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Resistance Cassette Replacement of Germination Genes

Two methods were employed to knock out germination genes: replacement by resistance cassettes and clean deletions. Resistance cassette (RC) knockouts were performed using long-flanking homology PCR (see Fig. 1). Single RC knockouts were created first; then they were combined to create multiple knockouts.

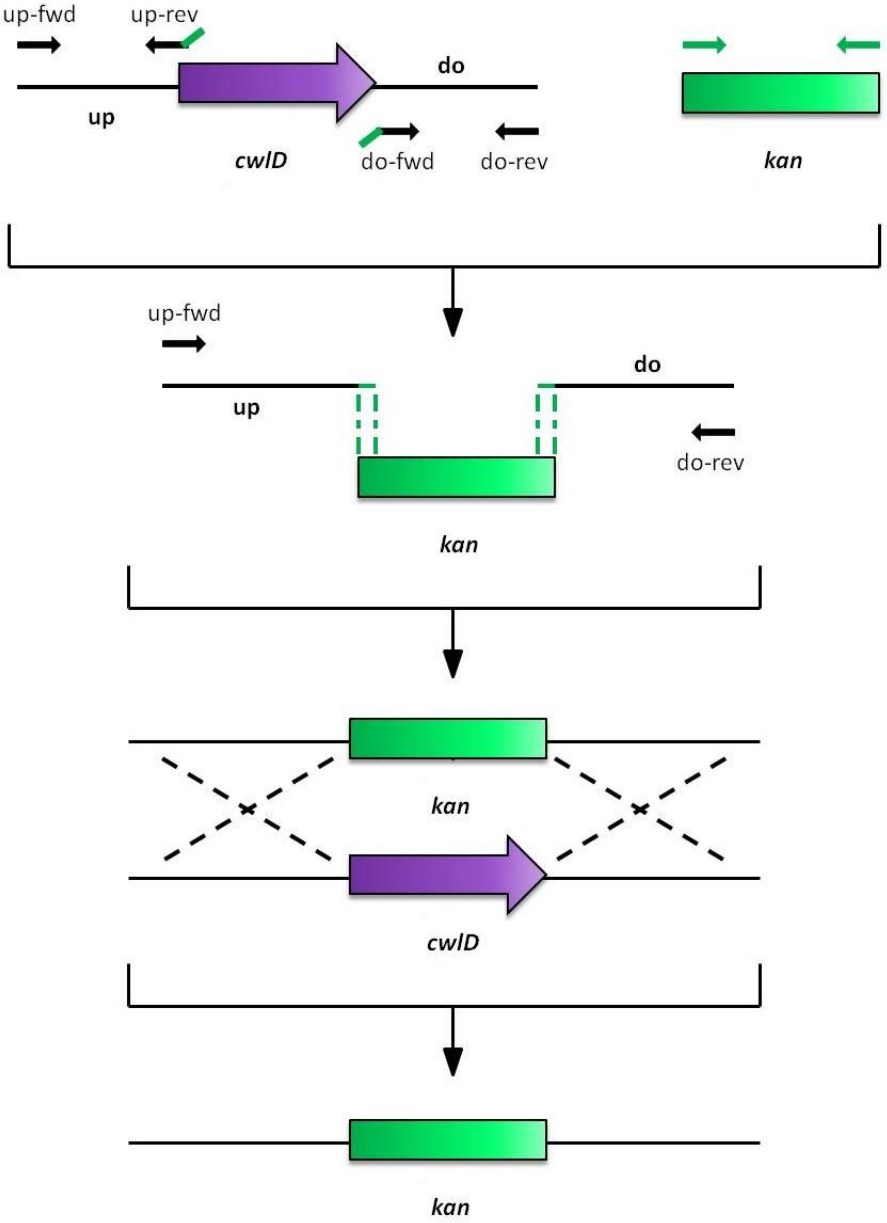

Fig. 1: Procedure of long-flanking homology PCR. As an example, replacement of *cwlD* by a kanamycin (kan) resistance cassette is shown. 1000 base-pair fragments flanking *cwlD* as well as the kan cassette were amplified. The up-reverse and down-forward primers have overhangs complementary to the kan cassette. The up and down *cwlD* fragments and the amplified kan cassette were fused in a PCR reaction. The result is a fragment containing the kan cassette flanked by the up- and downstream region *cwlD*. *B. subtilis* is transformed with the fragment and the replacement of *cwlD* by the kan cassette is checked by PCR.