

Supplementary Materials For:

A modified feeding RNAi method for simultaneous knock-down of more than one gene in *C. elegans*

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Plasmid construction

bli-1 RNAi vector.

We used the *C. elegans* feeding RNAi library (1).

gfp RNAi vector

First, we amplified *gfp* cDNA, which is not genomic DNA and does not contain any synthetic intron, by PCR using GFP-F-*Pst*I (5'-GGGCTGCAGATGAGTAAAGGA-

GAAGAACTTT-3') and GFP-R-*Hind*III (5'-GGAAGCTTACCCATGGAA-CAGGTA-3') primers. Next, we inserted 180 bp of the *gfp* cDNA fragment into the *C. elegans* feeding RNAi vector pPD129.36 (L4440; a gift from Andrew Fire).

bli-1 and *gfp* RNAi vector

The 180 bp of *gfp* cDNA fragment was subcloned into the *bli-1* RNAi vector.

dpy-5 RNAi vector

We amplified the full length of *dpy-5* cDNA (850 bp) by PCR using *dpy-5*-F-*Hind*III (5'-CCAAGCTTATGG-TAAAGGCCGTCGTCGG 3') and *dpy-5*-R-*Kpn*I (5' GG GTACCTTA-GACGCGTCTGCGCTTTC-3'). The amplified PCR fragment was subcloned into the pPD129.36 vector.

mes-3 RNAi vector

The full length of *mes-3* cDNA [from *C. elegans* ORFeome-Based RNAi Library (2)] was digested with *Nco*I restriction enzyme. Digested *mes-3* cDNA fragment (~900 bp) was subcloned into the pPD129.36 vector.

dpy-5 and *mes-3* RNAi vector

mes-3 cDNA fragment was digested with *Nco*I (see the "*mes-3* RNAi vector" section). This *mes-3* fragment was subcloned into the *dpy-5* RNAi vector. When *mes-3* was inserted into the vector, we obtained two kinds of *dpy-5-mes-3* RNAi vectors in which the orientation of the *mes-3* inserts were different: one had an opposite orientation to that of *dpy-5* (*dpy-5+mes-3* I) and the other, the same (*dpy-5+mes-3* II). The space between *dpy-5* and *mes-3* was 53 nucleotides.

oma-1 RNAi vector

oma-1 cDNA fragments were amplified by PCR using the primers: *oma-1*-F-*Hind*III (5'-CCAAGCTTGGCTC-

Supplementary Table S1. The result of *bli-1* and *gfp* double RNAi

	<i>bli-1</i>	<i>gfp</i>	(<i>bli</i>)+(<i>gfp</i>)	(<i>bli</i> + <i>gfp</i>)
Bli phenotype (%)	95.27%	0.00%	29.49%	96.32%
sd (%)	3.74%	0.00%	10.56%	2.22%
Total Number (<i>n</i>)	527	511	444	551
No GFP signal (%)	1.42%	99.42%	95.51%	98.17%
sd (%)	0.38%	0.54%	3.53%	0.85%
Total Number (<i>n</i>)	527	511	444	551

Supplementary Table S2. The dilution effect of *bli-1* and *gfp* RNAi

A. <i>bli-1</i> RNAi				
	Control (L4440)	<i>bli-1</i>	1:1 (<i>bli-1</i> :L4440)	1:2 (<i>bli-1</i> :L4440)
Bli phenotype (%)	0%	97.17%	72.88%	30.44%
sd (%)	0%	0.38%	4.93%	8.51%
Total Number (<i>n</i>)	210	207	208	182
B. <i>gfp</i> RNAi				
	Control (L4440)	<i>gfp</i>	1:1 (<i>gfp</i> :L4440)	1:2 (<i>gfp</i> :L4440)
No GFP signal (%)	0%	99.42%	94.44%	92.70%
sd (%)	0%	0.54%	3.61%	2.36%
Total Number (<i>n</i>)	373	511	588	598

