- Outline

 Small RNA sequencing
 Quality control
 Read mapping

Small RNAs

Three categories:

- piwi-interacting RNAs (piRNAs) 21-32 nt long, predominantly germline small RNAs that bind PIWI proteins (related to Argonautes). Found only in animals.
 - Essential roles in regulating transposons.
- 2. microRNAs (miRNAs) 20-24 nt long small RNAs expressed throughout development that bind to Argonaute proteins. Found in plants and animals.

 Regulate mRNAs throughout development.
- 3. Small interfering RNAs (siRNAs) 20-26 nt long small RNAs expressed in various tissues that bind to Argonaute proteins. Found in plants, some fungi, and animals.

 Various roles, including regulating transposons.

Unifying features: size and protein binding partners (Argonaute/Piwi).



Small RNA experiment		
wild type C. elegans	Small RNA-seq On 1 Small RNA-seq On 2 On 3 On	
prg-1 mutant C. elegans	CH 3 - 5' adapter figation Revense transcription and CDMA amplification	
Which small RNA pathway does PRG-1 function in?		

Small RNA data and	alysis pipeline	
Raw fastq data FastQC Trimmomatic	——— Quality statistics and plots	
Filtered fastq data Bowtie2	——— Map reads to reference genome	
Alignment data in sam format		
SamTools Processed data for visualization	Sum reads for specific small RNA loci	
[IGV	——— Data visualization	

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