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Protocol status: Working
We use this protocol and it's working

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Plasmid construction

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ABSTRACT

plasmid construction

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1 Amplify the insert gene fragment by PCR with primers including 21 nt of overlapping sequence with the target gene.

2 Linearize the backbone with restriction enzymes (NEB).

3 Treat linearized backbone with quick CIP (NEB).

4 Run PCR products and linearized backbone in an agarose gel to confirm the size.

5 Purify the DNA from gel using a Gel extraction kit (Bio Basic).

6 Ligate the linearized backbone and the insert with the T4 ligase (NEB).

7 Transform the ligation products into home made competent cells.

8 Perform colony PCR to screen for colons that with inserted gene.

9 Sequence to verify that the inserted gene is correct.