

Making Competent Cells

Need: 1-50 ml Falcon tube of CaCl_2
5-15 ml minicentrifuge tubes
2-15 ml Falcon tube

Night Before

Prepare Stock of DH5 α cells

1. Add 5 ml of LB broth into 15 ml Falcon tube
2. Add loop of cells from plate
3. Incubate in shaking incubator at 37°C O/N

Next Day

Add 100 μl DH5 α mixture + 9.9 ml LB broth in 15 ml Falcon tube
↓ 3-4 hours in shaking incubator

[Add 500 μl of vial into 1.7 ml minicentrifuge tubes] x 5

↓ centrifuge at 10000 RPM for 5 min
-if no pellet at base raise RPM or add more culture

Remove Supernatant x 5

↓

[Add 500 μl CaCl_2 - resuspend solution] x 5 → 15 min on ice

↓ centrifuge at 10000 RPM for 5 min

Remove supernatant x 5

↓

[Add 100 μl CaCl_2 - resuspend solution] x 5

↓

30 min on ice → add DNA within this time

Result 5 minicentrifuge tubes containing 100 μl competent DH5 α cells