DNA Fingerprinting

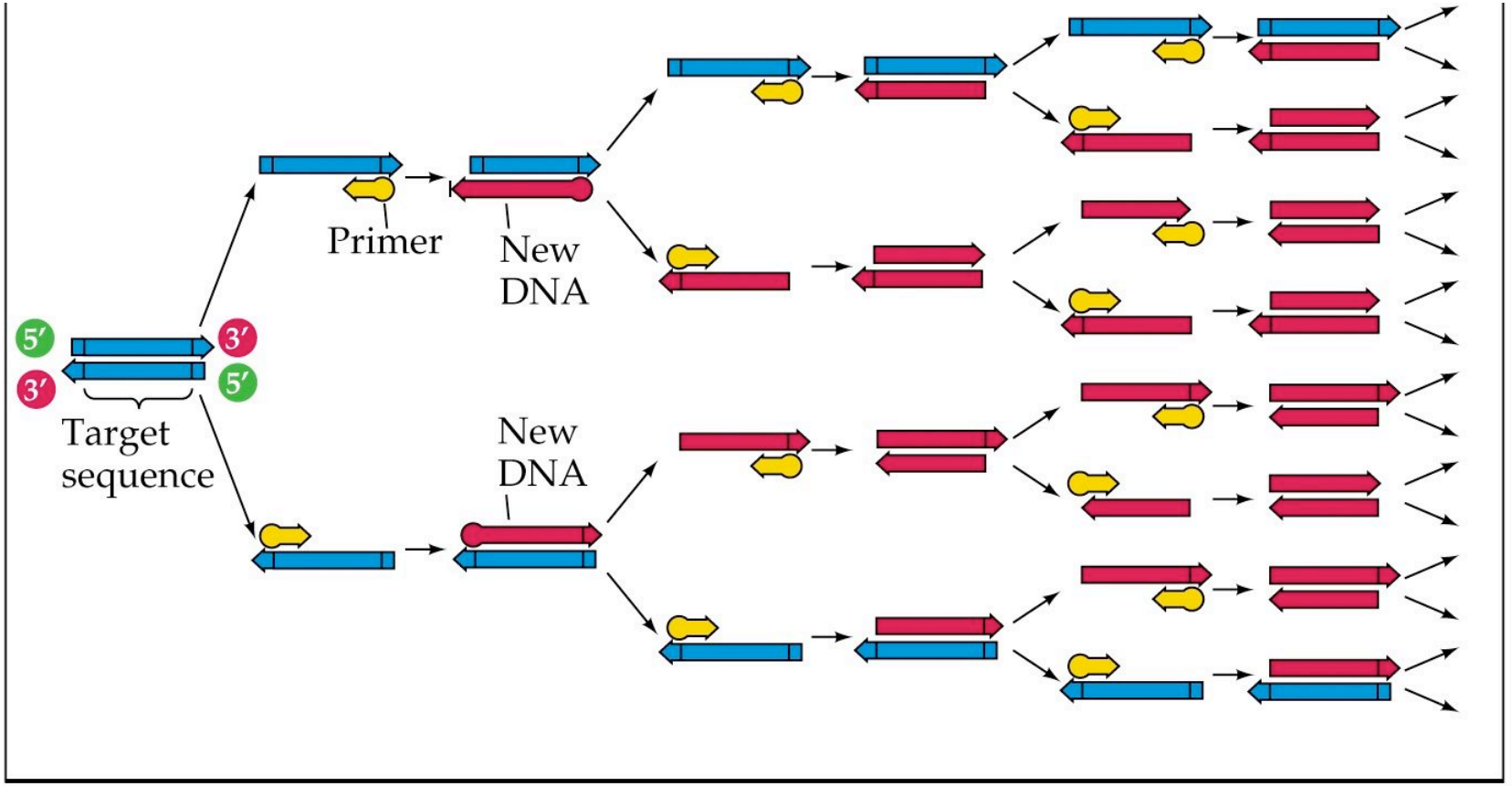
- Unless they are identical twins, individuals have unique DNA

- **DNA fingerprinting**
  - The name used for the unambiguous identifying technique that takes advantage of differences in DNA sequence

- The process of DNA fingerprinting begins by isolating DNA from
  - blood, semen, vaginal fluids, hair roots, skin, skeletal remains, or elsewhere

Polymerase Chain Reaction (PCR)

- If there is only a small amount of DNA available for DNA Fingerprinting
  - augment the amount of DNA using a technique called **PCR**
  - PCR is doing DNA replication in a test tube



Polymerase Chain Reaction (PCR)

- Like ALL DNA polymerases
- Taq polymerase can only add to the 3' end of an existing nucleotide
- A DNA primer that is complementary to the template is used to supply that 3' end

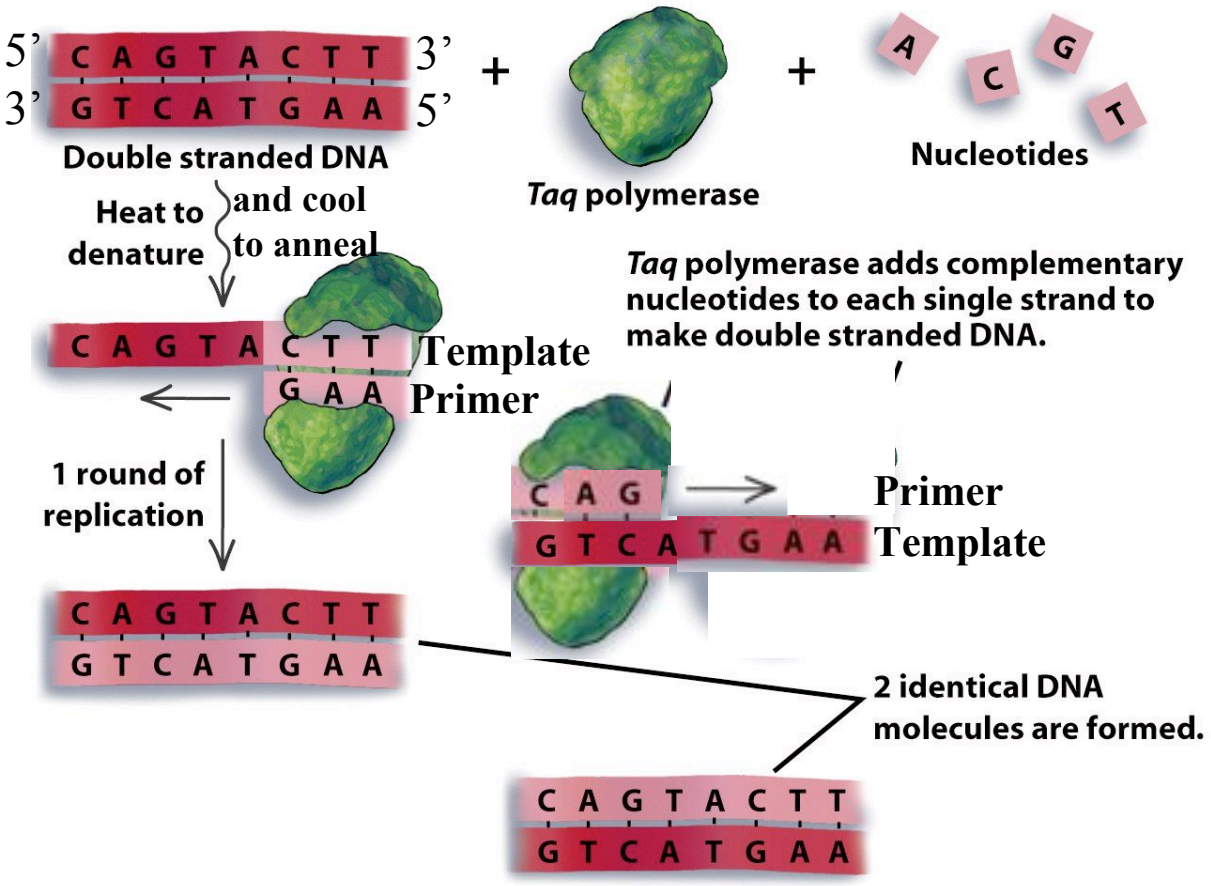


Figure 7-7a Biology: Science for Life, 2/e

DNA Fingerprinting

- After we isolate the DNA and amplify it with PCR
- Treat the DNA with **restriction enzymes**
  - cut DNA at specific sequences
  - Everyone's DNA is different, so everyone's DNA will cut at different sites
- This results in different sized fragments
- The different sized fragments are called **restriction fragment length polymorphisms**, or **RFLPs**
- We can observe the different sized fragments in an experiment that separates DNA based on fragment size called Gel Electrophoresis

RFLP Analysis

- Everyone has genetic sequences called **variable number tandem repeats**, or **VNTRs**
  - Everyone has different amounts of VNTRs
  - The VNTRs make the different sized RFLPs

