Copper Tolerance of *Listeria monocytogenes* strain DRDC8

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Appendix A

This sequence data was assembled as part of collaborative effort by F. Bell, M. Francis, M. Webster, A. Akya and C. Thomas. The sequence is available from the NCBI GenBank database (Accession Number U15554).

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LOCUS       LMU15554               37279 bp    DNA     linear   BCT 25-SEP-2007
DEFINITION  Listeria monocytogenes strain DRDC8 hypothetical protein (pCT0030)
            gene, partial cds; hypothetical protein (pCT0031), hypothetical
            protein (pCT0032), hypothetical protein (pCT0001), hypothetical
            protein (pCT0002), unknown (pCT0003), hypothetical protein
            (pCT0004), hypothetical protein (pCT0005), hypothetical protein
            (pCT0006), hypothetical protein (pCT0007), and hypothetical protein
            (pCT0008) genes, complete cds; insertion sequence ISS1-like
            hypothetical protein (pCT0009) gene, complete cds; unknown
            (pCT0010) gene, complete cds; insertion sequence ISS1-like
            hypothetical protein (pCT0011) gene, complete cds; hypothetical
            protein (pCT0012), hypothetical protein (pCT0013), and hypothetical
            protein (pCT0014) genes, complete cds; insertion sequence ISS1-like
            hypothetical protein (pCT0015) gene, complete cds; hypothetical
            protein (pCT0016), hypothetical protein (pCT0017), hypothetical
            protein (pCT0018), hypothetical protein (pCT0019), and CtpA (ctpA)
            genes, complete cds; transposon pCT0021 gene, complete sequence;
            and hypothetical protein (pCT0022), hypothetical protein (pCT0023),
            hypothetical protein (pCT0024), hypothetical protein (pCT0025),
            hypothetical protein (pCT0026), hypothetical protein (pCT0027),
            hypothetical protein (pCT0028), and hypothetical protein (pCT0029)
            genes, complete cds.
ACCESSION   U15554
VERSION     U15554.3  GI:157429038
KEYWORDS    .
SOURCE      Listeria monocytogenes
  ORGANISM  Listeria monocytogenes
            Bacteria; Firmicutes; Bacillales; Listeriaceae; Listeria.
REFERENCE   1  (bases 1 to 37279)
  AUTHORS   Francis,M.S. and Thomas,C.J.
  TITLE     The Listeria monocytogenes gene ctpA encodes a putative P-type
            ATPase involved in copper transport
  JOURNAL   Mol. Gen. Genet. 253 (4), 484-491 (1997)
   PUBMED   9037109
REFERENCE   2  (bases 1 to 37279)
  AUTHORS   Francis,M.S. and Thomas,C.J.
  TITLE     Mutants in the CtpA copper transporting P-type ATPase reduce
            virulence of Listeria monocytogenes
  JOURNAL   Microb. Pathog. 22 (2), 67-78 (1997)
   PUBMED   9049996
REFERENCE   3  (bases 1 to 37279)
  AUTHORS   Thomas,C.J., Bell,F.Y., Akya,A., Francis,M. and Webster,M.
  TITLE     Partial sequence analysis of a large plasmid from Listeria
            monocytogenes strain DRDC8
  JOURNAL   Unpublished
REFERENCE   4  (bases 1 to 37279)
  AUTHORS   Thomas,C.J.
  TITLE     Direct Submission
  JOURNAL   Submitted (05-OCT-1994) Microbiology and Immunology, University of
            Adelaide, Adelaide, South Australia 5005, Australia
REFERENCE   5  (bases 1 to 37279)
  AUTHORS   Thomas,C.J., Bell,F.Y., Akya,A., Francis,M. and Webster,M.
  TITLE     Direct Submission
  JOURNAL   Submitted (04-NOV-2005) Molecular and Biomedical Science, The
            University of Adelaide, Adelaide, SA 5005, Australia
  REMARK    Sequence update by submitter
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  AUTHORS   Thomas,C.J., Bell,F.Y., Akya,A., Francis,M. and Webster,M.
  TITLE     Direct Submission
  JOURNAL   Submitted (24-SEP-2007) Molecular and Biomedical Science, The
            University of Adelaide, Adelaide, SA 5005, Australia
  REMARK    Sequence update by submitter
COMMENT     On Sep 24, 2007 this sequence version replaced gi:83268869.

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CDS             8901..9260
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RBS             11972..11975
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                VKWSRGLKQKFSKGKKIDFLPDATIIGIYRPFTKKYLQYQVDIIESPGKYKDIFGDNN
                LSIYSTGAGASKGFSALITNAIPNFHFMDSGQGFYKNDNSSKDGFFVKDSSNLIENKL
                ELSQDDCFYYVYSVLHSSEYRKKYANDLQKTLPRIPLLKHREEYVEIGRKLADLHLNY
                EDQPIWEGVEVEISKPNYRVKKMKHPKKGILDTIIFNDSITIKNIPEKAYEYIVNGRP
                AIEWIIDQYQVKTDKKSGITDDPNDFSDDPKYILNLLLAVITVSMRTLELIDELPEFE
                LLEKE"
misc_feature    complement(19790..19849)
                /locus_tag="pCT0012"
                /note="PESYGIPKKDTGVDLVAEQK; Region: Catalytic
                endonuclease motif"
                /evidence=not_experimental
misc_feature    complement(19394..19450)
                /locus_tag="pCT0012"
                /note="RGQLIMAPGTGKTFTSLKI; Region: Helicase-like (DEAD
                motif) endonuclease motif I"
                /evidence=not_experimental
misc_feature    complement(19313..19360)
                /locus_tag="pCT0012"
                /note="FKVLYLVPSIQLLTQT; Region: Helicase-like (DEAD
                motif) endonuclease motif IA"
                /evidence=not_experimental
misc_feature    complement(19019..19042)
                /locus_tag="pCT0012"
                /note="FIISDEAH; Region: Helicase-like (DEAD motif)
                endonuclease motif II"
                /evidence=not_experimental
misc_feature    complement(18908..18934)
                /locus_tag="pCT0012"
                /note="RMYQTATPK; Region: Helicase-like (DEAD motif)
                endonuclease motif III"
                /evidence=not_experimental
misc_feature    complement(18476..18532)
                /locus_tag="pCT0012"
                /note="SSQFEEVVNEYISEAIEDE; Region: helicase-like (DEAD
                motif) endonuclease motif IV"
                /evidence=not_experimental
misc_feature    complement(18476..18532)
                /locus_tag="pCT0012"
                /note="SSQFEEVVNEYISEAIEDE; Region: helicase-like
                endonuclease motif IV"
                /evidence=not_experimental
misc_feature    complement(18305..18367)
                /locus_tag="pCT0012"
                /note="IVSNVRFLTEGIDIPTLDAVI; Region: Helicase-like (DEAD
                motif) endonuclease motif V"
                /evidence=not_experimental
misc_feature    complement(18245..18286)
                /locus_tag="pCT0012"
                /note="SQVDIVQAVGRIMR; Region: Helicase-like (DEAD motif)
                endonuclease motif V"
                /evidence=not_experimental
misc_feature    complement(17399..17449)
                /locus_tag="pCT0012"
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                /note="GIVFTPIEVVDFIVHSV; Region: Methylase motif Is"
                /evidence=not_experimental
misc_feature    complement(17300..17344)
                /locus_tag="pCT0012"
                /note="ILDPFTGTGTFIVRT; Region: methylase motif CM I"
                /evidence=not_experimental
misc_feature    complement(16979..17008)
                /locus_tag="pCT0012"
                /note="ITAIIGNPPY; Region: Methylase motif Cm II"
                /evidence=not_experimental
misc_feature    complement(16784..16840)
                /locus_tag="pCT0012"
                /note="FRWTSDRLKGNGIIGFVSN; Region: Methylase motif CM
                III"
                /evidence=not_experimental

gene            complement(20011..20553)
                /locus_tag="pCT0013"
                /evidence=not_experimental
CDS             complement(20011..20553)
                /locus_tag="pCT0013"
                /note="Similar to gram positive plasmid replication
                protein B, Listeria innocua (pli0022, NP_569176.1
                GI:18450305) and cryptic protein often found adjacent to
                replication protein genes, Lactococcus lactis (AAK71921.1
                GI:14669433)"
                /codon_start=1
                /evidence=not_experimental
                /transl_table=11
                /product="Hypothetical protein"
                /translation="MMSEKLKTIKELADEIGVSKQAVWQKIKKESSIDLRQFTSKKGN
                TVYVDGQKVIKSAFFNKTSTKKRQQKVFVDDNVNNSVDDNPEGNEEILFLRNLVSELQ
                SEKKDLHKLLDQQQRLALQDKKLLEEYKEEIKELKSLMVPVRKDDKDPMVRKSKDIDN
                TKEELHTKNKKWWHFGRNVK"

gene            complement(20550..21701)
                /locus_tag="pCT0014"
                /evidence=not_experimental
CDS             complement(20550..21701)
                /locus_tag="pCT0014"
                /note="Similar to plasmid replication initiation protein,
                Listeria innocua (pli0023, CAC42021.1  GI:16415807),
                Lactococcus lactis (AAD43535.1  GI:5453328) and repB
                replication protein, Lactococcus lactis (AAB52513.1
                GI:1944625)"
                /codon_start=1
                /evidence=not_experimental
                /transl_table=11
                /product="Hypothetical protein"
                /translation="MTSIPKNEQNQKQVQILNELSKRKVVEHNSLITSIAKMDKTPLK
                MFELAVSCIDTEEPPKDHTVYLSKEELFAFFKVSDNDKHSRFKQAVENMQKQAFFKIK
                EKKEHGFEFENIVPIPYVKWTDYHDEVTIRFSPEILPYLINLKKNFTQHALSDISELN
                SKHSIILYRWLSMNYNQYEHYSYKGGRREEQVEAYRNLSISIRELREMTDIVNEYKLF
                ADLEKWILKKPLEEINDHTSFTVTYDKVKKGRSIDSIVFHITKKRRADDNSYKLEDKV
                YQEDKARKAETEDMLTVQALKSPYTKLLMEHFLLSYLDLTDTKILSGLQAHVYPLYDE
                LKDLRGLDGVNDHLGYVSSKREEYSKHNIAKYLKKAIEQYLSTVKRQDL"
RBS             complement(21712..21715)
                /evidence=not_experimental
-10_signal      complement(21776..21781)
                /evidence=not_experimental
repeat_unit     21787..21799
                /rpt_type=Inverted
-35_signal      complement(21797..21802)
                /evidence=not_experimental
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repeat_unit     complement(21809..21839)
                /note="30 base direct repeat typical for promoter regions
                associated with repB genes"
                /rpt_type=Direct
repeat_unit     complement(21832..21861)
                /note="30 base direct repeat typical for promoter regions
                associated with repB genes"
                /rpt_type=Direct
repeat_unit     complement(21832..21843)
                /rpt_type=Inverted
repeat_unit     complement(21854..21868)
                /note="15 base imperfect repeat typical for promoter
                regions associated with repB genes"
                /rpt_type=Direct
repeat_unit     complement(21927..21944)
                /note="Inverted repeat (right) for ISS1 like element
                enclosing pCT0015"
                /rpt_type=Inverted

gene            complement(21981..22661)
                /locus_tag="pCT0015"
CDS             complement(21981..22661)
                /locus_tag="pCT0015"
                /note="Similar to transposase, Listeria innocua (pli0024,
                pli0071, CAC42022.1 GI:16415808, pli0063 CAC42061.1
                GI:16415846), Lactococcus lactis (Orf946, AAC64335.1
                GI:2895550)"
                /codon_start=1
                /evidence=not_experimental
                /transl_table=11
                /product="Hypothetical protein.  Similar to pCT0009,
                pCT0011."
                /translation="MSHFKGKQFKQDIIMVAVGYYLRYNLSYRDVSEILNERGIKVCH
                TTVYRWVQEYGSIIYCLWKKRNKSASDSWRMDETYIKVKGKWHYLYRAIDSTGLTLDI
                WLRKNRDKQAAYAFFKRLIKQFGEPRVFVTDKAPSLSSAFNRLKSEGLFSNTEHRTSK
                YLNNIIEQDHRPIKKRHKLYQSIRTASSTIKGIETIHALYKTSQRDFSLFGFSVIHEI
                NNLMGVPA"
RBS             complement(22670..22673)
                /evidence=not_experimental
-10_signal      22689..22694
                /evidence=not_experimental
repeat_unit     22716..22733
                /note="Inverted repeat (left) for ISS1 like element
                enclosing pCt0015"
                /rpt_type=Inverted

gene            23209..24534
                /locus_tag="pCT0016"
                /evidence=not_experimental
CDS             23209..24534
                /locus_tag="pCT0016"
                /note="Similar to Na+-driven multidrug efflux pump, MATE
                efflux family protein, Streptococcus suis (ZP_00332543.1
                GI:50591223), Streptococcus thermophilus (norN,
                ZP_00388590.1 GI:62527305)"
                /codon_start=1
                /evidence=not_experimental
                /transl_table=11
                /product="Hypothetical protein"
                /translation="MTRMLKNLIKLALPATVENILETSVGFVDSLMISQIGLFAVAGI
                GIANAILNVYIAVFIALGIGTSSLISRNIGAKNNEKAKLLLTIFTISNRLGLFWVGES
                SNRSTTLKLMGATKQTLNYSLQFFSIVGGGSVAIATMIILGSMLRAIGDTKTPMKIGL
                ITNILNISLDYILIFGLGPIPALGVVGTAIGTLVARLLGTILLYRKVHLSVLSFPLLS
                IFQRSNYQELLKLSFPAALERLVMRLGQVLYFGVIVAIGAKTYSAHSIAGSIESFVYM
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                PAYGLATAAATLAGNSIGKKDYAETKRVALYAVKYGVIVLSILGIGLFFGTPYIAPWF
                TIDAEAINQIVIALKIDAFNQPGLAISLILAGVLQGMGDTKTPLYSTAFGMWITRILG
                CILLGSIMGLGIAGVWLAIGLDLYVRSLFLTYHFKRNLQILNSNNELSS"

gene            25295..25789
                /locus_tag="pCT0017"
                /evidence=not_experimental
CDS             25295..25789
                /locus_tag="pCT0017"
                /note="Similar to negative transcriptional regulators
                associated with copper transport operons copR, Lactococcus
                lactis  (AAK04930.1  GI:12723756), CopY, Streptococcus
                mutans  (AAN58178.1  GI:24376801, AAG10085.1  GI:9965434)
                Streptococcus pneumoniae R6 copY (NP_358233.1
                GI:15902683)"
                /codon_start=1
                /evidence=not_experimental
                /transl_table=11
                /product="Hypothetical protein"
                /translation="MENKHQSNSNEVLMSQLPSDPKILKELNISDSELVIMRVVWSLG
                STTADEIGRELSETYQWSPSTIKTFLARLIKKGLLKNSRDGRKYVYIATCSEDEAICQ
                MTLSFLNKICAHKHANVILEMIDASSITAENKEAISEKLSSKNVVDEVTCDCINRLNC
                CDNN"
misc_feature    25379..25417
                /locus_tag="pCT0017"
                /note="Region: ISDSELVIMRVVW; consensus N-terminal DNA
                binding domain"
                /evidence=not_experimental
misc_feature    25475..25525
                /locus_tag="pCT0017"
                /note="Region: WSPSTIKTFLARLIKKG; N-terminal consensus DNA
                binding domain"
                /evidence=not_experimental
misc_feature    25748..25777
                /locus_tag="pCT0017"
                /note="Region: CDCINRLNCC; putative copper-binding motif"
                /evidence=not_experimental

