

A Whole-Genome RNAi Screen for *C. elegans* miRNA Pathway Genes

Devin H. Parry, Jinling Xu, and Gary Ruvkun

Supplemental Reference

S1. Kim, J.K., Gabel, H.W., Kamath, R.S., Tewari, M., Pasquinelli, A., Rual, J.F., Kennedy, S., Dybbs, M., Bertin, N., Kaplan, J.M., et al. (2005). Functional genomic analysis of RNA interference in *C. elegans*. *Science* 308, 1164–1167.

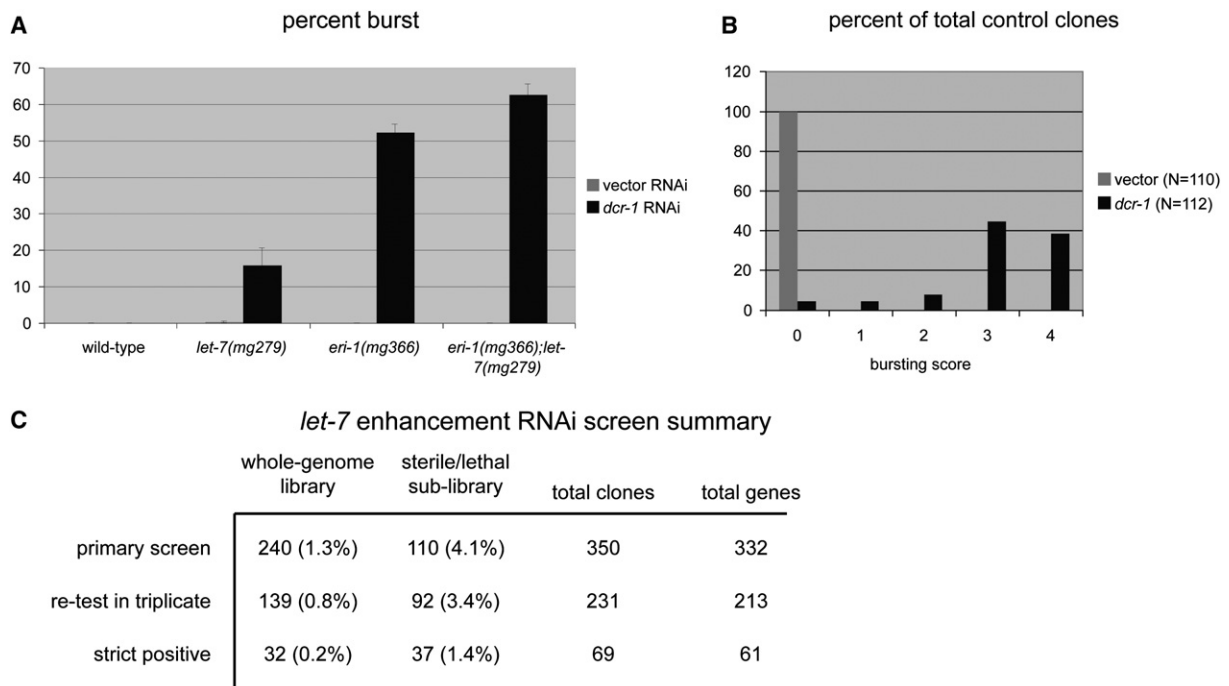


Figure S1. *let-7* Enhancement Screen Details

(A) *let-7(mg279)* is sensitized for defects in miRNA processing. Feeding of dsRNA targeting *dcr-1* induces significant bursting in the *let-7(mg279)* background as compared to wild-type N2. *dcr-1* RNAi also induces bursting in *eri-1(mg366)*, and this bursting is significantly increased in the *eri-1(mg366); let-7(mg279)* background (by chi-square test, p value = 9.15×10^{-9}). Each of these worm strains fed empty vector control showed negligible bursting.

(B) Positive and negative dsRNA feeding controls. Empty vector negative controls were consistently scored 0 in the *let-7* enhancement screen. *dcr-1*-positive controls were mostly scored as strong inducers of bursting (3 or 4), but a minority of these control clones were scored as inducing weak or no bursting. All controls were scored blindly.

