Chapter 4
Transformation, isolation and purification of plasmid DNA

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4.1 Transformation of pDNA in bacterial strain E. Coli Top 10

Chemically induced competence followed by transformation is a commonly used technique to introduce plasmids or other DNA fragments into E. coli. Depending on the genetic information it carries, the incoming DNA can be replicated as an independent entity or integrated into the host chromosome. To select for cells that incorporate the DNA, a plasmid is engineered to carry selectable markers such as antibiotic resistance genes. Plasmid uptake provides the host cell with the ability to survive on a selective media. Today, chemically induced transformation is frequently used to clone and amplify a fragment of a gene, a whole gene, or an entire DNA library. Transformation of cells with DNA is an invaluable technique that provides scientists with a way to introduce and manipulate genes.

Transformation of E. coli was first described by Mandel and Higa (1970) (1). Subsequent modifications to improve transformation efficiencies have included prolonged exposure of cells to $CaCl_2$ (2), substitution of calcium with other cations such as Rb^+ (3), Mn^{2+}, and K^+, and addition of other compounds such as dimethyl sulfoxide, dithiothreitol, and cobalt hexammine chloride (4). Transformation of E. coli was achieved by calcium chloride method. Calcium chloride gives good transformation efficiencies, is simple, one-step method, requires no special equipment, and allows storage of competent cells. Calcium chloride cause alteration in the permeability of the membranes allows DNA to cross the cell envelope of E. coli which is composed of an outer membrane, an inner membrane, and a cell wall. The outer membrane of E. coli can be understood by application of the fluid mosaic model for membranes and is composed of phospholipids, proteins, and lipopolysaccharides. Many channels or zones of adhesions are formed by the fusion of the outer membrane and the inner membrane through the cell wall layer. Although the transformation mechanism is not known, previous studies indicate that these channels allow for the transport of DNA molecules across the cell membrane (5, 6). The negative charges of the incoming DNA, however, are repelled by the negatively charged portions of the macromolecules on the bacterium's outer surface. The addition of $CaCl_2$ serves to neutralize the unfavorable interactions between the DNA and the polyanions of the outer layer. The DNA and competent cells are further incubated on ice for thirty minutes to stabilize the lipid membrane and allow for increased interactions between calcium ions and the negative components of the cell. The reaction mixture is then exposed to a brief period of heat-shock at 42^{o}C. The change in

temperature alters the fluidity of the semi-crystalline membrane state achieved at 0^{o}C thus allowing the DNA molecule to enter the cell through the zone of adhesion

4.1.1 Materials

Luria broths (LB), agar, Kanamycin, Ampicillin, calcium chloride, magnesium chloride were obtained from Himedia, Mumbai.

Bacterial Strain: Escherichia Coli strain (E. Coli Top 10).

Plasmid: piRIS2-EGFP CFTR plasmid (10 kb) & pCDNA-3 LUC-WT plasmid (7.4 kb).

4.1.2 Media

Sterile Luria broth (LB) for initial growth of culture (LB -2% w/v in water).

Sterile Luria broth (2% w/v in water) with appropriate antibiotic (Ampicillin- 100 µg/ml).

Sterile Luria broth (2% w/v in water) with appropriate antibiotic (Kanamicin- 50 µg/ml).

Sterile Luria broth agar plates (LB 2% w/v and Agar 1.5% w/v in water).

Sterile Luria broth agar plates (LB 2% w/v and Agar 1.5% w/v in water) with appropriate antibiotic (Ampicillin- 100 µg /ml).

Sterile Luria broth agar plates (LB 2% w/v and Agar 1.5% w/v in water) with appropriate antibiotic (Kanamicin- 50 µg/ml).