gene            25804..26175
                /locus_tag="pCT0018"
                /evidence=not_experimental
CDS             25804..26175
                /locus_tag="pCT0018"
                /note="Similar to conserved hypothetical proteins,
                Lactobacillus johnsonii (LJ1836, AAS09781.1  GI:41584175),
                Streptococcus pneumoniae R6 (spr0640, NP_358234.1
                GI:15902684)"
                /codon_start=1
                /transl_table=11
                /translation="MSQIIVLIIGLTLIAFIGWWFFGKHEVHQETAVISSDGQTATIV
                VNGGYNPAVLNLKKDVPVNLIFNRKDASSCLEKVIFPDFGVDADLPLNQDVSILIDTS
                KSGEFIYSCGMNMFHGKIIIK"

gene            26218..26457
                /locus_tag="pCT0019"
CDS             26218..26457
                /locus_tag="pCT0019"
                /note="Putative copper binding protein, similar to
                N-terminal region of P-type ATPase - probable copper
                transporter ctpA, Streptococcus pneumoniae R6  (AAK99445.1
                GI:15458226)"
                /codon_start=1
                /transl_table=11
                /product="Hypothetical protein"
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                /translation="MFGSKINNKVEVVVDGGYSPSKFKLKAGEPAEVSFTRVSDKGCA
                QQIIFNGELRNLPLNESVTFNFTPVEKGRHNWSAV"

gene            26528..28489
                /gene="ctpA"
                /locus_tag="pCT0020"
CDS             26528..28489
                /gene="ctpA"
                /locus_tag="pCT0020"
                /function="involved in cation transport"
                /note="copper-translocating ATPase: similar to copA,
                Lactobacillus plantarum (NP_786368.1  GI:28379476),
                Streptococcus pneumoniae R6 ctpA (AAK99445.1
                GI:15458226). Identified by similarity to Enterococcus
                hirae copper-translocating ATPase: Swiss-Prot Accession
                Number P32113, and Synechococcus sp. cation-transporting
                ATPase: Swiss-Prot Accession Number P37279"
                /citation=[1]
                /citation=[2]
                /codon_start=1
                /evidence=experimental
                /transl_table=11
                /product="CtpA"
                /translation="MSIKNRFIIGVIGSVPLLINMFMSLGGSMLGGDKYGVWILFAFG
                SLVYWFSGLPFLRTAVASFKNHHANMDTLVGLGTTIAYVYSLYAMFARPNETYFEAVA
                VVITLILLGSYFEERMKASASSAVDKLMGLQAKDAEVLRDGEFIKLPIEEIIVGDLIR
                VKPGEKVAVDGQIVEGTSTLDESMVTGESMPVEKGPGDNVIGATLNNTGSFTFEVTKV
                GADTMLSNIAEMVRHAQNSRAPIQKTVDRISNIFVPIVLMISILTFIVWYVFLGSTLV
                TAMIFSVSVMIIACPCALGIATPTALMVGTGRSAKLGILIKNAEVLEATHDIKTVVMD
                KTGTITVGKPQVTDIISIGRISENEILRIAAGLEDSSEHPLALAVINEAKDKKITPAV
                AKNFTAISGKGVQALIDGKQAFIGNDRLSDDFNMTDDLKVKMTSLQAQAKTVVLVGYD
                GQIIALIGIQDAPKSSSKAAIRAMQKSGFHTVMLTGDNRLVAQAIADDIGIDEVIADV
                MPGDKAQHIRKLQEKGAVAFVGDGINDAPALSTATVGIAMGSGSDIAIESGGIVLVKN
                DLMDVVTSLVLARKTYSRILINLFWAFIYNVIGIPVAAGIFSALGFTLSPELAGLAMA
                LSSITVVLSSLLLNYVRLPKSSETLIGNS"
misc_feature    27017..27124
                /gene="ctpA"
                /locus_tag="pCT0020"
                /note="Region: GEKVAVDGQIVEGTSTLDESMVTGESMPVEKGPGDN;
                Putative transduction domain"
                /evidence=not_experimental
misc_feature    27395..27424
                /gene="ctpA"
                /locus_tag="pCT0020"
                /note="Region: CPCALGIATP; Putative ion channel"
                /evidence=not_experimental
misc_feature    27494..27652
                /gene="ctpA"
                /locus_tag="pCT0020"
                /note="Region:
                EATHDIKTVVMDKTGTITVGKPQVTDIISIGRISENEILRIAAGLEDSSEHPL;
                Putative aspartyl kinase domain"
                /evidence=not_experimental
misc_feature    28055..28171
                /gene="ctpA"
                /locus_tag="pCT0020"
                /note="Region: PGDKAQHIRKLQEKGAVAFVGDGINDAPALSTATVGIAM;
                Putative hinge and ATP binding domain"
                /evidence=not_experimental
repeat_unit     28620..28650
                /note="Left Inverted repeat enclosing pCT0021"
                /evidence=not_experimental
                /rpt_type=Inverted
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gene            28770..30071
                /locus_tag="pCT0021"
                /note="CDS inactivated by mutations introducing
                termination codons located at positions 25810, 25840,
                25906, 26173, 26221"
                /evidence=not_experimental
CDS             28770..30071
                /locus_tag="pCT0021"
                /note="Similar to transposases used to facilitate
                insertion sequence transposition, Enterococcus faecium
                (EAN11060.1  GI:68196635), Staphylococcus aureus
                (CAD33702.1  GI:21436688)"
                /codon_start=1
                /evidence=not_experimental
                /transl_table=11
                /product="Hypothetical protein"
                /translation="MYHCTRKLLGLTDENLFFEEEWLETVEEDGFRTNLIHAKLSYIL
                SHCRKCGIKNEGQIIKNGSHKTKVQLLPYRATKTELRLVRTRFYCKECQSTFNAQTNL
                VDENCYLSKELKVQIALELAKNTIKKELPIAILYQT*SFCVSCTPV*KRIILVLIRYL
                RSFVLMSTNR*SPAVEKMSFVFMNGQTQQLIGVLENRRLTFLKPYFLNFTRKARANVK
                YVVMDMNAPYFELVKAVFPNAKIVTNRFHIVKQITRTLNQL*IKTMNRFQKTEPTKY*
                RLKRFWKLLLTHAYDLDSSDYQYDRFFRRPMTQKAMVDELLSYDEQLTRAYETCQLLL
                YHFKHKDNQSFFDTINSLDQCLPQWFCKKLTFLNKYKLGIQYALKPRYSNGALERTNN
                KIKVIKRVAYGYRNFHNFRARIYLIQGLIFQVKQKPVKHSA"
repeat_unit     complement(30104..30134)
                /note="Right Inverted repeat enclosing pCT0021"

gene            complement(30269..30865)
                /locus_tag="pCT0022"
                /evidence=not_experimental
CDS             complement(30269..30865)
                /locus_tag="pCT0022"
                /note="Hypothetical protein similar to YdhK, Bacillus
                subtilis (G69784  GI:7474963)"
                /codon_start=1
                /transl_table=11
                /product="Hypothetical protein"
                /translation="MNTVKKMIKLVFASLGIIVFLGACSNQSESNNSKSTNEESTSIA
                SSEMNSMEGMNHEGMVPSSMKDAANPKFPVGSNVILLGDHMEGMKGANAQVVGAFDTT
                MYEVSYEPKTGGPMVNNHRWVVQEELKDTETVANEGDTVILNADHMDGMMGAEAKVDK
                SITGTVYVVNYTPTDGQEEVKNHMWVTEDEMEYDENNE"

gene            complement(31107..32219)
                /locus_tag="pCT0023"
                /evidence=not_experimental
CDS             complement(31107..32219)
                /locus_tag="pCT0023"
                /note="Similar to two-component sensor histidine kinase,
                Clostridium perfringens (NP_563247.1 GI:18311313) and
                ATP-binding region, ATPase-like:Histidine kinase, HAMP
                region:Histidine kinase A, N-terminal, Clostridium
                thermocellum (EAM44605.1 GI:67848970)"
                /codon_start=1
                /evidence=not_experimental
                /transl_table=11
                /product="Hypothetical protein"
                /translation="MVWELTGKDLKSAQEKLKKNALKVSEKNSVKLDETIEVKKKLIN
                DGNEFGKVTFYYFGPFAYTEHDALFISSMKQSLMYVAIAALLVSFILASWISARLGLP
                LKHVSDFTHKLTRGEYADKIPQETSIIEINSLIDSLNDLSNQLEKQHGLRKRLTTDIS
                HELRTPLATLKGNVEGMIDGVWKITPERLQSCYDEIDRLTRLIGNIEIINKIEAKYDH
                LNKTEFNIYKLIESVIENFASKIESKNLHVEIQGDNINISADKDKMNQVVINLLNNAI
                KFTQKEGTIKFSISKNKDHVLLIVEDNGIGIEKDQQLHIFDRFYMADPSRSRALGGQG
                IGLAIVKSVVEAHKGSITVKSKLGLGTKFVIKLPFQ"
misc_feature    complement(31710..31757)
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                /locus_tag="pCT0023"
                /note="LTTDISHELRTPLATL; Region: Histidine kinase H-box
                motif"
                /evidence=not_experimental
misc_feature    complement(31383..31442)
                /locus_tag="pCT0023"
                /note="DKDKMNQVVINLLNNAIKFT; Region: Histidine kinase
                N-box motif"
                /evidence=not_experimental
misc_feature    complement(31263..31334)
                /locus_tag="pCT0023"
                /note="VLLIVEDNGIGIEKDQQLHIFDRF; Region: Histidine kinase
                D-box motif"
                /evidence=not_experimental
misc_feature    complement(31119..31223)
                /locus_tag="pCT0023"
                /note="QGIGLAIVKSVVEAHKGSITVKSKLGLGTKFVIKL; Region:
                Histidine kinase G-box motif"
                /evidence=not_experimental

gene            complement(32502..33182)
                /locus_tag="pCT0024"
                /evidence=not_experimental
CDS             complement(32502..33182)
                /locus_tag="pCT0024"
                /note="Complete; Similar to prokaryotic cytoplasmic
                two-component response regulators, Thermoanaerobacter
                tengcongensis (AAM25604.1 GI:20517480), Clostridium
                perfringens (BAB82038.1 GI:18145997)"
                /codon_start=1
                /evidence=not_experimental
                /transl_table=11
                /product="Hypothetical protein"
                /translation="MKILIVDDEEKILEIIDAYLVANHYSVYKAISGAMALEKFEKNN
                PDLVVLDLMLPDTDGLTVCRKIREISNVPIIMLTAKSDEDDILTGLKLGADDYMLKPF
                SPKELVARIQTVLRRTETLSNPNKLSSNNKELIVYPDSRQVYLHQVELNLTTTEFDIL
                HALMSTPNKVFSRSDLIEKVKGIEFDGLDRSIDSHIKNLRHKIENDAKSPMYILTVHG
                TGYRFGGH"
misc_feature    complement(32838..33179)
                /locus_tag="pCT0024"
                /note="Conserved N-terminal regulatory domain; PS50110;
                Region: Response regulatory domain"
                /evidence=not_experimental
misc_feature    complement(33015..33017)
                /locus_tag="pCT0024"
                /note="Phosphorylatable aspartate residue; phosphorylation
                site"
                /evidence=not_experimental
misc_feature    complement(32514..32786)
                /locus_tag="pCT0024"
                /note="C-terminal effector domain contains DNA and RNA
                polymerase binding sites; cd00383; Region: Response
                Regulator trans_reg_C effector domain"
                /evidence=not_experimental

gene            complement(33210..34796)
                /locus_tag="pCT0025"
CDS             complement(33210..34796)
                /locus_tag="pCT0025"
                /note="Similar to multi-copper oxidases. Bacillus
                coagulans 36D1 (ZP_01696949), Staphylococcus haemolyticus
                JCSC1435 (YP_252021)."
                /codon_start=1
                /evidence=not_experimental
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                /transl_table=11
                /product="Hypothetical protein"
                /translation="MWGDRHEISTYFFYGKYIIDMGDDGLKNNKYIWLAATTISIFTL
                GGIFILIQQQNKEEHAEVIYQEEQRQISSRMSNMMGDNQEADLKVVHQTDRKLLIPPV
                LEPSSKSKENVTYDIVTQNGEVQIMDGEKTETLGYNGDFLGPVIRLKKGQKVTINTTN
                NLDASTSFHWHGLKVASDADGGPHQIIEAGQKKSVTFEVDQEASTLWFHPHPEGETAS
                QVYKGLAGLMYIDDGNSKLLDLPSKYGVDDIPLIVQDKSFSSTNQINYENDFNSDGTK
                GETLLTNGTINPYVEIKSRWMRYRIVNGSNARNFTFNLDNDESFYQIATDGGFLNTSV
                KLSKLLLAPGERAEILVDTQNYKKGKVIHLLANNLVALTMRIENTIDNKEFNPTDSLN
                TISTLDEKKLEDLTRQSINLSGMSHMVNINNKQFDMERIDLYKKLGTQEIWEVNNISS
                MMGGMIHPFHIHGVQFQILSRDGNQPALNEQGWKDTVLVNPDETVELLVKFDREGIFM
                YHCHILEHEEYGMMGQMEIK"
misc_feature    complement(34092..34445)
                /locus_tag="pCT0025"
                /note="Copper oxidase-like domain not recognised by the
                pfam00394 model; pfam07732; Region: Cu-oxidase_3,
                Multicopper oxidase."
                /evidence=not_experimental
misc_feature    complement(33219..33533)
                /locus_tag="pCT0025"
                /note="Divergent copper oxidase-like domain not recognised
                by the pfam00394 model; pfam07731.  Contains the consensus
                signature pattern: H - C - H - x(3) - H - x(3) - [AG] -
                [LM]. The first 2 H's are copper type 3 binding residues]
                [The C, the third H, and L or M are copper type 1 ligands;
                Region: Cu-oxidase_2, Multicopper oxidase"
                /evidence=not_experimental

gene            complement(34807..35238)
                /locus_tag="pCT0026"
                /evidence=not_experimental
CDS             complement(34807..35238)
                /locus_tag="pCT0026"
                /note="Truncated.  Probably inactivated open reading
                frame.  Similar to heavy metal translocating P-type
                ATPase.  Desulfitobacterium hafniense Y51 (YP_518797),
                Desulfitobacterium hafniense DCB-2 (ZP_01369576),
                Pyrococcus furiosus DSM ~3638 (NP_578469)."
                /codon_start=1
                /evidence=not_experimental
                /transl_table=11
                /product="Hypothetical protein"
                /translation="MISKYNLYGMTCAVCATTIEKKIHELDGVYFAKVNLTTEVLKLE
                YDEGVLSNHTVITAIQDIGYDAEIRKKTEIKVFGISGMNCSGCATKVRNALEAEPTVS
                VKIVDLARGTVTFDADSKLTLTFLNQLLKNTKYTVTRDISI"
misc_feature    complement(35038..35229)
                /locus_tag="pCT0026"
                /note="Heavy-metal-associated domain (HMA) is a conserved
                domain of approximately 30 amino acid residues found in a
                number of proteins that transport or detoxify heavy
                metals, for example, the CPx-type heavy metal ATPases and
                copper chaperones. HMA domain contains two cysteine
                residues that are important in binding and transfer of
                metal ions, such as copper, cadmium, cobalt and zinc;
                Region: HMA Heavy metal associated domain"
                /evidence=not_experimental

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26161 atcatcatca aatagaatca caaattaaat actaatatac cctaaggagg atataaaatg
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33841 gattcgtcat tatccaaatt aaaagtgaag tttcttgcat tggatccatt aacaattcta
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    37141 tctaaggagg aagatttaca tggaagcatt acttaatctc ttaccnctac tattactact
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    37261 tatgcatgga gaagatcat
//
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Appendix B

ClustalX multiple sequence alignment of PCR products generated using the CuTrR/CuTrF oligonucleotide pair from a putative EGD-Rif transconjugant (Transconjugant) and strains DSE201 and EGD-Rif. ‘*’ indicates identical base pairs.

