University of Melbourne iGEM 2014 Lab Procedure

Procedure	Name:	RE-Based Colony Screening		
	Description:	Use to select colonies that were successfully transformed with desired insert. Protocol from Ken. NOTE: A possible alternative to this protocol is Colony PCR screening, which is not used in the Cheng lab but may save some miniprep resources. See here (iGEM Michigan 2010) for more information.		
	Trigger:	Do this after plating bacteria transformed with ligated plasmid.		
Last updated	Name:	Sheryl	Date:	03.08.14
You will need	Time:	1 – 2 days (overnight procedure)		
	PPE:	Lab coat, gloves		
	Equipment:	Micropipette tips Sample tubes Shaking incubator (37°C) Gel electrophoresis Microcentrifuge		
	Materials:	LB broth Antibiotic Miniprep kit Agarose gel Restriction enzymes - the same ones previously used to insert gene into plasmid (or any enzymes that will isolate/test for the desired gene).		
Step 1	Pick ~10 single colonies from agar plate with a micropipette tip and dispense into tubes of 10 mL LB+antibiotic, adding only one colony per tube. Label colony tubes accurately (or else you will not be able to tell which ones are successful later).			
Step 2	Leave to grow overnight (12 – 16 hours) in shaking incubator at ~250 rpm and 37°C.			
Step 3	Take a small portion of each colony and perform a miniprep according to kit instructions, again labelling the colonies carefully and keeping them separate. You should have 50 µL of each sample from the miniprep. Save the rest of the colony for later – do not throw out yet!			
Step 4	Keep 5 µL of each sample, and save to run on the gel later. This will act as a control.			
Step 5	Perform restriction digestion on the remaining amount of each colony miniprep sample with your selected restriction enzymes. For each 45 µL sample you should have: • 45 µL sample • 4 µL of **each** restriction enzyme • 0.65 µL BSA – this is to block random RE digestion at unwanted sites.			

	• 6.5 µL Buffer • 4.85 µL MilliQ water Total volume: 65 µL Let the reaction take place at 37°C (or as according to RE instructions) for 3 hours.
Step 6	Set up a slightly modified well comb before making the DNA gel. You need to combine wells by taping over two prongs on the comb, to allow for the larger 65 µL volume from Step 5.
Step 7	Perform DNA gel electrophoresis on the digested samples, along with a DNA ladder and the undigested samples from Step 4. Ensure you accurately record what has been loaded into each well.
Step 8	The results of the DNA gel should tell you which colonies have successfully taken up the plasmid, e.g. if you selected restriction enzymes that cut out the desired gene, the colonies that are 'successful' are those that will show two bands on the gel. The undigested plasmid samples will be highest on the gel, followed by single-band linearized plasmid, followed by fully digested plasmid from 'successful' colonies which will show two bands.
Step 9	Of the original colonies (left over from Step 3), keep only those that show successful ligation in the DNA gel, i.e. two bands. Dispose of the remaining colonies.
Version History	*Describe the changes made in each new version of the protocol here.*