Axiom Arrays and AgriSeq NGS Genotyping Offerings for Wheat

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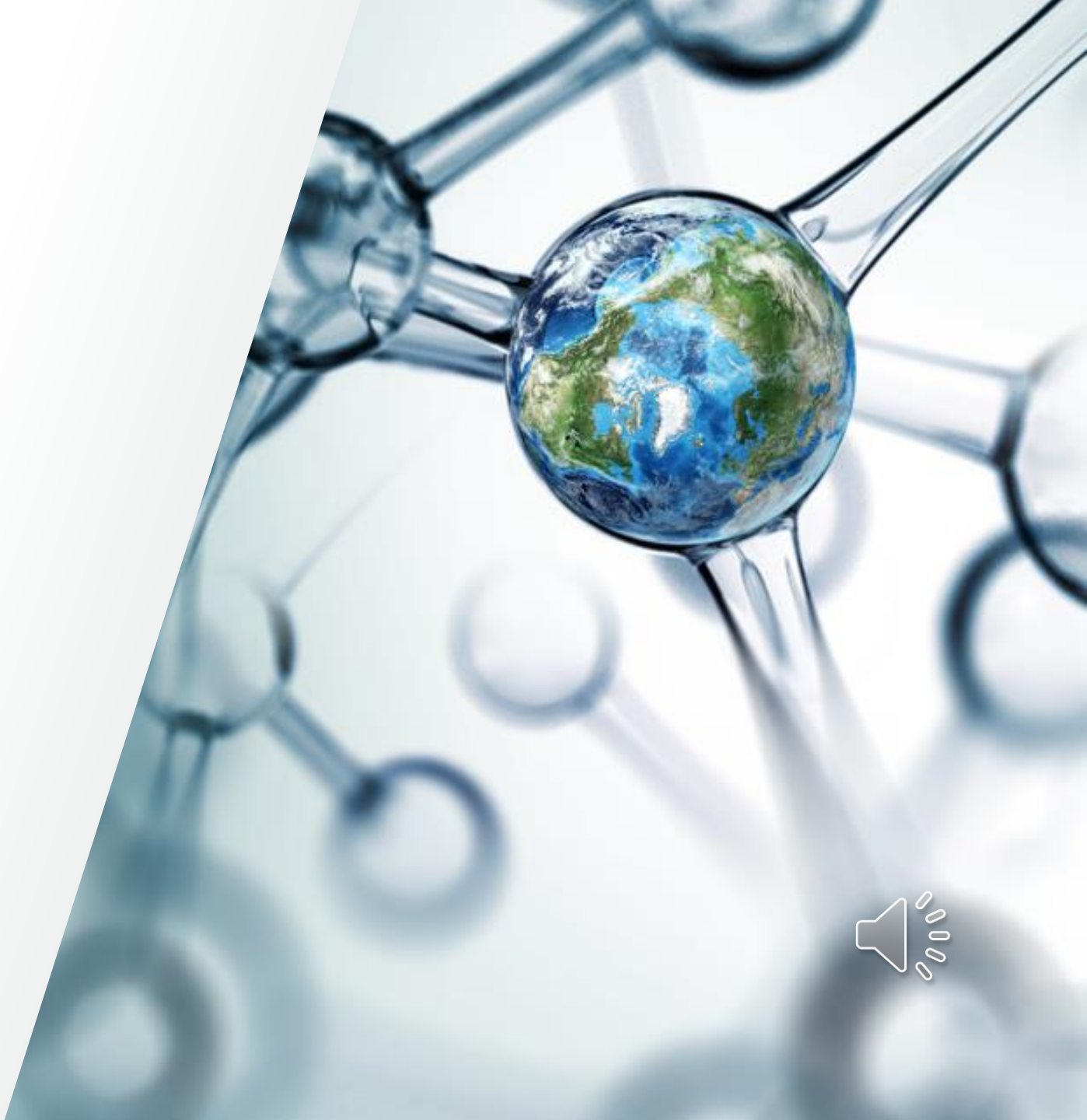


The world leader in serving science

Agenda

1. Introduction to Thermo Fisher Scientific
2. Solutions for Genotyping
3. Our offerings for Plant Genotyping
4. Customer Use Applications / Publications

Agribusiness at Thermo Fisher Scientific protecting our food supply

Genotyping for plants and animals

Participate in the improvement of plant and animal production through genetic selection

