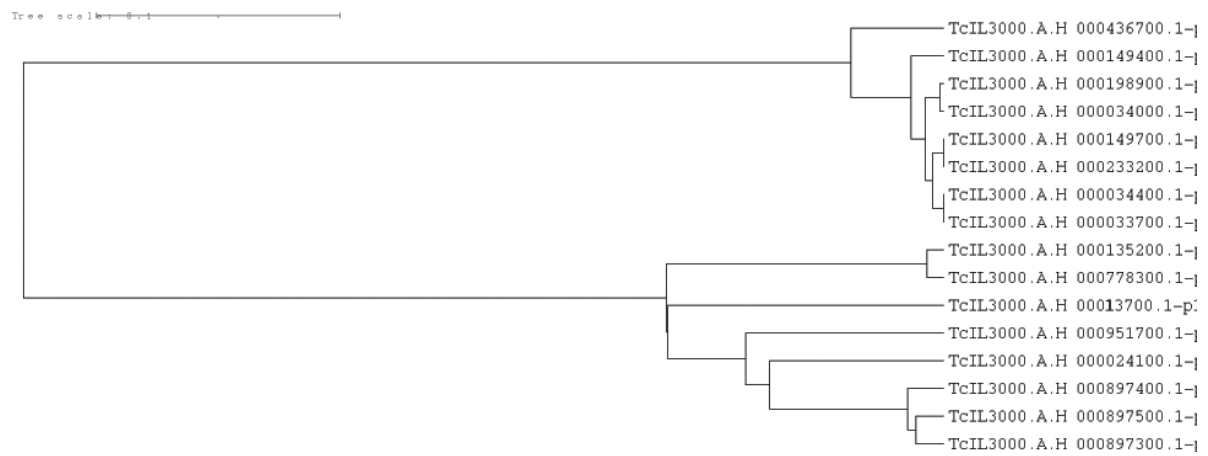


Supplemental Figure S1. Tree of nucleobase transporters from *T. congolense* IL-3000.



Tree was constructed by CLUSTAL Omega (1.2.3) multiple sequence alignment in TriTrypDB

