firefly® technical note
Nextera XT DNA Library Preparation (Volume-reduced)

Overview

- This technical note describes the 5× volume-reduced Illumina Nextera XT DNA Library Preparation workflow on SPT Labtech's firefly.
- The workflow reduces Nextera XT reagent consumption by five-fold while automating DNA dilution, DNA normalization, tagmentation and indexing, post-tagmentation bead cleanup, library quantification and final library normalization.
- The workflow consists of six protocols Detailed in Table 1.
- Instrument configuration: firefly 6 Head, Genomics
- Development software version: v1.8
- Deviation(s) from published method:
    - Reaction volumes are reduced 5× compared to the standard Illumina protocol.
    - Reservoir loading volumes include additional dead volume for automated pipetting.
    - Thermocycler programs and library preparation chemistry follow the standard Illumina Nextera XT protocol.

firefly protocols

Protocol number	Protocol name	Estimated run time (minutes)
Protocol 1 of 6	01. DNA dilution	33
Protocol 2 of 6	02. DNA normalization	22
Protocol 3 of 6	03. Tagmentation	17
Protocol 4 of 6	04. Post-tagmentation cleanup 05.	40
Protocol 5 of 6	DNA quantification for pooling 06.	15
Protocol 6 of 6	Normalization to 5 ng/µL	22
Total automated protocol time*		149 (2.5 hours)

Table 1. Protocols and estimated run times for the volume-reduced Nextera XT workflow on firefly.
***excluding external plate reading, thermocycling and any user-dependent pauses.**

Input variables

The supplied workflows are configured for a 96-sample run and do not require user-defined variables during execution. Protocols 01, 02 and 06 use file-defined MilliQ volumes generated from Qubit results. Review and change the input files before starting each normalization protocol.

1. For Research Use Only. Not for use in diagnostic procedures.

Reagents

Protocol	Reagent	Total volume (µL)	Volume per reservoir (µL)
01. DNA dilution	MilliQ water	Variable	Variable
	Qubit HS master mix	14,000	7,000 each
02. DNA normalization	MilliQ water	Variable	Variable
	Qubit HS master mix	11,120	5,560 each
03. Tagmentation	ATM	171	171
	NPM	363	363
	TDB	267	267
	NT	336	336
04. Cleanup	AMPure XP beads	651	651
	80% ethanol	19,680	9,840 each
	RSB	1,590	795 each
05. Quantification	Qubit HS master mix	11,456	5,728 each
06. Final normalization	Qubit HS master mix	11,120	5,560 each
	MilliQ water	Variable	Variable

Table 2. Reagents and dispense-head reservoir loading volumes. Reservoir volumes shown are the protocol setup volumes for a 96-sample run. Where two reservoirs are used, the total volume and the volume loaded into each reservoir are both shown.

Labware and accessories required

Labware / accessory	Supplier	Part number / kit	Combined quantity
96-well Hard-Shell PCR plate	Bio-Rad	HSP-9601	10
96-well F-bottom plate	Corning	3603	8
96-well index plate	N/A	N/A	1
96-well 2 mL deep-well plate	VWR	732-3325	1

Table 3. Combined labware required to execute all six protocols once.

2. For Research Use Only. Not for use in diagnostic procedures.

Accessory Type	Product Number(s)	Combined Quantity
Universal Tip Stand	3276-08075	4
Tip Loading Cassette	FFY-A-01-EZL-SL-5	4
Strip Tip Insert - 8 Channel	FFY-A-01-EZL-096-SC-8	4
Thermal Adapter for PCR Plate	3276-01065	1
Permagen X396 LE magnet	A040523	1

Table 4. **Combined labware required to execute all six protocols once.**

Consumables required

Pipette Head Consumable Type	Product Number(s)	P01	P02	P03	P04	P05	P06
35 µL tips, full rack, filtered	050-096-FF-FS	-	-	2x	1x	-	-
100 µL tips, full rack, filtered	125-096-FF-FS	1x	1x	-	3x	-	1x
100 µL filtered strip tips,	125-008-FF-FS	13	13	-	-	13	13

