Product Profile

QIAseq FX Single Cell DNA Library Kit

Obtain whole genome libraries with comprehensive genome coverage and exceptional sequence fidelity from single bacterial or eukaryotic cells

The proliferation of technologies that enabled the analysis of single, isolated cells has transformed research in neuroscience, cancer, reproduction and more. With technological breakthroughs in single cell isolation, whole genome amplification (WGA) and NGS library preparation, experiments using single cells are now possible – opening a wealth of exciting new insights for you to discover. However, challenges still exist. In particular, methods for the unbiased and complete amplification of a single genome and for the efficient conversion of that amplified DNA into a sequencer-compatible library face several technical limitations including incomplete amplification, the introduction of PCR errors, GC-bias and locus or allelic drop-out. With these considerations in mind, QIAGEN offers solutions for your research with the new QIAseq FX Single Cell DNA Library Kit.

The QIAseq FX Single Cell DNA Library Kit delivers:

- A complete, cell-to-library solution to incorporate unparalleled whole genome amplification and highly efficient QIAseq FX library preparations
- The highest genome coverage of any single cell technology
- Greater sequence fidelity than other comparable techniques
- Completely PCR-free cell-to-library protocol to minimize bias
- Libraries from single cells in under 4 hours using a streamlined protocol
- Compatibility with both eukaryotic and bacterial cells
- NGS libraries and amplified gDNA samples that can be archived for follow-up experiments or secondary analyses

PCR-free whole genome libraries from single cells in under 4 hours

The QIAseq FX Single Cell DNA Library Kit is designed to reliably produce high-quality, PCR-free whole genome libraries from single eukaryotic or bacterial cells in under 4 hours (Figure 1). The kit combines an optimized, high-fidelity phi29-based whole genome amplification with unbiased enzymatic DNA fragmentation and a hyper-efficient, proprietary single-tube end-polishing and ligation reaction to provide a single-kit solution for single cell DNA sequencing.

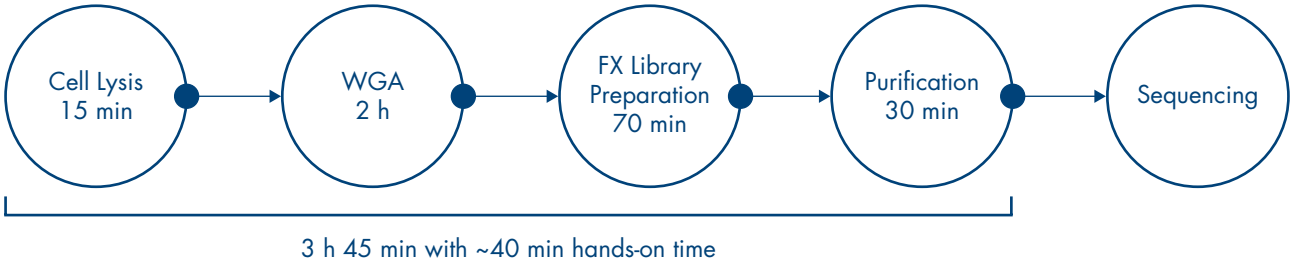

Figure 1. QIAseq FX Single Cell DNA workflow.

