Q&A

Webinar: Application of advanced genomics tools in aquaculture breeding programs

Our expert panelists from the 'Application of advanced genomics tools in aquaculture breeding programs' webinar received great questions. The answers from the experts have been assembled below into a supplementary resource.

How do you collect tissue for DNA extraction in finish/shrimp, control contamination, and tag the animals for phenotyping for association studies?

- There are a few methods of tissue collection that we are using. We singulate entire post-larvae (~1 week old) into 96-well plates. These babies fit perfectly and surprisingly no homogenisation is needed. This is a destructive method for sampling a spawn. For adults, we collect muscle tissue by clipping a pleopod or piece of tail fan. Phenotype data is collected on maps that match sample location in 96-well plates. Locally collected samples go right into a freezer while international samples are desiccated prior to shipping. 70% alcohol is used to disinfect tools between samples, and control samples are included in each plate to help detect contamination. Contamination is easy to spot when looking at the results from KASP™ reagents. Clusters are compacted and overlap. Controls are not what they are supposed to be. These tissue collection methods work well for KASP, where the chemistry is robust even with low DNA quantity and quality are good enough. For sequencing or SNP chips, I would want to measure and standardise samples, probably use a column based DNA extraction technique as opposed to our quick and dirty method. We have a team of ~8 people who collect tissue and phenotype data. Our instruments are faster than they can collect the samples. For tagging, we use custom-made labels that fit around the eyestalk of adults. The eyestalk tags are colored and labeled with a unique number so we can find the exact animal after processing its DNA. There are colored plastic elastomers that can be injected into the muscle but this is limited to the number of unique colors and may introduce pathogens into the animal. (Mitchell Lucas, Director of Genetics, American Penaeid)

- These are very standard procedures on each species. In finfish we use fin-clips and in shrimp pleopods to extract DNA. For sampling and preservation there are different approaches, but we generally use 96 deep well plates for these purposes. LGC also has the BioArk™ system, which we have tested with high success in shrimp. (Jose Manuel Yanez, Director of R&D and Faculty University of Chile)

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GENOMIC ANALYSIS BY LGC

Q&A

Webinar: Application of advanced genomics tools in aquaculture breeding programs

- We mark our fish with RFID pit-tags (injected under the skin). For DNA sampling, we use a fin clip (adipose fin in salmon) which we fix and preserve in 95% Ethanol. We keep the samples in separate 1.5 mL Eppendorf tubes to avoid cross contamination. (Marine Herlin, Global Genetics Manager Cooke Aquaculture)

- The above method is a very common DNA sampling/storage method we see across the industry. Last year we launched BioArk™ Sample Collection Kits to eliminate the ethanol storage buffer for fish fin clips, a partnership project we undertook with Hendrix Genetics. If ethanol is an issue for storage or shipment, these BioArk Fish Collection Kits are a great alternative. These, and the ethanol stored samples, are compatible with our sbeadex™ extraction chemistry, which is the same extraction chemistry we use in our KASP genotyping and SeqSNP™ targeted GBS service lab work. (Jason Hein, Strategic Development Manager, LGC, Biosearch Technologies)

What is the cost per sample DNA extracted and genotyped with 100 SNPs?

- The biggest dependency for cost is the number of samples you process against the 100 SNPs. The other dependency is whether you do this through a service offering, such as Biosearch Technologies' KASP all-inclusive genotyping services, or do this work in your own lab using automation and consumables. The choice of whether to use services or do this work in your own lab is also impacted by the number of samples you are analysing and how your projects (both in size and variety) will change over time. For a project of 1,000 samples, run as a service, you could pay between $10-15 per sample. Whereas a project with 10,000 samples, you could pay between $6-7 per sample. The best way to determine the cost, and what makes sense for your specific situation, is to reach out to our customer service team or local Client Executive and we can take you through a customised evaluation for the best fit for both your immediate and long-term needs. (Jason Hein, Strategic Development Manager, Biosearch Technologies)

Could you expand briefly on the practicalities of the SeqSNP platform? An indication of time to develop working assays from SNP sequence data and the scale that this approach is effective at i.e. recommended range of SNP's and individual samples.

- Typical timelines for the SeqSNP service are 6-8 weeks for upfront SNP library design and 2 weeks for processing (DNA to data). The method is optimally suited for 500-100k SNPs and up to about 2000 samples, beyond 2000 samples we have a high-throughput SeqSNP HT targeted sequencing service with more attractive pricing per sample. (Joris Parmentier, Strategic Marketing Manager, Biosearch Technologies)

Q&A

Webinar: Application of advanced genomics tools in aquaculture breeding programs

There is focus on genetic weight gains but very little info on taste of the aquaculture and genetic dependence of this....is this an area of development?

