Gel Purification of PCR Products

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- ***What is gel Purification of PCR Products?***

In molecular biology, gel extraction or gel isolation is a technique used to isolate a desired fragment of intact DNA from an agarose gel following agarose gel electrophoresis. After extraction, fragments of interest can be mixed, precipitated, and enzymatically ligated together in several simple steps. This process, usually performed on plasmids, is the basis for rudimentary genetic engineering.

- ***How does it run?***

Fifty µl of the PCR products will be separated on 2% agarose gel and then the expected bands will be purified using QIAquick Gel Extraction Kit (Qiagen, Germany). The DNA fragments will be excised from the agarose gel with a clean, sharp scalpel. The gel slices will be weighed in a 1.5 ml microcentrifuge tube. Then 3 volumes of buffer QG to 1 volume of gel will be added (100 mg ~100 µl). The tubes then will be incubated at 50 °C for 10 minutes, to help dissolve the gel, every 2-3 minutes the tubes will be vortexed during the incubation. After the gel slices become dissolved completely, 1 gel volume of Isopropanol will be added to each tube. Later, one QIAquick spin column will be placed in a provided 2 ml collection tube. To bind DNA, the sample will be applied to the QIAquick column and centrifugation at 17,900 x g will be performed for 1 minute and the flow through will be discarded. Next, 750µl of Washing Buffer (buffer PE) will be added to the column and centrifuged at 17,900 x g for 1 minute. The supernatant will be discarded and the column will be centrifuged again at 17,900 x g for 1 minute to dry the membrane. The DNA will be then eluted by adding 30µl of the Elution Buffer directly to the centre of the column, letting it stand for 1 minute and then centrifuging for 1 minute at 17,900 x g.

- ***Some tips for better gel extraction results***

1. **Trim the gel slice as much as possible**. Get rid of all the excess gel including that in front of or behind the DNA.

2. **Minimize exposure on the UV light.** The UV light causes DNA damage that can impact the clonability of the DNA. Cutting of the gel slice should be done quickly. If there are multiple bands to trim, it is suggested to work with one band at a time on the UV.

3. **Change to a new brand or bottle of agarose**. Sometimes, for some reasons, agarose actually causes enzyme inhibition. It may be that the agarose is old and the quality is no longer good. This may even be the case with certain brands.

4. **Run controls to determine if the problem is actually the gel extraction step**. It is suggested to run a control where empty vector cut with a single enzyme is digested and then the gel extraction is performed and again religation is performed. A vector cut with one enzyme should religate very easily and provide plenty of colonies on the plate.

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BA: benzyladenine; PSI: photosystem I; WT: wild type

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Acknowledgements

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A chapter in a book:

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A Report:

Makarewicz, J. C., T. Lewis and **P. Bertram.** 1995. *Epilimnetic phytoplankton and zooplankton biomass and species composition in Lake Michigan 1983-1992.* U.S. EPA Great Lakes National Program, Chicago, IL. EPA 905-R-95-009.

Conference proceedings:

Stock, A. 2004. 'Signal transduction in bacteria'. Proceedings of the 2004 Markey Scholars Conference, pp: 80-89.

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