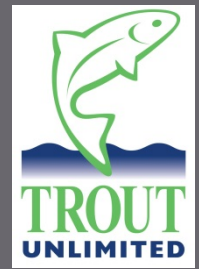




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A Comparison of Isolated Brook Trout Mitochondrial DNA from Pennsylvania

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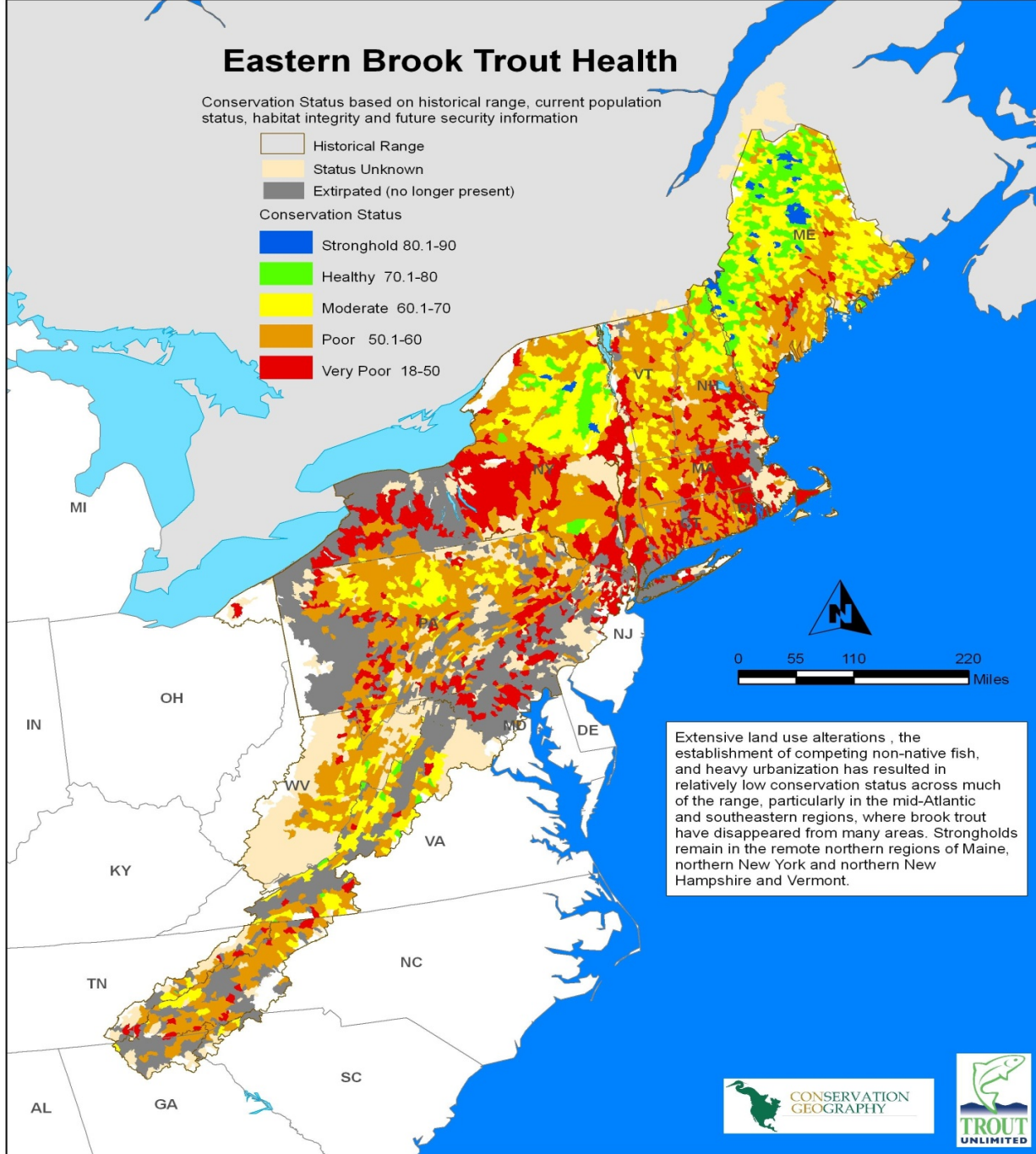
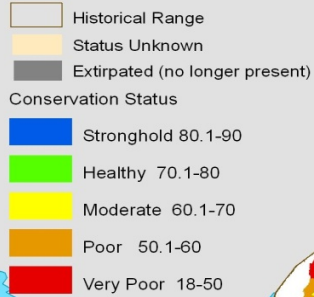
***Grove City College, **Trout Unlimited, Eastern Abandoned Mine Program**



