Application of advanced genomics tools in aquaculture breeding programs

Housekeeping

BIOSEARCH™ TECHNOLOGIES
GENOMIC ANALYSIS BY LGC

- All participants will be muted to prevent background noise during the presentation
- Q & A will follow the presentation
  - You may enter your question in the “Question” window at anytime during the event
  - We will answer as many questions as possible during the Q & A session. Any questions we don’t have time for will be answered via email
- An email with a link to the recorded event will be sent following the presentation.

Thanks for joining us today!

LGC

Meet our speakers

BIOSEARCH™ TECHNOLOGIES
GENOMIC ANALYSIS BY LGC

Marine Claire Herlin
Global Genomics Manager

Cooke Aquaculture

Dr. Jose Manual Yanez
Director of R&D and Faculty

University of Chile

Mitchell R. Lucas, PhD
Director of Genetics

American Penaeid Inc

Jason Hein
Strategic Development Manger

LGC, Biosearch Technologies

LGC

Development and application of tailor-made genomic tools in a commercial Atlantic salmon breeding program

M. Herlin, J.A.K. Elliott, K.P. Ang, F. Powell, L. Gonzalez, E.G. Boulding, T. Moen.

Palma de Mallorca, January 2021.

Scope of the presentation

- Introduction: Brief history of CAI salmon breeding program in east Canada.
- Selection for growth improvement at sea using traditional BLUP model
- 2014-2019: Development of a new molecular toolbox.
- Implementation of genomics selection in CAI commercial breeding program.
- Further developments.

- CAI manages its own breeding program for Atlantic salmon Saint John River Aquaculture Strain (SJR) since 1998.
- The breeding program is operated at Oak Bay Hatchery (OBH) in New Brunswick: a fresh water land-based facility with a production capacity of 30 million eggs per year.
- Traditionally, families were reared communally at OBH with future broodstock candidates being selected -at 3 years old- based mainly on growth (both in freshwater and at sea) and maturity.
- Family growth performances at sea are evaluated on sib groups reared until harvest size in commercial net pens.

- In 2014, CAI initiated a 3 year project in conjunction with the University of Guelph –with funding from Genome Canada/Genome Atlantic– to develop advanced genomics tools to apply in its breeding program: GAPP “Salmon and Chip” Project.
- In 2015, a 100 tank individual family rearing unit was set up in OBH.
- The same year, a customized HD 50K SNP Chip was successfully developed for CAI SJR aquaculture strain.
- In 2016, GEBVs for both SW growth and lice resistance were used for the first time in CAI breeding programme.




- In 2018, a second and improved version of the HD SNP chip (62K) was developed in collaboration with AquaGen AS.
- In 2019, a cost-effective low density SNP panel was fine tuned in collaboration with LGC, Biosearch Technologies.
- In 2019, construction a new “genomics” building at OBH with a rearing capacity of 250 families.
- In 2020, the low density SNP panel was validated for parentage analysis and continent of origin assignment.




Selection for growth improvement

- Growth is evaluated both in fresh water (selection candidates) and at sea (sib groups).
- Broodstock candidates are evaluated at 2 and 3 years old. Individual phenotypes are recorded for body weight, length, gender and maturity.
- Phenotypes (weight, length, gender) and genotypes are also collected from sib groups reared at sea (2,000 to 3,000 individuals per year class).
- Pedigrees are reconstructed using the genotype information from a panel of 245 SNP markers.

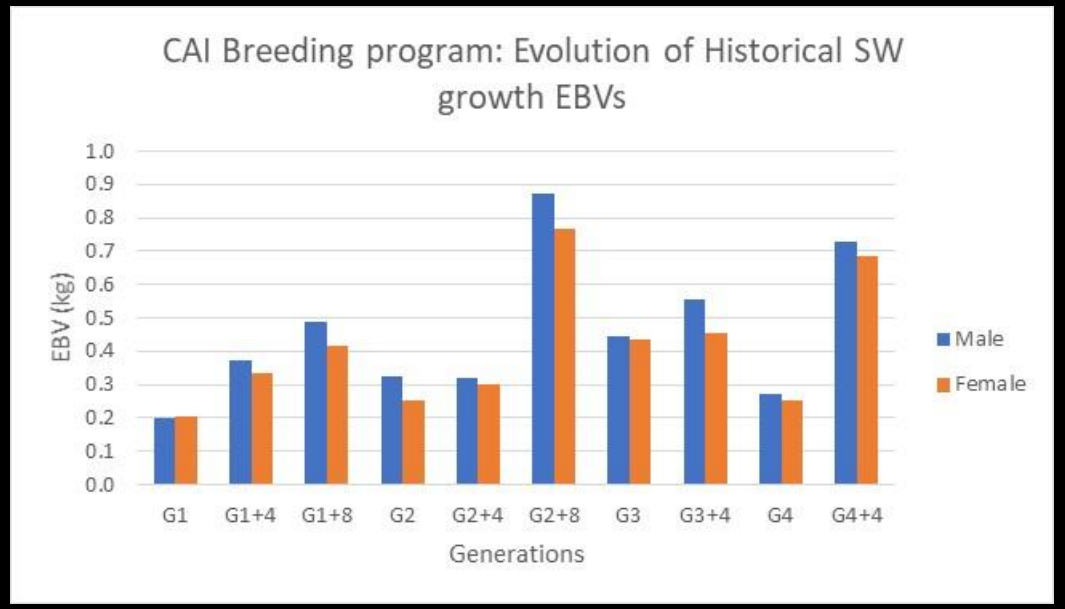
- Estimated breeding values are calculated using BLUP (best linear unbiased predictors) animal model.
- An eight traits' model is used to estimate individual breeding values for both male/female weight and length.

-> Evidences of a gender dimorphism for growth in NA Atlantic salmon strain.

- With this model, heritability estimates for growth at sea are as follow:

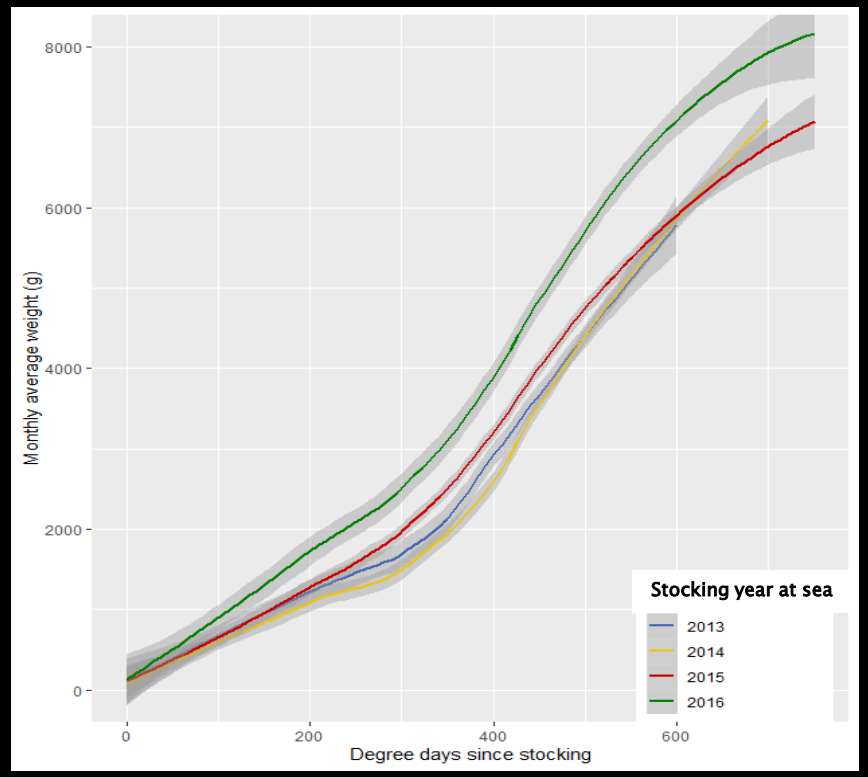
$$h2\ SWw\ F = 0.478 \pm 0.0367$$
$$h2\ SWw\ M = 0.441 \pm 0.0452$$

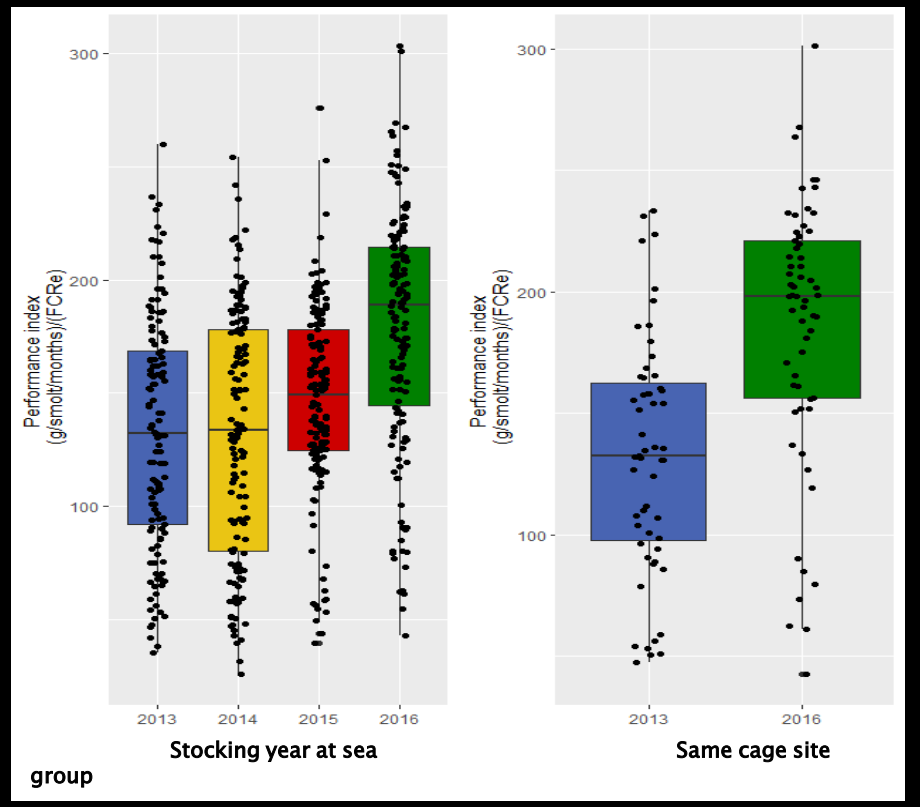
- Over the past twelve years, estimated genetic gains for growth at sea are on average +0.88kg for males and +0.77kg for females:



- Analysis of commercial data:

Spring entries at sea between 2013 and 2016





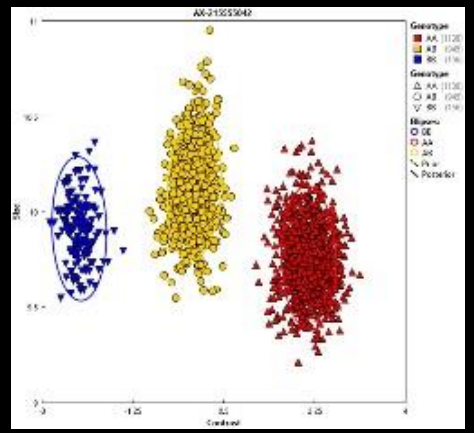
-> Genetic gains effectively transmitted to the commercial production.

