Illumina DNA Prep with Enrichment Checklist

Tagment Genomic DNA

- ☐ 1 Add 2–30 µl DNA to a 96-well PCR plate.
- ☐ 2 If DNA volume < 30 µl, add nuclease-free water to bring the volume to 30 µl.
- ☐ 3 Vortex eBLT (yellow cap) vigorously to resuspend.
- ☐ 4 For each sample, combine the following volumes.
  - eBLT (11.5 µl)
  - TB1 (11.5 µl)
- ☐ 5 Vortex the master mix to resuspend.
- ☐ 6 Divide the master mix volume into an 8-tube strip.
- ☐ 7 Transfer 20 µl to each well containing a sample.
- ☐ 8 Discard the 8-tube strip.
- ☐ 9 Pipette 10 times or shake at 1600 rpm for 1 minute.
- ☐ 10 Seal the plate, place on the thermal cycler, and run the TAG program.
- ☐ 11 Wait until the TAG program has reached the hold temperature before proceeding.

Post Tagmentation Cleanup

- ☐ 1 Let the plate stand at room temperature for 2 minutes.
- ☐ 2 Add 10 µl ST2 (red cap).
- ☐ 3 Pipette 10 times or shake at 1600 rpm for 1 minute.
- ☐ 4 Seal the plate and incubate at room temperature for 5 minutes.
- ☐ 5 Place the plate on the magnetic stand until liquid is clear.
- ☐ 6 Remove and discard supernatant.
- ☐ 7 Remove from the magnetic stand and add 100 µl TWB.
- ☐ 8 Pipette to resuspend, or shake at 1600 rpm for 1 minute.
- ☐ 9 Repeat steps 5–8 for a total of two washes.
- ☐ 10 Seal the plate and place on the magnetic stand until the liquid is clear.

Amplify Tagmented DNA

- ☐ 1 For each sample, combine the following volumes.
  - EPM (23 µl)
  - Nuclease-free water (23 µl)
- ☐ 2 Vortex, and then centrifuge the master mix at 280 × g for 10 seconds.
- ☐ 3 With the plate on the magnetic stand, remove and discard supernatant.
- ☐ 4 Remove from the magnetic stand.
- ☐ 5 Immediately add 40 µl master mix.
- ☐ 6 Immediately pipette to mix or shake at 1600 rpm for 1 minute.
- ☐ 7 Centrifuge at 280 × g for 3 seconds.
- ☐ 8 Centrifuge the index adapter plate at 1000 × g for 1 minute.
- ☐ 9 Prepare the index adapter plate.
- ☐ 10 Add 10 µl Index 1 (i7) and Index 2 (i5) index adapters.
- ☐ 11 Pipette 10 times or shake at 1600 rpm for 1 minute.
- ☐ 12 Centrifuge at 280 × g for 30 seconds.
- ☐ 13 Place on the thermal cycler and run the eBLT PCR program.

SAFE STOPPING POINT

If you are stopping, store at -25°C to -15°C for up to 30 days.

Clean Up Libraries

- ☐ 1 Shake the plate at 1800 rpm for 1 minute.
- ☐ 2 Place on the magnetic stand until the liquid is clear.
- ☐ 3 Transfer 45 µl supernatant to a new midi plate.
- ☐ 4 Vortex and invert AMPure XP Beads multiple times to resuspend.
- ☐ 5 For gDNA, blood, or saliva, perform the following steps.
  - ☐ a Add 77 µl nuclease-free water.
  - ☐ b Add 88 µl AMPure XP Beads.
  - ☐ c Pipette10 times or shake at 1800 rpm for 1 minute.
  - ☐ d Seal the plate and incubate for 5 minutes.
  - ☐ e Place on the magnetic stand until the liquid is clear.
  - ☐ f Vortex AMPure XP Beads, and then add 20 µl to a *new* midi plate.
  - ☐ g Transfer 200 µl supernatant to the new plate.
  - ☐ h Pipette 10 times or shake at 1800 rpm for 1 minute.
  - ☐ i Discard the first plate.
- ☐ 6 For extracted FFPE, perform the following steps.
  - ☐ a Add 81 µl AMPure XP Beads.
  - ☐ b Pipette 10 times or shake at 1800 rpm for 1 minute.
- ☐ 7 Incubate at room temperature for 5 minutes.
- ☐ 8 Place on the magnetic stand until the liquid is clear.
- ☐ 9 Remove and discard supernatant.
- ☐ 10 Wash two times with 200 µl fresh 80% EtOH.
- ☐ 11 Remove and discard residual EtOH.
- ☐ 12 Air-dry for 5 minutes.
- ☐ 13 Remove from the magnetic stand.
- ☐ 14 Add 17 µl RSB.
- ☐ 15 Seal the plate, and then shake at 1800 rpm for 2 minutes.
- ☐ 16 Incubate at room temperature for 2 minutes.
- ☐ 17 Place the plate on the magnetic stand until the liquid is clear.
- ☐ 18 Transfer 15 µl supernatant to a new plate.

