

# BHQplus® Probes and the Array Tape® Platform: A Successful Combination for Accurate and Economical SNP Genotyping

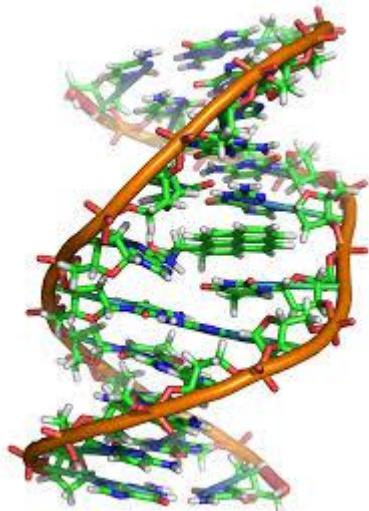
Cassie Keppel

October 30, 2014

# Presentation Outline

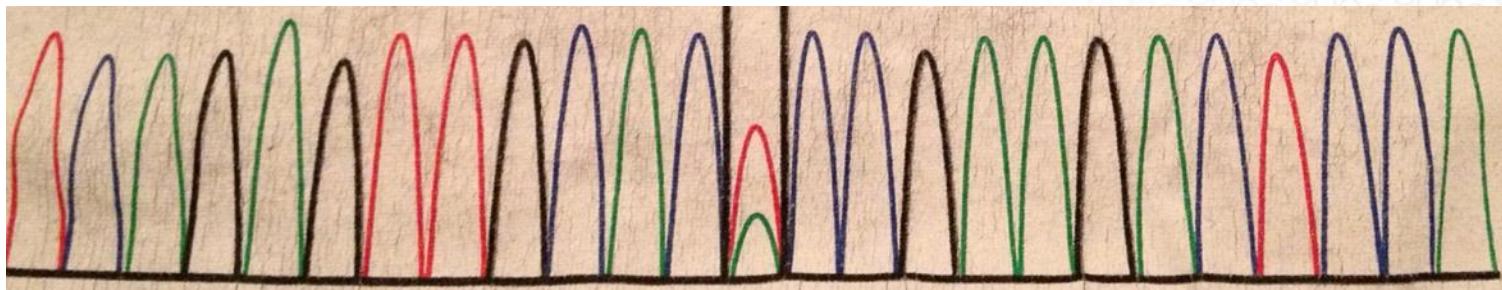
- SNP Genotyping Overview
- Methods for SNP Analysis
- BHQplus SNP Genotyping Assays
- Introduction to the Array Tape Platform
- Case Studies with BHQplus Probes and Array Tape
  - Human
  - Corn
  - Bovine
- Summary and Conclusions

# SNP GENOTYPING OVERVIEW



# SNP Definition

- SNPs are single nucleotide polymorphisms found in the sequence of genomic DNA that is otherwise identical
- SNPs are genomic DNA sequence variants between members of a biological species or paired chromosomes of an individual
- Alternate forms of genes or SNPs are called alleles





# SNP Genotyping Applications



# SNP Genotyping Applications

- Human research and diagnostics
  - Disease prediction
  - Pharmaceutical drug response prediction
  - Genetic research
- Ag-Biotech research and development
  - Marker assisted selection
  - Backcrossing plants or animals
  - Trait associations
  - New crop development and improvement

# SNP GENOTYPING METHODS

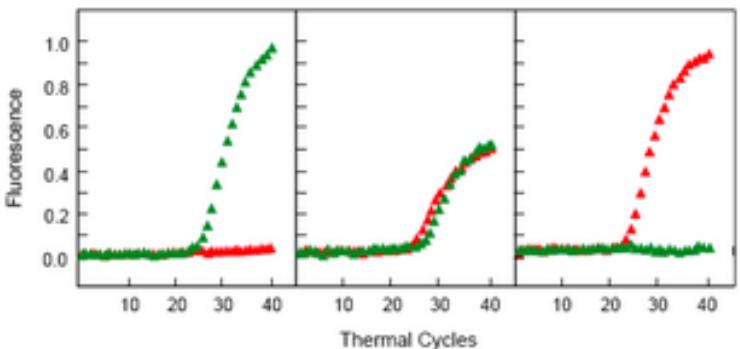
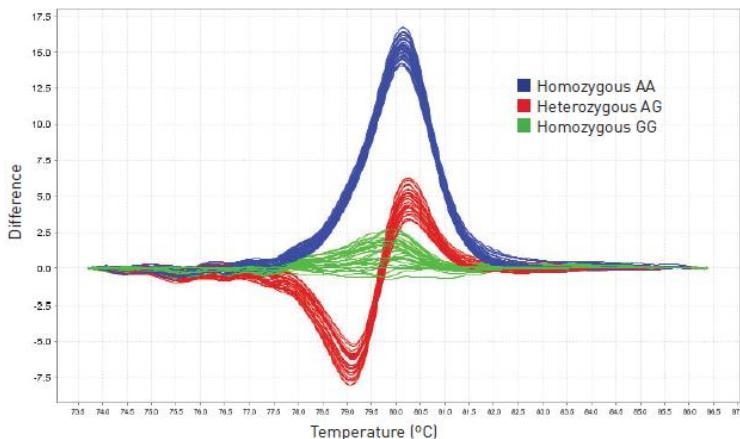


# Non-PCR Based SNP Genotyping Methods

- DNA Sequencing
  - Whole genome sequencing
  - Targeted genotyping by sequencing (GBS)
- Microarray Technologies
  - Affymetrix
  - Illumina
  - Exiqon

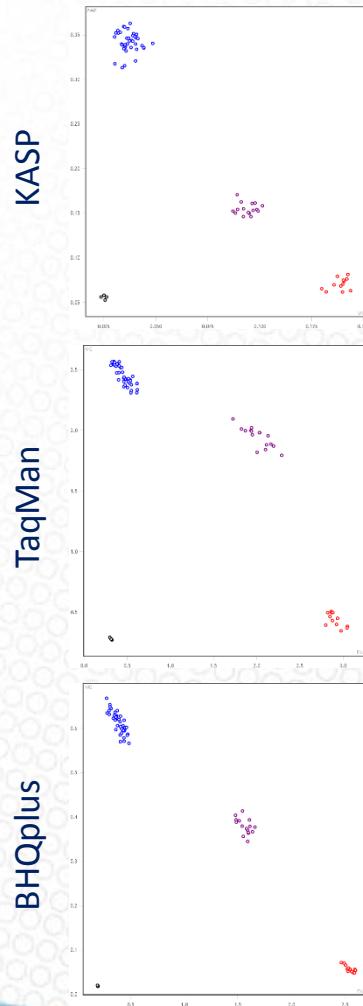
# SNP Genotyping with Real-Time PCR

- MeltDoctor or SYBR green reagents distinguish SNP alleles with melt curve analysis after PCR
- Molecular Beacons distinguish alleles in real time during PCR



# SNP Genotyping with Endpoint PCR

- Kompetitive Allele Specific PCR (KASP) assays from LGC Genomics are FRET-based
- TaqMan® assays from Life Technologies are hydrolysis probe-based with Minor Grove Binders (MGB)
- BHQplus® assays from Biosearch Technologies are hydrolysis probe-based with duplex stabilizing technology and Black Hole Quenchers®



# Considerations for SNP Genotyping with PCR

- Sample preparation protocol
  - Cost per sample
  - Speed and ease of use
  - Compatibility with assay and master mix
- Assay design
  - Purchase pre-designed, pre-validated assays
  - Design custom assays
- Reaction volume
  - Dependent upon the platform
- Cost of master mix
- Cost of assay

