

APPENDIX

DNA sequences and plasmid maps

A.1	pQE60_(ZRhis-ZE(gs) ₆ ELF)	A-2
A.2	pQE60_(ZEhis-ZRELF)	A-7
A.3	pQE60_ZE	A-11
A.4	pQE60_QKZE	A-14
A.5	pQE60_FNZE	A-17
A.6	pQE60_ZE-FN-ZE	A-20
A.7	pQE60_ZE-FN-ZE-FN-ZE	A-24
A.8	pQE60_RE ₂ RE -FN- RE ₂ RE.....	A-30
A.9	pQE60_RE ₂ RE -FN- RE ₂ RE-FN- RE ₂ RE.....	A-34
A.10	pQE9_PC ₁₀ A.....	A-40

A.1 pQE60_(ZRhis-ZE(gs)₆ELF)**Submitted by** Kechun Zhang**Date** 8 January 2007**Strain name** AF-IQ**Vector** pQE60_(ZRhis-ZE(gs)₆ELF)**Construction**

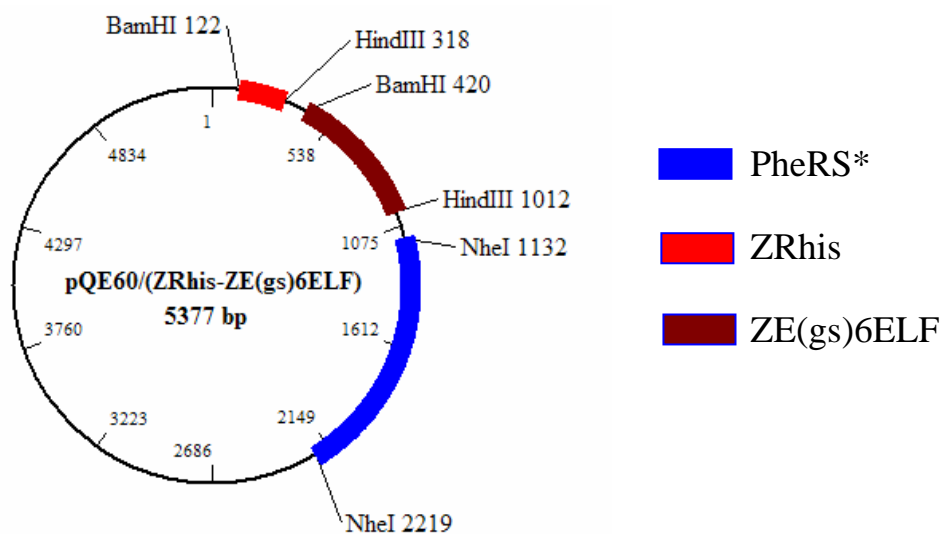
A *NheI* fragment encoding mutant *E. coli* PheRS (A294G) was inserted into expression plasmid pQE60 to yield pQE-FS*. Then the ZR gene was ligated into the *Bam*HI site of pQE-FS* to yield ZR-pQE-FS*. Finally, a DNA fragment encoding ZE(gs)₆ELF and containing a ribosome binding site was amplified and ligated into the *Bpu*1102I site of ZR-pQE-FS*. The resulting plasmid (ZRhis-ZE(gs)₆ELF) was transformed into phenylalanine auxotroph *E. coli* strain AF-IQ.

Available Sources:

1. 20% glycerol culture in Kechun Zhang's -80 °C freezer box
Ampicillin and chloramphenicol resistant.
2. Plasmid DNA in Kechun Zhang's -20 °C freezer box

Reference

Zhang, K. C.; Diehl, M. R.; Tirrell, D. A. *J. Am. Chem. Soc.* **2005**, *127*, 10136-10137.

