

Tryptic shaving of *Staphylococcus aureus* unveils immunodominant epitopes on the bacterial cell surface

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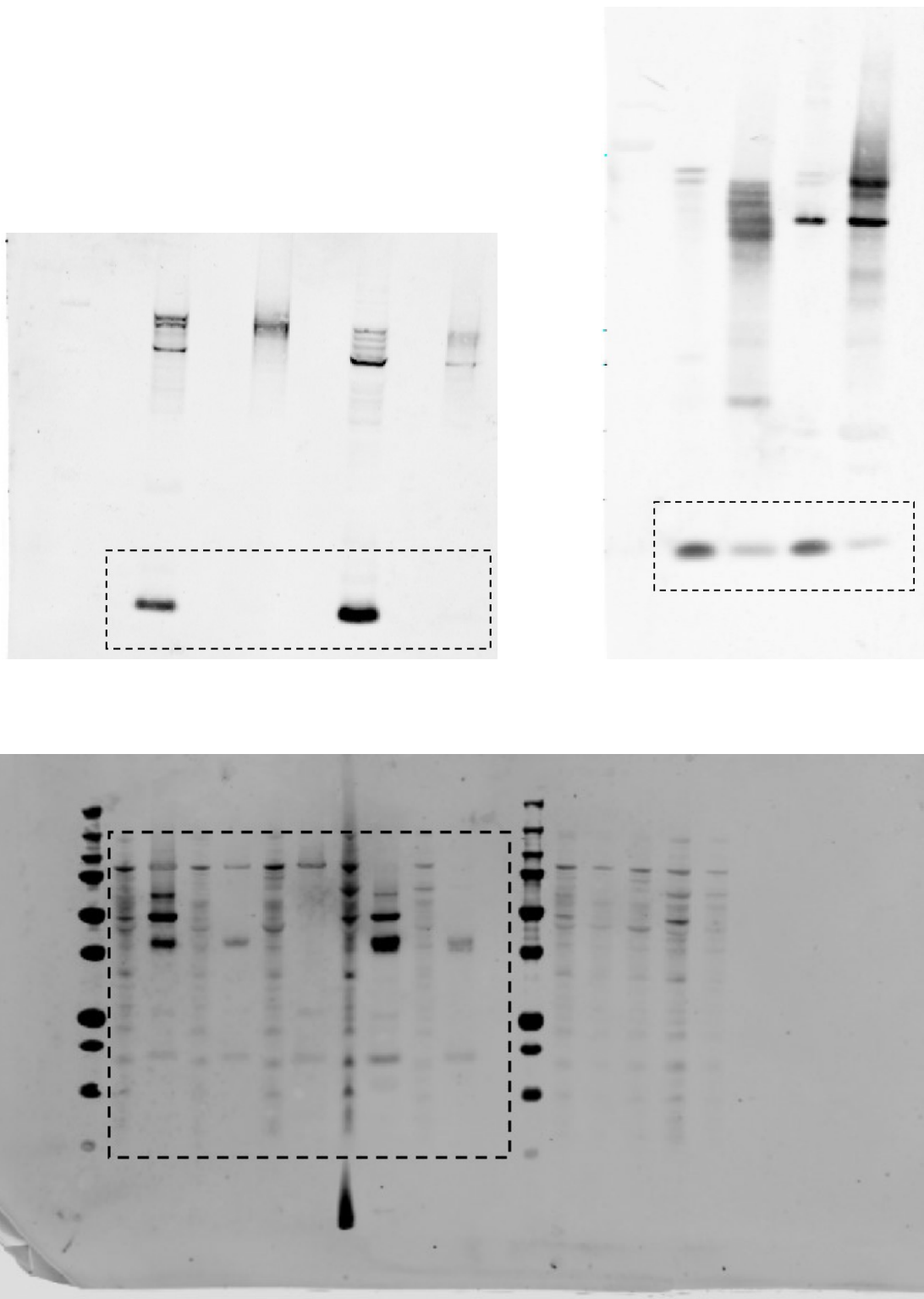
[†]Deceased

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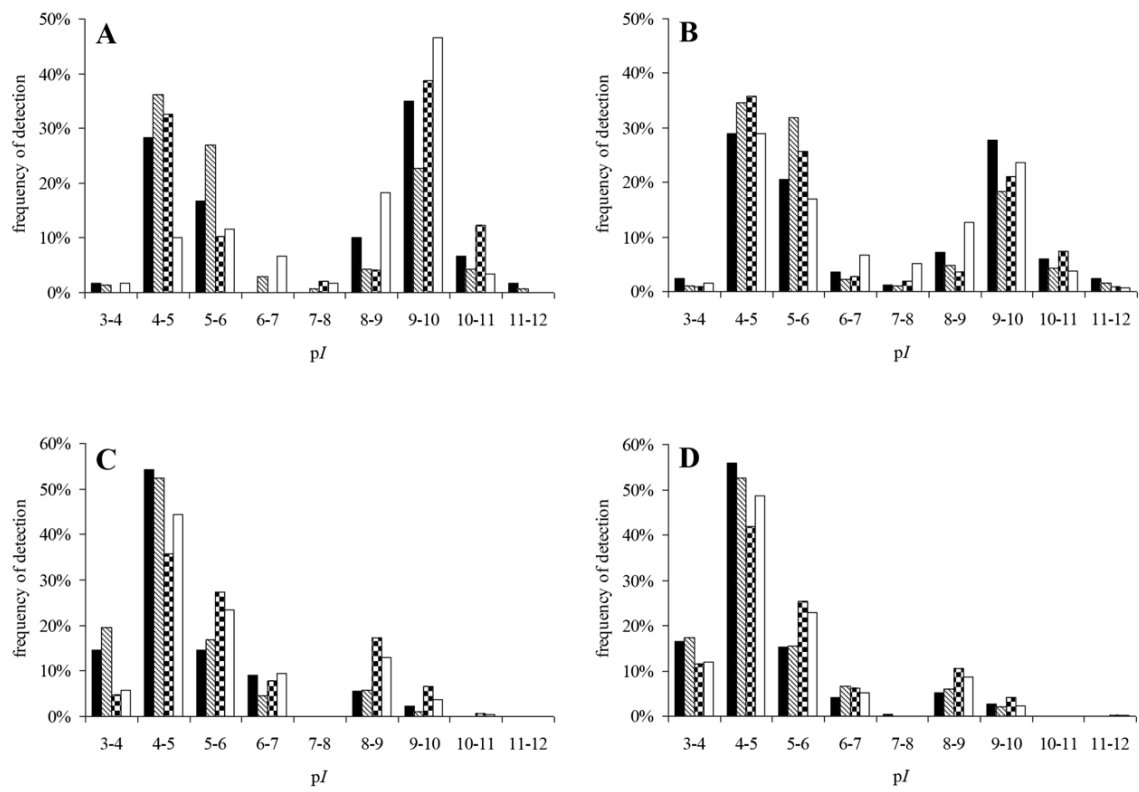
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Supplementary Figure S1.



Supplementary Figure S1. Original Western blots. Panel A shows the full blot used to generate Figures 3A, panel B shows the full blot used to generate Figure 3B, and panel C shows the full blot used to generate Figure 4B.

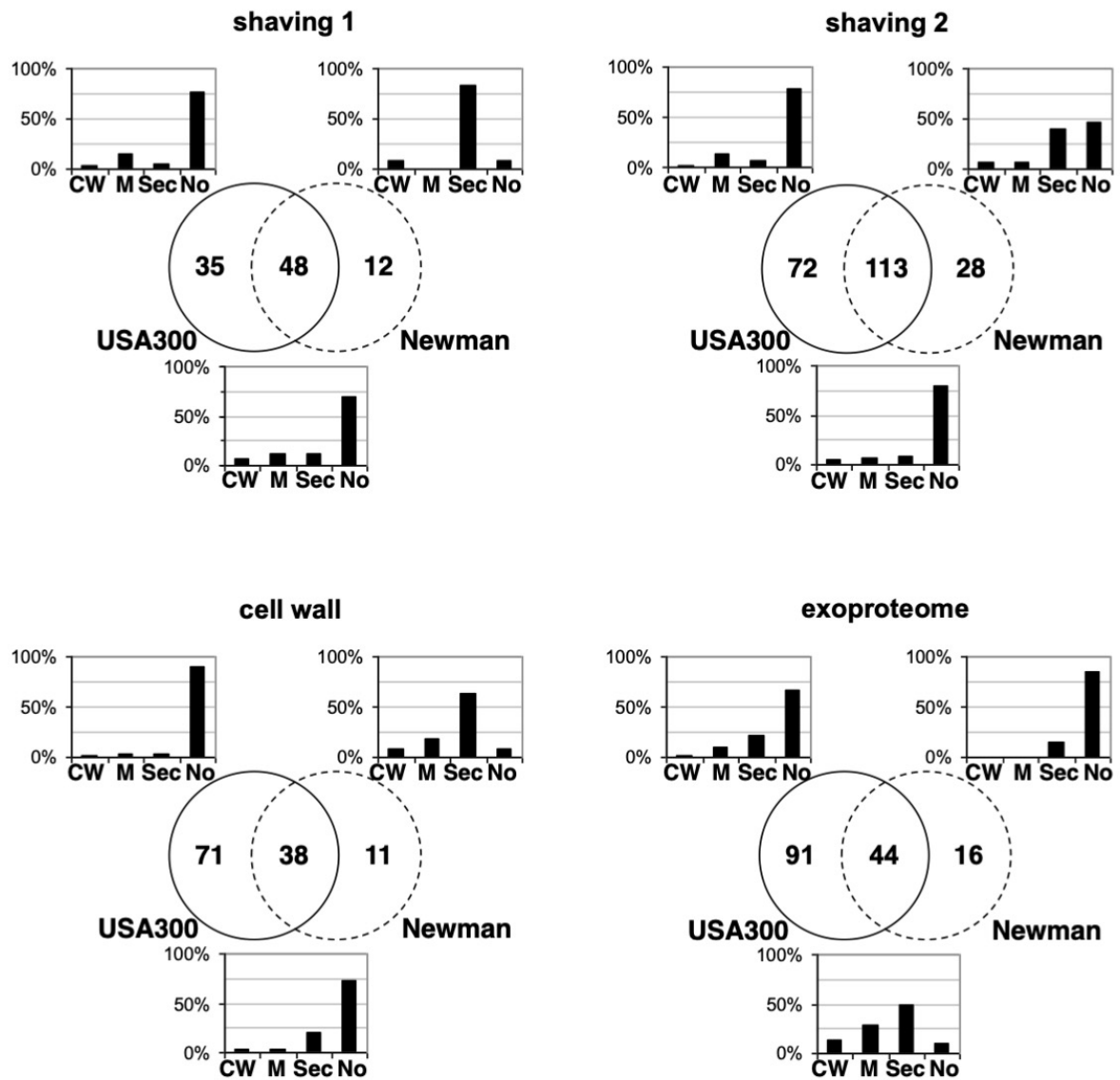
Supplementary Figure S2.



Supplementary Figure S2. Comparison of the pI values of identified proteins and peptides in the different sub-proteome fractions. The percentages of protein (A, B) and peptide (C, D) identifications with a certain pI are displayed for the different sub-proteome fractions of strains Newman (A, C) and USA300 (B, D).

■ shaving 1, ▨ shaving 2, ▩ cell wall, □ exoproteome.

Supplementary Figure S3.



Supplementary Figure S3. Predicted subcellular localization of the proteins identified with the different approaches for strains USA300 and Newman. The overlapping as well as unique proteins identified for the four different sub-proteome fractions from strains USA300 and Newman were analyzed regarding their predicted subcellular localization. CW, covalently cell wall-bound proteins; M, transmembrane- and lipoproteins; Sec, secreted proteins; and No, proteins with no predicted motif for subcellular localization.

Supplementary Figure S4



Supplementary Figure S4. Comparative overview of the results from the Pepscan analysis. The binding of antibodies from seven Epidermolysis bullosa patients to immobilized overlapping 15-mer peptide sequences from 54 different staphylococcal surface-exposed proteins were analyzed. For most of the proteins the overlap of consecutive peptides was 11 amino acids. For LytM and IsaA the overlap was 14 amino acids (high resolution, HR). Peptides were synthesized linearly and with the CLIPS™ technology. The quantified signals were normalized to the signal average per chip and are presented in a bee swarm plot, where the respective proteins are indicated on the y-axis and their normalized signal intensities on the x-axis. ● EB01, ● EB09, ● EB51, ● EB11, ● EB15, ● EB53, ● EB02.

Supplementary Figure S5.

