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7 Appendices

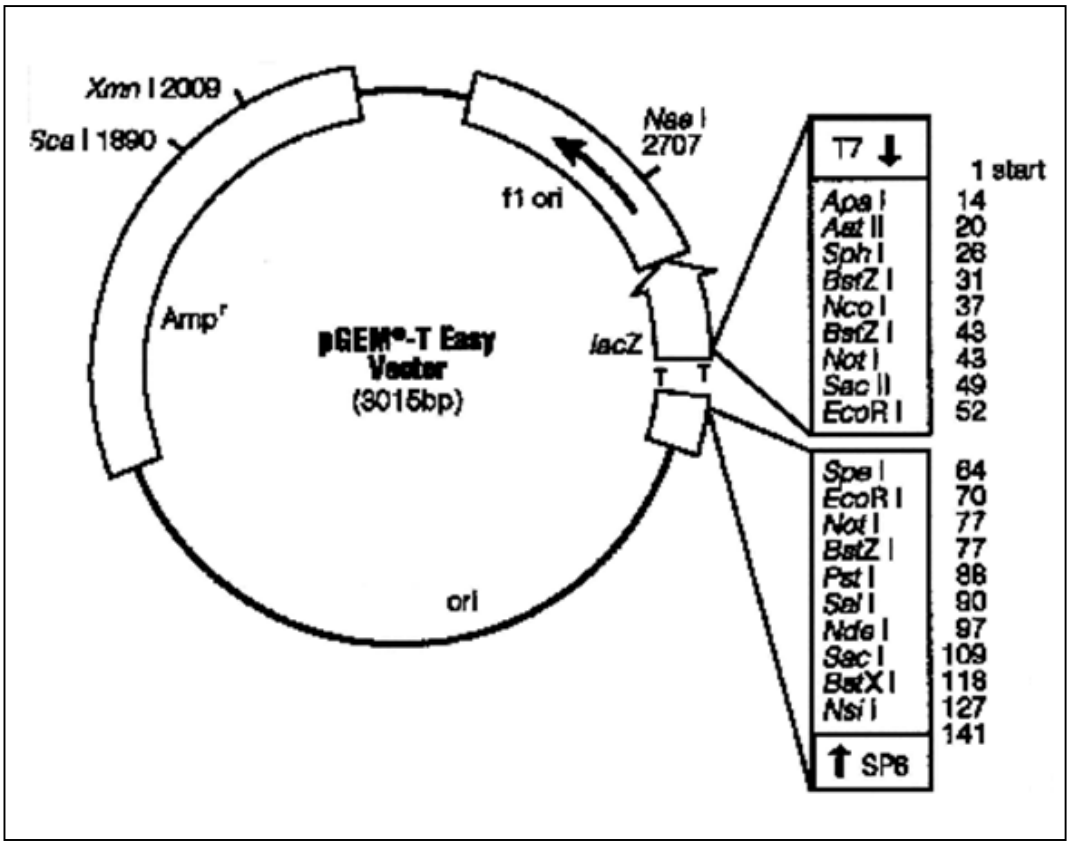

Figure A1: pGEM-T Easy vector (3015bp) with Ap^r.

