University of Melbourne iGEM 2014 Lab Procedure

Procedure	Name:	Restriction Digestion		
	Version:	3		
	Description:	Restriction digestion allows us to excise our gene of interest from a plasmid and linearise vector plasmids for ligation. Restriction Digestion Video		
Last updated	Name:	Robyn Esterbauer	Date:	22.06.14
You will need	Time:	1 ½ hours		
	PPE:	Lab coat Safety Glasses Gloves		
	Equipment:	P20 Pipette and tips Sample tubes and rack Ice and container		
	Materials:	Restriction digestion enzyme (e.g. Not1) Restriction digestion enzyme buffer (e.g. CutSmart Buffer) Vector Milli-Q Water Bovine Serum Albumin (BSA)		
Step 1	Aliquot 30µL of each vector being digested (i.e. pMK-RGD, pMK-Mag1, pET21d, pGEX6P-3) into separate microfuge tubes. Label the tubes appropriately eg. RD-pET-Mag1 22.06.14 RE iGEM			
Step 2	Pipette into each sample tube, to make a total 50µL (note, you should actually follow the instructions on the NEB restriction enzyme cards): ○ 12µL Milli-Q water ○ 5µL restriction digestion enzyme buffer (e.g. CutSmart Buffer) ○ 1-2µL restriction digestion enzyme (e.g Not1) ***Note: Adding the Milli-Q water first means that you can use the same pipette tip for each aliquot. For all following steps use a different tip to avoid cross-contamination.*** ***Note: Always add the restriction digestion enzyme last and leave it in the freezer until you are ready to use it.*** Note that you can decrease the total reaction volume if you want the concentration of DNA to be higher. Note that there is a spreadsheet on the dropbox in the iGEM protocols folder (search for "Restriction digest calculator.xlsx") which makes it easy to calculate new reaction volumes For double digestions, the concentration of restriction enzymes should not be greater than 10% by volume of the total reaction volume (see the NEB Double Digestion site, subheading "Setting up a Double Digestion"). For example, if the total reaction volume is 50 µL, then don't add more than 5 µL of restriction enzymes. Also, note that we have limited amounts of SpeI; so, consider adding less SpeI and incubating for longer.			
Step 3	Mix the contents in the tubes using pipette tips to move it up and down. Ensure that a different tip is used between tubes.			

Step 4	Spin using the desktop centrifuge on pulse for about 30 seconds to make sure all the contents are mixed with no bubbles
Step 5	Place in 37°C incubator for at least 1 hour (don't follow the instructions on the NEB cards for the reaction time). Note that if you are preparing the sample for a ligation reaction, you really want a thorough digest and cannot accept partial digestion of a parent plasmid. Thus, according to George, you should incubate for at least two hours. If partial digestion is acceptable, then you get away with much less time.
Version history	Version 3 created on 2014-07-30 by Sean Lowe. Version 2 created on 16.07.14 by Elizabeth Brookes o More detail added to all sections.