Name	TCR Sequence Determination by TraCeR
Version	A
Description	The variable sequences of the T cell receptor (TCR) are determined for single-cell whole transcriptome data with the C1™ Single-Cell mRNA Seq Protocol and the TraCeR data analysis pipeline.
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General Workflow	Single-cell libraries are generated with the mRNA Seq Protocol. TCR α and β chain sequences for individual cells are determined through the TraCeR analysis pipeline.
C1 Protocol	Using C1 to Generate Single-Cell cDNA Libraries for mRNA Sequencing (Fluidigm PN 100-7168). The reagents and IFC part numbers are specified in the protocol.
Data Analysis	The TraCeR data analysis pipeline uses artificial reconstructions of the recombined TCR genes to identify the recombination in each cell. This pipeline is used in parallel with standard whole transcriptome analysis techniques. The TCR gene and the whole transcriptome datasets are combined after the TraCeR analysis to present the clonotype and phenotype information at the single-cell level. (See Additional Documents to download the TraCeR application note.)
Special Instructions	The successful detection of TCR sequences will depend on the amount of relevant transcript available for isolation, which is dependent on the subtypes of T cells captured by the C1 IFC (integrated fluidic circuit) and on the sample preparation protocol. We used freshly isolated mouse splenic T cells and were able to detect a variety of subtypes, including activated proliferating T cells and central and effector memory T cells. Detecting transcripts in single, naïve T cells may be difficult due to their quiescent nature and low transcription rates. Recommended sequencing depth is 1 million reads per T cell. The sample handling method will also influence the amount of transcript available for amplification. Take the following precautions to minimize cell stress: • Avoid cryopreservation. • If cryopreserved samples must be used, implement a protocol that is optimized for cell recovery. • Minimize the amount of time between the C1 run and the cell harvest or thaw. • Conduct antibody labeling or other enrichment steps at 4 °C. • If you are pre-enriching by flow sorting, use a low-pressure and larger nozzle (100 µm) for reduced shear stress to the cells.