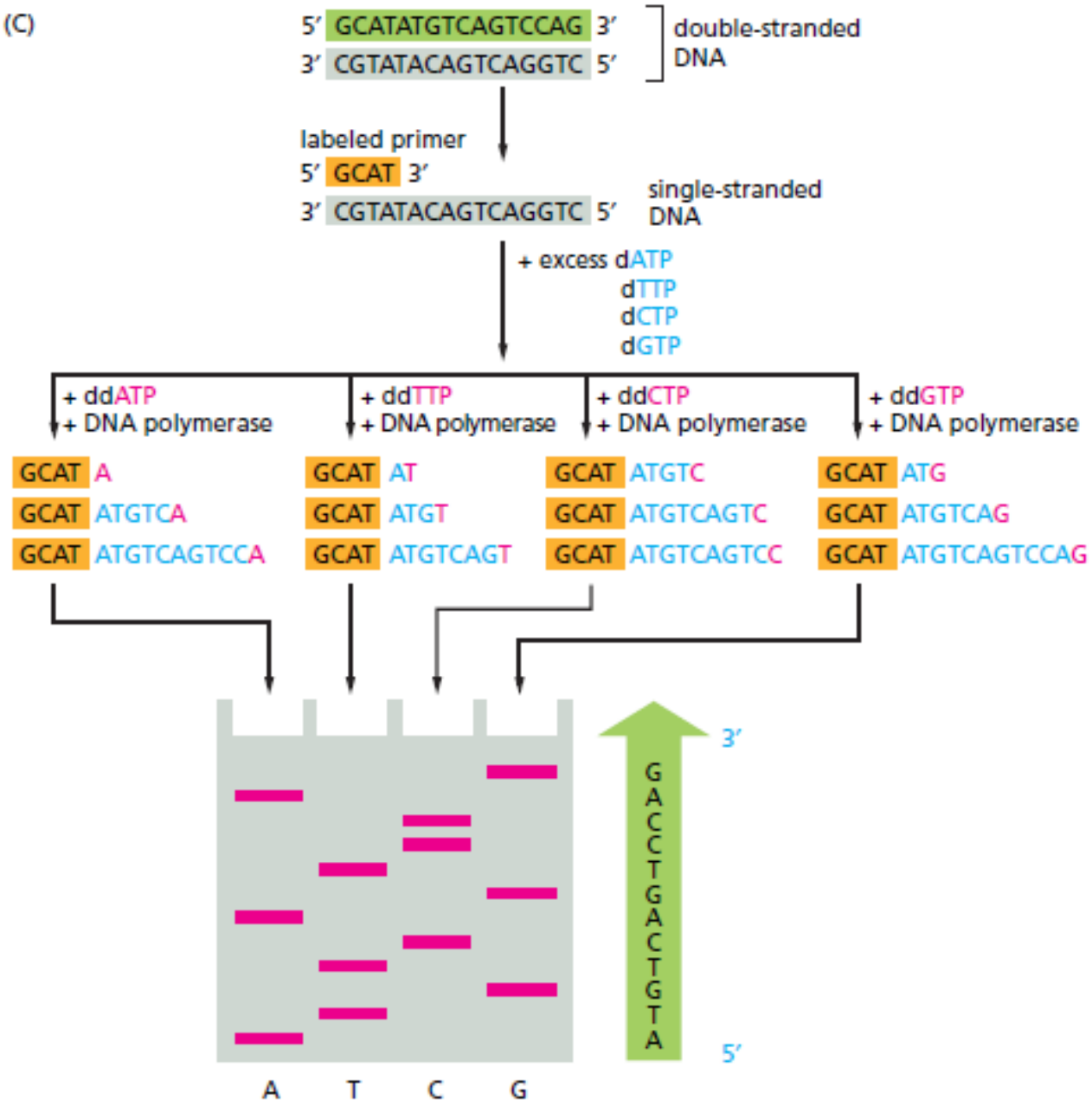


DNA sequence reading directly from the bottom of the gel upward, is

ATGTCAGTCCAG
1 12

DNA amplification in vitro: the polymerase chain reaction

If we know the sequence of at least some parts of the gene or sequence of interest, we can amplify it in a test tube. The procedure is called the **polymerase chain reaction (PCR).**

The process uses multiple copies of a pair of short chemically synthesized primers, from 15 to 20 bases long, each binding to a different end of the gene or region to be amplified.

