

Genomics and epigenetics measurements

Aleksandar Vojta



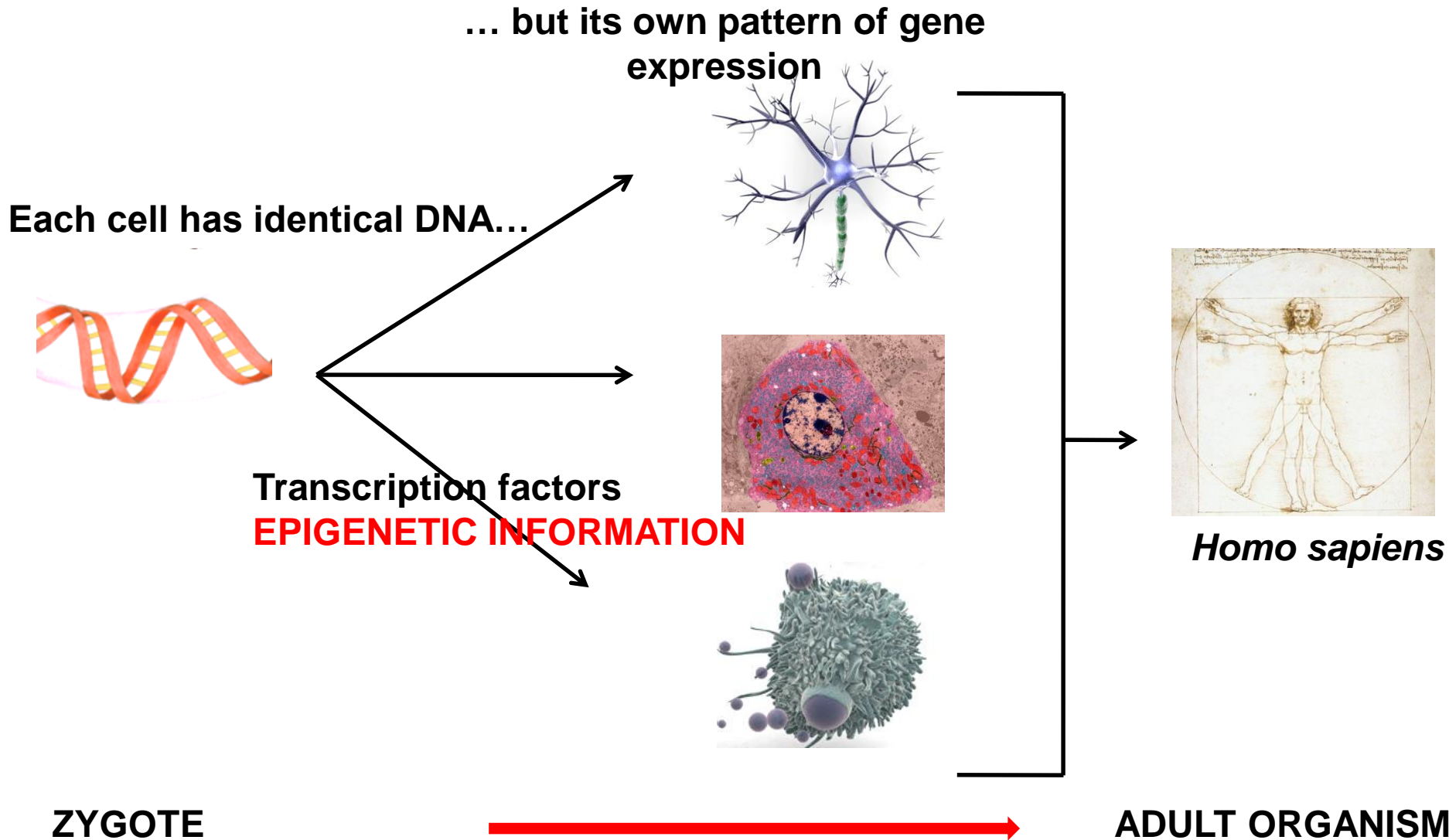
UNIVERSITY OF ZAGREB
FACULTY OF SCIENCE

Department of **Biology**

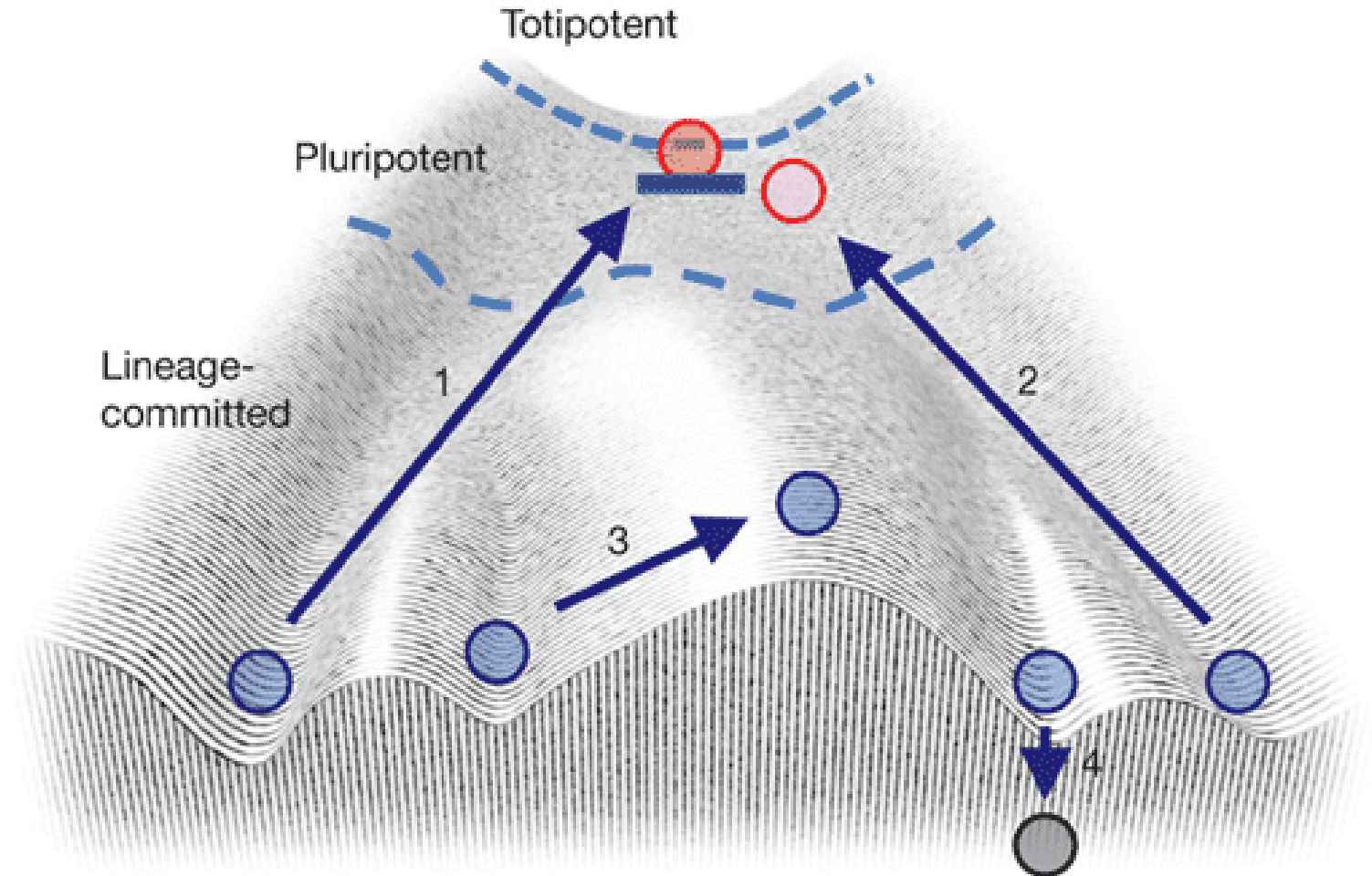
(0)

Introduction to epigenetics and the *big picture*

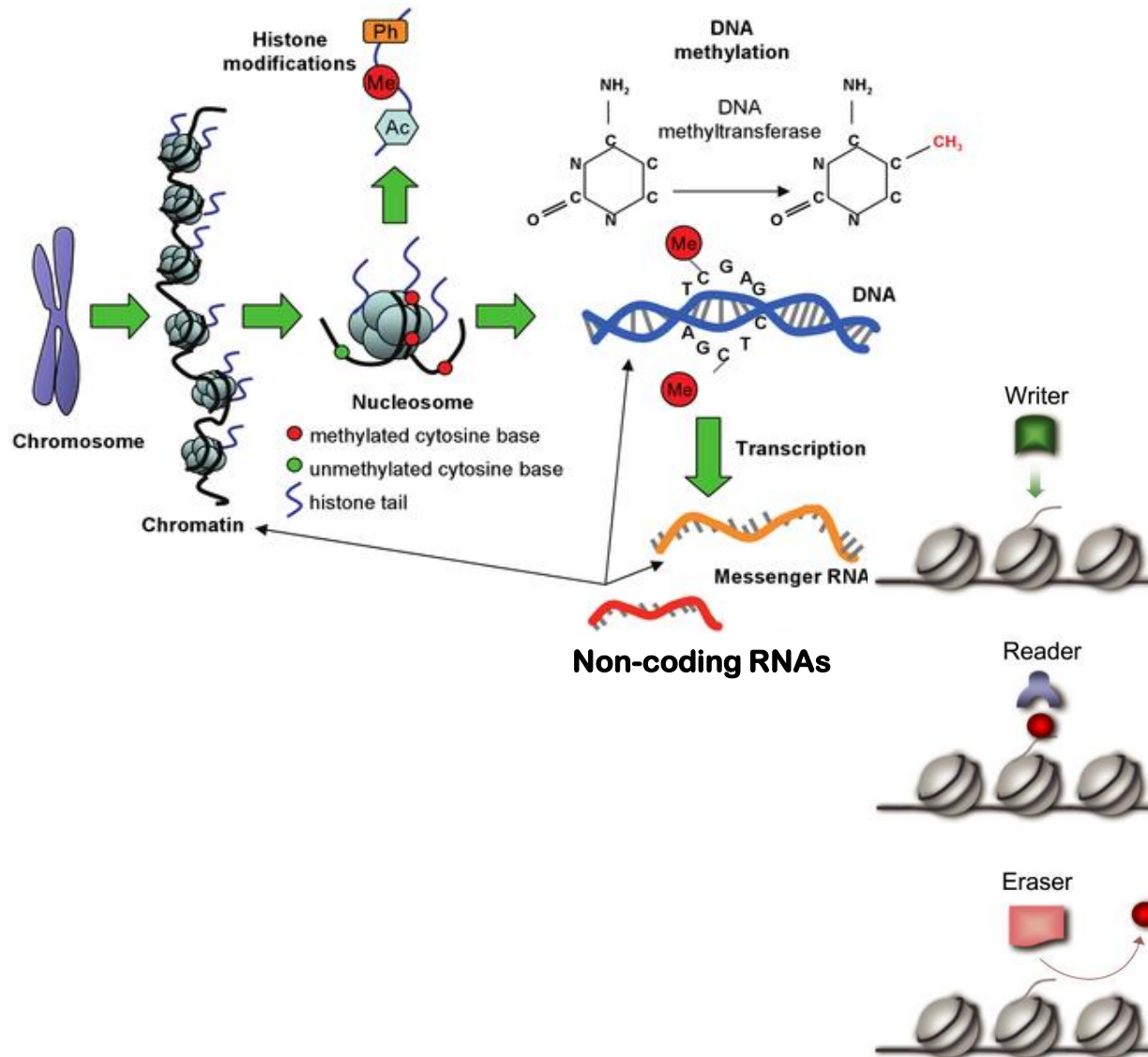
Somatic cells have identical DNA



Roles of the epigenome

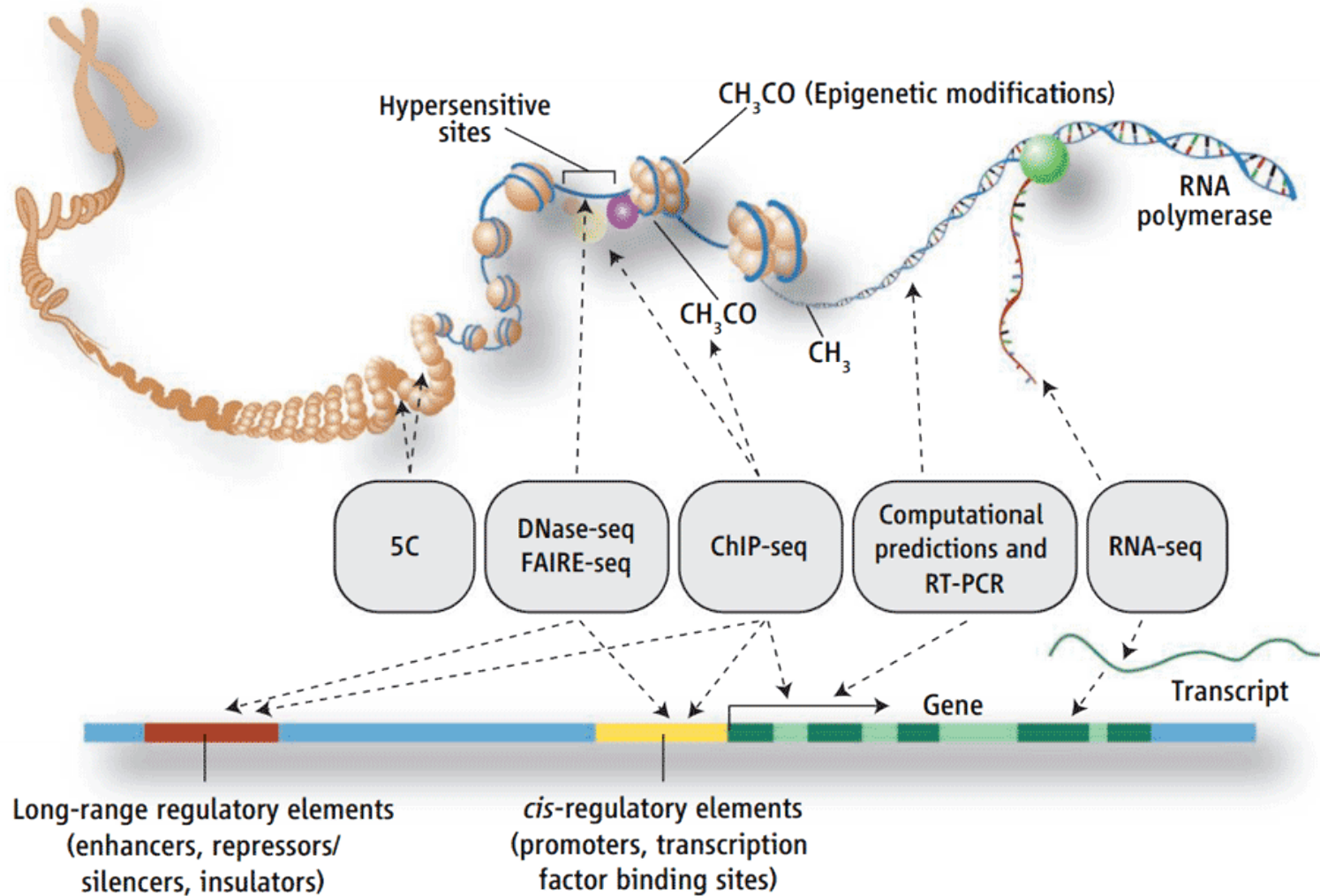


Epigenetic mechanisms



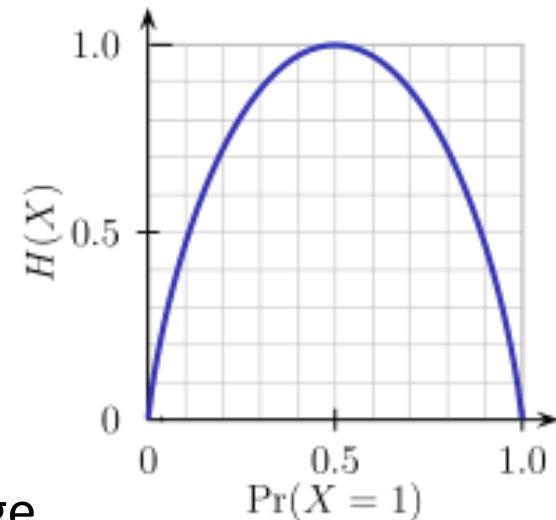
Writers	Ac	HATs
	Me	HMTs
	P	Kinases
Readers	Ac	Bromo
	Me	Chromo PHD Tudor PWWP MBT
	P	14-3-3, BRCT
Erasers	Ac	HDACs
	Me	DMTs
	P	Phosphatases

There is no *junk DNA*



Epigenome encodes information

- Entropy
 - a measure of uncertainty
- Information and entropy
 - connected but distinct
- Shannon entropy
 - quantifies the expected value of the information contained in a message



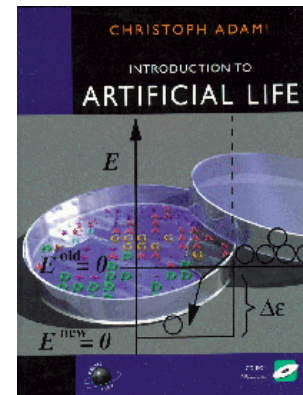
- The methylome: $H = -\sum p(x) \log p(x)$
- Shannon: $H = -\sum_i p_i \log_b p_i$
- Gibbs: $S = -k_B \sum_i p_i \ln p_i$
- Boltzmann:

$$S = k \cdot \log W$$

A definition of life

- Life is a property of an **ensemble** of units that **share information** coded in physical substrate and which, in the presence of **noise**, manages to keep its **entropy significantly lower** than the maximal entropy of the ensemble, on **timescales** exceeding the "natural" timescale of decay of the (information-bearing) substrate by many orders of magnitude

