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# Identification of some nucleotide mutations in *Waxy* gene (BGIOSGA022241) of a mutant rice line

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**Abstract:** *Waxy* genes of the original variety and its mutant type were sequenced by Sanger method and compared through Nucleotide Basic Local Alignment Search Tool (BLASTN) to clarify differences. BLASTN result showed four nucleotide mutations in coding regions and 59 nucleotide mutations in non-coding regions. Four point mutations in coding regions were: the deletion of T/- at position 34 and the insertion of -/T between positions 70 and 71 in exon 3; the substitution of C/T at position 14 in exon 4 and the substitution of T/C at position 115 in exon 9. In 59 mutant nucleotides in non-coding regions, some significant alterations were list: the deletion of nucleotide G at the first of intron 6 and the addition of 32 nucleotides "GGGCCTGCGAAGAACTGGGAGAATGTGCTCCT" at the end of intron 12. For the first trial, a new DNA marker was developed based on the mutation C/T at at position 14 in exon 4 and the substitution of T/C at position 115 in exon 9 to improve efficiency of rice breeding relevant to *Waxy* gene.

**Keywords:** *Waxy gene, BGIOSGA022241, amylose content, BLASTN, DNA marker development.*

## I. INTRODUCTION

Rice is a major staple and important food of population in over the world and the demand of it with good quality has been growing in the global market [1]. The endosperm starch is the important feature to assess the quality of rice [2, 3]. Two main properties of endosperm starch are amylose and amylopectine. The amylose is considered the most important predictor of sensory quality of rice and the low content of it is a desirable trait for rice breeding [4]. The synthesis of amylose is controlled mainly by *Waxy* gene, one of key genes [2,5]. Some alleles of *Waxy* gene were listed such as: *Wxa*, *Wxin*, *Wxb*, *Wxop*, and *wx* [6, 7, 8, 9]. Alleles *Wxa* and *Wxop* have been determined in Indica while alleles *Wxin*, *Wxb* and *wx* appeared in Japonica subspecies [9, 10, 11]. The amylose content of rice endosperm is controlled by complex pathways of the inhibition and the expression of *Waxy* gene through the synthesis of mRNA [12]. It was reported that the accumulation of 3.3 kb Wx mRNA (with intron 1) and 2.3 kb Wx mRNA (without intron 1) regulates the difference of amylose content [13]. Some mutations published to have effect on the cutting of intron 1 were the insertion of 16 nucleotides [6] or G/T substitution [14] at intron 1/ exon 1 junctions.

The SNP in exon 6, which is identified as *wx* allele, separates varieties with high and intermediate amylose content; and the SNP in exon 4 associating with opaque phenotype, is defined as the $Wx^{op}$ allele [9]. Some other SNPs at exon 6 (A/C) and exon 10 (C/T) in

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*Waxy* gene have been determined as the most significant impact on amylose content [15, 16, 17, 18]. Moreover, some changes of *Waxy* gene such as (C/T) SNP at point 2777 in boundary site of intron 7/exon 8 [19]; (A/G) SNP at position 497 from the start codon, leading to the Asp-165/Gly-165 substitution [3]; (G/T) SNP at position 497 (the Arg-158/His-158 substitution in exon 4) and (T/C) SNP at position 595 (Tyr-191/His-191 change in exon5) [20]; (GC/TT) SNP in intron 6, exon 7, intron 7, exon 8 and part of 3′ [21] or simple sequence repeats (CT)_(n) and (AATT)_(n) [1]... were considered to effect on amylose content.

It was reported that the diversity of *Waxy* genes among species of Oryzae genus was higher than among cultivations in the same subspecies [7]. Spontaneous mutations of *Waxy* gene were found in local rice cultivars from Asian and African countries [4, 10, 22, 23, 24]. In this study, we focused on clarifying the possible changes in *Waxy* gene (BGIOSGA022241) between two genotypes belonging to Oryza Sativa Indica subspecies: the original variety and its mutant type through some techniques such as: PCR, sequencing and, BLASTN.

## II. MATERIALS AND METHOD

### A. Materials

Rice samples were the original variety BT62 with high amylose content and its mutant type with lower amylose content.

### B. Methods

DNA extraction method: Total DNA of materials were extracted by DNeasy Plant Mini Kit (supplied by QIAGEN) [25].

PCR method: *Waxy* genes of original and mutant varieties were amplified by PCR method. The total 20 µl of PCR reaction contained 1 µl total DNA (1 ng/µl); 10 µl Prime STAR MAX DNA Polymerase mixture; 0.5 µl forward primer (20 pmol/µl or 20 µM); 0.5 µl reverse primer (20 pmol/µl or 20 µM); 8 µl $H_2O$. The PCR condition was: 98$^o$C - 2 minutes; 30 cycles of: 98$^o$C - 5 seconds, 60$^o$C - 5 seconds, 72$^o$C - 30 seconds; 72$^o$C - 5 minutes; Keep the sample at 4$^o$C. PCR products were analyzed on a 1.5% agarose gel and purified by QIAquick PCR Purification Kit (supplied by QIAGEN) [25].

Sequencing method: *Waxy* genes of original and mutant varieties were sequenced by Sanger method through the BigDye Terminator Sequencing Standard Kit (Thermofisher) with ABI PRISM 3100 Genetic Analyzer. Sequence reactions were conducted with the cocktail volume of 20 µl including 1 µl DNA (about 20 ng/µl); 4 µl Terminator Ready Reaction Mix; 4 pmol Primer; 11 µl $H_2O$ and the program: 94$^o$C - 2 minutes; 25 cycles of: 96$^o$C -10 seconds, 50$^o$C – 5 seconds, 60$^o$C – 4 minutes; Keep the sample at 4$^o$C. Sequence reactions were purified by DyeEx 2.0 Spin Kit (supplied by QIAGEN) [25] and read by ABI PRISM 3100 Genetic Analyzer.

