




ThermoFisher
S C I E N T I F I C

**High-Throughput Genotyping by Targeted Next-Generation Sequencing (NGS)
for Seed Purity
...AKA “Part II of Genetic Purity: Past, Present and Future”**

The world leader in serving science

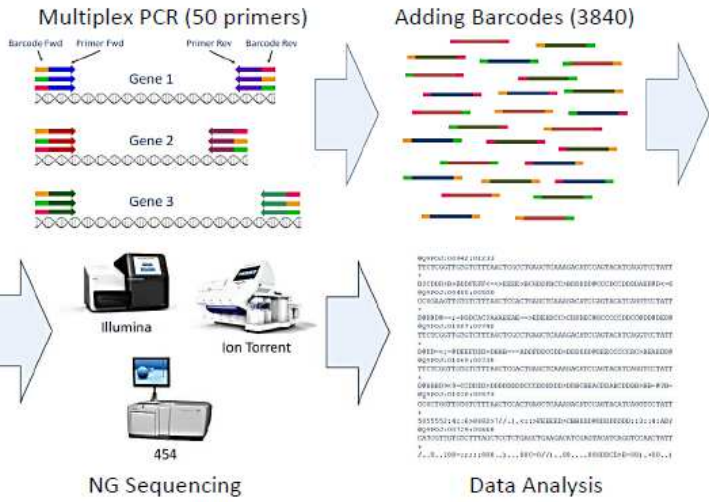
Picking up from where we left off Spring 2018....

Genetic Purity: Past, Present and Future: Dr. Farhad Ghavami- CSO Eurofins BDI


The Future of Genetic Purity 

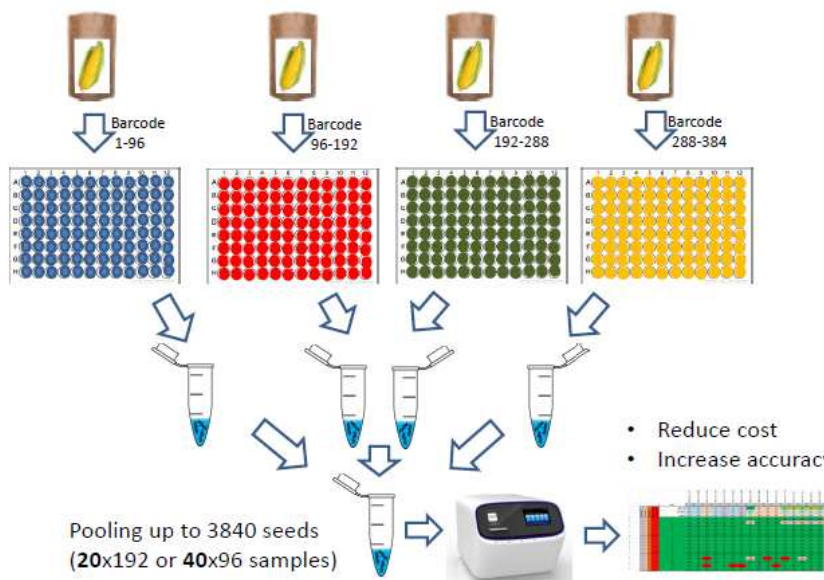
Amplicon based GBS using 50-100 targets (SNPs) can be used for Purity Purposes.

Multiplex PCR (50 primers) Adding Barcodes (3840)