The two primers bind to opposite DNA strands, with their 3 ends pointing at each other. Polymerases add bases to these primers, and the polymerization process shuttles back and forth between them, forming an exponentially growing number of double-stranded DNA molecules.

PCR Is a Powerful Technique in Medical Diagnostics, Forensics, and Studies of Molecular Evolution

PCR can provide valuable diagnostic information in medicine.

Selected DNA Sequences Can Be Greatly Amplified by the Polymerase Chain Reaction

In **1984, Kary Mullis** devised an ingenious method called the ***polymerase chain reaction*** *(PCR) for amplifying specific DNA sequences.*

Millions of the target sequences can be readily obtained by PCR if the flanking sequences of the target are known.

PCR is carried out by adding the following components to a solution containing the target sequence:

(1) a pair of primers that hybridize with the flanking sequences of the target, (2) all four deoxyribonucleoside triphosphates (dNTPs), and (3) a heat-stable DNA polymerase.

A PCR cycle consists of three steps.

1. *Strand separation. The two strands of the parent DNA molecule are* separated by heating the solution to 95ºC for 15 s.

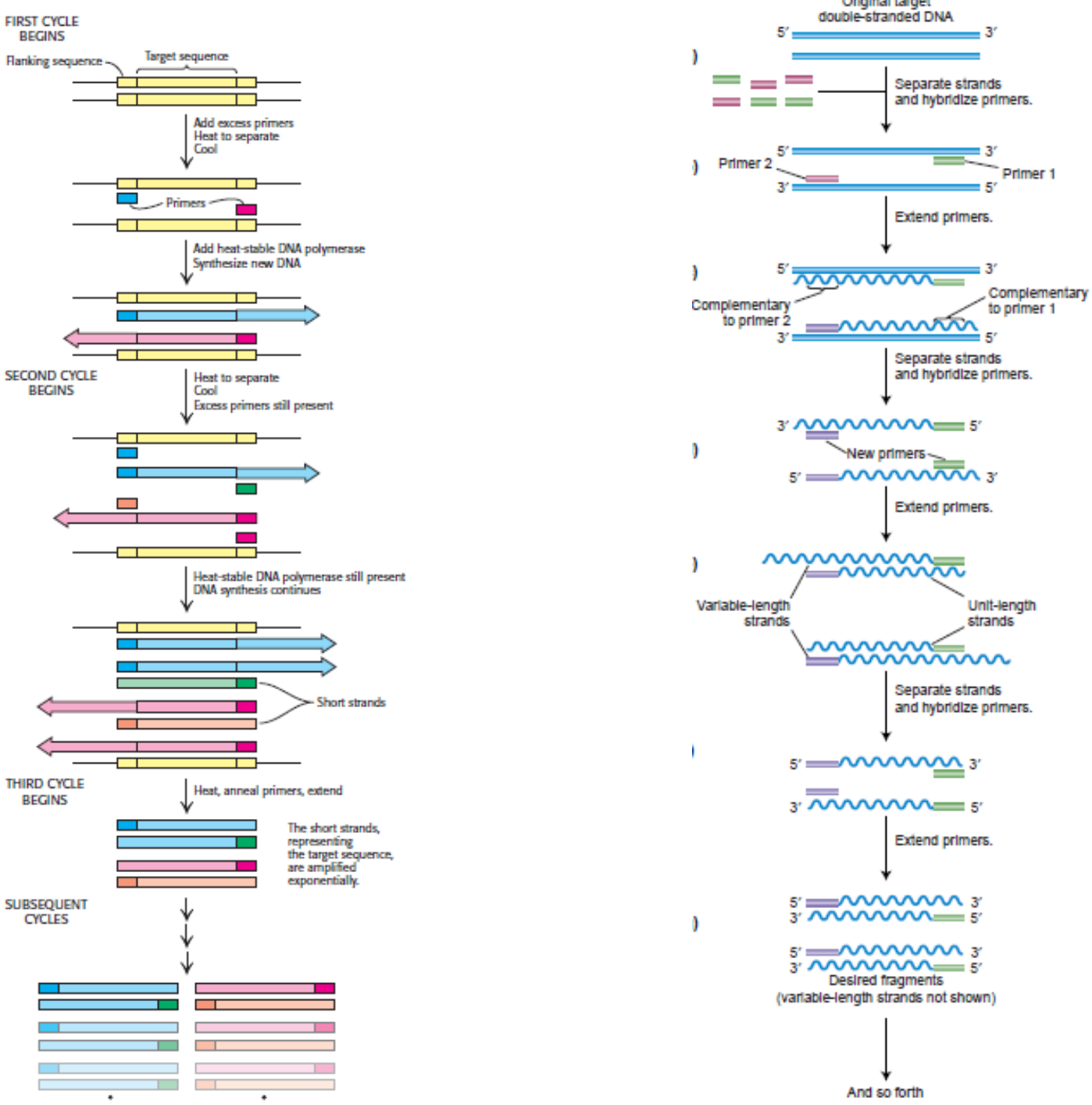
2. *Hybridization of primers. The solution is then abruptly cooled to 50-60°C* to allow each primer to hybridize to a DNA strand.

