PG-Seq™ Rapid Kit
Whole Genome Amplification and Barcoding for Ion Torrent® NGS

KIT CONTAINS: 96 RXNS
This product is for research use only. Not for use in diagnostic procedures.

PerkinElmer Health Sciences (Australia) Pty Ltd (PKI(A)) makes no representations and gives no warranties of any kind in relation to the contents of this document and all warranties, conditions and other terms implied by statute or common law are, to the fullest extent permitted by law, hereby excluded. In particular, PKI(A) assumes no responsibility for any errors or omissions that may appear in this document and makes no commitment to update or keep current the information contained in this document.

PKI(A) retains the right to make changes to this document (including any specifications contained herein) at any time without notice.

This document is confidential. No part of this document may be modified, copied, reproduced, republished, published, transmitted or distributed in any form or by any means without the prior written consent of PKI(A). The contents of this document are to be used solely for the purpose for which they are provided by PKI(A) and for no other purpose. All content, text, graphics and all other materials contained in this document are owned by PKI(A), and all proprietary and intellectual property (names, logos and trademarks) wherever arising in relation to this document, rest in PKI(A) (or its licensors) and all such rights are reserved.

The PG-Seq™ Rapid Kit is for Research Use Only and should not be used in diagnostic procedures. You are responsible for ensuring that you accurately follow the protocols provided in this Technical Data Sheet (user guide) and analysing and interpreting the results you obtain. PKI(A) does not guarantee any results obtained.
# PG-Seq™ Rapid Kit

## General Information

<table>
<thead>
<tr>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Product Overview</td>
<td>4</td>
</tr>
<tr>
<td>Kit Overview</td>
<td>5</td>
</tr>
<tr>
<td>Kit Contents and Storage</td>
<td>5</td>
</tr>
<tr>
<td>Revision History</td>
<td>5</td>
</tr>
<tr>
<td>Required Materials Not Provided</td>
<td>6</td>
</tr>
<tr>
<td>Warnings &amp; Precautions</td>
<td>6</td>
</tr>
</tbody>
</table>

## Sample Prep Setup

<table>
<thead>
<tr>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starting Materials</td>
<td>7</td>
</tr>
</tbody>
</table>

## Protocol | PG-Seq™ Rapid Kit

<table>
<thead>
<tr>
<th>Step</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1: Cell Lysis</td>
<td>8</td>
</tr>
<tr>
<td>1.2: Round 1 PCR</td>
<td>10</td>
</tr>
<tr>
<td>1.3: Round 2 PCR</td>
<td>12</td>
</tr>
<tr>
<td>1.4: Sample Purification</td>
<td>14</td>
</tr>
<tr>
<td>1.5: Sample Pooling</td>
<td>16</td>
</tr>
<tr>
<td>2.0: Setting Up the Run Software</td>
<td>17</td>
</tr>
<tr>
<td>2.1: Library Templating and Sequencing</td>
<td>18</td>
</tr>
<tr>
<td>2.2: Data Analysis</td>
<td>19</td>
</tr>
</tbody>
</table>

## Appendix A

<table>
<thead>
<tr>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Genome Amplification Quality Control</td>
<td>20</td>
</tr>
</tbody>
</table>

## Appendix B

<table>
<thead>
<tr>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sequencing Quality Control</td>
<td>23</td>
</tr>
</tbody>
</table>

## Appendix C

<table>
<thead>
<tr>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analysis Quality Control</td>
<td>24</td>
</tr>
</tbody>
</table>

## Appendix D

<table>
<thead>
<tr>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermal Cycler Ramp Rates</td>
<td>25</td>
</tr>
</tbody>
</table>
///// Product Overview

The PG-Seq™ Rapid Kit has been developed to analyze picogram quantities of DNA (single/multi-cells or low template DNA) from an embryo biopsy for preimplantation genetic testing. The kit utilizes whole genome amplification (WGA) and next generation sequencing (NGS) technology to accurately screen all 24 chromosomes for whole chromosome aneuploidy and sub-chromosomal abnormalities. From DNA to data, the kit includes all reagents required for whole genome amplification, barcoding plus PG-Find™ analysis software for automatic calling of aneuploidy and copy number variants. The kit is for Research Use Only and should not be used in diagnostic procedures.

The kit follows a single tube workflow, utilizing ready-mixes to minimize the pipetting steps needed and requires only three sample tube openings, reducing the risk of sample contamination. In the first step, a gentle but effective enzyme-based lysis procedure ensures robust cell lysis and a readily accessible DNA template for WGA. In the second step, a degenerate primer anneals at regular intervals across the whole genome, assisted by low stringency annealing conditions, and primer extension generates an array of fragments ready for amplification. Following this, the generated fragments are PCR amplified using high stringency annealing conditions to increase the yield available for barcoding. In the third step, primers containing a unique barcode sequence to allow multiplexing, plus the sequences necessary for Ion Torrent® instrument templating and sequencing anneal to each end of the amplified fragments, generating Ion Torrent® instrument templating and sequencing ready samples.

STEP 1: CELL LYSIS

STEP 2: WHOLE GENOME AMPLIFICATION

STEP 2a: Low Stringency Conditions

STEP 2b: High Stringency Conditions

STEP 3: PCR Barcoding
### Kit Overview

The PG-Seq™ Rapid Kit comes as a 96-reaction kit up to 96 sample throughput. The kit contains a 96-well plate containing 96 uniquely barcoded primers, allowing 96 sample throughput if required. Each well contains sufficient volume for single use in column format.

