Transformation of plasmids into a bacterial host

Recombinant DNA can be stably maintained in bacterial hosts as extrachromosomal plasmids, enabling clonal amplification ("molecular cloning") and, in some cases, expression of encoded transgenes.

Various non-pathogenic *Escherichia coli* strains have been engineered to optimize plasmid propagation or facilitate high-level protein expression. These strains can be rendered competent for DNA uptake through different transformation methods.

One widely used approach involves heat shock, where transformation efficiency is greatly enhanced in the presence of divalent cations (Inoue et al., 1990) such as $CaCl_2$ (*Alternative A*) or $MnCl_2$ (*Alternative B* and *Alternative C*), which neutralize the negative charges on DNA and cell surfaces. However, some *E. coli* strains with thicker cell walls may be less amenable to this method.

For a fast but less efficient approach, TSS transformation (Chung et al., 1989) (*Alternative D*) offers a convenient option, especially for wild-type *E. coli* strains that are sensitive to $CaCl_2$.

Electroporation (*Alternative E*) typically yields transformation efficiencies of 1×10^{9}–1×10^{10} cfu/µg DNA—significantly higher than chemical methods (1×10^{6}–1×10^{8} cfu/µg). A high-voltage pulse transiently permeabilizes the membrane, allowing DNA uptake. To avoid arcing, cells must be prepared in a low ionic-strength solution, which also makes it more sensitive to residual salts or impurities in the DNA preparation.

While freshly prepared competent cells yield the highest efficiencies, they can be prepared in bulk and stored at −80 °C in glycerol or DMSO with minimal loss of competence.

Risk assessment

– High-voltage power source

▷ Only trained users should operate the electroporator
▷ Follow the manufacturer's instructions for operation and shutdown of the electroporation apparatus
▷ DO NOT touch the sample chamber or cables during pulsing
▷ Wear gloves, safety glasses, lab coat

□ Collect and dispose liquid and solid waste after inactivation as REGULATED MEDICAL WASTE

In the event of ELECTRIC SHOCK:
▷ **Seek immediate medical attention, even if symptoms are mild or absent**

Reviewed: Feb 19, 2025

Procedures

›› ***Preparation of* E. coli *in logarithmic growth phase***

(1.) Inoculate a single colony into 1–5 mL LB medium. Grow overnight at 37 °C.

> ***Critical:*** Add antibiotics if needed. For example, to maintain BL21(DE3)pLysS, which carries a plasmid encoding T7 lysozyme, supplement with chloramphenicol. Many laboratory background strains of *E. coli* do not harbor antibiotic resistance markers. In that case, sterilize glassware and plastics used for growth.

(2.) Inoculate 1.0 vol LB or S. O. B. medium with 5 mL of the overnight culture per liter of final volume. The starting optical density (OD600) should be around 0.025 mL^{-1}.

> ***Hint:*** A 50 mL culture yields approximately 40–50 100 µL aliquots of competent cells.

(3.) Grow the culture at 18 °C, shaking at 150–200 rpm, until it reaches an OD600 of 0.4–0.6 mL^{-1}.

> ***Critical:*** Although growth at 37 °C for 2–3 h is faster, cold growth for at least three doublings is key for high competence. Do not allow the culture to overgrow! Harvest during log phase. Diluting stationary-phase cells is ineffective.

> ***This is why:*** Cold growth alters membrane composition—particularly lipid phase and surface charge—making the membrane more permeable to DNA during transformation.

(4.) Chill the culture on ice for 10 min. Pellet the cells in a refrigerated centrifuge at 1 500–2 000 × *g* for 5–10 min. Carefully decant the supernatant.

A › *Chemically competent* **E. coli** *K-12 and B strains for heat shock transformation*

(1.) *Optional:* Resuspend the cells in 0.5 vol ice-cold, sterile 100 mM $MgCl_2$ solution.

> ***This is why:*** Divalent cations like $MgCl_2$ can bridge negatively charged lipopolysaccharide (LPS) molecules in the outer membrane, stabilizing its structure. Paradoxically, when cells are chilled and exposed to ice-cold $MgCl_2$ solutions during preparation, this interaction is thought to temporarily loosen the membrane and aid plasmid DNA uptake. This effect is strain-dependent. *E. coli* K-12 W3110 has an outer membrane that responds poorly to Ca^{2+} or Mg^{2+} protocols. Alternative methods such as TSS transformation may yield better results for such strains.