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CLUSTAL X (1.81) multiple sequence alignment

Transconjugant  ATGCGGGTTATGACGCGGCGGAAAAAATGTCTAAAGAAGAAAAAGATGCTGTGTTAGAAA
DSE201          ATGCGGGTTATGACGCGGCGGAAAAAATGTCTAAAGAAGAAAAAGATGCTGTGTTAGAAA
EGD-Rif         ATGCGGGTTATGACGCTGCGGAAAAAATGTCCAAAGAAGAAAAAGATGCTGTATTAGAAA
                **************** ************** ******************** *******

Transconjugant  AGAATTTCAAAAAAGAAGTGAGACGTTTTATTCTTTCGGCGGTTCTTTCCTTACCATTAC
DSE201          AGAATTTCAAAAAAGAAGTGAGACGTTTTATTCTTTCGGCGGTTCTTTCCTTACCATTAC
EGD-Rif         AGAATTTCAAAAAAGAAGTGAGACGTTTTATTCTTTCGGCGCTTCTTTCCTTGCCATTAC
                ***************************************** ********** *******

Transconjugant  TTCTTACCATGGTGACACATATTCCGTACATTCATGAAATGGTGTTTGCGGAAACGATTG
DSE201          TTCTTACCATGGTGACACATATTCCGTACATTCATGAAATGGTGTTTGCGGAAACGATTG
EGD-Rif         TTCTTACCATGGTGACACATATTCCGTATATCCATGAAATGGCGTTTGCGGAAACGATAG
                **************************** ** ********** *************** *

Transconjugant  GTAACTGGATTAATCCAACCATCCAATTAGTACTCGCAACGATTGTTCAGTTTTATATTG
DSE201          GTAACTGGATTAATCCAACCATCCAATTAGTACTCGCAACGATTGTTCAGTTTTATATTG
EGD-Rif         GTAACTGGATTAATCCAACGATTCAATTAGTTCTTGCAACTATTGTTCAGTTTTATATTG
                ******************* ** ******** ** ***** *******************

Transconjugant  GTTGGCGGTTTTATGATGGGGCTTATAAAGCGCTTCGAGGTAAAAGTGCAAATATGGATG
DSE201          GTTGGCGGTTTTATGATGGGGCTTATAAAGCGCTTCGAGGTAAAAGTGCAAATATGGATG
EGD-Rif         GTTGGCGGTTTTACGATGGGGCTTATAAAGCGCTGCGAGGTAAAAGTGCGAATATGGATG
                ************* ******************** ************** **********

Transconjugant  TATTAGTCGCACTTGGGACTTCTGCTGCTTATTTCTATAGTGTAGTGGAATACATTCGTC
DSE201          TATTAGTCGCACTTGGGACTTCTGCTGCTTATTTCTATAGTGTAGTGGAATACATTCGTC
EGD-Rif         TATTAGTCGCACTTGGGACATCTGCTGCATACTTCTATAGTGTGGTAGAGTATGTCCGCC
                ******************* ******** ** *********** ** ** **  * ** *

Transconjugant  ACATGATTGATCCGAGCGTGATGCCGCATTACTACTTTGAAACAAGTGCTGTGTTAATCA
DSE201          ACATGATTGATCCGAGCGTGATGCCGCATTACTACTTTGAAACAAGTGCTGTGTTAATCA
EGD-Rif         ATATAATTGACCCAAGCGTGATGCCACATTACTACTTTGAAACAAGTGCCGTGCTAATCA
                * ** ***** ** *********** *********************** *** ******

Transconjugant  CCTTAATCTTGTTAGGTAAATTACTTGAATCATACGCAACATCGAGAACAACCGAATCTA
DSE201          CCTTAATCTTGTTAGGTAAATTACTTGAATCATACGCAACATCGAGAACAACCGAATCTA
EGD-Rif         CATTAATCTTATTAGGTAAACTGCTTGAATCATACGCAACTTCCAGAACAACGGAATCTA
                * ******** ********* * ***************** ** ******** *******
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Appendix C

Nucleotide sequence (5’ – 3’) (incomplete) for a 1883 bp region of ORF *cutR* amplified and sequenced using the CuTrM2F/CuTrM2R oligonucleotide pair.

```
   1  GCGAATGTCA ACTTAGTAAC GGAAAATGCA GCCGTATATT ATGACCCGGA
  51  AGTTACATCG ACAGAAGATT TGATTAAAGT TGTTAAACAT GCGGGTTATG
 101  ACGCGGCGGA AAAAATGTCT AAAGAAGAAA AAGATGCTGT GTTAGAAAAG
 151  AATTTCAAAA AAGAAGTGAG ACGTTTTATT CTTTCGGCGG TTCTTTCCTT
 201  ACCATTACTT CTTACCATGG TGACACATAT TCCGTACATT CATGAAATGG
 251  TGTTTGCGGA AACGATTGGT AACTGGATTA ATCCAACCAT CCAATTAGTA
 301  CTCGCAACGA TTGTTCAGTT TTATATTGGT TGGCGGTTTT ATGATGGGGC
 351  TTATAAAGCG CTTCGAGGTA AAAGTGCAAA TATGGATGTA TTAGTCGCAC
 401  TTGGGACTTC TGCTGCATAT TTCTATAGTG TAGTGGAATA CATTCGTCAC
 451  ATGATTGATC CGAGCGTGAT GCCGCATTAC TACTTTGAAA CAAGTGCTGT
 501  GTTAATCACC TTAATCTTGT TAGGTAAATT ACTTGAATCA TACGCAACAT
 551  CGAGAACAAC CGAATCTATT GCTGGTTTAC TTGAATTACA AGCAAAAGAA
 601  GCGACCGTTA TTCGAGAAGG AAAAGAATGG TTAGTGCCAG TAGATTCATT
 651  GAAAATTGGC GATATTATCC TTGTTCGTCC GGGTGAAAAA GTTCCGATGG
 701  ATGCCGAAAT TATTTCTGGT GAAACGAGTA TTGATGAAGC GATGATTACT
 751  GGGGAACCTG TACCAGTAGA GAAGAAACCA GGCGATTCTG TTATTGGCGC
 801  AACGATTAAC TTTGACGGGG CTTTCCAAGC AAAAATTACG AAACGAATGG
 851  AAGAAACCGT TTTAGAATCC ATTATTCGTT TGGTGGAAGA AGCGCAAGGT
 901  ATTAAAGCGC CAATTCAACG TTTGGCAGAT AGAATTTCCG GCATATTTGT
 951  ACCAATTGTA CTTGGGATTG CTGCTGTAAC CTTTATTATT TGGTATCTTG
1001  TTACTGGAAC GGTGGATGGT TCACTTGAAG CTGCGATTGC GGTATTAGTT
1051  ATCGCCTGTC CCTGTGCGCT TGGTCTTGCA ACGCCAACCG CTATCATGGC
1101  TGGAACTGGT AAAGGCGCCG AAAGTGGGAT ATTATTTAAA GGTGGCGAAC
1151  ATTTAGAACG TACTTCCAAA GTGGATACTA TCGTTTTTGA CAAAACTGGT
1201  ACTTTAACAG AAGGTAAACT AGAAGTGAGT GATAAAAAAG CAGCCAATGA
1251  TCACTTTTTC CCTTATTTAT TCTTAATGGA ACAACAATCA GAGCATCCGA
1301  TTGCGAAAGC GATTATTAAG ATGTTAGAGC CAGAGAATAT AGATGCTTCC
1351  GCAGTGAAAC AAGGGAAAAT CCGCGCGAAA GCTGGGCACG GCATGACCGG
1401  TAATCTGGAT GATAGTAAAG TGGAACTAGG TGCTTACCGC TATGTTTCTT
1451  CCCTTACAAC GATTCCAAAA GAAGATGATG AATTAATCGA AAGTTGGATG
1501  CATGCAGGAA AAACAGTCGT AGCAATGGCA ATTGATGGTG TATACGCAGG
1551  TGCCCTCGCT TTATCTGACA CACCACGACC AGAAGCAAAA GAAGCCATCC
1601  AAAAACTAAA AGCACAAGGT ATTAAAACAG CAATTTGTTC TGGGGACCAA
1651  TCTGTTGTCG TAGAAAATAT GGCTAAAGAT TTAGGTATTG ATATGTTCTT
1701  TGCCGAACAA CTACCAAATG ATAAGAGCGC CTTAGTCGAG AAATTACAGC
1751  AAGACGGTCA TATCGTTGCA TTCGTTGGTG ATGGCATTAA TGATGCTCCA
1801  GCTCTTGCGG CAAGTGATAT TGGGATTAGT ATCGGTACTG GAACTGACAT
1851  TGCGATTGAA ACAGGAGATG TAACACTTGT AAG
```

Appendix D

ClustalX multiple sequence alignment of the deduced polypeptide sequence of ORFs pCT0009, pCT0011 and pCT0015. ‘*’ indicates identical amino acids.

```
CLUSTAL X (1.81) multiple sequence alignment

pCT0009        MSHFKGKQFKQDIIMVAVGYYLRYNLSYRDVSEILNERGIKVCHTTVYRWVQEYGSIIYC
pCT0011        MSHFKGKQFKQDIIMVAVGYYLRYNLSYRDVSEILNERGIKVCHTTVYRWVQEYGSIIYC
pCT0015        MSHFKGKQFKQDIIMVAVGYYLRYNLSYRDVSEILNERGIKVCHTTVYRWVQEYGSIIYC
               ************************************************************

pCT0009        LWKKRNKSASDSWRMDETYIKVKGKWHYLYRAIDSTGLTLDIWLRKNRDKQAAYAFFKRL
pCT0011        LWKKRNKSASDSWRMDETYIKVKGKWHYLYRAIDFTGLTLDIWLRKNRDKQAAYAFFKRL
pCT0015        LWKKRNKSASDSWRMDETYIKVKGKWHYLYRAIDSTGLTLDIWLRKNRDKQAAYAFFKRL
               ********************************** *************************

pCT0009        IKQFGEPRVFVTDKAPSLSSAFNRLKSEGLFSNTEHRTSKYLNNIIEQDHRPIKKRHKLY
pCT0011        IKQFGEPRVFVTDKAPSLSSAFNRLKSEGLFSNTEHRTSKYLNNIIEQDHRPIKKRHKLY
pCT0015        IKQFGEPRVFVTDKAPSLSSAFNRLKSEGLFSNTEHRTSKYLNNIIEQDHRPIKKRHKLY
               ************************************************************

pCT0009        QSIRTASSTIKGIETIHALYKTSQRDFSLFGFSVIHEINNLMGVPA
pCT0011        QSIRTASSTIKGIETIHALYKTSQRDFSLFGFSVIHEINNLMGVPA
pCT0015        QSIRTASSTIKGIETIHALYKTSQRDFSLFGFSVIHEINNLMGVPA
               **********************************************
```

Appendix E

ClustalX multiple sequence alignment of the deduced polypeptide sequence of ORFs pCT0017 and pCT0027. ‘*’ indicates positions which have a single, fully conserved residue shared between all sequences, ‘**:**’ indicates amino acids that are in a 'strong' similarity group are fully conserved and ‘**.**’ indicates that the amino acids of a 'weaker' similarity group are fully conserved.

```
CLUSTAL X (1.81) multiple sequence alignment

pCT0017         MENKHQSNSNEVLMSQLPSDPKILKELNISDSELVIMRVVWSLGSTTADEIGRELSETYQ
pCT0027         -------------------MVLVEHKIQVSNSELDVLKFIWRYEPVTCGLITHGMQERNS
                                      : :::::*:*** :::.:*   ..*.. * : :.*  .

pCT0017         WSPSTIKTFLARLIKKGLLKNSRDGRKYVYIATCSEDEAICQMTLSFLNKICAHKHANVI
pCT0027         WHPSTTKTLIRRLLDKNVITFNTSKNQRYYTSLIKKQE-------------FLETEIQRL
                * *** **:: **:.*.::. . . .:  * :  .::*               ... : :

pCT0017         LEMIDASSITAENKEAISEKLSSKNVVDEVTCDCINRLNCCDNN
pCT0027         LSGMDEGCISEVS-------YYLNGLVKRSDTEEFNDFKI----
                *. :* ..*:  .          :.:*..   : :* ::
```

Appendix F

Nucleotide sequence and deduced amino acid sequence of ORF pCT0017 carrying an introduced *Bam*HI site. The nucleotide sequence is numbered relative to pCT100 sequence shown in Appendix A, page 310. Nucleotide changes (CGATTT → GGATCC) introduced using overlap extension PCR are shown (in bold). The *Bam*HI site (GGATCC) introduced as a result of these changes is indicated (underlined). These bases changes introduced two missense mutations resulting in the following amino acid changes: A → G and R → C (underlined and bold).

```
25295 - ATGGAAAATAAACATCAAAGTAATTCAAATGAAGTACTAATGAGCCAATTACCTTCTGAT
        M  E  N  K  H  Q  S  N  S  N  E  V  L  M  S  Q  L  P  S  D

25355 - CCTAAAATATTAAAAGAATTAAATATTTCGGACAGTGAATTAGTTATTATGCGTGTCGTT
        P  K  I  L  K  E  L  N  I  S  D  S  E  L  V  I  M  R  V  V

25415 - TGGTCTCTAGGGAGTACTACAGCAGATGAAATTGGACGTGAGCTTAGTGAAACTTATCAG
        W  S  L  G  S  T  T  A  D  E  I  G  R  E  L  S  E  T  Y  Q

25475 - TGGAGCCCTTCCACAATAAAAACATTTTTAGCGCGTTTAATTAAAAAAGGATTATTAAAA
        W  S  P  S  T  I  K  T  F  L  A  R  L  I  K  K  G  L  L  K

25535 - AACAGTCGTGATGGTCGTAAATATGTCTATATTGCAACTTGTTCTGAAGATGAAGGGATC
        N  S  R  D  G  R  K  Y  V  Y  I  A  T  C  S  E  D  E  G  I

25595 - CGCCAAATGACACTATCTTTTTTGAATAAAATTTGTGCACATAAACATGCCAATGTTATT
        C  Q  M  T  L  S  F  L  N  K  I  C  A  H  K  H  A  N  V  I

25655 - TTAGAAATGATTGACGCGAGTAGTATTACTGCTGAAAATAAAGAAGCTATCAGCGAAAAA
        L  E  M  I  D  A  S  S  I  T  A  E  N  K  E  A  I  S  E  K

25715 - CTAAGTAGCAAAAATGTTGTTGACGAAGTAACTTGTGATTGTATAAACAGATTAAATTGT
        L  S  S  K  N  V  V  D  E  V  T  C  D  C  I  N  R  L  N  C

25775 - TGTGATAATAATTAGAAAAGGAGAATAACATGTCTCAAATTATCG
        C  D  N  N  *
```

