iGEM2013 – Microbiology – BMB – SDU	
Title: Plasmid Miniprep **SOP number:** SOP0019_v01 **Version number:** 1	**Date issued:** 2013.07.01 **Review date:** **Written by:** MHK and SIS

1. Purpose

To extract plasmid from cells.

2. Area of application

All bacterial strains

3. Apparatus and equipment

Apparatus/equipment	Location (Room number)	Check points	Criteria for approval/rejection
Inoculation tubes	Micro storage hallway	●	
Mini centrifuge	Laboratory (class 1) - V18-403b-2	●	

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

Name	Components	Supplier / Cat.	Room (hallway	Safety

		#	storage)	considerations
Purple pipette tips		Contact lab-manager	Micro storage	
Green pipette tips		Contact lab-manager	Micro storage	
Plasmid Miniprep kit	Cell resuspension solution, cell lysis solution, neutralization solution, quantum prep matrix, wash buffer, spin filters, wash tubes and collection tubes	BIORAD	iGEM storage in hallway	
MiliQ water				
Ethanol			Laboratory (class 1) - V18-403b-2	
LB media				

5. QC – Quality Control

6. List of other SOPs relevant to this SOP

iGEM2013_SOP0009_v01_TSB transformation.pdf
iGEM2013_SOP0016_v01_Making LB and LA media.docx

7. Environmental conditions required

8. Procedure

1. Check that none of the kit solutions contain precipitate. In case it does warm the bottles to 37 deg with occasional mixing until dissolved.
2. Transfer 1-2 ml of the ON culture of plasmid containing cells to a microcentrifuge tube.
3. Centrifuge cells for 15-30 sec at maximum speed. Remove all the supernatant.
4. Add 200µl resuspension solution and vortex/pipet up and down until the pellet is completely resuspended.
5. Add 250µl of the cell lysis solution and mix by gently inverting the capped tube about 10 times(DO NOT VORTEX) when/if the lysis has occurred the solution should become slightly clear.

6. Add 250µl of the neutralization solution and mix by gently inverting the capped tube about 10 times(DO NOT VORTEX). A precipitate should form.
7. Pellet the cell debris for 5 min in a microcentrifuge, the debris pellet will form along the side or in the bottom of the tube. The supernatant (cleared lysate) contains the plasmid DNA.
8. While waiting for the centrifuge in step 7. Insert a spin filter into one of the 2 ml wash tubes from the kit. Mix the Quantum Prep matrix by vortexing or repeated shaking and inversion of the bottle to make sure it is completely suspended.
9. Transfer the cleared lysate(supernatant) from step 7 to the spin filter, add 200 µl of thoroughly suspended Quantum Prep matrix, then pipet up and down to mix. If you have multiple samples, transfer the lysates first, then add matrix and mix. When matrix has been added to all samples and mixed, centrifuge for 30 sec.
10. Remove the spin filter from the 2 ml tube, discard the filtrate at the bottom of the tube, and replace the spin filter in the same tube. Add 500 µl of wash buffer and wash the matrix by centrifugation for 30 sec.
11. Remove the spin filter from the 2 ml tube, discard the filtrate at the bottom of the tube and replace the spin filter in the same tube. Add 500 µl of wash buffer and wash the matrix by centrifugation for a full 2 mins to remove residual traces of ethanol.
12. Remove the spin filter and discard the microcentrifuge tube. Place the spin filter in one of the 1.5 ml collection tubes supplied with the kit or in any standard 1.5 ml microcentrifuge tube that accomodate the spin filter. Add 100 µl of deionized H2O or TE. Elute the DNA by centrifugation for 1 min at top speed.
13. Discard the spin filter and store the eluted DNA in the fridge.

9. Waste handling

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

10. Time consumption

- Total-time 30 min.
- Hands-on-time 30 min.

11. Scheme of development

Date / Initials	Version No.	Description of changes
13.07.01/SIS+MHK	01	The SOP has been written
	01	The SOP has been approved

12. Appendices