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Plasmid Construction and Gibson cloning

Elias Adriaenssens[1]

[1]Sascha Martens lab, University of Vienna, Max Perutz Labs - Vienna

ASAP Collaborative Research Network

Sascha Martens lab, University of Vienna, Max Perutz Labs - Vienna

Elias Adriaenssens

Sascha Martens lab, University of Vienna, Max Perutz Labs - ...

ABSTRACT

This protocol describes plasmid construction.

ATTACHMENTS

759-1927.pdf

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Protocol status: Working

We use this protocol and it's working

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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. 2h

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. 1h

8 
Grow transformed Gibson reactions Overnight on agar plates containing the appropriate selection marker (ampicillin, kanamycin, or chloramphenicol). 1h

9 
Pick single colonies, grown Overnight in liquid cultures, and pellet for DNA plasmid extraction using the GeneJet Plasmid Miniprep kit (Thermo Fisher). 1h

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).