Bacterial Conjugation in *Rhodobacter capsulatus*

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[Abstract] Bacterial conjugation of plasmids is the common method of introducing foreign DNA into *Rhodobacter capsulatus* because transformational systems have not been shown as efficient methods of introducing DNA to *R. capsulatus*. For *R. capsulatus* bacterial conjugation using an *Escherichia coli* donor can be used to introduce replicating vectors, and non-replicating vectors for targeted chromosomal modifications.

Materials and Reagents

1. *R. capsulatus* recipient strain
2. *Escherichia coli* donor strain (containing plasmid to be conjugated) capable of conjugation (*e.g.* S17-1) or *E. coli* donor strain containing plasmid to be conjugated and a helper strain containing the *tra* genes [*e.g.* HB101 (pRK2013)]. For a review on conjugation and *tra* genes see Willetts *et al.* (1984)
3. Plasmid to be conjugated into *R. capsulatus* (*e.g.* pXCA601; Tetracycline resistance)
4. Appropriate antibiotic (resistance specified by plasmids and bacterial strains)
5. Thiamine hydrochloride
6. H_3BO_3
7. $MnSO_4 \cdot H_2O$
8. $Na_2MoO_4 \cdot 2H_2O$
9. $ZnSO_4 \cdot 7H_2O$
10. $Cu(NO_3) \cdot 3H_2O$
11. D, L-malic acid
12. Na_2EDTA
13. $MgSO_4 \cdot 7H_2O$
14. $CaCl_2 \cdot 2H_2O$
15. $FeSO_4 \cdot 7H_2O$
16. 10 mM potassium phosphate buffer
17. 0.3% Difco yeast extract
18. 0.3% Difco bactopeptone
19. Bacto-tryptone

20. Yeast extract
21. Trace element solution (see Recipes)
22. RCV broth (see Recipes)
23. RCV agar (see Recipes)
24. LB broth (see Recipes)
25. LB agar (see Recipes)
26. YPS agar (see Recipes)

Equipment

1. 30 °C and 37 °C shakers
2. 30 °C and 37 °C incubator
3. Test tubes
4. Petri plates
5. Sterile 1.7 ml microcentrifuge tubes
6. Inoculation loop
7. Pipetmen (10 µl to 1 ml range) and appropriate tips
8. Graduated pipette (5 ml range) and aspiration bulb
9. Bench-top microcentrifuge with rotor for 1.7 ml microcentrifuge tubes

Procedure

1. Streak recipient *R. capsulatus* strain on RCV agar plate (with appropriate antibiotics) and incubate at 30 °C for 2-3 days.
2. Streak donor *E. coli* strain (and helper *E. coli*) on LB agar plate with appropriate antibiotics and incubate at 37 °C overnight.
3. Inoculate 4 ml of RCV broth (with appropriate antibiotics) with a single colony of the recipient *R. capsulatus* strain and incubate at 30 °C in a 200-250 rpm shaker for 2 days.
4. One day later inoculate 4 ml of LB broth (with appropriate antibiotics) with donor *E. coli* strain, and 4 ml of LB broth with helper *E. coli* strain if applicable (see Materials and Reagents for examples), and incubate at 37 °C in a 200-250 rpm shaker overnight.
5. In separate sterile microcentrifuge tubes transfer 100 µl of donor *E. coli* strain, 100 µl of helper *E. coli* strain (if applicable), and 200 µl of recipient *R. capsulatus* strain. Each strain should be in mid- to late- log phase.
6. Spin microcentrifuge tubes containing cultures at 3,500 *x g* for 1 min. in bench-top centrifuge.
7. Decant all supernatant from microcentrifuge tubes.

8. Resuspend cell pellets in 500 µl RCV broth per microcentrifuge tube to wash away residual antibiotics and LB broth.
9. Spin microcentrifuge tubes containing resuspended cultures at 4,000 *x g* for 1 min in bench-top centrifuge.
10. Decant all supernatant from microcentrifuge tubes.
11. Resuspend donor *E. coli* strain cell pellet in 50 µl RCV broth.
12. Transfer all of the resuspended donor *E. coli* strain to helper *E. coli* strain cell pellet and resuspend (if applicable).
13. Transfer all of the resuspended donor *E. coli* strain (and helper *E. coli* strain) to the recipient *R. capsulatus* strain and resuspend.
14. Aliquot 10 µl drops of donor-helper-recipient mix onto a dry RCV agar plate (no antibiotics) and allow for the drops to dry.
15. Incubate plate upside down at 30 °C for 1-2 days. *R. capsulatus* but not *E. coli* will grow on the RCV agar plate and the cells are ready when the conjugation spots have a red ring around it. The middle of the spot will likely be pale pink.
16. Streak the conjugation spots onto RCV agar plates containing appropriate antibiotic to select for the cell containing the plasmid (see Materials and Reagents for plasmid example). Do this by scraping the red ring around the conjugation spot up with an inoculation loop.
17. Incubate streaked plate at 30 °C for 3-4 days or until you see colonies.
18. Optional (this will also be done in step 20): Test the *R. capsulatus* colonies for plasmid using your choice method, such as colony PCR.
19. Restreak colony on YPS agar plate containing appropriate antibiotics and incubate at 30 °C for 2-3 days to ensure that it is “clean” of *E. coli* cells. Although *E. coli* does not grow on RCV, it can survive. *E. coli* will grow on YPS agar plates. This YPS agar plate will isolate *R. capsulatus* cells containing the conjugated plasmid from the *E. coli* survivors as individual colonies. You can visually identify single *R. capsulatus* colonies on this YPS agar plate. It will be pink/maroon in colour compared to the cream colored *E. coli* colonies.
20. Test the “non-contaminated” *R. capsulatus* colonies for the conjugated plasmid by colony PCR.

Recipes

1. Trace element solution (in 250 ml dH_2O)
   0.7 g H_3BO_3
   398 mg $MnSO_4 \cdot H_2O$

188 mg $Na_2MoO_4 \cdot 2H_2O$
60 mg $ZnSO_4 \cdot 7H_2O$
10 mg Cu $(NO_3) \cdot 3H_2O$

2. RCV broth/agar (Beatty *et al.,* 1981) (in 1 L; autoclaved)
   4 g D, L-malic acid
   1 g $(NH_4)_2SO_4$
   10 mM potassium phosphate buffer
   200 mg $MgSO_4 \cdot 7H_2O$
   75 mg $CaCl_2 \cdot 2H_2O$
   12 mg $FeSO_4 \cdot 7H_2O$
   20 mg Na_2EDTA
   1 ml trace element solution
   1 mg thiamine hydrochloride
   Adjust pH to 6.8 with NaOH before autoclaving
   (for agar add 1.5% Agar)
3. YPS broth/agar (Wall *et al.*, 1975) (autoclaved)
   0.3% Difco yeast extract
   0.3% Difco bactopeptone
   2 mM $CaCl_2$
   2 mM $MgSO_4$
   (for agar add 1.5% agar)
4. LB Broth (Sambrook *et al.,* 1989) (in 1 L; autoclaved)
   10 g Bacto-tryptone
   5 g Yeast extract
   10 g NaCl
   Adjust pH to 7.5 with NaOH before to autoclaving
   (for agar add 1.5% Agar)

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