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BABS UNSW iGEM Lab Protocol



Procedure	Name	Ligation				
	Description	Ligation of 2 fragments of digested DNA into a backbone				
Document	Name	Isabelle Capell-Hattam	Date	2/07/15	Version	1
Requirements	Time	1 hour				
	PPE	Gloves, Labcoat				
	Equipment	Pipettes and Tips Heat Block 1.5 mL tube				
	Materials	Digested plasmid backbone Digested insert fragments 10 x T4 DNA ligase buffer T4 DNA ligase MiliQ H2O				
Step 1	Add the following into a 1.5 mL tube <ul style="list-style-type: none">• 2 μL of digested plasmid (~25 ng)• no more than 3 μL of each digested fragment (equimolar)• 1 μL 10x T4 DNA ligase buffer• 0.5 μL of T4 DNA ligase Make the total volume up to 10 μ L with MiliQ H2O					
Step 2	Ligate at room temperature for 30 minutes - 1 hour					
Step 3	Heat inactivate the ligase at 80°C for 20 minutes					
Step 4	Transform ligated reaction mix OR freeze at -20°C till further use					
Notes	This protocol was adapted from the 2014 iGEM HQ protocol					
Version History						