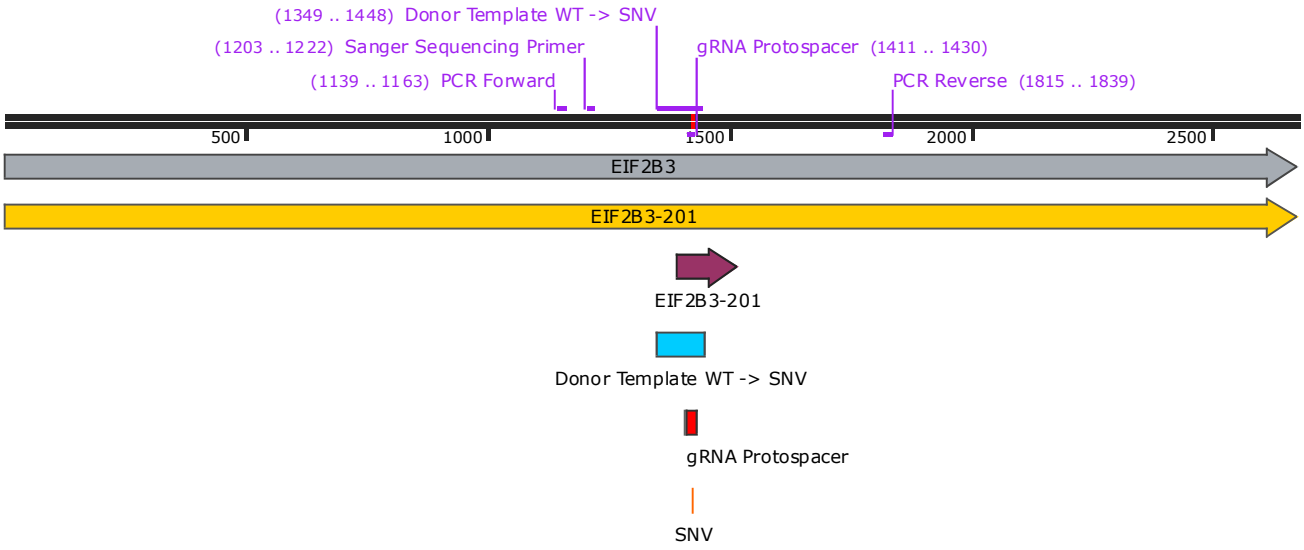

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2673 bp


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85
3′
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EIF2B3
EIF2B3-201
TGAGGCAGGAGAATCACTTGAACTGGGAGGTGGAGGTTGCGATAAGCCAAGATCGCACCGCTACACTCCAGCCTGGGCCACAGAG
170
ACTCCGTCCTCTTAGTGAACTTGACCCTCCACCTCCAACGCTATTCGGTTCTAGCGTGGCGATGTGAGGTCGGACCCGGTGTCTC
EIF2B3
EIF2B3-201
CGAGACTCCATCTCAAAAAAAAAAAAAAAAAAAAGAAAAAGAAAAAAAAAGTCAGGCACAGTGGCTCATGCCTGTAATCCCAGCACT
255
GCTCTGAGGTAGAGTTTTTTTTTTTTTTTTTTTCTTTTTCTTTTTTTTTCAGTCCGTGTCACCGAGTACGGACATTAGGGTCGTGA
EIF2B3
EIF2B3-201
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340
AATTCTCCGACGTCGTCCGCCTAGTGAACTCCGGTCCTCAAACTCTGGTCGAACCGGTTGTACCGTTTTGGGGTAGGGATGGTTT
EIF2B3
EIF2B3-201
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425
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EIF2B3
EIF2B3-201
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510
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EIF2B3
EIF2B3-201
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595
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EIF2B3
EIF2B3-201
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680
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EIF2B3
EIF2B3-201
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765
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EIF2B3
EIF2B3-201
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850
CTCAACTAATCCAGTCTAAAGAAAGTGACAGTATTAAAACAGTGACAATAAAAACGTTTCCACCAAAGTGGTGTTTTTCGAAAGC
EIF2B3
EIF2B3-201


TGATCTGGCCCCCTTGCCTACCTGATTAGCTTAACCTTCTGCTTTTCCTCTTAAACTCATACAGGCTACTGTCCATTGGATTATG
935
ACTAGACCGGGGGAACGGATGGACTAATCGAATTGGAAGACGAAAAGGAGAATTTGAGTATGTCCGATGACAGGTAACCTAATAC
EIF2B3
EIF2B3-201
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1020
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EIF2B3
EIF2B3-201
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1105
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EIF2B3
