PG-Seq™ Kit 2.0
Whole Genome Amplification
Library Preparation

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

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PG-Seq™ Kit 2.0
#4300-2300

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PROTOCOL | PG-SEQ™ KIT 2.0

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Product Overview

The PG-Seq™ Kit 2.0 has been developed to analyse pictogram 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 WGA, library preparation, and PG-Find™ analysis software for automatic calling of aneuploidy copy number variants. The kit is for research use only and should not be used in diagnostic procedures.

Kit Overview

The PG-Seq™ Kit 2.0 comes as a 96-reaction kit in a 2 x 48 reaction format. The kit contains a 96 well adapter plate containing 96 uniquely indexed adapters, allowing 96 sample throughput if required. Each well contains sufficient volume for a single use.

Kit Contents & Storage

<table>
<thead>
<tr>
<th>Kit Contents</th>
<th>Cap Color</th>
<th>Storage Temp.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PG-Seq™ Kit 2.0 WGA Reagents (4320-0020-P1)</strong></td>
<td></td>
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</tr>
<tr>
<td>PCR-grade H₂O</td>
<td>WHITE CAP</td>
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</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>
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<tr>
<td><strong>PG-Seq™ Kit 2.0 Library Preparation Reagents (4320-0020-P2)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fragmentation Buffer</td>
<td>CLEAR CAP</td>
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</tr>
<tr>
<td>Fragmentation Enzyme</td>
<td>CLEAR CAP</td>
<td>-20°C</td>
</tr>
<tr>
<td>Ligase Buffer</td>
<td>LIGHT PURPLE CAP</td>
<td>-20°C</td>
</tr>
<tr>
<td>Ligase Enzyme</td>
<td>LIGHT PURPLE CAP</td>
<td>-20°C</td>
</tr>
<tr>
<td>Library Amplification Mix</td>
<td>GREEN CAP</td>
<td>-20°C</td>
</tr>
<tr>
<td>Library Amplification Primer</td>
<td>GREEN CAP</td>
<td>-20°C</td>
</tr>
<tr>
<td><strong>PG-Seq™ Kit 2.0 Adapters (4320-0020-P3)</strong></td>
<td></td>
<td></td>
</tr>
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<td>1 x 96 reaction format</td>
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<tr>
<td><strong>PG-Seq™ Kit 2.0 Purification Beads (4320-0020-P4)</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>Nuclease-free Water</td>
<td>WHITE CAP BOTTLE</td>
<td>Room temp.</td>
</tr>
<tr>
<td>Resuspension Buffer</td>
<td>WHITE CAP BOTTLE</td>
<td>Room temp.</td>
</tr>
</tbody>
</table>

The PG-Seq™ Kit 2.0 contains a 96 well adapter plate containing 96 uniquely indexed adapters. Each well contains sufficient volume for single use in column format. Avoid repeated freezing and thawing of the plate. An excel spreadsheet of Index sequences can be accessed from the following website: perkinelmer-appliedgenomics.com/home/preimplantation-genetic-testing/. Contact support.au@perkinelmer.com for further assistance.
/// Required Materials Not Provided

Pre-PCR Laboratory Requirements

- Laminar flow cabinet
- Microcentrifuge
- Pipettes (2, 10, 20, 100, 200, 1000 µl)
- Cold block
- Thermocycler (with hot lid & programmable ramp rate to 0.25°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 seal
- 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, undenatured) to make 80% ethanol
- Molecular grade water
- Ice
- Thermocycler (with hot lid & programmable ramp rate to 0.25°C/sec)
- Tris-HCl 200 mM (pH 7.0)
- PhiX Sequencing Control v3 10nM (cat # FC-110-3001) diluted to 20 pM (Illumina®)
- LabChip® GXII Touch™ Nucleic Acid Analyzer and associated reagent kit (PerkinElmer)
- Qubit® Fluorometer and associated reagent kit (Thermo Fisher Scientific)
- Sodium hydroxide 1N
- Illumina® Sequencer (MiSeq® Instrument or MiniSeq® Instrument)
- Illumina® Sequencer related consumables
- Illumina® Sequencer reagent kit (select from):
  - MiSeq Reagent Kit v3 (150 cycle, cat # MS-102-3001)
  - MiSeq Reagent Micro Kit v2 (300 cycle, cat # MS-103-1002)
  - MiniSeq High Output Reagent Kit (75 cycles, cat # FC-420-1001)

/// Optional Materials Not Provided

- Agarose gel-electrophoresis apparatus
- Electrophoresis power supply
- UV transilluminator or gel documentation instrument
- Multi-channel pipette
- Multi-channel pipette reagent reservoirs

/// Revision History

<table>
<thead>
<tr>
<th>Version</th>
<th>Date</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>v3.0</td>
<td>September 2019</td>
<td>Updated protocol</td>
</tr>
<tr>
<td>v2.0</td>
<td>July 2019</td>
<td>Updated formatting</td>
</tr>
<tr>
<td>v1.3</td>
<td>April 2019</td>
<td>Initial release</td>
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//// Whole Genome Amplification Principle

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 kit employs the high processivity and fidelity of new generation, sequencing-grade polymerases, ensuring single base accuracy (1 error in $3.6 \times 10^6$ bases).