Development of new genomic tools

- **High density SNP chip:**

  - Two Affymetrix genechip arrays: 50K and 62K

  - Ongoing collaboration with NMBU, Cigene and Aquagen SA to produce a third version of the array using the genome reference for our NA strain.



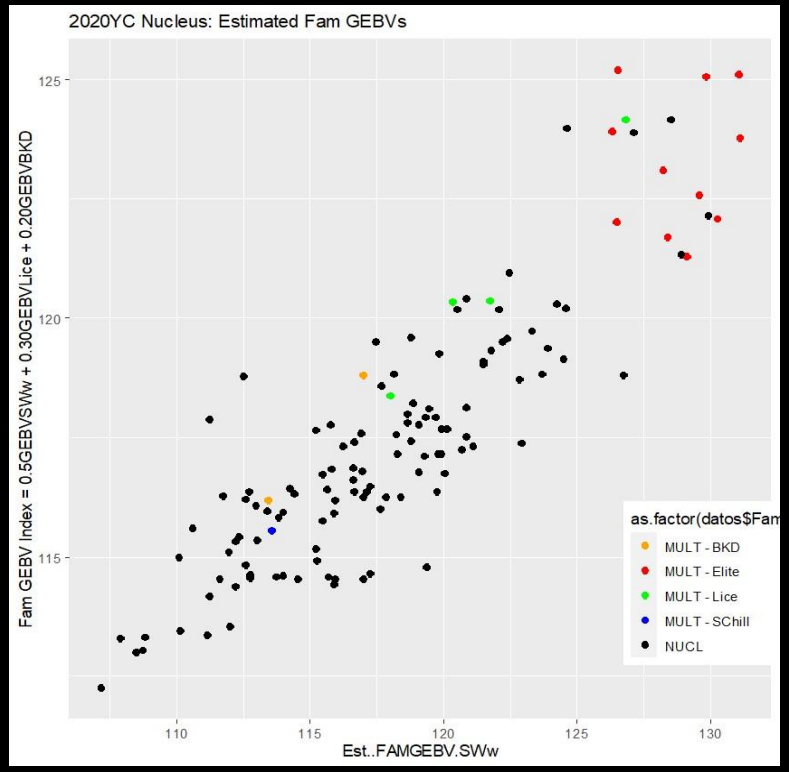
Norwegian University of Life Sciences

CIGENE Centre for Integrative Genetics

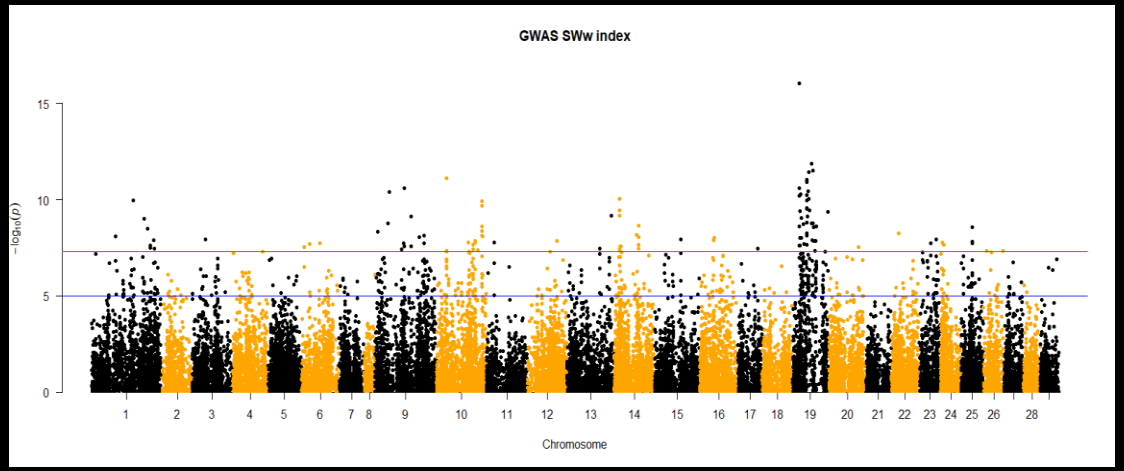
AquaGen

- Since 2014, 16K animals have been genotyped: broodstock candidates +SW eval and disease challenges animals.

- Since 2016, the company has selected candidate nucleus (NUCL) and multiplier (MULT) families for SW growth, bacterial kidney disease (BKD) and sea lice resistance based on estimated genomic breeding values (GEBVs).



- Preliminary association studies (GWAS) were carried out, last spring, using SNP positions from the European Atlantic salmon genome assembly (ICSASG_v2):

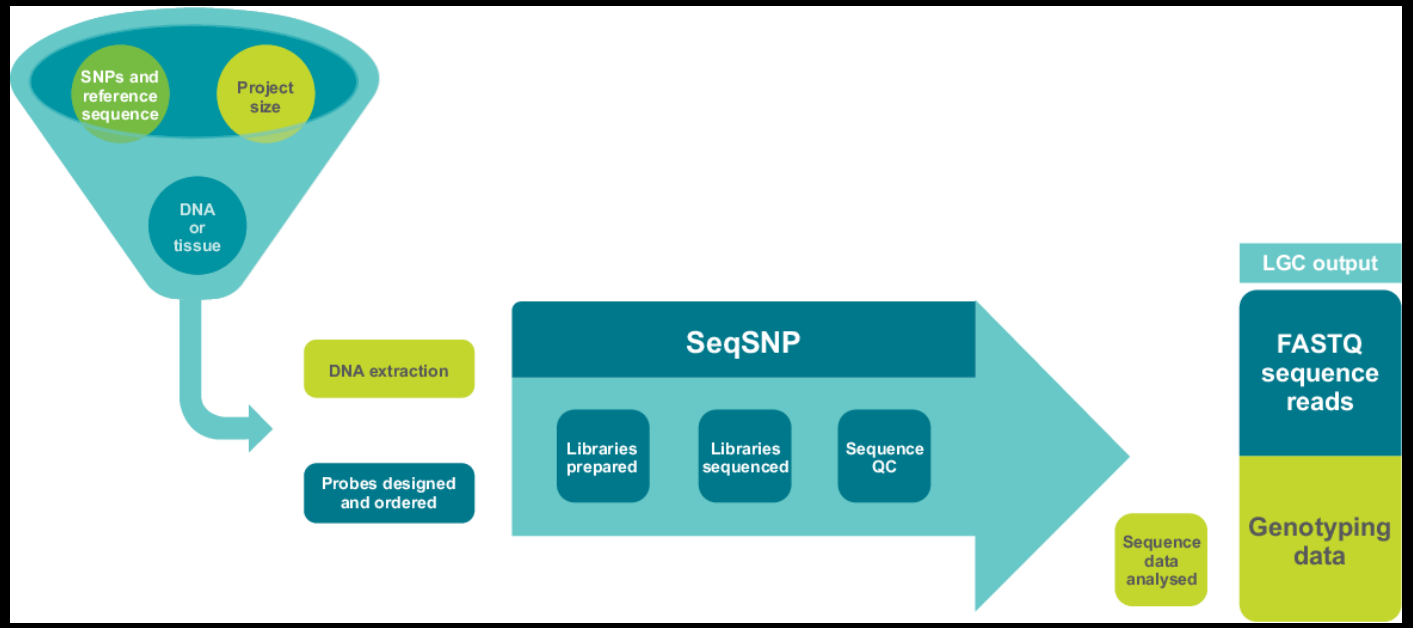


-> A list of 90 SNPs, with large effects on the observed phenotypic variation for major selection traits in CAI population, was produced.

- **Customized low density SNP assay (LGC, Biosearch Technologies):**

- Panel of 632 SNP markers specifically selected for parentage, Continent of Origin (COO) and gender assignments.

- Use of high-throughput cost-effective SeqSNP genotyping technology.



– First genotyping project successfully ran between April and June 2019:

- 4,275 candidates genotyped with an av. “no call” rate of 4.0% per candidate.
- Identification of 50 SNP markers with rates of incongruent allele calls between Affy. array and SeqSNP >10%.
- LD panel validated for both parentage and COO assignments on the 2016YC.

-> SeqSNP technology is a flexible genotyping platform: offers the possibility to include new informative SNP markers (future Implementation of Marker-Assisted Selection).

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AquaGen

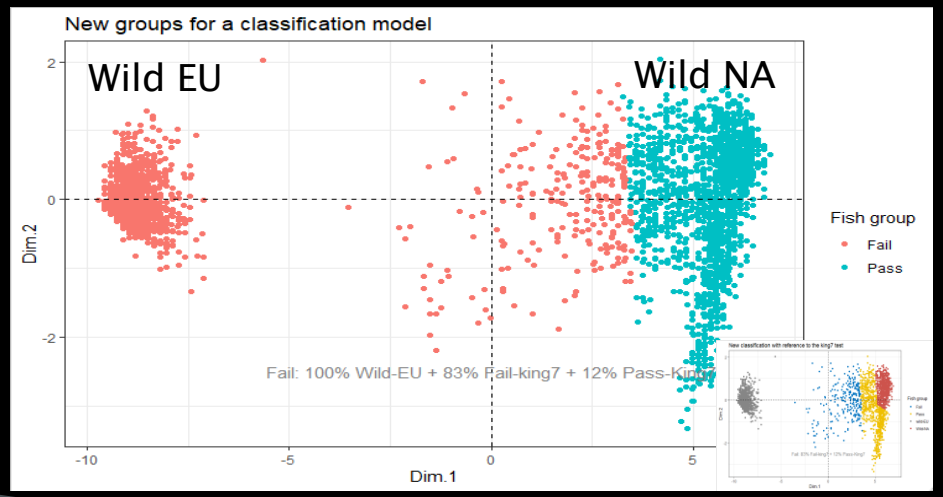
Integration of these genomic tools in CAI commercial breeding program

- **Parentage analysis (Biosearch Technologies' SeqSNP assay):**

– validation of a set of 243 SNPs for parentage assignment: 97.4% offspring successfully assigned to a family (2016YC).