Legend

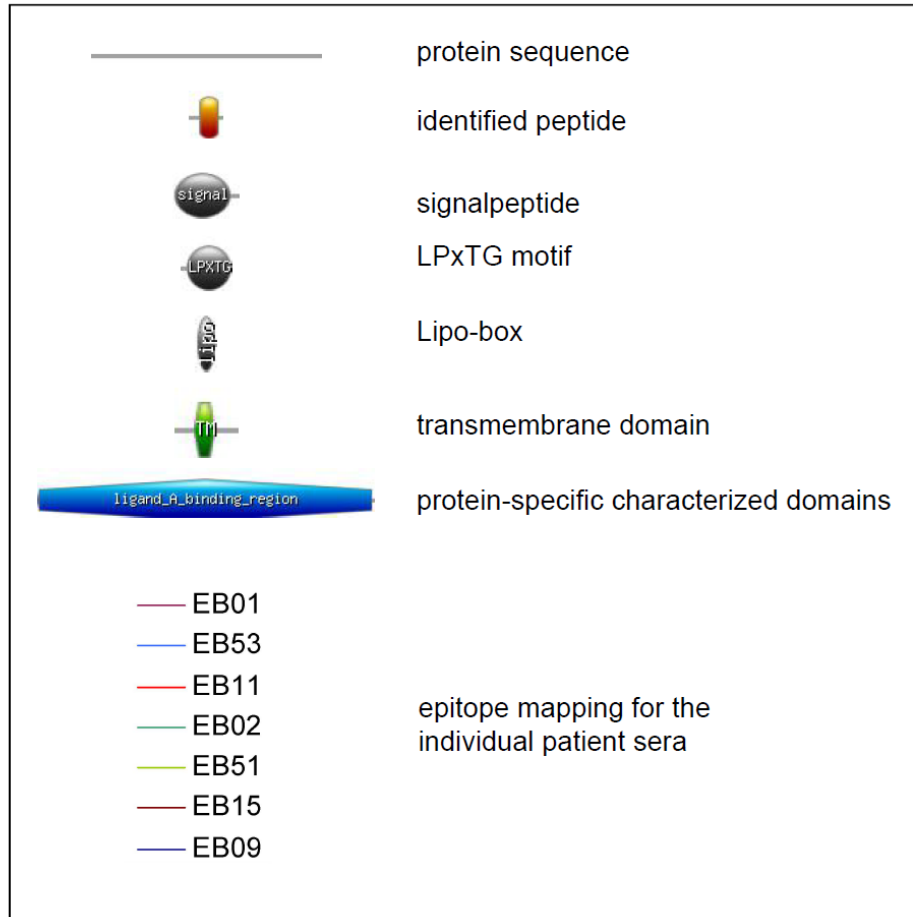
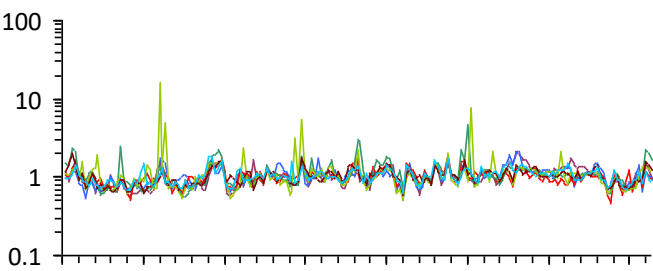
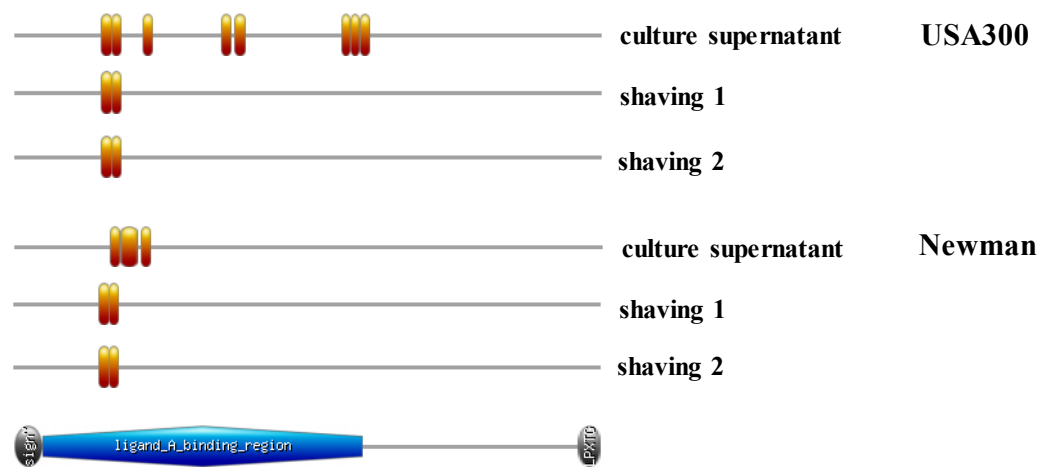


Figure S5. Comparison of the results from the proteomic studies and epitope mapping.

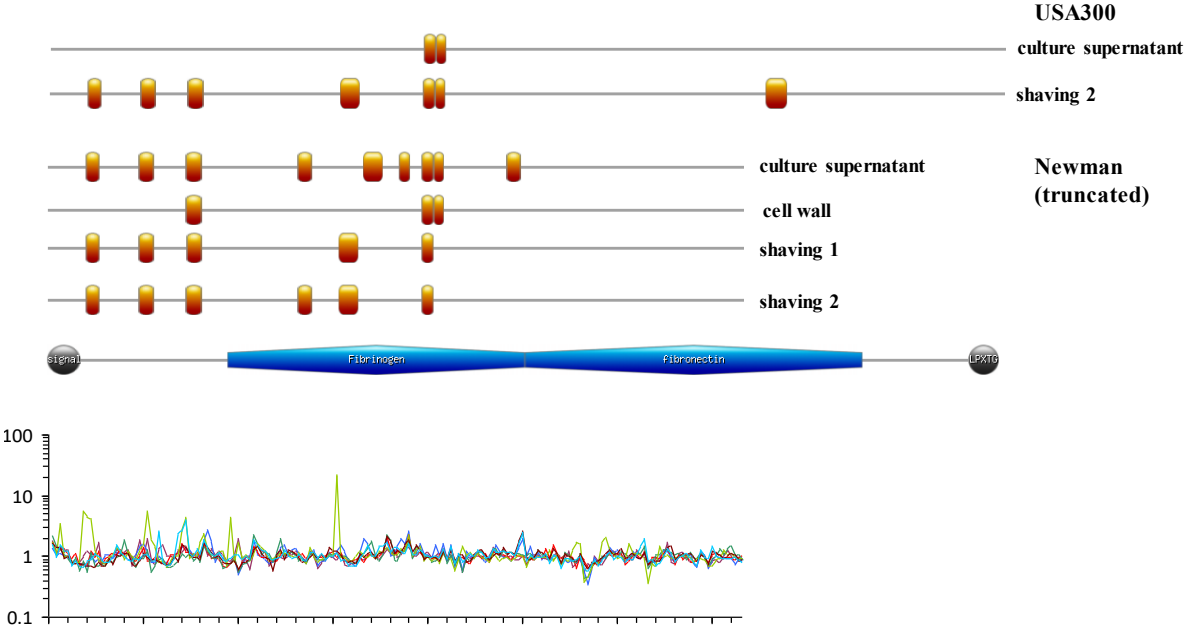
The locations of peptides in particular proteins as identified by the analyses of four different sub-proteome fractions of *S. aureus* strains USA300 and Newman are highlighted in the respective linearly depicted protein sequences. In addition, known protein domains are indicated. The graphs display the signals from the epitope mapping normalized to the median signal of the respective protein and serum for the seven different EB patients.

Cell Wall-Anchored Proteins

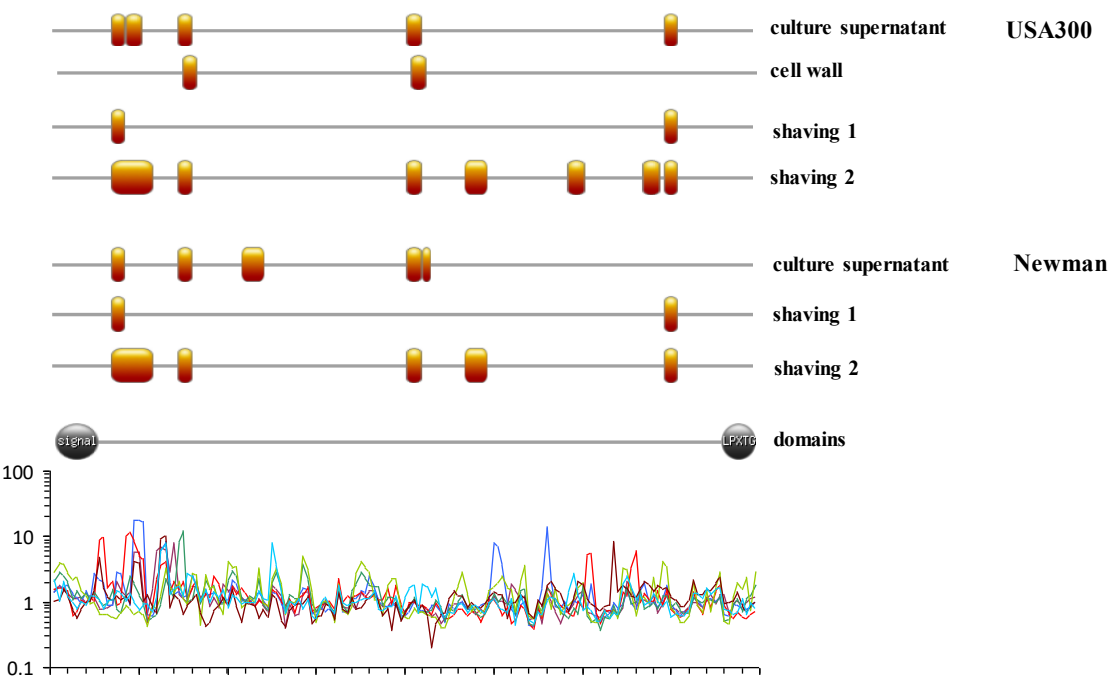
Clumping factor B, ClfB (Q2FDM9, CLFB)



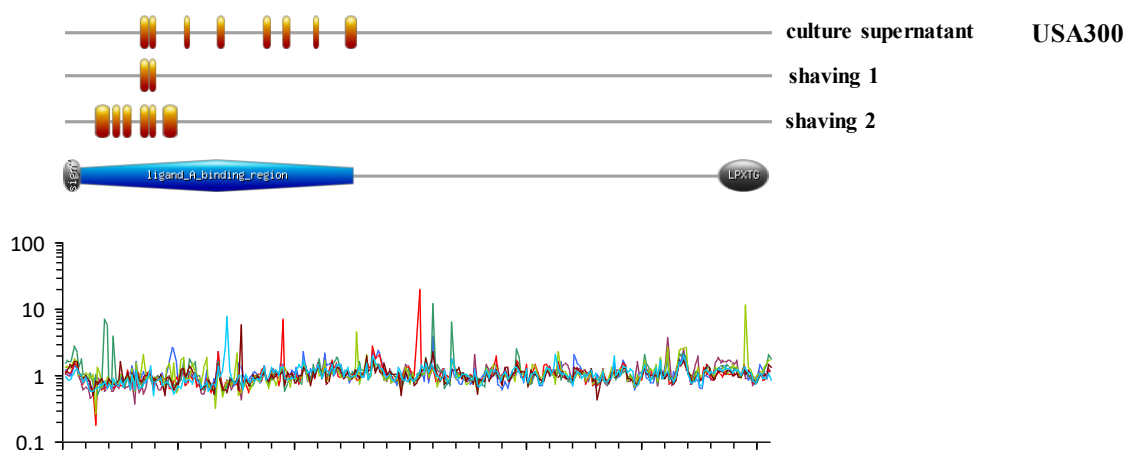
Fibronectin binding protein A, FnbA (Q2FE03, A6QJY9)



Iron-regulated surface protein B, IsdB (Q2FHV2, ISDB)

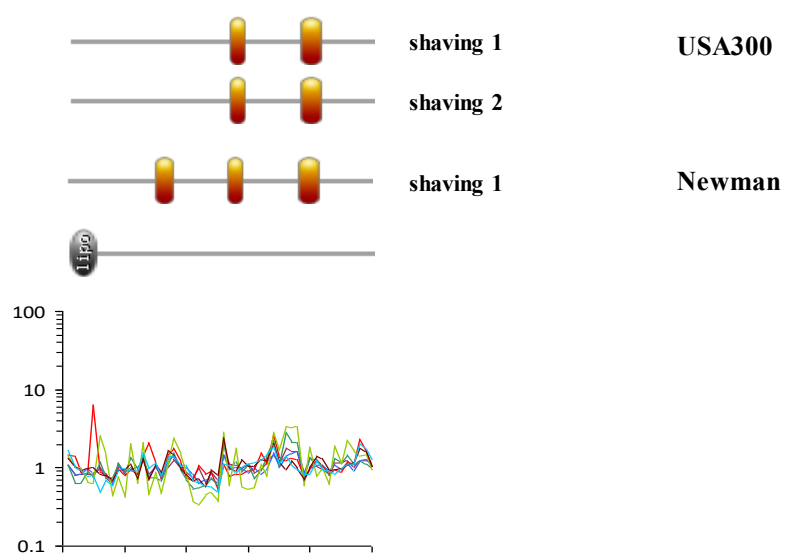


Serine-aspartate repeat-containing protein D, SdrD (Q2FJ78)

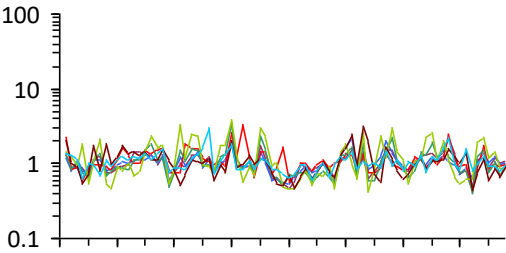
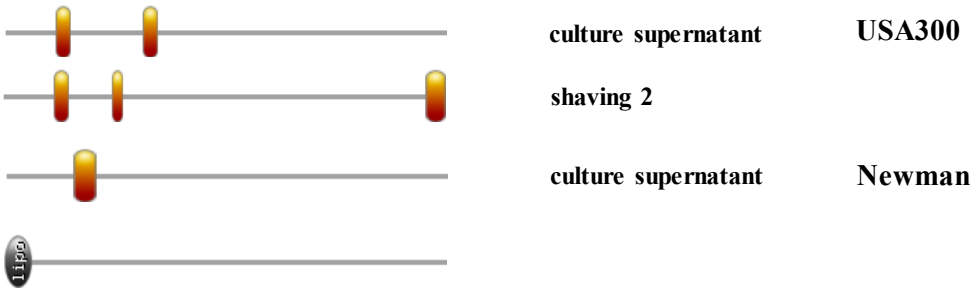


Lipoproteins

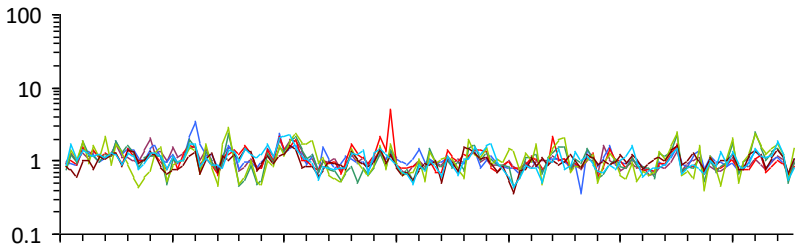
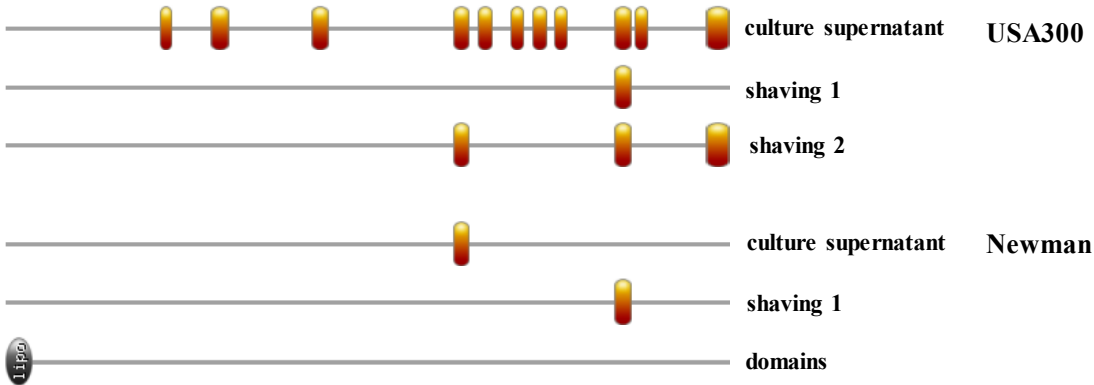
Cell wall binding lipoprotein (YkyA-like) (Q2FHY8 , A6QFU8)