The WGA is a single tube workflow, utilising ready-mixes to minimize the pipetting steps needed and requires only two 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, DOP-PCR based WGA begins with low temperature, low stringency cycles, facilitating 3’ primer annealing across the whole genome. High stringency PCR amplification of these fragments at an increased annealing temperature is then performed to increase the yield available for downstream applications.

**STEP 1: CELL LYSIS**

**STEP 2: LOW STRINGENCY PCR CYCLES**

**STEP 3: HIGH STRINGENCY PCR CYCLES**
////// 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 30pg/µL in 10mM Tris-HCl (pH 8.0) (no EDTA). 1 µL of 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.

////// Reagent Preparation

- Mix well and briefly centrifuge all reagents before use unless specified otherwise.
- Allow Purification Beads to come to room temperature and vortex the beads until the liquid appears homogeneous before every use.
- Adapters plates should be centrifuged at 280xg for 1 minute before removing the plate seal.

////// Warnings & Precautions

We strongly recommend that you read the following warnings and precautions. If you need further assistance, please contact your local distributor or contact us at Support.AU@perkinelmer.com.

- Do not use the kit past the expiration date.
- The Whole Genome Amplification kit is designed to amplify picograms quantities of DNA; therefore, extreme caution must be exercised to prevent the introduction of foreign DNA contaminants.
- Storage of PG-Seq™ 2.0 WGA Reagents, Cell Lysis and Mastermix setup for Whole Genome Amplification should be performed in a Clean (Pre-PCR) laboratory.
- Regularly clean pipettes and work surfaces.
- Use aerosol barrier pipette tips.
- Use a plate centrifuge to spin down plates before each use and after removal from a thermal cycler.
- Gently peel off plate seals to prevent splashing.
- Re-seal used wells, even if they appear empty.
- Never mix adapter plates using a vortex.
- Ensure pipettes are calibrated and there is a dedicated set for Whole Genome Amplification in a Clean/pre-PCR laboratory.
- 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.
- Do not freeze Purification Beads. Beads should be stored at 2-8°C and brought to room temperature before use.
- Unless otherwise specified, the hotlid for all PCR thermocycler steps should be set to 105°C.
SAMPLE PREP WORKFLOW

CELL LYSIS

WHOLE GENOME AMPLIFICATION

LIBRARY PREPARATION
ENZYMATIC FRAGMENTATION | END REPAIR | ADENYLATION

LIBRARY PREPARATION
ADAPTER LIGATION

LIBRARY PREPARATION
LIBRARY AMPLIFICATION

NEXT-GENERATION SEQUENCING

RESULT ANALYSIS
1.1: Cell Lysis

MATERIALS

- **WHITE CAP** - PCR-grade H₂O
  (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
- Cold Block
- 0.2mL or 0.5mL PCR Tubes

**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:

   - 6.5 µL PCR-grade Water
   - 1.0 µL Cell Lysis Enzyme

   **TOTAL** 7.5 µL

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. It is recommended that a master 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:

   **Volume for 1 Lysis Reaction**  
   **Component**
   
   | 2.7 µL | PCR-grade Water |
   | 0.15 µL | Cell Lysis Buffer |
   | 0.15 µL | Cell Lysis Enzyme Dilution 1 (from Step 1) |
   | **TOTAL** 3.0 µL |

5. Mix well then briefly centrifuge.

   **Cell Sample Preparation:**

6. 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. Do not touch the cell location with the pipette tip. Do not mix or vortex.

7. Briefly centrifuge if required to collect contents at the bottom of the tube.
NTC Preparation (recommended):

8. Add 3 µL of Cell Lysis Mix to one sterile PCR tube labelled NTC.
9. Add 1 µL of PCR-grade H₂O to the tube labelled NTC.
10. Briefly centrifuge.
11. Incubate all samples and NTC in a thermocycler programmed as follows:

<table>
<thead>
<tr>
<th>10 min</th>
<th>75°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 min</td>
<td>95°C</td>
</tr>
</tbody>
</table>

\{ \text{1 cycle} \}

\text{Cycling time is approximately 15 minutes}

Hold 4°C

12. Place the lysed samples in a cold block.
13. Proceed immediately with Whole Genome Amplification.

### 1.2: Whole Genome Amplification

**MATERIALS**

- **WHITE CAP** - PCR-grade H₂O
  (Thaw to room temperature. Briefly centrifuge.)
- **ORANGE CAP** - WGA PCR Buffer
  (Thaw to room temperature. Mix well then briefly centrifuge.)
- **BLUE CAP** - 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
- Cold Block
- 0.2mL or 0.5mL PCR Tubes

**Procedure**

\textbf{NOTE:} The following steps are to be performed in a Clean (Pre-PCR) Laboratory.