Salvelinus fontinalis

Eastern Brook Trout Health

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



Extensive land use alterations, the establishment of competing non-native fish, and heavy urbanization has resulted in relatively low conservation status across much of the range, particularly in the mid-Atlantic and southeastern regions, where brook trout have disappeared from many areas. Strongholds remain in the remote northern regions of Maine, northern New York and northern New Hampshire and Vermont.



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

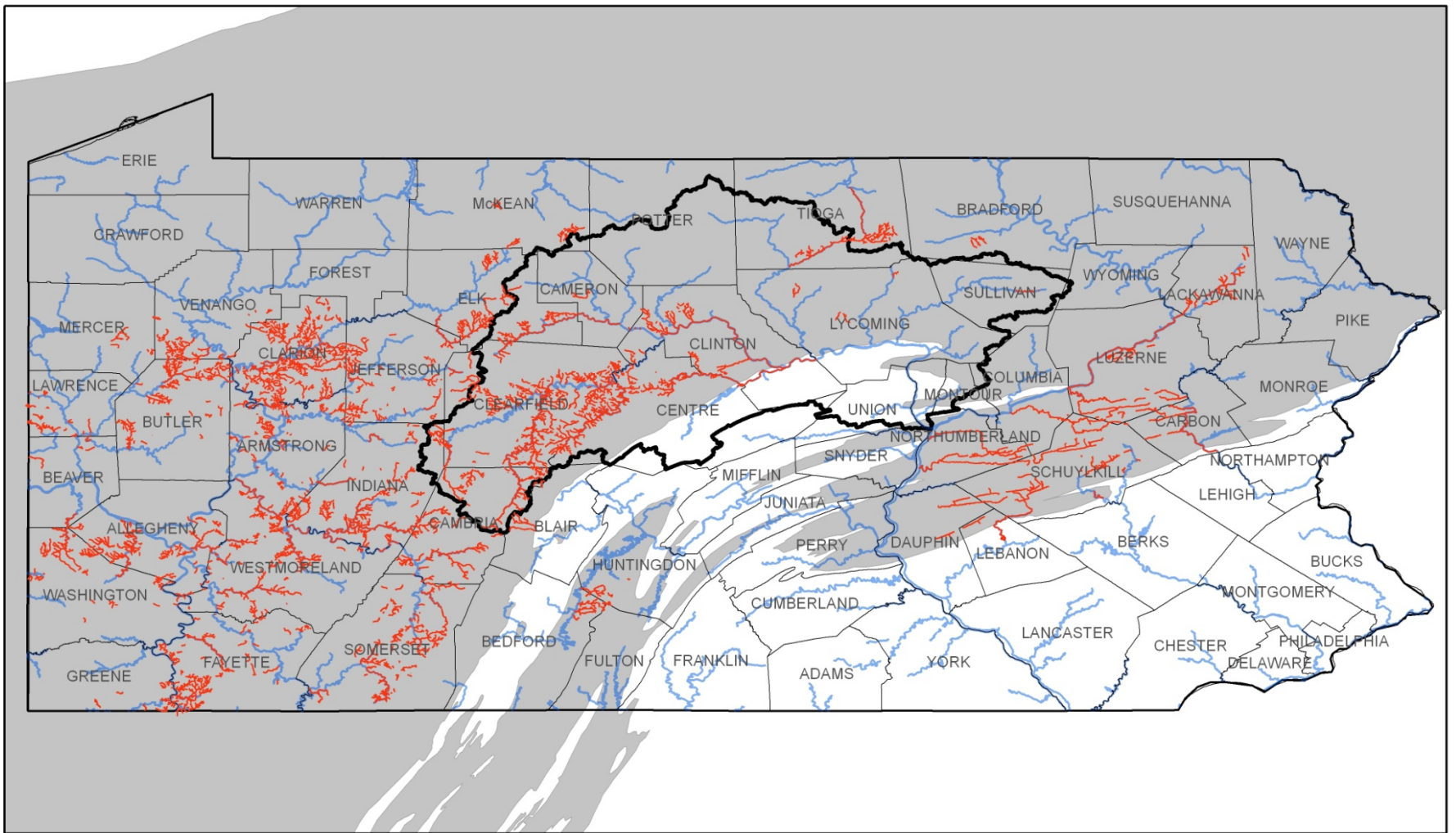
- **Coal mining and abandoned mine drainage**
 - Pyrite exposed in abandoned mines
 - Oxidation to sulfuric acid
 - Ground water contamination



