Use of the Applied Biosystems™ Axiom™ and Eureka™ genotyping platforms to fill a gap in low to medium-density genotyping

PAG 2019

Jason Nichols, Becky Breitinger

Key R&D centers across the world

Clinton
US

Greensboro
US

Research Triangle Park, US

Jealott's Hill
UK

Ghent
Belgium

Enkhuizen
Netherlands

Bad Salzuflen
Germany

Stein
Switzerland

Stanton
US

Slater
US

Woodland
US

Beijing
China

Goa
India

Over 100 R&D sites around the world supported by many field locations

Uberlândia
Brazil

Saint Sauveur
France

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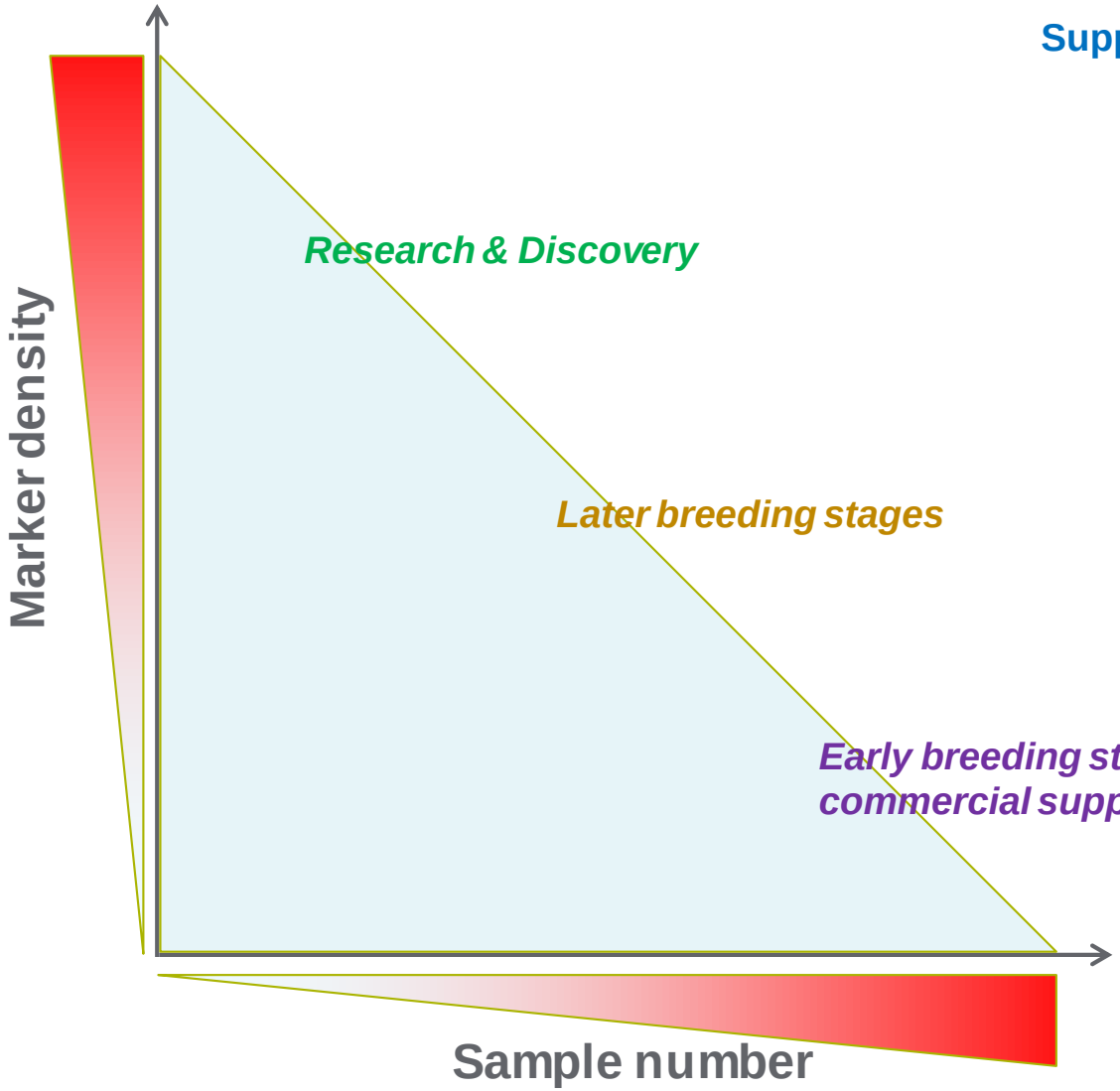
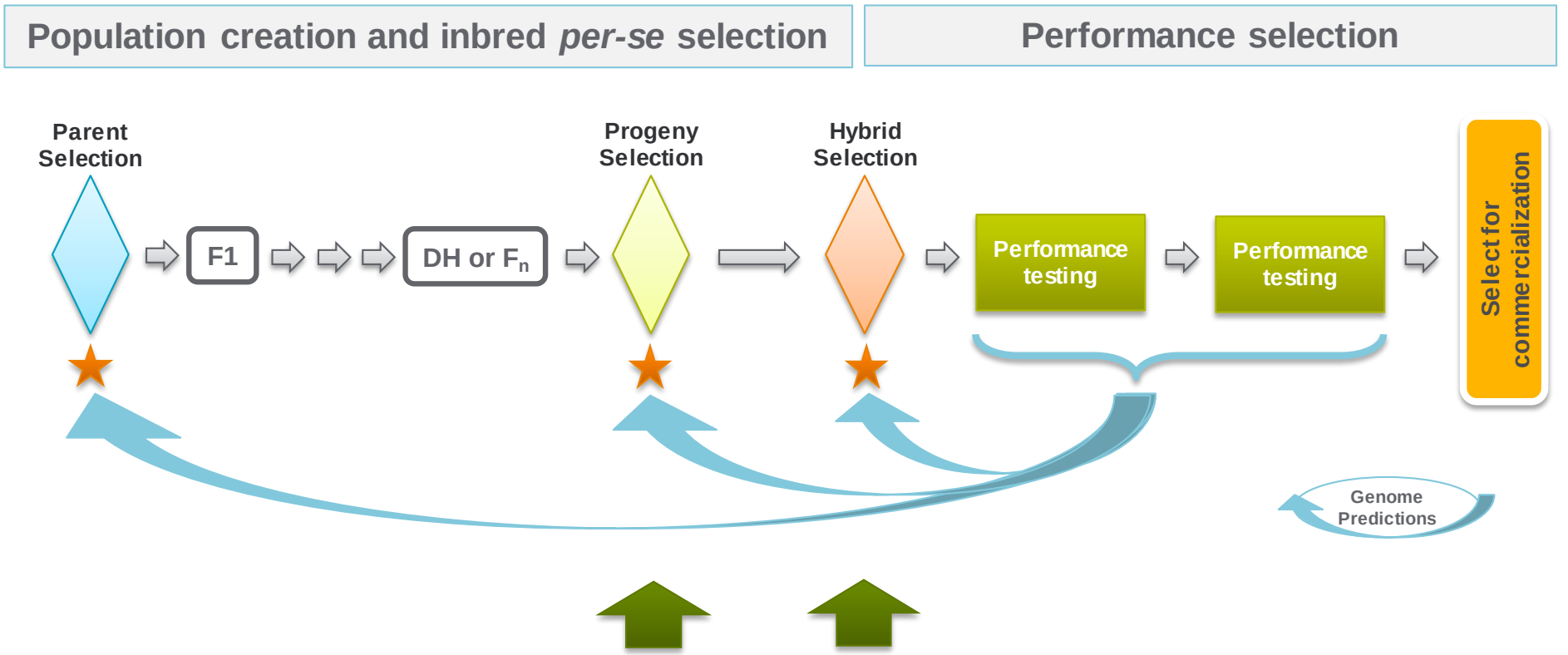
Genotypic data are used across Syngenta R&D



