About OMICS Group

OMICS Group International is an amalgamation of Open Access publications and worldwide international science conferences and events. Established in the year 2007 with the sole aim of making the information on Sciences and technology 'Open Access', OMICS Group publishes 400 online open access scholarly journals in all aspects of Science, Engineering, Management and Technology journals. OMICS Group has been instrumental in taking the knowledge on Science & technology to the doorsteps of ordinary men and women. Research Scholars, Students, Libraries, Educational Institutions, Research centers and the industry are main stakeholders that benefitted greatly from this knowledge dissemination. OMICS Group also organizes 300 International conferences annually across the globe, where knowledge transfer takes place through debates, round table discussions, poster presentations, workshops, symposia and exhibitions.

About OMICS Group Conferences

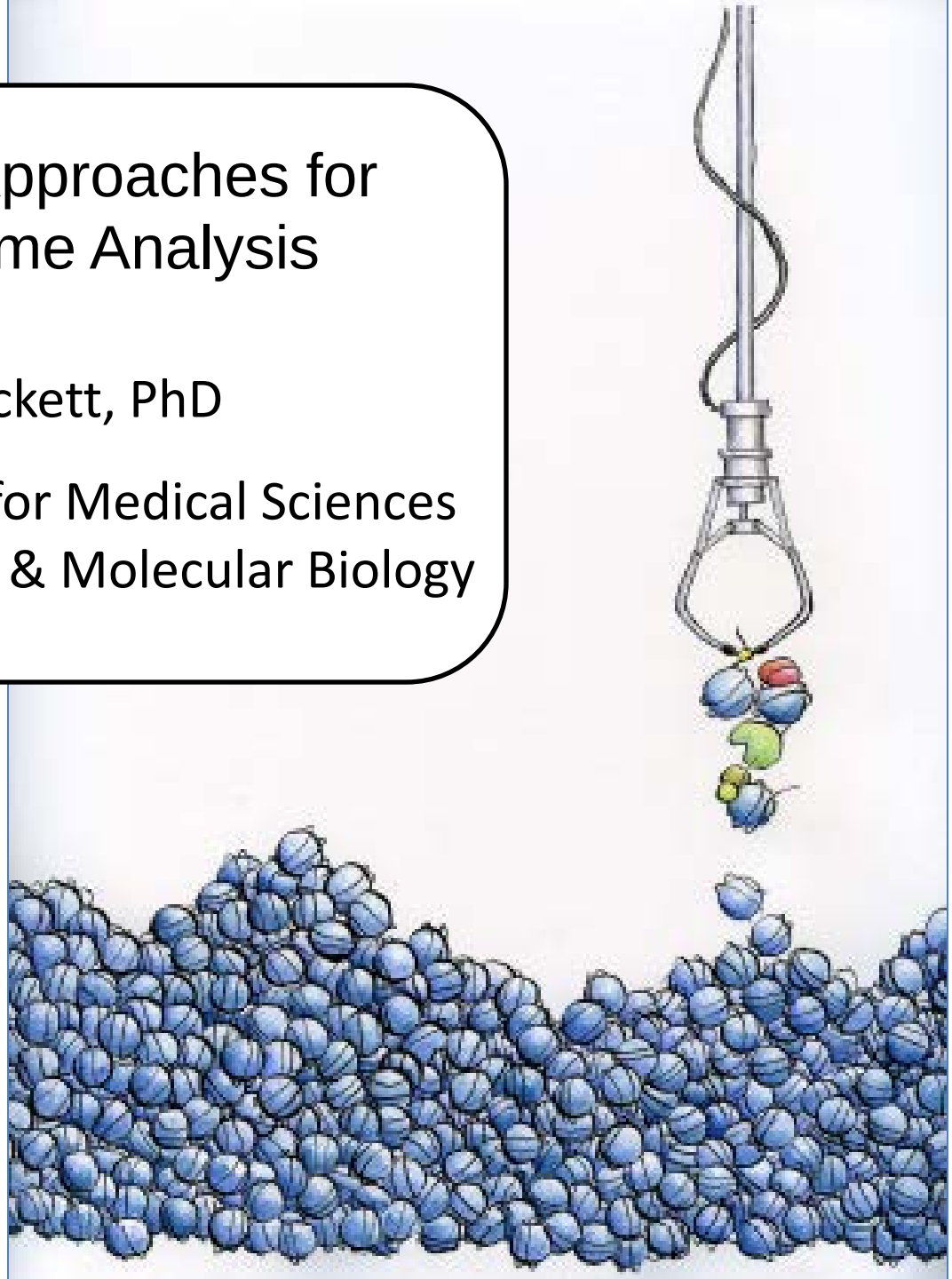
OMICS Group International is a pioneer and leading science event organizer, which publishes around 400 open access journals and conducts over 300 Medical, Clinical, Engineering, Life Sciences, Pharma scientific conferences all over the globe annually with the support of more than 1000 scientific associations and 30,000 editorial board members and 3.5 million followers to its credit.

OMICS Group has organized 500 conferences, workshops and national symposiums across the major cities including San Francisco, Las Vegas, San Antonio, Omaha, Orlando, Raleigh, Santa Clara, Chicago, Philadelphia, Baltimore, United Kingdom, Valencia, Dubai, Beijing, Hyderabad, Bengaluru and Mumbai.

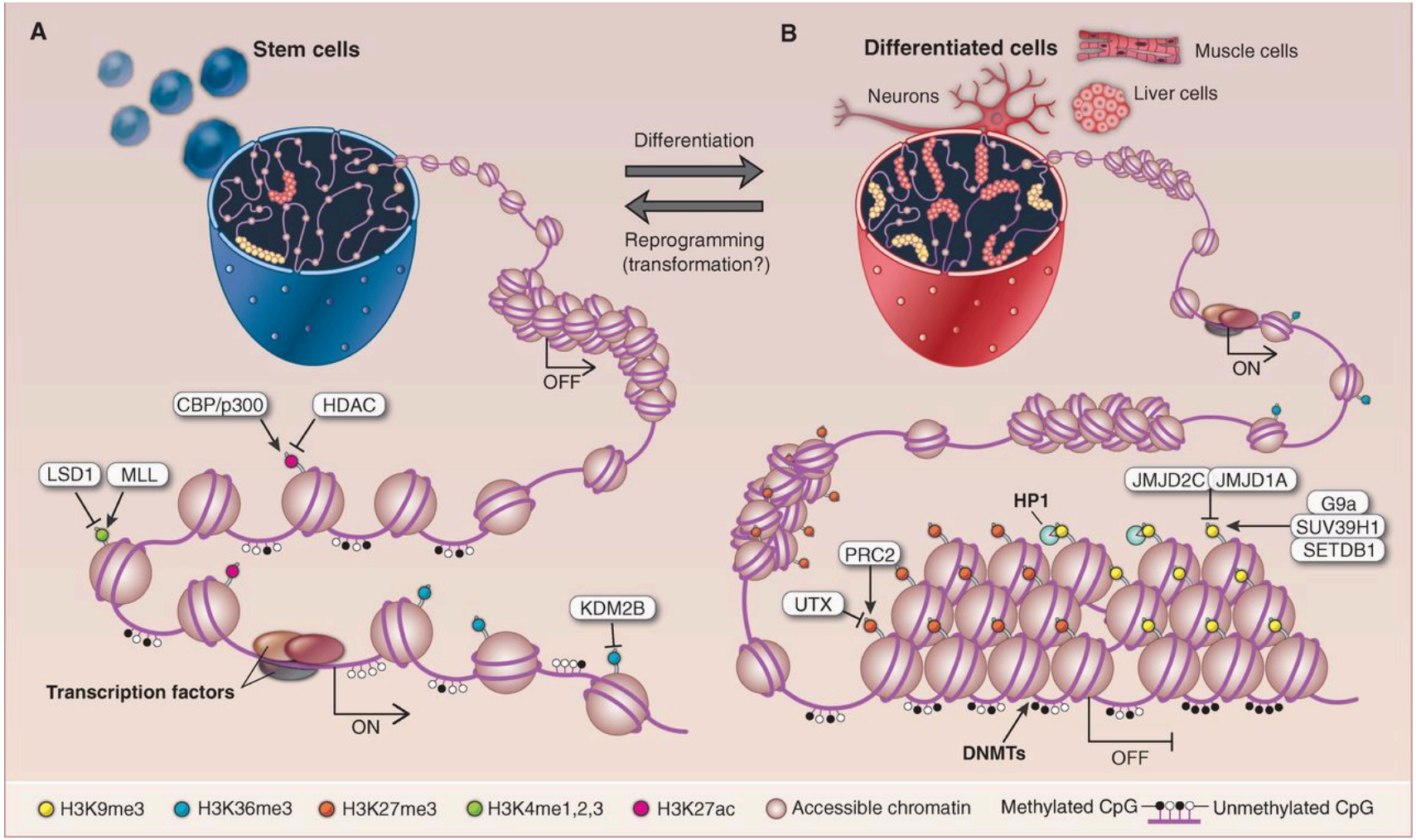
Proteomic Approaches for Epiproteome Analysis