Solutions for diseases

Rapidly find diseases that affect farm animals, plants, and the environment to secure global food sources

Helping to prevent disease spread

Detect zoonotic diseases to help prevent spread

Providing solutions for customers serving agriculture

Solutions Across the Agrigenomics Continuum

	Discovery GWAS	Genomic selection	Genomic prediction	Marker-assisted selection or breeding	Parentage	QC
Number of markers	1,000,000	100,000s	10,000s	1,000s	100s	10s
Technology						
Solutions						

- Known targets
- Mid to High density
- Multiplex

Dedicated to scientific partnership with customers

Genotyping balance

Breeding considerations

Genotyping solutions

- Fast
- Affordable
- Robust
- User-friendly
- **Targeted genomics**

Applied Biosystems™ Axiom™ Genotyping Solution

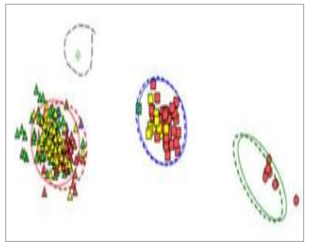
SNPs, indels, CNVs

SNP discovery initiatives

Applied Biosystems™ Axiom™ Reagent Kit
Robust and reliable assay

Target prep
Automated and manual protocols

Applied Biosystems™ GeneTitan™ MC Instrument
Automated, hands-free array processing



Applied Biosystems™ Axiom™ Analysis Suite Software
Automated genotyping

>10,000 high-density markers for GWAS, genomic selection and prediction, CNV, and polyploidy

Applied Biosystems™ Axiom™ arrays for Wheat

Partnership with a leading university

- Development of 2 wheat arrays
- Searchable SNP database at CerealsDB

Axiom™ Wheat HD Genotyping Array (819k SNPs)

- 100,000 SNPs from 10 elite cultivars
- 290k SNPs from elite cultivars and landraces
- 650k from hexaploid bread wheat, *T. urartu*, *Thinopyrum* spp., and *Aegilops* spp.

Axiom™ Wheat Breeder's Genotyping Array (35k SNPs)

- Most-utilized subset of SNP markers
- Based on international CIMMYT material

Axiom™ BreedWheat 35K Array (35k SNPs)

- Developed by E. Paux in France
- Diverse elite material from French and worldwide sources

Axiom Wheat Breeders 35k Array Cited in Literature

Contents lists available at ScienceDirect

Current Plant Biology

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

In silico quality assessment of SNPs—A case study on the Axiom® Wheat genotyping arrays

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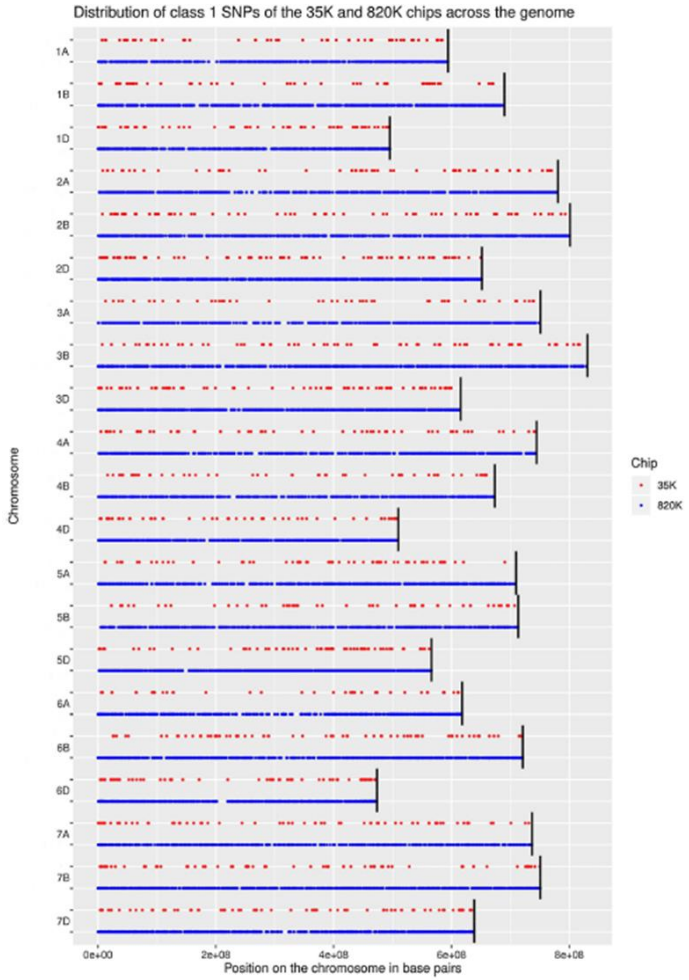
Genomic variant identification

