A Comparison of Isolated Brook Trout Mitochondrial DNA from Pennsylvania

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Eastern Brook Trout Health

Conservation Status based on historical range, current population status, habitat integrity and future security information

- **Historical Range**
- **Status Unknown**
- **Extirpated (no longer present)**

Conservation Status

- **Stronghold 80.1-90**
- **Healthy 70.1-80**
- **Moderate 60.1-70**
- **Poor 50.1-60**
- **Very Poor 18-50**

Map derived from TU's Conservation Success Index, a tool that incorporates federal, state and public data. See http://tucsi.spatialdynamics.com/ for more information. Brook Trout population data provided by the Eastern Brook Trout Joint Venture, www.easternbrooktrout.net.
Introduction

- Coal mining and abandoned mine drainage
  - Pyrite exposed in abandoned mines
  - Oxidation to sulfuric acid
  - Ground water contamination

http://www.tu.org/conservation/abandoned-mines/amd-101
Impacts of AMD on Aquatic Ecosystems and Brook Trout (*Salvelinus fontinalis*)

- **Aquatic Ecosystems:**
  - Poor water quality
  - Heavy metal contamination
  - Sedimentation

- **Brook Trout**
  - Isolated populations
  - Uninhabitable areas due to AMD
  - Genetic bottlenecks
  - Expected genetic variation
Methods of DNA Sequencing
Isolation

- Fin clips provided by Trout Unlimited
- Preserved in 70% ethanol
- Giagen Gentra PUREGENE® purification kit
Courtesy: Trout Unlimited
Amplification

- Polymerase Chain Reaction (PCR)
  - Rapid amplification
- D-loop region of mitochondrial DNA
  - 1000 base pair control region
- Primers designed from published DNA sequences
Verification

- 2% Agarose Gel Electrophoresis
  - Appearance of DNA bands verifies successful amplification
- NanoDrop ® Spectrophotometer ND-1000
  - Determine purity/concentration of DNA
Sequencing

- Di-deoxy preparation
- DyeEx Spin Kit purification
- Sequence using ABI 310 Genetic Analyzer
The diagram illustrates the Sanger sequencing process:

1. **Reagents**
   - Primer and DNA template
   - DNA polymerase
   - ddNTPs with fluorochromes
   - dNTPs (dATP, dCTP, dGTP, and dTTP)

2. **Primer Elongation and Chain Termination**
   - Primers are used to initiate the synthesis of DNA strands.
   - DNA polymerase extends the primers using dNTPs.
   - ddNTPs are added to the reaction mixture to act as chain terminators.

3. **Capillary Gel Electrophoresis**
   - DNA fragments are separated in a capillary gel based on size.

4. **Laser Detection and Sequence Analysis**
   - Laser detects the fluorochromes attached to the DNA strands.
   - Chromatograph displays the electropherogram, which is analyzed to determine the DNA sequence.
Results

- Initial primers yielded 500 base pair segment
- 99 to 100% homology with published genome
- Lack of sufficient data to determine possible genetic variations
Results Continued

• Current primers yielded 900 base pair segment
• Eight fish from five different locations have been sequenced
• Preliminary results show minor variations
Conclusions and Future Work

- Additional primer sets to analyze samples
- Increase sample size
- Expansion of project to include nuclear DNA