TRF_1v2

Pombe Transformation Protocol

(modified *S.cerevesiae* protocol; for preparation of 5-10 transformations)

by M.Rowley and S.Braun as of 9/17/07 based on Krogan lab protocol;
updated by PG on 2016/06/13

Day 1: Prepare pre-culture in YES medium

Day 2: Inoculate o/n culture in appropriate culture vol (e.g. 50 mL-100 mL) 1:500 -1:1000 (1:1000 dilution of saturated pre-culture takes 16-18 hours to reach mid-log phase)

Day 3: Measure OD_{600} – culture should be late log-phase (>0.4 and <1.0). If culture passed late log-phase (>1.0 but <2.0), dilute culture to OD of 0.2 in 100mL fresh media and grow to an OD of 0.8-1.0 (this may take 5 – 7 hrs; at least 2 doublings are required for optimal growth)

- Transfer 25-50 mL culture to 2 Falcon tubes and pellet cells (5min at 2K)
- Wash cells in 10 mL MQ-H_2O (resuspend pellet in water and spin down as before)
- Wash cells in 5 mL LiOAC /TE Solution (pH 8)
- Resuspend pellet in 0.5-1 mL LiOAc/TE Solution (pH 8)

For Each Transformation:

pre-incubation:

- Aliquot 100uL of cells into Eppendorf tube
- Add DNA (0.5 - 2.0 µg PCR product with 500 bp homology; at least 10 µg for PCR products with 100 bp homology
- Add 10 µl Carrier DNA (10 mg/mL Salmon Sperm), denatured
- Vortex
- Incubate on bench for 15 min
- Add 5x sample volume (cells + DNA + SS-DNA) PEG/LiOAc Solution – Mix by pipetting up and down or vortexing
- Incubate at 30°C for 30-50 min (optionally with nutating)

heat shock:

- Add 9/100 of the total µl DMSO – pipet up and down or vortex
- 42°C water bath (or thermoblock) for 10 min

Recovery:

- Pellet Cells – 3 min at 400 xg
- Remove Sup and wash by resupending cells in 500 µl YES – pellet again
- Remove Sup
- Resuspend in 100 µl YES
- Plate on YES – grow at 30°C for 2-3 days
- Replica-plate onto selective media

Solutions:

LiOAc/TE Sol'n – Total volume of 2 L:
1M LiOAc (pH 7.5) – 200 mL
1M Tris/HCl (pH 8) – 20 mL
0.5M EDTA (pH 8) – 4 mL
Water up to 2L – 1,776 mL

PEG/LiOAc Sol'n (PE) - Total volume 500mL
50% PEG – 400 mL
1M LiOAc – 50 mL
1M Tris/HCl (pH 8) – 5 mL
0.5M EDTA – 1 mL
Water up to 500mL – 44 mL

Velvet cleaning

3-4 x wash in hot water:EtOH (3:1)
2-3 x wash in dest. Water
dry it on air
wrap in alufoil
Atoklav: Progr. 1 (Festkörper)
Trockenschrank (ca. 2 days (or 2x 8h)
At 80 degr.)

Reserve at Marianne (shelf at desk, box
“Samt”