Heat Shock/Chemical transformation (TSS method)

The heat/chemical shock transformation method is a quick, economical method for transforming (inducing cell uptake of) self-propagating DNAs (plasmids) and possibly linear non-propagating DNAs under conditions favoring integration into resident DNA.
The TSS method starts by cooling cell culture to arrest growth in exponential phase, in which the cell wall is least developed, facilitating passage of DNA. The cells are stored in TSS, a solution of: the rich nutrient medium LB, an organic solvent DMSO to permeabilize the cell membranes, the viscous polymer polyethylene glycol (PEG) to reduce DNA diffusion and facilitate binding to the cell, and a divalent cation salt, magnesium chloride, to neutralize the divalent charge of DNA phosphodiesters and LPS charge and disrupt protein–membrane/LPS interactions that occlude DNA binding. Cold temperature crystallizes the membrane, stabilizing pores called adhesion zones. The DNA to be transformed is added and incubated to give time for binding and penetrance of cells, facilitated by a heat shock that supposedly creates a temperature differential that induces flow that carries shielded DNA through adhesion zones. Recovery in non-selective medium allows expression of antibiotic resistance from the transformed DNA, necessary for survival of plated transformants on selective medium.
The TSS method produces highly competent cell for most *E. coli* cloning strains, yielding **10^6–10^7 CFU/µg**pUC19 transformation efficiency, to 1/10,000–100,000 plasmid molecules transformed into 0.01–0.1% of viable cells.

Based on the Chung *et al.* protocol [1] . Modified by doubling cell concentration and adding heat-shock, which improve efficiency. Composed by Shyam Bhakta.

Competent Cell Preparation

For *n* transformations of *v* volume:

Materials

1. **Transformation/storage solution (TSS), *nv* volume**
   TSS: Lysogeny Broth (LB) 10% $_{m/V}$ PEG-3350, 5% $_{V/V}$ DMSO, 20 mM $MgCl_2$, pH 6.5.
   For 50 mL TSS from non-sterile components:
   - Dissolve 5.263 g PEG-3350 and
   - 0.3103 g $MgCl_2 \cdot 6H_2O$ or 1.053 mL 1 M $MgCl_2$
   - in a *final* 50 mL LB
   - Adjust pH to 6.5 using concentrated HCl. (pH 6.4–6.8 optimum)
   - Filter-sterilize. Transfer 47.5 mL to a sterile tube.
   - Last, add 2.5 mL DMSO (auto-sterile, filter-incompatible).
     Store at 4°C.

   For 50 mL TSS from sterile components (*cannot be pH-adjusted to optimum*)
   - 42.5 mL LB (autoclaved, RT)
   - 5 g PEG-3350 in 5 mL H_2O (filter-sterilized)
   - 1 mL 1 M $MgCl_2$ (autoclaved, RT)
   - 2.5 mL DMSO (auto-sterile)
     Store at 4°C.
2. Sterile rich growth medium (+antibiotic if necessary)
   20*nv* volume LB or 7*nv* volume TB or LB^{30}
   - *10–20 mM-Mg^{2+} in growth media considerably stimulates transformation efficiency* [(2)] [(3)] .
3. Culture tubes for precultures
4. Culture flasks large enough to hold growth medium volume (detergent-free).
5. Shaking incubator space for the culture flasks.
6. Ice bucket/tray large enough to swirl flasks in. Access to ice.
7. Chilled centrifuge bottles/lids or tubes appropriate for holding culture volumes and balancing. (detergent-free)
   - Bottles must be able to collectively hold culture(s) without any exceeding ¾ capacity. Filling bottles higher will result in leakage into rotor.
8. Chilled sterile water to balance multiple centrifuge bottles, if needed.
9. 4°C centrifuge and rotor compatible with centrifuge bottles.
10. (*opt.*) A serological pipette at -20°C for adding *nv* volume TSS before going to cold room.
11. Labeled cryoboxes for comp cell aliquots, prechilled at -80°C.
12. (*opt.*) Liquid nitrogen, dewar, and slotted ladle, if flash freezing.
13. Materials on a mobile lab bench cart for the cold room:
    a. *n* tubes (e.g. 200 µL tubes) for cell aliquots, labeled/marked and arranged in clean tube racks or tip rack+boxes. Blade, if planning to cut apart tube strips.
    b. Extra tubes or bag of tubes, for aliquotting any excess comp cells, if desired.

c. Serological pipette pump/controller and few 20 mL pipettes, for resuspending and transferring cells.
d. (*opt.*) Electronic repeater pipette and a 5 mL combitip per strain and some extras. Reservoirs, if opting to us a multichannel pipette.
e. A 50 mL tube per strain to hold resuspension.
f. Micropipettor and tips appropriate for aliquot volumes *v*. (Sometimes useful to aliquot remaining volume even when using electronic repeater pipette.)
g. Ethanol spray
h. Paper towels
i. Extra gloves
j. Trash bin/bag
k. (*opt.*) If flash freezing, box/bag to collect all tubes in before flash-freezing.