Alan Tackett, PhD

Univ of Arkansas for Medical Sciences
Dept Biochemistry & Molecular Biology

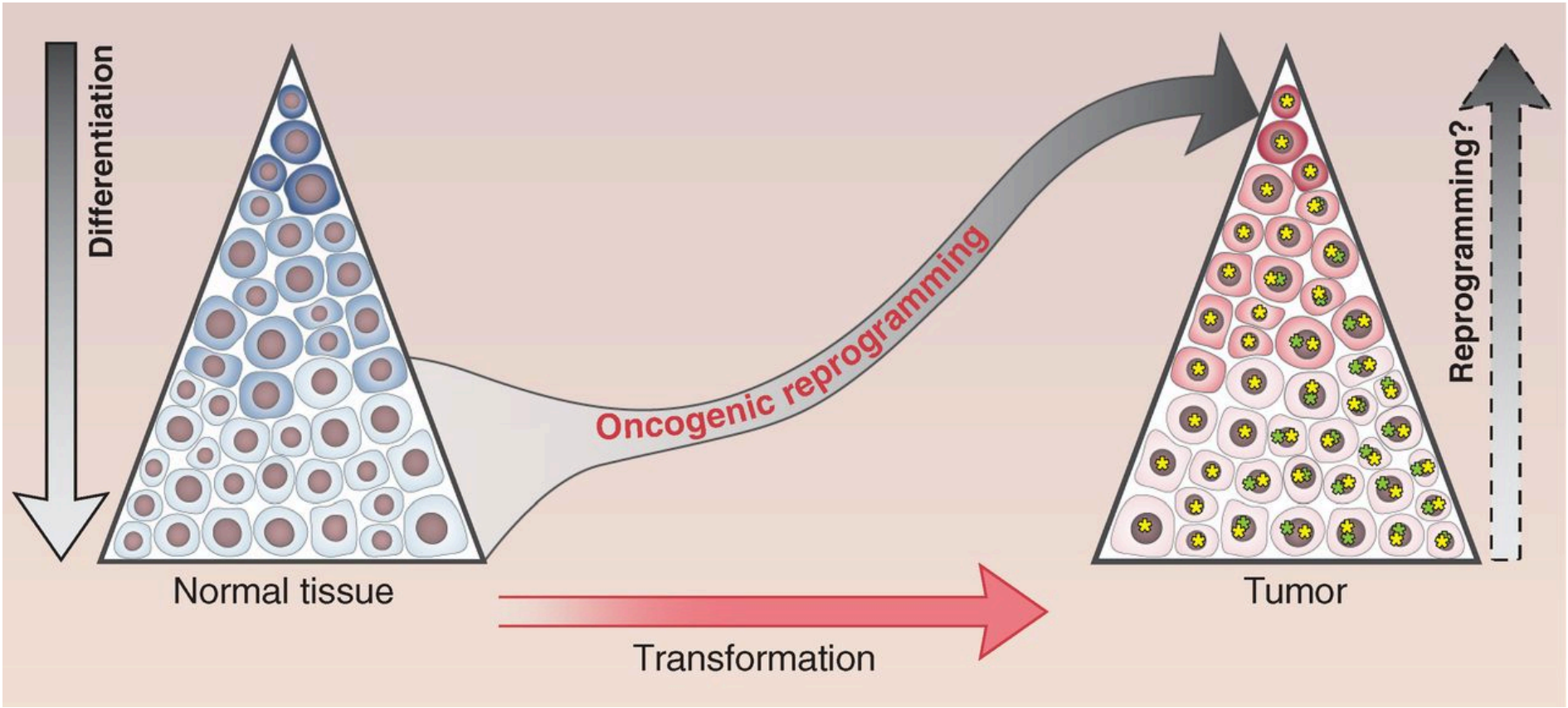


Epigenetics



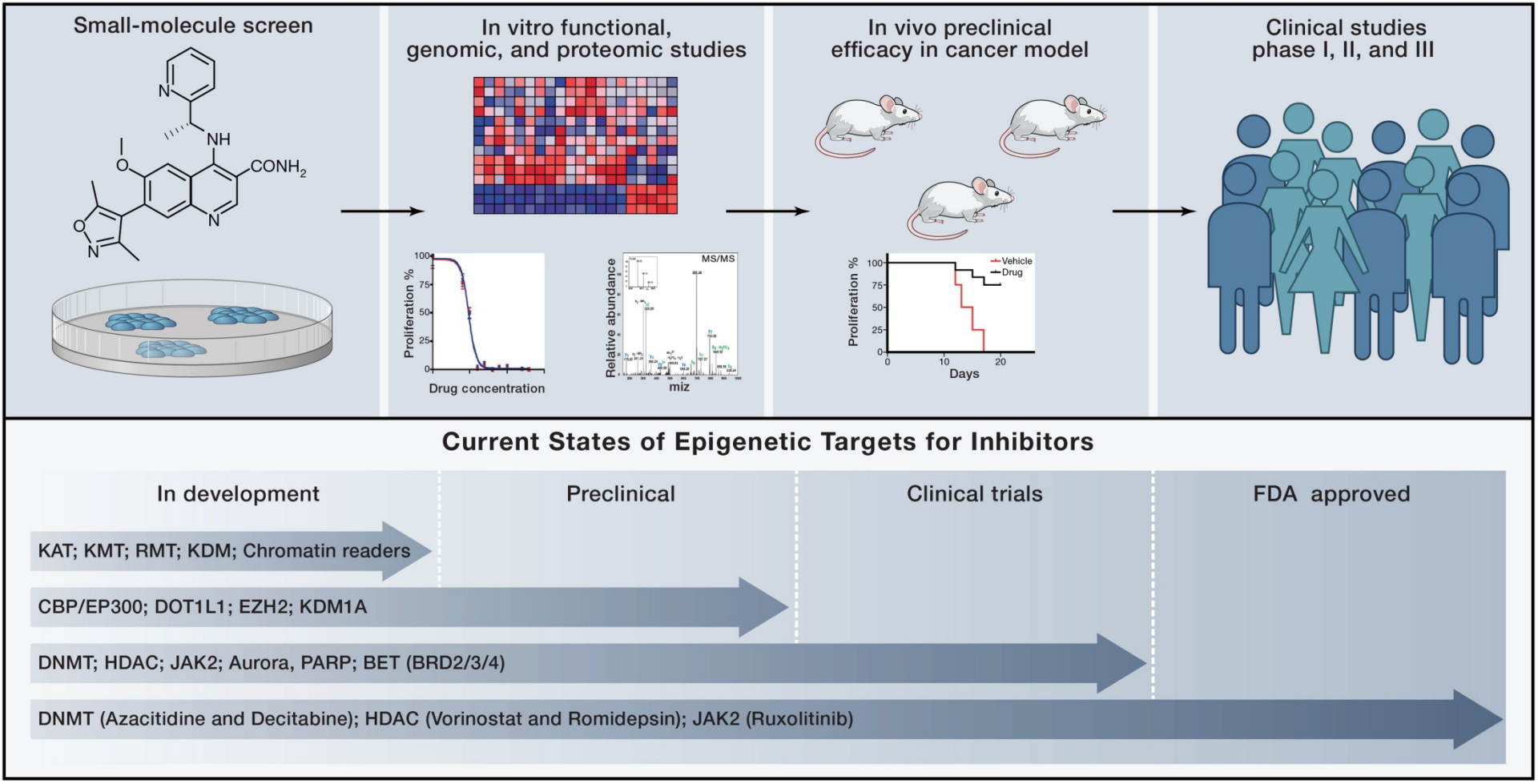
Science 29 March 2013: Vol. 339 no. 6127 pp. 1567-1570

Epigenetic Reprogramming in Cancer



Science 29 March 2013: Vol. 339 no. 6127 pp. 1567-1570

Epigenetic Inhibitors as Cancer Therapies



Source: Cell , Volume 150, Issue 1, Pages 12-27 (DOI:10.1016/j.cell.2012.06.013)