(C) *let-7* enhancement screen summary. The whole-genome library consists of the Ahringer library supplemented with clones from the Vidal Orfeome library for a total of ~17,900 clones and was screened in the second generation. The ~2700 sterile/lethal sublibrary clones have been previously described [S1] and were screened in the first generation. The primary screen was done in a single pass, the retesting was done in triplicate. A total of eight tests (for most clones) were used to calculate an average burst score (see Table S1), and those clones with an average of 2 or greater or that were scored as 3 or 4 on at least two separate occasions make up the strict positive class.

bursting enhancement test for *lin-1(e1777)*

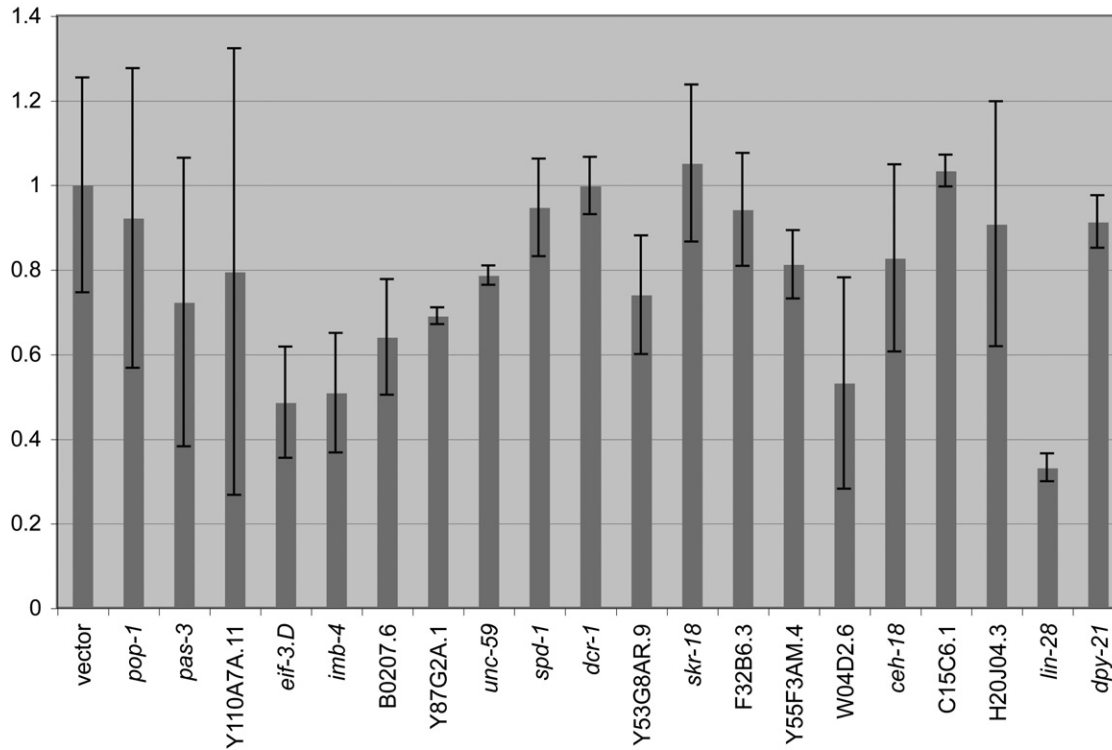


Figure S2. Bursting Enhancement Test with the General Vulval Development Mutant *lin-1(e1777)*

RNAi clones that induced *let-7(mg279)*-dependent bursting were fed to the *lin-1(e1777)* mutant, which has a multiple vulva phenotype and bursts at a low level on its own. The percent burst after dsRNA treatment was normalized to that seen for *lin-1(e1117)* fed empty vector control. Error bars indicate standard deviation of three replicates. None of the dsRNA treatments significantly enhanced the bursting of this vulval development mutant.

