



**Name of Mouse model or mutation:**

**hMAPT-A05-MOE4-EM3-129**

**hMAPT-A05-MOE4-EM4-129**

**Description:**

Correction of error in 5' LoxP site by CRISPR/Cas9 gene editing in existing ES-cell derived transgenic line (hMAPT-A05; HAR066Ai\_hMAPT\_A05).

**Type of mutation:**

Knock-in: Correction of error in 5' LoxP site

**Delivery method:**

Electroporation into 1-cell stage embryo.

**Genetic Background:**

129S9(/SvEV)

**Nuclease:**

Cas9 protein

**sgRNAs:**

Protospacer sequence	PAM sequence
TAGGAACTTCTTAGGGCCCG	<b>CGG</b>
CTAGAGAATAGGAACTTCTT	<b>AGG</b>
GACTGCAGCCAATATGGGAT	<b>CGG</b>
GTGCAATCCATCTTGTTCAA	<b>TGG</b>

**IssDNA donor sequence template (5'-3', NOTE donor delivered to embryo will be reverse complement (Codner et al., 2018: Figure 1)):**

LOCUS MOE\_5LoxFix\_dono 300 bp DNA linear 22-JUN-2022

FEATURES Location/Qualifiers

misc\_binding 82..115

/note="FRT site"

protein\_bind 139..172

/note="LoxP site"

misc\_feature 173..178

/note="Nrul RE site"

variation 70..71

/note="PAMs CC>tt in donor"

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misc_feature 1..69
    /note="5' homology arm"
misc_feature 152..159
    /note="Lox core FIXED (to match downstream LoxP)"
misc_feature 1..300
    /note="donor sequence"
variation 77..78
    /note="CCC>tg to delete PAMs"
variation 196..198
    /dnas_title="TCG > agt in donor"
    /note="Ser: TC*, AGT, AGC"
    /note="TCG > agt in donor"
variation 199..201
    /dnas_title="GCC > GCa in donor"
    /note="Ala: GC*"
    /note=""
misc_feature 205..300
    /note="3' homology arm"
PCR_primer complement(281..300)
    /note="MOE_5LoxFix_RNA"
CDS 190..300
    /note="NeoR"
source 1..300
    /dnas_title="MOE_5LoxFix_donor_1 lssDNA"

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#### ORIGIN

```

1 CCTACAGCTC CTGGGCAACG TGCTGGTTAT TGTGCTGTCT CATCATTTTG GCAAAGAATT
61 GATTTGATAt tGCGGGtgTA AGAAGTTCCT ATTCTCTAGA AAGTATAGGA ACTTCGTCGA
121 CATTAAATC ATTTAAATAT AACTTCGTAT AGCATAACATT ATACGAAGTT ATTCGCGACT
181 GCAGCCAATA TGGGAagtGC aATTGAACAA GATGGATTGC ACGCAGGTTC
TCCGGCCGCT
241 TGGGTGGAGA GGCTATTCGG CTATGACTGG GCACAACAGA CAATCGGCTG
CTCTGATGCC
//

```

#### Electroporation mixes:

Cas9 protein, sgRNAs and ssODNs were diluted and mixed in Electroporation buffer (EB; Gibco Opti-MEM I Reduced Serum Media – (Thermo Fisher Scientific)) to the working concentrations of 650 ng/μl, 130 ng/μl each and 400 ng/μl, respectively. Embryos were electroporated using the following conditions: 30 V, 3 ms pulse length, 100 ms pulse interval, 12 pulses. Electroporated embryos were re-implanted in CD1 pseudo-pregnant females. Host females were allowed to litter and rear F<sub>0</sub> progeny.

