A very brief intro to major single-cell protocols

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Contents

1 The Smart-seq2 protocol

1. First, the biological **sample of interest are collected**, and cells should be disaggregated into a single-cell suspension.
2. Cells are picked from the suspension and embedded in a **lysis** buffer containing a ribonuclease inhibitor that blocks RNA degradation, and primers for the reverse transcription step.
3. **Reverse transcription**. Single-stranded RNA molecules are converted into more stable double-stranded cDNA molecules.
4. The cDNA molecules are amplified using **PCR amplification**.
5. Construction of sequencing libraries using **tagmentation**. A transposase is used to **fragment** the cDNA molecules and simultaneously **ligate adapter sequences**.
6. Another cycle of PCR occurs (the enrichment PCR step). Sample barcodes may be added to allow multiplexing; a a dual-index strategy is often used, indices referred to as index 1 (i7) and index 2 (i5) in the Figure below.

2 The 10X Genomics Chromium protocol

- The 10X Genonics Chromium protocol is droplet-based, i.e., cells are embedded into nanodroplets within which library preparation steps occur.
- Within each droplet, gel beads along with reagents are encapsulated together with the cell.
- These gel beads contain millions of sequences on their surface. These sequences consist of a sequencing adapter ('TruSeq Read'), cell barcode ('10X Barcode'), molecular barcode ('UMI'), and a Poly(dT) to capture polyA'd mRNAs. As of v3, two additional capture sequences may be used for capturing additional molecules, e.g., guide RNAs.
- The beads are mixed with the cells of our sample, which are then emulated within oil to create nanodroplets. See 'Getting Started with Single Cell Gene Expression' video at 10:40 on the 10X Genomics website for a demonstration. These droplets are often called **GEMs**, for 'Gel beads in EMulsion'.
- Within these GEMs, the necessary library preparation steps occur, and the produced molecules each contain a cell barcode as well as a Unique Molecular Identifier (UMI), which may then be sequenced.

