Restriction Digest of Plasmid DNA

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Abstract

Please see the Addgene website for additional details.

Citation: Caroline LaManna Restriction Digest of Plasmid DNA. **protocols.io**
dx.doi.org/10.17504/protocols.io.cd2s8d
Published: 25 Sep 2014

Protocol

Step 1.
Select restriction enzymes to digest your plasmid.

NOTES
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For a list of many commonly used restriction enzymes, visit NEB.
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To determine which restriction enzymes will cut your DNA sequence (and where they will cut), use a sequence analysis program such as Addgene's Sequence Analyzer.

Step 2.
Determine an appropriate reaction buffer by reading the instructions for your enzyme.

NOTES
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If you are conducting a double digest (digesting with two enzymes at the same time), you will need to determine the best buffer that works for both of your enzymes. Most companies will have a compatibility chart, such as the double digest chart from NEB. If you cannot find a buffer that is appropriate for both of your enzymes, you will need to digest with one enzyme first in the buffer for enzyme 1, repurify the cut plasmid, and then conduct the second digest in the buffer for enzyme 2.

Step 3.
In a 1.5mL tube combine the following: DNA (all amounts are for a typical reaction; your amount may vary depending on the enzymes)

AMOUNT
1 µg Additional info:

NOTES
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The amount of DNA that you cut depends on your application. Diagnostic digests typically involve ~500ng of DNA, while molecular cloning often requires 1-3µg of DNA. The total reaction volume usually varies from 10-50µL depending on application and is largely determined by the volume of DNA to be cut.
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See Tip and FAQ section on the Addgene website for note on determination of restriction enzyme

volume to use.

Step 4.
Restriction Enzyme(s) (1ul of each Restriction enzyme)

NOTES

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Restriction enzymes MUST be placed in an ice bucket immediately after removal from the -20°C freezer because heat can cause the enzymes to denature and lose their function.

Step 5.
Buffer

AMOUNT

3 µl Additional info:

Step 6.
BSA (if recommended by manufacturer)

AMOUNT

3 µl Additional info:

Step 7.
dH2O up to total volume (up to 30ul for typical reaction)

Step 8.
Mix gently by pipetting.

Step 9.
Incubate tube at appropriate temperature (usually 37°C) for 1 hour.

DURATION

01:00:00

NOTES

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Depending on the application and the amount of DNA in the reaction, incubation time can range from 45 mins to overnight. For diagnostic digests, 1-2hr is often sufficient. For digests with >1µg of DNA used for cloning, it is recommended to digest for at least 4hr.

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f you will be using the digested DNA for another application (such as a digestion with another enzyme in a different buffer), but will not be gel purifying it, you may need to inactivate the enzyme(s) following the digestion reaction. This may involve incubating the reaction at 70°C for 15 mins, or purifying the DNA via a purification kit, such as a QIAGEN DNA cleanup kit. See the enzyme manufacturer's instructions for more details.

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To visualize the results of your digest, conduct gel electrophoresis.