- Christoph Adami,
Introduction to Artificial Life*



Information is shared entropy

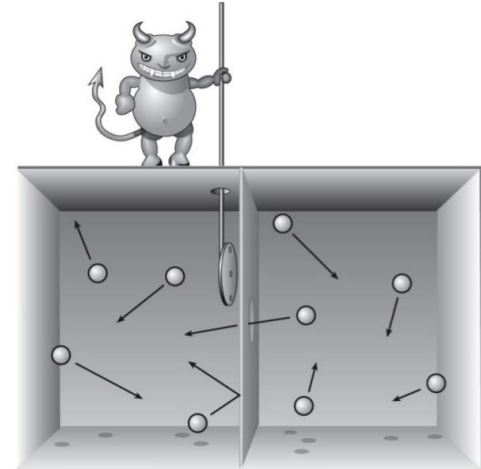
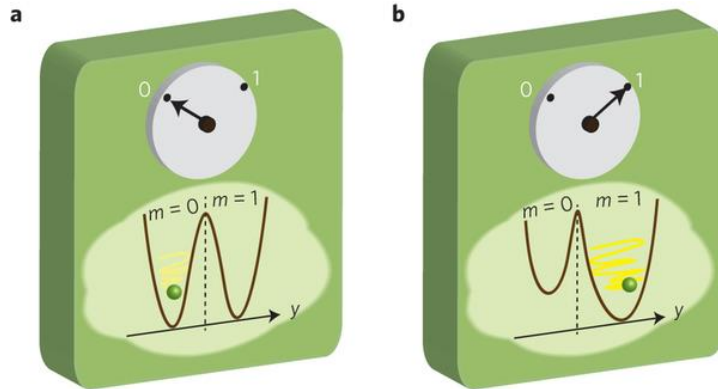
Information is correlated entropy (mutual entropy) between two random variables

$$H(X,Y) \leq H(X) + H(Y)$$

$$I(X:Y) = H(X) + H(Y) - H(X,Y)$$

Information is always **about** a system: it needs context

Information is physical



15 July 1996

PHYSICS LETTERS A

Physics Letters A 217 (1996) 188–193

The physical nature of information

Rolf Landauer¹

IBM T.J. Watson Research Center, P.O. Box 218, Yorktown Heights, NY 10598, USA

Received 9 May 1996

Communicated by V.M. Agranovich

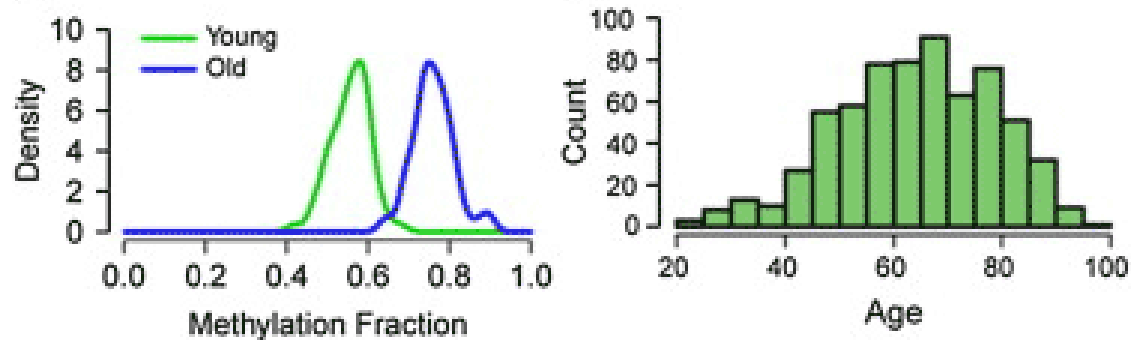
Abstract

Information is inevitably tied to a physical representation and therefore to restrictions and possibilities related to the laws of physics and the parts available in the universe. Quantum mechanical superpositions of information bearing states can be used, and the real utility of that needs to be understood. Quantum parallelism in computation is one possibility and will be assessed pessimistically. The energy dissipation requirements of computation, of measurement and of the communications link are discussed. The insights gained from the analysis of computation has caused a reappraisal of the perceived wisdom in the other two fields. A concluding section speculates about the nature of the laws of physics, which are algorithms for the handling of information, and must be executable in our real physical universe.

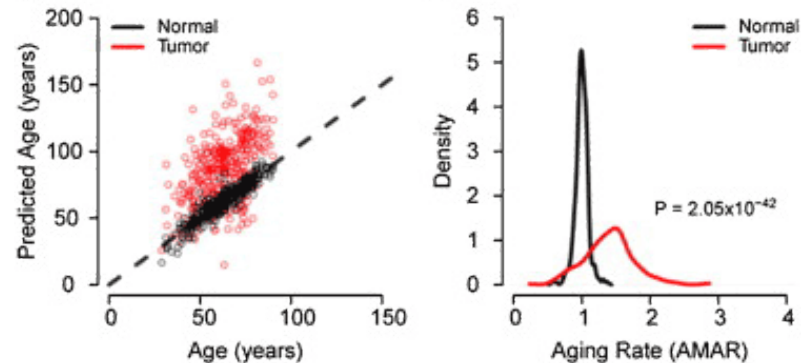
Erasing a bit of information
requires energy

Aging of the epigenome

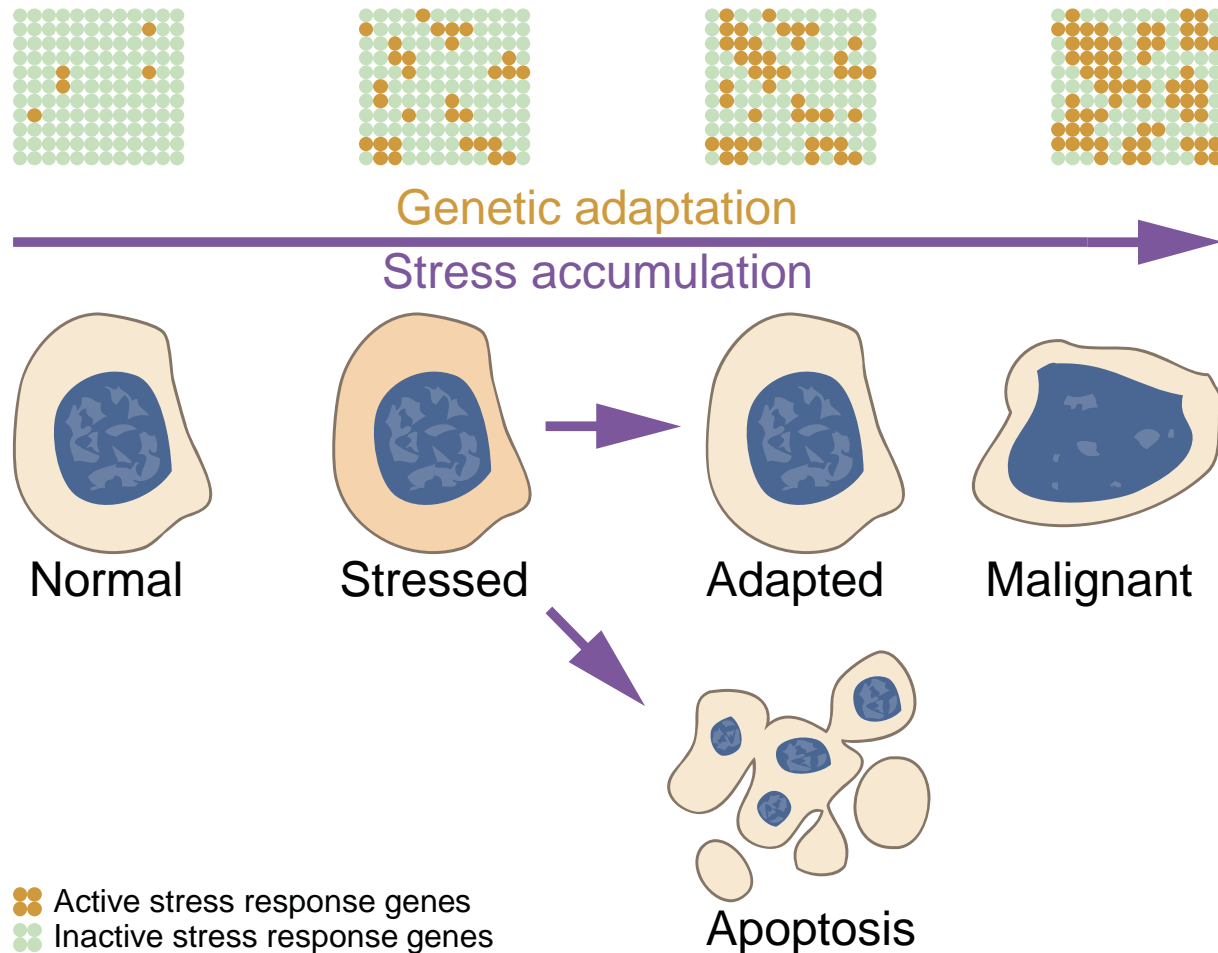
- Age could be predicted by measuring entropy of the methylome



- Tumor 'ages' quickly



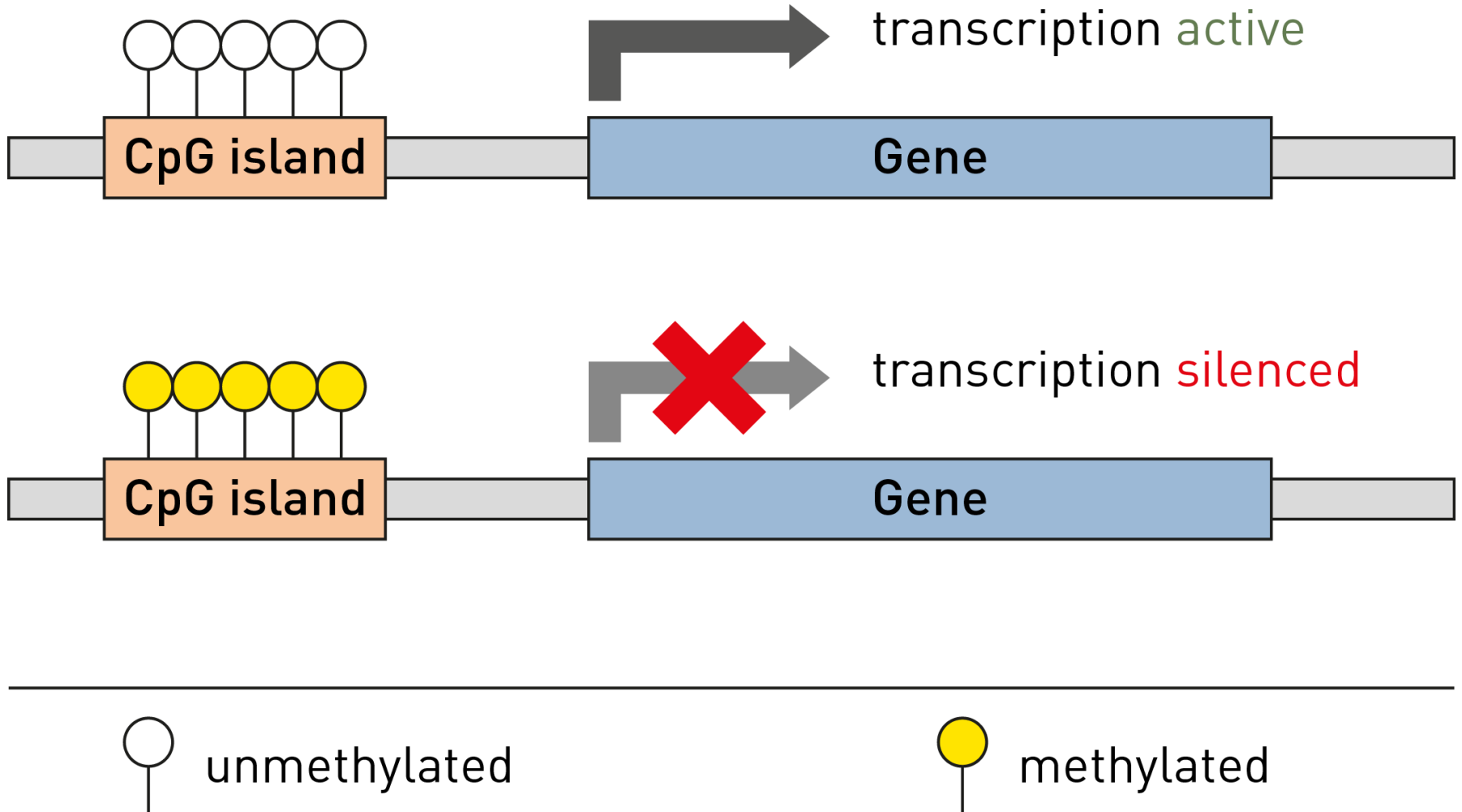
Physiological role of regulation



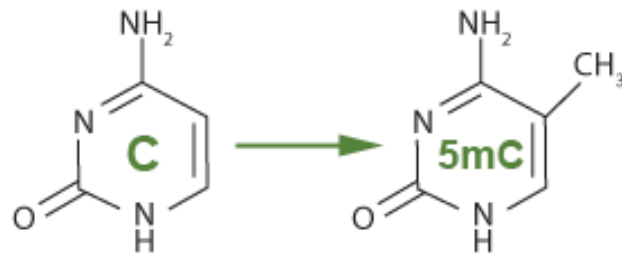
(1)