- **Continent of Origin assignment test (Biosearch Technologies' SeqSNP assay):**

– using a panel of 55 SNPs with high allele freq. differences between wild EU and wild NA stocks:



- **Gender assignment (Biosearch Technologies' SeqSNP assay):**

- 6 SNP markers validated with an overall 98% accuracy.

- **Genomic selection (Affymetrix HD SNP chip):**

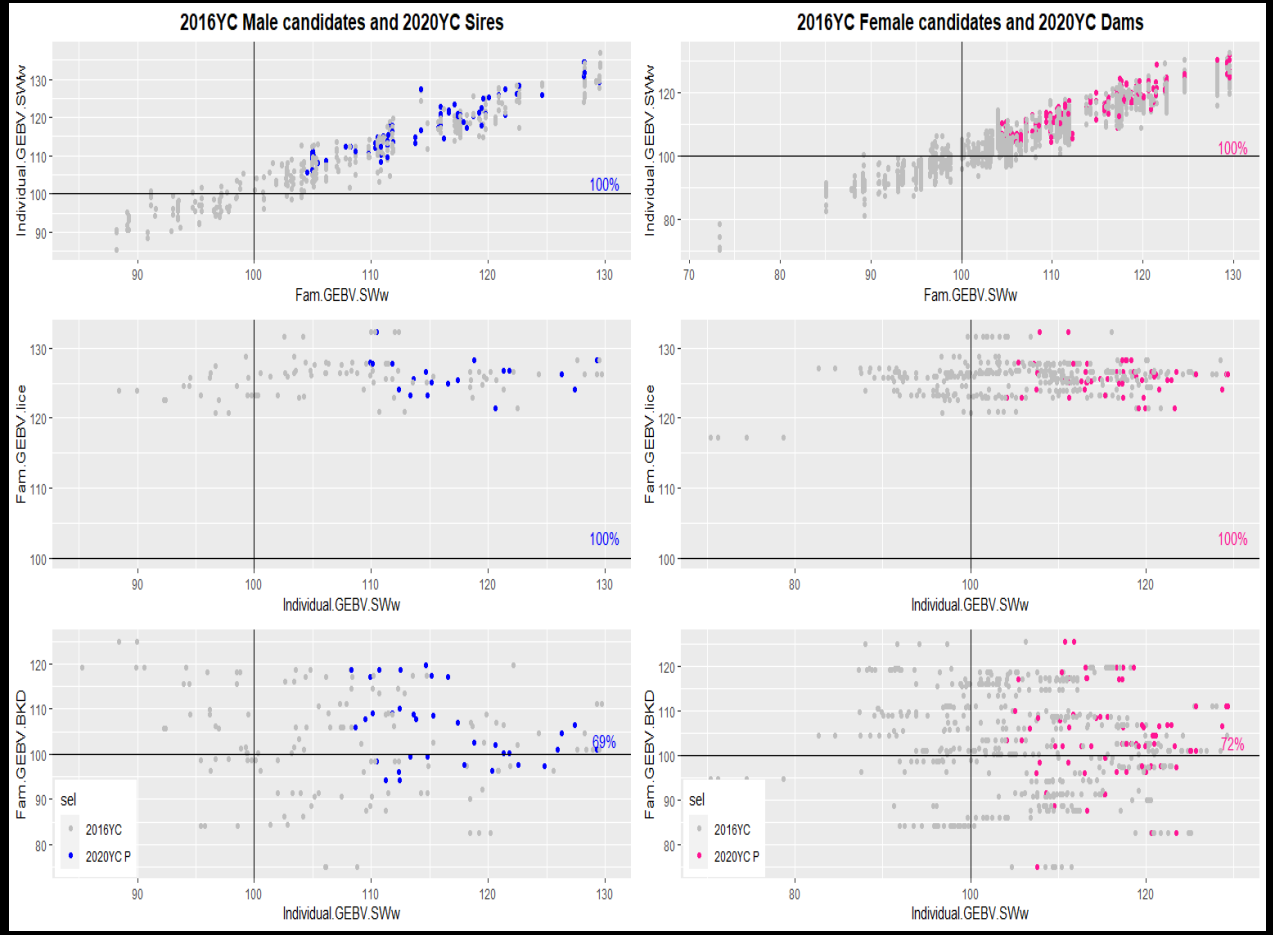
- Since 2016, GEBVs calculated for growth, lice and BKD resistance.

- In 2019, use a single step GBLUP analysis combining pedigree and molecular data.

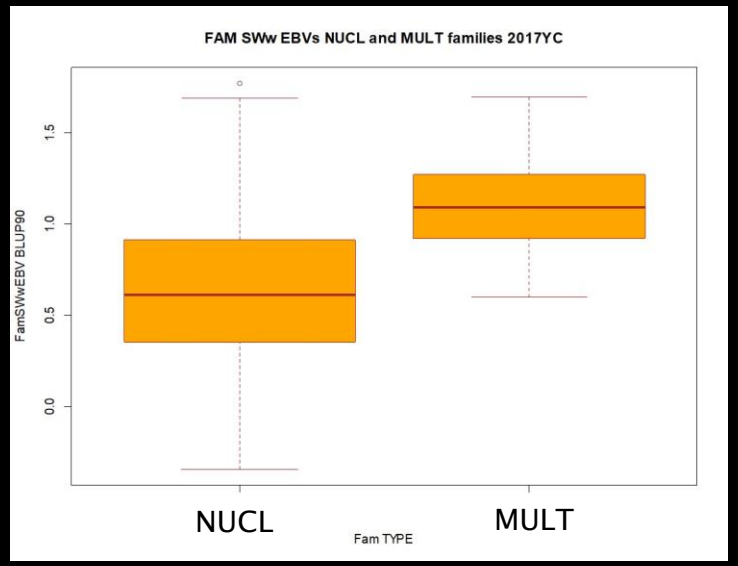
- Estimated heritabilities for Lice and BKD resistance suggest that significant genetic improvements are achievable:

	Lice	BKD
Lice	0.319	-0.037
BKD		0.324

- The use of genomic breeding values showed more within-family variation than BLUP breeding values. We expect it will have a positive impact on genetic gains.

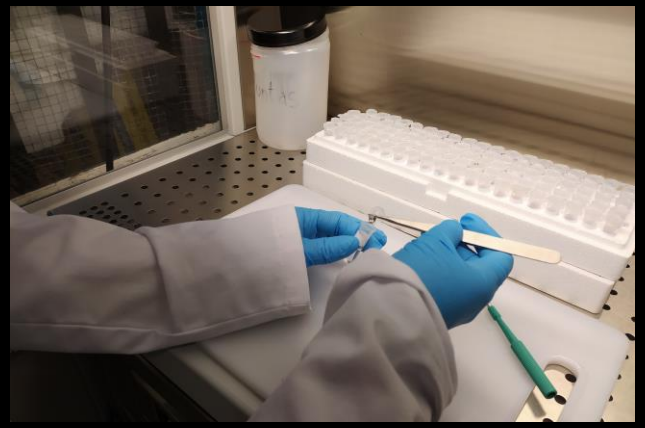


-> Since 2016, the parents used to produce the select crosses are among the "best" sibs from the "best" families:



Further developments

- Adding “large” effect pathogen SNP markers to **Biosearch Technologies’** SeqSNP assay.
- Combine genomic selection for the breeding nucleus and marker-assisted selection for the commercial production of juveniles.
- Develop a cost-effective KASP™ assay (max. 300 SNPs) for parentage analysis and COO assignment in collaboration with Biosearch Technologies.



Conclusion

- The introduction of genomic tools in our breeding program has significantly improved selection accuracy for both SW growth and disease resistance.
- The use of Biosearch Technologies' SeqSNP technology has allowed us to screen and evaluate more broodstock candidates used in our commercial egg production line.
- Over the past two years, Biosearch Technologies and CAI have developed a strong service provider/customer relationship.
- In 2021, Biosearch Technologies will develop new genomics tools for CAI seabass (*Dicentrarchus Labrax*) and seabream (*Sparus Aurata*) breeding division in the Mediterranean (Culmarex group).

Funding and partners

Thank you for attention!

ADVANCES IN GENETICS AND GENOMICS

REVIEWS IN Aquaculture

Reviews in Aquaculture, 1–14 doi: 10.1111/raq.12335

Advances in genetic improvement for salmon and trout aquaculture: the Chilean situation and prospects

Jean P. Lhorente[1], Marcelo Araneda[1,2], Roberto Neira[3] and José M. Yáñez[4,5]

ANIMAL GENETICS Immunogenetics, Molecular Genetics and Functional Genomics

REVIEW

doi: 10.1111/age.12989

Genomics to accelerate genetic improvement in tilapia

J. M. Yáñez*,† , R. Joshi‡ and G. M. Yoshida*

*Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Av Santa Rosa 11735, La Pintana, Santiago 8820808, Chile. †Núcleo Milenio INVASAL, Casilla 160-C, Concepción, Chile. ‡GenoMar Genetics AS, Bolette Brygge 1, Oslo 0252, Norway.

