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Product Information

ChIP Next Gen Seq Sepharose®

Catalog Number **S6576**

TECHNICAL BULLETIN

Synonym: Staph-Seq

Product Description

ChIP Next Gen Seq Sepharose are modified *Staphylococcus aureus* (Staph A) cells that are used to immunoprecipitate and purify chromatin complexes. ChIP Next Gen Seq Sepharose was developed as a novel component of Sigma's second generation chromatin immunoprecipitation (ChIP) kit, the Imprint® Ultra Chromatin IP Kit, Catalog Number CHP2. CHP2 was developed for maximum sensitivity and optimum next-generation sequencing results. Sequencing quality modified Staph A is achieved by employing a proprietary DNA-blocking technology to prevent Staph A DNA contamination of next-generation sequencing in ChIP-Seq experiments (Fig. 1). The DNA-blocked cells are ideally suited for studying recruitment of low abundance transcription factors (TF) in genome wide location analysis experiments such as ChIP-chip and ChIP-Seq. The high surface-density of endogenous protein A on the cell wall of Staph A serves as an excellent matrix to pull-down rare TF-associated chromatin complexes. This development allows researchers to explore the genome-wide binding sites of low abundance TF's as well as novel histone modifications.

The Imprint Ultra ChIP protocol has been adapted from, and validated in consultation with, the laboratory of Dr. Peggy Farnham, USC Los Angeles. It is optimized for ChIP reactions with chromatin from 10 million cells (up to ≈ 50 µg DNA), and can also be scaled up (or several preparations pooled) to accommodate 10^8 cells for genome-wide binding studies in ChIP-chip and ChIP-Seq applications. Our DNA-blocking treatment prevents blocked Staph A DNA from participating in any PCR-based amplification reactions, thereby virtually eliminating Staph A DNA contamination of Illumina-libraries prior to massively parallel next-generation sequencing (Figs.1-2). The blocking treatment does not alter the performance of ChIP Next Gen Seq Sepharose in ChIP-Seq assays.[2,3] This breakthrough results in dramatic improvement of the number of sequence reads that map back to the genome of interest in a BLAST search against the reference genome (Fig. 3).

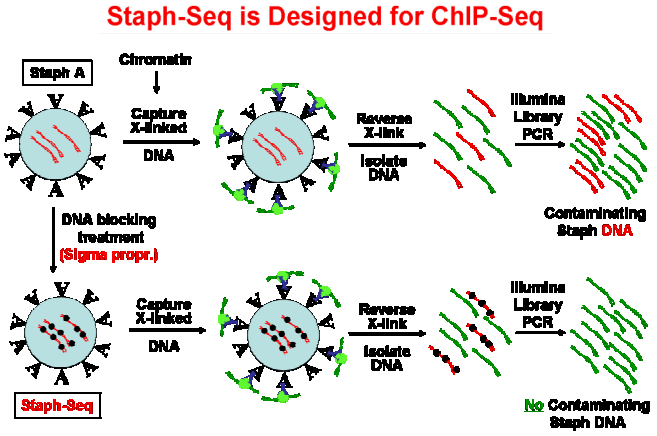

Fig. 1 Use of untreated (top panel) Staph A (blue circle) in a ChIP-Seq assay results in Staph A DNA sequences (red) contaminating the genomic ChIP'ed DNA sequences (green) of interest. Both the genomic DNA and the Staph A DNA are amplified in the Illumina library. Employing DNA-blocked Staph-Seq (lower panel) practically eliminates Staph A DNA contamination in Illumina-Sequencing data.

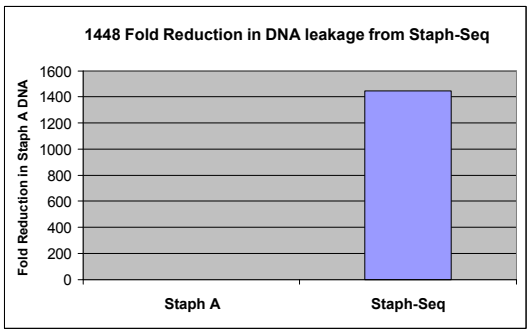

Fig. 2 Staph A DNA contamination is reduced using ChIP Next Gen Seq Sepharose. ChIPs were performed using ChIP Next Gen Seq Sepharose versus control Staph A (P7155) cells and the purified DNA was analyzed using primers targeting Staph A rDNA. A 1448 fold reduction in Staph A DNA contamination (a 10.5 Ct difference) was observed in comparison to control Staph A cells.

