

iGEM 2017 – Microbiology – BMB – SDU

Title: Electroporation Geobactor	Date issued: 2015.05.14
SOP number: SOP26	Review date: 2017.09.10
Version number: v02	Original by: Leang, Ching and Coppi, Maddalena 3-3
	Modified by: FN

1. **Purpose**

Make Electroporation for Geobactor

2. **Area of application**

Transfer DNA to an organism

- 3. Apparatus and equipment**
- 4. Materials and reagents – their shelf life and risk labelling**
- 5. QC – Quality Control**
- 6. List of other SOPs relevant to this SOP**
- 7. Environmental conditions required**
- 8. Procedure**

Electroporation of *Geobacter sulfurreducens*

Preparing Electrocompetent Cells:

1. Inoculate cells ~18 hours beforehand.
 - a. Inoculate cells to make a final OD₆₆₀ of 0.015 into 100ml prewarmed NBAF with cysteine and yeast extract
2. Incubate cells at 30°C overnight.
3. Take OD of culture
4. Stop overnight culture at midlog phase with an OD₆₆₀ of about 0.2 (0.13-0.35)
5. Figure out # of cells in the culture
 - a. Here is a rough table to use.
 - i. Too many cells is better than too few.
 - ii. Round down to nearest OD.

cells/ml	OD
7.69E + 06	0.04
2.78E + 07	0.08
4.43E + 07	0.12
7.01E + 07	0.16
8.54E + 07	0.2
1.02E + 08	0.24
1.29E + 08	0.28
1.50E + 08	0.32

1.84E + 08	0.36
2.06E + 08	0.4
2.53E + 08	0.44
3.13E + 08	0.48
3.99E + 08	0.52

- b. Calculate final resuspension volume.
 - i. You want 1×10^{11} cells/ml after the final resuspension.
 - c. Divide this volume by two to compensate for the volume that the cells occupy as well as the loss of cells during the washes.
 - d. This is the volume of buffer you will add to your final pellet.
7. (Because *G. sulfurreducens* was found to be particularly susceptible to shearing, pipetting of cell suspensions was minimized and, when necessary, performed with large-bore pipette tips.)
8. Set up centrifuge tubes on ice using sterile cannulas with gas flowing or in glove bag.
9. Pour cells into tubes.
 - a. Precooling the cells in ice water can increase electroporation efficiency.
 - b. Once the cells are put on ice, they must be kept as cold as possible.
 - c. Never carry them around without an ice bucket.
 - d. Never put them in an unchilled rotor.
10. Pellet cells by spinning at 6000rpm for 5 minutes at 4°C.
11. Move work into glove bag
12. Wash cell pellets with electroporation buffer
13. Repellet by spinning at 6000rpm for 5 minutes at 4°C.
14. Repeat steps 12-13
 - a. Keep cells ice cold at all the time!
15. Resuspend cells in 0.75ml buffer
16. Carefully transfer cells from centrifuge tubes to screw cap microfuge tubes.
17. Leave cells on ice for 3 minutes.
18. Harvest cells at 14,000rpm microfuge for exactly 1 minute, at 4°C.
19. Decant supernatant.
20. Resuspended cells in calculated buffer volume (An appropriate volume of a 60% dimethyl sulfoxide (DMSO)–40% electroporation buffer solution was added to the final cell suspension to achieve a final DMSO concentration of 10%.

The resulting electrocompetent cells were either electroporated immediately or stored at -70°C for future use.)

21. Aliquot 25 μl of these cells for each electroporation.
 - a. Keep cells on ice (or snip frozen) and ready to use.
22. Freeze unused cells in 10% DMSO.
 - a. Add 1/5 of cell volume (5 μl) of 60% DMSO to each aliquot.
 - b. Keep in the -70°C freezer.

Electroporation:

23. All electrotransformations were performed in 0.1-cm-gap microelectroporation chambers.
24. Electrocompetent *G. sulfurreducens* cells (100 μl), either freshly prepared or thawed on ice, were pulsed at 180 V (resistance = 200 Ω ; capacitance = 25 μF).
25. Immediately following electroporation the cells were then transferred to a prewarmed anaerobic pressure tube containing 20 ml of NBAFYE.
26. The electroporated cells were allowed to recover for 10 h at 30°C
27. Add the appropriate volume of antibiotic to a new tube.
27. Then the cells were transferred to tube containing 80 ml media with antibiotic at 37°C .

9. Waste handling

Chemical name	Concentration	Type of waste (C, Z...)	Remarks
One used Plastic		GMO	Yellow GMO Trash

10. Time consumption

11. Scheme of development

12. Appendices