PG-Seq™ Rapid Non-Invasive PGT 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.

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**PG-Seq™ Rapid Non-Invasive Kit**  
#4329-0010

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PRODUCT OVERVIEW

The PG-Seq™ Rapid Non-Invasive PGT Kit has been specifically developed to analyze picogram quantities of DNA (low template DNA) from spent embryo culture media or blastocoelic fluid samples (Non-Invasive samples) 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 two sample tube openings, reducing the risk of sample contamination. In the first 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 second 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.

GENERAL INFORMATION

<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>

STEP 1: WHOLE GENOME AMPLIFICATION

**STEP 1a: Low Stringency Conditions**
- Denaturation
- Low stringency primer annealing to template DNA
- Primer extension
- Denaturation & primer annealing to template DNA or amplicon DNA
- Primer extension
- After cycling

**STEP 1b: High Stringency Conditions**
- High stringency primer annealing
- Primer extension
- After cycling

**STEP 2: PCR Barcoding**
- Denaturation
- High stringency primer annealing
- Primer extension
- After cycling
/// Kit Overview

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

/// 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 Non-Invasive PGT Kit WGA Reagents</strong> (&lt;4329-0010-P1&gt;)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCR-grade H₂O</td>
<td>WHITE 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>Round 1 Primer</td>
<td>BLUE CAP</td>
<td>-20°C</td>
</tr>
<tr>
<td>dNTPs</td>
<td>GREEN CAP</td>
<td>-20°C</td>
</tr>
<tr>
<td><strong>PG-Seq™ Rapid Non-Invasive PGT Kit Barcoded Primers</strong> (&lt;4329-0010-P2&gt;)</td>
<td>96-well Plate</td>
<td>-20°C</td>
</tr>
<tr>
<td><strong>PG-Seq™ Rapid Non-Invasive PGT Kit Additional Reagents</strong> (&lt;4329-0010-P3&gt;)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PG-Seq™ Rapid Non-Invasive PGT Kit Purification Beads</td>
<td>WHITE CAP BOTTLE</td>
<td>2-8°C</td>
</tr>
<tr>
<td>PG-Seq™ Rapid Non-Invasive PGT Kit Resuspension Buffer</td>
<td>WHITE CAP BOTTLE</td>
<td>Room Temp.</td>
</tr>
</tbody>
</table>

*The PG-Seq™ Rapid Non-Invasive PGT Kit contains a 96-well primer plate containing 96 uniquely barcoded primers. Each well contains sufficient volume for single use. 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/pgt/](http://perkinelmer-appliedgenomics.com/home/pgt/).

*Contact* support.au@perkinelmer.com *for further assistance.*
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 picogram 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 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 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.
- Briefly centrifuge plates before use and 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)
- Pipette tips (low binding, barrier filter)
- Cold block (4°C)
- Thermal cycler (with hotlid & programmable ramp rate to 0.2°C/sec)
- PCR thin walled reaction tube (0.5mL or 0.2mL)
- PCR-grade 96-well plate to suit thermal cycler
- 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
- Pipettes (2, 10, 20, 100, 200, 1000 µL)
- Pipette tips (low binding, barrier filter)
- 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
- LabChip® GXII Touch™ Nucleic Acid Analyzer and associated reagent kit (PerkinElmer)
- Qubit® Fluorometer and associated reagent kit (Thermo Fisher Scientific)
- 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 S10-530™ Chip Kits
  - Ion P1™ Chip Kit

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
///// Starting Materials

Spent embryo culture media or blastocoelic fluid samples should be collected as soon as possible after the embryo has been removed from culture using a new, clean pipette tip for each and every media sample. The samples should be transferred to the bottom of a clean, sterile 0.2 mL or 0.5 mL PCR tube or 96-well PCR plate and immediately frozen at -20°C and if required, shipped with dry ice. It is recommended that sample collection, storage and shipping conditions are validated before use.

