
# DOPlify® kit

## Target Sequence Enrichment

## Technical Data Sheet

Version 1.6

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**DOPlify®** kit Target Sequence Enrichment Technical Data Sheet Version 1.6 July 2018

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**Contents**

# 1 Kit Contents

**DOPlify® Whole Genome Amplification (WGA) reagents – frozen at 20°C**

| Kit Codes | Component | Cap colour |
|---|---|---|
| DOPlify ®<br>RHS4050<br>50 reactions<br><br>Or<br><br>PG-Seq™ WGA Reagents<br>RHS5096-I-A<br>2x48 reaction format<br><br>Storage - Frozen at -20°C | PCR-grade $H_2O$ | White |
| | Cell Lysis Enzyme | Yellow |
| | Cell Lysis Buffer | Yellow |
| | WGA Polymerase | Red |
| | WGA PCR Buffer | Red |
| | Primer | Red |

# 2 Storage Information

**DOPlify®** kits (RHS4050) are shipped on ice packs. The **PG-Seq™** WGA reagents (RHS5096-I-A) are shipped as part of the **PG-Seq™** kit on dry ice. Whilst this TDS refers to the use of the DOPlify® kit, it is equally applicable to the PG-Seq™ WGA reagents.

Upon receipt, store the DOPlify® kit at -20°C in a constant temperature freezer (not frost-free).

Note that reagents stored at temperatures above -20°C are more prone to degradation and contamination; therefore storage at other temperatures is not recommended. It is not recommended to store reagents at -80°C. When stored under the correct conditions and handled correctly, full activity of the kit is retained until the expiry date indicated on the kit label.

The DOPlify® kit is designed to amplify picogram quantities of DNA; therefore extreme caution must be exercised to prevent the introduction of foreign DNA contaminants. Reagents should be stored in a clean laboratory away from potential PCR contaminants, including laboratories where PCR amplification is performed. Good laboratory practices for performing PCR should be followed when storing and handling reagents, as well as during the setup of PCR reactions.

# 3 Overview of workflow

**DOPlify® WGA and Target Sequence Enrichment**

Degenerate Oligonucleotide Primed PCR (DOP-PCR)-based Whole Genome Amplification (WGA) generates representative amplification of total DNA from small numbers of cells or their DNA equivalent. The DOPlify® kit DOP-PCR-based WGA reproducibly amplifies total DNA from single cells to produce microgram quantities of amplified DNA in less than 2 ½ hours. The DOPlify® kit can be used to amplify both cellular and purified genomic DNA inputs. This modified version of the DOPlify® WGA PCR protocol describes the additional feature of Target Sequence Enrichment (TSE) during WGA (Patent pending).

*Sample collection*

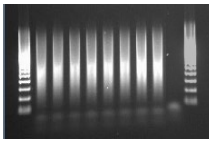
- Place the sample into a PCR tube in <2µl of compatible buffer (see below)
- Mark the sample location on the tube while the buffer is still visible

*Lysis*

- Add 3µl of Cell Lysis Mix above the sample
- Tap the PCR tube to allow the Cell Lysis Mix to roll over the sample
- Incubate for 15 min according to the Cell Lysis program

*Whole Genome Amplification and Target Sequence Enrichment*

- Add 22µl of WGA master mix to the lysed sample
- Perform low stringency cycles of the PKI (A) WGA PCR program
- Add the sequence specific primers during the PCR hold step
- Continue with the high stringency cycles of the PKI (A) WGA PCR program



- Assess the WGA products by agarose gel electrophoresis

- Confirm sequence enrichment using sequence specific PCR

# 4 Space, Equipment and Materials to be Supplied by User

**NOTE**: When working with chemicals, always wear a suitable lab coat and disposable gloves. For more information, please consult the appropriate safety data sheets (SDS), available from RHS upon request or other reagent suppliers if the material is not supplied with the DOPlify® kit.