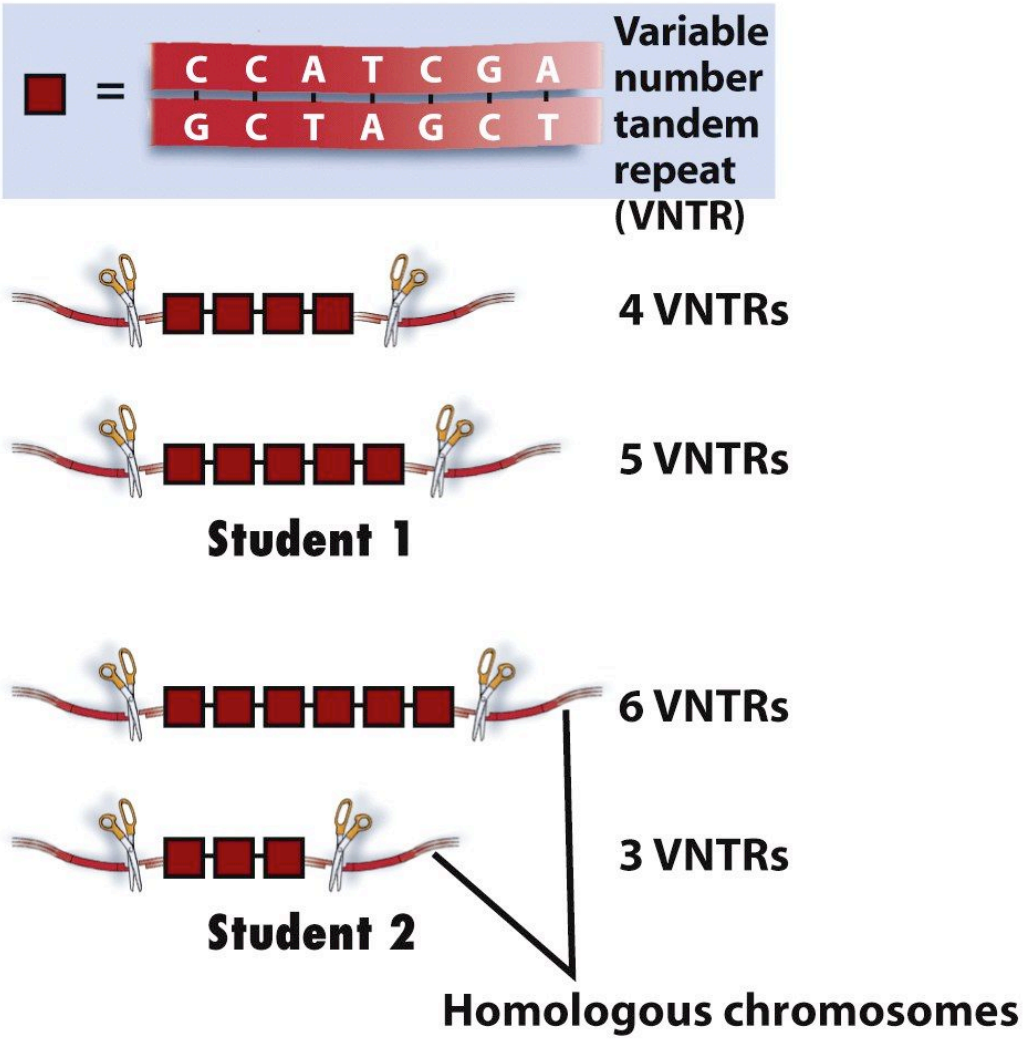


Figure 7-8 Biology: Science for Life, 2/e

Gel Electrophoresis

- Fragments of DNA from restriction enzyme cleavage are separated from each other when they migrate through a support called an **agarose gel**
  - It is similar to the yummy food Jell-O gelatin
  - It is actually made out of some of the same ingredients
- The size-based separation of Molecules of DNA separate based on size when an electric current is applied to an agarose gel
  - This is **gel electrophoresis**

Gel Electrophoresis

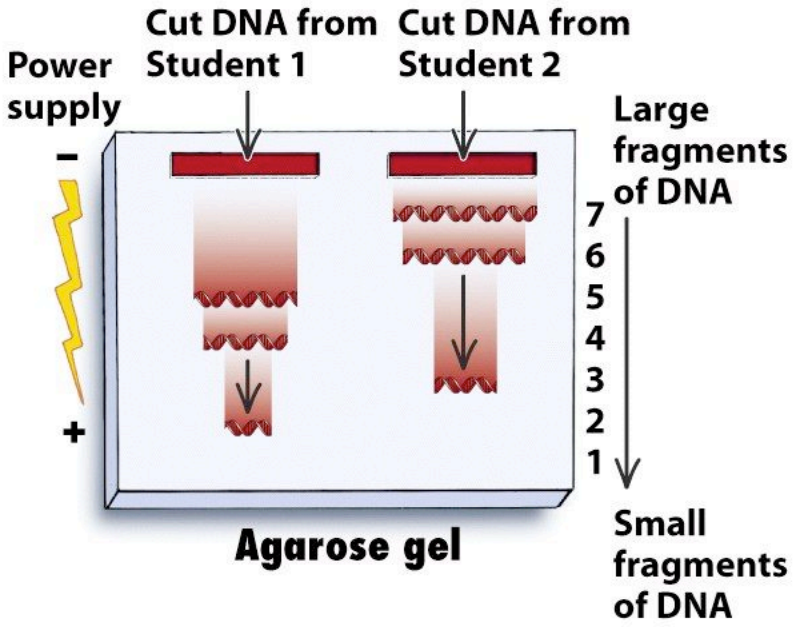


Figure 7-9a Biology: Science for Life, 2/e

DNA from two different individuals is cut with restriction enzymes and loaded on an agarose gel. When these fragments are subjected to an electric current, shorter fragments migrate through the gel faster than do larger fragments.

Student 1 has DNA sequences that carry 4 and 5 repeat sequences. Student 2 has 3 and 6 repeats. The remaining DNA is DNA that does not carry repeat sequences. Even though the DNA is visible in this figure, DNA is not visible with the unaided eye.

Gel Electrophoresis

- The separated DNA fragments are then drawn out of the gel using a nylon membrane
- The nylon membrane is treated with chemicals that break the hydrogen bonds in DNA and separate the strands
- The single stranded DNA is cross linked to the nylon membrane
  - By heat or UV light
- Incubate the nylon membrane with a radioactive **probe** of single stranded DNA complementary to the VNTRs

Gel Electrophoresis

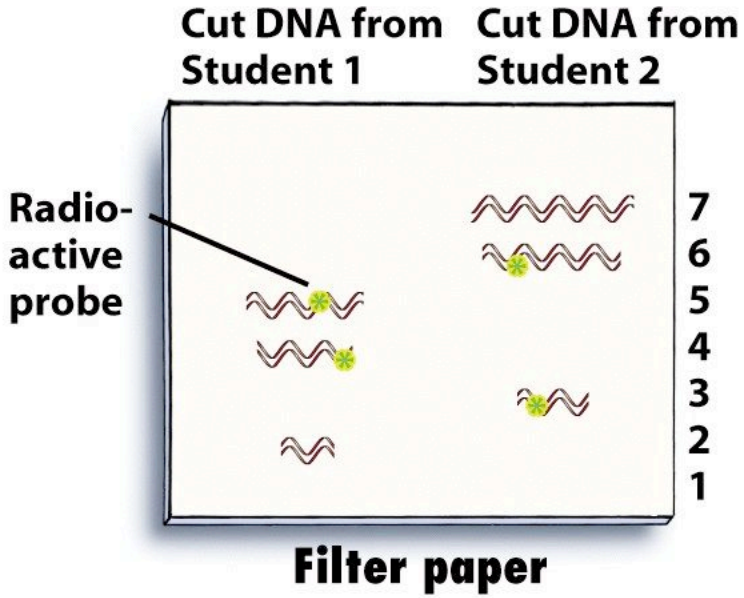


Figure 7-9b Biology: Science for Life, 2/e

The DNA is transferred from the gel onto filter paper and chemically treated to make it single stranded. The DNA is not visible on the filter paper and must be probed with single-stranded, radio-actively labeled DNA that is complementary to the repeat sequence. DNA that does not contain the repeat sequence will not bind to the probe.

