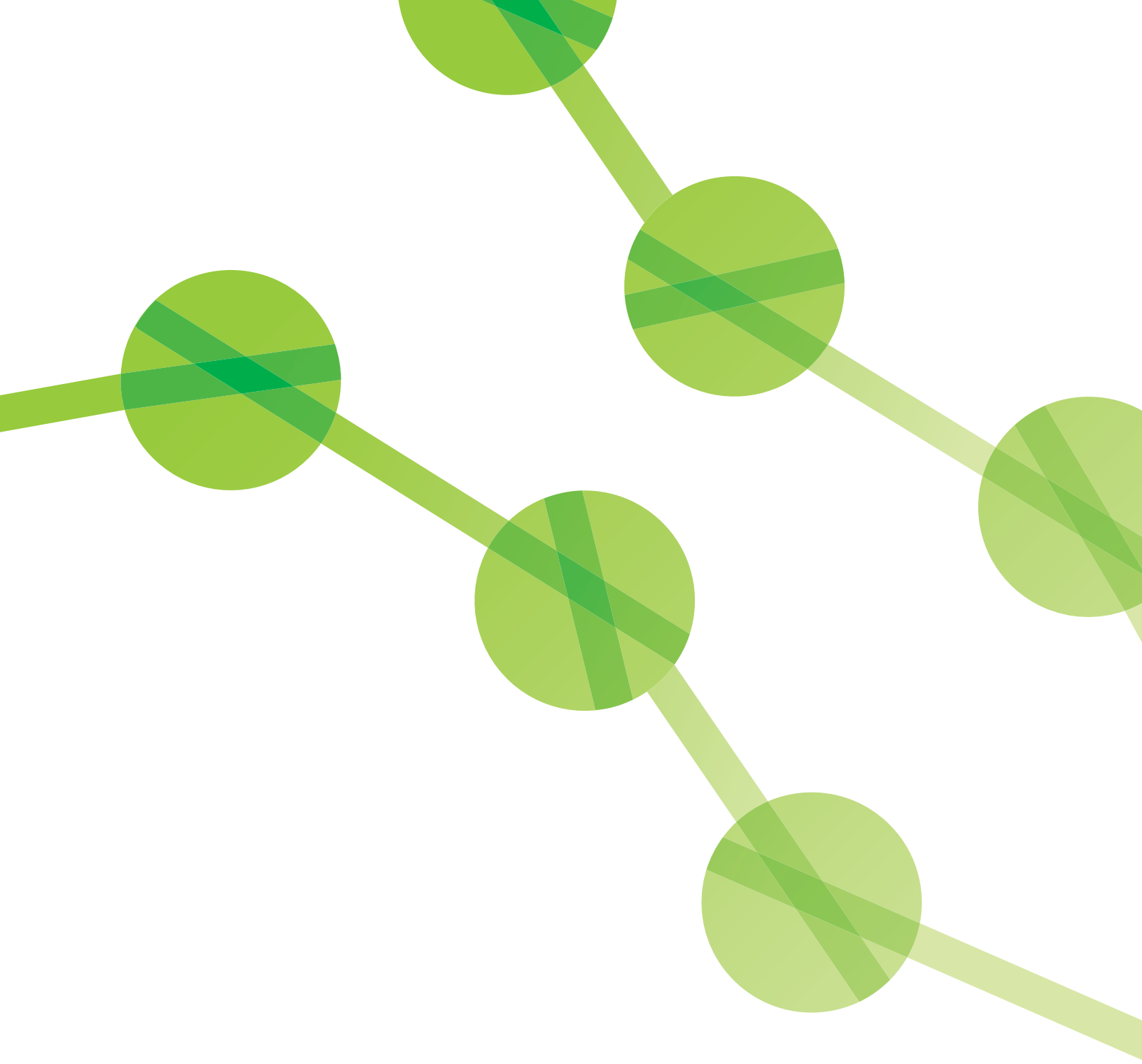


EpiCypher®

Bringing Epigenetics to Life

dCypher™

A service to discover novel chromatin binding interactions

dCypher™ A service to discover novel chromatin binding interactions

dCypher is a new epigenetics discovery service that combines the diversity of histone peptide array technology with the high sensitivity of AlphaScreen®, a bead-based no-wash proximity assay, to identify novel chromatin interactions