Appendix G

Nucleotide sequence and deduced amino acid sequence of ORF pCT0018 carrying an introduced *Bam*HI site. The nucleotide sequence is numbered relative to pCT100 sequence shown in Appendix A, page 310. The nucleotide change (GGATGC → GGATCC) introduced using overlap extension PCR is shown (in bold). The *Bam*HI site (GGATCC) introduced as a result of these changes is indicated (underlined). This base change introduced a missense mutation resulting in the following amino acid change: A → P (underlined and bold).

```
25804 - ATGTCTCAAATTATCGTACTTATTATTGGATTGACTCTAATTGCTTTTATTGGATGGTGG
        M  S  Q  I  I  V  L  I  I  G  L  T  L  I  A  F  I  G  W  W

25864 - TTCTTTGGCAAACATGAGGTCCATCAGGAAACTGCCGTTATCAGTTCTGATGGACAAACA
        F  F  G  K  H  E  V  H  Q  E  T  A  V  I  S  S  D  G  Q  T

25924 - GCTACTATTGTGGTAAATGGAGGATATAATCCAGCAGTCTTAAATTTAAAAAAAGATGTC
        A  T  I  V  V  N  G  G  Y  N  P  A  V  L  N  L  K  K  D  V

25984 - CCAGTTAACTTGATCTTTAACCGGAAAGATGCCTCATCTTGTCTTGAAAAAGTTATTTTT
        P  V  N  L  I  F  N  R  K  D  A  S  S  C  L  E  K  V  I  F

26044 - CCAGATTTTGGAGTGGATCCGGATTTACCACTCAATCAGGATGTGTCTATTTTGATTGAT
        P  D  F  G  V  D  P  D  L  P  L  N  Q  D  V  S  I  L  I  D

26104 - ACATCTAAGTCAGGCGAGTTTATATATTCATGTGGGATGAATATGTTCCATGGAAAAATC
        T  S  K  S  G  E  F  I  Y  S  C  G  M  N  M  F  H  G  K  I

26164 - ATCATCAAATAG
        I  I  K  *
```

Appendix H

Nucleotide sequence and deduced amino acid sequence of ORF pCT0019 carrying an introduced *Bam*HI site. The nucleotide sequence is numbered relative to pCT100 sequence shown in Appendix A, page 310. The nucleotide change (GGATC<u>A</u> → GGATC<u>C</u>) introduced using overlap extension PCR is shown (in bold). The *Bam*HI site (GGATCC) introduced as a result of these changes is indicated (underlined). This base change did not result in an amino acid change.

```
26218 - ATGTTCGGATCCAAAATTAATAATAAAGTAGAAGTAGTTGTTGACGGAGGTTACTCTCCT
        M  F  G  S  K  I  N  N  K  V  E  V  V  V  D  G  G  Y  S  P

26278 - AGTAAATTTAAGTTAAAAGCTGGAGAGCCAGCTGAAGTCTCTTTTACTCGTGTCTCTGAT
        S  K  F  K  L  K  A  G  E  P  A  E  V  S  F  T  R  V  S  D

26338 - AAAGGTTGTGCTCAGCAAATTATCTTCAATGGAGAACTTCGAAATTTACCCTTAAATGAA
        K  G  C  A  Q  Q  I  I  F  N  G  E  L  R  N  L  P  L  N  E

26398 - TCTGTCACTTTTAATTTCACTCCAGTTGAAAAAGGACGTCATAATTGGTCTGCGGTATGA
        S  V  T  F  N  F  T  P  V  E  K  G  R  H  N  W  S  A  V  *
```

Appendix I

Nucleotide and deduced amino acid sequence for ORF *cutR* carrying an introduced *Bam*HI site. The nucleotide sequence is numbered relative to the known *cutR* sequence shown in Appendix C, page 327. A nucleotide change (GGATGC → GGATCC) introduced using overlap extension PCR is shown (in bold). The *Bam*HI site (GGATCC) introduced as a result of this change is indicated (underlined). This base change also introduced a missense mutation resulting in the following amino acid change: A → P (underlined and bold).

```
  1 - GCGAATGTCAACTTAGTAACGGAAAATGCAGCCGTATATTATGACCCGGAAGTTACATCG
      A  N  V  N  L  V  T  E  N  A  A  V  Y  Y  D  P  E  V  T  S

 61 - ACAGAAGATTTGATTAAAGTTGTTAAACATGCGGGTTATGACGCGGCGGAAAAAATGTCT
      T  E  D  L  I  K  V  V  K  H  A  G  Y  D  A  A  E  K  M  S

121 - AAAGAAGAAAAAGATGCTGTGTTAGAAAAGAATTTCAAAAAAGAAGTGAGACGTTTTATT
      K  E  E  K  D  A  V  L  E  K  N  F  K  K  E  V  R  R  F  I

181 - CTTTCGGCGGTTCTTTCCTTACCATTACTTCTTACCATGGTGACACATATTCCGTACATT
      L  S  A  V  L  S  L  P  L  L  L  T  M  V  T  H  I  P  Y  I

241 - CATGAAATGGTGTTTGCGGAAACGATTGGTAACTGGATTAATCCAACCATCCAATTAGTA
      H  E  M  V  F  A  E  T  I  G  N  W  I  N  P  T  I  Q  L  V

301 - CTCGCAACGATTGTTCAGTTTTATATTGGTTGGCGGTTTTATGATGGGGCTTATAAAGCG
      L  A  T  I  V  Q  F  Y  I  G  W  R  F  Y  D  G  A  Y  K  A

361 - CTTCGAGGTAAAAGTGCAAATATGGATGTATTAGTCGCACTTGGGACTTCTGCTGCATAT
      L  R  G  K  S  A  N  M  D  V  L  V  A  L  G  T  S  A  A  Y

421 - TTCTATAGTGTAGTGGAATACATTCGTCACATGATTGATCCGAGCGTGATGCCGCATTAC
      F  Y  S  V  V  E  Y  I  R  H  M  I  D  P  S  V  M  P  H  Y

481 - TACTTTGAAACAAGTGCTGTGTTAATCACCTTAATCTTGTTAGGTAAATTACTTGAATCA
      Y  F  E  T  S  A  V  L  I  T  L  I  L  L  G  K  L  L  E  S

541 - TACGCAACATCGAGAACAACCGAATCTATTGCTGGTTTACTTGAATTACAAGCAAAAGAA
      Y  A  T  S  R  T  T  E  S  I  A  G  L  L  E  L  Q  A  K  E

601 - GCGACCGTTATTCGAGAAGGAAAAGAATGGTTAGTGCCAGTAGATTCATTGAAAATTGGC
      A  T  V  I  R  E  G  K  E  W  L  V  P  V  D  S  L  K  I  G

661 - GATATTATCCTTGTTCGTCCGGGTGAAAAAGTTCCGATGGATCCCGAAATTATTTCTGGT
      D  I  I  L  V  R  P  G  E  K  V  P  M  D  P  E  I  I  S  G

721 - GAAACGAGTATTGATGAAGCGATGATTACTGGGGAACCTGTACCAGTAGAGAAGAAACCA
      E  T  S  I  D  E  A  M  I  T  G  E  P  V  P  V  E  K  K  P

781 - GGCGATTCTGTTATTGGCGCAACGATTAACTTTGACGGGGCTTTCCAAGCAAAAATTACG
      G  D  S  V  I  G  A  T  I  N  F  D  G  A  F  Q  A  K  I  T
```

Continued next page ……

```
 841 - AAACGAATGGAAGAAACCGTTTTAGAATCCATTATTCGTTTGGTGGAAGAAGCGCAAGGT
       K  R  M  E  E  T  V  L  E  S  I  I  R  L  V  E  E  A  Q  G

 901 - ATTAAAGCGCCAATTCAACGTTTGGCAGATAGAATTTCCGGCATATTTGTACCAATTGTA
       I  K  A  P  I  Q  R  L  A  D  R  I  S  G  I  F  V  P  I  V

 961 - CTTGGGATTGCTGCTGTAACCTTTATTATTTGGTATCTTGTTACTGGAACGGTGGATGGT
       L  G  I  A  A  V  T  F  I  I  W  Y  L  V  T  G  T  V  D  G

1021 - TCACTTGAAGCTGCGATTGCGGTATTAGTTATCGCCTGTCCCTGTGCGCTTGGTCTTGCA
       S  L  E  A  A  I  A  V  L  V  I  A  C  P  C  A  L  G  L

1081 - ACGCCAACCGCTATCATGGCTGGAACTGGTAAAGGCGCCGAAAGTGGGATATTATTTAAA
       T  P  T  A  I  M  A  G  T  G  K  G  A  E  S  G  I  L  F  K

1141 - GGTGGCGAACATTTAGAACGTACTTCCAAAGTGGATACTATCGTTTTTGACAAAACTGGT
       G  G  E  H  L  E  R  T  S  K  V  D  T  I  V  F  D  K  T  G

1201 - ACTTTAACAGAAGGTAAACTAGAAGTGAGTGATAAAAAAGCAGCCAATGATCACTTTTTC
       T  L  T  E  G  K  L  E  V  S  D  K  K  A  A  N  D  H  F  F

1261 - CCTTATTTATTCTTAATGGAACAACAATCAGAGCATCCGATTGCGAAAGCGATTATTAAG
       P  Y  L  F  L  M  E  Q  Q  S  E  H  P  I  A  K  A  I  I  K

1321 - ATGTTAGAGCCAGAGAATATAGATGCTTCCGCAGTGAAACAAGGGAAAATCCGCGCGAAA
       M  L  E  P  E  N  I  D  A  S  A  V  K  Q  G  K  I  R  A  K

1381 - GCTGGGCACGGCATGACCGGTAATCTGGATGATAGTAAAGTGGAACTAGGTGCTTACCGC
       A  G  H  G  M  T  G  N  L  D  D  S  K  V  E  L  G  A  Y  R

1441 - TATGTTTCTTCCCTTACAACGATTCCAAAAGAAGATGATGAATTAATCGAAAGTTGGATG
       Y  V  S  S  L  T  T  I  P  K  E  D  D  E  L  I  E  S  W  M

1501 - CATGCAGGAAAAACAGTCGTAGCAATGGCAATTGATGGTGTATACGCAGGTGCCCTCGCT
       H  A  G  K  T  V  V  A  M  A  I  D  G  V  Y  A  G  A  L  A

1561 - TTATCTGACACACCACGACCAGAAGCAAAAGAAGCCATCCAAAAACTAAAAGCACAAGGT
       L  S  D  T  P  R  P  E  A  K  E  A  I  Q  K  L  K  A  Q  G

1621 - ATTAAAACAGCAATTTGTTCTGGGGACCAATCTGTTGTCGTAGAAAATATGGCTAAAGAT
       I  K  T  A  I  C  S  G  D  Q  S  V  V  V  E  N  M  A  K  D

1681 - TTAGGTATTGATATGTTCTTTGCCGAACAACTACCAAATGATAAGAGCGCCTTAGTCGAG
       L  G  I  D  M  F  F  A  E  Q  L  P  N  D  K  S  A  L  V  E

1741 - AAATTACAGCAAGACGGTCATATCGTTGCATTCGTTGGTGATGGCATTAATGATGCTCCA
       K  L  Q  Q  D  G  H  I  V  A  F  V  G  D  G  I  N  D  A  P

1801 - GCTCTTGCGGCAAGTGATATTGGGATTAGTATCGGTACTGGAACTGACATTGCGATTGAA
       A  L  A  A  S  D  I  G  I  S  I  G  T  G  T  D  I  A  I  E

1861 - ACAGGAGATGTAACACTTGTAAG
       T  G  D  V  T  L  V
```

Appendix J

Nucleotide sequence (5’ – 3’) for the *Bam*HI fragment of plasmid pCT800. The amino acid translation of the *erm*R gene is shown. Start and stop codons are indicated.

```
   1 - GATCCGGACTAGTCCGGGCCCTCGAGCTCAAGCTAGCTTGGTACCAGATCTGAGATCACG

  61 - CGTTCTAGAGGTACGTACCGCATGCGATGCGGCCGCATCGGCCTAGGCGATTTAGAAGCA

 121 - AACTTAAGAGTGTGTTGATAGTGCAGTATCTTAAAATTTTGTATAATAGGAATTGAAGTT

 181 - AAATTAGATGCTAAAAATTTGTAATTAAGAAGGAGTGATTACATGAACAAAAATATAAAA
   1 -                                           M  N  K  N  I  K
 241 - TATTCTCAAAACTTTTTAACGAGTGAAAAAGTACTCAACCAAATAATAAAACAATTGAAT
   7 - Y  S  Q  N  F  L  T  S  E  K  V  L  N  Q  I  I  K  Q  L  N

 301 - TTAAAAGAAACCGATACCGTTTACGAAATTGGAACAGGTAAAGGGCATTTAACGACGAAA
  27 - L  K  E  T  D  T  V  Y  E  I  G  T  G  K  G  H  L  T  T  K

 361 - CTGGCTAAAATAAGTAAACAGGTAACGTCTATTGAATTAGACAGTCATCTATTCAACTTA
  47 - L  A  K  I  S  K  Q  V  T  S  I  E  L  D  S  H  L  F  N  L

 421 - TCGTCAGAAAAATTAAAACTGAATACTCGTGTCACTTTAATTCACCAAGATATTCTACAG
  67 - S  S  E  K  L  K  L  N  T  R  V  T  L  I  H  Q  D  I  L  Q

 481 - TTTCAATTCCCTAACAAACAGAGGTATAAAATTGTTGGGAGTATTCCTTACCATTTAAGC
  87 - F  Q  F  P  N  K  Q  R  Y  K  I  V  G  S  I  P  Y  H  L  S

 541 - ACACAAATTATTAAAAAAGTGGTTTTTGAAAGCCATGCGTCTGACATCTATCTGATTGTT
 107 - T  Q  I  I  K  K  V  V  F  E  S  H  A  S  D  I  Y  L  I  V

 601 - GAAGAAGGATTCTACAAGCGTACCTTGGATATTCACCGAACACTAGGGTTGCTCTTGCAC
 127 - E  E  G  F  Y  K  R  T  L  D  I  H  R  T  L  G  L  L  L  H

 661 - ACTCAAGTCTCGATTCAGCAATTGCTTAAGCTGCCAGCGGAATGCTTTCATCCTAAACCA
 147 - T  Q  V  S  I  Q  Q  L  L  K  L  P  A  E  C  F  H  P  K  P

 721 - AAAGTAAACAGTGTCTTAATAAAACTTACCCGCCATACCACAGATGTTCCAGATAAATAT
 167 - K  V  N  S  V  L  I  K  L  T  R  H  T  T  D  V  P  D  K  Y

 781 - TGGAAGCTATATACGTACTTTGTTTCAAAATGGGTCAATCGAGAATATCGTCAACTGTTT
 187 - W  K  L  Y  T  Y  F  V  S  K  W  V  N  R  E  Y  R  Q  L  F

 841 - ACTAAAAATCAGTTTCATCAAGCAATGAAACACGCCAAAGTAAACAATTTAAGTACCGTT
 207 - T  K  N  Q  F  H  Q  A  M  K  H  A  K  V  N  N  L  S  T  V

 901 - ACTTATGAGCAAGTATTGTCTATTTTTAATAGTTATCTATTATTTAACGGGAGGAAATAA
 227 - T  Y  E  Q  V  L  S  I  F  N  S  Y  L  L  F  N  G  R  K  *

 961 - TTCTATGAGTCGCTTTTGTAAATTTGGAAAGTTACACGTTACTAAAGGGAATGTAGATAA

1021 - ATTATTAGGTATACTACTGACAGCTTCCAAGGAGCTAAAGAGGTCCCTAGCGCCTACGGG

1081 - GAATTTGTATCGATGATAAGCTGTCAAGCATGAGAATTATCGCATGCGCCTCCCCG
```

