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Title: Transcriptional Regulation by λBacteriophage Antitermination

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Abstract

As a regulator of transcription, antitermination works to allow for RNA polymerase to read through termination signals and express genes found downstream of these termination signals. The Q protein of phage λ displays this antitermination phenotype in *E.coli*. It works to allow for the expression of promoter distal genes by letting RNAP transcribe through termination signals that block these genes. Q function overlays the phage late gene promoter P_R' and requires the presence of a DNA element called the Q-utilization (*qut*) site. The *qut* site must contain Q-binding sequences and a specific pause site for the Q protein to be able to modify RNAP for the antitermination phenotype. Mutations in the *qut* site (-13/-15) have been shown to decrease antitermination efficiency by preventing Q protein binding. This research seeks to identify regions of the Q protein that interact with *qut* DNA, using a second site suppressor analysis to identify mutant Q genes that allow for antitermination phenotype on mutant (-13/-15) *qut* sites.

Key words: *qut* DNA, Q-binding sequences, σ^{70}, Second site suppressor, RNAP, β-flap domain

Introduction

In prokaryotic systems transcription regulation is necessary for cells to function properly. Regulation occurs in either the initiation or the elongation/termination phase of transcription. Regulation of initiation is dependent on RNA polymerase (RNAP) binding to promoter DNA and beginning transcription (Burgess et al. 1969). Termination is regulated by; RNAP reaching a termination signal that codes for a stop to transcription. Genes found promoter proximal to this termination signal will be expressed but genes found promoter distal will not. Antitermination is a type of regulation of transcription that allows for RNAP to read through termination signals (Fig. 1). This mechanism of regulation can be seen in the bacteriophage ʎ that infects *E.coli* (Yang et al. 1987). The phage's Q protein allows for the expression of late genes for lytic cycle because it allows RNAP to read through late gene terminators (Yang and Roberts 1989). The ʎQ gene of the bacteriophage produces ʎQ protein that interacts with *qut* site DNA that overlaps the P_R' promoter after this interaction occurs the ʎQ protein can interact with RNAP to create the antitermination phenotype (Yang et al. 1987).

The location of this protein-DNA interaction is called the Q-utilization site (*qut*) and overlaps the P_R' late promoter. A functional *qut* site requires a Q binding element (QBE) and a signal for transcription pausing by RNAP after synthesis of 16 nucleotide (Fig. 2) (Yang et al. 1987). The QBE spans the -35 to the -10 consensus elements and is the location for ʎQ protein interaction with DNA and the RNAP complex (Yarnell and Roberts 1992). The pause at +16 is recognized by the σ^{70} subunit that is retained by RNAP *E.coli* in the initial elongation stages of P_R' and is necessary to allow for ʎQ protein interaction with DNA and RNAP (Bar-Nahum and Nudler 2001, Ko et al. 1998). The modification of RNAP at the pause site is described as; region

4 of σ^{70} subunit becomes displaced from β-flap subunit causing a loss of function for region 4 (Deighan et al. 2008). ΛQ protein modifies RNAP by attaching region 4 to the DNA then ΛQ attaches itself to the now available β-flap subunit creating the antitermination complex (Deighan et al. 2008).

The interaction of the ΛQ protein and the *qut* DNA is not fully understood. Researchers know that a pause site at +16 and the QBE between the -35 and -10 consensus of the *qut* are necessary. The QBE allows the ΛQ protein to bind to DNA allowing for interactions between the protein and RNAP are necessary for antitermination (Yang et al. 1987). Mutations in pause site of *qut* do not allow for ΛQ protein to functionally bind to QBE and RNAP (Ko et al. 1998). Mutations of the QBE, notably between -13/-26, also decrease ΛQ protein's ability to attach to QBE, leading to reduced efficiency of transcription antitermination (Guo and Roberts 2004).

Research conducted by Guo and Roberts(2004) found that -13 and -15, of the *qut* site, are involved with ΛQ protein binding to QBE and when mutated greatly affected ΛQ protein's ability to bind and function as an antiterminator. Mutant ΛQ proteins were identified that were able to restore Q-binding function in the -13/-15 mutant *qut* site, but did not restore antitermination phenotype (Guo and Roberts 2004). These mutations were all clustered in the C-terminal region of the protein (Guo and Roberts 2004). with binding being restored but no antitermination phenotype it suggests more complex interactions between the *qut* DNA and ΛQ protein that need to be examined further in order to understand antitermination. This research seeks to identify regions of ΛQ protein that interact with *qut* DNA by using a second site suppressor approach for restored antitermination phenotype of the -13/-15 double point

mutation. By mutating ʎQ gene we will be able to find a mutant ʎQ protein that will restore antitermination function to -13/-15 *qut* mutant.