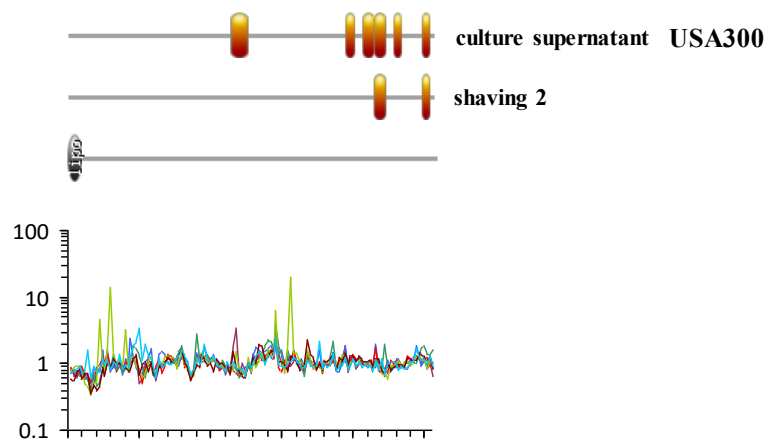
Foldase protein, PrsA (Q2FFQ5, PRSA)



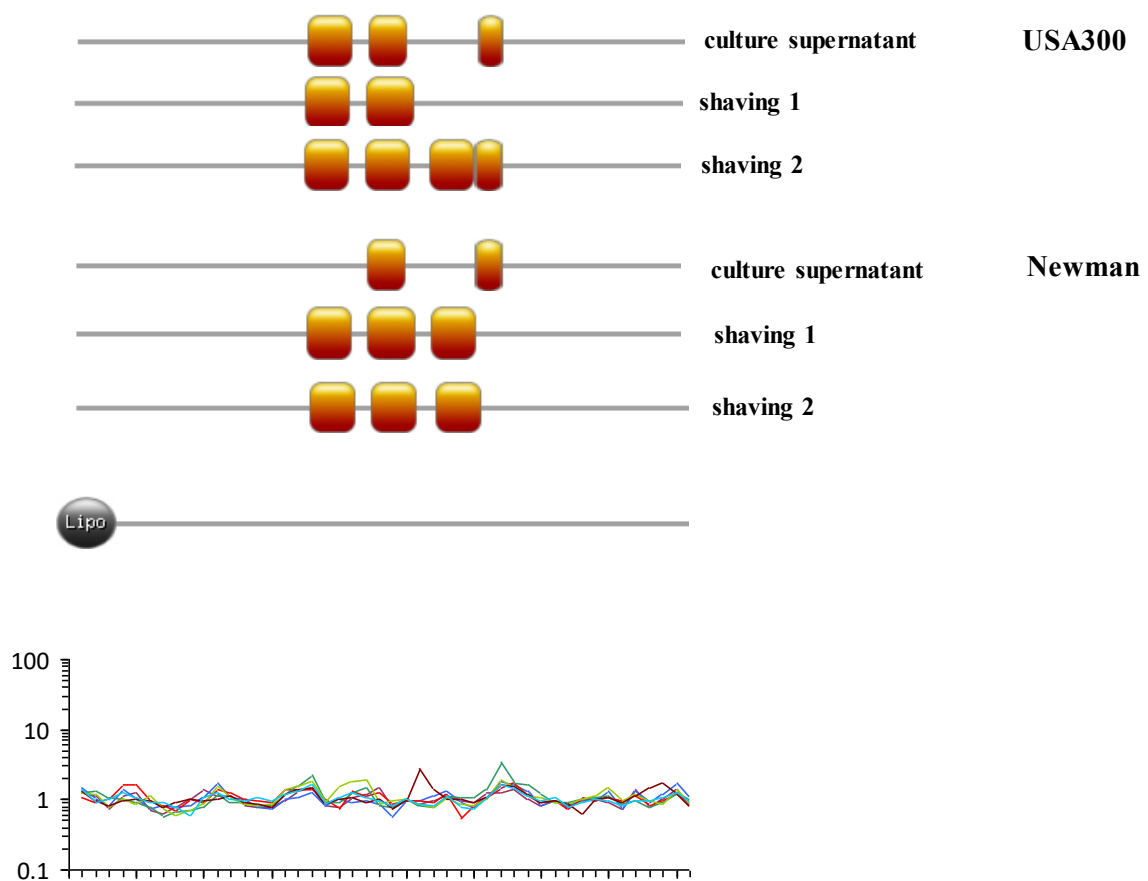
Oligopeptide permease peptide binding protein (Q2FE32, A6QJV4)



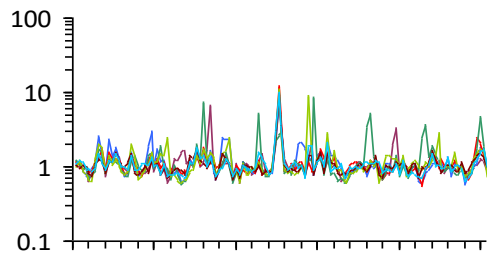
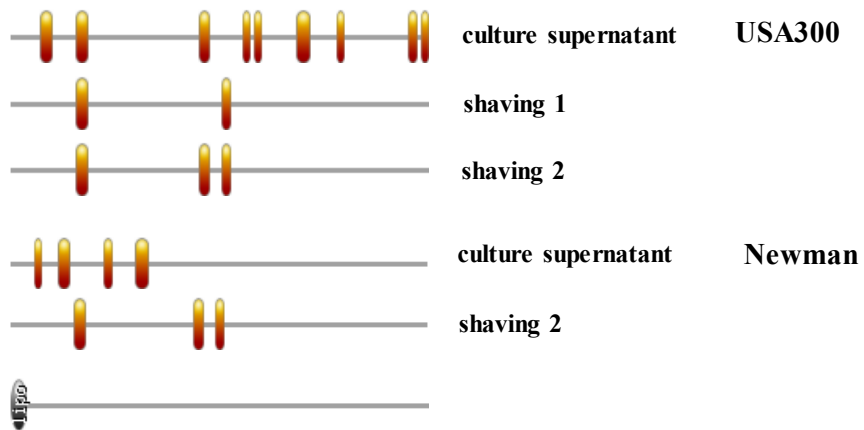
Peptide ABC transporter, peptide binding protein (Q2FKI7)



Putative Zinc-binding metalloprotease (Q2FJP2, A6QE54)

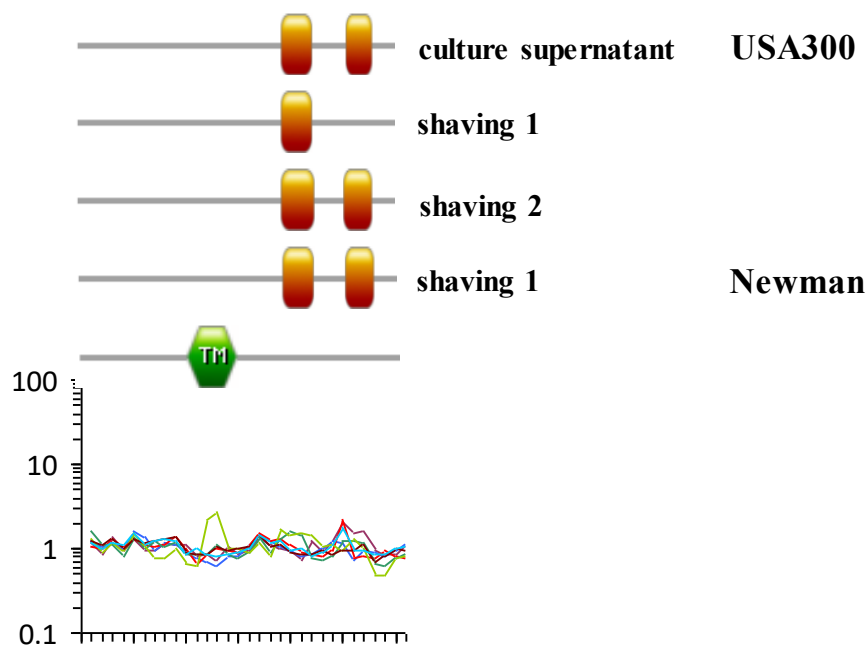


Zn-binding lipoprotein AdcA-like protein (Q2FE92, A6qjp6)

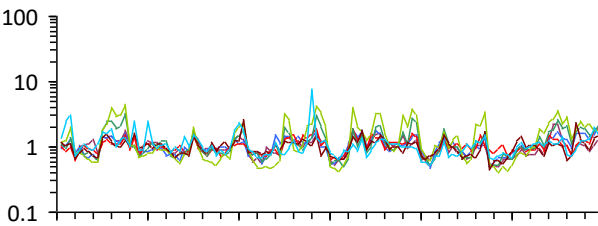
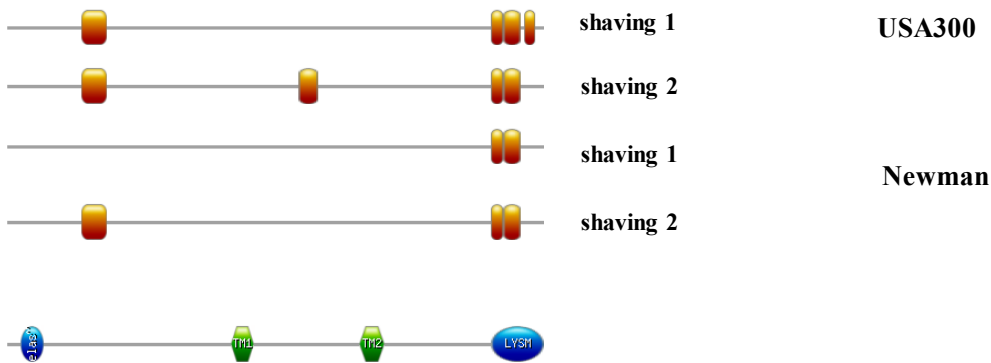


Membrane Proteins

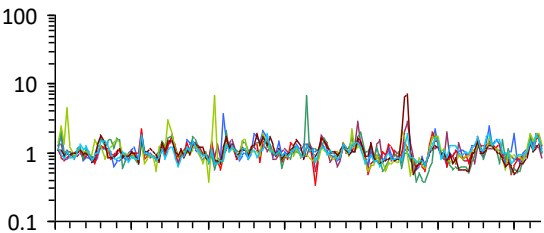
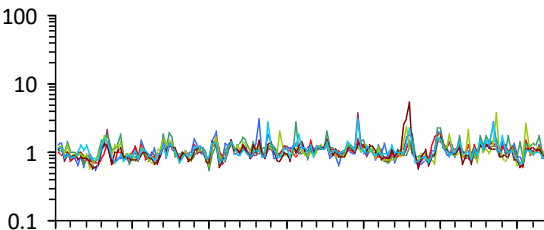
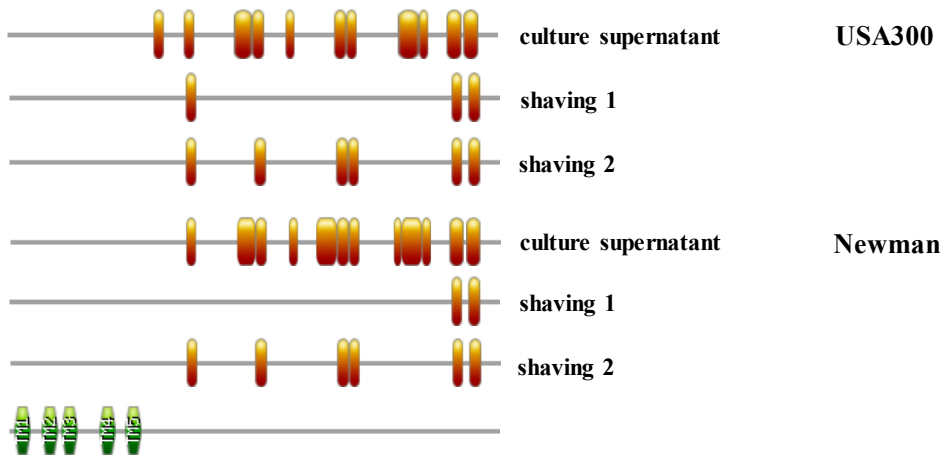
Cell division protein FtsL (Q2FHQ7, A6QG80)



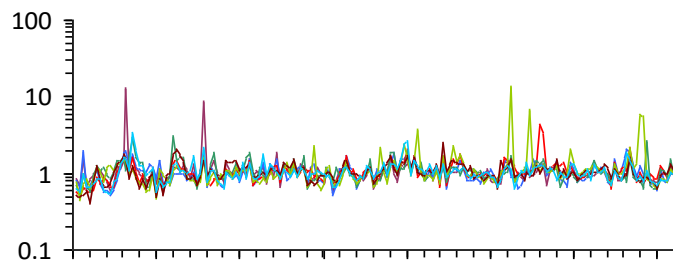
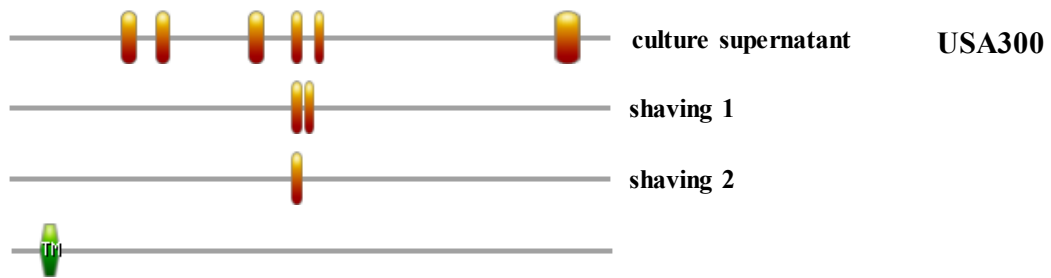
Elastin binding protein, EbpS (Q2FGW1, EBPS)



