Vibrio natriegens - DNA transformation by electroporation

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Abstract

Electroporation of V. natriegens cells is a rapid 2-hour protocol, from culture inoculation to plating. Each transformation produces >10^5 CFU / µg plasmid DNA. Each transformation requires 10mL of LB3 (high-salt) media, corresponding to 50uL of 200x concentrated cells.

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Before start

- Prepare 1M ice cold sorbitol
- Prepare "LB3" high-salt media as described below. To determine the volume of media you'll need, multiple the number of samples by 10mL. For example, if you want to transform 6 different samples, you need 60mL.

1. Short LB3 protocol: dissolve 25g LB Broth Miller (Fisher BP9723-500, ensure LB contains 10g of NaCl) and 20g NaCl in 1L total of deionized water, pH ~7.2. (alternatively, use the full LB3 protocl below).
2. Full LB3 protocol: dissolve 10g tryptone, 5g peptone, 30g NaCl in 1L total of deionized water. pH ~7.2.

- To make LB3 plates, use the protocl above and add agar to 1.5% final.

Protocol

Starter culture

Step 1.

- Start an overnight culture of V. natriegens (1-3ml) in LB3 media (with any required selection).

Wash overnight cells

Step 2.

- Use overnight culture corresponding to 1/100 of total media volume (For example, if you transform 6 samples you'll need a 600uL of overnight culture to inoculate 60ml media total). Wash the cells as follows:
- Transfer the appropriate volume into eppendorf tube
- Pellet cells at max centrifuge speed for 1 minute
- Dispense supernatant.

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- Resuspend cell pellet with same volume (i.e., 600uL) using fresh LB3 growth media

Inoculate culture & grow to exponential phase

Step 3.

Use the washed overnight cells to inoculate the appropriate volume of media and incubate the culture at 37C at 225rpm for 1 hour or until culture reaches OD600 0.4.

Use a baffled flask with total volume greater than 2x the media (For example, use a 250mL flask for up to 100mL culture).

preparation of cuvettes

Step 4.

In the meantime, prepare electrocuvettes with 50ng of plasmid DNA and place on ice. DNA volumen should not exceed 10ul. Also prepare 10mL of cold 1M sorbitol, chilled to 4C and placed on ice.

Cooling centrifuges

Step 5.

With 10-15 minutes before the hour, prepare the cold centrifuges:

- Set large cold centrifuge to 4C by spinning at 3500rpm for 5min at 4C (for harvesting cells from culture media).
- Set small cold centrifuge to 4C using “Fast Temp” setting (for eppendorfs).

Washing cells in osmoprotectant

Step 6.

- Once culture has reached OD 0.4, centrifuge at 3500rpm for 5 minutes at 4C (50ml tubes).
- Decant the supernatant, gently shaking to eliminate as much of the remaining media as possible.
- Resuspend the pellet with 1mL of cold 1M sorbitol, transfer to eppendorf tube.
- Pellet the cells at 4C at max speed for 1minute.
- Repeat sorbitol wash steps a total of 3 times.
- Resuspend the final cell pellet in 1/200th of the original culture volume. (i.e., use 300uL for 60mL of cell culture).
- Aliquot 50uL of concentrated cell solution into each electrocuvette (containing DNA.

Electroporation

Step 7.

To electroporate, wipe the outer surface of the electrocuvettes using kimwipe, place into the chamber and pulse with the following settings (BioRad electroporator)

- 0.4kV, 1kΩ, 25uF; time constants should be >12ms

Quickly, recover the cells by adding 1ml of LB3 media.

Recovery

Step 8.

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Incubate recovery tubes for 45 minutes at 37C at 220rpm.

- Shorter incubation time is sufficient if large colony number is not required.
- During recovery, dry agar plates in the incubator (upside down).

Plating

Step 9.

- Plate 100uL of the recovered culture on agar plate. Plate the remaining 900uL of the recovered culture by transfering the remaining volume into an eppendorf tube, pelleting, removing all but 100uL of the supernatant, resuspend and plate.
- Incubate overnight at 37C for colony formation. Incubation at 30C or room temperature is also possible, yet slower.

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