One primer hybridizes to the 5' end of the target on one strand, and the other primer hybridizes to the 3' end on the complementary target strand.

Parent DNA duplexes do not form, because the primers are present in large excess. Primers are typically from 20 to 30 nucleotides long.

3. *DNA synthesis. The solution is then heated to 72ºC, the optimal temperature* for *Taq DNA polymerase. This heat-stable polymerase comes* from *Thermus aquaticus, a thermophilic bacterium that lives in hot springs.*

The polymerase elongates both primers in the direction of the target sequence because DNA synthesis is in the 5'-to-3' direction.


FIRST CYCLE BEGINS
Flanking sequence
Target sequence
Add excess primers
Heat to separate
Cool
Primers
Add heat-stable DNA polymerase
Synthesize new DNA
SECOND CYCLE BEGINS
Heat to separate
Cool
Excess primers still present
Heat-stable DNA polymerase still present
DNA synthesis continues
Short strands
THIRD CYCLE BEGINS
Heat, anneal primers, extend
The short strands, representing the target sequence, are amplified exponentially.
SUBSEQUENT CYCLES
Original target double-stranded DNA
5′
3′
Separate strands and hybridize primers.
Primer 2
Primer 1
Extend primers.
Complementary to primer 2
Complementary to primer 1
Separate strands and hybridize primers.
New primers
Extend primers.
Variable-length strands
Unit-length strands
Separate strands and hybridize primers.
Extend primers.
Desired fragments
(variable-length strands not shown)
And so forth

PCR Is a Powerful Technique in Medical Diagnostics, Forensics, and Studies of Molecular Evolution

Bacteria and viruses can be readily detected with the use of specific primers.

For example, PCR can reveal the presence of human immunodeficiency virus in people who have not mounted an immune response to this pathogen and would therefore be missed with an antibody assay.

Finding *Mycobacterium tuberculosis bacilli in tissue specimens is slow* and laborious.

With PCR, as few as 10 tubercle bacilli per million human cells can be readily detected. PCR is a promising method for the early detection of certain cancers.