## Sequence details

### hMAPT-A05 (line to be corrected)

GCAGCGGAGGAGCGCTTCCGGCCGACGTCTCGTCGCTGATTGGCTTCTTTTCTCCCGCCGTGTGTG  
AAAACACAAATGGCGTGTTTTGGTTGGCGTAAGGCGCCTGTCAGTTAACGGCAGCCGGAGTGCGCA  
GCCGCCGGCAGCCTCGCTCTGCCACTGGGTGGGGCGGGAGGTAGGTGGGGTGAGGCGAGCTGG  
ACGTGCGGGCGCGGTCTGGCCTCTGGCGGGGCGGGGAGGGGAGGGGAGGGTACGCGAAAGTAGC  
TCGCGCGGAGCGGCCGCCACCCTCCCCTTCTCTGGGGGAGTCGTTTTACCCGCCGCCGGCCGGG  
CCTCGTCGTCTGATTGGCTCTCGGGGCCAGAAAAGTGGCCCTTGCCATTGGCTCGTGTTTCGTGCAA  
GTTGAGTCCATCCGCCGCCAGCGGGGGCGGCGAGGAGGCGCTCCCAGGTTCCGGCCCTCCCCTCG  
GCCCCGCGCCGAGAGTCTGGCCGCGCGCCCTGCGCAACGTGGCAGGAAGCGCGCGCTGGGGGC  
GGGGACGGGCAGTAGGGCTGAGCGGCTGCGGGGCGGGTGCAAGCACGTTTCCGACTTGAGTTGCC  
TCAAGAGGGGCGTGCTGAGCCAGACCTCCATCGCGCACTCCGGGGAGTGGAGGGAAGGAGCGAG  
GGCTCAGTTGGGCTGTTTTGGAGGCAGGAAGCACTTGCTCTCCCAAAGTCGCTCTGAGTTGTTATCA  
GTAAGGGAGCTGCAGTGGAGTAGGCGGGGAGAAGGCCGCACCCTTCTCCGGAGGGGGGAGGGGA  
GTGTTGCAATACCTTCTGGGAGTTCTCTGCTGCCTCTGGCTTCTGAGGACCGCCCTGGGCCTGGG  
AGAATCCCTTCCCCCTTCCCTCGTGATCTGCAACTCCAGTCTTCTAGCCGGGCCGGGCTGCAGA  
TCTGTAGGGCGCAGTAGTCCAGGGTTTCCTTGATGATGTCATACTTATCCTGTCCCTTTTTTTCCACA  
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CGCTCTAGTGGCCGTACGGGCCACCTGCCGGGCCACTTAATCGGGAAGTTCTATTCTCTAGAAAG  
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AATAGGGACTTTCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCACTTGGCAGTACAT  
CAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCCCCTGGCATT  
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ACCATGGTTCGAGGTGAGCCCCAGTCTGCTTCACTCTCCCCATCTCCCCCCCCTCCCCACCCCCAATT  
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CAATCAGAGCGGCGCGCTCCGAAAGTTTCTTTTATGGCGAGGCGGCGGCGGCGGCCCTATAA  
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GGGAGGGGCGCGGCGGCCCGGAGCGCCGGCGGCTGTGAGGCGCGGCGAGCCGCAGCCATTG  
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TCTGGGAGGCGCCGCCGCACCCCCTCTAGCGGGCGCGGGGCGAAGCGGTGCGGGCGCCGGCAGGA  
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GGGCTGTCCGCGGGGGACGGCTGCCTTCGGGGGGGACGGGGCAGGGCGGGGTTCCGGCTTCTGG  
CGTGTGACCGGCGGCTCTAGAGCCTCTGCTAACCATGTTTCATGCCTTCTTCTTTTCTACAGCTCCTG  
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GACCGACCTGTCCGGTGCCTGAATGAACTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCAC  
GACGGGCGTTCCTTGCGCAGCTGTGCTCGACGTTGCTACTGAAGCGGGAAGGGACTGGCTGCTATT  
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GCTGATGCAATGCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCATTGACCACCAAGCGAAAC  
ATCGCATCGAGCGAGCACGTA CTGGATGGAAGCCGGTCTTGTGATCAGGATGATCTGGACGAAG  
AGCATCAGGGGCTCGCGCCAGCCGAACTGTTCCGCCAGGCTCAAGGCGCGCATGCCCGACGGCGAG  
GATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTG  
GATTCATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTG  
ATATTGCTGAAGAGCTTGCCGGCGAATGGGCTGACCGCTTCTCGTGCTTTACGGTATCGCCGCTCC  
CGATTGCGAGCGCATCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGA

