CHAPTER TWENTY EIGHT

Transformation of Chemically Competent *E. coli*

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Abstract

To introduce DNA into *E. coli* cells.

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1. THEORY

Transformation of *E. coli* is an important step that allows the introduction of heterologous DNA using plasmid vectors or introducing mutations via homologous recombination events.

2. EQUIPMENT

Shaking incubator (37 °C)
UV/Vis spectrophotometer
Refrigerated low-speed centrifuge (4 °C)
Water bath (42 °C)
Incubator (37 °C)
Erlenmeyer flask, 500 ml (sterile)
10-cm Petri plates
0.2-μm filters
14-ml sterile polypropylene snap-cap tubes
Disposable cuvettes
50-ml sterile polypropylene tubes (e.g., Corning 430829)
2-ml sterile screw-capped conical tubes with no skirt (e.g., Phenix SCS-02 S)
5–10-ml glass vials or autoclavable screw-capped tubes
Glass spreader

3. MATERIALS

Antibiotic of choice
Bacto agar
Bacto tryptone
Bacto yeast extract
Calcium chloride ($CaCl_2$)
Magnesium chloride ($MgCl_2$)
Magnesium sulfate ($MgSO_4$)
Manganese chloride ($MnCl_2$)
3-(*N*-Morpholino)-propanesulfonic acid (MOPS)
Potassium acetate (KOAc)
Potassium chloride (KCl)
Rubidium chloride (RbCl)
Sodium chloride (NaCl)

Glucose
Glycerol
Acetic acid
Sodium hydroxide (NaOH)
Potassium hydroxide (KOH)
Dry ice
Ethanol
Liquid nitrogen

3.1. Solutions & buffers

Step 1 Psi Media

Component	Amount
Tryptone	20 g
Yeast extract	5 g
$MgCl_2$	5 g

Dissolve in 900 ml deionzed water. Adjust the pH to 7.6 with KOH. Add water to 1 l and autoclave to sterilize
Note: You can also use LB medium (low salt) supplemented with 4 mM $MgSO_4$ and 10 mM KCl or SOB (also commercially available)

Tfb I (Transformation Buffer I)

Component	Final concentration	Amount
Potassium acetate	30 mM	1.18 g
$RbCl_2$	100 mM	4.84 g
$CaCl_2{\cdot}2H_2O$	10 mM	0.59 g
$MnCl_2{\cdot}4H_2O$	50 mM	3.96 g
Glycerol	15% (v/v)	60 ml

Dissolve in 300 ml water. Adjust the pH to 5.8 with dilute acetic acid. Add water to 400 ml and pass through a 0.2-μm filter to sterilize

Tfb II (Transformation Buffer II)

Component	Final concentration	Amount
MOPS	10 mM	0.21 g
$CaCl_2{\cdot}2H_2O$	75 mM	1.1 g
$RbCl_2$	10 mM	0.12 g
Glycerol	15% (v/v)	15 ml

Dissolve in 75 ml water. Adjust the pH to 6.5 with dilute NaOH. Add water to 100 ml and pass through a 0.2-μm filter to sterilize

Step 2 LB Agar (Miller's high salt)

Component	Amount
Tryptone	10 g
Yeast extract	5 g
NaCl	10 g
Agar	15 g

Dissolve in 900 ml water. Adjust the pH to 7.2 with ~0.2 ml 5 N NaOH. Add water to 1 l and autoclave to sterilize. Cool to ~55 °C, add appropriate antibiotic and pour plates
Note: LB Agar is available commercially as a premixed powder

SOC media

Component	Final concentration	Stock	Amount
Yeast extract	0.5%	–	0.5 g
Tryptone	2%	–	2.0 g
NaCl	10 mM	3 M	0.33 ml
KCl	2.5 mM	1 M	0.25 ml
$MgCl_2$	10 mM	1 M	1 ml
$MgSO_4$	10 mM	1 M	1 ml
Glucose	20 mM	1.1 M	1.82 ml

Dissolve in 90 ml of deionized water and bring the volume to 100 ml. Dispense into 5–10 ml aliquots and autoclave to sterilize

4. PROTOCOL

4.1. Preparation

Pour selective agar plates and let harden.

Pick a single colony from a freshly streaked plate (without antibiotics!) and inoculate a small culture (2–5 ml) of Psi media in a sterile 14-ml snap-cap tube. Grow overnight at 37 °C with shaking at 250 rpm.

Store TfbI and TfbII at 4 °C to make sure they are chilled.

4.2. Duration

Preparation	About 15 min
Protocol	About 4–6 h

See Fig. 28.1 for the flowchart of the complete protocol.