Methods

Using a second site suppressor analysis, two companion plasmids were used to test for ʎQ antitermination function. The first plasmid was used as a regulated source of Q protein and the second plasmid was the means for testing of antitermination by the Q protein. The first plasmid, pPLQ^{ʎ}, contained a kanamycin resistance gene and the ʎQ gene that is expressed from the ʎ promoter P_L' and expression is regulated by a temperature sensitive mutant cI protein, cI857 (Fig. 3)(Breitling et al. 1990). ʎcI857 only allows for the ʎQ gene to be expressed when incubated at 42°C. the second plasmid, Chloramphenicol acetyltransferase (+49$t_{82}t_0$CAT) plasmid is the companion plasmid that contains an ampicillin resistance gene, *ʎ qut* site, two termination signals, and a chloramphenicol resistance (CAT) gene (Fig. 4). In this plasmid the chloramphenicol resistance will only be expressed if the ʎQ protein from pPLQ^{ʎ} is able to bind to the CAT plasmid *qut* site and have antitermination function.

pPLQ^{ʎ} plasmids were transformed into *E.coli* strain GM3135 (Rewinksi and Marinus 1987). Transformed cells were plated on agar containing LB+Kanamycin and incubated for 24 hours at 30°C selecting for cells containing the pPLQ^{ʎ} plasmid. pPLQ^{ʎ} was isolated directly from pooled transformants using a SV Wizard Miniprep Kit (Promega) the mutagenized plasmids were isolated from the selected cells for testing of -13/-15 *qut* site mutation suppression.

All experiments utilized *E.coli* strain DH5α (Hanahan 1985). Mutant pPLQ^{ʎ} plasmids were transformed into the cells containing +49(-13/-15)$t_{82}t_0$CAT plasmid (Table 1) and plated on agar

containing LB+Kanamycin+Ampicillin then incubated for 24 hours at 30°C to select for successful uptake of both companion plasmids. Successfully transformed cells were replica-plated on agar containing LB+Kanamycin+Ampicillin and 100µg/ml of chloramphenicol to select for Q mediated antitermination (First 2012). The plates were then incubated at 42°C until colonies appeared. Wild-type pPLQ$^{\lambda}$, pAPYC, and +49$t_{82}t_0$CAT were included for experimental controls (Table 1). The expected results of these controls were determined (Fig. 5).

Cells containing candidate mutant ʎQ for antitermination phenotype had the pPLQ$^{\lambda}$ plasmid isolated again using the SV Wizard Miniprep Kit (Promega). These plasmids were then tested using agarose gel electrophoresis to confirm the ability for cells with the -13/-15 *qut* mutation to grow in the presence of chloramphenicol was due to the Q plasmid. Phenotype expression of the purified mutant plasmids was also tested again with cells ability to grow on plates containing chloramphenicol. All successful plasmids were then sequenced.

Results

Second Site Suppressors

After screening 3000+ colonies seven possible mutant ʎQ plasmids were isolated (Fig. 6). These seven mutants were able to grow on media containing chloramphenicol at ability comparable to the wild-type ʎQ and +49$t_{82}t_0$CAT. It was then thought the seven ʎQ mutants were able to display the antitermination phenotype that allowed transcription through the termination signals on the +49$t_{82}t_0$CAT plasmid and allowing for the expression of the CAT gene. Mutant plasmids labeled 1, 2, 4, 5, 6, and 7 were purified from cells. Cells containing the

mutant labeled 3 were unable to be grown in broth containing LB+Kanamycin for purification. The phenotypes of the six purified plasmids could not be confirmed when retested.

Sequence Analysis

All 6 purified mutant ΛQ plasmids were sequenced. The sequences of 1, 2, 4, and 5 were compared to a known sequence of the wild-type ΛQ genome (GenBank). Mutant ΛQ plasmids 6 and 7 did not have readable sequences. The sequences of 1, 2, 4, and 5 all showed to have the same nucleotide changes in the ΛQ gene (Fig. 7). These nucleotide mutations resulted in a single change of amino acid 23 from alanine to threonine (Fig. 8). The amino acid mutation is not found in the primary region identified by Guo and Roberts that is responsible for ΛQ binding to DNA (2004).

