

Supplementary Figures

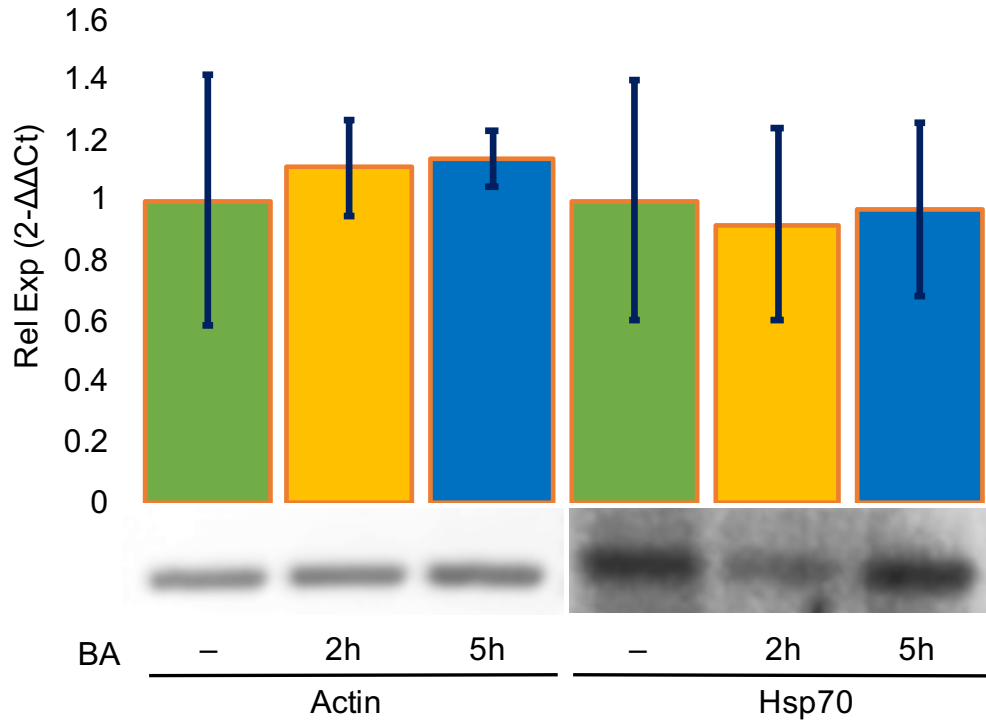


Figure S1. Boric Acid treatments do not affect the transcription of housekeeping genes. *E. histolytica* trophozoites were incubated in 5 mM H_3BO_3 for 2 or 5 h. RNA was extracted, and Actin and Hsp70 were amplified, visualized, and analyzed by qRT-PCR. Relative expression is compared to one in the HA control without BA. Expression profiles are statistically non-significant (ANOVA and Turkey test *a posteriori*). Standard deviations are shown as thin blue bars. Representative electrophoretic images of PRC products are shown.

Figure S2. Identification of TIA-1 binding motifs on flicRNA sequences from *Entamoeba histolytica*.

Selected homolog motifs: TIA1(Hs/Mm): uuuuubk, TIA1(Hs/Mm): uuuuuu.

Stringency level: High, conservation filter: On

(Paz I, Kosti I, Ares M, Jr., Cline M, Mandel-Gutfreund Y. RBPmap: a web server for mapping binding sites of RNA-binding proteins. Nucleic acids research. 2014;42(Web Server issue):W361-7. Epub 2014/05/16. DOI: 10.1093/nar/gku406. PubMed PMID: 24829458; PubMed Central PMCID: PMC4086114)

flicX13

Sequence Position	Motif	K-mer	Z-score	P-value
27	uuuuuu	uuuuua	2.523	5.82e-03 *
61	uuuuubk	auuuugu	2.598	4.69e-03 *
111	uuuuuu	uuuuua	1.947	2.58e-02 *

flic670i1

Sequence Position	Motif	K-mer	Z-score	P-value
70	uuuuuu	uuuuua	2.561	5.22e-03 *

flic670i2

=====				
Sequence Position	Motif	K-mer	Z-score	P-value
83	uuuuu	uuuuu	2.606	4.58e-03 *
148	uuuuubk	auuuuuu	3.422	3.11e-04 *
149	uuuuubk	uuuuuuu	3.735	9.39e-05 *
150	uuuuubk	uuuuuug	3.843	6.08e-05 *
151	uuuuubk	uuuuugu	3.539	2.01e-04 *

flic510

=====				
Sequence Position	Motif	K-mer	Z-score	P-value
77	uuuuu	uuuuu	1.917	2.76e-02 *
179	uuuuu	uuuuu	3.227	6.25e-04 *
180	uuuuu	uuuuu	3.227	6.25e-04 *
181	uuuuu	uuuuu	3.061	1.10e-03 *
202	uuuuu	uuuuu	3.061	1.10e-03 *

flic170

=====				
Sequence Position	Motif	K-mer	Z-score	P-value
26	uuuuu	uuuuu	2.947	1.60e-03 *
50	uuuuubk	auuuucu	2.667	3.83e-03 *