Plasmid map

Gene sequence

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A.2 pQE60_(ZEHIS-ZRELF)

Submitted by Kechun Zhang

Date 8 January 2007

Strain name AF-IQ

Vector pQE60_(ZEHIS-ZRELF)

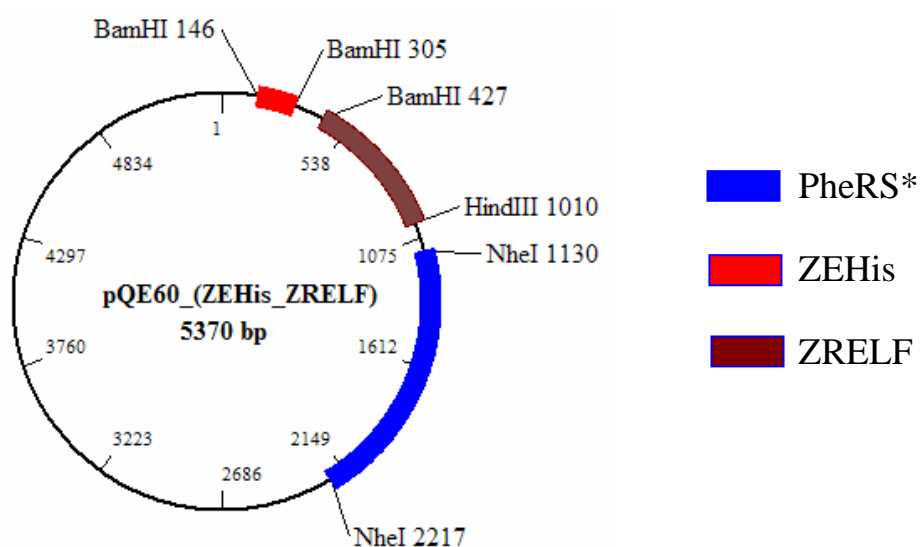
Construction

A *NheI* fragment encoding mutant *E. coli* PheRS (A294G) was inserted into expression plasmid pQE60 to yield pQE-FS*. Then the ZE gene was ligated into the *BamHI* site of pQE-FS* to yield ZE-pQE-FS*. Finally, a DNA fragment encoding ZRELF and containing a ribosome binding site was amplified and ligated into the *Bpu1102I* site of ZE-pQE-FS*. The resulting plasmid (ZEHIS-ZRELF) was transformed into phenylalanine auxotroph *E. coli* strain AF-IQ.

Available Sources:

1. 20% glycerol culture in Kechun Zhang's -80 °C freezer box
Ampicillin and chloramphenicol resistant.
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Plasmid map



Gene sequence

atgagaggatcgcatcaccatcaccatcacggatccctggaaatcgaagcggcggcgctg
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Full plasmid sequence

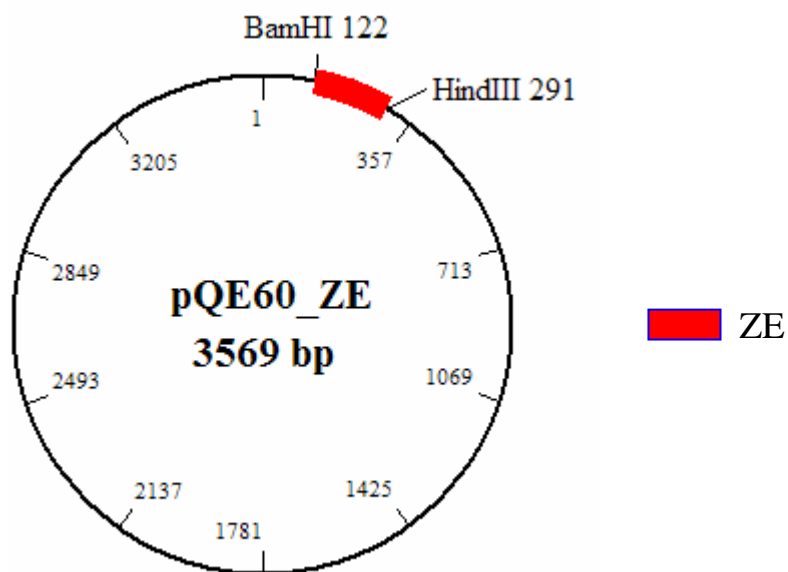
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A.3 pQE60_ZE**Submitted by** Kechun Zhang**Date** 8 January 2007**Strain name** AF-IQ**Vector** pQE60_ZE**Available Sources:**

