Conjugation (Modified *Rhodococcus-E. coli* Protocol (1)):

*Please note that this protocol requires the recipient bacteria to be have a natural resistance that is not present in the donor bacteria. In this protocol, oxytetracycline is used because *G.apicola* is naturally resistant (2).

Materials Needed:

TSA plates (*G. apicola* Bacterial Lawn Grown)
One TSA plate
Antibiotic of Interest & Oxytetracycline TSA Plate
Liquid Culture (*E. coli* SM10 With plasmid)
Centrifuge
1.5mL Eppendorf Tubes
TSB
Pipets

Protocol (estimated time 1 hour):

1. Begin by transforming *E. coli* SM10 (contains genes for conjunction) with a plasmid capable of mobilization or conjunction.
    a. Grow the cultures overnight in 5 ml LB with appropriate antibiotic.
2. The next morning pellet 400µL of the *E. coli* cells and remove the supernatant, resuspend the cells in TSB.
    a. wash removes the antibiotic from the cells (optional to do additional washes)
3. Remove the *G. apicola* from the plates by pipetting 2mL TSB onto the plates, followed by scraping the cells off of the TSA with an inoculation loop.
4. Once the bacteria are scraped off the plate and floating/suspended in the TSB, angle the plate to one side and pipet up 400µL the TSB (contains the cells) and mix it with the *E. coli*.
5. Pellet the cell mixture and dispose of supernatant. Resuspend the cells in 1000µL of TSB.
6. Plate 100µL of the cell mixture onto a TSA plate, and grow overnight at 37°C in a 5% CO_2 balanced with nitrogen environment.
7. The next day, remove the bacteria from the plates by pipetting 2mL TSB onto the plates, followed by scraping the cells off of the TSA with an inoculation loop.
8. Once the bacteria are scraped off the plate and floating/suspended in the TSB, angle the plate to one side and pipet up 125µL of the TSB (contains the cells) and plate it on a oxytetracycline plate and selective antibiotic for the plasmid.
    a. Oxytetracycline to select for *G. apicola*, the other antibiotic to select for the plasmid.

References

1. Van der Geize, R. et al. (2002). Molecular and functional characterization of kshA and kshB, encoding two components of 3-ketosteroid 9α-hydroxylase, a class IA

monooxygenase, in *Rhodococcus erythropolis* strain SQ1. *Mol. Microbiol, 45(4).* doi:0.1046/j.1365-2958.2002.03069

2. Kwong, W., Engel, P., Koch, H., and Moran, N. (2014). Genomics and host specialization of honey bee and bumble bee gut symbionts. *Proceedings of the National Academy of Sciences, 111, 11509-11514.*