HU-SOD1-FLOX-A4V-EM1-B6

Allele Description

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.

Samples are genotyped using qPCR copy counting with both a wild type and a mutant assay against a known reference assay (*Dot1l* on chromosome 10; 2 copies present). Samples for this line are genotyped using the following primers and probe:

- Wild type (WT) assay with probe and reverse primer binding to the WT bases mutated in the mutant allele.
- Mutant assay with probe and reverse primer binding to the G601R, F606Y and R609H point mutations.

For autosomal genes that have been targeted, the following results would be expected:

Genotype of the Modified allele	WT Assay	Mutant Assay
Wildtype	2	0
Heterozygous	1	1
Homozygous mutant	0	2

MU-SOD1-EX3-MUT1 WT assay (FAM labelled)

TGCCTGTTTTTATCGCCCCAGCACATACCAGGTCAGTGTTTGCTATGTTGGAGGTTGTAAGAATGCCT
GTGTTGTTACATATAGGGGTTTACTTCATAATCTGACTGCTGGTTTCTGGTAAATAGGCT**GTACCAGT**
GCAGGACCTCATTTTAATCCTCACTCTAAGA**AACATGGTGGCCCGGCGGATG**AAG**AGAGGTGAGC**
AGCACGCTCTGTATGCATGGTGGAGGAGAGGGGTCTGTGGAGGACCCCAGTAAGACAGAACTGCA
TGGCCTCCTGCCTCTGCTTTTGTGTTTGTTTCCATTCACCCAACTCACTCCCACAACCCCACGTGCTAG

Probe sequence is in bold and shaded grey.
Primer sequences are in bold and underlined.

Oligo	5' label	Sequence 5' → 3'	3' label	Oligo Type
MU-SOD1-EX3-MUT1-WT_F	n/a	GTACCAGTGCAGGACCTCAT	n/a	Wild type Forward
MU-SOD1-EX3-MUT1-WT_PROBE	FAM	AACATGGTGGCCCGGCGGATG	BHQ1	Wild type Probe
MU-SOD1-EX3-MUT1-WT_R	n/a	AGCGTGCTGCTCACCTCT	n/a	Wild type Reverse

HU-SOD1-EX3-MUT1 assay (FAM labelled)

TGCAGGTCAGCACTTTCTCCATGGGAAGTTTTAGCAGTGTTTCTTTTTAGAATGTATTTGGGAACTTT
AATTCATAATTTAGCTTTTTTTTCTTCTTCTTATAAATAGGCTGTACCA**GTGCAGGTCCTCACTTTAAT**
CCTCTATCCAGAAAACACGGTGGG**CCAAAGGATGAAGAGAGGTAACAAGATGC**TTAACT**CTTGTA**
ATAATGGCGATAGCTTTCTGGAGTTCATATGGTATACTACTTGTAAATATGTGCTAAGATAATTCCGT
GTTTCCCCCACCTTTGCTTTTGAACTTGCTGACTCATCTAAACCCCTGCTCCCAAATGCTGGAATGCTT

Lower case letters denote bases changed in the mutant allele.
Probe sequence is in bold and shaded grey.
Primer sequences are in bold and underlined.

Oligo	5' label	Sequence 5' → 3'	3' label	Oligo Type
HU-SOD1-EX3-MUT1_F	n/a	GTGCAGGTCCTCACTTTAATCC	n/a	Mutant Forward
HU-SOD1-EX3-MUT1_PROBE	FAM	CCAAAGGATGAAGAGAGGTAACAAGATGC	BHQ1	Mutant Probe
HU-SOD1-EX3-MUT1_R	n/a	CCAGAAAGCTATCGCCATTATTACAAG	n/a	Mutant Reverse

Dot1l internal control (VIC labelled)

CTGATGGGTGTGGGCAGATCCTACAGAGTCCCATTGGCCACCATGTGTGCTACGCCTGAAATAAAGCCTT**GCC CCAGCACGACCATT**CAGGG**CCAGCTCTCAAGTCG**ACTGTAA**GATGAAGCATAAGGATGCCAACTA**CTAACA GAAAACGACTAGAGGGGAAAAGAACAAGGAAACAGAAGACGCAGCACTCCGGCTTCCCTGGGTTGGCCAGT CACCCTATGA

Oligo Rabep2-S187AS191A	5' label	Sequence 5' → 3'	3' label	Oligo Type
Dot1l_Forward	n/a	**GCCCCAGCACGACCATT**	n/a	WT Forward
Dot1l_Probe	VIC	**CCAGCTCTCAAGTCG**	BHQ	WT Probe
Dot1l_Reverse	n/a	**TAGTTGGCATCCTTATGCTTCATC**	n/a	WT Reverse

