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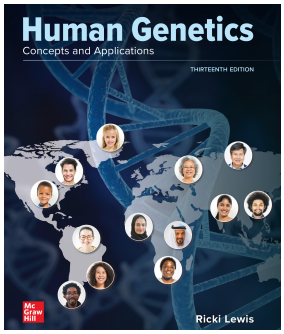

Chapter 21
DNA Technologies
Thirteenth Edition

Ricki Lewis

1

Learning Outcomes

1. State the criteria for a patentable invention.
2. Discuss the history of patenting organisms and DNA.
3. Distinguish between **recombinant DNA and transgenic organisms.**
4. Describe **applications of recombinant DNA technology**.
5. Explain how a **DNA microarray** is used to monitor gene expression.
6. Describe ways to decrease expression of a specific gene.
7. Explain how **genes and genomes** are edited.

2

Patenting DNA 1

- DNA is the language of life, the instruction manual for keeping an organism alive.
- **Biotechnology** is the use or alteration of cells or biological molecules for specific applications.
- A **transgenic organism** has DNA from different species.
- **Recombinant DNA** comes from more than one type of organism.
- All are possible because of the universality of the genetic code.

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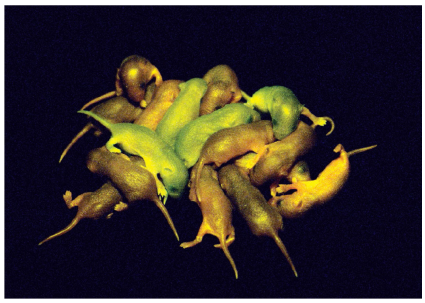
Patenting DNA 2

Mice containing the jellyfish gene for green fluorescent protein (GFP)



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What Is Patentable?

To qualify for patent protection, a transgenic organism must be new, useful, and non-obvious.

Patent law has had to evolve to keep up with modern biotechnology.

A DNA sequence alone does not warrant patent protection.

- It must be useful as a tool for research or as a novel or improved product, such as a diagnostic test or drug.

5

Patent Disputes

A DNA patenting war began in 2009, when several groups, including the ACLU challenged patents on two breast cancer genes (*BRCA1* and *BRCA2*)

- In 2010, a federal judge in the US ruled that seven patents on the *BRCA* genes were "improperly granted" because they are based on a "law of nature."
- In 2013 the US Supreme Court ruled that "a naturally occurring DNA segment is a product of nature and not patent eligible merely because it has been isolated."
  - The court did allow patenting of **complementary DNA (cDNA)**

Then, another issue arose: ownership of the gene editing technology CRISPR-Cas9.)

- UC Berkeley and the Broad Institute are still arguing!

6

Key Biotechnology Inventions

Invention	Function in Nature	Function as Technology
CAR-T (figure 20.18)	Combines T-cell receptor binding with antibody anti-cancer response	Directs antibody to cancer cells
CRISPR-Cas9 (figure 21.9)	Cuts across two DNA strands in bacteria to halt viral infection	Molecular scissors to remove, replace, or add genes to selected sites
Green fluorescent protein (figure 21.1)	Jellyfish gene provides bioluminescence, repelling predators	Attached to selected genes causes encoded proteins to glow, serving as "reporter"
Monoclonal antibodies (figure 19.16)	A natural antibody response is polyclonal (several antibody types)	An antibody response directed against a specific antigen

7

COVID Vaccines Development*

Inactivated or weakened virus vaccines, which use a form of the virus that has been inactivated or weakened so it doesn't cause disease, but still generates an immune response.

Protein-based vaccines, which use harmless fragments of proteins or protein shells that mimic the COVID-19 virus to safely generate an immune response.

Viral vector vaccines, which use a safe virus that cannot cause disease but serves as a platform to produce coronavirus proteins to generate an immune response.

RNA and DNA vaccines, a cutting-edge approach that uses genetically engineered RNA or DNA to generate a protein that itself safely prompts an immune response.

