



# THE PATH LESS TRAVELED JUST GOT EASIER

## C<sub>1</sub> Single-Cell Auto Prep System for mRNA Sequencing

- **Complete**—End-to-end workflow for whole transcriptome analysis of individual cells
- **Highest throughput**—Unprecedented parallel processing of 96 single cells per run
- **Easier**—Streamlined sample preparation directly from single cells with only 3 hours of hands-on time, and no fragmentation or purification steps
- **Affordable**—One-eighth the cost of other library preparation systems

Researchers are analyzing the transcriptome at greater depth to uncover new mechanisms of cell development, metabolism and disease. mRNA sequencing is a valuable tool to help researchers understand how subpopulations respond to signals and other environmental cues at critical stages of cell-fate determination or when they acquire aberrant phenotypes. Studying these gene expression patterns in single cells already has dramatically advanced cell biology.

Most methods, however, are impractical for single-cell analysis; they require large numbers of cells, are based on complex workflows that are too slow or generate highly variable results, and are too costly to generate significant results from single-cells.

## THE C<sub>1</sub> SINGLE-CELL mRNA SEQUENCING APPLICATION

- Measure expression levels of genes, alleles, and spliced variants
- Compare expression profiles between individual cells and populations
- Map transcription initiation sites
- Characterize alternate splicing patterns
- Evaluate post-transcriptional activity
- Discover new transcripts and gene fusions

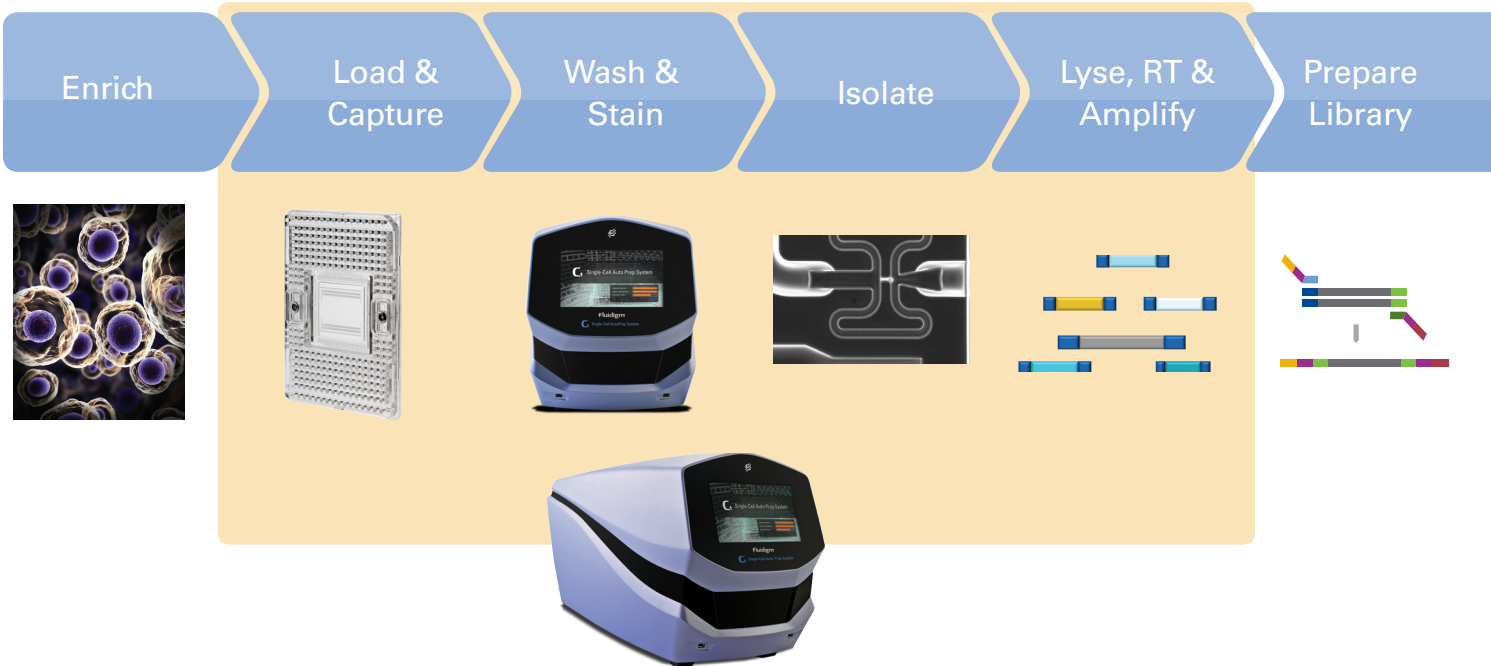
The Fluidigm C<sub>1</sub> Single-Cell Auto Prep System for mRNA Sequencing is designed specifically for detailed expression profiling in diverse cell populations. The C<sub>1</sub> Single-Cell Auto Prep System takes an entirely new approach, based on innovative microfluidic technology with nanoliter-scale reaction volumes. It delivers consistent results with the lowest sample requirements at a fraction of the cost of traditional library preparation methods.

