

Preliminary Studies of the Agena Bioscience HFE Genotyping Panel

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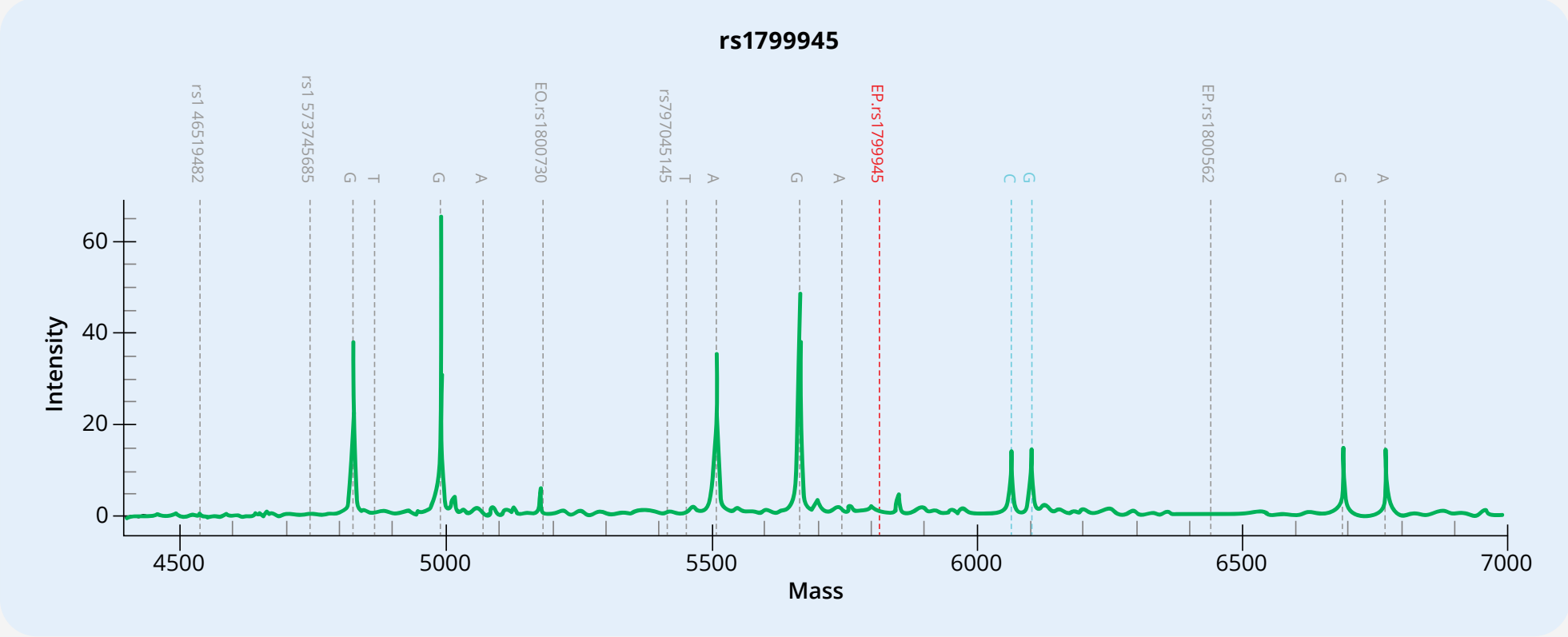
INTRODUCTION

The Agena Bioscience *HFE* Genotyping Panel (Table 1) detects six variants in the *HFE* gene in a single well. A preliminary panel was designed, tested for feasibility internally and sent to three volunteer laboratories for evaluation.

Table 1: *HFE* Genotyping Panel

NP_000401.1	NM_000410.4	rsID#	Variant frequencies
p.His63Asp	c.187C>G	rs1799945	3-15%
p.Ser65Cys	c.193A>T	rs1800730	0.1-3%
p.Glu168Ter	c.502G>T	rs146519482	0-0.002%
p.Trp169Ter	c.506G>A	rs797045145	N/A
p.Cys282Tyr	c.845G>A	rs1800562	0.02-7%
N/A	c.1006+1G>A	rs573745685	0-0.05%

Figure 1: Raw Validation Study Data Output



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MATERIALS & METHODS

Assays were designed using the online Agena Assay Design Suite (ADS, v2.0). Designed primer sequences were then aligned against GRCh38. PCR primers causing interactions were redesigned and re-analyzed until all interactions between primers in the same well were non-existent. Feasibility studies were performed by multiple operators on DNA extracted from buccal swabs, saliva, and whole blood samples using 2 different Agena MassARRAY Systems on different days using both the 96- and 384-well format. Additionally, multiple thermal cyclers were used. The following parameters were guard banded to determine optimum parameters and robustness: annealing temperature (52-62°C), PCR ramp rate (3-6°C/s), PCR reaction mix (0.5-2X), unincorporated extension primers (0.75-1.25X), MgCl₂ concentration (0.5-1X) and recommended sample inputs (0.1-662 ng). Reagents were sent to 3 external laboratories to determine accuracy, analytical sensitivity and specificity from orthogonally tested samples.

