CSB1-ADH5-DKO, ADH5-B6J

Allele Description

These mice were imported as double mutants for ADH5 and CSB1.

ADH5 (GSNOR) is Adh5tm1Stam (MGI 3033711)
CSB (Ercc6) is Ercc6tm1Gvh (MGI 1932102)

Stockcode:
CSB1-ADH5-DKO-IC (RQ:003118)

The ADH5 (GSNOR) allele was origially described here:

J:88337 Liu L, et al., Essential roles of S-nitrosothiols in vascular homeostasis and endotoxic shock. Cell. 2004 Feb 20;116(4):617-28

The CSB allele was originally described here:

J:40211 van der Horst GT, et al., Defective transcription-coupled repair in Cockayne syndrome B mice is associated with skin cancer predisposition. Cell. 1997 May 2;89(3):425-35

The two alleles ADH5 and CSB1 are independently inherited (not linked). Each of them has Neo cassette inserted a part of the mutant allele.

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 wildtype loss of allele (WT-LOA) and a mutant assay (Neo) against a known reference assay (*Dot1l* on chromosome 10; 2 copies present). Samples for this line are genotyped using the following primers and probe:

- For ADH5, homologous recombination was used to replace exons 5 and 6 with a neomycin resistance gene. ADH5 wildtype specific qPCR assay was designed to detect the wildtype sequence which was deleted and replaced by Neo.
- For CSB insertion of a floxed neomycin resistance cassette into exon 5 near a site corresponding to a known human mutation disrupted the Ercc6 gene. CSB wildtype specific qPCR assay was designed across the region of insertion, so that the presence of Neo cassette would disrupt the wildtype amplicon.
- Both ADH5 and CSB mutant alleles were created by insertion of Neo to disrupt the wildtype allele. Neo qPCR mutant assay was therefore a mutant assay for both lines. For this reason, Neo copy number could be up to 4 copies if the samples were double HOMs.

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

Adh5-LOA-wt2 assay (FAM labelled)

CTTGCATGGAGAAGTCTAGAAAGCTCCTTTGCAGCCTTTCTGATAGTTGTCATTCTCACTGGAATGACTTGGTA
TGTTGTGGTTTGAATTAGTGATGTTGAACAGTTTTTCATGTATCTATTTGCTGTCTGTGTCTGGTTTTGAGAAAT
GTTGTTTTCTATCCCTTGCCCAGGCTGTTTGGTTTTGGGGAGTTTCCTATAAAATCTGGGTACCAGCCCATCATC
AGACGCCTGT**GCTCTGCCCCAACTGTTTC**CCTC**TGCTATGCAGAGGTCTTTCGTTTGC**TGTGCCCCATCT**GTTT**
ATTTCTGCTTTCGCCTCCCACGTTGTTTAAGATCTTACCCAAAAAAAAAAGTCTCGCCATTCAATGTCTTTATTTC
CCTTATATTTTCTTCTAGTAGTTTGAAAGTCTCAGGTCTGATATTT

All of the sequence shown above is deleted in the mutant allele.

(Note, this assay is labelled as wt2 because other assays called Adh5-Tm1a-WT 1 and Adh5-Tm1b-WT 1 had previously been used in the Genotyping Core for an IMPC Adh5 construct but would not have been suitable for this line.)

Oligo Name	5' label	Sequence 5' → 3'	3' label	Oligo Type
Adh5-LOA-wt2	n/a	**GCTCTGCCCCAACTGTTTC**	n/a	WT Forward
Adh5-LOA-wt2	6-FAM	**TGCTATGCAGAGGTCTTTCGTTTGC**	ZEN/IBFQ	WT Probe
Adh5-LOA-wt2	n/a	**GGGAGGCGAAAGCAGAAATAAAC**	n/a	WT Reverse

Ercc6-LOA-wt1 (renamed as CSB1-LOA-wt1) assay (FAM labelled)

