Simple Whole Genome Amplification Technique Developed on the Basis of SD DNA Polymerase

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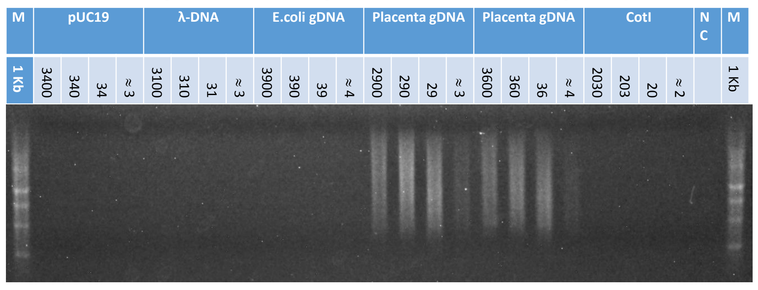
Introduction:

Whole Genome Amplification (WGA) techniques are used for non-specific amplification of low-copy number of genomic DNA; WGA provides the only possibility to work with single-cells genomes so far. There are a number of WGA methods that have been developed. Degenerate Oligonucleotide-Primed PCR (DOP-PCR) is one of the simplest, fast and inexpensive WGA techniques. Although DOP-PCR has been regarded as one of the pioneering methods for WGA, it only provides low genome coverage and a high allele dropout rate when compared to more modern techniques. Here we describe an Improved DOP-PCR with enhanced WGA performance (iDOP-PCR) based on a BIORON's novel DNA polymerase with a strong strand-displacement activity (SD Polymerase, Patent US 9,896,671).

Several commercially available WGA kits were compared with the newly developed BIORON's One Step WGA Kit. The amplified WGA DNA libraries were evaluated by different ways including NGS data.

iDOP-PCR WGA provided comparable or better quality of the amplified DNA libraries compared to the other WGA methods tested, the workflow is more simple, user-friendly and not labor-intensive. Thus, the new technique based on SD Polymerase can be used as a simple and convenient alternative for the Single Cells WGA in research and clinical practice.

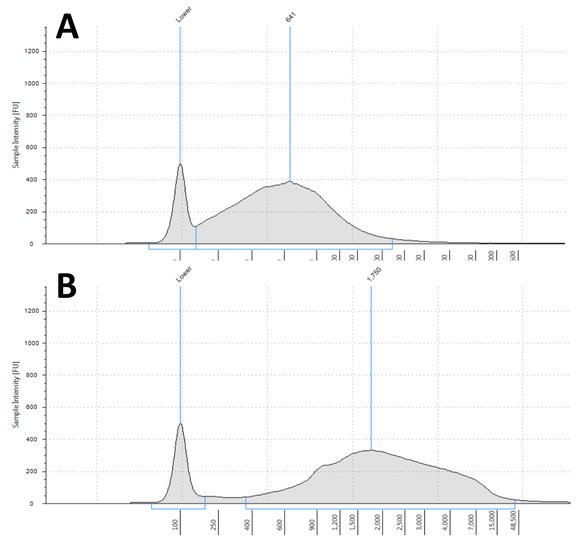
Template dependent amplification



Amplification of different templates shows no amplification with plasmid DNA (**pUC19**); **λ-Phage DNA, *E. Coli* gDNA or Cot I fraction of human DNA**. Human **Placenta gDNA** from different preparations shows uniform amplification even with low copy numbers.

Size distribution of fragments amplified by WGA

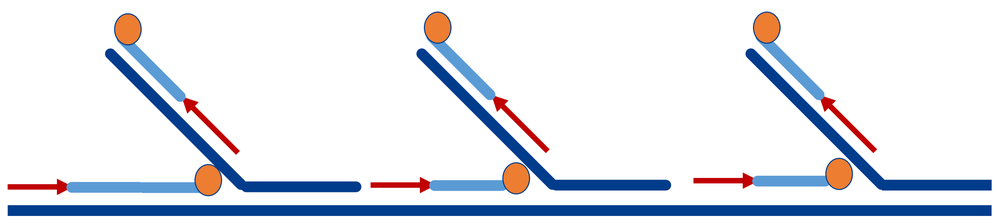
Amplifications of human genomic DNA done according to the corresponding manuals with **A:** Rubicon PicoPlex WGA Kit; **B:** BIORON One Step WGA Kit analysed with Agilent Tapestation shows an increase in the average fragment lengths for the BIORON Kit.



