Making Heat-Shock Competent Cells

Overview:

This protocol is generously provided by the lab. We use DH5α *E. coli* strains.

Materials:

- 2xTY media: 1 L

| Component | Volume & Mass | Procedure | |
|---------------|---------------|---|--|
| Bactotryptone | 16 g | Adjust volume to 1 L Sterilize by autoclave | |
| Yeast Extract | 10 g | | |
| NaCl | 5 g | | |

- SOB: 1 L

| Final [c] | Component | Volume & Mass | Procedure |
|-----------|---------------|--------------------|---------------------------------|
| 2 % | Bactotryptone | 20 g | |
| 0,5 % | Yeast Extract | 5 g | 1) Add NaOH until |
| 10 mM | NaCl | 2 ml (5 M stock) | pH is 6.7, adjust volume to 1 L |
| 2,5 mM | KCI | 2.5 ml (1 M stock) | 2) Sterilize by autoclave |
| 10 mM | MgCl2 | 10 ml (1 M stock) | 33.55.5.76 |
| 10 mM | MgSO4 | 10 ml (1 M stock) | |

- Transformation Broth (TB): 1 L

| Final [c] | Component | Volume & Mass | Procedure |
|-----------|-------------------|---------------|--|
| 10 mM | Pipes | 3.02 g | 1) Mix the Pipes, CaCl ₂ , |
| 15 mM | CaCl ₂ | 2.21 g | and KCl in 900 mL of millipore water. |
| 250 mM | KCI | 18.64 g | 2) Add NaOH until pH is 6.7 (Don't worry, dust |
| 55 mM | MnCl ₂ | 10.89 g | disappear after the pH is adjusted) 3) Add MnCl2 (see above), stir, adjust volume to 1 L 4) Filter sterilize 5) Store at 4°C |

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Procedure:

DAY ONE:

- 1) Grow 12 ml overnight culture of your favorite strain of *E. coli* in 2XTY (preheat medium at 37°C before inoculation)
- 2) Make SOB and TB.

DAY TWO:

- 1) Keep 5mL of SOB for initial OD.
- Inoculate 1 L SOB with 12 ml overnight culture.
- 3) Grow culture at 18C (this temperature is really important as we see a 10-fold decrease in competency when we grow them at room temperature).

DAY THREE:

1) Grow cells until A₆₀₀ 0.5-0.7.

Subsequent steps should be carried out in the cold room on ice:

- 2) Put flask on ice for 10 minutes, then spin cells down at 2500xg (3350 RPM)
- 3) Pour off supernatant
- 4) Resuspend gently first in 25 ml TB, then add remaining 295 ml (Final 320mL)
- 5) Leave on ice for 5 minutes
- 6) Spin down again at 2500xg for 10 minutes
- 7) Resuspend cells in 40 ml TB
- 8) Add 3 ml of DMSO dropwise while gently shaking [final DMSO concentration is 7%].
- 9) Aliquot in 100 µl aliquots (you will need about 450 pre-chilled 0.5 ml tubes).
- 10) Flash freeze in liquid nitrogen.
- 11) Store at -80C.

Heat-Shock Transformation of chemically competent bacteria

- 1) Take competent cells out of -80°C and thaw on ice (approximately 20-30min).
- 2) Take agar plates (containing the appropriate antibiotic) out of 4°C to warm up to room temperature or place in 37°C incubator.
- Label one 2.0mL microcentrifuge tube for each transformation and then pre-chill by placing the tubes on ice.
- 4) Pipet 1 to 5 μ L of DNA (usually 10pg to 100ng) into each microcentrifuge tube. For each transformation, use a separate tube.
- 5) Pipet 50 μL of competent cells into each tube. Flick the tube gently with your finger to mix. Incubate on ice for 30 minutes. Pre-heat waterbath now to 42°C.
- 6) Heat shock each transformation tube by placing the bottom 1/2 to 2/3 of the tube into a 42°C water bath for 45 seconds. Do not mix. Be careful to keep the lids of the tubes above the water level, and keep the ice close by.
- 7) Put the tubes back on ice for 5 min. Do not mix. This helps the cells recover.
- 8) Add 200µl LB+glucose media (20mM final) **without antibiotic** and grow in 37°C shaking incubator at 180 rpm for 60min.
- 9) Prepare the agar plates during this time: label them, and add sterile glass beads if using beads to spread the mixture.
- 10) Plate some or all of the transformation onto a 10 cm LB agar plate containing the appropriate antibiotic.
- 11) **Note:** We recommend that you plate 50 µL on one plate and the rest on a second plate. This gives the best chance of getting single colonies, while allowing you to recover all transformants.
- 12) Incubate plates overnight at 37°C. Position the plates so the agar side is facing up, and the lid is facing down.
- 13) Pipette 5 ml of LB media containing the correct concentration of antibiotic into sterile tubes
- 14) Pick an isolated bacterial colony with a tip and place the tip with bacteria on it into one of the tubes containing LB liquid media.
- 15) Culture the tubes overnight in an incubated orbital shaker at 37°C at 180rpm. The next day you can continue with miniprep protocol.