Supplementary Table S3. The result of endogenous gene RNAi

A. <i>dpy-5</i> and <i>mes-3</i> double RNAi					
	<i>dpy-5</i>	<i>mes-3</i>	(<i>dpy-5</i>)+(<i>mes-3</i>)	(<i>dpy-5</i> + <i>mes-3</i>)I	(<i>dpy-5</i> + <i>mes-3</i>)II
Dpy phenotype (%)	78.97%	0.00%	0.00%	66.06%	58.06%
sd (%)	7.16%	0.00%	0.00%	14.83%	4.13%
Total Number (<i>n</i>)	247	176	301	366	228
Mes phenotype (%)	0.00%	76.81%	57.89%	68.61%	61.46%
sd (%)	0.00%	8.85%	10.73%	2.08%	16.06%
Total Number (<i>n</i>)	103	115	217	255	126
B. <i>dpy-5</i> , <i>oma-1</i> , and <i>oma-2</i> double RNAi					
	<i>dpy-5</i>	<i>oma-1</i>	<i>oma-2</i>	(<i>oma-1</i>)+(<i>oma-2</i>)	
Dpy phenotype (%)	97.18%	0.00%	0.00%	0.00%	
STDEV (%)	3.61%	0.00%	0.00%	0.00%	
Total Number (<i>n</i>)	451	75	64	392	
Oma phenotype (%)	0.00%	1.39%	0.00%	92.88%	
sd (%)	0.00%	1.96%	0.00%	4.95%	
Total Number (<i>n</i>)	269	75	64	296	
	(<i>oma-1</i> + <i>oma-2</i>)	(<i>dpy-5</i>) +(<i>oma-1</i>) +(<i>oma-2</i>)	(<i>dpy-5</i> + <i>oma-2</i> + <i>oma-1</i>)	(<i>dpy-5</i> + <i>oma-1</i> + <i>oma-2</i>)	
Dpy phenotype (%)	0.00%	71.43%	89.72%	84.69%	
STDEV (%)	0.00%	13.42%	3.93%	8.50%	
Total Number (<i>n</i>)	86	893	492	286	
Oma phenotype (%)	96.15%	93.30%	94.84%	96.75%	
sd (%)	8.16%	5.50%	6.83%	5.63%	
Total Number (<i>n</i>)	86	489	210	187	

GAATCTAAGAC-3') and *oma-1*-R-*KpnI* (5' GGGGTACCCACAAATTGAGATGCTTGGTC-3'). The length of *oma-1* cDNA fragment is 865 nucleotides. This fragment was inserted into the pPD129.36 vector using *HindIII* and *KpnI* restriction enzymes.

oma-2 RNAi vector

oma-2 feeding RNAi vector was used in the *C. elegans* ORFeome-Based RNAi Library (2). The size of *oma-2* cDNA in vector is ~1100 nucleotides.

oma-1 and *oma-2* RNAi vector

The *oma-1* cDNA was digested by *HindIII* and *KpnI* enzymes. This fragment was subcloned into the *oma-2* RNAi vector. *oma-1* was inserted in the opposite orientation to that of *oma-2*. The space between *oma-1* and *oma-2* was 35 nucleotides.

dpy-5, *oma-1*, and *oma-2* RNAi vector.

To make *dpy-5*, *oma-1*, and *oma-2* RNAi vector, we digested *oma-1* and *oma-2* RNAi vector (see above) with *KpnI*. The digested *oma* fragment was subcloned into the *dpy-5* RNAi vector. We obtained two different

vectors. We named vectors according to the orders of genes. The first one was (*dpy-5*+*oma-2*+*oma-1*) and the other was (*dpy-5*+*oma-1*+*oma-2*).

RNAi experiment

Bacteria containing each RNAi clone were cultured in Luria Broth (LB) containing 50 µg/mL ampicillin for 8–14 h. We measured the OD₆₀₀ values of each RNAi clone by electrophotometry (SmartSpec Plus, Bio-Rad, CA, USA) and cultured RNAi clone to the same OD₆₀₀ values. The OD₆₀₀ value of the bacterial cultures which we used was within the range of 1.8–2.8. To make a mixture of bacteria that expressed different dsRNA, we mixed the same volumes of bacteria. Bacteria were spotted in an NGM medium containing 1 mM IPTG and 50 µg/mL ampicillin. The RNAi media were incubated for 1–2 days to induce dsRNA at room temperature.

To perform the RNAi, synchronized L1 worms (for *bli-1*, *gfp*, *dpy-5*, *oma-1*, and *oma-2* RNAi) were placed in the RNAi media. After 2–3 days, the RNAi phenotypes were analyzed. To observe the Bli and Dpy phenotypes, the worms were analyzed

using a dissecting microscope (Leica S6E, Leica, Wetzlar, Germany). To check *gfp* expression, worms were observed using a fluorescence microscope (Zeiss Axioplan 2, Zeiss, Osnabrück, Germany). To analyze the Oma phenotype, adult worms were observed (Zeiss Axioplan 2).

To examine the Dpy and Mes phenotypes, L4 worms were placed in the RNAi media. After 1 or 2 days, gravid adults were transferred to the new RNAi media and left to lay eggs for 2–3 h. After 2–3 days, the Dpy phenotypes were analyzed (Leica S6E). To check Mes phenotypes, adult worms were analyzed (Zeiss Axioplan 2).

References

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