Applications

- Reader binding interaction discovery
- Drug target identification and validation
- Binding domain inhibitor screening

dCypher advantages over current technologies

- Unmatched sensitivity vs. peptide pulldown → more hits using less starting material
- Nearly 300 modified histone peptides (single and combinatorial PTMs)
- Works in liquid phase with all peptides individually synthesized, HPLC purified and mass spec validated according to EpiCypher's rigorous quality control standards
- dCypher is also compatible with EpiCypher's designer Nucleosomes (dNucs)
- Easily customizable with additional user-specified targets

Available in both histone peptide and nucleosome substrate formats

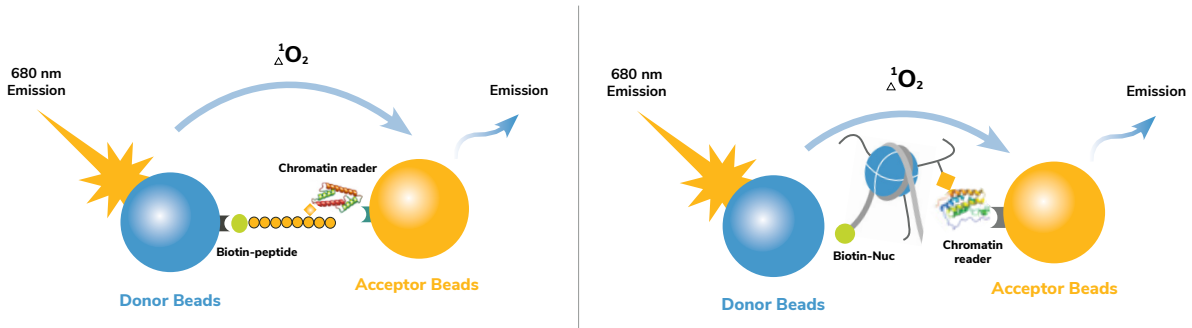


FIGURE 1

Schematic representation of the dCypher platform. Each well contains a peptide or a nucleosome with a different histone modification. Streptavidin Donor Beads capture biotinylated nucleosomes or peptides and a tagged (6xHIS, GST) chromatin reader of interest is bound to Acceptor Beads (Nickel-chelate, Glutathione). When the reader protein binds to its target, the Acceptor and Donor Beads are brought within proximity. Laser excitation of the Donor Beads at 680 nm generates a short-lived singlet oxygen molecule that triggers an emission of light from nearby Acceptor Beads.

WHAT DOES YOUR DOMAIN BIND TO?

Let dCypher tell you!

dCypher™ A service to discover novel chromatin binding interactions

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

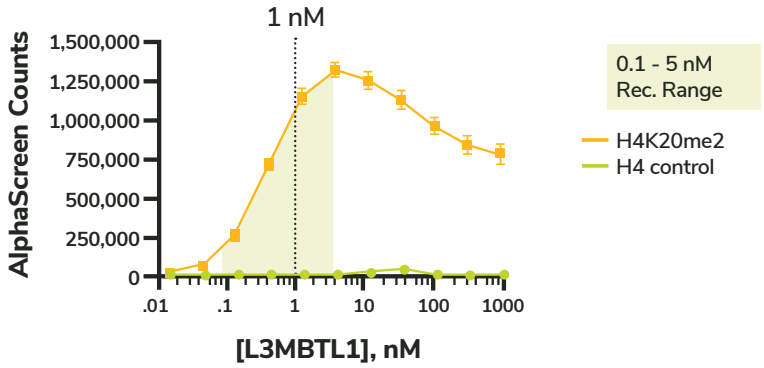
This highly sensitive method results in lower assay background, enabling the identification of putative 'hits' that could be otherwise missed using peptide arrays. Whether your reader is known to interact with a PTM or not (*i.e.* blind candidate testing), dCypher can be used to identify novel chromatin interactions.