Analysis method: The sequences of *Waxy* gene of original and mutant varieties were analyzed through Nucleotide Basic Local Alignment Search Tool (BLASTN).

## III. RESULTS

### A. Primer design and *Waxy* gene (BGIOSGA022241) amplification

The sequence of *Waxy* gene (BGIOSGA022241) was mined from database of Oryza sativa Indica [26]. It locates on chromosome 6 (from 1.931.535 to 1.935.014 forward strand) with 3479 bp including 13 exons and 12 introns (Fig. 1).

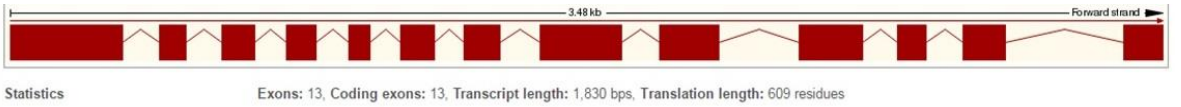


**Fig. 1.** The structure of *Waxy* gene (BGIOSGA022241) mined from database (Source: http://www.gramene.org)

Based on the mined information of *Waxy* gene, eight primer pairs (sixteen primers) were designed to amplify and sequence (Table I).

The full length of the *Waxy* gene (BGIOSGA022241) was amplified by forward primer Wx-1F and reverse primer Wx-8R with the size between 3 kb and 4 kb (Fig. 2).

**Table I.** The information of primers for *Waxy* gene study.

| **Name** | **Sequence (5'-3')** | **Name** | **Sequence (5'-3')** |
|---|---|---|---|
| Wx-1F | ACAGCAACAGCTAGACAACCACCAT | Wx-5F | AAGTACGACGCAACCACGGTAAGAA |
| Wx-1R | CTAATCGATCTTGTGATGATCTGA | Wx-5R | GTGGACTAGACGATCTGGGTTCAAA |
| Wx-2F | TGTGGTGCAATTCATTGCAGATCAA | Wx-6F | TTAGCCGGAAGACCTCTGAGCATTT |
| Wx-2R | CATCATGGATTCCTTCGAAGAAAGT | Wx-6R | GTAGTGTACCGACTTATCGGTATTA |
| Wx-3F | TGACAACAGGTGAGGATGTTGTGTT | Wx-7F | GTCTCAGCGTCGACGTAAGCCTATA |
| Wx-3R | ACGATGGACAGTAGTGCAGGGTTGT | Wx-7R | CCAGTTCTTCGCAGGCCCCTGAAAT |
| Wx-4F | CATCGACGGGTATGAGTAAGATTCT | Wx-8F | GAACAAGACGAACGGTCAAACATGT |
| Wx-4R | TTCGCCTCGATTGCCTGAAATTTGT | Wx-8R | CATATGTAGATCTCAGGCTCTTCAA |

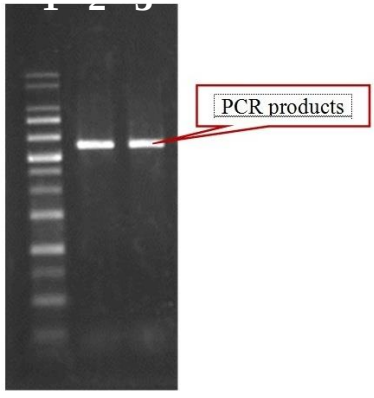


**Fig. 2.** PCR products of *Waxy* gene on agarose gel 1.5%
(1: DNA ladder 1kb; 2: PCR product of the original type; 3: PCR product of mutant type)

**B. Sequence the *Waxy* gene (BGIOSGA022241)**

The sequencing was conducted by Thermofisher's BigDye Terminator Sequencing Standard Kit and read by ABI PRISM 3100 Genetic Analyzer and results were shown in Fig. 3.

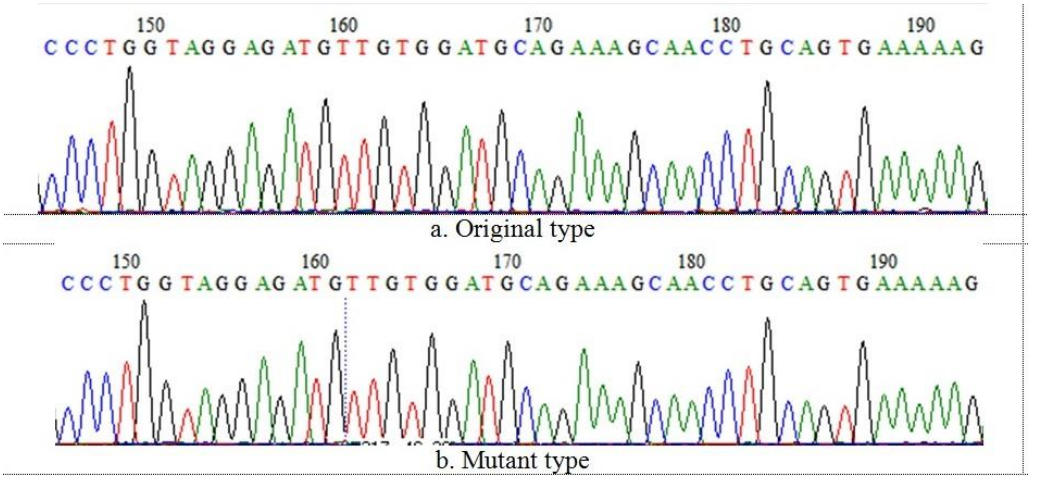


**Fig. 3.** The result of sequencing *Waxy* gene by Wx-3R primer
(a. Original type – a part sequences of *Waxy* gene in the original type; b. Mutant type - a part sequences of *Waxy* gene in the mutant type)

**C. The identification of mutant nucleotides in *Waxy* gene (BGIOSGA022241)**

The result of comparations between *Waxy* genes of the original and the mutant variety was shown in Fig. 4; Fig. 5.

