

pGEX-4T-3-hAgo4, N-terminal framing is right. Joined cloning site: Sall/XhoI is good.

Sequence details:

pGEX-4T-1 XhoI and NotI

Insert: template: pOZ-Ago4 (perfectly sequenced), PCR: supermix

Cut: Sall and NotI

Framing

Ago4 [NM_017629.3](#)

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>pGEX-A4-4-pGEX5_C09.ab1
NNNNNNNNNNNNNNNNNNNTCGNNCTGGTTCCGGCTGGATCCCCG GAA TTC CCG GGT CGA CTC GAC GAGGCGCTGGG
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CCAATCATTTTCAGGTTTCAGATTCTAAAATAGATGTGTATCACTATGATGGGATTTAAGCCTGAAAAACGGCCTCGT
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AGGGTAAAGACCAACATTTAAAGTGTCTGTTCAAGTGGTGTGAGTGTGAGCCTTCANTTGCTTTTNNAGCTTTGGCTG
GGCACTTGAATGAAGTCCAGATGACTCAGTACAAGCACTTATGTTATCACAAAGACCTTCCCTCCATGAGGTACACC
CCAGTGGGCGTTCCTTTTCTCACCCCGGAAGGTTACTACCCTCTGGGANGGGCAGGGAGGTCTGGTTGGTTT
TCATCAGTCTGTGAGACCTGCCATGTGGAATATGATGCTCAACATTGATGTATCTGCAACTGCTTTCTACCGNGTCAAC
CTATCATTGAGTTTCATGTGTGAGGNTTANACNTTTCAGAACATCNATGANCAGACCANACCTCTAACAGACTCCAGCGT
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AATGTGACTAAACNGNCANNNNNTCATCAAACNTTCCCTTTGCNNCTAGAAANNGGTCAANNATGNAATGTACAGTAG
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Query 70 GAGGCGCTGGGACCCGNNCTCCGGCTAGCCTGTTTCAGCCACCTCGTCTGCTGGCCTT 129
Sbjct 194 GAGGCGCTGGGACCCGGACCTCCGGCTAGCCTGTTTCAGCCACCTCGTCTGCTGGCCTT 253

Query 130 GGAAGTGTGGAAAACCAATTTCGACTGTAGCCAATCATTTTCAGGTTTCAGATTCCTAAA 189
Sbjct 254 GGAAGTGTGGAAAACCAATTTCGACTGTAGCCAATCATTTTCAGGTTTCAGATTCCTAAA 313

Query 190 ATAGATGTGTATCACTATGATGTGGATATTAAGCCTGAAAAACGGCCTCGTAGAGTCAAC 249
Sbjct 314 ATAGATGTGTATCACTATGATGTGGATATTAAGCCTGAAAAACGGCCTCGTAGAGTCAAC 373

Query 250 AGGGAGGTAGTAGATACAATGGTGCAGCACTTCAAGATGCAAAATATTTGGTGTATCGGCAG 309
Sbjct 374 AGGGAGGTAGTAGATACAATGGTGCAGCACTTCAAGATGCAAAATATTTGGTGTATCGGCAG 433

Query 310 CCTGGGTATGATGGCAAAGAAACATGTACACAGCACATCCACTACCAATTGGACGGGAT 369
Sbjct 434 CCTGGGTATGATGGCAAAGAAACATGTACACAGCACATCCACTACCAATTGGACGGGAT 493

Query 370 AGGGTTGATATGGAGGTGACTCTTCCAGCGAGGGTAAAGACCAACATTTAAAGTGTCT 429
Sbjct 494 AGGGTTGATATGGAGGTGACTCTTCCAGCGAGGGTAAAGACCAACATTTAAAGTGTCT 553

Query 430 GTTCAGTGGGTGTCAGTTGTGAGCCTTCANTTGCTTTTNNNA-GCTTTGGCTGGGCACTTG 488
Sbjct 554 GTTCAGTGGGTGTCAGTTGTGAGCCTTCAGTTGCTTTTAGAAGCTTTGGCTGGGCACTTG 613

Query 489 AATGAAGTCCCAGATGACTCAGTACAAGCACTTGTATGTTATCACAAAGACACCTTCCCTCC 548
Sbjct 614 AATGAAGTCCCAGATGACTCAGTACAAGCACTTGTATGTTATCACAAAGACACCTTCCCTCC 673

