Nucleosome Remodeling Assay by EpiDyne® Restriction Enzyme Accessibility

Fully defined nucleosome substrates engineered for chromatin remodeling studies

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Overview

Nucleosome remodeling (or repositioning) regulates DNA access on chromatin and thus impacts gene expression and genome repair. Many ATP-dependent remodeling enzymes (and their multi-subunit complexes) are associated with human disease but are notoriously challenging targets to study biochemically due to the requirement for nucleosome-based substrates. EpiCypher has addressed this need by developing EpiDyne®, a family of recombinant nucleosome substrates to monitor nucleosome remodeling activity in vitro. Here, we describe the use of EpiDyne Nucleosome Remodeling Substrates in a Restriction Enzyme Accessibility (REA) assay.

Chromatin Remodeling Enzymes as Therapeutic Targets

Aberrant nucleosome organization can severely disrupt gene expression, DNA repair and cellular differentiation, playing major roles in many human disorders (e.g. cancer, inflammation, autoimmunity, schizophrenia, cardiovascular disease, and intellectual disability). Remarkably, nearly 20% of all cancers contain mutations in subunits from the ATP-dependent SWI/SNF family of chromatin remodeling complexes. These enzyme complexes regulate local genome access by 'pumping' the DNA around histone octamers, thus repositioning nucleosomes along DNA [1]. Recurrent somatic mutations in SWI/SNF subunits are observed in multiple cancers, supporting a driver role in tumorigenesis [2]. The mutated complexes are desirable therapeutic targets, since further compromising their ATPase activity promotes cancer cell death but spares normal cells [2, 3]. This phenomenon is known as synthetic lethality and identifying inhibitors to exploit it may lead to drugs with cancer specificity [4, 5].

Nucleosome Remodeling Assays Using Recombinant Mononucleosome Substrates

EpiCypher has developed the EpiDyne family of Nucleosome Remodeling Substrate to study the activity of chromatin re-modeling enzymes. The nucleosome, 147 base pairs of DNA wound around a core histone octamer, represents the fundamental repeating unit of chromatin. EpiDyne nucleosomes consist of a recombinant human histone octamer wrapped with DNA containing a terminal nucleosome positioning sequence (147 base pairs) adjacent to a 70 base pair downstream acceptor region. The positioned histone octamer shields a restriction enzyme recognition site (e.g. DpnII [GATC] or Mfe1 [CAATTG]) which is exposed by ATP-dependant nucleosome remodeling to allow DNA cleavage (Figure 1).

This first generation EpiDyne substrate is also compatible with a high-throughput readout, such as radiolabeling with the GATC-specific DAM methyltransferase [6, 7]. We are currently developing next-generation remodeling substrates compatible with other HTS platforms, including FRET [8], fluorescence polarization and AlphaScreen® Assays.

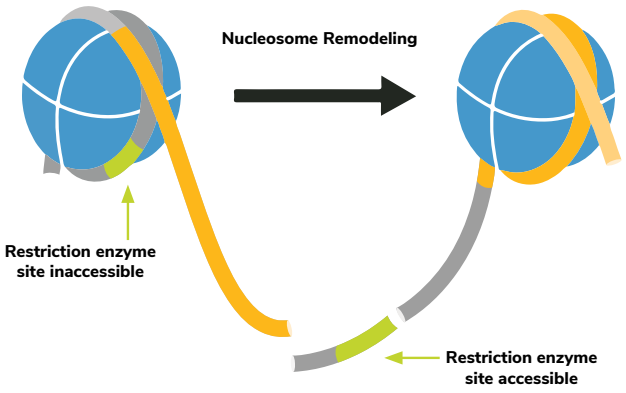

FIGURE 1: EPIDYNE® NUCLEOSOME REMODELING SUBSTRATES
EpiDyne Nucleosome Remodeling Substrates consist of a human histone octamer wrapped with a strong nucleosome positioning sequence (Widom 601) shielding a target motif (green box). The target motif is either a restriction enzyme recognition site or a DAM methylation sequence. The orange DNA sequence represents the 70 based pair acceptor region. Subsequent to the action of an ATP-dependent remodeler (e.g. Yeast RSC, or a human SWI/SNF ATPase such as SMARCA2/4), the target site is exposed, whereupon it can either be cut by a restriction enzyme or methylated by DAM. The latter is immediately compatible with HTS applications.