Gel Electrophoresis

- The radioactive probe shows up on photographic film
  - Because as it decays it gives off light
  - The light leaves a dark spot on the film

- Different individuals have different patterns of bands
  - these make up the fingerprint

Gel Electrophoresis

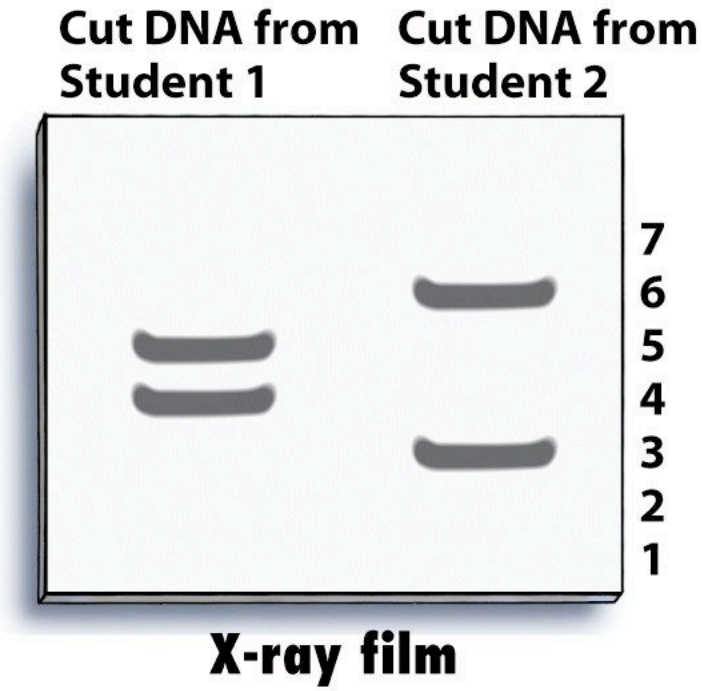
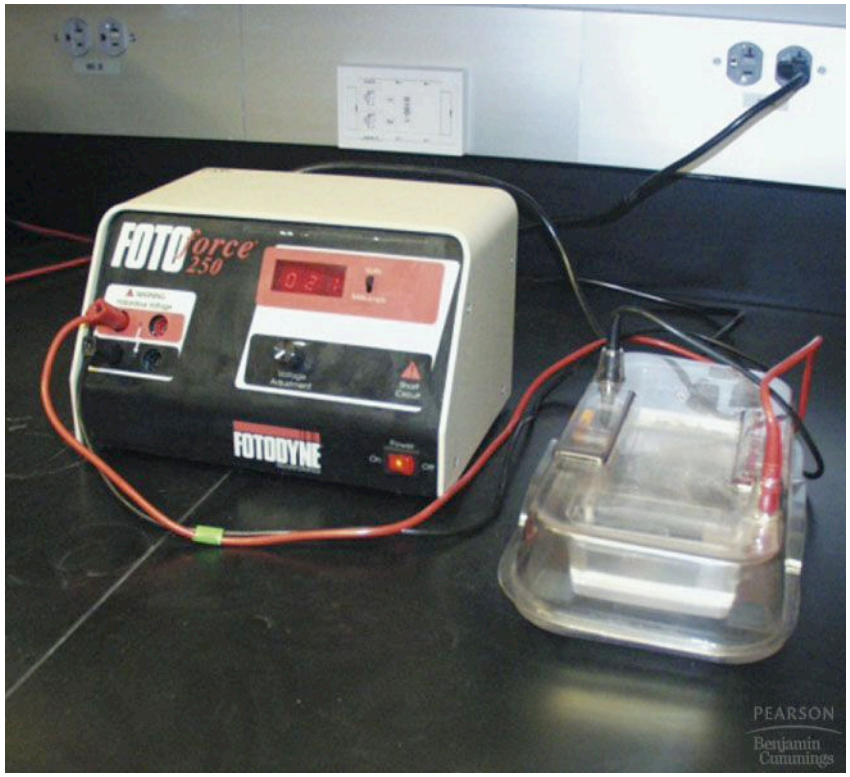


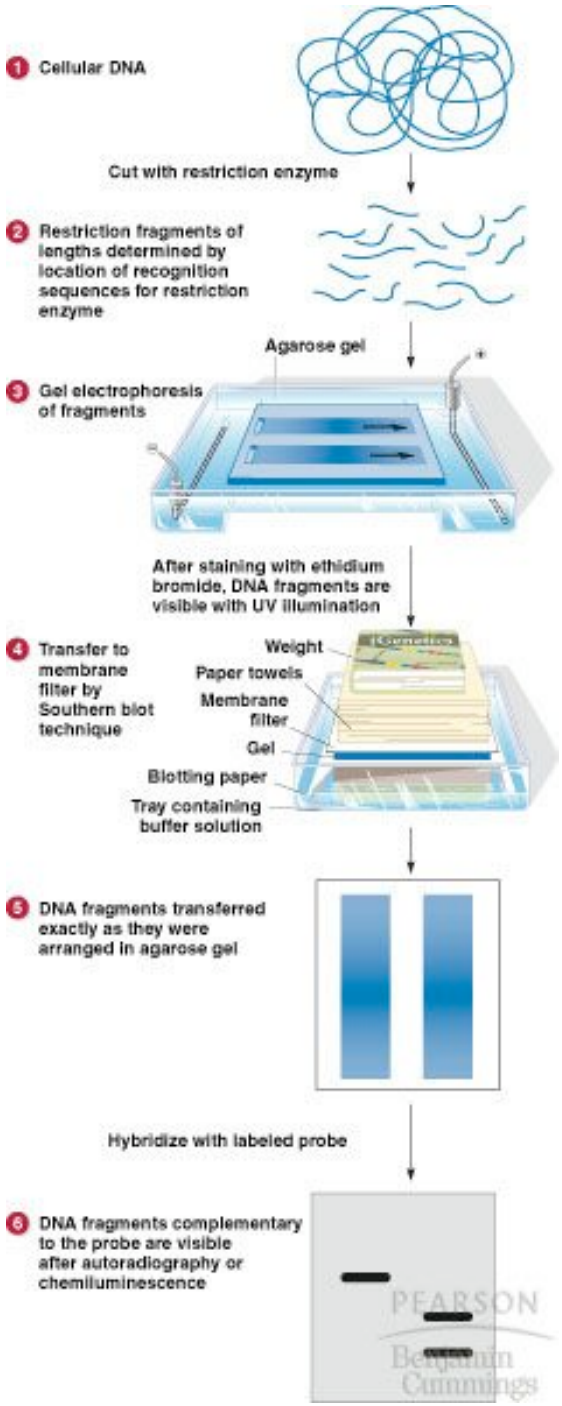
Figure 7-9c Biology: Science for Life, 2/e

When the filter is exposed to X-ray film, radioactive DNA sequences (where the probe is bound) produce a characteristic banding pattern, or DNA fingerprint. Student 1's DNA has 4 and 5 VNTRs, and Student 2's DNA has 3 and 6 VNTRs. The two bands represent the number of VNTR repeats.

This Protocol is known as Southern Blotting

Southern Blotting





DNA Fingerprinting

- DNA fingerprints can be used to determine which bone fragments belong to which individual

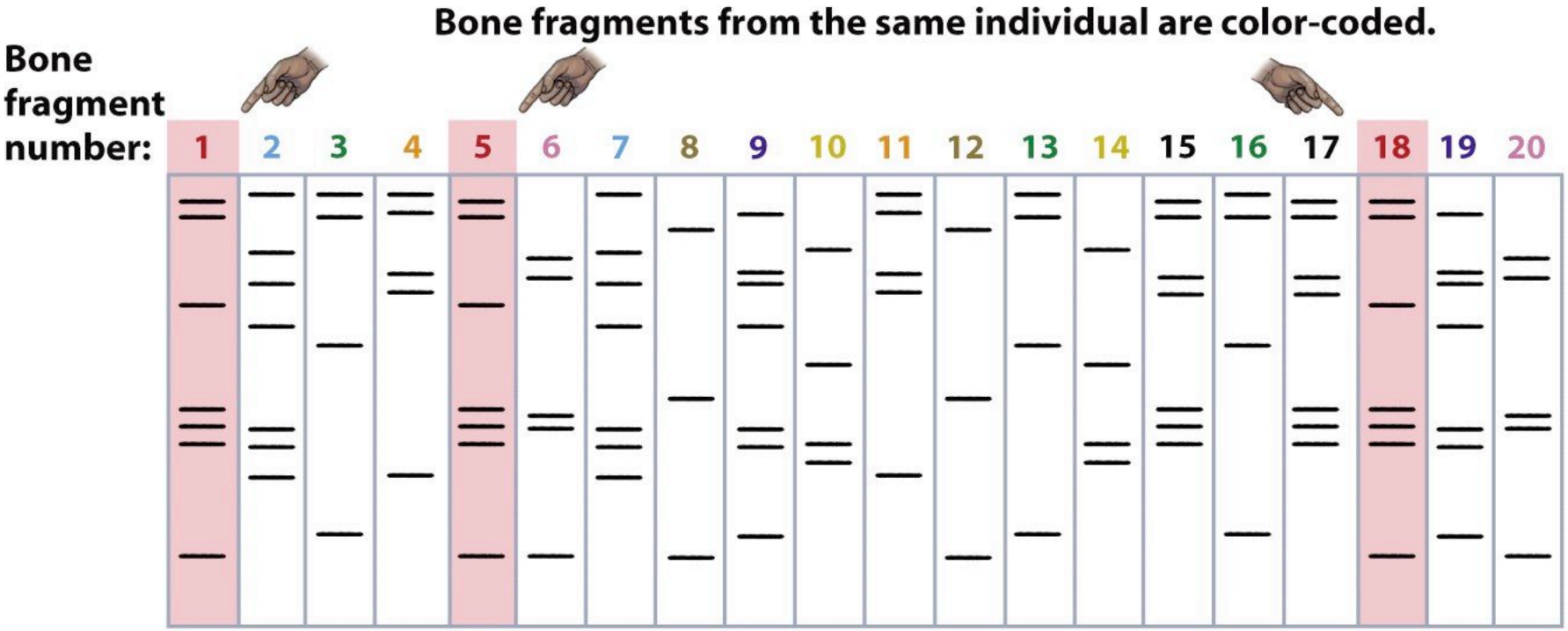


Figure 7-10 Biology: Science for Life, 2/e

DNA Fingerprinting

- DNA fingerprints of children should be similar to the those of parents
- DNA fingerprinting can show which individuals are the parents of specific children

