PG-Seq™ Core Panel

PGT-M Library Preparation Kit for Illumina® and Element® instruments

KIT CONTAINS: 24 RXNS

#5340-0124 (24 rxn, Barcodes 1-24)

#5341-0124 (24 rxn, Barcodes 25-48)

#5342-0124 (24 rxn, Barcodes 49-72)

#5343-0124 (24 rxn, Barcodes 73-96)

PG-Seq™ Core Panel

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GENERAL INFORMATION

Revision History

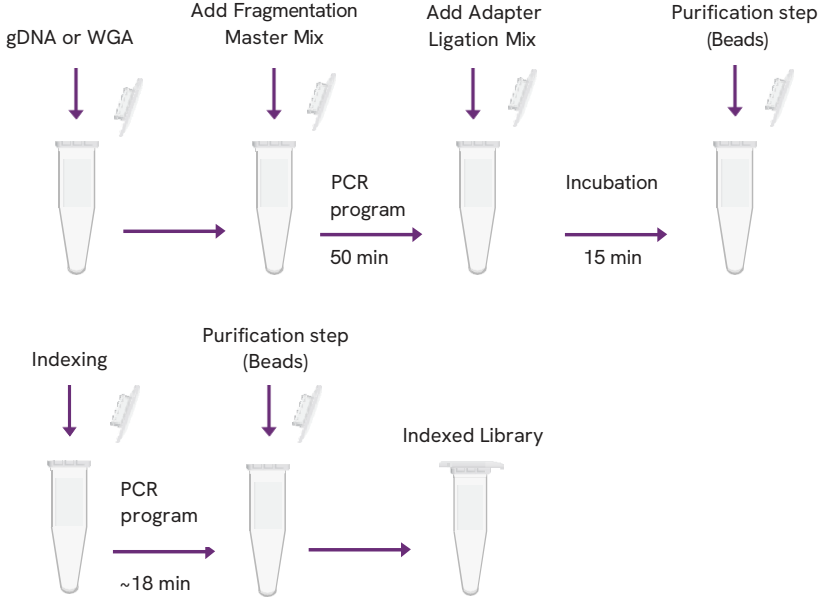
Version	Date	Description
v1.0	March 2025	Product Launch

Product Overview

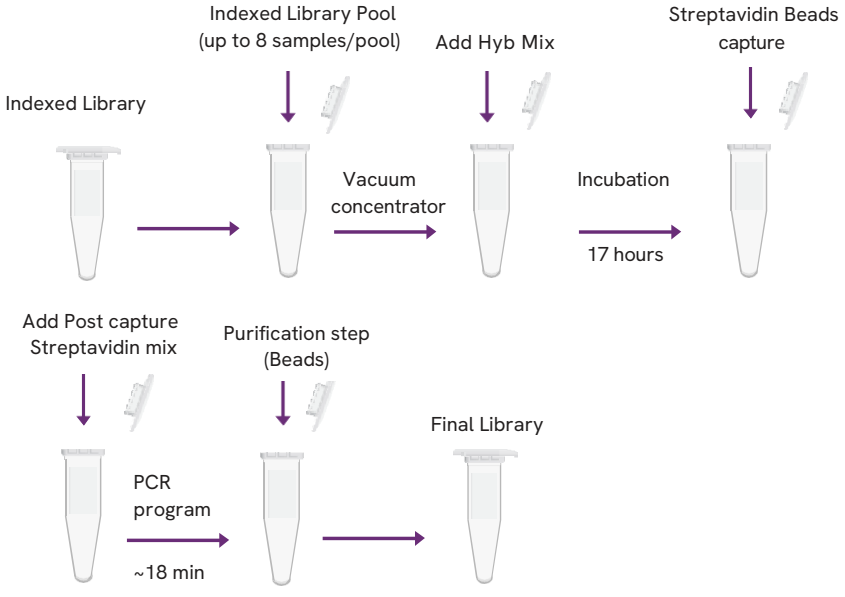
The PG-Seq™ Core Panel analyzes 8 commonly targeted genes in monogenic disorders for PGT-M studies: ***BRCA1***, ***BRCA2***, ***CFTR***, ***DMD***, ***F8***, ***FMR1***, ***GJB2*** and ***HBB***. Starting with an input of 50 ng of genomic DNA (gDNA) or whole genome amplified DNA (WGA) from an embryo biopsy, the kit provides all the reagents for library preparation hybridization, capture and data analysis for implementation of PGT-M on Illumina® and Element Biosciences® sequencing platforms. The kit is for Research Use Only and should not be used in diagnostic procedures.

The protocol follows a standard hybridization enrichment workflow that can be divided in 2 parts. In the first part, libraries are generated from the DNA sample. In the second part, libraries are hybridized with probes designed to target the regions of interest, followed by capture with streptavidin beads and amplified before sequencing.

PART 1: LIBRARY GENERATION FROM gDNA OR WGA PRODUCT



PART 2: TARGET ENRICHMENT



Kit Contents and Storage

The PG-Seq™ Core Panel kit contains reagents to prepare 24 samples for Illumina® and Element Biosciences® sequencing platforms. 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
Fragmentation Enzyme	CLEAR	-20°C	240 µL
Fragmentation Buffer	CLEAR	-20°C	120 µL
Ligase Enzyme Mix	PURPLE	-20°C	240 µL
Ligase Buffer Mix	PURPLE	-20°C	480 µL
Universal Adapter	PURPLE	-20°C	120 µL
UDI Primers	N/A (Plate)	-20°C	10 µL per well
Amplification Mix	GREEN	-20°C	675 µL
Hybridization Mix	YELLOW	-20°C	60 µL
Core Panel	YELLOW	-20°C	12 µL
Hybridization Boost	YELLOW	-20°C	60 µL
Universal Blocker	YELLOW	-20°C	36 µL
Blocking Solution	GREY	-20°C	15 µL
Purification Beads	BOTTLE	2-8°C	3.8 mL
Streptavidin Beads	BLUE	2-8°C	300 µL
Binding Buffer	YELLOW	-20°C	(2) 1200 µL
Wash Buffer 1	YELLOW	-20°C	600 µL
Wash Buffer 2	YELLOW	-20°C	(2) 1050 µL
Amplification Primers	GREEN	-20°C	1200 µL

