

Biology

A group of penguins is shown swimming underwater. The water is a deep blue-green color. The penguins are in various positions, some swimming towards the camera and others away. Their bodies are sleek and dark, with some showing lighter patches on their chests. Bubbles are visible around them, suggesting they are breathing or moving through the water.

Concepts and Applications | 9e
Starr | Evers | Starr

Chapter 15

Biotechnology

15.1 What is DNA Cloning?

- Cut and paste
 - How to identify a single base among thousands or millions was a huge challenge
 - Arber and Smith discovered how some bacteria resist infection by bacteriophage
 - These bacteria have enzymes that chop up injected viral DNA
 - Enzymes restrict viral growth

What is DNA Cloning? (cont'd.)

- Restriction enzymes
 - Cuts DNA wherever a specific nucleotide sequence occurs
 - Allows researchers to cut DNA into manageable chunks
 - Allows for combining DNA fragments from other organisms
 - Leave single-stranded tails
 - Complementary tails will base-pair together

What is DNA Cloning? (cont'd.)

- Making recombinant DNA
 - A restriction enzyme recognizes a specific base sequence in DNA from any source
 - Enzyme cuts DNA from two sources into fragments; leaves “sticky ends”
 - DNA fragments are mixed together, matching sticky ends base-pair with each other
 - DNA ligase seals the base-paired DNA fragments
 - Molecules of recombinant DNA result

Animation: Base-pairing of DNA fragments

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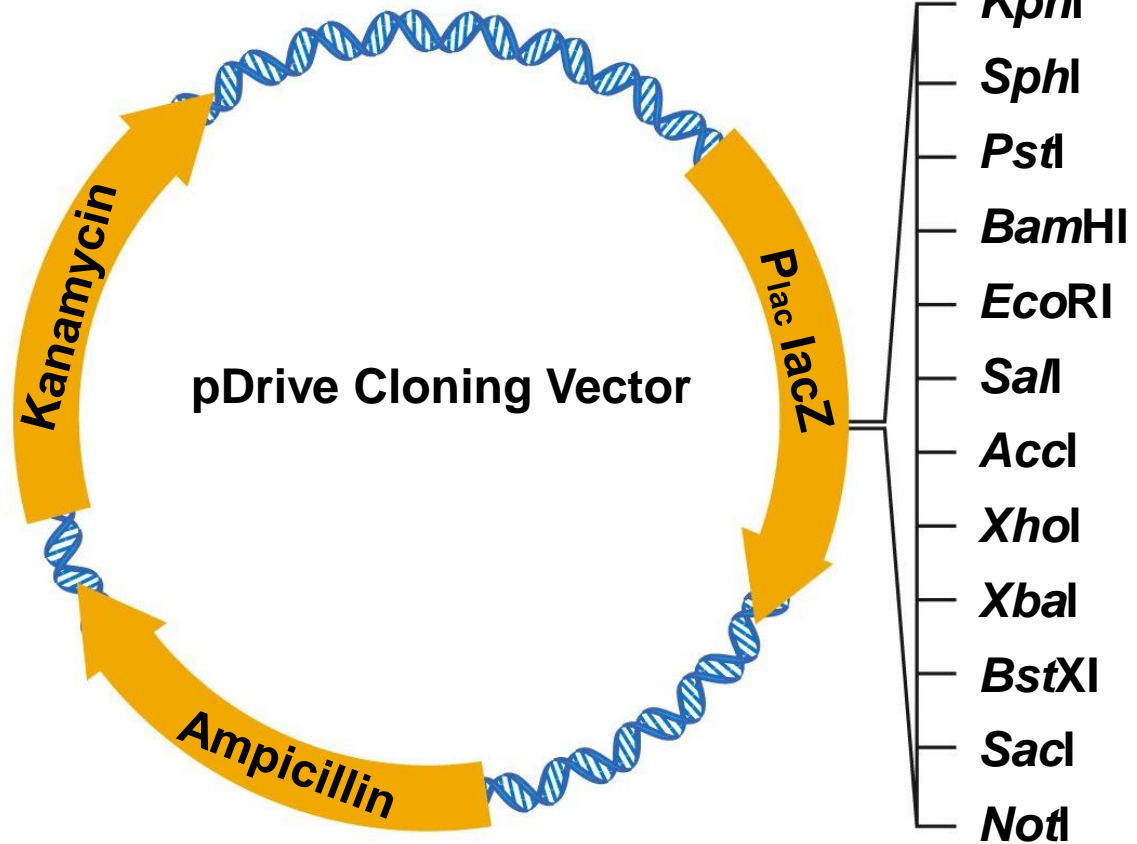
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What is DNA Cloning? (cont'd.)

