ChrX-DEL57Kb-EM1-B6

Allele Description

This is a CRISPR/Cas9 induced mutation characterised by a 57,975 nt deletion on chromosome X.

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 assay with probe and forward and reverse primers binding to the WT critical region.
- Mutant assay with probe and forward and reverse primers that only amplify in the presence of the 57,975 nt deletion on chromosome X.

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

ChrX-DEL57kb-WT1 WT assay (FAM labelled)

TTTGTAAAAAGGGGATAGATAGGAGATCCTAAGAAGCAAAGTAAGTACACCTTTATGTTAACAGCTC
ACATAACTATTGGAGTTTTGAAAAAAAAGTTCATGTCTTCAGGCAAGACCAGATCGAAAACAACTCA
TGGGGGCTG**GAGAGATGGCTCAGTGGTTAAG**AGCAC**TCACTGCTCTTCCAGAGGTCCTGA**GTTCA
ATTCCCAGCAACCACATGGTGACTCACAACCATCTGTAATG**GTATCCAATGCAGTCTTCTGGT**GTGT
CTGAACAGAGCAACAGTGTACTCTCATACATAAAATAAATAAATAAATCTTTTTTAAAAGATTACTAT
GTTACATAACTGAAATAATTTATATAAAAAAGAAAGAAAACAACTCACAGAATTGTTAAGCCATTTCT

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

Oligo	5' label	Sequence 5' → 3'	3' label	Oligo Type
ChrX-DEL57kb-WT1_F	n/a	GAGAGATGGCTCAGTGGTTAAG	n/a	Wild type Forward
ChrX-DEL57kb-WT1_PROBE	FAM	TCACTGCTCTTCCAGAGGTCCTGA	Zen-IBFQ	Wild type Probe
ChrX-DEL57kb-WT1_R	n/a	ACCAGAAGACTGCATTGGATAC	n/a	Wild type Reverse

ChrX-DEL57kb-MUT1 MUT assay (FAM labelled)

ACTATCTTGCAATAAGGAAGATATGAACGTAGAGTCCTTTTCTAATTTAGGTAGAGAGAGTCTCTGA
AGTGCTATGTGTTTGAGGTGAGACCCGAAAATTATGAGAGATAGGCCAGGGTATAACTATAGAGTC
T**AAGCTCTCAGGACAGAAGAATAAA**TTATCCCAAGGATGGTAATTGGG**ATAGGTGGACCTGGGAT
AGGCTCA**GCACAGCCAGGGTCTCCAGTCCTGTT**GTG**[57,975_nt_del]**CAATTATCTAGTCCATATT
TTTAGTTC**TTTCCTGTCTCATTTTTTTTTTTTTGTCTAGTAAGCAAATGCACATGCGGCCCCCTTTGTAC
GATGATATTTTTGAAGTTAGAAATTGTGTTAATGCCCTATTGTTAATGT

Probe sequence is in bold and shaded grey.
Primer sequences are in bold and underlined.
Deletion is indicated in red.

Oligo	5' label	Sequence 5' → 3'	3' label	Oligo Type
ChrX-DEL57kb-MUT1_F	n/a	AAGCTCTCAGGACAGAAGAATAAA	n/a	Mutant Forward
ChrX-DEL57kb-MUT1_PROBE	FAM	ATAGGTGGACCTGGGATAGGCTCA	Zen-IBFQ	Mutant Probe
ChrX-DEL57kb-MUT1_R	n/a	GAACTAAAAATATGGACTAGATAATTGCAC	n/a	Mutant Reverse

Dot1l internal control (VIC labelled)

CTGATGGGTGTGGGCAGATCCTACAGAGTCCCATTGGCCACCATGTGTGCTACGCCTGAAATAAAGCCTT**GCC CCAGCACGACCATT**CAGGG**CCAGCTCTCAAGTCG**ACTGTAA**GATGAAGCATAAGGATGCCAACTA**CTAACA GAAAACGACTAGAGGGGAAAAGAACAAGGAAACAGAAGACGCAGCACTCCGGCTTCCCTGGGTTGGCCAGT CACCCTATGA

