

## Transformation of DH5 alpha cells

Take 1 vial of competent cells (50  $\mu$ l) for plasmid transformation, or 2 vials of competent cells (100  $\mu$ l) for ligation mix transformation.

Use 1  $\mu$ l of 1:50 dilution of a miniprep plasmid DNA or 1  $\mu$ l of 10  $\mu$ l ligation mix for one transformation.

Incubate cells and DNA in a 12 ml opaque off-white tube and incubate on ice for 20 min. Heat-shock at 42°C for exactly 2 min. The temperature and time is very critical.

Sit on ice for 2 min.

Add 1 ml of SOC medium and incubate at 37°C with slow shake (100 rpm) for 45–60 min.

Transfer cells into an Eppendorf tube. Spin full-speed on bench-top centrifuge for 1–2 min. Aspirate supernatant but leave ~ 300  $\mu$ l of medium behind. Resuspend cells in the residual medium. Plate 100  $\mu$ l and 200  $\mu$ l of cells onto two LB+ amp plates. Incubate at 37°C overnight.