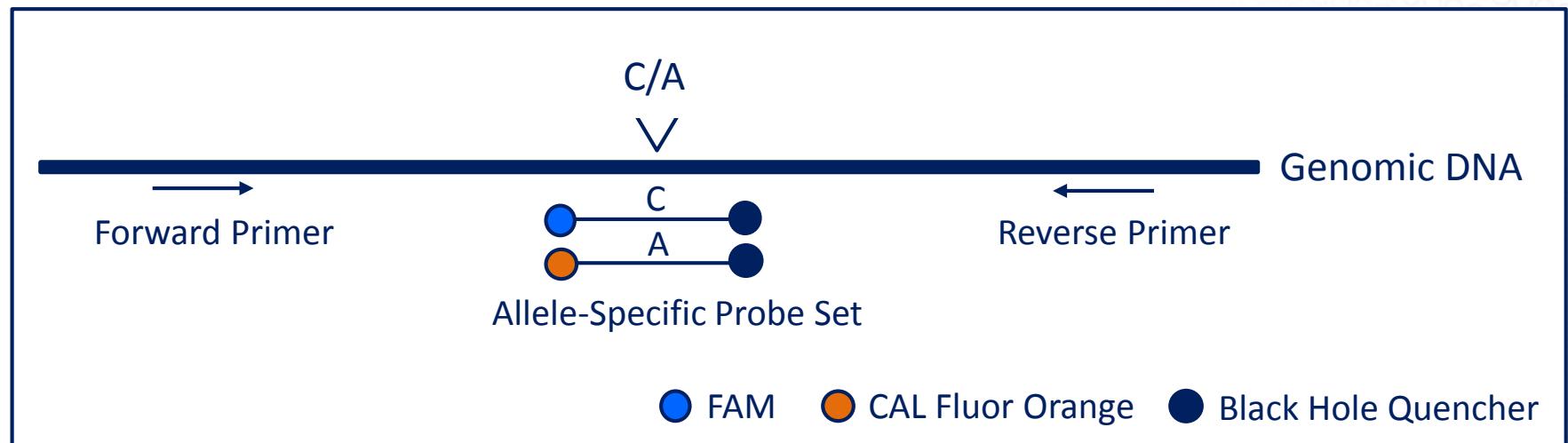
# SNP GENOTYPING WITH BHQPLUS ASSAYS



# BHQplus Probes and SNP Genotyping Assays

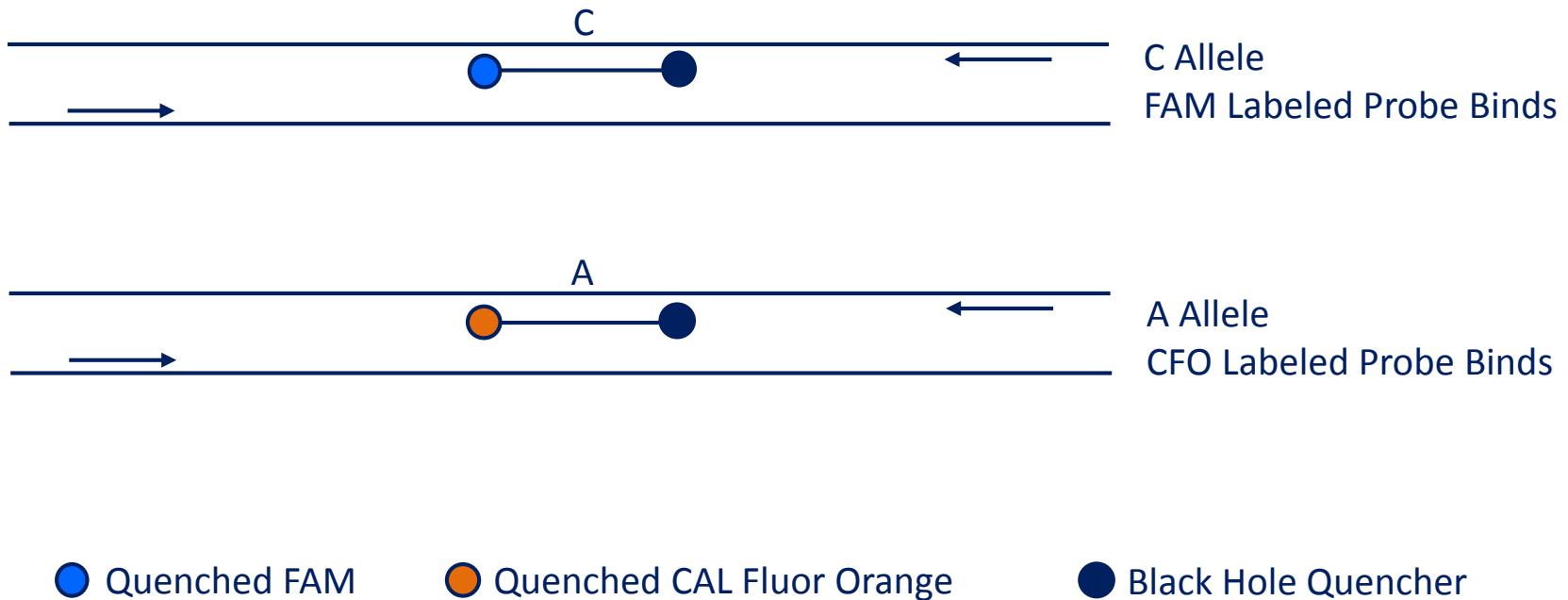
- BHQplus probes form highly stable duplexes with DNA targets
  - Allows shorter probes enhanced target specificity
  - Ideal for SNP discrimination
- Black Hole Quenchers do not interfere with fluorescent dye signal after hydrolysis
- BHQplus assays are easy to design and order with RealTimeDesign software available on the Biosearch Technologies website
- BHQplus probes provide a cost-effective method for SNP genotyping with hydrolysis probe-based assays

# BHQplus SNP Genotyping Assays



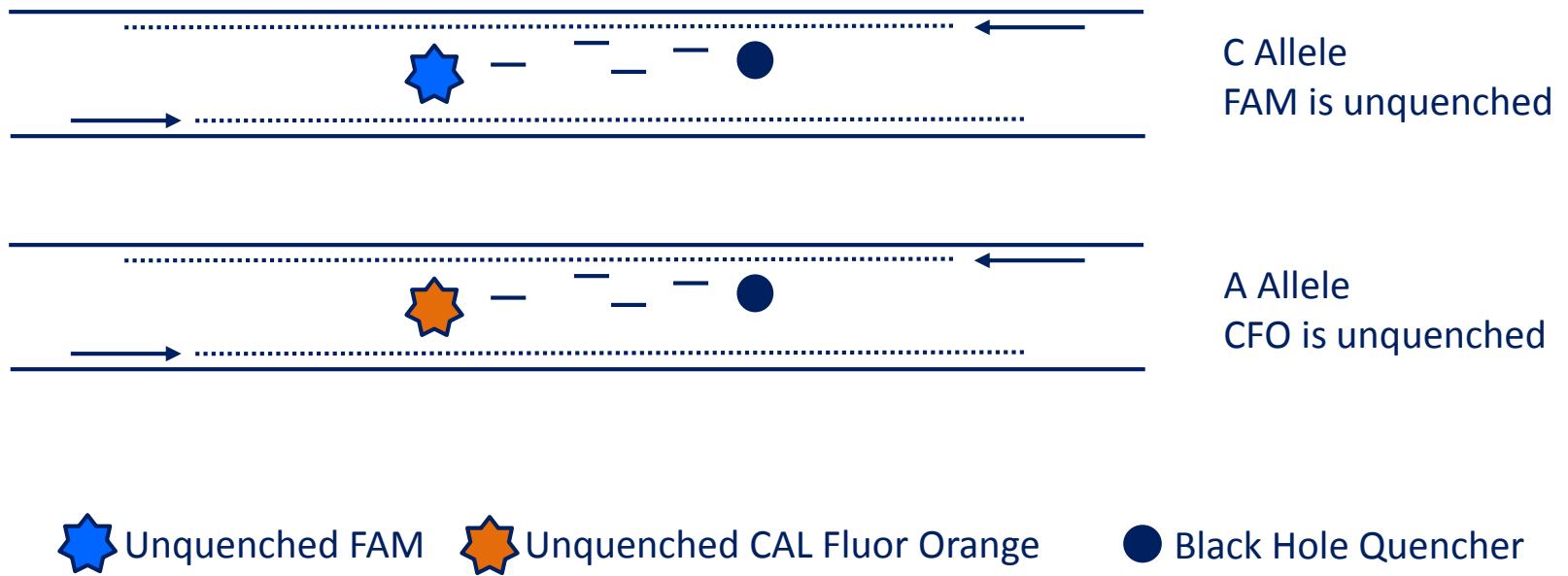
# BHQplus SNP Genotyping Mechanism

## Primer and Allele-Specific Probe Annealing

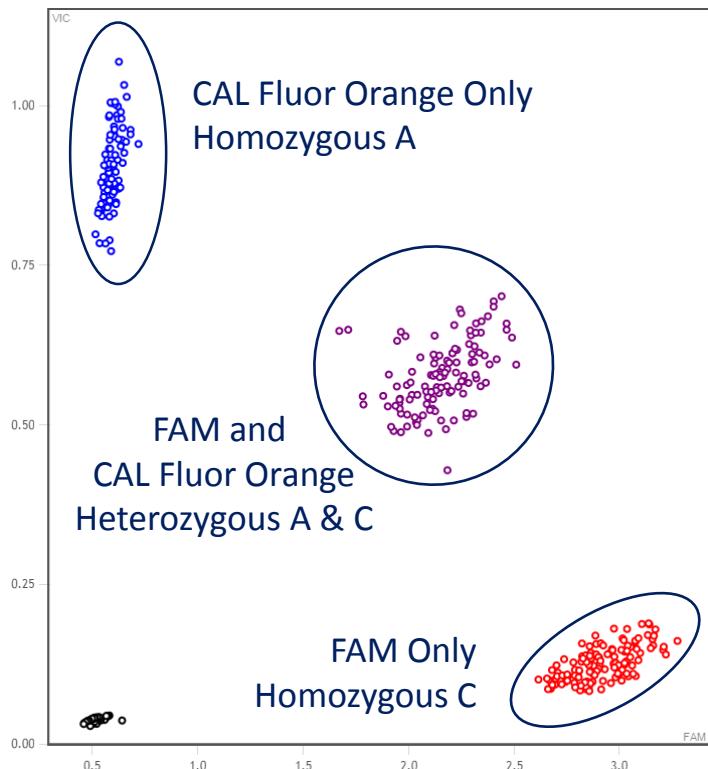


# BHQplus SNP Genotyping Mechanism, Continued

## Primer Extension and Allele-Specific Probe Hydrolysis

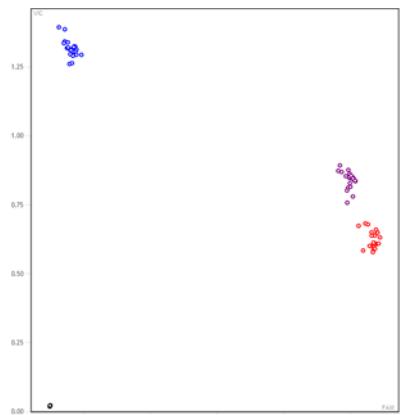


# SNP Genotyping Assay Cluster Plot Analysis

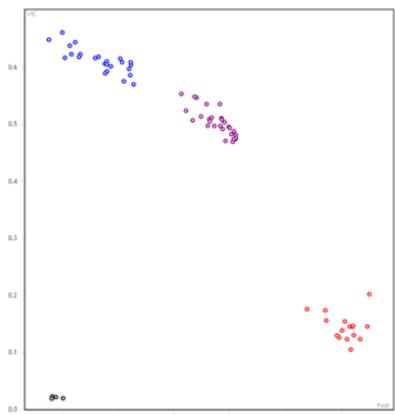


# Varied Cluster Plot Characteristics

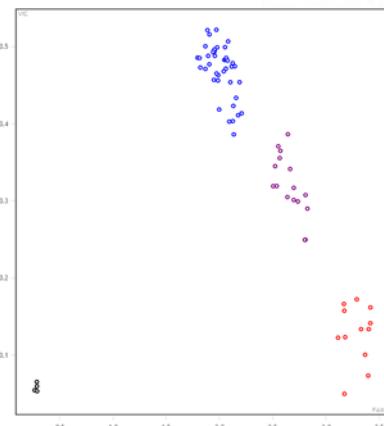
Het cluster near  
homozygous FAM  
cluster



