

Nnt (C57BL/6J)

Gene: Nnt ENSMUSG00000025453

Description

nicotinamide nucleotide transhydrogenase [Source:MGI Symbol;Acc:MGI:109279 [↗](#)]

Gene Synonyms

4930423F13Rik

Location

Chromosome 13: [119,335,448-119,408,997](#) reverse strand.
GRCm38:CM001006.2

About this gene

This gene has 5 transcripts ([splice variants](#)), [270 orthologues](#), [1 paralogue](#), is a member of [1 Ensembl protein family](#) and is associated with [6 phenotypes](#).

Transcripts

Hide transcript table

Show/hide columns (1 hidden)							
Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Nnt-202	ENSMUST00000099149.9	3464	835aa	<div><div></div>Protein coding</div>	CCDS84067	Q8BGK0	TSL:1 GENCODE basic APPRIS P2
Nnt-203	ENSMUST000000109204.7	3198	713aa	<div><div></div>Protein coding</div>	-	E9Q8F4	TSL:5 GENCODE basic
Nnt-201	ENSMUST00000069902.12	3122	721aa	<div><div></div>Protein coding</div>	-	Q8C9V5	TSL:1 GENCODE basic APPRIS ALT2
Nnt-204	ENSMUST000000133627.7	2340	No protein	<div><div></div>Retained intron</div>	-	-	TSL:1
Nnt-205	ENSMUST000000144599.1	1885	No protein	<div><div></div>Retained intron</div>	-	-	TSL:1

Summary

Name

[Nnt](#) (MGI Symbol)

CCDS

This gene is a member of the Mouse CCDS set: [CCDS84067.1](#)

Ensembl version

ENSMUSG00000025453.18

Gene type

Protein coding

Annotation method

Annotation for this gene includes both automatic annotation from Ensembl and Havana manual curation, see [article](#).

Alternative genes

This gene corresponds to the following database identifiers:

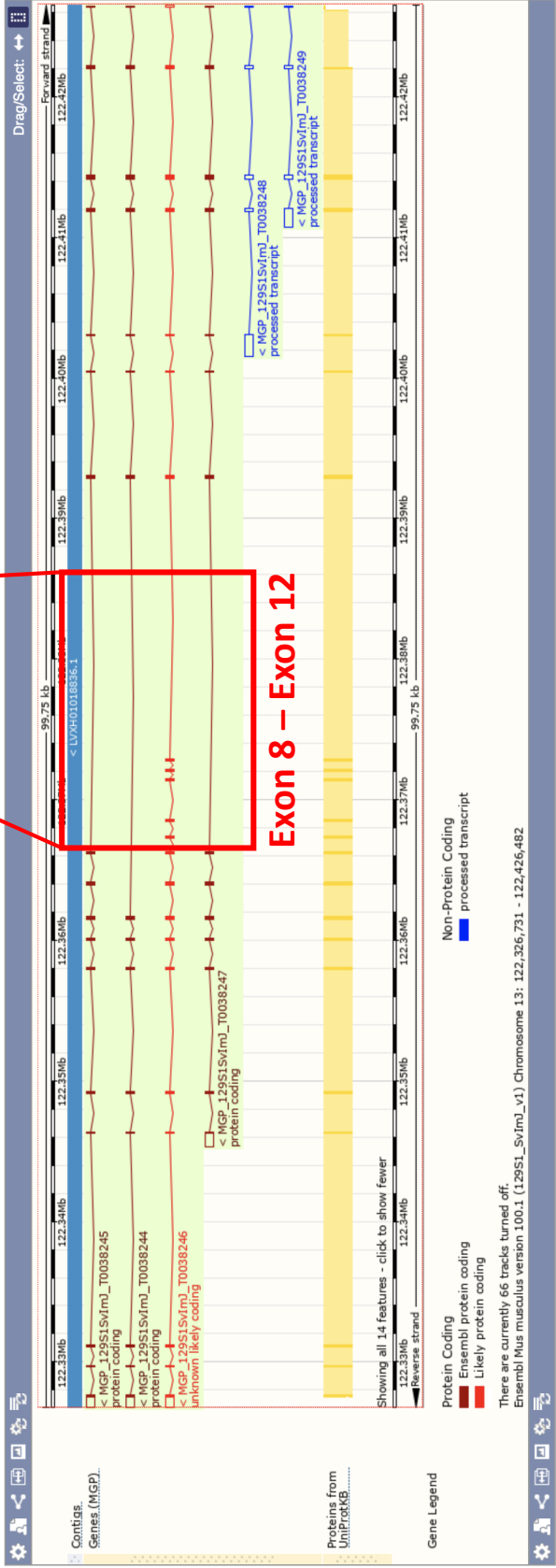
Havana gene: [OTTMUSG00000021068](#)

