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

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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.

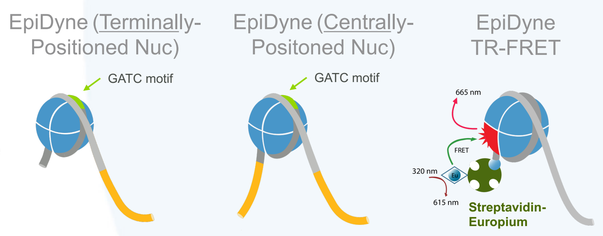
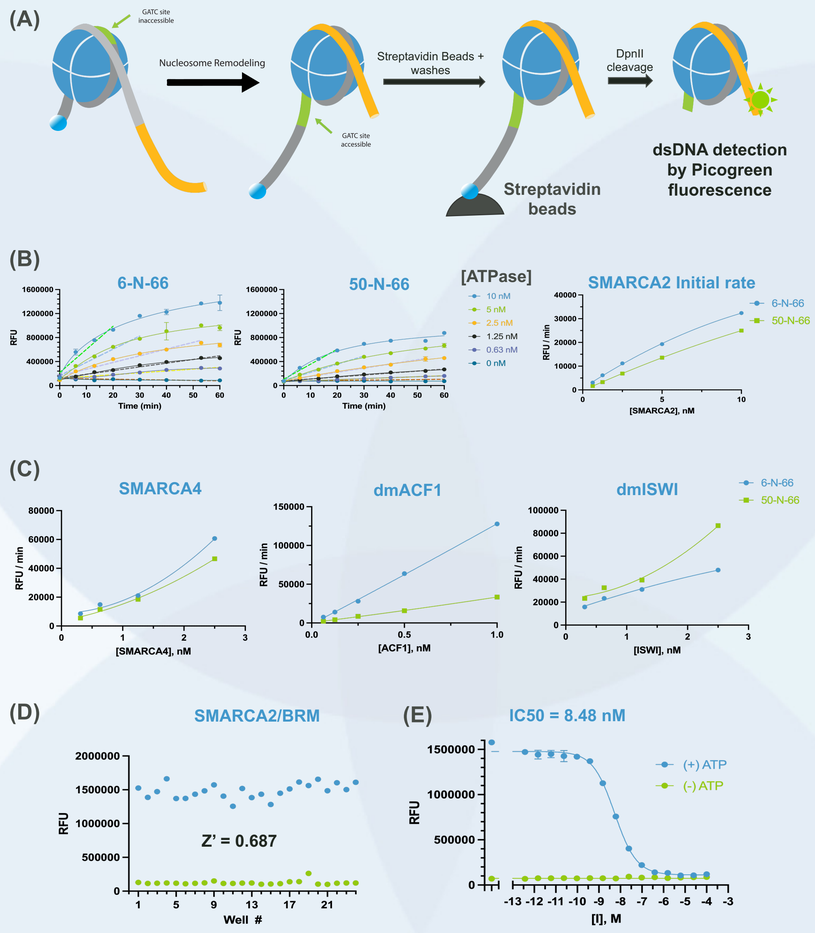


Figure 1. EpiDyne Nucleosome remodeling substrates

EpiDyne remodeling Assay comparisons

	PicoGreen	TR-FRET
Suitable Nucleosome	**terminal/central**	terminal
Assay readout	end point	**real time / end point**
Operation time	4-5 hr [2 hr hands on]	**~3 hr [0.5 hr hands on]**
S/B window	**5 ~ 20**	1.8 ~ 2.5
Z' with SMARCA2/4/5	> 0.7	> 0.8
Fluorescence interference	**Very low**	low
Assay format	384-well plate based	384-well plate based
Reagent cost / well	$2.88	**$1.61**

