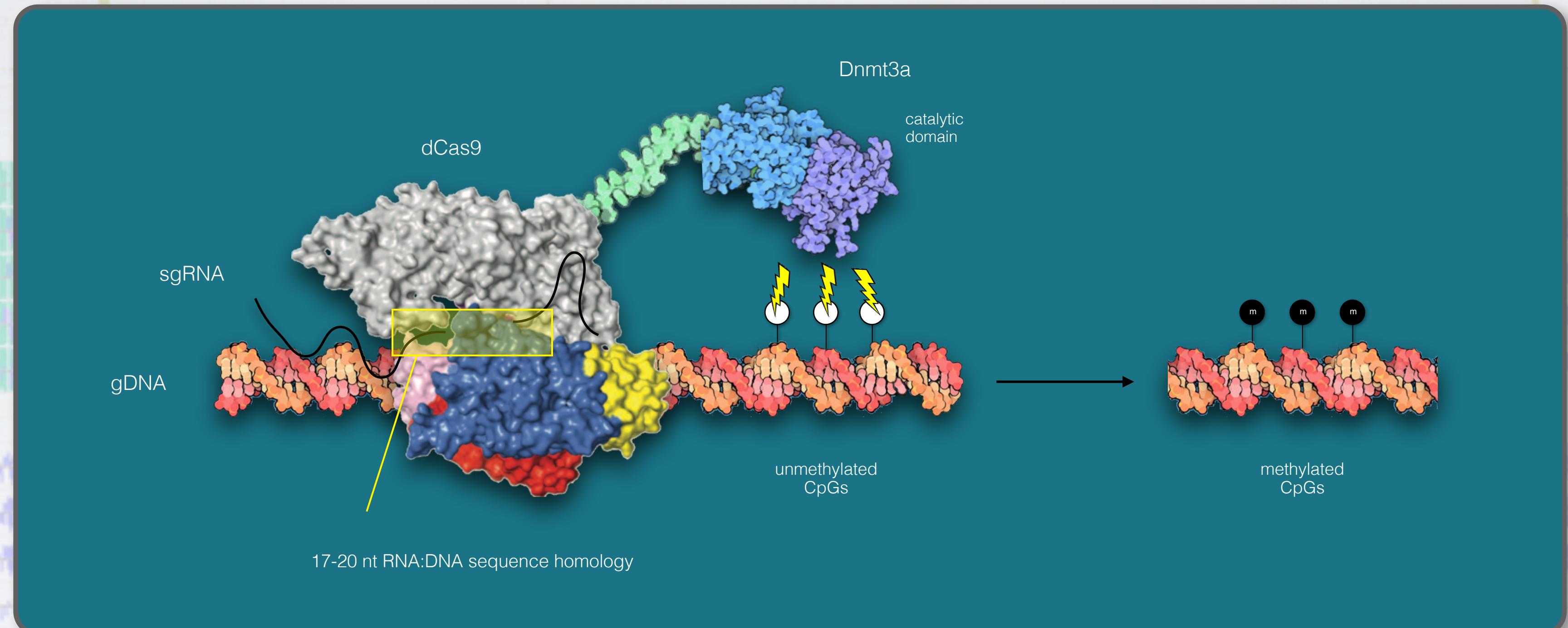


Building a CRISPR/dCas9 tool for targeted epigenome editing

SCIE5508 Workshop



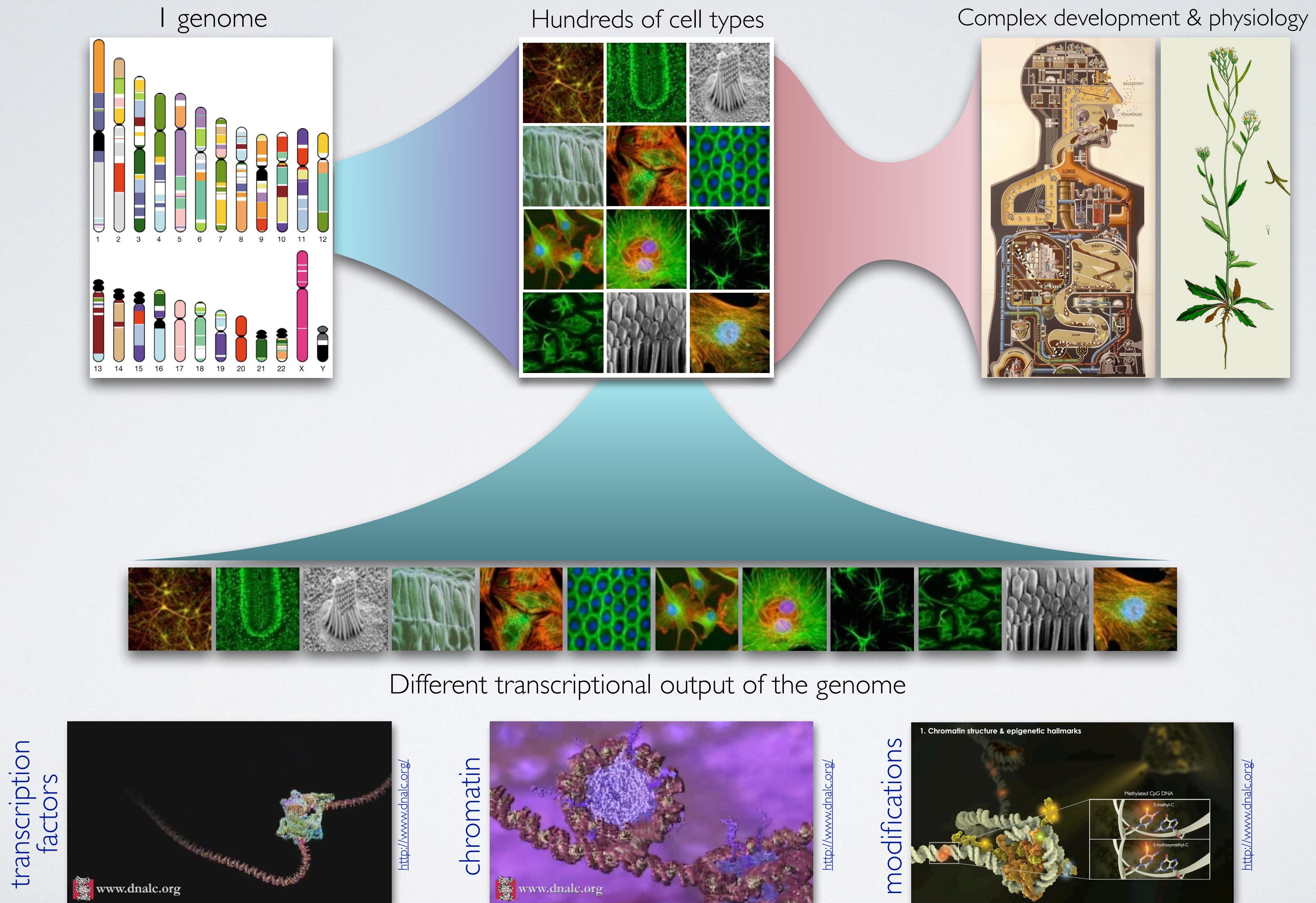
Ryan Lister

listerlab.org

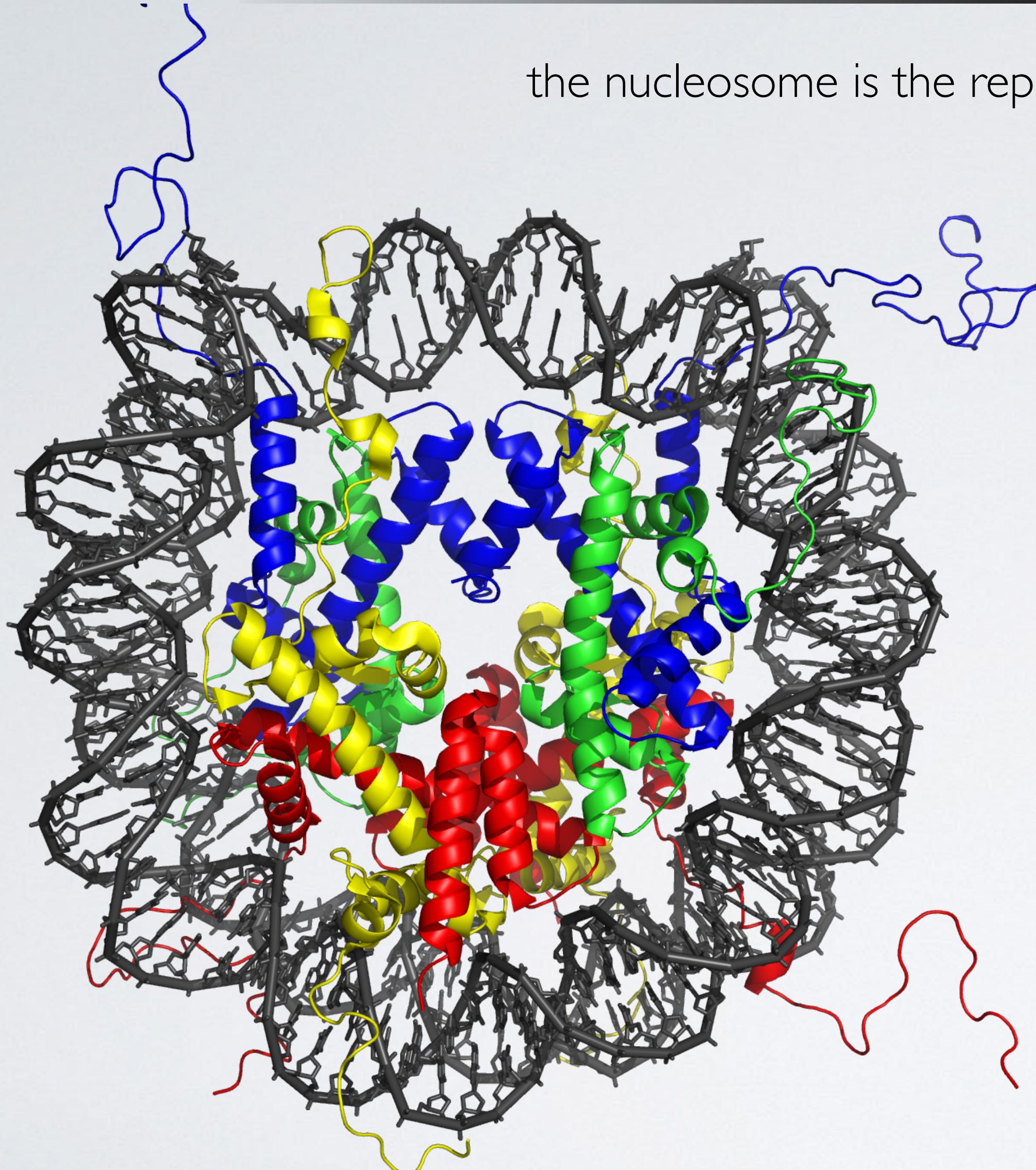


The University of
Western Australia

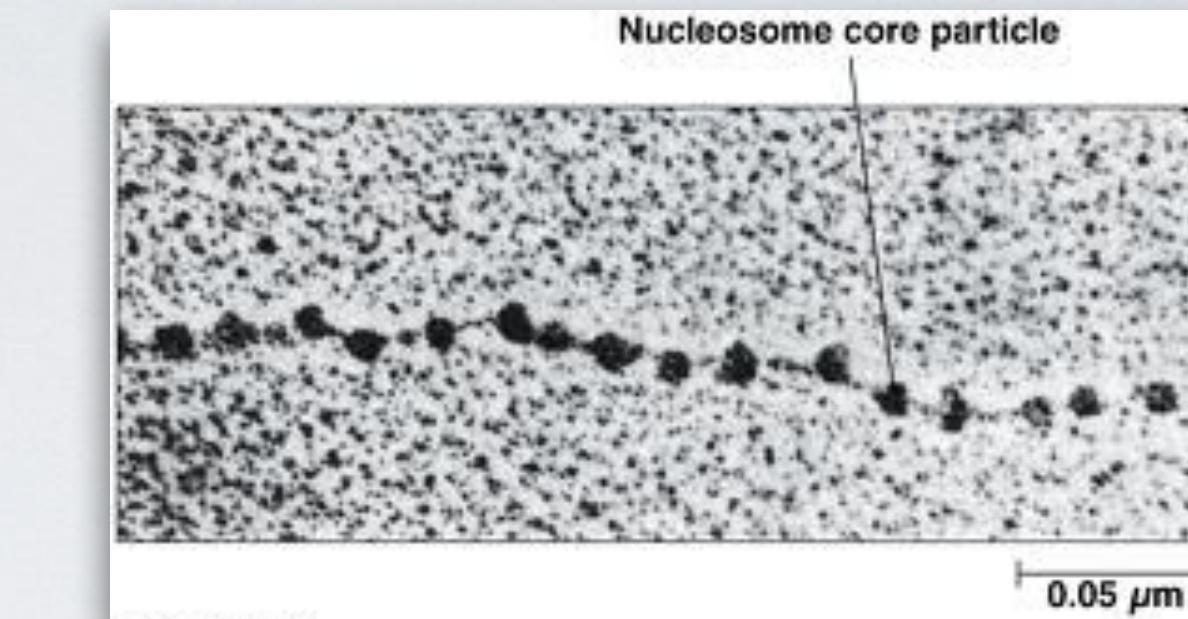
Transcriptional output of the genome



DNA is packaged with protein - the nucleosome



the nucleosome is the repeating unit of chromatin



DNA binds to histone proteins H2A, H2B, H3, H4 (2 each), tightly wrapping around the histone octamer to form a nucleosome

H2A, H2B, H3, H4 share the histone fold, but have diverse tails

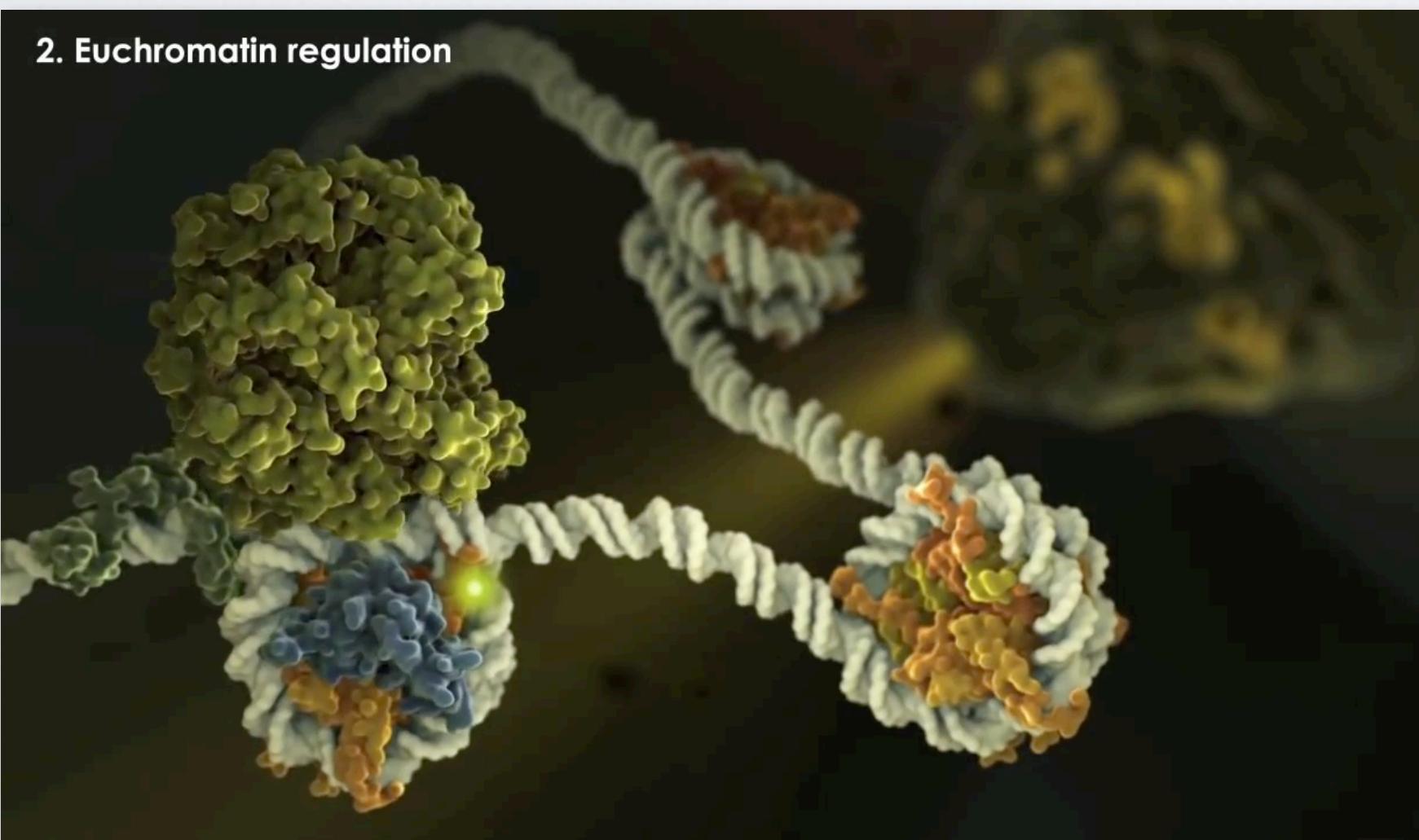
The N-terminal tails protrude away from the nucleosome core

