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Plasmid Construction and Gibson cloning

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol describes plasmid construction.

Attachments



[759-1927.pdf](#)

43KB



Plasmid Construction

5h

- 1 Obtain the sequences of all cDNAs by amplifying existing plasmids, HAP1 cDNA, or through gene synthesis (Genscript).
- 2 For insect cell expressions, the sequences are codon optimized and the gene is synthesized (Genscript).
- 3 With the exception of the NAP1-6xAla mutant, which was obtained through gene synthesis (Genscript), all other plasmids were generated by Gibson cloning.
- 4 For Gibson cloning, generate inserts and vector backbones by PCR amplification or excise from agarose gels after restriction enzyme digestion at  37 °C for  02:00:00 .
- 5 Purify the inserts and plasmid backbones with Promega Wizard SV gel and PCR Cleanup System (Promega).
- 6 Mix the purified inserts and backbones in a molar 3:1 ratio, respectively, supplemented by a 2x NEBuilder HiFi DNA assembly enzyme mix (New England Biolabs). 
- 7 Incubate Gibson reactions for  01:00:00 at  50 °C and then transform into DH5-alpha competent *E. coli* cells. 
- 8 Grow transformed Gibson reactions  Overnight on agar plates containing the appropriate selection marker (ampicillin, kanamycin, or chloramphenicol). 
- 9 Pick single colonies, grown  Overnight in liquid cultures, and pellet for DNA plasmid extraction using the GeneJet Plasmid Miniprep kit (Thermo Fisher). 
- 10 Submit the purified plasmid DNA for DNA Sanger sequencing (MicroSynth AG) to verify insert sequences by Sanger sequencing. 
- 11 Further analyze positive clones by whole plasmid sequencing (Plasmidsaurus). 