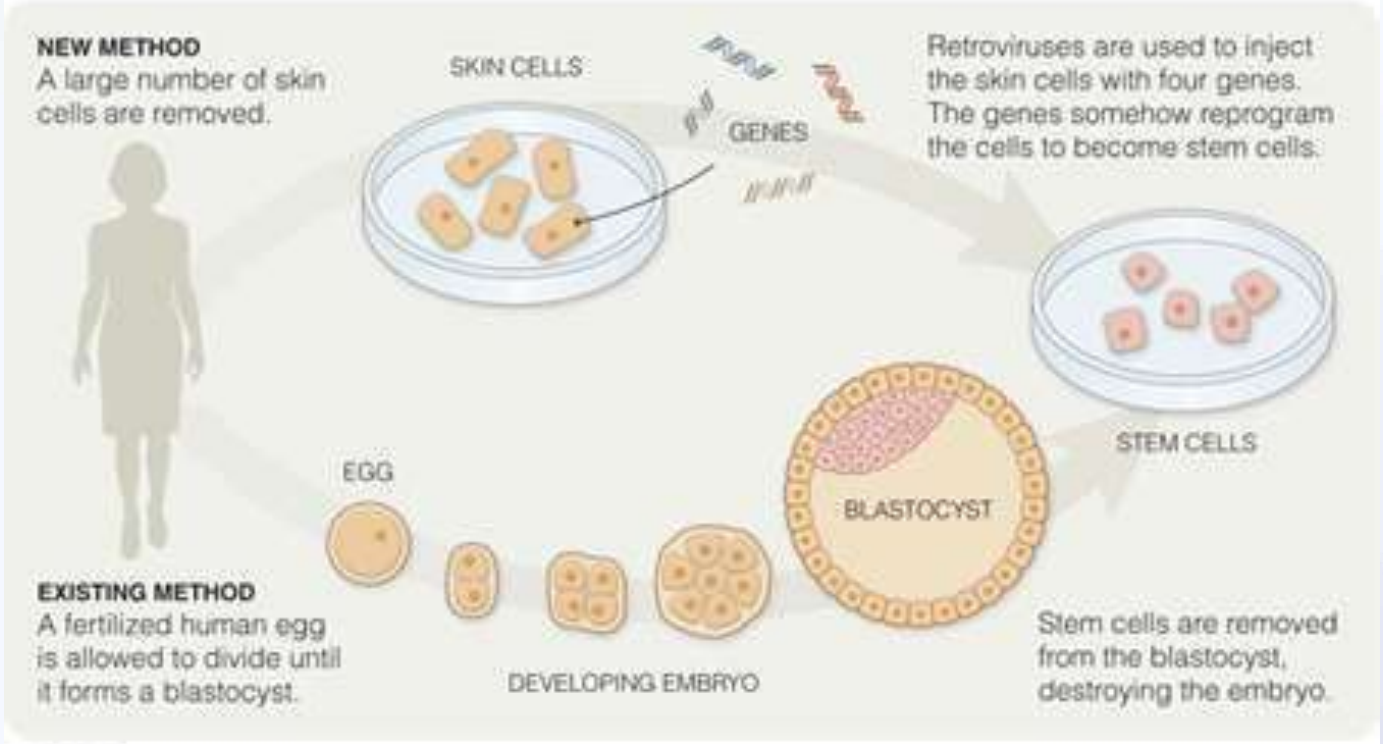
Reprogramming Human Skin Cells

Researchers have developed a technique for creating stem cells without the controversial use of human eggs or embryos. If the method can be perfected, it could quell the ethical debate troubling the field.



TIMELINE

1995 1996 1997 1998 1999 2000 2001 2002 2003 2004 2005 2006 2007

July 1995 Congress bans federal financing of research on human embryos.

July 1996 Dolly is born. The lamb is the first clone of an adult mammal.

Nov. 1998 First isolation and cultivation of embryonic stem cells. The cells are derived from fertilized human eggs.

