DNA Extraction, PCR, and Error Analysis for Mystery Meat Sample

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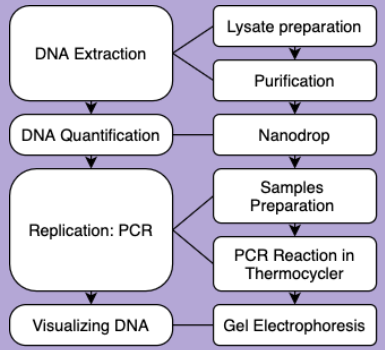
Introduction

1. Motivation: To identify the content of the mystery meat sample.
2. Purpose: To learn about performing the DNA extraction procedure and the PCR procedure, analyzing the data from the experiments and making error analysis of the process.
3. Primary Research Question: Can we use DNA extraction and PCR to extract and identify the species of the meat sample?

Materials

DNA Extraction	Lysate Preparation	Digestion Buffer
		Proteinase K
		RNase A
		Lysis/Binding Buffer
	Purification	Wash Buffer 1
		Wash Buffer 2
		Elution Buffer
		Collection Tubes with/without Spin Columns
PCR	Solutions	Standard Reaction Buffer
		dNTPs
		Taq DNA Polymerase
		MgCl2
		DNA Ladder
	Primers	Universal Forward
		Beef Reverse
		Chicken Reverse
	Thermal Cycler	

Protocol



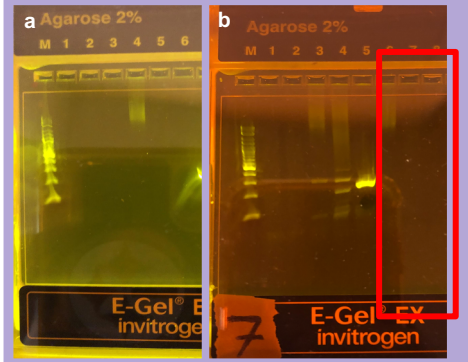
Demonstration

We basically followed the pipeline of DNA extraction & quantification, DNA amplification via PCR, and gel electrophoresis.
No significant procedural error was discovered when reviewing the recordings. However, there are some possible issues with the PCR component of the protocol:

- Water was added as our last component, this may have caused proteins and dNTPs to form a gel at the bottom of the sample tubes, which would cause PCR to be unsuccessful.
- The cocktail was not immediately iced after adding the Taq polymerase
- We only centrifuged the cocktail mix, but did not centrifuge each PCR tube

Result

DNA extraction result: 260/280 : 1.70
Reference: good if in range 1.65-1.85



a. Lane 1-6: first run - **Lane 1-3**: positive control **& lane 4-6**: sample with beef, chicken and pork primers respectively
b. Second run of sample DNA in **lane 6-8**

Error Report

1. PCR failed to amplify the DNA, which is possibly caused by the fact that we added water to the PCR tubes at the very end. The proteins and dNTPs may 'gelify' and clump together. We should add water before the DNA sample and primers.
2. We did not Immediately ice the cocktail after adding Taq polymerase, which might cause the Taq polymerase to degrade some of the reactants, which is undesirable. We should keep the reactants on ice before we start the reaction.
3. We centrifuged the cocktails but not each PCR tubes. In the future, all PCR tubes should be centrifuged.
4. Unknown incidents might have happened to the 6 PCR tubes because they were left unattended for 20 mins. We should keep an eye on the tubes at all time during the experiment.
5. No distinguishable results for all lanes can be caused by measuring error while mixing the cocktail. We should double check materials and measurement before adding them to the cocktail.

Analysis

The lanes containing the positive controls and the experimental samples with chicken and pork reverse primers did not display any DNA bands. In fact, there was no fluorescence at all, leading to the conclusion that these samples did not contain a significant amount of DNA.

The lane containing the experimental sample with beef primer did display some fluorescence, but not distinctly or in a clear band, so it, too, did not contain a significant amount of DNA.

These results are likely due to a failure to replicate DNA during PCR, since the DNA extraction results were within the desired range.

Conclusion

Due to the limitation of a lack of clear evidence from gel electrophoresis, we are unable to identify the species of the meat sample.

In future experiments, we would repeat this procedure, but we would be careful during the 'samples preparation' step to add water before adding the DNA or reverse primers, in order to prevent 'gel-ing' before PCR. We should also follow the protocols exactly and do some steps more timely. Additionally, we would be sure to double check all materials and measurements before adding them to the cocktail.

Reference: Thermo Fisher Scientific: Thermo-Scientific-NanoDrop-Products-Nucleic-Acid-Technical-Guide-EN.pdf; New England Biolabs Inc. : Protocol for a Routine *Taq* PCR