Discussion

I identified seven cell colonies with ΛQ gene mutants that were able to that could grow on chloramphenicol. Of the seven mutants, six mutant plasmids were able to be purified. The antitermination function of these plasmids could not be confirmed by further testing. From the plasmids purified four of the mutants with readable sequences were used for comparison with the wild-type sequence of the ΛQ gene.

Only six mutant plasmids were purified because mutant plasmid 3's inability to grow in broth containing LB+kanamycin. The cells containing mutant plasmid 3 may have been an artifact in their ability to grow on the plates containing chloramphenicol due to long incubation times. The cells also may not have been viable at the time they were transferred into the broth for purification. The phenotypes of the six purified plasmids could not be confirmed because

cells they were transformed into showed no growth on media containing chloramphenicol. Controls used also showed no growth on media containing chloramphenicol suggesting a problem with media used.

The four sequences used for comparison; mutants 1, 2, 4, and 5. It was found that all consisted of the same nucleotide mutations that did not result in an amino acid changes in the region primarily responsible for ΛQ interaction with DNA (Guo and Roberts 2004). A hypothesis for the returned antitermination phenotype is that the mutation that occurred in these four mutant ΛQ plasmids caused an up-regulation of the ΛQ promoter allowing for a higher rate of production for ΛQ protein. This hypothesis would follow the findings of Deighan and Hochschild (2007) in that with a greatly increased amount of Q present the *qut* site is not required for Q binding.

Future work should be done to confirm the up-regulation of ΛQ promoter hypothesis with these mutations. The mutant plasmids that were sequenced will be transformed into *E.coli* cells containing the +49$t_{82}t_0$CAT plasmid. These cells can then be compared to cells with the wild-type pPLQ$^{\Lambda}$ and +49$t_{82}t_0$CAT plasmids for growth on media containing chloramphenicol. If the rate of growth for cells containing the mutant plasmids are greater than the cells containing the wild-type plasmid then the upregulation hypothesis is viable.

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Table 1. Plasmids used for second site repressor experiments

Q plasmid	*qut* plasmid	Controls and Variable
pPLQ$^{\lambda}$	+49$t_{82}t_0$CAT	Positive control
pACYC	+49(-13/-15)$t_{82}t_0$CAT	Negative controls
	+49$t_{82}t_0$CAT	
	+49(-13/-15)$t_{82}t_0$CAT	
pPLQ$^{\lambda}$ (mutant)	+49(-13/-15)$t_{82}t_0$CAT	Experimental

