DATA SHEET

TruSeq™ ChIP Library Preparation Kit

Proven TruSeq data quality delivers comprehensive and accurate profiling of target protein-DNA interactions

- Comprehensive and accurate profiling of target protein–DNA interactions
- Robust results from just 5 ng of input DNA from various sample sources
- Enhanced scalability with a simple, streamlined workflow
- Optimized sequencing output distribution across samples, reducing cost per sample

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Introduction

Determining how protein–DNA interactions regulate gene expression is essential for fully understanding many biological processes and disease states. This epigenetic information is complementary to DNA sequencing, genotyping, gene expression, and other forms of genomic analysis. Chromatin immunoprecipitation sequencing (ChIP-Seq) harnesses next-generation sequencing (NGS) to quickly and efficiently determine the distribution and abundance of DNA-bound protein targets of interest across the genome. ChIP-Seq has become one of the most widely applied NGS-based applications, enabling researchers to reliably identify binding sites of a broad range of targets across the entire genome with high resolution.

As the output of NGS systems has increased, ChIP-Seq researchers increasingly require a combination of highly multiplexed sequencing and simple, streamlined workflows. TruSeq ChIP Library Preparation Kits meet those demands, offering a simple, cost-effective solution for obtaining visibility into the mechanics of gene regulation. Library generation from ChIP-derived DNA includes the addition of indexed adapters, enabling the optimal distribution of sequencing output based on coverage needs. An optimized, highly scalable library preparation workflow and master-mixed reagents reduce hands-on time and support an automation-friendly format for parallel processing of up to 48 samples. Samples with different indexes can be mixed and matched to maximize experimental throughput. A low sample input requirement (5 ng) ensures robust results even when input DNA availability is limited, providing flexibility in the choice of sample source and target proteins for analysis.

Simple, streamlined workflow

TruSeq ChIP Library Preparation Kits provide a significantly improved library preparation workflow compared to other methods. The TruSeq workflow reduces the number of purification, sample transfer, pipetting, and clean-up steps. A universal adapter design incorporates an index sequence at the initial ligation step for improved workflow efficiency and more robust multiplexed sequencing (Figure 1). The workflow begins with enrichment of specific cross-linked DNA-protein complexes using an antibody against a protein of interest (Figure 1A-B). The stretches of DNA bound to the target protein are then isolated and used as input DNA for TruSeq ChIP library preparation.

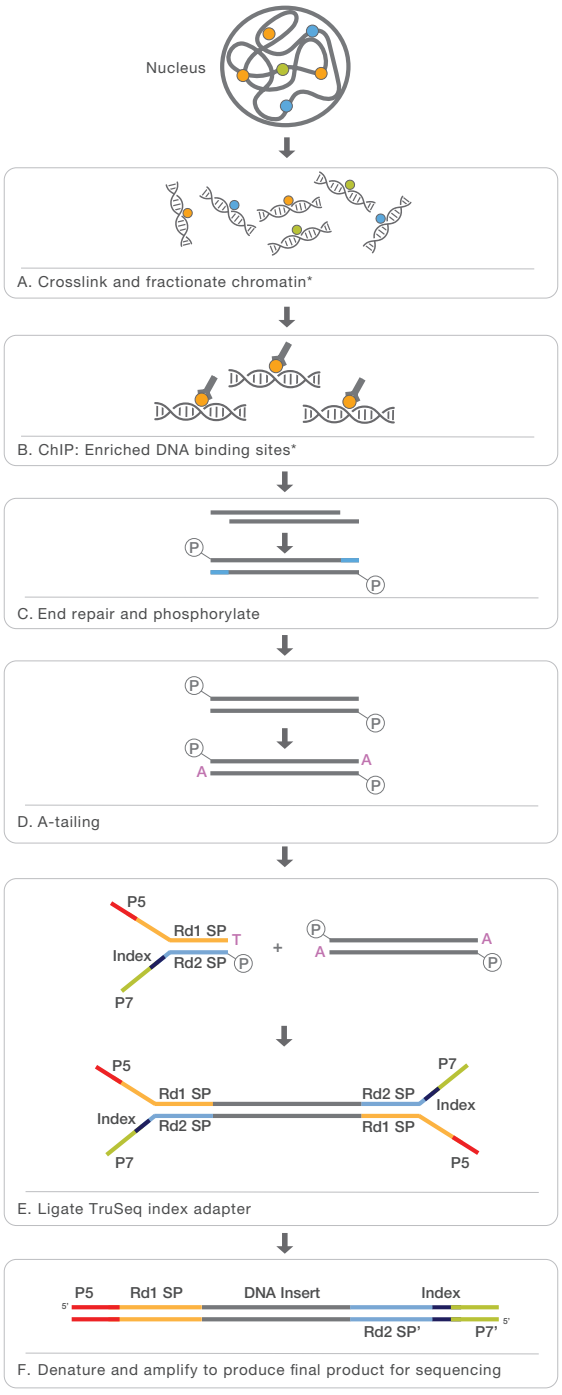

Figure 1: TruSeq ChIP-Seq workflow—The simple, streamlined workflow (steps C-F) reduces hands-on time and speeds analysis; TruSeq universal adapters improve workflow efficiency and enable robust multiplexed sequencing.

