

Supplemental Material to:

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C. elegans RNA-binding protein GLD-1 recognizes its multiple targets using sequence, context, and structural information to repress translation

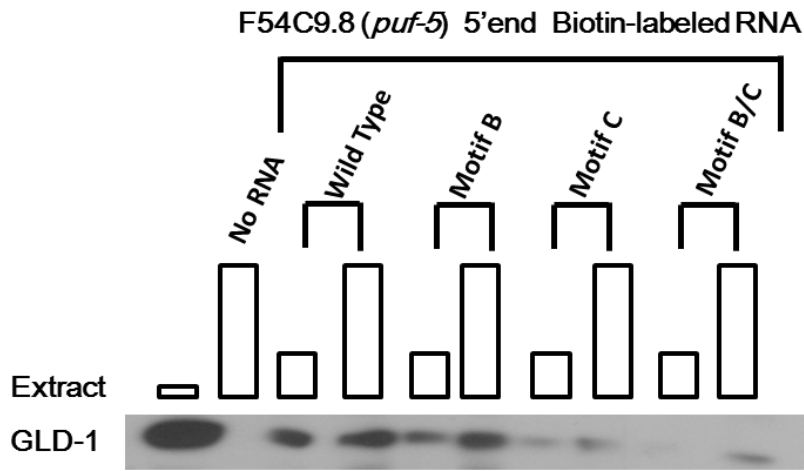
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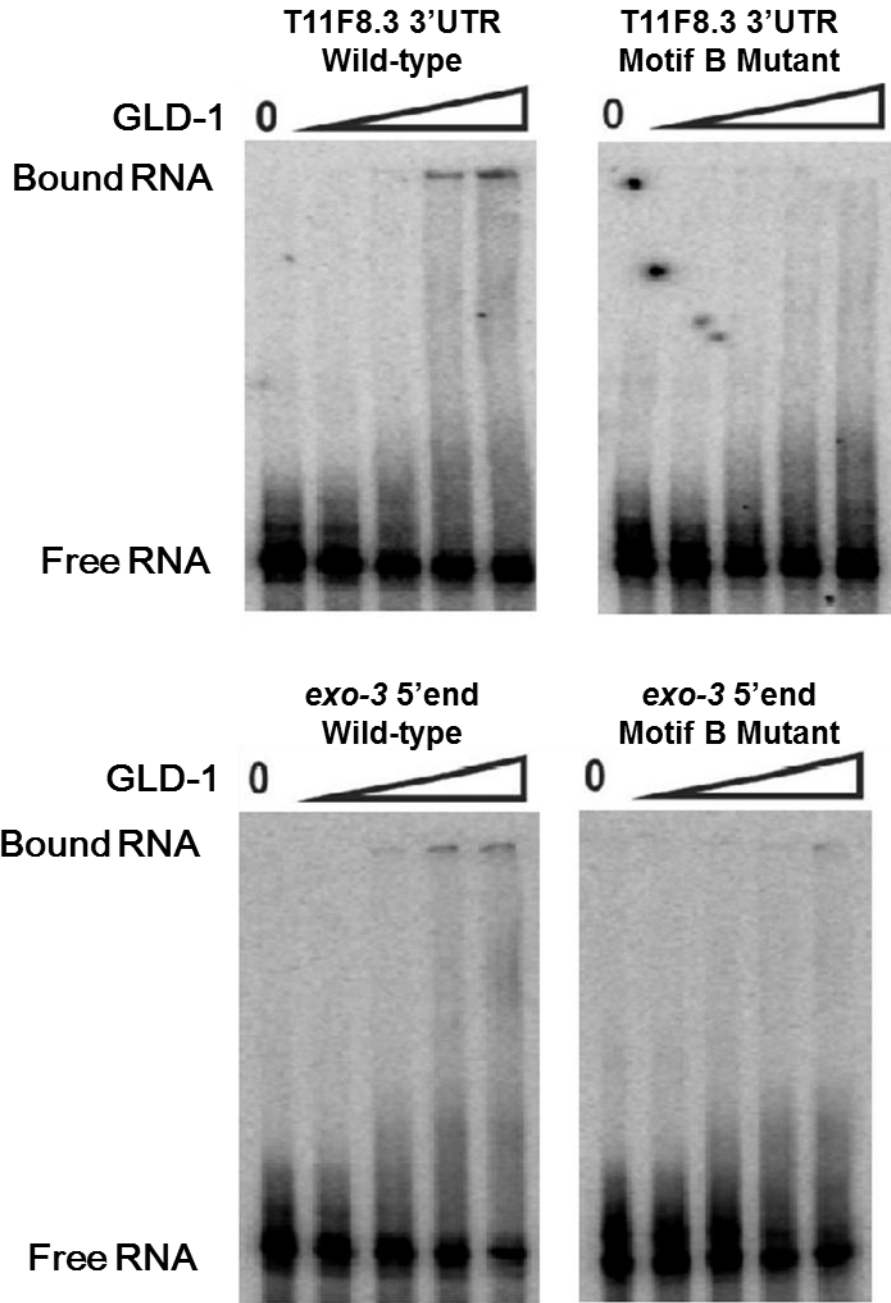
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***puf-5* (F54C9.8) 5'end**

