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 such as SMARCA2/4/5 (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 responses. However, traditional genomic approaches have significant issues: *e.g. ChIP-seq* demands high cell numbers and sequencing depths, and is unable to effectively map ATPases without heavily modified protocols; while *ATAC-seq* to map open regions deals poorly with cross-linking that could stabilize transient states of interest. To these ends, we optimized the CUTANA™ CUT&RUN approach to efficiently capture the localization of all major classes of chromatin remodelers with high signal to background in multiple tissue cultures, enabling evaluation of specificity and/or efficiency from remodeler targeting inhibitors or degraders. We have also adopted NicE-seq for chromatin accessibility profiling in cross-linked material that captures landscape changes in response to remodeling disruptions. As complementary tools to the EpiDyne platform, CUT&RUN and NicE-seq facilitate epigenomic research on chromatin remodelers in cancer therapeutic intervention.

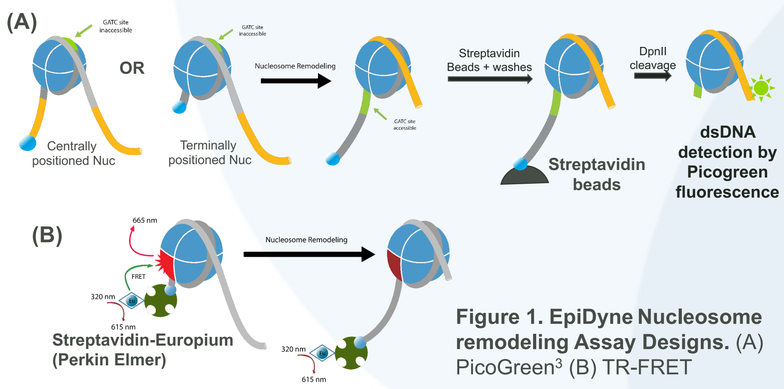


Figure 1. EpiDyne Nucleosome remodeling Assay Designs. (A) PicoGreen[3] (B) TR-FRET

EpiDyne-picogreen (HTS compatible) reveals remodeler substrate preference

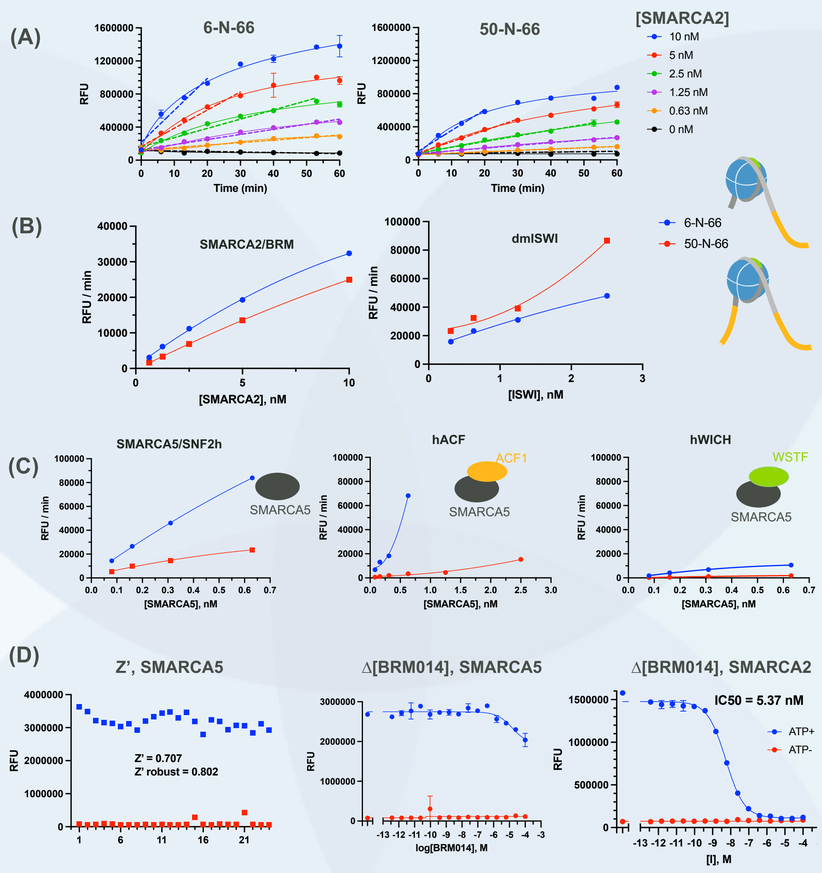


Figure 2. EpiDyne-PicoGreen remodeling assay. (A) Enzyme-, ATP- and time-dependent remodeling reactions by SMARCA2 (BRM). **(B)** Initial rates for terminally [6-N-66] or centrally [50-N-66] positioned nucleosomes by various ATPases[4], plotted for reactions within linear ranges. **(C)** Substrate preference for various SMARCA5 remodeling complexes in EpiDyne-picogreen. **(D)** Z' analysis and tool compound[5,6] dose response for SMARCA5/SNF2H compared to SMARCA2/BRM remodeling with EpiDyne-PicoGreen.