## 4.1 Laboratory Set Up

### Clean Laboratory

Cell lysis and master mix set-up for Whole Genome Amplification (WGA) should both be performed in a contained and dedicated clean laboratory equipped with a laminar flow hood, dedicated pipettes and a PCR thermocycler.

It is imperative to maintain a clean, tidy work space with regular decontamination in order to limit the risk of DNA contamination of the DOPlify® kit reaction.

All DOPlify® kit WGA reagents should be stored in a -20°C freezer located in the clean laboratory.

### General Laboratory

Agarose gel electrophoresis and handling of amplified DNA should be performed in a post-PCR laboratory away from the clean laboratory and any master mix set-up.

## 4.2 Other Laboratory Items

The cell lysis and WGA steps are recommended to be performed in 0.2 or 0.5mL PCR tubes (supplied by user). A cold block at a temperature of approximately 4°C should be used when preparing all master mixes and for storing samples during set up. Sequence specific PCR primers are to be supplied by the user. Note that if primers are to be multiplexed, it is advised that optimisation of the multiplex reaction be performed prior to using them in the DOPlify® kit TSE protocol. It is also advised that the primer sets are validated prior to use in the DOPlify® kit TSE protocol.

## 4.3 Input Specifications

### Number of Cells

The DOPlify® kit is suitable for amplifying the genomes of single cells or their DNA equivalent, as well as small numbers of cells (e.g. <10 cells).

### Cell Collection Method

Flow sorting, dilution and micromanipulation are collection methods that are compatible with the DOPlify® kit. Single cells should be transferred to a PCR tube with minimal transfer buffer (<2µl). The location of the cell in the tube should be marked with a dot on the outside of the tube using a permanent marker pen while the transfer buffer is still visible so as to enable easy cell location for the lysis step.

### DNA Dilution

It is recommended that genomic DNA is diluted to a final concentration of 30pg/µl in 10mM Tris-HCl (pH 8.0) (no EDTA).

### Compatible Buffers

Recommended cell transfer buffers include 10mM Tris-HCl (pH 8.0) (no EDTA) and PBS ($Mg^{2+}$, $Ca^{2+}$ free and BSA free).

# 5 Protocols: Whole Genome Amplification

## 5.1 Cell Lysis

Estimated Time for 4 samples + 1 No Template Control (NTC)

- Hands on: 10 minutes
- Thermocycler: 15 minutes
- Total duration: 25 minutes

In this step, cells are lysed and DNA is made soluble with the addition of a Cell Lysis Mix and a short incubation in a PCR thermocycler.

Consumables:

- 0.2mL or 0.5mL sterile PCR tubes
- DOPlify® kit PCR-grade $H_2O$
- DOPlify® kit Cell Lysis Enzyme
- DOPlify® kit Cell Lysis Buffer
- Cell or DNA samples

Preparation:

- Remove the PCR-grade $H_2O$ and Cell Lysis Buffer from the freezer and thaw to room temperature.
- Remove the Cell Lysis Enzyme from the freezer and store in a cold block at 4°C.
- Mix reagents well then briefly centrifuge to collect contents at the bottom of the tube.
- Calculate volumes of reagents required for the Cell Lysis Mix. Enough Cell Lysis Mix should be prepared for all samples and one NTC plus 1-2 reactions extra to allow for pipetting loss.

Procedure:

**NOTE**: The following steps are to be performed in a **Clean Laboratory**.

1. Prepare **Cell Lysis Enzyme Dilution 1** in a 4°C cold block by combining the following reagents:

| Component | Volume | Cap Colour |
|---|---|---|
| DOPlify® kit PCR-grade $H_2O$ | 6.5 µl | White |
| DOPlify® kit Cell Lysis Enzyme | 1.0 µl | Yellow |
| **Total volume** | **7.5 µl** | |

   Note that this is sufficient Cell Lysis Enzyme Dilution 1 for use in 50 lysis reactions. The Cell Lysis Enzyme Dilution 1 must be made up fresh for each use. There are sufficient reagents in the **DOPlify®** kit for the above dilution to be made 20 times.