1. Calculate the volumes of reagents required to prepare the WGA PCR master mix for all samples, one NTC plus 10% extra.
2. Prepare WGA PCR master mix for the required number of reactions by combining the following reagents in the order they are listed below:

<table>
<thead>
<tr>
<th>Volume for 1X WGA PCR reaction</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.5 µL</td>
<td>PCR-grade Water</td>
</tr>
<tr>
<td>12.5 µL</td>
<td>WGA PCR Buffer</td>
</tr>
<tr>
<td>2.5 µL</td>
<td>Primer</td>
</tr>
<tr>
<td>0.5 µL</td>
<td>WGA Polymerase</td>
</tr>
<tr>
<td>22 µL</td>
<td>TOTAL</td>
</tr>
</tbody>
</table>
3. Mix very well then briefly centrifuge.

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

4. Transfer 22 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>5 min</td>
<td>95°C</td>
<td>1 cycle</td>
</tr>
<tr>
<td>Denaturation</td>
<td>20 sec</td>
<td>98°C</td>
<td>8 cycles</td>
</tr>
<tr>
<td>Annealing</td>
<td>1 min 30 sec</td>
<td>25°C</td>
<td></td>
</tr>
<tr>
<td>Extension</td>
<td>0.25°C/sec</td>
<td>Ramp to 72°C</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 min</td>
<td>72°C</td>
<td></td>
</tr>
<tr>
<td>Denaturation</td>
<td>20 sec</td>
<td>98°C</td>
<td>21 cycles</td>
</tr>
<tr>
<td>Annealing</td>
<td>1 min</td>
<td>58°C</td>
<td></td>
</tr>
<tr>
<td>Extension</td>
<td>1 min</td>
<td>72°C</td>
<td></td>
</tr>
<tr>
<td>Final extension</td>
<td>1 min</td>
<td>72°C</td>
<td>1 cycle</td>
</tr>
<tr>
<td>Cooling</td>
<td>Hold</td>
<td>4°C</td>
<td></td>
</tr>
</tbody>
</table>

*Cycling time is approximately 2 hours, 20 minutes.*

7. It is recommended that users analyze samples using agarose gel electrophoresis. See Appendix A for quality control information. *(Optional QC)* Users can analyse samples using Qubit® dsDNA HS Assay (Life Technologies) for yield quantification.

**SAFE STOPPING POINT:**

If you are stopping sample preparation here, seal the plate with an adhesive seal and store WGA PCR Products at -20°C.
1.3: WGA DNA Purification

MATERIALS

- CLEAR CAP TUB - Nuclease-free Water
- CLEAR CAP TUB - Resuspension Buffer
- CLEAR CAP TUB - Purification Beads

(Allow purification beads to reach room temperature. Vortex the purification beads for 30 seconds to ensure homogeneous resuspension.)

User Supplied

- Sample WGA DNA (from Step 1.2)
- Absolute ethyl alcohol (EtOH, undenatured)
- Molecular grade water
- 96 well plate

NOTE: Always prepare a fresh solution of 80% ethanol using molecular grade water. For example, prepare 25ml 80% ethanol to purify up to 48 samples.

1. For each sample, combine the following reagents in a 96 well plate: 2-10 µL of Sample WGA DNA Product (minimum 30ng) + 16 µL of Purification Beads and topped up with Nuclease-free Water for a total volume of 36 µL. (Recommend using 10 µL Sample WGA DNA Product if the sample showed a weak amplification on the gel).

2. Mix thoroughly by pipetting up and down at least 15-20 times.

3. Incubate the plate at room temperature for 5 minutes.

4. Place the plate on a magnetic stand to capture the beads, incubate for 2-5 minutes or until the liquid is clear. Keep the plate on the stand for the following steps.

5. Using a multichannel pipette remove and discard all supernatant from each well of the plate.

6. Add 200 µL of 80% ethanol to each sample well, do not resuspend the beads.

IMPORTANT: Always use freshly prepared 80% ethanol and do not incubate the bead pellet with 80% ethanol for extended periods.

7. Incubate the plate at room temperature for 30 seconds.

8. Carefully remove the ethanol using a multichannel pipette.

9. Repeat the wash by adding 200 µL of 80% ethanol to each sample well.

10. Incubate the plate at room temperature for 30 seconds.

11. Using a multichannel pipette carefully remove the ethanol solution. Remove all residual ethanol without disturbing the beads.

12. Air dry the beads at room temperature for 3-5 minutes or until the beads are completely dry.

13. Remove the plate from the magnetic stand.

14. Add 36 µL of Resuspension buffer to each well

15. Resuspend the beads by gentle vortexing and/or pipetting up and down at least 15-20 times.

16. Incubate at room temperature for 2 minutes.

17. Place the plate on a magnetic stand to capture the beads, incubate for 2 minutes or until the liquid is clear.

18. Transfer 34 µL of each supernatant to the wells of a new 96 well plate suitable for PCR, avoid carry over of any purification beads.

NOTE: The WGA Master Mix should be added to samples in a General (Post-PCR) Laboratory.
1.4: Fragmentation, End-Repair & Adenylation

MATERIALS

- CLEAR CAP - Fragmentation Buffer
  (Remove Fragmentation Buffer from the freezer and thaw. Mix well then briefly centrifuge.)

