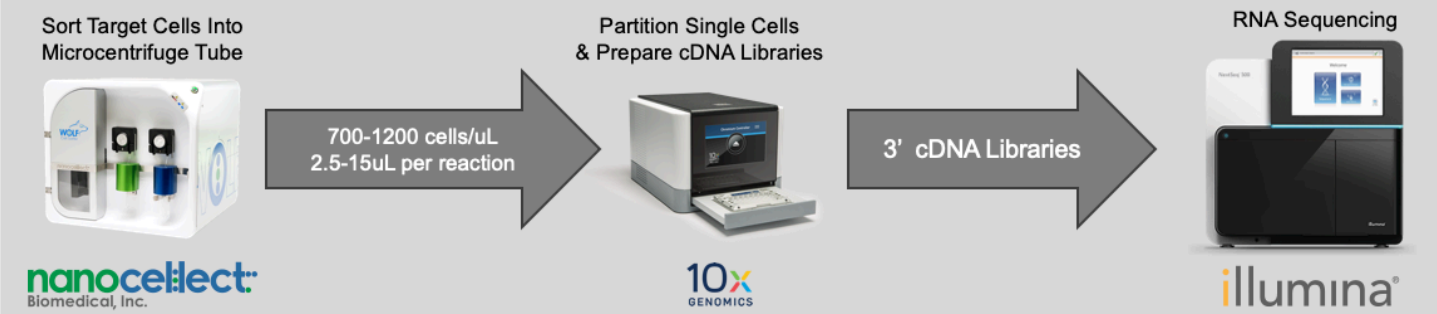
Seamlessly Transition from Cell Sorter to Single Cell Library Preparation with the WOLF®

Introduction: Single cell RNA-Sequencing has led to many novel discoveries such as the detection of rare cell populations, microbial populations and cancer mutations. The WOLF Cell Sorter and N1 Single Cell Dispenser, developed by NanoCellect®, is a novel microfluidic-based cell sorter compatible with several RNA-sequencing platforms. At less than 2 psi, the WOLF is more gentle than any other conventional cell sorter, enabling healthier cells post sort and higher RNA integrity. Low stress during cell sorting avoids potential gene expression changes induced by traditional sorters. In addition, the WOLF excels at excluding dead cells and debris; therefore, maximizing the data generated per dollar spent on sequencing reagents and analysis time. Furthermore, the WOLF's microfluidic cartridges are completely disposable, everything the sample and sheath fluid touches is sterile and free from sample to sample contamination enabling more accurate sequencing results. The N1 can dispense 1 to 100 cells directly into a 96 or 384 PCR plate, seamlessly transitioning from cell sorter to RNA library preparation. Moreover, with 5 parameters of detection, the WOLF and N1 provide higher rates of singlet detection and live/dead discrimination compared to cell printers and limiting dilution. Here, we explain the simplicity and ease of using the WOLF upstream of two common methods for single cell RNA-seq assays.

Bulk cell sorting for 10x Genomics assays: The WOLF generates suspensions of viable cells, which can be used as input for 10x Genomics' single cell assays. We have demonstrated compatibility using the Single Cell Gene Expression Solution. A single cell suspension of target cells can be isolated using the WOLF and then loaded into the Chromium Controller. The Chromium Controller encapsulates single cells with Gel Beads-in-emulsion where each individual cell undergoes automated reverse transcription and barcoding. This is then followed by cDNA library preparation and sequencing (Figure 1). When isolating your target population for downstream use in the Chromium Controller workflow it is critical that you take the proper steps to maximize cell viability. The total number of cells required should be determined by the user, however, for Single Cell Gene Expression, 10x Genomics recommends that your cell population should contain more than 90% viable cells at a cell concentration of 700-1200 cells per/uL. Single cell expression kits are designed to profile 500-10,000 cells in 2.5 to 15ul of buffer per sample/reaction. After isolating your cell population on the WOLF, your sample should be loaded into the Chromium chip within 30 minutes to avoid cell clumping and death. Each chip can run 8 reactions/samples at once. Refer to 10x Genomics technical documentation for additional guidance on sample cleanliness and viability.

Figure 1: WOLF to Chromium Controller Workflow



Sorting into 96-well plates for QIAGEN scRNA-seq kits: The WOLF is compatible with QIAGEN's QIAseq RNA library preparation kits. Combining the WOLF Cell Sorter and QIAseq kits, single cells can be directly dispensed into PCR plates preloaded with cell lysis buffer. This allows for a continuous transition from cell sorter to single cell library preparation using the QIAseq UPX 3' Transcriptome kit or the QIAseq FX Single Cell RNA Library Kit (Figure 2).

The QIAseq UPX 3' Transcriptome kit allows for library construction of 1 to 100 cells in a simplified workflow. Cells are dispensed into wells of PCR plates (approximately 7uL) pre-loaded with 3 uL of Cell Lysis Premix. Reverse transcription is then performed with a Unique Molecular Index (UMI) and well-specific cell ID (up to 384 wells) allowing all cDNAs to be combined into one tube that enables simplified, single-tube library construction that can then be sequenced (Figure 2). This kit is ideal for high-throughput next-generation sequencing (4608-18,432 samples per sequencing lane) from extremely minute amounts of RNA.

The QIAseq FX Single Cell Library Kit is perfect for generating libraries that require extreme accuracy and deep transcriptome analysis. This kit allows for library construction of 1-1000 cells in a PCR free workflow. Multiple Displacement Amplification (MDA) with a REPLI-g SensiPhi DNA Polymerase allows users to amplify cDNA with minimal sequencing bias. This results in higher yields of unaltered amplified cDNA with negligible false positive and negative results. This kit requires preloading a PCR plate with 4uL of Cell Lysis. Cells are then captured into the PCR plate with a droplet size of ~7 uL of buffer into each well. Reverse transcription is then performed followed by library construction. Adaptor barcodes are then added allowing for multiplexing and then sequencing (Figure 2).

Figure 2: WOLF to QIAseq scRNA-Sequencing Workflow