Below we highlight two examples comparing the performance of dCypher and histone peptide arrays

Example 1

dCypher screen using a reader protein with known PTM interactions

- L3MBTL1 is a pan me1/2 binding protein that performs poorly on peptide arrays
- dCypher assay shows remarkable S/N over a range of L3MBTL1 concentrations



- dCypher screen using an optimized L3MBTL1 concentration (1 nM) uncovers many interactions missed by peptide array (top table)
- Numbers in table reflect hits observed vs. number of peptides tested carrying me1/2

	H2A	H2B	H3	H4
dCypher (1 nM)	0/20	2/9	65/199	23/60
Peptide Array (2 µM)	0/15	2/4	16/129	8/43

NOTE: dCypher uses 2000-fold less material vs. peptide arrays

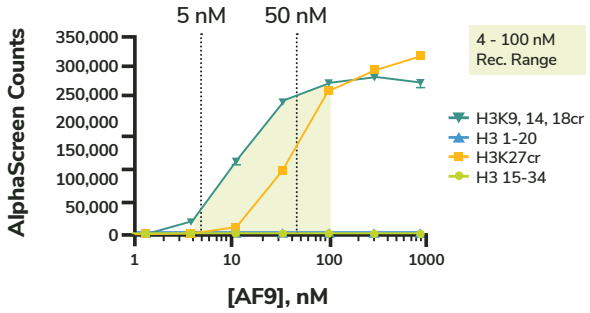
- dCypher identifies L3MBTL1 hits that agree with known role as pan me1/me2 binding protein (bottom table)

	H4K20me1/2	H3K4me1/2	H3K9me1/2	H3K27me1/2
dCypher (1 nM)	4/4	16/16	19/19	5/6
Peptide Array (2 µM)	2/3	9/14	9/16	1/4

Example 2

dCypher™ screen when reader-PTM interactions are unknown (*i.e.* blind candidate testing)

For blind candidate testing, the dCypher screen is performed at a high and a low concentration to maximize hit discovery. These assay conditions are ideal starting points for readers with wide-ranging Kd values (see graph).



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

	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., (2016) *Mol Cell*:62: 181-193

Only a small amount of material is required for dCypher, making it more economical to perform multiple screens

DOMAIN	REFERENCE PROTEIN(S)	RECOMMENDED TEST RANGE*
3xMBT	L3MBTL1	0.1 - 5 nM
PHD	RAG2	0.1 - 2 nM
CHD	HP1ß	0.05 - 1 nM
BD	ATAD2B BRD4-BD1	0.2 - 1 nM 0.2 - 5 nM
YEATS	AF9	4 - 50 nM

*Recommended probing concentration ranges and materials required for chromatin readers using dCypher

NEW! **dCypher™** Nucleosome Panels

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

dCypher Nucleosome Panels represent unprecedented 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 physiologic substrates)

dCypher Nucleosome Full Panel layout

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	H3K20me1	H3K18ac	H3K27cr	tetraAc-H3/ H4	H3R2me2s	H4R3me2s		H3.3K9M	H2AZ.1	
H3K4me1	H3K27me3	H3K20me2	H3K18bu	H3K27ac + S28ph	H4K20ac	H3R8me1	H2AK119ub		H3.3K27M	H2AZ.1	
H3K4me2	H3K36me1	H3K20me3	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+ K4/9/14/18ac	H34K8ac	H2AR3me2a	H3R17me1	H3S10ph		H3.3G34W		
H3K9me2	H3K79me1	H3K9bu	H3K23ac	H4K12ac	H2AR3me2s	H3R17me2a			H3.3G36M		