- CLEAR CAP - Fragmentation Enzyme
  (Transfer Fragmentation Enzyme from the freezer to ice. The Fragmentation Enzyme is very viscous, mix well then briefly centrifuge. Do not vortex.)

User Supplied

- Purified WGA DNA (in a total volume of 34 µL) (from Step 1.3)
- Adhesive plate seals
- Thermocycler
- Ice

1. While on ice, add 5 µL of Fragmentation Buffer and 11 µL of Fragmentation Enzyme to each sample.
2. Briefly centrifuge the sample plate.
3. Set the multichannel pipette to 30 µL and mix the Fragmentation Buffer and Fragmentation Enzyme into the sample by pipetting up and down at least 15-20 times. To avoid contamination use a new pipette tip for each sample.

**NOTE:** For optimal fragmentation, it is vital that the fragmentation reagents are mixed well with each sample. Ensure you are diligent with pipette mixing for this step. Do not vortex the final fragmentation reaction. It is important to mix the reaction on ice.

4. Briefly centrifuge then return the plate to ice.
5. Transfer the plate from ice to the thermocycler once the temperature reaches 35°C for the following PCR program:

<table>
<thead>
<tr>
<th>Step</th>
<th>Time</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fragmentation</td>
<td>12 min</td>
<td>35°C</td>
</tr>
<tr>
<td>End-Repair &amp; Adenylation</td>
<td>30 min</td>
<td>65°C</td>
</tr>
<tr>
<td>Cooling</td>
<td>Hold</td>
<td>4°C</td>
</tr>
</tbody>
</table>

**Hot lid 85°C**

6. Proceed immediately to Adapter Ligation.
1.5: Adapter Ligation

MATERIALS

- **LIGHT PURPLE CAP** - Ligase Buffer
  (Thaw Ligase Buffer to room temperature and vortex for 5-10 seconds. Do NOT spin down the tube, as this may cause components of the mix to separate.)

- **LIGHT PURPLE CAP** - Ligase Enzyme
  (Transfer Ligase Enzyme from the freezer to ice. The Ligase Enzyme is very viscous, mix well then briefly centrifuge. Do not vortex.)

- **ADAPTERS**
  Remove the Adapter plate from the freezer and thaw. Centrifuge at 280xg for 1 minute then place on ice.

User Supplied

- Fragmented DNA (from Step 1.4)
- Adhesive plate seals
- Ice
- Thermocycler

1. Calculate the volumes of reagents required to prepare the Adapter Ligation master mix for all samples, plus at least 10% extra.

2. Prepare the Adapter Ligation master mix by adding the components according to the following table:

<table>
<thead>
<tr>
<th>Volume for 1x Adapter Ligation reaction</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>44.5 µL</td>
<td>Ligase Buffer</td>
</tr>
<tr>
<td>3 µL</td>
<td>Ligase Enzyme</td>
</tr>
<tr>
<td>47.5 µL</td>
<td>TOTAL</td>
</tr>
</tbody>
</table>

3. Mix well by inverting and pipetting mixing up and down at least 15-20 times

4. Briefly centrifuge.

5. While on ice, dispense 47.5 µL of the Adapter Ligation master mix into individual wells of the Adapter Plate, for the number of samples required. Each adapter well is single use only.
6. Mix well using a multichannel pipette then transfer the entire volume into corresponding wells of the sample plate containing fragmented DNA from Step 1.4.

![Diagram]

NOTE: Be sure to track the correspondence of each sample and the adapter used to prevent sample misidentification.

7. Using a multichannel pipette, mix the samples well by pipetting up and down at least 15-20 times. To avoid contamination use a new tip for each sample.

8. Briefly centrifuge the sample plate.

9. Transfer the plate from ice to the thermocycler once it reaches 20°C for the following PCR program:

<table>
<thead>
<tr>
<th>Adapter Ligation</th>
<th>15 min</th>
<th>20°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooling</td>
<td></td>
<td>4°C</td>
</tr>
<tr>
<td>Hold</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Do not use a hot lid for this step

10. Proceed immediately to Adapter Purification.
1.6: Adapter Purification

MATERIALS

- WHITE CAP BOTTLE - Nuclease-free Water
- WHITE CAP BOTTLE - Resuspension Buffer
- PURIFICATION BEADS
  (Allow purification beads to reach room temperature. Vortex the purification beads for 30 seconds to ensure homogeneous resuspension.)

User Supplied

- Adapter ligated library (from Step 1.5)
- Molecular grade water
- Absolute ethyl alcohol (EtOH, undenatured)
- 96 well PCR plate

NOTE: Prepare a fresh solution of 80% ethanol using Absolute ethyl alcohol (EtOH, undenatured) and Molecular grade water.