Required Materials Not Provided

- Absolute ethyl alcohol (EtOH, undenatured) to make 80% ethanol
- Molecular grade water
- Sterile, molecular grade tubes (1.5mL or larger)
- PCR thin-walled reaction tube (0.5mL or 0.2mL)
- Cold block 1.5-mL and 96-well compatible (optional)
- Magnetic stand for 96-well plates or 1.5mL tubes
- 96-well plate, compatible with magnetic stand
- Qubit dsDNA High Sensitivity Quantitation Assay or equivalent
- Qubit dsDNA Broad Range Quantitation Assay or equivalent
- Agilent High Sensitivity DNA Kit or equivalent
- Agilent DNA 7500 Kit or equivalent
- Pipettes (P10, P20, P200, P1000 µL)
- Pipette tips (low binding barrier filter)
- Vortex
- Minicentrifuge
- Thermomixer for 1.5-mL tubes (or equivalent)
- Thermal cycler (with hot lid)
- Fluorometer (Qubit 3.0 or equivalent)
- LabChip® GXII Touch™ Nucleic Acid Analyzer or equivalent

Warnings & Precautions

We strongly recommend that you read the following warnings and precautions. Periodically, optimizations and revisions are made to the components and manual. Therefore, it is important to follow the protocol included with the kit. If you need further assistance, you may contact your local distributor, or contact us at https://www.revvity.com/contact-us/technical-support and choose the "Next Gen Sequencing" category.

- Do not use the kit past the expiration date.
- Try to maintain a laboratory temperature of 20-25°C.
- Wear appropriate protective equipment (lab coat, gloves, and protective glasses or goggles) always when performing this protocol.
- For best results, read this document before performing the protocol, and follow the instructions provided.
- This library preparation method may yield more material than needed for target enrichment. Excess product can be stored at –20°C for later use.
- Do NOT mix or combine the same reagents from the different lots.
- 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.
- Do not freeze Beads. Beads should be stored at 2-8 °C.
- Allow Beads to come to room temperature and vortex the beads until the liquid appears homogeneous before every use.
- Test the compatibility of your thermal cycler and PCR tubes by incubating at 95°C for up to 5 minutes to ensure the PCR tubes do not crack under heat and pressure. Adjust the tightness of the thermal cycler lid and/or use a spacer specific to the thermal cycler model.

Starting Materials

The PG-Seq™ Core Panel has been developed using as input 50 ng of either gDNA or purified WGA (generated by the PG-Seq™ Rapid v2 Kit or DOPlify® v2 Whole Genome Amplification Kit), offering a combined PGT-A and PGT-M analysis from a single research sample. Purified WGA, free of cations and chelating agents, should be diluted to a final concentration of 5 ng/µL. If required, purified gDNA samples can be used and should be diluted to a final concentration of 5 ng/µL with water or 10 mM Tris-HCl pH 8. 10 µL of freshly diluted DNA can be then added to the Enzymatic Fragmentation step. As in the previous case, purified gDNA should be free of cations and chelating agents.

PART 1 - LIBRARY GENERATION FROM gDNA OR WGA PRODUCT

Step 1: Fragmentation, End-repair & Adenylation

Reagents Required

- DNA sample: purified gDNA or WGA product (50 ng)
- Molecular grade water (chilled)
- Qubit dsDNA Broad Range Quantitation Assay (or equivalent)
- Fragmentation Enzyme
- Fragmentation Buffer
- 10 mM Tris-HCl pH 8 (Optional)

Before you begin

- Thaw Fragmentation Enzyme and gDNA samples on ice, then mix by flicking the tube with a finger.
- Thaw Fragmentation Buffer on ice, then mix by pulse vortexing for 2 seconds.

Procedure

1. Program the thermal cycler with the following conditions. Set the temperature of the heated lid to 70°C. Start the program to pre-chill the thermal cycler.

Step	Time	Temperature	
1	HOLD	4°C	Total cycle time is approx. 50 minutes
2	10 mins	32°C	
3	30 mins	95°C	
4	HOLD	4°C	

2. Use the Qubit dsDNA Broad Range Quantitation Assay or equivalent to determine the concentration of your gDNA samples.
3. Dilute the gDNA or WGA samples to 5 ng/µL in water or 10 mM Tris-HCl pH 8. Mix well with gentle pipetting.
4. Add 10 µL of each diluted gDNA or WGA sample (50 ng total DNA) into a thin-walled PCR tube or 96-well plate, and place on ice.
5. Pulse-spin to ensure all the solution is at the bottom of the tube or 96-well plate.
6. Prepare an enzymatic fragmentation master mix in a 1.5mL microcentrifuge tube on ice. Use the volumes listed below. Mix thoroughly by gentle pipetting.

Component	Volume
Water (chilled)	25 µL
Fragmentation Buffer	5 µL
Fragmentation Enzyme	10 µL
Total	40 µL

Prepare a master mix for multiple reactions. Add 10% overage to compensate for pipetting loss.

7. Add 40 µL enzymatic fragmentation master mix from previous step to each 10 µL DNA sample well or tube and mix well by gentle pipetting. Cap the tube and keep the reaction on ice.
8. Pulse-spin the sample plate or tubes and immediately transfer to the pre-chilled thermal cycler.
9. Proceed to steps 2–4 of the thermal cycler program (32°C step of the thermocycler program in Step 1 above).

! IMPORTANT: While the thermal cycler program is running, prepare the reagents for Step 2: Adapter Ligation

10. When the thermal cycler program is complete and the sample block has returned to 4°C, remove the samples from the block and place on ice.
11. Proceed immediately to Step 2. Adapter Ligation.

Step 2: Adapter Ligation

Reagents Required

- dA-tailed DNA fragments (from Step 1)
- Ethanol
- Molecular grade water (chilled)
- Ligase Enzyme Mix
- Ligase Buffer Mix
- Universal Adapter
- Purification Beads
- 10 mM Tris-HCl pH 8 (Optional)

Before you begin

- Thaw on ice: Ligase Enzyme Mix, Ligase Buffer Mix, and Universal Adapter.
- Prepare 1 mL 80% ethanol for each sample (for use in Steps 2 and 3 of the protocol).
- Equilibrate Purification Beads to room temperature for at least 30 minutes (for use in Steps 2 and 3 of the protocol).
- Program a thermal cycler to incubate the samples at 20°C with the heated lid set to minimum temperature or turned off. Start the program so that the cycler is at 20°C when the samples are prepared.