117	uuuuubk	auuuuug	3.206	6.73e-04 *
118	uuuuubk	uuuuugu	3.176	7.47e-04 *
181	uuuuu	uuuuu	2.871	2.05e-03 *
182	uuuuu	uuuuu	2.758	2.91e-03 *

Supplementary Figure S3.

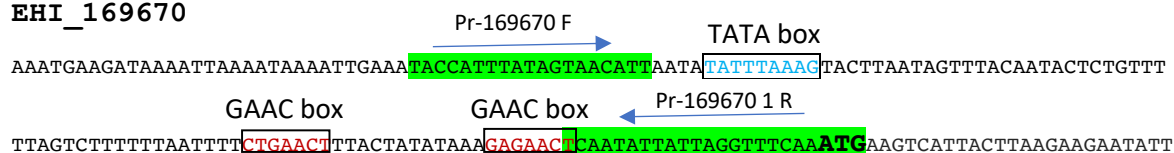
Identification of promoter elements in *Entamoeba histolytica* genes.

Inr: Initiator

RabX13 EHI_065790



EHI_169670



EH1_014170

CAACAATGTATATTGCTCCAACCAATGACTTTAGTGCTATTGATTCTTATATAACCTTAAACCAACTTC
Pr-014170A F

AGAACAACCTCATCTACGCTGTTTAAGAAAATCACTATTCATTTCAAACAACAACACTGTCACTCA
Pr-014170B F GAAC box
GTCCATATTGTTTCATTATTACTTCTGAAGAAGCTTTATCTGATGTAGTTTAAAGACAAAAGAACTTC

AAACATTACGTAGTTATCACGTTCAATTTGTTTTATTAGGAGATGTATTAAATCCATTAAGCCATACCCG
Pr-014170A R
TTCCTTAGTTCGTTCTTTGGTTAACGACAAAGTCGTGGATGGGGAGTAACTGAAAACGATTTAACAAAA

ACAAATCCTTATGAGCGTTTAATGATGAGAATGTCTGAAGGCATTATCATTTTGCCTCGTCGTAAAGTAT
Pr-014170D F
TAAAACAATTACCTCATATGAGAGCTGGACAAGTTCGTGTGAAAGTAAAGCTGGCAAATTTATTTTCATG

TAAAGTAATTACACATATGACAAATGATACAGTTTCAATGATTCCAGTACCAATTATTTCTAAACAAAAG
Pr-014170B R
AATTTAAGCACTATCAGATGTGGTAAATCAAATATAGTTGATGGAAAATGTGTGTCTTCAGAAGAACGAG

GAATGCTTTTCTTGGTTGAATTATCTCCGATTTTATTTAAATTGATTGGAAAACCGAAAATGAGATAGA
TATA box
Pr-014170E F
CCATTGACAATATTTTATGGTGAGACAAGTTGTAAGCAGATAAAAAAGGTAAATATAACTTCTTAATT

TTTGAAAGGTAATAAACCAAATGGTAGTCTTACTAAAACAATTTATTGGACTACTTTGGATACAAAAGAAT
Pr-014170D R

TTTGACGGTTTTTGAAAAAGCTATGAGTCAAGATGTCCACAAAATTATATAAAAATTATGCAAACTAT
TTGGAATGATAATAAGAAGACATTAAGTAGTATGGAAGAAAAATATATGAGTATGAAGAGGGAAAAAGAA
Pr-014170F F Pr-014170E R
ATTGTGAACAAAATGGATGAATATGTAAAAGGTGTTCTTAAACAGAACTAAATAGAAAAGTAAGAAATG

AAGCTGATAAAAAACAGAACCAAAAGAGCCAAGTACTAATTAATAAATAAAATGTCCAAATTTTTTGCT
AATCATTCACGTACATTTATTTTTTTGTTATTTAATAACTGGTTAATTTTGTATTAGGAAAAGAATAATA
Pr-014170G F
AAATAAGCACTTAATTAAATGTTAATAAACTCTATTTTTTTTATTCCTTGTTCAAGTGACACAAATCCTG

