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.

Application:

* Sample thawing
* Bacteriological examination
* waste water laboratory
* Clinical laboratory
* Cell cultivation
* Food and cosmetic technology laboratory.

(water bath)

PRACTICAL

(CENTRIFUGE MACHINE)

Principle:

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

Application:

* Laboratory separation.
* Isotope separation
* Aeronautics and aesteronautics.
* Synthesis of material.
* Commercial uses.
* Separator
* Soil machines.

(centrifuge machine)

Practical:

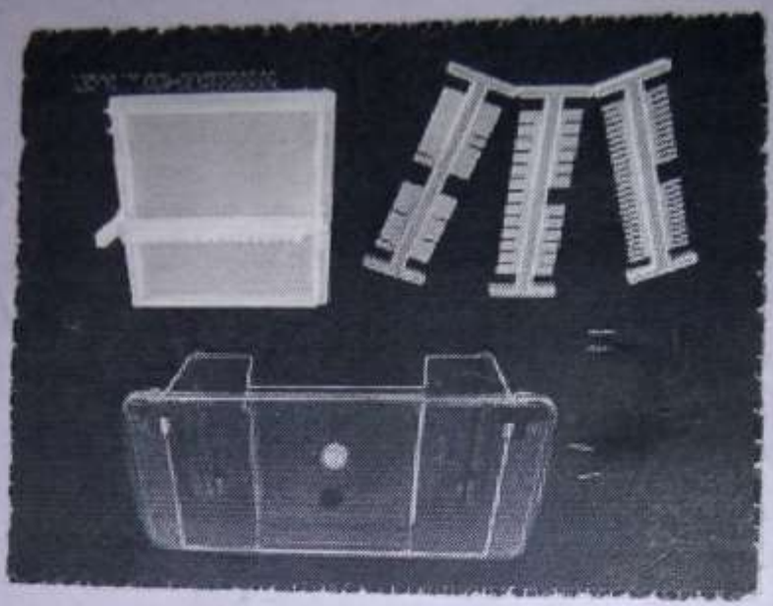
(ELECTROPHORESIS APPARATUS)

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.



Electrophoresis apparatus

Gel

Practical

(MICROPIPETTE)

Principle:

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

Application:

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

Micropipette & Tips

PRACTICAL

(Gel documentation apparatus)

Principle.

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

* Stained with ethidiumbromide.

Application:

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

(Gel documentation apparatus)

PRACTICAL #

(POLYMERASE CHAIN REACTION MACHINE)

Principle:

"It is used in molecular biology to amplify a single copy 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"

Application:

* Seled DNA isolation.
* Amplification and quantification of DNA.
* Genetic testing.
* Infectious disease application.
* DNA fingerprinting.
* DNA sequencing.

(PCR- machine)

PRACTICAL

DNA extraction from fish flesh by inorganic salt - based extraction.

Material:

Fish, blade, gloves, mask, eppendrof, H_2O bath, centrifuge machine, micropippelle, tips, chemicals.

CHEMICALS.

* Lysis buffer
* Proteinase - K
* NaCl (solution)
* Ethanol.

PROCEDURE:

* I took fish meat protein and crush it.
* Added proteinase - K and lysis buffer.
* for 12 hours at 50°C the sample was kept.
* After that NaCl solution is added and centrifuged for 10 mins at 12000rpm.
* The supernant had removed and ethanol added.
* For precipitation of DNA the sample was look to freeze for 2 hours.
* Again ethanol added and centrifuges
* White pellet was shown at the bottom after washing.

* The pellet was dissolved in distilled water and stored in refrigerator.

DNA Confirmation:

* The conformation of DNA in the sample was done by Gel electrophoresis method.
* First TAE buffer and agarose was requireed.
* we formed gel and run the sample in gel electrophoresis apparatus.
* Formation of band was shown.

So, the DNA was confirmed.

PRACTICAL #

(POLYMERASE CHAIN REACTION)

Ingredients required:

* primess.
* DDW (Double distilled water).
* $MgCl_2$
* $MgCl_2$ buffer.
* Enzyme (Polymerase)
* dNTPs

Reaction held in PCR:

* Polymerase chain reaction was used for the amplification of DNA which was consisted on different chains at different time duration.

Basically it consists on three cycles.

-> Denaturation is with denaturating of H-bonding
-> Anneling Enzyme attachment held.
-> Elongation - dNTPs attached.

and in some proof reading is there.

* We had designed primess and took (DNA confirmed) sample.
* We prepared complete reaction (chemicals) and putted them in PCR tubes.

* Runed the sample in PCR.
* After the duration completed the sample was runned on the gel in electrophoresis apparatus along with the ladder.
* The bands was shown in gel documentation apparatus.

PRACTICAL #

(GEL ELECTROPHORESIS)

Chemicals:

Agarose , TAE (Tris acetic acid EDTA) buffer.

Apparatus:-

* Electrophoresis tub
* Power supply
* tray
* Combs
* tray blockers.

Method:

* TAE buffer was took and agarose mixed in it.
* On oven heated it and boiled.
* After the suitable temperature reached the solution poured in the tray and comb putted in the gel.
* The gel hardened and placed in tub
* Power supply attached and the sample putted in valved.
* After the time duration passed the gel placed in the gel documentation apparatus and the bands were shown.

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 water

Procedure:

-> Took the sample of fish meat protein portion 1g from the upper part of the dorsal line.
(here the fat portion is lower.
-> Homogenized the sample. So, that the chemical action took placed efficiently.
-> Added 600ul lysis buffer and 7ul 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 600ul Nacl and centrifuged the sample at 12000 rpm for 10 mins.
-> The ~~supernat~~ separated supernant added 700 ul Ethanol.

-> For the precipitation of the DNA, the sample (supernant + Ethanol) was took to freeze for 2 hrs at -20°c temp.
-> 600 ul ethanol added and centrifuged again at 12000 rpm for 10 mins.
-> The white colored pellet was appeared at the bottom.
-> Discarded the supernant and washed the pellet with distilled water.
-> on the side of eppendorf gently dropped the water and washed it
-> Distilled the pellet in distilled water and stored at 4°c in refrigerator.

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 slender.

PROCEDURE:

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

-> Placed the gel in tub, and loaded the samples in the valves.
-> For loading the sample mixed it with loading dye. (which increased the density and helped in view).
-> Attached the powersupply. Setted the voltage and time.
-> After 2 hrs (the sample travelled suitable length). Putted the gel off from the tub.
-> placed the gel in documentation apparatus and viewed in U.V light.
-> Bands appeared and confirmed the DNA.

PRACTICAL

(PCR APPARATUS)

Polymerase 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 confirmed DNA)

PROCEDURE.

We programmed the PCR apparatus and selted 4 cycles at different temperature
-> Total 36 cycles was programmed.
-> In fust cycle denaturation of H- bonding held.
-> In anealing - 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 on the gel.
-> After completion of duration, in documentation apparatus different bands was shown.