4.1.3 Reagents

0.1 M $MgCl_2$: 0.1 M $MgCl_2$ was prepared by dissolving 11.1 g $MgCl_2$ in 1 L purified water followed by sterilization by filtration

0.1M $CaCl_2$: 0.1 M $CaCl_2$ was prepared by dissolving 11.1 g $CaCl_2$ in 1 L purified water followed by sterilization by filtration

20 % Glycerol: 20 % Glycerol was prepared by dissolving 20 g glycerol in 100 mL purified water followed by sterilization by using autoclave.

4.1.4 Method for transformation of pDNA in bacterial strain E. Coli Top 10 (7)

1. Initially a single bacterial colony was grown on a Luria broth agar plate using the streak plate method and the plate incubated overnight at 37°C.
2. Then single bacterial colony was picked from a plate and transferred into 10 ml Luria broth medium in a 50 ml flask. The flask was incubated for 3-4 hours at 37°C with vigorous agitation, monitoring the growth of the culture. As a guideline, 1 OD_{600} of a culture of E. coli strain Top 10 contains approx.10^9 bacteria/ml.

3. The bacterial cells were recovered by centrifugation in a Remi centrifuge at 5000 RPM at 4 ºC for 10 min.
4. Supernatant was removed from the cell pellets and cell pellet was re-suspended by swirling in 3 mL ice-cold $MgCl_2$ (0.1M) solution.
5. Bacterial cells were recovered by centrifugation in a sigma centrifuge at 5000 RPM at 4 ºC for 10 min.
6. Decant the medium from the cell pellets and re-suspend each pellet by swirling in 0.4 mL ice-cold $CaCl_2$ (0.1M) solution.
7. Incubate for 10 min.
8. Recover bacterial cells by centrifugation in a sigma centrifuge at 5000 RPM at 4 ºC for 10 min and re-suspend each pellet by swirling in 0.4 mL ice-cold $CaCl_2$ (0.1M) solution.
9. Incubate in ice bath for 45 min.
10. Add up to of plasmid DNA (10-100 ng) into new microcentrifuge tubes. Chill on ice for 2 min.
11. Add the prepared competent cells to each tube containing DNA, mix and incubate on ice for 45 min. The prepared competent cells to each tube without DNA is used for positive and negative control also incubated on ice for 45 min.
12. Transfer the tubes in a preheated 42°C water bath for exactly 90 seconds without shaking.
13. Rapidly transfer the tubes to an ice bath. Allow the cells to chill for 10 minutes.
14. Then 1 mL sterile LB was added to each tube and allow to stand for 1 hr.
15. Recover bacterial cells by centrifugation in a sigma centrifuge at 5000 RPM at 4 ºC for 10 min.
16. Again re-suspend the bacterial cells in 0.1 mL sterile LB.
17. Then transfer the transformed competent cells (with and without pDNA) on LB agar plate containing appropriate antibiotics (Kanamycin for piRIS2-EGFP CFTR plasmid & Ampicillin for pCDNA-3 LUC-WT plasmid).
18. Incubate the plate overnight at 37°C.
19. Plates used are
    - + Control: Prepare competent cells and grow on LB plate not containing antibiotic. (Without plasmid DNA)

- – Control: Prepare competent cells and grow on LB plate containing antibiotic. (Without plasmid DNA)
- Plasmid Transformation Plate: Prepare competent cells, transformed with plasmid DNA and grow on LB plate containing antibiotic.

Obtained transformed cells were then used for plasmid amplifications and isolation.

4.2 Plasmid Amplification and Isolation

Isolation of the plasmid from bacteria was done by Alkaline Lysis method. The alkaline lysis procedure (8, 9) is the most commonly used miniprep. Plasmid DNA is prepared from small amounts of many different cultures (1 to 24) of plasmid-containing bacteria. Bacteria are lysed by treatment with a solution containing sodium dodecyl sulfate (SDS) and NaOH (SDS denatures bacterial proteins, and NaOH denatures chromosomal and plasmid DNA). The mixture is neutralized with potassium acetate, causing the covalently closed plasmid DNA to reanneal rapidly. Most of the chromosomal DNA and bacterial proteins precipitate as does the SDS, which forms a complex with potassium and are removed by centrifugation. The reannealed plasmid DNA from the supernatant is then concentrated by ethanol precipitation.