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Reagents and Materials Required for the Assay
Note: store protein aliquots @ -80°C, avoid freeze/thaw

- Yeast RSC Remodeling Complex [9] (substrate is also compatible with human SMARCA2/4 / BRM/BRG1 complexes; not shown)
- EpiDyne Remodeling Substrate (EpiCypher Catalog No. 16-4101)
- Positive Control naked DNA template, GATC1 (EpiCypher Catalog No. 18-4101)
- Negative control naked DNA template, GATC0 (EpiCypher Catalog No. 18-4100)
- Restriction enzyme (for cleavage at GATC, use DpnII (NEB Catalog No. R0543T); for cleavage at CAATTG, use MfeI (NEB Catalog No. R3589S)
- Assay Buffer (20 mM Tris pH 7.5, 50 mM KCl, 3 mM MgCl2 and 0.1 mg/ml BSA (Sigma Catalog No. A3059)
- Assay Quench Buffer (make fresh: 10 mM Tris pH 7.5, 40 mM EDTA, 0.6% SDS and 50 µg/ml Proteinase K (Worthington Bio-chem. Corp. Catalog No. PROKS).
- 8% Native Polyacrylamide Gel (37.5:1) assembled / resolved in 1XTBE
- Ethidium Bromide (EtBr, 10 mg/ml)
- Imaging device for EtBr-stained DNA gel

Standard Protocol

1. Add EpiDyne REA substrate, or control DNA (positive or nega-tive) to 0.5 ml Eppendorf tubes (recommended to run all three in parallel). Concentrations of EpiDyne will vary based on your specific ATPase. For RSC, use 20 nM nucleosome.
2. Add assay buffer to predetermined volume (typical reactions are 20 µL, equivalent to ~100 ng DNA)
3. Add Chromatin Remodeling Enzyme (e.g. RSC, 10 nM)
4. Add 50 units of desired restriction enzyme (e.g. DpnII)
5. Initiate reactions by addition of 2 mM ATP
6. Incubate reactions at 23-37°C (30°C for RSC) for the desired timepoints
7. Quench the reaction by adding an equal volume of Assay Quench Buffer and incubate at 50°C for 20 min
8. Resolve samples on non-denaturing acrylamide gel
9. Stain the gel with EtBr (0.2 µg/ml) in 1XTBE for 10 min
10. Destain the gel in 1XTBE and visualize on an appropriate imaging device

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Functional Validation using Restriction Enzyme Accessibility Assay

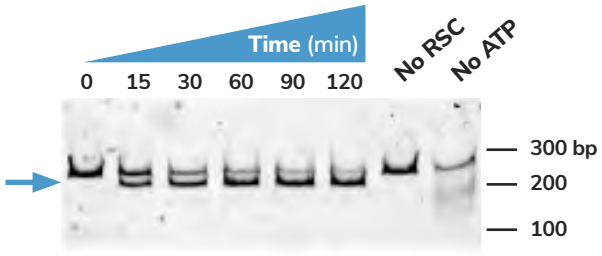

FIGURE 2: RSC ATP-DEPENDENT NUCLEOSOME REMODELING REACTION IN THE PRESENCE OF DpnII.
EpiDyne Nucleosome Remodeling Substrates (20 nM) were incubated for the indicated time with or without (No RSC) the ATP-dependent chromatin remodeler RSC (10 nM) in the presence of 2 mM ATP (or No ATP as indicated) and 50U of the restriction enzyme DpnII. Reactions were quenched and then resolved on an 8% polyacrylamide gel and stained with ethidium bromide. Red arrow indicates the mobility of the fragment cleaved from the EpiDyne nucleosomal DNA after remodeling.

References

1. *Hamiche A et al. (1999) ATP-dependent histone octamer sliding mediated by the chromatin remodeling complex NURF. Cell 97(7):833-42.*
2. *Vangamudi B et al. (2015) The SMARCA2/4 ATPase Domain Surpasses the Bromo-domain as a Drug Target in SWI/SNF-Mutant Cancers: Insights from cDNA Rescue and PFI-3 Inhibitor Studies. Cancer Res 75(18):3865-78.*
3. *Wu Q et al. (2016) Targeting the chromatin remodeling enzyme BRG1 increases the efficacy of chemotherapy drugs in breast cancer cells. Oncotarget 7(19):27158-75.*
4. *Helming KC, Wang X, Roberts CW. (2014) Vulnerabilities of mutant SWI/SNF complexes in cancer. Cancer Cell 26(3):309-17.*
5. *Karnezis AN et al. (2016) Dual loss of the SWI/SNF complex ATPases SMARCA4/BRG1 and SMARCA2/BRM is highly sensitive and specific for small cell carcinoma of the ovary, hypercalcaemic type. J Pathol 238(3):389-400.*
6. *Marinus MG, Morris NR. (1973) Isolation of deoxyribonucleic acid methylase mutants of Escherichia coli K-12. J Bacteriol 114(3):1143-50.*
7. *Geier GE, Modrich P. (1979) Recognition sequence of the dam methylase of Escher-ichia coli K12 and mode of cleavage of Dpn I endonuclease. J Biol Chem 254(4):1408-13.*
8. *Shahian T, Narlikar GJ. (2012) Analysis of changes in nucleosome conformation using fluorescence resonance energy transfer. Methods Mol Biol 833:337-49.*
9. *Clapier CR et al. (2016) Regulation of DNA Translocation Efficiency within the Chromatin Remodeler RSC/Sth1 Potentiates Nucleosome Sliding and Ejection. Mol Cell 62(3):453-61.*

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