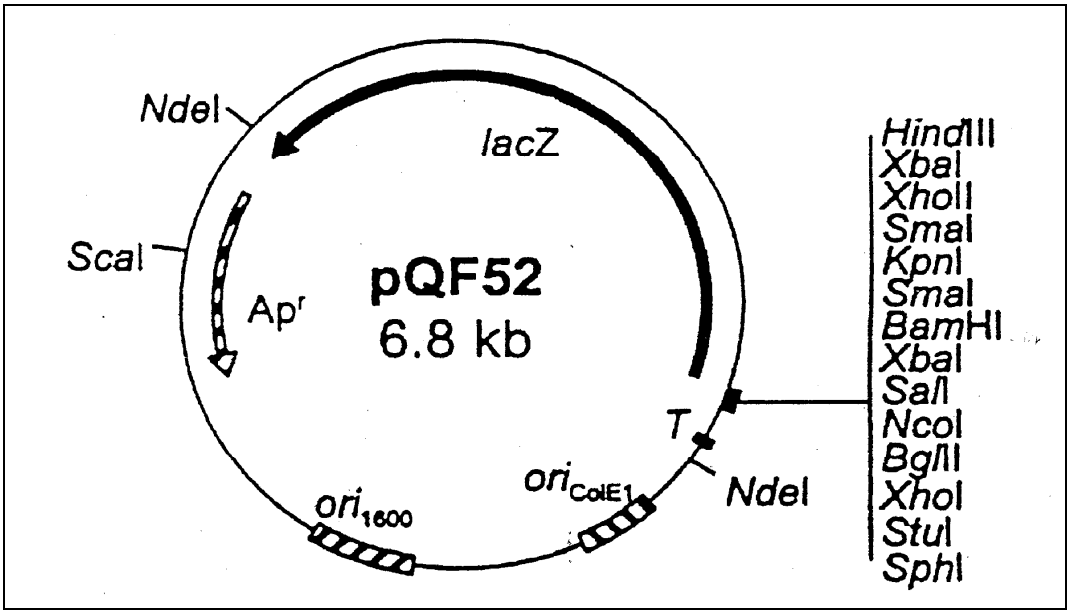

Figure A2: pQF52 plasmid (6800 bp) with Ap^r and promoterless *lacZ*.