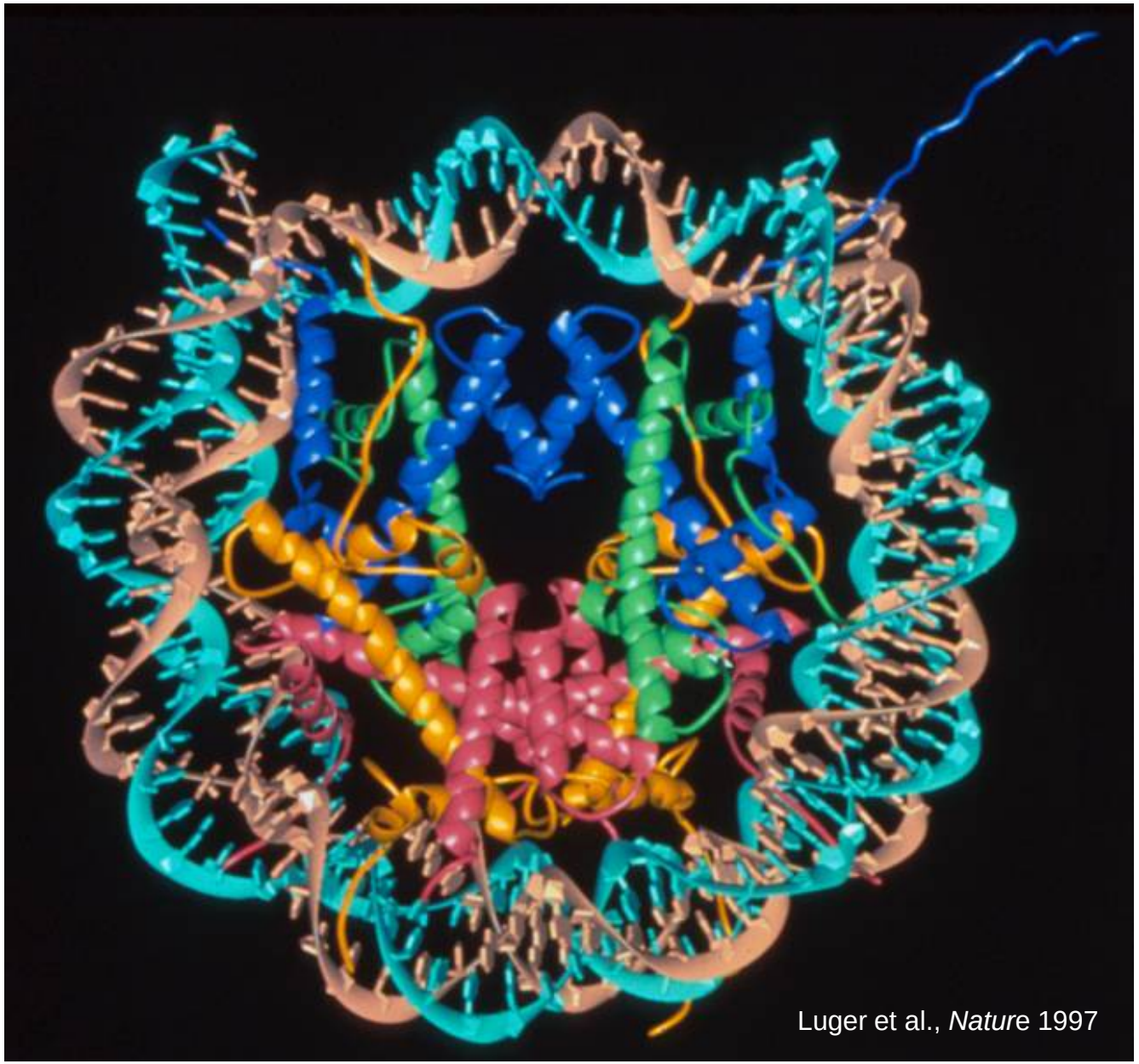
Histone epigenetics

H3

H4

H2A

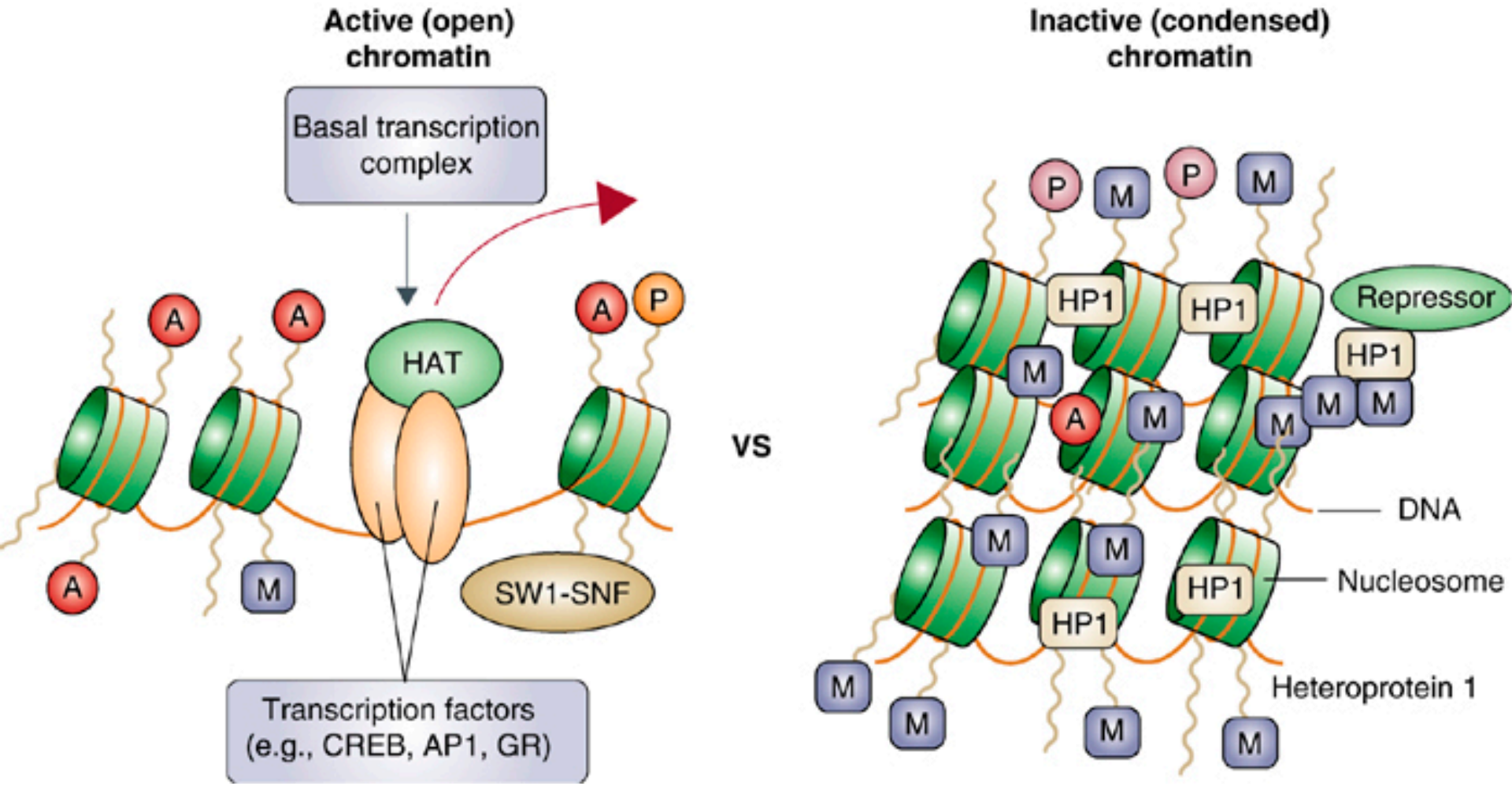
H2B



Histone Code Hypothesis

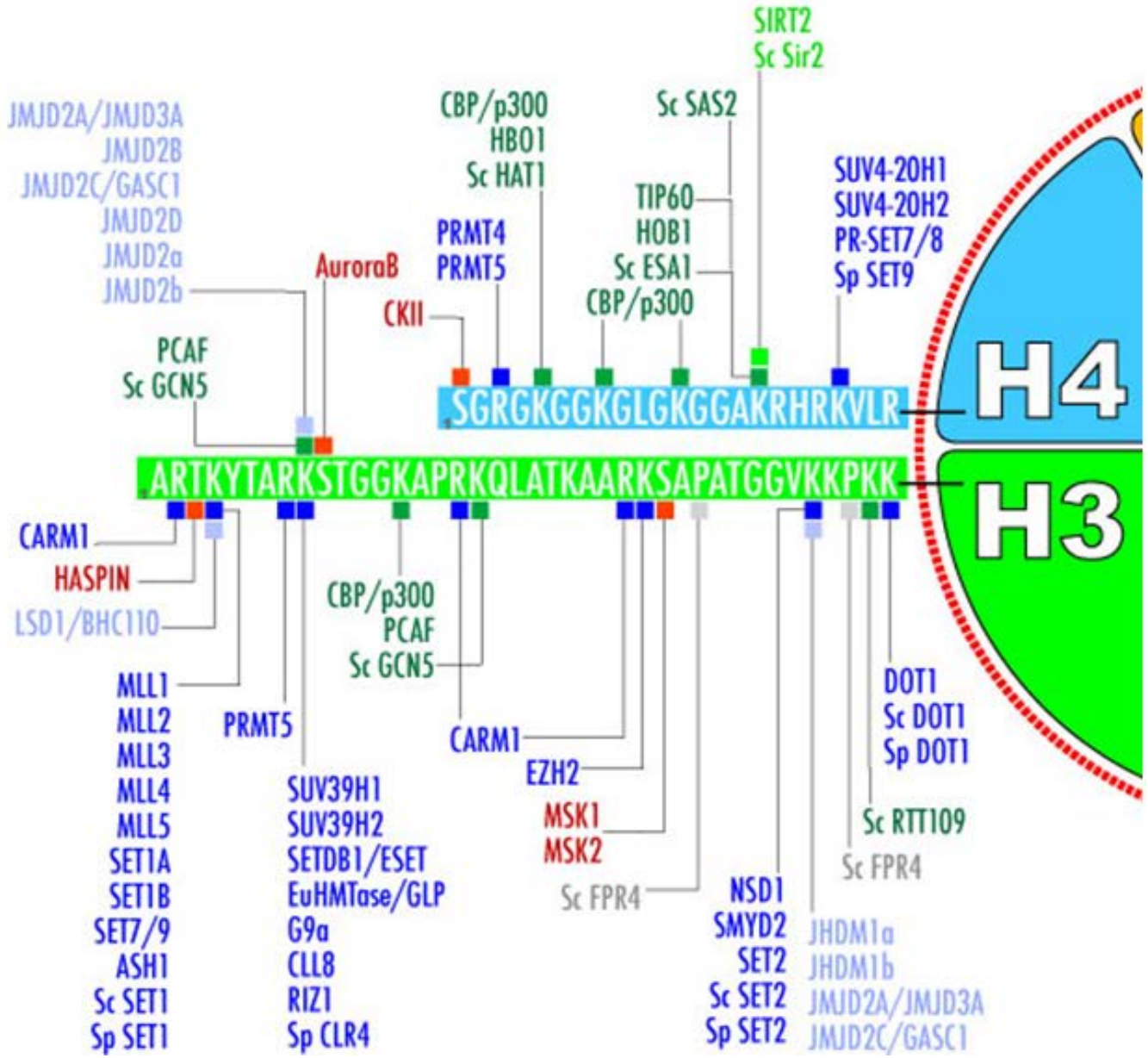


Gene Transcription in the Context of Chromatin



http://www.nature.com/npp/journal/v33/n1/images/1301544f3.jpg

Numerous Histone Epigenetic Targets



http://en.wikipedia.org/wiki/File:NucleosomeKG.jpg

Cancer Mutations Affecting Epigenetic Regulators Involved in Histone Acetylation

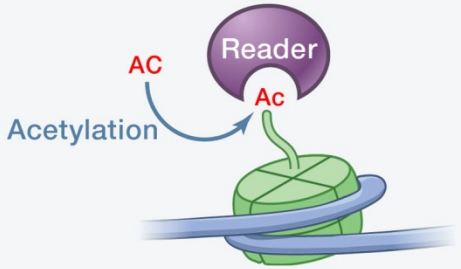
Acetyltransferases

Enzyme	Mutation	Tumor
KAT3A (CBP)*	T, N, F, M	AML, ALL, DLBCL, B-NHL, TCC
KAT3B (p300)*	T, N, F, M	AML, ALL, DLBCL, TCC, Colorectal, Breast, Pancreatic
KAT6A (MOZ)+	T	AML, MDS
KAT6B (MORF)+	T	AML, Uterine leiomyoma

Readers

Reader	Mutation	Tumor
BRD1**	T	ALL
BRD3*	T	Midline carcinoma
BRD4*	T	Midline carcinoma
TRIM33*+	T	Papillary thyroid
PBRM1*	N, F, M, S, D	Renal, Breast

* = Bromodomain
+ = PHD Finger



