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DOPlify® Kit - A New Generation of Whole Genome Amplification

Whole genome amplification: no method is the same

Currently, single cell and low copy number DNA templates require whole genome amplification (WGA) prior to downstream DNA analysis. There are a number of commercially available kits, each with distinct ability to generate representative coverage of the complete genome. Ideally, the amplification should be robust, scalable and automation ready for high throughput analysis. More importantly, non-biased coverage should be achieved, with a resolution compatible with single nucleotide variant (SNV) and copy number variation (CNV) detection.

There are a small number of single cell whole genome amplification kits available on the market. It is seldom known or appreciated that the method of whole genome amplification used in these kits is not consistent and that each method has its own mechanism by which it replicates the DNA template with different amplification biases due to the processivity and fidelity of enzymes used.

AIM - The purpose of this review is to compare and contrast the 4 main whole genome amplification technologies currently available in kits, including their hands on and incubation times.

Methods for cell lysis, as well as the primer and polymerase characteristics, and protocol steps for multiple displacement amplification (MDA), linker adapter PCR, MALBAC, and degenerate oligonucleotide primed PCR (DOP-PCR) methods were compared (Table 1). The original published DOP-PCR (1) and advances possible with the use of new generation engineered polymerase technology to DOP-PCR. Metrics for comparison included amplified DNA fragment length, DNA yield and consequent suitability for specific downstream DNA analysis platforms.

The time frame to complete the work flow for each kit is summarized in Figure 1 and the identification of each intervention step highlighted. Due to the nature of the different technologies some kits require multiple hands-on steps in contrast to the 2-step methods requiring only cell lysis and amplification. The shortest protocols can generate > 1µg DNA in less than 3 hours. In combination, the number of hands-on steps and total protocol length also impact on laboratory resourcing.

WGA kit comparison						
Kit name	GenomePlex	PicoPlex	Repli-G	MALBAC	DOPlify	Telenius DOP
WGA technology	Linker Adapter-PCR	Linker Adapter-PCR	Multiple Displacement Amplification	Hybrid	Advanced DOP-PCR	DOP-PCR
Launched	Feb-06	Jun-09	Oct-12	Oct-14	Jun-16	Jul-92
Lysis	Proteinase K digestion	Enzyme- based	Alkaline	Enzyme- based	Enzyme- based	Nil
Lysis volume (µl)	10	10	7	6	5	na
Lysis incubation time (mins) (a)	64	14	10	60	15	0
Template preparation	Yes	Yes	No	Yes	No	No
Template preparation incubation time (mins)	2	45	0	62	0	0
Cumulative volume (µl)	13	15	10	37	5	0
Amplification						
Cumulative volume (µl)	74	75	47	67.8	27	50
Amplification incubation time (mins) (b)	137.5	33.5	483	66	103	138
Total incubation time (mins) (a+b)	203.5	92.5	493	134	118	138
Yield from single cells	65-130ng/µl	25-65ng/µl	Up to 800ng/µl	30-55ng/µl	60-80ng/µl	Up to 140ng/µl
Fragment size range	0.1-1kb	0.2-1kb	2-100kb	0.3-2kb	0.2-2kb	0.1-1kb

Table 1 Information sourced from Manufacturer's protocols and information sheets available on their websites. Protocol times exclude PCR machine ramping duration.

DNA generated following whole genome amplification of limited template, such as single cells has specific characteristics that dictate its suitability for higher resolution analyses. Each of the kits and methods tested differed in the yield and fragment size (200bp to >10kbp) generated from limited DNA template. The very long fragments (approx. 2-100kb) amplified using MDA are well suited to genotyping due to the likelihood that a single amplicon of continuous DNA will contain a large region of a gene of interest and will span a significant number of SNVs. Limitations include amplification bias and template-independent primer interactions limiting the detection of exogenous DNA contamination. PCR-based amplification methods generating fragments < 2kbp, generate a more uniform coverage of the genome and are more suited to CNV detection. With the use of new generation reagents and a high fidelity polymerase, the DOPlify® kit is suited to applications requiring either CNV and SNV (2-4). Using RHS' patent pending Target Sequence Enrichment (TSE) protocol, a method for combined CNV and high resolution targeted analysis is possible.

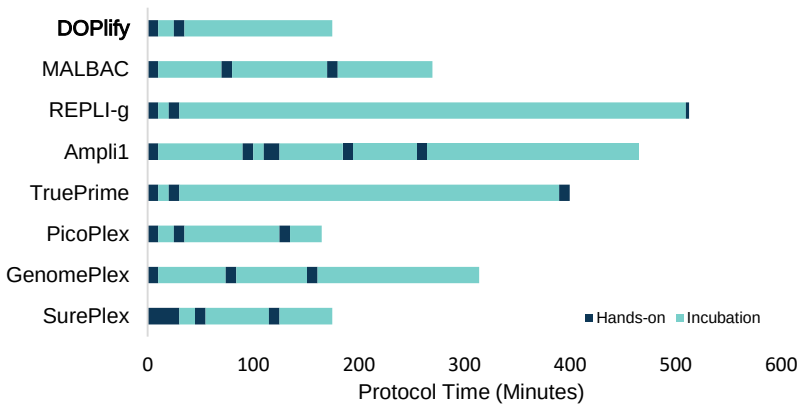

Figure 1 Comparison of WGA kit protocols

Conclusions

- The characteristics and suitability of the WGA products need to be considered with respect to the choice of downstream technologies and resolution required.
- With the use of new generation reagents and a high fidelity polymerase, the DOPlify® kit is suited to application requiring both SNV and CNV detection.

Reference
1. Telenius et al (1992) Genomics 13: 718
2. Deleye et al (2017) Scientific Reports 7: 3422
3. Deleye et al (2018) Scientific Reports 8: 1255
4. Fraser et al (2017) PGDIS Valencia, Spain P-21

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