### Kit Contents and Storage

<table>
<thead>
<tr>
<th>Kit Contents</th>
<th>Cap Color</th>
<th>Storage Temp.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PG-Seq™ Rapid Kit WGA Reagents (4327-0010-P1)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCR-grade H$_2$O</td>
<td>WHITE CAP</td>
<td>-20°C</td>
</tr>
<tr>
<td>Cell Lysis Enzyme</td>
<td>YELLOW CAP</td>
<td>-20°C</td>
</tr>
<tr>
<td>Cell Lysis Buffer</td>
<td>GREEN CAP</td>
<td>-20°C</td>
</tr>
<tr>
<td>WGA Polymerase</td>
<td>RED CAP</td>
<td>-20°C</td>
</tr>
<tr>
<td>WGA PCR Buffer</td>
<td>ORANGE CAP</td>
<td>-20°C</td>
</tr>
<tr>
<td>Primer</td>
<td>BLUE CAP</td>
<td>-20°C</td>
</tr>
<tr>
<td><strong>PG-Seq™ Rapid Kit Barcoded Primers (4327-0010-P2)</strong></td>
<td>96-well Plate</td>
<td>-20°C</td>
</tr>
<tr>
<td><strong>PG-Seq™ Rapid Kit Additional Reagents (4327-0010-P3)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purification Beads</td>
<td>WHITE CAP BOTTLE</td>
<td>2-8°C</td>
</tr>
<tr>
<td>Resuspension Buffer</td>
<td>WHITE CAP BOTTLE</td>
<td>Room Temp.</td>
</tr>
</tbody>
</table>

The PG-Seq™ Rapid Kit contains a 96-well primer plate containing 96 uniquely barcoded primers. Each well contains sufficient volume for single use in column format. Avoid repeated freezing and thawing of the plate. An excel spreadsheet containing the sequences of the PG-Seq™ Rapid Kit Barcoded Primers can be accessed from the following website: perkinelmer-appliedgenomics.com/home/preimplantation-genetic-testing/ Contact support.au@perkinelmer.com for further assistance.

### Revision History

<table>
<thead>
<tr>
<th>Version</th>
<th>Date</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>v1.0</td>
<td>September 2019</td>
<td>Product Launch</td>
</tr>
</tbody>
</table>
Warnings & Precautions

We strongly recommend that you read the following warnings and precautions. Contact your local distributor or support.au@perkinelmer.com for further assistance.

- Do not use the kit past the expiration date.
- The kit is designed to amplify picograms quantities of DNA; therefore, extreme caution must be exercised to prevent the introduction of foreign DNA contaminants.
- Ensure pipettes are calibrated and there is a dedicated set for Cell Lysis and Round 1 and 2 PCR setup in a Clean (Pre-PCR) laboratory.
- Regularly clean pipettes and work surfaces.
- Use aerosol barrier pipette tips.
- Storage of PCR Reagents, Cell Lysis and master mix setup for the Round 1 PCR and Round 2 PCR should be performed in a Clean (Pre-PCR) laboratory.
- Unless otherwise specified, mix well and briefly centrifuge all reagents before use.
- Unless otherwise specified, prepare all master mixes and store all samples in a cold block or ice.
- Take special care to mix all enzyme solutions thoroughly. Do not vortex. Mix by pipetting up and down at least 15-20 times or by inverting and flick mixing the tube.
- Take special care to thaw the WGA PCR Buffer to room temperature and mix thoroughly before use.
- Gently peel off plate seals to prevent splashing.
- Re-seal plates and used wells, even if they appear empty using a clean plate seal.
- Never mix the Barcoded Primer plate using a vortex.
- Avoid repeated freezing and thawing of the Barcoded Primer plate.
- Do not freeze Purification Beads. Beads should be stored at 2-8 °C.
- Allow Purification Beads to come to room temperature and vortex the beads until the liquid appears homogeneous before every use.

Required Materials Not Provided

Pre-PCR Laboratory requirements
- Laminar flow cabinet
- Minicentrifuge
- Pipettes (2, 10, 20, 100, 200, 1000 µL)
- Cold block (4°C)
- Thermal cycler (with hotlid & programmable ramp rate to 0.2°C/sec)
- Pipette tips (low binding, barrier filter)
- PCR thin walled reaction tube with flat cap (0.5mL or 0.2mL)
- Molecular grade tubes (1.5mL)
- 96-well plate centrifuge
- Adhesive 96-well plate seals
- Vortex

Post-PCR Laboratory requirements
- Magnetic stand for 96-well plates
- 96-well plate, to suit Magnetic stand
- Adhesive 96-well plate seals
- Absolute ethyl alcohol (EtOH, unedenatured) to make 80% ethanol
- Molecular grade water
- Ice

LabChip® GXII Touch™ Nucleic Acid Analyzer and associated reagent kit (PerkinElmer)
Qubit® Fluorometer and associated reagent kit (Thermo Fisher Scientific)
Agarose gel-electrophoresis apparatus
Electrophoresis power supply
UV transilluminator or gel documentation instrument
Multi-channel pipette
Multi-channel pipette reagent reservoirs
Ion Torrent® Sequencer (PGM® Instrument, S5® Instrument or Proton Instrument) (Thermo Fisher Scientific)
Ion Torrent® Sequencer related consumables (Thermo Fisher Scientific)
Ion Torrent® Sequencer reagents and solutions (Thermo Fisher Scientific)
Ion Torrent® Sequencer Chip Kits (Thermo Fisher Scientific)
- Ion 316-318™ Chip Kits
- Ion 510-530™ Chip Kits
- Ion P1™ Chip Kit