NG Sequencing Data Analysis

The Future of Genetic Purity 

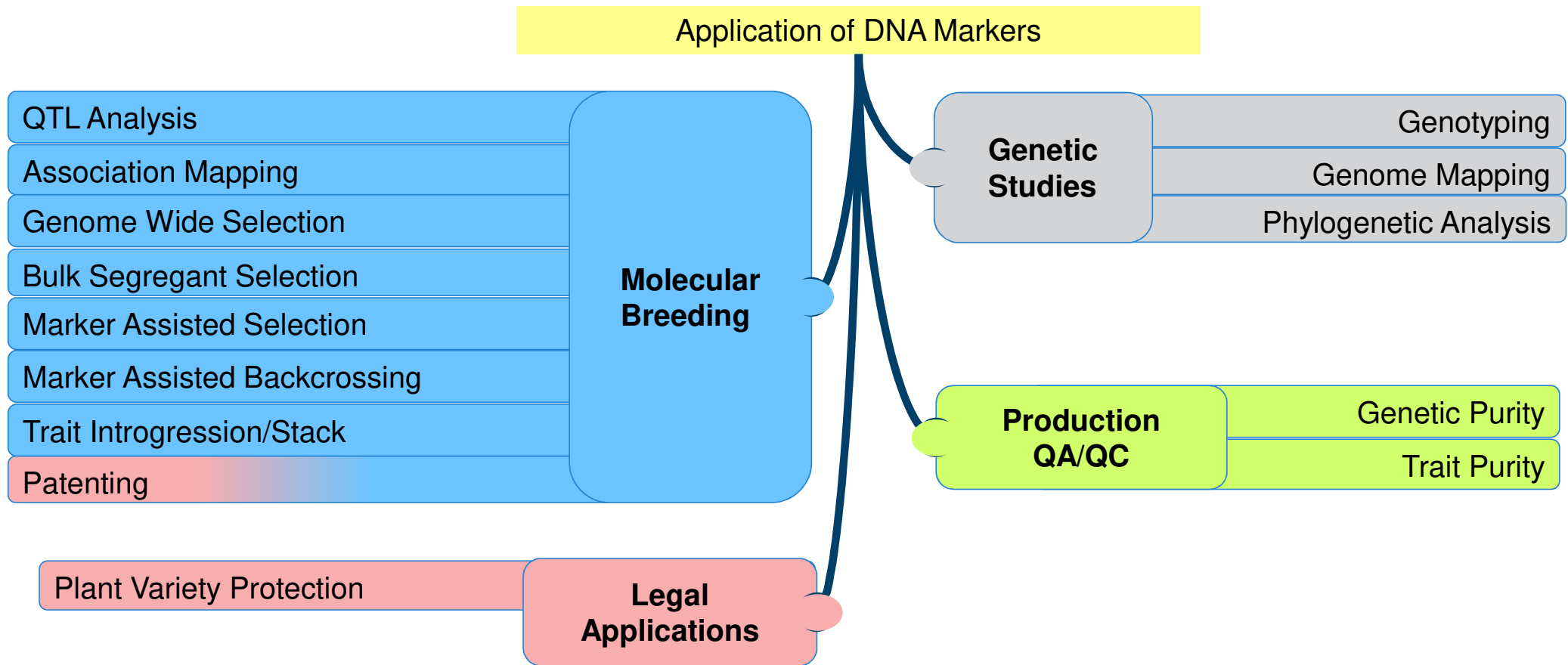


- Reduce cost
- Increase accuracy

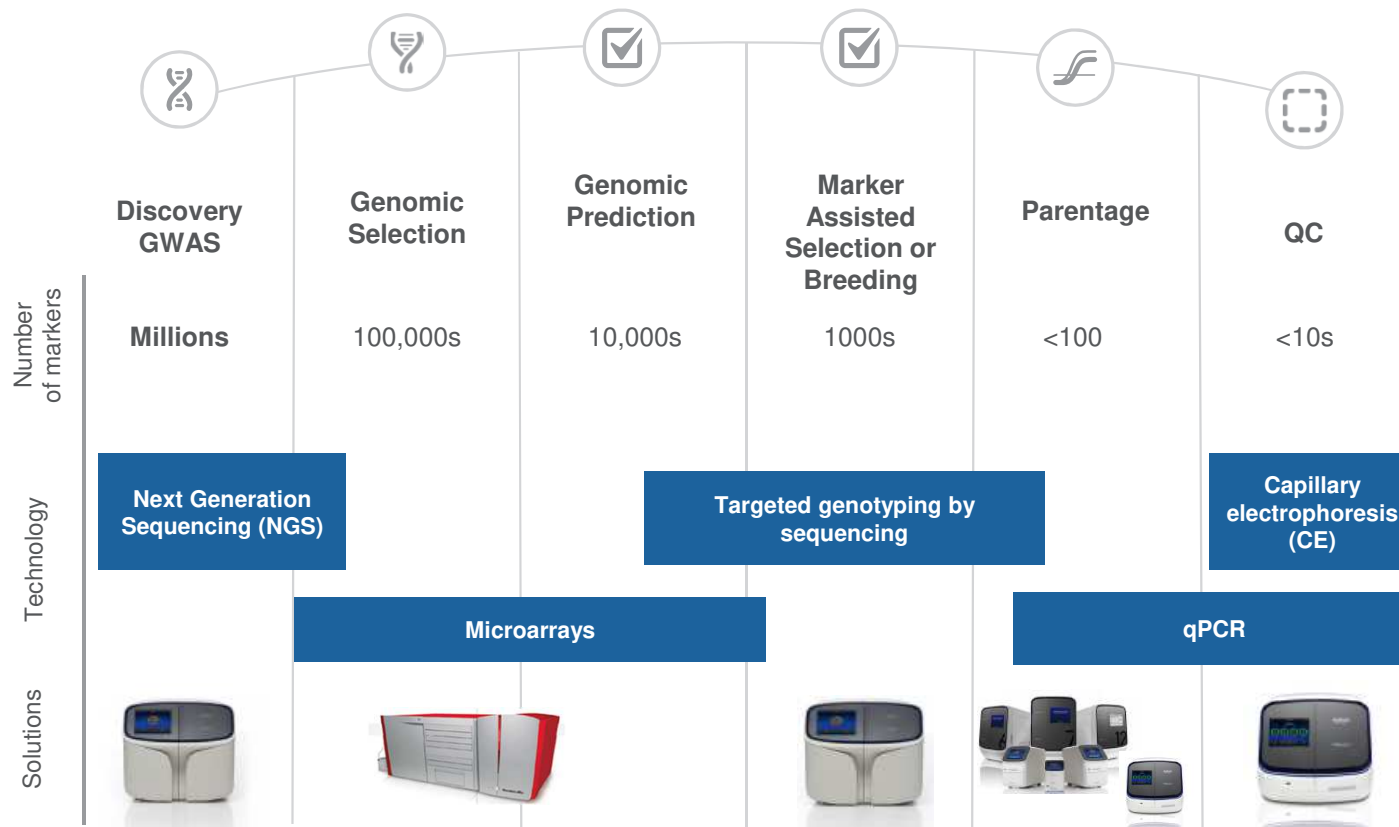
Pooling up to 3840 seeds (20x192 or 40x96 samples)

Customer Benefits: Reduced Costs and Increased Accuracy

