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Electroporatic transformation of Rhodobacter sphaeroides

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ABSTRACT

The expression host for *R. sphaeroides DrshI* wasconstructed by deleting Type II restriction enzyme RshI (locus tag RSP_3759; recognition sequence CGATCG) from strain DD11 is suitable for transformation by either chemical or electrical procedures.

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MATERIALS TEXT

- 15 ml sterile tubes
- 1 ml microcentrifuge tubes
- Sterile glass beads
- Competent cells (Drsh1)
- Ice
- Electroporation tube
- Incubator at 33^0C
- Media plate with appropriate Antibiotic selection
- GYCC liquid medium
- Centrifuge

1 In a microfuge tube on ice, mix **40 µL** cells with 100 ng of plasmid DNA.

2 Turn on the electroporator and select the voltage to 2500 V with other setting of 25uF and 400 ohm.

3 Transfer the cells-DNA mixture to a chilled **2 mm** electroporation cuvette on ice.

4 Transfer the cuvette to the pulse chamber and shock to desired number of pulses (only once) 1m (if doing multiple pulses, do first pulse and return cuvette to ice for one minute before doing next (and additional) pulses. **00:01:00** e on ice between pulses.

5 Return the cuvette to ice and add 1ml GYCC **immediately**; record the time constant and the voltage achieved for each pulse.

6 Transfer cells/GYCC from electroporation cuvette to a Falcon 2059 culture tube (15ml). 4h Incubate at **35 °C** with slow shaking (**150 rpm**) for a **04:00:00** outgrowth period.

7 Pellet the cells in microcentrifuge tube and spread onto GYCC agar containing appropriate antibiotic. Incubate plates at **33 °C** for 2-4 days.

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