



Optimization of quality and quantity of DNA recovery from an agarose gel

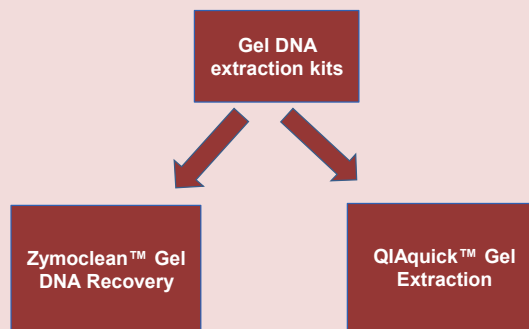
Kevon Seay, Varsha C. Anche, Stylianos Fakas,
Department of Food & Animal Sciences, Alabama A & M University, Normal, AL 35762

Abstract

Gel purification is a procedure used to isolate and purify linear DNA fragments from an agarose gel. These DNA fragments are produced either by PCR or restriction digestion reactions and can be further used for restriction enzyme-based cloning. The efficiency of a gel DNA purification method depends on several factors, including the initial DNA concentration, the agarose content of the gels, weight of the excised agarose gel with DNA sample, sample incubation time and temperature with dissolution buffer, volume, and temperature of the buffer and type of kit utilized for extraction process. Although gel DNA recovery is a widely used procedure, there is a need to optimize the protocol for a maximized DNA yield. The main objective of this study was to optimize the protocol for improved yield of DNA from agarose gels. Gels containing 0.8-1% agarose gel were used to separate the linear fragments by electrophoresis. Following electrophoresis, the DNA bands on the agarose gel were visualized under UV light and the desired DNA fragment was excised from the gel. The excised gel was processed with Zymoclean™ Gel DNA Recovery kit and QIAquick™ Gel Extraction Kit DNA recovery kits using the manufacturer's protocol. The quality and quantity of the eluted DNA from the two kits was assessed using Nanodrop and the efficiency of the gel extraction procedure was estimated by comparing the quantity of the DNA analyzed by gel electrophoresis and the quantity of the DNA extracted from the agarose. From the preliminary results, 90ng of DNA was recovered out of the 1000ng of DNA loaded on the gel using the Qiagen Kit, indicating that there was only 10% recovery of the DNA loaded on the agarose gel.

Materials & Methods

Experimental Design



Results

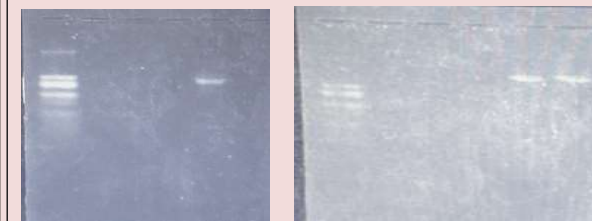


Fig A. Gel images of the DNA prior to the gel excision

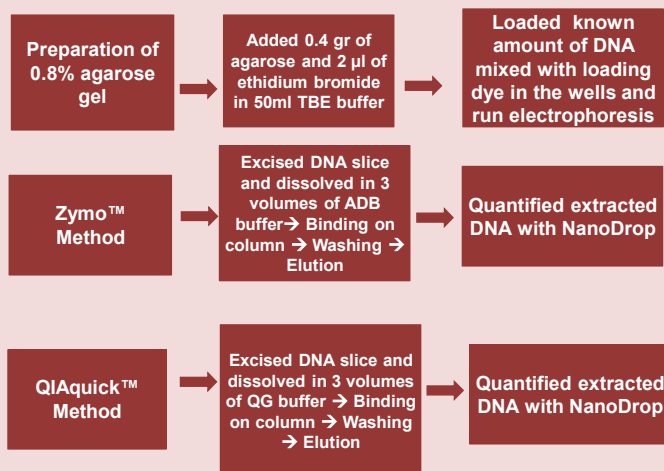
DNA quantification

Nucleic acid concentration (Zymo™) = 85.1 ng/μl
Nucleic acid concentration (Qiagen™) = 0

Introduction

- Gel extraction, or gel purification, is a process in which a fragment of DNA is isolated from agarose gel (Addgene, 2022).
- This process involves the extraction of DNA for the downstream applications like restriction cloning, ligation, transformation etc.
- The purpose of gel extraction is to recover as much DNA as possible for further use.
- Generally, four methods are used for gel extraction: electroelution, elution by diffusion, gel dissolution, and extrusion of DNA by gel compression. The choice of the method depends on several factors, such as the type of **gel matrix and DNA size** (Smith, 2004).

Methodology



Conclusions

- In comparison to the Qiagen™ kit, Zymo™ Kit showed more promising results in terms of DNA quality and yield from the agarose gels.

References

- Smith, H. O. (2004, January 7). [46] Recovery of DNA from Gels. Methods in Enzymology. <https://www.sciencedirect.com/science/article/abs/pii/S0076687980650484>
- Purifying DNA from an Agarose gel. Addgene. (2022). <https://www.addgene.org/protocols/gel-purification/>

Objectives

- The objective of this study was to evaluate commercial gel extraction kits to improve the quality and quantity of the DNA extracted from the gel.

Acknowledgements

- NSF HBCU- Excellence in Research grant 2100980
- Food Biotechnology Lab members