May 2026

QIAseq® xHYB Trinity™ Reagent DNA/RNA Kit Handbook

For library preparation and hybridization capture–based enrichment for comprehensive genomic profiling on Element Biosciences™ Trinity flow cell using the QIAseq Multimodal Library Kits and QIAseq xHYB CGP DNA/RNA Panels

Sample to Insight

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Kit Contents

QIAseq xHYB Trinity™ Reagent DNA/RNA Kit	(24)	(96)
Catalog no.	336002	336005
No. of hybridization reactions	3	12
Typical no. of samples	24	96
Box 1 of 3		
One-4-All Blocking Solution	2 × 15 µL	2 × 60 µL
E Dry hybridization Buffer	2 × 3 tubes	2 × 12 tubes
Vapor-Lock	500 µL	2 × 500 µL
E DNA Binding Reagent	20 µL	70 µL
E RNA Binding Reagent	20 µL	70 µL
QIAseq RNA Read 1 Primer E	120 µL	470 µL
QIAseq RNA Read 2 Primer E	70 µL	275 µL
QIAseq RNA Index 1 Primer E	70 µL	275 µL
HiFi Ultra Buffer	2 × 384 µL	2 × 1536 µL
HiFi Ultra Polymerase	2 × 315 µL	3 × 850 µL
MM E F-R Primer Mix	280 µL	2 × 550 µL
E UMI Adapter	70 µL	290 µL
Multimodal RT Enhancer	12 µL	48 µL
MM E RNA RT Primer	50 µL	185 µL
MM E RNA TSO Primer	50 µL	185 µL
Box 2 of 3		
QIAseq Beads	10 mL	10 mL
Nuclease-Free Water	1.5 mL	1.5 mL

QIAseq xHYB Trinity™ Reagent DNA/RNA Kit	(24)	(96)
Catalog no.	336002	336005
No. of hybridization reactions	3	12
Typical no. of samples	24	96
Box 3 of 3		
EDNA-24X (QIAseq Multimodal EDNA Index Plate 24) Each plate allows indexing of 24 DNA samples. Each well in the plate is single-use and contains 9 µL of UDI index pairs for DNA libraries.	1	N/A
ERNA-24X (QIAseq Multimodal ERNA Index Plate 24) Each plate allows indexing of 24 RNA samples. Each well in the plate is single-use and contains 9 µL of UDI index pairs for RNA libraries.	1	N/A
EDNA-96AX (QIAseq Multimodal EDNA Index 96 Set A) Each plate allows indexing of 96 DNA samples. Each well in the plate is single-use and contains 9 µL of UDI index pairs for DNA libraries.	N/A	1
ERNA-96AX (QIAseq Multimodal ERNA Index 96 Set A) Each plate allows indexing of 96 RNA samples. Each well in the plate is single-use and contains 9 µL of UDI index pairs for RNA libraries.	N/A	1

QIAseq xHYB Trinity Reagent DNA Kit	(24)	(96)
Catalog no.	336012	336015
No. of hybridization reactions	3	12
Typical no. of samples	24	96
Box 1 of 3		
One-4-All Blocking Solution	15 µL	60 µL
E Dry hybridization Buffer	3 tubes	12 tubes
Vapor-Lock	500 µL	500 µL
E DNA Binding Reagent	20 µL	70 µL
HiFi Ultra Buffer	384 µL	1536 µL
HiFi Ultra Polymerase	315 µL	2 × 850 µL
MM E F-R Primer Mix	280 µL	550 µL
E UMI Adapter	70 µL	290 µL
Box 2 of 3		
QIAseq Beads	10 mL	10 mL
Nuclease-Free Water	1.5 mL	1.5 mL
Box 3 of 3		
EDNA-24X (QIAseq Multimodal EDNA Index Plate 24) Each plate allows indexing of 24 DNA samples. Each well in the plate is single-use and contains 9 µL of UDI index pairs for DNA libraries.	1	N/A
EDNA-96AX (QIAseq Multimodal EDNA Index 96 Set A) Each plate allows indexing of 96 DNA samples. Each well in the plate is single-use and contains 9 µL of UDI index pairs for DNA libraries.	N/A	1

QIAseq xHYB Trinity Reagent RNA Kit	(24)	(96)
Catalog no.	336022	336025
No. of hybridization reactions	3	12
Typical no. of samples	24	96
Box 1 of 3		
One-4-All Blocking Solution	15 µL	60 µL
E Dry hybridization Buffer	3 tubes	12 tubes
Vapor-Lock	500 µL	500 µL
E RNA Binding Reagent	20 µL	70 µL
QIAseq RNA Read 1 Primer E	120 µL	470 µL
QIAseq RNA Read 2 Primer E	70 µL	275 µL
QIAseq RNA Index 1 Primer E	70 µL	275 µL
HiFi Ultra Buffer	384 µL	1536 µL
HiFi Ultra Polymerase	315 µL	2 × 850 µL
MM E F-R Primer Mix	280 µL	550 µL
Multimodal RT Enhancer	12 µL	48 µL
MM E RNA RT Primer	50 µL	185 µL
MM E RNA TSO Primer	50 µL	185 µL
Box 2 of 3		
QIAseq Beads	10 mL	10 mL
Nuclease-Free Water	1.5 mL	1.5 mL
Box 3 of 3		
ERNA-24X (QIAseq Multimodal ERNA Index Plate 24) Each plate allows indexing of 24 RNA samples. Each well in the plate is single-use and contains 9 µL of UDI index pairs for RNA libraries.	1	N/A
ERNA-96AX (QIAseq Multimodal ERNA Index 96 Set A) Each plate allows indexing of 96 RNA samples. Each well in the plate is single-use and contains 9 µL of UDI index pairs for RNA libraries.	N/A	1

QIAseq Multimodal DNA/RNA Library Kit Catalog no. Number of samples	(24) 334842 24	(96) 334845 96
10× FX Buffer	48 µL	225 µL
5× WGS FX Mix	96 µL	475 µL
QIAseq FastSelect® –rRNA HMR (8)	12 µL	12 µL
Side Reaction Reducer	48 µL	192 µL
FG Solution	170 µL	170 µL
ATP Solution	36 µL	290 µL
PAP Enzyme	96 µL	96 µL
PAP Dilution Buffer	192 µL	192 µL
T4 Polynucleotide Kinase	125 µL	125 µL
DNA Ligase	2 ×120 µL	1100 µL
UPH Ligation Buffer, 2.5×	1152 µL	3 × 1500 µL
Buffer GE2 gDNA elimination buffer	72 µL	250 µL
US RT Buffer, 5×	150 µL	450 µL
100 mM DTT	20 µL	80 µL
10 mM dNTP	55 µL	235 µL
EZ Reverse Transcriptase	36 µL	2 × 96 µL
RNase Inhibitor	12 µL	96 µL
Nuclease-Free Water	10 mL	10 mL
QIAseq Beads	10 mL	55 mL

QIAseq Multimodal DNA Kit-EnzFrag Catalog no. Number of samples	(24) 335022 24	(96) 335025 96
10× FX Buffer	48 µL	225 µL
5× WGS FX Mix	96 µL	475 µL
Side Reaction Reducer	48 µL	192 µL
FG Solution	170 µL	170 µL
DNA Ligase	2 × 120 µL	1100 µL
UPH Ligation Buffer, 2.5×	1152 µL	3 × 1500 µL
Nuclease-Free Water	10 mL	10 mL
QIAseq Beads	10 mL	55 mL

QIAseq Multimodal DNA Kit-MechFrag Catalog no. Number of samples	(24) 335032 24	(96) 335035 96
ERA Buffer, 10×	60 µL	480 µL
ERA Enzyme	120 µL	960 µL
DNA Ligase	2 × 120 µL	1100 µL
UPH Ligation Buffer, 2.5×	1152 µL	3 × 1500 µL
Nuclease-Free Water	10 mL	10 mL
QIAseq Beads	10 mL	55 mL

QIAseq Multimodal RNA Kit Catalog no. Number of samples	(24) 335012 24	(96) 335015 96
10× FX Buffer	48 µL	225 µL
QIAseq FastSelect® –rRNA HMR (8)	12 µL	12 µL
ATP Solution	36 µL	290 µL
PAP Enzyme	96 µL	96 µL
PAP Dilution Buffer	192 µL	192 µL
T4 Polynucleotide Kinase	125 µL	125 µL
Buffer GE2 gDNA elimination buffer	72 µL	250 µL
US RT Buffer, 5×	150 µL	450 µL
100 mM DTT	20 µL	80 µL
10 mM dNTP	55 µL	235 µL
EZ Reverse Transcriptase	36 µL	2 × 96 µL
RNase Inhibitor	12 µL	96 µL
Nuclease-Free Water	10 mL	10 mL
QIAseq Beads	10 mL	55 mL

QIAseq xHYB CGP DNA/RNA Panels	**(24)**	**(96)**
Catalog no.	**335122**	**335125**
No. of hybridization reactions	**3**	**12**
Typical no. of samples	**24**	**96**
xHYB Probe panel for DNA and RNA target enrichment for comprehensive genomic profiling	12 µL	48 µL

QIAseq xHYB CGP DNA Panel	**(24)**	**(96)**
Catalog no.	**333122**	**333125**
No. of hybridization reactions	**3**	**12**
Typical no. of samples	**24**	**96**
xHYB Probe panel for DNA target enrichment for comprehensive genomic profiling	12 µL	48 µL

QIAseq xHYB CGP RNA Panel	**(24)**	**(96)**
Catalog no.	**334122**	**334125**
No. of hybridization reactions	**3**	**12**
Typical no. of samples	**24**	**96**
xHYB Probe panel for RNA target enrichment for comprehensive genomic profiling	12 µL	48 µL

Shipping and Storage

The QIAseq xHYB Trinity Reagent DNA/RNA Kit is shipped on dry ice and a cool pack (4°C). Store the kit immediately upon receipt at –30°C to –15°C in dry ice box, and 2–8°C in cool pack box.

QIAseq xHYB CGP Panels are shipped on dry ice. Store panels immediately upon receipt at –30°C to –15°C in a constant-temperature freezer.

QIAseq Multimodal DNA/RNA Library Kit (except QIAseq Beads and Nuclease-Free Water) is shipped on dry ice and should be stored immediately upon receipt at –30°C to –15°C in a constant-temperature freezer. QIAseq Beads and Nuclease-Free Water are shipped in a separate box on cold packs and should be stored immediately upon receipt at 2–8°C.

Under these conditions, the components are stable until the expiry date indicated on the label.

Intended Use

QIAseq xHYB Panels, QIAseq xHYB Trinity Reagent Kit, QIAseq Multimodal DNA/RNA Library and Index Kits are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety** where you can find, view, and print the SDS for each QIAGEN kit and kit component.

Quality Control

In accordance with QIAGEN ISO-certified Quality Management System, each lot of QIAseq xHYB Trinity Reagent DNA/RNA Kit is tested against predetermined specifications to ensure consistent product quality.

Introduction

The QIAseq xHYB Trinity Reagent DNA/RNA Kit workflow supports comprehensive genomic profiling with a simplified workflow designed for cancer research through its multiomic and multimodal capabilities.

