

Solutions to a problem of the CRISPR MR vectors

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We were told that pG3GB411-BWM and pG4GB411-BWM were contaminated with small plasmids. We re-extracted the CRISPR MR vectors including pG3GB411-BWM, pG4GB411-BWM, and pC2GB411-BWM, finding that they were indeed contaminated with small plasmids whereas our original plasmids were shown to have no contamination (Figure 1A and 1B).

We reasoned that the small plasmids were derived from degraded original plasmids during successive cultivations and gradually prevailed in the cultures. We re-transformed pG4GB411-BWM and selected the clones on the Amp LB agar plate. We observed two types of colonies (Figure 1C). We streaked with the colonies on two parallel LB agar plates, Kan and Amp plates, respectively, finding that the large colonies were Kan sensitive. We extracted plasmids from the large and small colonies, finding that the large colonies harbor a small plasmid whereas the small colonies harbor the original plasmid. We successively cultivated the correct colonies, and found that in the 3rd time successive culture the small plasmid re-appeared (Figure 1C). We sequenced the small plasmid from the large colonies, finding that the small plasmid was derived from homologous recombination between the two 46-bp homologous arms located at ApR downstream site and pUC_ori upstream site of the original plasmid, respectively.

Based on the above observations, we suggest that the plasmids should be extracted from original cultures rather than the cultures successively grown for many times. If possible, Bsal-ApR-Bsal fragment should be replaced with other fragments, such as Bsal-ccdB-Bsal.

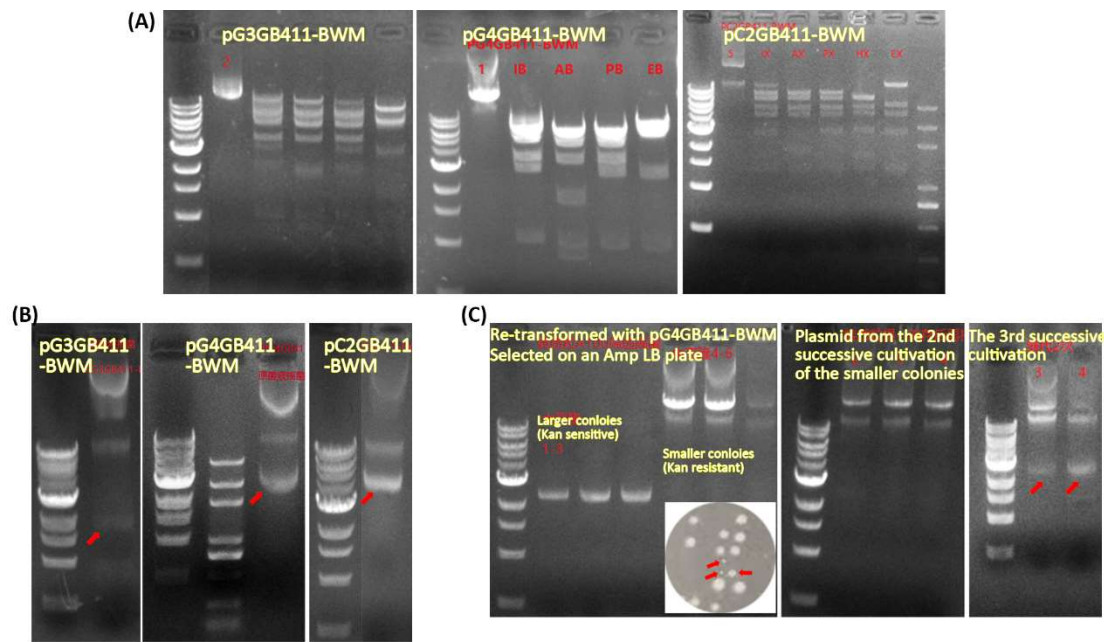


Figure 1. The successive cultivation resulted in the generation of small plasmids.

- (A) Original plasmids and digestion analysis with restriction enzymes.
- (B) Plasmids extracted from successive cultures were contaminated with small plasmids.
- (C) Successive cultivations of correct colonies harboring the newly transformed plasmid resulted in the small plasmids. The large colonies obtained from the re-transformed plasmid harbor the small plasmid and are Kan-sensitive whereas the small colonies harbor the original plasmid.