Source: Cell , Volume 150, Issue 1, Pages 12-27 (DOI:10.1016/j.cell.2012.06.013)

Cancer Mutations Affecting Epigenetic Regulators Involved in Histone Methylation

Methyltransferases

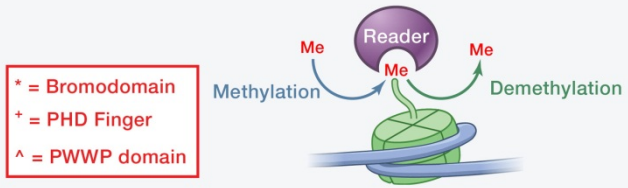
Enzyme	Mutation	Tumor
KMT2A (MLL1*+)	T, PTD	AML, ALL, TCC
KMT2B (MLL2*)	N, F, M	Medulloblastoma, Renal, DLBCL, FL
KMT2C (MLL3*)	N	Medulloblastoma, TCC, Breast
KMT3A (SETD2)	N, F, S, M	Renal, Breast
KMT3B (NSD1+^)	T	AML
NSD2+^	T	Multiple myeloma
NSD3^	T	AML
KMT6 (EZH2)	M	DLBCL, MPD, MDS

Readers

Reader	Mutation	Tumor
TRIM33+*	T	Papillary thyroid
ING1+	M, D	Melanoma, Breast
ING4+	D	HNSCC
MSH6^	M, N, F, S	Colorectal

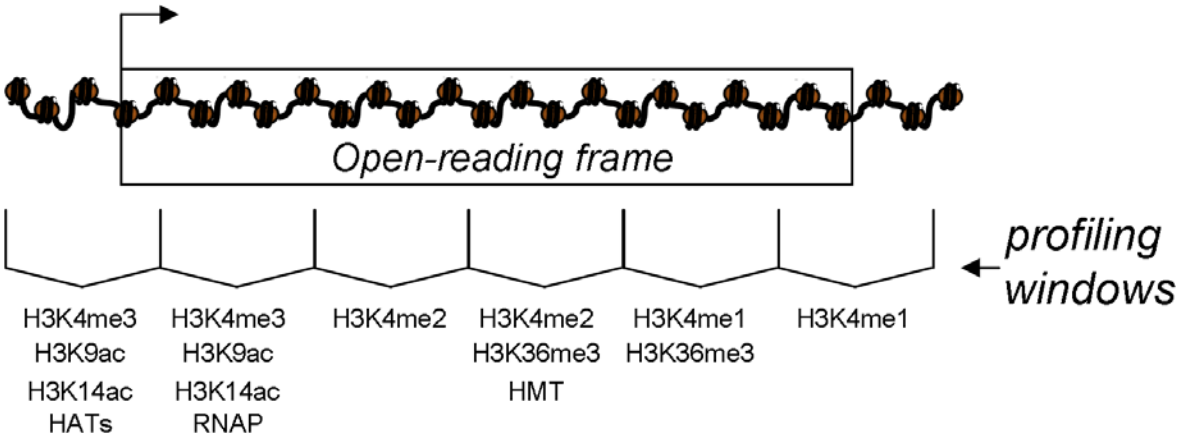
Demethylases

Enzyme	Mutation	Tumor
KDM5A (JARID1A)+	T	AML
KDM5C (JARID1C)+	N, F, S	Renal
KDM6A (UTX)	D, N, F, S	AML, TCC, Renal, Oesophageal, Multiple myeloma

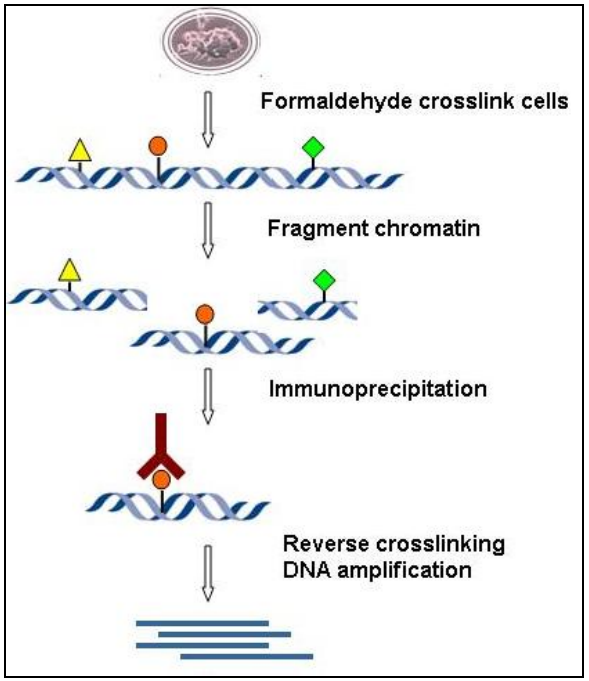


Source: Cell , Volume 150, Issue 1, Pages 12-27 (DOI:10.1016/j.cell.2012.06.013)

