University of Toronto

Faculty of Applied Science and Engineering

BME440 Biomedical Engineering Technology and Investigation

Lab 3: PCR Amplification & Gel Electrophoresis

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1.0 Introduction & Scientific Objective

Polymerase Chain Reaction (PCR) is a laboratory method that is used to amplify small amounts of DNA sequences exponentially in order to create more copies for analysis [1]. Agarose Gel Electrophoresis is a technique that is used to separate DNA and RNA fragments by size [1]. Agarose Gel Electrophoresis method is commonly applied to evaluate PCR reaction success, along with intercalating agents or dyes to visualize the amplified fragments [2]. In addition , the use of positive controls that consist of a segment of DNA of known size, preferably of the same size as the target of amplicon, are necessary to provide a reference point for the expected outcomes [3].

The specific problem that will be addressed in this lab is the presence or absence of the Alu sequence within the PV92 locus on chromosome 16 in the genomic DNA of the individuals. The scientific objective of this lab is to determine whether the template male and female genomic DNA is heterozygous or homozygous based on the Alu sequence at the PV92 locus, by using PCR amplification and Agarose Gel Electrophoresis to visualize the amplification products and positive controls to compare. Through using PCR and Agarose Gel Electrophoresis in this experiment, we expect to observe that if the homozygosity is present for the Alu sequence to display a single band at 941 base pairs, if absent to display a single band at 641 base pairs and if heterozygous to exhibit both 641 and 941 base pair bands on the gel.

2.0 Materials and Methods

In this lab, we started by collecting DNA samples from our cheek cells by swishing a saline (NaCl) solution and using a mouth swab. We then transferred 200 μL of saliva to a tube and spun it down using a centrifuge at 5000 RPM for 5 minutes. We then discarded the supernatant, added 200 μL of the InstaGene solution, incubated our sample at 90°C for 10 minutes and centrifuged the sample once again.

The reagents that were used in this lab were: MiliQ Water, PCR Mastermix consisting of Taq Polymerase, MgCl2, DNTPs and primers; DNA samples and PV92 positive controls. To prepare the PCR reaction, we added the reagents into 6 tubes (please refer to the lab manual for exact concentrations), spun them using the microfuge and incubated them in the Thermocycler for 90 minutes. We then proceeded to cast an Agarose Gel, as we waited for the PCR reaction to be complete. We dissolved 2 0.5g Agarose tablets in 100 mL of 0.5x TAE buffer, microwaved it for 1.5 minutes and cooled it down before pouring it into the gel cast tray. Once the gel was placed into the electrophoresis chamber and the PCR was completed, we mixed the samples with the loading dye and loaded each sample and the DNA ladder into separate wells and ran electrophoresis at 135 V for 25 minutes. Once complete, we removed the gel and recorded images of the DNA bands. Due to a technical issue with the Thermocycler, causing the PCR to fail, we weren't able to obtain any results. Therefore, a provided example gel will be used instead, for the results and discussion sections of this report.

3.0 Results

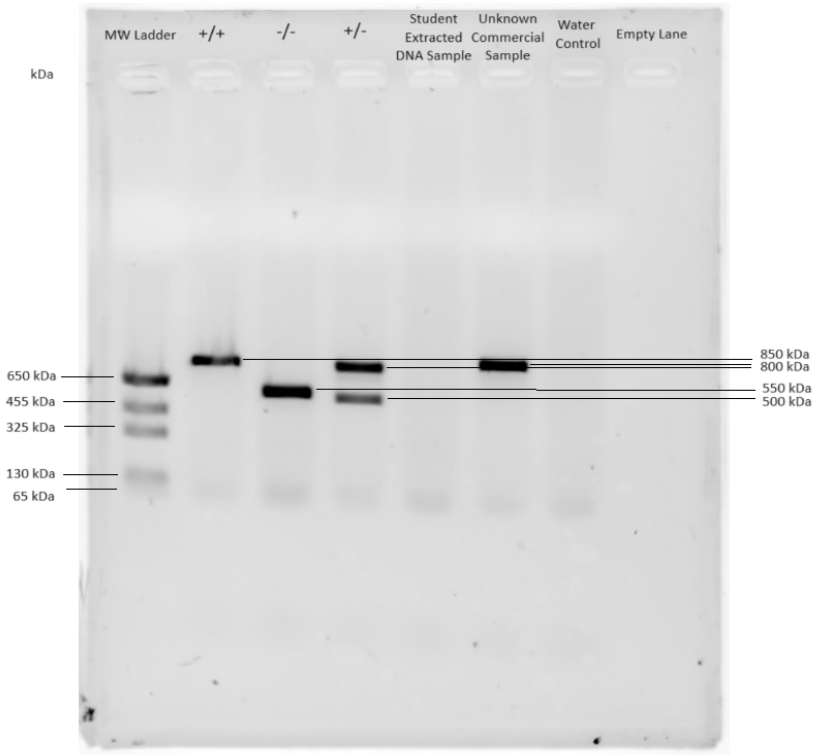

Figure 1: Agarose Gel Electrophoresis of PCR amplification of the PV92 locus on chromosome 16 in DNA samples, in order to detect Alu sequence presence.
MW ladder contains 10µL of Bio-Rad EZ Load Precision Molecular Mass Ruler. All except MW ladder contain equal amounts of water (4.5µL) and PCR Mastermix (5µL). All positive controls contain equal amounts (0.5µL) of the corresponding positive control DNA with homozygous for the presence (+/+), absence (-,-) and heterozygous (+/-) of the Alu sequence at 92 locus. Student extracted and commercial DNA samples contain equal amounts (0.5µL) of their respective DNA. Electrophoresis was carried out at room temperature and was run at 135V for 25 minutes.

