

Genomic sequence: hg38 chr12:4367226-4380728 rev

cDNA sequence formatted with Showseq (from the EMBOSS package)

### Table of primers (for re-PCR)

Exon2: 104 bp, Product size 241

Exon3: 441 bp, Product size 576

| OLIGO  | start | len | tm    | gc%   | any  | 3'   | rep   | seq                  |
|--|-------|-----|-------|-------|------|------|-------|----------------------|
| LEFT PRIMER  | 1056  | 20  | 59.73 | 50.00 | 4.00 | 2.00 | 11.00 | AAGGAAAAGGCCAGTAAGGC |
| RIGHT PRIMER   | 1424  | 19  | 59.81 | 63.16 | 3.00 | 3.00 | 11.00 | CTCCCAAGCCTCTGTAG    |
| PRODUCT SIZE: 369, PAIR ANY COMPL: 4.00, PAIR 3' COMPL: 1.00 |       |     |       |       |      |      |       |                      |
| TARGETS (start, len)*: 1111,281                              |       |     |       |       |      |      |       |                      |

1020 AGTCTAGGATCCTCACACCAGCTACTTGCAAGGGAGAAGGAAAAGGCCAGTAAGGCCTGG  
.....>>>>>>>>>>>>>>>>

1080 GCCAGGAGAGTCCCGACAGGAGTGTGAGTTTCAATCTCAGCACCAGCCACTCAGAGCAG  
XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

1140 GGCACGATGTTGGGGGCCCGCCTCAGGCTCTGGGTCTGTGCCTTGTGCAGCGTCTGCAGC  
XX

1200 ATGAGCGTCCTCAGAGCCTATCCCAATGCCTCCCCACTGCTCGGCTCCAGCTGGGGTGGC  
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1260 CTGATCCACCTGTACACAGCCACAGCCAGGAACAGCTACCACCTGCAGATCCACAAGAAT  
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1320 GGCCATGTGGATGGCGCACCCCATCAGACCATCTACAGTGAGTAGGGCTTCAGGCTGGGA  
\*\*\*\*\*XX

1380 AGAAGGGGAGCACCTTGTGTCCATCTACAGGAGGCTTGGGGAGGTTGGGGACTAGACT  
XX

1440 GGAGGGCTAATCCAACCCTCCTAGCTTTCTGCCAGGAACCACTTATTGTCTTtgtgtgt

## ADDITIONAL OLIGOS

|               | start | len  | tm    | gc%          | any  | 3'   | rep         | seq                   |
|---------------|-------|------|-------|--------------|------|------|-------------|-----------------------|
| 1 LEFT PRIMER | 1056  | 20   | 59.73 | 50.00        | 4.00 | 2.00 | 11.00       | AAGGAAAAGGCCAGTAAGGC  |
| RIGHT PRIMER  | 1461  | 19   | 60.28 | 57.89        | 3.00 | 2.00 | 11.00       | AGGAGGGTTGGATTAGCCC   |
| PRODUCT SIZE: | 406,  | PAIR | ANY   | COMPL: 3.00, | PAIR | 3'   | COMPL: 2.00 |                       |
| 2 LEFT PRIMER | 1056  | 20   | 59.73 | 50.00        | 4.00 | 2.00 | 11.00       | AAGGAAAAGGCCAGTAAGGC  |
| RIGHT PRIMER  | 1458  | 19   | 60.28 | 57.89        | 4.00 | 3.00 | 11.00       | AGGGTTGGATTAGCCCTCC   |
| PRODUCT SIZE: | 403,  | PAIR | ANY   | COMPL: 3.00, | PAIR | 3'   | COMPL: 3.00 |                       |
| 3 LEFT PRIMER | 1056  | 20   | 59.73 | 50.00        | 4.00 | 2.00 | 11.00       | AAGGAAAAGGCCAGTAAGGC  |
| RIGHT PRIMER  | 1449  | 21   | 59.72 | 57.14        | 4.00 | 0.00 | 12.00       | TTAGCCCTCCAGTCTAGTCCC |
| PRODUCT SIZE: | 394,  | PAIR | ANY   | COMPL: 3.00, | PAIR | 3'   | COMPL: 2.00 |                       |
| 4 LEFT PRIMER | 1056  | 20   | 59.73 | 50.00        | 4.00 | 2.00 | 11.00       | AAGGAAAAGGCCAGTAAGGC  |
| RIGHT PRIMER  | 1455  | 20   | 58.60 | 55.00        | 4.00 | 1.00 | 11.00       | GTTGGATTAGCCCTCCAGTC  |
| PRODUCT SIZE: | 400,  | PAIR | ANY   | COMPL: 3.00, | PAIR | 3'   | COMPL: 3.00 |                       |

