Using PCR Assay to Detect Dermo Disease in Eastern Oysters in Maryland Waterways

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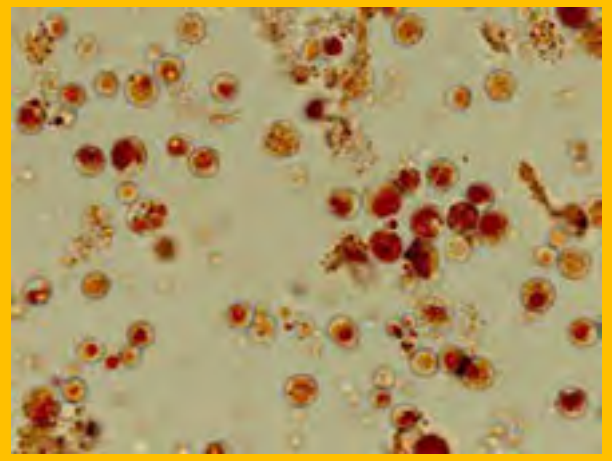
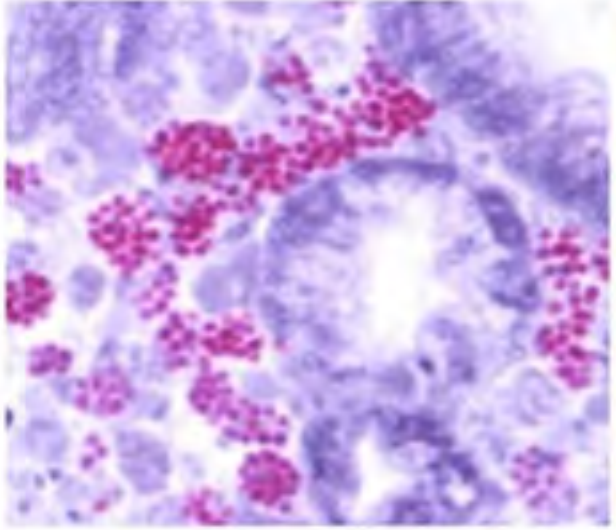
Background : A World Without Oysters

1. Source of food for humans and wildlife
2. Oyster reefs construct habitats for other organisms
3. Economic Value
4. Improve our waters quality

THREATS

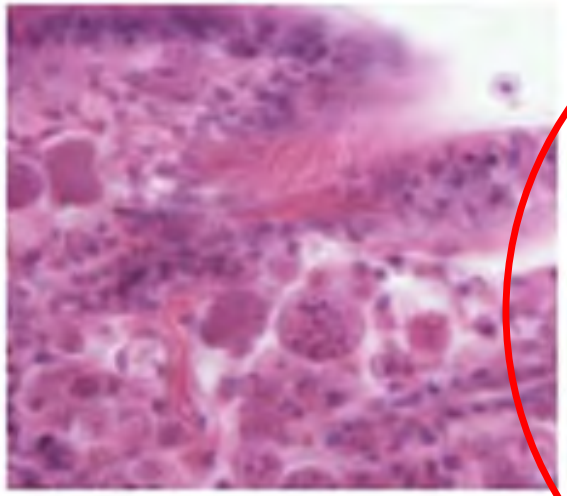
- Habitat Degradation
- Predation
- Mass Harvesting
- Climate Change
- Disease



