Optimization of mtDNA Extraction from Challenging Matrices



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INTRODUCTION

Obtaining high quality DNA is an essential process in molecular analysis, impacting the reliability of applications like amplification, sequencing and genetic profiling. Although many protocols exist for nuclear DNA, mitochondrial DNA (mtDNA) has emerged as a crucial alternative target in situations where nuclear DNA is degraded, scarce, or missing. While it offers benefits, extracting mtDNA from forensic and environmental samples poses considerable methodological difficulties, because samples often show signs of DNA degradation, fragmentation, and contain inhibitory substances which may hinder DNA extraction and Polymerase Chain Reaction (PRC) amplification. This study seeks to assess how key extraction parameters (lysis temperature, incubation time, lysis method, buffer formulation,extraction chemistry) affect the recovery of mitochondrial DNA from difficult biological matrices relevant to forensic and environmental applications, using commercially available extraction kits that focus solely on nuclear DNA extraction.

OBJECTIVES

- Compare exraction protocols
- Test and evaluate different conditions
- Modify protocols
- Evaluate PCR amplifiability

RESULTS

DNA EXTRACTION RESULTS & PCR

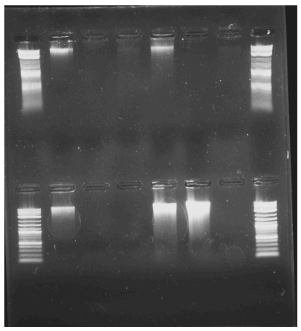
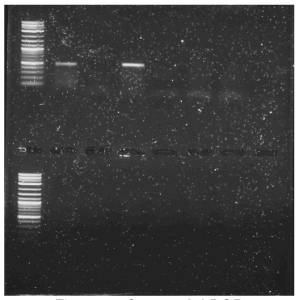


Figure 1: Animal tissue extraction using the NucleoSpin Kit.



Firuge 5: Sucessful PCR results for ITS2 and failed PCR attempts for Rbcl4 in plants.

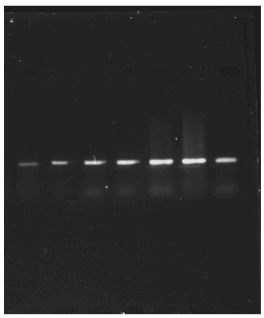


Figure 3: Sucessful PCR results for 16S in plants.

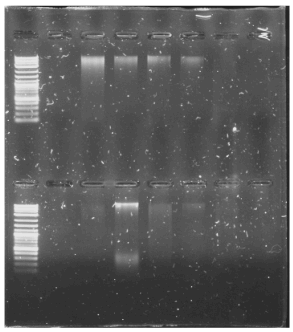


Figure 2: Plant tissue extraction using the PureLink kit under modified conditions.

SUCESS:
✓ 16S
✓ Dloop
✓ MHCI
FAILED:
✗ Rbcl4

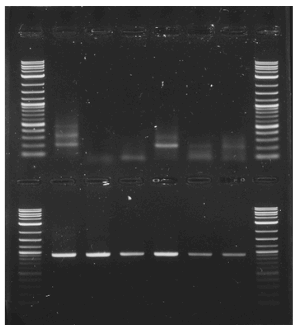


Figure 4: Sucessful PCR results for MHCI and Dloop in turtles.

METHODOLOGY

- Different sample types were collected
- DNA extraction was performed and protocol was modified where needed
- Performed PCR to determine sucessful mtDNA extraction

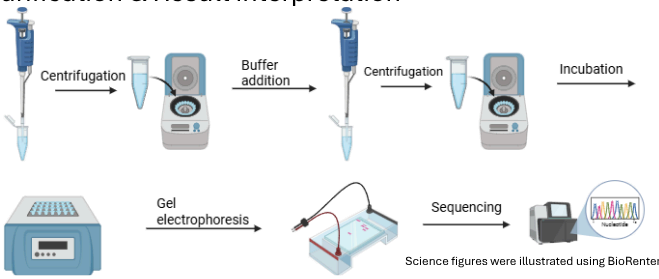
Sample Collection



Lysis



Purification & Result interpretation



Science figures were illustrated using BioRenter Illustration software

Kit	Application
NucleoSpin Tissue Extraction Kit (Macherey-Nagel)	Animal & Plant tissue
PureLing Genomic DNA Mini Kit (Invitrogen)	Modified Plant Extraction

SEQUENCING RESULTS

Marker	Sequencing Outcome
16S	Sucessful mitochondrial amplification
MHCI	82-90% sequence identity confirmed
Dloop	Sucessful mitochondrial amplification
ITS2	Sucessful nuclear amplification
Rbcl4	No sucessful amplification obtained

CONCLUSIONS

- Commercial kits can recover amplifiable mtDNA from degraded matrices
- Modified lysis conditions improved extraction efficacy
- PCR optimization is marker-dependable
- mtDNA analysis remains valuable in forensic and environmental applications
- Future work should focus on optimization of Rbcl4 primers, use of shorter amplicons, and expand to additional degraded matrices

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