Plasmid Midiprep: A Method to Purify Plasmids for Recombinant DNA Studies

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Introduction: A fundamental aspect of molecular biology involves exploring the properties and functions of specific genes. The rise of recombinant DNA technology has vastly improved and simplified functional studies by allowing scientists to isolate specific genes using restriction enzymes and plasmids providing greater precision. First discovered naturally existing in bacteria providing additional survival advantages, plasmids have grown to be a fundamental element of recombinant DNA technology. Plasmids take great significance in downstream studies, which is why quantity and quality of the plasmids purified is important.

Objective: To isolate and purify recombinant plasmids in microgram quantities, and confirm the yield, quantity and quality using spectral and size fractionation methods. Finally, to assess the plasmids for downstream studies.

Methods: An *E. Coli* colony transformed with recombinant plasmids were grown in Luria Agar (LA) and later single colony selected and propagated in Luria Broth (LB) media at 37^{o}C under appropriate antibiotic selection. A Promega Midiprep kit was used to isolate plasmid DNA from overnight grown culture, further purified using phenol chloroform method. Concentration and quality were analyzed using Nanodrop. Agarose gel electrophoresis was employed to visualize and estimate the size. The plasmids were transfected into mammalian cell lines to observe GFP expression under a fluorescence microscope.

Results: The isolated plasmids were isolated with a good yield ranging from 1-1.5 mg/ml. The quality, which is a ratio of A260/A280, was in a range of 1.8-1.9 confirming it to be high quality plasmids with little or no impurities. The spectral analysis was performed using Nanodrop. The high yield and purity suggest the Promega Midiprep kit is effective in producing high quality plasmids. Agarose gel electrophoresis confirmed the size, integrity, and concentration. The multiple bands on the agarose gel represented different forms of the plasmid DNA (covalently closed circular, nicked, and linear). Mammalian cells transiently transfected with the plasmids showed GFP expression under the fluorescence microscope.

Discussion: The maintenance of high purity and integrity are the main criteria of sophisticated and highly purified plasmid samples. We isolated and purified recombinant plasmids for transient transfection and used GFP to confirm the expression levels of the target gene. Further

high scale techniques like, CsCl gradient are also used for plasmid purification. We need to optimize for high concentration, integrity of the plasmids, while minimizing contamination.

Conclusions: The plasmids that were isolated and purified showed high quantity and quality. However, one sample did show a contaminant when analyzed on a gel. The samples used for this study can be applied for downstream investigations involving molecular assays for disease risk management or treatment.

Source of funding: Development grant to MKT, from School of Medicine, UTRGV, TX.
Acknowledgements: Dr. Subhash Chauhan (Chairman), Dr. Neeraj Chauhan (Assistant Research Scientist), Benilde Adriano.