Probe sequence is in bold and shaded grey
Primer sequences are in bold and underlined

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

Applied Biosystems GTX Taqman master mix	5 µl
Dot1l_Forward (20 µM)	0.225 µl
Dot1l_Reverse (20 µM)	0.225 µl
Dot1l_Probe (5 µM)	0.2 µl
FAM Assay (probe 5 µM & primers 15 µM each)	0.3 µl
ddH20	1.55 µl
DNA (1:10 dilution of ABI Sample-to-SNP prep)	2.5 µl

Each sample is ran in technical duplicate. Seven WT and/or mutant controls are also included in duplicate along with non-template controls.

qPCR cycling conditions

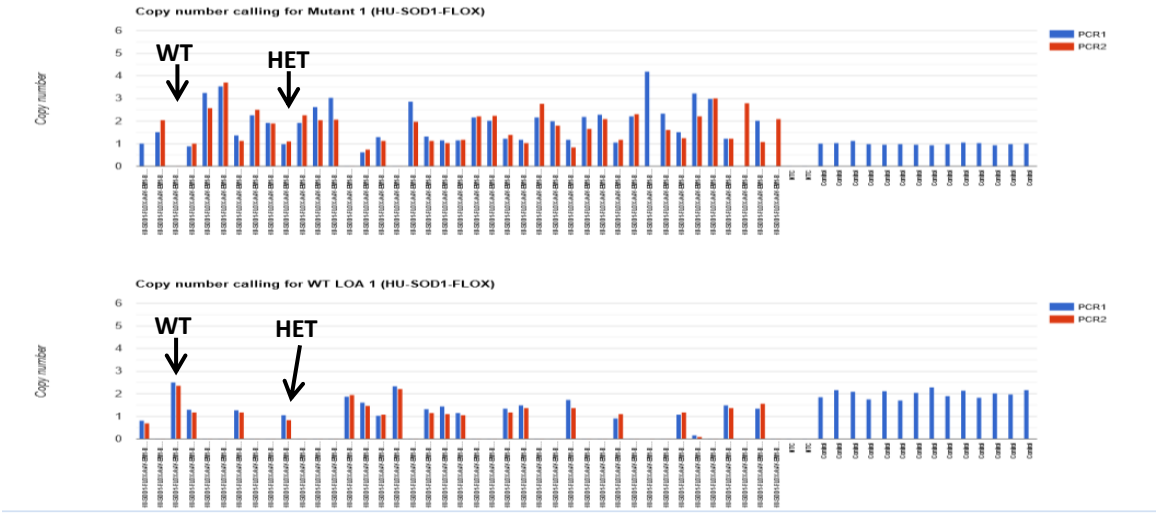
qPCR instrument: Applied Biosystems 7500/7900 or ThermoFisher QuantStudio 7

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 CopyCaller software v2.0 from Applied Biosystems or in-house software that is based on CopyCaller v2.0.

HU-SOD1-FLOX-B6J-WT and HU-SOD1-FLOX-B6J-MUT assays copy called results, image showing copy number chart for WT and MUT assays (from task 313182).



Allele Description

HU-SOD1-FLOX-A4V is a point mutation project targeting exon 1 on chromosome 16. The targeted point mutation is introduced by a 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

HU-SOD1-FLOX-A4V Allelic Discrimination assay

HU-SOD1-FLOX-A4V-AD1 WT sequence

CTACCCTCCGCCCCGCCCCCAGCGTGCCCCGCGGCCAGGGAGCTCCACGAAGGGCGGGCGGAGGCCGCGGG
TAGCGATTGGTTCCGTGCCAAGGTGGGCGTGGTCAGACTCAGGCCTATAAAAGCTCCGTGGCGCCAGGGCCT
CGTTTTTTTGCGCGGTCCTTTCCTGCGGCGCCTTCCGTCCGTCGGC**TTCTCGTCTTGCTCTCTCTG**GTCCCTCCG
GAGGAGGCCGCCGCGCGTCTCCCGGGGAAGCATGGCGAC**GAAGGcCGTGTGCGT**GCTGAAGGGCGACGGC
CCAGTGCAGGG**CATCATCAATTTCGAGCAGAAGG**CAAGGGCTGGGACGGAGGCTTGTTTGCGAGGCCGCTC
CCACCCGCTCGTCCCCCCGCGCACCTTTGCTAGGAGCGGGTCGCCCGCCAGGCCTCGGGGCCGCCCTGGTCCA
GCGCCCGGTCCCGGCCCGTGCCGCCCGGTCGGTGCCTTCGCCCCCAGCGGTGCGGTGCCCAAGTGCTGAGTC

