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PG-Seq™ Rapid Kit v2

Whole Genome Amplification & Indexing
for Illumina® NGS

KIT CONTAINS : 48 RXNS

#4340-0248

#4341-IL48A

#4341-IL48B

PG-Seq™ Rapid Kit v2

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GENERAL INFORMATION

Revision History

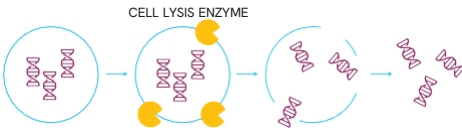
| Version | Date | Description |
|---------|--------------|--------------------|
| v1.0 | June 2022 | v2 Launch |
| V2.0 | October 2023 | Rebrand to Revvity |

Product Overview

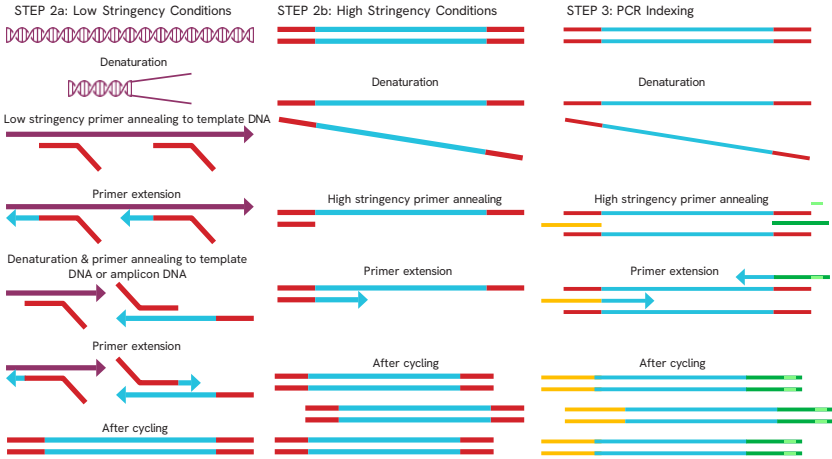
The PG-Seq™ Rapid Kit v2 has been developed to analyze picogram quantities of DNA (single/multi-cells or low template DNA) from an embryo biopsy for preimplantation genetic testing. The kit utilizes whole genome amplification (WGA) and next generation sequencing (NGS) technology to accurately screen all 24 chromosomes for whole chromosome aneuploidy and sub-chromosomal abnormalities. From DNA to data, the kit includes all reagents required for cell lysis, WGA, indexing, and software analysis with the PG-Find™ 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 protocol follows a simple workflow with minimal sample handling steps, utilizing ready-mixes to minimize pipetting steps, and 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 amplified using high stringency annealing conditions to increase the yield available for indexing. In the third step, primers containing a unique index sequence to allow for multiplexing, plus the sequences necessary for Illumina® instrument sequencing anneal to each end of the amplified fragments, generating sequencing ready libraries.

STEP 1: CELL LYSIS



STEP 2: WHOLE GENOME AMPLIFICATION



Kit Contents and Storage

The PG-Seq™ Rapid Kit v2 contains enough reagents to prepare 48 samples for Illumina® Sequencing. The shelf life of all reagents is at least 6 months when stored according to the specified storage temperatures.

| Kit Contents | Cap Color | Storage Temp. | Tube Volume |
|--|------------|------------------|-------------|
| PG-Seq™ Rapid Kit v2, 48rxns (cat # 4340-0248) | | | 48 rxns |
| Cell Lysis Mix | GREEN CAP | -20°C | 240 µL |
| PCR 1 Ready-mix | ORANGE CAP | -20°C | 960 µL |
| PCR 1 Primer | BLUE CAP | -20°C | 720 µL |
| PCR 2 Ready-mix | RED CAP | -20°C | 600 µL |
| Clean-up Beads | BROWN CAP | 2-8°C | 960 µL |
| Resuspension Buffer | CLEAR CAP | Room Temperature | 1200 µL |

PG-Seq™ Indexing Primers for Illumina® sequencing

A separate catalogued indexing primer plate is required for the complete protocol and one or both of the below options should be acquired depending on your sequencing needs.

| Catalogue Number | Kit Contents | Storage Temp. |
|------------------|--|---------------|
| 4341-IL48A | 10 µL x 48 uniquely indexed primers, suitable for Illumina® Sequencing instruments. Set A Illumina® Indexes 1-48. | -20°C |
| 4341-IL48B | 10 µL x 48 uniquely indexed primers, suitable for Illumina® Sequencing instruments. Set B Illumina® Indexes 49-96. | -20°C |

Up to 384 sample multiplexing is available by custom request.