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

Histone Lysine Acylation (K-AcylStat) Panel

Unmodified
H2A K5,8,13,15ac
H3K4ac
H3K9ac
H3K9bu
H3K9cr
H3K14ac
H3K18ac
H3K18bu
H3K18cr
H3 K4,9,14,18ac
H3K23ac
H3K27ac
H3K27bu
H3K27cr
H3K27ac + S28ph
H3K36ac
H4K5ac
H4K8ac
H4K12ac
H4K16ac
H4 K5,8,12,16ac
H4K20ac

K-MetStat + OncoStat Panel

Unmodified
H3K4me1
H3K4me2
H3K4me3
H3K9me1
H3K9me2
H3K9me3
H3K27me1
H3K27me3
H3K36me1
H3K36me2
H3K36me3
H3K79me2
H4K20me1
H4K20me2
H4K20me3
H3.3 WT
H3.3K4M
H3.3K9M
H3.3K27M
H3.3G34R
H3.3G34V
H3.3G34W
H3.3K36M

Histone Arginine Methylation (R-MetStat) Panel

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

Product Name	Catalog No.	Price
dCypher® Nucleosome Full Panel	16-9001	$2,495
dCypher® Nucleosome K-MetStat & OncoStat Panel	16-9002	$1,995
dCypher® Nucleosome K-AcylStat Panel	16-9003	$1,995
dCypher® Nucleosome R-MetStat	16-9004	$1,795

dCypher™ Histone Modification Screening Services

EpiCypher's dCypher Histone Modification Screening services are incredibly versatile, capable of being utilized with either synthetic histone peptides or modified designer nucleosomes.

dCypher Peptide Screening Service

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 Screening Service

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.

FIGURE 2

dCypher Histone Modification 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.

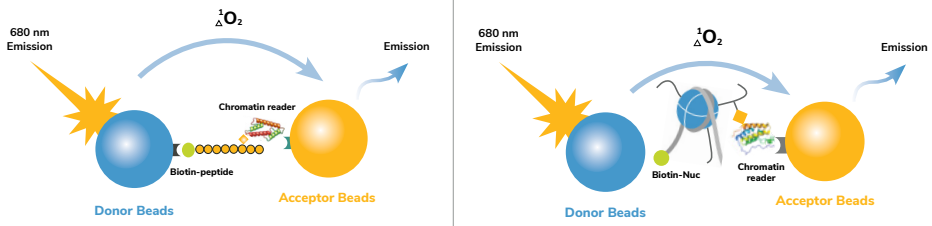
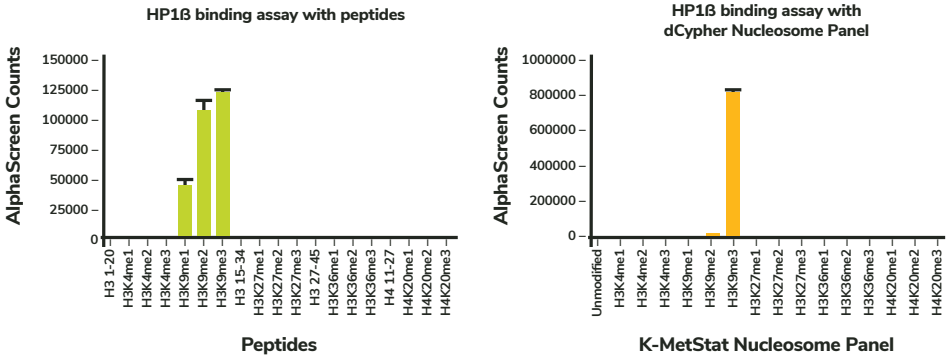


FIGURE 3

dCypher Histone Modification Screening 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. Note that the nucleosome binding profile for HP1ß is restricted to H3K9me3, whereas the peptide binding results indicate that H3k9me2 and, too a lesser extent, H3K9me1 are bound by HP1ß.*



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