Biochemical and genomic approaches for high throughput drug discovery in chromatin remodeling research

PDF poster download

***Tessa M. Firestone*[1]**, Lu Sun[1], Hannah Willis[1], Catherine E. Smith[1], Matthew R. Marunde[1], Vishnu U. Sunitha Kumary[1], Matthew J. Meiners[1], Saarang Gopinath[1], Jonathan M. Burg[1], Bryan J. Venters[1], Allison Hickman[1], Zu-Wen Sun[1], Martis W. Cowles[1], Pierre Esteve[2], Hang Gyeong Chin[2], Chaithanya Ponnaluri[2], Sriharsa Pradhan[2] & Michael-Christopher Keogh[1]

[1] EpiCypher Inc, Durham, NC 27709

[2] New England Biolabs, Ipswich, MA 01938

EpiDyne® remodeling assay and genomic approaches in remodeler research

Chromatin remodeling is mediated by ATP-dependent enzymes that play key roles regulating gene expression and genome replication / repair. Aberrant nucleosome organization from dysregulated chromatin remodeling can severely alter chromatin accessibility and disrupt these important processes, thereby driving various cancers. Remarkably, nearly 20% of all human cancers contain mutations in subunits from the SWI/SNF family of chromatin remodeling complexes, making them of great interest to basic research and therapeutic intervention[1,2].

In vitro studies on the remodeling enzymes (and their multi-subunit complexes) are challenging, partially due to the strong preference for nucleosome-based substrates (the physiological target of these enzymes). We have created the **EpiDyne®** nucleosome portfolio to examine chromatin remodeler activity in biochemical assays, and here present the development of novel readouts (-**PicoGreen™** and **-TR-FRET**). These nonradioactive plate-based assays are automation adaptable, ready for high-throughput inhibitor screening, and can be customized for various remodeling enzymes that exhibit preferences in nucleosome composition (*e.g.* histone type or DNA linker length).

For parallel *in vivo* studies we note that genome-wide remodeler localization and open chromatin mapping are fundamental for understanding the function / activity of these enzymes in cancer development and inhibitor response. However, traditional genomic approaches have significant issues: *e.g. ChIP-seq* is unable to effectively map ATPases without heavily modified high-noise protocols; while *ATAC-seq* to map open regions cannot deal with cross-linking that could stabilize transient states on interest. To this end, we have optimized the CUTANA™ **CUT&RUN** approach to efficiently capture the localization of all major classes of chromatin remodelers with high signal to background. We have also adopted NicE-seq for chromatin accessibility profiling in cross-linked material. As complementary tools to the EpiDyne platform, CUT&RUN and NicE-seq facilitate epigenomic research on chromatin remodelers in cancer therapeutic intervention.

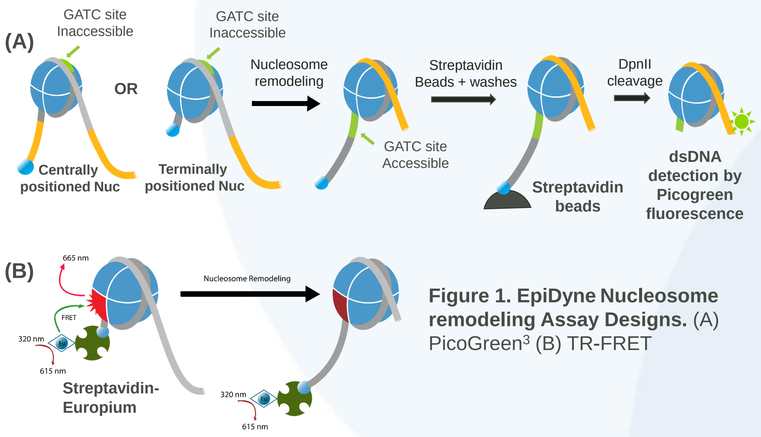
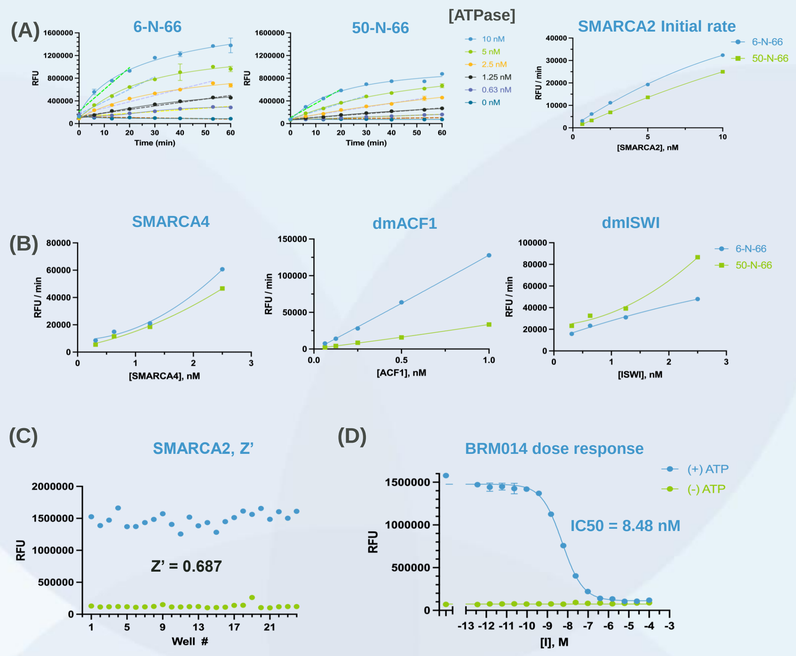