DNA complexed with protein is called **chromatin**

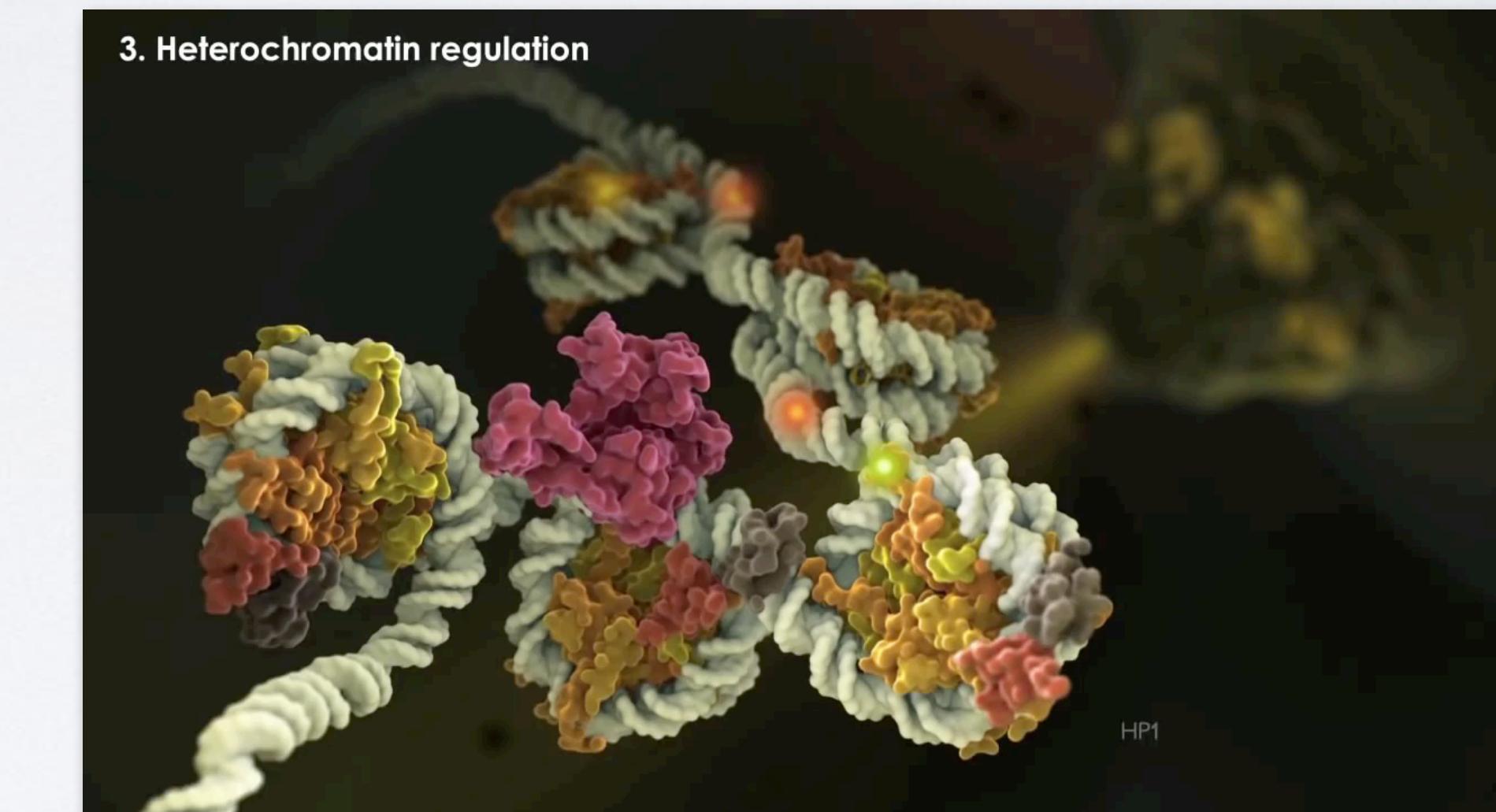
Euchromatin and heterochromatin

But if the genome was always all highly compressed, it would be inaccessible to transcriptional machinery

Euchromatin - relaxed chromatin,
transcriptionally active



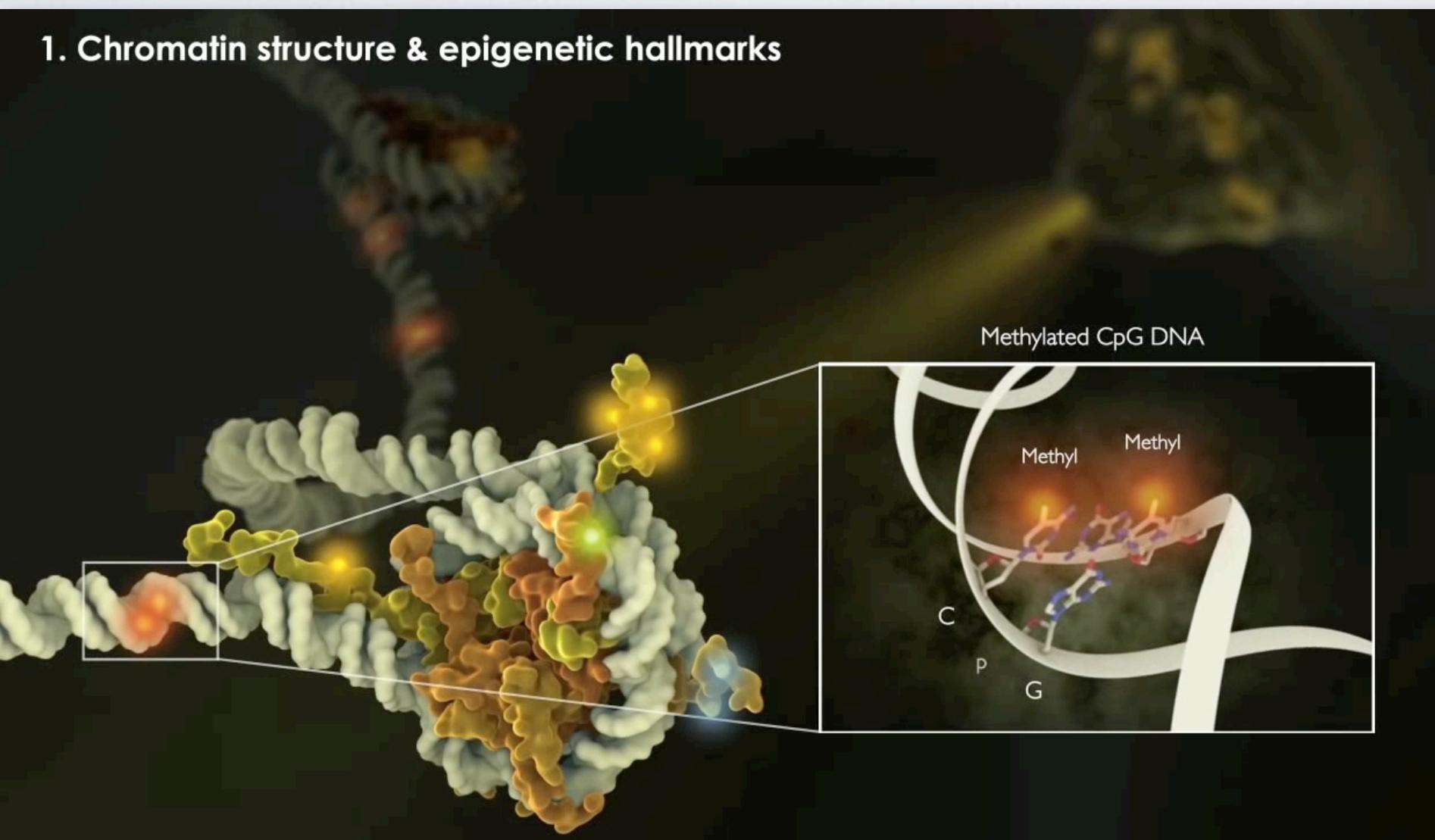
Heterochromatin - compressed
chromatin, transcriptionally inactive



<https://www.youtube.com/watch?v=nygyUMODV7Y>

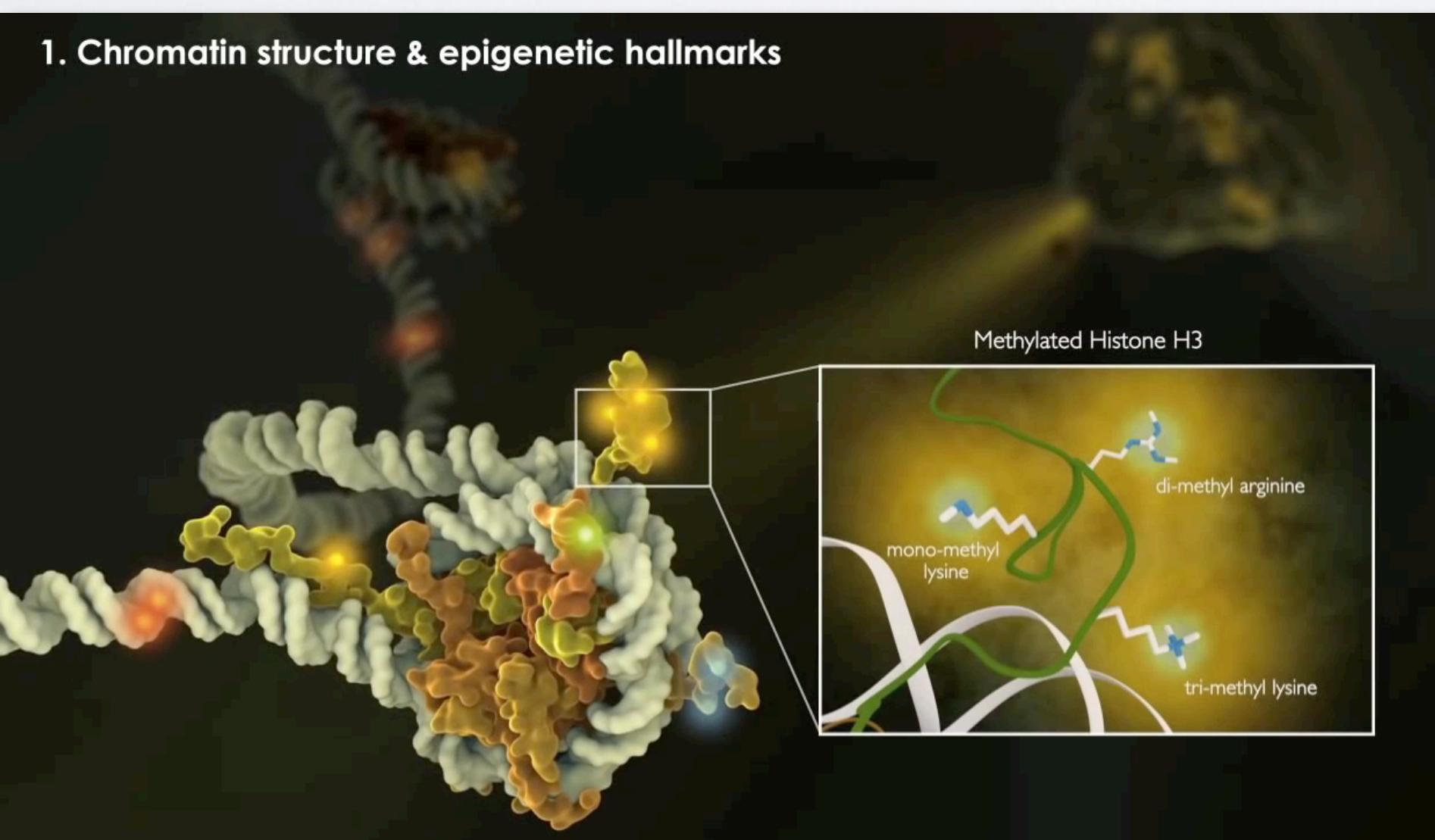
The cell can dynamically modulate a region of genome between euchromatin and heterochromatin state

