Sinorhizobium meliloti gene knockout through a triple mating protocol

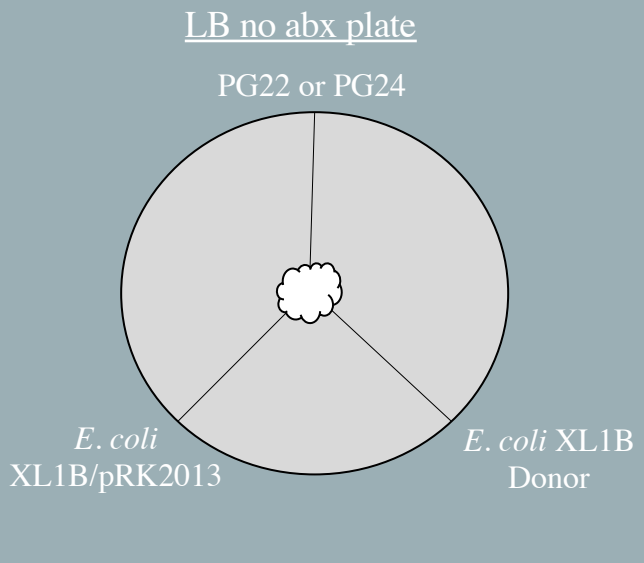
Castleton University
Ryanne Mix & Preston P. Garcia, Ph.D.

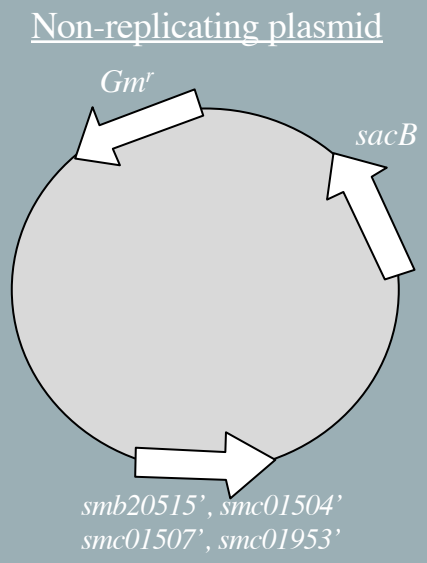
Table 1. Mutant *S. meliloti* strains

PG22	**PG24**
Histidine Kinase (*sma0113* knockout)	Response Regulator (*sma0114* knockout)

Table 2. *E. coli* XL1B Donor

Non-replicating plasmid	**Truncated gene**
pPG200	*smb20515'*
pPG201	*smc01504'*
pPG202	*smc01507'*
pPG203	*smc01953'*





S. meliloti contains a two-component signal transduction system encoded by genes *sma0113*/*sma0114*, *sma0113* encodes a histidine kinase, while *sma0114* encodes a response regulator. Our goal is to integrate the plasmid into the chromosome, then knock out the truncated gene along with the non-truncated gene, as they are believed to encode proteins that carry out the same function as genes *sma0113*/*sma0114* in *S. meliloti*. Using the triple mating method, PG22 or PG24 is transformed by the non-replicating plasmid with the assistance of the *E. coli* XL1B/pRK2013 helper plasmid.