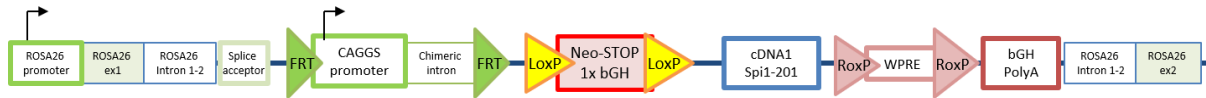


# R26-MOE4-Spi1-EM10-B6J

## Allele Description

This is a CRISPR/Cas9 induced knock-in line made by Embryonic stem cell (ESC) to mouse conversion followed by CRISPR-Cas9 targeting into 1-cell stage embryo.



MOE (Multi-level Over Expression) allele with Spi1 (ENSMUST00000002180.8 Spi1-201) cDNA.

- Conditional expression via Cre-LoxP excisable STOP cassette.
- Promoter switchable via Flp-FRT recombination to remove CAG promoter and depend on lower ROSA26 promoter expression.
- WPRE (Woodchuck hepatitis virus posttranscriptional regulatory element) transcript stabilising element excisable via Dre-RoxP recombination

## 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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## R26-WT2 assay (FAM labelled)

TCTGGGAGTTCTCTGCTGCCTCCTGGCTTCTGAGGACCGCCCTGGGCCTGGGAGAATCCCTTCCCCCTCT**TCCC**  
**T**CGTGATCTGCAAC**CCAGTCTTTCTAGAAGATGGGCGGGA**GTCTTCTGGGCAGGCTTAAAGG**CTAACCTGG**  
**TGTGTGGGCGTT**GTCTGTCAGGGGAATTGAACAGGTGTAAATTGGAGGGACAAGACTTCCACAGATTTTC  
 GGTTTTGTCGGGAAGTTTTTAATAGGGGCAAATAAGGAAAATGGGAGGATAGGTAGTCATCTGGGGTTTTA

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        |
|---------------|----------|------------------------------------|----------|-------------------|
| R26-WT2_F     | n/a      | <b><u>TCCCTCGTGATCTGCAAC</u></b>   | n/a      | Wild type Forward |
| R26-WT2_PROBE | FAM      | <b>CAGTCTTTCTAGAAGATGGGCGGGA</b>   | BHQ1     | Wild type Probe   |
| R26-WT2_R     | n/a      | <b><u>AACGCCACACACCAGGTTAG</u></b> | n/a      | Wild type Reverse |

## MOE-Spi1-MUT1 assay (FAM labelled)

TGGGGCTCGAAAACATGCATATAACTTCGTATAGCATACATTATACGAAGTTATATCCGGGGGTACCGCGTCG  
 AGGCTAGATCCAATTGTTGTACGCGTTAAGTGCAACACGATCCCGCCACCA**gttacaggcgtgcaaaatggaagggtt**  
**tcctcaccgccccctccat**cgatgacttggttacttacgattcagagctataccaacgtccaatgcatgactactactccttcgtgggcagcga**tg**  
 gagaaag**ccatagcgatcactactgggatttc**ccgcacaccatgtccacaacaacgagtttgagaacttcctgagaaccacttcacagagct

Lower case letters denote Spi1 cDNA sequence.  
 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     |
|--------------------|----------|-----------------------------------------|----------|----------------|
| MOE-Spi1-MUT_F     | n/a      | <b><u>GTTACAGGCGTGCAAAATGGAAG</u></b>   | n/a      | Mutant Forward |
| MOE-Spi1-MUT_PROBE | FAM      | <b>TTCCCTCACCGCCCCTCCAT</b>             | Zen-IBFQ | Mutant Probe   |
| MOE-Spi1-MUT_R     | n/a      | <b><u>GAAATCCCAGTAGTGATCGCTATGG</u></b> | n/a      | Mutant Reverse |

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## Dot1l internal control (VIC labelled)