Local Epiproteome



Methods for locus-specific epiproteome measurements

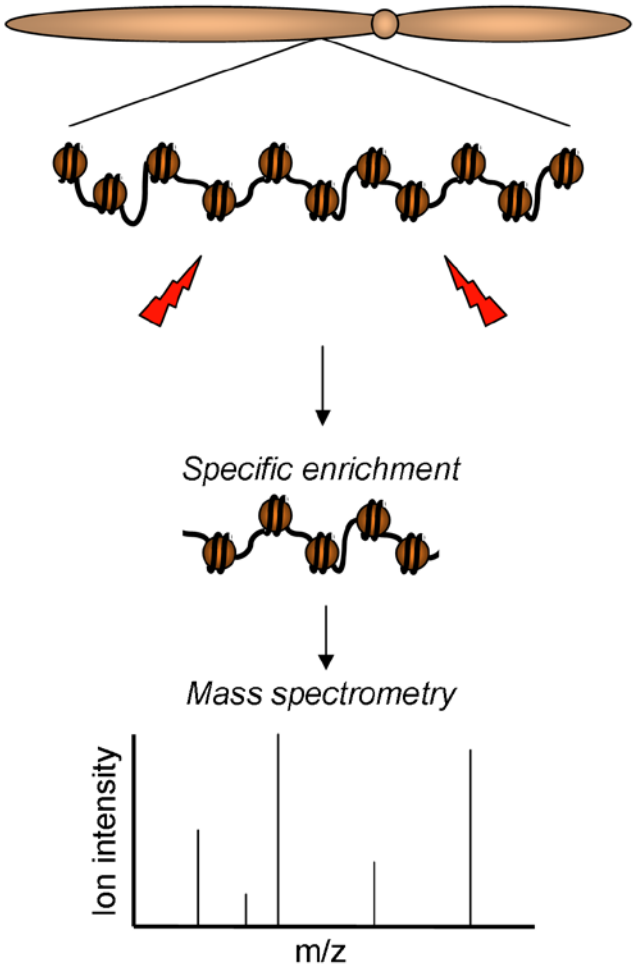


ChIP

Limitations with Standard ChIP

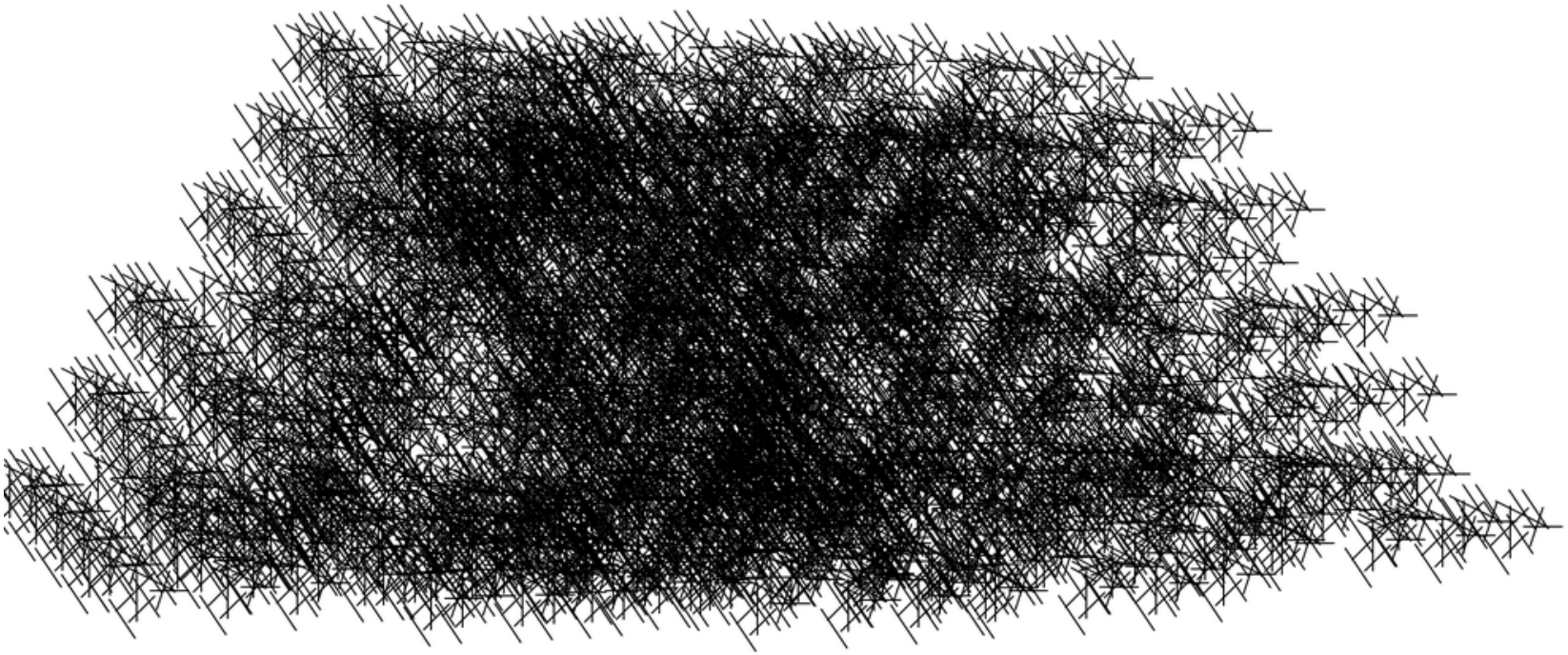
1) Target one protein/PTM at a time
2) Co-localization of proteins/PTMs can only be inferred
3) No information of the combinatorial set of PTMs on single histone molecules
4) You have to know what you are targeting

Chromatin Affinity Purification with Mass Spectrometry

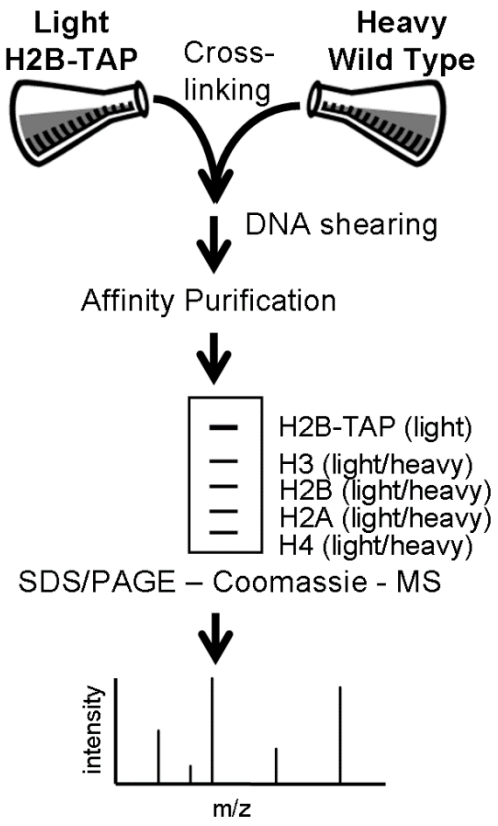


Needle in a Haystack (1:3,000,000 – that's a lot of straw)

Let's start with budding yeast 1:12,000

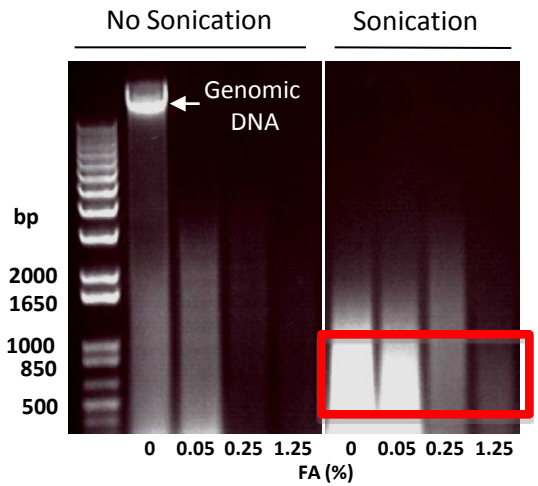


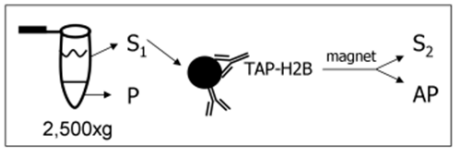
Preventing Histone Exchange During Chromatin Isolation

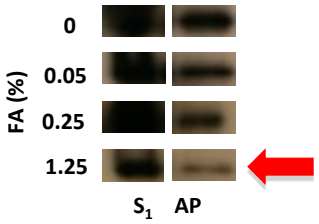


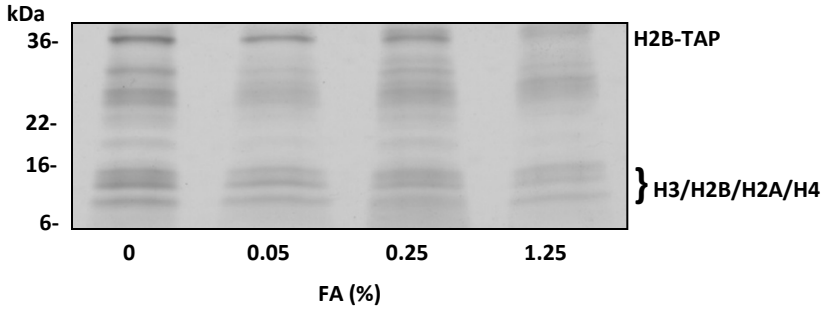
Transient I-DIRT (isotopic differentiation of interactions as random or targeted)