Procedure

1. Add 5 µL Universal Adapter into each sample well or tube containing the dA-tailed DNA fragments for Step 1. 11. Mix gently by pipetting and keep on ice.
2. Prepare the ligation master mix in a 1.5-mL microcentrifuge tube on ice as indicated below. Mix well by gentle pipetting.

Component	Volume
Water (chilled)	15 µL
Ligase Buffer Mix	20 µL
Ligase Enzyme Mix	10 µL
Total	45 µL

Prepare a master mix for multiple reactions. Add 10% overage to compensate for pipetting loss.

3. Add 45 µL of the ligation master mix to the sample from Step 2.1 and mix well by gentle pipetting.
4. Seal or cap the tubes and pulse-spin to ensure all solution is at the bottom of the tube
5. Incubate the ligation reaction at 20°C for 15 minutes in the thermal cycler, then move the samples to the bench top.

! IMPORTANT: Turn off the heated lid or set to minimum temperature. While the thermal cycler program is running, prepare the reagents for Step 3 (see Step 3: Post-Ligation PCR).

6. Vortex the pre-equilibrated Purification Beads until well mixed.
7. Add 80 µL (0.8x) of homogenized Purification Beads to each ligation sample. Mix well by vortexing.
8. Transfer the entire volume of each ligation mixed with the Purification Beads to a clean 1.5-mL microcentrifuge tube for each sample.
9. Incubate the samples for 5 minutes at room temperature.
10. Place the samples on a magnetic stand for 1 minute or until the supernatant is clear.
11. The Purification Beads form a pellet, leaving a clear supernatant. Without removing plate or tubes from the magnetic stand, remove and discard the supernatant.
12. Wash the bead pellet by gently adding 200 µL freshly prepared 80% ethanol (do not disturb the pellet). Incubate for 1 minute, then remove and discard the ethanol.
13. Repeat the wash once, for a total of two washes, while keeping the samples on the magnetic stand.
14. Carefully remove all remaining ethanol with a 10-µL pipet, making sure not to disturb the bead pellet.

! IMPORTANT: Before pipetting, the bead pellet may be briefly spun to collect ethanol at the bottom of the plate or tube and returned to the magnetic plate.

15. Air-dry the bead pellet on the magnetic stand for 5 minutes or until the bead pellet is dry. Do not overdry the bead pellet.
16. Remove the plate or tubes from the magnetic stand and add 17 µL of water or 10 mM Tris-HCl pH 8 to each sample. Mix by pipetting until homogenized.
17. Transfer the entire volume of each 1.5-mL microcentrifuge tube to a clean thin-walled PCR 0.2-ml strip-tube or well of a 96-well thermal cycling plate.
18. Incubate at room temperature for 2 minutes.
19. Place the plate or tubes on a magnetic plate and let stand for 3 minutes or until the beads form a pellet.
20. Transfer 15 µL of the clear supernatant containing the ligated and indexed libraries to a clean thin-walled PCR 0.2-mL strip-tube or well of a 96-well thermal cycling plate, making sure not to disturb the bead pellet.
21. Proceed immediately to Step 3: Post-Ligation PCR.

Step 3: Post-Ligation PCR

Reagents Required

- Ligated, adapted libraries (from Step 2)
- 80% Ethanol
- Equilibrated Purification Beads
- Molecular grade water
- 2xAmplification Mix
- UDI Primers
- 10 mM Tris-HCl pH 8 (Optional)

Before you begin

- Thaw on ice: UDI Primers (single use primers), Amplification Mix

Procedure

1. Program a thermal cycler with the following conditions. Set the temperature of the heated lid to 105°C.

Step	Time	Temperature	Cycles	
Initial Denaturation	45 sec	98°C	1 cycle	
Denaturation	15 sec	98°C		
Annealing	30 sec	60°C	6 cycles	Cycling time is approximately 18 minutes
Extension	30 sec	72°C		
Final Extension	1 min	72°C	1 cycle	
Final Hold	HOLD	4°C	-	

2. Add 10 µL of a unique UDI Primer from the 96-well plate to each of the gDNA libraries from Step 2.20 and mix well by gentle pipetting.

! IMPORTANT: For index selection refer to Appendix A.

3. Add 25 µL of 2x Amplification Mix to the gDNA libraries from Step 3.2 and mix well by gentle pipetting.
4. Pulse-spin sample plate or tube and immediately transfer to the thermal cycler. Start the program.
5. Remove the sample(s) from the block when the thermal cycler program is complete. Proceed to purification.
6. Vortex the pre-equilibrated Purification Beads until mixed.
7. Add 50 µL (1x) of homogenized Purification Beads to each ligation sample from Step 3.5. Mix well by vortexing.

8. Incubate the samples for 5 minutes at room temperature.
9. Place the samples on a magnetic plate for 1 minute.
10. The Purification Beads form a pellet, leaving a clear supernatant. Without removing plate or tubes from the magnetic plate, remove and discard the supernatant.
11. Wash the bead pellet by gently adding 200 µL freshly prepared 80% ethanol (do not disturb the pellet), incubate for 1 minute, then remove and discard the ethanol.
12. Repeat this wash once, for a total of two washes, while keeping the samples on the magnetic plate.
13. Carefully remove all remaining ethanol with a 10-µL pipet, making sure not to disturb the bead pellet.

    ! IMPORTANT: Before pipetting, the bead pellet may be briefly spun to collect ethanol at the bottom of the plate or tube and returned to the magnetic plate.

14. Air-dry the bead pellet on the magnetic plate for 5 minutes or until the bead pellet is dry. Do not over dry the bead pellet.
15. Remove the plate or tubes from the magnetic plate and add 22 µL water or 10 mM Tris-HCl pH 8 to each sample. Mix by pipetting until homogenized.
16. Incubate at room temperature for 2 minutes.
17. Place the plate or tubes on a magnetic plate and let stand for 3 minutes or until the beads form a pellet.
18. Transfer 20 µL of the clear supernatant containing the Amplified Indexed Libraries to a clean thin-walled PCR 0.2-mL strip-tube or well of a 96-well thermal cycling plate, making sure not to disturb the bead pellet.
19. Quantify and validate the size range of each library using the Thermo Fisher Scientific Qubit dsDNA Broad Range Quantitation Assay or equivalent and LabChip® GXII Touch™ Nucleic Acid Analyzer or equivalent.Final concentration values should be ≥80 ng/µL, and average fragment length should be 375–425 bp using a range setting of 150–1000 bp (Figure 1).