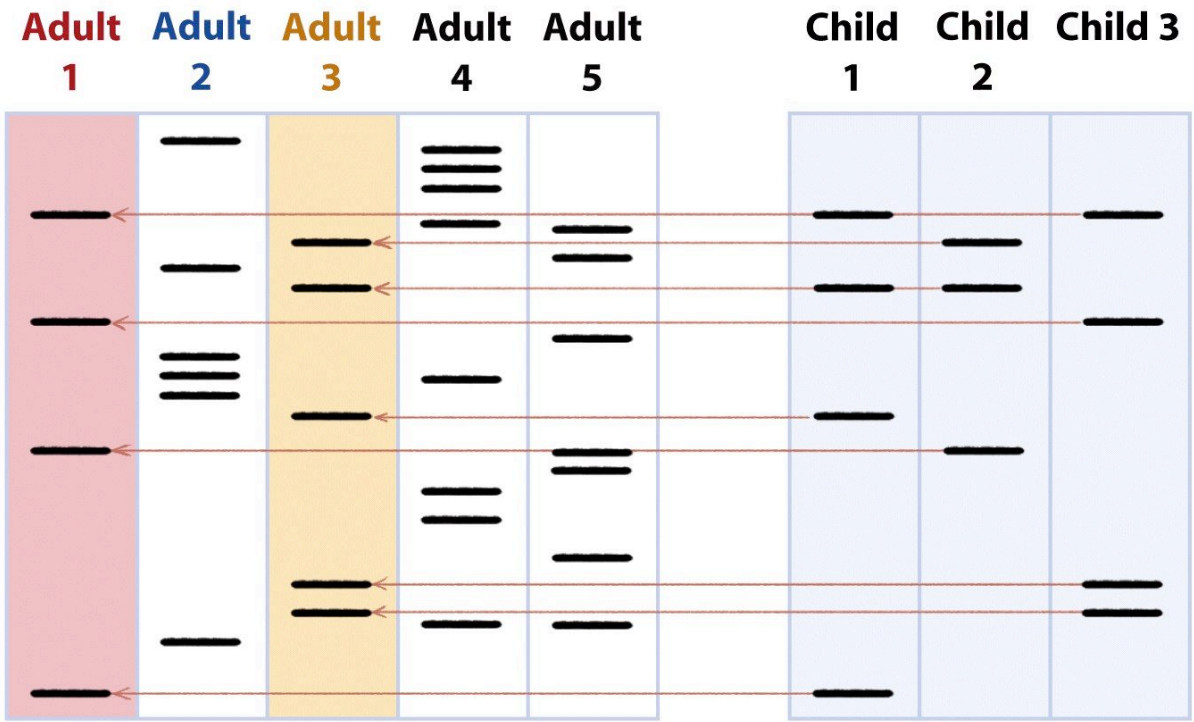


Figure 7-12 Biology: Science for Life, 2/e

Northern Blot Analysis

- Northern blotting analyzes RNA much the same way that Southern blotting does DNA:
  - RNA is extracted from the cell, undergoes gel electrophoresis, and is bound to a filter.
  - Hybridization between bound cellular RNA and a labeled probe occurs. The sizes of the RNA fragments detected by the probe can be determined

Northern Blot Analysis

- Northern blot analysis is used for determining:
  - The size(s) of mRNA encoded by a gene. Northern blots have shown that different mRNA species arise from the same region of DNA, suggesting differential use of promoters and terminators, and/or alternative mRNA processing.
  - Whether a specific mRNA is present in a cell type, and if so, at what levels. Gene activity is measured in this way, and RNA sampling is widely used to study development, tissue specialization, or the response of cells to various physiological stimuli.

Producing Recombinant Proteins

- The first step in the production of *rBGH* protein (or insulin) is
  - to transfer the *BGH* gene (or human insulin gene) from the nucleus of a cow cell (or human cell) into a bacterial cell
- How do we do that????
- 3 steps are involved in turning a cow *BGH* gene into a recombinant BGH (rBGH) gene in a bacterial cell
  - *rBGH* gene means that this product is genetically engineered
  - with the *r* indicating recombinant

Producing Recombinant Proteins

- 5 steps are involved in turning a cow *BGH* gene into a recombinant BGH (rBGH) gene in a bacterial cell

1. Make lots of copies of the cow BGH gene in the lab in a test tube
2. Cut cow BGH gene with restriction enzymes
3. Insert this cow BGH gene into bacterial DNA = rBGH
4. Inject the bacterial DNA containing the rBGH into bacteria
5. Grow up lots of these genetically engineered bacteria and purify the rBGH cow protein they are making

Cloning a Gene Using Bacteria

Step 1. Make lots of copies of the BGH Gene

- Use PCR to amplify only the cow BGH gene from the cow chromosomes
- Remember, PCR is just replicating DNA in the laboratory in a test tube
- End up with lots and lots of copies of the cow BGH gene DNA in a test tube

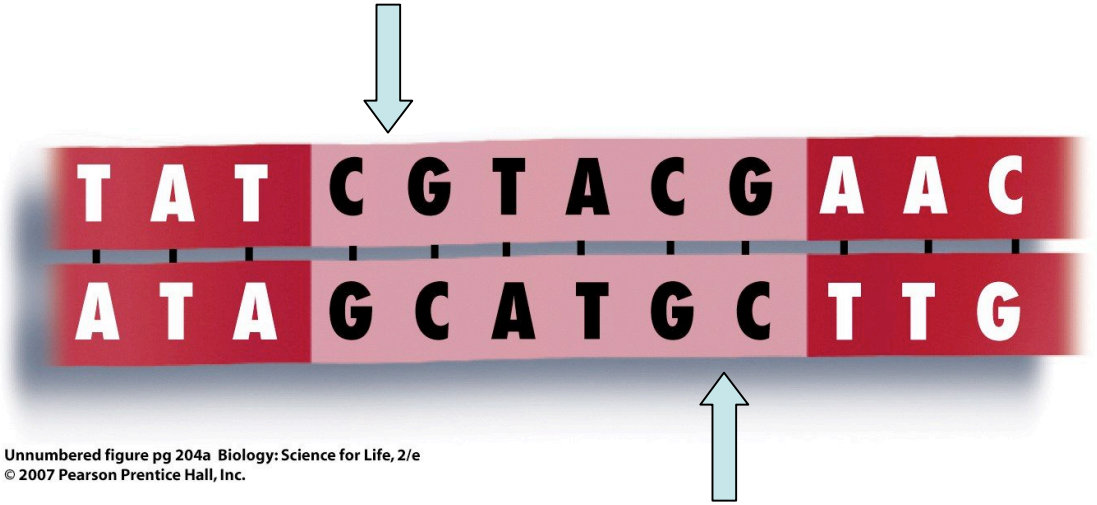
Cloning a Gene Using Bacteria

Step 2. Prepare the cow BGH Gene for inserting into bacterial DNA

- The cow BGH gene ends are sliced using **restriction enzymes**
- Restriction enzymes cut DNA only at specific sequences that leave the double-stranded DNA jagged or “sticky” on the ends

Unnumbered figure pg 206 Biology: Science for Life, 2/e

Restriction enzymes cut the DNA in a staggered pattern, leaving "**sticky ends**"…



