Allele Description

HDLBP-EX14SV is a point mutation project targeting exon ENSMUSE00000283636 on chromosome 1. The targeted point mutation is introduced by a non-coding G > A conversion.

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. Homozygotes are samples having only wild type (WT) or mutant (MUT) alleles. Heterozygotes are samples having both WT and MUT alleles.

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

- Forward and reverse primers common to both WT and MUT alleles
- WT probe binding to the WT base mutated in the mutant allele
- Mutant probe binding to the SNP

HDLBP-EX14SV Allelic Discrimination assay

HDLBP-EX14SV WT sequence

GTAGTTGTGAGTTCTGAATTCAGGTCACCAGGATCAGTGCCAAACACCCTTACCCACTGATCTCTATCTTGCTT
TCCCAAAACTTAGTCTTAGAATAATTGTGCACTGTCTTCTGATGATTTGTTTTAGGTTATCATCAACTTTCCAGA
CCCAGCACAAAAAAGTGATATTGTACAACTCAGAGGCCCCAAGAA**TGAGGTGGAGAAGTGCACTAAGTAT**A
TGCAGAAGATGGTCG**CAGACCTGgTAGGTGAC**TTTGTGCACCCTCTGGAGATGGGGAGCGGCTCCCATGGAT
TTGTGAGTGCATGCAGGACCTCTGCCCTAAGGGTGGTCTGCTGCCTCAGGGGCTTAGAACAAAGAAGTGGTA
AAACCTCTCTCCTTGATGCCTCGCTTACACGGTCCTTATGATCAACTAGTATGTGGGAGATTGCTGTTTACCATA

HDLBP-EX14SV mutant sequence

GTAGTTGTGAGTTCTGAATTCAGGTCACCAGGATCAGTGCCAAACACCCTTACCCACTGATCTCTATCTTGCTT
TCCCAAAACTTAGTCTTAGAATAATTGTGCACTGTCTTCTGATGATTTGTTTTAGGTTATCATCAACTTTCCAGA
CCCAGCACAAAAAAGTGATATTGTACAACTCAGAGGCCCCAAGAA**TGAGGTGGAGAAGTGCACTAAGTAT**A
TGCAGAAGATGGTCG**CAGACCTGaTAGGTGAC**TTTGTGCACCCTCTGGAGATGGGGAGCGGCTCCCATGGAT
TTGTGAGTGCATGCAGGACCTCTGCCCTAAGGGTGGTCTGCTGCCTCAGGGGCTTAGAACAAAGAAGTGGTA
AAACCTCTCTCCTTGATGCCTCGCTTACACGGTCCTTATGATCAACTAGTATGTGGGAGATTGCTGTTTACCATA

SNP details:
WT = G
MUT = A

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
HDLBP-EX14SV-AD _F	n/a	**TGAGGTGGAGAAGTGCACTAAGTAT**	n/a	Common forward primer
HDLBP-EX14SV-AD _WT_PROBE	VIC	**CAGACCTGGTAGGTGAC**	NFQ	Wild type Probe
HDLBP-EX14SV-AD-MUT_PROBE	FAM	**CAGACCTGATAGGTGAC**	NFQ	Mutant probe
HDLBP-EX14SV-AD _R	n/a	**CATCTCCAGAGGGTGCACAAA**	n/a	Common reverse primer