Ion T orrent® Sequencer (PGM® Instrument, S5® Instrument or Proton Instrument) (Thermo Fisher Scientific)
Ion T orrent® Sequencer related consumables (Thermo Fisher Scientific)
Ion T orrent® Sequencer reagents and solutions (Thermo Fisher Scientific)
Ion 316-318™ Chip Kits
Ion 510-530™ Chip Kits
Ion P1™ Chip Kit
///// Starting Materials

Blastomere or trophectoderm embryo biopsy samples should be transferred to the bottom of a PCR clean, sterile 0.2 mL or 0.5 mL PCR tube with minimal transfer buffer (<2 µL). Recommended cell transfer buffers include 10 mM Tris-HCl (pH 8.0) (no EDTA) or PBS (Mg²⁺, Ca²⁺ free and BSA free). PBS with a maximum concentration of 0.1% PVA can be used if required. The location of the cell in the tube should be marked on the outside of a tube using a permanent marker to enable the cells to be located for cell lysis. After cell to tube transfer, the embryo biopsy sample should be immediately frozen and if required, shipped with dry ice. It is recommended that sample collection, storage and shipping conditions are validated before use.

If required, DNA samples can be used in Whole Genome Amplification and should be diluted to a final concentration of 30 pg/µL in 10mM Tris-HCl (pH 8.0) (no EDTA). 1 µL of freshly diluted DNA can be added to the Cell Lysis step. To avoid contamination, ensure DNA samples or diluted DNA is not brought into the Clean (Pre-PCR) Laboratory.

///// Workflow

SAMPLE PREP SETUP

Add cell/template DNA       Add cell Lysis Mix       Add Round 1 WGA Master Mix       Add Barcoded Primers       Add Round 2 WGA Master Mix

15 minute incubation       1 hour 20 minute PCR

Bead Purification       Pool
1.1: Cell Lysis

MATERIALS

- **WHITE CAP** - PCR-grade Water
  (Thaw to room temperature. Briefly centrifuge.)
- **YELLOW CAP** - Cell Lysis Enzyme
  (Place in a cold block. Mix well then briefly centrifuge. Do not vortex.)
- **GREEN CAP** - Cell Lysis Buffer
  (Thaw to room temperature. Mix well then briefly centrifuge.)

User Supplied

- Cell or DNA samples
- Thermocycler
- Minicentrifuge
- Cold Block (4°C)
- 0.2mL or 0.5mL PCR Tubes

Procedure

**NOTE:** The following steps are to be performed in a Clean (Pre-PCR) Laboratory.

1. Prepare Cell Lysis Enzyme Dilution 1 by combining the following reagents:

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR-grade Water</td>
<td>6.5 µL</td>
</tr>
<tr>
<td>Cell Lysis Enzyme</td>
<td>1.0 µL</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>7.5 µL</td>
</tr>
</tbody>
</table>

**NOTE:** The Cell Lysis Enzyme Dilution 1 should be prepared fresh each use.

2. Mix well then briefly centrifuge.

3. Calculate the volumes of reagents required to prepare Cell Lysis Mix for all samples, one no template control (NTC) plus 10% extra to allow for loss during pipetting. It is recommended that the Cell Lysis Mix is prepared for a minimum of 10 samples to prevent pipetting low volumes.

4. Prepare Cell Lysis Mix for the required number of reactions by combining the following reagents:

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume for 1x Cell Lysis Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR-grade Water</td>
<td>2.7 µL</td>
</tr>
<tr>
<td>Cell Lysis Buffer</td>
<td>0.15 µL</td>
</tr>
<tr>
<td>Cell Lysis Enzyme Dilution 1</td>
<td>0.15 µL</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>3.0 µL</td>
</tr>
</tbody>
</table>

5. Mix well then briefly centrifuge.
6. Prepare Samples as described below:

Cell Sample Preparation:

- Add 3 µL of Cell Lysis Mix above the cell sample located in a PCR tube. Make sure that the Cell Lysis Mix rolls over the sample location as marked on the tube by gently tapping the tube on the benchtop.

NOTE: Do not touch the cell location with the pipette tip. Do not mix or vortex.
- Briefly centrifuge if required to collect contents at the bottom of the tube.
- Repeat with other samples.

Genomic DNA / Positive Control Preparation:

NOTE: Steps involving genomic DNA are to be performed in a General (Post-PCR) Laboratory.

- Add 3 µL of Cell Lysis Mix to the required number of sterile empty PCR tubes.
- Add 1 µL of 30 pg/µL DNA sample to each tube containing Cell Lysis Mix. DNA should be freshly diluted to 30 pg/µL.
- Briefly centrifuge.

NTC Preparation (recommended):

- Add 3 µL of Cell Lysis Mix to one sterile PCR tube labelled NTC.
- Add 1 µL of PCR-grade H₂O to the tube labelled NTC.
- Briefly centrifuge.

7. Incubate all samples and NTC in a thermal cycler programmed as follows:

```
<table>
<thead>
<tr>
<th>Cycling stage</th>
<th>Temperature</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 cycle</td>
<td>95°C</td>
<td>5 min</td>
</tr>
<tr>
<td>Hold</td>
<td>≤15°C</td>
<td></td>
</tr>
<tr>
<td>75°C</td>
<td>10 min</td>
<td></td>
</tr>
</tbody>
</table>
```

Cycling time is approximately 15 minutes

8. Place the lysed samples in a cold block.

9. Proceed immediately with Round 1 PCR.
1.2: Round 1 PCR

MATERIALS

- **WHITE CAP** - PCR-grade Water
  (Thaw to room temperature. Briefly centrifuge.)