Supported by an array of technologies

Molecular Breeding at Syngenta



Need: A cost-effective, medium-density option for genome-wide fingerprinting

Need: a lost cost, fast-turn around option for low-density marker panels

Rapid, low-plex genotyping of wheat and other cereals

J. D. Curry, V. Joshi, Q. Wan, H. Koshinsky
Affymetrix, Inc., Santa Clara, CA

affymetrix
part of Thermo Fisher Scientific

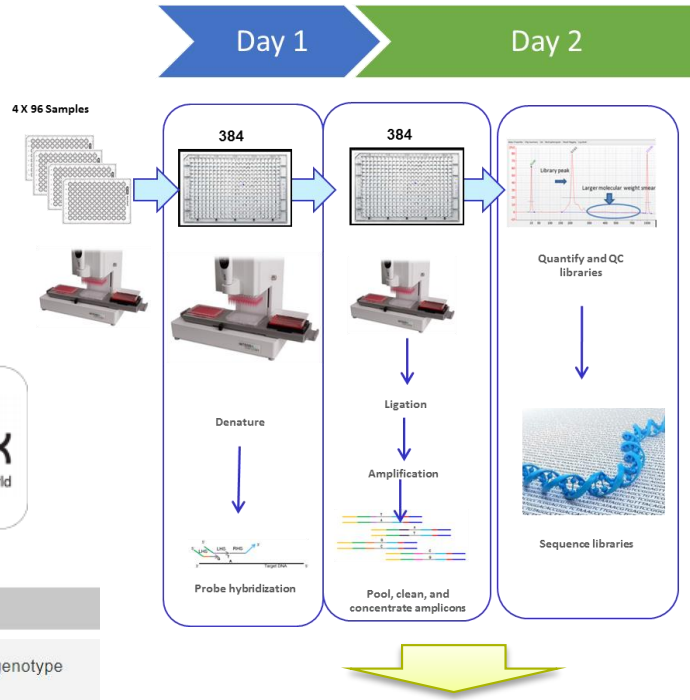
Introduction
Eureka™ Genotyping Solution has utility in plant breeding and regulatory

