Long Lab, Stanford University
http://cmgm.stanford.edu/biology/long/

Triparental Matings

Quick mating to transfer a plasmid from E. coli into R. meliloti:
1) Patch together colonies of the E. coli donor strain, the E. coli helper strain (e.g. MT616, Cmr), and the R. meliloti recipient strain (e.g. Rm1021, Smr) on an LB plate. Also do the pairwise controls. Incubate overnight at 30°C.
2) From each patch, take a large swab and streak out on M9 sucrose plates with antibiotics.

More efficient mating of a plasmid from E. coli into R. meliloti:
1) Grow up liquid cultures of the E. coli donor strain, the E. coli helper strain (e.g. MT616, Cmr), and the R. meliloti recipient strain (e.g. Rm1021, Smr).
2) Pellet 1.5 ml and wash once with LB to remove the antibiotics.
3) Resuspend cells in 150 μl LB (1/10 volume).
4) Mix 45 μl R. meliloti cells and 15 μl of the E. coli helper cells (e.g. MT616) and donor cells. Also do the pairwise controls. Plate on LB. Incubate overnight at 30°C.
5) Flood each plate with 4 ml of 10 mM MgSO4 and scrape up the cells. Transfer to a test tube and lightly vortex to resuspend the cells. Serially dilute 1:10, 1:100, and 1:1000 in 10 mM MgSO4. Plate 100 μl of undiluted cells and of each dilution on M9 sucrose (E. coli cannot metabolize sucrose) with antibiotics. For the pairwise controls, plating the undiluted cells and the 1:10 dilution is sufficient

Mating a plasmid from R. meliloti into E. coli:
The plasmid in R. meliloti may either be a multicopy plasmid or may be integrated into the chromosome.
1) Grow up liquid cultures of the R. meliloti donor strain, the E. coli helper strain (e.g. MT616, Cmr), and the E. coli recipient strain (e.g. DH5a, Nalr).
2) Pellet 1.5 ml and wash once with LB to remove the antibiotics.
3) Resuspend in 150 μl LB (1/10 volume).
4) Mix 45 μl R. meliloti cells and 15 μl of the E. coli helper cells (e.g. MT616) and recipient cells. Also do the pairwise controls. Plate on LB. Incubate overnight at 30°C.
5) Flood each plate with 4 ml of 10 mM MgSO4 and scrape up the cells. Transfer to a test tube and lightly vortex to resuspend the cells. Serially dilute 1:10, 1:100, and 1:1000 in 10 mM MgSO4. Plate 100 μl of undiluted cells and of each dilution on selective medium. For the pairwise controls, plating the undiluted cells and the 1:10 dilution is sufficient. Incubate at 37°C. The 37°C growth temperature is sufficient to select against R. meliloti cells.
n.b. Note that the plasmid could move into MT616 (MM294A/pRK600) unless an antibiotic is used to which MM294A is sensitive or the plasmid is incompatible with pRK600.

Tips:
Doing matings by mixing cells from cultures instead of by patching colonies together is useful when either the exconjugant is going to be rare (e.g. if the plasmid needs to integrate into the chromosome) or if a large number of colonies are needed to perform a secondary screen (e.g. when exchanging Tn5 for another derivative with a different marker).
Use high levels of antibiotics during selection (e.g. spectinomycin and neomycin at 200 μg/ml).