WHO regions and malaria endemic countries

Not applicable or malaria-free	Eastern Mediterannean region	Region of the Americas
African region	European region	South-East Asia region
		Western Pacific region

Source: World Health Organization
Map Production: Global Malaria Programme (GMP), World Health Organization

Figure 1. Malaria endemic countries divided according by WHO regions.
Source: [3].

Figure 2. Life cycle of *P. falciparum* in mammal and vector hosts.
 Source: [7].

Selection of 5 sets of primers to amplify 16s rDNA in Bacteria

Independent BLAST search with primers sequences

First cleaning of sequences based in dual alignment of primers

Sequences with only one aligned primer were eliminated

Second cleaning based on repeated sequences

Analysis of sequences shared by all five sets of primers

Unmatched sequences were eliminated

Selection of three sets of primers

Elimination of outliers

ANOVA one-way test for differences in alignment parameters

Selection of primers

Figure 3. General overview of the *in silico* analysis of primers for 16s rDNA.

In Silico Analysis

Winsley's Primers
PCR program: 95°C (5 min), 35 cycles of 95°C (30s), annealing gradient 55-65°C (30 s), and 72°C (60 s), final extension of 72°C (5 min). Total volume of reaction: 50 µl
MgCl₂: 1.5mM- 3mM
Taq Polymerase: Qiagen/Phire Hot Start Taq Polymerase
dNTP's: 2.5mM, 5 µg Bovine Serum Albumin (BSA), 10 pmol each primer, 10 µl of Buffer 10X (Qiagen Taq) or 5 µl of 5X Phire Hot Start Taq Polymerase Buffer.
DNA template: 2µl of *M. tuberculosis* (10ng/µl)

Herlemann's Primers
PCR program: 95°C (5 min), 40 cycles of 95°C (40s), annealing gradient 58°C (40 s), and 72°C (60 s), final extension of 72°C (7 min). Total volume of reaction: 50 µl
MgCl₂: 2mM
Taq Polymerase: Qiagen/Phire Hot Start Taq Polymerase
dNTP's: 2.5mM, 5 µg Bovine Serum Albumin (BSA), 10 pmol each primer-adaptor, 10µl of Buffer 10X (Qiagen Taq) or 5 µl of Phire Hot Start Taq Polymerase Buffer.
DNA template: 2µl of *M. tuberculosis* (10ng/µl) or

Successful amplification

Lane 1-50: Qiagen Taq Polymerase. Lane 51-101: Hot Start Taq DNA Polymerase
Lane 1-12 gradient 55-65oC. MgCl₂: 1,5mM
Lane 13: Ladder 100bp. Lane 14-25: Gradient 55-65°C. MgCl₂ 2mM
Lane 26-38: Gradient 55-65°C. MgCl₂ 2,5mM.
Lane 37: Ladder 100bp. Lane 39-50: Gradient 55-65°C. MgCl2 3mM
Lane 51-63: Gradient 55-65°C. MgCl₂ 1,5mM.
Lane 60: Ladder 100bp. Lane 64-76: Gradient 55-65°C. MgCl₂ 2 mM
Lane 77-89: Gradient 55-65°C. Mgcl₂ 2,5mM
Lane 84: Ladder 100bp. Lane 100-111: Gradient 55-65ºC. Mgcl₂ 3 mM
Lane 112: ladder 100bp.

Successful amplification with adapters.

Lane 1: An. Stephensi HT.
Lane 2: An. Stephensi ABD.
Lane 3: *M. tuberculosis* 0,4x10⁻⁴ µg/µl
Lane 4: *M. tuberculosis* 0,4x10⁻⁵µg/µl
Lane 5: *M. tuberculosis* 0,4x10⁻⁶µg/µl
Lane 6: *M. tuberculosis* 0,4x10⁻⁷µg/µl
Lane 7: Negative control

Adjust to very low concentration of DNA:
2 µl of 0,4x10⁻⁵ µg/µl (1680 copies)
2 µl of 0,4x10⁻⁶ µg/µl (168 copies)
2 µl of 0,4x10⁻⁷ µg/µl (16.8 copies)
2 µl of 0,4x10⁻⁸ µg/µl (1.68 copies)
Annealing temperature: 52-54oC
MgCl2: 3,5 mM.
45 cycles of amplification

Lane 1-12: Gradient 52-54°C. MgCL₂ 3,5mM DNA template 0,4x10⁻⁵ µg/µl (1680 copies/µl)
Lane 13: Ladder 10 bp. Lane 14-25: Gradient 52-54°C, MgCL₂ 3,5mM,
DNA template: 0,4x10⁻⁶ µg/µl (168 copies/µl). Lane 26-38: Gradient 52-54°C, MgCL₂ 3,5mM
DNA template: 0,4x10⁻⁷ µg/µl (16,8 copies/µl). Lane 37: Ladder 100bp.
Lane 39-50: Gradient 52-54°C, MgCL₂ 3,5mM, DNA template: 0,4x10-8 μg/μl (1,68 copies/μl)

New batches of primers, dNTP's and BSA:
Successful amplification of *An. stephensi* and false positives.
MgCL₂: 3.5mM
Annealing temperature: 52ºC
45 cycles of amplification.

Unsuccessful amplification with adapters

Lane 1-3: Negative controls. Lane 4: Negative control DNA extraction.
Lane 5: *An. stephensi* head/thorax. Lane 6: *An. stephensi* abdomen.
Lane 7: False positive head/thorax. Lane 8: False positive abdomen
Lane 9: CSP ELISA negative mosquito head/thorax. Lane 10: Ladder 100bp
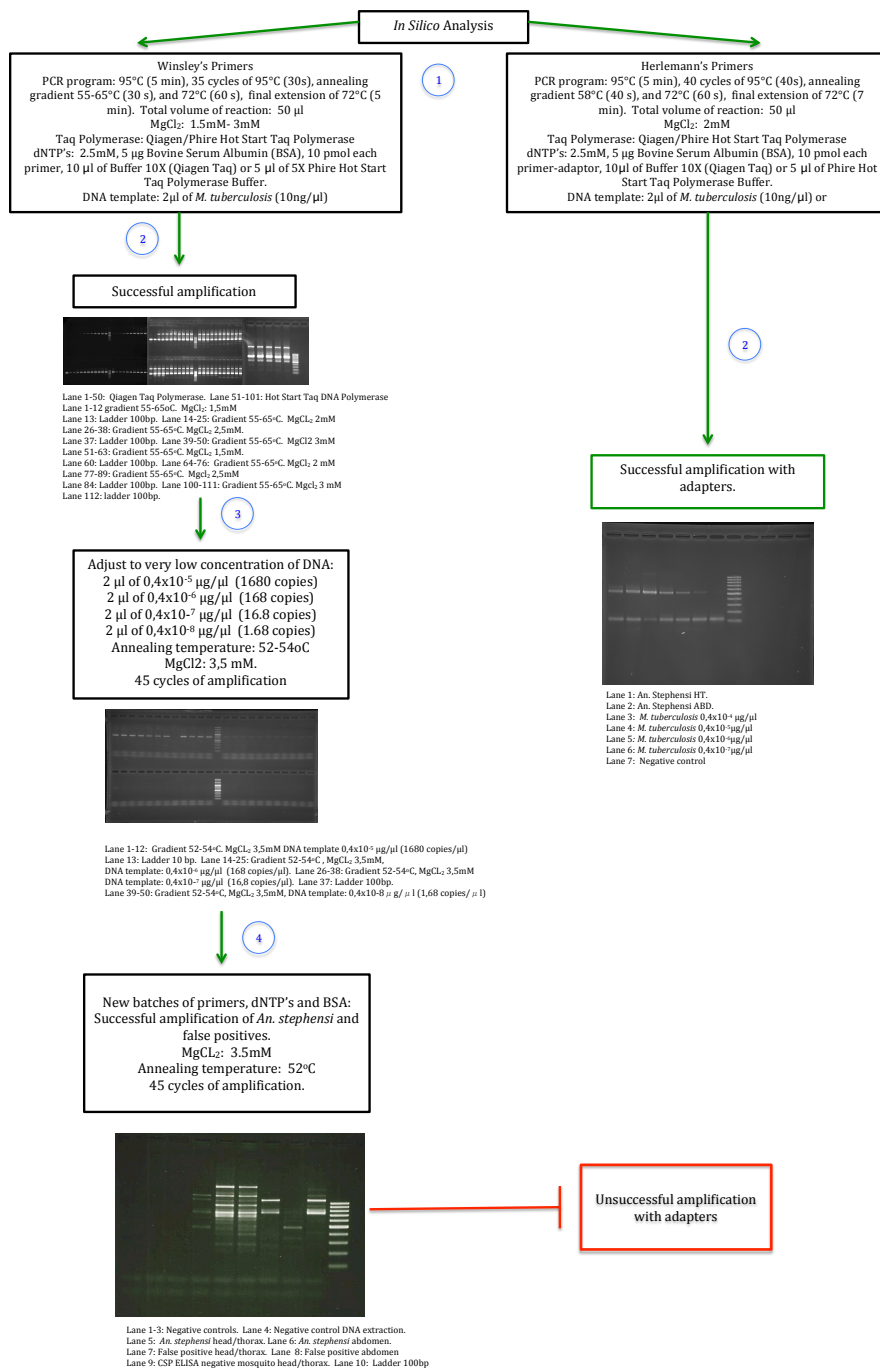
Figure 4. Standardization of PCR protocol through modifications in reagent concentrations and annealing temperature.
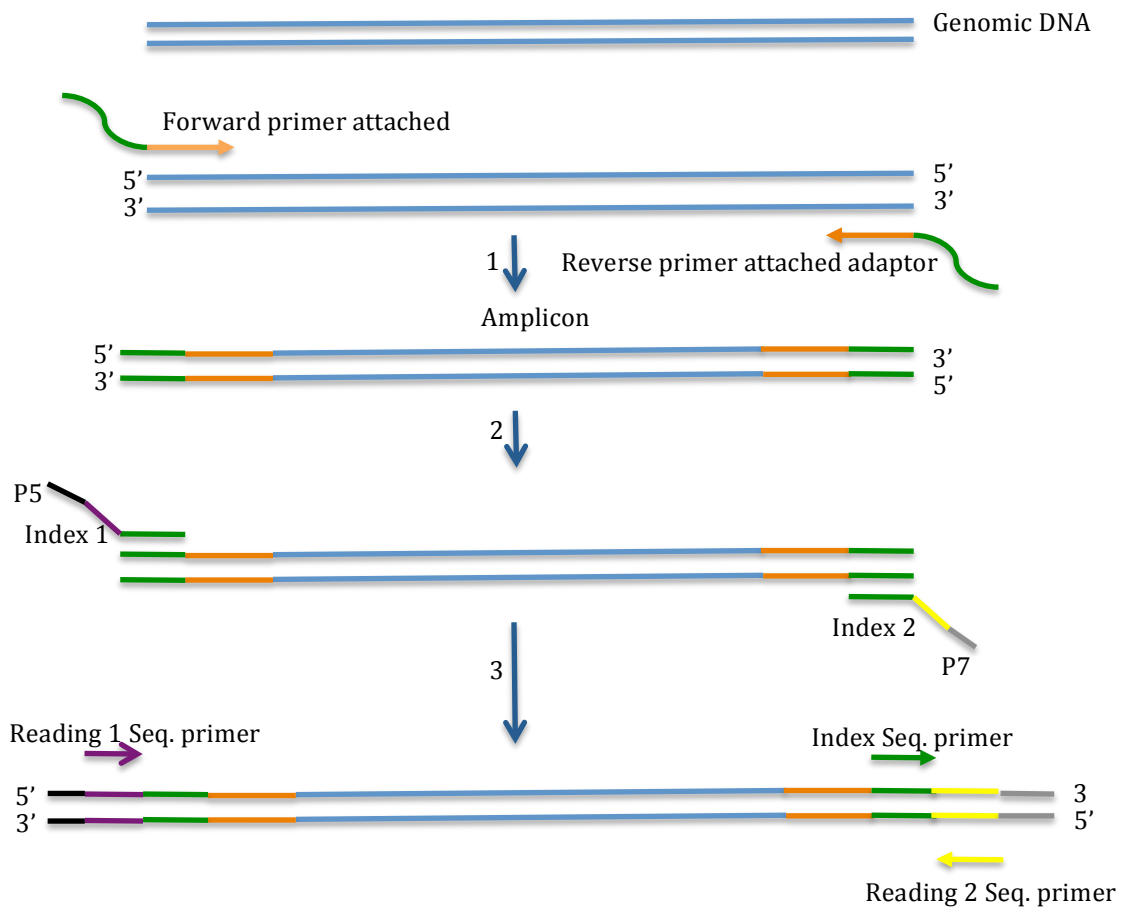
Figure 5. Overview of the steps followed prior to sequencing reaction through Illumina®️ platform.

