

ENSE0...

SOD1-201

Donor Template WT -> SNV

gRNA Protospacer

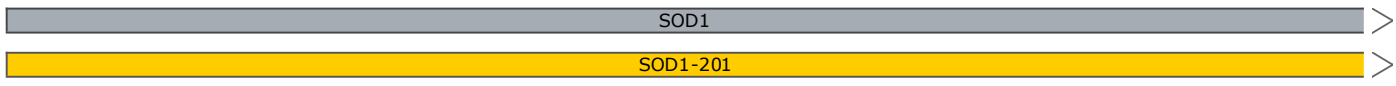
SNV

CZK2J00160_SOD1_D91A_C05_BB
1395 bp

5'
3'

CCACCTTTGCTTTTGAACCTGCTGACTCATCTAAACCCCTGCTCCCAAATGCTGGAATGCTTTTACTTCCTGGGCTTAAAGGAAT
GGTGGAAACGAAAACCTGAACGACTGAGTAGATTTGGGGACGAGGGTTACGACCTTACGAAAATGAAGGACCCGAATTTCTTA

85

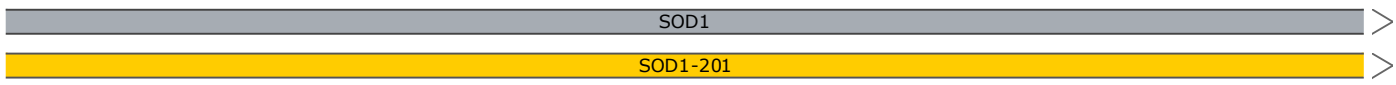


PCR Forward

tacaagtgccaaaggggaactaata

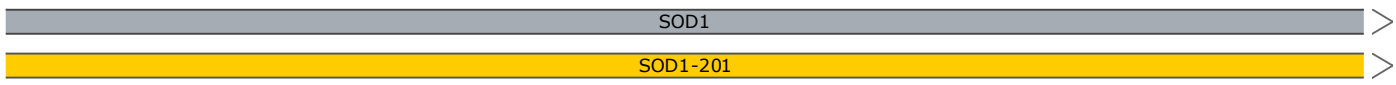
TGACAAATGGGGACACTTAAAACGATTTGGTTTTGTAGCATTTATTGAATATAGAACTAATACAAGTGCCAAAGGGGAACATA
ACTGTTTACCCCTGTGAATTTGCTAAACCAAACATCGTAAATAACTTATATCTTGATTATGTTTCACGGTTTCCCCTTGATTAT

170



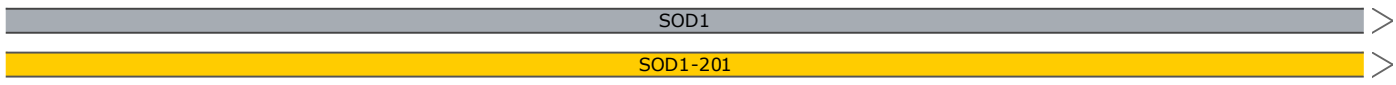
CAGGAAATGTCATGAACAGTACTGTCAACCACTAGCAAAATCAATCATCATTGTGAAACATAGGAAGCTTCTGTAGATAAAAAA
GTCCTTTACAGTACTTGTGATGACAGTTGGTGATCGTTTTAGTTAGTAGTAACACTTTGTATCCTTCGAAGACATCTATTTTTT

255



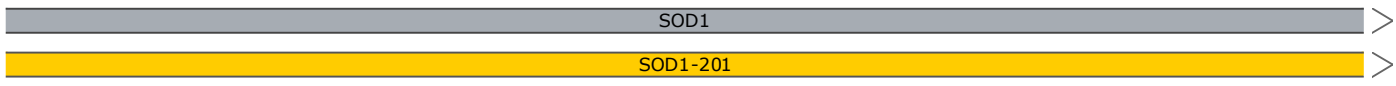
AAAATTGATACTGAAAACCTAGTCGAGACTCCATTTATATGTGTATGTTTTCTGAAAGCCTTTCAGAAAAATATTAATTTAAGGA
TTTTAACTATGACTTTTGATCAGCTCTGAGGTAATATACACATACAAAAGACTTTCGGAAAGTCTTTTTATAATTTAAATTCCT

340



CAAGATTTTTATATCAGAGGCCCTTGGGACATAGCTTTGTTAGCTATGCCAGTAATTAACAGGCATAACTCAGTAACTGAGAGTT
GTTCTAAAAATATAGTCTCCGGAACCCTGTATCGAAACAATCGATACGGTCATTAATTGTCGGTATTGAGTCATTGACTCTCAA