If required, control 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). One microlitre of freshly diluted DNA can be added to 5 μL PCR-grade H2O for the Round 1 PCR step. To avoid contamination, ensure DNA samples or diluted DNA is not brought into the Clean (Pre-PCR) Laboratory.

///// Workflow

SAMPLE PREP SETUP

Add template DNA → Add Round 1 WGA Master Mix → Add Barcoded Primers → Add Round 2 WGA Master Mix → 25 minute PCR → Pool → Bead Purification → 1 hour 20 minute PCR
1.1: 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.)
- GREEN CAP - dNTPs
  (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

- Samples
- Thermal cycler
- Minicentrifuge
- Cold block (4°C)
- 1.5 mL molecular grade tube
- 0.2 mL or 0.5mL PCR tubes

Procedure

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

1. Calculate the volume of reagents required to prepare the Round 1 PCR Master Mix for all samples, one NTC plus add 1-2 samples extra to the total sample number 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 listed below:

<table>
<thead>
<tr>
<th>Volume for 1x Round 1 PCR Reaction</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.2 µL</td>
<td>PCR-grade Water</td>
</tr>
<tr>
<td>10.0 µL</td>
<td>WGA PCR Buffer</td>
</tr>
<tr>
<td>3.8 µL</td>
<td>Round 1 Primer</td>
</tr>
<tr>
<td>0.5 µL</td>
<td>dNTPs</td>
</tr>
<tr>
<td>0.5 µL</td>
<td>WGA Polymerase</td>
</tr>
<tr>
<td></td>
<td><strong>TOTAL 21 µL</strong></td>
</tr>
</tbody>
</table>

No Template Control (NTC) Preparation (recommended):

3. Add 6 µL of PCR-grade H2O to the tube or plate well label as NTC.

Non-Invasive sample Preparation:

4. Add 6 µL of spent culture media / blastocoelic fluid to sterile empty PCR tubes or plate wells.
**NOTE**: Steps involving genomic DNA are to be performed in a *General (Post-PCR) Laboratory*.

Positive Control Preparations (optional):
5. Add 1 µL of 30 pg/µL DNA sample and 5 µL of PCR-grade H₂O to the tube or plate well label as Positive Control. DNA should be freshly diluted to 30 pg/µL.
6. Mix well and briefly centrifuge.
7. Transfer 21 µL of WGA PCR Master Mix to the individual tubes containing template (sample or NTC).
8. Mix well and briefly centrifuge.
9. Incubate samples and NTC in a thermal cycler programmed as follows:

<table>
<thead>
<tr>
<th>Step Type</th>
<th>Time</th>
<th>Temperature</th>
<th>Cycle</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>0.2°C/sec</td>
<td>Ramp to 50°C</td>
<td>8 cycles</td>
</tr>
<tr>
<td></td>
<td>1°C/sec</td>
<td>Ramp to 72°C</td>
<td></td>
</tr>
<tr>
<td></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>1 min</td>
<td>58°C</td>
<td>16 cycles</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>

10. Place Round 1 PCR samples in a cold block and proceed with Round 2 PCR.
11. If preferable, transfer samples into a 96-well PCR plate for the following steps.

**SAFE STOPPING POINT**:

Round 1 PCR product can be stored for up to 1 week at 4°C before proceeding to Round 2 PCR.
1.2: 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** - PG-Seq™ Rapid Non-Invasive PGT 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 1.1)
- 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 1-2 samples 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 listed below:

<table>
<thead>
<tr>
<th>Volume for 1x Round 2 PCR Reaction</th>
<th>Component</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.42 µL</td>
<td>PCR-grade Water</td>
</tr>
<tr>
<td>40.50 µL</td>
<td>WGA PCR Buffer</td>
</tr>
<tr>
<td>1.08 µL</td>
<td>WGA Polymerase</td>
</tr>
<tr>
<td>46 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 8 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 46 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>4 cycles</td>
</tr>
<tr>
<td>Annealing</td>
<td>30 sec</td>
<td>50°C</td>
<td></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>6 cycles</td>
</tr>
<tr>
<td>Annealing</td>
<td>30 sec</td>
<td>58°C</td>
<td></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

(Recommended QC) It is recommended that users analyze samples using agarose gel electrophoresis. See Appendix A for quality control information.