- **ORANGE CAP** - WGA PCR Buffer
  (Thaw to room temperature. Mix well then briefly centrifuge.)

- **BLUE CAP** - Round 1 Primer
  (Thaw to room temperature. Mix well then briefly centrifuge.)

- **RED CAP** - WGA Polymerase
  (Place in a cold block. Mix well then briefly centrifuge. Do not vortex.)

User Supplied

- Lysed samples and NTC (from Step 1.1)
- Thermocycler
- Minicentrifuge
- Cold block (4°C)
- 1.5 mL molecular grade tube

Procedure

**NOTE:** The following steps are to be performed in a **Clean (Pre-PCR) Laboratory.**

1. Calculate the volumes of reagents required to prepare the Round 1 PCR Master Mix for all samples, one NTC plus 10% extra volume to allow for loss during pipetting.
2. Prepare Round 1 PCR Master Mix for the required number of reactions by combining the following reagents in a 1.5 mL molecular grade tube in the order they are listed below:

<table>
<thead>
<tr>
<th>Volume for 1x Round 1 PCR Reaction</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.95 µL</td>
<td>PCR-grade Water</td>
</tr>
<tr>
<td>7.5 µL</td>
<td>WGA PCR Buffer</td>
</tr>
<tr>
<td>2.25 µL</td>
<td>Round 1 Primer</td>
</tr>
<tr>
<td>0.3 µL</td>
<td>WGA Polymerase</td>
</tr>
<tr>
<td>12 µL</td>
<td>TOTAL</td>
</tr>
</tbody>
</table>

3. Mix very well then briefly centrifuge.
4. Transfer 12 µL of WGA PCR Master Mix to the individual tubes containing lysed template (sample or NTC in Cell Lysis Mix).

**NOTE:** To prevent removal of any DNA from the sample, do not insert the pipette tip into lysed sample mix.

**NOTE:** Do not mix or vortex the PCR tubes.

5. Briefly centrifuge.
6. Incubate samples and NTC in a thermocycler programmed as follows:

<table>
<thead>
<tr>
<th>Step</th>
<th>Time</th>
<th>Temperature</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Denaturation</td>
<td>3 min</td>
<td>95°C</td>
<td>1 cycle</td>
</tr>
<tr>
<td>Denaturation</td>
<td>20 sec</td>
<td>98°C</td>
<td></td>
</tr>
<tr>
<td>Annealing</td>
<td>1 min</td>
<td>25°C</td>
<td></td>
</tr>
<tr>
<td>Extension</td>
<td></td>
<td>0.2°C/sec</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ramp to 50°C</td>
<td>8 cycles</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1°C/sec</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ramp to 72°C</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>30 sec</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>72°C</td>
<td></td>
</tr>
<tr>
<td>Denaturation</td>
<td>20 sec</td>
<td>98°C</td>
<td>16 cycles</td>
</tr>
<tr>
<td>Annealing</td>
<td>1 min</td>
<td>58°C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30 sec</td>
<td>72°C</td>
<td></td>
</tr>
<tr>
<td>Cooling</td>
<td>Hold</td>
<td>≤15°C</td>
<td></td>
</tr>
</tbody>
</table>

Ramping from 25°C to 50°C is approximately 2 minutes and ramping from 50°C to 72°C is approximately 20 seconds. See Appendix D for setup instructions.

Total cycling time is approximately 1 hour, 28 minutes.

7. Place the Round 1 PCR samples in a cold block and proceed with Round 2 PCR.

8. If preferable, transfer samples into a 96-well PCR plate for the following steps.

**NOTE:** Round 1 PCR product can be stored for up to 1 week at 4°C before proceeding to Round 2 PCR.
1.3: Round 2 PCR

MATERIALS

- **WHITE CAP** - PCR-grade Water
  (Thaw to room temperature. Briefly centrifuge.)
- **ORANGE CAP** - WGA PCR Buffer
  (Thaw to room temperature. Mix well then briefly centrifuge.)
- **RED CAP** - WGA Polymerase
  (Place in a cold block. Mix well then briefly centrifuge. Do not vortex.)
- 96-well Plate - Barcoded Primer
  (Thaw then centrifuge at 280 x g for 1 minute before removing the plate seal.)

User Supplied

- Round 1 samples and NTC (from Step 1.2)
- Thermocycler
- Cold block (4°C)
- 1.5 mL molecular grade tube

Procedure

**NOTE:** The Round 2 Master Mix should be prepared in a **Clean (Pre-PCR) Laboratory.**

1. Calculate the volumes of reagents required to prepare the Round 2 PCR Master Mix for all samples, one NTC plus 10% extra.

2. Prepare Round 2 PCR Master Mix for the required number of reactions by combining the following reagents in a 1.5 mL molecular grade tube in the order they are listed below:

<table>
<thead>
<tr>
<th>Volume for 1x Round 2 PCR Reaction</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.4 µL</td>
<td>PCR-grade Water</td>
</tr>
<tr>
<td>22.5 µL</td>
<td>WGA PCR Buffer</td>
</tr>
<tr>
<td>0.6 µL</td>
<td>WGA Polymerase</td>
</tr>
<tr>
<td>25.5 µL</td>
<td>TOTAL</td>
</tr>
</tbody>
</table>

3. Mix well then briefly centrifuge.

**NOTE:** The Round 2 Master Mix should be added to samples in a **General (Post-PCR) Laboratory.**

4. Transfer 4.5 µL of an appropriate Barcoded Primer from the 96-well Plate to individual samples from step 1.2. Record the corresponding barcode used for each sample to ensure correct identification during downstream processing.

