preparation of KCM competent cells

Used instruments

glass beads or pipette
2x 5L Erlenmeyer flask
2x 250 mL Erlenmeyer flask
2x 100 mL Erlenmeyer flask
1x 100 mL bottle
1x 2L bottle
1x 1L bottle
1x 100 mL graduated cylinder
1x 2L graduated cylinder
1x 1L graduated cylinder
1x 50 mL graduated cylinder
big centrifuge & rotor for 250 mL flasks
6x centrifuge tubes (250 mL) with grey lids — autoclavable, labelled HRP
BIG Iceboxes
pipette boy
sterile pipettes — 5mL, 10 mL, 25 mL
pipette and sterile tips — 100 uL
lots of sterile 1.5 mL Eppendorf vials — approx. 100 per freezer box

Day 1

prepare plate with single clone

- prewarm BAB plate w/o antibiotics at 37°C for approx. 1h
- thaw 100 uL aliquot of desired cell line on ice for 10 min
- dilute 1 uL of that aliquot in 500 uL TSB medium and streak out 25 uL on plate

Day 2

look for overnight plate

- single clones obtainable?
-

prepare media and sterile instruments (forenoon)

- solve 50 g of LB broth in 2L milliQ (in 2L bottle)
- weight in and solve:

chemical	**stored at**	**amount**	**volume MilliQ**	**vessel**
$MgSO_4$ (anhydrous)	wood cupboard of balance room	12.04g	100 mL	Erlenmeyer flask
$MgCl_2$ * 6 H_2O	wood cupboard	20.80g	100 mL	Erlenmeyer flask

- prepare “competency solution”
  - 800 mL of broth to 1 L bottle with magnetic stirrer
  - adjust pH to 6.1 (with HCl or NaOH)
  - add
    - 50 mL DMSO
    - 10 mL of $MgSO_4$ solution
    - 10 mL of $MgCl_2$ solution
    - 100 g PEG6000 (stored under big balance)
  - stir until everything is resolved
- prepare glycerol
  - fill 100 mL of 85% glycerol in 250 mL bottle
- split LB broth
  - 2x 500 mL in each 5L Erlenmeyer flask, apply “bomullskorkar” to opening

    - 2x 35 mL in each 250 mL Erlenmeyer flask
- prepare for autoclave by putting aluminum foil and autoclaving tape on each opening
    - competency solution
    - glycerol
    - 5L Erlenmeyer flasks
    - 250 mL Erlenmeyer flasks
- prepare centrifuge tubes
    - add approx. 5 mL H_2O in them
    - close lids slightly
- autoclave this stuff (ask Mehri for starting)
    - takes approx. 3h so make sure to start in time

inoculate overnight culture

- take a single colony from overnight plate and inoculate one of the 250 mL Erlenmeyer flasks
    - approx. 16-17h
- put it into 37°C room for shaking
- also put the uninocculated 250 mL Erlenmeyer flask into the 37°C room (as negative control)

Day 3

prepare solutions

- place in cold room
    - centrifuge tubes
    - competency solution
- precool centrifuge and suitable rotor to 4°C

growth culture

- add 12.5 mL of overnight culture to each of the 5L Erlenmeyer flasks (approx. 8-9 a.m.)
- measure OD_{600} after 15-30 minutes and in regular intervals until it reaches approx. 0.6
    - doubling time = 20 mins

make cells competent

- cool cultures in ice bath in cooling room as soon as they reach OD_{600} of 0.6
- refill cultures to centrifuge tubes (after emptying H_2O, of course)
- spin at 3000 rcf and 4°C for 5 min
- carry rotor to cold room
- discard supernatant and resuspend culture in 50 mL competency solution in total
- add 7 mL of 85% glycerol
- cool on ice for 10 min
- aliquot per 100 uL in sterile 1.5 mL Eppendorf vials
- store vials at -80°C