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BABS UNSW iGEM Lab Protocol



Procedure	Name		Preparation of Streptococcal Regeneration Media			
	Description		Media used for cell recuperation after lactococcus transformation by electroporation			
Document	Name	Mackenzie Labine-Romain	Date	7/07/15	Version	1
Requirements	Time					
	PPE		Gloves, Labcoat			
	Equipment		Scales Magnetic Mixer Autoclave 1 L schott bottle			
	Materials		10 grams Tryptone 5 grams Yeast Extract 200 grams Sucrose 10 grams Glucose 25 grams Gelatin 15 grams Agar 2.5 mM MgCl <sub>2</sub> 2.5 mM CaCl <sub>2</sub> (pH 6.8) 1g Antibiotic required for selection			
Step 1	Weigh out the components (except sucrose and glucose) and add to the 1 L bottle. Make up to with 650mL with RO H <sub>2</sub> O.					
Step 2	Mix with the magnetic mixer until fully dissolved,					
Step 3	Make glucose solution by dissolving 10 grams with Milli-Q water up to 50mL					
Step 4	Make sucrose solution by dissolving 200 grams with Milli-Q water up to 300mL.					
Step 5	Seal all bottles and autoclave.					
Step 6	After autoclaving this agar can either be cooled for future use or poured direct. Before using, add glucose and sucrose solutions and antibiotic.					

Notes	*not using this probably Adapted from: Holo, H., & Nes, I. F. (1989). High-frequency transformation, by electroporation, of <i>Lactococcus lactis</i> subsp. <i>cremoris</i> grown with glycine in osmotically stabilized media. <i>Applied and Environmental Microbiology</i> , 55(12), 3119-3123.
Version History	