Chemical/Heat-Shock transformation (CCMB80 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 CCMB80 method starts by growing cultures at an unusually cool 20°C, to minimize membrane stiffness from growth at higher temperatures. Cell culture is cooled to arrest growth in exponential phase, in which the cell wall is least developed, facilitating passage of DNA. The cells are washed and stored in CCMB80 solution, a solution of: the rich nutrient medium LB, glycerol as a cryoprotectant to allow freezing and a viscous agent to reduce DNA diffusion and facilitate binding to the cell, and three divalent cation salts: calcium, manganese, and magnesium chlorides, 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 [(2)]. The DNA to be transformed is added and incubated to give time for cation-association and binding and penetrance of cells, facilitated by a heat shock that supposedly creates a temperature differential that induces flow that carries the charge-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.

Transformation efficiency with the CCMB80 method is best for *E. coli* K-12 str. MC1061/DH10B derivatives, yielding **1×10^8–10^9 CFU/µg** pUC19 (0.1–1% of plasmid molecules into 1–5% of viable cells).

Based on Tom Knight's OpenWetware adaptation [(6)] of the Hanahan *et al.* calcium/manganese-based transformation protocol [(5)], which is modified from the Hanahan protocol mainly by omitting the frozen stock preparation on SOB agar and broth, restoring the Mg in SOB, lowering the growth temp to 20°, and doubling the transformation cell concentration. Composed by Shyam Bhakta.

Competent Cell Preparation

For *n* transformations of *v* volume:

Materials

1. **CCMB80 Buffer, 9*nv* volume**
   10 mM KOAc, 80 mM $CaCl_2$, 20 mM $MnCl_2$, 10 mM $MgCl_2$, 10% $_{V/V}$ glycerol, pH 6.4.

$CaCl_2$	80 mM	11.76 g/L (·2HO)	Dissolve with det-free stir bar.
$MnCl_2$	20 mM	3.96 g/L (·4HO)	
$MgCl_2$	10 mM	2.03 g/L (·6HO) 0.95 g/L (anhyd.)	
CH_3CO_2K (KOAc)	10 mM	0.98 g/L	
Glycerol, or *50% Glycerol, det-free*	10% *V/V* *20% V/V*	126 g/L *200 mL/L*	
0.1 M HCl, det-free	pH 6.4		Adjust pH.
Filter-sterilize*. Store at 4°C * Detergent-free membranes (PES, NYL, CN) are preferred for filter-sterilization.			

   Allow each salt to dissolve before adding next. pH adjustment to 6.4 prevents MnO_2 precipitation. During storage, the pH of the solution drifts down to a final value of 6.1–6.2 but then stabilizes. During prolonged storage, a faint precipitate of a tan-colored substance may appear, but does not seem to affect the efficiency of transformation [(7)].

2. **25*n v* volume SOB** (+antibiotic if necessary)
   20 g/L tryptone, 5 g/L yeast extract, 10 mM NaCl, 2.5 mM KCl, 10 mM $MgCl_2$, 10 mM $MgSO_4$, pH 7.0

Tryptone	–	20 g/L	Dissolve with stir bar.
Yeast extract	–	5 g/L	
NaCl	10 mM	0.584 g/L	
KCl	2.5 mM	0.186 g/L	
Milli-Q water		Reserve Mg soln volumes	

Transformation

Summary for frozen lab aliquots

1. **Thaw** comp cell aliquots (50 µL) slowly on ice or above cold block for a few min.
   Use one aliquot 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.
3. **Incubate cold** on 1°C block / ice for 5 min, best 30 min.
4. **Heat shock** 42°, 30 s in a heat block or thermocycler.
5. **Return to cold** immediately for 1–5 min. Can repeat steps 4–5.
6. **Recovery**: remove from ice and add 150 µL SOC. **Flick** to mix. Incubate at growth temp (*e.g.* 37°C), 45–60 min stationary.
7. **Plate** on selective agar and incubate.