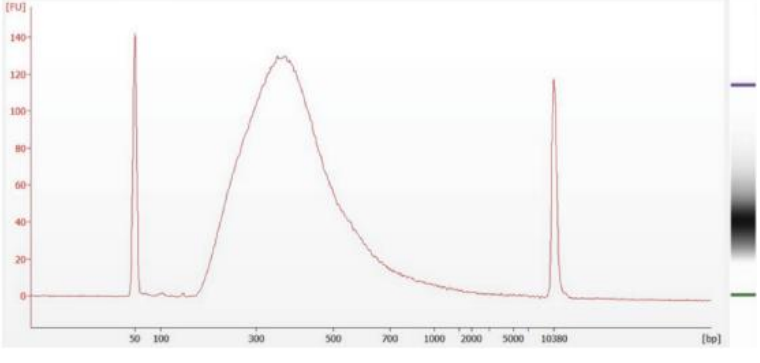


Figure 1. Electropherogram of library samples that were prepared as described. Note the single prominent peak at ~400 bp.

! IMPORTANT: If the average fragment length is not in the range of 375–425 bp:

- Input DNA concentration may be inaccurate. Typically, using too much DNA leads to shorter fragments, while not using enough DNA leads to larger fragments.
- The presence of cations and chelators may also affect the average fragment length.
- If neither of the above factors apply, optimize the 32°C fragmentation in Step 1.1 by changing the time in 3 minute increments - increase time to produce shorter fragments, and decrease time to produce longer fragments.

STOPPING POINT. If not proceeding immediately to Target Enrichment store the amplified indexed libraries at –20°C.

PART 2 - TARGET ENRICHMENT

Step 1: Prepare Libraries for Hybridization

Reagents Required

- Amplified, indexed libraries (from previous Step)

Before you begin

This step involves aliquoting the appropriate amount of amplified, indexed libraries (generated previously in library preparation) and preparing the hybridization reaction solution.

Procedure

! IMPORTANT: This protocol supports a single or multiplex (up to 8-plex) hybridization capture. The amount of indexed library to use depends on the number of indexed samples per pool.

1. Use the concentration of each amplified, indexed library to calculate the volume (in µL) of each library needed for hybridization:
    - Determine the amount of each indexed library per pool from the table below.
    - Divide the amount of each indexed library per pool by the concentrations measured in ng/µL from the library preparation.

    For example, if multiplexing eight libraries per hybridization reaction, the amount of each library will be 187.5 ng, and the total mass of the pool will be 1,500 ng.

Number of indexed samples per pool	Amount of each indexed library	Total mass per pool
1	500 ng	500 ng
2	500 ng	1,000 ng
3	500 ng	1,500 ng
4	375 ng	1,500 ng
8	187.5 ng	1,500 ng

! IMPORTANT: More than 1,500 ng (1.5 µg) total DNA can be used; do not, however, use more than 4 µg total DNA as this might lead to reduced performance of the enrichment.

2. Transfer the calculated volumes from each amplified indexed library to an indexed library pool reaction tube for each hybridization being performed. Clean, thin-walled PCR 0.2-ml strip-tube or well of a 96- well thermal cycling plate are recommended to avoid unnecessary transfers in downstream steps.

   ! IMPORTANT: Check for a proper seal on the tube(s) as evaporation may occur leading to decreased performance.

3. Pulse-spin the indexed library pool tube(s) to minimize the amount of bubbles present.
4. Dry the indexed library pool(s) using a vacuum concentrator using low or no heat.

STOPPING POINT. If not proceeding immediately to Step 2 store the amplified indexed libraries at –20°C.

Step 2: Hybridize Capture Probes with Pools

Reagents Required

- Dry-indexed library pool tube(s) (from Step 1.4)
- Hybridization mix
- Core Panel
- Blocking solution
- Universal Blocker
- Hybridization Boost

Before you begin

- Thaw on ice: Hybridization Mix, Blocking solution, Universal Blocker, Hybridization Boost. Then pulse-vortex for 2 seconds to mix and pulse-spin.
- Program a thermal cycler or heat block to incubate Hybridization Mix at 65°C with the heated lid set to minimum temperature or turned off. Start the program so that the cycler is at 65°C.
- Program a 96-well thermal cycler to 95°C and set the heated lid to 105°C.

Procedure

1. Heat the Hybridization Mix at 65°C in the heat block for 10 minutes, or until all precipitate is dissolved, then cool to room temperature on the benchtop for 5 minutes.
2. Prepare a probe solution per pool in a clean thin-walled PCR 0.2-mL strip-tube or well of a 96-well thermal cycling plate as indicated in the table below. Mix by flicking the tube(s).

Component	Volume per pool
Water	4 µL
Core Panel	4 µL
Hybridization Mix	20 µL
Total	28 µL

! IMPORTANT:
- Hybridization Mix is very viscous. Pipette slowly to ensure accurate pipetting.
- Small white particles may be present in the Core Panel tube. This will not affect the final capture product.

3. Resuspend the dried indexed library pool by adding the reagents described below. Mix by flicking the tube(s).

Reagent	Volume per pool
Dried Indexed Library Pool	-
Blocking Solution	5 µL
Universal Blocker	7 µL
Total	12 µL

4. Heat the probe solution to 95°C for 2 minutes in a thermal cycler with the lid at 105°C, then immediately cool on ice for 5 minutes.
5. While probe solution is cooling on ice, heat the tube containing the resuspended indexed library pool at 95°C for 5 minutes in a thermal cycler with the lid at 105°C, then equilibrate both the probe solution and resuspended indexed library pool to room temperature on the benchtop for 5 minutes.
6. Vortex and spin down the probe solution, then transfer the entire volume to the resuspended indexed library pool. Mix well by vortexing.
7. Pulse-spin the tube(s) to ensure all solution is at the bottom of the tube(s).
8. Add 30 µL Hybridization Boost to the top of the entire capture reaction.
9. Pulse-spin the tube(s) to ensure there are no bubbles present.