The QIAseq xHYB CGP DNA/RNA panels target more than 720 genes and provide coverage of coding regions, enabling assessment of tumor mutational burden (TMB) and microsatellite instability (MSI) markers for tumor profiling and biomarker identification. Its compatibility with cell-free DNA (cfDNA) supports detection of gene fusions relevant to cancer biology. The panel's multiomic capability integrates both DNA and RNA analyses, to further enable exploration of tumor heterogeneity and gene expression dynamics. Customization options allow users to add specific targets or utilize an optional RNA panel that includes more than 270 genes.

The QIAseq Multimodal library preparation supports DNA and RNA, DNA-only and RNA-only workflows. Flexible hybridization times and automation options support workflow adaptability for diverse research applications.

The Trinity workflow eliminates all post hybridization steps in the standard workflow, including streptavidin bead capture, washing, library amplification, and bead clean up. After a 30-minute hybridization, the reaction can be loaded directly onto the Trinity flow cell, resulting in a faster CGP panel workflow compared with traditional methods.

In summary, the QIAseq xHYB Trinity Reagent DNA/RNA Kit workflow enables comprehensive multiomic genomic analysis for oncology research. The workflow supports research leading to the discovery of new biomarkers and therapeutic targets in cancer. It also provides accessible genomic profiling solutions with rapid turnaround time for cancer research.

Principle and procedure

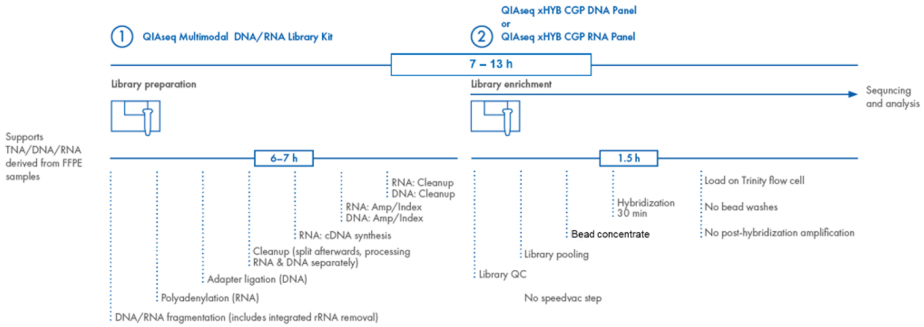

Figure 1. Workflow principle of the QIAseq xHYB Trinity Reagent DNA/RNA Kit. Step 1: Generation of indexed libraries using QIAseq Multimodal DNA/RNA Library Kit and QIAseq xHYB Trinity Reagent DNA/RNA Kit. **Step 2:** Enrichment of xHYB CGP Targets and sequencing on Trinity flow cell.

Step 1: Generation of indexed libraries

QIAseq xHYB Trinity Reagent DNA/RNA Kit combined with the QIAseq Multimodal DNA/RNA Lib Kit enables construction of multimodal WGS/WTS libraries, as well as separate WGS and WTS libraries, fully compatible with the Element Biosciences™ Aviti™ sequencer. We recommend using a minimum of 40 ng DNA and 80 ng RNA of good quality for CGP DNA/RNA Panels. The following reactions occur in a streamlined, single-tube workflow.

Step 2: Enrichment of human xHYB targets for Trinity

During xHYB enrichment, indexed library fragments bind to biotinylated double-stranded DNA capture probes, with flexible hybridization times ranging from 30 minutes to 4 hours of incubation. After hybridization, the reaction is diluted with Trinity Fast Hyb Loading Buffer and loaded directly onto the Trinity flow cell. This eliminates lengthy, variable post hybridization steps, such as streptavidin bead binding, washing, amplification, and bead clean up. This

workflow reduces variability to specificity and uniformity associated with post-hybridization processing.

Since the hybridization reaction is loaded directly onto Trinity flow cell without any post-hybridization steps, sequencing reads reflect the library amount in the hybridization reaction. As a result, the required library input needed in hybridization for Trinity flow cell is higher than in a regular manual hybrid-capture workflow.

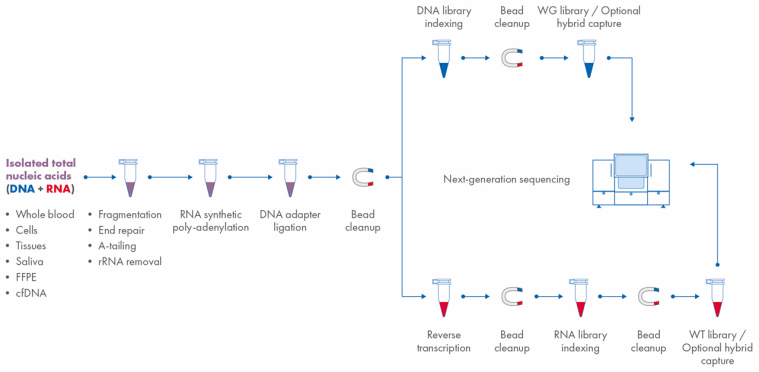

Figure 2. Workflow details of DNA and RNA libraries construction using the QIAseq Multimodal DNA/RNA Library Kit

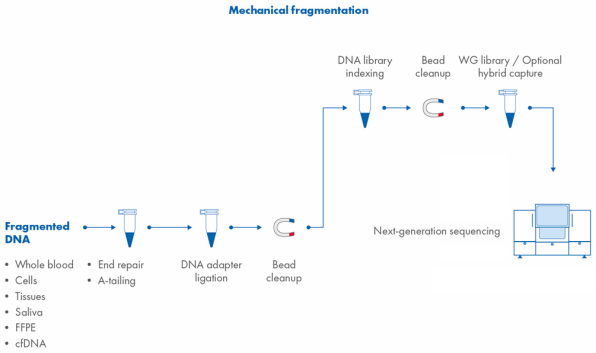

Figure 3. Workflow details of DNA library preparation with mechanical fragmentation using the QIAseq Multimodal DNA Kit-MechFrag Kit.

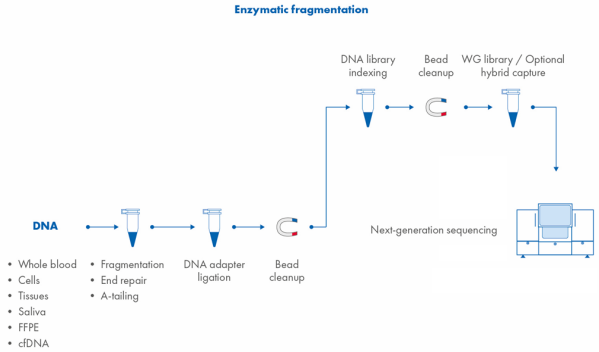

Figure 4. Workflow details of DNA library preparation with enzymatic fragmentation using the QIAseq Multimodal DNA Kit-EnzFrag Kit.

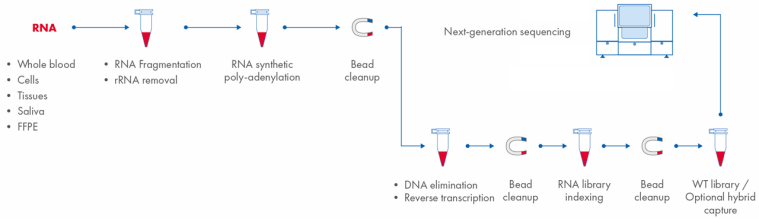

Figure 5. Workflow details of RNA library construction using the QIAseq Multimodal RNA Kit.

QIAseq xHYB CGP DNA/RNA Panels

QIAseq xHYB CGP DNA panel is a comprehensive genomic profiling assay covering 724 genes and enabling assessment of all DNA variant types, including SNVs, CNVs and indels, as well as MSI and TMB. It can be combined with QIAseq xHYB CGP RNA panel to target 274 genes for fusion detection. This analysis supports advanced cancer research applications.

Description of protocols

The QIAseq xHYB Trinity Reagent DNA/RNA Kit combined with the QIAseq Multimodal DNA/RNA Library Kit are for use to generate whole genome and whole transcriptome libraries compatible to Trinity flow cell using an optimized workflow.

The QIAseq xHYB Trinity Reagent DNA/RNA Kit and QIAseq Multimodal DNA/RNA Library Kit can also be used for the generation of DNA-only or RNA-only libraries if needed.

The QIAseq xHYB CGP Panel protocol describes the hybridization capture workflow for human xHYB panel and is suitable for input libraries generated using the QIAseq xHYB Trinity Reagent DNA/RNA Kit and the QIAseq Multimodal DNA/RNA Library Kit.

Equipment and Reagents to Be Supplied by User

- 100% ethanol (ACS grade)
- Nuclease-Free Water
- Buffer EB (cat. no. 19086)
- PCR tubes or plates
- Pipette tips and pipettes
- DNA LoBind tubes 1.5 mL or 2 mL (from Axygen™ or Eppendorf®)
- Vortexer
- Microcentrifuge
- Magnetic racks for magnetic beads separation (e.g., Thermo Fisher Scientific DynaMag™ Magnet [cat. no.12321D] for 1.5 mL or 2 mL tubes; [cat. no. 12331D] for 96-well plate or PCR tube)
- Thermal cycler with heated lid
- Capillary electrophoresis device, e.g., QIAGEN QIAxcel®, Agilent® Bioanalyzer®, TapeStation®, or similar to evaluate the DNA fragmentation profile

The following sequencing kit and reagents are needed to run the libraries on the Element Biosciences Aviti:

- Trinity 2 × 150 Sequencing Kit (cat. no. 860-00020)
- Trinity Fast Hyb Loading Buffer (1 run) (cat. no. 830-00030)
- Trinity PhiX Control (cat. no. 830-00031)

The following controls can be used to verify the performance of the QIAseq xHYB CGP DNA/RNA Panels on Trinity workflow:

- Horizon Mimix™ OncoSpan, FFPE Reference Standard (cat. no. HD832)
- Horizon Mimix OncoSpan, cfDNA Reference Standard (cat. no. HD833)
- Horizon Mimix OncoSpan, gDNA Reference Standard (cat. no. HD827)
- SeraSeq® Lung & Brain CNV Mix, +6 Copies (cat. no. 0710-0415)
- Seraseq Myeloid Mutation DNA Mix (cat. no. 0710-0408)
- Seraseq gDNA TMB Mix Score 20 (WT) (cat. no. 0710-1324)
- Seraseq gDNA TMB Mix Score 20 (tumor) (cat. no. 0710-1324)
- Seraseq FFPE Tumor Fusion RNA v4 Reference Material (cat. no. 0710-0496)
- Seraseq Fusion RNA Mix v4 (cat. no. 0710-0497)
- Seraseq Whole Transcriptome RNA Seq Mix (cat. no. 0710-2129)
- NA12878 (from Coriell Institute, control for copy number detection)

Important Notes

General precautions

- Use good laboratory practices to minimize cross-contamination of nucleic acid products.
- Always use PCR tubes, microcentrifuge tubes, and pipette tips that are certified sterile and DNase-free and RNase-free.
- Before starting, wipe down the work area and pipettes with an RNase and DNA cleaning product.
- For consistent hybridization capture and amplification, ensure the thermal cycler used in this protocol is in good working condition and has been calibrated within the manufacturer's specifications.
- Read the entire protocol before beginning. Take note of stopping points where samples can be frozen at –30°C to –15°C and plan your workflow accordingly.

