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Gibson Assembly

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We use this protocol and it's working

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Abstract

This protocol details how to clone DNA plasmids by Gibson assembly (New England Biolabs, E2621), an exonuclease-based method to easily assemble DNA in the correct order.

Materials

- Wizard SV Gel and PCR Clean-Up System **Promega Catalog #**A9281
- NEBuilder HiFi DNA Assembly Master Mix **New England Biolabs Catalog #**E2621

Purification of DNA Fragments

1 Prepare the DNA fragments by digestion with restriction enzyme or PCR amplification.

Note

- DNA fragments can be also synthesized by companies like Twist Bioscience.
- Ensure that the DNA fragments have overlapping ends of 15-20 base pairs.

2 Purify the DNA fragments by agarose gel electrophoresis, followed by excision of the corresponding band and extracting the DNA from the gel piece using a clean-up system like the Wizard SV Gel and PCR Clean-Up System.

3 Determine the DNA concentrations with a Nanodrop spectrophotometer.

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1h

4 For a reaction with 2-3 fragments, prepare the following mixture On ice, Molar ratio vector:insert = 1:2

- 50 ng of vector (or bigger DNA fragment) + double in pmol of insert(s) + 10 µL NEBuilder HiFi DNA Assembly Master Mix + to 20 µL deionized H_2O.

Note

- pmols = (weight in ng) x 1,000 / (base pairs x 650 Daltons)
- For other number of fragments and other detailed information please refer to, https://www.neb.com/en/protocols/2014/11/26/nebuilder-hifi-dna-assembly-reaction-protocol
- Other total reaction volume can be set as far as the Master Mix (2x) is diluted to a final concentration of 1x.

5 Incubate at 50 °C for 01:00:00.

1h

6 Place the reaction On ice .

7 Transform 50 µL of competent bacteria with 2 µL of the Gibson assembly reaction.

Note

Use of NEB Stable Competent E. coli (High Efficiency) increases the efficiency of the cloning.