- Axiom Wheat HD Genotyping Array (819k)
- Axiom Wheat Breeder's Genotyping Array (35k)
- Wheat polyploid (hexaploid 2x = 6n = 42)
- **Good equidistant genome coverage**
- **Future utility of Axiom Wheat Breeder's Genotyping Array for use in genotyping hexaploid wheat**



35k red

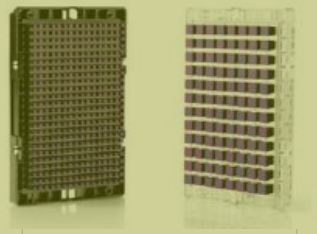
819k blue

Chromosome coverage

AgriSeq NGS and Axiom array workflow

	Technology	Number of markers	Workflow	Applications
  **Applied Biosystems™ AgriSeq™ NGS**	**Targeted genotyping with next-generation sequencing (NGS)**	**50–5,000**; targeted genomic regions Pilot program provides support	1. Custom panel design 2. Library prep—manual or automated 3. Template on Ion Chef™ system 4. Sequencing to data in 2.5 hours 5. Data collection	**Targeted** • Applied Biosystems™ TaqMan™ single-marker assay • QTL mapping and discovery • Genomic association • Genomic selection • **Routine screening** • **Lower cost per data point**
  **Applied Biosystems™ Axiom™ arrays**	**Microarrays**	**Up to 660,000** • Targeted probe • Custom design • Catalog design • 600k in 96-well format • 70k in 384-well format	1. Design custom array or choose from catalog 2. Run in-house or in a service lab 3. Data turnaround from service lab	**Comprehensive discovery** • **Discovery > SNP selection** • Catalog designs available • Over 400 custom designs • QTL mapping • GWAS • Single-marker TaqMan Assay • Genomic selection • Genomic prediction

Solutions across the agrigenomics continuum

	Discovery GWAS	Genomic selection	Genomic prediction	Marker-assisted selection or breeding	Parentage	QC
Number of markers	1,000,000	100,000s	10,000s	1,000s	100s	10s
Technology						
Solutions						

- Known targets
- Mid-density
- Multiplex

Dedicated to scientific partnership with customers

AgriSeq GBS is custom-designed for your targets

Multiplex 50–5,000 markers in a single tube

Compatible with:
- Single-nucleotide polymorphisms (SNPs)
- Multi-nucleotide polymorphisms (MNPs)
- Indels

Design maximizes:
- Analytical specificity
- Efficiency
- Multiplex compatibility

Dedicated bioinformatics team experienced with agricultural genomes

Flexibility to update panels—add or delete markers

Success with a wide variety of species
Significant species with successful panel designs

Plants

- Barley
- Cacao
- Canola
- Chilies
- Chickpea
- Corn
- Cucumber
- Eucalyptus
- Oats
- Oil palm
- Onion
- Pearl millet
- Pine
- Rice
- Sorghum
- Soybean
- Spinach
- Spruce
- Sunflower
- Tomato
- Watermelon
- Wheat
- Groundnut
- Coconut