GAGGAGTCTGGGTCCTCTCCCACCGATGGGGAGGAGGAGGAAGAACAAGAGGAGGAGGAAGGGGTAGCCA
GCCTGTCTTCTGATGACGTTAGCTATGAGCTGAAGCCTCTTCGGAAGAGGCAGAAATACCAGAAGAAAGTCCC
AGTTCAGGAGATCGACGATGACTTCTTTCCAAGTTCTGAGGAGGAAGATGAAGCTATGGAAGGAAGA**GGAG**
GAGGCCGAAAGGTAG**CCAGG**[Neo_insert]**CGCCAAGACGATGGA**GATGAGGATTATTATAAGCAGCGGTT
AAGGTTAGTGTGTACGGGGGGTAGGGGGTGGGGGGTGAGAGGTGGGGGCTCTGCTGCTTACGGGAGGCCA
CCCTAAAGATCTTAGCATTTTAGATCCCTAGGTCCCCTAGCTGTGAGACGCTGGACTTGCTGCCTTAGCCCACA
AGAACCTGGACATGGAGTCCTTAGTGGAGCTGGCCCCAAAGGTTTCAGTCTTTATTCTAAT

The WT probe is designed to span the location of the Neo insert, which is larger than 500bp, so the amplicon would be disrupted if Neo is present.

Oligo Name	5' label	Sequence 5' → 3'	3' label	Oligo Type
Ercc6-LOA-wt1 renamed to CSB1-LOA-wt1	n/a	**GGAGGAGGCCGAAAGGTA**	n/a	WT Forward
Ercc6-LOA-wt1 renamed to CSB1-LOA-wt1	6-FAM	**CCAGGCGCCAAGACGATGGA**	ZEN/IBFQ	WT Probe
Ercc6-LOA-wt1 renamed to CSB1-LOA-wt1	n/a	**CACACTAACCTTAACCGCTGCTTAT**	n/a	WT Reverse

Neo assay (FAM labelled)

ATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTG**GGTGGAGAGGCTATTCGGC**TATGAC**TGGGCA**
CAACAGACAATCGGCTGCT**CTGATGCCGCCGTGTTC**CGGCTGTCAGCGCAGGGGCGCCCGGTTCTTTTTGTCA
AGACCGACCTGTCCGGTGCCCTGAATGAACTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACG
GGCGTTCCTTGCGCAGCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTG
CCGGGGCAGGATCTCCTGTCATCTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCT

Oligo Name	5' label	Sequence 5' → 3'	3' label	Oligo Type
Neo	n/a	GGTGGAGAGGCTATTCGGC	n/a	Mutant Forward
Neo	6-FAM	TGGGCACAACAGACAATCGGCTG	ZEN/IBFQ	Mutant Probe
Neo	n/a	GAACACGGCGGCATCAG	n/a	Mutant Reverse

Dot1l internal control (VIC labelled)

CTGATGGGTGTGGGCAGATCCTACAGAGTCCCATTGGCCACCATGTGTGCTACGCCTGAAATAAAGCCTT**GCC**
CCAGCACGACCATTCAGGG**CCAGCTCTCAAGTCG**ACTGTAA**GATGAAGCATAAGGATGCCAACTA**CTAACA
GAAAACGACTAGAGGGGAAAAGAACAAGGAAACAGAAGACGCAGCACTCCGGCTTCCCTGGGTTGGCCAGT
CACCCTATGA

Oligo Name	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

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 5µM & primers 15µM 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. Non-template controls are also run.

