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Restriction Digest of Plasmid DNA V.3

Addgene the Nonprofit Plasmid Repository[1]

[1]Addgene

1 Works for me dx.doi.org/10.17504/protocols.io.63shgne

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ABSTRACT

This protocol is for restriction digest of plasmid DNA. To see the full abstract and additional resources, please visit http://www.addgene.org/plasmid_protocols/restriction_digest/.

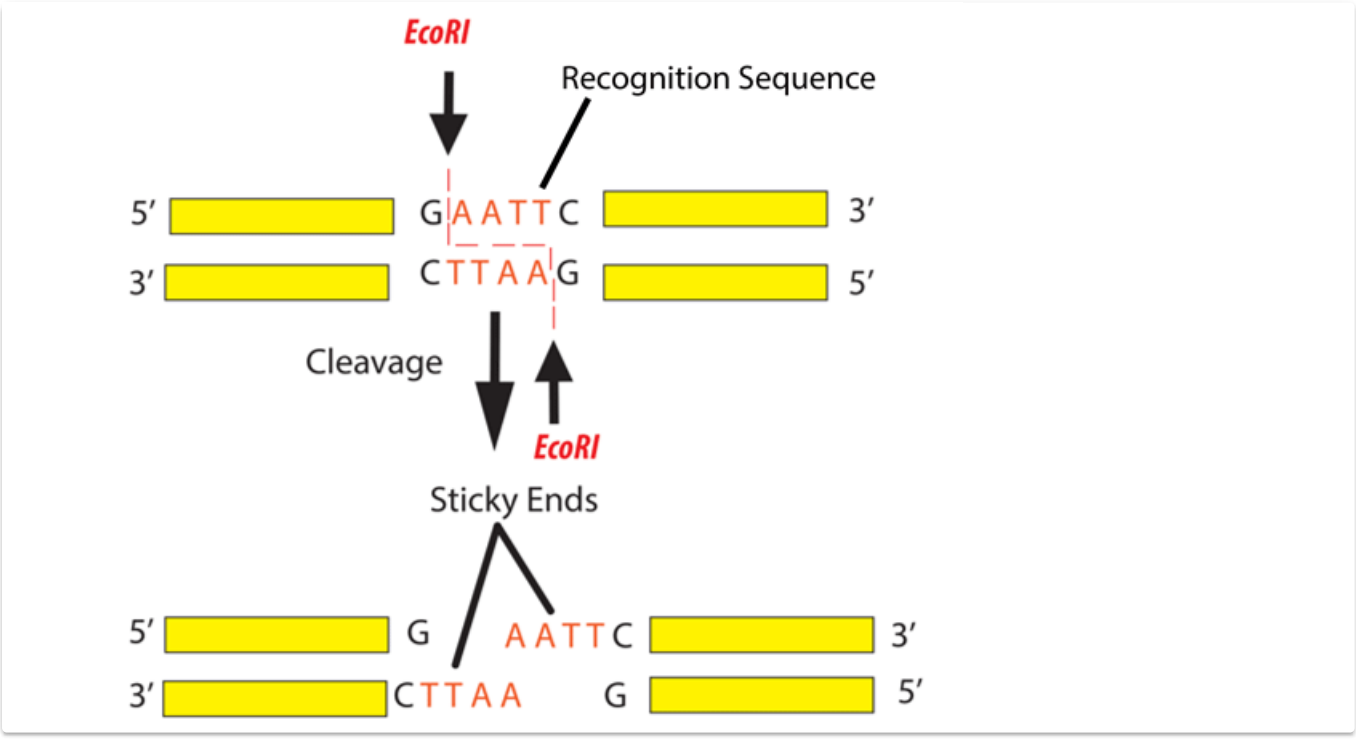
EXTERNAL LINK

http://www.addgene.org/plasmid_protocols/restriction_digest/

GUIDELINES

Tips and General Guidelines

- 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.



- If you are having difficulty finding an enzyme that cuts your vector's multiple cloning site (MCS), but does not cut your insert, you could try using two different enzymes that have compatible sticky ends. See NEB's compatible cohesive ends chart.
- If you cannot find compatible sticky ends, you will need to fill in the overhangs and conduct a blunt end ligation. Use T4 DNA Polymerase or Klenow DNA Polymerase I for 3' overhang removal and 5' overhang fill-in.
- If you are using blunt ends or a single enzyme to cut the vector, you will need to use a phosphatase to prevent re-circularization of the

vector if you are cloning in an insert. CIP (calf alkaline phosphatase) or SAP (shrimp alkaline phosphatase) are commonly used. Follow the manufacturer's instructions.

