

# Znrf3-MYCHIS

## Allele Description

This is a CRISPR/Cas9 induced knock-in of MycHis tag specific to transcript Znrf3-201. 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

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## Znrf3-MYCHIS-WT1 assay (FAM labelled)

TCAGGAAAGGAGCCATTGCTGCCCCCTTATCACACAGAGCCAAATCACCATTGTGAACTATCACATC  
CAAGGGCCCTATGGATGGGCTAGGGATGCTCAAGGACCTTACCTGATACGTACTACCCAAAGCCTG  
CTGTGCTGGCTCTCT**CCAGACCCTTGATGACTGTTCTCCCTCTTTCTCCAGGACCAGG**ATCTGGCCC  
AGGCATCGGTACGGG**AGCTTGAGCTCAAAAGGA**ACTCTTATGTAGAAATCGTGAAGTGTACGGAG  
ACTTCAAAGTCTGGCTTCCCTCAAACACTACTATACACACACACACACACACACACACACACACACA

Probe sequence is in bold and shaded grey

Primer sequences are in bold and underlined

Oligo Znrf3-MYCHIS	5' label	Sequence 5' → 3'	3' label	Oligo Type
Znrf3-MYCHIS-WT_F	n/a	<b><u>CCAGACCCTTGATGACTGTTCT</u></b>	n/a	WT Forward
Znrf3-MYCHIS-WT_PROBE	FAM	<b>TCCCTCTTTCTCCAGGACCAGG</b>	BHQ1	WT Probe
Znrf3-MYCHIS-WT_R	n/a	<b><u>AAGAGTTCCTTTGAGCTCAAGCT</u></b>	n/a	WT Reverse

## Znrf3-MYCHIS-MUT1 assay (FAM labelled)

CAAGGGCCCTATGGATGGGCTAGGGATGCTCAAGGACCTTACCTGATACGTACTACCCAAAGCCTG  
CTGTGCTGGCTCTCT**CCAGACCCTTGATGACTGTTCTCCCTCTTTCTCCAGGACCAGG**ATCTGGCCC  
AGGCATCGGTACGGGAGCTgaaCAAAACTCATCTCAGAAGAG**GATCTGaatagcgcctcga**cCACCAT  
CATCACCATCACTGAGCTCAAAGGAACTCTTATGTAGAAATCGTGAAGTGTACGGAGACTTCAAAG

KI sequence is in lower case

Probe sequence is in bold and shaded grey

Primer sequences are in bold and underlined

Oligo Znrf3-MYCHIS	5' label	Sequence 5' → 3'	3' label	Oligo Type
Znrf3-MYCHIS-MUT_F	n/a	<b><u>CCAGACCCTTGATGACTGTTCT</u></b>	n/a	MUT Forward
Znrf3-MYCHIS-MUT_PROBE	FAM	<b>TCCCTCTTTCTCCAGGACCAGG</b>	BHQ1	MUT Probe
Znrf3-MYCHIS-MUT_R	n/a	<b><u>TCGACGGCGCTATTCAGATC</u></b>	n/a	MUT Reverse

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## Dot1l internal control (VIC labelled)

CTGATGGGTGTGGGCAGATCCTACAGAGTCCCATTGGCCACCATGTGTGCTACGCCTGAAATAAAGCCTT**GCC**  
**CCAGCACGACCATT**CAGGG**CCAGCTCTCAAGTCG**ACTGTAA**GATGAAGCATAAGGATGCCAACT**ACTAACA  
GAAAACGACTAGAGGGGAAAAGAACAAGGAAACAGAAGACGCAGCACTCCGGCTTCCCTGGGTTGGCCAGT  
CACCTATGA

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

Oligo Znrf3-MYCHIS	5' label	Sequence 5' → 3'	3' label	Oligo Type
Dot1l_Forward	n/a	<b><u>GCCCCAGCACGACCATT</u></b>	n/a	WT Forward
Dot1l_Probe	VIC	<b>CCAGCTCTCAAGTCG</b>	BHQ	WT Probe
Dot1l_Reverse	n/a	<b><u>TAGTTGGCATCCTTATGCTTCATC</u></b>	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

