WGA AND NGS READ LENGTH SIGNIFICANTLY IMPACT MITOCHONDRIAL CHARACTERISATION

PG-Seq™ A novel complete NGS solution for PGS and PGD

The mtDNA genome is 16,571bp in length and there are multiple copies per cell. It contains mutations linked to diseases such as cancer, diabetes and deafness. These attributes make mtDNA an ideal model to evaluate performance metrics of whole genome amplification (WGA) technologies. Additionally, recent data suggests that mitochondrial genome load may 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 WGA methodology.

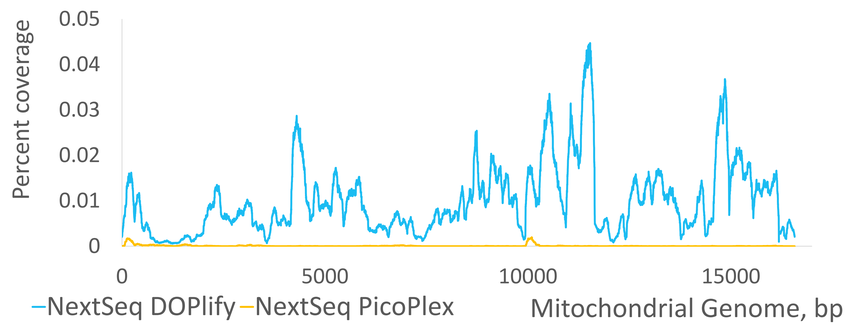
Aim: This study aimed to compare two different commercially available WGA kits using short and mid-range read length NGS; evaluating overall mtDNA genome coverage along with coverage of 23 common mitochondrial mutations.

Method: Single cells or Trophectoderm biopsies were amplified with either PicoPlex® (Rubicon), SurePlex (Illumina) or DOPlify™ (RHS) WGA kits and sequenced using standard VeriSeq (Illumina: 1x36bp, 24 sample multiplex), PG-Seq™ (RHS: 1x75bp, 48 sample multiplex) or a custom NextSeq (2x150bp, 23 sample multiplex) protocols. Bioinformatics was used to truncate the 75bp read lengths from PG-Seq™ to 36bp. All samples were aligned to hg19 then analysed to determine mitochondrial genome coverage.

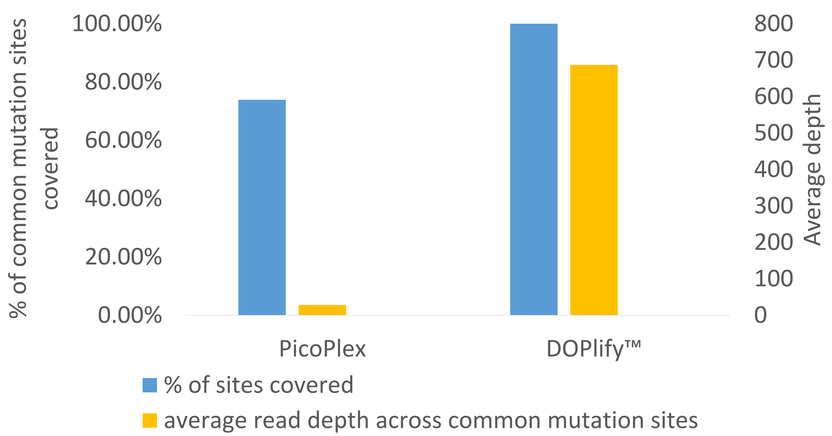
Results: DOPlify™ samples following NextSeq 2x150bp NGS generated 53x greater coverage compared with PicoPlex, even though there was almost half the number of mapped reads.

	PicoPlex NextSeq 2x150	DOPlify NextSeq 2x150
# samples multiplexed	23	23
# reads mapped to hg19	13,030,000	7,349,000
mtDNA Coverage	33x	1763x

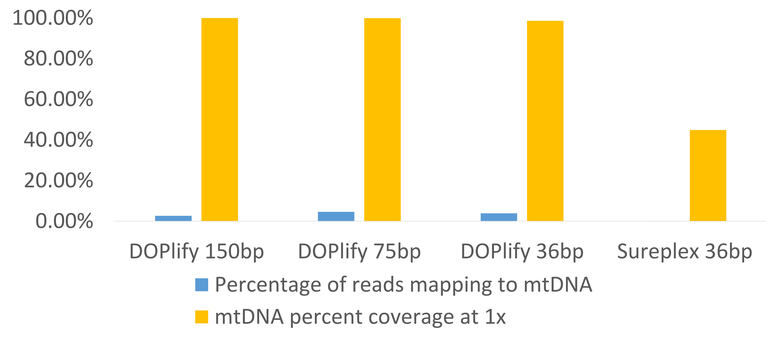
Not only was there more depth of coverage, but there was greater breadth of coverage with DOPlify™ achieving 100% mtDNA coverage compared with 79% for PicoPlex.



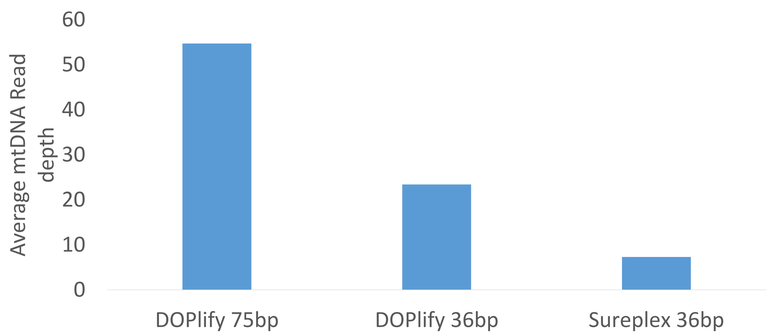
DOPlify™ amplified cells sequenced on a NextSeq also displayed superior coverage and read depth over 23 common mitochondrial mutation sites.



When reducing the read length from 150bp (NextSeq) to 75bp (PG-Seq) to 36bp (VeriSeq); the DOPlify™ mtDNA breadth of coverage is slightly decreased, however DOPlify™ 36bp still showed greater percent coverage compared to Sureplex 36bp.



When observing average read depth across the mitochondrial genome, the difference between the WGA technologies is normalised when the NGS read length is cut to 36bp with more than 50% of DOPlify™ read depth data being lost.



Conclusions

The WGA methodology and sequencing read length significantly impacts the amount of data generated by NGS. Using DOPlify™ at 75bp (48 sample run) provides significantly broader mitochondrial genome coverage and increased average read depth compared to either DOPlify™ at 36bp (48 sample run) or SurePlex at 36bp (24 sample run).

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