   ! IMPORTANT: Seal the tube(s) tightly to prevent excess evaporation over the 16-hour incubation.

10. Incubate the hybridization reaction at 70°C for 16 hours in a thermal cycler with the lid at 85°C.

    ! IMPORTANT: Halting hybridization between 15-17 hours will not affect downstream capture quality.

11. Proceed to Step 3. Bind Hybridized Targets to Streptavidin Beads.

Step 3: Bind Hybridized Targets to Streptavidin Beads

Reagents Required

- Hybridization reactions (from Step 2.10)
- Amplification Mix
- Amplification Primers
- Binding Buffer
- Wash Buffer 1
- Wash Buffer 2
- Streptavidin Beads
- Purification Beads

Before you begin

- Preheat the following tubes at 48°C until any precipitate is dissolved:
  - Binding Buffer
  - Wash Buffer 1
  - Wash Buffer 2
- For each hybridization reaction:
  - Equilibrate 800 µL Binding Buffer to room temperature.
  - Equilibrate 200 µL Wash Buffer 1 to room temperature.
  - Leave 700 µL Wash Buffer 2 at 48°C.
- Equilibrate the Streptavidin Beads to room temperature for at least 30 minutes.
- In preparation for Step 4, thaw on ice:
  - Amplification Mix
  - Amplification Primers
- Equilibrate Purification Beads to room temperature for at least 30 minutes.

Procedure

! IMPORTANT: Prepare the washed Streptavidin Beads before hybridization ends.

12. Vortex the pre-equilibrated Streptavidin Beads until mixed.
13. Add 100 µL Streptavidin Beads to a 1.5-mL microcentrifuge tube. Prepare one tube for each hybridization reaction.
14. Add 200 µL Binding Buffer to the tube(s) and mix by pipetting.
15. Place the tube(s) on a magnetic stand for 1 minute, then remove and discard the clear supernatant. Make sure to not disturb the bead pellet. Remove the tube from the magnetic stand.
16. Repeat the wash (Steps 3.13 and 3.14) two more times for a total of three washes.
17. After removing the clear supernatant from the third wash, add a final 200 µL Binding Buffer and resuspend the beads by vortexing until homogenized. Now, the washed

Streptavidin Beads are prepared.

18. After the hybridization (Step 2.10) is complete, open the thermal cycler lid and directly transfer the volume of each hybridization reaction into a corresponding tube of washed Streptavidin Beads from Step 3.6. Mix by pipetting and flicking.

! IMPORTANT: Rapid transfer directly from the thermal cycler at 70°C is a critical step for minimizing off-target binding. Do not remove the tube(s) of hybridization reaction from the thermal cycler or otherwise allow it to cool to less than 70°C before transferring the solution to the washed Streptavidin Beads. Allowing to cool to room temperature for less than 5 minutes will result in as much as 10–20% increase in off-target binding.

19. Mix the tube(s) of the hybridization reaction with the Streptavidin Beads for 30 minutes at room temperature on a shaker, rocker, or rotator at a speed sufficient to keep the solution mixed.

! IMPORTANT: Do not vortex. Aggressive mixing is not required.

20. Remove the tube(s) containing the hybridization reaction with Streptavidin Beads from the mixer and pulse-spin to ensure all solution is at the bottom of the tube(s).
21. Place the tube(s) on a magnetic stand for 1 minute.
22. Remove and discard the clear supernatant including the Hybridization Boost. Do not disturb the bead pellet.

! IMPORTANT: Some Hybridization Boost may be visible after supernatant removal and throughout each wash step. It will not affect the final capture product.

23. Remove the tube(s) from the magnetic stand and add 200 µL Wash Buffer 1. Mix by pipetting.
24. Pulse-spin to ensure all solution is at the bottom of the tube(s).
25. Transfer the entire volume from Step 3.13 (~200 µL) into a new 1.5-mL microcentrifuge tube, one per hybridization reaction. Place the tube(s) on a magnetic stand for 1 minute.

! IMPORTANT: This step reduces background from non-specific binding to the surface of the tube.

26. Remove and discard the clear supernatant. Make sure to not disturb the bead pellet.
27. Remove the tube(s) from the magnetic stand and add 200 µL of 48°C Wash Buffer 2. Mix by pipetting, then pulse-spin to ensure all solution is at the bottom of the tube(s).
28. Incubate the tube(s) for 5 minutes at 48°C.
29. Place the tube(s) on a magnetic stand for 1 minute.
30. Remove and discard the clear supernatant. Make sure to not disturb the bead pellet.
31. Repeat the wash (Steps 3.13–3.14) two more times, for a total of three washes.
32. After the final wash, use a 10 µL pipette to remove all traces of supernatant. Proceed immediately to the next step. Do not allow the beads to dry.

! IMPORTANT: Before removing supernatant, the bead pellet may be briefly spun to collect supernatant at the bottom of the tube or plate and returned to the magnetic plate.

33. Remove the tube(s) from the magnetic stand and add 45 µL water. Mix by pipetting

until homogenized, then incubate this solution, hereafter referred to as the Streptavidin suspension, on ice.

34. Proceed to Step 4. Post-capture PCR amplify, purify and perform QC.

Step 4: Post-capture PCR amplify, purify and perform QC

Reagents Required

- Streptavidin suspension (from Step 3.33)
- Ethanol
- Molecular biology grade water
- Purification Beads
- 2xAmplification Mix
- Amplification Primers
- 10 mM Tris-HCl pH 8 (Optional)
- LabChip® GXII Touch™ Nucleic Acid Analyzer or equivalent
- Thermo Fisher Scientific Qubit dsDNA High Sensitivity Quantitation Assay

Before you begin

- Prepare 500 µL 80% ethanol for each Streptavidin suspension to be processed.

Procedure

1. Program a thermal cycler with the following conditions. Set the heated lid to 105°C.

Step	Time	Temperature	Cycles	
Initial Denaturation	45 sec	98°C	1 cycle	
Denaturation	15 sec	98°C		
Annealing	30 sec	60°C	13* cycles	Cycling time is approximately 18 minutes
Extension	30 sec	72°C		
Final Extension	1 min	72°C	1 cycle	
Final Hold	HOLD	4°C	-	

* If Single-plex is performed, the number of cycles is 14.