 1. Amplification with 5'overhanging adaptors from Illumina®️ attached to forward and reverse primers targeting V3-V4 of 16s r DNA is performed. 2. Attaching of Illumina®️ default index1 (P7) and index 2(P5). 3. The final contruct contains sequences P5/P7 for binding the surface flow and also sequencing primers to read in both directions of the amplicon. Adapted from [61].
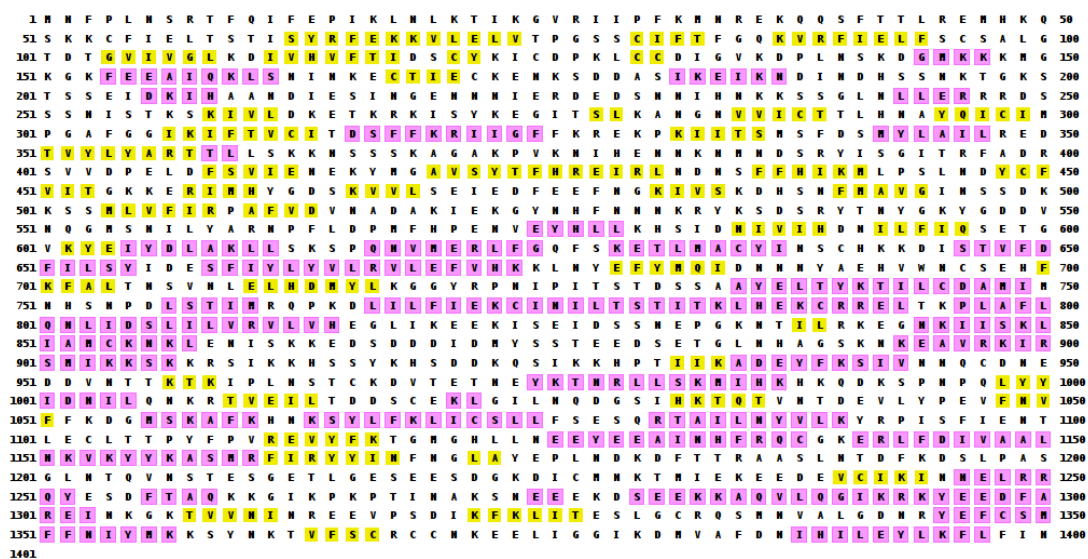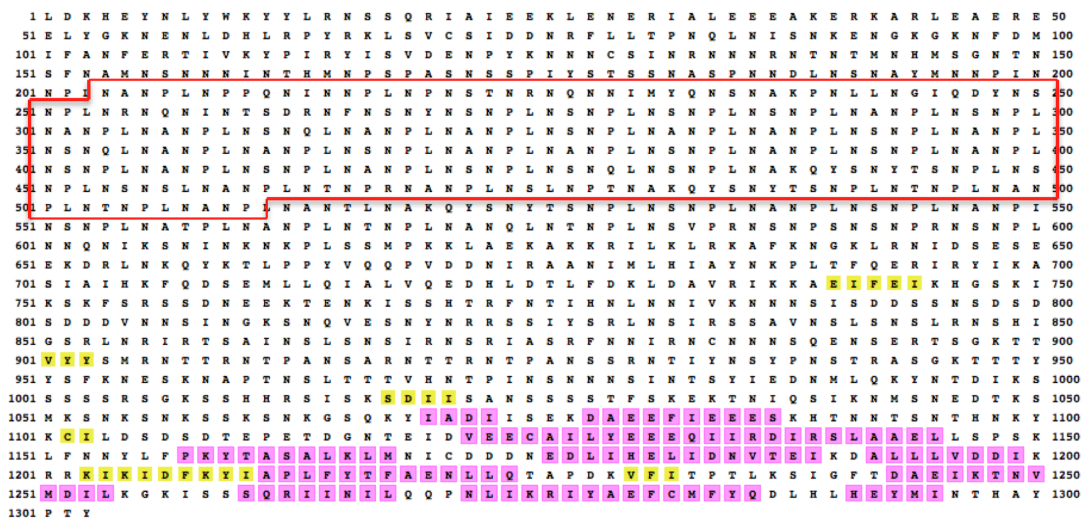
Figure 6. Secondary structure prediction of EJW02257.1 retrieved from PSIRED.
Red lines indicate the region from 1605 to 1962 aminoacid residues where the NANPL repetitions spans into the protein. Pink squares denote Helix structure while yellow squares indicates sheet structures.
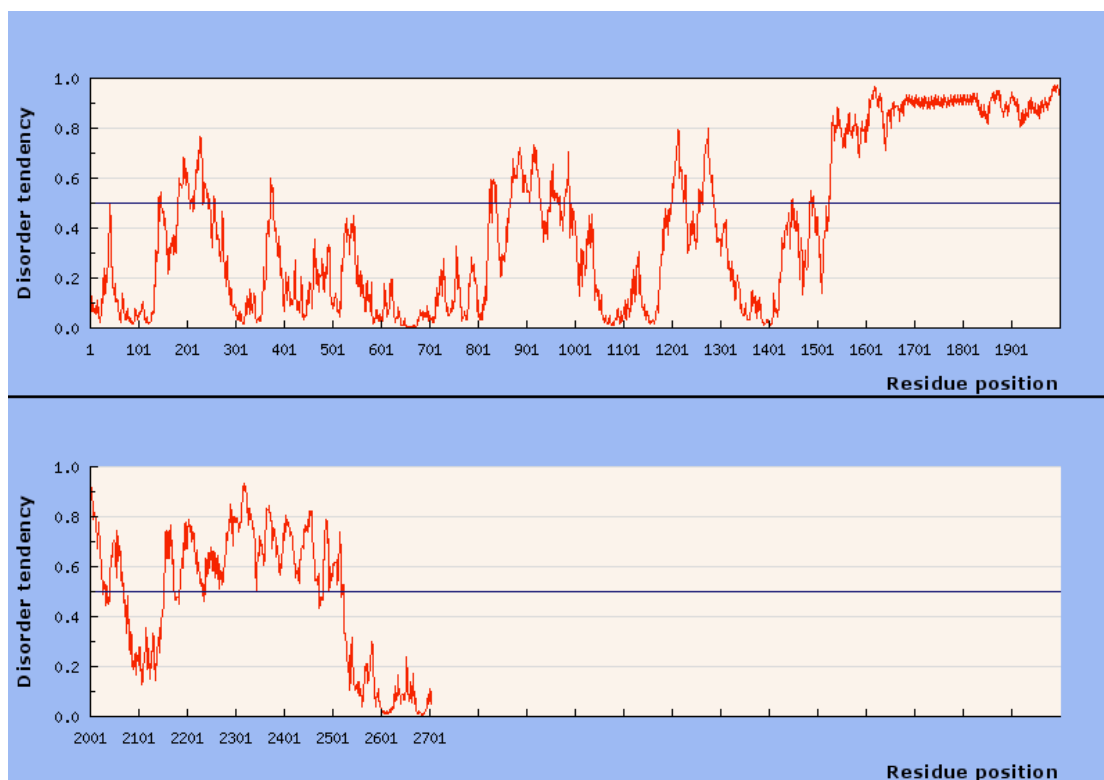
Figure 7. IUPred disorder prediction of EJW02257.1.
1605 to 1962 aminoacid residues show disorder tendency score higher than 0.8,
indicating high degree of disorder.
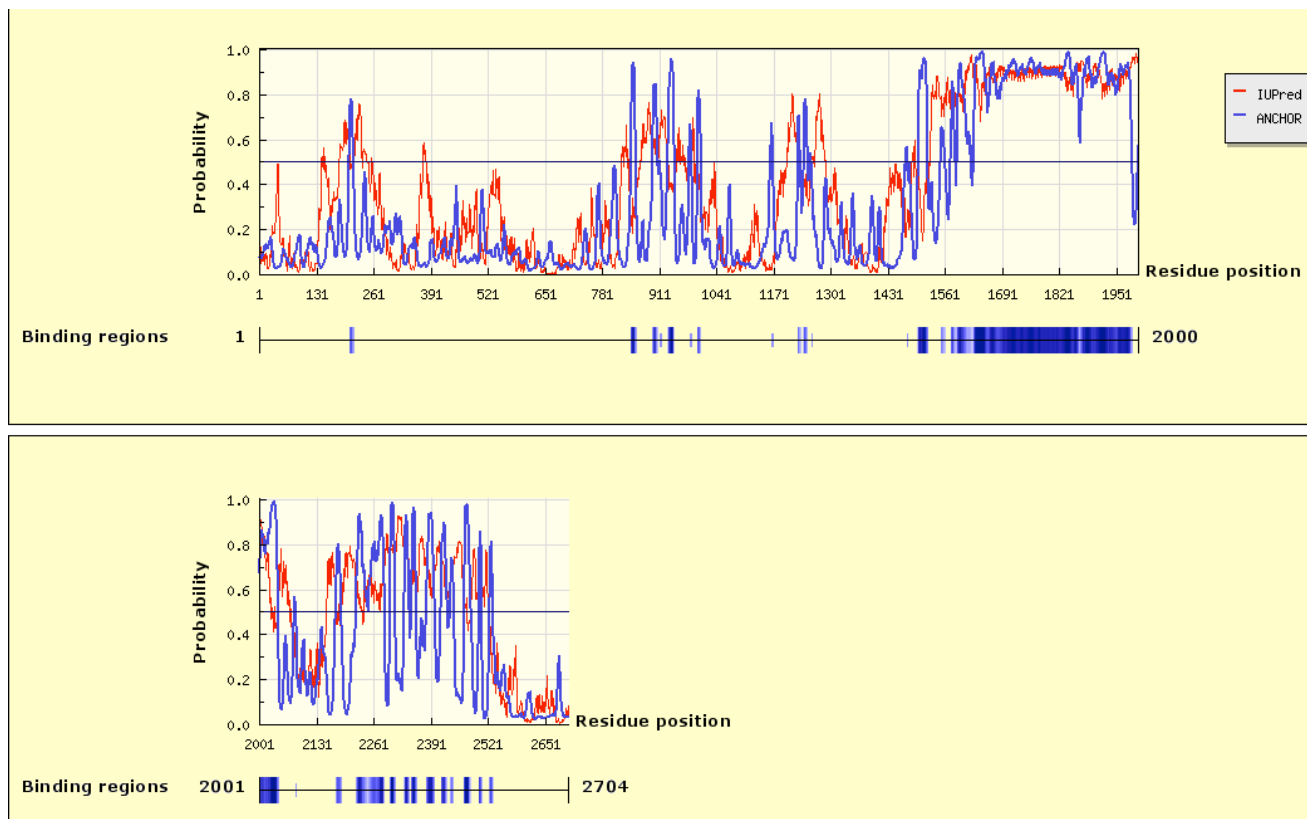
Figure 8. Anchor prediction of EJW02257.1.
Disordered binding sites below the curves are showed by blue bars. Blue bars means sites that are predicted to be prone to bind to another protein.
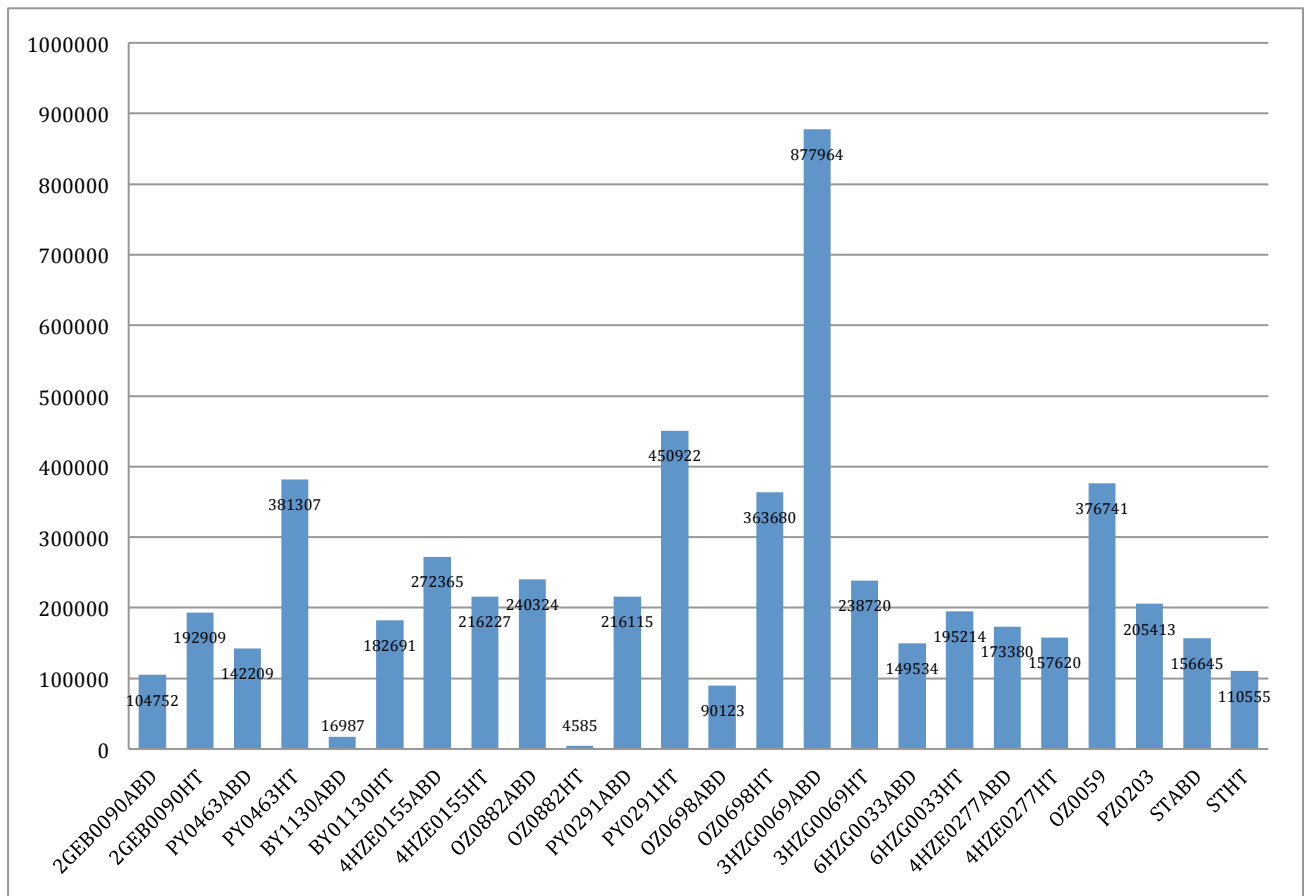
Figure 9. Number of readings identified in samples and controls.
X=identification of the sample. Y= Number of readings identified through Illumina®
sequencing.
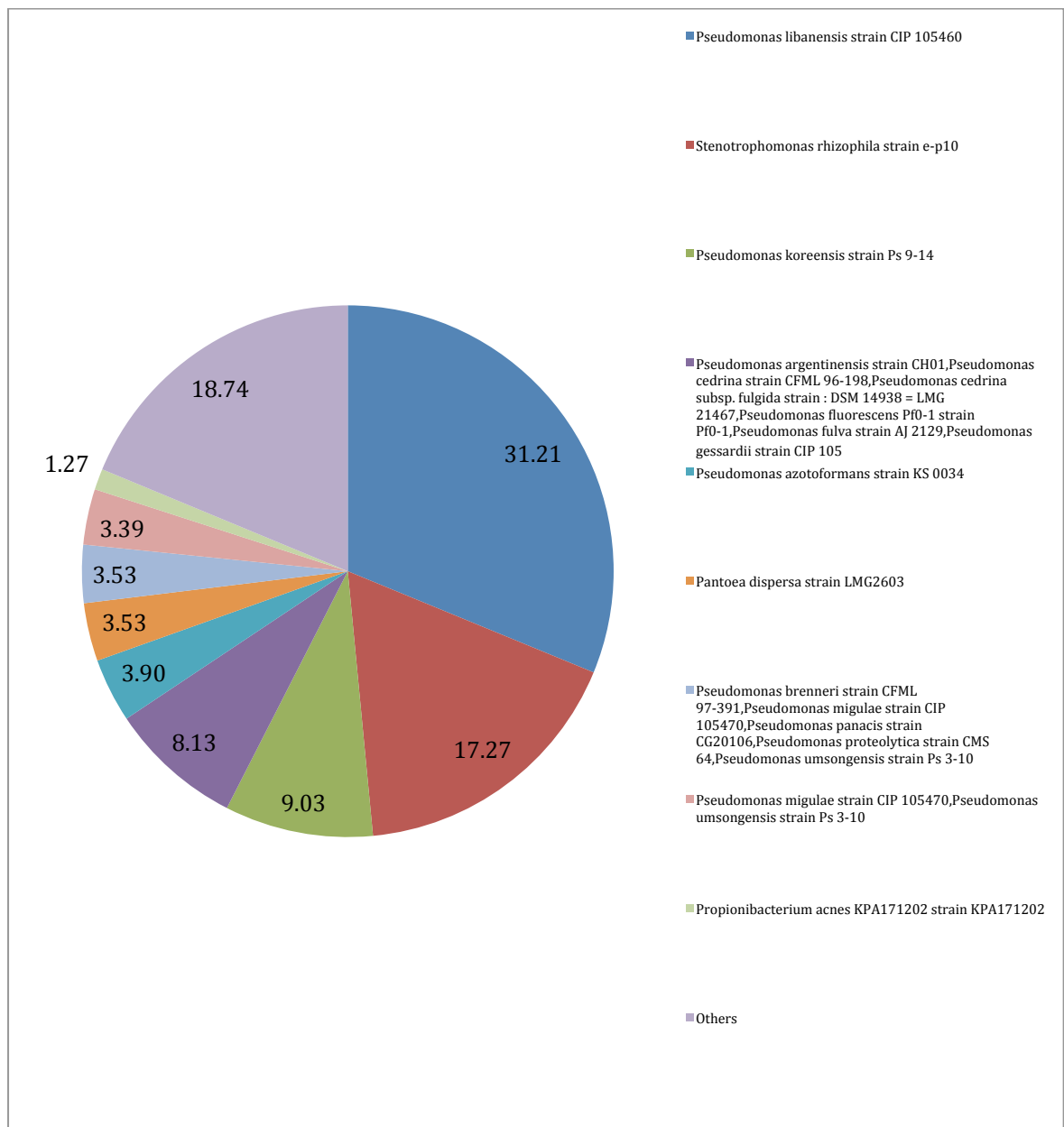
Figure 10. Bacterial composition of abdomen portions of False positive samples identified by Next Generation Sequencing.