**hMAPT-A05-MOE4-EM3-129 & hMAPT-A05-MOE4-EM4-129 (see also Genbank including features of allele in its entirety)**

GCAGCGGAGGAGCGCTTCCGGCCGACGTCTCGTCGCTGATTGGCTTCTTTTCTCCCGCCGTGTGTG  
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GCCGCCGGCAGCCTCGCTCTGCCACTGGGTGGGGCGGGAGGTAGGTGGGGTGAGGCGAGCTGG  
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TCGCGCGGAGCGGCCGCCACCCTCCCCTTCTCTGGGGGAGTCGTTTTACCCGCCGCCGGCCGGG  
CCTCGTCGTCTGATTGGCTCTCGGGGCCAGAAAAGTGGCCCTTGCCATTGGCTCGTGTTTCGTGCAA  
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TCAAGAGGGGCGTGCTGAGCCAGACCTCCATCGCGCACTCCGGGGAGTGGAGGGAAGGAGCGAG  
GGCTCAGTTGGGCTGTTTTGGAGGCAGGAAGCACTTGCTCTCCCAAAGTCGCTCTGAGTTGTTATCA  
GTAAGGGAGCTGCAGTGGAGTAGGCGGGGAGAAGGCCGCACCCTTCTCCGGAGGGGGGAGGGGA  
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TCTGTAGGGCGCAGTAGTCCAGGGTTTCTTGATGATGTCATACTTATCCTGTCCCTTTTTTTTCCACA  
GCTCGCGTTGAGGACAAACTCTTCGCGGTCTTTCCAGTGGGGATCGACGGTATCGATAAGCTGGC  
CGCTCTAGTGGCCGTACGGGCCACCTGCCGGGCCACTTAATCGGGAAGTTCCTATTCTCTAGAAAG  
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CAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCCGCTGGCATT  
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ACCATGGTCGAGGTGAGCCCCACGTTCTGCTTCACTCTCCCCATCTCCCCCCCCTCCCCACCCCCAATT  
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CCGGGCGGGGCGGGGCGGGGCGAGGGGCGGGGCGGGGCGAGGCGGAGAGGTGCGGCGGCAGC  
CAATCAGAGCGGCGCGCTCCGAAAGTTTCTTTTATGGCGAGGCGGCGGGCGGCGGCCCTATAA  
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CGCTCGCGCCGCCCGCCCCGGCTCTGACTGACCGCGTACTCCCACAGGTGAGCGGGCGGGACGG  
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AAAGCCTTGAGGGGCTCCGGGAGGGCCCTTGTGCGGGGGAGCGGCTCGGGGGGTGCGTGCGT  
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GCGGCGCGGGGCTTTGTGCGCTCCGCAGTGTGCGCGAGGGGAGCGCGGCCGGGGGCGGTGCCCC  
GCGGTGCGGGGGGGGCTGCGAGGGGAACAAAGGCTGCGTGC GGGTGTGTGCGTGGGGGGGTG  
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CCTTTTATGGTAATCGTGCAGAGGGGCGCAGGGACTTCTTTGTCCCAAATCTGTGCGGAGCCGAAA  
TCTGGGAGGCGCCGCCGACCCCCCTAGCGGGCGCGGGGCGAAGCGGTGCGGCGCCGGCAGGA  
AGGAAATGGGCGGGGAGGGCCTTCGTGCGTCGCCGCGCCGCGTCCCCTTCTCCTCTCCAGCCTCG  
GGGCTGTCCGCGGGGGACGGCTGCCTTCGGGGGGGACGGGGCAGGGCGGGGTTCCGGCTTCTGG  
CGTGTGACCGCGGCTCTAGAGCCTCTGCTAACCATGTTTCATGCCTTCTTCTTTTCTACAGCTCCTG  
GGCAACGTGCTGGTTATTGTGCTGTCTCATATTTTGGCAAAGAATTGATTTGATACCGCGGGtgTAA  
GAAGTTCCTATTCTAGAAAGTATAGGAACTTCGTCGACATTTAAATCATTAAATATAACTTCGTA  
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CGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCGCAGGGGCGCCCGTTCCTTTTGTCAAGACC  
GACCTGTCCGTGCCCTGAATGAACTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGAC  
GGGCGTTCCTTGCAGCTGTGCTCGACGTTGCTACTGAAGCGGGAAGGGACTGGCTGCTATTGGG  
CGAAGTGCCGGGGCAGGATCTCCTGTCTCTCACCTTGTCTCCTGCCGAGAAAGTATCCATCATGGCT  
GATGCAATGCGGCGGCTGCATACGCTTATCCGGCTACCTGCCATTTCGACCACCAAGCGAAACATC  
GCATCGAGCGAGCACGTA CTGGATGGAAGCCGGTCTTGTGATCAGGATGATCTGGACGAAGAG  
CATCAGGGGCTCGCGCCAGCCGAACTGTTCCGAGGCTCAAGGCGCGCATGCCCGACGGCGAGGA  
TCTCGTCGTGACCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGA  
TTCATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGAT  
ATTGCTGAAGAGCTTGGCGGCAATGGGCTGACCGCTTCTCGTGCTTTACGGTATCGCCGCTCCCG  
ATTCGAGCGCATCGCCTTCTATCGCCTTCTGACGAGTTCCTTCTGA

Please note hMAPT-A05-MOE4-EM3-129 and hMAPT-A05-MOE4-EM4-129 have the same allele sequence but were derived from different founders.

### **hMAPT-A05-MOE4-EM3-129 & hMAPT-A05-MOE4-EM4-129 full allele sequence at Rosa26**

LOCUS Rosa26 12865 bp DNA linear 23-JUN-2022

FEATURES Location/Qualifiers

misc\_feature 424..566  
/note="Rosa 26 Exon 1 ENSMUSE00000815063%09"

misc\_feature 1..423  
/note="5' UTR"

PCR\_primer 459..478  
/note="AiME\_5'integr\_F1"

PCR\_primer 491..507  
/note="AiME\_5'integr\_F2"

PCR\_primer 544..563  
/note="Ai2\_PW\_Rosa26\_1F"

misc\_feature 567..700  
/note="Rosa 26 Intron 1-2"

misc\_feature 4821..4854  
/note="LoxP"

misc\_feature 4553..4777  
/note="bGH poly(A)-signal"

misc\_feature 6361..6949  
/note="WPRE"