EIF2B3-201
PCR Forward
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1190
TTCATATTTATTTTTGTATTTATTTTTTGTGTGTGACTTGATTTCTAGTCCAGGGAAGAAGGGGATCATGTCTAAGGACTAGTGA
EIF2B3
EIF2B3-201
Sanger Sequencing Primer
CCTGTCTCAGTACCCACCAG
TAGACCCAACATCCTGTCTCAGTACCCACCAGGAAGCACTCTGCTTCTCCGAACTGCCAAGTCAATGCCACATTTGATCTCTGAC
1275
ATCTGGGTTGTAGGACAGAGTCATGGGTGGTCCTTCGTGAGACGAAGAGGCTTGACGGTTCAGTTACGGTGTAAACTAGAGACTG
EIF2B3
EIF2B3-201
Donor Template WT -> SNV
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1360
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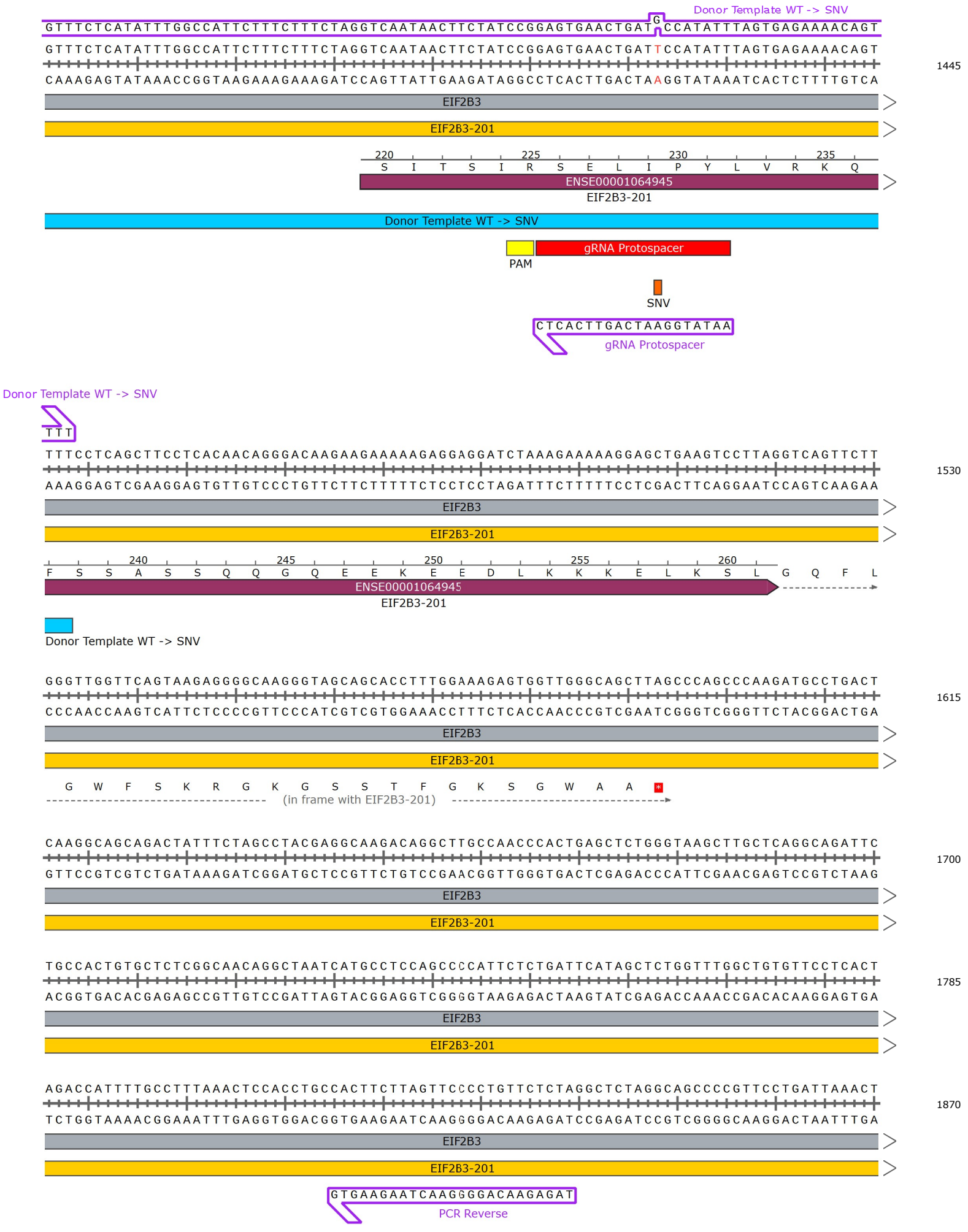
EIF2B3
EIF2B3-201
Donor Template WT -> SNV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1445
EIF2B3
EIF2B3-201
220 225 230 235
S I T S I R S E L I P Y L V R K Q
ENSE00001064945
EIF2B3-201
Donor Template WT -> SNV
gRNA Protospacer
PAM
SNV
CTCACTTGACTAAGGTATAA
gRNA Protospacer
Donor Template WT -> SNV
TTT
TTTCCTCAGCTTCCTCACAACAGGGACAAGAAGAAAAAGAGGAGGATCTAAAGAAAAAGGAGCTGAAGTCCTTAGGTCAGTTCTT
AAAGGAGTCGAAGGAGTGTTGTCCCTGTTCTTCTTTTTCTCCTCCTAGATTTCTTTTTCCTCGACTTCAGGAATCCAGTCAAGAA
1530
EIF2B3
EIF2B3-201
240 245 250 255 260
F S S A S S Q Q G Q E E K E E D L K K K E L K S L G Q F L
ENSE00001064945
EIF2B3-201
Donor Template WT -> SNV
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CCCAACCAAGTCATTCTCCCCGTTCCCATCGTCGTGGAAACCTTTCTCACCAACCCGTCGAATCGGGTCGGGTTCTACGGACTGA
1615
EIF2B3
EIF2B3-201
G W F S K R G K G S S T F G K S G W A A *
(in frame with EIF2B3-201)
CAAGGCAGCAGACTATTTCTAGCCTACGAGGCAAGACAGGCTTGCCAACCCACTGAGCTCTGGGTAAGCTTGCTCAGGCAGATTC
GTTCCGTCGTCTGATAAAGATCGGATGCTCCGTTCTGTCCGAACGGTTGGGTGACTCGAGACCCATTCGAACGAGTCCGTCTAAG
1700
EIF2B3
EIF2B3-201
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ACGGTGACACGAGAGCCGTTGTCCGATTAGTACGGAGGTCGGGGTAAGAGACTAAGTATCGAGACCAAACCGACACAAGGAGTGA
1785
EIF2B3
EIF2B3-201
AGACCATTTTGCCTTTAAACTCCACCTGCCACTTCTTAGTTCCCCTGTTCTCTAGGCTCTAGGCAGCCCCGTTCCTGATTAAACT
TCTGGTAAAACGGAAATTTGAGGTGGACGGTGAAGAATCAAGGGGACAAGAGATCCGAGATCCGTCGGGGCAAGGACTAATTTGA