qPCR cycling conditions

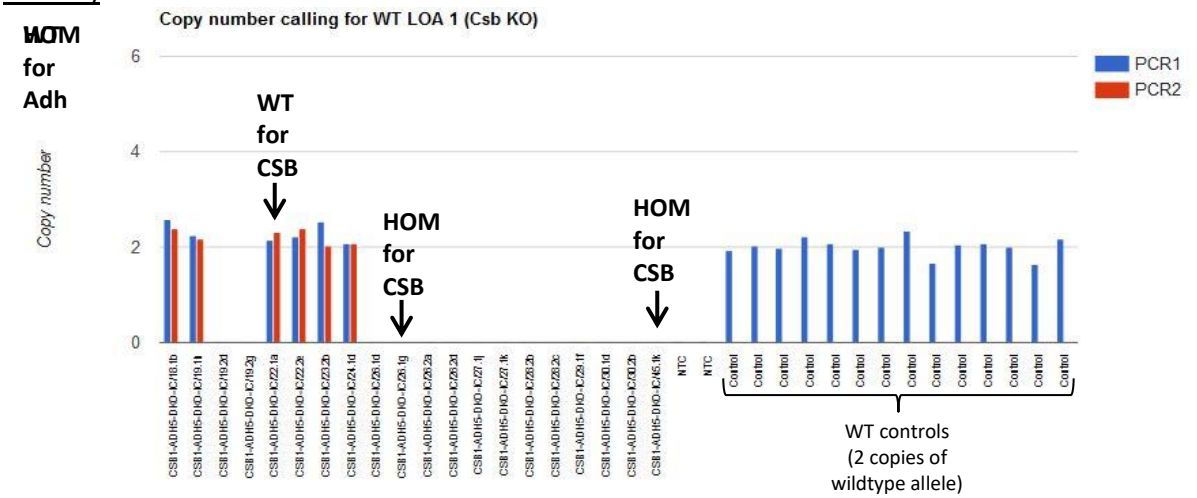
qPCR instrument: Applied Biosystems 7500/7900 or ThermoFisher QuantStudio 7

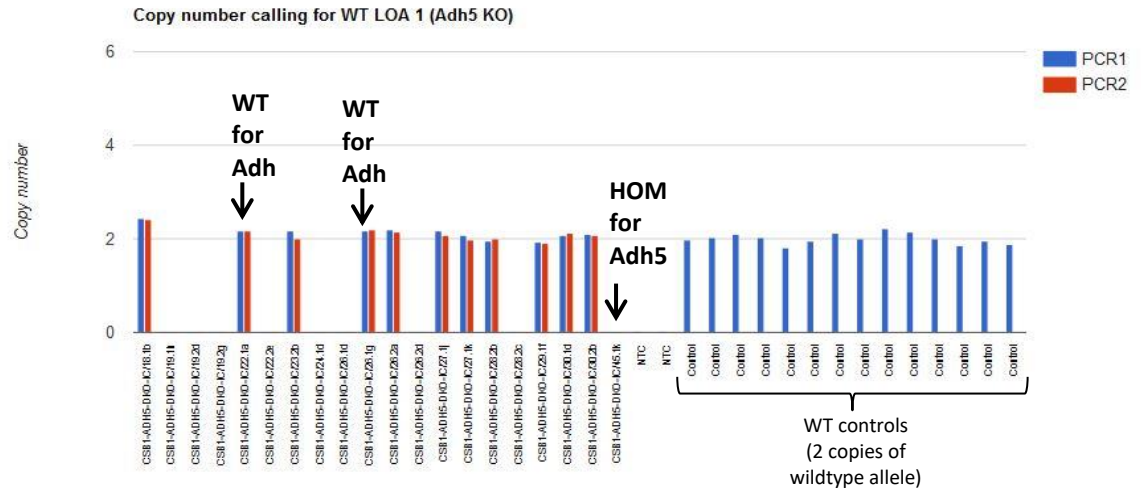
95^{o}C for 20 sec
Then 40 cycles of;
95^{o}C for 3 sec
60^{o}C for 30 sec