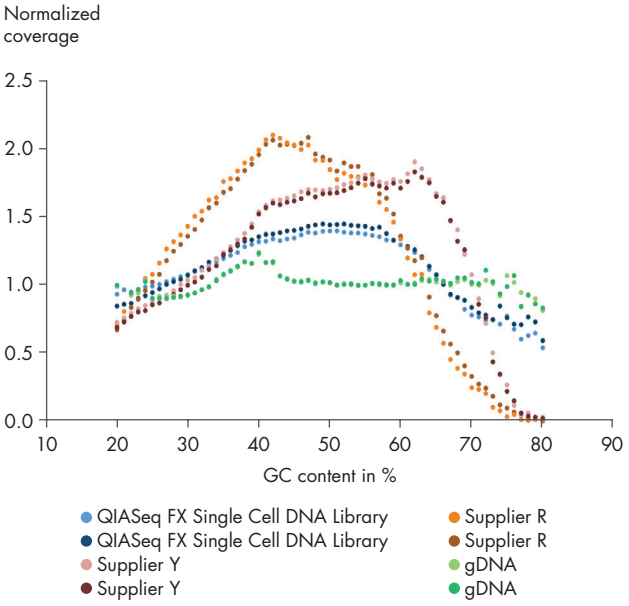

Figure 2. GC-bias of single cell whole genome sequencing kits. Libraries were generated from either bulk gDNA using the QIAseq FX DNA Library Kit or from single peripheral blood mononuclear cells (PBMC) using the QIAseq FX Single Cell DNA Library Kit or kits from two other suppliers.

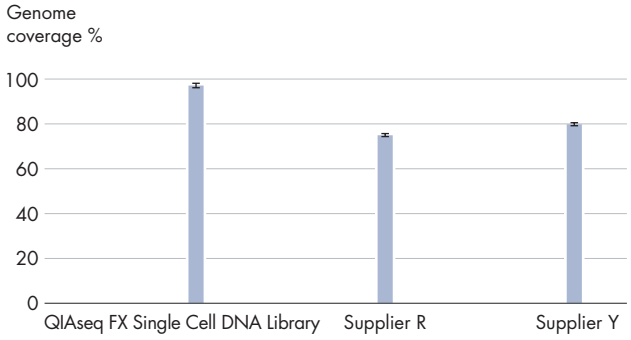

Figure 3. Average genome coverage for several single cell libraries. Libraries were generated from single PBMC using the QIAseq FX Single Cell DNA Library Kit or kits from two other suppliers and sequenced at low depth using an MiSeq. Data were analyzed according to Zhang, CZ, et al. (2015) Nat. Commun. 6, 6822. Computed maximum achievable coverage by unlimited sequencing capacity are plotted.

The entire process, from isolated single cell to sequencer-ready library (Figure 1), is performed without PCR, completely eliminating the possibility of generating PCR duplicates, which at moderate to high read depths, cannot be distinguished from biologically meaningful data. The extensive use of PCR during both whole genome amplification and library preparation also introduces substantial GC-bias, and leads to underrepresenting GC-rich regions of the genome (Figure 2).

Maximize genome coverage and your discoveries

The optimized multiple displacement annealing-based whole genome amplification technology employed by the QIAseq FX Single Cell DNA Library Kit provides the most complete representation of the genome (Figure 3). This comprehensive, uniform amplification ensures even representation of the input DNA, providing an effective use of available sequencing depth and reducing locus drop-out, where coverage of biologically meaningful regions of the genome is not available for a subset of samples in your dataset.

This exceptional genome coverage enables powerful new types of experiments, such as low-pass consensus variant calling for the identification of rare mutations in cancer samples at extremely low sequencing depth. Additionally, the QIAseq FX Single Cell DNA Library Kit is the only kit compatible with both eukaryotic and bacterial cells, enabling novel applications such as de novo genome assembly from unculturable bacteria in microbiome samples. For more information on current advances in single cell research, see our Single Cell Knowledge Hub under Knowledge Area at **www.qiagen.com**.

Ultra-high sequence fidelity reduces background when analyzing sequence variants

Mutation detection from single cells is critical when examining heterogeneous tissues such as tumor samples, where variants specific to a low proportion of cells in a population are diluted to the point of non-detection when analyzing bulk samples. However, the amplification technologies employed in single cell workflows often introduce an unacceptably high number of artificial sequence variants. During analysis, the reads containing these sequence errors contribute substantially to background noise and in some instances can pass variant calling algorithms and generate false positives, wasting valuable analysis efforts and obscuring the true biological meaning of the dataset. The MDA employed by the QIAseq FX Single Cell DNA Library Kit has been proven both in-house and by external researchers to deliver the highest fidelity dataset (Figure 4), ensuring that you can have confidence that the sequence variants detected are true mutations in the cell and not artifacts.

