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Purifying DNA from an Agarose Gel

Addgene The Nonprofit Plasmid Repository[1]

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

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ABSTRACT

This protocol is for purifying DNA from an agarose gel. To see the full abstract and additional resources, please visit https://www.addgene.org/protocols/gel-purification/.

EXTERNAL LINK

https://www.addgene.org/protocols/gel-purification/

GUIDELINES

Tips and FAQ

How do you get better resolution of bands?
A couple simple ways to increase the resolution (crispness) of your DNA bands include: a) running the gel at a lower voltage for a longer period of time; b) using a wider gel comb; or c) loading less DNA in the well.

How do you get better separation of bands?
If you have similarly sized bands that are running too close together you can adjust the gel percentage to get better separation. A higher percentage agarose gel will help resolve smaller bands from each other, and a lower percentage gel will help separate larger bands.

10% Rule:
For each sample you want to load on a gel, make 10% more volume than needed because several microliters can be lost in pipetting. For example, if you want to load **1 µg** in **10 µl**, make **1.1 µg** in **11 µl**.

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Follow the agarose gel electrophoresis protocol with the following amendments:

- Gel purification is most efficient with lower % agarose gels, so you will want to stay in the 0.7-0.8% range if possible.
- You will want nice crisp bands. This can be achieved by using a wider gel comb and running the gel at a lower voltage.
- You will want to have enough space around each band to cut without having DNA in other lanes contaminating your sample. To accomplish this, it is best to skip lanes between samples and between the ladder and nearest sample.
- To minimize the risk of DNA damage, it is best to limit the UV exposure of the DNA. Therefore, it is a bad idea to use a gel imager to take a picture of the gel before cutting out the bands and you will want to use long-wavelength UV for as short a time as possible to get the bands cut out.

2 Once you have run your gel, move it to an open UV box.

be sure to wear proper UV protection - especially for your eyes!, remove it from any gel tray as plastic will block much of the UV and with a clean, sterile razor blade, slice the desired DNA fragment from the gel.

Notes:

- To protect the UV box, it is a good idea to place the gel on a glass plate if available. Unlike the plastic tray, this will not significantly reduce the UV, but will protect the UV box from being cut by the razor blade.
- Try to get as little excess gel around the band as possible. To do so, it is often important to take the excised band, lay it down on the UV box and trim the top, bottom and sides with the razor blade. This is especially important during the DNA purification step, as many kits cannot handle more than a certain total volume of gel per reaction.

3 Place the gel in a labeled microfuge tube.

4 Using a scale, weigh the tube with the gel fragment after zeroing the scale with an empty tube. Alternatively, you can just subtract the weight of the empty tube from the weight of the tube with the gel fragment. The weight of the gel is directly proportional to its liquid volume and this is used to determine how much of each buffer to add during the DNA isolation step.

5 Finally, you will want to isolate the DNA from the gel. This is most commonly done with a commercial gel purification kit, such as the QIAquick Gel Extraction Kit. Always follow the manufacturer's instructions.

It is usually important to determine the concentration of the DNA that you isolated before proceeding to your next intended step with the now gel purified DNA. Find more information about DNA quantification here.