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

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***Lu Sun***[1], Tessa Firestone[1], Hannah Willis[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.

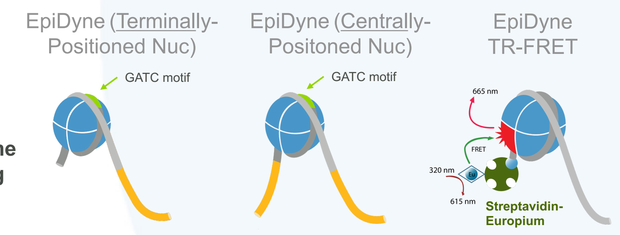
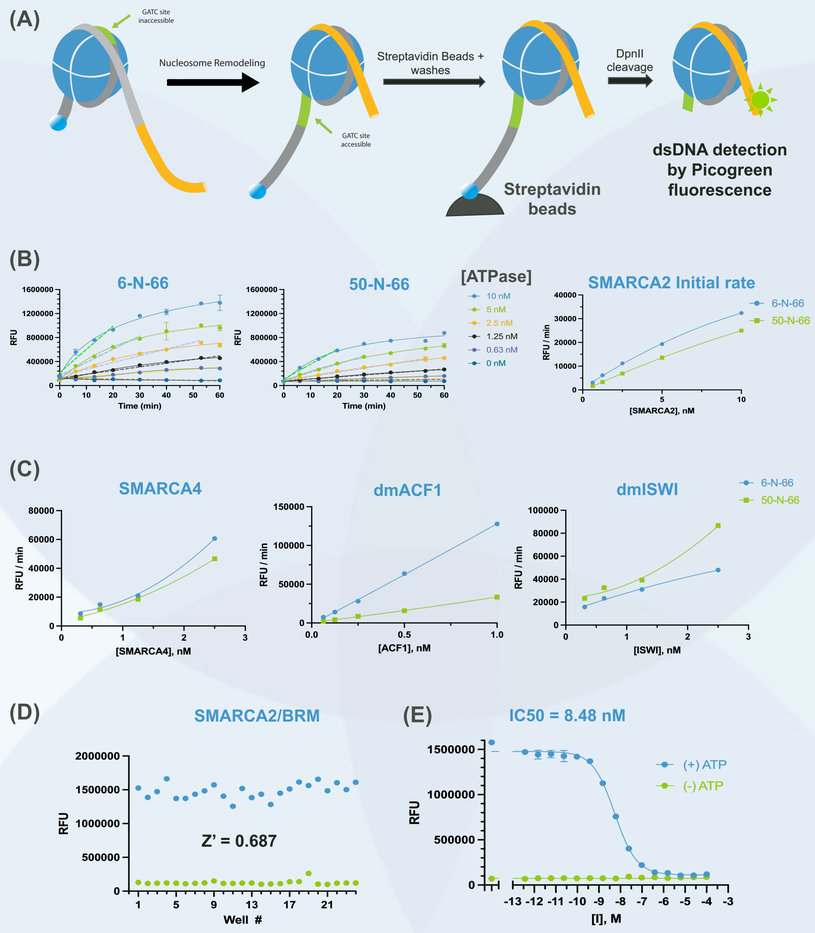



**Figure 1. EpiDyne Nucleosome remodeling substrates**

## 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

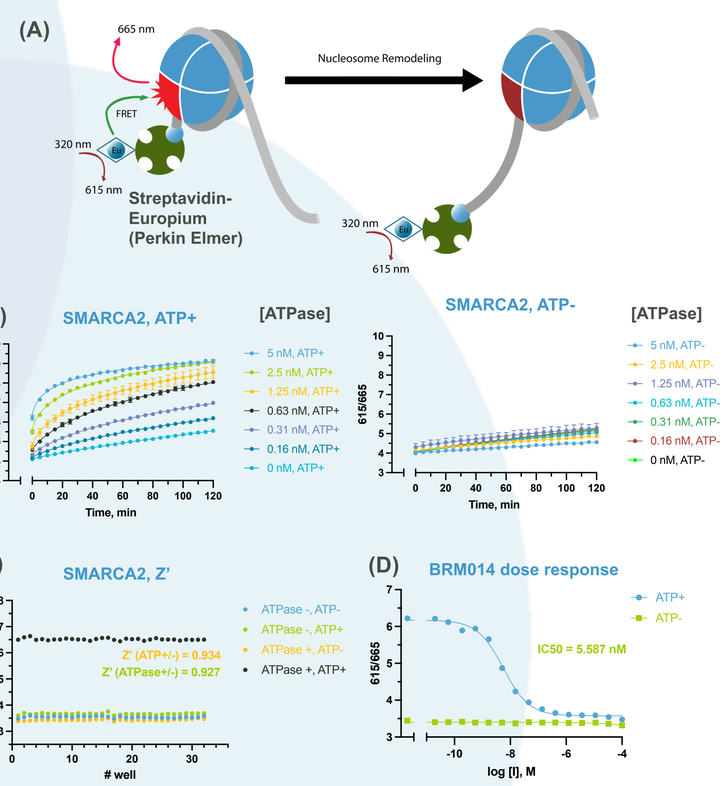



| ATPase | EpiCypher cat# | [ATPase] | Z', N=24 | IC50, [BRM014] |
|---|---|---|---|---|
| SMARCA4/BRG1 | 15-1014 | 1.25 nM | 0.614 | 6.58 nM |
| SMARCA2/BRM | 15-1015 | 2.5 nM | 0.687 | 5.37 nM |
| dmACF1 | 15-1013 | 0.5 nM | 0.761 | N.D. |
| SMARCA5/Snf2h | 15-1024 | 0.625 nM | 0.772 | N.D. |

