TRANSFORMATION OF BACTERIA WITH PLASMID DNA :-

INTRODUCTION :-

Bacteria transformation is process of horizontal gene transfer by which some bacteria take up foreign genetic material from the environment.

Transformation process allows a bacterium to take up genes from its surrounding environment that is transformation involves the direct up take of fragments of DNA by a recipient cell and the ecquisition of new genetic characteristic There are two major paramters involved in efficiently tranforming a bacterial organism. The first is the method used to induce competence for transformation. The second major parameter is the genetic constitution of the host strain of the orgnism being transformed. Copetent cells are capable of up taking DNA from the environment.

E. Colie cells are stored at -80°C in aliquots of 500 µl. Then one vial on ice. Generally need 100 µl for each transformation. It is the bedt not to refreez.

For each Transformation pipet 100 µl of component cells into a sterile 1.5 ml microfuge tube.

Some of keys application of RFLP are listed below.

1. To determine the status of genetic disease such as cystic Fibrosis in an an individual
2. To determine the status or confirm the source of DNA sample such as in paternity tests or crimind investigation.
3. In genetic mpping to determine recognization that show the genestis distance b/w loci'

DESADVATAGE OF RFLP:-

Since its invention, RFLP has been a widly used genome analysis techniques employed in fossis, medicine, and genetic studies. However, its has become almost obsolete with the advent of relatively simple and less expensive DNA profiling teenique such as the polymerase chain reaction.

7 Centrifuge at 6000 g for 5 min at 4°C
8 Pour off supernatant and resuspend each resuspend in 21.5 ml ice cold 50 mM CaCl2
9 Add 3.5 ml sterile glycerol 10%
10 Store in -80°C Freezer. Use with in 6 month possible.

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How Does It Work?

RFLP is performed using a series of steps briefly outlined below.

DNA Extraction :-

To begin with, DNA is extracted from blood, saliva or other samples and purified.

DNA Fragmets :-

DNA Purified DNA is digested using restriciton endonucleases. The recognition sites of these enzyme are generally 4 to 6 base pairs in Length. The shorter the sequence recognized the greater the number of fragments genrated from digestion.

Gel Electrophoresis :-

The restriction are negatively charged and can be easily seprated by electrophoresis which seprates molecules based on their size and charge. The fragmented DNA samples are placed in the chamber containing the electrophoretic gel and two electrod when an eletric fieldd is applied, the Fragmen migrate towards the positive electrodes. Smaller fragments move faster through the gel Leving the large ones behind thus the DNA samples are seprated.

Application Of RFLP :-

RFLP has been used for seral genetic analysis application since its invention

Detection Of Mutation By Restriction Fragment Lenght Polymorphoism:-

Restriction Fragments length polymorphism is a technique invented in 1984 the english scientist Alec Jeffrayd during reserch into hereditary diseses. It is used for the analysis of unique patterns in DNA Fragments in order to genetically differentiate between orgnisms these patterns are Called Variable Numbers of Tandem Repeats. Genetic polymorphism is defined as the inherital genetic differenes amoxg individuals in over 1% normal population The RFLP techiaue exploits thes diffrences in ove 1 % of normal population. The RFLP Techique explotic these diffrenes in DNA Seqauenes to recognize and study both.

Principle

Restriction endonucleases are enzyme that cut lenghth DNA into short pieces. Each restriction endonucleses targets different nucleotide Sequences in DNA Strand and therefor cuts at diffrent sites. The distane between the clevge sited of a certain restriction endonuclease differ between individuals. Hence the lenght of the DNA fragments produced by restriction endonuclede will differ across individual orgnisms and species

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MATERIAL :-

- Four microtubes
- 1 Microtube rack
- 20 µl micropipette
- waterproof
- Beaker or foam cup with Crushed ice for the following
- 20 µl of 0.4 µg/µl DNA
- µl BamHI restriction enzyme
- µl EcoRI restriction
- 2.5 µl HindIII
- restriction enzyme
- 10 µl distilled
- water
- Gloves
- 5000 ml beaker
- Electrophoresis Chamber

COMMON MATERIAL :-

- Container with TBE Solution
- 37°C water bath
- Floating rack
- 60°C water bath
- Cooler with
- Crushed ice
- Freezer
- Camera

Types Of Polymerase Chain Reaction And Application :-

Types Of Polymerase Chain Reaction- PCR.

Several application of PCR methods have been developed to enhance the utility of this method in diagnostic setting based on their application. Some of the common types of PCR are.

① Real time PCR ② Reverse-Transcription ③ Gradiant PCR ④ Touch down PCR ⑤ Mutiplex PCR ⑥ Nested PCR ⑦ High fidelity PCR ⑧ Fast PCR

Appliction Of PCR :-

① Identification and characterization of infection
② Direct detection of microorganism grown in cultr
③ Identification of microorganism in cultr.
④ Investigation of strain relatendce
⑤ Cloning genes
⑥ PCR Seqvencing.