Figure 1. EpiDyne Nucleosome remodeling Assay Designs. (A) PicoGreen[3] (B) TR-FRET

EpiDyne-PicoGreen (HTS compatible) confirms remodeler substrate preference

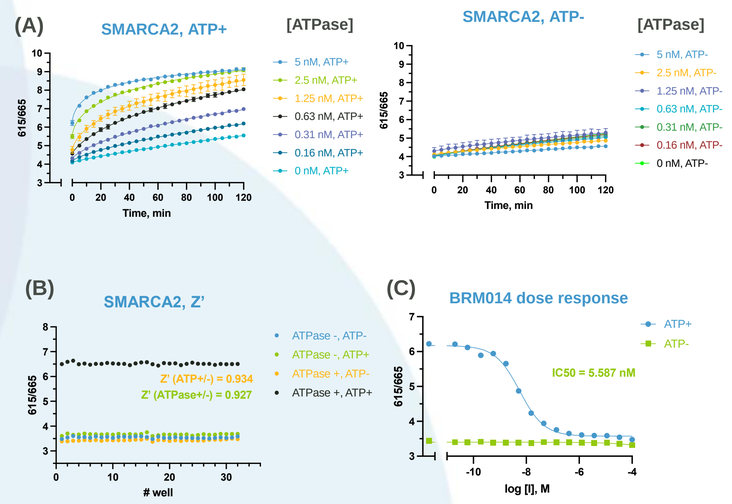


ATPase	EpiCypher cat#	[ATPase]	Z', N=24	IC50, [BRM014]
SMARCA4/BRG1	15-1014	1.25 nM	0.614	6.58 nM
SMARCA2/BRM	15-1015	2.5 nM	0.687	5.37 nM
dmACF1	15-1013	0.5 nM	0.761	N.D.
SMARCA5/Snf2h	15-1024	0.625 nM	0.772	N.D.

Table 1. HTS compatibility of remodeling enzymes (all human *vs*. *Drosophila* ACF1[4]) in EpiDyne-PicoGreen.

Figure 2. EpiDyne-PicoGreen remodeling assay. (A) Enzyme-, ATP- and time-dependent remodeling reactions by SMARCA2 (BRM). Initial rates were plotted for reactions within linear ranges. **(B)** Initial rates for terminally [6-N-66] or centrally [50-N-66] positioned nucleosomes by various ATPases. **(C)** Z' analysis and **(D)** tool compound[5,6] inhibition of SMARCA2/BRM remodeling with EpiDyne-PicoGreen.

EpiDyne-TR-FRET (HTS compatible) for real time remodeler studies



ATPase	EpiCypher cat#	[ATPase]	Z', N=24	IC50, [BRM014]
SMARCA4/BRG1	15-1014	1.25 nM	0.904	6.999 nM
SMARCA2/BRM	15-1015	2.5 nM	0.934	5.587 nM
SMARCA5/Snf2h	15-1024	0.625 nM	0.912	N/A

Table 2. HTS compatibility of various remodeling enzymes in EpiDyne-TR-FRET.