Aug. 2001 President Bush announces that federal money will pay for research on existing stem cell lines, but not new lines.

Nov. 2004 California voters approve a measure to spend $3 billion over 10 years on embryonic stem cell research.

Nov. 2007 New Jersey voters reject a measure to borrow $450 million for stem cell research.

THE NEW YORK TIMES

Stem Cell Research Timeline

- 1981 – embryonic stem cells are isolated from mice
- 1995 – embryonic stem cells isolated in Rhesus macaques
- 1998 – embryonic stem cells isolated in humans from aborted fetuses and IVF leftovers
- 2001 – President Bush halts federal funding for stem cell research
- 2001 – Advanced Stem Cell Technology clones a human embryo. Evidence is controversial
- 2004 – South Korean scientists clone a human embryo. Their data is later revealed to be fraudulent
- 2004 – New Jersey and California are the first states to fund stem cell research on the state level
- 2005 – Scientists find injecting neural stem cells in mice helps them walk again

Stem Cell Research Timeline

- 2006 – Advanced Cell Technology finds a way to create embryonic stem cell lines without destroying the embryo
- 2006, 2007 – President Bush vetoes two bills funding stem cell research
- 2007 – iPSCs created
- 2009 – President Obama overturns President Bush's embryonic stem cell funding ban
- 2010 – Isolation of adult hematopoetic stem cells
- 2012 – Geron discontinues the first FDA-approved clinical trial of human adult neural stem cells for treating spinal cord injuries

Barriers to stem cell therapies

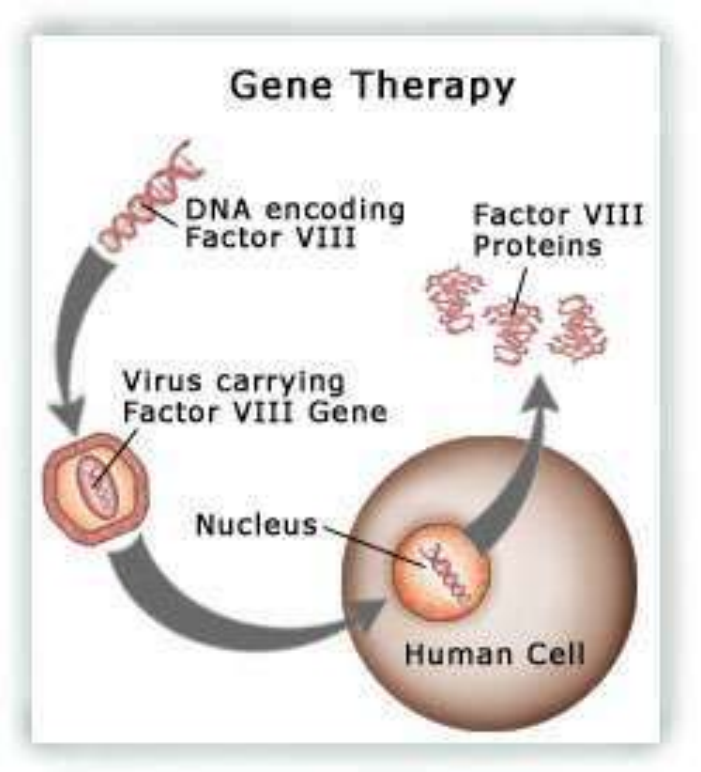
- Politics
- Immunogenicity
- Tumorigenicity
- Implantation and migration
- Correct maturation
- Zoonosis
  - Development of Xenofree systems

