1 Supplementary Figures:

Fig S1. Relative standard curves for RT qPCR analysis of selected genes at the ess locus. RT-qPCR standard curves for: (A) 16S rRNA; (B) *esxA*; (C) *esaA*; (D) *essC* and (E) *esxB*. Standard curves were prepared as described in the materials and methods section using serial 10-fold dilutions of RN6390 genomic DNA. Data points represent individual results from three technical repeats at each dilution. Regression curves to calculate the efficiency of each primer pair were calculated using MxPro software (Stratagene).

8

9 Fig S2. EsaA, EssB and EssC presence in RN6390 and individual ess deletion strains.

10 The RN6390 wild-type (wt) or isogenic deletion strains, as indicated, were cultured in TSB 11 medium until an OD₆₀₀ of 2 was reached. Whole cell samples were prepared as described in 12 the Methods section, adjusted to a calculated cell density of OD 1 in LDS sample buffer and 13 separated on bis-Tris gels (8 % for EsaA and EssC blots, 10% for EssB and 15% for TrxA). 14 Immunoblotting was undertaken with anti-EsaA, anti-EssB, anti-EssC or anti-TrxA 15 antibodies. An equivalent of 10 µl of cells from an OD₆₀₀ of 1 was loaded for each lane. 16 Molecular weight markers are indicated to the left of each blot, and the specific protein band 17 recognised by each antibody is indicated by an arrow to the right. The asterisk indicates a 18 non-specific cross-reacting band.

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Fig S3. EsxA and EsxC secretion are restored to the *esaA* mutant strain by *in trans* expression of a his-tagged allele of *esaA*.

The RN6390 wild-type strain (wt), or the isogenic *esaA* mutant strain containing either pRMC2 empty vector (*esaA*), or pRMC2 encoding an N-terminally hexahistidine-tagged EsaA variant (*esaA* pesaA) were cultured in TSB medium. When an OD₆₀₀ of 0.5 was reached, one culture of *esaA* pesaA was supplemented with 100 ng/ml anhydrotetracycline

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26 to induce plasmid-encoded overexpression of his-tagged EsaA (+++). All strains were 27 subsequently grown until they reached OD₆₀₀ of 2. The cells were subsequently spun down 28 and the supernatant (sn) was retained as the secreted protein fraction, while the pellet was 29 retained as the cellular fraction. (A) Samples of the supernatant and cellular fractions (An 30 equivalent of 250 µl of supernatant and 10 µl of cells adjusted to OD1) were separated on 31 bis-Tris gels and immunoblotted using the anti-EsxA or EsxC antisera, or control antisera 32 raised to TrxA (cytoplasmic protein). (B) Samples of the cellular fractions were separated on 33 bis-Tris gels and immunoblotted using the anti-EsaA polyclonal antisera. The asterisk 34 indicates a non-specific cross-reacting band.

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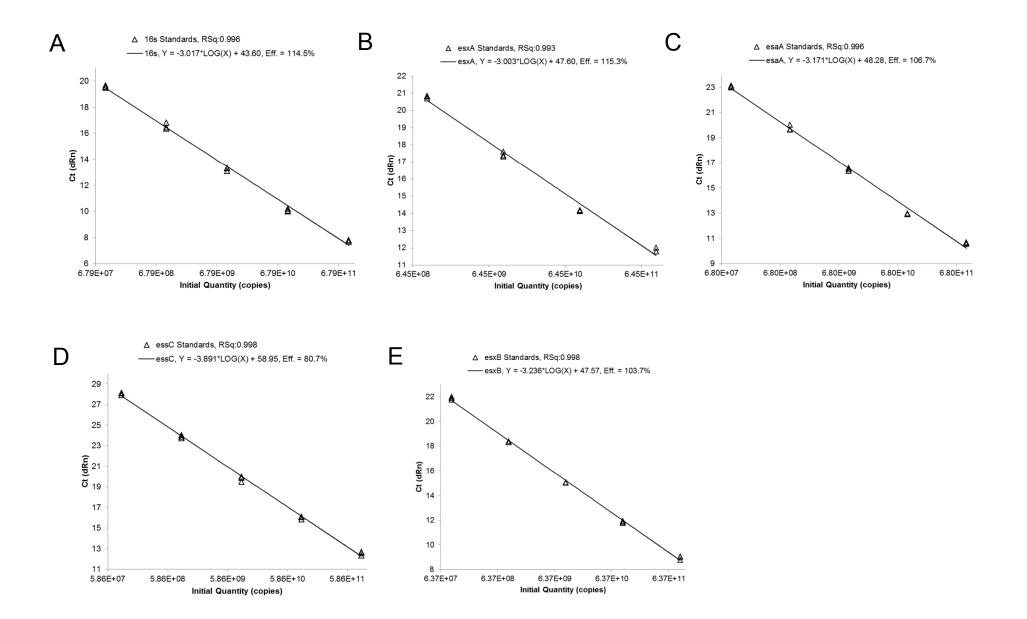
36 Fig S4. Deletion of the 12 gene ess locus has no effect on S. aureus RN6390 growth.

The RN6390 wild-type (wt) or complete *ess* deletion (Δess) strains were inoculated into 100 μ l volumes of either complex (TSB) or defined (RPMI) growth media in 96 well plates and cultured aerobically at 37°C for the indicated time period. Note that in this growth format, optical density at 600nm did not exceed 1 unit, whereas in batch culture it routinely reaches $OD_{600} > 6$.

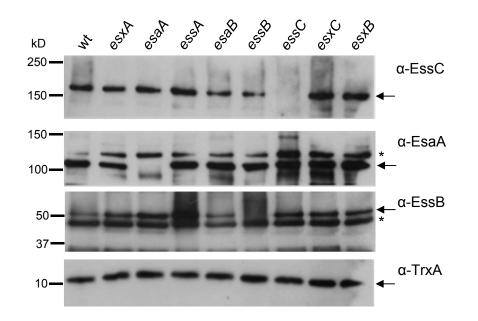
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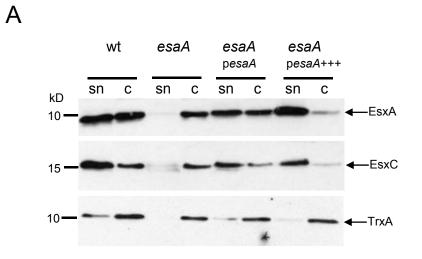
43 Fig S5. The ESS system is dispensable for S. aureus virulence in the wax moth larvae 44 infection model. Kaplan-Meier survival curves showing the survival of Galleria Mellonella 45 larvae over seven days following infection with (A) S. aureus strain RN6390 and the isogenic 46 ess deletion (Δ ess) strain and (B) S. aureus strain COL and the isogenic ess deletion (Δ ess) 47 strain. Curve comparisons are not statistically significant using the log rank test (p=0.6 for 48 RN6390 and p=0.5 for COL). Curves are generated from n=30 larvae in each group 49 (experiments performed as 10 in each group repeated 3 times). Larvae injected with 10µl of 50 phosphate-buffered saline as a control (not shown) demonstrated no mortality over 1 week 51 of observation (*n*=10).

