iGEM2016 – Microbiology – BMB – SDU	
Title: Ca^{++} transformation **SOP number:** SOP0023_v01 **Version number:** 01	**Date issued:** 2016.05.20 **Review date:** 2016.05.20 **Written by:** Brian Kenn Baltzar

1. Purpose

To transform E. coli cells with plasmid using Ca^{++} competent E. coli from freeze-stock

2. Area of application

Ca^{++} competent E. coli from freeze-stock

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	
LB			Autoclave room	
Plasmid				

C++ competent E. coli			-80ºC freezer #11	

5. QC – Quality Control

Colony PCR on transformed cells using primers for the plasmid.

6. List of other SOPs relevant to this SOP

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7. Environmental conditions required

8. Procedure

8.1. Put 100µL of competent cells in a microtube.
8.2. Add 1µL of plasmid (remember + and - control)
8.3. Keep at ice for 30 min.
8.4. Transfer directly to a heating block at 42°C for 2 min.
8.5. Add 900µL LB.
8.6. Phenotypical expression at 37°C with shaking (0-2 hours).
8.7. Spin for 5 min. at 3500 G.
8.8. Remove 800-900 µL supernatant and dissolve pellet in the remaining supernatant.
8.9. 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 1-3 hours.
- Hands-on-time 30 min.

11. Scheme of development

Date / Initials	Version No.	Description of changes
16.05.20 / BKB	01	The SOP has been written

	01	The SOP has been approved

12. **Appendices**