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DOPlify™ A New Generation of Whole Genome Amplification

Mitochondrial genome coverage for copy number determination and detection of disease; the impact of WGA

Mutations in the mitochondrial genome (mtDNA) have been linked to diseases such as cancer, diabetes and deafness. Additionally, recent data suggests that mitochondrial genome load can impact implantation potential of euploid embryos. The selection of embryos for IVF transfer using the additional information from mitochondria requires an accurate and high coverage whole genome amplification (WGA) methodology. Additionally, since the mtDNA genome is 16,571bp in length and there are multiple copies per cell, this provides a model to evaluate performance of WGA technologies.

Aim – This study aimed to compare two different commercially available WGA kits; PicoPlex® (Rubicon Genomics) and DOPlify™ (RHS Ltd), evaluating overall mtDNA genome coverage along with coverage of 23 common mitochondrial mutations using NGS of the whole genome amplified single cells.

Methods – Single cells sorted from an aneuploid cell line (Coriell Institute for Medical Research) were subjected to WGA using DOPlify™ (n=2) and PicoPlex® (n=2) according to manufacturer's instructions. Nextera libraries (Illumina) were prepared from 50ng WGA DNA with an additional 19 samples (total 23) subsequently multiplexed and sequenced on a NextSeq platform according to standard 2x150bp protocol (Illumina). The sequencing data was bioinformatically aligned to hg19 then analysed to determine mitochondrial genome coverage.

Results – Despite normalising DNA concentrations prior to pooling, the total mapped reads for PicoPlex® amplified cells were considerably higher than the DOPlify™ amplified cells. Down sampling was not performed. Overall coverage of the mitochondrial genome was on average 65x for PicoPlex® and 3525x for DOPlify™ (Table 1). Reads for the two DOPlify™ samples covered 100% of the mitochondrial genome, far greater coverage than the PicoPlex® samples (Figure 1). Evaluation of DOPlify™ amplified cells confirmed coverage at all 23 common mitochondrial mutation sites, with a minimum read depth of 120 reads and an average depth of 678 reads. For the two PicoPlex® amplified cells, reads mapped to 16/23 and 11/23 of the common mutations, however only 7/23 and 2/23 mutations in each cell had a read depth higher than 50 reads (Table 2 and Figure 2).

Table 1 – NGS metrics and coverage

	PicoPlex® 1	PicoPlex® 2	DOPlify™ 1	DOPlify™ 2
Total mapped reads to human genome (hg19)	16,200,000	9,800,000	6,500,000	8,200,000
mtDNA Coverage (read length x reads/genome size)	x 101	x 29	x 3348	x 3702
mtDNA Percent coverage at x1	92 %	67 %	100 %	100 %

Figure 1 – The 16,571bp mitochondrial genome (hg19) showing the read depth at each base position as a percentage of total mapped reads for PicoPlex® and DOPlify™ whole genome amplified single cells