Traits that are difficult to measure…

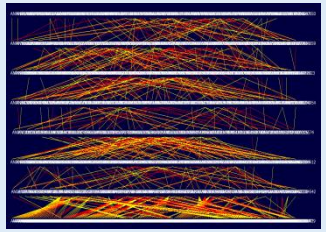
Disease resistance

Family-based selection

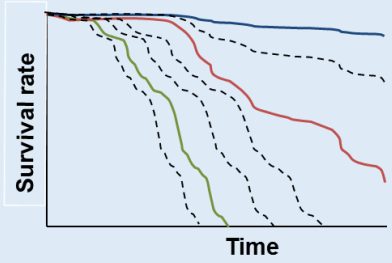
- ↓ EBVs accuracies
- Selection intensity ↔ inbreeding
- Limited genetic progress

Carcass quality

Pedigree data



Phenotypes



SNPs

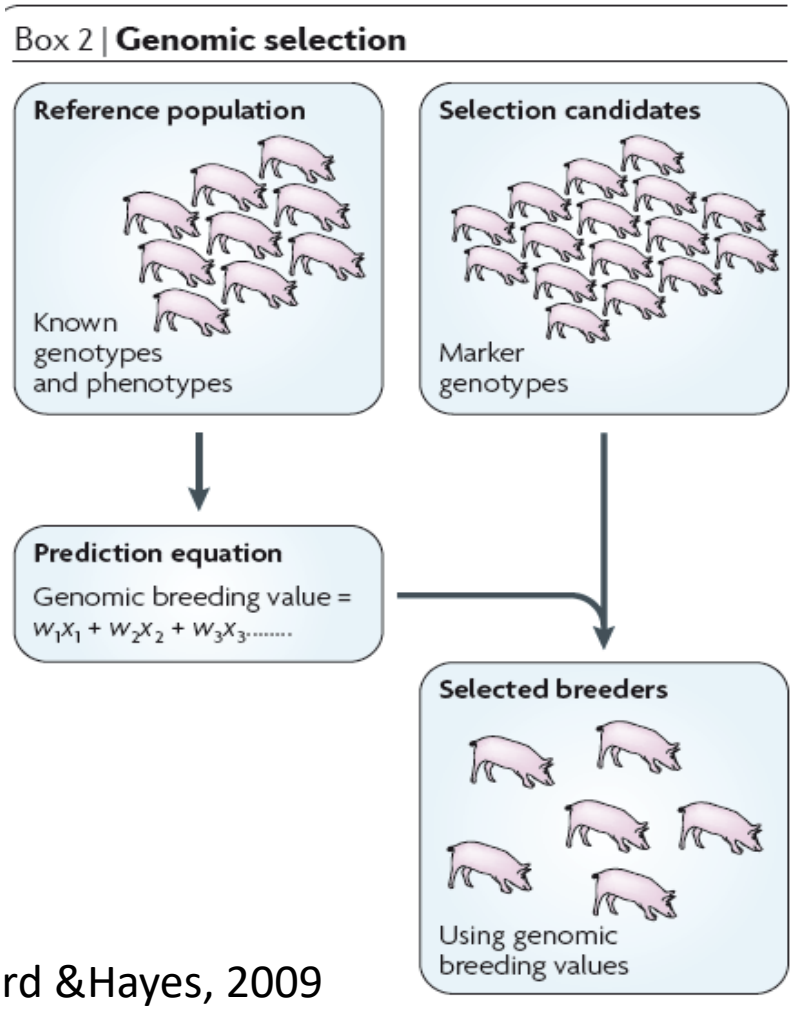
Marker effects

$$y_i = \mu + \sum_j^n X_{ij} a_j + e_i$$

Genomic selection

- ↑EBVs accuracies
- ↑ Response to selection
- Accelerates genetic progress

GENOMIC PREDICTIONS

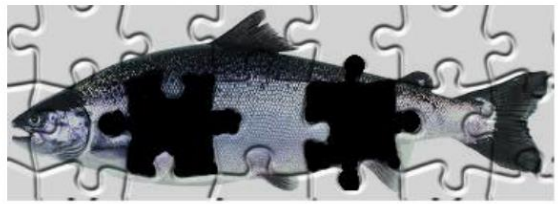


Goddard &Hayes, 2009

IMPUTATION OF GENOTYPES

- Prediction of missing genotypes using information from a reference population
- Low-density SNP panels are cheaper than high-density ones

Reference



Target

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REFERENCE2  1 0 1 1 1 0 0 0 1 0 2 1 1 0 1 1 1 1 1 0 1 0 2 0 0 0 0 0 0 1 0 0 0 0 1 1 0 0 0 2 0 0 1 1 0 2 1 0 1 1
REFERENCE3  0 0 0 1 0 0 0 0 0 0 0 0 0 0 0 1 1 1 0 1 1 2 0 0 1 1 1 0 1 0 0 0 0 0 1 1 0 1 1 2 0 1 0 2 1 1 1 0 0 1
REFERENCE4  1 1 1 2 0 0 1 0 0 0 0 0 1 0 0 1 1 0 1 2 0 1 0 0 0 0 0 1 2 1 0 0 0 0 1 1 0 2 1 0 0 0 1 1 1 1 1 1 1
REFERENCE5  1 1 0 2 0 0 1 0 0 1 0 1 1 1 1 1 1 1 0 2 1 1 1 0 0 2 0 0 0 2 0 0 0 0 2 0 1 0 2 0 0 0 1 2 1 0 1 2 0 0
TARGET1     1 1 1 1 0 0 ? 0 0 0 0 0 1 0 0 ? 1 0 0 2 0 1 0 0 1 0 1 0 1 1 0 0 0 1 2 0 0 1 0 1 0 0 2 1 2 2 1 2 1
TARGET2     1 1 0 0 0 0 ? 0 0 2 0 0 1 0 0 2 0 ? 1 2 1 0 0 0 2 2 1 1 2 ? 0 0 0 1 1 0 0 2 1 0 0 0 2 1 2 1 0 1 0
TARGET3     1 0 1 2 0 1 ? 0 0 0 0 0 2 0 0 1 0 0 1 1 0 1 0 0 1 1 0 0 2 1 1 0 0 0 1 1 1 1 1 0 0 0 2 0 2 0 1 0 0
TARGET4     0 1 1 0 0 0 ? 0 0 1 0 0 0 0 1 2 1 1 0 0 1 0 1 1 1 0 1 0 0 ? 0 0 0 1 1 0 0 2 0 1 1 2 1 1 1 0 1 0 0
TARGET5     1 1 0 0 0 0 ? 0 0 1 1 0 2 0 0 1 1 0 1 1 0 2 0 1 0 0 2 ? 0 0 1 1 0 0 0 1 ? 2 1 0 0 0 0 0 0 0 0 1 0
TARGET6     1 1 1 1 0 1 ? 0 0 1 0 1 1 0 0 1 0 0 1 0 2 1 1 0 2 0 2 0 1 0 0 0 0 1 0 0 1 0 1 1 ? 1 0 0 2 2 1 2 1
TARGET7     0 2 1 0 0 1 ? 0 0 1 0 0 0 0 0 1 1 0 0 0 0 ? 0 2 0 0 0 0 1 0 0 0 0 1 1 1 1 2 0 0 0 1 0 0 1 1 0 0 0
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IMPUTATION IN ATLANTIC SALMON

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Accuracy of genotype imputation and genomic predictions in a two-generation farmed Atlantic salmon population using high-density and low-density SNP panels

Grazyella M. Yoshida[a,b], Roberto Carvalheiro[b], Jean P. Lhorente[c], Katharina Correa[c], René Figueroa[c], Ross D. Houston[d], José M. Yáñez[a,c,e,*]

Table 1
Imputation accuracy from low-density (LD) to high-density (HD) panel in Atlantic salmon using groups with different numbers of animals in reference and validation set and different scenarios of available genotypic information.

Scenario	Reference			Size	Validation[b]	LD0.5 K	LD3 K	LD6 K
	Sires n = 19	Dams n = 34	Sibs[a]			Mean	Mean	Mean
Group A								
A1	HD	HD	HD	1015	LD	0.948	0.980	0.983
A2	HD	–	HD	981	LD	0.863	0.967	0.973
A3	–	HD	HD	996	LD	0.864	0.962	0.971
A4	–	–	HD	962	LD	0.851	0.976	0.982
A5	HD	HD	–	53	LD	0.829	0.942	0.951

- Low-density SNP panels (from 0.5 to 6K) can be used to accurately impute to higher density genotypes (50K).
- **LGC, Biosearch Technologies' KASP™** and **SeqSNP targeted GBS** are good options to genotype low density SNP panels.

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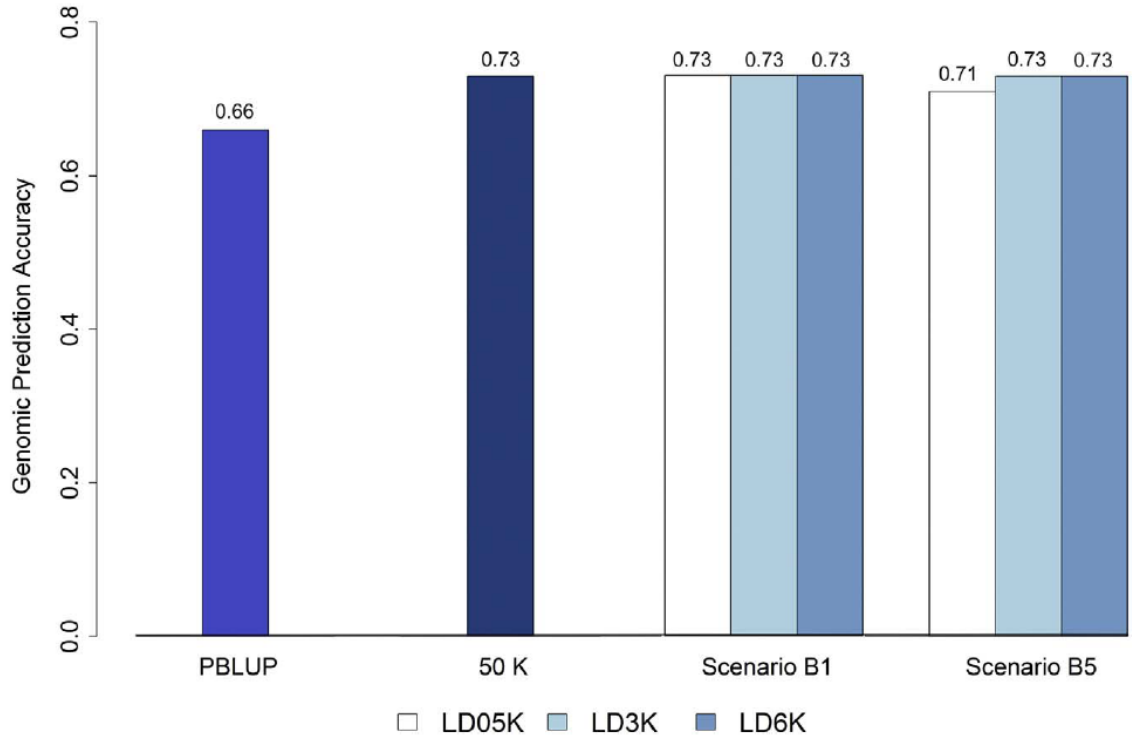


Imputation from low-density to higher density SNP panels did not considerably decrease accuracy of genomic selection.