Quality control of input libraries

- Check quality of each indexed library using a capillary electrophoresis method such as using High Sensitivity DNA chip with the QIAxcel Connect System, Agilent 2100 Bioanalyzer, or TapeStation.
- A typical indexed whole genome library has an average size distribution centered around 400 bp and is free of adapter dimers at a typical yield of 4–6 µg on Nanodrop (refer to Figure 3 in "Recommendations: Library QC and Quantification").
- Libraries from physically sheared DNA will reflect the size distribution of the sheared DNA input.

Indexing recommendations

- Use QIAseq xHYB Trinity Reagent DNA/RNA Kit to generate whole genome and/or whole transcriptome libraries compatible with Element Aviti, and for hybridization capture sequencing on the Element Trinity flow cell.
- Use different sample indexes for all samples within a hybridization capture pool.
- Consider using different sample indexes between hybridization capture pools to allow post-capture multiplexing, depending on sequencing requirement.

Protocol: Nucleic Acid Fragmentation and End Repair

Important points before starting

- This protocol describes fragmentation of nucleic acids samples.
- Recommended starting amounts of nucleic acid:
  - For **fresh DNA**, use a minimum of 40 ng in good quality with the QIAseq xHYB Trinity CGP DNA workflow. For **FFPE DNA** with high QuantiMIZE quality, input 40–100 ng. With low QuantiMIZE quality, input 100–250 ng.
  - For **fresh RNA**, use a minimum of 80 ng in good quality with the QIAseq xHYB Trinity CGP RNA workflow. For **FFPE RNA** with high quality (DV200>=60%), input 80–250 ng. With low quality (DV200<60%), input 250–500 ng.
  - For **cfDNA** samples, use the recommended starting amount of 20 ng.
- Set up reactions on ice.
- Use a 96-well plate with 250 µL capacity to set up reaction with FFPE sample, this capacity is needed for bead cleanup after ligation.
- Unless specifically indicated, do not vortex any reagents or reactions.

Procedure

1. Thaw nucleic acid sample(s) on ice. Gently mix, briefly centrifuge to collect residual liquid from the sides of the tubes, and then return to ice.

2. Prepare the reagents required for the fragmentation.

   a. Thaw 10× FX Buffer and QIAseq FastSelect –rRNA HMR (8) at room temperature (15–25°C).

   b. Mix each reagent by flicking the tube, and then centrifuge briefly.

      Note: Just before use, remove 5× WGS FX Mix and Side Reaction Reducer from the freezer and place them on ice. After use, immediately return the enzymes to the freezer.

3. Dilute an aliquot of QIAseq Fastselect –rRNA HMR 1:10 with Nuclease-Free Water. Mix by flicking the tube, and then centrifuge briefly.

4. On ice, prepare the fragmentation mix according to Table 1. Briefly centrifuge, mix by pipetting up and down 20 times with the pipette volume close to 20 µL, and then briefly centrifuge again.

   Note: If setting up more than one reaction, prepare a volume of master mix 10% greater than what is required for the total number of reactions.

Table 1. Reaction mix for fresh, FFPE samples, cfDNA, or mechanically fragmented DNA

	Volume/reaction			
Component	**Both DNA and RNA**	**DNA-only**	**RNA-only**	**cfDNA or mechanically fragmented DNA**
DNA (see input recommendation in the "Important points before starting" section)*†	Variable	Variable	N/A	Variable
RNA (see input recommendation in the "Important points before starting" section)* ‡	Variable	N/A	Variable	N/A
10× FX Buffer	2 µL	2 µL	2 µL	N/A
5x WGS FX Mix	4 µL	4 µL	4 µL	N/A
ERA Buffer, 10×	N/A	N/A	N/A	2 µL
ERA Enzyme	N/A	N/A	N/A	4 µL
QIAseq FastSelect – rRNA HMR (diluted 1:10)	1 µL	N/A	1 µL	N/A
Side Reaction Reducer	1.6 µL	N/A	1.6 µL	N/A
Nuclease-Free Water	Variable	Variable	Variable	Variable
Total	**20 µL**	**20 µL**	**20 µL**	**20 µL**

*Instead of adding DNA and RNA separately, total nucleic acid containing both DNA and RNA can be added. If adding total nucleic acid, base the input amount on DNA.

† For FFPE DNA with high QuantiMIZE quality, input 40–100 ng; with low QuantiMIZE quality, input 100–250 ng. For cfDNA samples, the recommended starting amount is 20 ng.

‡ For FFPE RNA with high quality (DV200>=60%) , input 80–250 ng. With low quality (DV200<60%), input 250–500 ng.

5. Using the instrument's heated lid, program the thermal cycler according to:

   a. Table 2a for both DNA/RNA or RNA-only input

   b. Table 2b for DNA only input

   c. Table 2c for cfDNA or mechanically fragmented DNA

   Important: The thermal cycler must be prechilled and paused at 4°C (as indicated in step 1 of Table 2a/2b/2c).

Table 2a. DNA/RNA or RNA-only input: Incubation conditions for nucleic acid fragmentation

Step	Incubation temperature (°C)	Incubation time for fresh DNA/RNA (min)	Incubation time for FFPE DNA/RNA (min)
1	4	1	1
2	32	16	12
3	65	30	30
5	60	2	2
6	55	2	2
7	37	5	2
8	25	5	2
9	4	Hold	Hold

Table 2b. DNA-only input: Incubation conditions for nucleic acid fragmentation

Step	Incubation temperature (°C)	Incubation time for fresh DNA (min)	Incubation time for FFPE DNA (min)
1	4	1	1
2	32	16	12
3	65	30	30
4	4	Hold	Hold

Table 2c. cfDNA or mechanically fragmented DNA: Incubation conditions for nucleic acid end repair

Step	Incubation temperature (°C)	Incubation time for cfDNA or mechanically fragmented DNA (min)
1	4	1
2	20	15
3	65	15
4	4	Hold

6. Transfer the tubes/plate prepared in step 4 to the prechilled thermal cycler and resume the program.
7. Upon completion, allow the thermal cycler to return to 4°C.
8. Place the samples on ice and immediately proceed to:
   a. "Protocol: RNA Polyadenylation" if input is either DNA and RNA, or RNA-only; or
   b. "Protocol: DNA Ligation" if input is DNA-only.

Protocol: RNA Polyadenylation

Important points before starting

- The product from "Protocol: Nucleic Acid Fragmentation and End Repair " is the starting material for this protocol if input is both DNA and RNA, or RNA-only.
- For DNA-only input, skip this protocol and go directly to "Protocol: DNA Ligation".
- Set up reactions on ice.
- Do not vortex any reagents or reactions.

Procedure

1. Prepare the reagents required for the polyadenylation.
    a. Thaw PAP Dilution Buffer and ATP Solution on ice.
    b. Mix each reagent by flicking the tube approx. 7 to 8 times, and then centrifuge briefly.

    Note: Just before use, remove T4 Polynucleotide Kinase and PAP Enzyme from the freezer, and place them on ice. After use, immediately return the enzymes to the freezer.
2. Dilute PAP Enzyme from 5 U/µL to 1 U/µL as follows:
    a. Prepare 1× PAP Dilution Buffer by diluting 2 µL of the 10× PAP Dilution Buffer with 18 µL Nuclease-free Water.
    b. Use the 1× PAP Dilution Buffer to dilute an aliquot of the PAP Enzyme from 5 U/µL to 1 U/µL (if RNA input <100 ng) or 2 U/µL (if RNA input >=100 ng). Briefly centrifuge, mix by pipetting up and down 20 times, and then briefly centrifuge again.

3. Prepare the RNA polyadenylation mix according to Table 3. Briefly centrifuge, mix by pipetting up and down 20 times, and then briefly centrifuge again.

   Note: If setting up more than one reaction, prepare a volume of master mix 10% greater than what is required for the total number of reactions.

Table 3. Reaction mix for RNA polyadenylation

	Volume/reaction (µL)	
Component	**RNA input <100 ng**	**RNA input >=100 ng**
Fragmentation reaction (already in tube)	20	20
ATP Solution	1.25	1.25
T4 Polynucleotide Kinase	1	1
Diluted PAP Enzyme (1 U/µL)*	1	N/A
Diluted PAP Enzyme (2 U/µL)*	N/A	1
Nuclease-Free Water	1.75	1.75
Total	**25**	**25**

* Ensure PAP Enzyme has been diluted from its stock 5 U/µL concentration to 1 U/µL or 2 U/µL using 1× PAP Dilution Buffer.

4. Incubate the reactions in a thermal cycler according to Table 4. Use the instrument's heated lid.

Table 4. Incubation conditions for RNA polyadenylation

Step	Temperature (°C)	Time (min)
1	4	1
2	30	10
3	4	Hold

5. Upon completion, place the reactions on ice and proceed to
    a. “Protocol: DNA Ligation” if input is both DNA and RNA; or
    b. “Protocol: Clean up Ligation or Polyadenylation with QIAseq bead” if input is RNA-only.

Protocol: DNA Ligation

Important points before starting

- The product from "Protocol: RNA Polyadenylation" is the starting material for this protocol if input is both DNA and RNA.
- For RNA-only input, skip this protocol and go directly to "Protocol: Clean up Ligation or Polyadenylation with QIAseq bead".
- The product from "Protocol: Nucleic Acid Fragmentation and End Repair" is the starting material for this protocol if input is DNA-only.
- Set up reactions on ice.
- Do not vortex any reagents or reactions.
- Prepare fresh 80% ethanol daily.
- Use a 96-well plate with 250 µL capacity for bead clean-up of FFPE sample.
- Ensure that the QIAseq Beads are thoroughly mixed at all times. This necessitates working quickly and resuspending the beads immediately before use. If a delay in the protocol occurs, simply vortex the beads before proceeding.

Procedure

1. Prepare the reagents required for the DNA ligation.
    a. Thaw E UMI Adapter and UPH Ligation Buffer, 2.5×, at room temperature.

        Note: The E UMI Adapter contains a UMI.

    b. Mix each reagent by flicking the tube approx. 7 to 8 times, and then centrifuge briefly.

Note: Just before use, remove DNA Ligase from the freezer and place them on ice. After use, immediately return the enzyme to the freezer.

2. Prepare the DNA ligation mix according to Table 5. Briefly centrifuge, mix by pipetting up and down 20 times with pipette volume close to 100 µL, and then briefly centrifuge again.

   Note: If setting up more than one reaction, prepare a volume of master mix 10% greater than what is required for the total number of reactions.

Table 5. Reaction mix for DNA ligation (both DNA and RNA or DNA-only input)

	Volume/reaction (µL)			
	Both DNA and RNA input		DNA-only input	
Component	DNA <50 ng	DNA >=50 ng	DNA <50 ng	DNA >=50 ng
RNA polyadenylation reaction (already in tube)	25	25	N/A	N/A
Fragmentation or end repair reaction (already in tube)	N/A	N/A	20	20
UPH Ligation Buffer, 2.5×	40	40	40	40
E UMI Adapter*	1.25	2.5	1.25	2.5
DNA Ligase	10	10	10	10
Nuclease-Free Water	23.75	22.5	28.75	27.5
Total	**100**	**100**	**100**	**100**

* For low quality FFPE DNA samples with input <100 ng, use UMI Adapter with 1:10 dilution.

