EpiCypher®

Bringing Epigenetics to Life

dCypher™

Products & services to discover novel chromatin binding interactions

dCypher™ A service to discover novel chromatin binding interactions

dCypher™ is a new epigenetics discovery service for the high-throughput identifi cation of reader domain binding interactions.

- High sensitivity relative to peptide arrays
- Nucleosome and peptide based formats

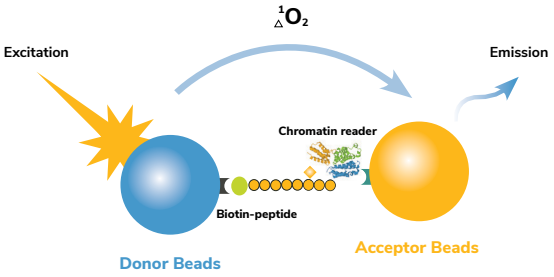
Applications

- Discover new binding domain interactions
- Binding domain inhibitor development
- Determine how modifications impact enzyme activity
- See how dCypher has been used to uncover novel epigenetic signaling pathways
  Weinberg et *al.*, *Nature* 573, 281–286 (2019)

FIGURE 1

Schematic representation of the dCypher platform, available using either peptides or nucleosomes as the interaction substrates. Based on AlphaScreen, Streptavidin Donor Beads capture biotinylated nucleosomes or peptides. An epitope-tagged chromatin reader domain of interest is bound to Acceptor Beads. Interaction of the reader domain with the substrate brings the Acceptor and Donor Beads within proximity. Excitation of the Donor Beads generates a singlet oxygen molecule that triggers emission of light from nearby Acceptor Beads.

dCypher - Peptide



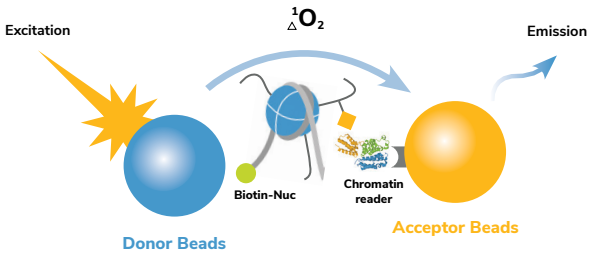
Advantages

- Screen > 300 unique peptides
- Examine combinatorial modifications
- Ideal for interaction discovery screening

With **dCypher - Peptide**, you can interrogate hundreds of single and combinatorial modifications in a single experiment.

dCypher - Peptide is ideal for discovering novel interactions and for examining the role of combinatorial modifications, and is a good starting point for situations where the binding substrate is not known.

dCypher - Nucleosome



Advantages

- Physiologically relevant substrates
- Screen > 80 modified nucleosomes
- Ideal for determining biological relevance

With **dCypher - Nucleosome**, you will examine interactions with modifications within the context of a nucleosome - over 80 different nucleosomes are available to screen.

dCypher - Nucleosome can help identify biologically relevent interactions of proteins with modified nucleosomes. It is useful in determining nucleosome-specific binding modes and serves as an obvious follow-up to peptide interaction studies.

