BABS UNSW iGEM Lab Protocol

Procedure	Name	Lactococcus Transformation				
	Description	Transformation of Lactococcus cells using electroporation				
Doccument	Name	Mackenzie Labine-Romain	Date	8/07/15	Version	3
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 GM17 with 40 mmol/l threonine (2ml per transformation) 8mL per sample of pre-treating solution: 100mM lithium acetate, 10mM dithiothreitol, 0.6M sucrose, 10mM Tris-HCl (pH7.5) SGM17 (GM17 with 1% w/v sucrose) SGM17 with selection antibiotic SGM17 agar plates with selection antibiotic Milli-Q water 50 mM EDTA 0.3 mol/L sucrose solution Purified plasmid DNA (1ug per sample)				
Step 1	Grow overnight cultures of lactococcus in GM17 broth supplemented with 40 mmol/l threonine. (2ml broth in 50mL falcon tube, in 30˚C shaking 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	Suspend 10^9 cells (use OD→ cell density calculator) for 30 mins at room					

	temp in 8mL of pre-treating solution in 15 mL falcon tube. **if OD600 is ~0.3, add ~4ml cells
Step 5	Centrifuge at 3,220 g (max on benchtop centrifuge) for 15 mins.
Step 6	Remove supernatant.
Step 7	Wash cells with 2mL Milli-Q water, centrifuge for 5 mins at 3,220 g and discard supernatant
Step 8	Wash cells with 1mL Milli-Q water, centrifuge for 5 mins at 3,220 g and discard supernatant
Step 9	Wash cells with 1mL 50mmol/L EDTA, centrifuge for 5 mins at 3,220 g and discard supernatant
Step 10	Wash cells with 1mL Milli-Q water, centrifuge for 5 mins at 3,220 g and discard supernatant
Step 11	Wash cells with 1mL 0.3 mol/L sucrose, centrifuge for 5 mins at 3,220 g and discard supernatant
Step 12	Suspend in 300uL of 0.3mol/L sucrose
Step 13	Add 1 ug plasmid DNA then transfer to a 2mm gap disposable cuvette.
Step 14	Electroporate immediately (single pulse at 2.5kV, 200 Ohms, 25 uF)
Step 15	Transfer immediately to falcon tube containing 5 mL GSM17 with antibiotic at recommended concentration
Step 16	Incubate for 2h at 30C under 5% CO2
Step 17	Centrifuge for 7 minutes at 3,220 g and 30˚C, remove supernatant (leave 200ul)
Step 18	Resuspend pellet and spread 200ul aliquots on GSM17 agar plates (supplemented with antibiotic)
Step 19	Incubate overnight at 30˚C.
Notes	Adapted from: Papagianni, M., Avramidis, N., & Filioussis, G. (2007). High efficiency electrotransformation of Lactococcus lactis spp. lactis cells pretreated with lithium acetate and dithiothreitol. *BMC biotechnology*, *7*(1), 15. *include controls checking for absence of transformants due to either electric pulse and plasmid DNA omission
Version History	Referred to new paper with more recent/efficient method due to advice by Jeff Welch