PDE4D-S185A

Allele Description

This is a CRISPR/Cas9 induced mutation creating a point mutations; S185A in exon 4 ENSMUSG00000021699 of *PDE4D*. The stock was generated at MRC Harwell via microinjection of CRISPR/Cas9 reagents into 1-cell stage embryos.

qPCR Copy Counting Genotyping Strategy

The genotyping strategy presented here has been optimized for reagents and conditions used by the Genotyping Core at MRC Harwell. To genotype animals, we recommend researchers validate the assay independently. PCR cycling temperature and times may require additional optimization based on the specific genotyping reagents used.

An Allelic Discrimination assay is used to detect two possible variants of a Single Nucleotide Polymorphism (SNP). It is a multiplexed assay (with two primer/probe pairs) with data being collected at the completion of the PCR process. The relative level of fluorescence from each probe is used to determine the genotype of an animal.

Samples for this line are genotyped using the following primers and probe:

- Forward and reverse primers common to both Wild Type (WT) and mutant alleles
- WT probe binding to the WT base mutated in the mutant allele.
- Mutant probe binding to the SNP.

PDE4D-S185A Allelic Discrimination assay

PDE4D WT sequence

AGGCCAATGCTCCCACCAACGTAACTCTCTTCCTCTATTCTCAGTTTCGATGTGGACAATGGCACATC
AGCGGGACGGAGTCCCTTGGATCCCATGACCAGCCCA**GGGTCTGGGCTGATTC**TCCAAGCAAACTT
TGTCCACAGTCAACGCCGg**GAGtCCTTCCTGTACCGATCT**GACAG**CGACTATGACCTCTCTCCAA**AGT
CTATGTCCAGGAACTCCTCGATTGCCAGTGATATGTAAGTATAAGGGTGGGCAGAAAAGGCAGAAG
TACCTAGAAAAACCTGAGCAGATTCATCTCTGGAGTAGATGACGTCATAAATGATGATTTCATCCA

PDE4D-S185A mutant sequence

AGGCCAATGCTCCCACCAACGTAACTCTCTTCCTCTATTCTCAGTTTCGATGTGGACAATGGCACATC
AGCGGGACGGAGTCCCTTGGATCCCATGACCAGCCCA**GGGTCTGGGCTGATTC**TCCAAGCAAACTT
TGTCCACAGTCAACGCCGa**GAGgCCTTCCTGTACCG**ATCTGACAG**CGACTATGACCTCTCTCCAA**AG
TCTATGTCCAGGAACTCCTCGATTGCCAGTGATATGTAAGTATAAGGGTGGGCAGAAAAGGCAGAA
GTACCTAGAAAAACCTGAGCAGATTCATCTCTGGAGTAGATGACGTCATAAATGATGATTTCATCCA

SNP details:
WT= T
MUT= G

Lower case letters denote SNP position.
Probe sequence is in bold and shaded grey.
Primer sequences are in bold and underlined

Oligo Name	5' label	Sequence 5' → 3'	3' label	Oligo Type
PDE4D-S185A_F	n/a	**GGGTCTGGGCTGATTC**	n/a	Common forward primer
PDE4D-S185A_WT_PROBE	FAM	**AGATCGGTACAGGAAGGACTC**	BHQ-plus	Wild type Probe
PDE4D-S185A_Mutant_PROBE	TET	**CGGTACAGGAAGGCCTC**	BHQ-plus	Mutant probe
PDE4D-S185A_R	n/a	**TTGGAGAGAGGTCATAGTCG**	n/a	WT Reverse

DNA extraction method

DNA is extracted from ear clips using Applied Biosystems Taqman Sample-to-SNP Kit and qPCR run using 1:10 dilution from the crude preparation.

qPCR master mix 1X

ABI GTX Taqman master mix	5µl
Assay (Probes 5 µM each & Primers 15µM each)	2µl
ddH2O	0.5µl
DNA (1/10 dilution of ABI Sample-to-SNP prep)	2.5µl

qPCR cycling conditions

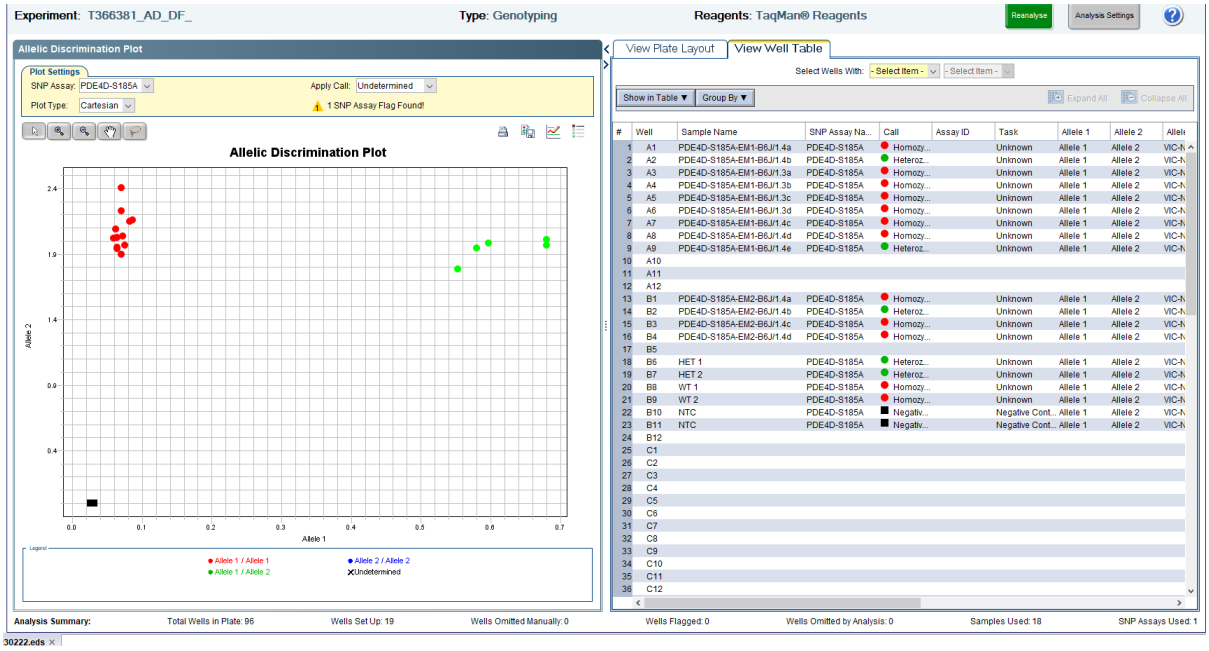
qPCR instrument: Applied Biosystems 7500

95^{o}C for 20 sec
Then 40 cycles of;
95^{o}C for 3 sec
60^{o}C for 30 sec

Analysis

The results are analysed using 7500 software v2.0.6 from Applied Biosystems

PDE4D-S185A Allelic Discrimination assay results (Task 366381 results)



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