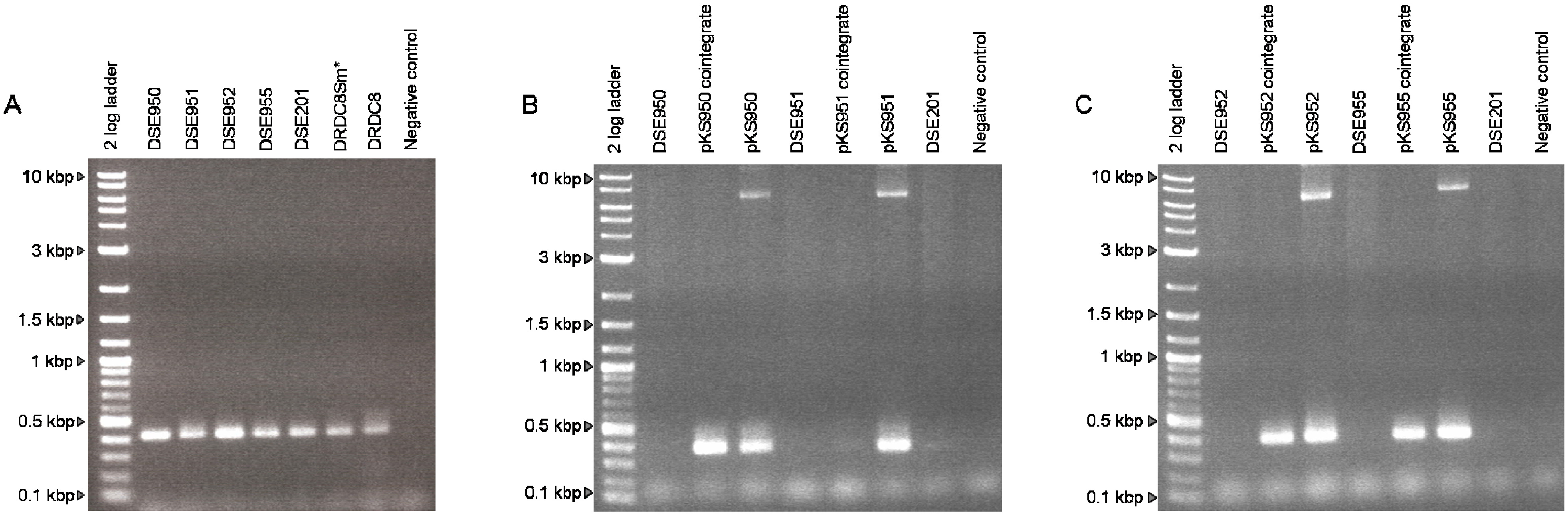
Appendix K

Panel A.

PCR analysis using the p234 and p319 oligonucleotide pair showed amplification of a 417 bp fragment of the *hly* gene from DNA extracted from the putative mutant strains DSE950, DSE951, DSE952 and DSE955, as well as the positive control strains DRDC8Sm* and DRDC8. No amplicon was obtained for the no DNA negative control.

Panel B and C.

PCR analysis using the catPF and catPR oligonucleotide pair showed amplification of a 391 bp fragment of the catP gene from DNA extracted from *L. monocytogenes* strains pKS950 cointegrate, pKS951 cointegrate, pKS952 cointegrate and pKS955 cointegrate and from plasmids pKS950, pKS951, pKS952 and pKS955. Amplicons were not obtained from DNA extracted from the putative mutant *L. monocytogenes* strains DSE950, DSE951, DSE952 and DSE955. PCR products were also absent from the negative control strain DSE201 and the no DNA negative control.

A
2 log ladder
DSE950
DSE951
DSE952
DSE955
DSE201
DRDC8Sm*
DRDC8
Negative control
10 kbp
3 kbp
1.5 kbp
1 kbp
0.5 kbp
0.1 kbp
B
2 log ladder
DSE950
pKS950 cointegrate
pKS950
DSE951
pKS951 cointegrate
pKS951
DSE201
Negative control
10 kbp
3 kbp
1.5 kbp
1 kbp
0.5 kbp
0.1 kbp
C
2 log ladder
DSE952
pKS952 cointegrate
pKS952
DSE955
pKS955 cointegrate
pKS955
DSE201
Negative control
10 kbp
3 kbp
1.5 kbp
1 kbp
0.5 kbp
0.1 kbp

Appendix L

Nucleotide sequence (5' – 3') for the 1496 bp PCR product generated using the p2037/ermR2 oligonucleotide pair from strain DSE950 DNA. The *Bam*HI site flanking the 3' end of the *erm* insertion (shaded in grey) in ORF pCT0017 is underlined. ORF pCT0017 stop codon is shown in bold.

1 GTACCGTTAC TTATGAGCAA GTATTGTCTA TTTTTAATAG TTATCTATTA
51 TTTAACGGGA GGAAATAATT CTATGAGTCG CTTTTGTAAA TTTGGAAAGT
101 TACACGTTAC TAAAGGGAAT GTAGATAAAT TATTAGGTAT ACTACTGACA
151 GCTTCCAAGG AGCTAAAGAG GTCCCTAGCG CCTACGGGGA ATTTGTATCG
201 ATGATAAGCT GTCAAGCATG AGAATTATCG CATGCGCCTC CCCGGATCCG
*Bam*HI
251 GATCCGCCAA ATGACACTAT CTTTTTTGAA TAAAATTTGT GCACATAAAC
301 ATGCCAATGT TATTTTAGAA ATGATTGACG CGAGTAGTAT TACTGCTGAA
351 AATAAAGAAG CTATCAGCGA AAAACTAAGT AGCAAAAATG TTGTTGACGA
401 AGTAACTTGT GATTGTATAA ACAGATTAAA TTGTTGTGAT AATAAT**TAG**A
pCT0017 Stop
451 AAAGGAGAAT AACATGTCTC AAATTATCGT ACTTATTATT GGATTGACTC
501 TAATTGCTTT TATTGGATGG TGGTTCTTTG GCAAACATGA GGTCCATCAG
551 GAAACTGCCG TTATCAGTTC TGATGGACAA ACAGCTACTA TTGTGGTAAA
601 TGGAGGATAT AATCCAGCAG TCTTAAATTT AAAAAAAGAT GTCCCAGTTA
651 ACTTGATCTT TAACCGGAAA GATGCCTCAT CTTGTCTTGA AAAAGTTATT
701 TTTCCAGATT TTGGAGTGGA TGCGGATTTA CCACTCAATC AGGATGTGTC
751 TATTTTGATT GATACATCTA AGTCAGGCGA GTTTATATAT TCATGTGGGA
801 TGAATATGTT CCATGGAAAA ATCATCATCA AATAGAATCA CAAATTAAAT
851 ACTAATATAC CCTAAGGAGG ATATAAAATG TTCGGATCAA AAATTAATAA
901 TAAAGTAGAA GTAGTTGTTG ACGGAGGTTA CTCTCCTAGT AAATTTAAGT
951 TAAAAGCTGG AGAGCCAGCT GAAGTCTCTT TTACTCGTGT CTCTGATAAA
1001 GGTTGTGCTC AGCAAATTAT CTTCAATGGA GAACTTCGAA ATTTACCCTT
1051 AAATGAATCT GTCACTTTTA ATTTCACTCC AGTTGAAAAA GGACGTCATA
1101 ATTGGTCTGC GGTATGAAAA TGATCCTGGG GAGCTATTCA GTTAAATAAA
1151 AAACTATTTT TAATGTAGTT ACAAAAGGAG ATACTTTATG TCAATAAAAA
1201 ATCGTTTTAT CATAGGTGTC ATCGGATCAG TCCCATTGCT TATCAATATG
1251 TTTATGAGCT TGGGCGGTTC CATGCTTGGA GGCGATAAAT ATGGTGTTTG
1301 GATTCTGTTT GCCTTTGGCT CATTAGTTTA CTGGTTCTCA GGATTGCCAT
1351 TCTTGCGTAC TGCAGTCGCT T

Appendix M

Panel A.

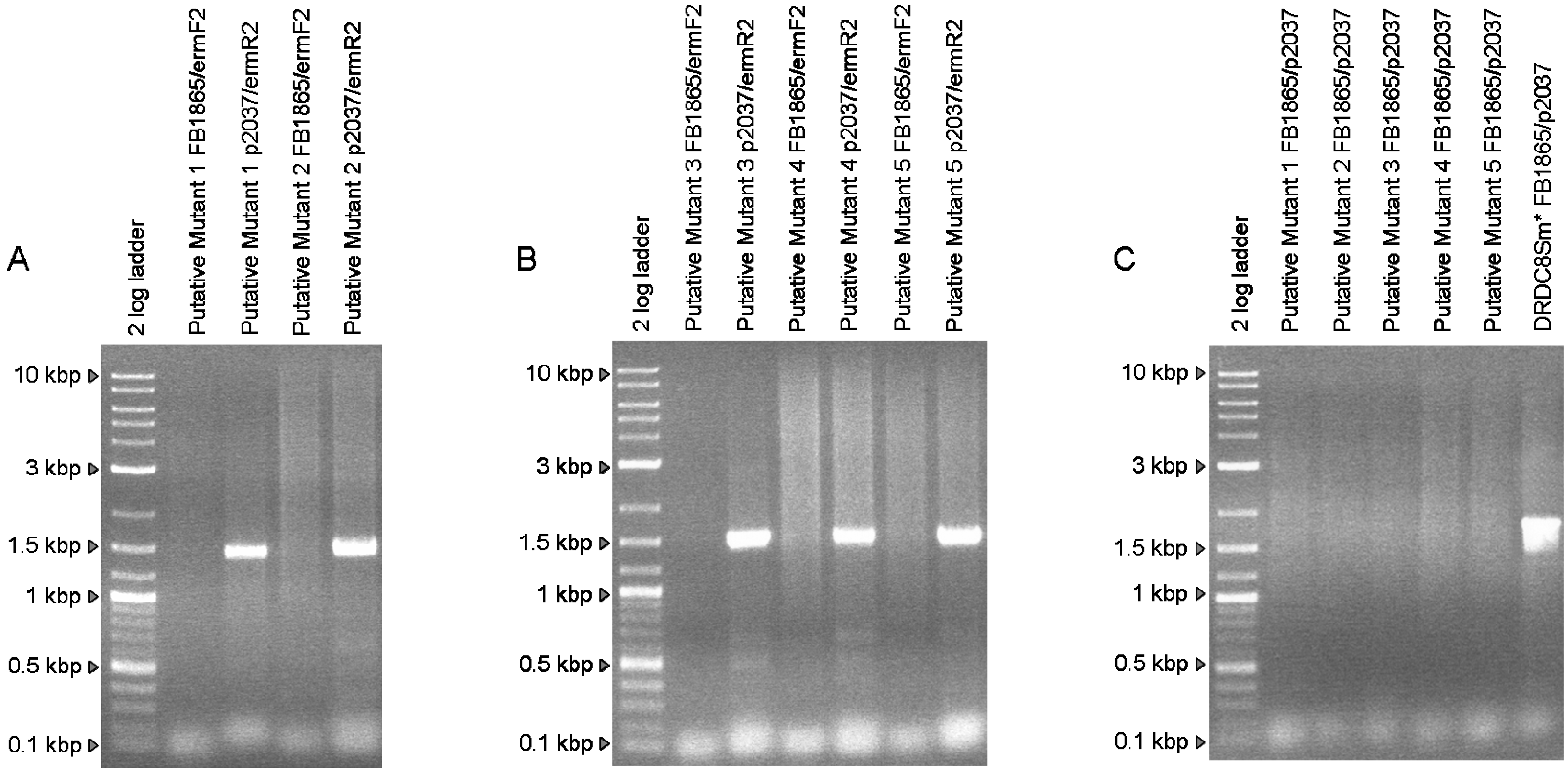
PCR analysis of DNA extracted from Putative Mutant 1 and 2 showed no amplification of DNA using the FB1865/ermF2 oligonucleotide pair. A 1496 bp product was amplified using the p2037/ermR2 oligonucleotide pair for Putative Mutant 1 and 2.

Panel B.

PCR analysis of DNA extracted from Putative Mutant 3, 4 and 5 showed no amplification of DNA using the FB1865/ermF2 oligonucleotide pair. A 1496 bp product was amplified using the p2037/ermR2 oligonucleotide pair for Putative Mutants 3, 4 and 5.

Panel C.

No PCR products were amplified from DNA extracted from Putative Mutant 1, 2, 3, 4, or 5 using the FB1865/p2037 oligonucleotide pair. The expected 1723 bp bp PCR product was amplified from DNA extracted from the positive control strain DRDC8Sm*.


A
2 log ladder
Putative Mutant 1 FB1865/ermF2
Putative Mutant 1 p2037/ermR2
Putative Mutant 2 FB1865/ermF2
Putative Mutant 2 p2037/ermR2
10 kbp
3 kbp
1.5 kbp
1 kbp
0.5 kbp
0.1 kbp
B
2 log ladder
Putative Mutant 3 FB1865/ermF2
Putative Mutant 3 p2037/ermR2
Putative Mutant 4 FB1865/ermF2
Putative Mutant 4 p2037/ermR2
Putative Mutant 5 FB1865/ermF2
Putative Mutant 5 p2037/ermR2
10 kbp
3 kbp
1.5 kbp
1 kbp
0.5 kbp
0.1 kbp
C
2 log ladder
Putative Mutant 1 FB1865/p2037
Putative Mutant 2 FB1865/p2037
Putative Mutant 3 FB1865/p2037
Putative Mutant 4 FB1865/p2037
Putative Mutant 5 FB1865/p2037
DRDC8Sm* FB1865/p2037
10 kbp
3 kbp
1.5 kbp
1 kbp
0.5 kbp
0.1 kbp

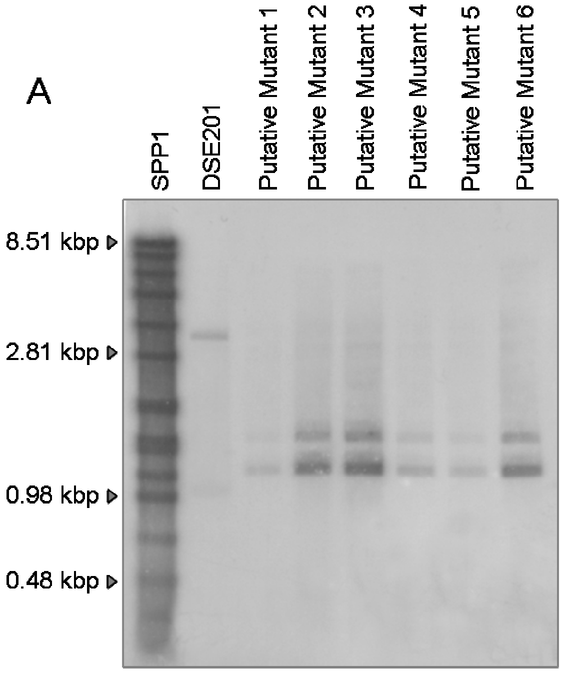
Appendix N

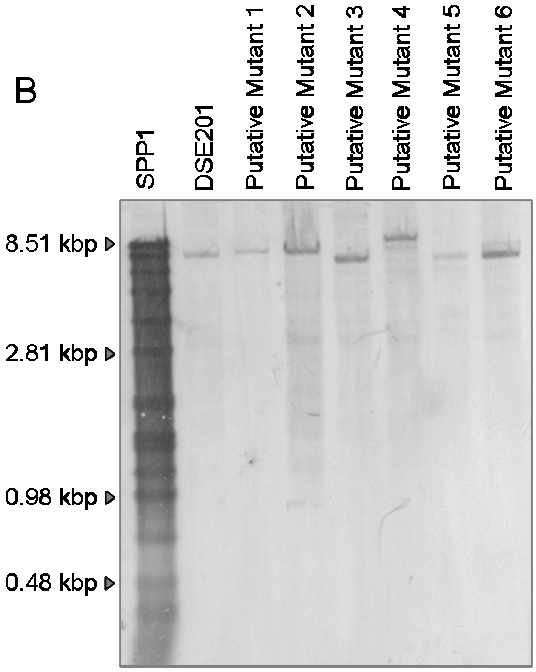
Panel A.

