APPLICATION NOTE

Utility of DOPlify® WGA kit for molecular analysis.

The DOPlify® kit uses an advanced Degenerate Oligonucleotide Primed PCR (DOP-PCR) for WGA:

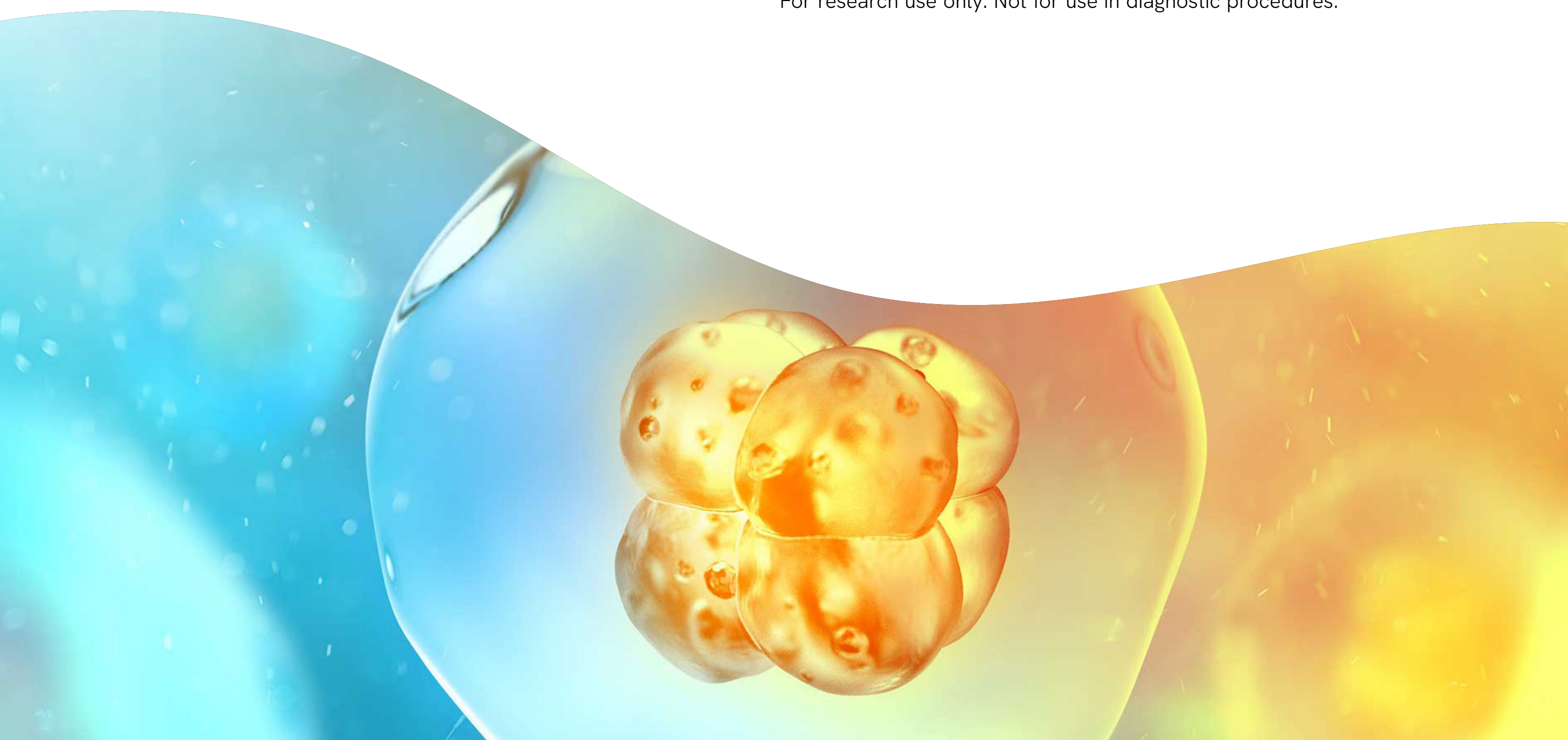
- Optimised specifically to amplify the DNA from single cells and limited template
- No GC bias provides even amplification across the template
- Uses best-in-class high processivity and fidelity new generation reagents, providing single base accuracy
- Reagents are stable during shipping (no need for dry ice and unaffected by repeat freeze thaws)

Whole Genome Amplification (WGA) is used to obtain sufficient quantities of DNA from limited template samples and has become an essential first step for testing single cells. Following WGA, the detection of genetic changes from these samples, including copy number variants (CNV) and mutation analysis is achieved using downstream genomic applications such as Massively Parallel Sequencing (MPS), genotyping and array-based technologies. WGA methods must be critically assessed for their suitability to their intended use, as some methodologies can introduce inaccuracies resulting in representation bias, induce errors, and inconsistent yield leading to analytical variation.

Some applications which may require WGA are pre-implantation genetic testing (PGT), molecular karyotyping, SNP and STR genotyping, qPCR- and PCR-based mutation detection, next generation sequencing, and microsatellite analysis.

The DOPlify® kit from Revvity is a whole genome amplification kit designed and optimized specifically to amplify the genome from single cells and limited DNA templates.