Made by CLUSTAL Omega (1.2.3) multiple sequence alignment in TriTrypDB

TcIL3000.A.H_000436700.1-p1	MASDTSFATKTYVCVTCFILDVCAYWAVETRFLAPIFMASHYKYITGNEHAEPSSVLFWL
TcIL3000.A.H_000149400.1-p1	MASDTSFATKTYVCVTCFILDVCAYWAVETRFLAPIFMASHYKYITGNEHAEPSSVLFWL
TcIL3000.A.H_000034000.1-p1	MASDTSFATKTYVCVTCFILDVCAYWAVETRFLAPIFMASHYKYITGNEHAEPSSVLFWL
TcIL3000.A.H_000198900.1-p1	MASDTSFATKTYVCVTCFILDVCAYWAVETRFLAPIFMASHYKYITGNEHAEPSSVLFWL
TcIL3000.A.H_000033700.1-p1	MASDTSFATKTYVCVTCFILDVCAYWAVETRFLAPIFMASHYKYITGNEHAEPSSVLFWL
TcIL3000.A.H_000034400.1-p1	MASDTSFATKTYVCVTCFILDVCAYWAVETRFLAPIFMASHYKYITGNEHAEPSSVLFWL
TcIL3000.A.H_000233200.1-p1	MASDTSFATKTYVCVTCFILDVCAYWAVETRFLAPIFMASHYKYITGNEHAEPSSVLFWL
TcIL3000.A.H_000149700.1-p1	MASDTSFATKTYVCVTCFILDVCAYWAVETRFLAPIFMASHYKYITGNEHAEPSSVLFWL
TcIL3000.A.H_000778300.1-p1	MALGFSSAAEVVVYTCTIFLGMSILLPLNTRYSAFMFMINYKYVTGDENAEPNSKLFWA
TcIL3000.A.H_000135200.1-p1	MALGFSSAAEVVVYTCTIFLGMSILLPLNTRYSAFMFMINYKYVTGDENAEPNSKLFWA
TcIL3000.A.H_000113700.1-p1	MALGFSSAAEVVVYTCTIFLGMSILLPLNTRYSAFMFMINYKYVTGDENAEPNSKLFWA
TcIL3000.A.H_000951700.1-p1	MALGLSSSAAEVVVYTCLFLGVSIILLPLNTRYSAFMFMINYKYVTGDENAKPNSVLFWA
TcIL3000.A.H_000024100.1-p1	MALGLSSSAAEVVVYTCTIFLGVSILLPSSTRYSAPMFMVNYKYVTGDENAEPNSVLFWA
TcIL3000.A.H_000897400.1-p1	MALGFSSAAEVVVYTCLLLGMSILLPLNTRYSAFMFMVNYYKYVTGDENAEPNSVLFWA
TcIL3000.A.H_000897300.1-p1	MALGFSSSAAEVVVYTCLLLGVSILLPLNTRYSAFMFMVNYYKYVTGDENAEPNSVLFWA
TcIL3000.A.H_000897500.1-p1	MALGFSSSAAEVVVYTCLLLGVSILLPLNTRYSAFMFMVNYYKYVTGDENAEPNSVLFWA
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TcIL3000.A.H_000436700.1-p1	NIFTFHNLILFAARALAERPELERFVRGWGLRLRFTLSIVLILAEALIFFLPVLSMPLP
TcIL3000.A.H_000149400.1-p1	NIFTFHNLILFAARALAERPELERFVRGWGLRLRFTLSIVLILAEALIFFLPVLSMPLP
TcIL3000.A.H_000034000.1-p1	NIFTFYNLILFAARALAERPELERLRVGWLRLRFTLSIVLILAEALIFFLPVLSMSPP
TcIL3000.A.H_000198900.1-p1	NIFTFYNLILFAARALAERPELERLRVGWLRLRFTLSIVLILAEALIFFLPVLSMSPP
TcIL3000.A.H_000033700.1-p1	NIFTFHNLILFAARALAERPELERLRVGWLRLRFTLSIVLILAEALIFFLPVLSMSPP
TcIL3000.A.H_000034400.1-p1	NIFTFHNLILFAARALAERPELERLRVGWLRLRFTLSIVLILAEALIFFLPVLSMSPP
TcIL3000.A.H_000233200.1-p1	NIFTFHNLILFAARALAERPELERLRVGWLRLRFTLSIVLILAEALIFFLPVLSMSPP
TcIL3000.A.H_000149700.1-p1	NIFTFHNLILFAARALAERPELERLRVGWLRLRFTLSIVLILAEALIFFLPVLSMSPP
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TcIL3000.A.H_000135200.1-p1	NILTFYGAISLVLIQSLVGPTVLTQAARRLSLSVRFTLSITSMIMEVFVVLMPVIKVSQT
TcIL3000.A.H_000113700.1-p1	NILTFYGVISLVTQSLIGTAVFTSTICRVLSLSRFALSTIFMMIEFFVVMPIKVSQT
TcIL3000.A.H_000951700.1-p1	NILTFYTVISLVTQSFGVPTVLTQTVRRLSLSLRFALSTITLMMTEVFVVLMLPITIKVSQT
TcIL3000.A.H_000024100.1-p1	NILTFYTVISLVTQSFGVPTVLTQTVRRLSLSLRFTLSITLMMVEVFVVLMLPVIKVSQT
TcIL3000.A.H_000897400.1-p1	NILTFYNVISLVTQSLIGTPTVLTPTVRRLSLSLRFALSTIFMMMEVFVVLMLPVIKVSQT