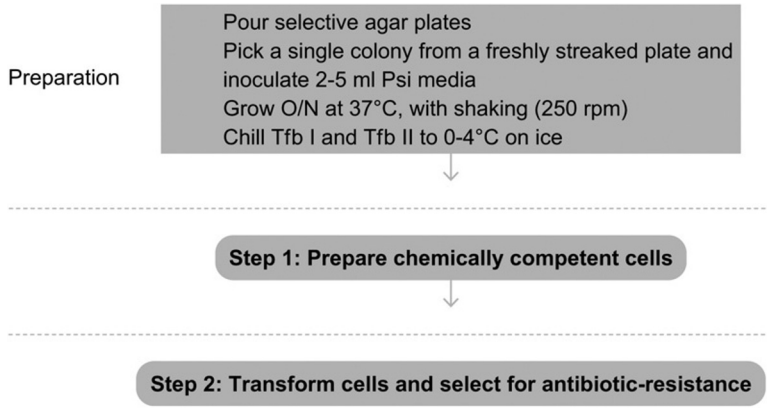

Figure 28.1 Flowchart of the complete protocol, including preparation.

5. STEP 1 PREPARE COMPETENT CELLS

5.1. Overview

Grow cells to mid-log phase and make competent by chemical treatment.

5.2. Duration

3–5 h

1.1 Inoculate 100 ml of Psi media with 0.5 ml of the overnight culture and incubate at 37 °C with vigorous shaking.

1.2 When A_{600} reaches 0.4–0.5, place on ice and chill for 5–10 min.

1.3 Transfer the cells to 50-ml sterile chilled polypropylene centrifuge tubes. Pellet cells at $5000 \times g$, 4 °C for 5 min.

1.4 Discard supernatant carefully and gently resuspend the cell pellet in 0.4 volume of ice-cold Tfb I (20 ml for each 50 ml tube). Do not vortex; keep on ice while resuspending.

1.5 Incubate the cells on ice for 15 min.

1.6 Pellet the cells at $2000 \times g$, 4 °C for 10 min.

1.7 Discard the supernatant carefully and gently resuspend in 0.02 volume of ice-cold TfbI II (1 ml for 50 ml of culture). Keep the tube on ice.

1.8 Aliquot 50 μl into 2-ml prechilled sterile screw-capped tubes with conical bottoms.

1.9 Flash-freeze in a dry ice-ethanol bath or liquid nitrogen and store at −80 °C.

5.3. Tip

Use sterile technique at all times; the bacteria are not antibiotic-resistant at this point.

5.4. Tip

If reusable tubes are used to pellet the cells, they must be very clean and free of soap residue.

5.5. Tip

Cells and transformation buffers should be kept cold at all times. It is also preferable to use chilled pipettes and do everything in the cold room if possible.

5.6. Tip

The incubation in Tfb I can vary from 5 min to 1–2 h without any harm. The cells must be kept on ice.

5.7. Tip

Do not use standard 1.5-ml conical microcentrifuge tubes – they do not work well in the heat shock step of the transformation.

See Fig. 28.2 for the flowchart of Step 1.

6. STEP 2 TRANSFORM COMPETENT CELLS

6.1. Overview

Introduce DNA into competent cells and select for antibiotic-resistant bacteria.

6.2. Duration

2 h

2.1 Equilibrate a water bath to 42 °C. A dry heating block will work if the tube fits snugly, but it is not as good as a water bath.

2.2 Thaw one vial of competent cells on ice for each transformation. Handle gently since cells are sensitive to temperature changes and mechanical lysis.

2.3 Add 1–5 μl of DNA (10 pg to 100 ng) to a vial of thawed competent cells. DO NOT VORTEX OR PIPETTE UP AND DOWN.

2.4 Incubate on ice for 30 min.

2.5 Heat-shock cells for 30 s at 42 °C. Do not go any longer or shake the tube.

2.6 Remove the tube from the water bath and place on ice for 2 min.

2.7 Add 250 μl of SOC media to each vial.

2.8 Make sure that the cap is tight and incubate the tube on its side in a 37 °C shaking incubator (200–250 rpm) for 1 h.

