Nextera XT DNA Library Prep Checklist

Tagment Genomic DNA

- ☐ 1 Add the following volumes to a new PCR plate.
  - TD (10 µl)
  - 1 ng DNA (5 µl)
- ☐ 2 Pipette to mix.
- ☐ 3 Add 5 µl ATM.
- ☐ 4 Pipette to mix.
- ☐ 5 Centrifuge at 280 × g at 20°C for 1 minute.
- ☐ 6 Place on the thermal cycler and run the TAG program. Immediately proceed to step 7.
- ☐ 7 Add 5 µl NT.
- ☐ 8 Pipette to mix.
- ☐ 9 Centrifuge at 280 × g at 20°C for 1 minute.
- ☐ 10 Incubate at room temperature for 5 minutes.

Amplify Libraries

- ☐ 1 Add the appropriate to index adapter volumes per sample according to your index adapter kit type.
- ☐ 2 Add 15 µl NPM.
- ☐ 3 Pipette to mix.
- ☐ 4 Centrifuge at 280 × g at 20°C for 1 minute.
- ☐ 5 Place on the thermal cycler and run the NXT PCR program.

SAFE STOPPING POINT

If you are stopping, seal the plate and store at 2°C to 8°C for up to 2 days. Alternatively, leave on the thermal cycler overnight.

Clean Up Libraries

- ☐ 1 Centrifuge at 280 x g at 20°C for 1 minute.
- ☐ 2 Transfer 50 µl supernatant.
- ☐ 3 If you are using standard DNA input, add 30 µl AMPure XP beads.
- ☐ 4 If you are using small PCR amplicon sample input, add the AMPure XP beads volume according to your input size.
- ☐ 5 Shake at 1800 rpm for 2 minutes.
- ☐ 6 Incubate at room temperature for 5 minutes.
- ☐ 7 Place on the magnetic stand until liquid is clear.
- ☐ 8 Remove and discard all supernatant.
- ☐ 9 Wash two times with 200 µl 80% EtOH.
- ☐ 10 Use a 20 µl pipette to remove and discard residual EtOH.
- ☐ 11 Air-dry on the magnetic stand for 15 minutes.
- ☐ 12 Remove from the magnetic stand.
- ☐ 13 Add 52.5 µl RSB.
- ☐ 14 Seal the plate, and then shake at 1800 rpm for 2 minutes.
- ☐ 15 Incubate at room temperature for 2 minutes.
- ☐ 16 Place on the magnetic stand until liquid is clear.
- ☐ 17 Transfer 50 µl supernatant.

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 7 days.

Check Library Quality

- ☐ 1 Run 1 µl undiluted library on an Agilent Technology 2100 Bioanalyzer using a High Sensitivity DNA kit.

Normalize Libraries

- ☐ 1 Transfer 20 µl supernatant.
- ☐ 2 For each sample, combine the following volumes in a 15 mL conical tube.
  - LNA1 (46 µl)
  - LNA2 (8 µl)
- ☐ 3 Pipette to mix.
- ☐ 4 Pour the LN master mix into a trough.
- ☐ 5 Transfer 45 µl LN master mix.
- ☐ 6 Shake at 1800 rpm for 30 minutes.
- ☐ 7 Place on the magnetic stand until liquid is clear.
- ☐ 8 Remove and discard all supernatant.
- ☐ 9 Wash two times with 45 µl LNW1.
- ☐ 10 Add 30 µl 0.1 N NaOH.
- ☐ 11 Shake at 1800 rpm for 5 minutes.
- ☐ 12 Add 30 µl LNS1 to each well of a new 96-well PCR plate labeled SGP.
- ☐ 13 After the 5 minute elution completes, make sure that all samples are resuspended. If they are not, resuspend as follows.
  - ☐ a Pipette to mix.
  - ☐ b Shake at 1800 rpm for 5 minutes.
- ☐ 14 Place on a magnetic stand until liquid is clear.
- ☐ 15 Transfer 30 µl supernatant from the midi plate to the SGP plate.
- ☐ 16 Centrifuge at 1000 × g for 1 minute.

SAFE STOPPING POINT

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Dilute Libraries to the Starting Concentration

- ☐ 1 Calculate the molarity value of the library or pooled libraries.
- ☐ 2 Using the molarity value, calculate the volumes of RSB and library needed to dilute libraries to the starting concentration.
- ☐ 3 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 each diluted library to a tube.
- ☐ 4 Dilute to the final loading concentration.

Acronyms

Acronym	Definition
ATM	Amplicon Tagment Mix
HT1	Hybridization Buffer
LNA1	Library Normalization Additives 1
LNB1	Library Normalization Beads 1
LNS1	Library Normalization Storage Buffer 1
LNW1	Library Normalization Wash 1
NT	Neutralize Tagment Buffer
NPM	Nextera PCR Master Mix
RSB	Resuspension Buffer
SGP	Storage Plate
TD	Tagment DNA Buffer
UD	Unique Dual Index