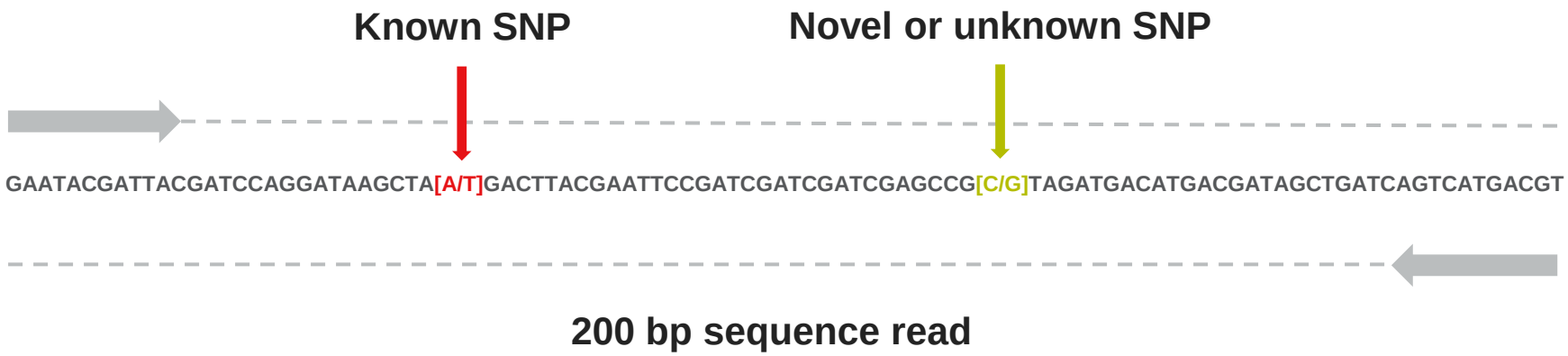
Animals

- Bovine
- Canine
- Feline
- Equine
- Ovine
- Chicken
- Porcine
- Salmon
- Trout
- Black fly
- Turbot

>300 custom panel designs for over 70 species in plants, animals, and aquaculture

Identification of novel SNPs by AgriSeq GBS

AgriSeq GBS generates ~200 bp of flanking sequence



If desired, additional variants can be utilized for additional analysis

- Confirmation of novel variants
- Utility for additional linkage analysis
- Generation of microhaplotypes for improved marker specificity or enhanced discrimination in parentage testing and/or traceability analysis

Applied Biosystems™ AgriSeq™ targeted GBS

Sample prep

Custom panels

Standardized manufacturing—scalable from 6k to 200k reactions/lot

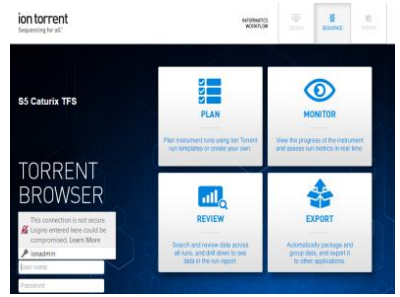
Library prep

Increased throughput of 3,072 samples/day/sequencer

Sequencing

Ion 550™ Chip enables 120M reads, at 2 runs/day/sequencer

Data analysis



Applied Biosystems™ AgriSum™ Toolkit plugin: data summarization and visualization; polyploid and structural variants

Interpretation made easy with the AgriSum Toolkit

Panel summary

Number of markers	535
SNP	476
DEL	40
INS	14
MNP	3
COMPLEX	2
Number of amplicons	**513**
Mean coverage	**533x**
Mean call rate (Mean CR)	**94%**

