

SUPPLEMENTARY DATA

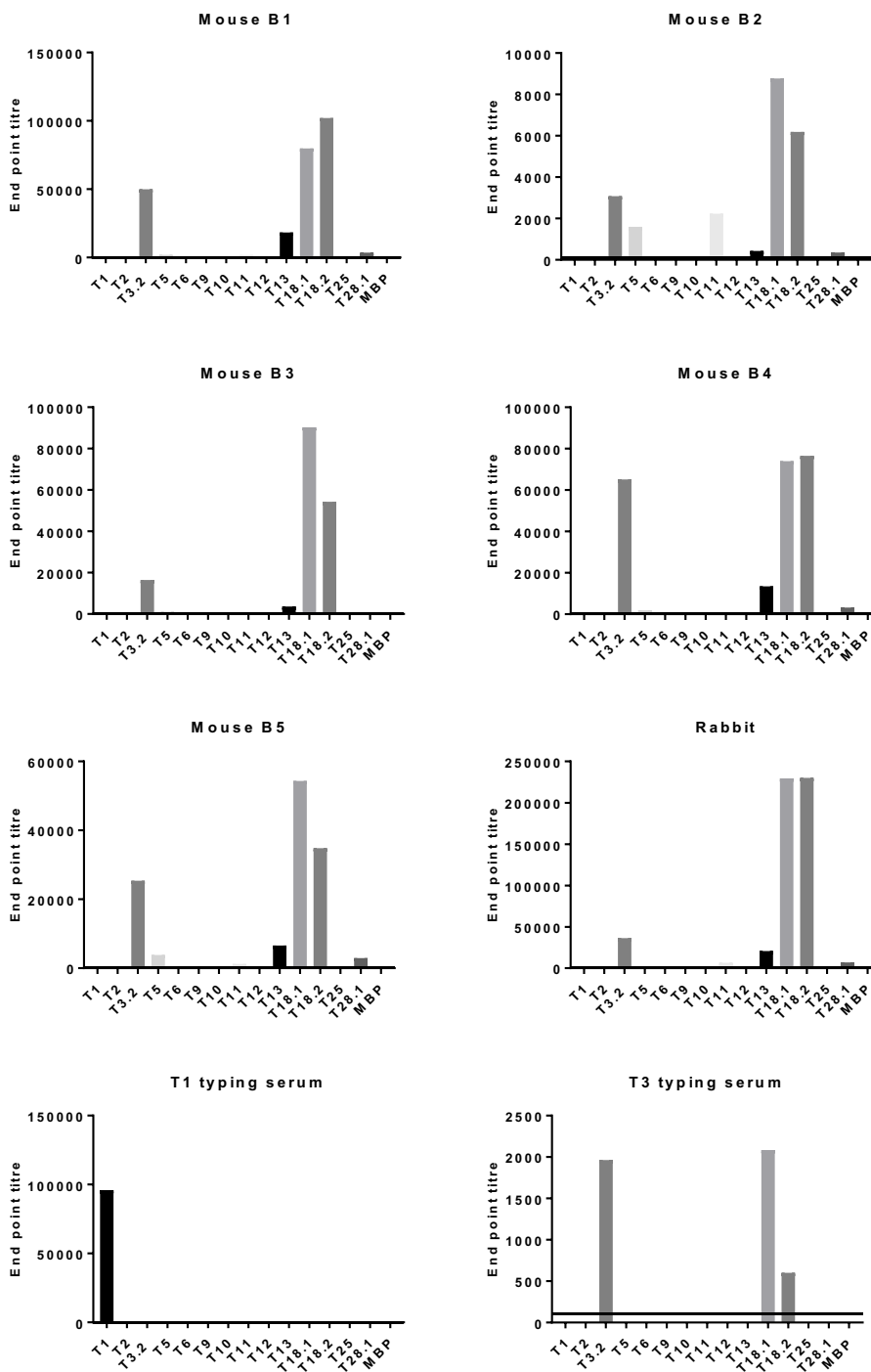


FIG S1: Endpoint titers of the anti-T-antigen sera to the T-antigen panel measured by ELISA. The endpoint titer was defined as the highest serum dilution above the control (absorbance of pre-immune rabbit serum +3 times the standard deviation).

