Supplementary Figure S1

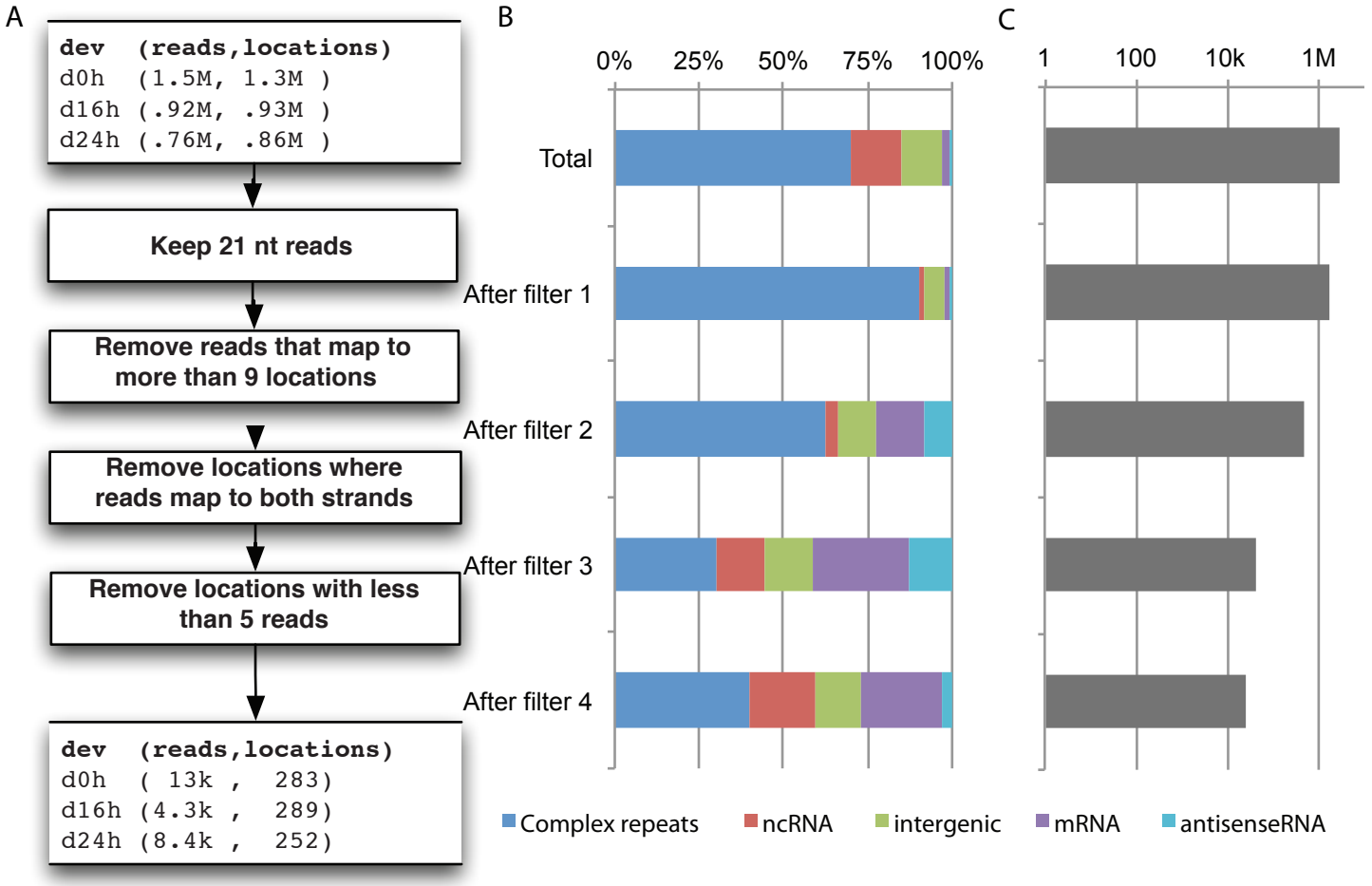


Figure S1. Small RNA filter scheme. (A) The different filter steps used for reducing the number of reads of putative miRNAs in each library, i.e. cDNA from growing cells (d0h), 16 h developed cells (d16h), and 24h developed cells (d24h). Number of reads and their genomic locations are depicted as reads and location, respectively. (B) Relative number of reads of different classes of small RNAs after the different filtration steps. (C) Number of genomic locations, from where the small RNAs originate, after the different filter steps.