5. Transfer 25.5 µL of Round 2 PCR Master Mix to each sample tube then briefly centrifuge.
6. Incubate samples and NTC in a thermocycler programmed as follows:

<table>
<thead>
<tr>
<th>Step</th>
<th>Time</th>
<th>Temperature</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Denaturation</td>
<td>3 min</td>
<td>95°C</td>
<td>1 cycle</td>
</tr>
<tr>
<td>Denaturation</td>
<td>20 sec</td>
<td>98°C</td>
<td></td>
</tr>
<tr>
<td>Annealing</td>
<td>30 sec</td>
<td>50°C</td>
<td>4 cycles</td>
</tr>
<tr>
<td>Extension</td>
<td>30 sec</td>
<td>72°C</td>
<td></td>
</tr>
<tr>
<td>Denaturation</td>
<td>20 sec</td>
<td>98°C</td>
<td></td>
</tr>
<tr>
<td>Annealing</td>
<td>30 sec</td>
<td>58°C</td>
<td>6 cycles</td>
</tr>
<tr>
<td>Extension</td>
<td>30 sec</td>
<td>72°C</td>
<td></td>
</tr>
<tr>
<td>Cooling</td>
<td>Hold</td>
<td>≤15°C</td>
<td></td>
</tr>
</tbody>
</table>

Total cycling time is approximately 24 minutes.

(Optional QC) It is recommended that users analyze samples using agarose gel electrophoresis. See Appendix A for quality control information.
1.4: Sample Purification

**NOTE:** For high throughput runs, sample purification can be automated. Contact support.au@perkinelmer.com for further assistance.

**MATERIALS**

- CLEAR CAP TUB - Purification Beads (Allow Purification beads to reach room temperature. Vortex the Purification Beads for 30 seconds to ensure homogeneous resuspension.)
- CLEAR CAP TUB - Resuspension Buffer

**User Supplied**

- Sample WGA DNA (from Step 1.3)
- Absolute ethyl alcohol (EtOH, undenatured)
- Molecular grade water
- 96-well plate
- Adhesive plate seals
- Reagent reservoirs
- Magnetic stand

**Procedure**

**NOTE:** Always prepare a fresh solution of 80% ethanol using absolute ethyl alcohol (EtOH, undenatured) and molecular grade water. For example, prepare 25 mL 80% ethanol to purify up to 48 samples.

1. Add 30 µL of each amplified and barcoded sample to a 96-well plate.
2. Add 27 µL of Purification Beads to each sample.
3. Mix thoroughly by vortexing and/or pipetting up and down at least 15-20 times.
4. Incubate the plate at room temperature for 5 minutes.
5. Place the plate on a magnetic stand to capture the beads, incubate for 2-5 minutes or until the liquid is clear.

**NOTE:** Keep the plate on the stand for steps 6-13.

6. Using a multichannel pipette, remove and discard (or store until the success of the purification has been confirmed by quantification) all supernatant from each well of the plate.
7. Add 200 µL of 80% ethanol to each sample well, do not resuspend the beads.

**NOTE:** Do not incubate the bead pellet with 80% ethanol for extended periods.

8. Incubate the plate at room temperature for 30 seconds.
9. Carefully remove the ethanol using a multichannel pipette.
10. Repeat the wash by adding 200 µL of 80% ethanol to each sample well, do not resuspend the beads.
11. Incubate the plate at room temperature for 30 seconds.
12. Using a multichannel pipette carefully remove the ethanol solution. Remove all residual ethanol without disturbing the beads.
13. Air dry the beads at room temperature for 3-5 minutes or until the beads are completely dry.
14. Remove the plate from the magnetic stand.
15. Add 50 µL of Resuspension Buffer to each well
16. Resuspend the beads by gentle vortexing and/fullstop or pipetting up and down at least 15-20 times.
17. Incubate at room temperature for 2 minutes.
18. Place the plate on a magnetic stand to capture the beads, incubate for 2 minutes or until the liquid is clear.
19. Transfer 45 µL of each supernatant to the wells of a new 96-well plate, avoid carry over of any Purification Beads.
20. Quantify the concentration of each purified sample by Qubit® dsDNA HS Assay (Life Technologies).
1.5: Sample Pooling

**IMPORTANT:** Due to variations in laboratory quantification analysis techniques, it is vital that the final pooling concentration is optimized.

**User Supplied**
- Purified samples (from step 1.4)
- Molecular grade water
- 1.5 mL molecular grade tube

**Preparation**
- Download and open the PG-Seq™ Rapid Kit 4 nM Pool Calculator from the following website: [perkinelmer-appliedgenomics.com/pgt](http://perkinelmer-appliedgenomics.com/pgt).
  Contact support.au@perkinelmer.com for further assistance.