dCypher™ A service to discover novel chromatin binding interactions

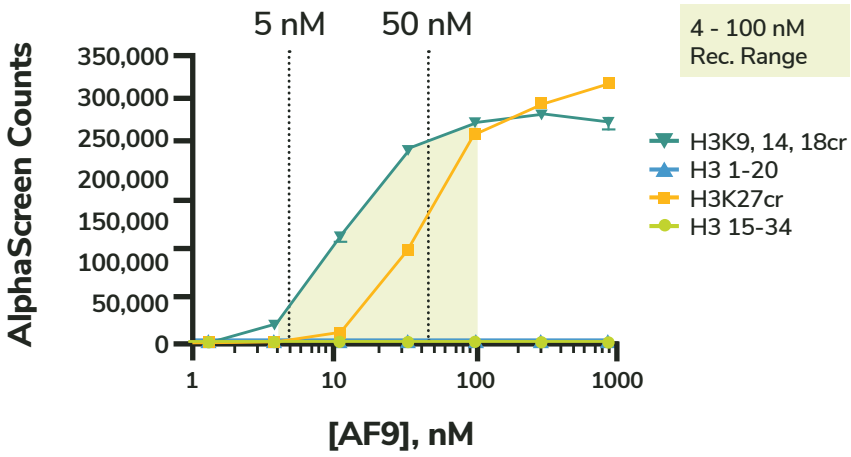
dCypher - Peptide Screening service uses nearly 300 single and combinatorially modified histone peptides to further "dCypher" the Histone Code

- Highly sensitive method - orders of magnitude better than peptide arrays
- Lower assay background leads to remarkable S/N ratios
- Identify 'hits' that would be missed using peptide arrays
- Facilitate "Orphan" candidate domain testing - no known PTM interaction
- Much lower quantities of protein required per assay

dCypher can be used to identify true hits missed by peptide arrays

FIGURE 2

dCypher chromatin reader titration using 6HIS AF9 YEATS (Catalog No. 15-0071). AF9 was titrated with biotinylated histone peptides containing acyl modifications or the related unmodified controls. Two concentrations were selected for a full dCypher-peptide screen. At 5 nM AF9, very high affinity interactions were identified (stringent screening conditions). At 50 nM AF9, a greater number of hits were observed due to capturing lower affinity interactions.



- AF9 YEATS interacts with many acyl modifications on histone H3*
- dCypher screen reveals more AF9-H3 interactions than peptide array (see Table 1)
- AF9 interactions identified by dCypher (but missed by peptide arrays) have been confirmed in the literature*

TABLE 1

Using a 40 - 400-fold lower protein concentration, AF9 YEATS dCypher-peptide screen identified all the same hits plus additional, biologically verified hits (Li et al., 2016) compared to a traditional histone peptide array.

AF9	H2A	H2B	H3	H4
dCypher (5 nM)	0/20	0/9	33/199	0/60
dCypher (50 nM)	4/20	0/9	61/199*	6/60
Peptide Array (2 µM)	0/20	0/9	3/199	6/60

*Li et al., *Mol Cell* 62, 181-193 (2016)

dCypher™ A service to discover novel chromatin binding interactions

EpiCypher's dCypher™ screening services for histone modifi cation binding are incredibly versatile, capable of being utilized with either synthetic histone peptides or modified designer nucleosomes.

dCypher - Peptide

Use dCypher Peptide Screening when you are uncertain of what your protein binds to, or if you want to determine the effect of nearby modifications. With over 300 peptides included, this offers tremendous diversity of potential binding substrates.

dCypher - Nucleosome

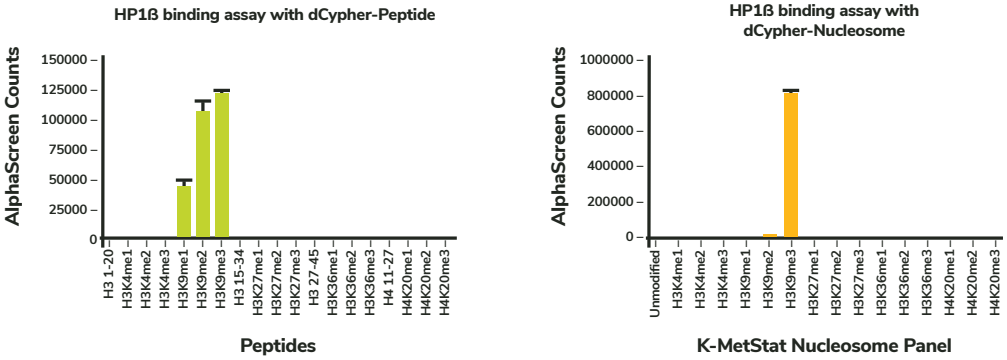
Nucleosomes possess additional interaction surfaces that some proteins utilize for determining the selectivity of binding. Use dCypher nucleosome screening when you want to test your protein using the most physiologically relevant substrate available. EpiCypher currently has > 80 nucleosomes available for services testing -- new modifications are added every month.