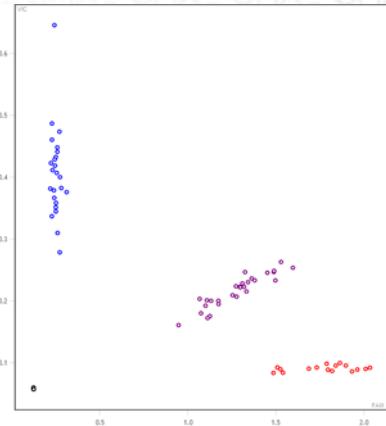
Het cluster near  
homozygous VIC  
cluster



Clusters large and  
spread out



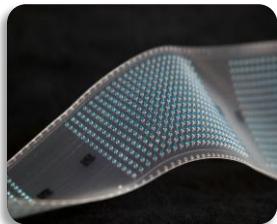
Elongated clusters



# THE ARRAY TAPE® PLATFORM



# The Array Tape Platform



## Array Tape®

- » Flexible microplate replacement
- » Reduced reaction volumes
- » Total volume = 2 $\mu$ L
- » Optically clear seal

## Nexar®

- » Liquid handler optimized for Array Tape
- » Dispenses 800nL DNA, 384 samples at once
- » Dispenses 800nL Master Mix, 384 wells in 38 sec
- » Seals Array Tape for thermal cycling

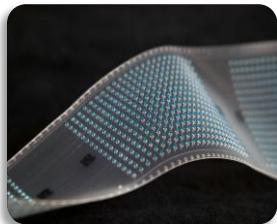
## Soellex®

- » High capacity water bath PCR; holds up to 600 arrays
- » Optimized for Array Tape
- » Three tanks for PCR protocol optimization
- » Touchdown, two step, or three step protocols

## Araya®

- » Endpoint fluorescence scanner
- » Three channels: FAM; HEX, VIC, & CFO; ROX
- » Scans 384 wells in 28 sec
- » Data ready for analysis in Intellics™ or other software

# The Array Tape Platform Workflow



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**Array Tape®**

**Nexar®**

**Soellex®**

**Araya®**

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Microplate  
Replacement

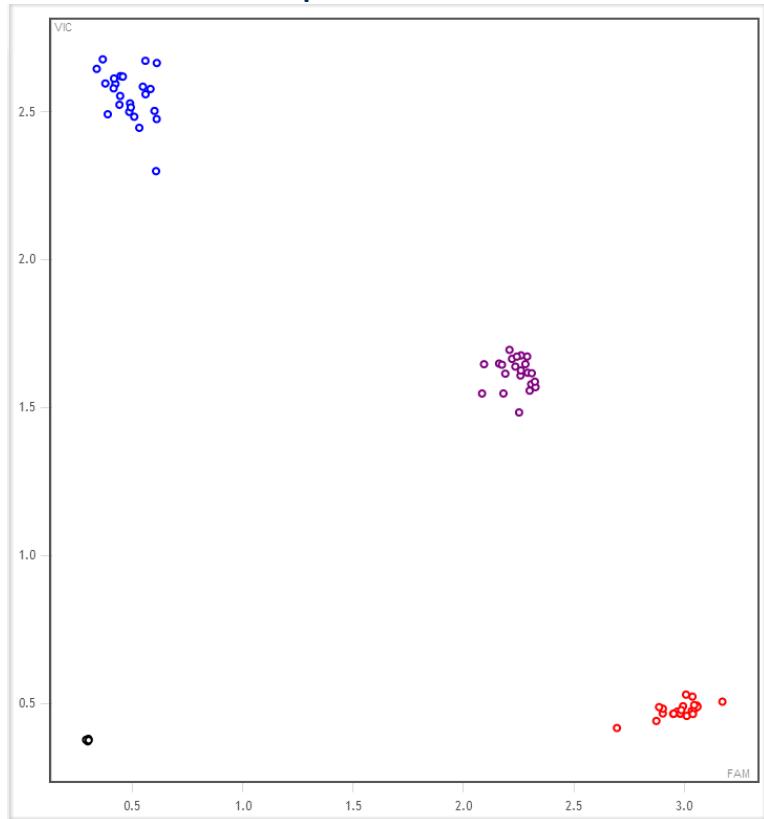
Liquid  
Handling

Thermal  
Cycling

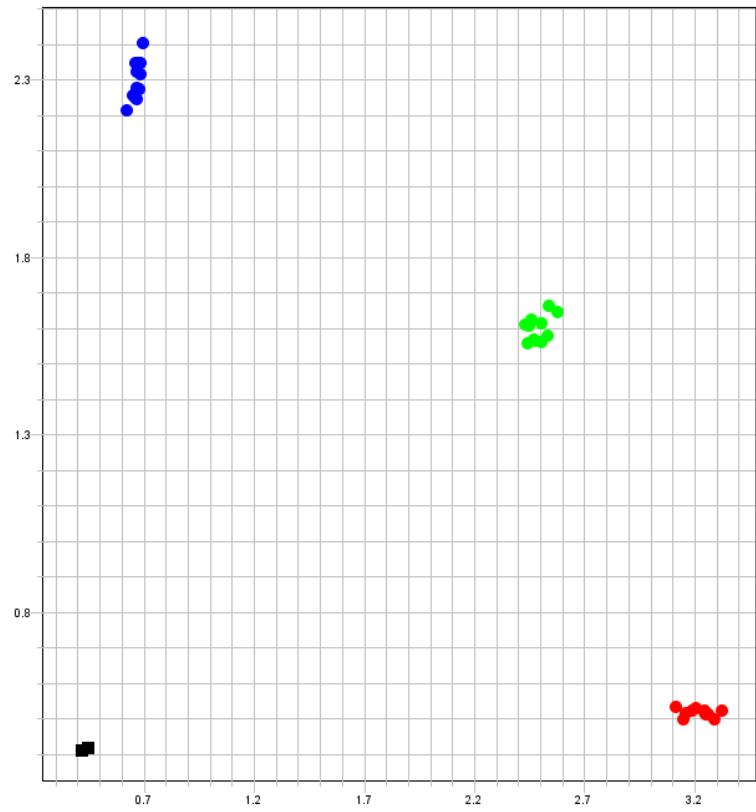
Fluorescence  
Detection

# SNP Genotyping Result Comparison

Array Tape Platform  
1.6 $\mu$ L Reactions



Real Time PCR Instrument  
5 $\mu$ L Reactions



# **SNP GENOTYPING CASE STUDIES WITH BHQPLUS ASSAYS AND ARRAY TAPE**

# HUMAN SNP GENOTYPING



# Supplies for Human SNP Genotyping Study

Reagent	Supplier
Human Genomic DNA Samples	34 purified human DNA cell line samples from Coriell Cell Repositories ➤ Samples were diluted to 5ng/ $\mu$ L in water before use
SNP Genotyping Assays	Pre-designed TaqMan SNP genotyping assays from Life Technologies Custom BHQplus SNP genotyping assays designed with RealTimeDesign software ➤ SNP assays were chosen because of their pharmacogenetic significance
Master Mix	TaqMan Genotyping master mix by Life Technologies
Instruments	ViiA7 by Life Technologies with 384 well plates (5 $\mu$ L total volume reactions) Array Tape Platform with 384 well arrays (1.6 $\mu$ L total volume reactions)

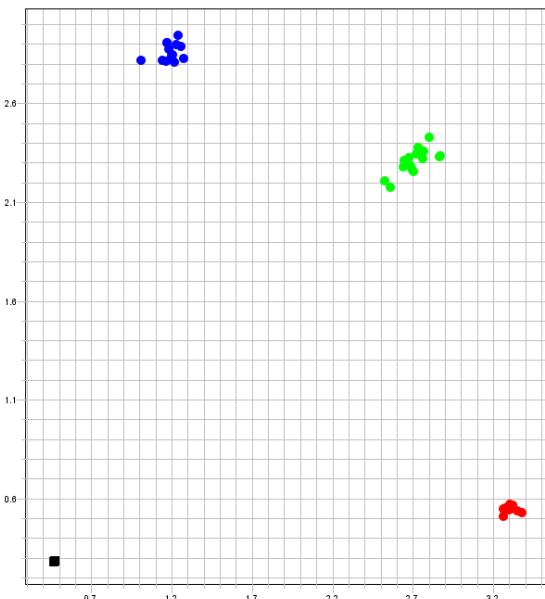
# Human SNP Information

SNP Name	rs Number	Chromosomal Location	Pharmacogenetic Significance
VKORC1	rs9923231	Chr16 (p11.2)	Genetic variability impacts metabolism and dosage of warfarin
CYP4F2	rs2108622	Chr19 (p13.12)	Genetic variability impacts metabolism and dosage of warfarin
KIF6	rs20455	Chr6 (p21.2)	Genetic variability impacts the absolute risk reduction in coronary heart disease by statin therapy

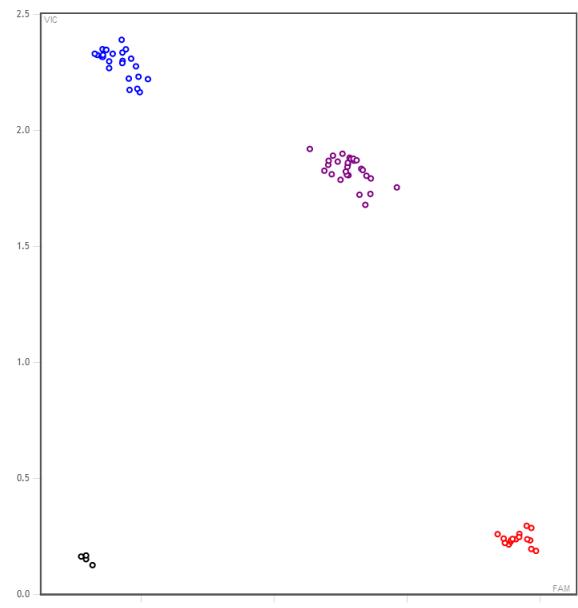
The consensus genotype calls for the 34 Coriell genomic DNA samples in this study are publicly available  
Genotypes for VKORC1 are published by Pratt, et. al., JMD November 2010, Vol. 12, No. 6  
Genotypes for CYP4F2 and KIF6 are published in the accompanying supplemental materials by Pratt, et. al.