Nnt transcripts

C57BL/6J

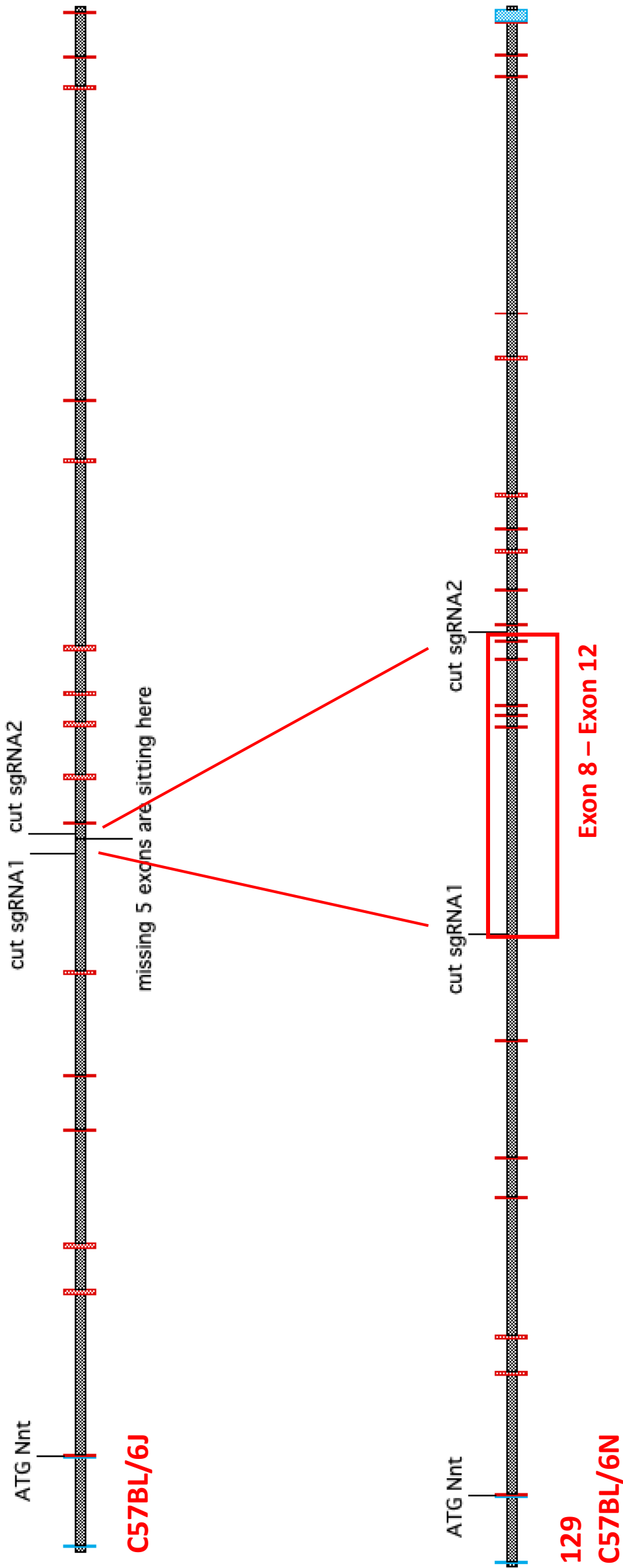


129
C57BL/6N



Exon 8 – Exon 12

Nnt gene structures



Positions of sgRNAs Nnt (shown on 6J genomic DNA)

