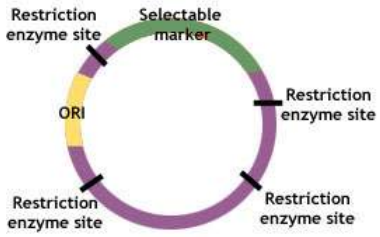
AGAROSE GEL ELECTROPHORESIS AND RESTRICTION MAPPING OF PLASMID DNA

Aim1: Estimation of purity/ quality of plasmid DNA
Aim2: Estimation of length of plasmid in base pairs
Aim3: Restriction Enzyme map of plasmid DNA

Theory: Agarose gel electrophoresis involves the principle of movement of DNA or RNA from negative to positive electrode in an electric field, as DNA/RNA are negatively charged macromolecules due to the presence of phosphates in them. Molecules of linear duplex DNA travel through agarose gels at a rate which is inversely proportional to the log of their molecular weight / No. of base pairs. Thus, shorter DNA migrates to longer distance in the gel and vice versa.

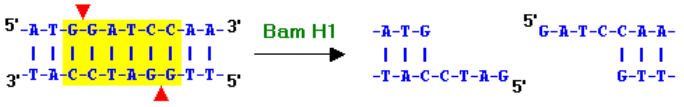
Relative mobility (Mr) = 1/log (bp).



Plasmid DNA

Agarose is obtained from the red algae *Gelidium&Gracilaria.* Its gels have sieving property, which is used as the supporting matrix for the separation of DNA molecules. Agarose gels are prepared after boiling agarose powder with 1x TAE buffer and casting on a gel tray. A comb on the gel tray is kept to obtain uniform gel pockets (wells). Upon solidification, DNA sample can be loaded into the wells.

Restriction enzymes bind to a fixed palindromicsequence of DNA (sequences that read same on the two strands of DNAwhen read in 5'-3' direction),and make a cut into it. The restriction enzyme EcoRI binds and cuts GAATTC,whereasBamHI binds and cuts GGATCC (see the schematic below).



APPARATUS: Gel electrophoresis unit, Casting tray, glass frame, conical flask, Microwave oven, U.V Trans illuminator, Micropipette

MATERIAL OR CHEMICALS REQUIRED AND THEIR USE:

You are given the following materials and reagents for your experiments

a. Plasmid DNA: 200 nano gram per microliter
b. Restriction Enzymes EcoRI and BamHI
c. Reaction buffers for restriction enzymes EcoRI and BamHI
d. Agarose powder
e. Ethidium Bromide (it intercalates between DNA double helical strands and gives fluorescence under ultra violet light at 260nm wavelength).
f. DNA Ladder / Ruler (this is a mixture of small fragments of DNA starting from 100 base pairs to 10,000 base pairs)
g. 6x DNA Loading Dye (in this Bromophenol blue is used as a tracking dyewhichmigrates around 300bp of linear DNA; glycerol is added to increases the density of the sample so that your DNA sinks and settles in the wells of agarose gel).
h. TAE Buffer (Stock of 50x TAE (Tris Acetate EDTA) buffer (pH-8.0)).
   Prepare 1L of 1x TAE buffer by diluting 50x stock solution with distilled water.

PROCEDURE:

This experiment will be done jointly by 4 students

1. Set up restriction digestion of given plasmid DNA by adding the following components in 0.5 ml tubes:

Sample no. /components	**1. Undigested**	**2. EcoRI* digestion**	**3. BamHI* digestion**	**4. EcoRI*+BamHI* digestion**
DNA	*2µl*	*2µl*	*2µl*	*2µl*
10X Buffer	*1µl*	*1µl*	*1µl*	*1µl*
*Enzyme**	*0µl*	*2µl*	*2µl*	*4µl (2µlEcoRI+2µlBamHI)*
H2O	*7µl*	*5µl*	*5µl*	*3µl*
Total	*10µl*	*10µl*	*10µl*	*10µl*

2. Add the components in the following sequence: First add *4µl* DNA to each tube. Then add 10x buffer to each tube. Then add water to each tube and finally add the different restriction enzymes to each tube.
3. Gently tap the tubes to mix the components and spin down for 10 seconds.
4. Incubate the tubes at 37C for 1 hour.
5. While the restriction digestions are going on, make the Agarose gel. 1 agarose gel per bench of 4 students. Make 0.7%agarose in 100 ml of **1X TAE buffer**. The volume of gels depends on size of the gel unit. Ask teaching assistants for this detail.
6. Heat the mixture in microwave oven for 2 min. Swirl the flask gently to allow the agarose to dissolve. Allow the solution to cool down to 60°C.
7. Obtain help from lab assistants and add 2µl of Ethidium Bromide[1] to the agarose solution. Mix. Wear gloves. Pour the solution into the gel-casting tray having comb. (Final concentration of Ethidium bromide in gel = 0.5µg/ml)
8. Let the gel solidify for half an hour.
9. Wear gloves. Transfer the gel into gel electrophoresis tank and fill the tank with **1X TAE** buffer till the gel is submerged.Gently take out the comb.
10. Add 2µl of 6x DNA loading dye to the samples. In one lane you would need to have the marker DNA for sizing.
11. Using micropipette load the samples into the gel wells. Note down position of your samples.
12. Run it at a constant voltage of 100V for 45min-1 hour.
13. Take out the gel from electrophoresis unit and observe in gel-doc (UV illuminator). Record picture of your gel.
14. Compare DNA bands with DNA ladder to estimate lengths of DNA fragments in various samples.
15. Based on the DNA fragments obtained with restriction enzymes, put the sites onto a circular plasmid to generate a restriction map of the plasmid.

Caution:

(1) Ethidium bromide is notoriously unsafe. Not only is it a very strong mutagen, it may also be a carcinogen or teratogenic. Its MSDS documents state that it is harmful if swallowed and very toxic by inhalation, as well as being irritating to the eyes, respiratory system and skin. Additionally it carries the risk of irreversible effects. Ethidium bromide can therefore pose a major safety problem for the researcher. (Taken from Web).

(2) Protect yourself from any exposure to UV light. It can severely damage your DNA.