FIGURE 3

dCypher Screening Services are available using peptides (left) or nucleosome (right) substrates. Each assay point contains a peptide or a nucleosome with a different histone modification. See Figure 1 for more information.

FIGURE 4

dCypher Screening Services performed using the chromodomain from human HP1ß (Catalog No. 15-0058). ***Left Panel:*** *Binding results from study using dCypher peptide screening.* ***Right panel:*** *Binding results using the K-MetStat nucleosome panel for interaction screening (Catalog No. 16-9002). Note that the nucleosome binding profile for HP1ß is restricted to H3K9me3, whereas the peptide binding results indicate that H3K9me2 and H3K9me1 are also bound by HP1ß.*



NEW! **dCypher™** Nucleosome Panels

EpiCypher now offers all of our recombinant modified nucleosomes in convenient microtitre plate-based collections for your assay needs.

dCypher™ Nucleosome Panels allows access to epigenetic diversity in a physiologically relevant nucleosome context. Included in the panels are single and combinatorially-modified nucleosomes in either the full nucleosome panel, or focused panel sets (lysine acylation, arginine methylation and the lysine-methylation / oncostat panel).

Applications - in native nucleosome context:

- Epigenetic reader binding interactions
- Antibody specificity testing
- Enzyme activity assays (preferred physiological substrates)

dCypher™ Nucleosome Full Panel layout

Catalog No. **16-9001**

147x601 rNuc (H3.1 unmod.)	H3K9me3	H3K79me2	H3K9cr	H3K27ac	H4K16ac	H3R2me1	H4R3me1		H3.3 WT	H2AX	
H3.1ND32 (tailless)	H3K27me1	H3K79me3	H3K14ac	H3K27bu	tetraAc-H4 (K5/8/12/16ac)	H3R2me2a	H4R3me2a		H3.3K4M	H2AXS139ph	
H4ND15 (tailless)	H3K27me2	H4K20me1	H3K18ac	H3K27cr	tetraAc-H3/H4	H3R2me2s	H4R3me2s		H3.3K9M	H2AZ.1	
H3K4me1	H3K27me3	H4K20me2	H3K18bu	H3K27ac + S28ph	H4K20ac	H3R8me1	H2AK119ub		H3.3K27M	H2AZ.2	
H3K4me2	H3K36me1	H4K20me3	H3K18cr	H3K36ac	tetraAc-H2A (K5/8/13/15ac)	H3R8me2a	H2BK120ub		H3.3G34R	187x601 rNuc (linker DNA)	
H3K4me3	H3K36me2	H3K4ac	tetraAc-H3 (K4/9/14/18ac)	H4K5ac	H2AR3me1	H3R8me2s	H3R2,8,17cit		H3.3G34V	Hemi-me (linker DNA)	
H3K9me1	H3K36me3	H3K9ac	H3K4me3+ K9/14/18ac	H34K8ac	H2AR3me2a	H3R17me1	H3S10ph		H3.3G34W		
H3K9me2	H3K79me1	H3K9bu	H3K23ac	H4K12ac	H2AR3me2s	H3R17me2a			H3.3K36M		

Full panel key	dNuc	OncoNucs	vNucs	methyl DNA Nucs	TOTAL
Number	63	8	4	2	77

Additional panels available (see page 6 for details):

Histone Lysine Acylation (K-AcylStat) Panel
K-MetStat + OncoStat Panel
Histone Arginine Methylation (R-MetStat) Panel