2. Mix well then briefly centrifuge.

3. Prepare **Cell Lysis Mix** in a 4°C cold block for the required number of reactions by combining the following reagents:

| Component | Volume for 1 lysis reaction | Recommended minimum volume (6.7x master mix) | Cap colour |
|---|---|---|---|
| DOPlify® kit PCR-grade $H_2O$ | 2.7 µl | 18 µl | White |
| DOPlify® kit Cell Lysis Buffer | 0.15 µl | 1.0 µl | Yellow |
| Cell Lysis Enzyme Dilution 1 | 0.15 µl | 1.0 µl | - |
| **Total volume** | **3.0µl** | **20.0 µl** | |

**Steps for Lysing Single Cells/Multi Cell samples**

a. Add 3µl of Cell Lysis Mix directly above the cell sample location in the PCR tube. Make sure that the Cell Lysis Mix rolls over the sample location by gently tapping the tube on the benchtop. **Do not mix or vortex.**

b. Briefly spin in a mini centrifuge if required to collect contents at the bottom of the tube.

c. Repeat with other samples.

**Steps for NTC Preparation**

a. Add 3 µl of Cell Lysis Mix to a sterile PCR tube labelled NTC.

b. Add 1 µl of PCR-grade $H_2O$ to the tube labelled NTC.

**Recommended:** Lyse cell samples and NTC in a **Clean Laboratory** thermocycler to avoid DNA contamination (according to Step 4 below).

**Steps for DNA Sample Preparation prior to WGA PCR (if required)**

a. Add 3 µl of Cell Lysis Mix to the required number of sterile empty PCR tubes.

**NOTE**: The following steps are to be performed in a **General Laboratory**.

b. Add 1µl of 30pg/µl DNA sample to each tube containing Cell Lysis Mix.

c. Proceed to Step 4.

4. Incubate all samples and NTC in a thermocycler programmed as follows:

Cell Lysis program

| Process | Temperature | Duration | Cycles |
|---|---|---|---|
| Lysis | 75°C | 10 min | 1 |
| Heat inactivation | 95°C | 5 min | |
| | 4°C | Hold | |

5. Place the lysed samples in a cold block.

## 5.2 Whole Genome Amplification and Target Sequence Enrichment

Estimated Time for 4 samples + 1 NTC

- Hands on: 10 minutes
- Thermocycler: 2 hours 20 minutes
- Total duration: 2 hours 30 minutes

In this step a master mix is created and added to the lysed samples before Degenerate Oligonucleotide Prime PCR (DOP-PCR) based Whole Genome Amplification (WGA). This generates representative amplification of total DNA from cells or their DNA equivalent. Between the low and high stringency PCR cycles, sequence specific primers and/or linked marker primers are added to facilitate targeted amplification of regions of interest. This provides Target Sequence Enrichment.

Consumables

- 1.5mL tube
- PCR-grade $H_2O$
- DOPlify® WGA PCR Buffer
- DOPlify® Primer
- DOPlify® WGA Polymerase
- Lysed samples and NTC (from step 5.1 Cell Lysis)
- Sequence-Specific or Linked Marker Primers (user supplied)

Preparation:

- Dilute Sequence-Specific Primers or Linked Marker Primers to the required concentration in user supplied PCR-grade $H_2O$.
    - **See Appendix 1 for detailed instructions on how to optimise the concentration for each set/multiplex of primers for TSE.**
- For multiplexing Sequence-Specific or Linked Marker Primers combine all primers in equal volumes in a single primer pool.
- Remove PCR-grade $H_2O$, WGA PCR Buffer and Primer from storage and thaw to room temperature.
- Remove WGA Polymerase from storage and store in a cold block at 4°C.
- Mix reagents well then briefly centrifuge to collect contents at the bottom of the tube.
- Calculate volumes of reagents required for the WGA master mix. Enough WGA master mix should be prepared for all samples and 1 NTC plus 1-2 reactions extra. Do not add the Sequence-Specific Primers or Linked Marker Primers to this mastermix.