For research use only. Not for use in diagnostic procedures.



Why use DOPlify® technology?

The DOPlify® kit is unique in because it uses advanced DOP-PCR, employing the high processivity and fidelity of new generation, sequencing-grade polymerases. The kit uses ready-mixes to minimize the pipetting steps needed to set up an amplification reaction and requires only two sample tube openings, reducing the risk of sample contamination. The DOPlify® kit can representatively amplify DNA from the 6 pg found in a single cell and comprehensively amplifies the mitochondrial genome. Revvity's proprietary Target Sequence Enrichment (TSE) protocol provides an amplification system flexible enough to concurrently perform WGA on a sample while specifically amplifying genes of interest, reducing the risk of allele drop-out.

Effective amplification with reduced hands-on time

A gentle but effective enzyme-based lysis procedure ensures robust cell lysis and a readily accessible DNA template for whole genome amplification.

The DOPlify® kit reduces the chance of contamination with a streamlined workflow. The DOPlify® workflow only requires 2 hands-on steps. A short 2 ½ hour PCR cycle time provides sufficient amplified DNA for multiple downstream molecular assays. The use of ready-mixes in the DOPlify® kit simplifies the workflow and minimizes the pipetting steps. With only two sample tube openings, the DOPlify® kit reduces the risk of contamination.

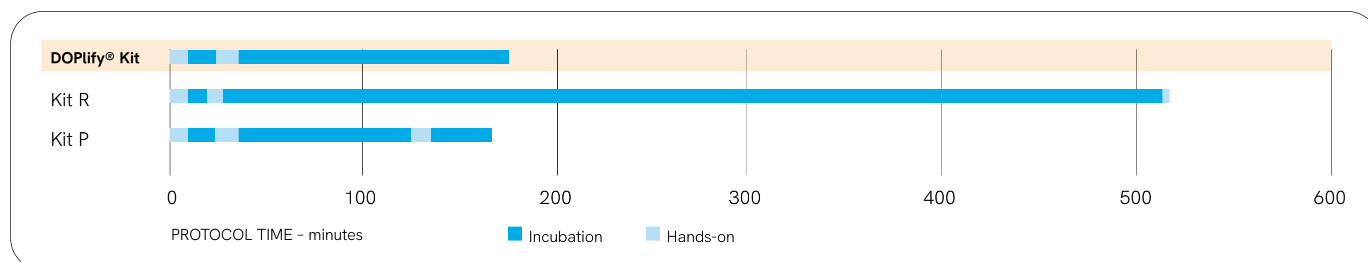


Figure 2: Illustration of commercially available WGA methods depicting time required to complete the protocol, in minutes, and number of manual steps necessary to complete reaction set up, depicted in light blue.

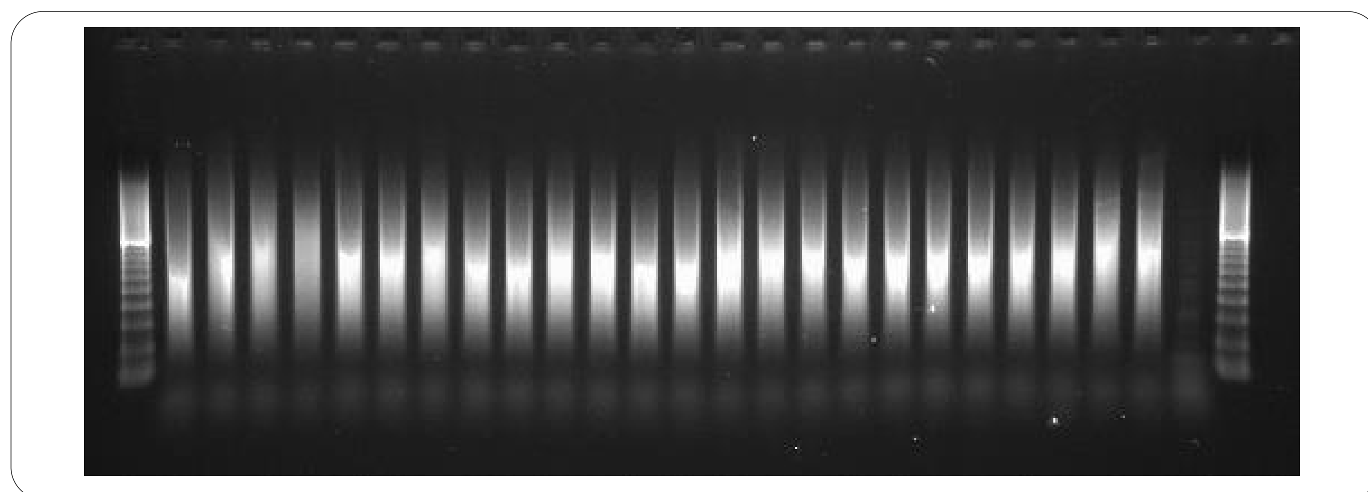


Figure 3: Gel electrophoresis examination of DOPlify® kit amplified samples. Lanes 1 and 27 contain a 100 bp increment DNA size ladder (DMW-100M, GeneWorks, Australia), lanes 2-25 are WGA 5 cell samples and lane 26 is a no template control (NTC) where no DNA was added to the reaction.

