

Practical

(Centrifuge machine)

Applications and principle:-

"A centrifuge is a piece of equipment that puts an object in rotation around a fixed axis, applying a potentially strong force perpendicular to the axis of spin."

Application:-

- * Laboratory separation
- * Isotope separation
- * Aeronautics and astronautics.
- * Synthesis of material
- * Commercial uses.
- * Separation
- * Soil mechanics.

Practical

(Gel documentation apparatus)

Application and principle:-

Principle:-

"Gel documentation apparatus is used for the imaging and documentation of nucleic acid and protein suspended in polyacrylamide / agarose gel."

* Stained with ethidium bromide.

Applications:-

- * includes UV light and camera for image capture.
- * Gel and blot imaging
- * Colony counting
- * Immunoassay
- * Multiplex protein detection.
- * two-D electrophoresis.

Practical

(Micropipette)

Application and principle

Principle :-

"Micropipettes are tools used to handle volumes of liquid in the microliter scale pipettes."

Applications :-

- * used in biology, biochemistry, less commonly used in chemistry for taking few ml. of sample used in labs.
- * Widely used in molecular genetics, genetics labs.
- * Used for mixing, spreading and holding chemicals in labs.

Practical

(Electrophoresis apparatus)

Application and principle:-

Principle:-

"Gel electrophoresis is a method for the separation and analysis of macromolecules (DNA, RNA and proteins) and their fragments, based on their size and charge. It is used in clinical chemistry to separate proteins by charge or size." (Agarose)

Application:-

- * Analysis of PCR products.
- * Separation of restricted genomic DNA
- * Used in forensic molecular biology.
- * Genetic labs
- * Microbiology labs
- * Biochemistry labs.
- * Estimation of size of DNA molecules.

Practical

(Polymerase chain reaction machine)

Application and principle.

Principle:-

"Used in molecular biology to amplify a single copy to or a few copies of a segment of DNA's across several order of magnitude, generating thousands to millions of copies of a particular DNA sequence."

Applications:-

- * ~~Selective~~ DNA isolation
- * Amplification and quantification of DNA
- * Genetic testing
- * Infectious disease application.
- * DNA fingerprinting
- * DNA sequencing

Practical :-

Equipments :-

(Water bath)

Principle :-

Laboratory equipment made from a container filled with heated water. It is used to incubate sample in water at a constant temperature for a long period of time.

Applications :-

- + Sample thawing
- + Bacteriological Dept examinations
- + Waste water laboratory
- + Clinical laboratory
- + Cell cultivation
- + Food and cosmetic technology laboratory

Practical:-

DNA confirmation by Gel electrophoresis method.

For the confirmation of DNA in the sample gel electrophoresis method is used.

Chemicals and apparatus:-

Electrophoresis tub, power supply, Tray, combs, Agarose, TAE buffer, distilled water, Flask, microwave, measuring stand.

Procedure:-

- * We measured the volume of the gel and tub, which was 1400 ml.
- * Took 14 ml TAE Buffer and raised the volume upto 1400 ml with distilled water.
- * Took 200 ml of solution and added 2g of agarose for 1% gel.
- * For the dissolving the agarose, we took the flask in microwave and gently heated it for dissolving the agarose. (Difference in weight was filled with D.W).
- * Cooled the solution up to certain temperature. Gently poured the solution in Tray.
- * After some time, the gel solidified.

- * Took the gel in tub, set and loaded the samples in the wells.
- * For loading the sample mixed it with loading dye. (which increased the density and helped in view).
- * Attached the power supply, set the voltage and time.
- * After 2 hrs. (the sample travelled suitable length), pulled the gel off from the tub.
- * One pulled the gel in documentation apparatus and viewed in U-V light.
- * Bands appeared and confirmed the DNA.

Practical:-

(PCR apparatus)

Polymersase Chain reaction for the amplification of the DNA was held in PCR apparatus

Ingredients required:-

- * Primers
- * DDW
- * $MgCl_2$
- * Buffer
- * dNTPs
- * Enzyme (Taq polymerase)
- * Sample (which have conformed DNA).

Procedure:-

- * We programmed the PCR apparatus and setted 4 cycles at different temperatures
- * Total 36 cycles was programmed.
- * In first cycle denaturation of H-bonding held.
- * In annealing - enzyme attached to the stand.
- * In elongation - dNTPs attached and the copies formed.
- * Proof reading to point out the mistakes.
- * After 3 hrs the reaction was completed and putted the PCR tubes off

- * The sample was loaded in gel and runned it off on the gel.
- * After the completion of duration, in the documentation apparatus different bands was shown.



12/4/17

Practical

DNA extraction by Inorganic salt-based extraction.

Material:-

Fish, Blade, gloves, mask, eppendorf, water bath, centrifuge machine, micropipette, tips, lysis Buffer, proteinase K, NaCl, Ethanol, distilled H₂O

Procedure:-

- * Took the sample of fish meat portion ^(3g) from the upper part of the dorsal line. (here the fat portion is lower).
- * Homogenized the sample, so, that the chemical action took place effectively.
- * Added 600 μ l lysis buffer and 7 μ l proteinase K.
- * Kept the sample in water bath for over night (12 hrs) at 50°C. (so, that the enzyme and chemicals worked effectively).
- * Took the supernant in separate eppendorf and discarded the left over. Before separation of supernant, for better lysis added 600 μ l NaCl and centrifuged the sample at 12000 rpm for 10 mins.

In separated supernant added 700 μ l ethanol.

* For the precipitation of the DNA, the sample (supernatant + ethanol) was took to freezer for 2 hrs at -20°C temperature.

* 600 μl ethanol added and centrifuged again at 12000rpm for 10 mins.

* The white colored pellet was appeared at the bottom.

* Discarded the supernatant and the washed the pellet with distilled water.

* On the side of ependorf gently dropped the water and washed it.

* Dissolved the pellet in distilled water and stored at 4°C in refrigerator.