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Restriction enzyme cut

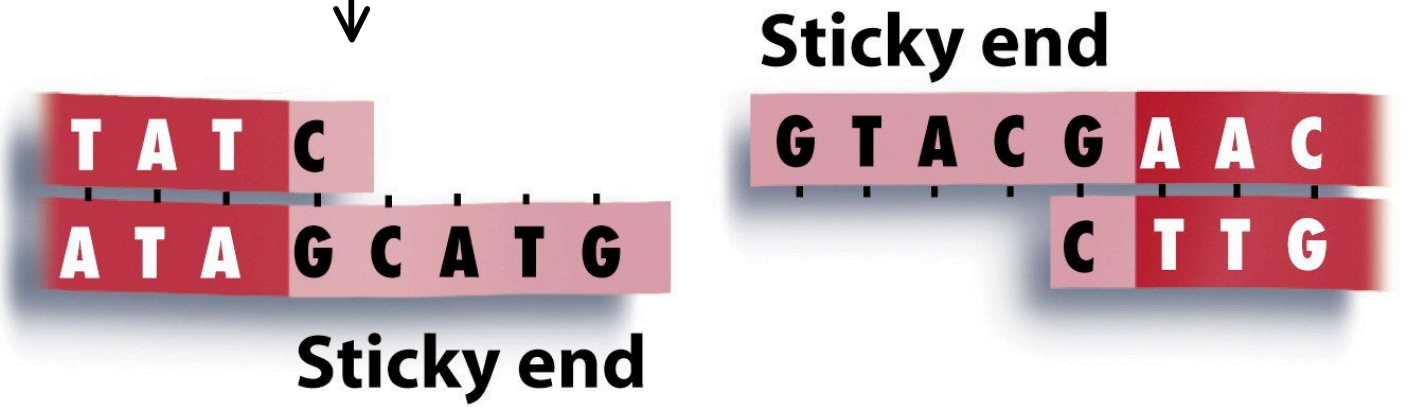

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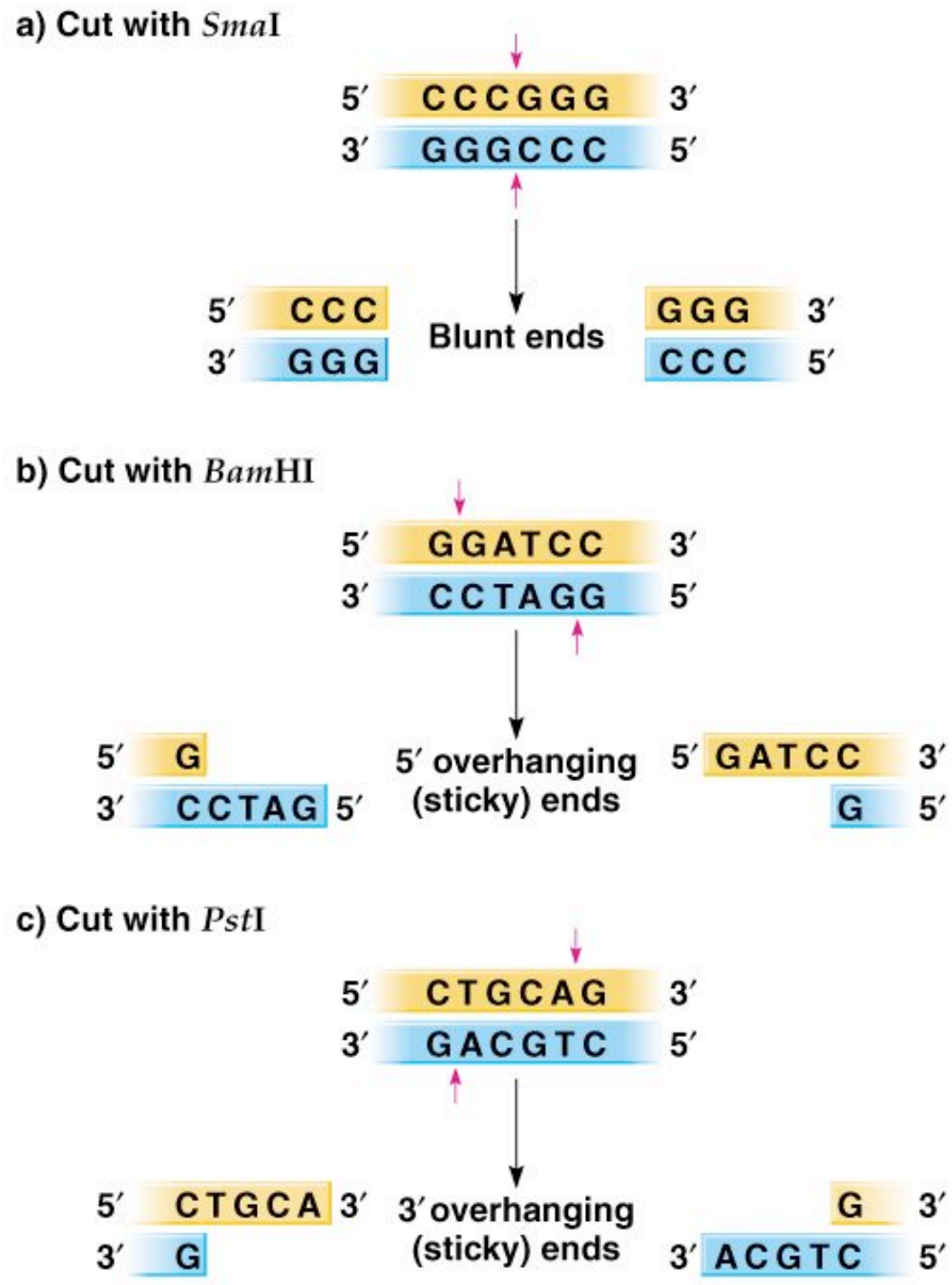
Fig. 16.2
Examples of how different restriction enzymes cleave DNA

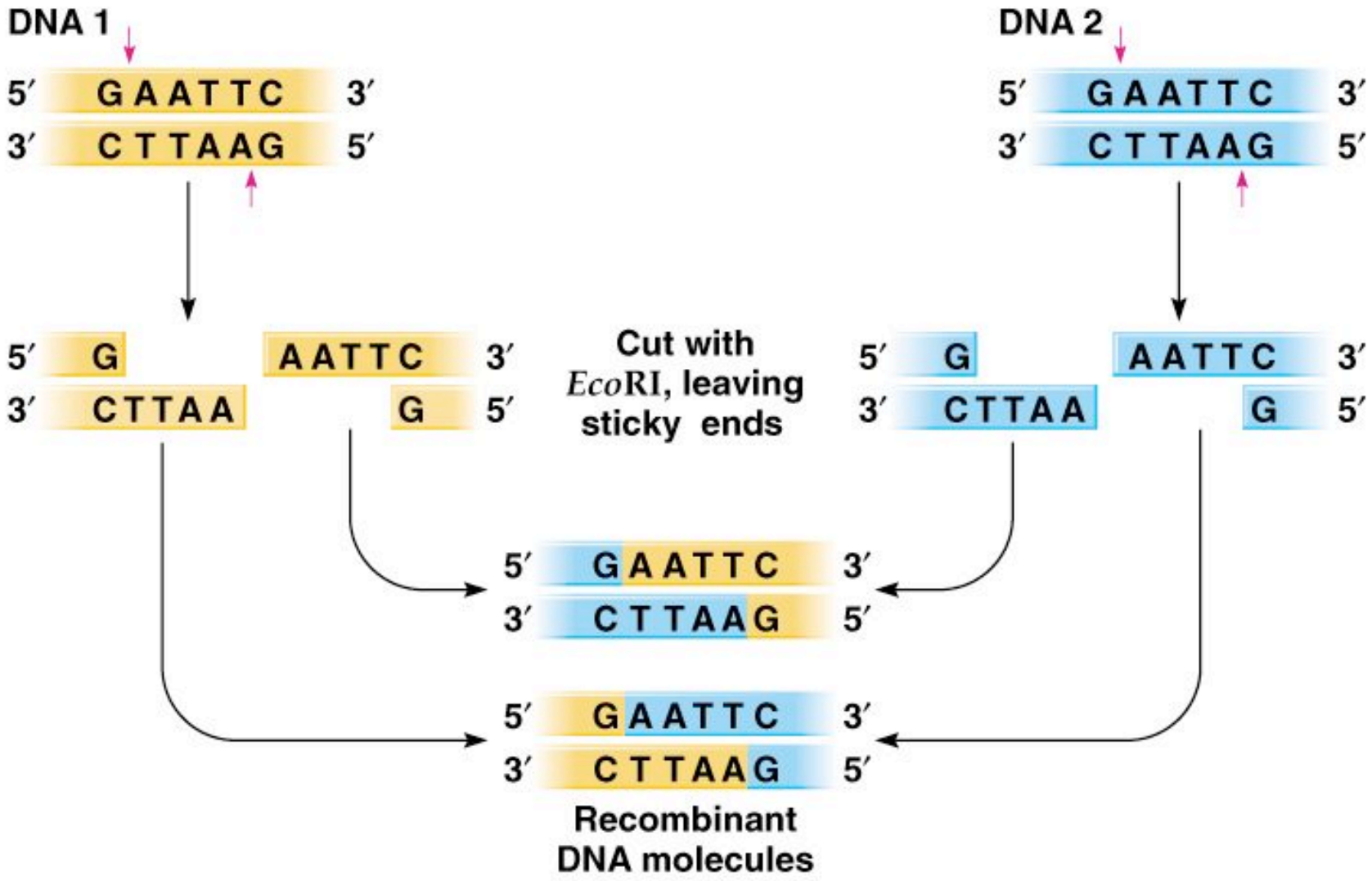


Cloning a Gene Using Bacteria

Step 3. Insert the *BGH* Gene into the Bacterial Plasmid

- The bacterial plasmid is also cut with the restriction enzyme, leaving sticky ends
  - A plasmid is a small circular DNA that is separate from the bacterial genome
- Sticky ends of the cut BGH DNA attach by complementary base pairing to the sticky ends of the cut plasmid DNA
- This is now recombinant DNA

