

Supplementary materials

Accession Codes for phylogenetic tree:

Trichomonas vaginalis

TvagENT1 (TVAG_166380), TvagENT2 (TVAG_192810), TvagENT3 (TVAG_271560), TvagENT4 (TVAG_441760), TvagENT5 (TVAG_483030), TvagENT6 (TVAG_053320) TvagENT7 (TVAG_101510), TvagENT8 (TVAG_271570), TvagENT9 (TVAG_341290)

Trypanosoma brucei brucei

TbNT2 (Tb.927.2.6150), TbNT3 (Tb.927.2.6200), TbNT4 (Tb.927.2.6220), TbNT5 (Tb.927.2.6240), TbNT6 (Tb927.2.6320), TbNT7 (Tb.927.6280), TbNBT8.1 (Tb927.11.3610), TbNBT8.2 (Tb927.11.3620), TbNBT8.3 (Tb.927.11.3620), TbNBT8.3(Tb.927.11.3630), TbNT9 (Tb.927.6.220), TbNT10 (Tb.927.9.7470), TbAT1 (Tb927.5.286b), TbNT12 (Tb927.3.590), TbNT11 (Tb927.3.590)

Leishmania donovani

LdNT1.1 (LdBPK_151230.1), LdNT1.2 (LdBPK_151250.1), LdNT2 (LdBPK_362040.1), LdNT3 (LdBPK_131110.1), LdNT4 (LdBPK_110520.1)

hENT1 (Q99808-1), hENT2 (Q14542-1), hENT3 (Q9BZD2), hENT4 (BC047592.1)

PfNT1 (PF3D7_1347200), PfNT2 (PF3D7_0824400), PfNT3 (PF3D7_1469400), PfNT4 (PF3D7_0103200)

Supplemental Figure S1. Multiple alignment of the protein sequences of the TvagENTs, generated by CLUSTAL Omega (1.2.4). For TvagENT9, trans-membrane segments are highlighted (TrichDB).