Table 5. **Pipette head consumables required to execute all six protocols once. Tip quantities reflect the protocol definitions and do not include additional contingency stock.**

Dispense Head Consumable Type	Product Number(s)	P01	P02	P03	P04	P05	P06
Standard syringes	4150-07200	4x	4x	-	4x	2x	4x
Ultra-low retention syringes	4150-07208	-	-	4x	1x	-	-
Standard reservoirs	4150-07103	4x	4x	-	2x	2x	4x
Low dead volume reservoirs	4150-07202	-	-	4x	3x	-	-

Table 6. **Dispense-head consumables required for eachprotocol.**

3. For Research Use Only. Not for use in diagnostic procedures.

Protocol details

Figure 1. **Workflow overview for the volume-reduced Nextera XT method on firefly. Bright pink steps are automated on firefly; Dark pink step includes an off-instrument step for quantification or thermocycling, and the grey step represents downstream pooling and sequencing.**

01. DNA dilution

The protocol dilutes extracted DNA with MilliQ water, mixes the diluted samples and prepares two Qubit HS assay plates. A 0-10 ng standard stock plate is transferred into column 1 of each assay plate, while diluted samples are added to the remaining wells. The plates are then ready for fluorescence measurement.

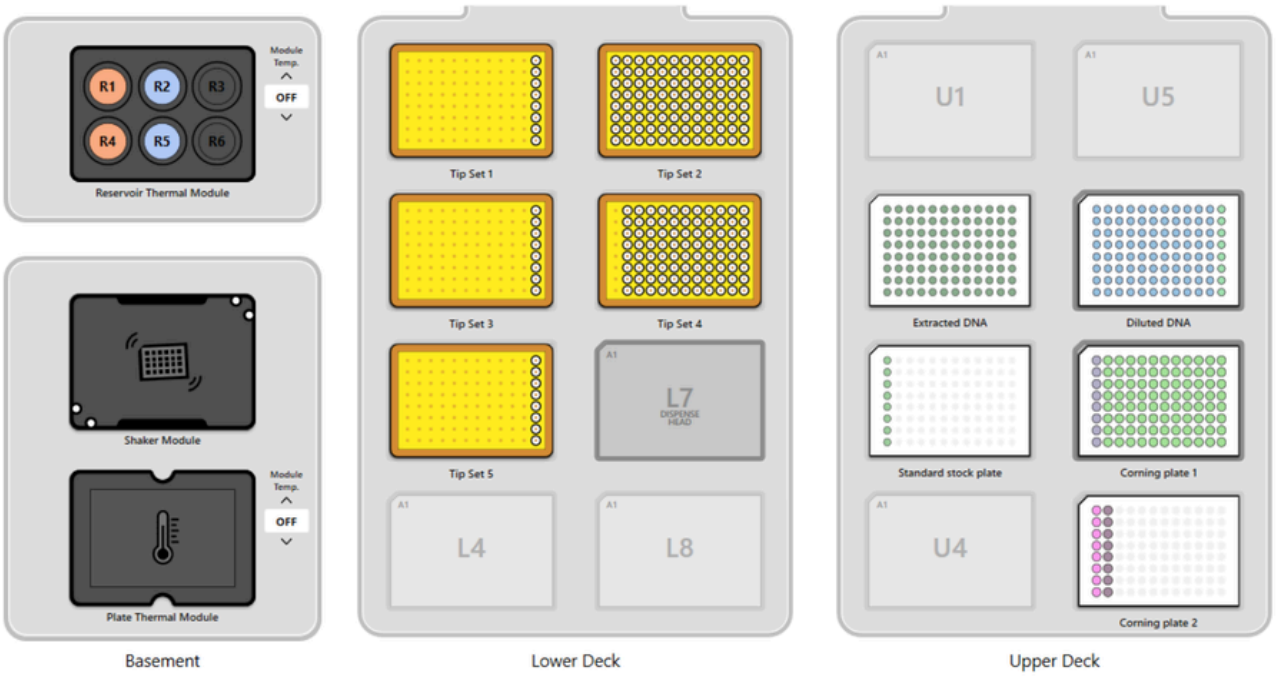

Figure 2. **Protocol 1 of 6 – DNA dilution and Qubit assay setup deck layout.**

02. DNA normalization

Based on the initial Qubit results, the protocol uses file-defined water volumes to normalize DNA to 0.2 ng/µL. It then prepares two Qubit HS assay plates using a 0-1 ng standard series and the normalized DNA for concentration confirmation.

