13.2: Biotechnology

Basic techniques to manipulate genetic material (DNA and RNA)

To evaluate genetic disorders a variety of biochemical techniques can be used. The type, kind, and size of the projected genetic variation will determine what approach is taken.

Following DNA extraction there are a variety of techniques that can be employed. The lowest resolution technique for evaluating the genome is the karyotype followed by high-resolution banding. From here, smaller genomic changes can be observed using comparative genome hybridization, fluorescence in situ hybridization (FISH) analysis, or microarrays. Finally, specific nucleotide changes can be examined by whole genome sequencing.

DNA and RNA extraction

To study or manipulate nucleic acids, one must first isolate or extract the DNA or RNA from the cells. Most nucleic acid extraction techniques involve steps to break open the cell and use enzymatic reactions to destroy all macromolecules that are not desired. Enzymes such as proteases that break down proteins inactivate macromolecules, and ribonucleases (RNAses) that break down RNA are inhibited to ensure sample stability. Using alcohol precipitates the DNA. Human genomic DNA is usually visible as a gelatinous, white mass. One can store the DNA samples frozen at −80°C for several years (figure 13.8).
Scientists perform RNA analysis to study gene expression patterns in cells. RNA is naturally very unstable because RNAses are commonly present in nature and very difficult to inactivate. Similar to DNA, RNA extraction involves using various buffers and enzymes to inactivate macromolecules and preserve the RNA.

### Karotype and high-resolution banding

Karyotyping can be used to look at general chromosome morphology and chromosome number. To do this, cells are harvested and arrested in metaphase allowing for the chromosomes to be fixed, spread on slides, and stained by one of several techniques. Giemsa banding (G banding) is the gold standard for the detection and characterization of structural and numerical genomic abnormalities in clinical diagnostic settings for both constitutional (postnatal or prenatal) and acquired (cancer) disorders.

The pattern of light and dark bands on each chromosome is numbered on each arm from the centromere to the telomere, and comparison of a patient sample to a standard map can be used to precisely identify changes in chromosome structure. Microdeletion syndromes can be detected with this technique (figure 13.9).

![Male karyotype with G-banding patterns](https://med.libretexts.org/Bookshelves/Basic_Science/Cell_Biology_Genetics_and_Biochemistry_for_Pre-Clinical_Students/13/13.09.png)

**Figure 13.9:** Male karyotype with G-banding patterns.

### Fluorescence in situ hybridization (FISH)

FISH is a targeted approach using a sequence-specific probe to detect the presence or absence of a particular DNA
sequence or for evaluating the number or organization of a chromosome or chromosomal region in situ.

This technique has several advantages and can be used to identify a variety of different chromosomal changes:

1. DNA probes specific for chromosomal regions, or genes, can be labeled with different fluorochromes and used to identify particular chromosomal rearrangements.
2. Repetitive DNA probes allow detection of satellite DNA or other repeated DNA elements localized to specific chromosomal regions.

Microarrays

Although FISH can detect chromosome changes, microarrays can simultaneously query the whole genome to detect relative copy number variations, gains, or losses by hybridizing a control genome to one of a patient. In looking at the results, an excess of sequences from one genome would represent an overrepresentation in a gene locus within an individual (duplication). This technique can also be used to look at single nucleotide polymorphisms to determine allele frequency.

DNA sequencing techniques

Sanger sequencing is commonly referred to as the dideoxy chain termination method. The method is based on the use of chain terminators, the dideoxynucleotides (ddNTPs). The ddNTPSs differ from the deoxynucleotides by the lack of a free 3’ OH group on the five-carbon sugar. If a ddNTP is added to a growing DNA strand, the chain cannot be extended any further because the free 3’ OH group needed to add another nucleotide is not available. By using a predetermined ratio of deoxyribonucleotides to dideoxynucleotides, it is possible to generate DNA fragments of different sizes.

The DNA sample to be sequenced is denatured (separated into two strands by heating it to high temperatures). The DNA is divided into four tubes in which a primer, DNA polymerase, and all four nucleoside triphosphates (A, T, G, and C) are added. In addition, limited quantities of one of the four dideoxynucleoside triphosphates (ddCTP, ddATP, ddGTP, and ddTTP) are added to each tube respectively. The tubes are labeled as A, T, G, and C according to the ddNTP added. For detection purposes, each of the four dideoxynucleotides carries a different fluorescent label. Chain elongation continues until a fluorescent dideoxy nucleotide is incorporated, after which no further elongation takes place. After the reaction is over, electrophoresis is performed. Even a difference in length of a single base can be detected (figure 13.10).