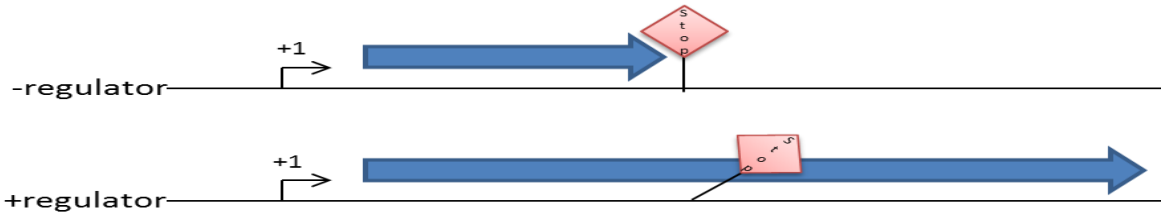

Figure 1. Example of antitermination regulation with regulator being ʎQ protein

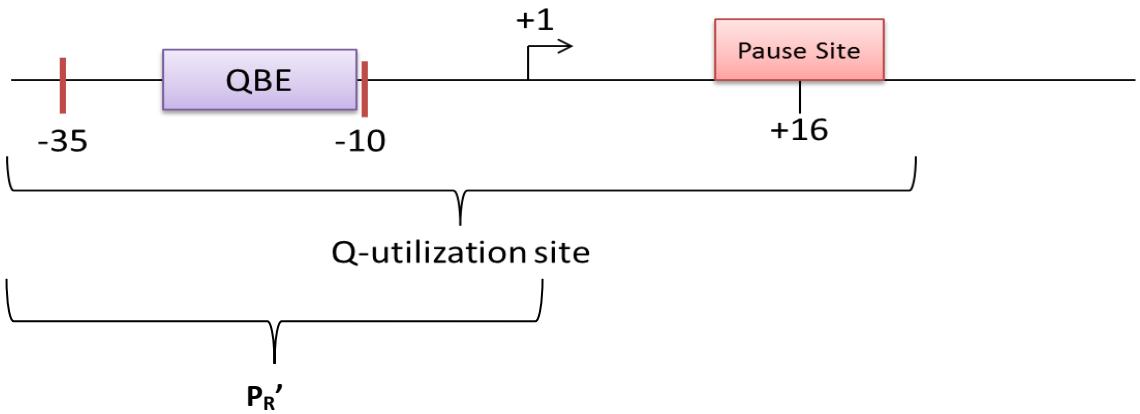

Figure 2. Q-utilization site (*qut*) with pause and Q-binding sequence regions

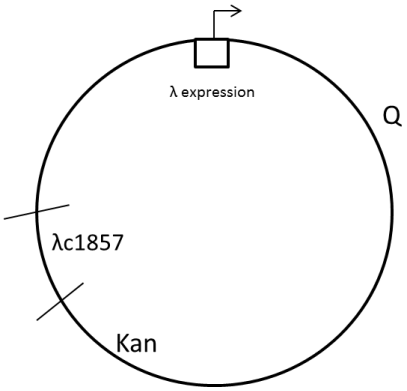

Figure 3. pPLQ$^{\lambda}$ Plasmid with Q gene, Kanamycin resistant gene, and ΛcI1857

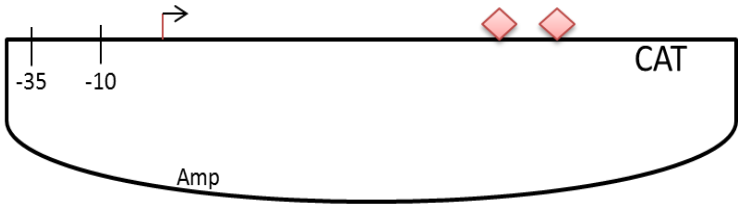

Figure 4. Chloramphenicol acetyltransferase (+49$t_{82}t_0$CAT) plasmid containing ampicillin resistance gene, *Λ qut* site, termination signals, and CAT gene