1. 20% glycerol culture in Kechun Zhang's -80 °C freezer box
Ampicilin and chloramphenicol resistant.
2. Plasmid DNA in Kechun Zhang's -20 °C freezer box

Plamsid map

Gene sequence

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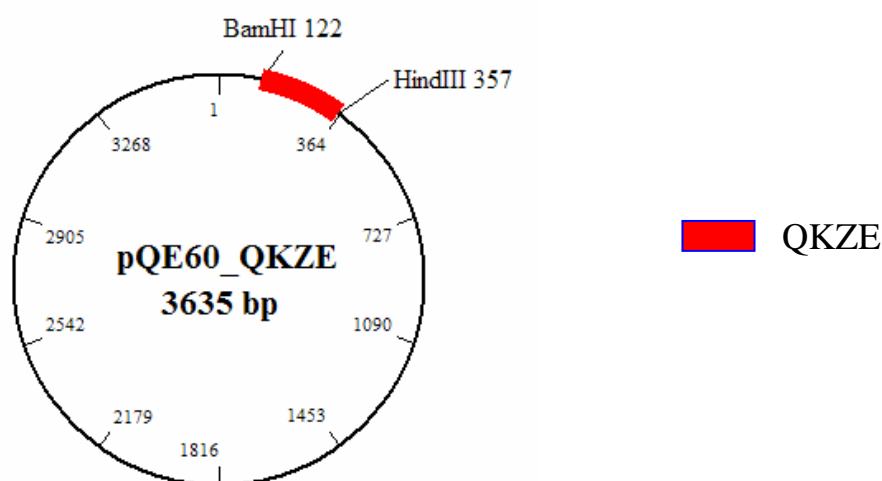
Full sequence

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A.4 pQE60_QKZE**Submitted by** Kechun Zhang**Date** 8 January 2007**Strain name** AF-IQ**Vector** pQE60_QKZE**Available Sources:**

1. 20% glycerol culture in Kechun Zhang's -80 °C freezer box
Ampicilin and chloramphenicol resistant.
2. Plasmid DNA in Kechun Zhang's -20 °C freezer box

Plamsid map**Gene sequence**

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L E T E V A E L E Q E V Q R L E N I V S
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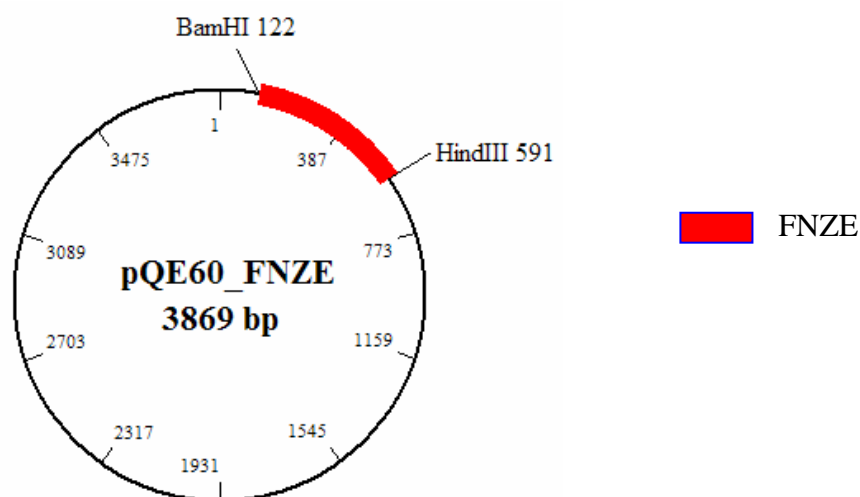
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A.5 pQE60_FNZE**Submitted by** Kechun Zhang**Date** 8 January 2007**Strain name** AF-IQ**Vector** pQE60_FNZE**Available Sources:**

1. 20% glycerol culture in Kechun Zhang's -80 °C freezer box
Ampicilin and chloramphenicol resistant.
2. Plasmid DNA in Kechun Zhang's -20 °C freezer box