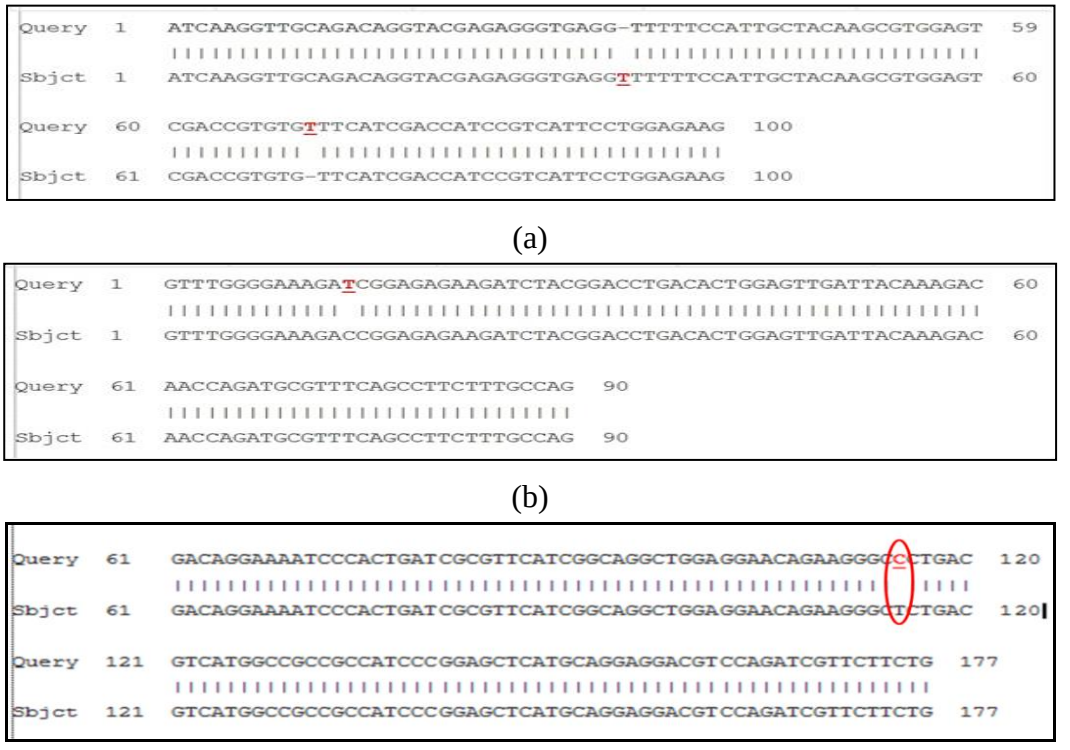


**Fig. 4.** BLASTN to identify mutation in coding region of *Waxy* gene
(a): Mutation(s) in exon 3; (b): Mutation(s) in exon 4; (c): Mutation(s) in exon 9
*(Note: Query- mutant type; Subject- original type)*

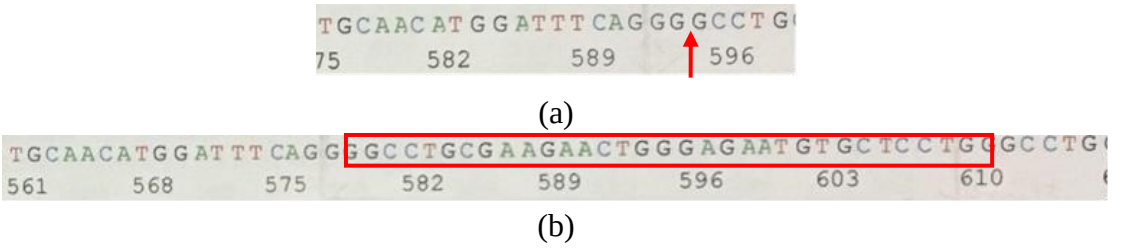


**Fig. 5.** The insertion of 32 nucleotides at the splipcing point of intron 12
(a): original sequences; (b): mutant seuqences

Total 3480 nucleotides of *Waxy* gene were analyzed via BLASTN and the result was shown in table II.

**D. Development of new DNA marker for rice mutation breeding**

Based on these point mutations, new DNA marker was developed to improve effeciency of rice mutation breeding (Table III).

**Table II.** The discovery of mutation in *Waxy* gene through BLASTN.

| **Gene region** | **Total** | **Identities (%)** | **Gaps (%)** | **Reference** |
|---|---|---|---|---|
| Exon | 1810 | 1806 (99,8%) | 4 (0,2%) | - Exon 3: 34 (T/-); 71 (-/T)<br>- Exon 4: 14 (C/T)<br>- Exon 9: 115 (T/C) |
| Intron | 1670 | 1611 (96,5%) | 59 (3,5%) | - Intron 3: 29 (T/-); 31 (T/-)<br>- Intron 5: 9 (T/C)<br>- Intron 6: Exon6/intron6 junction (G/-); 53 (T/-); 59 (T/-); 63 (T/-)<br>- Intron 8: 29 (A/G); 46 (-/T)<br>- Intron 9: 81 (A/G); 95 (A/G); 99 (-/TAA); 139 (G/A); 142 (A/G); 148 (C/T); 161 (A/G); 165 (C/T); 177 (G/C); 193 (G/A)<br>- Intron 11: 41 (T/C); 58 (A/G)<br>- Intron 12: 83 (A/T); 98 (G/A); 134 (A/C); intron12/exon13 junction (insertion of 32 nucleotides) |
| **Total** | **3480** | **3417 (98,2%)** | **63 (1,8%)** | |