LOW-COST GENOMIC PREDICTIONS FOR TILAPIA

G3 Genes | Genomes | Genetics

GENOMIC PREDICTION

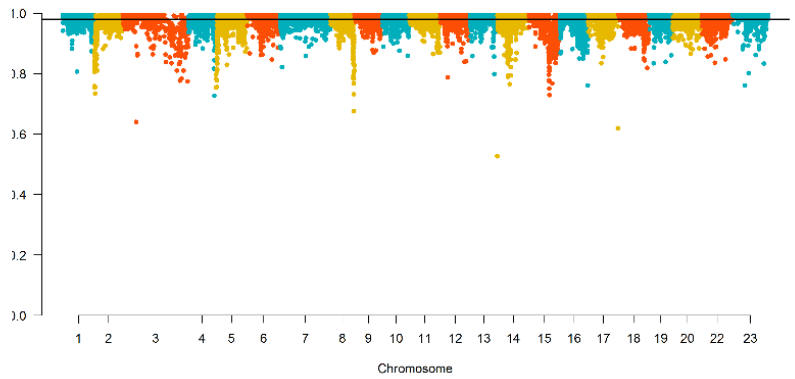
Genome-Wide Association Study and Cost-Efficient Genomic Predictions for Growth and Fillet Yield in Nile Tilapia (*Oreochromis niloticus*)

Grazyella M. Yoshida,*,† Jean P. Lhorente,† Katharina Correa,† Jose Soto,‡ Diego Salas,‡ and José M. Yáñez*,†

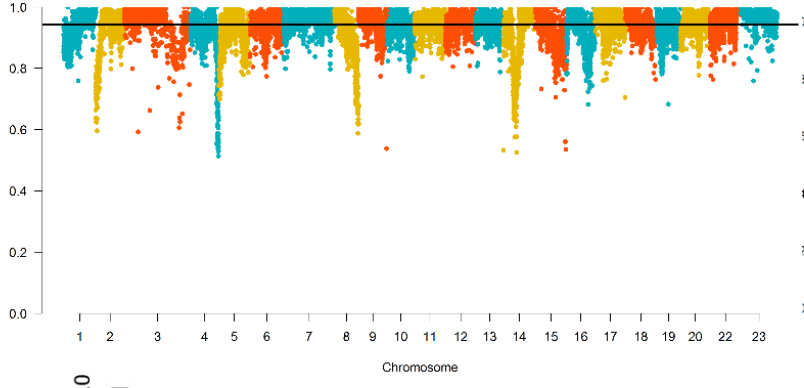
*Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santiago, 8820808 Chile, †Benchmark Genetics Chile, Puerto Montt, Chile, and ‡Grupo Acuacorporacion Internacional (GACI), Cañas, Costa Rica

ORCID IDs: 0000-0002-6788-7369 (G.M.Y.); 0000-0002-6612-4087 (J.M.Y.)

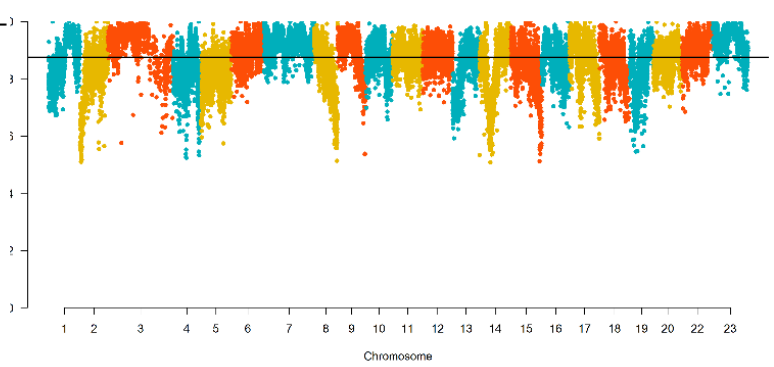
From 3K to 32K

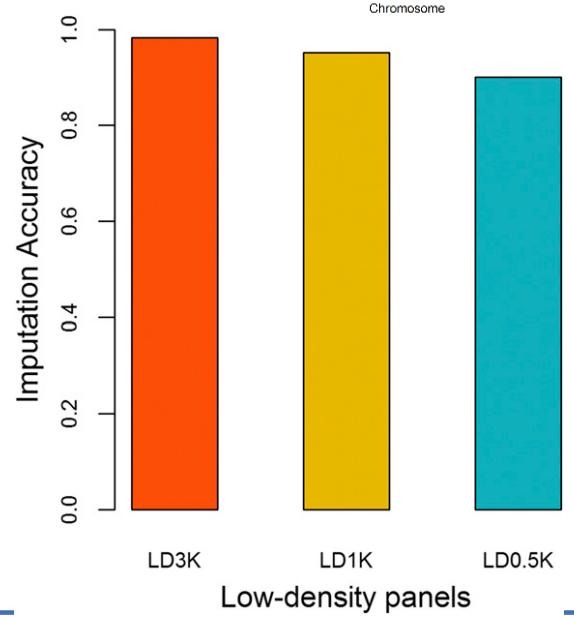


From 1K to 32K



From 0.5K to 32K





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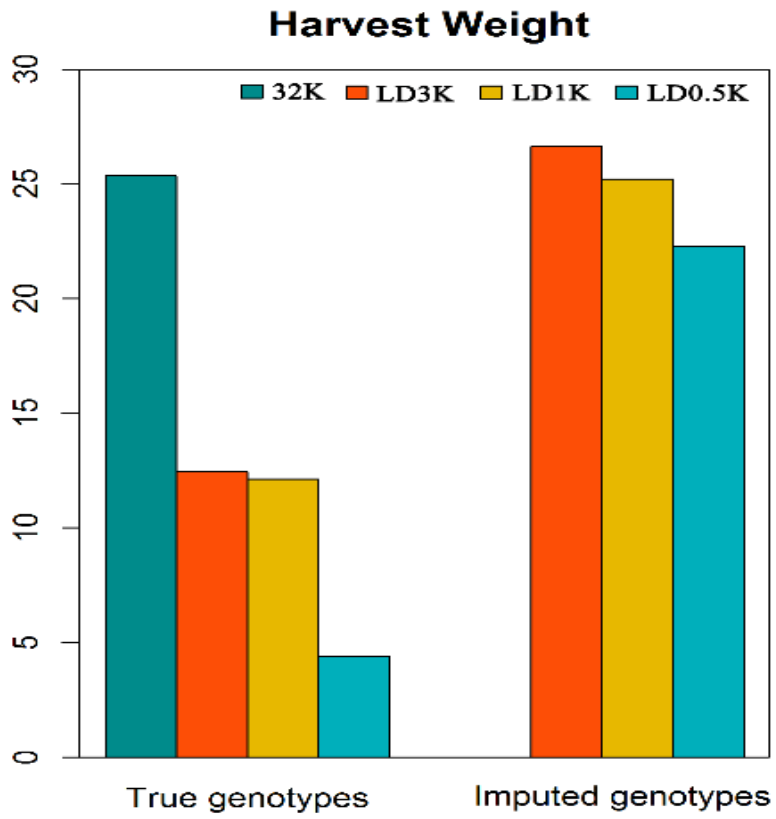

Genomic prediction accuracy

COST REDUCTION IN GENOMIC SELECTION

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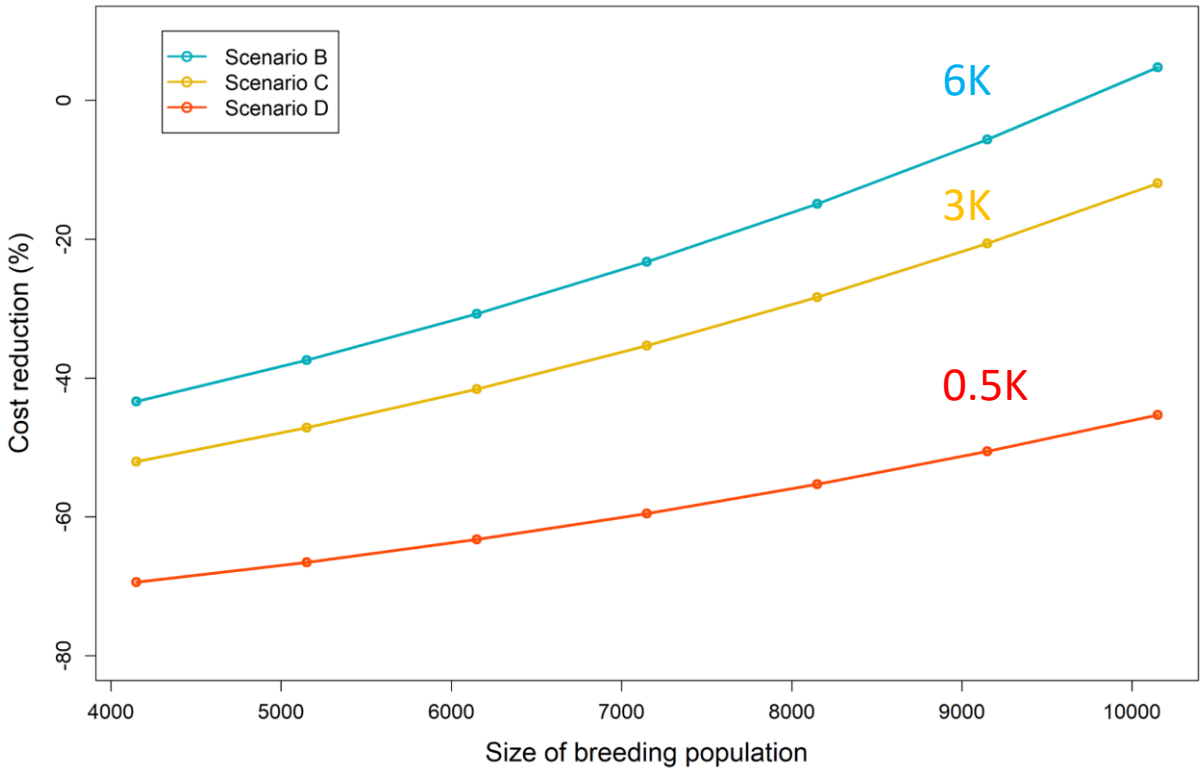


50K = $ 50; 6K = $ 25; 3K = $ 20; 0.5K = 10$.