2. If the Streptavidin suspension has settled, mix by pipetting.
3. Transfer 22.5 µL of the Streptavidin suspension to a 0.2-ml thin-walled PCR strip-tube(s). Keep on ice until ready to use in the next step.

! IMPORTANT: Store the remaining 22.5 µL water/ Streptavidin suspension at –20°C for future use.

4. Prepare a PCR mixture by adding the following reagents to the tube(s) containing the Streptavidin suspension. Mix by pipetting.

Component	Volume per pool
Streptavidin Suspension (from previous step)	22.5 µL
Amplification Primers	2.5 µL
Amplification Mix	25 µL
Total	50 µL

5. Pulse-spin the tubes, transfer them to the thermal cycler and start the cycling program.
6. When the thermal cycler program is complete, remove the tube(s) from the block and immediately proceed to the Purify step.
7. Vortex the pre-equilibrated Purification Beads until well mixed.
8. Add 50 µl (1.0x) homogenized Purification Beads to the tube(s) from Step 4.6. Mix well by vortexing.

! IMPORTANT: It is not necessary to recover supernatant or remove Streptavidin Beads from the amplified PCR product.

9. Incubate for 5 minutes at room temperature.
10. Place the tube(s) on a magnetic plate for 1 minute or until the supernatant is clear.
11. The Purification Beads form a pellet, leaving a clear supernatant. Without removing the plate or tube(s) from the magnetic plate, remove and discard the clear supernatant.
12. Wash the bead pellet by gently adding 200 µL freshly prepared 80% ethanol (do not disturb the pellet). Incubate for 1 minute, then remove and discard the ethanol.
13. Repeat this wash once, for a total of two washes, while keeping the tube on the magnetic plate.
14. Carefully remove all remaining ethanol using a 10 µL pipette, making sure to not disturb the bead pellet.

! IMPORTANT: Before pipetting, the bead pellet may be briefly spun to collect ethanol at the bottom of the plate or tube and returned to the magnetic plate.

15. Air-dry the bead pellet on the magnetic plate for 5 minutes or until the bead pellet is dry. Do not over dry the bead pellet.
16. Remove the tube(s) from the magnetic plate and add 32 µl water or 10 mM Tris-HCl pH 8 to each capture reaction. Mix by pipetting until homogenized.
17. Incubate at room temperature for 2 minutes.
18. Place the plate or tube(s) on a magnetic plate and let stand for 3 minutes or until the beads fully pellet.
19. Transfer 30 µL of the clear supernatant containing the enriched library to a clean thin-walled PCR 0.2-ml strip-tube or well of a 96-well thermal cycling plate, making sure to not disturb the bead pellet.
20. Validate and quantify each enriched library using LabChip® GXII Touch™ Nucleic Acid Analyzer or equivalent and a Thermo Fisher Scientific Qubit dsDNA High Sensitivity Quantitation Assay (Figure 2).

! IMPORTANT: When using the Agilent Bioanalyzer High Sensitivity DNA Kit, load 0.5 µL of the final sample. Average fragment length should be 375–425 bp using a range setting of 150–1,000 bp. Final concentration may vary and is dependent on panel size, library input, hybridization reaction size, and the number of PCR cycles.

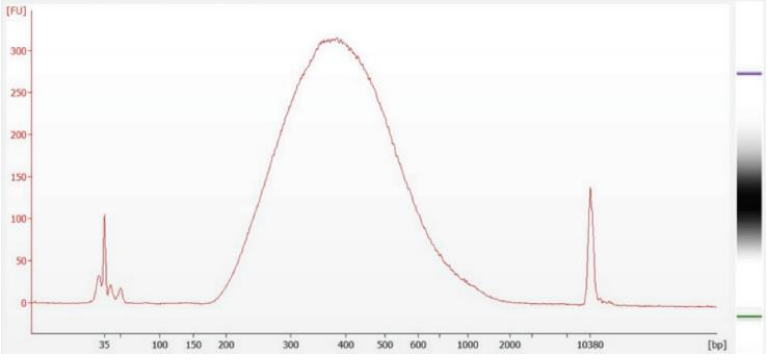


Figure 2. Electropherogram of final library samples that were prepared as described. Note the single prominent peak at ~400 bp.

STOPPING POINT. If not proceeding immediately, store the enriched library sample at –20°C for up to 24 hours.

RUN SETTING

Denaturate and load your libraries as indicated in the Illumina® System Guide. 2x150 bp configuration is recommended to achieve high mapping percentages. Recommended minimum reads per sample is 1.4 M (paired end reads) to achieve recommended coverage and uniformity (as verified/tested with embryonic samples). Lower values may compromise the results. This means that on a Miseq v2 flow cell (that generates 30 M paired-end reads) can be loaded up to 21 samples, on a Miseq v3 flow cell up to 35.

To set the run, login to Basespace, then Runs > New Run > Run Planning

Run Settings	• Write the "Run Name" • Select "Instrument Platform" • In "Secondary Analysis select" *Local.*
Configuration	• Application "*DRAGEN Enrichment – 4.2.*" • Library Prep "Add Custom Library Prep Kit" and name it as you want. Same with Index Adapter Kit • Index Reads "2 indexes" • Read Type "Paired End" • Read Lengths 151 (read 1 & 2) 10 (index 1 & 2) • Add to the table the samples you want to run
Analysis Settings	• FASTQ Compression Format "*gzip*" • Reference Genome "Homo sapiens [1000 Genomes] hg38 Alt Masked v3" • Targeted Regions "*PGSeqCorePanel-hg38.240806.bed*" • Variant Caller "Germline" • Enable CNV calling "No" • Map/Align Output Format "BAM" • Keep FASTQ Files "Yes"

Following Illumina® sequencing, the potential genetic mutations of the samples for the targeted genes are analyzed and visualized with the PG-Seq™ Core Panel software. The gVCF files generated by the sequencer are used as input. Please refer to the software manual for additional information regarding data analysis.

If you need assistance, please contact us at https://www.revvity.com/contact-us/technical-support and choose the "Next Gen Sequencing" category.