This new, simplified workflow leverages the Clontech® SMARTer™ Ultra Low RNA Input Kit for cDNA synthesis and the Illumina® Nextera™ XT DNA Sample Preparation Kit

to provide the most comprehensive workflow with minimal hands-on time. Most traditional methods require total RNA as input which adds time, labor and cost. The C<sub>1</sub> Single-Cell mRNA Sequencing application streamlines the process; it works directly from single cells, so you can eliminate the extra workflow steps of extensive RNA purification beforehand, such as magnetic beads, gel size selection or filter columns.

With the C<sub>1</sub> Single-Cell mRNA Sequencing application, you can parallel process up to 96 cDNA libraries from multiple individual cells, for relative quantitation of mRNA expression on any Illumina sequencer. Key features of this system include:

- **Highest throughput**—get higher efficiency with a massively parallel, sequence preparation of 96 individual cells and in-line barcoding to maximize multiplexing capacity during sequencing.
- **Easiest to use**—less than 3 hours total hands-on time, working directly from single cells, with no RNA fragmentation and purification step.



C<sub>1</sub> Single-Cell Auto Prep System

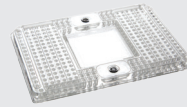
- **Ultra sensitive**—compatible with single-cell input as little as 10 picograms per reaction
- **More mappable reads**—over 70% of total reads mapped to RefSeq
- **Full gene analysis**—gives better read coverage across the entire transcript for better detailed analysis of alternative transcript isoforms and identification of single nucleotide polymorphisms
- **Cost effective**—delivers one-eighth the cost of standard single-cell library prep due to innovative nanoliter reaction scale
- **Compatible with any Illumina® system**—supported on HiSeq®, HiScan®, GAll and MiSeq® sequencing platforms



Any Illumina System

## THE C<sub>1</sub> SINGLE-CELL AUTO PREP SYSTEM

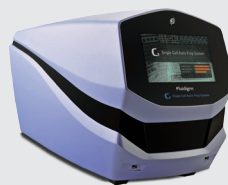
*The complete solution for mRNA sequencing for single cells*



- **C<sub>1</sub> Single-Cell Auto Prep IFCs for mRNA Sequencing**—our proprietary array and script for capture and cDNA amplification of 96 individual cells



- **C<sub>1</sub> Single-Cell Auto Prep Kit for mRNA Seq**—our reagent kit for lysis, wash, and stain of individual cells



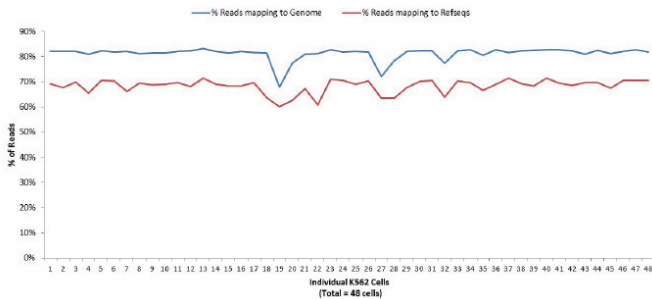
- **C<sub>1</sub> Single-Cell Auto Prep System**—isolate, process and profile individual cells for analysis



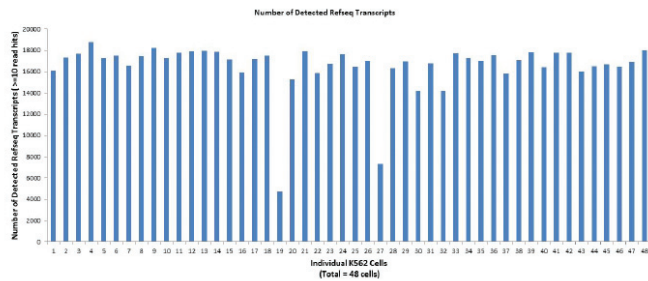
- **Clontech SMARTer Ultra Low RNA Kit**—reverse transcribe and amplify cDNA