# Cluster Plots for VKORC1

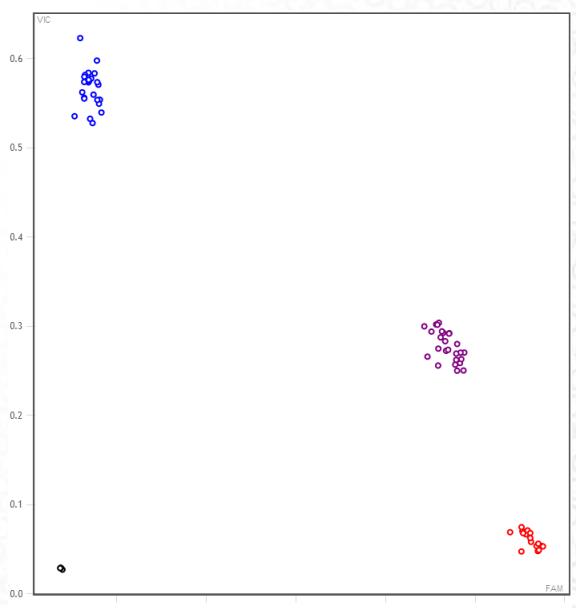
TaqMan Assay  
ViiA7 Real Time PCR Instrument



TaqMan Assay  
Array Tape Platform



BHQplus Assay  
Array Tape Platform

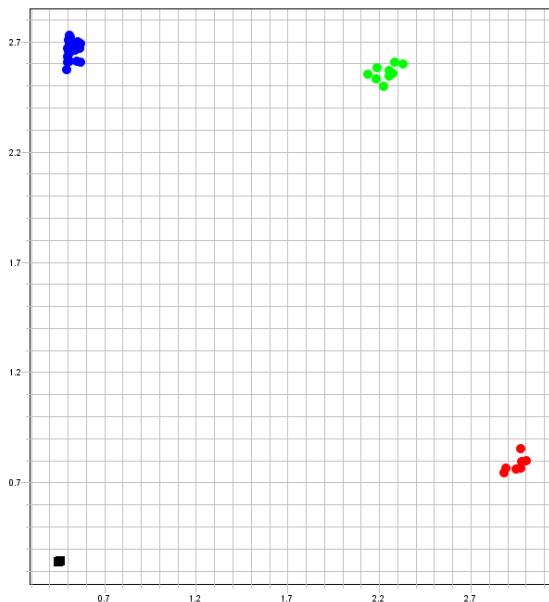


# VKORC1 Results

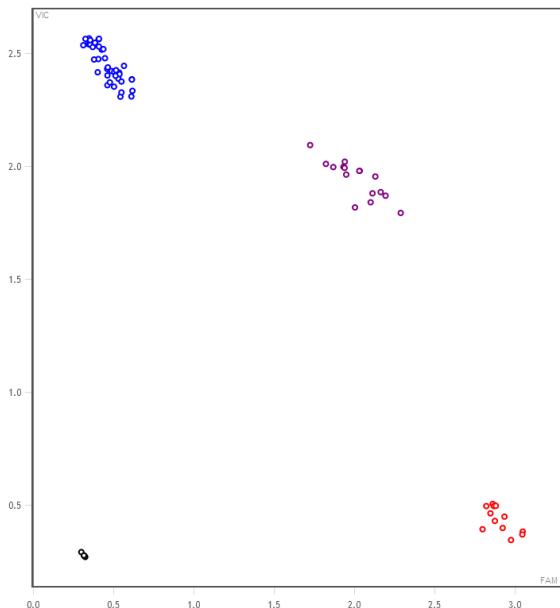
Cell Line	Published/ Expected	TaqMan ViiA7	TaqMan Array Tape	BHQplus Array Tape
7439	G/G	G/G	G/G	G/G
10005	G/G	G/G	G/G	G/G
12244	G/G	G/G	G/G	G/G
12273	G/G	G/G	G/G	G/G
17039	G/G	G/G	G/G	G/G
17052	A/A	A/A	A/A	A/A
17057	A/G	A/G	A/G	A/G
17058	A/A	A/A	A/A	A/A
17084	A/G	A/G	A/G	A/G
17114	G/G	G/G	G/G	G/G
17115	G/G	G/G	G/G	G/G
17119	G/G	G/G	G/G	G/G
17129	G/G	G/G	G/G	G/G
17130	G/G	G/G	G/G	G/G
17203	A/G	A/G	A/G	A/G
17204	A/A	A/A	A/A	A/A
17210	A/A	A/A	A/A	A/A
17221	A/G	A/G	A/G	A/G
17227	A/G	A/G	A/G	A/G
17235	G/G	G/G	G/G	G/G
17240	A/A	A/A	A/A	A/A
17246	A/G	A/G	A/G	A/G
17247	A/G	A/G	A/G	A/G
17248	A/A	A/A	A/A	A/A
17252	A/G	A/G	A/G	A/G
17272	A/A	A/A	A/A	A/A
17280	G/G	G/G	G/G	G/G
17281	A/G	A/G	A/G	A/G
17289	A/A	A/A	A/A	A/A
17293	A/G	A/G	A/G	A/G
17296	A/G	A/G	A/G	A/G
17298	A/G	A/G	A/G	A/G
17300	A/G	A/G	A/G	A/G
2016	A/G	A/G	A/G	A/G

# Cluster Plots for CYP4F2

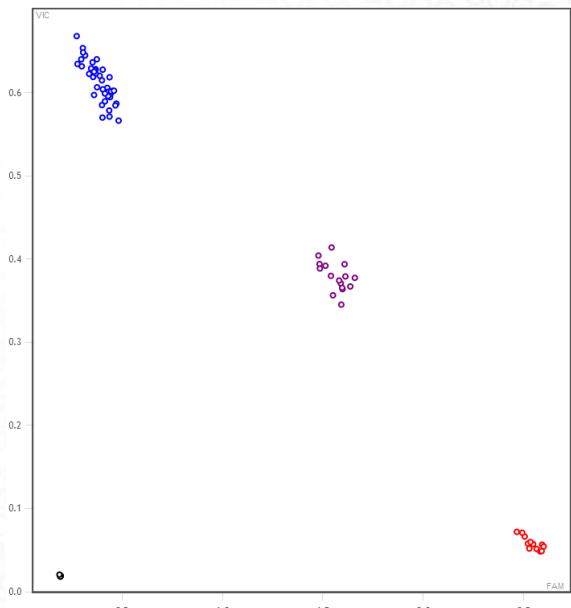
TaqMan Assay  
ViiA7 Real Time PCR Instrument



