Tools of the trade

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Profiling the total transcriptome of single nuclei in archived samples with snRandom-seq

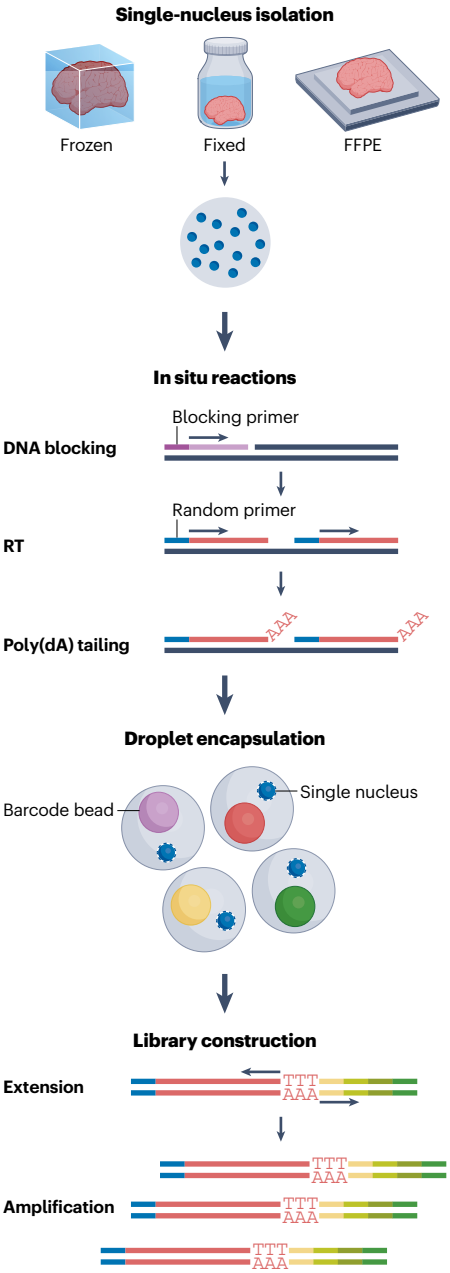

Fig. 1 | Schematic workflow of snRandom-seq. The main stages of snRandom-seq are single-nucleus isolation, in situ reactions, droplet encapsulation and library construction. FFPE, formalin-fixed paraffin-embedded; RT, reverse transcription.

Single-cell RNA-sequencing (scRNA-seq) technologies, such as the Chromium Single Cell platform from 10x Genomics, are widely used to uncover cellular diversity, with a primary focus on distinguishing the expression patterns of coding genes. However, understanding complex disease mechanisms and biological processes requires the exploration of a broader spectrum of RNA species, including small or long non-coding or circular RNAs. Traditional scRNA-seq methods, which rely on poly(dT) primers or probes to capture polyadenylated or target-specific RNAs, respectively, exhibit inherent biases, such as a preference for the 3′ ends of transcripts and the inability to capture non-polyadenylated or non-target RNAs. Moreover, these methods face challenges when applied to archived samples, especially formalin-fixed paraffin-embedded (FFPE) samples, which represent a valuable resource for both clinical practice and research.

To address these limitations, we developed snRandom-seq, a high-throughput, high-sensitivity single-nucleus total RNA sequencing technology optimized for archived samples, including frozen, fixed and FFPE specimens (Fig. 1). The workflow of snRandom-seq involves four main stages. First, the samples are pre-processed and dissociated to isolate single nuclei. Second, the single-nucleus suspension undergoes three in situ reactions, including blocking of single-stranded DNA, cDNA synthesis using random primers, and poly(dA) tailing at the 3′ ends of cDNAs. Third, single nuclei, barcode beads and the reagent mix are encapsulated into droplets by a microfluidic platform. Specific barcodes are added to the cDNAs in each droplet through an extension reaction. Finally, the droplets are disrupted, and the barcoded cDNAs are amplified and sequenced. To increase throughput, a pre-indexing strategy is applied during reverse transcription, in which nuclei are distributed into separate tubes with pre-indexed random primers and then pooled for the subsequent reaction. The entire snRandom-seq workflow, excluding sequencing and data analysis, can be completed in 2 days.

The optimized single-nucleus isolation and random primer-based RNA capture strategies enable snRandom-seq to generate comprehensive total transcriptome profiles at the single-nucleus level across various archived sample types. We have demonstrated that snRandom-seq captures a diverse array of RNA biotypes, including abundant non-coding RNAs, and exhibits minimal 3′- or 5′-end biases across gene bodies. The detection of extensive unspliced transcripts in snRandom-seq results, as demonstrated in mouse testis samples, enhances its effectiveness for velocity analysis. Additionally, snRandom-seq is well suited for archived samples, including FFPE specimens stored for extended periods. This capability is particularly advantageous for rare clinical samples and retrospective studies involving large-scale datasets collected over decades. For instance, snRandom-seq provided comprehensive molecular insights into the single-nucleus landscape of various glioma subtypes, including rare clinical cases and matched primary–recurrent glioblastomas.

> “a high-throughput, high-sensitivity single-nucleus total RNA sequencing technology optimized for archived samples”

To ensure reliable performance and improve consistency during the application of snRandom-seq, we have further developed automated systems for its key steps, including single-nucleus isolation and droplet encapsulation. This automation not only reduces variability but also lowers the technical barrier for adoption, making the technology more accessible and reproducible across different settings. These advantages in RNA detection performance and practical applications make snRandom-seq a powerful tool to explore comprehensive transcript diversity in various samples and enable large-scale integrative and retrospective clinical research. Emerging

technologies, such as robotics and artificial intelligence, offer considerable opportunities to modernize traditional laboratory workflows and experimental approaches. Our next step is to establish a fully automated, sample-to-result single-nucleus total RNA-seq platform, enabling streamlined, scalable applications for both research and clinical settings.

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Competing interests
Z.X. and Y.W. are co-inventors on a patent (CN114507711B) related to the snRandom-seq method.

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