Introduction to gene regulation by CpG methylation

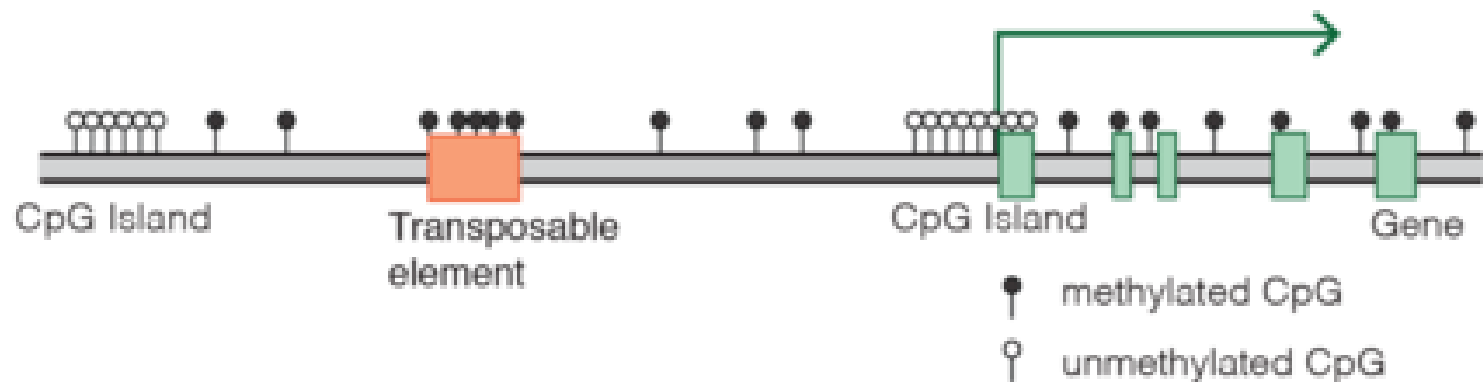
DNA methylation



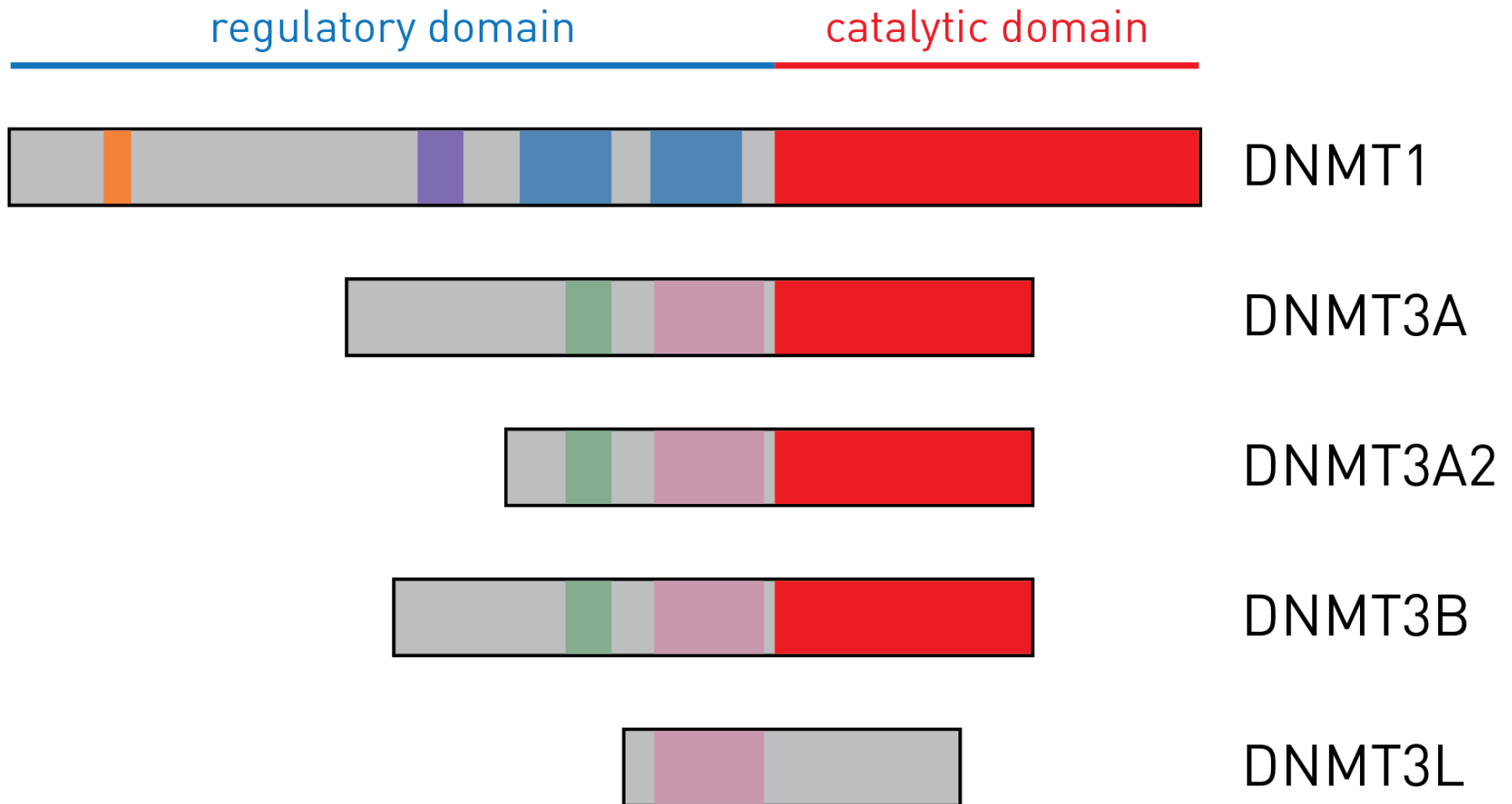
Cytosine methylation



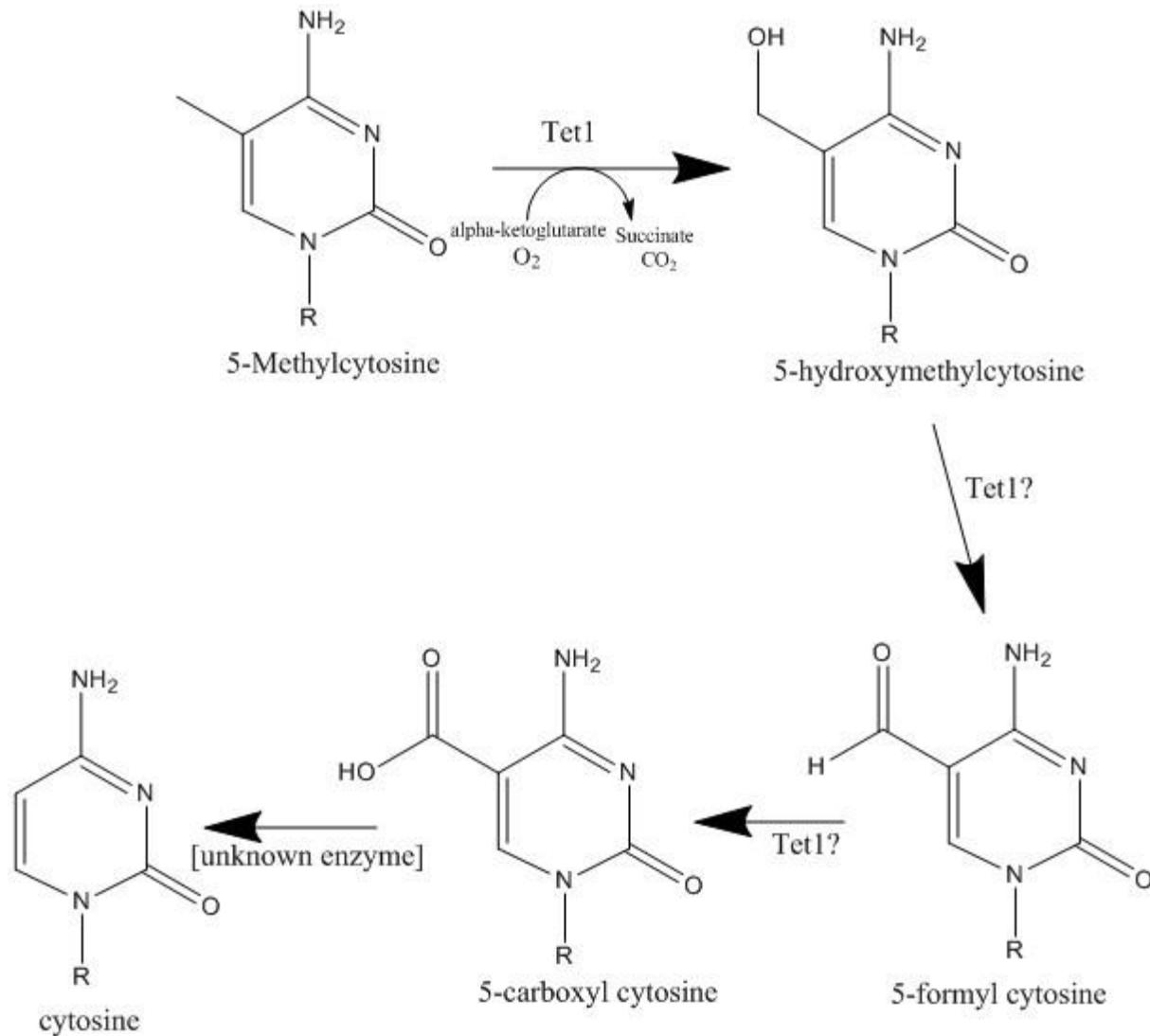
Typical mammalian DNA methylation landscape



DNMT3A gene family



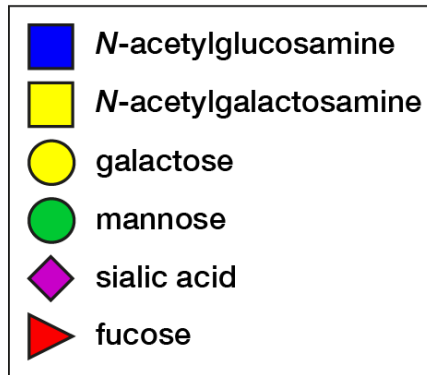
TET1-mediated demethylation



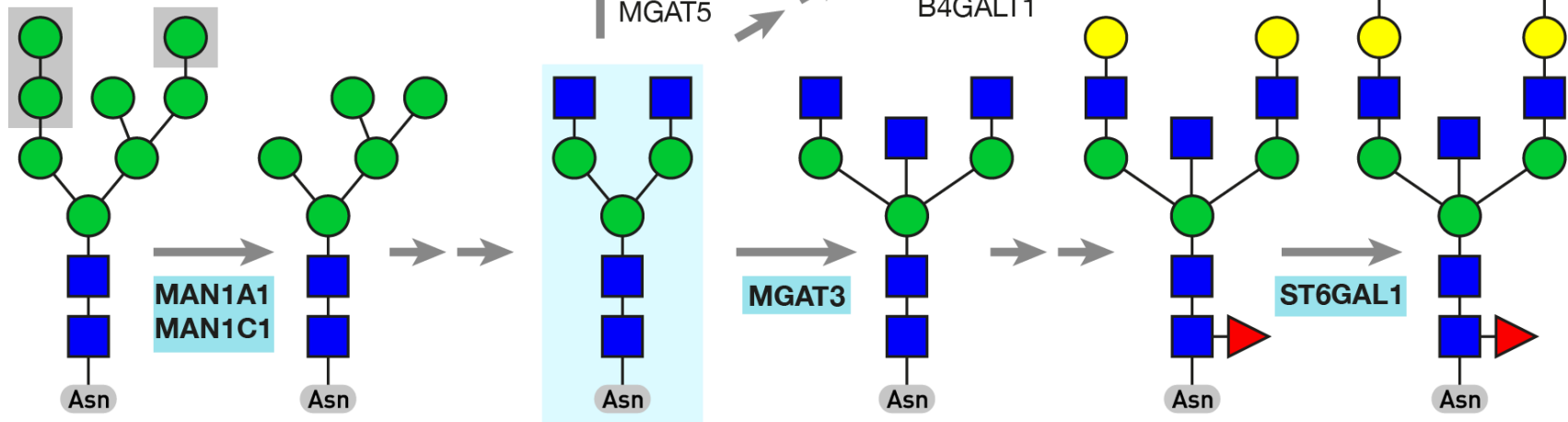
(1.1)

Phenotype: protein glycosylation

Protein *N*-glycosylation



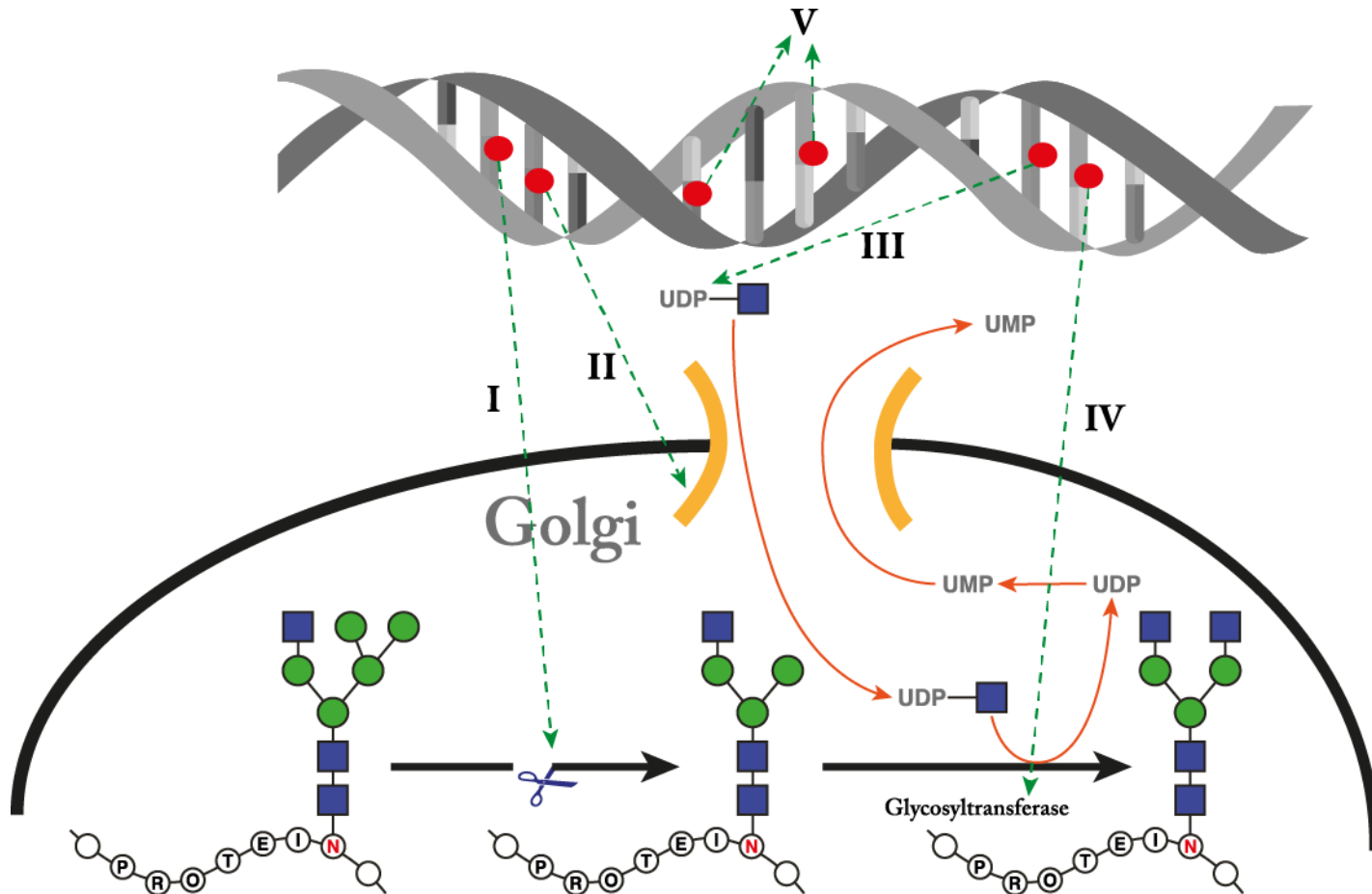
N-linked glycosylation



trimming to core structure

antennae elongation

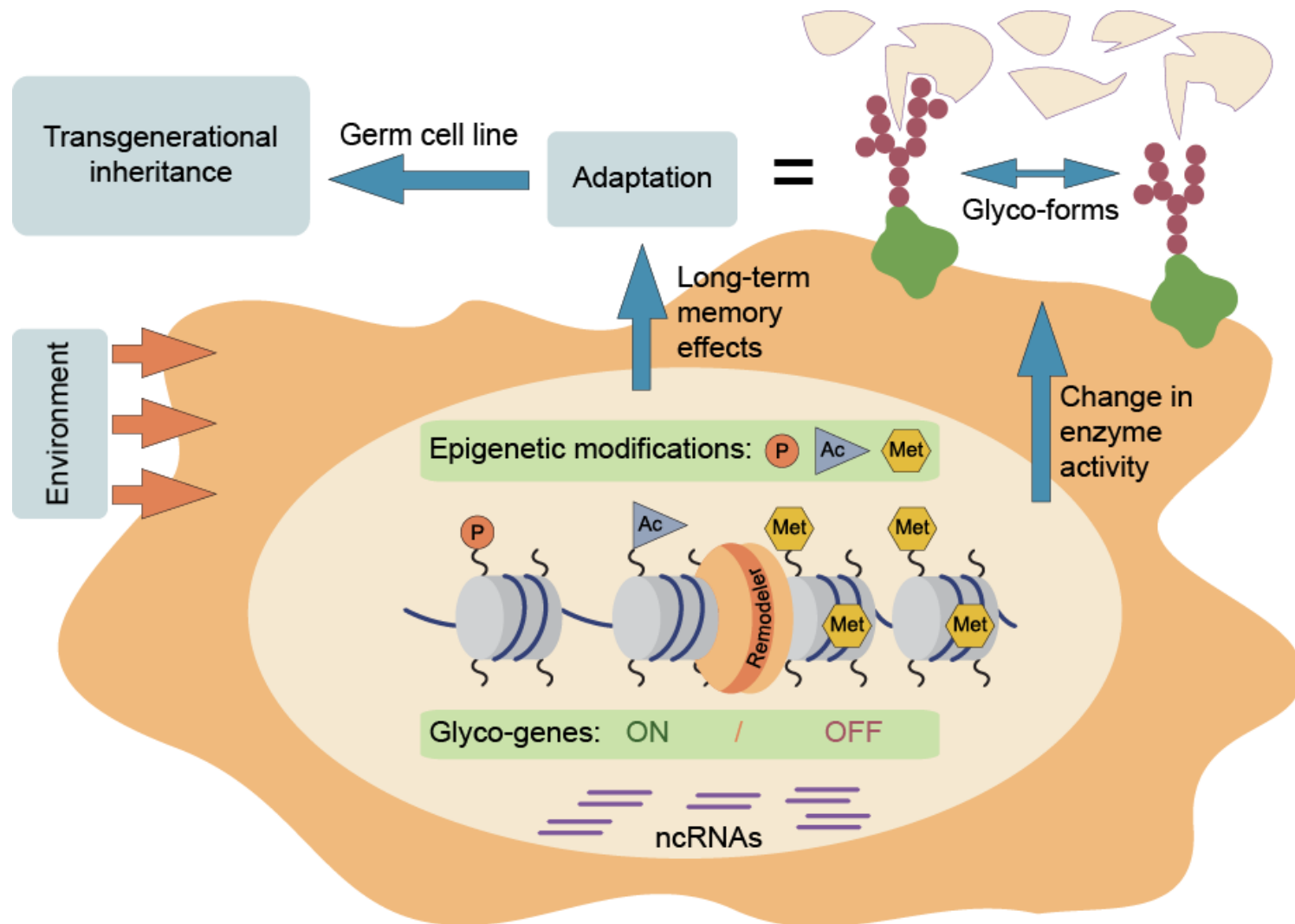
Epigenetics and Protein Glycosylation



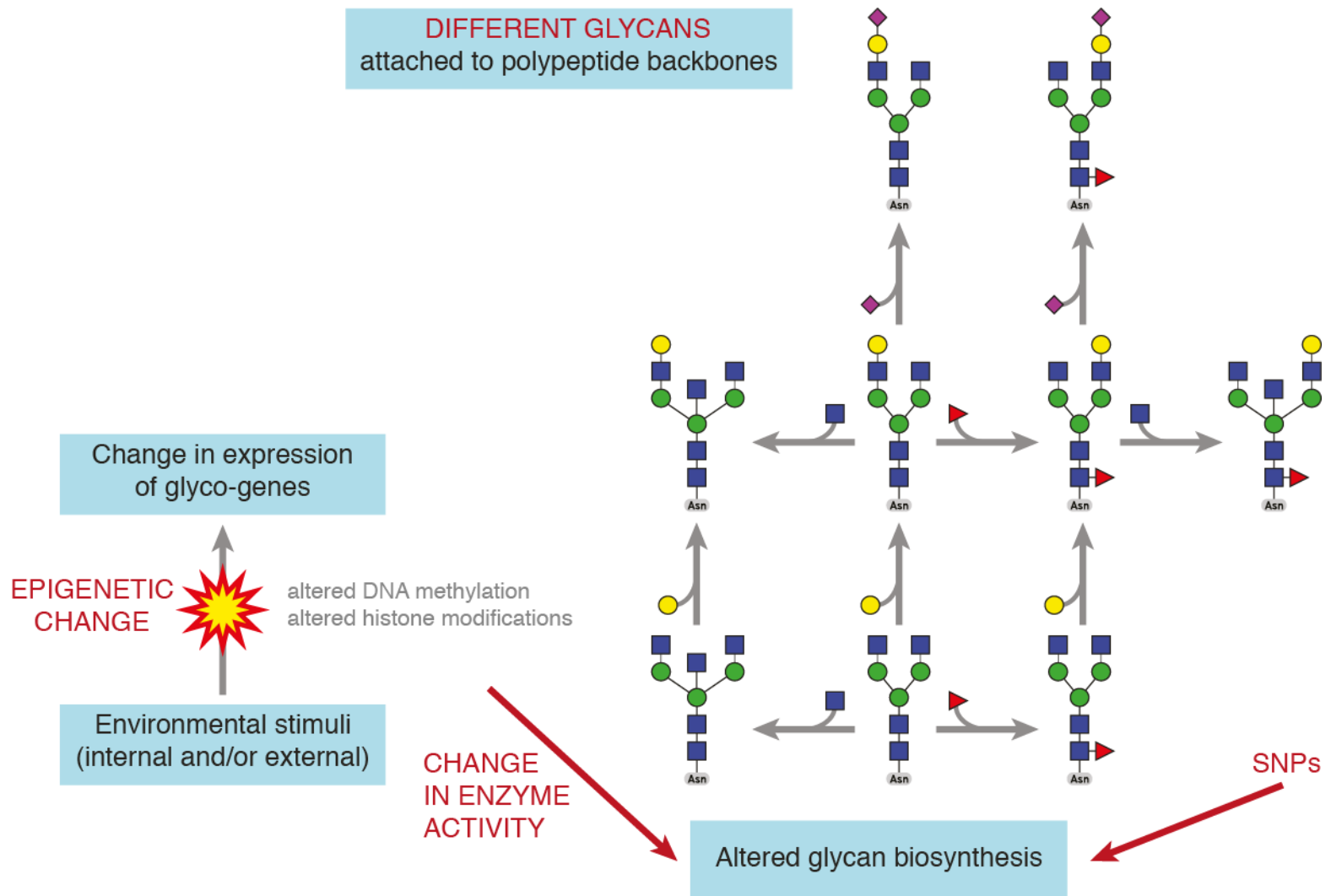
- I Genes for glycosydases
- II Genes for transporters of nucleotide-activated sugars
- III Genes for enzymes involved in biosynthesis of nucleotide-activated sugars
- IV Genes for glycosyltransferases
- V Other genes directly or indirectly involved in glycosylation