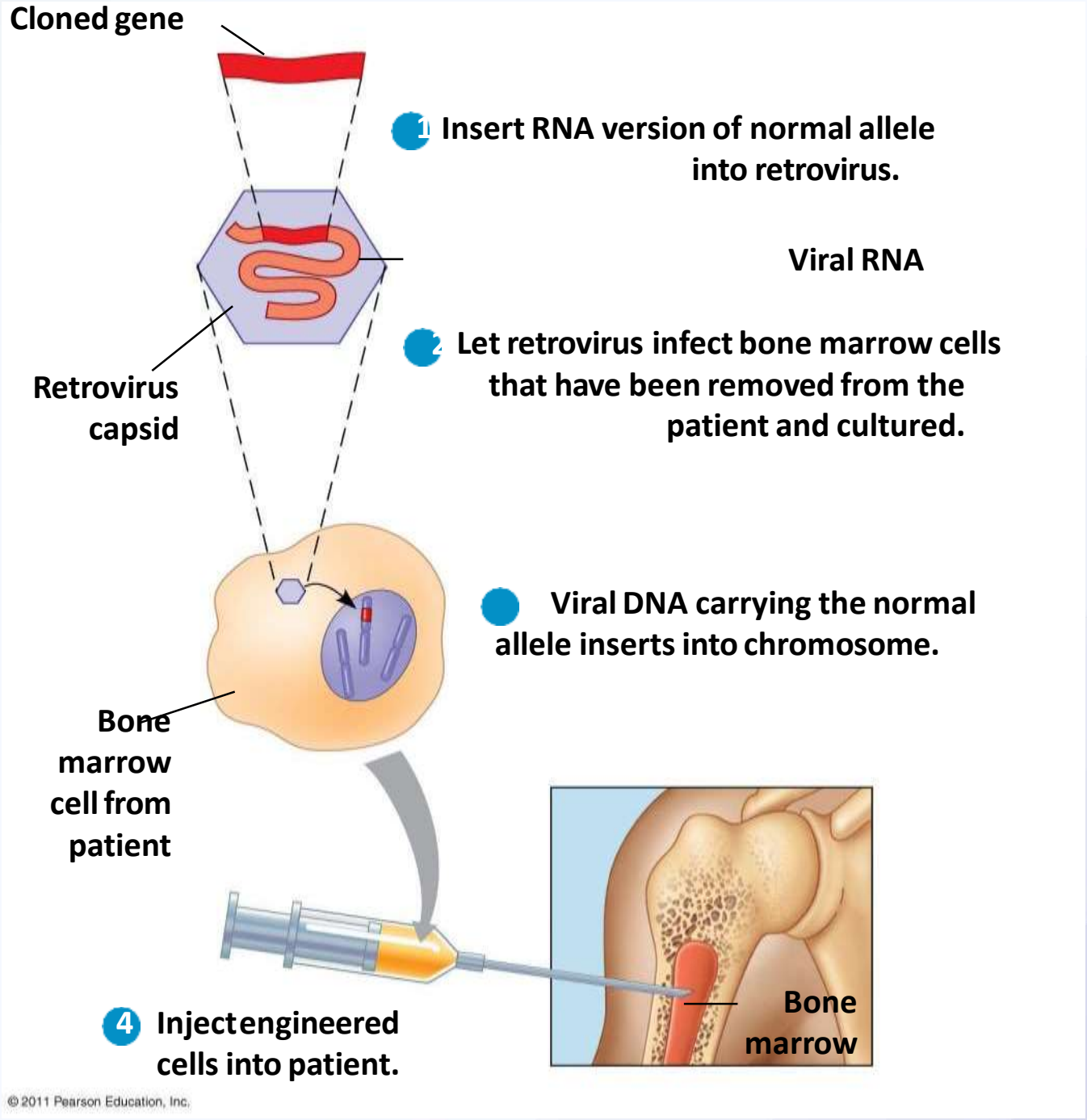
Cancer and stem cells

- Cancer cells may ‘reactivate’ genes stem cells use to grow rapidly and safely
  - Telomerase
- Cancer growth may be driven by a small subpopulation of ‘cancer stem cells’ that survive when chemotherapy kills off normal cancer cells
- CSCs may drive tumor growth the same way ESCs drive organism growth

