



Allele Description

This is a CRISPR/Cas9 induced mutation deleting 22 nt from *Sts* gene, within a critical exon to introduce a frameshift resulting in a premature stop codon/null allele. 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 wildtype loss of allele (WT-LOA) 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:

- Universal probe and Universal primer designed 5' of the deleted region.
- Wildtype specific primer situated within the deleted region.
- Mutant specific primer that either bridges the junction designed for the CRISPR mutant allele or is 3' of the junction

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



STS-DEL22-EM2-WT1 assay (FAM labelled)

GAGGCCTGACACAGGCAGGAAGTGACATCACGGACGACAGGAAGTGGGGCCTCACACAGACGGG
 AAGTGACATCACAGGGGGACAGGAAGTGGGGC**CTCACACGGACAGGAAGT**GACCCCGCGACCCC
 GCAGGACGCCGCACCTCGACCGCCTGGCCCGGAGGGGGTGAAGC**tgacgcagcac**ctggcgccgcCCC
 GCTCTGCACCCCCAGCCGCGCCGCC**TTCTGACCGGGCG**CTAC

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

Oligo STS-DEL22-EM2	5' label	Sequence 5' → 3'	3' label	Oligo Type
STS-DEL22-EM2-UNI_F	n/a	CTCACACGGACAGGAAGT	n/a	Universal Forward
STS-DEL22-EM2-WT_PROBE	FAM	TGA+C+G+CAG+C+AC	Zen-IBFQ	Wildtype Probe
STS-DEL22-EM2-UNI_R	n/a	CGCCCGGTCAGGAA	n/a	Universal Reverse

The + symbol in the probe sequence stands for 'LNA (locked nucleic acid) linker

STS-DEL22-EM2-MUT1 assay (FAM labelled)

GAGGCCTGACACAGGCAGGAAGTGACATCACGGACGACAGGAAGTGGGGCCTCACACAGACGGG
 AAGTGACATCACAGGGGGACAGGAAGTGGGGC**CTCACACGGACAGGAAGT**GACCCCGCGACCCC
 GCAGGACGCCGCACCTCGACCGCCTGGCCCGGAGGGGGTGA**AAGC**[22ntdeletion]**CCC**GCTCTGC
 ACCCCAGCCGCGCCGCC**TTCTGACCGGGCG**CTAC

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

Oligo STS-DEL22-EM2	5' label	Sequence 5' → 3'	3' label	Oligo Type
STS-DEL22-EM2-UNI_F	n/a	CTCACACGGACAGGAAGT	n/a	Universal Forward
STS-DEL22-EM2-MUT_PROBE	FAM	AAGC+C+C+CGCT	Zen-IBFQ	Mutant Probe
STS-DEL22-EM2-UNI_R	n/a	CGCCCGGTCAGGAA	n/a	Universal Reverse

The + symbol in the probe sequence stands for 'LNA (locked nucleic acid) linker



Dot1l internal control (VIC labelled)

CTGATGGGTGTGGGCAGATCCTACAGAGTCCCATTGGCCACCATGTGTGCTACGCCTGAAATAAAGCCTT**GCC**
CCAGCACGACCATTCAGGG**CCAGCTCTCAAGTCG**ACTGTAA**GATGAAGCATAAGGATGCCAACT**ACTAACA
GAAAACGACTAGAGGGGAAAAGAACAAGGAAACAGAAGACGCAGCACTCCGGCTTCCCTGGGTTGGCCAGT
CACCTATGA

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

Oligo STS-DEL22-EM2	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

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
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. Non-template controls are also run.

qPCR cycling conditions

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

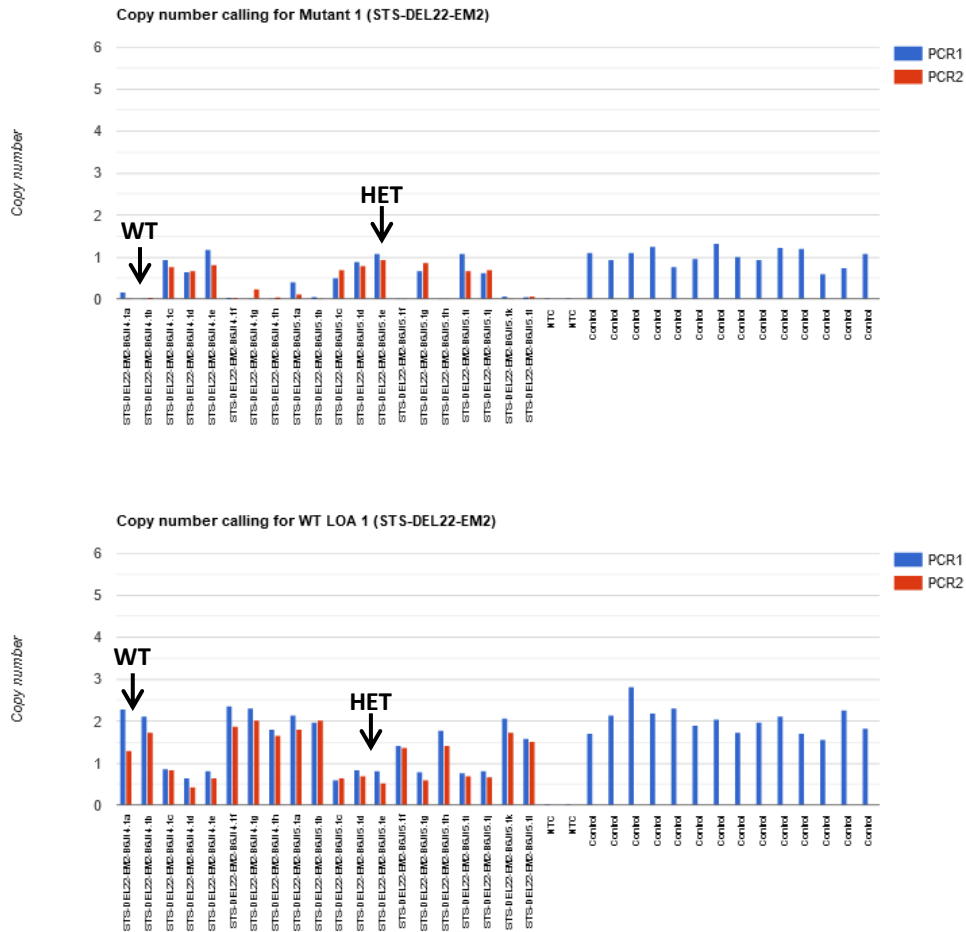
95°C for 20 sec
Then 40 cycles of;
95°C for 3 sec
60°C for 30 sec



Analysis

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

STS-DEL22-EM2-WT1 and STS-DEL22-EM2-MUT1 copy called result, image showing copy number chart for WT and Mutant assays (Task 360448 results)



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