Preparation of Single-Cell RNA-Seq Libraries for Next Generation Sequencing

John J. Trombetta,David Gennert,Diana Lu,Rahul Satija,Alex K. Shalek,Aviv Regev

Abstract

For the past several decades, due to technical limitations, the field of transcriptomics has focused on population-level measurements that can mask significant differences between individual cells. With the advent of single-cell RNA-Seq, it is now possible to profile the responses of individual cells at unprecedented depth and thereby uncover, transcriptome-wide, the heterogeneity that exists within these populations. Here, we describe a method that merges several important technologies to produce, in high-throughput, single-cell RNA-Seq libraries. Complementary DNA (cDNA) is made from full-length mRNA transcripts using a reverse transcriptase that has terminal transferase activity. This, when combined with a second “template-switch” primer, allows for cDNAs to be constructed that have two universal priming sequences. Following preamplification from these common sequences, Nextera XT is used to prepare a pool of 96 uniquely indexed samples ready for Illumina sequencing.

Citation: John J. Trombetta,David Gennert,Diana Lu,Rahul Satija,Alex K. Shalek,Aviv Regev Preparation of Single-Cell RNA-Seq Libraries for Next Generation Sequencing. **protocols.io**

dx.doi.org/10.17504/protocols.io.pbhdij6

Published: 27 Jun 2018

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PROTOCOLS

1. Preparation of Single-Cell RNA-Seq Libraries for Next Generation Sequencing
CONTACT: Anita Bröllochs

2. Smart-seq2 single-cell RNA-Seq modified method
CONTACT: Anita Bröllochs

3. Single-cell RNA-Seq expression analysis
CONTACT: Anita Bröllochs