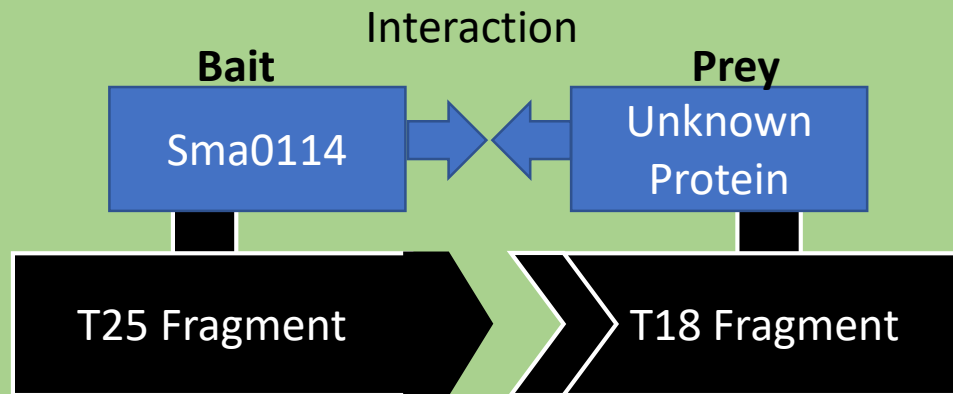
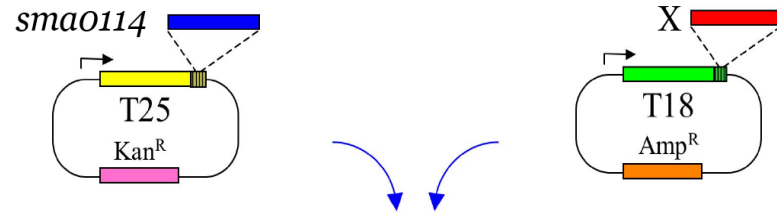


Using the Bacterial Adenylate Cyclase Two-Hybrid System (BACTH) for Identification of Proteins Associated with the *Sma0114* Response Regulator in *Sinorhizobium meliloti*.

Anthony Laniewski | Breanna Babiarz | Preston Garcia, Phd

Step 1 Clone *sma0114* into pKT25 Create genomic library using pUT18C



Step 2

Co-transform plasmids into DHM1

Screening

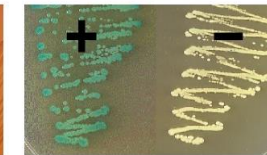
Plate transformants on :
- MacConkey/maltose,
- LB/X-gal + IPTG
+ antibiotics (Amp, Kan)

Incubate 24-72 h at 30 °C

McConkey/maltose



LB/X-gal

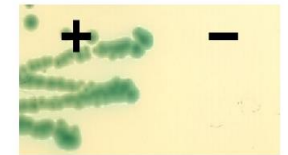


Selection

Plate transformants on :
M63/maltose
+ X-gal + IPTG
+ antibiotics (Amp, Kan)

Incubate 4-6 days at 30 °C

M63/maltose, X-gal



- : DHM1/ pKT25/ pUT18C + : DHM1/ pKT25-zip/ pUT18C-zip

sma0114 and X interact → Red colonies on McConkey/maltose

→ Blue colonies on LB/X-gal

→ Growth on M63/maltose

Step 3

Characterization of positive clones

- Retransformation
- β -galactosidase (and/or cAMP) assays
- Sequence analysis
- *in vitro* studies of physical interaction