1. Add 65 µL of Nuclease-free water and 35 µL of Purification Beads to each sample in a 96 Well plate.
2. Mix the plate thoroughly pipetting up and down at least 15-20 times.
3. Incubate the plate at room temperature for 5 minutes.
4. Place the plate on a magnetic stand to capture the beads, incubate for 2-5 minutes or until the liquid is clear. Keep the plate on the stand for the following steps.
5. Using a multichannel pipette remove and discard all supernatant from each well of the plate.
6. Add 200 µL of 80% ethanol to each sample well, do not resuspend the beads. IMPORTANT: Always use freshly prepared 80% ethanol and do not incubate the bead pellet with 80% ethanol for extended periods.
7. Incubate at room temperature for 30 seconds.
8. Carefully remove the ethanol using a multichannel pipette.
9. Repeat the wash by adding 200 µL of 80% ethanol to each sample well.
10. Incubate the plate at room temperature for 30 seconds.
11. Using a multichannel carefully remove the ethanol solution. Remove all residual ethanol without disturbing the beads.
12. Air dry the beads at room temperature for 3-5 minutes or until the beads are completely dry.
13. Remove the plate from the magnetic stand.
14. Add 28 µL of Resuspension Buffer to each well.
15. Resuspend the beads by vortexing and/or pipetting up and down at least 15-20 times.
16. Incubate samples at room temperature for 2 minutes.
17. Place the 96 well plate on the magnetic stand to capture the beads, incubate for 2 minutes or until the liquid is clear.
18. Transfer 23 µL of each supernatant to the wells of a new 96 well PCR plate, avoid carry over of any purification beads.

SAFE STOPPING POINT
If you are stopping sample preparation here, seal the plate with an adhesive seal and store WGA PCR Products at -20°C.
1.7: Library Amplification

MATERIALS

- GREEN CAP - Library Amplification Mix
  (Transfer Library Amplification Mix from the freezer to ice. Mix well then briefly centrifuge.)
- LIBRARY AMPLIFICATION PRIMER
  (Remove Library Amplification Primer from the freezer and thaw. Mix well then briefly centrifuge.)

User Supplied

- Purified Adapter Ligated DNA (from Step 1.6)
- Adhesive plate seals
- Ice
- Thermocycler

1. Calculate the volumes of reagents required to prepare the Library Amplification master mix for all samples, plus at least 10% extra.

2. Prepare the Library Amplification master mix by adding the reagents according to the following table:

<table>
<thead>
<tr>
<th>Volume for 1x Library Amplification</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 µL</td>
<td>Library Amplification Mix</td>
</tr>
<tr>
<td>2 µL</td>
<td>Library Amplification Primer</td>
</tr>
<tr>
<td>27 µL</td>
<td>TOTAL</td>
</tr>
</tbody>
</table>

3. Mix well then briefly centrifuge.

4. Dispense 27 µL of the Library Amplification Master Mix into each sample well containing purified adapter ligated library.

5. Use a multichannel pipette set to 30 µL and pipette mix up and down at least 15-20 times.

6. Briefly centrifuge the sample plate.

7. Incubate the sample plate in a thermocycler according to the following program:

<table>
<thead>
<tr>
<th></th>
<th>Time</th>
<th>Temp</th>
<th>Cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial denaturation</td>
<td>30 sec</td>
<td>98°C</td>
<td>1 cycle</td>
</tr>
<tr>
<td>Denaturation</td>
<td>15 sec</td>
<td>98°C</td>
<td></td>
</tr>
<tr>
<td>Annealing</td>
<td>30 sec</td>
<td>65°C</td>
<td>4 cycles</td>
</tr>
<tr>
<td>Extension</td>
<td>30 sec</td>
<td>72°C</td>
<td></td>
</tr>
<tr>
<td>Final extension</td>
<td>2 min</td>
<td>72°C</td>
<td>1 cycle</td>
</tr>
<tr>
<td>Cooling</td>
<td></td>
<td>4°C</td>
<td></td>
</tr>
</tbody>
</table>

Cycling time is approximately 10 minutes.

SAFE STOPPING POINT
If you are stopping sample preparation here, seal the plate with an adhesive seal and store WGA PCR Products at -20°C.
1.8: Purification of Final Libraries

MATERIALS

- WHITE CAP BOTTLE - Resuspension Buffer
- PURIFICATION BEADS
  (Allow purification beads to reach room temperature. Vortex the purification beads for 30 seconds to ensure homogeneous resuspension.)

User Supplied

- Amplified library samples (from Step 1.7)
- Molecular grade water
- Absolute ethyl alcohol (EtOH, undenatured)
- 96 well plate
- Adhesive plate seals
- Reagent reservoirs
- Magnetic stand -96

NOTE: Prepare a fresh solution of 80% ethanol using Absolute ethyl alcohol (EtOH, undenatured) and Molecular grade water.

1. In a 96 well plate, add 45 µL of Purification Beads to each amplified library sample from the previous step 1.7.

NOTE: Mix or vortex the Purification Beads between each aliquot to ensure homogeneous resuspension

2. Mix the Purification beads and sample thoroughly by pipetting up and down at least 15-20 times.

NOTE: Mix or vortex the Purification Beads between each aliquot to ensure homogeneous resuspension.

3. Incubate the plate at room temperature for 5 minutes.

4. Place the 96 well plate on a magnetic stand to capture the beads, incubate for 2-5 minutes or until the liquid is clear. Keep the plate on the stand for the following steps.

5. Using a multichannel pipette remove and discard all supernatant from each well of the plate.

6. Add 200 µL of 80% ethanol to each sample well, do not resuspend the beads.

IMPORTANT: Always use freshly prepared 80% ethanol and do not incubate the bead pellet with 80% ethanol for extended periods.