PCR\_primer 6738..6758  
/note="AiME\_3'integr\_F1"

PCR\_primer 6751..6774  
/note="AiME\_3'integr\_F2"

misc\_feature 6965..6996  
/note="RoxP"

misc\_feature 6997..7002  
/note="BstBI"

misc\_feature 7008..7215  
/note="bGH poly(A) signal"

misc\_feature 7218..7225  
/note="AscI"

misc\_feature 7228..11568  
/note="3'HA Rosa26 in AiME (from Ai9)"

PCR\_primer 6993..7014  
/note="AiME\_3'integr\_F5"  
/note=""

PCR\_primer 9028..9049  
/note="AiME\_3'integr\_F3"

PCR\_primer 9073..9095  
/note="AiME\_3'integr\_F4"

PCR\_primer complement(9464..9483)  
/note="AiME\_3'integr\_R3"

PCR\_primer complement(9298..9317)  
/note="AiME\_3'integr\_R4"

misc\_feature 11569..11827  
/note="Rosa 26 Intron 1-2"  
PCR\_primer complement(11755..11778)  
/note="AiME\_3'integr\_R2"  
PCR\_primer complement(11581..11602)  
/note="AiME\_3'integr\_R5"  
/note=""  
misc\_feature 11828..12865  
/note="Rosa 26 Exon2 %09ENSMUSE00000801245%09"  
PCR\_primer complement(11885..11905)  
/note="AiME\_3'integr\_R1"  
PCR\_primer 4855..4875  
/note="LoxPF"  
misc\_feature 4884..4889  
/note="Mfel"  
misc\_feature 4918..4926  
/note="Kozak"  
misc\_feature 4924..6249  
/note="protein coding sequence of human MAPT-205"  
misc\_feature 4924..5056  
/note="Exon 2: ENSE00003549966 (only protein coding  
coding sequence)"  
misc\_feature 5057..5143  
/note="Exon 3: ENSE00003598892"  
misc\_feature 5144..5230  
/note="Exon 4: ENSE00002331131"  
misc\_feature 5231..5296  
/note="Exon 5: ENSE00003658894"  
misc\_feature 5297..5352  
/note="Exon 6: ENSE00003611335"  
misc\_feature 5353..5479  
/note="Exon 7: ENSE00003682122"  
misc\_feature 5210..5229  
/note="Ai\_Mt\_centre\_UPL64\_Fwd"  
misc\_feature 5294..5314  
/note="Ai\_Mt\_Centre\_UPL64\_Rev"  
misc\_feature 5214..5232  
/note="AiME\_MAPT-205\_MUT1\_Fwd"  
misc\_feature 5292..5310  
/note="AiME\_MAPT-205\_MUT1\_Rev"  
misc\_feature 5262..5284  
/note="AiME\_MAPT-205\_MUT1\_Probe"  
misc\_feature 5480..5745  
/note="Exon 8: ENSE00003634058"  
misc\_feature 5746..5838  
/note="Exon 9: ENSE00002409189"  
misc\_feature 5839..5920  
/note="Exon 10: ENSE00002405491"  
misc\_feature 5921..6033

/note="Exon 11: ENSE00003570976"  
misc\_feature 6277..6284  
/note="Fsel"  
misc\_feature 6289..6320  
/note="RoxP"  
PCR\_primer complement(6321..6340)  
/note="LoxPR"  
misc\_feature 6034..6249  
/note="Exon 12: ENSE00001291240 (exclusive 3'UTR)"  
misc\_feature 701..1601  
/note="MOE KI 5' homology arm Rosa 26"  
intron 701..1601  
/note="Rosa26 intron 1-2"  
misc\_feature 1602..1609  
/note="SrfI RE site"  
Site 1625..1728  
/note="Splice acceptor site"  
misc\_signal 1681..1700  
/note="splice acceptor 100% confidence NetGene2 10/10"  
misc\_binding 1801..1834  
/note="FRT site"  
misc\_feature 1837..1844  
/note="PacI RE site"  
enhancer 1930..2233  
/note="CMV enhancer"  
misc\_structure 1814..1821  
/note="FRT core"  
promoter 2235..2360  
/note="B-actin promoter"  
intron 2513..3529  
/note="chimeric intron"  
misc\_binding 3604..3637  
/note="FRT site"  
misc\_feature 3695..3700  
/note="NruI RE site"  
misc\_feature 3523..3591  
/note="5' homology arm"  
PCR\_primer 3245..3264  
/note="Geno\_MOE\_5LoxFix\_F1"  
PCR\_primer 3211..3230  
/note="Geno\_MOE\_5LoxFix\_F2"  
PCR\_primer 3210..3230  
/note="MOE\_5LF-specific\_F1"  
misc\_signal 3520..3539  
/note="splice acceptor 97% confidence NetGene2 10/10"  
misc\_feature 3584..3598  
/note="hMAPT-MOE-5'-PRE-MUT1"  
misc\_feature 3695..3708  
/note="hMAPT-MOE-5'-PRE-MUT1"