Plamsid map**Gene sequence**

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Full sequence

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A.6 pQE60_ZE-FN-ZE

Submitted by Kechun Zhang

Date 8 January 2007

Strain name AF-IQ

Vector pQE60_ZE-FN-ZE

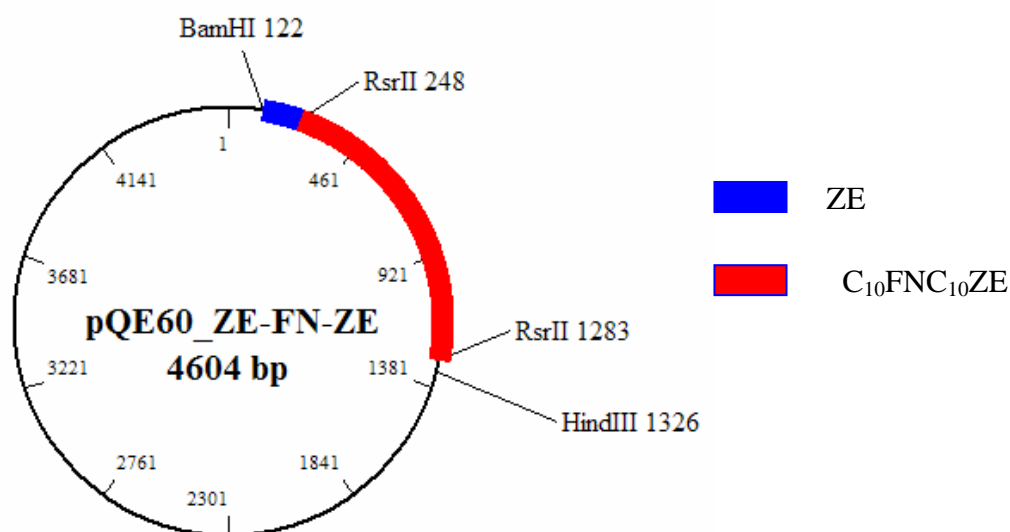
Construction

A previously constructed plasmid (pQE60_FNZE-FN-ZE) was digested with RsrII to obtain the FNC₁₀ZE fragment. This fragment was ligated into pQE60_ZE to yield pQE60_ZE-FN-ZE

Available Sources:

1. 20% glycerol culture in Kechun Zhang's -80 °C freezer box
Ampicilin and chloramphenicol resistant.
2. Plasmid DNA in Kechun Zhang's -20 °C freezer box