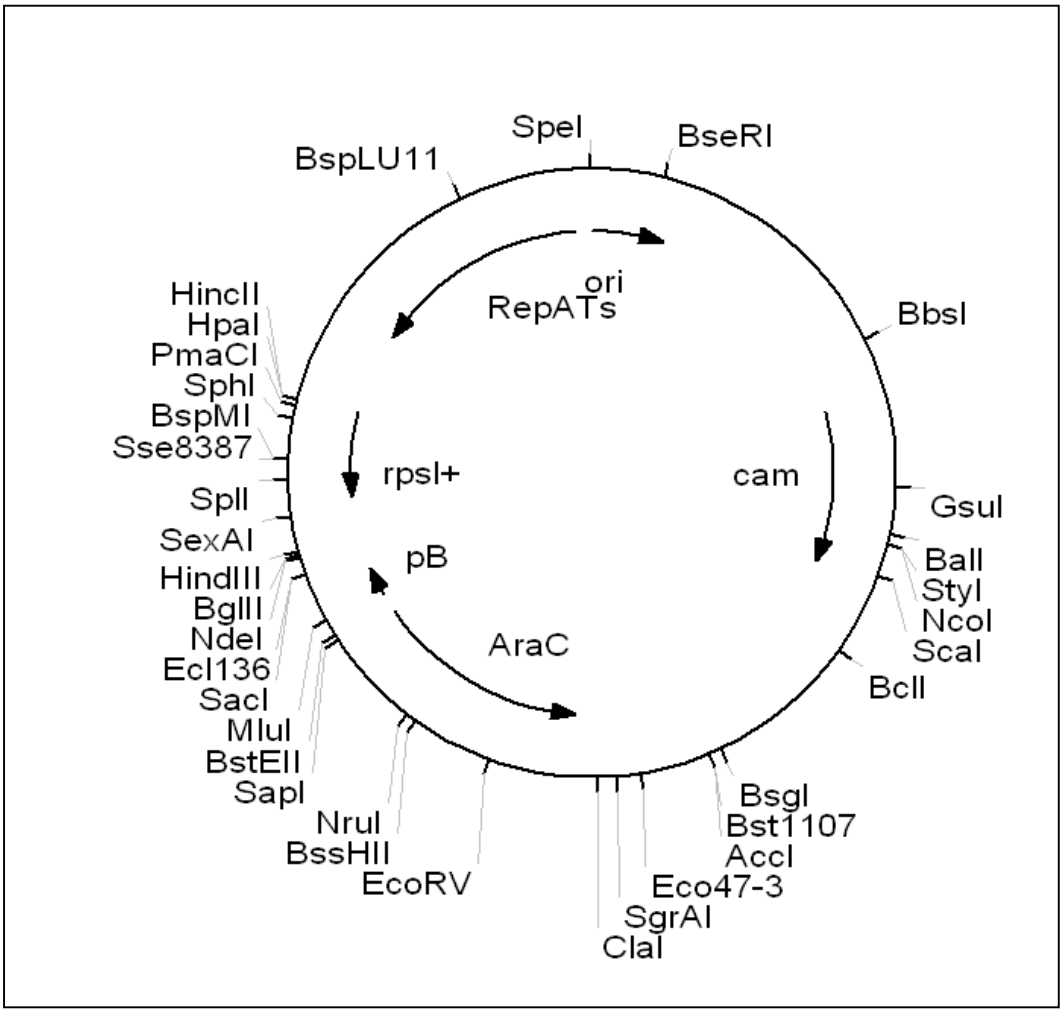

Figure A3: pLNBAD plasmid (6585 bp) with Cm^r and P_{BAD} promoter

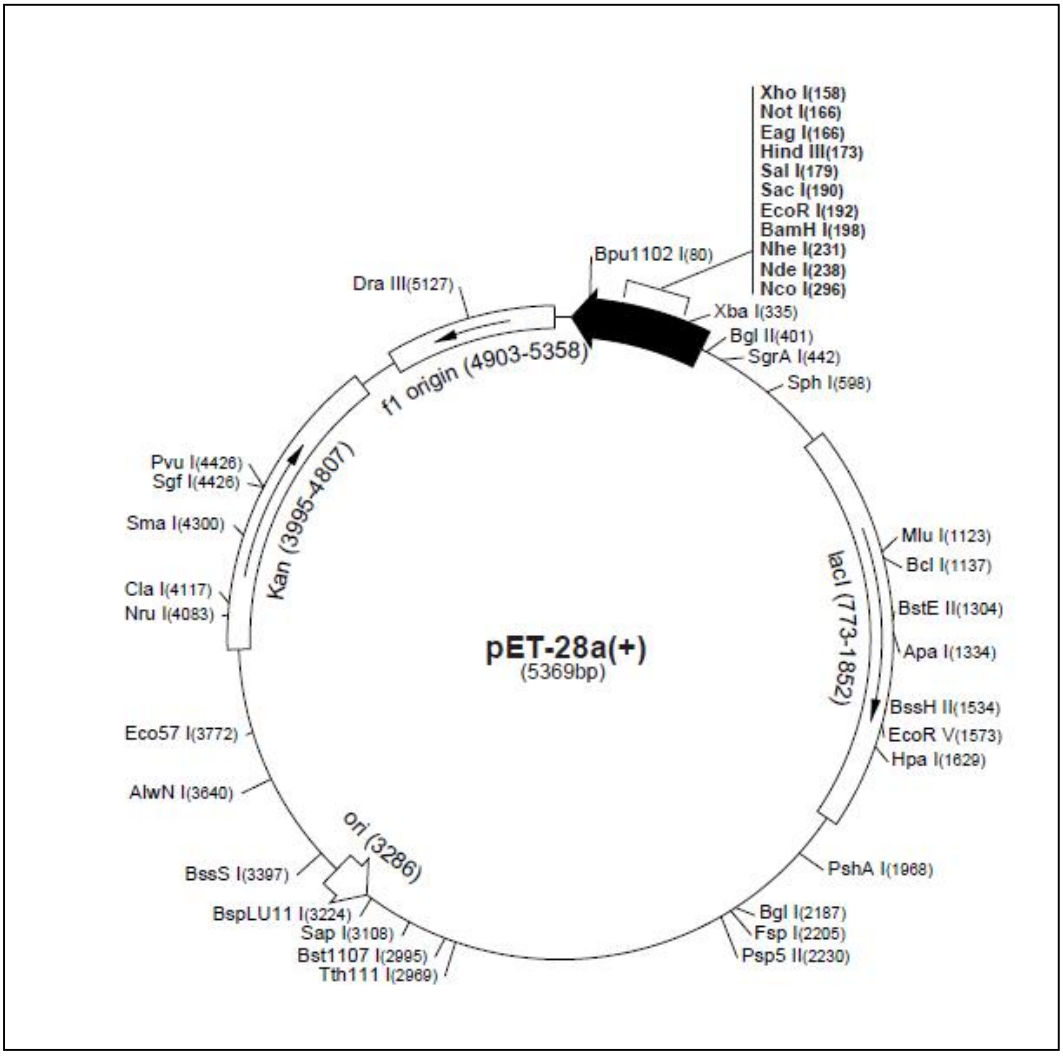

Figure A4: pET28a vector (5369 bp) with Km^r and T7 promoter

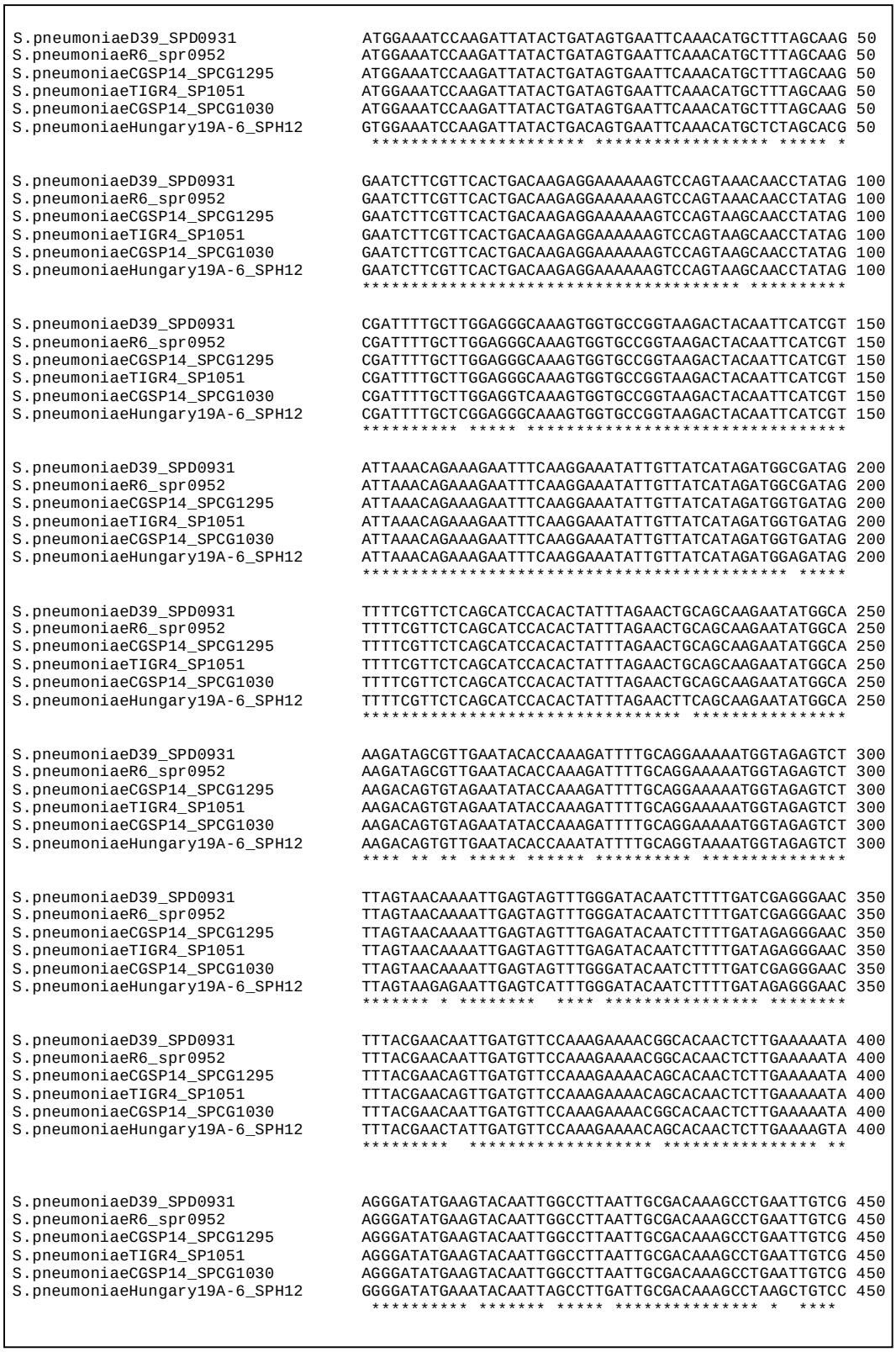

Figure B1: (continued) Multiple nucleotide sequences alignment of the PezT homologues in annotated *S. pnuemoniae* strains. The "*" indicates identical residues; ":" indicates conserved substitution; and "." indicates semi-conserved substitution.

```
S.pneumoniaeD39_SPD0931             TATCTAAGTACTCTTATCCGTTATGAAGAACTGTACATTATCAATCCAAA 500
S.pneumoniaeR6_spr0952              TATCTAAGTACTCTTATCCGTTATGAAGAACTGTACATTATCAATCCAAA 500
S.pneumoniaeCGSP14_SPCG1295         TATCTAAGTACTCTTATCCGTTATGAAGAACTGTACATTATCAATCCAAA 500
S.pneumoniaeTIGR4_SP1051            TATCTAAGTACTCTTATCCGTTATGAAGAACTGTACATTATCAATCCAAA 500
S.pneumoniaeCGSP14_SPCG1030         TATCTAAGTACTCTTATCCGTTATGAAGAACTGTACATTATCAATCCAAA 500
S.pneumoniaeHungary19A-6_SPH12      TATCTGAGCACCCTTATCCGATACGAAGAACTGTACGCTATTAACCCAAA 500
                                    ***** ** ** ******** ** ************  *** ** *****

S.pneumoniaeD39_SPD0931             TCAAGCACGCGCAACTCCAAAAGAACATCATGATTTCATTGTAAATCATC 550
