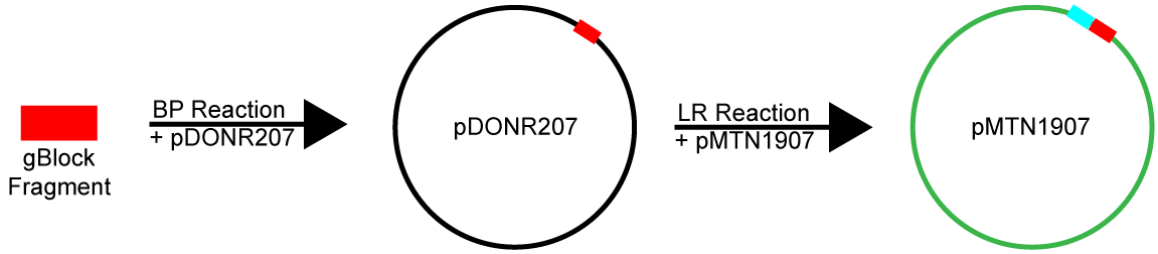
Creation of the *algW* SNP in the ancestral *P. stutzeri* background

Sequence of the synthesized gBlock containing the *algW* SNP

GGGGACAAGTTTGTACAAAAAAGCAGGCTCCTTGCTGGTAGGCCTGCTAGTGGCCCTGCTGAT
CATTCAGCGCTACCCGCATCTGGTAGGCCTGAGCGACGAGCCGGAATATAAGCTGCAGCAAAC
GCCGTTAGTGGGCATACCGCAACAAGGCCCCTACTCCTATGCCAATGCGGTCAGCAGTGCAGC
GCCCGCCGTTGCCAATCTCTACACCACCAAGGTGATCGACAGCTCCAGCCAGCCGCCGATGCTC
AAGGACGATCCGCTGTTCCGGCGATTCTACGGCGATAACCTGCCACGGCAGCGCCGCATGGAG
TCGAGCCTGGGCTCAGCGGCGATCATGAGCCCAGAGGGTTATCTGCTGACCAACAACCACGTT
ACGGCCAACGCCGAGCAGATCGTCGTGGCCTTGCGCGATGGCCGGGAAACCCTTGCGCGGGTG
ATAGGCAGCGACCCGGAGACGGATCTGGCACCCAGCTTTCTTGTACAAAGTGGTCCCC

Red: BP sites
Green: *algW* fragment
Yellow: SNP site (T>C)

The gBlock fragment was cloned in a similar method as presented in https://doi.org/10.6084/m9.figshare.3204175.v1 using Gateway BP/LR procedures.



Once the fragment is introduced into the destination vector (pMTN1907) it can then be recombined into the recipient chromosome *P. stutzeri* with pMPPla107 (DBL 494) using the TetR/SacB (Blue rectangle in pMTN1907) counter selection method mentioned in https://doi.org/10.6084/m9.figshare.3204175.v1. Tetracycline resistant colonies were selected for proper integration of the construct into the chromosome. Those colonies were then selected for flipping out the TetR/SacB cassette by streaking to 5% sucrose plate. Sucrose resistant colonies were then tested for the presence of *algW* using PCR with primer BAS 7/8.

BAS 7 primer: ATGATCAATGCCCTGCGTTTT

BAS 8 Primer: TTATTGAGGGCTCTTCTCTACCA

Thermocycler settings used were as follows:

