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Transforming the marine bacterium Ruegeria pomeroyi using tri-parental mating

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DISCLAIMER

Please reference the following publication:

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ABSTRACT

This protocol describes a tri-parental mating method for transforming the model marine bacterium *Ruegeria pomeroyi* DSS-3 with a desired recombinant plasmid. This transformation method is reliable, easy-to-use, and does not require specialized equipment. Using this method, we have successfully transformed *R. pomeroyi* with large plasmids (up to 14.833 kb) which enabled the construction of the first fluorescent reporter strains of *R. pomeroyi* (described in doi.org/10.1038/s41467-020-15693-z).

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We use this protocol and it's working

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Keywords: Ruegeria pomeroyi, genetic engineering, cloning, transformation, triparental mating, fluorescence reporter

ATTACHMENTS

Transforming the marine bacterium Ruegeria pomeroyi using tri-parental mating.pdf

MATERIALS

Materials:

- E. coli donor strain, containing the plasmid to be transformed into R. pomeroyi
- E. coli helper strain, containing the helper plasmid pRK600 plasmid (ampicillin resistant)
- R. pomeroyi, wildtype
- 1/2 YTSS liquid medium (in 500 ml^{-1} : 2 g yeast extract (BD Biosciences), 1.25 g tryptone (BD Biosciences), 10 g sea salts (Sigma-Aldrich))
- 1/2 YTSS agar plate (in addition to 500 ml liquid medium, 7.5 g agar (Bacto Agar, BD Biosciences)), pre-warmed to 30 °C
- Luria Broth (LB) liquid medium
- Antibiotics kanamycin and chloramphenico
- Potassium tellurite (Fluorochem)
- Sterile L-shape cell spreader
- Parafilm
- Eppendorf tubes (1.5 ml; sterile)
- Incubators (with shakers) at 30 °C and 37 °C
- Table-top centrifuge

SAFETY WARNINGS

Potassium tellurite is used to select for R. pomeroyi, and against E. coli. Potassium tellurite is toxic if swallowed, and causes skin and serious eye irritation. Furthermore, it is possible that R. pomeroyi mediates the formation of volatile tellurides which may be highly toxic. Thus, care should be used to avoid contact with the substance, and colony picking may best be performed in a fume hood to protect against the potential presence of volatile tellurides.

BEFORE START INSTRUCTIONS

Before using this protocol, the user should have designed and built the plasmid desired for transforming into R. pomeroyi. The authors have built and successfully transformed reporter plasmids on vector backbones pBBR1MCS-KanR (GenBank U23751) and pRK415 (GenBank EF437940). Furthermore, if the desired plasmid is small (empirically, the authors suggest less than 11 kb), electroporation may be a faster method for transforming R. pomeroyi. Recommendations for plasmid design and electroporation method for R. pomeroyi are available in detail in Cherry Gao's PhD Thesis (https://hdl.handle.net/1721.1/129213).

Execution

5m

1

Prepare Overnight 5 mL liquid cultures of the three bacterial strains for tri-parental mating:

(1) wildtype R. pomeroyi (1/2 YTSS medium, 30 °C with shaking);

(2) helper E. coli containing the pRK600 plasmid (LB medium amended with [M] 15 µg/mL chloramphenicol, 37 °C with shaking)

(3) donor E. coli containing the constructed plasmid (LB medium amended with [M] 50 µg/mL kanamycin, 37 °C with shaking).

2

After overnight growth, wash the two E. coli cultures twice in 1/2 YTSS medium to eliminate antibiotics.

3

5m

To wash, aliquot 1 mL overnight cultures into Eppendorf tubes, pellet the bacterial cells through gentle centrifugation 3000 rpm, 00:05:00 (on a tabletop centrifuge), discard the supernatant, and resuspend in 1 mL 1/2 YTSS medium.

4

Pellet the E. coli cells again through centrifugation, discard the supernatant, then resuspend in 1 mL YTSS medium.

5

In a clean Eppendorf tube, combine the following: 2 mL overnight culture R. pomeroyi, 200 µL washed overnight culture helper E. coli and

200 µL washed overnight culture of donor E. coli .

6 Gently centrifuge this mixture to pellet the bacterial cells. Discard the supernatant and resuspend in 100 µL 1/2 YTSS medium .

7 Deposit 50 µL concentrated bacterial mixture onto the center of a pre-warmed 1/2 YTSS plate. Place the lid onto the plate.

8 Incubate the plate (lid-side up) Overnight at 30 °C to allow mating to occur.

9 For selection of plasmid-containing R. pomeroyi, pick up most of the biomass (now dry) that is at the center of the mating plate with a sterile L-shaped cell spreader, and evenly spread this biomass onto a 1/2 YTSS plate amended with [M] 50 µg/mL kanamycin and [M] 50 µg/mL potassium tellurite .

Note

Kanamycin and potassium tellurite stock solutions (1000× concentration) should be prepared by dissolving desired amounts in milliQ water and filter sterilizing (0.2 µm; not autoclaved). Stock solutions can be added to melted 1/2 YTSS agar medium before pouring into plates, or spread directly onto solidified 1/2 YTSS agar plates. To directly spread onto solidified agar plates, place ~20 µl of the 1000x stock solution in the middle of the plate and spread evenly using a sterile L-shaped cell spreader. Let the compounds diffuse through the agar medium for at least 1 hour at room temperature before applying bacteria.

10 Incubate the selection plate (lid-side down) at 30 °C until colonies of transformed R. pomeroyi form. Typically, small colonies become visible within 48–72 hours. Due to the presence of potassium tellurite, R. pomeroyi colonies appear dark brown.

11 To avoid evaporation during this long incubation time, secure the lid to the plate with Parafilm.

12 Confirm successfully transformed R. pomeroyi through colony PCR and sequencing. If possible, 16S sequencing to confirm the identity of R. pomeroyi is recommended.