**Table III.** The information of new developed DNA marker.

| **Sequence (5'- 3')** | **Target mutation** | **Annealing temperature** | **Expected size** |
|---|---|---|---|
| Wx-F: GATTTCAGGTTTGGGGAAAGA**T** | Nucleotide **T** at position 14 in exon 4 | 49.4 °C | 1271 bp |
| Wx-R: TGGCGGCGGCCATGACGTCAG**G** | Nucleotide **C** at position 115 in exon 9 | | |

*(Bold and underline character – mutation point)*

## IV. DISSCUSION

### A. Amplify and sequence the full length of *Waxy* gene

Sixteen primers were designed in Table I with lengths from 24 to 25 nucleotides. The Wx-1F primer was designed at boundary of 5'-UTR/exon 1 and the Wx-8R primer was designed at boundary of exon 13/3'-UTR. There was no failure in amplifying *Waxy* genes of both original variety and its mutant by Wx-1F and Wx-8R. It was indicated that there was no difference at junction sites. In agarose gel, there is only one band of PCR products and this band is bold and densitic (Fig. 2). These criteria are very important for the accuracy of sequencing.

Results in Fig. 3 were good at reading: no sequences were miss-calls (N), high concentration, no spaced peaks, only one color for each peak and lack of baseline (noise). The full *Waxy* genes of original type and mutant type were sequenced successfully by sixteen primers (Table I).

### B. Identify mutation in *Waxy* gene between the original type and its mutant type

Sequences of thirteen exons (coding regions) and twelve introns (non-coding regions) of *Waxy* gene from original and mutant lines were compared via BLASTN to identify mutation(s). The result in table II shown that, mutant rate in the non-coding region (3,5%) was higer than that in the coding region (0,2%). In coding region, there were 1806 identities (99.8%) and 4 gaps (0.2%) in coding region. Four gaps mean point mutations including: the deletion of T nucleotide (T/-) at point 34 and insertion of T (-/T) between points 70 and 71 (in exon 3); the substitution (C/T) at position 14 in exon 4 and the substitution T/C at position 115 in exon 9. In total of 1670 non-coding nucleotides compared, it was shown 1611 identities (96.5%) and 59 gaps (3.5%) (table II). The changes were listed: deletions (T/-) at positions 29 and 31 in intron 3; the change (T/C) at position 9 in intron 5; the deletions (T/-) at position 53, 59 and the deletion (A/-) at the position 63 of intron 6; the change (A/G) at position 29 and the insertion (-/T) between positions 45 and 46 in intron 8; the substitutions (A/G) at positions 81, 95, 142, 161, the changes (G/A) at positions 139 and 193, the changes (C/T) at positions 148 and 165, the change (G/C) at position 177 and the insertion (-/TAA) between positions 98 and 99 in intron 9; the alterations (T/C) at position 41 and (A/G) at position 58 in intron 11; the alterations (A/T) at position 83, (G/A) at position 98, (A/C) at position 134, (G/T) at position 206 and the addition of 32 nucleotides "GGGCCTGCGAAGAACTGGGAGAATGT GCTCCT" at the end of intron 12.

Four point mutations collected in coding regions (exons) (Fig. 4) will result the effect on translation directly. Because information of proteins for life is coded by triplets, thus with every mRNA there are three frame of translation. In theoretical, the structure of DNA is double strands, thus there are total six frame of reading. Based on the C/T mutation at position 14 in exon 4 will cause the replacement of "T" in the original type to "I" the in mutant type; or "P" to "S". The T/C mutation at position 115 in exon 9 resulted substitution of amino acid sequences "XAXNKX" in original type to "KALNKE" in mutant type; or "XXXTRX" to "RR*TRR"; or "XXX" to "GAE". Mutations in exon 3, the deletion of T nucleotide at point 34 and insertion of T at point between 70 and 71, will create the change of amino acids starting from the mutant site.

In total 59 gaps identified in non-coding regions, two types of mutation with more frequency than other ones were the deletion (T/-) (with five observations) and the substitution (A/G) (with six observations). Mutations at intron/exon junctions were also determined and listed: the deletion G/- at the first of intron 6 and the insertion of 32 nucleotides at the end of intron12 (fig. 5). These results leading to us the next research to interpret that if these changes are effective on the cutting of intron 6 and intron 12 or not and how they regulate on the amylose content.

### C. Development of new DNA marker for rice breeding relevant to amylose content

The forward primer Wx-F was designed based on the substitution C/T at position 14 in exon 4 with the length of 22 nucleotides and 40.9% GC content. The reverse primer Wx-R was designed based on the substitution T/C at position 115 in exon 9 with the length of 22 nucleotides and 72.7% GC content. The expected size of PCR product which is amplified by this new primer pair is 1271 bp and the recommended annealing temperature is 49.4$^{o}$C (table III).

The new developed DNA marker which was designed with both point mutations at 3' of two primers in pair: the forward primer Wx-F (5'- GATTTCAGGTTTGGGGAAAGA**T** - 3') with the change C/T at position 14 in exon4 (nucleotide T – bold and underline) and the reverse primer Wx-R (5' - TGGCGGCGGCCATGACGTCAG**G** - 3') with the substitution T/C at position 115 in exon 9 (nucleotide G – bold and underline). The 3' of primer which will be bind to the DNA strand firstly in transcription is better in conservating. Thus, the mutations were set in the first triplet of 3' to engage of the accuracy of mutant screening in PCR. This new developed DNA marker will be studied in further by being used back directly for its mutant population before applying for selection.

## V. CONCLUSIONS

(1) Four point mutations in coding regions (exon 3, exon 4 and exon 9) of *Waxy* gene would lead to the difference of amino acids in polypeptide in obvious.

(2) Some alterations at the first of intron 6 and the end of intron 12 will be done in more experiments to clarify their impact on expression of *Waxy* gene.