Procedure:

**NOTE**: The following steps are to be performed in a **Clean Laboratory**.

1. Prepare WGA master mix for the required number of reactions by combining the following reagents in a 1.5mL tube in the order they are listed below:

| Component | Volume for 1 WGA reaction | Cap colour |
|---|---|---|
| PCR-grade $H_2O$ | 6.5 µl | White |
| DOPlify® kit WGA PCR Buffer | 12.5 µl | Red |
| DOPlify® kit Primer | 2.5 µl | Red |
| DOPlify® kit WGA Polymerase | 0.5 µl | Red |
| **Total volume** | **22 µl** | |

2. Mix very well then briefly spin down in a mini centrifuge.
3. Transfer 22µl of WGA master mix to the individual tubes containing lysed template (sample or NTC in Cell Lysis Mix). To prevent removal of any DNA from the lysed sample, do not insert the pipette tip into the lysed sample mix. **Do not mix or vortex the PCR tubes.** Briefly centrifuge or spin in a mini centrifuge to collect contents at the bottom of the tube.

NOTE: The following steps are to be performed in a **General Laboratory** for DNA handling

4. (If required for genomic DNA amplification) Transfer 22 µl of WGA master mix to the individual tubes containing DNA template in Cell Lysis Mix. To prevent removal of any DNA from the sample, do not insert the pipette tip into lysed sample mix. Mix well then briefly centrifuge to collect contents at the bottom of the tube.
5. Incubate all samples and NTC in a thermocycler programmed as follows:

**PKI(A) WGA with TSE PCR program:**

| Step | Temperature | Duration | Cycles |
|---|---|---|---|
| Initial denaturation | 95°C | 5 min | 1 |
| Denaturation | 98°C | 20 sec | 8 |
| Annealing | 25°C | 1 min 30 sec | |
| Extension | Ramp to 72°C | 1°C/4 sec | |
| | 72°C | 1 min | |
| Cooling | 4°C | Hold | |
| Add sequence specific primers to samples according to protocol step 6. | | | |
| Continue PCR program | | | |
| Denaturation | 98°C | 20 sec | 21 |
| Annealing | 58°C | 1 min | |
| Extension | 72°C | 1 min | |
| Final extension | 72°C | 1 min | 1 |
| Cooling | 15°C | Hold | |

6. At the HOLD step during the PCR program, transfer 2.8 µl of the sequence specific primer pool to each tube containing the DNA template in the WGA PCR mix, including the NTC. To prevent removal of any sample DNA, do not insert the pipette tip into the sample mix. Instead, pipette the primers onto the side of the PCR tube, just above the sample master mix level.
7. Briefly centrifuge to collect contents at the bottom of the tube.
8. Resume the WGA with TSE PCR program.
9. On completion of the PCR, store the DNA either at 4°C short term or -20°C longer term, or proceed directly to step 4.6 WGA Quality Control.

## 5.3 WGA Quality Control

To confirm amplification of the DNA sample and to check for contamination in the NTC, run an agarose gel.

Consumables:

- Gel Loading Buffer
- DNA Ladder 100-3000+bp (Geneworks DMW-100M)
- Agarose
- 0.5x TBE
- WGA products
- Gel Red (Biotium 41003)

Preparation:

- Create and pour a 1% agarose gel by combining 1g agarose, 100mL 0.5x TBE and 5µL Gel Red. Adjust the volumes to suit the size of your electrophoresis system.

Procedure:

1. Apply 2µl of PCR product in gel loading buffer to the gel.
2. Load DNA Ladder.
3. Electrophorese for ~30 min at 100 volts.

**Steps to determine quality of WGA DNA**

a. The WGA amplification products should appear as a smear, ranging in size from approximately 200 bp - 2000 bp. The NTC should appear clean, with the presence of primer dimers (See Figure 1).

