

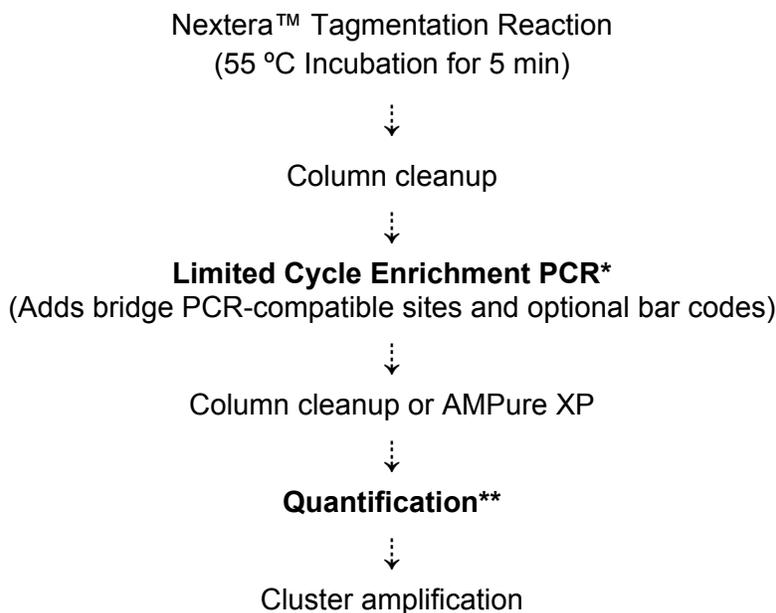


Nextera™ DNA Sample Prep Kit Update

KAPA Library Amplification Kits and KAPA Real-Time Library Amplification Kits are compatible with the Nextera™ DNA Sample Prep Kit from Illumina. The protocol has been validated internally as well as externally by collaborators. The protocol is now included in the updated versions of the Technical Data Sheets. The improved enzyme bias characteristics of the KAPA Library Amplification Kits are a direct benefit to the Nextera™ system.

Nextera™ technology is an elegant solution for library preparation that employs *in vitro* transposition to simultaneously fragment and tag DNA (tagmentation) in a single tube reaction, eliminating the need for canonical DNA library preparation involving fragmentation, end-repair, adenylation, and ligation. The method is amenable to very low amounts of starting DNA (~50 ng).

Workflow for Nextera™ DNA Sample Preparation:



*The KAPA Library Amplification Kit and KAPA Real-Time Library Amplification Kit are compatible at this step. The reaction set up and protocol in the Nextera™ technical data sheet can be used, however minor protocol modifications to denaturation time and temperature are recommended for optimal performance using the KAPA Library Amplification Kits.

**KAPA Library Quantification Kits are recommended for quantifying low concentration Nextera™ libraries.



The following protocol is recommended for Nextera™ library enrichment PCR:

Reaction setup:

| | |
|-------|--|
| 18 µL | Nuclease-free water |
| 25 µL | KAPA HiFi HS ReadyMix (2X) or KAPA HiFi HS Real-Time PCR Master Mix (2X) |
| 1 µL | Nextera™ Primer Cocktail |
| 1 µL | Nextera™ Adaptor 2 (or bar coded adaptor) |
| 5 µL | Recovered DNA Fragment Library |
| <hr/> | |
| 50 µL | Total reaction volume |

Cycling protocol:

72 °C for 3 min*
98 °C for 30 sec**

9 cycles*** of:
98 °C for 45 sec
62 °C for 30 sec
72 °C for 3 min

Hold at 4 °C

*It is critical to perform this step prior to denaturation. This step is specific to the Nextera™ workflow.
**Nextera™ protocol recommends 95 °C. 98 °C is recommended for KAPA HiFi for optimal performance.
***Increased yields from KAPA HiFi may require cycle number optimization. KAPA Real-Time Library Amplification Kit is recommended for cycle number optimization.

Note: Nextera™ protocol is subject to change based on technological improvements currently being implemented by Illumina.