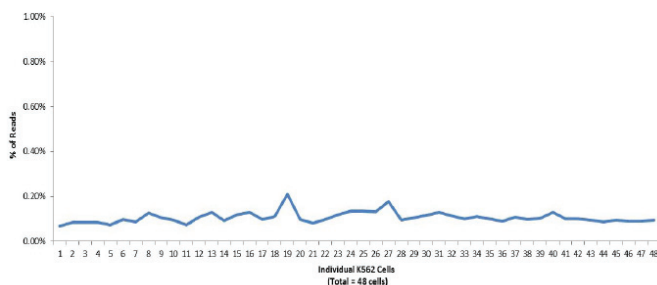
- **Illumina Nextera XT DNA Sample Preparation Kit**—simple, transposase based method to streamline tag incorporation and amplification with in-line barcoding



**Figure 1. Percent of mapped reads to reference as a function of the total number of reads.** The data was generated from 48 individual K562 cells input to the SMARTer Ultra Low RNA Kit, processed on the C<sub>1</sub> Single-Cell System followed by library construction using the Nextera XT kit. Average read depth is 3 million reads, >95% cells had >500k total read.

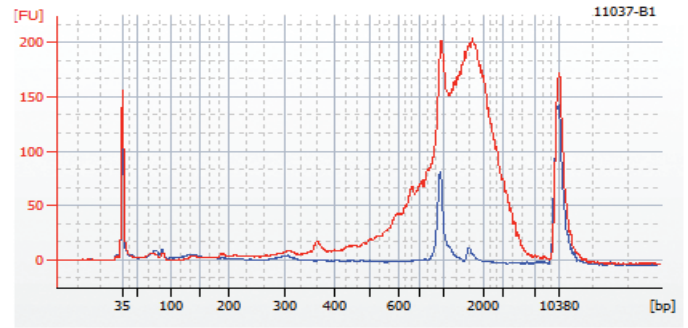


**Figure 2. Number of detected RefSeq transcripts.** Number of detected transcripts across 48 single K562 cells with at least 10 reads per transcript. Number of transcripts detected is read depth dependent; average read depth for these samples is ~3 million reads.

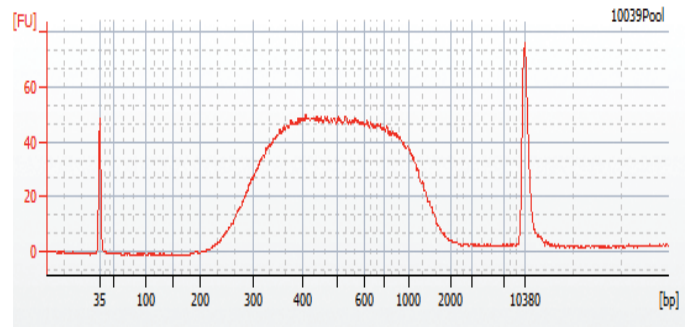


**Figure 3. Percent of mapped reads to ribosomal RNA.** Non-rRNA sequences are preferentially primed and amplified, reducing the number of reads from rRNA sequences to less than 0.25% for each of 48 single K562 cells.

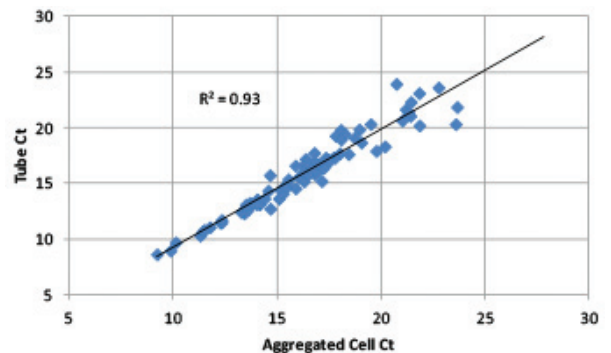
**Figure 4a: Size distribution of cDNA after harvest from C<sub>1</sub> Array**