TCTTTTATCTTATTTTTTTTATTAGGAAAAGAAAAAGAGAAAAAATAACAAAATAAAATAATAAAAAAT
TATATATAAATGTAATTTTTATTTGAAGAAAGGAAAAGAAAGTCAAAAATTTTGTAAATAAAA **TATA box**
Pr-014170F R
AAATGAAACGAATACGATTAAAAGAAGTTAAGGAATGTTGAGTCATACTAACGTAATACAGAAAAGAAGAA
Inr
GAGATCTTGAAATT **AAAAAAGGAATA** AAGA **ATG**AAATCATTATTAAAGAAGAAATATTAAACAAGACTATA
Pr-014170G R
CCATTGAATTTAAACATATTAAAG **SACTAGATATTGGAGACAATA** TTGAAGTTGTTTTAAATGGAGAAG

EHI_192510

Pr-192510A F **GAAC box**
AAA **ATGGTTACGCTTGAATG** AGGGGTG **ATATGAACT** TTCTCGCAAATATTTCCATGACGACAAGATCCAA
Pr-192510B F
CTTTAGAGAAGAACTCACAGAGA **TTGAGACTTTCCATCAATAACT** AAAAACTATTTTTCGTTTTGACAAT
TATA box
TTTTAATTTTTTAATTTTTTTATCAGAGTTTTAAATTATTAATTTT **TTTAAAG** AATGATGTCTCAGTTTTT
Inr **Inr**
TTAATAAAAAGTGAACATCTGCTAA **AAAAATTAA** GAT **AAATTTCAT** TTAATA **ATG** TCTTCTCAAAAAA **TA**
Pr-192510A R **Pr-192510B**
GTTAGTGTGGAATTGAT TTAGGAACTACTTGTTGTTATGT **TGCTTGTATTGAAGATGGTAAT** GAACCAA
AAATACTTCTAATGAATAATGAAGACTATTTACCTTCATGGATTTCTCTTTCAAATGTTAAGAATGGTTT

EhRNF EHI_098590

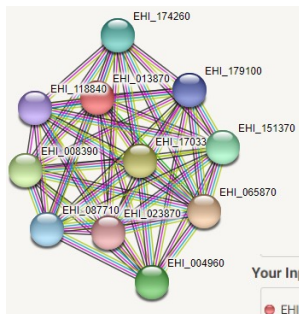
Pr-098590 F
AAT **TAAACAAGATTCTCCAGGTG** TTGATTGGTCAATTTCAATCTATTCTGACAAAGAAAAAGATCCTTCA
TATA box
TTTTATAATGTTTATCAGTGTTTACATTACGTC **CACCTTTAAAC** CAACACAACATCATTAATCCTG
AATTTAAATTTGAGTATGAAAATGCTATTGATGTTAGTGCGTTTACTGGAAATAAAGAAAACTTTGCAGT
AAAACGATTATTTTCTTCAATGGTATCAATAAACTTTGTAATTTTTTATTCACATTTTCTTATTATT
Inr **GAAC box**
TTGTT **AAAAATTCT** GTTGAATAATAAAAGGGGAAAAA **AAGAACT** TATTTTTTTTATTCTTTATCTTTTT
Pr-098590 R
CTT **ATG** GGTGCTGAACCTTCAAAAC **CAGTTATTTCTAATGATGTTAATAGAA** TGTGTTCAACTATTACAG

EhNudC EHI_023890

TATTAAATCATCCAAACATTAAGAATTTGCCAAAAGCAATTATTTGTGCAGAGGAGACTTTGTTTTGGAGATACCAAAATA
P-nudc S 500
TAAGTAATACTAACTTATCAACTATTGAGGCTATCTATTATTTTATAGAGATTATTATACTTGTATGAATGGTAAATATA
ATGGAGAATGTGATGATCTTTTATATTTCTTCACCCATATTTATCATCGTGTCCAAGACTTTTATAACGAACATCCAGATA
AAACGTTTCATCCACAAAGATGGATATATTCAATACAAAACACCAGAGCTTGAAAAACAAAAGAAGAATACAAAAGA
P-nudc S 250
GGGAAGAGATGATTGAATCTAAAAACAATCTAAAGCACAAAAATAATTAAATATTTTGTATTTTAAATGGTTTGCAT
P-nudc AS 250
TACTCTTATAATTTTTTTTAAAGAACAAAAAGAATGAAGAAAGATTAAATGTTGTTTCGTATTAATAATCTCAATCACAA
GAAC box
TGAACAAGTTGGTACTAAAAATCTAAAAAGAAAAAGGATGATCATCACCATGTCAGTCCACGTCATTTCATTTCGTGAAAG
TATA box Inr P-nudc As1
ATAATATTTTAAACATAGAACTTAGACAAAAAAACAATTCGTTGCGTTATGGCTGGATGGAACCATCTCCTTATGTTAA
TATGGATATACCGAAAAGAAGTATAAATGGACTCAGACACCTGAAGATGTAAACATTAACAATAGAATTACCTGATGAAAA
TTAAAAACAAAAACAGATATTCAAATAATAATGAAAACAAATAAATTTGAAATGATTATTAAAGGAGAAGTTTATATTGGA
GGAGAATTAGAACATGCTATTAATGTAGATGATTCTACATGGACT