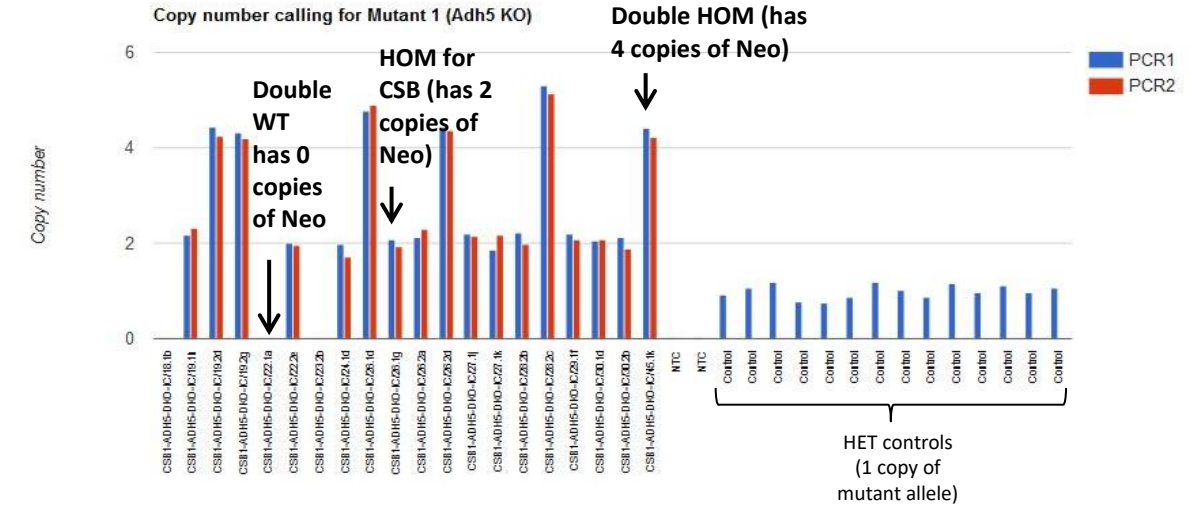
Analysis

The results are analysed using CopyCaller Software v2.0 from Applied Biosystems.

Adh5-LOA-wt2, Ercc6-LOA-wt1 renamed to CSB1-LOA-wt1 and Neo Assay copy called result, image showing copy number chart for both WT assays and Neo Mutant assay (Task T266615 results)





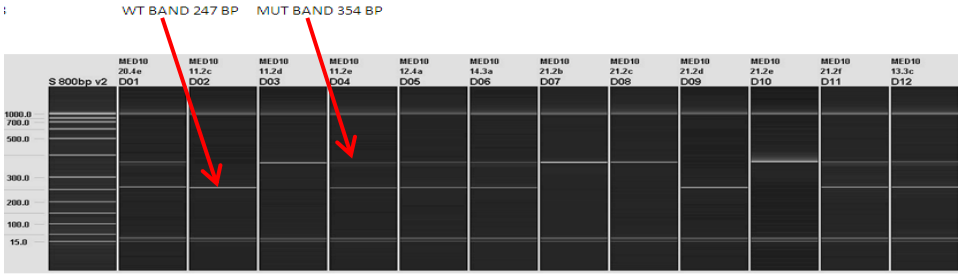


Version No.	1
Date:	11.08.2021
Created/Updated by:	KB
Approved by:	30/11/2021 DF

ADH5 Genotyping Strategy

Introduction

The gel based assays are normally run on the Qiagen QIAxcel. This is a capillary based system that provides clearer resolution and is quicker than running standard agarose gels. Different size ladders maybe loaded onto runs depending on the fragment sizes being analysed. Typically samples are run with a 50-800bp size ladder.



PCR is performed using KAPA fast Taq polymerase, although alternatives may be used.

ADH5 gel based primers

GSNORINT63	TCAAGAGGTGAGGCTACAAGTT
GSNORINT65	GGCATGTCTTCATTTAGCTCAC
GSNORE7	CCTGAAGCAGGTACTCCCACTACCAC
GSNORNEO3	TCTTGACGAGTTCTTCTGAGG

PCR mix

KAPA Taq PCR master mix	5µl
GSNORINT63	0.5µl
GSNORINT65	0.5µl
GSNORE7	0.5µl
GSNORNEO3	0.5µl
H_2O	2.0µl
DNA	1µl

60TM30FA

1. 95ºC 1min.
2. 95ºC 10sec.
3. **60**ºC 10sec.
4. 72ºC 1sec.
5. Go to 2 for 29 cycles
6. 72ºC 30sec.
7. 16 ºC forever
8. end