**Figure 4b: Size distribution after library prep**



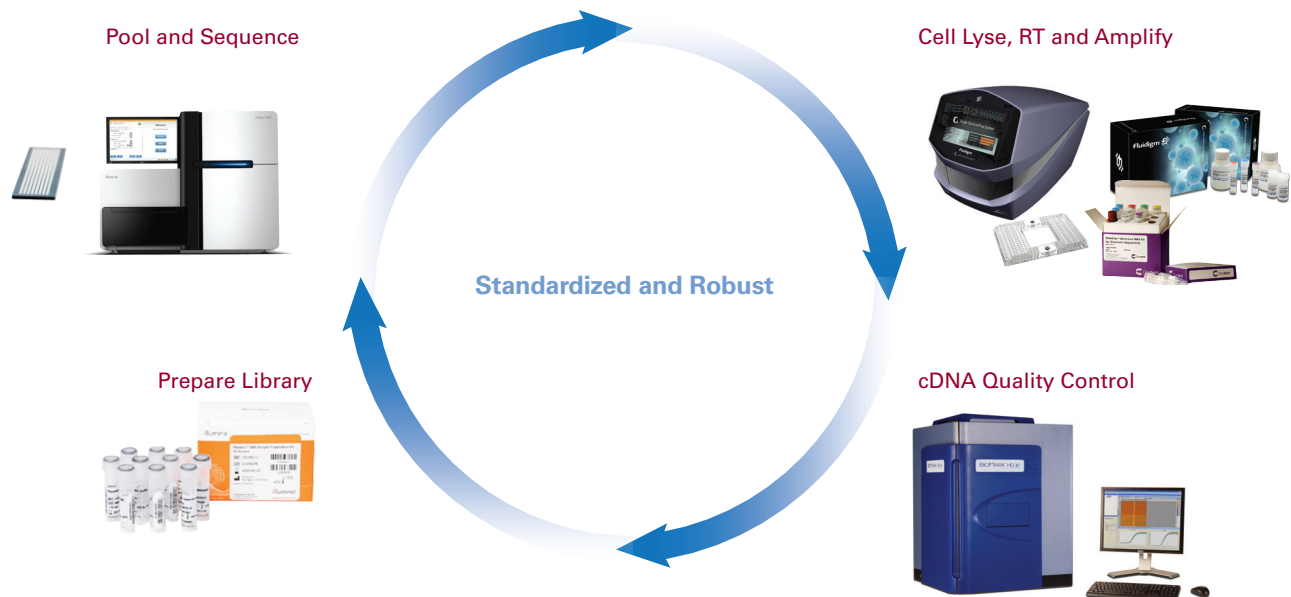
**Figure 4. Size distribution of cDNA and libraries.** a). Bioanalyzer trace of cDNA product obtained from K562 cells using the DNA High Sensitivity Chip on the Agilent® Technologies Bioanalyzer® 2100. The average cDNA is approximately 2 Kb long as measured on the Bioanalyzer 2100. The red line corresponds to the cDNA library of a single cell; blue corresponds to “no cell”. The 800 bp fragment corresponds to RNA Spike Internal Control. b). Bioanalyzer traces of one single-cell library generated by Nextera XT kit and one round of clean up by solid-phase reverse immobilization (SPRI).



**Figure 5. Correlation plot of aggregated cell C<sub>t</sub>s vs tube C<sub>t</sub>s.** Single-cell cDNA libraries and a multi-cell tube control were evaluated on the BioMark™ HD System using DELTAgene™ Assays. Data aggregated from single-cell cDNA libraries correlate closely with data from a multi-cell tube control (R<sup>2</sup> = 0.93). Incorporating the BioMark HD System into the routine analysis provides a rapid assessment of yield and quality of each library.

## FAST AND EASY QUALITY CONTROL OF cDNA LIBRARIES—THE BIOMARK HD SYSTEM

The quality of single-cell mRNA sequencing data is directly proportional to the quality of each cDNA library. In order to produce the most reliable sequence data, a routine quality control step should be implemented. The BioMark HD System assesses the quality of each cDNA amplification, ensuring that each library is free of contamination and is not degraded. The system also will quantitate the level of each transcript.



## GET A CLEARER PICTURE WITH SINGLE-CELL ANALYSIS

Assuming that every cell in your sample behaves exactly the same is a dangerous gamble; taking averages of pooled cells masks dramatic variations in gene expression. Recognizing cellular variations in what appear to be homogenous populations has become crucial to advances in stem cell research, understanding cancer cells, identifying immune responses, determining the effectiveness of biological therapies, and discovering the mechanisms of neurodegenerative diseases. The drawbacks become particularly apparent in mRNA sequencing.

The C<sub>1</sub> Single-Cell Auto Prep System enables you to rapidly and reliably isolate, process and profile individual cells for analysis. Our family of instruments, arrays, assays, software and kits are designed to take you from cellular isolation and extraction, through reverse transcription and amplification and ultimately to detection and analysis of cell activity using just one technology. The system opens the doors to studying cell differentiation, measuring individual cell responses to specific stimuli, verifying critical disease biomarkers, and conducting candidate drug screens.

## FIND THE PATHWAY LESS TRAVELED—WITH EASE

Great discoveries require blazing new trails. Single-cell analysis has the potential to find new transcriptome pathways, which could uncover new cellular roles in development, metabolism and disease. Finally, you can mRNA sequence more individual cells, with reduced technical noise and greater statistical significance. Discover how easy the path less traveled is to find at [www.fluidigm.com/C1system](http://www.fluidigm.com/C1system).