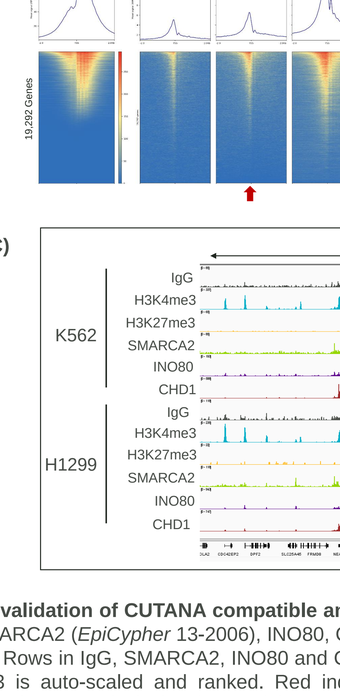
Figure 3. EpiDyne-TR-FRET remodeling assay. (A) Enzyme-, ATP- and time-dependent remodeling reactions by SMARCA2. **(B)** Z' analysis and **(C)** tool compound[5,6] inhibition of SMARCA2/BRM remodeling in EpiDyne-TR-FRET.

Acknowledgement

We are grateful to Dr. Dmitry Fyodorov (*Albert Einstein College of Medicine*) for the kind gift of dm ISWI[3]. This work was supported by multiple awards from the National Institutes of Health (R44GM116584, R44GM123869, R44DE029633, R44HG010640, and R44HG011006).

Functional epigenomic approaches in remodeler research

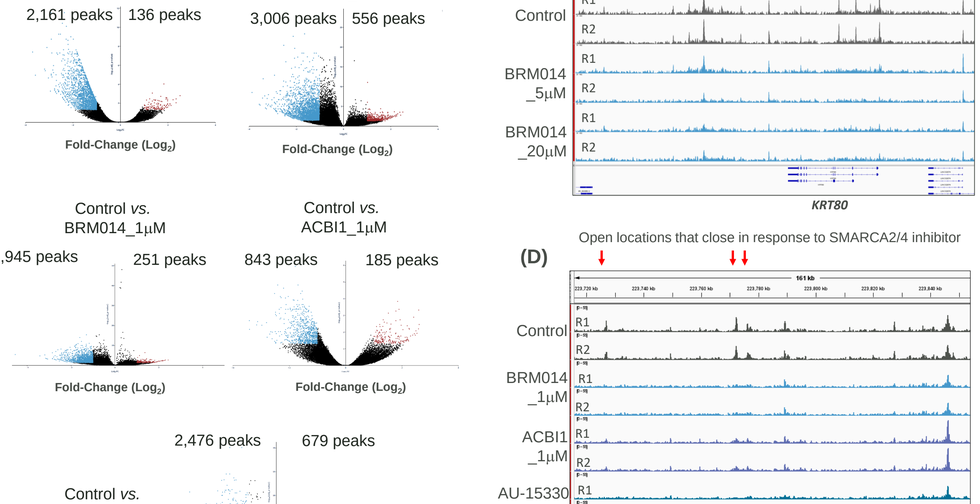
CUT&RUN Workflow



CUT&RUN *vs*. ChIP-seq

Platform Comparison	ChIP-seq	CUTANA™ CUT&RUN
Required Cells	>1 million	5,000-500,000
Ideal for Profiling	Histone PTMs, TFs	Histone PTMs, TFs & chromatin remodelers
Sequencing Depth (Reads)	>30 million	3-8 million
Experimental Throughput	Low	High
Signal-to-Noise	Low	High
Assay Automation	Difficult	Yes

One-pot Uni-NicE-seq vs. ATAC-seq



Platform Comparison	ATAC-seq	NicE-seq
Required Cells	Single-50k nuclei	25-50k nuclei
Sequencing Depth (Reads)	>50 million	30-35 million
Strengths & limitations	• Field standard • Sensitive	• Robust and sensitive • Low mitochondrial contamination (<5%) • **Works with fixed and FFPE tissue**