S.pneumoniaeR6_spr0952              TCAAGCACGCGCAACTCCAAAAGAACATCATGATTTCATTGTAAATCATC 550
S.pneumoniaeCGSP14_SPCG1295         TCAAGCACGCGCAACTCCAAAAGAACATCATGATTTCATTGTAAATCATC 550
S.pneumoniaeTIGR4_SP1051            TCAAGCACGCGCAACTCCAAAAGAACATCATGATTTCATTGTAAATCATC 550
S.pneumoniaeCGSP14_SPCG1030         TCAAGCACGCGCAACTCCAAAAGAACATCATGATTTCATTGTAAATCATC 550
S.pneumoniaeHungary19A-6_SPH12      TCAAGCACGCGCAACTCCAAAAGAACATCATGATTTCATTGTAAATCATC 550
                                    ************************************************

S.pneumoniaeD39_SPD0931             TAGTTGATAACACACGAAAATTGGAAGAACTAGCTATCTTTGAAAGAATT 600
S.pneumoniaeR6_spr0952              TAGTTGATAACACACGAAAATTGGAAGAACTAGCTATCTTTGAAAGAATT 600
S.pneumoniaeCGSP14_SPCG1295         TAGTTGATAACACACGAAAATTGGAAGAACTAGCTATCTTTGAAAGAATT 600
S.pneumoniaeTIGR4_SP1051            TAGTTGATAACACACGAAAATTGGAAGAACTAGCTATCTTTGAAAGAATT 600
S.pneumoniaeCGSP14_SPCG1030         TAGTTGATAACACACGAAAATTGGAAGAACTAGCTATCTTTGAAAGAATT 600
S.pneumoniaeHungary19A-6_SPH12      TAGTTGATAATACACGACAATTGGAAGAACTAGCTATCTTTGAAAGAATT 600
                                    ********** ****** ********************************

S.pneumoniaeD39_SPD0931             CAAATTTACCAACGAGATAGAAGTTGTGTATATGATTCAAAAGAAAATAC 650