Motif B ↑
ag**auacu**aaccuguaacuccugugacucuguuucaauAUGAGUGACAGUACUGGAA**GAAUCAAC**
UCGAAAGCUUCGGAUUCAUCGUCGAUCAGCGAUCACCAAACUGCCGAUCUCAGCAUUUUAACGG
AUCCUUCGAUGGAGGUGCAUUCUCAUCGUCCAACAUUCCUCUGUUAACUUCAUGGGA
Motif C ↑



S. Figure 1. Motif C, but not Motif B, is important for GLD-1 binding in the *puf-5* (F54C9.8) 5'end. Single and double mutations of Motif C significantly reduce GLD-1 binding to the *puf-5* 5'end. The RNA sequence of the *puf-5* 5' end is shown (top). Sequence in lower-case represents the 5'UTR and in upper-case the first 150 nucleotides of the ORF. The Motif B and C are depicted in the sequence. The four most conserved nucleotides were mutated, where the Motif B is mutated to "UAGAUU" while the Motif C is mutated "CUUAGGA". Biotin-RNA pull down assays were performed with the wild-type and mutated *puf-5* 5' ends as described previously.



S. Figure 2. Purified, recombinant GLD-1 binds directly to the Motif B in *rme-2* 3'UTR and *exo-3* 5'end RNAs. EMSA was conducted to examine the ability of purified recombinant GLD-1 to bind to the wild-type and mutated Motif B in *rme-2* 3'UTR (A) and *exo-3* 5'end (B) in the absence of other RNAs and proteins. Binding reactions of 20 μ L were set up with constant amount of radiolabeled wild-type or mutated GLD-1 binding regions and in increasing concentrations of GLD-1 (1, 100, 1000, 3000, and 5000 nM), and resolved on 4.8% polyacrylamide gel (acrylamide: bis-acrylamide = 37.5:1) in 0.25 \times TBE buffer.

S. Table 1. Putative GLD-1 Targets

Sequence Name	Gene name	Previously Identified	MicroarrayFL AG/IgG	Microarrayp value	Real time FLAG/IgG
ZC513.6	<i>oma-2</i>	Yes	13.47	2.43E-05	27.86
T05G5.7	<i>rmd-1</i>	Yes	8.75	1.13E-03	28.84
T11F8.3	<i>rme-2</i>	Yes	8.36	7.81E-05	13.00
Y75B12B.1		Yes	8.01	7.88E-04	22.63
C50B6.2	<i>nasp-2</i>		7.66	6.27E-04	10.56
C07G2.1	<i>cej-1</i>	Yes	7.40	2.27E-04	19.03
F32B6.5	<i>sss-1</i>		6.10	1.01E-02	35.51
F56A3.2	<i>slx-1</i>		5.59	3.10E-03	9.85
F44D12.4	<i>gipc-2</i>		5.11	5.94E-03	10.93
R01B10.4			4.90	1.97E-04	6.96
H02I12.1	<i>cbd-1</i>		4.87	1.84E-04	3.73
R09B3.1	<i>exo-3</i>	Yes	4.83	1.08E-03	20.39
T01H8.1	<i>rskn-1</i>		4.69	9.77E-04	8.88
F44E7.2			4.57	2.18E-04	6.73
C18G1.9			4.43	1.40E-02	16.56
F25H9.6			4.33	1.25E-02	6.06
C35D10.2	<i>gipc-1</i>		4.20	1.11E-02	14.42
C09G9.6	<i>oma-1</i>	Yes	4.17	8.13E-07	3.31
ZK829.5	<i>tbx-36</i>		4.11	5.55E-03	7.46
ZK632.13	<i>lin-52</i>		4.05	8.71E-03	6.73
F14B4.2		Yes	4.04	2.62E-02	10.20
Y44E3B.1	<i>zip-4</i>		4.00	3.70E-03	19.03
T23G11.2	<i>gna-2</i>	Yes	3.99	3.82E-04	3.86
W09C5.2	<i>unc-59</i>		3.90	4.22E-03	5.66
C04F12.7			3.87	3.61E-02	30.91
T07A9.6	<i>daf-18</i>		3.84	7.03E-04	7.21