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

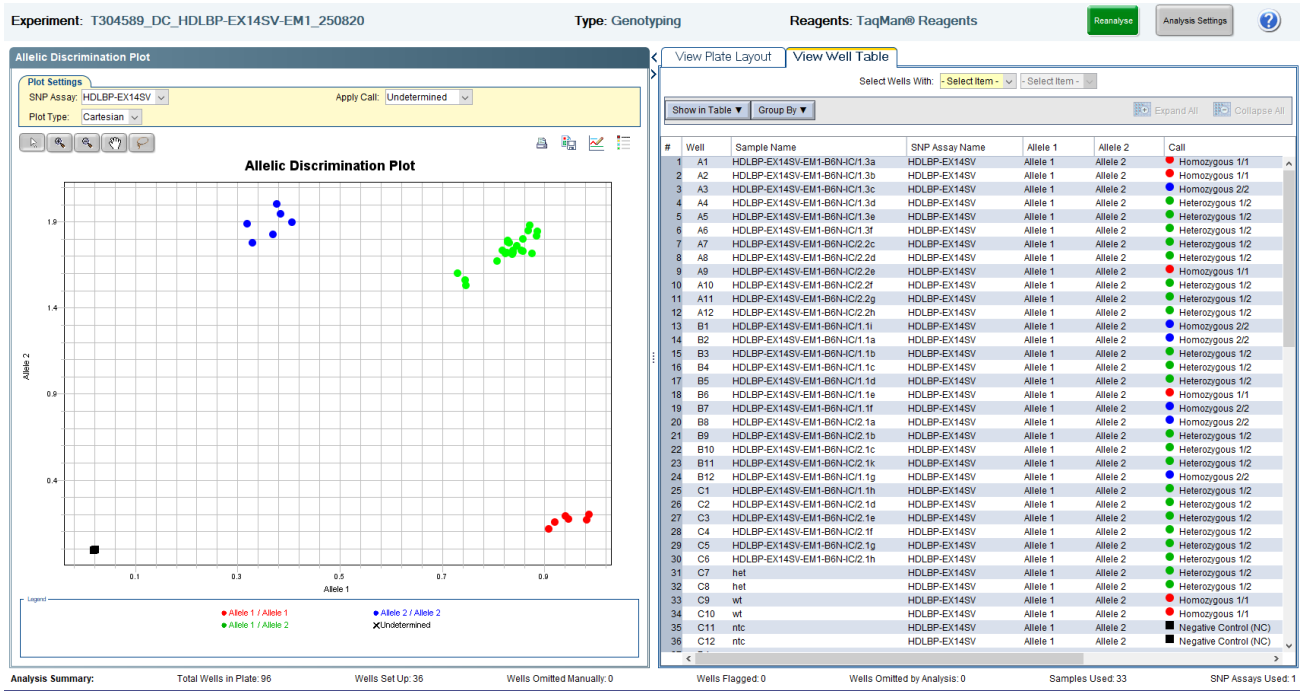
qPCR instrument: Applied Biosystems 7500

95°C for 20 sec
Then 40 cycles of;
95°C for 3 sec
60°C for 30 sec

Analysis

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

Example of HDLBP-EX14SV Allelic Discrimination Assay Results (Task 304589)