- Some product quality traits are also included into the breeding goal for different species. For instance, flesh color and appearance traits (skin color) are included in Atlantic salmon and rainbow trout breeding programs. These are traits related with consumer preferences. What you mention about taste is very interesting and as you said, there is little or null information on the presence of genetic variation and genomic regions associated with taste in aquaculture species. Without any doubt is something worthy to explore it! (Jose Manuel Yanez, Director of R&D and Faculty University of Chile)

- Unfortunately, no. I doubt we have significant genetic variation for these traits in our germplasm. It is also very difficult to control environmental impacts when trying to breed for these traits. Environmental factors (different feeds, photoperiod, light intensity, water quality, temps) do have a big impact on culinary traits. Manipulation of these inputs at the production level is where I would start. If we were to pursue the work from a genetic perspective, I would want to bring in some new germplasm that is known to be different for these attributes, providing heritable elements we could breed into our commercial germplasm. I hope we won't completely follow the mistakes of tomato and strawberry breeding where flavor and color have been compromised in favor of production traits. It is extremely difficult to manage these priorities with growers who may not benefit from improvements that are extracted by downstream distributors. Completely integrated companies are probably best positioned to commercialise these types of value-added products. Growers take a lot of risk and focus on the basics to secure their future. Products bringing culinary improvements still need to perform for yield and disease tolerance. (Mitchell Lucas, Director of Genetics, American Penaeid)

- For the moment no, maybe in the future? (Marine Herlin, Global Genetics Manager Cooke Aquaculture)

What is done to produce YY inbred male tilapia?

- Chromosomal manipulations are used to generate YY males in tilapia. (Jose Manuel Yanez, Director of R&D and Faculty University of Chile)

Q&A

Webinar: Application of advanced genomics tools in aquaculture breeding programs

Dr. Lucas: Have you found significant genotype environment interactions by running these analyses on the same families in different systems?

- That is a huge motivation for our work. Eventually we want to prescribe varieties based on a grower's system; resulting from replicated trialing of different genetics. During 2019, trialing was not organised enough. We drew some conclusions but lacked replication and fair comparisons. We found family performance was similar in Indonesia and India, while slightly different genetics won these studies in China. We had a good start in 2020, really focusing on Asia, but coronavirus dramatically limited our ability to collect samples and data once the animals made it out of the hatcheries. Our genetics are better organised and we have trialing partnerships in place for 2021, so we expect to get good data even if we cannot travel. We have an ongoing study to compare varieties in biofloc and RAS systems for high-value, specialty, shrimp production. We're testing mixes of 4 hybrids with lots of replication in each environment and hope to get the final data within a month. When we did this on families for parent line development, performance differences were not that large between environments. I think that study was limited because the genetics were not production caliber F1 hybrids. Thanks for the question. (Mitchell Lucas, Director of Genetics, American Penaeid)

How to use SNP in wild broomstick?

- SNPs will work on wild shrimp broodstock for vannamei and monodon where target sequences are publicly available. If you are working with a lesser studied species, you may need to first discover SNPs by using some sequencing techniques. It is not that difficult or expensive. Biosearch Technologies offer this as part of their GBS sequencing services. Once SNPs are in hand, I would first use them to characterise different genetic types you can collect from the wild. Are genotypes unique to a location or season? Which animals are most closely related and which are distant relatives? This can help you decide which animals to breed with. (Mitchell Lucas, Director of Genetics, American Penaeid)

- SNPs are suitable for genetic studies in wild populations. How to use them depends on the objective of the study. (Jose Manuel Yanez, Director of R&D and Faculty University of Chile)

What SNP platform and marker density do you use in genomic selection?

- For genomic selection, we are using a 62K SNP array from Affymetrix. (Marine Herlin, Global Genetics Manager Cooke Aquaculture)

Q&A

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How many SNP markers and which panel(s) are used for the BKD and the sea lice analysis?

- We are using the 62K data for genomic predictions on our select broods. We plan to use a reduced panel to 15-20 SNP markers per disease to screen our commercial production breeders (Marine Herlin, Global Genetics Manager Cooke Aquaculture)

What are basics early carrier geneticists to set up genetic lab in countries where technology is still at the infant stage?

- No matter where you are working, your team is most important to business success. Everyone wants to enjoy whom they work with and be happy, it is second to nothing. Plan to spend a lot of time and resources to hire and develop your team. Seek cooperation from vendors to help plan your lab and workflow from the start. They have a lot of exposure to different operations and can find you large efficiency and cost gains. When equipment breaks down you will need to ship in replacement parts and maybe even a technician; this is not cheap or convenient. This should influence what you buy and whom you buy it from. (Mitchell Lucas, Director of Genetics, American Penaeid)