Required Materials Not Provided

Pre-PCR Laboratory requirements

- Laminar flow cabinet
- Minicentrifuge
- Pipettes (P10, P20, P200, P1000 μL)
- Pipette tips (low binding, barrier filter)
- Cold block (4°C)
- Thermocycler (with hot lid & programmable ramp rate to $0.3^{\circ}\text{C}/\text{sec}$)
- PCR thin-walled reaction tube (0.5 mL or 0.2 mL)
- Sterile, molecular grade tubes (1.5 mL or larger)

Post-PCR Laboratory requirements

- Single and multichannel pipettes
- Minicentrifuge
- Magnetic stand for 96-well plates or 1.5mL tubes
- 96-well plate, compatible with magnetic stand
- Adhesive 96-well plate seals
- Absolute ethyl alcohol (EtOH, undenatured) to make 80% ethanol
- Molecular grade water
- Thermocycler
- 96-well plate centrifuge

Illumina Sequencing requirements

- Tris-HCl 200 mM (pH 7.0)
- PhiX Sequencing Control v3 10 nM (cat # FC110-3001) diluted to 20 pM (Illumina®)
- 1.0 N Sodium hydroxide (NaOH)
- 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)

Warnings & Precautions

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

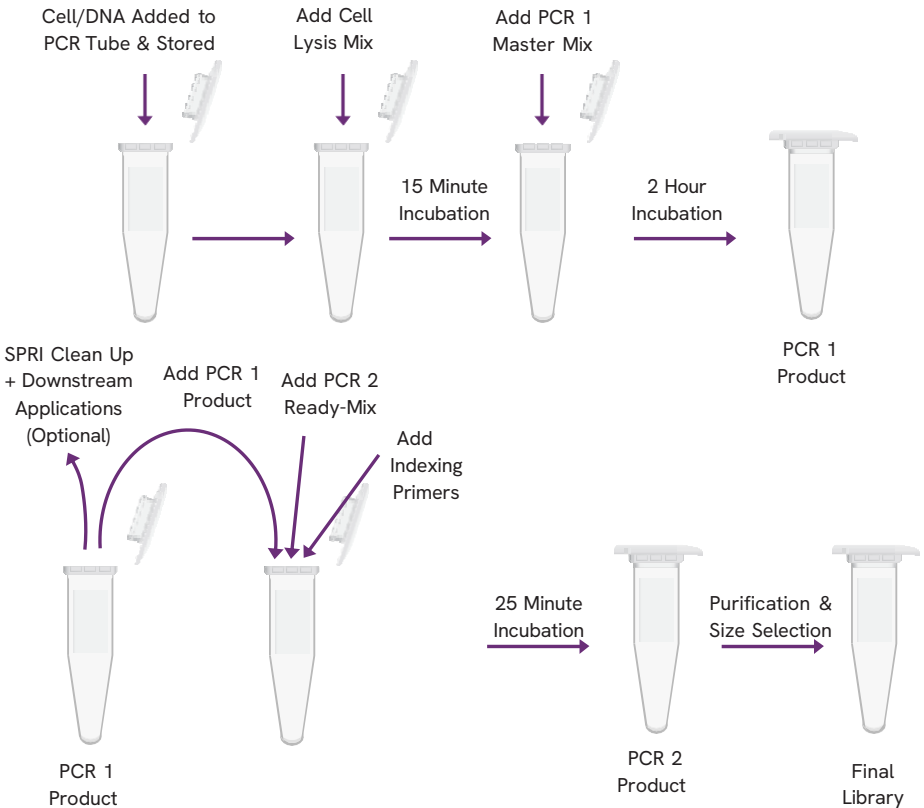
- Do not use the kit past the expiration date.
- Try to maintain a laboratory temperature of 20 - 25°C.
- The kit is designed to amplify picograms quantities of DNA; therefore, extreme caution must be exercised to prevent the introduction of foreign DNA contaminants.
- Unless otherwise specified, the hot lid for thermocyclers should be set to 105°C.
- Ensure pipettes are calibrated and there is a dedicated set for Cell Lysis and PCR 1 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 WGA PCR 1 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.
- Re-seal plates and used wells, even if they appear empty, using a clean plate seal.
- Never mix the Indexing Primer plate using a vortex.
- Do not freeze Clean Up Beads. Beads should be stored at 2-8 °C.
- Allow Clean Up Beads to come to room temperature and vortex the beads until the liquid appears homogeneous before every use.

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 (Mg^{2+} , Ca^{2+} 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 the 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 WGA and should be diluted to a final genomic concentration of 30 $\text{pg}/\mu\text{L}$ 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 are not brought into the Clean (Pre-PCR) Laboratory.

Workflow



PROTOCOL | PG-Seq™ Rapid Kit v2

Step A: Cell Lysis

MATERIALS

- GREEN CAP - Cell Lysis Mix

(Thaw to room temperature. Mix well then briefly centrifuge.)