Procedure

1. Grow preculture to saturation: inoculate 1/100 culture volume of rich medium (+selection, if necessary) with colony or frozen stock chunk, and incubate at growth temp (*e.g.* 37°C) shaking for 8 hr for LB.
    - *Use a fresh, trusted source of the strain. For the most reliable results, inoculate a seed culture from colonies from relatively fresh plates streaked from a frozen stock. Don't obtain from old plates; retransform necessary plasmid(s) or obtain directly from a frozen stock.*
2. Meanwhile, prepare growth medium and warm to growth temperature.
   Prepare the mobile lab bench cart for the cold room as described.
3. Inoculate 20*nv* mL pre-warmed LB with 0.2% seed culture (or 7*nv* mL TB/LB^{30} with same).
   Incubate 37°C, shaking 250 rpm for flasks or 800 rpm for blocks.
    - *10–20 mM-Mg^{2+} in growth media considerably stimulates transformation efficiency* [(2)] [(3)] .
    - *Lower growth temperature reduces efficiency considerably with the TSS method, based on comparisons of 22°, 30°, and 37° culturing [Shyam].*

   Grow LB culture to OD=0.2–0.35 (early exponential phase). For TB/LB^{30} OD1–2. It requires 1.5–3 hours at 37°C, 250 rpm in a large flask, a bit slower at 30°C. Monitor with periodic OD measurement.
    - *Transformation efficiency from LB cultures decreases linearly between OD=0.3–0.6.* [(1)]
    - *Several tenfold lower-efficiency at stationary phase (10^5–10^7/µg DNA), but still fine for transforming pure plasmid or the simplest cloning, supposedly.*

   In the meantime, prechill both the mobile lab bench cart (with its contents) and the centrifuge to 4°C 45 min before cell harvest (next step).
4. When in early log phase (OD0.2–0.35), swirl culture vessel in ice water bath for several minutes *until 4°C* to rapidly stop growth. It takes up to 20 min for ~1 L volumes.
5. When culture is ice-cold, decant into prechilled centrifuge bottles and balance them. Use chilled sterile water if necessary.
   Centrifuge cells 2500×*g*, 5–20 min (depending on volume) in a prechilled 4°C centrifuge.
6. Check for a small pellet. Gently decant medium away from pellet, shaking the bottle to drain. Absorb the last of the medium on the lip with a paper towel. Return bottles to ice.

   Perform remaining steps with pre-chilled materials in the cold room for maximum efficiency.
7. To the cell pellets, add 5% the original culture volume of 4°C TSS (*nv* mL). For TB/LB^{30} culture, 14% volume TSS.
   Gently resuspend pellet in TSS using serological pipette, or P1000 tip if small volume.
   For large volumes, decant cells into 5 or 50 mL conical tubes on ice for easier access with pipette for aliquotting.
    - *Flat-bottom centrifuge bottles allow easier gentle resuspension of pellets by pipetting against wall.*
    - *Original protocol instructs 1–10-fold concentration in TSS. 20-fold as here, or even 40-fold increases transformation efficiency, but requires larger culture with diminishing returns in efficiency.*

8. Aliquot into tubes in the cold room (for top efficiency) or on cold block or ice. You can use a repeater pipette or multichannel pipette from a chilled reservoir.
9. Cap the tubes while they are racked, and slice apart if using tube strips. Minimize touching the tubes to keep them cool. Check that all caps are fully in the tube.
   Dislodge tubes from racks into a bag/box without touching the bottom (your hands are warm). This is easiest by pushing them out from the bottom using a spare rack or tip box.
10. (*opt.*) Flash-freeze in liquid nitrogen, supposedly for maximum efficiency.
    - *Slow freezing works well, too, maybe even better. You can likely omit this step.*
    - *Residual ethanol from a dry-ice-ethanol bath fails to dry from tubes/boxes at -80°C. Flash-freeze tubes in a bag if using this method, and pour tubes into box.*
11. Quickly move tubes to prechilled, labeled -80°C freezer boxes, either directly from 4°C, or if flash freezing, directly ladled out of liquid nitrogen dewar or dry-ice/ethanol bag (so as not to heat tubes).
    If not freezing, proceed to transformation right away.