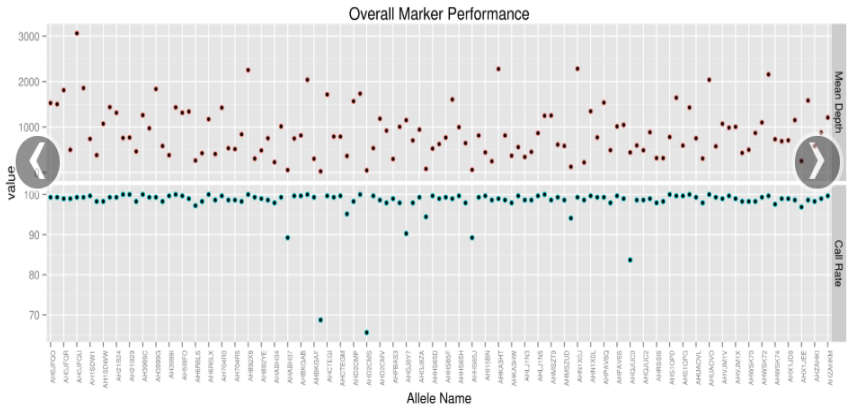
Marker summary

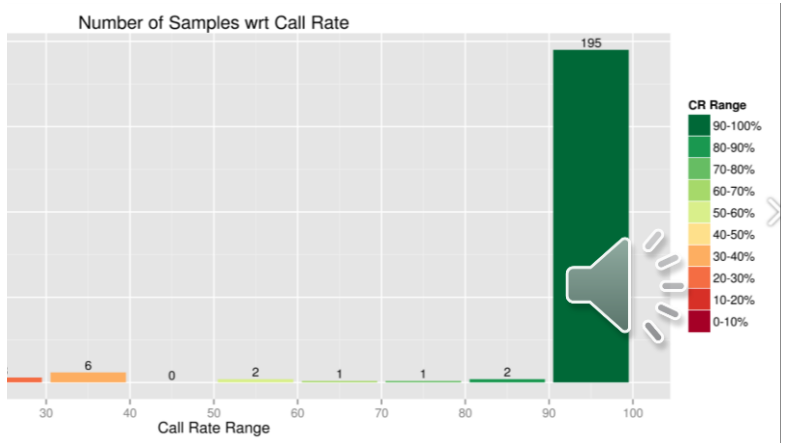
Number of markers	535
# markers = 100% CR	**0**
# markers = 98–100% CR	**0**
# markers = 95–98% CR	**154**
# markers = 90–95% CR	**369**
# markers = 80–90% CR	**7**
# markers = 50–80% CR	**5**
# markers = 10–50% CR	**0**
# markers = 0–10% CR	**0**
# markers = 0% CR	**0**

Sample summary

Samples run	288
# markers = 100% CR	**0**
# markers = 98–100% CR	**46**
# markers = 95–98% CR	**103**
# markers = 90–95% CR	**64**
# markers = 80–90% CR	**39**
# markers = 50–80% CR	**19**
# markers = 10–50% CR	**5**
# markers = 0–10% CR	**11**
# markers = 0% CR	**1**

- **Genotype matrix**
- **Top/bottom format**
- **Parentage format**





Supporting genotyping continuum at ICRISAT

ThermoFisher SCIENTIFIC

International Crops Research Institute for the Semi-Arid Tropics (ICRISAT)

Dr. Rajeev Varshney

Needs from ICRISAT

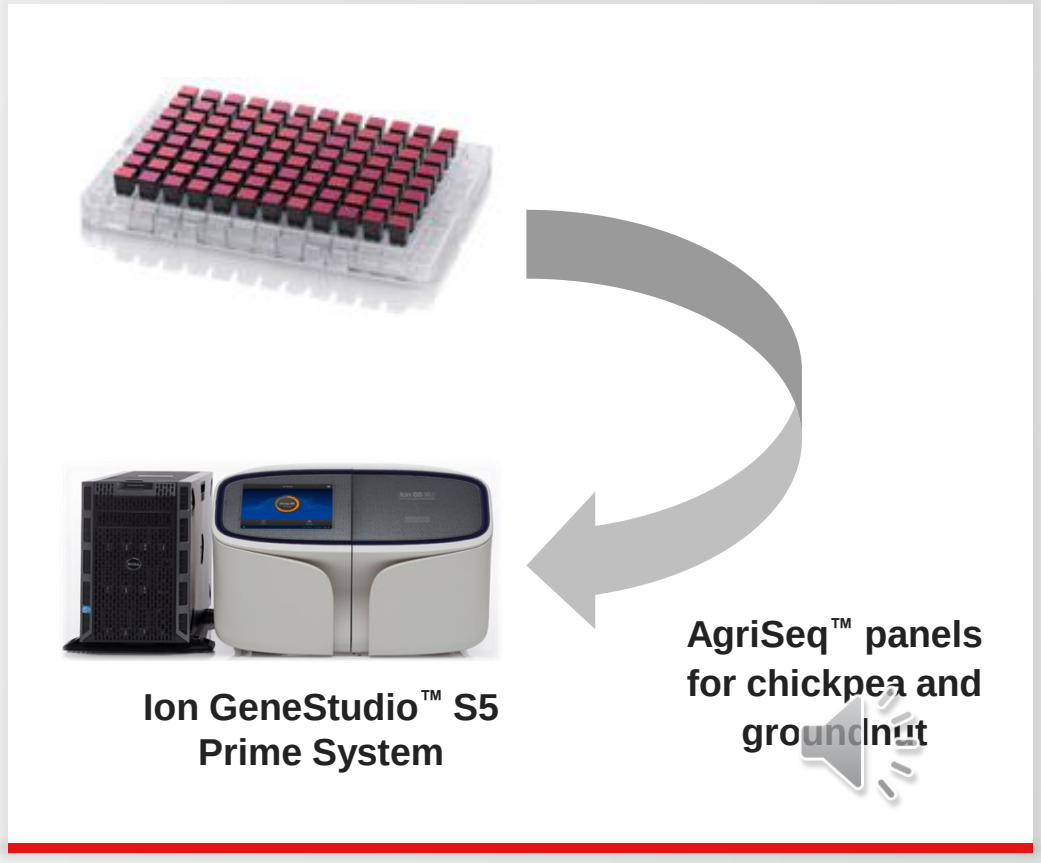
- Studying crops from semi-arid regions: chickpea, groundnut, and pigeon pea
- Exploring highly customizable and affordable SNP genotyping solutions
- Developing panels for marker-assisted selection for their breeding teams