Detailed

1. Thaw -80°C comp cell aliquots on ice or atop cold block for a few min. Or use comp cells within hours of comp cell prep. Keep at 1–4°C except for heat shock and recovery.
   - *Slow thawing seems to give somewhat better efficiency; can thaw in the deli sitting on some plastic surface or scatter tubes on top of cold block, putting them in the metal wells after thawed.*
   - *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.*
   - ***Colony transformation** with purified plasmid: pick several colonies or a clump of cells using a pipette tip, toothpick, or inoculation loop, and disperse cells into 50–100 µL aliquot of CCMB80, TFB, or FSB. Incubate 1°C 10 min.*
2. **Add DNA**, aiming for 10% comp cell volume.
   Flick five times to mix.
   - *Do not vortex or triturate (pipette-mix).*
3. **Incubate** at 1°C (ice or cold block) for 5–30 min, with efficiency maximized by 30 min[(1)].
4. **Heat shock** at 42°C for 15–45 s in a heated block, thermocycler, or water bath.
   - *20–30 s optimal for NEB Turbo and DH10B/10-beta [(Shyam, NEB1, NEB2)].*
   - *45–90 s purportedly better for different strains.*
   - *Metal bead bath either needs a significantly different heat shock time or is inferior with too low thermal conductivity.*
5. Immediately return to 1°C (ice or cold block) for 1–5 min.
   - *Commercial NEB competent cells instruct 5 min, which may be better.*

   Optionally **repeat** steps 4–5 for twofold higher efficiency[(11)].
6. **Recovery:** Move to room-temp rack. Add 2–10 volumes room-temp recovery medium (no antibiotic); normally 3 volumes: 150

NaOH, 1 M		pH 7.0	Check pH. typically not needed
Autoclave 15 min 1 L, 20 min 1–1.4 L. Or filter sterilize* after adding components below.			
$MgCl_2$ sterile	10 mM	10 mL/L 1 M	autoclaved or filtered
$MgSO_4$ sterile	10 mM	10 mL/L 1 M	autoclaved or filtered
Glucose sterile	20 mM 3.6 g /L	20 mL/L 1 M 7.2 mL/L 50% 18 mL/L 20%	*For SOC only.* Can be spiked-in.
Store at 4° for best long-term preservation of nutrients. Aliquots of SOC can be frozen to prevent contaminant growth. * Detergent-free membranes (PES, NYL, CN) are preferred for filter-sterilization.			

3. Culture tubes for precultures (detergent-free).
4. Culture flasks large enough to hold growth medium volume (detergent-free).
5. *Refrigerated* shaking incubator space for the culture flasks.
    - Only a refrigerated shaker can sustain 18–23°C temperature.
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 80% 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 *n v* volume CCMB80 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. 250 µL PCR tubes) for cell aliquots, labeled/marked and arranged in clean tube racks or tip rack+boxes. Blade, if planning to cut apart tube strips.
    PCR tubes are more space-efficient and allow multichanneling cells /DNA and thermocycling for incubation and heat shock. 3 volumes recovery medium is sufficient and can fit with a 50 µL cell aliquot.
    b. Extra tubes or bag of tubes, for aliquotting any excess comp cells, if desired.
    c. Serological pipettor and few pipettes appropriate for resuspending and transferring cells.
    d. A 5 or 50 mL tube per strain to hold resuspension.
    e. (*opt.*) Electronic repeater pipette and a 5 mL combitip per strain and some extras. Reservoirs, if opting to us a multichannel pipette.
    f. Micropipette 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

