



University of Melbourne iGEM 2014 Lab Procedure

Procedure	Name:	Transformation		
	Version:	2		
	Description:	How to transform competent <i>E. coli</i> cells.		
	Trigger:	Use this procedure to transform ligated plasmids into competent cells		
Last updated	Name:	Elizabeth Brookes	Date:	29.07.14
You will need	Time:			
	PPE:	Gloves Lab coat		
	Equipment:	37°C Incubator Shaker Sterile Microtubes Ice Box Heat Block		
	Materials:	Competent Cells LB Broth		
Step 1	Thaw the appropriate amount of competent cells on ice. Also pre-chill the required number of empty 1.5mL microcentrifuge tubes.			
Step 2	Pipet 50 μ L aliquots of cells into the pre-chilled tubes.			
Step 3	Add 1-5 μ L of a ligation reaction mixture or 5ng of pure plasmid DNA into each tube. Mix the tube gently.			
Step 4	Incubate the tubes on ice for 30 minutes . However, George says everyone in Cheng lab only does 10 minutes and it's all right.			
Step 5	Heat shock the tubes for 45 seconds at 42°C. Similar results are obtained by giving the cells a 2 minute heat shock at 37°C.			
Step 6	Place the tubes immediately on ice for at least 2 minutes.			
Step 7	<p>Add 800μL of LB medium to each tube and incubate for 30 min-1 hour at 37°C in the shaking incubator at 200 rpm.</p> <p><i>Note: when using pure plasmid DNA for the transformation, plate out 100μL of the suspension directly onto LB agar plates containing the appropriate antibiotic.</i></p> <p>Web protocols: OCW says "at least 30 mins" incubation http://ocw.mit.edu/courses/biological-engineering/20-109-laboratory-fundamentals-in-biological-engineering-fall-2007/labs/mod1_3/</p> <p>Addgene says 45 mins http://www.addgene.org/plasmid_protocols/bacterial_transformation/</p>			
Step 8	Spin for 1 minute at 6000 rpm.			
Step 9	You should now have 855-860 μ L of solution. Remove 750 μ L of the supernatant and resuspend the pellet with the remaining fluid by pipetting up and down. This results in a concentrated slurry of cells that can now be plated out.			
Step 10	Plate out the suspension on an LB agar plate containing the appropriate antibiotic.			

Version history

V2: Updated based on discussion with Ken clarifying key details.