S.pneumoniaeR6_spr0952              CAAATTTACCAACGAGATAGAAGTTGTGTATATGATTCAAAAGAAAATAC 650
S.pneumoniaeCGSP14_SPCG1295         CAAATTTACCAACGAGATAGAAGTTGTGTATATGATTCAAAAGAAAATAC 650
S.pneumoniaeTIGR4_SP1051            CAAATTTACCAACGAGATAGAAGTTGTGTATATGATTCAAAAGAAAATAC 650
S.pneumoniaeCGSP14_SPCG1030         CAAATTTACCAACGAGATAGAAGTTGTGTATATGATTCAAAAGAAAATAC 650
S.pneumoniaeHungary19A-6_SPH12      CAAATTTACCAACGAGATAGAGGTTGTGTATATGATGCAAAAGAAAATAC 650
                                    ********************* ************** *************

S.pneumoniaeD39_SPD0931             AACTTCAGCAGCAGATGTTCTTCAAGAGTTACTCTTTGGGGAGTGGAGTC 700
S.pneumoniaeR6_spr0952              AACTTCAGCAGCAGATGTTCTTCAAGAGTTACTCTTTGGGGAGTGGAGTC 700
S.pneumoniaeCGSP14_SPCG1295         AACTTCAGCAGCAGATGTTCTTCAAGAGTTACTCTTTGGGGAGTGGAGTC 700
S.pneumoniaeTIGR4_SP1051            AACTTCAGCAGCAGATGTTCTTCAAGAGTTACTCTTTGGGGAGTGGAGTC 700
S.pneumoniaeCGSP14_SPCG1030         AACTTCAGCAGCAGATGTTCTTCAAGAGTTATTCTTTGGGGAGTGGAGTC 700
S.pneumoniaeHungary19A-6_SPH12      AACTTCAGCGGCAGATGTTCTTCAAGAGTTACTGTTTGGGGAGTGGAGTC 700
                                    ********* ********************** * ****************