SAFE STOPPING POINT

If you are stopping, seal the plate with Microseal 'B' adhesive seal or Microseal 'F' foil seal and store at -25°C to -15°C for up to 30 days.

Qualify Pre-Enriched Libraries

- ☐ 1 Run 1 µl of the individual library on one of the following instruments:
  - Advanced Analytical Fragment Analyzer with the HS-NGS High Sensitivity 474 kit
    - Add 1 µl of RSB to the library to achieve the 2 µl volume required for the Fragment Analyzer
  - Agilent Technology 2100 Bioanalyzer using a DNA 1000 kit

Pool by Volume

☐ 1 Record the indexes for the libraries you plan to pool in this step.

☐ 2 Pool pre-enriched libraries based on the sample volumes in the following table.

Library Pool Plexity	Each Pre-Enriched Library Volume (µl)	Total Volume (µl)
1-plex	14	30 (with 16 RSB)
12-plex	2.5	30

SAFE STOPPING POINT

If you are stopping, cap the 1.5 ml microcentrifuge tube and store at -25°C to -15°C for up to 30 days.

Pool by Mass

☐ 1 Run 1 µl of the pre-enriched libraries using the Qubit dsDNA BR Assay Kit to quantify library concentration (ng/µl).

Table 1 Expected Pre-Enriched Library Yield

Sample Input Type	Pre-Enriched Library Yield
10–49 ng gDNA	≥ 100 ng
50–1000 ng gDNA, blood, saliva	≥ 250 ng

☐ 2 Prepare each pre-enriched library to 500 ng.

☐ 3 Record the indexes for the libraries you plan to pool in this step.

☐ 4 Combine 500 ng of each library in a 1.5 ml microcentrifuge tube into the plexities shown in the following table.

Library Pool	Each Library Sample (ng)	Total DNA Library Mass (ng)
1	500	500
12	500	6000

☐ 5 Perform one of the following based on the total volume of the pooled pre-enriched libraries:

- If pre-enriched library volume = 30 µl, proceed to *Hybridize Probes*.
- If pre-enriched library volume < 30 µl, add RSB to reach 30 µl total volume.
- If pre-enriched library volume > 30 µl, concentrate the pooled sample.

SAFE STOPPING POINT

If you are stopping, cap the 1.5 ml microcentrifuge tube and store at -25°C to -15°C for up to 30 days.

Hybridize Probes

- ☐ 1 Add the following reagents to a new PCR plate or 8-tube strip.
  - Pre-enriched library sample or pool (30 µl)
  - NHB2 (blue cap) (50 µl)
  - Enrichment probe panel (10 µl)
  - EHB2 (10 µl)
- ☐ 2 Pipette 10 times.
- ☐ 3 Centrifuge at 280 × g for 30 seconds.
- ☐ 4 Place on the thermal cycler and run the NF-HYB program.
- ☐ 5 Proceed immediately to the next procedure when the NF-HYB program ends.