Several species in each piece of the chart correspond to readings that match with more than one bacterial species.

Figure 11. Bacterial composition of False Positive samples in head/thorax portion identified by Next Generation Sequencing.

Several species in each piece of the chart correspond to readings that match with more than one bacterial species.
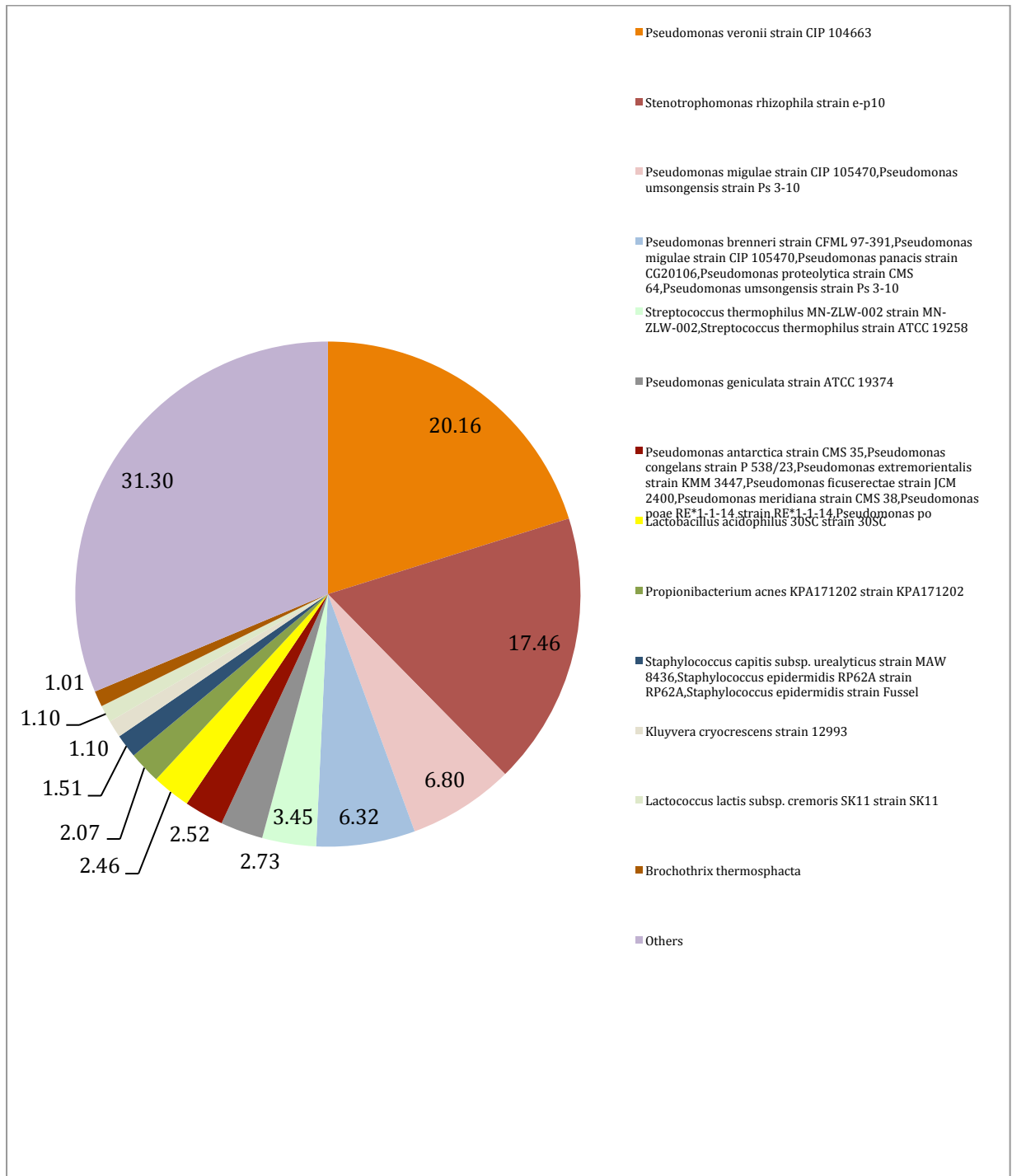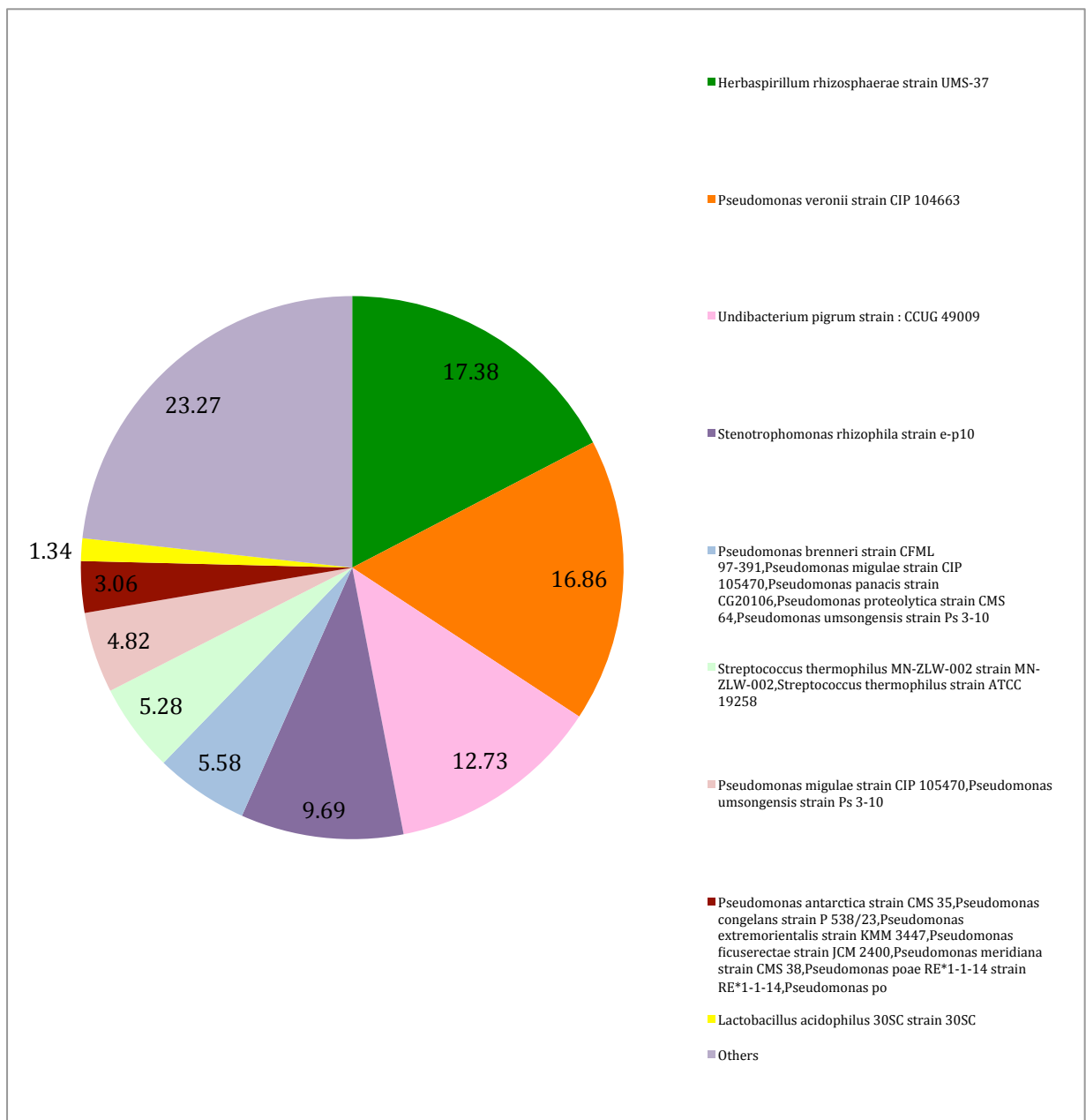
Figure 12. Bacterial composition of True Negatives identified by Next Generation Sequencing.