	Control "Staph A"	Treated "Staph-Seq"
Total Reads	**45,368,364**	**34,942,887**
Mapped Reads	**29,878,289 (65.9 %)**	**31,074,058 (88.9 %)**
Unique	**17,171,199**	**22,200,188**

Fig. 3 Summary of ChIP-Seq experiments done with ChIP Next Gen Seq Sepharose vs Staph A cells.
EZH2 ChIPs were performed with Staph A and ChIP Next Gen Seq Sepharose and cross-linked chromatin from 200 million DU145 cells. Illumina sequencing was performed by the UC Davis Genome Center (http://genomecenter.ucdavis.edu/dna_technologies. Bioinformatics analysis was done using "SOLE Search" program (Farnham Lab; http://havoc.genomecenter.ucdavis.edu/cgi-bin/chipseq.cgi). Compared to regular Staph A cells, DNA-blocked cells gave a 23% gain in mappability of reads when blasted against the human genome (HG19 build).

Components
Five vials of lyophilized ChIP Next Gen Seq Sepharose are packaged in one bag and shipped at RT.

Bridging Antibody Requirement
The protein A present on the surface of ChIP Next Gen Seq Sepharose binds efficiently to antibodies derived from rabbits. For ChIP assays with antibodies derived from other animals, such as monoclonals (from mouse), rat, chicken, etc. an appropriate bridging antibody (raised in rabbits) should be used as instructed in the CHP2 protocol.

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage and Stability
Lyophilized ChIP Next Gen Seq Sepharose is stable for up to 12 months when stored at RT.

Care must be taken not to introduce DNases. We recommend the use of DNase-free pipette tips, preferably those having an aerosol barrier. Wear latex gloves and change them frequently. Keep bottles and tubes closed when not in use.

Planning the ChIP experiment and chromatin/cell requirements
Depending on the relative abundance of the target DNA-binding protein [Transcription factor (TF), chromatin regulator/modifier or histone modification, DNA methylation] and the downstream DNA detection application, grow and cross-link the appropriate number of cells before starting to prepare the chromatin. See ChIP data for low-abundance (EZH2), medium-abundance (RNAP II) and high-abundance (H3K27me3) targets with chromatin from 10 million and 2 million cells to help determine your cell number requirements (Figs. 7-9 in Appendix of CHP2 User Guide[2]). Follow the guidelines indicated in the table below:

TF abundance	Application	Number of cells
Histone modification or abundant TF	qPCR	0.5-2 x 10^6
	Microarray	10^4-10^6
	Sequencing	10^5-10^7
Medium abundance TF (e.g. RNAP II)	qPCR	2-10 x 10^6
	Microarray	10^5-10^6
	Sequencing	10^6-10^7
Rare TF (Low abundance TF and co-activator/co-repressor that does not bind DNA directly e.g. EZH2)	qPCR	5-10 x 10^6
	Microarray	50 x 10^6 (5 ChIPs)
	Sequencing	100-200 x 10^6 (10-20 ChIPs)

Table 1: Guidelines for number of cells required, depending on the relative abundance of ChIP target and downstream application.

If you are interested in performing ChIPs with fewer cells (10^4-10^5) we recommend following the MicroChIP protocol[7] which is compatible with ChIP Next Gen Seq Sepharose and the reagents provided in the CHP2 kit. The WGA DNA amplification kits will have to be purchased separately.