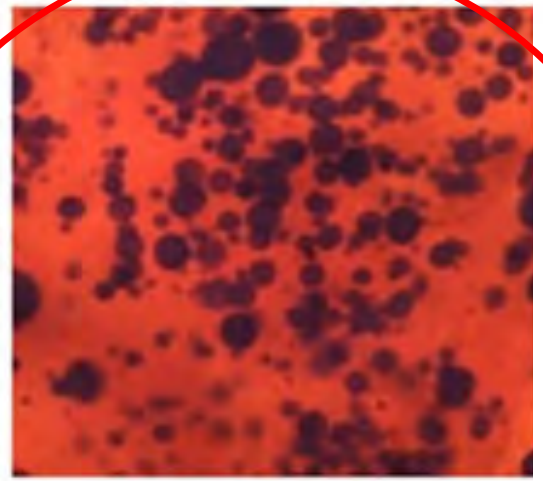


SSO

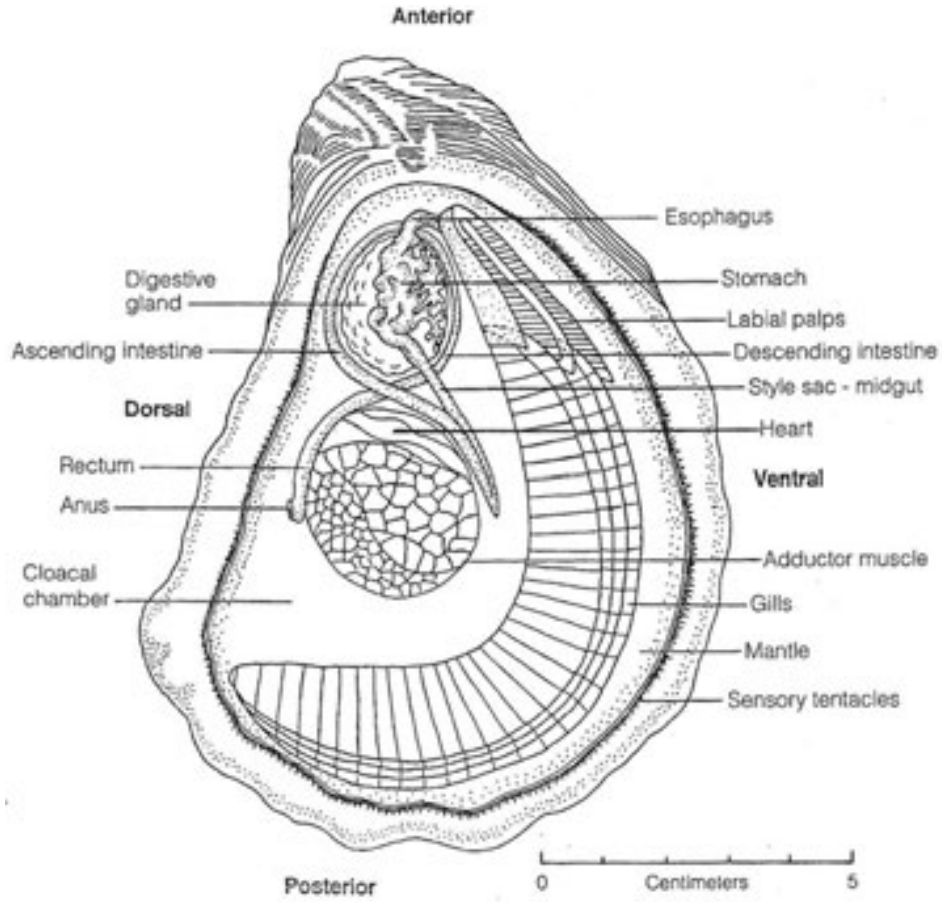
ROD



MSX



Dermo



https://portal.ct.gov/DOAG/Aquaculture1/Aquaculture/Oyster-and-Clam-Diseases

Purpose of study

- Despite the dermo prevalence has been reported to decline in recent years, it remains detected in oyster populations in Delaware Bay,
  https://hsrl.rutgers.edu/SAWreports/index.htm
- Assess if the mortality in our oysters are caused by disease infection.

Objectives

Investigate if there is any Dermo infection in Maryland oysters.

