Abstract

DNA extraction and polymerase chain reaction (PCR) are the basic techniques employed in the molecular laboratory. This short overview covers various physical and chemical methods used for DNA extraction so as to obtain a good-quality DNA in sufficient quantity. DNA can be amplified with the help of PCR. The basic principle and different variants of PCR are discussed.

**Keywords:** DNA extraction, Polymerase chain reaction, real time PCR

**DNA Extraction**

DNA extraction is a method to purify DNA by using physical and/or chemical methods from a sample separating DNA from cell membranes, proteins, and other cellular components. Friedrich Miescher in 1869 did DNA isolation for the first time.

The use of DNA isolation technique should lead to efficient extraction with good quantity and quality of DNA, which is pure and is devoid of contaminants, such as RNA and proteins. Manual methods as well as commercially available kits are used for DNA extraction. Various tissues including blood, body fluids, direct Fine needle aspiration cytology (FNAC) aspirate, formalin-fixed paraffin-embedded tissues, frozen tissue section, etc., can be used for DNA extraction.

DNA extraction involves lysing the cells and solubilizing DNA, which is followed by chemical or enzymatic methods to remove macromolecules, lipids, RNA, or proteins.

DNA extraction techniques include organic extraction (phenol–chloroform method), nonorganic method (salting out and proteinase K treatment), and adsorption method (silica–gel membrane).

**Organic extraction**

This method is labor intensive and time consuming.

Cell lysis can be done using nonionic detergent (sodium dodecyl sulfate), Tris–Cl, and Ethylene diamine tetraacetic acid (EDTA), and this step is followed by removal of cell debris by centrifugation. Protease treatment is then used to denature proteins. Organic solvents such as chloroform, phenol, or a mixture of phenol and chloroform (phenol/chloroform/ isomyl alcohol ratio is 25:24:1) are used for denaturation and precipitation of proteins from nucleic acid solution, and denatured proteins are removed by centrifugation and wash steps. RNAse treatment is done for the removal of unwanted RNA. Precipitation with ice-cold ethanol is performed for concentrating DNA. Nucleic acid precipitate is formed, when there is moderate concentration of monovalent cations (salt). This precipitate can be recovered by centrifugation and is redissolved in TE buffer or double-distilled water.

Other methods include silica-based technology (DNA absorbs to silica beads/particles at a specific pH in presence of specific salts), magnetic separation (DNA binds reversibly to magnetic beads, which are coated with DNA-binding antibody), anion exchange technology, salting out, and cesium chloride density gradients.

Assessing the quality and yield of DNA: The quality and yield of DNA are assessed by spectrophotometry or by gel electrophoresis. Spectrophotometry involves estimation of the DNA concentration by measuring the amount of light absorbed by the sample at specific wavelengths. Absorption peak for nucleic acids is at ~260 nm. The $A_{260}/A_{280}$ ratio is ~1.8 for dsDNA. A ration of less than 1.7 indicates protein contamination.
Polymerase Chain Reaction

Polymerase chain reaction (PCR) is a robust technique to selectively amplify a specific segment of DNA in vitro.\(^1\) PCR is performed on thermocycler and it involves three main steps: (1) denaturation of dsDNA template at 92–95°C, (2) annealing of primers at 50–70°C, and (3) extension of dsDNA molecules at approx. 72°C. These steps are repeated for 30–40 cycles.

Various chemical components of PCR include MgCl\(_2\), buffer (pH: 8.3–8.8), Deoxynucleoside triphosphates (dNTPs), PCR primers, target DNA, and thermostable DNA polymerase.\(^2\)

Target sequence is the sequence within the DNA template, which will be amplified by PCR.\(^2\)

PCR primers are single-stranded DNA (usually 18–25 nucleotides long), which match the sequences at the ends of or within the target DNA, and these are required to start DNA synthesis in PCR.\(^2\)

Various types of PCR

- Conventional (qualitative) PCR
- Multiplex PCR
- Nested PCR
- Reverse transcriptase PCR and Quantitative Real-time PCR
- Quantitative PCR
- Hot-start PCR
- Touchdown PCR
- Assembly PCR
- Colony PCR
- Methylation-specific PCR
- LAMP assay.

Multiplex PCR: It is used to amplify multiple targets in a single PCR permitting their simultaneous analysis.

Nested PCR: It is a modified PCR intended to decrease nonspecific binding of products because of amplification of unexpected primer-binding sites. It involves two PCR steps. In the first PCR reaction, one pair of primers is used to produce DNA products, which act as a target for the second PCR reaction. It helps to increase the specificity of DNA amplification.\(^[3,4]\)

Reverse transcriptase PCR: RT-PCR involved mRNA as the starting material and it uses reverse transcriptase to convert mRNA into the complementary DNA (cDNA). This cDNA is then amplified with the help of regular PCR.

Quantitative PCR: It is used to quantitate the amount of target DNA (or RNA) in a particular sample.

Hot-start PCR: The main advantage of hot-start PCR is to decrease nonspecific amplification of DNA at lower temperature steps of PCR. Reaction components are manually heated before adding Taq polymerase to the DNA-melting temperature (i.e. 95°C).\(^4\)

Touchdown PCR: Annealing temperature during the first two cycles of amplification is set at approximately 3–10°C above estimated \(T_m\) and the temperature is slowly decreased in the subsequent cycles. Higher annealing temperature in two initial cycles leads to more specificity for primer binding, and the lower temperatures allow more efficient amplification later on.\(^4\)

Assembly PCR: Assembly PCR helps in synthesis of long DNA segments by doing PCR on a pool of long oligonucleotides having short overlapping segments and in turn assembling more DNA segments into one segment.

Methylation specific PCR: This PCR involves sodium bisulfite treatment and is used to identify patterns of DNA methylation at cytosine guanine islands in genomic DNA.

LAMP assay (loop-mediated isothermal amplification): It is another modification of PCR, which uses 3:6 primers sets, one of which is a loop-like primer. This technique utilizes Bst-polymerase.

Real-time PCR: It allows quantitative estimation of PCR product, as the amplification progresses. It uses nonspecific dye such as SYBR\(^\text{®}\) green I or fluorescence resonance energy transfer.

PCR products are then sequenced to determine the order of bases in the DNA segment.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References

1. Lo AC, Feldman SR. Polymerase chain reaction: Basic concepts and clinical applications in dermatology. J Am Acad Dermatol 1994;30:250-60.
2. Clark DP, Pazdernik NJ. Molecular Biology, Polymerase Chain Reaction. 2\(^{nd}\) ed. United States of America (USA): Elsevier BV; 2013. Chap. 6, p. 163-93.
3. Niemz A, Ferguson TS, Boyle DS. Point-of-care nucleic acid testing for infectious diseases. Trends Biotech 2011;29:240-50.
4. Lorenz TC. Polymerase chain reaction: Basic protocol plus troubleshooting and optimizing strategies. J Vis Exp 2012;63:e3998.