Isolating Genes

Huntington's disease causes nerve cells in the brain to break down. The onset of Huntington's often begins midlife, with no physical hints of the disease before symptoms arise. For those who have a parent with Huntington's disease, a Punnett square or pedigree analysis may provide a probability of having the disease, but not a definitive diagnosis. For Huntington's and many other diseases, genetic material can be tested to determine whether a person has, or is a carrier of, a specific disease.

Gather Evidence Would you undergo tests to determine your likelihood of having certain diseases? Why or why not? If you did, what would you want to happen to your genetic information? Should it be shared with scientific researchers, your health insurer, or your future employers? Explain your reasoning.

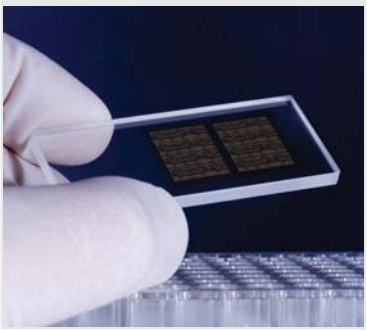
Genetic Testing

Genetic testing is the analysis of a person's DNA to determine the risk of having or passing on a genetic disorder. Geneticists test for abnormalities in genetic material, from entire chromosomes down to individual genes. It is also possible to test for proteins that indicate a particular disease. Since proteins reflect the DNA patterns of genes, this is an indirect method of testing genetic material. Genetic testing is a powerful tool to screen for genetic disorders. However, not all diseases can be found through genetic testing.

Analyze Why can't genetic testing identify all diseases? How does inheriting cystic fibrosis differ from developing cardiovascular disease due to poor diet and exercise?

There are thousands of genetic tests available, each targeting a specific gene or genomic region. DNA microarrays are tools that allow scientists to study many genes, or their expression, at once. A microarray is a small chip that is dotted with all of the genes being studied. The genes are laid out in a grid pattern. Each block of the grid is so small that a one-square-inch chip can hold thousands of genes. Microarrays, such as the one shown in Figure 2, help researchers find which genes are expressed in which tissues, and under what conditions.

FIGURE 2: DNA microarrays are used in genetic testing.



Collaborate In a group, discuss the benefits, risks, and limitations of genetic testing. Why is it important to identify carriers of a genetic disease? How should genetic information be used and safeguarded?

Replicating Genes

Genetic tests are useful for genes that have been linked to a disease, but identifying specific genes that cause disease is not simple. Scientists spend years finding genes that are associated with a particular disease among the 20,000–25,000 genes in the human genome. Small quantities of target sequences collected from patients must be amplified many times to produce the amount needed for testing. The invention of the polymerase chain reaction (PCR) was a turning point, making it possible to obtain the large amounts of DNA needed for genetic testing in hours instead of days.

FIGURE 3: The steps of the polymerase chain reaction.

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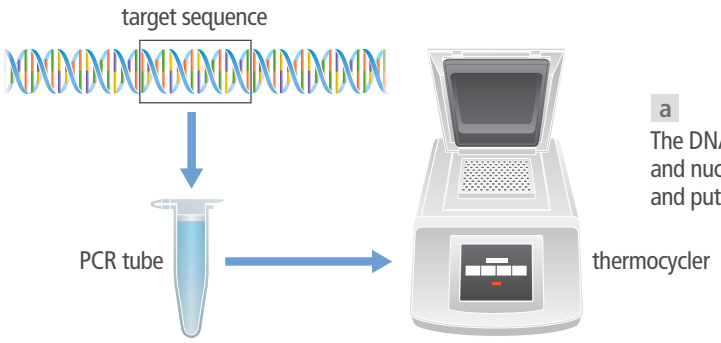
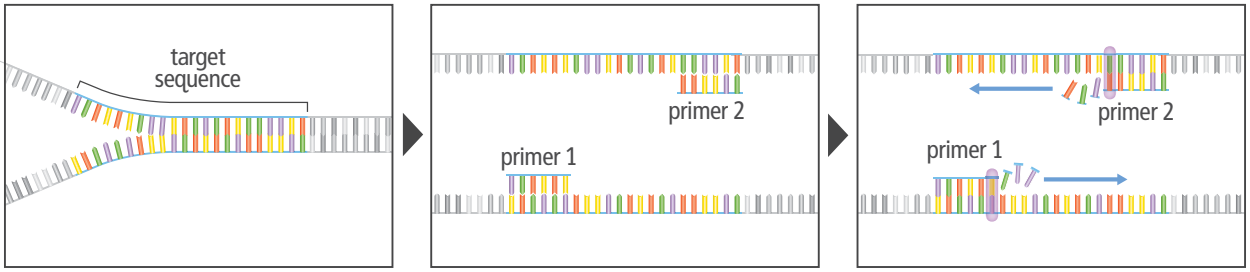


a The DNA sample, primers, DNA polymerase, and nucleotides are placed in the PCR tube and put in the thermocycler.