- DNA cloning mass produces specific DNA fragments
 - Fragments to be copied are inserted into plasmids or other cloning vectors and inserted into host cells such as bacteria
- Host cells divide and make identical copies (clones) of the foreign DNA
- A huge population of clones can be grown

What is DNA Cloning? (cont'd.)

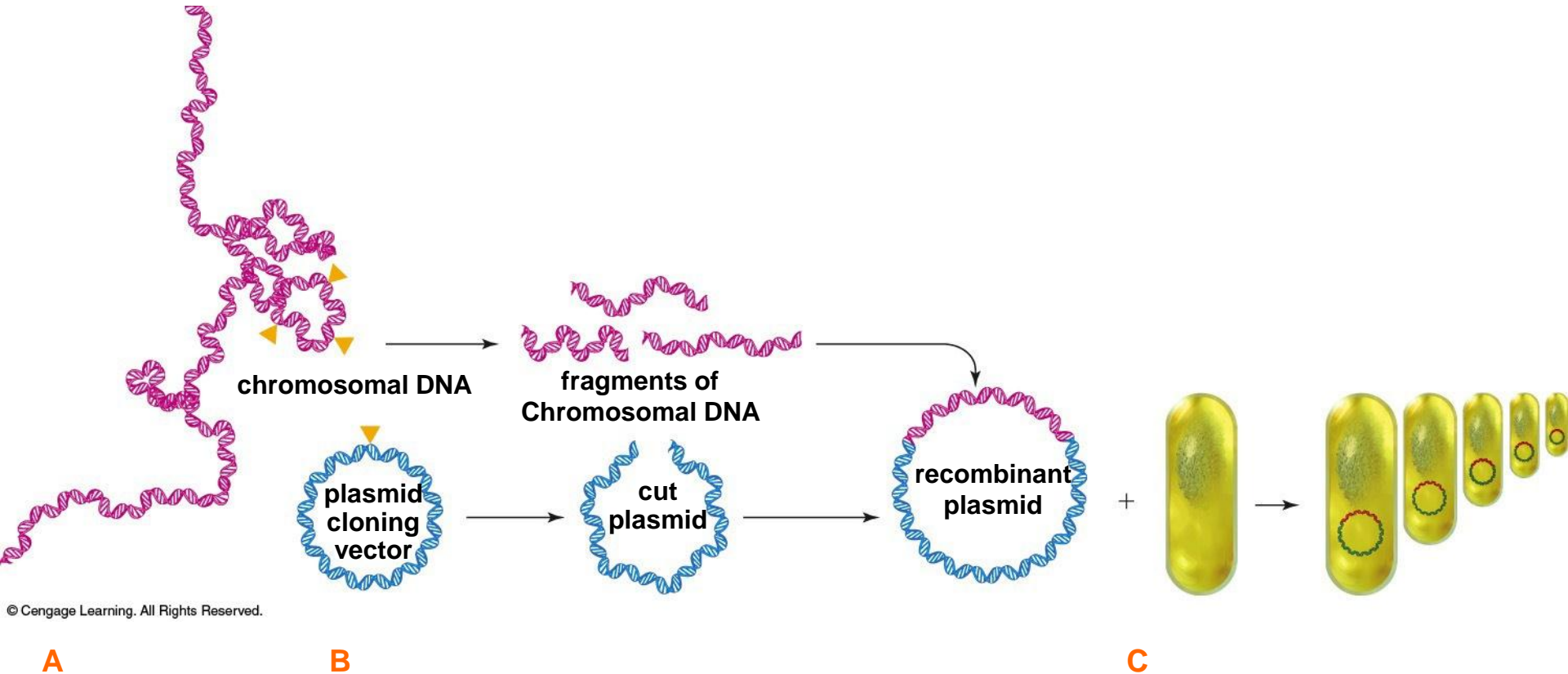


Professor Stanley Cohen/Science Source; (2B) With permission of © QIAGEN, Showing a reduced pDrive Cloning Vector.

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What is DNA Cloning? (cont'd.)



What is DNA Cloning? (cont'd.)

- cDNA cloning
 - Researchers who study eukaryotic genes and their expression work with mRNA transcripts of genes
 - RNA cannot be cloned directly
 - *Reverse transcriptase*, a viral enzyme, is used to transcribe single-stranded RNA into *complementary DNA (cDNA)* for cloning

15.2 How Do Researchers Study One Gene In The Context of Many?

- Genome
 - The entire set of genetic material of an organism
 - Consists of thousands of genes

How Do Researchers Study One Gene In The Context of Many? (cont'd.)

