BABS UNSW iGEM Lab Protocol

Procedure	Name	Restriction Enzyme Digestion				
	Description	Enzyme Digestion of DNA				
Doccument	Name	Isabelle Capell-Hattam	Date	2/07/15	Version	1
Requirements	Time	1.5 - 2 hours				
	PPE	Gloves, Labcoat				
	Equipment	Pipettes and Tips Heat Block 1.5 mL tubes Ice bucket				
	Materials	NEB Buffer 2 BSA EcoR1 Pst1 Spe1 Xba1 MiliQ H2O Dpn1 (optional)				
Digestion of a single fragment for insertion into backbone						
Step 1	Add into each tube: • 250 ng DNA • 2.5 µL of NEB Buffer 2 • 0.5 µL BSA • 0.5 µL EcoR1 • 0.5 µL Pst1 Make the total volume in the tube up to 20 µL with MiliQ H2O					
Step 2	Incubate at 37℃for 30 minutes					
Step 3	Heat inactivate at 80℃for 20 minutes					
Step 4 (optional)	Run digested products on a gel to verify size					
Step 5 (optional)	Ligate fragments together					

Digestion of 2 fragments for directional assembly into a backbone	
Step 1	Add into each tube: ● 250 ng DNA ● 2.5 µL of NEB Buffer 2 ● 0.5 µL BSA Make the total volume in the tube up to 19 µL with MiliQ H2O
Step 2	In the backbone tube add: ● 0.5 µL EcoR1 ● 0.5 µL Pst1 In the fragment 1 tube add: ● 0.5 µL EcoR1 ● 0.5 µL Spe1 In the fragment 2 tubes add: ● 0.5 µL Xba1 ● 0.5 µL Pst1
Step 3	Incubate at 37℃for 30 minutes
Step 4	Heat inactivate at 80℃for 20 minutes
Step 5 (optional)	Run digested products on a gel to verify size
Step 6 (optional)	Ligate fragments together
Notes	This protocol was adapted from the 2014 iGEM HQ protocol. Enzymes should be kept on ice at all times 0.5 µL of Dpn1 can be added to the linearized backbone digest to prevent background colonies from being observed after ligation and transformation
Version History	