Solubilizing and Purifying Cognate Chromatin Sections



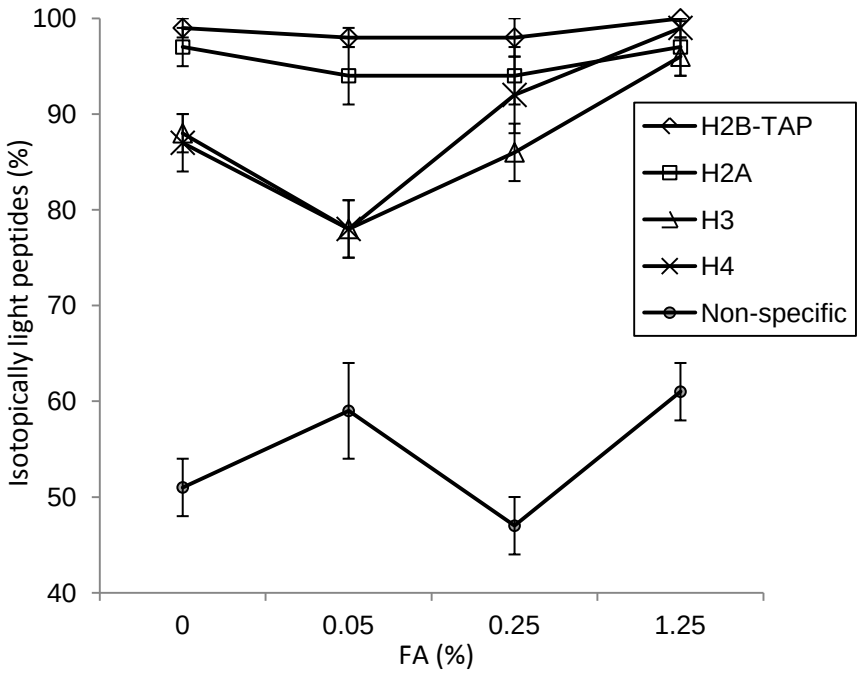






Efficient purification of chromatin sections requires optimized *in vivo* cross-linking

Cross-linking is Necessary to Prevent Histone Exchange



1.25% formaldehyde cross-linking is necessary to prevent histone exchange in yeast synthetic media – level is dependent on reactive content of media

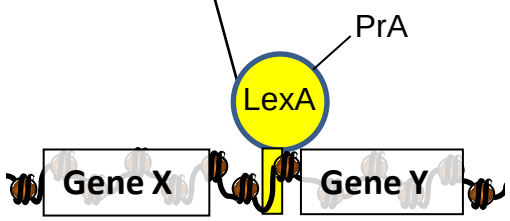
ChAP Technology

Three Platforms for Single Locus Purification

1st Generation

ChAP-MS

Genomic Engineering of a LexA binding site



2nd Generation

TAL-ChAP-MS

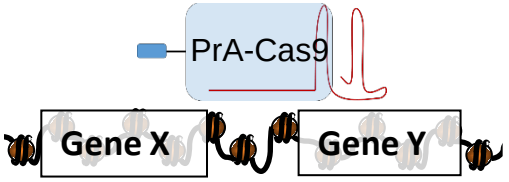
- No genomic engineering
- Transcription activator-like effector nuclease (TALEN)
- 18 tandem domains of 34 amino acids each recognize single nucleotides
- Targets 18mer



3rd Generation

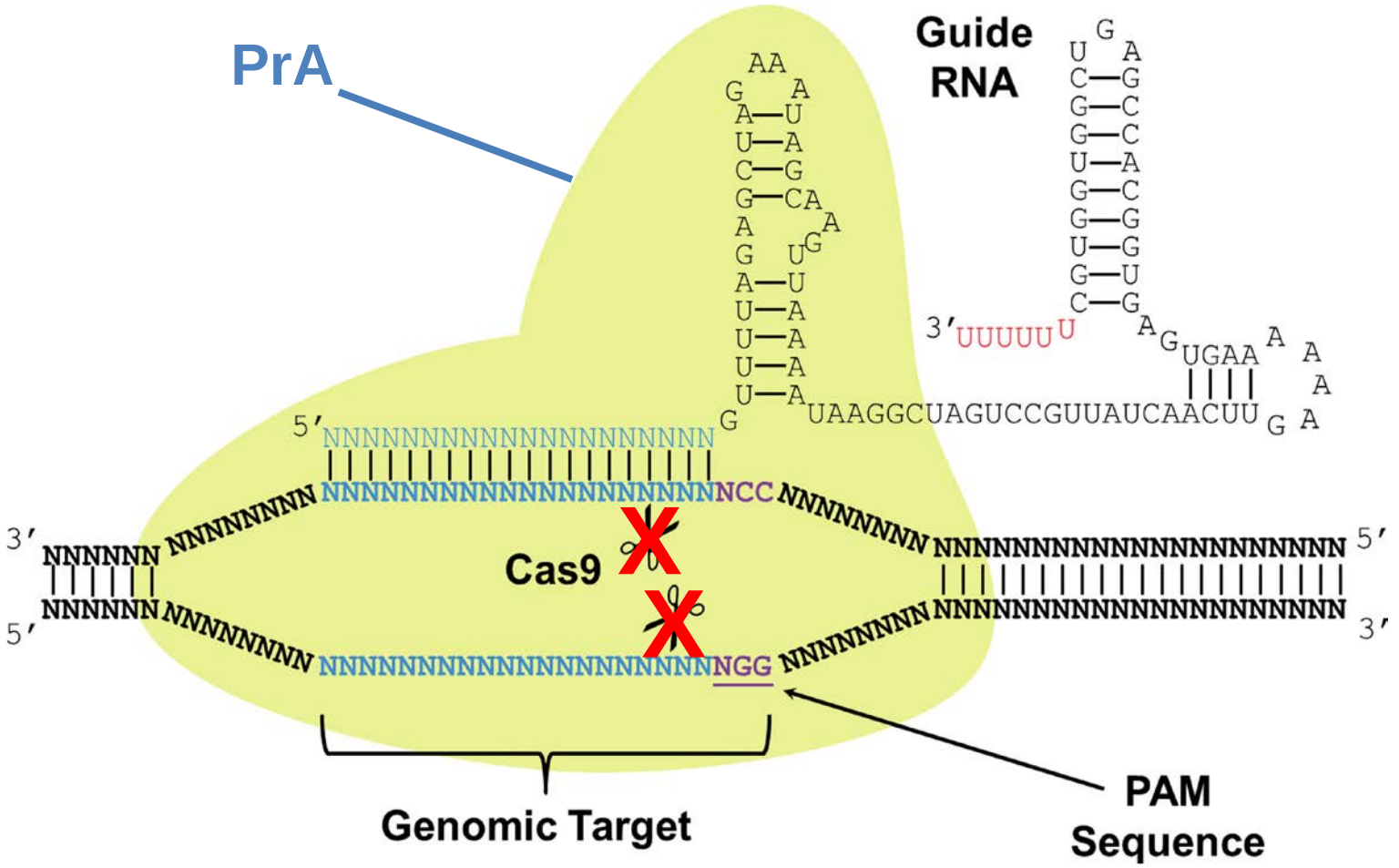
CRISPR-ChAP-MS

- No genomic engineering
- Clustered regularly interspaced short palindromic repeats (CRISPR)
- RNA-guided nuclease
- Catalytically dead nuclease available
- Targets 20mer