For **Scenario A**, a decrease in 10% of the cost per 1K animals was assumed.

Scenario A (base case): All animals genotyped with 50K SNPs

Scenario B: All parents (n=150) and 10% of the offspring genotyped with 50K SNPs and the remaining animals with 6K SNPs (KASP or SeqSNP targeted GBS)

Scenario C: All parents (n=150) and 10% of the offspring genotyped with 50K SNPs and the remaining animals with 3K SNPs (KASP or SeqSNP targeted GBS)

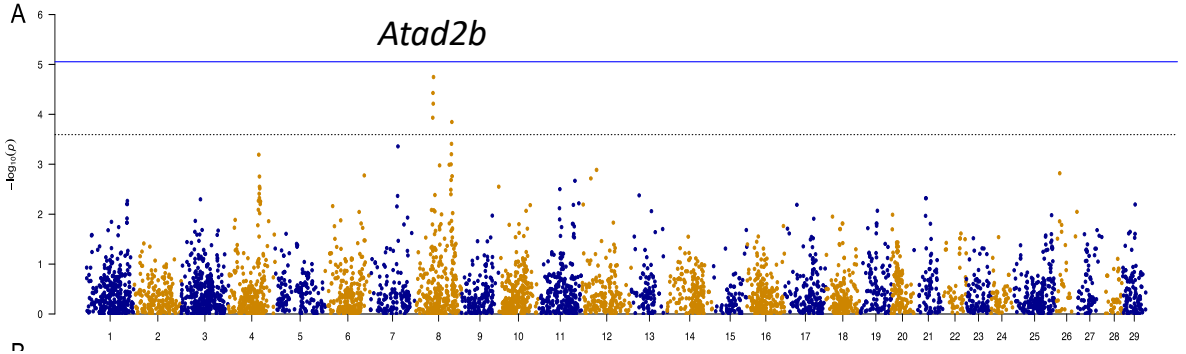
Scenario D: All parents (n=150) and 10% of the offspring genotyped with 50K SNPs and the remaining animals with 0.5K SNPs (KASP or SeqSNP targeted GBS)

Jousepth Gallardo-Hidalgo

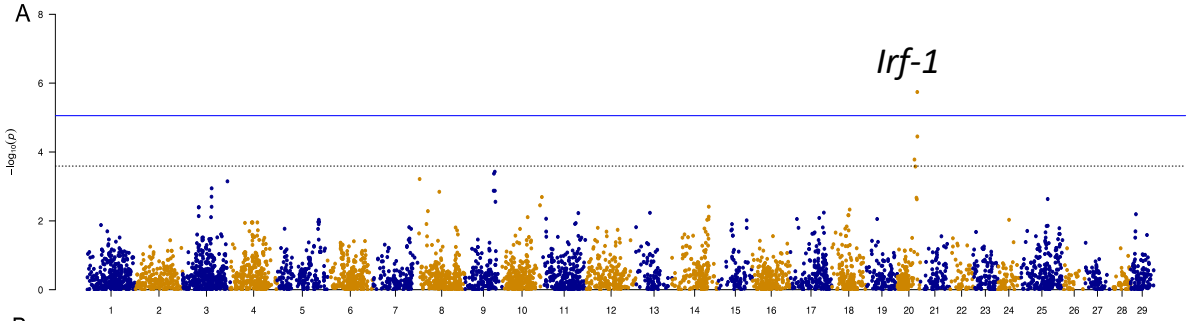
GWAS FOR GROWTH AT EXTREME TEMPERATURES IN TROUT

- 192 fish genotyped with a 20K SeqSNP panel
- 614 fish genotyped with a 1K SeqSNP panel
- Imputation from 1K to 20K SNPs
- *Atad2b* → Thermal stress in catfish, bluefin tuna, arctic charr and longjaw Mudsucker (Gillichthys mirabilis)
- *Irf-1* → mediates cell growth inhibition and its expression is regulated by Growth Hormone

Average daily gain at 22 °C



Average daily gain at 7 °C



HOW TO MAXIMIZE ACCURACY OF GENOMIC PREDICTIONS

Pérez-Enciso *et al. Genetics Selection Evolution* (2015) 47:43
DOI 10.1186/s12711-015-0117-5

GSE Genetics Selection Evolution

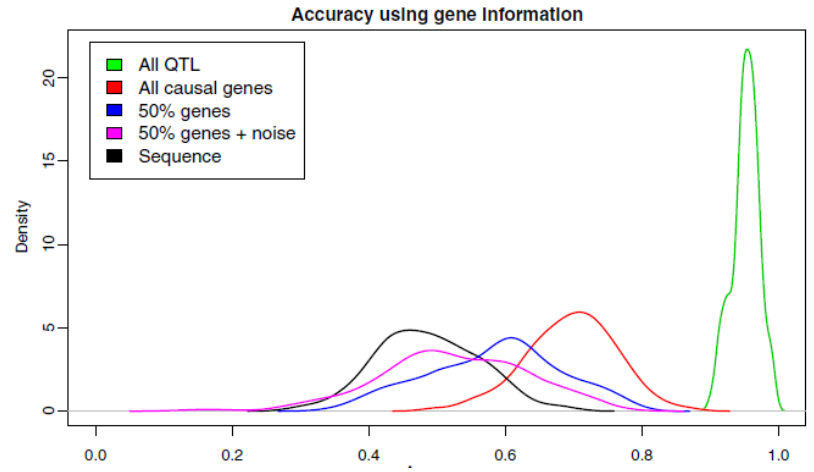
RESEARCH Open Access

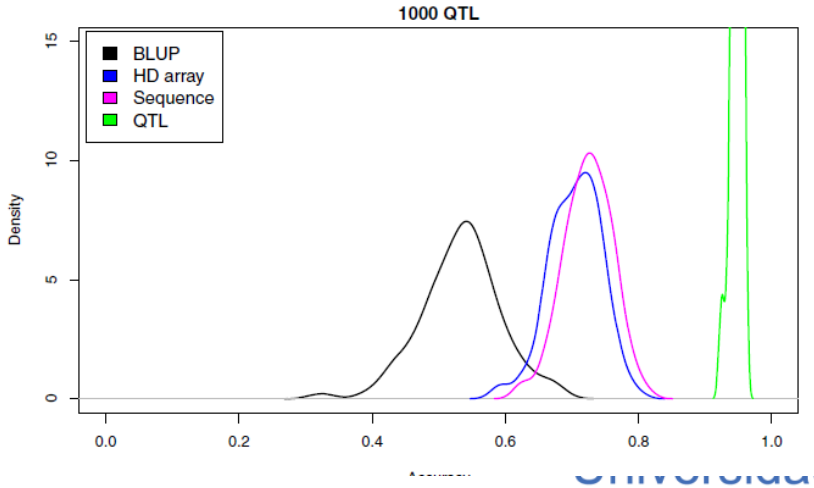
Sequence- vs. chip-assisted genomic selection: accurate biological information is advised

Miguel Pérez-Enciso[1,2,3*], Juan C Rincón[1,4] and Andrés Legarra[5]

Table 3 Accuracy obtained with different strategies

Method	Number of SNPs*	Number of QTN	
		20	100
Pedigree BLUP	-	0.38 (0.09)	0.43 (0.09)
RAD	11,000	0.28 (0.10)	0.27 (0.09)
Medium-density array	7,500	0.45 (0.09)	0.45 (0.09)
High-density array	17,000	0.47 (0.08)	0.47 (0.08)
Sequence	335,000	0.49 (0.10)	0.49 (0.08)
Causal SNPs	20/100	0.98 (0.01)	0.95 (0.02)





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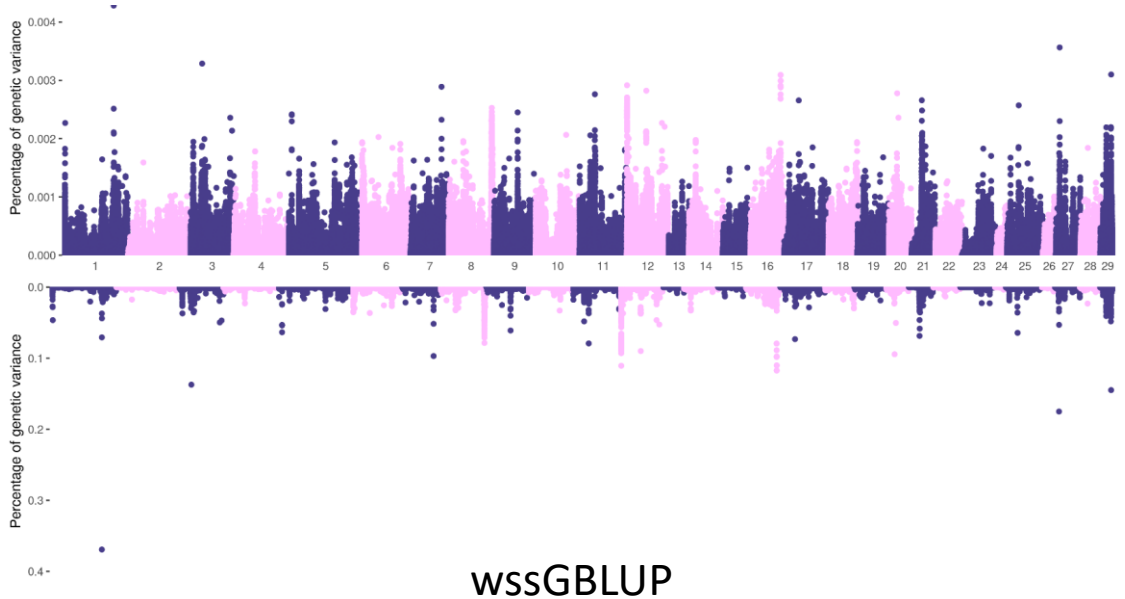
PRIORITIZATION OF SNPS FOR GENOMIC PREDICTIONS

Grazyella Yoshida

- 192 fish genotyped with a 20K SeqSNP panel
- 614 fish genotyped with a 1K SeqSNP panel
- Imputation from 1K to 20K SNPs
- WGS data on >100 rainbow trout parents (10X)
- 22 million SNPs
- Body weight after 2 months of termal stress (>20°C)
- Fish phenotyped = 1,800
- Fish genotyped = 800
- Genotypes imputed to 1.4 million SNPs
- Weighted single-step GBLUP

wssGBLUP



wssGBLUP

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PRIORITIZATION OF SNPS FOR GENOMIC PREDICTIONS

Grazyella Yoshida

- **Accuracy of genomic predictions:**
  - **PBLUP:** No SNPs
  - **50K:** randomly distributed
  - **WGS:** imputed 1.4 million SNPs
  - **22K SNP:** prioritized based on the variance explained in wssGBLUP (descending order)

Method	Mean	SD	% Increase
PBLUP	0.667	0.074	0.000
50K_Random	0.705	0.117	5.674
WGS	0.708	0.112	6.198
22K_wssGBLUP	0.817	0.039	22.554

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CONCLUSIONS

- Genomic selection accelerates the genetic progress for disease resistance and other important traits in aquaculture species.
- Imputation from low-density SNP panels (e.g. KASP or SeqSNP) to high-density genotypes decreases the costs of genomic selection in aquaculture.
- wssGBLUP and ultra dense SNP information can be used to increase genomic prediction accuracy by means of SNP prioritization.