Touch Down PCR :-

Touch down (TD) PCR offers a simple and rapid meas to optimize PCR, incresing Specificity, Sensivity. and Yield, withalt the need for lengty optimization and the Re designing of primers. TD-PCR employs an intial annealing temperatur above the projected melting (TM) of the primers beiny used. then progressively transition to a lover more permissive annealing (TM) over the course of Successive cycle.

TD PCR CYCLING CONDITION :-

The suggested cycling program had two phase The first phase of touchdown programming used a (TM) That a approximately 10°c above the calculated (TM). The temperatur is reduced by 1°c every successive cycle until the calculated (TM) range is reached. This is done for a total of phase 2 follow generic PCR amplification of up to 20-25 cycle using the final annealing (TM) Reached inthe touchdown phase.

GENRAL TIPS FOR TD-PCR :-

Keeping all reaction cold until thermal cycling starts is Cruicd to avoding non-Specific Priming even with TD-PCR.

A hot start setup is performed. Since the main aim of TD-PCR is to elimineton non-Specific intreraction during the intials cycles. it is important to use a hot-start set-up TD-PCR Can address problem with monoplex reaction better then mutiplex reaction.

method used in the restriction of enzyme analysis of DNA. This Laboratory had been used effectively in both introductory and advanced Undergraduate courses. The use of predigested DNA Fragments helps to ensure the success of the experiment and to reduce Cost. Restriction mapping exercises are designed to provide students with tangible experience in the abstract reasoning required for accurate interpretation of result. These exercise are meant to be carried out during electrophoresis of student samples.

PRINCIPLE :-

Restriction enzyme, also called restriction endonuclease, recognize specific base sequences in dubble-helical DNA and clove at specific places both strands containing the recognized sequence. Many restriction enzyme recognize specific sequence of 4 pairs.

AIM OF THE PRACTICAL :-

① Understand the DNA Restriction enzym. ② Learn tube micropipette. ③ Learn to Separate DNA on agarose gel using electroporesis. ④ Understand how to use a restriction digestion map to identify a sample DNA.

Restriction Digestion Of DNA And Preparation Of Restriction Maps :-

Introduction:-

Restriction enzyme mapping is a powerful tool for the analysis of DNA. This technique relies on restriction endonucleases, hunderds of which are now available, each other one recongnizing and reproducibly cleving a specific base pair sequence in double-stranded DNA thus genrating fregments of varying sizes. Since the rate at which a DNA momoleule moves through an agarode gel during electrophoresis is inversely proportional to its size 1 the length of thede DNA fregments can be ascertained and this information used to determine the position of clerage sites in a DNA molecule. Comparatives analysis of Fregments genrater by clerage with two diffrent restriction enelonucleas enable the moleale bialogeist to determine the relative location of particular recognization sequen in DNA moleale. Restriction mapping has widely publicized application. theste include DNA figepointing the dotectation of genetic defects and the sequencing of human genome. Although students redily appreciate the importance of this new methodology, they often find it difficult to understand the tecniqve and logic involved. The experiments and mapping ~~exe~~ exercises described below introduce students to

QUATIFICATION OF RNA BY NANODROP.

NANDROP :-

The most Common teeniajue to determine DNARNA yield and purity is meansurment of absorbed using Nandrop. A Spectophotometer is able to determine the averge Concentration of the nucleic acids DNA or RNA present in a mixture, as well as theire purity. The absorbed method is a Spectrophotometer equipped with a UV Lamp, and a Solution of purified DNA/RNA Sample. DNA Concentration is extimated by measuring the absorbed at 260 nm. To evalute DNA purity, measure absorbed From 230nm to deatect athere possiable Contamination. The most common purity calculation is the ratio of the absorbed at 260 nm divided by reading at 280 nm. A Good-qualtiy DNA will have an $A_{260/280}$ - is widly Concentrated ~ 1.8 and for RNA is ~ 2.0. A reading of 1.7, 1.6 etc does not render the DNA Unsuitable for any application but Lower ratios indiciate more Contamination are poesent

QUATIFICATION OF DNA BY GEL ELECTROPHORESIS

DNA QUANTFICATION:

DNA yield can be assessed using various method including absorbance, agarose gell electrophoresis

AGAROSE GEL ELECTROPHORESIS

Agrose gel electrophoresis in another way to quickly estimate DNA condentration. To use this meathod. A horeizonatal gel electrophoresis tank with an external power supply, analytical-grade agarose, an appropriate running buffer and an intercalating DNA dye along with approximatily sized DNA standards are recquired A sample of the isolated DNA is loaded into well of agarose gel and then exposed to an electric field. For the quantification and qualty of DNA. 0.8% Agrose gel was prepared by gel was prepared by boiling of Agrose in 100 ml of 1x TAE buffer. As the temprature of gel solution reduced to approximatly 55°C Ethimum bromide add at the rate of 5 ul/100 ml of gel solution. When the gel get solidify transfer into electrophoresis tank filled with 1x TBE buffer. The wells carefully Load with 5 ul DNA mixed with 3 ul 3x Bromophenol blue. Standard DNA da Ladder Load along with the DNA Samples in Seprate wall. Electrophorsi's carried out 100V for 30-60 mint.