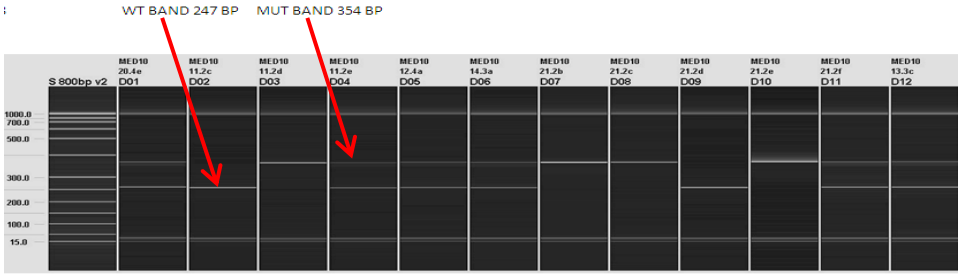
WT band = 200 bp Mut band = 800 bp

Version No.	1
Date:	30/11/2021
Created/Updated by:	Daniel Ford
Approved by:	Rumana Zaman

CSB Genotyping Strategy

Introduction

The gel based assays are normally run on the Qiagen QIAxcel. This is a capillary based system that provides clearer resolution and is quicker than running standard agarose gels. Different size ladders maybe loaded onto runs depending on the fragment sizes being analysed. Typically samples are run with a 50-800bp size ladder.



PCR is performed using KAPA fast Taq polymerase, although alternatives may be used.

CSB gel based primers

CSBfEX5	GTCTTCTGATGACGTTAGGTATGAG
CSBneo	ATCTGCGTGTTCGAATTCGCCAATG
CSBrEX5	GCTGCTTATAATAATCCTCATCTCC

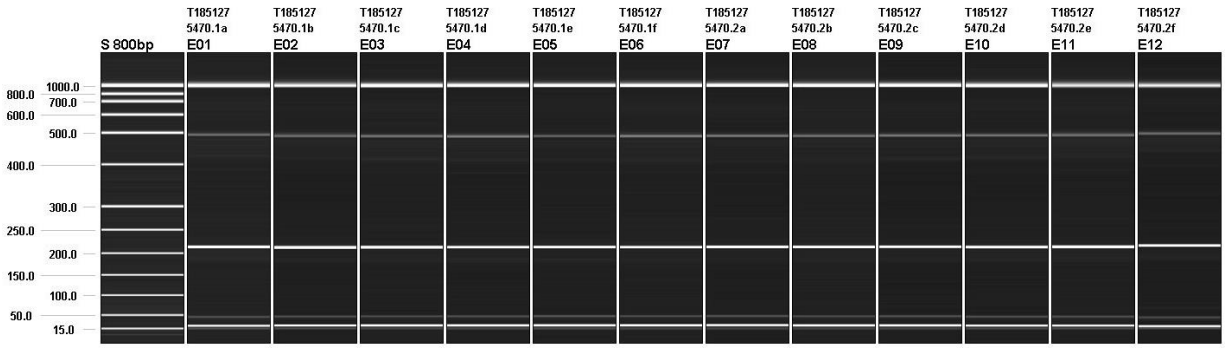
PCR mix

KAPA Taq PCR master mix	5µl
CSBfEX5	0.5µl
CSBneo	0.5µl
CSBrEX5	0.5µl
H_2O	2.5µl
DNA	1µl

60TM30FA

1. 95ºC 1min.
2. 95ºC 10sec.
3. **60**ºC 10sec.
4. 72ºC 1sec.
5. Go to 2 for 29 cycles
6. 72ºC 30sec.
7. 16 ºC forever
8. end
7. 16 ºC forever
8. end

Example of a CSB gel based assay (T226570)



WT band = 210 bp Mut band = 550 bp

Version No. 1

Date: 26/02/19

Created/Updated by: Daniel Ford

Approved by: Rumana Zaman