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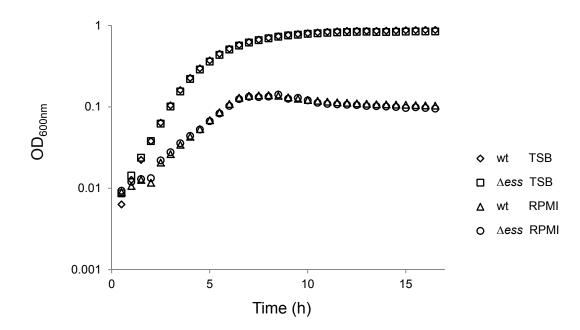
Kneuper et al. Fig S1



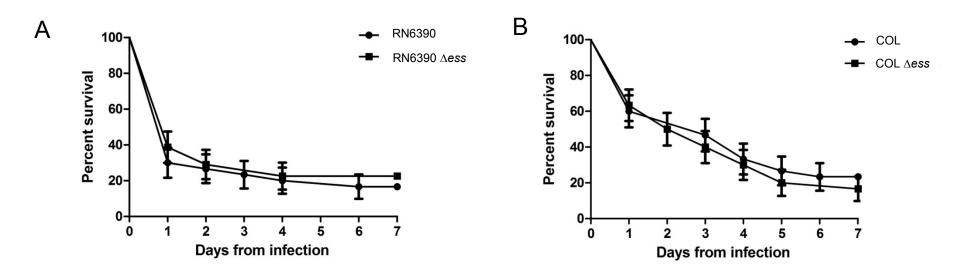




Kneuper et al. Fig S3



Kneuper et al. Fig S4.



Kneuper et al. Fig S5.

