

# DOPlify® A new generation of whole genome amplification

## Embryo biopsy to tube transfer – How much PBS is too much?

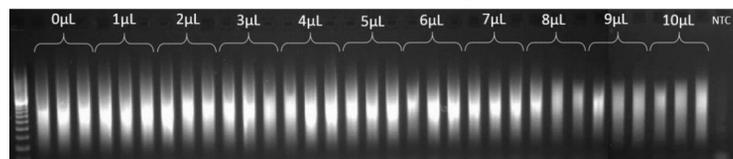
When an embryo biopsy is performed prior to Preimplantation Genetic Testing for Aneuploidy (PGT-A), the biopsy is washed in PBS or similar buffer to remove residual culture media before being placed in a tube for Whole Genome Amplification (WGA). The amount of buffer transferred to the tube can vary from the minimal recommended amount (< 2  $\mu\text{L}$ ) to a larger volume. It is unknown what effect increased volumes of transfer buffer have on the WGA and subsequent PGT-A outcomes.

**Aim** – This study aimed to determine the maximum volume of PBS that can be transferred into a tube with an embryo biopsy without compromising WGA using the DOPlify® kit.

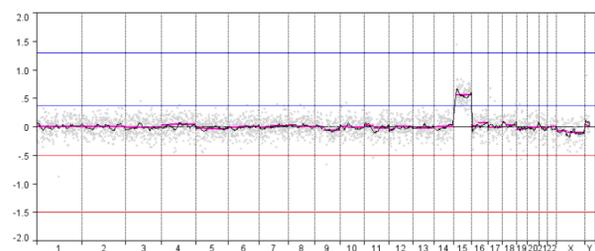
**Methods** – To replicate the effect of transferring different volumes of buffer with an embryo biopsy, a 5-cell equivalent amount of human genomic DNA (30 pg, Promega) had incremental volumes of PBS (0-10  $\mu\text{L}$ ) added before standard DOPlify® kit WGA (25  $\mu\text{L}$ ) (PerkinElmer). This was then repeated with 5-cell aliquots manually sorted from various cell lines (Coriell Institute) to determine the effect on cells representing a trophectoderm biopsy. Larger volume (40  $\mu\text{L}$ ) DOPlify® kit reactions were also performed on 5-cell aliquots with an additional 0-20  $\mu\text{L}$  of PBS added. WGA DNA was processed for PGT-A using the standard PG-Seq™ 48 sample protocol for MiSeq® instrument sequencing. WGA DNA yield and molecular karyotype after sequencing were analysed.

### Results

✓ A WGA DNA smear was visualised for all 25  $\mu\text{L}$  reaction amplifications from the genomic DNA templates with yields decreasing significantly ( $P < 0.05$ ) when 5  $\mu\text{L}$  or more of PBS was added prior to the standard DOPlify® kit WGA (Figure 1).

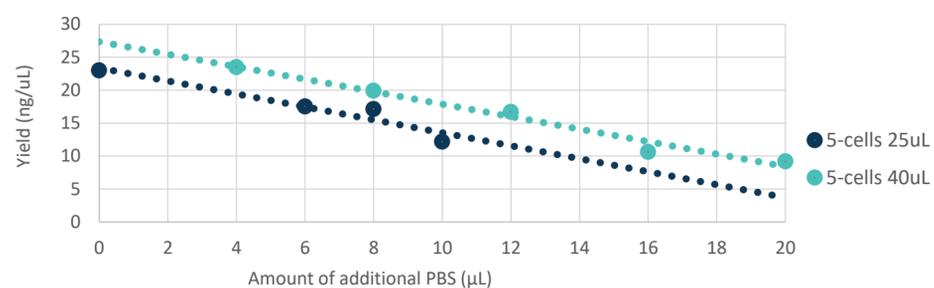


**Figure 1.** Genomic DNA (30 pg) amplified using DOPlify® kit following the addition of excess transfer buffer (0-10  $\mu\text{L}$ ).