- If your enzyme did not cut, check to make sure that it isn't methylation sensitive. Plasmids grown in Dam or Dcm methylase positive strains will be resistant to cleavage at certain restriction sites. See NEB's table of methylation sensitive restriction sites.

- Sometimes enzymes cut sequences which are similar, but not identical, to their recognition sites. This is due to "Star Activity" and can happen for a variety of reasons, including high glycerol concentration. Learn more at NEB's website about star activity.

- If you are digesting a large number of plasmids with the same enzyme(s) (for instance, in a diagnostic digest), you can create a "Master Mix" consisting of all of the reaction components except for the DNA. Aliquot your DNA into individual tubes and then add the appropriate amount of Master Mix to each tube. This will save you time and ensure consistency across the reactions.

MATERIALS TEXT

Equipment

- Electrophoresis chamber
- Pipetman

Reagents

- Liquid DNA aliquot of your plasmid of interest (see below for recommend amounts)
- Appropriate restriction enzyme (see manufacturer's instructions for proper ammount)
- Approrpriate restriction digest buffer (see manufacturer's instructions)
- Gel loading dye
- Electrophoresis buffer
- Pipet tips

1 Select restriction enzymes to digest your plasmid.

Notes:
- For a list of many commonly used restriction enzymes, visit NEB.
- 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.

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

3 In a 1.5mL tube combine the following (typically **50 µl** reaction):

- **1 µg** DNA (all amounts are for a typical reaction; your amount may vary depending on the enzymes)

Notes:
- The amount of DNA that you cut depends on your application. Diagnostic digests typically involve ~ **500 ng** of DNA, while molecular cloning often requires **1 µg** - **3 µg** of DNA. The total reaction volume usually varies from **10 µl** - **50 µl** depending on application and is largely determined by the volume of DNA to be cut.
- See the Tips and FAQ in the guidelines section for determining what volume to use for restriction enzymes.

- **1 µl** of each restriction enzyme

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.

- **3 µl** of Buffer
- **3 µl** of BSA (if recommended by manufacturer)
- dH_2O up to total volume (up to **30 µl** for typical reaction)

4 Mix gently by pipetting.

5 Incubate tube at appropriate temperature (usually **37 °C**) for **01:00:00** .

Pro-Tips
- Depending on the application and the amount of DNA in the reaction, incubation time can range from **00:45:00** to overnight. For diagnostic digests, **01:00:00** - **02:00:00** is often sufficient. For digests with > **1 µg** of DNA used for cloning, it is recommended to digest for at least **04:00:00** .
- If 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 **00:15:00** , or purifying the DNA via a purification kit, such as a QIAGEN DNA cleanup kit. See the enzyme manufacturer's instructions for more details.

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

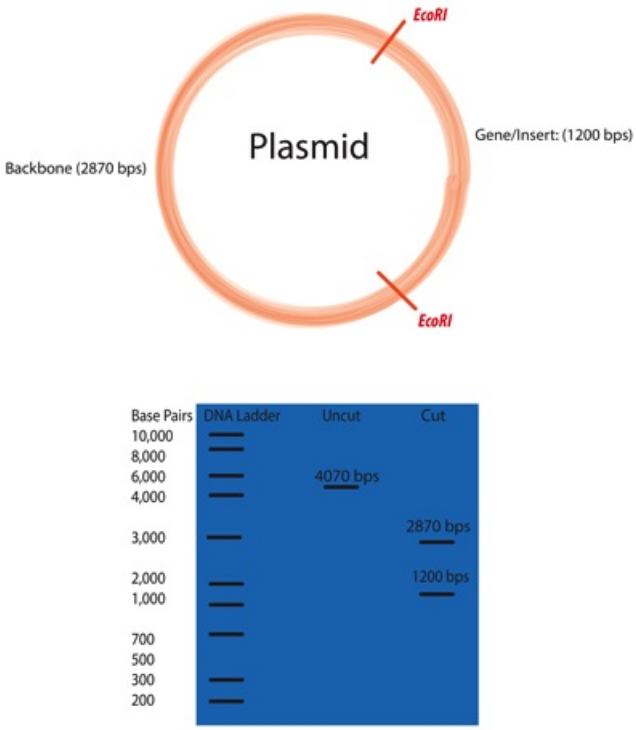


Figure 1: example restriction digest run on an agarose gel after gel electrophoresis.