DNA fragments are end-repaired and an 'A'-base added to the blunt ends of each strand, preparing them for ligation to the sequencing adapters (Figure 1C-D). Each TruSeq adapter contains a 'T'-base overhang on the 3'-end providing a complementary overhang for ligating the adapter to the A-tailed fragmented DNA (Figure 1E). Final product is created (Figure 1F) and after size selection, all of the ChIP DNA fragments are simultaneously sequenced.

For maximum flexibility, TruSeq ChIP Library Preparation Kits can be used to prepare samples for single-read or paired-end sequencing, and are compatible with any Illumina sequencing system.

TruSeq data quality

Proven TruSeq data quality delivers the comprehensive and accurate profile of target protein–DNA interactions, enabling an optimal percentage of passing filter reads, percent alignable reads, and coverage uniformity, as well as high sensitivity to detect low-abundance hits.

Robust multiplex performance

TruSeq ChIP Library Preparation Kits provide up to 24 total indexes to increase throughput and consistency without compromising results. The TruSeq universal adapters ligate to sample fragments during library construction, allowing samples to be pooled and individually identified during downstream analysis. This indexing capability improves workflow efficiency and enables robust multiplexed sequencing. By enhancing study design flexibility, indexing aids researchers in maximizing the value of each run by efficiently distributing read output based on optimal per-sample read depth requirements.

Flexible range of targets

TruSeq ChIP Library Preparation Kits enable libraries to be generated using as little as 5 ng input DNA and provide a high-quality, cost-efficient, and high-throughput solution across a broad array of ChIP study designs. ChIP-Seq is a versatile application that has been successfully applied against a wide range of protein targets, including transcription factors and histones, the building blocks of chromatin. ChIP studies targeting transcription factors are useful in elucidating the specific modulators and signal transduction pathways contributing to disease states, stages of development, or across other conditions, while histone "marks" can be used to better understand how chromatin modifications and local structural changes impact local gene expression activity.[1-8]

Detecting peaks across the genome

To demonstrate the performance of the TruSeq ChIP Library Preparation Kit, a library was generated for transcription factor MafK using 5 ng of input DNA (Figure 2) derived from a ChIP performed in HELA cells.

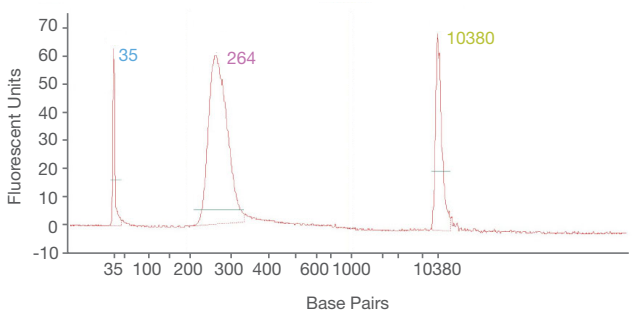

Figure 2: Bioanalyzer trace of MafK library—Bioanalyzer trace data for a library generated for transcription factor target MafK using the TruSeq ChiP Library Preparation Kit with 5 ng of input DNA. The center peak indicates robust yield within the desired insert size range.

Sequencing data were generated using a single run on a MiSeq™ System. Quality-filtered, BAM output files were then entered into the MACS peak finder software, with the identified peaks then screened for enrichment using MEME motif finder software. Results show sensitive and reliable detection of DNA-protein interactions, with a representative, identified peak corresponding to a MafK binding site included in the ENCODE project database (Figure 3).

Enrichment for the known, MafK binding motif was detected as expected (Table 1), again in concordance with data generated using MafK peak data available through ENCODE.[9] The ability to robustly detect peaks across the genome with low starting input amounts is critical to ensuring successful ChIP studies. TruSeq ChIP Library Preparation Kits provide the flexibility to target any protein target of interest, offering a streamlined, cost-efficient solution for studies requiring a broad range of reads per sample including transcription factors (Figure 3), and histone marks, such as H3K4Me3 (Figure 4).