Procedure

Blocking ChIP Next Gen Seq Sepharose (The evening before you start the ChIPs)

Pre-requisites:

- Pre-cool microcentrifuge to 4°C
- Reconstitute AEBSF (A8456, -20°C): add 1.04 mL water (W4502, RT) to a 25 mg bottle to make a 0.1 M stock solution. Mix and vortex until dissolved, and keep on ice. After use, store at –20°C
- Reconstitute 250 mg BSA (A7638, 4°C) with 12.5 mL of water (W4502, RT), mix by vortexing and inverting to make a 20 mg/mL solution and keep on ice. After use, make 1 ml aliquots and store at –20°C
- Prepare Dialysis buffer – 2 mM EDTA, 50 mM Tris-HCl, pH 8
- Thaw sonicated salmon sperm DNA (D9156, –20°C) and keep on ice
- Prepare the required amount of blocked ChIP Next Gen Seq Sepharose depending on the number of ChIP experiments planned within the next two weeks. You will need 20 µL of blocked ChIP Next Gen Seq Sepharose per ChIP reaction with chromatin from 10^7 cells.

Note: After blocking, each vial of ChIP Next Gen Seq Sepharose will be reconstituted with 200 µL of Dialysis buffer + 2 µL of 0.1M AEBSF. Blocked ChIP Next Gen Seq Sepharose is stored at 4°C and used within two weeks. The procedure below is outlined for blocking one vial, it may be scaled proportionately depending on the amount required.

Note: For all subsequent steps requiring removing supernatant from ChIP Next Gen Seq Sepharose pellets, please aspirate gently with a P1000 or P200 pipette. **DO NOT USE VACUUM TO ASPIRATE**, as you may lose the ChIP Next Gen Seq Sepharose.

Note: Do not allow pellets to dry out, add fresh wash buffer to each tube immediately after the previous wash buffer has been removed.

1. Rehydrate 1 vial of lyophilized ChIP Next Gen Seq Sepharose with 400 µL of water (W4502, RT) for 15 minutes at RT, vortex briefly and resuspend with a pipette until no clumps are visible. Brief hydration helps make a uniform suspension. Overnight blocking (step 3 below) facilitates hydration leading to uniform suspensions and low backgrounds in ChIPs.

Note: For ChIP-Seq experiments just add the BSA and AEBSF in step 2 below. No foreign DNA should be added.

2. Transfer the suspension into a 0.5 mL tube, add 20 µL of BSA (20 mg/mL), 40 µL of salmon sperm DNA (10 mg/ml), 4 µL of 0.1 M AEBSF and mix by pipette. Final concentration of BSA is ≈ 1 mg/mL and salmon sperm DNA is ≈ 1 mg/mL.

   Note: If only a portion of the ChIP Next Gen Seq Sepharose suspension is blocked, the remaining suspension may be stored at 4 °C for up to a month, then blocked and used successfully in ChIPs.

3. For best results, incubate on rotating platform at 4 °C overnight (recommended), or at least for 3 hours at 4 °C. If time constrained, this incubation may be shortened to 2 hrs at RT.

4. Transfer to a 1.5 mL tube and pellet at 14,000 rcf, 4 °C, for 3 minutes and remove supernatant.

5. Wash the pellet **twice** by resuspending in 1 mL Dialysis Buffer and repelleting (14,000 rcf, 4 °C, 3 minute).

6. After the last wash, resuspend the pellet in half the starting volume of the suspension using Dialysis buffer (i.e. use 100 µL Dialysis Buffer for 200 µL of starting suspension or 200 µL Dialysis Buffer for 400 µL of starting suspension, i.e., entire vial). Add 1/10th volume of 0.1 M AEBSF (i.e. 1 µL of 0.1 M AEBSF to 100 µL of suspension or 2 µL of 0.1 M AEBSF to 200 µL of suspension) and mix by pipetting. Washed and blocked ChIP Next Gen Seq Sepharose may be stored at 4 °C and used for up to two weeks.

7. To pull-down the antibody-bound chromatin complexes in ChIP, use 5 µL (for chromatin from ≤ 2 x 10^6 cells) or 10 µL (for chromatin from > 2 x 10^6 cells) of blocked cells to each chromatin-antibody sample. Incubate on the rotating platform for **exactly** 15 minutes (**no longer!**) at RT.

Results

Successful performance of ChIP Next Gen Seq Sepharose has been demonstrated in ChIP-Seq experiments using the Imprint Ultra Chromatin IP Kit, and in collaboration with the Farnham lab (Ref. 4 and Palhan, et al., manuscript in preparation[9]).

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