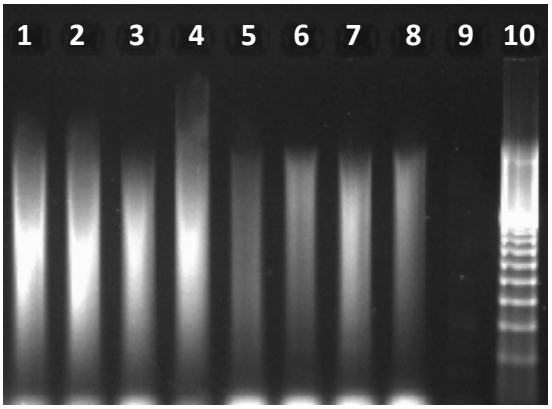



**Figure 1: Target sequence enrichment example**

Lanes 1-4: WGA+TSE using 5-cells

Lanes 5-8: WGA+TSE using single-cells

Lane 9: NTC

Lane 10: DNA Ladder (100bp)

b. TSE amplified samples typically have a slightly lower PCR yield compared to standard DOPlify® kit WGA samples. This is because the sequence specific primers have a small inhibitory effect on the WGA.

c. A **failed** WGA amplification is indicated by the presence of primer dimers, but no evidence of the larger amplification products (see Figure 2, lane 4). Possible causes are that the sample was not successfully transferred to the PCR tube or that the sample was located in the PCR tube above the lysis and PCR reagents. Failed samples should be discarded.

d. **Poor** WGA amplification is indicated by smears with lower intensity or with PCR products that are notably larger or smaller than the expected size range observed for the other samples on the same agarose gel (see Figure 2, lane 6). It is recommended that these samples are discarded but if this is

not possible, the results from these samples should be interpreted with caution because they are more likely to provide false positive results due to the poorer template amplification.

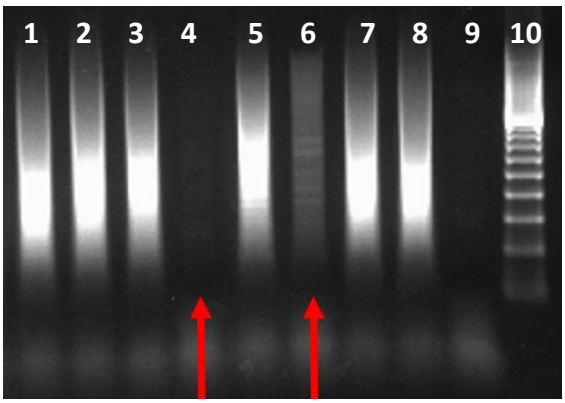



**Figure 2: WGA example**

Lanes 1, 2, 3, 5, 7, 8: Amplified single cells

Lane 4: Failed WGA reaction

Lane 6: Poor WGA reaction

Lane 9: NTC

Lane 10: DNA Ladder (100bp)

**Steps to confirm enrichment of target region of interest**

Depending on the end-point analysis for WGA-TSE samples, if using gene-specific PCR and electrophoretic sizing of PCR products, it may be necessary to dilute the WGA-TSE DNA sample to a concentration lower than the typical recommended input concentration for the PCR. Due to the enrichment of the target sequence in the WGA, using the standard recommended DNA concentration can result in the appearance of additional weakly amplified PCR products that are probably generated from incomplete DNA fragments. To reduce electrophoretic banding of these partial PCR products, titrate the WGA TSE sample concentrations for the end-point assay. Typically this should be performed in the range of 1:20-1:10 of the template standard concentration.

a. Confirm sequence enrichment using semi-quantitative sequence specific PCR (where a low number of PCR cycles are used so that the target band is just visible on a gel) of WGA+TSE compared to WGA only as the PCR template (see Figure 3, lane 2-5 vs lane 6-9). **Do not run the PCR to plateau phase**. Alternate methods can also be used; e.g. a nested PCR directed towards the target regions can be used to confirm that the target sequence enrichment has occurred.