Capture Hybridized Probes

- ☐ 1 Centrifuge at 280 × g for 30 seconds.
- ☐ 2 Transfer 100 µl to a new midi plate or 1.5 ml microcentrifuge tube.
- ☐ 3 Add 250 µl SMB, and then mix thoroughly as follows.
  - [Plate] Shake at 1200 rpm for 4 minutes.
  - [Tube] Vortex at high speed 3 times for 10 seconds each.
- ☐ 4 Place on the microheating system and incubate for 15 minutes at the applicable temperature:
  - [FFPE] 58°C
  - [CEX panel] 58°C
  - [Somatic variant calling] 58°C
  - [All others] 62°C
- ☐ 5 Preheat EEW (amber tube).
  - [FFPE] 58°C
  - [CEX panel] 58°C
  - [Somatic variant calling] 58°C
  - [All others] 62°C
- ☐ 6 Immediately centrifuge the sample plate or tube at 280 × g for 30 seconds.
- ☐ 7 Immediately place on a magnetic stand until the liquid is clear.
- ☐ 8 Remove and discard all supernatant.
- ☐ 9 Remove from the magnetic stand.
- ☐ 10 Add 200 µl preheated EEW (amber tube).
  - [Plate] Shake at 1800 rpm for 4 minutes.
  - [Tube] Vortex at high speed 3 times for 10 seconds each. Do not centrifuge.
- ☐ 11 Place on the microheating system and incubate for 5 minutes at the following temperature:
  - [FFPE] 58°C
  - [CEX panel] 58°C
  - [Somatic variant calling] 58°C
  - [All others] 62°C
- ☐ 12 Immediately place on a magnetic stand until the liquid is clear.
- ☐ 13 Remove and discard all supernatant.
- ☐ 14 Repeat steps 9–13 two times for a total of 3 washes.
- ☐ 15 Remove from the magnetic stand.
- ☐ 16 Add 200 µl preheated EEW (amber tube).
  - [Plate] Shake at 1800 rpm for 4 minutes.
  - [Tube] Vortex at high speed 3 times for 10 seconds each. Do not centrifuge.
- ☐ 17 Transfer 200 µl to a new midi plate or tube.
- ☐ 18 Place on the microheating system and incubate for 5 minutes at the applicable temperature:
  - [FFPE] 58°C
  - [CEX panel] 58°C
  - [Somatic variant calling] 58°C
  - [All others] 62°C
- ☐ 19 Immediately place on a magnetic stand and wait until the liquid is clear.
- ☐ 20 Remove and discard all supernatant.
- ☐ 21 Centrifuge at 280 × g for 30 seconds.
- ☐ 22 Place on a magnetic stand for 10 seconds.
- ☐ 23 Remove and discard residual liquid.
- ☐ 24 For each sample, combine the following volumes. Multiply each volume by the number of samples being processed.
  - EE1 (28.5 µl)
  - HP3 (1.5 µl)
- ☐ 25 Vortex, and then centrifuge the elution mix at 280 × g for 10 seconds.
- ☐ 26 Remove from the magnetic stand.

- ☐ 27 Add 23 µl elution mix.
  - [Plate] Shake at 1800 rpm for 2 minutes.
  - [Tube] Vortex at high speed 3 times for 10 seconds each.
- ☐ 28 Incubate at room temperature for 2 minutes.
- ☐ 29 Centrifuge at 280 × g for 30 seconds.
- ☐ 30 Place on a magnetic stand until the liquid is clear.
- ☐ 31 Transfer 21 µl supernatant to a new 96-well PCR or new 8-tube strip.
- ☐ 32 Add 4 µl ET2 to each to each well or tube containing 21 µl supernatant.
- ☐ 33 Slowly pipette 10 times.
- ☐ 34 Centrifuge at 280 × g for 30 seconds.

Amplify Enriched Library

- ☐ 1 Add 5 µl PPC to each well containing a sample.
- ☐ 2 Add 20 µl EPM to each well containing a sample.
  - [Plate] Shake at 1200 rpm for 1 minute.
  - [Tube] Pipette 10 times, and then cap the 8-tube strip.
- ☐ 3 Centrifuge at 280 × g for 30 seconds.
- ☐ 4 Place on the thermal cycler and run the AMP program.

SAFE STOPPING POINT

If you are stopping, store at 2°C to 8°C for up to two days. Alternatively, leave on the thermal cycler for up to 24 hours.