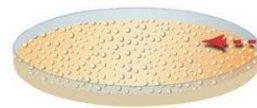
- DNA libraries are sets of cells that host cloned DNA fragments
 - A *genomic* library collectively contains all DNA in a genome
 - A *cDNA* library contains only those genes being expressed when mRNA was harvested
- DNA libraries and the polymerase chain reaction (PCR) help researchers isolate particular DNA fragments

How Do Researchers Study One Gene In The Context of Many? (cont'd.)

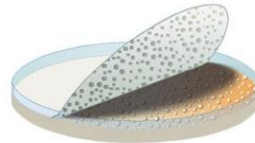
- Probes
 - A cell that contains a particular DNA fragment of interest is mixed up with millions that do not
 - One way to find the clone involves using a fragment of DNA or RNA
 - Fragment is labeled with a tracer
 - Tracers can be radioactive nucleotides

How Do Researchers Study One Gene In The Context of Many? (cont'd.)

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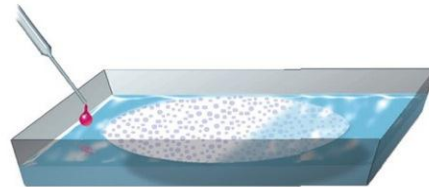
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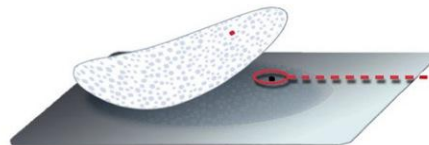
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How Do Researchers Study One Gene In The Context of Many? (cont'd.)

- Polymerase chain reaction (PCR)
 - Technique used to mass-produce copies of sections of DNA without having to clone in living cells
 - Uses *primers*: a short strand of DNA designed to hybridize with a DNA fragment

How Do Researchers Study One Gene In The Context of Many? (cont'd.)

- Polymerase chain reaction (PCR) (cont'd.)
 - Can be used on any sample of DNA with at least one molecule of a target sequence
 - Each cycle of a PCR reaction doubles the number of copies of a section of DNA
 - Thirty cycles can make a billion copies

How Do Researchers Study One Gene In The Context of Many? (cont'd.)

- Polymerase chain reaction (PCR) (cont'd.)
 - DNA template is mixed with primers, nucleotides, and heat-tolerant Taq DNA polymerase
 - Mixture is heated
 - The double-stranded DNA template separates into single strands