TaqMan Assay  
Array Tape Platform



BHQplus Assay  
Array Tape Platform

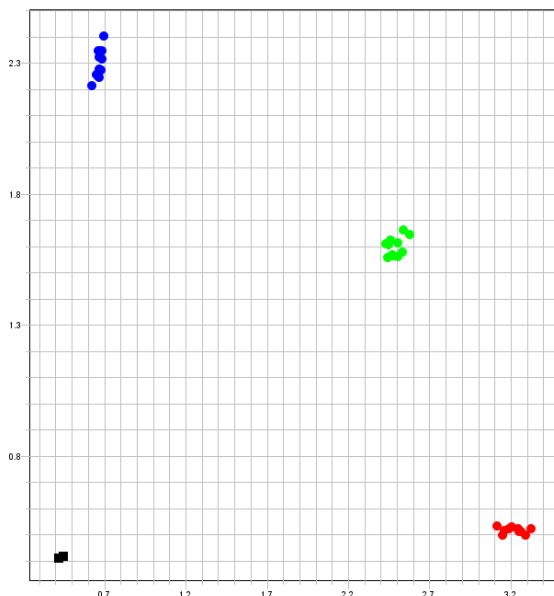


# CYP4F2 Results

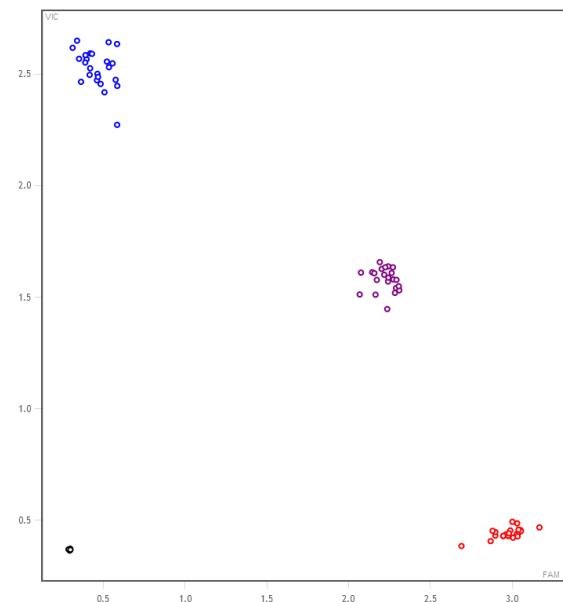
Cell Line	Published/ Expected	TaqMan ViiA7	TaqMan Array Tape	BHQplus Array Tape
7439	G/G	G/G	G/G	G/G
10005	A/G	A/G	A/G	A/G
12244	G/G	G/G	G/G	G/G
12273	A/G	A/G	A/G	A/G
17039	G/G	G/G	G/G	G/G
17052	G/G	G/G	G/G	G/G
17057	A/A	A/A	A/A	A/A
17058	G/G	G/G	G/G	G/G
17084	A/A	A/A	A/A	A/A
17114	G/G	G/G	G/G	G/G
17115	G/G	G/G	G/G	G/G
17119	G/G	G/G	G/G	G/G
17129	G/G	G/G	G/G	G/G
17130	G/G	G/G	G/G	G/G
17203	G/G	G/G	G/G	G/G
17204	G/G	G/G	G/G	G/G
17210	A/A	A/A	A/A	A/A
17221	A/G	A/G	A/G	A/G
17227	A/G	A/G	A/G	A/G
17235	G/G	G/G	G/G	G/G
17240	G/G	G/G	G/G	G/G
17246	G/G	G/G	G/G	G/G
17247	A/A	A/A	A/A	A/A
17248	A/G	A/G	A/G	A/G
17252	G/G	G/G	G/G	G/G
17272	A/A	A/A	A/A	A/A
17280	G/G	G/G	G/G	G/G
17281	G/G	G/G	G/G	G/G
17289	A/G	A/G	A/G	A/G
17293	G/G	G/G	G/G	G/G
17296	A/A	A/A	A/A	A/A
17298	A/G	A/G	A/G	A/G
17300	A/G	A/G	A/G	A/G
2016	G/G	G/G	G/G	G/G

# Cluster Plots for KIF6

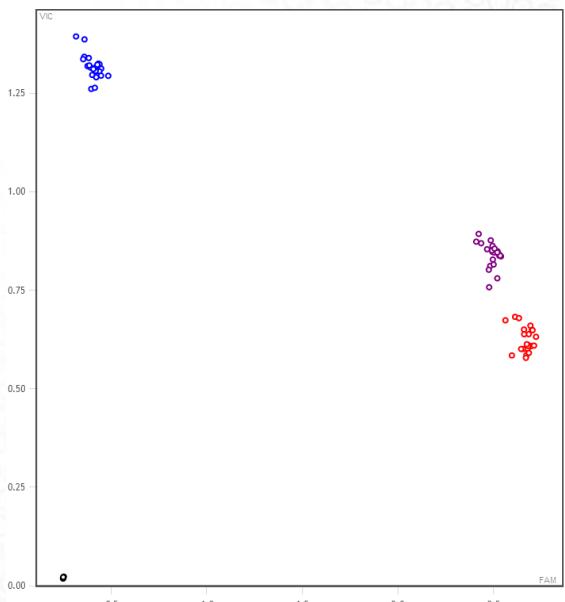
TaqMan Assay  
ViiA7 Real Time PCR Instrument



TaqMan Assay  
Array Tape Platform



BHQplus Assay  
Array Tape Platform



# KIF6 Results

Cell Line	Published/ Expected	TaqMan ViiA7	TaqMan Array Tape	BHQplus Array Tape
7439	C/C	C/C	C/C	C/C
10005	C/T	C/T	C/T	C/T
12244	T/T	T/T	T/T	T/T
12273	C/T	C/T	C/T	C/T
17039	C/C	C/C	C/C	C/C
17052	T/T	T/T	T/T	T/T
17057	C/C	C/C	C/C	C/C
17058	C/T	C/T	C/T	C/T
17084	C/T	C/T	C/T	C/T
17114	C/T	C/T	C/T	C/T
17115	C/C	C/C	C/C	C/C
17119	C/C	C/C	C/C	C/C
17129	C/C	C/C	C/C	C/C
17130	C/C	C/C	C/C	C/C
17203	T/T	T/T	T/T	T/T
17204	T/T	T/T	T/T	T/T
17210	C/T	C/T	C/T	C/T
17221	T/T	T/T	T/T	T/T
17227	T/T	T/T	T/T	T/T
17235	C/T	C/T	C/T	C/T
17240	C/T	C/T	C/T	C/T
17246	C/T	C/T	C/T	C/T
17247	T/T	T/T	T/T	T/T
17248	T/T	T/T	T/T	T/T
17252	C/C	C/C	C/C	C/C
17272	T/T	T/T	T/T	T/T
17280	T/T	T/T	T/T	T/T
17281	C/C	C/C	C/C	C/C
17289	T/T	T/T	T/T	T/T
17293	C/T	C/T	C/T	C/T
17296	T/T	T/T	T/T	T/T
17298	C/T	C/T	C/T	C/T
17300	C/C	C/C	C/C	C/C
2016	C/C	C/C	C/C	C/C

# Human SNP Study Summary and Conclusions

- Custom BHQplus SNP genotyping assays
  - Produced the expected calls for each sample and assay
  - Performed equivalently to pre-designed TaqMan assays
  - Were successfully miniaturized to 1.6µL reactions
- The Array Tape Platform
  - Produced the expected calls for each sample and assay
  - Performed equivalently to the real-time PCR instrument
- The combination of BHQplus SNP genotyping assays and the Array Tape Platform matches the performance of pre-designed reagents and a real time PCR instrument

# CORN SNP GENOTYPING



# Corn SNP Information

DZm SNP ID	GenBank Accession Number	Chromosome
2575115	AC185516.4	1
2490176	AC196688.4	3
2571611	AC219032.4	4

All SNPs in this study were identified as highly polymorphic as published by Mammadov et. al., Theor Appl Genet (2010) 121:577-588

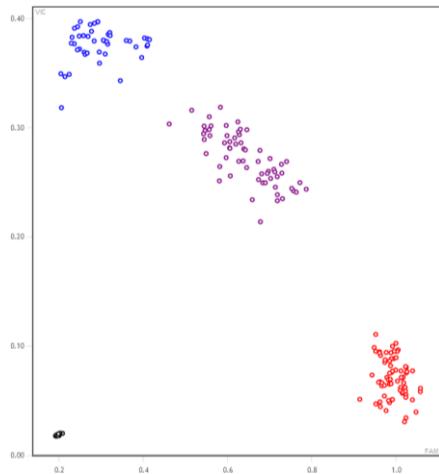
The SNPs used in this study are not associated with any specific corn traits. They were chosen because of their polymorphic nature and were used to demonstrate the impact of master mix, sample prep, reaction volume, and instrumentation on cluster plot quality and call rates.

# Supplies for Corn SNP Genotyping Study

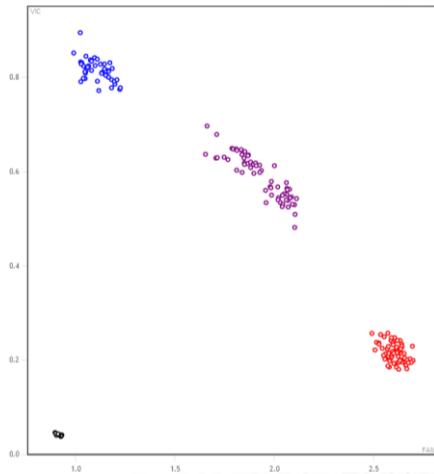
Reagent	Supplier
Corn Samples	<p>Yellow field corn samples were obtained from 11 sources in central Minnesota</p> <ul style="list-style-type: none"> <li>➤ 8 individual kernels were used from each source, for 88 total samples</li> <li>➤ Crude preps were prepared from individual kernels with sodium hydroxide</li> </ul>
SNP Genotyping Assays	<p>Custom BHQplus SNP genotyping assays designed with RealTimeDesign software</p> <ul style="list-style-type: none"> <li>➤ SNP assays were chosen because of their polymorphic nature</li> </ul>
Master Mixes	<p>KlearKall Master Mix from LGC Genomics</p> <p>TaqMan GTxpress Master Mix from Life Technologies</p> <p>PerfeCTa qPCR ToughMix from Quanta BioSciences</p> <p>SsoAdvanced Universal Probes Supermix from Bio-Rad Laboratories</p>
Instruments	<p>ViiA7 by Life Technologies with 384 well plates (5µL total volume reactions)</p> <p>Array Tape Platform with 384 well arrays (1.6µL total volume reactions)</p>