Query 549 ATGAGGTACACCCAGTGGGCGTTCCTTTTCTCACCCCGGAAGGTTACTACCACCT 608
Sbjct 674 ATGAGGTACACCCAGTGGGCGTTCCTTTTCTCACCCCGGAAGGTTACTACCACCT 733

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Home 3/18/11 11:13 AM
Comment: It is AC

Home 3/18/11 11:13 AM
Comment: It is a G , agaa is there


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Query 154 GTGGAAATATGATGCTCAACATTGATGTATCTGCAACTGCTTTCTACCGGGCTCAGCCTAT 213
Sbjct 790 GTGGAAATATGATGCTCAACATTGATGTATCTGCAACTGCTTTCTACCGGGCTCAGCCTAT 849

Query 214 CATTGAGTTCATGTGTGAGGTTTGTAGACATTGAGAACATCAATGAACAGACCAAACCTCT 273
Sbjct 850 CATTGAGTTCATGTGTGAGGTTTGTAGACATTGAGAACATCAATGAACAGACCAAACCTCT 909

Query 274 AACAGACTCCCAGCGTGTCAAATTTACCAAGAAATCAGAGGTCTCAAAGTTGAGGTGAC 333
Sbjct 910 AACAGACTCCCAGCGTGTCAAATTTACCAAGAAATCAGAGGTCTCAAAGTTGAGGTGAC 969

Query 334 CCACTGTGGACAGATGAAACGAAAATACCGAGTTTGTAAATGTGACTAGACGGCCAGCCAG 393
Sbjct 970 CCACTGTGGACAGATGAAACGAAAATACCGAGTTTGTAAATGTGACTAGACGGCCAGCCAG 1029

Query 394 TCATCAAACCTTTTCCTTTGCAGCTAGAAAACGGTCAAGCTATGGAATGTACAGTAGCTCA 453
Sbjct 1030 TCATCAAACCTTTTCCTTTGCAGCTAGAAAACGGTCAAGCTATGGAATGTACAGTAGCTCA 1089

Query 454 ATATTTTAAAGCAAAAGTATAGTCTGCAACTGAAATACCCCATCTTCCCTGTCTCCAAGT 513
Sbjct 1090 ATATTTTAAAGCAAAAGTATAGTCTGCAACTGAAATACCCCATCTTCCCTGTCTCCAAGT 1149

Query 514 GGGACAAGAACAAAAGCATACTACTTGCCTCGAGGTCTGTAATATAGTGGCAGGACA 573
Sbjct 1150 GGGACAAGAACAAAAGCATACTACTTGCCTCGAGGTCTGTAATATAGTGGCAGGACA 1209

Query 574 GCGATGTATCAAGAAGCTCACAGACAATCAACTTCCACAATGATCAAAGCTACAGCAAG 633
Sbjct 1210 GCGATGTATCAAGAAGCTCACAGACAATCAGACTTCCACAATGATCAAAGCTACAGCAAG 1269

Query 634 ATCTGCTCCTGACAGACAGGAAGAGATCAGTAGACTGGTGAAGAGCAACAGTATGGTGGG 693
Sbjct 1270 ATCTGCTCCTGACAGACAGGAAGAGATCAGTAGACTGGTGAAGAGCAACAGTATGGTGGG 1329

Query 694 TGGACCTGATCCATACCTTAAAGAATTTGGTATTGTTGTCCACAATGAAATGACAGAGCT 753
Sbjct 1330 TGGACCTGATCCATACCTTAAAGAATTTGGTATTGTTGTCCACAATGAAATGACAGAGCT 1389

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Sbjct 1390 CACAGGCAGGGTACTTCCAGCACCATGCTGCAATATGGAGGCCGAATAAAACAGTAGC 1449

Query 814 CACACCCAACCGAGGTGTCTGGGACATGCGAGGAAAGCAGTTTTATGCTGGCATTGAAAT 873
Sbjct 1450 CACACCCAACCGAGGTGTCTGGGACATGCGAGGAAAGCAGTTTTATGCTGGCATTGAAAT 1509

Query 874 TAAAGTTTGGGCAGTTGCTNGTNTTNCACCTCAGAA-CAATGTAGGGAANANTTACTAAA 932
Sbjct 1510 TAAAGTTTGGGCAGTTGCTTGTGTCACCTCAGAAACAATGTAGGGAAGATTTACTAAA 1569