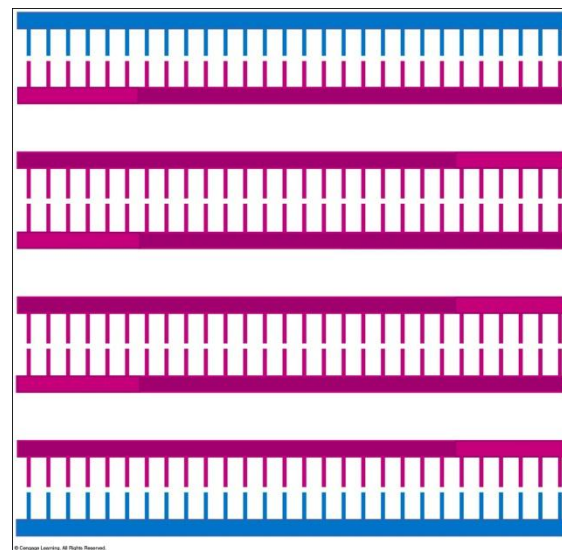
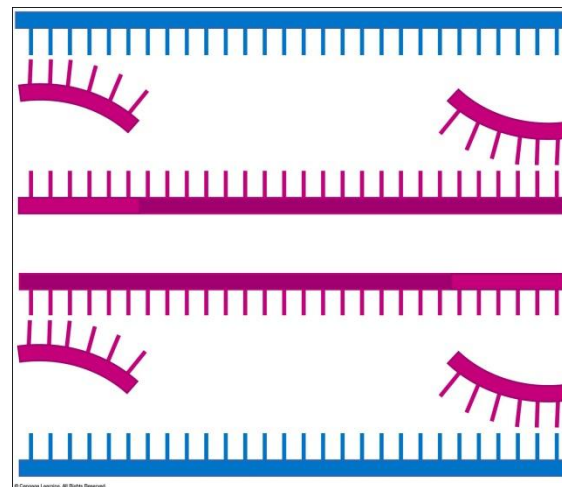
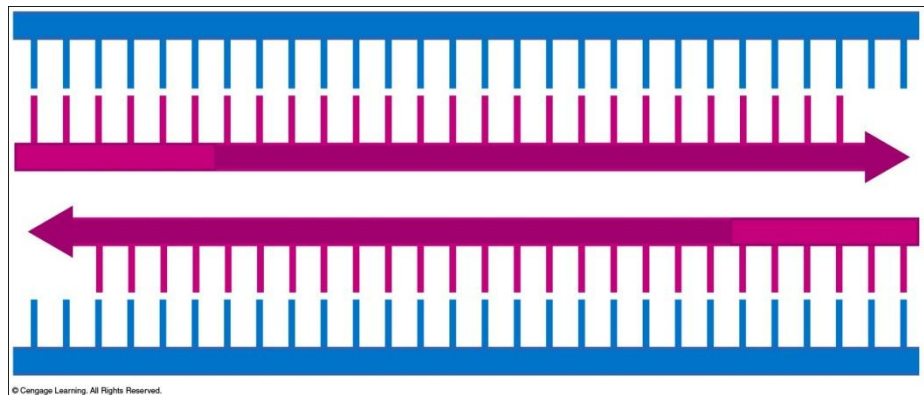
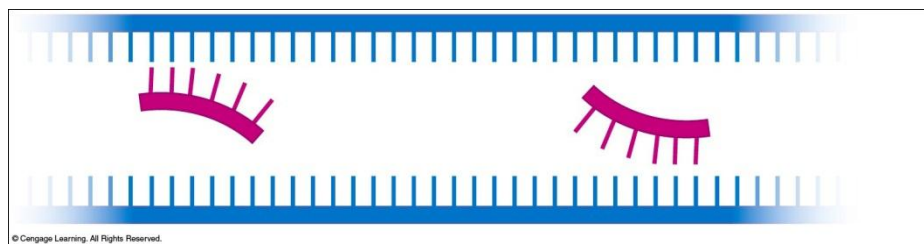
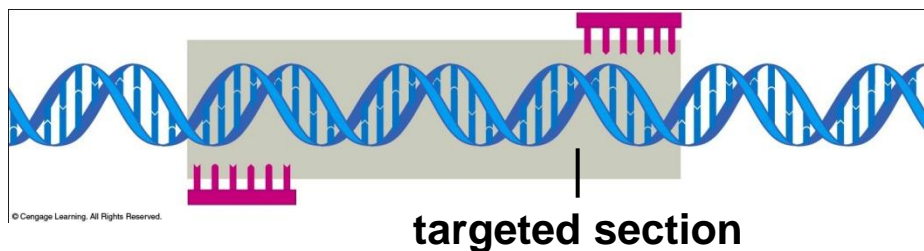
How Do Researchers Study One Gene In The Context of Many? (cont'd.)

- Polymerase chain reaction (PCR) (cont'd.)
 - When it is cooled, some of the primers base-pair with the template DNA
 - *Taq* polymerase begins DNA synthesis at primers, so complementary DNA strands form on single-stranded templates

How Do Researchers Study One Gene In The Context of Many? (cont'd.)

- Polymerase chain reaction (PCR) (cont'd.)
 - The mixture is heated again; double-stranded DNA separates into single strands
 - When it is cooled, primers base pair with old and new DNA strands
 - Each round of PCR reactions doubles the number of copies of the targeted DNA section

How Do Researchers Study One Gene In The Context of Many? (cont'd.)



15.3 DNA Sequencing

- Sequencing
 - Reveals the order of bases in DNA
 - Determines the order of nucleotides in DNA
 - DNA polymerase partially replicates a DNA template
 - Produces a mixture of DNA fragments of different lengths, separated by electrophoresis
 - Has been done in entire genomes of several organisms

DNA Sequencing (cont'd.)

- Electrophoresis
 - Separates fragments by length into bands
 - Electric field pulls DNA fragments through semisolid gel
 - Fragments of different sizes move at different rates
 - Shorter fragments slip through the tangled molecules of the gel faster than longer fragments

Five Steps of DNA Sequencing

1. DNA polymerase adds a nucleotide only to the hydroxyl group on the 3' carbon of a DNA strand
 - Each kind (A,C,G,T) is labeled with a colored pigment
 - During the reaction, the polymerase randomly adds with either a regular or modified nucleotide to the end of the growing DNA strand

Five Steps of DNA Sequencing (cont'd.)

2. The reaction produces millions of DNA fragments of different lengths
 - The fragments are incomplete complementary copies of the starting DNA
3. Electrophoresis separates DNA fragments into bands according to length and color
 - Fragments move through the gel at different rates

Five Steps of DNA Sequencing (cont'd.)