This technique can identify mutations of certain growth-control genes, such as the *ras genes*

A reaction that cycles 20 times will amplify the specific target sequence 1-million-fold.

PCR is also having an effect in forensics and legal medicine. An individual DNA profile is highly distinctive because many genetic loci are highly variable within a population.

For example, variations at a specific one of these locations determines a person's HLA type (human leukocyte antigen type;); organ transplants are rejected when the HLA types of the donor and recipient are not sufficiently matched.

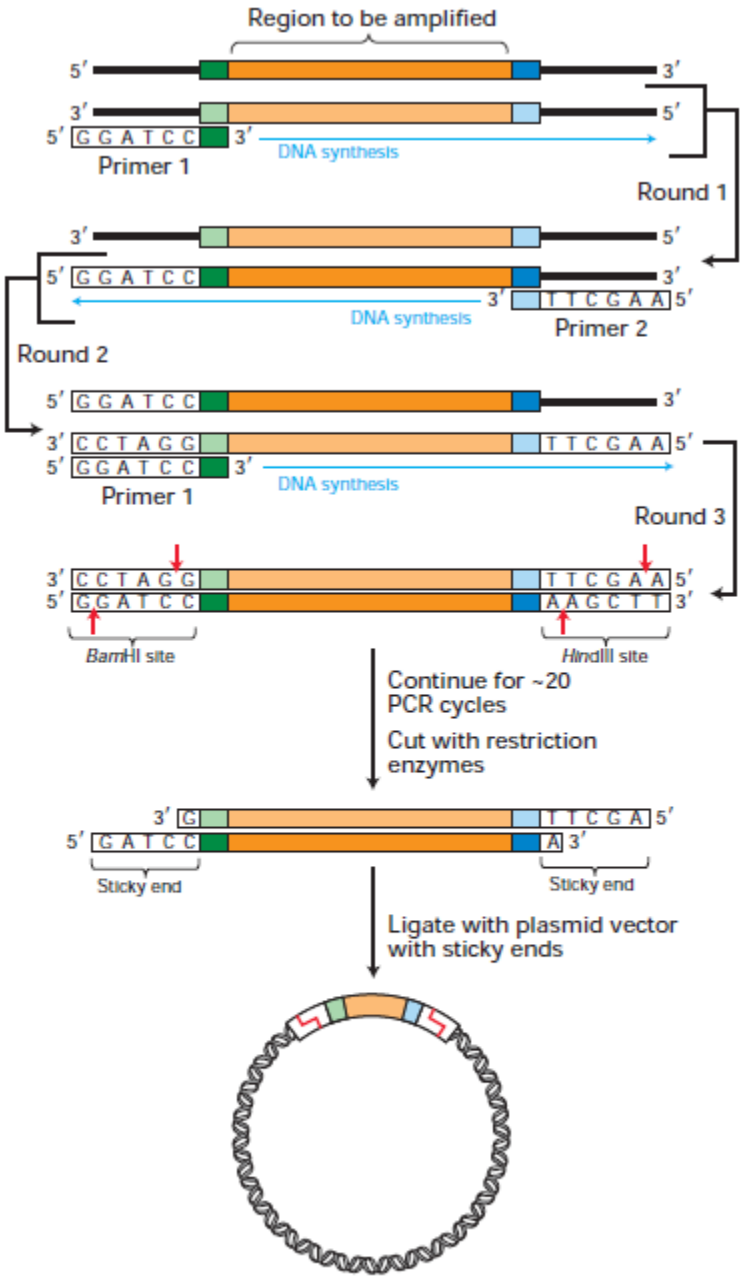
PCR amplification of multiple genes is being used to establish biological parentage in disputed paternity and immigration cases.

Analyses of blood stains and semen samples by PCR have implicated guilt or innocence in numerous assault and criminal cases.

