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Diagnostic Restriction Digest

Addgene The Nonprofit Plasmid Repository[1]

[1]Addgene

1 Works for me dx.doi.org/10.17504/protocols.io.4gvgtw6

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ABSTRACT

This protocol is for running a diagnostic restriction digest. To see the full abstract and other resources, visit the Addgene protocol page.

EXTERNAL LINK

https://www.addgene.org/protocols/diagnostic-digest/

GUIDELINES

For detailed protocols as well as tips and tricks see the Restriction Digest and Gel Electrophoresis protocol pages.

MATERIALS TEXT

Equipment

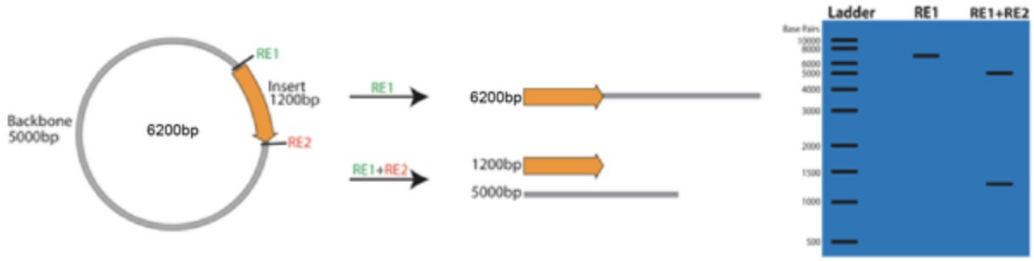
- Electrophoresis chamber
- Pipetman
- Pipet tips

Reagents

- Liquid DNA aliquot of your plasmid of interest (see protocol steps for recommended amounts)
- Appropriate restriction enzyme (see manufacturer's instructions for proper amount)
- Appropriate restriction digest buffer (see manufacturer's instructions)
- Gel loading dye
- Electrophoresis buffer

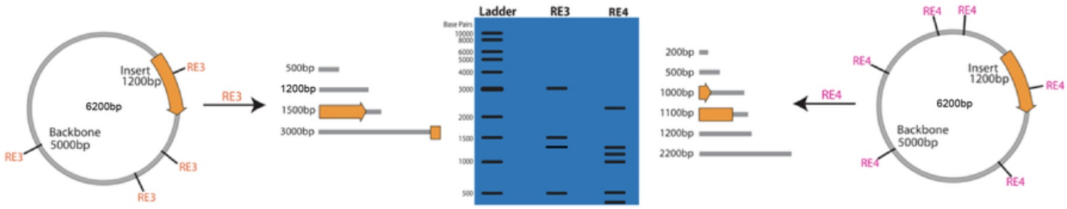
Verifying Total Plasmid Size -OR- Insert and Backbone Size

1 The simplest form of diagnostic digest is one in which you just want to verify that the plasmid that you have is the expected size, or that it is composed of a backbone and insert of expected sizes. This is frequently done after performing either PCR - or restriction enzyme -based cloning to test individual clones before use of more expensive forms of plasmid verification, such as DNA sequencing. In the example image below, digestion with enzyme RE1 will linearize the 6200bp plasmid into one single 6200bp fragment. Alternatively, digestion with both RE1 and RE2 will result in two bands; the 1200bp insert and the 5000bp backbone.



Verifying Plasmid Identity by "Plasmid Fingerprinting"

2 Often, it will be enough to know that you have a 1,200bp insert in a 5,000bp backbone, but there are many plasmids out there that, when digested with restriction enzymes common to multiple cloning sites, will result in similar sized bands, thus making this simple digest less informative. This is particularly true if you receive a plasmid from someone in another lab, or dig one out of the freezer and you are not 100% sure it is what you are looking for, but you have a map and know exactly what it should be. A useful restriction enzyme based technique for verifying plasmids like this is "plasmid fingerprinting", where you cut the plasmid into 3-8 pieces such that all (or most) fragments are small enough to be accurately sized on a gel and also such that they are different enough in size to be easily resolved from each other. However, by choosing an enzyme or enzymes that will cut your plasmid into multiple fragments, you can get a very unique pattern that will distinguish one 5kb backbone with a 1.2kb insert from all others.



When choosing restriction enzymes for this approach, it is often a good idea to choose two different enzymes that will give you unique but distinct patterns so that you get double confirmation. In the example above, digestion with either RE3 or RE4 will give a very predictable pattern of bands on the gel, but by digesting with both and seeing both patterns you can be incredibly confident that you have the correct plasmid.

Verifying Insert Orientation by Restriction Digest

3 If you clone an insert into a vector by a single restriction enzyme, you will need to verify that it has be cloned in the correct orientation - this can be done by restriction digest. Although this is never an ideal cloning strategy, sometimes it cannot be avoided. If you do have to do so, there is no way to control which orientation the insert is ligated into the vector backbone and therefore the only thing left to do is identify the clone(s) in which the insert is in the correct orientation.

In the example below we want to know how to differentiate between two clones that differ only in the orientation of the insert. By choosing an enzyme (or enzymes) that cuts within the insert, but significantly off center (ideally around 1/3 of the way from one end), and also cuts in the backbone on one side of the insert, you can get a very obvious verification of the orientation so long as the expected products from each orientation are different sizes. Simply run the digest products on a gel and those clones with the appropriately sized products should have the correct orientation.