(3) It is important to study, utilize these mutants and new developed DNA marker to improve the efficiency of rice breeding with low amylose content.

## ACKNOWLEGMENT

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[25] https://www.qiagen.com

[26] http://www.gramene.org

## APPENDIX

The comparison between *Waxy* genes of original and mutant types

### Exon1

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 627 bits(339) | 0.0 | 339/339(100%) | 0/339(0%) | Plus/Plus |

```
Query  1    ATGTCGGCTCTCACCACGTCCCAGCTCGCCACCTCGGCCACCGGCTTCGGCATCGCCGAC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    ATGTCGGCTCTCACCACGTCCCAGCTCGCCACCTCGGCCACCGGCTTCGGCATCGCCGAC  60

Query  61   AGGTCGGCGCCGTCGTCGCTGCTCCGCCACGGGTTCCAGGGCCTCAAGCCCCGCAGCCCC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   AGGTCGGCGCCGTCGTCGCTGCTCCGCCACGGGTTCCAGGGCCTCAAGCCCCGCAGCCCC  120

Query  121  GCCGGCGGCGACGCGACGTCGCTCAGCGTGACGACCAGCGCGCGCGCGACGCCCAAGCAG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  GCCGGCGGCGACGCGACGTCGCTCAGCGTGACGACCAGCGCGCGCGCGACGCCCAAGCAG  180

Query  181  CAGCGGTCGGTGCAGCGTGGCAGCCGGAGGTTCCCCTCCGTCGTCGTGTACGCCACCGGC  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  CAGCGGTCGGTGCAGCGTGGCAGCCGGAGGTTCCCCTCCGTCGTCGTGTACGCCACCGGC  240

Query  241  GCCGGCATGAACGTCGTGTTCGTCGGCGCCGAGATGGCCCCCTGGAGCAAGACCGGCGGC  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  GCCGGCATGAACGTCGTGTTCGTCGGCGCCGAGATGGCCCCCTGGAGCAAGACCGGCGGC  300

Query  301  CTCGGTGACGTCCTCGGTGGCCTCCCCCCTGCCATGGCT  339
```

```
            ||||||||||||||||||||||||||||||||||||||
Sbjct  301  CTCGGTGACGTCCTCGGTGGCCTCCCCCCTGCCATGGCT  339
```

## Exon 2

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 150 bits(81) | 2e-42 | 81/81(100%) | 0/81(0%) | Plus/Plus |

```
Query  1   GCGAATGGCCACAGGGTCATGGTGATCTCTCCTCGGTACGACCAGTACAAGGACGCTTGG  60
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1   GCGAATGGCCACAGGGTCATGGTGATCTCTCCTCGGTACGACCAGTACAAGGACGCTTGG  60

Query  61  GATACCAGCGTTGTGGCTGAG  81
           |||||||||||||||||||||
Sbjct  61  GATACCAGCGTTGTGGCTGAG  81
```

## Exon 3

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 174 bits(94) | 2e-49 | 99/101(98%) | 2/101(1%) | Plus/Plus |

```
Query  1   ATCAAGGTTGCAGACAGGTACGAGAGGGTGAGG-TTTTTCCATTGCTACAAGCGTGGAGT  59
           |||||||||||||||||||||||||||||||| ||||||||||||||||||||||||||
Sbjct  1   ATCAAGGTTGCAGACAGGTACGAGAGGGTGAGGTTTTTTCCATTGCTACAAGCGTGGAGT  60

Query  60  CGACCGTGTGTTTCATCGACCATCCGTCATTCCTGGAGAAG  100
           |||||||||| ||||||||||||||||||||||||||||||
Sbjct  61  CGACCGTGTG-TTCATCGACCATCCGTCATTCCTGGAGAAG  100
```

## Exon 4

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 161 bits(87) | 1e-45 | 89/90(99%) | 0/90(0%) | Plus/Plus |

```
Query  1   GTTTGGGGAAAGATCGGAGAGAAGATCTACGGACCTGACACTGGAGTTGATTACAAAGAC  60
           |||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1   GTTTGGGGAAAGACCGGAGAGAAGATCTACGGACCTGACACTGGAGTTGATTACAAAGAC  60

Query  61  AACCAGATGCGTTTCAGCCTTCTTTGCCAG  90
           |||||||||||||||||||||||||||||
Sbjct  61  AACCAGATGCGTTTCAGCCTTCTTTGCCAG  90
```

## Exon 5

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 119 bits(64) | 4e-33 | 64/64(100%) | 0/64(0%) | Plus/Plus |

```
Query  1   GCAGCACTCGAGGCTCCTAGGATCCTAAACCTCAACAACAACCCATACTTCAAAGGAACT  60
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1   GCAGCACTCGAGGCTCCTAGGATCCTAAACCTCAACAACAACCCATACTTCAAAGGAACT  60
```

```
Query  61  TATG  64
           ||||
Sbjct  61  TATG  64
```

## Exon 6

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 187 bits(101) | 3e-53 | 101/101(100%) | 0/101(0%) | Plus/Plus |

```
Query  1    GTGAGGATGTTGTGTTCGTCTGCAACGACTGGCACACTGGCCCACTGGCGAGCTACCTGA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    GTGAGGATGTTGTGTTCGTCTGCAACGACTGGCACACTGGCCCACTGGCGAGCTACCTGA  60

Query  61   AGAACAACTACCAGCCCAATGGCATCTACAGGAATGCAAAG  101
            |||||||||||||||||||||||||||||||||||||||||
Sbjct  61   AGAACAACTACCAGCCCAATGGCATCTACAGGAATGCAAAG  101
```