EsxAdA1 AATGTGCGAATT_CTGACCAC Amplification of EsxAdB1 TTGTTATTGCATTGCCATAACTAGAAACC esxA flanking EsxAdB2 ATGGCAATGCAATAACCATTCTGAAATTG into pIMAY EssAdA1 TAGGCAATCGCAATAACCATTCTGAAATTG into pIMAY EssAdA2 AAAGTGTTTCTTTTTTCATGTCTATTTCC essA flanking EssAdA2 AAAGTGTTTCTTTTTTTCATGTCTATTTCC essA flanking EssAdA2 AAGTGAATAACATCAACATTAGAATG Amplification of EssAdA1 TGATGAATAGCAACTTTAGGAATG Amplification of EssAdB1 GTCATAATGGAATCCAACATTAGGAATTG essA flanking EssAdB2 ATGTAATGAAACATCAACATTAGGAATTGC into pIMAD EssAdB3 GTCATAATGAATCAACATTAGGAATTGC essA flanking EssAdB4 ACGATAATGAATCAACAATGACCATTG into pMAD EssBdA1 ACTATAGTAAATCAACTAAGGAGCAAAATAG into pMAD EssBdA2 CTATTTTTTTTTATGGATTGTTTTCCC essB flanking EssBdA1 AAGGCGCATCAACTAATGCAAATGAAATAG regions for cloning EssBdA2 CTATTTTTTTTTTTTTTTTTCTGCCT essB flanking EssBdA1 AAGGCGATCAACAATAAGCATGAG Amplification of	Primer	Sequence (5'-3')	Usage
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EsaBdB1AAGGATCCTTCAACTAAAGCATCAAATG EsaBdB2regions for cloning into pMADEsaBdB2ACATTTGATTTACTATAGGAGGAAAAATAGinto pMADEssBdA1AATGATGCGAATTCTACTATGGCAGAmplification of essB flankingEssBdA2CTATTTTTTTTAACCATCTATTTTCCTCessB flankingEssBdB1ATGCCACTGAATTCGACTCregions for cloning into pMADEssBdB2ATGGTTAAAAAAAATAGTATAGGACTGAGinto pMADEssCdA1AGTACTGAATTCGTATGATGAmplification of essCdA2EssCdB1AGGGATCCGTTGCGTTTGCTTTGCCTTessC flanking regions for cloningEssCdB2ATGCATAAAAGATGGTTTAAATAGCAATGinto pIMAYEssCdB1AGGGATCCAGCACAGATGATTTCATAACATAGCAATGinto pIMAYEssCdB2ATGCATAAAAGATGGTTTAAATAGCAATGinto pIMAYEssCdB1TCATGAATTCATAAAATTCATAACATACCTCCessC flanking regions for cloning issCdB2EssCdB2ATGCATAAAAGATTCATAAATTTAACATAGGCGCregions for cloning issCdB2EssCdB2ATGAATTTTATGAATTTACAATGGCGCregions for cloning issBdA1AGGGTGGAGATAGGTGAACCCATCATCTCCACCCATACTTCCACCCCCessC flankingEssBdA2CACCCTATCTCCACCCATACTTCACCTCessC flankingEssBdB2ATGGGTGGAGATAGGTGAAACCCesson for cloning into pMADEssBdB2ATGGGTGGAGATAGGTGAACCCATGATGinto pMADEssBdB2ATGGGTGGAGATAGGTGAACCCATGATGATGATGATGATGATGATGATGAACCTCTCCACCACAAATTCTCCATGCCACCAAmplification of essAdA1AAGTGCGGGGGAGATAAGGTGAACCACCACGAATCATGGCGATGAmplification of ess operon flanking regions for cloning into pMADEsxdB1GAGGCCCATGGAAGAATAAA	EsaBdA1	ACTAATA <u>GAATTC</u> CACCATTG	Amplification of
EsaBdB2ACATTTGATTTACTATAGGAGGAAAAATAGinto pMADEssBdA1AATGATGCGAATTCTAAGCAGAmplification ofEssBdA2CTATTTTTTTTAACCATCTATTTTCCTCessB flankingEssBdB2ATGCCACTGAATTCGACTCregions for cloningEssBdB2ATGCTAAAAAAAAATGGATATAGGACTGAGinto pMADEssCdA1AGTACTGAATTCGTATGATGAmplification ofEssCdA2AAACCATCTTTTATGCATTGTCTTTGCCTCessC flankingEssCdB1AGGGATCCGTTGCGTTGCGTTTGCATTGACATGregions for cloningEssCdB2ATGCATAAAAGATGGTTTAAATAGCAATGinto pIMAYEssCdA2TAACCACCACGACAATGATTCATACAGAmplification ofEssCdB2ATGCATAAAAGATGGTTTAAATAGCAATGregions for cloningEssCdB2ATGCATAAAAGATGATTTCATAAATGGCGCregions for cloningEssCdB2ATGAATTCATAAAATTCATAAATTTACAATGGCGCregions for cloningEsxCdB1TCATGAATTCGTATTTCCACCCAACAATTCAmplification ofEsxCdB2ATGAATTTTATGAATTATATATTGAGGTGAAGinto pMADEsxBdA1AGGAATCGTAATAATTCTCGACAACAATCCessB flankingEsxBdA2CACCCTATCTCCACCCATACTCACCACAmplification of essEsxBdB1TCGGATCGAAATAATTCTGGACACAmplification of essEsxdB2ATGGCTGAGAATAGGTGAAACCATGGTGAAACCoperon flankingEsxdB2ATGGCAATGGAAGAATAAATCTGTAAATGregions for cloningEsxdB2ATGGCAATGGAAGAATAAATCTGTAACTAGAAACCoperon flankingEsxdB2ATGGCAATGGAAGAATAAATCTGGAAGAACCoperon flankingEsxdB1GAAGAGCTCCATAACGAGGTGAACCATGGCGATGHis-tag insertionNhisinsAAAGATCT	EsaBdA2	CTATAGTAAATCAAATGTTACTTTTACGTG	esaB flanking
EssBdA1AATGATGCGAATTCTAAGCAGAmplification ofEssBdA2CTATTTTTTTTTAACCATCTATTTTTCCTCessB flankingEssBdB1ATGCCACTGAATTCTGACTCregions for cloningEssBdB2ATGGTTAAAAAAAATAGTATAGGACTGAGinto pMADEssCdA1AGTACTGAATTCGTATGATGAmplification ofEssCdA2AAACCATCTTTTATGCATTGTCTTTGCCTCessC flankingEssCdB1AGGGATCCGTTGCGTTTGCTTTGACATGregions for cloningEssCdB2ATGCATAAAAGATGGTTTAAATAGCAATGinto pIMAYEssCdB2ATGCATAAAAGATGGTTTAAATAGCAATGinto pIMAYEsxCdA1AACGGATCCAGCAATGATTTCATACGessC flankingEsxCdB1TCATGAATTCGAATATTACAATGGCGCregions for cloningEsxCdB1TCATGAATTCGAATATTACAATGGCGCregions for cloningEsxCdB2ATGAATTTCGAATATTACAATGGCGCregions for cloningEsxCdB2ATGAATTCGAATATTACAATGGCGCregions for cloningEsxBdA2CACCCTATCTCCACCCCATATCTTCACCTCesxB flankingEsxBdB1TCGGATCCAAATAATTCGTATAATGGCGCregions for cloningEsxBdB2ATGGGTGGAGATAGGGTGAACCCATGATGinto pMADEsxBdB2ATGGGTGGAGATAGGGTGAACCCATGATGamplification of essEsxdA1AAGGACTCAAATAGGATGATGATGATGATGCapplification of essEsxdA2TTATTCTCCATTGCATAACTAGAAACCoperon flankingEsxdB1GAAGAGCTCAAAATGAATATTGAACAGCregions for cloningEsxdB2ATGGCGAATGGAAGAATAAACTATCTTAATGinto pIMADEsxdB1GAAGAGCTCATAATGAATGATGATGATGATGATGATGCapplification of essesxdA1AAGGCAATGGAAGAAGATGAACCACCCCGC <td>EsaBdB1</td> <td>AA<u>GGATCC</u>TTCAACTAAAGCATCAAATG</td> <td>regions for cloning</td>	EsaBdB1	AA <u>GGATCC</u> TTCAACTAAAGCATCAAATG	regions for cloning