![Dye-labeled dideoxynucleotides are used to generate DNA fragments of different lengths](https://med.libretexts.org/Bookshelves/Basic_Science/Cell_Biology_Genetics_and_Biochemistry_for_Pre-Clinical_Students/13%20DNA%20Sequencing/Figure_13_10.png)
Nucleic acid fragment amplification by polymerase chain reaction (PCR)

DNA analysis often requires focusing on one or more specific genome regions. Polymerase chain reaction (PCR) is a technique that scientists use to amplify specific DNA regions for further analysis (figure 13.11). Researchers use PCR for many purposes in laboratories, such as cloning gene fragments to analyze genetic diseases, identifying contaminant foreign DNA in a sample, and amplifying DNA for sequencing. More practical applications include determining paternity and detecting genetic diseases.

Scientists use polymerase chain reaction, or PCR, to amplify a specific DNA sequence. Primers are short pieces of DNA complementary to each end of the target sequence combined with genomic DNA, Taq polymerase, and deoxynucleotides.

Reverse transcriptase PCR (RT-PCR) is similar to PCR, but cDNA is made from an RNA template before PCR begins. DNA fragments can also be amplified from an RNA template in a process called reverse transcriptase PCR (RT-PCR). The first step is to recreate the original DNA template strand (called cDNA) by applying DNA nucleotides to the mRNA. This process is called reverse transcription. This requires the presence of an enzyme called reverse transcriptase. After the cDNA is made, regular PCR can be used to amplify it.

Gel electrophoresis

Gel electrophoresis is a technique used to separate DNA fragments of different sizes. Usually the gel is made of a
chemical called agarose or polyacrylamide depending on the sample being used. The DNA has a net negative charge and moves from the negative electrode toward the positive electrode. The electric current is applied for sufficient time to let the DNA separate according to size; the smallest fragments will be farthest from the well (where the DNA was loaded), and the heavier molecular weight fragments will be closest to the well. Once the DNA is separated, the gel is stained with a DNA-specific dye for viewing it.

Hybridization, southern blotting, and northern blotting

Different types of electrophoresis can be used to look at various changes at the level of the DNA (genome), RNA (transcriptome), or protein (proteome). In all cases, a sample (DNA, RNA, protein) is run on a gel (electrophoresis) and is then examined using a probe specific to the sample.

![Image](https://onlinelibrary.wiley.com/cms/92864.png)

Figure 13.12: Schematic of southern blotting technique.

Southern blots are designed to examine changes in DNA. DNA, typically genomic DNA, is probed with a DNA probe complementary to the region of interest in the genome (figure 13.12).

Northern blots are designed to examine changes in RNA. RNA is probed with a DNA probe complementary to the transcript of interest. This will detect changes in gene expression.

Western blots are designed to examine changes in protein size and amount. Cell lysates or protein isolates are probed with an antibody specific to the protein of interest. This will detect changes in protein expression.

References and resources

Text

Clark, M. A. Biology, 2nd ed. Houston, TX: OpenStax College, Rice University, 2018, Chapter 10: Cell Reproduction, Chapter 11: Meiosis and Sexual Reproduction, Chapter 13: Modern Understandings of Inheritance, Chapter 17: Biotechnology and Genomics.


Figures

Grey, Kindred, Figure 13.8 Basic process for DNA extraction. 2021. CC BY 4.0. Adapted from Biology 2e. Figure 17.3 This diagram shows the basic method of DNA extraction. CC BY 4.0. From OpenStax. Added Test Tube by Victoria Codes from the Noun Project.

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Grey, Kindred, Figure 13.12 Schematic of Southern Blotting technique. 2021. CC BY 4.0. Adapted from Biology 2e. Figure 17.6 Scientists use Southern blotting to find a particular sequence in a DNA sample. CC BY 4.0. From OpenStax.

Lieberman M, Peet A. Figure 13.11 Overview of polymerase chain reaction. Adapted under Fair Use from Marks' Basic Medical Biochemistry. 5th Ed. pp 329. Figure 17.10 Polymerase chain reaction (PCR). 2017.

National Cancer Institute. Figure 13.9 Male karyotype with G-banding patterns. Karyotype (normal). Public domain. From Wikimedia Commons.