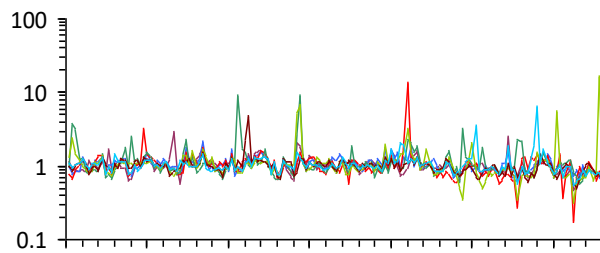
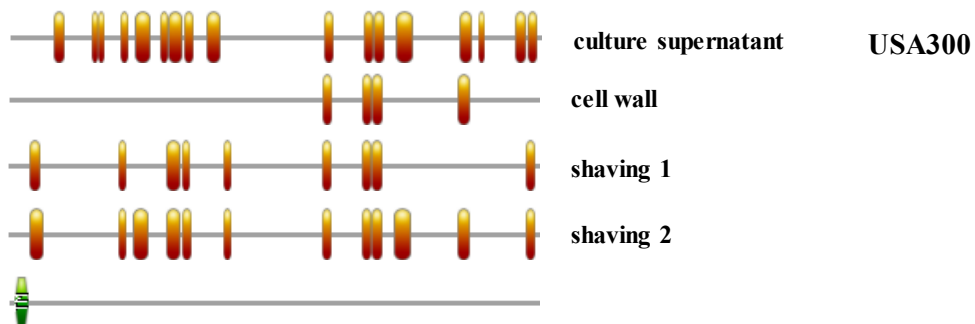
Glycerol phosphate lipoteichoic acid synthase, LtaS (Q2FIS2, A6QF27)



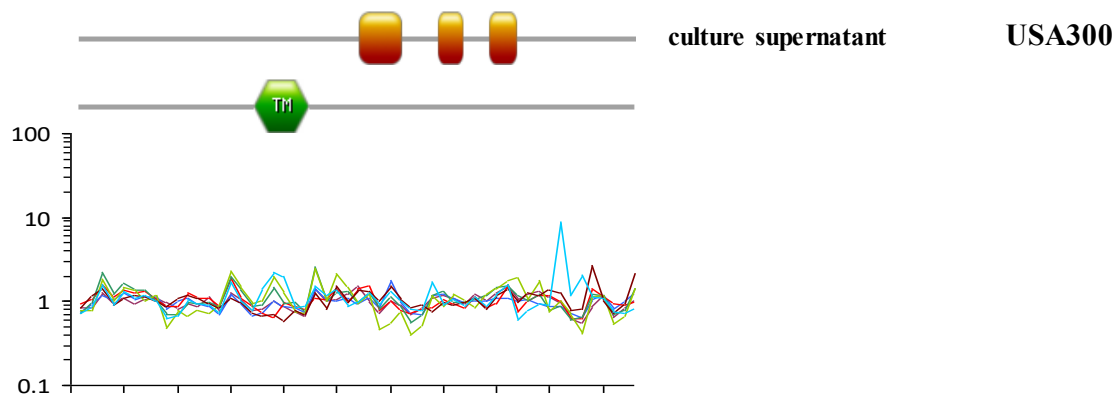
Penicillin binding protein 2, Pbp2 (Q2FGZ0)



Penicillin binding protein 2', Pbp2A (Q2FKM6)

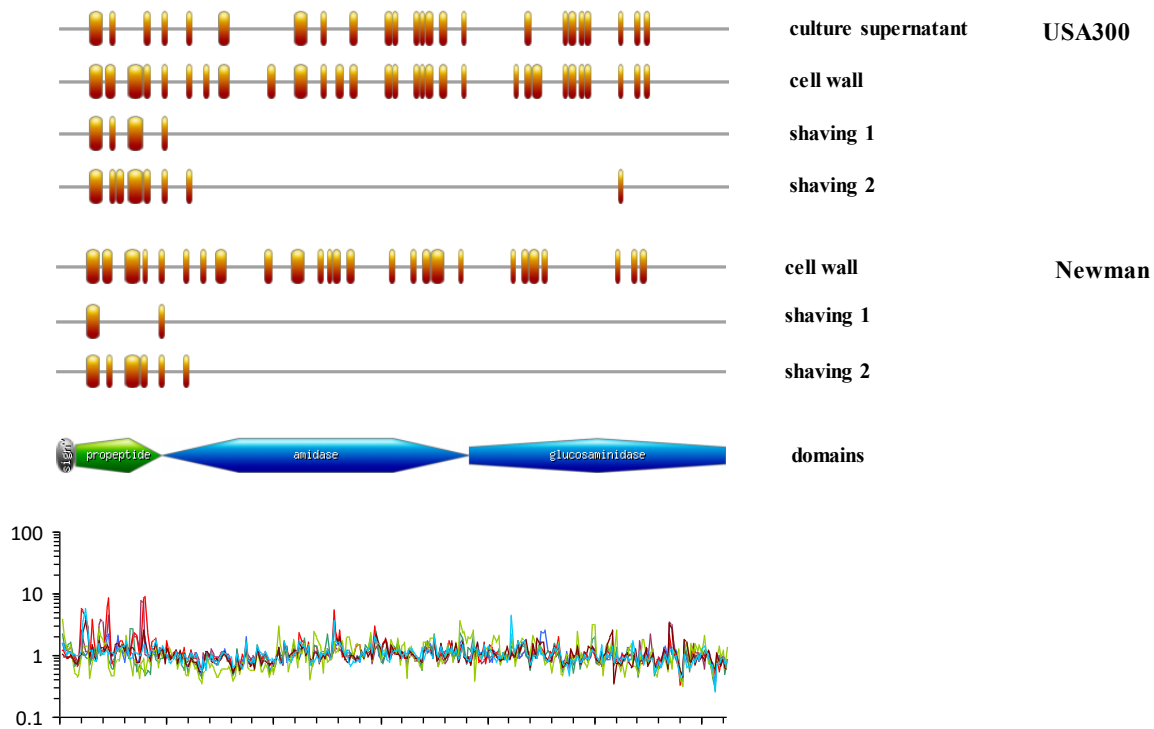


Uncharacterized protein (Q2FE65)

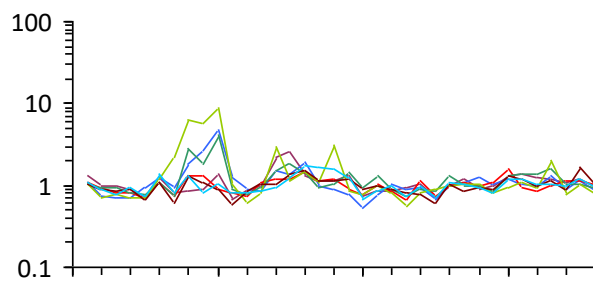
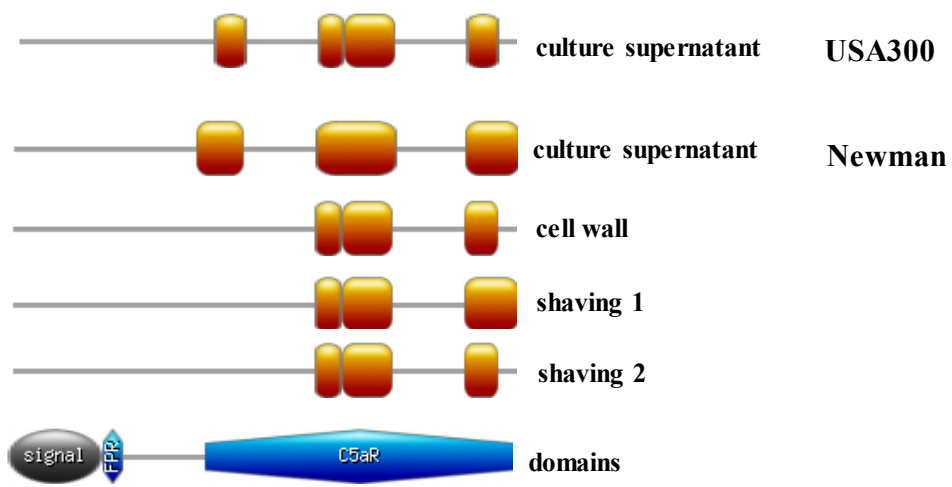


Secreted Proteins

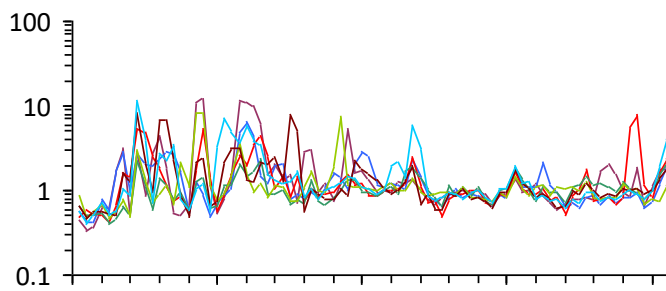
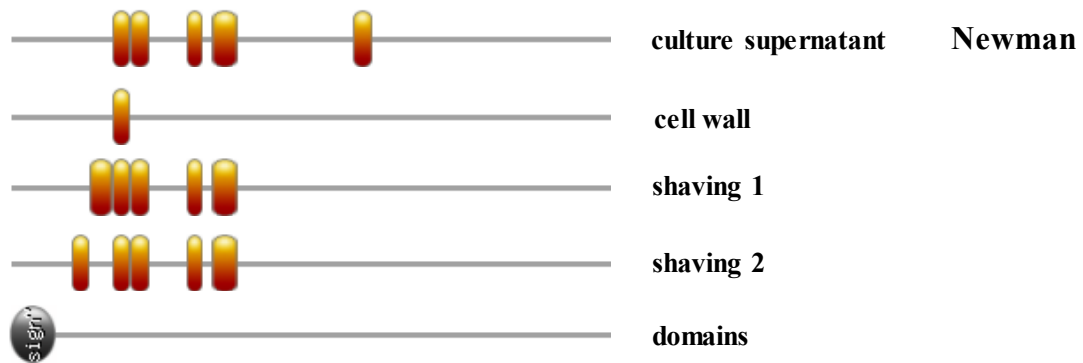
Bifunctional autolysin (Q2FI25, A6QFR2)



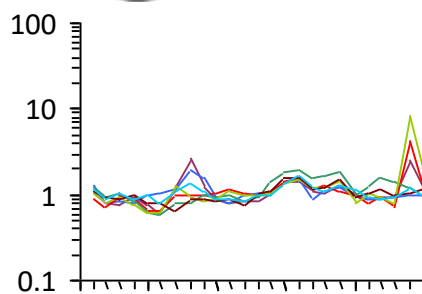
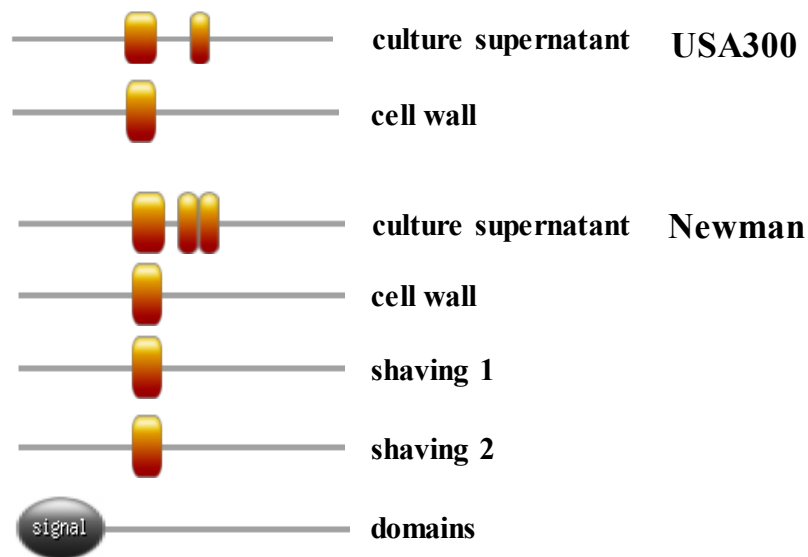
Chemotaxis inhibitory protein, CHIPS (Q2FFF7, CHIPS)



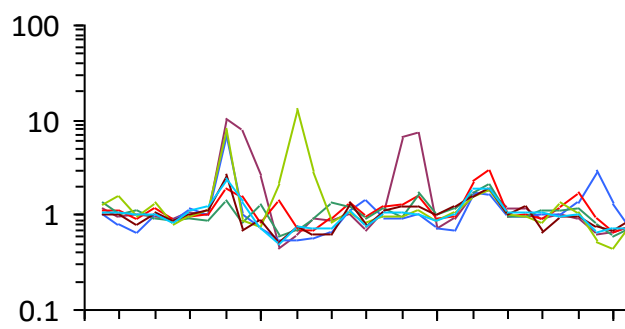
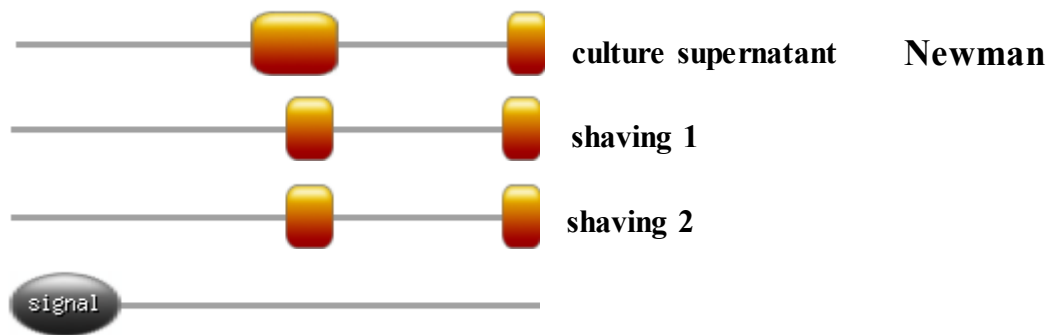
Extracellular matrix binding protein, Emp(EMP)



