Mapping Bacterial Transcriptomes from RNA-Seq Expression Data

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The importance of mapping bacterial transcriptomes

Defining transcribed regions and how they change in different environments gives us clues about bacterial physiology and evolution
Using RNA-Seq to map bacterial transcriptomes

RNA-Seq

- Convert RNA into cDNA libraries

- Generates sequencing “reads”

- Reads are aligned to a reference genome (when available) with nucleotide resolution

- Number of reads in region correlates to expression of RNA from region
The challenges of mapping bacterial transcripts by RNA-Seq

- Biases introduced during cDNA library construction
- Complexity of bacterial transcriptomes
The complexity of bacterial transcriptomes

Many adjacent genes in bacteria are independently transcribed...

Many others are coordinately transcribed as part of a single mRNA (operons)
The challenges of mapping bacterial transcripts by RNA-Seq: an example
Developing an algorithm for using RNA-Seq data to define *E. coli* putative transcription units (PTUs)

1. Smooth RNA-Seq coverage data
2. Average coverage within “bricks”
3. Group adjacent bricks into PTUs
4. Refine PTU start and end sites
5. Compare PTUs to transcripts, operons
The algorithm in action

Known transcription units identified, the algorithm worked

Raw data

Data smoothed, converted to bricks

Bricks grouped, boundaries refined
Operon missed, so close yet so far...
How do we systematically measure the performance of our algorithm?

- Success of the algorithm is measured by sensitivity and precision.

- **Sensitivity** - the total number of PTUs whose 5’ end is between 0 and 200bp upstream of the nearest annotated gene

- **Precision** – percent of total number of PTUs identified whose 5’ end is between 0 and 200bp upstream of the nearest annotated gene
Measuring the success of our algorithm

Sensitivity: 608 PTUs
Precision: 74.0 %
Candidates for previously unknown transcripts: an example
Conclusions

• We have developed an algorithm for mapping bacterial transcriptomes with RNA-Seq data

• The algorithm successfully identified many previously annotated transcription units, failed to identify others, and identified numerous novel PTUs
Going Forward

• Further fine tuning of the algorithm for higher precision and sensitivity

• Testing how decreasing the depth of RNA-Seq data effects the algorithm’s performance

• Testing the algorithm on RNA-Seq data from different bacteria
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