



Allele Description

The stock was generated at MRC Harwell via pronuclear injection 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 binds to the inserted LoxP sequence

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



GJA10-Flox-WT1 assay (FAM labelled)

WT assay has been designed but not tested at MRC Harwell.

GTCCACTCCCACTCCACTATAGTGGGGAAGATCTGGCTGACCATCCTCTTCATCTTCCGAATGCTGGT
ACTTGGTGTGCTGCTGAGGACGTCTGGGATGATGAGCAGTCCGCCTTGCCTGCAACACCCAGCA
GCCCCGTTGCAACAATATCTGTTACGATGATGCTTCCCATCTCTTTGATCAGATTCTGGGTTTTGC
AGATCATCTTTGTGTCTTCCCCTTCTTGGTGTATATGGGCCATGCCCTTTATAGACTCAGGGACTTTG

Probe sequence is in bold and shaded grey

Primer sequences are in bold and underlined

Oligo GJA10-Flox	5' label	Sequence 5' → 3'	3' label	Oligo Type
GJA10-Flox-WT1_F	n/a	<u>CTTGGTGTGCTGCTGAG</u>	n/a	WT Forward
GJA10-Flox-WT1_PROBE	FAM	AAAGGCGGACTGCTCATCATCCC	Zen-IBFQ	WT Probe
GJA10-Flox-WT1_R	n/a	<u>GGGAAAGCATCATCGTAACAGA</u>	n/a	WT Reverse

GJA10-Flox -MUT1 assay (FAM labelled)

GTCCACTCCCACTCCACTATAGTGGGGAAGATCTGGCTGACCATCCTCTTCATCTTCCGAATGCTGGT
ACTTGGTGTGCGCCGCCGAGgtaagtaataacttcgtatagcatacattatacgaagtattcaaggtagaagacaggttta
aggagac**ccaatagaaactgggcttgc**gagacagagaag**actcttgcgtttctgataggcacct**attggtcttact**gacatccactt**
tgccataacttcgtatagcatacattatacgaagttatttctctccacagGACGTCTGGGATGATGAGCAGTCCGCCTT
TGCTGCAACACCCAGCAGCCCCGTTGCAACAATATCTGTTACGATGATGCTTCCCATCTCTTTGA

Lower case letters denote the DECAI cassette

Probe sequence is in bold and shaded grey

Primer sequences are in bold and underlined

Oligo GJA10-Flox	5' label	Sequence 5' → 3'	3' label	Oligo Type
GJA10-Flox-UNI_F	n/a	<u>CCAATAGAAACTGGGCTTGT</u>	n/a	Universal Forward
GJA10-Flox-UNI_PROBE	FAM	ACTCTTGC GTTCTGATAGGCACCT	Zen-IBFQ	Universal Probe
GJA10-Flox-MUT_R	n/a	<u>AGTTATGGCAAAGTGGATGT</u>	n/a	Mutant Reverse



Dot1l internal control (VIC labelled)

CTGATGGGTGTGGGCAGATCCTACAGAGTCCCATTGGCCACCATGTGTGCTACGCCTGAAATAAAGCCTT**GCC**
CCAGCACGACCATTCAGGG**CCAGCTCTCAAGTCG**ACTGTAAGATGAAGCATAAGGATGCCAACTACTAACA
GAAAACGACTAGAGGGGAAAAGAACAAGGAAACAGAAGACGCAGCACTCCGGCTTCCCTGGGTTGGCCAGT
CACCTATGA

Oligo GJA10-Flox	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

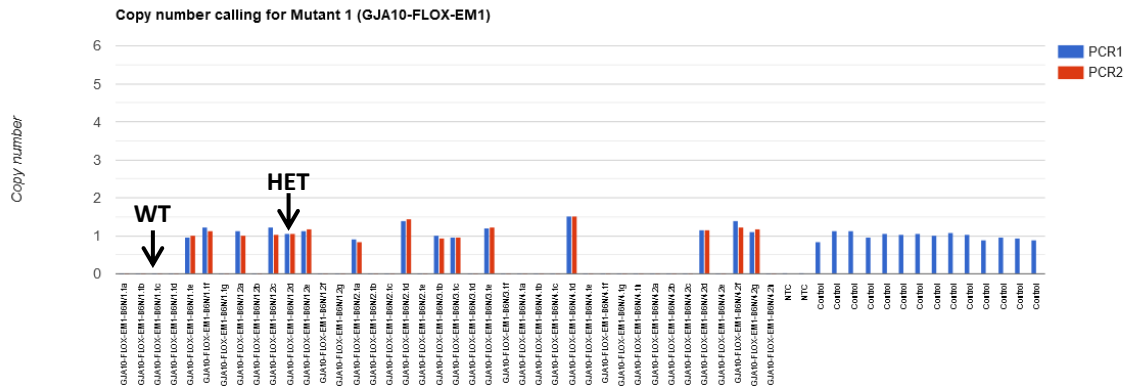
95°C for 20 sec
Then 40 cycles of;
95°C for 3 sec
60°C for 30 sec



Analysis

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

GJA10-Flox-MUT1 assays copy called results, image showing copy number chart for Mutant assays (Task 327687 results)



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