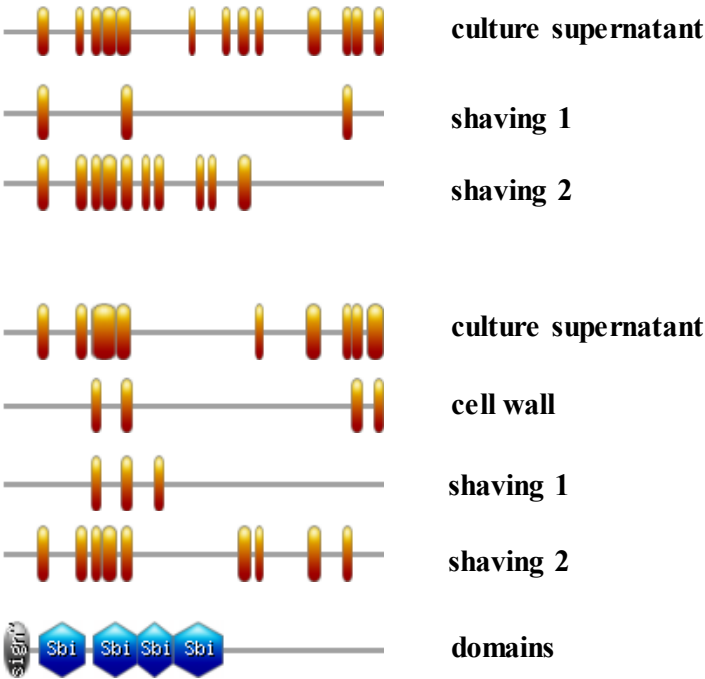
Fibrinogen binding related protein (Q2FHS8, A6QG56)



FPRL1 inhibitory protein, FLIPR (FLIPR)

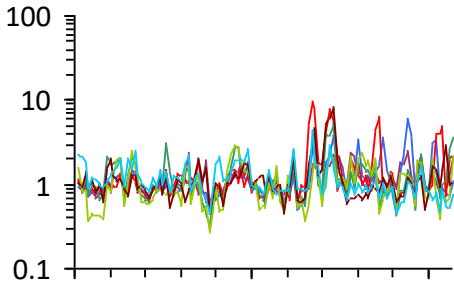


IgG binding protein, Sbi (Q2FE79, SBI)



USA300

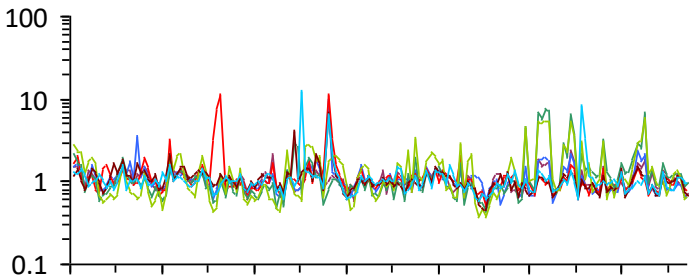
Newman



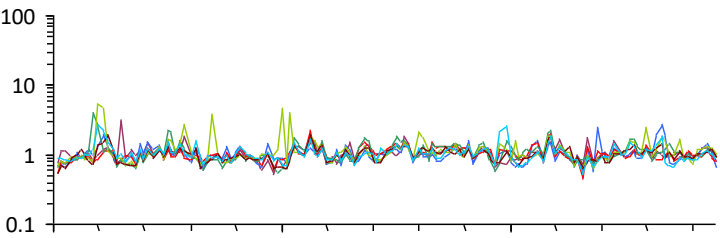
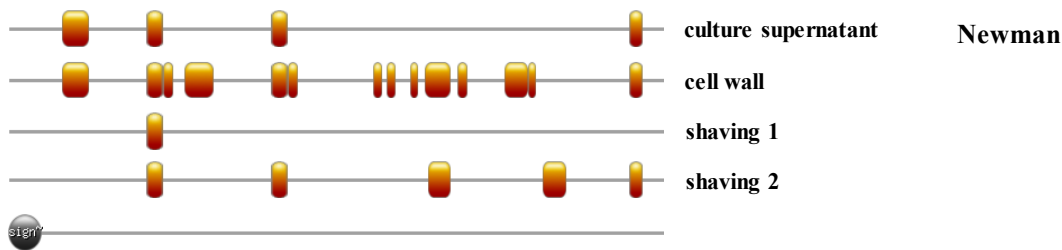
Lipase, Lip (Q2FDJ1)



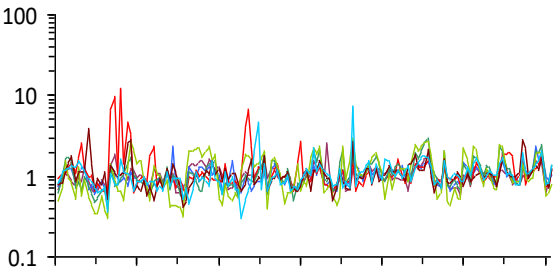
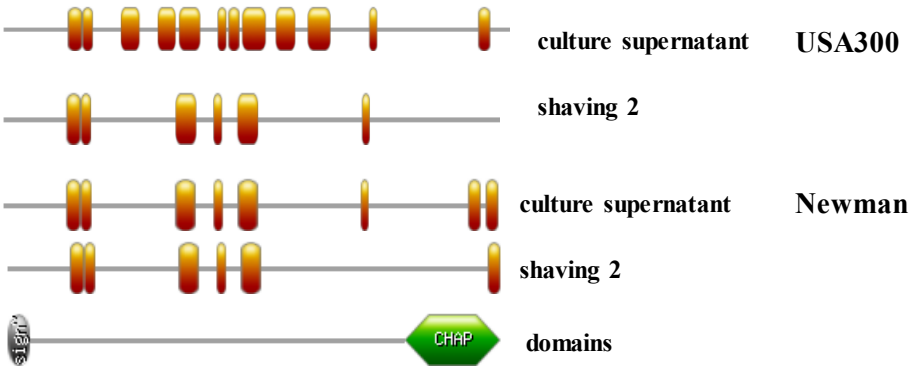
USA300



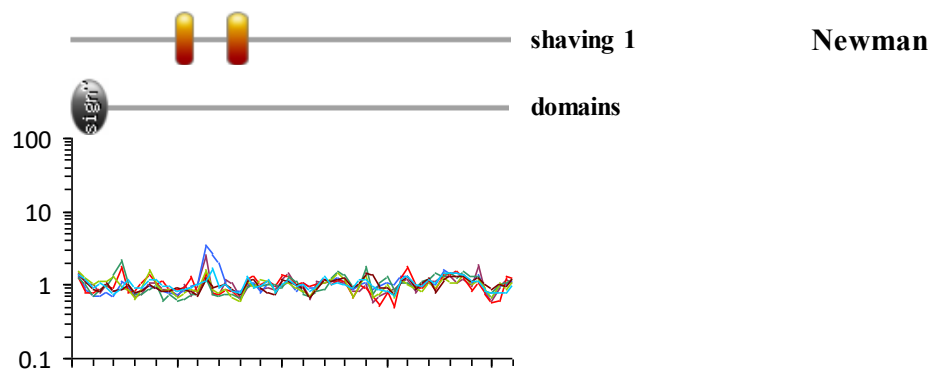
MHC class II analog protein, Omp7 (OMP7)



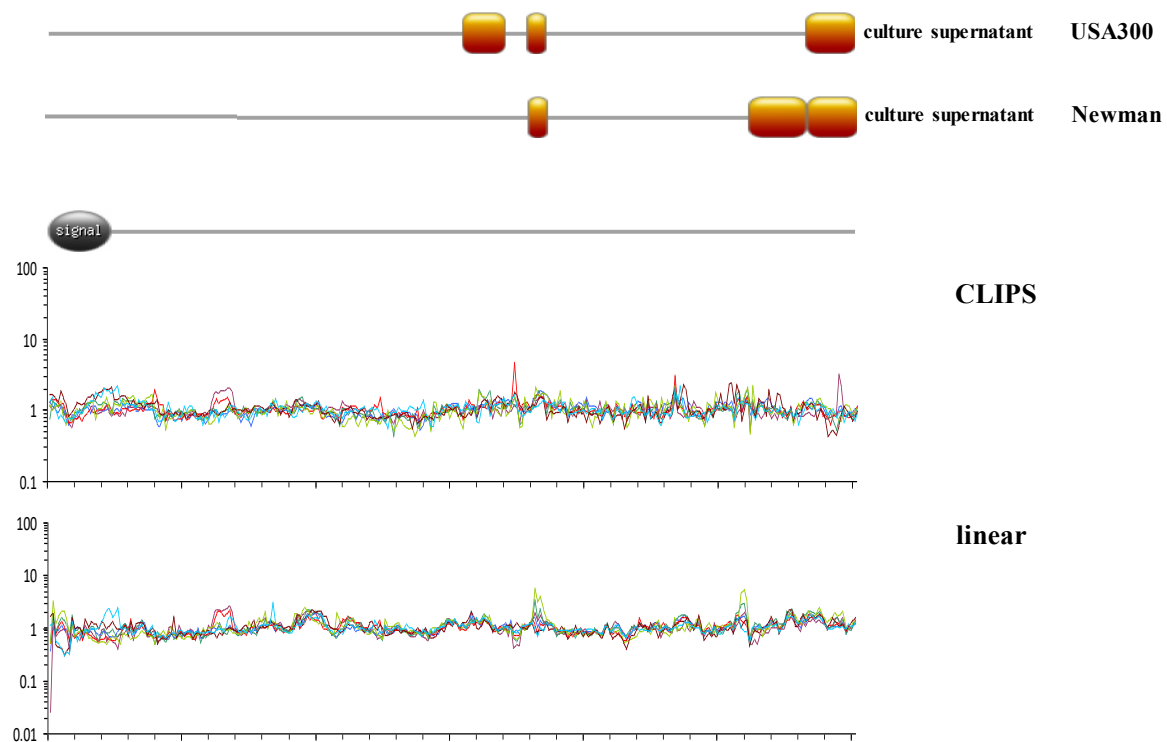
N-acetylmuramoyl-L-alanine amidase domain proteins (Q2FDL5, A6QKD3)



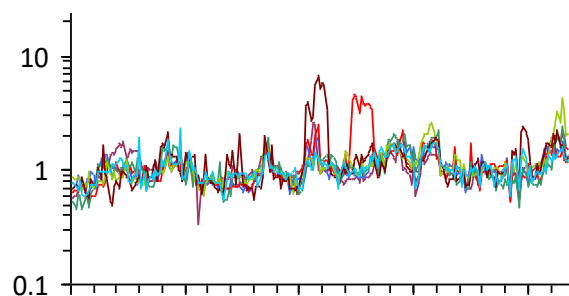
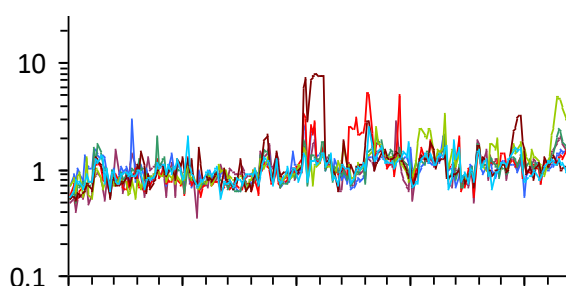
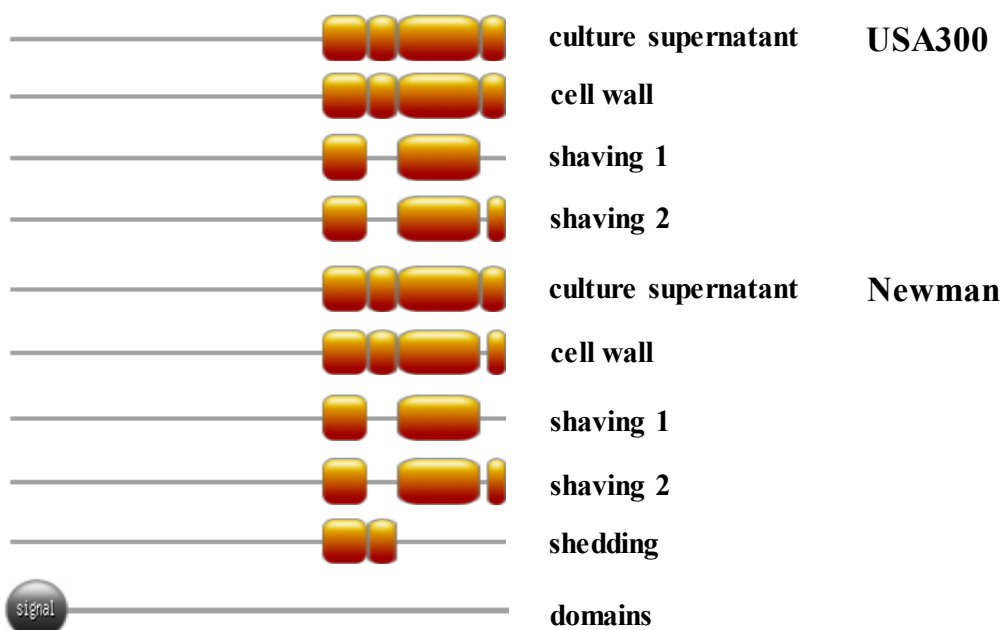
N-acetylmuramoyl-L-alanine amidase, family 4 (A6QJE7)



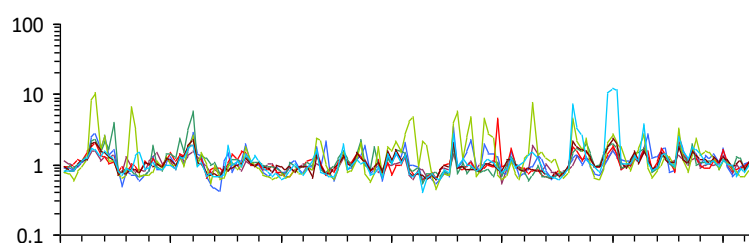
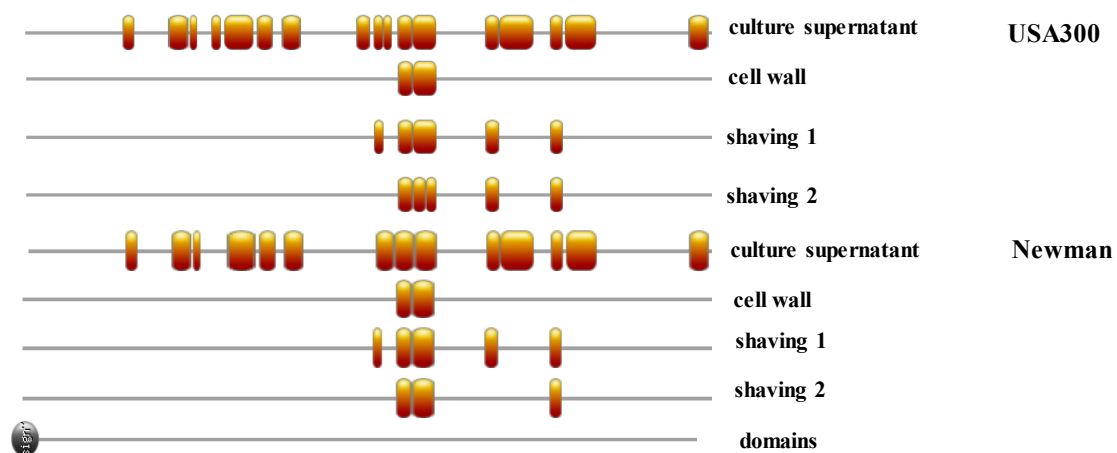
Peptidoglycan hydrolase, LytM (Q2FJZ4, A6QDQ0)