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

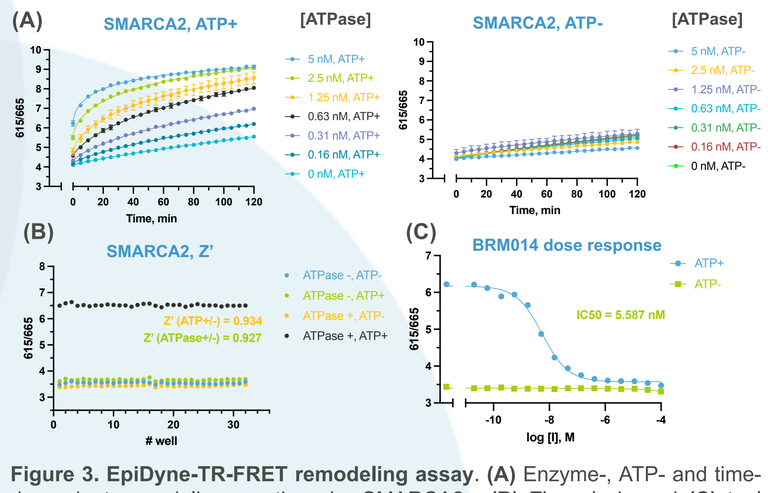


Figure 3. EpiDyne-TR-FRET remodeling assay. **(A)** Enzyme-, ATP- and time-dependent remodeling reactions by SMARCA2. **(B)** Z' analysis and **(C)** tool compound[5,6] inhibition of SMARCA2/BRM remodeling in EpiDyne-TR-FRET.

	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**	1.5 ~ 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

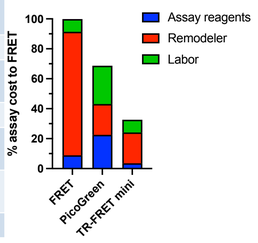


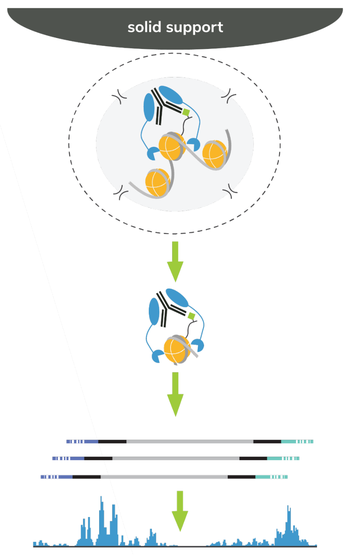
Figure 4. Performance comparisons of EpiDyne remodeling assays.

Acknowledgement

We are grateful to Dr. Dmitry Fyodorov (*Albert Einstein College of Medicine*) for the kind gift of dm ISWI[4] and human SMARCA5 complexes. 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