1870
EIF2B3
EIF2B3-201
GTGAAGAATCAAGGGGACAAGAGAT
PCR Reverse


CAACCTCTTTTATCAAGGGAAGGAAAGTGAAGGAATGTACAATTGCAGTCCAACCACAGGCCAACCCAGGTGTCTCCGTGCCAGG
GTTGGAGAAAATAGTTCCCTTCCTTTCACTTCCTTACATGTTAACGTCAGGTTGGTGTCCGGTTGGGTCCACAGAGGCACGGTCC
1955
EIF2B3
EIF2B3-201
ATCTGAAGATCGGATCTGTCTTGTTTGCTGTCATATCCCTAATCCCCGGCCCAGTCGTGGACTCATAGTAGATATTCTGAAAAAG
TAGACTTCTAGCCTAGACAGAACAAACGACAGTATAGGGATTAGGGGCCGGGTCAGCACCTGAGTATCATCTATAAGACTTTTTC
2040
EIF2B3
EIF2B3-201
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2125
EIF2B3
EIF2B3-201
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CTGACGGAGAATGATCGTAACACAGAAAAAAAAAAAAATAAAAAAACTCTGCCTCAGAGGGAGACAGCGGGTCCGACCTCCCGTC
2210
EIF2B3
EIF2B3-201
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ACCGCGTTAGAGCCGAGTGACGTTCGAGGCGGAGGGCCCAAGTGCGGTAAGAGGACGGAGTCGGAGGGGTCGTCGATCCTGATGT
2295
EIF2B3
EIF2B3-201
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CCGTGTGCGGTGGTGCGGGCCGATTAAAAACATAAAAATCATCTCTGCCCCAAAGAGACACAATCGGTCCTACCATAGCTAGAGG
2380
EIF2B3
EIF2B3-201
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ACTGGAGTACTAGGTGGGCGGAGCCGGAGGGTTTCACGACCCTAATGTCCGTACTCGGTGGCACGGGCCGGTCGTAACACAGAAC
2465
EIF2B3
EIF2B3-201
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TCCGTCCAATGAGTCAGGAGACTCGGGGTCAAAAAAATACTCTTTCATTTTCTAGAGTTTCCTAGCAACATTCTCGACTTTTTGA
2550
EIF2B3
EIF2B3-201
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TCTCGGGACCATGAGACCCGAGGTTCAAGACATGAAAGACAGTATCGTGTCGATGGTCTATACCATTATCTCTGATCGTCGTGTT
2635
EIF2B3
EIF2B3-201
AGTCAACCTGGCTGGCCACTAAAGCCTGGATGCTGGCT 3′
2673
TCAGTTGGACCGACCGGTGATTTCGGACCTACGACCGA 5′
EIF2B3
EIF2B3-201

	Feature			Location			Size			Type
✓	**EIF2B3**			1	..	2673	2673 bp		→	gene
	/note	=	gene ENSG00000070785 Protein coding							
✓	**EIF2B3-201**			1	..	2673	2673 bp		→	prim_transcript
	/note	=	primary transcript ENST00000360403							
	EIF2B3-203			1	..	2673	2673 bp		→	prim_transcript
	/note	=	primary transcript ENST00000372183							
	EIF2B3-204			1	..	2673	2673 bp		→	prim_transcript