S.pneumoniaeD39_SPD0931             AGGTAGAGAAGGAGATGTTGCAGGTGGGGGAAAAGAGACTTAATGAATTA 750
S.pneumoniaeR6_spr0952              AGGTAGAGAAGGAGATGTTGCAGGTGGGGGAAAAGAGACTTAATGAATTA 750
S.pneumoniaeCGSP14_SPCG1295         AGGTAGAGAAGGAGATGTTGCAGGTGGGGGAAAAGAGACTTAATGAATTA 750
S.pneumoniaeTIGR4_SP1051            AGGTAGAGAAGGAGATGTTGCAGGTGGGGGAAAAGAGACTTAATGAATTA 750
S.pneumoniaeCGSP14_SPCG1030         AGGTAGAGAAGGAGATGTTGCAGGTGGGGGAAAAGAGACTTAATGAATTA 750
S.pneumoniaeHungary19A-6_SPH12      AAGTAGAGAAGGATATGCTTAAATCTGGAGAAGAAAGATTGAAAGATTTA 750
                                    * *********** *** *  *    ** *** * *** * ** ** ***

S.pneumoniaeD39_SPD0931             CTTGAAAAATAA 762
S.pneumoniaeR6_spr0952              CTTGAAAAATAA 762
S.pneumoniaeCGSP14_SPCG1295         CTTGAAAAATAA 762
S.pneumoniaeTIGR4_SP1051            CTTGAAAAATAA 762
S.pneumoniaeCGSP14_SPCG1030         CTTGAAAAATAA 762
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```

Figure B1: (continued)

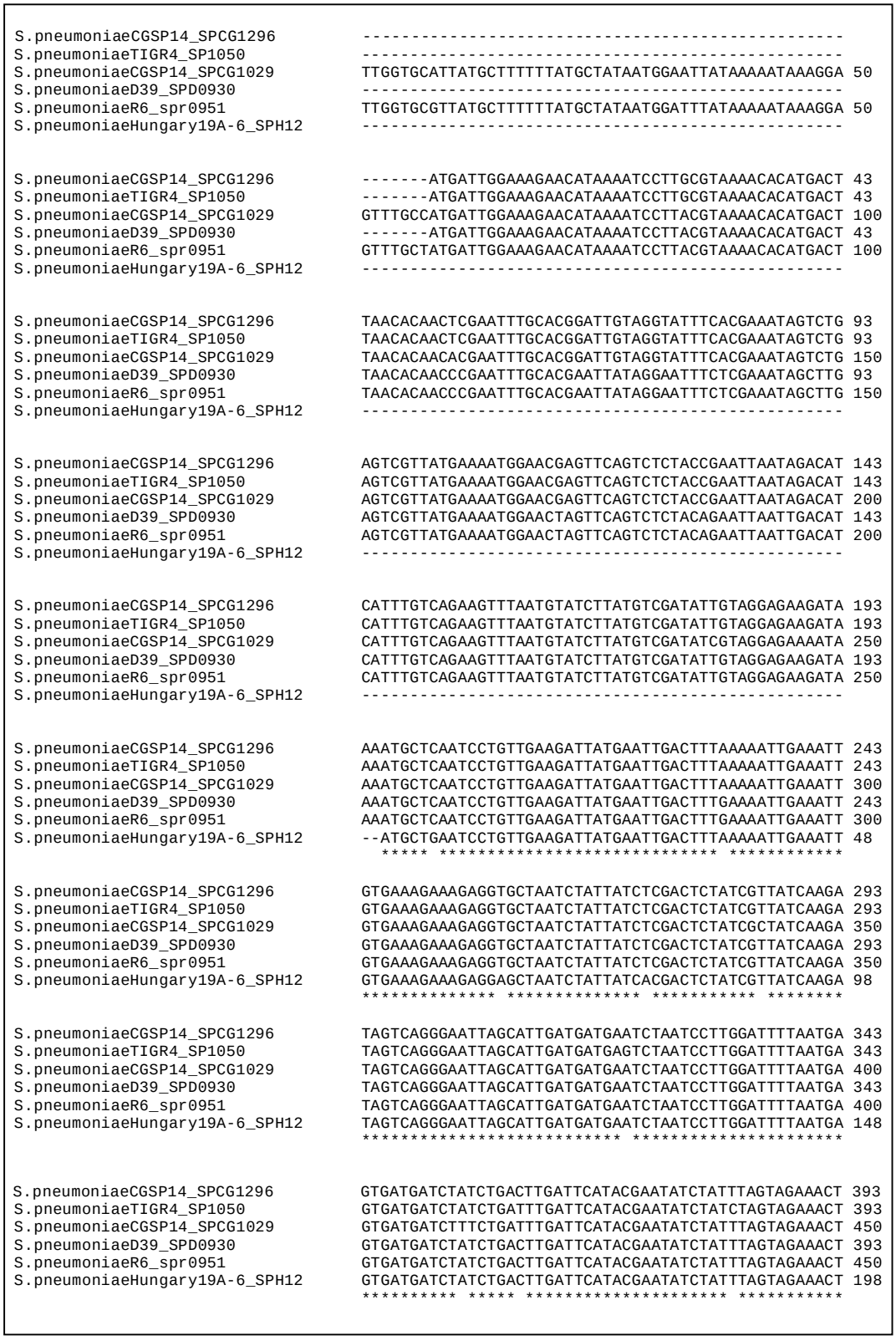

Figure B2: Multiple nucleotide sequences alignment of the PezA homologues in annotated *S. pnuemoniae* strains. The "*" indicates identical residues; ":" indicates conserved substitution; and "." indicates semi-conserved substitution.