Southern hybridisation analysis of *Dra*I digested DNA extracted from Putative Mutants 1 – 6. A digoxigenin-labelled 645 bp *erm*-specific DNA probe hybridised to two *Dra*I DSE201 DNA fragments 1014 bp and 3347 bp in size. Probe DNA also hybridised with two *Dra*I DNA fragments *ca*. 1215 bp and 1718 bp in size for Putative Mutants 1 – 6.

Panel B.

Southern hybridisation analysis of *Eco*RV digested DNA extracted from Putative Mutants 1 – 6. Digoxigenin-labelled *erm*-specific probe DNA hybridised to a 7273 bp *Eco*RV DSE201 DNA fragment. Probe DNA also hybridised with a single *Eco*RV DNA fragment ranging from *ca*. 6900 to 8600 bp in size for Putative Mutants 1 – 6.





Appendix O

Nucleotide sequence (5' – 3') for the PCR product amplified using the FB1865/p2037 oligonucleotide pair from strain DSE951 DNA. The *Bam*HI sites flanking the 1136 bp *erm* insertion (shaded in grey) in ORF pCT0018 are underlined. ORF pCT0018 start and stop codons are shown in bold.

1 TACTTTGTGT AGTAGCATAG ATGATTATCT AATATCAGCA ATATGTTAAT
51 TCGTAATCAA CCTTATATGA CTAAACGAAA AAAGGTTTTT TTCTCTATTA
101 AATTTATCAT CTACAAATGT AAATTTTTAG TTGACAAATT AAATCTACAA
151 GAGTAGACTT TGGTCATACC ATAAAAATTG AGAGGAGAAA TCATGGAAAA
201 TAAACATCAA AGTAATTCAA ATGAAGTACT AATGAGCCAA TTACCTTCTG
251 ATCCTAAAAT ATTAAAAGAA TTAAATATTT CGGACAGTGA ATTAGTTATT
301 ATGCGTGTCG TTTGGTCTCT AGGGAGTACT ACAGCAGATG AAATTGGACG
351 TGAGCTTAGT GAAACTTATC AGTGGAGCCC TTCCACAATA AAAACATTTT
401 TAGCGCGTTT AATTAAAAAA GGATTATTAA AAAACAGTCG TGATGGTCGT
451 AAATATGTCT ATATTGCAAC TTGTTCTGAA GATGAAGCGA TTTGCCAAAT
501 GACACTATCT TTTTTGAATA AAATTTGTGC ACATAAACAT GCCAATGTTA
551 TTTTAGAAAT GATTGACGCG AGTAGTATTA CTGCTGAAAA TAAAGAAGCT
601 ATCAGCGAAA AACTAAGTAG CAAAAATGTT GTTGACGAAG TAACTTGTGA
651 TTGTATAAAC AGATTAAATT GTTGTGATAA TAATTAGAAA AGGAGAATAA
701 C**ATG**TCTCAA ATTATCGTAC TTATTATTGG ATTGACTCTA ATTGCTTTTA
pCT0018 Start
751 TTGGATGGTG GTTCTTTGGC AAACATGAGG TCCATCAGGA AACTGCCGTT
801 ATCAGTTCTG ATGGACAAAC AGCTACTATT GTGGTAAATG GAGGATATAA
851 TCCAGCAGTC TTAAATTTAA AAAAAGATGT CCCAGTTAAC TTGATCTTTA
901 ACCGGAAAGA TGCCTCATCT TGTCTTGAAA AAGTTATTTT TCCAGATTTT
951 GGAGTGGATC CGGGGAGGCG CATGCGATAA TTCTCATGCT TGACAGCTTA
*Bam*HI
1001 TCATCGATAC AAATTCCCCG TAGGCGCTAG GGACCTCTTT AGCTCCTTGG
1051 AAGCTGTCAG TAGTATACCT AATAATTTAT CTACATTCCC TTTAGTAACG
1101 TGTAACTTTC CAAATTTACA AAAGCGACTC ATAGAATTAT TTCCTCCCGT
1151 TAAATAATAG ATAACTATTA AAAATAGACA ATACTTGCTC ATAAGTAACG
1201 GTACTTAAAT TGTTTACTTT GGCGTGTTTC ATTGCTTGAT GAAACTGATT
1251 TTTAGTAAAC AGTTGACGAT ATTCTCGATT GACCCATTTT GAAACAAAGT
1301 ACGTATATAG CTTCCAATAT TTATCTGGAA CATCTGTGGT ATGGCGGGTA
1351 AGTTTTATTA AGACACTGTT TACTTTTGGT TTAGGATGAA AGCATTCCGC
1401 TGGCAGCTTA AGCAATTGCT GAATCGAGAC TTGAGTGTGC AAGAGCAACC
1451 CTAGTGTTCG GTGAATATCC AAGGTACGCT TGTAGAATCC TTCTTCAACA
1501 ATCAGATAGA TGTCAGACGC ATGGCTTTCA AAAACCACTT TTTTAATAAT
1551 TTGTGTGCTT AAATGGTAAG GAATACTCCC AACAATTTTA TACCTCTGTT
1601 TGTTAGGGAA TTGAAACTGT AGAATATCTT GGTGAATTAA AGTGACACGA
1651 GTATTCAGTT TTAATTTTTC TGACGATAAG TTGAATAGAT GACTGTCTAA
1701 TTCAATAGAC GTTACCTGTT TACTTATTTT AGCCAGTTTC GTCGTTAAAT
1751 GCCCTTTACC TGTTCCAATT TCGTAAACGG TATCGGTTTC TTTTAAATTC
1801 AATTGTTTTA TTATTTGGTT GAGTACTTTT TCACTCGTTA AAAAGTTTTG
1851 AGAATATTTT ATATTTTTGT TCATGTAATC ACTCCTTCTT AATTACAAAT
1901 TTTTAGCATC TAATTTAACT TCAATTCCTA TTATACAAAA TTTTAAGATA
1951 CTGCACTATC AACACACTCT TAAGTTTGCT TCTAAATCGC CTAGGCCGAT
2001 GCGGCCGCAT CGCATGCGGT ACGTACCTCT AGAACGCGTG ATCTCAGATC
2051 TGGTACCAAG CTAGCTTGAG CTCGAGGGCC CGGACTAGTC CGGATCCGGA
*Bam*HI

Continued next page ……

2101 TTTACCACTC AATCAGGATG TGTCTATTTT GATTGATACA TCTAAGTCAG
2151 GCGAGTTTAT ATATTCATGT GGGATGAATA TGTTCCATGG AAAAATCATC
2201 ATCAAA**TAG**A ATCACAAATT AAATACTAAT ATACCCTAAG GAGGATATAA
pCT0018 Stop
2251 AATGTTCGGA TCAAAAATTA ATAATAAAGT AGAAGTAGTT GTTGACGGAG
2301 GTTACTCTCC TAGTAAATTT AAGTTAAAAG CTGGAGAGCC AGCTGAAGTC
2351 TCTTTTACTC GTGTCTCTGA TAAAGGTTGT GCTCAGCAAA TTATCTTCAA
2401 TGGAGAACTT CGAAATTTAC CCTTAAATGA ATCTGTCACT TTTAATTTCA
2451 CTCCAGTTGA AAAAGGACGT CATAATTGGT CTGCGGTATG AAAATGATCC
2501 TGGGGAGCTA TTCAGTTAAA TAAAAAACTA TTTTTAATGT AGTTACAAAA
2551 GGAGATACTT TATGTCAATA AAAAATCGTT TTATCATAGG TGTCATCGGA
2601 TCAGTCCCAT TGCTTATCAA TATGTTTATG AGCTTGGGCG GTTCCATGCT
2651 TGGAGGCGAT AAATATGGTG TTTGGATTCT GTTTGCCTTT GGCTCATTAG
2701 TTTACTGGTT CTCAGGATTG CCATTCTTGC GTACT

Appendix P

Nucleotide sequence (5’ – 3’) for the PCR product amplified using the FB1865/p2037 oligonucleotide pair from strain DSE952 DNA. The *Bam*HI sites flanking the 1136 bp *erm* insertion (shaded in grey) in ORF pCT0019 are underlined. ORF pCT0019 start and stop codons are shown in bold.

1 ACCTGCTACT AAAAATTTAC TTTGTGTAGT AGCATAGATG ATTATCTAAT
51 ATCAGCAATA TGTTAATTCG TAATCAACCT TATATGACTA AACGAAAAAA
101 GGTTTTTTTC TCTATTAAAT TTATCATCTA CAAATGTAAA TTTTTAGTTG
151 ACAAATTAAA TCTACAAGAG TAGACTTTGG TCATACCATA AAAATTGAGA
201 GGAGAAATCA TGGAAAATAA ACATCAAAGT AATTCAAATG AAGTACTAAT
251 GAGCCAATTA CCTTCTGATC CTAAAATATT AAAAGAATTA AATATTTCGG
301 ACAGTGAATT AGTTATTATG CGTGTCGTTT GGTCTCTAGG GAGTACTACA
351 GCAGATGAAA TTGGACGTGA GCTTAGTGAA ACTTATCAGT GGAGCCCTTC
401 CACAATAAAA ACATTTTTAG CGCGTTTAAT TAAAAAAGGA TTATTAAAAA
451 ACAGTCGTGA TGGTCGTAAA TATGTCTATA TTGCAACTTG TTCTGAAGAT
501 GAAGCGATTT GCCAAATGAC ACTATCTTTT TTGAATAAAA TTTGTGCACA
551 TAAACATGCC AATGTTATTT TAGAAATGAT TGACGCGAGT AGTATTACTG
601 CTGAAAATAA AGAAGCTATC AGCGAAAAAC TAAGTAGCAA AAATGTTGTT
651 GACGAAGTAA CTTGTGATTG TATAAACAGA TTAAATTGTT GTGATAATAA
701 TTAGAAAAGG AGAATAACAT GTCTCAAATT ATCGTACTTA TTATTGGATT
751 GACTCTAATT GCTTTTATTG GATGGTGGTT CTTTGGCAAA CATGAGGTCC
801 ATCAGGAAAC TGCCGTTATC AGTTCTGATG GACAAACAGC TACTATTGTG
851 GTAAATGGAG GATATAATCC AGCAGTCTTA AATTTAAAAA AAGATGTCCC
901 AGTTAACTTG ATCTTTAACC GGAAAGATGC CTCATCTTGT CTTGAAAAAG
951 TTATTTTTCC AGATTTTGGA GTGGATGCGG ATTTACCACT CAATCAGGAT
1001 GTGTCTATTT TGATTGATAC ATCTAAGTCA GGCGAGTTTA TATATTCATG
1051 TGGGATGAAT ATGTTCCATG GAAAAATCAT CATCAAATAG AATCACAAAT
1101 TAAATACTAA TATACCCTAA GGAGGATATA AA**ATG**TTCGG ATCCGGGGAG
pCT0019 start *Bam*HI
1151 GCGCATGCGA TAATTCTCAT GCTTGACAGC TTATCATCGA TACAAATTCC
1201 CCGTAGGCGC TAGGGACCTC TTTAGCTCCT TGGAAGCTGT CAGTAGTATA
1251 CCTAATAATT TATCTACATT CCCTTTAGTA ACGTGTAACT TTCCAAATTT
1301 ACAAAAGCGA CTCATAGAAT TATTTCCTCC CGTTAAATAA TAGATAACTA
1351 TTAAAAATAG ACAATACTTG CTCATAAGTA ACGGTACTTA AATTGTTTAC
1401 TTTGGCGTGT TTCATTGCTT GATGAAACTG ATTTTTAGTA AACAGTTGAC
1451 GATATTCTCG ATTGACCCAT TTTGAAACAA AGTACGTATA TAGCTTCCAA
1501 TATTTATCTG GAACATCTGT GGTATGGCGG GTAAGTTTTA TTAAGACACT
1551 GTTTACTTTT GGTTTAGGAT GAAAGCATTC CGCTGGCAGC TTAAGCAATT
1601 GCTGAATCGA GACTTGAGTG TGCAAGAGCA ACCCTAGTGT TCGGTGAATA
1651 TCCAAGGTAC GCTTGTAGAA TCCTTCTTCA ACAATCAGAT AGATGTCAGA
1701 CGCATGGCTT TCAAAAACCA CTTTTTTAAT AATTTGTGTG CTTAAATGGT
1751 AAGGAATACT CCCAACAATT TTATACCTCT GTTTGTTAGG GAATTGAAAC
1801 TGTAGAATAT CTTGGTGAAT TAAAGTGACA CGAGTATTCA GTTTTAATTT
1851 TTCTGACGAT AAGTTGAATA GATGACTGTC TAATTCAATA GACGTTACCT
1901 GTTTACTTAT TTTAGCCAGT TTCGTCGTTA AATGCCCTTT ACCTGTTCCA
1951 ATTTCGTAAA CGGTATCGGT TTCTTTTAAA TTCAATTGTT TTATTATTTG
2001 GTTGAGTACT TTTTCACTCG TTAAAAAGTT TTGAGAATAT TTTATATTTT
2051 TGTTCATGTA ATCACTCCTT CTTAATTACA AATTTTTAGC ATCTAATTTA
2101 ACTTCAATTC CTATTATACA AAATTTTAAG ATACTGCACT ATCAACACAC

Continued next page ……

2151 TCTTAAGTTT GCTTCTAAAT CGCCTAGGCC GATGCGGCCG CATCGCATGC
2201 GGTACGTACC TCTAGAACGC GTGATCTCAG ATCTGGTACC AAGCTAGCTT
2251 GAGCTCGAGG GCCCGGACTA GTCCGGATCC AAAATTAATA ATAAAGTAGA
*Bam*HI
2301 AGTAGTTGTT GACGGAGGTT ACTCTCCTAG TAAATTTAAG TTAAAAGCTG
2351 GAGAGCCAGC TGAAGTCTCT TTTACTCGTG TCTCTGATAA AGGTTGTGCT
2401 CAGCAAATTA TCTTCAATGG AGAACTTCGA AATTTACCCT TAAATGAATC
2451 TGTCACTTTT AATTTCACTC CAGTTGAAAA AGGACGTCAT AATTGGTCTG
2501 CGGTA**TGA**AA ATGATCCTGG GGAGCTATTC AGTTAAATAA AAAACTATTT
pCT0019 stop
2551 TTAATGTAGT TACAAAAGGA GATACTTTAT GTCAATAAAA AATCGTTTTA
2601 TCATAGGTGT CATCGGATCA GTCCCATTGC TTATCAATAT GTTTATGAGC
2651 TTGGGCGGTT CCATGCTTGG AGGCGATAAA TATGGTGTTT GGATTCTGTT
2701 TGCCTTTGGC TCATTAGTTT ACTGGTTCTC AGGATTGCCA TTCTTGCGTA
2751 CTGCAGTCGC TTCGTTCAAA AATCATCATG

