Transforming the marine bacterium *Ruegeria pomeroyi* using tri-parental mating

Full Author List

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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).

Keywords

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

Before Start

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).

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.

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 chloramphenicol

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

Steps

1. Prepare overnight liquid cultures (5 ml) 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 15 µg ml^{-1} chloramphenicol, 37 °C with shaking); and (3) donor *E. coli* containing the constructed plasmid (LB medium amended with 50 µg ml^{-1} kanamycin, 37 °C with shaking).
2. After overnight growth, wash the two *E. coli* cultures twice in 1/2 YTSS medium to eliminate antibiotics. To wash, aliquot 1 ml of overnight cultures into Eppendorf tubes, pellet the bacterial cells through gentle centrifugation (~3000 rpm for 5 min on a table-top centrifuge), discard the supernatant, and resuspend in 1 ml of 1/2 YTSS medium. Pellet the *E. coli* cells again through centrifugation, discard the supernatant, then resuspend in 1 ml of 1/2 YTSS medium.
3. In a clean Eppendorf tube, combine the following: 2 ml overnight culture of *R. pomeroyi*, 200 µl of washed overnight culture of helper *E. coli*, and 200 µl of washed overnight culture of donor *E. coli*.
4. Gently centrifuge this mixture to pellet the bacterial cells. Discard the supernatant and resuspend in 100 µl of 1/2 YTSS medium.
5. Deposit 50 µl of this concentrated bacterial mixture onto the center of a pre-warmed 1/2 YTSS plate. Place the lid onto the plate.
6. Incubate the plate (lid-side up) overnight at 30 °C to allow mating to occur.
7. 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 kanamycin (50 µg ml^{-1}) and potassium tellurite (50 µg ml^{-1}).
    a. 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.

8. 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. To avoid evaporation during this long incubation time, secure the lid to the plate with Parafilm.
9. Confirm successfully transformed *R. pomeroyi* through colony PCR and sequencing. If possible, 16S sequencing to confirm the identity of *R. pomeroyi* is recommended.

Citations

Gao, C., Fernandez, V.I., Lee, K.S. *et al.* Single-cell bacterial transcription measurements reveal the importance of dimethylsulfoniopropionate (DMSP) hotspots in ocean sulfur cycling. *Nat Commun* **11,** 1942 (2020). https://doi.org/10.1038/s41467-020-15693-z

Gao, C. Ecological insights through single-cell measurements of marine bacteria. Ph. D. Thesis, Massachusetts Institute of Technology, Department of Biological Engineering, September 2020. https://hdl.handle.net/1721.1/129213