## Exon 7

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 226 bits(122) | 9e-65 | 122/122(100%) | 0/122(0%) | Plus/Plus |

```
Query  1    TTTTCACTGCAGGTTGCTTTCTGCATCCACAACATCTCCTACCAGGGCCGTTTCGCTTTC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    TTTTCACTGCAGGTTGCTTTCTGCATCCACAACATCTCCTACCAGGGCCGTTTCGCTTTC  60

Query  61   GAGGATTACCCTGAGCTGAACCTCTCCGAGAGGTTCAGGTCATCCTTCGATTTCATCGAC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   GAGGATTACCCTGAGCTGAACCTCTCCGAGAGGTTCAGGTCATCCTTCGATTTCATCGAC  120

Query  121  GG  122
            ||
Sbjct  121  GG  122
```

## Exon 8

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 451 bits(244) | 6e-132 | 244/244(100%) | 0/244(0%) | Plus/Plus |

```
Query  1    GTATGACACGCCGGTGGAGGGCAGGAAGATCAACTGGATGAAGGCCGGAATCCTGGAAGC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    GTATGACACGCCGGTGGAGGGCAGGAAGATCAACTGGATGAAGGCCGGAATCCTGGAAGC  60

Query  61   CGACAGGGTGCTCACCGTGAGCCCGTACTACGCCGAGGAGCTCATCTCCGGCATCGCCAG  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   CGACAGGGTGCTCACCGTGAGCCCGTACTACGCCGAGGAGCTCATCTCCGGCATCGCCAG  120

Query  121  GGGATGCGAGCTCGACAACATCATGCGGCTCACCGGCATCACCGGCATCGTCAACGGCAT  180
```

```
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  GGGATGCGAGCTCGACAACATCATGCGGCTCACCGGCATCACCGGCATCGTCAACGGCAT  180

Query  181  GGACGTCAGCGAGTGGGATCCCAGCAAGGACAAGTACATCACCGCCAAGTACGACGCAAC  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  GGACGTCAGCGAGTGGGATCCCAGCAAGGACAAGTACATCACCGCCAAGTACGACGCAAC  240

Query  241  CACG  244
            ||||
Sbjct  241  CACG  244
```

## Exon 9

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 322 bits(174) | 2e-93 | 176/177(99%) | 0/177(0%) | Plus/Plus |

```
Query  1    GCAATCGAGGCGAAGGCGCTGAACAAGGAGGCGTTGCAGGCGGAGGCGGGTCTTCCGGTC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    GCAATCGAGGCGAAGGCGCTGAACAAGGAGGCGTTGCAGGCGGAGGCGGGTCTTCCGGTC  60

Query  61   GACAGGAAAATCCCACTGATCGCGTTCATCGGCAGGCTGGAGGAACAGAAGGGCCCTGAC  120
            |||||||||||||||||||||||||||||||||||||||||||||||||||| |||||
Sbjct  61   GACAGGAAAATCCCACTGATCGCGTTCATCGGCAGGCTGGAGGAACAGAAGGGCTCTGAC  120

Query  121  GTCATGGCCGCCGCCATCCCGGAGCTCATGCAGGAGGACGTCCAGATCGTTCTTCTG  177
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  GTCATGGCCGCCGCCATCCCGGAGCTCATGCAGGAGGACGTCCAGATCGTTCTTCTG  177
```

## Exon 10

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 355 bits(192) | 3e-103 | 192/192(100%) | 0/192(0%) | Plus/Plus |

```
Query  1    GGTACTGGAAAGAAGAAGTTCGAGAAGCTGCTCAAGAGCATGGAGGAGAAGTATCCGGGC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    GGTACTGGAAAGAAGAAGTTCGAGAAGCTGCTCAAGAGCATGGAGGAGAAGTATCCGGGC  60

Query  61   AAGGTGAGGGCCGTGGTGAAGTTCAACGCGCCGCTTGCTCATCTCATCATGGCCGGAGCC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   AAGGTGAGGGCCGTGGTGAAGTTCAACGCGCCGCTTGCTCATCTCATCATGGCCGGAGCC  120

Query  121  GACGTGCTCGCCGTCCCCAGCCGCTTCGAGCCCTGTGGACTCATCCAGCTGCAGGGGATG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  GACGTGCTCGCCGTCCCCAGCCGCTTCGAGCCCTGTGGACTCATCCAGCTGCAGGGGATG  180

Query  181  AGATACGGAACG  192
            ||||||||||||
Sbjct  181  AGATACGGAACG  192
```

## Exon 11

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 161 bits(87) | 1e-45 | 87/87(100%) | 0/87(0%) | Plus/Plus |

```
Query  1   CCCTGTGCTTGCGCGTCCACCGGTGGGCTCGTGGACACGGTCATCGAAGGCAAGACTGGT  60
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1   CCCTGTGCTTGCGCGTCCACCGGTGGGCTCGTGGACACGGTCATCGAAGGCAAGACTGGT  60

Query  61  TTCCACATGGGCCGTCTCAGCGTCGAC  87
           |||||||||||||||||||||||||||
Sbjct  61  TTCCACATGGGCCGTCTCAGCGTCGAC  87
```

## Exon 12

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 239 bits(129) | 1e-68 | 129/129(100%) | 0/129(0%) | Plus/Plus |

```
Query  1    TGCAAGGTGGTGGAGCCAAGCGACGTGAAGAAGGTGGCGGCCACCCTGAAGCGCGCCATC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    TGCAAGGTGGTGGAGCCAAGCGACGTGAAGAAGGTGGCGGCCACCCTGAAGCGCGCCATC  60

Query  61   AAGGTCGTCGGCACGCCGGCGTACGAGGAGATGGTCAGGAACTGCATGAACCAGGACCTC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   AAGGTCGTCGGCACGCCGGCGTACGAGGAGATGGTCAGGAACTGCATGAACCAGGACCTC  120

Query  121  TCCTGGAAG  129
             |||||||||
Sbjct  121  TCCTGGAAG  129
```

