DH10Bac Cells Protocol

*Use autoclaves glass/plastics

*Keep everything under a flame

Buffers:

TFB1 (200mL)

30mM KOAC
100mM RbCl
10mM $CaCl_2$
50mM $MnCl_2$
15% glycerol
pH to 5.8 by 1M HAC (acetic acid)
filter
store at 4C

TFB2 (50mL)
10mM MOPS
75mM $CaCl_2$
10mM RbCl
15% glycerol
pH to 6.5 1/ 2N HCl
filter
store at 4C

Plates→ LB + Bactoagar (to make 100mL aka 5 plates: 2g LB pellets + 0.75g Bactoagar)
100ul tetracycline + 50ul kanamycin after agar cools, pour plates

"Transformation"→Scrape some cells off frozen pellet (from 'Cells for Cloning' rack-DH10Bac box-Blue top tube)
Add cells to 1mL of SOC in 1.5mL tube, plunging pipette tip up and down
Rotate for 1 hour at 37C
Plate 100ul and 200ul of that on 2 plates
Incubate o/n at 37C

Next day:
Put plate in cold room first thing in the morning (wrap in plastic wrap)
Set up overnight: 5mL LB broth + 5ul tetracycline + 2.5ul kanamycin + 3 colonies in a 50mL conical tube. Can do up to three tubes to ensure growth in at least one.
Shake overnight at 37C

Next day:

Prepare 40mL LB broth in a 500mL glass flask (2x)
Add 40ul tetracycline + 20ul kanamycin + 200ul from overnight growth
Grow to OD 0.6-0.8 (should take 3-4 hrs, sometimes 5hrs)
Chill cells on ice for 10min, transfer to 50mL Falcon tubes
Spin down @ 3,500rpm @ 4C for 10min
Resuspend cells gently with 16mL TFB1 Buffer in each of the tubes. DO NOT vortex. Leave on ice for 5min
Spin down 3,500rpm @ 4C for 10min
Resuspend cells with 1.6mL TFB2 Buffer (in each of the 2 tubes)
Aliquot cells by 50ul in 1.5mL tubes
Freeze in liquid nitrogen
Store in -80C

Final step→ Ask someone to check the cells and compare growth to last time. DO NOT distribute cells to the lab until they're checked.

DH10Bac Cells Protocol (basic version)

6/3/99, from Blobel Lab, Ning Zheng based on protocol from Zhiping

Basically, 40 mL LB is used for one batch of preparation of one type of cells. 2X40 ml could be used too. 40 ml LB prep gives 1.6mL competent cells, which will be aliquoted into 50 uL. Each aliquot will be used for one transformation reaction.

Two buffers will be used: TFB1, TFB2.
Other stuff needed: liquid **N_2,** ice,

- Sterile TFB1(RbC1 transformation), total 200 ml

30 mM KOAC
100 mM RbC1
10 mM $CaC1_2$
50 mM $MnC1_2$
15 % glycerol(v/v)
adjust pH to 5.8 by 1M acetic acid (~80 uL) sterilize by filtration, store at 4°C indefinitely

- Sterile TFB2, total 50 mL 10mM MOPS

75 mM $CaCl_2$ 10 mM RbCl

0.105 g
0.551 g ($CaC1_2.2H_2O$)

0.060 g
7.5 m1(100%)

15% glycerol(v/v)
adjust pH to 6.5 with 2N HCl (~10 uL)

0.588 g
2.42 g
0.296 g ($CaC1_2.2H_2O$) 1.98 g ($MnC1_2$. $4H_2O$)

30 m1(100%)

sterilize by filtration, store at 4 °C indefinitely

Inoculate (3 colonies) into 40 ml culture, grow at 37°C until OD_{600} = 0.6 – 0.8

Chill the cells on ice for 10 mm, transfer to 50 mL falcon tubes (prechill and mark microcentrifuge tubes)

Spin down at 3500 rpm at 4°C for 10 min.

Resuspend the cells gently by 16 mL (2/5 v/v) TFB1 buffer and incubate 5 min on ice.

Spin down at 3500 rpm at 4°C for 10 min. Resuspend the cells by 1.6 mL (1/25) TFB2 buffer.

Aliquot the cells into 50 uL in microcentrifuge tubes. Freeze the cells in liquid N_2. Store at -80 °C.