425

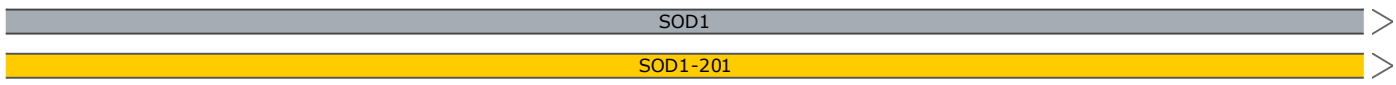


Sanger Sequencing Primer

ccccatctttcttcccagag

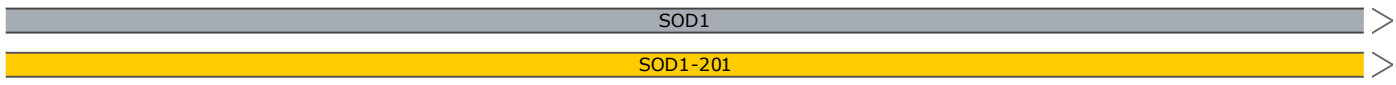
ACCCTTTGGTACTTCTGAAATCAGGTGCAGCCCATCTTTCTCCAGAGCATTAGTGTGTAGACGTGAAGCCTTGTGTTGAAGAG
TGGGAAACCATGAAGACTTTAGTCCACGTCGGGGTAGAAAGAAGGGTCTCGTAATCACACATCTGCACTTCGGAACAAACTTCTC

510



CTGTATTTAGAATGCCTAGCTACTTGTGTTGCAAATTTGTGTCTACTCAGTCAAGTTTTAATTTAGCTCATGAACTACCTTGATGT
GACATAAATCTTACGGATCGATGAACAAACGTTTAAACACAGATGAGTCAGTTCAAATTAATCGAGTACTTGATGGAACACTACA

595



gRNA Protospacer

GACTGCTGACAAAGAT

TTAGTGGCATCAGCCCTAATCCATCTGATGCTTTTTTCATTATTAGGCATGTTGGAGACTTGGGCAATGTGACTGCTGACAAAGAT
AATCACCGTAGTCGGGATTAGGTAGACTACGAAAAAGTAATAATCCGTACAACCTCTGAACCCGTTACACTGACGACTGTTTCTA

680

SOD1

SOD1-201

H V G D L G N V T A D K D

ENSE00003555033

SOD1-201

Donor Template WT -> SNV

gRNA Protospacer

SNV

ACCTCTGAACCCGTTACACTGACGACGTTTCTA

Donor Template WT -> SNV

gRNA Protospacer

GGTG

GGTGTGGCCGATGTGTCTATTGAAGATTCTGTGATCTCACTCTCAGGAGACCATTGCATCATTGGCCGCACACTGGTGGTAAGTT
CCACACCGGCTACACAGATAACTTCTAAGACACTAGAGTGAGAGTCCTCTGGTAACGTAGTAACCGGCGTGTGACCACCATTCAA

765

SOD1

SOD1-201

G V A D V S I E D S V I S L S G D H C I I G R T L V V S

ENSE00003555033

SOD1-201

Donor Template WT -> SNV

PAM

gRNA Protospacer

CCACACCGGCTACACAGATAACTTCTAAGACACTAGAGTGAGAGTCCTCTGGTAACGTAGTAACCG

Donor Template WT -> SNV

TTCATAAAAGGATATGCATAAACTTCTTCTAACATACAGTCATGTATCTTTTCACTTTGATTGTTAGTCGCGGTTTCTAAAGAT
AAGTATTTTCTATACGTATTTGAAGAAGATTGTATGTCAGTACATAGAAAAGTGAACCTAACAATCAGCGCCAAAGATTCTA

850

SOD1

SOD1-201

F H K R I C I K L L L T Y S H V S F H F D C *
(in frame with SOD1-201)

CCAGATAAACTGTACTTGCAGTTCAAATTAGGAAAAGCAATTTTATTGGACAATTACGGTGAAAATGAATTATTTTATCTAGGTC
GGTCTATTTGACATGAACGTCAAGTTAATCCTTTTCGTTAAAATAACCTGTTAATGCCACTTTTACTTAATAAAAATAGATCCAG

935

SOD1

SOD1-201

AGTTAAGAACACTGTTCTGCTAAGATGCAGTAAAAAGCAGGTTACATTTGACCATATTAGATCTGAGTTTGGAAAACAGAAGTAG
TCAATTCCTGTGACAAGACGATTCTACGTCATTTTCGTCCAATGTAACTGGTATAATCTAGACTCAAACCTTTTGTCTTCATC

1020

SOD1

SOD1-201

TCTTTAGTTTTAAAATGGCCAGATTTTCTTGCCAGGATTGGGTTTCTCACTTGTTAAACAGAACATTTTGTAAAGTTTAAAACCT
AGAAATCAAATTTTACCGGTCTAAAAGAACGGTCCTAACCCAAAGAGTGAACAATTTGTCTTGTTAAAACAATTCAAATTTTGG