Isolation Of RNA By Trizol Method

RNA Extraction By Trizol Reagents :-

① Take 200 ul Sampled in a 1.5 ml eppendroff and add 800 ul Trizol and 200 ul chloroform mixed and centrifuge for 15 mintue at 12,000g. ② Transfer the upper equad layer to a new ep endorf ③ Add 500 ul isopropanal and phace at 4°C for ½ hour ④ Centrifuge at 12000g for 10 minute ⑤ Discard supernantant and add 1 mL 75% ethonal and centrifuge at 7500g for 5 mints ⑥ Dicards supernatant and add 1ml 100% ethonl and centrifuge at 7500g for 5 mits. ⑦ Dry and add 20ul DEPC water.

RNA Extraction By Trisol Reagents:

Collect 250 ul samples to a 1.5 ml eppendorff and add 750 uL Trizol and samples is overtex for 15 sec, incubate at room temperature for 7 mint. ② Add 200uL 100% chloroform to the sample, vortex for 15sec and incubate at room temperature for 7 mint. ③ Plus spin to remove liquid form the tube lid. ④ Centrifuge at 12,000g for minutes at room temperature. ⑤ Transfer 450ul of the upper aqueous layer to a separate microcentrifuge tube. ⑥ Add 500l 100% isopropanol, invert tube several time to mix add and hold at room temperature for 10 mint. ⑦ Discard supernatant. Care should be taken to assure that the RNA pellet is not

distribute: ⑧ Add one mL of 80% ethanol. Dot mix gently. ⑨ Centrifuge at 10,000g for 5 minutes at 4°C. ⑩ Discard ethanol. Invert tube on a clean tissue wipe and allow to air dry for 10 minutes. It is important not to let the RNA pellet over dry as this will decrease its solubility. ⑪ Hydrate pellet in 50uL of RNase free water and allow sitting at 4°C for 1hr to overnight. Briefly vortex to resuspend pellet before pipetting.

6 DILUTE THE PELLET :—

① Add 50-100 uL of Low T.E. (TRIS HCl 10 mL. EDTA 0.2mM) OR DepC water.

② Place the tube in shaking water bath at 70°C for one hour so that nucleases were inactivated. Finally DNA will store at ~~200~~ -20 °C.

Isolation Of DNA Part B.

3. Phase Sepration Step.

① In this step, we can remove the digested protein throug. ② Phenol-chloroform isoamyl alcohol ③ DNA released into iso solution is extracted with PCI to remove proteinaceous material. ④ Add equal volume of Phenol-chloroform-isoamyl alcohol Mix gently for 2 min and centrifuge for 10 minutes at 10000 rpm at 4C. ⑤ Carefully remove the top phase containing the DNA used a 1000 Ul pipette transfer to a new tube.

4 DNA Precipitation.

① Precipitation of DNA with absolute isopropanol and inverted the tubes gentle till DNA threads become visible and then Left the tubes at room temperature for 10 mint.

② Centrifuged at 8000 rpm for 10 mintues. and discarded the supernatant carefully and white pellect of DNA may visiable at the bottom of tube.

5 Washing With Ice Cold Ethanol.

① washed DNA pellet with 1 ml of 70-100% ethanal, breakk and mix the pellet.

② Then centrifuges at 8000 rpm for 10 mints. and discarded the supernatant carefully.

③ Air dried the DNA pellect at room temperatu

1. DNA Extraction

Steps in Organic DNA Extraction:

1- Lysis of red Blood cells, RBC ② Digestion step.
③ Phase separation step ④ Discard the supernant
⑤ Break the pellet formed at the bottom of eppendrof by taping it gently. Add 1 ml TE buffer and mixed it gently. ⑥ Centrifuged at 5000 rpm for 10 min. this step may be repeated until pellet become light pink.

2. Digestion Step

Lysis of White Blood Cells, WBC

① Pellet obtained after lysis of RBC re-suspended
② 400 ul Buffer TNE
③ 200 ul 10% SDS
④ 50 ul Proteinase K
⑤ Homogenize the tube with gentle rotation
⑥ Samples incubated at overnight in 50°C Shark water bath.
⑦ Next Day.