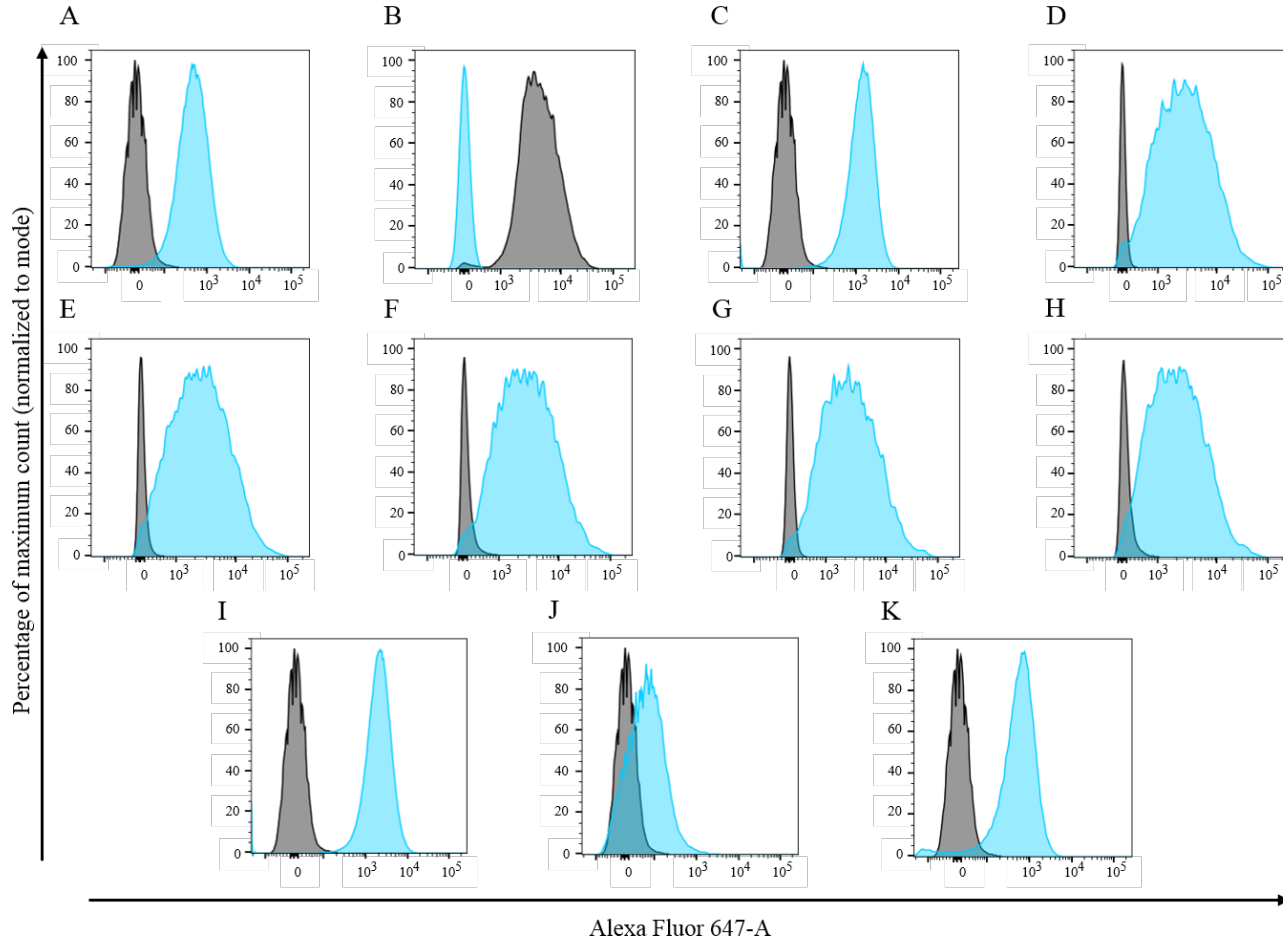


FIG S2: Binding of animal sera and affinity purified antibodies to the full T1 (black) and T18.1 (blue) pili expressed on the surface of *L. lactis* measured by flow cytometry. (A) T18.1 immunized rabbit (B) T1 typing serum (C) T3 typing serum (D) B1 mouse (E) B2 mouse (F) B3 mouse (G) B4 mouse (H) B5 mouse (I) T18.1 immunized rabbit serum passed over T18.1-coupled resin (J) T18.1 immunized rabbit serum passed over T3.2-coupled resin and (K) T18.1 immunized rabbit serum passed over T13-coupled resin.