Several species in each piece of the chart correspond to readings that match with more than one bacterial species.

Figure 13. Bacterial composition of *An. stephensi* in abdomen portions identified by Next Generation Sequencing.
Several species in each piece of the chart correspond to readings that match with more than one bacterial specie.
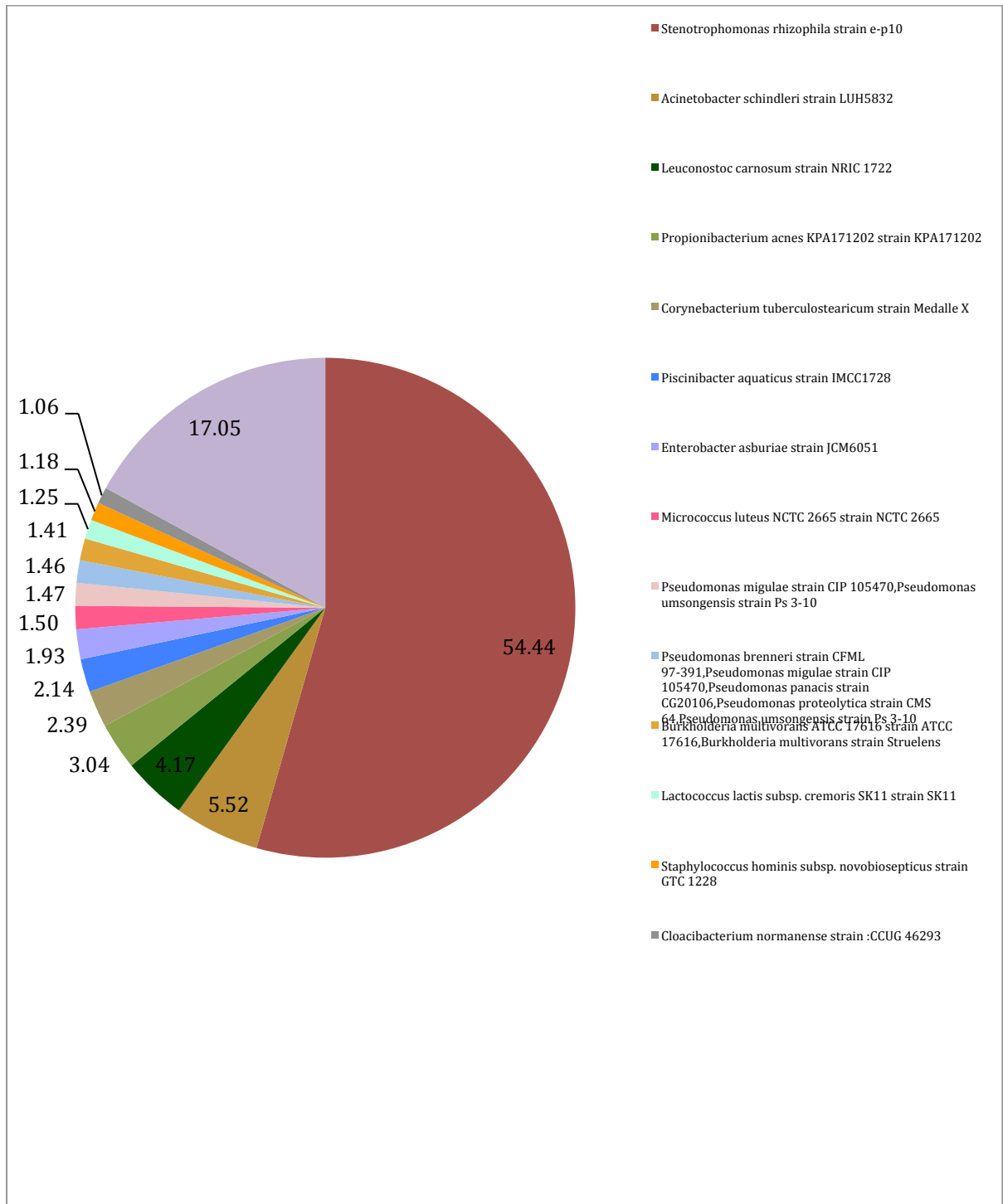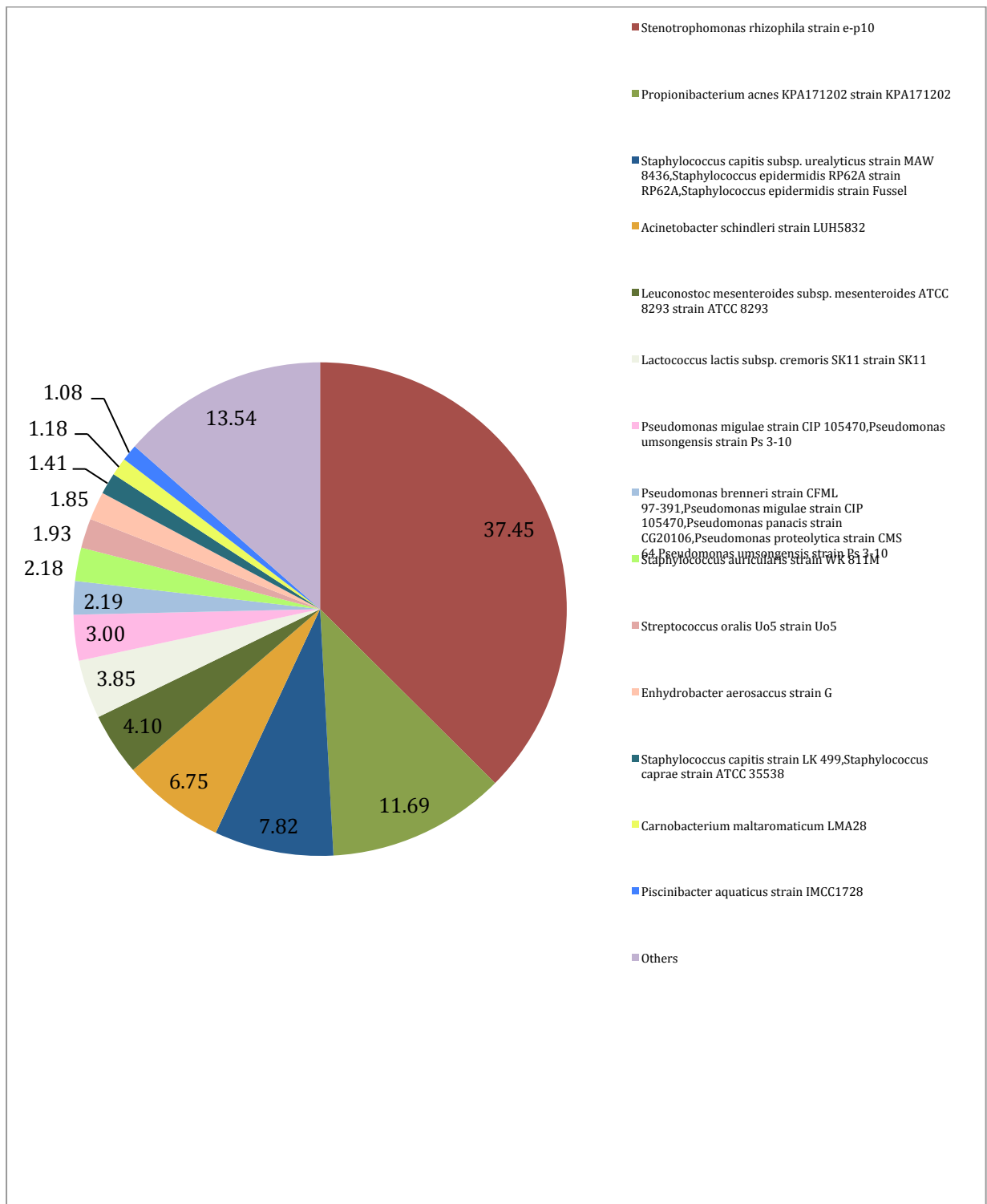
Figure 14. Species composition of *An. stephensi* head/thorax portion identified by Next Generation Sequencing.
Several species in each piece of the chart correspond to readings that match with more than one bacterial species.

Figure 15. Western Blot of the false positives samples pool.
 Lane 1: Ladder, Lane 2: False positive pool, Lane 3: Negative control, Lane 4: Positive control.

Figure 16. SDS gel stained with Coomassie Blue.
Lane 1: Positive control, Lane 2: Negative control, Lane 3: pool of samples. Lane 4: Ladder. Red arrows indicate bands present in the sample and not in the controls.