## Exon 13

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 158 bits(85) | 2e-44 | 85/85(100%) | 0/85(0%) | Plus/Plus |

```
Query  1   GGGCCTGGGCGTCGCCGGCAGCGCGCCGGGGATCGAAGGCGACGAGATCGCGCCGCTCGC  60
           |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1   GGGCCTGGGCGTCGCCGGCAGCGCGCCGGGGATCGAAGGCGACGAGATCGCGCCGCTCGC  60

Query  61  CAAGGAGAACGTGGCTGCTCCTTGA  85
           |||||||||||||||||||||||||
Sbjct  61  CAAGGAGAACGTGGCTGCTCCTTGA  85
```

## Intron 1

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 209 bits(113) | 8e-60 | 113/113(100%) | 0/113(0%) | Plus/Plus |

```
Query  1    GTAAGCACACACAAACTTCGATCGCTCGTCGTCGCTGACCGTCGTCGTCTTCAACTGTTC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    GTAAGCACACACAAACTTCGATCGCTCGTCGTCGCTGACCGTCGTCGTCTTCAACTGTTC  60
```

```
Query  61   TTGATCATCGCATTGGATGGATGTGTAATGTTGTGTTCTTGTGTTCTTTGCAG  113
            ||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   TTGATCATCGCATTGGATGGATGTGTAATGTTGTGTTCTTGTGTTCTTTGCAG  113
```

## Intron 2

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 198 bits(107) | 2e-56 | 107/107(100%) | 0/107(0%) | Plus/Plus |

```
Query  1    GTAGGAGCATATGCGTGATCAGATCATCACAAGATCGATTAGCTTTAGATGATTTGTTAC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    GTAGGAGCATATGCGTGATCAGATCATCACAAGATCGATTAGCTTTAGATGATTTGTTAC  60

Query  61   ATTTCGCAAGATTTTAACCCAAGTTTTTGTGGTGCAATTCATTGCAG  107
            |||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   ATTTCGCAAGATTTTAACCCAAGTTTTTGTGGTGCAATTCATTGCAG  107
```

## Intron 3

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 169 bits(91) | 1e-47 | 96/98(98%) | 2/98(2%) | Plus/Plus |

```
Query  1    GTGGAGTCATCATTAGTTTACCtttttt-g-tttttACTGAATTATTAACAGTGCATTTA  58
            |||||||||||||||||||||||||| | ||||||||||||||||||||||||||||||
Sbjct  1    GTGGAGTCATCATTAGTTTACCTTTTTTTGTTTTTTTACTGAATTATTAACAGTGCATTTA  60

Query  59   GCAGTTGGACTGAGCTTAGCTTCCACTGGTGATTTCAG  96
            ||||||||||||||||||||||||||||||||||||||
Sbjct  61   GCAGTTGGACTGAGCTTAGCTTCCACTGGTGATTTCAG  98
```

## Intron 4

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 182 bits(98) | 1e-51 | 98/98(100%) | 0/98(0%) | Plus/Plus |

```
Query  1    GTCAGTGATTACTTCTATCTGATGATGGTTGGAAGCATCACGAGTTTACCATAGTATGTA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    GTCAGTGATTACTTCTATCTGATGATGGTTGGAAGCATCACGAGTTTACCATAGTATGTA  60

Query  61   TGGATTCATAACTAATTCGTGTATTGATGCTACTGCAG  98
            ||||||||||||||||||||||||||||||||||||||
Sbjct  61   TGGATTCATAACTAATTCGTGTATTGATGCTACTGCAG  98
```

## Intron 5

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 165 bits(89) | 1e-46 | 91/92(99%) | 0/92(0%) | Plus/Plus |

```
Query  1    GTGAGTTACAATTGATCTCAAGATCTTATAACTTTCTTCGAAGGAATCCATGATGATCAG  60
            |||||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||
```

```
Sbjct  1   GTGAGTTATAATTGATCTCAAGATCTTATAACTTTCTTCGAAGGAATCCATGATGATCAG  60

Query  61  ACTAATTCCTTCCGGTTTGTTACTGACAACAG  92
           ||||||||||||||||||||||||||||||||
Sbjct  61  ACTAATTCCTTCCGGTTTGTTACTGACAACAG  92
```

## Intron 6

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 130 bits(70) | 3e-36 | 78/81(96%) | 3/81(3%) | Plus/Plus |

```
Query  1   GTCTATGCTTGTTCTTGCCATACCAACTCAAATCTGCATGCACACTGCATT-CTGTT-CA  58
           |||||||||||||||||||||||||||||||||||||||||||||||||| ||||| ||
Sbjct  2   GTCTATGCTTGTTCTTGCCATACCAACTCAAATCTGCATGCACACTGCATTTCTGTTTCA  61

Query  59  G-AAACTGACTGTCTGAATCT  78
           | ||||||||||||||||||
Sbjct  62  GAAAACTGACTGTCTGAATCT  82
```

## Intron 7

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 224 bits(121) | 3e-64 | 121/121(100%) | 0/121(0%) | Plus/Plus |

```
Query  1    GTATGAGTAAGATTCTAAGAGTAACTTACTGTCAATTCGCCATATATCGATTCAATCCAA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    GTATGAGTAAGATTCTAAGAGTAACTTACTGTCAATTCGCCATATATCGATTCAATCCAA  60

Query  61   GATCCTTTTGAGCTGACAACCCTGCACTACTGTCCATCGTTCAAATCCGGTTAAATTTCA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   GATCCTTTTGAGCTGACAACCCTGCACTACTGTCCATCGTTCAAATCCGGTTAAATTTCA  120

Query  121  G  121
            |
Sbjct  121  G  121
```

