COMPETENT CELLS (based on Hanahan 1983 J Mol Biol 166, 557–580)

- STREAK a fresh plate the day before inoculation from parent stock (NOT previous
- batch of competent cells: glycerol stock only) with appropriate antibiotic using sterile
- technique and grow O/N 37°C.
    - XL1 or 2 Blue=TetR.
    - TOP-10=StrepR (plate without antibiotic for TOP-10).
    - Other strains: check with parent company (websites).
- INOCULATE a single colony into 5 ml **TYM** + antibiotic in a 14 ml Falcon 2059 tube; grow in shaker incubator at 37°C, 225 rpm.
- INOCULATE 1 ml O/N culture into 500 ml **TYM** prewarmed to 37°C in a 2 l Erlenmeyer flask (NO antibiotic!!); grow in shaker incubator at 37°C, 225 rpm.
- GROW until OD 550=0.5 (approximately 2.5 h); take sample to measure OD but keep the culture shaking and at 37°C.
- COOL culture rapidly in ice water, 2 min with swirling.
- TRANSFER culture to pre-chilled 50 ml conical tubes.
- SPIN 3,000 rpm, 10 min, 4°C: NO BRAKE! (pre-cooled swinging bucket rotor)
- REMOVE SUP, CAREFULLY tap pellet and GENTLY resuspend in 20 ml **Tfb1**/tube. Try to get rid of clumps, but always be gentle.
- INCUBATE ON ICE in cold room for 1.5 h.
- SPIN 3,000 rpm, 10 min, 4°C: NO BRAKE!
- REMOVE SUP, CAREFULLY tap pellet and GENTLY resuspend in 1.5 ml.
- ALIQUOT 200 µl to pre-labeled and pre-chilled Eppendorf tubes.
- FREEZE ON DRY ICE and STORE immediately at -70°C.
- Calculate cfu/µg DNA using 3 serial 1/10 dilutions of 1 ng/µl pUC18.

TYM: autoclave a solution containing

2% Bacto-Tryptone
0.5% Bacto-Yeast
0.1 M NaCl
0.01 M $MgCl_2$

Tfb1: filter sterilize, store at 4°C

Make from autoclaved stocks to give final concentrations of
30 mM KOAc
50 mM $MnCl_2.4H_20$
100 mM KCl
10 mM $CaCl_2$
15% w/v glycerol
Mix well, pH to 5.8 with 0.2 M acetic acid

Tfb2: filter sterilize, store at 4°C

Make from autoclaved stocks to give final concentrations of
10 mM Na-MOPS pH7.0
10 mM KCl
75 mM $CaCl_2$
15% ***w/v*** glycerol