1	ATAGTCACTT	TTATTCTTTA	AAGGCTATCT	AAAGAAAGCT	GGACAGAACA	CTCTGTTGGC	TTTTTTTTTT
71	TTTTTTTTAA	ATAGGATCAT	TGTAAAAGCT	AACTTAAGAC	TGGAAACTTA	GCTCTCTTAA	ATTATCCAAA
141	AACTAAAACA	CATGCCCCGT	CTGCAATTTG	AGGTGGCTAG	CTTGACCCTT	CAGGCACTGG	AATTAGGAAG
211	TAGTGGAGGA	TATTCAGGAA	TTGTAAAGTG	TCTTGTGGG	AAGGAAGATA	TTCCTCGATT	GTA AATTATA
281	ACATCTTAAT	CCCGGTTAGA	GAGCAGTATG	TTTTGTGTTT	ATCCTAGTTA	GGGTCTATAA	AATATCCCTG
351	TCACCGGACA	CTTAAATCCT	CCACAACCTGG	GGAAGAGTTG	GGAAGGAGGT	GTTTTCCTCT	GATGGGACTC
421	AGCCTAAGAT	GTTGCAGTCA	CATAGAGCCA	TAAATGATTC	TGCCCTCTGAT	GCTGCCCCATT	TTTTCTTGAA
491	TGTATCTTCA	TAGGATAGGA	TGACAGTCTT	GAAAAGATGG	CATTGCAGAC	AGGAGACTAG	TCCAGCGGTA
561	ACTGGCACTT	GCTGGTGGAA	TTCCGCTGAG	AGAACTCTTG	CTCTGGCAAC	TGTACAAAAG	CCAGAAGAGT
631	TTATCATTCA	GAGGGTTAGT	TTCTTCTCTG	TGCACATTAT	GATTTTAGAA	GTCAAAAC TA	CATTGTGTAG
701	GTCAGCAACT	CCCAATGTTT	TGGAAGTTGT	ACTTCTTTTT	TATTTTGAGT	GCTGATACG	TCCTTCATGG
771	TCCCCCTCCCT	TCCATTTAGT	TTATGACGCT	ATCGAATTCT	AAAATATTGC	TTCCACACGA	CCACTTATT C
missing 5 exons are sitting here							
841	TGAGAATTCT	GAAAGACAGC	AAGAAATGCT	TCGATGTTAC	AACACTGCTT	TGAGTGCAGA	ACCAATCCTT
911	CCATACCCCC	AGCCACACAG	TAATGTTCTG	CTAGAAGCTA	ACTGGGAAGT	CATGGAATGC	ATAGTTTTGG
981	ATAACATTGA	CTTCCACTGT	CACCATGACA	TTTGTA AATG	AGTACAAC TG	CAGATAAGAT	TTTAGTTTAT
1051	TGCCTTATCA	CAATGTTGTA	TGTTCCCTGAA	AACTGAAAGG	CTAGCACATC	ACTCATGTAT	GACACTCATC
1121	TGATGCTATT	TTATAGCTTA	AAATTCTTTA	GTGGGTCCAA	GGTAACTGTT	GATACAGTAT	GTGTTCTTTA
1191	TCATTTGCAA	CAGAGTTTGA	TAAACAGAGT	AACCTATCAG	AGAAAAAATG	CTGATTTT TG	ACCC

Guides to delete exon 8 – exon 12 of Nnt (in 6N strain)

Nnt sgRNA1

CATGCCCCCGT CTGCAATTG

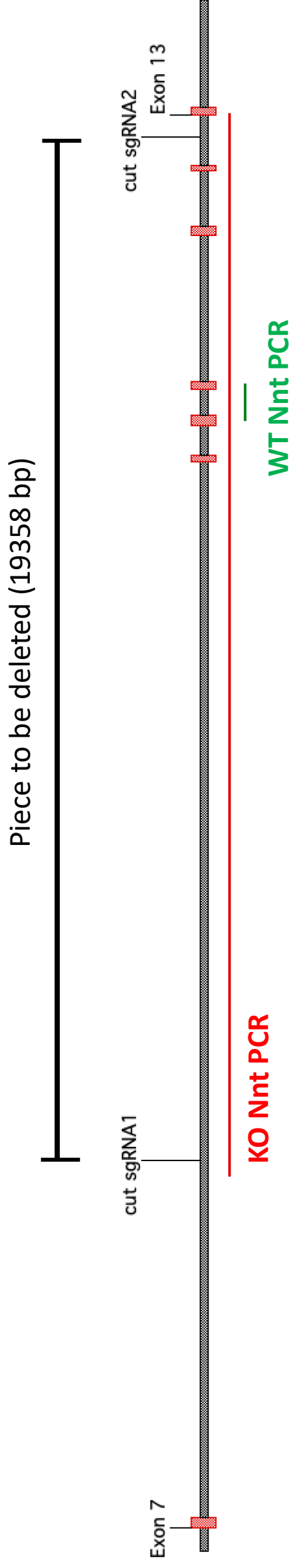
This guide cuts 6761 bp downstream of Exon 7 and 13210 bp upstream of Exon 8 of Nnt on C57BL/6N