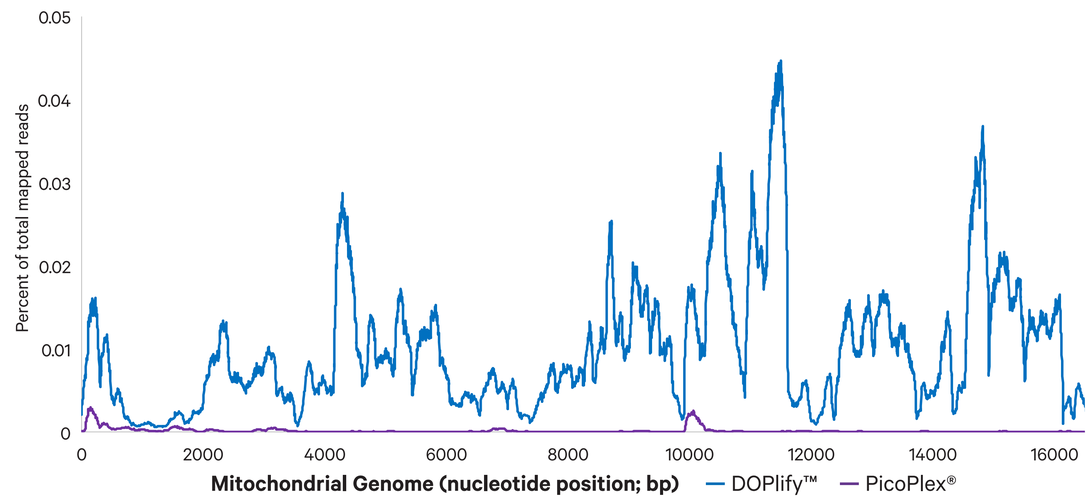

Table 2 – Depth of coverage across 23 common mitochondrial mutation sites

mtDNA Mutation position (bp) (1)	Depth of Coverage (x) PicoPlex® 1	PicoPlex® 2	DOPlify™ 1	DOPlify™ 2
1555	93	31	126	161
1624	75	23	156	179
3243	53	8	422	374
3271	50	11	484	423
3460	17	10	340	353
4300	4	2	2140	2248
5545	4	0	819	854
7445	2	0	120	136
7472	2	0	154	192
8344	1	0	513	593
8356	3	0	722	906
8993	8	7	804	829

mtDNA Mutation position (bp) (1)	Depth of Coverage (x) PicoPlex® 1	PicoPlex® 2	DOPlify™ 1	DOPlify™ 2
9176	19	8	1451	1463
10158	255	84	700	985
10191	225	64	727	922
10197	160	34	600	747
11777	19	0	248	287
11778	19	0	251	286
13513	5	2	925	1006
14484	0	2	465	440
14459	0	2	387	400
14487	0	2	467	436
14709	4	0	2045	2315

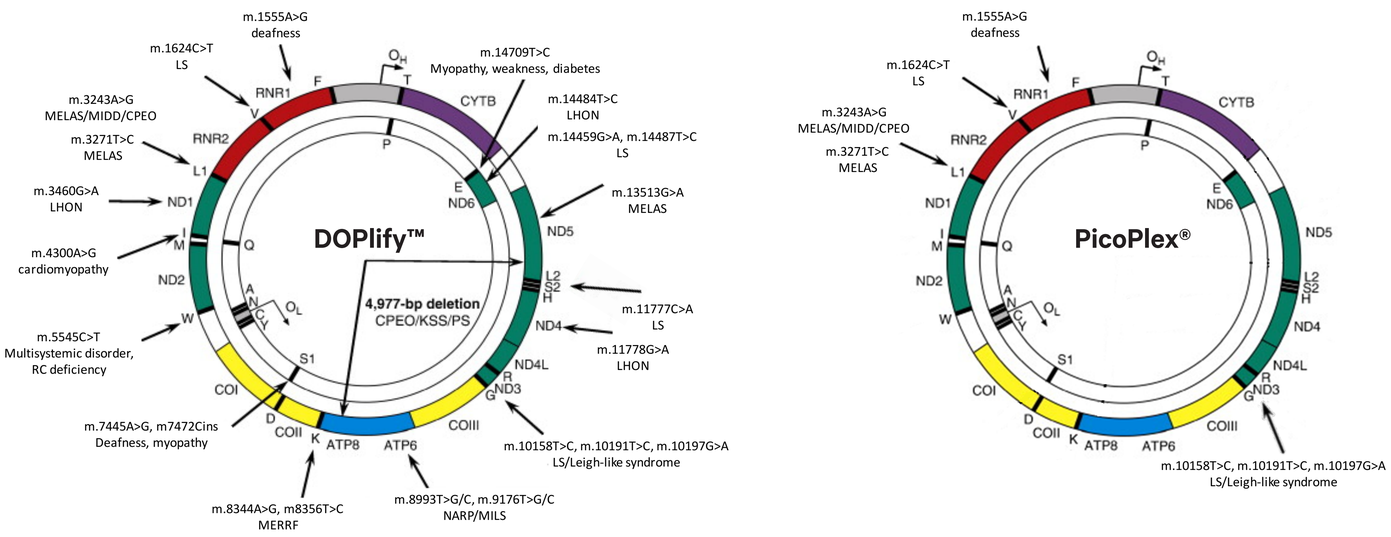

Figure 2 – The mitochondrial genome showing locations of common mutations (1) which have a read depth greater than 50x for DOPlify™ sample 1 and Picoplex® sample 1

Conclusions

- DOPlify™ whole genome amplification resulted in significantly greater breadth and depth of coverage over the mitochondrial genome when compared to PicoPlex®
- DOPlify™ amplified all 23 common mitochondrial genome mutations from single cells

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1. Tuppen, H., Blakely, E., Douglass, M., Taylor, R. 2010. Mitochondrial DNA Mutations and human disease. Biochimica et Biophysica Acta – Bioenergetics. Vol 1797, Issue 2, pg 113-128.