TcIL3000.A.H_000897300.1-p1	NILTFYNVISLVTQSLIGTPTVLTPTVRRLSLSLRFALSTIFMMMEVFVVLMLPVIKVSQT
TcIL3000.A.H_000897500.1-p1	NILTFYNVISLVTQSLIGTPVLTPTVRRLSLSLRFALSTIFMMMEVFVVLMLPVIKVSQT
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TcIL3000.A.H_000436700.1-p1	NAIVSLCFTTILGTIGKQFLGRTHTLWDFFLTEKAGGVIRLTAFLVAIVHATLCIIVVV
TcIL3000.A.H_000149400.1-p1	NAIVSLCFTTILGTIGKQFLGRTHTLWDFFLTEKAGGVIRLTAFLVAIVHAMLCIIVVV
TcIL3000.A.H_000034000.1-p1	YAIVSLCFTTILGTIGKQFLGRTHTLWDFFLTEKAGGVMRFTAFLVAIVHATLCIIVVV
TcIL3000.A.H_000198900.1-p1	YAIVSLCFTTILGTIGKQFLGRTHTLWDFFLTEKAGGVMRFTAFLVAIVHATLCIIVVV
TcIL3000.A.H_000033700.1-p1	YAIVSLCFTTILGTIGKQFLGRTHTLWDFFLTEKAGGVIRLTAFLVAIVHAMLCIIVVV
TcIL3000.A.H_000034400.1-p1	YAIVSLCFTTILGTIGKQFLGRTHTLWDFFLTEKAGGVIRLTAFLVAIVHAMLCIIVVV
TcIL3000.A.H_000233200.1-p1	YAIVSLCFTTILGTIGKQFLGRTHTLWDFFLTEKAGGVIRLTAFLVAIVHATLCIIVVV
TcIL3000.A.H_000149700.1-p1	YAIVSLCFTTILGTIGKQFLGRTHTLWDFFLTEKAGGVIRLTAFLVAIVHATLCIIVVV
TcIL3000.A.H_000778300.1-p1	TAIVFFVIVTIIVGGIGKCYLEATCYTLVAPLPKPFKMSTIMFGICFCGVVSTIQCI IKAS
TcIL3000.A.H_000135200.1-p1	TAIVFFVIVTIIVGGIGKCYLEATCYTLVAPLPKPFKMSTIMFGICFCGVVSTIQCI IKAS
TcIL3000.A.H_000113700.1-p1	TAIVFFVIVTIIVGGIKWCYLEATCYTLVAPHVPKPFKMSTIMFSCFCGAISTIRCI IKAS
TcIL3000.A.H_000951700.1-p1	TAIVFFVIATILGGMGKPCLEATSYTLAASMPKEKFTSTIMFGISICGLLTSMMQCIVKLS
TcIL3000.A.H_000024100.1-p1	VAIVFFVIVTIIVGGIGKCYLEATCYTLVAPLPKPFKMSTIMFGISICGVLTTSTMQCIIKVS
TcIL3000.A.H_000897400.1-p1	VAIVFFVIVTIIVGGMGKSYLEATSYTLAASMPKFKMSTIMFGISICGVLTTSTMQCIIKVS
TcIL3000.A.H_000897300.1-p1	VAIVFFVIVTIIVGGMGKCYLEATSYTLAASMPKFKMSTIMFGISFCGMTLSTMQCIIKVS
TcIL3000.A.H_000897500.1-p1	VAIVFFVIVAILGGMGKCYLEATSYTLAASMPKFKMSTIMFGISICGVLTTSTMQCIIKVS
	***: :. *: : * * *: : : : :. :. :. **:
TcIL3000.A.H_000436700.1-p1	P GTTGESRENTLSLHTVVYAVVLVIALVMVLSLSLIRCTREGFRSAQRAAQKGRVEGINH
TcIL3000.A.H_000149400.1-p1	P GTTGESRENTLSLHTVVYAVVLVIALVMVLSLSLIRCTREGFRSAQRAAQKGRVEGINH
TcIL3000.A.H_000034000.1-p1	P GTTGESRENTLSLHTVVYAVVLVIALVMVLSLSLIRCTREGFRSAQRAAQKGRVEGINH
TcIL3000.A.H_000198900.1-p1	P GTTGESRENTLSLHTVVYAVVLVIALVMVLSLSLIRCTREGFRSAQRAAQKGRVEGINH
TcIL3000.A.H_000033700.1-p1	P GTTGESRENTLSLHTVVYAVVLVIALVMVLSLALIRCTREGFRSAQRAAQKGRVEGINH
TcIL3000.A.H_000034400.1-p1	P GTTGESRENTLSLHTVVYAVVLVIALVMVLSLALIRCTREGFRSAQRAAQKGRVEGINH
TcIL3000.A.H_000233200.1-p1	P GTTGESRENTLSLHTVVYAVVLVIALVMALSLSLIRCTREGFRSAQRAAQKGRVEGINH
TcIL3000.A.H_000149700.1-p1	P GTTGESRENTLSLHTVVYAVVLVIALVMALSLSLIRCTREGFRSAQRAAQKGRVEGINH
TcIL3000.A.H_000778300.1-p1	MEDTYESHILTQSYIYFSALLFMMAALVMALSRLRNSYAQMVAEYRVLKQKQEGQKV EP
TcIL3000.A.H_000135200.1-p1	MEDTYESHILTQSYIYFSALLFMMAALVMALSRLRNSYAQMVAEYRVLKQKQEGQKV EP
TcIL3000.A.H_000113700.1-p1	MEDTYESHILTQSYIYFSALLIMAVALVMVLTLYHNSYAQQHVAEFRVLKQKH DENNV EP
TcIL3000.A.H_000951700.1-p1	