EssBdA2CTATTTTTTTAACCATCTATTTTCCTCessB flankingEssBdB1ATGCCACTGAATTCTGACTCregions for cloningEssBdB2ATGGTTAAAAAAAAATAGTATAGGACTGAGinto pMADEssCdA1AGTACTGAATTCGTATGATGAmplification ofEssCdA2AAACCATCTTTTATGCATTGTCTTTGCCTCessC flankingEssCdB1AGGGATCCGTTGCGTTTGCTTTGACATGregions for cloningEssCdB2ATGCATAAAAGATGGTTTAAATAGCAATGinto pIMAYEssCdB2ATGCATAAAAGATGGTTTAAATAGCAATGinto pIMAYEssCdB2ATGCATAAAAGATGGTTTAAATAGCAATGessC flankingEssCdB2TCATCATAAAATTCATAACATACCTCCessC flankingEssCdB2ATGAATTCGAAATATCATAACATACCTCCessC flankingEssCdB2ATGAATTTCGAATTTACAAAGGGTGAAGinto pIMAPEssCdB2ATGAATTTCGAATTTACAACGCCregions for cloningEssCdB2ATGAATTTCGAATTTCCACACCAACAATTCAmplification ofEssRbA1AGGAATCGAATAATTCTCAACTAGAAACCessX flankingEssRbB2ATGGCGGGAATAGGGTGAACCCATGATGinto pMADEssAdB1TCGGGACTCCAAATTGCCACACACACCAmplification of essEssAdB1GCGGAATGGAAGAATAAACTACTGAAACCoperon flankingEssAdB2ATGGCAATGGAAGAATAAACTACTGAAGACCoperon flankingEssAB2ATGGCAATGGAAGAATAAACTACTTAATGinto pIMAYPRMC2seq1ATTGGACCCCCCCGAGTTCATGHis-tag insertionNhisinsAAAGATCTCCACAAGAAAATTGGATTATGesaA cloning intoPAACAACTCTCAGACAATGAACCCCCCGCGgenon pRMC2esaA nhis fwGCGCGCTCGACAATGGCGATGAntibody productionGC <td>EsaBdB2</td> <td>ACATTTGATTTACTATAGGAGGAAAAATAG</td> <td>into pMAD</td>	EsaBdB2	ACATTTGATTTACTATAGGAGGAAAAATAG	into pMAD
EssBdB1ATGCCACTGAATTCTGACTCregions for cloning into pMADEssBdB2ATGGTTAAAAAAAAATGTATAGGACTGAGinto pMADEssCdA1AGTACTGAATTCGTATGATGAmplification of essCdB1EssCdB2AACCATCTTTTATGCATTGTCTTTGCCTCessC flankingEssCdB1AGGGATCCGTTGCGTTTGCTTTGACATGregions for cloning into pIMAYEssCdB2ATGCATAAAAGATGGTTTAAATAGCAATGinto pIMAYEssCdB2TAATCATAAAAGATGGATTTAATAGCAATGinto pIMAYEsxCdA1AACGGATCCAGCAATGATTTAAACATACCTCCessC flanking regions for cloning into pIMAYEsxCdB1TCATGAAATTCATAAAATTCATAACATACCTCCessC flanking regions for cloning into pIMAPEsxCdB2ATGAATTTCGTATTTACAATGGCGCregions for cloning into pMADEsxCdB2ATGAATTTCGTATTTCAGACCAACAATTCAmplification of esxB flanking regions for cloning into pMADEsxBdA1AGGAATTCGTATTTCAGACCAACAATTCAmplification of esxB flanking regions for cloning into pMADEsxBdB1TCGGGTGGAGATAGGGTGAACCCATGATG into pMADregions for cloning into pMADEsxBdB2ATGGGTGGAGATAGGGTGAACCCATGATG into pMADoperon flanking regions for cloning into pMADEsxdB2ATGGCAATGGAAGAATAATTCTGAAAACCTATCTTAATG into pMADnot plMAPEsxdB2ATGGCAATGGAAGAATAAACTATCTTAAGAAACC into pIMAYoperon flanking regions for cloning into pIMAYpRMC2seq1ATTGGATCCCCCTCGAGTTCATG ATACTAGAAACCTCCTGGTATCATGATGATGATGATGATGATGATGATGATGATGATGATGA	EssBdA1	AATGATGC <u>GAATTC</u> TAAGCAG	Amplification of
EssBdB2ATGGTTAAAAAAAAATGGTATAGGACTGAGinto pMADEssCdA1AGTACTGAATTCGTATGATGAmplification ofEssCdA2AAACCATCTTTTATGCATTGTCTTTGCCTCessC flankingEssCdB1AGGGATCCGTTGCGTTTGCTTTGACATGregions for cloningEssCdB2ATGCATAAAAGATGGTTTAAATAGCAATGinto pIMAYEsxCdA1AACGGATCCAGCAATGATTTCATCAGmplification ofEsxCdA2TTAATTCATAAAATTCATAACATACCTCCesxC flankingEsxCdB1TCATGAATTCGAATAGTATATAACATAGGCGCregions for cloningEsxCdB2ATGAATTTTATGAATATATTGAGGTGAAGinto pMADEsxBdA1AGGAATTCGTATTTCAGACCAACAATTCAmplification ofEsxBdA2CACCCTATCTCCACCCATATCTTCACCTCesxB flankingEsxBdB1TCGGATCCAAAAAAATAGTCTCGTATAAATGregions for cloningEsxBdB2ATGGGTGGAGATAGGGTGAACCCATGATGinto pMADEsxBdB1TCGGATCCAAATAATTCTGGAACACAATTCAmplification of essEsxdA2TTATTCTTCCATTGCCATACCACAmplification of essEsxdB1GAAGAGCTCATAATGGCGAACCACTGGTGoperon flankingEsxdB2ATGGCAATGGAAGAATAACTATCTTAATGinto pIMADEsxdB1GAAGAGCTCATAATGAATGATGATGATGATGATGATGATGATGATGATG	EssBdA2	CTATTTTTTTTTTTTAACCATCTATTTTTCCTC	essB flanking
EssCdA1AGTACTGAATTCGTATGATGAmplification ofEssCdA2AAACCATCTTTTATGCATTGTCTTTGCCTCessC flankingEssCdB1AGGGATCCGTTGCGTTTGCTTTGACATGregions for cloningEssCdB2ATGCATAAAAGATGGTTTAAATAGCAATGinto pIMAYEsxCdA1AACGGATCCAGCAATGATTTCATCAGAmplification ofEsxCdA2TTAATTCATAAAAATTCATAACATACCTCCesxC flankingEsxCdB1TCATGAATTCGAATATTTCATAACATACCTCCesxC flankingEsxCdB2ATGAATTTCGAATATTTGAGGTGAAGinto pMADEsxCdB2ATGAATTTCGAATATTTCAGACCAACAATTCAmplification ofEsxCdB2ATGAATTTCGTATTAGAATATTTGAGGTGAAGinto pMADEsxBdA1AGGAATTCGTATTTCAGACCAACAATTCAmplification ofEsxBdB1TCGGATCCAAATAATTCTCGTATAAATGregions for cloningEsxBdB1TCGGATCCAAATAATTCTGGAACACCesxB flankingEsxdB2ATGGGTGGAGATAGGGTGAACCCATGATGinto pMADEsxdB1GAAGAGCTCATAATGGCAACACACoperon flankingEsxdB1GAAGAGCTCATAATGGATTTTGTACAGCregions for cloningEsxdB2ATGGCAATGGAAGAAATAACTATCTTAATGinto pIMAYpRMC2seq1ATTGGATCCCCTCGAGTTCATGHis-tag insertionNhisinsAAAGATCTTCAAAAGAAAAATTGGATTATGesaA cloning intoesaA nhis revGGTGGAGCCAATGGCGATGAntibody productionGGGGTTACTGCAGGCAATGCGCATGpET27bmodEsxAGCGCCCTCGACAATGGCGATGAntibody productionCCGAntibody productionCCGAntibody productionCCC <t< td=""><td>EssBdB1</td><td>ATGCCACT<u>GAATTC</u>TGACTC</td><td>regions for cloning</td></t<>	EssBdB1	ATGCCACT <u>GAATTC</u> TGACTC	regions for cloning
