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Plasmid construction

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We use this protocol and it's working

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Abstract

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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 clones that with inserted gene.
- 9 Sequence to verify that the inserted gene is correct.