Cluster plots of two loci produced with Eureka Genotyping Solution in *Hordeum vulgare* (barley) and a 400+-plex genotyping panel

Application of analysis tools from Affymetrix on Eureka™ Genotyping Solution to provide accurate and automated animal and crop genotypes

S. N. Malakshah, V. Joshi, A. Pirani
Affymetrix, Inc., Santa Clara, CA 95051

affymetrix
Biology for a better world

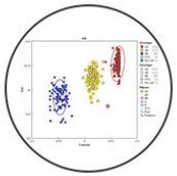


Genotype any species, genome size, or ploidy level with your own proprietary markers or markers from recent sequencing initiatives on the fully customizable Eureka genotyping platform.

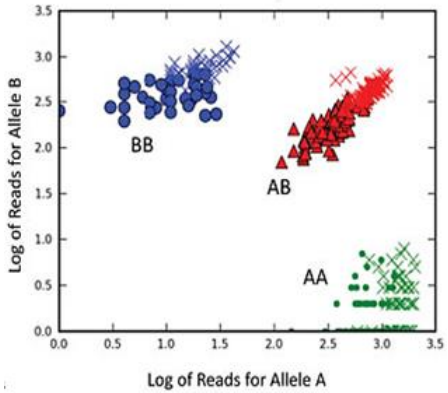
- **Rapid:** Two-day workflow goes from extracted DNA to genotypes for thousands of samples per week.
- **Targeted:** The custom design process and assay workflow ensures that all informative loci are targeted across all samples and across all manufacturing batches.
- **Flexible:** Eureka genotyping panels can easily be updated to add or remove variants.
- **Affordable:** The low cost per base of NGS is achieved through an intelligent combination of locus and allelic barcode and sample multiplexing.

Benefits

Fast turnaround time	Automated genotype calling



Data scored as scatterplots



Source: Thermo Fisher

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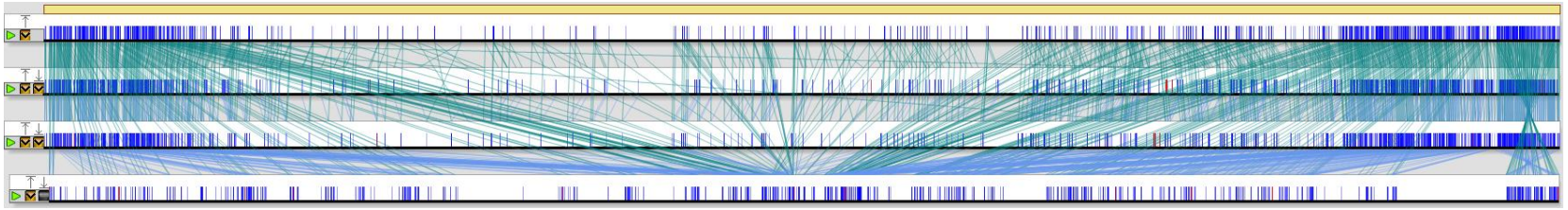
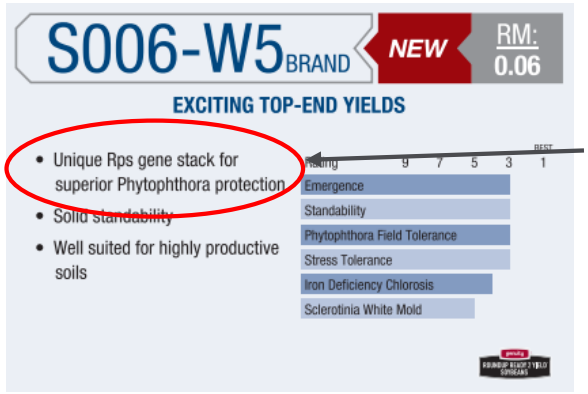
Eureka panel development



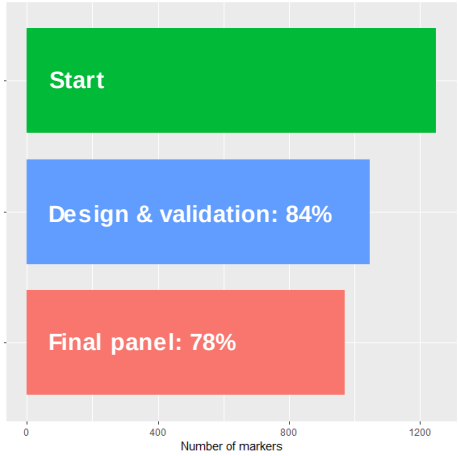
Mostly "anonymous" markers for genome-wide selection