EssCdA2AAACCATCTTTTATGCATTGTCTTTGCCTCessC flankingEssCdB1AGGGATCCGTTGCGTTTGCTTTGACATGregions for cloningEssCdB2ATGCATAAAAGATGGTTTAAATAGCAATGinto pIMAYEsxCdA1AACGGATCCAGCAATGATTTCATCAGAmplification ofEsxCdA2TTAATTCATAAAATTCATAACATACCTCCesxC flankingEsxCdB1TCATGAATTTGAATTAATATGAGGTGAAGinto pMADEsxCdB2ATGAATTTCGTATTTCAGACCAACAATTCAmplification ofEsxCdB2ATGAATTTGTGTATTTCAGACCAACAATTCAmplification ofEsxBdA1AGGAATTCGTATTTCAGACCAACAATTCAmplification ofEsxBdA2CACCCTATCTCCACCCATATCTTCACCTCesxB flankingEsxBdB1TCGGATCCAAATAATCTCGTATAAATGregions for cloningEsxBdB2ATGGGTGGAGATAGGGTGAACCCATGATGinto pMADEsxAdA1AATGTGCGAATTCTGACCACAmplification of essEsxdA2TTATTCTTCCATTGCCATAACTAGAAACCoperon flankingEsxdB1GAAGAGCTCATAATGGATGAACACACoperon flankingEsxdB2ATGGCAATGGAAGAATAAACTACTTAATGinto pIMAYpRMC2seq1ATTTGGATCCCCTCGAGTTCATGHis-tag insertionNhisinsAAGATCTTCAAAAGAAAAATTGGATGATGATGATGATGATGATGATGAopRMC2esaA nhis fwGGAAGATCTAAAAAGAAAAAATGGATTAATGGATTTATGesaA cloning intogggpRMC2hgpET27bmodEsxAGCGCGCTCGACAATGGCGATGAntibody productionCCGAntibody productionCCGAntibody production	EssBdB2	ATGGTTAAAAAAAAATAGTATAGGACTGAG	into pMAD
EssCdB1AGGGATCCGTTGCGTTTGCTTTGACATG regions for Coning into pIMAYEssCdB2ATGCATAAAAGATGGTTTAAATAGCAATG into pIMAYEsxCdA1AACGGATCCAGCAATGATTTCATCAG SxCdA2Amplification of esxC flanking regions for cloning into pMADEsxCdB1TCATGAATTCGTAAAATTCATAACATACCTCC GAATTTTATGAATTACAATGGCGC regions for cloning into pMADesxC flanking regions for cloning into pMADEsxCdB2ATGAATTTCGTAATTAGAATAATTGAGGTGAAG SxCdB2into pMADEsxBdA1AGGAATTCGTATTTCAGACCAACAATTC ACCCTATCTCCACCCATATCTTCACCTC esxB flanking regions for cloning into pMADEsxBdB1TCGGATCCAAATAATTCTCGTATAAATG regions for cloning into pMADEsxBdB2ATGGGTGGAGATAGGGTGAACCCATGATG into pMADEsxAdA1AATGTGCGAATTC AATGTGCGAATTCTGACAC sxdB1GAAGAGCTCATAATGATTTTGTACAGC peron flanking fesxdB2operon flanking regions for cloning into pIMAYpRMC2seq1ATTGGCAATGGAAGAATAAACTATCTTAATG AACTAGAAACCTCCTGGAGTTCATG ATAACTAGAAACCTCCTGGATGATGATGATGATGATG ATAACTAGAAACCTCCTGGATGACGC ATAACTAGAAACCTCCTGGTACCGC insertion pRMC2esaA nhis fwGGAAGATCTAAAGAAAAATTGGATTATG GgpFT27bmodEsxAGCGCCGTCGACAATGGCGATG GCGCCTCGAGTTACTGCAGGCCAAAGTTGTGT Antibody production Antibody production Antibody production Antibody production	EssCdA1	AGTACT <u>GAATTC</u> GTATGATG	Amplification of
EssCdB2ATGCATAAAAGATGGTTTAAATAGCAATGinto pIMAYEsxCdA1AACGGATCCAGCAATGATTTCATCAGAmplification ofEsxCdA2TTAATTCATAAAATTCATAACATACCTCCesxC flankingEsxCdB1TCATGAATTCGAATATTTACAATGGCGCregions for cloningEsxCdB2ATGAATTTTATGAATTAATATTGAGGTGAAGinto pMADEsxBdA1AGGAATTCGTATTTCAGACCAACAATTCAmplification ofEsxBdA2CACCCTATCTCCACCCATATCTTCACCTCesxB flankingEsxBdB1TCGGATCCAAATAATTCTCGTATAAATGregions for cloningEsxBdB2ATGGGTGGAGATAGGGTGAACCCATGATGinto pMADEsxAdA1AATGTGCGAATTCTGACCACAmplification of essEsxdB1GAAGGCTCATAATGGCATAACTAGAAACCoperon flankingEsxdB2ATGGCAATGGAAGAATAAACTATCTTAATGinto pIMAYPRMC2seq1ATTTGGATCCCCTCGAGTTCATGHis-tag insertionNhisinsAAAGATCTTCCTAAAAGAAAAATTGGATTTATGesaA cloning intopRMC2bestGGAGAGCTCATAAAGAAAAAATTGGATTTATGesaA cloning intopRMC2hGGmodelpET27bmodEsxAGCGCGTCGACAATGGCGATGAntibody productionCCCAntibody production	EssCdA2	AAACCATCTTTTATGCATTGTCTTTGCCTC	essC flanking
EsxCdA1AACGGATCCAGCAATGATTTCATCAGAmplification ofEsxCdA2TTAATTCATAAAATTCATAACATACCTCCesxC flankingEsxCdB1TCATGAATTCGAATATTTACAATGGCGCregions for cloningEsxCdB2ATGAATTTTATGAATTATATATATGAGGTGAAGinto pMADEsxBdA1AGGAATTCGTATTTCAGACCAACAATTCAmplification ofEsxBdA2CACCCTATCTCCACCCATATCTTCACCTCesxB flankingEsxBdB1TCGGATCCAAATAATTCTCGTATAAATGregions for cloningEsxBdB2ATGGGTGGAGATAGGGTGAACCCATGATGinto pMADEsxAdA1AATGTGCGAATTCTGACCACAmplification of essEsxdB1GAAGAGCTCATAATGCTGACCACAmplification of essEsxdB2ATGGCAATGGAAGAATAACTAGAAACCoperon flankingEsxdB1GAAGAGCTCATAATGATTTTGTACAGCregions for cloningEsxdB2ATGGCAATGGAAGAATAAACTATCTTAATGinto pIMAYpRMC2seq1ATTTGGATCCCCTCGAGTTCATGHis-tag insertionNhisinsAAAGATCTTCCTGAATGATGATGATGATGATGATGATGATGN-terminal his-taginsertion pRMC2esaA nhis fwGGAAGATCTAAAAAGAAAAATTGGATTTATGesaA cloning intogpFT27bmodEsxAGCGCGTCGACAATGGCGATGAntibody productionCCCAntibody production	EssCdB1	AG <u>GGATCC</u> GTTGCGTTTGCTTTTGACATG	regions for cloning