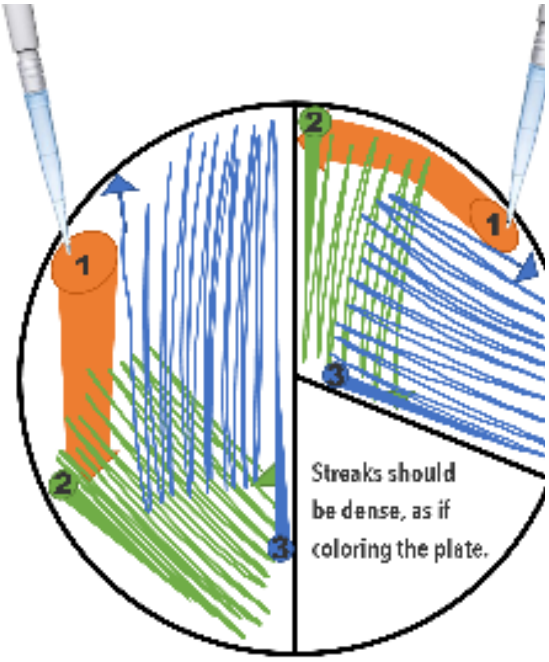
µL medium to 50 µL comp cells + DNA. Flick to mix. Trituration or very brief vortexing are fine, too.
    - *Open tubes without touching the insides, which have cells on them. Touching multiple tube lids can cross-contaminate the transformations.*
    - *Adding 3 volumes (150 µL) SOC to lab standard 50 µL transformation aliquots in the original 250 µL tubes, flicking, and incubating stationary 37° gives about as good efficiency as recovering in 10 volumes SOC in a test tube incubated 37° shaking (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 rich media with added 20 mM Mg^2 and 20 mM glucose, are best. 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 ~tenfold less efficiency without recovery, so don't necessarily require it.*
7. **Recovery:** Incubate at growth temp (often 37°C) for 1 hour, *opti onally* shaking. Recovery time can be reduced at the expense of efficiency.
Meanwhile, you may incubate selective agar plates inverted to warm them.
    - *Lower efficiency obtained with 15–30 min recovery, no rich medium addition, poor rich medium addition (LB), or sometimes no aeration during recovery.*
    - *Crack open the plate lids 30–45 min while incubating to dry them slightly, such that cell suspension liquid is quickly absorbed after plating.*
    - *Transformants of plasmids with temperature-sensitive replicons will need to be grown at a lower, permissive temperature.*
    - *Greater volume recovery medium and shaking not found to increase typical efficiency. See below.*
8. **Plating:** 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.
    - *Prewarming/drying the plate is said to slightly improve transformation efficiency, but the main advantage is the quick absorption of plated cell suspension, which may otherwise flow around the surface when moved. Wait until it is absorbed before inverting the plates. You may leave the plates partially uncovered in the incubator and cover and invert them once dried, 15–30 min. However, simply incubating them covered and non-inverted is also an option without any noticeable negative consequence, allowing you to not further monitor the plates.*
    - *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.*
9. **Incubate** agar plates inverted in an incubator or room of the appropriate growth temperature until there is sufficient growth.
    - *Pickable colonies form with MG1655, JS006, or NEB Turbo in 9–10 hr. Other strains like DH10B, other Rec^- strains, and Mach1 require at least 15–16 hr.*

k. (*opt.*) If flash freezing, box/bag to collect all tubes in before flash-freezing.

Procedure

1. **Grow preculture** to saturation:
   Inoculate 1/400 culture volume, **0.05*nv*** , SOB (+selection, if necessary) with colonies (best) or a scrape of frozen stock, and incubate shaking 18–22°C 12–18 hr until saturated.
   - *Use a fresh, trusted source of the strain. For most reliable results, inoculate a seed culture from colonies /patch from <2 week-old plates streaked from a frozen stock. Plasmid-bearing strains ought to be used from even fresher plates to minimize plasmid mutations.*
   - *Incubation of preculture at 30° or 37°C seems to be fine for 10^9/µg efficiency , though 20–23°C is recommended in the original protocol.*
   - *NEB Turbo grown in 20°C SOB saturates at OD3.5.*
2. **Grow culture:** Measure preculture OD. Inoculate **25*nv*** mL SOB to a final OD0.01, 0.2% seed culture at a saturated OD6. Incubate **18–22°C**, shaking 250 rpm for flasks.
   Grow culture to **OD_{600}=0.2–0.26** (early exponential phase), **9–15 hr**. Periodically monitor OD starting at 8 hr.
   - *Transformation efficiency from LB cultures decreases linearly between OD=0.3–0.6.* [(1)] *The same might apply here. Do not allow the culture to grow to an OD $_{600}$ > 0.26 for maximum efficiency.*
   - *Several tenfold lower-efficiency at stationary phase, but supposedly still fine for transforming pure plasmid or the simplest cloning.*
   - *12–15 hr required for 18° culturing, less for higher temperatures.*
3. **Prepare** the mobile lab bench cart for the cold room as described.
   Prechill both the mobile lab bench cart (with its contents) and the centrifuge with rotor to 2°C 45 min before cell harvest (next step) *[measured with infrared thermometer]* .
4. **Harvest:** when culture reaches **OD_{600}=0.2–0.26**, immediately harvest cells: decant into prechilled centrifuge bottles and balance them. Use chilled sterile water if necessary. Centrifuge 1000×*g*, 10–15 min (depending on volume) in a prechilled 4°C centrifuge.
5. 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.
6. **Resuspend** cells *gently* in culture volume (8*nv* mL) of chilled CCMB80 buffer, by swirling. Combine volumes to reduce vessels.
7. **Incubate** suspension on ice 20 min.
8. **Harvest** cells as before (step 4–5).
9. **Resuspend** *gently* in 1/25 volume (*nv* mL) chilled CCMB80 buffer by swirling and striking.
   - *Flat-bottom centrifuge bottles allow easier gentle resuspension of pellets.*
   - *Original Hanahan / CSH protocol instructs 12-fold concentration in CCMB80 buffer. The OpenWetware version instructs 25-fold concentration.*

Perform remaining steps with pre-chilled materials in the cold room for maximum efficiency.