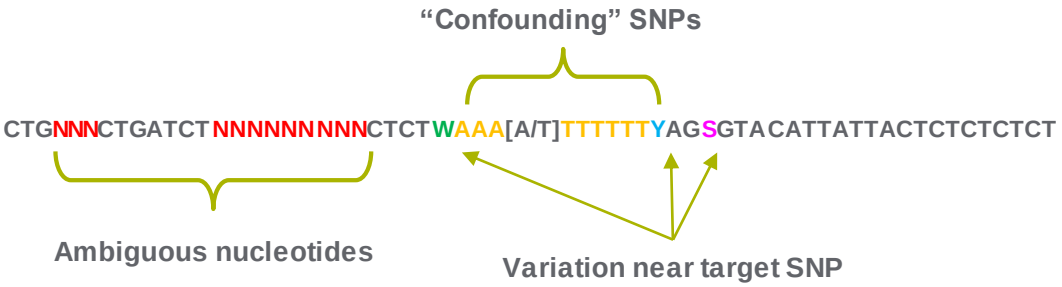
Source: http://www.syngenta-us.com/seed-guides/_assets/nk-northern-region-2016.pdf

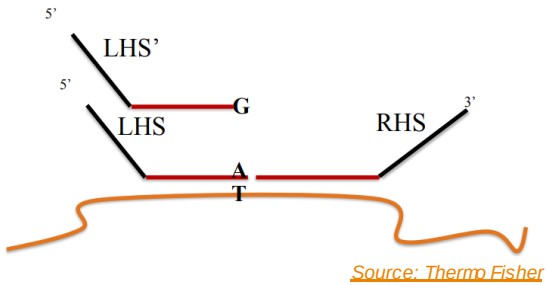
Flexibility for "must-haves"

Good designability rate…



… but some assays are not designable or fail during validation





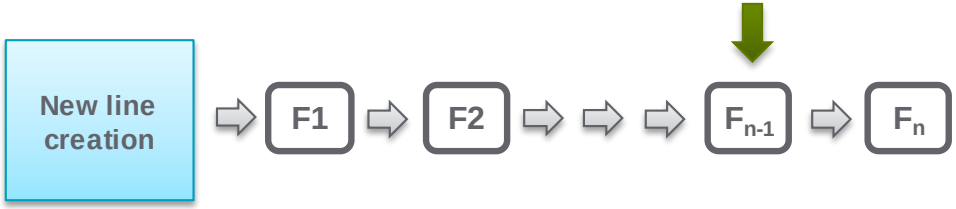
Source: Thermo Fisher

Fixed probe position limits options for challenging footprints

Able to apply genomic selection at an earlier stage

Cost and fast data turn-around time enable progeny selection before replicated field trials