(2.) Collect by centrifugation as above. Discard the supernatant.

(3.) Resuspend the cells in 0.5 vol ice-cold, sterile 50 mM $CaCl_2$ solution. Chill for 15 min on ice.

(4.) Collect by centrifugation as above. Discard the supernatant.

(5.) Resuspend in 0.07 vol ice-cold, sterile 50 mM $CaCl_2$ 15% glycerol solution.

B › *Highly efficient chemically competent* **E. coli** *K-12 and B strains for heat shock transformation*

- ☐ RF1, 200 mL ⟨*R*⟩
- ☐ RF2, 200 mL ⟨*R*⟩

(1.) Resuspend the cells in 0.3 vol ice-cold RF1. Chill the suspension for 15 min on ice.

(2.) Collect by centrifugation as above. Discard the supernatant.

(3.) Resuspend in 0.08 vol ice-cold RF2. Chill for 15 min on ice.

C › *Simple and efficient chemically competent* **E. coli** *K-12 and B strains for heat shock transformation*

- ☐ Simple & efficient buffer, 200 mL ⟨*R*⟩

(1.) Resuspend in 0.3 vol ice-cold SEB. Chill the suspension for 10 min on ice.

(2.) Collect by centrifugation as above. Discard the supernatant.

(3.) Resuspend in 0.04 vol ice-cold SEB. While swirling the tube, add 0.006 vol DMSO in a drop-wise manner. Chill for 10 min on ice.

> ***Note:*** Mixing of DMSO is an exothermic process; this way, the suspension stays cold.

D › *TSS-competent* **E. coli** *cells*

- ☐ 2 × Transformation & storage medium, 50 mL ⟨*R*⟩

(1.) Resuspend in 0.025 vol cold LB medium.

> ***Hint:*** Recovery in additional medium is not required since TSS is based on LB and supports outgrowth directly after transformation. So, a small volume (200 µL) of freshly grown culture in exponential phase (inoculated 90–180 min before transformation) can be directly used.

(2.) Add an equal volume of ice-cold 2 × TSS solution. Mix gently by pipetting.

> ***Critical:*** Do not exceed 30 min incubation on ice. Longer exposure may lower transformation efficiency, possibly due to membrane recovery or PEG toxicity.

E › *Electrocompetent* **E. coli**

(1.) Resuspend in 0.5 vol ice-cold, sterile, reagent-grade water.

(2.) Collect by centrifugation as above. Discard the supernatant.

(3.) Resuspend in 0.2 vol ice-cold, sterile, reagent-grade water.

(4.) Collect by centrifugation as above. Discard the supernatant.

(5.) Resuspend in 0.008 vol ice-cold, sterile 10% glycerol.

> ***Hint:*** The OD600 of this suspension should range 40–50 mL^{-1} which is about 10-fold higher than for chemically competent cells. For *E. coli* and some other bacteria, increasing the cell concentration increases the yield of transformants. For diverse library transformations, the cell density can be increased to 200–400 mL^{-1} by resuspending in 0.001 vol.

›› ***Frozen aliquots for long-term storage***

(1.) Pre-chill all containers that will be used for storage.

> ***Critical:*** Keep materials on ice and proceed quickly. The shorter this process, the better.

(2.) Dispense into 50–200 µL aliquots.

> ***Hint:*** This is about 50×10^6–100×10^6 chemically competent cells per aliquot and 250×10^6 electrocompetent cells. One aliquot is sufficient for up to four transformations.

(3.) Freeze aliquots in liquid nitrogen or in an ethanol/dry ice bath.

(4.) Store at −80 °C for up to two years. Freeze-thawing will significantly decrease transformation efficiency.

›› ***Preparing the transformation mixture***

(1.) Thaw cells on ice. Do not warm the tube in your hands, as even brief warming reduces competence. Homogenize the suspension by gently flicking the tube.

(2.) *Optional:* If necessary, dispense the thawed suspension into additional pre-chilled tubes.

(3.) To 20–50 µL suspension, add 0.1–100 ng plasmid DNA. The added material should not exceed 0.2 vol of the total volume. Mix the contents by gently flicking the tube.

A,B,C › ***Transformation of chemically competent bacteria***

(1.) Incubate the mixture for 5–30 min on ice.

(2.) Place the tubes in a water or sand bath of 42 °C for 30–45 s with no shaking.

(3.) Place the tubes on ice for 2 min.

