

Table S1: Primers generated for SOE cloning of HA-hMC4R point mutants and polymorphisms

Primer	Purpose	Nucleotide sequence
HA-hMC4RForR1	Binding pcDNA3.1 multiple cloning site upstream of the HA-epitope (NheI, PmeI AflIII HindIII section of plasmid)	5' gctagcgtttaacttaagc
HA-hMC4RRevR2	Binding pcDNA3.1 multiple cloning site downstream of the HA-epitope (XhoI, XbaI Apa section of plasmid)	5' ggccctctgactcgagtta
HA-hMC4RForR3	Amplification of full length mutant HA-hMC4R (Asp7181 and KpnI section of plasmid)	5' cttgtaccaccatgtacc
HA-hMC4RRevR3	Amplification of full length mutant HA-hMC4R (3' HA-hMC4R and XhoI section of plasmid)	5' gactcgagttaatatctgc
HA-hMC4RR7HForR2	Introducing CGT codon change to CAT (His)	5' aactccaccaccatgggatgcacacttc
HA-hMC4RR7HRevR1		5' gaagtgtgcatccatgggtgggtggagtt
HA-hMC4RR18LForR2	Introducing CGC codon change to CTC (Leu)	5' gcacctctggaacctcagcagttacagac
HA-hMC4RR18LRevR1		5' gtctgtaactgctgaggtccagagggtgc
HA-hMC4RH76RForR2	Introducing CAT codon change to CGT (Arg)	5' gaacaagaatctgcgttcacccatgtac
HA-hMC4RH76RRevR1		5' gtacatgggtgaacgcagattcttgttc
HA-hMC4RD90NForR2	Introducing GAT codon change to AAT (Asn)	5' cttggctgtggctaataatgctggtagcg
HA-hMC4RD90NRevR1		5' cgctcaccagcatattagccacagccaag
HA-hMC4RV103IForR2	Introducing GTC codon change to ATC (Ile)	5' gaaaccattatcatcaccta
HA-hMC4RV103IRevR1		5' tagggtgatgataatggtttc
HA-hMC4RD146NForR2	Introducing GAC codon change to AAC (Asn)	5' cctgcttcaattgcagtgaaacaggtac
HA-hMC4RD146NRevR1		5' gtacctgttctactgcaattgaaagcagg
HA-hMC4RT150IForR2	Introducing ACT codon change to ATT (Ile)	5' gcagtgagcaggtactttattatcttctatgctctc
HA-hMC4RT150IRevR1		5' gagagcatagaagataataaagtacctgttcactgc
HA-hMC4RA154DForR2	Introducing GCT codon change to GAT (Asp)	5' ctatcttctatgatctccagttacc
HA-hMC4RA154DRevR1		5' ggtactggagatcatagaagatag
HA-hMC4RH158RForR2	Introducing CAT codon change to CGT (Arg)	5' gctctccagtaccgtaacattatgacag
HA-hMC4RH158RRevR1		5' ctgtcataatgttacggtactggagagc
HA-hMC4RP230LForR2	Introducing CCC codon change to CTC (Leu)	5' aagaggattgctgtcctcctggcactgggtg
HA-hMC4RP230LRevR1		5' caccagtgccgaggaggacagcaatcctctt
HA-hMC4RL250QForR2	Introducing CTG codon change to CAG (Gln)	5' ccttgaccatccagattggcgtctttg
HA-hMC4RL250QRevR1		5' caaagacccaatctggatgggtcaagg
HA-hMC4RI251LForR2	Introducing ATT codon change to CTG (Leu)	5' accatctgcttggcgtcttt
HA-hMC4RI251LRevR1		5' aaagacccaagcaggatgggt
HA-hMC4RF280LForR2	Introducing TTC codon change to TTG (Leu)	5' ccatattgtgtgtcttgatgtctcac
HA-hMC4RF280LRevR1		5' gtgagacatcaagcacacacaatatgg
HA-hMC4RS295PForR2	Introducing TCA codon change to CCA (Pro)	5' gtgtaatccaatcatcgatcctctg
HA-hMC4RS295PRevR1		5' cagaggatcgatgattggattacac
HA-hMC4RR305SForR2	Introducing CGG codon change to AGC (Ser)	5' gatttatgactcagcagtcagaactgagg
HA-hMC4RR305SRevR1		5' cctcagttctgactgctgagtgataaatc
HA-hMC4RS127LForR2	Introducing TCG codon change to TTG (Leu)	5' atgtcattgacttggatctgtagctcc
HA-hMC4RS127LRevR1		5' ggagctacagatcaccaagtcaatgacat