4.2.1 Materials

Tris-HCl solution (1 M), EDTA solution (0.5 M), Tris-EDTA buffer (pH 8), Alkaline Lysis I solution, Alkaline Lysis II solution, Alkaline Lysis III solution. Phenol, Chloroform, Isoamyl alcohol, Ethidium Bromide, 70 % Ethanol, Lithium chloride, polyethylene glycol 8000, Absolute Isopropanol are obtained from Sigma-Aldrich, India. Bromophenol Blue Dye, Lysozyme, DNase-free RNase-A are obtained from Himedia, Mumbai.

4.2.2 Reagents

a) Tris - HCl (1 M): 121.1 g of Tris base was dissolved in 800 mL of water. pH was adjusted to 8.0 by adding 42 mL of concentrated Hydrochloric acid. Final pH was adjusted and the volume was made up with water to 1 L and sterilized by autoclaving.
b) EDTA 0.5 M (pH 8.0): 186.1 g of disodium EDTA. $2H_2O$ was dissolved in 800 ml of water with vigorous stirring on a magnetic stirrer, pH was adjusted to 8.0 with sodium hydroxide and the volume was made up with water to 1 liter and sterilized by autoclaving.
c) 1 M NaCl: 58.4 g of NaCl was dissolved in 900 mL of water with vigorous shaking and the volume was made up with water to 1 L and sterilized by autoclaving.

d) 10X Tris - EDTA (TE):100 mM of Tris- Cl (pH 8.0) and 10 mM EDTA (pH 8.0) in water was prepared and the solution was sterilized by autoclaving and stored at room temperature.
e) 10 X Sodium Chloride – Tris – EDTA (STE) buffer (pH 7.4): 100 mM of Tris- Cl (pH 8.0), 10 mM EDTA (pH 8.0) and 1 M NaCl in water was prepared and the solution was sterilized by autoclaving and stored at room temperature.
f) Alkaline Lysis I: 25 mM of Tris- Cl (pH 8.0), 10 mM EDTA (pH 8.0) and 50 mM of Glucose in water was prepared and the solution was sterilized by autoclaving and stored at 4 ^{o}C
g) Alkaline Lysis II: Freshly 0.2 M of sodium hydroxide and 1 % w/v of sodium lauryl sulphate in freshly autoclaved water was prepared and discarded after use.
h) 5 M Potassium Acetate: 490.5 g of Potassium Acetate was dissolved in 500 mL of autoclaved water and the volume was made up with water to 1 L and stored at 4 C.
i) Alkaline Lysis III: 60 mL of 5 M potassium acetate and 11.5 mL of glacial acetic acid were mixed and the volume was made up with autoclaved water to 100 mL and the solution was stored at 4 ^{o}C.
j) 50 X Tris-Acetate- EDTA buffer (TAE): 242 g of Tris base was dissolved in 500 mL of autoclaved water and was mixed with 100 mL of 0.5 M EDTA (pH 8.0) and 37.1 mL of glacial acetic acid and the volume was made to1 L with autoclaved water and was stored at 4 ^{o}C
k) Phenol pH 8: 500 g of crystalline phenol is initially after melting is mixed with 0.1 % of hydroxyquonoline and is vacuum distilled. This distilled phenol is to be stored in dark container with tight closure. The phenol for DNA purification is prepared by equilibrating the melted pure phenol with Tris Buffer pH 10.0 (200 mL) for 2 times followed by equilibration with Tris buffer pH 8.0, until pH of phenol is near 8.0. The liquid phenol is kept under the layer of Tris buffer until further used.
l) Phenol Chloroform Isoamyl Alcohol: The solution is freshly prepared before use by mi xing Phenol:Chloroform:Isoamyl Alcohol in the ratio of 25:24:1 (v/v). The solution is used for purifying the pDNA from soluble proteins and chromosomal DNA.
m) Ethidium Bromide: 10 mg of Ethidium Bromide was dissolved in 1 mL of sterile water. The solution was covered in eppendorf tube by aluminum foil and stored in cool and dark place.
n) Gel Loading Dye (Bromophenol Blue): The gel loading dye is prepared by preparing 0.25 % w/v solution of Bromophenol Blue in 30 % v/v glycerol in water.