EsxCdA2TTAATTCATAAAATTCATAACATACCTCCesxC flankingEsxCdB1TCATGAATTCGAATATTTACAATGGCGCregions for cloningEsxCdB2ATGAATTTCGTATTTCAGAATAATTGAGGTGAAGinto pMADEsxBdA1AGGAATTCGTATTTCAGACCAACAATTCAmplification ofEsxBdA2CACCCTATCTCCACCCATATCTTCACCTCesxB flankingEsxBdB1TCGGATCCAAATAATTCTCGTATAAATGregions for cloningEsxBdB2ATGGGTGGAGATAGGGTGAACCCATGATGinto pMADEsxAdA1AATGTGCGAATTCTGACCACAmplification of essEsxdB1GAAGACTCATATGTGACCACAmplification of essEsxdB2ATGGCAATGGCAATAGGTGAACCCATGAACCoperon flankingEsxdB1GAAGACTCATAATGATTTTGTACAGCregions for cloningEsxdB2ATGGCAATGGAAGAATAACTATCTTAATGinto pIMAYpRMC2seq1ATTTGGATCCCCTCGAGTTCATGHis-tag insertionNhisinsAAAGATCTTCTCAAAAAGAAAAATTGGATTATGesaA cloning intoesaA nhis fwGGAAGATCTAAAAAGAAAAATTGGATTTATGesaA cloning intoggggpET27bmodEsxAGCGCGTCGACAATGGCGATGAntibody productionCCGAntibody production	EssCdB2	ATGCATAAAAGATGGTTTAAATAGCAATG	into pIMAY
EsxCdB1TCATGAATTCGAATATTTACAATGGCGCregions for cloning into pMADEsxCdB2ATGAATTTTATGAATTATATATGAGGTGAAGinto pMADEsxBdA1AGGAATTCGTATTTCAGACCAACAATTCAmplification of esxBdA2EsxBdA2CACCCTATCTCCACCCATATCTTCACCTCesxB flanking regions for cloning into pMADEsxBdB1TCGGATCCAAATAATTCTCGTATAAATGregions for cloning into pMADEsxBdB2ATGGGTGGAGATAGGGTGAACCCATGATGinto pMADEsxAdA1AATGTGCGAATTCTGACCACAmplification of ess operon flanking regions for cloning into pMADEsxdB1GAAGAGCTCATAATGATTTTGTACAGCregions for cloning into pIMAYpRMC2seq1ATTGGCAATGGAAGAATAAACTATCATGHis-tag insertion NhisinsAAAGATCTTCCGAGATCATGATGATGATGATGATGATGATGATGATGATGATGATGA	EsxCdA1	AAC <u>GGATCC</u> AGCAATGATTTCATCAG	Amplification of
EsxCdB2ATGAATTTATGAATTATATATATTGAGGTGAAGinto pMADEsxBdA1AGGAATTCGTATTTCAGACCAACAATTCAmplification ofEsxBdA2CACCCTATCTCCACCCATATCTTCACCTCesxB flankingEsxBdB1TCGGATCCAAATAATTCTCGTATAAATGregions for cloningEsxBdB2ATGGGTGGAGATAGGGTGAACCCATGATGinto pMADEsxAdA1AATGTGCGAATTCTGACCACAmplification of essEsxdA2TTATTCTTCCATTGCCATAACTAGAAACCoperon flankingEsxdB1GAAGAGCTCATAATGATTTTGTACAGCregions for cloningEsxdB2ATGGCAATGGAAGAATAAACTATCTTAATGinto pIMAYpRMC2seq1ATTTGGATCCCCTCGAGTTCATGHis-tag insertionNhisinsAAAGATCTTCCTGAATGATGATGATGATGATGATGCN-terminal his-tagATAACTAGAAACCTCCTGGTACCGCinsertion pRMC2esaA nhis revGGAGATCTAAAAAGAAAAATTGGATTTATGesaA cloning intoggGGAntibody productionCCCAntibody production	EsxCdA2	TTAATTCATAAAATTCATAACATACCTCC	esxC flanking
EsxBdA1AGGAATTCGTATTTCAGACCAACAATTCAmplification ofEsxBdA2CACCCTATCTCCACCCATATCTTCACCTCesxB flankingEsxBdB1TCGGATCCAAATAATTCTCGTATAAATGregions for cloningEsxBdB2ATGGGTGGAGATAGGGTGAACCCATGATGinto pMADEsxAdA1AATGTGCGAATTCTGACCACAmplification of essEsxdA2TTATTCTTCCATTGCCATAACTAGAAACCoperon flankingEsxdB1GAAGAGCTCATAATGATTTTGTACAGCregions for cloningEsxdB2ATGGCAATGGAAGAATAAACTATCTTAATGinto pIMAYpRMC2seq1ATTTGGATCCCCTCGAGTTCATGHis-tag insertionNhisinsAAAGATCTTCCTGAATGATGATGATGATGATGATGATGATGN-terminal his-tagaTAACTAGAAACCTCCTGGTACCGCinsertion pRMC2esaA nhis fwGGAAGATCTAAAAGAAAAATTGGATTTATGesaA cloning intoggGCGCCTCGACAATGGCGATGAntibody productioncCCAntibody production	EsxCdB1	TCAT <u>GAATTC</u> GAATATTTACAATGGCGC	regions for cloning
EsxBdA2CACCCTATCTCCACCCATATCTTCACCTCesxB flankingEsxBdB1TCGGATCCAAATAATTCTCGTATAAATGregions for cloningEsxBdB2ATGGGTGGAGATAGGGTGAACCCATGATGinto pMADEsxAdA1AATGTGCGAATTCTGACCACAmplification of essEsxdA2TTATTCTTCCATTGCCATAACTAGAAACCoperon flankingEsxdB1GAAGAGCTCATAATGATTTTGTACAGCregions for cloningEsxdB2ATGGCAATGGAAGAATAAACTATCTTAATGinto pIMAYpRMC2seq1ATTTGGATCCCCTCGAGTTCATGHis-tag insertionNhisinsAAGATCTTCCTGAATGATGATGATGATGATGATGCN-terminal his-tagaTAACTAGAAACCTCCTGGTACCGCinsertion pRMC2esaA nhis fwGGAAGATCTAAAAAGAAAAATTGGATTATTGesaA cloning intogFT27bmodEsxAGCGCGTCGACAATGGCGATGAntibody productionCCCAntibody production	EsxCdB2	ATGAATTTTATGAATTAATATTGAGGTGAAG	into pMAD
EsxBdB1TCGGATCCAAATAATTCTCGTATAAATG TGGGTGGAGATAGGGTGAACCCATGATGregions for cloning into pMADEsxBdB2ATGGGTGGAGATAGGGTGAACCCATGATGinto pMADEsxAdA1AATGTGCGAATTCTGACCACAmplification of essEsxdA2TTATTCTTCCATTGCCATAACTAGAAACCoperon flankingEsxdB1GAAGAGCTCATAATGATTTTGTACAGCregions for cloningEsxdB2ATGGCAATGGAAGAATAAACTATCTTAATGinto pIMAYpRMC2seq1ATTTGGATCCCCTCGAGTTCATGHis-tag insertionNhisinsAAAGATCTTCCTGAATGATGATGATGATGATGATGATGATGATGAN-terminal his-tag insertion pRMC2esaA nhis fwGGAAGATCTAAAAAAAAAAAAAAAATTGGATTTATGesaA cloning into pRMC2hpET27bmodEsxAGCGCGTCGACAATGGCGATG GCGCCTCGAGTTACTGCAGGCCAAAGTTGTTGAntibody production Antibody production Antibody production	EsxBdA1	AG <u>GAATTC</u> GTATTTCAGACCAACAATTC	Amplification of