Flexibility to add/remove assays over time is also key

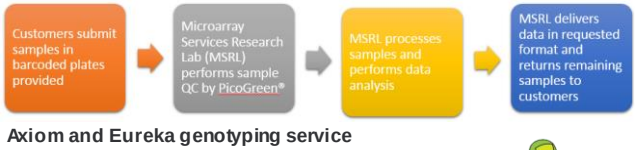


Still, some obstacles to overcome for implementation

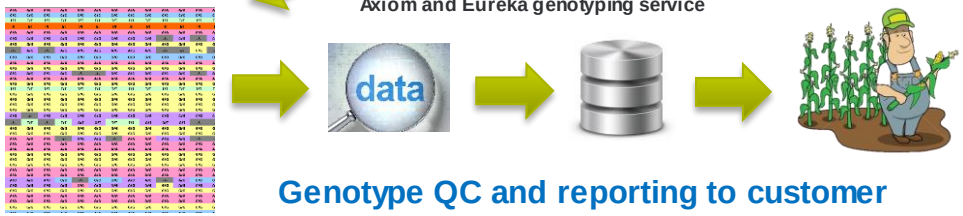
Adapting to a production workflow

Sample management & DNA preparation

- **Track and trace**
- **Capacity management**

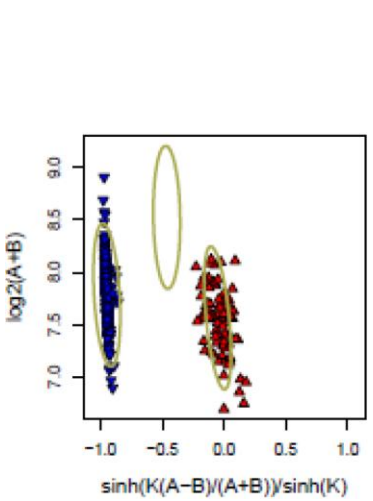


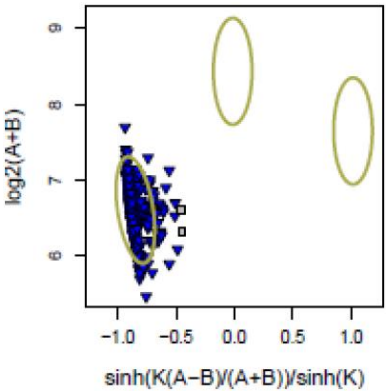
Axiom and Eureka genotyping service



Genotype QC and reporting to customer

Scoring algorithms for atypical clusters





Source: Thermo Fisher

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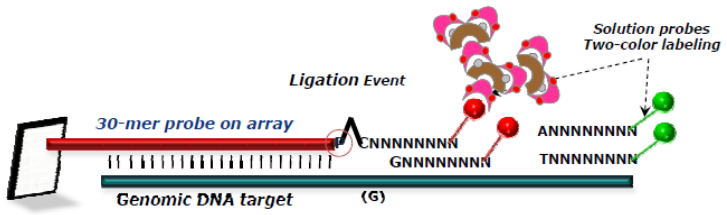
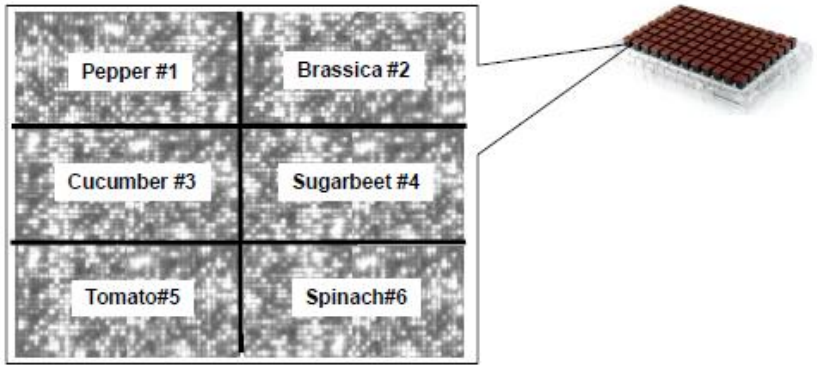
An alternative for medium densities

- Eureka is a good fit for lower density panels, e.g., for ~1k markers
- Other crops with lower LD, higher genetic diversity, etc. require higher densities
  - Example: Corn genomic selection
- Axiom 384 HT provides an alternative for medium-density panels, e.g., 3-5k markers



Source: Thermo Fisher

Ability to multiplex crops



Flexibility in array design

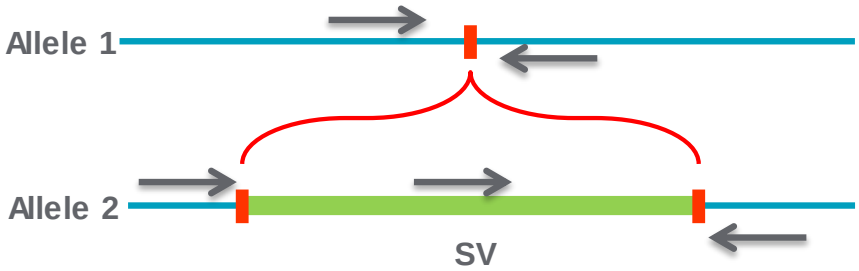
Typically 1 probeset per variant

Source: Thermo Fisher

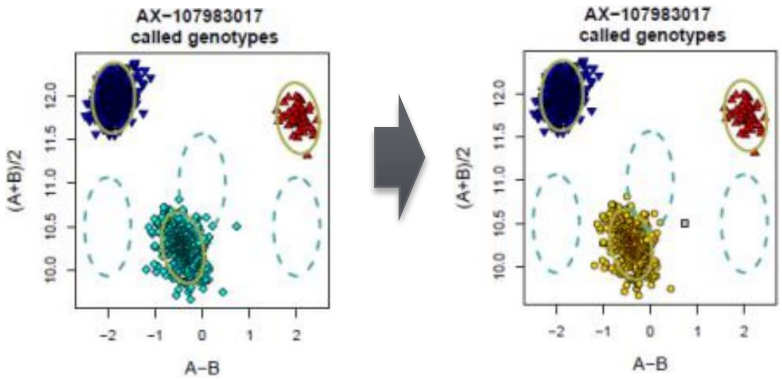
For more complex or "Must-have", both directions are used

Best probeset can vary

Different handling for larger SVs



Many factors contribute to results quality & consistency



Credit: Thermo Fisher

Footprint quality matters!