(4.) Add 200–500 µL pre-warmed SOC or LB medium.

D › ***Transformation of TSS competent bacteria***

(1.) Vortex the suspension. Incubate for 20–30 min on ice.

> ***Critical:*** Longer incubation time will reduce transformation efficiency.

> ***This is why:*** It is not necessary to add more non-selective growth medium during recovery as TSS medium is based on LB.

E › *Transformation of electrocompetent bacteria*

(1.) Pre-chill the cuvette for electroporation on ice.

> ***Hint:*** A 0.2 cm gap cuvette is sufficient for routine transformations. Larger gaps may be required to accommodate higher-volume library transformations.

(2.) Pulse with a field strength of 7.5–10 kV/cm, which corresponds to 1.5–2.5 kV for a 0.2 cm cuvette.

> ***Hint:*** For *E. coli*, the pulse controller should be set to 200–400 Ω resistance, 25 µF capacitance, and deliver a 20 ms pulse. Optimal electro-transformation conditions might differ for other bacteria.

> ***Quality assurance:*** A successful pulse yields a time constant between 4–8 ms. If arcing occurs, discard both sample and cuvette.

> ***Safety:*** Turn off the pulse apparatus according to the manufacturer's instructions to fully discharge the capacitors!

(3.) Add 1 mL pre-warmed SOC or LB medium to the cuvette. Gently pipette up and down to mix. Transfer the suspension to a clean tube for recovery.

(4.) *Optional:* Rinse the cuvette with 70% ethanol. It can be reused after thorough drying.

> ***Note:*** Discard cuvettes if time constant drops or discoloration is visible.

›› *Selection of transformed clones*

(1.) Incubate the transformed suspension in non-selective growth medium for at least 45–60 min at 37 °C. 60 min

> ***Hint:*** This recovery time allows for expression of the selection marker. For ampicillin and other markers that do not inhibit the cellular metabolism directly, this time can be shortened to 10–20 min or omitted.

(2.) Spread 0.1–1.0 vol of the recovered suspension onto a selective LB agar plate. Allow the plate to dry and place it upside-down at 37 °C in an incubator. Colonies will be visible after 12–18 h.

> ***Hint:*** For plasmid re-transformations, 0.1 vol is sufficient to obtain enough single colonies. After restriction cloning or other recombineering techniques, plate 0.1 vol and the remainder separately, for example, on each half of a Petri dish.

Analyses

○ Test the transformation efficiency with 10 pg supercoiled pUC19:

- Rescue 100 µL competent cells in 1 mL growth medium. Prepare 1:10 and 1:100 dilutions.
- Plate 100 µL of all three concentrations on LB agar plates supplemented with 50–100 µg/mL ampicillin; incubate overnight at 37 °C.
- Count the number of colonies on each plate. Do not consider plates with less than 100 colonies.
- Calculate colony forming units (cfu) per microgram of transformed DNA. Account for the dilution factor from plating a fraction of the total recovery volume.

Troubleshooting

Transformation of chemically competent bacteria

In Step 2:

– Low transformation efficiency
  - Verify competence with pUC19 standard. Competent cells should yield at least 1×10^6.
  - Do not exceed 42 °C during heat shock. Even 1–2 °C above optimal temperature dramatically reduces efficiency.
  - Ensure cells were grown to mid-log phase (OD600 0.4–0.6) before harvesting. Stationary-phase cells cannot be made competent by dilution.

Transformation of electrocompetent bacteria

In Step 2:

– Arcing during electroporation
  - Reduce DNA volume or desalt the DNA preparation. Arcing indicates excess ionic strength in the cuvette.
  - Ensure cells were washed thoroughly in ice-cold water or 10% glycerol to remove all salts.

Selection of transformed clones

In Step 2:

– No or very little colonies after transformation
  - Verify the antibiotic resistance and origin of the plasmid. Some bacterial strains have restriction-modification (RM) systems that degrade foreign DNA unless methylation pattern match the host. When cloning foreign DNA, it's best to use an *E. coli* strain that lacks the Mcr and Mrr systems.
  - Verify cell competence with the pUC19 standard.
  - Purify the DNA. Specifically, electroporation is sensitive to impurities and buffer salts.
  - Analyze the DNA by gel electrophoresis to check for degradation.
  - Some bacteria, for example *Pseudomonas*, require different transformation conditions. Gram-positive species such as *Staphylococcus*, *Streptococcus*, or *Bacillus*, require additional glycine treatment to weaken the cell wall.
– Lawn growth or satellite colonies
  - For ampicillin selection, satellites indicate beta-lactamase secretion from resistant colonies depleting local antibiotic. Restreak individual colonies onto fresh selective plates.
  - Consider using carbenicillin as a more stable alternative to ampicillin.