Thermocycler Heat/Cold Shock Protocol

Thermocycling may somewhat increase transformation efficiency compared to heat shocks performed between cold and heated metal tube blocks. With a simple single heat shock, good competent cells produce hundreds of colonies for even complex assemblies with low yield. However, thermocycling may at times be convenient for more unattended time.

After incubating cells + DNA on ice or cold block, add to thermocycler block prechilled to the first step and proceed to the next step. This way, it's less taxing on the thermocycler, not built for sustained low-temp holds.

Unheated lid	
1°C	5–30 min *Try to do this cold incubation on ice/cold block instead. Then start this program and add to thermocycler **after it reaches 1°C**. Proceed to next step .*
42°C	30 s
1°C	1–2 min
42°C	30 s ; *beep*
1°C	1–5 min ; *beep*
END	Add recovery medium. Place in incubator bead bath (steps 8–11).

Recovery

Transformation recovery is generally prescribed to consist of adding the transformation suspension to 9–10 volumes prewarmed recovery medium and shaking for 1 hr.
Home-made CCMB comp cells appear to do as well if not better with just 2–3 volumes recovery medium added to the 250 µL "PCR" tubes that transformations are often performed in for space efficiency, thermocycling flexibility, and multichannelability of DNA in tube strips into comp cell aliquots.

Decant or pipette cells into 5 or 50 mL conical tubes on ice for easier access with pipette for aliquotting. Finish gently resuspending any visible remaining cell clumps using P1000 tip if necessary.

10. **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.
    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.
11. **Flash freeze** (*opt.*) in liquid nitrogen for reportedly higher efficiency.
    - *Flash freezing is reported to retain higher competence of cells in the literature, but simply freezing is not estimated to greatly reduce competence, maybe within twofold* [SPB].
    - *For a dry-ice-ethanol bath, residual ethanol fails to dry from tubes/boxes at -80°C. Freeze tubes in a bag if using this method, and pour tubes into box.*
12. **Store:** 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.

According to the Cold Spring Harbor protocol [7] : "Detergents and organic contaminants are strong inhibitors of transformation reactions. To avoid problems caused by residual detergent in glassware, use disposable plastic tubes and flasks wherever possible for preparation and storage of all solutions and media used in transformation. Organic contaminants present in the H O used to prepare transformation buffers can reduce the efficiency of transformation of competent bacteria. H O obtained directly from a well-serviced Milli-Q filtration system (Millipore) usually gives good results. If problems should arise, treat the deionized H O with activated charcoal before use."

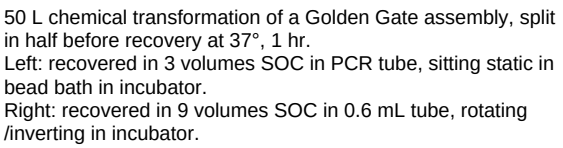
Choice of Culture Medium

ZymoBroth™ [3] , containing only 0.5–5% yeast extract and tryptone (LB components) and 10 mM MgCl, improves many strains' competence using an unspecified protocol, 13-fold for TG1, the NEB Turbo parent. MgCl in the growth medium may thus be more effective than the 20 mM MgCl 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."

These enhancements were later found to be more specific to strain MC1061 and derivatives like DH10B, and not strains derived from Hoffman 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] .

Ligation Adjustment Buffer (5× LAB)

We have never used or tried ligation-adjustment buffers, nor have felt the need to, as simply using at most one-tenth the volume of Golden



50 L chemical transformation of a Golden Gate assembly, split in half before recovery at 37°, 1 hr.
Left: recovered in 3 volumes SOC in PCR tube, sitting static in bead bath in incubator.
Right: recovered in 9 volumes SOC in 0.6 mL tube, rotating /inverting in incubator.

Gate assembly reaction (5 µL) as comp cell volume (50 µL) seems to be reliable enough to get hundreds of colonies.

