Nextera™ DNA Flex Library Prep Checklist

Tagment Genomic DNA

- ☐ 1 Save the following TAG program on the thermal cycler:
  - Choose the preheat lid option and set to 100°C
  - Set the reaction volume to 50 µl
  - 55°C for 15 minutes
  - Hold at 10°C
- ☐ 2 Add 2–30 µl DNA to a 96-well PCR plate.
- ☐ 3 If DNA volume < 30 µl, add nuclease-free water to bring the volume to 30 µl.
- ☐ 4 Vortex BLT vigorously to resuspend.
- ☐ 5 Combine the following volumes.
  - BLT (11 µl)
  - TB1 (11 µl)
- ☐ 6 Vortex the master mix to resuspend.
- ☐ 7 Divide the master mix volume into an 8-tube strip.
- ☐ 8 Transfer 20 µl to each well containing a sample. Pipette to resuspend.
- ☐ 9 Seal the plate, place on the thermal cycler, and run the TAG program.

Post Tagmentation Cleanup

- ☐ 1 Add 10 µl TSB .
- ☐ 2 Slowly pipette to resuspend the beads.
- ☐ 3 Seal the plate and incubate at 37°C for 15 minutes on a thermal cycler with heated lid.
  - Set the thermal cycler lid at 100° C
  - Set the reaction volume to 60 µl
  - Hold at 10° C
- ☐ 4 Place the plate on the magnetic stand until solution is clear.
- ☐ 5 Using a multichannel pipette, remove and discard supernatant.
- ☐ 6 Wash with 100 µl TWB.
- ☐ 7 Wash again with 100 µl TWB.
- ☐ 8 Remove from the magnetic stand and add 100 µl TWB.
- ☐ 9 Pipette to resuspend
- ☐ 10 Seal the plate and place on the magnetic stand until the solution is clear.

Amplify Tagmented DNA

- ☐ 1 Seal the plate and run the following program in the thermal cycler (heated lid at 100°C, volume reaction of 50 µl):
  - 68°C, 3 min
  - 98°C, 3 min
  - Repeat these conditions for the number of cycles in *Tagment Genomic DNA*:
    - 98°C, 45 sec
    - 62°C, 30 sec
    - 68°C, 2 min
  - 68°C, 1 min
  - 10°C hold

Table 1 PCR Cycle Recommendations

Total DNA Input (ng)	Recommended # of PCR Cycles
1–9	12
10–24	8
25–49	6
50–99	5
100–500	5
Blood/Saliva	5

- ☐ 2 Prepare PCR master mix in a 1.7 ml microcentrifuge tube.
  - EPM (22 µl)
  - Nuclease-free water (22 µl)
- ☐ 3 Vortex and centrifuge at 280 × g for 10 seconds.
- ☐ 4 With the plate on the magnetic stand, use a 20 µl multichannel pipette to remove and discard supernatant.
- ☐ 5 Remove the plate from the magnet.

☐ 6 Add 40 µl of the PCR master mix to each sample well.
☐ 7 Immediately pipette to mix. Alternatively, seal the plate and use a plate shaker at 1600 rpm for 1 minute.
☐ 8 Add the appropriate index adapters to each sample.

Index Kit Type	Kit Configuration	Volume of Index Adapter per Sample
24 plex (dual index)	Individual tubes	5 µl i5 adapter 5 µl i7 adapter
96 plex (dual index)	96-well plate	10 µl primer mix

☐ 9 Using a pipette set to 40 µl, pipette 10 times.

Table 2 DNA Input Recommendations

Total DNA Input (ng)	Recommended # of PCR Cycles
1–9	12
10–24	8
25–49	6
50–99	5
100–500	5
Blood/Saliva	5

☐ 10 Seal the plate with Microseal 'B', and centrifuge at 280 × g for 30 seconds.
☐ 11 Place on the thermal cycler and run the BLT PCR program.
☐ 12 Remove the plate when the PCR program completes.
☐ 13 Centrifuge at 280 × g for 1 minute.

SAFE STOPPING POINT

If you are stopping, seal the plate with a Microseal 'B' adhesive seal, and store at 2°C to 8°C for up to 3 days.