```
S.pneumoniaeCGSP14_SPCG1296         TTTGATGAAATAGAGAGATATAGTGGCTATTTGGATGGAATTGAACGTAT 443
S.pneumoniaeTIGR4_SP1050            TTTGATGAAATAGAGAGATATAGTGGCTATTTGGATGGAATTGAACGTAT 443
S.pneumoniaeCGSP14_SPCG1029         TTTGATGAAATAGAGAGATATAGTGGCTATTTGGATGGAATTGAACGTAT 500
S.pneumoniaeD39_SPD0930             TTTGATGAAATAGAGAGATATAGTGGCTATTTGGATGGAATTGAACGTAT 443
S.pneumoniaeR6_spr0951              TTTGATGAAATAGAGAGATATAGTGGCTATTTGGATGGAATTGAACGTAT 500
S.pneumoniaeHungary19A-6_SPH12      TTTGATGAAATAGAGAGATATAGCGGCTATTTGGATGGAATTGAACGTAT 248
                                    ************************ *************************

S.pneumoniaeCGSP14_SPCG1296         GTTAGAGATATTTGAAAAACGGATGGCAGCCTAA 477
S.pneumoniaeTIGR4_SP1050            GTTAGAGATATCTGAAAAACGGATGGTGGCCTAA 477
S.pneumoniaeCGSP14_SPCG1029         GTTAGAGATATCTGAAAAGCGGATGGTAGCTTAA 534
S.pneumoniaeD39_SPD0930             GTTAGAGATATCTGAAAAGCGGATGGTAGCTTAA 477
S.pneumoniaeR6_spr0951              GTTAGAGATATCTGAAAAGCGGATGGTAGCTTAA 534
S.pneumoniaeHungary19A-6_SPH12      GTTAGAGATATCTGAAAAACGGATGGTAGCCTAG 282
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Figure B2: (continued)

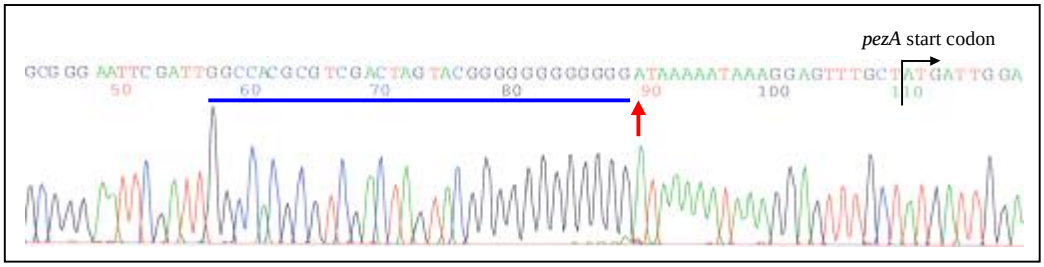

Figure C1: Chromatogram showing the transcriptional start site of the *pezAT* operon. The 5'-RACE abridged anchor primer is underlined in blue. The *pezA* start codon is indicated. The transcriptional start site of the *pezAT* operon is dipicted with a red arrow.

PUBLICATIONS:

1. **Chan, W.T.**, Nieto, C., Harikrishna, J.A., Khoo, S.K., Othman, R.Y., Espinosa, M., and Yeo, C.C. (2011) Genetic regulation of the *yefM-yoeB*$_{Spn}$ toxin-antitoxin locus of *Streptococcus pneumonia*. (in preparation for submission to a Tier 1 ISI cited journal)

2. Khoo, S.K., Loll, B., **Chan, W.T.**, Shoeman, R.L., Ngoo, L., Yeo, C.C., and Meinhart, A. (2007) Molecular and structural characterization of the PezAT chromosomal toxin-antitoxin system of the human pathogen *Streptococcus pneumoniae*. *J Biol Chem*. **282:** 19606-19618.