Oligo	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

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
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 along with non-template controls.

qPCR cycling conditions

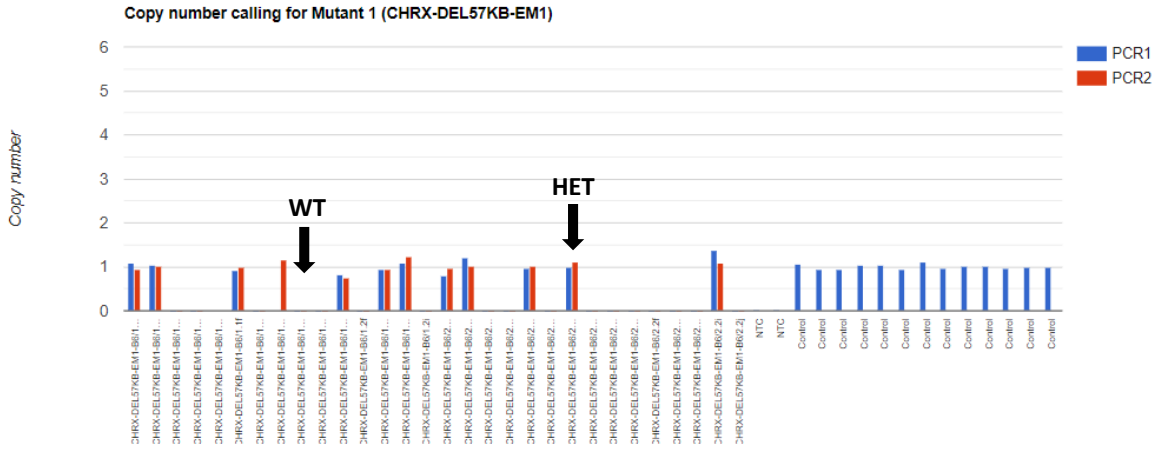
qPCR instrument: Applied Biosystems 7500/7900 or ThermoFisher QuantStudio 7

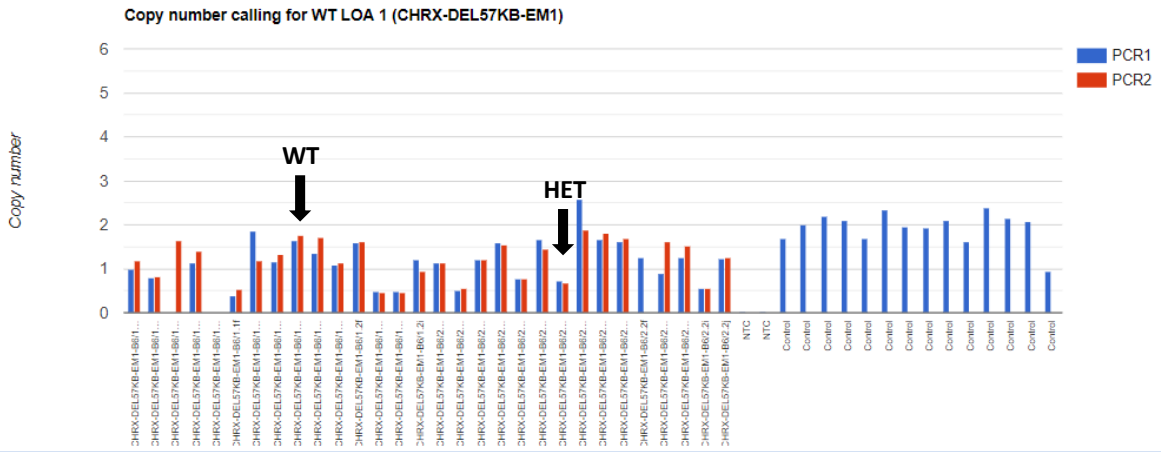
95°C for 20 sec
Then 40 cycles of;
95°C for 3 sec
60°C for 30 sec

Analysis

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

ChrX-DEL57kb-WT1 and ChrX-DEL57kb-MUT1 assays copy called results, image showing copy number chart for WT and MUT assays (task 311686).





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