## Statistics

Pair Stats:

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Exon2: 104 bp

8220 TTCCATTTGCAGTGTACCCTGGTGACCTGTTTCCACTGATACTTCATGCAGTCTAAGTAT

## ADDITIONAL OLIGOS

|               | start | len  | tm    | gc%          | any  | 3'             | rep seq                          |
|---------------|-------|------|-------|--------------|------|----------------|----------------------------------|
| 1 LEFT PRIMER | 7971  | 20   | 60.50 | 50.00        | 5.00 | 2.00           | 12.00 GTGCTTGAAGGTGGGAATTG       |
| RIGHT PRIMER  | 8202  | 25   | 58.81 | 36.00        | 6.00 | 1.00           | 12.00 TCTATGTCGTGTCAAAGAAATTAGG  |
| PRODUCT SIZE: | 232,  | PAIR | ANY   | COMPL: 4.00, | PAIR | 3' COMPL: 0.00 |                                  |
| 2 LEFT PRIMER | 7962  | 20   | 60.37 | 50.00        | 5.00 | 3.00           | 12.00 TTTCAGGAGGTGCTTGAAGG       |
| RIGHT PRIMER  | 8203  | 26   | 59.54 | 38.46        | 6.00 | 1.00           | 12.00 GTCTATGTCGTGTCAAAGAAATTAGG |
| PRODUCT SIZE: | 242,  | PAIR | ANY   | COMPL: 4.00, | PAIR | 3' COMPL: 0.00 |                                  |
| 3 LEFT PRIMER | 7971  | 20   | 60.50 | 50.00        | 5.00 | 2.00           | 12.00 GTGCTTGAAGGTGGGAATTG       |
| RIGHT PRIMER  | 8203  | 26   | 59.54 | 38.46        | 6.00 | 1.00           | 12.00 GTCTATGTCGTGTCAAAGAAATTAGG |
| PRODUCT SIZE: | 233,  | PAIR | ANY   | COMPL: 4.00, | PAIR | 3' COMPL: 0.00 |                                  |
| 4 LEFT PRIMER | 7962  | 20   | 60.37 | 50.00        | 5.00 | 3.00           | 12.00 TTTCAGGAGGTGCTTGAAGG       |
| RIGHT PRIMER  | 8201  | 24   | 57.15 | 37.50        | 4.00 | 1.00           | 12.00 CTATGTCGTGTCAAAGAAATTAGG   |
| PRODUCT SIZE: | 240,  | PAIR | ANY   | COMPL: 4.00, | PAIR | 3' COMPL: 0.00 |                                  |

## Statistics

Pair Stats:

<https://ihq.helmholtz-muenchen.de/cgi-bin/primer/ExonPrimerR.pl?dir=/srv/www/tmp/exonprimer219141516996096&times=1>

|  |       |     |       |       |      |      |       |                      |
|--|-------|-----|-------|-------|------|------|-------|----------------------|
| OLIGO  | start | len | tm    | gc%   | any  | 3'   | rep   | seq                  |
| LEFT PRIMER  | 9877  | 18  | 60.35 | 66.67 | 4.00 | 0.00 | 12.00 | AGGAGGAGCTGGGGAGTG   |
| RIGHT PRIMER   | 10452 | 20  | 61.02 | 60.00 | 7.00 | 3.00 | 11.00 | GACCTGGTCCTTGGGAAGAG |
| PRODUCT SIZE: 576, PAIR ANY COMPL: 5.00, PAIR 3' COMPL: 0.00 |       |     |       |       |      |      |       |                      |
| TARGETS (start, len)*: 9910,511                              |       |     |       |       |      |      |       |                      |

|               | start | len  | tm    | gc%          | any  | 3'   | rep         | seq                  |
|---------------|-------|------|-------|--------------|------|------|-------------|----------------------|
| 1 LEFT PRIMER | 9882  | 17   | 60.19 | 70.59        | 4.00 | 0.00 | 12.00       | GAGCTGGGGAGTGGGTG    |
| RIGHT PRIMER  | 10452 | 20   | 61.02 | 60.00        | 7.00 | 3.00 | 11.00       | GACCTGGTCCTTGGGAAGAG |
| PRODUCT SIZE: | 571,  | PAIR | ANY   | COMPL: 3.00, | PAIR | 3'   | COMPL: 0.00 |                      |
| 2 LEFT PRIMER | 9877  | 18   | 60.35 | 66.67        | 4.00 | 0.00 | 12.00       | AGGAGGAGCTGGGGAGTG   |
| RIGHT PRIMER  | 10451 | 19   | 59.09 | 57.89        | 5.00 | 3.00 | 11.00       | ACCTGGTCCTTGGGAAGAG  |
| PRODUCT SIZE: | 575,  | PAIR | ANY   | COMPL: 5.00, | PAIR | 3'   | COMPL: 0.00 |                      |
| 3 LEFT PRIMER | 9877  | 18   | 60.35 | 66.67        | 4.00 | 0.00 | 12.00       | AGGAGGAGCTGGGGAGTG   |
| RIGHT PRIMER  | 10449 | 20   | 62.26 | 60.00        | 4.00 | 2.00 | 11.00       | CTGGTCCTTGGGAAGAGCTG |
| PRODUCT SIZE: | 573,  | PAIR | ANY   | COMPL: 4.00, | PAIR | 3'   | COMPL: 0.00 |                      |
| 4 LEFT PRIMER | 9876  | 17   | 59.40 | 70.59        | 4.00 | 0.00 | 12.00       | CAGGAGGAGCTGGGGAG    |
| RIGHT PRIMER  | 10452 | 20   | 61.02 | 60.00        | 7.00 | 3.00 | 11.00       | GACCTGGTCCTTGGGAAGAG |
| PRODUCT SIZE: | 577,  | PAIR | ANY   | COMPL: 5.00, | PAIR | 3'   | COMPL: 0.00 |                      |

## Statistics

|       | con<br>sid<br>ered | too<br>many<br>Ns | in<br>tar<br>get | in<br>excl<br>reg | bad<br>GC% | no<br>clamp | tm<br>too<br>low | tm<br>too<br>high | high<br>any<br>compl | high<br>3'<br>compl | high<br>lib<br>sim | poly<br>X | high<br>end<br>stab | ok |
|-------|--------------------|-------------------|------------------|-------------------|------------|-------------|------------------|-------------------|----------------------|---------------------|--------------------|-----------|---------------------|----|
| Left  | 386                | 0                 | 0                | 0                 | 17         | 16          | 0                | 334               | 0                    | 0                   | 0                  | 2         | 0                   | 17 |
| Right | 342                | 0                 | 0                | 0                 | 0          | 22          | 16               | 225               | 1                    | 1                   | 0                  | 0         | 0                   | 77 |

## Pair Stats:

considered 34, unacceptable product size 27, ok 7

primer3 release 1.1.1

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