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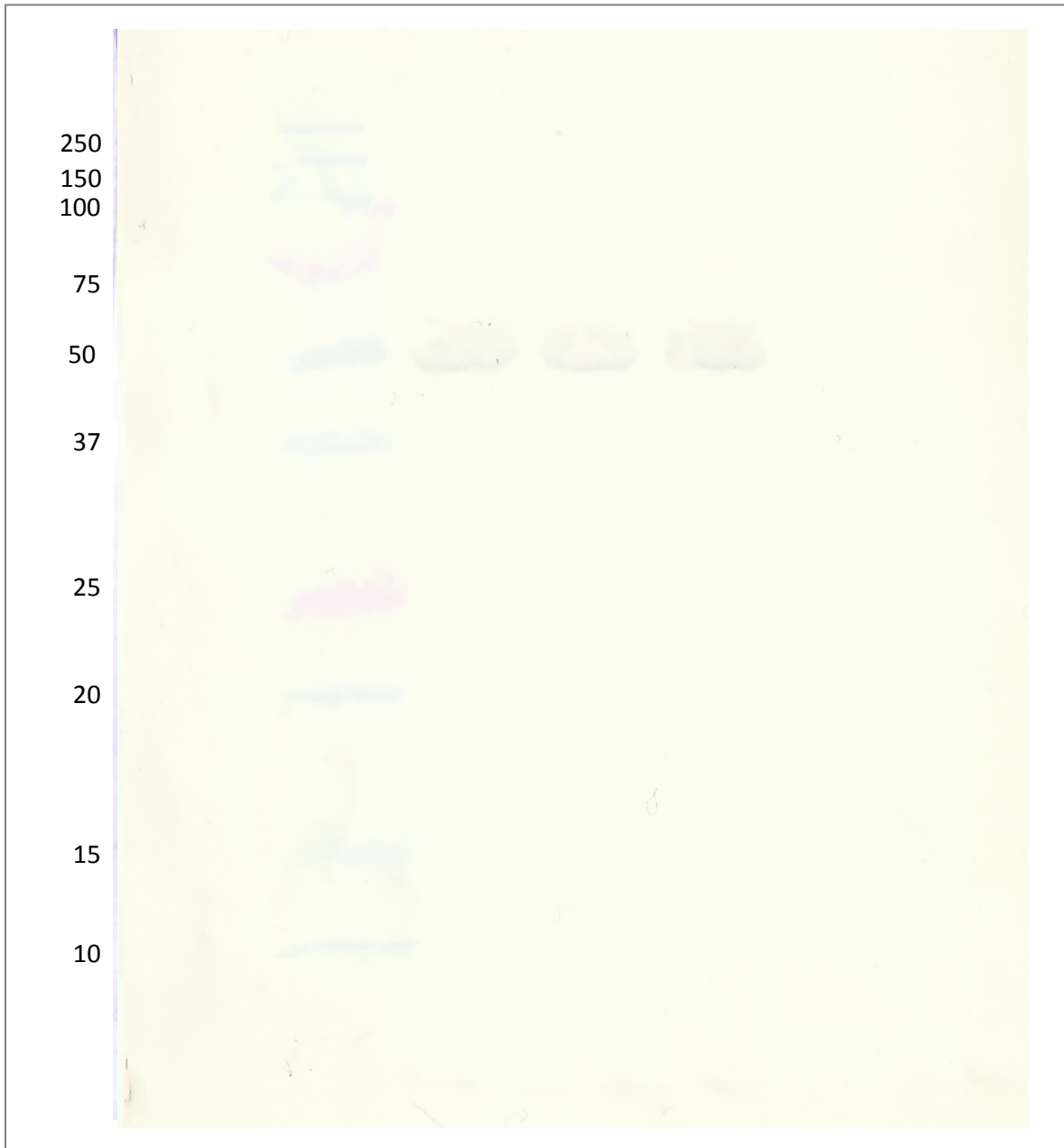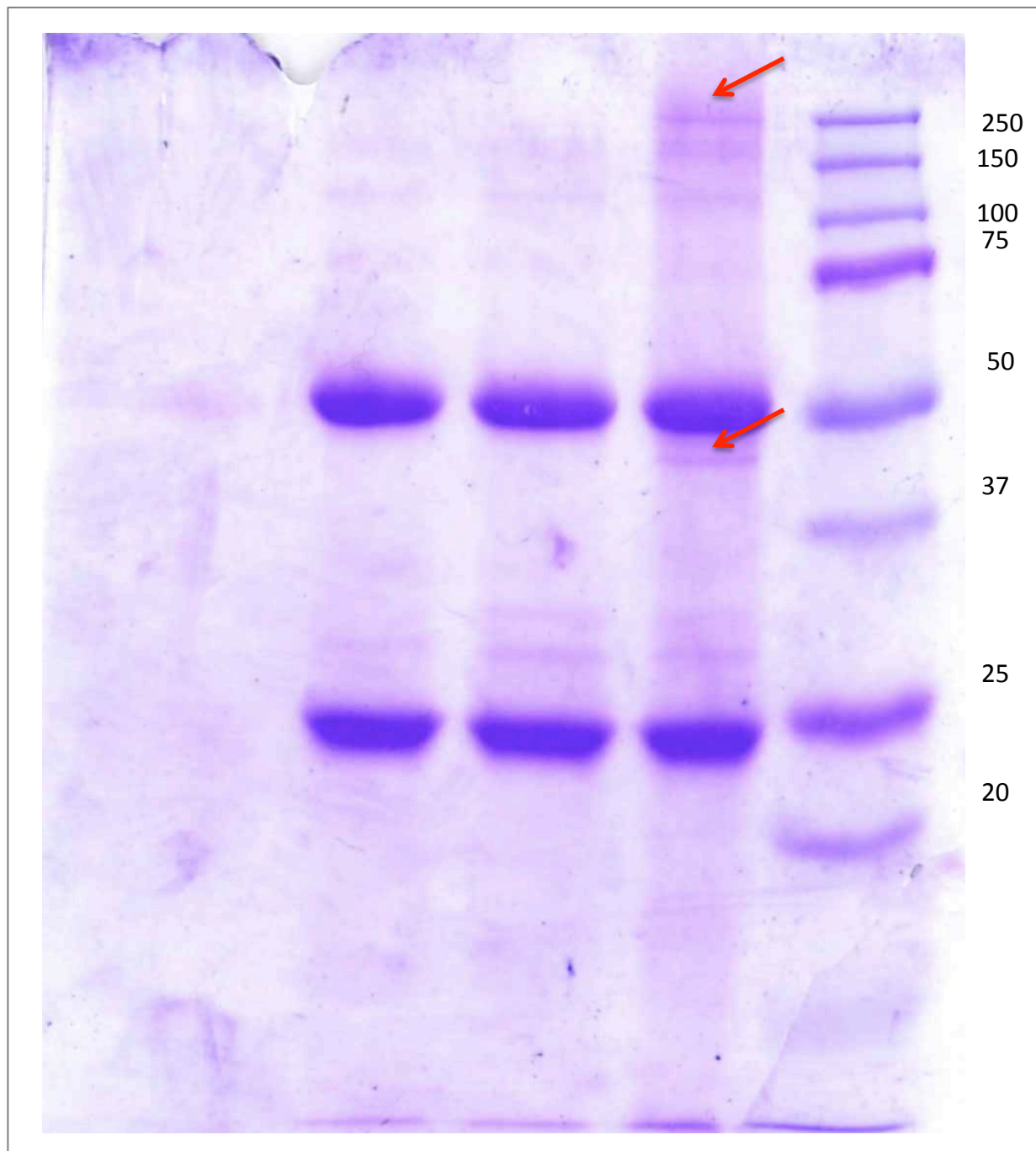
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gi|6636344|gb|AAF20152.1|AF209    AP--LNPKANREKMTQIMFETFNTPAMYVAIQAVLSLYGSGRTTGIVMDS  62
gi|194376310|dbj|BAG62914.1|      AP--LNPKANREKMTQIMFETFNTPAMYVAIQAVLSLYASGRTTGIVMDS 127
gi|62897625|dbj|BAD96752.1|       AP--LNPKANREKMTQIMFETFNTPAMYVAIQAVLSLYASGRTTGIVMDS 155
gi|62897409|dbj|BAD96645.1|       AP--LNPKANREKMTQIMFETFNTPAMYVAIQAVLSLYASGRTTGIVMDS 155
gi|14485767|gb|AAK63074.1|        AP--LNPKANREKMTQIMFETFNTPAMYVAIQAVLSLYASGRTTGIVMDS  79
gi|10442727|gb|AAG17452.1|        AP--LNPKANREKMTQIMFETFNTPAMYVAIQAVLSLYASGRTTGIVMDS 155
gi|158668333|gb|ABW76683.1|       --------------------------------------------------
gi|499050836|ref|XP_004575933.    AP--LNPKANREKMTQIMFETFNTPAMYVAIQAVLSLYASGRTTGIVMDS 155
gi|187968772|gb|ACD44579.1|       AP--LNPKANREKMTQIMFETFNTPAMYVAIQAVLSLYASGRTTGIVLDS  63
gi|11191964|dbj|BAB18035.1|       AP--LNPKANREKMTQIMFETFNTPAMYVAIQAVLSLYASGRTTGIVMDS  92
gi|311222955|gb|ADP76556.1|       AP--LNPKANREKMTQIMFETFNTPAMYVAIQAVLSLYASGRTTGIVMDS 151
gi|478497115|ref|XP_004422896.    AP--LNPKINREKM---MFEAFNTPAMYVAIQAVLSLYASGRTTGIVMDS 111
gi|478497113|ref|XP_004422895.    AP--LNPKINREKM---MFEAFNTPAMYVAIQAVLSLYASGRTTGIVMDS 153
gi|410948613|ref|XP_003981025.    AP--LNPKINREKMTQIMFEAFNTPAMYVAIQAVLSLYASGRTTGIVMDS 156
gi|403267534|ref|XP_003925881.    AP--LNPKINREKMTQIMFEAFNTPAMYVAIQAVLSLYASGRTTGIVMDS 156
gi|402871595|ref|XP_003899742.    AP--LNPKINREKMTQIMFEAFNTPAMYVAIQAVLSLYASGRTTGIVMDS 156
gi|505801926|ref|XP_004608507.    AP--LNPKINREKMTQIMFEAFNTPAMYVAIQAVLSLYASGRTTGIVMDS 114
gi|505801923|ref|XP_004608506.    AP--LNPKINREKMTQIMFEAFNTPAMYVAIQAVLSLYASGRTTGIVMDS 156
gi|8895917|gb|AAF81173.1|         AP--LNPKANREKMTQIMFETFNTPAMYVAIQAVLSLYASGRTTGIVMDS  66
gi|8895853|gb|AAF81141.1|         AP--LNPKANREKMTQIMFETFNAPAMYVAIQAVLSLYASGRTTGIVLDS  66
gi|8895901|gb|AAF81165.1|         AP--LNPKANREKMTQIMFETFNAPAMYVAIQAVLSLYASGRTTGIVLDS  66
gi|8895795|gb|AAF81112.1|         AP--LNPKGNREKMTQIMFETFNAPAMYVSIQAVLSLYASGRTTGIVLDS  66
gi|156388857|ref|XP_001634709.    AP--LNPKANREKMTQIMFETFNSPAMYVAIQAVLSLYASGRTTGIVMDS 155
gi|84873373|gb|ABC67746.1|        --------------------------------------------------
gi|303306184|gb|ADM13664.1|       AP--LNPKANREKMTQIMFETFNSPAMYVAIQAVLSLYASGRTTGIVFDS 156
gi|38176182|gb|AAR13014.1|        AP--LNPKANREKMTQIMFETFNSPAMYVAIQAVLSLYASGRTTGIVFDS 156
gi|1703130|sp|P53473.1|ACTB_ST    AP--LNPKANREKMTQIMFETFNSPAMYVAIQAVLSLYASGRTTGIVFDS 156
gi|60391982|gb|AAX19287.1|        AP--LNPKANREKMTQIMFETFNSPAMYVAIQAVLSLYASGRTTGIVLDS 155
gi|405974534|gb|EKC39169.1|       AP--LNPKANREKMTQIMFETFNSPAMYVAIQAVLSLYASGRTTGIVLDS 156
gi|6693629|dbj|BAA89429.1|        AP--LNPKANREKMTQIMFETFNCPAMYVAIQAVLSLYASGRTTGIVLDS 155
gi|319893880|gb|ADV76250.1|       AP--LNPKANREKMTQIMFETFNCPAMYVAIQAVLSLYASGRTTGIVMDS 155
gi|157106887|ref|XP_001649530.    AP--LNPKANREKMTQIMFETFNSPAMYVAIQAVLSLYASGRTTGIVLDS 156
gi|187282496|ref|NP_001119777.    AP--LNPKANREKMTQIMFETFNAPAMYVAIKAVLSLYASGRTTGIAXXX 156
gi|260785919|ref|XP_002588007.    AP--LNPKSNREKMTQIMFETFNSPAMYVNIQAVLSLYASGRTTGIVMDS 155
gi|432875503|ref|XP_004072874.    AP--LNPKANREKMTQVMFETFNSPAMYVAIQAVLSLYSSSRVTGIVMES 152
gi|160215|gb|AAA29554.1|          ANPNANPNANPNANPNANPNANPNANPNANPNANPNANPNANPNANPNAN 200
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gi|12697220|emb|CAC28248.1|    GDGVTHTVPIYEGYALPHAILRLDLAG-RDLTEYLMKILMERGYSFNTSA 117
gi|12697246|emb|CAC28270.1|    GDGVTHTVPIYEGYALPHAILRLDLAG-RDLTDYLMKILMERGYSFSTSA 117
gi|209402327|gb|ACI45913.1|    GDGVTHTVPIYEGYALPHAILRLDLAG-RDLTDYLMKILMERGYSFSTTA 117
gi|55982464|gb|AAV69654.1|     GDGVTHVVPIYEGFALPHAISRVDMAG-RDLTDYLMKILAERGYTFSTTA 117
gi|169865532|ref|XP_001839365. GDGVTHTVPIYEGFALPHAILRLDLAG-RDLTDYLIKCLGERGYPFTTTA 204
gi|393228332|gb|EJD35981.1|    GDGVTHTVPIYEGFALPHAILRLDLAG-RDLTDYLIKILMERGYPFTTTA 204
gi|505756334|gb|EOR00494.1|    GDGVSHTVPIYEGYALPHAIIRLDLAG-RDLTDYLIKILMERGYPFTTTA 204
gi|6636344|gb|AAF20152.1|AF209 GDGVTHTVPIYEGYALPHAILRLDLAG-RDLTDYLMKILTERGYSFTTTP 111
gi|194376310|dbj|BAG62914.1|   GDGVTHTVPIYEGYALPHAILRLDLAG-RDLTDYLMKILTERGYSFTTTA 176
gi|62897625|dbj|BAD96752.1|    GDGVTHTVPIYEGYALPHAILRLDLAG-RDLTDYLMKILTERGYSFTTTA 204
gi|62897409|dbj|BAD96645.1|    GDGVTHTVPIYEGYALPHAILRLDLAG-RDLTDYLMKILTERGYSFTTTA 204
gi|14485767|gb|AAK63074.1|     GDGVTHTVPIYEGYALPHAILRLDLAG-RDLTDYLMKILTERGYSFTTTA 128
gi|10442727|gb|AAG17452.1|     GDGVTHTVPIYEGYALPHAILRLDLAG-RDLTDYLMKILTERGYSFTTTA 204
gi|158668333|gb|ABW76683.1|    --------------------------------------------------
gi|499050836|ref|XP_004575933. GDGVSHTVPIYEGYALPHAILRLDLAG-RDLTDYLMKILTERGYSFTTTA 204
gi|187968772|gb|ACD44579.1|    GDGVSHTVPIYEGYALPHAILRLDLAG-RDLTDYLMKILTERGYSFTTTA 112
gi|11191964|dbj|BAB18035.1|    GDGVTHTVPIYEGYALPHAILRLDLAG-RDLTDYLMKILTERGYSFTTTA 141
gi|311222955|gb|ADP76556.1|    GDGVTHTVPIYEGYALPHAILRLDLAG-RDLTDYLMKILTERGYSFTTTA 200
gi|478497115|ref|XP_004422896. GDGVTHTVPIYEGYALPHAILRLDLAG-RDLTDYLMKILTERGYNFTTTA 160
gi|478497113|ref|XP_004422895. GDGVTHTVPIYEGYALPHAILRLDLAG-RDLTDYLMKILTERGYNFTTTA 202
gi|410948613|ref|XP_003981025. GDGVTHTVPIYEGYALPHAILRLDLAG-RDLTDYLMKILTERGYNFTTTA 205
gi|403267534|ref|XP_003925881. GDGVTHTVPIYEGYALPHAILRLDLAG-RDLTDYLMKILTERGYNFTTTA 205
gi|402871595|ref|XP_003899742. GDGVTHTVPIYEGYALPHAILRLDLAG-RDLTDYLIKILTERGYNFTTTA 205
gi|505801926|ref|XP_004608507. GDGVTHTVPIYEGYALPHAILRLDLAG-RDLTDYLMKILTERGYNFTTTA 163
gi|505801923|ref|XP_004608506. GDGVTHTVPIYEGYALPHAILRLDLAG-RDLTDYLMKILTERGYNFTTTA 205
gi|8895917|gb|AAF81173.1|      GDGVTHTVPIYEGYALPHAILRLDLAG-RDMTDYLMKIMTERGYSFTTTA 115
gi|8895853|gb|AAF81141.1|      GDGVTHTVPIYEGYALPHAILRLDLAG-RDLTDYLMKILTERGYSFTTTA 115
gi|8895901|gb|AAF81165.1|      GDGVTHTVPIYEGYALPHAILRLDLAG-RDLTDYLVKILTERGYSFTTTA 115
gi|8895795|gb|AAF81112.1|      GDGVSHTVPIYEGYALPHAILRLDLAG-RDLTDYLMKILTERGYSFTTTA 115
gi|156388857|ref|XP_001634709. GDGVTHTVPIYEGYALPHAIIRLDLAG-RDLTDYLMKILTERGYSFTTTA 204
gi|84873373|gb|ABC67746.1|     --------------------------------------------------
gi|303306184|gb|ADM13664.1|    GDGVSHTVPIYEGYALPHAILRLDLAG-RDLTDYLMKILTERGYSFTTTA 205
gi|38176182|gb|AAR13014.1|     GDGVSHTVPIYEGYALPHAILRLDLAG-RDLTDYLMKILTERGYSFTTTA 205
gi|1703130|sp|P53473.1|ACTB_ST GDGVSHTVPIYEGYALPHAILRLDLAG-RDLTDYLMKILTERGYSFTTTA 205
gi|60391982|gb|AAX19287.1|     GDGVSHTVPIYEGYALPHAIMRLDLAG-RDLTDYLMKILTERGYSFTTTA 204
gi|405974534|gb|EKC39169.1|    GDGVSHTVPIYEGYALPHAIMRLDLAG-RDLTDYLMKILTERGYSFTTTA 205
gi|6693629|dbj|BAA89429.1|     GDGVSHTVPIYEGYALPHAILRLDLAG-RDLTDYLMKILTERGYSFTTTA 204
gi|319893880|gb|ADV76250.1|    GDGVSHTVPIYEGYALPHAILRLDLAG-RDLTDYLMKILTERGYSFTTTA 204
gi|157106887|ref|XP_001649530. GDGVSHTVPIYEGYALPHAILRLDLAG-RDLTDYLMKILTERGYSFTTTA 205
gi|187282496|ref|NP_001119777. GDGVTHTVPIYEGYALPHAILRLDLAG-RDLTDYLMKILTERGYSFTTTA 205
gi|260785919|ref|XP_002588007. GDGVTHTVPIYEGYALPHAIERLDLAG-RDLTDNLMKILTERGYGLVNSA 204
gi|432875503|ref|XP_004072874. GDGVSQIVPIYEGKVLTHATIRLDLAG-RDLTDYLMKILTERGYRFTPTA 201
gi|160215|gb|AAA29554.1|       PNANPNANPNVDPNANPNANPNANPNANPNANPNANPNANPNANPNANPN 250