EpiDyne-picogreen (HTS compatible) confirms remodeler substrate preference

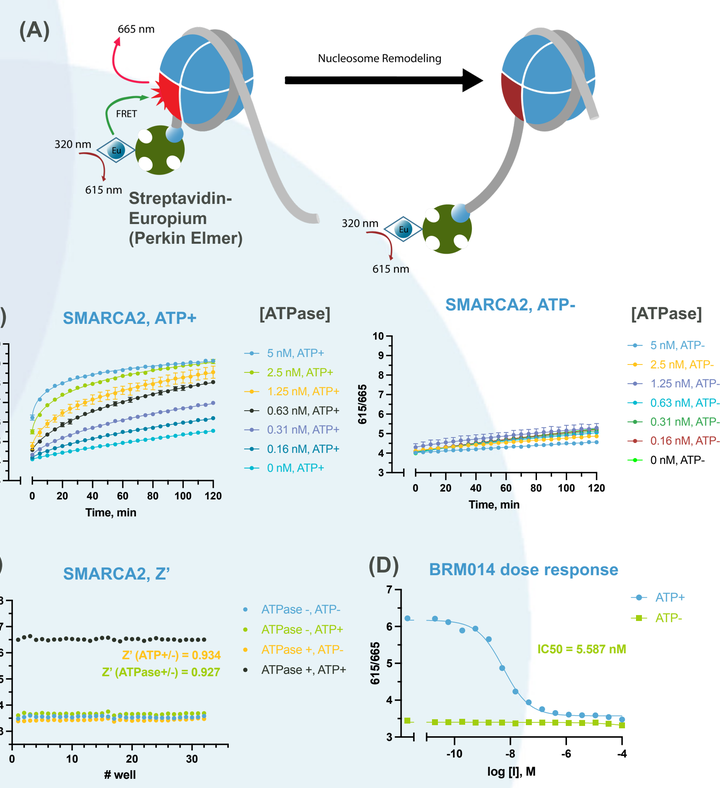


ATPase	EpiCypher cat#	[ATPase]	Z', N=24	IC50, [BRM014]
SMARCA4/BRG1	15-1014	1.25 nM	0.614	6.58 nM
SMARCA2/BRM	15-1015	2.5 nM	0.687	5.37 nM
dmACF1	15-1013	0.5 nM	0.761	N.D.
SMARCA5/Snf2h	15-1024	0.625 nM	0.772	N.D.

Table 2. HTS compatibility of remodeling enzymes (all human *vs.* *Drosophila* ACF1[3]) in EpiDyne-PicoGreen.

Figure 2. EpiDyne-PicoGreen remodeling assay. (A) Assay design[4]. **(B)** Enzyme-, ATP- and time- dependent remodeling reactions by SMARCA2 (BRM). Initial rates were plotted for reactions within linear ranges. **(C)** Initial rates for terminally [6-N-66] or centrally [50-N-66] positioned nucleosomes by various ATPases. **(E)** Z' analysis and 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 3. HTS compatibility of various remodeling enzymes in EpiDyne-TR-FRET.

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

Acknowledgement

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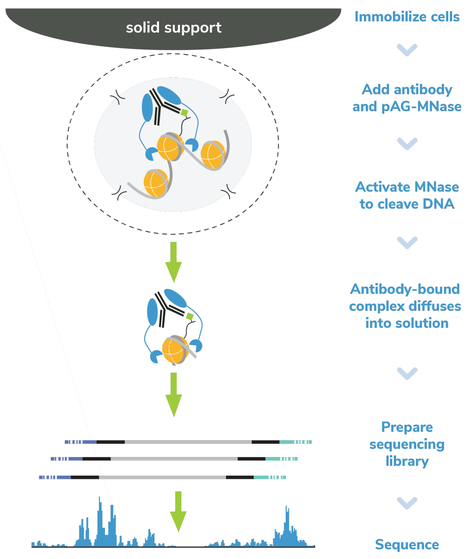
Functional epigenomic approaches in remodeler research