Practical No 1

Preparation of Stock and Working Solution:

The following genral intruction are applicable in the prepration of all regents. Use graduated cylinders or pipettes closet to the volume being measured for preparing Liquid regents. Store all regents in sterile Containers unless otherwise noted Label all regents with name of regents, date prepared, intials of Scientist that prepared regent, lot number, and expirationdate. Record each prepration in Lab's Logbook.

1M Tris-Hcl

(Tris Hydroxy methyl methane) PH 8
Tris base 121.1 g
H_2O to 800 ml
Adjust to desired PH with Concentrated Hcl.
Mix and add H_2O to 1 Liter
Store at room temprature

TE (Tris 10mM-EDTA 2mM) PH 8.0 (Lysis Buffer)

1 M Tris-Hcl PH 8.0 10 ml
0.5 M EDTA PH 8.0 4 ml
H_2O 1 Liter

Proteinase K (100mg/ml)

Proteinase K 100 mg lyophilized Powder in ultra pure H_2O to 10 Make Liquid and store at 25 °C

0.5 M EDTA

(ETHylendiamine Tetra acetic Acid) PH. 8.0)
$Na_2EDTA \cdot 2H_2O$ 186.1 g
Adjust PH at 8.0 with 10 M NaoH (almost) 50ml
Mix and add H_2O to 1 L
Store at Room tempera

SDS 10% w/v

Sodium dodecyl Sulfate 1
H_2O to 700 ml Heat to approximatley 65 °C to dissolve Bring to a find Volume of 1.0L with Ultra pure water.
store at 25 °C

Ten buffer

(10mM Tris, 2mM EDTA, 400 mM NaCl)

1 M Tris-Hcl pH 8.0 10ml

5 M NaCl 80ml

0.5 M EDTA 4ml

H_2O to 1 Liter

store at 25 °C

50x TAE

(Tris-Acetate-EDTA)

Electrophoresis stock buffer

Tris base 242g

Glacial acetic acid 57.1ml

0.5 M EDTA PH 8.0 100ml

H_2O to 1 Leter

Store at 25°C

1x TAE

(Tris-Acetate EDTA) 2mM)

Electrophoresis working buffer

50x TAE 10ml H_2O to

500 ml the PH diluted buffer

is 8.3.

Store at 25 °C

5M Sodium Chloride

Sodium Chlorride 292.2g

H_2O to 1 liter

Store at 25 °C

Low TE

(Tris 10mM EDTA 0.2mM) PH 8.0

1 M Tris-Hcl pH 8.0 10ml

0.5 M EDTA PH 8.0 0.4ml

H_2O to 1 Liter

Store at 25 °C

10x TBE

(Tris 90mM-Borate 90mM EDTA 2mM) P

Electrophoresis buffer

Trite base 108g

Boric acid 55 g

0.5 M EDTA (PH 8.0) 40ml

H_2O to 1 Liter at 25 °C

2x Gel Loading Dye

2% Bromophenole blue .2ml

2x Xylene Cyanol 0.25ml

Glycerol 7ml

H_2O 10ml

Store at 25 °C

6 M Sodium Chlorrd

Sodium Chloride 351 g

H_2O to 1 Leter.

Store at 25 °C.

Isolation of DNA

Source of DNA

Purified DNA is required for a variety of molecular biological application. DNA can be purified from any living organism and its living organs / parts.

Origin of Samples.

① Human tissues ② Blood ③ Hair ④ Radiant tissues ⑤ Leaf
⑥ Bacteria ⑦ Yeast ⑧ Fungi ⑨ Insect ⑩ Stool
⑪ Body fluids ⑫ Spore ⑬ Soil ⑭ Clinical Samples ⑮ Forensic
DNA extraction is used to isolate.

[1] Types of DNA [2] Mitochondrial [3] DNA Genomic DNA
[4] Plasmid DNA.

DNA Extraction From Whole Blood Materials.

① Lysis buffer ② Proteinase K ③ Phenol-Chloroform isoamyl alcohol
④ SDC 10%. ⑤ Ten buffer ⑥ Isopropanol ⑦ Ice cold
⑧ Ultra pure (DNA- & DNase-Free) water.

Lab equipment needed.

① Pipettes ② 1.5ml sterile microcentrifuge tubes or 15/50 ml falcon ③ Racks ④ Tips ⑤ Vortex ⑥ Freezer
⑦ Centrifuge.

Blood Samples.

Blood collection in anticoagulant Ethylenediamide tetra acetic acid (0.5 M EDTA) Containing tube 1.5ml eppendrof or 15 ml Falcone tube.

Storage of Blood Samples.

Fresh blood samples should be place on ice immediatly after their collection store at Freezer -20°C before.