iGEM2017 – Microbiology – BMB – SDU

Title: TSB transformation	**Date issued:** 2013.06.17
SOP number: SOP06	**Review date:** 2017.05.01
Version number: 02	**Original by:** PRA
	Written by: SJ

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)	Micro Storage	●	
Heating block	Laboratory 1. Floor	●	
Ice	Across V18-403b-2	●	

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

Name	Components	Supplier / Cat. #	Room (hallway storage)	Safety considerations
Purple pipette tips		Contact lab-manager	Micro storage	
Green pipette tips		Contact lab-manager	Micro storage	
Blue pipette tips		Contact lab-manager	Micro storage	
Fort. LB		The new	Autoclave room	
Polyethylene glycol (PEG) 3,350		Sigma Aldrich	Micro chemical room	
Dimethyl sulfoxid (DMSO)		Sigma Aldrich	Micro chemical room	
Magnesium chloride (MgCl2) 1M		The new	Autoclave room	
Sterile filter (Pref. Blue)		Contact lab-manager	Micro storage	
Plasmid				
15 mL falcon tube		Contact lab-manager	Micro storage	
10 mL 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

1. Preparation of E. coli culture:
   1.1. Add at least 5 mL fort. LB (depending on amount of transformation to perform) to a bulb
   1.2. With a blue pipette tip add E. coli culture from agar plate to the LB media or use an ON culture that is diluted 100 x.
   1.3. Grow culture to an OD600 of 0.3 to 0.5

2. Preparation of TSB buffer
   2.1. Add the following components to a 15 mL falcon tube:
      2.1.1. PEG 3,350 1 g
      2.1.2. DMSO 500 µL
      2.1.3. $MgCl_2$ (1M) 200 µL
      2.1.4. Fort. LB →10 mL
   2.2. When everything is completely dissolved, transfer it to a new (sterile) falcon tube through
      a sterile filter using a syringe

3. TSB Transformation
   3.1. Spin 0.5-1.0 mL culture for 5 min. at 4000 rpm.
   3.2. Remove supernatant
   3.3. Dissolve pellet in 200 µL TSB buffer
   3.4. Add plasmid (varying amount)
   3.5. Keep at ice for 30 min.
   3.6. Transfer directly to a heating block at 42°C for 2 min.
   3.7. Add 1 mL fort. LB
   3.8. Phenotypical expression at 37°C (0-2 hours)
   3.9. Spin for 5 min. at 4000 rpm.
   3.10. Remove most supernatant and dissolve pellet in the remaining supernatant (50-150 µL)
   3.11. Plate on agar plate with appropriate antibiotic

9. Waste handling

Chemical name	Concentration	Type of waste (C, Z…)	Remarks
ON Culture		Liquid bacterial waste	
Once use plastic		GMO yellow waste	

10. Time consumption

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

11. Scheme of development

Date / Initials	Version No.	Description of changes
13.06.18 / PRA	01	The SOP has been written
13.06.18 / AK	01	The SOP has been approved
17.04.28 / SJ	02	The SOP has been modified
17.05.01 / EG	02	The SOP has been approved

12. Appendices