

## PROTOCOL: Proof of transformation of *Escherichia coli*

### **Material and chemicals:**

PureYield Plasmid Miniprep System from Promega kit

Fresh overnight culture of transformed *E.coli*

Restriction enzymes EcoRI and HindIII from NEB (in our case), appropriate buffer(CutSmart)

Electrophoresis equipment - marker, loading buffer, 1x TAE, Ethidium Bromide or SybrSafe

### **Workflow:**

1. Prepare overnight culture in 10 ml of LB.
2. Centrifuge the overnight culture for 3 min, 9000 rpm and 20°C.
3. Isolate plasmid with PureYield Plasmid Miniprep System from Promega kit.
4. Measure concentration and purity on Nanodrop.
5. Prepare restriction reaction: For 500 ng of input plasmid DNA → 0.5 µl of EcoRI + 0.5 µl of HindIII + 2 µl of buffer (CutSmart).
6. Digest 2 h at 37°C.
7. Heat inactivate restriction enzymes in reaction mixtures at 80°C for 15 min, then keep at room temperature for 5 min.
8. Visualize product sizes on 0.6% agarose gel → we ran agarose electrophoresis for 40 minutes and 140 V in 1x TAE buffer.
9. Use Ethidium Bromide or SybrSafe for bend visualization.

### **Note:**

- Do not forget to use DNA Marker in your electrophoresis for comparing band length.