User Supplied

- Cell or DNA samples
- Thermocycler
- Minicentrifuge
- PCR Tubes

Procedure

! NOTE: The following steps should be performed in a pre-PCR area inside a clean laminar flow cabinet.

Cell Sample Preparation:

- Add 5 μL of Cell Lysis Mix above a cell sample located in a PCR tube. Make sure the Cell Lysis Mix rolls over the sample location by gently tapping the tube on the benchtop.

! NOTE: Do not touch the cell location with the pipette tip. Do not mix or vortex.

Genomic DNA / Positive Control Preparation:

! NOTE: To prevent contamination, genomic DNA should be stored and handled in post-PCR location, separate to Cell Lysis and WGA reagent storage and master mix set up.

- Add 5 μL of Cell Lysis Mix to the required number of sterile empty PCR tubes.
- Add 1 μL of 30 $\text{pg}/\mu\text{L}$ DNA sample to each tube containing Cell Lysis Mix. DNA should be freshly diluted to 30 $\text{pg}/\mu\text{L}$ in 10 mM Tris pH 8 (no EDTA).

Negative Control Preparation (recommended):

- Add 5 μL of Cell Lysis Mix to one sterile negative control PCR tube.
- Add 1 μL of PCR-grade H₂O to the negative control tube.
 - Briefly centrifuge all samples, genomic DNA (if included), and negative control to collect the contents in the bottom of the tube.
 - Incubate in a thermocycler programmed as follows:

| Time | Temperature | |
|---------|-------------|--|
| 10 mins | 56°C | Total incubation time is approx. 15 minutes |
| 5 mins | 95°C | |
| HOLD | 4 °C | |

Step B: WGA PCR 1

MATERIALS

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

User Supplied

- Lysed samples and negative control (from previous step)
- Thermocycler (with hot lid & programmable ramp rate to 0.3°C/sec)
- Minicentrifuge
- Molecular grade tube

Procedure

! NOTE: The following steps should be performed in a pre-PCR area inside a clean laminar flow cabinet.

1. Calculate the volumes of reagents required to prepare the PCR 1 Master Mix for all samples and one negative control.
2. Add 10% to each calculated volume to allow for loss during pipetting.
3. Prepare WGA PCR 1 Master Mix for the required number of reactions by combining the following reagents in a molecular grade tube:

| <u>Component</u> | <u>Volume</u> |
|------------------|---------------|
| PCR 1 Ready-mix | 20 μ L |
| PCR 1 Primer | 15 μ L |
| Total | 35 μ L |

4. Mix well then briefly centrifuge.
5. Transfer 35 μ L of PCR 1 Master Mix to each individual tube containing lysed template (sample or negative control 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.

6. Briefly centrifuge each PCR tube.
7. Incubate samples and negative control in a thermocycler programmed as follows:

| Step | Time | Temperature | Cycles | |
|----------------------|--------------|--------------------|-----------|---|
| Initial Denaturation | 5 min | 95°C | 1 cycle | |
| Denaturation | 20 sec | 98°C | | |
| Annealing | 1 min 30 sec | 25°C | | |
| | 0.3°C/sec | Slow ramp to 72°C* | 8 cycles | Total cycle time is approx. 1 hour 54 minutes |
| Extension | 1 min | 72°C | | |
| Denaturation | 20 sec | 98°C | | |
| Annealing | 1 min | 58°C | 15 cycles | |
| Extension | 1 min | 72°C | | |
| Final Extension | 1 min | 72°C | 1 cycle | |
| Cooling | Hold | 4°C | | |

**The slow ramping step is critical to the success of the amplification. Ramping from 25°C to 72°C at 0.3°C/sec takes approximately 3 minutes, 15 seconds on an Eppendorf Mastercycler® Nexus PCR thermal cycler. If your thermocycler requires the slow ramp to be inputted as a percentage of the instruments maximum ramp rate, an approximate percentage should be set, and the time taken to ramp from 25°C to 72°C should be timed and adjusted to match the expected time.*

Optional QC: Quantify and visualize the PCR 1 WGA [Recommended: Qubit® dsDNA HS Assay and LabChip® GXII Touch™ DNA Assay]

PCR 1 WGA product can be used for both copy number analysis (by following the steps below for Indexing PCR 2), or as input for alternate downstream processing such as amplicon or hybridization panels. For alternative downstream processes, it is recommended the PCR 1 WGA product is purified with a 1x SPRI purification.

Step C: Indexing PCR 2

MATERIALS

- PCR 2 Ready-Mix - **RED CAP**
(Thaw to room temperature. Mix well then briefly centrifuge.)
- Indexing Primers - 96 well plate with pierce seal (Thaw at room temperature then centrifuge at 280 x g for 1 minute before use. Place new seal over pierce holes after use)

User Supplied

- PCR 1 samples and NTC (from previous step)
- Thermocycler
- Minicentrifuge
- 96 well PCR plate, PCR strip tubes or individual PCR tubes
- 96-well plate centrifuge
- Adhesive plate seals

Procedure

! NOTE: The following steps should be performed in a post-PCR area.