o) 10 mM Tris (pH 8.0): 1 mL of 1 M Tris buffer was diluted to 100 mL with sterile water with pH maintained to 8.0.

p) 70 % Ethanol: Absolute ethanol diluted to 70% v/v by water.

q) Absolute Isopropanol

r) Lithium chloride 5 M: 21.2 g of LiCl was dissolved in 100 mL of water sterilized by passing it through a 0.22 μm filter and stored at 4°C.

s) Polyethylene Glycol 8000 (PEG 8000) (% w/v): Appropriate concentration of PEG 8000 was dissolved in sterile water with warming if necessary. Sterilized by passing it through a 0.22 - μm filter and the solution was stored at room temperature.

t) Sodium Acetate 3.0 M: 408.3 g of sodium acetate.3H2O was dissolved in 800 mL of water pH was adjusted to 5.2 with glacial acetic acid or to pH 7.0 with dilute acetic acid final volume was adjusted with water to 1 liter and sterilize by autoclaving.

u) Lysozyme (10 mg/mL): Lysozyme solution (10 mg/mL) was prepared in 10 mM Tris-HCl buffer pH 8.0. The solution was freshly prepared before every use.

v) DNase free RNase - A (1 mg/mL): DNase free RNase-A solution (1 mg/mL) was prepared in 10 mM Tris-HCl buffer pH 8.0. The solution was freshly prepared before every use, heated to 60 ^{o}C, for 1 hour to destroy DNAse, allowed to cool and then used.

4.2.3 Medium

LB Media with Kanamycin antibiotic (50 μg/mL of culture) for piRIS2-EGFP CFTR plasmid), LB Media with Ampicillin antibiotic (100 μg/mL of culture) for pCDNA-3 LUC-WT plasmid

Culture: Transformed E. coli bacterial culture containing piRIS2-EGFP CFTR plasmid or pCDNA-3 LUC-WT plasmid

4.2.4 Method for Plasmid Amplification and Isolation by Maxi-precipitation (7)

1. Initially, 30 mL of rich medium (LB) was inoculated with the appropriate antibiotic either with a single colony of transformed bacteria.
2. Then, culture was incubated at 37°C with vigorous shaking until the bacteria reach late log phase (OD_{600} = approx. 0.6).
3. 500 mL of LB medium containing the appropriate antibiotic in a 2 L flask was inoculated with 25 mL of the late-log-phase culture. The culture was incubated for approximately 12-16 hours at 37°C with vigorous shaking on a shaker.