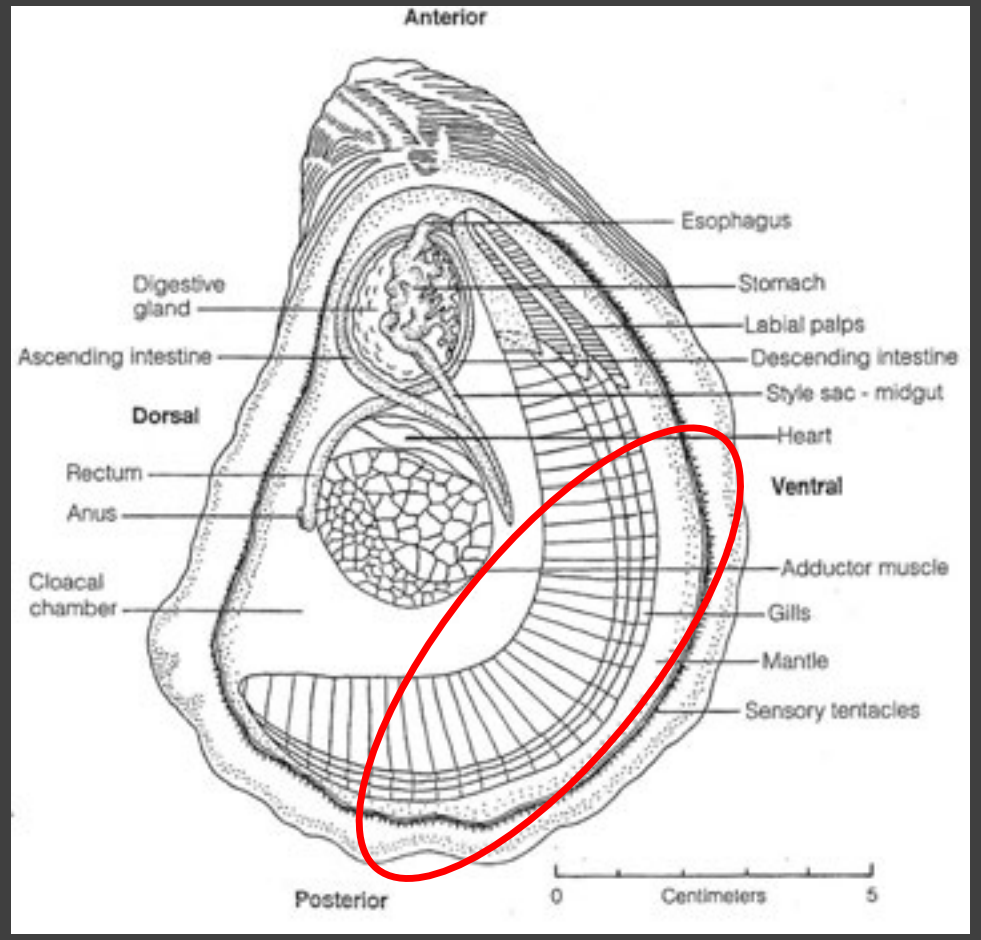
Determine if there is any prevalence difference among different oyster lines.

Materials and Methods

Study Region

Sampling

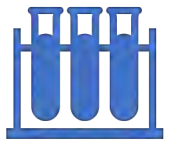
Identification	Line	Amount
Patuxent River	D and B	12
New Jersey	B	10
Potomac River	Commercial	12
Eastern Shore	Commercial	12
	Total	**46**



