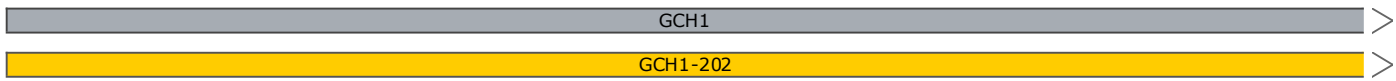


**INK2J00053\_GCH1\_G201E\_E08\_AB**  
1249 bp

5'  
3'

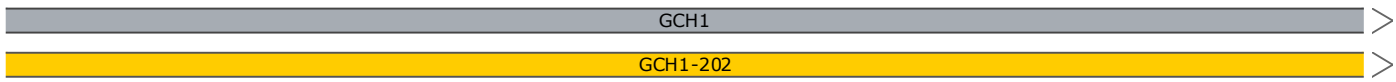
TCCAGAAGAAATCACTGTTGATTTGCACATTCCTTCACATTTTCATGCATATACCTGCATTTTCAAATCAATTTATTTGGATTA  
AGGTCTTCTTTAGTGACAACATAAACGTGTAAGGAAGTGTAAAAGTACGTATATGGACGTAAAAGTTTAGTTAAATAAACCTAAT

85



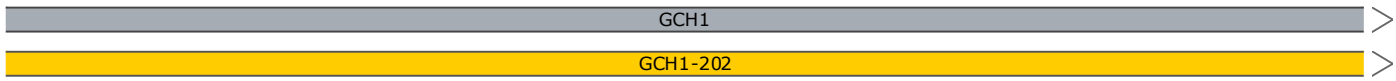
TGTTTAAACAAAAATGGGCTCATTTTATACATAATCAGCCTTGCTTTTTTCCCTGCTTTTTTTCATTTAACAAATCTTAAACATT  
ACAAATTTGTTTTTACCCGAGTAAAATATGTATTAGTCGGAACGAAAAAGGGACGAAAAAAGTAAATTGTTTAGAATTTGTAA

170



TTTGCCACATCCATGTAACATCTCATTCTGGTGGCTGTCTAGAATCTACCGTATATGCCATGGCTTGGCGTAACCTATA  
AAACGGTGTAGGTACATTGATAGAGTAAGTAAGACCACCGACAGATCTTAGGATGGCATATACGGTACCGAACCGCATTGGATAT

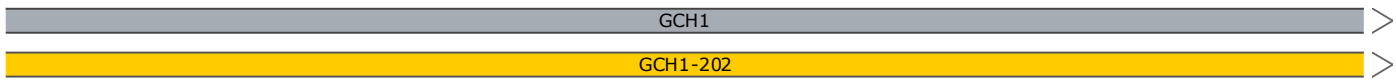
255



PCR Forward  
GAC

CCAGCTCTGATAGATACTGGGGGGTTTCATCAGTTTTCAAATAGATGCAGTGTGCAATGAATATTCCTGTATGAATAAAAGAC  
GGTCGAGACTATCTATGACCCCCCAAAGTAGTCAAAGTTTATCTACGTACAGACGTTACTTATAAGGACATACTTAATTTTTCTG

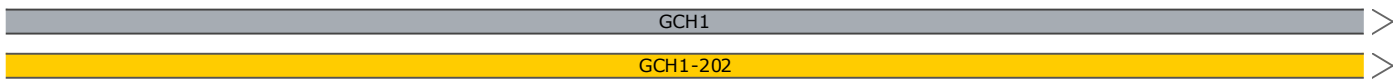
340



PCR Forward  
AAGTCATTTTATTCAGGACAGG

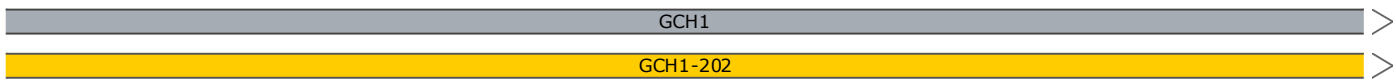
AAGTCATTTTATTCAGGACAGGAACATATATTATTTAGGTTAAATTGTTGGTTTTCAACATTGCTCTAAGCACAATTCAATAATTG  
TTCAGTAAAATAAGTCCTGTCTTGGATATAATAAATCCAATTTAACAACCAAAGTTGTAACGAGATTCTGTGTTAAGTTATTAAC

425



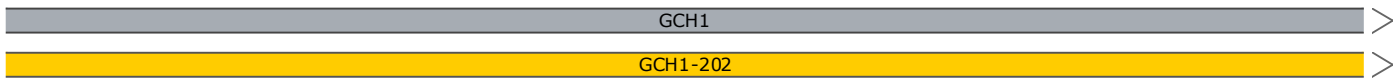
GACTGCAGCCCCTTTGTAAACCCTGTTGCAGGCTATTTATTTGCTGCACTACTTTACCTCCATGGAGGTGCTTGGTAACCAGTAC  
CTGACGTGGGGAAACAATTGGGACAACGTCCGATAAATAAACGACGTGATGAAATGGAGGTACCTCCACGAACCATTGGTCATG