EsxBdB2ATGGGTGGAGATAGGGTGAACCCATGATGinto pMADEsxAdA1AATGTGCGAATTCTGACCACAmplification of essEsxdA2TTATTCTTCCATTGCCATAACTAGAAACCoperon flankingEsxdB1GAAGAGCTCATAATGATTTTGTACAGCregions for cloningEsxdB2ATGGCAATGGAAGAATAAACTATCTTAATGinto pIMAYpRMC2seq1ATTTGGATCCCCTCGAGTTCATGHis-tag insertionNhisinsAAAGATCTTCCTGAATGATGATGATGATGATGATGATGATGATGATGATGA	EsxBdA2	CACCCTATCTCCACCCATATCTTCACCTC	esxB flanking
EsxAdA1AATGTGCGAATTCTGACCACAmplification of essEsxdA2TTATTCTTCCATTGCCATAACTAGAAACCoperon flankingEsxdB1GAAGAGCTCATAATGATTTTGTACAGCregions for cloningEsxdB2ATGGCAATGGAAGAATAAACTATCTTAATGinto pIMAYpRMC2seq1ATTTGGATCCCCTCGAGTTCATGHis-tag insertionNhisinsAAGATCTTCCTGAATGATGATGATGATGATGATGATGCN-terminal his-taginsertion pRMC2esaA nhis fwGGAAGATCTAAAAAGAAAAATTGGATTTATGesaA cloning intopET27bmodEsxAGCGCGTCGACAATGGCGATGAntibody productionCCCAntibody production	EsxBdB1	TC <u>GGATCC</u> AAATAATTCTCGTATAAATG	regions for cloning
EsxdA2TTATTCTTCCATTGCCATAACTAGAAACC GAAGAGCTCATAATGATTTTGTACAGCoperon flanking regions for cloning into pIMAYEsxdB2ATGGCAATGGAAGAATAAACTATCTTAATGinto pIMAYpRMC2seq1ATTTGGATCCCCTCGAGTTCATGHis-tag insertionNhisinsAAAGATCTTCCTGAATGATGATGATGATGATGATGATGATG ATAACTAGAAACCTCCTGGTACCGCN-terminal his-tag insertion pRMC2esaA nhis fwGGAAGATCTAAAAAAGAAAAATTGGATTTATG GesaA cloning into pRMC2hpET27bmodEsxAGCGCGTCGACAATGGCGATG GCGCCTCGAGTTACTGCAGGCCAAAGTTGTTG CAntibody production Antibody production	EsxBdB2	ATGGGTGGAGATAGGGTGAACCCATGATG	into pMAD
EsxdB1GAAGAGCTCATAATGATTTTGTACAGC ATGGCAATGGAAGAATAAACTATCTTAATGregions for cloning into pIMAYpRMC2seq1ATTTGGATCCCCTCGAGTTCATG ATGGATCTTCCTGAATGATGATGATGATGATGATGATGATG AAAGATCTTCCTGAATGATGATGATGATGATGATGC ATAACTAGAAACCTCCTGGTACCGC esaA nhis fwHis-tag insertion N-terminal his-tag insertion pRMC2 esaA cloning into pRMC2hesaA nhis fw esaA nhis revGGAGCTCATTAGATTAATCTCTCTTTATG GesaA cloning into pRMC2hpET27bmodEsxAGCGCGTCGACAATGGCGATG GCGCCTCGAGTTACTGCAGGCCAAAGTTGTTG CAntibody production Antibody production	EsxAdA1		Amplification of ess
EsxdB2ATGGCAATGGAAGAATAAACTATCTTAATGinto pIMAYpRMC2seq1ATTTGGATCCCCTCGAGTTCATGHis-tag insertionNhisinsAAAGATCTTCCTGAATGATGATGATGATGATGATGCN-terminal his-tag insertion pRMC2esaA nhis fwGGAAGATCTAAAAAGAAAAATTGGATTTATGesaA cloning into pRMC2hesaA nhis revGGTGAGCTCATTAGATTAATCTCTCTTTTCTTAAA GPET27bmodEsxAGCGCGTCGACAATGGCGATG GCGCCTCGAGTTACTGCAGGCCAAAGTTGTTGAntibody production Antibody production			· •
pRMC2seq1ATTTGGATCCCCTCGAGTTCATGHis-tag insertionNhisinsAAAGATCTTCCTGAATGATGATGATGATGATGATGCN-terminal his-tag insertion pRMC2esaA nhis fwGGAAGATCTAAAAAGAAAAATTGGATTTATGesaA cloning into pRMC2hesaA nhis revGGTGAGCTCATTAGATTAATCTCTCTTTTCTTAAApRMC2hgGCGCCTCGACAATGGCGATGAntibody production Antibody productioncCCAntibody production			
NhisinsAAAGATCTTCCTGAATGATGATGATGATGATGATGC ATAACTAGAAACCTCCTGGTACCGCN-terminal his-tag insertion pRMC2 esaA nhis fw GGTGAGCTCATTAGATTAATCTCTCTTTTCTTAAA GN-terminal his-tag insertion pRMC2 esaA cloning into pRMC2hpET27bmodEsxAGCGCGTCGACAATGGCGATG GCGCCTCGAGTTACTGCAGGCCAAAGTTGTTG CAntibody production Antibody production			·
ATAACTAGAAACCTCCTGGTACCGCinsertion pRMC2esaA nhis fw esaA nhis revGGAAGATCTAAAAAGAAAAATTGGATTTATG GGTGAGCTCATTAGATTAATCTCTCTTTCTTAAA GesaA cloning into pRMC2hpET27bmodEsxAGCGCGTCGACAATGGCGATG GCGCCTCGAGTTACTGCAGGCCAAAGTTGTTG CAntibody production Antibody production	•		U
esaA nhis fw GGAAGATCTAAAAAGAAAAATTGGATTTATG esaA cloning into esaA nhis rev GGTGAGCTCATTAGATTAATCTCTCTTTTCTTAAA pRMC2h pET27bmodEsxA GCGCGTCGACAATGGCGATG Antibody production GCGCCTCGAGTTACTGCAGGCCAAAGTTGTTG C Antibody production	Nhisins		v
esaA nhis rev GGTGAGCTCATTAGATTAATCTCTCTTTCTTAAA pRMC2h G G Antibody production pET27bmodEsxA GCGCGTCGACAATGGCGATG Antibody production GCGCCTCGAGTTACTGCAGGCCAAAGTTGTTG C Antibody production			•
G G pET27bmodEsxA GCGC <u>GTCGAC</u> AATGGCGATG Antibody production GCGC <u>CTCGAG</u> TTACTGCAGGCCAAAGTTGTTG Antibody production C C			•
pET27bmodEsxA GCGCGTCGACAATGGCGATG Antibody production GCGCCTCGAGTTACTGCAGGCCAAAGTTGTTG Antibody production C Antibody production	esaA nhis rev		pRMC2h
GCGC <u>CTCGAG</u> TTACTGCAGGCCAAAGTTGTTG Antibody production C			
C	pET27bmodEsxA		
			Antibody production
pET27bmodEsaA GCGCGTCGACACAGACCGTGAAAGAAACCTG Antibody production			
	pE127bmodEsaA	GCGC <u>GTCGAC</u> ACAGACCGTGAAAGAAAACCTG	Antibody production