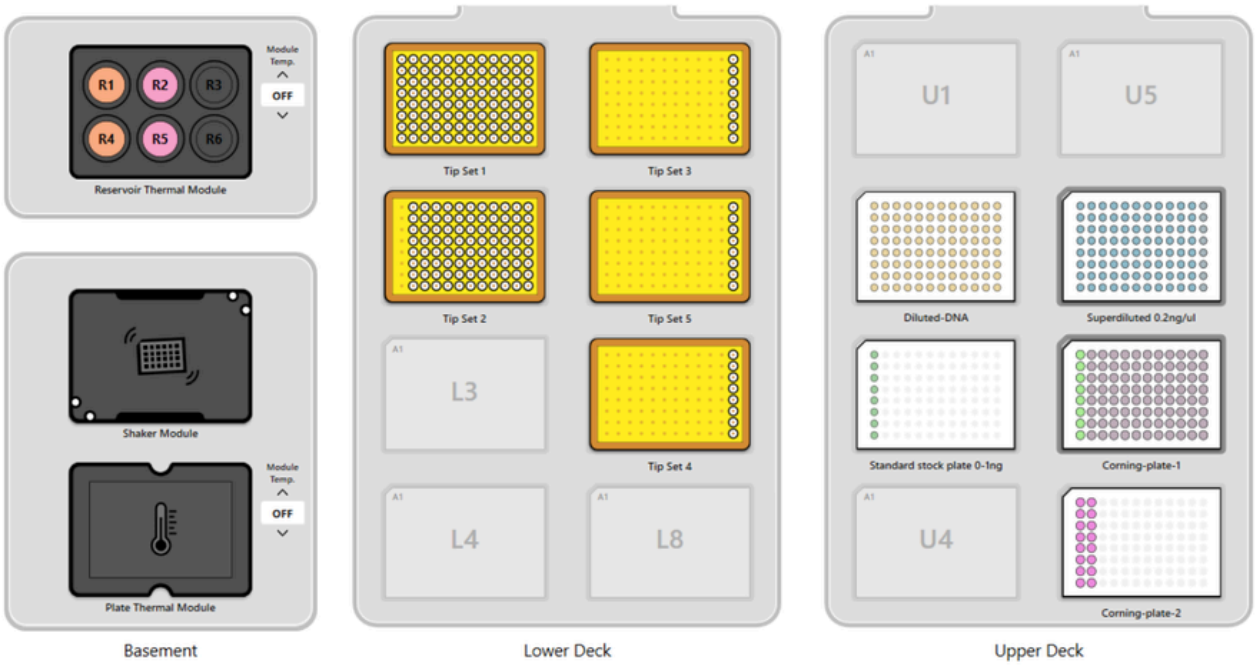

Figure 3. **Protocol 2 of 6 – DNA normalisation deck layout.**

03. Tagmentation

TDB, normalized DNA and ATM are dispensed into a PCR plate. Following the external tagmentation incubation, NT is added to stop the reaction. NPM and index primers are then added before the external index PCR step. The plate thermal and shaker modules support controlled reagent handling and mixing.