7. Incubate at room temperature for 30 seconds.

8. Carefully remove the ethanol using a multichannel pipette.

9. Repeat wash by adding 200 µL of 80% ethanol to each sample well.

10. Incubate at room temperature for 30 seconds.

11. Using a multichannel carefully remove the ethanol solution. Remove all residual ethanol without disturbing the beads.

12. Air dry the beads at room temperature for 3-5 minutes or until the beads are completely dry.

13. Remove the 96 well plate from the magnetic stand.

14. Add 33 µL of Resuspension Buffer to each well.

15. Resuspend the beads by vortexing and/or pipetting up and down at least 15-20 times.

16. Incubate at room temperature for 2 minutes.

17. Place the 96 well plate on the magnetic stand to capture the beads, incubate for 2 minutes or until the liquid is clear.

18. Transfer 30 µL of supernatant to the wells of a new plate, avoid carry over of any purification beads. This is your final sequencing library.

19. Quantify the concentration of each sample using a Qubit® dsDNA HS Assay (Life Technologies). (Optional QC) Check the size distribution of each sample using a LabChip® GXII Touch™ DNA Assay (PerkinElmer). See Appendix B for quality control information.

SAFE STOPPING POINT
If you are stopping sample preparation here, seal the plate with an adhesive seal and store WGA PCR Products at -20°C.
1.9: Sample Pooling

User Supplied

- Purified samples (from step 1.8)
- Molecular grade water
- 1.5 mL molecular grade tube

Preparation

- Download and open the PG-Seq™ Kit 2.0 4nM Pool Calculator from the following website: [perkinelmer-appliedgenomics.com/home/preimplantation-genetic-testing/](http://perkinelmer-appliedgenomics.com/home/preimplantation-genetic-testing/). Contact support.au@perkinelmer.com for detailed instructions.

Procedure

1. Enter the sample concentration (determined in Step 1.8 – 19) into the “Concentration (ng/µL)” column.
2. From the PG-Seq™ Kit 2.0 4nM 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. See Appendix B for quality control information.

**NOTE:** Quantify sample pool 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 1 µL of pool is loaded in a 20 µL final volume made up with buffer. 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™ Kit 2.0 4nM Pool Calculator.
   a. If the final pool is < 3 nM, the pooling calculations should be checked, and the pool needs to be remade.
   b. If the final pool is ≤ 4.4 nM, then proceed to step 2.0.
   c. If the final pool is > 4.4 nM, adjust to 4 nM by adding additional molecular grade water (see example box) before proceeding to step 2.0.

**NOTE:** Quantifying the diluted final pool in duplicate using a sample volume of 10 µL is recommended.

**EXAMPLE**

Using the 4nM Pool Calculator, it was determined that the final pool concentration was **4.9 nM**.

The final pool was adjusted to **4 nM** by diluting by a factor of \( \frac{4.9}{4} \) = **1.23**.

The pool was in **600 µL**, so **600 * 1.23 - 600 = 138 µL** molecular grade water was added.
2.0: Library Denaturation & MiSeq Sample Loading

User Supplied

- 4nM pool (from step 1.9)
- Illumina® HT1 (Hybridization Buffer)
- Suitable MiSeq® or MiniSeq® Reagent kit:
  - 1-96 Samples: MiSeq® Reagent Kit v3, 150 cycle, catalogue # MS-102-3001
  - 1-96 Samples: MiniSeq™ High Output Reagent Kit (75-cycles), catalogue # FC-420-1001
  - 1-8 Samples: MiSeq® Reagent Micro Kit v2, 300 cycles, catalogue # MS-103-1002
- 1.0 N NaOH
- 200 mM Tris-HCl (pH 7.0)
- Molecular grade water
- 1.5 mL molecular grade tube
- Ice
- Vortex
- (Optional) 20 pM PhiX

Procedure

**NOTE:** Dilute the NaOH to 0.2 N by combining 80 µL of molecular grade water with 20 µL of 1.0N NaOH. This dilution should be made freshly before each use.

**NOTE:** Remove HT1 from the freezer and thaw on ice, store in ice at 2-8 °C until ready to use.

Denature & Dilute:

1. Use the following calculation to determine the volume of pool required to denature:

   \[
   \text{Volume of pool to Denature (µL)} = \frac{20}{\text{Pool concentration (nM)}}
   \]

   **EXAMPLE**
   
   The final pool concentration was **3.6 nM**.
   
   To dilute to **20 pM**, \( \frac{20}{3.6} = 5.55 \) µL of pool was added.
   
   Subsequently, **5.55 µL of NaOH** and **Tris-HCl** was used.

2. Add the volume calculated above of pooled library to a 1.5 mL tube.
3. Add an equal volume of 0.2 N NaOH to the same tube then vortex briefly.
4. Centrifuge at 280 x g for 1 minute.
5. Incubate for 5 minutes at room temperature.
6. Place on ice.
7. Add an equal volume of 200 mM Tris-HCl (pH 7.0).
8. Dilute to 20 pM by increasing the total volume to 1000 µL using HT1.

