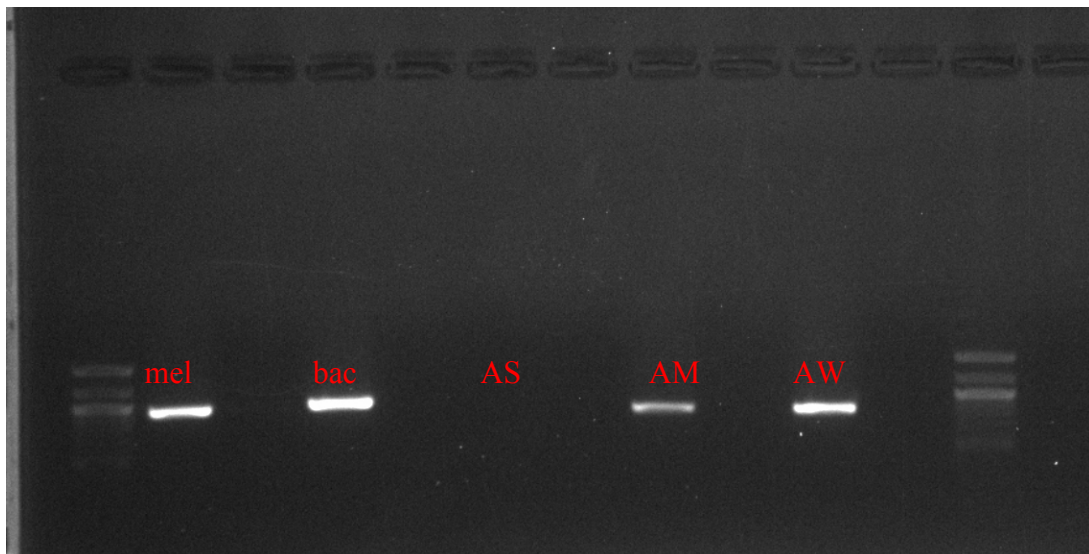
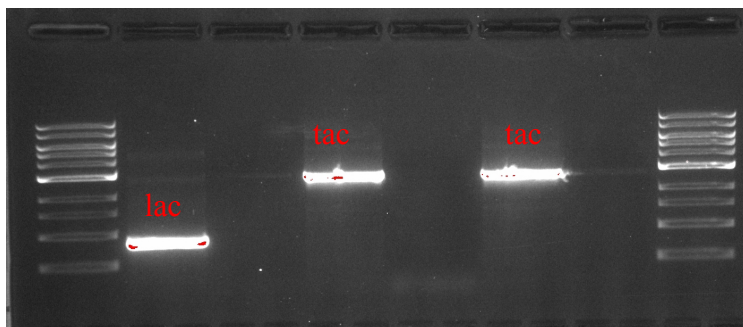


Amplification of Promoters to Drive Recombinase Expression

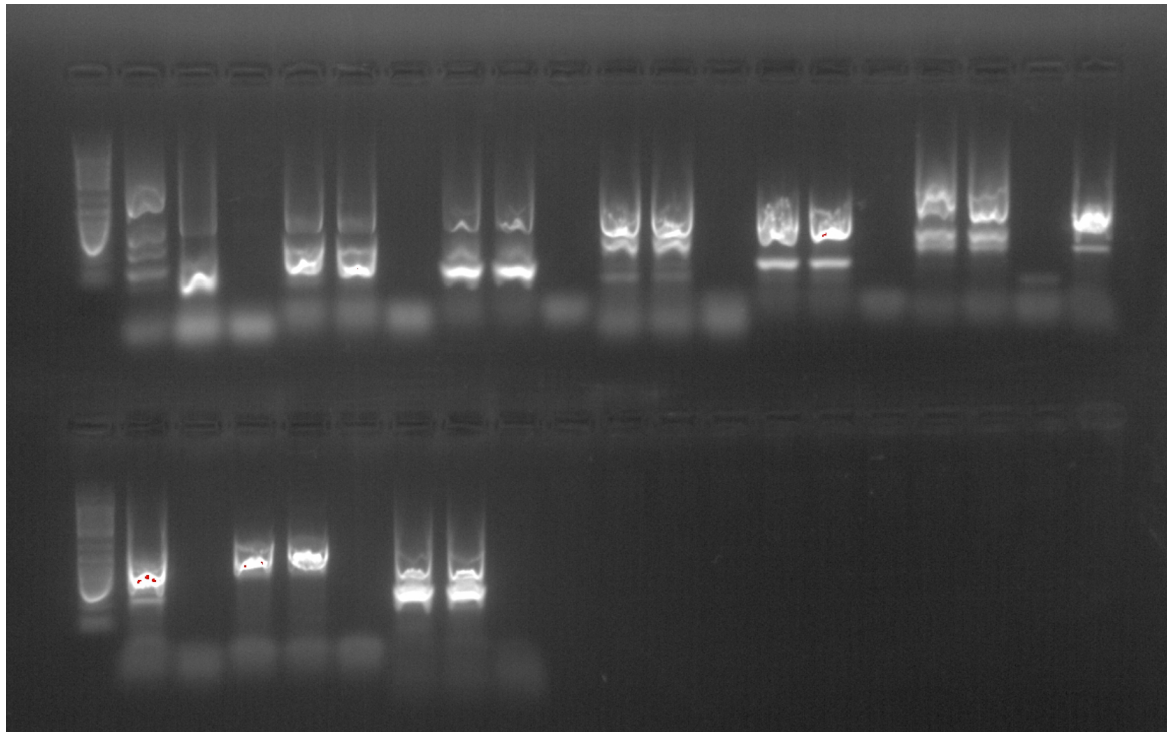
Promoters that work in Rhizobia are necessary to drive and control the expression of the recombinases. With constitutive promoters, we can make sure that the recombinase can be expressed in Rhizobia; with the inducible promoters, we can control the expression of the recombinase. Inducible promoters *mel* and *bac* were PCR amplified from *S. meliloti* 356, *tac* and *lac* from *E. coli*, and three constitutive Anderson promoters (Strong, Medium, Weak) were received from the Berkeley iGEM team. In the figures below, gel electrophoresis verified that the promoters were of expected size, with the exception of Anderson Strong, which showed no bands.