8

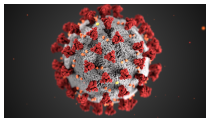
Current COVID-19 Vaccines*

Pfizer – mRNA vaccine

Moderna – mRNA vaccine

Johnson and Johnson – viral vector

Astrazeneca – double stranded DNA



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21.2 Modifying DNA

Recombinant DNA technology adds genes from one type of organism to the genome of another.

- First gene modification biotechnology, and was initially done in bacteria to produce peptides and proteins useful as drugs.

Recombinant DNA technology is also known as gene cloning.

- "Cloning" in this context refers to making many copies of a specific DNA sequence.

10

Recombinant DNA Technology

The story begins in February 1975 when molecular biologists convened to discuss the safety and implications of combining g"|}ns.

The scientists discussed restricting the types of organisms and viruses used, and "physical containment" and "biological containment"

- However, the technology turned out to be safer than expected.
- It also spread to industry faster and in more diverse ways than imagined.

11

Creating Recombinant DNA Molecules

Manufacturing recombinant DNA molecules requires three components

- **Restriction enzymes,** which are enzymes that cut DNA at specific sequences
- **Cloning vectors,** which are pieces of DNA used to deliver specific DNA sequences to cells
- **Recipient cells,** such as bacteria or cultured single cells

Selected DNA is inserted into vectors, and then the loaded vectors are delivered to cells.

- The cells then use the added genetic instructions to manufacture the desired protein.

12

Restriction Enzymes 1

Hundreds of types of restriction enzymes are naturally found in bacteria

- Where they cut DNA of infecting viruses, protecting the host cell.

These enzymes cut DNA at sites that are palindromic*.

- GAATTC
- CTTAAG

The cutting action of many of these enzymes generate single-stranded extensions called “sticky ends.”

- They are complementary to each other, forming hydrogen bonds as their bases pair
  - Regardless of the original DNA source

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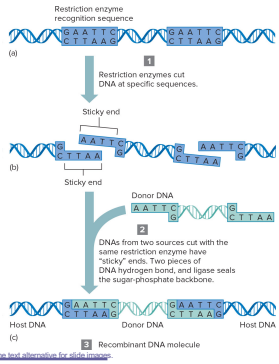
Restriction Enzymes 2

Recombining DNA Uses Restriction Enzymes to Insert a Foreign DNA Sequence



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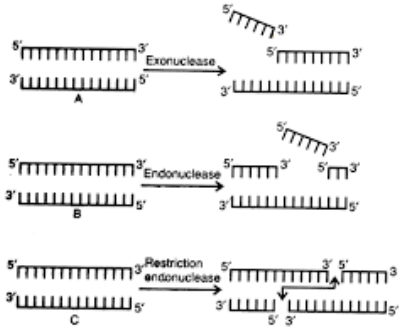
Restriction Enzyme*



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Cloning Vectors

Commonly used vectors include:

- **Plasmids:** the most common vector of plasmids is *E Coli*
- Bacteriophages
- Disabled retroviruses
- Artificial chromosomes from bacteria and yeast

Cloning vectors can hold up to about 2 million DNA bases.

DNA from donor and cloning vector are cut with the same restriction enzyme, put together, and sealed with ligase enzyme

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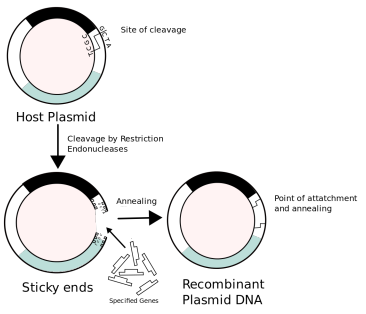

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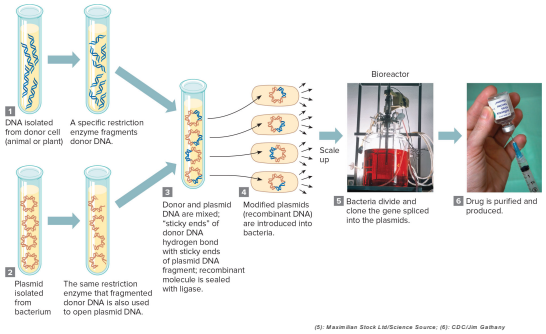
Recombinant DNA 1



