dCypher™: A High-Throughput Service for Chromatin Interaction Discovery

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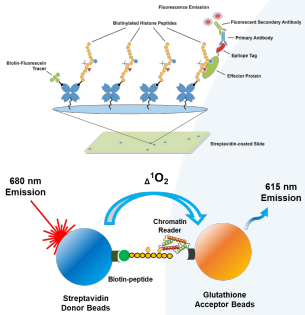
EpiCypher™ Bringing Epigenetics to Life

Background

Histone post-translational modifications (PTMs) maintain diverse cellular functions, including transcriptional activation / repression, DNA damage repair and mitotic chromosome transmission. Many epigenetic regulators recognize the specific PTM state of chromatin through evolutionarily conserved histone-binding domains ('readers'), thereby recruiting nuclear complexes ('effectors') to specific genomic loci. While significant advances have been made in understanding how many reader domains distinguish and bind specific histone PTMs, the majority of putative binding motifs and their substrates remain uncharacterized. In response to this need, EpiCypher® has developed dCypher™, a high-throughput discovery service for the rapid interrogation of effector protein, antibody, and enzyme interactions. This platform is based on a no-wash bead-based proximity assay (AlphaScreen®) and employs a comprehensive library of singly- or combinatorially-modified histone peptides (~300) encompassing ~100 unique PTMs (*e.g.* lysine methylation / acylation, arginine methylation, and serine / threonine phosphorylation) on the four core histones and several histone variant. Major advantages of dCypher over the current-gold-standard histone peptide arrays include: **(1)** much less starting material (>100-fold); **(2)** much higher sensitivity (>10-fold); and **(3)** faster throughput (> 80% time saving / screen). We are currently expanding this service platform to compatibility with our recombinant designer nucleosomes (dNucs) and combinatorial versaNucs™, to provide a powerful and physiological high-throughput capability for chromatin biology research and drug discovery.

Platform Comparison - EpiTriton™ vs. dCypher™

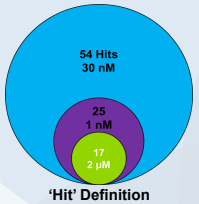


dCypher combines Peptide Array and Alpha Technology

- 288 modified (single → combinatorial) biotinylated histone peptides
- Peptides individually synthesized / purified / validated
- PTMs : Kmethyl (me1-2-3); Rmethyl (1-2a-2s); Kacyl (acetyl, butyryl, crotonyl, propionyl); S/T/Y-phos
- Robust
- Homogenous (no wash) format
  - Highly sensitive
  - Rapid (~ 2 hours)

BRD4 BD-1

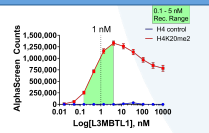
2 µM – Peptide Array
30 nM, 1 nM – dCypher



'Hit' Definition
EpiTriton: ' > *background* '
dCypher: ≥ 10% max signal

dCypher + Kmethyl readers - Approach: pre-test to candidate PTMs

L3MBTL1 3xMBT



90 hits

	H2A	H2B	H3	H4
dCypher 0.4 nM	0/20	2/9	65/199	23/60
Peptide Array 2 µM	0/15	2/4	16/129	8/43

L3MBTL1: pan Kme1 + Kme2 reader

Kd (µM)	unmodified	monomethyl	dimethyl	Trimethyl
H4K20	NB	138±6	211±6	NB
H1bK26	NB	136±20	134±9	NB
H3K4		85±0.2	192±16	
H3K9		412±17	303±35	
H3K27		462±51	463±19	

NB: No detectable binding

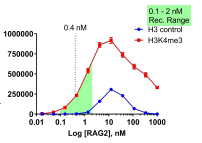
Min et al (2007) NSMB

dCypher detected L3MBTL1 binding to peptides containing Kme1 & Kme2

Contained	dCypher	Array
H4K20me1/2	4/4	2/3
H3K4me1/2	16/16	9/14
H3K9me1/2	19/19	9/16
H3K27me1/2	5/6	1/4

Missing: + S28p

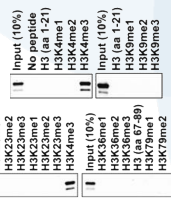
RAG2 PHD



36 hits

	H2A	H2B	H3	H4
dCypher 0.4 nM	0/20	0/9	36/199	0/60
Peptide Array 2 µM	0/21	0/11	25/160	0/55

RAG2: H3K4me3 reader

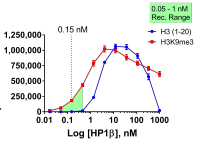


Peptide pulldown data from: Matthews et al (2007) Nature

dCypher detected RAG2 binding specifically to peptides containing H3K4 me3 / -me2/ -me1

Contained	Bound/ Present	Missing peptides contained
H3K4me3	27/34	T3p(4), T6p(2), highly acetylated (1)
H3K4me2	7/10	T6p(2), highly acetylated (1)
H3K4me1	2/6	Combinatorial peptides

HP1β CHD

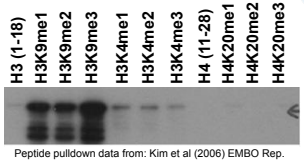


20 hits

	H2A	H2B	H3	H4
dCypher 0.15 nM	0/20	0/9	20/199	0/60
Peptide Array 2 µM	0/10	0/4	16/98	0/15

HP1β: H3K9me3 / -me2/ -me1 reader

Modified peptides

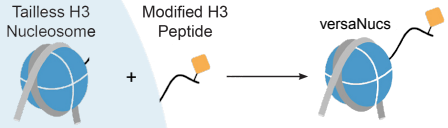


Peptide pulldown data from: Kim et al (2006) EMBO Rep.

