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Home-made TOP10 competent heat-shock cells

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1 Works for me dx.doi.org/10.17504/protocols.io.pbtdinn

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ABSTRACT

The standard protocol to propagate TOP10 Competent cells for Heatshock transformation method

EXTERNAL LINK

openwetware.org

BEFORE STARTING

Make TSS buffer:

- 5g PEG 8000
- 1.5 mL 1M MgCl2 (or 0.30g MgCl2*6H20)
- 2.5 mL DMSO
- Add LB to 50 mL
- Filter sterilize (0.22 µm filter)

Note 1: PEG 3350 can be used instead of PEG 8000. According to the original CT Chung paper, PEG 3350 produces better efficiency, and other sized PEGs can be used as well albeit with a slight loss in efficiency.
Note 2: pH from original Chung CT paper calls for pH to be acidic (pH=6.5)... be sure to pH solution before sterilization.
Note 3: If using non-chemically resistant filters (e.g., cellulose nitrate), add DMSO after sterilization. DMSO should be sterile in and of itself, so it may be prudent it add it afterwards if you are unsure about the compatibility of your filters.

1 Grow a 5ml overnight culture of cells in LB media. In the morning, dilute this culture back into 25-50ml of fresh LB media in a 200ml conical flask. You should aim to dilute the overnight culture by at least 1/100.. Grow the diluted culture to an OD600 of 0.2 - 0.5. (You will get a very small pellet if you grow 25ml to OD600 0.2)

2 Put eppendorf tubes on ice now so that they are cold when cells are aliquoted into them later. If your culture is X ml, you will need X tubes. At this point you should also make sure that your TSS is being chilled (it should be stored at 4oC but if you have just made it fresh then put it in an ice bath).

3 Split the culture into two 50ml falcon tubes and incubate on ice for 10 min.

4 Centrifuge for 10 minutes at 3000 rpm and 4oC.

5 Remove supernatant. The cell pellets should be sufficiently solid that you can just pour off the supernatant if you are careful. Pipette out any remaining media.

6 Resuspend in chilled TSS buffer. The volume of TSS to use is 10% of the culture volume that you spun down. You may need to vortex gently to fully resuspend the culture, keep an eye out for small cell aggregates even after the pellet is completely off the wall.

7 Add 100 µl aliquots to your chilled eppendorfs and store at − 80oC.The original paper [1] suggests freezing the cells immediately using a dry ice bath. I (BC) have used liquid nitrogen quite successfully instead of dry ice. Simply placing the cells at − 80oC also seems to work well (Jkm)If you run a control every time you clone (i.e. a vector-only ligation), you can as well freeze cells in 200 µl aliquots. Unused cells can be frozen back once and reused, albeit with some loss of competence.

8 It is a good idea to run a positive control on the cells.