TEAM SVCE CHENNAI

CFastDx

EXPERIMENT PROTOCOL

DNA ELUTION

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PROTOCOL FOR DNA ELUTION

MATERIALS REQUIRED:

1. Ammonium Acetate
2. Cholroform
3. Ethanol
4. Ethidium Bromide
5. LMT Elution Buffer
   - 20 mM Tris- Cl (pH 8.0)
   - 1 mM EDTA (pH 8.0)
6. Phenol : Choloroform (1:1)
7. Phenol (Equilibrated pH 8.0)

STEPS

1. Prepare a 1.5 % Agarose Gel containing an appropriate concentration of low melting agarose in 1X TAE with 0.5 µg/mL of Ethidium Bromide (EtBr).
2. Cool the gel to room temperature and transfer it to the horizontal gel casting boat.
3. Mix the samples of DNA with gel loading buffer and load them in the appropriate slots followed by running the electrophoresis with 4-7 V/cm.
4. The Plasmid DNA Band is Visualized in UV Trans-illuminator and transferred in a microfuge tubes.

5.Then Cut out the region of the gel containing desired DNA. Transfer the gel slice into a microfuge tube.

6.Add to the gel slice about 5 volumes of LMT elution buffer and incubate at 65° C for 15 Minutes.

7.Bring the sample to room temperature and add equal volume of equilibrated phenol followed by 20 sec vortexes. The solution will be centrifuge at 6000 rpm for 10 mins at 20 ° C for phase separation.

8.Remove the aqueous phase and extract with phenol: Chloroform followed by chloroform (The solution will be centrifuge at 6000 rpm for 10 mins at 20 ° C for phase separation subsequently).

9.Transfer the aqueous phase to a fresh tube and add 0.2 volume of 10 M Ammonium acetate and 2 volumes of absolute ethanol at 4 ° C for 10 min and centrifuge at 6000 rom for 20 mins at 4 ° C.

10.Wash the DNA Pellet with 70 % ethanol and dissolve the DNA in TE buffer. Repeat the step 1-4 for further confirmation

REFERENCES:

Life Technologies, Inc., gibco-BRL Focus 7(4): 1 - 2, 1985. Sambrook, J., et al., Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory Press, 1989.