Plasmid map



Gene sequence

```
atgaaaggatccctggaaatcgaagcggcggcgctggaacaggaaaacaccgcgctggaa
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accgaagtgcggaactggaacaggaagttcagcgtctggaacacatcgtttctcagtac
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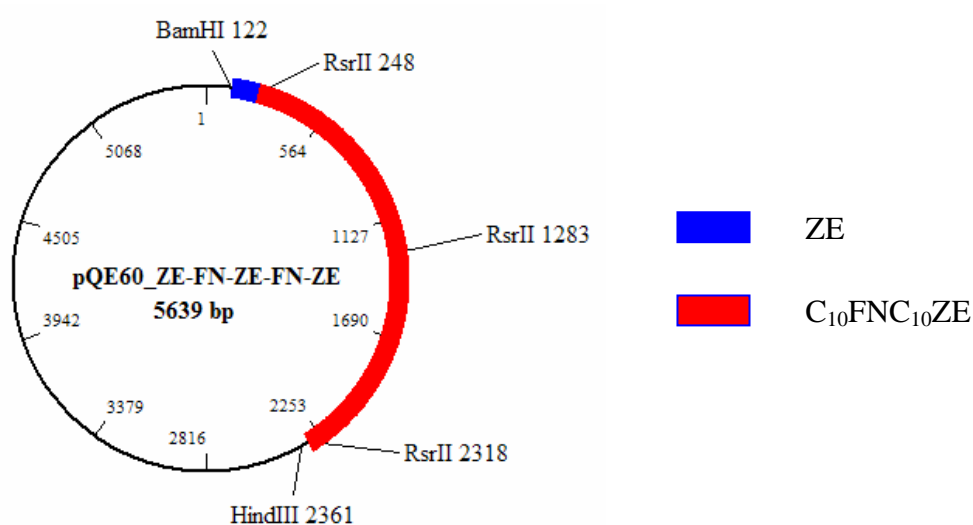
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A.7 pQE60_ZE-FN-ZE-FN-ZE**Submitted by** Kechun Zhang**Date** 8 January 2007**Strain name** AF-IQ**Vector** pQE60_ZE-FN-ZE-FN-ZE**Construction**

A previously constructed plasmid (pQE60_FNZE-FN-ZE) was digested with RsrII to obtain the FNC₁₀ZE fragment. This fragment was multimerized by ligating monomers with T4 DNA ligase. The resulting dimer was ligated into pQE60_ZE to yield pQE60_ZE-FN-ZE-FN-ZE.

Available Sources:

1. 20% glycerol culture in Kechun Zhang's -80 °C freezer box
Ampicilin and chloramphenicol resistant.
2. Plasmid DNA in Kechun Zhang's -20 °C freezer box

Plasmid map

Gene sequence

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Full sequence

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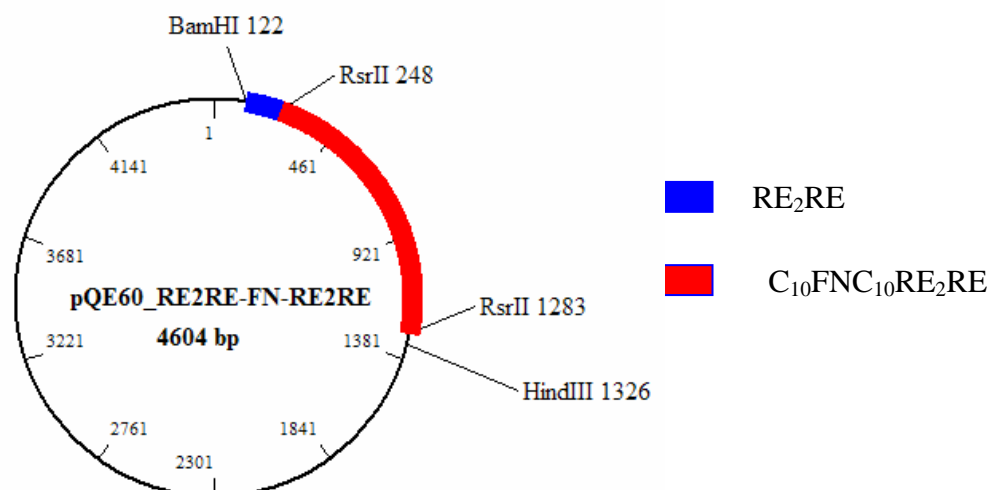
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A.8 pQE60_RE₂RE -FN- RE₂RE**Submitted by** Kechun Zhang**Date** 8 January 2007**Strain name** AF-IQ**Vector** pQE60_RE₂RE -FN- RE₂RE**Available Sources:**

1. 20% glycerol culture in Kechun Zhang's -80 °C freezer box
Ampicilin and chloramphenicol resistant.
2. Plasmid DNA in Kechun Zhang's -20 °C freezer box

Plasmid map**Gene sequence**

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Full sequence

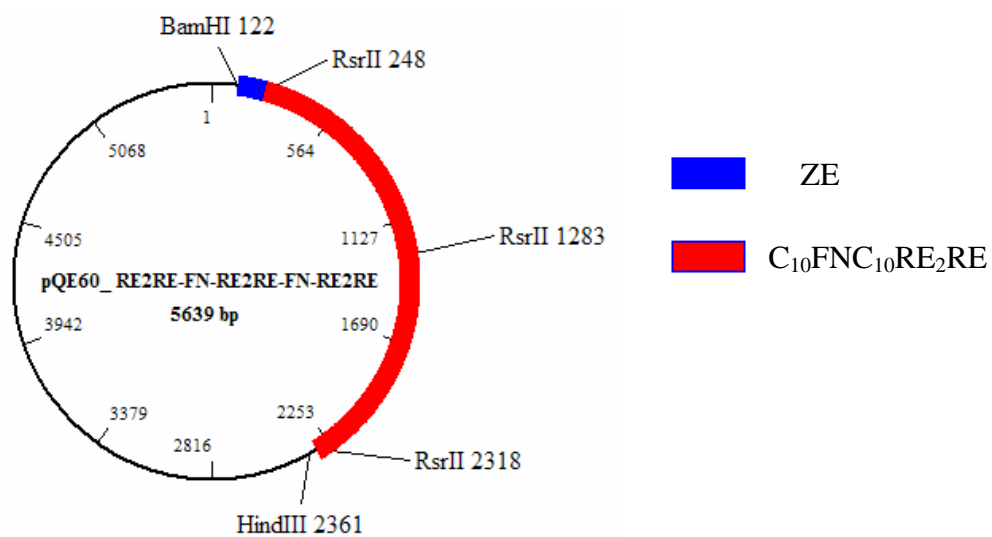