**Procedure**
1. Enter the sample concentration (determined in Step 1.4 - 20) into the “Concentration (ng/µL)” column.
2. From the PG-Seq™ 2.0 Kit 4 nM Pool Calculator record the volumes for each sample and water to add to the final pool.
3. Add the specified volume of molecular grade water to a 1.5 mL molecular grade tube.
4. Add the specified volume of each sample to the same 1.5 mL molecular grade tube.
5. Mix well then briefly centrifuge.
6. Quantify the concentration of the final pool by Qubit® dsDNA HS Assay.
   **NOTE:** Quantifying samples in duplicate using a sample volume of 10 µL is recommended.
7. Quantify the average fragment size of the final pool by LabChip® GXII Touch™ DNA Assay.
   **NOTE:** Typically 2 µL of pool is loaded in a 20 µL final volume made up with buffer buffer (DNA NGS 3K Assay Kit). For detailed instructions refer to the LabChip® GXII Touch™ user guide. Contact support.au@perkinelmer.com for further assistance.
8. Determine the nM concentration of the final pool using the “final pool concentration calc” tab of the PG-Seq™ Rapid Kit 4 nM Pool Calculator.
   a. If the final pool is < 0.75 nM, the pooling calculations should be checked, and the pool needs to be remade.
   b. If the final pool is ≤ 1.1 nM, then proceed to step 2.0.
   c. If the final pool is > 1.1 nM, adjust to 4 nM by adding additional molecular grade water (see example box) before proceeding to step 2.0. Requantify the diluted final pool before proceeding to step 2.0.

**EXAMPLE:**
Using the PG-Seq™ Rapid Kit for Ion Torrent Pool Calculator, it was determined that the final pool concentration was 1.3 nM. The final pool was adjusted to 1 nM by diluting by a factor of \( \frac{1.3}{1} = 1.3 \). The pool was in 600 µL, so \( 600 \times 1.3 = 180 \) µL molecular grade water was added.
2.0: Setting Up the Run Software

**NOTE:** The PG-Seq™ Rapid Kit for Ion Torrent NGS has been validated using an Ion Chef™ instrument for templating and an Ion SS™ instrument for sequencing. We recommend using these instruments, and the following instructions are presented for this specific setup. If Ion Torrent instruments other than these are used, such as the Ion OneTouch™ instrument for templating, or the Ion Proton™ or Ion PGM™ instruments for sequencing, then for instructions please refer to the user guide of the instruments used. Contact support.au@perkinelmer.com for further assistance.

Add a Barcode Set

**NOTE:** If the barcode set has already been added to the Torrent Suite™ Software then the following steps can be skipped.

1. Download the PG-Seq™ Rapid Barcode Set from the following link: perkinelmer-appliedgenomics.com/pgt and save on your local storage.
2. Sign in to the Torrent Server in Torrent Suite™ Software.
3. Click “Settings” > “References”, then click “Barcode Sets” in the left navigation menu.
4. In the “Barcode Sets” screen, click “Add new DNA Barcodes”.
5. In the “Add New DNA Barcodes” dialog box, in “Barcode Set Name”, enter the name “PG-Seq™ Rapid”.
6. Click “Choose File”, select the downloaded PG-Seq™ Rapid Barcode Set CSV file from your local storage, then click “Open”.
7. Click “Upload File”.

Load a Planned Run Template

**NOTE:** If the template has already been added to the Torrent Suite™ Software then the following steps can be skipped.

1. Download the PG-Seq™ Rapid - Ion SS System template from the following link: perkinelmer-appliedgenomics.com/pgt and save on your local storage.
2. Sign in to the Torrent Server in Torrent Suite™ Software.
3. Click the “Plan” tab, then click “Upload” on the right side of the screen and select “Upload Template” from the dropdown.
4. Click “Choose File”, select the downloaded PG-Seq™ Rapid - Ion SS System CSV file from your local storage, then click “Open”.
5. Click “Load”.

Create a Planned Run

**NOTE:** Contact support.au@perkinelmer.com for further assistance.

1. **(optional) Download and set up the PG-Seq™ Rapid Samples Table Template from the following link:** perkinelmer-appliedgenomics.com/pgt and save on your local storage.

Complete the following information and save:

- Barcode
- Sample Name
2.1: Library Templating and Sequencing

User Supplied

- 1 nM pool (from step 1.5)
- Ion Chef™ S5 Series Chip Balance
- Ion 510™, Ion 520™, or Ion 530™ chip
- Molecular grade water
- 1.5 mL molecular grade tube
- Ice

Procedure

1. Pipet 4 µL of the 1 nM pooled library into the Library Sample Tube (barcoded tube) from Position A of the Reagents cartridge. Add 46 µL Nuclease-free Water to give a concentration of 80 pM. Pipet up and down 5 times to mix.

2. Cap the Library Sample Tube and store on ice until you are ready to load the tube into the Reagents cartridge and the Ion Chef™ Instrument.

3. Load the Ion Chef™ Instrument, start the Ion Chef™ run and unload the chip for sequencing following the instructions in the Ion 510™ & Ion 520™ & Ion 530™ Kit – Chef user guide (Thermo Fisher Publication MAN0016854).

4. Initialize the sequencer and start the sequencing run following the instructions in the Ion S5™ and Ion S5™ XL Instrument user guide (Thermo Fisher Publication MAN0010811).

5. Please refer to Appendix B in this user guide for quality metrics to aid assessment of the sequencing run using the Run Report.
2.2: Data Analysis

**NOTE:** If using the provided template, by default, the Torrent Suite™ Software will align all reads to the reference. To download the aligned reads (BAM) files:

1. In the “Data” tab, click “Completed Runs & Reports”.
2. In the list of runs, find the run of interest, then click the link in the “Report Name” column.
3. In the left navigation menu, click “Output Files” or scroll to the “Output Files” section, then select BAM as the file type to download.

Contact support.au@perkinelmer.com for further assistance.

User Supplied

- Sample BAM files.