1. In a 96 well PCR plate, PCR suitable strip tubes or individual PCR tubes, combine the following for each individual PCR 1 sample, and record the corresponding index used for each sample to ensure correct identification during downstream processing:

| <u>Component</u> | <u>Volume</u> |
|------------------|---------------|
| PCR 2 Ready-mix | 12.5 μ L |
| Indexing Primers | 10 μ L |
| PCR 1 sample | 2.5 μ L |
| Total | 25 μ L |

2. Mix each sample by pipetting up and down 10 times then briefly centrifuge.
3. Incubate samples and NTC and a thermocycler programmed as follows:

| Step | Time | Temperature | Cycles |
|---|--------|-------------|----------|
| Initial Denaturation | 3 min | 95°C | 1 cycle |
| Denaturation | 20 sec | 98°C | |
| Annealing | 30 sec | 50°C | 4 cycles |
| Extension | 30 sec | 72°C | |
| Total incubation time is approx. 25 minutes | | | |
| Denaturation | 20 sec | 98°C | |
| Annealing | 30 sec | 62°C | 6 cycles |
| Extension | 30 sec | 72°C | |
| Cooling | Hold | 4°C | |

Step D: Purification and Size Selection

MATERIALS

- Clean Up Beads – **BROWN CAP** (Allow beads to reach room temperature. Vortex before use to ensure homogeneous resuspension)
- Resuspension Buffer – **CLEAR CAP**

User Supplied

- PCR 2 product (from the previous step)
- Absolute ethyl alcohol (EtOH, undenatured)
- Molecular Grade Water
- 1.5mL tube or 96-well plates
- Reagent reservoirs
- Magnetic tube stand or magnetic plate stand

Procedure

! NOTE: Always prepare a fresh solution of 80% ethanol using undenatured EtOH and molecular grade water.

! NOTE: Optimization of bead 1 and bead 2 volumes may be required to ensure fragments are of a suitable size for sequencing.

Option 1:

Single tube purification and size selection

If consistent template is used and reliable yields are achieved following PCR 1 and 2 reactions, individual samples can be pooled prior to size selection. Where “Bead 1” and “Bead 2” are referred to in the protocol below, the following bead volumes should be used:

Bead 1: $0.75 \times$ the sample pool volume. E.g., if your sample pool volume is 200 μL , add 150 μL of Clean Up Beads.

Bead 2: $0.12 \times$ the transferred supernatant volume. E.g., if the volume of supernatant transferred is 300 μL , add 36 μL of Clean Up Beads.

1. Combine 4-10 μL of each sample into a 1.5 mL tube and note the final volume.
2. Calculate and add the required Bead 1 volume and vortex / mix thoroughly.
3. Briefly spin down the tube then incubate at room temperature for 5 minutes.
4. Place the tube on a tube magnet to capture the beads. Incubate for 2-5 minutes or until the liquid is clear.
5. Transfer the supernatant to a new 1.5 mL tube and note the volume transferred.

! IMPORTANT: Do not discard the supernatant.

6. Calculate and add the required Bead 2 volume to the supernatant and vortex / mix thoroughly.
7. Briefly spin down the tube then incubate at room temperature for 5 minutes.
8. Place the supernatant tube on a tube magnet to capture the beads. Incubate for 2-5 minutes or until the liquid is clear.
9. Carefully remove the supernatant and discard.
10. Add 500 µl of 80% ethanol to the tube, do not resuspend the beads.
11. Incubate the tube at room temperature for 30 seconds.
12. Carefully remove the ethanol.
13. Repeat the wash by adding 500 µL of 80% ethanol to the tube, do not resuspend the beads.
14. Incubate the tube at room temperature for 30 seconds.
15. Remove the ethanol, briefly spin down the tube, place the tube back on the magnet then remove remaining ethanol with a pipette.
16. Air dry the beads at room temperature for 3 minutes or until the beads are dry.

! IMPORTANT: for successful size selection, ensure all traces of ethanol are removed/evaporated from the wells.

17. Remove the plate from the magnetic stand and add a volume of Resuspension Buffer equal to your initial sample pool volume.
18. Resuspend the beads and incubate at room temperature for 3 minutes.
19. Place the tube on a tube magnet to capture the beads. Incubate for 2 minutes or until the liquid is clear.
20. Transfer as much of the supernatant as practical to a new tube, avoiding carryover of any Clean Up Beads. This is your final library pool.
21. Quantify the concentration of the pool

Recommended: Determine the average fragment size of the pool using the Revvity LabChip® GXII Touch™ Nucleic Acid Analyzer or equivalent.