E.g., variation closely flanking the SNP of interest

GGTATTTTTTTGACCATTASGGCTNNCTCTKA[A/G]TTYAGWGGTGTCTAAAKKGAGATAGGAACTT

Didion *et al. BMC Genomics* 2012, **13**:34
http://www.biomedcentral.com/1471-2164/13/34

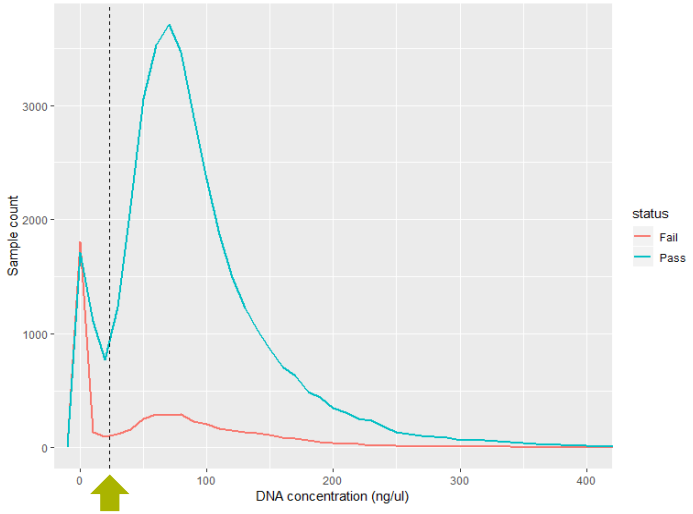
BMC Genomics

METHODOLOGY ARTICLE Open Access

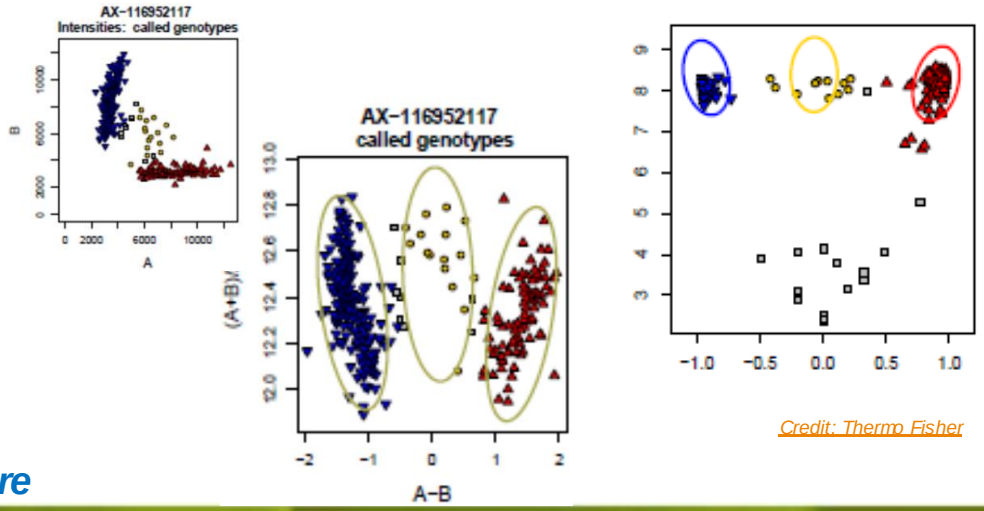
Discovery of novel variants in genotyping arrays improves genotype retention and reduces ascertainment bias

John P Didion[1,2,3†], Hyuna Yang[4†], Keith Sheppard[5], Chen-Ping Fu[6], Leonard McMillan[6], Fernando Pardo-Manuel de Villena[1,2,3*] and Gary A Churchill[5*]

DNA quantity is important…



Complex or bulked samples can have poor clusters



Credit: Thermo Fisher

… but doesn't always correlate with success/failure

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The use of priors can improve scoring