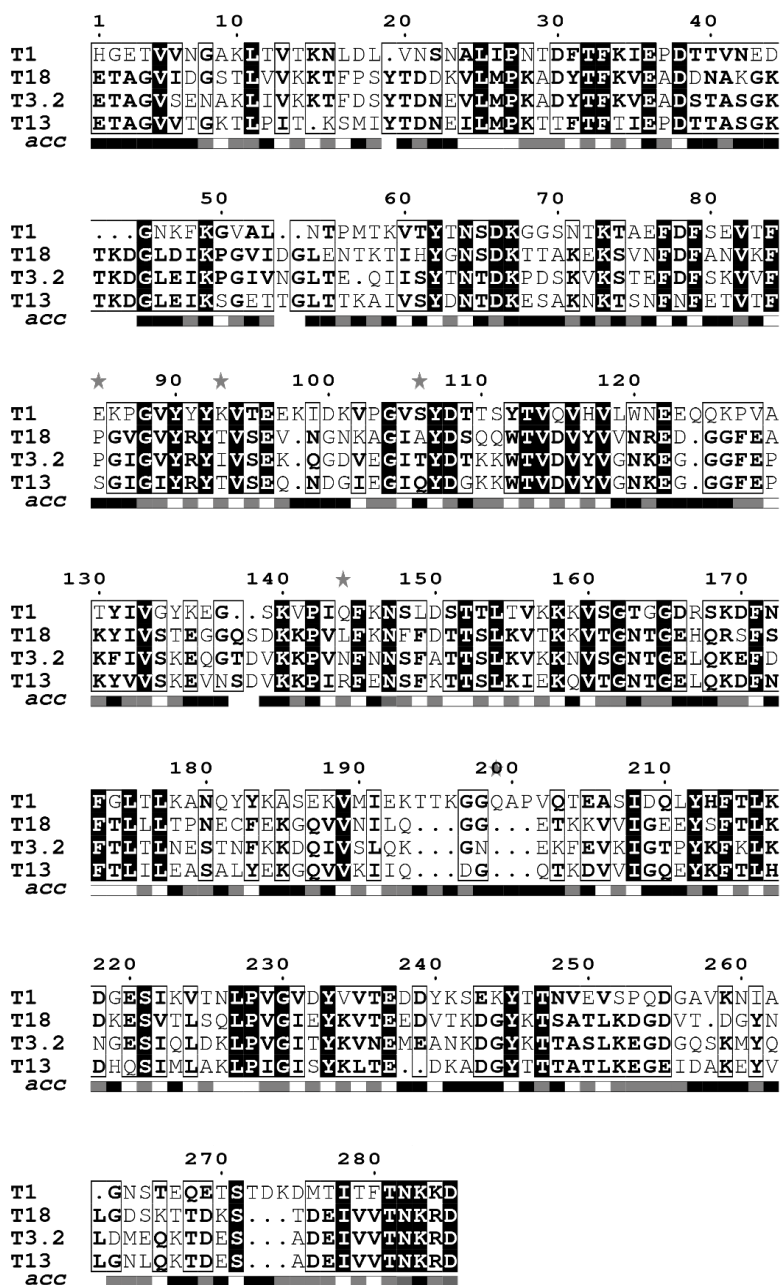


FIG. S3 Amino acid sequence alignment of T1, T3.2, T18.1 and T18.2. The sequences in the alignment represent the mature recombinant T-antigens used in this study. The residues are numbered according to the sequence of T1 and solvent accessibility (acc), predicted from the structure of T1, is shown as a bar below the sequences. Darker shading indicates more accessible residues.

