***Eubacterium limosum* Competent Cells Prep**

1) Procedure Objective

Prepare electrocompetent cells from *E. limosum* cultures to perform genetic transformation by electroporation.

2) Health and Safety

Lab coat, closed toed shoes, gloves, safety glasses should be worn for this procedure.

3) Materials List

- 50mL tubes
- Eppendorf tubes
- Large refrigerated centrifuge
- Sucrose
- Glycerol
- DSMZ 135 medium (see media recipes)
- *E. limosum*
- Ice/ice bucket
- Autoclave and/or 0.22um filter cups
- Anaerobic chamber

4) Pre-lab Steps

- Ensure that all plastics, reagents, and media have been in the anaerobic chamber for at least 48 hours to become totally anoxic. The ice is an exception to this rule.
- Prepare 270mM sucrose solution and pass into chamber
- Prepare 270mM sucrose solution + 20% v/v glycerol and pass into chamber

5) Procedure Steps

1) Inside the anaerobic chamber, transfer 50mL sterile DSMZ 135 medium without sulfide to a sterile 50mL centrifuge tube (with o-ring cap) and inoculate with a stab from an *E. limosum* cryostock.
    a. Grow up culture overnight at 37C in the anaerobic chamber incubator.
    b. Should grow up for 18-19 hours so that the culture is just starting to become opaque. Ideal procedure is to inoculate at 1 or 2pm and the perform competent cells prep at 9am the following day.
2) Pass a large ice bucket filled with ice into the chamber.
3) Place the culture in the tube on the ice and fully submerge.
    a. Leave in the ice for ~20 minutes or until it becomes ice cold.

4) Pass the tube out of the chamber through the glove hole
5) Spin down culture at 15,000xg for 10 minutes at 4C in a pre-chilled centrifuge and pass back into the chamber.
6) Decant supernatant and resuspend fully in 10mL ice cold 270mM sucrose solution
7) Repeat centrifugation step and resuspend fully in 10mL ice cold 270mM sucrose solution+20% v/v glycerol
8) Centrifuge again and decant supernatant.
9) Resuspend cells in the leftover liquid – this should be about ~1mL total volume
10) Aliquot 50uLs into pre-chilled Eppendorf tubes and store at -80C for future use.

6) References

[1] "Genome Engineering of *Eubacterium limosum* Using Expanded Genetic Tools and the CRISPR-Cas9 System" Shin et.al. 2019

[a] "A genetic System for *Clostridium ljungdahlii*: a chassis for Autotrophic Production of Biocommodites and a Model Homoacetogen" Leang et.al. 2013

7) Revision Table

Revision No.	Date of Change	Change Description	Person Responsible
0	1/21/21	Initial Development	PAS
1	2/1/22	Modified to grow culture in 50mL tube from start and increased speed to 15xkg	PAS

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