Query 933 GAGTTTCACTGACCANNTCGCTAAAATCTCTANG-ATGCNNNA-TGCCATCCANNGTCA 990
Sbjct 1570 GAGTTTCACTGACCAGCTGCGTAAAATCTCTAAGGATGCAGGAATGCCATCCAGGTCA 1629

Query 991 GCCATGTTTCTGCAAGTATG 1010
Sbjct 1630 GCCATGTTTCTGCAAGTATG 1649

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Home 3/18/11 11:26 AM
Comment: The N is G on trace file

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>pGEX-Ago4-4-Ago4seq2_H04.ab1
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AAACCTTTCCAATCTTTCGCTGAAGATAAATGCAAACTTGGAGGAATTAACAATGTGCTTGTGCTCATCAAAGGCC
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GCTGCTGTGGTTGGCAGTATGGATGGCCACCCAGCCGTTACTGTGCCACCGTTCCGGTGCANACTTCCCAGGAGAGAT
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 CANNNGAACNNTTNCNNTGNCANANNNAAACANNAANNGNN

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Query   54      t g a t c a a a g c t a c a g c a a g a - n n n n n n c t g n c a g a c a g g a a g a g a t c a g t a g a c t g g t g a      112
          | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct  1251     T G A T C A A A G C T C A C A G C A A G A T C T G C T C C T G A C A G A C A G G A A G A G A T C A G T A G A C T G G T G A      1310

Query   113     A G A G C A A C N G N A T G G T G G G T G G A C C T G A T C C A T A C C T T A A A G A A A T T G G T A T T G T T G T C C      172
          | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct  1311     A G A G C A A C A G T A T G G T G G G T G G A C C T G A T C C A T A C C T T A A A G A A A T T G G T A T T G T T G T C C      1370

Query   173     A C A A T G A A A T G A C A G A G C T C A C A G G C A G G G T A C T T C C A G C A C C A A T G C T G C A A T A T G G A G      232
          | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct  1371     A C A A T G A A A T G A C A G A G C T C A C A G G C A G G G T A C T T C C A G C A C C A A T G C T G C A A T A T G G A G      1430

Query   233     G C C G G A A T A A A A C A G T A G C C A C A C C C A A C C A G G G T G T C T G G G A C A T G C G A G G A A A G C A G T      292
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Sbjct  1431     G C C G G A A T A A A A C A G T A G C C A C A C C C A A C C A G G G T G T C T G G G A C A T G C G A G G A A A G C A G T      1490

Query   293     T T T A T G C T G G C A T T G A A A T T A A A G T T G G G C A G T T G C T T G T T T T G C A C C T C A G A A C A A T      352
          | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct  1491     T T T A T G C T G G C A T T G A A A T T A A A G T T G G G C A G T T G C T T G T T T T G C A C C T C A G A A A C A A T      1550

Query   353     G T A G G G A A G A T T T A C T A A A G A G T T T C A C T G A C C A G C T G C G T A A A A T C T C T A A G G A T G C A G      412
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Sbjct  1551     G T A G G G A A G A T T T A C T A A A G A G T T T C A C T G A C C A G C T G C G T A A A A T C T C T A A G G A T G C A G      1610

Query   413     G A A T G C C C A T C C A G G G T C A G C C A T G T T T C T G C A A G T A T G C A C A A G G T G C A G A C A G T G T G G      472
          | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct  1611     G A A T G C C C A T C C A G G G T C A G C C A T G T T T C T G C A A G T A T G C A C A A G G T G C A G A C A G T G T G G      1670

Query   473     A G C C T A T G T T T A A A C A T C T G A A A A T G A C T T A T G T G G G C C T A C A G C T A A T A G T G G T T A T C C      532
          | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct  1671     A G C C T A T G T T T A A A C A T C T G A A A A T G A C T T A T G T G G G C C T A C A G C T A A T A G T G G T T A T C C      1730

Query   533     T G C C T G G A A A G A C A C C A G T A T A T G C G G A G G T G A A A C G T G T T G G A G A T A C C C T T C T A G G T A      592
          | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct  1731     T G C C T G G A A A G A C A C C A G T A T A T G C G G A G G T G A A A C G T G T T G G A G A T A C C C T T C T A G G T A      1790

