RISK ASSESSMENT – TASK BASED
IGEM 2016

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

Description of Activity: **4.3 Restriction Enzyme Digest** **SWP 4.3**	
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**
DNA digestion with restriction enzyme digest	Skin contact with chemicals in buffer (Tris, potassium acetate, Magnesium acetate, dithiothreitol), irritant	1x3x1	Personal Protective Equipment ; training	1	3	0.1	0.3	Low risk
	TOTAL	3.		TOTAL			0.3	Low risk

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 4.3 Restriction Enzyme Digest
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: 1/2/2016 Updated : February 2016, Review by: February 2018
Introduction	Restriction Enzyme Digest
Principles / Scope	DNA digestion by specific enzymes
Risk Management	***Risk assessments have been prepared and are available in the Risk Register (or attached to the SWP).*** **Raw Risk:Residual Risk:** Low Risk
Safety Management	**Hazards:**Always wear appropriate personal protective equipment **Risk Controls:** Administrative, PPE
Licences / Permits	N/A
Training / Competency	All team members must be inducted to the use of any equipment used.
Equipment	Heat Block Vortex Benchtop centrifuge 1.5 mlL eppendorf tubes
Protoco **Step 1** **Step 2**	**Reagents** DNA to be cut 10 x buffer 10 x BSA Restriction enzyme **Methods** Clean up PCR product using gel purification. (See section 3.5 of the Gooley Laboratory Manual) Set up the following reaction mixture as shown below: Note: Add enzyme last to prevent enzyme denaturation due to extreme conditions such as high salt concentrations. Note: Follow the instructions on the NEB restriction enzyme cards if available. The concentration of restriction enzymes should not be greater than 10% by volume of the total reaction volume. Vector Digestions. ** It is recommended to also set up a single enzyme digestion to serve as a positive ligation control in subsequent steps.

	<table><tr><td>Reagent</td><td>Volume (µL)</td></tr><tr><td>DNA template (Vector DNA only)</td><td>3–10</td></tr><tr><td>10 x buffer</td><td>2</td></tr><tr><td>Restriction enzyme 1</td><td>1</td></tr><tr><td>Restriction enzyme 2</td><td>1</td></tr><tr><td>Milli Q</td><td>5–12</td></tr><tr><td>TOTAL</td><td>20</td></tr></table>
Step 3	Vortex BRIEFLY to mix components.
Step 4	Spin contents briefly to pull all components to the bottom of the tube.
Step 5	Incubate according to specifications of enzyme, typically 2-3 hours at 37 ºC.
Step 6	Add 1 µL of shrimp alkaline phosphatase to the vector ONLY control tube and incubate at 37 ºC for approximately 15 mins.
Step 7	Load the whole vector reaction onto 1 % agarose gel in separate lanes to assess the digestion and purify the product. (gel purification is required for the vector to purify away the DNA of the MCS that may contaminate ligations. Purify the vector according to the method outlined in 3.5.
Step 8	Load a small amount of the digested PCR product (insert) onto an appropriate % agarose gel to assess the quality of the DNA. Loading some uncut insert can indicate in some cases if the insert has been digested.
Step 9	If necessary purify the remaining digested insert using ethanol precipitation.
Controls / Calibration	N/A
Waste Disposal	biohazard bin
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 plentyof 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	
Authorised By	Amber Willems Jones