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Recombinant DNA 2

1. DNA isolated from donor cell (animal or plant)
   - A specific restriction enzyme fragments donor DNA.
2. Plasmid isolated from bacterium
   - The same restriction enzyme that fragmented donor DNA is also used to open plasmid DNA.
3. Donor and plasmid DNA are mixed; "sticky ends" of donor DNA hydrogen bond with sticky ends of plasmid DNA fragment; recombinant molecule is sealed with ligase.
4. Modified plasmids (recombinant DNA) are introduced into bacteria.
5. Bacteria divide and clone the gene spliced into plasmids.
6. Drug is purified and produced.

 19

19

Drugs Made from Recombinant DNA 1

Drug	Use
Atrial natriuretic peptide	Dilates blood vessels, promotes urination
Colony-stimulating factors	Help restore bone marrow after marrow transplant; restore blood cells following cancer chemotherapy
Deoxyribonuclease (DNase)	Thins secretions in lungs of people with cystic fibrosis
Epidermal growth factor	Accelerates healing of wounds and burns; treats gastric ulcers
Erythropoietin (EPO)	Stimulates production of red blood cells in cancer patients
Factor VIII	Promotes blood clotting in treatment of hemophilia A
Glucocerebrosidase	Corrects enzyme deficiency in Gaucher disease
Human growth hormone	Promotes growth of muscle and bone in people with very short stature due to hormone deficiency
Insulin	Allows cells to take up glucose in treatment of type 1 diabetes

 20

20

Drugs Made from Recombinant DNA 2

Drug	Use
Interferons	Treat genital warts, hairy cell leukemia, hepatitis B and C, Kaposi sarcoma, multiple sclerosis
Interleukin-2	Treats kidney cancer recurrence
Lung surfactant protein	Helps lung alveoli to inflate in infants with respiratory distress syndrome
Renin inhibitor	Lowers blood pressure
Somatostatin	Decreases growth in muscle and bone in pituitary gigantism
Superoxide dismutase	Prevents further damage to heart muscle after heart attack
Thrombin	Stops postsurgical bleeding
Tissue plasminogen activator	Dissolves blood clots in treatment of heart attack, stroke, and pulmonary embolism

 21

21

Products from Recombinant DNA Technology

Recombinant DNA is also used to mass-produce protein-based drugs and vaccines

Drugs

- Insulin, which is produced in *E. coli* cells
- Erythropoietin (EPO)
  - A built-in blood booster or performance-enhancing drug? (See **Bioethics** on p. 400)

Vaccines

- Influenza vaccine
- Covid-19 vaccine
- Malaria vaccine

 22

22

Transgenic Organisms 1

An even more efficient way to express some recombinant genes is in a body fluid of a **transgenic animal**.

Transgenic sheep, cows, and goats have all expressed human genes in their milk.

- Clotting factors
- Clot busters
- Collagen
- Antibodies

 23

23

Transgenic Organisms 2

Several techniques are used to insert DNA into cells to create transgenic animals:

- Chemicals and brief jolts of electricity open transient holes in plasma membranes that admit "naked" DNA
- Gunlike device is used to shoot tiny metal particles coated with DNA inside cells
- Liposomes (tiny fat bubbles) that carry DNA into cells
- Plant cells can be genetically modified using a natural delivery system, the bacterium *Agrobacterium tumefaciens*.

 24

24

Transgenic Organisms 3

Finally, an animal must develop from the fertilized ovum in a surrogate mother.

- If the trait is dominant, the transgenic animal must express it in the appropriate tissue at the right time in development.
- If the trait is recessive, crosses between heterozygotes may be necessary to yield homozygotes that express the trait.