#	Well	Sample Name	SNP Assay Name	Allele 1	Allele 2	Call
1	A1	HDLBP-EX14SV-EM1-B6N-IC/1.3a	HDLBP-EX14SV	Allele 1	Allele 2	Homozygous 1/1
2	A2	HDLBP-EX14SV-EM1-B6N-IC/1.3b	HDLBP-EX14SV	Allele 1	Allele 2	Homozygous 1/1
3	A3	HDLBP-EX14SV-EM1-B6N-IC/1.3c	HDLBP-EX14SV	Allele 1	Allele 2	Homozygous 2/2
4	A4	HDLBP-EX14SV-EM1-B6N-IC/1.3d	HDLBP-EX14SV	Allele 1	Allele 2	Heterozygous 1/2
5	A5	HDLBP-EX14SV-EM1-B6N-IC/1.3e	HDLBP-EX14SV	Allele 1	Allele 2	Heterozygous 1/2
6	A6	HDLBP-EX14SV-EM1-B6N-IC/1.3f	HDLBP-EX14SV	Allele 1	Allele 2	Heterozygous 1/2
7	A7	HDLBP-EX14SV-EM1-B6N-IC/2.2c	HDLBP-EX14SV	Allele 1	Allele 2	Heterozygous 1/2
8	A8	HDLBP-EX14SV-EM1-B6N-IC/2.2d	HDLBP-EX14SV	Allele 1	Allele 2	Heterozygous 1/2
9	A9	HDLBP-EX14SV-EM1-B6N-IC/2.2e	HDLBP-EX14SV	Allele 1	Allele 2	Homozygous 1/1
10	A10	HDLBP-EX14SV-EM1-B6N-IC/2.2f	HDLBP-EX14SV	Allele 1	Allele 2	Heterozygous 1/2
11	A11	HDLBP-EX14SV-EM1-B6N-IC/2.2g	HDLBP-EX14SV	Allele 1	Allele 2	Heterozygous 1/2
12	A12	HDLBP-EX14SV-EM1-B6N-IC/2.2h	HDLBP-EX14SV	Allele 1	Allele 2	Heterozygous 1/2
13	B1	HDLBP-EX14SV-EM1-B6N-IC/1.1i	HDLBP-EX14SV	Allele 1	Allele 2	Homozygous 2/2
14	B2	HDLBP-EX14SV-EM1-B6N-IC/1.1a	HDLBP-EX14SV	Allele 1	Allele 2	Homozygous 2/2
15	B3	HDLBP-EX14SV-EM1-B6N-IC/1.1b	HDLBP-EX14SV	Allele 1	Allele 2	Heterozygous 1/2
16	B4	HDLBP-EX14SV-EM1-B6N-IC/1.1c	HDLBP-EX14SV	Allele 1	Allele 2	Heterozygous 1/2
17	B5	HDLBP-EX14SV-EM1-B6N-IC/1.1d	HDLBP-EX14SV	Allele 1	Allele 2	Heterozygous 1/2
18	B6	HDLBP-EX14SV-EM1-B6N-IC/1.1e	HDLBP-EX14SV	Allele 1	Allele 2	Homozygous 1/1
19	B7	HDLBP-EX14SV-EM1-B6N-IC/1.1f	HDLBP-EX14SV	Allele 1	Allele 2	Homozygous 2/2
20	B8	HDLBP-EX14SV-EM1-B6N-IC/2.1a	HDLBP-EX14SV	Allele 1	Allele 2	Homozygous 2/2
21	B9	HDLBP-EX14SV-EM1-B6N-IC/2.1b	HDLBP-EX14SV	Allele 1	Allele 2	Heterozygous 1/2
22	B10	HDLBP-EX14SV-EM1-B6N-IC/2.1c	HDLBP-EX14SV	Allele 1	Allele 2	Heterozygous 1/2
23	B11	HDLBP-EX14SV-EM1-B6N-IC/2.1k	HDLBP-EX14SV	Allele 1	Allele 2	Heterozygous 1/2
24	B12	HDLBP-EX14SV-EM1-B6N-IC/1.1g	HDLBP-EX14SV	Allele 1	Allele 2	Homozygous 2/2
25	C1	HDLBP-EX14SV-EM1-B6N-IC/1.1h	HDLBP-EX14SV	Allele 1	Allele 2	Heterozygous 1/2
26	C2	HDLBP-EX14SV-EM1-B6N-IC/2.1d	HDLBP-EX14SV	Allele 1	Allele 2	Heterozygous 1/2
27	C3	HDLBP-EX14SV-EM1-B6N-IC/2.1e	HDLBP-EX14SV	Allele 1	Allele 2	Heterozygous 1/2
28	C4	HDLBP-EX14SV-EM1-B6N-IC/2.1f	HDLBP-EX14SV	Allele 1	Allele 2	Heterozygous 1/2
29	C5	HDLBP-EX14SV-EM1-B6N-IC/2.1g	HDLBP-EX14SV	Allele 1	Allele 2	Heterozygous 1/2
30	C6	HDLBP-EX14SV-EM1-B6N-IC/2.1h	HDLBP-EX14SV	Allele 1	Allele 2	Heterozygous 1/2
31	C7	het	HDLBP-EX14SV	Allele 1	Allele 2	Heterozygous 1/2
32	C8	het	HDLBP-EX14SV	Allele 1	Allele 2	Heterozygous 1/2
33	C9	wt	HDLBP-EX14SV	Allele 1	Allele 2	Homozygous 1/1
34	C10	wt	HDLBP-EX14SV	Allele 1	Allele 2	Homozygous 1/1
35	C11	ntc	HDLBP-EX14SV	Allele 1	Allele 2	Negative Control (NC)
36	C12	ntc	HDLBP-EX14SV	Allele 1	Allele 2	Negative Control (NC)

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