

Supplemental File 1A. Primer sequences

Sequences of primers used in this study for cloning, genotyping, mutagenesis, and RT-PCR. Where relevant, gene specific sequences are underlined.

Primer name	Purpose	Sequence
Gateway primers		
attB1F-comta	pDONR221-comta(NS)	GGGGACAAGTTTGTACAAAAAAGCAGGCTgcaaacATGCTGTGGGTTGTGTTGGC
attB1F-HA-GGSG-tomt_45-259	pDONR221-HA-tomt_45-259(NS)	GGGGACAAGTTTGTACAAAAAAGCAGGCTgcaaacATGTACCCATACGATGTTCCAGATTACGCT gggtggtctggtAGTCGAGAGGGAACGGGCC
attB1F-mgat1a_1-110	pDONR221-mgat1a_1-110(NS)	GGGGACAAGTTTGTACAAAAAAGCAGGCTgcaaacATGCTCCGCAAGAGAAGTCCTC
attB1F-Mmu.Tomt	pDONR221-Mmu.Tomt(NS)	GGGGACAAGTTTGTACAAAAAAGCAGGCTgcaaacATGTCCCCTGCCATTGC
attB1F-tmie	pDONR221-tmie-HA	GGGGACAAGTTTGTACAAAAAAGCAGGCTgtaaacATGAGACGCGGGAGAAGAAG
attB1F-tomt	pDONR221-tomt(NS); pDONR221-tomt-HA; pDONR221-tomt_1-45(NS)	GGGGACAAGTTTGTACAAAAAAGCAGGCTAAACATGGTATCTCCTGCGATCGC
attB2F-lhfp15a	pDONR P2R-P3-lhfp15a	GGGGACAGCTTCTTGTACAAAGTGGCGATGGCGAAAATGCTATCTGCC
attB2R-comta	pDONR221-comta(NS)	GGGGACCACCTTTGTACAAGAAAGCTGGGTcTCCTAAGAAGACCGATTTCTCCAG
attB2R-HA+st	pDONR221-tmie-HA; pDONR221-tomt-HA	GGGGACCACCTTTGTACAAGAAAGCTGGGTcttaAGCGTAATCTGGAACATCGTATGG
attB2R-mgat1a_1-110	pDONR221-mgat1a_1-110(NS)	GGGGACCACCTTTGTACAAGAAAGCTGGGTcCGGAGCTGTGTGTTCAACTTCTG
attB2R-Mmu.Tomt	pDONR221-Mmu.Tomt(NS)	GGGGACCACCTTTGTACAAGAAAGCTGGGTcGCCGGTCCAGTATAGGTG
attB2R-tomt	pDONR221-tomt(NS); pDONR221-HA-tomt_45-259(NS)	GGGGACCACCTTTGTACAAGAAAGCTGGGTcCCTTGATGCCGATGTAAGTGAGC
attb2r-tomt_1-45	pDONR221-tomt_1-45(NS)	GGGGACCACCTTTGTACAAGAAAGCTGGGTcACTGATGCCCGATAAACAGTCC
attB3R-lhfp15a	pDONR P2R-P3-lhfp15a	GGGGACAACCTTTGTATAATAAAGTTGGTCATGCTTCCTCTTCTTCTC
Cloning and RT-PCR		
lrrc51_ORF-F	RT-PCR for lrrc51	CATGTTCCGGATCTTCAGTGGAC
lrrc51_ORF-R	RT-PCR for lrrc51	TCAGTCTGATTTGCTGTAACTGTG
Mmu.Tomt_H183A-F	Site directed mutagenesis to make pDONR221-Mmu.Tomt_H183A	CCGAGGTCGGGCTGCCAGGAGCACCAGATCT
Mmu.Tomt_H183A-R	Site directed mutagenesis to make pDONR221-Mmu.Tomt_H183A	AGATCTGGTGCTCCTGGCAGCCCGACCTCGG
pcdh15a ^{th263b} -F	genotyping pcdh15a ^{th263b}	AGGGACTAAGCCGAAGGAAG
pcdh15a ^{th263b} -R	genotyping pcdh15a ^{th263b}	TCCGGCTGTA AACATCAGC
tmc2b ^{sa8817} -F	genotyping tmc2b ^{sa8817}	CAAGTTATGAAATCATTAAAGTCACTCTG
tmc2b ^{sa8817} -R	genotyping tmc2b ^{sa8817}	AAGAACTTAAACAGTGAACATAATGTTGTA
tmie-F	clone tmie ORF	gtaaacATGAGACGCGGGAGAAGAAG
tmie-HA-R	clone tmie ORF C-terminally tagged with an HA epitope (lowercase)	agcgtaatctggaacatcgatgggtaacctccaccTTTCTTCGAGGCTTCTTGGC
tmie-R	clone tmie ORF	TCATTTCTTCGAGGCTTCTTG
tomt_exon1-F	genotyping tomt ^{tk256c} and tomt ^{nl16} alleles	TGTGTATTGCAGGTCAGTGTG
tomt_exon1-R	genotyping tomt ^{tk256c} and tomt ^{nl16} alleles	AAGCGTTTTTCTGGGTGTTG
tomt_ORF-F	RT-PCR for tomt	GTGTATTGCAGGTCAGTGTGGAAG
tomt_ORF-R	RT-PCR for tomt	GCCATATAAATGTGAGCAATGATG
tomt-HA-R	clone tomt ORF C-terminally tagged with an HA epitope (lowercase)	agcgtaatctggaacatcgatgggtaacctccaccCTTGATGCCGATGTAAGTGAGC
NheI-kozak-Mm-Tomt	clone mouse Tomt into pcDNA3.1+	cacaGCTAGCgccaccATGTCCCCTGCCATTGC
XhoI-Mm-Tomt-HA	clone mouse Tomt-HA into pcDNA3.1+	cacaCTCGAGtcaagcgtaatctggaacatcgatgggtaacctccaccGCCGGTCCAGTATAGGTGAG
NheI-kozak-Mm_Comt	clone mouse Comt into pcDNA3.1+	cacaGCTAGCgccaccATGCTGTTGGCTGCTGTCT
XhoI-Mm-Comt-HA	clone mouse Comt-HA into pcDNA3.1+	cacaCTCGAGtcaagcgtaatctggaacatcgatgggtaacctccaccGGACTTCACGGGGCTG