DNA and histones can be chemically modified

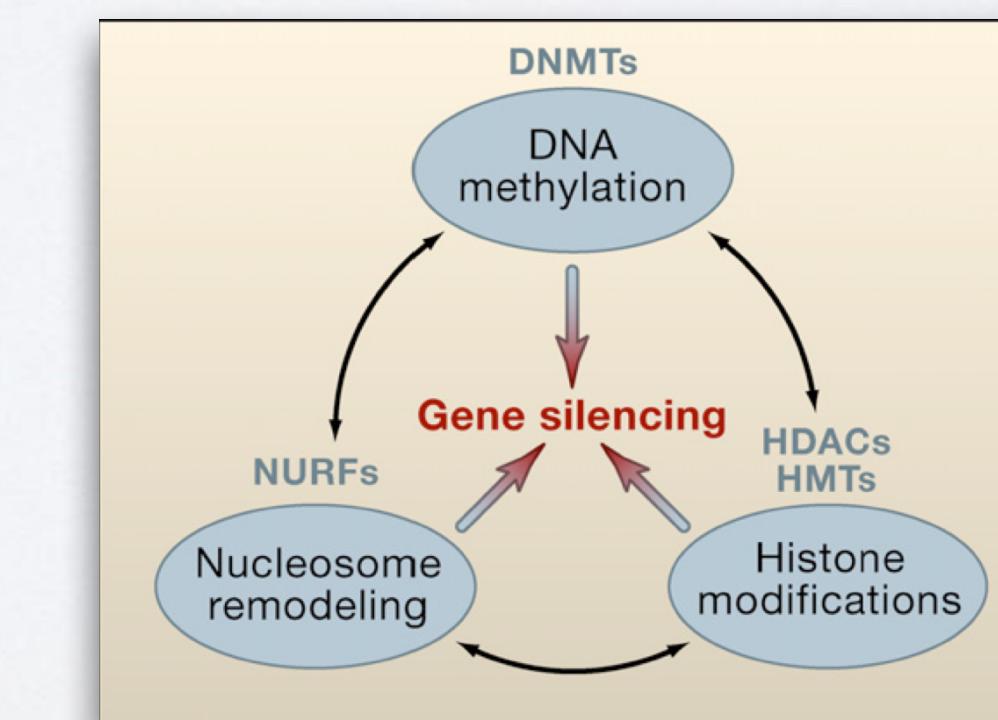


DNA methylation

<https://www.youtube.com/watch?v=nygyUMODV7Y>

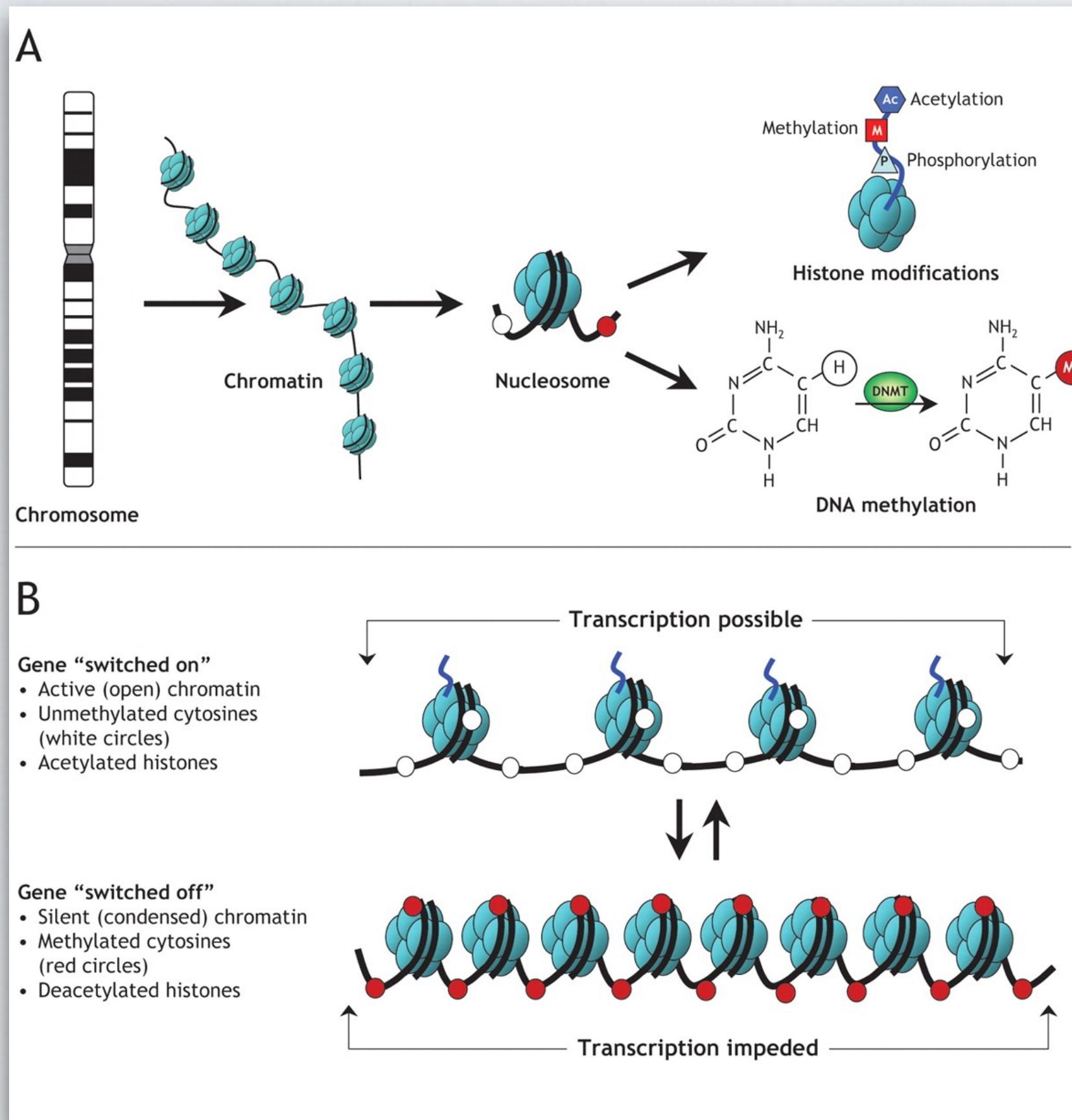


Histone post-translational modification



Epigenetic modifications of the genome

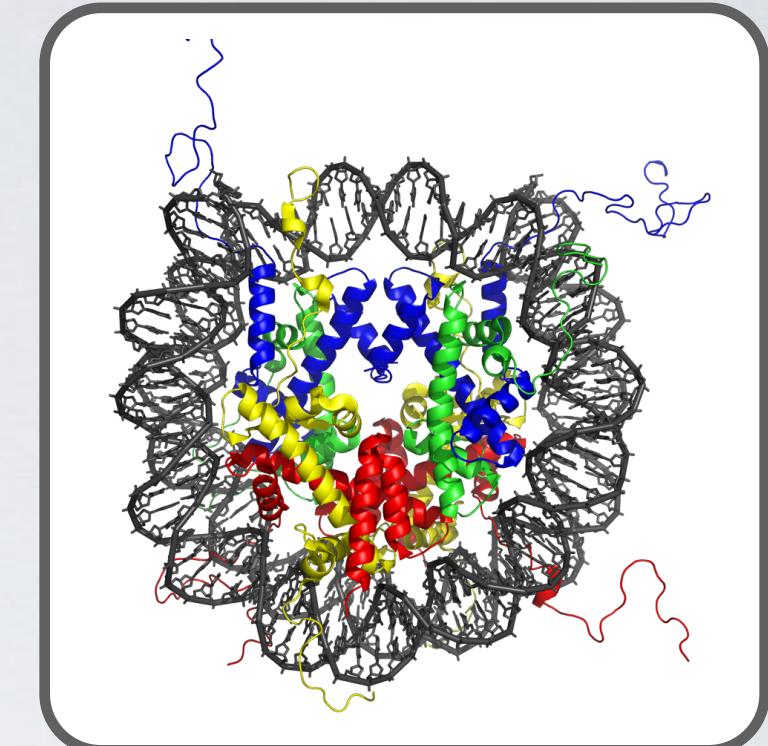
Epigenetic states are “*the structural adaptation of chromosomal regions so as to register, signal or perpetuate altered activity states*” (Adrian Bird)



Histone modifications

Methylation (H3K4me1, H3k4me3, etc)

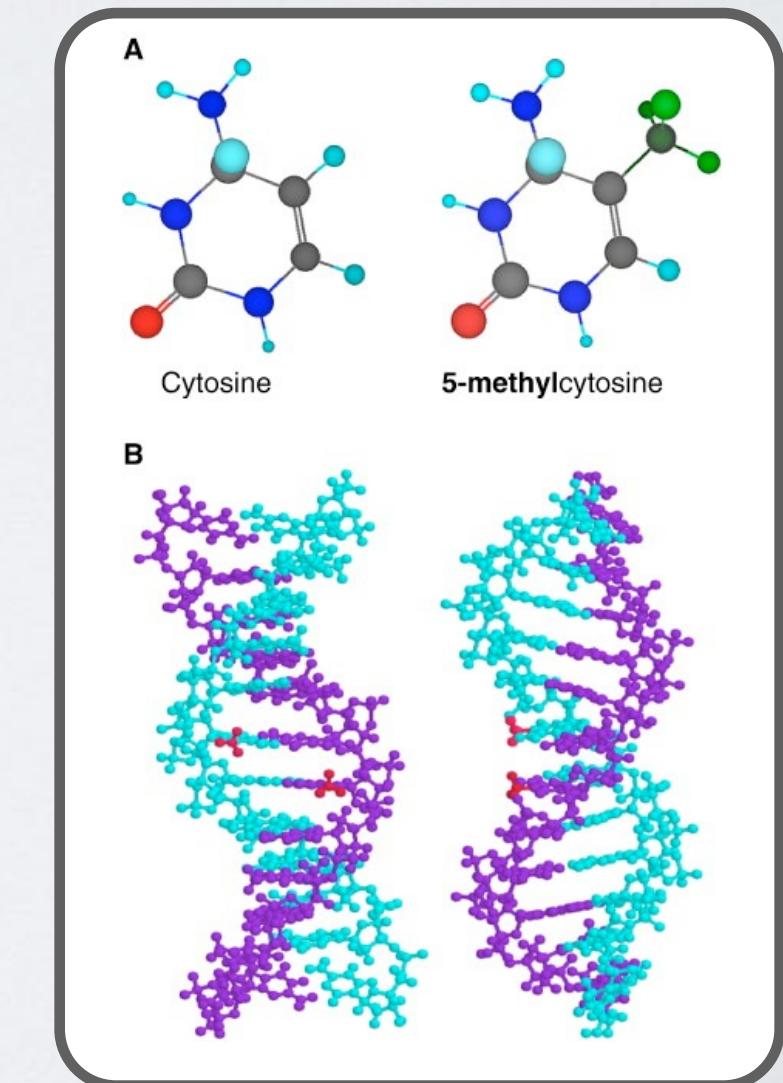
Acetylation (H3K27ac), phosphorylation etc.



DNA methylation

5-methylcytosine (mC)

5-hydroxymethylcytosine (hmC)

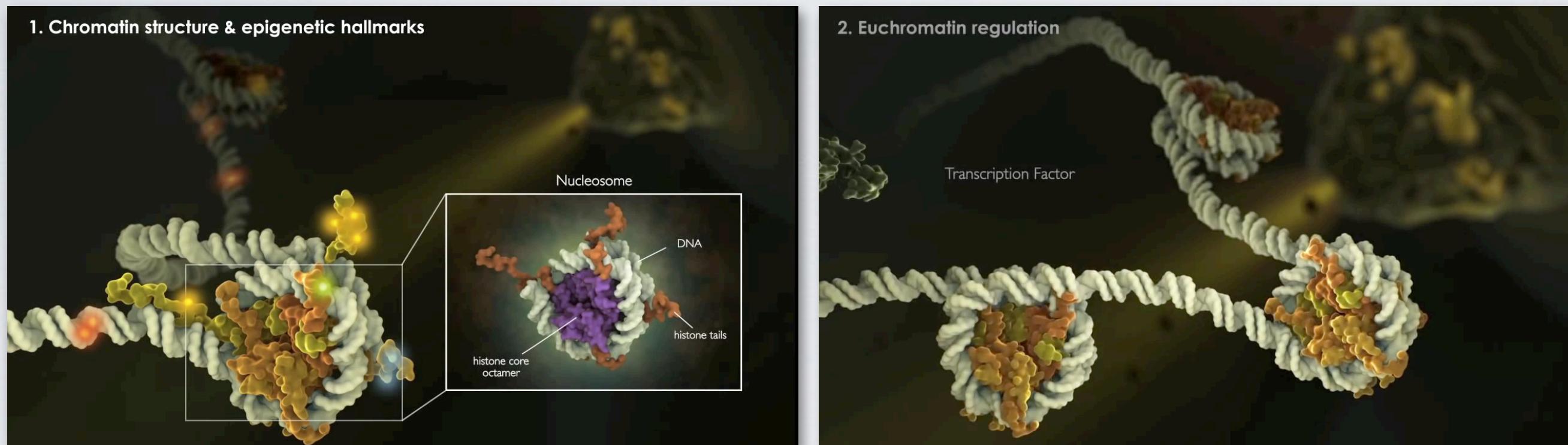


Roles:

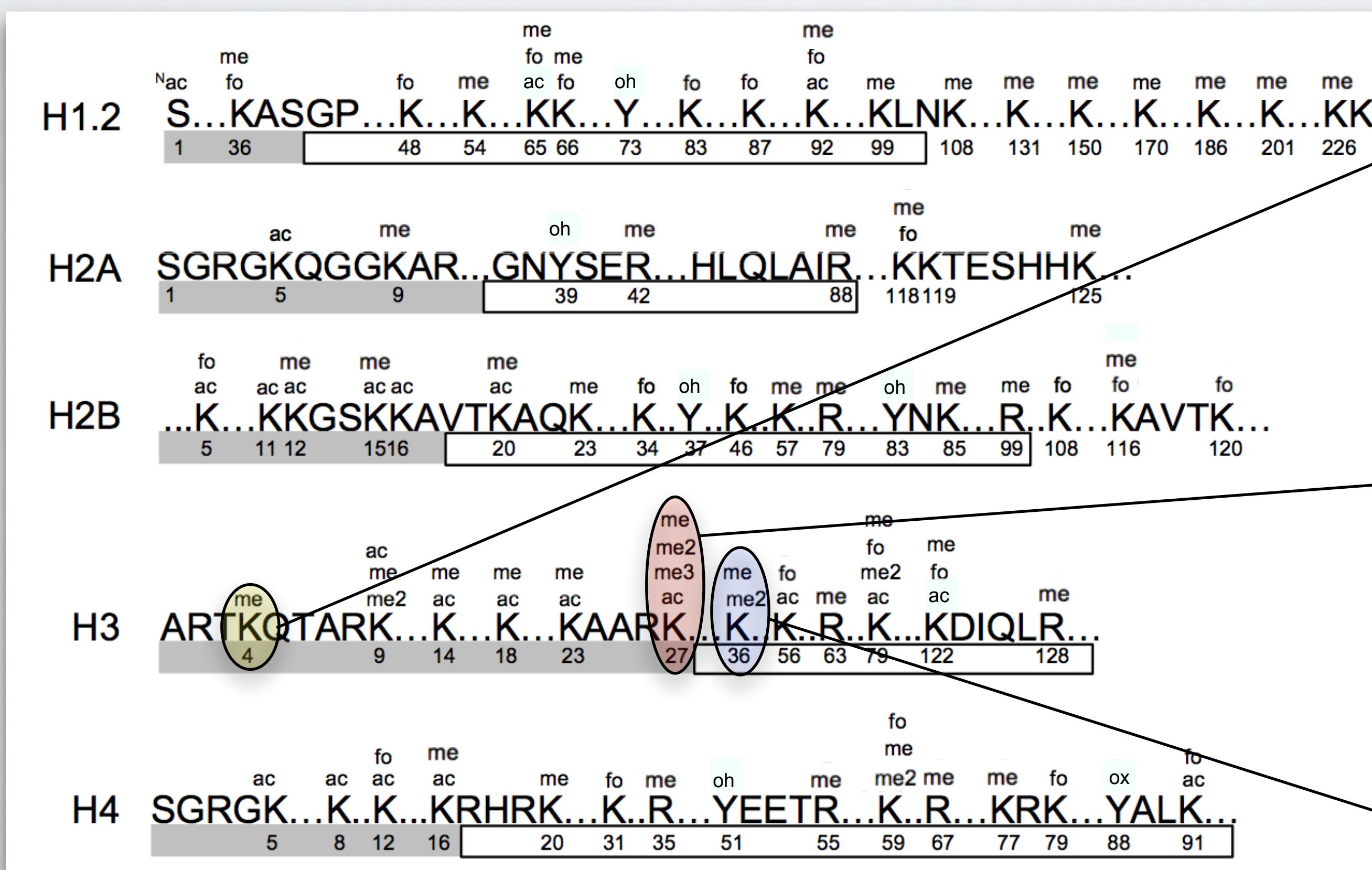
- chromatin compaction to affect transcription
- modulation of DNA-protein interactions
- heritable modification of transcription
- control of gene expression & differentiation
- transposon silencing
- development

Prokhartchouk and Defossez 2008 BBA

Diverse post-translational modification of histone tails



me	monomethylation
me2	dimethylation
me3	trimethylation
fo	formylation
ac	acetylation
oh	hydroxylation
cr	crotonylation



Histone 3 (H3) Lysine 4 (K4)

H3K4 me

- monomethylation (H3K4me1)
- dimethylation (H3K4me2)
- trimethylation (H3K4me3)

H3K4me1: enhancers
H3K4me3: active promoters

Histone 3 (H3) Lysine 27 (K27)

H3K27 me/ac

- monomethylation (H3K27me1)
- dimethylation (H3K27me2)
- trimethylation (H3K27me3)
- acetylation (H3K27ac)

H3K27me3: repressive, heterochromatin
H3K27ac: active enhancers

Histone 3 (H3) Lysine 36 (K36)

H3K36 me

- monomethylation (H3K36me1)
- dimethylation (H3K36me2)

