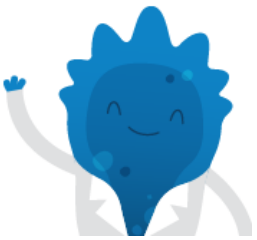
Dec 31, 2019 Version 3

Restriction Digest of Plasmid DNA V.3



DOI
dx.doi.org/10.17504/protocols.io.63shgne

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

Protocol Citation: Addgene The Nonprofit Plasmid Repository 2019. Restriction Digest of Plasmid DNA. **protocols.io** https://dx.doi.org/10.17504/protocols.io.63shgne

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

Created: September 03, 2019

Last Modified: December 31, 2019

Protocol Integer ID: 27474

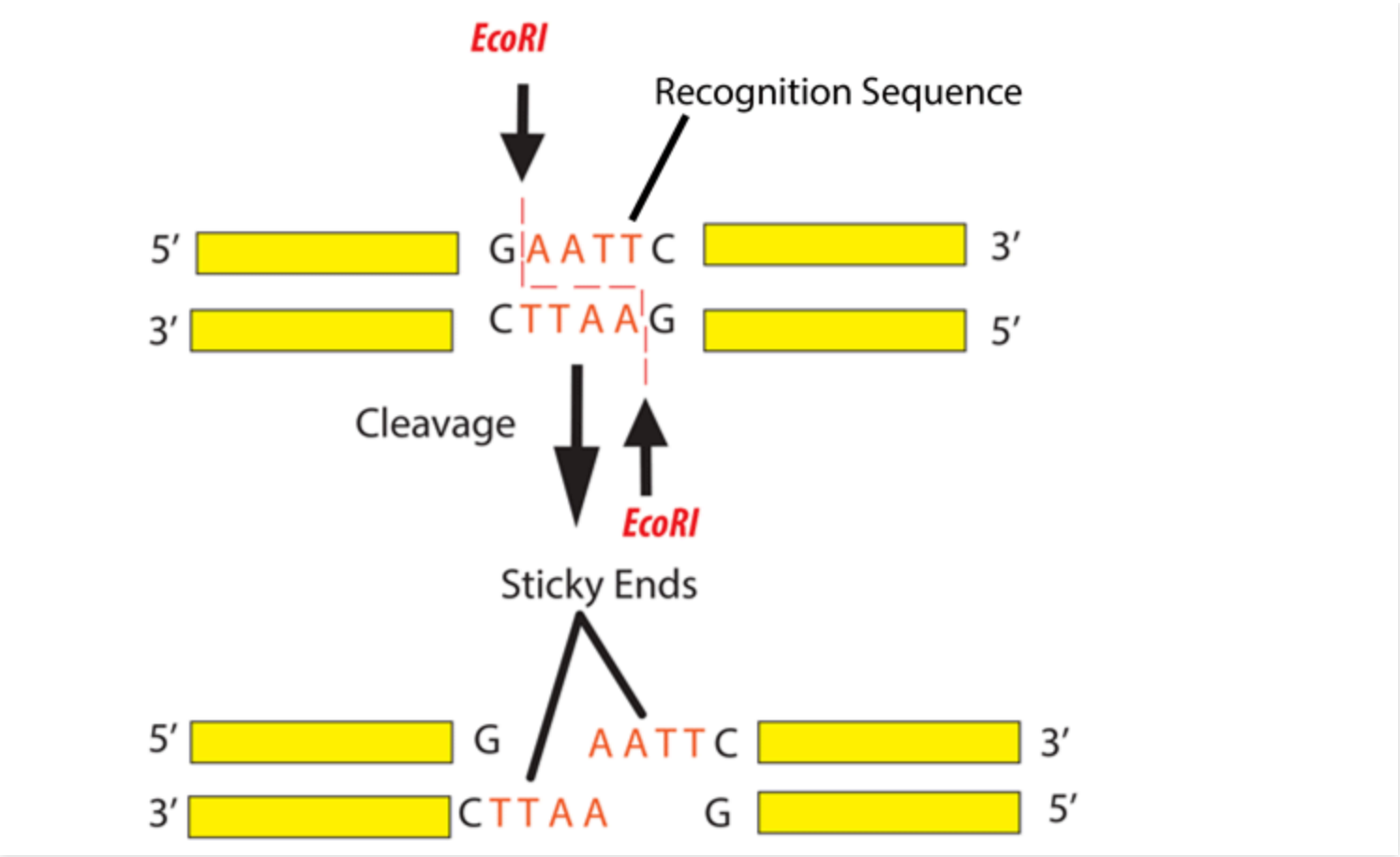
Abstract

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/.

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

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.

Note

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.

Note

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.

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)

Note

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

Note

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.

Note

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.

Note

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

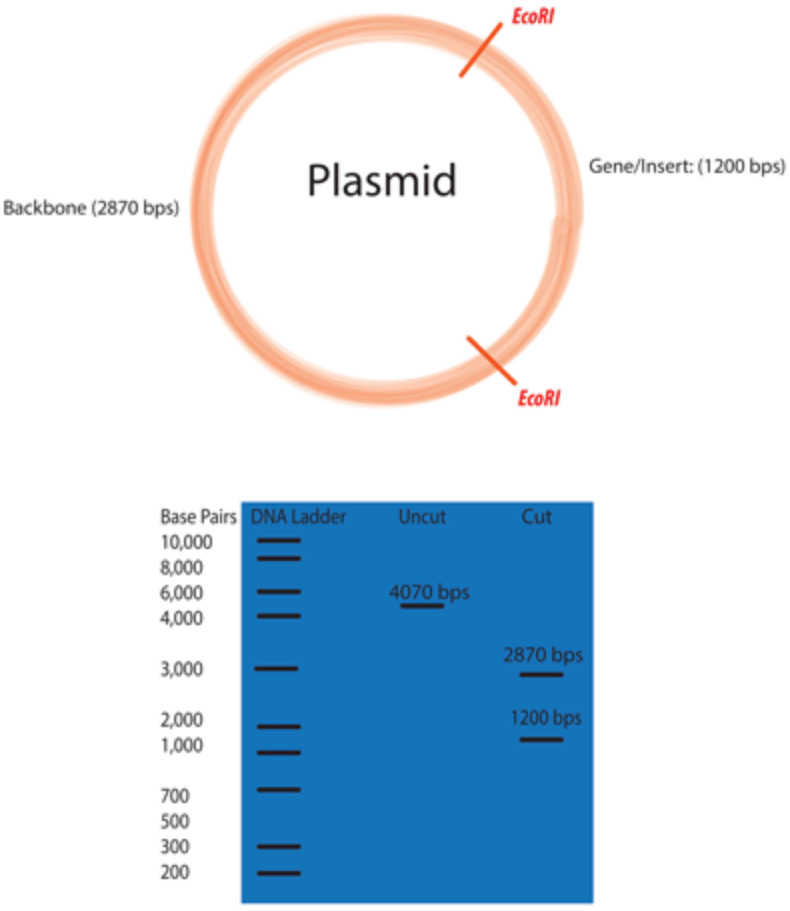


Figure 1: example restriction digest run on an agarose gel after gel electrophoresis.