510



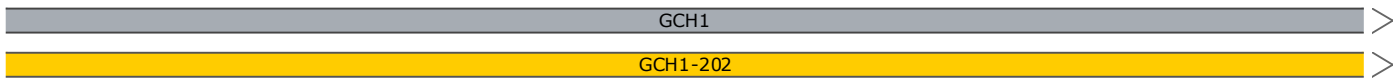
TGTGTTGTTCTAACTGCACAGGTATGAGATAATTGCTATTCTAATTCCTTCCCGCAGTGATGCTGGAGGGCTTCAGGGTGTCTG  
ACACAACAAGATTGACGTGTCCATACTCTATTAACGATAAGATTAAGGAAGGGCGTCACTACGACCTCCCGAAGTCCCACAAGAC

595



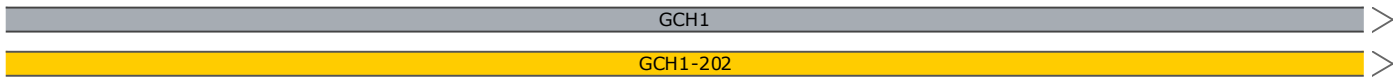
AGATCTCTTTAGCCCTGTACTTCAGGTGAGGGAGTTGAATCCGGCCTCTCTGTGGCCAGTCAGCAGACAAAGCAGTCTGGTAA  
TCTAGAGAAATCGGGACATGAAGTCCACTCCCTCAACTTAGGCCCGGAGAGACACCGGGTCAGTCGTCTGTTTCGTGACACCATT

680



AAGCTGGTGTGTCTTGGCTCTTAAATCTCACAGAAGTCTGATTTTTAAGATTTCAAATGTTCTAGAGACAGAAAAAGCTTCCAGC  
TTCGACCACACAGAACCGAGAATTTAGAGTGTCTTCAGACTAAAAATTCTAAAGTTTACAAGATCTCTGTCTTTTTTCGAAGGTGC

765



Donor Template WT -> SNV

CCACAGTTCAGGAGCGCCTTACAAAACAAATTGCTGTAGCAATCAC

TGTTTGTGTCAGACTCTCAAAGTCTCCTTATCACATCCACAGTTCAGGAGCGCCTTACAAAACAAATTGCTGTAGCAATCAC  
ACAAAACACAGTCTGAGAGTTTACTCGAGGAATAGTGTAGGTGTCAAGTCTCGCGGAATGTTTTGTTAACGACATCGTTAGTG

850

GCH1

GCH1-202

Donor Template WT -> SNV

Q E R L T K Q I A V A I T

ENSE00003472475

GCH1-202

Donor Template WT -> SNV

GGAAGCCTTGCAGCCTGCTGAGTTCGGGGTAGTGGTTGAAGCAACGTAAGTCTG

GGAAGCCTTGCAGCCTGCTGAGTTCGGGGTAGTGGTTGAAGCAACGTAAGTCTGCATCTGCCTTTAGTAACGTCATAATGGTGCA  
CCTTCGGAACGCCGGACGACCTCAGCCCCATCACCAACTTCGTTGCATTACAGACGTAGACGGAAATCATTGCAGTATTACCACGT

935

GCH1

GCH1-202

Donor Template WT -> SNV

E A L R P A G V G V V V E A T

ENSE00003472475

GCH1-202

PAM gRNA Protospacer

SNV

CGACCTCAGCCCCATCACCA  
gRNA Protospacer

CTAGAAGTGATCTTGTATTTAGTCTTCTCATATTTTGTAGCACCAGGTGATGCCACACAACCTGATATGATAACTGTAGATTTT  
GATCTTCACTAGAACGATAAATCACGAAGAGTATAAAACATCGTGGTCCACTACGGTGTGTTGACTATACTATTGACATCTAAAG

1020

GCH1

GCH1-202

CCACTACGGTGTGTTGACTA  
Sanger Sequencing Primer

CATCCCTGAGCCTAAAGCTCTTTTACCACCTTTCATTTACTGTTACTGATATAGGAACAGAATATGGGAGAAGTATTTTTTGGGG  
GTAGGGACTCGGATTTTCGAGAAAATGGTGAAGTAAATGACAATGACTATATCCTTGTCTTATACCCTCTTCATAAAAAAAGTCCC

1105

GCH1

GCH1-202

TAGGATGGGGCTTGTGGAGGCATAGGGAGTTAAAAGCACAGGTGTAGAGATTTGCATAATCAATAGCCTTTTTTTTTTTGAGAT  
ATCCTACCCCGAACACCTCCGTATCCCTCAATTTTCGTGTCCACATCTCTAAACGTATTAGTTATCGGAAAAAAAAAAAAAAGTCTA