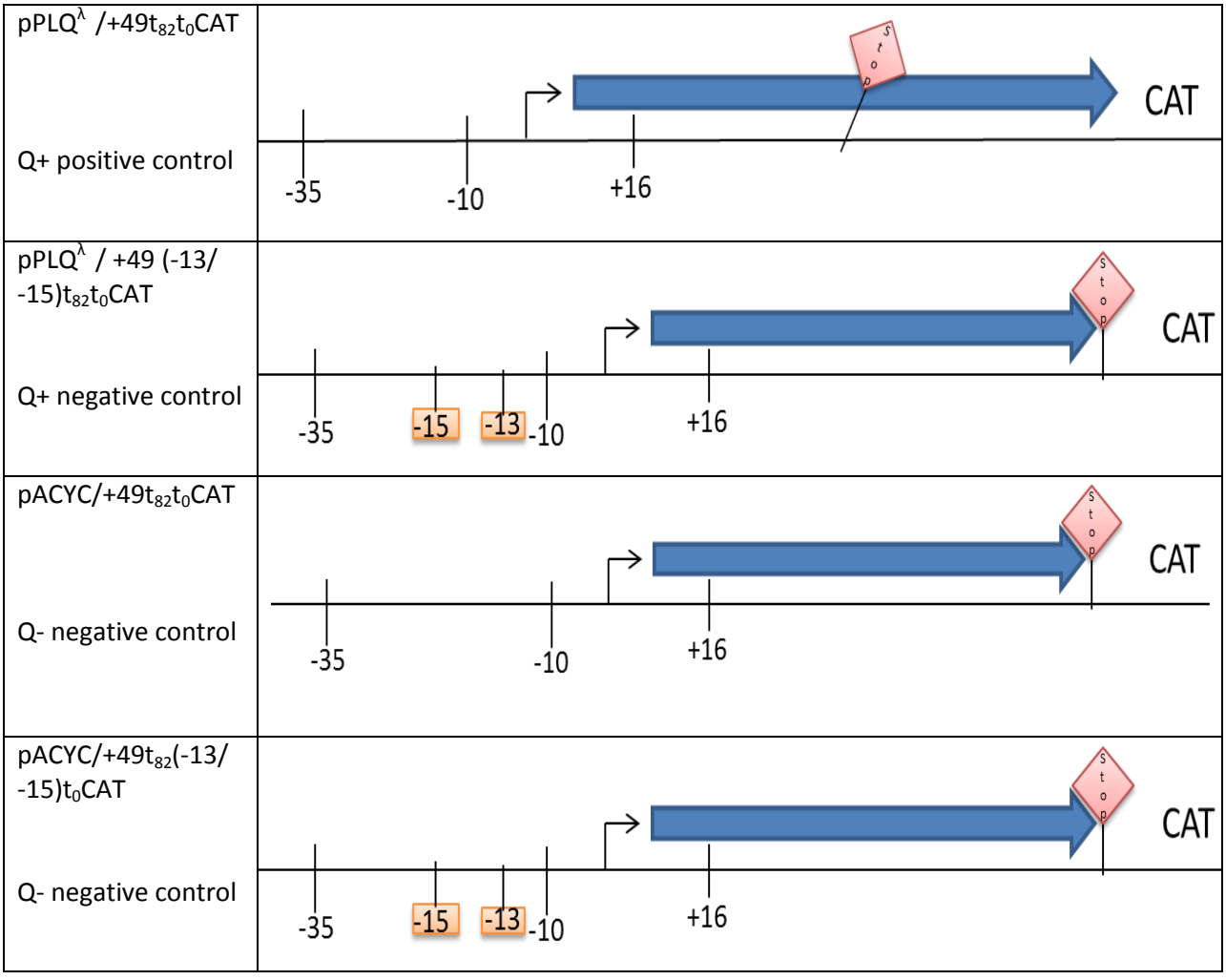

Figure 5. Expected antitermination phenotypes with control plasmids for the expression of the CAT gene

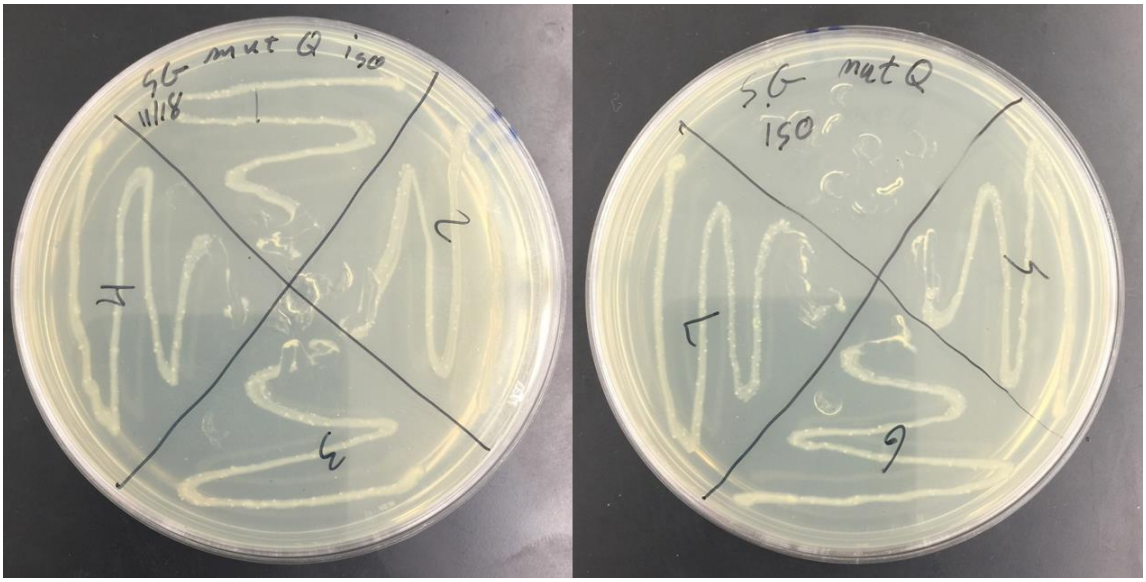

Figure 6. Showing cells containing the seven mutant ΛQ plasmids that displayed growth on chloramphenicol