Especially with 1 or 2 clusters

Previous performance

Updated priors

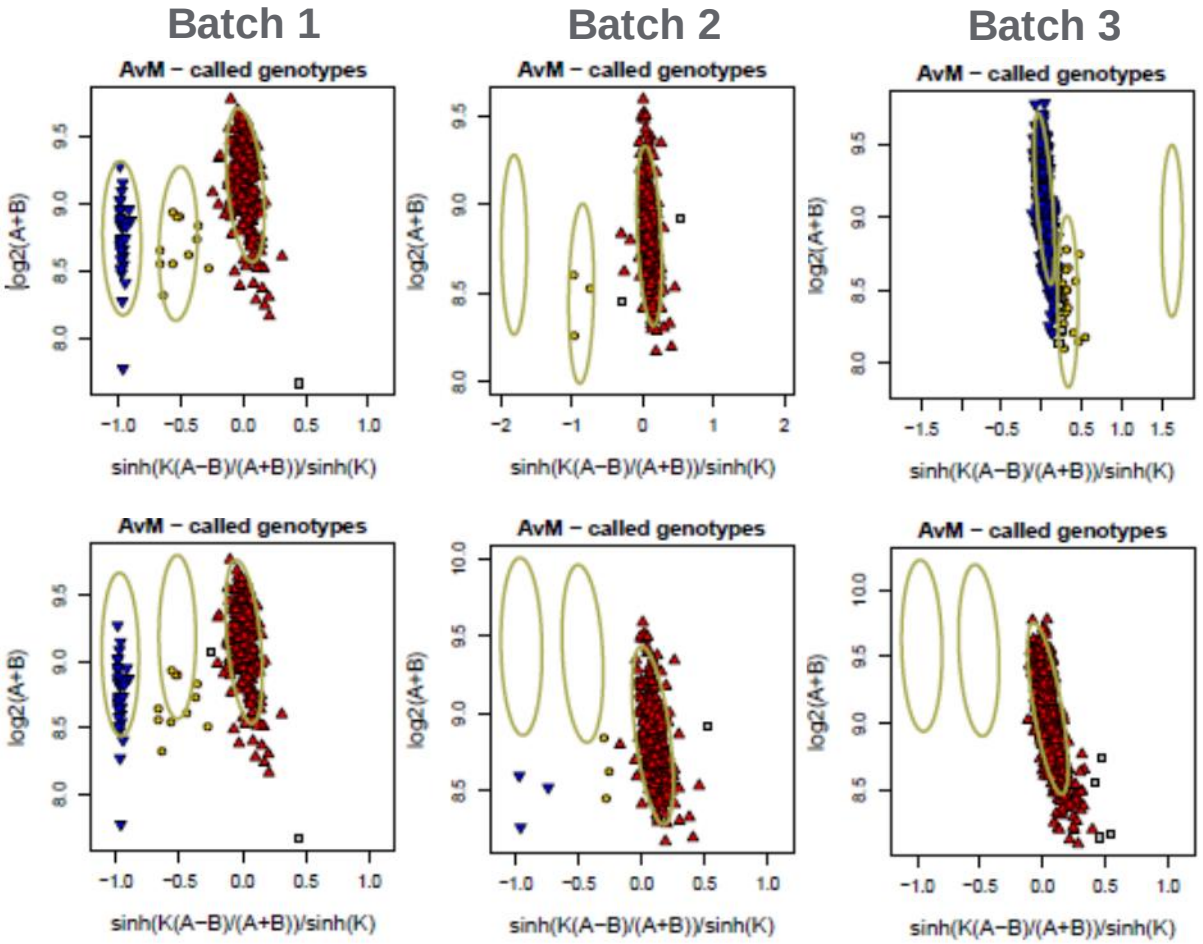

Figure credit: Thermo Fisher

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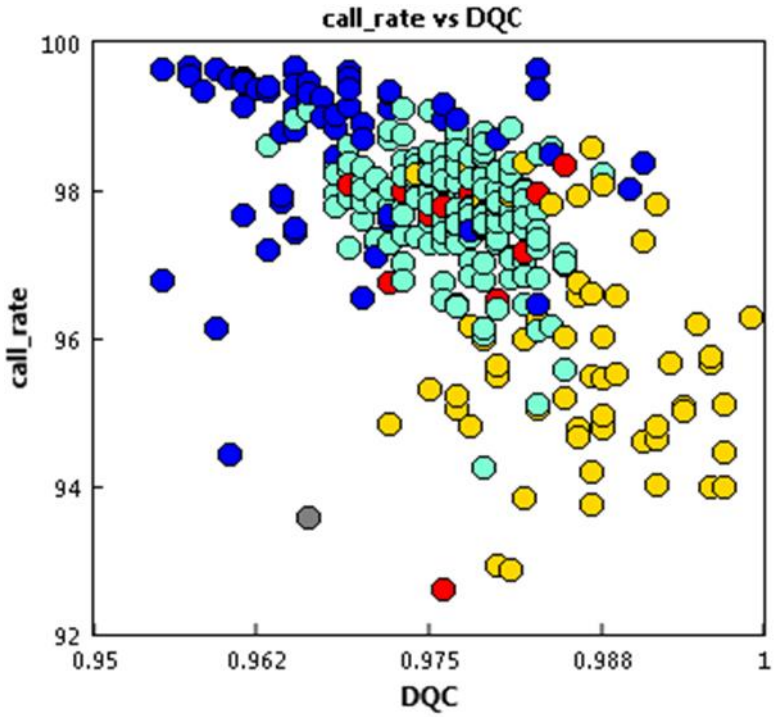
Accounting for population structure can also improve scoring

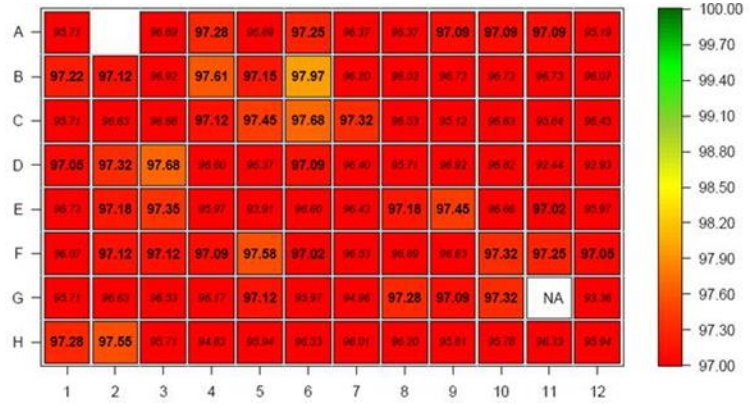
Example crop 1



Probesets selected for QC during validation may not be optimal for divergent populations