TvagENT1	MNSDDEAVTIDIEAQSPPKSPPRSPNTKSNLLDPFSFYSKGNSNVDTPKTPQKQKNSTEK	60
TvagENT6	MSQEDPQVVEDVESGEVKKDSEYT-----S---	25
TvagENT3	MNQEDPETMHDLEAEPEKSDE-----	21
TvagENT2	-----	0
TvagENT8	MTQEDPAILHDVEAEPEKSES-----	21
TvagENT7	-----	0
TvagENT9	-----MK---KELN	6
TvagENT4	-----	0
TvagENT5	-----	0
TvagENT1	QPITPTKQSNADANSVHSR-DIEIPNEDEDKITFGIEAIFMLLGTNVLFSYNTFINGLDF	119
TvagENT6	----DDKKSEESSTQLDNKDSEKKDDNIENRVTFTEIEMFFFLIGTNIILYSYNTFLNGNDF	81
TvagENT3	-----DLSKEMDE-----KKDDIDSRTIGIQLVFFFFIGINILFSYNTFLSGTDF	66
TvagENT2	-----	0
TvagENT8	-----YSSKDLDNKS-DDKKSEDDVKISLGIQLIFFLIGTNIILFSYNTFLSGTDF	70
TvagENT7	---M---ADMKEDPLLTPE-S-TPNKPSKKNENSESLMFFFLGNTSLLAFNIIINAIDI	51
TvagENT9	IPLM---SDSISDQLLEES---EAKKKAMPANPE <u>SLMFFFLGNTSLLCFNIIINAIDI</u>	58
TvagENT4	---M---TLLDNRQLLEEK-DILISDYSEMTDPDQALFFWLGNASLLVYNVINAIDI	52
TvagENT5	-----MFIDANKLIETS-SGAGEDVRTVSKSESWLFFWLGNVSLLVFNIAINAIDI	51
TvagENT1	YDTLFPGKN-----APTNIARAYNITASLIY--IFSLPFIERFT-L---VTRFYFSS	165
TvagENT6	FVKLFPE-----RDIGMELSRVLTISSGVCY--LITLFPFIEQFT-L---VSRMYAST	127
TvagENT3	YESITIGQKYTYGSLAKIQRDLPRVLILTSEFCN--LCSLPFIERFT-L---VSRLYFSL	120
TvagENT2	-----MFLRTSATLNGKSVTLNGRTSFR-L---VSRLYFAM	32
TvagENT8	YGSLVGHQRYTYGGKVNIGRDLPRVLIITSETVN--LCSLPFIESFR-L---ISRLYFSM	124
TvagENT7	YAKKTNRSD-----MASLLNRSYNI PNALMALFLCIFKPTNYKISLISALASLTFIM	103
TvagENT9	YNKLTGRTD----- <u>MAGILNRTYNFPNALMALLCFFKPTNYKISIIIFALGSLSFIM</u>	110
TvagENT4	YIHLSHRKS-----VGNDLARAYNFPCLIALVLCFIKIPNQKILFIIISLLVLPFDL	104
TvagENT5	YAIITNDKN-----IGNDLNRAYNIPSSIAALILCFVTIRNHKRSFIISITSLIIV	103
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TvagENT1	IGITIMMFFTFLYSNIGTPIYGVIIIGAAVFAALFSGILFGTTMGFAGLFGANCSVCTAG	225
TvagENT6	ILMAAVQIIVWIYVNVGTPKYQVYVYTLAALTSVAQSVLYGTSMGFAGLFGKTSAMANTG	187
TvagENT3	TMMAIVQIVIFYVNFNGPQYYIYLLAALTSAAQSVFMFGSSMGFAGLFGKTSALANTG	180
TvagENT2	TLMAIVQIVIFFYVNFNGPQYYIYLLAALTSAAQSVLFGSSMGFAGLFGDKTSALANTG	92
TvagENT8	TIMAIVQIVIFYVNFNGDPQYYIYLLAALTSAAQSVIFGSSMGFAGLFGDKTSALANTG	184
TvagENT7	CFLPIFLII---H-LNANAFLYLTLVVIGLTVISSLFSSIFSFASQFGPISSAMASSG	159
TvagENT9	<u>CFLPIFILII---D-IDDRVMFYLTLSIIGLTVISSLFSSVFSMASQFSPVSSAMASSG</u>	166
TvagENT4	LAFPLLIII---P-MSESVVYWGTTAAITVSGVFSSIMSGSFAVSTQFADETAGFISAG	160
TvagENT5	SVMAASMIII---K-MPAKIVVYISVVISGLSGVFSAILSSSFALATQFAPESSTSVSSG	159
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TvagENT1	LALGGLVTSIVRVLSKLM-----GKGEGWFYFGFTVVFNTCSVIAFILFKRRPIAIRRI	279
TvagENT6	VALGGLISSILRMISKTF-----PRGDGWFYFAFAVICTSSSAICFHIFNKTEICQERV	241
TvagENT3	VALGGLITSILWVIAKNAFNGS--LKKQGLFYLFVSVLVTISTLITFHFFFSKTEIGQERL	238
TvagENT2	VALGGLITSLLWVAKAAFPNS--VKNQGVFYLFVSCFVTVKTALTFHFFSRTEAAQKKL	150
TvagENT8	VALGGLITSLLWILAKGVFPNS--VRNQGFYLFVSCFVSIATAVTFHFFSRTEIAQKRL	242
TvagENT7	CGCCGVIASVLRITKAAAVTDRANLYSTCAYFFISAGIIFLTLVFFLFKMQKPEIRLKM	219
TvagENT9	<u>CGCCGVLASVLRITKAAIATGKANLYSTAAAYFFISAAIIFFTLIFVIRNPEIKEKL</u>	226
TvagENT4	NGLCGILAAVARIITKGLFSSESQKLISSIVYFALAALTLGLTILFFILKLRNPDISNRF	220
TvagENT5	NGLCGVLAALRITTKAVFSSESLKYSSFCYFILTAIILATLIYFIKKVNQPSISSKF	219
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TvagENT1	SHSHTSTDFLDRMKRIKGVFLKIWPVLEACLMMITLTLFPGYACSIKSKHGLSKDWVT	339
TvagENT6	KLAQTSSNFLVRMKRIGGVFKKIWPVLEGGFNMAITLTLFPGYAFYVGDHNN-LGDWYM	300