Dilute to a final loading concentration - MiSeq® Instrument V3

- a. Further dilute the pooled library by adding 450 µL of the 20 pM pool to a 1.5 mL tube.
- b. Add 145 µL of pre-chilled HT1. This results in a 15 pM library.
- c. Add 5 µL 20 pM PhiX.

Dilute to a final loading concentration - MiSeq® Instrument V2 Micro

- a. Further dilute the pooled library by adding 240 µL of the 20 pM pool to a 1.5 mL tube.
- b. Add 357.4 µL of pre-chilled HT1. This results in a 8 pM library.
- c. Add 2.6 µL 20 pM PhiX.
Dilute to a final loading concentration - MiniSeq® Instrument
  a. Further dilute the pooled library by adding 52.5 µL of the 20 pM pool to a 1.5 mL tube.
  b. Add 446.9 µL of pre-chilled HT1. This results in a 2.1 pM pool.
  c. Add 0.6 µL 20 pM PhiX.

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

Sequencing - MiSeq® Instrument

1. Ensure the MiSeq® Reporter Software is installed on the MiSeq® Instrument
2. Download and set up the run Sample Sheet according to the PKI(A) Sample Sheet Template from the following website: [perkinelmer-appliedgenomics.com/home/preimplantation-genetic-testing/](http://perkinelmer-appliedgenomics.com/home/preimplantation-genetic-testing/). Contact support.au@perkinelmer.com for support. Complete the following information:
   - Date
   - Sample_ID
   - Sample_Name
   - I7_Index_ID
   - index

**NOTE:** The sample sheet is sensitive to spacebars and special characters other than underscores, sample names should use alphanumeric characters and underscores only. Contact support.au@perkinelmer.com for detailed instructions.

**NOTE:** Adapter sequences can also be installed into Illumina® Experiment Manager. Contact support.au@perkinelmer.com for detailed instructions.

3. Sequence your library as indicated in the Illumina MiSeq® System Guide using 1x75 bp read lengths.

Sequencing - MiniSeq® Instrument

1. Ensure the MiniSeq® Local Run Manager Software is installed on the MiniSeq® Instrument
2. Load the PG-Seq™ Library Prep Kit by following the instructions under “Add a Library Prep Kit”

**NOTE:** An excel file ready to load into Local Run Manager can be provided. Contact support.au@perkinelmer.com for detailed instructions.

3. Set up your sequencing run in Local Run Manager by using the Create Run command and selecting the module Resequencing.
4. Sequence your library using the run you set up in Local Run Manager, as indicated in the Illumina MiniSeq® System Guide using 1x75 bp read lengths.
2.1: Data Analysis

! **NOTE:** Fastq files generated during the sequencing run need to be aligned to generate BAM files ready for data analysis. If using the provided PG-Seq sample sheet, alignment of Fastq files will be automatically queued via MiSeq® Reporter or Local Run Manager software. The BAM files can be located in the following directory: **Data\Intensities\BaseCalls\Alignment** on the sequencing instrument. If using the Illumina® BaseSpace website, alignment will need to be manually queued at the completion of the run.

Contact [support.au@perkinelmer.com](mailto:support.au@perkinelmer.com) for detailed instructions.

**User Supplied**
- Sample BAM Files

**Procedure**
1. The PG-Find™ software setup application can be downloaded from the following link [installers.biodiscovery.com/PG-Find/Release%201.1/Windows64/VM/PG-Find.exe](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](mailto:support.au@perkinelmer.com).

! **NOTE:** Dilute the NaOH to 0.2 N by combining 80 μL of molecular grade water with 20 μL of

7. Please refer to the PG-Find™ software user guide for detailed information and instructions for data analysis.
Whole Genome Amplification Quality Control

Electrophoresis

The WGA DNA products should appear as a smear, ranging in size from 200 bp - 3000 bp. The NTC should appear clean, with the presence of primer dimers (Figure 1: Agarose gel Electrophoresis). (Figure 2: LabChip® Electropherogram).

Figure 1: Agarose gel electropherogram of WGA DNA products. Samples were run on a 0.5x TBE gel at 100 V for 30 minutes. Lanes 1 and 23: 100bp DNA Marker (DMW-100M, Geneworks), Lanes 2-21: WGA DNA products, Lane 22: No Template Control.

Figure 2: LabChip electropherogram of a sample which showed good amplification. The sample was diluted to 1 ng/µL before being analysed using the LabChip® DNA High Sensitivity Reagent Kit.

Concentration dsDNA ng/µL

Yield of the WGA DNA products should be 20-40 ng/µL using fluorometric DNA quantification.
Troubleshooting

A failed WGA amplification is indicated by the presence of primer dimers, but no evidence of the smear of amplification products (Agarose Gel Electropherogram: Figure 3, LabChip Electropherogram: 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 lysis and PCR reagents. Failed samples should be discarded.

Figure 3: Agarose gel electropherogram of WGA DNA products. Samples were run on a 0.5x TBE gel at 100 V for 30 minutes. Lanes 1: 100bp DNA Marker (DMW-100M, Geneworks), Lanes 2-11: WGA DNA products, Lane 12: No Template Control. Lanes 4 and 6 showed a failed WGA reaction.