F43G6.1	<i>dna-2</i>		3.68	5.06E-03	4.59
K09H11.7			3.63	7.74E-04	1.15
F22B3.4			3.62	6.79E-04	5.10
T10C6.8	<i>hpo-42</i>		3.61	5.87E-03	6.50
F10C2.4			3.59	3.99E-03	5.66
C17E4.3			3.51	9.88E-04	5.86
F54C9.8	<i>puf-5</i>	Yes	3.45	1.51E-04	2.93
C01G8.1			3.41	3.66E-03	2.55
T01C3.2			3.38	1.46E-02	4.29
C48E7.7			3.38	3.48E-02	40.79
C28D4.3	<i>gln-6</i>		3.36	1.07E-03	4.14
F26D10.10	<i>gln-5</i>	Yes	3.32	8.08E-05	2.93
R12C12.7			3.26	1.58E-02	3.86
C53A3.2			3.25	2.02E-03	4.44
F22B7.6	<i>polk-1</i>		3.25	9.40E-04	2.30
C02B10.2			3.24	1.87E-02	2.46
T03F6.3			3.24	3.21E-03	
F18A1.7			3.24	2.41E-03	3.36
F59A3.4			3.23	5.92E-04	2.00
K08F9.2	<i>aipl-1</i>		3.20	1.69E-02	2.46
T03D8.1	<i>num-1</i>		3.19	1.08E-03	3.25
T27E9.3	<i>cdk-5</i>		3.13	8.32E-03	3.73
C18G1.5	<i>hil-4</i>		3.10	2.13E-03	
F43D9.4	<i>sip-1</i>		2.99	1.03E-02	
C55A6.1			2.95	3.13E-02	0.90
T23B12.6			2.91	1.16E-03	
Y49E10.14	<i>pie-1</i>		2.90	6.26E-03	5.66
ZC317.6			2.84	4.28E-02	3.25
ZK973.6	<i>anc-1</i>		2.83	4.05E-03	0.84
Y39E4B.5			2.79	1.04E-03	3.25

K07H8.9		2.74	7.26E-03	4.29
K07A1.8	<i>ile-1</i>	2.70	8.44E-03	3.36
T19B10.11	<i>mxl-1</i>	2.70	3.69E-02	2.64
F27C8.6	<i>trcs-1</i>	2.68	8.12E-04	2.30
F07H5.3		2.68	4.91E-03	
C39E9.13	<i>rfc-3</i>	2.65	3.93E-03	1.80
C28C12.2	<i>mesp-1</i>	2.62	3.43E-03	2.46
ZK973.a	<i>pdp-1</i>	2.61	4.84E-03	0.97
C33H5.14	<i>ntp-1</i>	2.59	6.00E-03	2.73
F07H5.10		2.59	1.84E-03	3.61
F55G1.6		2.58	5.01E-03	3.03
C50F4.5	<i>his-41</i>	2.57	2.56E-03	3.48
Y47G6A.8	<i>crn-1</i>	2.55	3.01E-03	2.38
Y73F4A.1		2.55	4.73E-03	3.25
F19B6.2	<i>ufd-1</i>	2.55	3.61E-03	3.48
F35G12.4		2.54	4.57E-05	
ZK863.4		2.54	1.71E-03	
T13F2.9		2.53	2.06E-02	
Y43E12A.1	<i>cyb-2.1</i>	2.50	5.86E-04	
R02F2.4		2.49	1.96E-03	
F42G9.5	<i>alh-11</i>	2.45	5.01E-03	
C36B1.11		2.44	3.80E-03	
B0280.10	<i>pot-1</i>	2.42	6.55E-04	
K01C8.3	<i>tdc-1</i>	2.39	3.31E-03	
ZK593.8	<i>fic-1</i>	2.38	5.08E-04	
F35C11.5		2.38	1.11E-04	
ZK1058.3		2.38	1.71E-03	
M04F3.1	<i>rpa-2</i>	2.37	1.01E-03	
C30F12.4		2.36	5.74E-04	
F59B2.3		2.36	7.61E-03	

