A PCR-free protocol for single-cell DNA library construction

Ioanna Andreou, Nan Fang, Annika Piotrowski, Mary von Langsdorff and Devika Mathur
QIAGEN GmbH, Hilden, Germany

Introduction

Single-cell genomic analysis enables researchers to gain novel insights across a diverse set of applications in developmental biology, tumor heterogeneity, and disease pathogenesis and progression. Typically, conducting single-cell genomic analysis using next-generation sequencing (NGS) methods is challenging because the amount of genomic DNA present in a single cell is very limited. PCR-based whole genome amplification methods tend to have high error rates, low coverage uniformity, extensive allelic drop-outs and limited amplification yields.

We describe a streamlined workflow for single-cell NGS library construction that uses Multiple Displacement Amplification (MDA) to amplify the whole genome with high uniformity and fidelity, combined with high adaptor ligation efficiency library construction. The entire procedure generates high-quality sequencing libraries without PCR amplification, thereby eliminating PCR-related bias and errors and reducing handling steps. Our data demonstrate that this PCR-free method for single-cell sequencing library preparation affords highly uniform sequence coverage and high library complexity.

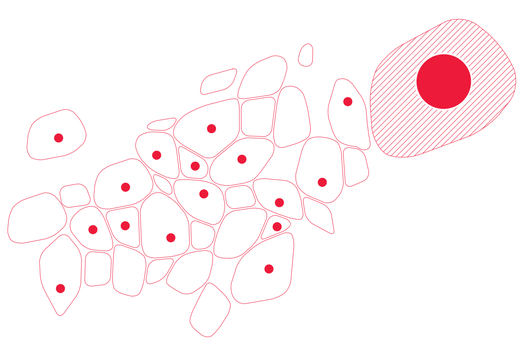

Figure 1. The unique genetic makeup of a single cell can hold key insights into cellular diversity, tissue heterogeneity and mechanisms of disease. Capturing the genome of single cells with uniform sequence coverage and high fidelity is essential for a range of applications.

Principle of the method

The protocol relies on the REPLI-g® Single Cell DNA Library Kit, which leverages QIAGEN's unique Multiple Displacement Amplification (MDA) technology and efficient GeneRead™ library construction technology to prepare a sequencing library with high fidelity and minimal bias, while retaining the sample's genomic diversity.

- Amplification occurs via isothermal genome amplification, constant-temperature strand displacement synthesis and additional priming events. Supported by a unique buffer/polymerase combination, it generates DNA fragments up to 100 kb without sequence bias.
- Cell lysis and DNA denaturation is achieved using gentle alkaline incubation that yields genomic DNA with uniform representation of genomic loci and sequences and very low fragmentation or generation of abasic sites.
- High-quality library preparation delivers NGS-ready libraries without need for any enrichment steps. The high WGA yields and high ligation efficiency of the library construction reagents remove the need for PCR-based amplification.

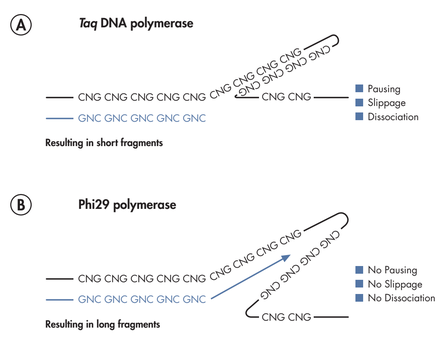

Figure 2. Uniform amplification with Phi29 polymerase. Ⓐ Upon encountering secondary DNA structures, Taq polymerase may pause synthesis, slip or dissociate from the template. This can lead to inaccurate DNA amplification, incomplete loci coverage and short fragment sizes. Ⓑ REPLI-g kits use Phi29 polymerase, which displaces secondary structures enabling more accurate and uniform amplification of the entire genome.

Simple, five-hour procedure for amplification and library construction

In the first step of the WGA procedure, the cell sample is lysed and the DNA is denatured. A neutralization buffer stops denaturation, then a master mix containing buffer and DNA polymerase is added. The isothermal amplification reaction proceeds for 3 hours at 30°C. The resulting DNA can be stored long-term at –20°C with no negative effects, or used directly to generate sequencing libraries.

For library construction, samples consisting of longer DNA fragments are first sheared into a random library of fragments. The median fragment sizes depend on the applications and sequencing read length. Following end-repair and A-addition, platform-specific adaptors with sequences essential for binding the library to a flow cell and binding sequencing primer are ligated to both ends of the DNA fragments.

