Optimizing Microsatellite Primers for the *Dallia pectoralis* (Alaska Blackfish)

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Drawing by H.L. Todd

Introduction

Dallia pectoralis (Alaska Blackfish):

•A species of fresh water fish native to the arctic.

•There is believed to be one large population along the Bering Coast from the Colville River delta south to central Alaska Peninsula near Chignik; then upstream into the Yukon River drainage (Page *et al*).

•The Blackfish aren't salt water tolerant enough to survive in an oceanic habitat.

•Because the water by the Brooks Range makes the pass unsuitable for the Blackfish to pass, we believe that in the place of one clad of Blackfish, there are two genetically different populations.

•The lack of gene flow between the two populations is thought to have allowed them to mutate into two genetically varying populations.

•Using primers Dpec 14 F02, and Dpec 34 B05, we will develop microsatellite markers for the study of my mentor: Mathew Campbell.

•We will test our primers on samples from Fairbanks (Ballaine pond), Togiak, Bethel, and Palmer (Palmer hay flats).

We've hypothesized that if we manipulate our PCR (polymerase chain reaction) cocktail, we will produce clear DNA bands using gel electrophoresis. We assume the DNA samples that we are testing are accurate representatives of each of their regions; we also assume that the populations weren't introduced to the area, and that they all have the microsatellite loci.

What effect will the following changes make?:

• Level of Magnesium

•Annealing temperature

•Type of gel matrix (agarose or polyacrylamide)

•Intensity of volts running DNA during electrophoresis

•Length of time electrophoresis is applied to DNA samples

Methods and materials

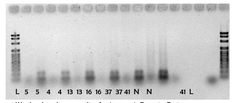
DNA samples:
Collected by Megan Hoffman, Mark Lisac, Peter Abraham and Mathew Campell.
Collected from individuals from Ballaine pond, Bethel, Alaska, Togiak, Alaska, and the Palmer hay flats.
Primers:
Dpec 14 F02 and Dpec 34 B05 provided by Andreas Lopez; amplified using a polymerase chain reaction (PCR) in an Eppendorf vapo-protect thermocycler (Barker).
Dpec primers B05 AND F02 were cloned and developed by Monica Arakaki, Tulli Makinen and Andres Lopez.
For the first reaction we used 96.6uL of ddH2O, 15uL of 2.5 mM dntp, 15uL of 2.5 mM Mg, 7.5uL of forward primer, 7.5uL of reverse primer, 1uL of *Taq* polymerase, 1 uL of each DNA sample. We ran our PCR results through a 1.5% agarose gel for one hour, at 110 volts.
Because our products of the gel electrophoresis showed unclear bands of DNA we optimized the cocktail. Using the same amount of all the ingredients, not including the concentration of magnesium (now 2 nM) and the addition of betaine (40uL). We ran our results in a 2% agarose gel for 45 minutes at 110 volts.
We ran a gradient PCR using 40uL of betaine, we set the annealing temperatures to 45.2, 47.9 and 51.7 degrees C. We ran our results in a 2% gel for 25 minutes at 125 volts.
We cycle sequenced DNA samples 4 and 37 from Ballaine pond and Bethel, Alaska; unfortunately our results we unreadable. We purified DNA samples 14 and 37 with shrimp alkaline phosphorace; we ran purified samples in a 2% gel for 45 minutes at 145 volts.
We ran samples 6,7,8,9,23,24,26,28,42,45,15 and 26 through a 3% gel for one hour at 95 volts.
*All of our PCR results were visualized then soaked in ethidium bromide, following a viewing under a UV light.

*Numbers correspond to individuals from various areas

Sample locations:

Ballaine pond: 4,5,6,7,8,9
Togiak, Ak: 13,16,16,20
Palmer hay flats: 42,45
Bethel: 23,24,26,28,37,41

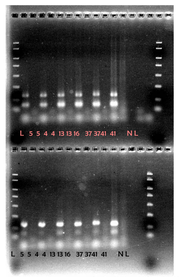
A.



* Weaker bands are results of primer pair Dpec 34 B05
Stronger bands are results from primer pair Dpec 14 F02

•5 mM concentration of Magnesium
•Annealing temperature: 52° C
•Run in a 1.5% agarose gel
•Ran for 40 minutes at 100 volts

B.



* 5 mM concentration of Mg

* 2 mM concentration of Mg

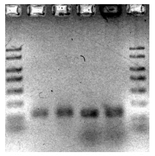
* Weaker bands are results of primer pair Dpec 34 B05
Stronger bands are results from primer pair Dpec 14 F02

•5 mM and 2 mM concentration of Magnesium
•Annealing temperature of 45°C
•Ran in a 2% agarose gel
•Ran for 45 minutes at 110 volts

C.

•2 mM concentration of Magnesium
•A gradient PCR: annealing temperatures of 45.2°C 47.9°C and 51.7°C
•With the addition of betaine (first three wells of each row)
•Ran in a 2% agarose gel
•Ran for 15 minutes at 125 volts

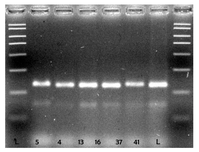
D.



* Weaker bands are results of primer pair Dpec 34 B05
Stronger bands are results from primer pair Dpec 14 F02

•Two samples purified using shrimp alkaline phosphorace
•2 mM concentration of Magnesium
•Annealing temperature of 52°C
•Ran in a 2% agarose gel
•Ran for 45 minutes at 125 volts

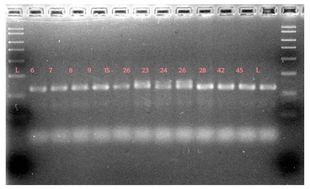
E.



Primer pair Dpec 14 F02

•2 mM concentration of Magnesium
•A gradient PCR: annealing temperatures of 45.2°C 47.9°C and 51.7°C
•Ran in a 2% agarose gel
•For for 45 minutes at 80 volts (in a small gel rig)

F.



Primer pair Dpec 14 F02

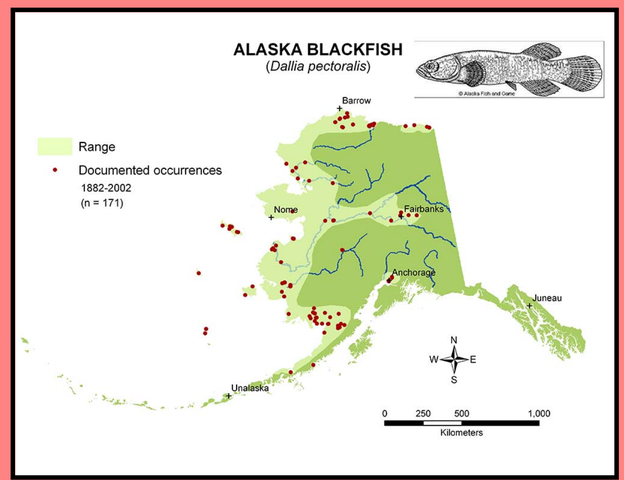
•1.5 mM concentration of Magnesium
•Annealing temperature of 45°C
•Run in a 3% agarose gel
•Run for one hour at 95 volts

Conclusion

•We found that changing the concentration of magnesium gave us less distinguishable bands when higher, and clearer results when lower.

•The addition of betaine and a higher annealing temperature increased the specificity of the amplification

•Higher voltage was used when we ran the samples for a shorter amount of time, when we increased the amount of time our gels ran, we generally decreased the amount of voltage; an increase in run time gave us more distinguishable bands, and more variation in length



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