1190

GCH1

GCH1-202

CCCTCAATTTTCGTGTCCACATCTC  
PCR Reverse

GGAGCCTCACTCTGTCGCCAGGCTGGAGTGCAGTGGTGAATCTCGGCTCACTGCAAC  
CCTCGGAGTGAGACAGCGGGTCCGACCTCACGTACCACGTTAGAGCCGAGTGACGTTG

3'

1249

5'

GCH1

GCH1-202

Feature	Location	Size	Start	End	Type
✓ <b>GCH1</b>	1 .. 1249	1249 bp	■	➔	gene
/note = gene <a href="#">ENSG00000131979</a> Protein coding					
<b>GCH1-201</b>	1 .. 1249	1249 bp	■	➔	prim_transcript
/note = primary transcript <a href="#">ENST00000254299</a> protein_coding_CDS_not_defined					
✓ <b>GCH1-202</b>	1 .. 1249	1249 bp	■	➔	prim_transcript
/note = primary transcript <a href="#">ENST00000395514</a>					
<b>GCH1-203</b>	1 .. 1249	1249 bp	■	➔	prim_transcript
/note = primary transcript <a href="#">ENST00000395521</a> protein_coding_CDS_not_defined					
<b>GCH1-204</b>	1 .. 1249	1249 bp	■	➔	prim_transcript
/note = primary transcript <a href="#">ENST00000491895</a>					
<b>GCH1-205</b>	1 .. 1249	1249 bp	■	➔	prim_transcript
/note = primary transcript <a href="#">ENST00000536224</a>					
<b>GCH1-206</b>	1 .. 1249	1249 bp	■	➔	prim_transcript
/note = primary transcript <a href="#">ENST00000543643</a>					
✓ <b>Donor Template WT -&gt; SNV</b>	805 .. 904	100 bp	■	⌊	misc_feature
✓ <b>GCH1-202</b>	811 .. 895	85 bp	■	➔	CDS
/codon_start = 1					
/note = coding sequence <a href="#">ENSP00000378890</a>					
/translation = QERLTKQIAVAITEALRPAGVGVVVEA 28 amino acids = 2.8 kDa					
<b>GCH1-204</b>	811 .. 895	85 bp	■	➔	CDS
/codon_start = 1					
/note = coding sequence <a href="#">ENSP00000419045</a>					
/translation = QERLTKQIAVAITEALRPAGVGVVVEA 28 amino acids = 2.8 kDa					
<b>GCH1-205</b>	811 .. 895	85 bp	■	➔	CDS
/codon_start = 1					
/note = coding sequence <a href="#">ENSP00000445246</a>					
/translation = QERLTKQIAVAITEALRPAGVGVVVEA 28 amino acids = 2.8 kDa					
<b>GCH1-206</b>	811 .. 895	85 bp	■	➔	CDS
/codon_start = 1					
/note = coding sequence <a href="#">ENSP00000444011</a>					
/translation = QERLTKQIAVAITEALRPAGVGVVVEA 28 amino acids = 2.8 kDa					
✓ <b>PAM</b>	864 .. 866	3 bp	■	⌊	misc_feature
✓ <b>gRNA Protospacer</b>	867 .. 886	20 bp	■	⌊	misc_feature
✓ <b>SNV</b>	871 .. 871	1 bp	■	⌊	misc_feature
/note = WT = G SNV = A					

Primer	Length		Binding Sites	↕	Tm	Date Added
✓ <b>PCR Forward</b>	25-mer		338 .. 362	→	55°C	Jul 18, 2023
/sequence = GACAAGTCATTTTATTCAGGACAGG 40% GC / 7705.1 Da						
✓ <b>Donor Template WT -&gt; SNV</b>	100-mer		805 .. 904	→	78°C	Jul 18, 2023
/sequence = CCACAGTTCAGGAGCGCCTTACAAAACAAATTGCTGTAGCAATCACGGAAGCCTTGCGGCCTGCTGAAGTCGGGGTAGTGGTTGAAGCAACGTAAGTCTG 52% GC / 30,981.1 Da						
✓ <b>gRNA Protospacer</b>	20-mer		867 .. 886	←	63°C	Jul 18, 2023
/sequence = ACCACTACCCCGACTCCAGC 65% GC / 5951.9 Da						
✓ <b>Sanger Sequencing Primer</b>	20-mer		983 .. 1002	←	58°C	Jul 18, 2023
/sequence = ATCAGTTGTGTGGCATCACC 50% GC / 6108.0 Da						
✓ <b>PCR Reverse</b>	25-mer		1130 .. 1154	←	60°C	Jul 18, 2023
/sequence = CTCTACACCTGTGCTTTTAACTCCC 48% GC / 7478.9 Da						