Recipes

RF1, pH 5.8

Amount	Ingredient		Stock	Final
2.42 g	RbCl	[7791-11-9]	120.92 g/mol	100 mM
1.62 g	$MnCl_2 \cdot 2\ H_2O$	[20603-88-7]	161.87 g/mol	50 mM
2 mL	Sodium acetate (NaOAc), pH 5.2	R0045	3 M	30 mM
2 mL	Calcium chloride ($CaCl_2$)	R0012	1 M	10 mM
60 mL	Glycerol	R0022	50%	15%
To 200 mL	Water, reagent-grade			

Adjust pH with 0.2 M acetic acid. Filter sterilize. Store at 4 °C.

RF1

100 mM RbCl, 50 mM $MnCl_2$, 30 mM NaOAc, 10 mM $CaCl_2$, 15% Glycerol, pH 5.8

WARNING

Acute oral toxicity; Brain toxicity upon repeated exposure; Hazardous to the aquatic environment

Date: Sign: R0185

RF2, pH 6.8

Amount	Ingredient		Stock	Final
0.24 g	RbCl	[7791-11-9]	120.92 g/mol	10 mM
0.42 g	4-Morpholinepropanesulfonic acid (MOPS)	[1132-61-2]	209.27 g/mol	10 mM
2 mL	Calcium chloride ($CaCl_2$) R0012		1 M	10 mM
60 mL	Glycerol R0022		50%	15%
To 200 mL	Water, reagent-grade			

Adjust pH with 1 M sodium hydroxide. Filter sterilize. Store at 4 °C.

RF2

10 mM RbCl, 10 mM MOPS, 10 mM $CaCl_2$, 15% Glycerol, pH 6.8

Date: Sign: R0186

Simple & efficient buffer (SEB), pH 6.7

Amount	Ingredient		Stock	Final
0.60 g	1,4-Piperazinediethanesulfonic acid (PIPES)	[6525-37-6]	302.37 g/mol	10 mM
3.73 g	KCl	[7447-40-7]	74.55 g/mol	250 mM
1.78 g	$MnCl_2 \cdot 2\ H_2O$	[20603-88-7]	161.87 g/mol	55 mM
3 mL	Calcium chloride ($CaCl_2$) R0012		1 M	15 mM
To 200 mL	Water, reagent-grade			

Adjust pH with 5 M potassium hydroxide before adding manganese chloride; filter sterilize. Store at 4 °C.

Simple & efficient buffer (SEB)

10 mM PIPES, 250 mM KCl, 55 mM $MnCl_2$, 15 mM $CaCl_2$, pH 6.7

WARNING

Acute oral toxicity; Brain toxicity upon repeated exposure; Hazardous to the aquatic environment

Date: Sign: R0187

Transformation & storage medium (TSS), pH 6.5, 2×

Amount	Ingredient		Stock	Final
5 mL	Magnesium chloride ($MgCl_2$) R0031		1 M	100 mM
10 g	Polyethylene glycol 4000 [25322-68-3]		3 500–4 500 g/mol	20%
5 mL	Dimethyl sulfoxide (DMSO), reagent-grade [67-68-5]			10%
To 50 mL	LB medium (Miller)			

Filter sterilize. Store at 4 °C.