Cycle 1

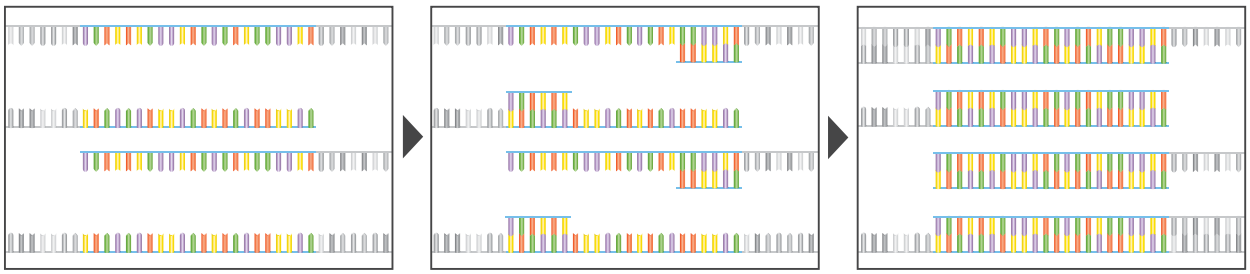


b Separating
The temperature is raised to 95 °C (203 °F) to separate the DNA strands.

c Binding
The temperature is cooled to 55 °C (131 °F), and the primers bind to the DNA strands.

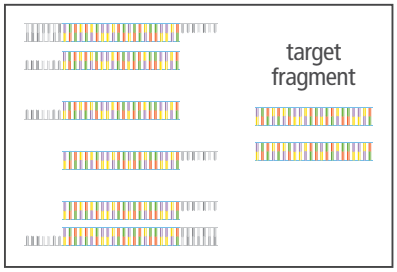
d Copying
The temperature is heated to 72 °C (152 °F). DNA polymerase locates the primers and begins synthesizing a complementary strand. It continues to synthesize the DNA strand until it reaches the end of the strand.

Cycle 2 The same three steps occur in Cycle 2 and each subsequent cycle: separating, binding, and copying.



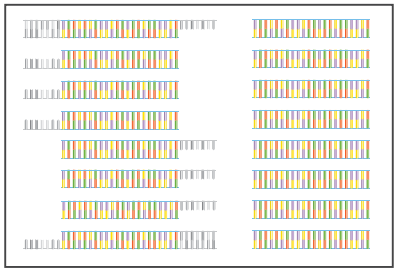
Separating **Binding** **Copying**

Cycle 3



At the end of Cycle 3, fragments that include only the target DNA have been synthesized.

Cycle 4



By the end of Cycle 4, eight target fragments have been synthesized.

Cycle 30

After 30 cycles, more than a billion fragments have been synthesized.

Figure 3a shows the beginning of a PCR run. DNA is extracted from cell nuclei and added to a PCR tube, along with primers, DNA polymerase, and nucleotides. The tube is placed inside a thermocycler, which automatically regulates the temperature of the solution.

The polymerase chain reaction occurs in three steps:

Separating The thermocycler heats the sample until the complementary strands of DNA separate (Figure 3b). Separation occurs around 95 °C (203 °F).

Binding The thermocycler then cools to around 55 °C (131 °F) (Figure 3c), and primers bind to the separated DNA strands. Primers are short nucleotide segments that allow a specific type of DNA polymerase to attach to the DNA strands. Two primers are required for each reaction. One primer attaches to the beginning of the target segment on one strand of DNA. The other primer attaches to the beginning of the target segment on the complementary strand of DNA.

Copying The thermocycler heats to 72 °C (162 °F) (Figure 3d). At this temperature, DNA polymerase attaches to the primer segments and begins adding complementary nucleotides. The free nucleotides added to the solution act as building materials for the new strands of DNA. DNA synthesis continues until the DNA polymerase reaches the end of the strand and detaches. A complementary strand of DNA is produced, and the first PCR cycle is complete.

Collaborate With a partner, take turns explaining and modeling how the three steps of PCR produce DNA sequences. While you walk your partner through the steps, explain the significance of the following terms: *DNA polymerase*, *nucleotides*, *primers*, *DNA separation*, *primer binding*, *DNA synthesis*, and *thermocycler*. Then, your partner explains and models the process to you. Continue to take turns until both of you feel comfortable with the steps of PCR.

The cycle is repeated a second time. The thermocycler heats to 95 °C and the DNA strands separate. The thermocycler cools to 55 °C and primers bind to the target sites. Finally, the thermocycler heats to 72 °C. DNA polymerase attaches to primer segments and synthesizes a complementary strand of DNA using the free nucleotides.

The thermocycler continues to heat and cool the solution automatically. The first fragment of the target DNA sequence is synthesized after the third cycle. More than one billion fragments of target DNA are synthesized after thirty cycles. PCR cycles continue until an adequate amount of the target DNA is produced.

Analyze Why is it necessary to keep changing the temperature in the PCR process? Use evidence to support your claim.

The polymerase chain reaction was invented by Kary Mullis in 1983, who shared the Nobel Prize in Chemistry in 1993. This invention solved two problems Mullis was facing. First, his lab was trying to create a new use for the oligonucleotides, or short DNA segments, they produced. PCR uses oligonucleotides as primers. Second, genetic testing and other DNA-related tests took weeks to perform. PCR greatly decreased the time required to amplify a DNA sample.

Explain Describe the relationship between genetic testing and the polymerase chain reaction. How has the PCR technique made genetic testing possible on a large scale?

Patterns

DNA replication produces a complementary strand of DNA, while PCR amplifies a target section of DNA by copying just that section. How else are DNA replication and PCR similar? How are they different?