CTGATGGGTGTGGGCAGATCCTACAGAGTCCCATTGGCCACCATGTGTGCTACGCCTGAAATAAAGCCTT**GCC**  
**CCAGCACGACCATT**CAGGG**CCAGCTCTCAAGTCG**ACTGTAAG**GATGAAGCATAAGGATGCCAACTACTAACA**  
GAAAACGACTAGAGGGGAAAAGAACAAGGAAACAGAAGACGCAGCACTCCGGCTTCCCTGGGTTGGCCAGT  
CACCTATGA

| Oligo Name    | 5' label | Sequence 5' → 3'                       | 3' label | Oligo Type |
|---------------|----------|----------------------------------------|----------|------------|
| Dot1l_Foward  | n/a      | <u><b>GCCCCAGCACGACCATT</b></u>        | n/a      | WT Forward |
| Dot1l_Probe   | VIC      | <b>CCAGCTCTCAAGTCG</b>                 | BHQ      | WT Probe   |
| Dot1l_Reverse | n/a      | <u><b>TAGTTGGCATCCTTATGCTTCATC</b></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_Foward (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

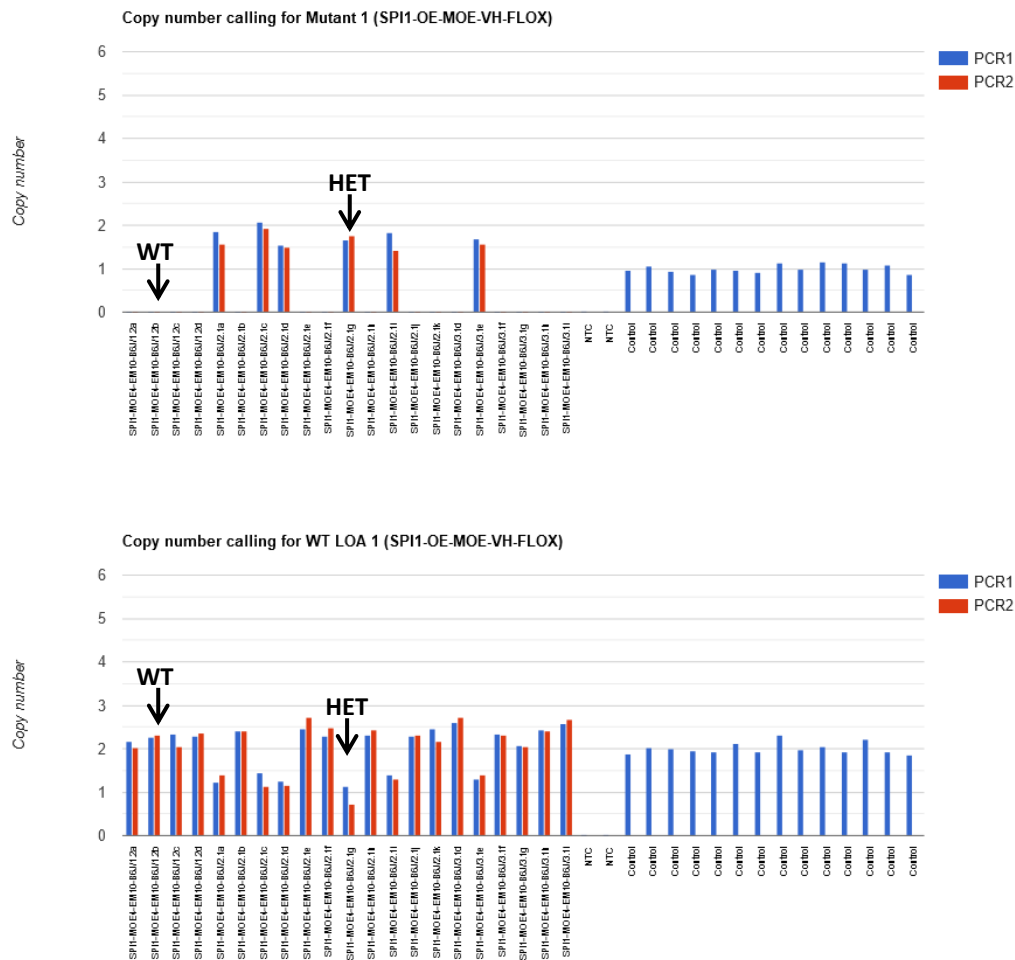
60°C for 30 sec

## Analysis

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

# R26-MOE4-Spi1-EM10-B6J

R26-WT2 and MOE-Spi1-MUT1 assays copy called results, image showing copy number chart for WT and Mutant assays (Task 405116 results)



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Created/Updated by: DF

Approved by: DJW