Nnt sgRNA2

CAGTTACCTT GGACCCACTA

This guide cuts 527 bp downstream of Exon 12 of Nnt and 420 bp upstream of Exon 12 of Nnt on C57BL/6N

Nnt KO genotyping strategy



- | | |
|--------------------|------------------------------------|
| WT MICE: | WT PCR works / KO PCR doesn't work |
| HETEROZYGOUS MICE: | WT PCR works / KO PCR works |
| HOMOZYGOUS MICE: | WT PCR doesn't work / KO PCR works |

WT genotyping PCR Nnt

PCR = 702 bp

part of exon 9

```
1 AG GCC ATC AGC CCT GAC AAG GAC AAT TTT CAC TTT GAA GTG AAG GAT GAC TTT GAC TTT GGT
P1
63 ACA ATG AGT CAC GTC ATT CGA GGG ACC GTG ATG AAG GTAGAGGTCC CCCTTTTTTGT GTTCTTCCTG
132 TGACCCCTGGA CTTAGGACTT CTGTCCCTTGA AGAATTCCAT TATGGAATAG AGATGTGTTT GAATAAAGTA
202 TTGGCATGTC AGTGGAATTT GTGTTAAAT GGAGAGTGAC TTAAGGAAAG CATTTCAAAG ACTCAAGTAT
272 GTATGAGTCA TTGAAAACAC ACCTCACTTT TGAAAACCAG GGCAGAAAT TTAATAAAGT AAAAATGAAT
342 TGTTCCTCCC ATGTACATAC TTTTCAGGGT TTGAACAGTA TTCCTCTATA TGTGTCCCAG ACATACAAAA
412 ATTACATGTG TGCAGCACAA ACATTATTCA CTTATATACC CATTTTGTA ATTATATGAA TTTATATGCA
482 TTAAAATTAT AGTTACAGTT TTTTTCCTCAG CATGCACTCT CTTCTGTCAG TCATTTTGAA ATGCTAATTT
Exon 10
552 GACTTTAAAA AACATTATTT CCTAATATTG GTTAG GAT GGC AAA GTG ATT TTC CCA GCT CCC ACA CCA
620 AAA AAT ATT CCC GAA GAA GCC CCA GTC AAA CCG AAG ACC GTG GCT GAG CTG GAA GCC GAG AAA
683 GCA GGC ACC GTC TCC ATG TA
P2
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→ = genotyping oligo

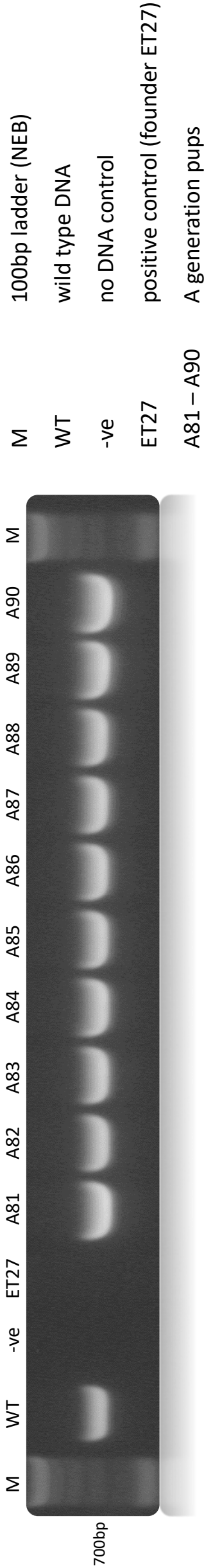
Red sequence shown is part of exon 9 and Exon 10 of Nnt, sequence in back is intronic sequence

Genotyping strategy - wild type PCR

Primers P1 (5’ AGGCCATCAGCCCTGACAAG 3’) and P2 (5’ TACATGGAGACGGTGCTGC 3’) will amplify a product of 702bp from the wild type allele. Sequence analysis was used to determine the exact nature of modification events.

The assay was performed using AccuStart II GelTrack PCR SuperMix (Quantabio)

Reagents		Cycling parameters			
2X reaction buffer	15.0µl	Denature	94°C	2min	x1 cycle
Forward primer (10µM)	1.2µl	Denature	94°C	20sec	x35 cycles
Reverse primer (10µM)	1.2µl	Annealing	64°C	20sec	