3. Nieto, C., Cherny, I., Khoo, S.K., de Lacoba, M.G., **Chan, W.T.**, Yeo, C.C., Gazit, E., and Espinosa, E. (2007) The *yefM-yoeB* toxin-antitoxin systems of *Escherichia coli* and *Streptococcus pneumoniae*: Functional and structural correlation. *J. Bacteriol.* **189:** 1266-1278.

GRANTS & AWARDS:

Universiti Malaya fellowship (2007)

Perdana Scholarship (2007-2011)

European Molecular Biology Organization (EMBO) short term fellowship (Jun-Nov 2008)

Peruntukan Penyelidikan Pascasiswazah (PPP) grant PS150-2009A (2007)

Peruntukan Penyelidikan Pascasiswazah (PPP) grant PS039-2007C (2009)

BEST OVERALL POSTER AWARD: 18th Malaysian Society for Molecular Biology and Biotechnology (MSMBB) Scientific Meeting. **Chan, W.T.**, Nieto, C., Espinosa, M., Harikrishna, J.A., Othman, R.Y., and Yeo, C.C. Elucidation of the DNA binding sites for the autoregulation of the chromosomal *yefM-yoeB* toxin-antitoxin locus of *Streptococcus pneumoniae.* 18-20 Aug 2009. The Saujana Kuala Lumpur, Malaysia.

BEST MICROBIAL MOLECULAR BIOLOGY/BIOTECHNOLOGY POSTER AWARD: 18th Malaysian Society for Molecular Biology and Biotechnology (MSMBB) Scientific Meeting. Yeo, C.C., **Chan, W.T.**, Espinosa, M., Othman, R.Y., and Harikrishna, J.A. 2009. Genetic regulation of the chromosomal *yefM-yoeB* toxin-antitoxin locus of *Streptococcus pneumoniae.* 18-20 Aug 2009. The Saujana Kuala Lumpur, Malaysia.

BEST POSTER AWARD: 32nd Annual Conference of The Malaysian Society for Biochemistry and Molecular Biology (MSBMB). **Chan, W.T.**, Khoo, S.K., Nieto, C., Espinosa, M., Harikrishna, J.A., and Yeo, C.C. 2007. Genetic regulation of the chromosomal *yefM-yoeB*$_{spn}$ toxin-antitoxin locus of *Streptococcus pneumoniae.* Poster presentation. 5-6 Sep 2007. Eastin Hotel, Petaling Jaya, Malaysia.

ORAL PRESENTATION:

12th Biological Sciences Graduate Congress. **Chan, W.T.**, Khoo, S.K., Nieto, C., Espinosa, M., Harikrishna, J.A., and Yeo, C.C. 2007. The genetic regulation of the chromosomal *yefM-yoeB*$_{Spn}$ toxin-antitoxin locus of *Streptococcus pneumoniae.* 17-19 Dec 2007. University of Malaya, Kuala Lumpur, Malaysia.

POSTER PRESENTATION:

34th Annual Conference of The Malaysian Society for Biochemistry and Molecular Biology (MSBMB) in conjunction with the 3rd ASEAN Biochemistry Conference. **Chan, W.T.**, Nieto, C., Espinosa, M., Harikrishna, J.A., Othman, R.Y., and Yeo, C.C. 2009. Negative autoregulation of the *yefM-yoeB* toxin-antitoxin locus of *Streptococcus pneumoniae*: the YefM antitoxin functions as a transcriptional repressor with the YoeB toxin as a co-repressor. 7-8 Oct 2009. Prince Hotel, Kuala Lumpur, Malaysia.

34th Annual Conference of The Malaysian Society for Biochemistry and Molecular Biology (MSBMB) in conjunction with the 3rd ASEAN Biochemistry Conference. Lim, C.K., Ngoo, L., **Chan, W.T.**, Meinhart, A., and Yeo, C.C. 2009. Cross-reactivities between the chromosomally-encoded PezAT toxin-antitoxin system of *Streptococcus pneumoniae* and the plasmid pSM19035-enocded ε-ζ toxin-antitoxin system of *Streptococcus pyogenes.* 7-8 Oct 2009. Prince Hotel, Kuala Lumpur, Malaysia.

18th Malaysian Society for Molecular Biology and Biotechnology (MSMBB) Scientific Meeting. **Chan, W.T.**, Nieto, C., Espinosa, M., Harikrishna, J.A., Othman, R.Y., and Yeo, C.C. Elucidation of the DNA binding sites for the autoregulation of the chromosomal *yefM-yoeB* toxin-antitoxin locus of *Streptococcus pneumoniae.* 18-20 Aug 2009. The Saujana Kuala Lumpur, Malaysia.

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