■ N-acetylglucosamine
● Mannose

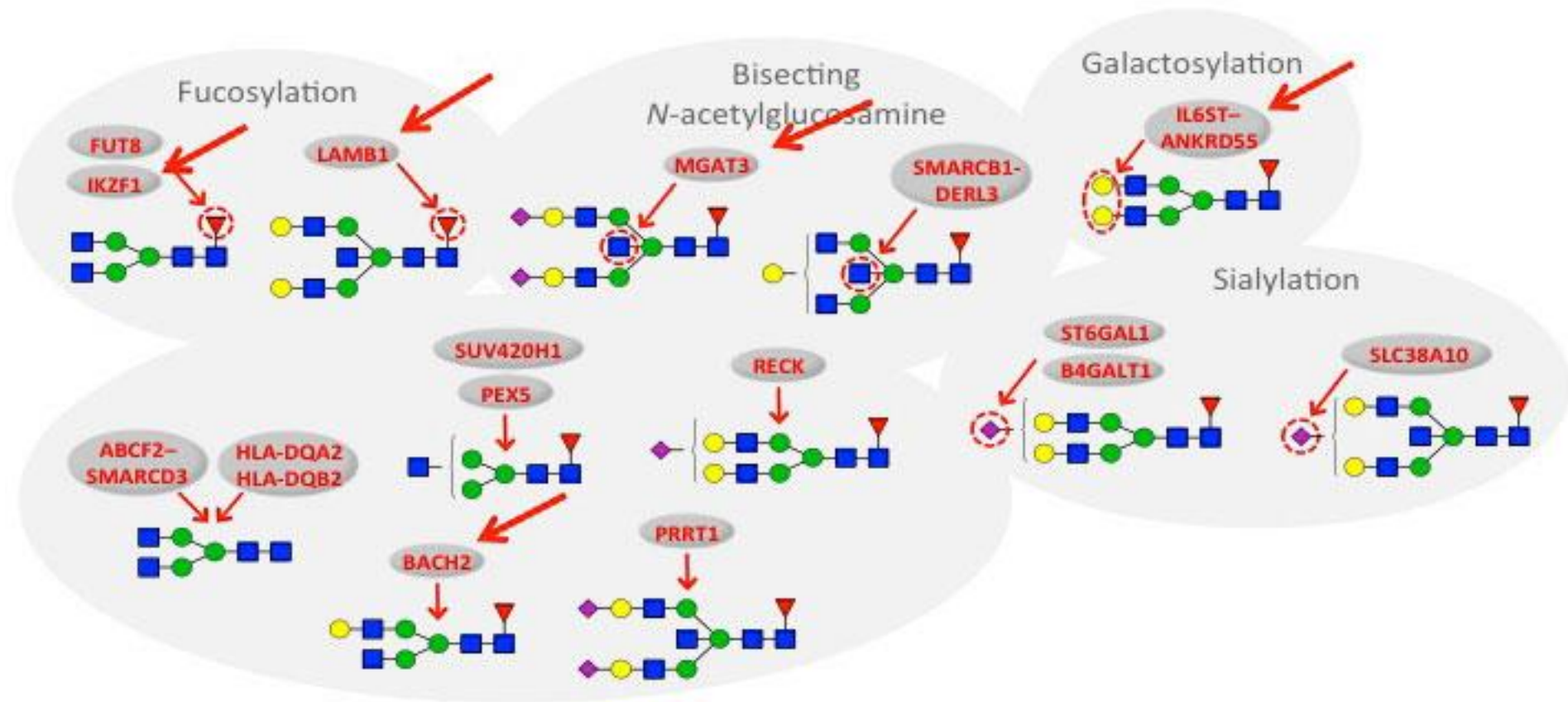
Glycosylation in adaptation



Genes for IgG glycosylation



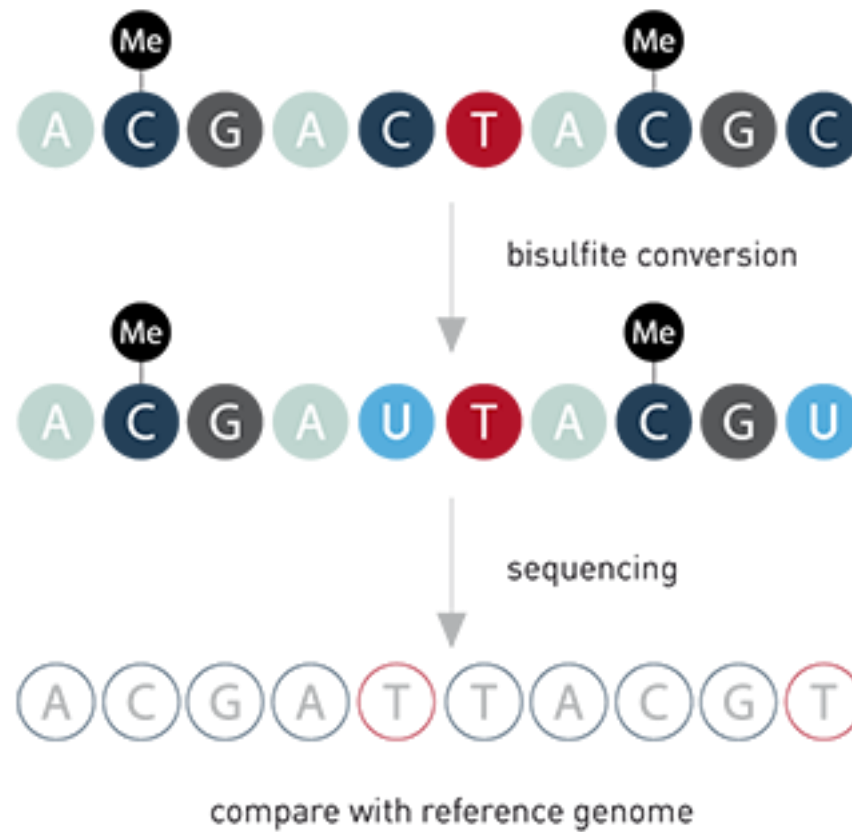
GWAS hits in IBD (IgG glycosylation)



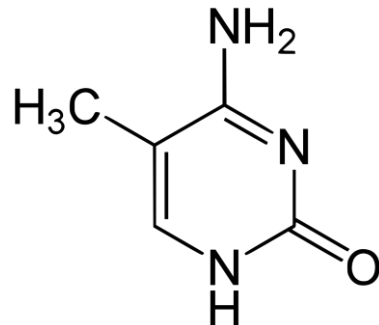
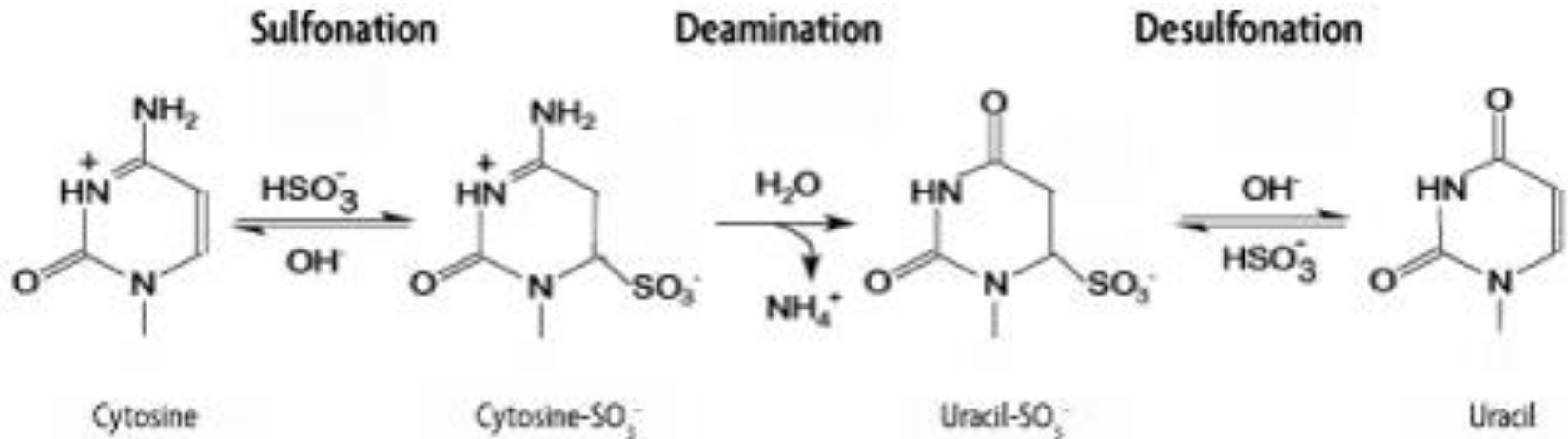
(2)

Analysis of DNA by pyrosequencing

Bisulfite conversion



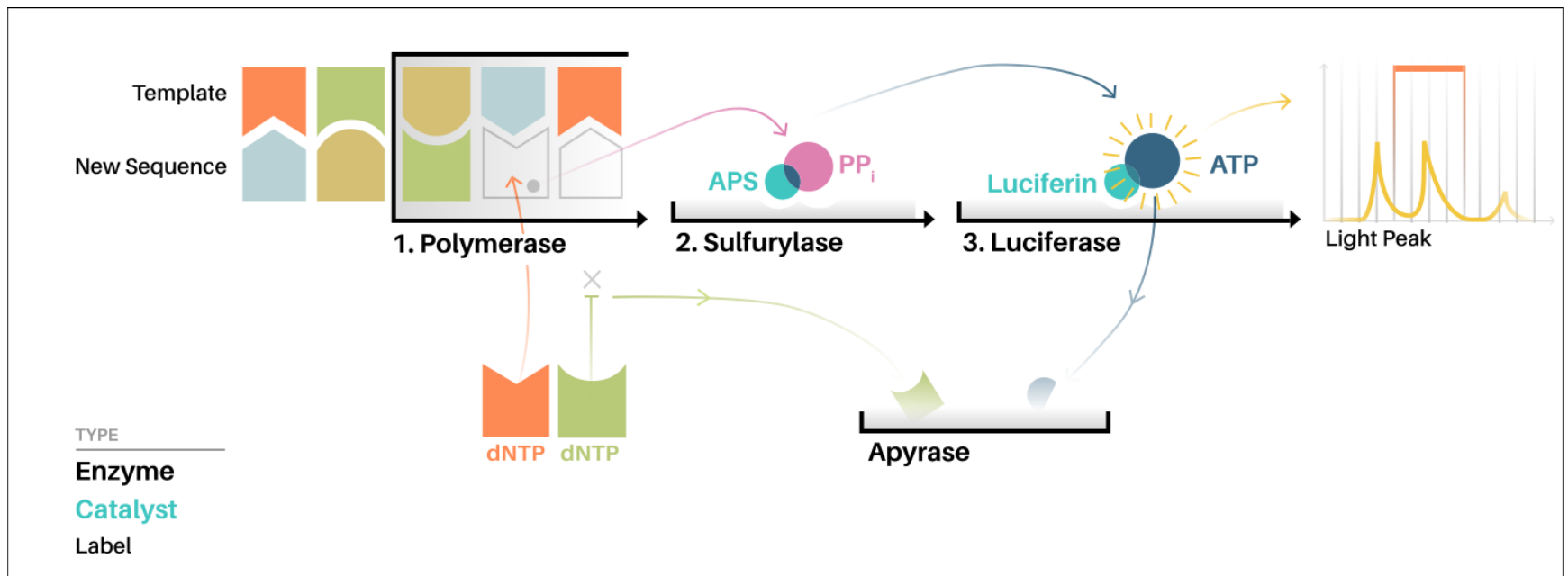
Conversion reaction



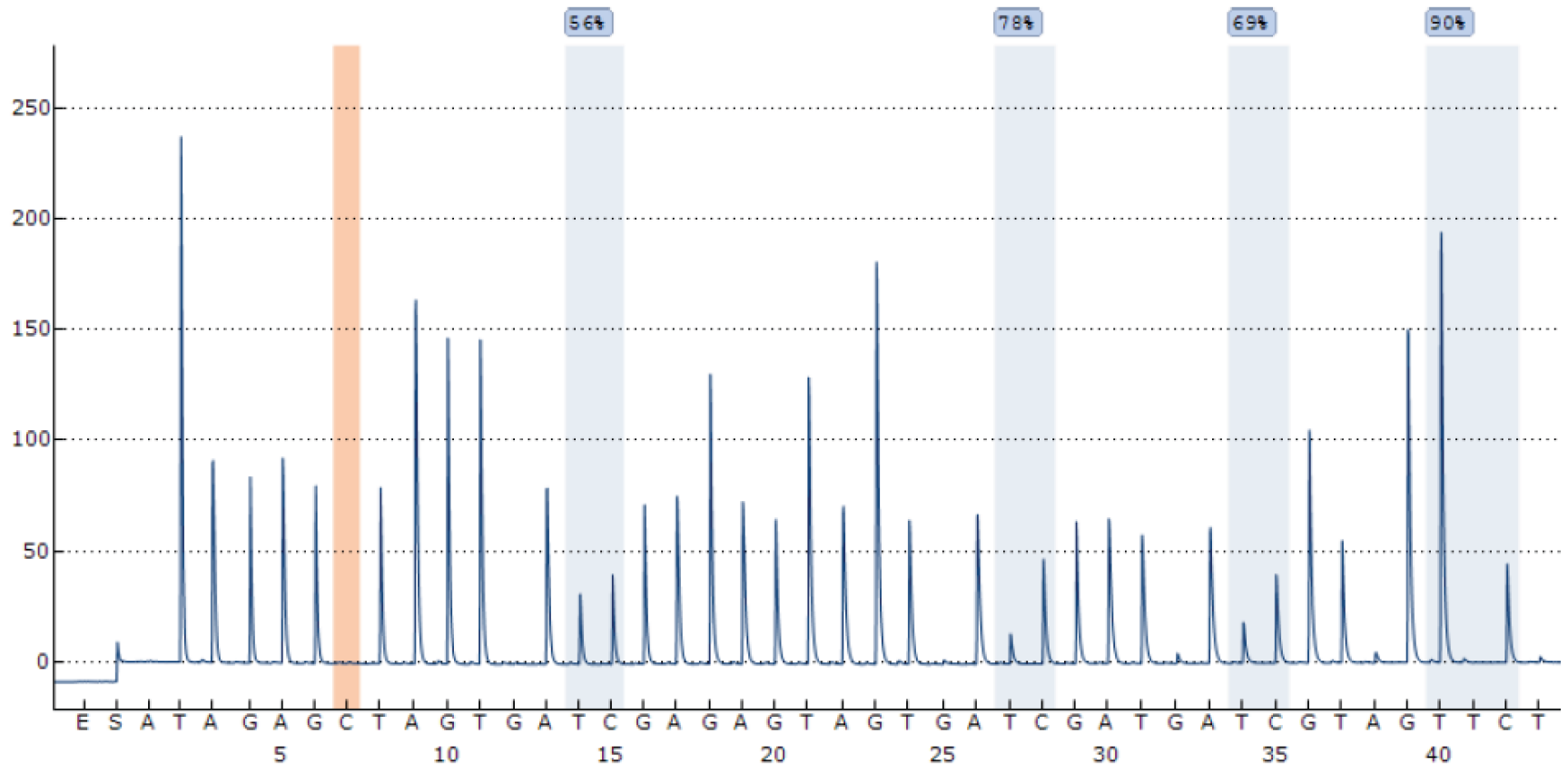
5-methylcytosine

Not susceptible to bisulphite conversion

Pyrosequencing



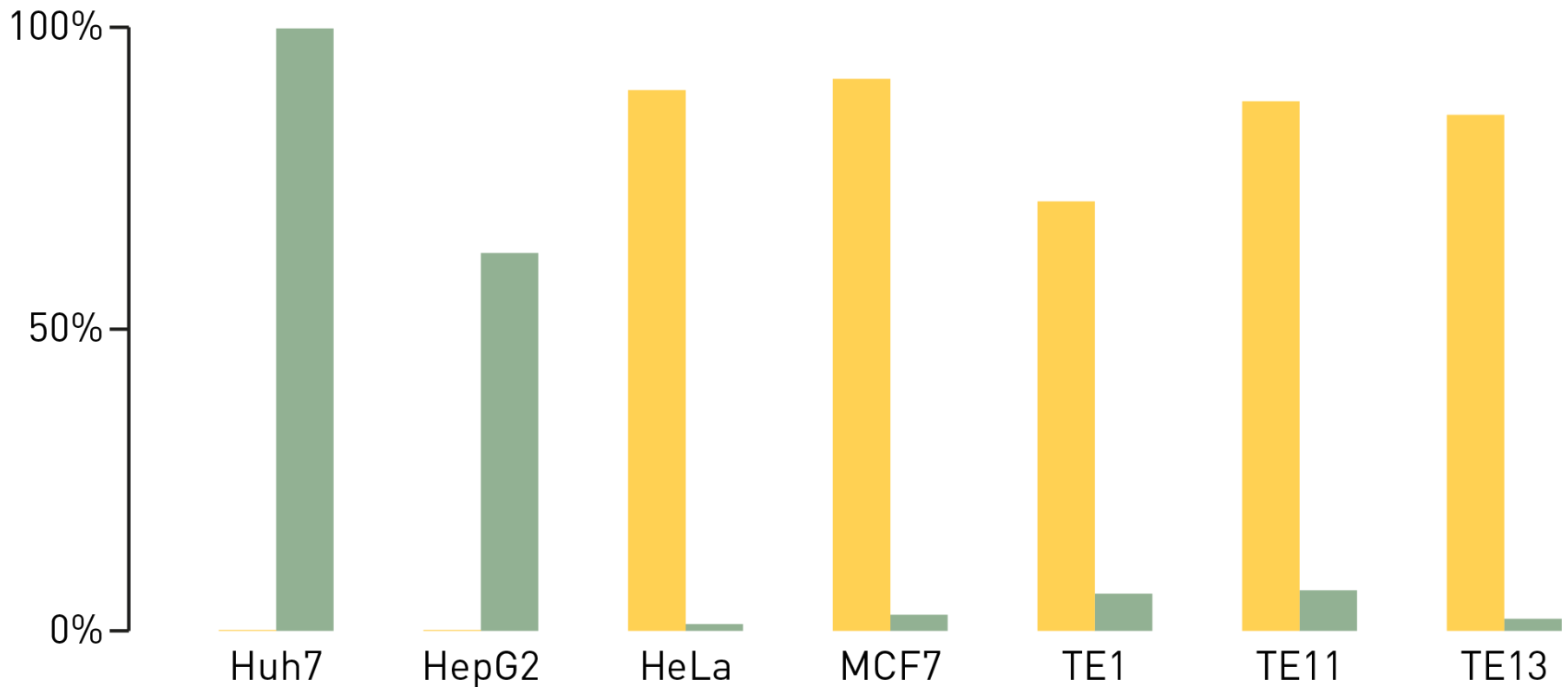
Interpreting pyrosequencing data



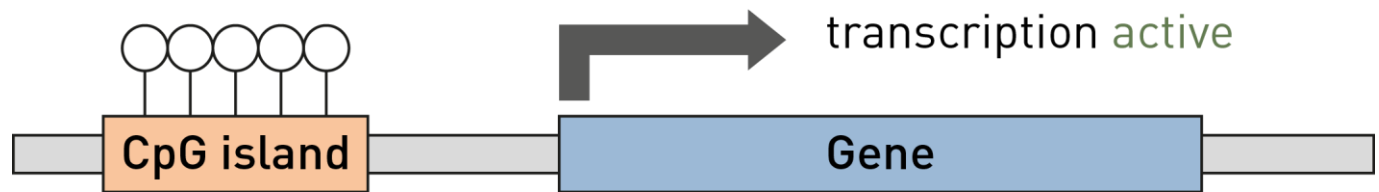
(3)