3. Incubate the reactions in a thermal cycler according to Table 6.

   Important: Do not use the heated lid.

Table 6. Incubation conditions for DNA ligation

Step	Temperature (°C)	Time (min)
1	4	1
2	20	15
3	4	Hold

4. After ligation reaction finished, proceed to "Protocol: Clean up Ligation or Polyadenylation with QIAseq bead", next section.

Protocol: Clean up Ligation or Polyadenylation with QIAseq bead

Important points before starting

- The product from "Protocol: DNA Ligation" is the starting material for this protocol if input is both DNA and RNA, or DNA-only.
- The product from "Protocol: RNA Polyadenylation" is the starting material for this protocol if input is RNA-only.
- Prepare fresh 80% ethanol daily.
- Use a 96-well plate with 250 µL capacity for bead clean-up of FFPE sample.
- Ensure that the QIAseq Beads are thoroughly mixed at all times. This necessitates working quickly and resuspending the beads immediately before use. If a delay in the protocol occurs, simply vortex the beads before proceeding.

Procedure

1. For ligation product from both DNA and RNA, or DNA-only input, add QIAseq beads according to Table 7 for cleanup.

   For RNA-only input, add water and QIAseq beads as Table 7 to finished polyadenylation for cleanup.

   Mix by vortexing or by pipetting up and down 20 times with pipette volume close to maximum volume.

Table 7. Clean up ligation or polyadenylation with QIAseq bead

Step	Fresh DNA-only or Both DNA/RNA (µL)	FFPE DNA-only or DNA/RNA (µL)	Fresh RNA-only (µL)	FFPE RNA-only (µL)
From DNA ligation	100	100	NA	NA
From RNA polyadenylation	NA	NA	25	25
Nuclease-Free Water	NA	NA	75	75
QIAseq Beads	90	130	90	130
Total	**190**	**230**	**190**	**230**

2. Incubate for 5 min at room temperature.
3. Place the tubes/plate on a magnetic rack for 5 min (for tubes) or 10 min (for plates).

   Once the solution has cleared, with the beads still on the magnetic stand, carefully remove and discard the supernatant.

   Important: Do not discard the beads because they contain the DNA of interest.

   Note: For plates, the following may improve performance. After 8 min, remove 90 µL supernatant. Leave it on the magnetic stand for 2 min and remove 90 µL supernatant. Then, briefly centrifuge the plate, return it to the magnetic stand for 1 min, and use a 10 µL pipette to remove the remaining supernatant.

   Note: Do not dry bead after removing supernatant.
4. After removing supernatant, add 50 µL of QIAseq bead, and then mix either by vortexing or by pipetting up and down 20 times with pipette volume close to 50 µL.
5. Add 50 µL of Nuclease-Free Water for fresh sample or 41 µL of Nuclease-Free Water for FFPE sample, and then mix by vortexing or by pipetting up and down 20 times with pipette volume close to 90 µL.
6. Place the tubes/plate on a magnetic rack for 5 min (for tubes) or 10 min (for plates).

Once the solution has cleared, with the beads still on the magnetic stand, carefully remove and discard the supernatant.

Important: Do not discard the beads because they contain the DNA/RNA of interest.

7. With the beads still on the magnetic stand, add 200 µL of 80% ethanol. Rotate the tube 2–3 times or move the plate side to side between the 2 column positions of the magnet to wash the beads. Carefully remove and discard the wash.
8. Repeat the ethanol wash.

   Important: Completely remove all traces of the ethanol after the second wash. Remove the ethanol with a 200 µL pipette tip first, spin down briefly, and then use a 10 µL pipette tip to remove any residual ethanol.
9. Dry beads at room temperature for 10 min.

   Note: Visually inspect the pellet to confirm that it is completely dry. If the pellet is not completely dry, additional drying time may be required.
10. Elute the DNA from the beads by adding 16 µL Nuclease-Free Water. Mix well by pipetting.
11. Return the tube/plate to the magnetic rack until the solution has cleared.
12. For each sample, aliquot 6.8 µL of the eluate into two separate clean tubes/plate wells.

    Aliquot 1: Proceed to “Protocol: DNA Library Indexing”.

    Aliquot 2: Proceed to “Protocol: RNA Reverse Transcription”.

    For DNA-only workflow, the whole 13.6 µL elution without split can be used for "Protocol: DNA Library Indexing". This is recommended for fresh DNA below 40 ng input or all FFPE DNA.

    Alternatively, the samples can be stored at –30°C to –15°C in a constant-temperature freezer.

Protocol: DNA Library Indexing

Important points before starting

- The starting material is a either a 6.8 µL sample aliquot or the whole 13.6 µL from "Protocol: DNA Ligation".
- Set up all reactions on ice.
- Do not vortex any reagents or reactions.
- Use QIAseq Multimodal EDNA index plates, either EDNA-24X or EDNA-96AX.
- Prepare fresh 80% ethanol daily.
- Ensure that the QIAseq Beads are thoroughly mixed at all times. This necessitates working quickly and resuspending the beads immediately before use. If a delay in the protocol occurs, simply vortex the beads before proceeding.

Procedure

1. Prepare the reagents required for DNA library indexing.
    a. Thaw HiFi Ultra Buffer, 5×; MM E F-R Primer Mix; and EDNA-24X or EDNA-96AX index plate at room temperature.
    b. Mix by either flicking the tube or vortexing the index plate, and then centrifuge briefly. Centrifuge the plate at 1000 × *g* for 1 min.

    Note: Just before use, remove HiFi Ultra Polymerase from the freezer and place them on ice. After use, immediately return the enzyme to the freezer.
2. Prepare the reactions on ice according to Table 8. Briefly centrifuge, mix by pipetting up and down 20 times with pipette volume close to 80 µL, and then briefly centrifuge again.

    Note: If setting up more than one reaction, prepare a volume of master mix 10% greater than what is required for the total number of reactions.

Table 8. Reaction mix for DNA indexing

Component	Volume/reaction (µL)	
	Both DNA and RNA or DNA-only input	DNA-only input with whole ligation product
Sample aliquot (from "Protocol: DNA Ligation")	6.8	13.6
HiFi Ultra Buffer, 5×	16	16
MM E F-R Primer Mix	4.8	4.8
HiFi Ultra Polymerase	12	12
Index from EDNA-24X or EDNA-96AX index plate well*	2.5	2.5
Nuclease-Free Water	37.9	31.1
Total	**80**	**80**

*Ensure proper technique to prevent cross-contamination. Additionally, ensure that every sample has a unique index and that no well is used twice.

3. Program a thermal cycler as described in Table 9, using cycle numbers described in Table 10.

Important: Set up the ramp rate of the thermal cycler at ≤2°C/s.

Table 9. Cycling conditions for DNA indexing

Step	Time	Temperature (°C)
Hold	2 min	98
3-step cycling		
Denaturation	20 s	98
Annealing	1 min	60
Extension	1 min	72
Cycle number	See Table 10	
Hold	3 min	72
Hold	∞	4

Table 10. Cycle number recommendations for DNA indexing, based on original sample input

DNA Input	Fresh sample	FFPE sample (cycles)
40 ng	15	16
100 ng	14	15
250 ng	13	14

4. After the reaction is complete, clean up reaction with QIAseq beads. Alternatively, experienced user of this workflow can use quick protocol in "Appendix B" if input DNA are of same amount and similar quality among samples.
5. For bead clean up, add 72 µL QIAseq Beads, and then mix by vortexing or pipetting up and down 20 times with pipette volume close to 152 µL.
6. Incubate for 5 min at room temperature.
7. Place the tubes/plate on a magnetic rack for 5 min (for tubes) or 10 min (for plates) to separate the beads from the supernatant.

   After the solution has cleared, with the beads still on the magnetic stand, carefully remove and discard the supernatant.

   Important: Do not discard the beads because they contain the DNA of interest.
8. With the beads still on the magnetic stand, add 200 µL of 80% ethanol. Rotate the tube 2–3 times or move the plate side to side between the 2 column positions of the magnet to wash the beads. Carefully remove and discard the wash.
9. Repeat the ethanol wash once, for a total of 2 washes.

   Important: Completely remove all traces of the ethanol after the second wash. Remove the ethanol with a 200 µL pipette tip first, spin down briefly, and then use a 10 µL pipette tip to remove any residual ethanol.
10. Dry beads at 60°C thermal cycler or heat block for 1–2 min, or air-dry at room temperature for 10 min.

Note: Visually inspect the pellet to confirm that it is completely dry. If the pellet is not completely dry, additional drying time may be required.

11. Elute the DNA from the beads by adding 24 µL Nuclease-Free Water. Mix well by pipetting.
12. Return the tube/plate well to the magnetic rack until the solution has cleared.
13. Transfer 22 µL of the supernatant to clean tubes/plate wells.
14. The whole genome (WGS) library is now ready for sequencing or hybrid capture. Proceed to "Recommendations: Library QC and Quantification".

    The library is in very high concentration, it is recommended to take 1 µL to dilute 20-fold for QC. Alternatively, the samples can be stored at –30°C to –15°C in a constant-temperature freezer.
15. For targeted hybrid capture, go to "Protocol: Target Enrichment from Indexed whole genome or whole transcriptome Libraries Using QIAseq xHYB Human Hybrid Capture CGP Panels for Element Biosciences Trinity Flow Cell". For WGS or WTS library sequencing, follow protocol in "Appendix A: Sequencing WGS and/or WTS library on Aviti sequencer".

Protocol: RNA Reverse Transcription

Important points before starting

- The starting material is a 6.8 µL sample aliquot from "Protocol: Clean up Ligation or Polyadenylation with QIAseq bead".
- If input is DNA-only, skip this protocol and go to "Protocol: Target Enrichment from Indexed whole genome or whole transcriptome Libraries Using QIAseq xHYB Human Hybrid Capture CGP Panels for Element Biosciences Trinity Flow Cell"
- Set up reactions on ice.
- Do not vortex any reagents or reactions.
- Prepare fresh 80% ethanol daily.
- Ensure that the QIAseq Beads are thoroughly mixed at all times. This necessitates working quickly and resuspending the beads immediately before use. If a delay in the protocol occurs, simply vortex the beads before proceeding.

Procedure

1. Prepare the reagents required for sample pre-treatment.

   Note: Just before use, remove Buffer GE2 gDNA elimination buffer from the freezer and place them on ice. After use, immediately return the tube to the freezer.

2. Prepare the sample pretreatment mix on ice according to Table 11. Briefly centrifuge, mix by pipetting up and down 20 times with pipette volume close to 8.4 µL, and then briefly centrifuge again.

   Note: If setting up more than one reaction, prepare a volume of master mix 10% greater than what is required for the total number of reactions.

Table 11. Reaction mix for sample pretreatment

Component	Volume/reaction (µL)
Sample aliquot (from "Protocol: Clean up Ligation or Polyadenylation with QIAseq bead ")	6.8
Buffer GE2 gDNA elimination buffer	1.6
Total	**8.4**

3. Incubate the reactions in a thermal cycler according to Table 12. Use the instrument's heated lid.

Table 12. Incubation conditions for sample pretreatment

Step	Incubation temperature (°C)	Incubation time (min)
1	42	5
2	75	10
3	4	Hold

4. Prepare the reagents required for reverse transcription.
   a. Thaw US RT Buffer, 5×; DTT (100 mM); dNTP (10 mM); MM E RNA RT Primer; and MM E RNA TSO at room temperature.
   b. Mix by flicking the tubes and then centrifuge briefly.

