



University of Melbourne iGEM 2014 Lab Procedure

Procedure	Name:	A3 Making LB Agar Plates		
	Version:	2		
	Description:	How to make LB agar plates		
	Trigger:	Use this procedure when agar plates are required for bacterial growth		
Last updated	Name:	Robyn	Date:	4/9/14
You will need	Time:	30 minutes		
	PPE:	Gloves Lab coat		
	Equipment:	Microwave Bunsen Burner Sterile Petri Dishes Sterile measuring bottle/flask/tube		
	Materials:	LB Agar Antibiotics (dependant on plasmid): (CL) Chloramphenicol for pSB (KA) Kanmycin for pMK (Amp) Ampicillin for pGEX		
Step 1	<p>The LB agar (stored in minibar fridge) should a solid gel in a glass bottle.</p> <p>Completely melt the LB agar in a microwave in its glass bottle – this will take several minutes and you will need to stop occasionally and invert the bottle gently to mix while it melts.</p> <p>SAFETY: Ensure that the lid of the glass bottle is loosened to let out any excess steam; AND be careful when handling – the bottle will become AND do not walk away while microwaving – you need to keep an eye on it to stop it if it threatens to boil over.</p>			
Step 2	Allow the medium to cool slightly at room temperature until the bottle is cool enough to be held without burning.			
Step 3	<p>Measure out the amount of agar required into a lidded measuring container</p> <p>NOTE: You will need about 20ml per plate, plus up to 50ml extra for pouring error</p>			
Step 4	<p>Add 1µl of antibiotic per 1ml of agar and invert gently to mix</p> <p>NOTE: The antibiotic required will vary for the plasmid you wish to grow on the plate (see materials section)</p>			
Step 5	<p>Close to a flame, pour the medium into sterile petri dishes until the dishes are half full.</p> <p>Use sterile technique and ensure that the dishes are open for a minimal amount of time.</p>			
Step 6	Allow the plates to set at room temperature. Once the plates are set and have reached room temperature they may be used or stored.			

Step 7	Allow an excess LB agar to cool down to room temperature in its original bottle. Place the bottle back in the bar fridge for storage.
Step 8	Label plates with the antibiotic name abbreviation, the date, and your initials. Eg. CL 4/9 RE
Step 9	Store unused plates in the bar fridge.
Version history	<i>2. 4/9/14 Added steps for addition of antibiotics - Robyn</i> <i>1. 16/07/14 Original version created - Elizabeth Brooks</i>