Supplemental File 1B. Expression constructs generated for this study

Gateway entry vectors

pDONR221-tomt(NS)
pDONR221-tomt-HA
pDONR221-tomt_1-45(NS)
pDONR221-HA-tomt_45-259(NS)
pDONR221-mgat1a_1-110(NS)
pDONR221-comta(NS)
pDONR221-Mmu.Tomt(NS)
pDONR221-Mmu.Tomt_H183A(NS)
pDONR221-tmie-HA
pDONR221-tmc2b-GFP
pDONR221-tmc1-GFP
pDONR P2R-P3-lhfp15a

Expression constructs

pDESTCG2-myo6b:tomt-GFP
pDESTCG2-hsp70l:tomt-emGFP
pDESTCG2-myo6b:tomt-HA-pA
pDESTCG2-myo6b:Mmu.Tomt-emGFP
pDESTCG2-myo6b:Mmu.Tomt_H183A-emGFP
pDESTCG2-myo6b:comta-emGFP
pDESTCG2-myo6b:tomt_1-45-emGFP
pDESTCG2-myo6b:mgat1a_1-110-mKate2
pDESTCG2-myo6b:GFP-lhfp15a
pDESTCG2-myo6b:tmie-HA-pA
pDESTCG2-myo6b:tmc2b-GFP-pA
pDESTCR-myo6b:tmc1-GFP-pA
pDESTCR-myo6b:tomt-p2a-nls-mCherry
pDESTCG2-myo6b:HA-tomt_45-259-emGFP
pDESTCG2-myo6b:mKate2-KDEL-pA
pcDNA3.1+Tomt-HA
pcDNA3.1+Tomt(H183A)-HA
pcDNA3.1+Comt-HA

Supplemental File 1C. Stable transgenic lines generated for this study

Transgenic Fish Lines
Tg(hsp70l:tomt-emGFP)
Tg(myo6b:comta-emGFP)
Tg(myo6b:GFP-lhfpl5a)
Tg(myo6b:mgat1a_1-110-mKate2)
Tg(myo6b:Mmu.Tomt_H183A-emGFP)
Tg(myo6b:Mmu.Tomt-emGFP)
Tg(myo6b:tmc1-GFP-pA)
Tg(myo6b:tmc2b-GFP-pA)
Tg(myo6b:tmie-HA-pA)
Tg(myo6b:tomt_1-45-emGFP)
Tg(myo6b:tomt-GFP)
Tg(myo6b:tomt-HA-pA)*

Single copy of transgene unless noted with an *