

Purpose: To insert plasmid DNA into bacteria

Transformations (Electroporation or Heat Shock)

To thaw:

1. Competent cells (on ice) ... Found in -80 freezer
2. SOC medium (@ room temp.) ... Found in -80 freezer
3. Turn on shaking incubator
4. Turn on water bath (Heat shock only)
5. Agar plates in incubator
6. The DNA (@ room temp)

Electroporation

1. Combined 40 μL of electrically competent DH5a cells and 1 μL of ligated DNA to an Eppendorf tube.
2. Transferred the contents of the Eppendorf tube to a cuvette and lightly tapped the cuvette on the table to evenly distribute the contents and to get rid of air bubbles.
3. Placed the cuvette into the Bio-Rad MicroPulser and delivered the electric shock.
4. **Immediately after**, added 900 μL SOC medium to the cuvette and micropipette mixed the solution.
5. Transferred the solution from the cuvette to a shaker tube and placed in the shaker at 37°C at 200 rpm for 1 hour.
6. After shaking for 1 hour, streaked 150 μL of the solution onto an agar plate with the respective antibiotics.
7. Incubated plates at 37°C for at least 24 hours.

Heat Shock

1. Thawed One Shot TOP10 chemically competent cells on ice (50 μL).
2. Added 2 μL of DNA sample into competent cells
3. Incubated the cells on ice for 35 minutes.
4. After the ice incubation, placed the samples into a 42° C water bath for 30 seconds.
5. **Quickly** took them out and **immediately** added 250 μL of SOC medium
6. Placed the samples into a 37° C shaking water incubator for 1 hour at 200 rpm.
7. After shaking for 1 hour, streaked 150 μL of the solution onto an agar plate with the respective antibiotics.
8. Incubated plates at 37°C for at least 24 hours.