Uniform amplification and consistent GC coverage

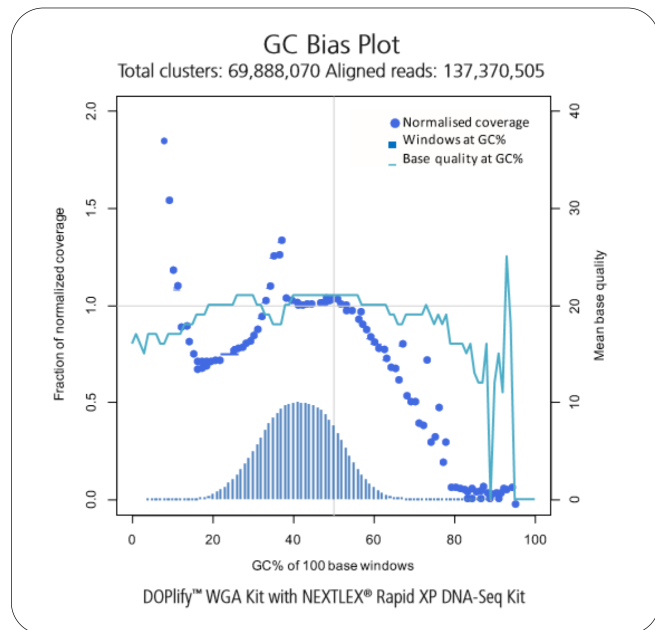


Figure 4: GC bias analysis of libraries constructed using the PG-Seq™ kit which uses DOPlify® reagents for WGA.

GC content is variable between different organisms and even between different chromosomes within a specific genome. This variability is caused by variation in selection, mutational bias, and biased recombination-associated DNA repair.

The average GC-content in human genomes ranges from 35% to 60% across 100 Kb fragments, with a mean of 46.1%.[3]

As a measure of amplification fidelity and genome coverage, GC content was bioinformatically reviewed post whole genome sequencing and compared to unamplified genomic DNA (Fig. 4). **The results show the GC content resulting from DOPlify® kit whole genome amplification is consistent and the base quality remains high across the sequence.**

The GC content of DNA amplified with the DOPlify® kit matches the GC content of unamplified genomic DNA (Fig. 4) indicating the DOPlify® WGA kit delivers libraries with:

No bias | Even amplification | Consistent base quality across the range of GC content sequenced

The DOPlify® kit provides the fidelity and processivity required for robust WGA enabling accurate detection of copy number changes with the added benefit of consistent mitochondrial DNA amplification and target sequence enrichment, separating the DOPlify® kit from other commercially available kits.

Preservation of mitochondrial DNA during amplification

The human mitochondrial genome is composed of 16,569 base pairs and encodes for 37 genes. Mitochondrial DNA (mtDNA) analysis examines the DNA present in the mitochondria of a cell, rather than the nuclear DNA most tests are designed for. The mitochondrial genome can be used for identification through its maternal inheritance and is also the site of a range of disease associated mutations.

In samples with degraded or absent nuclear DNA, the multiple copies of mtDNA provides a target for identification from samples such as hair, bones, and teeth. Recent data suggest an association between mitochondrial genome load in euploid embryos and implantation potential[3]. The DOPlify® kit provides complete mitochondrial genome coverage, which not only more accurately quantifies the mitochondrial load but also provides a genetic signature for sample tracking through the use of informative Single Nucleotide Variants (SNVs) (Revvity, unpublished) in high throughput labs. Through uniform amplification and preservation of mtDNA, the DOPlify® kit can enable the detection of disease associated mitochondrial mutations.

Sample & WGA	Detection of 23 common mitochonrial mutations
DOPlify® Kit WGA, sample 1	23
DOPlify® Kit WGA, sample 2	23
PicoPLEX® Kit WGA, sample 1	16
PicoPLEX® Kit WGA, sample 2	11

Either the DOPlify® kit or Takara® Bio PicoPLEX® WGA kit was used to amplify five cell samples (Table 1). Mitochondrial DNA coverage was assessed by bioinformatically analyzing human mitochondrial specific sequences across 23 common mutations. Sequencing revealed the DOPlify® kit amplified samples provided superior mitochondrial DNA sequence coverage and mutation detection compared to the Takara® Bio PicoPLEX® WGA kit.

Table 1: Mitochondrial DNA coverage in samples amplified with either the DOPlify® kit or Takara® Bio PicoPLEX® WGA kit.

	PicoPLEX® Kit WGA, sample 1[1]	PicoPLEX® Kit WGA, sample 2	DOPlify® Kit WGA, sample 1	DOPlify® Kit WGA, sample 2
mtDNA Coverage (read length x reads/genome size)	x101	x29	x3348	x3702
mtDNA Percent coverage at x1	92%	67%	100%	100%

Target sequence enrichment during WGA

In certain molecular biology applications, TSE can be used to ensure sufficient amplification and abundance of specific genes or sequences requiring analysis. Preimplantation Genetic Testing for Monogenic disorders or PGT-M is one such method requiring detailed genetic analysis of specific mutations linked with disease from IVF embryo biopsies of 1-10 cells in size. A benefit of the DOPlify® WGA kit is TSE can be achieved while performing whole genome amplification through the simple addition of sequence specific primers part way through the WGA process. The addition of the sequence specific primers improves the presence/ yield of the sequence specific targets, reduces the incidence of Allele Drop Out (ADO) and allows for improved analysis of the DNA.