One Step WGA Kit:

10 µl Master Mix
1 µl SD Polymerase
X µl Sample (1-14 µl)

One Step → PCR amplification

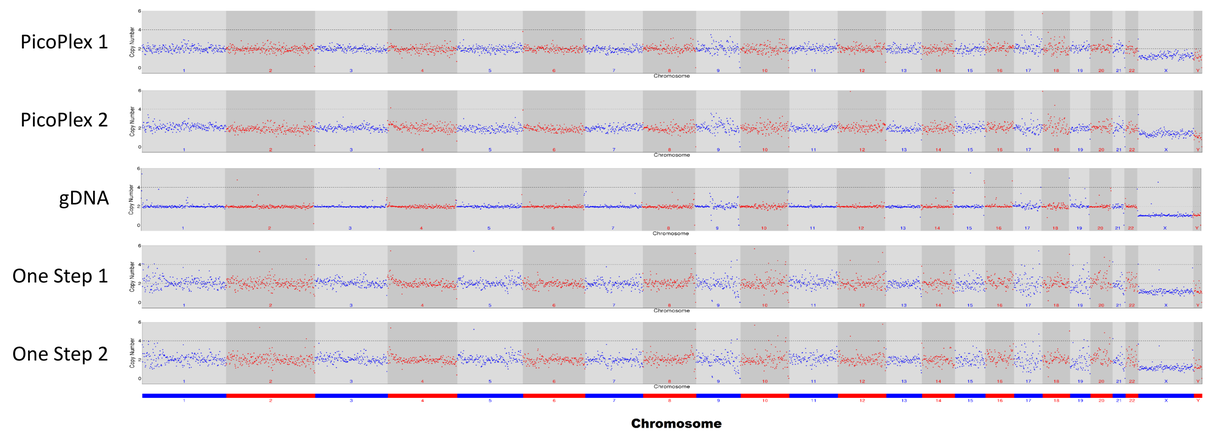


Comparison of key characteristics

WGA method	Sample	Genome coverage	Unmappable sequences	Reproducibility
PicoPlex	1	51.0 %	42.4 %	98.1 %
	2	50.8 %	44.6 %	
One Step WGA Kit	1	61.5 %	31.5 %	97.6 %
	2	60.7 %	33.1 %	
PicoPlex +One Step WGA Kit		80 %		

Key characteristics of the WGA methods (reproducibility of the methods, genome coverage, the rate of unmappable sequences) were compared by NGS analysis of two PicoPlex and two iDOP-PCR WGA libraries. Each library was obtained from 15 pg (about 2.5 copies) Human gDNA (Blagodatskikh et al., 2017). The combination of both Kits shows 80 % genome coverage suggesting the amplification of different regions so the individual Kits can complete each other. There is no other known technology reaching this coverage at the moment.

Copy number Variation Analysis comparison



Copy number variations of diploid human genome from single genome copies amplified by PicoPlex and iDOP-PCR WGA methods. Digitized copy numbers across the genome are plotted for two PicoPlex and two iDOP-PCR WGA samples as well as the non-amplified gDNA sample for control. Raw data at a sequencing depth of 8× with a bin size of 1,000 kb are mapped to the human reference genome. The chromosomes are shown in alternating red and blue colors.

Conclusion

There are a lot of different techniques on the market for Whole Genome Amplification with individual properties depending on the performed subsequent analyses. The combination of optimised Primers and the heat stable Taq capable of doing strand displacement making the handling of the presented Kit as easy as setup a regular PCR. There is no need for special equipment or time consuming processing steps each increasing the risk for contamination. In combination with the Library generation system ‚Fragmentation Through Polymerisation' it can be a powerful tool for analysing single cells by NGS.

References:
Harris et al., 2013; Polymerase Chain Displacement Reaction; Biotechniques, 54(2):93-7
Ignatov et al., 2018; Fragmentation Through Polymerization (FTP): A New Method to Fragment DNA for Next-Generation Sequencing; bioRxiv, 2018
Blagodatskikh et al., 2017; Improved DOP-PCR (iDOP-PCR): A robust and simple WGA method for efficient amplification of low copy number genomic DNA; PLoS ONE 12(9)