STEP 1: Tissue Sampling

Step 2: DNA Extraction

Step 3: Reaction Mixture



PCR Buffer

dNTP – Deoxynucleotide Triphosphate

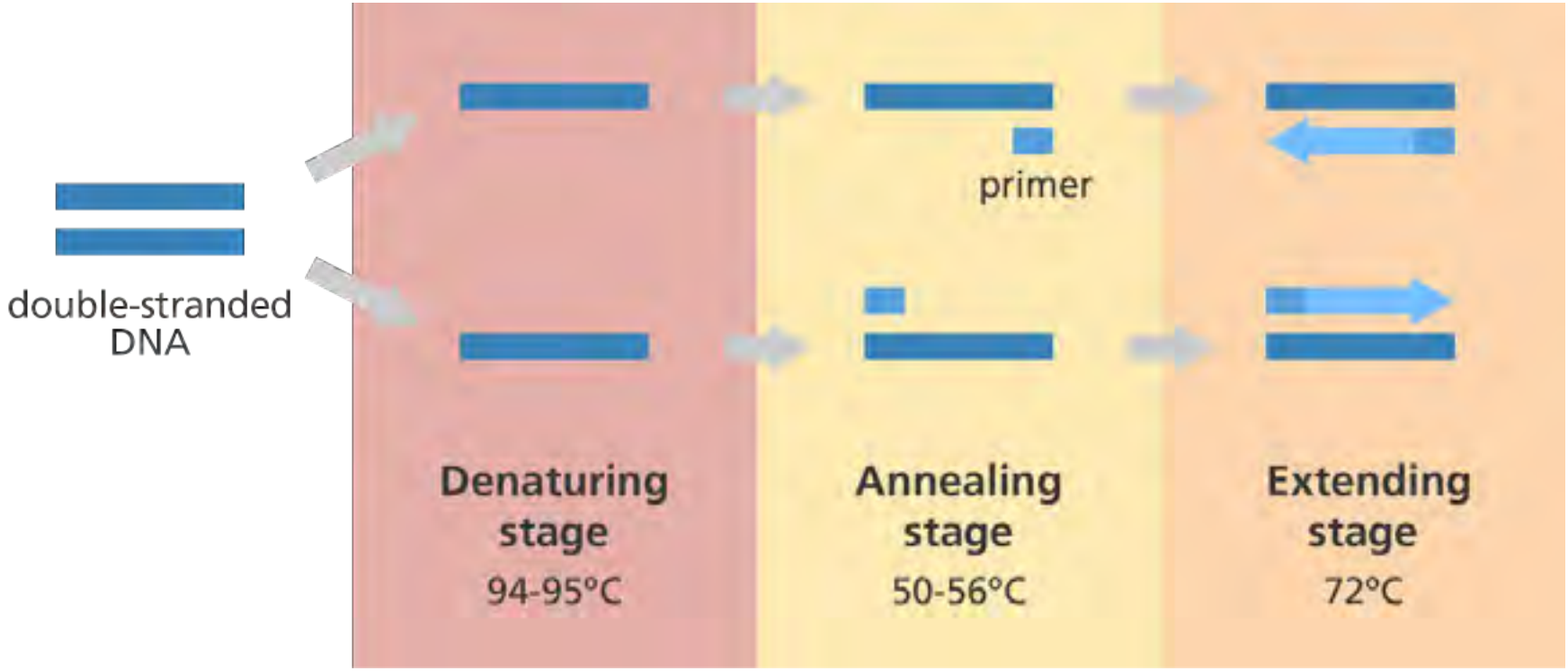
Forward and Reverse Primer

Taq DNA Polymerase