Library enrichment is generally unnecessary because the yield from WGA is so high, but an optional high-fidelity amplification step can also be performed if required.

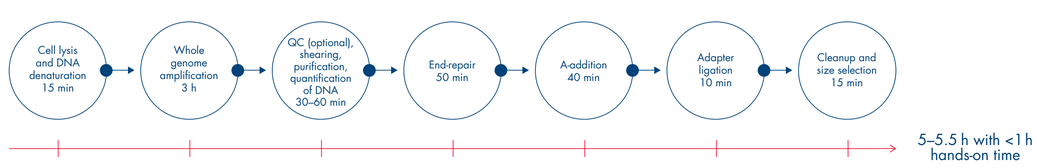

Figure 3. The time-saving, streamlined protocol for ready-to-use library generation.

Results: high-quality libraries

This protocol, which uses the REPLI-g Single Cell DNA Library Kit, delivers high-quality libraries within one day. Independent of the amount of starting material and the incubation time, the libraries have a high percentage of mapped reads and a very low percentage of duplicates.

The results confirm that the yields of the WGA step and the ligation efficiency of the library construction reagents are sufficiently high, so that PCR-based library amplification, which can introduce coverage bias and reduce library diversity, is unnecessary.

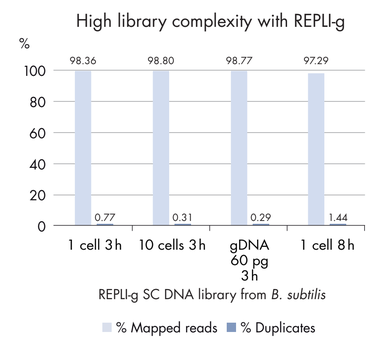

Figure 4. High-quality libraries generated with the REPLI-g Single Cell DNA Library Kit. For analysis, 1 cell, 10 cells or gDNA were used as starting material. WGA was carried out for 3 h or 8 h. The complexity of libraries prepared using the REPLI-g Single Cell DNA Library Kit was very high, as indicated by the extremely low percentage of the duplicates detected, enabling efficient use of the sequencing capacity. Similar results were obtained independent of the amount of starting material and incubation time.

Results: High coverage uniformity, regardless of GC content

The protocol outperformed PCR-based single cell library preparation protocols using kits from another supplier.

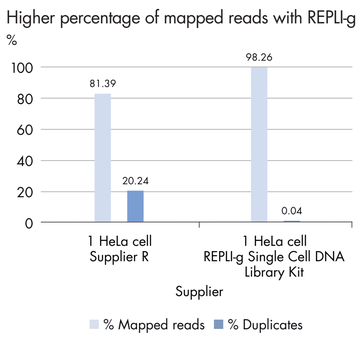

Figure 5. The REPLI-g Single Cell DNA Library Kit outperforms PCR-based single cell library preparation. Data from libraries constructed from one HeLaS3 cell is shown. Compared to a PCR based product from another supplier, the REPLI-g Single Cell DNA Library Kit provided a higher percentage of mapped reads and an extremely low percentage of duplicates.

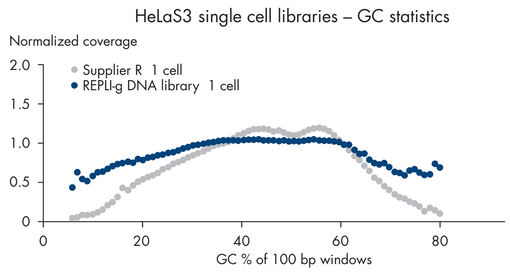

Figure 6. Uniform coverage over a wide range of % GC content. Compared to a product from another supplier, libraries generated using the REPLI-g Single Cell DNA Library Kit provided greater and more uniform coverage over a wide range of GC content (expressed as a pecentage).

Conclusion

The REPLI-g Single Cell DNA Library Kit offers an efficient, PCR-free method for next-generation sequencing library construction from single cells. The kit combines QIAGEN's unique Multiple Displacement Amplification (MDA) technology and efficient GeneRead library construction technology to overcome the challenges of working and analyzing single cells by allowing preparation of a sequencing library with high fidelity and minimal bias, while ensuring retention of the sample's genomic diversity.

For more information, visit **www.qiagen.com/Single-Cell-DNA**

1095137 05/2015

Sample to Insight