K04F10.1			2.33	2.14E-03	
R01H2.3	<i>egg-2</i>	Yes	2.32	1.27E-02	
C50E3.12			2.32	2.66E-03	
Y43C5A.5	<i>thk-1</i>		2.32	9.13E-04	
C52E12.3	<i>sqv-7</i>		2.30	1.12E-03	
C10F3.1	<i>cpg-4</i>		2.30	1.80E-04	
C26E6.7	<i>eri-9</i>		2.29	3.10E-03	
T22C1.6			2.29	2.82E-02	
C01F6.4	<i>fem-3</i>		2.25	2.54E-03	2.00
T25G3.2	<i>chi-1</i>		2.25	1.97E-03	
F36F2.4	<i>syx-7</i>		2.24	3.90E-02	
R01E6.3	<i>cah-4</i>		2.24	1.05E-02	
F10C2.2	<i>kup-1</i>		2.22	4.84E-02	
T27F2.3	<i>bir-1</i>		2.21	2.66E-03	
Y48G1BL.3	<i>puf-10</i>	Yes	2.19	1.57E-03	
R07E5.8	<i>cku-80</i>		2.18	1.51E-02	
F35B12.5	<i>sas-5</i>		2.16	5.51E-05	
R12E2.10	<i>egg-5</i>		2.16	4.75E-03	
ZK177.5	<i>ccp-44</i>		2.16	1.03E-03	
F32G8.6	<i>cat-4</i>		2.15	2.11E-02	
R05H5.4			2.15	4.10E-03	
F54C8.2	<i>cpar-1</i>		2.15	1.78E-03	
F56C9.6			2.14	4.41E-03	
F01F1.12	<i>aldo-2</i>		2.14	8.81E-03	
R03D7.7	<i>nos-1</i>		2.14	2.64E-03	
T25B6.2	<i>nep-22</i>		2.13	5.54E-03	
F30A10.6	<i>sac-1</i>		2.13	1.79E-02	
C32E8.8	<i>ptr-2</i>		2.13	1.19E-03	
C14B1.9			2.11	1.75E-03	
Y45F10A.2	<i>puf-3</i>		2.11	1.32E-03	

T05F1.2			2.07	3.16E-03	
W02F12.4			2.06	1.83E-03	
C34G6.5	<i>cdc-7</i>		2.06	2.93E-03	
Y75B12B.3			2.06	1.93E-02	
F08H9.7	<i>clec-56</i>		2.04	3.00E-02	
F58A4.4	<i>pri-1</i>		2.03	5.31E-03	2.22
F57B10.12	<i>mei-2</i>		2.02	1.30E-03	
Y62H9A.6			2.02	1.26E-02	
H02I12.5		Yes	2.02	5.23E-03	
C17G1.3	<i>ugt-23</i>		2.02	4.28E-02	
F54F2.2	<i>zfp-1</i>		2.02	5.44E-03	
F47D12.7	F47D12.7		2.01	1.05E-02	
C34H4.3			2.00	2.30E-02	
T05G5.3	<i>cdk-1</i>		1.20	0.05768	1.07
H12I13.4	<i>fbf-1</i>		1.04	0.60626	1.11
F21H12.5	<i>fbf-2</i>		1.09	0.40439	1.32