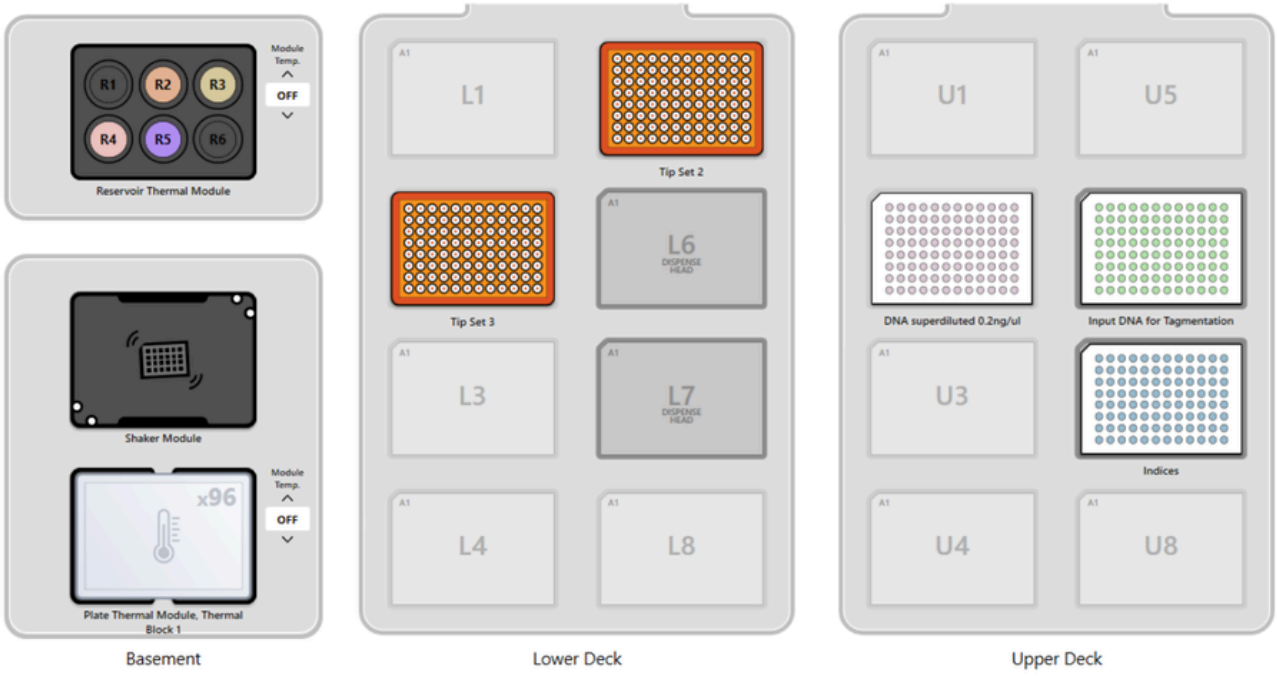

Figure 4. **Protocol 3 of 6 – Tagmentation and indexing deck layout.**

04. Post-tagmentation cleanup

AMPure XP beads are added to the amplified libraries and mixed. Following magnetic separation, supernatant is removed and the beads are washed twice with freshly prepared 80% ethanol. After drying, DNA is eluted in RSB and transferred into a clean Hard-Shell plate.