Fig. 16.3
Cleavage of DNA by the restriction enzyme *Eco*RI



Cloning a Gene Using Bacteria

Step 4. Insert the Recombinant Plasmid into a Bacterial Cell

- The recombinant plasmid containing the rBGH is then placed into bacterial cells

Step 5. Grow up bacteria and purify rBGH protein

- Large numbers of these *rBGH* plasmids are copied in each bacteria and each bacteria copies itself
  - Can grow up billions and billions of bacteria in the lab
  - All containing the rBGH plasmid
- Each plasmid will generate rBGH protein through transcription and translation
- Isolate the BGH protein from the bacterial proteins
- TaDa! Recombinant proteins

Cloning a Gene Using Bacteria

- How can bacteria produce the cow BGH protein?
- This works because bacteria use the same genetic code as cows (and all living things)
- Other proteins are made in this way:
  - Insulin for diabetics
  - Clotting factors for hemophiliacs
  - Cancer treatment drugs

Cloning a Gene Using Bacteria

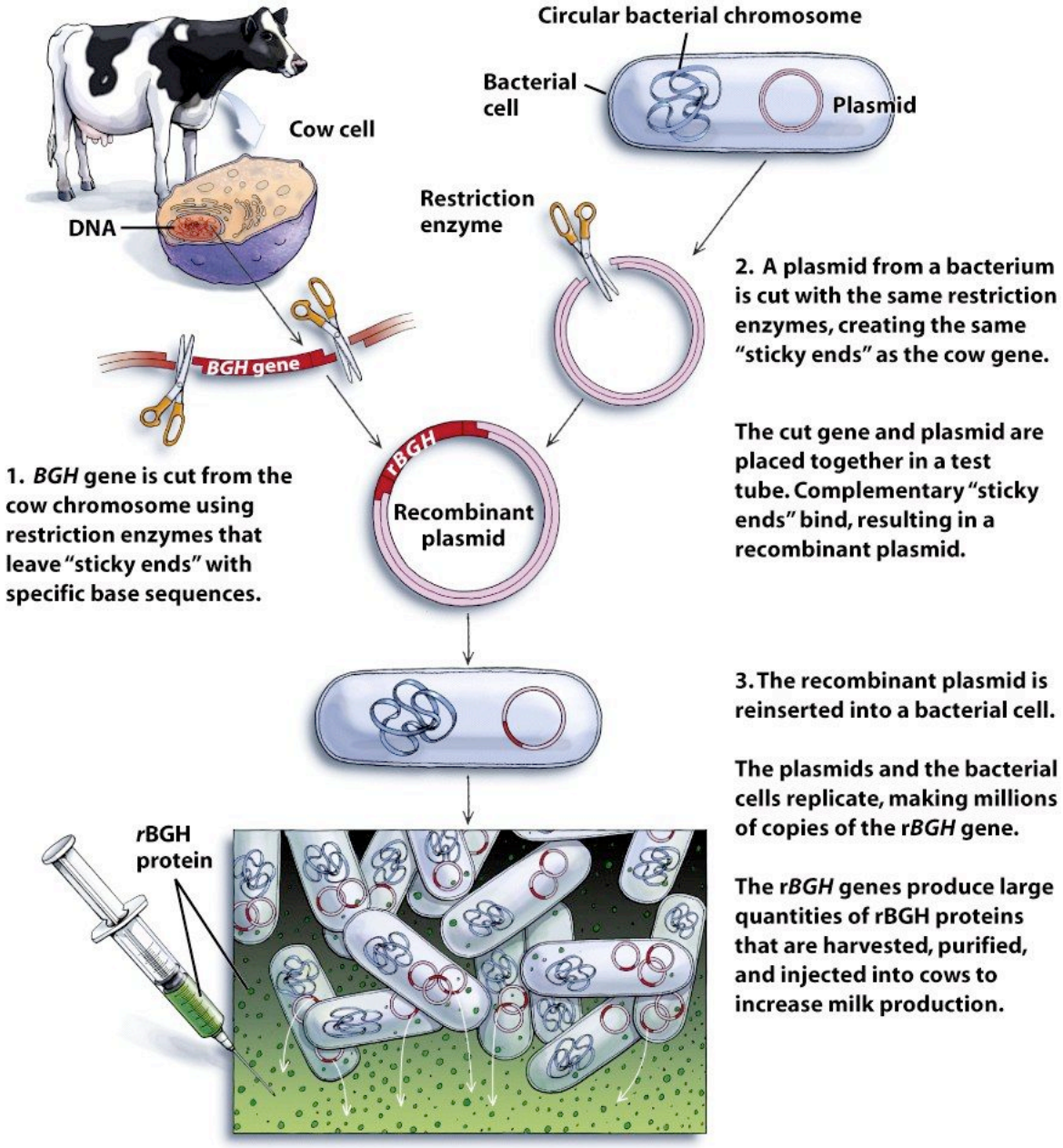


Figure 8-12 Biology: Science for Life, 2/e

Using the lacZ gene as a reporter of gene expression

- Reporter gene – protein encoding gene whose expression in the cell is quantifiable by techniques of protein detection.

- Fusion of reporter gene to cis acting (DNA) regulatory regions (like promoters) allows assessment gene activity by monitoring amount of reporter gene product

Fusion used to perform genetic studies of the regulatory region of gene X

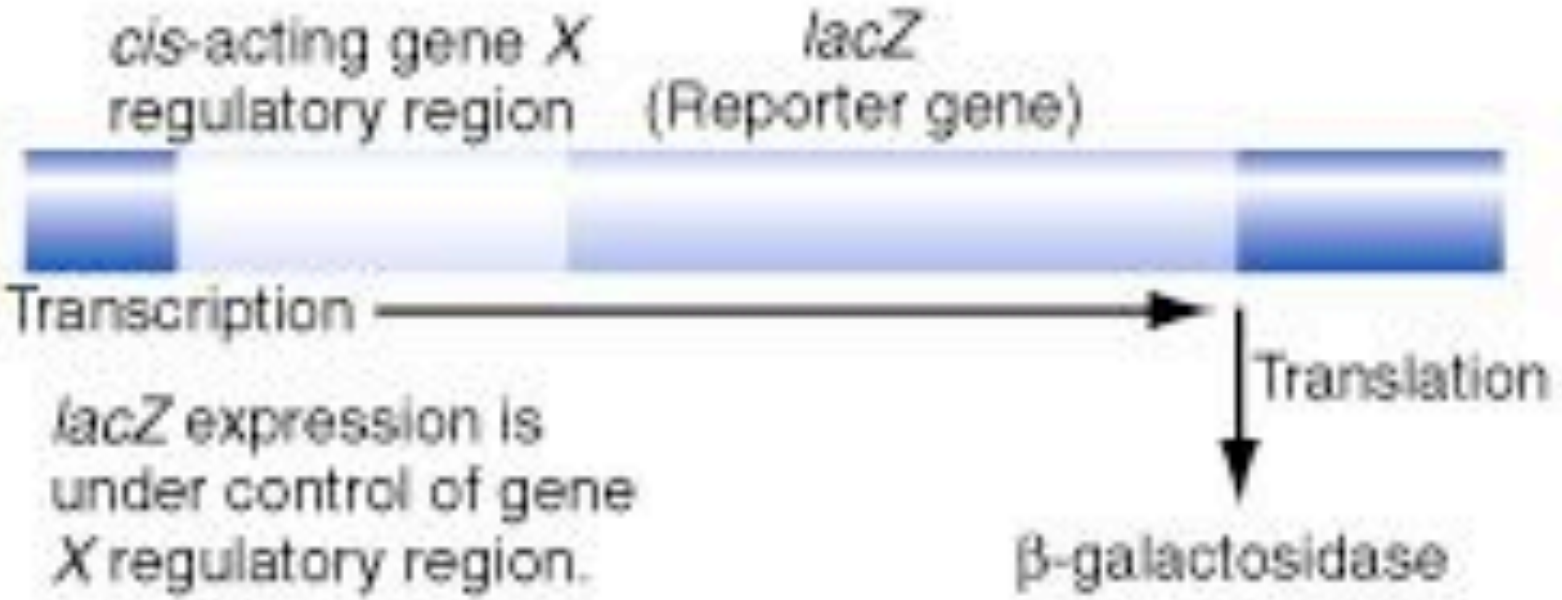


Fig. 16.18 a

Cloning is Genetic Engineering

- **Cloning** is the making of entire organisms using genetic engineering
- Has been done in cattle, goats, mice, cats, pigs, rabbits, and sheep
- Has never been done in humans

Cloning is Genetic Engineering

- Dolly the sheep was the first animal to be cloned
- The DNA (all 46 chromosomes) from an adult sheep mammary gland were fused with an unfertilized egg cell without any DNA inside
- The treated egg was placed in the uterus of an adult sheep
  - that had received hormone treatments to support pregnancy
- There were 277 failures before this **nuclear transfer** technique succeeded
  - Dolly was successfully created in 1997