Example crop 2

Original: pass rate = 39%



Re-processed with new QC controls

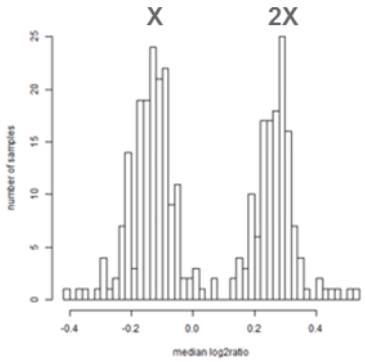
New: pass rate = 95%

Figure credit: Thermo Fisher

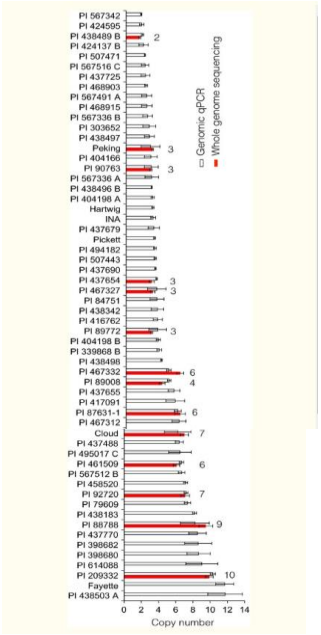
Opportunities

Structural variants

E.g., quantifying copy # variation

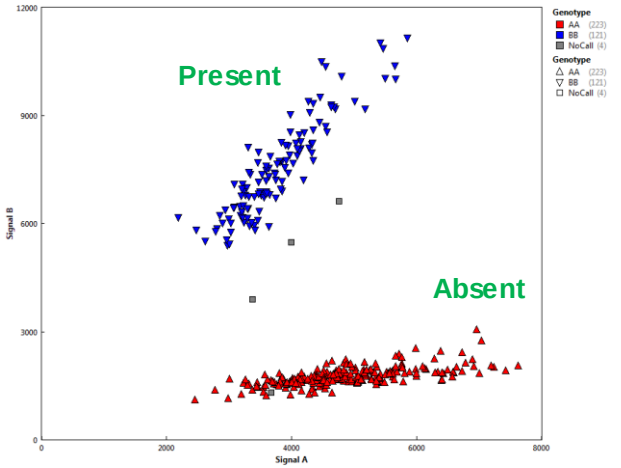


Credit: Thermo Fisher



https://onlinelibrary.wiley.com/doi/full/10.1111/mec.13138

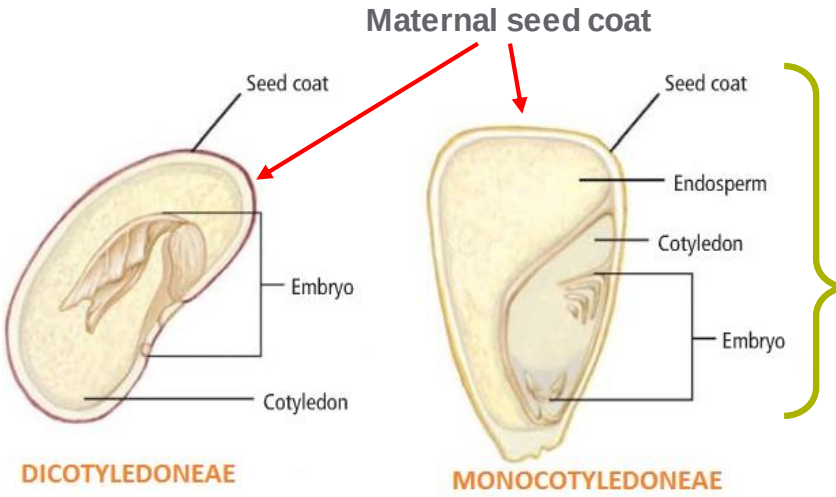
Dominant markers

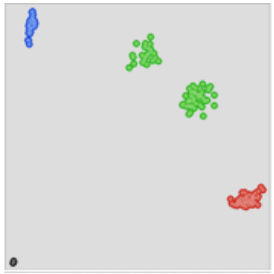


Seeds and other tissues

Composition complexity:

- Starch
- Oils
- Polyphenols
- Etc





https://biologigonz.blogspot.com/2010/01/angiospermae-gymnospermae.html

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Summary

- Eureka and Axiom are in use for production-scale genotyping for most crops for early genomic selection.
- Invested significant effort to properly adapt to and integrate with Syngenta's existing infrastructure for production workflows.
- Ability to handle high sample volumes with short turn-around times offers the possibility to extend Eureka to other, more demanding applications.

Bringing plant potential to life

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