4. All fragments in a given band have the same modified nucleotide at their ends
 - Pigment labels now impart distinct colors on the bands
5. A computer detects and records the color of successive bands on the gel
 - The order of colors represents the sequence of the template DNA

Animation: Automated DNA sequencing

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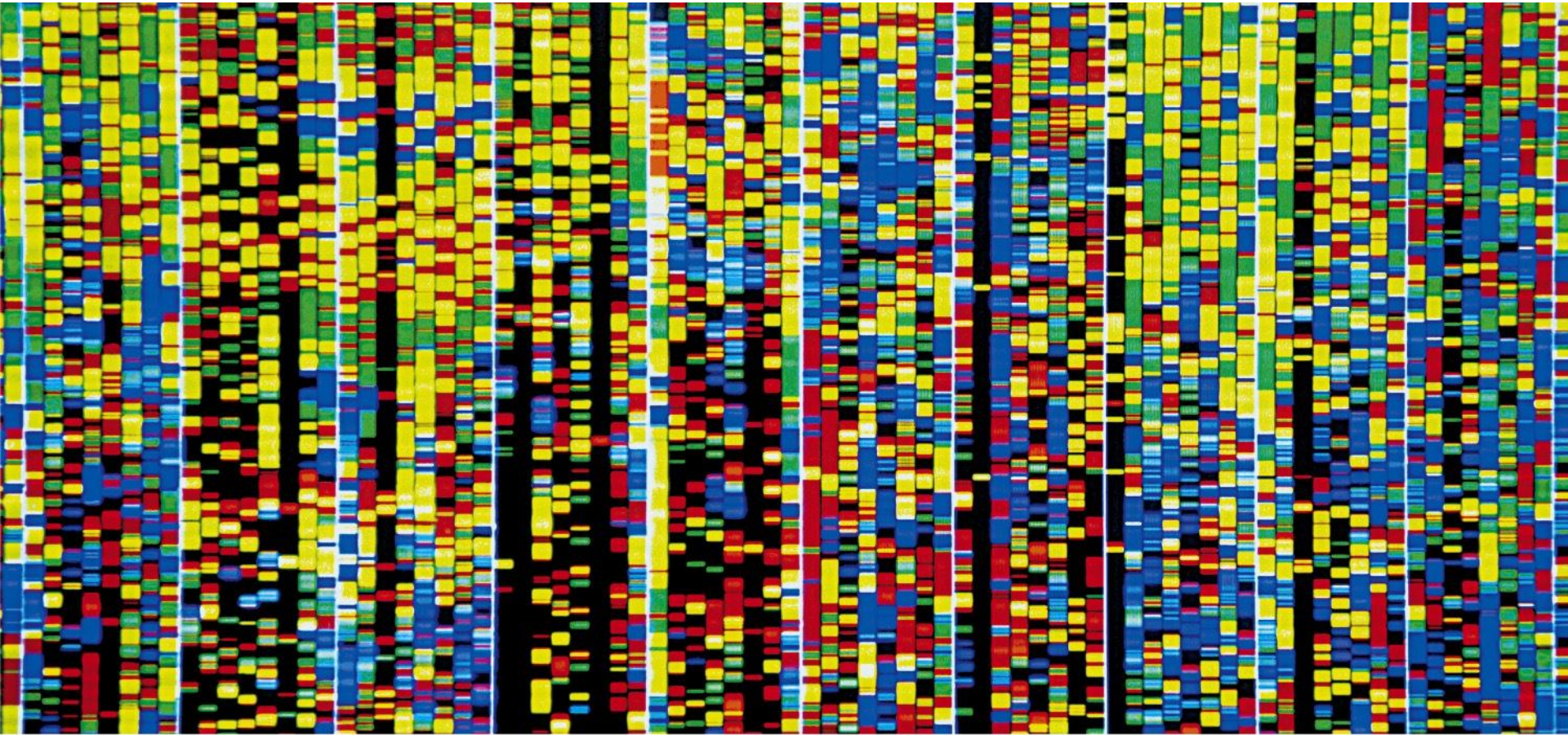
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DNA Sequencing (cont'd.)

- The Human Genome Project
 - Human genome consists of about three billion nucleotide bases
 - Celera Genomics invented faster methods of sequencing genomic DNA
 - Human genome sequence completed in 2003, but could not be patented
 - 50 years after discovery of DNA structure, human genome was complete

DNA Sequencing (cont'd.)



Patrick Landmann/Science Source.

15.4 How Do We Use What Researchers Discover About Genomes?

- Genomics: the study of genomes
 - First time to sequence human genome took 15 years; now it takes about a day
 - Includes:
 - Whole-genome comparisons
 - Structural analysis of gene products
 - The study of small-scale variation
 - Provides insights into evolution and medical benefits

How Do We Use What Researchers Discover About Genomes? (cont'd.)