Cloning is Genetic Engineering

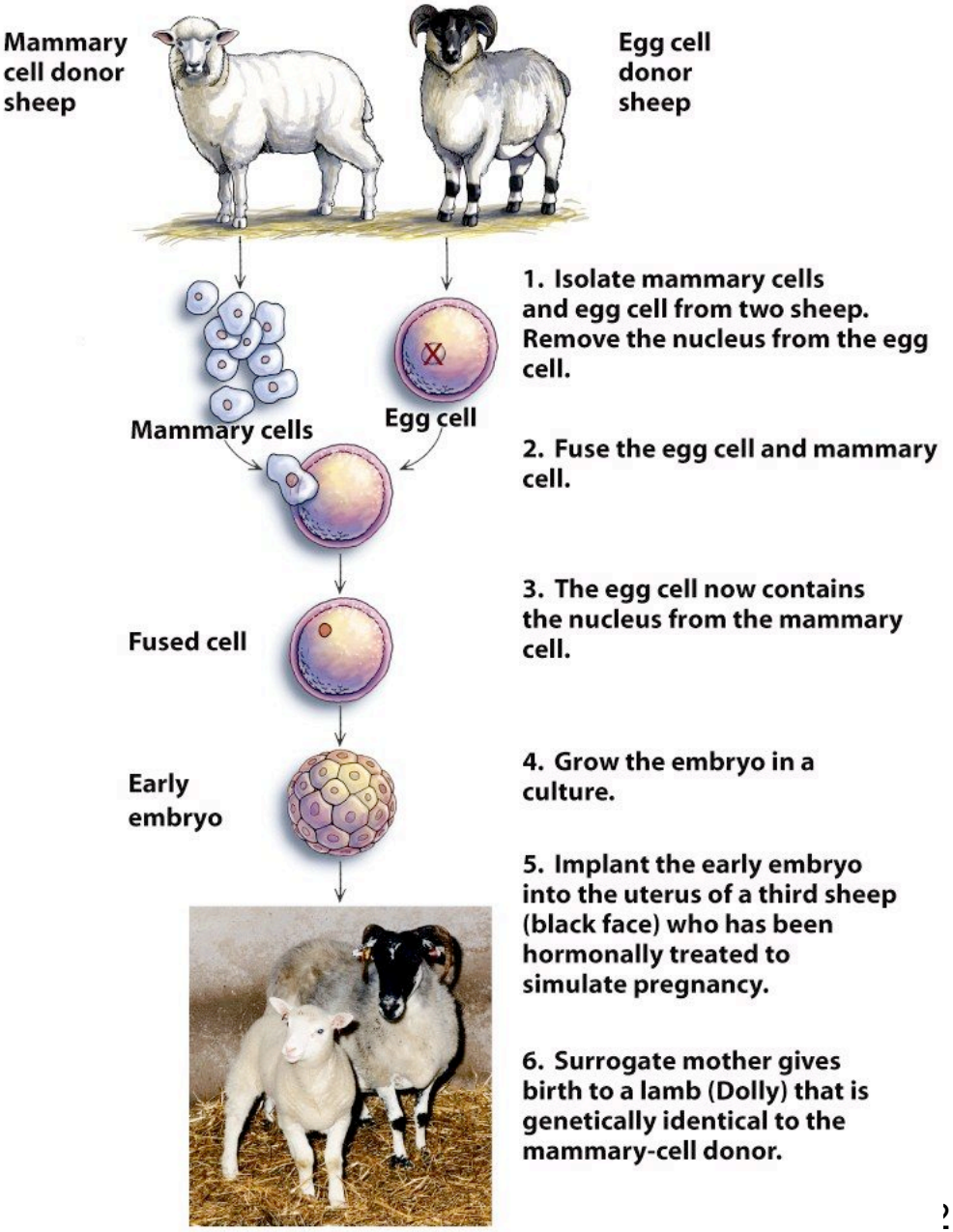


Figure 8-21 Biology: Science for Life, 2/e

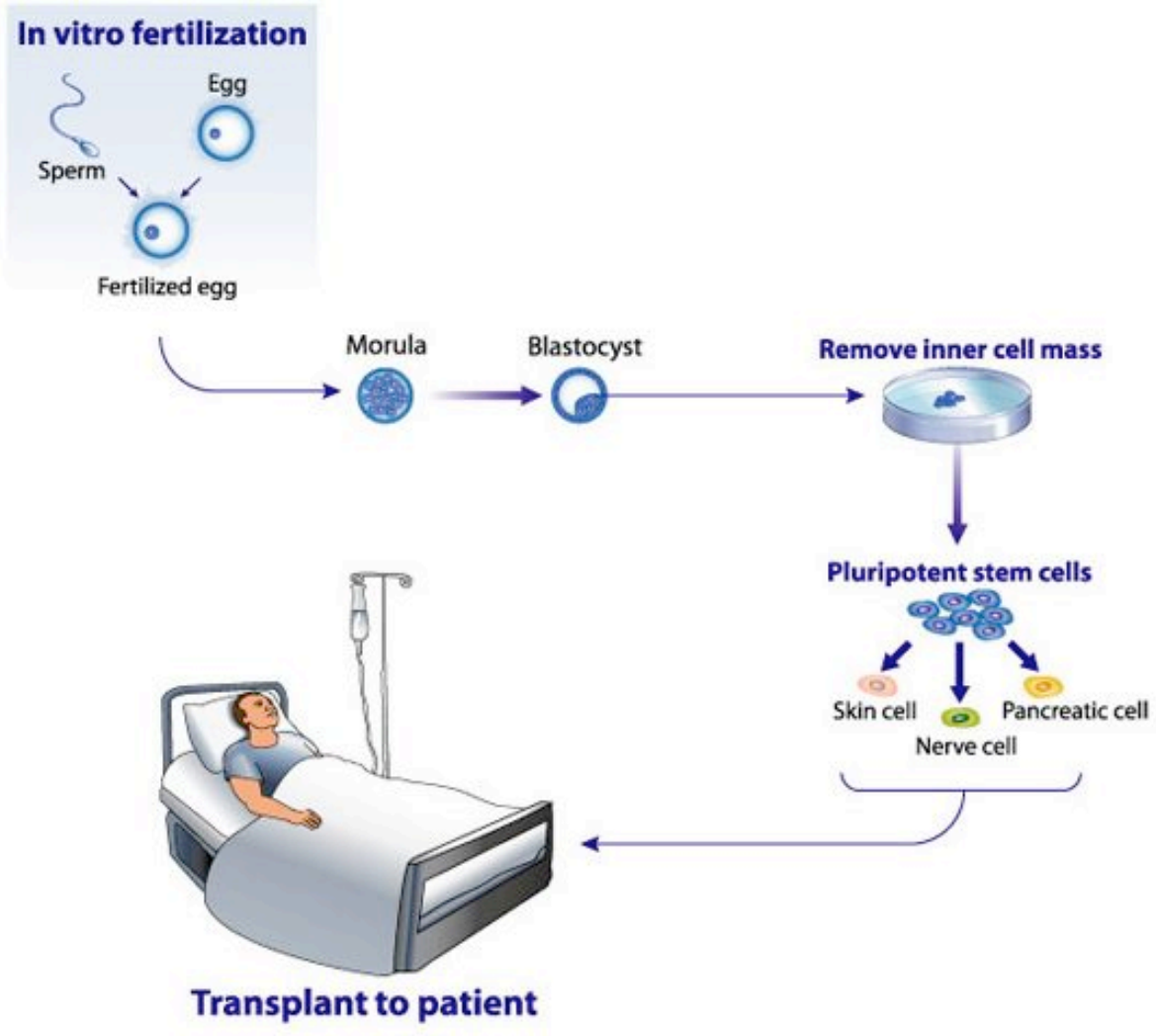
Cloning is Genetic Engineering

- Dolly was put to sleep at the age of 6 in 2003
  - Because of health problems
  - She was suffering from arthritis and a progressive lung disease
  - These are usually only seen in old sheep
- Cloned animals seem to age prematurely and show signs of other health problems that are normally associated with aging
- Hypothesis:
  - Egg and Sperm DNA is “reprogrammed” and does not reflect the age of the parents
  - Adult donor DNA is not “reprogrammed” in the egg and reflects the age of the donor
  - Using adult DNA to create new organisms results in organisms that remain at the age of their donor DNA at birth

Therapeutic Cloning

- Not cloning of entire organisms, but cloning of specific tissues or cells
  - Pancreatic cells to produce insulin in diabetics
  - Spinal cord cells in parallized patients
- **Stem cells** are induced in the laboratory to turn into specific tissue cells
- What are Stem Cells?
  - Cells that can be induced to turn into every type of cell in the human body
- Where are Stem Cells found?
  - In embryos
  - The original fertilized egg grows into an entire human being.
  - These cells can make every cell type

