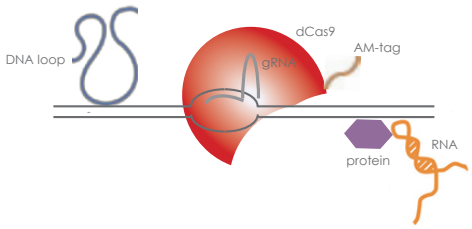
ACTIVE MOTIF®
Enabling Epigenetics Research

enChIP

Determine CRISPR/Cas9 specificity with enChIP

To enable you to assess Cas9 specificity, Active Motif's enChIP Kit (Engineered DNA-binding molecule-mediated Chromatin Immunoprecipitation) offers a modified ChIP assay that utilizes the CRISPR/Cas9 system to target a specific DNA locus for immunoprecipitation (Figure 1). Using the enChIP Kit for your genome editing experiments enables you to biologically validate each target sequence for specificity. Sequences demonstrating off-target effects can be excluded, saving valuable time and resources.

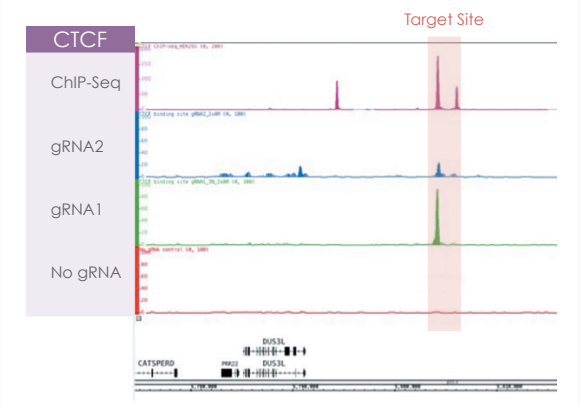
- ✓ Identify off-target binding events for each gRNA sequence
- ✓ Evaluate *cis*- and *trans*-interacting chromosomal looping events
- ✓ ChIP-optimized AM-tag design ensures highly efficient dCas9 enrichment
- ✓ Works well with open chromatin regions to detect promoter, enhancer and insulator elements



▲ FIGURE 1: Illustration of enChIP design.

How does it work?

A guide RNA (gRNA) containing 20 nucleotides complementary to the desired genomic region is expressed in combination with an enzymatically inactive Cas9 protein (dCas9) tagged with an AM-tag that is specifically designed for ChIP. The gRNA directs dCas9 to the target sequence and an RNA-DNA heteroduplex is formed. Chromatin immunoprecipitation is then performed using an antibody directed against the dCas9 AM-tag to enrich for genomic sequences bound by the gRNA/dCas9 complex. By using the enChIP Kit, off-target gRNA binding sites can be identified (Figure 2). This provides valuable information regarding the quality of gRNA design prior to use in genome editing experiments. Additionally, the enChIP Kit can be used to study *cis*- and *trans*-interacting chromosomal looping events. For more complete information, please visit us at www.activemotif.com/enchip.



▲ FIGURE 2: enChIP validates specificity of gRNA for a CTCF binding site.
Two gRNAs were designed targeting different 20 nt sequences within a 500 bp region of a CTCF binding site on chromosome 19. Each gRNA sequence was cloned into the pAM_dCas9 vector and transfected into HEK293T cells. A 'No gRNA' negative control was also performed. Chromatin was prepared and immunoprecipitated according to the enChIP Kit. Enriched DNA was analyzed by Next-generation sequencing and background was subtracted using the 'No gRNA' control. Data was compared to ChIP-Seq data for CTCF in the same cell line. Results show gRNA1 had a strong peak and specific binding at the target location. Data for gRNA2 revealed a large number of off-target binding events outside of the target location. This confirms that enChIP provides biological validation for CRISPR/Cas9 gRNA specificity.

ORDERING INFORMATION

Product	Format	Cat. No.
enChIP Kit	16 rxns	53125
pAM_gRNA Vector	10 µg	53121
pAM_dCas9 Vector	10 µg	53122
pAM_gRNA_CTCF Vector (Positive control)	10 µg	53123
pAM_dCas9_CTCF Vector (Positive control)	10 µg	53124