Variety of applications using DNA markers in plant and animal breeding



Thermo Fisher Scientific Offers Solutions Across the Agrigenomics Continuum



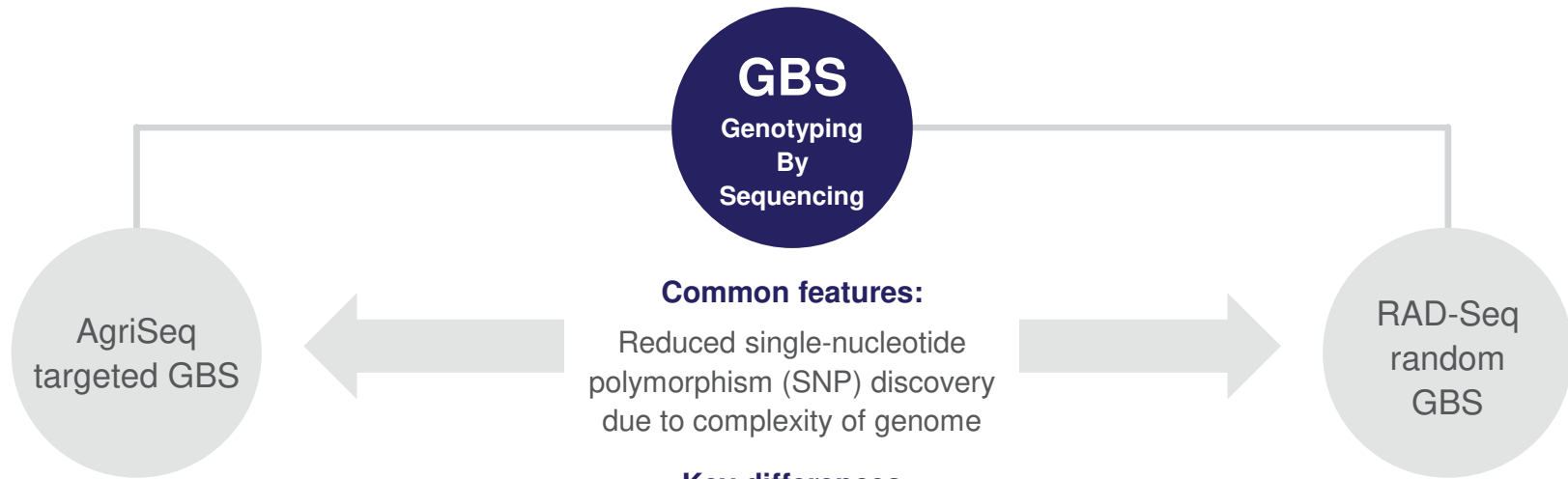
Thermo Fisher Scientific Agrigenomics business

