Dealing with Small-RNA Data

CHALLENGES AND WORKAROUNDS

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Customized Method Development
From Design to Results

Experimental Design

Method Development and Data Analysis

Results

Test
Control
Use of small-RNA data

Understand Eukaryotic Gene Regulation

Provides Insights into post-transcriptional modulation of gene expression patterns

Small-RNA are implicated in several biological processes including development, regeneration and cancer

Disruption of small-RNA expression patterns is a strong indication of ectopic gene expression – cellular diseases.
Challenges in dealing with small-RNA

Small-RNA is, well, small!!

Small-RNA molecules, sometimes as small as 15 – 20 bases, can be exactly replicated in the genome.

Sporadic degradation of longer RNA might show up as small RNA.

How do we know if the presence of a small RNA is biologically interesting?
Statistics holds the key

Tools are available to analyse small-RNA

Often, the analysis moves outside the ambit of what the tools offer.

An understanding of what to do at these points underlines the possibility of extracting biologically meaningful information.

Statistically valid interpretation is of paramount importance
How many reads mapping to a site is enough?

Many authors / scientists go by an arbitrary number: 5 reads, 10 reads!!

Here is the problem:
- 5 reads mapping to a point out of 10 Million Reads is not the same as 5 reads mapping out of 50 million reads.

What happens when the amount of data between replicates varies widely? (10 million vs. 25 million reads)

*How do we deal with this?*
How to tell apart small-rna from fragmented rna?

Sometimes, despite a good RIN, fragmented RNA will contribute to small-RNA data. Practically, many times, PI’s are forced to deal with bad raw data due to limitations in time / budget

How can we distinguish between the two? – Are there any Caveats?!!

*Widening the scope of analysis often gives a better idea*
How to Identify small-RNA in Non-Model Organisms?

In non-model organisms, there is very little or no information about small-RNA / micro-RNA

Role of Replicates in Identifying possible small-RNA loci.

In case of global signals, one can use k-mer preferences at the loci of interest.

*Case in Point: Identifying 3P-sites in non-model organisms.*
Thank You!!

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