dCypher detected HP1β binding specifically to peptides containing H3K9me3 / -me2/ -me1

Contained	Bound/ Present	Missing peptides contained
H3K9me3	8/12	T6p, S10p, highly acetylated
H3K9me2	9/14	S10p, highly acetylated, [K4A, K4me3]
H3K9me1	3/5	S10p, highly acetylated

versaNucs™ - Increase access to novel and diverse screening sets



Nucleosome Customization

- DNA template (e.g.)
  - 147x601 (± biotin)
  - 187x601 (± biotin)
  - Fluor DNA (e.g. EpiDyne-FRET)
  - Custom template (single NPS or arrays)
- Histone (e.g.)
  - NΔ32 H3.1, H3.2, Heterotypic H3s (R&D)
  - H2A-Cy5 (e.g. EpiDyne-FRET)
  - Histone variants (e.g. H2A.Z)
  - PTMs on H2A, H2B or H4

Personalization of H3-tail (residues 1 – 23)

- N-terminal tagging (fluorophore, biotin, etc.)
- PTMs (methylation, acylation, phosphorylation, etc.)
- Gives access to recently identified or combinatorial PTMs on histone H3

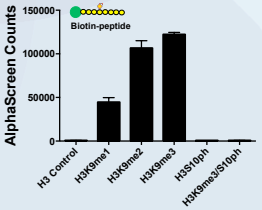
Versatility imparted by modular treatment of NCP precursor(s)

- Create appropriate 'H3 tail-less' as custom ligation substrate(s)
- Ligate peptide(s) individually (or in parallel) → purify → deliver

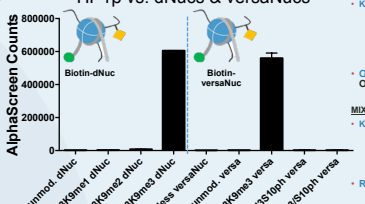
- versaNucs give access to novel PTMs on H3 (residues 1 – 23); combinatorial PTMs on H3 (*in cis*); or combinatorial nucleosome libraries (*in cis* / *in trans*)
- versaNucs are rapidly generated and purified, can fulfill needs for small-scale screening efforts, and pass EpiCypher's rigorous QC metrics
- versaNucs increase available substrate diversity (DNA modifications, histone subtype, etc.)
- A focused versaNuc panel, including combinatorial and unique modifications, was rapidly created to probe HP1β CHD specificity for use on the dCypher platform

dCypher + dNucs + versaNucs™ - Deeper investigation of HP1β CHD specificity

HP1β vs. Modified peptides



HP1β vs. dNucs & versaNucs



EpiCypher's dNuc catalog includes…

AVAILABLE NOW

- **K-MetStat: Lysine Methylation**
  - Mono-, di- & tri-methylation
  - 15 uniquely modified nucleosomes
  - Used in our recent Mol Cell publication (see Shah *et al*)
- **OncoStat: Histone H3.3 Oncogenic mutations**
  - 7 unique mutants

MIXED AVAILABILITY

- **K-AcylStat: Lysine Acylation**
  - Acetylation, butyrylation, & crotonylation
  - Single & combinatorial modifications (tetra-acetyl, proximal phosphorylation)
  - 24 uniquely modified nucleosomes
- **R-MetStat: Arginine Methylation**
  - Mono, symmetric, & asymmetric methylation
  - 14 uniquely modified nucleosomes
- **K-UbStat: Lysine Ubiquitination**
  - H2A & H2B ubiquitylated nucleosomes

- dCypher is able to recapitulate the HP1β CHD specificity profile on modified peptide substrates
- The platform is also directly compatible with EpiCypher's expanding dNuc portfolio, allowing nucleosomal investigation
- HP1β CHD specificity is strikingly altered when presented with nucleosomal substrates, challenging the current paradigm
- Nucleosome screening set was expanded to include combinatorial modifications via versaNuc product line
- HP1β CHD H3K9me3 binding activity is negatively affected by H3S10ph and recapitulates specificity noted on peptide substrates

Conclusions

1. dCypher provides a simple yet powerful high-throughput platform for chromatin biology research and drug discovery
2. dCypher is remarkably more sensitive and specific vs. histone peptide arrays
3. Assays are less time consuming (> 80% reduction vs. peptide arrays) and allow for quick follow up studies
4. Assays require dramatically less starting material (>100-fold reduction [concentration]) vs. histone peptide arrays
5. Chromatin binding proteins can be comprehensively and rapidly characterized with dNucs and versaNucs on platform
6. HP1β CHD binding capability is drastically restricted when presented with a nucleosomal substrate

References

1. Kim *et al* (2006) Tudor, MBT and chromo domains gauge the degree of lysine methylation. *EMBO Rep* **7**:397.
2. Matthews *et al* (2007) RAG2 PHD finger couples histone H3 lysine 4 trimethylation with V(D)J recombination. *Nature* **450**:1106.
3. Min *et al* (2007) L3MBTL1 recognition of mono- and dimethylated histones. *Nat Struct Mol Biol* **14**:1229.
4. Fischle *et al* (2005) Regulation of HP1–chromatin binding by histone H3 methylation and phosphorylation. *Nature* **438**.1116.
5. Shah *et al* (2018) Examining the Roles of H3K4 Methylation States with Systematically Characterized Antibodies. *Mol Cell* In Press.