SAFE STOPPING POINT:
If you are stopping sample preparation here, store WGA PCR Products at -20°C
1.3: Sample Purification

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

**MATERIALS**

- CLEAR CAP TUB - PG-Seq™ Rapid Non-Invasive PGT Purification Beads. Allow Purification beads to reach room temperature. Vortex the Purification Beads for 30 seconds to ensure homogeneous resuspension.
- CLEAR CAP TUB - PG-Seq™ Rapid Non-Invasive PGT Resuspension Buffer

**User Supplied**

- Sample WGA DNA (from Step 1.2)
- Absolute ethyl alcohol (EtOH, undenatured) to make up 80% Ethanol
- Molecular grade water
- 96-well plate
- Adhesive plate seals
- Reagent reservoirs
- Magnetic stand
- Qubit® dsDNA HS Assay reagents

**Procedure**

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

1. Add 40 µL of each amplified and barcoded sample to a 96-well plate.
2. Add 36 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 all supernatant from each well of the plate (or store until the success of the purification has been confirmed by quantification).
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.
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 dry.
14. Remove the plate from the magnetic stand.
15. Add 30 µL of Resuspension Buffer to each well.
**1.4: Sample Pooling**

**User Supplied**
- Purified samples (from step 1.3)
- Molecular grade water
- 1.5 mL molecular grade tube
- Qubit dsDNA HS Assay reagents

**Preparation**
- Download and open the **PG-Seq™ Rapid Non-Invasive PGT Kit for Ion Torrent Pool Calculator** from the following website: perkinelmer-appliedgenomics.com/pgt/. Contact support.au@perkinelmer.com for further assistance.

**Procedure**
1. Enter the sample concentration (determined in Step 1.3 - 20) into the “Concentration (ng/µL)” column.
2. From the **PG-Seq™ Rapid Non-Invasive PGT Kit for Ion Torrent 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. Do not add failed samples in the pool.
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. (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 Non-Invasive PGT Kit for Ion Torrent Pool Calculator**.
   a. If the final pool is < .075 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 1 nM by adding additional molecular grade water (see example box) before proceeding to step 2.0.

   **EXAMPLE:**
   Using the 4 nM 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 (1.3 / 1) = 1.3.
   The pool was in 600 µL, so 600 * 1.3 - 600 = 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/](http://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 S5 System** template from the following link: 

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 S5 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: [https://perkinelmer-appliedgenomics.com/home/preimplantation-genetic-testing/](https://perkinelmer-appliedgenomics.com/home/preimplantation-genetic-testing/) and save on your local storage.

   Complete the following information and save:
   
   a. Barcode
   b. Sample Name
   c. Sample ID (optional)
   d. Sample Description (optional)

2. Click the “Plan” tab, then click “Whole Genome” from the list of applications on the left side of the screen.

3. Select **PG-Seq™ Rapid - Ion S5 System** from the list under “Template Name”.

4. The **PG-Seq™ Rapid - Ion S5 System** Planned Run template auto-populates settings in the Application, Kits, Plugins, and Plan steps appropriately.

5. In the Kits step, verify the selections and select the chip type you are using.

6. Proceed to the Projects step and complete your selections.

7. Proceed to the Plan step and enter or make the following selections:
   
   a. Enter a new Run Plan Name, if desired.

   b. If uploading a Samples Table Template, click “Load Samples Table”. Click “Choose File”, select the Samples Table CSV file from your local storage, then click “Open”. Click “Load”. If not uploading a Samples Table Template, enter the number of barcodes you are using in your sample set, then in the table ensure the correct barcodes are listed (selected barcode can be changed by clicking the dropdown), and assign sample details if desired.