Gene Therapy

- The replacement of a defective gene or set of genes with a functional copy
- Used to largely treat **monogenic** diseases





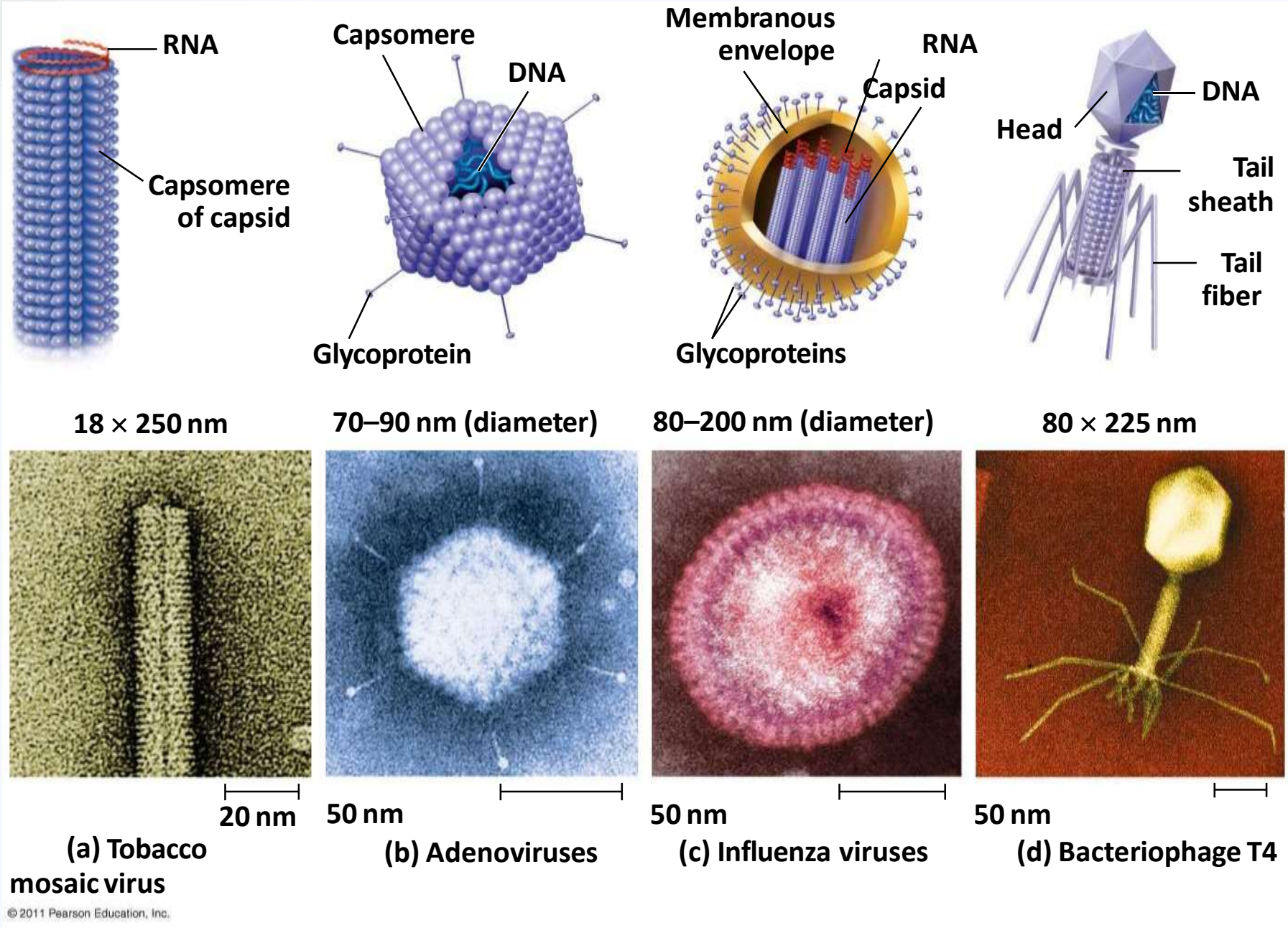
Vectors in gene therapy

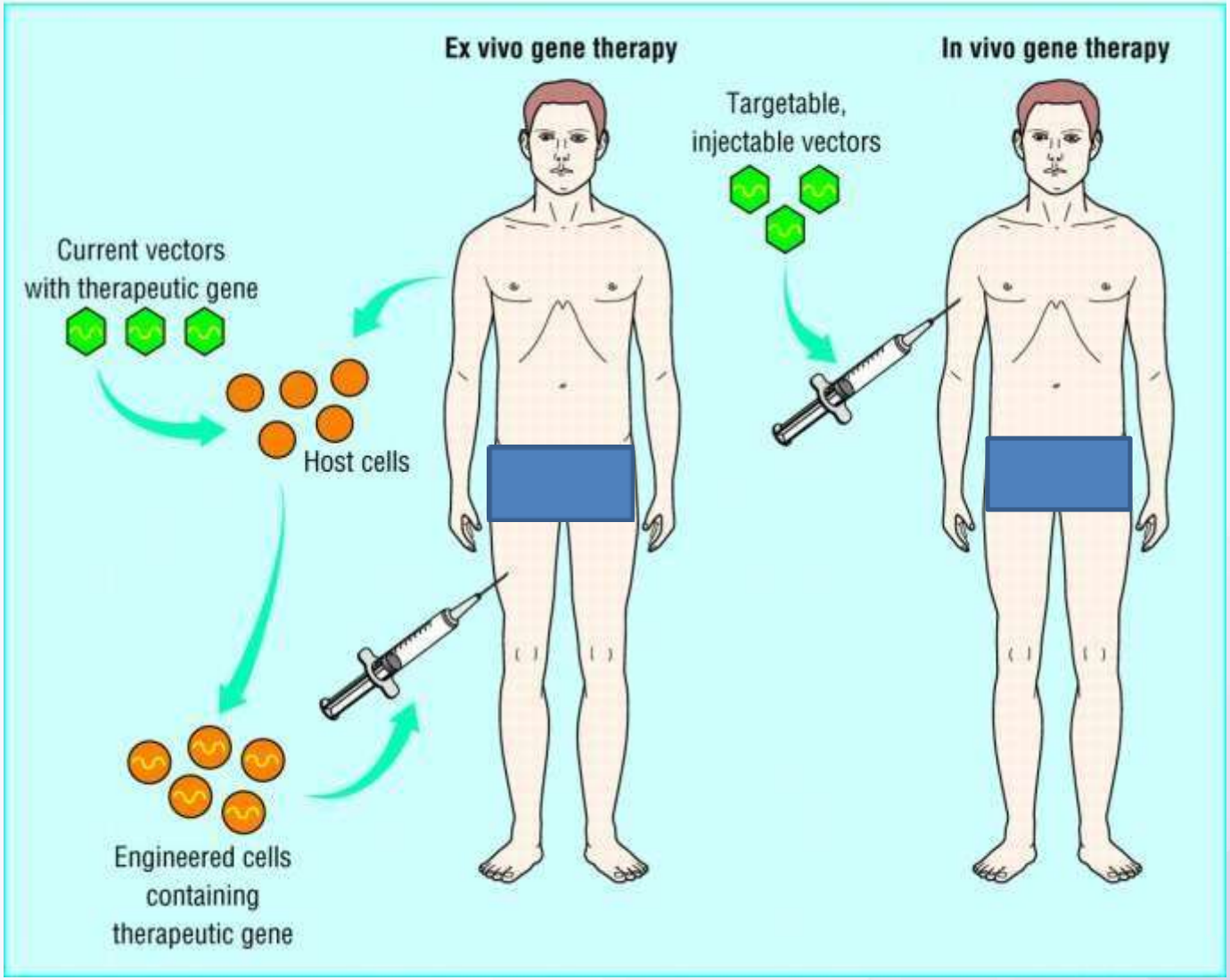
- A **virus** is a small infectious agent that can only replicate within living cells
  - Millions of different types infecting every organism on the planet
- Have three possible components:
  - Genome (ssDNA, dsDNA, ssRNA, dsRNA)
  - Protein coat
  - Lipid membrane (optional)
- Two main life cycles
  - Lysogenic viruses
  - Lytic viruses