      Note: Just before use, remove the RNase Inhibitor and EZ Reverse Transcriptase from the freezer and place them on ice. After use, immediately return the enzymes to the freezer.

5. To the treated sample, prepare the reverse transcription mix on ice according to Table 13. Briefly centrifuge, mix by pipetting up and down 20 times with pipette volume close to 20 µL, and then briefly centrifuge again.

   Note: If setting up more than 1 reaction, prepare a volume of master mix 10% greater than what is required for the total number of reactions.

Table 13. Reaction mix for reverse transcription

Component	Volume/reaction (µL)
GE2 Treated Sample	8.4
US RT Buffer, 5×	4
DTT (100 mM)	0.5
dNTP (10 mM)	2
MM E RNA RT Primer	1.6
MM E RNA TSO	1.6
Multimodal RT Enhancer	0.4
RNase Inhibitor	0.5
EZ Reverse Transcriptase	1
Total	**20**

6. Incubate the reactions in a thermal cycler according to Table 14. Use the instrument's heated lid.

Table 14. Incubation conditions for reverse transcription

Step	Incubation temperature (°C)	Incubation time (min)
1	4	1
2	25	5
3	42	90
4	70	10
5	4	1
6	4	Hold

7. After reaction is completed, add 20 µL Nuclease-Free Water and 44 µL QIAseq Beads and mix by vortexing or by pipetting up and down 20 times with pipette volume close to 84 µL.
8. Incubate for 5 min at room temperature.
9. Place the tubes/plate on a magnetic rack for 5 min (for tubes) or 10 min (for plates).
10. After the solution has cleared, with the beads still on the magnetic stand, carefully remove and discard the supernatant.

    Important: Do not discard the beads because they contain the DNA of interest.
11. With the beads still on the magnetic stand, add 200 µL of 80% ethanol. Rotate the tube 2–3 times or move the plate side to side between the 2 column positions of the magnet to wash the beads. Carefully remove and discard the wash.
12. Repeat the ethanol wash.

    Important: Completely remove all traces of the ethanol after the second wash. Remove the ethanol with a 200 µL pipette tip first, spin down briefly, and then use a 10 µL pipette tip to remove any residual ethanol.
13. Dry beads at 60°C thermal cycler or heat block for 1–2 min, or air-dry at room temperature for 10 min.

    Note: Visually inspect the pellet to confirm that it is completely dry. If the pellet is not completely dry, additional drying time may be required.
14. Elute the DNA from the beads by adding 42 µL Nuclease-Free Water.
15. Return the tube/plate to the magnetic rack until the solution has cleared.
16. Transfer 40 µL of the eluate to clean tubes/plate.
17. Add 44 µL QIAseq Beads and mix by vortexing or by pipetting up and down 20 times with pipette volume close to 84 µL.

18. Incubate for 5 min at room temperature.
19. Place the tubes/plate on a magnetic rack for 5 min (for tubes) or 10 min (for plates). After the solution has cleared, with the beads still on the magnetic stand, carefully remove and discard the supernatant.

    Important: Do not discard the beads because they contain the DNA of interest.
20. With the beads still on the magnetic stand, add 200 µL of 80% ethanol. Rotate the tube 2–3 times or move the plate side to side between the 2 column positions of the magnet to wash the beads. Carefully remove and discard the wash.
21. Repeat the ethanol wash.

    Important: Completely remove all traces of the ethanol after the second wash. Remove the ethanol with a 200 µL pipette tip first, spin down briefly, and then use a 10 µL pipette tip to remove any residual ethanol.
22. Dry beads at 60°C thermal cycler or heat block for 1–2 min, or air-dry at room temperature for 10 min.

    Note: Visually inspect the pellet to confirm that it is completely dry. If the pellet is not completely dry, additional drying time may be required. Ethanol carryover to the next universal PCR step will affect PCR efficiency.
23. Elute the DNA from the beads by adding 22 µL Nuclease-Free Water.
24. Return the tube/plate to the magnetic rack until the solution has cleared.
25. Transfer 20 µL of the eluate to clean tubes/plate.
26. Proceed to "Protocol: RNA Library Indexing". Alternatively, the samples can be stored at –30°C to –15°C in a constant-temperature freezer.

Protocol: RNA Library Indexing

Important points before starting

- The starting material is the 20 µL sample from "Protocol: RNA Reverse Transcription".
- Set up all reactions on ice.
- Do not vortex any reagents or reactions.
- Use ERNA index plates, either ERNA-24X or ERNA-96AX.
- Prepare fresh 80% ethanol daily.
- Ensure that the QIAseq Beads are thoroughly mixed at all times. This necessitates working quickly and resuspending the beads immediately before use. If a delay in the protocol occurs, simply vortex the beads before proceeding.

Procedure

1. Prepare the reagents required for RNA Library indexing.

   a. Thaw HiFi Ultra Buffer, 5×; MM E F- R Primer Mix, and ERNA- 24X or ERNA- 96AX index plate at room temperature, place them on ice after thawing.

   b. Mix by either flicking the tube or vortexing the index plate, and then centrifuge briefly. Plate should be centrifuged at 1000 × *g* for 1 min.

   Note: Just before use, remove the HiFi Ultra Polymerase from the freezer and place them on ice. After use, immediately return the enzyme to the freezer.

2. Prepare the reactions on ice according to Table 15. Briefly centrifuge, mix by pipetting up and down 20 times with pipette volume close to 80 µL, and then briefly centrifuge again.

   Note: If setting up more than one reaction, prepare a volume of master mix 10% greater than what is required for the total number of reactions.

Table 15. Reaction mix for RNA indexing

Component	Volume/reaction (µL)
Sample aliquot (from ""Protocol: RNA Reverse Transcription" on page 41")	20
HiFi Ultra Buffer, 5×	16
MM E F-R Primer Mix	4.8
HiFi Ultra Polymerase	12
Index from ERNA-24X or ERNA-96AX index plate*	2.5
Nuclease-Free Water	24.7
Total	**80**

*Ensure proper technique to prevent cross-contamination. Additionally ensure that every sample has a unique index and that no well is used twice.

3. Program a thermal cycler as described in Table 16, using cycle numbers described in Table 17.

Important: Set up the ramp rate of the thermal cycler at ≤2°C/s.

Table 16. Cycling conditions for RNA indexing

Step	Time	Temperature (°C)
Hold	2 min	98
2-step cycling		
Denaturation	20 s	98
Annealing	1 min	60
Extension	1 min	72
Cycle number	See Table 17	
Hold	3 min	72
Hold	∞	4

Table 17. Cycle number recommendations for RNA indexing, based on original sample input

RNA Input (ng)	Fresh sample or FFPE DV200>=60% (cycle)	FFPE sample DV200<60% (cycle)
80	25	27
200	23	25
500	22	24

4. After the reaction is complete, add 72 µL QIAseq Beads, and then mix by vortexing or pipetting up and down 20 times with pipette volume close to 152 µL.
5. Incubate for 5 min at room temperature.
6. Place the tubes/plate on a magnetic rack for 5 min (for tubes) or 10 min (for plates) to separate the beads from the supernatant.

   After the solution has cleared, with the beads still on the magnetic stand, carefully remove and discard the supernatant.

   Important: Do not discard the beads because they contain the DNA of interest.
7. With the beads still on the magnetic stand, add 200 µL of 80% ethanol. Rotate the tube 2–3 times or move the plate side to side between the 2 column positions of the magnet to wash the beads. Carefully remove and discard the wash.
8. Repeat the ethanol wash once, for a total of 2 washes.

   Important: Completely remove all traces of the ethanol after the second wash. Remove the ethanol with a 200 µL pipette tip first, spin down briefly, and then use a 10 µL pipette tip to remove any residual ethanol.
9. Dry beads at 60°C in a thermal cycler or on a heat block for 1–2 min, or air-dry at room temperature for 10 min.

   Note: Visually inspect the pellet to confirm that it is completely dry. If the pellet is not completely dry, additional drying time may be required.

10. Elute the DNA from the beads by adding 24 µL Nuclease-Free Water. Mix well by pipetting.
11. Return the tubes/plate to the magnetic rack until the solution has cleared.
12. Transfer 22 µL of the supernatant to clean tubes/plate.
13. The library is now ready for sequencing or hybrid capture. Proceed to "Recommendations: Library QC and Quantification".

    Note: Due to the high concentration of the library, it is recommended to take 1 µL to dilute 20-fold for Quality Check (QC). Alternatively, the samples can be stored at –30°C to –15°C in a constant-temperature freezer.

Protocol: Target Enrichment from Indexed whole genome or whole transcriptome Libraries Using QIAseq xHYB Human Hybrid Capture CGP Panels for Element Biosciences Trinity Flow Cell

Pool libraries and concentrate the indexed library with bead

Pooling of amplified, indexed whole genome or whole transcriptome libraries enables processing multiple libraries in a single hybridization-capture reaction. We recommend pooling a minimum of 8 indexed libraries for one hybridization capture reaction of CGP DNA or RNA panel. The amount of indexed libraries to be pooled depends on the number of samples per pool. Make sure to only use PCR-amplified libraries for hybridization capture

Things to do before starting

- Before pooling library, thaw the Trinity sequencing cartridge protected from light, and in a water bath at room temperature for 2.5–3 h. Alternatively, the Trinity sequencing cartridge can be thawed at 4°C for 24 h. Once thawed, store the cartridge at 4°C before sequencing.
- Quantify the amplified, indexed libraries with Nanodrop (see guidelines in “Important Notes”).
- Thaw the xHYB Probe Set, One-4-All Blocking Solution, and either E DNA Binding Reagent or E RNA Binding Reagent on ice. Then, pulse-vortex and pulse-spin.
- Equilibrate the Vapor-Lock reagent and E Dry hybridization Buffer to room temperature.

Procedure

1. For each hybrid capture pool, determine the per-library amount (ng) according to the estimated reads of each library in Table 18 and Table 19, and pooling size referring to the guidelines in Table 20 and Table 21 for library and panel type.

   Important: The number of reads on Trinity flow cell is directly related to input library amount and hybridization panel size used.

   Note: For Trinity flow cell, it is required to start with a higher library amount in the hybridization reaction than in a regular manual hybrid-capture workflows.

   Note: Adjusting library multiplexing to achieve specific library reads on Trinity flow cell differs from conventional sequencing. In conventional sequencing, doubling the reads for each library can be achieved by loading half the number of libraries on the sequencing flow cell. In contrast for the Trinity flow cell, achieving double the reads requires not only reducing the number of libraries by half, but also doubling the each library input to the hybridization reaction.

