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PROTOCOL

Re-shearing of decrosslinked immunoprecipitated DNA using the Bioruptor® Pico

INTRODUCTION

ChIP-seq is a common tool used to study protein-DNA interactions in cells and tissues on a genome-wide scale. The size of the immunoprecipitated DNA fragments is a key parameter for successful library preparation and reliable ChIP-seq data. Re-shearing of the purified de-crosslinked DNA after immunoprecipitation and before library preparation enable enrichment of fragments in the desired optimal size range suitable for next-generation sequencing (Figure 1). This approach eliminates a size-selection step from a conventional library prep workflow and increases the yield of immunoprecipitated DNA available for a library. It improves the sensitivity and/or resolution of standard ChIP-seq experiments, especially for inactive histone marks (Laczik et al, 2016). The chromatin that represents inactive marks is more resistant to sonication. As a consequence, these regions are relatively more present among the longer fragments after the initial shearing step, and re-shearing shifts these fragments to smaller sizes which are more efficiently incorporated into sequencing libraries. ChIP-seq on active histone marks, transcriptional factors or chromatin modifying proteins benefits from the re-shearing approach by increasing the number of fragments available for library generation for sequencing and permitting reduction of the starting amount of cells.

REQUIRED MATERIALS AND EQUIPMENT

- Bioruptor® Pico (Diagenode, Cat. No. B01060001)
- 0.2ml tube holder (Diagenode, Cat. No. B01200044) and corresponding 0.2 ml microtubes for Bioruptor® Pico (Diagenode, Cat. No. C30010020) or 0.1 ml tubes holder (Diagenode, Cat. No. B01200041) and corresponding 0.1 ml microtubes for Bioruptor® Pico (Diagenode, Cat. No. C30010015)
- Library preparation Kit (e.g. MicroPlex Kit, Diagenode, Cat. No. C05010012 or iDeal Library Preparation Kit, Diagenode, Cat. No. C05010020).
- (Optional) Fragment Analyzer™ (Advanced Analytical) or BioAnalyzer (Agilent) with high sensitivity reagents

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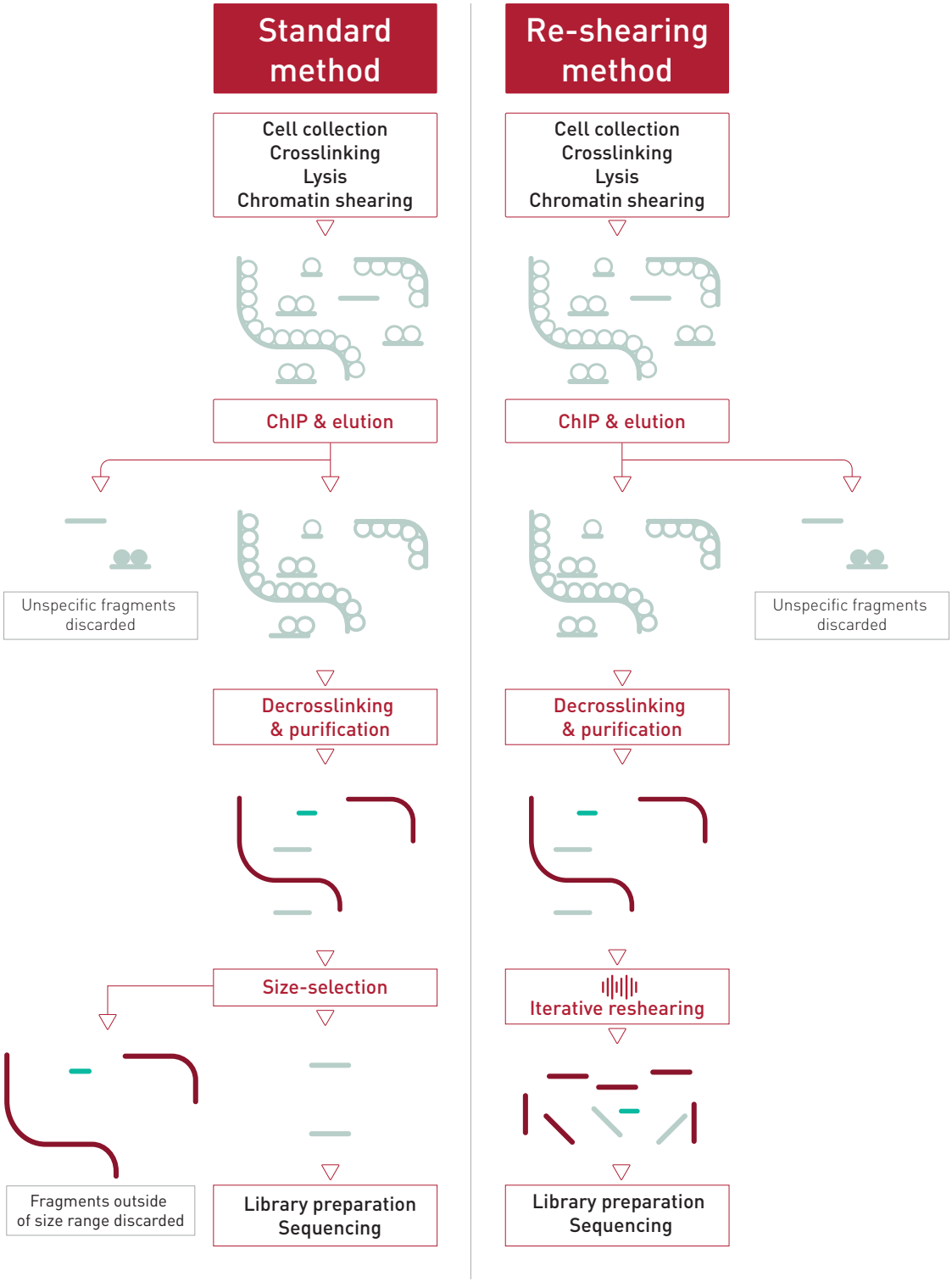