TABLE S1. Construct details for the T-antigens used in this study.

Tee type	Strain No.	NCBI accession	emm type	Cloned sequence	Vector	PCR PRIMERS	Structure PDB accession
18.1	MGAS8232	NP_606439.1	emm18	ETAGVIDGSTLVVKKTFPSYTDKVLMPKADYTFKVEADDNAKGKT KDGLDIKPGVIDGLENTKTIHYGNSDKTTAKEKSNFDFANVKFPG VGVYRYTVSEVNGNKAGIAYDSQQWTVDVYVNVNREDDGGFEAKYIVS TEGGQSDKKPVLFKNFDTTSLKVTKKVTGNTGEHQRSFSFTLLLT PNECFEKQGVNVLQGGETKKVVIGEEYSFTLKDKESVTLSQLPVG IEYKVTEEDVTKDGYKTSATLKDGDVTDGYNLGDSTTDKSTDEIV VTNKR	pProEX Hta and pProEX Hta-AviTag	TEE18FWD: GTATTTTCAGGGCGCC GAGACAGCAGGAGTGATTGATGGTTCA TEE18REV: GACTGCAGGCTCTAGATTAGTCACGCT TATTGTGACAACGATTTTCGTC	6NOA This study
13	GAS131465	KJ816975	emm53	ETAGVVVGKTLPIITKSMIYTDNEILMPKTTFTTIEPDTTASGKTK DGLEIKSGETGLTTKAIIVSYDNTDKESAKNKTSNFNFETVTFSGI GIYRYTVSEQNDGIEGIQYDGKKWTVDVYVGNKEGGGFEPKYVVS EVNSDVKKPIRFENSFKTTLKIEKQVTGNTGELQKDFNFTLILEA SALYEKQVVKLIQDGGTKDQVIGQYKFTLHDHQSIMLAKLPIGI SYKLTEDKADGYTTTATLKEGEIDAKEYVLGNLQKTDESADIVVT NKR	MBP-pProEX Hta	M53_222FWD: AAAGGCGCCGAGACGGCAGGGGTGTGA AC M53_222REV: AAAGAATTCCTTAGTCACGCTTATTGT GACAACG	6BBT This study
3.2	GAS13637	KJ816984	emm65	ETAGVSENAKLIVKKTFFDSYTDNEVLMKADYTFKVEADSTASGKT KDGLEIKPGIVNGLTEQIISYTNTPDKPDSKVKSTEFDFSKVVFPGI GVYRYIVSEKQGDVEGITYDTKKWTVDVYVGNKEGGGFEPKFIIVSK EQGTDVKKPVNFNNSFATTSKLVKKNVSGNTGELQKEFDFTLTLNE STNFKKDQIVSLQKGNKFEVKIGTPYKFKLKNGESIQDLKLPVGI TYKVNEMANKDGYKTASLKEGDGQSKMYQLDMEQKTDESADIV VTNKR	MBP-pProEX Hta	M65104FWD: AAAGGCGCCGAGACGGCAGGAGTGTC G M65_104REV: AAAGAATTCCTTAGTCACGCTTATTGT GACAACG	6BBW This study
1	SF370	NP_268517.1	emm1	ATTVHGETVVNGAKLTVTKNLDLVNSNALIPNTDFTFKIEPDTTVN EDGNKFKGVALNTPMTKVITYTNSDKGGSNTKTAEFDFSEVTFEKP VYYYKVTTEKIDKVPVGSYDTSYTVQVHVLWNEEQKPVATYIVG YKEGSKVPIQFKNSLDSTLLTVKKVSGTGGDRSKDFNFGTLKAN QYYKASEKVMIEKTTKGGQAPVQTEASIDQLYHFTLKDGESIKVTN LPVGVYVVTEDDYKSEKYTTNVEVSPQDGAVKNIAGNSTEQETST DKDMTITFTNKKFE	pGEX-3c	TEE1FWD: CGGGATCCGCTACAACAGTTCACGG TEE1REV: CGGAATTCCTTATCAAGACTTTTTTA TTTG	3B2M <i>Kang et al. 2007</i> (1)
2	MGAS10270	ABF33174.1	emm2	EDTRVPSQTPDKTTVNIYKLGADFSKQPEGIKNENGEPIIDITKL KDTFGTAVTYLPGVKFKYKVNYSSTDDVLKSIKTVEQADSKTDL LDVAGAKETEATDQSGKVSIDLPSNDKVKYLFVSSNQDTVNKVVG YTAVPFIHLPLVSNNGKGYDEVNVPKNTTVNEPKVDKDVTKLG KDDDTYQIGDKITWFLKSTVPSNIKTLDKFGFTDNLNKLGSFIGDK TQTVTKVQFGTTVLSPD TDYTVVEILDSKLTVSLTSAGIEKVSGLVA SKQLITEAEKLYKAEDNTDEAAFLSVEVNAKLNADAVMGSRIENDV ELDYGHESDIYKSKVPTNEVPEVHTGGARFEKVDATNQTDLQDAE FGLYSNIEATEVVKWTEELLKANEAINAGKFESNTTVGTPIIFK SASDGSFEIKGLRYGDDSTNTRSDGTGTAETKGTYYIKELVAP KGYVVSQDIVQFDVYSSYYKDPKVTGLGTEAGDAAPTSTVKNKRP SIPNTGG	pProEX Htb	TEE2FWD: CGGGATCCGAGGACACCAGAGTGCCCT CTC TEE2REV: CGAAGCTTTTAACCACCAGTATTAGGG ATTGAAGG	-