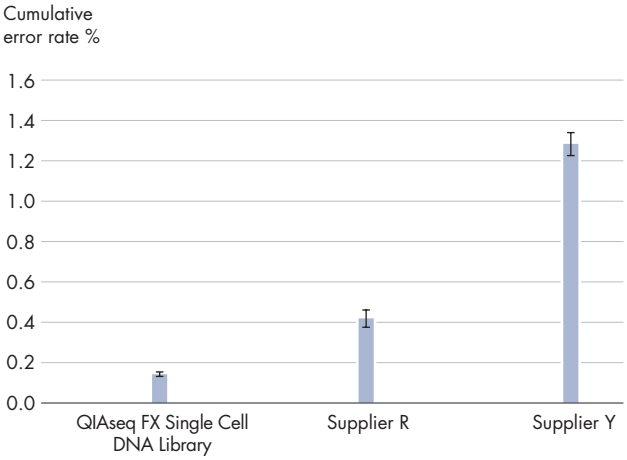

Figure 4. Sequence error rates of several single-cell NGS methods. Single cell libraries were prepared from isolated PBMCs using QIAseq FX Single Cell DNA Library Kit or kits from two other suppliers and sequenced with an Illumina MiSeq. Reads were mapped to the human genome (hg19) and sequence mismatches between NGS data and the reference were computed. All analysis was performed with CLC Genomic workbench 8.5.1. Data plotted are the mean proportion of sequence differences +/- standard deviation for 3 individual libraries prepared with each kit.

The only choice for combined mutation and copy-number variation detection

While the sequence variants of each single cell provide a great deal of biological meaning, copy number and other structural variations can also add a valuable insight to any analysis. In addition to best-in-class genome coverage, minimal bias and fewer false positive sequence variants, the QIAseq FX Single Cell DNA Library Kit enables the detection of both aneuploidy and sub-chromosomal copy number variations (Figure 5). Additionally, with the most complete genome coverage, you can be sure that smaller structural variants are not missed due to low coverage over certain genomic regions.

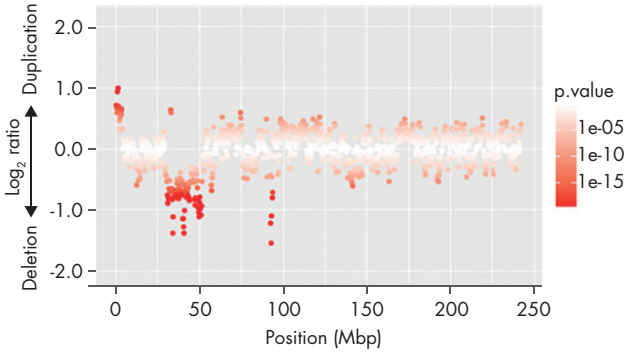

Figure 5. Detection of small copy-number variations. Methods and specifics (chromosome, size of CNV). Single cell libaries from PBMCs and Jurkat cell were prepared using QIAseq FX Single Cell DNA Library Kit and sequenced at Depth 0.1x. Reads were mapped using BWA mem to human genome (GRCh38) and copy number variation of Jurkat vs PBMCs (control diploid cells) was assessed using the script published in: Chao Xie, Martti T Tammi, "CNV-seq, a new method to detect copy number variation using high-throughput sequencing", BMC Bioinformatics, 2009,10:80, DOI: 10.1186/1471-2105-10-80. Plotted is the Log2 ratio(Jurkat/PBMC) of coverage using a window size of 500Kb for chromosome 2 from a cell with an approximately 2.5 Mbp deletion.

Ordering Information

Product	Contents	Cat. no.
QIAseq FX Single Cell DNA Library Kit (24)	For 24 reactions: Buffers and reagents for cell lysis, whole genome amplification, and library preparation including DNA fragmentation, end-repair and adapter ligation. Includes a plate containing 24 barcoded adapters for use with Illumina instruments.	180713
QIAseq FX Single Cell DNA Library Kit (96)	For 96 reactions: Buffers and reagents for cell lysis, whole genome amplification and library preparation including DNA fragmentation, end-repair and adapter ligation. Includes a plate containing 96 barcoded adapters for use with Illumina instruments.	180715

These products are intended for molecular biology applications. These products are not intended for the diagnosis, prevention or treatment of a disease.

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.

Visit **www.qiagen.com/goto/QIAseq-FX-SC-DNA** for more information!