Introduction to sequencing: Sequencing technology and preprocessing of sequencing data

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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.

1.0.1 The 10X Genomics protocol

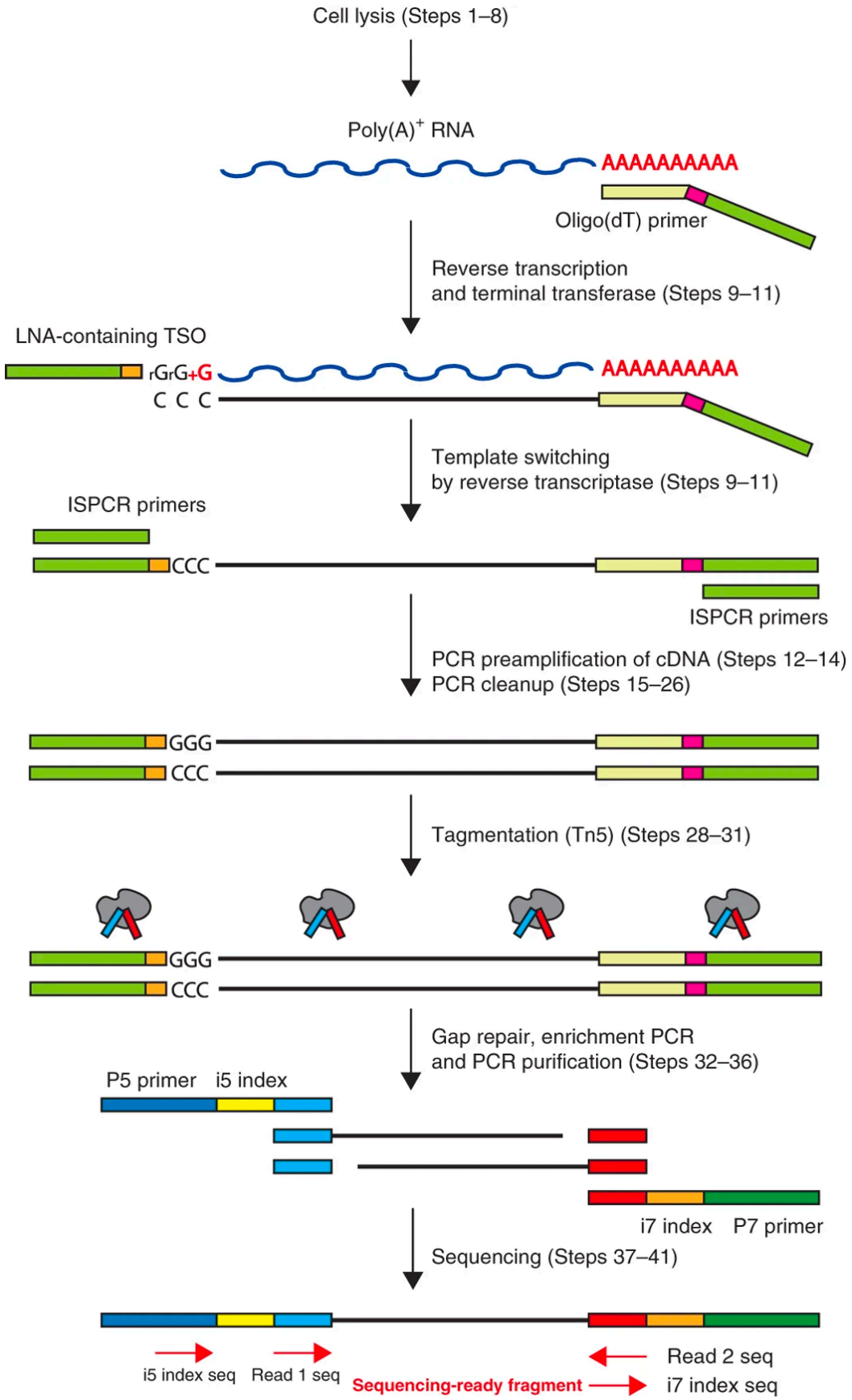

Figure 1: Figure: The SMART-Seq2 library prep workflow. Image from Picelli *et al.* (2014).