biostate.AI

Affordable and Flexible RNAseq with BIRT+PERD Technology

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Biostate's solution for RNAseq

Obtaining accurate gene expression data from RNAseq workflows is both costly and complex. Traditional depletion or enrichment steps used to target RNAs of interest rely on expensive enzymes, introduce a variety of biases, or exclude lncRNAs (long noncoding RNAs). To address these challenges, Biostate has developed an innovative solution that integrates two proprietary technologies: BIRT (Barcode-Integrated Reverse Transcription) for sample multiplexing and PERD (Probes for Excess RNA Depletion) for nonenzymatic depletion of ribosomal RNA (rRNA). BIRT+PERD offers compelling advantages: a 16-fold increase in sample throughput, a 90% reduction in cost, the measurement of lncRNAs, and the ability to serve clients with more challenging samples or budget constraints. By adopting BIRT+PERD, NGS service providers can expand their client base, increase operational efficiency, and deliver superior value across research, clinical, and pharmaceutical applications.

BIRT: Barcode-Integrated Reverse Transcription

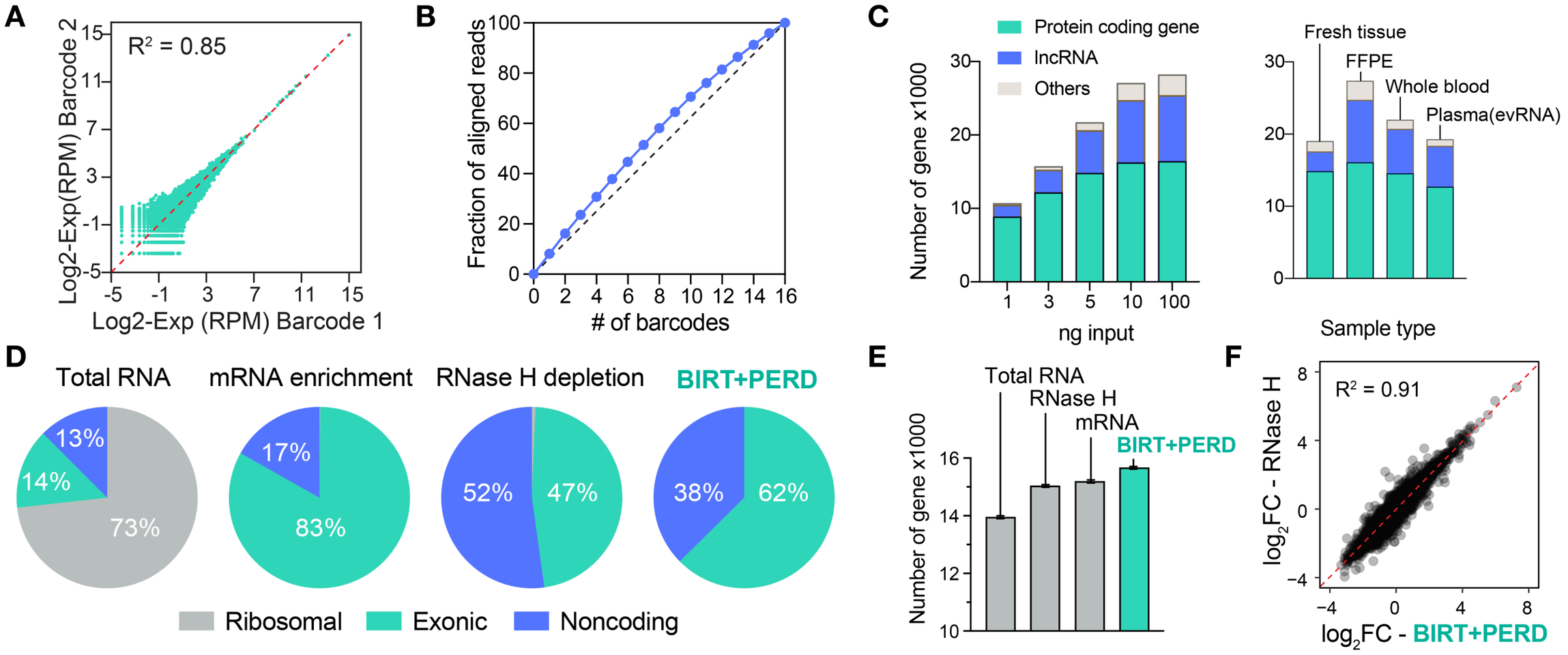
Patent-pending BIRT technology (Patent App. #63/570082) integrates sample-specific barcodes directly during the reverse transcription (RT) step using proprietary hairpin primers. Instead of traditional oligo(dT) priming, BIRT uses random polyN priming, which helps generate cDNAs from highly degraded samples, avoids 3'-end bias, and captures RNAs with short or no polyadenylation such as lncRNAs. In conventional RNAseq workflows, samples are processed individually until late stages of library preparation. BIRT revolutionizes this process by enabling the pooling of barcoded cDNAs from up to 16 samples immediately after the RT step. This pooling reduces hands-on time, reagent use, and costs, as downstream processes (e.g., adapter ligation and purification) can be performed collectively. The benefits of barcoding are realized without sacrificing reproducibility: expression data generated by different barcodes are highly concordant (Fig. 1A, R^2= 0.85). And all 16 barcodes have a similar barcoding rate—each barcode represents, on average, 4.7% of the total reads (Fig. 1B). Further, because BIRT uses random priming and avoids lossy enzymatic or bead-capture steps, it reduces the amount of RNA required from 1 µg to just 10 ng (Fig. 1C, left). This enables BIRT to work effectively with sample types that yield very small amounts of highly degraded RNA, such as Formalin-Fixed Paraffin-Embedded (FFPE) tissue on histopathology slides. BIRT delivers strong results using just a single 4-micron-thick FFPE slide (Fig. 1C, right), whereas typical approaches might require sacrificing multiple slides. Here, BIRT presents an opportunity for pharmaceutical customers to leverage their vast FFPE archives, both accelerating R&D and boosting RNAseq sales for NGS providers. Widely available but low-RNA-content plasma samples provide another opportunity: BIRT's small RNA input requirement allows more practical measurement of RNA from plasma, such as the extracellular evRNA fraction (Fig. 1C, right).