Top: Gel electrophoresis of lac and tac promoters with 1kb ladders. The sizes of the promoters are 1.2kb for lac and 1.9kb for tac. The bands were of expected sizes.

Bottom: Gel electrophoresis of mel, bac, AS, AM, and AW promoters with of 100 bp ladder. The sizes of the promoters are ~200bp for mel and bac, and ~100bp for AS, AM, The bands were of expected sizes with the exception of Anderson Strong, which showed no band.

Amplification of Recombinases



Gel electrophoresis of recombinase g-blocks (2 wells each + 1 neg. control).

From left to right:

G1: Lambda A6UAS9 – 1 kb

G2: Lambda B5ZVM8 – 1 kb

G3: Lambda H0FXW6 – 960bp

G4: Lambda K0PZW0 – 950bp

G11: D3ER_Q9MC70 – 750 bp

G12: Phage Protein J0G213 – 1.1kb

G13: Phage Protein N6VDJ4 – 1.1kb

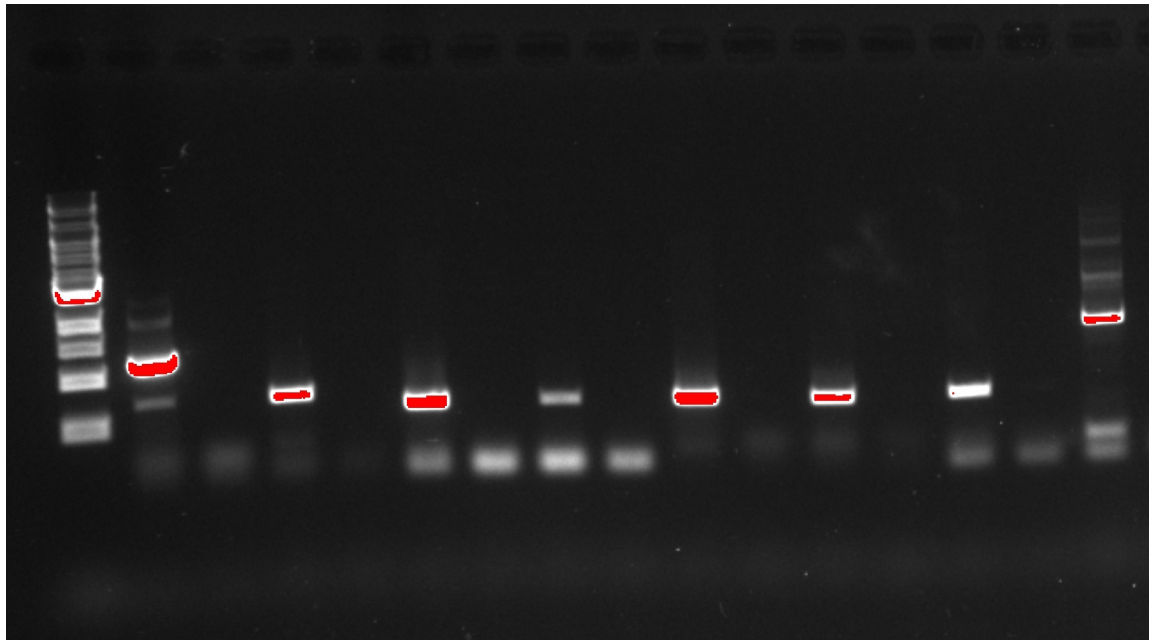
