CONJUGAL TRANSFER OF DNA USING *RHODOCOCCUS* SP. B264-1

(Mating protocol for transferring pB264-based plasmids from Rhodococcus *B264-1 donors to recipient cells)*

Preparation of Donor and Recipient Cultures

1. Transform pB264-based plasmid into the B264-1 strain of *Rhodococcus* (see separate protocol for electroporation of *Corynebacterium* and *Rhodococcus* sp. B264-1); verify transformants
2. Inoculate a small volume (2-10 ml) culture of the donor strain (B264-1) carrying the plasmid in LB medium supplemented with an appropriate antibiotic; also inoculate negative controls (e.g. B264-1 lacking a plasmid) in an appropriate medium; grow at 30°C (2-3 days)
3. Inoculate a small volume culture (2-10 ml) of the recipient (strain into which the plasmid will be transferred) in LB; grow at an appropriate temperature (e.g. 37°C for *Rhodococcus* sp. I24; 30°C for other *Rhodococcus* strains) for 2-3 days

Plates

4. Prepare LB plates (no antibiotics) as well as LB plates containing antibiotics appropriate for selecting transconjugants

Mating (once the donor and recipient cultures have grown)

5. Onto the center of each of an appropriate number of LB plates (one per donor/recipient pair) place one 25 mm mixed cellulose ester (MCE) filter (e.g. Millipore cat no. HAWP02500); drip onto the center of this filter 100-200µl of 95% ethanol; place these plates at 30°C with the lids askew to dry for 30-40min.
6. Transfer 0.5 ml of each donor culture (including controls) to a separate, sterile 1.6 ml microcentrifuge tube
7. Briefly (30s-1min) centrifuge the culture to pellet the cells; discard the supernatant by drawing it off with a Pipetman (be careful not to contaminate the culture)
8. Pipette 500µl of fresh LB (no antibiotics) onto the pellet; vortex to resuspend the cells
9. Briefly (30s-1min) centrifuge the culture to pellet the cells; discard the supernatant by drawing it off with a Pipetman; (this step serves to wash residual antibiotics out of the cell slurry)
10. Pipette onto this pellet a 500µl aliquot of the recipient cell culture
11. Briefly (30s-1min) centrifuge the culture to pellet the cells; discard the supernatant by drawing it off with a Pipetman (you may notice a two-color cell pellet at this point)

12. Pipette ~100µl of fresh LB onto the pelleted cells; resuspend the cells together by pippetting up and down repeatedly until you get a uniform slurry
13. Using a Pipetman, pipette the cell slurry (mating mixture) out of the microcentrifuge tube and drip it onto the very center of the dried MCE filters (prepared in step 5, above)
14. Incubate the plates at 30°C overnight

Selection

15. Dry the plates that will be used for selection for ~30min.
16. Using sterilized forceps (flamed with ethanol), pry the MCE filters from the LB plates and place each in a separate 50ml conical tube (e.g. a blue-capped falcon tube)
17. Pipette 500µl LB into each tube; use the Pipetman and/or vortexing to wash the mating mixture from the MCE filter and resuspend the cells in the LB
18. Transfer as much of the mating mixture slurry to the center of a dried selection plate; suing a sterilized glass spreader, spread the cell slurry until the liquid is fully absorbed into the agar
19. Incubate at an appropriate temperature

Notes on selection criteria

Selection must be carried out in such a way that donor cells do not grow while transconjugants do. For example, conjugal transfers between *Rhodococcus* strains B264-1 (donor) and SQ1 (recipient) can be selected by adding to the plate (1) an antibiotic to select for the presence of the plasmid and (2) an antibiotic to which the recipient strain is resistant (for SQ1, rifampicin or streptomycin would work) but to which the donor strain is sensitive. In matings between *Rhodococcus* strains B264-1 (donor) and I24 (recipient), an antibiotic must still be used to select for the appearance of the plasmid, but instead of using an antibiotic to select for the recipient strain, temperature may be used to select against the donors. That is, while I24 will grow at 37°C, B264-1 will not. This strategy will only work if the plasmid being transferred can replicate stably at 37°C, though.