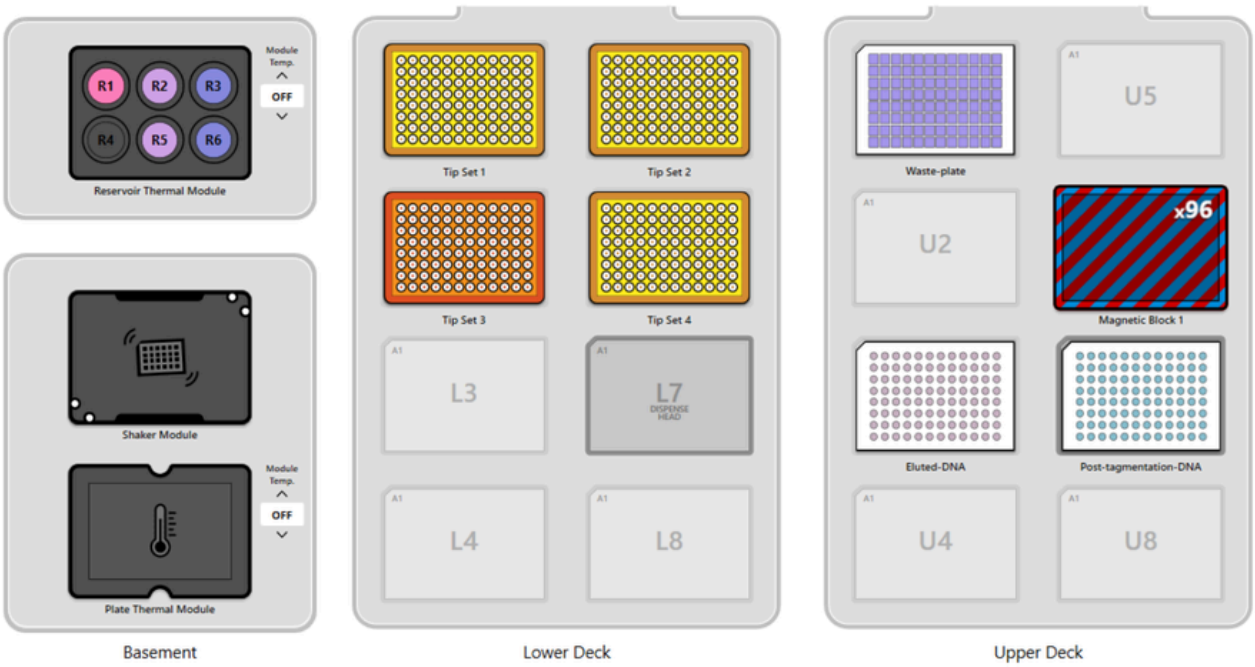

Figure 5. **Protocol 4 of 6 – Post-tagmentation cleanup deck layout.**

05. DNA quantification for pooling

The cleaned libraries and a 0-10 ng standard stock plate are transferred into duplicate Qubit HS assay plates. The resulting fluorescence measurements provide the concentrations used for final library normalization and pooling calculations.

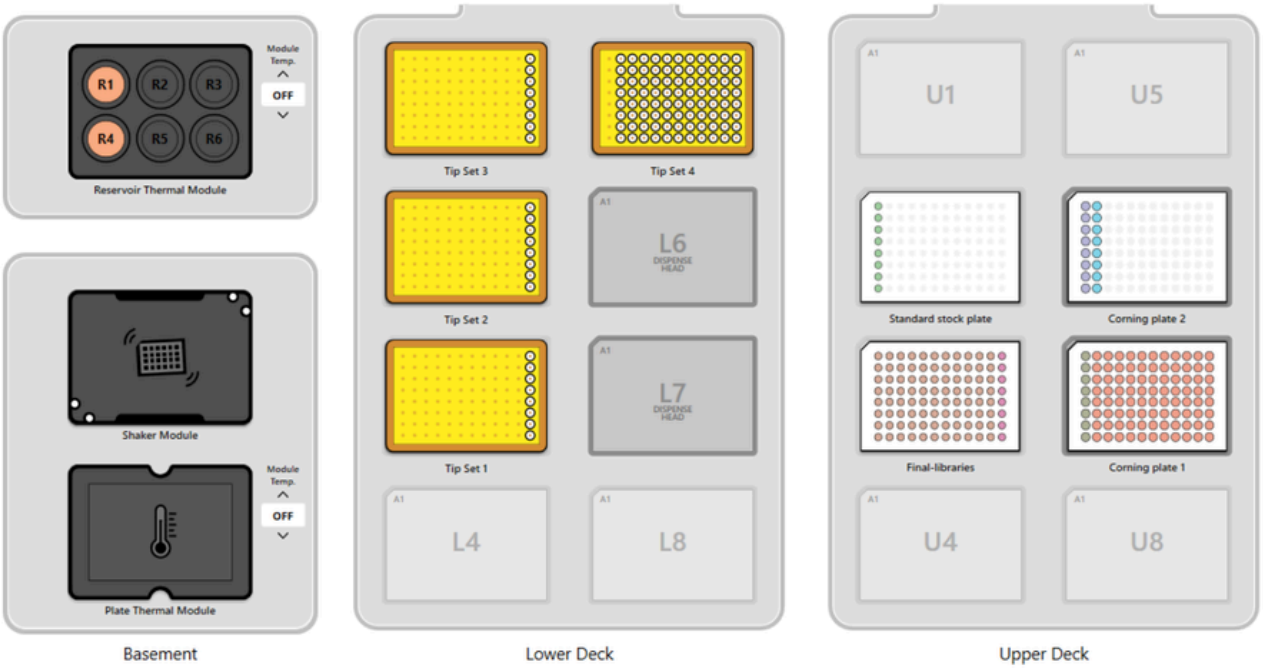

Figure 6. **Protocol 5 of 6 – Library quantification deck layout.**

06. Normalization to 5 ng/µL

File-defined MilliQ volumes are dispensed to create a final library plate at 5 ng/µL. Duplicate Qubit HS assay plates are prepared to confirm the normalized concentrations before pooling.

