Levin et al. Genome Biology 2010, 11(Suppl 1):P26
http://genomebiology.com/2010/11/S1/P26

Genome Biology

POSTER PRESENTATION Open Access

Development and evaluation of RNA-seq methods

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From Beyond the Genome: The true gene count, human evolution and disease genomics
Boston, MA, USA. 11-13 October 2010

RNA-seq provides insights at multiple levels into the transcription of the genome as it yields sequence, splicing and expression-level information. We have been developing and comparing a wide range of RNA-seq methods for their ability to annotate transcribed genomic regions, identify differences between normal and cancer states, and quantify mRNA expression levels. We will present results in two areas: (i) strand-specific RNA-seq; and (ii) RNA-seq starting from total RNA.

Strand-specific RNA-seq is a powerful tool for novel transcript discovery and genome annotation because it enables the identification of the strand of origin for non-coding RNA and anti-sense RNA, as well as defining the ends of adjacent or overlapping transcripts transcribed in different directions. Using the well-annotated *Saccharomyces cerevisiae* transcriptome as a benchmark, we directly compared seven library construction protocols, including both published and our own novel methods. We found marked differences in strand-specificity, library complexity, evenness and continuity of coverage, agreement with known annotations, and accuracy for expression profiling. Weighing the performance and ease of conducting each method, we identified the dUTP second strand marking [1] and the Illumina RNA ligation methods as the leading protocols, with the former benefitting from the current availability of paired-end sequencing. Our analysis provides a comprehensive benchmark, and our computational pipeline is applicable for assessment of future protocols in other organisms.

RNA-seq methods that do not require the purification of mRNA will be valuable for several applications, including samples with low input amounts and/or partial degradation. In these experiments, it is necessary to reduce the fraction of sequencing reads derived from ribosomal RNA. We will present results from multiple approaches, including the use of Not-So-Random (NSR) primers for reverse transcription [2] and the NuGEN Ovation RNA-Seq kit.

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Published: 11 October 2010

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doi:10.1186/gb-2010-11-S1-P26
Cite this article as: Levin *et al.*: Development and evaluation of RNA-seq methods. *Genome Biology* 2010 **11**(Suppl 1):P26.

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