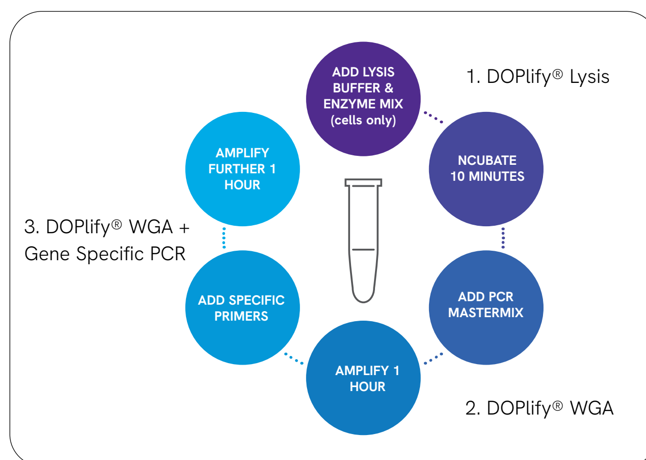
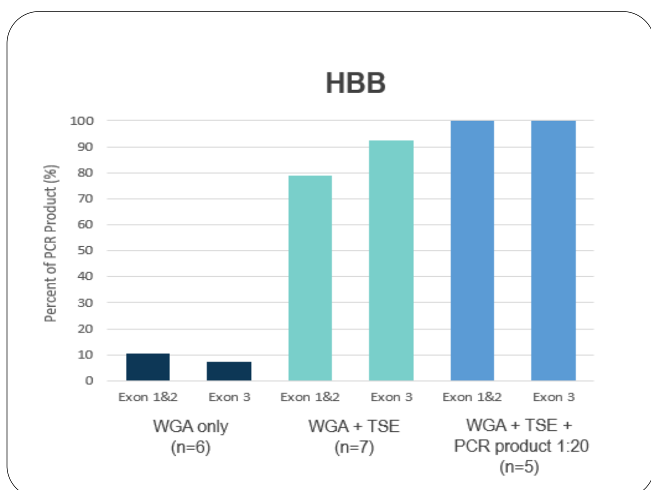


Figure 5: DOPlify® WGA kit workflow.



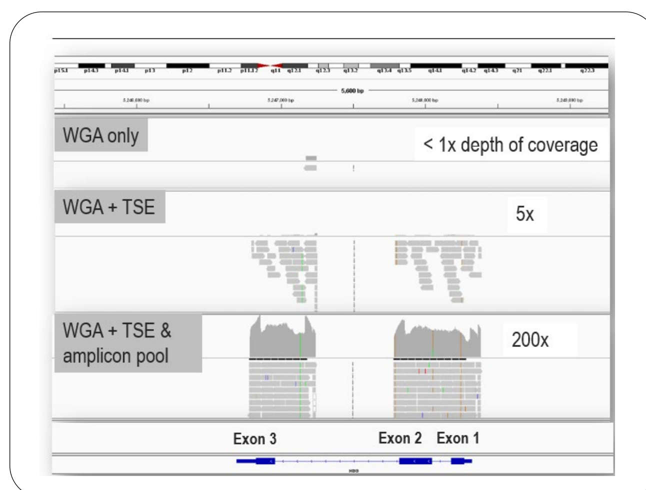


Figure 6: TSE using the DOPlify® WGA kit to amplify the DNA both with and without β-thalassemia specific primers.

An example for TSE using the DOPlify® WGA kit to amplify the DNA is shown (Fig. 6). Using WGA without sequence specific primers, in this with low pass NGS example for β-thalassemia, yielded relatively low abundance of the β-thalassemia fragments in exon 1,2 & 3, which is to be expected with low pass NGS. Through primer addition during WGA, the percentage of HBB PCR product increases from 10% to 90% for exon 3. By seeding β-thalassemia-enriched PCR products from the WGA & TSE into a further HBB specific PCR, this increased the percentage of HBB PCR product to achieve sufficient breadth and depth of gene coverage to allow diagnosis.

The NGS protocol without TSE used in this study is typical for low pass Preimplantation Genetic Testing for Aneuploidy (PGT-A) with resolution of approximately 10kb and was not expected to yield the depth of reads required for PGT-M in the absence of enrichment. Target sequence enrichment provided the sequencing reads necessary for PGT-M without requiring costly deep sequencing of the entire genome.

The addition of the HBB primer set during WGA did not impact the PGT-A result and demonstrates the capability of the DOPlify® kit to increase the target sequence while continuing to amplify the whole genome.