# Cluster Plots for SNP 115

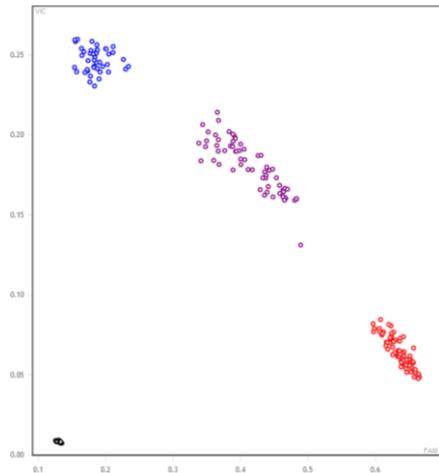
KlearKall



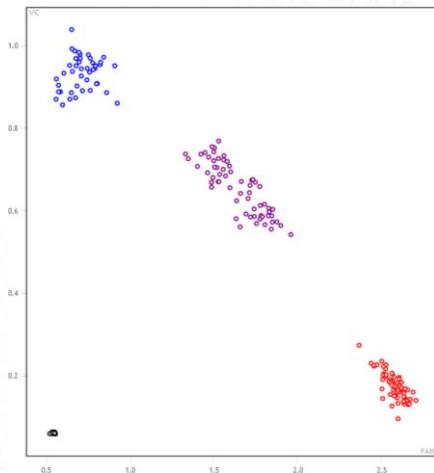
GTxpress



PerfeCTa



SsoAdvanced

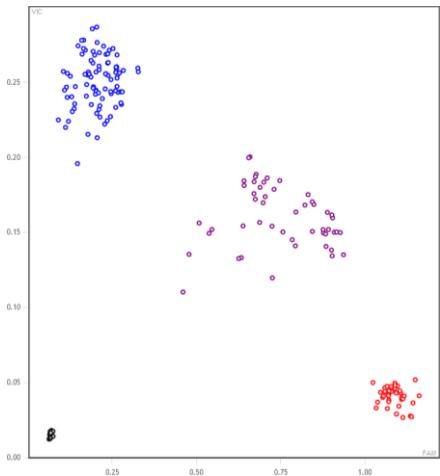


# Summary of Results for SNP 115

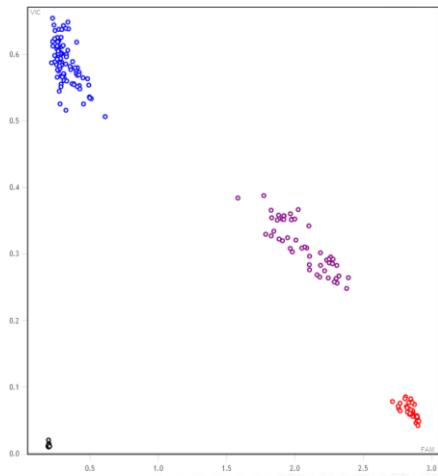
Genotype	Master Mix	Array Tape Platform				ViiA7
		KlearKall	TaqMan GTxpress	PerfeCTa ToughMix	Bio-Rad SsoAdvanced	TaqMan GTxpress
FAM (A/A)		35	35	35	35	35
HET (A/T)		31	31	31	31	31
CAL Fluor Orange (T/T)		22	22	22	22	22
Indeterminate		0	0	0	0	0
Call Rate (%)		100	100	100	100	100

# Cluster Plots for SNP 176

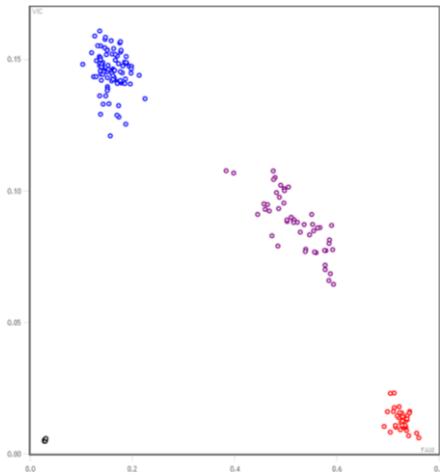
KlearKall



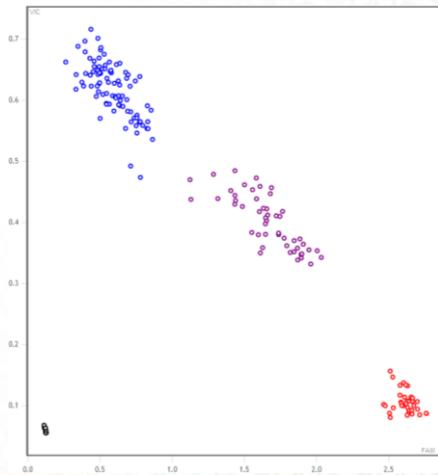
GTxpress



PerfeCTa



SsoAdvanced

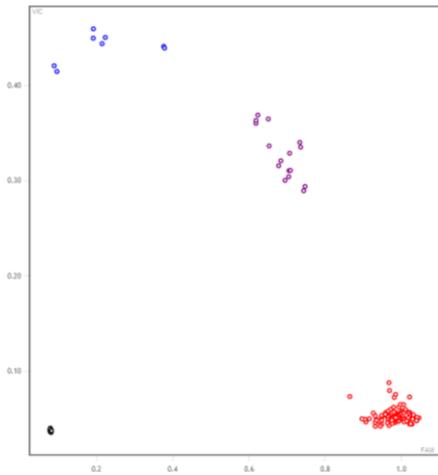


# Summary of Results for SNP 176

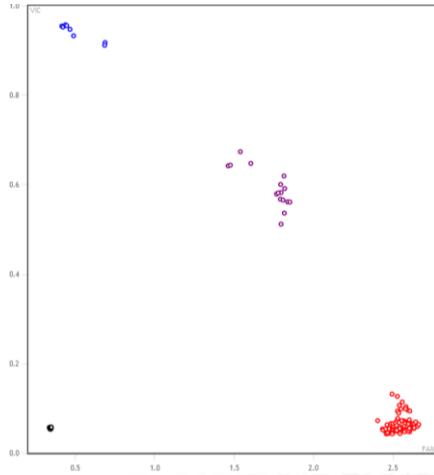
Genotype	Master Mix	Array Tape Platform				ViiA7
		KlearKall	TaqMan GTxpress	PerfeCTa ToughMix	Bio-Rad SsoAdvanced	TaqMan GTxpress
FAM (C/C)		18	18	18	18	18
HET (C/T)		25	25	25	25	25
CAL Fluor Orange (T/T)		45	45	45	45	45
Indeterminate		0	0	0	0	0
Call Rate (%)		100	100	100	100	100

# Cluster Plots for SNP 611

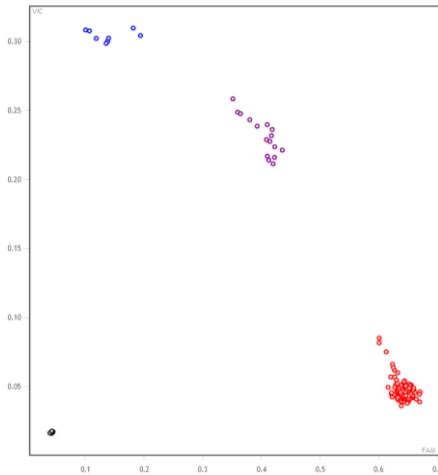
KlearKall



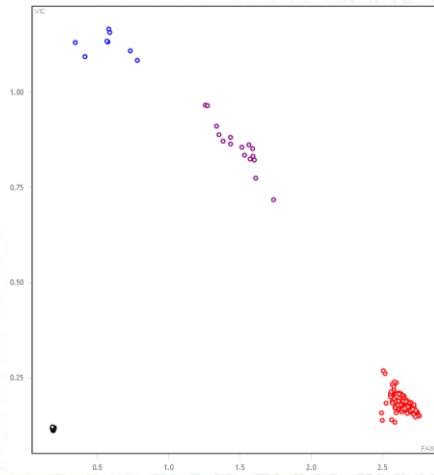
GTxpress



PerfeCTa



SsoAdvanced



# Summary of Results for SNP 611

Genotype	Master Mix	Array Tape Platform				ViiA7
		KlearKall	TaqMan GTxpress	PerfeCTa ToughMix	Bio-Rad SsoAdvanced	TaqMan GTxpress
FAM (G/G)		76	76	76	76	76
HET (G/T)		8	8	8	8	8
CAL Fluor Orange (T/T)		4	4	4	4	4
Indeterminate		0	0	0	0	0
Call Rate (%)		100	100	100	100	100

## Corn SNP Study Summary and Conclusions

- Crude DNA samples are compatible with BHQplus probe SNP assays and the Array Tape Platform
- SNP genotype calls were consistent with multiple master mixes and across instruments
- Call rates were 100% for each assay
- Genotype calls matched for Array Tape and the ViiA7
- When combined, inexpensive, crude preparation protocols and miniaturized reactions enable incredible cost savings for SNP genotyping analysis of agricultural samples

# BOVINE SNP GENOTYPING



# Supplies for Bovine SNP Genotyping Study

Reagent	Supplier
Bovine Samples	29 beef samples were obtained from various grocery stores in Minneapolis, MN
Sample Preparation Protocols	Qiagen DNeasy Blood and Tissue Kit Quanta BioSciences Extracta™ DNA Prep for PCR-Tissue Sodium hydroxide crude prep protocol
SNP Genotyping Assays	Custom BHQplus SNP genotyping assays designed with RealTimeDesign software ➤ SNP assays were chosen because for commercial importance to the beef industry
Master Mixes	TaqMan GTxpress Master Mix from Life Technologies TaqMan Genotyping Master Mix from Life Technologies PerfeCTa qPCR ToughMix from Quanta BioSciences SensiFAST Genotyping Master Mix from Bioline SensiMix II Probes Master Mix from Bioline SsoAdvanced Universal Probes Supermix from Bio-Rad Laboratories
Instrument	Array Tape Platform with 384 well arrays (1.6µL total volume reactions)

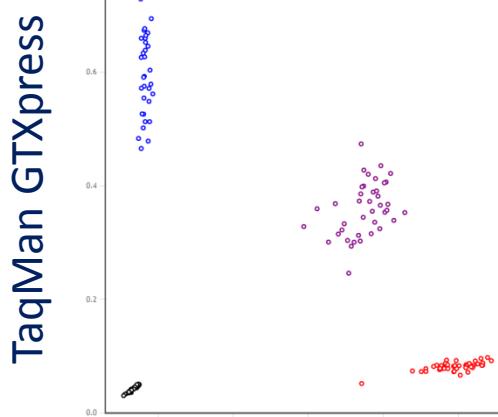
# Bovine SNPs Analyzed in Array Tape

SNP Name	GenBank Accession Number	Chromosome	Commercial Importance
Leptin-E2FB	JQ711179.1	4	SNP linked to body fat in cattle CC Allele: lean cattle, not favorable CT Allele: economically intermediate TT Allele: fatter cattle, favorable
CAPN1-4751	AF248054.2	29	SNP linked to meat tenderness TT Allele: less tender, not favorable TC Allele: intermediate CC Allele: favorable for tenderness
UoGCAST	AY834770.1	7	SNP linked to meat tenderness GG Allele: less tender, not favorable CG Allele: less tender; dominant G allele CC Allele: most tender, favorable

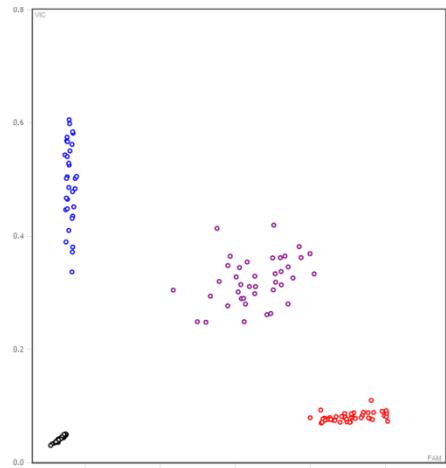
# DNA Sample Preparation Comparison

## CAPN1-4751 SNP assay

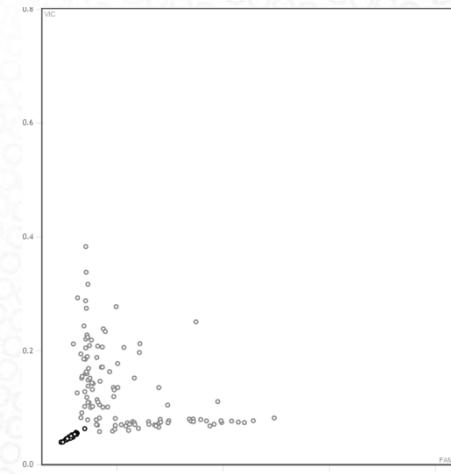
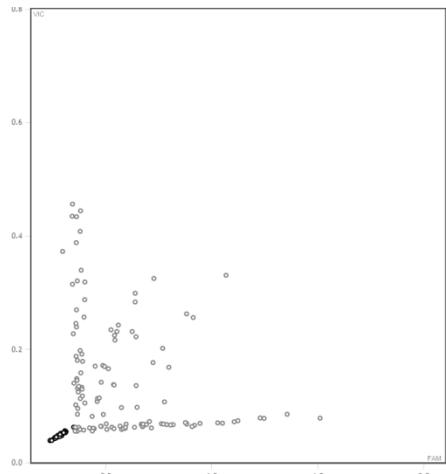
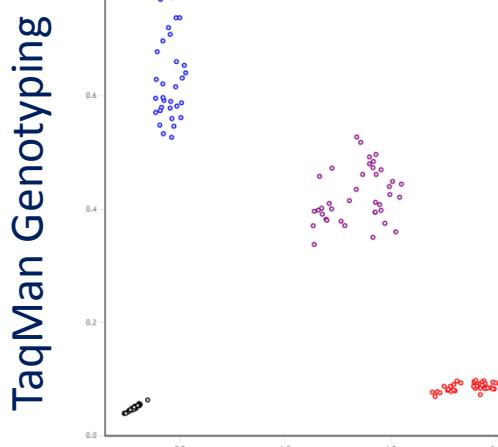
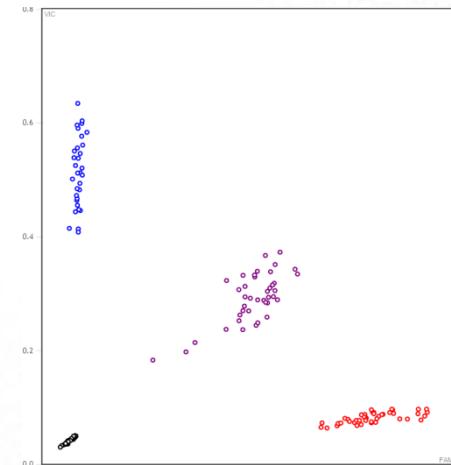
Purified DNA



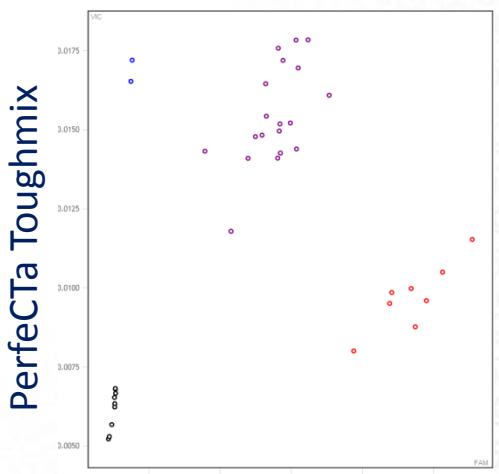
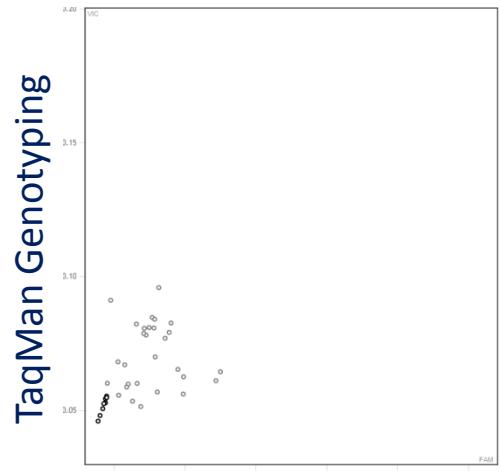
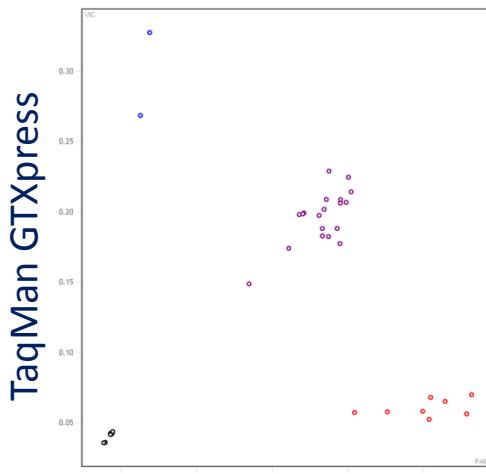
Extracta Kit



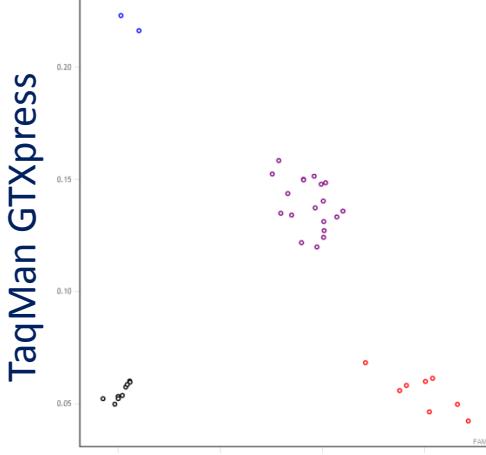
NaOH Crude Prep



# Leptin SNP Assay: Master Mix Comparison, NaOH Crude Prep

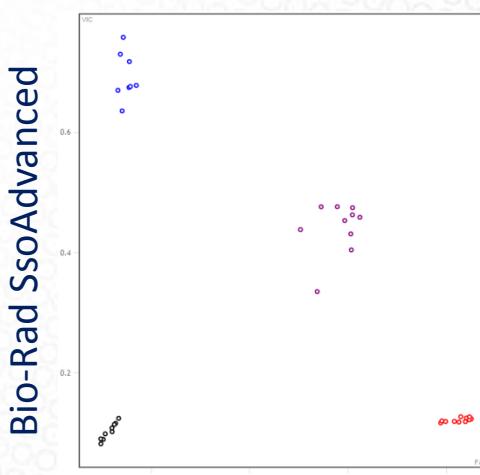
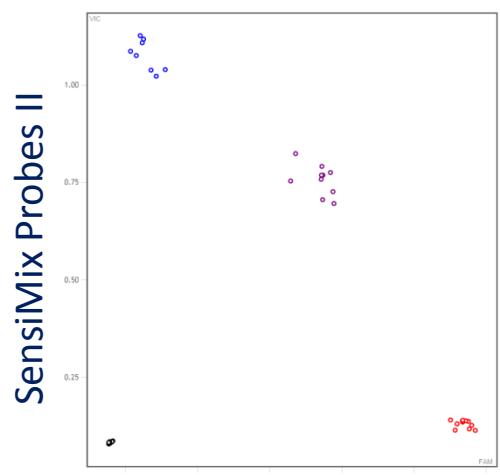
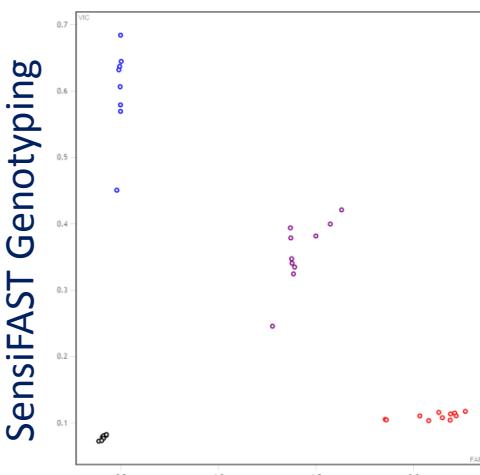
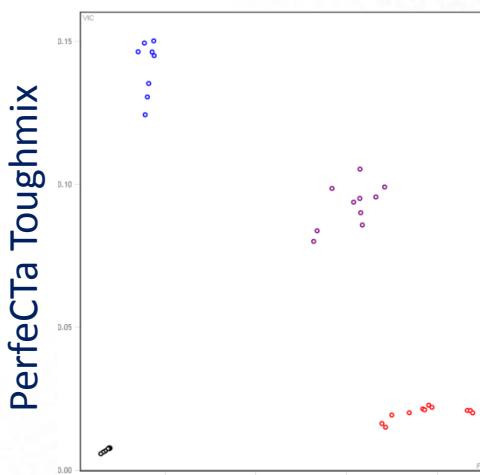
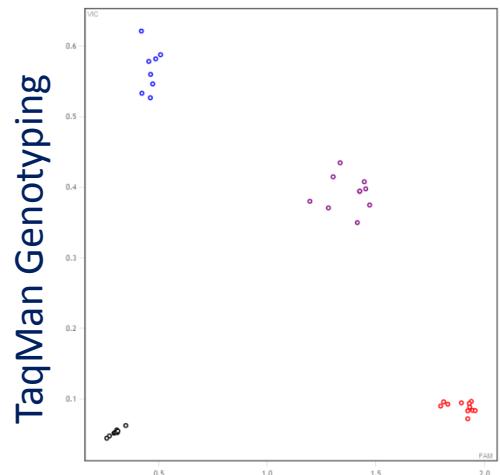
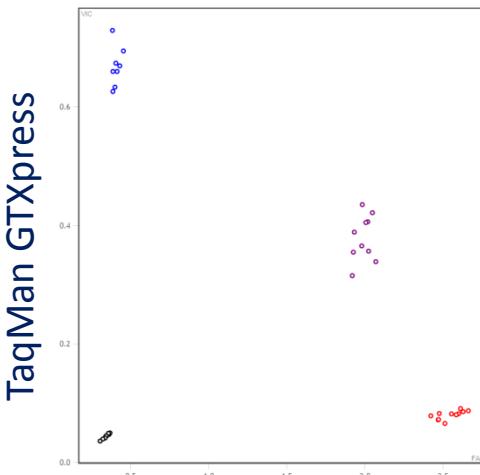


# Leptin SNP Assay: Master Mix comparison, purified DNA

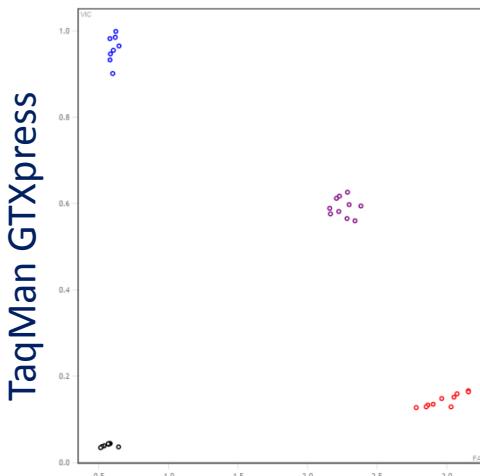


# CAPN1-4751 SNP Assay:

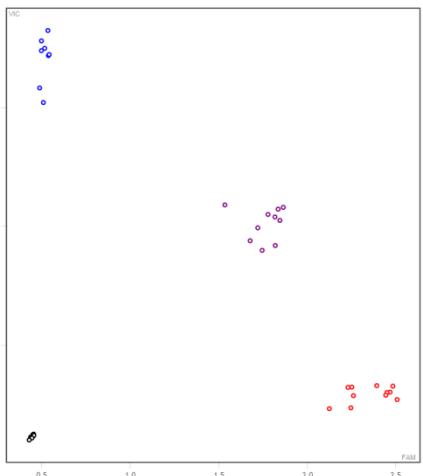
## Master Mix comparison, purified DNA



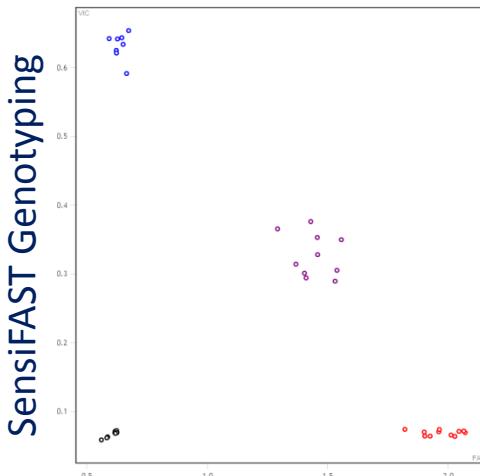
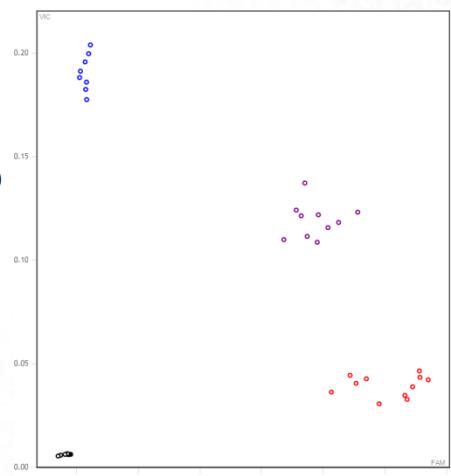
# UoGCAST SNP Assay: Master Mix comparison, purified DNA



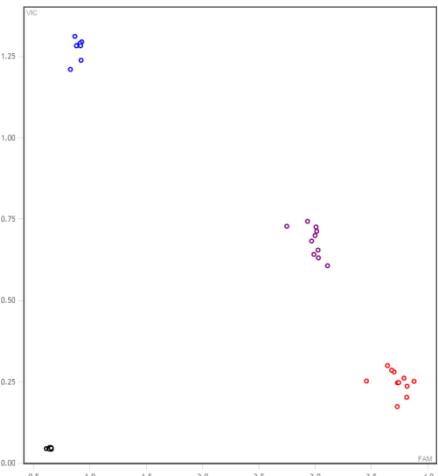
TaqMan Genotyping



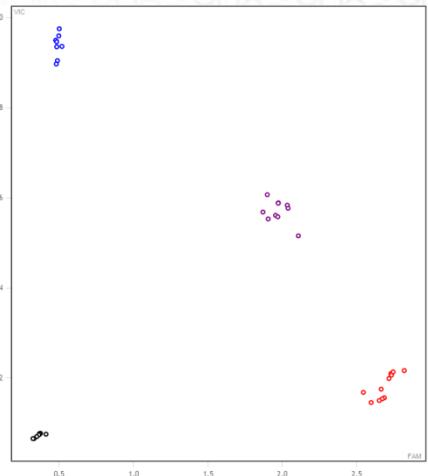
PerfeCTa Toughmix



SensiMix Probes II



Bio-Rad SsoAdvanced



# Summary of Bovine Sample SNP Genotyping

- None of samples had the ideal combination of SNP alleles
- 31% of the samples contained two of the three ideal alleles
- Several packages contained both highly favorable and highly unfavorable samples
- The sample preparation method and master mix must be compatible

Sample	Leptin	CAPN4751	UoGCAST
1-B	TT	CT	CC
3-A	TC	CC	CC
4-B	TC	CC	CC
7-A	TC	CC	CC
7-B	TC	CC	CC
8-A	TC	CC	CC
8-C	TC	CT	CC
2-B	TC	TT	CC
4-A	TT	CC	GC
5	TT	CC	GC
11	TT	CC	GC
1-C	TC	TT	GC
9-A	TC	TT	GC
9-B	TC	TT	GC
9-C	TC	TT	GC
9-D	TC	TT	GC
2-A	CC	CT	GC
1-A	CC	TT	GC
12-A	TT	CT	GG
12-B	TT	CT	GG
12-C	TT	CT	GG
3-B	TT	TT	GG
6-A	TC	CT	GG
6-B	TC	CT	GG
6-C	TC	CT	GG
8-B	TC	CT	GG
10-A	TC	TT	GG
10-B	TC	TT	GG
10-D	TC	TT	GG

# SUMMARY AND CONCLUSIONS



# Master Mix and Assay Cost Considerations

Reaction Volume	Master Mix	TaqMan Clinical Panel	TaqMan Standard Assay	Custom BHQplus Assay
5uL Real Time PCR Instrument	GTxpress	100.0%	58.6%	35.2%
	TaqMan Genotyping	99.2%	57.8%	34.4%
	Sso Advanced	97.5%	56.1%	32.7%
	KlearKall	95.1%	53.7%	30.3%
	SensiMix II Probes	92.1%	50.7%	27.4%
	PerfeCTa ToughMix	90.7%	49.3%	25.9%
1.6uL Array Tape Platform	GTxpress	32.0%	18.8%	11.3%
	TaqMan Genotyping	31.8%	18.5%	11.0%
	Sso Advanced	31.2%	17.9%	10.5%
	KlearKall	30.4%	17.2%	9.7%
	SensiMix II Probes	29.5%	16.2%	8.8%
	PerfeCTa ToughMix	29.0%	15.8%	8.3%

## Summary and Conclusions

- BHQplus probes are effective and accurate for SNP genotyping in a wide variety of samples
- The Array Tape Platform allows researchers to miniaturize and automate PCR-based analyses such as SNP genotyping with accuracy and high call rates
- The combination of BHQplus probes and Array Tape enables accurate and reliable SNP genotyping results while providing significant cost savings

# Thank You!