International Symposium of Genetics in Aquaculture XIV

28 November – 3 December 2021

Puerto Varas - Chile

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1. Biotechnology and Functional Genomics
2. Sex Control
3. Genomic Prediction
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8. Genetics of Nutrition
9. Epigenetics
10. Genomes and Metabiomes
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Using KASP to Breed Hybrid Shrimp with Improved Survival

LGC, Biosearch Technologies' Aquaculture Webinar

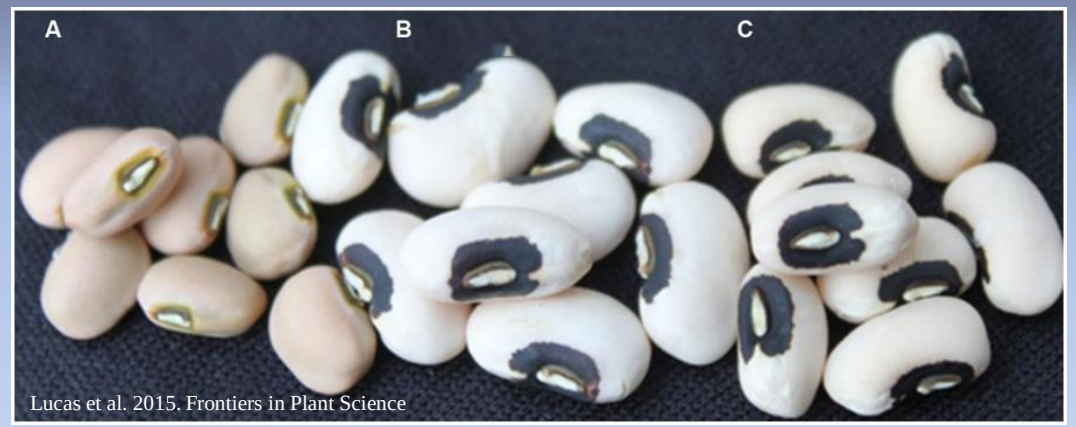
Mitch Lucas

January 12, 2021

Mitchell R. Lucas, Ph.D.
mlucas@sunshrimp.com
Director of Genetics

American Penaeid Inc.
9703 Stringfellow Road
Saint James City, FL - 33956

Breeding with KASP



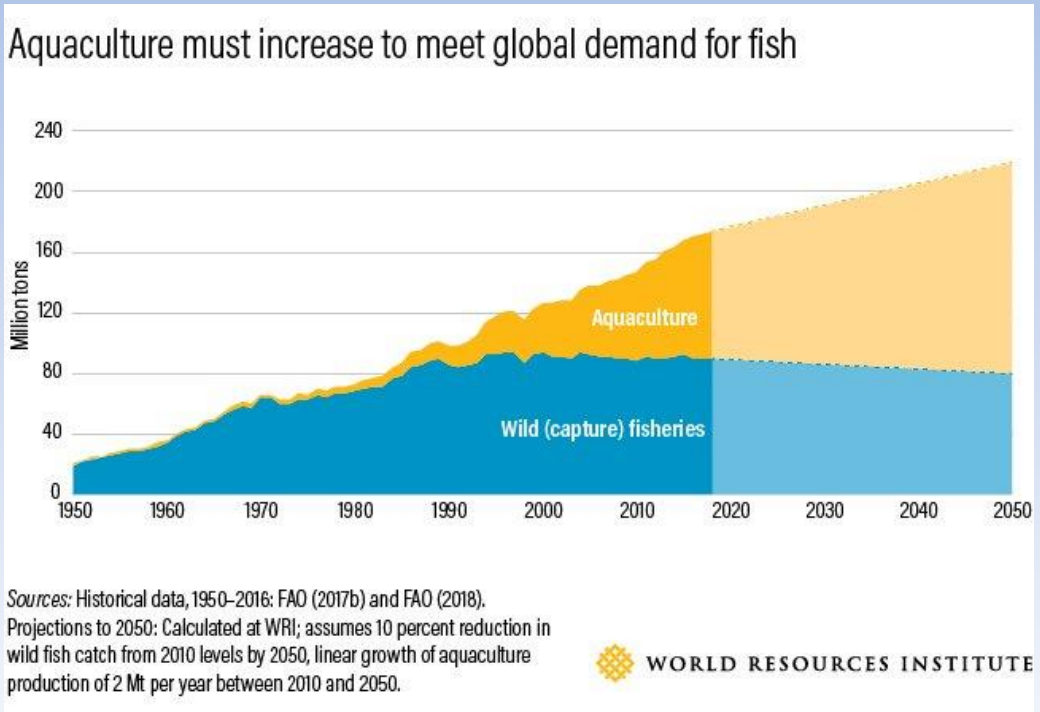
Lucas et al. 2015. Frontiers in Plant Science

UC Riverside
Blackeyed Pea
Seed Size

Syngenta
Bell Peppers
Virus Resistance

American Penaeid Inc
P. vannamei Shrimp
Survival

Opportunity in Aquaculture



- Limited Domestication History
- Many newly farmed species, large spawns
- Extremely variable farms
- Customized genetic needs

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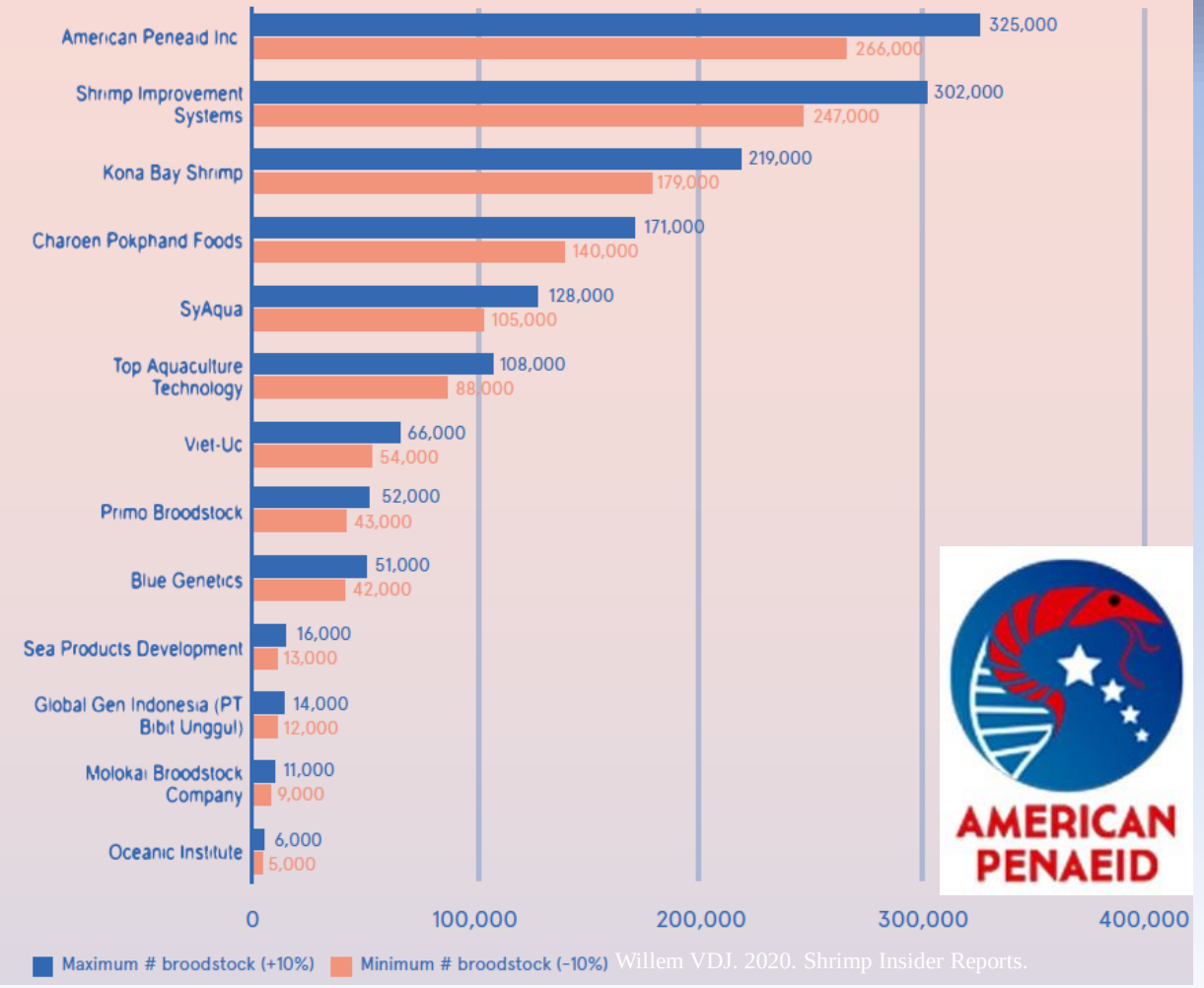
SUN SHRIMP®
Fresh from Florida

Fresh, Clean, Sustainable US Shrimp

FIGURE 1: *SHRIMP INSIGHTS* ESTIMATE OF MARKET SHARES IN 2019[2, 3]



Willem VDJ. 2020. Shrimp Insider Reports.

Broodstock and Post-Larvae Supplier
Custom Genetics Programs

Building an On-Farm Genetics Lab

Sample preparation

We work in multiples of 384 samples, DNA extraction is extremely easy in shrimp and other marine species. Sample processing and analysis within 3 days, usually 1 day DNA extraction/inventory, 1 day PCR and clustering, 1 day analysis for breeding decision.