protein\_bind 3661..3694  
/note="LoxP site"  
misc\_feature 3674..3681  
/note="Lox core FIXED (to match downstream LoxP)"  
misc\_feature 3599..3600  
/dnas\_title="PAMs to modify in donor"  
/note="CCC>tg"  
PCR\_primer 3587..3608  
/dnas\_title="MOE\_5LF-postfix\_F3"  
/note=""  
CDS 3712..4515  
/note="NeoR"  
variation 3721..3723  
/dnas\_title="GCC > GCa in donor"  
/note="Ala: GC\*"  
/note="GCC > GCa in donor"  
misc\_feature 3727..3822  
/note="3' homology arm"  
PCR\_primer complement(4119..4138)  
/note="Geno\_MOE\_5LoxFix\_R1"  
PCR\_primer complement(4192..4211)  
/note="Geno\_MOE\_5LoxFix\_R2"  
PCR\_primer complement(3803..3822)  
/note="MOE\_5LoxFix\_RNA"  
misc\_feature complement(3709..3721)  
/note="hMAPT-MOE-5'-PRE-MUT1"  
misc\_feature 3718..3720  
/dnas\_title="TCG > agt in donor"  
/note=""  
PCR\_primer complement(4091..4110)  
/dnas\_title="MOE\_5LF-postfix\_R3"  
/note=""  
PCR\_primer complement(3713..3734)  
/dnas\_title="MOE\_5Lox-postfix\_R2"  
/note=""  
PCR\_primer complement(3716..3737)  
/dnas\_title="MOE\_5Lox-postfix\_R1"  
/note=""  
misc\_feature 3583..3600  
/note="hMAPT-MOE-5POST-MUT1\_F"  
misc\_feature complement(3688..3706)  
/note="hMAPT-MOE-5POST-MUT1\_R"  
misc\_feature 3615..3644  
/note="hMAPT-MOE-5POST-MUT1\_P"  
misc\_feature 3684..3708  
/note="hMAPT-MOE-5PRE-MUT1\_P"  
source 1..12865  
/dnas\_title="Rosa26 with MOE4 5LoxFix hMAPT allele in mouse"