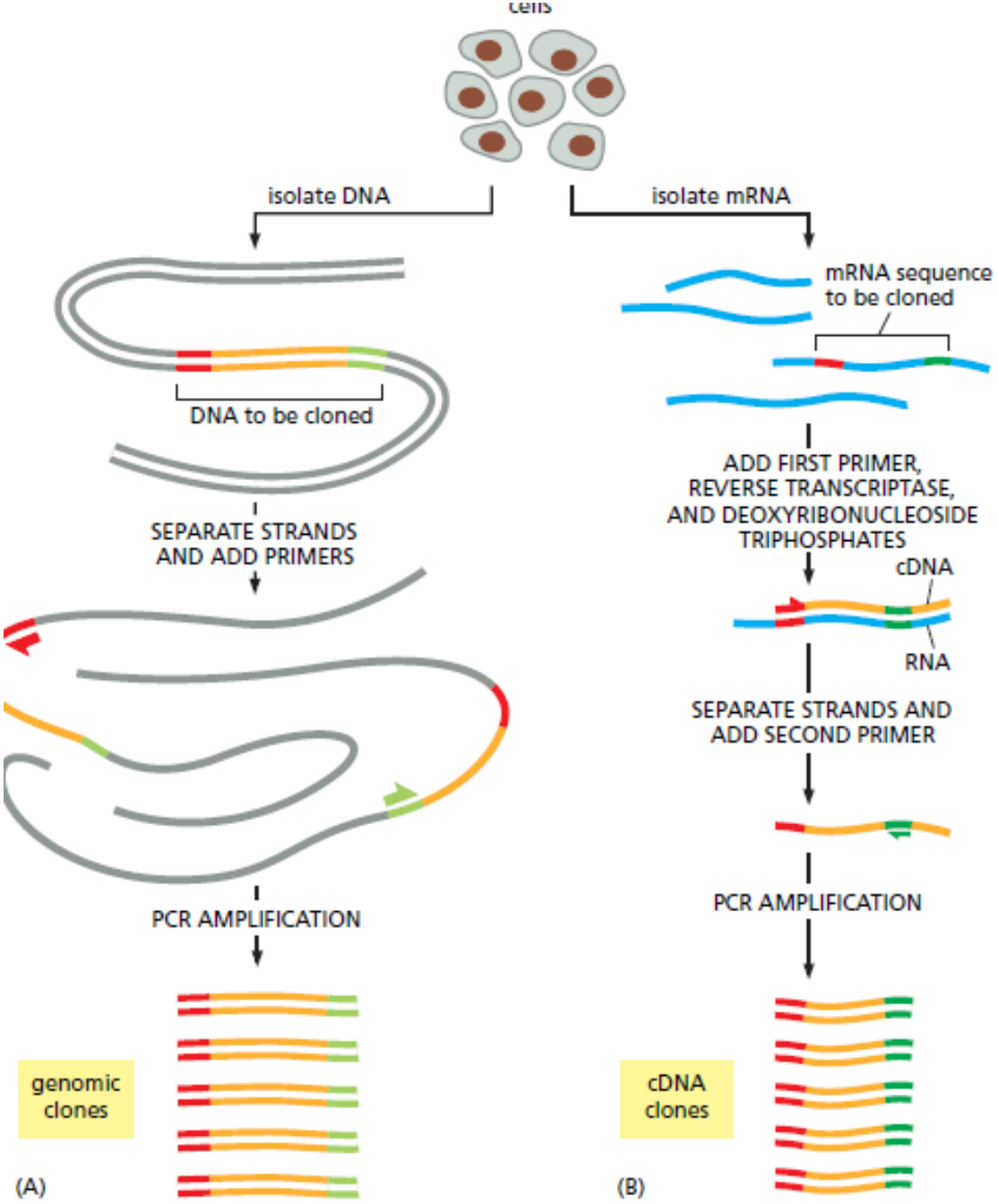
The root of a single shed hair found at a crime scene contains enough DNA for typing by PCR.