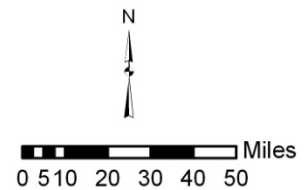


Impacts of AMD on Aquatic Ecosystems and Brook Trout (*Salvelinus fontinalis*)

- **Aquatic Ecosystems:**
 - Poor water quality
 - Heavy metal contamination
 - Sedimentation
- **Brook Trout**
 - Isolated populations
 - Uninhabitable areas due to AMD
 - Genetic bottlenecks
 - Expected genetic variation



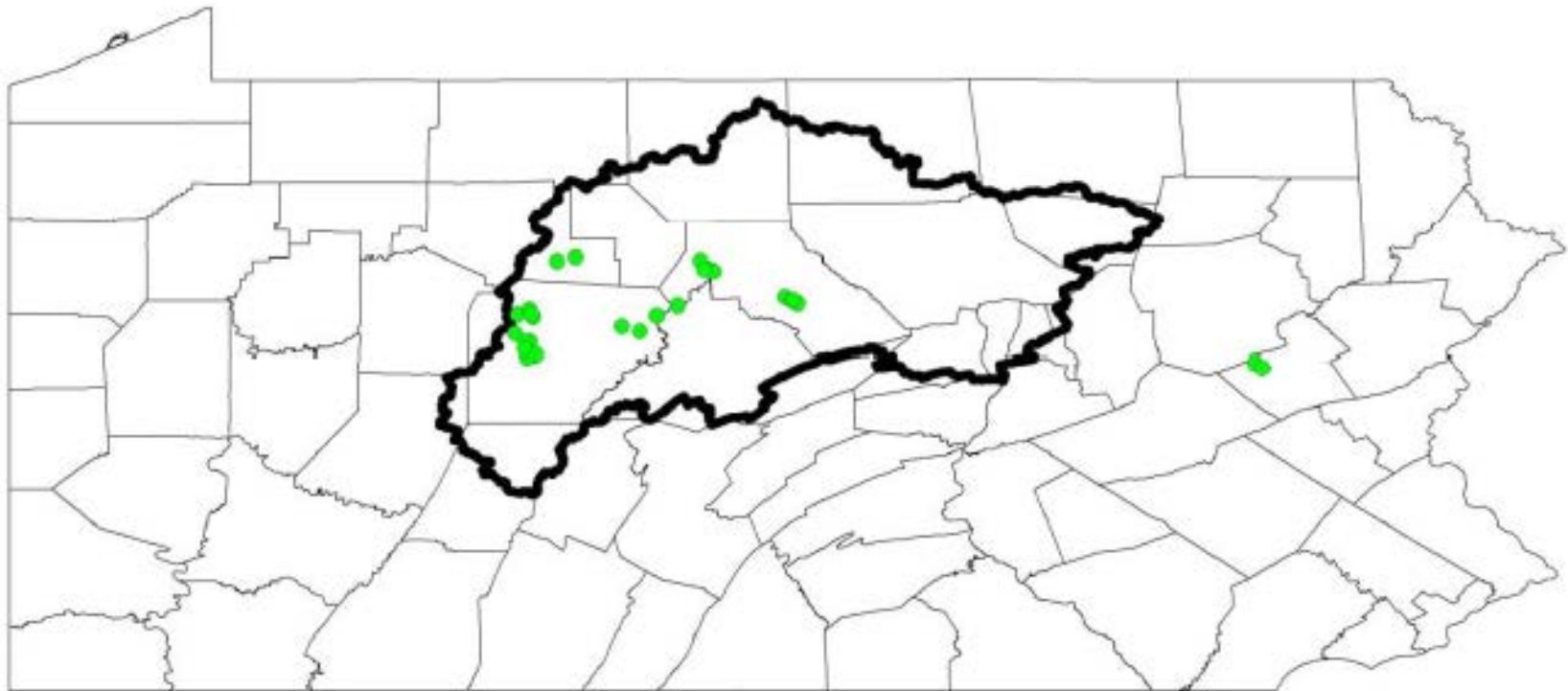
- AMD Impaired Streams
- West Branch Susquehanna Watershed
- Marcellus Shale