Supplementary Figure S2

A

	d0h	d16h	d24h	total
Locations	283	289	252	567

Identify pre-miRNA structures using MiRfold

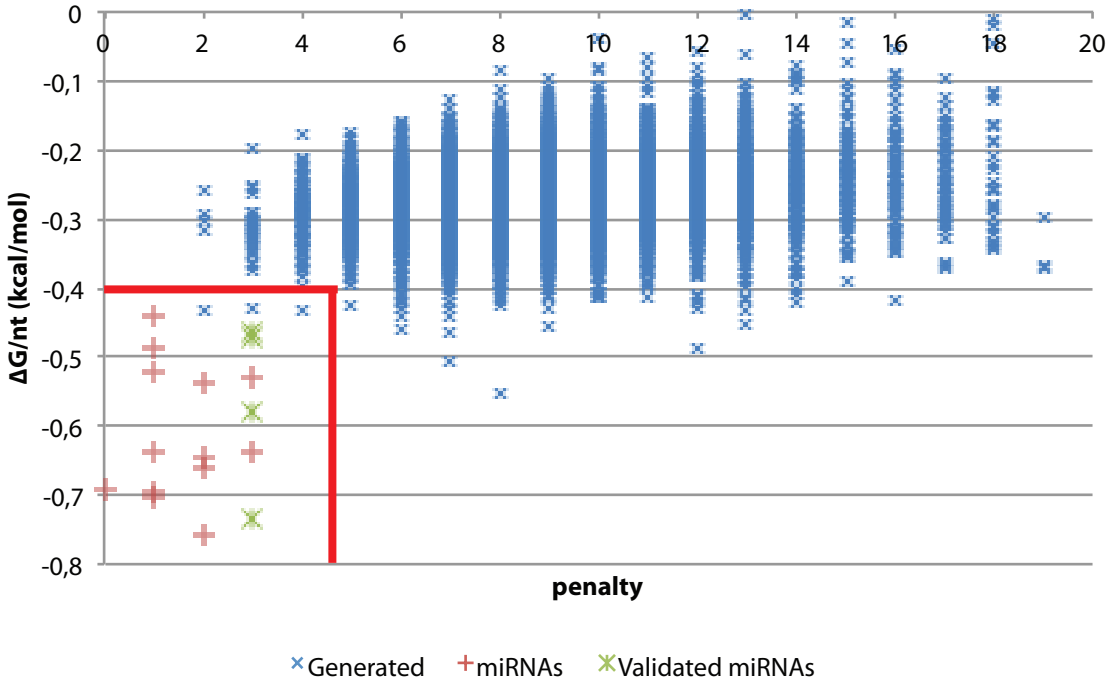
ΔG/nt < -0.4 kCal/mol
penalty < 5

Identify human-like pre-miRNA structures using miRabela

score > 0

	d0h	d16h	d24h	total
miRNAs	9	9	13	17
pre-miRNAs	10	15	20	45

B



C

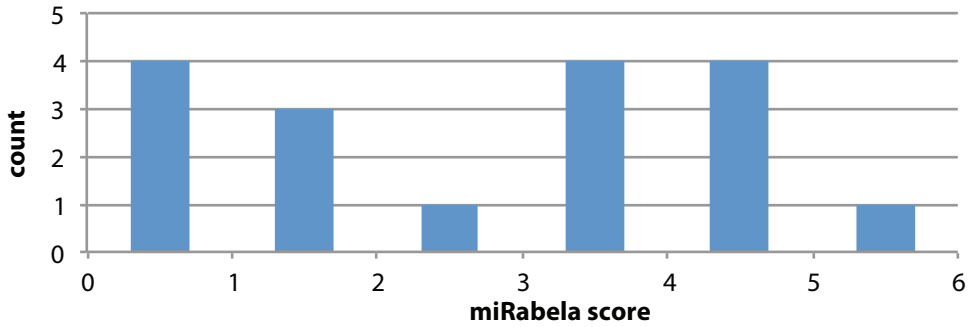


Figure S2. Identification of pre-miRNA structures. (A) The different filter steps used to analyze the sequences surrounding the putative miRNA locations (Location) using MiRfold and miR-abela and the number of resulting miRNAs and their pre-miRNAs. The number of miRNAs from growing cells (d0h), 16 h developed cells (d16h), and 24h developed cells (d24h) as well as the total number is presented. Note that the same miRNA often was identified in the different libraries. (B) Distribution of MiRfold scores. Red crosses and green stars depict the *D. discoideum* miRNAs described in this study. Green stars represent the four miRNAs that were experimentally verified. Two of the miRNAs (green upper stars) received almost identical scores and hence overlap. Blue crosses represent the distribution of 10 000 generated sequences. Red lines mark filter cutoff for the MiRfold step. (C) The miR-abela scores for the 17 different miRNAs.