Harnessing Template Switching Technology for Preparation of Low-Input Ligation-Free Sequencing Libraries

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Abstract
Preparation of sequencing libraries from minute amounts of DNA can be extremely challenging. Most methods rely on inefficient adapter ligation and require an input of 50-100 ng of high-quality, double-stranded DNA. Template switching has gained renewed attention as an alternative to ligation for NGS library production due to the ability to provide step adapter addition and inherent sensitivity for amplifying picogram quantities of material. However, until now, template switching has been limited to use only with RNA samples. Here, we report a modified template switching reaction that accepts DNA instead of RNA as a template. This new technology accommodates either double-stranded or single-stranded DNA templates, thus making it very amenable to a wide range of applications.

We have created a single-tube workflow allowing the use of DNA template switching for sequencing on Illumina platforms. The workflow can be completed in 4 hours and works with 100 pg to 10 ng of double-stranded or single-stranded DNA. To validate the performance of the technology, we applied it to chromatin immunoprecipitation (ChIP) sequencing (ChIP-seq). We used histone H3 trimethylated (H3K4me3)-pull down on HEK293T cells, and found that over 85% of the peaks identified with the DNA template switching method overlap with peaks identified from data generated by the ENCODE project. Furthermore, we found 86% overlap between the peaks identified when comparing H3K4me3 pull down experiments performed with 1 x 10⁶ cells and 1 x 10⁵ cells. Input contamination of as low as 500 pg yielded a non-redundant rate >85%, meeting standards established by the ENCODE consortium. These data illustrate that DNA template switching technology provides a robust and reliable tool for preparation of sequencing libraries from challenging samples such as ChIP DNA, particularly at low inputs.

Introduction

The ChIP Elute Kit is a fast and very simple tool to replace the slow and tedious DNA recovery steps performed at the end of ChIP experiments. It is compatible with any upstream ChIP protocol, and efficiently isolates, purifies, and concentrates ChIP DNA in less than one hour. Cell nucleic acid that is directly compatible with the DNA SMART™ ChIP-Seq Kit that uses an adaptation of SMART™ template switching technology for use with DNA. High-quality libraries can be generated using these two products in an efficient, time-saving manner.

Materials and Methods

Template switching was performed on HEK 293T cells with ChIP-grade antibodies according to standard procedures. After washes, DNA-protein complexes immobilized to Protein A/G agarose beads were processed using the traditional method or the ChIP Elute Kit. For the traditional method, DNA-protein complexes were eluted from the beads in SDS buffer (1% SDS, 0.1 M NaHCO₃) and cross-links were reversed in the presence of 100 µg/ml of EDTA, followed by a two-hour Proteinase K treatment. The DNA was purified and concentrated with a Mini NucleoSpin Gel and PCR CLEAN-Up Kit (using Buffer NTB). Samples processed with the ChIP Elute Kit were handled according to the kit protocol (15 min Proteinase K treatment).

ChIP-seq libraries were generated using the DNA SMART ChIP-Seq Kit according to the user manual with size selection using Option 1 or Option 4. Sequencing was carried out on Illumina MiSeq® or HiSeq® 3500 instruments. All runs were performed with paired-end sequencing; the Custom Read Seq Primer from the DNA SMART ChIP-Seq Kit was used for some runs.

Mapping of reads (unpaired) to the human genome (hg19) was performed using Bowtie with default settings (allowing of the first 3′ nucleotides of the reads obtained with the Read Primer 1). Uniquely mapping reads were selected and the SAM files were sorted and converted to BAM files using SAMTOOLS. Peaks were identified using MACS version 1.4 (default settings except the p-value cutoff set at 1 x 10⁻⁶) with a non-enriched (control) chromatin input sample as a control. For quantitative peak overlap comparison to data generated by the ENCODE consortium (293 cells, anti-H3K4me3 antibody, U. Washington), raw data were analyzed and compared to the ENCODE project. The number of overlapping peaks was determined using 85% area of overlap of genome. Table 1: Sequence Metrics from Different Cross-Linking Reversal Methods

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<th>Table 1: Sequence Metrics from Different Cross-Linking Reversal Methods</th>
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Conclusions
The DNA SMART ChIP-Seq Kit generates sequencing libraries for Illumina platforms that maintain the complexity of the ChIP input sample, even when used with low input amounts. This sensitive, ligation-independent method expands the range of input samples that can be used for ChIP-seq experiments. It is particularly well suited for histones like that generated by the fast and efficient ChIP Elute Kit.

- Efficient workflows-The ChIP Elute Kit provides a fast DNA elution and cross-linking reversal method, and the DNA SMART ChIP-Seq Kit adds a SMART™ template switching and ligation-independent amplification as the same time as library amplification.
- Compatible with low-input samples-Libraries can be generated from as little as 100 pg of fragmented input DNA.
- Preservation of library complexity-Excellent non-redundant rate from 95% to 99%, even at low input amounts of 4-10 ng (ChIP DNA generated using anti-H3K4me3 or anti-CTCF antibodies)

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