**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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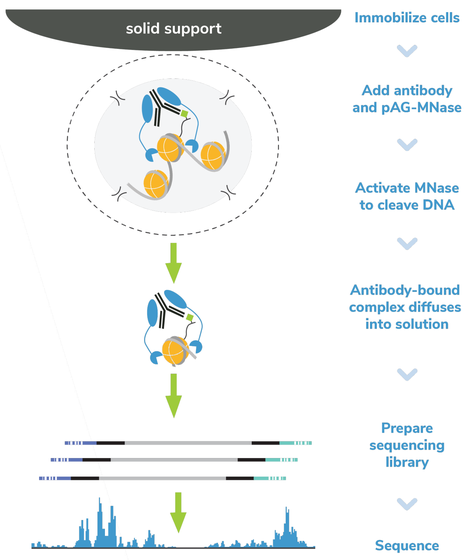
# Functional epigenomic approaches in remodeler research

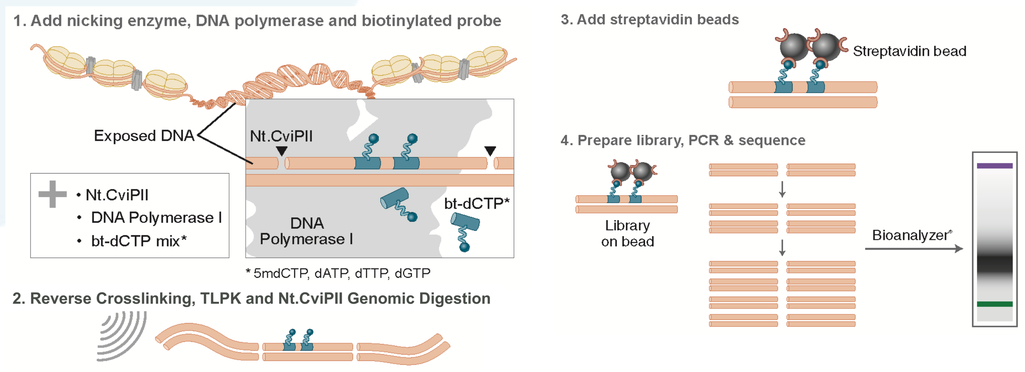
### CUT&RUN Workflow




### 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<br>• Sensitive | • Robust and sensitive<br>• Low mitochondrial contamination (<5%)<br>• **Works with fixed and FFPE tissue** |

## Remodeler mapping optimization by CUTANA CUT&RUN

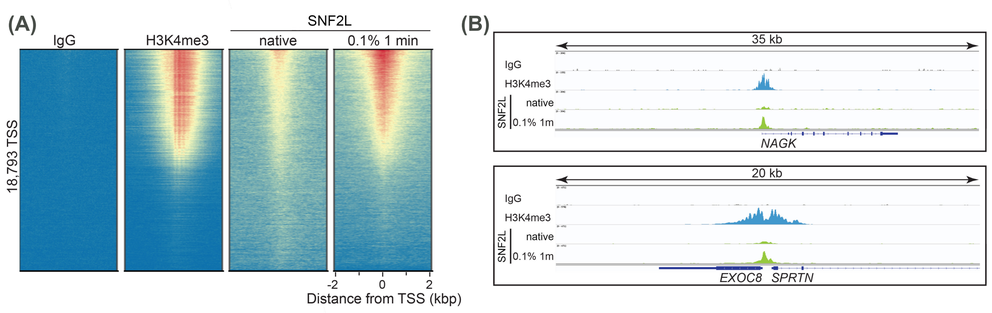



**Figure 4. CUT&RUN validation of CUTANA compatible antibodies against remodelers. (A)** Heatmaps show that anti-SNF2L (*EpiCypher* 13-2005) CUT&RUN signal is improved by light crosslinking at transcription start sites. Rows are aligned and ranked by intensity (top to bottom) and colored such that red 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

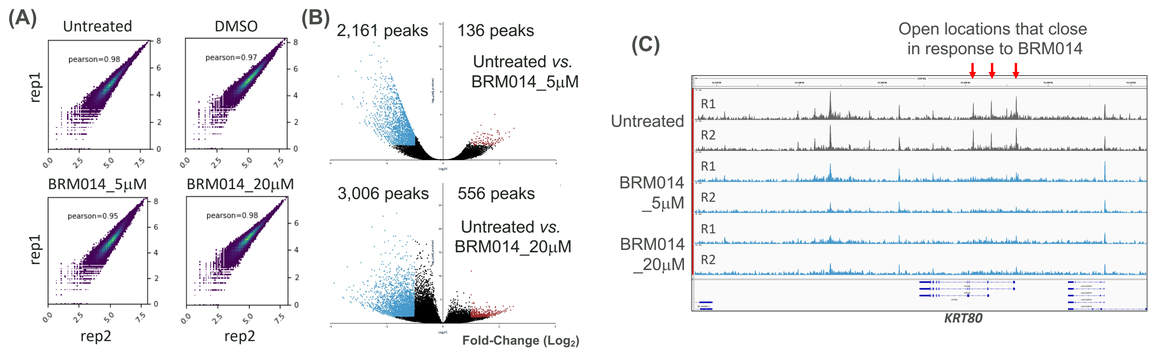



**Figure 5. NicE-seq[8,9] measures chromatin response to SMARCA2/4 remodeling inhibitor. (A)** Pearson Correlation analyses with biological replicates (rep1-rep2). Peaks were called from 100k formaldehyde-fixed NCI-H1299 cells (untreated / DMSO / + BRM014[5,6]). **(B)** Volcano plots of differential 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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