4. The grown bacterial cells were harvested by centrifugation at 5000 rpm for 10 minutes at 4°C. Supernatant was discarded and bacterial cell pellet were re-suspended in 200 mL ice-cold TE buffer and bacterial cells were collected by centrifugation at 5000 rpm for 10 minutes at 4°C.
5. Then pellet was re-suspended in 18 mL of alkaline lysis-I solution with gentle vortexing and a freshly prepared 2 mL solution of (10mg/mL) lysozyme and RNase-A (1mg/mL) was added.
6. After 5 min. incubation at room temperature, freshly prepared 40 mL alkaline lysis-II solution was added and the contents were mixed thoroughly by inverting the centrifuge tubes 10 times. Further incubate for 5 minutes at room temperature.
7. Then 20 mL ice-cold alkaline lysis-III solution was added and the contents were mixed thoroughly by inverting the centrifuge tubes 10 times. Tubes were then placed in ice for 10 minutes.
8. Centrifugation of the bacterial lysate was carried out at 6000 rpm for 10 minutes at 4°C. After the completion of centrifugation, clear supernatant was decanted into a centrifuge tubes and the pellet was discarded.
9. To the supernatant, equal amount of Phenol:Chloroform:Isoamyl alcohol (25:24:1) was added. The tubes were vortexed for a minute and allowed to separate. The Tubes were centrifuged at 6000 rpm for 5 min at 4 ^{o}C. The supernatant was removed and again treated with Chloroform alone to remove residual traces of phenol and the supernatant was collected.
10. Equal volume of isopropanol was added to the collected supernatant and mixed well and stored for 30 minutes at room temperature.
11. The precipitated nucleic acids were recovered by centrifugation at 15,000 rpm for 30 minutes at 4 ^{o}C.
12. Supernatant was carefully removed and the pellet was rinsed with 70% ethanol and then ethanol was drained off by inverting the tubes on a pad of paper towels for a few minutes at room temperature and dried in air.
13. The pellet was dissolved in sufficient volume of Tris–EDTA (TE) buffer.
14. The purity of plasmid preparations was determined by using agarose gel electrophoresis.
15. DNA concentration was measured by UV absorption at 260 nm. The purity of pDNA was estimated by measuring the optical density at 260 nm and 280 nm and calculating the ratio of

Abs 260 nm/Abs 280 nm which was generally 1.8 to 2.0, also by gel electrophoresis to ensure the absence of RNA.

4.3 Plasmid Purification (7)

4.3.1 Method

The isolated plasmid DNA was purified by Polyethylene Glycol-Lithium Chloride Precipitation technique as follow,

1. Prepared crude plasmid was transferred of to a centrifuge tube and the solution was chilled to 0°C in an ice bath.
2. Then ice-cold 5M lithium chloride (LiCl) solution was added to the crude plasmid preparation, mixed well, and centrifuged at 14,000 rpm for 15 minutes at 4°C.
3. Supernatant was transferred to a fresh centrifuge tube and an equal volume of isopropanol was added with proper mixing. Precipitate of nucleic acids was recovered by centrifugation at 14,000 rpm for 20 minutes at room temperature.
4. Supernatant was removed and pellet was rinsed with 70% ethanol at room temperature and ethanol was carefully discarded.
5. Pellet of nucleic acid was dissolved in 1X TE (pH 8.0) with RNase-A and stored it for 30 minutes at room temperature.
6. The plasmid-RNase mixture extracted once with phenol:chloroform and once with chloroform and then the DNA was recovered by standard ethanol precipitation.
7. The pellet of plasmid DNA dissolved in sterile H_2O, and then add PEG-$MgCl_2$ solution, store the solution for 10 minutes at room temperature, and then collect the precipitated plasmid DNA by centrifugation.
8. Remove traces of PEG by resuspending the pellet of nucleic acid in 70% ethanol. Collect the nucleic acid by centrifugation.
9. Remove the ethanol by aspiration and store the open tube on the bench for 10-20 minutes to allow the ethanol to evaporate.
10. Dissolve the damp pellet in TE (pH 8.0). Measure the OD260 of a 1:100 dilution in TE (pH 8.0) of the solution, and calculate the concentration of the plasmid DNA assuming that 1 OD260 = 50 µg of plasmid DNA/ml.
11. Store the DNA in aliquots at -20°C.

4.4 Gel retardation assay

The purity of pDNA was determined by 0.8% agarose gel electrophoresis. pDNA along with DNA ladder was loaded on the agarose gel (10 µl of the sample containing 0.5 µg of pDNA). A 1:5 dilution of loading dye was added to each sample and electrophoresis was carried out at a constant voltage of 150 V for 20 min in TBE buffer (1M Tris–base, 0.01M sodium EDTA, 0.9 M boric acid) containing 0.5 g/ml ethidium bromide (EtBr). The pDNA bands were then visualized under a UV transilluminator.