5	GAS10514	KJ816997	emm82	ETAGVVTGKSLQVTKTMTYDDEEVLMPETAFTFTTIEPDMTASGKEG DLIDKNGIVEGLDKQVTVKYKNTDKTSQKTKIAQPFDFSKVFPFAIG VYRYMVSEKNDKDDGIRYDDKKTVDVYVGNKANNEEGFEVLYIVS KEGTSSTKPKIEFTNSIKTTSLSKIEKQITGNAGDRKKSFNFTLTLQ PSEYYKTGSVVKIEQDGSKKDVTIGTPYKFTLGHGKSVMLSKLPIG INYYLSEDEANKDGYTTTATLKEQGKEKSSDFTLSTQNQKTDESAD EIVVTNKR	pProEX Hta	TEE5FWD: AAAGGATCCTGAGACGGCAGGAGTTGT AAC TEE5REV: AAAGAATTCTTAGTCACGCTTATTTGT GACAACGAT	Modelled
6	MGAS10394	ABF33174.1	emm2	KDDTAQLKITNIEGGPTVTLYKIGEGVYNTNGDSFINFKYAEGVSL TETGPTSQEI TTIANGINTGKIKPFSTENVSISNGTATYNARGASV YIALLTGATDGRTYNPILLAASYNGEGLVTKNIDSKSNLYGQTS VAKSSLPSITKKVTGTIDVNNKTTSLGSVLSYSLTFELPSYTKEA VNKTVYVSDNMSEGLTFNFSNLTVEWKGKMANITEDGSMVENTKI GIAKEVNNGFNLSFIYDSLESISPNI SYKAVVNNKAI VGEENPNK AEFFYSNNPTKGNTYDNLDKKPKDNGITSKEDSKIYVYTYQIAFRK VDSVSKTPLIGAI FGVYDTSNKLIDIVTTNKNGYAISTQVSSGKYK IKELKAPKGYSLNTEYETITANVWTATVKTSANSKSTTYTSDKNKA TDNSEQVGLKNGIFYSIDSRPTGNDVKEAYIESTKALTDGTTFSK SNEGSGTVLLETDIPNTKL	pProEX Htb	TEE6FWD: CGGGATCCTTATCAAAGATGATACTG CACAAAC TEE6REV: CGAAGCTTTTATCCACCTGTCGAAGGT AATTCACC	4POD Young et al., 2014 (2)
9	GAS11262	KJ816988	emm74	EGGVSTGSILNVKKTFFSSYNDIEVLMFNATFTFKIQADTVKNGEKD KKSGLDIKTGIMGEGLDVQIVTYTNDSPKVDKEKNVNFDFSKVEFP NVGIYRYKVEEKGNAVGRYDDKTWTVDVVSENGNFIPKYIIS TTTENDKKPIVFDNEFTTSLIVKKQVLGNSGDKTEGDFDFTLLLKE NSLFEKQVSLIKITSDQKEEKVVTIGEKYDFKLDGEGVQLDK LPIGINYQVNEKDANTNGYTTAAILEGNGTSQPYTLDSLRETDL IDTITVTNKR	pProEX Hta	TEE9FWD: AAAGGATCCAGAAGGTGGAGTGAGTAC TGG TEE9REV: AAAGAATTCTTAGTCACGCTTATTTGT GACTGTAAT	-