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1>ATGAGACTCGAAAGCGTAGCTAAATTTCACTCGCCAAAAAGCCCGATGATGAGCGACTCACCACGGACCACGGCTTCTGACTCTCTTTCCGGTACTGATG>100
1>ATGAGACTCGAAAGCGTAGCTAAATTTCATTCGCCAAAAAGCCCGATGATGAGCGACTCACCACGGGCCACGGCTTCTGACTCTCTTTCCGGTACTGATG>100
                *         *         *         *         *         *         *         *         *         *
101>TGATGGCTGCTATGGGGATGGCGCAATCACAAGCCGGATTCGGAATGGCTGCATTCTGCGGTAAGCATGAACTCAGCCAGAACGACAAACAAAAGGCTAT>200
101>TGATGGCTGCTATGGGGATGGCGCAATCACAAGCCGGATTCGGTATGGCTGCATTCTGCGGTAAGCACGAACTCAGCCAGAACGACAAACAAAAGGCTAT>200
                *         *         *         *         *         *         *         *         *         *
201>CAACTATCTGATGCAATTTGCACACAAGGTATCGGGGAAATACCGTGGTGTGGCAAAGCTCGAAGGAAATACTAAGGCAAAGGTACTGCAAGTGCTCGCA>300
201>CAACTATCTGATGCAATTTGCACACAAGGTATCGGGGAAATACCGTGGTGTGGCAAAGCTTGAAGGAAATACTAAGGCAAAGGTACTGCAAGTGCTCGCA>300
                *         *         *         *         *         *         *         *         *         *
301>ACATTCGCTTATGCGGATTATTGCCGTAGTGCCGCGACGCCGGGCGCAAGATGCAGAGATTGCCACGGTACAGGCCGTGCGGTTGATATTGCCAAAACAG>400
301>ACATTCGCTTATGCGGATTATTGCCGTAGTGCCGCGACGCCGGGGGCAAGATGCAGAGATTGCCATGGTACAGGCCGTGCGGTTGATATTGCCAAAACAG>400
                *         *         *         *         *         *         *         *         *         *
401>AGCTGTGGGGGAGAGTTGTTGAGAAAGAATGCGGAAGATGCAAAGGTGTCGGCTATTCAAGAATGCCAGCAAGCGCCGCATATCGCGCTGTAACGATGCT>500
401>AGCTGTGGGGGAGAGTTGTCGAGAAAGAGTGCGGAAGATGCAAAGGCGTCGGCTATTCAAGGATGCCAGCAAGCGCAGCATATCGCGCTGTGACGATGCT>500
                *         *         *         *         *         *         *         *         *         *
501>AATCCCAAACCTCACCCAACCCACCTGGTCACGCACTGTTAAGCCGCTGTATGACGCTCTGGTGGTGCAATGCCACAAGGAAGAGTCAATTGCAGACAAC>600
501>AATCCCAAACCTTACCCAACCCACCTGGTCACGCACTGTTAAGCCGCTGTATGACGCTCTGGTGGTGCAATGCCACAAAGAAGAGTCAATCGCAGACAAC>600
                *         *
601>ATTTTGAACGCGGTCACACGTTAG>624
601>ATTTTGAATGCGGTCACACGTTAG>624
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Figure 7. Showing aligned nucleotide sequences for wild-type ΛQ gene (top) and the found mutant ΛQ gene (bottom) with mutations underlined

MRLESVAKFHSPKSPMMSDSPRTTASDSLSGTDVMAAMGMAQSQAGFGMAAFCGKHELSQNDKQKAINYLMQFA
MRLESVAKFHSPKSPMMSDSPR<u>A</u>TASDSLSGTDVMAAMGMAQSQAGFGMAAFCGKHELSQNDKQKAINYLMQFA

HKVSGKYRGVAKLEGNTKAKVLQVLATFAYADYCRSAATPGARCRDCHGTGRAVDIAKTELWGRVVEKECGRCKGVG
HKVSGKYRGVAKLEGNTKAKVLQVLATFAYADYCRSAATPGARCRDCHGTGRAVDIAKTELWGRVVEKECGRCKGVG

YSRMPASAAYRAVTMLIPNLTQPTWSRTVKPLYDALVVQCHKEESIADNILNAVTR*
YSRMPASAAYRAVTMLIPNLTQPTWSRTVKPLYDALVVQCHKEESIADNILNAVTR*

Figure 8. Amino acid sequences of wild-type ʎQ gene (top) and the mutant ʎQ gene (bottom) with a single amino acid change underlined