- Significant history of innovation and applying genomics to agriculture—microarrays, qPCR, and capillary electrophoresis (CE)
- Dedicated Agrigenomics R&D resources
- Focused on technologies for advancing plant and animal genotyping research

Dedicated to scientific partnership with customers

* GWAS = Genome-wide association studies.

Genotyping by Sequencing (GBS) Can Represent Different Approaches



Key differences

Mapping and/or screening	Best fit	Discovery
Amplicon	Mediated by	Restriction enzyme
Targeted—user selected	Type of markers	Random—Non reproducible
High >95%	Consistency of markers called between samples	Med (20–80%)
Up to 5,000	Number of SNPs interrogated/sample	Up to 100,000s

AgriSeq Targeted GBS—a Flexible, Powerful, Highly Accurate Genotyping System



Construct library

- Single-tube, amplicon-based ultrahigh multiplexing
- 50–5,000 variants targeted in 1 reaction



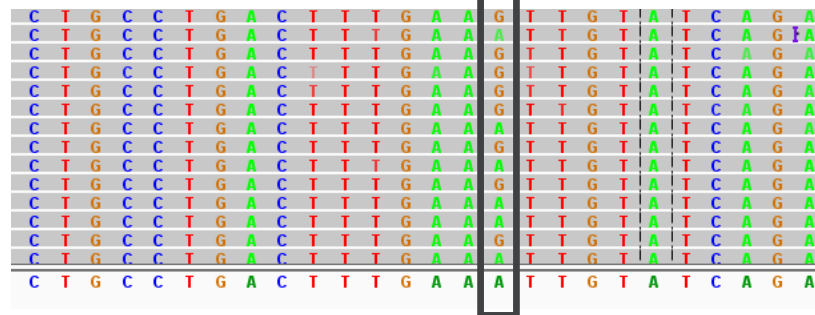
Run sequence

- Barcode up to 768* samples per run
- Combine different panels or species in the same run