Appendix A – Index Sequences

A complete list of index sequences can be found at https://resources.revvity.com/pdfs/bar-pg-seq-core-panel-96-barcodes-sequences.xlsx

If you need assistance, please contact us at https://www.revvity.com/contact-us/technical-support and choose the "Next Gen Sequencing" category.

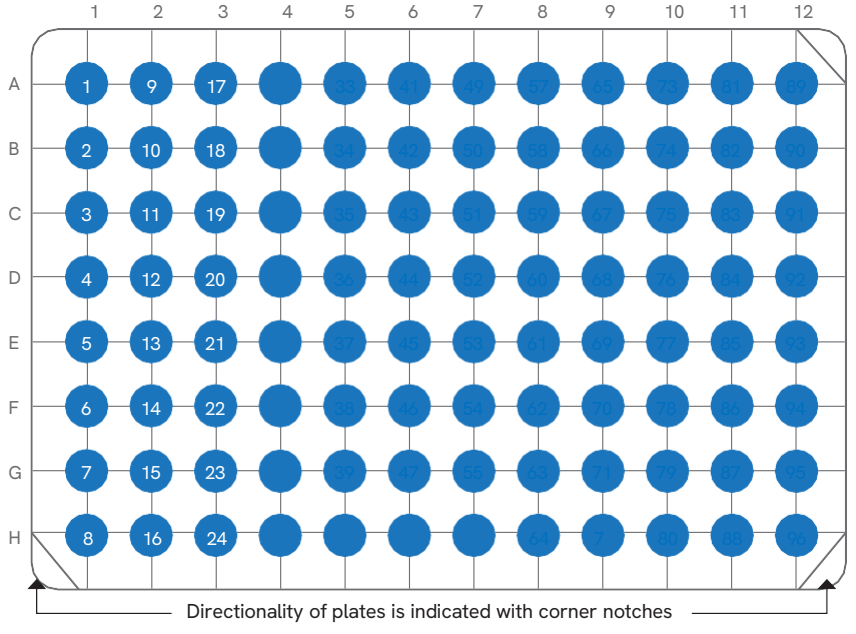
Index Number	i5 index sequence (5'-3')	i5 index reverse sequence (5'-3')	i7 index sequence (5'-3')
1	CGGAGGAATG	CATTCCTCCG	TCGAAGTCAA
2	GAGTCAGCCA	TGGCTGACTC	CGAGGCCTAT
3	GGAATTAGGC	GCCTAATTCC	TCCTGAAGTG
4	TTCGCCACAC	GTGTGGCGAA	AATCCTTACC
5	CCTCGCTTAC	GTAAGCGAGG	TTGTTGCAGA
6	ACAGCGTGTG	CACACGCTGT	AATCTAGGCC
7	TTCCGCTTCT	AGAAGCGGAA	GGCTCTACTG
8	CAGCGTCATT	AATGACGCTG	GTCCACGTTG
9	CCGTAGAACA	TGTTCTACGG	CTCCGCAGTT
10	CGGTTATCGT	ACGATAACCG	AGAACAGTGA
11	TCTGGTATCA	TGATACCAGA	GCTCTTATTG
12	AAGTATGCGT	ACGCATACTT	TGTAGACGAA
13	TTCCTTCGAG	CTCGAAGGAA	CTTGTCGTCG
14	GCTATGGATA	TATCCATAGC	TCGTCTTACA
15	AGGTACCATT	AATGGTACCT	GAGAGGAGGA
16	TTACGGAGTC	GACTCCGTAA	GTTAGATACC
17	TGAGGACTTA	TAAGTCCTCA	GGCTTAAGAA
18	TTGAGTTGCC	GGCAACTCAA	TCTGGTACAA
19	AGCTTCGCGA	TCGCGAAGCT	GTGAATTCGG
20	CATACGCCAG	CTGGCGTATG	GAATGGAGAA
21	CAAGACCAGC	GCTGGTCTTG	AGTCAATTGG
22	GATAGACAGT	ACTGTCTATC	CGCATCACCT
23	CGCTCGTGAA	TTCACGAGCG	TATTGACACC
24	TCTCTAACAG	CTGTTAGAGA	AGACTGTCGG
25	ACCTAGGAGG	CCTCCTAGGT	ATCTGGACTC
26	TCTGTACCTT	AAGGTACAGA	GAGAATAAGG
27	CTCAGGCCAT	ATGGCCTGAG	TGTTGTCGCC

28	TTGTGCAGCC	GGCTGCACAA	CTGCGGTGTT
29	TAGCCGAATC	GATTCGGCTA	GATAACTCCG
30	AAGCCTGTTA	TAACAGGCTT	ATCCTTGTAC
31	TGTACAGTAG	CTACTGTACA	TACGCGTATA
32	CGATTCTGCC	GGCAGAATCG	CCACCAATTG
33	TTGCTAAGGA	TCCTTAGCAA	TGTGAAGGCC
34	ACTCCTTGGC	GCCAAGGAGT	CCTTGACTGC
35	GAAGGCGAAC	GTTCGCCTTC	AATGCGTCGG
36	CAATACCTTG	CAAGGTATTG	AAGACTACAC
37	CGACGACAAG	CTTGTCGTCG	GTCAGTGCAG
38	GAACCTGACC	GGTCAGGTTC	CTCACCAGAA
39	TTGCCTCGCA	TGCGAGGCAA	TCTCGTACTT
40	TTCGTGTCGA	TCGACACGAA	TCAGATTAGG
41	TGGATGGCAA	TTGCCATCCA	CACTCAAGAA
42	TTCACCAGCT	AGCTGGTGAA	AGAGCCATTC
43	CCTGAGTAGC	GCTACTCAGG	CACGATTCCG
44	AGGTGTCCGT	ACGGACACCT	TTGGAGCCTG
45	GTCTGGTTGC	GCAACCAGAC	TTACGACTTG
46	CTCTTAGATG	CATCTAAGAG	TTAAGGTCGG
47	TATCACCTGC	GCAGGTGATA	GGTTCTGTCA
48	CAGAGGCAAG	CTTGCCTCTG	GATACGCACC
49	CCGGTCAACA	TGTTGACCGG	TCGCGAAGCT
50	TCACGAGGTG	CACCTCGTGA	GTTAAGACGG
51	CCATAGACAA	TTGTCTATGG	CCGGTCATAC
52	GAGCTTGGAC	GTCCAAGCTC	GTCAGCTTAA
53	TACGGTGTTG	CAACACCGTA	ACCGCGGATA
54	TTCAACTCGA	TCGAGTTGAA	GTTGCATCAA
55	AAGGCAGGTA	TACCTGCCTT	TGTGCACCAA
56	CGGCCAATTC	GAATTGGCCG	ATCTGTGGTC
57	CAACCGGACA	TGTCCGGTTG	CACAAGATCC
58	AACTTGGCCG	CGGCCAAGTT	CTGCTAGCTG
59	TGGAACATAG	CTATGTTCCA	ACCGGTCGAA
60	TTCGGATCTA	TAGATCCGAA	GCACGTTCTA
61	CGGAATCGTG	CACGATTCCG	AAGGAAGGAA
62	TCTAATCGGT	ACCGATTAGA	AGAGAGATAG
63	GCTGGAATTA	TAATTCCAGC	GGTTCCTATT
64	CGCTTCTCAC	GTGAGAAGCG	TTCACGAGCG