Then the organisms must pass the characteristic on to the next generation.

 25

25

Drugs Made in Transgenic Animals

Animal	Product	Used to Treat
Chicken	Recombinant enzyme made with egg white protein (albumin)	Lysosomal acid lipase deficiency
Cow	Antibodies in plasma	Infectious disease treatment
Goat	Antithrombin (clotting factor) in milk	Prevents blood clots
Rabbit	Recombinant C1 esterase inhibitor in milk	Hereditary angioedema

 26

26

Animal Models 1

Transgenic animals are far more useful as models of human diseases.

- Example: Inserting the mutant human beta globin gene that causes sickle cell anemia into mice

Drug candidates can be tested on these animal models *before* testing on humans.

- Will be abandoned if they cause significant side effects

 27

27

Animal Models 2

Transgenic animal models have limitations:

- Researchers cannot control where a transgene inserts, and how many copies do so.
- The level of gene expression necessary for a phenotype may differ in the model and humans.
- Animal models may not mimic the human condition exactly because of differences in development or symptoms.

 28

28

Animal Models 3

 Courtesy MRC Harwell 29

29

Genetically Modified Foods 1

Form of genetic modification based on phenotype, such as taste or appearance

Genetically modified organisms—Organisms altered to have genes from other species or to over- or underexpress their own genes

Crops are genetically modified to protect them against specific pests, weeds, natural diseases, and toxins such as pesticides

- Example: Golden rice

 30

30

Genetically Modified Foods 2

Genetic modification can introduce interesting traits from unusual source species:

- Lettuce plants produce more vitamin C when they are transgenic for a gene from rats.
- Apples resist fire blight, a bacterial infection, thanks to a transgene from a moth.
- Potatoes given genes from moths and bees resist potato blight, a fungal infection.
- Grapes with a silkworm gene resist Pierce's disease, a bacterial infection.

31

Genetically Modified Foods 3

Genetically modified foods have been controversial.

- Some nations outlaw GM foods, but not the US

People object to GM foods for a variety of reasons.

- Field tests may not adequately predict the effects on ecosystems
- Overreliance may lead to genetic uniformity
- Some GM organisms can disrupt natural ecosystems.

32

Engineered Crops

Plant	Altered Trait	Method
Alfalfa	Decreased lignin extends feeding season for cattle	RNAi suppresses enzyme
Apple	Less browning	Transgene from one apple species suppresses expression of browning enzyme genes in another apple species
Cassava	Increased iron and zinc	Transgenes from mustard weed block transporter proteins, increasing levels of these minerals
False flax	Seeds make more omega-3 fatty acids, normally eaten in fish oil	Transgenes for omega-3 fatty acid production from alga, moss, and water mold
Potato	Less bruising/browning, less acrylamide (carcinogen)	RNAi blocks synthesis of enzyme (polyphenol oxidase)
Rice	Resistance to drought-related stress	Transgene from mustard weed encodes enzyme that enables a sugar to accumulate in cells, retaining water during drought
Soybean	Oil without trans fats, increased oleic acid, extended shelf life	TALENS (gene editing)

33

Bioremediation

Transgenic organisms can provide processes as well as products

Bioremediation: The use of bacteria or plants to detoxify environmental pollutants

- Nickel-contaminated soils
- Mercury-tainted soils
- Trinitrotoluene (TNT) in land mines

Bioremediation is also used to decrease the amount of phosphorus in pig manure

34

Monitoring Gene Function 1

Microarrays (gene chips) are devices that detect and display the mRNAs in a cell.

- Piece of glass or plastic that is about 1.5 centimeters square

Many small pieces of DNA of known sequence are attached to one surface, in a grid pattern.

In many applications, a sample from an abnormal situation is compared to a normal control.