G14: SPP1_GP35 – 860bp

G15: Lambda Phage F7X3D1 – 950bp

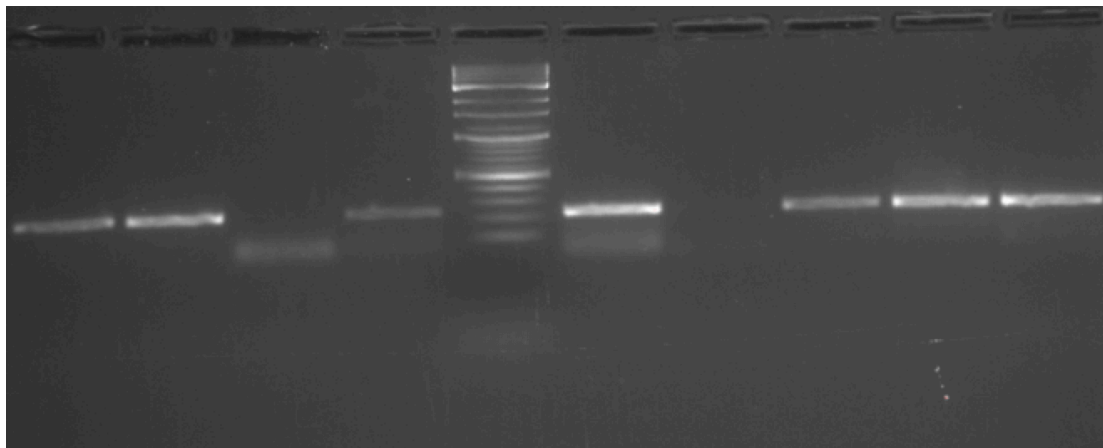
Lambda beta - 790bp.

Assembly of Promoter-Citrine and Promoter-Recombinase Constructs

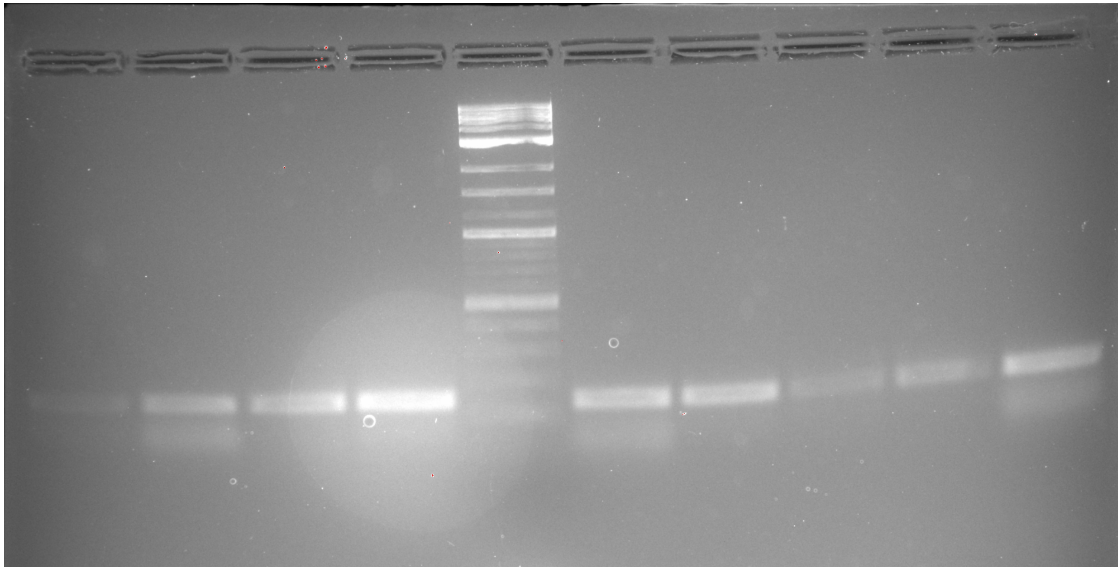
Promoter-Cit-Term constructs:



Gel electrophoresis of promoter-citrine constructs. The expected sizes of the of the desired bands are as follows: mel, bac - 1060bp; AS, AM, AW - 1kb; tac - 2.8kb.



Gel electrophoresis of mel promoter with recombinase overhangs. The size of mel promoter is 200bp; the expected sizes of the bands are size of mel promoter + size of recombinase (listed above).



Gel electrophoresis of Anderson Medium promoter with recombinase overhangs. The size of AM promoter is 100bp; the expected sizes of the bands are size of AM promoter + size of recombinase (listed above).