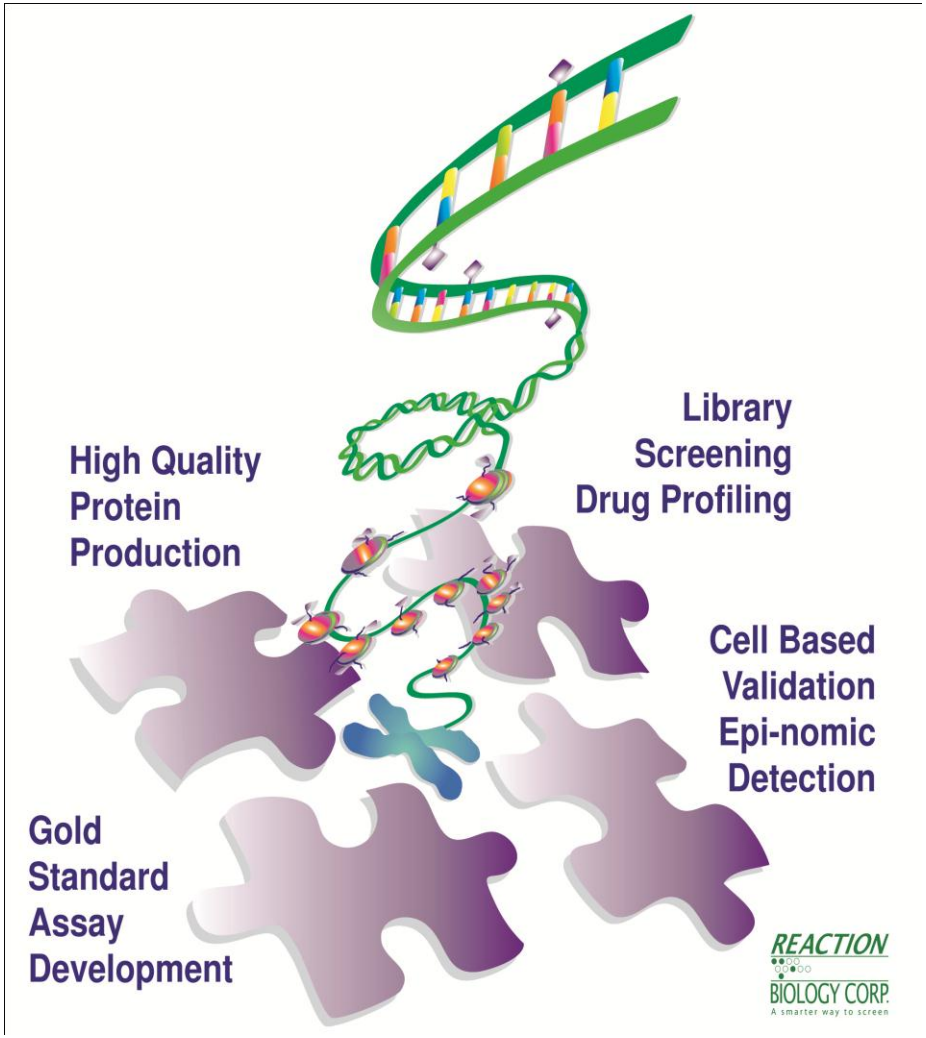
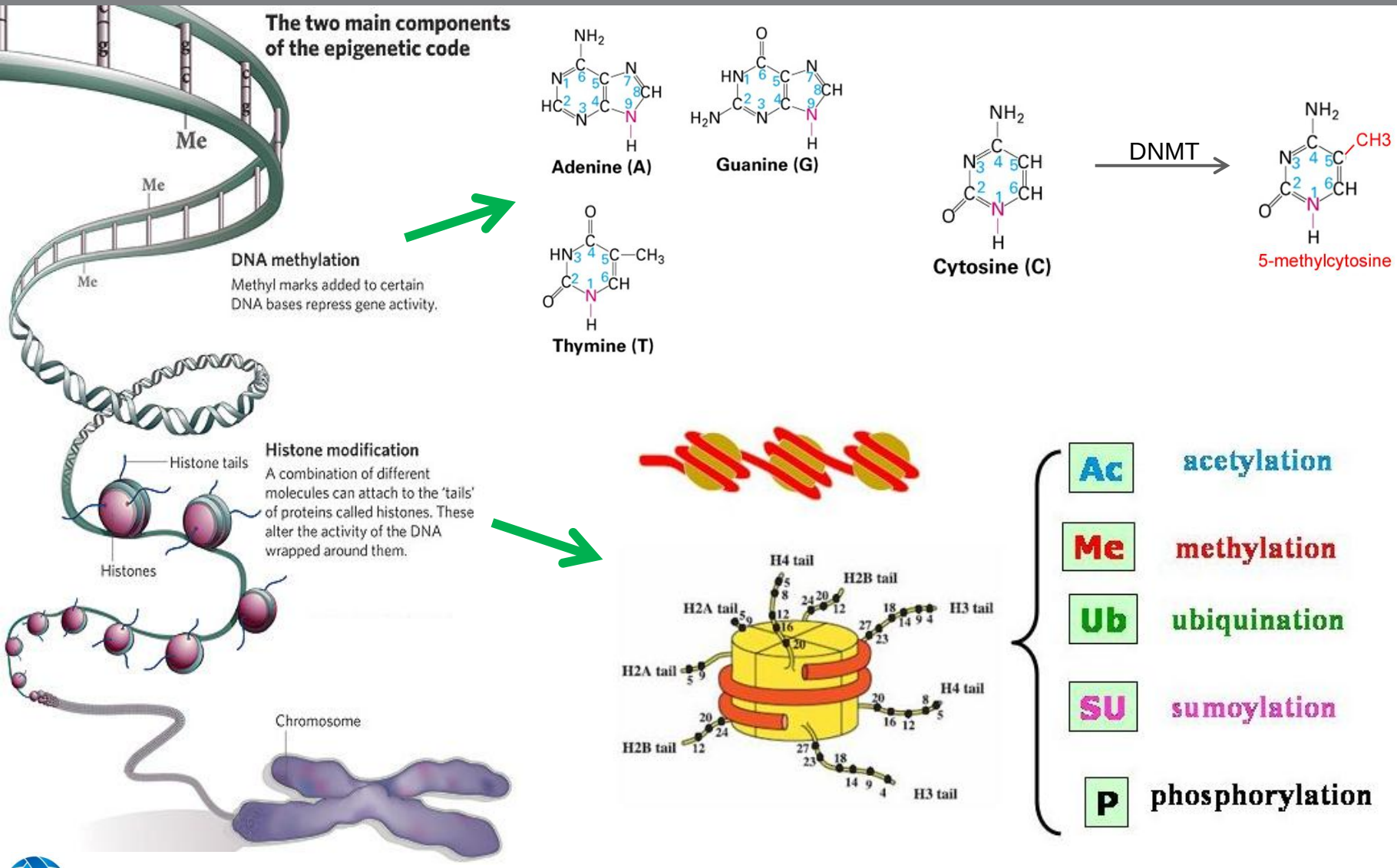
REACTION BIOLOGY CORP.