Detergent Residue

According to Tom Knight [(4)] : Detergent is a major inhibitor of competent cell growth and transformation. Glass and plastic must be detergent-free for these protocols. The easiest way to do this is to avoid washing glassware and simply rinse it out. Autoclaving glassware filled ¾ with deionized water is an effective way to remove most detergent residue. Media and buffers should be prepared in detergent-free glassware and cultures should be grown in detergent-free glassware.

Choice of Culture Medium

Shyam found that competent cells prepared from a TB culture produced far more satellite colonies in a transformation of pUC19[Amp^R], compared to competent cells prepared from an LB culture, both plated on LB-amp^{100} plates. The effect was mostly apparent after plates sat at room temperature for several days.

ZymoBroth™ [(3)] , containing only 0.5–5% yeast extract and tryptone (LB components) and 10 mM $MgCl_2$, improves many strains' competence using an unspecified protocol, 13-fold for TG1, the NEB Turbo parent. $MgCl_2$ in the growth medium may thus be more effective than the 20 mM $MgCl_2$ in TSS is alone. Even before, Hanahan (1983) [(2)] found that "the presence of 10 to 20 mM Mg^{2+} in all growth media considerably stimulates transformation efficiency," as well as "incubation of the cells at 0°C in a solution of Mn^{2+} , Ca^{2+} , Rb^+ or K^+ , dimethyl sulfoxide, dithiothreitol, and hexamine cobalt(III)."

These enhancements were later found to be more specific to strain MC1061 and derivatives like DH10B, and not strains derived from Hoffma n Berling strain 11008 (*e.g.*, MM294, DH1, DH5) as well as from many other strains (*e.g.*, HB101, C600), for which Mg^{2+} is *not* beneficial in the growth medium, and the addition of either DMSO or DTT to the transformation buffer reduces competence [(5)] .

Transformation

Summary for frozen lab aliquots

1. **Thaw** comp cell aliquots (50 µL) o*nice* for a few min.
   Use one per assembly reaction.
   An aliquot can be split/distributed; 5 µL is sufficient to transform a purified plasmid.
2. **Add DNA** 5 µL. Flick five times to mix. Don't vortex or triturate (pipette-mix).
3. **Incubate on ice** for 5 min, best 30 min.
4. **Heat shock** 42° for 30 s in a heat block or water bath.
5. **Return to ice** immediately for 1–2 min.
6. **Recovery**: remove from ice and add **150**–200 µL room-temp non-selective recovery medium (e.g. SOC). Flick to mix.
   Incubate at growth temp (*e.g.* 37°C) 45–90 min stationary.
7. **Plate.**

Detailed

1. Thaw -80°C comp cell aliquots on ice or cold block for a few min. Or use comp cells within hours of comp cell prep. Keep at 4°C except for heat shock and recovery.
   - *Unused volume of comp cells can be frozen back once and reused, albeit with some loss of competence. Lab aliquots are*

small/abundant enough not to merit this.