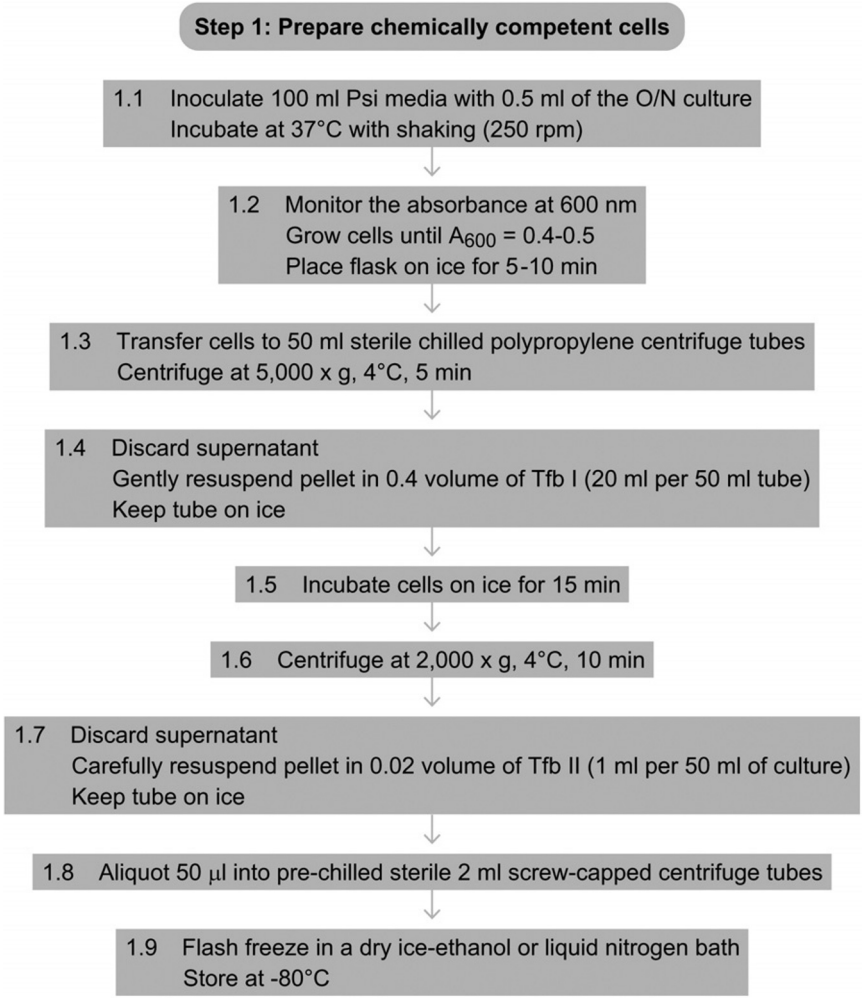

Figure 28.2 Flowchart of Step 1.

2.9 Spread from 20 to 200 μl on an appropriate selective plate. The plates should be at room temperature or prewarmed to 37 °C. Incubate overnight at 37 °C.

6.3. Tip

Validate the chemically competent cells by plating untransformed cells on LB plates (without antibiotic – should have a lawn of cells) and LB plates containing antibiotic (should not have any colonies growing on them).

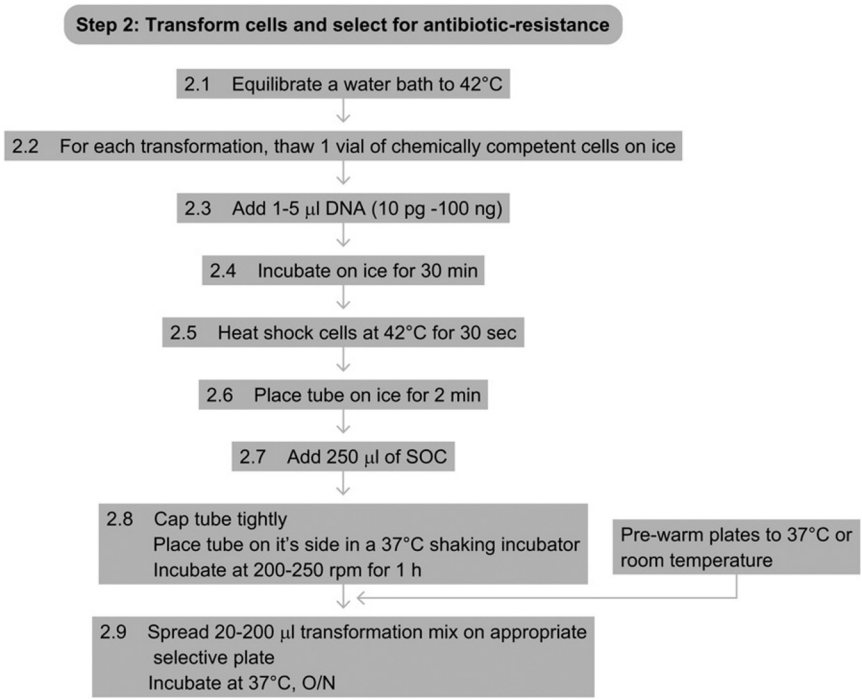

Figure 28.3 Flowchart of Step 2.

6.4. Tip

Transform the cells with 10, 30, and 100 pg of supercoiled plasmid DNA and determine the transformation efficiency (# colonies per microgram DNA).

6.5. Tip

Bacteria are transformed more efficiently using supercoiled DNA than ligated DNA.

6.6. Tip

Transformed cells can be stored at 4 °C for 24–48 h with minimal loss of viability. Transformation efficiency varies depending on DNA.

See Fig. 28.3 for the flowchart of Step 2.

REFERENCES

Related Literature

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