Finding regulatory sites


Case study: HNF1A gene regulation



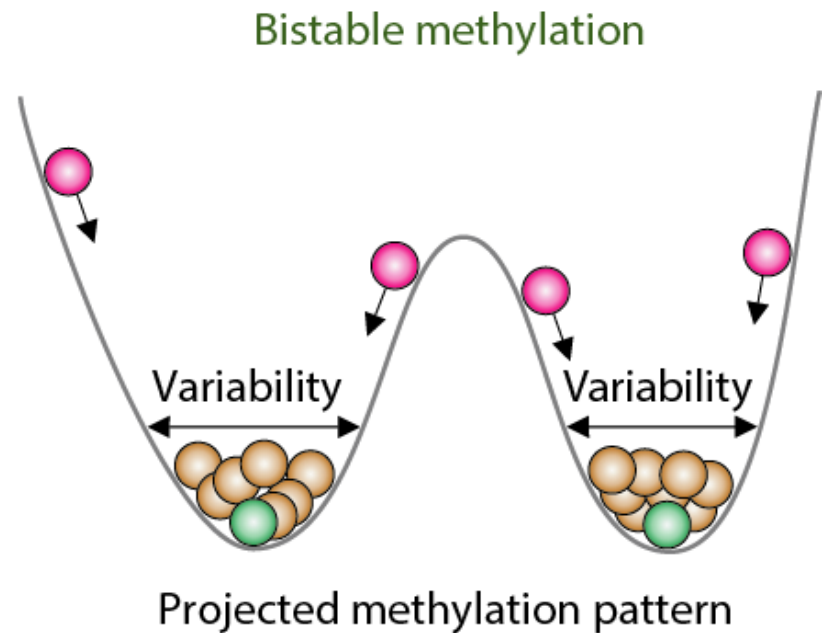
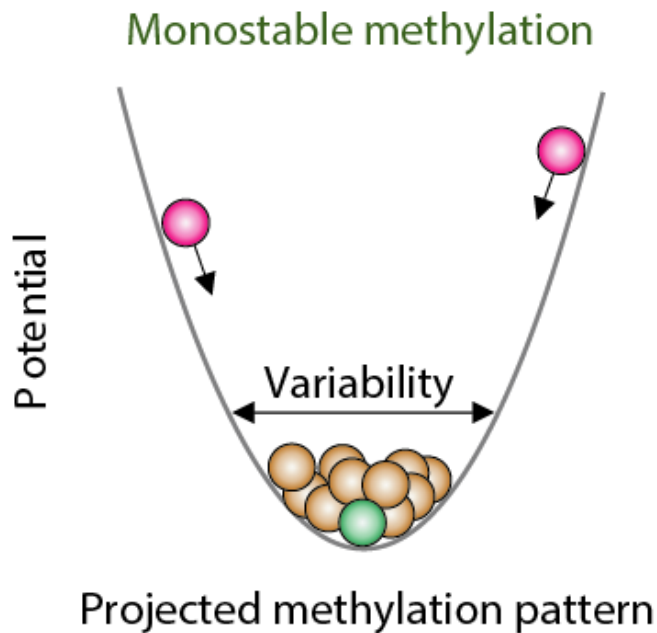
From correlation to direct manipulation



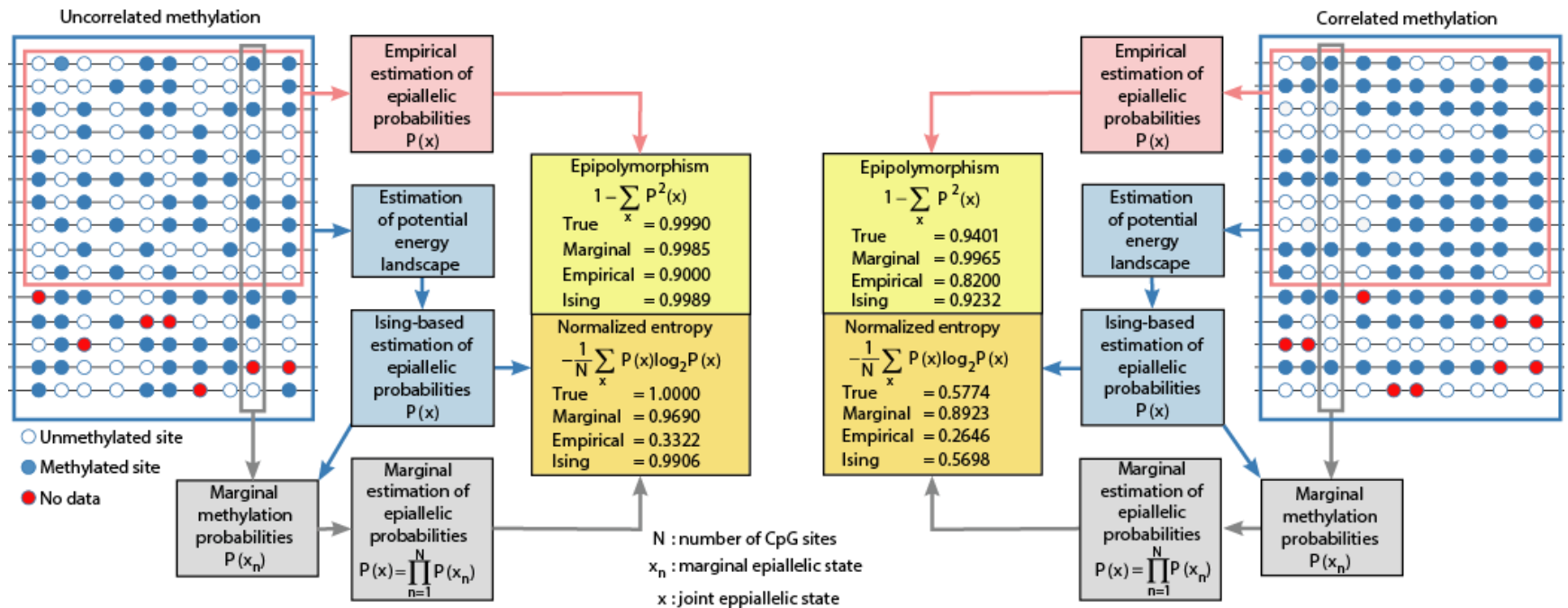
 unmethylated

 methylated

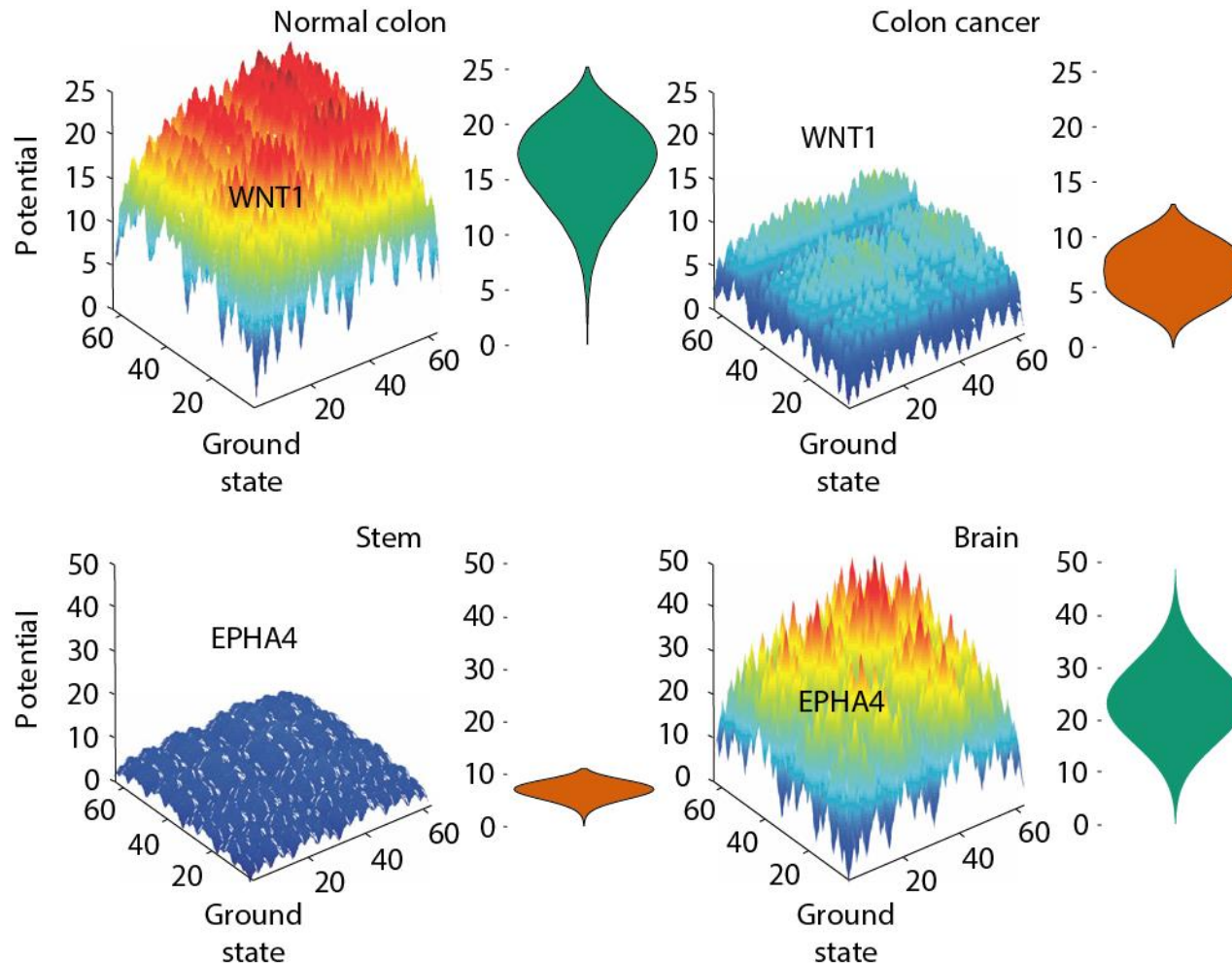
Regulated or stochastic?



Finding regulatory sites



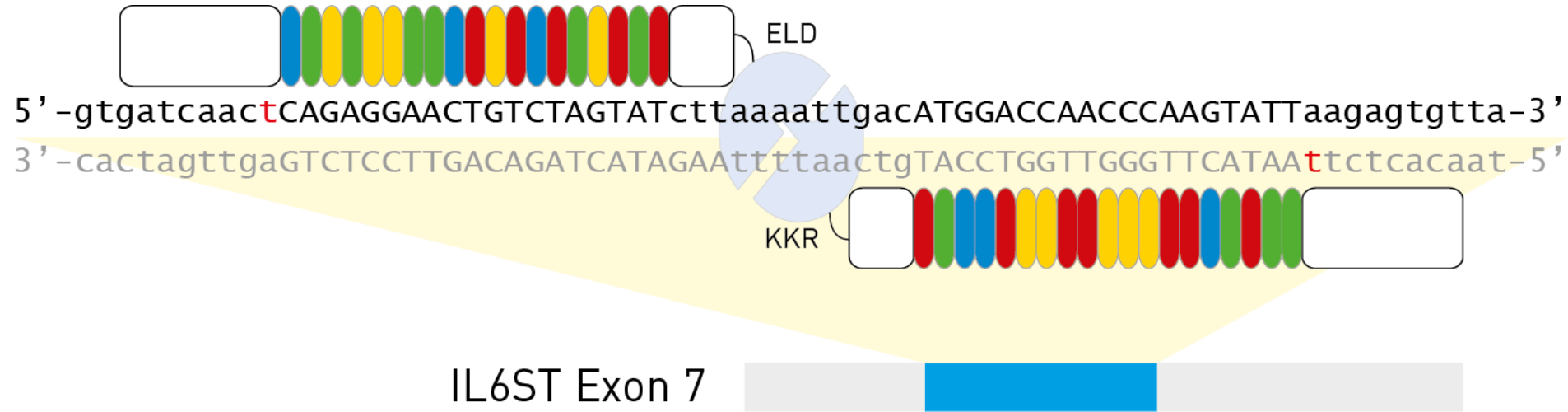
Example in colon cancer



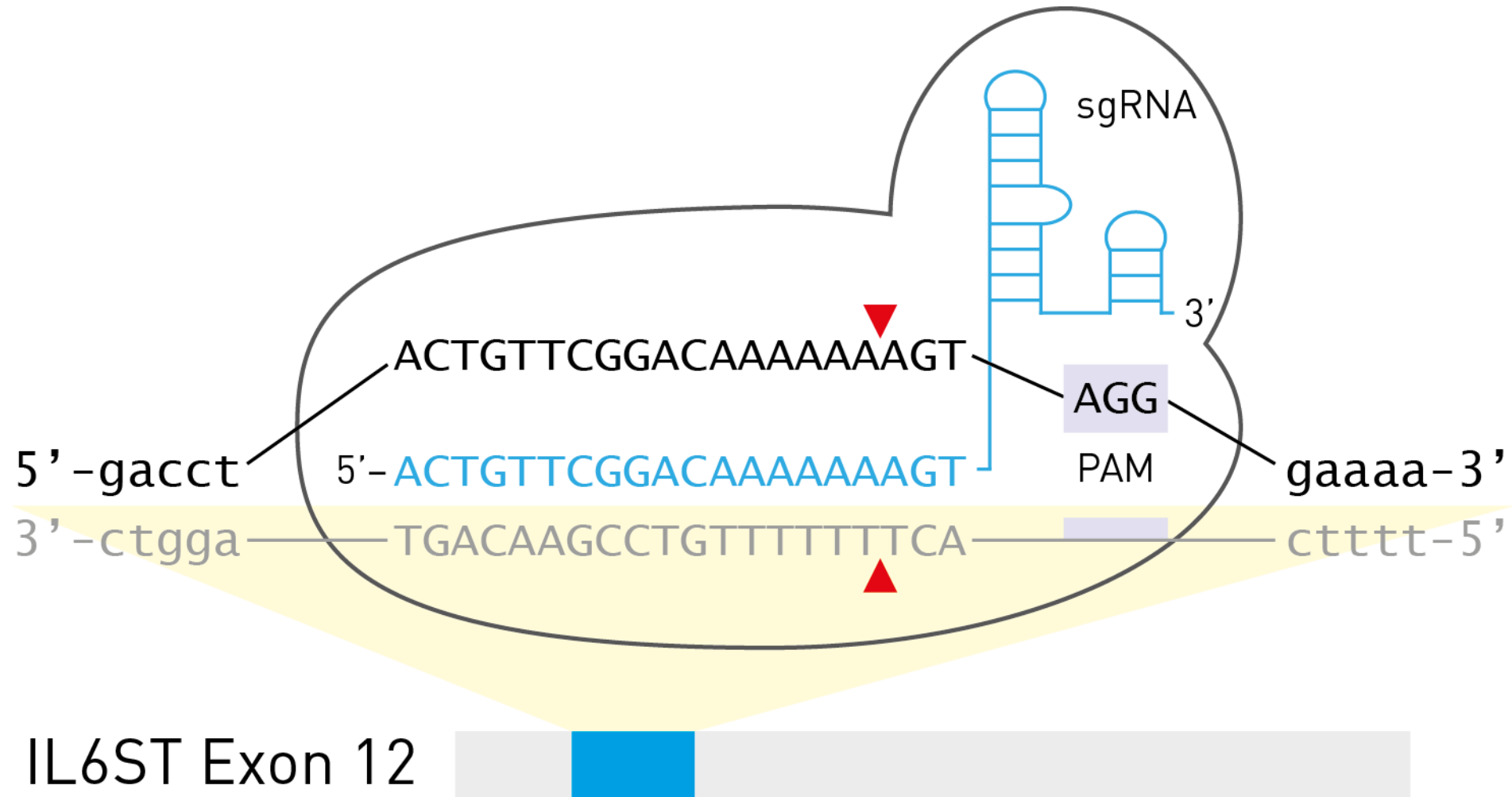
(4)