22. Determine the molar concentration of the pool using the following calculation:

$$\text{nM} = \frac{\text{pool concentration (ng/}\mu\text{L)}}{\text{average fragment size (bp)} \times 660} \times 1,000,000$$

23. Proceed to Library Denaturation and MiSeq®/MiniSeq® Sample Loading.

Option 2:

Individual sample purification and size selection

If performing the protocol for the first time, or if inconsistent sample types and yields are achieved from the PCR 1 and 2 steps, individual sample purification and size selection is recommended to allow sample normalization prior to pooling.

1. Combine 20 µl of sample and 15 µL of Clean Up Beads in a 96 well plate and mix thoroughly.

2. Incubate the plate at room temperature for 5 minutes.
3. Place the plate on a magnetic stand to capture the beads. Incubate for 2-5 minutes or until the liquid is clear.
4. Transfer the supernatant to a second 96-well plate.

! IMPORTANT: Do not discard the supernatant.

5. Add 4 μL of Clean Up Beads to each well of the transferred supernatant and mix thoroughly.
6. Incubate the supernatant plate at room temperature for 5 minutes.
7. Place the supernatant plate on a magnetic stand to capture the beads. Incubate for 2-5 minutes or until the liquid is clear.
8. Carefully remove the supernatant and discard.
9. Add 200 μL of 80% ethanol to each sample well, do not resuspend the beads.
10. Incubate the plate at room temperature for 30 seconds.
11. Carefully remove the ethanol.
12. Repeat the wash by adding 200 μL of 80% ethanol to each sample well, do not resuspend the beads.
13. Incubate the plate at room temperature for 30 seconds.
14. Remove the ethanol and air dry the beads at room temperature for 3 minutes or until the beads are dry.

! IMPORTANT: for successful size selection, ensure all traces of ethanol are removed/evaporated from the wells.

15. Remove the plate from the magnetic stand and add 25 μL of Resuspension Buffer.
16. Resuspend the beads and incubate at room temperature for 3 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 20 μL of each supernatant to the wells of a new 96-well plate, avoid carryover of any Clean Up Beads.
19. Quantify the concentration of each purified sample [recommended: Qubit® dsDNA HS Assay]

Recommended: Determine the average fragment size of each sample or a selection of samples using the Revvity LabChip® GXII Touch™ Nucleic Acid Analyzer, Agilent® Bioanalyzer® or Agilent® TapeStation®.

20. Determine the molar concentration of each sample using the following formula:

$$\text{nM} = \frac{\text{sample concentration (ng/}\mu\text{L)}}{\text{average fragment size (bp)} \times 660} \times 1,000,000$$

21. Pool an equimolar volume of each sample. See the instructions and table below as a guide. Determine the volume of each sample to add to the pool by dividing a constant volume by each sample's nM value. e.g.

| Sample Name | nM | Constant Volume (μL) | Calculation to determine volume of each sample to add to pool: | Volume of sample to add to pool (μL): |
|-------------|-------|-----------------------------------|--|--|
| Sample 1 | 7.15 | 40 | $= 40 / 7.15$ | 5.59 |
| Sample 2 | 10.14 | 40 | $= 40 / 10.14$ | 3.94 |
| Sample 3 | 8.80 | 40 | $= 40 / 8.80$ | 4.55 |
| Sample 4 | 6.03 | 40 | $= 40 / 6.03$ | 6.63 |

22. Quantify the concentration of the pool.
23. Determine the molar concentration of the pool using the following calculation:

$$\text{nM} = \frac{\text{pool concentration (ng}/\mu\text{L)}}{\text{average fragment size (bp)} \times 660} \times 1,000,000$$

24. Proceed to Library Denaturation and MiSeq®/MiniSeq® Sample Loading.

Library Denaturation and MiSeq®/MiniSeq® Sample Loading

MATERIALS

Pooled final library samples

User Supplied

- 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
 - 1-16 Samples: MiSeq® Reagent Micro Kit v2, 300 cycles, catalogue # MS-103-1002
- 1.0 N NaOH
- 200 mM Tris-HCl (pH7.0)
- Molecular grade water
- (Optional) PhiX diluted to 20pM or 1.8pM
- 1.5 mL tubes
- Ice
- Vortex
- Minicentrifuge

Instructions for Illumina® MiSeq® Instrument

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 (provided in the Illumina® Sequencing reagent kit) from the freezer and thaw on ice, store in ice at 2-8°C until ready to use.