10	emm89_198	KJ817015.1	emm89	ETAGVVSSGQLTIKKSITNFNDTLLMPKATFTFDVDPVDVTGEEK DTASGLKIQKGIAGVASQTIQYDNTDKPTNKEKAVNDFSTVTFPN VGVIYRYEVSEQAGDVKGITYDSKKWTVDVVYVNNENKFTPKYIVSK ETDSSKKPIVFNELKTTSLTIKKEVTGNSGDKTSDFTFTLLLKE NTQFETGQVKATKKKSGAEEETVEVTIGKQYFTTLKSEELILDK LPIGITYQVDETDKNKEQYETSAMTETSKEAQGYTLNLDLKTDET ADTITVTNKR	pET101/D- TOPO	TEE10TOPOF2: CACCATGGAGACGGCAGGGGTTG TEE10TOPOR: GTTTCTCTTATTGGTAACAGTGAT	Modelled
11	GAS05151	KJ817016	emm89	EVNYVKSGVIDGAKLEIHKTIKNDYDDKVLMPNVIFKFNIPDSDV VAGKKEEKSGLDIKPGIVDGLTSEKVSYSYNSDKPNSKDKYTYFDF SQVKFTNNGIYRYVVEEVQDTVSGIHYDSQKWYIDVYVVEGDNGFV PKYIVSSKLTLETKEPVLFSNSFDTTSLVVKKEVTGNTGDKTKNFKF QLLLKENAYFSAGQKVSVTITSEKASEVTTATIGQPLIFELKHNEQ LKLKLPVGIYQIDETSKNSDSYTTATI QEGEQSQNSYVLNSDK ETDKSPDIITVTNKR	pProEX Hta	TEE11FWD: AAAGGCGCCGAAGTAAATATGTAAAA TC TEE11REV: AAAGAATTCTTAGTCACGCTTATTTGT GACTG	Modelled
12	GAS12303	KJ816951	emm12	ETAGVVSSGQLTIKKSITNFNDTLLMPKTDYTFVSNPDSAAATGTE SNLPIKPGI AVNNQDIKVSYSNTDKTSGKEKQVVVDFMKVTFPSVG IYRYVVTENKGTAEGVYDDTKWLVDVYVGNNEKGLLEPKYIVSKK GDSATKEPIQFNNSFETTSLSKIEKEVTGNTGDHKKAFNFTLTLQPN EYVEASSVVKIEENGQTKDVKIGEAYKFTLNSQSIVLSKLPVGIN YKVEEAANQGGYTTTATLKDGEKLSYNYLQGEHKTDKTADEIVVT NNRD	pProEX Hta	TEE12FWD: AAAGGATCCTGAGACGGCAGGGGTTGT TAG TEE12REV: AAAGAATTCTTAGTCACGCTTATTTGT GACAACGAT	Modelled

