***E. limosum* Electroporation Protocol**

1) Procedure Objective

Transform *E. limosum* with foreign DNA by electroporation.

2) Health and Safety

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

3) Materials List

- *E. limosum* electrocompetent cells (competent cells prep described in another protocol)
- Integrative DNA
- Ice/ice bucket
- Anaerobic chamber
- Electroporator
- 2mm gap width electroporation cuvette (sterile)
- DSMZ 135 media
- RCM+selection agar plate
- RCM only agar plate

4) Pre-lab Steps

Prepare all media, plates, DNA, and plastics ahead of time and place them in the anaerobic chamber at least 48 hours in advance to become anoxic.

5) Procedure Steps

1) Remove a 50uL stock of electrocompetent *E. limosum* from the -80C freezer and pass into the anaerobic chamber on ice – IMPORTANT the competent cells must be kept on ice at all times.
2) Once thawed, add DNA in <3uL volume to the tube and mix by flicking gently several times
    a. Higher concentrations of DNA work best, but too much DNA can cause arcing, especially when using plasmids. Ideally you should use ~400 ng in 2uL. You can get away with a lot more when using linear DNA without arcing (I am not sure why).
3) Pipette mixture into pre-chilled 2mm gap width electroporation cuvette and tap down gently to settle on bottom of cuvette.

4) Incubate on ice for 2-5 minutes
5) Wipe the cuvette and place in shock pod and pulse with exponential decay protocol
    a. 2500v, 600Ω, 25uF
6) Immediately add 950uL of room temperature DSMZ 135 media to the cuvette and pipette up and down several times gently with extreme care
7) Place cuvette in 37C incubator in anaerobic chamber and allow to recover for 5-6 hours.
8) Resuspend fully by pipetting and plate out 200uL of transformation mixture onto agar plates containing RCM and appropriate selection marker.
9) Incubate plates at 37C in the anaerobic chamber
    a. It usually takes 3-4 days for colonies to form

NOTE: While the manufacturer suggests the cuvettes are single use, they can actually be reused several times. Bleach and wash thoroughly with soap and water after every use. Cuvette and cap can be sterilized with 70% ethanol before use. Do not autoclave as the plastic an adhesive are not autoclave safe.

6) References

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

[2] "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	10/8/2021	Modified recovery media type and temperature along with recovery time to reflect actual lab practices. Moved ice incubation step to after cells are transferred to cuvette.	PAS
1.1	2/1/2022	Added details for improved clarity.	PAS

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