Methods of DNA Sequencing

Isolation

- **Fin clips provided by Trout Unlimited**
- **Preserved in 70% ethanol**
- **Qiagen Genra PUREGENE[®] purification kit**



0 25 50 100 Kilometers

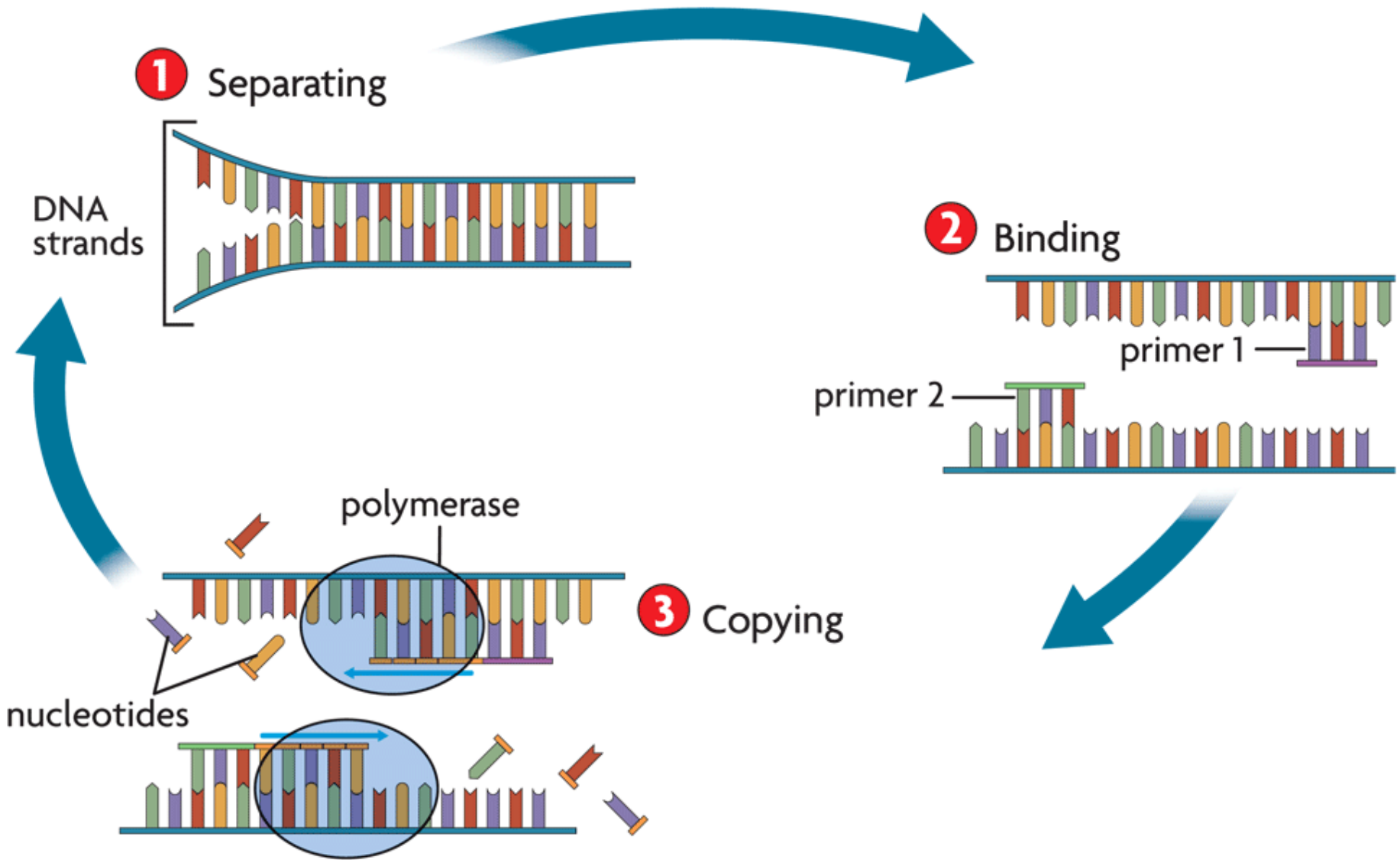