H3K36me3: transcribed gene bodies

Enzymes that catalyze DNA and histone modifications

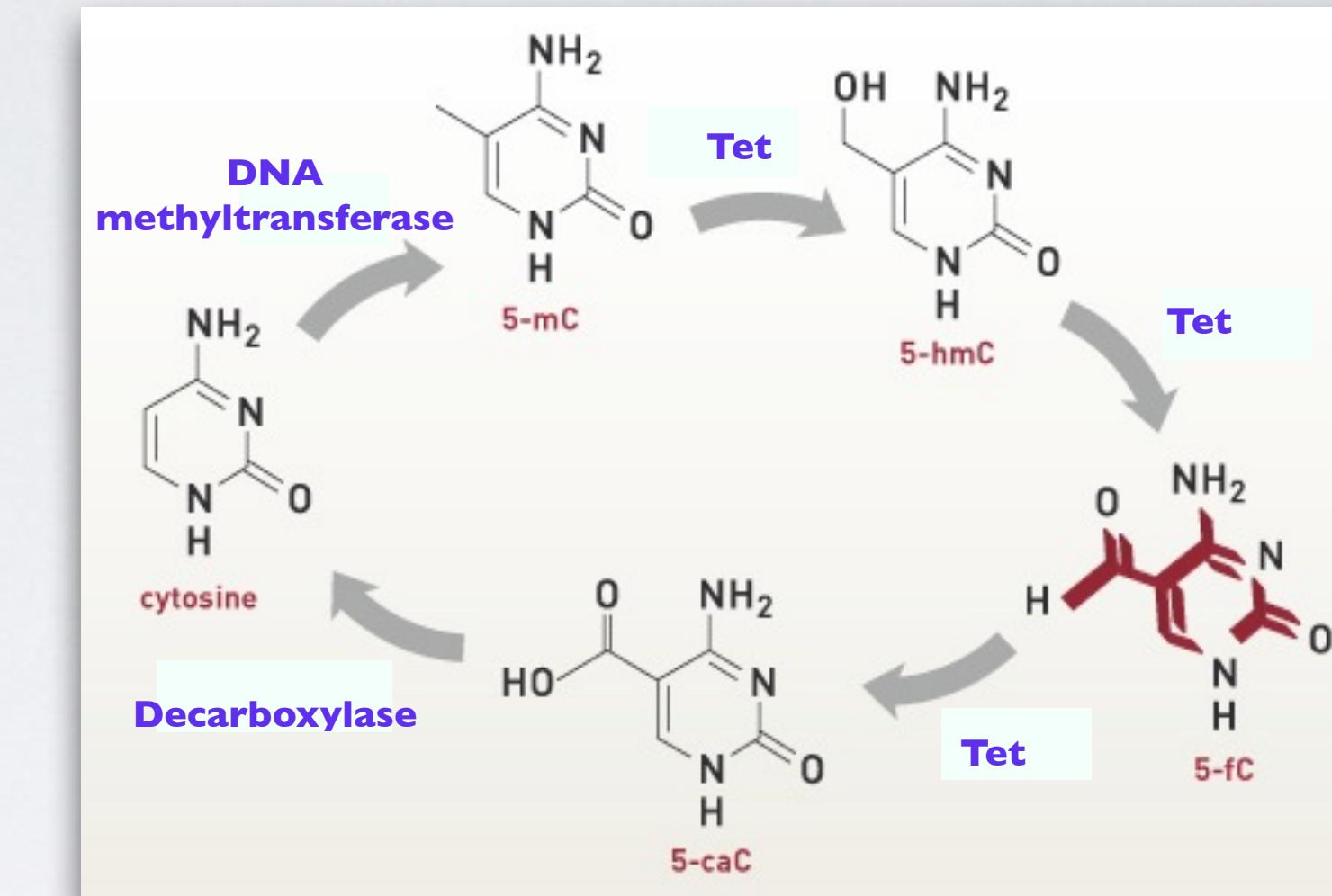
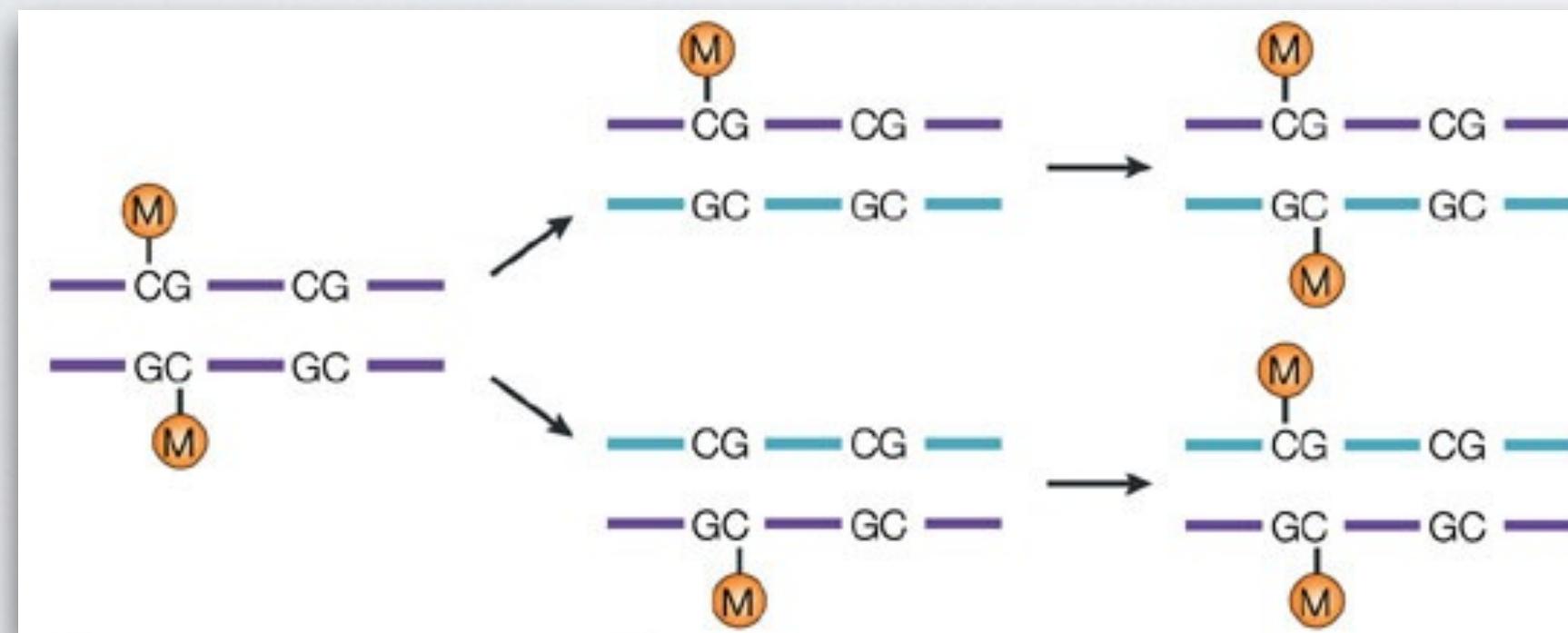
There are a wide range of enzymes catalyze the addition or removal of modifications of DNA and histones

DNA methylation

DNA methyltransferases (DNMTs):

DNMT3a, DNMT3b - establish cytosine DNA methylation, converting cytosine into methylcytosine (**mC**)

DNMT1 - recognizes hemimethylated DNA at CG sites after genome replication, methylates CG in new daughter DNA strand



DNA demethylation

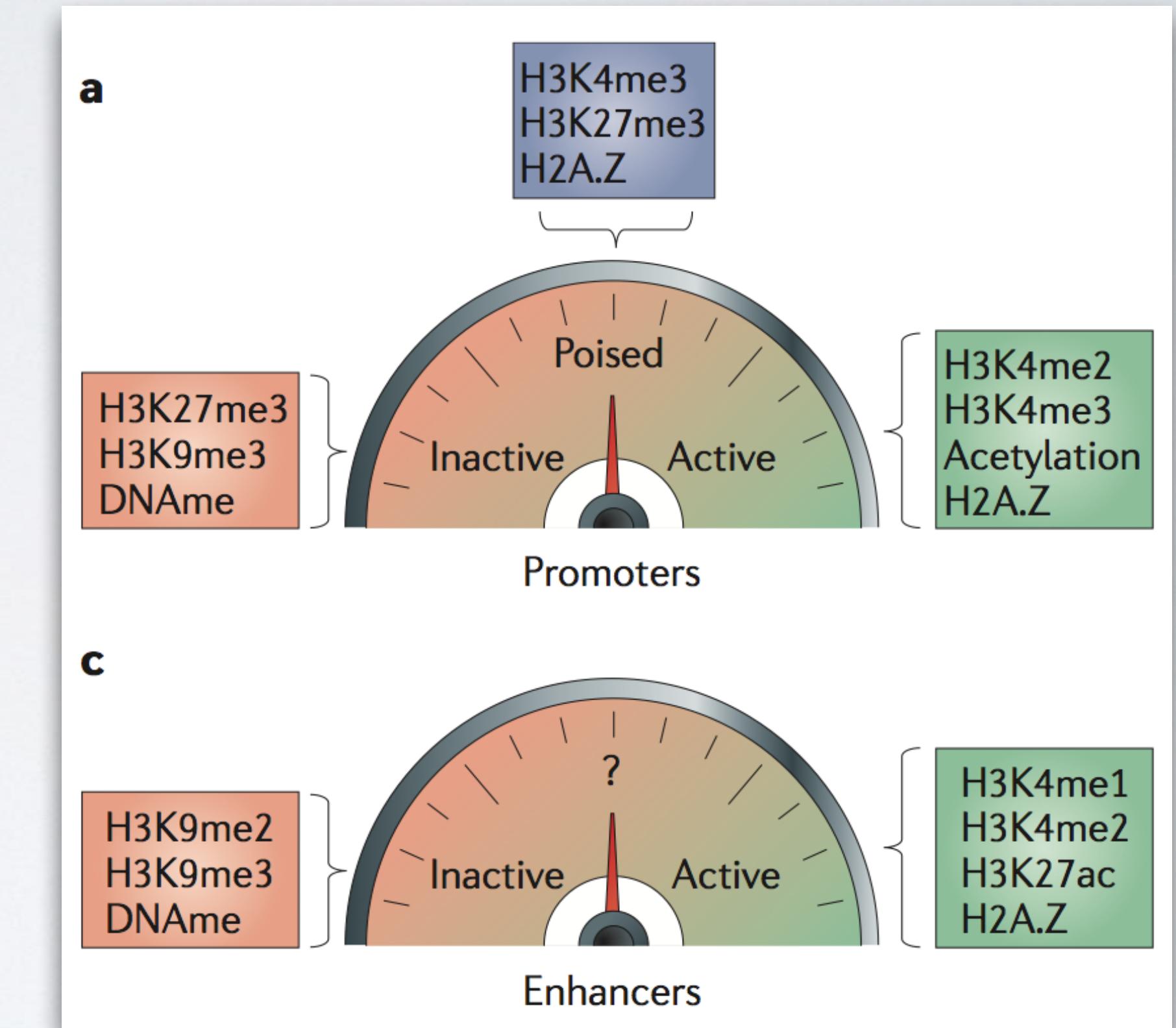
TET1, TET2, TET3 - methylcytosine (mC) hydroxylases that convert mC into hydroxymethylcytosine (hmC), then to 5-formylcytosine, then to 5-carboxylcytosine

Histone modifications

Histone acetyltransferases (HATs), histone deacetylases (HDACs), histone methyltransferases (HMTs), histone demethylases, kinases, phosphatases, etc

DNA and histone modifications together regulate gene expression

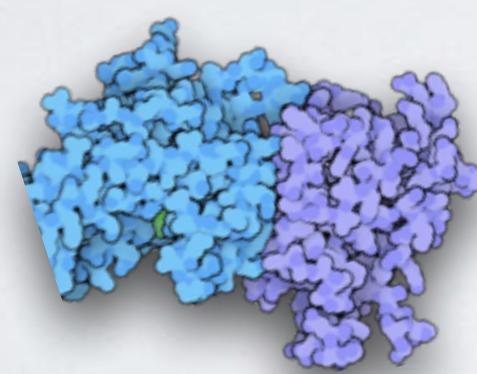
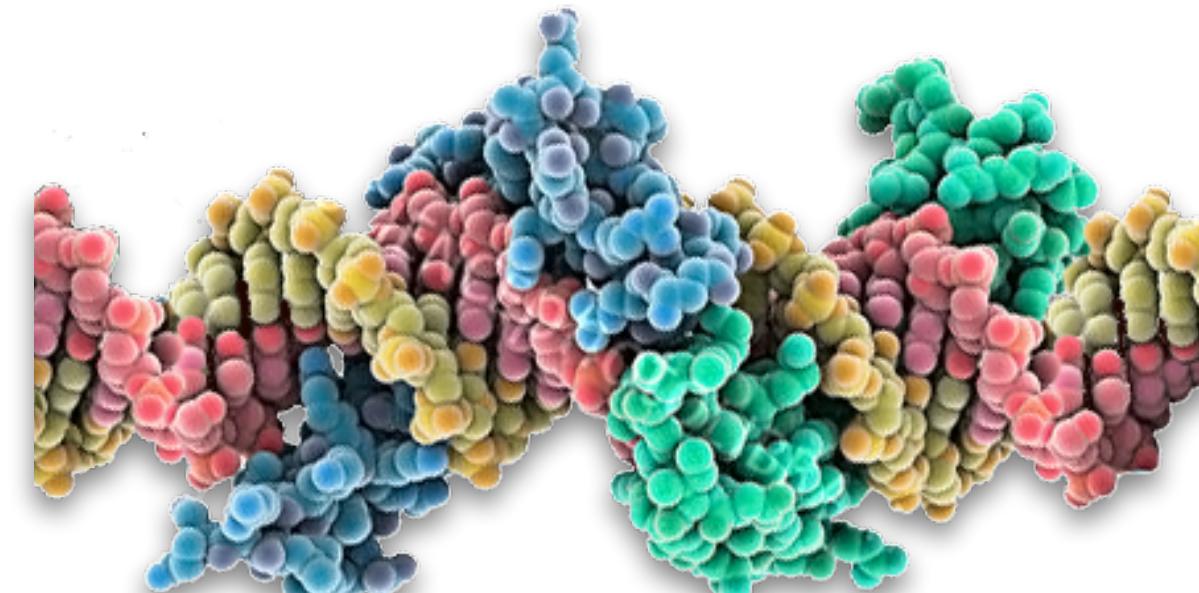
Hierarchical and feedback relationships between DNA methylation and histone modifications



Different combinations of modifications are observed in different genomic regions and activity states

Targeted editing of the epigenome

Zinc Fingers



Dnmt3a: DNA cytosine methyltransferase

ZF: Rivenbark 2012

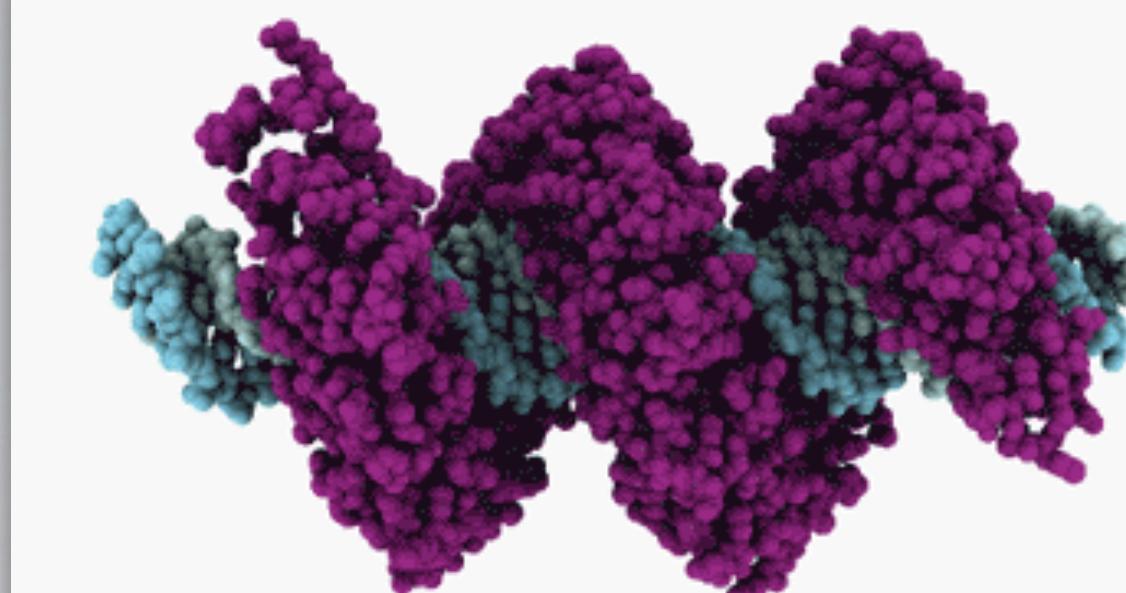
Cas9: Vojta 2015

Cas9: Liu 2016

Cas9: Huang 2017

Cas9: Galonska, Pflueger 2018

TALENs



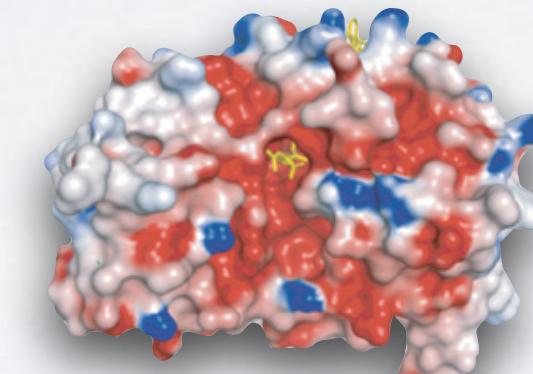
Tet: DNA methylcytosine dioxygenase

ZF: Chen 2013

TALE: Maeder 2013

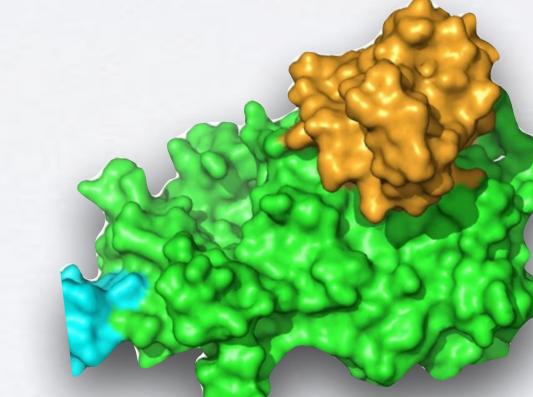
Cas9: Amabile, Liu 2016

Cas9: Morita 2017



p300: histone acetyltransferase (H3K27ac)