Clean Up Amplified Enriched Library

- ☐ 1 Centrifuge at 280 × g for 30 seconds.
- ☐ 2 Transfer 50 µl supernatant to a new midi plate or 1.5 ml microcentrifuge tube.
- ☐ 3 Vortex and invert AMPure XP Beads multiple times.
- ☐ 4 Add 45 µl AMPure XP Beads.
  - [Plate] Shake at 1800 rpm for 1 minute.
  - [Tube] Vortex at high speed 3 times for 10 seconds each.
- ☐ 5 Incubate at room temperature for 5 minutes.
- ☐ 6 Centrifuge at 280 × g for 1 minute.
- ☐ 7 Place on a magnetic stand until liquid is clear.
- ☐ 8 Remove and discard all supernatant.
- ☐ 9 Wash two times with fresh 200 µl 80% EtOH.
- ☐ 10 Remove and discard residual EtOH.
- ☐ 11 Air-dry for 5 minutes.
- ☐ 12 Remove from the magnetic stand and add 32 µl RSB.
- ☐ 13 Mix thoroughly as follows.
  - [Plate] Shake at 1800 rpm for 1 minute.
  - [Tube] Vortex at high speed 3 times for 10 seconds each.
- ☐ 14 Incubate at room temperature for 5 minutes.
- ☐ 15 Centrifuge at 280 × g for 30 seconds.
- ☐ 16 Place on a magnetic stand until liquid is clear.
- ☐ 17 Transfer 30 µl supernatant to a new 96-well PCR plate or 1.5 ml microcentrifuge tube.

SAFE STOPPING POINT

If you are stopping, seal the plate with Microseal 'B' adhesive seal or Microseal 'F' foil seal or cap the tube and store at -25°C to -15°C for up to 7 days.

Check Enriched Libraries

- ☐ 1 Run 1 µl of the enriched libraries using the Qubit dsDNA BR Assay Kit to quantify library concentration.
- ☐ 2 Run 1 µl of the pooled library or the individual libraries on the Bioanalyzer using a High Sensitivity DNA kit.

Dilute Libraries to the Starting Concentration

- ☐ 1 For sequencing, Illumina recommends setting up a paired-end run with 101 cycles per read (2 × 101) and 10 cycles per index read.
- ☐ 2 Calculate the molarity value of the library or pooled libraries.
- ☐ 3 Using the molarity value, calculate the volumes of RSB and library needed to dilute libraries to the starting concentration.

Sequencing System	Starting Concentration (nM)	Final Loading Concentration (pM)
HiSeq 2500 and HiSeq 2000 Systems (high output modes)	2	16–18
HiSeq 2500 System (rapid run mode)	2	7–8
HiSeq 4000 and HiSeq 3000 Systems	2–3	150–200
iSeq 100 System	2	100
MiniSeq System	2	1.7–1.8
MiSeq System (v3 reagents)	4	10–12
NextSeq 550 and NextSeq 500 Systems	2	1.4–1.5
NextSeq 2000 System	2	1000
NovaSeq 6000 System (standard workflow)	2	175–185

- ☐ 4 Dilute libraries using RSB:
  - **Libraries quantified as a multiplexed library pool**—Dilute the pool to the starting concentration.
  - **Libraries quantified individually**—Dilute each library to the starting concentration. Add 10 µl of each diluted library to a tube.
- ☐ 5 Dilute to the final loading concentration by following the denature and dilute instructions for your system.
- ☐ 6 Dilute to the final loading concentration.

Acronyms

Acronym	Definition
BLB	Blood Lysis Buffer
BLT	Bead-Linked Transposome
eBLT	Enrichment BLT
CEX	Coding Exome Oligos
EE1	Enrichment Elution Buffer 1
EEW	Enhanced Enrichment Wash
EHB2	Enrich Hyb Buffer 2
EPM	Enhanced PCR Mix
ET2	Elute Target Buffer 2
EtOH	Ethanol
HP3	2 N NaOH
IEM	Illumina Experiment Manager
NHB2	Hyb Buffer 2 + IDT NXT Blockers
NXT	Nextera
PK1	Proteinase K
PPC	PCR Primer Cocktail
QCP	QC Primer Reagent
QCT	QC Template Reagent
RSB	Resuspension Buffer
SMB	Streptavidin Magnetic Beads
ST2	Stop Tagment Buffer 2
TB1	Tagmentation Buffer 1
TOO	TruSight One Oligos
TOE	TruSight One Expanded
TSHC	TruSight Hereditary Cancer
TWB	Tagment Wash Buffer
UD	Unique Dual