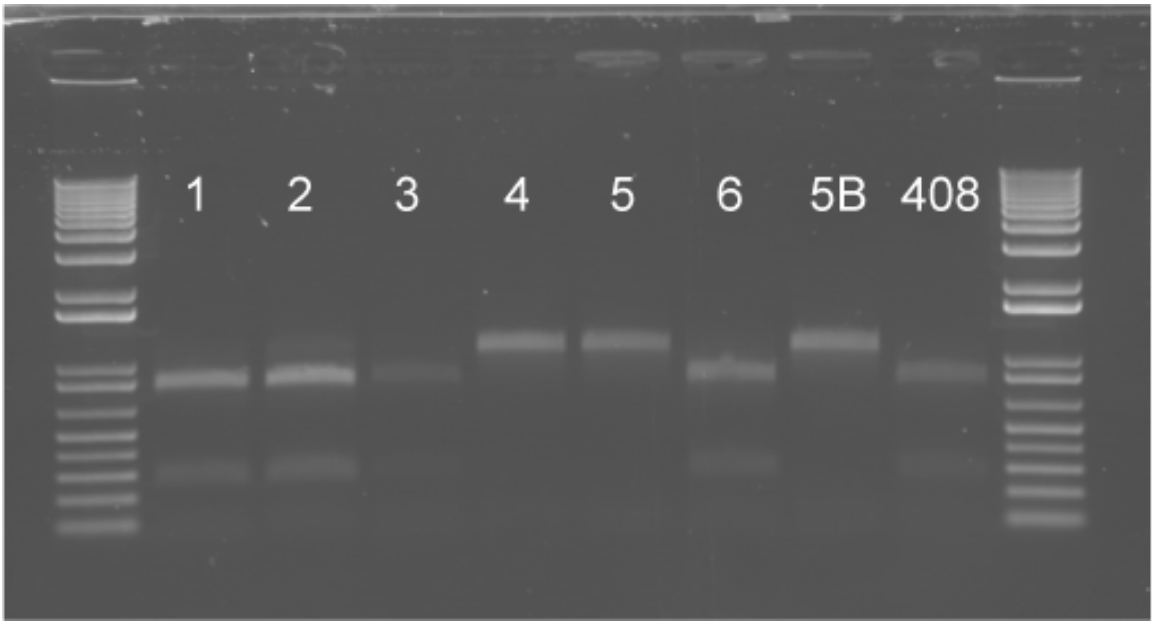
1. 95C˚ 5:00 min
2. 95C˚ 15 sec

3. 55C˚ 30 sec
4. 72C˚ 1:30 min
5. 72˚C 5:00 min

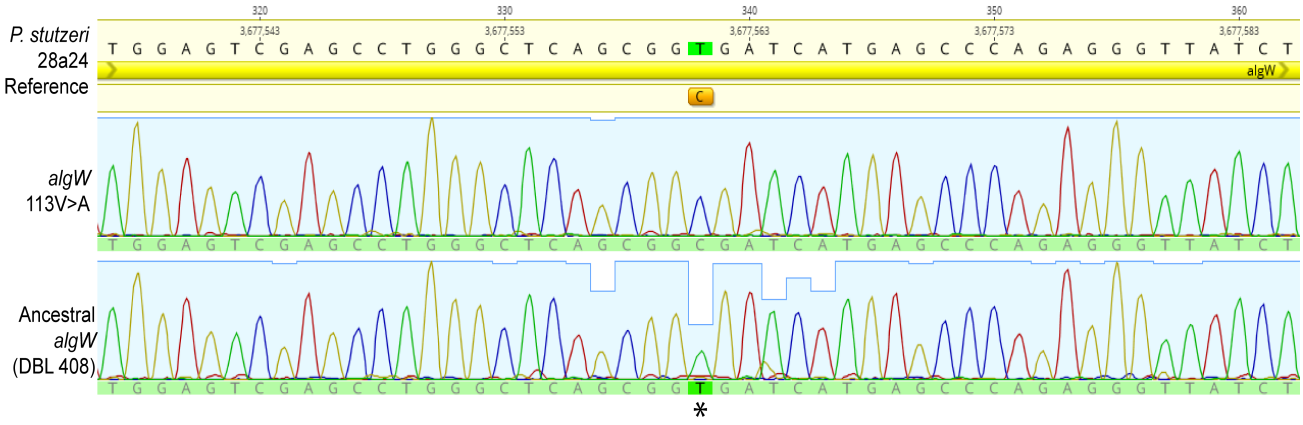
Repeat steps 2-4 34x

Confirming successful recombination of *algW* into a *P. stutzeri* ancestral background

Those that amplified successfully were cut with the restriction enzyme BslI. BslI will cut the SNP site of ancestral *algW* while the evolved 5B SNP will not cut. Therefore, we can select for proper recombination of the *algW* SNP by observing differences in fragment size. DBL408 is the *P. stutzeri* ancestral strain and is used here as a positive control.



Isolated TetR/SucR colonies 4 and 5 were resistant to restriction digest with BslI along with 5B, indicating proper recombination of the *algW* 5B SNP. To further confirm the presence of the SNP was properly introduced to ancestral *P. stutzeri* we used Sanger Sequencing on PCR products of *algW*.



Sanger Sequencing confirmed successful recombination and the presence of the SNP (*) in ancestral *P. stutzeri* with pMPPla107 (DBL 494) when compared to the reference sequence (yellow) and the PCR product of ancestral *algW*.