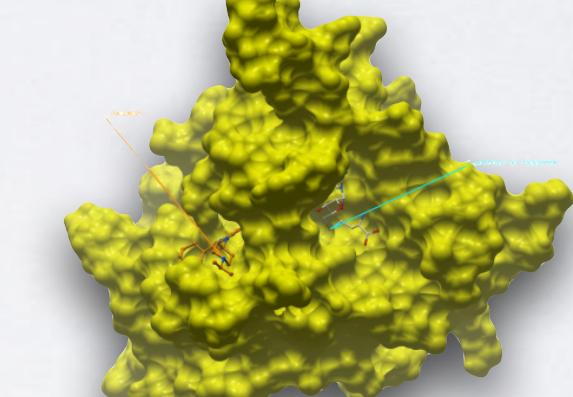
ZF, TALE, Cas9: Hilton 2015



LSD1: histone lysine demethylase H3K4me

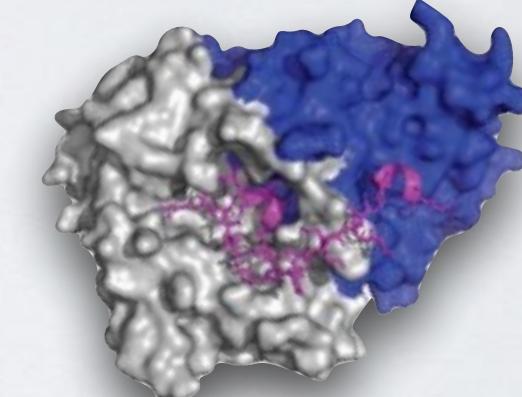
TALE: Mendenhall 2015

Cas9: Kearns 2015



G9a: histone lysine methyltransferase H3K9me2

ZF: Heller 2014



KRAB: increased H3K9me3

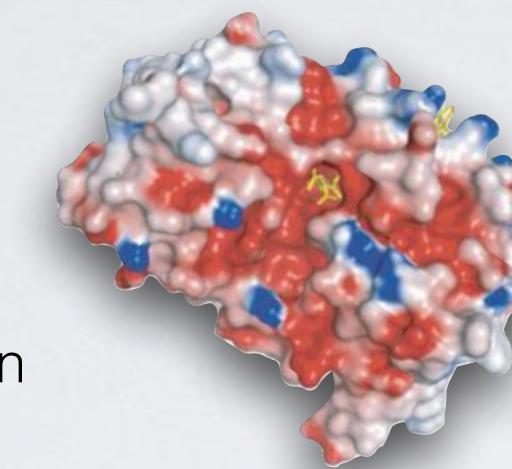
ZF: Beerli 1998, **TALE:** Cong 2012, **Cas9:** Thakore 2015

Targeted editing of the epigenome

H3K4 acetylation

p300 histone
acetyltransferase
catalytic domain

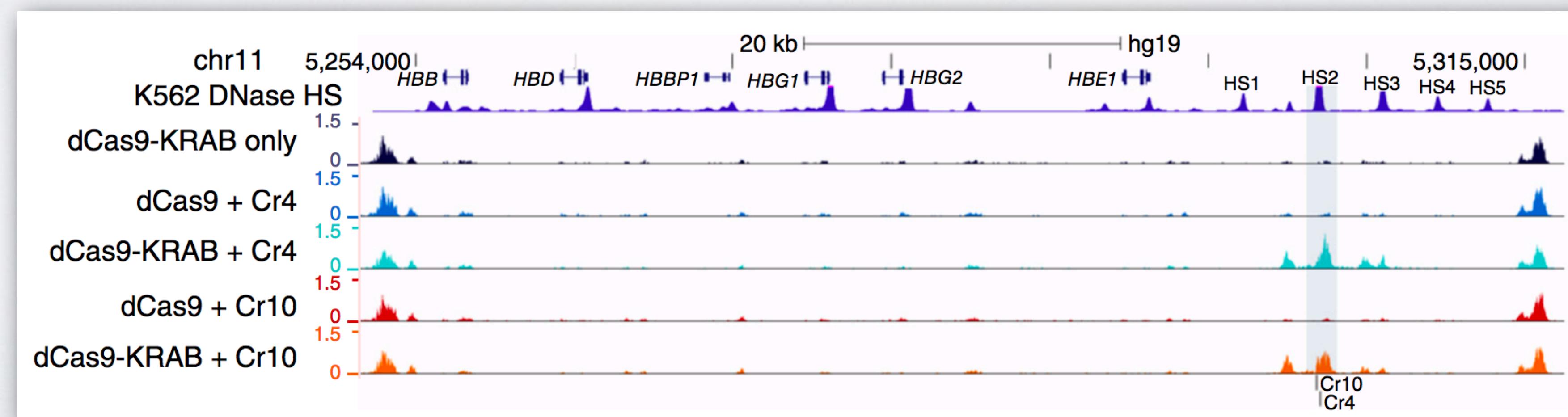
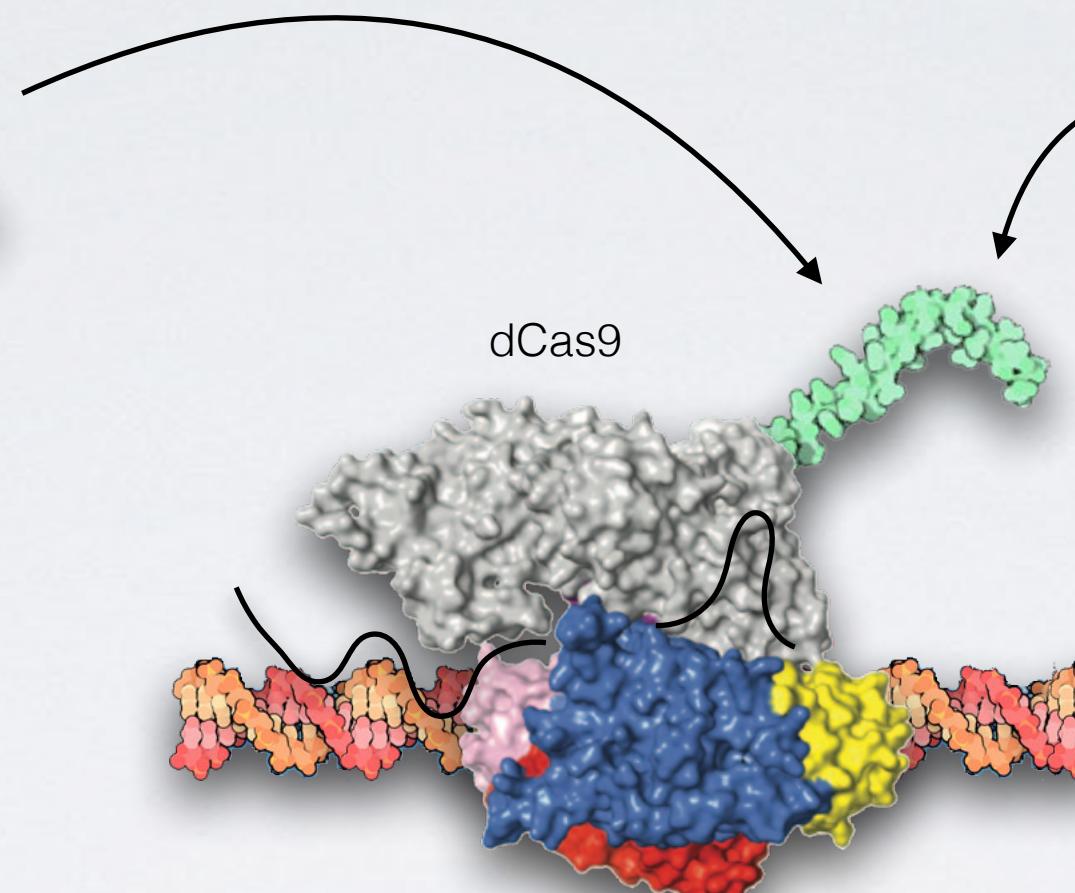
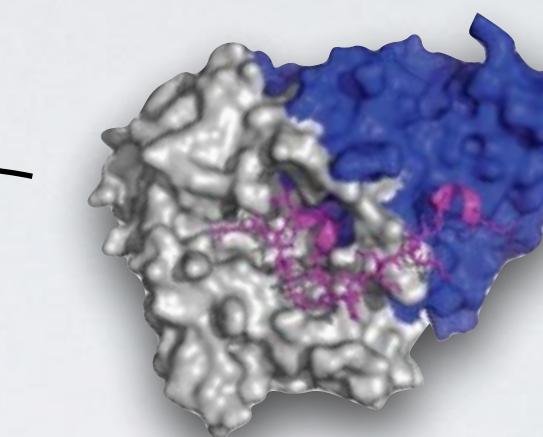
histone lysine acetylation



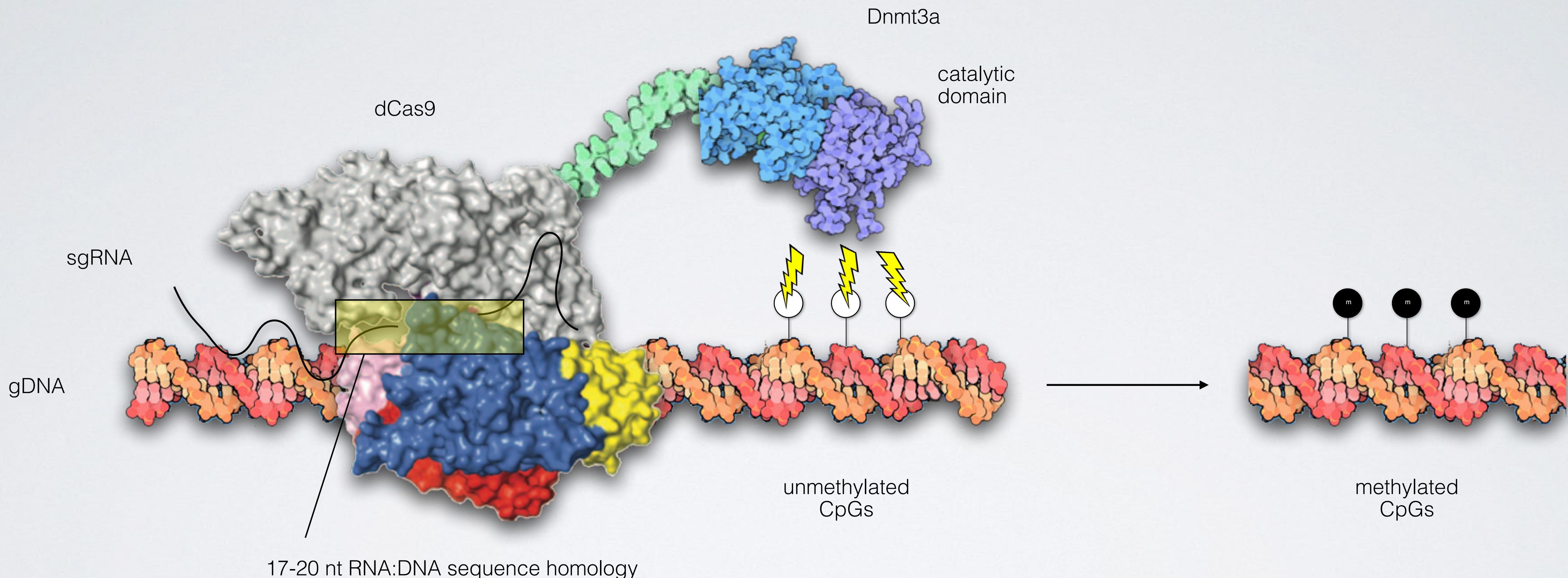
H3K4 methylation

KRAB

recruits heterochromatin-forming complex, induces histone methylation and deacetylation

