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NEXTFLEX® Rapid XP DNA-Seq Kit
Alternative Protocol for low input samples

Starting Material

The minimum recommended input for low input gDNA samples is 100 pg in up to 34 µL nuclease-free water. Note that the quality of DNA extracted from samples may affect results.

Summary of Alterations to Standard Protocol

1) Follow protocol as described using the recommended fragmentation times, adapter dilution, and PCR cycles.

Protocol Changes

In Step 1A or 2A (Fragmentation, End-Repair & Adenylation)

In step 2.

Apply adhesive PCR plate seal an incubate on a thermal cycler using the following program:

1 min	4 °C
See Fragmentation Table	35 °C
30 min	65 °C
end	4 °C

Note: The initial 4 °C step is to pre-chill the instrument temperature. Place samples into the thermal cycler after the temperature reaches 4 °C and follow the program. A full one-minute incubation at 4 °C is not necessary.

The following table lists the recommended incubation times as a guideline for fragmentation. The mode fragment size can be adjusted by changing the duration of incubation at this 35 °C step. These times are recommendations only, and incubation time may need to be optimized for different sample inputs and types to obtain desired mode fragment size.

Input DNA	**Target Fragment Peak Size**			
	200 – 300	300 – 400	400 – 500	500 – 600
	Fragment Time (min) at 35 °C			
< 1 ng – 100 pg	50	30	20	8

Note: The final library size will be approximately 120 bp larger than the fragment size.

The procedure may be safely stopped at this step with samples stored at -20 °C if needed. To restart the protocol, thaw frozen samples on ice before proceeding to **Step B1** or **B2**.

In Step B1 or B2 (Adapter Ligation)

In Step 2.

The following table lists recommended barcoded adapter concentration dilutions for various input amounts for all listed barcoded adapter except ChIP-Seq barcoded adapters:

Input DNA	Desired Adapter	Adapter Dilution Concentration
< 1 ng – 100 pg	0.1 µM	1 / 250

* ChIP-Seq barcoded adapter may be used for only 1ng inputs. Please inquire for details.

Each sample will require 2.5 µL of barcoded adapter to be added. Perform barcoded adapter dilutions if necessary with Nuclease-free Water, depending on input amount and starting barcoded adapter concentration.

In Step C1 or C2 (PCR Amplification)

At Step 2.

Note: The NEXTflex® Primer Mix that is included in the NEXTflex® NGS Barcodes are NOT compatible with this kit and should NOT be used in place of the Primer Mix XP.

*The following table lists recommended PCR cycles:

	Number of PCR cycles to product	
Input DNA	**100 ng libraries**	**1 µg libraries**
< 1 ng – 100 pg	16 – 17	19 – 20

Apply adhesive PCR plate seal and place in thermal cycler for the following PCR cycles:

30 sec	98 °C
15 sec	98 °C
30 sec	65 °C
30 sec	72 °C
2 min	72 °C

Repeat as suggested in above table

Continue the protocol as stated in the protocol guide for the rest of the standard protocol to completion.