Difficulties with gene therapy

- Replacing genes in humans is a lot easier said than done
  - Deliver the gene to the necessary tissues
  - Ensure the gene is stably integrated into the genome
  - Make sure the gene is expressed
  - Immune response
  - Tumorigenesis

Figure 19.3




Ex vivo gene therapy
In vivo gene therapy
Targetable,
injectable vectors
Current vectors
with therapeutic gene
Host cells
Engineered cells
containing
therapeutic gene

Gene Therapy Timeline

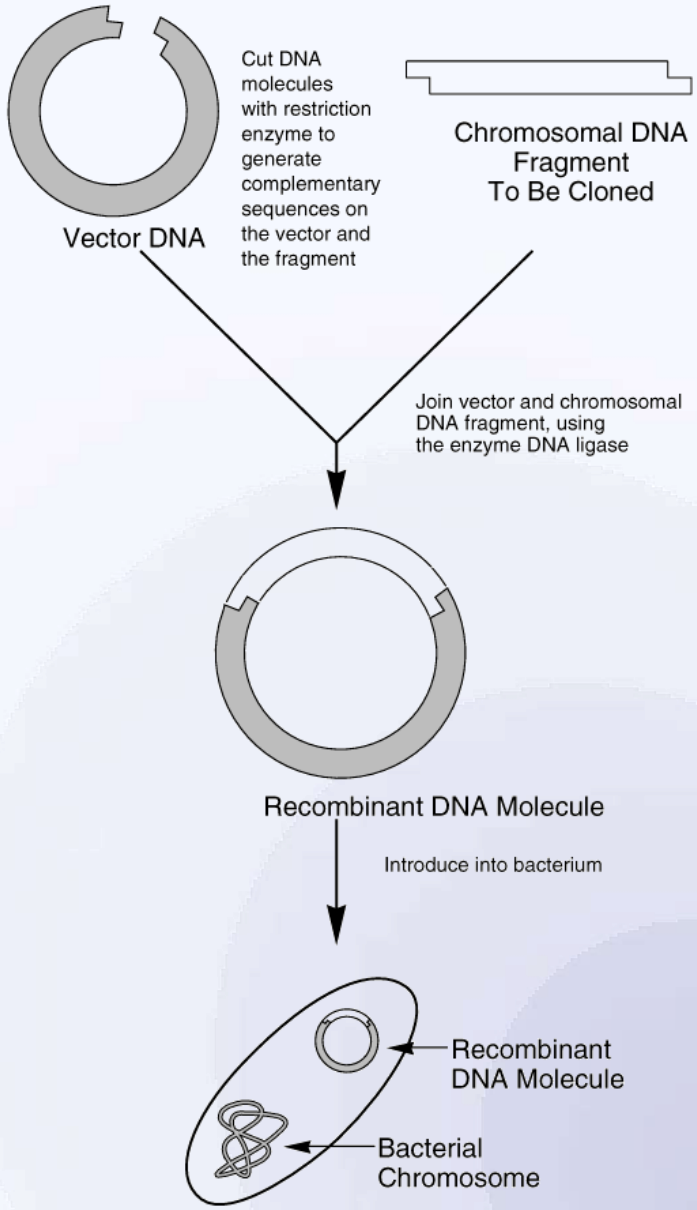
- 1990 – Ashanti DeSilva X-SCID
- 1992 – Treatment for SCID causes leukemia in 20% of patients
- 1999 – Jesse Gelsinger dies after being treated for OTC
- 2003 – RNA interference using gene therapy developed
- 2006 – Two patients cured of melanoma after their T cells are engineered to target cancer
- 2008 – Congenital blindness in children treated using an adeno-associated virus
- 2009 – Adrenoleukodystrophy cured using a vector derived from HIV
- 2011 – A man is cured of HIV infection when engineered hematopoetic stem cells are used to replace his bone marrow