ORIGIN

1 CCCAACGCGG CGCCACGGCG TTTCTGGCC GGAATGGCC CGTACCCGTG AGGTGGGGT  
61 GGGGGCAGA AAAGCGGAG CGAGCCGAG GCGGGGAGGG GGAGGGCCAG GGGCGAGGG  
121 GGCCGGCACT ACTGTGTTGG CGGACTGGCG GACTAGGGC TCGTGAGTC TCTGAGCGCA  
181 GGGGGCGGC GGCCGCCCT CCCC GGCGG CGGCAGCGGC GGCAGCGGC GCAGCTCACT  
241 CAGCCGCTG CCCGAGCGGA AACGCCACTG ACCGCACGGG GATTCCAGT GCCGGCGCCA  
301 GGGCACGCG GGACACGCC CCTCCGCGG CGCCATTGGC CTCTCCGCC ACCGCCAC  
361 ACTTATTGGC CGGTGCGCC CCAATCAGCG GAGGCTGCC GGGCCGCTA AAGAAGAGG  
421 TGTGCTTGG GGCTCCGGCT CTCAGAGAG CCTCGGCTAG GTAGGGGATC GGGACTCTGG  
481 CGGGAGGGCG GCTTGGTGC TTTGCGGGGA TGGGCGGCC CGGCAGGCC TCCGAGCGTG  
541 GTGGAGCCGT TCTGTGAGAC AGCCGGGTAC GAGTCGTGAC GCTGGAAGGG GCAAGCGGT  
601 GGTGGCAGG AATGCGTCC GCCCTGCAGC AACCGGAGGG GGAGGGAGAA GGGAGCGGAA  
661 AAGTCTCCAC CGGACGCGG CATGGCTCG GGGGGGGGG GCAGCGGAGG AGCGTTCCG  
721 GCCGACGTCT CGTCGCTGAT TGGCTTCTT TCCTCCGCC GTGTGTGAAA ACACAAATG  
781 CGTGTTTTGG TTGGCGTAA GCGCCTGTCA GTTAACGGCA GCCGAGTGC GCAGCCGCC  
841 GCAGCCTCG TCTGCCACT GGTGGGGCG GGAGGTAGGT GGGTGAGGC GAGCTGGAC  
901 TGCGGGCGCG GTCGGCTCT GCGGGGCGG GGGAGGGGAG GGAGGGTCAG CGAAAGTAG  
961 TCGCGCGCA GCGGCCGCC ACCCTCCCT TCCTCTGGGG GAGTCGTTT ACCCGGCC  
1021 GGCCGGCCT CGTCGTCTGA TTGGCTCTG GGGCCAGAA AACTGGCCT TGCCATTGGC  
1081 TCGTGTTCGT GCAAGTTGAG TCCATCCGCC GGCCAGCGG GCGGCGAGG AGGCGTCCC  
1141 AGTTCCGGC CCTCCCTCG GCGCGCGCC GCAGAGTCTG GCCGCGGCC CCTGCGCAAC  
1201 GTGGCAGGAA GCGCGCGCTG GGGGCGGGGA CGGGCAGTAG GGCTGAGCG CTGCGGGCG  
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1321 TCGCRACTC CGGGAGTGG AGGGAAGGAG CGAGGGCTCA GTTGGGCTGT TTTGGAGGCA  
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1441 GTAGGCGGGG AGAAGGCCG ACCCTTCTCC GGAGGGGGGA GGGAGTGTT GCAATACCT  
1501 TCTGGGAGTT CTCTGCTGCC TCCTGGCTT TGAGGACCGC CCTGGGCTG GGAGAATCCC  
1561 TCCCCCTCT TCCTCGTGA TCTGCACTC CAGTCTTCT AGCCGGGCC GGGCTGCAGA  
1621 TCTGTAGGGC GCAGTAGTCC AGGTTTCT TGATGATGTC AACTTATCC TGTCCTTTT  
1681 TTTCCACAG CTCGCGGTTG AGGACAACT CTTCGCGTC TTTCCAGTGG GGATCGACGG  
1741 TATCGATAAG CTGGCGCTC TAGTGCCGT ACGGGCCAC CTGCCGGCC ACTTAATCGG  
1801 GAAGTTCCTA TTCTCTAGAA AGTATAGGAA CTCCCTTAA TTAACGTTT AAACAATTCT  
1861 GCAGGAATCT AGTTATTAAT AGTAATCAAT TACGGGTCA TTAGTTCATA GCCCATATAT  
1921 GGAGTCCGC GTTACATAAC TTACGTAAG TGGCCGCCT GGCTGACCG CCAACGACC  
1981 CCGCCATTG ACGTCAATA TGACGTATGT TCCCATAGTA ACGCAATAG GACTTTCCA  
2041 TTGACGTCAA TGGGTGGAGT ATTTACGGTA AACTGCCAC TTGGCAGTAC ATCAAGTGA  
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2221 CGCTATTACC ATGGTCGAGG TGAGCCCCAC GTTCTGCTT ACTCTCCCA TCTCCCCC  
2281 CTCCCACCC CCAATTTTGT ATTTATTTAT TTTTAATTA TTTTGTGAG CGATGGGGG  
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2401 GAGGCGGAGA GGTGCGGCG CAGCAATCA GAGCGGCGG CTCCGAAAGT TTCCTTTAT  
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2521 TGCGGCTGC CTTGCCCCG TGCCCCGCT CGCCCGGCC TCGCGCGCC CGCCCCGCT  
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2701 GGGGCTCCG GAGGGCCCT TGTGCGGGG GAGCGGCTCG GGGGTGCGT GCGTGTGTG  
2761 GTGCGTGGG AGCGCCCGT GCGGCTCCG GCTGCCCGG GGCTGTGAG GCTGCGGGG  
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3421 CGGCTGCCTT CGGGGGGGAC GGGGCAGGGC GGGGTTGCGC TTCTGGCGTG TGACCGGCGG  
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4561 TCTAGTTGCC AGCCATCTGT TGTTTGCCCC TCCCCGTGC CTTCTTGAC CTTGGAAGGT  
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4861 GGGTACCGCG TCGAGGCTAG ATCCAATTGT TGTACGCGTT AAGTGCAACA CGATCCCGCC  
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5581 ACCCGGGAGC CCAAGAAGGT GGCAGTGGTC CGTACTCCAC CCAAGTCGCC GTCTTCGCC  
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12781 GCTTACAAAT GTGGCTTGAC TTGCTACTGT GCTTGTTTTA GTTATGTGAA AGTTTGGCAA  
12841 TAAACCTATG TCCTAAATAG TCAAA

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## Nucleotide Alignment:

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*      2420      *      2440      *      2460      *      2480      *      2500      *      2520      *      2540      *
hMAPT_A05      : CCGGGCGGGGCGGGCCGCTCGGGCCGGGAGGGCTCGGGGGAGGGCGCGGGCGGGCCCGGGAGCGCGGGCGGCTGTGAGGGCGGGCGAGCCGAGCCATTGCCCTTTTATGGTAATCGTGCAGAGGGGCGAGGACTTCCTTTGTCC
hMAPT_MOE4_EM3 : CCGGGCGGGGCGGGCCGCTCGGGCCGGGAGGGCTCGGGGGAGGGCGCGGGCGGGCCCGGGAGCGCGGGCGGCTGTGAGGGCGGGCGAGCCGAGCCATTGCCCTTTTATGGTAATCGTGCAGAGGGGCGAGGACTTCCTTTGTCC