Procedure

1. The PG-Find™ software setup application can be downloaded from the following link http://installers.biodiscovery.com/PG-Find/Release%201.1/Windows64/VM/PG-Find.exe.
2. To install the PG-Find™ software, run the setup application and follow the prompts.
3. After installation, the software requires activation using an activation key.
4. To obtain the activation key, open the PG-Find™ software and click “Request activation key via email”.
5. Complete the Activation request data form and click “Launch Mail Client”.
6. This should open your email client with a pre-filled request (if an email client is not launched, then click “Copy to Clipboard” and paste the pre-filled request in an email). Send the request email to support.au@perkinelmer.com.

**NOTE:** Please allow 1-2 days for the supply of the activation key.

7. Please refer to the PG-Find™ software user guide for detailed information and instructions for data analysis.
8. Please refer to Appendix C in this user guide for quality metrics to aid quality assessment of individual sample sequence data in PG-Find™.
APPENDIX A

///// Whole Genome Amplification Quality Control

Electrophoresis

The PG-Seq™ Rapid Kit WGA DNA products should appear as a smear, ranging in size from approximately 200 bp - 700 bp with a mean fragment size of approximately 360 bp. The NTC should appear clean, with the presence of primer dimers (Figures 1 and 2). After sample purification, the fragment size should remain consistent with no primer dimers present (Figure 3).

Figure 1: Agarose gel electropherogram of unpurified PG-Seq™ Rapid Kit WGA DNA products of 5-cell aliquots from a fibroblast cell culture. Samples were run on a 0.5x TBE 1% agarose gel at 100 V for 30 minutes. Lane 1: DNA Marker (DMW-P1, Geneworks), Lane 2: No Template Control, Lanes 3-14: WGA DNA products.

Figure 2: LabChip® electropherogram of an unpurified PG-Seq™ Rapid Kit WGA sample with expected amplification. The sample was diluted to 1 ng/µL before being analyzed using the LabChip 3K NGS Reagent Kit.
Concentration dsDNA ng/µL

Typical yield of the PG-Seq™ Rapid Kit amplified WGA DNA products post purification is 20-40 ng/µL using Qubit fluorometric DNA quantification. Samples with poor quality, degraded template or containing PCR inhibitors are likely to generate a reduced yield.

Troubleshooting

A failed WGA amplification is indicated by the presence of primer dimers, but no evidence of a smear of amplification products (Figure 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 Cell Lysis and Round 1 PCR reagents. Failed samples should be discarded.

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 (Figure 5). The results from these samples should be interpreted with caution and it is recommended that these samples are removed from further analysis.
<table>
<thead>
<tr>
<th>Problem</th>
<th>Potential Cause</th>
<th>Suggested Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>No template control (NTC) generates PCR products evident on the agarose gel</td>
<td>PCR-grade H₂O used to seed the NTC is contaminated with DNA</td>
<td>Replace PCR-grade H₂O</td>
</tr>
<tr>
<td>Work area is contaminated with DNA</td>
<td>Kit reagents have been contaminated</td>
<td>Clean work area thoroughly and use dedicated PCR pipettes and tips</td>
</tr>
</tbody>
</table>

**Figure 5: Agarose gel electropherogram of PG-Seq™ Rapid Kit WGA DNA products.** Samples were run on a 0.5x TBE 1% agarose gel at 100 V for 30 minutes. Lane 1: DNA Marker (DMW-P1, Geneworks), Lanes 2-5: WGA DNA products, Lane 6 shows a weak WGA reaction.
/// Sequencing Quality Control

There are several sequencing metrics which can influence the overall sequencing run and sample quality. The values in the table below are presented as a guide for runs with templating on the Ion Chef™ instrument and sequencing on the Ion S5™ instrument; the values for these metrics can be found in the “Run Report” under “Run Summary”.

<table>
<thead>
<tr>
<th>Metric</th>
<th>520 chip</th>
<th>530 chip</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ISP loading</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A percentage of chip wells that contain an Ion Sphere™ Particle (ISP; templated and non-templated, or live and dud ISPs). This percentage value considers only the potentially addressable wells and is a result of the software well classification step. Optimal ISP loading results in high data output.</td>
<td>&gt;85%</td>
<td>&gt;85%</td>
</tr>
<tr>
<td><strong>Total Reads</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The total number of filtered and trimmed reads independent of length reported in the output BAM file. Determined by the number of chip wells that contain an ISP that pass filter. Less than the expected number of reads results in a lower output which may affect the accuracy of the analysis.</td>
<td>&gt;3 million</td>
<td>&gt;15 million</td>
</tr>
<tr>
<td><strong>% polyclonal</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage of polyclonal ISPs. An ISP is polyclonal if its DNA fragments are cloned from two or more original templates. A high percentage of polyclonal ISPs can indicate over loading and will result in lower total reads, in turn affecting the accuracy of the analysis.</td>
<td>&gt;40%</td>
<td>&gt;40%</td>
</tr>
<tr>
<td><strong>% low quality</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage of ISPs with a low or unrecognizable signal. Having a high percentage of low quality ISPs will result in lower total reads, in turn affecting the accuracy of the analysis.</td>
<td>&lt;25%</td>
<td>&lt;25%</td>
</tr>
<tr>
<td><strong>Mean Read Lenght (for 250 flows)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average length after trimming, in base pairs, of called reads. Shorter reads can indicate problems with templating or settings for filtering and trimming.</td>
<td>120-140 bp</td>
<td>120-140 bp</td>
</tr>
</tbody>
</table>

If Ion Torrent instruments other than the Ion Chef™ and Ion S5™ were used for templating or sequencing, then for relevant quality control metrics please refer to the user guide of the instruments used.
### Analysis Quality Control

Sample level quality control assesses the data quality of each individual sample through alignment to copy number calling. The values in the table below are presented as a guide for samples being used for detecting whole chromosome aneuploidy using an Ion S5 instrument; the values for these metrics can be found in the “Data Set” tab of the PG-Find™ Software. See the PG-Find™ Software user guide for more information.