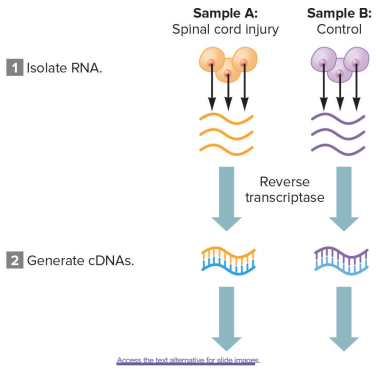
35

Monitoring Gene Function 2

- Messenger RNAs are extracted from the samples and cDNAs are made.
- These are differentially-labeled and then applied to the microarray.
- The pattern and color intensities of the spots indicate which genes are expressed.
- A laser scanner detects and computer algorithms interpret the results.

36

A DNA Microarray Experiment 1



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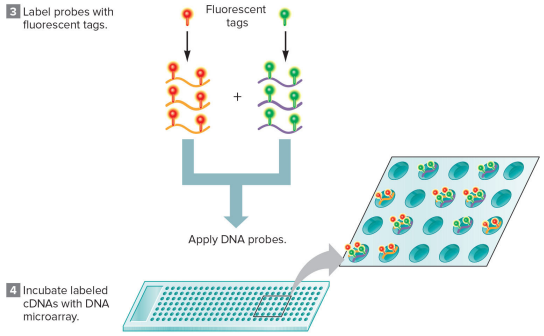
A DNA Microarray Experiment 2



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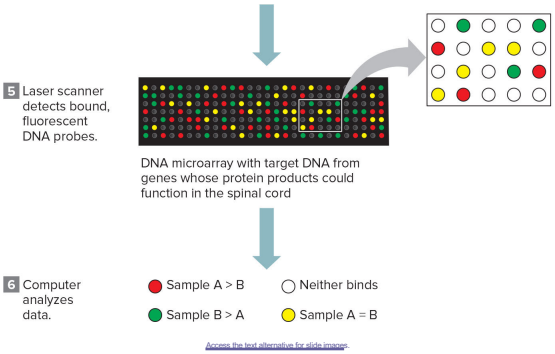
A DNA Microarray Experiment 3



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39

Gene Expression Profiling Chronicles Repair after Spinal Cord Injury

Time After Injury (rats)	Type of Increased Gene Expression
Day 1	Protective genes to preserve remaining tissue
Day 3	Growth, repair, cell division
Day 10	Repair of connective tissues
	Angiogenesis
Days 30 to 90	Blood vessels mature
	New type of connective tissue associated with healing

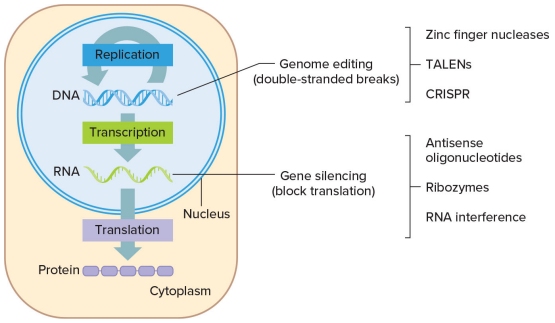
40

21.4 Gene Silencing and Genome Editing 1

- Since the 1960s, researchers have been trying to diminish ("knock down") or silence ("knock out") the expression of specific genes
- **Gene silencing** techniques block synthesis of, or degrade, mRNA.
- **Genome editing** techniques create double-stranded breaks in the DNA double helix, enabling insertion of a desired DNA sequence or removal of a sequence.

41

Gene Silencing and Genome Editing 2



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42

Gene Silencing by Antisense Technology

- Block gene expression by introducing RNA that is complementary to the gene's mRNA transcript.
- The introduced RNA, called antisense RNA, binds to the mRNA, preventing its translation into a protein.
- An early application of antisense technology was a tomato intended to stay fresh longer because the antisense RNA blocked the ripening enzyme

43

Gene Silencing by Use of Morpholinos

A **morpholino** consists of 25 DNA bases bonded to each other by organic groups that are not the sugar-phosphate ones in DNA.

The morpholinos can block splice-site mutations that would otherwise delete entire exons.

One use is in Duchenne muscular dystrophy.