1105

SOD1

SOD1-201

GGGATGGACTTAAGTATTCATGTTTCATTCATGTTTCATTCAGGACTGCAGGTTATCATGACTTGTTTAACTTGTGGGAAGCTGTTG
CCCTACCTGAATTCATAAGTACAAGTAAGTACAAGTAAGTCCTGACGTCCAATAGTACTGAACAAATTGAACACCCTTCGACAAC

1190

SOD1

SOD1-201

TCCCAAGTTATCCTGGGGAAGTGCATCTGGTTCTTGCAAAACACCAAGTAGACAGGCTCTCTTTTACCTCCCCTTGAGGGCATT
AGGGTTCAATAGGACCCCTTGACGTAGACCAAGAAGCTTTTGTGGTTCATCTGTCCGAGAGAAAATGGAGGGGAAGTCCCCTAAT

1275

SOD1

SOD1-201

gttcatctgtccgagagaaaatgga

PCR Reverse

ACATTCAGTAGTCACTTCCATTCAGTTAACCCCTTTATTTTTATGGTTTTTCTTGAGCCATAGTTGTAAAGCAGAAAAATCATT
TGTAAGTCATCAGTGAAGGTAAGTCAATTGGGAAATAAAAATACAAAAAGAAGTCCGGTATCAACATTTTCGTCTTTTTAGTAAAT

1360

SOD1

SOD1-201

TAAAGGTTTGTGTAACAAAATTCAAAATACTGTTG
ATTTCAAACAACCTGTTTTAAGTTTTATGACAAC

3'

1395

5'

SOD1

SOD1-201

Feature	Location	Size	Start	End	Type
✓ SOD1	1 .. 1395	1395 bp	■	→	gene
/note	= gene ENSG00000142168 Protein coding				
✓ SOD1-201	1 .. 1395	1395 bp	■	→	prim_transcript
/note	= primary transcript ENST00000270142				
SOD1-202	1 .. 1395	1395 bp	■	→	prim_transcript
/note	= primary transcript ENST00000389995				
SOD1-203	1 .. 1395	1395 bp	■	→	prim_transcript
/note	= primary transcript ENST00000470944 protein_coding_CDS_not_defined				
SOD1-204	1 .. 724	724 bp	■	→	prim_transcript
/note	= primary transcript ENST00000476106 protein_coding_CDS_not_defined				
	111 .. 630	520 bp	■	←	gene
/note	= gene ENSG00000273271 lncRNA				
	111 .. 630	520 bp	■	←	prim_transcript
/note	= primary transcript ENST00000609934 lncRNA				
✓ SOD1-201	641 .. 758	118 bp	■	→	CDS
/codon_start	= 1				
/note	= coding sequence ENSP00000270142				
/translation	= HVGDLGNVTADKDGADVSIEDSVISLSGDHCCIIGRTL 39 amino acids = 4.0 kDa				
SOD1-202	641 .. 758	118 bp	■	→	CDS
/codon_start	= 1				
/note	= coding sequence ENSP00000374645				
/translation	= HVGDLGNVTADKDGADVSIEDSVISLSGDHCCIIGRTL 39 amino acids = 4.0 kDa				
✓ Donor Template WT -> SNV	647 .. 746	100 bp	■		misc_feature
✓ gRNA Protospacer	665 .. 684	20 bp	■		misc_feature
✓ SNV	673 .. 673	1 bp	■		misc_feature
/note	= WT = A SNV = C				
✓ PAM	685 .. 687	3 bp	■		misc_feature

Primer	Length	Binding Sites	Tm	Date Added
✓ PCR Forward /sequence = tacaagtgccaaaggggaactaata 40% GC / 7732.1 Da	25-mer	146 .. 170 →	56°C	Nov 7, 2023
✓ Sanger Sequencing Primer /sequence = ccccatctttcttcccagag 55% GC / 5963.9 Da	20-mer	456 .. 475 →	56°C	Nov 7, 2023
✓ Donor Template WT -> SNV /sequence = GCCAATGATGCAATGGTCTCCTGAGAGTGAGATCACAGAATCTTCAATAGACACATCGGCCACACCATCTTTGGCAGCAGTCACATTGCCCAAGTCTCCA 49% GC / 30,684.0 Da	100-mer	647 .. 746 ←	76°C	Nov 7, 2023
✓ gRNA Protospacer /sequence = GACTGCTGACAAAGATGGTG 50% GC / 6206.1 Da	20-mer	665 .. 684 →	57°C	Nov 7, 2023
✓ PCR Reverse /sequence = aggtaaaagagagcctgtctacttg 44% GC / 7730.1 Da	25-mer	1235 .. 1259 ←	58°C	Nov 7, 2023