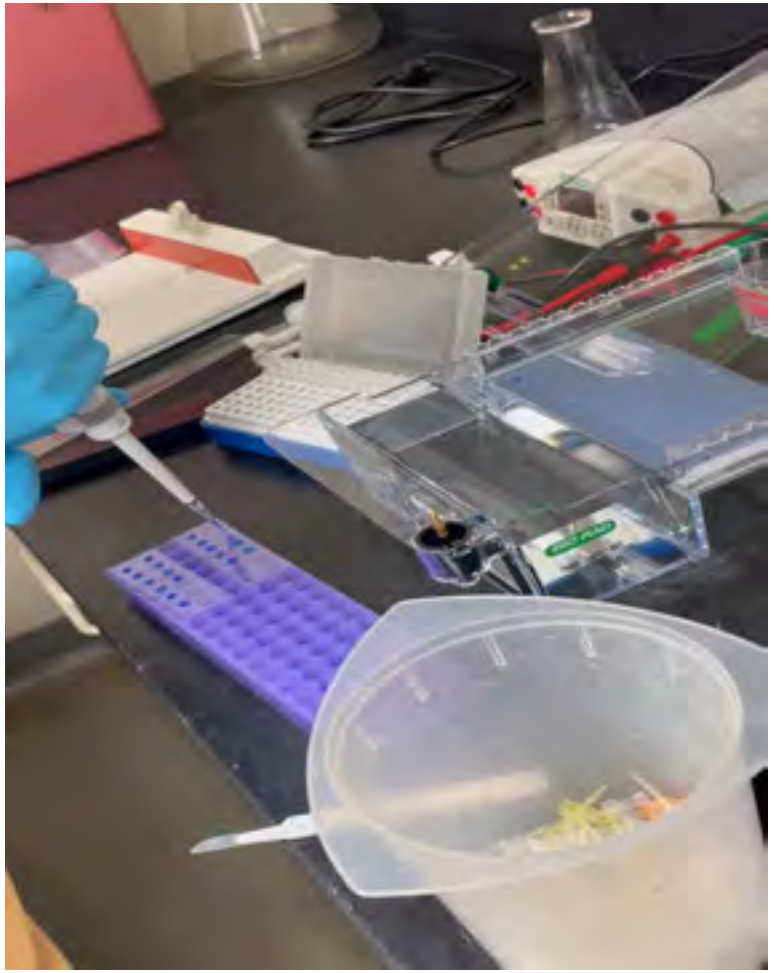
H2O

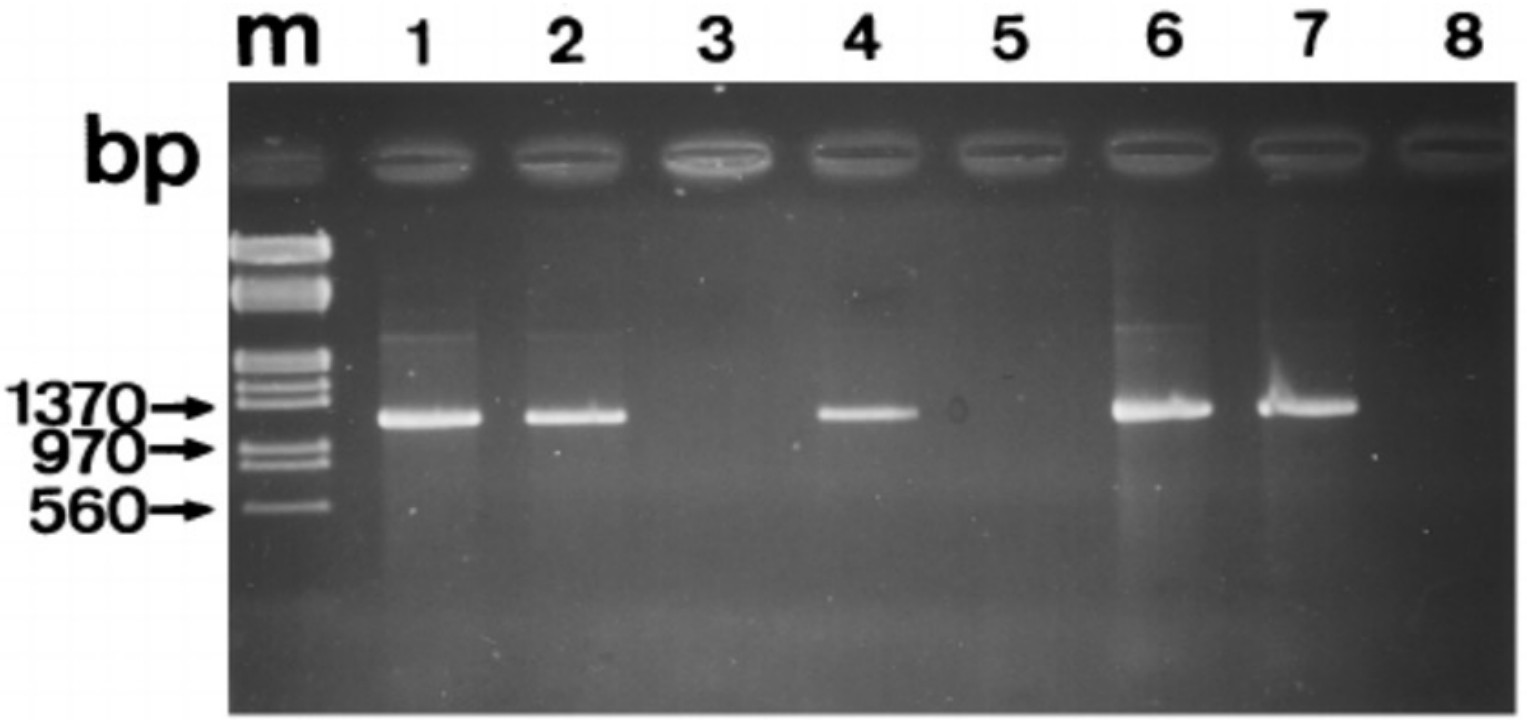
Polymerase chain reaction is a technique used to make multiple copies of a segment of a specific DNA fragment resulting in many copies from a small initial sample

