BABS UNSW iGEM Lab Protocol

Procedure	Name	Preparation of Lactococcus Competent Cells				
	Description	Making competent cells for immediate use or storage at -85˚C.				
Doccument	Name	Mackenzie Labine-Romain	Date	6/07/15	Version	1
Requirements	Time					
	PPE	Gloves, Labcoat				
	Equipment	30˚C incubator Cold centrifuge -85˚C freezer				
	Materials	Liquid SGM17 media (GM17 with 0.5M sucrose) supplemented with glycine. 0.5M sucrose with 10% glycerol				
Step 1	Innoculate 1mL liquid SGM17 media with lactococcus colony in 50mL falcon tube and grow at 30˚C to an optical density of 0.5-0.8 at 600nm.					
Step 2	Dilute 100-fold in SGM17 (supplemented with glycine) and grow at 30˚C to an optical density of 0.2-0.7 at 600nm.					
Step 3	Harvest by centrifuging at 4˚C at 5,000 x g for 5 minutes.					
Step 4	Wash twice in ice-cold 0.5M sucrose with 10% glycerol. *to make 1L of 0.5					
Step 5	Suspend in 1/100 culture volume of washing solution.					
Step 6	Transfer to microcentrifuge tubes in aliquots of 1mL and store at -85˚C until use.					
Notes	Adapted from: Holo, H., & Nes, I. F. (1989). High-frequency transformation, by electroporation, of Lactococcus lactis subsp. cremoris grown with glycine in osmotically stabilized media. *Applied and Environmental Microbiology*, *55*(12), 3119-3123. *not tested					
Version History						