Histone Lysine Acylation (K-AcylStat) Panel

Components Present in Triplicate

Catalog No. 16-9003

unmodified	H3K18cr	H4K5ac		unmodified	H3K18cr	H4K5ac		unmodified	H3K18cr	H4K5ac	
H3K4ac	tetraAc-H3 (K4/9/14/18ac)	H4K8ac		H3K4ac	tetraAc-H3 (K4/9/14/18ac)	H4K8ac		H3K4ac	tetraAc-H3 (K4/9/14/18ac)	H4K8ac	
H3K9ac	H3K23ac	H4K12		H3K9ac	H3K23ac	H4K12		H3K9ac	H3K23ac	H4K12	
H3K9bu	H3K27ac	H4K16ac		H3K9bu	H3K27ac	H4K16ac		H3K9bu	H3K27ac	H4K16ac	
H3K9cr	H3K27bu	tetraAc-H4 (K5/8/12/16ac)		H3K9cr	H3K27bu	tetraAc-H4 (K5/8/12/16ac)		H3K9cr	H3K27bu	tetraAc-H4 (K5/8/12/16ac)	
H3K14ac	H3K27cr	H4K20ac		H3K14ac	H3K27cr	H4K20ac		H3K14ac	H3K27cr	H4K20ac	
H3K18ac	H3K27ac+ S28ph	tetraAc-H2A (K5/8/13/15ac)		H3K18ac	H3K27ac+ S28ph	tetraAc-H2A (K5/8/13/15ac)		H3K18ac	H3K27ac+ S28ph	tetraAc-H2A (K5/8/13/15ac)	
H3K18bu	H3K36ac			H3K18bu	H3K36ac			H3K18bu	H3K36ac		

K-MetStat + OncoStat Panel

Components Present in Triplicate

Catalog No. 16-9002

unmodified	H3K27me2	H3.3 WT		unmodified	H3K27me2	H3.3 WT		unmodified	H3K27me2	H3.3 WT	
H3K4me1	H3K27me3	K4M		H3K4me1	H3K27me3	K4M		H3K4me1	H3K27me3	K4M	
H3K4me2	H3K36me1	K9M		H3K4me2	H3K36me1	K9M		H3K4me2	H3K36me1	K9M	
H3K4me3	H3K36me2	K27M		H3K4me3	H3K36me2	K27M		H3K4me3	H3K36me2	K27M	
H3K9me1	H3K36me3	G34R		H3K9me1	H3K36me3	G34R		H3K9me1	H3K36me3	G34R	
H3K9me2	H4K20me1	G34V		H3K9me2	H4K20me1	G34V		H3K9me2	H4K20me1	G34V	
H3K9me3	H4K20me2	G34W		H3K9me3	H4K20me2	G34W		H3K9me3	H4K20me2	G34W	
H3K27me1	H4K20me3	K36M		H3K27me1	H4K20me3	K36M		H3K27me1	H4K20me3	K36M	

Histone Arginine Methylation (R-MetStat) Panel

Components Present in Triplicate

Catalog No. 16-9004

unmodified	H3R8me2a			unmodified	H3R8me2a			unmodified	H3R8me2a		
H2AR3me1	H3R8me2s			H2AR3me1	H3R8me2s			H2AR3me1	H3R8me2s		
H2AR3me2a	H3R17me1			H2AR3me2a	H3R17me1			H2AR3me2a	H3R17me1		
H2AR3me2s	H3R17me2a			H2AR3me2s	H3R17me2a			H2AR3me2s	H3R17me2a		
H3R2me1	H4R3me1			H3R2me1	H4R3me1			H3R2me1	H4R3me1		
H3R2me2a	H4R3me2a			H3R2me2a	H4R3me2a			H3R2me2a	H4R3me2a		
H3R2me2s	H4R3me2s			H3R2me2s	H4R3me2s			H3R2me2s	H4R3me2s		
H3R8me1				H3R8me1				H3R8me1			

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