	GATAAAC	
	GCGC <u>CTCGAG</u> TTACGCGTGCAGTTTGTTCACG TTGTTATC	Antibody production
pET27bmodEsaC	GCGC <u>GTCGAC</u> AATGAACTTTAACGATATTGAAA CG	Antibody production
	GCGC <u>CTCGAG</u> TTAGTTCATCGCTTTGTTAAAAT ATTCGCTCG	Antibody production
pET27bmodEssB	GCGC <u>GTCGAC</u> ACAGGATATGCTGACCCCGCTG GATG	Antibody production
	GCGC <u>CTCGAG</u> CTACACGGTATGGCCCACTTTG CGCAC	Antibody production
pET27bmodEssC	GCGC <u>GTCGAC</u> GAAGATCTGGTGGAAACCG GCGC <u>CTCGAG</u> TTATTTAAACCAGCGAATTTTCT	Antibody production Antibody production
	G	
region-1-f	CAGGAGGTTTCTAGTTATGGC	RT-PCR
region-1-r	GTTCTTGAACGGCATCAGC	RT-PCR
region-2-f	TTACGGGCAAGGTTCAGACC	RT-PCR
region-2-r	GTAAATAATTCCGGGAAGTCG	RT-PCR
region-3-f	CTAAGACAGGTAAAATCTATCGG	RT-PCR
region-3-r	TGCTTCTTCAGCATCTCTAAAGGCG	RT-PCR
region-4-f	GCATATGTACGCAAAGTAGGAC	RT-PCR
region-4-r	TCGTTAGTTGCTCTTGAGTTC	RT-PCR
region-5-f	CTTGAACATTTTATTTGTCGGC	RT-PCR
region-5-r	AGCCACTTTAAAACCTGCATC	RT-PCR
region-6-f	ATGCAGGTTTTAAAGTGGCTAC	RT-PCR
region-6-r	CGCATCATCCATTGTTGTATCT	RT-PCR
region-7-f	TGGGTCAAAACATAAAGCGTGC	RT-PCR
region-7-r	TCGCATGATGTCCATGGTTC	RT-PCR
esxA-GSP1	GTTCTTGAACGGCATCAGC	5' RACE
esxA-GSP2	ACGGCATCAGCAGTGCTATTC	5' RACE
esxA-GSP3	CGCGCTCGAGATTTCTTCTAATAATTGTGC	5' RACE
esaA-GSP1	TCAAACCAGACTCAGCAAGG	5' RACE
esaA-GSP2	AATGCTTGACCCAGCTCAAC	5' RACE
esaA-GSP3	CGCGAATTCCCTTTTTCTGATTGATCTCC	5' RACE
Anchor primer1	GACCACGCGT <u>ATCGAT</u> GTCGACTTTTTTTTTT TTTTV	5' RACE
Anchor primer2	GACCACGCGT <u>ATCGAT</u> GTCGAC	5' RACE
M13-F	GTAAAACGACGGCCAGT	5' RACE
esxA-QPCR-F	TGGCAATGATTAAGATGAGTCC	RT-qPCR
esxA-QPCR-R	TCTTGTTCTTGAACGGCATC	RT-qPCR
esaA-QPCR-F	TGGCTATAGAGCGAAATTCATC	RT-qPCR
esaA-QPCR-R	CCAAGCCTATAGGATGCTCTG	RT-qPCR
essC-QPCR-F	TTTCGATGTTGCAAGACACC	RT-qPCR
essC-QPCR-R	GACATGCGGAATTGTTTCAC	RT-qPCR
esxB-QPCR-F	GGTATTAAAGCAGATGGTGGCAAG	RT-qPCR
esxB-QPCR-R	GTCAGCCATCGGTTGTACTAATTC	RT-qPCR
16S rRNA-F	GTGCACATCTTGACGGTACCTA	RT-qPCR

Table S1. Oligonucleotide primers used in this study. Restriction enzyme sites are underlined.