S. Table 2. Functional analysis of GLD-1 targets

Function	Gene Size	Observed Genes	Significance	Genes(symbol)
embryonic development ending in birth or egg hatching (GO:0009792)	2338	46	1.52E-10	C50F4.6, cyb-2.1, egg-4, F22B3.4, cdk-5, H02112.1, gln-6, mei-2, C02B10.2, sqv-7, F56A3.2, ptr-2, F18A1.7, pie-1, zfp-1, 04F3.1, C14B1.9, C17E4.3, H02112.5, sas-5, T23B12.6, C28C12.2, R12C12.7, rmd-1, F19B6.1, Syntaxin, esterase, crn-1, thk-1, hum-1, dna-2, nasp-2, cpar-1, F10C2.4, rme-2, chs-1, unc-59, F01F1.12, exo-3, F30A10.6, pri-1, bir-1, gna-2, fem-3, rfc-3, puf-3
cytokinesis (GO:0000910)	105	9	8.78E-08	cyb-2.1, F22B3.4, ptr-2, C28C12.2, Syntaxin, F10C2.4, pri-1, gna-2, puf-3
protein binding (GO:0005515)	2098	38	1.04E-07	H02112.1, gln-6, mei-2, hexokinase, F25H9.6, F56A3.2, rskn-1, pie-1, zfp-1, M04F3.1, sss-1, C17E4.3, F44D12.4, H02112.5, 55G1.6, sas-5, C35D10.2, T23B12.6, R01B10.4, F19B6.1, F47D12.7, Syntaxin, sss-1, esterase, crn-1, beta-transducin, hum-1, oma-2, cat-4, F01F1.12, T03F6.3, bir-1, R05H5.4, fem-3, num-1, F59B2.3, rfc-3, mxl-1
embryonic cleavage (GO:0040016)	181	10	1.02E-06	cyb-2.1, F22B3.4, ptr-2, H02112.5, C28C12.2, Syntaxin, F10C2.4, pri-1, gna-2, puf-3
DNA replication (GO:0006260)	47	6	1.27E-06	mucb, crn-1, thk-1, F10C2.4, pri-1, rfc-3
chitin metabolic process (GO:0006030)	13	3	0.000109	cej-1, H02112.1, R02F2.4
DNA repair (GO:0006281)	104	6	0.000127	F56A3.2, M04F3.1, mucb, crn-1, exo-3, rfc-3
transferase activity (GO:0016740)	594	14	0.000139	F22B3.4, cdk-5, hexokinase, F19B6.1, rskn-1, C34G6.5, mucb, thk-1, UDP-glucosyltransferase, F10C2.4, pri-1, ZK1058.3, R05H5.4, gna-2
nuclease activity (GO:0004518)	36	4	0.000144	F56A3.2, crn-1, F10C2.4, exo-3
chitin binding (GO:0008061)	16	3	0.000211	cej-1, H02112.1, R02F2.4
nucleotidyltransferase activity (GO:0016779)	43	4	0.00029	mucb, F10C2.4, pri-1, ZK1058.3
glutamate-ammonia ligase activity (GO:0004356)	5	2	0.000543	gln-6, gln-5
glutamine biosynthetic process (GO:0006542)	5	2	0.000543	gln-6, gln-5
centrosome (GO:0005813)	23	3	0.000642	pie-1, oma-1, oma-2
P granule (GO:0043186)	24	3	0.000729	pie-1, oma-1, oma-2
oocyte maturation (GO:0001556)	9	2	0.001916	cyb-2.1, oma-1
cleavage furrow (GO:0032154)	9	2	0.001916	Syntaxin, unc-59
nitrogen compound metabolic process (GO:0006807)	11	2	0.002899	gln-6, gln-5
cell cycle (GO:0007049)	83	4	0.003432	cyb-2.1, mei-2, sas-5, unc-59
protein domain specific binding (GO:0019904)	12	2	0.003462	num-1, mxl-1
embryonic development (GO:0009790)	136	5	0.003512	mei-2, ptr-2, lin-52, crn-1, exo-3
endonuclease activity (GO:0004519)	13	2	0.004071	crn-1, exo-3
carbohydrate metabolic process (GO:0005975)	144	5	0.004478	F22B3.4, F19B6.1, T03F6.3, ZK1058.3, F59B2.3
reproduction (GO:0000003)	949	15	0.004696	egg-4, F22B3.4, cdk-5, H02112.1, sqv-7, F19B6.1, ptr-2, Syntaxin, crn-1, oma-1, rme-2, chs-1, unc-59, gna-2, fem-3
DNA-directed DNA polymerase activity (GO:0003887)	14	2	0.004727	mucb, F10C2.4
catalytic activity (GO:0003824)	597	11	0.005056	tdc-1, gln-6, F25H9.6, phosphatase, crn-1, oma-1, cat-4, C53A3.2, F01F1.12, ZK1058.3, gln-5
phosphoric monoester hydrolase activity (GO:0016791)	93	4	0.005151	phosphatase, egg-4, daf-18, C53A3.2
morphogenesis of an epithelium (GO:0002009)	233	6	0.008029	sqv-7, daf-18, M04F3.1, crn-1, cpar-1, unc-59
cell division (GO:0051301)	58	3	0.009251	cyb-2.1, cdk-5, mei-2
nucleosome assembly (GO:0006334)	58	3	0.009251	C50F4.6, hil-4, cpar-1
nucleosome (GO:0000786)	59	3	0.009694	C50F4.6, hil-4, cpar-1
kinase activity (GO:0016301)	319	7	0.009974	cdk-5, hexokinase, F19B6.1, rskn-1, C34G6.5, thk-1, R05H5.4

