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

Starting Material

The minimum recommended input for FFPE samples is 50ng in up to 34 µL nuclease-free water. Note that the quality and length of DNA extracted from FFPE samples may affect results.

Summary of Alterations to Standard Protocol

1) Treat DNA from FFPE samples as 1/50 of their mass going into the protocol.

As an example, if using 50 ng of DNA from FFPE samples, treat as if 1 ng of quality gDNA input for the protocol.

2) Fragmentation time locked to 24 minutes for all samples regardless of concentration.

Protocol Changes

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

1. For each sample, combine the following reagents on ice in a nuclease-free 96 well PCR plate:

__ µL	Nuclease-free Water
__ µL	**FFPE DNA (at least 50 ng)**
5 µL	NEXTFLEX® Fragmentation Buffer
39 µL	**TOTAL**

Ensure thorough mixing by pipetting up and down. Proceed with adding the enzyme.

39 µL	DNA + NEXTFLEX® Fragmentation Buffer mixture
11 µL	NEXTFLEX® Fragmentation Enzyme Mix (DO NOT VORTEX)
50 µL	**TOTAL**

Note: Do NOT vortex the final NEXTFLEX® Fragmentation reaction. Mix by pipette only. It is important to mix the reaction on ice.

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

1 min	4 °C
24 min	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.

3. 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**.

Continue the protocol as stated in the protocol guide, treating the samples as **1/50 amount of Input DNA** for the rest of the standard protocol to completion (such as for adapter dilution and PCR cycle number guidelines).