## Intron 8

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 204 bits(110) | 4e-58 | 115/117(98%) | 1/117(0%) | Plus/Plus |

```
Query  1    GTAAGAACGAATGCATTCTTCACAAGATGTGCAATCTGAATTTTCTTTGAAAAAGAAATT  60
            |||||||||||||||||||||||||| ||||||||||||||| ||||||||||||||
Sbjct  1    GTAAGAACGAATGCATTCTTCACAAGATATGCAATCTGAATTTTC-TTGAAAAAGAAATT  59

Query  61   ATCATCTGTCACTTCTTGATTGATTCTGACAAGGCAAGAATGAGTGACAAATTTCAG  117
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||
```

```
Sbjct  60   ATCATCTGTCACTTCTTGATTGATTCTGACAAGGCAAGAATGAGTGACAAATTTCAG  116
```

## Intron 9

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 375 bits(203) | 4e-109 | 231/243(95%) | 3/243(1%) | Plus/Plus |

```
Query  1    GTATAATATAATACACTACAAGACACACTTGCACGATATGCCAAAAATTCAGAACAAATT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    GTATAATATAATACACTACAAGACACACTTGCACGATATGCCAAAAATTCAGAACAAATT  60

Query  61   CAGTGGCaaaaaaaaaaCTCGAATATTAGGGAAGGACCTAATAATATCAAATAATTAGAA  120
            |||||||||||||||||| |||||||||||||  |||   ||||||||||||||||||||
Sbjct  61   CAGTGGCAAAAAAAAAACTCAAATATTAGGGAAGAACC---TAATATCAAATAATTAGAA  117

Query  121  GGGGTGAGGCTTTGAACCCAGATCGTCTAGTCCACCACCTTGTGGAGTTAGCCGGAAGAC  180
            |||||||||||||||||||| || ||||| |||||||||||| ||| ||||||||||||
Sbjct  118  GGGGTGAGGCTTTGAACCCAGGTCATCTAGCCCACCACCTTGTAGAGCTAGCCGGAAGAG  177

Query  181  CTCTGAGCATTTCTCAATTCAGTGGCAAATGATGTGTATAATTTTGATCCGTGTGTGTTT  240
            ||||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  178  CTCTGAGCATTTCTCGATTCAGTGGCAAATGATGTGTATAATTTTGATCCGTGTGTGTTT  237

Query  241  CAG  243
            |||
Sbjct  238  CAG  240
```

## Intron 10

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 196 bits(106) | 5e-56 | 106/106(100%) | 0/106(0%) | Plus/Plus |

```
Query  1    GTATACAATTTCCATCTATCAATTCGATTGTTCGATTTCATCTTTGTGCAATGCAATGCA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    GTATACAATTTCCATCTATCAATTCGATTGTTCGATTTCATCTTTGTGCAATGCAATGCA  60

Query  61   ATTGCAAATGCAAATGCATGATGATTTTCCTTGTTGATTTCTCCAG  106
            |||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   ATTGCAAATGCAAATGCATGATGATTTTCCTTGTTGATTTCTCCAG  106
```

## Intron 11

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 191 bits(103) | 3e-54 | 107/109(98%) | 0/109(0%) | Plus/Plus |

```
Query  1    GTAAGCCTATACATTTACATAACAATCAGATATGACACATCCTAATACCGATAAGTCGGT  60
            ||||||||||||||||||||||||||||||||||||||| ||||||||||||||||| ||
Sbjct  1    GTAAGCCTATACATTTACATAACAATCAGATATGACACATCCTAATACCGATAAGTCGGT  60
```

```
Sbjct  1    GTAAGCCTATACATTTACATAACAATCAGATATGACACATTCTAATACCGATAAGTCAGT  60

Query  61   ACACTACTACACATTTACATGGTTGCTGGTTATATGGttttttGGCAG  109
            |||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   ACACTACTACACATTTACATGGTTGCTGGTTATATGGTTTTTTTGGCAG  109
```

## Intron 12

| Score | Expect | Identities | Gaps | Strand |
|---|---|---|---|---|
| 638 bits(345) | 0.0 | 353/357(99%) | 0/357(0%) | Plus/Plus |

```
Query  1    GTATAAATTACGAAACAAATTTAACCCAAACATATACTATATACTCCCTCCGCTTCTAAA  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    GTATAAATTACGAAACAAATTTAACCCAAACATATACTATATACTCCCTCCGCTTCTAAA  60

Query  61   TATTCAACGCCGTTGTCTTTTTTAAATATGTTTGACCATTCGTCTTATTaaaaaaaTTAA  120
            |||||||||||||||||||||| ||||||||||||| ||||||||||||||||||||||
Sbjct  61   TATTCAACGCCGTTGTCTTTTTAAAATATGTTTGACCGTTCGTCTTATTAAAAAAATTAA  120

Query  121  ATAATTATAAATTCTTTTCCTATCATTTGATTCATTGTTAAATATACTTATATGTATACA  180
            |||||||||||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  ATAATTATAAATTATTTTCCTATCATTTGATTCATTGTTAAATATACTTATATGTATACA  180

Query  181  TATAGTTTTACATATTTCATAAAATTTTTTGAACAAGACGAACGGTCAAACATGTGCTAA  240
            |||||||||||||||||||||| ||||||||||||||||||||||||||||||||||||
Sbjct  181  TATAGTTTTACATATTTCATAAAAGTTTTTGAACAAGACGAACGGTCAAACATGTGCTAA  240

Query  241  AAAGTTAACGGTGTCGAATATTCAGAAACGGAGGGAGTATAAACGTCTTGTTCAGAAGTT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  AAAGTTAACGGTGTCGAATATTCAGAAACGGAGGGAGTATAAACGTCTTGTTCAGAAGTT  300

Query  301  CAGAGATTCACCTGTCTGATGCTGATGATGATTAATTGTTTGCAACATGGATTTCAG  357
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  301  CAGAGATTCACCTGTCTGATGCTGATGATGATTAATTGTTTGCAACATGGATTTCAG  357
```