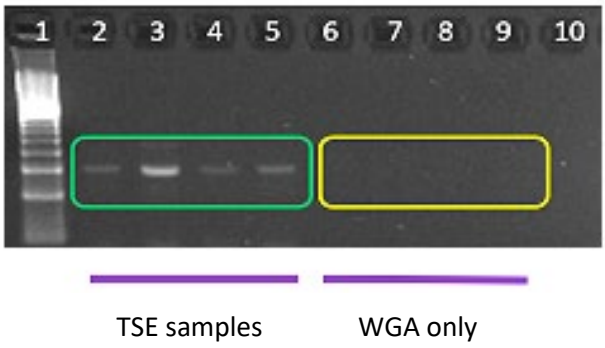



**Figure 3: Sequence-specific PCR reamplification**

Lane 1: DNA ladder (100bp)

Lanes 2, 3, 4, 5: Target sequence enriched samples

Lanes 6, 7, 8, 9: Control WGA samples with no TSE

Lane 10: NTC

b. WGA+TSE DNA and the sequence-specific PCR products generated using it as a template are compatible with a range of methods including next generation sequencing (NGS), PCR, electrophoresis & Sanger sequencing.

c. To increase the depth of coverage of enriched sequence for NGS analysis, the sequence-specific PCR product can be pooled with the WGA+TSE DNA and barcoded/indexed as a single sample. The sequence-specific PCR product or products can be diluted with the DNA in a range of 1:20 to 1:1 depending on the depth of coverage required for NGS analysis. Sequencing can then be performed following the standard PG-Seq™ kit protocol or similar.

# 6 Optimisation of the TSE protocol sequence-specific primer concentration

It is recommended that you optimise the concentration of specific individual or multiplex primers to add to the DOPlify® for the TSE protocol using 30pg gDNA as template prior to using the TSE protocol on test samples. This only needs to be done once and the same concentration can be used for all subsequent TSE amplifications with that primer set. This process needs to be performed for each individual or multiplex primer set.

The following protocol is to be used as a guide for the primer concentration optimisation.

## 6.1 Cell Lysis

1. Label 11 sterile empty PCR tubes with the following labels:

| Tube Label |
|---|
| A-1 |
| B-1 |
| C-2 |
| D-2 |
| E-5 |
| F-5 |
| G-10 |
| H-10 |
| I-0 |
| J-0 |
| K-NTC |

2. Prepare enough Cell Lysis Mix for 12 samples according to the **DOPlify®** kit TSE protocol.
3. Add 3 µl of Cell Lysis Mix to the sterile empty PCR tubes.
4. Add 1 µl of 30 pg gDNA diluted in PBS or PCR-grade $H_2O$ to each tube except the NTC.
5. Add 1 µl of PCR-grade $H_2O$ to the NTC.
6. Incubate all tubes in a thermocycler according to the program: RHS Lysis.

## 6.2 Whole Genome Amplification and Target Sequence Enrichment

1. Dilute all forward and reverse sequence-specific primers and pool all primers in equal volumes if primers are being multiplexed. Note that the concentration of each primer set to add to the multiplex can be further optimised by comparing the amount of sequence-specific product generated in step 8 below. Dilute the primer pool to working stock concentrations of 1µM, 2µM 5µM and 10µM with PCR-grade $H_2O$.
2. Prepare enough WGA mastermix for 12 samples according to the **DOPlify®** kit TSE protocol. Note that the sequence-specific primers are not added to this mastermix.
3. Transfer 22 µl of WGA mastermix to the 11 tubes. There will be WGA mastermix left over that is discarded.
4. Incubate all tubes in a thermocycler according to the program: RHS WGA with TSE (page 10).
5. At the hold step during the PCR program, add 2.8 µl of the corresponding sequence-specific primer dilution to each tube.