- We have learned the function of human genes by studying counterpart genes in other species
 - A human version of a mouse gene, APOA5, encodes a lipoprotein
 - Mice with APOA5 have four times normal level of triglycerides in their blood
 - Correlation between APOA5 mutations and high triglyceride levels in humans

How Do We Use What Researchers Discover About Genomes? (cont'd.)

758	GATAATCCTGTTTTGAACAAAAGGTCAAATTGCTGAATAGAAA-GTCTTGATTAACTAAAAGATGTACAAAGTGGGAATTA	836	Human
752	GATAATCCTGTTTTGAACAAAAGGTCAAATTGCTGAATAGAAA-GTCTTGATTAACTAAAAGATGTACAAAGTGGGAATTA	830	Mouse
751	GATAATCCTGTTTTGAACAAAAGGTCAAATTGCTGAATAGAAA-GTCTTGATTAACTAAAAGATGTACAAAGTGGGAATTA	829	Rat
754	GATAATCCTGTTTTGAACAAAAGGTCAAATTGCTGAATAGAAA-GTCTTGATTAACTAAAAGATGTACAAAGTGGGAATTA	832	Dog
782	GATAATCCTGTTTTGAACAAAAGGTCAAATTGCTGAATAGAAA-GTCTTGATTAACTAAAAGATGTACAAAGTGGGAATTA	860	Chicken
758	GATAATCCTGTTTTGAACAAAAGGTCAAATTGCTGAATAGAAA-GTCTTGATTAAGTAAAAGATGTACAAAGTGGGAATTA	836	Frog
823	GATAATCCTGTTTTGAACAAAAGGTCAAGATTGCTGAATAGAAAAGGCTTGATTAAGCAGAGATGTACAAAGTGGACCGCA	902	Zebrafish
763	GATAATCCTGTTTTGAACAAAAGGTCAAATTGTTGAATAGAGACGCTTTGATAAAGCGGAGGAGGTACAAAGTGGGACC-	841	Pufferfish

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How Do We Use What Researchers Discover About Genomes? (cont'd.)

- DNA profiling
 - Identifies a person by their DNA
 - 99% of DNA is same among all humans
 - A base pair variation >1% of population is called a *single-nucleotide polymorphism* (SNP)
 - Examples include determining an individual's array of SNPs on microscopic arrays

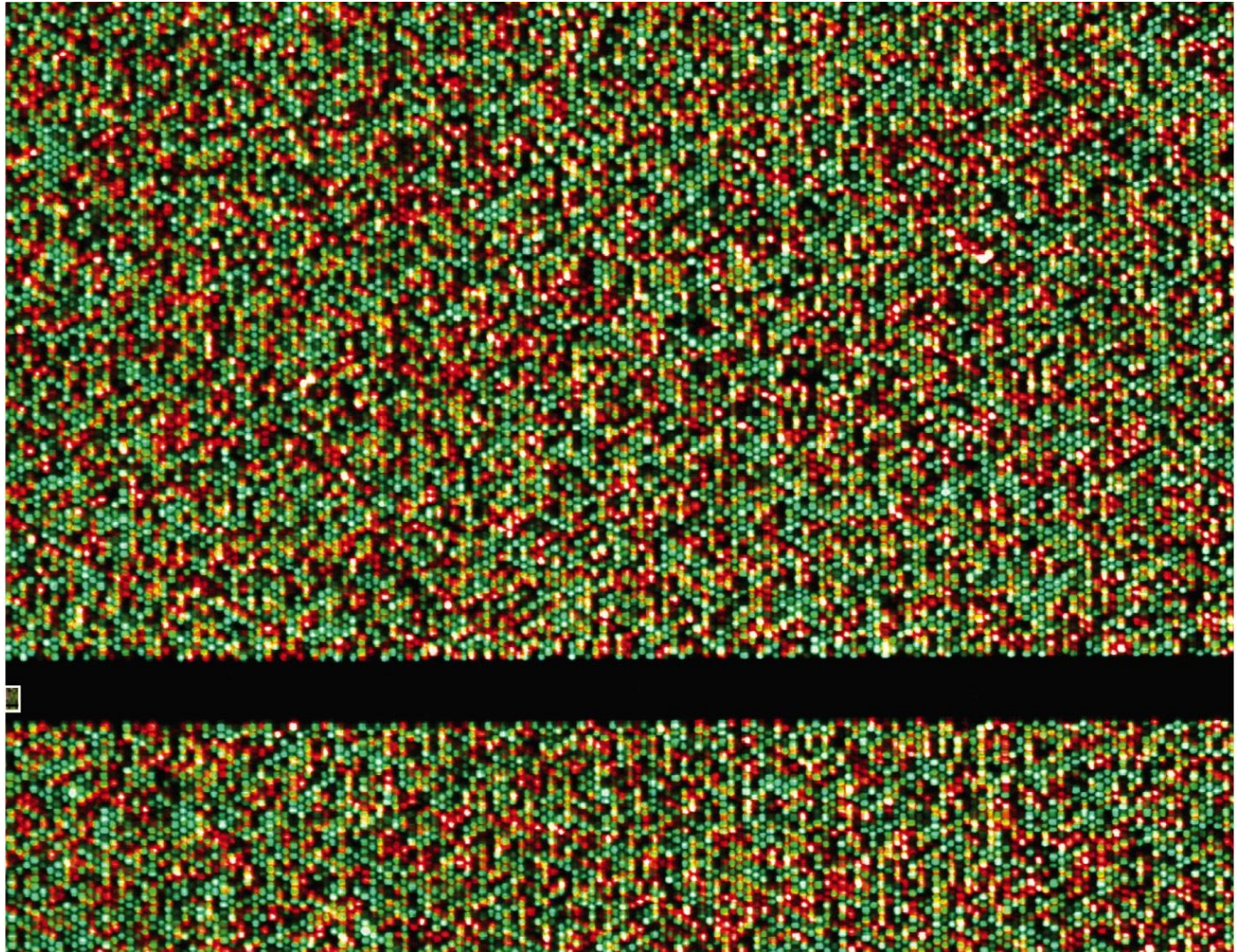
How Do We Use What Researchers Discover About Genomes? (cont'd.)