gi|12697220|emb|CAC28248.1|    EREIVRDIKEKLCYVALDFEQEMQTAASSSSALEKSYELPDGQVITIGNER 167
gi|12697246|emb|CAC28270.1|    EREIVRDIKEKLCYVALDFEQEMQTAVSSSSALEKSYELPDGQVITIGNER 167
gi|209402327|gb|ACI45913.1|    EREIVRDIKEKLCYVALDFEQEMQTAASSSSALEKSYELPDGQVITIGNER 167
gi|55982464|gb|AAV69654.1|     EREIVRDIKEKLCYVALDFEQEIQTASQSSSLEKSYELPDGQVITIGNER 167
gi|169865532|ref|XP_001839365. EREIVRDIKEKLCYVALDFEQEMQTAAQSSSSLEKSYELPDGQVITIGNER 254
gi|393228332|gb|EJD35981.1|    EREIVRDIKEKLCYVALDFEQEMQTAAQSSSALEKSYELPDGQVITIGNER 254
gi|505756334|gb|EOR00494.1|    EREIVRDIKERLCYVALDFEQELQTANQSSALEKSYELPDGQVITIGNER 254
gi|6636344|gb|AAF20152.1|AF209 EREIVRDIKEKLSYVALDFEQEMATAASSSSLEKSYELPDGQVITIGNER 161
gi|194376310|dbj|BAG62914.1|   EREIVRDIKEKLCYVALDFEQEMATAASSSSLEKSYELPDGQVITIGNER 226
gi|62897625|dbj|BAD96752.1|    EREIVRDIKEKLCYVALDFEQEMATAASSSSLEKSYELPDGQVITIGNER 254
gi|62897409|dbj|BAD96645.1|    EREIVRDIKEKLCYVALDFEQEMATAASSSSLEKSYELPDGQVITIGNER 254
gi|14485767|gb|AAK63074.1|     EREIVRDIKEKLCYVALDFEQEMATAASSSSLEKSYELPDGQVITIGNER 178
gi|10442727|gb|AAG17452.1|     EREIVRDIKEKLCYVALDFEQEMGTAASSSSLEKSYELPDGQVITIGNER 254
gi|158668333|gb|ABW76683.1|    ---------------------QEMGTAASSSSLEKSYELPDGQVITIGNER 30
gi|499050836|ref|XP_004575933. EREIVRDIKEKLCYVALDFEQEMQTAASSSSLEKSYELPDGQVITIGNER 254
gi|187968772|gb|ACD44579.1|    EREIVRDIKEKLCYVALDFEQEMATAASSSSLEKSYELPDGQVITIGNER 162
gi|11191964|dbj|BAB18035.1|    EREIVRDIKEKLCYVALDFEQEMATAASSSSLEKSYELPDGQVITIGNER 191
gi|311222955|gb|ADP76556.1|    EREIVRDIKEKLCYVALDFEQEMATAASSSSLEKSYELPDGQVITIGNER 250
gi|478497115|ref|XP_004422896. EREIVRDVKEKLCYVALDFQQEMVSAATSSSSLERSYELPDGQVITIGNER 210
gi|478497113|ref|XP_004422895. EREIVRDVKEKLCYVALDFQQEMVSAATSSSSLERSYELPDGQVITIGNER 252
gi|410948613|ref|XP_003981025. EREIVRDVKEKLCYVALDFEQEMVSASASSSLERSYELPDGQVITIGNER 255
gi|403267534|ref|XP_003925881. EREIVRDVKEKLCYVALDFAQEMVSAVASSSLERSYELPDGQVITIGNER 255
gi|402871595|ref|XP_003899742. EREIVRDVKEKLCYVALDFEQEMVRAAASSSLERSYELPDGQVITIGNER 255
gi|505801926|ref|XP_004608507. EREIVRDVKEKLCYVALDFEQEMVNAAASSALEKSYELPDGQVITIGNER 213
gi|505801923|ref|XP_004608506. EREIVRDVKEKLCYVALDFEQEMVNAAASSALEKSYELPDGQVITIGNER 255
gi|8895917|gb|AAF81173.1|      EREIVRDIKEKLCYVALDFDQEMQMAASSSSLEKSYELPDGQVITIGNER 165
gi|8895853|gb|AAF81141.1|      EREIVRDIKEKLCYVALDFEQEMATAASSSSLEKSYELPDGQVITIGNER 165
gi|8895901|gb|AAF81165.1|      EREIVRDIKEKLCYVALDFEQEMATAASSSSLEKSYELPDGQVITIGNER 165
gi|8895795|gb|AAF81112.1|      EREIVRDIKEKLCYVALDFEQEMATAASSSSLEKSYELPDGQVITIGNER 165
gi|156388857|ref|XP_001634709. EREIVRDIKEKLCYVALDFEQEMETAASSSSLEKSYELPDGQVITIGNER 254
gi|84873373|gb|ABC67746.1|     --------------------------------SLEKSYELPDGQVITIGNER 20
gi|303306184|gb|ADM13664.1|    EREIVRDIKEKLCYVALEFDQEMQTAASSSSLEKSYELPDGQVITIGNER 255
gi|38176182|gb|AAR13014.1|     EREIVRDIKEKLNYVALDFEQEMQTAASSSSLEKSYELPDGQVITIGNER 255
gi|1703130|sp|P53473.1|ACTB_ST EREIVRDIKEKLCYVALDFEQEMQTAASSSSLEKSYELPDGQVITIGNER 255
gi|60391982|gb|AAX19287.1|     EREIVRDIKEKLAYIALDFEQEMQTAASSSSLEKSYELPDGQVITIGNER 254
gi|405974534|gb|EKC39169.1|    EREIVRDIKEKLCYVALDFEQEMQTAASSSSLEKSYELPDGQVITIGNER 255
gi|6693629|dbj|BAA89429.1|     EREIVRDIKEKLSYVALDFEQEMQTAAQSSNLEKSYELPDGQVITIGNER 254
gi|319893880|gb|ADV76250.1|    EREIVRDIKEKLCYVGLDFEQEMQTAASSSSLEKSYELPDGQVITIGNER 254
gi|157106887|ref|XP_001649530. EREIVRDIKEKLCYVALDFEQEMATAAASTALEKSYELPDGQVITIGNER 255
gi|187282496|ref|NP_001119777. EREIVRDIKEKLCYTALNFEREMATAAASSSLEKSYELPDGQVITIGNER 255
gi|260785919|ref|XP_002588007. EREIVRDIKEKLCYAALDFEQEMQTAASSSSLEKSYELPDGQVITIGNER 254
gi|432875503|ref|XP_004072874. EHEIVREIKEKLCYVALDFEQEMQTAASSSSLEKSYELPDGQVITIGNER 251
gi|160215|gb|AAA29554.1|       ANPNANPNANPNANPNANPNANPNANPNANPNANPNANPNANPNANPNAN 300
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gi|12697220|emb|CAC28248.1|      FRA---PEALFQPSLLGLEGVGIHETTFNSIKKGDVDIRRDLYGNIVMSG 214
gi|12697246|emb|CAC28270.1|      FRA---PEALFQPSFIGLESVGIHETTFNSIMKGDVDIRRDLYGNIVMSG 214
gi|209402327|gb|ACI45913.1|      FRA---PEALFQPTLLGLEAVGVHETTYNSIMKGDVDIRRDLYGNIVMSG 214
gi|55982464|gb|AAV69654.1|       FRA---PEALFQPSVLGLESGGIHVTTFNSIMKCDVDVRKDLYGNIVMSG 214
gi|169865532|ref|XP_001839365.   FRA---PEALFQPAFLGLEAAGIHETTYNSIFKCDLDIRRDLYGNVVLSG 301
gi|393228332|gb|EJD35981.1|      FRA---PEALFQPSFLGLEASGIHETTYNSIFKCDLDIRRDLYGNVVLSG 301
gi|505756334|gb|EOR00494.1|      FRA---PEGLFKPSLLGLEAAGIHETTYNSIMKCDLDIRRDLYGNVVMSG 301
gi|6636344|gb|AAF20152.1|AF209   FRC---PEALFQPSFLGMESCGIHETTFNSIMKCDVDIRRDLYANTVLSG 208
gi|194376310|dbj|BAG62914.1|     FRC---PEALFQPSFLGMESCGIHETTFNSIMKCDVDIRRDLYANTVLSG 273
gi|62897625|dbj|BAD96752.1|      FRC---PEALFQPSFLGMESCGIHETTFNSIMKCDVDIRRDLYANTVLSG 301
gi|62897409|dbj|BAD96645.1|      FRC---PEALFQPSFLGMESCGIHETTFNSIMKCDVDIRRDLYANTVLSG 301
gi|14485767|gb|AAK63074.1|       FRC---PEALFQPSFLGMESCGIHETTYNSIMKCDVDIRRDLYANTVLSG 225
gi|10442727|gb|AAG17452.1|       FRC---PEALFQPSFLGMESCGIHETTFNSIMKCDVDIRRDLYANTVLSG 301
gi|158668333|gb|ABW76683.1|      FRC---PEALFQPSFLGMESCGIHETTFNSIMKCDVDIRRDLYANTVLSG 77
gi|499050836|ref|XP_004575933.   FRC---PEALFQPSFLGMESAGIHETTYNSIMKCDVDIRRDLYANTVLSG 301
gi|187968772|gb|ACD44579.1|      FRC---PEALFQPSFLGMESCGIHETTYNSIMKCDVDIRRDLYANTVLSG 209
gi|11191964|dbj|BAB18035.1|      FRC---PEALFQPSFLGMESCGIHETTFNSIMKCDVDIRRDLYANTVLSG 238
gi|311222955|gb|ADP76556.1|      FRA---PEALFQPSFLGMEISGIHETTFNSIMKCDVDIRRDLYANTVLSG 297
gi|478497115|ref|XP_004422896.   FRC---PEAIFQPSFLGIESNGIHETTFNSIMKCDVDIRRDLYANTVLSG 257
gi|478497113|ref|XP_004422895.   FRC---PEAIFQPSFLGIESNGIHETTFNSIMKCDVDIRRDLYANTVLSG 299
gi|410948613|ref|XP_003981025.   FRC---PESIFQPSFLGIESSGIHETTFNSIMKCDVDIRRDLYANTVLSG 302
gi|403267534|ref|XP_003925881.   FRC---PEAIFQPSFLGIESSGIHETTFNSIMKCDVDIRRDLYANTVLSG 302
gi|402871595|ref|XP_003899742.   FRC---PEAIFQPSFLGIESSGIHETTFNSIMKCDVDIRRDLYANTVLSG 302
gi|505801926|ref|XP_004608507.   FRC---PEAIFQPSFLGIESGIHEATFNSIMKCDVDIRRDLYANTVLSG 260
gi|505801923|ref|XP_004608506.   FRC---PEAIFQPSFLGIESSGIHEATFNSIMKCDVDIRRDLYANTVLSG 302
gi|8895917|gb|AAF81173.1|        FRA---PEATFQPSFLGMESAGIHETTYNSIMKCDVDIRRDLYANTVLSG 212
gi|8895853|gb|AAF81141.1|        FRC---PESLFQPSFLGMESAGIHETTYNSIMKCDVDIRRDLYANTVLSG 212
gi|8895901|gb|AAF81165.1|        FRC---PESLFQPSFLGMESAGIHETTYNSIMKCDVDIRRDLYANTVLSG 212
gi|8895795|gb|AAF81112.1|        FRS---PESMFQPSFLGMESSGIHETTYNSIMKCDVDIRRDLYANTVLSG 212
gi|156388857|ref|XP_001634709.   FRC---PEAMFQPSFLGMESAGIHETTYNSIMKCDVDIRRDLYANTVLSG 301
gi|84873373|gb|ABC67746.1|       FRC---PEAMFQPSFLGMESAGIHETCYNSIMKCDVDIRRDLYANTVLSG 67
gi|303306184|gb|ADM13664.1|      FRA---PEAMFQPSFLGMESAGIHETTYNSIMKCDVDIRRDLYANTVLSG 302
gi|38176182|gb|AAR13014.1|       FRA---PEAMFQPSFLGMESAGIHETTYNSIMKCDVDIRRDLYATTVLSG 302
gi|1703130|sp|P53473.1|ACTB_ST   FRC---PEALFQPAFLGMESAGIHETCYNSIMKCDVDIRRDLYANTVLSG 302
gi|60391982|gb|AAX19287.1|       FRC---PEALFQPSFLGMESAGIHETTYNSIMKCDVDIRRDLYANTVLSG 301
gi|405974534|gb|EKC39169.1|      FRC---PEALFQPSFLGMESSGIHETTYNSIMKCDVDIRRDLYANTVLSG 302
gi|6693629|dbj|BAA89429.1|       FRC---PETLFQPSFIGMESAGIHETTYNSIMKCDVDIRRDLYANTVLSG 301
gi|319893880|gb|ADV76250.1|      FRC---PETLFQPSFIGMESAGVHETTFNSIMKCDVDIRRDLYANTVLSG 301
gi|157106887|ref|XP_001649530.   FRC---PESLFQPSFLGMESCGIHETVYNSIMKCDVDIRRDLYANIVMSG 302
gi|187282496|ref|NP_001119777.   FRC---PETLFQPAFIGMESAGIHETTYNSIMKCDIDIRRDLYANTVLSG 302
gi|260785919|ref|XP_002588007.   FRC---PETLFQPSFIGMESVGIHELTYNSIMKCDIDIRRDLYANTVLSG 301
gi|432875503|ref|XP_004072874.   FRC---PEALFQPPFLGIEIAGIHEMTYNSIMKCNVDIHENLYANMVLSG 298
gi|160215|gb|AAA29554.1|         PNANPNANPNANPNANPNKNNQGNGQGHNMPNDPNRNVDENANANNAVKN 350

