EXPERIMENT 7

DNA EXTRACTION AND DNA ANALYSIS USING ELECTROPHORESIS

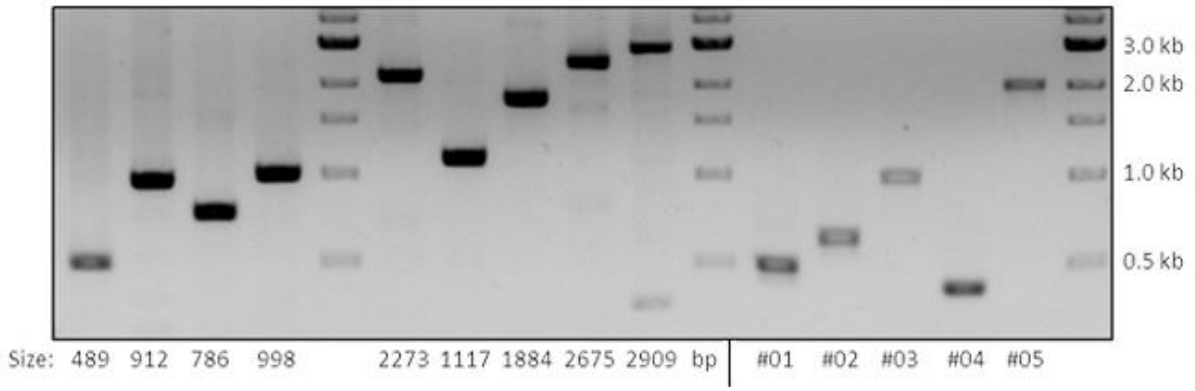

Figure 1: DNA gel electrophoresis (Source: https://www.thermofisher.com/my/en/home/life-science/cloning/gene-synthesis/gene-strings-dna-fragments.html)

OBJECTIVES:

Student should be able to

1. To investigate and explain DNA extraction using different methods.
2. To conduct and explain DNA by analytical methods.

MATERIAL

- DNA buffer (5 mL of dishwashing clear liquid, 1.5 g NaCl, 5 g NaHCO3, 120 mL distilled water)
- Microbial culture (centrifuge to obtain cells) 15 mL x 2 for each group
- 100 % Ethanol
- TE buffer (10 mMTris pH 8, 10 mM EDTA)
- 10 % SDS
- Alcohol solution A (phenol/chloroform/isoamyalcohol) (25:24:1)

METHODOLOGY

i) DNA isolation I

i) Add 1 ml of DNA Buffer to the tube containing cells. Mix the content well by inverting 2 or 3 times.

ii) Next, add2 mL of ethanol by pipetting it slowly down the side of tube to form a layer that floats on top of the sample while the tube is held at a slight angle. DO NOT MIX OR INVERT THESE TUBES. Gently place tube on the table.

iii) Observe any precipitate forms between two layers of the liquid. Use the glass rod provided to spool out the DNA clumps.

ii) DNA isolation II

i) Add 1 mL of TE buffer and centrifuge to wash the cells.
ii) Add in 100 uL of TE, 50 uL of 10 % SDS and incubate for 30 min at 65 C. Then, centrifuged and discard the supernatant.
iii) Heat the tube in a microwave with low intensity (900W) for 1 min or 750W for 1 min, 3 times.
iv) Add in 200 uL of TE and 200 uL of alcohol solution A, and incubated for 15 min.
v) Centrifuge to obtain the supernatant and add in 100 % Ethanol with the same volume.
vi) Centrifuge again to obtain pellet. Dissolve the pellet with 50 uL of TE.

iii) Electrophoresis (Follow the instructions given by demonstrator and write the standard operation procedure (SOP) in your report.

QUESTIONS

1) Explain the differences between chromosomal DNA and plasmid DNA.
2) Describe other method of DNA extraction.