CXCR4-CRE

Allele Description

This is a CRISPR/Cas9 induced knock-in of a Cre cassette into *CXCR4*. The stock was generated at MRC Harwell via pronuclear injection of CRISPR/Cas9 reagents into 2-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

Cxcr4-Cre-ERT2-BP-WT1 assay (FAM labelled)

AACTCCATGAGCAGAGGCTCCAGCCTCAAGATCCTTTCCAAAGGAAAGCG**GGGTGGACACTCTTCC**
GTCTCCACGGAGTCAGAATCCTCCAGTTTTCACTCCAGCTAACACTTATGCAAAGACATATATAATAT
ATATATATATATATATGATAAAGAACTTTTTTATGTTA**CACATTTTCCAGATATAAGAGACTGACC**AG

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

Oligo CXCR4-CRE	5' label	Sequence 5' → 3'	3' label	Oligo Type
CXCR4-CRE-WT_F	n/a	**GGGTGGACACTCTTCCGTCT**	n/a	WT Forward
CXCR4-CRE-WT_PROBE	FAM	**CCACGGAGTCAGAATCCTCCAGTT**	BHQ	WT Probe
CXCR4-CRE-WT_R	n/a	**GGTCAGTCTCTTATATCTGGAAAATGTG**	n/a	WT Reverse

Cxcr4-Cre-ERT2-5'-MUT1 assay (FAM labelled)

AACTCCATGAGCAGAGGCTCCAGCCTCAAGATCCTTTCCAAAGGAAAGCGGGGGTGGACACTCTTC**C**
GTCTCCACGGAGTCAGAATCCAGCAGCTTTCACTCCAGCTAAgaattccgcccctctccctcccccccccctaac
gttactggccgaagccgcttggaataaggccggtgtgcgtttgtctatatgttattttccaccatattgccgtcttttggcaatgtgagg

Lower case letters denote the inserted sequence
Probe sequence is in bold and shaded grey
Primer sequences are in bold and underlined

Oligo CXCR4-CRE	5' label	Sequence 5' → 3'	3' label	Oligo Type
CXCR4-CRE-MUT_F	n/a	**CGTCTCCACGGAGTCAGAATC**	n/a	Mutant Forward
CXCR4-CRE-MUT_PROBE	FAM	**CAGCAGCTTTCACTCCAGCTAACC**	BHQ	Mutant Probe
CXCR4-CRE-MUT_R	n/a	**AGCGGCTTCGGCCAGTAAC**	n/a	Mutant Reverse

Dot1l internal control (VIC labelled)

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

Oligo CXCR4-CRE	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

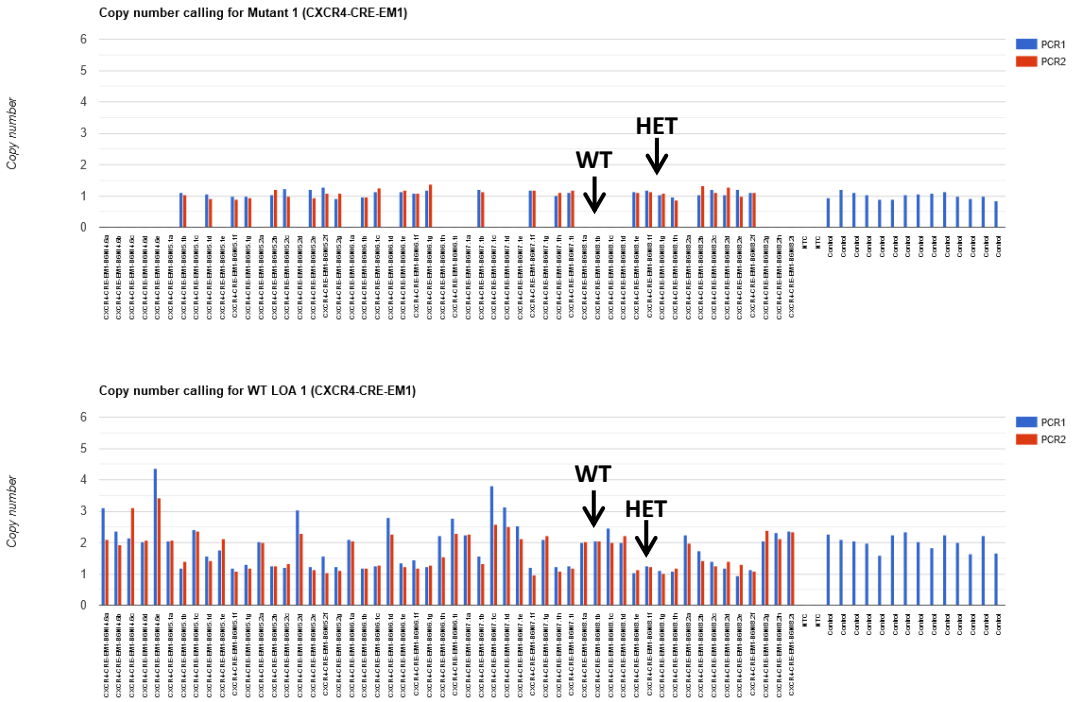
qPCR instrument: Applied Biosystems 7500/7900 or ThermoFisher QuantStudio 7

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.

CXCR4-CRE'-WT1 and CXCR4-CRE-MUT1 assays copy called results, image showing copy number chart for WT and Mutant assays (Task 335092 results)



Version No.	1
Date:	09/09/2021
Created/Updated by:	Daniel Ford
Approved by:	Rumana Zaman