RISK ASSESSMENT – TASK BASED
IGEM 2016

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

Description of Activity:
4.8 Preparing Chemically Competent E.coli
SWP No: 4.8

Is there past experience with the Activity that may assist in the risk assessment? **NO**
Incidents & Near-hits, Incident Investigations, Workplace Inspections, Training, Standards, Legislation & Codes, Uni Guidance Material, Existing Controls, Industry Standards.

1. TASK	**2. HAZARD**	**3. Estimated RAW RISK SCORE C x E x L**	**4. CONTROLS**	**5. Residual Risk SCORE C x E x L**			**6. Residual Risk**	
Preparing Chemically Competent E.coli	Skin contact with Potassium chloride, Calcium Chloride, MOPS, MgCl2, liquid nitrogen, dry ice which may cause skin dryness and cracking, irritating, burns	25x3x1	Personal Protective Equipment ; training	25	3	0.1	7.5	low
Bench top centrifuge	Samples unbalanced, manual handling of rotors	15x3x1	Adequate training	15	3	0.1	4.5	
	TOTAL	120		TOTAL		12	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 4.8 Preparing Chemically Competent *E.coli*
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	The methods outlined in the following describe how to make competent cells
Principles / Scope	This method shows how to make E.coli cell competent for transformation
Risk Management	***Risk assessments have been prepared and are available 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 Sterile 50 ml Falcon Tubes Benchtop centrifuge Ice box Dry ice Liquid nitrogen Reagents TFB1 MOPS KCl $CaCl_2$ $MnCl_2$ Glycerol Hepes TFB2 HEPES KCl $CaCl_2$ Glycerol SOB medium Bacto-tryptone Yeast extract NaCl KCl $MgCl_2$ $MgSO_4$

<table>
<tr><td>Protocol
Step 1:

Step 2:

Step 3:

Step 4:

Step 5:

Step 6

Step 7

Step 8

Step 9

Step 10

Step 11</td><td>Grow 130 ml LB culture to OD_{600} of 0.45.

Pour cultures into sterile 50 mL falcon tubes.

Pellet cells at 4 ºC, 3000 rpm for 5 min.

Discard supernatant.

Resuspend pellet in total volume of 50 ml ice-cold TFB1.

Chill on ice for 5 mins.

Pellet cells at 4 ºC, 3000 rpm for 5 mins.

Discard supernatant.

Resuspend pellet in 5 ml ice-cold TFB2.

Chill on ice for 15 mins.

Aliquot on dry ice, then snap-freeze, then store at -80 ºC.
Recipes
TFB1 (Transformation Buffer 1) components (500 mL):
<table><tr><th>Reagent</th><th>Amount</th></tr><tr><td>10 mM MOPS</td><td>1.045 g</td></tr><tr><td>100 mM KCl</td><td>25 mL of 2 M sterile stock</td></tr><tr><td>10 mM $CaCl_2$</td><td>2.5 mL 2 M sterile stock</td></tr><tr><td>50 mM $MnCl_2$</td><td>12.5 mL of 2 M sterile stock</td></tr><tr><td>15 % Glycerol</td><td>75 mL</td></tr></table>Dissolve MOPS and glycerol and pH to 5.8 with acetic acid and KOH in final volume of 460 mL then autoclave. Add the salts after autoclaving.
TFB2 (Transformation Buffer 2) components (50 mL):
<table><tr><th>Reagent</th><th>Amount</th></tr><tr><td>10 mM HEPES</td><td>0.11915 g</td></tr><tr><td>10 mM KCl</td><td>25 µL of 2 M sterile stock</td></tr><tr><td>75 mM $CaCl_2$</td><td>1.875 mL of 2 M sterile stock</td></tr><tr><td>15 % Glycerol</td><td>7.5 mL</td></tr></table>Dissolve HEPES and glycerol and pH to 6.5 with KOH in final volume of 48.1 mL.
SOB medium (500 mL)
<table><tr><th>Reagent</th><th>Amount (g)</th></tr><tr><td>Bacto-tryptone</td><td>10</td></tr><tr><td>Yeast extract</td><td>2.5</td></tr><tr><td>10 mM NaCl (0.6 g/L)</td><td>0.3</td></tr><tr><td>2.5 mM KCl (0.18 g/L)</td><td>0.09</td></tr><tr><td>10 mM $MgCl_2$ ($MgCl_2$-$6H_2O$ 2.03 g/L)</td><td>1.015</td></tr><tr><td>10 mM $MgSO_4$ ($MgSO_4$-$7H_2O$ 2.46 g/L or anhydrous 1.35 g/L 1.35 g/L)</td><td>1.23/0.675</td></tr></table>pH to 7.0 with NaOH, autoclave and store at 4 ºC.</td></tr>
<tr><td>Controls / Calibration</td><td>N/A</td></tr>
<tr><td>Waste Disposal</td><td><u>Disposal requirements:</u>
Follow PC I guidelines for handling, cleaning and when necessary, disposal of bacterial culture and solid wastes.</td></tr>
<tr><td>Emergency Procedures</td><td>First aid measures
Eye contact: Immediately flush eyes with plenty of water for at least 20</td></tr>
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	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