One Stop-Shop for Epigenetics Drug Discoveries

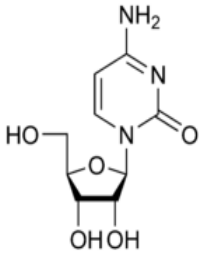


Reaction Biology Corp.

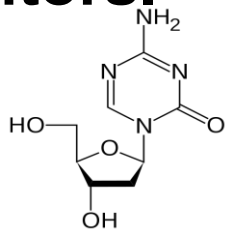


DNA Methyltransferases

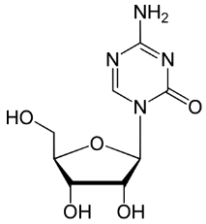
Current DNA methyltransferases inhibitors are nucleotide analogues, instead of DNMT inhibitors.



Cytidine



Dacogen: Decitabine or 5-aza-2'-deoxycytidine



Vidaza: Azacitidine or 5-azacytidine,

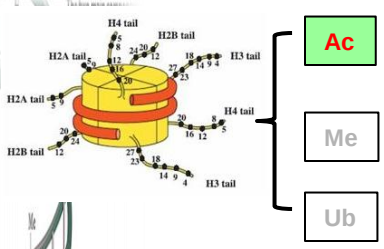
Indications:

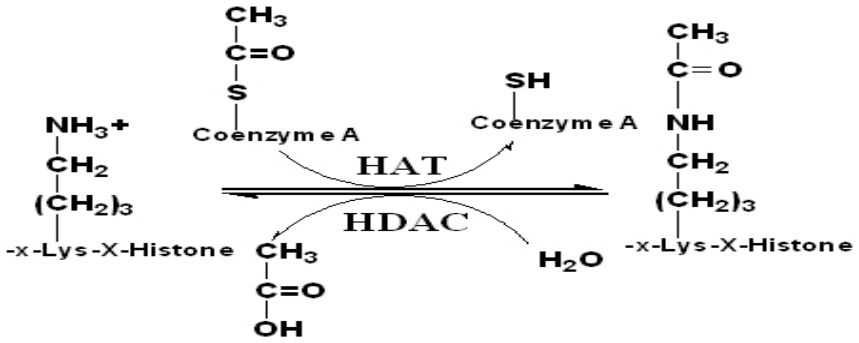
FDA approved for acute myeloid leukemia (AML) and myelodisplastic syndrome (MDS).
Neurological disorder: Rett Syndrome
Diabetes
Immunal disorder: Lupus, ICF syndrome