4.5 Confirmation of plasmid by restriction enzymes:

The confirmation of the transformed plasmid was done by digestion of plasmid using restriction endonuclease enzymes, which when compared with the molecular marker run along with plasmid on 0.8 % agarose gel shows the bands according to molecular weight.

4.5.1 Materials

1 Kb DNA ladder (Invitrogen), Restriction enzymes (Pst I & Xho I, Hind III, BamH I, EcoR I, 1X NEBuffer 3 [50 mM Tris-HCl, 100 mM NaCl, 10 mM MgCl2, 1 mM Dithiothreitol, pH 7.9], 1X NEBuffer 2 [10 mM Tris-HCl, 50 mM NaCl, 10 mM MgCl2, 1 mM Dithiothreitol, pH 7.9], Bovine Serum Albumin (BSA) obtained from New England BioLab's Inc.

4.5.2 Plasmid digestion method piRIS2-EGFP CFTR plasmid

1. To the isolated plasmid DNA, BSA, 1X NE Buffer 3 and restriction endonuclease enzyme (Pst I or Xho I) was added and volume made to 20 µl autoclaved distilled water.
2. The above cocktail was incubated at 37 ºC for 1 Hr.
3. The above cocktail was loaded along with molecular marker and supercoiled pDNA in three different wells into 0.8 % agarose gel

4.5.3 Plasmid digestion method pCDNA-3 LUC-WT plasmid

1. To the isolated plasmid DNA, BSA, 1X NE Buffer 3, restriction endonuclease enzyme (Hind III & BamH I or EcoR I) was added and volume made to with 20 µl autoclaved distilled water.
2. The above cocktail was incubated at 37 ºC for 1 Hr.
3. The above cocktail was loaded along with molecular marker and supercoiled pDNA in three different wells into 0.8 % agarose gel

4.6 Results and discussion

4.6.1 Transformation of pDNA in bacterial strain E. Coli Top 10

The transformation of piRIS2-EGFP CFTR plasmid & pCDNA-3 LUC-WT plasmid into prepared competent bacterial cells was confirmed by the growth of the competent bacterial cells on agar plates (with and without appropriate antibiotic), which acts as positive and negative control. During transformation of plasmid, 3 agar plates were streaked. The observations of experiment are shown in Table 4.1.

Table 4.1: Observations of bacterial growth on agar plate after transformation

Agar Plate	Observation	Inference
+ **Control:** Competent cells grown on LB plate not containing antibiotic	High bacterial cell growth observed throughout plate.	Competent bacterial cells are not damaged and are able to grow on agar plate.
– **Control:** Competent cells grown on LB plate containing appropriate antibiotic.	No bacterial cell growth observed.	No growth of competent bacterial cells observed due to sensitivity of cells towards the used antibiotic.
Test Plate: Competent cells transform with plasmid grown on LB plate containing appropriate antibiotic.	Discrete colonies of bacterial cells are observed throughout the plate	Transformed bacterial cells with plasmid DNA shows antibiotic resistance and are able be grow on agar plate.

The prepared competent bacterial cells were found to grow on agar plate without antibiotic; however, on agar plates with antibiotics, no bacterial cell growth was observed due to sensitivity towards antibiotics. When transformed cells contain the plasmid containing Antibiotic resistance marker base pairs, which enable them to grow on agar plate with antibiotics. These transformed bacterial cells were further sub-cultured and used for DNA isolation.

4.6.2 Plasmid amplification, isolation and purification

The plasmid DNA was isolated from the E. coli transformed strains using the alkaline lysis method. The plasmid was purified by PEG-LiCl method. The purity of the plasmid was ascertained by agarose gel assay and UV spectrophotometry for absorbance detection at 260 and

280 nm. The ratio of absorbance was observed between 1.8-2.0 at 260 and 280 indicated pure plasmid devoid of protein and RNA. The concentration of plasmid is determined by absorbance at 260 nm by comparing with the standard equation: 1 OD 260 = 50 µg of plasmid DNA/mL. Figure 4.1 depict the purity of plasmid by gel electrophoresis. Both plasmids are pure devoid of proteins and RNA. Both isolated plasmids are pure, show distinct band on gel electrophoresis, devoid of any RNA or protein.