The lanes, from left to right, MW ladder (Bio-Rad EZ Load Precision Molecular Mass Ruler) formed bands at 650 kDa (1,000 bp), 455 kDa (700 bp), 325 kDa (500 bp), 130 kDa (200 bp) and 65 kDa (100 bp); a positive control sample with +/+ (homozygous presence of Alu at PV92) genotype formed a band approximately at 850kDA (1308 bp); a positive control sample with -/- (homozygous absence of Alu at PV92) genotype formed a band approximately at 550kDA (846 bp); a positive control sample with +/- (heterozygous for the PV92 locus) genotype formed 2 bands, one at approximately 800kDA (1231 bp) and one at approximately 500kDA (769 bp); student extracted DNA sample formed no visible bands; unknown commercial DNA sample formed a single band at at approximately 800kDA (1231 bp); water control and empty lane formed no visible bands.

4.0 Discussion

Positive and negative controls are needed to ensure the validity and the reliability of our results [3]. In this experiment, the positive controls contained DNA with known genotypes, which served as a reference to what bands should look like if the Alu sequence is present or absent, thereby helping us to interpret the patterns in the samples. Having negative controls help with troubleshooting, as they indicate if the bands are appearing due to the sample DNA or other factors. The negative controls, water control and empty lane, did not show any bands as expected, suggesting that there was no DNA contamination and the reagents were clean. In our experiment the positive controls, although bands did not align with the expected base pair lengths, worked as expected since they produced bands indicating that the PCR and electrophoresis procedures were fundamentally operational and provided a reference for our samples.

The unknown commercial sample produced a single band at approximately 800kDA (1231 bp). Although this band does not align with the expected 941 bp size, by comparing the band pattern with the positive controls, we observe that it is the most similar to the (+,+) control, which corresponds to the sample with homozygous Alu sequence presence at the PV92 locus.

Neither our positive control nor the commercial DNA sample matched the expected molecular weight values of 941 and 641 bp since we observe band formations at around 1308 and 846, using the MW ladder as a reference. Since we observe the same discrepancy across all bands with DNA, this is likely either due to PCR issues in the amplification process, which may lead to unexpected sizes, or inaccuracy of the ladder. To improve the results, we can optimize the PCR conditions by ensuring primers are specific to the target and that the reagents have not expired.

If I had to run this experiment again, I would ensure the thermocycler is calibrated by running a test PCR with known samples. I would ensure that the student-extracted samples were of good quality by using NanoDrop to measure the purity of the DNA [4]. I would adjust the PCR process by optimizing the concentration of the reagents in the PCR Mastermix and the annealing temperature [5]. I would also ensure that the MW ladder has been stored properly and not expired.

In the example gel, no bands showed up for the student extracted DNA sample. This could be due to insufficient cell concentration during the extraction process, or the loss or degradation of DNA during the purification steps. If the concentration of the DNA is too low, we would not observe bands on the gel if even the PCR is run successfully. Another potential deviation from the protocol could be issues with the PCR reaction due to the nonspecific primer to that sample, inhibitors in the sample, or issues with other reagents such as Taq polymerase or dNTPs. This deviation would affect the results, as a poor PCR reaction would not amplify the DNA fragments as desired, resulting in no visible bands for analysis.

Our key finding from this experiment is that the commercial DNA sample was homozygous for Alu sequence presence at the PV92 locus. Our initial hypothesis was not confirmed due to issues with the experimental procedure and discrepancies in the molecular weight of the observed bands. However, we were still able to detect the Alu sequence presence in the unknown sample with the use of the positive controls. Therefore we can conclude the importance of equipment calibration, sample preparation and PCR condition optimization when running an experiment.

5.0 References

[1] 2023-2024 BME440H Lab 3: PCR Amplification & Gel Electrophoresis, Lab III, University of Toronto, Department of Biomedical Engineering, 2023.

[2] S;, W. P. Agarose gel electrophoresis to assess PCR product yield: Comparison with spectrophotometry, fluorometry and qPCR https://pubmed.ncbi.nlm.nih.gov/35311378/#:~:text=Agarose%20gel%20electrophoresis%20is%20a,the%20concentration%20of%20the%20amplicons. (Accessed Nov 3, 2023).

[3] Quality control: Molecular diagnostics - who/OMS extranet systems https://extranet.who.int/hslp/who-hslp-download/package/501/material/217 (Accessed Nov 3, 2023).

[4] García-Alegría, A. M.; Anduro-Corona, I.; Pérez-Martínez, C. J.; Guadalupe Corella-Madueño, M. A.; Rascón-Durán, M. L.; Astiazaran-Garcia, H. Quantification of DNA through the NanoDrop spectrophotometer: Methodological validation using standard reference material and Sprague Dawley rat and human DNA https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7719535/#:~:text=One%20of%20the%20most%20commonly,process%20%5B3%2C%204%5D. (Accessed Nov 3, 2023).

[5]PCR setup-six critical components to consider https://www.thermofisher.com/ca/en/home/life-science/cloning/cloning-learning-center/invitrogen-school-of-molecular-biology/pcr-education/pcr-reagents-enzymes/pcr-component-considerations.html (Accessed Nov 3, 2023).