BACILLUS SUBTILIS TRANSFORMATION PROTOCOL

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Day 1

1. Set up a 4ml O/N culture of PB047 *(B. subtilis* 168 wt) in plain LB
2. Grow at 37°C, 225 rpm for 14-18 hours
3. Ensure clean, autoclaved 250ml conical flasks as well as all of the stock solutions listed in the recipe section below are available for use tomorrow

Day 2

1. Make fresh SM1 in a sterile 125ml conical flask and pre-warm at 37°C
2. Transfer 1ml of saturated O/N culture to 15ml pre-warmed SM1. Final OD_{600} should be between 0.4-0.6. Adjust concentration as necessary, ensuring the final culture volume is close to 15ml
3. Incubate culture flask at 37°C, 225rpm until the culture departs log phase. I suggest taking OD_{600} readings of 1:4 diluted aliquots every hour for the first 3 hours, then every 20 min thereafter. It usually takes between 4-5 hours, depending on the incubator with a final 1/4 OD_{600} between around 0.5-0.7.
4. Once the culture reaches stationary phase, add an equal volume of pre-warmed SM2 and incubate a further 90 minutes. At this point, and for the next 2 or so hours (haven't tested beyond that) the cells are highly competent.
5. Remove 500 µl of cell culture and combine with 5 µl of plasmid.
6. Incubate at 37°C for 30 min on an end over end rotator.
7. Add 300 µl LB and incubate for further 30 min at 37°C rotating.
8. Plate desired volume of cells on selective media (transformation efficiencies of $2x10^4$-$2x10^5$ transformed cells per ug plasmid from DH5α prepped DNA have been observed in our lab).

*Note: to freeze competent cells; after **step 4**, add glycerol to 10% (v/v), freeze 500ul aliquots at -80°C. You should expect a 2-fold or greater reduction in transformation efficiency upon freezing. Long term viability has been a problem, but for short term storage (weeks to a month) this works great.*

BACILLUS SUBTILIS COMPETENT CELL PREP RECIPES

1X ST Base (1L)

- Ammonium sulfate 2g
- K_2HPO_4 12g
- KH_2PO_4 6g
- Sodium citrate dihydrate 1g
- ddH_2O up to 1L
- pH should be ~7.0
- Autoclave

Supplements (Stocks) – unless indicated otherwise store all solutions at room temp

1. $MgSO_4 \cdot 7H_2O$ (0.3M)
    - Dissolve 7.40g in 100ml ddH_2O
    - 100X in SM1, 400X in SM2
2. $CaCl_2 \cdot 2H_2O$ (0.3M)
    - Dissolve 4.41g in 100ml ddH_2O
    - 100X in SM2, not used in SM1
3. 50X YECA
    - 5g Bacto yeast extract
    - 0.625g Casamino acids
    - Make up to 50ml with dH_2O
    - May need heating at 60°C to fully go into solution
    - Filter sterilize
    - 50X in SM1, 100X in SM2
4. Tryptophan (10mg/ml)
    - Dissolve 500mg of L-tryptophan in 50ml ddH_2O
    - Filter sterilize, store at 4°C
    - 100X in all solutions
5. 50% Glucose
    - Dissolve 25g glucose (Dextrose) in 50ml ddH_2O
    - Filter sterilize
    - 100x in all solutions

To make SM1 and SM2 combine the following

Reagent	**SM1 (15ml)**	**SM2 (15ml)**
ST Base	15 ml	15 ml
$MgSO_4$ (0.3M)	37.5 µl (.75mM)	150 µl (3mM)
50X YECA	300 µl	150 µl
Tryptophan (10mg/ml)	150 µl (.49mM)	150 µl (.49mM)
50% Glucose	150 µl (27.75mM)	150 µl (27.72mM)
$CaCl_2$ (0.3M)	-	75 µl (1mM)

Notes
Calcium chloride final concentrations >2mM cause $CaPO_4$ precipitate to form
P. Levin lab recommends final pH of base should be 7.5 - it may be adjusted by adding more K_2HPO_4 and less KH_2PO_4 to make solution more basic