<table>
<thead>
<tr>
<th>Metric</th>
<th>Each Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Reads</strong></td>
<td>≥155,000</td>
</tr>
<tr>
<td>The total number of reads assigned to a particular sample after demultiplexing. The maximum number of samples recommended to use in a single run is 96 with a 530 chip or 24 with a 520 chip.</td>
<td></td>
</tr>
<tr>
<td><strong>% Mapped Reads</strong></td>
<td>&gt;93 %</td>
</tr>
<tr>
<td>The percentage of reads which successfully align to the human genome reference sequence (version hg19). Lower values can indicate non-human contamination in your final library DNA.</td>
<td></td>
</tr>
<tr>
<td><strong>Usable Reads</strong></td>
<td>≥143,000</td>
</tr>
<tr>
<td>The percentage of reads available for copy number analysis after PG-Find™ Software filtering. Reads aligning to anomalous, unstructured and highly repetitive sequence are filtered from the analysis.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of usable reads</th>
<th>Bin Width (bp)</th>
<th>Quality Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>150,000</td>
<td>500,000</td>
<td>&lt; 0.365</td>
</tr>
<tr>
<td>300,000</td>
<td>500,000</td>
<td>&lt; 0.290</td>
</tr>
<tr>
<td>600,000</td>
<td>500,000</td>
<td>&lt; 0.224</td>
</tr>
</tbody>
</table>

**Quality**

The bin to bin variance in each sample before smoothing. A lower quality score indicates more reliable data while a higher quality score indicates less reliable data. The quality score will vary based on the bin width selected and the number of usable reads per sample and should be determined and validated in each laboratory. A high quality score can indicate low quality DNA or issues during sample lysis and preparation.
APPENDIX D

The Thermal Cycler Ramp Rates

When setting ramp rates on the thermal cycler, variables such as sample volume and tube size need to be considered. Furthermore, different thermal cyclers perform differently, and equivalent ramp rate settings can produce different ramp rate results. Some thermal cyclers allow entering of the ramp rate in °C/sec while others require it to be entered as a percentage of the instrument’s maximum ramp rate, which requires knowledge of the maximum ramp rate and subsequent calculation of the required percentage. To alleviate these complications, we provide the following table containing recommended ramp rate settings for some common thermal cyclers:

<table>
<thead>
<tr>
<th>Thermal Cycler</th>
<th>Ramp Rate Setting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.2°C/sec</td>
</tr>
<tr>
<td>Applied Biosystems ('Thermo Fisher Scientific')</td>
<td></td>
</tr>
<tr>
<td>Veriti</td>
<td>8%</td>
</tr>
<tr>
<td>Eppendorf Mastercycler Nexus (standard aluminum block)</td>
<td>0.2°C/sec</td>
</tr>
</tbody>
</table>

To confirm the ramp rate settings for a thermal cycler not listed above, the time taken to ramp to each temperature can be timed and adjusted to match the ramping times listed in Step 1.2.

Some thermal cyclers will also not allow two consecutive ramp steps or a 0 sec hold, in which case holding for 1 sec at 50°C between the two ramp steps is acceptable and will not negatively impact the data. Contact support.au@perkinelmer.com for further assistance.
This product is for research use only. Not for use in diagnostic procedures. PerkinElmer Health Sciences (Australia) Pty Ltd (PKI(A)) makes no representations and gives no warranties of any kind in relation to the contents of this document and all warranties, conditions and other terms implied by statute or common law are, to the fullest extent permitted by law, hereby excluded. In particular, PKI(A) assumes no responsibility for any errors or omissions that may appear in this document and makes no commitment to update or keep current the information contained in this document.

PKI(A) retains the right to make changes to this document (including any specifications contained herein) at any time without notice.

This document is confidential. No part of this document may be modified, copied, reproduced, republished, published, transmitted or distributed in any form or by any means without the prior written consent of PKI(A). The contents of this document are to be used solely for the purpose for which they are provided by PKI(A) and for no other purpose. All content, text, graphics and all other materials contained in this document are owned by PKI(A), and all proprietary and intellectual property (names, logos and trademarks) wherever arising in relation to this document, rest in PKI(A) (or its licensors) and all such rights are reserved.

You are responsible for ensuring that you accurately follow the protocols provided in this Technical Data Sheet (TDS) and analysing and interpreting the results you obtain. PKI(A) does not guarantee any results obtained.
To reorder or learn more, visit perkinelmer-appliedgenomics.com/pg-seq-rapid

PerkinElmer Health Sciences (Australia) Pty Ltd.
40-46 West Thebarton Road | Thebarton, SA, 5031 AUSTRALIA
P: +61 (0)8 8152 9348  |  F: +61 (0)8 8152 9474
www.perkinelmer-appliedgenomics.com

Copyright ©2019, PerkinElmer, Health Sciences (Australia) Pty Ltd. All rights reserved. PerkinElmer® is a registered trademark of PerkinElmer, Inc. All other trademarks are the property of their respective owners.