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Gel Purification

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Abstract

Gel purification is used to recover DNA fragments after electrophoretic separation. DNA recovery from an agarose gel includes three basic steps: binding, washing and eluting from a silica column. DNA is believed to bind to silica in the presence of high salt via a salt bridge. Following binding, DNA is washed of impurities and eluted under low salt conditions disrupting this interaction.

This video goes through a step-by-step, generalized procedure for cutting out a band from the gel, gel solubilization, purification through binding to a silica column, and elution of purified DNA. In addition, the presentation discusses several tips for ensuring successful gel purification, including the importance of running an agarose gel with a marker or ladder that has DNA of known sizes.

Transcript

Gel-purification is a standard procedure performed to recover desired DNA fragments from agarose gels after electrophoretic separation. After dissolving the gel fragment and running it through a specialized filter, this procedure yields DNA freed from impurities such as salts, free nucleotides and enzymes, suitable for downstream applications.

The basic principle behind DNA recovery from agarose gel involves a sequence of bind, wash, and elute steps. Once the gel is in solubilizing buffer, it is applied onto a “spin column,” which, upon centrifugation, allows DNA molecules to selectively bind to a silica-filter while the impurities flow through into a collection tube.

DNA is able to bind to silica thanks to a high salt concentration in the gel solubilization buffer. This buffer is believed to disrupt the hydration structure around the filter and create a cation salt bridge between the strong negative charges on the filter and negative charges on the DNA. Residual impurities are removed by washing with ethanol.

Water or low salt buffer is added to the column and will “elute,” or free, the DNA from it, presumably by disrupting the cation bridge. The DNA is now purified from the gel.

The first step in the gel purification procedure involves casting the agarose gel and performing electrophoresis of the DNA samples. Once the gel-run is complete, desired DNA fragments are visualized against UV light and fragments are selected after comparing against a molecular weight standard.

If the gel is unstained, the band location can be approximately determined based on a comparison to the DNA ladder. While cutting the gel with a razor blade, one must take care to recover as much DNA as possible with as little agarose as possible.

When handling ethidium bromide stained gels and working in front of UV light, gloves and protective eyewear should be used. After cutting the desired DNA from the gel, dispose of the gel and running buffer properly, in compliance with institutional safety protocols.

Once isolated, the piece of gel is placed in a microfuge tube and weighed on a balance. Using the approximation that 100 mg of gel occupies 100 l, a volume of solublilization buffer that is 4X the gel weight is added to the gel piece. After being placed in buffer, the gel piece is incubated at around 50 C to melt the agarose.

Once melted, the solubilized gel is added onto a spin column and the solution is centrifuged, which will cause all of the DNA and other particulates to stick to the filter.

Next, the bound DNA is washed by adding 70% ethanol to the filter, followed by centrifugation, which will remove residual impurities from the filter. Flow through is discarded, and this washing step is then generally repeated up to three times. The empty filter is spun again to remove residual ethanol, and the silica filter is allowed to dry at room temperature. Water or elution buffer is added to the filter, and with another round of centrifugation, purified DNA is collected in the bottom of the tube.

The method you’ve just seen applies to gel purification with silica spin column filters. Other methods exist that make use of the same basic principles of DNA binding to silica followed by washing and elution steps. For example, silica can be mixed with DNA in a suspension called “glassmilk,” which can be pelleted and washed, and later eluted. Also, suction can be used to pull DNA through silica filters and, later, elute it. Be sure to understand your lab’s gel purification procedures.

Now that you have learned how to recover DNA from agarose gels, let us examine a few downstream applications that use DNA obtained from gel-purification.

For example, gel-purification is an intermediate step in Chromatin Immunoprecipitation, a technique that aims to isolate the regulatory proteins that bundle genomic DNA, in order to identify which sequences are being regulated. The isolated fragments are gel-purified and sequenced, in order to be mapped onto the individual chromosome regions.

Subcloning - the process of moving a gene in one vector to another - can involve gel purification. For example, gene sequences from one vector can be digested from one construct, and assembled into chimeric sequences via PCR, after which they are gel purified and put into other constructs.

Perhaps the simplest application of gel-purification is its use after long-term storage at -80 ˚C of excised DNA bands following electrophoresis.

You have now learned how to extract DNA fragments from agarose gel, the variations of bind, wash, and elute procedures followed as per individual user-preference, and finally some of the possible downstream applications of this method. As always, thank you for watching.