EhSMC EHI_187190

TAAACAAAATGAAAAATTTGCCCAATAATTTAATTAAAGAGAATGCCATGAATGAAAAATGATCCAATAAATAACGTTA
TTCCAATTATATAAATAATTGCAGTTTCTACTTATAAATTATAATTCAATCAATTAAATAAAATATACAACTTTTATCT
GACATTGTTGTAAATCAGGAAATCCTAAGTAATTTGCAACTGCATGGCATAAAATGCATGAAATTACAGATTTTGTACTG
AAGAAAACATATGACGAATACATTCCAAATAATGTAGTAAATCCTACTTGTATCATTGTATTGATGATAGCAGCTTTATCA
ATAACATCTTCTGTTATTAGGTGGTTGATGTGTGCAAGTCCAAAAGCATTGAACAATAGATAAATGTAAATATTGATGTG
P-smc S 500
CATCCGTTTGTCAACAGGTATGGTATAAACACTCCTCTAAATAATGTTTCTTCAATTATTGGTGCAACAACAAGTGCCCGT
ATGATGAAAAGTGAATTAGGACAATATATTGATCAGTTTGTGATAAACACAATATATAAATCCAATAAAAAGAATGACG
ATTTGGGTTAGATGAAATAACGTTGATAAACAGGAATATTAAAGTTAAGAGGGACATCAAGAATAATAGTGACAAATACA
P-smc S 250
CAATATCCTATTCCAAATGATCATTCTTATTATTGCTATTTTATTTTCGTCATTTATCTATGTGAAGCTATACCCAAAAA
P-smc AS 250 TATA box
GCAAGAAGAATGACTGTCAATGTTAATGTTATGATAATACTCATAAACCATCCAACAATAATCATTAAACATATAAATTAT
Inr P-smc As1
GTATTTGTTTTTTTTTAATTTCCAAATAAAGTGATAGTAATAAATATAGTAAATTTGCTATAATTTACTACTATAAACT
ATATAATAAAAATGAAAAATAAACCATCATTACATGGATAAGACTCAATCTGATGTGCTTGCTCACTTCCTTGAACAGGAA
CTAAGTGTGACCAAAAAGTAAAGAGGATACAAAGAAAGGTAGCAGAGTATATGAAAACTATAATTATGATGAATATGTA



EHI_013870 (METTL3 ortholog): rRNA adenine N(6)-methyltransferase;
Dimethyladenosine transferase

Your Input:

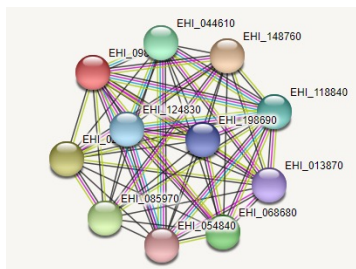
EHI_013870 rRNA adenine N(6)-methyltransferase; Dimethyladenosine transferase, putative; Belongs to the class I-like SAM-binding methyltransferase superfamily. rRNA adenine N(6)-methyltransferase family (295 aa)

Predicted Functional Partners:

	Neighborhood	Gene Fusion	Cooccurrence	Coexpression	Experiments	Databases	Textmining	(Homology)	Score
EHI_065870	Rna-binding protein pno1; RNA-binding protein, putative			•	•	•			0.996
EHI_170330	RIO1 family protein			•	•	•			0.996
EHI_008390	Pre-rna-processing protein tsr1; Uncharacterized protein			•	•	•			0.995
EHI_004960	Essential nuclear protein 1; Bystin, putative			•	•	•			0.994
EHI_151370	Periodic tryptophan protein 2; WD domain containing protein			•	•	•			0.994
EHI_174260	Hypothetical protein; Uncharacterized protein			•	•	•			0.994
EHI_087710	Rna-binding protein nob1; Belongs to the NOB1 family			•	•	•			0.991
EHI_179100	U3 small nucleolar RNA-associated protein 22; Uncharacterized protein			•	•	•			0.989
EHI_118840	Rma 2'-o-methyltransferase fibrillarin; Fibrillarin, putative			•	•	•			0.988
EHI_023870	U3 small nucleolar RNA-associated protein 4; WD domain containing protein			•	•	•			0.982

Your Current Organism:

Entamoeba histolytica
NCBI taxonomy Id: 5759
Other names: E. histolytica



EHI_098730: Trna (cytidine32/guanosine34-2'-o)-methyltransferase;
Ribosomal RNA methyltransferase

Entamoeba histolytica protein-protein associations

associations are meant to be specific and meaningful, i.e. proteins jointly contribute to a shared function; this does not necessarily mean they are physically binding to each other.