Step 3: Polymerase Chain Reaction – PCR



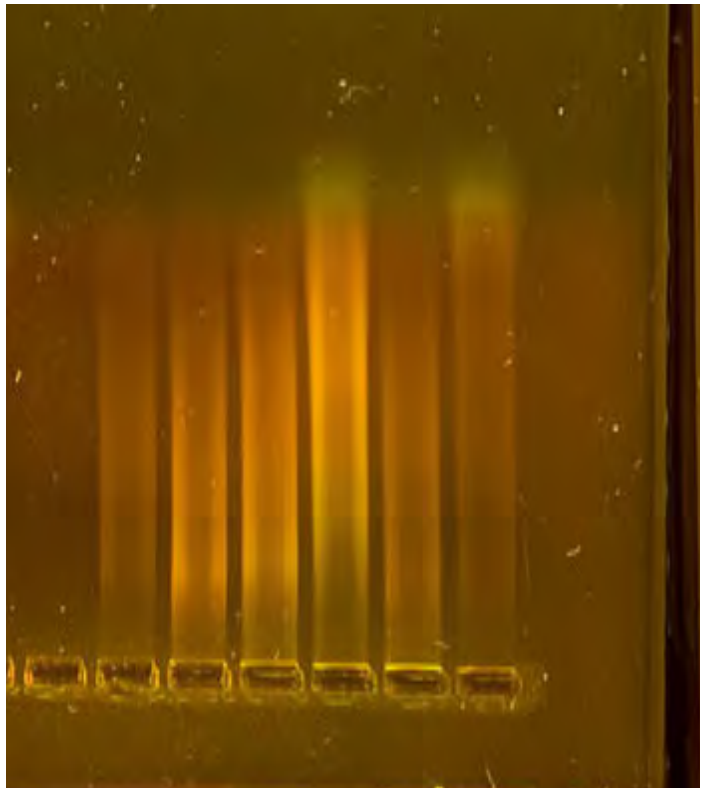
STEP 4: Gel Electrophoresis



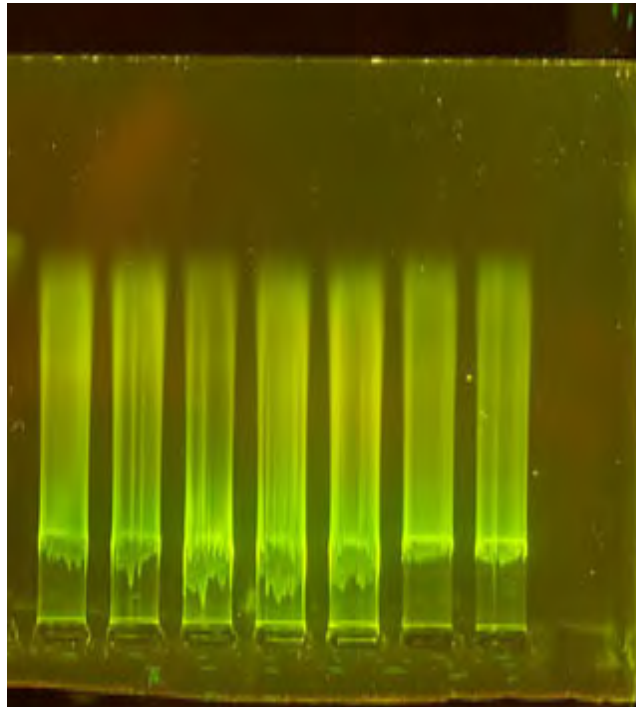


Results

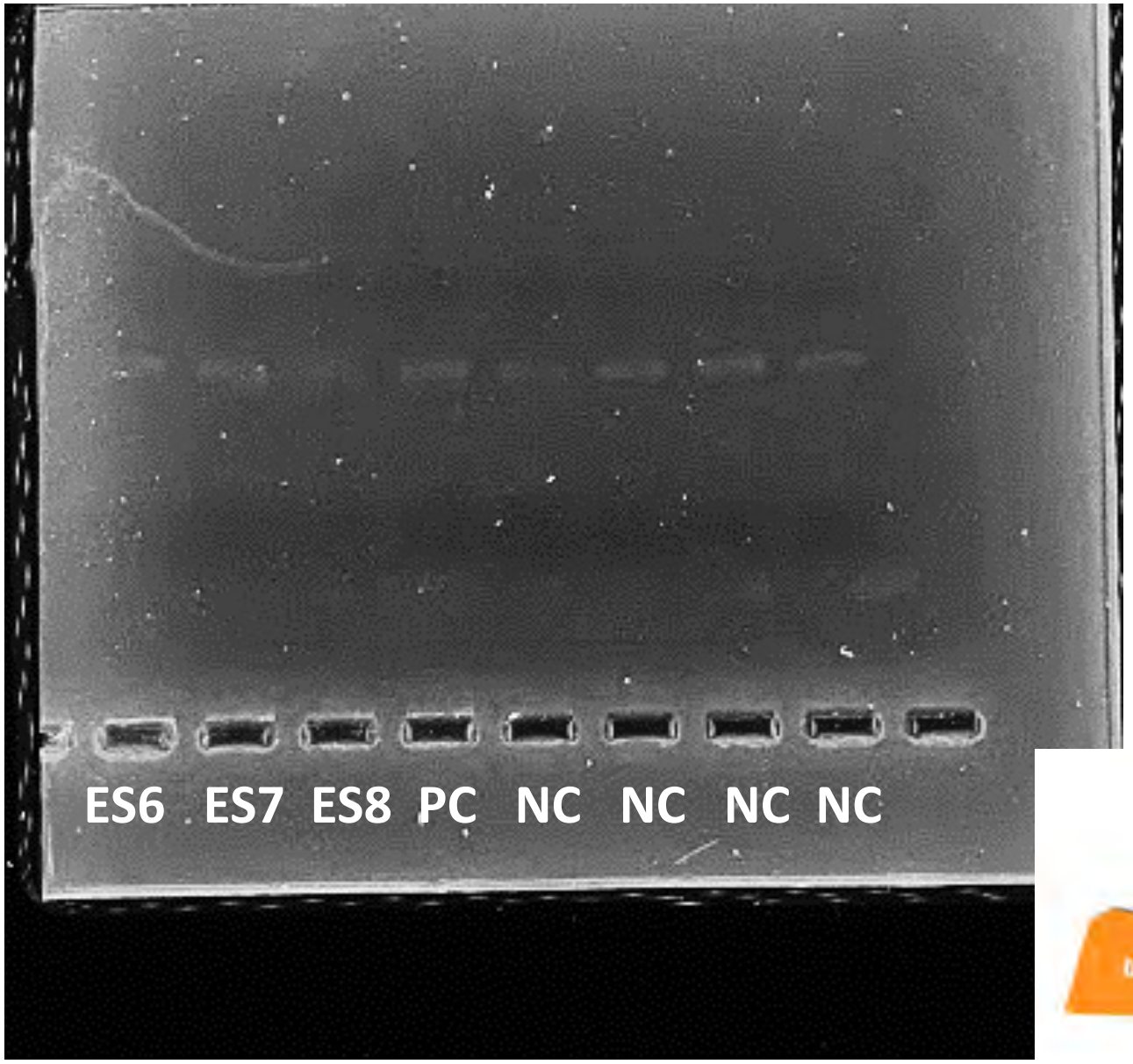
DNA Quality Comparisons between two methods



Chelex Method



E.Z.N.A. Mollusc DNA Kit Method



Primer Dimer Interruption

Primer Dimer PCR product size :50 – 100bp

Dermo PCR product is :85 bp

Solutions to Exclude Primer Dimer

1. Increased annealing temperature
2. Revising the primer sequence

    Forward primer Sequence from literature

    5’ CGC CTG TGA TGA TCT CTC GA 3’

    Forward primer sequence from another source

    5’ CGC CTG TGA TGA TCT CTC AG 3’

PC PC PC PC PC NC
NC NC

Data Interpretation

Through our efforts we were able to determine proper primer sequence to exclude interference of primer dimer.

Advancement and Future Endeavors

1

Continue this project using the new primer to achieve desired results.

2

Use new positive control extract.

3

Investigate prevalence of Dermo at other hatcheries and farms in the Chesapeake Bay watershed.

References

Ewart, John W., and Susan E. Ford. History and Impact of MSX and Dermo Disease on Oyster Stocks I the Northeast Region. 1993.

Dungan, Christopher F, et al. *Diseases & Parasites of the Eastern Oyster, Crassostrea Virginica, in Chesapeake Bay : An Illustrated Guide*. College Park, Maryland, Sea Grant, 2020.

Virginia Institute Of Marine Science. *Oyster Disease of the Chesapeake Bay*.


Questions?

THANK YOU

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Youtube Channel -FarAsMyaCanSea