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A.9 pQE60_RE₂RE-FN-RE₂RE-FN-RE₂RE**Submitted by** Kechun Zhang**Date** 8 January 2007**Strain name** AF-IQ**Vector** pQE60_RE₂RE-FN-RE₂RE-FN-RE₂RE**Available Sources:**

1. 20% glycerol culture in Kechun Zhang's -80 °C freezer box
Ampicilin and chloramphenicol resistant.
2. Plasmid DNA in Kechun Zhang's -20 °C freezer box

Plasmid map**Gene sequence**

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Full sequence

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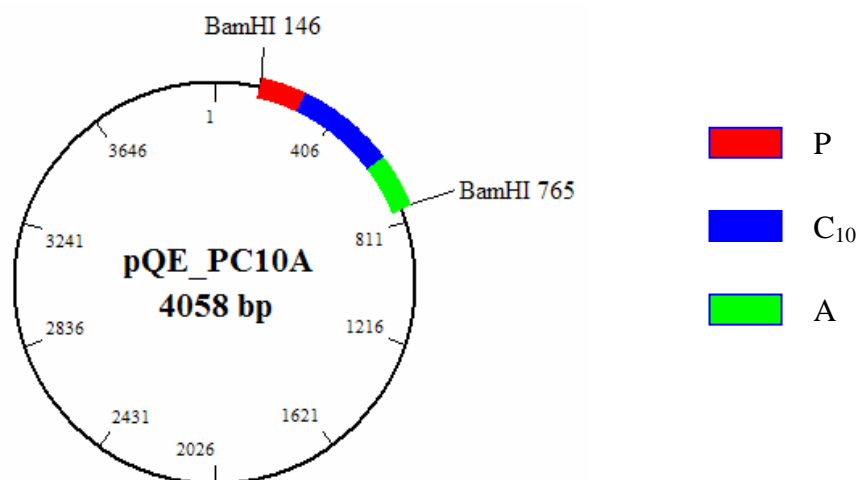
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AAGTGCCACCTGACGTCTAAGAAACCATTATTATCATGACATTAACCTAT
AAAAATAGGCGTATCACGAGGCCCTTTCGTCTTAC

A.10 pQE9_PC₁₀A**Submitted by** Kechun Zhang**Date** 8 January 2007**Strain name** AF-IQ**Vector** pQE9_PC₁₀A**Available Sources:**

1. 20% glycerol culture in Kechun Zhang's -80 °C freezer box
Ampicilin and chloramphenicol resistant.
2. Plasmid DNA in Kechun Zhang's -20 °C freezer box

Plasmid map**Gene sequence**

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M R G S H H H H H G S G D L A P Q M L
cgtgaactgcaggaaaccaacgcggcgctgcaggacgttcgtgaactgctgcgtcagcag
R E L Q E T N A A L Q D V R E L L R Q Q
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V K E I T F L K N T V M E S D A S G T S
tatcgcgatccgatgggtgcccggcgctggtgcccggcccgaagggtgcaggcgtggtgcg
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 D E A A E L E Q K V S R L K N E I E D L
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Full sequence

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