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gi|12697220|emb|CAC28248.1|      GTTMYPG---IADRMQKEITAIAPSSMKIKIVAP-------PE---RKYSV 252
gi|12697246|emb|CAC28270.1|      GTTMYPG---IADRMQKEITAIAPSSMKIKIVAP-------PE---RKYSV 252
gi|209402327|gb|ACI45913.1|      GTTMYPG---IADRMQKEITAIAPSSMKVEIVAP-------PE---RKYSV 252
gi|55982464|gb|AAV69654.1|       GTTMYPG---ISDRMQKEVTAIAPSSMKVKIIAP-------PE---RKYSV 252
gi|169865532|ref|XP_001839365.   GTTMFSG---IADRMQKELLAISPASMRVKIVAP-------LE---RKYSV 339
gi|393228332|gb|EJD35981.1|      GTTMYPG---IADRMQKELTSISPSSVKVKIVAP-------PE---RKYSV 339
gi|505756334|gb|EOR00494.1|      GTTMYHG---IPDRMQKELTGIAPSSMKVKIVAP-------PE---RKYSV 339
gi|6636344|gb|AAF20152.1|AF209   GTTMYPG---IADRMQKEITAIAPSTMKIKIAP--------PE---RKYSV 246
gi|194376310|dbj|BAG62914.1|     GTTMYPG---IADRMQKEITAIAPSTMKIKIIAP-------PE---RKYSV 311
gi|62897625|dbj|BAD96752.1|      GTTMYPG---IADRMRKEITAIAPSTMKIKIIAP-------PE---RKYSV 339
gi|62897409|dbj|BAD96645.1|      GTTMYPG---IADRMQKEITAIAPSTMKIKIIAP-------PE---RKYSV 339
gi|14485767|gb|AAK63074.1|       GTTMYPG---IADRMQKEITAIAPSTMKIKIIAP-------PE---RKYSV 263
gi|10442727|gb|AAG17452.1|       GTTMYPG---IADRMQKEITSIVPSTMKIKIIAP-------PE---RKYSV 339
gi|158668333|gb|ABW76683.1|      GTTMYPG---IADRMQKEITAIAPTTMKIKI------------------- 105
gi|499050836|ref|XP_004575933.   GTTMYPG---IADRMQKEITAIAPPTMKIKVCYLDLLS-SPRAALQEFVE 347
gi|187968772|gb|ACD44579.1|      GTTMYPG---IADRMQKEITAIAPSTMKIIAP------------------ 240
gi|11191964|dbj|BAB18035.1|      GTTMYPG---IADRMQKEITAIAPSTMKIKIAP--------PE---RKYSV 276
gi|311222955|gb|ADP76556.1|      GTTMYPG---IADRMQKEITAIAPSTMKIKIAP--------PE---RKYSV 335
gi|478497115|ref|XP_004422896.   GSTMYPG---IADRMQKEIVTIAPGTMKIKIIAP-------PE---RKYSV 295
gi|478497113|ref|XP_004422895.   GSTMYPG---IADRMQKEIVTIAPGTMKIKIIAP-------PE---RKYSV 337
gi|410948613|ref|XP_003981025.   GSTMYPG---IADRMQKEIVTIAPSTMKIKIIAP-------PE---RKYSV 340
gi|403267534|ref|XP_003925881.   GSTMYPG---IADRMQKEIITIAPSNMKIKIIAP-------PE---RKYSV 340
gi|402871595|ref|XP_003899742.   GSTMYPG---IADRMQKEITTIAPSTMKIKIIAP-------PE---RKYSV 340
gi|505801926|ref|XP_004608507.   GSTMYPG---IADRMQKEIVIIAPGTMKIKIIAP-------PE---RKYSV 298
gi|505801923|ref|XP_004608506.   GSTMYPG---IADRMQKEIVIIAPGTMKIKIIAP-------PE---RKYSV 340
gi|8895917|gb|AAF81173.1|        GSTMFPG---IADRMQKEISSIAPPTMKIIAP--------PE---RKYSV 250
gi|8895853|gb|AAF81141.1|        GTTMFPG---IADRMQKEIISIAPSTIKVKIIAP-------PE---RKYSV 250
gi|8895901|gb|AAF81165.1|        GTTMFPG---IADRMQKEITSIAPSTMKIKIIAP-------PE---RKYSV 250
gi|8895795|gb|AAF81112.1|        GTTMFPG---IADRMQKEITAIAPTTMKIKIIAP-------PE---RKYSV 250
gi|156388857|ref|XP_001634709.   GSTMYPG---IADRMQKEITSIAPPTMKIKIIAP-------PE---RKYSV 339
gi|84873373|gb|ABC67746.1|       GSTMYPG---IADRMQKEIANIAPPTMKIKIIAP-------PE---RKYSV 105
gi|303306184|gb|ADM13664.1|      GSTMFPG---IADRMQKEITAIAPPTMKIKIIAP-------PE---RKYSV 340
gi|38176182|gb|AAR13014.1|       GSTMFPG---IAARMQKEITAIAPPTMKIKIIAP-------PE---RKYSV 340
gi|1703130|sp|P53473.1|ACTB_ST   GSTMFPG---IADRMQKEITAIAPPTMKIKIIAP-------PE---RKYSV 340
gi|60391982|gb|AAX19287.1|       GTTMFPG---IADRMQKEITAIAPATMKIKIIAP-------PE---RKYSV 339
gi|405974534|gb|EKC39169.1|      GTTMYPG---IADRMQKEITSIAPQTMKIKVIAP-------PE---RKYSV 340
gi|6693629|dbj|BAA89429.1|       GTTMYPG---IADRMQKEITAIAPPTMKIKIIAP-------PE---RKYSV 339
gi|319893880|gb|ADV76250.1|      GTTMYPG---IADRMQKEITAIAPPTMKIKIIAP-------PE---RKYSV 339
gi|157106887|ref|XP_001649530.   GTTMYPG---IADRMQKEITAIAPSTIKIKIIAP-------PE---RKYSV 340
gi|187282496|ref|NP_001119777.   GTSMYPG---IADRMQKEITAIAPSSVKIKIIAP-------PE---RKYSV 340
gi|260785919|ref|XP_002588007.   GTSMFPG---IADRMQKEITAIAPSTMKIKIIAP-------PE---RKYSV 339
gi|432875503|ref|XP_004072874.   GTTMLSG---LADRMQKEITAIAPPTMNIDISAP------PD---RKYSV 336
gi|160215|gb|AAA29554.1|         NNNEEPSDKHIEQYLKKIQNSISTEWSPCSVTCG---NG------IQVRI 391