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The Sanger Institute. Wellcome Images

B



Wellcome Trust Sanger Institute.

How Do We Use What Researchers Discover About Genomes? (cont'd.)

- Short tandem repeats
 - Sequences of 4 or 5 bases repeated multiple times in a row
 - Analyze using PCR
 - Electrophoresis is used to reveal individual's unique array
 - “DNA fingerprint profile” in criminal investigation

ANIMATED FIGURE: Who did it?

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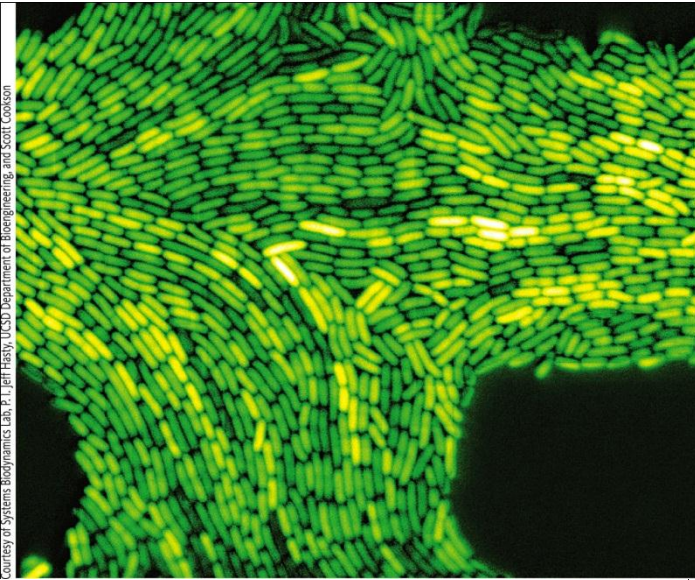
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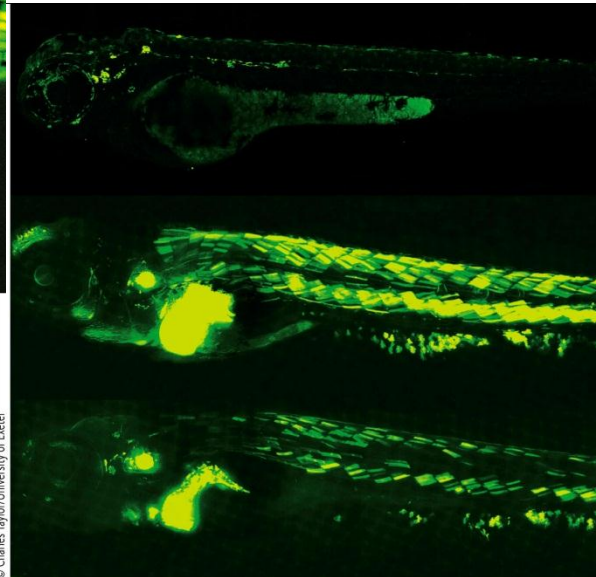
15.5 What is Genetic Engineering?