Cloning

- Duplication of biological material
  - Creating copies of DNA fragments
  - Creating multiple cells
- Three types of cloning
  - DNA cloning
  - Research/therapeutic
  - Reproductive

DNA cloning

- "Recombinant DNA technology," "DNA cloning," "molecular cloning"
- Transfer of a gene or other DNA fragment from an organism to a vector, such as a plasmid
  - Transform that plasmid into a bacteria
  - Bacteria multiplies, creating millions of copies of the plasmid



Therapeutic cloning

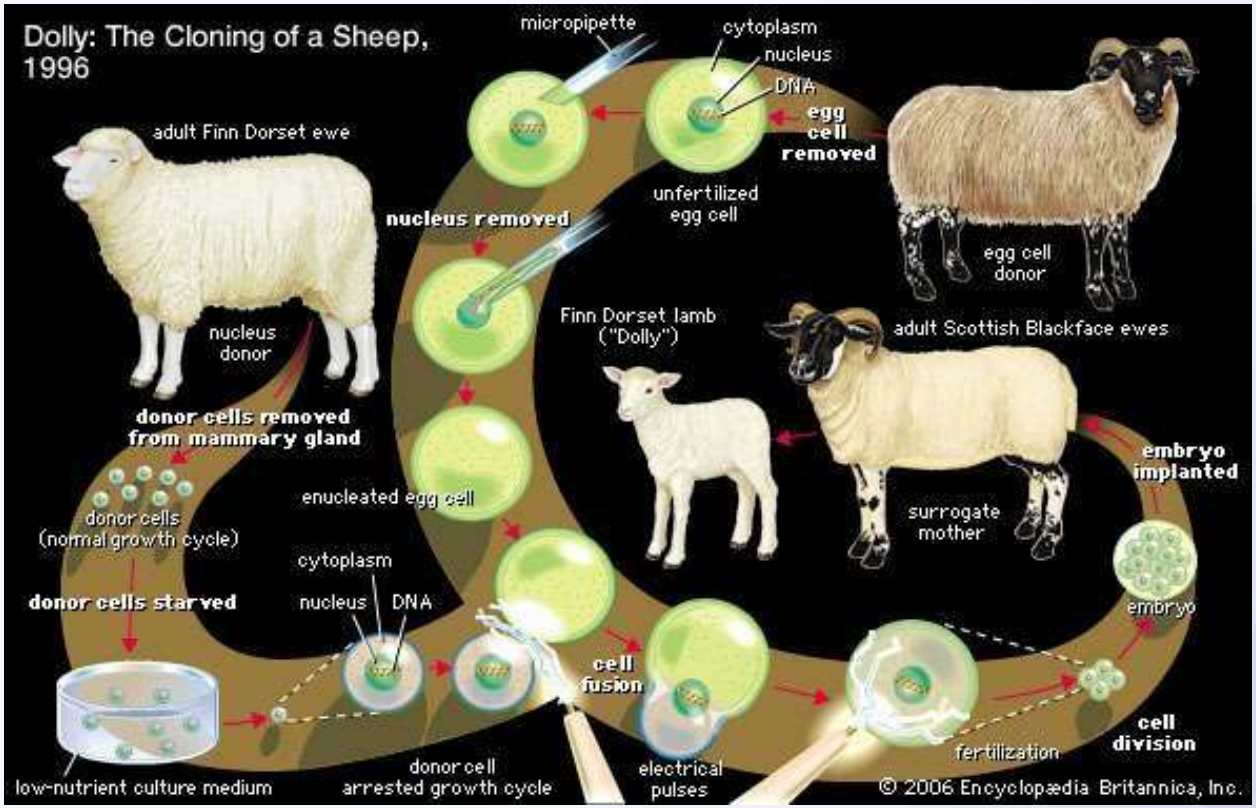
- AKA embryo cloning – production of human embryos and tissue for use in research
- Used to generate stem cells that can be harvested for stem cell research
- Might also be used one day to create organs for transplant
- Very, very low success rate (<90%) and very expensive