Embryonic Stem Cells



http://sps.k12.ar.us/massengale/images/ivf.jpg

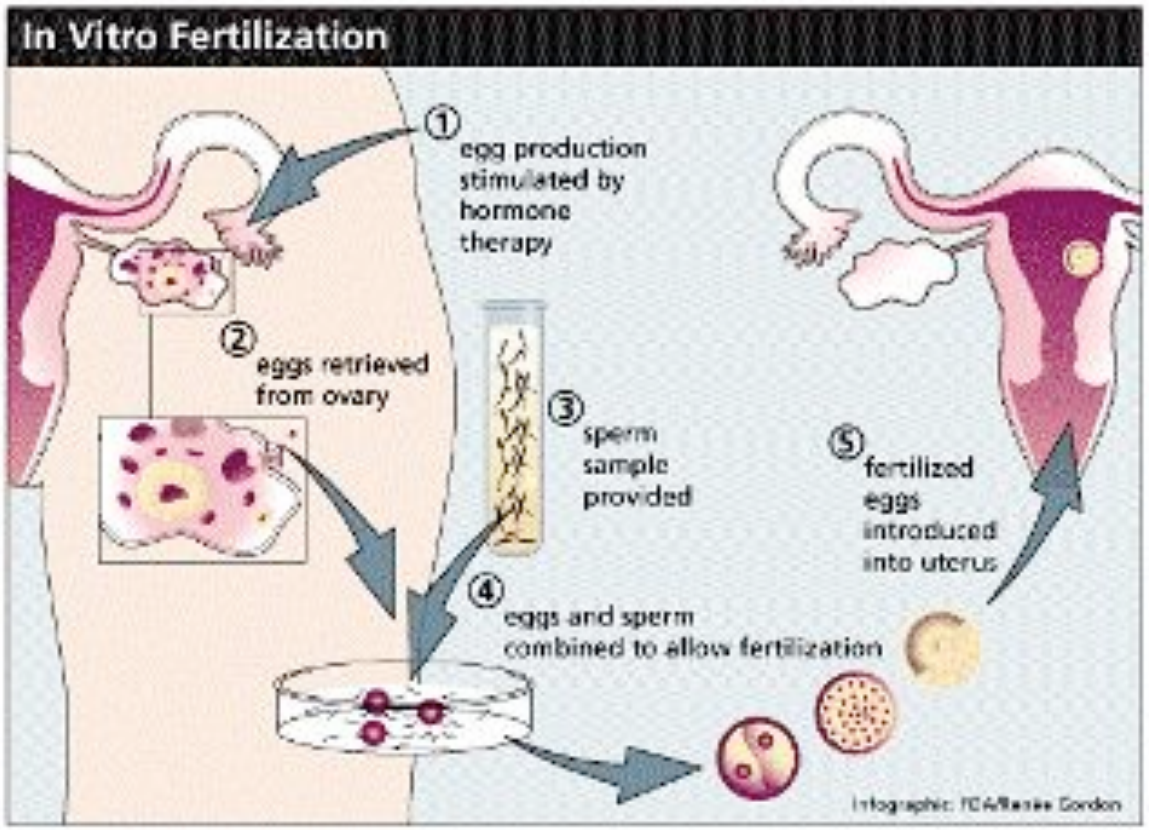
Stem Cells

- How do scientists acquire Stem Cells?
- Human eggs are fertilized by human sperm in vitro
  - (in a test tube)

- Fertilized egg grows and divides by mitosis to an embryo which is just a ball of 8 cells at this point
  - These cells can now be frozen as stem cells for research
  -
- This is the same process that is used by couples that cannot conceive a baby
  - Called in vitro fertilization (IVF)

IVF

- in vitro fertilization (IVF)
  - The ball of 8 cells is implanted into the female's uterus
  - She has been treated with hormones to simulate pregnancy to accept the embryo



http://pregnancyandbaby.com/pregnancy/baby/Facing-infertility-5476.htm

Stem Cells

- Couples using IVF generally generate 15-30 frozen embryos and use only 3-9 of them
- The remaining embryos can either be thrown away or donated to stem cell research
- Stem cells can NEVER be acquired by abortion or miscarriage
  - There are no embryonic stem cells left, they have already changed
  - The 8 cell stage is before implantation in the uterus
  - Before anyone could even know they have conceived
- Stem cell researchers use more donated embryos rather than ones created in the laboratory specifically for research
- Most stem cell research uses embryonic stem cell lines
  - Cells that originally came from an 8 cell embryo, but have been manipulated in the lab to continue growing as separate cells in a flask
  - They do not form any tissues, they just grow as individual stem cells
  - Researchers can grow millions and millions of these in the lab to perform studies that may someday save lives and cure diseases

8 Cell Stage Embryonic Stem Cells

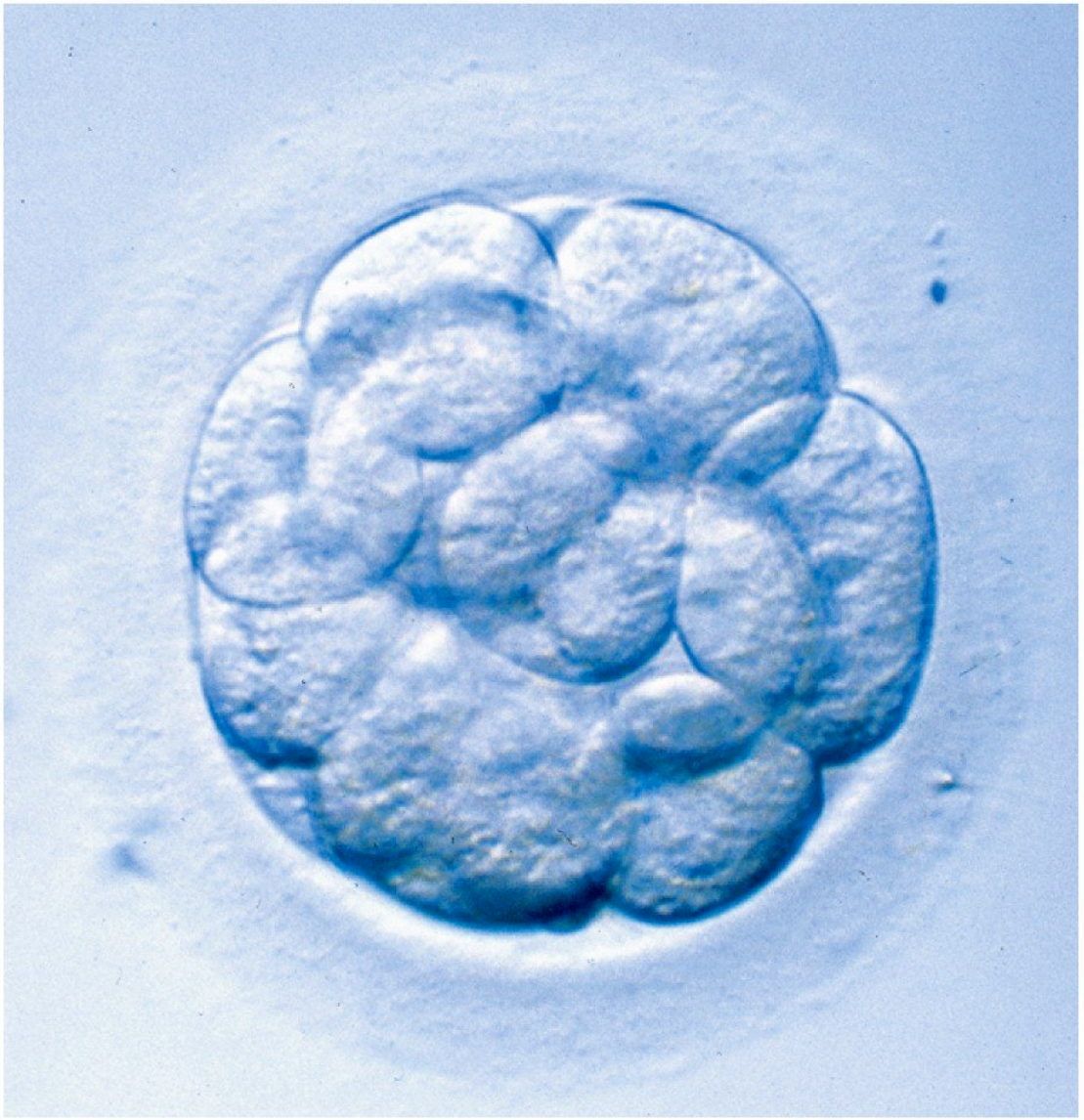


Figure E8-1 Biology: Science for Life, 2/e

Stem Cells

- The use of embryonic stem cells in research fuels a heated national debate
  - Mostly because of scientific ignorance
- Embryonic stem cells are valued by researchers because they are **totipotent**, or able to become any other cell
  - With increased study, these could potentially treat or cure any type of disease and cancer
- In 2001, President Bush banned federal funding for reaching using embryonic stem cells
  - Because he never took Bio 360!!!
  - (or any biology for that matter)