Known Interactions

from curated databases
experimentally determined

Predicted Interactions

gene neighborhood
gene fusions
gene co-occurrence

Others

textmining
co-expression
protein homology

Your Input:

EHI_098730 Trna (cytidine32/guanosine34-2'-o)-methyltransferase; Ribosomal RNA methyltransferase, putative (231 aa)

Predicted Functional Partners:

	Neighborhood	Gene Fusion	Cooccurrence	Coexpression	Experiments	Databases	Textmining	(Homology)	Score
EHI_148760	Trna (guanine26-n2/guanine27-n2)-dimethyltransferase; N2,N2-dimethylguanosine tRNA methyltransferase, putative			•	•	•			0.988
EHI_023130	Trna (guanine10-n2)-methyltransferase; Uncharacterized protein			•	•	•			0.987
EHI_085970	Trna (adenine58-n1)-methyltransferase non-catalytic subunit; Uncharacterized protein			•	•	•			0.986
EHI_068680	Nucleolar phosphoprotein Nopp34, putative			•	•	•			0.979
EHI_044610	Rma small subunit pseudouridine methyltransferase nep1; Ribosome biogenesis protein NEP1, putative			•	•	•			0.976
EHI_118840	Rma 2'-o-methyltransferase fibrillarin; Fibrillarin, putative			•	•	•			0.976
EHI_124830	Hypothetical protein; Uncharacterized protein			•	•	•			0.974
EHI_198690	MAK16 protein, putative; Belongs to the MAK16 family			•	•	•			0.971
EHI_013870	rRNA adenine N(6)-methyltransferase; Dimethyladenosine transferase, putative; Belongs to the class I-like SAM-binding m...			•	•	•			0.965
EHI_054840	U3 small nucleolar RNA-associated protein 5; Uncharacterized protein			•	•	•			0.962

Figure S4. String analysis of loci EHI_013870 and EHI_098730. Protein products of these loci were identified as members of pre-mRNA ribonucleoparticles [Valdés *et al.*, Proteomic analysis of *Entamoeba histolytica* *in vivo* assembled pre-mRNA splicing complexes. J Proteomics. 2014]. Whereas only locus EHI_013870 codes for the METTL ortholog involved in adenine N(6)-methyltransferase, locus EHI_098730 codes for a tRNA (cytidine32/guanosine34-2'-o)-methyltransferase, but its predicted protein-protein interactions show association with the EhMETTL ortholog.

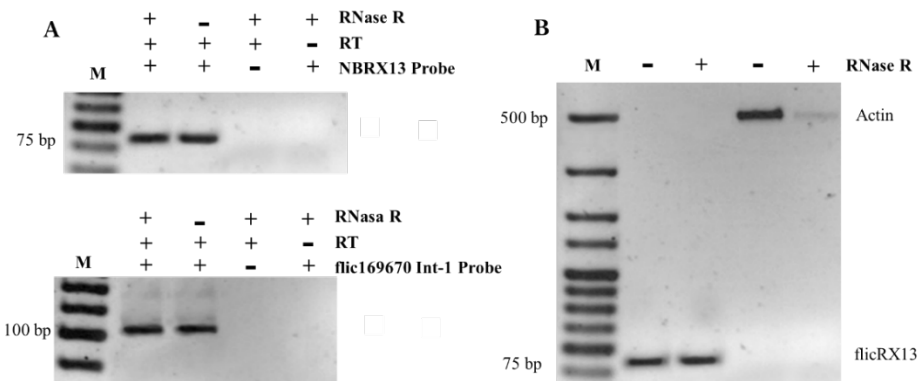


Figure S5. Validation of flicRNAs with RNase R treatment. (A) Primers that span the 5'-3'ss junctions were used to identification flicRX13 and flic670i1 by divergent RT-PCR with purified RNA treated with RNase R. Amplicons were resolved in 2.5% agarose gel. (B) Analysis of linear RNA degradation efficiency by exoribonuclease R. Amplification of the constitutive transcript Actin versus flicRX13 from treated RNA was compared. M, molecular weight marker.