Clean Up Libraries

☐ 1 Place the plate on the magnetic stand until the liquid is clear.
☐ 2 Transfer 45 µl supernatant from each well to the corresponding well of a fresh midi plate.
☐ 3 Vortex and invert PB multiple times to resuspend.
☐ 4 Combine the following volumes.
- PB (45 µl)
- Nuclease-free water (40 µl)

☐ 5 Thoroughly vortex the diluted PB.
☐ 6 Pipette mix a minimum of 10 times or until thoroughly mixed.
☐ 7 Add 85 µl diluted PB to each well containing supernatant.
☐ 8 Pipette 10 times to mix, or seal the plate and shake at 1600 rpm for 1 minute.
☐ 9 Incubate the sealed plate at room temperature for 5 minutes.
☐ 10 Place on the magnetic stand until supernatant is clear.
☐ 11 During incubation, thoroughly vortex the PB, and then add 15 µl to a *new* midi plate.
☐ 12 Transfer 125 µl supernatant from the first midi plate to the second midi plate.
☐ 13 Pipette 10 times to mix.
☐ 14 Apply the seal and incubate at room temperature for 5 minutes.
☐ 15 Place on a magnet and wait until clear.
☐ 16 Without disturbing the beads, remove and discard supernatant.
☐ 17 Wash with 200 µl of fresh 80% ethanol.
☐ 18 Wash again with 200 µl of fresh 80% ethanol.
☐ 19 Remove and discard residual EtOH from the wells.
☐ 20 Air-dry.

☐ 21 Remove from the magnetic stand and add 32 µl RSB to the beads.
☐ 22 Pipette to resuspend.
☐ 23 Incubate at room temperature for 2 minutes.
☐ 24 Place on the magnetic stand until the liquid is clear.
☐ 25 Transfer 30 µl supernatant to a new plate.

SAFE STOPPING POINT

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

Pool Libraries

☐ 1 **For DNA inputs of 100–500 ng**, pool 5 µl per sample of up to 96 libraries into a single 1.7 ml microcentrifuge tube. Mix by vortexing then spin down in a microcentrifuge.
☐ 2 Quantify the single pooled library using a dsDNA specific fluorescent dye method such as Qubit or PicoGreen.

Dilute Libraries to the Starting Concentration

Table 3 Recommended Read Length on Illumina Systems with Nextera CD Indexes (8 base pair index codes)

Sequencing System	Read Length
NovaSeq 6000, HiSeq X, HiSeq 3000 and HiSeq 4000, NextSeq 500 and NextSeq 550, MiSeq, MiniSeq, iSeq 100	2 x 151
HiSeq 2000, HiSeq 2500 (high output)	2 x 126
HiSeq 2500 (rapid run)	2 x 101

☐ 1 Calculate the molarity value of the library or pooled libraries using the following formula.
- For libraries qualified on a Bioanalyzer, use the average size obtained for the library.
- For all other qualification methods, use 600 bp as the average library size.

$$\frac{ng/\mu l \times 10^6}{660\frac{g}{mol} \times average\ library\ size\ (bp)} = Molarity\ (nM)$$

☐ 2 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 4000 and HiSeq 3000	2–3	150–200
iSeq 100 System	2	100

Sequencing System	Starting Concentration (nM)	Final Loading Concentration (pM)
MiniSeq System	2	1.7–1.8
MiSeq	4	7–8
NextSeq 550 and NextSeq 500	2	1.4–1.5
NovaSeq 6000	2	150–175

☐ 3 Dilute libraries using RSB:

- **Libraries quantified as a pool**—Dilute the pool to the starting concentration.
- **Libraries quantified individually**—Dilute each library to the starting concentration. Add 10 µl each diluted library to a tube.

☐ 4 Follow the denature and dilute instructions for your system, diluting to the final loading concentration listed in the preceding table.

Acronyms

Acronym	Definition
BLB	Blood Lysis Buffer
BLT	Bead Linked Transposome
EPM	Enhanced PCR Mix
EtOH	Ethanol
PK1	Proteinase K
RSB	Resuspension Buffer
PB	Purification Beads
TB1	Tagmentation Buffer 1
TSB	Tagment Stop Buffer
TWB	Tagment Wash Buffer