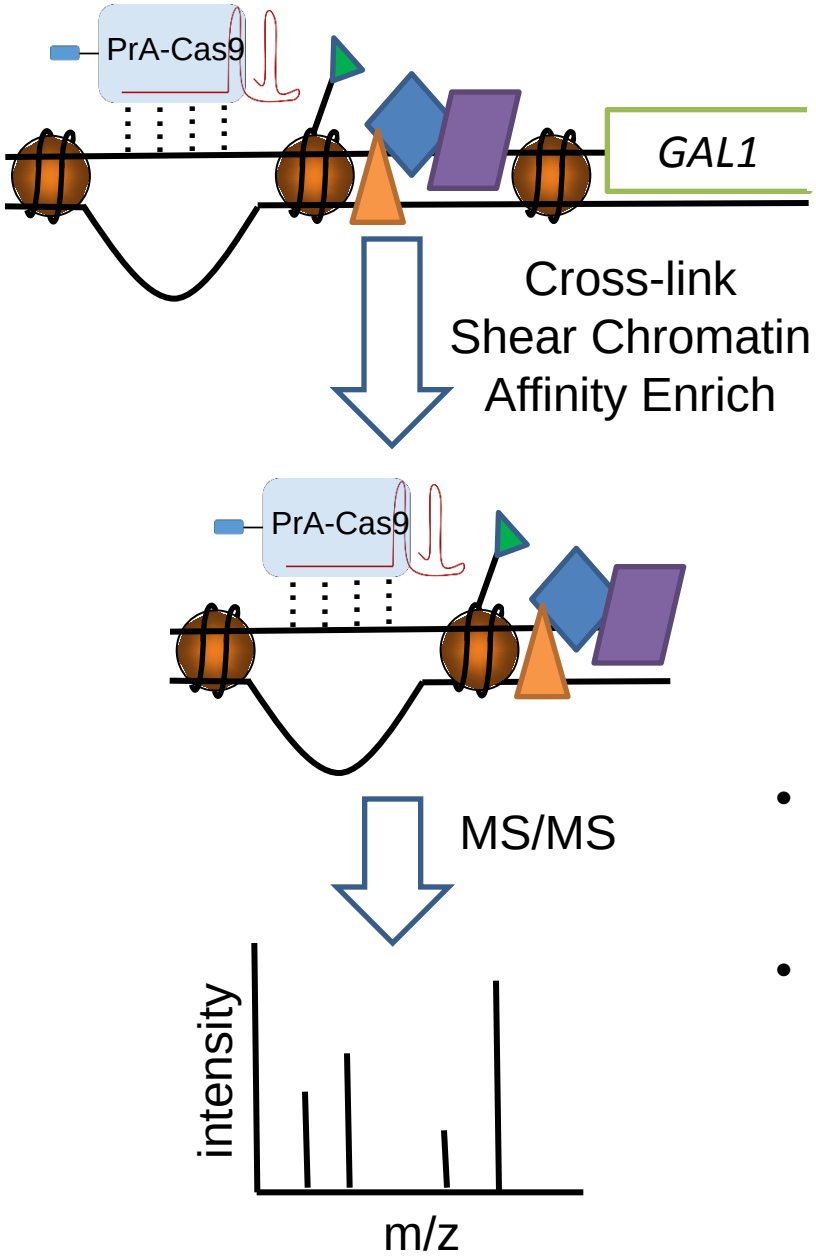
Byrum et al (2011) *JIOMICS*
Byrum et al (2011) *J Clin Bioinfor*
Byrum et al (2012) *Cell Reports*
Waldrip et al (2014) *Epigenetics*
Byrum et al (2013) *Nuc Acids Res*
Byrum et al (2014) *Meth Mol Biol*
US Patent 61/726,936
R01DA025755 & R01GM106024 (AJT)

CRISPR-ChAP-MS



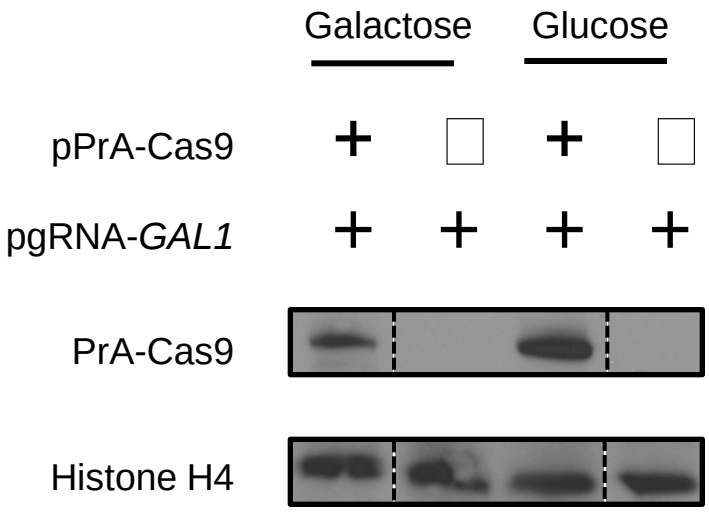
4336–4343 Nucleic Acids Research, 2013, Vol. 41, No. 7

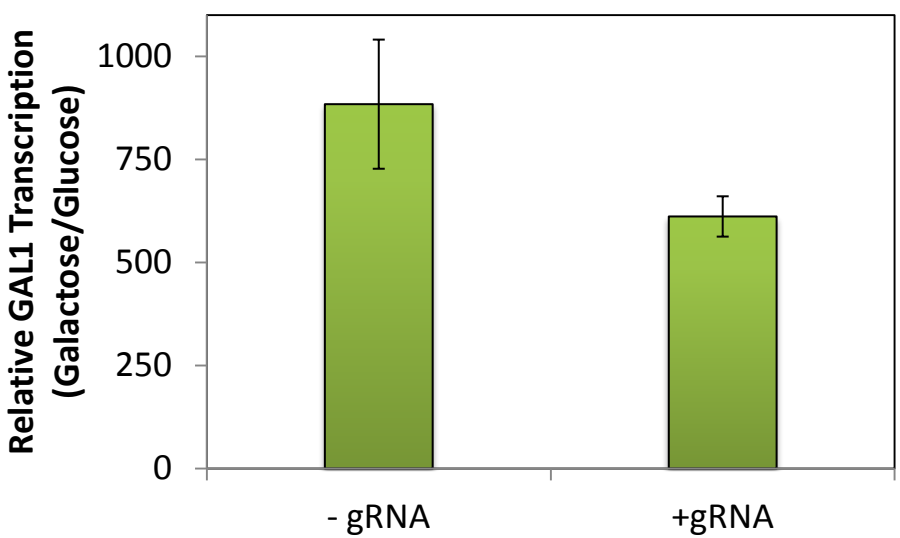
Measurement of Epiproteome at *GAL1* Promoter Chromatin Using CRISPR-ChAP-MS



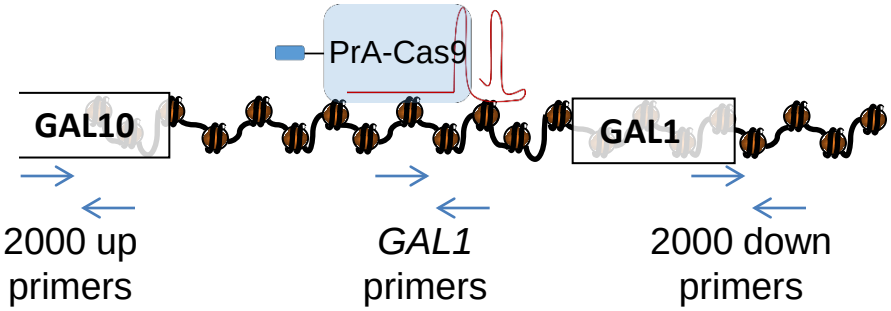
- Glucose sets an inactive chromatin state
- Galactose sets transcriptionally active