Table 18. Estimated number of reads for each sample on Trinity flow cell based on library type, panel, and library input amount

Library type	Panel	Panel size (~Mb)	Library input (µg)	Estimated paired reads per library on Trinity flow cell (M)
WGS				
	CGP DNA	2	2.5	240
			1.25	120
			0.63	60
	[**Custom xHYB Panel**]	1	2.5	120
	[**Custom xHYB Panel**]	4	0.63	120
WTS				
	CGP RNA	0.2	2.5	240
			1.25	120
			0.63	60
	[**Custom xHYB Panel**]	0.1	2.5	120
	[**Custom xHYB Panel**]	0.4	0.63	120

Table 19. Recommended paired reads for CGP DNA and RNA panel to reach optimal panel performance

	DNA + RNA library	DNA-only library	RNA-only library
CGP DNA library reads (M)	75	75	–
Reads for CGP RNA library (M)	50	–	50

Table 20. Recommended library pooling strategy for sequencing of CGP DNA-only or RNA-only library for hybridization capture and loading on one Trinity flow cell

No. of libraries	No. of Hybridization Pool	Library type	Panel	Library input (µg)*	Estimated total paired reads on Trinity flow cell (M)
8	1	WGS	CGP DNA	2.5	1920
16	2	WGS	CGP DNA	1.25	1920
24	3	WGS	CGP DNA	0.83	1920
8	1	WTS	CGP RNA	2.5	1920
16	2	WTS	CGP RNA	1.25	1920
24	3	WTS	CGP RNA	0.83	1920
32	4	WTS	CGP RNA	0.63	1920

* Do not exceed a total WGS library input of 24 µg or WTS library input of 12 µg per pool.

Note: To maintain equal representation of libraries during sequencing, use the same input amount for all libraries in the hybridization pool. For optimal passing filter, it is recommended to load less than 2B paired reads due to the unique read structure of QIAseq MM library with UMI.

Table 21. Recommended library pooling strategy for sequencing of both CGP DNA and RNA libraries for hybridization capture and loading on one Trinity flow cell

Total library number	DNA-only or RNA-only library number	No. of hybridization pool	Library input (µg)	Estimated total paired reads on Trinity flow cell (M)
16	8 (DNA)	CGP DNA (1)	1.25	960
	8 (RNA)	CGP RNA (1)	1.25	960
24	16 (DNA)	CGP DNA (2)	0.94	1440
	8 (RNA)	CGP RNA (1)	0.63	480
32	16 (DNA)	CGP DNA (2)	0.83	1280
	16 (RNA)	CGP RNA (2)	0.42	640

Note: To maintain equal representation of libraries during sequencing, use the same input amount for all libraries in the same hybridization pool.

2. Calculate the required volume (µL) of each amplified library for pooling by dividing the desired DNA input by the library concentration.

   For example, to add 2.5 µg of indexed library in a concentration of 200 ng/µL, pipette 12.5 µL into the capture pool.

3. Transfer the calculated volumes for each library to a 1.5 mL LoBind tube for each hybridization pool. Use multiple tubes if performing multiple hybridization reactions at once.

4. Add QIAseq Beads in an amount equal to 2.5 times the library volume. For example, if a pooled library volume is 100 µL, then add 250 µL QIAseq Beads. Mix by vortexing.
5. Incubate for 5 min at room temperature.
6. Place the tubes on a magnetic rack for 5 min to separate the beads from the supernatant. After the solution has cleared, with the beads still on the magnetic stand, carefully remove and discard the supernatant.

   Important: Do not discard the beads because they contain the DNA of interest.
7. With the beads still on the magnetic stand, add 200 µL of 80% ethanol. Rotate the tube 2–3 times. Carefully remove and discard the wash.
8. Repeat the ethanol wash, for a total of 2 washes.

   Important: Completely remove all traces of the ethanol after the second wash. Remove the ethanol with a 200 µL pipette tip first, spin down briefly, and then use a 10 µL pipette tip to remove any residual ethanol.
9. Dry beads at room temperature for 10 min.

   Note: Visually inspect the pellet to confirm that it is completely dry. If the pellet is not completely dry, additional drying time may be required.
10. While the beads are drying, refer to Table 22 to prepare the elution mix for each hybrid capture pool. Mix by pipetting up and down 20 times with pipette volume close to 25 µL.

Table 22. Hybridization reaction mix

	Volume/reaction (µL))	
Component	**CGP DNA**	**CGP RNA**
xHYB Probe Set (CGP DNA or RNA panel)*	4	4
One-4-All Blocking Solution	5	5
E DNA Binding Reagent†	5	N/A
E RNA Binding Reagent†	N/A	5
Nuclease-Free Water	11	11
Total	**25**	**25**

*Use the CGP DNA probe set for DNA library pools and the CGP RNA panel for RNA library pools.

†Use E DNA Binding Reagent for WGS library hybridization with CGP DNA probe and use E RNA Binding Reagent for WTS library hybridization with CGP RNA probe.

11. After the beads have dried, add 25 µL hybridization reaction mix to elute the DNA from the beads. Mix well by pipetting up and down 20 times with pipette volume close to 25 µL.
12. Incubate 5 min at room temperature.
13. Return the tube to the magnetic rack until the solution has cleared.
14. Take the whole 25 µL elute. Minor bead carryover is not a problem.
15. For each hybrid capture pool, obtain one hybridization tube containing the E Dry hybridization Buffer.
16. Dispense the eluate onto the dried buffer pellet. Do not pipet to mix.
17. Close the tube with its lid.
18. Set a thermal cycler to 95°C with the heated lid switched on.
19. Place the hyb tube at 95°C for 5 min.

20. Take the tube out and vortex for 1 min at maximum speed.
21. Repeat steps 18 and 19 twice for a total of 3 times.
22. Pulse spin the tube and add 30 µL of Vapor Lock. Close the tube tightly.
23. Proceed immediately to next step (“Hybridization capture”).

Hybridization capture

During the hybridization step, QIAseq xHYB Probes bind specifically to targeted library fragments present in the pool. The flexible hybridization protocol allows adjusting the duration of hybridization from as little as 30 min to 4 h.

Important: Do not incubate more than 4 h for hybridization.

Things to do before starting

- When performing the hybridization capture reaction for 30 min, start preparing sequencing reagents needed for sequencing on Trinity flow cell after hybridization as described in the next chapter.

Procedure

1. Program a thermal cycler with a heated lid according to Table 23.

   Important: The heated lid must be set to 85°C or higher to prevent evaporation.

Table 23. Conditions for hybridization capture

Step	Incubation temperature (°C)	Incubation time
1	95	1 min
2	95	5 min
3	71	30 min–4 h*
4	71	Hold†

* 30 min hybridization is enough for optimal result, hybridization time should not be extended to more than 4 h.

† Program the thermal cycler to maintain 71°C after hybridization is complete. Do not allow the hybridization reaction to cool down as this negatively affects off-target rate.

2. Start the program. When the thermal cycler block reaches 95°C, pause the program in step 1.

3. Transfer the tube with hybridization reaction mixed with hybridization buffer in step 22 of "Pool libraries and concentrate the indexed library with bead" into the thermal cycler. Continue the program at 95°C.

   Important: Make sure the heated lid is set to 85°C or higher and the tube is tightly sealed with lid. Make sure to use the correct lid spacers for your thermal cycler.

   Important: During hybridization, start preparing the Trinity sequencing as described in "Protocol: Trinity sequencing run on an Aviti System with QIAseq xHYB CGP Panel hybridization", next section.

4. After hybridization, immediately add 145 µL Trinity Fast Hyb Loading Buffer to the finished hybridization reaction, then mix by pipetting 7–8 times with pipette volume close to 200 µL. Place the diluted hybridization reaction on ice.

   Important: Do not allow the hybridization reaction to incubate more than 4 h.

5. Proceed immediately to "Protocol: Trinity sequencing run on an Aviti System with QIAseq xHYB CGP Panel hybridization".

   Alternatively, diluted hybridization product can be stored at –30°C to –15°C for up to 7 days.

Protocol: Trinity sequencing run on an Aviti System with QIAseq xHYB CGP Panel hybridization

Run preparation

Library hybridized with xHYB CGP DNA or RNA panel can be sequenced directly on Trinity flow cell on an Aviti System.

Things to do before starting

- Always use Trinity 2 × 150 Sequencing Kit.
- Add QIAseq RNA Index 1 Primer E, QIAseq RNA Read 1 Primer E, and QIAseq RNA Read 2 Primer E custom sequencing primers to the sequencing cartridge when sequencing libraries hybridized with CGP RNA panel.
- Ensure that the Trinity sequencing cartridge is completely thawed.

Procedure

1. For each sequencing flow cell, prepare the sequencing solution based on the number of hybridization pools to be sequenced in one flow cell, as described in Table 24, in a 5 mL tube. Mix by pipetting, flicking tube, and then place on ice.

   Important: Load all 200 µL diluted hybridization of each pool to reach the estimated reads in Table 18, Table 20, and Table 21.

Table 24. Sequencing solution

	Volume (µL)			
Component	**One Hyb pool**	**Two Hyb pools**	**Three Hyb pools**	**Four Hyb pools**
Trinity Sequencing Reagent	72	72	72	72
Trinity Phix Control	8	8	8	8
Trinity Fast Hyb Loading Buffer	1920	1720	1520	1320
Total	**2000**	**1800**	**1600**	**1400**

Run parameter set up

Procedure

2. On Aviti sequencer home screen, select **New Run** to initiate a sequencing run. Then select **Sequencing**.
3. Select which side for sequencing: **Side A**, **Both**, or **Side B**. For chemistry type, select **Trinity**, and then select **Next**.
4. In the run set up, choose either:
    a. Select **Planned run** to input the run configured in advance on Element biocloud. Once you have chosen this option, skip steps 5–9 and proceed to "Library loading and sequencing run".
    b. Set up manually as below (step 5–9).
5. In the **Run Name** field, enter a unique name. To import the run manifest, select **Browse** and navigate to the run manifest.
6. In the **Storage** drop-down menu, select a storage location.
7. In the **Sequencing Kit** drop-down menu, select the appropriate Trinity Sequencing Kit, 2×150 Trinity.

8. In the **Panel** drop-down menu, select **Other**.
9. In the **Cycles** fields, enter the number of cycles for each read:
    - **Index1 (I1)**: 13
    - **Index2 (I2)**: 10
    - **Read1 (R1)**: 151
    - **Read2 (R2)**: 151

    Then select **Next**.

Library loading and sequencing run

Procedure

10. Gently invert the Trinity Sequencing Cartridge 10 times to mix reagents.
11. Tap the cartridge base on the benchtop to remove any large droplets from the tube tops.
12. Place the cartridge into a clean sequencing basket and lock the clips.
13. Refer to Table 25 to prepare library loading mix in the 5 mL tube containing sequencing solution prepared in step 1. Mix by pipetting or flicking tube and keep on ice.

Table 25. Library loading mix

	Volume (µL)			
Component	**One Hyb pool**	**Two Hyb pools**	**Three Hyb pools**	**Four Hyb pools**
Sequencing solution from step 1	2000	1800	1600	1400
Total diluted Hyb pool	200	400	600	800
Total	**2200**	**2200**	**2200**	**2200**

14. If sequencing only DNA panel hybridization pool, skip steps 15–16 and go directly to step 17 for library loading.
15. If sequencing hybridization pool with RNA panel or mixed pools with both DNA and RNA panel hybridization, using a new 1 mL pipette tip, pierce the center of the applicable I1, R1, and R2 wells of Trinity Sequencing Cartridge to create one hole. Push the foil to the edges. Discard the pipette tip and use new tip for each well.
16. Refer to Table 26 on where to add the applicable volume of 100 µM custom primer to each pierced well. Pipette the content of each tube 15 times to mix. Avoid losing existing primer volume.