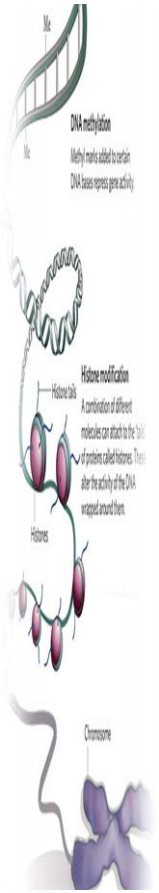
RBC Is Offering (gold standard radioisotope based assays)**:**

DNMT1,
DNMT3a,
DNMT3b,
DNMT3b/3L

Histone Acetyltransferases







Indication:

Asthma and COPD
Alzheimer (AD)
Diabetes
Cancer
Hyperlipidaemia
Heart Failure

RBC Is Offering (gold standard radioisotope based assays)**:**

P300/CBP family:

- CBP (CREB Binding Protein): All Histones
- P300: All Histones

MYST Family:

- KAT5 (TIP60) (Tat Interacting Protein 60): H4, H3, H2A
- KAT6B (MYST4): H4
- KAT7 (MYST2): H4

GNAT family:

- GCN5: H3K14, H4K8, H4K16
- pCAF (p300/CBP Associated Factor): free H3, H4 and nucleosomal H3

Histone Deacetylases

Classification:

- Class I: HDAC1, 2, 3 and 8. Nucleus localization.
- Class II: HDAC4,5, 6,7, 9 and 10. In nucleus and cytoplasm. 2-times larger than Class I. HDAC6 has 2-deacetylase domains in C- and N-terminals.
- Class III: also known as Sirtuins, Sirt 1 to 7. In nucleus and cytoplasm. NAD-dependent enzyme.
- Class IV: HDAC 11.

Indication:

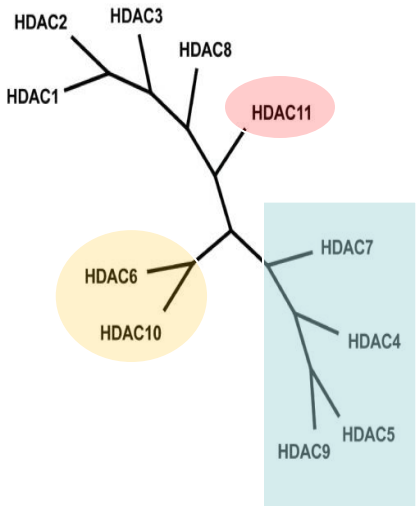
Cancer:

Vorinostat (SAHA), 2006 for CTCL (cutaneous T cell lymphoma), trials for AML, MDS, CML etc.

Romidepsin, 2009, for CTCL

Skin disorders: lupus, contact dermatitis

Neurodegenerative diseases: Alzheimer (AD), and Huntingtoı Disease

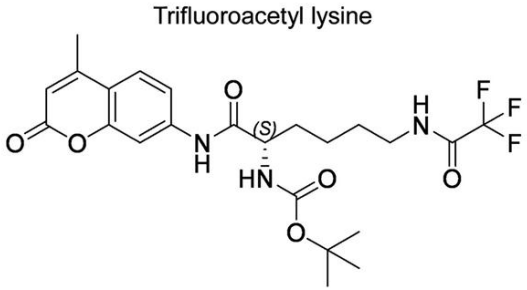


Histone Deacetylases

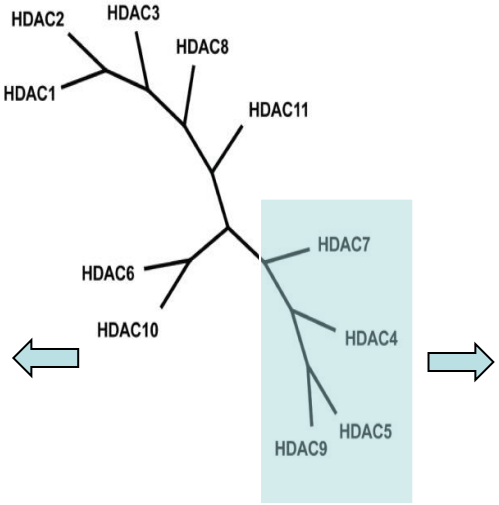
RBC Is Offering:

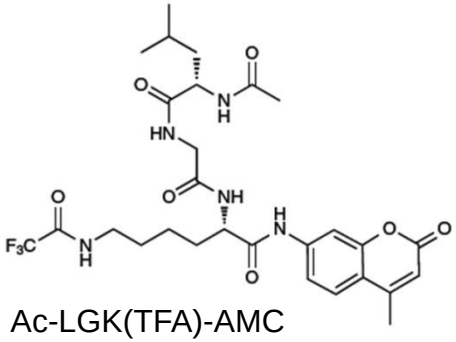
- Fluorescence based assay, All 11 isoforms with peptide substrates: p53 peptides (RHKKAc), and (RHKAcKAc).
- Sirt 1,2 and 3 with p53 peptides (RHKKAc), and (QPKKAc).

RBC Is Offering: Class IIa specific substrates as options:



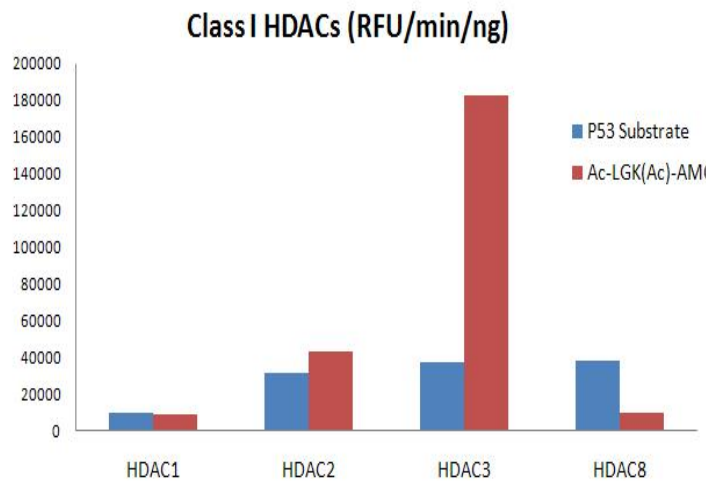
Lahm et al 2007, PNAS

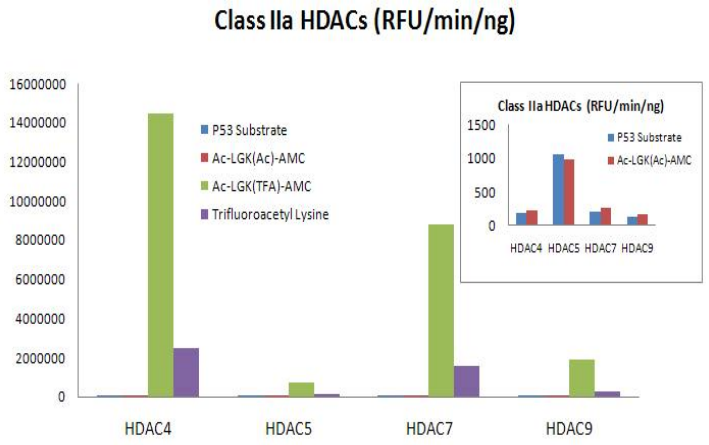


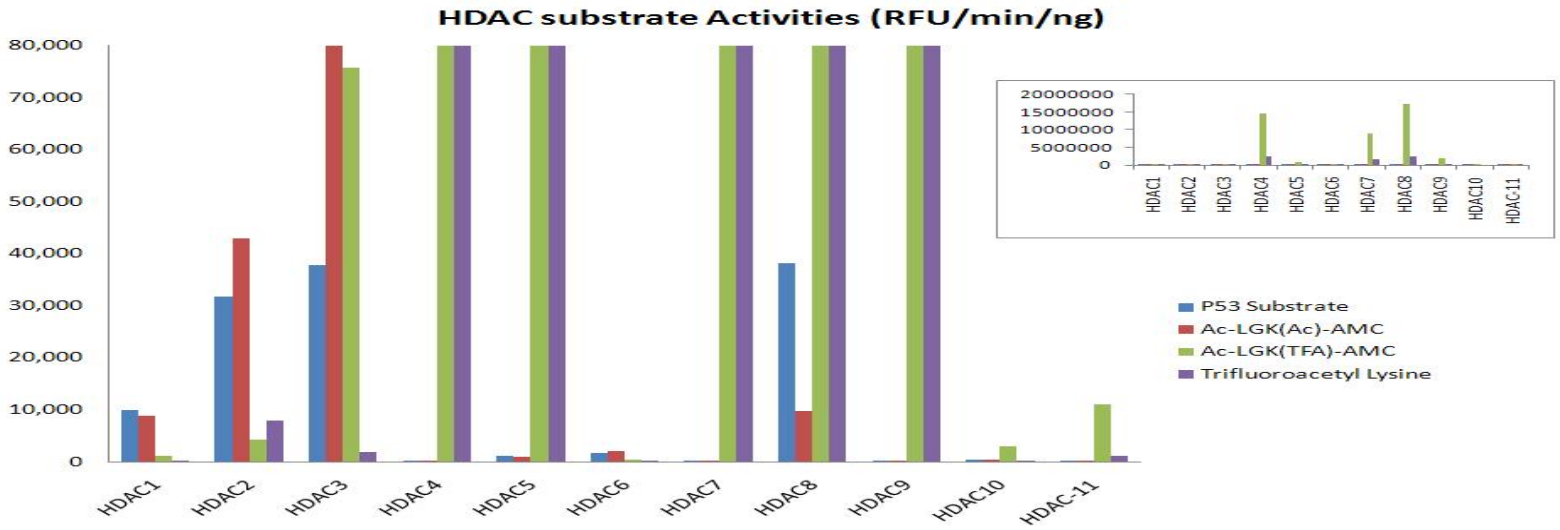


Ac-LGK(TFA)-AMC
Bradner et al, 2010, Nat. Chem. Bio.

Histone Deacetylases



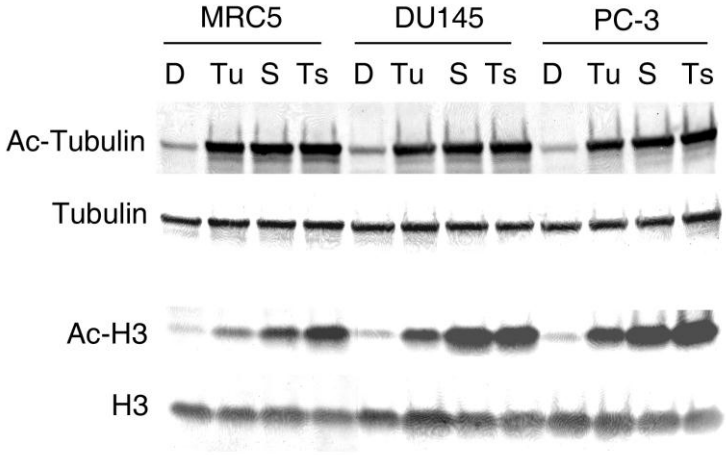




HDACs Cell Based Assays

Sample Assays:

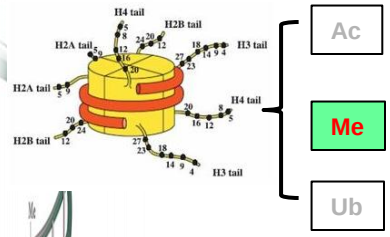
Ac-tubulin & Tubulin; Ac-H3 & H3; Ac-H4 & H4
Viability;
Anti-ChK2-S68-p (activated in response to DNA damage in both G1 and G2 checkpoint activation);
Anti-PARP (Poly(ADP-ribose)polymerase activated in response to DNA damage)

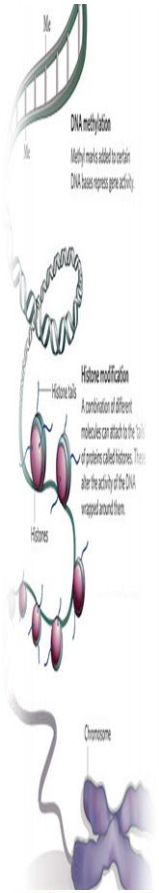


Human fibroblast cell line, MRC5, and human prostate cancer cell lines, DU145 and PC-3, were treated with 10uM Tubastatin A (Tu), 5uM SAHA (S), 1uM TSA (Ts) or DMSO (D) as control in the presence of 10% FBS for 22 hrs. Acetylation is detected by anti-Acetylated a–Tubulin, anti-a-Tubulin, anti-acetylated Histone H3 and anti-Histone H3.