S. Table 3. The Half-motif B is present in many of the 32 GBRs that contain a highly conserved Motif B.

GLD-1 BINDING REGIONS ^a	MOTIF B AND SURROUND SEQUENCES ^b	Spacing ^c
<i>rme-2</i> 3'UTR	NNTTCTAC TAC AAAAT TACTAA ATCAGATGTCTG	2
<i>rme-2</i> 5'end	CCACTTTT TA TTTCACA TACTAA TCAAGAACACNN	2
<i>exo-3</i> 5'end	NNNNGCC TAC TCGACT TACTAA CCTGTTGCTGAAA	2
<i>tra-2</i> 3'utr	TATT TA ATTTCTTATC TACTCA TATCTANNNNNN	6
<i>dna-2</i> 3'end	TCA TA TTTTCTATTCC TACTCA ACTG TA AAATTC	6 4
<i>dna-2</i> 3'end	NATTCCCGCCTTTTT TACTCA TT TA TTAGATTT	2
<i>hcp-3</i> 3'end	NAACA TA TTTGCA TACTCA TTTTTCGATCTT	3
F14B4.2 3'end	NCATGCATCCTCCCT TACTAA CTCCTTTCTTCA	N/A
<i>oma-1</i> 3'end	TATTCGTGTCCCCCA TACTAA CTAA TAAT GCCT	1 4
<i>lin-45</i> 5'UTR	CGCCCATCGT TA TTTT TACTAA TCATTTCTCGTCN	N/A
<i>oma-1</i> 5'UTR	GATTGGAGATTTGGT TACTCA AACTGCT TA ACTTG	6
<i>cbd-1</i> 5'end	CCGTCTCGCTGCTTC TACTAA CATTTTTTTTCGAA	N/A
<i>rskn-1</i> 3'end	TTCTTTTT TAA ATAC TACTAA CATT TA TCCAATT	1 4
<i>rskn-1</i> 3'end	AATAAC TA ACTTTATT TACTCA TCACACTCATCC	4
T01C3.2 3'end	TCGAATTTCGTCACAAC TACTAA CAAGAAATGATT	N/A
<i>sss-1</i> 5'end	GCGAATTTTCATCGCTC TACTAA CCA ACTA CAATC	N/A
<i>sss-1</i> 5'end	CAAC TA CAATCAACTC TACTAA CAC TA TGGTCAA	6 3
<i>nasp-2</i> 5'end	GCGCTTTTGCTGATTC TACTAA CAT TA TCGCTCA	3
<i>nasp-2</i> 5'end	CCACTGATTCGCCAC TACTAA CGAGNNNNNNN	N/A
<i>cdk-5</i> 3'end	CAC TA TGCACTTTCC TACTCA CATTGTTTTCTC	7
<i>sas-5</i> 3'end	TA ATGTCTGTGAATTG TACTAA AACCAT TA CTTC	6
<i>fem-3</i> 3'end	GTTTGAAATCTCTCGC TACTCA CCTC TA GATGTA	4
<i>cpg-1</i> 3'end	TAATC TA TCAAATGCC TACTCA TCCCAGTGCCAT	5
<i>gna-2</i> 5'UTR	CATTCACTTCT TA TTA TACTCA CTTCAANNNNN	N/A
<i>cep-1</i> 3'end	NNNATTTCTCACT TA G TACTCA AACTGACATTTG	N/A
<i>pri-1</i> 3'end	NNNAAGCACTTTC TA AG TACTAA CCCGGAAAACT	N/A
<i>cye-1</i> 3'end	TAT TA ATGTTTTGTTTT TACTCA TCTCACTTCCNN	7

^a GBRs

^b Stringent Motif Bs (red highlights) and surrounding UA dinucleotide (yellow highlights) are listed.

^c The spacing between the UA dinucleotide of the Half-motif B and Motif B within each GBR.