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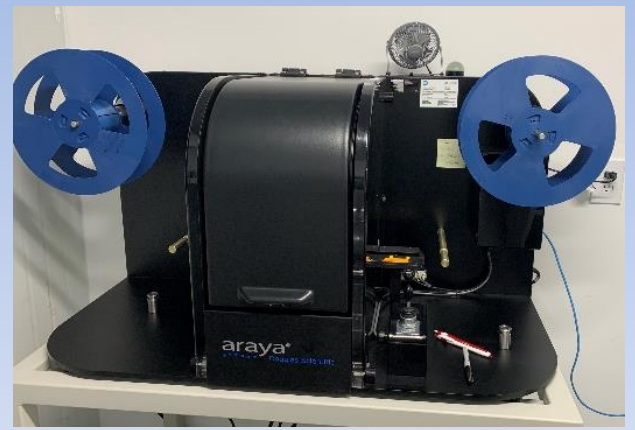
Building an On-Farm Genetics Lab

Nexar™ modular inline liquid handling and assay processing system can process **153K SNP data points per day** at the lowest cost per sample in the industry.

Hydrocycler™ thermal cycler increases throughput.



Araya™ is an inline assay detection instrument that can scan a 384-well array in the Array Tape™ consumable in 28 seconds.

High-throughput, scalable SNP genotyping system

1 scientist routinely process 4,000 samples/day (2,000 DNA, 2,000 PCR) against a panel of genome wide SNPs, with essentially no error.

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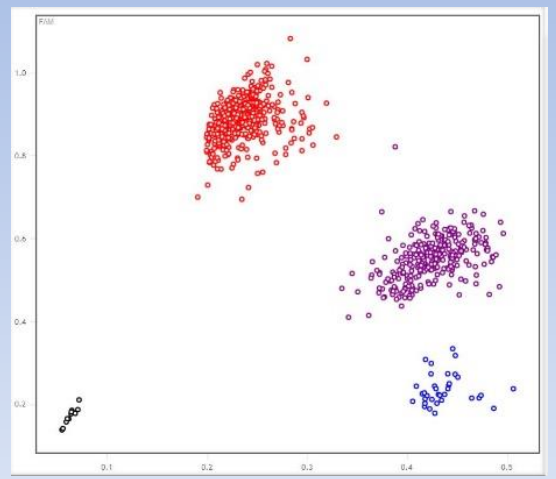
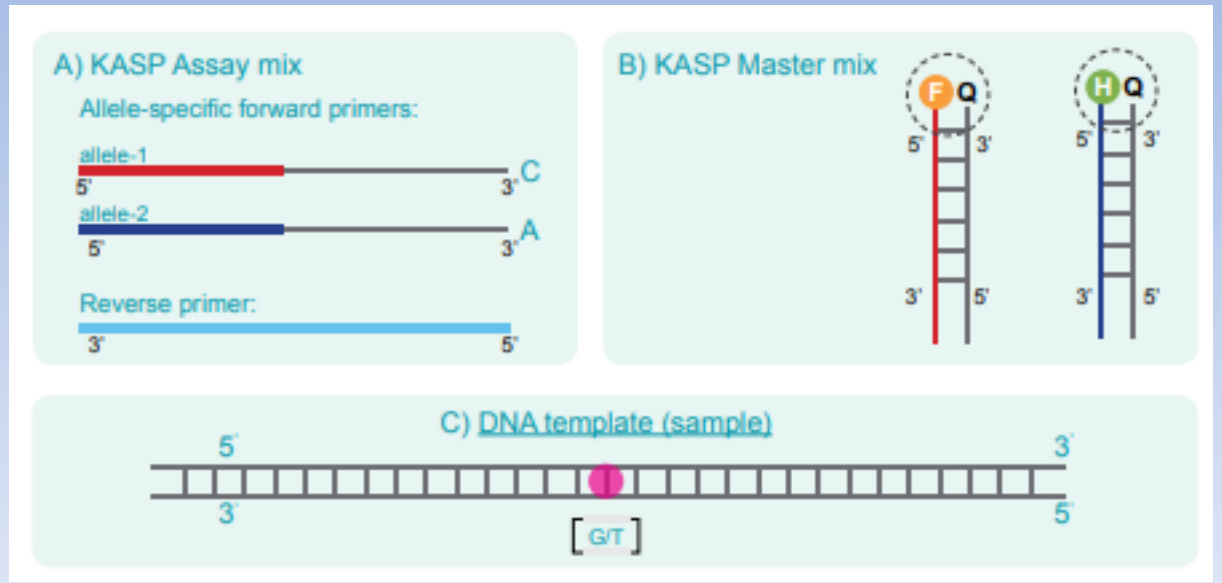
Building an On-Farm Genetics Lab





KASP™ genotyping chemistry

- Off-the-shelf, repeatable, fast, easy to analyze, and tolerant of sample variation.
- Ideal for commercial breeding programs due to gold standard for breeders across industries for its workhorse capability.

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Applications of KASP in Shrimp Breeding

~500,000 Unique Animals Genotyped in <2 years

Hybrid Breeding Program – Germplasm Management

- Manage inbreeding, maximize heterosis and uniformity

Quality Control

- Validate production identity and movements
- Sample every spawn at several timepoints

Trait-Associated Markers

- QTLs, perfect markers

Competitor Analysis

- Who has what and where is it working?

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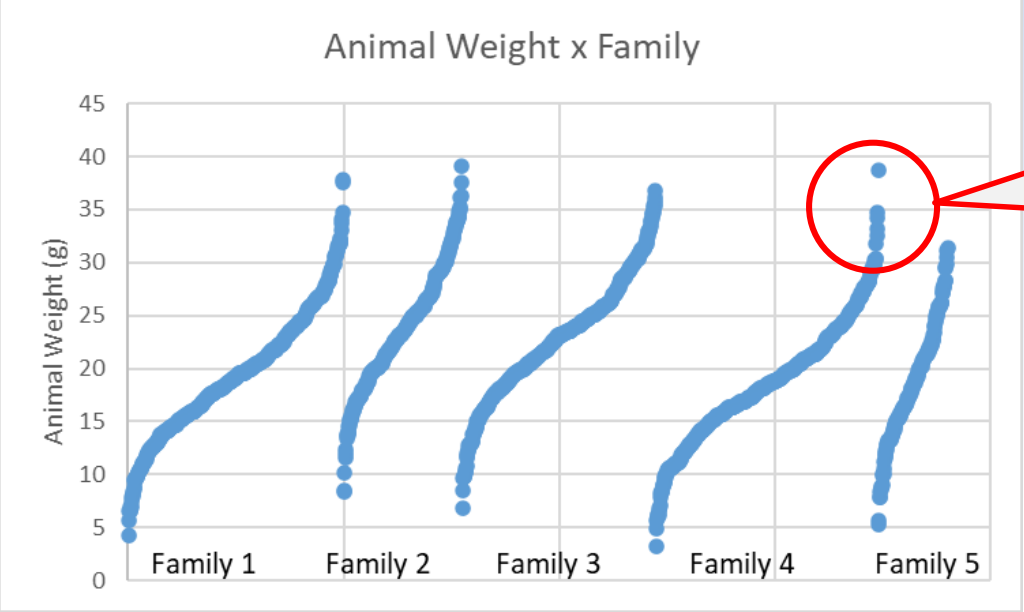
Breeding for Survival 1st, Growth 2nd

Multi-Family Testing

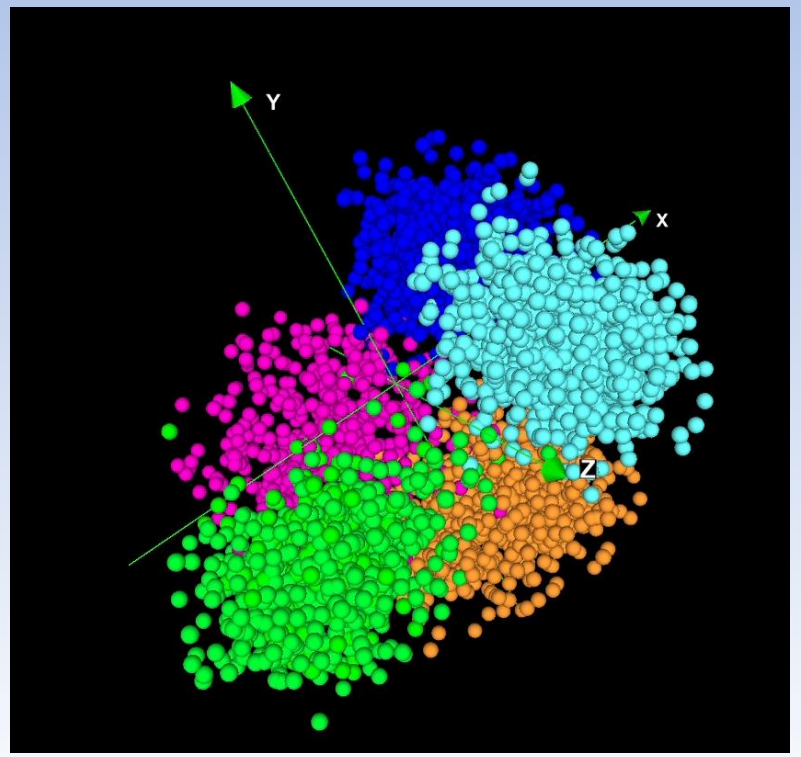
Family	PL Count	PL Proportion	Adult Count	Adult Proportion	Change in Proportion	Relative Survival	Avg. Animal Weight (g)
1	855	0.176	906	0.188	0.012	101.2	19.4
2	926	0.190	520	0.108	-0.083	91.7	23.7
3	1321	0.271	1062	0.220	-0.052	94.8	22.8
4	1014	0.208	1497	0.310	0.102	110.2	18.6
5	751	0.154	845	0.175	0.021	102.1	18.7



~20% Survival Difference
~20% Growth Difference

API Broodstock Selections

5 Family PCA and STRUCTURE



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Thank You!

American Penaeid Inc

Robin Pearl, Margaret Barlow, Miguel Artilles, Tim Morris, Suzanne Li

Contact us for your shrimp and genetic program needs!

LGC, Biosearch Technologies

Liyan Pang and Webinar Team

Jo Eakin, Mike Salentine, Erin Steer, Andres Truong - Sales

Erin Steer, Sean Cantleberry, Dan Harms, Mike New - Lab Setup

Travis Bruns and Intellics Support Team

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