	/note	=	primary transcript ENST00000439363							
	EIF2B3-210			1	..	2673	2673 bp		→	prim_transcript
	/note	=	primary transcript ENST00000620860							
✓	**Donor Template WT -> SNV**			1349	..	1448	100 bp		⊢⊣	misc_feature
✓	**EIF2B3-201**			1393	..	1520	128 bp		→	CDS
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	/note	=	coding sequence ENSP00000353575							
	/translation	=	SITSIRSELIPYLVRKQFSSASSQQGQEEKEEDLKKKELKSL 42 amino acids = 4.8 kDa							
	EIF2B3-203			1393	..	1520	128 bp		→	CDS
	/codon_start	=	1							
	/note	=	coding sequence ENSP00000361257							
	/translation	=	SITSIRSELIPYLVRKQFSSASSQQGQEEKEEDLKKKELKSL 42 amino acids = 4.8 kDa							
	EIF2B3-204			1393	..	1520	128 bp		→	CDS
	/codon_start	=	1							
	/note	=	coding sequence ENSP00000396985							
	/translation	=	Q*LLSGVN*FHI**ENSFPQLPHNRDKKKKRRI*RKRS*SP* 42 codons (6 internal stop codons)							
	EIF2B3-210			1393	..	1520	128 bp		→	CDS
	/codon_start	=	1							
	/note	=	coding sequence ENSP00000483996							
	/translation	=	SITSIRSELIPYLVRKQFSSASSQQGQEEKEEDLKKKELKSL 42 amino acids = 4.8 kDa							
✓	**PAM**			1408	..	1410	3 bp		⊢⊣	misc_feature
✓	**gRNA Protospacer**			1411	..	1430	20 bp		⊢⊣	misc_feature
✓	**SNV**			1423	..	1423	1 bp		⊢⊣	misc_feature
	/note	=	WT = T SNV = G							

	Primer	Length	Binding Sites		Tm	Date Added
✓	**PCR Forward**	25-mer	1139 .. 1163	→	58°C	Sep 29, 2023
	/sequence = ACTGAACTAAAGATCAGGTCCCTTC 44% GC / 7610.0 Da					
✓	**Sanger Sequencing Primer**	20-mer	1203 .. 1222	→	59°C	Sep 29, 2023
	/sequence = CCTGTCTCAGTACCCACCAG 60% GC / 5997.9 Da					
✓	**Donor Template WT -> SNV**	100-mer	1349 .. 1448	→	71°C	Sep 29, 2023
	/sequence = CCAGACAGTCAGGTTTCTCATATTTGGCCATTCTTTCTTTCTAGGTCAATAACTTCTATCCGGAGTGAACTGATGCCATATTTAGTGAGAAAACAGTTTT 38% GC / 30,742.1 Da					
✓	**gRNA Protospacer**	20-mer	1411 .. 1430	←	49°C	Sep 29, 2023
	/sequence = AATATGGAATCAGTTCACTC 35% GC / 6100.1 Da					
✓	**PCR Reverse**	25-mer	1815 .. 1839	←	57°C	Sep 29, 2023
	/sequence = TAGAGAACAGGGGAACTAAGAAGTG 44% GC / 7837.2 Da					