Tubastatin A is a potent HDAC 6 inhibitor, SAHA and TSA are pan HDAC inhibitors

Histone Methyltransferases





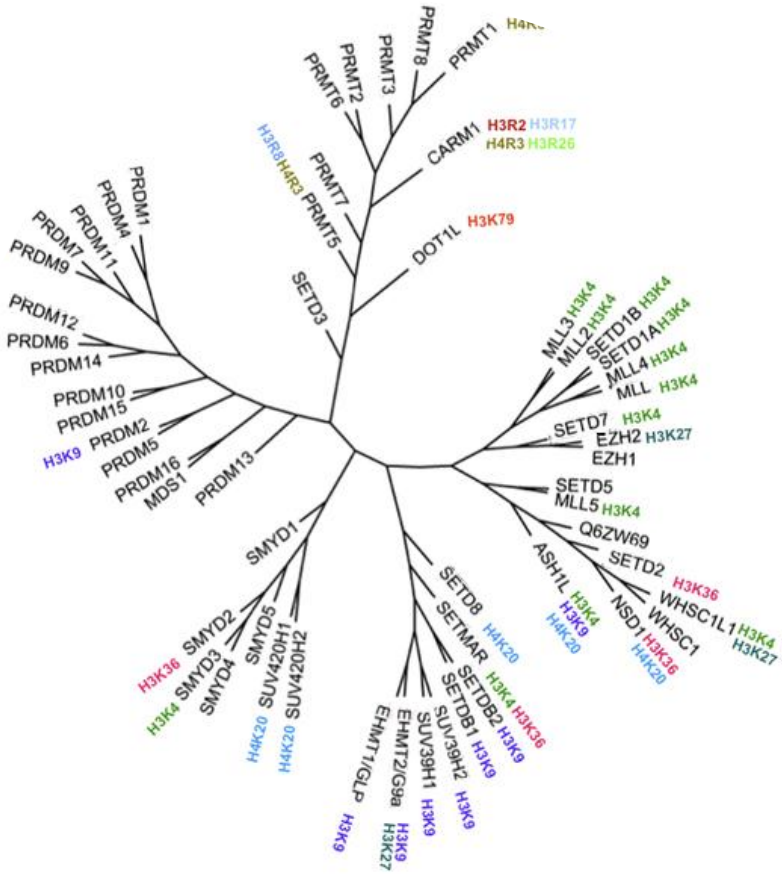
Class

> 70 members,

Indications

Cancers: such as Leukemia, Prostate, Breast, Lung, Liver, Colon, Gastric Lymphoma

Neurodegenerative: Huntington's Disease



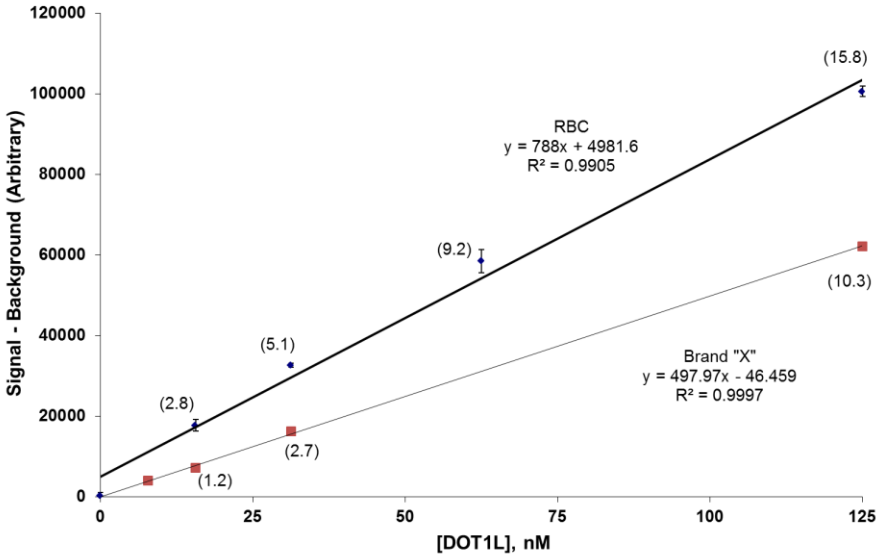
Modified based on Wu , et al. (*2010*) *PLoS ONE* 5(1): e8570

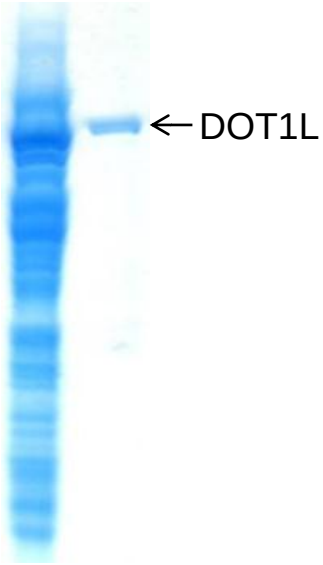
Histone Methyltransferases

RBC Is Offering:

- Protein Production:

Signal as a function of [DOT1L]
0.05 mg/mL Core Histones, 1 µM [^{3}H]-SAM, 60 min., 30° C
() = Signal/Background

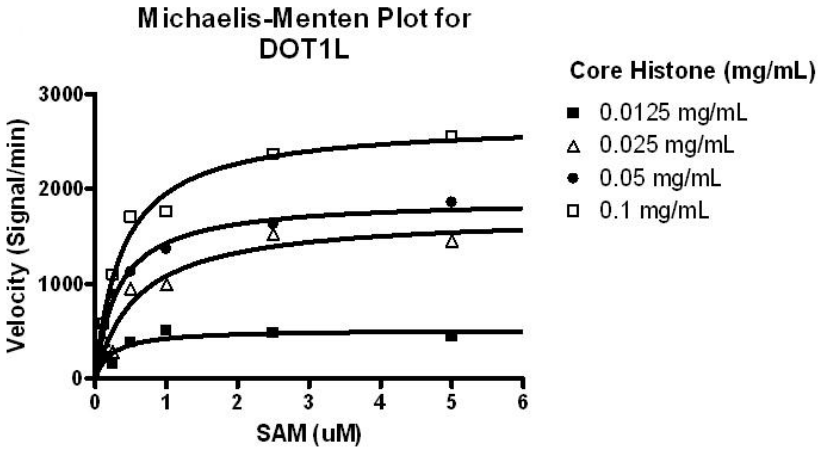


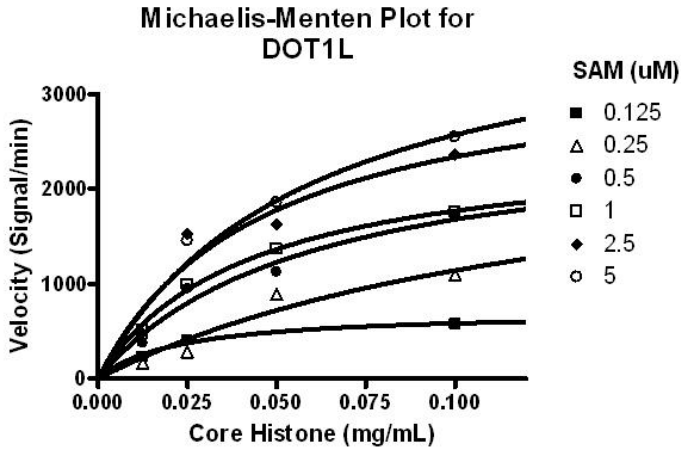


Histone Methyltransferases

RBC Is Offering:

- Assay development: “Gold Standard” Radiolabeled HotSpot^{SM} Assay ([^{3}H]-SAM)
- Substrates: Nucleosome, core Histone, Histone protein, Histone-derived peptide.





Histone Methyltransferases

RBC Is Offering: HTS and Profiling (gold standard radioisotope based assays)**:**

Histone Lysine Methyltransferase (Lysine residue modification: mono, di and trimethylation):

- EZH1, EZH2, DOT1, EHMT2/G9a, SET7, SET8, SETMAR, SMYD2, SUV39H1, SUV39H2,

Histone Arginine Methyltransferase (mono, di-methylation, symmetric and asymmetric):

- Type 1: PRMT1, PRMT3, CARM1/PRMT4, PRMT6
- Type II: PRMT5/MEP50

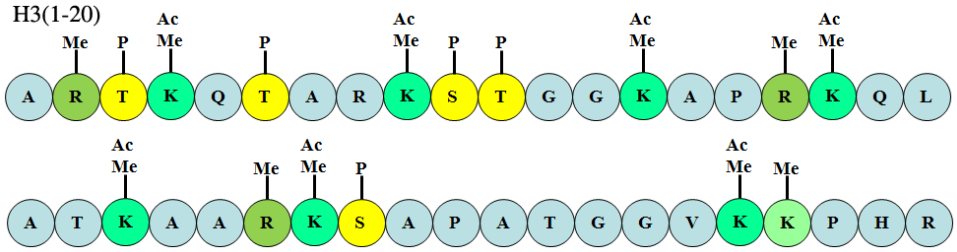
Assays under development: MLL1, SMYD3, NSD1, NSD2, NSD3, PRMD2, GLP (EHMT1), etc