Tools for targeted methylation and
demethylation

Genome editing: TALEN



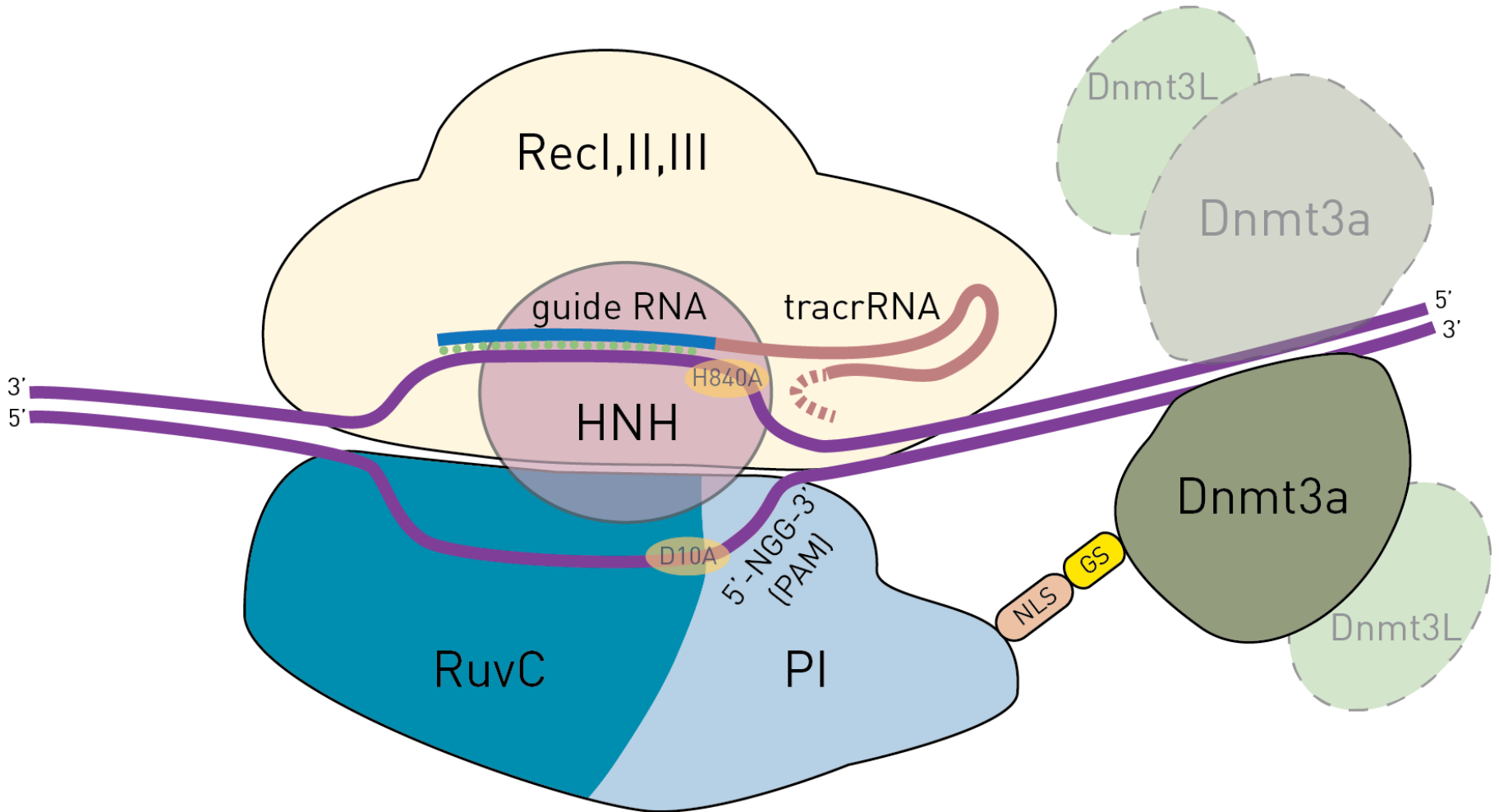
Genome editing: CRISPR/Cas9



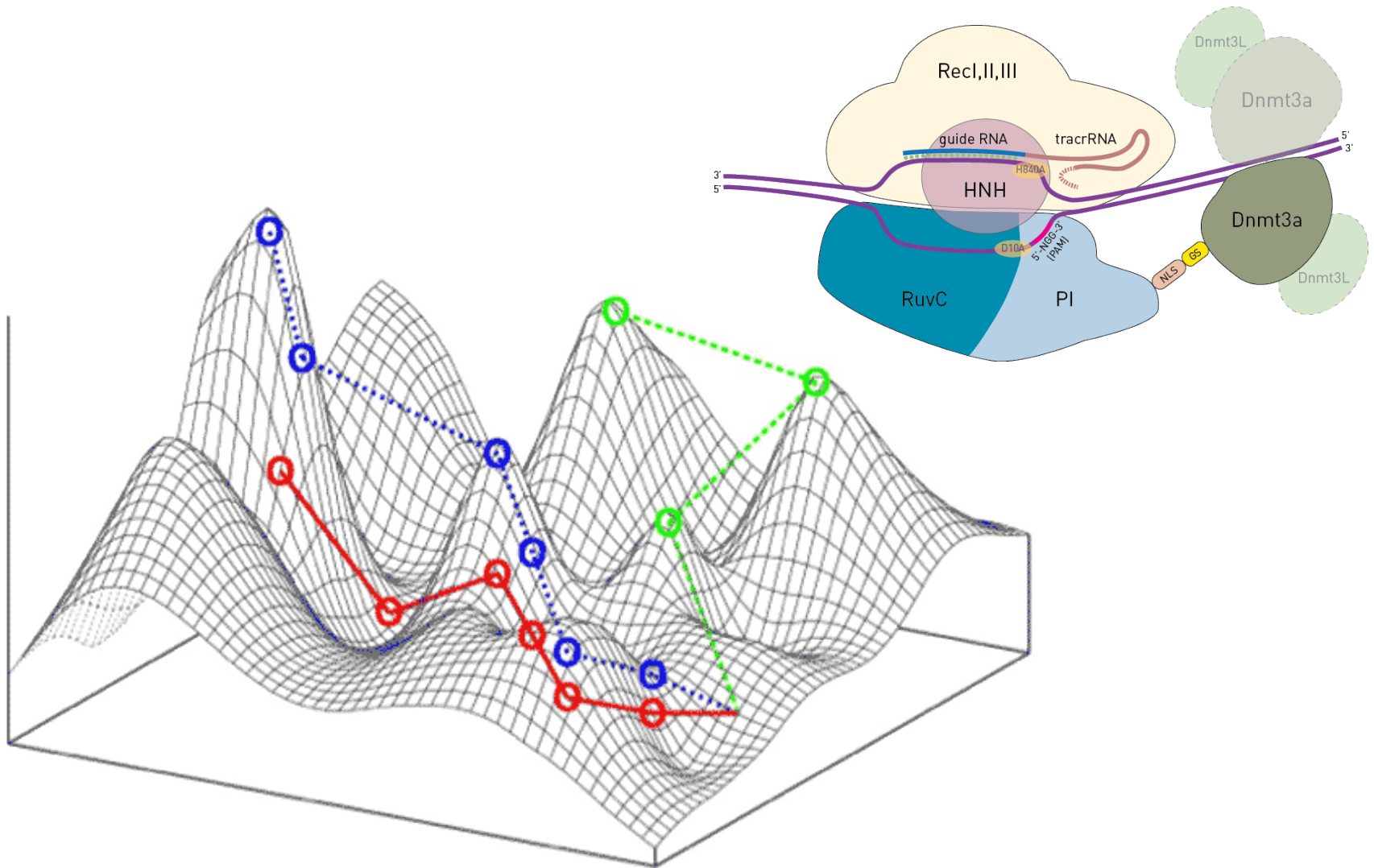
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CRISPR/Cas9 tools for epigenome editing

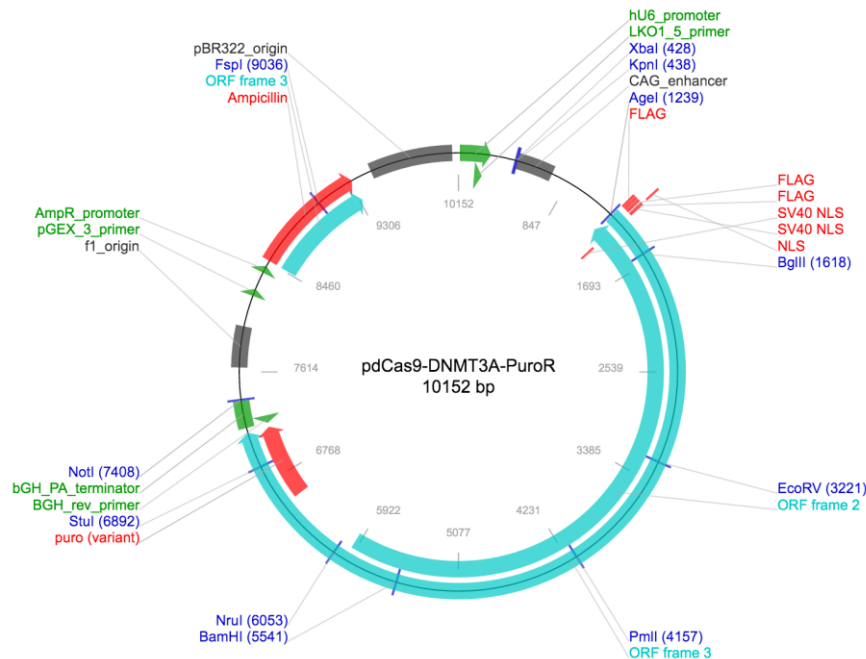
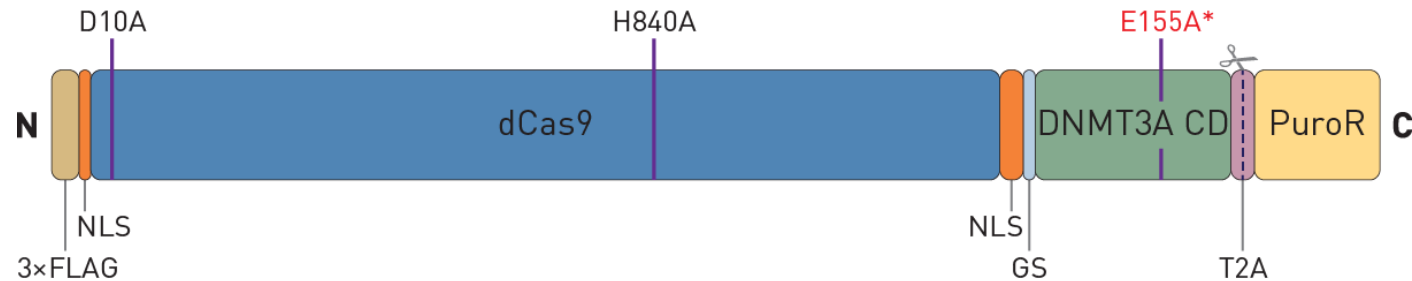
Cas9-DNMT3A construct



Epigenome editing



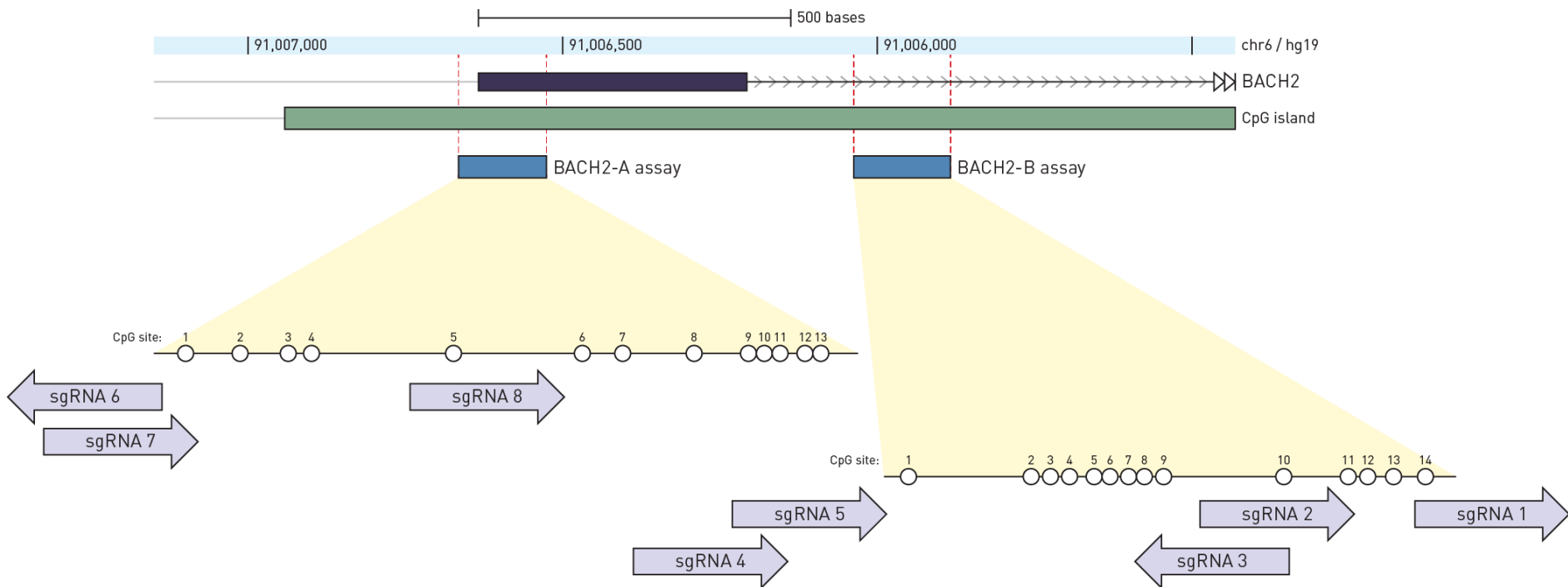
The Construct



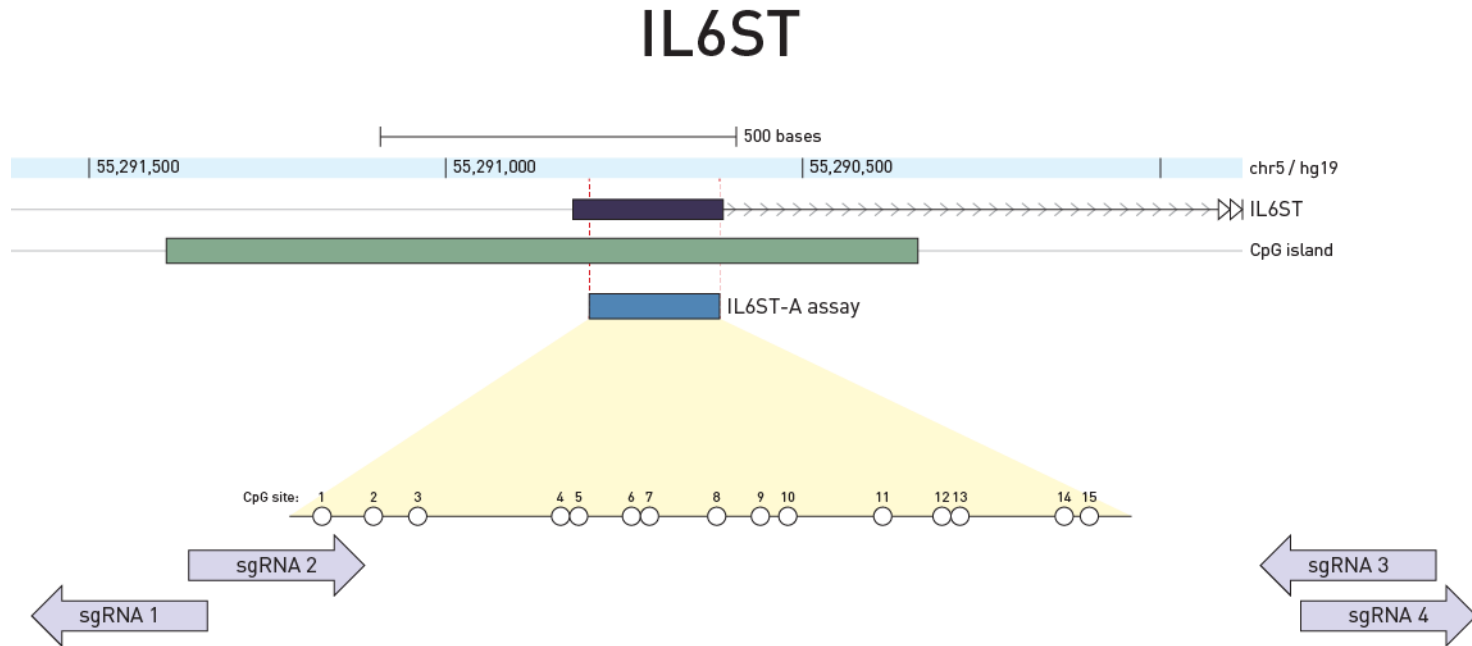
Based on Addgene Plasmid #48141
(pX462)

Genome engineering using the CRISPR-Cas9 system. Ran FA, Hsu PD, Wright J, Agarwala V, Scott DA, Zhang F. Nat Protoc. 2013 Nov;8(11):2281-308.