Denature and Dilute

1. Dilute the pool to 4 nM in a final volume of 20 µL using the following calculations:

$$\text{Volume of sample pool to add } (\mu\text{L}) = \frac{80}{\text{concentration of sample pool (nM)}}$$

Volume of Molecular grade water to add (µL) = 20 µL - volume of sample pool added (µL)

2. Add 5 µL of the 4nM pool to a 1.5mL tube.
3. Add 5 µL of 0.2 N NaOH to the pool 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 then add 5 μ L of 200 mM Tris-HCl (pH 7.0).
7. Dilute to 20 pM by adding 985 μ L of chilled HT1.
8. Dilute to a final loading concentration of between 10-15pM. For example, the below calculations result in a 12 pM final library.
 - a) Add 360 μ L of the 20 pM pool to a 1.5 mL tube.
 - b) Add 235 μ L of chilled HT1.
 - c) Add 5 μ L of 20 pM PhiX.

! IMPORTANT: Due to variations in laboratory quantification analysis techniques, it may be necessary to optimize the loading concentration to achieve optimal cluster density and sequencing run metrics. Optimal final loading concentrations for the MiSeq[®] are likely to be between 10-15 pM.

9. Ensure the MiSeq[®] Reporter Software v2.6 or later is installed on the MiSeq[®] Instrument. This will allow alignment of your fastq files using BWA-MEM. Ensure on your samplesheet that next to 'RunBwaAln' you have 0 as your value.
10. Contact NGS@revvity.com to Download and set up the run sample sheet according to the Revvity Sample Sheet Template.

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

11. Sequence your library as indicated in the Illumina[®] MiSeq[®] System Guide.

! NOTE: 1 x 75bp reads lengths are recommended to achieve high mapping percentages while maintaining a run time of approximately 9 hours. Longer read lengths and sequencing runs using paired-end reads are compatible with PG- Seq[™] Rapid samples.

Instructions for Illumina[®] MiniSeq[®] Instrument

Procedure

! NOTE: Dilute the NaOH to 0.1 N by combining 90 μ L of molecular grade water with 10 μ L of 1.0N NaOH. This dilution should be made freshly before each use.

! NOTE: Remove HT1 (provided in the Illumina[®] Sequencing reagent kit) from the freezer and thaw on ice, store in ice at 2-8°C until ready to use.

Denature and Dilute

1. Dilute the pool to 1 nM in a final volume of 50 μ L using the following calculations:

$$\text{Volume of sample pool to add } (\mu\text{L}) = \frac{50}{\text{concentration of sample pool (nM)}}$$

Volume of Molecular grade water to add (μL) = $50 \mu\text{L}$ - volume of sample pool added (μL)

2. Add $5 \mu\text{L}$ of the 1 nM pool to a 1.5 mL tube.
3. Add $5 \mu\text{L}$ of 0.1 N NaOH to the same tube then vortex briefly.
4. Centrifuge at $280 \times g$ for 1 minute.
5. Incubate for 5 minutes at room temperature.
6. Place on ice then add $5 \mu\text{L}$ of 200 mM Tris-HCl ($\text{pH } 7.0$).
7. Dilute to 5 pM by adding $985 \mu\text{L}$ of chilled HT1.
8. Dilute to the final loading concentration. For example, the below calculations result in a 1.4 pM final library.
 - d) Add $140 \mu\text{L}$ of the 5 pM pool to a 1.5 mL tube.
 - e) Add $350 \mu\text{L}$ of chilled HT1.
 - f) Add $10 \mu\text{L}$ of 1.8 pM PhiX .

! IMPORTANT: Due to variations in laboratory quantification analysis techniques, it may be necessary to optimize the loading concentration to achieve optimal cluster density and sequencing run metrics. Optimal final loading concentrations for the MiniSeq® are likely to be between $1\text{-}2 \text{ pM}$.

9. Ensure the MiniSeq® Local Run Manager Software is installed on the MiniSeq® Instrument.
10. Load the PG-Seq™ Rapid Library Prep Kit by following the instructions under "Add a Library Prep Kit".
11. Set up your sequencing run in Local Run Manager by using the Create Run command and selecting the module Resequencing.
12. Sequence your library using the run you set up in Local Run Manager, as indicated in the Illumina® MiniSeq® System Guide using $1 \times 75 \text{ bp}$ read lengths.

Data Analysis with PG-Find™ Software

! NOTE: Fastq files generated from sequencing should be aligned to the human reference genome hg19 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 NGS@revvity.com for further assistance.

Please refer to the PG-Find™ software user guide for detailed information on download, installation and instructions for data analysis. Please contact NGS@revvity.com for a copy of the PG-Find™ software user guide.

