Optimizing DNA Barcoding Protocols for Identifying Invertebrates

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Introduction

- Purpose of this research:
  - Efficiently identify organisms
  - Can be applied to all organisms
- Why invertebrates?
  - Important for the transfer of energy
  - Can tell the overall health of a population or ecosystem
- How?
  - We used a lab culture of the zooplankton species *Daphnia magna* to develop a robust protocol for DNA Barcoding zooplankton
    - Can be applied to other Crustaceans as well
  - Protocol for other organisms was modified using different primers

Methods

Sample Collection
- Collect organisms from lab cultures
  - *Daphnia magna* control
  - Lab Culture Unknown

DNA Extraction
- Follow Qiagen's DNeasy Blood & Tissue Kit Protocol, with modifications

PCR
- Make PCR master solution according to polyermase master solution
- Run PCR cycle according to modified recommended protocols
- Check PCR products with Gel Electrophoresis

Sequencing
- Clean PCR product and submit for sequencing

Analyze Sequence Results
- Use sequencing results to identify species through a DNA barcoding library

Search sequence in Bold Systems database for identification to the species level → Yes → Search complete
Search sequence in Bold Systems database for identification to the species level → No → Blast search sequence in GenBank database

Results

- DNA Sequencing Results for Positive Control and for Lab Culture Unknown (Figure 1 and Figure 2)
- We have developed the protocol for the DNA extraction of individuals from mixed cultures as confirmed by Nanodrop readings
- We have optimized the protocols for PCR master solutions and cycles as confirmed by Gel Electrophoresis
- We have identified individual organisms belonged to several different families in lab cultures and environmental samples (listed in Table 1)

Table 1:List of identified organisms using optimized protocols

Family	Source of sample(s)	Number of Organisms ID'd	Species
Daphniidae	South Carolina (Lab Culture), Keystone	4	*Daphnia magna*
Diaptomidae	Keystone	1	*Arctodiaptomus cf. dorsalis3*
Dogielinotidae	South Carolina (Lab Culture)	3	*Hyalella sp. Tbird, Hayaella azteca*

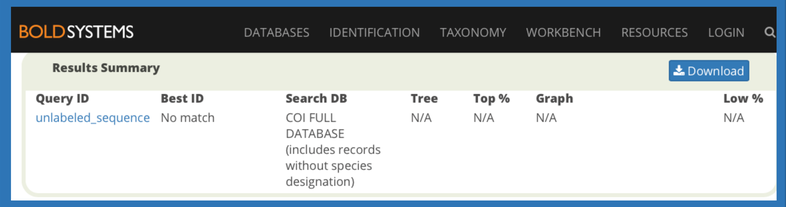

Figure 1: Bold Systems search results for Lab Culture Unknown

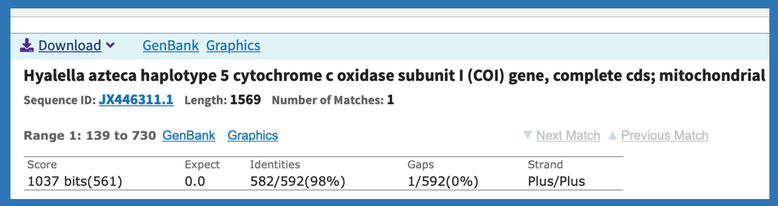

Figure 2: GenBank blast search results for Lab Culture Unknown

Conclusion

- We successfully developed a method including extraction, amplification, and analysis of individuals of different organisms.
- We are developing multiple protocols that works for different organisms, and have one completely optimized protocol that works efficiently for freshwater crustaceans.
- Protocols for organisms that are not freshwater crustaceans, such as horse hairworms and algae, are still being developed and optimized

Future Directions

- Optimize protocols for identifying:
  - All species in a mixed culture using next generation sequencing (NGS)
  - Species of Zooplankton from resting eggs.
  - Species of horse hairworms, earthworms, and cysts
  - Species of algae

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References

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