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. 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.

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 alternative, 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.

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

□ Dispose 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: Nov 2, 2026

Procedures

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

□ LB medium (Miller) ◇ R0030 □ S. O. B. medium ◇ R0212

(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 (OD_{600}) should be around 0.025.

> *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 OD_{600} = 0.4–0.6.

> *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*

- ☐ 100 mM $MgCl_2$, sterile (*optional*)
- ☐ 50 mM $CaCl_2$, sterile
- ☐ 50 mM $CaCl_2$ / 15% glycerol, sterile

(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, pH 5.8 ◇ R0185
- ☐ RF2, pH 6.8 ◇ R0186

(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*

- ☐ SEB, pH 6.7 ◇ R0187
- ☐ DMSO

(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 0.08 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 × TSS, pH 6.5 ◇ R0188

(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.

> ***Hint:*** Chill for 5–60 min on ice. The timing is not critical; efficiency peaks near 30 min and does not drop within the first hour.

E › ***Electrocompetent* E. coli**

- ☐ 10% Glycerol, sterile

(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 OD_{600} of this suspension should range 40–50 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, resuspend in less glycerol to reach OD_{600} = 100–150.

›› ***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:*** An electrocompetent suspension holds about 1×10^{10}–3×10^{10}/mL cells.

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

(4.) Store at −80 °C, where the cells stay competent for at least six months. Freeze-thawing will significantly decrease transformation efficiency.

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

(1.) Thaw cells on ice. Do not warm the tube by hand, 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.

> ***Hint:*** A ligation mixture can be used without purification at a twofold loss in transformants. Purify it when the number of transformants matters, as for a library.

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

- ☐ S. O. C. medium ◇ R0044
  - • *or*: LB medium (Miller) ◇ R0030

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

> ***Hint:*** Expect a twofold loss in transformants for every 10 min this step is shortened.

(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 5–60 min on ice.

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*

- ☐ Electroporation cuvette, 0.2 cm gap
- ☐ Electroporator
- ☐ S. O. C. medium ◇ R0044
  - *or*: LB medium (Miller) ◇ R0030
- ☐ 70% Ethanol (*optional*)

(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 12.5–15 kV/cm, which corresponds to 2.5–3.0 kV for a 0.2 cm cuvette.

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

> ***Quality assurance:*** A successful pulse yields a time constant of about 5 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.

> ***Critical:*** Add the medium without delay. Transformants are lost within the first minutes after the pulse.

(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*

- ☐ Selective LB agar plate, selection antibiotic
- ☐ Selection antibiotic

(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. SOC gives about twice the transformation efficiency of LB. Expect a twofold loss in transformants for every 15 min the step is shortened. For ampicillin it can be omitted; other markers need at least 20–30 min.

(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.
- Calibrate the heat shock for the tubes in use. Both the temperature and the duration depend on the volume and the vessel: 30 s suits most strains in a tube, BL21 wants exactly 10 s, and a 96-well plate needs its own calibration again.
- Ensure cells were grown to mid-log phase (OD_{600} = 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.

Materials

Preparation of* E. coli *in logarithmic growth phase

- ☐ **LB medium (Miller)** ◇ R0030
- ☐ **S. O. B. medium** ◇ R0212

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

- ☐ **Magnesium chloride ($MgCl_2$)**, 100 mM, sterile (*optional*)
- ☐ **Calcium chloride ($CaCl_2$)**, 50 mM, sterile
- ☐ **50 mM $CaCl_2$ / 15% glycerol**, sterile

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

- ☐ **RF1**, pH 5.8 ◇ R0185
  Store at 4 °C.
- ☐ **RF2**, pH 6.8 ◇ R0186
  Store at 4 °C.

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

- ☐ **Simple & efficient buffer (SEB)**, pH 6.7 ◇ R0187
  Store at 4 °C.
- ☐ **Dimethyl sulfoxide (DMSO)**

TSS-competent* E. coli *cells

- ☐ **Transformation & storage medium (TSS)**, 2 ×, pH 6.5 ◇ R0188
  Store at 4 °C.