Peer reviewed, independent DOPlify® benchmarking study

Revvity's DOPlify® kit has been independently validated to reliably amplify copy number variants down to 3Mb in size from 1, 3 or 5 cell templates. According to published data, it is the only commercially available kit with equivalent accuracy to the Takara® Bio PicoPLEX® WGA kit. 4

The DOPlify® kit WGA resulted in a yield of 21.1 +/- 2.6 ng/µL (in 30 µL) and fragment lengths of 957.3 + or - 87.2 bp in the independent assessment allowing for downstream analysis of CNV variant as shown below:

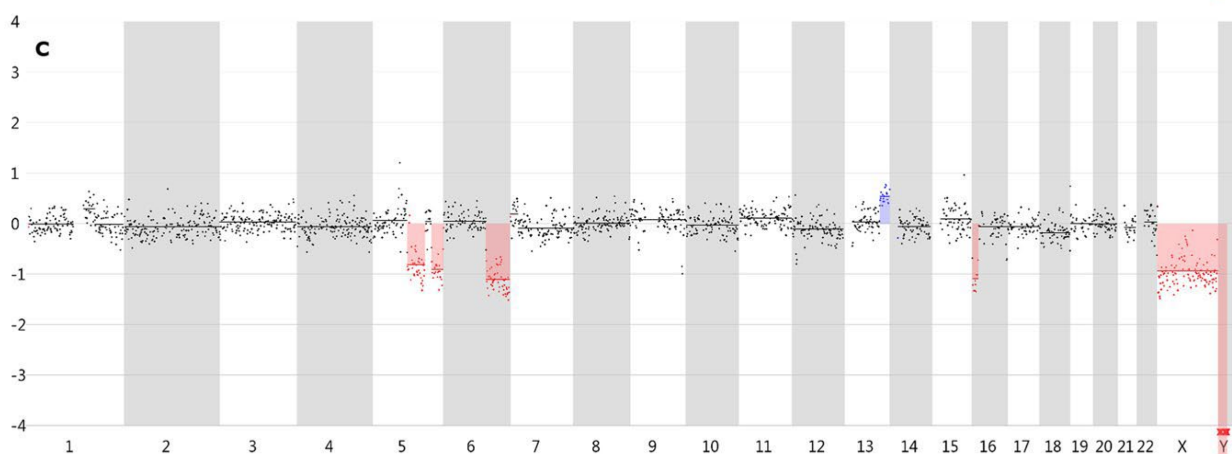


Figure 7: A CNV profile of a DOPlify® kit amplified 3-cell Loucy cell line sample, demonstrating the detection of the expected karyotype consisting of deletion of an entire X-chromosome, two deletions of 45 Mb (consists of 6 Mb and a 36.5 Mb deletion interspersed by a 2.5 Mb normal ploidy region) and 30 Mb on respectively 5q14.3-q31.1 and 5q33.1-q35.3, a deletion of 60 Mb on 6q21-q27, a deletion of 3 Mb on 12p13.31-p13.2, a 26 Mb duplication of 13q31.3-q34, and two deletions of 16 Mb and 3 Mb on respectively 16p13.3-p13.11 and 16q24.2q24.3. CNV lines were generated using a 1Mb window providing a resolution of >3Mb4.

	Specificity (%)	Positive predictive value (%)
Rubicon Genomics® PicoPLEX® DNA-Seq Kit	100	100
Revvity® DOPlify® Kit	100	100
Silicon Biosystems® Ampli-1™ Kit	93.8±8.6	93.2±13.7
Qiagen® RepliG® Kit	96.3±7.3	90.5±10.1

"Picoseq and DOPlify excelled, leading to the highest number of detected true positives without detection of false positives."
Deleye et al. 2017

Validation of the DOPlify® WGA Kit for Molecular Karyotyping Applications

The Revvity® KaryoLite® BoBs® assay uses probes generated from selected Bacterial Artificial Chromosomes (BACs) immobilized onto Luminex® xMAP® encoded beads for aneuploidy screening and copy number analysis down to the chromosome arm level. The DOPlify® WGA kit was used with the KaryoLite® BoBs® assay for Preimplantation genetic testing for aneuploidy (PGT-A) applications. 5-cell aliquots of lymphocyte and fibroblast cells were used as the input samples to mimic trophectoderm biopsies. Aliquots from each cell line were amplified using the Takara® Bio PicoPLEX® WGA kit or the DOPlify® WGA kit.

