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General Protocol for Chromatin Shearing Analysis

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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 a method of choice

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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.

- Purify DNA using a method of choice (eg. DNA purification kit, spin columns or phenol-chloroform extraction) and 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

Technical Support

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