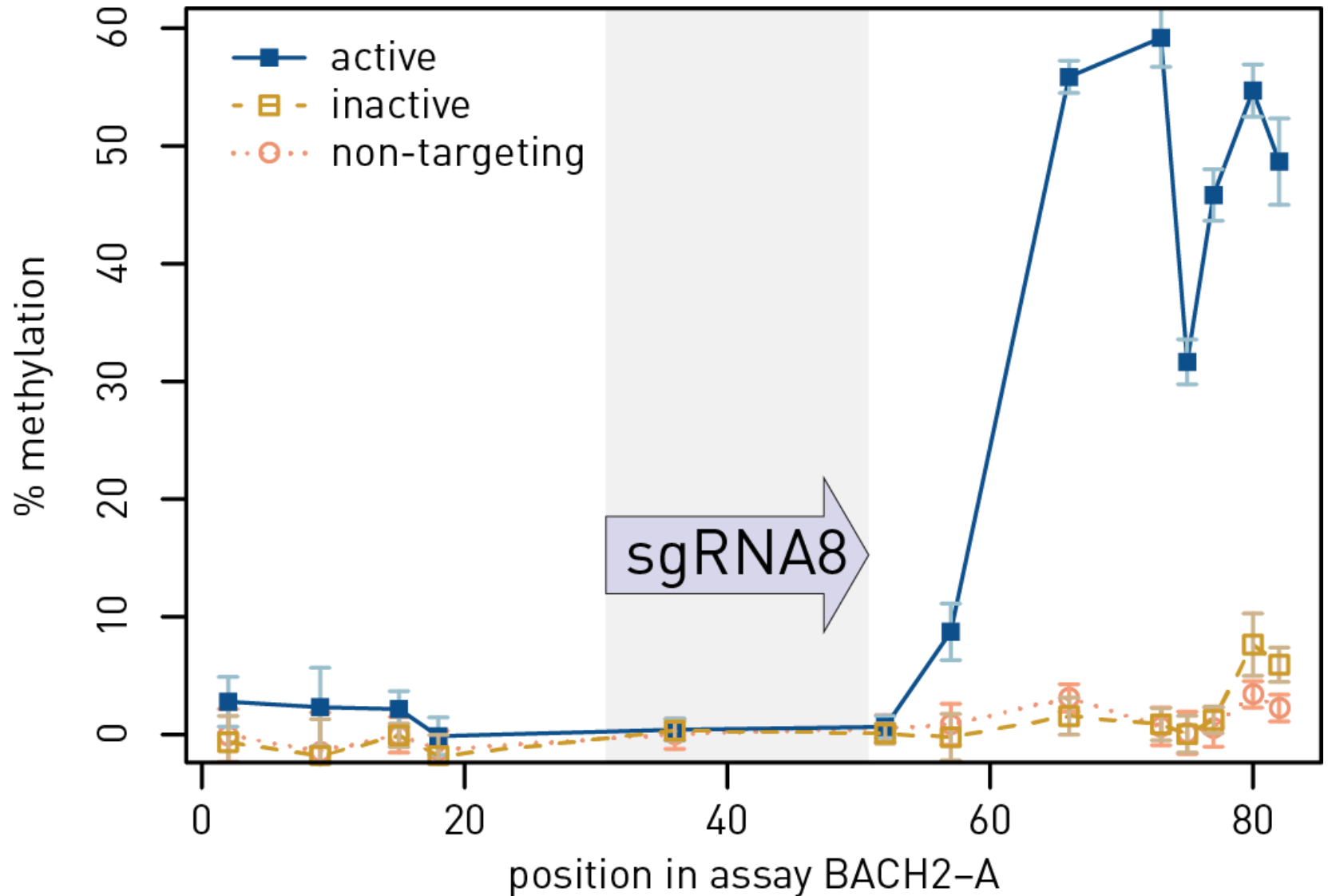
Targeting to BACH2



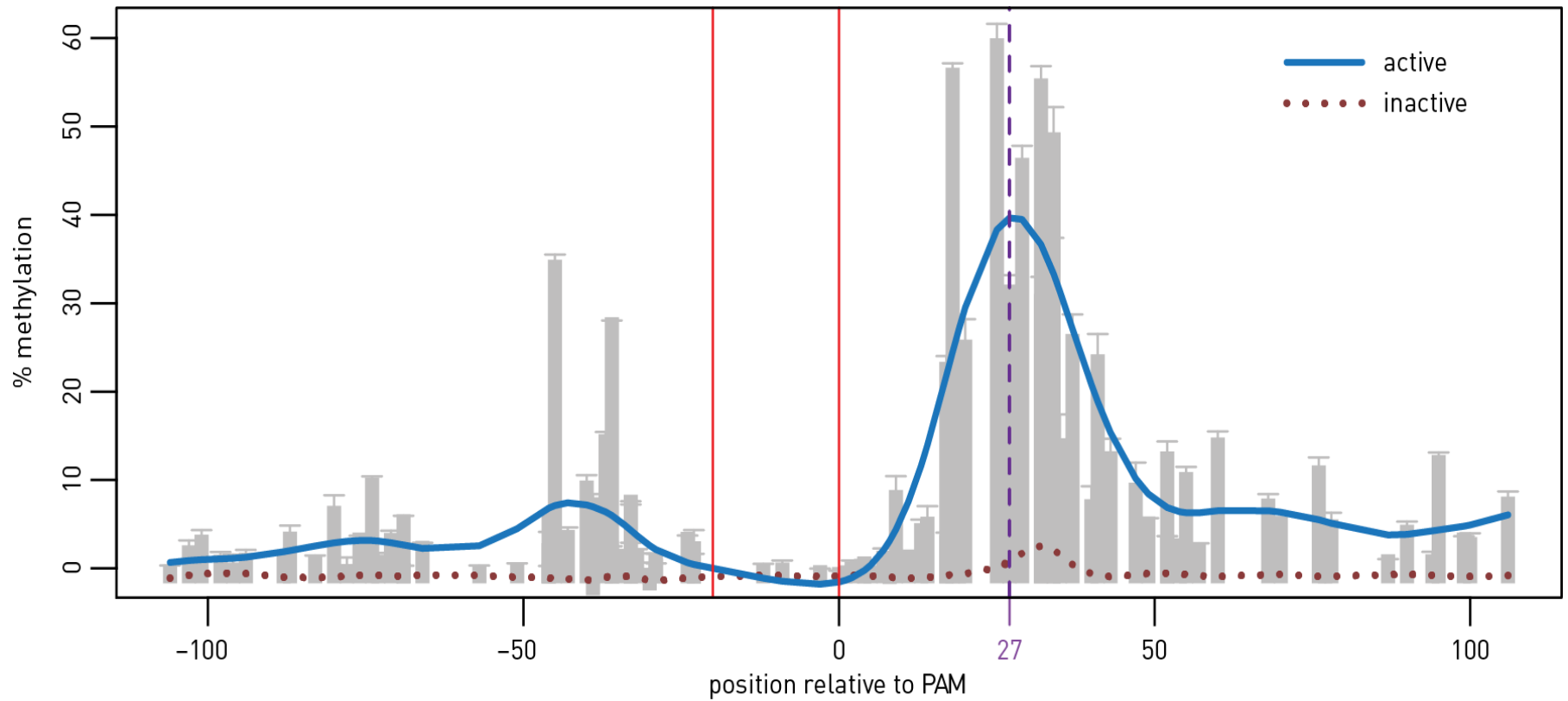
Targeting to IL6ST



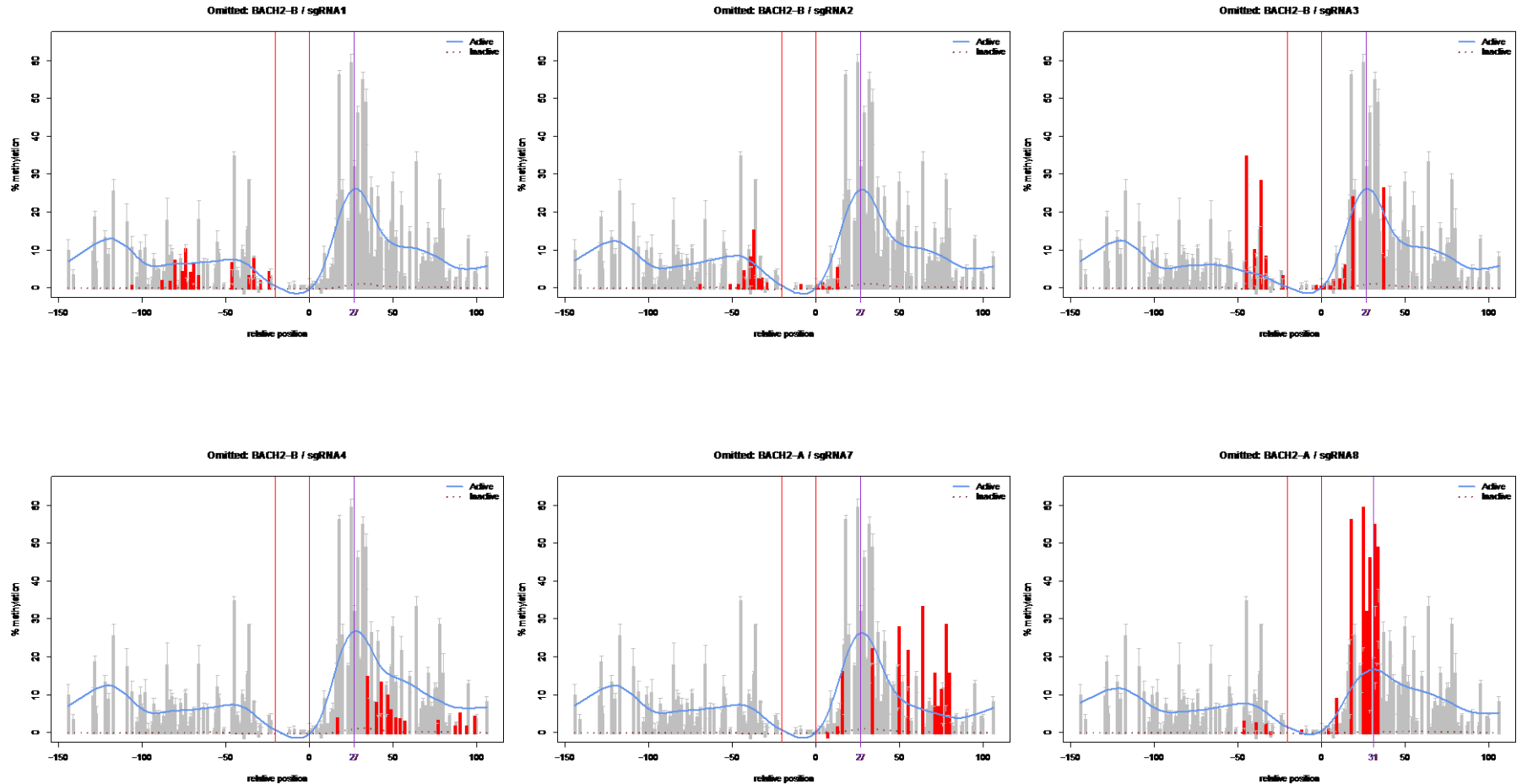
Construct activity



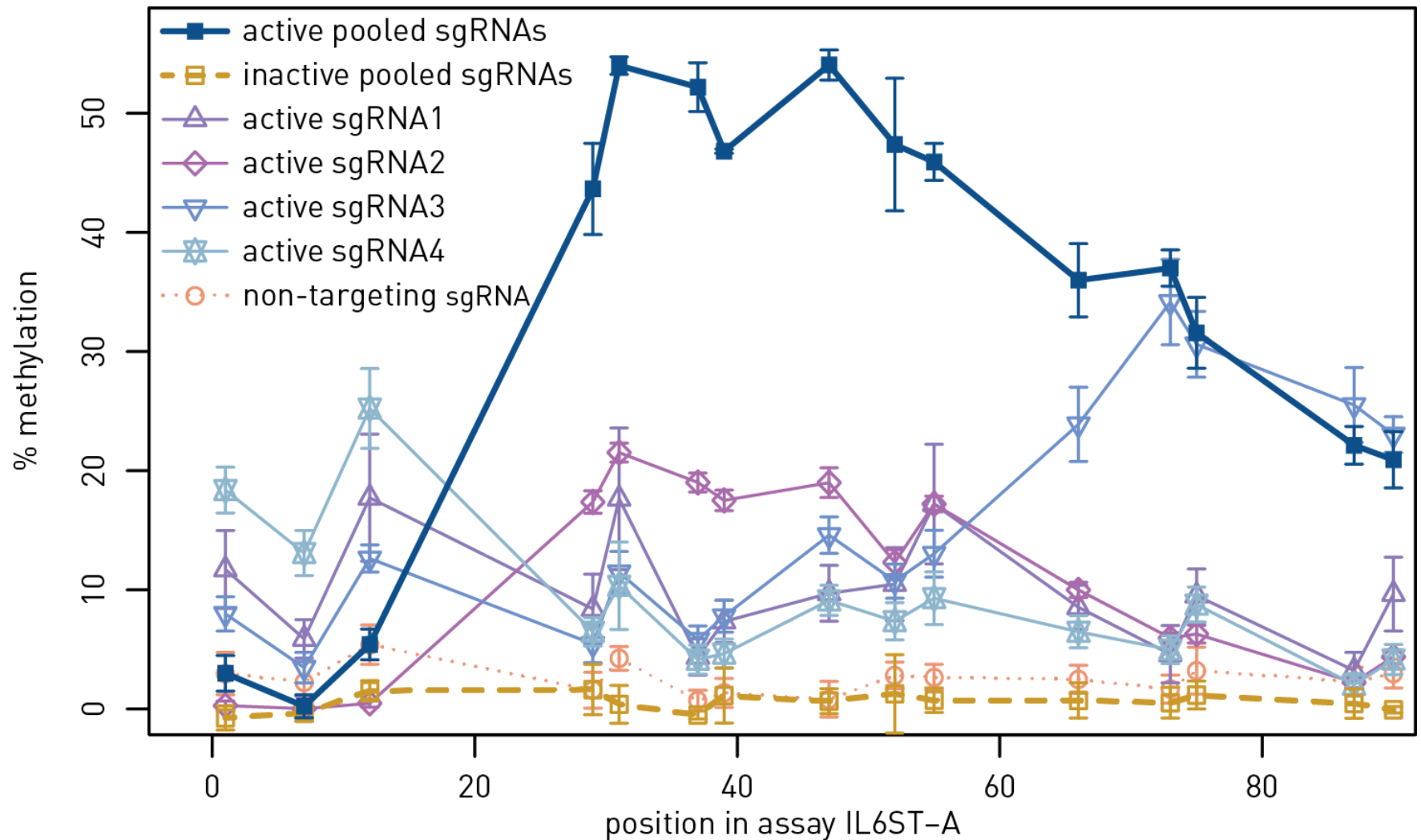
Activity profile



Profile Robustness

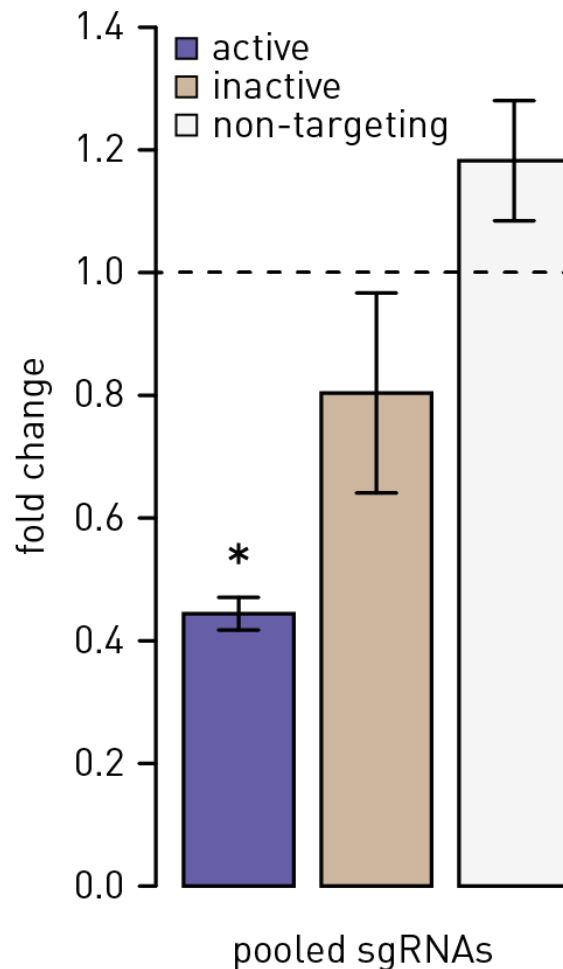


Multiple targeting

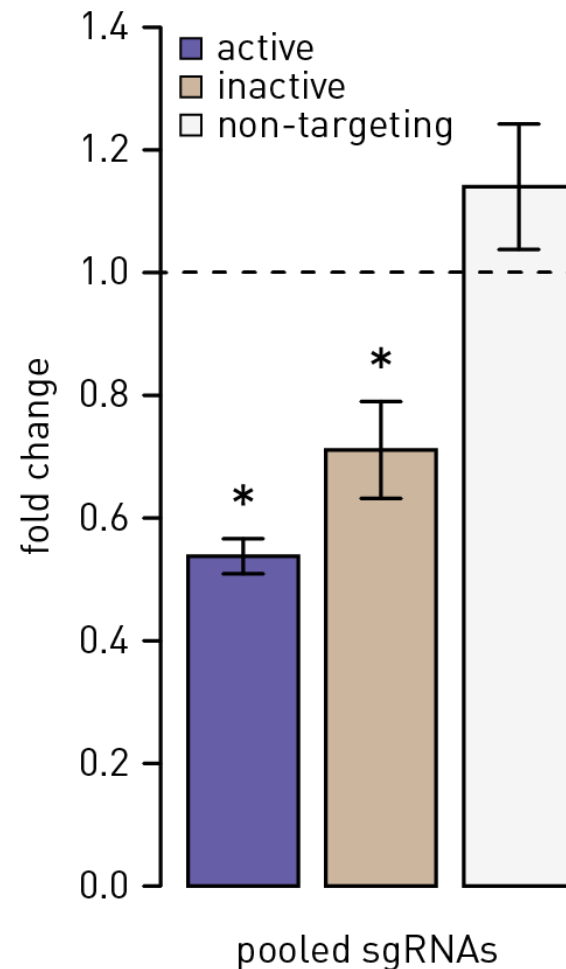


Effect on expression

IL6ST

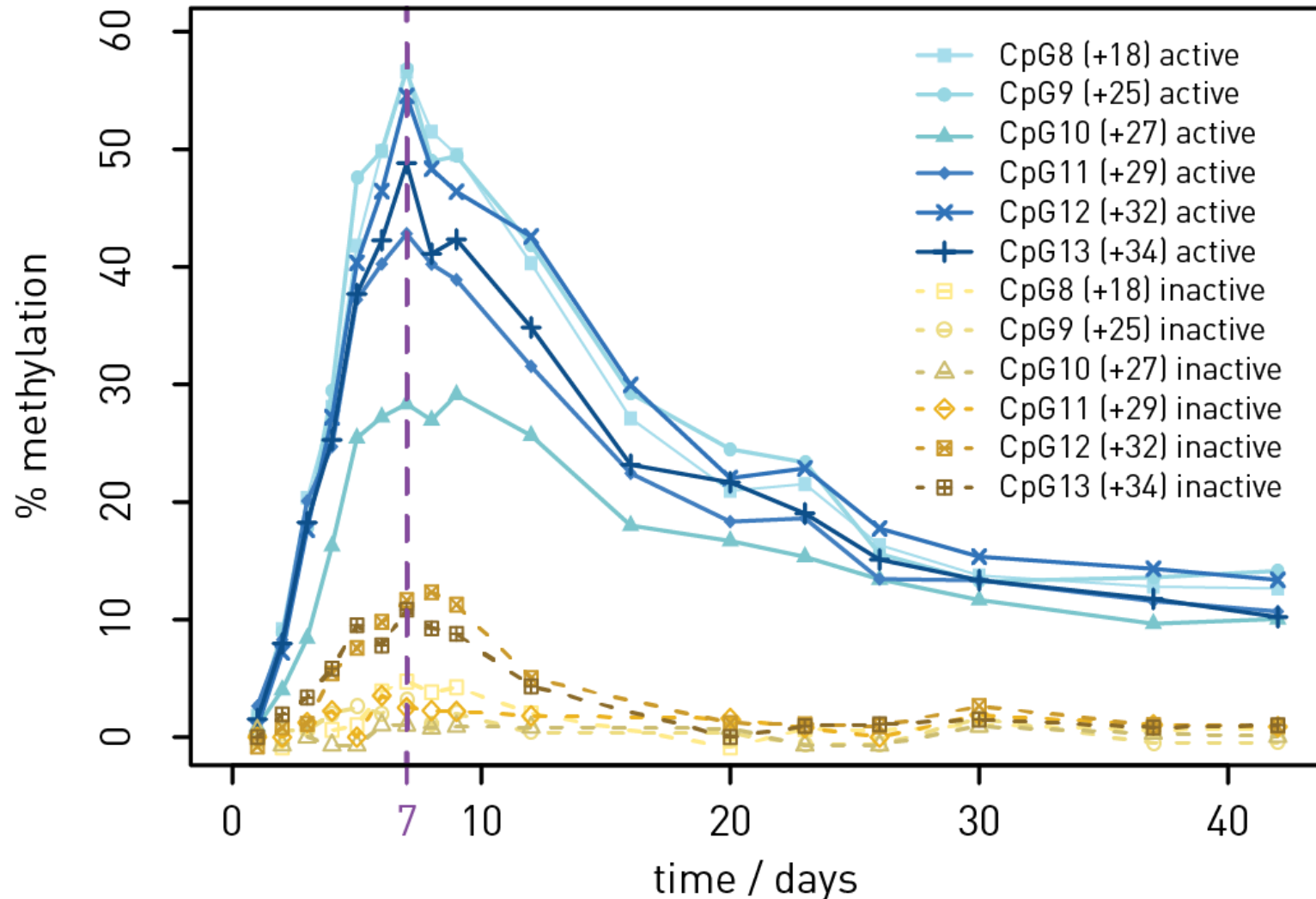


BACH2

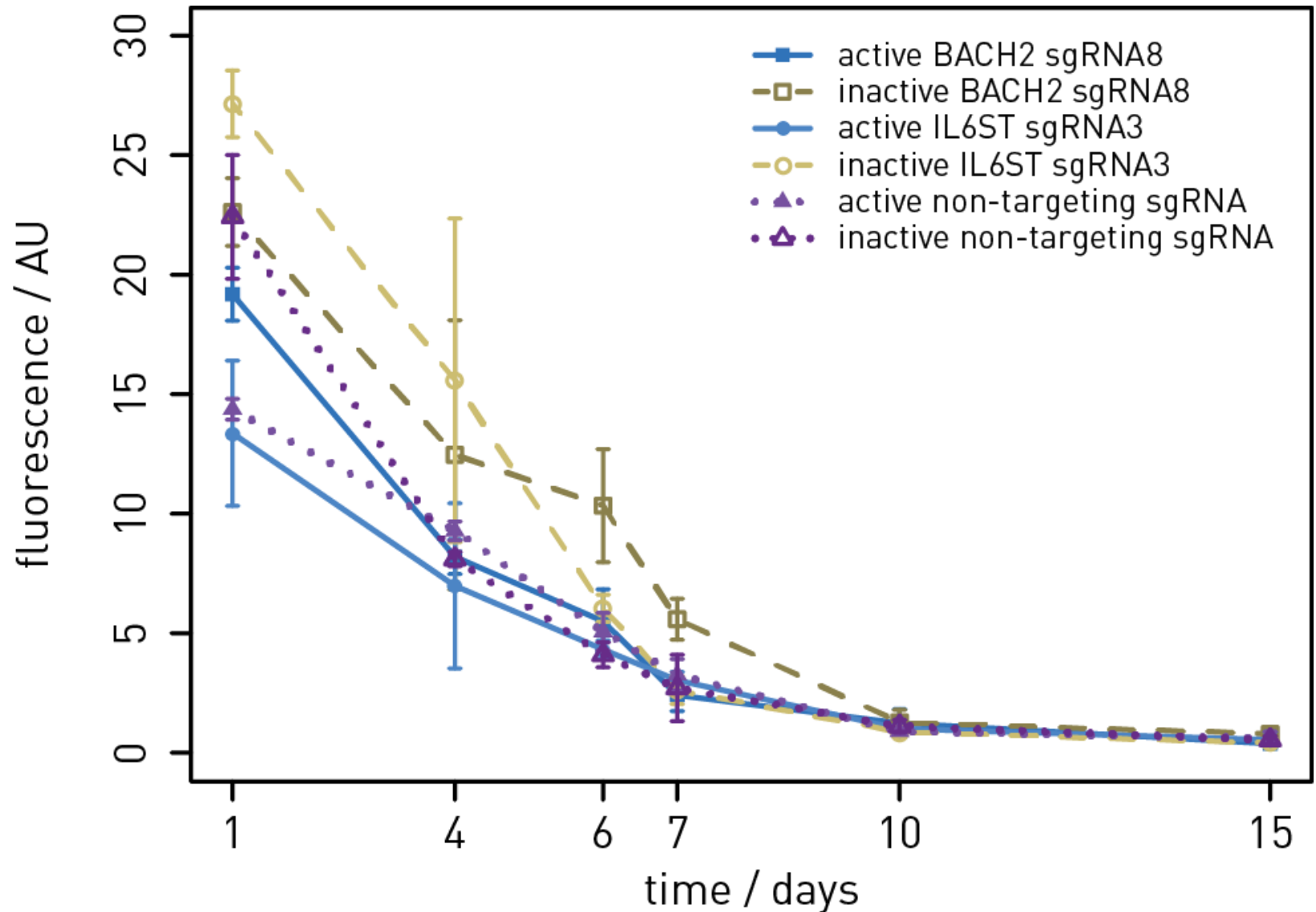


Time course experiment