Analyze data

- Unambiguous sequence data
- Automated accurate genotype calls

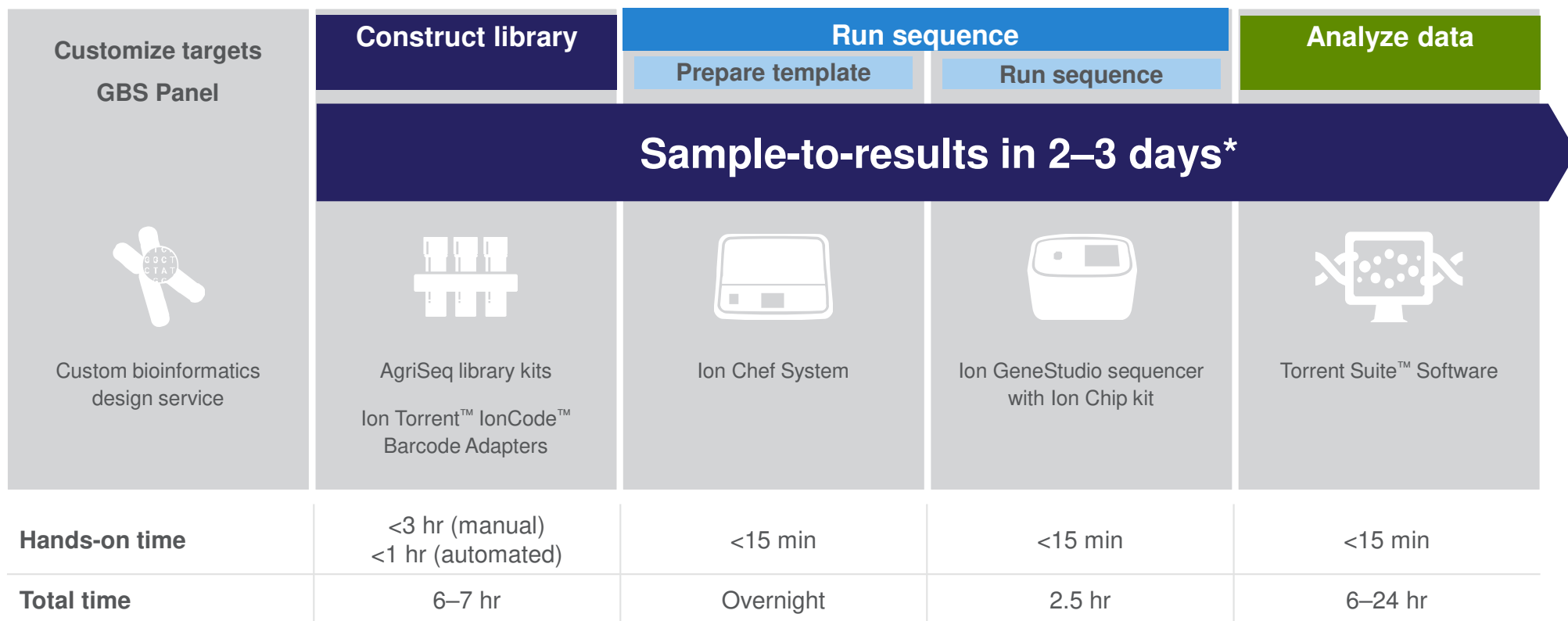


Unambiguous sequence-based genotyping

AgriSeq technology delivers sequencing-based genotyping compared to a reference sequence. Unlike an indirect measurement such as fluorescence-based methods, this is a true sequence-based method.

* Additional barcodes available as early access.

Rapid AgriSeq Workflow with Minimal Hands-On Time

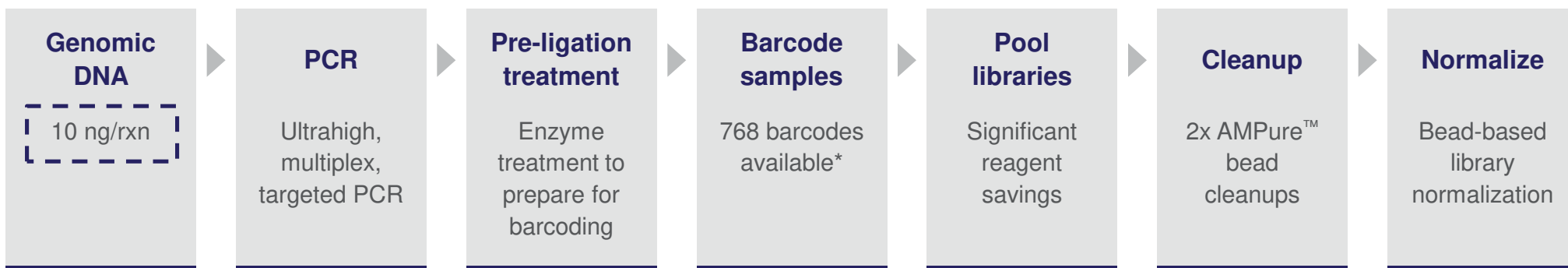


* Depends on customer implementation or varies depending upon system used and samples/chip.

