Title

A set of miniaturized high-throughput library protocols for RNA sequencing.

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increase of protein coding transcripts, from 5% in the existing kit to 55% in RiboMinus™ Bacteria 2.0. In addition, rRNA removal increases sensitivity for detection of noncoding transcripts. Finally, we also demonstrate that the newly developed RiboMinus™ Bacteria 2.0 Kit utilizing the new RiboMinus™ Pan-Prokaryote Probe Mix exhibits high ribosomal removal efficiency when tested with Salmonella enterica total RNA.

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A set of miniaturized high-throughput library protocols for RNA sequencing

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Several studies have demonstrated that increasing the number of biological replicates, even without increasing the total amount of sequencing, significantly improves experimental power to detect differentially expressed genes by RNA sequencing. However, the high cost of Illumina library production is a critical factor that limits the number of biological replicates performed, leading to underpowered experiments. Here we describe a set of protocols for Illumina library preparation from bulk RNA at miniaturized reaction volumes. Methods for preparation of long RNA-seq libraries (using either polyadenylated RNA enrichment or rRNA depletion) and small RNA-seq libraries were established on a mosquito pipetting robot that enables accurate liquid transfers at nanoliter to microliter volumes. Reaction volumes were scaled down between 5-fold and 20-fold compared to the kit manufacturers' protocols, while RNA input was kept constant. The miniaturized protocols were assessed for their performance with respect to numbers of transcripts detected and other standard RNA-seq quality metrics. Through a dramatic reduction in the cost of library preparation, this approach enables cost-effective high-throughput projects with increased numbers of biological replicates.

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Monitoring the Impact of Pre-analytical Parameters on cfDNA Quality

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Cell-free DNA (cfDNA) has become an important source for potential new biomarkers. Via liquid biopsies it is easily accessible and can be a non-invasive way for genetic information. Nevertheless, it represents a challenging sample type due to its low yield and complex fragment size distribution. Controlling and monitoring pre-analytical parameters, including sample collection, storage conditions, and extraction methods, is crucial for the experimental success of any downstream application like next generation sequencing (NGS). A new automated electrophoresis assay was used to compare different kits tailored for cfDNA extraction. Three independent human blood plasma samples were processed in parallel with nine extraction kits from eight different vendors. The extracted cfDNA samples were analyzed with the new Cell-free DNA ScreenTape assay in combination with the Agilent TapeStation systems. The Cell-free DNA ScreenTape assay offers total DNA quantification, separate cfDNA quantification, and a %cfDNA quality score. A pre-set, customizable cfDNA region is assigned to analyze cfDNA from high molecular weight (HMW) DNA. The presence of HMW DNA can negatively affect library yield and sequencing quality of cfDNA samples. The %cfDNA quality score is provided as an additional quality parameter, determining the percent cfDNA subcomponents in the total DNA sample. The analyzed data for the different extraction kits and plasma samples revealed significant differences in total DNA and cfDNA concentration as well as %cfDNA. The choice for the most suitable cfDNA extraction kit depends on the requirements for downstream analyses as well as the expected cfDNA concentration and occurrence of HMW DNA.

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Pushing the limits of single-cell RNA-seq with SMART-Seq single cell technology

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Using droplet sequencing and full-length mRNA information in parallel has become an emerging requirement to help generate and better understand rich single-cell datasets. To address this need, we developed the SMART-SeqÂ® Single Cell Kit (SSsc) using new chemistry with unparalleled sensitivity and a highly scalable and easily automatable workflow. These features make SSsc chemistry extremely useful for difficult cells-e.g., clinical research samples that often have very low RNA content-making it ideal for highly detailed characterization of precious samples.

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Miniaturization of Ribosomal RNA Depletion and Total RNA Library Preparation in Single Cells

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Miniaturization of library preparation methods has been shown to significantly decrease both costs and labor associated with next generation DNA sequencing, without