Statistical analysis of small RNA-seq data

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- Piwi-interacting RNAs (“piRNAs”).

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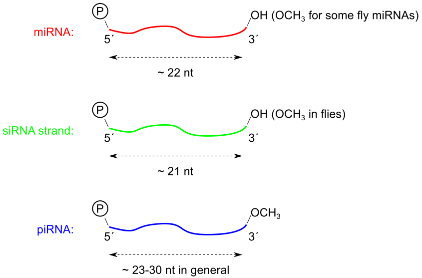

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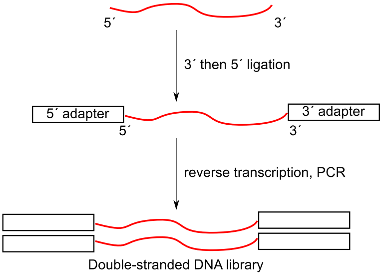

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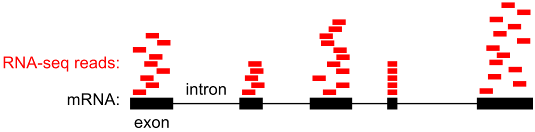

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Ligation biases were measured in Jayaprakash *et al.* (2011) *Nucleic Acids Research* 39(21): e141.

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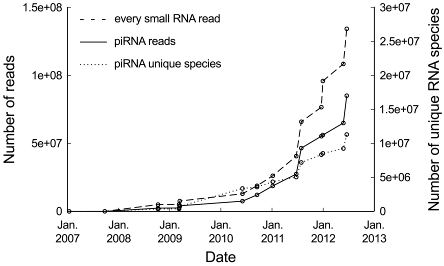

(Sergeeva *et al.* (2013) *Biochemistry (Moscow)* 78(6): 613)

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piRNAs and siRNAs are pooled (by targeted transposon, or by genomic locus) in order to get decent numbers to analyze.

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	Condition 1	Condition 2
Reads matching miRNA of interest	100	300
Reads not matching miRNA of interest	9,999,900	19,999,700

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	Condition 1	Condition 2
Reads matching miRNA of interest	1	3
Reads not matching miRNA of interest	99,999	199,997

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(p-value $= 1$)

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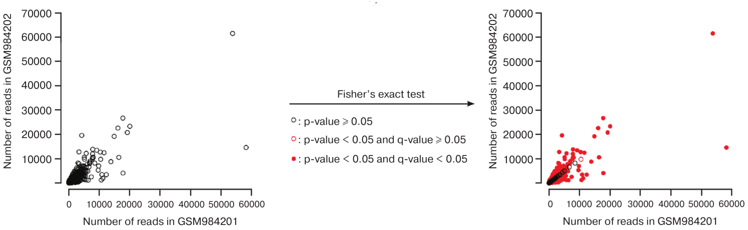
Comparison of two biological replicates (*Drosophila melanogaster* first instar larva small RNAs, modENCODE project):



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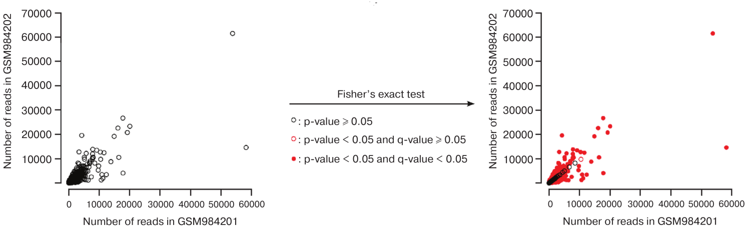

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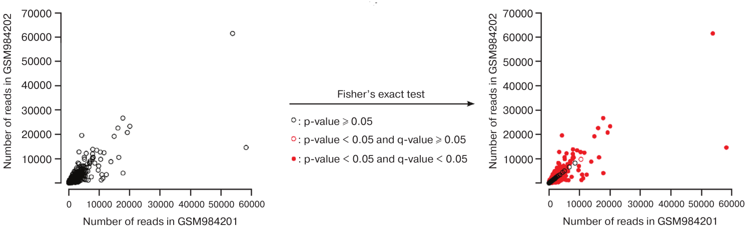

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Inter-individual variability is detected. Confounding effect, when comparing two genotypes.

Assessing differential expression

Detailed protocol for differential expression analysis:
Anders *et al.* (2013) *Nature Protocols* 8(9): 1765.
(developed for RNA-seq, not small RNA-seq)

Laboratory effects in small RNA-seq

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Sequencing the same collection of RNA samples in 7 sequencing facilities, to estimate laboratory effects: 't Hoen *et al.* (2013) *Nature Biotechnology* 31(11): 1015.

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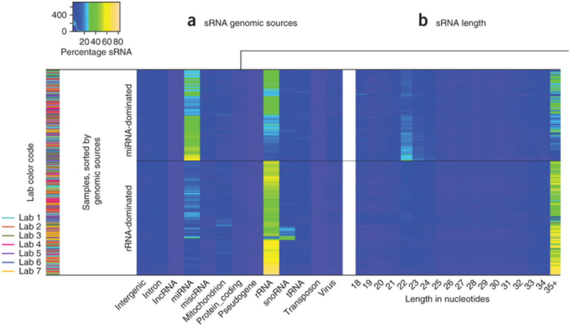
Results are consistent despite independent library preparation (most variation comes from the RNA extraction step; *e.g.*, rRNA fragment contamination). Main difference: % GC.



Absolute RNA quantification using RNA-seq

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Goal: absolute measurement of mRNAs (number of molecules per cell).

(Natalia Pinzón Restrepo)

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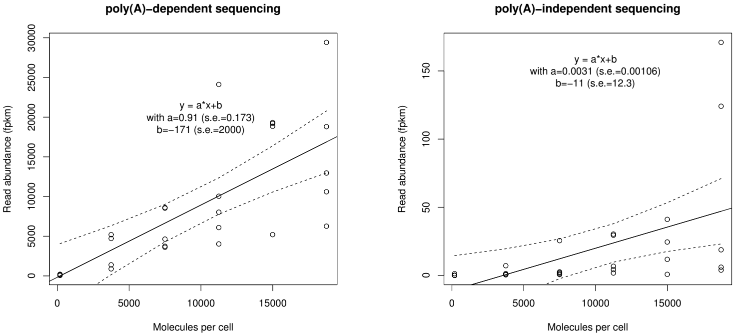

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When cautiously analyzed, small RNA-seq results are robust (even across laboratories).