DNA is a remarkably stable molecule, particularly when relatively shielded from air, light, and water. Under such circumstances, large fragments of DNA can remain intact for thousands of years or longer.

PCR provides an ideal method for amplifying such ancient DNA molecules so that they can be detected and characterized



▲ EXPERIMENTAL FIGURE 9-25 A specific target region in total genomic DNA can be amplified by PCR for use in cloning. Each primer for PCR is complementary to one end of


cells
isolate DNA
isolate mRNA
DNA to be cloned
mRNA sequence to be cloned
SEPARATE STRANDS AND ADD PRIMERS
ADD FIRST PRIMER, REVERSE TRANSCRIPTASE, AND DEOXYRIBONUCLEOSIDE TRIPHOSPHATES
cDNA
RNA
SEPARATE STRANDS AND ADD SECOND PRIMER
PCR AMPLIFICATION
PCR AMPLIFICATION
genomic clones
cDNA clones
(A)
(B)

Detecting human disease alleles: molecular genetic diagnostics

- A contributing factor in more than 500 human genetic diseases is a recessive mutant allele of a single gene.
- It is also necessary to be able to detect homozygous progeny early, ideally in the fetal stage, so that doctors can apply therapies early.
- In the future, there may even be the possibility of gene therapy. Dominant disorders also can require genetic diagnosis.
- For example, people at risk for the late-onset Huntington disease need to know whether they carry the disease allele before they have children.
- Widely used tests are able to detect homozygous defective alleles in fetal cells.

- The fetal cells can be taken from the amniotic fluid, separated from other components, and cultured to allow the analysis of chromosomes, proteins, enzymatic reactions, and other biochemical properties.

- This process, **amniocentesis** , can identify a number of known disorders;

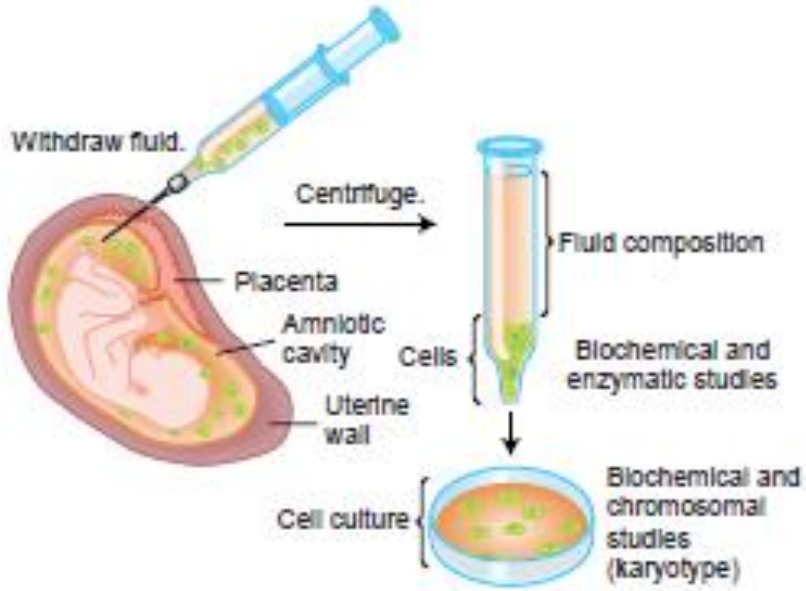
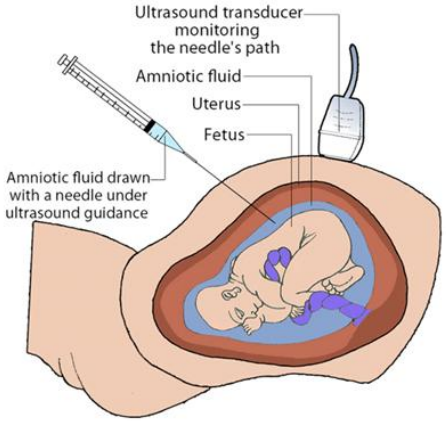
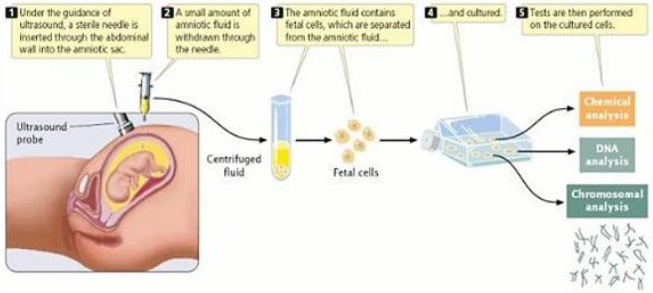


