RISK ASSESSMENT – TASK BASED
IGEM 2016

Location:	**Building Number:**	**Date:**	**Assessed By:**	**Health & Safety Representative:**
Room W301, Medical Building	**181**	March 2016	Amber Willems-Jones	**Vincé Kalangi**

Description of Activity: **4.7 Plasmid Mini-Prep using Favorgen's FavorPrep™ Plasmid DNA Extraction Mini Kit** **SWP No: 4.7**	
Is there past experience with the Activity that may assist in the risk assessment? Incidents & Near-hits, Incident Investigations, Workplace Inspections, Training, Standards, Legislation & Codes, Uni Guidance Material, Existing Controls, Industry Standards.	**NO**

1. TASK	**2. HAZARD**	**3. Estimated RAW RISK SCORE C x E x L**	**4. CONTROLS**	**5. Residual Risk Score RISK SCORE C E L C x E x L**				**6. Residual Risk**
Plasmid purification	Skin contact with NaOH (corrosive), SDS (irritant) in buffers.	5x3x1	Personal Protective Equipment ; training	5	3	0.1	1.5	low
Bench top centrifuge	Samples unbalanced	15x3x1	Adequate training and induction	15	3	0.1	4.5	low
	TOTAL	60		TOTAL			6	low

Name & Signature of Laboratory Head/Supervisor or Delegate	Amber Willems-Jones	Date	
Name & Signature of Person Performing Activity or Task		Date	

Number and Title	PRG 4.7 Plasmid Mini-Prep using Favorgen's FavorPrepTM Plasmid DNA Extraction Mini Kit Purification System
Name of Laboratory/Department	The University of Melbourne IGEM Team Laboratory, Department of Biochemistry
Author, Date Prepared & Date of Review	Author: Ella Bocquet-Gaylard Date: 22/2/2016 Updated : March 2016, Review by: March 2018
Introduction	The methods outlined in the following describe how to purify plasmid DNA from an inoculated media broth.
Principles / Scope	Plasmid Mini-Prep using Favorgen's FavorPrepTM Plasmid DNA Extraction Mini Kit.
Risk Management	***Risk assessments have been prepared and are available in the Risk Register (or attached to the SWP).*** **Raw Risk: low Residual Risk:low**
Safety Management	**Hazards:** Always wear appropriate personal protective equipment. When handling hot materials **Risk Controls:** Administrative , PPE
Licences / Permits	N/A
Training / Competency	All team members must be inducted into the use of any equipment used.
Equipment	Materials Bench top centrifuge 1.5 mL microcentrifuge tubes Reagents FAPD1 Buffer (containing RNase A) FAPD2 Buffer FAPD3 Buffer W1 Buffer Wash Buffer Elution Buffer
Protocol **Step 1** **Step 2** **Step3** **Step4**	Note: This system works most efficiently when the plasmid is less than 12,000 bp in size. Harvest 1-4 mL of the 4567 overnight bacterial culture by centrifugation for 1 mins at 13 000 rpm in a tabletop centrifuge in a 1.5mL microcentrifuge tube. Pour off the supernatant and blot the inverted tube on a paper towel to remove excess media. If more plasmid is desired then add another 2 mL from the overnight culture to the same tube and repeat centrifugation. This obtains a bigger pellet. Add 200 µL of FAPD1 buffer (RNase A added), and completely resuspend the pellet by pipetting up and down, until no cell pellet is visible.

Step 5	Note: Ensure thorough resuspension by expelling solutions close to the pellet to gradually disturb the pellet and then pipetting up and down until solution does not contain any lumps of pellet (check by holding up to the light). Add 200 µL of FAPD2 Buffer,and gently invert the tube 10 times to lyse cells. Do not vortex. Note: It is important to keep the cell solution in round bottomed tubes to ensure proper mixing by inversion.
Step6	Incubate for 2 minutes at room temperature (but for no more than 5 min).
Step7	Add 300 µl of FAPD3 Buffer and mix by inverting imediately but gently 10 times. This helps avoid uneven precipitation.
Step 8	Centrifuge at full speed (13,000 rpm) for 5 minutes.
Step 9	Transfer the supernatant carefully to a FAPD Column (ensure the column is inside its collecting tube), being very careful not to transfer any of the white precipitate.
Step 10	Centrifuge for 30 seconds at 13,000 rpm. Discard flow through and place Column back in the Collection Tube.
Step 11	Add 400 µl of W1 Buffer to FAPD column.
Step 12	Centrifuge for 30 seconds at 13,000 rpm. Discard flow through and place Column back in the Collection Tube.
Step 13	Add 600 µl of Wash Buffer (ethanol added) to FAPD column.
Step 14	Centrifuge for 30 seconds at 13,000 rpm. Discard flow through and place Column back in the Collection Tube.
Step 15	Centrifuge again for an additional 3 minutes at 13,000 rpm. Discard flow through.
Step 16	Place FAPD column in a sterile, labeled 1.5mL microcentrifuge tube.
Step 17	Add 50-100 µl of Elution Buffer to the membrane center of the FAPD column. Stand the column for 2 minutes.
Step 18	Centrifuge for 1 min at 13,000 rpm to elute plasmid DNA.
Step 19	Store plasmid DNA at 4 °C or -20 °C.

Controls / Calibration	N/A
Waste Disposal	***Disposal requirements:*** Follow PC I guidelines for handling, cleaning and when necessary, disposal of bacterial culture and solid wastes.
Emergency Procedures	First aid measures Eye contact: Immediately flush eyes with plenty of water for at least 20 minutes and get medical attention. Skin contact: In case of contact, immediately flush skin with plenty of water for at least 20 minutes. Inhalation: Move exposed person to fresh air. If not breathing, if breathing is irregular or if respiratory arrest occurs, provide artificial respiration or oxygen by trained personnel. Get medical attention. Ingestion: Wash out mouth with water. Do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Call medical doctor or poison control centre immediately.
References	Favorgen Manufacturers Guide for the FavorPrepTM Plasmid DNA Extraction Mini Kit
Authorised By	Amber Willems-Jones