Remodeler mapping optimization by CUTANA CUT&RUN

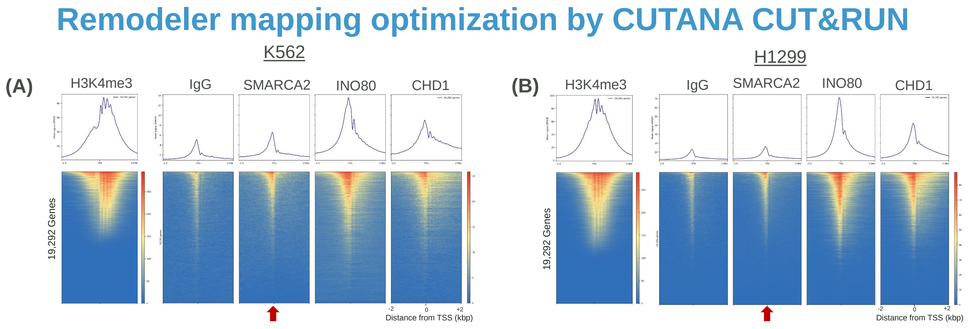


Figure 4. CUT&RUN validation of CUTANA compatible antibodies against remodelers. (A,B) Heatmaps for K562 & H1299 cells showing anti-SMARCA2 (*EpiCypher* 13-2006), INO80, CHD1 signal enrichment aligned at transcription start sites (TSS) of protein coding genes. Rows in IgG, SMARCA2, INO80 and CHD1 heatmaps are ranked and scaled by SMARCA2 intensity (top to bottom). H3K4me3 is auto-scaled and ranked. Red indicates high localized enrichment and blue denotes background signal. **(C)** Representative gene loci showing enrichment, peak structure, and overlap for SMARCA2, INO80, CHD1 with H3K4me3 and H3K27me3 peaks, consistent with their reported functions as families of chromatin remodeler complexes[7].

Open chromatin mapping as a remodeling readout by NicE-seq

Figure 5. NicE-seq[8,9] measures chromatin response to SMARCA2/4 remodeling inhibitor. Peaks were called from 100k formaldehyde-fixed NCI-H1299 cells (untreated / DMSO / + BRM014[5,6] for 24Hr) & 100K formaldehyde-fixed K562 cells (DMSO / +BRM014 / +AU-15330[10] / +ACBI1[11] for 24Hr) . **(A)** Volcano plots of differential peak occupancy between untreated and H1299 drug treated cells. **(B)** Representative biomarker (*KRT80*; locus functionality is reliant on SMARCA2/BRM function[6]) showing open chromatin changes in response to BRM014 treatment. **(C)** Volcano plots of differential peak occupancy between DMSO- and drug- treated K562 cells. **(D)** Gene loci showing open chromatin changes in response to compound treatment.

References

1. Hoffman *et al* (2014) Functional epigenetics approach identifies BRM/SMARCA2 as a critical synthetic lethal target in BRG1-deficient cancers. *PNAS* **111:**3128.
2. Hodges *et al* (2016) The Many Roles of BAF (mSWI/SNF) and PBAF Complexes in Cancer. *Cold Spring Harbor Perspect Med* **6:**a026930.
3. Andreyeva *et al* (2022) *Drosophila* SUMM4 complex couples insulator function and DNA replication control. *Elife* **11:**e81828.
4. Fyodorov DV & Kadonaga JT (2003) Chromatin assembly *in vitro* with purified recombinant ACF and NAP-1. *Methods Enzymol* **371:**499.
5. Papillon *et al* (2018) Discovery of Orally Active Inhibitors of Brahma Homolog (BRM)/SMARCA2 ATPase Activity for the Treatment of Brahma Related Gene 1 (BRG1)/SMARCA4-Mutant Cancers. *J Med Chem* **61:**10155.
6. Jagani *et al* (2019) In-Depth Characterization and Validation in BRG1-Mutant Lung Cancers Define Novel Catalytic Inhibitors of SWI/SNF Chromatin Remodeling. *bioRxiv* (10.1101/812628v1)
7. Wysocka J et al (2006) A PHD finger of NURF couples histone H3 lysine 4 trimethylation with chromatin remodelling. *Nature*. 2006 Jul 6;442(7098):86-90.
8. Chin *et al* (2020) Universal NicE-seq for high-resolution accessible chromatin profiling for formaldehyde-fixed and FFPE tissues. *Clin Epigenet* **12:** 143.
9. Vishnu *et al* (2021) One-pot universal NicE-seq: all enzymatic downstream processing of 4% formaldehyde crosslinked cells for chromatin accessibility genomics. *Epigenetics & Chromatin* **14:**53.
10. Xiao et al. (2021) Targeting SWI/SNF ATPases in enhanced-addicted prostate cancer. *Nature.* **601**, 434-439.
11. Farnaby *et al* (2019) BAF complex vulnerabilities in cancer demonstrated via structure-based PROTAC design. *Nat. Chem Biol,* **15,** 672-680.