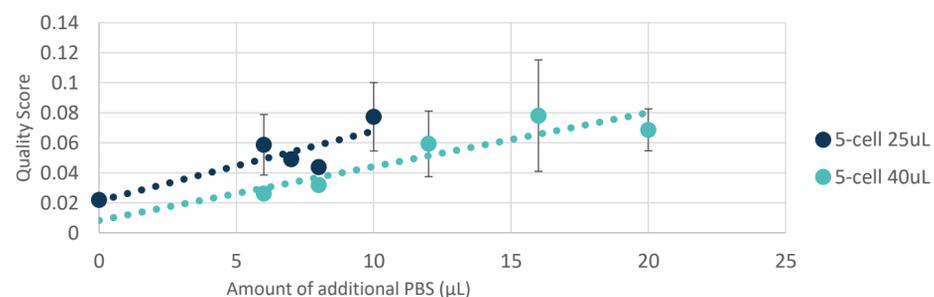
**Figure 2.** Example of cell line GM07189 (Coriell Institute) Karyotype 47,XY,+15 (25  $\mu\text{L}$  reaction volume WGA).

✓ A similar result was observed with the 5-cell aliquots, with the DNA yield decreasing as the volume of additional PBS increased; from a standard of 23 ng/ $\mu\text{L}$  to 12 ng/ $\mu\text{L}$  after 10  $\mu\text{L}$  of PBS was added (Figure 3).



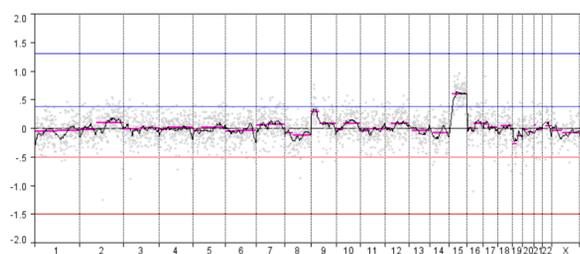
**Figure 3.** Yield of Whole Genome Amplified 5-cell samples from 25  $\mu\text{L}$  and 40  $\mu\text{L}$  reactions after 0.8x bead purification.\*  $P < 0.05$

✓ The correct karyotype results were obtained for 5-cell aliquots following the addition of 10  $\mu\text{L}$  PBS to the standard DOPlify® kit reaction, suggesting that a reduction in WGA DNA yield does not necessarily compromise the PGT-A outcome (Figure 2 compared to Figure 5A). The additional volume of transfer buffer did however result in noisier sample profiles, indicated by an increase in the software Quality Score which may lead to difficulty interpreting results and the reporting of “no-result” due QC fail (Figure 4).

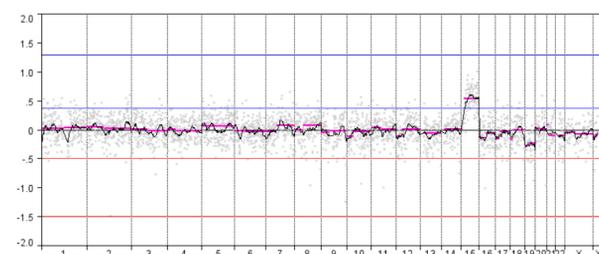


**Figure 4.** Quality score of 5-cell samples from 25  $\mu\text{L}$  and 40  $\mu\text{L}$  reactions after PG-Seq™ kit recommended sequencing.

✓ Expected WGA DNA yields and correct karyotypes were obtained for the 40  $\mu\text{L}$  DOPlify® kit reactions for all additional PBS volumes up to 20  $\mu\text{L}$ , indicating the additional WGA kit reaction volume improves result outcomes (Figure 5B).



**Figure 5A.** Example of cell line GM07189 (Coriell Institute) Karyotype 47,XY,+15 after addition of 10  $\mu\text{L}$  PBS prior to 25  $\mu\text{L}$  reaction volume WGA.



**Figure 5B.** Example of cell line GM07189 (Coriell Institute) Karyotype 47,XY,+15 after addition of 20  $\mu\text{L}$  PBS prior to 40  $\mu\text{L}$  reaction volume WGA.

### Conclusions

The efficiency of the WGA PCR is impacted by the volume of transfer buffer accompanying the embryo biopsy. Transfer buffer volumes of up to 5  $\mu\text{L}$  can be transferred into a tube with an embryo biopsy with no adverse result after standard 25  $\mu\text{L}$  DOPlify® kit WGA. However, for transfer buffer volumes greater than 5  $\mu\text{L}$  it is recommended that the WGA reaction volume is increased to 40  $\mu\text{L}$ .