Targeted Sequencing for Accurate Mutation Detection at the Single-Cell Level

Kathrin Wolf, Silke Huebner, Karim Benyaa, Ioanna Andreou, Jennifer Kastner, Frank Narz, Nan Fang
QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, Germany

Introduction

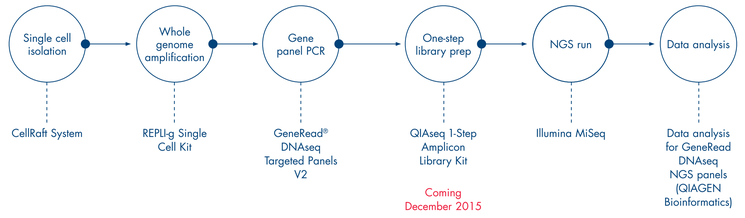
Genomic analyses at the single-cell level shed light on the genes and their functions in each individual cell and give valuable information on the fundamental mechanisms of cellular functions. Recent developments in single-cell sequencing technologies have enabled single-cell genomic analysis at single nucleotide resolution and revealed the cellular heterogeneity among presumably homogeneous cell populations. Single-cell sequencing has become a powerful tool in cancer research (for early detection and monitoring of rare non-identical circulating tumor cells), in immunology, and stem cell research.

Targeted sequencing, the selective enrichment and sequencing of a panel of genes of interest, is a sensitive and cost-effective method to analyze functionally relevant genes. The targeted sequencing at single-cell level is especially useful in cancer research, due to the high heterogeneity of the tumor cells and relatively small numbers of significantly mutated genes (SMGs) in all cancer types. We present here an optimized protocol for streamlined targeted sequencing at the single-cell level that combines reliable, multiple displacement amplification (MDA)-based, single-cell whole genome amplification (WGA), multiplex PCR-based targeted enrichment, and a fast one-step sequencing library construction protocol.

REPLI-g® Single Cell Kit.

Materials and Methods

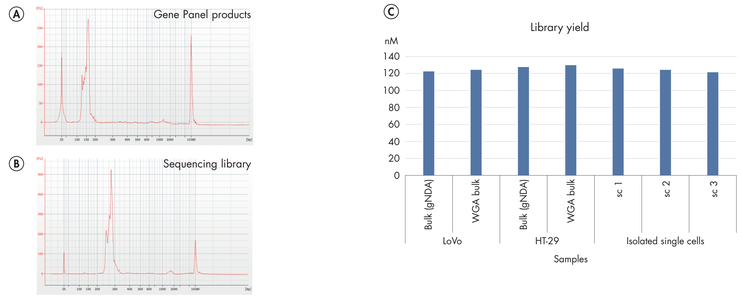
Single cells were isolated from colorectal cancer cell lines (HT-29 and LoVo) using CellRaft™ Arrays (Cell Microsystems). The CellRaft Array is composed of 12,000 (200 x 200 microns) individual microwells with releasable individual carriers or "rafts". Cells were plated on the CellRaft Array in the same manner as a standard tissue culture dish. After settlement onto the rafts, single cells were isolated using a piercing device under microscopic control. Released rafts carrying individual cells were transferred to PCR tubes for further processing using a magnetic wand. Genomic DNA was amplified with a MDA-based approach using the REPLI-g Single Cell Kit (QIAGEN). A multiplex PCR target enrichment panel for cancer-relevant genes (which amplifies coding regions of 24 genes known to harbor clinically relevant mutations) was used to enrich DNA from WGA single-cell DNA and followed by a one-step sequencing library construction. The libraries were then sequenced on a MiSeq® instrument (Illumina) to detect cancer-relevant genes at the single-cell level. A similar experiment was carried out on DNA from bulk cells or bulk-cell DNA that underwent WGA.



High-Quality, Convenient Library Preparation Method

The new QIAseq 1-Step Amplicon Library Kit (cat. no.180412) is designed specifically for multiplex PCR products (e.g., from GeneRead DNAseq Targeted Panels). Sequencing adaptors can be added onto PCR amplicons in one 30-minute, room-temperature incubation step.

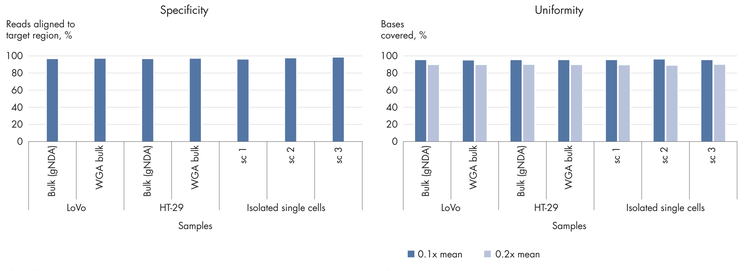
The BioAnalyzer trace shows the expected size shift (+120 bp) for Illumina libraries after library preparation and amplification. Optimized size selection efficiently removes adaptor–dimers without decreasing library yield. Libraries are free of nonspecific products.



Ⓐ BioAnalyzer trace of GeneRead Gene Panel PCR products. Ⓑ BioAnalyzer trace of sequencing library prepared with new QIAseq 1-Step Amplicon Library Kit. Ⓒ Library yield after purification and library amplification.

Good Sequencing Metrics

Basic sequencing metrics of bulk and single cell samples: Outstanding specificity, uniformity and coverage were achieved on sequencing results of all samples. Samples were either DNA from individual cells that underwent WGA, DNA from bulk cells or DNA from bulk cells that underwent WGA.

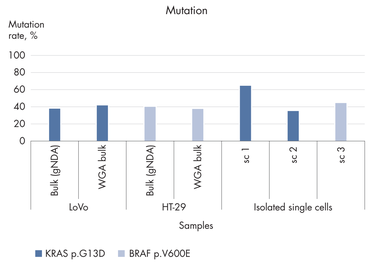


Specificity is calculated as percentage of reads mapped to targeted region of interest out of total number of reads per run.

Coverage uniformity is measured as percentage of bases in the region of interest covered at least 0.1x or 0.2x mean coverage depth.

Detection of Variants in Single Cells

The figure shows the mutation rates for various DNA samples detected by the GeneRead Targeted Human Clinically Relevant Cancer Panel V2. Previously known variants in LoVo cell (KRAS G13D) and HT29 cell (BRAF V600E) were detected by next-generation sequencing (NGS). It was possible to determine mutations even in samples with only 5% of mutated gDNA, showing high method sensitivity.



Mutation rate of single cells, bulk DNA and mixtures of bulk DNA.

Results and Conclusions

- Single-cell isolation is made easy and visually controllable with the CellRaft System.
- Previously reported mutations were successfully detected with this single-cell targeted sequencing method.
- Compared to sequencing of bulk DNA with a mixed population, single-cell sequencing demonstrates higher sensitivity and less ambiguity in mutation detection.
- The complete procedure, from single cell to sequencing-ready library uses less than 2 hours hands-on time, greatly simplifying the single-cell sequencing workflow.
- Taken together, the single-cell target sequencing procedure helps researchers to gain more insights into genomic alterations of single cells among heterogeneous cell populations.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

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Sample to Insight