Query   593     T G G C C A C A C A G T G T G T C C A G G T A A A A A T G T A G T G A A G A C C T C A C C T C A A A C C C T T T C C A      652
          | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct  1791     T G G C C A C A C A G T G T G T C C A G G T A A A A A T G T A G T G A A G A C C T C A C C T C A A A C C C T T T C C A      1850

Query   653     A T C T T T G C C T G A A G A T A A A T G C A A A A C T T G G A G A A T T A A C A A T G T G C T T G T G C C T C A T C      712
          | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct  1851     A T C T T T G C C T G A A G A T A A A T G C A A A A C T T G G A G A A T T A A C A A T G T G C T T G T G C C T C A T C      1910

Query   713     A A A G G C C C T C G G T G T T C C A G C A G C C T G T C A T C T T C C T G G G A G C G G A T G T C A C A C A C C C C C      772
          | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct  1911     A A A G G C C C T C G G T G T T C C A G C A G C C T G T C A T C T T C C T G G G A G C G G A T G T C A C A C A C C C C C      1970

Query   773     C A G C A G G G A N G G G A A G A A A C C T T C - A T T G C T G C T G T G G T T G G C A G T A T G G A T G G C C A C C      831
          | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct  1971     C A G C A G G G A T G G G A A A A C C T T C C A T T G C T G C T G T G G T T G G C A G T A T G G A T G G C C A C C      2030

Query   832     C C A G C C G G T A C T G T G C C A C C G T T C G G G T G C A N A C T T C C C G G C A G G A G A T C T C C C A A G A G C      891
          | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct  2031     C C A G C C G G T A C T G T G C C A C C G T T C G G G T G C A G A C T T C C C G G C A G G A G A T C T C C C A A G A G C      2090

Query   892     T C C T C T A C A G T C A A G A N G T C N T C C N N G A C C T G A C N A A C N T G G T T C N A G A G C T G C T G A T - C      950
          | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct  2091     T C C T C T A C A G T C A A G A G T C A T C C A G G A C C T G A C T A A C A T G G T T C G A G A G C T G C T G A T T C      2150

Query   951     A G T T C T A C A A - T C C N N N G C T - C A A - C C C A C T C G G A T C A T C      988
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Sbjct  2151     A G T T C T A C A A A T C C A C A C G C T T C A A A C C C A C T C G G A T C A T C      2191

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TAAAAAATGAGTGAAGACCTCAGTCAAACCCTTCCAATCTTGCCTGAAGATAAATGCAAAACTGGAGGAATTAAC
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Query	35	TCTG-AAATGACTTATGTAGGAGCATACAGCTAATAGTGGTTATCCTGCCTGTNNAGACA	93
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Query	94	CCAGTATATGCGGAGGTGAAACGTGTGGAGATACCCTTCTAGGTATGGCCACACAGTGT	153
Sbjct	1745	CCAGTATATGCGGAGGTGAAACGTGTGGAGATACCCTTCTAGGTATGGCCACACAGTGT	1804
Query	154	GTCCAGGTAATAAAATGTAGTGAAGACCTCACCTCAAACCCCTTCCAATCTTGCCTGAAG	213
Sbjct	1805	GTCCAGGTAATAAAATGTAGTGAAGACCTCACCTCAAACCCCTTCCAATCTTGCCTGAAG	1864
Query	214	ATAAATGCAAACTTGGAGGAATTAACAATGTGCTTGTGCCTCATCAAAGGCCCTCGGTG	273
Sbjct	1865	ATAAATGCAAACTTGGAGGAATTAACAATGTGCTTGTGCCTCATCAAAGGCCCTCGGTG	1924
Query	274	TTCCAGCAGCCTGTCATCTTCTGGGAGCGGATGTACACACCCCCAGCAGGGGATGGG	333
Sbjct	1925	TTCCAGCAGCCTGTCATCTTCTGGGAGCGGATGTACACACCCCCAGCAGGGGATGGG	1984
Query	334	AAGAAACCTTCCATTTGCTGTGTTGGCAGTATGGATGGCCACCCAGCCGCTACTGT	393
Sbjct	1985	AAGAAACCTTCCATTTGCTGTGTTGGCAGTATGGATGGCCACCCAGCCGCTACTGT	2044
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Query	454	GAGGTCATCCAGGACCTGACTAACATGGTTCGAGAGCTGCTGATTCAAGTCTACAATCC	513
Sbjct	2105	GAGGTCATCCAGGACCTGACTAACATGGTTCGAGAGCTGCTGATTCAAGTCTACAATCC	2164
Query	514	ACACGCTTCAAACCCACTCGGATCATCTATTACCGTGGAGGGGTATCTGAGGGACAATG	573
Sbjct	2165	ACACGCTTCAAACCCACTCGGATCATCTATTACCGTGGAGGGGTATCTGAGGGACAATG	2224
Query	574	AAACAGGTAGCTTGGCCAGAATAATAGCAATTCGAAAGGCATGTATTANCTGGAAGAA	633
Sbjct	2225	AAACAGGTAGCTTGGCCAGAATAATAGCAATTCGAAAGGCATGTATTAGCTTGGGAAGAA	2284
Query	634	GATTACCGGCCAGGAATAACTTATATTGTGGTGCAAAAAGACATCACACAGCTCTTC	693
Sbjct	2285	GATTACCGGCCAGGAATAACTTATATTGTGGTGCAAAAAGACATCACACAGCTCTTC	2344
Query	694	TGTGCAGATAAAAACAGAAAGGGTAGGGAAAAGTGGCAATGTACCAGCAGGCCTACAGTG	753
Sbjct	2345	TGTGCAGATAAAAACAGAAAGGGTAGGGAAAAGTGGCAATGTACCAGCAGGCCTACAGTG	2404
Query	754	GATAGTACCATCACACATCCACTGTGAGTTTGTACTTTTACCTCTG ^N AGTCATGCAGGAATT	813