TvagENT3 KLAQTSNDFMFRMKRIKGVFLKIWPFVIEGWLHFAITLTFPPSYMFYAGNQHFKEFGDFI 298
TvagENT2 KLAQTSNDFMYRVRRIKGVFLKIWPFVIEGWLHFAITLTFYPGYMFLAGNQHFKEFGWFT 210
TvagENT8 KLAQTSNDFFRLLKRIKGVFLKIWPFVIEGWLHLTITLTFYPGYMFLAGNQHFKEFGWFT 302
TvagENT7 IPASKSEKVAIFNRETLVVIKSIWVSWLSVFANFLITLSIFPGYVANT-RATKQIGDWT 278
TvagENT9 VPKV-EEKVSIFSRRETITVIKSIWVSWLSVFANFLITLSIFPGYVTGT-YTPPKIRDWTF 284
TvagENT4 IFNSYQKENTAFISQIFTTLKSIIWLLWIAEALTYFITLIIFPGYVCSG-PE-GPLKSWTF 278
TvagENT5 IFPAETVDIKSFLRSLKGTFKHISLLWIGCVLDFMITLTIFPGYVCAG-PE-GALKSWNP 277

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TvagENT1 TLVTSFYMVGDFGRFLFTRWWAWPSAKWLWVPHISRLIFFVLYIPIESVFL-EDDIFIY 398
TvagENT6 TIILFMFMVGDFVGRFLITRWFWSWPSAKYLWIPHLRLLIFIVLYVCPVEGVFL-QDDIFID 359
TvagENT3 TAVIFCYNVGDVFLGRFITRWFPPKPKYLWIPHLRLLFIPLIVVSAEVPKF-RSDIYMC 357
TvagENT2 TVMILCYNIGDFVGRFMTRFFSWPKPKYLWIPHALRLLFIPLIVVSVVEVPKL-RSDILMC 269
TvagENT8 TVMILCYNIGDFLGRFVTRFFLWPKPKYLWIPHALRLLFIPLIVVSVVEVPKL-RSDVYMC 361
TvagENT7 VIVVTIFCVFDWVGRAGPGLFIWPPRKFAPWIPIVLRFLSYPIFIVSIQHKFK-AEPWTF 337
TvagENT9 VIVVAIFCVFDWVGRAGSGLKVPRLFAWIPIVLRFFSFPFIIFSISIQKRFK-AEPWTF 343
TvagENT4 VLITTVFCIFDFIGRFVASKFIWPSLNMSPLASVFRIFIFIPLEIISIQKIVNFREPWFTL 338
TvagENT5 VCITTFFCIFDWLGRWLPKFLWPSLNLTIFPILRLLFFPIEIIISLQKVLNLGEPWFTL 337

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TvagENT1 FVTLALALTGGFWIGLCITYTAKDEKLEEEDEVELAVFCTSLALNLGIFIGSWLTYALPTH 458
TvagENT6 FVTLALSALTGGYWGGLCITYTATSEKLEKEEIDLAVFCTVLATNLGVFAGSWLTFAAQAG 419
TvagENT3 VMAFLLAVTTGYFGGLCIVYTATCENLATEEIDLGVFTTVLATNLGVFTGVWLTFLSNL 417
TvagENT2 IMAFLLSVTTGYFGGLCIVYTATSEKLEKEEIDLGVFTTVLATNLGVFKGVWLTFLSNL 329
TvagENT8 IMSFLLAVTTGYFGGLCIVYTATSEKLEKEEIDLGVFTTVLATNLGVFTGVWLTFLSNL 421
TvagENT7 GWMIPFALSNGYFGTVQMIYGSNPDELTLQKRFAGFLMSFAVNAGILCAMGLTFLLP 397
TvagENT9 AWMIPFAISNGYFGTVQIIYGSNPDELNSEQKKFAGFIISFAVNAGILCAMGLTFLMPK 403
TvagENT4 ALQIPFALTNGYVGTILMIYGSNHPDLSEKKLKLAGYLMTFAINVGI I I AMFLTFLPKP 398
TvagENT5 FMQIPFAITNGYFGTTVMIIYASIHKPLTDEQKKTAGFMMSFAINFGI I I AMFLTYAMP 397