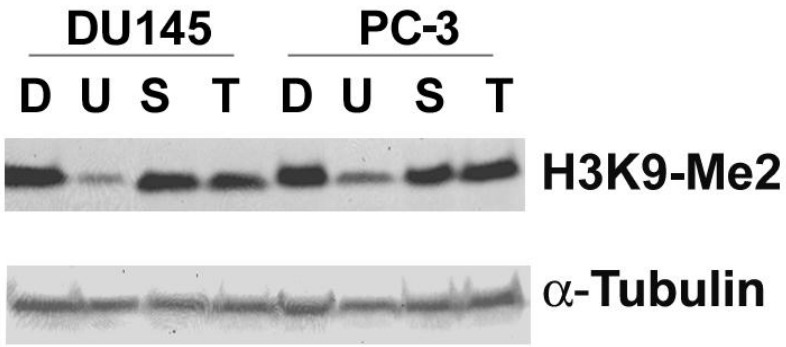
HMTS	CANCERS
CARM1/ PRMT4	Breast and prostate cancers
DOT1L	MLL-rearranged leukemias
EZH1	Breast
EZH2	Breast, prostate, colon, gastric, bladder, liver, melanoma, lymphoma
G9a/EHMT 2	Lung, prostate and hepatocellular carcinoma
MLL1	Leukemia, gastric
MLL2	Breast, Lung
MLL4	Pancreatic
NSD1	Acute myeloid leukemia
PRMD10	Melanoma, Oral, Ovarian, Colorectal,
PRDM14	Breast cancers
PRMT5	Lymphoma
SETD7	Breast cancers
SMYD3	Breast, liver, colon and gastric cancers
SUV39H1	Colon cancer Lung cancer Sarcoma
SUV39H2	Head/neck
WHSC1/N SD2	Myeloma
WHSC1L1/ NSD3	Lung and breast cancers, and childhood acute myeloid leukemia

HMT Cell Based Assays

Cell Based Protein Methylations Detection

Western and ELISA to detect Epi-nomic changes





UNC0638 is a G9a/GLP selective histone methyltransferase inhibitor (HMT). Prostate cancer cell lines, DU145 and PC-3, were treated with 10uM UNC0638 (U), 5uM SAHA (S), 1uM TSA (T) or DMSO (D) control for 2 days in the presence of 10% fetal bovine serum. Western blot analysis with anti-dimethyl-Histone H3K9 antibody or control antibody anti-a-Tubulin.

Histone Demethylases

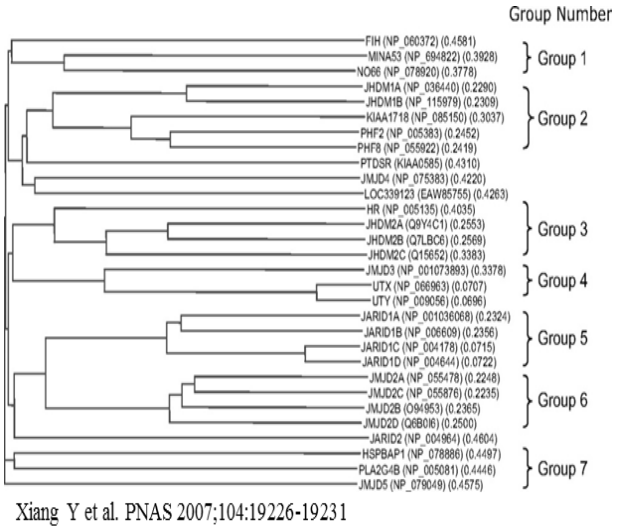
Histone Demethylases ~30 members

LSD (**Offered by RBC**)

JmjC domain-containing proteins (~27 members) (**Working On**)

LSD1 Indications

Cardiovascular Diseases
Herpes virus Infection
Cancer



Xiang Y et al. PNAS 2007;104:19226-19231

Cancer	
LSD1	Overexpressed in prostate cancer, high levels correlated with cancer and tumor relapse during therapy.
JMJD2C	Overexpressed in esophageal squamous carcinoma.
PLU-1	Overexpressed in breast cancer and testis cancer.
MINA53	Highly expressed in esophageal squamous carcinoma and colon cancer.
MAPJD	Overexpressed in non-small-cell lung cancer (NSCLC), trans-activates genes that are relevant to proliferation of lung cancer cells.
FBXL10	Potential tumour suppressor.
JMJD5	Potential tumour suppressor.
Neurological disorders	
SMCX	Multiple missense mutations in X-linked mental retardation
PHF8	Nonsense mutations in X-linked mental retardation.

Modified from Shi, 2007, Nature Rev. Genetics