TECHNICAL NOTE

Esben Bjørn Madsen, Jesper Tholstrup, Marie Just Mikkelsen, and Thomas Kvist
Samplix ApS, Birkerød, Denmark

Samplix

Improved whole genome amplification through unbiased single-molecule amplification with Xdrop®

Summary

- We compared the whole genome amplification results using the Xdrop workflow and three other methods.
- Only the Xdrop workflow produced consistently sensitive results with unbiased, even coverage and accurate representation of the genome.

Introduction

Whole genome amplification (WGA) typically uses some form of isothermal and/or PCR-based system. One such method is bulk multiple displacement amplification (MDA).[1] Unfortunately, bulk amplifications generate errors, artifacts, and biases, including false mutations and allelic dropout.[2,3] For example, during the amplification reaction, two similar molecules can recombine. If taken as a template for further amplification, this produce intermolecular chimeric products.[1,4] Bulk MDA can also lead to allelic dropout, when one of the alleles present in a heterozygous sample is not amplified.[2]

Xdrop ensures even coverage of the entire genome. It enables independent amplification reactions to be run in highly stable droplets containing individual DNA fragments from the samples (Figure 1).

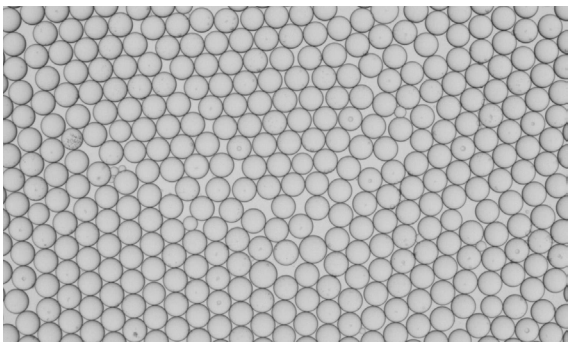

Figure 1. Single-emulsion droplets produced with Xdrop. Each droplet contains reagents and primers for droplet multiple displacement amplification (dMDA). Any DNA present in the droplet, regardless of sequence, is amplified using a high-fidelity proofreading phi29 enzyme. The gentle isothermal reaction leaves the DNA template intact for downstream analysis.

Experimental setup

Chromosomal DNA from *Escherichia coli* was used to compare the performance of Xdrop dMDA and three selected bulk MDA methods (listed here as A, B and C). Single-emulsion droplets containing DNA and reaction mix were produced with Xdrop and the Xdrop SE85 Cartridge. The droplets are stable and can be incubated in a thermal cycler as long as required for the amplification. Following the manufacturer's instructions, MDA solutions from three alternative suppliers were used to amplify concentrations of *E. coli* DNA ranging from 1 pg to 1 ng. This was followed by library preparation and sequencing on an Illumina® instrument.

Sensitive results without misleading background

Only the Xdrop workflow produced consistently sensitive results without background noise. This is shown by the measure of output DNA from the amplifications for a serial dilution of input *E. coli* DNA (Figure 2). The bulk solutions either struggled to amplify the low-input samples or generated measurable product in the no-template control.

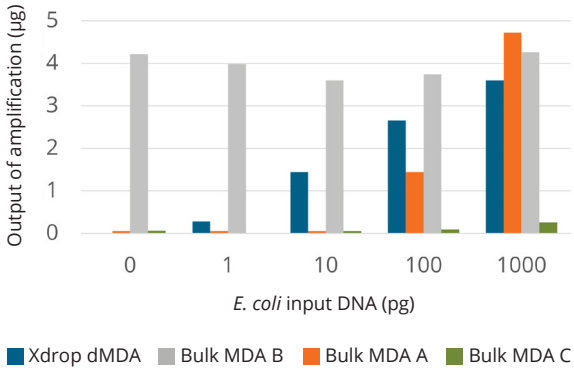

Figure 2. Output DNA (µg) after WGA using increasing amounts of input DNA (pg) with Samplix Xdrop droplet MDA (dMDA) and three bulk MDA methods from other suppliers.

Unbiased and even coverage of the genome

Xdrop dMDA generated unbiased amplification outputs that yielded even coverage in the sequencing libraries. The spread in relative coverage over the sequenced genome is minor for Xdrop (Figure 3). 99% of the target genome was covered more than once by sequencing reads from libraries generated with Xdrop dMDA output, even with an input amount as low as 1 pg (Figure 4).