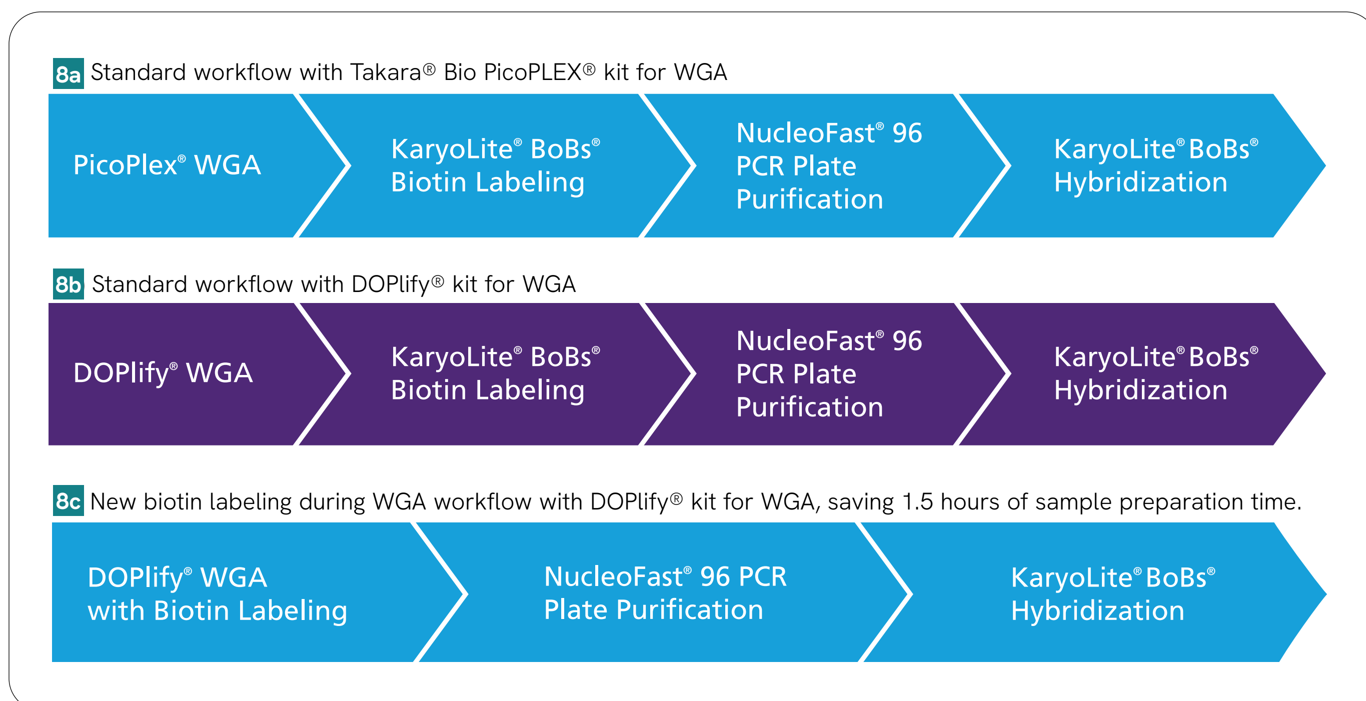


Figure 8: Summary of the compared Karyolite BoBs workflows.

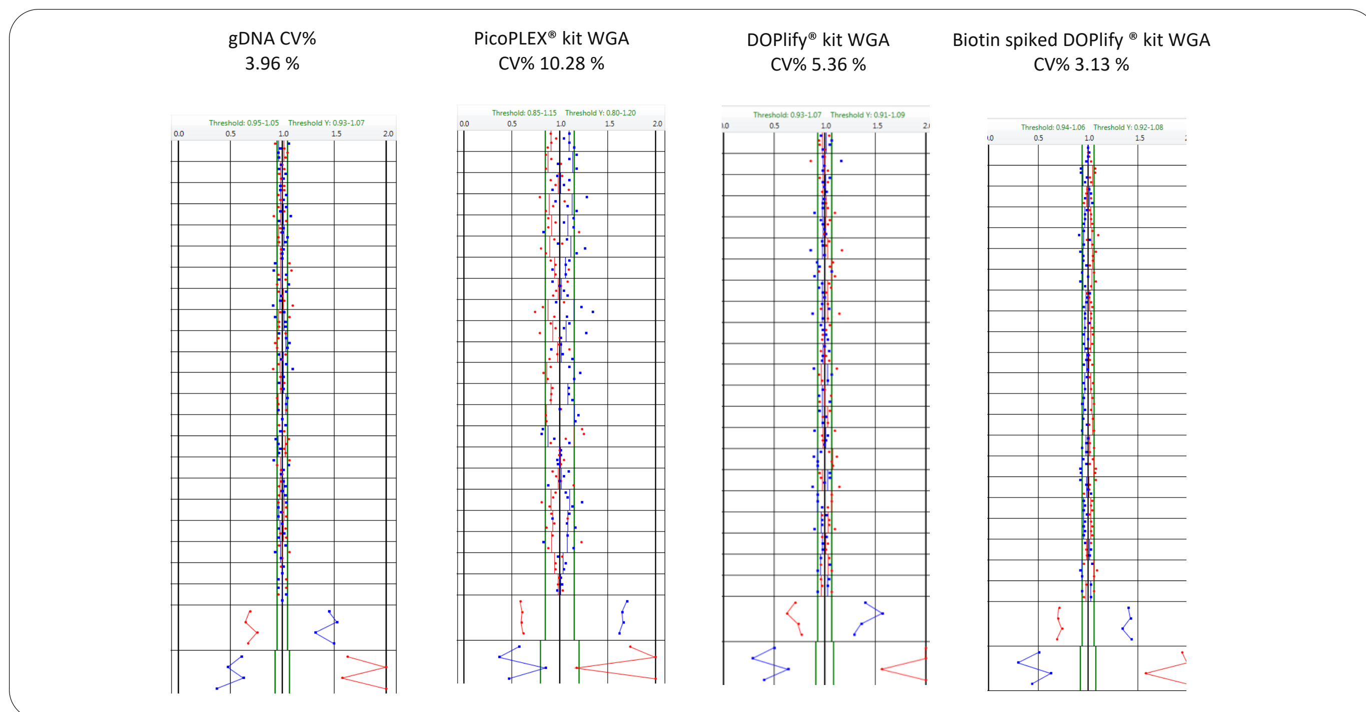


Figure 9: Karyolite® BoBs® assay results for unamplified genomic DNA and 5 cell aliquots from a euploid female cell line prepared using 3 different WGA protocols.