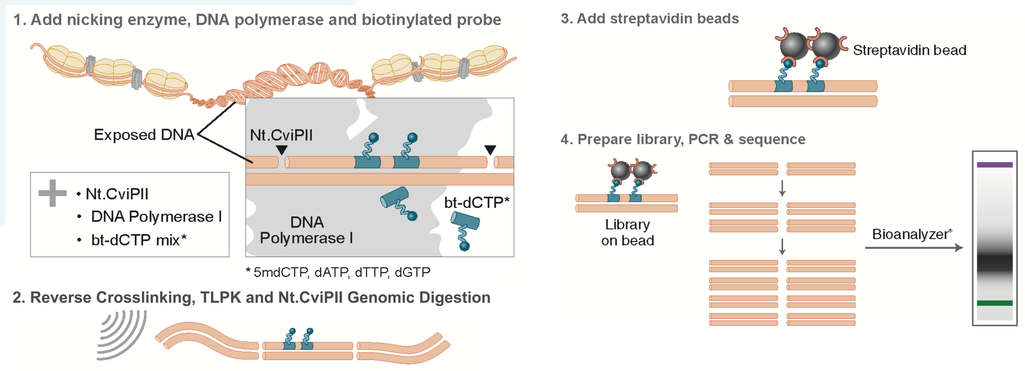
CUT&RUN Workflow



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

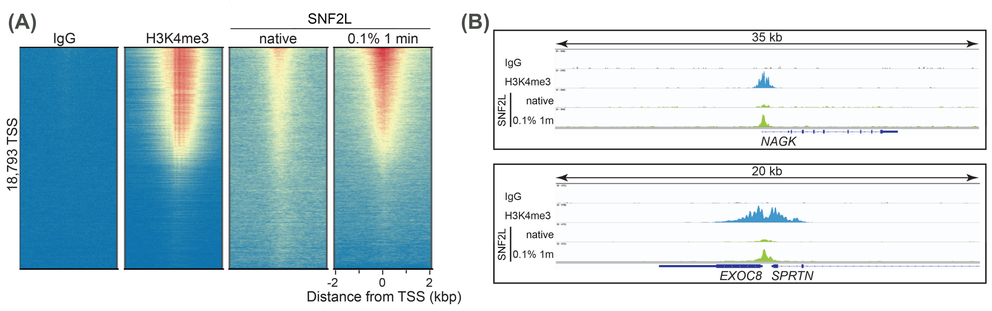


Figure 4. CUT&RUN validation of CUTANA compatible antibodies against remodelers. (A) Heatmaps show that anti-SNF2L (*EpiCypher* 13-2005) CUT&RUN signal is improved by light crosslinking at transcription start sites. Rows are aligned and ranked by intensity (top to bottom) and colored such that red indicates high localized enrichment and blue denotes background signal. **(B)** Two gene loci show overlap of SNF2L and H3K4me3 peaks, consistent with its reported function as a member of the NURF ISWI chromatin remodeler complex[7].

Open chromatin mapping as a remodeling readout by NicE-seq

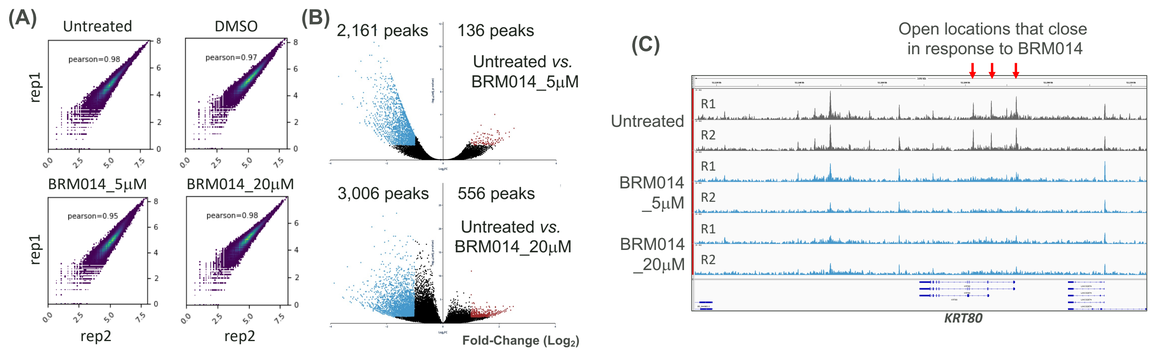


Figure 5. NicE-seq[8,9] measures chromatin response to SMARCA2/4 remodeling inhibitor. (A) Pearson Correlation analyses with biological replicates (rep1-rep2). Peaks were called from 100k formaldehyde-fixed NCI-H1299 cells (untreated / DMSO / + BRM014[5,6]). **(B)** Volcano plots of differential peak occupancy between untreated and drug treated cells. **(C)** Representative biomarker (*KRT80*; locus functionality is reliant on SMARCA2 (BRM) function[6]) showing open chromatin changes in response to BRM014 treatment.

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