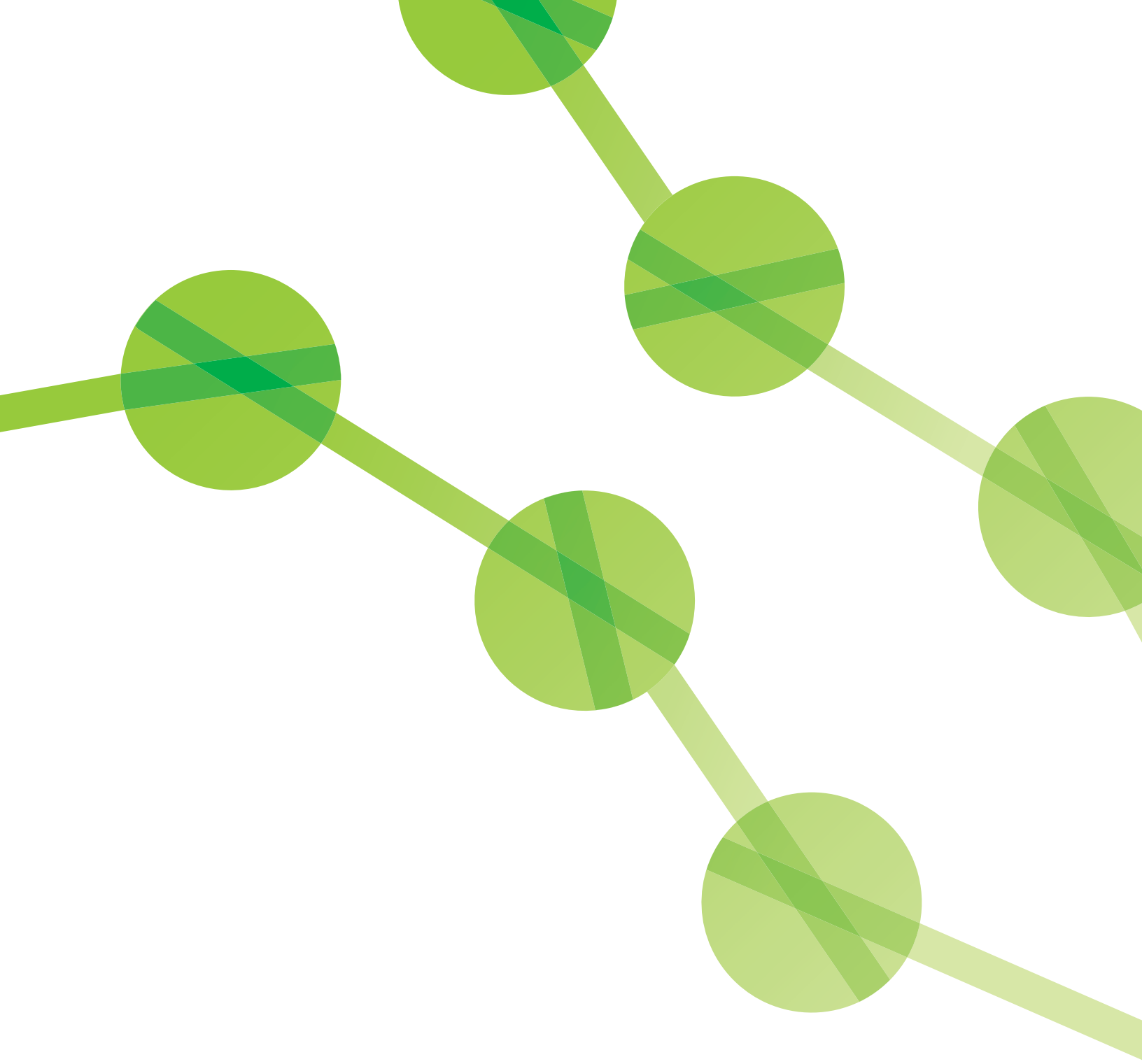


EpiCypher®
Bringing Epigenetics to Life

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 interactions 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

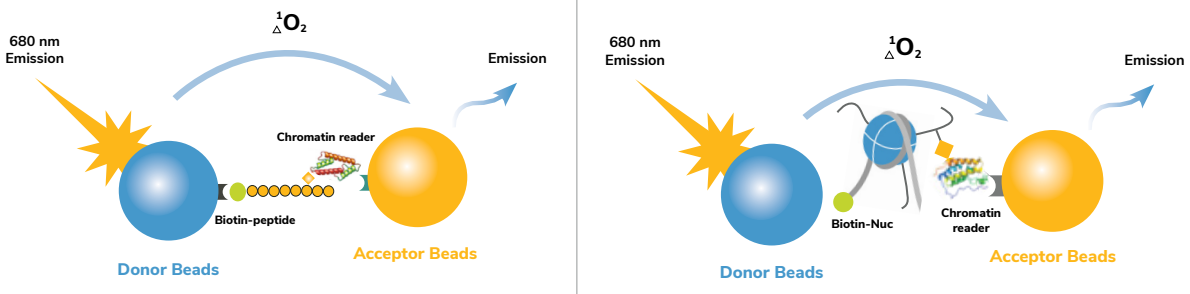


FIGURE 1

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 futher "dCypher" the Histone Code

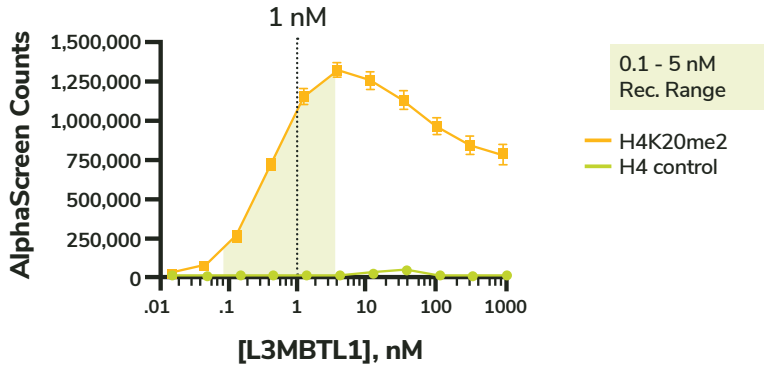
This highly sensitive method results in lower assay background, enabling the identification of putative 'hits' that could be otherwise missed using peptide arrays. Whether your reader is known to interact with a PTM or not (*i.e.* blind candidate testing), dCypher can be used to identify novel chromatin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

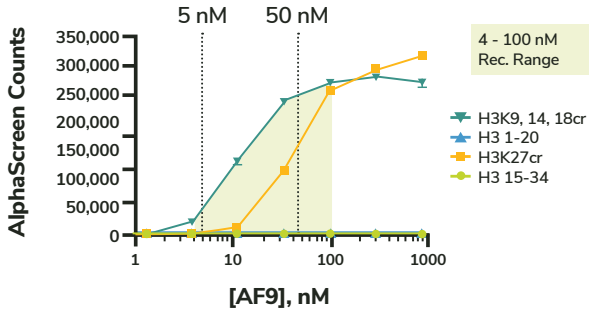
- dCypher identifies L3MBTL1 hits that agree with known role as pan me1/me2 binding protein (bottom table)

	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*

ORDERING INFO

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