

Bacteroides/Porphyromonas Electroporation

derived from CJ Smith, Genetic Transformation of Bacteroides by Electroporation,
Methods Mol Biol. 1995;47:161-9

Making electrocompetant cells \rightarrow pre-warmed & pre-reduced TSBHK

1. Inoculate a ^{25~ (3 ml x 2) & (35 ml x 2)} 10 ml culture of bacteria from a fresh plate, into pre-reduced complex media, incubate overnight. (Try to inoculate cells around one o'clock in the afternoon, so the culture is in late log the next morning.)
2. Inoculate ^{25~} 100 ml of pre-warmed, reduced media with the overnight culture, to a starting OD₆₀₀ ^{~0.4} ~0.3.
3. Allow the culture to reach an absorbance of ^{0.6~0.8} ~0.4-0.6 at OD₆₀₀. Harvest cells by centrifugation at 4000g for 15 minutes at 4°C, in a sterile centrifuge tube.
4. Resuspend cells in ³⁵ 100 mls cold EP buffer, pellet cells, repeat with ³⁵ 50 mls of EP buffer. Resuspend the final pellet in ^{500 ml} 1 ml EP buffer. Cells can be aliquoted and frozen at -70 °C at this stage, or used fresh.

Electroporation

1. For each electroporation, pre-chill a 0.2 cm cuvette on ice, then add 100 ul of cell suspension*. Add 2-25 ul (0.5- 2 ug)[†] of DNA in TE buffer, and tap gently. Incubate on ice 5 minutes.
2. Place cuvette in electroporation chamber and pulse with controller set to:
2.5 kV
5 msec (up to 10 msec)
400 Ω
3. Immediately after electroporation, add 1 ml of pre-reduced media directly to the cuvette, then transfer the cells to a culture tube. Place the cells in the anaerobic chamber at 37°C for at least 3 hours, preferably overnight.
4. Plate 50-200 ul aliquots on selective media.
Colonies should appear in 2-3 days for Bacteroides, 4-7 days for Porphyromonas.

* cells and DNA combined in microcentrifuge tube then added to cuvette

† Up to 2 ug of linear DNA, but 5 ug plasmid

Electroporation Buffer (EP):

<u>Final Concentration</u>	<u>500 mls</u>	<u>Stock</u>
10% Glycerol	50 mls	100%
1 mM MgCl ₂	500 uls	1 M

Filter sterilize and store at 4°C. (Pre-rinse the filter with sterile water before use.)

Notes:

This protocol makes 10 aliquots of competent cells from 100 mls of culture. It can be scaled up or down as necessary.

Extra competent cells can be stored at -70°C for at least 3 months and used for subsequent transformations.

DNA for electroporation should be of high quality, with **no salt**. Purification of DNA from DH5α with Qiagen kits gives good results.

When making gene replacement mutants by transformation, linearize the DNA to be transformed by restriction digest, then purify over a Qiagen or Promega column to remove salt. Use ~1- 2 ug of linear DNA for transformation.

When possible, purify DNA from DH5alpha

* Caution!

W83 cells are like slush.