Workflow is achievable with 1 FTE

Highly scalable, with automation to help reduce hands-on time

AgriSeq Library Prep



96- and 384-well protocols



AgriSeq library prep can be performed manually or automated with liquid-handling robotics



6–7 hr total time
(<3 hr hands-on time)



Requires thermal cycler, plate magnet, and AMPure beads



Pool samples postligation to simplify sample handling; reduce tip usage, cleanup, reagent costs

* Additional barcodes available as early access.

AgriSeq GBS Can Generate up to 2.6M Data Points/Day



Ion 550™ Chip

- Sequencing time: 2.5 hr
- Read length: Up to 200 bp
- Reads: 100M–130M
- Coverage 1000x

Throughput

		Number of samples		
		Per chip	Per day	Per year
Number of markers	1,000	115	230	57,500
	500	230	460	115,000
	250	460	920	230,000
	100	1,150*	2,300	575,000

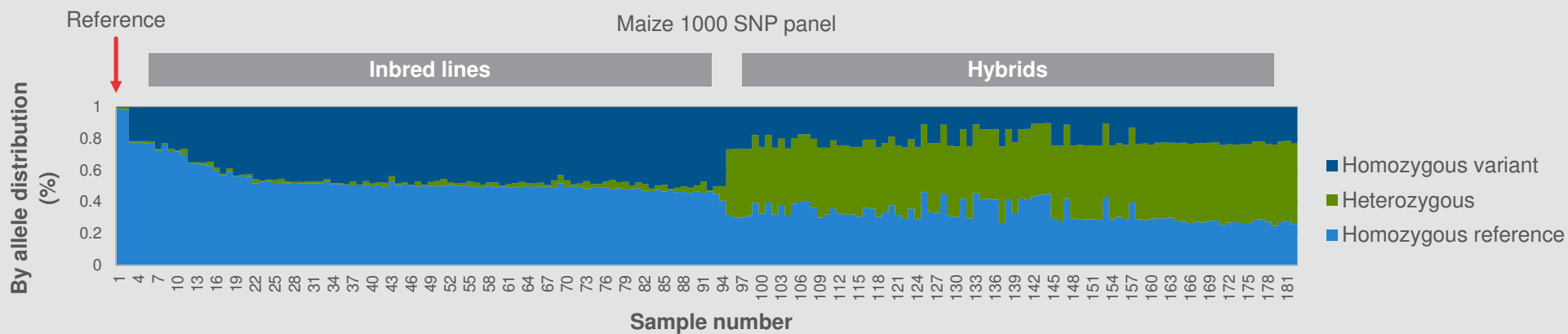
- 1 FTE, single Ion Chef instrument, and Ion GeneStudio™ S5 Prime System
- * Additional barcodes available as early access