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TcIL3000.A.H_000198900.1-p1      LIERVGKTR-KTLESIRVRLHAQSDPILRNEVQKEHQAKRETVEAQEDIRRR----- 292
TcIL3000.A.H_000033700.1-p1      LIERVGKTR-KTLESIRVRLHAQSDPILRNEVQKEHQAKRETVEAQEDIRRR----- 292
TcIL3000.A.H_000034400.1-p1      LIERVGKTR-KTLESIRVRLHAQSDPILRNEVQKEHQAKRETVEAQEDIRRR----- 292
TcIL3000.A.H_000233200.1-p1      LIERVGKTR-KTLESIRVRLHAQSDPILRNEVQKEHQAKRETVEAQEDIRRR----- 292
TcIL3000.A.H_000149700.1-p1      LIERVGKTR-KTLESIRVRLHAQSDPILRNEVQKEHQAKRETVEAQEDIRRR----- 292
TcIL3000.A.H_000778300.1-p1      Q--PVADVPEKVEKPTDGEED----PQ----NKEENKGEGEVGMTTAEQLAATAVMPVVK 290
TcIL3000.A.H_000135200.1-p1      Q--PVADVPTGKPTCGEEG----PQ----NKEESKGEGEVGMTTAEQLAATAVMPVVK 290
TcIL3000.A.H_000113700.1-p1      Q--PVADVPEKVEKPTGCEDEK---C-----DVDSKGEGCMTTAEQLAATPIMPVVR 251
TcIL3000.A.H_000951700.1-p1      Q--TAADVPTGKPTDVEDK----S-----DVDSRGEGGMMTTAEQLVATPIMPVVR 286
TcIL3000.A.H_000024100.1-p1      Q--PVADVPTGKPTSCCEDEK---C-----DVDSKEEGCITTAEQLSPTPIMPVVR 286
TcIL3000.A.H_000897400.1-p1      Q--PVADVPTGKPTGVEDQ----C-----EVDNKGEGGMMTTAEQLAATPIMPVVR 286
TcIL3000.A.H_000897300.1-p1      Q--PVADVPTGKPTGVEDQ----C-----EVDNSGEGGMMTTAEQLAATPIMPVVR 286
TcIL3000.A.H_000897500.1-p1      Q--PVADVPTGKPTGVEDQ----C-----EVDNKGEGGMMTTAEQLAATPIMPVVR 286
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TcIL3000.A.H_000149400.1-p1      LKDEASISAL-----SRLAEFEAEWARPLPPEASSDGGNRDVAYSQPQAPDVV 339
TcIL3000.A.H_000034000.1-p1      LKDEASISAL-----SRLAEFEAEWARPLPPEASSDGGNRDVAYSQPQAPDVV 339
TcIL3000.A.H_000198900.1-p1      LKDEASISAL-----SRLAEFEAEWARPLPPEASSDGGNRDVAYSQPQAPDVV 339
TcIL3000.A.H_000033700.1-p1      LKDEASISAL-----SRLAEFEAEWARPLPPEASSDGGNRDVAYSQPQAPDVV 339
TcIL3000.A.H_000034400.1-p1      LKDEASISAL-----SRLAEFEAEWARPLPPEASSDGGNRDVAYSQPQAPDVV 339
TcIL3000.A.H_000233200.1-p1      LKDEASISAL-----SRLAEFEAEWARPLPPEASSDGGNRDVAYSQPQAPDVV 339
TcIL3000.A.H_000149700.1-p1      LKDEASISAL-----SRLAEFEAEWARPLPPEASSDGGNRDVAYSQPQAPDVV 339
TcIL3000.A.H_000778300.1-p1      MIHMMLLSCLGFFVTIFIFPNLIIPIDRTHKWFATIAALIFNIG-KSIGSFSTS----- 344
TcIL3000.A.H_000135200.1-p1      MIHMMLLSCLGFFVTIFIFPNLIIPIDRTHKWFATIAALIFNIG-KSIGSFSTS----- 344
TcIL3000.A.H_000113700.1-p1      IIRTVLISCLGFFLTIFIFPTLVVPIRVDWDFSTIAALVYNIG-YSIGSFSTS----- 305
TcIL3000.A.H_000951700.1-p1      IIRMLLISCLGFFLTIFIFPSLIPIDRADDWDFSTIAALIFNIG-NSIGSFSTS----- 340
TcIL3000.A.H_000024100.1-p1      IIRVMLISCLGFFLTIFIFPSFGIPIDRVDDWDFSTIAALIFNVG-KSIGSFSTS----- 340
TcIL3000.A.H_000897400.1-p1      IIRMLLISCLGFFLTIFIFPSLVIPIDRVDDWDFSTIAALVYNIG-NSIGSFSTS----- 340
TcIL3000.A.H_000897300.1-p1      IIRMLLISCLGFFLTIFIFPSLVIPIDRVDDWDFSTIAALIFNIG-NSIGAFSTS----- 340
TcIL3000.A.H_000897500.1-p1      IIRMLLISCLGFFLTIFIFPSLVIPIDRVDDWDFSTIAALIFNIG-NSIGAFSTS----- 340
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TcIL3000.A.H_000436700.1-p1      LRQKSTPRPLASS----- 352
TcIL3000.A.H_000149400.1-p1      ----- 339
TcIL3000.A.H_000034000.1-p1      ----- 339
TcIL3000.A.H_000198900.1-p1      ----- 339
TcIL3000.A.H_000033700.1-p1      ----- 339
TcIL3000.A.H_000034400.1-p1      ----- 339
TcIL3000.A.H_000233200.1-p1      ----- 339
TcIL3000.A.H_000149700.1-p1      ----- 339
TcIL3000.A.H_000778300.1-p1      FKMFMYPRGVVLYGSIVRFLLIIPFMLSIIYKHIPGHTVPYIFSFILGLTHYFPVLSMVY 404
TcIL3000.A.H_000135200.1-p1      FKMFMYPRGVVLYGSIVRFLLIIPFMLSIIYKHIPGHTVPYIFSFILGLTHYFPVLSMVY 404
TcIL3000.A.H_000113700.1-p1      FETFRYPRRVVLYGS----- 320
TcIL3000.A.H_000951700.1-p1      FETFRYPRRVVLYGSIVRFLLIIPFMLSIIYKHIPGHTVPYIFSFILGLTHYFPVLSMVY 400
TcIL3000.A.H_000024100.1-p1      SEMFMHPRGVALYWSIVRFLLIIPFMLSIIYKHITGHVVPYIFSFILGLTHQVSVLSMVY 400
TcIL3000.A.H_000897400.1-p1      FETFRYPRRVVLYGSIVRFLLIIPFVLSIIYKIIPGHVGPYIFSFILGLTHQVAVLSMVY 400
TcIL3000.A.H_000897300.1-p1      FETFRYPRRVVLYGIIIVRFLLIIPFMLSIIYKIIPGHVGPYIFSFILGLTHQVAVLSMVY 400
TcIL3000.A.H_000897500.1-p1      FETFRYPRRVVLYGSIVRFLLIIPFVLSIIYKIIPGHVGPYIFSFILGLTHQVAVLSMVY 400

