

Purification of plasmid DNA from *E.coli* bacteria cells and DNA gel electrophoresis

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Abstract

The main purpose of this experiment was to learn how to use a commercial kit for the purification of plasmid DNA from *E. coli* and run agarose gel electrophoresis after. Once the gel results were obtained, it could be seen that plasmid 3.3 was the purest, while plasmids 3.1 and 3.4 were the most contaminated.

Introduction

The purification of plasmid DNA is one of the most crucial techniques in biochemistry. This method allows scientists to measure the DNA concentration accurately and use it in cloning, protein synthesis, gene therapies, and much other influential scientific research.

During purification, a bacterial cell is lysed making the DNA free. Then, the lysate is cleared and purified on the QIAprep silica membrane. In this experiment, the following material was used for the *E.coli* cells containing plasmid pSpCas9(BB)-2A-Puro (PX459) V2.0 purification: buffer P1, P2, N3, PE, EB, QIAprep Spin Columns, and Buffer TE. The P1 buffer is necessary for the cell's resuspension, while buffer P2 is used to open the cell wall. Buffer N3 is necessary to stick the DNA to the silica gel membrane in the small column. PE buffer is used to keep the plasmid DNA on the column and wash it. Finally, the EB buffer is responsible for eluting the DNA from silica salt. Thus, all these buffers are sufficient for the plasmid DNA purification. However, it is hard to determine if the plasmid has been obtained by the naked eye. In this case, a 1% agarose gel is used. Nowadays, gel electrophoresis is the most effective technique for DNA fragment separation. The procedures include loading the DNA into the well in the gel and applying a current. Since the phosphate backbone of the DNA is negatively charged, fragments will migrate to the positively charged anode. However, the charge is not the only aspect

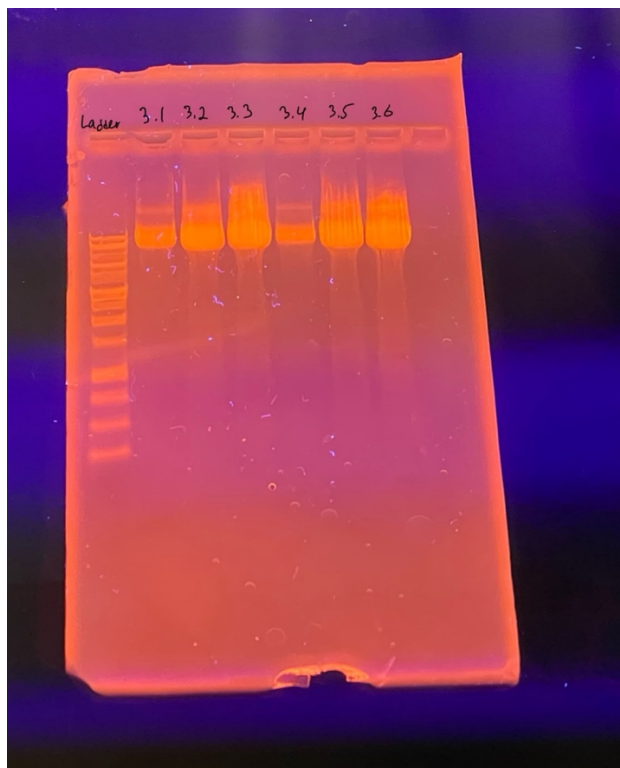
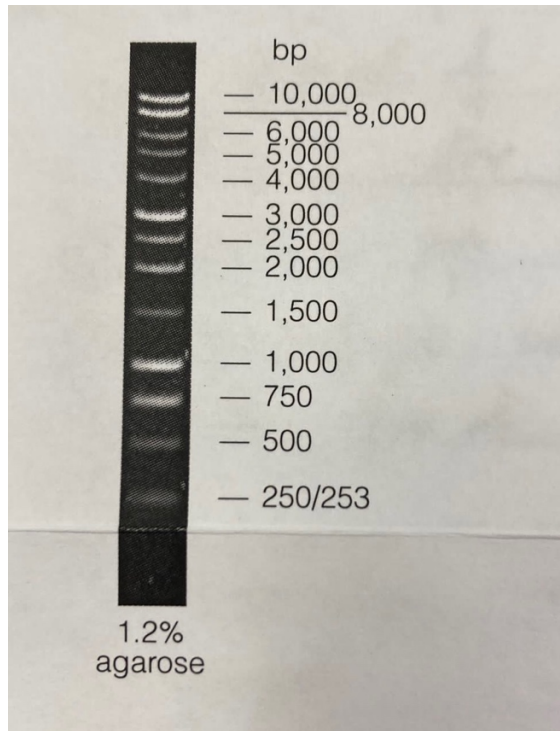
by which the DNA molecules can be separated- it also includes separating the molecules by size as smaller molecules migrate faster. Thus, based on obtained results from the gel electrophoresis, plasmids 3.1 and 3.4 were relatively larger than plasmids 3.3, 3.5, and 3.5 and they traveled the smallest distance.

