

PCR cloning of a streptomycin phosphotransferase (aphE) gene from *Streptomyces griseus* ATCC 12475

Michael K.Trower* and Kevin G.Clark[†]

MRC Molecular Genetics Unit, Hills Road, Cambridge CB2 2QH, UK

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The aminoglycoside phosphotransferases are prokaryotic antibiotic resistance proteins that achieve inactivation of their antibiotic substrates by phosphorylation. To assist in our structure/function investigations of this group of kinases a gene encoding a streptomycin phosphotransferase (aphE) was cloned from the chromosomal DNA of the streptomycin producing *Streptomyces griseus* ATCC 12475 by the polymerase chain reaction (PCR). Although this is the second streptomycin phosphotransferase isolated from this strain of *S. griseus* (1), the deduced primary structures are significantly different (<20% homologous). The PCR cloned gene shows however an almost identical nucleotide and deduced amino acid sequence to the streptomycin-3''-phosphotransferase isolated from *Streptomyces griseus* N2-3-11 (2). Only 4 nucleotides differ; a C to G at base 151, A to G at 363, C to T at 479 and C to T at 750. Two of

these base changes are silent whilst the other two generate the amino acid substitutions; proline to alanine at residue 51 (CCG to GCG) and alanine to valine at 160 (GCT to GTT).

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REFERENCES

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1   ATGAGTGATCACCCCGGGCCGGGGCCGTCACGCCGAGCTGTTCGGCGTGGGCGGCGACTGGCTGGCC
    M S D H P G P G A V T P E L F G V G G D W L A   23
70  GTCACCGCGGGCGAATCGGGCCCTCCGTCTTTCGCGCCGCGGACGCCACCCGGTACGCCAAGTGCCTG
    V T A G E S G A S V F R A A D A T R Y A K C V   46
139 CCCGCCGCGGACGCGGCCGGTCTTGAGGCGGAACCGGACCGGATCGCCTGGCTGAGCGGGCAGGGCGTA
    P A A D A A G L E A E R D R I A W L S G Q G V   69
208 CCGGGCCCCCGCTCCTCGACTGGTACGCCGGTACGCGGGCGCCTGCCTGGTACCCCGTCCCGTCCCC
    P G P R V L D W Y A G D A G A C L V T R A V P   92
277 GCGGTACCCGCTGATCGGGTGGGCGCGATGACCTTCGCACTGCTGGGGGGCGTCGCGGACGCGGTC
    G V P A D R V G A D D L R T A W G A V A D A V   115
346 CGTCGGCTGCACGAGGTGCCCGTGGCCCTCGTGTCCGTTCCGCCGGGGGCTGGACTCCGTGGTTCGACGCC
    R R L H E V P V A S C P F R R G L D S V V D A   138
415 GCCCCTGACGTGGTGGCCCGTGGCGCGGTGCATCCGGAGTTCCTGCCGGTGGAGCAGCGGCTCGTTCCC
    A R D V V A R G A V H P E F L P V E Q R L V P   161
484 CCGGCGGAGCTGCTGGCCCGGCTACCGGGGAGCTCGCCCGTCCGCGGATCAGGAGGCCCGGACAGC
    P A E L L A R L T G E L A R R R D Q E A A D T   184
553 GTCGTCTGCCACGGTATCTCTGCCTGCCCAACATCGTCTCCATCCGGAGACCTGGAGGTGTCGGGC
    V V C H G D L C L P N I V L H P E T L E V S G   207
622 TTCATCGACCTGGGACGGCTCGGGGCGGCCGACGCCACGCGGACCTGGCGCTGCTGCTGGCCAACGCG
    F I D L G R L G A A D R H A D L A L L L A N A   230
691 CGCGAGACCTGGGTGGACGAGGAGCGGGCGCGGTTCGCCGACGCGGCGTTCGCCGAGCGTTACGGCATC
    R E T W V D E E R A R F A D A A F A E R Y G I   253
760 GCCCCGACCCGGAACGGCTGCGCTTCTACCTCCATCTCGATCCGCTCACCTGGGGCTAG
    A P D P E R L R F Y L H L D P L T W G *   272

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* To whom correspondence should be addressed

[†]On student placement from Nottingham Polytechnic, Nottingham, UK.