Duck Tales:

Using NGS to Unravel the Genetic Diversity of the Mallard Duck Population

Flor Hernández (left) and Philip Lavretsky, Ph.D. from the Lavretsky Lab. Mallard ducks are studied for a unique opportunity to understand how hybridization impacts population structure.

Quantabio sparQ PureMag Beads help optimize workflows for both Illumina® and Oxford Nanopore sequencing systems.

> *"The Quantabio sparQ PureMag Beads provided us with a far more efficient and cost-effective replacement for the AMPure XP® and SPRIselect® beads. The easy-to-use product enabled us to improve the PCR cleanup and size selection for our ddRAD protocol on the Illumina system and the PCR cleanup steps for the Oxford Nanopore workflow. We are now able to manually process up to 250 samples in less than 16 hours, with identical if not better performance and for 38% less cost than before."*

The research program in the Lavretsky Lab at the University of Texas at El Paso (UTEP) is quickly transforming our understanding of the population and evolutionary genetics of ducks and other wildlife. The team is using next-generation sequencing technologies to determine the extent to which adaptive and non-adaptive genetic diversity shapes population structure. This same methodology is now being used to study a variety of organisms, from ducks to mosquitoes to whales.

We recently caught up with Philip Lavretsky, PhD, Assistant Professor of Biological Sciences at UTEP, to discuss his current National Science Foundation (NSF) project and how Quantabio products have enabled him to optimize his next-generation sequencing workflow.

Adaptive Impacts of Hybridization on Animal Populations

In September 2020, Lavretsky's lab secured a $1 million-dollar National Science Foundation (NSF) grant to investigate what happens when domestic and wild animals interbreed in nature, along with the complicated consequences that can occur when people release their pets in the wild. His team is looking at how interbreeding or hybridization can lead to negative results in their populations if certain domestic traits are not adaptive in the wild. Specifically, the team is exploring: the genes that are responsible for geographic adaption versus alternative selective pressures, how contemporary pressures influence a species' adaptive landscape, and how best to use this information to establish better management and conservation practices.

For the NSF project, Lavretsky's team is focusing on the mallard duck because of its unique behavior and evolutionary history. Mallards are also one of the best-studied organisms because of the management implications for the waterfowl hunting community. The eastern population of mallards provides an interesting case study for this project because it has been declining steadily for the last 20 years. Lavretsky's team has been able to demonstrate that the mallard case is similar to any salmon or trout stocking situation. When the fish are bred in tanks, they are all fed and the majority survive. However, once this stock is released into the wild, those recessive traits can explode and significantly impact the overall population rather quickly.

Thankfully for Lavretsky's research, ducks have been mounted in museums since the early days of our country. His team has been able to partner with museums, as well as private and public organizations across North America to determine when hybridization between domestic and wild mallards became a problem.

"Not only is the mallard important culturally, ecologically, and agriculturally, I've always been interested in studying these birds because of their unique behavior and evolutionary history," Lavretsky said. "The mallard duck is a study system that I have been working on over the last decade, and I believe that this species offers a unique chance to understand the impact on the adaptive potential of wild populations when they breed with their domestic version."

The Mallard Model

Lavretsky's team uses a combination of next-generation sequencing techniques to generate genome-wide markers and to link genetic variation to species or population traits of interest. To look closely at the population genetics at the landscape level with hundreds of thousands of samples, the team employs a double digest restriction-site associated DNA sequencing (ddRADseq) methodology on the Illumina HiSeq® systems for partial genome sequencing. To understand which genes may be important in the survival of the species, the lab is using Oxford Nanopore sequencing technology for comparison looking at 125 full genome sequences, which includes 5–10 pure parental wild and domestic, as well as varieties of hybrids. They are also analyzing phenotypic and ecological data to look at the genetic traits that are associated with the genes that have been passed on from generation to generation. Additionally, Lavretsky's team is using ancient DNA techniques to analyze hundreds of historical mallards to track the establishment of the wild x domestic mallard hybrid swarm now present in eastern North America.

"We are really trying to understand what happened by going back in time with biological collections. We are looking closely at the genomes for genetic variation that may be important in the adaptive potential of wild mallards, while also uncovering mechanisms that may explain some of the recent declining trends in North American mallards," Lavretsky said. "Specifically, if they cannot eat, they cannot survive through the winter and migrate as well as nest properly, and so with enough generations of less fit individuals you expect a population decline."

Quantabio Offers Significant Time and Cost Savings

The team is using the Quantabio sparQ PureMag Beads to optimize the size selection and PCR cleanup steps for both the ddRADseq application on the Illumina sequencers and the PCR cleanup with the Oxford Nanopore protocols. The sparQ PureMag Beads were designed for seamless integration into existing NGS workflows and to be a cost-effective alternative for both AMPure XP and SPRIselect. For this custom ddRADseq protocol, the sparQ PureMag Beads replaced the SPRIselect beads for size selection and the AMPure XP beads for purification. For the Oxford Nanopore protocol, the sparQ PureMag Beads replaced the AMPure XP beads for the PCR cleanup process.

The sparQ PureMag Beads are a fast and reliable nucleic acid purification system for reaction cleanup and size selection in NGS workflows. Based on the reversible nucleic acid-binding properties of magnetic beads, this product can be used to quickly remove primers, primer-dimers, unincorporated nucleotides, salts, adapters, and adapter-dimers from NGS library prep reactions to improve downstream sequencing performance. sparQ PureMag Beads allow excellent recovery of fragments greater than 100 bp without centrifugation or filtration. Consistent and reliable size selection can be achieved by simply adjusting the beads to sample ratio.

In 2013, it took the team about two months to analyze 120 samples. With the new optimized protocols, Lavretsky's team is now able to manually run 250 samples in only 16 hours, all at a very cost-effective rate. In fact, the Quantabio technology has resulted in a 38% cost savings compared to the previous workflow. The team is also able to consistently recover the same quantity and types of loci as with the alternative AMPure XP bead technologies. The decision to use the sparQ PureMag Beads for the Oxford Nanopore cleanup applications was an easy one after seeing how well they performed with the

Illumina protocol. As expected, the overall performance has been identical if not better than the AMPure XP beads. In the future, Lavretsky is hoping to use the Oxford Nanopore technology and protocols in the field and be one of the first researchers to sequence waterfowl samples on remote islands across the country.

Education Leads to Improved Conservation

Importantly, Lavretsky hopes his team's work will educate the general public about the negatives of releasing their pet ducks, or generally, any domestic animal.

"The results from our research will shed light on the consequences of interbreeding between wild individuals and their domesticated versions," Lavretsky said. "In short, many traits that are great in a domestic setting are simply non-adaptive in the wild. We will look to answer the question 'What are the genetic and adaptive consequences from such events?' Not only are the results important to understand the impact from the interbreeding between wild and domestic creatures, but also will help better guide the conservation of the wild population."

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