Procedures

First, *E. coli* cells with plasmid pSpCas9(BB)-2A-Puro (PX459) V2.0, buffer P1, P2, N3 PE, and EN from Qiagen, QIAprep Spin Columns, and buffer TE were obtained. After that, the *E. coli* cells were centrifuged for 2-3 minutes at 13,000 rpm. The next step included resuspending pelleted bacterial cells using 250 microliters of buffer P1. Later, 250 microliters of P2 were added to the tube, and it was gently inverted 6 times for mixing. Once the process was done, 350 microliters of buffer N3 were added. The tube was inverted again 6 times and then centrifuged for 10 minutes at 13,000 rpm. When the centrifuging was over, the supernatants from the previous step were applied to the QIAprep column using a micropipette, and the mixture was centrifuged. After centrifuging, the flow-through was discarded into biological hazard waste. Later, the QIAprep spin column was washed with 0.75 ml of buffer PE and centrifuged for 1 minute. The flow-through was discarded, and the mixture was centrifuged again for 2 more minutes to the removal of residual wash buffer. Next, the QIAprep column was placed in a clean 1.5 ml microfuge tube, and, to start the DNA elution, 50 microliters of buffer EB were added. The mixture stood for 2 minutes and then was centrifuged for another 2 minutes. Once the preparation procedures were done, a 1% agarose gel was used to determine if the plasmids have been gotten. The plasmid DNA was saved and

the relative amount of it from the intensity of the EB fluorescence in the gel was estimated.

Results



Discussion/ Conclusion

Based on the obtained gel electrophoresis results, plasmid 3.3 had the highest quality, while plasmids 3.1 and 3.4 were damaged. Plasmids 3.2, 3.5, and 3.5 were relatively pure, but still obtained contaminations.

In general, the gel electrophoresis results should give 2 bands: the major and the minor. The major band is responsible for the representation of pure and high-quality supercoiled plasmid. Usually, this band migrates faster. On the other hand, the minor band represents a tiny species of plasmid with nick, single-strand break, which means that a plasmid is damaged. Usually, a minor band migrates slower.

Thus, since plasmid 3.3 showed only one strand, which was the major band, it can be concluded that the obtained plasmid is pure. Inversely, plasmids 3.1 and 3.4 are contaminated as 2 strands can be distinctly visible. Next, looking at plasmids 3.2, 3.5, and 3.6, two strands can be noticed as well. However, compared to samples 3.1 and 3.4, only the traces of the minor bands can be visible. As follows, those plasmids are partially contaminated.

In addition, plasmids 3.2, 3.3, 3.5, and 3.6 have a smaller size than plasmids 3.1 and 3.4 as they migrated further.

The possible source of error could be partial removal or damaging of a plasmid DNA from the column by touching the liquid with the micropipette's tip during the process of buffer addition. To avoid the same mistake in the future, a tip of a micropipette should not be submerged into the column with a sample.

Overall, the experiment was completed successfully, and the basic skills of purifying a plasmid DNA were obtained, as well as the ability to use the gel electrophoresis and read its results.

References

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Notebook

Lab 2 Plasmid Purification

- P₁ - resuspend

RNAse A - breaks down RNA

- P₂ - open/lyse the cell wall

NaOH

- N₃ - neutralize NaOH & G-lycero^{salt} chloride, which denatures proteins

- EB - elutes the DNA from the silica gel

• PE - washes DNA

- Source of error - plasmid could be removed

- Gel - pictures

Centrifuge - #3