2. *optional:* Add 4°C 5× KCM to 1×.
    - *Twofold lower efficiency for NEB Turbo. Neutral for DH10B and OmniMAX. Somewhat improves transformation efficiency of DB3.1 [Shyam].*
3. Add DNA, aiming for 10% comp cell volume.
4. Flick five times to mix. Do not vortex or triturate.
5. Incubate at 4°C (ice or cold block) for 5–30 min.
    - *Efficiency maximizes by 30 min, supposedly.*
6. Heat shock at 42°C for 30–45 s in a heated block or water bath.
    - *15–30 s optimal for NEB Turbo (3.0E7 CFU/µg in water bath). Efficiencies fell for longer heat shocks: 28% loss for 45 s, 31% for 60 s, and 61% for 90 s [Shyam].*
    - *45 s purportedly close to optimal for many strains. Some strains/preparations/methods might do well with up to 90 s.*
    - *78% loss in efficiency in metal bead bath than water bath for 45 s heat shock of NEB Turbo [Shyam].*
    - *People on OpenWetware find heat shock after ice incubation improves efficiency 10–20 fold [(4)] .*
7. Immediately return to 4°C (ice or cold block) for at least 1 min.
8. Remove from 4°C. Add 1–10 volumes room-temp recovery medium (non-selective). Flick to mix (trituation or gentle vortexing may be ok).
    - *Adding 3 volumes (150 µL) SOC to lab aliquot standard of 50 µL NEB Turbo transformations in the original 200 µL tubes, flicking, and incubating stationary 37° gave just as good efficiency as recovering in 10 volumes SOC in a test tube incubated in a 37° horizontal shaker [Shyam].*
    - *Fewer volumes of recovery medium can prevent having to switch to larger tubes than aliquot tube, but better recovery medium is then probably more important. Efficiency purportedly maximized by approaching 10 volumes recovery medium to improve transformation mix dilution, though shown otherwise.*
    - *SOC or TB/2×YT/LB^{30} + glucose/Mg^2 are best; LB+glucose ±Mg^2 is good. Media with poor carbon sources (LB, 2×YT) are ok. Mg^2, part of SOC, is said to improve efficiency by stabilizing outer membrane.*
    - *-lactam resistance transformations (ampicillin, carbenicillin) have good efficiency without recovery, but still benefit from it.*
9. Incubate at growth temp (often 37°C) for 45–90 min, *optionally* shaking.
    - *Lower efficiency obtained with 30 min recovery, no rich medium addition, poor rich medium addition (LB), or sometimes no aeration during recovery.*
    - *Adding 3 volumes SOC to lab stock standard of 50 µL NEB Turbo transformations in the original 200 µL tubes, flicking, and incubating stationary 37° gave just as good efficiency as recovering in 10 volumes SOC in a test tube incubated 37°C, shaking [Shyam].*
    - *Transformants of plasmids with temperature-sensitive replicons will typically need to be grown at a lower, permissive temperature.*
10. Spread/streak a fraction of the transformation on selective agar medium (plates), optimally room-temp or warmed to growth temperature (often 37°C).
    Store the remainder at 4°C in case later needed due to finding too few or too dense colonies on the transformation plate.
    - *Spread or streak only as much as can fit on the plate/sector without either flowing into neighboring sectors or inhibiting streaking dilution such that single colonies are not obtainable (the case with higher efficiency transformations).*
    - *Save resources; use half to a third the number of plates by plating transformations on half/third-sectors of plates.*

Original Protocol from Literature

From Chung *et al*. [(1)]

1. Prepare transformation/storage solution (TSS), if not already prepared. (LB, 10% (W/V) PEG-3350, 5% (V/V) DMSO, 20–50 mM $MgCl_2$/$MgSO_4$, pH 6.5)
2. Grow seed culture.
3. Inoculate prewarmed LB 1:100 with saturated culture, and incubate 37°C, 225 rpm to an OD_{600} 0.3–0.4.
    - *Tenfold less efficiency at OD_{600} 0.55.*
    - *Several tenfold lower efficiency in stationary phase, but still ok.*
4. Add equal volume ice-cold **2×** TSS to a final 1×, or concentrate culture in 1 – $\frac{1}{10}$ volume ice-cold 1× TSS
    - *10× concentration is twice as efficient as 1×.*
    - *Adding 2× TSS will half the cell concentration.*
5. For long-term storage, flash-freeze in a dry ice/ethanol bath and store at -80°C.
6. For transformation, pipet 100 µL into a cold polypropylene tube containing 1 µL (100 pg) plasmid. Mix gently. (When frozen cells are used, cells are thawed slowly on ice and used immediately.)
7. Incubate cell/DNA mixture 5–60 min at 4°C.

- Efficiency maximizes by 30 min, supposedly 4–5-fold over 5 min incubation.

8. Allow cell recovery in equal volume TSS (or LB 20 mM glucose).

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1. Chung, C. T., Suzanne L. Niemela, and Roger H. Miller. "One-step preparation of competent Escherichia coli: transformation and storage of bacterial cells in the same solution." *Proceedings of the National Academy of Sciences* 86.7 (1989): 2172-2175. doi: 10.1073/pnas.86.7.2172
2. Hanahan, Douglas. "Studies on transformation of Escherichia coli with plasmids." *Journal of molecular biology* 166.4 (1983): 557-580. doi: 10.1016/S0022-2836(83)80284-8
3. ZymoBroth https://www.zymoresearch.com/zymobroth
4. “Transforming chemically competent cells.” *OpenWetWare*, openwetware.org/wiki/Transforming_chemically_competent_cells.
5. Hanahan, Douglas, Joel Jessee, and Fredric R. Bloom. "[4] Plasmid transformation of Escherichia coli and other bacteria." *Methods in enzymology*. Vol. 204. Academic Press, 1991. 63-113. doi: 10.1016/0076-6879(91)04006-A
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