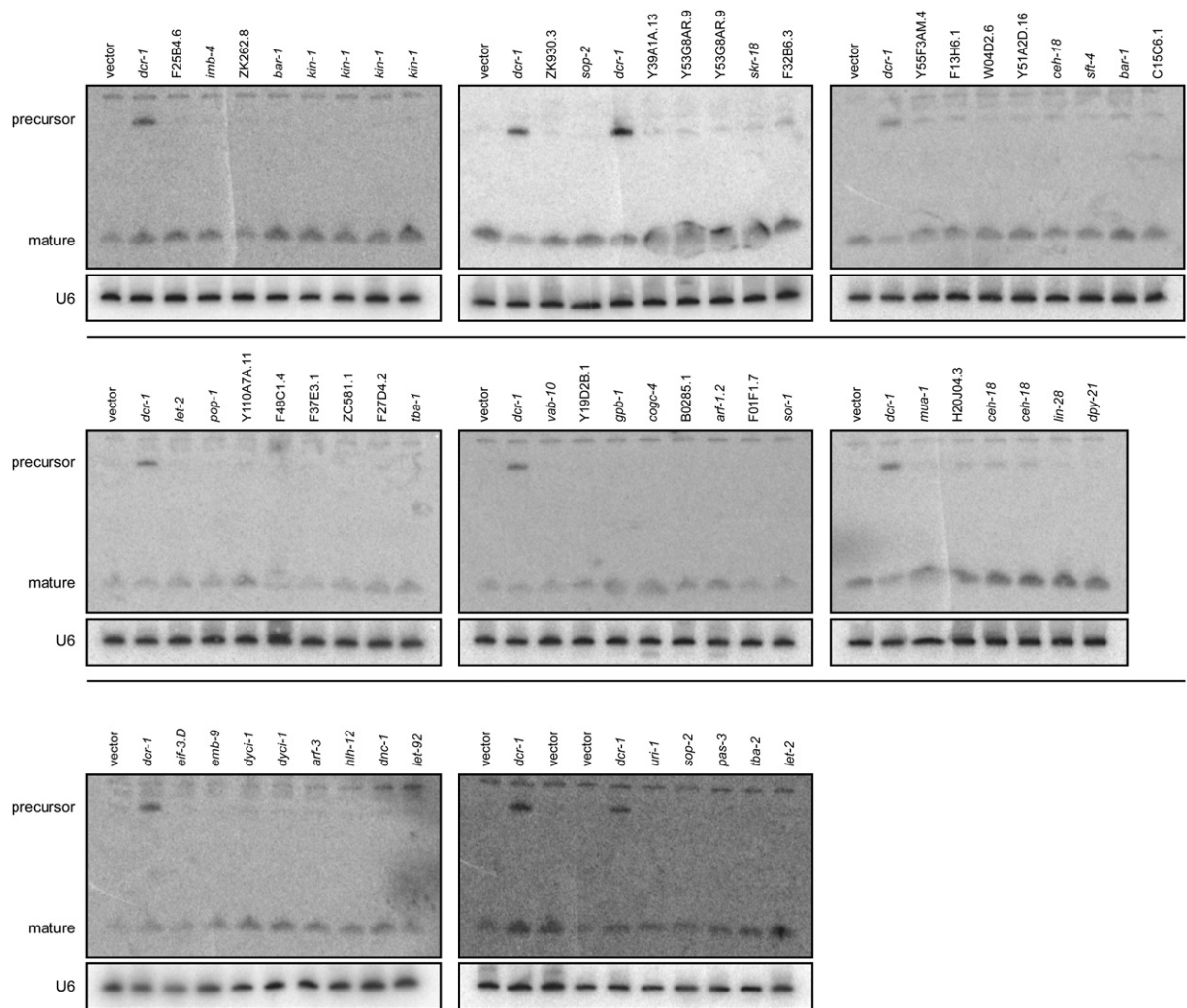


Figure S3. *let-7* Northern Blots

Total RNA was isolated from young adults fed RNAi. Each blot contains an empty vector-fed negative control and a *dcr-1* (RNAi)-positive control. Blots were reprobbed with a U6 snRNA probe as a loading control. Only *dcr-1* (RNAi) shows a significant increase in precursor accumulation, indicating a defect in *let-7* miRNA processing.