Figure 4: LabChip electropherogram of a sample which failed WGA. The sample was diluted to 1 ng/µL before being analysed using the LabChip DNA High Sensitivity Reagent Kit.
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 (Agarose Gel Electropherogram: Figure 5, LabChip Electropherogram: Figure 6). The results from these samples should be interpreted with caution and it is recommended that these samples are removed from further analysis.

**Figure 5: Agarose gel electropherogram of WGA DNA products.** Samples were run on a 0.5x TBE gel at 100 V for 30 minutes. Lanes 1: 100bp DNA Marker (DMW-100M, Geneworks), Lanes 2-18: WGA DNA products, Lane 19: No Template Control. Lanes 4 and 12 show a weak WGA reaction with a smaller than expected fragment size.

**Figure 6: LabChip® electropherogram of a sample which showed weak amplification.** This sample presented a smaller than expected smear with reduced intensity. The sample was diluted to 1 ng/µL before being analysed using the LabChip® DNA High Sensitivity Reagent Kit.
Library Preparation Quality Control

Electrophoresis

Final library samples should have a size distribution of approximately 200-1000 bp with a mode fragment size of 350-450 bp (Agarose Gel Electropherogram: Figure 7, LabChip® Electropherogram: Figure 8).

Figure 7: Agarose gel electropherogram of final library products. Samples were run on a 0.5x TBE gel at 100 V for 30 minutes. Lanes 1: 100bp DNA Marker (DMW-100M, Geneworks), Lanes 2-13: final library products.

Figure 8: Sample LabChip® electropherogram of a final library. The sample was run using the LabChip® NGS 3K reagent kit. The mode fragment size is 384 bp.
/// Sequencing Run Quality Control

There are a number of sequencing metrics which can influence the overall sequencing run and sample quality. The values for the below metrics can be found in the “Run summary” or “Indexing QC” tabs in BaseSpace or in the “Summary” or “Indexing” tabs in Sequence Analysis Viewer (SAV).

<table>
<thead>
<tr>
<th>Metric</th>
<th>MiSeq® Reagent Kit v3 (150 cycle)</th>
<th>MiSeq® Reagent Kit v2 micro (300 cycle)</th>
<th>MiniSeq® High Output Reagent Kit (75-cycles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cluster Density</td>
<td>1000-1600 K/mm²</td>
<td>1000-1200 K/mm²</td>
<td>170-220 K/mm²</td>
</tr>
<tr>
<td>Clusters detected</td>
<td>A measure of the density of clusters detected during image analysis. It is influenced by the concentration of DNA loaded and the size of the DNA fragments within the library. An optimal cluster density results in good image resolution for the sequencer, high quality data and high data output.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reads</td>
<td>25 million - 30 million</td>
<td>4 million</td>
<td>25 million – 30 million</td>
</tr>
<tr>
<td>Reads PF</td>
<td>&gt;19 million</td>
<td>&gt;3.4 million</td>
<td>&gt;19 million</td>
</tr>
<tr>
<td>% ≥Q30</td>
<td>&gt;90 %</td>
<td>&gt;90 %</td>
<td>&gt;90 %</td>
</tr>
<tr>
<td>Reads identified (PF) per sample (%)</td>
<td>Samples</td>
<td></td>
<td></td>
</tr>
<tr>
<td>For a well balanced library the percentage of reads attributed to each sample should be approximately equal. Higher or lower percentages could indicate that the sample quantification was inaccurate or there was a problem with the library preparation.</td>
<td>8</td>
<td>~ 12%</td>
<td>~ 1%</td>
</tr>
<tr>
<td>16</td>
<td>~ 6%</td>
<td>~ 4%</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>~ 4%</td>
<td>~ 2%</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>~ 2%</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>96</td>
<td>~ 1%</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>~ 6%</td>
<td>~ 4%</td>
<td>~ 1%</td>
</tr>
</tbody>
</table>
Sample Quality Control

Sample level quality control assesses the data quality of each individual sample through alignment to copy number calling. The values for the below metrics can be found in the “Data Set” tab of the PG-Find™ Software.

### Total Reads
The total number of reads assigned to a particular sample after demultiplexing. Ideally samples will have approximately 500,000 total reads to achieve a resolution of 7Mb.

<table>
<thead>
<tr>
<th>Total Reads</th>
<th>Each Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>500,000</td>
<td></td>
</tr>
</tbody>
</table>

### % Mapped Reads
The percentage of reads which successfully align to hg19. Lower values can indicate non-human contamination in your final library DNA.

>95 %

### % Usable Reads
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.

>90 %

### 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 reads per sample and should be determined and validated in each laboratory. A high quality score can indicate low quality DNA or issues during the WGA.

<table>
<thead>
<tr>
<th>Number of Reads</th>
<th>Bin Width (bp)</th>
<th>Quality Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>500,000</td>
<td>500,000</td>
<td>&lt;0.08</td>
</tr>
<tr>
<td>500,000</td>
<td>1,000,000</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>
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