Table 1: Motif-finder analysis of peaks using TruSeq ChIP

Name	% top peaks with MafK motif
TruSeq ChIP	95%
ENCODE HELA	92%
ENCODE HES	86%

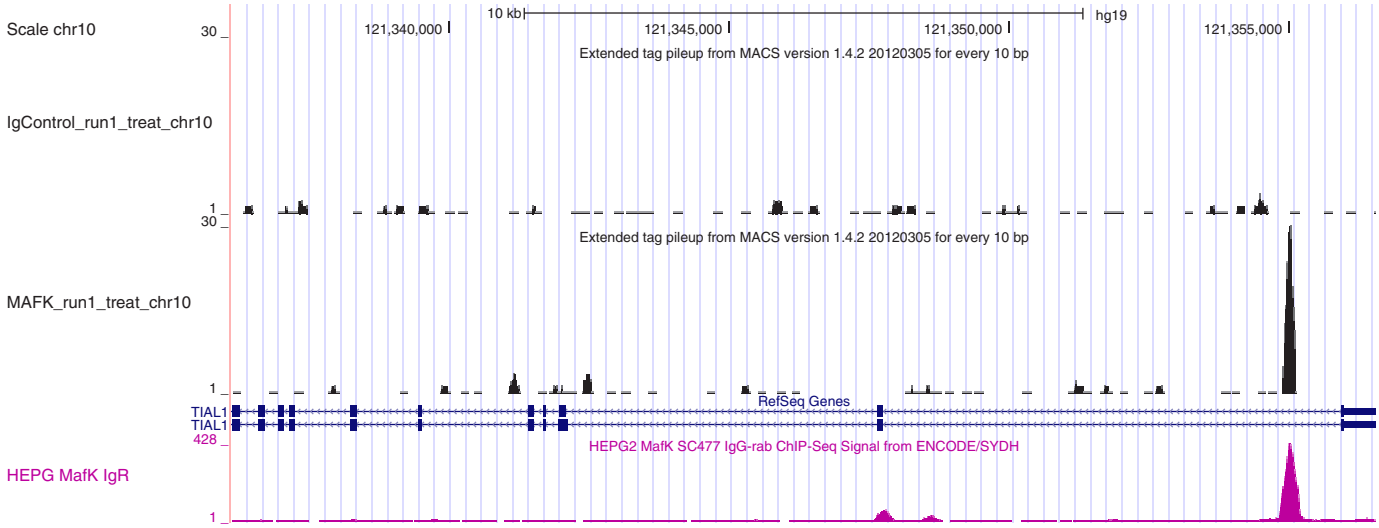

Figure 3: Peak finding output for MafK—TruSeq ChIP Library Preparation Kits enable the generation of libraries across a broad range of study designs. Above is peak data for a negative Ig control, the transcription factor target MafK, and a reference peak for MafK from the ENCODE database.

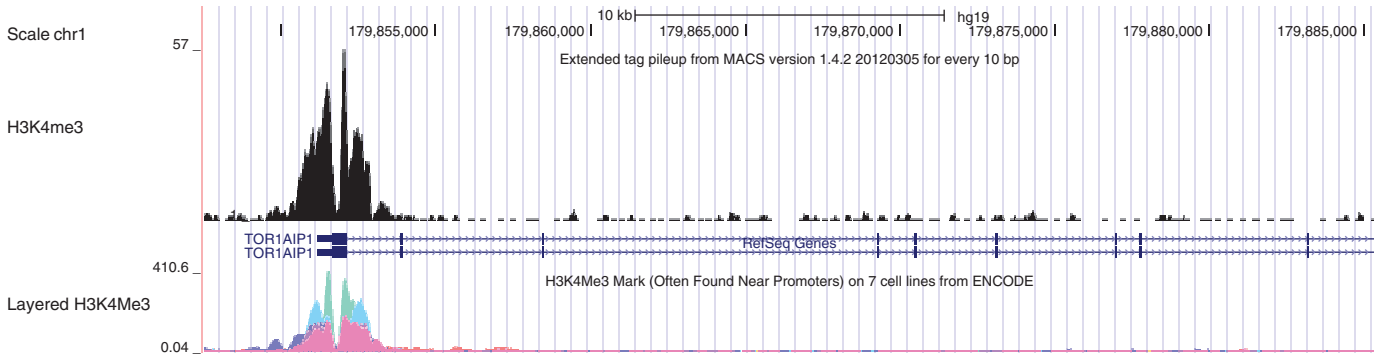

Figure 4: Peak finding output for MafK—The peak results for the H3K4me3 target compare favorably with the ENCODE annotation data for this well-characterized target, with a representative peak for the histone mark target H3K4me3 and a corresponding ENCODE reference peak.

Illumina sequencing solutions

TruSeq ChIP Library Preparation Kits are compatible with all Illumina sequencing by synthesis (SBS)–based NGS systems. Data compatibility is ensured whichever system is chosen.

Summary

TruSeq ChIP Library Preparation Kits offer proven TruSeq accuracy, and a simple, streamlined workflow, enabling highly multiplexed, cost-effective ChIP sequencing. Supporting analysis of a broad range of targets across the genome even from low sample input, the kits provide a complete, accurate profile of DNA-protein binding interactions and enhanced visibility to the mechanics of gene regulation.

Ordering information

Product	Catalog No.
TruSeq ChIP Library Preparation Kit, Set A (12 indexes, 48 samples)	IP-202-1012
TruSeq ChIP Library Preparation Kit, Set B (12 indexes, 48 samples)	IP-202-1024

References

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