RESULTS

The assay performance was robust at all levels of guard banding. The Maine Molecular Quality control resulted in the expected for p.His63Asp, p.Ser65Cys, and p.Cys282Tyr. The external laboratory results (106 samples) were 100% concordant with previously tested results for 100% accuracy. The analytical sensitivity is 100% (95% CI; 92.1-100%) and the analytical specificity is 100% (95% CI; 97.8-100%).

SUMMARY OF VALIDATION METRICS

Table 2: *HFE* Genotyping Panel Validation Summary

96 well format validation		Operator 1	Operator 2
Lot 1	Sample Call Rate (%)	100	100
	NTC Pass Rate (%)	100	100
	Concordance (%)	100	100
Lot 2	Sample Call Rate (%)	98.4	100
	NTC Pass Rate (%)	97.1	97.1
	Concordance (%)	100	100
Lot 3	Sample Call Rate (%)	100	100
	NTC Pass Rate (%)	100	100
	Concordance (%)	100	100

384 well format validation		Operator 1	Operator 2
Lot 1	Sample Call Rate (%)	100	100
	NTC Pass Rate (%)	100	97.1
	Concordance (%)	100	100
Lot 2	Sample Call Rate (%)	100	100
	NTC Pass Rate (%)	100	100
	Concordance (%)	100	100
Lot 3	Sample Call Rate (%)	100	100
	NTC Pass Rate (%)	100	97.1
	Concordance (%)	100	100



CONCORDANCE

All results were referenced against genotypes available on Ensembl database: https://asia.ensembl.org/Homo_sapiens/Info/Index

Table 3: Samples and Results Used In Internal Studies

Sample ID	Sample Type	rs 1799945	rs 1800730	rs 146519482	rs 797045145	rs 1800562	rs 573745685
HG00106	HAPMAP	C	T	G	G	G	G
HG00237	HAPMAP	CG	A	G	G	G	G
HG00264	HAPMAP	C	A	G	G	A	G
HG01403	HAPMAP	G	A	G	G	G	G
HG01438	HAPMAP	C	A	G	G	A	G
HG02087	HAPMAP	C	A	G	G	G	GA
HG02121	HAPMAP	C	A	G	G	G	GA
HG02130	HAPMAP	C	A	G	G	G	GA
NA12889	HAPMAP	CG	A	G	G	GA	G
NA19684	HAPMAP	C	A	G	G	GA	G
NA20810	HAPMAP	G	A	G	G	G	G
NA20814	HAPMAP	C	TA	G	G	G	G
gBlock rs146519482	In house gBlock mix	C	A	GT	G	G	G
gBlock rs797045145	In house gBlock mix	C	A	G	GA	G	G
pG20010	Maine Molecular Control	CG	A	G	G	GA	G
pG20013	Maine Molecular Control	C	T	G	G	G	G

CONCLUSION

Agena Bioscience has developed a robust panel for *HFE* targeted gene testing. This more broadly ethnic panel was designed to enhance *HFE* research. While the commonly tested *HFE* variants (p.His63Asp, p.Ser65Cys, and p.Cys282Tyr) were evaluated by external studies, the rare variants (p.Glu168Ter, p.Trp169Ter, and c.1006+1G>A) were not. A reference DNA is available for c.1006+1G>A; the other rare variants do not have any reference DNA.

The validation and verification experiments showed that the *HFE* Genotyping Panel fulfils the requirements of a research assay with ≥99% accuracy and precision. Repeat rate, no template control, and signal intensity are appropriate for routine use.

Overall, the Agena Bioscience *HFE* Genotyping Panel performed well in internal and external studies. The *HFE* Genotyping Panel is commercially available as Research Use Only.

ACKNOWLEDGEMENTS

The entire Agena Team: Aaron Florece, Aleksey Nakorchevsky, Amber Lane, Glenn Wise, Maggie Lowe, Heath Metzler, Nichole Rupp, Sharron Ohgi, Shelley Spisak, Wayne Ge, Darryl Irwin, Paul Yates