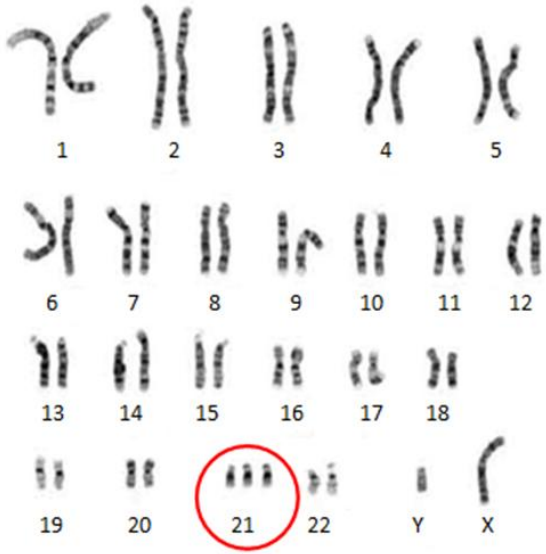
Figure 11-23 Amniocentesis

Table 11-1 Some Common Genetic Diseases

Inborn errors of metabolism	Approximate incidence among live births
1. Cystic fibrosis (defective chloride channel protein)	1/1600 Caucasians
2. Duchenne muscular dystrophy (defective muscle protein, dystrophin)	1/3000 boys (X linked)
3. Gaucher disease (defective glucocerebrosidase)	1/2500 Ashkenazi Jews; 1/75,000 others
4. Tay-Sachs disease (defective hexosaminidase A)	1/3500 Ashkenazi Jews; 1/35,000 others
5. Essential pentosuria (a benign condition)	1/2000 Ashkenazi Jews; 1/50,000 others
6. Classic hemophilia (defective clotting factor VIII)	1/10,000 boys (X linked)
7. Phenylketonuria (defective phenylalanine hydroxylase)	1/5000 Celtic Irish; 1/15,000 others
8. Cystinuria (defective membrane transporter of cystine)	1/15,000
9. Metachromatic leukodystrophy (defective arylsulfatase A)	1/40,000
10. Galactosemia (defective galactose 1-phosphate uridyl transferase)	1/40,000
11. Sickle-cell anemia (defective β-globin chain)	1/400 U.S. blacks. In some West African populations, the frequency of heterozygotes is 40 percent.
12. Thalassemia (reduced or absent globin chain)	1/400 among some Mediterranean populations


Ultrasound transducer monitoring the needle's path
Amniotic fluid
Uterus
Fetus
Amniotic fluid drawn with a needle under ultrasound guidance


1 Under the guidance of ultrasound, a sterile needle is inserted through the abdominal wall into the amniotic sac.
2 A small amount of amniotic fluid is withdrawn through the needle.
3 The amniotic fluid contains fetal cells, which are separated from the amniotic fluid...
4 ...and cultured.
5 Tests are then performed on the cultured cells.
Ultrasound probe
Centrifuged fluid
Fetal cells
Chemical analysis
DNA analysis
Chromosomal analysis


1 2 3 4 5
6 7 8 9 10 11 12
13 14 15 16 17 18
19 20 21 22 Y X