Template	2.0µl	Extension	72°C	60sec	
Water	10.6µl	Extension	72°C	5min	x1 cycle
Total	30.0µl				



KO genotyping for Nnt

PCR = around 764 bp

(WT PCR band with these oligos is 20122 bp, this PCR won't work)

1

ACAGGCTAAT

CTGGGTGAGG

CACTACCTCA

TCTGAGATT

CCTCGTACTT

CAAACACAGT

AGTGGCTCAG

71

CAAAATATTGT

TCAACTCATA

ATCCTGTA

TGTTCCCTTAG

GTCTTGGTTG

GCATAGTCAC

TTTTATTCTT

141

TAAAGGCTAT

CTAAGAAAG

CTGGACAGAA

CACTCTGTTG

GCCTTTTTTT

TTTTTTTTTT

TTAAATAGGA

211

TCATTGTAGA

AGCTAACTTA

AGACTGGAAA

CTTAGCTCTC

TTAAATTATC

CAAAAACATA

AACACATGCC

281

CCGTCTGCAA

TTGGGTCCAA

GGTAACTGTT

GATACAGTAT

GTGTTCTTTA

TCATTTGCAA

CAGAGTTTGA

351

TAAACAGAGT

AACCTATCAG

AGAAAAAATG

CTGATTTTGT

ACCTTTTGTA

TTTCAAGCC

TTTAAATGGC

421

TATTTACTTT

TAGTCATGCA

GAAACAGTTG

GCCCTACGTT

CTGATAGCAA

GTTTTCTTCC

TAACTGTATC

491

TAGGGTTCCC

CGTATACTTC

ATCCACCCTTA

AGGGAACCTG

CTTTAATAAT

AGAGTGGCTT

TTGCTTTTGA

561

GGTTCAAACT

GTTACTTGAA

ATAATAGCAT

CACCTTGTA

TACTTAGTGA

TAAAGTATA

CTTAAAATAA

631

ATATGCTGTA

GCAGACGGAA

AAAAGTCATG

CTGTCTGACG

TGACACCTCT

TGCCGTGTTG

TGCTTCTCTT

701

GCTTCGGTAA

GGT GGT TTT CTG GTG ACT CAG AGA ATG CTG GAC ATG TTC AAG CGA CCC ACG GAC

Exon 13

P3

P4

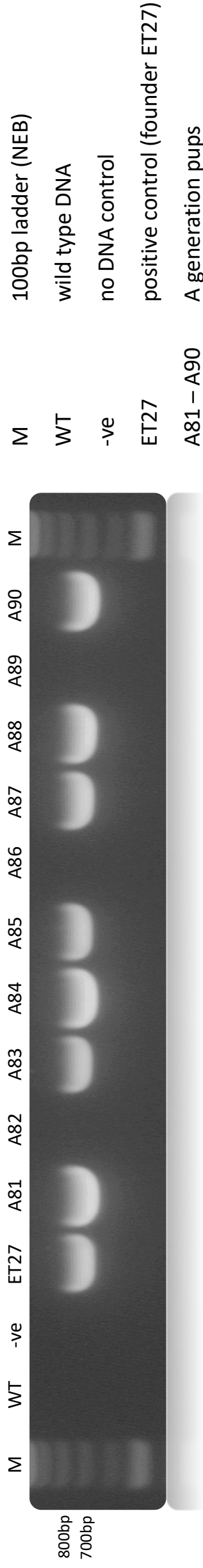
= genotyping oligo

Genotyping strategy - knockout PCR

Primers P3 (5' ACAGGCTAATCTGGTGAGGC 3') and P4 (5' GTCCGTGGTCTGCTGAACA 3') will amplify a product of 764bp from the correctly modified allele. Sequence analysis was used to determine the exact nature of modification events.

The assay was performed using AccuStart II GelTrack PCR SuperMix (Quantabio)

Reagents		Cycling parameters		
2X reaction buffer	15.0µl	Denature	94°C	2min
Forward primer (10µM)	1.2µl	Denature	94°C	20sec
Reverse primer (10µM)	1.2µl	Annealing	64°C	20sec
Template	2.0µl	Extension	72°C	60sec
Water	10.6µl	Extension	72°C	5min
Total	30.0µl			



Genotyping strategy

Primer P4 (5' GTCCGTGGTCTGCTGAACA 3') is used to sequence the knock out PCR product amplified using primers P3 and P4

Sequencing chromatogram for animal ET27