   **NOTE:** we strongly recommend that you assign unique sample names for each barcode and experiment.

8. Click “Plan Run” in the lower right corner to save the Planned Run. The run is listed on the “Planned Runs” screen under the name you entered.
2.1: Library Templating and Sequencing

User Supplied

- 1 nM pool (from step 1.4)
- Ion Chef™ S5 Series Chip Balance
- Ion 510™, Ion 520™, or Ion 530™ chip

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.

4. Initialize the sequencer and start the sequencing run following the instructions in the Ion S5™ and Ion S5™ XL Instrument user guide.

**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.
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 Non-Invasive Kit WGA DNA products should appear as a smear, ranging in size from approximately 200 bp - 800 bp with a mean fragment size of approximately 380 bp. The NTC should appear clean, with the presence of primer dimers (Figures 1 and, Figure 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 Non-Invasive Kit WGA DNA products of spent embryo culture media of 3 different culturing protocols (Illumina example). 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 2-9: WGA DNA products (media sample set 1), Lane 210-17: WGA DNA products (media sample set 2), Lanes 18-19: WGA DNA products (media sample set 3), Lane 20: positive control, Lane 21: No Template Control, Lanes 3-14: WGA DNA products, Lane 22: 100bp DNA Marker (DMW-100M, Geneworks).

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

Figure 3: LabChip® electropherogram of a purified PG-Seq™ Rapid Non-Invasive Kit WGA sample which showed expected amplification. The sample was diluted to 1 ng/µL before being analyzed using the LabChip 3K NGS Reagent Kit.
Concentration dsDNA ng/µL

The typical yield of the PG-Seq™ Rapid Kit Non-Invasive Kit amplified WGA DNA products post purification is 30 ng/µL WGA 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 contained no or un-amplifiable DNA. 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 4). The results from these samples should be interpreted with caution and it is recommended that these samples are removed from further analysis.

Figure 4: Agarose gel electropherogram of PG-Seq™ Rapid Kit WGA DNA products (Illumina example). 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 2-4 and 6-7: WGA DNA products, Lane 5 shows a failed WGA reaction.

<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></td>
<td>Clean work area thoroughly and use dedicated PCR pipettes and tips</td>
</tr>
<tr>
<td>Kit reagents have been contaminated</td>
<td></td>
<td>Discard unused reagents and open a new kit</td>
</tr>
</tbody>
</table>
# 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>&gt;85%</td>
<td>&gt;85%</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></td>
<td></td>
</tr>
<tr>
<td><strong>Total Reads</strong></td>
<td>&gt;3 million</td>
<td>&gt;15 million</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></td>
<td></td>
</tr>
<tr>
<td><strong>% polyclonal</strong></td>
<td>&gt;40%</td>
<td>&gt;40%</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></td>
<td></td>
</tr>
<tr>
<td><strong>% low quality</strong></td>
<td>&lt;25%</td>
<td>&lt;25%</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></td>
<td></td>
</tr>
<tr>
<td><strong>Mean Read Length (for 250 flows)</strong></td>
<td>120-140 bp</td>
<td>120-140 bp</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></td>
<td></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.
APPENDIX C

///// Analysis Quality Control

Sample level quality control assesses the data quality of each individual sample through alignment to copy number calling. 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.
### APPENDIX D

#### 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 instruments 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 0.2°C/sec</th>
<th>Ramp Rate Setting 1°C/sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Applied Biosystems (Thermo Fisher Scientific) Veriti</td>
<td>8%</td>
<td>40%</td>
</tr>
<tr>
<td>Eppendorf Mastercycler Nexus (standard aluminum block)</td>
<td>0.2°C/sec</td>
<td>1°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.
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