TcIL3000.A.H_000436700.1-p1      ----- 352
TcIL3000.A.H_000149400.1-p1      ----- 339
TcIL3000.A.H_000034000.1-p1      ----- 339
TcIL3000.A.H_000198900.1-p1      ----- 339
TcIL3000.A.H_000033700.1-p1      ----- 339
TcIL3000.A.H_000034400.1-p1      ----- 339
TcIL3000.A.H_000233200.1-p1      ----- 339
TcIL3000.A.H_000149700.1-p1      ----- 339
TcIL3000.A.H_000778300.1-p1      PTPGLDDGQKLMAGQLMGIALLVGVSAAASVVAMIVVIFLPQD 447
TcIL3000.A.H_000135200.1-p1      PTPGLDDGQKLMAGQLMGIALLVGVSAAASVVAMIVVIFLPQD 447
TcIL3000.A.H_000113700.1-p1      ----- 320
TcIL3000.A.H_000951700.1-p1      PVTHGLNDGQKLMAGRLMGMAALLGASAAASVVAMTVVIFLP-- 441
TcIL3000.A.H_000024100.1-p1      PITGLNDGQKLIIEGQLIGIALLVGASAAASVAMVVVIFLPD 443
TcIL3000.A.H_000897400.1-p1      PITPGLNDGQKLMGTGQLMGIALLVGASAAASVVAMIVVIFLP-- 441
TcIL3000.A.H_000897300.1-p1      PITPGLNDGQKLMAGQLMGIALLAGATAASVSAMIVVIFLP-- 441
TcIL3000.A.H_000897500.1-p1      PITPGLNDGQKLMAGQLMSIALLAGASAAASVVAMIVVIFLP-- 441