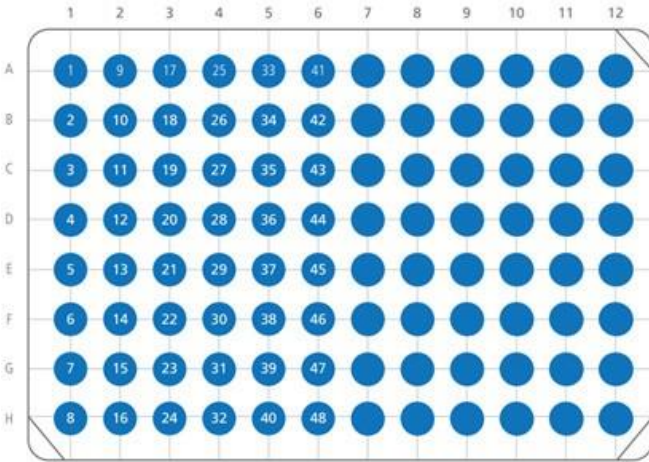
APPENDIX A

Appendix A - Index Sequences

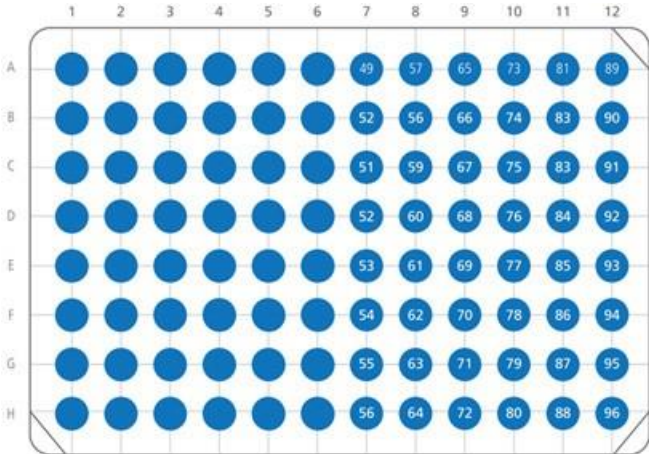
A complete list of index sequences can be found at https://resources.revity.com/pdfs/PG_Seq_Rapid_V2_96_Index_set.xlsx

For additional information, please email NGS@revity.com

4341-IL48A



4341-IL48B



| Plate Location | Index Number | 17 Index Sequence (5'-3') |
|----------------|--------------|---------------------------|
| A1 | 1 | TCTCTGTG |
| B1 | 2 | TGTACGTG |
| C1 | 3 | ATCGTCTG |
| D1 | 4 | TAGCTCTG |
| E1 | 5 | AGTATCTG |
| F1 | 6 | TCGAGCTG |
| G1 | 7 | TCATACTG |
| H1 | 8 | TACGACTG |
| A2 | 9 | ACTCACTG |
| B2 | 10 | AGAGTATG |
| C2 | 11 | AGCTGATG |
| D2 | 12 | TATCGATG |
| E2 | 13 | ATGCGATG |
| F2 | 14 | ACGTCATG |
| G2 | 15 | TCATGTCCG |
| H2 | 16 | TAGCGTCG |
| A3 | 17 | ATGACTCG |
| B3 | 18 | ATCTATCG |
| C3 | 19 | ACAGATCG |
| D3 | 20 | ATACTGCG |
| E3 | 21 | TATATGCG |
| F3 | 22 | TGCTCGCG |
| G3 | 23 | ATCGCGCG |
| H3 | 24 | TAGTAGCG |
| A4 | 25 | AGATAGCG |
| B4 | 26 | TGTGAGCG |
| C4 | 27 | TCACAGCG |
| D4 | 28 | ACTGTACG |
| E4 | 29 | TGCGTACG |
| F4 | 30 | TCGCTACG |
| G4 | 31 | TACTGACG |
| H4 | 32 | AGACGACG |
| A5 | 33 | TGTAGACG |
| B5 | 34 | ACGAGACG |

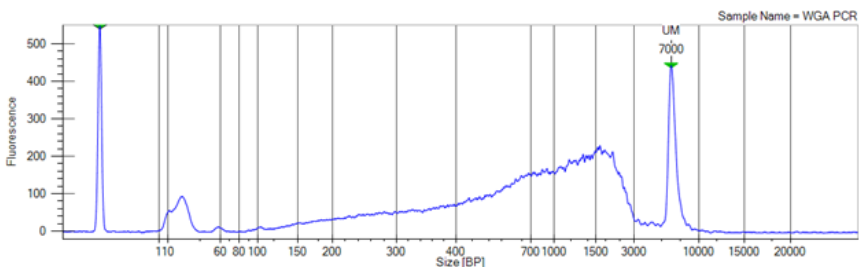
| | | |
|----|----|----------|
| C5 | 35 | ATATCACG |
| D5 | 36 | TCAGCACG |
| E5 | 37 | TAGACACG |
| F5 | 38 | AGCACACG |
| G5 | 39 | ATGTGTAG |
| H5 | 40 | ACTCGTAG |
| A6 | 41 | TGCAGTAG |
| B6 | 42 | TGATCTAG |
| C6 | 43 | TACGCTAG |
| D6 | 44 | TCGTATAG |
| E6 | 45 | AGACATAG |
| F6 | 46 | AGCGTGAG |
| G6 | 47 | ATGATGAG |
| H6 | 48 | ACATCGAG |
| A7 | 49 | TCTGCGAG |
| B7 | 50 | ATAGAGAG |
| C7 | 51 | TATCAGAG |
| D7 | 52 | ACAGTCAG |
| E7 | 53 | TCTATCAG |
| F7 | 54 | TAGTGCAG |
| G7 | 55 | TGACGCAG |
| H7 | 56 | TGCTACAG |
| A8 | 57 | AGTGACAG |
| B8 | 58 | ACTGTGTC |
| C8 | 59 | TACATGTC |
| D8 | 60 | ATGACGTC |
| E8 | 61 | AGCGAGTC |
| F8 | 62 | TCGCAGTC |
| G8 | 63 | ATACAGTC |
| H8 | 64 | TGCGTCTC |
| A9 | 65 | TCACTCTC |
| B9 | 66 | ATCTGCTC |
| C9 | 67 | TGTAGCTC |
| D9 | 68 | ACGTACTC |
| E9 | 69 | TCTGACTC |