Home 3/18/11 11:36 AM
Comment: The N is G from Trace file, and it is confirmed by pGEXReverse sequence below


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Query 416  TGATGGTACTATCCACTGTAGTGCCTGCTGGTACATTGCCACTTTCCCTACCCTTCTG 475
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Sbjct 2417  TGATGGTACTATCCACTGTAGTGCCTGCTGGTACATTGCCACTTTCCCTACCCTTCTG 2358

Query 476  TTTTATCTGCACanaanaaTCGTGTGTGATGTCTTTTTGCACCACAATATAAGTTATTC 535
          |||
Sbjct 2357  TTTTATCTGCACAGAAGACTCGTGTGTGATGTCTTTTTGCACCACAATATAAGTTATTC 2298

Query 536  CTGGCCGGTAATCTTCTTCCAAGCTAATACATGCCTTCAAATGCTATTANNCTGGCC 595
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Sbjct 2297  CTGGCCGGTAATCTTCTTCCAAGCTAATACATGCCTTCAAATGCTATTAGTTCTGGCC 2238

Query 596  AANCTACCTGTTTCATTTGTCCCTCANATACCCCTCCACGGTAATAGATGATCCGAGTGG 655
          |||
Sbjct 2237  AAGCTACCTGTTTCATTTGTCCCTCAGATACCCCTCCACGGTAATAGATGATCCGAGTGG 2178

Query 656  GTTGAANCCTGTGGATTGTGANNACTGAATCAGCANCTCTCGAACCATGTTNAGTCANG 715
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Sbjct 2177  GTTGAAGCGTGTGGATTGTGAACTGAATCAGCAGCTCTCGAACCATGTT-AGTCAGG 2119

Query 716  TCCTGNATGACCTCTTGACTGTANANGAGCTCTTGGGANATCTCCTGCCGGGAAGTCTGC 775
          |||
Sbjct 2118  TCCTGGATGACCTCTTGACTGTAGAGGAGCTCTTGGGAGATCTCCTGCCGGGAAGTCTGC 2059

Query 776  ACCCGAACGGTGGNNCAGTACCGGNTGGGGTGGGCATCCATACTGCCAAC-ACAGCANCA 834
          |||
Sbjct 2058  ACCCGAACGGTGGCACAGTACCGGCTGGGGTGGCCATCCATACTGCCAACACAGCAGCA 1999

Query 835  ATGGAAGGTT-CN-CCCATCCC-TGCTGGGGGTGTGTGANATCNGCTCCN-G-ANNANG 889
          |||
Sbjct 1998  ATGGAAGGTTCTTCCCATCCCCTGCTGGGGGTGTGTGACATCCGCTCCAGGAAGATG 1939

Query 890  ANAGGCTGCTGGAANNC-GANGGCCTT 915
          |||
Sbjct 1938  ACAGGCTGCTGGAACACCGAGGCCTT 1912

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