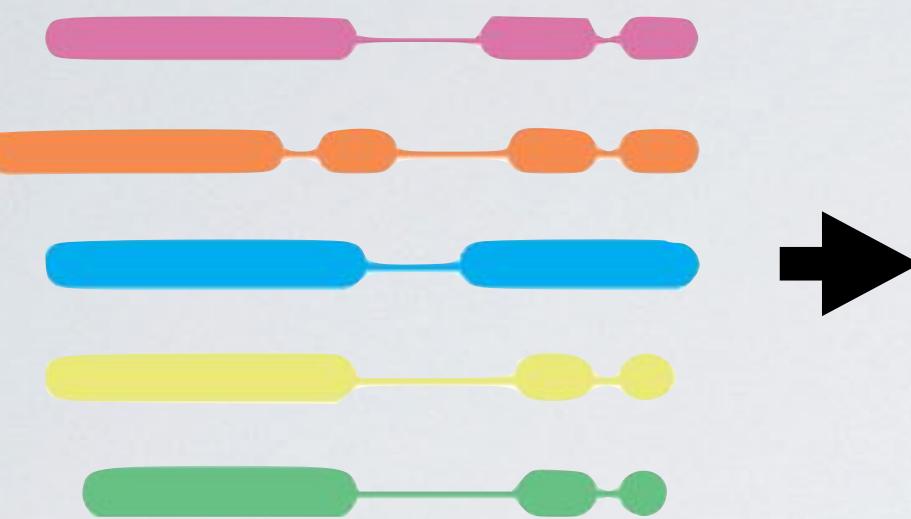


Targeted editing of the epigenome

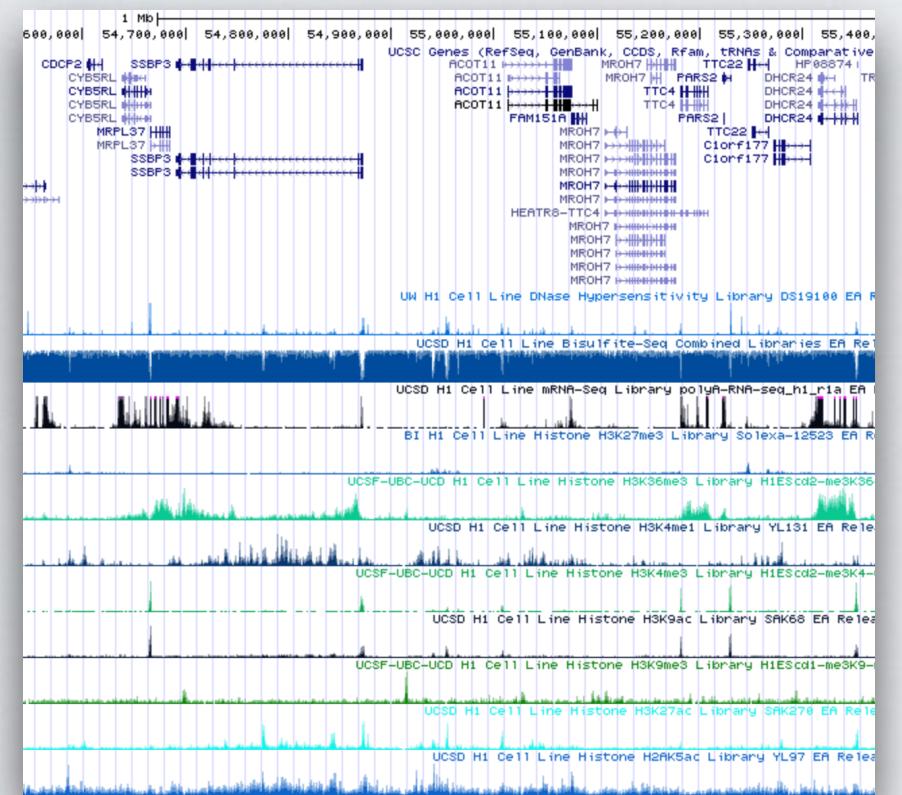


Workshop: building a new tool for targeted epigenome editing

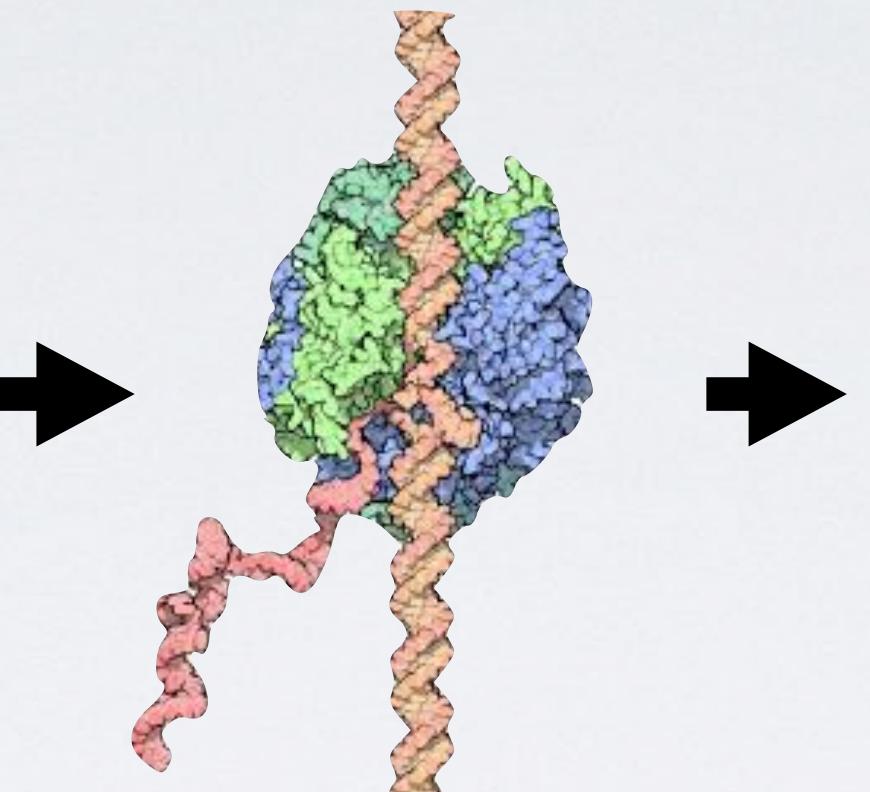
1. Each group assigned a gene



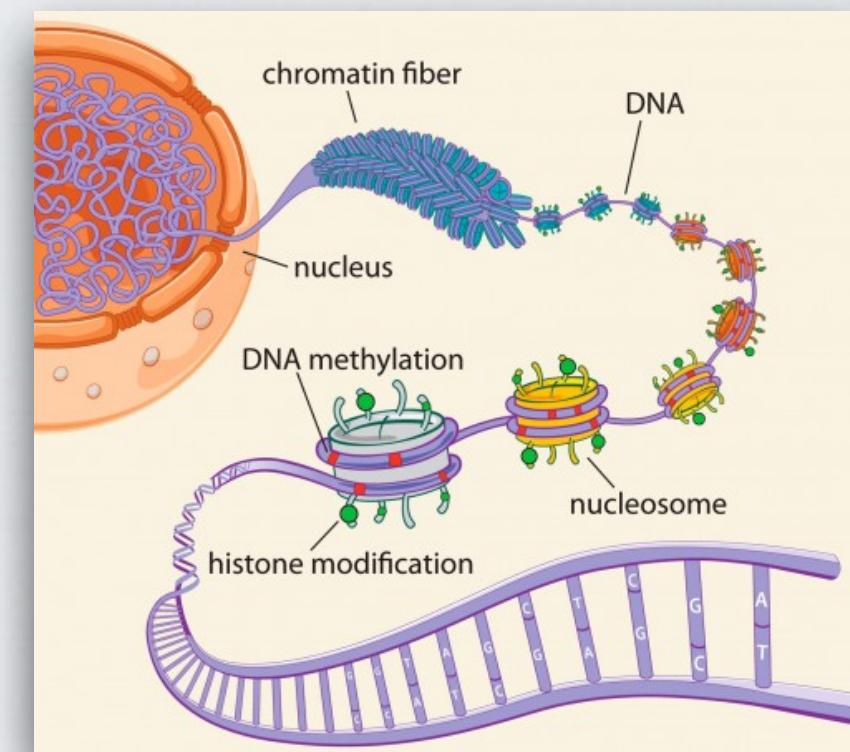
2. Inspect it in Human Epigenome browser



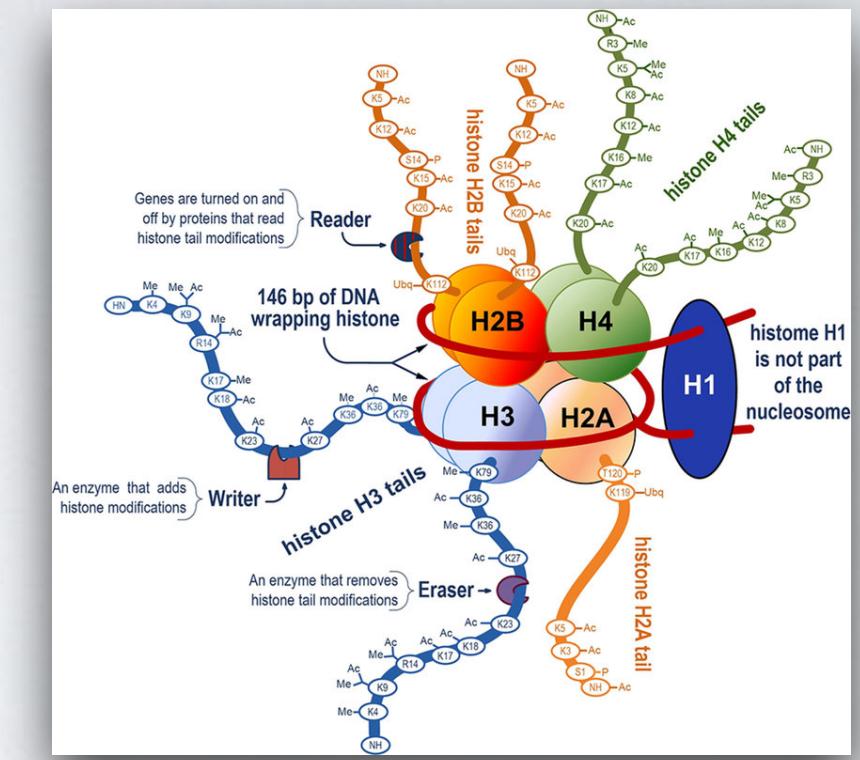
3. Expressed or not in human ES cells?



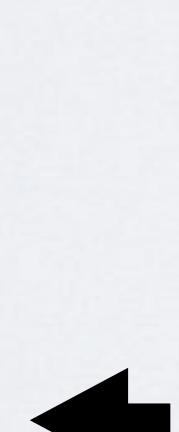
4. What epigenome modifications are present?



5. Identify the modification(s) you would like to edit to change gene expression



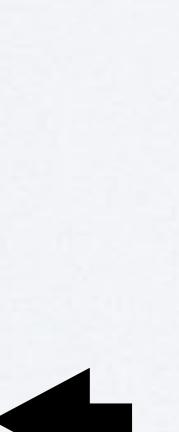
10. Geneious: add sgRNA target sequence



9. Find suitable sgRNA target site at gene



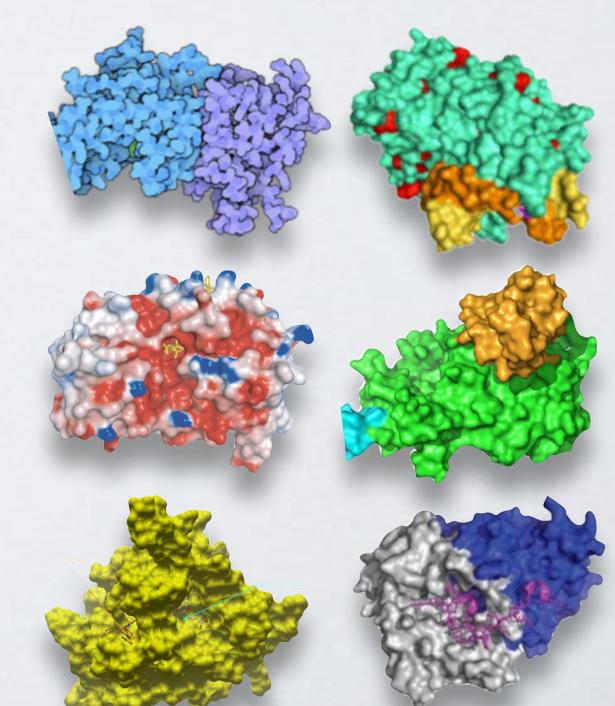
8. Geneious: design dCas9-fusion protein



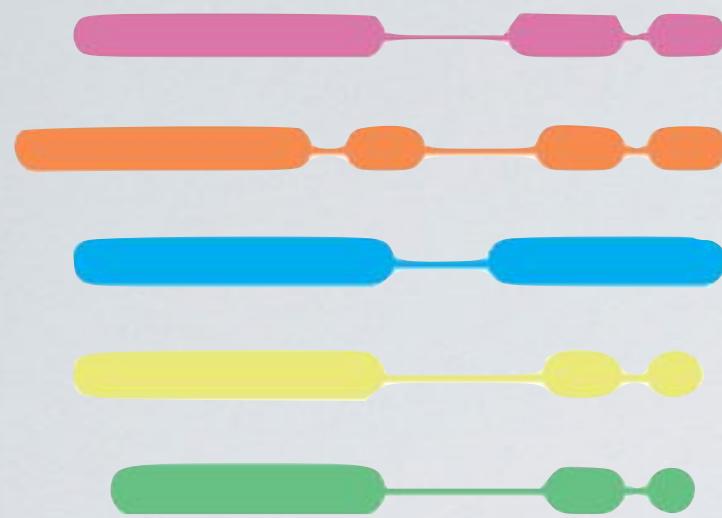
7. Find the protein coding sequence



6. Figure out which epigenome modifying enzyme you could use



I. Each group is assigned a gene



WNT7B

SRRM1

NCKAP1L

WDR43

ACVRL1

NANOG

FAIM2

SENPI

GRHL3

KRT8

ITGB7

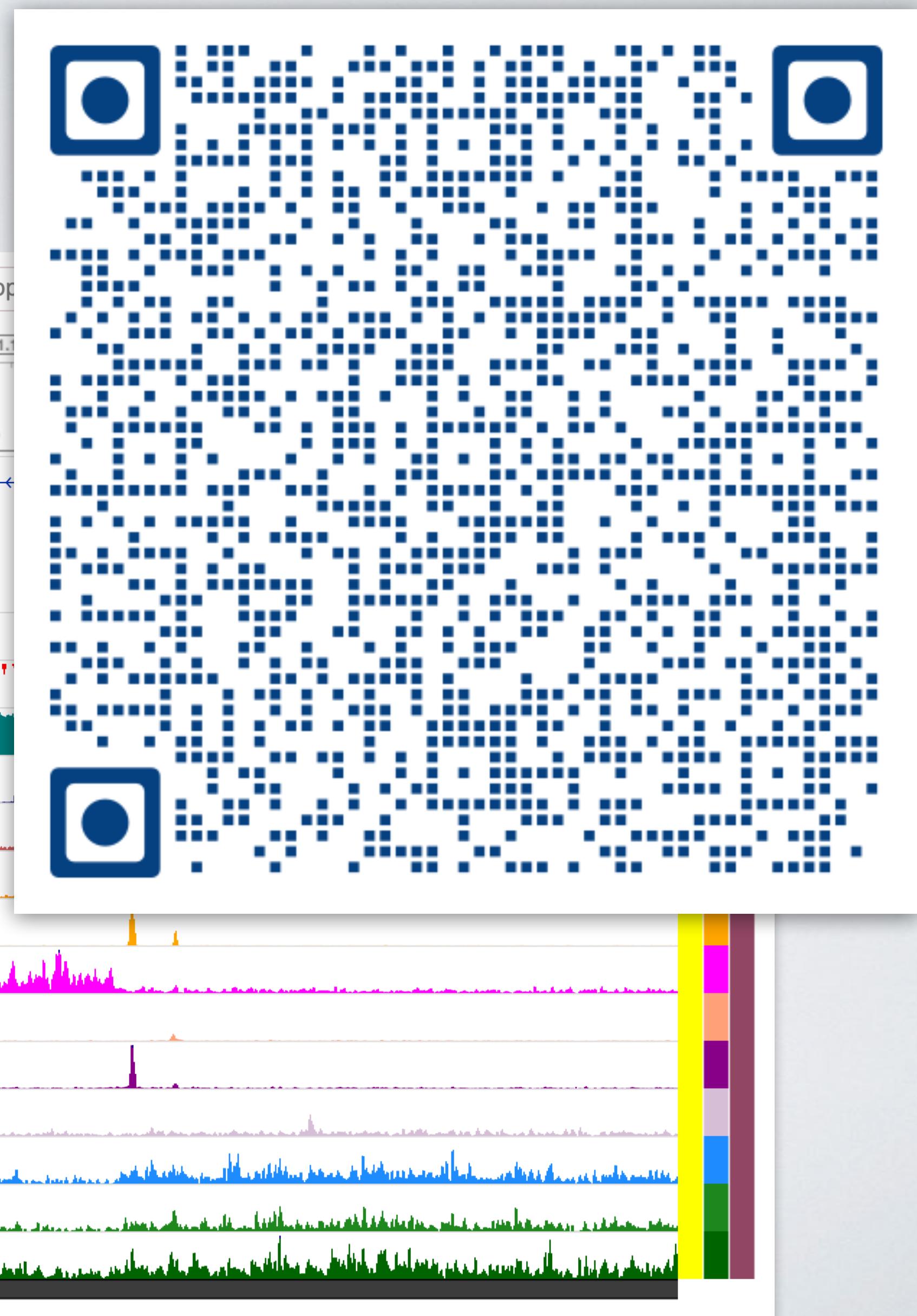
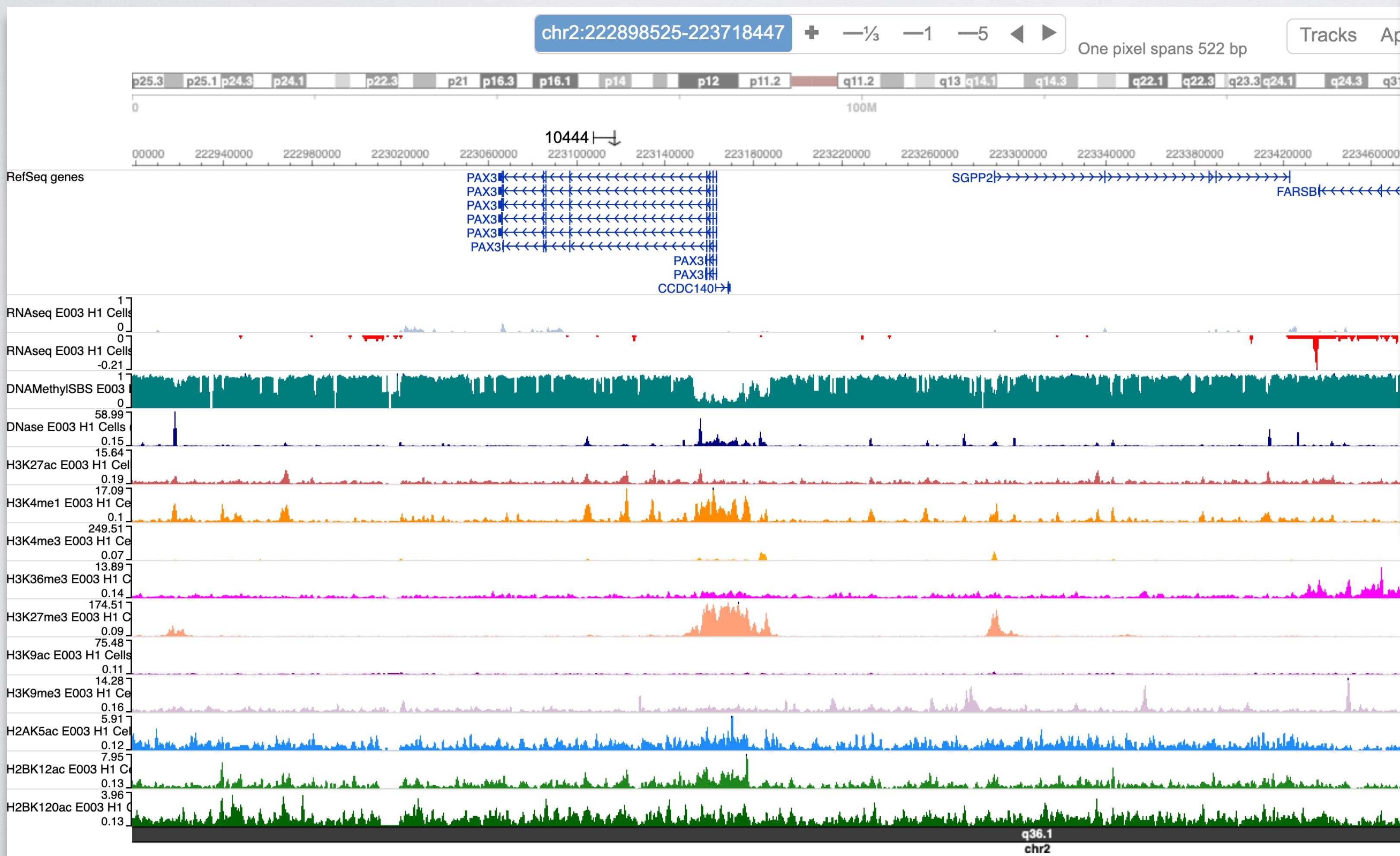
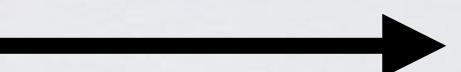
PPP1CC

