Zymo-Seq Cell Free DNA WGBS Library Kit with FFPE DNA Samples

Considerations Before Starting and Performing Bisulfite Conversion

If the formalin-fixed, paraffin-embedded (FFPE) derived DNA input is sufficient (≥20 ng), we recommend performing sonication to shear the FFPE DNA to an average size of 200-500 bp for best results. This should yield enough sheared DNA for the 5 ng minimum input required for the kit (concentration of the DNA may need to be performed for best results). Perform **Section 1: Bisulfite Conversion** as normal, then refer to the **FFPE DNA Protocol** below for required adjustments to **Sections 2 and 3**.

If sonication is not possible or the FFPE DNA input is insufficient for sonication, the DNA may still be processed directly by performing the bisulfite conversion as instructed in the **EZ DNA Methylation-Gold Kit** (D5005, D5006) instead of **Section 1: Bisulfite Conversion**:

1. The following reagents will need to be purchased separately to perform the **EZ DNA Methylation-Gold Kit** protocol. For users who plan to process all 24 or 96 preps with this protocol, please refer to the following table for ordering information:

Product Name	24 Preps	96 Preps
CT Conversion Reagent	3 x D5001-1	2 x D5001-1-50
M-Dilution Buffer	1 x D5005-2	1 x D5006-2
M-Dissolving Buffer	1 x D5005-6	1 x D5005-6
M-Desulphonation Buffer	1 x D5001-5	2 x D5001-5

2. Perform the bisulfite conversion as instructed in the **EZ DNA Methylation-Gold Kit** manual. Use ≤50 ng of FFPE DNA input, with higher input yielding best results. Elute the bisulfite-converted DNA in 18 µL of the provided **DNA Elution Buffer**.

FFPE DNA Protocol

After the bisulfite conversion process by either **Section 1: Bisulfite Conversion** or with the **EZ DNA Methylation-Gold** protocol, continue the **Zymo-Seq Cell Free DNA WGBS Library Kit** protocol as instructed with the following adjustments:

1. In **Section 2, Step 11**: After the 1-hour adapter ligation reaction, add 85 µL of **DNA Elution Buffer** to the sample to bring the volume up to 135 µL and mix well by pipetting.

2. In **Section 2, Step 12**: Follow the clean-up protocol in **Appendix A** on pg. 13 using 50 µL of **Select-a-Size MagBeads**. Allow the **Select-a-Size MagBeads** to equilibrate to room temperature for 30 minutes prior to use. For elution, resuspend the beads in 15 µL of **DNA Elution Buffer** and aspirate all 15 µL eluate into a new tube after separation from the beads.

3. In **Section 3, Step 3**: The recommended number of PCR cycles may be increased to the following depending on the FFPE DNA sample input amount and type used:

Sample Input	Sonicated	Not Sonicated
5 ng	9-10 cycles	14-15 cycles
10 ng	8-9 cycles	13-14 cycles
20 ng	-	12-13 cycles
30 ng	-	11-12 cycles
50 ng	-	9-10 cycles

4. In **Section 3, Step 4**: Follow the clean-up protocol in **Appendix A** on pg. 13 using 40 µL of **Select-a-Size MagBeads**. Allow the **Select-a-Size MagBeads** to equilibrate to room temperature for 30 minutes prior to use. For elution, resuspend the beads in 20 µL of **DNA Elution Buffer** and aspirate all 20 µL eluate after separation from the beads.