***Electrocompetent* E. coli**

- ☐ **Glycerol**, 10%, sterile

Transformation of chemically competent bacteria

- ☐ **Recovery medium**
  - **S. O. C. medium** ◇ R0044
  - *or* **LB medium (Miller)** ◇ R0030

Transformation of electrocompetent bacteria

- ☐ **Electroporation cuvette**, 0.2 cm gap
- ☐ **Electroporator**
  - *e.g.* **Gene Pulser Xcell™**
    Bio-Rad
- ☐ **Recovery medium**
  - **S. O. C. medium** ◇ R0044
  - *or* **LB medium (Miller)** ◇ R0030
- ☐ **Ethanol**, 70% (*optional*)

Selection of transformed clones

- ☐ **Selective LB agar plate**, selection antibiotic
  - **LB agar plate**
- ☐ **Selection antibiotic**
  - *e.g.* **Ampicillin (Amp)**, 100 g/L, 1 000–5 000 × ◇ R0004
  - *e.g.* **Kanamycin (Kan)**, 50 g/L, 1 000 × ◇ R0029

Recipes

RF1, pH 5.8

Amount	Ingredient		Stock	Final
2.42 g	RbCl	[7791-11-9]	120.92 g/mol	100 mM
1.98 g	$MnCl_2 \cdot 4\ H_2O$	[13446-34-9]	197.90 g/mol	50 mM
	Serious eye damage (H318); Organ toxicity, repeated exposure (H373)			
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



Organ damage through prolonged or repeated exposure

- ☐ Collect as HEAVY METAL WASTE
- ☐ DO NOT wash into sewer

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
15 mL	Calcium chloride ($CaCl_2$) R0012	1 M	75 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, 75 mM $CaCl_2$, 15% Glycerol, pH 6.8



No GHS hazards at the indicated concentrations

☐ Hold for EHS review before disposal

Date: Sign: R0186

Simple & efficient buffer (SEB), pH 6.7

Amount	Ingredient	Stock	Final
0.60 g	1,4-Piperazinediethanesulfonic acid (PIPES) [5625-37-6]	302.37 g/mol	10 mM
3.73 g	KCl [7447-40-7]	74.55 g/mol	250 mM
2.18 g	$MnCl_2 \cdot 4\ H_2O$ [13446-34-9]	197.90 g/mol	55 mM
	Serious eye damage (H318); Organ toxicity, repeated exposure (H373)		
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



Organ damage through prolonged or repeated exposure

☐ Collect as HEAVY METAL WASTE
☐ DO NOT wash into sewer

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 3350 [25322-68-3]	3 000–3 700 g/mol	20%
5 mL	Dimethyl sulfoxide (DMSO), reagent-grade [67-68-5]		10%
To 50 mL	LB medium (Miller)		

Adjust to pH 6.5 with hydrochloric acid; filter sterilize. Store at 4 °C.

2× Transformation & storage medium (TSS)