65	TAGACTCCTG	CAGGAGTCTA	GGCACAACCT
66	CCGTTGATTG	CAATCAACGG	TGACTCAGAA
67	CGAACCTCCA	TGGAGGTTCG	CGATCTCAGG
68	TTGGAAGTTG	CAACTTCCAA	CCTGCTGGAA
69	CCAGGAGTAC	GTACTCCTGG	GAGCTGTATA
70	AGGTTCGTCG	CGACGAACCT	AACCTGACGG
71	GACCTGAAGA	TCTTCAGGTC	AAGCTCGTGG
72	TTAACGCACA	TGTGCGTTAA	GTCCAAGCTC
73	TCGGAGTTGG	CCAACTCCGA	CTAGACTTCG
74	CGATGACTCC	GGAGTCATCG	TCCAAGGTAA
75	TATAGGTTGG	CCAACCTATA	CTTGGTAGCA
76	GACAAGTGTT	AACACTTGTC	AACGAGGCGT
77	TTCTCCGGAA	TTCCGGAGAA	CAGAAGATGG
78	ACACACTCCG	CGGAGTGTGT	TGATACATCC
79	CTGGTCACTA	TAGTGACCAG	GCGCGTAGTT
80	TTCGTGCCAC	GTGGCACGAA	GTTGTCTGCG
81	AGATCATGGA	TCCATGATCT	CTTAGCGCTG
82	GAGTATGTAC	GTACATACTC	ATCAGCCTCC
83	TAGAACACCT	AGGTGTTCTA	TGCAGTGCTC
84	CCAGTTAAGA	TCTTAACTGG	GAGCTCAGAC
85	CGCTTATCTG	CAGATAAGCG	ACCTGGACAA
86	GAGCTCTTAC	GTAAGAGCTC	CAACTTCCAA
87	TCTCAAGGCG	CGCCTTGAGA	CCATCCTGTG
88	CTAAGTACCA	TGGTACTTAG	GGCAGTTAGA
89	TCGACAAGCC	GGCTTGTCGA	TCACATGAGA
90	TTCGACATCA	TGATGTCGAA	TATTCGTTGG
91	AGTGGTACTT	AAGTACCACT	AGCGGTCTTC
92	TTGCACTTGT	ACAAGTGCAA	GCGACCGATT
93	GTCTTCGCAG	CTGCGAAGAC	GATCTCGTCC
94	CAGGCTCCAA	TTGGAGCCTG	CCATTATAGG
95	CCAGGTTACG	CGTAACCTGG	ACAGACCACG
96	CAATCGCCTA	TAGGCGATTG	ATTCCACACA

Appendix B – Plate Format

All barcodes in 96-well plate; 10 µL (1 reaction) / well
Representative Orientation



5340-0124 Contains barcodes 1-24, arrayed in columns 1-3
5341-0124 Contains barcodes 25-48, arrayed in columns 1-3
5342-0124 Contains barcodes 49-72, arrayed in columns 1-3
5343-0124 Contains barcodes 73-96, arrayed in columns 1-3

Appendix C - Selecting Samples for PGT-M Analysis

PG-Seq™ technology employs linkage analysis to investigate familial allele inheritance patterns. Linkage analysis is used to identify disease-causing mutation by tracking the inheritance patterns of genetic markers, specifically SNPs, near the mutation of interest. This approach is based on the fact that markers that are physically close on a chromosome are more likely to be inherited together due to reduced probability of recombination events occurring between them. The key to successful linkage analysis lies in careful sample selection for study inclusion.

This guide provides a comprehensive overview of sample selections. As a general recommendation the ideal scenario is to include the couple seeking PGT-M, a previous child (whether affected, carrier* or non-carrier) and/or the parents of the couple. This combination allows for the most informative analysis of inheritance patterns. However, when this ideal scenario is not feasible, we propose the following minimum sample requirements, distinguishing between the three Mendelian inheritance patterns: autosomal recessive, autosomal dominant and X-linked inheritances.

A critical consideration in sample selection for PGT-M studies is ensuring that each sample has undergone prior genetic testing to determine its status (non-carrier, carrier or affected). This pre-screening is essential for the correct interpretation of the linkage analysis results.

Autosomal dominant inheritance:

- The couple.
- At least, one parent of the affected individual (proband).

Rationale: This combination allows for the identification of the disease-associated haplotype in the affected individual and its transmission pattern.

Autosomal recessive inheritance:

- The couple
- At least, one parent of the carrier member of the couple. If both members are carrier, at least one parent from each side of the couple.

Rationale: This setup enables the tracing of carrier haplotypes from both parental lineages, crucial for identifying compound heterozygosity or homozygosity in affected offspring.

X-linked inheritance:

- The couple.
- One parent of the carrier female.

Rationale: This combination allows tracking of the X-linked haplotype through the maternal line.

* In autosomal recessive inheritance, if both parents are carriers of the same variant a carrier child cannot be used to perform linkage analysis. Only affected or non-carrier

individuals provide unambiguous information.

Note: When using an unaffected family member for linkage analysis, it's essential to rule out the possibility that the variant is de novo in the IVF couple. If the variant originated spontaneously in one of the parents, it would not be linked to an inherited haplotype in the unaffected family members, potentially leading to misleading results.

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