| | | |
|-----|----|----------|
| F9 | 70 | ACGCTATC |
| G9 | 71 | ATCATATC |
| H9 | 72 | TCGTGATC |
| A10 | 73 | TGACGATC |
| B10 | 74 | TGCTCATC |
| C10 | 75 | TATGCATC |
| D10 | 76 | ACAGCATC |
| E10 | 77 | AGTACATC |
| F10 | 78 | AGTGCTGC |
| G10 | 79 | TGCGATGC |
| H10 | 80 | ATGCATGC |
| A11 | 81 | TCACATGC |
| B11 | 82 | AGAGTCGC |
| C11 | 83 | ACTATCGC |
| D11 | 84 | TAGATCGC |
| E11 | 85 | TCATGCGC |
| F11 | 86 | TACTACGC |
| G11 | 87 | ATATACGC |
| H11 | 88 | TGTCACGC |
| A12 | 89 | AGTCTAGC |
| B12 | 90 | ATGTGAGC |
| C12 | 91 | TAGCGAGC |
| D12 | 92 | ACACGAGC |
| E12 | 93 | TCTAGAGC |
| F12 | 94 | CACAGAGA |
| G12 | 95 | CACGTACA |
| H12 | 96 | CAGACGAT |

Appendix B - Quality Control

Whole Genome Amplification PCR 1

The PG-Seq™ Rapid kit v2 WGA PCR 1 generates whole genome amplified products appearing as a smear, ranging in size from 200 - 3000 bp with a mode fragment size of approximately 1500 bp. The NTC should appear clean, with the presence of primer dimers.



Typical yield following WGA PCR 1 for a 5-10 cell sample is approximately 90 ng/μL or between 2 - 4 μg total DNA (quantified using Qubit® 4 Fluorometer and Qubit® 1X dsDNA HS Assay Kit). Yield may vary depending on the sample type, sample DNA quality, volume and composition of the transfer buffer used to place the cells in the tube

A failed WGA PCR 1 result is indicated by the presence of primer dimers, but no evidence of a smear of amplification products. It is recommended that failed samples are discarded. Poor WGA PCR 1 amplification is indicated by smears with lower intensity, lower yield or products that are notably larger or smaller than the expected size range. The results from these samples should be interpreted with caution.

Causes of failed or weak WGA may include:

- Cells not transferred correctly into the bottom of the PCR tube.
- Cell/DNA damage.
- Too much transfer buffer added to the PCR tube during cell to tube transfer.
- PCR inhibitors present in the sample (e.g. EDTA, Ca⁺, proteins, embryo culture media, ethanol).

Indexing PCR 2

The PG-Seq™ Rapid Kit v2 Indexing PCR 2 generates products appearing as a smear with a similar size to the WGA PCR 1 products. After size selection, individual samples or a pool of samples are expected to have a yield of approximately 3-8 ng/μL (quantified using Qubit® 4 Fluorometer and Qubit® 1X dsDNA HS Assay Kit) with an average fragment size of approximately 420 bp.

Analysis

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 chromosomal aberrations down to 7Mb in size. 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.

| Metric | Each Sample | | |
|--|-----------------|--|---------------|
| <p>Total Reads</p> <p>The total number of reads assigned to a particular sample after demultiplexing. Ideally samples will have 500,000 total reads or greater to achieve a resolution of 7Mb.</p> | | 500,000 | |
| <p>% Mapped Reads</p> <p>The percentage of reads which successfully align to hg19. Lower values can indicate non-human contamination in your final library DNA</p> | | > 95% | |
| <p>% Useable Reads</p> <p>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.</p> | | > 80% | |
| <p>Quality</p> <p>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 sample lysis and preparation.</p> | Number of Reads | Bin width (PG-Find Self Reference Algorithm) | Quality Score |
| | 500,000 | 500,000 bp | <0.1200 |



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