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Protocol for Chromatin Shearing Analysis (Purification Beads)

1. Overview

We recommend using an agarose gel analysis or the Fragment Analyzer (Advanced Analytical) for the size assessment. Although the microfluidics technology (Agilent BioAnalyzer 2100, Agilent 2200 Tape Station System, Perkin Elmer Caliper LabChip GX Touch) is widely used for size assessment of DNA fragments before library preparation for NGS, this technology is less optimal for sheared chromatin resulting in a regular over-estimation of high molecular weight fragments.

For accurate size determination of the chromatin fragments, reverse crosslinking, including RNase treatment followed by DNA purification, is advised. Size estimation of chromatin fragments without reverse crosslinking is not precise. The presence of the crosslinks retards electrophoretic migration resulting in a misinterpretation of fragment size. RNase treatment significantly reduces background caused by degraded RNA and improves visual assessment of shearing..

RNAse treatment (1 h, optional but highly recommended for an accurate size assessment)

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Reverse crosslinking (4 h or overnight)

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DNA purification using Purification Beads (30 minutes)

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Fragment size assessment (agarose gel or Fragment Analyzer from Advanced Analytical) (1 h)

2. Protocol

2.1 RNAse Treatment.

- RNase cocktail not supplied.

2.1.1 Take 50 µl of sheared chromatin and transfer to a 1.5 ml microtube.
2.1.2 Dilute RNase cocktail in ChIP-seq grade water.
2.1.3 Add 2 µl of diluted RNase cocktail to the aliquot of sheared chromatin.
2.1.4 Incubate for 1 hour at 37°C.

2.2 Reverse cross-linking.

2.2.1 Add 50 µl of Elution Buffer E1 to the sample.
2.2.2 Add 4 µl of Elution Buffer E2, mix thoroughly.
2.2.3 Incubate samples at 65°C for 4 hours (or overnight).

2.3 DNA Purification.

- The protocol below describes DNA purification using the Purification Beads included in the kit. Other methods of DNA purification (columns-based DNA clean-up, e.g. spin columns or a phenol–chloroform extraction followed by ethanol precipitation) can be used also.

2.3.1 Add 2 µl of carrier to the sample.
2.3.2 Add 100 µl of 100% isopropanol to the sample.
Δ Note: Following the addition of isopropanol the solution may become cloudy. This is not detrimental to your experiment and will not influence the quality or quantity of your purified DNA.
2.3.3 Resuspend by vortexing the Purification Beads and add 20 µl to the sample.
2.3.4 Incubate samples for 10 minutes at room temperature on the rotator.
2.3.5 Briefly spin the tubes, place in the magnetic rack, wait 1 minute and discard the buffer.
2.3.6 Add 100 µl of Wash Buffer 1 per tube. Close the tubes and vortex well until the beads pellet is completely resuspended. Incubate for 30 seconds at room temperature. Briefly spin the tubes, place in the magnetic rack, wait 1 minute and discard the buffer.

2.3.7 Add 100 µl of Wash Buffer 2 per tube. Close the tubes and vortex well until the beads pellet is completely resuspended. Incubate for 30 seconds at room temperature. Briefly spin the tubes and place them into the magnetic rack, wait 1 minute and discard the buffer.

2.3.8 Spin the tubes again and place them on the magnetic rack. Discard the remaining Wash Buffer 2 if necessary. Resuspend the beads pellet in 25 µl of Buffer C. Incubate at room temperature for 15 minutes on the rotator.

2.3.9 Spin the tubes and place them into the magnetic rack. Wait 1 minute and transfer the supernatants into a new 1.5 ml tube. Discard the beads.

2.4 Fragment Size Assessment.

- Analyze the purified DNA on a 1.5% agarose gel. Load around 300 ng of DNA for an optimal separation and visualization. Alternatively, you can use a Fragment Analyzer.

3. Notes

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