Figure 1. *An overview of standard method (left) and re-shearing method (right)*

PROTOCOL

1. Perform chromatin immunoprecipitation using an appropriate protocol (e.g. Diagenode iDeal ChIP-seq Kit for Histones (Cat. No. C01010051) or iDeal ChIP-seq Kit for Transcription Factors (Cat. No. C01010055) and ChIP-seq grade antibodies (www.diagenode.com/categories/antibodies). Ensure that an expected enrichment is achieved.
2. Transfer 20 µl of immunoprecipitated DNA to Bioruptor® microtubes (0.2 ml or 0.1 ml).
3. Vortex and spin down samples and proceed to sonication in the Bioruptor® Pico with the following settings:

   Sonication cycle: 30 sec ON/30 sec OFF
   Total sonication time: 5 -10 cycles
   Temperature: 4°C

   Note: To guarantee homogeneity of sonication, the tube holder should be always completely filled with tubes.
4. (Optional) if DNA concentration is sufficient (at least 2-3 ng/µl), DNA fragment size distribution before preparing libraries can be assessed on the Fragment Analyzer™ (Advanced Analytical) using the DNF-474 High Sensitivity NGS Fragment Analysis Kit or BioAnalyzer (Agilent) using High Sensitivity DNA Analysis kit.
5. Proceed to the library preparation using re-sheared DNA.

   Note: A size selection step can be skipped.

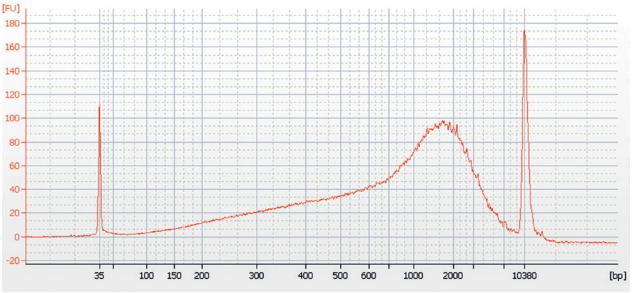

Figure A

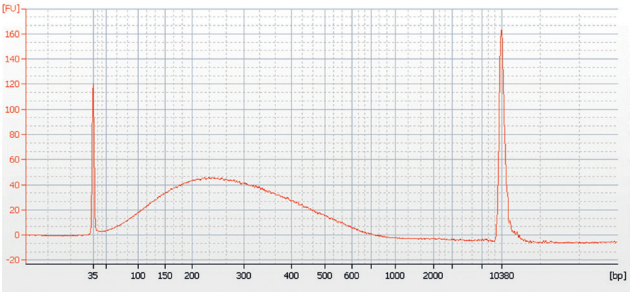

Figure B

Figure 2. The re-shearing method enriches fragments in an optimal size range suitable for next-generation sequencing. *Shearing profile of immunoprecipitated chromatin before (Figure A) and after (Figure B) re-shearing. Chromatin from HeLa cells was prepared according to the iDeal ChIP-seq Kit for Histones and sheared using the Bioruptor® Pico for 8 cycles. ChIP was performed with an H3K27me3 antibody (Diagenode, Cat. No. C15410195). De-crosslinked and purified DNA was analysed on a BioAnalyzer. The observed fragment range is compatible with ChIP-seq library preparation.*

LIST OF DIAGENODE ANTIBODIES SUCCESSFULLY TESTED USING THE RE-SHEARING METHOD:

Product	Cat. No.
H3K27me3	Cat. No. C15410195
H3K4me3	Cat. No. C15410195
H3K4me1	Cat. No. C15410194
p53	Cat. No. C15410083
H3K27me3S28p	Cat. No. C15410091
H4K20me3	Cat. No. C15410057
HDAC2	Cat. No. C15200201
EZH2	Cat. No. C15200180
LSD1	Cat. No. C15410028
CTCF	Cat. No. C15410210

Product	Cat. No.
NFkB	Cat. No. C15310255
E2F6	Cat. No. C15410314
RNF2	Cat. No. C15410313
OCT4	Cat. No. C15410305
H3K36me3	Cat. No. C15410058
NFYB	Cat. No. C15410241
KAP1	Cat. No. C15410236
H3K9me3	Cat. No. C15410193
CBX8	Cat. No. C15410333

REFERENCES

Laczik et al. Iterative Fragmentation Improves the Detection of ChIP-seq Peaks for Inactive Histone Marks. *Bioinformatics and Biology Insights* 2016:10 209–224 doi: 10.4137/BBI.S40628

Mokry M, Hatzis P, de Bruijn E, Koster J, Versteeg R, Schuijers J, et al. (2010) Efficient Double Fragmentation ChIP-seq Provides Nucleotide Resolution Protein-DNA Binding Profiles. PLoS ONE 5(11): e15092. doi:10.1371/journal.pone.0015092