BABS UNSW iGEM Lab Protocol

Procedure	Name	Simple Lactococcus Transformation				
	Description	Transformation of Lactococcus cells using electroporation (low efficiency)				
Doccument	Name	Mackenzie Labine-Romain	Date	13/07/15	Version	1
Requirements	Time	2 days (20 minutes on day 1, 4-5 hours on day 2)				
	PPE	Gloves, Labcoat				
	Equipment	Electroporation cuvette (2mm gap) BioRad Gene pulser Centrifuge Spectrophotometer Hood 30˚C shaking incubator 30˚C still incubator				
	Materials	GM17 liquid media GM17 agar plates + selection antibiotic SGM17 (GM17 with 0.5M sucrose) SGM17 with selection antibiotic Milli-Q water Purified plasmid DNA				
Step 1	Grow overnight cultures of lactococcus in GM17 broth in 30˚C **still** incubator					
Step 2	Dilute overnight cultures in 23 ml of GM17 (1:12.5 dilution).					
Step 3	Measure optical density - should be between 0.26 and 0.38.					
Step 4	Centrifuge at 6000rpm (10 min/4˚C)					
Step 5	Wash pellet in 0.5M in 5ml 0.5M sucrose					
Step 6	Divide 5 x 1 ml in eppendorf tubes					
Step 7	Spin and resusped in 5 x 100 µl Milli-Q					

Step 8	Take 50 µl for each electroporation and mix with 0.1-1.0 µg plasmid DNA (<5 µl)
Step 9	Transfer to 2mm cuvette
Step 10	Electroporate (2.5 kV, 25 µF, 200 ohms, time constant 5ms)
Step 11	Immdiately add 1ml cold SGM17 w selection antibiotic
Step 12	Incubate 2 hours in 1.5 ml eppendorf tube at 30˚C (not shaking)
Step 13	Plate 300 ul on GM17 plates
Notes	M. Harvey
Version History	Referred to simpler method by Jeff Welch