**Restriction Digestion**

Take a 10 micro litres pipette and adjust the volume to 5 microlitres.

Insert a fresh tip.

Take the vial labeled as Deionized water, kept in the ice box.

Open the vial and pipette out 5 microlitres of deionized water.

Keep it back in the ice box.

Take the vial labeled as Vial 1.

Open the cap and transfer 5 microlitres of deionized water into it.

Keep the vial back on the rack.

Discard the used tip.

Adjust the volume of micropipette to 1 microlitre.

Insert a fresh tip.

Take the vial labeled as 10 X (\* read as 10 X) Reaction Buffer, kept in the ice box.

Open the vial and pipette out 1 microlitre of Buffer.

Recap the vial and keep it back in the ice box.

Take the vial labeled as Vial 1.

Open the cap and transfer 1 microlitre of 10X (\* read as 10 X) Reaction Buffer into it.

Close the vial and keep it back on the rack.

Discard the used tip.

Adjust the volume of micropipette to 3 microlitres.

Insert a fresh tip.

Take the vial labeled as Plasmid, kept in the ice box.

Open the vial and pipette out 3 microlitres of plasmid.

Recap the vial and keep it back in the ice box.

Take the vial labeled as Vial 1.

Open the cap and transfer 3 microlitres of plasmid into it.

Keep the vial back on the rack.

Discard the used tip.

Adjust the volume of micropipette to 0.5 microlitre.

Insert a fresh tip.

Remove the vial labeled as EcoR1 (\* read as E co R 1) enzyme from the ice box.

Open the vial and pipette out 0.5 microlitre of EcoR1 (\* read as E co R 1) enzyme.

Recap the vial and kept it back in the ice box.

Take the vial labeled as Vial 1.

Open the cap and transfer 0.5 microlitre of EcoR1 (\* read as E co R 1) enzyme into it.

Keep the vial back on the rack.

Discard the used tip.

Adjust the volume of micropipette to 0.5 microlitre.

Insert a fresh tip.

Take the vial labeled as BamH1 (\* read as Bam H 1) enzyme, kept in the ice box.

Open the vial and pipette out 0.5 microlitre of BamH1 (\* read as Bam H 1) enzyme.

Recap the vial and keep it back in the ice box.

Take the vial labeled as Vial 1.

Open the cap and transfer 0.5 microlitre of BamH1 (\* read as Bam H 1) enzyme into it.

Tap the vial at the bottom so as to mix the contents.

Open the centrifuge.

Place the vial in the rotor and place another vial with an equal volume of water exactly opposite to the first vial in the rotor to balance it.

Close the micro centrifuge.

Spin for a few seconds to mix the contents.

Open the micro centrifuge.

Now take out the vial.

Keep the vial in a rack.

Incubate the restriction digestion mixture in water bath at 37˚C (\* 37 degree Celsius) for 1 hour.

After 1 hour, take out the vial from the water bath.

Now, either you can perform Agarose Gel Electrophoresis or kept the cryo box with the vial in 4°C (\* 4 degree Celsius) freezer for short term storage.

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