UNIVERSITY OF MIAMI
ROSENSTIEL SCHOOL of MARINE, ATMOSPHERIC & EARTH SCIENCE

Assessment of family lineage in *Lobotes surinamensis* aquaculture stocks

Nikki Lyons, John Stieglitz
Rosenstiel School of Marine, Atmospheric, and Earth Science, University of Miami, FL
njl84@miami.edu

Marine Genomics Lab

Introduction:

- Genomic sequences can be aligned to determine single nucleotide polymophisms (SNPs), and these variable DNA sites can be used to determine parental lineage
- We can enhance aquaculture stock of Atlantic tripletail (*Lobotes surinamensis*) offspring at the University of Miami Experimental Hatchery (UMEH) at the Rosenstiel School by defining successful parents
- My research isolated genomic DNA and created a genotype-by-sequencing (GBS) library to define SNPs and determine parental lineages

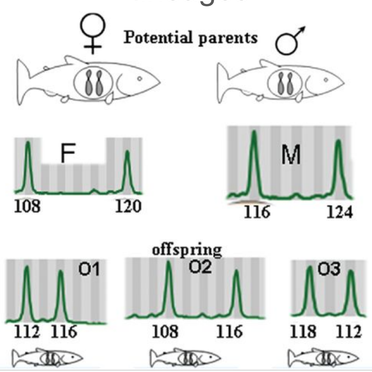
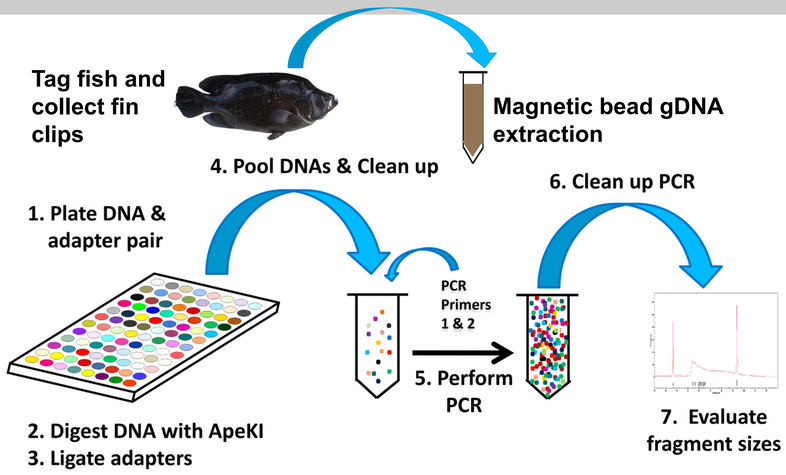


Figure 1: Principal of parentage analysis using the exclusion method.The numbers under the peaks are the allele sizes in bp. This allele analysis provides an example of how a collection of varied offspring genotypes can be compared to the F1 generation to identify the parents of individuals. In aquaculture, this can be done with alleles of desired traits for optimized breeding

Methods:



Align sequences and identify SNPs to determine parentage

Results:

- Library: ~600 bp sequences
- Library concentration: 37.8 ng/µL
- Library volume: 30 µL

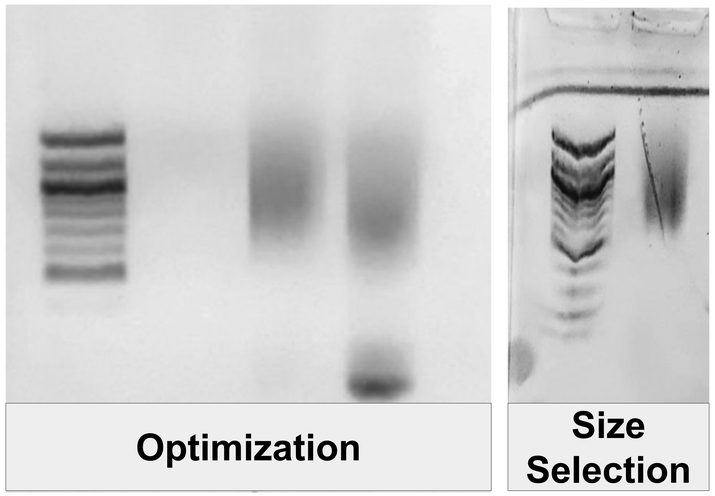


Figure 2: The right gel shows the size distribution of library PCR product (with initial gDNA concentrations of 1 µL, 2 µL, and 4 µL) before size selection. 6 more replicates of the 4 µL PCR were completed and the pooled volume was size selected for a range of 400-700 bp, seen on the right gel. The size selection for shorter base-pair fragments was necessary for successful next-generation sequencing

Discussion:

The gDNA was isolated, digested with restriction enzyme ApeK1, then bar codes used to identify individuals were ligated to the size selected DNA fragments (500-700 bp). Analysis using gel electrophoresis showed that the library had a range from 1000-500 pb before size selection, which was reduced to a range of about 500-700 bp post-size selection. Each individual has a unique DNA barcode, so that SNPs for each individual can be identified using a GBS pipeline. From the SNPs, the full parental lineage of the offspring generation will be determined and used to achieve improved breeding outcomes.

Conclusion / Future Goals:

- By identifying the parental lineage of the offspring generation, it may be possible to improve broodstock management regimes
- Future breeding can be improved by accounting for genotype information
- One of the key first steps in establishing breeding programs for aquaculture species is genotyping of broodstock and identification of family lineage
- Presented techniques have potential to reduce the expense and time required to establish an effective breeding program in broodstock fish

References:

Vesal, S. E. (2024). Molecular Parentage Analysis in Aquaculture: Principles, Applications, and Challenges: A Review. *Asian Journal of Fisheries and Aquatic Research*, *26*(3), 62–75.
Yue, G.H. and Xia, J.H. (2014), Practical Considerations of Molecular Parentage Analysis in Fish. J World Aquacult Soc, 45: 89-103.
Elshire RJ, Glaubitz JC, Sun Q, Poland JA, Kawamoto K, Buckler ES, Mitchell SE. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PLoS One. 2011 May 4;6(5):e19379.

Acknowledgements:

Special Thanks To…
Douglas Crawford
Marjorie Oleksiak
John Stieglitz
Ronald Hoenig
Samantha Sierra-Martinez
Liza Merly
Funding:
SURGE Award Funding
Tripletail Aqua, LLC
All animal handling and sampling procedures conducted in accordance with IACUC Protocol: 23-132