All samples were 100% concordant with the expected karyotype. The CV, which is used as a measure of noise across the result, was lower with the DOPlify® kit amplified samples than with the Takara® Bio PicoPLEX® WGA kit amplified samples. A novel protocol incorporating biotin during WGA with the DOPlify® kit gave correct results with the lowest CV (figures 8 and 9).

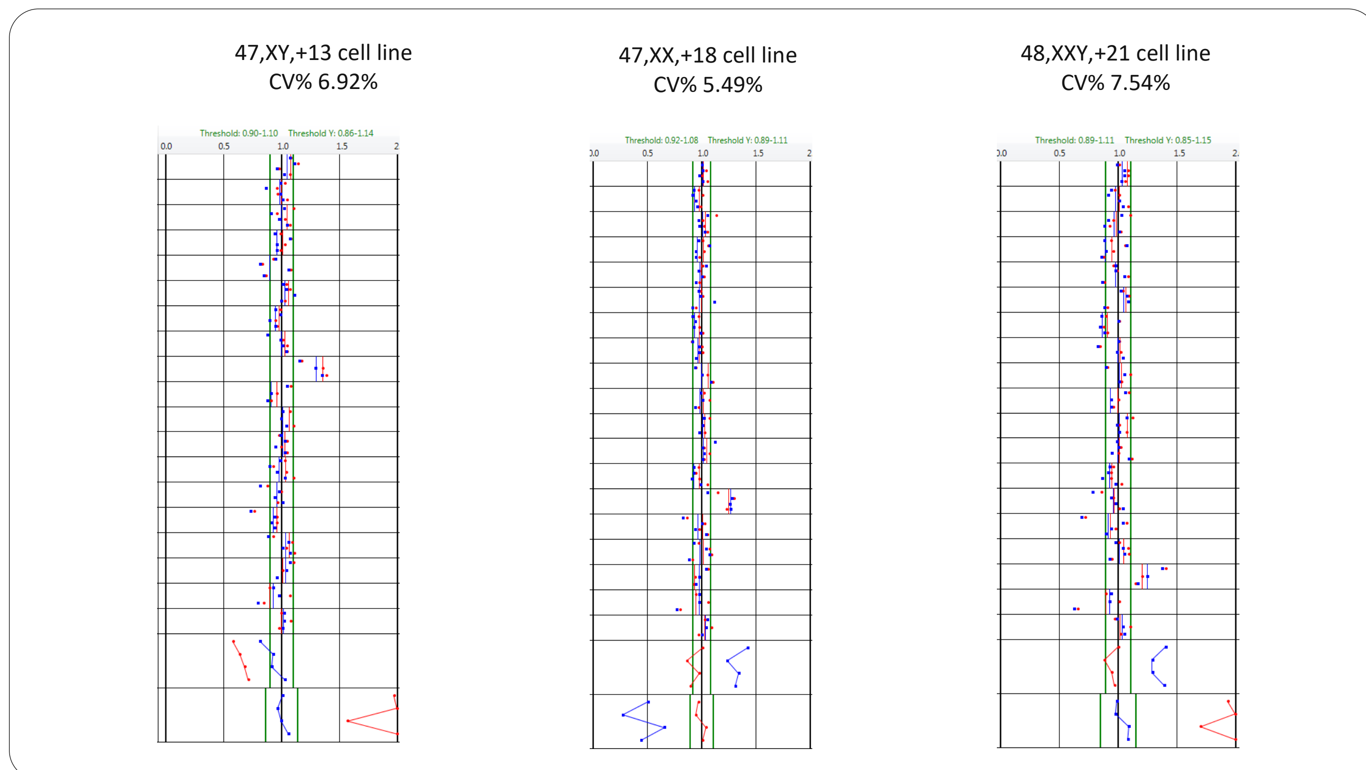


Figure 10: Analysis of a range of chromosomal abnormalities using the Karyolite® BoBs® assay with the new biotin labeling workflow with the DOPlify® kit for WGA (Workflow 8c).

The DOPlify® WGA kit workflow incorporating biotin labelling reduced sample preparation time by 1.5 hours and provided accurate aneuploidy results when used in the KaryoLite® BoBs® assay.

Low-pass, whole genome wequencing using PG-Seq™ on the Illumina® MiSeq® instrument

To further illustrate the robustness and repeatability of the DOPlify® kit for whole genome amplification, cell lines were used as template for the DOPlify® kit and then low-pass whole genome sequencing was performed. The performance was assessed by determining the accuracy of detecting a range of whole chromosomal abnormalities.

