Rubidium Chloride Competent Cell Protocol

Protocol used for the Lab Job of making competent cells.

Materials:

Prepare following solutions:

TFB1	Concentration	FW
Rubidium Chloride	100 mM	120.92
Manganese Chloride (MnCl2 H20)	50 mM	197.91
Potassium Acetate	30 mM	98.14
Calcium Chloride (CaCl2H2O)	10 mM	147.02
Glycerol	15%	

Adjust to pH 5.8 with dilute (0.2%; 1.0 M) acetic acid. Do NOT overshoot. Filter sterilize. Store at room temp but bring to 4C before use.

TFB2	Concentration	FW
MOPS	10 mM	209.3
Rubidium Chloride	10 mM	120.92
Calcium Chloride	75 mM	147.02
Glycerol	15%	

Adjust pH to 6.5 with KOH. Do NOT overshoot. Filter sterilize. Store at room temp but bring to 4C before use.

2X YT media

16 g Bacto tryptone

10 g Bacto yeast extract

5g NaCl

Add 900 mL water and adjust to pH 7.0 with 5N NaOH.

Bring up to 1000L and autoclave.

Or TEKZR097 in CCF 48h delivery (~$40)

Methods:

Step 1: Culture bacteria.

Streak/plate bacteria of choice on LB agar plate.

Inoculate single colony into starter culture of 20 mL SOC media in 125mL Erlenmeyer flask.

Incubate overnight in 30C or 37C shaker.

Inoculate growth culture 1:100 with starter culture. (2.5 mL of starter into 250 mL 2X YT media in 1L Erlenmeyer flask). Put in 37C shaker.

Grow until OD600 reaches 0.4 to 0.6 (~5h)

After this point, keep everything cold. Work in cold room and pre-chill all supplies.

Step 2: Collect and treat bacteria.

Transfer bacteria to culture flasks and spin down. 5000 x g, 4C, 10 mins.

Pour out supernatant.

Gently rinse flasks and pellet with small aliquot of TFB1 to remove all traces of media.

Add 100 mL TFB1 per 250 mL of growth culture and resuspend using 10ml serological pipette.

Incubate in wet ice 5 minutes.

Spin down 5000 x g, 4C, 5 mins.

Remove all supernatant.

Add 10 mL TFB2 per 250 mL growth culture and gently resuspend by serological pipette.

Incubate in wet ice 15-60 mins.

Step 3: Dispense and freeze bacteria

Having another person will be helpful at this point

Dispense 100 ul into pre-chilled 1.5 mL microcentrifuge tubes and snap freeze in LN2.

Store at -80C in freezer boxes without dividers.