- Genetic engineering
 - Process by which an individual's genome is deliberately modified
 - Produces a genetically modified organism (GMO)
 - A gene may be altered and reinserted into an individual of the same species
 - A gene from one species may be transferred to another to produce an organism that is transgenic
 - Most common GMOs are bacteria

What is Genetic Engineering? (cont'd.)



A



B



C

Courtesy of Systems Biodynamics Lab, P. L. Jeff Hasty, UCSD Department of Bioengineering, and Scott Coakson

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What is Genetic Engineering? (cont'd.)

- Engineered microorganisms
 - GM bacteria:
 - Produce medically important proteins such as insulin
 - Produce enzymes used in food manufacturing, such as chymotrypsin
 - Are used to improve taste of beer and fruit juice and slow bread from staling

What is Genetic Engineering? (cont'd.)

- Genetically modified animals
 - Mice: used for medical research
 - Goats: have proteins that treat cystic fibrosis, heart attacks, nerve gas exposure, etc.
 - Rabbits: make interleukin-2
 - Pigs: modified for heart-healthy fat and lower phosphate feces
 - Trout and salmon: bigger versions
 - Farm animals: produce more meat or milk

What is Genetic Engineering? (cont'd.)

- Designer plants
 - Genetically engineered crop plants are widespread in the U.S.
 - Genes introduced by a tumor-inducing (Ti) plasmid from bacteria
 - Transfers foreign or modified genes into food crop plants such as soybeans, squash, and potatoes
 - May be resistant to diseases, offer improved yields, or make a protein (Bt) that is toxic to some insect larvae

ANIMATED FIGURE: Transferring genes into plants

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What is Genetic Engineering? (cont'd.)

- GMO crops
 - Engineered for drought tolerance and nutrition
 - Example: rice plants engineered to make β -carotene, a precursor of vitamin A, in their seeds
 - Most widely planted GMO crops include:
 - Corn, sorghum, cotton, soy, canola, and alfalfa

What is Genetic Engineering? (cont'd.)



The Bt and Non-Bt corn photos were taken as part of field trial conducted on the main campus of Tennessee State University at the Institute of Agriculture and Environmental Research. The work was supported by a competitive grant from the CSREES, USDA titled "Southern Agricultural Biotechnology Consortium for Underserved Communities," (2000-2005). Dr. Fisseha Fegegne and D. Ahmad Aziz served as Principal and Co-principal Investigators respectively to conduct the portion of the study in the State of Tennessee.



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What is Genetic Engineering? (cont'd.)

- Concerns with GMOs
 - Transgenes can (and do) escape into the environment
 - Many people are opposed to any GMO crops or “Frankenfoods”
 - Controversy raised by GMO use invites you to read the research and form your own opinions

15.6 Can We Genetically Modify People?

- Gene therapy
 - Gene is transferred into body cells to correct a genetic defect or treat a disease
 - Tested as a treatment for heart attack, sickle-cell anemia, cystic fibrosis, hemophilia A, Parkinson's and Alzheimer's diseases, several cancers, and inherited diseases
- Weigh potential benefits of genetically modifying humans against potential risks

Can We Genetically Modify People? (cont'd.)

- Gene therapy (cont'd.)
 - SCID-X1
 - A severe X-linked disorder of the *IL2RG* gene, which codes for an immune-system receptor protein
 - Affected children can't fight infections, and only survive in germ-free isolation tents

Can We Genetically Modify People? (cont'd.)

- Gene therapy and SCID-X1
 - In the 1990s, 20 boys with SCID-X1 were treated with gene therapy
 - Researchers used a genetically engineered virus to insert unmutated copies of *IL2RG* into cells taken from their bone marrow
 - 18 were cured; five treated with gene therapy for SCID-X1 developed leukemia, and one died

Can We Genetically Modify People? (cont'd.)

- Eugenics
 - Idea of deliberately improving the genetic qualities of the human race
 - Uses methods to select the most desirable human traits
 - Raises ethical issues
 - Engineer cuter babies, or “superhumans”?
 - Prevent of obesity, aggressiveness, or homosexuality?

15.7 Personal Genetic Testing



© Oli Scarff/Getty Images.

Personal Genetic Testing (cont'd.)

- Do you want to know your SNP profile?
 - Companies can extract DNA from saliva and analyze it using a SNP-chip
 - Results indicate estimated risks of developing certain diseases
 - Personalized genetic testing is revolutionizing medicine
 - Can cause people to make beneficial lifestyle changes