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TvagENT1 ----- 458
TvagENT6 HK----- 421
TvagENT3 HEKYSAP 424
TvagENT2 HEKYSAP 336
TvagENT8 HEKYSPO 428
TvagENT7 PKFN--- 401
TvagENT9 PEYNQ-- 408
TvagENT4 SI----- 400
TvagENT5 KA----- 399

Supplemental Table 1: Primers for qRT-PCR amplification of native TvagENTs

Gene	primer	F / R	Sequence
TvagENT1	HKD908	F	CGCCCACCAAGCAGAGTAA
TvagENT1	HDK909	R	TCGGTATTTCTATGTCACGACTATGC
TvagENT2	HDK910	F	GTGTCGGTGGGAAGTCCCTAAAC
TvagENT2	HDK911	R	CAGGAATGCCATAATGCACATC
TvagENT3	HDK912	F	CTCGCTGTGACAACGGGATA
TvagENT3	HDK913	R	TTCGCACGTAGCCGTATATACAA
TvagENT4	HDK914	F	GCGGAGGCACTTACATACTTCA
TvagENT4	HDK915	R	CGGTCCGCTGCACACAT
TvagENT5	HDK916	F	GGGTAGGTGGCTTCCATCAA
TvagENT5	HDK917	R	GCAAGAGAATCGGGAATATCGT
TvagENT6	HDK918	F	CGCTGGCGGCATTAACA
TvagENT6	HDK919	R	GCCCATGGAGGTACCATAAAGA
TvagENT7	HDK920	F	TGAAGATGATACCAGCCAGCAA
TvagENT7	HDK921	R	CAATGTTTCGCGGTTGAAAA
TvagENT8	HDK922	F	GGCCACGGAGGAGATTGA
TvagENT8	HDK923	R	CCAAGGTTGGTTGCCAGAAC
TvagENT9	HDK924	F	CGTTTTTTTTCCTCCCCATT
TvagENT9	HDK925	R	CACCACGGCTCGGCTTTA

Supplemental Table 2: Primers to amplify TvagENTs to insert into pHD1336

Gene	Number	F / R	Sequence
TvagENT1	HKD1127	F	CAGTCTAGAATGAACAGTGACGACGAAGCTGTGA
TvagENT1	HDK1128	R	CAGGGATCCATGCGTGGGGAGAGCGTACG
TvagENT2	HDK1129	F	CAGTCTAGAATGTTTCTTCGGACTAGTGCCACC
TvagENT2	HDK1130	R	CAGGGATCCTGGGGCTGAGTATTTCTCATGGAG
TvagENT3	HDK1131	F	CAGTCTAGAATGAACCAAGAAGACCCTGAGACT
TvagENT3	HDK1132	R	CAGGGATCCGGGGCAGAATACTTCTCGTG
TvagENT4	HDK1133	F	CAGTCTAGAATGACTCTGTTAGATAATAGGCAGTTAC
TvagENT4	HDK1134	R	CAGGGATCCTATGGATGGCTTGGGCAGTATAAAT
TvagENT5	HDK1135	F	CAGTCTAGAATGTTTATAGACGCTAACCAAGCTCATC
TvagENT5	HDK1136	R	CAGGGATCCGGCCTTAGGAATCGGCATAG
TvagENT6	HDK1137	F	CAGTCTAGAATGTCGCAGGAGGACCCTCA
TvagENT6	HDK1138	R	CAGGGATCCTTTATGTCCGGCCTGTGCGG
TvagENT7	HDK1139	F	CAGTCTAGAATGGCAGATATGAAGGAAGACCCG
TvagENT7	HDK1140	R	CAGGGATCCAATTGAACTTCGGTGGCGTAGGC
TvagENT8	HDK1141	F	CAGTCTAGAATGACTCAGGAAGACCCCGCTAT
TvagENT8	HDK1142	R	CAGGGATCCTTACTGAGGACTGTACTTCTCATGAAGATT
TvagENT9	HDK1143	F	CAGTCTAGAATGAAGAAGGAATTGAATATTCCTTG
TvagENT9	HDK1144	R	CAGGGATCCCTGGTTATATTCAGGGGGCTTC