FGR

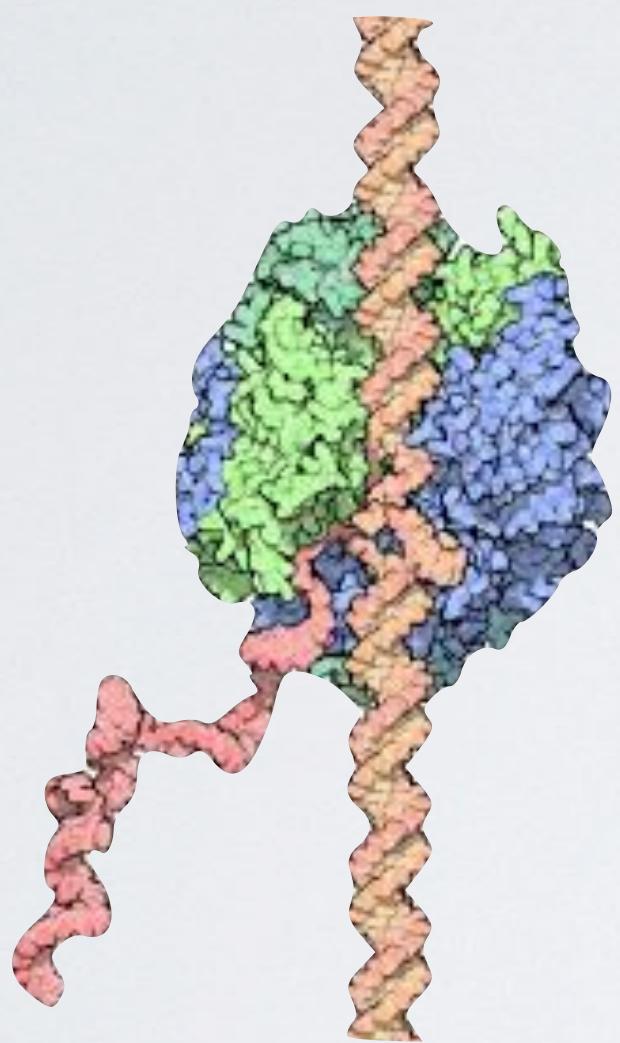
SGPP2

2. Inspect gene in human epigenome browser

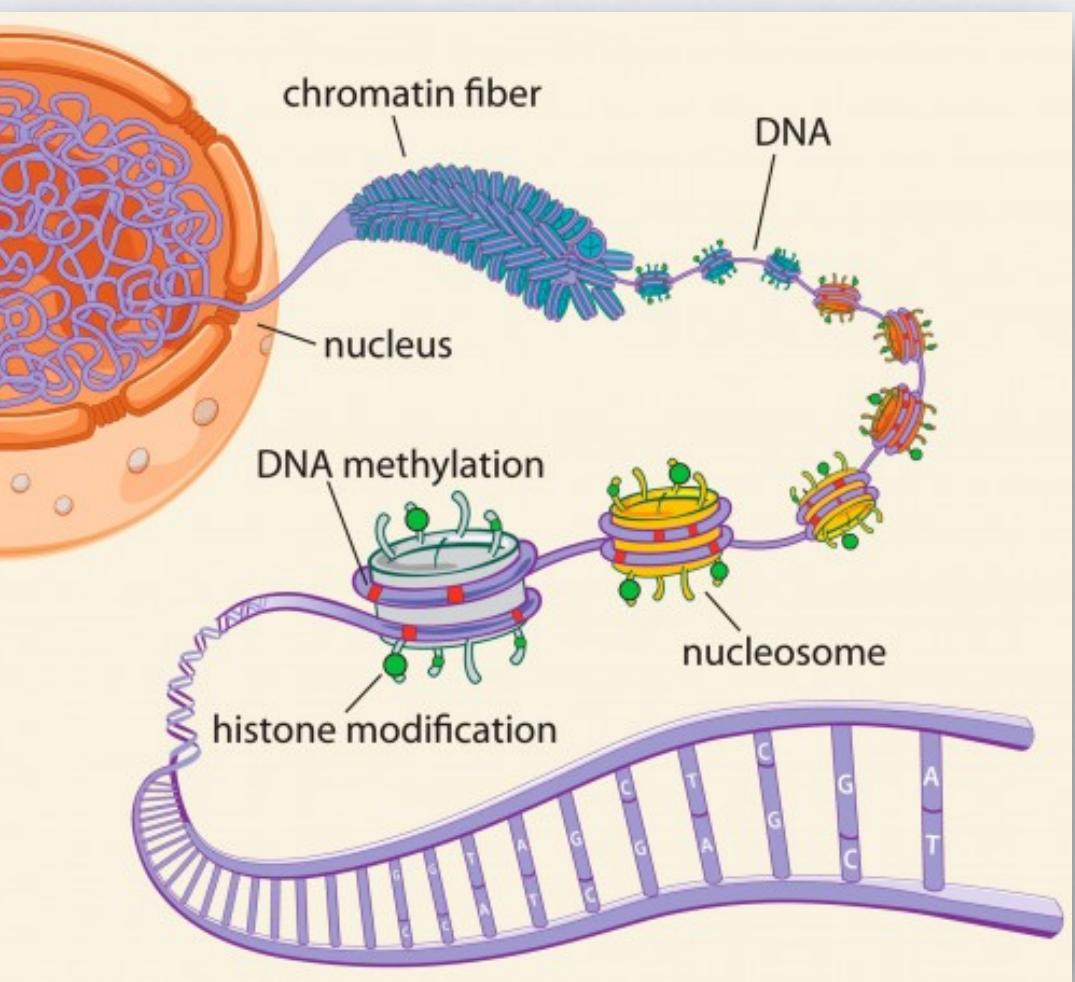
Scan QR code to get link to epigenome browser



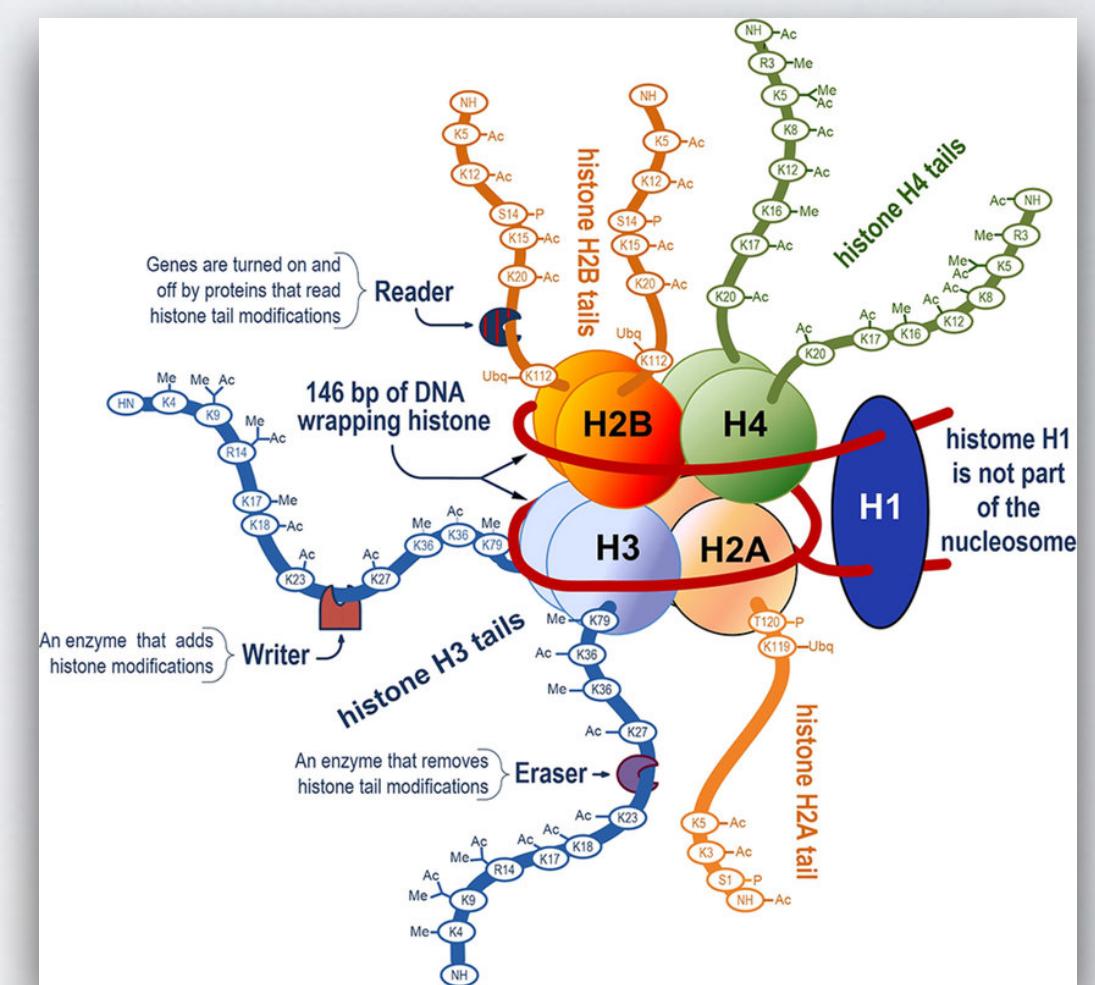
3. Expressed or not in human ES cells?



4. What epigenome modifications are present?



5. Identify the modification(s) you would like to edit to change gene expression



6. Figure out which epigenome modifying enzyme you could use

Look up database of epigenome modifying enzymes to identify one that could edit the modification

<https://epifactors.autosome.ru/>

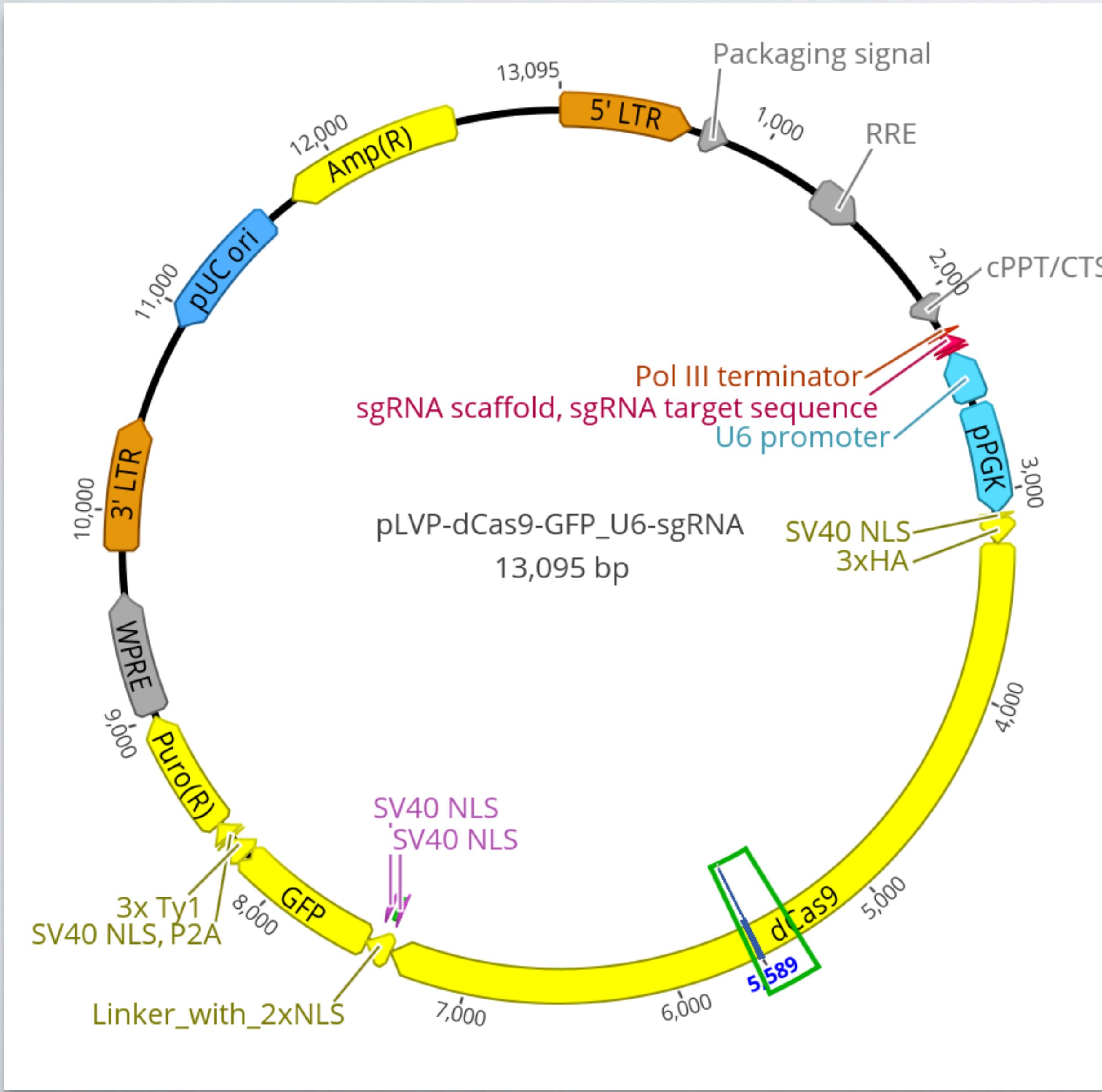
Search genes for keywords, find modifier, then click on *Uniprot ID (human)* link

HGNC approved symbol	HGNC approved name	UniProt ID (human)	Pfam domains	UniProt ID (mouse)	Function	Modification	Protein complex	Target entity	Product
SMBT2 (details)	Scm-like with four mbt domains 2	SMBT2_HUMAN	DUF3588 PF12140 (527-646) MBT PF02820 (78-152, 191-263, 304-380, 412-485) SAM_1 PF00536 (822-885)	SMBT2_MOUSE	Histone modification read, Polycomb group (PcG) protein, TF	TF repressor	#	H3K9me2, H3K9me3, H3K27me3, H4K20me2, H4K20me3	H3, H4
LRWD1 (details)	leucine-rich repeats and WD repeat domain containing 1	LRWD1_HUMAN	LRR_1 PF00560 (48-69) Pfam-B_28625 PB028625 (274-344) Pfam-B_39252 PB039252 (191-239) WD40 PF00400 (382-421)	LRWD1_MOUSE	Chromatin remodeling	#	#	H3K9me3, H3K27me3	#
KDM6B (details)	lysine (K)-specific demethylase 6B	KDM6B_HUMAN	JmjC PF02373 (1377-1485) Pfam-B_5108 PB005108 (101-843) Pfam-B_992 PB000992 (1-99)	KDM6B_MOUSE	Histone modification erase	Histone methylation	#	H3K27me2, H3K27me4	H3K28
KDM6A (details)	lysine (K)-specific demethylase 6A	KDM6A_HUMAN	JmjC PF02373 (1133-1241) Pfam-B_604 PB000604 (631-769) Pfam-B_972 PB000972 (771-1109) TPR_1 PF00515 (130-163, 318-351, 352-380) TPR_17 PF13431 (272-304) TPR_8 PF13181 (205-238)	KDM6A_MOUSE	Histone modification erase	Histone methylation	CHD8, MLL2/3, MLL4/WBP7, COMPASS-like MLL3,4	H3K27me2, H3K27me3	H3K27
JARID2 (details)	jumonji, AT rich interactive domain 2	JARD2_HUMAN	ARID PF01388 (618-709) JmjC PF02373 (916-1031) JmjN PF02375 (558-591) Pfam-B_728 PB000728 (1-122) zf-C5HC2 PF02928 (1139-1193)	JARD2_MOUSE	Histone modification write cofactor	Histone methylation	PRC2	H3K27, H3K9	#
GFI1B (details)	growth factor independent 1B transcription repressor	GFI1B_HUMAN	zf-C2H2 PF00096 (220-242) zf-H2C2_2 PF13465 (177-203, 262-287, 290-314)	GFI1B_MOUSE	Histone modification cofactor	#	#	#	#
EZH2 (details)	enhancer of zeste 2 polycomb repressive complex 2 subunit	EZH2_HUMAN	EZH2_WD-Binding PF11616 (39-68) SET PF00856 (623-727)	EZH2_MOUSE	Histone modification write, Polycomb group (PcG) protein	Histone methylation	PRC2	H3K27	H3K27me1, H3K27me2, H3K27me3
EZH1 (details)	enhancer of zeste 1 polycomb repressive complex 2 subunit	EZH1_HUMAN	EZH2_WD-Binding PF11616 (39-68) SET PF00856 (624-728)	EZH1_MOUSE	Histone modification write, Polycomb group (PcG) protein	Histone methylation	PRC2	H3K27	H3K27me1, H3K27me2, H3K27me3

