| BABS UNSW iGEM Lab Protocol | | | | | | |
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| Procedure | Name | Lactococcus Transformation | | | | |
| | Description | Transformation of Lactococcus cells using electroporation | | | | |
| Doccument | Name | Mackenzie Labine-Romain | Date | 7/07/15 | Version | 1 |
| Requirements | Time | | | | | |
| | PPE | Gloves, Labcoat | | | | |
| | Equipment | Electroporation cuvette (2mm gap)<br>BioRad Gene pulser<br>Ice | | | | |
| | Materials | DNA sample dissolved in 10mM Tris-HCl-EDTA<br>SGM17MC (SGM17 with 20mM $MgCl_2$ and 2mM $CaCl_2$)<br>Selection antibiotic<br>Streptococcal regeneration medium agar plates | | | | |
| Step 1 | Thaw competent cells on ice. | | | | | |
| Step 2 | Mix 40µl of cells with 1µl of DNA dissolved in Tris-EDTA. | | | | | |
| Step 3 | Transfer suspension to ice-cooled electroporation cuvette and expose to a single pulse (gene pulser set at 25µF and at 2.0kV). | | | | | |
| Step 4 | Immediately after, mix suspensions with 0.96ml of ice-cold SGM17MC and leave on ice for 5 mins. | | | | | |
| Step 5 | Dilute cells in SGM17MC and incubate at 30˚C for 2h. | | | | | |
| Step 6 | Spread 100µl portions on streptococcal regeneration medium plates containing 1µg/ml of antibiotic. | | | | | |
| Step 7 | Enumerate transformants after 1-2 days of incubation at 30˚C. | | | | | |
| 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. | | | | | |
| Version History | | | | | | |