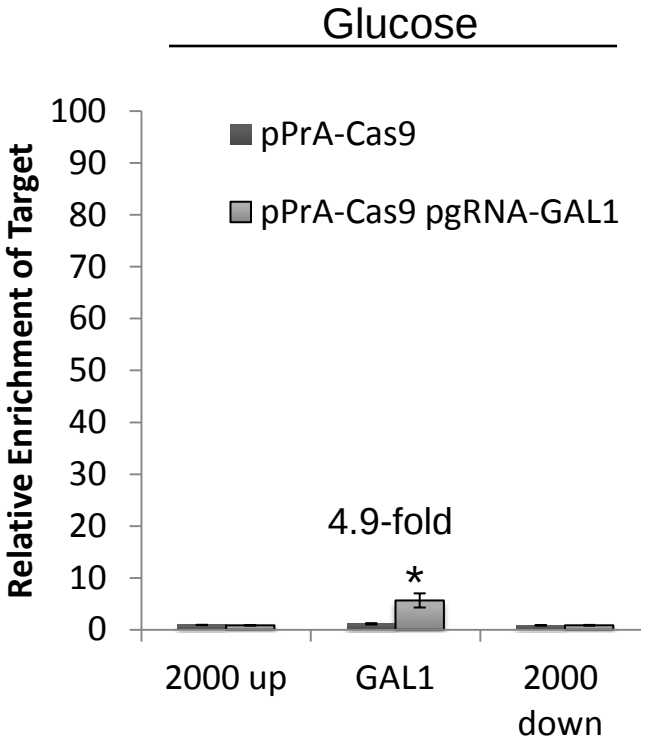
Expression of the PrA-Cas9/gRNA



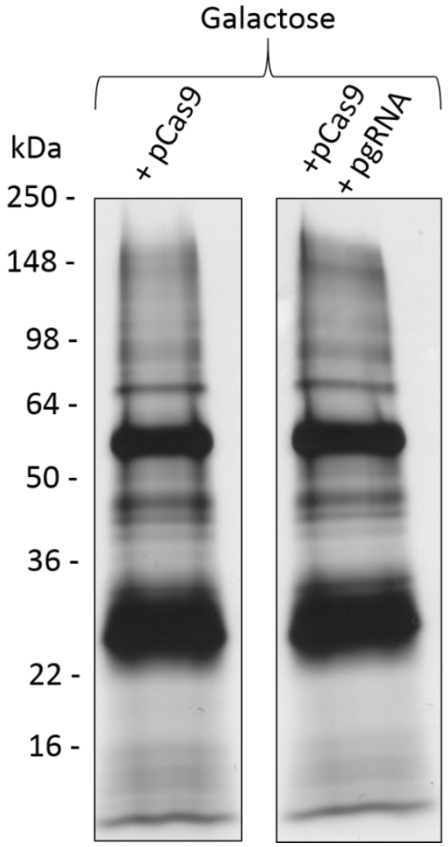


Chromatin Enrichment by PrA-Cas9/gRNA



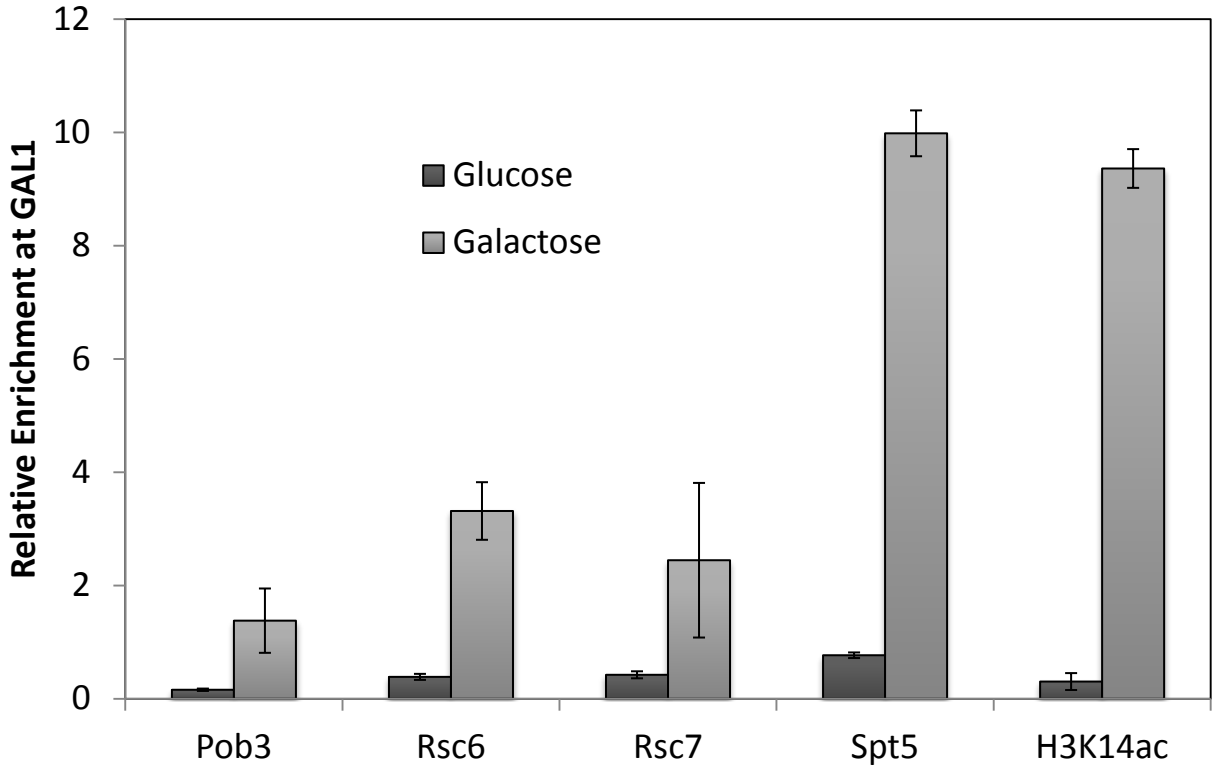


Proteomic Analysis of *GAL1* Promoter Chromatin



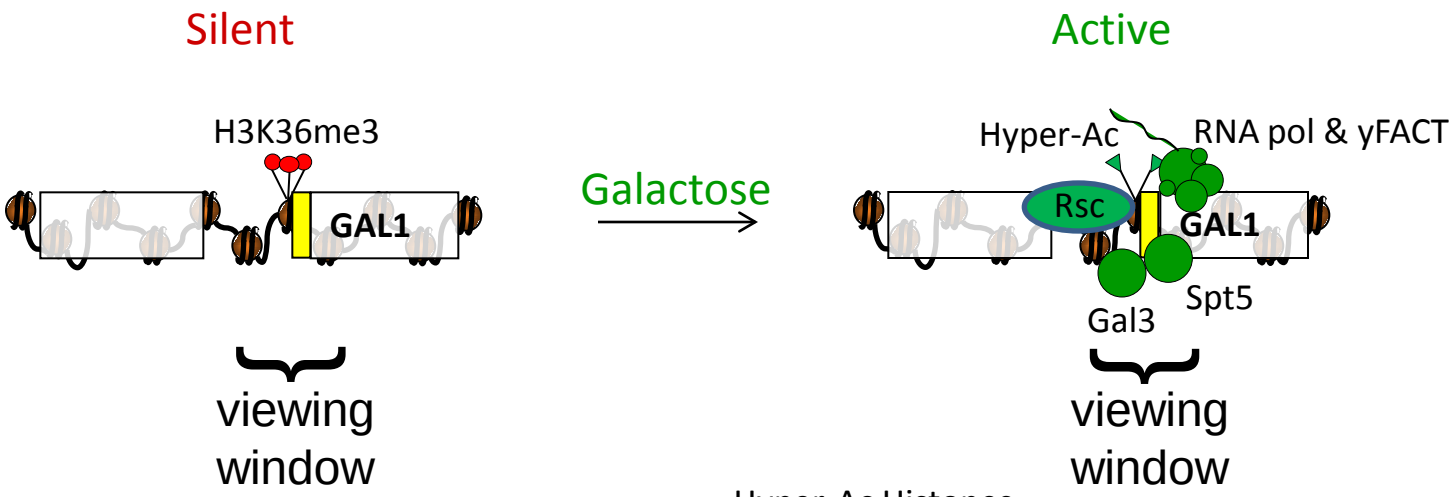
- Chromatin isolated from 10^9 cells
- 1832 proteins identified
- Spectral count comparison revealed 86 specific
- 11 of the 86 had annotated functions on chromatin
- H3K23ac and H3K14ac

ChIP Validation of CRISPR-ChAP-MS Results



Chromatin Upstream of *GAL1*

All Three ChAP-MS Platforms



Hyper-Ac Histones

H3K14ac

H3K23ac

H3K9acK14ac

H3K18acK23ac

H4K5acK8ac

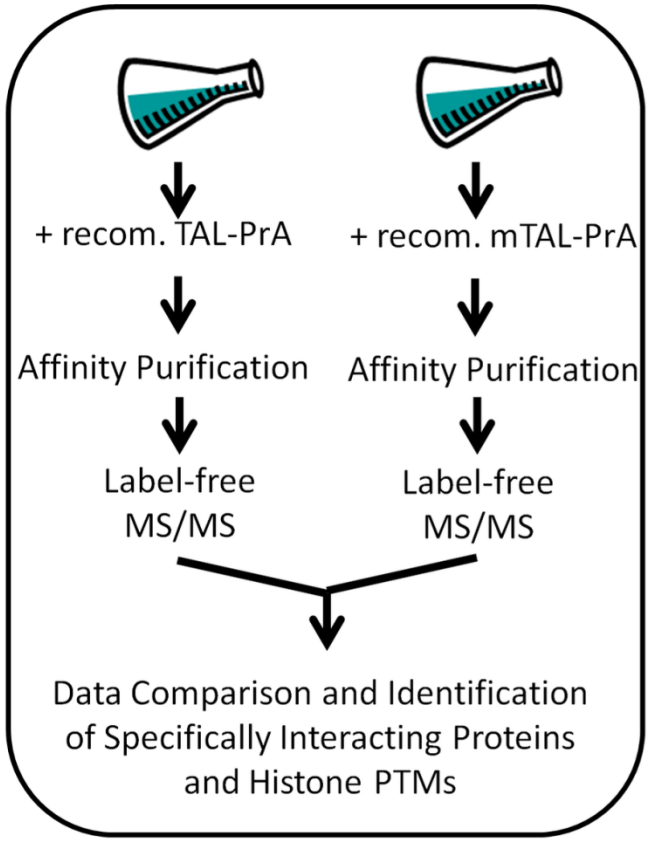
H4K12acK16ac

} Only approach to obtain combinatorial info

Application of ChAP-MS to Melanoma Cells

- Currently targeting promoter chromatin of
  - EZH2
  - RUNX3
  - E-cadherin
  - DAB2IP
  - BCL2
- WM115 vs WM266-4 melanoma cell lines
- FFPE patient melanoma
  - Fourth Generation ChAP-MS

rTAL-ChAP-MS



People Responsible…..

<u>Tackett Lab</u>
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Deepa Sengupta
Zach Waldrip
Kirk West
Lauren Davis
Lisa Orr

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Jennifer Hunt, MD

<u>UAMS Proteomics Facility</u>
Rick Edmondson, PhD
Sam Mackintosh, PhD
Linley Moreland, MS
Lisa Orr

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NIH R33CA173264(AJT)

Kevin Raney's Lab
UAMS

Sean Taverna's Lab
at Johns Hopkins
Medical School



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