Legend

- Survey Sites
- ▭ West Branch Susquehanna River Watershed

Amplification

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

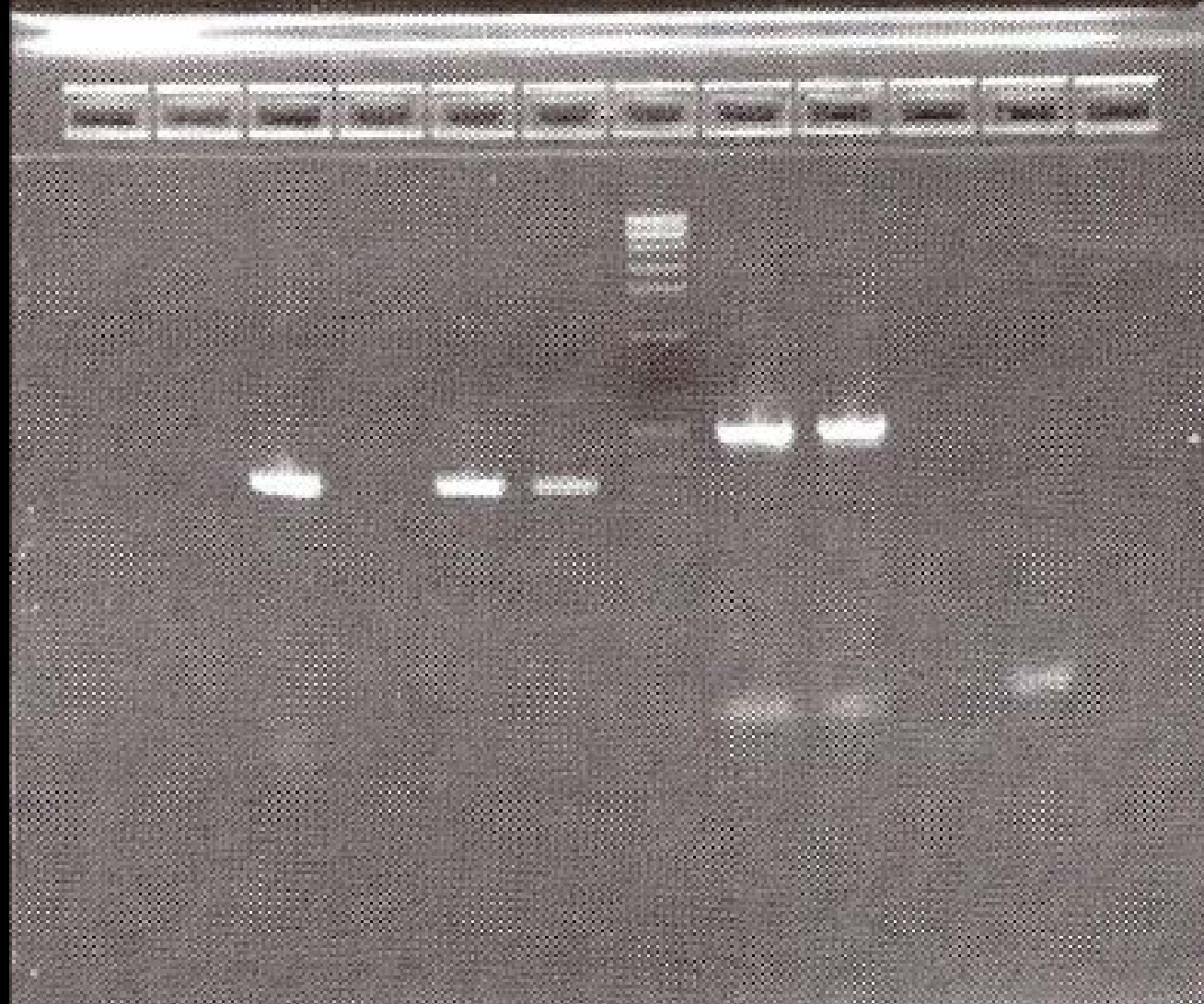


Verification

