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Vector Digestion and Purification

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

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Abstract

Protocol for plasmid digestion and purification

Materials

Reagents:

- Restriction Enzymes (New England Biolabs)
- 10x Cutsmart Buffer (New England Biolabs)
- Agarose
- EtBr
- Promega Wizard SV Gel and PCR Purification Kit (A9282)

Isolate Digested Vector:

3h 2m

1 *Gibson Assembly technology uses homologous recombination to assemble adjacent DNA fragments that share end-terminal homology. The optimal length of the homologous fragment ends region depends on*
the number and length of the fragments in the assembly reaction.

2 1. Digest Vector with New England Biolabs Restriction Enzymes:

3h 2m

Component	Volume (uL)
DNA Plasmid	X uL for 10 ug of Plasmid
10x Cutsmart Buffer	5
Enzyme 1	2.5
Enzyme 2	2.5
Nuclease Free H20	40-X

2. Incubate for 02:00:00 at 37 °C .

3. Add 10 µL of 6x loading buffer to reaction and vortex briefly to mix.

4. Make 1% low melt-agarose gel.

a) Mix 1 g of Agar with 100 mL of TAE Buffer.

b) Microwave to boil agarose and let cool until you can touch bottle, but gel is not solid.

c) Add 1.5 µL of EtBr to agarose and pour into DNA gel mold with 10 well comb.

d) Let gel solidify.

5. Load 60 µL of reaction into well of gel

6. Run gel for 00:45:00 at 120V.

7. Visualize band with UV light and cut out section of gel with band with new razor blade and place in 1.5 mL tube.

8. Purify Band from gel with Promega Wizard SV Gel and PCR Purification Kit (A9282)

a) https://www.promega.com/products/nucleic-acid-extraction/clean-up-and-concentration/wizard-sv-gel-and-pcr-clean-up-system/?catNum=A9281

b) Weigh DNA gel fragment and add 10 µL of Membrane Binding Solution per 10 mg of gel slice.

c) Incubate mixture at 65 °C for 00:10:00 or until gel is completely melted.

d) Add melted gel mixture to SV minicolumn in Collection Tube and incubate at room temperature for 00:01:00 .

e) Centrifuge at max speed for 00:01:00 . Discard flowthrough and reinsert column into tube.

f) Add 700 µL of Membrane Wash Solution. Centrifuge at max speed for 00:01:00. Discard flowthrough and reinsert column into tube.

g) Add 500 µL of membrane wash solution. Centrifuge at max speed for 00:01:00. Discard flowthrough and reinsert column into tube.

h) Spin empty column for 00:01:00 at max speed to remove excess ethanol.

i) Transfer column to labelled 1.5 mL tube and add 35 µL of NF H_20. Incubate for 00:01:00 at room temperature.

j) Centrifuge at max speed for 00:01:00. Keep eluate and store at -20 °C.