According to the CSH protocol: "Ligation mixtures generally contain high concentrations of DNA and, depending on the termini of the insert and vector, a variety of ligation products: linear and recircularized vector, linear insert, dimers, linear and circular vector:insert recombinants, recirculized vectors, etc. In addition, other components of the ligation mixture (e.g. DNA ligase, polyethylene glycol) may interfere with uptake of DNA by competent bacteria.

"A common mistake is to add too much of the ligation mixture to the competent cells. To avoid problems, use no more than 1 µL of a standard ligation reaction in each transformation assay. Better still,

- precipitate the DNA in the ligation mixture with 2 volumes of ethanol. Recover the DNA by centrifugation and dissolve it in 50 µL of TE (pH 7.6)
- or dilute the ligation mixture fivefold. Use 1 µL of the diluted reaction mixture for transformation
- or adjust the buffer composition of the ligation reaction to more closely resemble that of the CCMB80 buffer by adding 0.2 volumes of ligation adjustment buffer to the ligation mixture immediately before using the products of ligation for transformation. After adjustment, the pH of the ligation mixture should be 6.3–6.5.

40 mM KOAc, 400 mM $CaCl_2$, 100 mM $MnCl_2$, 46.8% $_{V/V}$ glycerol , pH 6.3–6.5 with HOAc.

$CaCl_2$	400 mM	44.392 g/L (·$2H_2O$)
$MnCl_2$	100 mM	12.584 g/L (·$4H_2O$)
CH_3CO_2K (KOAc)	40 mM	3.926 g/L
Glycerol, or 50% Glycerol	46.8% *V* / *V* 20% *V* / *V*	468 mL/L, 126 g/L 200 mL/L (50%)
0.1 M HOAc	pH 6.4	12.8 mL/L 10% acetic acid
Filter-sterilize*. Store at 4°C		

- Shyam thinks the glycerol makes more sense at 30% for 5×, which makes a 10% enzyme volume reaction (5% glycerol) a final 10% glycerol, like CCMB80 buffer.

Potential Transformation Enhancers

A patent from Stratagene[8] describes transformation efficiency being enhanced by adding a final 110 mM NaCl and 50 mM 2-mercaptoethanol (2-sulfanylethanol) to comp cells and incubating 10 min on ice before adding DNA and further incubating. Elsewhere, however, NaCl is described as being inhibitory to transformation [9] .

NEB writes [10] : Addition of 2-sulfanylethanol to cells at a final 24 mM from a high-purity, sterile 1.5 M stock has been shown to increase the pUC19 transformation efficiency of NEB 5-alpha by 40%. After comp cells are thawed on ice, add 0.8 µL 2-mercaptoethanol to cells, flick five times to mix, and incubate 10 min on ice before proceeding with transformation and adding DNA.

Hanahan[2] found that 7% DMSO with either 75 mM dithiothreitol or 25 mM 2-sulfanylethanol act as potent transformation enhancers. One might thus surmise that they may have some enhancing effect when added to CCMB80 comp cells upon thawing, perhaps 10–30 min before adding DNA.

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. https://doi.org/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. https://doi.org/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
6. “TOP10 Chemically Competent Cells.” *OpenWetWare* , openwetware.org/wiki/TOP10_chemically_competent_cells.
7. Green, Michael R., and Joseph Sambrook. "The Hanahan method for preparation and transformation of competent Escherichia coli: high-efficiency transformation." *Cold Spring Harbor Protocols* 2018.3 (2018): pdb-prot101188. https://doi.org/10.1101/pdb.prot101188
8. Greener, Alan Lewis, and Bruce Douglas Jerpseth. "Highly transformable bacterial cells and methods for producing the same." U.S. Patent No. 6,706,525. 16 Mar. 2004. https://patents.google.com/patent/US6706525B1/en
9. Pope, Brian, and Helen M. Kent. "High efficiency 5 min transformation of Escherichia coli." *Nucleic acids research* 24.3 (1996): 536-537. https://doi.org/10.1093/nar/24.3.536
10. NEB FAQ. https://www.neb.com/faqs/0001/01/01/how-can-i-increase-transformation-efficiency
11. Panja, Subrata, et al. "How does plasmid DNA penetrate cell membranes in artificial transformation process of Escherichia coli?." *Molecular membrane biology* 25.5 (2008): 411-422. DOI: 10.1080/09687680802187765