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Supplemental Table S1. *T. congolense* nucleobase gene family

IL3000 (2019) sequences and IDs from TriTrypDB (<https://tritrypdb.org/tritrypdb/app>)

TMD prediction: <https://services.healthtech.dtu.dk/service.php?TMHMM-2.0>

E value for ENT family from Panther via TriTrypDB

TriTrypDB gene ID	a.a.	TMDs	E-value for ENT
TcIL3000.A.H_000436700.1-p1	352	6	1.7E-19
TcIL3000.A.H_000149400.1-p1	339	6	2.0E-19
TcIL3000.A.H_000034000.1-p1	339	6	1.6E-21
TcIL3000.A.H_000198900.1-p1	339	6	1.6E-21
TcIL3000.A.H_000033700.1-p1	339	6	2.0E-19
TcIL3000.A.H_000034400.1-p1	339	6	2.0E-19
TcIL3000.A.H_000233200.1-p1	339	6	2.0E-19
TcIL3000.A.H_000149700.1-p1	339	6	2.3E-19
TcIL3000.A.H_000778300.1-p1	447	11	0
TcIL3000.A.H_000135200.1-p1	447	11	0
TcIL3000.A.H_000113700.1-p1	320	7	4.5E-95
TcIL3000.A.H_000951700.1-p1	441	10	0
TcIL3000.A.H_000024100.1-p1	443	10	0
TcIL3000.A.H_000897400.1-p1	441	11	0
TcIL3000.A.H_000897300.1-p1	441	11	0
TcIL3000.A.H_000897500.1-p1	441	11	0

Supplementary Table S2: List of primers used, their sequences and purposes

Primer	Sequence	Function	Restriction site
HDK1761	CTTCCACTTGTC AAGCGAATT CCATATG ATGCTCGGTTTTGA ATCCATTTCCG	Forward primer for TcoAT1	<i>NdeI</i>
HDK1762	CTCAGTGGTGGTGGTGGTGGT GCTCGAG CCACTCTGGCAG GGCC	Reverse primer for TcoAT1	<i>XhoI</i>
HDK1763	CTTCCACTTGTC AAGCGAATT CCATATG ATGGCAGTGTTAG GGTTTGAGTCC	Forward primer for TvY486_0202110	<i>NdeI</i>
HDK1764	CTCAGTGGTGGTGGTGGTGGT GCTCGAG CAGCGTCTTGCTC AGTCCTATTGC	Reverse primer for TvY486_0202110	<i>XhoI</i>
HDK1765	CTTCCACTTGTC AAGCGAATT CCATATG ATGCTAGGCTTCGA GTCCACCTC	Forward primer for TvY486_1112030	<i>NdeI</i>
HDK1766	CTCAGTGGTGGTGGTGGTGGT GCTCGAG AAGCAACTTGCT CAGTCCTATTGC	Reverse primer for TvY486_1112030	<i>XhoI</i>
HDK1767	CTTCCACTTGTC AAGCGAATT CCATATG ATGGGGTTACTGG GGTTTGAGTCG	Forward primer for TvY486_0043680	<i>NdeI</i>
HDK1768	CTCAGTGGTGGTGGTGGTGGT GCTCGAG CAAGTGCTTCTCC AAACCCAGTGC	Reverse primer for TvY486_0043680	<i>XhoI</i>
HDK1769	CTTCCACTTGTC AAGCGAATT CCATATG ATGGGGTTGCTGG GTTTGAGTCG	Forward primer for TvY486_0014570	<i>NdeI</i>
HDK1770	CTCAGTGGTGGTGGTGGTGGT GCTCGAG CCGTCCGCGGGA TGGGTG	Reverse primer for TvY486_0014570	<i>XhoI</i>
HDK1734	CGACCTTACACCTCTACTTGTC	Reverse primer for pNUS-HcN plasmid	
HDK340	CGTGGAGCAGCTGAAGGACA	Reverse primer for pNUS-HcN plasmid	

Sequence in bold represent restriction sites. Sequence in lower case represent intended mutation.