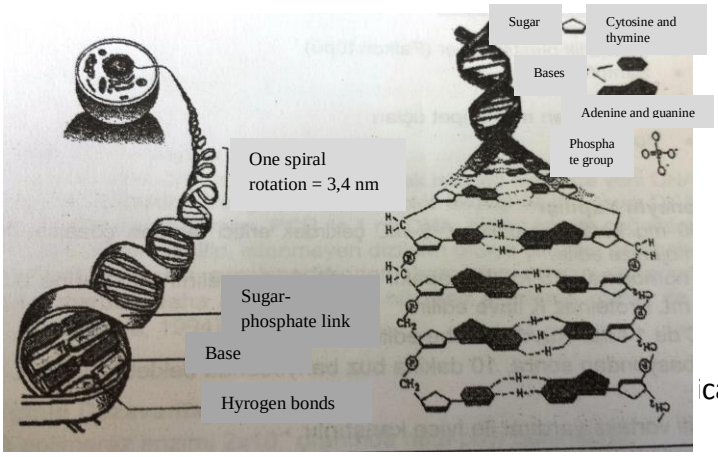
EXPERIMENT : 2

THE ISOLATION OF DNA MOLECULE FROM BIOLOGICAL MATERIAL AND ANALYSES

A) General information:

The characteristics of all living organisms are carried to the offspring by genetic material called genes. Genes are localized in the chromosome and consisted of nucleotide sequences. The building blocks of nucleic acids, nucleotides are composed of a nitrogenous base, a five-carbon sugar (ribose or deoxyribose), and at least one phosphate group. Nucleotides together form nucleic acids which are called DNA and RNA. DNA is the molecule that is responsible for the control of biological (such as genetic, physiological and biochemical) functions in the cell and it carries the genetic information to the offspring.



The sources fo

Blood, tissue (ical samples, embryo, postmortem tis

Purposes of DNA analyses

1. Determination of mutations and polymorphisms in genes encoding enzymes for the investigation of individual susceptibility to diseases including cancer.

2. Determination of mechanisms of genetic diseases such as autoimmune disorders, hypertension or coronary artery diseases.

3. Determination of genes responsible for the interindividual differences in alcohol and xenobiotic metabolism.

4. Diagnosis of genetic disorders.

5. Diagnosis of genetic disorders before birth.

6. Genetic consulting.

7. Forensic medicine

8. Microbiology studies

B) Materials, devices and solutions used in the assay

All materials, devices and solutions used in the assay should be sterilized.

Solutions:

Nuclei lysis buffer solution (pH: 8.2): (Stored in room temperature)

(10 mM Tris-HCl, 0.4 M NaCl, 2 mM Na2EDTA, distilled water up to 100 ml)

SDS (Sodyum dodecyl sulphate) solution (pH: 7.2): (Stored in room temperature)

(10% SDS, distilled water up to 10 ml)

NaCl solution: (Stored in room temperature)

(6 M NaCl, distilled water up to 50 ml)

Proteinase K: (Stored in -20^{0}C)

İsopropanol: (Stored in room temperature)

Materials and devices

Micropipette, 15ml plastic tubes, centrifuge, incubator, pipette tips (blue and yellow).

C) Procedure

1. 100mg liver tissue homogenized with 5ml nuclei lysis solution in ice bath.

2. Transfer the homogenate to 15ml tube; add 0.5ml SDS and 0.2ml Proteinase K

3. Incubate 1hour at 55^{0}C.

4. Wait 10 minutes in ice bath after incubation. Add 2ml 6M NaCl solution.

5. Vortex vigorously

6. Centrifuge at high speed for 10 min

7. Transfer the supernatant to a clean plastic tube without disturbing the pellet.

8. Add equal volume of isopropanol to supernatant. Gently mix the tube up and down to make DNA visible.

PCR Technique

It is one of the mostly preferred techniques in molecular biology. PCR (Polymerase chain reaction) technique is designed by Kerry Mullis in 1987. PCR is the *in vitro* amplification of a specific DNA part by primers. This technique is preferred to have the specific DNA part generously, making its molecular analyses and to be used in gene transfer to make recombinant organisms.

Mechanism: Each PCR cycle have 3 stages. These are:

1) The separation of the DNA strands (Denaturation)

2) The adhesion of primers (Primer annealing)

3) Synthesis of DNA molecule (Synthesis)

In PCR, the annealing of two oligonucletide primers to the target DNA series and the synthesis of the new DNA molecule is the main principle. In high temperatures (92-96^{0}C), the template DNA strands are denaturized and after that, primers go and anneal to the appropriate DNA parts by hybridization. Hybridization process is occurred between 37^{0}C and 65^{0}C. Then, with the help of appropriate buffer and dNTPs (deoxynucleotide triphosphate), DNA polymerase enzyme helps the primer to lengthen so that, the synthesis of a new DNA molecule comes off. DNA isolation method must be done before the PCR method as a pre-operation.

The components of PCR method:

1) DNA molecule

2) DNA polymerase enzyme

3) Primers

4) dNTP mixture

5) Reaction buffer

The Advantages of PCR:

1) It is possible to get new DNA molecule generously from a small amount of genomic DNA.

2) It is a fast and quick method. For example, in Southern blot technique, with 5-10µg DNA it is possible to make the DNA analysis in 6-7 days but with PCR method, it is possible to make the analysis with 1ng DNA in only 2-3 hours.

3) It is possible for the researcher to work with only the specific part of DNA, not with the whole DNA molecule.

4) It is not needed to purify the DNA before PCR method. No extra time is wasted.

The Disadvantages of PCR:

1) There may be a margin of error in results from the *Taq* DNA polymerase enzyme (approximately 2x10^4)

2) A specific probe and primers are needed for both of the two ends of the DNA that will be amplified.

3) Undesirable nucleotides may join to the amplified DNA serie.

4) Following the incorrect connection of the primers or occurrence of the primer-dimers, non-specific products may consist.

5) There is a risk of contamination.

6) Because of its expensiveness, PCR method cannot be used in routine analyses.

Application Area of PCR Method

PCR method can be preferred in molecular and genetic fields. The method is used in the development of genetic researches, molecular analyses, rapid analyses of DNA sequencing, development of human genome analyses and in gene-activity studies.

Important application areas of PCR method can be listed as:

- The determination of gene deletions

- The determination of specific point mutations

- Constituting of the gene maps of the specific DNA sequences and enzymes

- The determination of the genotype distribution of enzymes giving rise to genetic polymorphisms in the population and researching the sensitivity of individuals to the cancer and the other diseases.

- Population epidemiology

- The clinical diagnosis of the diseases

- The study of the genetic factors giving rise to diseases

- To bring out the family tree

- The genetic consultancy

- Cases in forensic medicine

- Microbiological studies

- Basic biological studies