In the validation study, a total of 442 five-cell and single cell aliquots of cultured fibroblasts and lymphocytes were manually isolated from cells lines, amplified with the DOPlify® kit and 48 samples per run sequenced on the Illumina® MiSeq® sequencing instrument using the Illumina® MiSeq® V3 Reagent Kit:

- 98.4% whole chromosome aneuploidy sensitivity in 5-cell samples
- 95.8% whole chromosome aneuploidy sensitivity in single cell samples
- 98.3% segmental aberration sensitivity in 5-cell samples

Further details can be found in the PG-Seq™ kit validation app note available at Revvity-appliedgenomics.com/pg-seq.

A subsequent clinical comparison was made between the Illumina® VeriSeq® kit and the PG-Seq™ kit using repeat biopsies from aneuploid embryos donated to research.

Below are some examples of results demonstrating the resolution of the PG-Seq™ kit from the clinical validation study.

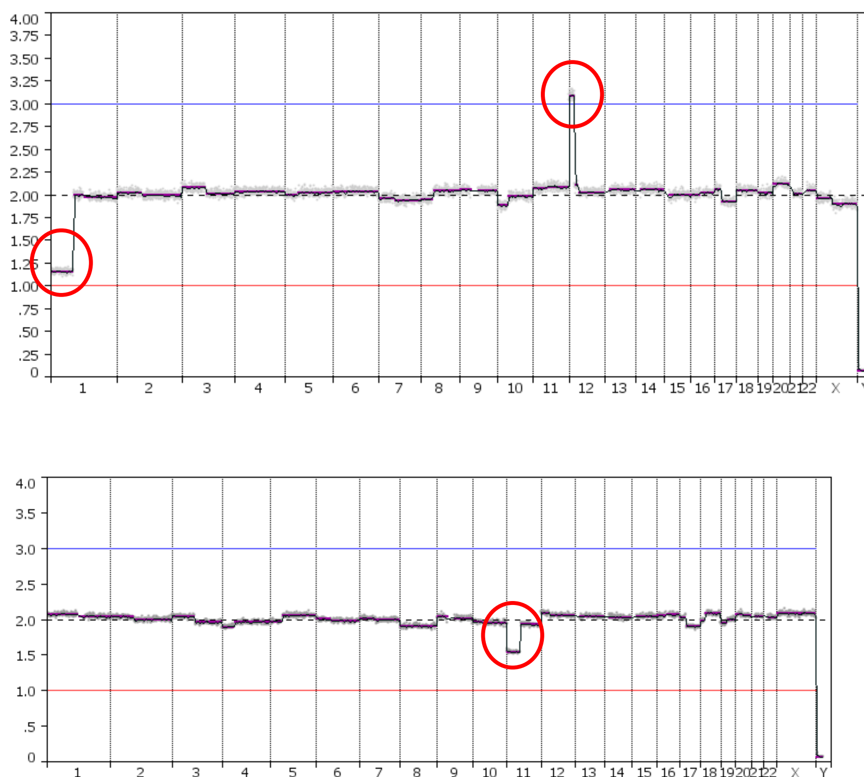


Figure 11: Segmental copy number variant detected through PGT-A using the PG-Seq™ kit. Additional information on the validation study can be found at: www.revvity.com

Segmental copy number variant (CNV), gain on chromosome 12 (21Mb) and loss of chromosome 1 (86Mb), detected through Preimplantation Genetic Testing for Aneuploidy, PGT-A, using Revvity's PG-Seq™ kit, which is based on the DOPlify® kit (Fig. 11). The 50% mosaic loss in chromosome 11 (53 Mb) was accurately detected using the PG-Seq™ kit.

References

1. (2018). Short Tandem Repeat analysis after Whole Genome Amplification of single B-lymphoblastoid cells. Scientific Reports,8(1). doi:10.1038/s41598-018-19509-5.

2. Plaetsen, A. V., Deleye, L., Cornelis, S., Tilleman, L., Nieuwerburgh, F. V., & Deforce, D. (2017). STR profiling and Copy Number Variation analysis on single, preserved cells using current Whole Genome Amplification methods. Scientific Reports, 7(1). doi:10.1038/s41598-017-17525-5.

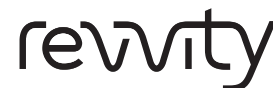
3. Romiguier, Jonathan; Ranwez, Vincent; Douzery, Emmanuel J. P.; Galtier, Nicolas (2010). "Contrasting GC-content dynamics across 33 mammalian genomes: Relationship with life-history traits and chromosome sizes". Genome Research. 20 (8): 1001– 1009. doi:10.1101/gr.104372.109. ISSN 1088-9051. PMC 2909565. PMID 20530252.

4. Deleye L, et al. (2017). Performance of four modern whole genome amplification methods for copy number variant detection in single cells. Sci Rep, 7:3422 doi:10.1038/s41598-017-03711-y.

5. Comparing the Reproducibility of Different Aneuploid Findings webinar (2018). Viewable at www.revvity.com.

Ordering information

Please contact your local distributor or Revvity representative for ordering information.
For technical support please contact www.revvity.com



Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA

(800) 762-4000
www.revvity.com

For a complete listing of our global offices, visit www.revvity.com
Copyright ©2023, Revvity, Inc. All rights reserved.