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

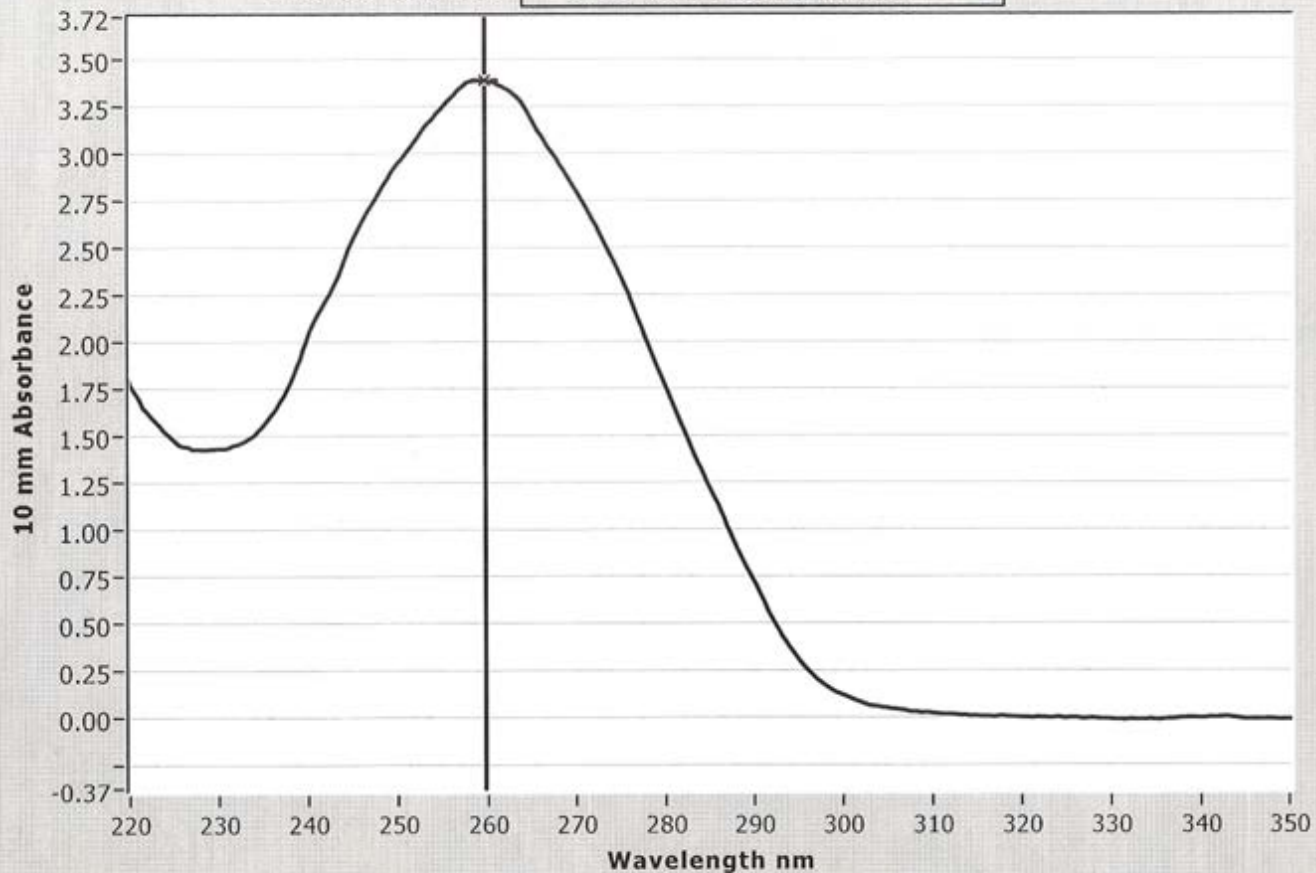
2% Agarose (GP)

1 2 3 4 5 6 7 8 9 10 11 12



Overlay control

Clear graph each Sample ▼



Sample Type

DNA-50

Sample ID

A14a

Sample #

6

λ

260

Abs.