50 mM $MgCl_2$, 10% Polyethylene glycol 3350, 5% DMSO, pH 6.5



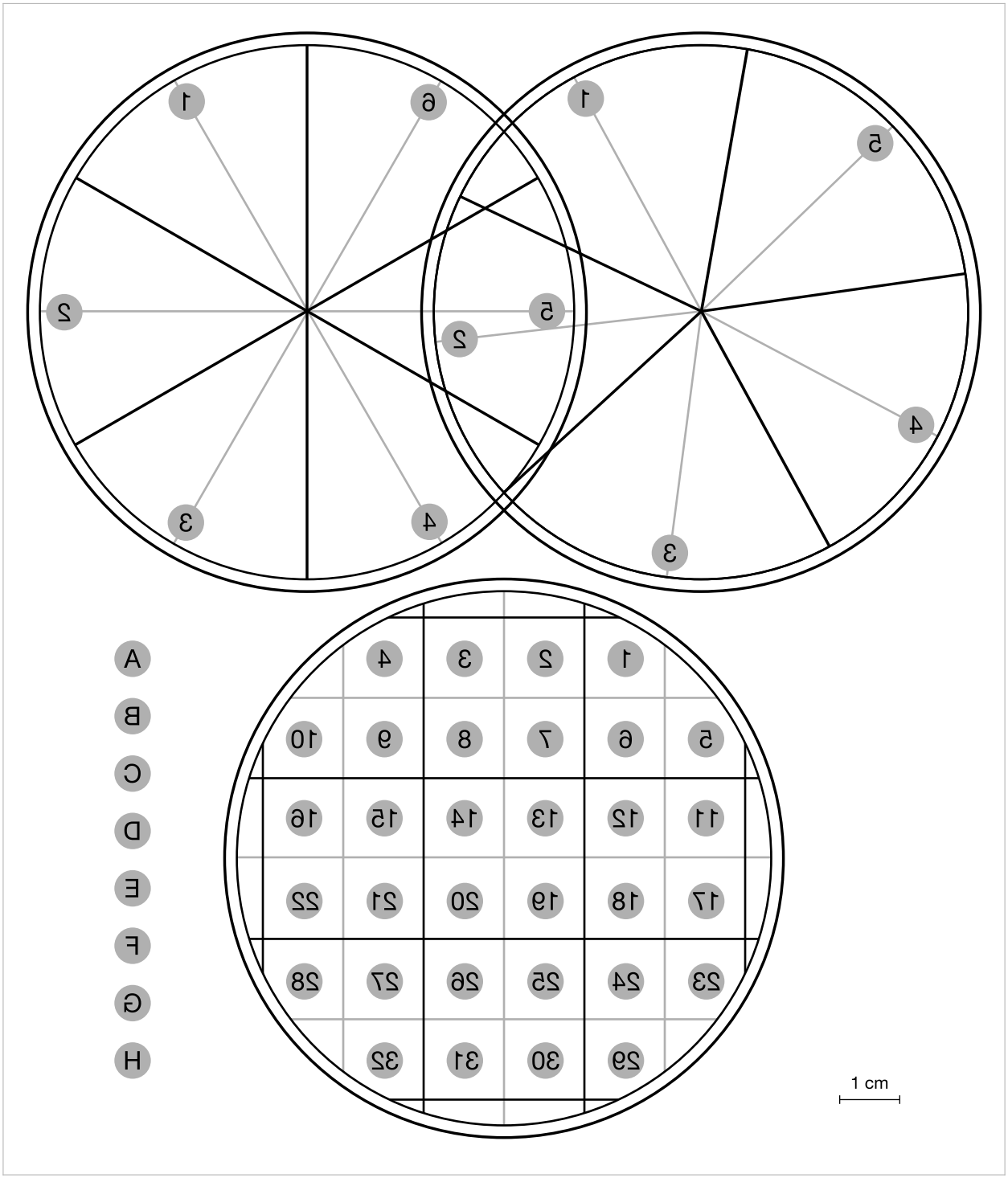
No GHS hazards at the indicated concentrations

☐ Collect as HAZARDOUS WASTE
☐ DO NOT wash into sewer

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

2026-06-11 **Jeff Coller, Konrad Herbst, Brinja Kosel, Alice Pawlowski, Benjamin C. Buchmuller** Version 1, 2012 to 2026. Chemically competent HB101 after Im, Sambrook & Russell, "The Inoue Method for Preparation and Transformation of Competent E. coli", Bio-protocol e143 (2011); heat shock, electroporation and high-efficiency competent cells from the Michael Knop lab; electrocompetent cells for library transformation and TSS transformation from the Daniel Summerer lab. Modularized and adapted as an SOP in 2025.

2026-11-02 **Benjamin C. Buchmuller** Version 2. Transformation conditions change: heat-shock and recovery windows calibrated to the vessel, with BL21 at ten seconds, and a 3,000 to 3,700 gram per mol polyethylene glycol. Credits: the Lamitina lab; New England Biolabs for the losses measured on a shortened incubation or recovery. Also corrected the electroporation settings and cell density, the Inoue resuspension volume, the TSS incubation window and the storage figures; SOC is noted as twice as efficient as LB.

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e258dec