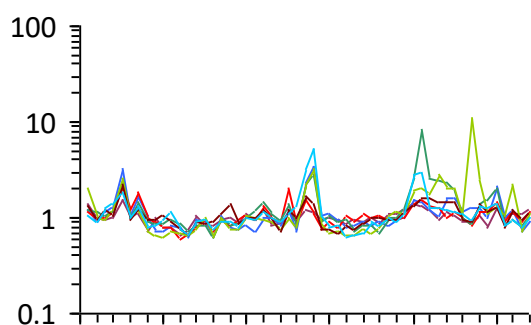
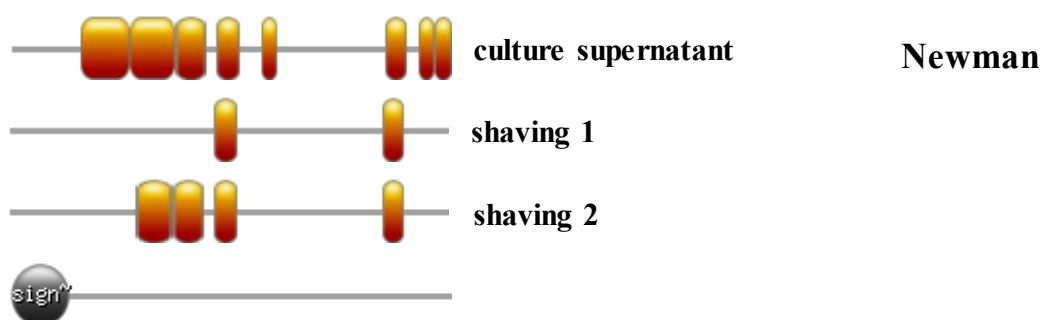
Probable transglycosylase A, IsaA (Q2FDT8, ISAA)



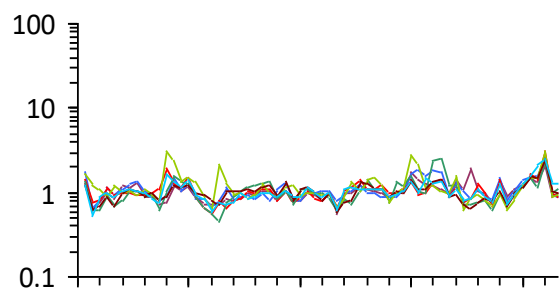
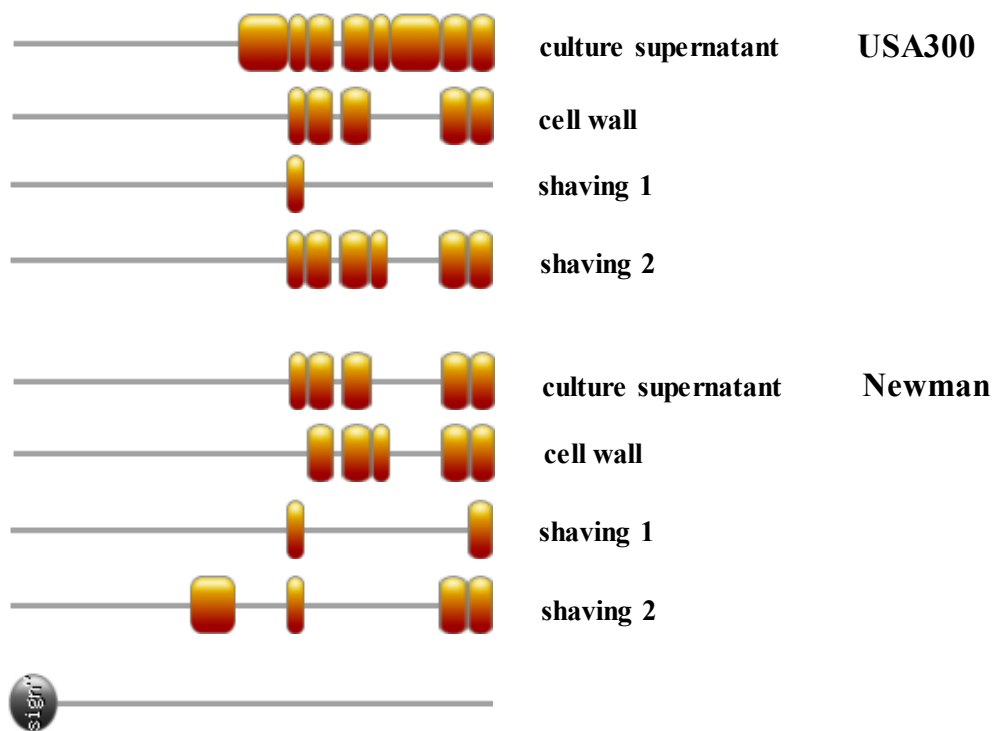
Staphylocoagulase, Coa (Q2FK40, A6QDK6)



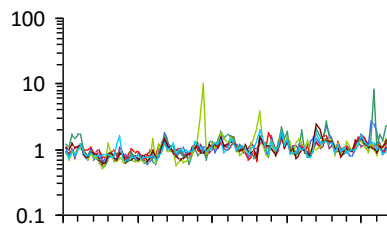
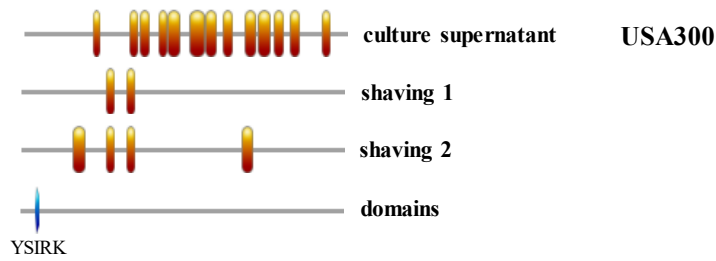
Staphylococcal enterotoxin-like toxin (A6QE90)



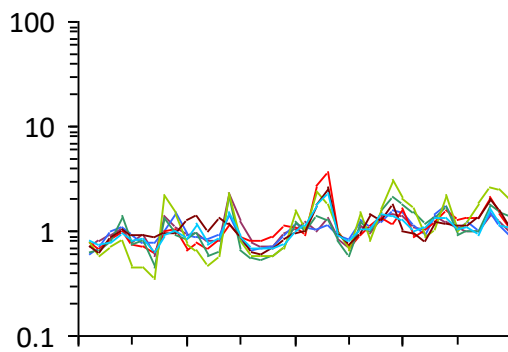
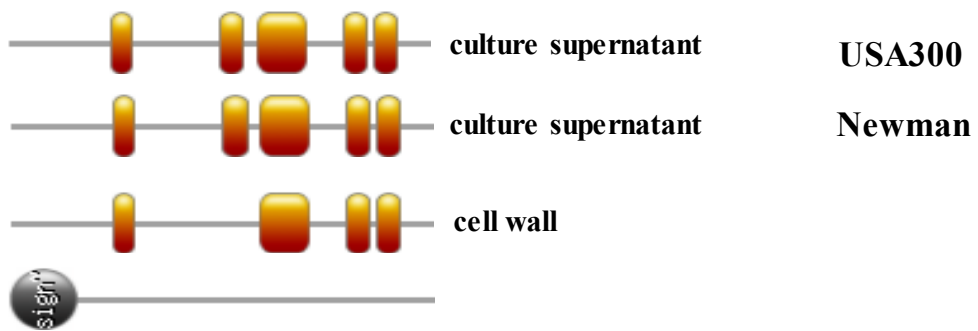
Staphylococcal secretory antigen, SsaA (Q2FEJ4, A6QJD9)



Truncated putative cell wall surface anchor protein, truncated SasG (Q2FE08)



uncharacterized protein (Q2FJ23, A6QES5)



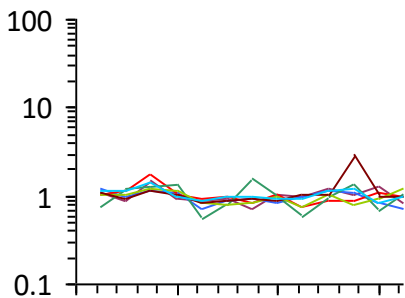
“Cytosolic” Proteins

CsbD-like protein (A6QHG7)

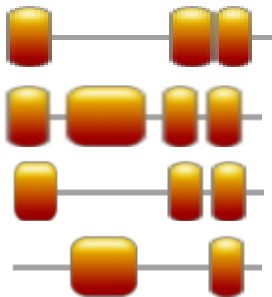


shaving 2

Newman



CsbD-like superfamily protein (Q2FIG2, A6QFC3)



shaving 1

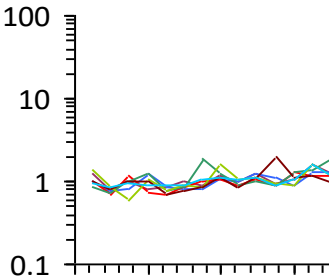
USA300

shaving 2

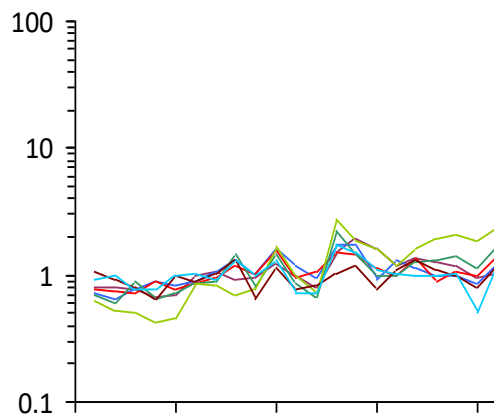
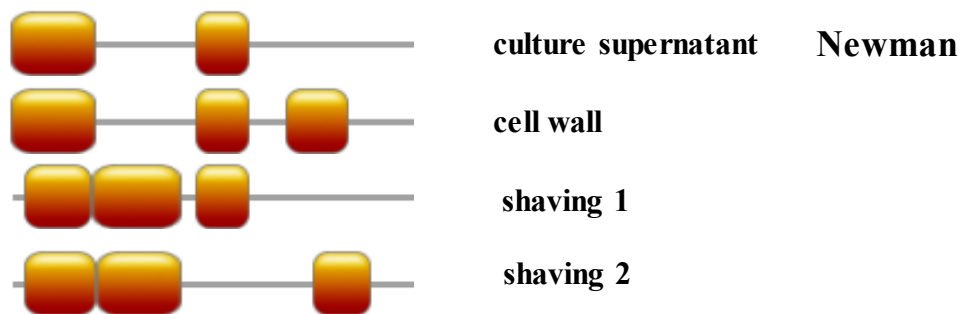
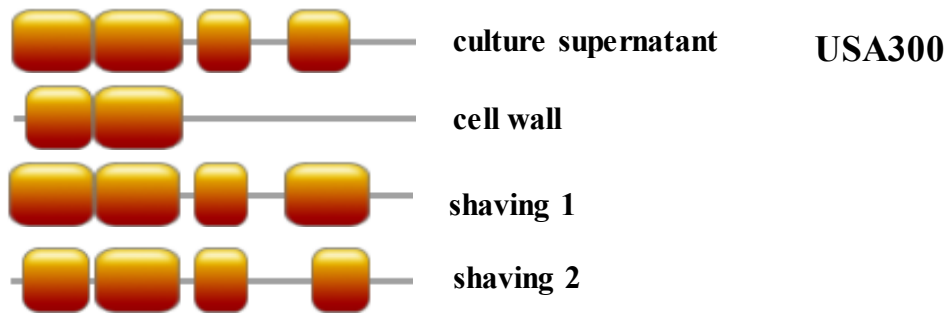
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Newman

