Competent Cells E.coli

Name:

Date:

***E.coli* strain to make competent:**

Materials and Equipment

Please book the Equipment listed below via our online booking system PPMS or by scanning the QR code on the system itself. You don't have a PPMS account yet? Feel free to contact us.
Please ensure the following is at hand and prepared in sufficient amounts.

- [ ] S1 Eppendorf 5920R
- [ ] Incubator Innova S441
- [ ] S1 Laminar Flow
- [ ] 30mL TFB1 for 100mL LB
- [ ] 2mL TFB2 for 100mL LB
- [ ] 100mL LB per strain
- [ ] selection marker if needed:
- [ ] 2x 50mL Falcon Tube per 100mL LB, chilled in -80°C
- [ ] Eppis
- [ ] liquid Nitrogen
- [ ] glycerol stock of strain of interest:
- [ ] original vial of strain of interest:

Buffers

TFB1	500mL	1000mL
30 mM K-acetate	1.47g	2.94g
50 mM MnCl	4.94g	9.88g
100 mM RbCl	6.04g	12.08g
10 mM $CaCl_2$	0.735g	1.126g
15 % Glycerol (v/v)	75mL	150mL

table 01 - TFB1 buffer composition

set pH to 5.8 with 0.2 M acetic acid, sterilize with filtering

TFB2	500mL
10 mM MOPS	1.07 g
75 mM $CaCl_2$	5.5 g
10 mM RbCl	0.6 g
15 % Glycerol (v/v)	75 mL

table 02 - TFB2 buffer composition

set pH to 7.0 with NaOH, sterilize with filtering

keep buffers at 4°C for storage

Day 1, date:

- inoculate 10mL LB in 50mL flasks from original *E.coli* stock or from glycerol stock
- add 10µL of your desired selection marker
- please note: if there is no selection marker needed, work as sterile as possible
- growth at 37°C, 180 RPM overnight

Day 2, date:

- inoculate 100mL LB with ___ mL overnight culture (e.g. 3-5mL)
- incubate at 37°C, 180 RPM until OD_{600} = 0.5 – 0.6
- please note: depending on the strain this may take 1.5 – 2.5h

time							
OD_{600}							

- transfer to 50mL Falcon Tubes, pre-chilled in -80°C
- 1st centrifugation at 3484xg, 20min. at 4°C
- resuspend pellets carefully in 15mL ice cold TFB1 buffer each (no vortexing)
- total volume should be 30mL from 100mL culture
- incubate on ice for 10 min.

- 2nd centrifugation at 23484xg, 20min. at 4°C
- resuspend pellets carefully in 1mL ice cold TFB2 buffer each (no vortexing)
- aliquot in 50 µL portions 1.5 mL Eppis, pre-chilled in -80°C, and freeze in liquid Nitrogen
- store at -80°C
- perform test transformation to see if procedure was successful (also include a blank transformation)

Day 3, date:

- check plates