7. Find the protein coding sequence

Under Sequences section select UniParc link, select Ensembl link, select cDNA in left menu, click CCDS link

8. Geneious: design dCas9-fusion protein

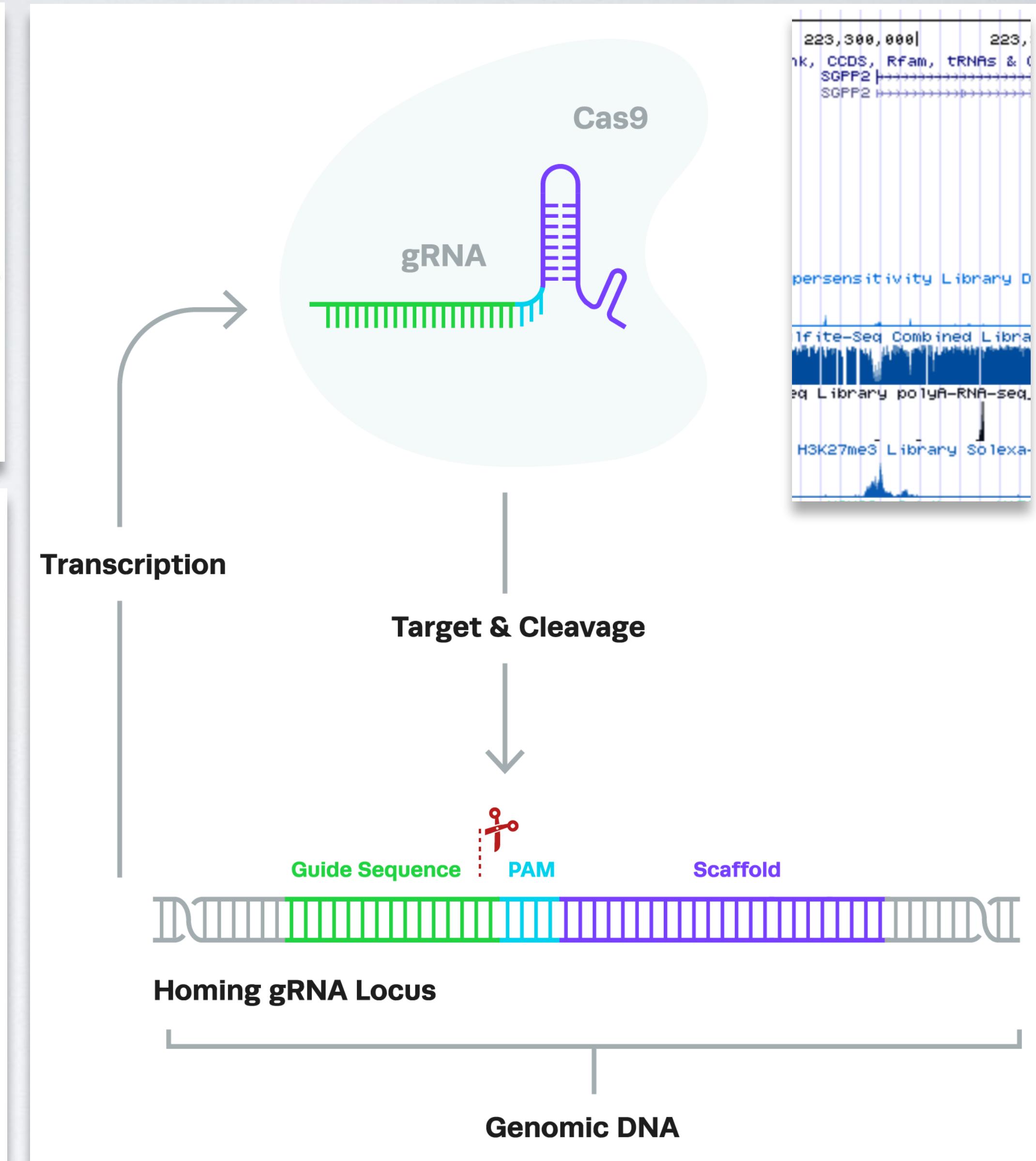
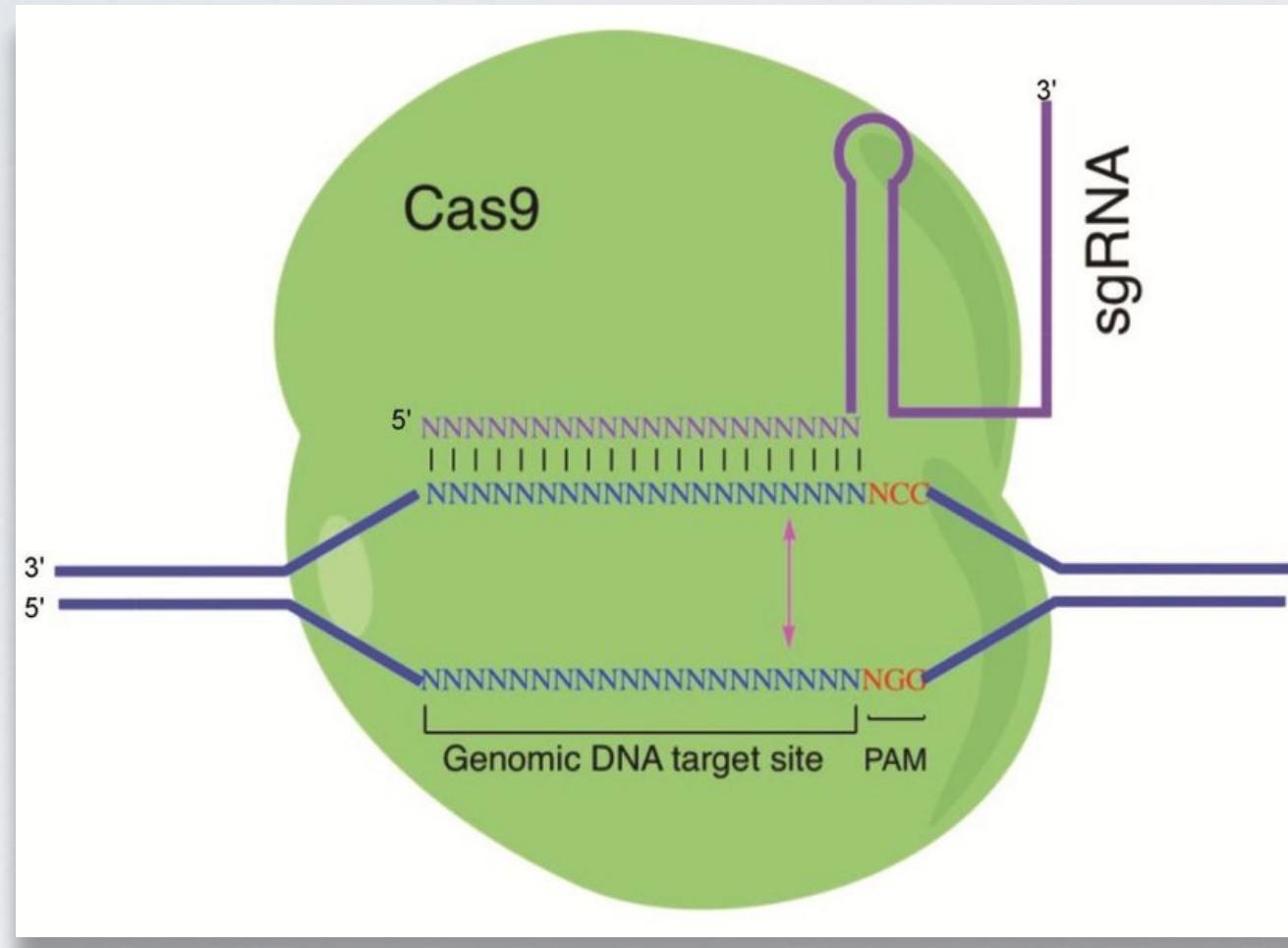
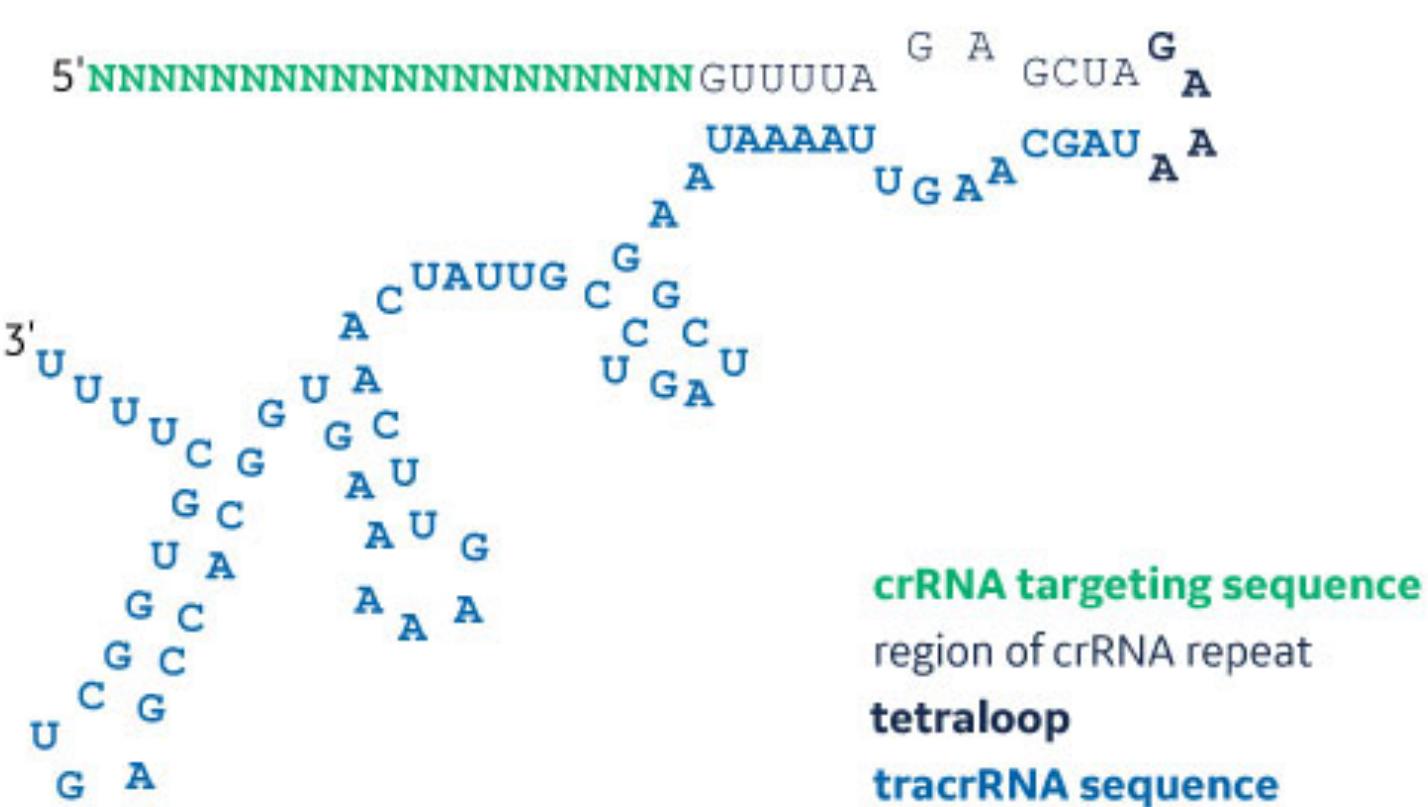


Open vector file (available in LMS) in Geneious

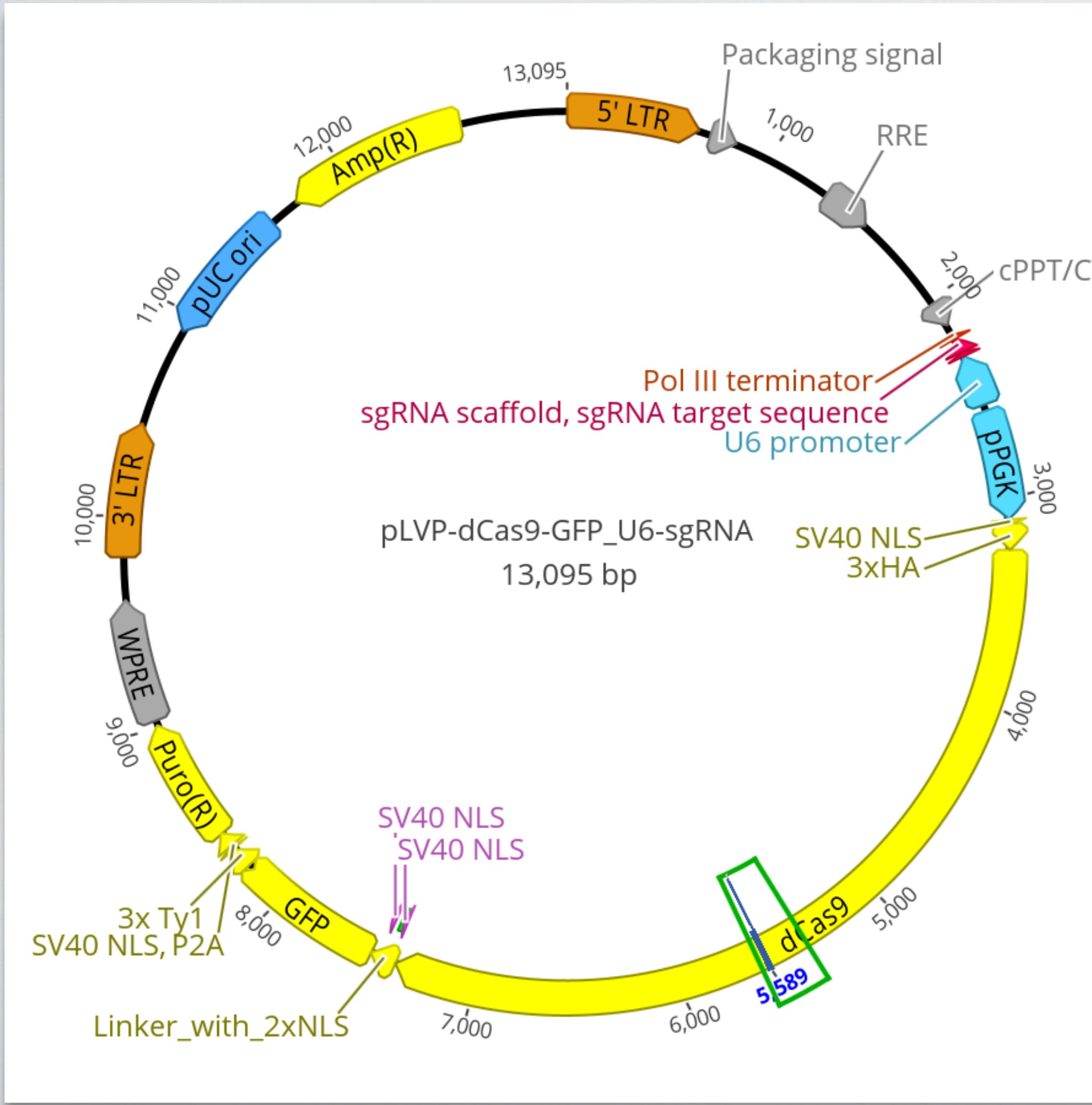
pLVP-dCas9-GFP_U6-sgRNA.geneious

Edit the construct sequence to incorporate the sequence encoding your selected epigenome modifier fused to dCas9

9. Find suitable sgRNA target site at gene, for dCas9-editor to bind and change modification



10. Geneious: add sgRNA target sequence to sgRNA scaffold



Use the same vector file (available in LMS) in Geneious where you already incorporated the sequence encoding your selected epigenome modifier fused to dCas9

Edit the construct sequence to introduce the sgRNA target sequence in the appropriate place in the sgRNA scaffold

Check your CRISPR/dCas9-based epigenome editing construct is correct and has no problems

Outputs

Screenshot of the Epigenome Browser showing your target gene, the relevant epigenome modifications including the one(s) you want to change, and the location of the sgRNA target site

The epigenome modifications you selected to change, and why

The epigenome modifying enzyme you chose, and why

The sgRNA target site and an explanation of why you chose it

Your final Geneious construct, annotated to show the new components added