HU-SOD1-FLOX-A4V-AD1 mutant sequence

CTACCCTCCGCCCCGCCCCCAGCGTGCCCCGCGGCCAGGGAGCTCCACGAAGGGCGGGCGGAGGCCGCGGG
TAGCGATTGGTTCCGTGCCAAGGTGGGCGTGGTCAGACTCAGGCCTATAAAAGCTCCGTGGCGCCAGGGCCT
CGTTTTTTTGCGCGGTCCTTTCCTGCGGCGCCTTCCGTCCGTCGGC**TTCTCGTCTTGCTCTCTCTG**GTCCCTCCG
GAGGAGGCCGCCGCGCGTCTCCCGGGGAAGCATGGCGA**CGAAGGtCGTGTGCGT**GCTGAAGGGCGACGGC
CCAGTGCAGGG**CATCATCAATTTCGAGCAGAAGG**CAAGGGCTGGGACGGAGGCTTGTTTGCGAGGCCGCTC
CCACCCGCTCGTCCCCCCGCGCACCTTTGCTAGGAGCGGGTCGCCCGCCAGGCCTCGGGGCCGCCCTGGTCCA
GCGCCCGGTCCCGGCCCGTGCCGCCCGGTCGGTGCCTTCGCCCCCAGCGGTGCGGTGCCCAAGTGCTGAGTC

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
HU-SOD1-FLOX-A4V-AD1_F	n/a	TTCTCGTCTTGCTCTCTCTG	n/a	Common forward primer
HU-SOD1-FLOX-A4V-AD1_WT_PROBE	TET	ACGCACACGGCCTTC	BHQ-1 plus	Wild type Probe
HU-SOD1-FLOX-A4V-AD1_MUT_PROBE	FAM	ACGCACACGACCTTCG	BHQ-1 plus	Mutant probe
HU-SOD1-FLOX-A4V-AD1 _R	n/a	CCTTCTGCTCGAAATTGATGATG	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

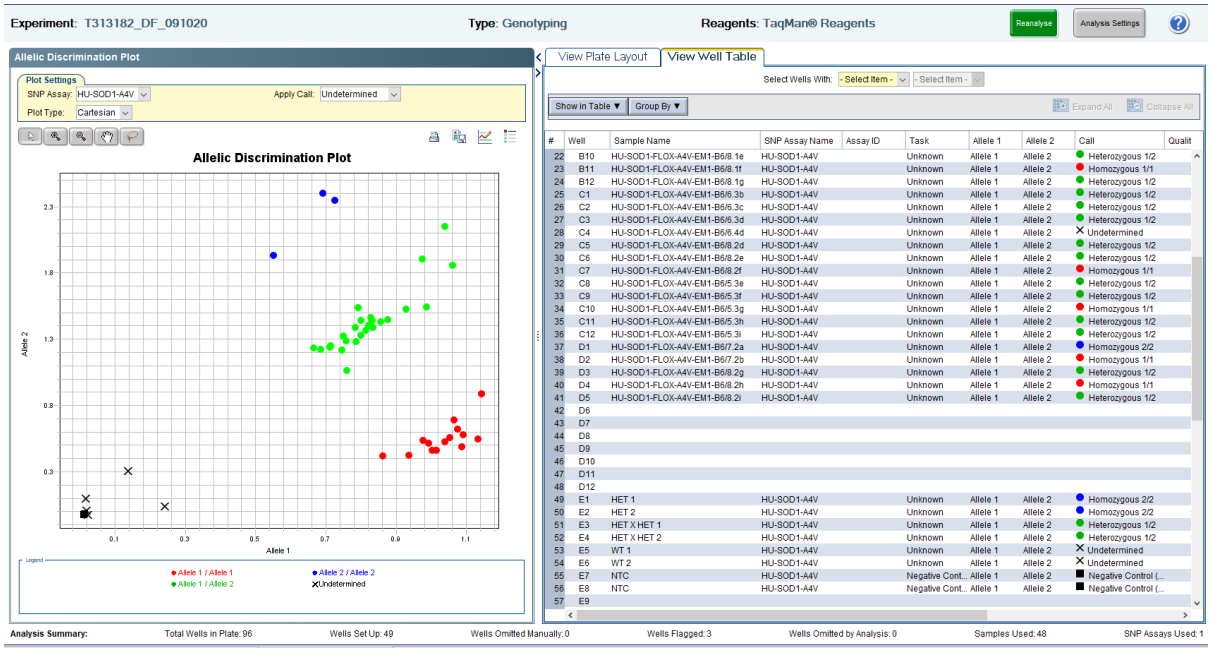
qPCR instrument: Applied Biosystems 7500

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 HU-SOD1-FLOX-A4V Allelic Discrimination Assay Results (from task 313182).



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