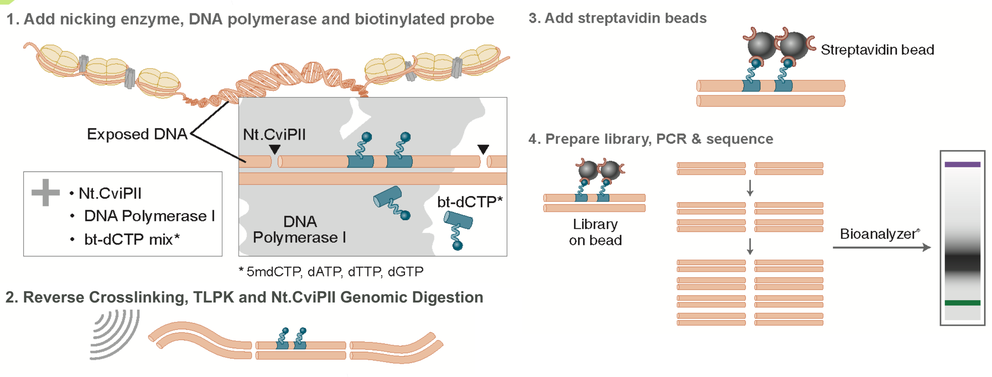
CUT&RUN Workflow



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**

Distinct remodeler mapping responses to SMARCA2/4 inhibitors in H1299 cells

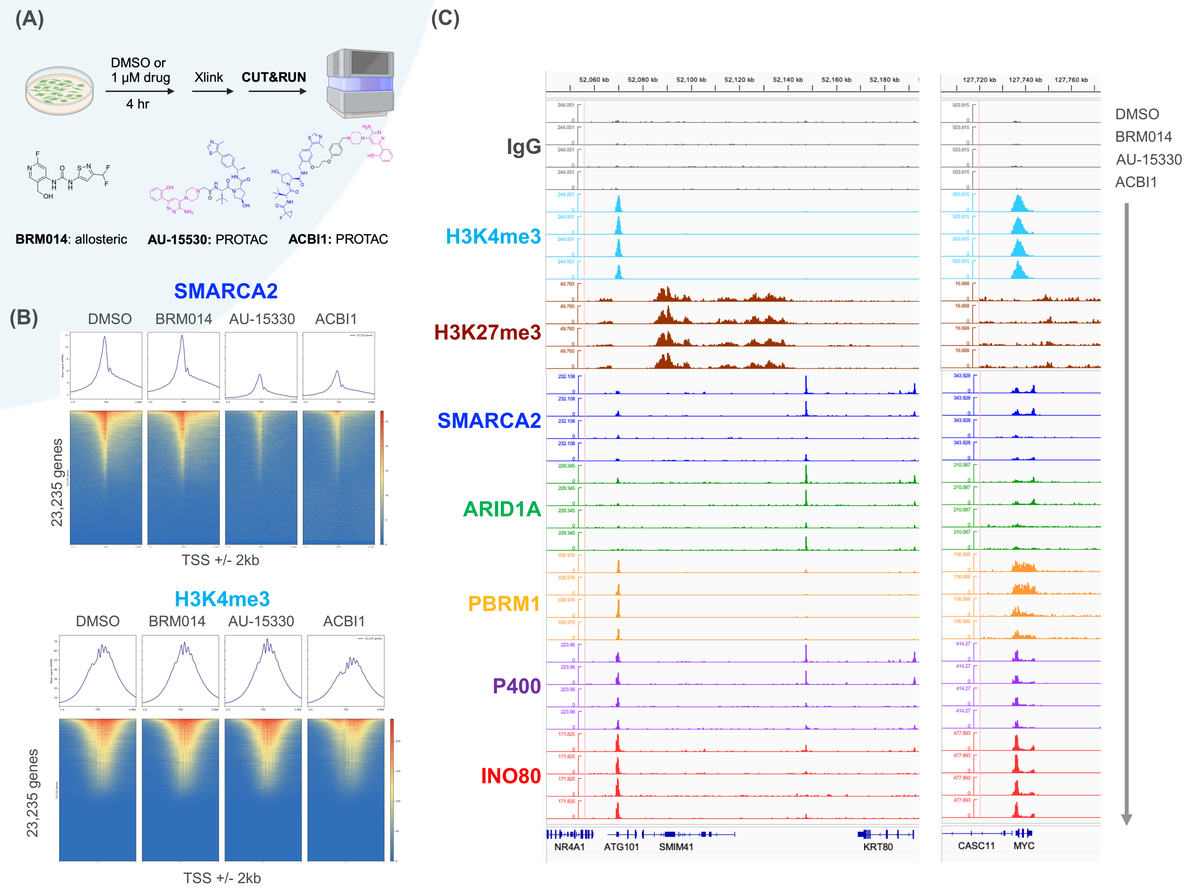


Figure 4. CUT&RUN validation of CUTANA compatible antibodies against remodelers in drug treatments. (A) Experimental workflow. Proliferating H1299 cells were treated with 1 μM of SMARCA2/4 targeting BRM014[5,6], AU-15330[9] , ACBI1[10], or 0.1% DMSO vehicle control for 4 hours before light crosslinked and harvested for CUT&RUN mapping. **(B)** Heatmaps showing SMARCA2 and H3K4me3 enrichment at TSS and their responses to variant drug treatments. Rows are aligned, ranked and group scaled by intensity (top to bottom) in respective DMSO controls and colored such that red indicates high localized enrichment and blue denotes background signal. **(C)** Representative chromatin regions showing enrichment of core or accessory remodeler proteins in response to DMSO, various allosteric or PROTAC based drugs, group scaled by individual targets.

SMARCA2/4 disruption alters chromatin accessibility

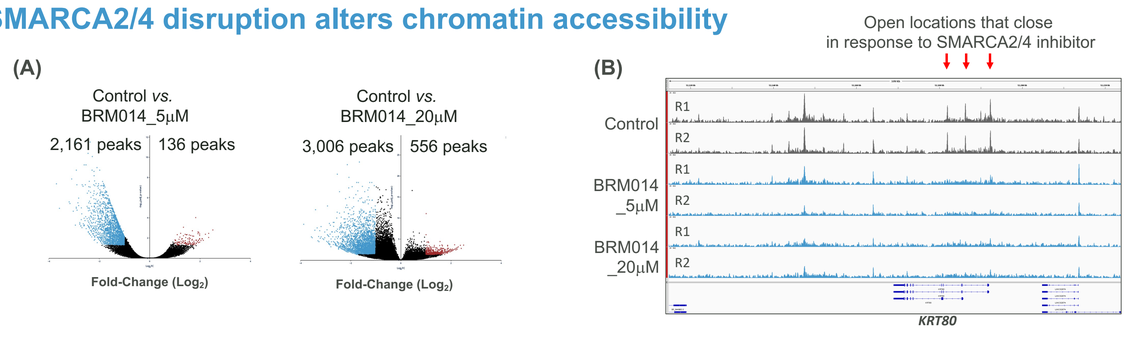


Figure 5. NicE-seq[8,9] measures chromatin response to SMARCA2/4 remodeling inhibitor. Peaks were called from 100k formaldehyde-fixed NCI-H1299 cells (untreated / DMSO / + BRM014[5,6]). **(A)** Volcano plots of differential peak occupancy between untreated and H1299 drug treated cells. **(B)** Representative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

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