- The morpholino blocks an exon site necessary to produce effective dystrophin.

44

Gene Silencing by Use of Ribozymes

- **Ribozyme** is a RNA in the ribosome that has a catalytic activity like enzymes.
- It fits the shapes of certain RNA molecules, and can cut the RNA molecule.
- Because it can cut the RNA molecule, it could used to destroy RNA from pathogens, such as HIV.

45

Gene Silencing by Use of RNA interference (RNAi) 1

- **RNA interference (RNAi)** is a gene silencing technique based on the fact that RNA molecules can fold into short, double-stranded regions where the base sequence is complementary .
- Short double-stranded RNAs sent into cells separate into single strands, one of which binds to its complement in mRNA, preventing it from being translated.
- RNAi involves the use of double-stranded RNA and several proteins.
- An enzyme termed "dicer" cuts double stranded RNA in to pieces of 21 to 24 nucleotides.

46

Gene Silencing by Use of RNA interference (RNAi) 2

- These short segments of double-stranded RNA attach to protein complexes, termed RNA-induced silencing complex (RISC).
- One strand of the double-stranded short RNA, called the guide strand finds attaches to the protein complex of the RISC.
- As part of the RISC, the guide strand finds its complementary RNA and binds.
- Another part of the RISC, a protein component, acts as a nuclease enzyme degrading targeted RNA, preventing its translation into a protein.

47

RNA Hairpins (Double-Stranded RNA)

RNA molecules can fold into short, double-stranded regions where the base sequence is complementary.

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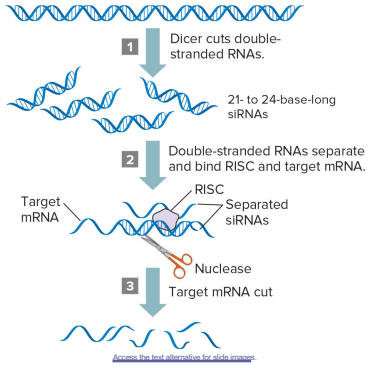
48

RNA Interference 1



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RNA Interference 2

1. Dicer cuts double- stranded RNAs.
2. Double-stranded RNAs separate and bind RISC and target mRNA.
3. Target mRNA cut.

50

Applications of RNA Interference

RNA interference can be used to:

- Create vaccines by knocking down expression of key genes in viruses that cause diseases
- Treat cancer by silencing oncogenes
- Engineer a better-tasting decaf coffee by silencing an enzyme required for caffeine synthesis.

Another agricultural application of RNAi is to engineer crops to make RNAi molecules that a specific pest species eats.

51

Genome Editing 1

- Genome editing uses restriction endonucleases to cut and paste DNA molecules in patterns that might not exist in nature.
  - Genome editing: Restriction endonucleases precisely cut
  - Recombinant DNA technology: inserts DNA randomly
- This technology can be used on somatic cells or germline cells (developing oocytes and sperm).
- The three major genome editing techniques are ZFNs, TALENs, and CRISPR-Cas9.

52

Genome Editing 2

Abbreviation	Full Name
CRISPR-Cas9	Clustered regularly interspaced short palindromic repeats-CRISPR-associated protein 9
TALENs	Transcription-activator-like effector nucleases
ZFNs	Zinc finger nucleases

ZFNs and TALENS cut can only one gene at a time

53

Zinc Finger Nuclease Technology (ZFN)

- This technology uses protein motifs (parts of proteins that have characteristic shapes) called zinc fingers.
- A zinc finger is a protein consisting of a beta-pleated sheet linked to an alpha helix by a zinc atom.
- Different zinc fingers bind different three-base DNA sequences.
- If the zinc finger binds, then a nuclease cuts the DNA.

54

Transcription-Activator-Like Effector Nuclease (TALEN) Technology

In this technology, a restriction enzyme from a bacterium (*Xanthomonas*) that infects plants cuts DNA on both strands.

TALENs also work on animal cells.