                                 ...   .   :   ::*  *   .      .:
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```
gi|12697220|emb|CAC28248.1|      WIGGS------------ILASLSTFQQMW-------------------- 269
gi|12697246|emb|CAC28270.1|      WIGGS------------ILASLSTFQQMW-------------------- 269
gi|209402327|gb|ACI45913.1|      WIGGS------------ILASLSTFQQMW-------------------- 269
gi|55982464|gb|AAV69654.1|       WIGGS------------ILASLSTFQQMWISKQEYDESG--PSIV----- 283
gi|169865532|ref|XP_001839365.   WIGGS------------ILASLSTFQNLWCSKQEYDESG--PGIVHRKCF 375
gi|393228332|gb|EJD35981.1|      WIGGS------------ILASLSTFQNLWCSKQEYDESG--PGIVHRKCF 375
gi|505756334|gb|EOR00494.1|      WIGGS------------ILASLSTFQQMWISKQEYDESG--PSIVHRKCF 375
gi|6636344|gb|AAF20152.1|AF209   WIGGS------------ILASLSTFQQMWI-------------------- 264
gi|194376310|dbj|BAG62914.1|     WIGGS------------ILASLSTFQQMWISKQEYDESG--PSIVHRKCF 347
gi|62897625|dbj|BAD96752.1|      WIGGS------------ILASLSTFQQMWISKQEYDESG--PSIVHRKCF 375
gi|62897409|dbj|BAD96645.1|      WIGGS------------VLASLSTFQQMWISKQEYDESG--PSIVHRKCF 375
gi|14485767|gb|AAK63074.1|       WIGGS------------ILASLSTFQQMWI-------------------- 281
gi|10442727|gb|AAG17452.1|       WIGGS------------ILASLSTFQQMWVSKQEYDESG--PSIVHRKCF 375
gi|158668333|gb|ABW76683.1|      -------------------------------------------------
gi|499050836|ref|XP_004575933.   WCDSSTLELNVSNTKEMVVTPLTLFTLHFNGSPLGGASAAFPAGLRRRRS 397
gi|187968772|gb|ACD44579.1|      -------------------------------------------------
gi|11191964|dbj|BAB18035.1|      WIGGS------------ILASL--------------------------- 286
gi|311222955|gb|ADP76556.1|      WIGGS------------ILASLSTFQQMWI-------------------- 353
gi|478497115|ref|XP_004422896.   WIGGS------------ILASLSTFQQMWISKQEYDEAG--PPIAHRKCF 331
gi|478497113|ref|XP_004422895.   WIGGS------------ILASLSTFQQMWISKQEYDEAG--PPIAHRKCF 373
gi|410948613|ref|XP_003981025.   WIGGS------------ILASLSTFQQMWISKQEYDEAG--PPIVHRKCF 376
gi|403267534|ref|XP_003925881.   WIGGS------------ILASLSTFQQMWISKQEYDEAG--PPIVHRKCF 376
gi|402871595|ref|XP_003899742.   WIGGS------------ILASLSTFQQMWISKQEYDEAG--PPIVHRKCF 376
gi|505801926|ref|XP_004608507.   WIGGS------------ILASLSTFQQMWISKQEYDEAG--PPIVHRKCF 334
gi|505801923|ref|XP_004608506.   WIGGS------------ILASLSTFQQMWISKQEYDEAG--PPIVHRKCF 376
gi|8895917|gb|AAF81173.1|        WIGGS------------ILASLS--------------------------- 261
gi|8895853|gb|AAF81141.1|        WIGGS------------ILASLS--------------------------- 261
gi|8895901|gb|AAF81165.1|        WIGGS------------ILASLS--------------------------- 261
gi|8895795|gb|AAF81112.1|        WIGGS------------ILASLS--------------------------- 261
gi|156388857|ref|XP_001634709.   WIGGS------------ILASLSTFQQMWISKQEYDESG--PSIVHRKCF 375
gi|84873373|gb|ABC67746.1|       WIGGS------------ILASLSTFQQMWISKQEYDESG--PS------- 134
gi|303306184|gb|ADM13664.1|      WIGGS------------ILASLSTFQQMWISKQEYDESG--PSIVHRKCF 376
gi|38176182|gb|AAR13014.1|       WIGGS------------ILASLSTFQQMWISKQEYDESG--PSIVRRKCF 376
gi|1703130|sp|P53473.1|ACTB_ST   CIGGS------------ILASLSTFQQMWISKQEYDESG--PSIVHRKCF 375
gi|60391982|gb|AAX19287.1|       WIGGS------------ILASLSTFQQMWISKQEYDESG--PSIVHRKCF 375
gi|405974534|gb|EKC39169.1|      WIGGS------------ILASLSTFQQMWISKQEYDESG--PSIVHRKCF 376
gi|6693629|dbj|BAA89429.1|       WIGGS------------ILASLSTFQQMWISKQEYDESG--PSIVHRKCF 375
gi|319893880|gb|ADV76250.1|      WIGGS------------ILASLSTFQQMWISKQEYDESG--PSIVHRKCF 375
gi|157106887|ref|XP_001649530.   WIGGS------------ILASLSTFQQMWISKQEYDESG--PGIVHRKCF 376
gi|187282496|ref|NP_001119777.   WIGGS------------ILASLSTFQQMWISKQEYXXSG--ASIVHRKCF 376
gi|260785919|ref|XP_002588007.   WIGGS------------ILGSLSTFQQMWISKQEYDESG--PSIVHRKCF 375
gi|432875503|ref|XP_004072874.   WLGGS------------IMASLPTFQQMWISKQEYEESG--PGIVHRKCD 372
gi|160215|gb|AAA29554.1|         KPGSADKPKDQLDYENDIEKKICKMEKCSSVFNVVNSSIGLIMVLSFLFL 441
```

```
gi|12697220|emb|CAC28248.1|      ---------------
gi|12697246|emb|CAC28270.1|      ---------------
gi|209402327|gb|ACI45913.1|      ---------------
gi|55982464|gb|AAV69654.1|       ---------------
gi|169865532|ref|XP_001839365.   ---------------
gi|393228332|gb|EJD35981.1|      ---------------
gi|505756334|gb|EOR00494.1|      ---------------
gi|6636344|gb|AAF20152.1|AF209   ---------------
gi|194376310|dbj|BAG62914.1|     ---------------
gi|62897625|dbj|BAD96752.1|      ---------------
gi|62897409|dbj|BAD96645.1|      ---------------
gi|14485767|gb|AAK63074.1|       ---------------
gi|10442727|gb|AAG17452.1|       ---------------
gi|158668333|gb|ABW76683.1|      ---------------
gi|499050836|ref|XP_004575933.   ATFVPKATLLFSSSR 412
gi|187968772|gb|ACD44579.1|      ---------------
gi|11191964|dbj|BAB18035.1|      ---------------
gi|311222955|gb|ADP76556.1|      ---------------
gi|478497115|ref|XP_004422896.   ---------------
gi|478497113|ref|XP_004422895.   ---------------
gi|410948613|ref|XP_003981025.   ---------------
gi|403267534|ref|XP_003925881.   ---------------
gi|402871595|ref|XP_003899742.   ---------------
gi|505801926|ref|XP_004608507.   ---------------
gi|505801923|ref|XP_004608506.   ---------------
gi|8895917|gb|AAF81173.1|        ---------------
gi|8895853|gb|AAF81141.1|        ---------------
gi|8895901|gb|AAF81165.1|        ---------------
gi|8895795|gb|AAF81112.1|        ---------------
gi|156388857|ref|XP_001634709.   ---------------
gi|84873373|gb|ABC67746.1|       ---------------
gi|303306184|gb|ADM13664.1|      ---------------
gi|38176182|gb|AAR13014.1|       ---------------
gi|1703130|sp|P53473.1|ACTB_ST   ---------------
gi|60391982|gb|AAX19287.1|       ---------------
gi|405974534|gb|EKC39169.1|      ---------------
gi|6693629|dbj|BAA89429.1|       ---------------
gi|319893880|gb|ADV76250.1|      ---------------
gi|157106887|ref|XP_001649530.   ---------------
gi|187282496|ref|NP_001119777.   ---------------
gi|260785919|ref|XP_002588007.   ---------------
gi|432875503|ref|XP_004072874.   ---------------
gi|160215|gb|AAA29554.1|         N-------------- 442
```

Figure 17. CLUSTALW2 alignment of the sequences retrieved from 45kDa protein identified through MALDI TOF/TOF and CSP from *P. falciparum*.

Asterisks denotes 100% conserved residues. Dots represent conserved but different residues. Double dots indicate less conserved substitutions. Red rectangles indicate positions of 100% conserved residues. Blue rectangles indicate conserved residues in CSP *P. falciparum* that could not be aligned with all the sequences due to gaps.

```
gi|187282496|ref|NP_001119777.   MCDEDVAALVVD-----------------NGSGMCKTGFAGDDAPRAVFP 33
gi|156388857|ref|XP_001634709.   -MDDDVAALVID-----------------NGSGMCKAGFAGDDAPRAVFP 32
gi|260785919|ref|XP_002588007.   -MSDEDIALVVD-----------------NGSGMCKAGFAGDDAPRAVFP 32
gi|160215|gb|AAA29554.1|          -MMRKLAILSVSSFLFVEALFQEYQCYGSSSNTRVLNELNYDNGTNLYN 49
                                          .  *  :.                      ...        :    *:::      ::

gi|187282496|ref|NP_001119777.   SIVG----------RPRHBGVMVGMGQK------DSYVGDEAQSKRGILT 67
gi|156388857|ref|XP_001634709.   SIVG----------RPRBQGVMVGMGQK------DSYVGDEAQSKRGILT 66
gi|260785919|ref|XP_002588007.   SLVG----------RPRYQPQMIGMGNK------DSYVGDEAQSKRGILT 66
gi|160215|gb|AAA29554.1|          ELEMNYYGKQENWYSLKKNSRSLGENDDGDNDNGNNNNGNNNNGDNGREG 99
                                   .:          :  : :  :*  .:.     :.    *:: :..*

gi|187282496|ref|NP_001119777.   LKYPIEBGIVTNWDDMEKIWHHTFYN----------ELRVAPEEHPVLLT 107
gi|156388857|ref|XP_001634709.   LKYPIEBGIVTNWDDMEKIWHHTFYN----------ELRVAPEEHPVLLT 106
gi|260785919|ref|XP_002588007.   LKYPIEBGVVTNWDDMEKIWHHTFYN----------ELRVAPEEHPVLLT 106
gi|160215|gb|AAA29554.1|          KDEDKRDGNNEDNEKLRKPKHKKLKQPGDGNPDPNANPNVDPNANPNVDP 149
                                   .    ..*:  :  :.:*    *:.: :           :  *: :*: : .

gi|187282496|ref|NP_001119777.   EAPLNPKANREKMTQIMFETFNAPAMYVAIKAVLSLYASGRTTGIAXXXG 157
gi|156388857|ref|XP_001634709.   EAPLNPKANREKMTQIMFETFNSPAMYVAIQAVLSLYASGRTTGIVMDSG 156
gi|260785919|ref|XP_002588007.   EAPLNPKSNREKMTQIMFETFNSPAMYVNIQAVLSLYASGRTTGIVMDSG 156
gi|160215|gb|AAA29554.1|          NANPNANPNANPNANPNANPNANPNANPNANPNANPNANPNANPNANPNA 199
                                   :*  *.:.*  :   ::     :.    *       :.   .  *.  .:. .    .

gi|187282496|ref|NP_001119777.   --DGVTHTVPIYEGYALPHAILRLDLAG-RDLTDYLMKILTERGYSFTTT 204
gi|156388857|ref|XP_001634709.   --DGVTHTVPIYEGYALPHAIIRLDLAG-RDLTDYLMKILTERGYSFTTT 203
gi|260785919|ref|XP_002588007.   --DGVTHTVPIYEGYALPHAIERLDLAG-RDLTDNLMRILTERGYGLVNS 203
gi|160215|gb|AAA29554.1|          NPNANPNANPNVDPNANPNANPNANPNANPNANPNANPNANPNANPNANP 249
                                   :.  .::  :  *  *:*  .:   .:    .    . ...

gi|187282496|ref|NP_001119777.   AEREIVRDIKEKLCYTALNFEREMATAAASSSLEKSYELPDGQVITIGNE 254
gi|156388857|ref|XP_001634709.   AEREIVRDIKEKLCYVALDFEQEMETAASSSSLEKSYELPDGQVITIGNE 253
gi|260785919|ref|XP_002588007.   AEREIVRDIKEKLCYAALDFEQEMATAAQSSSLEKSYELPDGQVITIGNE 253
gi|160215|gb|AAA29554.1|          NANPNANPNANPNANPNANPNANPNANPNANPNANPNANPNANPNANPNA 299
                                   .   ..    :  .      : : :  :..    :.      *:.:   :    *

gi|187282496|ref|NP_001119777.   RFRCP===ETLFQPAFIGMESAGIHETTYNSIMKCDIDIRKDLYANTVLS 301
gi|156388857|ref|XP_001634709.   RFRCP===EAMFQPSFLGMESAGIHETTYNSIMKCDVDIRKDLYANTVLS 300
gi|260785919|ref|XP_002588007.   RFRCP===ETLFQPSFIGMESVGIHELTYNSIMKCDIDIRKDLYANTVLS 300
gi|160215|gb|AAA29554.1|          NPNANPNANPNANPNANPNKNNQGNGQGHNMPNDPNRNVDENANANNAVK 349
                                   .  ..    :.   :*     :.    . : ::  :.     :  ::    **. .:.

gi|187282496|ref|NP_001119777.   GGTSMYPG===========IADRMQREITALAPSS======VRIKIIAPPER 336
gi|156388857|ref|XP_001634709.   GGSTMYPG===========IADRMQREITSLAPPT======MKIKIIAPPER 335
gi|260785919|ref|XP_002588007.   GGTSMFPG===========IADRMQREITALAPST======MKIKIIAPPER 335
gi|160215|gb|AAA29554.1|          NNNNEEPSDKHIEQYLKKIQNSLSTEWSPCSVTCGNGIQVRIKPGSADKP 399
                                   ....   *:          *  : :..*  :. :        :*** :. :

gi|187282496|ref|NP_001119777.   KYSVWIGGSILASLSTFQQMWISKQEYXXSGASIVHRKCF=== 376
gi|156388857|ref|XP_001634709.   KYSVWIGGSILASLSTFQQMWISKQEYDESGPSIVHRKCF=== 375
gi|260785919|ref|XP_002588007.   KYSVWIGGSILGSLSTFQQMWISKQEYDESGPSIVHRKCF=== 375
gi|160215|gb|AAA29554.1|          KDQLDYENDIEKKICKMEKCSSVFNVVNSSIGLIMVLSFLFLN 442
                                   *  .:   ..*   .:..:::             :     *     *:   .  :
```

Figure 18. CLUSTALW2 alignment of the sequences retrieved from MALDI TOF/TOF and CSP from P. falciparum that were also present in the BLAST search of CSP of *P. falciparum*. Asterisk denotes 100% conserved residues. Dots represent conserved but different residues. Double dots indicate less conserved substitutions. Red rectangles indicate positions of 100% conserved residues.