Table 26. Custom primers on sequencing cartridge

Custom primer	Volume (µL)	Add to wells on cartridge
QIAseq RNA Index 1 Primer E	19	I 1
QIAseq RNA Read 1 Primer E	32.5	R 1
QIAseq RNA Read 2 Primer E	19	R 2

17. Using a 1 mL pipette tip, pierce the library well on the sequencing cartridge.
18. Transfer the 2200 µL of prepared library loading mix to the library well in the sequencing cartridge.
19. On the screen of sequencer, select **Invert cartridge** > **Insert into basket** > **Load hybrid reaction checkbox** > **Next** to confirm reagent preparation.
20. Open the reagent bay door and remove any used material.
21. Slide the basket containing the Trinity Sequencing Cartridge with loaded hybrid library into the reagent bay.
22. Slide the buffer bottle into the reagent bay until it stops.
23. Close the reagent bay door, and then select **Next**.

24. Open the waste bay door, remove the waste bottle, and close the transport cap.
25. Open the transport cap and the vent cap, then empty the waste bottle.
26. Close the vent cap, then reload the empty waste bottle.
27. Select **Next** to start priming. Bring a new flow cell to room temperature in the package during priming.
28. When priming is complete, select **Next**. The nest door opens automatically.
29. Remove the used flow cell from the nest.
30. Unpackage the new flow cell and load it onto the nest.
31. Select **Close Nest**, and then select **Next**.
32. Review the run parameters to ensure correct setup.
33. Select **Run** to start sequencing.

Recommendations: Library QC and Quantification

NGS Library QC

Perform QC with the QIAxcel Connect or similar instrument. Check for the correct size distribution of library fragments (~400–500 bp median size) and for the absence of adapters or adapter–dimers (~<200 bp) (Figure 6, next page).

Preferred library quantification method

Quantify the WGS or WTS library using Nanodrop if proceeding to "Hybrid Capture", or Qubit-based method if proceeding to sequencing.

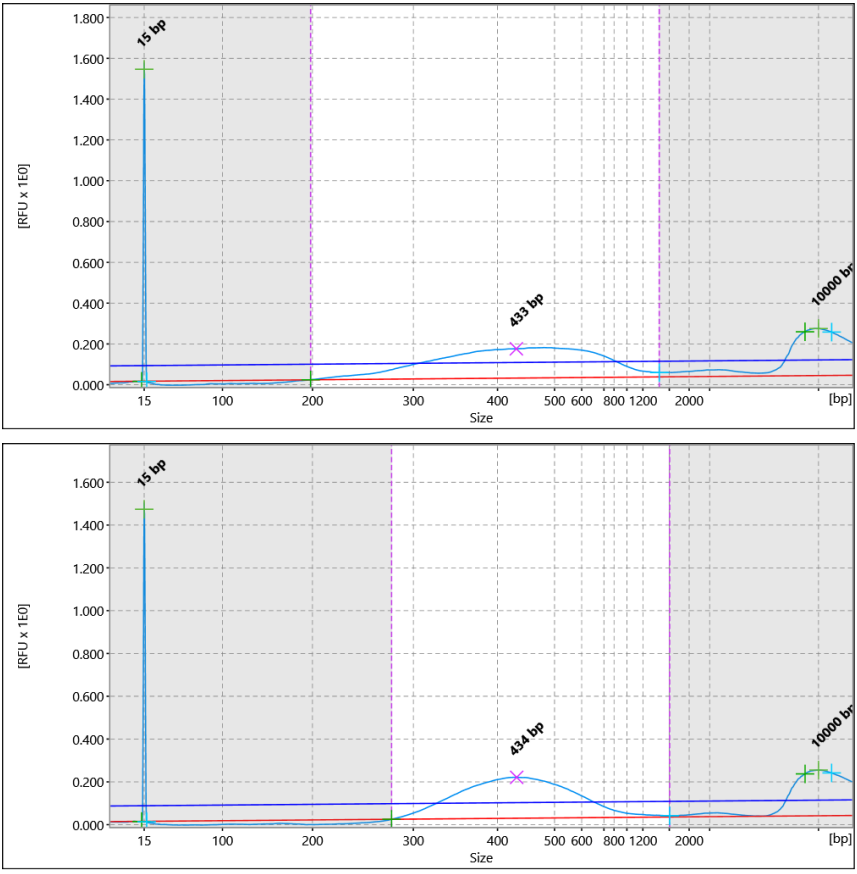

Figure 6. QIAseq Multimodal WGS DNA (upper, 20-fold diluted) and WTS RNA (lower, 20-fold diluted) Libraries.

Troubleshooting guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, visit **www.qiagen.com/FAQ/FAQList.aspx** for Frequently Asked Questions at our Technical Support Center (for contact information, visit **www.qiagen.com**).

	Comments and suggestions
Low CGP Trinity sequencing reads	
a) Suboptimal hybrid capture input quality or amount	Use high-quality clean indexed whole genome or whole transcriptome libraries as input that have been checked using a capillary electrophoresis and quantified accurately.
b) Library amount into hybridization is low.	Sequencing reads on Trinity flow cell is directly related to library amount into the hybridization. When increasing each library reads on the Trinity flow cell by adjusting library multiplexing, reduce the number of libraries while simultaneously increasing the amount of each library added into the hybridization.
c) Hybridization time too long	Sequencing reads might decrease on Trinity flow cell if QIAseq CGP panel hybridization condition exceeds 4h.
d) Inappropriate hybridization temperature	Sequencing reads might decrease on Trinity flow cell if QIAseq CGP panel hybridization condition below 71°C.
Decreased target region specificity or uniformity of CGP DNA or RNA libraries	
a) Incorrect use of Trinity binding reagent	Only use E DNA Binding Reagent in hybridization reaction for CGP DNA library as outlined in the protocol. For CGP RNA panel hybridization, use E RNA Binding Reagent as indicated in the protocol.
b) Suboptimal hybridization temperature	Check thermal cycler programs and ensure thermal cycler is properly calibrated.
Unequal read representation of libraries within a hybrid capture pool	
a) Unequal hybrid capture input library used	Use equal amounts of indexed whole genomes libraries to be pooled for a hybrid capture reaction. Quantify indexed whole genome libraries before hybrid capture by using a capillary electrophoresis method such as the QIAxcel Advanced System or the Agilent 2100 Bioanalyzer using High Sensitivity DNA chips.

	Comments and suggestions
b) Suboptimal library enrichment conditions	Do not use PCR-free whole genome libraries. Always follow the library enrichment protocol outlined in the respective library preparation protocol using QIAGEN HiFi PCR Master Mix for library enrichment. Quantify indexed whole genome libraries using Qubit for sequencing or Nanodrop for hybridization input. Assess library quality via capillary electrophoresis.

Appendix A: Sequencing WGS and/or WTS library on Aviti sequencer

Run preparation

WGS and/or WTS libraries generated with QIAseq Multimodal DNA/RNA Library Kit and QIASeq xHYB Trinity Reagent DNA/RNA Kit can be sequenced directly on Cloudbreak Freestyle™ flow cell on an Aviti System.

Things to do before starting

- Always use Aviti 2 × 150 Sequencing Kit CB Freestyle™ (cat. no. 860-00011, 860-00012, 860-00013)
- Add QIAseq RNA Index 1 Primer E, QIAseq RNA Read 1 Primer E, and QIAseq RNA Read 2 Primer E custom sequencing primers to sequencing cartridge when sequencing WTS libraries. No custom sequencing primers are needed for WGS library.
- Set up number of cycles for each read:
  - **Index1**: 13
  - **Index2**: 10
  - **Read1**: 151
  - **Read2**: 151
- Ensure sequencing cartridge is completely thawed.

Procedure

1. Thaw the cartridge of an Aviti 2 × 150 Sequencing Kit CB Freestyle as described in *Cloudbreak™ Sequencing User Guide* by either using room temperature water bath for 2.5 h, or in a refrigerator for 24 h while protecting the cartridge from light.
2. Perform library dilution and pooling when the cartridge is in thawing stage.
3. Dilute each library to1 nM, pool diluted 1 nM libraries into one pool that will be sequenced in one run. Make sure pooled volume is more than 8 µL.
4. Prepare freshly made 0.2 N NaOH.
5. Start the sample denature step after the cartridge has been fully thawed and ready to use. In the meantime, if sequencing WTS library, thaw 100 µM custom index 1, read 1, and read 2 custom sequencing primers at room temperature and place on ice after thawing.
6. Combine 8.0 µL of the pooled library at 1 nM with 0.4 µL of Cloudbreak PhiX Control Library, then add 8.4 µL of 0.2N NaOH. Mix and incubate at room temperature for 5 min to denature.
7. After 5 min denature, immediately add 8.4 µL 0.2 M Tris-HCL pH7 to the denatured sample. Mix well by pulse vortexing followed by spin down. Keep it on ice.
8. Prepare final library loading solution as Table 27, mix by pipetting and keep on ice. This corresponds to 0.5 pM loading for WGS and WTS library.

Table 27. Final library loading for WGS and WTS library

Component	WGS library (µL)	WTS library (µL)
Denatured library pool	2.1	2.1
Library loading buffer	1397.9	1397.9
Total	**1400**	**1400**

Run parameter set up

Procedure

9. On Aviti sequencer home screen, select **New Run** to initiate a sequencing run. Then select **Sequencing**.
10. Select which side for sequencing: **Side A, Both**, or **Side B**. For chemistry type, select **Cloudbreak**, and then select **Next**.
11. In the run set up, choose either:
    a. Select **Planned run** to input the run configured in advance on Element biocloud. Once you have chosen this option, skip steps 12–16 and proceed to "Library loading and sequencing run".
    b. Set up manually as below (step 12–16).
12. In the **Run Name** field, enter a unique name. To import the run manifest, select **Browse** and navigate to the run manifest.
13. In Library type, select **Elevate** and for Library structure, select **Linear**.
14. In the **Storage** drop-down menu, select a storage location.
15. In the **Sequencing Kit** drop-down menu, select the appropriate Cloudbreak Freestyle Sequencing Kit, 2×150.
16. In the Cycles fields, enter the number of cycles for each read:
    - **I1**: 13
    - **I2**: 10
    - **R1**: 151
    - **R2**: 151

    Then select **Next**.

Library loading and sequencing run

Procedure

17. Gently invert the Cloudbreak Sequencing Cartridge 10 times to mix reagents.
18. Tap the cartridge base on the benchtop to remove any large droplets from the tube tops.
19. Place the cartridge into a clean sequencing basket and lock the clips.
20. If sequencing only WGS library pool: skip steps 21–22 and go directly to step 23 for library loading.
21. If sequencing WTS library or mixed pools with both WGS and WTS libraries: use a new 1 mL pipette tip, then pierce the center of the applicable I1, R1, and R2 wells of sequencing cartridge to create one hole each. Push the foil to the edges. Discard the pipette tip and use a new tip for each well.
22. Refer to Table 28 on which pierced well to add the applicable volume of 100 µM custom sequencing primer. Pipette the content of each tube 15 times to mix. Avoid losing existing primer volume.