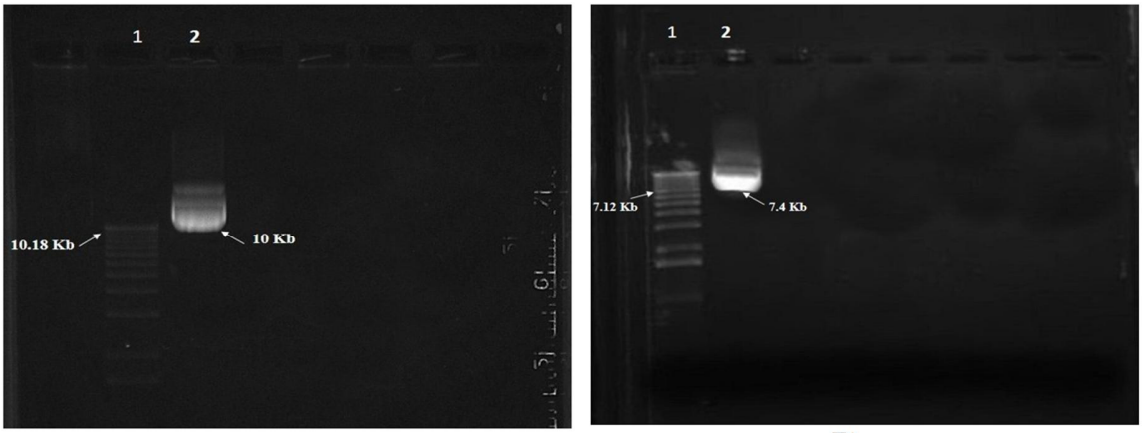

Figure 4.1: Agarose gel electrophoresis for (A) piRIS2-EGFP CFTR plasmid (Lane 1: Marker DNA, Lane 2: Isolated Plasmid); (B) pCDNA-3 LUC-WT plasmid (Lane 1: Marker DNA, Lane 2: Isolated Plasmid)

4.6.3 Plasmid Digestion

The isolated plasmid was digested by restriction endonuclease enzymes for confirming the pDNA with the transformed DNA. The isolated pDNA after linearizing shows a single strong band on the agarose gel corresponding to its molecular weight. Both plasmids showed the molecular weight similar to their theoretical weight on basis of the map provided by supplier. The molecular weights were confirmed by the molecular weight markers, which were run alongside the plasmids. In piRIS2-EGFP CFTR plasmid, CFTR gene is reside between Pst I site. When plasmid is digested with Pst I enzyme, it show two bands, one for CFTR and other for plasmid construct at 4.7 Kb and 5.3 Kb respectively. When plasmid is digested with Xho I, it showed a linear band at 10 Kb confirming the isolated plasmid (Figure 4.2).

In case of pCDNA-3 LUC-WT plasmid, Luciferase is reside between Hind III site and BamH I site. When plasmid is digested with Hind III & BamH I enzyme, it show two bands, one for Luciferase and other for plasmid construct at 1.95 Kb and 5.44 Kb respectively. When plasmid is digested with EcoR I, it showed a linear band at 7.4 Kb confirming the isolated plasmid (Figure 4.3)