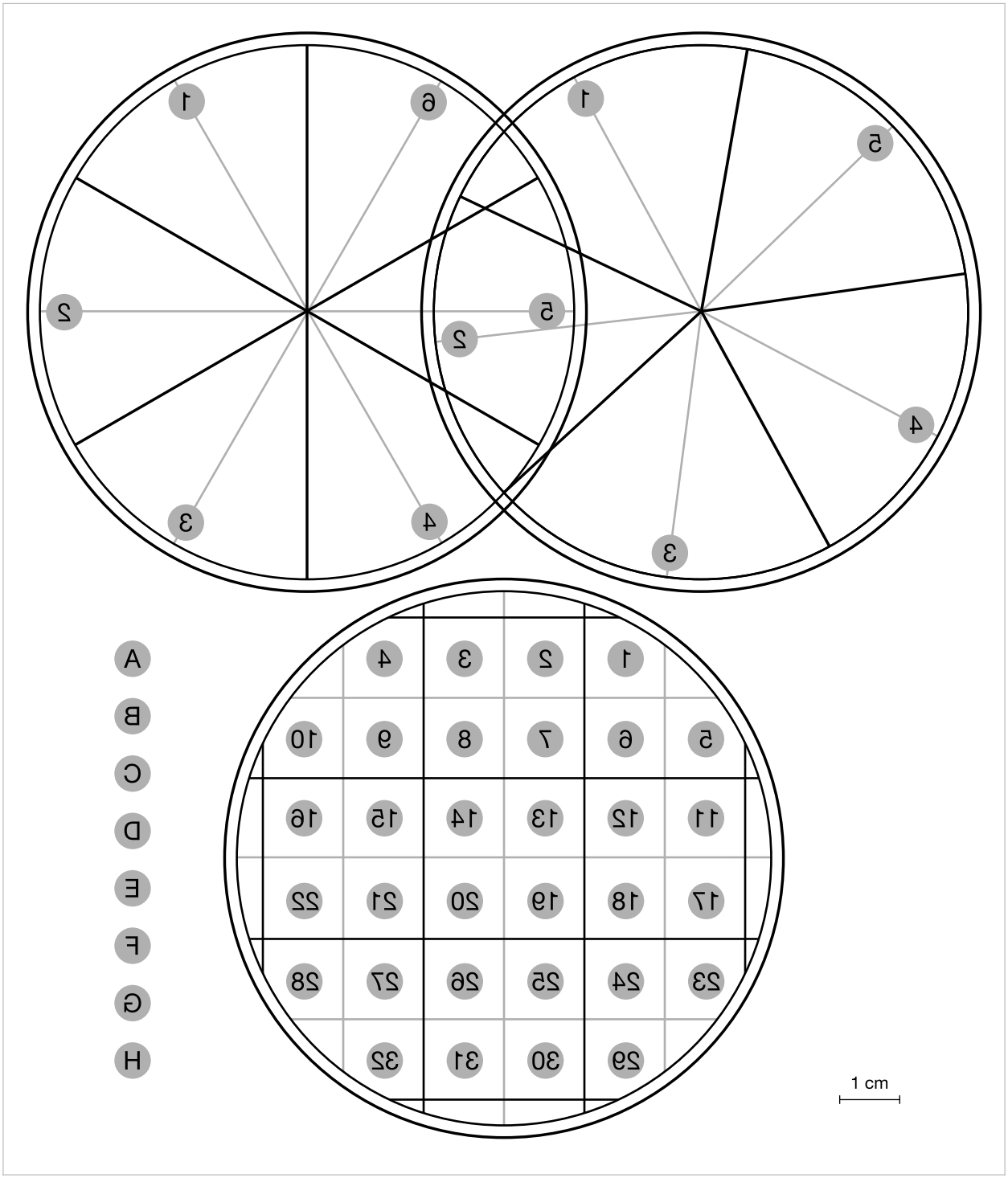
2× Transformation & storage medium (TSS)

100 mM $MgCl_2$, 20% Polyethylene glycol 4000, 10% DMSO, pH 6.5

Date: Sign: R0188

Resources

Selection of transformed clones



In Step 2: Sector templates to evenly distribute 2, 3, 4, 5, 6, 9, 10, 12, or 32 clones on 10 cm Petri dishes with a typical inner diameter of 88 mm. Mirrored letters will appear correctly oriented when traced on the back of the plate.

List of references

C. Chung, S. Niemela, and R. Miller, *Proc. Natl. Acad. Sci. U.S.A.* **86**(7), 2172—2175 (1989).

H. Inoue, H. Nojima, and H. Okayama, *Gene* **96**(1), 23—28 (1990).

Change log

2012-02-06	Jeff Coller	Initial protocol for preparation of chemically competent HB101.
2015-11-03	Konrad Herbst	Integration of heat shock and electroporation methods; high-efficiency competent cell protocol.
2019-11-27	Brinja Kosel	Initial protocol for preparation of electrocompetent cells for library transformation; Daniel Summerer lab.
2020-03-25	Alice Pawlowski	TSS transformation of non-competent E. coli cells.
2025-02-19	Benjamin C. Buchmuller	Modularization and adaptation as SOP; added troubleshooting and efficiency guidelines.