University of Melbourne iGEM 2014 Lab Procedure

Procedure	Name:	Ligation		
	Version:	2		
	Description:	Ligation Ligation Video		
	Trigger:	Having finished purifying the DNA fragments obtained from DNA gel electrophoresis		
Last updated	Name:	Robyn Esterbauer	Date:	22/06/14
You will need	Time:	1hr 30 min to overnight		
	PPE:	Lab coat Gloves Safety glasses		
	Equipment:	Sample tubes and rack Pipette and tips Ice and container		
	Materials:	DNA ligase enzyme, on ice T4 DNA ligase enzyme buffer, on ice Insert (e.g. RGD or Mag1) Digested vector (e.g. pET21d or pGEX6p-3) Note that you have to be aware of your concentrations. You roughly want the concentration of insert to be three times that of the vector. Also, there needs to be at least 60 ng of the vector, according to the NEB protocol.		
Step 1	For each sample being ligased, take one sample tube and label with L (for ligased)-the E. coli vector-the DNA construct, date, your initials, iGEM eg. L-pET-Mag1 22/06/14 RE iGEM			
Step 2	Pipette into each sample tube, changing tips each time: • 15µL of insert (or at least 3x higher than vector) • 2µL of digested vector, being sure to add the correct vector to the correct insert • 2.5µL of T4 DNA ligase enzyme buffer • 4µL water • 1µL enzyme (always last) – watch carefully to make sure the enzyme leaves the pipette as this is a very small amount			
Step 3	Spin for a few seconds to make sure all the ingredients are mixed			
Step 4	Leave at room temperature for 1-1.5 hours or Leave at 16°C overnight			
Version history	Version 2 updated on 16.07.14 by Elizabeth Brookes			