Reproductive cloning

- Used to generate an animal that has the same nuclear DNA as another animal
  - Impossible under normal conditions – animals reproduce sexually
- "Somatic cell nuclear transfer"
  - Remove the nucleus from an egg and replace it with the nucleus from an adult cell
- Cloned animals tend to be less healthy and die earlier – about 4% of genes are abnormally expressed due to abnormal methylation
- Cloned meat does not have to be labeled at the grocery store

Dolly the Sheep

- Proved that a highly differentiated cell could eventually be reprogrammed to create an entire organism
- Researchers ‘deleted’ the nucleus out of a sheep egg and transferred the nucleus of a mammary cell
- Dolly lived for 6 years and died of respiratory issues

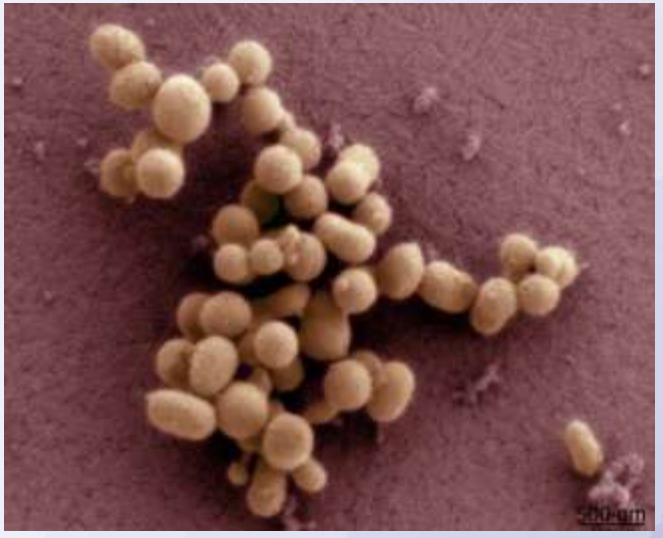


Synthetic Life

- In May 2010, Craig Venter announced the creation of the first synthetically created genome
- We are currently capable of producing lots of short <100bp **oligonucleotides** on a commercial level
- The team ordered millions of oligonucleotides from a biotech company, then stitched them together through homologous recombination in yeast

Synthetic Life

- Venter chose *Mycoplasma genitalium* for its tiny genome
- Deleted the genome out of another bacterial strain, and inserted the synthetic genome
- Successfully “booted up” the cell
- Is this really synthetic life?



Additional Information

- Molecular biology of the cell, Garland Science , 2008.

- Lots of very good information on bt, its uses, and concerns
  - http://www.bt.ucsd.edu/
- More on IPSCs
  - http://stemcells.nih.gov/info/Regenerative_Medicine/2006chapter10.htm
- More on cloning
  - http://www.ornl.gov/sci/techresources/Human_Genome/elsi/cloning.shtml

Vocabulary

- Embryonic stem cells, adult stem cells, iPSCs
  - Totipotent, pluripotent, multipotent
- Gene therapy
- Vector
- Immunogenesis, tumorigenesis
- biotechnology
- bt toxin
- A. tumefaciens
- Ti plasmid
- Biolistics
- Roundup
- Biofuels
- 1st generation, 2nd generation, 3rd generation biofuels
- Ethanol biofuels
- Biodiesel
- Virus
- Cloning
- DNA cloning, therapeutic cloning, reproductive cloning
- Dolly the sheep