*      2560      *      2580      *      2600      *      2620      *      2640      *      2660      *      2680      *      2700
hMAPT_A05      : CAAATCTGTGCGGAGCCGAAATCTGGGAGGCGCGCCGCAACCCCTCTAGCGGGCGGGGGCGAAGCGGTGCGGGCGGGCGAGGAAGAAATGGGCGGGGAGGGCCCTCGTGCCTGCGCGCGCCGCGCTCCCTTCTCCCTCCAGCCT
hMAPT_MOE4_EM3 : CAAATCTGTGCGGAGCCGAAATCTGGGAGGCGCGCCGCAACCCCTCTAGCGGGCGGGGGCGAAGCGGTGCGGGCGGGCGAGGAAGAAATGGGCGGGGAGGGCCCTCGTGCCTGCGCGCGCCGCGCTCCCTTCTCCCTCCAGCCT

*      2720      *      2740      *      2760      *      2780      *      2800      *      2820      *      2840      *
hMAPT_A05      : CGGGGCTGTCCGCGGGGGACGGCTGCCCTCGGGGGGACGGGGCAGGGCGGGGTTCCGGCTTCTGGCGTGTGACCGGGCGGCTCTAGAGCCTCTGCTAACCATGTTTCATGCCTTCTTCTTTTCTTCTACAGCTCCTGGGCAACGTGCCTGGTT
hMAPT_MOE4_EM3 : CGGGGCTGTCCGCGGGGGACGGCTGCCCTCGGGGGGACGGGGCAGGGCGGGGTTCCGGCTTCTGGCGTGTGACCGGGCGGCTCTAGAGCCTCTGCTAACCATGTTTCATGCCTTCTTCTTTTCTTCTACAGCTCCTGGGCAACGTGCCTGGTT

*      2860      *      2880      *      2900      *      2920      *      2940      *      2960      *      2980      *      3000
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hMAPT_MOE4_EM3 : ATTGTGCTGTCTCATCTTTTGGCAAAGAATTGATTTGATACCGGGGtgTAAGAAGTTCCTATTCTCTAGAAAGTATAGGAAGTTCGTCGACATTTAAATCATTTAAATATAAAGTTCGTATAgcaTcaTTATACGAAGTTATTCGGC

*      3020      *      3040      *      3060      *      3080      *      3100      *      3120      *      3140      *
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hMAPT_MOE4_EM3 : ACTGCAGCAATATGGGAagtGCATTGAACAAGATGGATTGCACGCAAGTTCCTCCGGCCGCTTGGGTGGAGAGGCTATTCCGGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCCGCGTTCGGCTGTCCAGCGCAGGGG

*      3160      *      3180      *      3200      *      3220      *      3240      *      3260      *      3280      *      3300
hMAPT_A05      : CGCCCGGTTCTTTTGTCAAGACCGACCTGTCCGGTGCCCTGAATGAAGTGCAGGACGAGGCGAGCGGGCTATCGTGGCTGGCCACGACGGGGCTTCTTGCAGCTGTGCTCGACGTTGTCACCTGAAGCGGGGAGGGACTGGCTGCTA
hMAPT_MOE4_EM3 : CGCCCGGTTCTTTTGTCAAGACCGACCTGTCCGGTGCCCTGAATGAAGTGCAGGACGAGGCGAGCGGGCTATCGTGGCTGGCCACGACGGGGCTTCTTGCAGCTGTGCTCGACGTTGTCACCTGAAGCGGGGAGGGACTGGCTGCTA

*      3320      *      3340      *      3360      *      3380      *      3400      *      3420      *      3440      *
hMAPT_A05      : TTGGGCGAAGTGCCGGGCGAGGATCTCCTGTCACTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGGCGGCTGCATACGCTTGATCCGGCTACCTGCCATTCGACCACCAAGCGAAACATCGCATCGAGCGA
hMAPT_MOE4_EM3 : TTGGGCGAAGTGCCGGGCGAGGATCTCCTGTCACTCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGGCGGCTGCATACGCTTGATCCGGCTACCTGCCATTCGACCACCAAGCGAAACATCGCATCGAGCGA

*      3460      *      3480      *      3500      *      3520      *      3540      *      3560      *      3580      *      3600
hMAPT_A05      : GCACGTACTCGGATGGAAGCCGGTCTTGTGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACGTTTCGCCAGGCTCAAGGCGCGCATGCCCGACGGCGAGGATCTCGTGTGACCCATGGCGATGCTGCTTG
hMAPT_MOE4_EM3 : GCACGTACTCGGATGGAAGCCGGTCTTGTGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACGTTTCGCCAGGCTCAAGGCGCGCATGCCCGACGGCGAGGATCTCGTGTGACCCATGGCGATGCTGCTTG