PERD: Probes for Excess RNA Depletion

Complementing BIRT, our patent-pending PERD technology (Patent App. #63/679268) technology innovatively solves one of the biggest inefficiencies in RNA-Seq: the overwhelming abundance of ribosomal RNA. In typical mammalian cells, ribosomal RNA (rRNA) comprises over 80% of total RNA content. Thus RNA-Seq on total RNA (Fig. 1D) wastes significant sequencing capacity on rRNA, driving up costs. PERD probes solve this issue by competitively inhibiting BIRT primers on rRNA transcripts, preventing them from being converted to cDNA in the first place. Compared with other methods (Fig.1D, RNaseH depletion), PERD reduces the proportion of rRNA reads as effectively—or better—by up to 99.8% (Fig. 1D, BIRT+PERD) while delivering more than twice the noncoding reads as mRNA enrichment. Finally, PERD's workflow is both enzyme-free and requires no bead-capture steps, substantially reducing cost.

BIRT+PERD performance

Conventional mRNA enrichment and rRNA depletion techniques are designed to deliver expression data on more unique genes than total RNA, and here BIRT+PERD outperforms both methods (Fig. 1E). In the process of delivering more unique genes, it is important that a method not distort the differential expression information in the sample. When comparing measured differences in gene expression (DEG), BIRT+PERD correlates as well with "undistorted" total RNA as does conventional RNase H; the two depletion methods give even more similar DEG results when compared against each other (Fig. 1F), suggesting that they reveal similar DEG patterns for low abundance transcripts.



"Biostate AI's customer support has been outstanding—they were responsive, knowledgeable, and made the entire process hassle-free. Their BIRT technology is also incredibly cost-effective, providing high-quality RNA-seq results without breaking the budget."

- Xiaonan Han, Dept. of Medicine (Case Western Reserve University)