Table 28. Custom primers on sequencing cartridge

Custom primer	Volume (µL)	Add to wells on cartridge
QIAseq RNA Index 1 Primer E	19	I 1
QIAseq RNA Read 1 Primer E	32.5	R 1
QIAseq RNA Read 2 Primer E	19	R 2

23. Using a 1 mL pipette tip, pierce the library well on the sequencing cartridge.
24. Transfer the 1400 µL of prepared library loading mix from step 8 to the library well in the sequencing cartridge.

25. On the screen of sequencer, select **Invert cartridge** > **Insert into basket** > **Load library checkbox** > **Next** to confirm reagent preparation.
26. Open the reagent bay door and remove any used material.
27. Slide the basket containing the Cloudbreak Sequencing Cartridge with loaded library into the reagent bay.
28. Slide the buffer bottle into the reagent bay until it stops.
29. Close the reagent bay door, and then select **Next**.
30. Open the waste bay door, remove the waste bottle, and close the transport cap.
31. Open the transport cap and the vent cap, then empty the waste bottle.
32. Close the vent cap and reload the empty waste bottle.
33. Select **Next** to start priming. Bring a new flow cell to room temperature in the package during priming.
34. When priming is complete, select **Next**. The nest door opens automatically.
35. Remove the used flow cell from the nest.
36. Unpackage the new flow cell and load it onto the nest.
37. Select **Close Nest**, and then select **Next**.
38. Review the run parameters to ensure correct setup. Select **Run** to start sequencing.

Appendix B: DNA-only library with similar DNA quality and input for Target Enrichment Using QIAseq xHYB Human Hybrid Capture CGP DNA Panels for Element Biosciences Trinity Flow Cell

Library pooling

For an experienced user of this workflow, WGS libraries generated with QIAseq Multimodal DNA Library Kit and QIASeq xHYB Trinity Reagent DNA/RNA Kit on samples with the same quality and input amount can be pooled without performing bead clean up after DNA index library amplification.

Things to do before starting

- Before pooling library, thaw the Trinity sequencing cartridge protected from light, and in a water bath at room temperature for 2.5–3 h. Alternatively, the Trinity sequencing cartridge can be thawed at 4°C for 24 h. Once thawed, store the cartridge at 4°C before sequencing.
- Quantify the amplified, indexed libraries with Nanodrop.
- Thaw the xHYB Probe Set, One-4-All Blocking Solution, and E DNA Binding Reagent on ice. Then, pulse-vortex and pulse-spin.
- Equilibrate the Vapor-Lock reagent and E Dry hybridization Buffer to room temperature.

Procedure

1. After DNA index library amplification in “Protocol: DNA Library Indexing” step 4, without bead clean up, take 1 µL library and dilute it to 20 µL with Nuclease-Free Water. Use diluted library to assess library quality by Tapestation and quantity by Nanodrop.
2. On Tapestation, a clear and robust library peak should be present. The dimmer peak is normal without bead clean up; it will be removed during concentrate step with beads after library pooling. Library overamplification is not an issue.

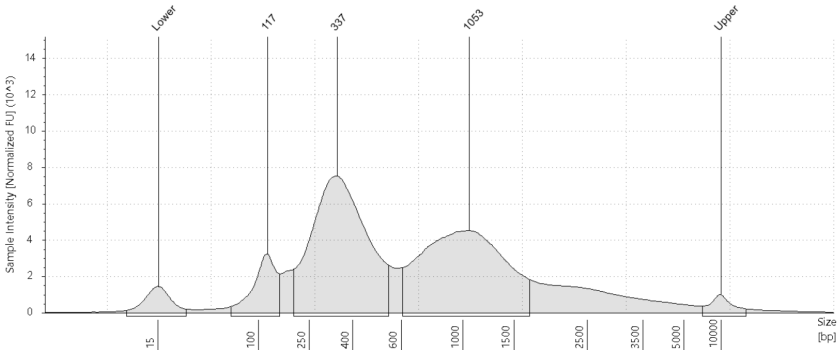

Figure 7. QIAseq Multimodal WGS DNA library after index amplification without bead clean up. Dimmer around 110bp is normal and overamplification is not an issue.

3. The library concentration measured by Nanodrop without bead cleanup is not an accurate library concentration, but can be used to normalize libraries for pooling. For each hybrid capture pool, refer to Table 29 to determine the per-library amount (µg) of each library and pooling size based on Nanodrop measurement.

Table 29. Recommended library pooling strategy for no bead cleaned WGS library for sequencing of CGP DNA-only library for hybridization capture and loading on one Trinityflow cell

No. of libraries	No. of pools	Library type	Panel	Each library input (µg)*	Estimated total reads on Trinity flow cell (M)
8	1	WGS	CGP DNA	76	960
16	2	WGS	CGP DNA	38	960
24	3	WGS	CGP DNA	25	960

* The library amount is not the actual library amount; the Nanodrop numbers used here mainly for normalization among libraries.

4. Calculate the required volume (µL) of each amplified library for pooling by dividing the desired DNA input by the library concentration. For a library concentration of 1600 ng/µL, 47.5 µL is required to obtain 76 µg of indexed library for the capture pool.
5. Transfer the calculated volumes for each library to a 1.5 mL LoBind tube for each hybridization pool. Use multiple tubes if performing multiple hybridization reactions at once.
6. Add 0.9× volume of QIAseq Beads. For example if a pooled library volume is 400 µL, then add 360 µL QIAseq Beads. Mix by vortexing.
7. Incubate for 5 min at room temperature.
8. Place the tubes on a magnetic rack for 5 min to separate the beads from the supernatant. After the solution has cleared, with the beads still on the magnetic stand, carefully remove and discard the supernatant.

   Important: Do not discard the beads because they contain the DNA of interest.
9. With the beads still on the magnetic stand, add 200 µL of 80% ethanol. Rotate the tube 2–3 times. Carefully remove and discard the wash.
10. Repeat the ethanol wash, for a total of 2 washes.

Important: Completely remove all traces of the ethanol after the second wash. Remove the ethanol with a 200 µL pipette tip first, spin down briefly, and then use a 10 µL pipette tip to remove any residual ethanol.

11. Dry beads at room temperature for 10 min.

    Note: Visually inspect the pellet to confirm that it is completely dry. If the pellet is not completely dry, additional drying time may be required.

12. After the beads have dried, elute the DNA from the beads by adding 12 µL Nuclease-Free Water. Mix well by vortexing.

13. Incubate 5 min at room temperature.

14. Return the tube to the magnetic rack until the solution has cleared.

15. Take the whole 12 µL elute, some beads carrying is not a problem. Take 1 µL and dilute 20-folds with Nuclease-Free Water for checking on Tapestation.

16. Refer to Table 30 to make the hybridization reaction. Mix by pipetting up and down at least 20 times.

Table 30. Hybridization reaction mix

Component	Volume/reaction (µL) (CGP DNA)
Cleaned and concentrated pooled library	11
xHYB Probe Set (CGP DNA panel)	4
One-4-All Blocking Solution	5
E DNA Binding Reagent	5
Total	**25**

17. For each hybrid capture pool, obtain 1 hybridization tube containing the E Dry hybridization Buffer.

18. Dispense the 25 µL hybridization reaction mix onto the dried buffer pellet. Do not pipet to mix.
19. Close the tube with its lid.
20. Set a thermal cycler to 95°C with the heated lid switched on.
21. Place the hyb tube at 95°C for 5 min.
22. Take the tube out and vortex for 1 min at maximum speed.
23. Repeat steps 21 and 22 two more times for a total of 3 times.
24. Pulse spin the tube and add 30 µL of Vapor Lock. Close the tube tightly.
25. Proceed immediately to “Hybridization capture protocol”.

Ordering Information

Product	Contents	Cat. no.
QIASeq xHYB Trinity Reagent DNA/RNA Kit (24)	DNA and RNA indexes for 24 samples for a total of 48 reactions plus reagents required for hybrid capture-based target enrichment for Trinity flow cell. Kit contains 6 hybrid capture reactions, which are typically sufficient for 24 DNA and 24 RNA libraries.	336002
QIASeq xHYB Trinity Reagent DNA/RNA Kit (96)	DNA and RNA indexes for 96 samples for a total of 192 reactions plus reagents required for hybrid capture-based target enrichment for Trinity flow cell. Kit contains 24 hybrid capture reactions, which are typically sufficient for 96 DNA and 96 RNA libraries.	336005
QIASeq xHYB Trinity Reagent DNA Kit (24)	DNA indexes for 24 samples for a total of 24 reactions plus reagents required for hybrid capture-based target enrichment for Trinity flow cell. Kit contains 3 hybrid capture reactions, which are typically sufficient for 24 DNA libraries.	336012
QIASeq xHYB Trinity Reagent DNA Kit (96)	DNA indexes for 96 samples for a total of 96 reactions plus reagents required for hybrid capture-based target enrichment for Trinity flow cell. Kit contains 12 hybrid capture reactions, which are typically sufficient for 96 DNA libraries.	336015
QIASeq xHYB Trinity Reagent RNA Kit (24)	RNA indexes for 24 samples for a total of 24 reactions plus reagents required for hybrid capture-based target enrichment for Trinity flow cell. Kit contains 3 hybrid capture reactions, which are typically sufficient for 24 RNA libraries.	336022
QIASeq xHYB Trinity Reagent RNA Kit (96)	RNA indexes for 96 samples for a total of 96 reactions plus reagents required for hybrid capture-based target enrichment for Trinity flow cell. Kit contains 12 hybrid capture reactions, which are typically sufficient for 96 RNA libraries.	336025
QIAseq xHYB CGP DNA/RNA Panel (24)	xHYB probe panel for DNA and RNA target enrichment for comprehensive genomic profiling, fixed panel for 24 DNA and 24 RNA libraries	335122
QIAseq xHYB CGP DNA/RNA Panel (96)	xHYB probe panel for DNA and RNA target enrichment for comprehensive genomic profiling, fixed panel for 96 DNA and 96 RNA libraries	335125
QIAseq xHYB CGP DNA Panel (24)	xHYB probe panel for DNA target enrichment for comprehensive genomic profiling, fixed panel for 24 DNA libraries	333122

Product	Contents	Cat. no.
QIAseq xHYB CGP DNA Panel (96)	xHYB probe panel for DNA target enrichment for comprehensive genomic profiling, fixed panel for 96 DNA libraries	333125
QIAseq xHYB CGP RNA Panel (24)	xHYB probe panel for RNA target enrichment for comprehensive genomic profiling, fixed panel for 24 RNA libraries	334122
QIAseq xHYB CGP RNA Panel (96)	xHYB probe panel for RNA target enrichment for comprehensive genomic profiling, fixed panel for 96 RNA libraries	334125
QIAseq Multimodal DNA/RNA Lib Kit (24)	Reagents (except indexes) for multimodal (DNA and RNA) library preparation of 24 samples: 24 DNA and 24 RNA libraries	334842
QIAseq Multimodal DNA/RNA Lib Kit (96)	Reagents (except indexes) for multimodal (DNA and RNA) library preparation of 96 samples: 96 DNA and 96 RNA libraries	334845

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Document Revision History

Date	Changes
05/2026	Initial release.

Limited License Agreement for QIAseq® xHYB Trinity™ Reagent DNA/RNA Kit

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Notes.