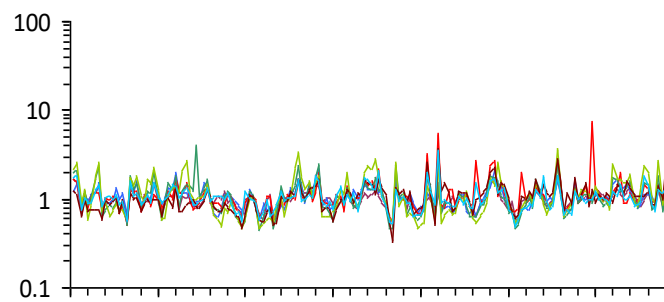
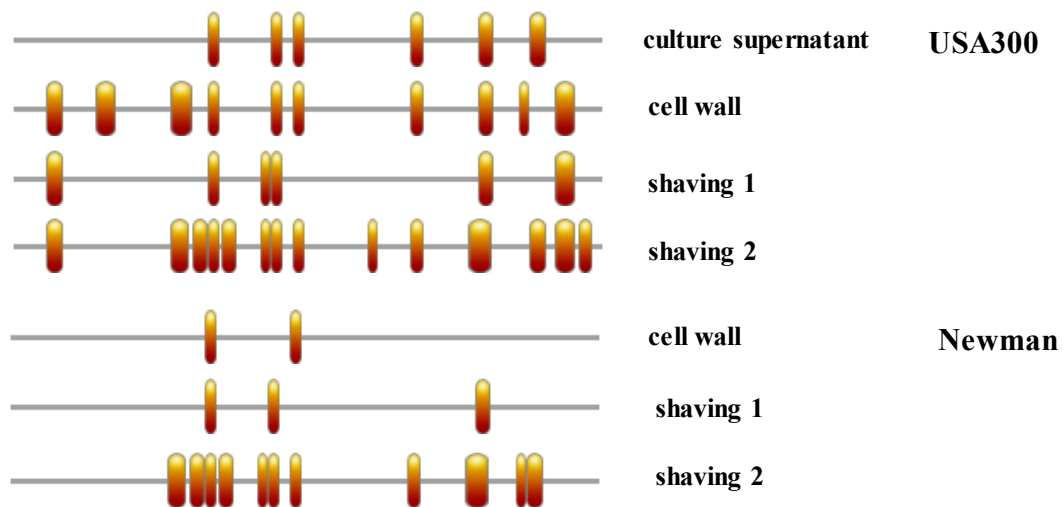
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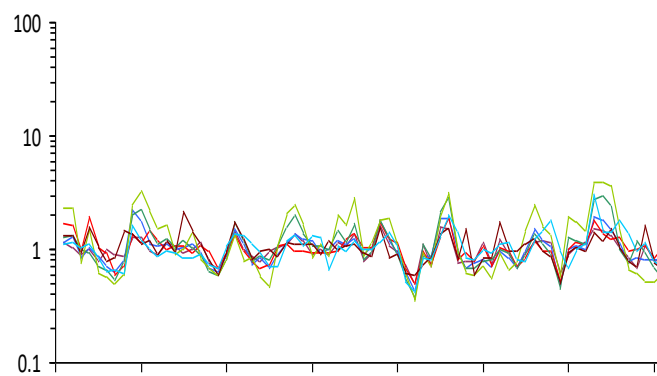
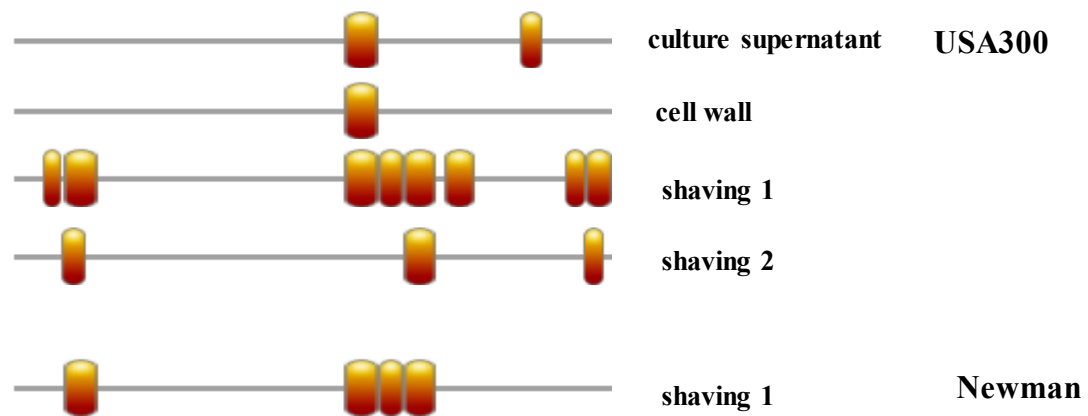
DNA-binding protein HU (Q2FGW9, A6QH22)



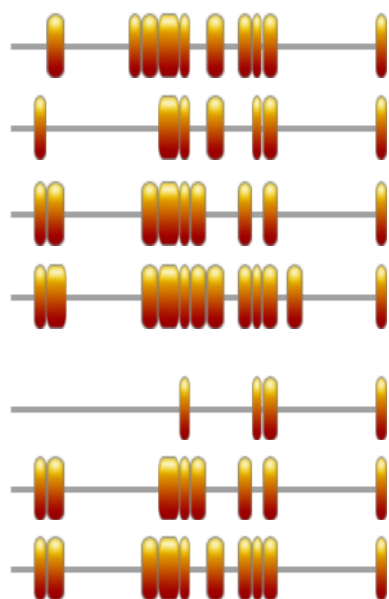
Elongation factor, Ef-G (Q2FJ93, EFG)



Elongation factor Ts, Ef-Ts (Q2FHI1, EFTS)



Elongation factor Tu, Ef-Tu (Q2FJ92, EFTU)



culture supernatant

USA300

cell wall

shaving 1

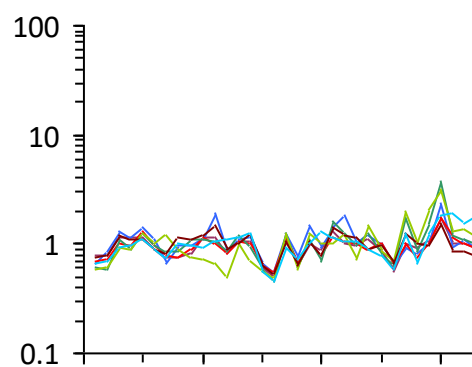
shaving 2

cell wall

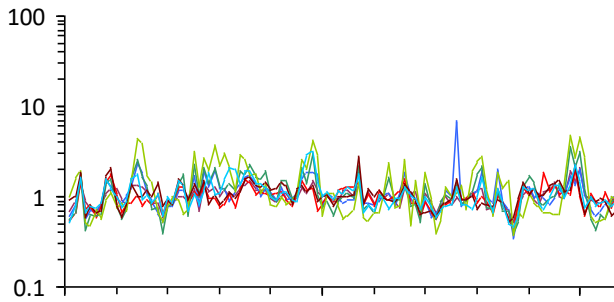
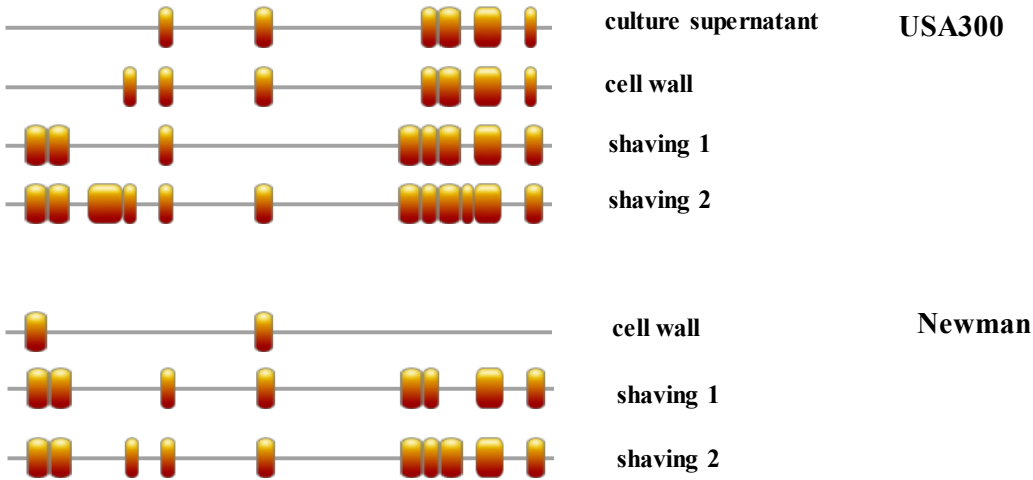
Newman

shaving 1

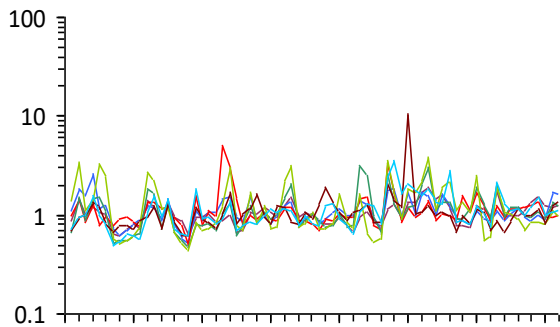
shaving 2



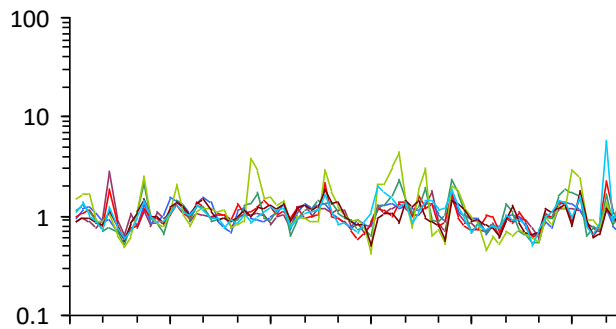
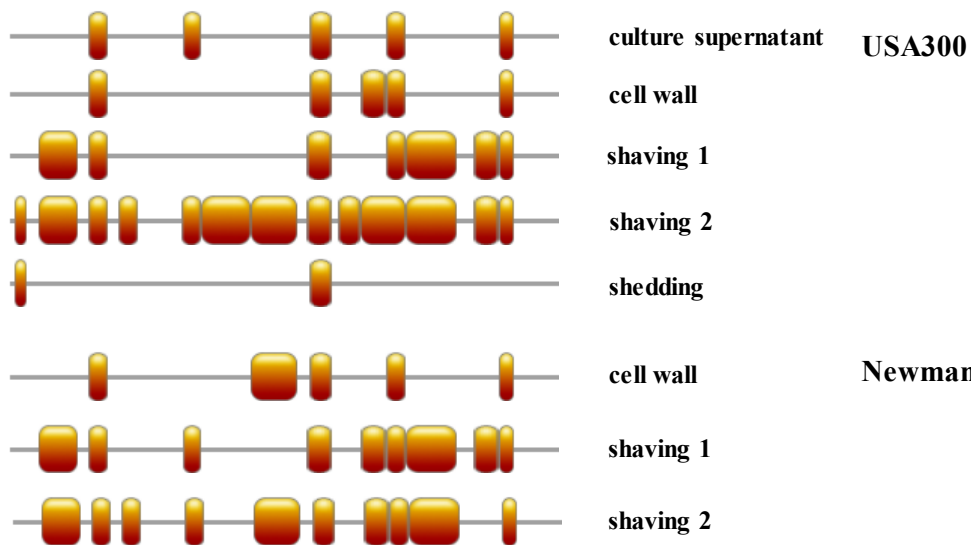
Enolase, Eno (Q2FIL7, ENO)



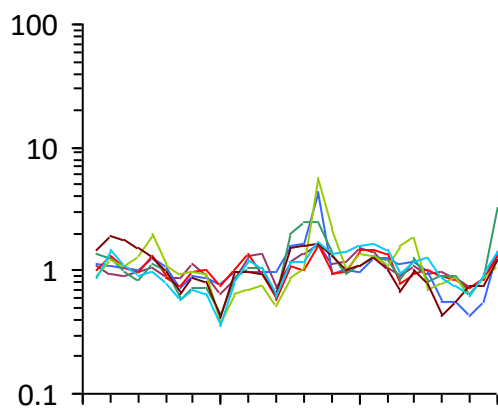
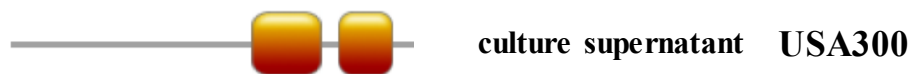
Fructose-bisphosphate-aldolase, Alf1 (Q2FF03, A6QIW9)



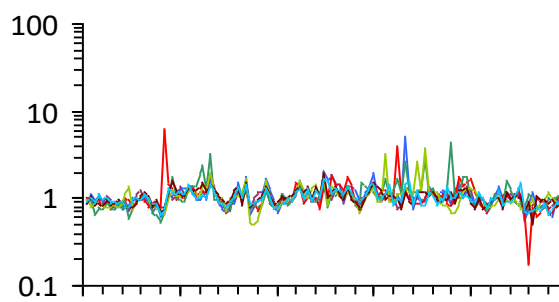
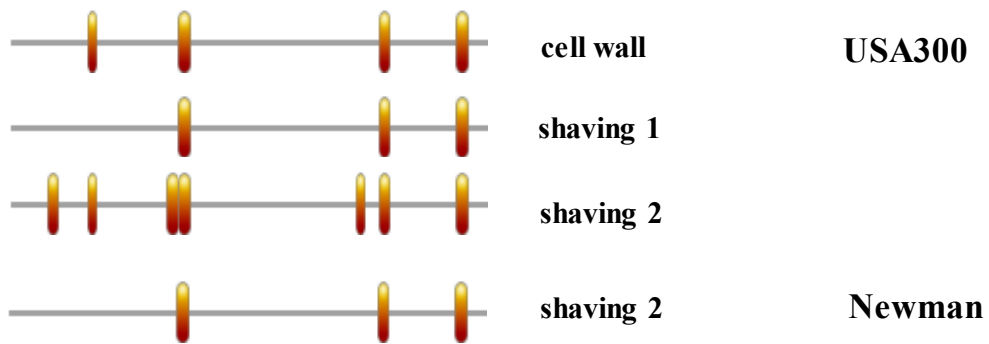
Glyceraldehyde 3-phosphate dehydrogenase GapDH (Q2FIM1, A6QF81)



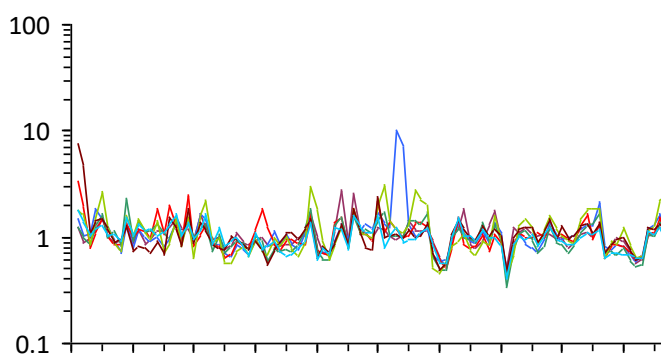
Glycine cleavage system H protein, GcsH (Q2FII7)



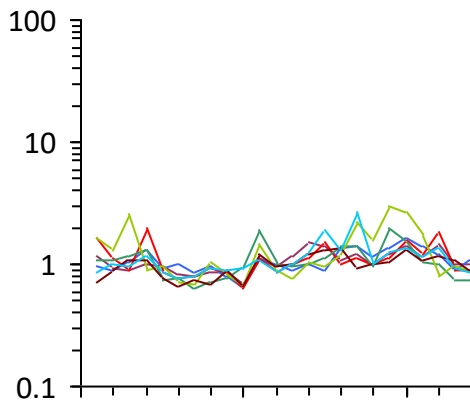
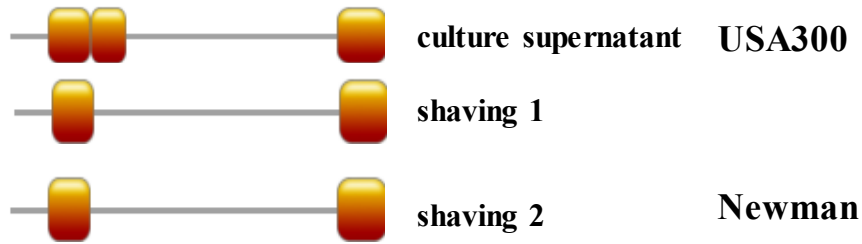
Mqo malate:quinone oxidoreductase (Q2FDQ3, A6QK94)