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Grey = LoxP site, Red = corrected nucleotides, Yellow = silent changes/changes within backbone sequence of allele introduced to prevent re-processing of engineered allele by CRISPR/Cas9

**QC strategy employed at Harwell to check the edited allele:**

Genomic DNA was extracted from ear clip biopsies and amplified in a PCR reaction using the following conditions/primer sequences:

Geno_MOE_5LoxFix_F1 (5'-3')	TTGTCCCAAATCTGTGCGGA
Geno_MOE_5LoxFix_R1 (5'-3')	GATGTTTCGCTTGGTGGTCG
Taq Polymerase used	ThermoFisher SuperFi II PCR Kit
Annealing Temperature (°C)	60
Elongation time (min)	0.5
WT product size (bp)	894
Mutant product size (bp)	894
Notes	Will amplify both 'unfixed' and the 'corrected' allele.

Geno_MOE_5LoxFix_F1 (5'-3')	TTGTCCCAAATCTGTGCGGA
MOE_5Lox-postfix_R2 (5'-3')	TCTTGTTCAATtGCactTCCCA
Taq Polymerase used	ThermoFisher SuperFi II PCR Kit
Annealing Temperature (°C)	60
Elongation time (min)	0.33
WT product size (bp)	
Mutant product size (bp)	490
Notes	Screening PCR for 'corrected' allele.

MOE_5LF-postfix_F3 (5'-3')	TGATAttGCGGGtgTAAGAAGT
MOE_5LF-postfix_R3 (5'-3')	GGTAGCCGGATCAAGCGTAT
Taq Polymerase used	ThermoFisher SuperFi II PCR Kit
Annealing Temperature (°C)	60
Elongation time (min)	0.5
WT product size (bp)	
Mutant product size (bp)	524



Notes	Screening PCR for 'corrected' allele.
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All amplicons were sent for Sanger sequencing to check for integration of the donor oligo sequence at the target site. F1 sequences should be heterozygous unless on sex chromosome.

**Off-target site with  $\leq 2$  mismatches for guide(s) used were checked with the following primers:**

Off-target site	Sequence	Type	Primers used (5'-3')
<a href="#">13:9534896-9534918</a>	CAGGAACTTCTTAGGTCCCG TGG	Intronic	MOE_5LoxFix_OT1_F1 (AATGGAAGTGCTGTGTCCCA) MOE_5LoxFix_OT1_R1 (AGCAACATACAGGACGGATGA)

All amplicons were sent for Sanger sequencing.

No off-target activity was detected in the animals selected to establish the colony.

#### **Additional integrations of the donor sequence**

Copy counting of the donor sequence was carried out by ddPCR at the F1 stage to confirm donor oligos were inserted once on target into the genome. The following Taqman assay was used to copy count the donor sequence compared against a VIC-labelled reference assay for Dot1l:

Assay name	hMAPT-MOE-5PRE-MUT1
Forward Primer (5'-3')	ATTTGATACCGCGGGCC
Reverse Primer (5'-3')	CCGATCCCATATT
Probe (5'-3')	TACGAAGTTATTCGCGACTGCAGCC
Label	FAM

This ddPCR assay is specific to the ES cell-derived erroneous allele and only the erroneous alleles are expected to be recognised by this assay. Therefore, 129 WT controls are expected to call at 0 copies, animals carrying the erroneous hMAPT allele are expected to call at 1 copy and a single integration for the correct mutation is expected to call at 0 copy for F1 (HET) animals.

Assay name	hMAPT-MOE-5POST-MUT1
Forward Primer (5'-3')	GATTTGATACCGCGGGtg
Reverse Primer (5'-3')	CTGCAGTCGCGAATAACTT
Probe (5'-3')	TCTCTAGAAAAGTATAGGAACTTCGTCGACA
Label	FAM

This ddPCR assay is specific to the donor used to create the engineered mutation and only the corrected alleles are expected to be recognised by this assay. Therefore, 129 WT controls are expected to call at 0 copies, animals carrying the erroneous hMAPT allele are expected to call at 0 copies and a single integration for a correct mutation is expected to call at 1 copy for F1 (HET) animals.

Reference Assay Name	Dot1l
Forward primer (5'-3')	GCCCCAGCACGACCATT
Reverse primer (5'-3')	TAGTTGGCATCCTTATGCTTCATC
Probe (5'-3')	CCCAACAGGCCTGGATTCTCAATGC
Label	VIC

VIC-labelled reference assay for Dot1l gene.

No additional donor integrations were detected in the animals taken forward to establish the colony.