18.2	NZ131 Spy49_0114	ACI60465	emm49	ETAGVIDGSTLVVKKTFPSYTDNVLMPKADYSFKVEADDNAKGKT KDGLDIKPGVIDGLENTKTIIRYSNSDKITAKEKSVNFEFANVKFPG VGVYRYTVAEVNNGKAGITYDSQQWTVDVVVVNKEGGGFVVKYIVS TEVVGQSEKKPVLFKNSFDTTSLKIEKQVTGNTGEHQRLFSFTLLLT PNECFEKQVNVNFLQGGETKKVVIGEYSF TL KDKESV TL SQIPVG IEYKLT EED VTKDGYKTSATLKDGEQSSTYELGKDHKTDKSADEIV VTNKRDTQVPT	pProEX Hta	M49FWD: AAAGCGCCGAGACAGCAGGAGTGATT GATG M49REV: AAAGAATCCTTAAGTTGGAACCTTGAGT GTCACGC	Modelled
25	LN144	EU725507	emm75	IPSSTVRAETLQDR TQ KTIPEATEV VIT KLQADDYNDV KP NGKAN ENGLP INN L GEL GRNVKPLSDVTFVAYKIPEGIAEEKVKELKTKQT VVDVENYLNAQNLKIEKTVLTKTDGNGQTTFTVQKSSYGKVFVVED MTATGTPETISKAYAVPFTLELPISASDGTGYLTKVNIYPKNVTSS LPKPGKDVKELGLNHSSYNI GER FSWFLKGTVPKNMLDYEKYSFTD TLDSQLDFISVKS VK YGSQILEKNNDYTFSEPTAQNRTLKVELTEA GIKKVAGLYPDRQEVLDTEIEAIKENTDQKPFLEVEFETNINSTVI LGKPV TNE VKIEFDNKPDKIAKPV TTP SDNPEVHTGGKRFVKVAA GNDATKLGGAEFDLLTEANQPINWTAELIRANNKSEYIVGTPQEGQ PVK LK SDTDGSFEIKGLAYAIDA EAT GAGVKYK LK ETKAPAGYVIP EAPIEF AV NQTSYNKTP TT IDVDKADAEPQKVENNRPE	pProEX Hta	TEE25FWD: AAAGGATCCAATACCAAGTAGTACGGT AAGGG TEE25REV: AAAGAATCCTTATTCAGGACGTTTGT GTTTTCTAC	-
28.2	GAS11209	KJ817028	emm91	ETAGV TNG AQLTIKKTIAN YND SEVLMPKAI FT FV KP DNSV TG VE KTVDGLTIKAGIAEGLVKTGNVEYSNTDKVENKDKT TT FD F STVKF PEVGVYRYTVSETDSK VSG IKYDTKTWIVDVVVNDGNGGFKAQYI VSKEKQNDKKPVV FENS FKT TS LKVEKQVTGNTGELKKDFNFTLL INPNDNFVAGQVIKLEKGGIKADV KI GEPYK FAL KNGEKV TL SKLP VGITYSIIEDDAGKDGK YK TTAILKDGEQSSTYELGKNQK TD ESADE IVVTNNRE	pProEX Hta	TEE28.1FWD: AAAGGATCCTGAGACGGCAGGGGTAAC TAA TEE28.1REV: AAAGAATCCTTATTCACGGTTATTTGT GACAACGAT	Modelled

REFERENCES

1. Kang HJ, Baker EN. 2009. Intramolecular Isopeptide Bonds Give Thermodynamic and Proteolytic Stability to the Major Pilin Protein of *Streptococcus pyogenes*. *Journal of Biological Chemistry* 284:20729-20737.
2. Young PG, Moreland NJ, Loh JM, Bell A, Atatoa Carr P, Proft T, Baker EN. 2014. Structural conservation, variability, and immunogenicity of the T6 backbone pilin of serotype M6 *Streptococcus pyogenes*. *Infect Immun* 82:2949-57.