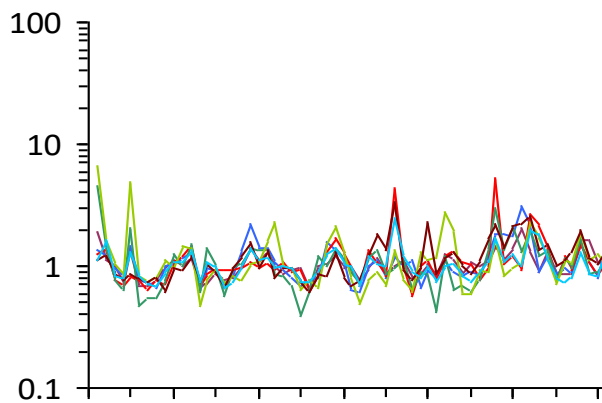
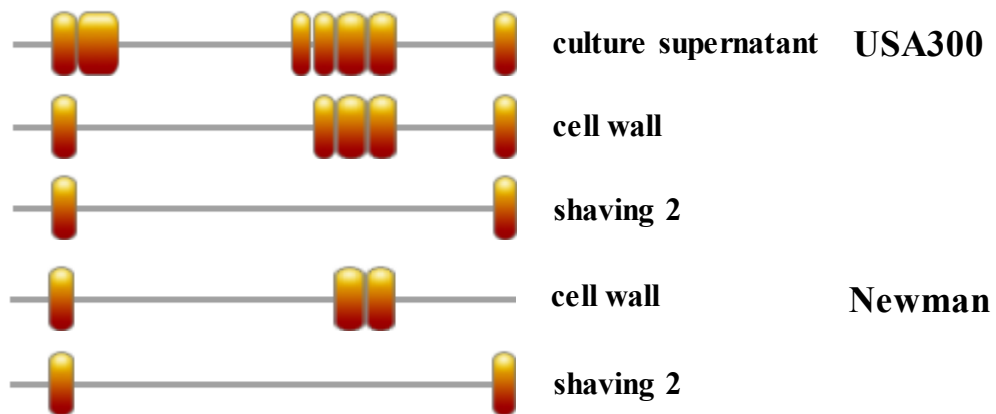
Phosphoglycerate kinase, Pgk (Q2FIM0)



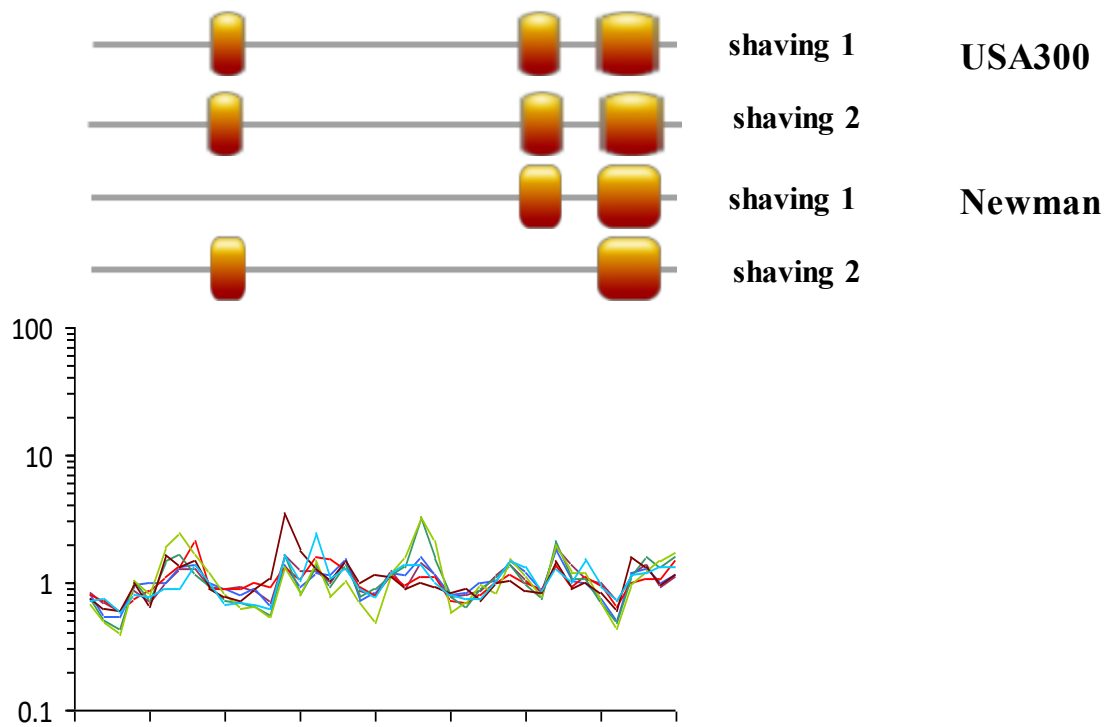
Thioredoxin like (Q2FII9, A6QFB4)



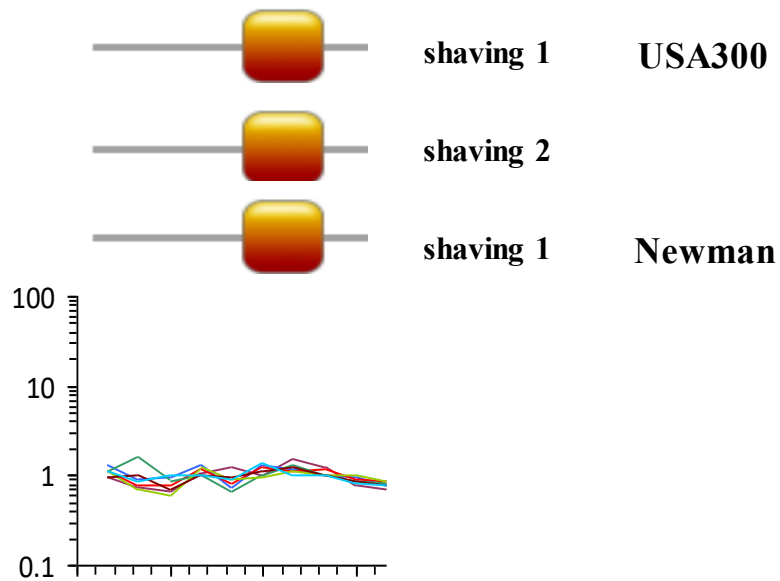
Triosephosphate isomerase, TpiS (Q2FIL9, TPIS)



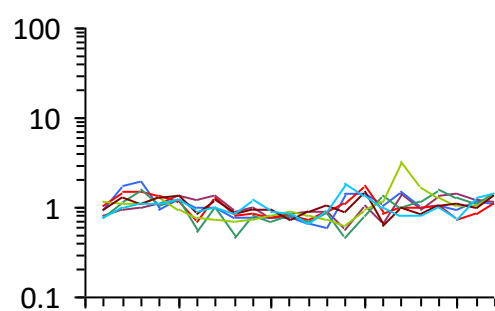
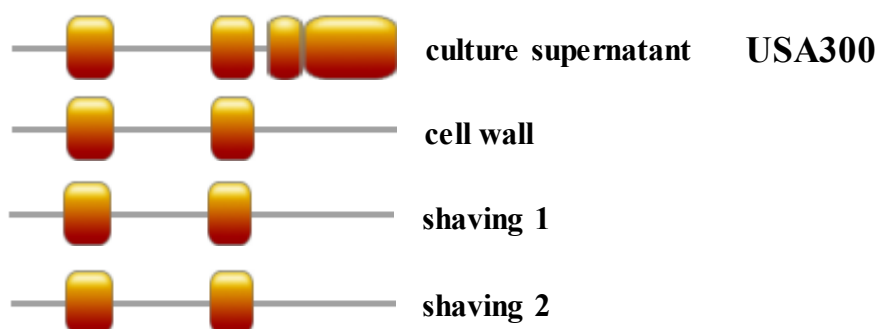
universal stress protein, Y1819 (Q2FG28, A6QHP4)



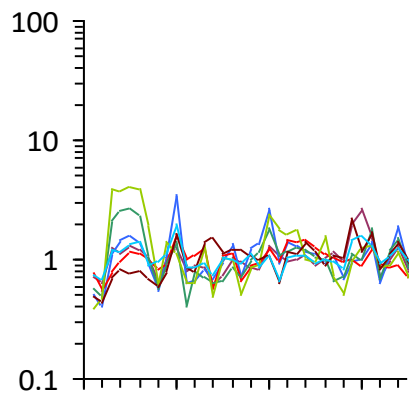
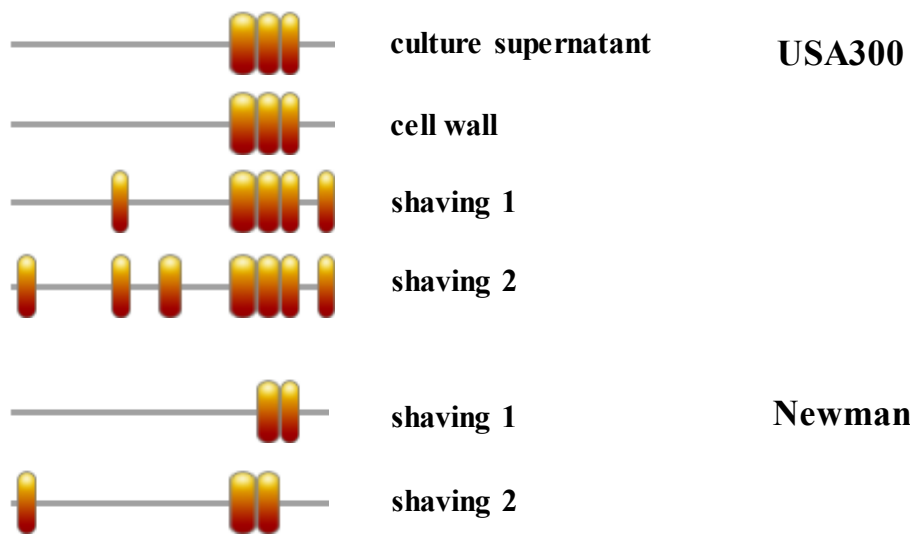
UPF0337protein (A6QHG6)



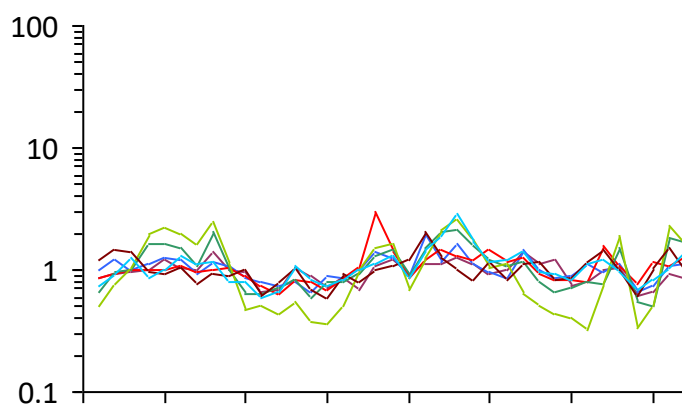
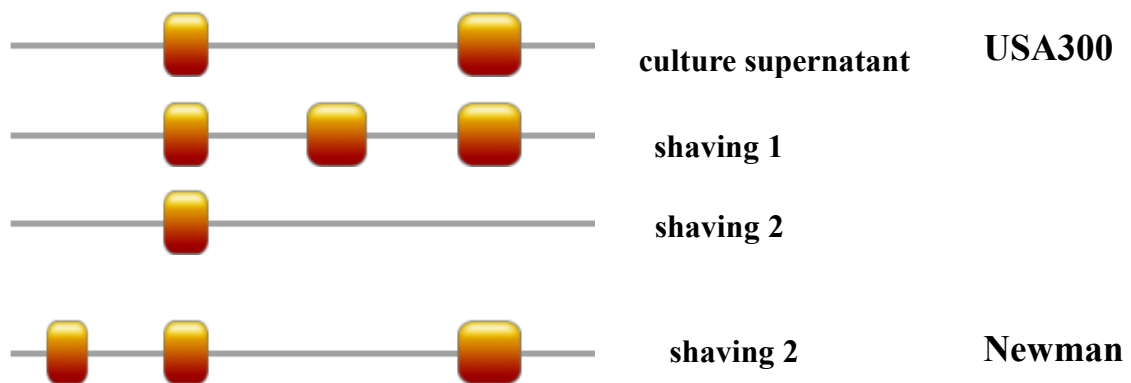
Virulence factor, EsxA (Q2FJY6)



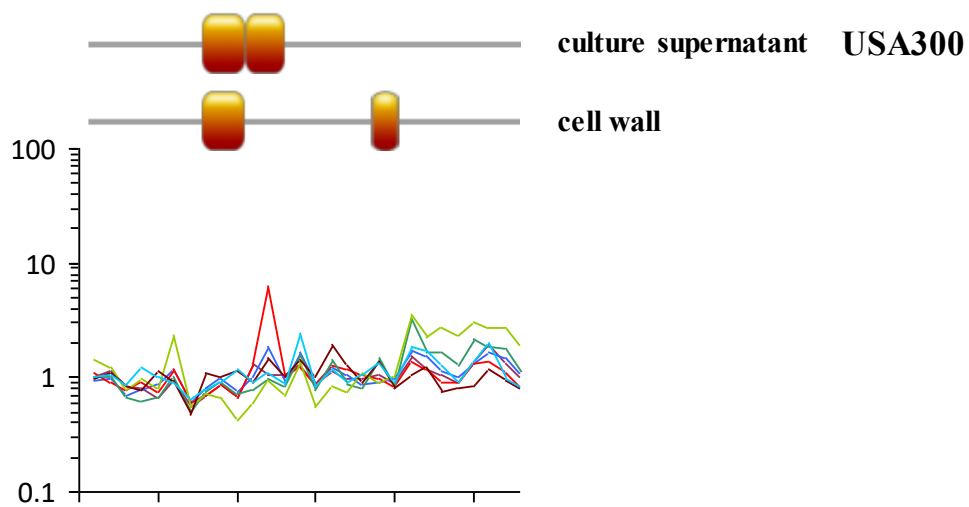
30S ribosomal protein S5, RS5 (Q2FEQ6, RS5)



30S ribosomal protein S7, RS7 (Q2FJ94, RS7)



30S ribosomal protein S13, RS13 (Q2FER3)



50S ribosomal protein L25 (Ctc) (Q2FJE0, A6QEF4)

