iGEM2013 – Microbiology – BMB – SDU

Title: TSB transformation	**Date issued:** 2013.06.17
SOP number: SOP0009_v01	**Review date:** 2013.06.17
Version number: 01	**Written by:** Patrick Rosendahl Andreassen

1. Purpose

To transform *E. coli* cells with plasmid using TSB buffer

2. Area of application

All *E. coli* cells

3. Apparatus and equipment

Apparatus/equipment	Location (Room number)	Check points	Criteria for approval/rejection
Pipettes (p1000,200,10)		•	
Heating block		•	
Ice	Across V18-403b-2	•	

4. Materials and reagents – their shelf life and risk labelling

Name	Components	Supplier / Cat. #	Room (hallway storage)	Safety considerations
Green pipette tips		Contact lab-manager	Micro storage	
Blue pipette tips		Contact lab-manager	Micro storage	
Purple pipette tips		Contact lab-manager	Micro storage	
Fort. LB		The new Anne-mette	Autoclave room	
Polyethylene glycol (PEG) 3,350		Sigma Aldrich	Micro chemical room	
Dimethyl sulfoxid (DMSO)		Sigma Aldrich	Micro chemical room	
Magnesium chloride ($MgCl_2$) 1M		The new Anne-mette?	Autoclave room	
Sterile filter (Pref. Blue)		Contact lab-manager	Micro storage	
Plasmid				
15mL falcon tube		Contact lab-manager	Micro storage	
10mL syringe		Contact lab-manager	Micro storage	
Long needle for syringe		Contact lab-manager	Micro storage	

5. QC – Quality Control

Colony PCR on transformed cells using primers for the plasmid.

6. List of other SOPs relevant to this SOP

7. Environmental conditions required

8. Procedure

8.1 Preparation of *E. coli* culture:
- 8.1.1 Add at least 5mL fort. LB (depending on amount of transformation to perform) to a bulb
- 8.1.2 With a blue pipette tip add *E. coli* culture from agar plate to the LB media
- 8.1.3 Grow culture to a OD_{600} of 0.3 to 0.5

8.2 Preparation of TSB buffer
- 8.2.1 Add the following components to a 15mL falcon tube:
  - 8.2.1.1 PEG 3,350 1g
  - 8.2.1.2 DMSO 500µL
  - 8.2.1.3 $MgCl_2$ (1M) 200 µL
  - 8.2.1.4 Fort. LB →10 mL
- 8.2.2 When everything is completely dissolved, transfer it to a new (sterile) falcon tube through a sterile filter using a syringe

8.3 TSB transformation
- 8.3.1 Spin 0.5-1.0mL culture for 5 min. at 4000 rpm.
- 8.3.2 Remove supernatant
- 8.3.3 Dissolve pellet in 200µL TSB buffer
- 8.3.4 Add plasmid (varying amount)
- 8.3.5 Keep at ice for 30 min.
- 8.3.6 Transfer directly to a heating block at 42°C for 2 min.
- 8.3.7 Add 1 mL fort. LB
- 8.3.8 Phenotypical expression at 37°C (0-2 hours)
- 8.3.9 Spin for 5 min. at 4000 rpm.
- 8.3.10 Remove most supernatant and dissolve pellet in the remaining supernatant (50-150µL)
- 8.3.11 Plate on agar plate with appropriate antibiotic

9. Waste handling

Chemical name	Concentration	Type of waste (C, Z…)	Remarks

10. Time consumption

- Total-time 4-6 hours.
- Hands-on-time 45 min.

11. Scheme of development

Date / Initials	Version No.	Description of changes
13.06.17 / PRA	01	The SOP has been written
13.01.02 / TJK	01	The SOP has been approved

12. Appendixes