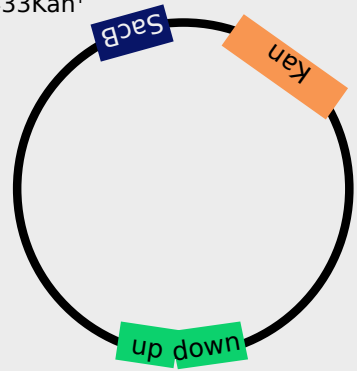
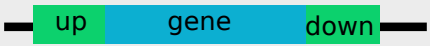
Knockouts using pCM433Kan^T

See Puri 2015: Genetic Tools for the Industrially Promising Methanotroph Methylomicrobium buryatense (AEM)

pCM433Kan^T



genomic DNA

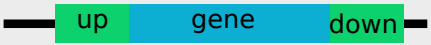


Step 1: Select for recombinants using Kan.
Can get upstream or downstrem crossovers

single crossover upstream:

single crossover downstream:

Step 2: Select for 2nd recombination using sucrose counter-selection.
Two main possibilities:

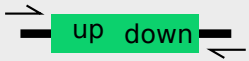


wild type:
Kan but not Sucrose sensitive

desired mutant: Kan but not sucrose sensitive

Step 3: (optional) Select for colonies that are Kan sensitive (requires plates w/ and w/o Kan). Include if you expect your knockout to be difficult or impossible to obtain. See warning below.

Step 4: Colony PCR to separate the WT revertants from your desired KOs



Warning: if you select for sucrose resistant colonies but your colonies are still Kan resistant, you likely have a SacB gene/promoter/RBS mutant

BAD: