



# WEATHERBYS

## SCIENTIFIC

Development of an AgriSeq™ targeted GBS panel for Equine SNP  
parentage verification and Sire/Dam allocation

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ISAG 8<sup>th</sup> July 2019

# Weatherbys Scientific - History

**Weatherbys** published first Equine Thoroughbred General Stud Book (UK and IRE) in 1791.

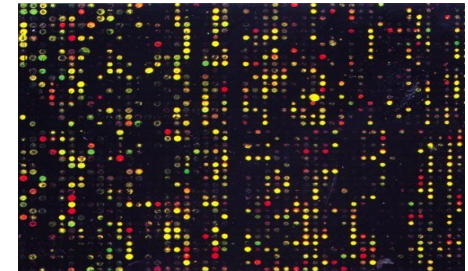
**Weatherbys Scientific** commenced parentage testing thoroughbred horses using blood typing technology in **1985**

In **2000**, the technology for parentage verification changed to **Microsatellite (STR)** markers and Weatherbys Scientific genotypes ~ 30K horses per year using STR technology.

In **2010** our laboratory commenced genotyping cattle using **SNP Microarray** technology and currently supports global wide cattle genotyping projects via genotyping ~**25,000 cattle/week**.

We are an institutional member of the scientific body **ISAG**, also accredited by **ICAR** and hold **ISO17025** accreditation within various SNP Microarray genotyping platforms.

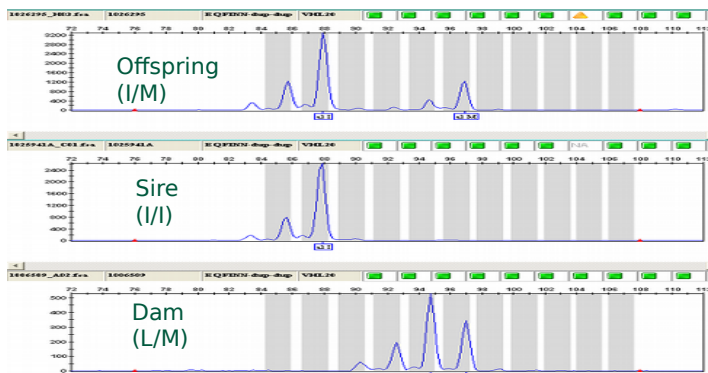
In October 2018 Weatherbys Scientific began providing services to livestock industries in **Australia and the Asia-Pacific region**, thanks to a partnership with the **University of Adelaide**.



# STR to SNP – Equine Transition ?



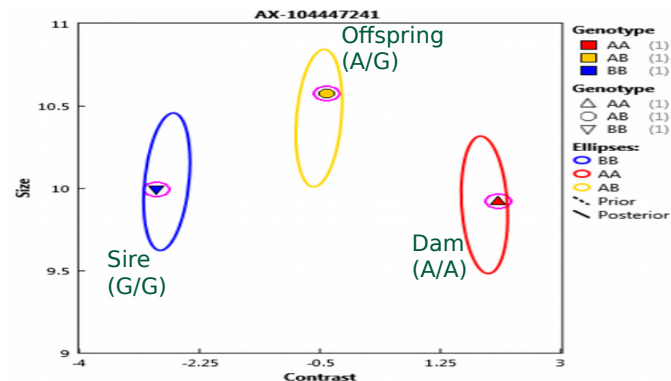
Horse 1: agctattgtttatc**gcgc**tcactgagga  
 Horse 2: agctattgtttatc**gcgcgc**tcactgagga



~10-20 markers per individual

ID	VHL 20	HTG 4	AHT 4	HMS 7	AHT 5	HMS 6	ASB 2	HTG 10	HMS 3	HMS 2	ASB 17	ASB2 3
Horse	IM	KK	HL	LO	KK	LM	KN	MQ	MM	HO	NN	SS

Horse 1: agctattgtttatc**g**tcactgaggaagat  
 Horse 2: agctattgtttatc**a**tcactgaggaagat



~1000's markers per individual

SNP Name	Sample ID	Allele1 - Top	Allele2 - Top
rs1000087	NA12878	A	G
rs10000875	NA12878	A	A
rs10000908	NA12878	A	A

# Project Overview

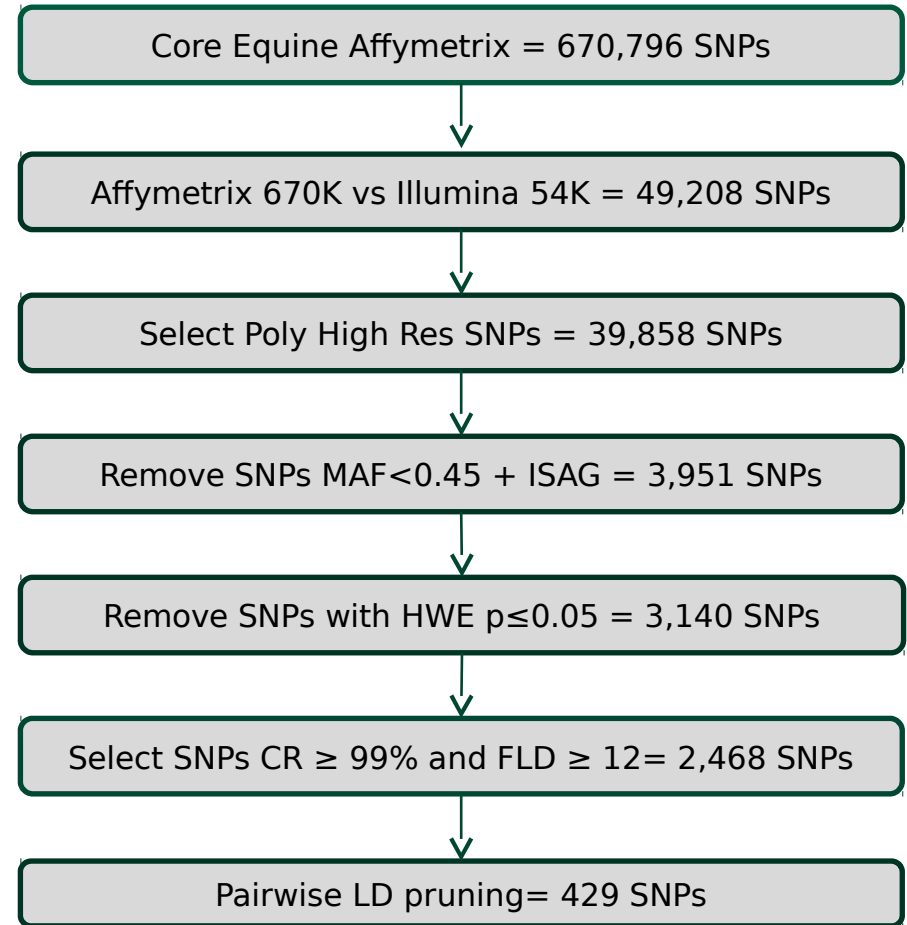
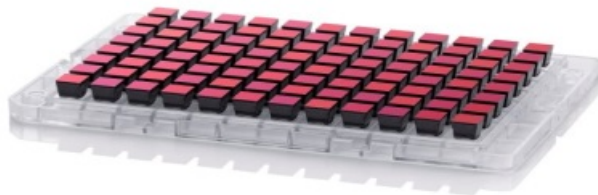
- Develop Genotyping By Sequencing Panel
  - Thermo Fisher Scientific AgriSeq™ \*
- ISAG 2017+2019
  - Genotype Performance + Concordance
- Equine Thoroughbreds
  - 64 Trio Cases - Offspring/Sire/Dam
  - Archived Blood Samples - Chelex DNA
- SNP Parentage Verification
- Simulated Exclusion Mismatch Counts



# Genotyping By Sequencing - Panel Discovery

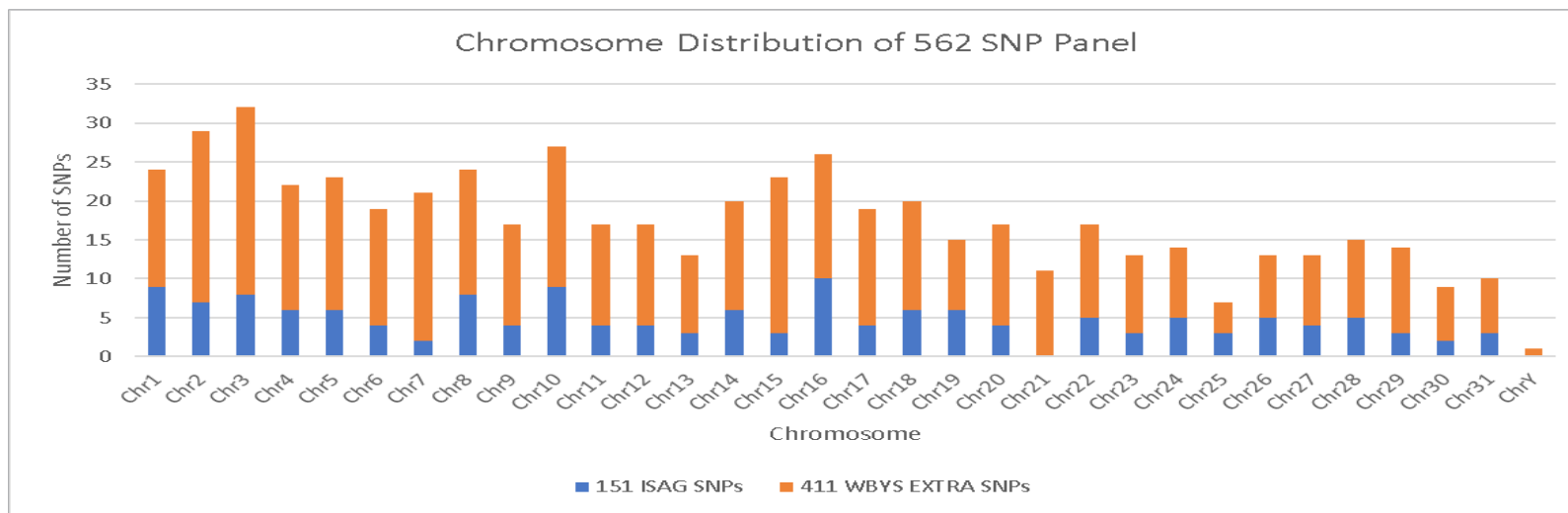
- 186 Samples
- 9 Equine Breeds
- Affymetrix 670K

Equine Breed/Group	N
Thoroughbred (TB)	50
Irish Draft (IDHS)	24
Sport Horse (HIS)	33
Connemara Pony (CP)	37
Standard Bred (STAG)	25
Harness Racing Breed (HRA)	6
Nooitgedacht (NO)	8
Basuto Pony (BP)	1
Arabian (AN)	2
<b>TOTAL</b>	<b>186</b>



# Genotyping By Sequencing - Panel Design

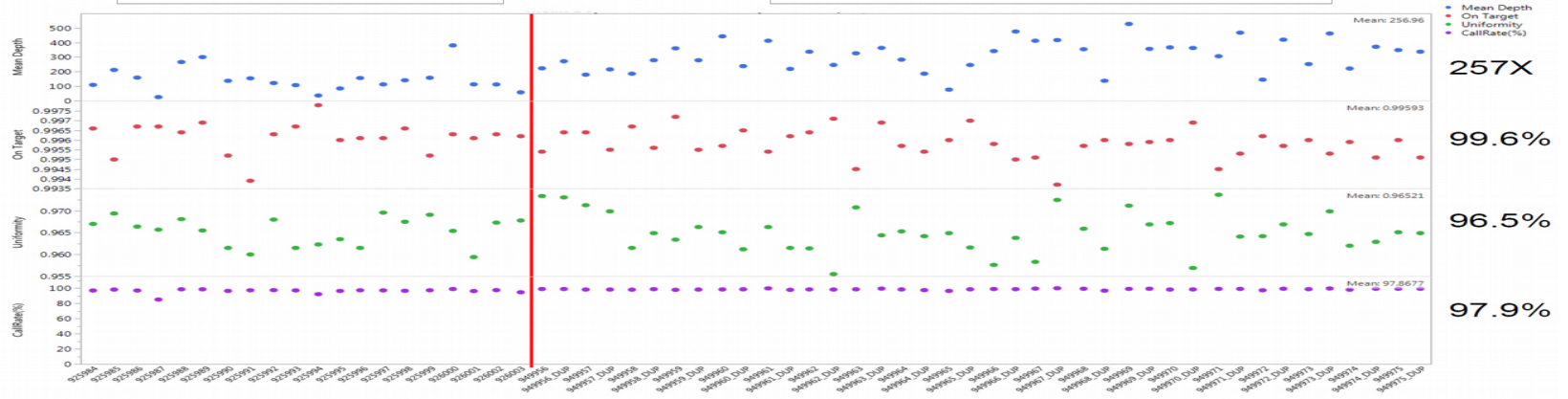
SNP Panel	Number SNPs Submitted	Number of SNPs Synthesised	ISAG Drop Out SNPs
ISAG Etalon	101	98	BIEC2-189666
ISAG Tozaki	53	53	BIEC2-245923
WBYS Extra Autosomal	429	410	BIEC2-476920
WBYS Extra Y-Chr	2	1	-
<b>Total</b>	<b>585</b>	<b>562</b>	<b>96% Conversion</b>



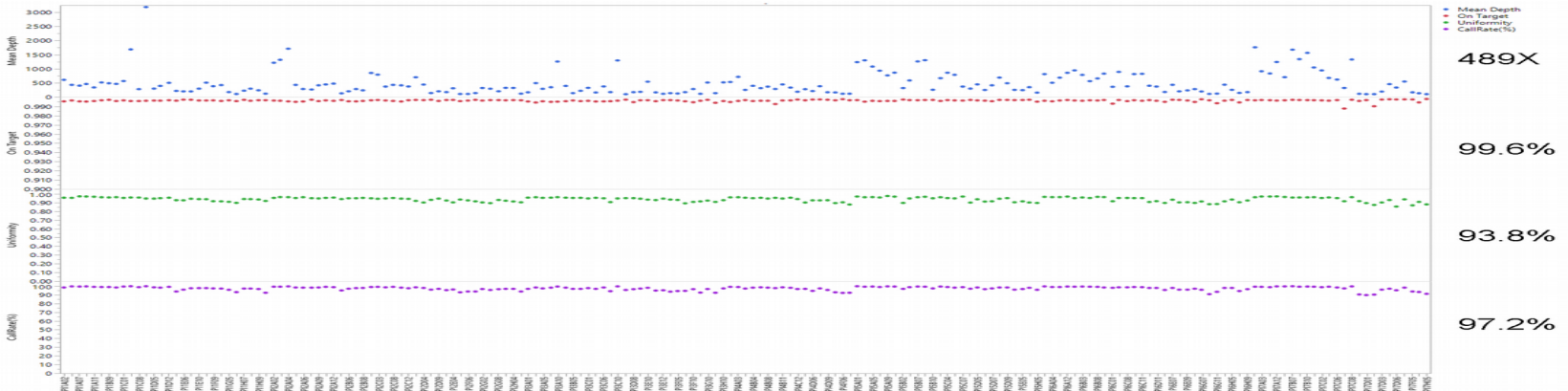
# Panel Performance – ISAG and Trio Cases

20 ISAG 2017 Samples

20 ISAG 2019 Samples Duplicated



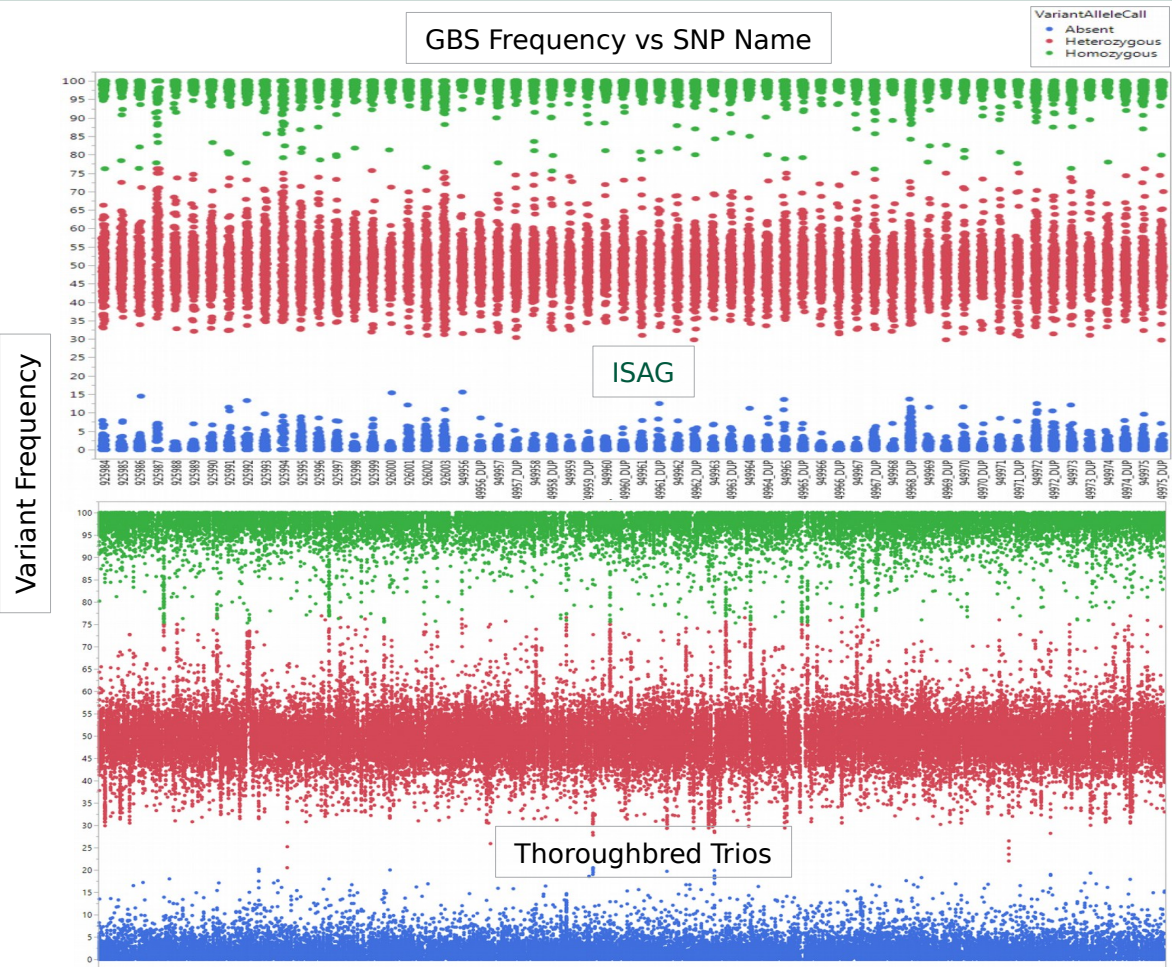
183 Thoroughbred Samples (64 Trio Cases)





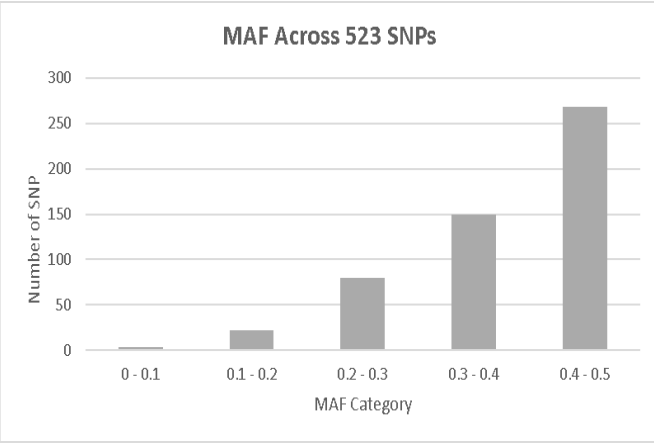


# Panel Performance – SNP Frequencies



## Minor Allele Frequencies 183 Thoroughbreds

SNP Panel	Number SNPs	Average MAF
ISAG	145	0.34
Extra	378	0.39
Y Chr	1	N/A
<b>Overall</b>	<b>523</b>	<b>0.38</b>



# Panel Performance – Genotype Concordance

<b>Concordance Test</b>	<b>Number of Samples</b>	<b>Number of SNPs</b>	<b>Platform Comparison</b>	<b>% Genotype Concordance</b>
<b>ISAG 2017</b>	<b>20</b>	<b>99</b>	<b>Axiom Equine 670K vs Equine GBS Panel</b>	<b>99.0</b>
<b>ISAG 2019</b>	<b>20</b>	<b>562</b>	<b>Duplicates using Equine GBS Panel</b>	<b>99.8</b>
<b>ISAG 2019</b>	<b>20</b>	<b>147</b>	<b>Inter Laboratory vs Equine GBS Panel</b>	<b>99.2</b>



# SNP Parentage Verification

- 183 Thoroughbreds
  - 64 Offspring
  - 64 Dams
  - 55 Sires
- 524 SNPs per Animal
  - 145 ISAG, 378 Extra and 1 Y Chr
- 1.5% Mismatch Acceptance

## 63 Offspring Qualify Against Nominated/STR Verified Parents

- 1 Offspring had 8 Mismatches
  - Call Rate = **90.8%**

## 13 SNPs > 5 Mismatches

- **BIEC499860** (ISAG - 94.9%)
- **BIEC2-183251** (ISAG - Prob)
- **BIEC2-667195** (ISAG - 100%)



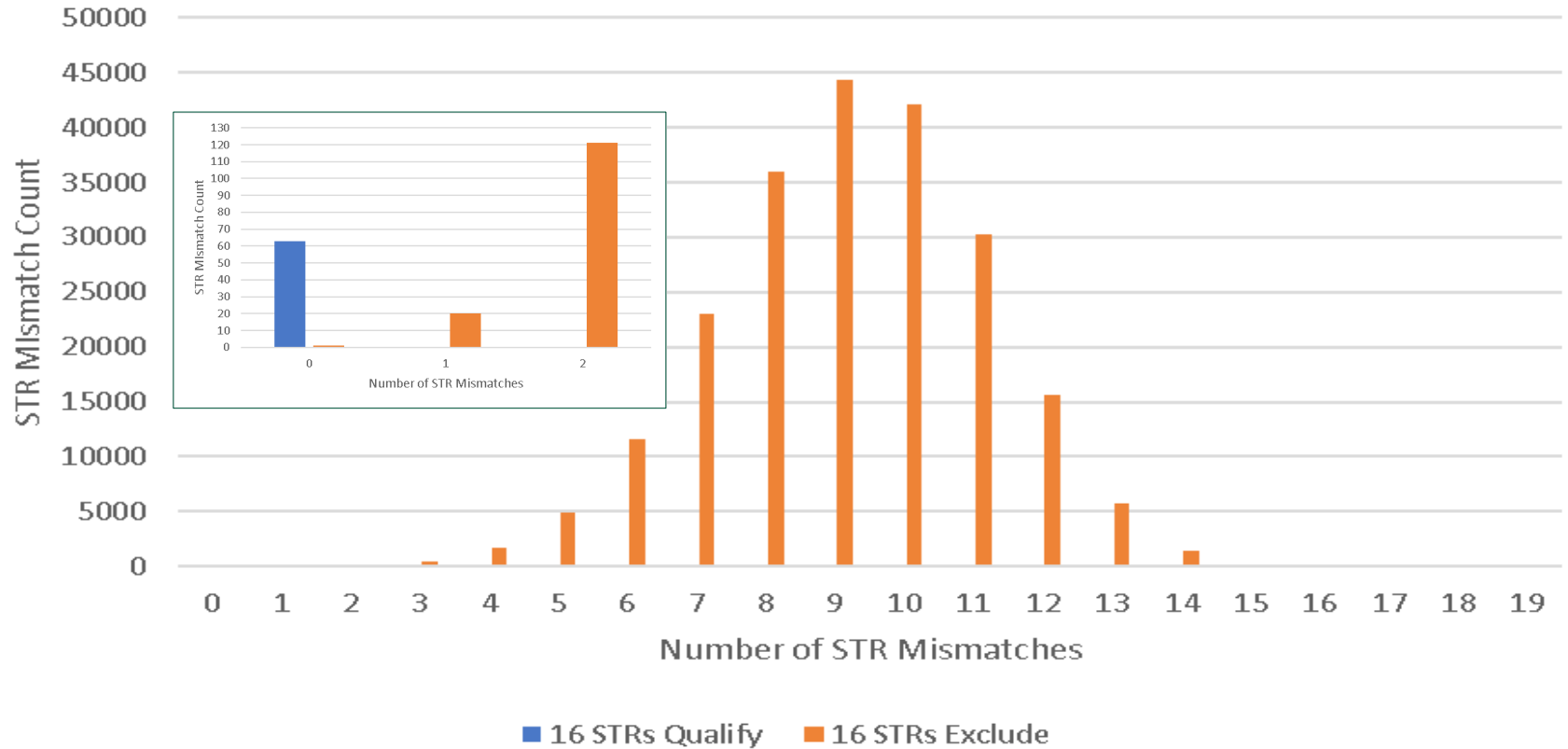
*SIMMULATION TESTING*



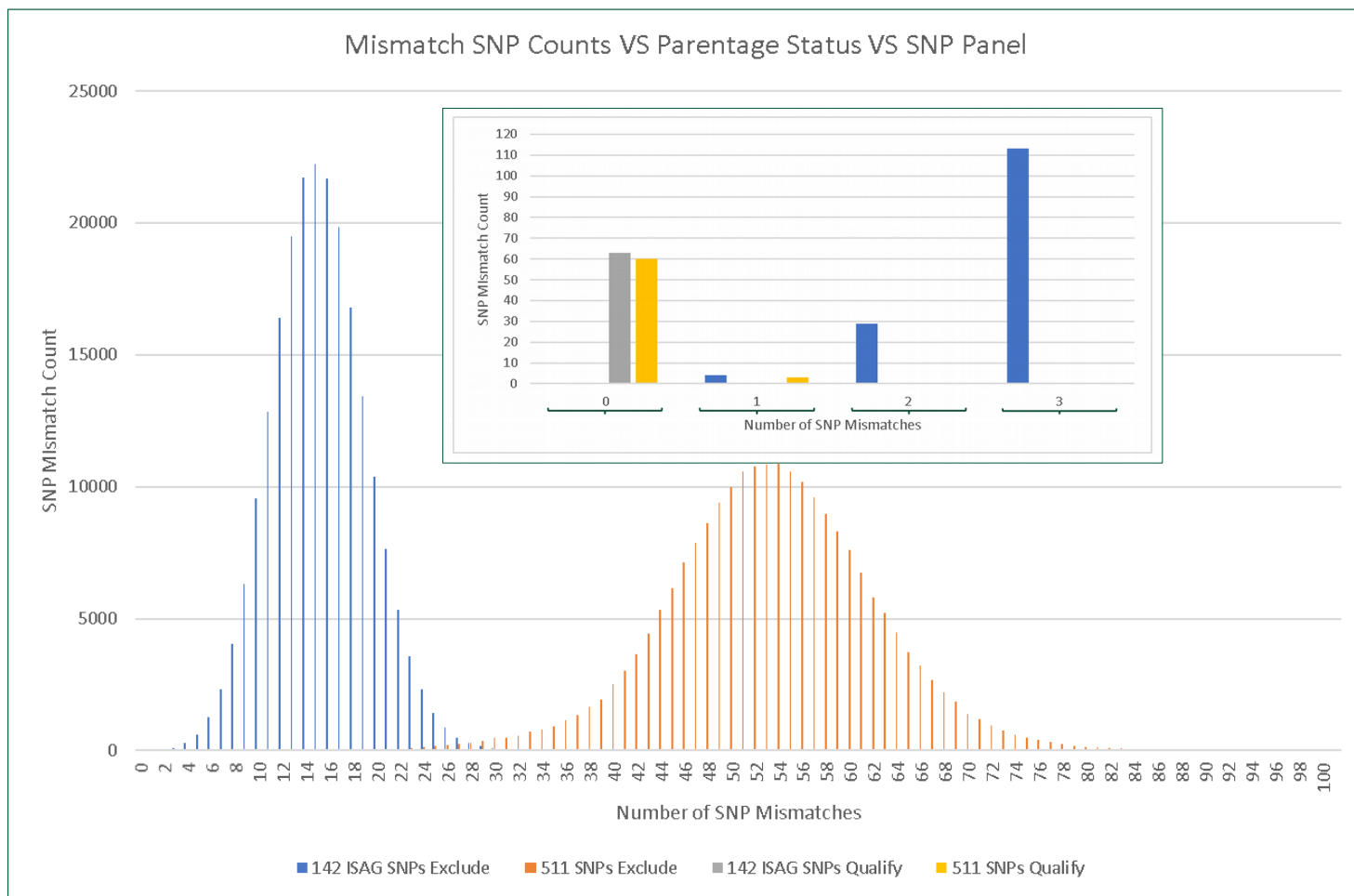
- 182 Thoroughbreds
  - **63 Offspring**
  - 64 Dams
  - 55 Sires
- **511 SNPs per Animal**
  - 142 ISAG, 368 Extra and 1 Y Chr

# Simulated STRs Trios – 221,760 Combinations

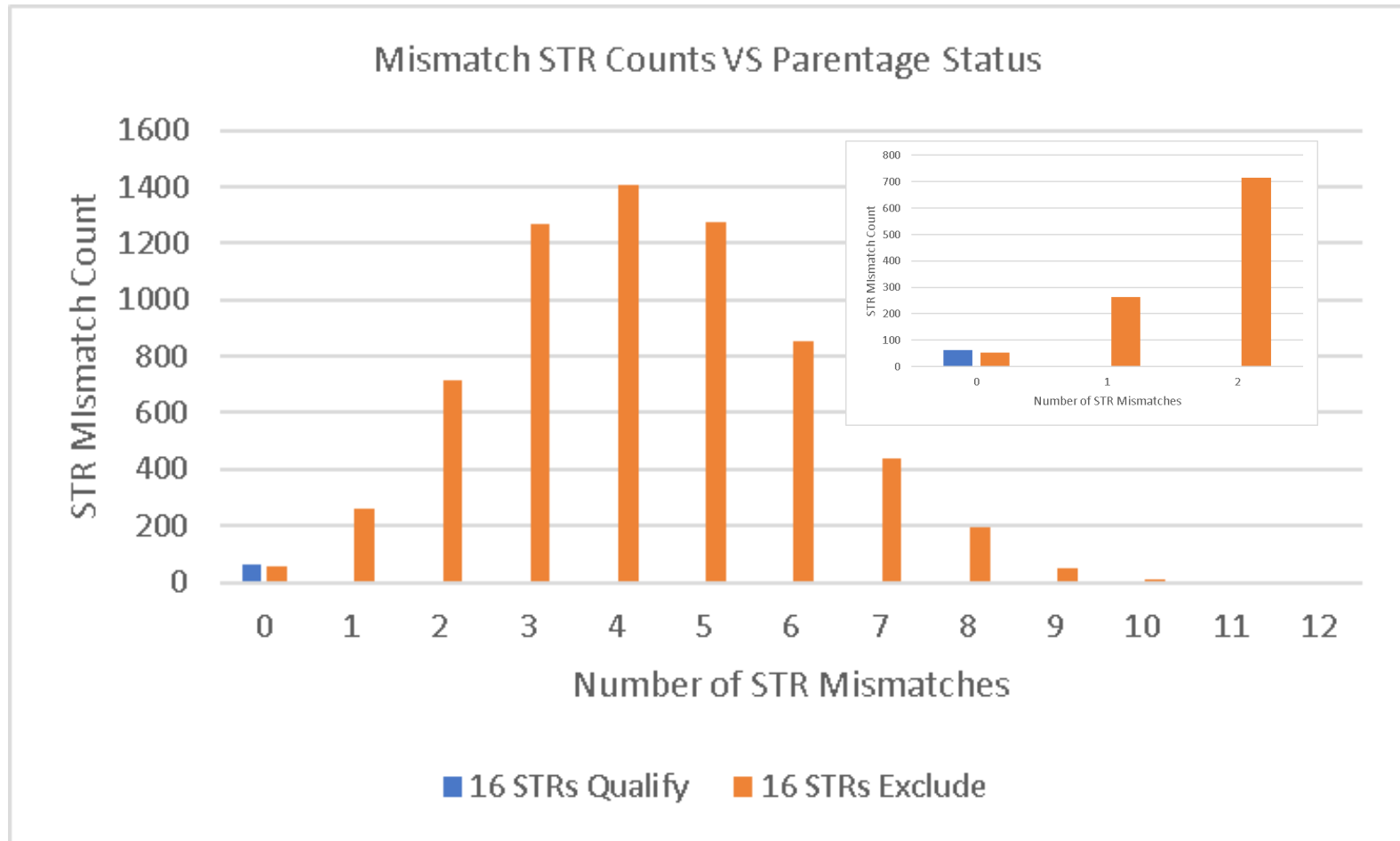
Mismatch STR Counts VS Parentage Status



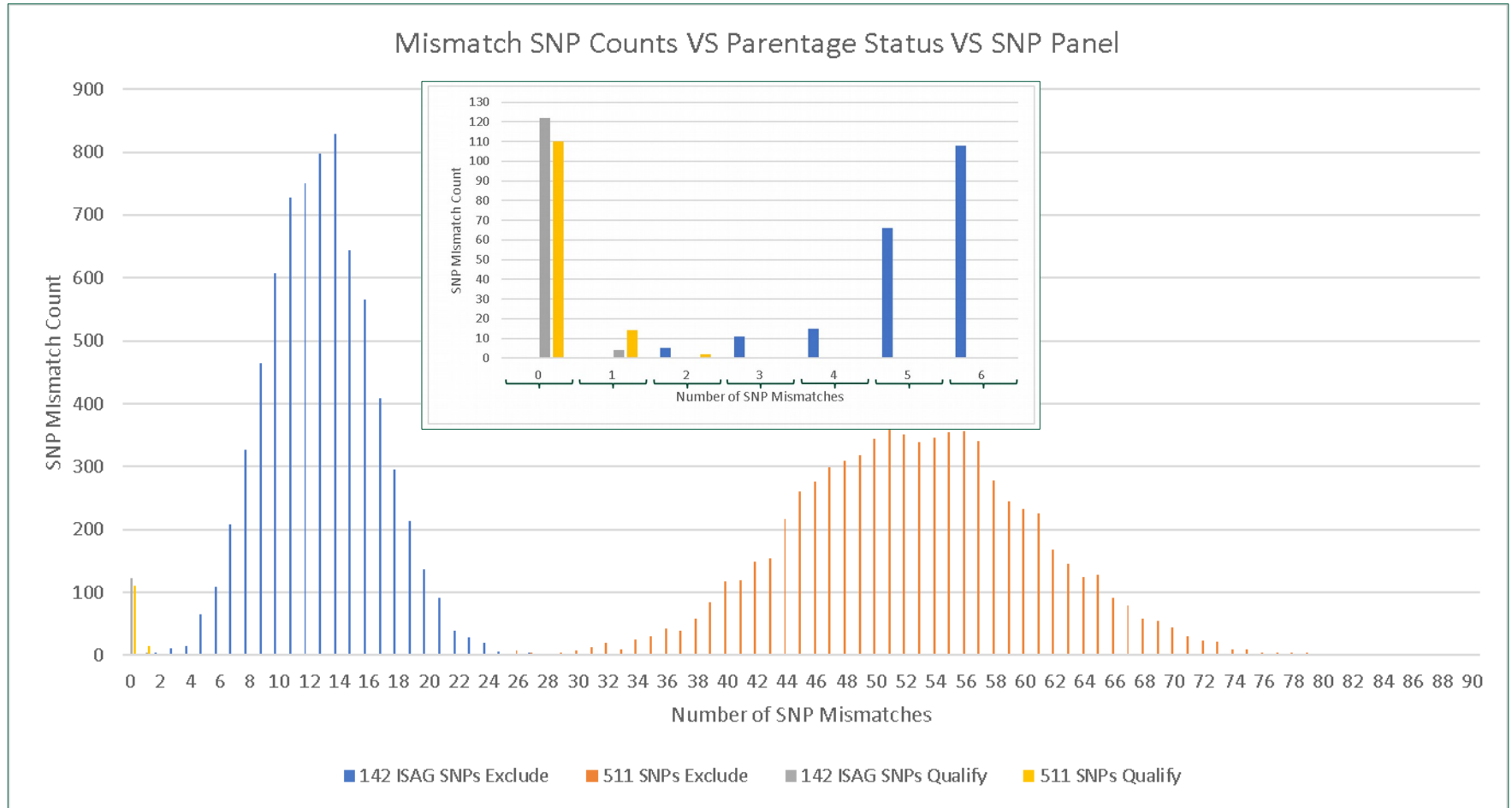
# Simulated SNPs Trios – 221,760 Combinations



# Simulated STRs Sire/Dam – 7,497 Combinations



# Simulated SNPs Sire/Dam – 7,497 Combinations





# Conclusions

- Thermo Fisher Scientific AgriSeq™ GBS technology proficient in generating Low Density Equine SNP profiles - achieved using Chelex Based DNA.
- Average Call Rate and Genotype Concordance across Samples was 97.6% and 99.3% respectively.
- SNP PV POC successful for ISAG and WBYS EXTRA SNP Panels, with simulated parentage assignment anomalies observed for STRs, to lesser extent for ISAG and not observed for WBYS Extra SNP panels.
- Future development - ISAG SNP drop outs, Additional Y-Chr and Diagnostic Traits.

# Thank You