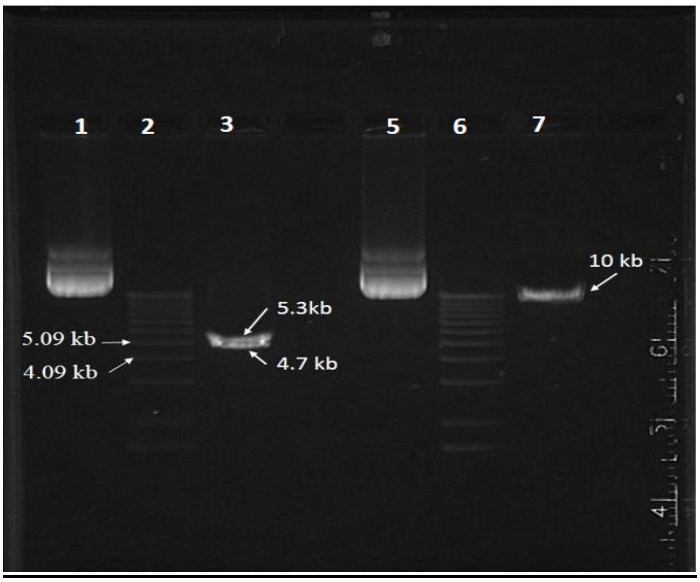

Figure 4.2: Digestion of piRIS2-EGFP CFTR plasmid by Pst I and Xho I restriction endonuclease enzyme. (Lane 1: Undigested Plasmid, Lane 2: Marker DNA, Lane 3: Digested with Pst I, Lane 5: Undigested Plasmid, Lane 6: Marker DNA, Lane 7: Digested with Xho I)

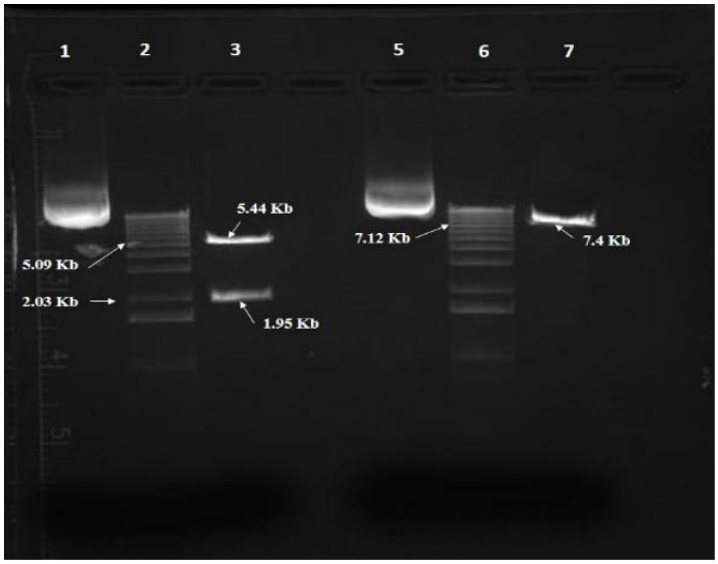

Figure 4.3: Digestion of pCDNA-3 LUC-WT plasmid by Hind III, BamH I and EcoR 1 restriction endonuclease enzyme. (Lane 1: Undigested Plasmid, Lane 2: Marker DNA, Lane 3: Digested with Hind III & BamH I, Lane 5: Undigested Plasmid, Lane 6: Marker DNA, Lane 7: Digested with EcoR I)

4.7 Conclusion

The piRIS2-EGFP CFTR plasmid & pCDNA-3 LUC-WT plasmids were transformed in bacterial cells using CaCl2 method and transformation was confirmed by the growth of the bacterial cells on agar plates containing antibiotics. From the transformed colonies pDNA was isolated using alkaline lysis method and was confirmed for correct transformation by restriction endonuclease digestion. Confirmed plasmids were amplified using maxi-precipitation technique using the principle of alkaline lysis. The isolated pDNA was purified using phenol chloroform extraction followed by PEG 8000-Lithium Chloride precipitation. The purity of the pDNA was assessed by calculating the ratio of UV absorbance at 260 and 280 nm. The ratio in between 1.8-2.0 indicates pure pDNA devoid of protein and RNA.

4.8 References

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