

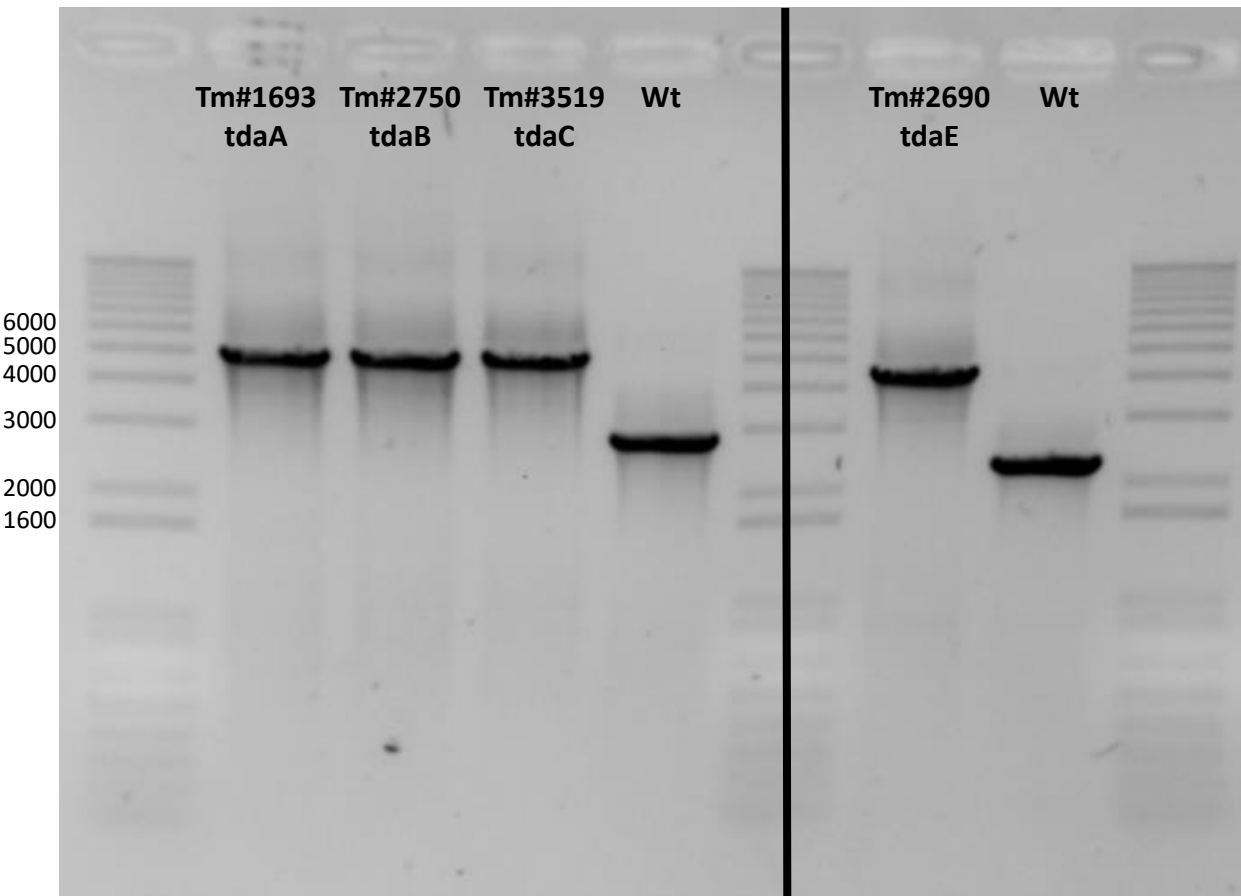
A: Authenticity of transposon mutants

PCR-assay of transposon mutants

Former plasmid curing experiments of *P. inhibens* DSM 17395 showed that the homogeneity of mutants might be impeded either by residual wild type cells that are attached to the mutant or by a heterogeneous plasmid population that still harbors genuine plasmids without the transposon insertion. Accordingly, we cultivated the transposon mutants in liquid Marine Broth (MB) medium with 120 µg/ml kanamycin (kan), plated the cultures under antibiotic selection on MB plates and streaked out single colonies on kan-plates three additional times for separation. Passaged mutants were grown in MB medium without antibiotics and total DNA was isolated with the DNeasy® Blood & Tissue Kit of QIAGEN. The absence of wild type cells and plasmids was documented with a sensitive PCR-assay.

OneTaq Polymerase

initial denat.	94°C	30sec	
denat.	94°C	30 sec	x 30
annealing	56/57°C	30 sec	x 30
extension	68°C	4,5 min	x 30
final extension	68°C	10 min	



P1491	TCGCTATGACGCCACCTATGT	P1495	GTCAGGGCGCTTCGGTAAAAC
P1492	CCAACCAAAACCGCTCCATCC	P1496	GGGAGTGCCATCAGCGTAAGC
Annealing temperature	56°C	Annealing temperature	57°C
Wildtype band	2787 bp	Wildtype band	2249 bp
Mutant band	4788 bp	Mutant band	4250 bp

Conclusion

The PCR-assay documented the presence of the EZ-Tn5 transposon in the four TDA genes by a size shift of 2,001 bp compared to the respective band of the wild type. The sensitive test also unequivocally showed the absence of any residual wild type signal in the mutant strains and thus validated the integrity of the mutants.