Development path

- **Whole-genome sequencing**—build native-variety reference genomes
- **Axiom microarrays**—GWAS and genomic selection
- **AgriSeq™ targeted genotyping by sequencing (GBS)**—low-density 5K panels for diversity and selection

What resonated with ICRISAT and why?

- **Flexible content**—helps provide low- to medium-density genotype data
- **Affordable cost and turnaround time**—support their breeding needs
- **Collaborative partnership**—developing panels and analysis tools



USDA Wheat 5k AgriSeq Panel

5.1k Markers with 212 Trait Markers

- **Developed by 4 USDA Genotyping Labs NA**
- Winter and Spring Wheat
- Several Market Classes
  - Soft White, Hard Red, Soft Red
- 5,174 SNPs and INDELs
  - Coverage 2.9 Mbp
  - 294 SNPs per Chromosome
- 212 Trait Markers
  - Disease, Agronomic, Quality Traits

Chromosome	SNPs	Average Distance
Chr1A	286	2,089,406
Chr1B	300	2,326,800
Chr1D	201	2,488,088
Chr2A	245	3,214,442
Chr2B	332	2,450,638
Chr2D	214	3,063,823
Chr3A	491	2,722,065
Chr3B	332	2,565,597
Chr3D	475	4,350,685
Chr4A	704	3,279,589
Chr4B	202	3,346,202
Chr4D	96	5,378,628
Chr5A	309	2,305,916
Chr5B	376	1,905,327
Chr5D	163	3,506,626
Chr6A	227	2,747,197
Chr6B	289	2,516,512
Chr6D	154	3,229,386
Chr7A	330	2,260,558
Chr7B	248	3,091,370
Chr7D	210	3,068,650
Totals	**294**	**2,947,976**

Trait Category	# SNP Markers	Trait Description
Agronomic	11	Rht genes, maturity, earliness, plant height
Pathology	38	Resistance genes for Leaf rust, Stripe rust (Yr5/15), Stem rust, FHB, Cre8CCN Nematode R
Physiology	73	Vernalization, Photoperiod sensitivity, Al tolerance, Alpha amylase
Quality	16	Gluten content/strength, PPO, PPD
Other	74	CAD, Cfn, GLG, Jsc, KASP, mAsSNP, QYr, TSn
Total	212	