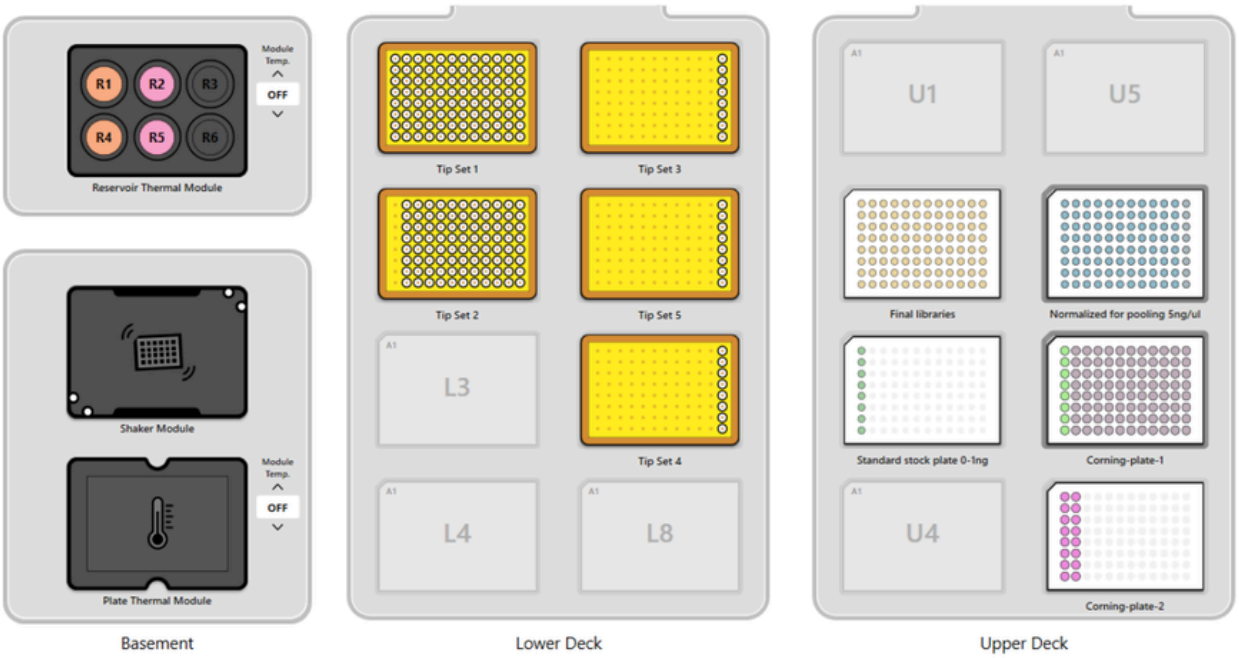

Figure 7. **Protocol 6 of 6 – Final library normalization deck layout.**

Important workflow notes

- External fluorescence measurements arerequired after protocols 01, 02, 05 and 06. The resulting concentration data must be used to generate the appropriate file-defined dilution volumes.
- Confirm that plates are free of bubbles before fluorescence measurement and centrifuge briefly where required. Thermal cycling is performed off-deck during protocol 03. The stated run time represents the firefly protocol time and excludes external thermocycler programmes.
- Fresh 80% ethanol should be loaded when prompted during the cleanup protocol.

Protocol set covered by this note

Protocol	firefly protocol name
01	NextXT DNA dilution
02	NexteraXT Normalization
03	NexteraXT Tagmentation
04	NexteraXT Cleanup
05	NexteraXT DNA quantification for pooling
06	Normalization to 5 ng/µL

Table 5. **Supplied firefly protocol files used to prepare this technical note.**