

U2AF2-C429S

Allele Description

This is a CRISPR/Cas9 induced mutation creating a series of point mutations; C429S in exon ENSMUSE00000300490 of *U2AF2*. 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.

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 |

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U2AF2-C429S-WT1 assay (FAM labelled)

CAATCAGACACCTGTGACCCTTCAAGTGCCCGGCCTGATGAGCTCTCAGGTGCAGATGGGCGGTCA
 CCCAACTGAGGTCCTGTGCCTCATGAACATGGTGCTGCCTGAGGAGCTGCTGGAC**CGATGAGGAGTA**
TGAGGAGATTGTAGAGGACGTACGAGACG**AGTGCAGCAAGTATGGGTTGGTCA**AATCCATTGAAA
 TTCCCCGCCcGTGGACGGCGTCGAGGTGCCTGG**CTgtGGAAAGGTCAGGGTG**CCCTTTTATCTTTT
 GTGAACAAATCCCTTAGCACCTGTCTCTCAGTACCTGACTAGTTCTGAACAAGGGTGACGCAGA

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 U2AF2-C429S | 5' label | Sequence 5' → 3' | 3' label | Oligo Type |
|----------------------|----------|---------------------------------------|----------|-------------------|
| U2AF2-C429S-WT_F | n/a | <u>CGATGAGGAGTATGAGGAGATTG</u> | n/a | Wild type Forward |
| U2AF2-C429S-WT_PROBE | FAM | AGTGCAGCAAGTATGGGTTGGTCA | ZEN/IBFQ | Wild type Probe |
| U2AF2-C429S-WT_R | n/a | <u>CACCCTGACCTTTCCACAG</u> | n/a | Wild type Reverse |

U2AF2-C429S-MUT1 assay (FAM labelled)

CAATCAGACACCTGTGACCCTTCAAGTGCCCGGCCTGATGAGCTCTCAGGTGCAGATGGGCGGTCA
 CCCAACTGAGGTCCTGTGCCTCATGAACATGGTGCTGCCTGAGGAGCTGCTGGAC**GATGAGGAGTA**
 TGAGG**GATTGTAGAGGACGTACGAGAC**G**AGTGCAGCAAGTATGGGTTGGTCA**AATCCATTGAA
 ATTCCCCGCCcGTGGACGGCGTCGAGGTGCCTGGCT**ccGGAAAGGTCAGGGTG**CCCTTTTATCTTT
 TGTGAACAAATCCCTTAGCACCTGTCTCTCAGTACCTGACTAGTTCTGAACAAGGGTGACGCAG

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 U2AF2-C429S | 5' label | Sequence 5' → 3' | 3' label | Oligo Type |
|-----------------------|----------|--------------------------------------|----------|----------------|
| U2AF2-C429S-MUT_F | n/a | <u>GATTGTAGAGGACGTACGAGAC</u> | n/a | Mutant Forward |
| U2AF2-C429S-MUT_PROBE | FAM | AGTGCAGCAAGTATGGGTTGGTCA | BHQ | Mutant Probe |
| U2AF2-C429S-MUT_R | n/a | <u>CACCCTGACCTTTCCGG</u> | n/a | Mutant Reverse |

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Dot1l internal control (VIC labelled)

CTGATGGGTGTGGGCAGATCCTACAGAGTCCCATTGGCCACCATGTGTGCTACGCCTGAAATAAAGCCTT**GCC**
CCAGCACGACCATTCAGGG**CCAGCTCTCAAGTCG**ACTGTAAGATGAAGCATAAGGATGCCAACTACTAACA
 GAAAACGACTAGAGGGGAAAAGAACAAGGAAACAGAAGACGCAGCACTCCGGCTTCCCTGGGTTGGCCAGT
 CACCCTATGA

| Oligo U2AF2-C429S | 5' label | Sequence 5' → 3' | 3' label | Oligo Type |
|-------------------|----------|--|----------|------------|
| Dot1l_Forward | n/a | <u>GCCCCAGCACGACCATT</u> | n/a | WT Forward |
| Dot1l_Probe | VIC | CCAGCTCTCAAGTCG | BHQ | WT Probe |
| Dot1l_Reverse | n/a | <u>TAGTTGGCATCCTTATGCTTCATC</u> | 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 |
| ddH2O | 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

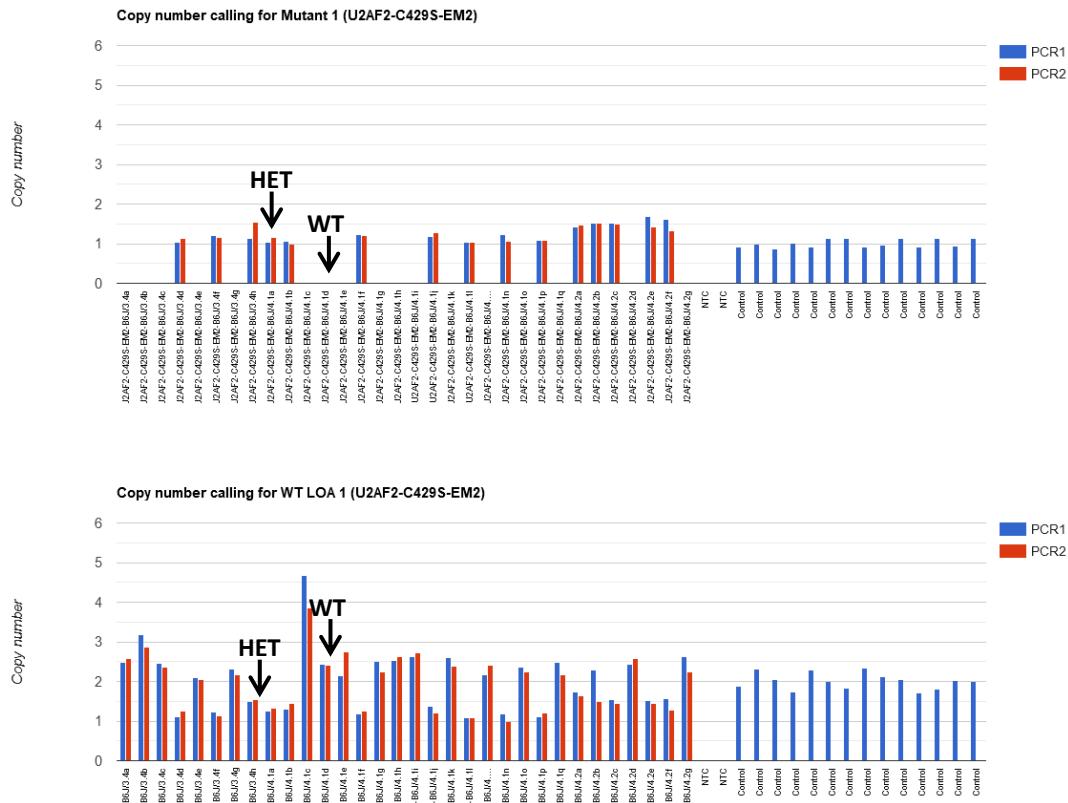
95°C for 20 sec
 Then 40 cycles of;
 95°C for 3 sec
 60°C for 30 sec

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Analysis

The results are analysed using CopyCaller software v2.0 from Applied Biosystems or in-house software that is based on CopyCaller v2.0.

U2AF2-C429S-WT1 and U2AF2-C429S -MUT1 assays copy called results, image showing copy number chart for WT and Mutant assays (Task 372132 results)



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 Created/Updated by: Daniel Ford
 Approved by: Debbie Williams