Power of experience—AgriSeq GBS pilot program

Run a proof-of-concept pilot project to see for yourself

Statement of work

***In silico* design review**

1

Project scoping
1–2 weeks

2

Custom panel design
4–6 weeks

3

Panel manufacturing
2–3 weeks

4

Wet-lab verification
2–3 weeks

Data review

End-to-end project management

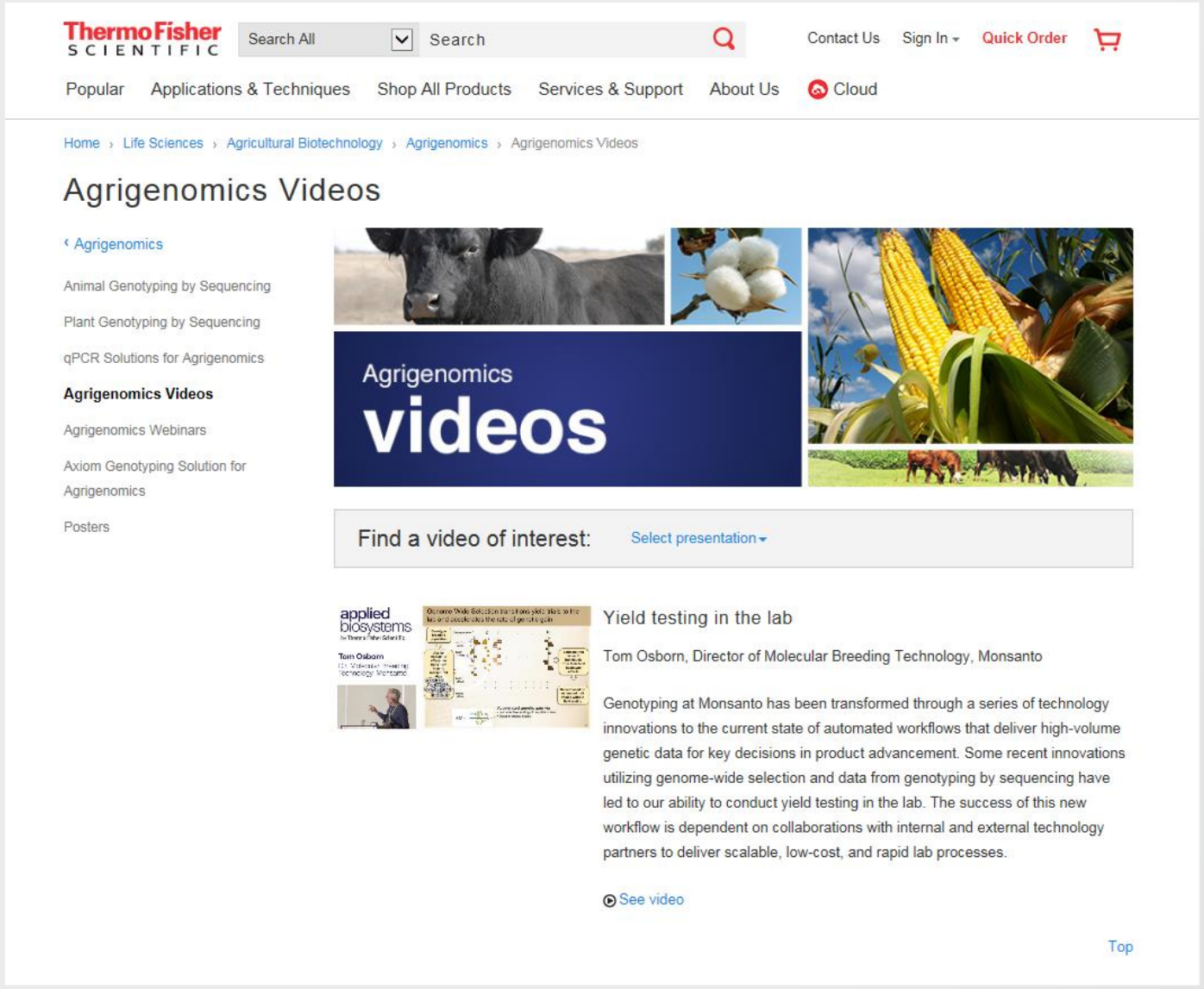
Catalog AgriSeq panel development

Consortia-based catalog crop panels

- **We are Working with Several Government Agencies**
- **Wheat, Canola, and Soy Consortia Groups**
- **Developing AgriSeq targeted sequencing panels**
- **Publicly available for major crops**

  - Wheat
  - Maize
  - Soybean
  - Barley
  - Canola
  - Rice
  - Strawberry
  - Tomato
  - Pepper
  - Melon
  - Coffee
  - Peanut

Watch our videos for customer testimonials



Empowering Global Agriculture

Advancing vegetable breeding with AgriSeq targeted genotyping by sequencing

- Hans Peter Koelewijn, Scientist, Genetics, **Bayer Vegetable Seeds**

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

- Jason Nichols, PhD, Principal Scientist, Molecular Analytics, **Syngenta Crop Protection**, LLC

Advancement in sunflower breeding using an optimal genotyping-by-sequencing AgriSeq panel designed for QTL mapping and genomic selection

- Farhad Ghavami, PhD, Vice President, Agrigenomics, **Eurofins** BioDiagnostics Inc.

AgriSeq technology: T-GBS as a potential tool for molecular breeding approaches—a practical overview by GDM Seeds

- Gaspar Malone, PhD, Biotechnology Research Manager, **GDM Seeds**

50K Axiom SNP microarray allows high-quality genotyping of coffee, cashew, cassava, Brazilian pine, and eucalyptus

- Dario Grattapaglia, PhD, Senior Research Scientist and Project Leader, Brazilian Agricultural Research Corporation (**EMBRAPA**)

Determination of purity and quantification of varietal components through AgriSeq targeted GBS

- Dr. Carlos Azambuja, Director, **GENIA**

Questions?

Thank you