Nextera XT DNA Library Prep Checklist

illumina

Step 1: Tagment Genomic DNA

1. Add the following volumes to a new 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.

Step 2: Amplify Libraries

1. Add the following 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.

Step 3: Clean Up Libraries

1. Centrifuge at 280 × g at 20°C for 1 minute.
2. Transfer 50 µl supernatant.
3. If you are using standard DNA input, add 30 µl Illumina Purification Beads.
4. If you are using small PCR amplicon sample input, add the Illumina Purification Beads volume.
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.

Step 4: Check Library Quality

1. Run 1 µL undiluted library on an Agilent Technology 2100 Bioanalyzer with a High Sensitivity DNA kit.

Step 5: 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

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.

Step 6: Dilute Libraries to the Starting Concentration

1. Calculate the molarity value of the library or pooled libraries using the following formula.
2. Using the molarity value, calculate the volumes of RSB and library needed to dilute libraries to the starting concentration for your system.
3. Dilute libraries using RSB as follows.
    - For libraries quantified as a multiplexed library pool, dilute the pool to the starting concentration.
    - For 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