3.380

A-260 10 mm path

3.380

A-280 10 mm path

1.774

260/280

1.91

260/230

2.36

ng/uL

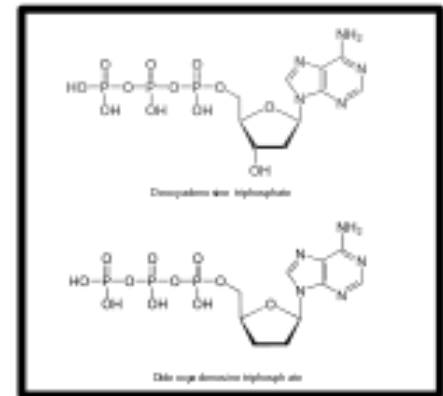
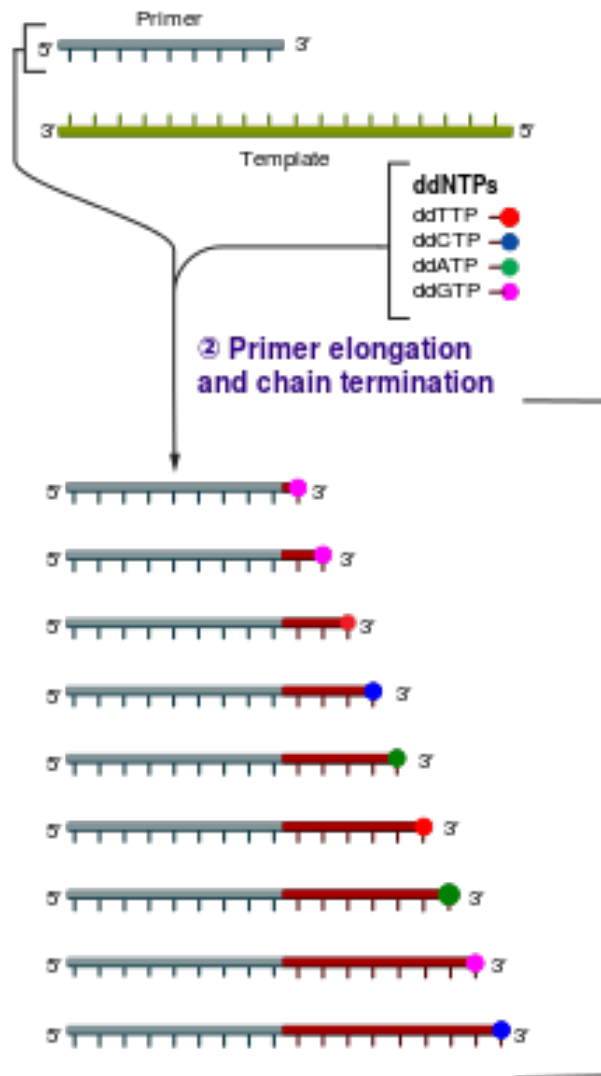
169.0

Sequencing

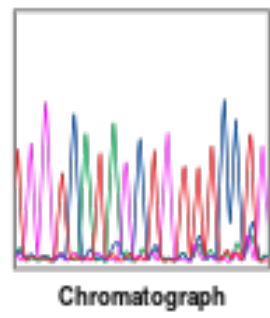
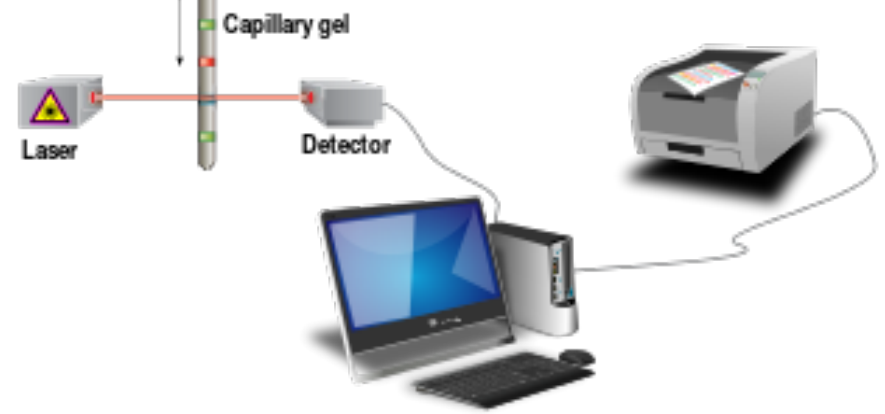
- **Di-deoxy preparation**
- **DyeEx Spin Kit purification**
- **Sequence using ABI 310 Genetic Analyzer**

