Increasing the Sensitivity of Transcriptome Profiling in Eukaryote and Blood samples by depleting abundant RNAs.

Genomics

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The large dynamic range of transcript expression within a total RNA sample presents a challenge in whole-transcriptome sequencing. Highly expressed transcripts with minimal biological interest can dominate readouts, masking detection of more informative lower abundance transcripts. Here, we present a method to enrich for RNAs of interest by eliminating unwanted RNAs before sequencing. This method is based on hybridization of probes to the targeted RNA and subsequent enzymatic degradation of the selected RNAs.

We optimized this method to remove cytoplasmic and mitochondrial ribosomal RNA (rRNA) from human, mouse, and rat total RNA samples. This includes removal of the internal and external transcribed spacers (ITS & ETS) in human primary rRNA transcripts, which accounts for up to ~10% of transcripts in certain sample types. We have also incorporated new enzymes and streamlined the method to make it more robust and specific. Additionally, and to address the presence of highly abundant globin transcripts in blood samples, we expanded the probe set to remove adult, fetal and embryonic globin transcripts from blood RNA from which globin can constitute up to ~60% of mRNA transcripts.

Using strand-specific RNA sequencing we measured depletion efficiency, library complexity and transcript expression before and after depletion. We achieved high depletion efficiency (up to 99%) for both rRNA and globin mRNAs while maintaining transcript abundance and complexity. The method works efficiently with a wide range of inputs (10ng-1ug total RNA), as well as degraded total RNA including that from formalin-fixed-paraffin-embedded (FFPE) samples.

We conclude that removal of abundant transcripts using this simple and efficient method greatly increases the sensitivity of RNA-seq studies by enabling the detection of lower abundance transcripts and true biological variations. Furthermore, this method is amenable to high throughput sample preparation and robotic automation for easy implementation in a clinical setting.