| Tube Label | TSE Primer addition |
|---|---|
| A-1 | 2.8uL of 1µM pool |
| B-1 | 2.8uL of 1µM pool |
| C-2 | 2.8uL of 2µM pool |
| D-2 | 2.8uL of 2µM pool |
| E-5 | 2.8uL of 5µM pool |
| F-5 | 2.8uL of 5µM pool |
| G-10 | 2.8uL of 10µM pool |
| H-10 | 2.8uL of 10µM pool |
| I-0 | 2.8uL of PCR-grade $H_2O$ |
| J-0 | 2.8uL of PCR-grade $H_2O$ |
| K-NTC | 2.8uL of PCR-grade $H_2O$ |

6. Resume the PKI(A) WGA with TSE PCR program.
7. To confirm amplification of the DNA sample and determine the optimal concentration of sequence specific primers to add for TSE, run an agarose gel. Compare the yield of the WGA PCR with TSE samples to the control samples with no sequence-specific primers added I-0 and J-0 (Figure 4).

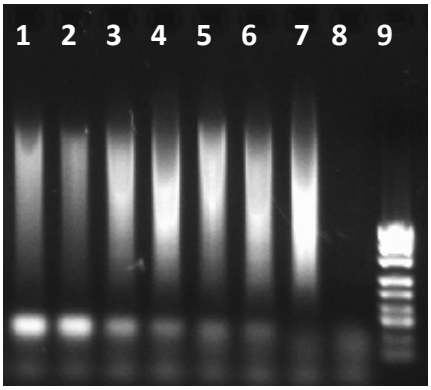



**Figure 4: WGA example with TSE**

Lanes 1, 2: 10 µM

Lanes 3, 4: 5 µM

Lanes 5, 6: 2.5 µM

Lane 7: Control WGA sample with no TSE primers

Lane 8: NTC

Lane 9: DNA ladder

8. To evaluate the enrichment of your target sequence, perform a PCR with the same sequence-specific primers and use DNA from each of the WGA TSE PCRs as the template (tubes A-1, B-1, C-2, D-2, E-5, F-5, G-10 H-10, I-0 and J-0). If necessary, dilute each WGA TSE amplicon sample (approximately 1:20-1:10 as a guide) or use neat as a template for sequence-specific PCR amplification.

9. The optimal concentration of sequence-specific primers will be the highest concentration that has minimal impact on WGA yield and generates detectable PCR product in the second sequence-specific PCR using the WGA TSE amplicons as template (Figure 3).

# 7 Troubleshooting and Technical Support

| Problem | Potential cause | Suggested Solution |
|---|---|---|
| No amplification | Sample was not present in tube | Ensure that method of cell collection consistently results in the sample being accurately transferred to a tube. Position the sample at the bottom of the tube and mark the location with a permanent marker. |
| Low amplification yield | Poor template quality | The DNA in the sample is likely to have been degraded or of poor quality. This could be due to incorrect storage.<br>Poor amplification could also be caused by the sample being placed too high in the tube so that the lysis buffer did not cover the sample and it was not completely lysed. Ensure that the sample is placed at the bottom of the PCR tube when collected and that the sample location is marked on the tube.<br>If the concentration of sequence-specific primers was too high, reduce their concentration in the TSE protocol. This may require a repeat of the primer optimisation steps (Section 6). |
| No template control (NTC) generates PCR product evident on the agarose gel | PCR-grade $H_2O$ used to seed the NTC is contaminated with DNA | Replace PCR-grade $H_2O$. |
| | Work area is contaminated with DNA | Clean work area and equipment thoroughly and use dedicated PCR pipettes and tips. |
| | DOPlify® kit reagents have been contaminated | Discard unused reagents and open a new DOPlify® kit. |

For technical support please contact: **info.au@perkinelmer.com**

# 8 Ordering Information

Please contact PKI(A) or your local distributor for ordering information. For more information on the products use, limitations, and licenses: www.perkinelmer-appliedgenomics.com

The **DOPlify®** kit is a Research Use Only product and is not to be used for diagnostic procedures

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