Accurate representation of the genome

Compartmentalized dMDA minimizes the risk of the errors seen with bulk MDA. Nearly every read in the downstream sequencing of the DNA amplified with Xdrop mapped to the target genome (Figure 5).

Conclusion

The Xdrop dMDA workflow enables highly sensitive, accurate and unbiased whole genome amplification, even from picogram DNA input.

For more information about Xdrop products and applications, visit samplix.com.

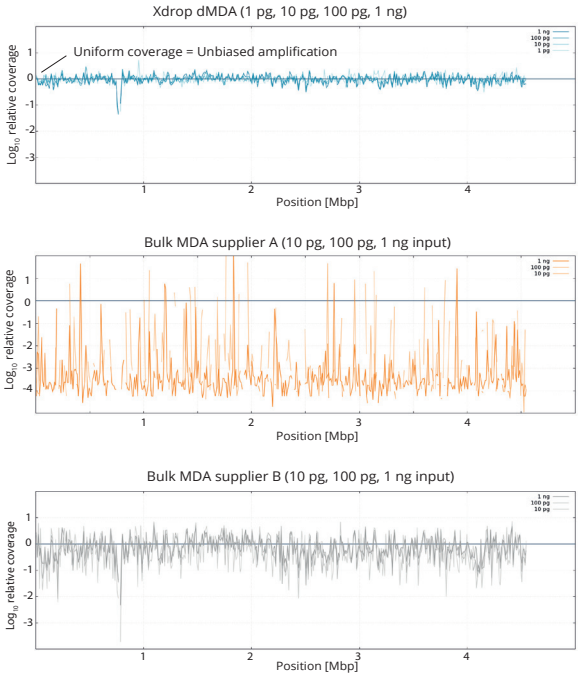

Figure 3. Graphs showing the spread in relative coverage over the sequenced genome after WGA with the Xdrop, supplier A and supplier B methods. The wider spread indicates less uniform coverage.

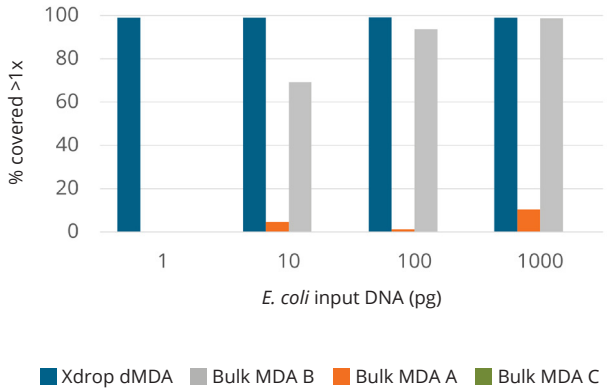

Figure 4. Percentage of the target genome covered more than once by sequencing reads from libraries generated with MDA output.

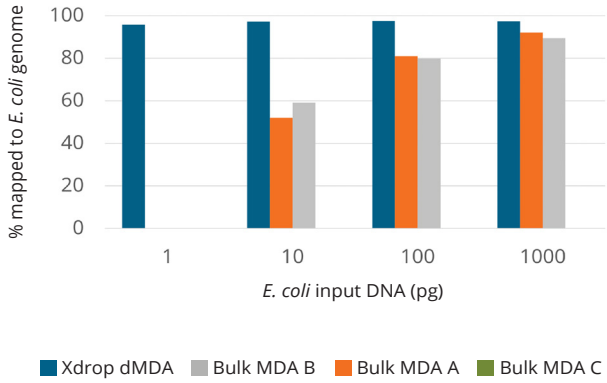

Figure 5. Percentage of the reads from libraries generated with the MDA output that mapped accurately to the ***E. coli*** genome.

References

1. Lasken, R.S. 2009. Genomic DNA amplification by the multiple displacement amplification (MDA) method. Biochem. Soc. Trans. 37: 450.

2. Huang, L., et al. 2015. Single-cell whole-genome amplification and sequencing: methodology and applications. Annu. Rev. Genomics Hum. Genet. 16: 79

3. Rhee, M., et al. 2016. Digital droplet multiple displacement amplification (ddMDA) for whole genome sequencing of limited DNA samples. PLoS One 11: e0153699.

4. Lasken, R.S. and Stockwell, T.B. 2007. Mechanism of chimera formation during the multiple displacement amplification reaction. BMC Biotechnol. 7: 19.

SX-000743 - TN – ver. 01