- Introduce a gene from a golden orb-web spider into the silkworm spider, enabling the recipient to produce a more elastic silk in greater amounts.

 55

55

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPRs) 1

- These are short sequences of DNA that include several repeats.
- They are natural components of the genomes of certain bacteria, where they provide an action similar to an immune response.
- CRISPRs enable bacteria to deploy a restriction enzyme called Cas9.
- Cas9 enzyme recognizes and cuts out DNA sequences from infecting viruses that have inserted into the viral genome.

 56

56

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPRs) 2

- The CRISPR DNA sequences along with the viral sequences are transcribed into CRISPR RNAs.
- These RNA serve as guides that bring Cas9 to other sites in the bacterial genome where viral DNA has inserted and then cuts both strands.
- CRISPR-Cas9 is very versatile; researchers can design and synthesize guide RNAs to direct which DNA sequences are removed.
- New DNA sequences can be placed into the gene and even new genes can be added.

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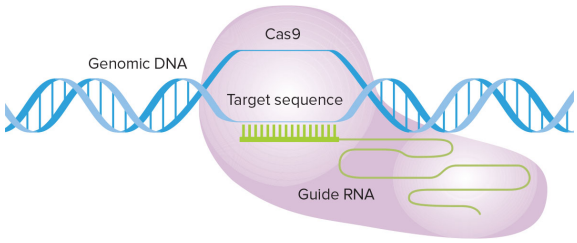
Clustered Regularly Interspaced Short Palindromic Repeats (CRISPRs) 3



 Source: Adapted from Chemical & Engineering News 93(24): 29–21. 58

58

Innovative Uses of CRISPR-Cas9 Genome Editing

Application	Example
GMO containment	Deleting genes that encode hormones that catfish require to reproduce, so that catfish with other genetic modifications can be raised on farms where the hormones are added to the water, but not survive in the wild.
Bringing back extinct species	Replace genes with counterparts from extinct relatives, such as mammoth genes in an elephant.
Limiting spread of infectious diseases	Introduce genes into disease vectors such as mosquitoes and ticks that make them infertile.
Avoiding allergy to vaccines	Remove the gene that encodes albumin, the egg white protein, which makes some people react to vaccines grown in hen eggs.
Adding genes to resist disease	Swapping in a CCR5 mutation to provide resistance to HIV (see the chapter opener of chapter 19).
Creating organ donors	Introduce human genes into pig genomes to create cell surfaces that the human immune system will not reject.
Adding traits to show animals	Introduce genes for valued traits (such as fur color, body size, and stamina) into pets or show animals in just one or two generations.

 59

59

Gene Drive 1

Gene drive is the application of genome editing to kill, alter, or render infertile an pathogen.

It is based on a natural form of DNA repair, called homing.

- Homing is a process which removes one copy of a pair of alleles of a selected gene and replaces it with another copy of the remaining allele.
  - Homing genes are found in certain single-celled organisms.

A gene drive counters Mendel's first law, of segregation.

- Has been applied to the mosquitoes that transmit malaria.

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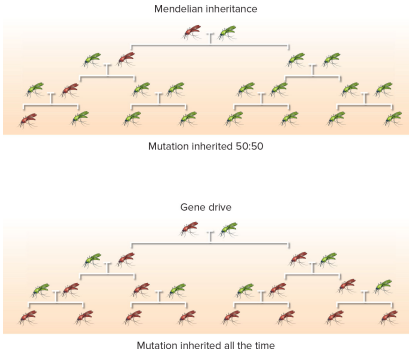
Gene Drive 2



61

Editing the Human Germline

Although many researchers have agreed not to attempt to edit the human germline, attempts have been made to do so.

- Experiments introduced CRISPR at the moment of fertilization, creating germline-edited embryos because the change was transmitted to every cell.
  - Correction of a dominant mutation that causes heart disease

Genetics organizations do not object to the use of CRISPR on gametes and embryos if appropriate permissions are granted.

- However, they do object to the use of the technology that leads to a human pregnancy

62

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