Appendix Q

Nucleotide sequence (5’ – 3’) for the 3019 bp PCR product generated using the CuTrMF2/CuTrMR2 oligonucleotide pair from strain DSE955 DNA. The *Bam*HI sites flanking the 1136 bp *erm* insertion (shaded in grey) in ORF *cutR* are underlined.

```
   1  TGACCCGGAA GTTACATCGA CAGAAGATTT GATTAAAGTT GTTAAACATG
  51  CGGGTTATGA CGCGGCGGAA AAAATGTCTA AAGAAGAAAA AGATGCTGTG
 101  TTAGAAAAGA ATTTCAAAAA AGAAGTGAGA CGTTTTATTC TTTCGGCGGT
 151  TCTTTCCTTA CCATTACTTC TTACCATGGT GACACATATT CCGTACATTC
 201  ATGAAATGGT GTTTGCGGAA ACGATTGGTA ACTGGATTAA TCCAACCATC
 251  CAATTAGTAC TCGCAACGAT TGTTCAGTTT TATATTGGTT GGCGGTTTTA
 301  TGATGGGGCT TATAAAGCGC TTCGAGGTAA AAGTGCAAAT ATGGATGTAT
 351  TAGTCGCACT TGGGACTTCT GCTGCATATT TCTATAGTGT AGTGGAATAC
 401  ATTCGTCACA TGATTGATCC GAGCGTGATG CCGCATTACT ACTTTGAAAC
 451  AAGTGCTGTG TTAATCACCT TAATCTTGTT AGGTAAATTA CTTGAATCAT
 501  ACGCAACATC GAGAACAACC GAATCTATTG CTGGTTTACT TGAATTACAA
 551  GCAAAAGAAG CGACCGTTAT TCGAGAAGGA AAAGAATGGT TAGTGCCAGT
 601  AGATTCATTG AAAATTGGCG ATATTATCCT TGTTCGTCCG GGTGAAAAAG
 651  TTCCGATGGA TCCGGACTAG TCCGGGCCCT CGAGCTCAAG CTAGCTTGGT
                BamHI
 701  ACCAGATCTG AGATCACGCG TTCTAGAGGT ACGTACCGCA TGCGATGCGG
 751  CCGCATCGGC CTAGGCGATT TAGAAGCAAA CTTAAGAGTG TGTTGATAGT
 801  GCAGTATCTT AAAATTTTGT ATAATAGGAA TTGAAGTTAA ATTAGATGCT
 851  AAAAATTTGT AATTAAGAAG GAGTGATTAC ATGAACAAAA ATATAAAATA
 901  TTCTCAAAAC TTTTTAACGA GTGAAAAAGT ACTCAACCAA ATAATAAAAC
 951  AATTGAATTT AAAAGAAACC GATACCGTTT ACGAAATTGG AACAGGTAAA
1001  GGGCATTTAA CGACGAAACT GGCTAAAATA AGTAAACAGG TAACGTCTAT
1051  TGAATTAGAC AGTCATCTAT TCAACTTATC GTCAGAAAAA TTAAAACTGA
1101  ATACTCGTGT CACTTTAATT CACCAAGATA TTCTACAGTT TCAATTCCCT
1151  AACAAACAGA GGTATAAAAT TGTTGGGAGT ATTCCTTACC ATTTAAGCAC
1201  ACAAATTATT AAAAAAGTGG TTTTTGAAAG CCATGCGTCT GACATCTATC
1251  TGATTGTTGA AGAAGGATTC TACAAGCGTA CCTTGGATAT TCACCGAACA
1301  CTAGGGTTGC TCTTGCACAC TCAAGTCTCG ATTCAGCAAT TGCTTAAGCT
1351  GCCAGCGGAA TGCTTTCATC CTAAACCAAA AGTAAACAGT GTCTTAATAA
1401  AACTTACCCG CCATACCACA GATGTTCCAG ATAAATATTG GAAGCTATAT
1451  ACGTACTTTG TTTCAAAATG GGTCAATCGA GAATATCGTC AACTGTTTAC
1501  TAAAAATCAG TTTCATCAAG CAATGAAACA CGCCAAAGTA AACAATTTAA
1551  GTACCGTTAC TTATGAGCAA GTATTGTCTA TTTTTAATAG TTATCTATTA
1601  TTTAACGGGA GGAAATAATT CTATGAGTCG CTTTTGTAAA TTTGGAAAGT
1651  TACACGTTAC TAAAGGGAAT GTAGATAAAT TATTAGGTAT ACTACTGACA
1701  GCTTCCAAGG AGCTAAAGAG GTCCCTAGCG CCTACGGGGA ATTTGTATCG
1751  ATGATAAGCT GTCAAGCATG AGAATTATCG CATGCGCCTC CCCGGATCCC
                                                  BamHI
1801  GAAATTATTT CTGGTGAAAC GAGTATTGAT GAAGCGATGA TTACTGGGGA
1851  ACCTGTACCA GTAGAGAAGA AACCAGGCGA TTCTGTTATT GGCGCAACGA
1901  TTAACTTTGA CGGGGCTTTC CAAGCAAAAA TTACGAAACG AATGGAAGAA
1951  ACCGTTTTAG AATCCATTAT TCGTTTGGTG GAAGAAGCGC AAGGTATTAA
2001  AGCGCCAATT CAACGTTTGG CAGATAGAAT TTCCGGCATA TTTGTACCAA
2051  TTGTACTTGG GATTGCTGCT GTAACCTTTA TTATTTGGTA TCTTGTTACT
2101  GGAACGGTGG ATGGTTCACT TGAAGCTGCG ATTGCGGTAT TAGTTATCGC
2151  CTGTCCCTGT GCGCTTGGTC TTGCAACGCC AACCGCTATC ATGGCTGGAA
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Continued next page ……

2201 CTGGTAAAGG CGCCGAAAGT GGGATATTAT TTAAAGGTGG CGAACATTTA
2251 GAACGTACTT CCAAAGTGGA TACTATCGTT TTTGACAAAA CTGGTACTTT
2301 AACAGAAGGT AAACTAGAAG TGAGTGATAA AAAAGCAGCC AATGATCACT
2351 TTTTCCCTTA TTTATTCTTA ATGGAACAAC AATCAGAGCA TCCGATTGCG
2401 AAAGCGATTA TTAAGATGTT AGAGCCAGAG AATATAGATG CTTCCGCAGT
2451 GAAACAAGGG AAAATCCGCG CGAAAGCTGG GCACGGCATG ACCGGTAATC
2501 TGGATGATAG TAAAGTGGAA CTAGGTGCTT ACCGCTATGT TTCTTCCCTT
2551 ACAACGATTC CAAAAGAAGA TGATGAATTA ATCGAAAGTT GGATGCATGC
2601 AGGAAAAACA GTCGTAGCAA TGGCAATTGA TGGTGTATAC GCAGGTGCCC
2651 TCGCTTTATC TGACACACCA CGACCAGAAG CAAAAGAAGC CATCCAAAAA
2701 CTAAAAGCAC AAGGTATTAA AACAGCAATT TGTTCTGGGG ACCAATCTGT
2751 TGTCGTAGAA AATATGGCTA AAGATTTAGG TATTGATATG TTCTTTGCCG
2801 AACAACTACC AAATGATAAG AGCGCCTTAG TCGAGAAATT ACAGCAAGAC
2851 GGTCATATCG TTGCATTCGT TGGTGATGGC ATTAATGATG CTCCAGCTCT
2901 TGCGGCAAGT GATATTGGG

Appendix R

Amino acid translation of ORF pCT0017 from plasmid pETCF confirmed by protein mass spectrometry sequencing (see Appendix S, page 349). The introduced *Nde*I (CATATG) site at the 5’ end and the *Xho*I (CTCGAG) at the 3’end are indicated (underlined and bold). Introduction of the *Xho*I site at the 3’ end of the ORF resulted in an animo acid change from N → L (underlined) and the addition of an E (underlined). Translation of the His-Tag (HHHHHH) is indicated in bold.

```
  1 - CCCGCGAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTA

 61 - GAAATAATTTTGTTTAACTTTAAGAAGGAGATATACATATGGAAAATAAACATCAAAGTA
  1 -                                       M  E  N  K  H  Q  S  N

121 - ATTCAAATGAAGTACTAATGAGCCAATTACCTTCTGATCCTAAAATATTAAAAGAATTAA
  9 -   S  N  E  V  L  M  S  Q  L  P  S  D  P  K  I  L  K  E  L  N

181 - ATATTTCGGACAGTGAATTAGTTATTATGCGTGTCGTTTGGTCTCTAGGGAGTACTACAG
 29 -   I  S  D  S  E  L  V  I  M  R  V  V  W  S  L  G  S  T  T  A

241 - CAGATGAAATTGGACGTGAGCTTAGTGAAACTTATCAGTGGAGCCCTTCCACAATAAAAA
 49 -   D  E  I  G  R  E  L  S  E  T  Y  Q  W  S  P  S  T  I  K  T

301 - CATTTTTAGCGCGTTTAATTAAAAAAGGATTATTAAAAAACAGTCGTGATGGTCGTAAAT
 69 -   F  L  A  R  L  I  K  K  G  L  L  K  N  S  R  D  G  R  K  Y

361 - ATGTCTATATTGCAACTTGTTCTGAAGATGAAGCGATTTGCCAAATGACACTATCTTTTT
 89 -   V  Y  I  A  T  C  S  E  D  E  A  I  C  Q  M  T  L  S  F  L

421 - TGAATAAAATTTGTGCACATAAACATGCCAATGTTATTTTAGAAATGATTGACGCGAGTA
109 -   N  K  I  C  A  H  K  H  A  N  V  I  L  E  M  I  D  A  S  S

481 - GTATTACTGCTGAAAATAAAGAAGCTATCAGCGAAAAACTAAGTAGCAAAAATGTTGTTG
129 -   I  T  A  E  N  K  E  A  I  S  E  K  L  S  S  K  N  V  V  D

541 - ACGAAGTAACTTGTGATTGTATAAACAGATTAAATTGTTGTGATAATCTCGAGCACCACC
149 -   E  V  T  C  D  C  I  N  R  L  N  C  C  D  N  L  E  H  H  H

601 - ACCACCACCACTGAGATCCGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTG
169 -   H  H  H  *

