Published in final edited form as:
Methods Enzymol. 2013 ; 529: 329–336. doi:10.1016/B978-0-12-418687-3.00028-8.

Chemical Transformation of *E. coli*

Rachel Green and **Elizabeth J. Rogers**
Johns Hopkins School of Medicine/HHMI, Molecular Biology and Genetics, PCTB 704, 725 N. Wolfe Street, Baltimore, MD 21205

Purpose

To introduce DNA into *E. coli* cells

Theory

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

Equipment

4°C Centrifuge

42°C Water Bath

37°C Shaking Incubator

Materials

Bacto Agar

Bacto Tryptone

Bacto Yeast Extract

Selective Agar Plates

Calcium Chloride

Magnesium Chloride

Magnesium Sulfate

Manganese Chloride

MOPS

Potassium Acetate

Potassium Chloride

Rubidium Chloride

Corresponding Author, eroger11@jhmi.edu, ragreen@jhmi.edu.

Sodium Chloride

Glucose

Glycerol

Acetic Acid

Sodium Hydroxide

Potassium Hydroxide

50 ml sterile polypropylene centrifuge tubes (*i.e* Corning 430829)

2 ml sterile, screw cap, conical bottom tubes (i.e. Phenix SCS-02S)

5–10 ml glass vials or autoclaveable screw cap tubes

Solutions & buffers	***Step 1*** **Psi Media**
Component	**Amount/liter**
Tryptone	20 g
Yeast Extract	5 g
$MgCl_2$	5 g
Adjust to pH 7.6 with KOH Add water to 1 liter and autoclave	

Tip You can also use LB medium (low salt) supplemented with 4 mM MgSO4 and 10 mM KCl or SOB (also commercially available)

Tfb I (Transformation Buffer I)			
Component	**Final concentration**	**Stock**	**Amount/400 ml**
Potassium acetate	30 mM	-	1.18 g
$RbCl_2$	100 mM	-	4.84 g
$CaCl_2$ -$2H_2O$	10 mM	-	0.59 g
$MnCl_2$	50 mM	-	3.96 g
glycerol	15% v/v	-	60 ml
pH 5.8 with dilute acetic acid. Add water to 400 ml and filter sterilize			

Tfb II (Transformation Buffer II)			
Component	**Final concentration**	**Stock**	**Amount/100 ml**
MOPS	10 mM	-	0.21 g

Tfb II (Transformation Buffer II)			
Component	**Final concentration**	**Stock**	**Amount/100 ml**
$CaCl_2$-$2H_2O$	75 mM	-	1.1 g
$RbCl_2$	10 mM	-	0.12 g
Glycerol	15% v/v	-	15 mls
pH 6.5 with dilute NaOH. Add water to 100 ml and filter sterilize			

Step 2	
LB Agar (Miller's high salt)	
Component	**Amount/liter**
Tryptone	10 g
Yeast Extract	5 g
NaCl	10 g
Agar	15 g
Adjust to pH 7.2 (~0.2 ml of 5 N NaOH) Add water to 1 liter and autoclave, add appropriate antibiotic when cool and pour plates	

Tip LB Agar is available in premixed commercial preps

SOC media			
Component	**Final concentration**	**Stock**	**Amount/100 mls**
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
MgCl2	10 mM	1 M	1 ml
MgSO4	10 mM	1 M	1 ml
Glucose	20 mM	1.1 M	1.82 ml
Add water to 100 mls aliquot in 5–10 ml samples and autoclave			

Protocol

Duration		
	Preparation	about 15 minutes
	Protocol	about 4–6 hours
Preparation	Make sure you have selective agar plates on hand.	
	Pick a single colony from a freshly streaked plate and inoculate a small culture (2–5 mls). Grow overnight at 37°C.	

TfbI and TfbII should be stored at 4°C to make sure they are chilled

Step 1	**Prepare competent cells**
Overview *Duration*	Grow cells to mid-log and make competent by chemical treatment 3–5 hours
1.1	Inoculate 100 mls of Psi broth with 0.5 ml of 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 5–10 minutes
1.3	Transfer cells to 50 ml sterile chilled polypropylene centrifuge tubes. Pellet cells at 4°C for 5 minutes at 5,000 × g
Tip	*If using other tubes they must be very clean and free of soap residue. Cells and transformation buffers should be kept cold at all times. It is also preferable to use chilled pipets and do everything in the cold room if possible.*
1.4	Discard supernatant carefully and gently resuspend cell pellet in 0.4 volume ice cold TfbI (20 mls for each 50 ml tube). Do not vortex and keep on ice while resuspending.
1.5	Incubate cells on ice for 15 minutes.
Tip	*Some protocols incubate for only 5 minutes and cells can be left on ice for longer periods (i.e. 1–2 hrs) without any harm*
1.6	Pellet cells at 4°C for 10 minutes at 2,000 × g
1.7	Discard supernatant carefully and gently resuspend in 0.02 volume (1 ml for 50 mls of culture) TfbII while keeping on ice.
1.8	Aliquot 50 microliters into 2 ml sterile screw cap tubes with conical bottom and no skirt. Do not use standard 1.5 ml conical microfuge tubes – they don't work well in the heat shock step of the transformation.
1.9	Flash freeze in dry ice ethanol bath or liquid nitrogen and store at −80°C
Step 2	**Transform competent cells**
Overview *Duration*	Introduce DNA into competent cells 2 hours
2.1	Equilibrate a water bath to 42°C. A dry block will work if the tube fits snugly, but is not as good as the water bath.
2.2	Thaw 1 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 to 5 microliters of DNA (10 pg to 100 ng) to a vial of thawed competent cells. DO NOT VORTEX OR PIPETT UP AND DOWN. Supercoiled DNA is transformed more efficiently than ligated DNA.
2.4	Incubate on ice for 30 minutes
2.5	Heat shock cells for 30 seconds at 42°C. Do not go any longer or shake the cells
2.6	Remove from the water bath and place on ice for 2 minutes
2.7	Add 250 microliters of SOC media to each vial
2.8	Make sure cap is tight and incubate tube on its side in a 37°C shaking incubator (200–250 rpm) for 1 hour.
2.9	Spread from 20 – 200 microliters on an appropriate selective plate. The plates should be at room temperature or prewarmed to 37°C. Incubate overnight at 37°C.
Tip	*Transformed cells can be stored at 4° C for 24–48 hours with minimal loss of viability. Transformation efficiency varies depending on DNA.*

References

1. Hanahan D. Studies on transformation of Escherichia coli with plasmids. J. Mol. Biol. 1983; 166:557–580. [PubMed: 6345791]
2. Hanahan D, Jessee J, Bloom FR. Plasmid transformation of Escherichia coli and other bacteria. Methods in Enzymology. 1991; 204:63–114. [PubMed: 1943786]