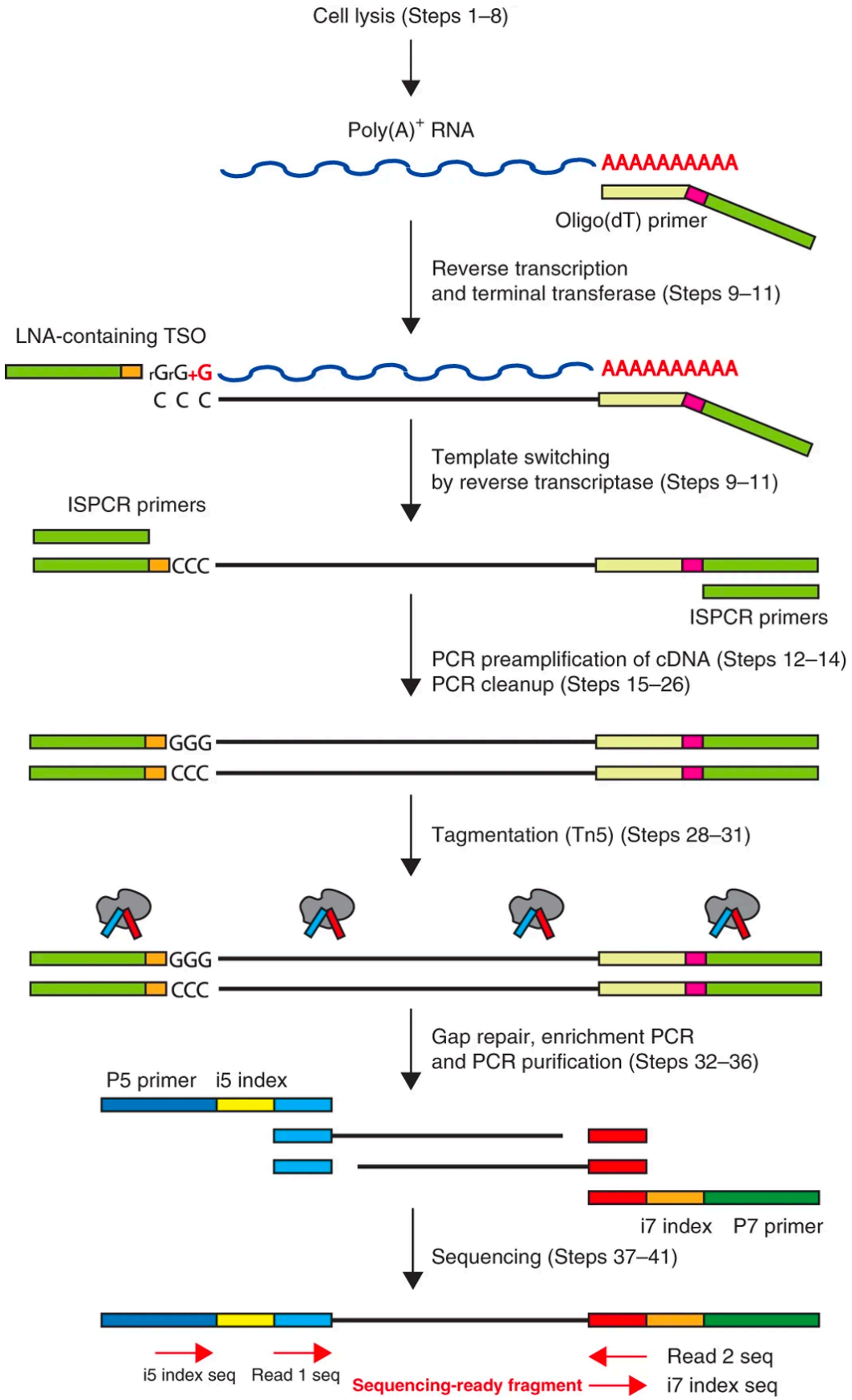
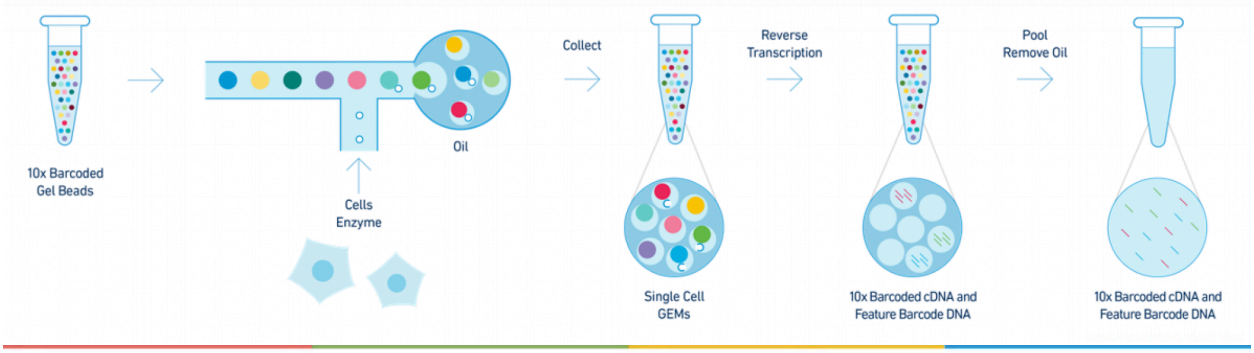
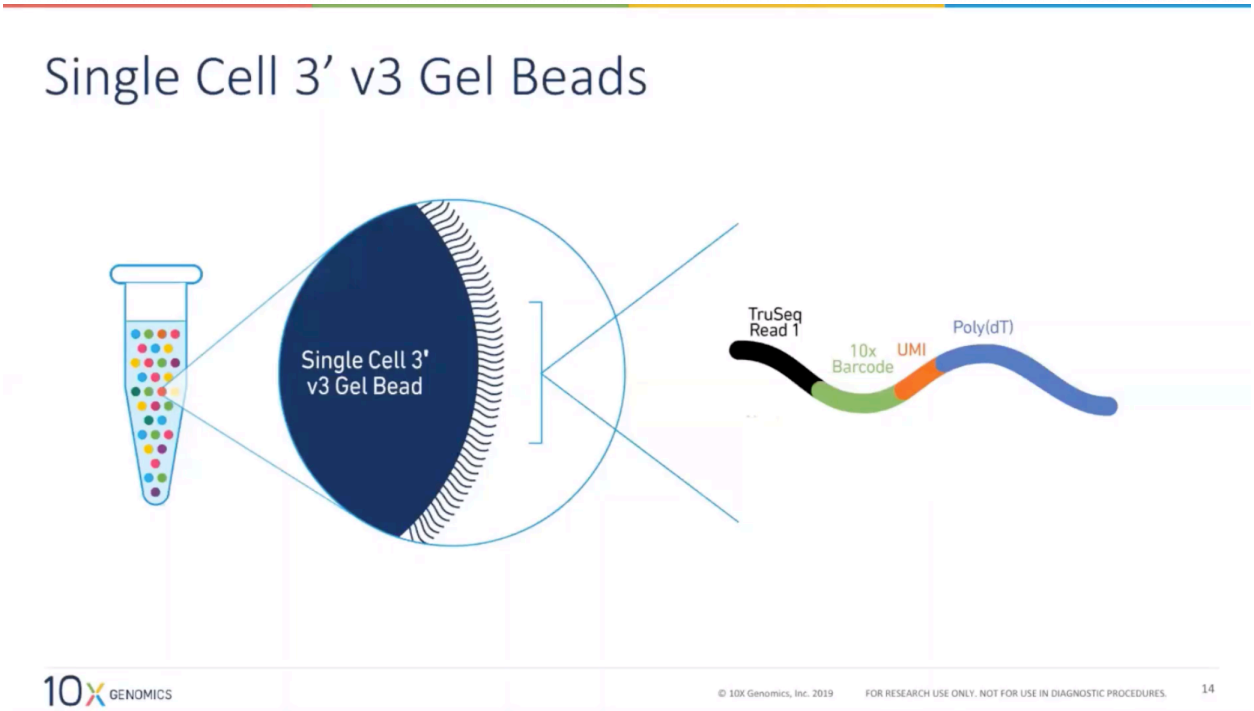


Figure 1: Figure: The SMART-Seq2 library prep workflow. Image from Picelli *et al.* (2014).





The Single Cell 3' dual index libraries generated using the standard and the HT assay have the same configuration.