661 - CCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTT
```

Appendix S

Protein mass spectrometry sequencing results for His-tagged pCT0017 protein.

The MS spectra obtained for the band of interest. The peak masses were matched to the provided sequence as follows:

Peak number	Meas. M/z	Intensity	Ion score	Range	Missed cleavage	Sequence
peak 46	2026.878	142.654	-	5 – 22	0	HQSNSNEVLMSQLPSDPK 10: Oxidation (M)
peak 36	1872.976	118.6	-	23 – 38	1	ILKELNISDSELVIMR
peak 39	1888.981	1459.776	-	23 – 38	1	ILKELNISDSELVIMR 15: Oxidation (M)
peak 9	1518.724	1889.144	-	26 – 38	0	ELNISDSELVIMR
MSMS 11	1534.727	14943.89	532	26 – 38	0	ELNISDSELVIMR 12: Oxidation (M)
MSMS 17	1590.763	24263.278	274	39 – 53	0	VVWSLGSTTADEIGR
MSMS 27	1668.748	1547.37	1083	54 – 67	0	ELSETYQWSPSTIK
peak 52	2257.087	545.001	-	54 – 72	1	ELSETYQWSPSTIKTFLAR
peak 72	2900.259	223.246	-	87 – 110	1	KYVVYIATCSEDEAICQMTLSFLNK 17: Oxidation (M)
peak 68	2772.171	797.402	-	88 – 110	0	YVVYIATCSEDEAICQMTLSFLNK 16: Oxidation (M)
peak 47	2071.965	824.969	-	116 – 134	0	HANVILEMIDASSITAENK 8: Oxidation (M)
peak 63	2729.277	480.044	-	116 – 140	1	HANVILEMIDASSITAENKEAISEK 8: Oxidation (M)
peak 18	1593.649	6970.638	-	145 – 157	0	NVVDEVTCDCINR 8: Carbamidomethyl (C) 10: Carbamidomethyl (C)
MSMS 35	1859.693	5038.121	540	158 – 171	0	LNCCDNLEHHHHHH 3: Carbamidomethyl (C) 4: Carbamidomethyl (C)

MS/MS analysis of the 1534.727, 1590.763 and 1668.748 parent ions produced clear spectrum that confirmed the peptide assignments (Figure 2). The majority of the peaks in the spectra could be assigned to the protein sequence, thus this appears to be the only protein present in the band. The level of coverage of the modified protein sequence was 84.2% by MS analysis and 32.7% by MS/MS analysis.

Figure 1.
The MS spectra obtained for the band of interest. The peaks corresponding to the peptides that were matched to the provided sequence are shown. Blue peak masses indicate that the peptide contains an oxidized methionine residue.

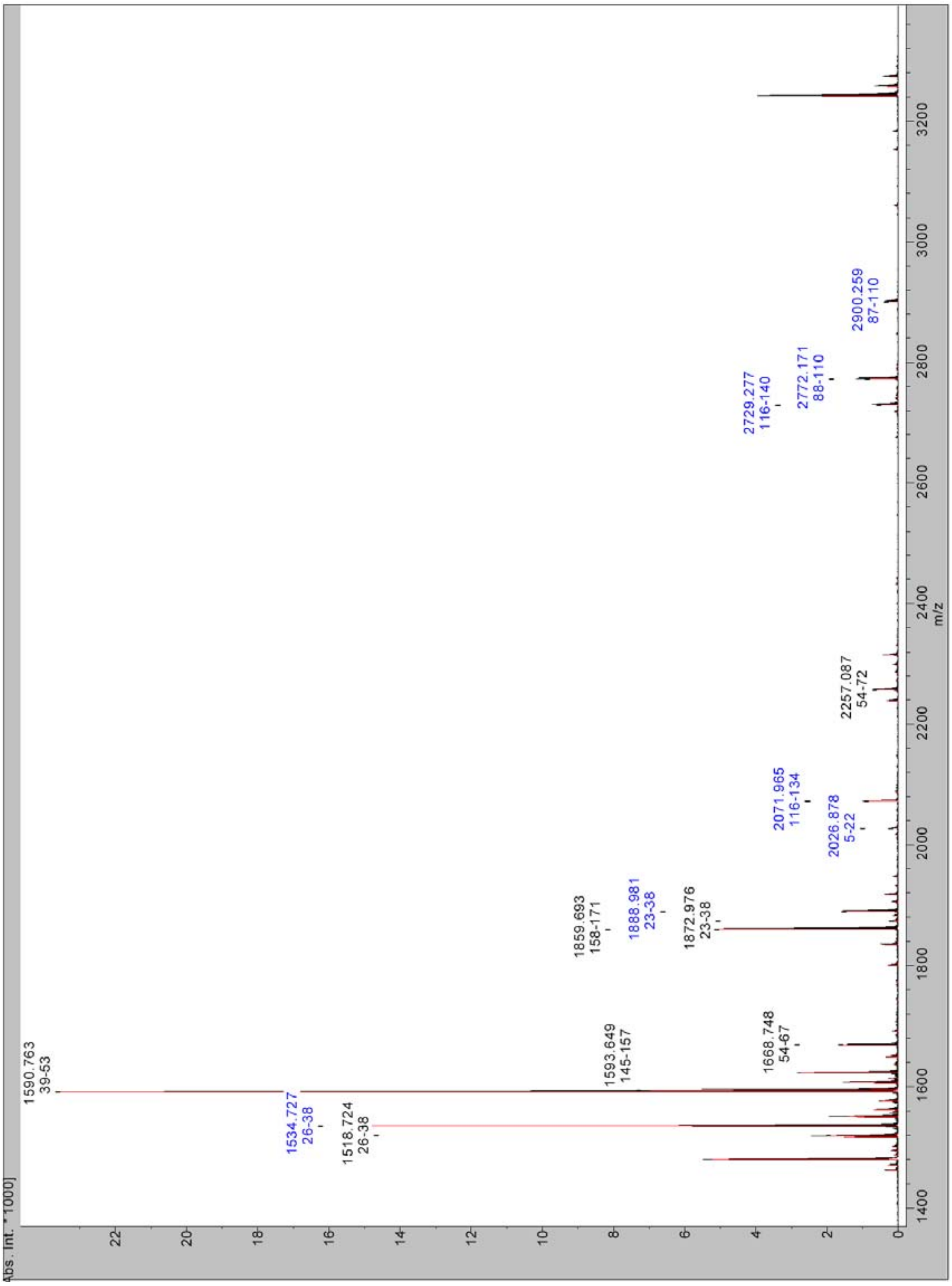
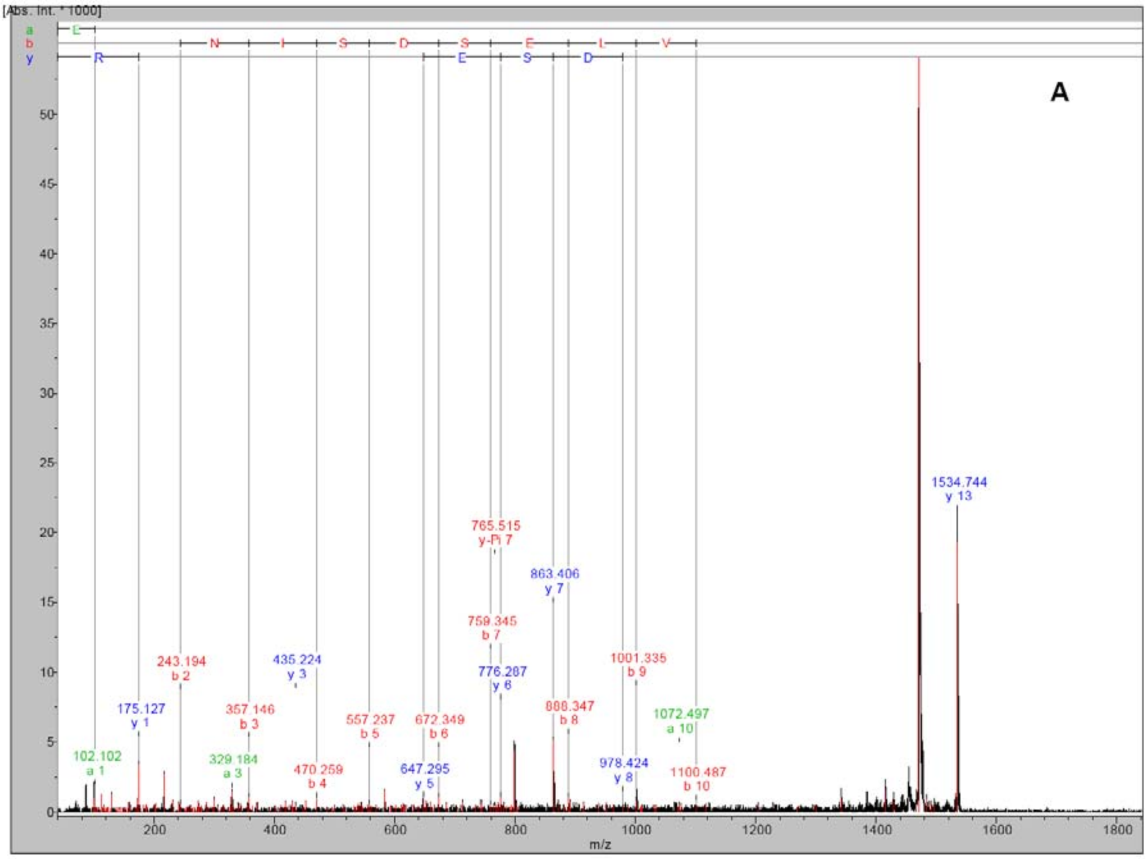
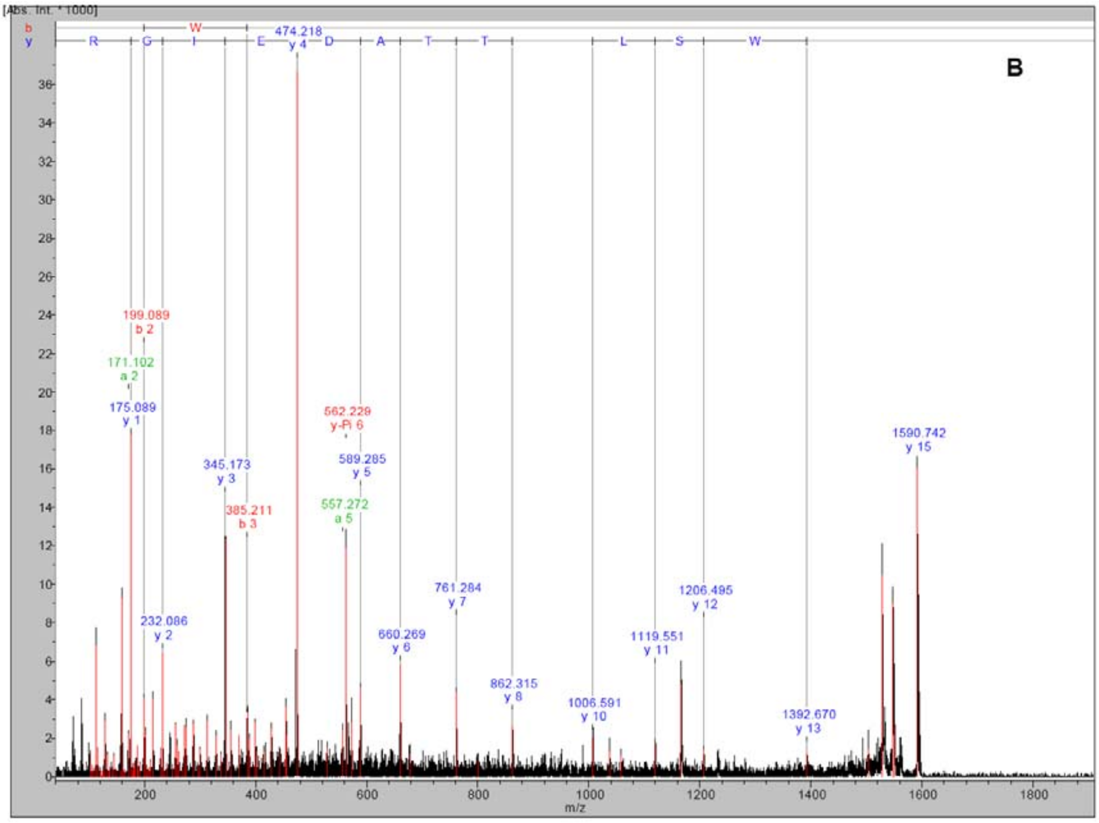


Figure 2.
The MS/MS spectra of the 1534.727 (A), 1590.763 (B) and 1668.748 (C) parent ions.




[Abs. Int. * 1000]
b
y
W
R G I E D A T T L S W
B
474.218
y 4
199.089
b 2
171.102
a 2
175.089
y 1
562.229
y-Pi 6
589.285
y 5
345.173
y 3
385.211
b 3
557.272
a 5
1590.742
y 15
761.284
y 7
1206.495
y 12
232.086
y 2
660.269
y 6
1119.551
y 11
862.315
y 8
1006.591
y 10
1392.670
y 13
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
200
400
600
800
1000
1200
1400
1600
1800
m/z

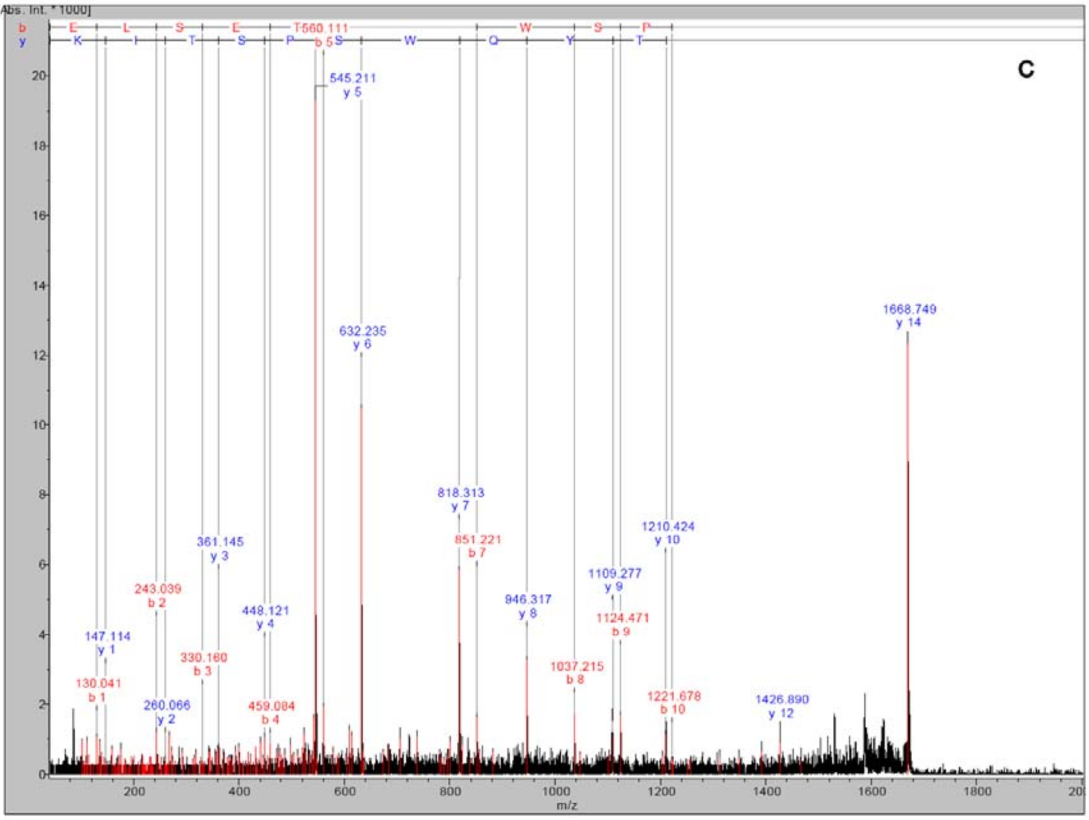

[Abs. Int. * 1000]
b
y
E L S E T W S P
K I T S P S W Q Y T
560.111
b 5
C
545.211
y 5
632.235
y 6
1668.749
y 14
818.313
y 7
851.221
b 7
1210.424
y 10
361.145
y 3
1109.277
y 9
243.039
b 2
946.317
y 8
448.121
y 4
1124.471
b 9
147.114
y 1
330.160
b 3
1037.215
b 8
130.041
b 1
1221.678
b 10
260.066
y 2
459.084
b 4
1426.890
y 12
0
2
4
6
8
10
12
14
16
18
20
200
400
600
800
1000
1200
1400
1600
1800
m/z

Figure 3.
The MS/MS spectra of the 1859.693 parent ion showing the sequence of the C-terminal peptide.

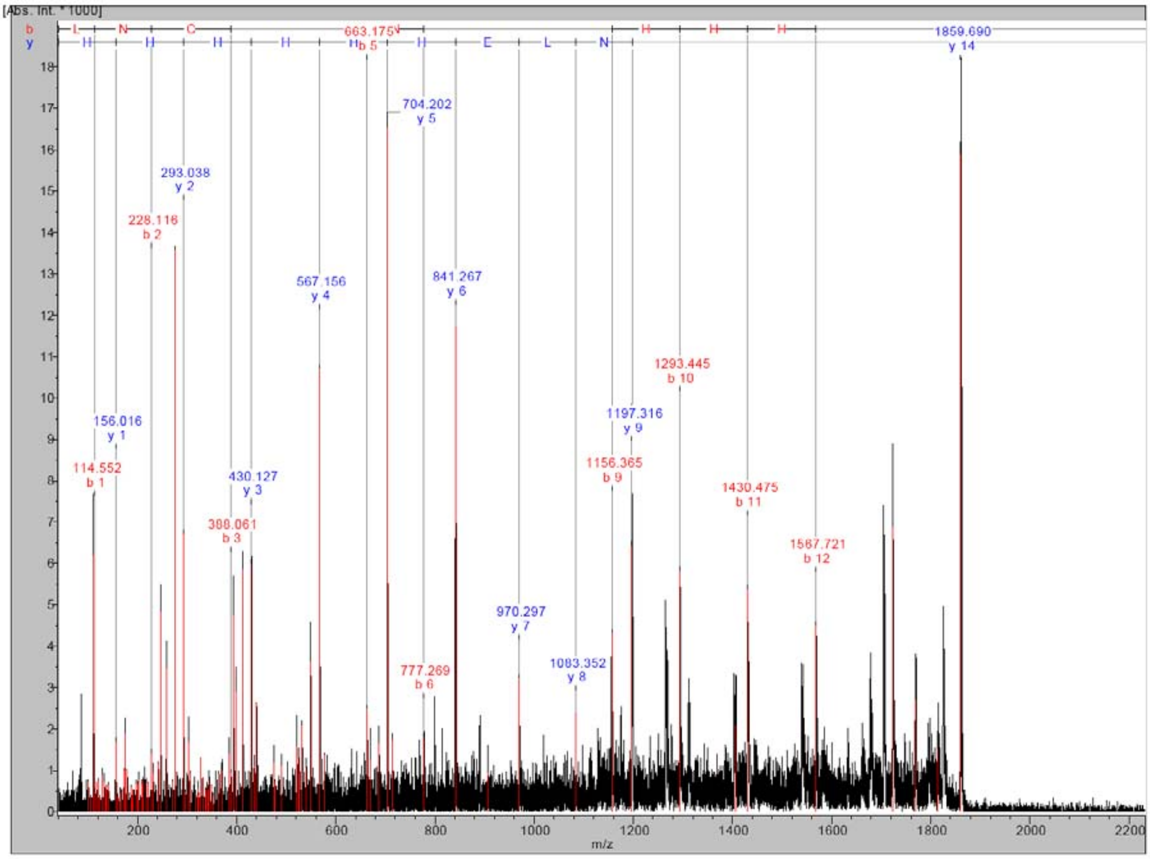


Figure 4.

The regions covered by the MS data are shown with the grey bars, whereby the depth of the grey colour corresponds to the peak's intensity in the spectra. The red boxes show the amino acids that were sequenced by MS/MS analysis, whereby the upper row of boxes represents the b-ion and the lower the y-ion series. The regions of sequence that were not identified contained peptides that were too small to be detected by the mass spectrometer (i.e. <800).