① Reaction mixture

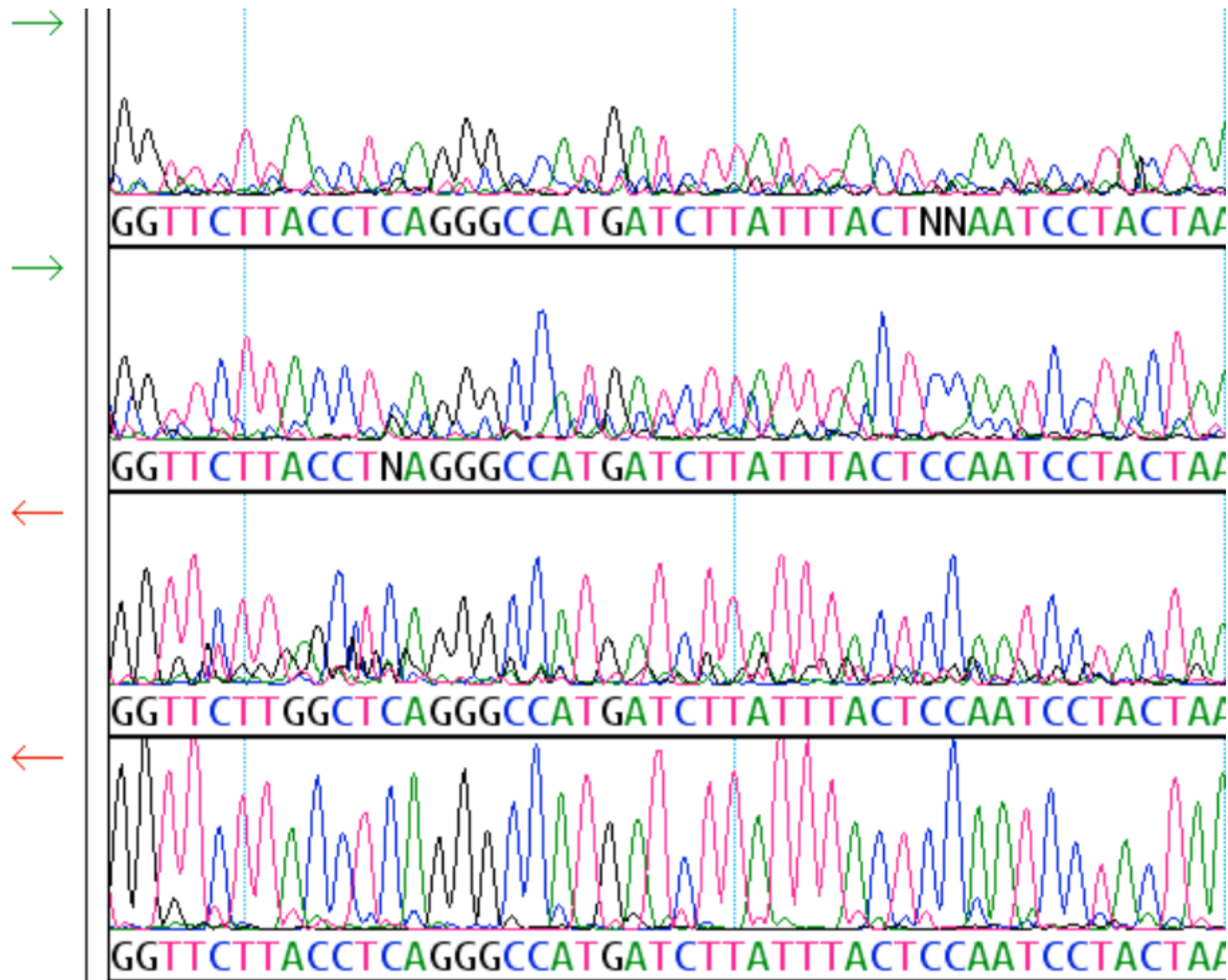
- ▶ Primer and DNA template
- ▶ DNA polymerase
- ▶ ddNTPs with flouorchromes
- ▶ dNTPs (dATP, dCTP, dGTP, and dTTP)



③ Capillary gel electrophoresis separation of DNA fragments



④ Laser detection of flouorchromes and computational sequence analysis



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**