Output Ready for Genotyping Analysis

Standard Genotyping TVC output

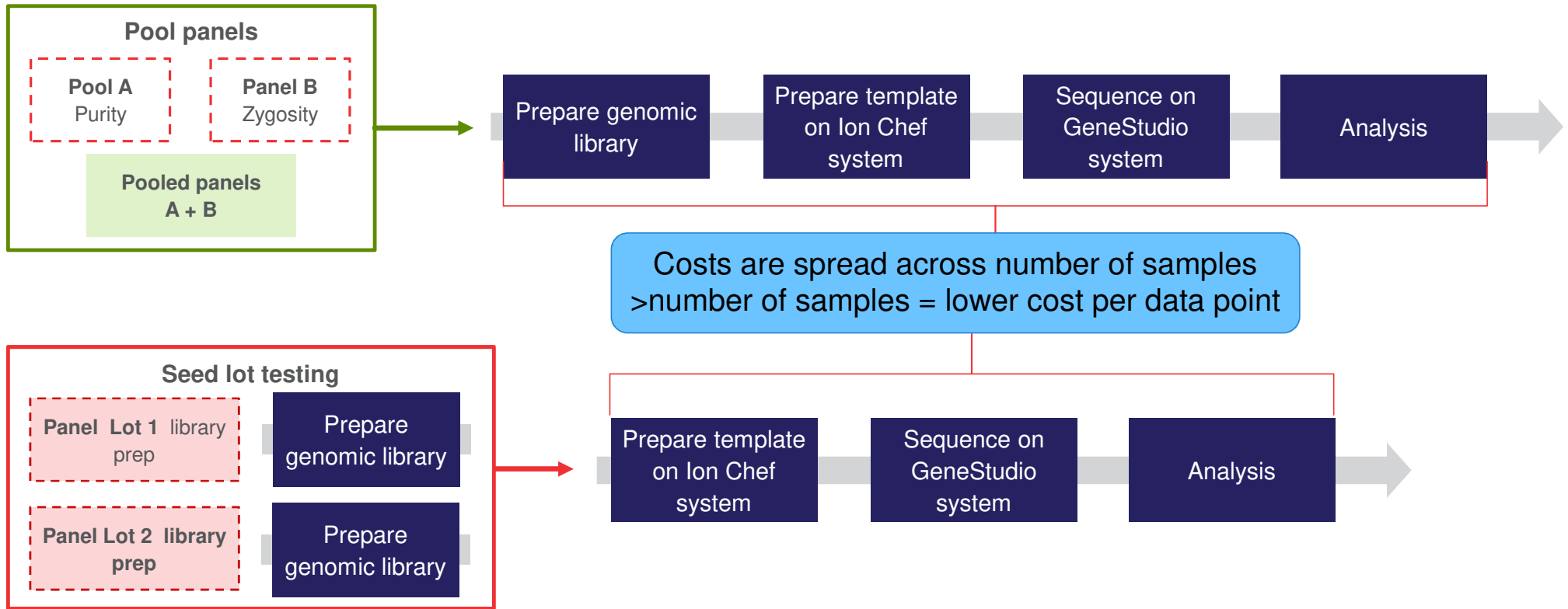
Sample by marker	101	102	103	104	105	106	107
ARS-USMARC-Parent-DQ381153-rs29012842	G/T	G/T	T/T	G/T	G/T	G/G	T/T
ARS-BFGL-BAC-27364	T/G	T/G	T/G	T/G	T/T	./	T/G
ARS-USMARC-Parent-DQ451555-rs29010795	A/G	A/G	A/G	A/A	A/G	A/G	A/A
ARS-BFGL-NGS-86662	G/T	G/T	G/T	G/T	G/G	G/T	G/G
BTB-01285245	T/C	T/C	C/C	T/C	./	T/C	C/C
ARS-USMARC-Parent-DQ404149-no-rs	T/C	T/C	C/C	T/C	C/C	T/C	T/C
BTB-01057979	T/C	T/C	T/C	C/C	C/C	T/C	T/C
ARS-USMARC-Parent-AY761135-rs29003723	A/T	A/T	A/A	A/T	A/A	A/T	A/T
ARS-USMARC-Parent-AY943841-rs17871566	A/G	A/G	A/A	G/G	A/G	A/G	A/G
ARS-USMARC-Parent-DQ404151-rs29019282	C/C	T/C	C/C	T/C	T/C	T/C	T/C

Data suitable for complex analysis and further insights



AgriSeq Target Enrichment Provides Workflow Flexibility

Panels can be pooled and/or markers can be added (spiked into existing panels) to meet customers' needs.



Thermo Fisher Scientific Is Dedicated to Continuous Improvement

>70 custom panel designs for over 29 species and growing...

Plant

- Barley
- Cacao
- Canola
- Corn (maize)
- Cucumber
- Eucalyptus
- Oats
- Onion
- Pine
- Rice
- Sorghum
- Soybean
- Spinach
- Spruce
- Sunflower
- Tomato
- Wheat

Animals

- Bovine
- Canine
- Chicken
- Equine
- Feline
- Porcine
- Salmon

Design improvements

- Complex markers (large indels)
- Faster design and analysis pipeline

Workflow improvements

- Automation scripts
- Compatible with liquid-handling automation

Partnership development

- More predesigned off-the-shelf panels
- Service provider expansion
- KOL partnership and application development



Thank you

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