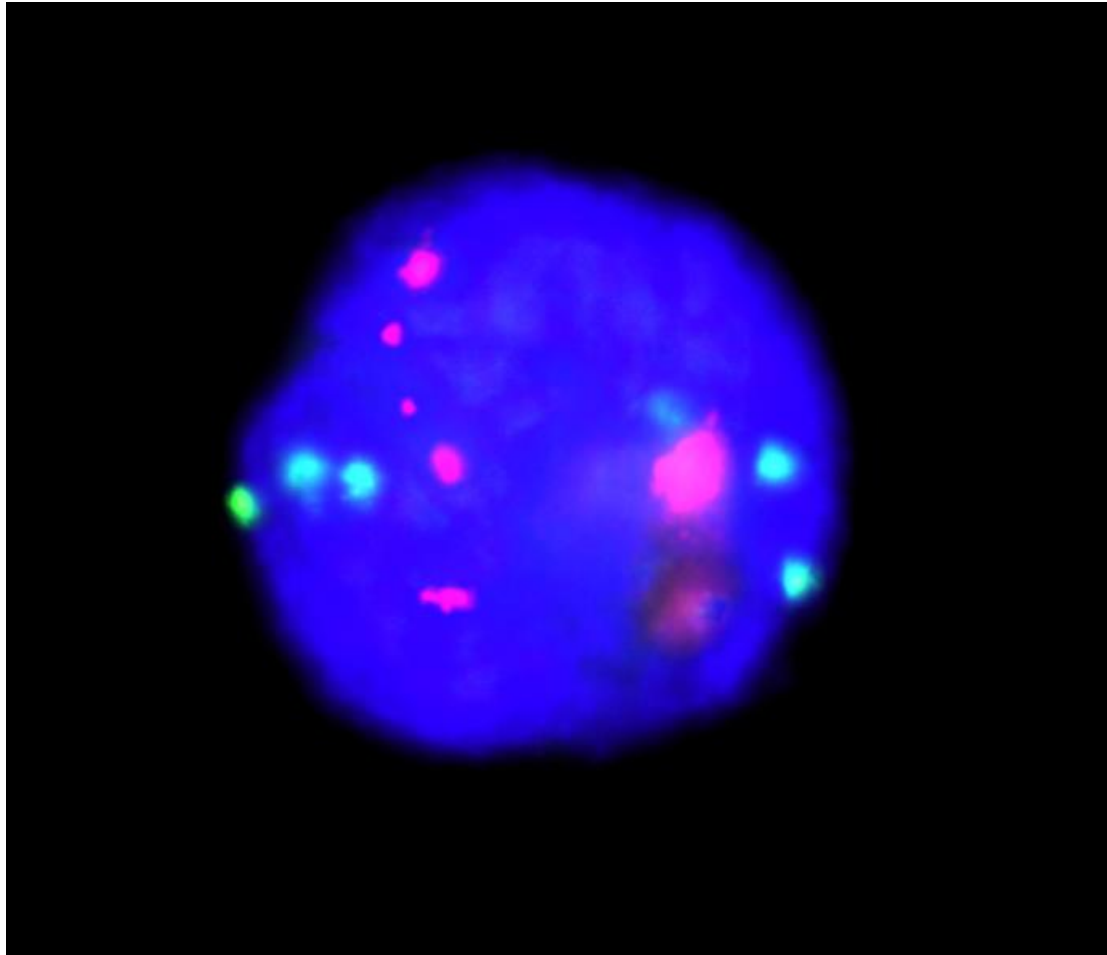
BACH2-A sgRNA8



Plasmid persistence



Plasmid during cell division



FISH

Chromosome 7

Plasmid DNA

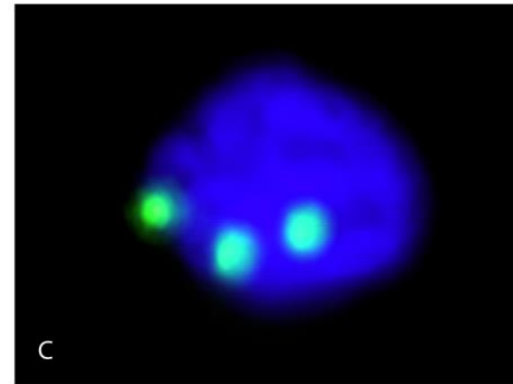
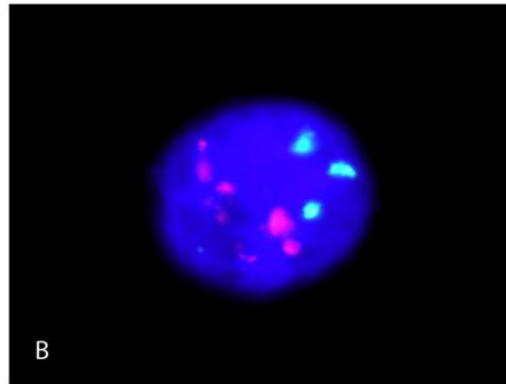
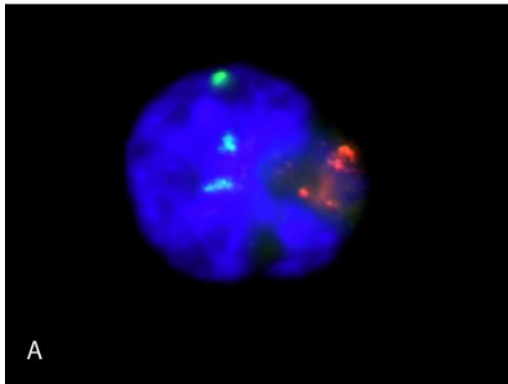
Nucleus (DAPI)

Cas9 DNA and Expression

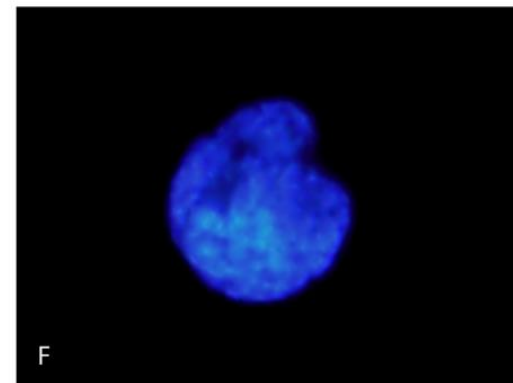
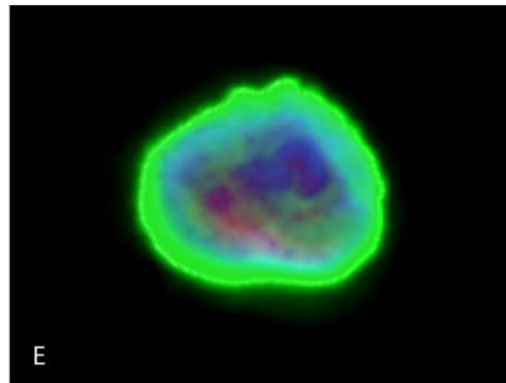
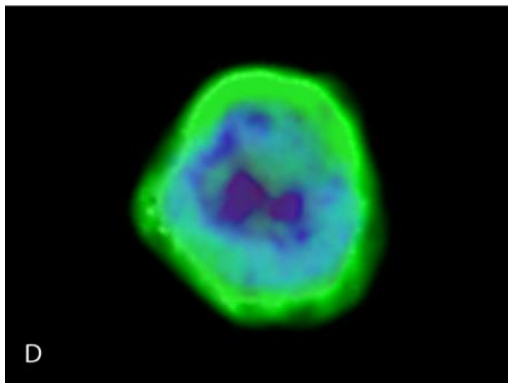
BACH2-sgRNA8

BACH2-sgRNA8 ANV

Mock

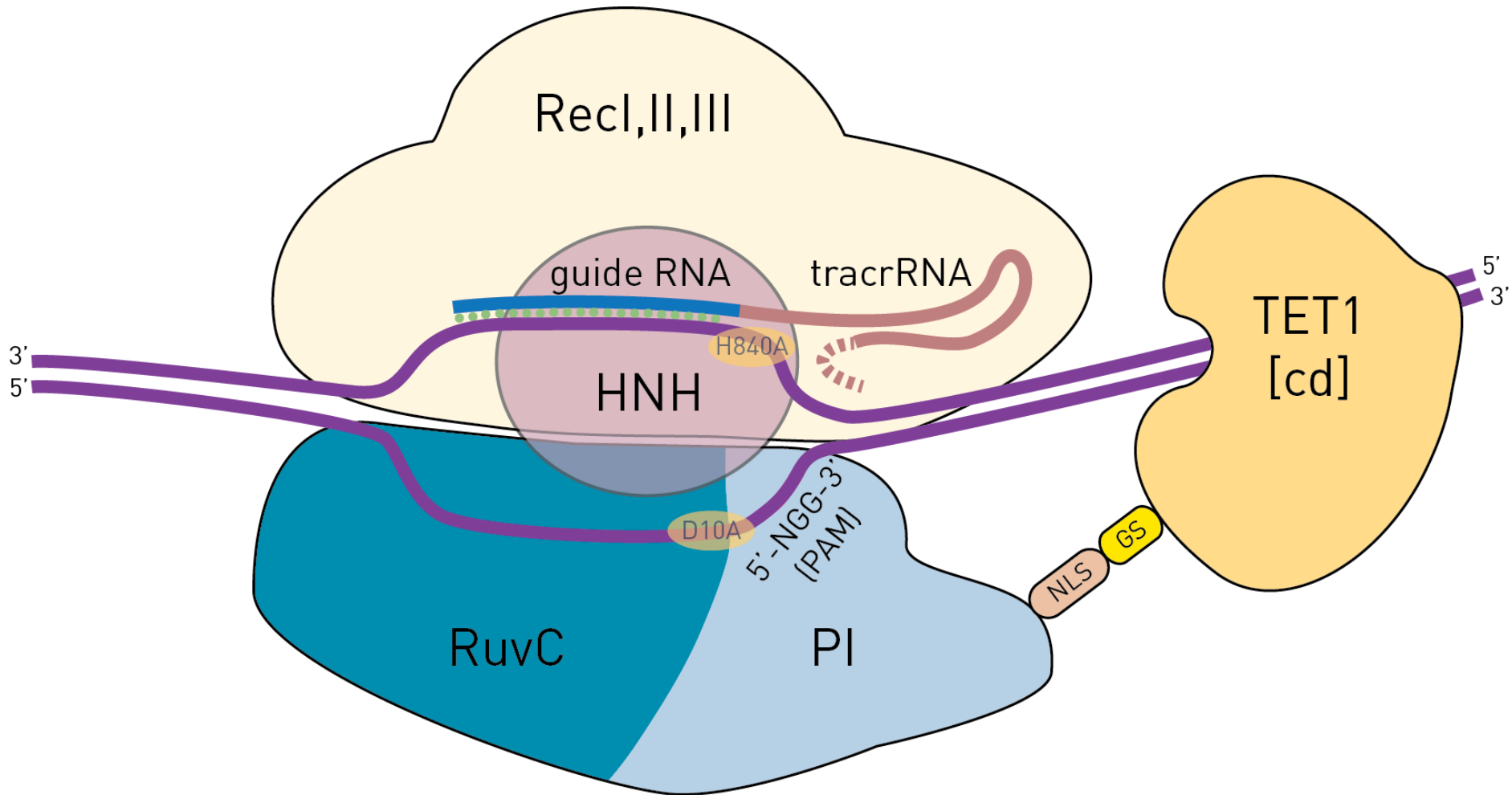


FISH:
Chr7
Cas9
DAPI

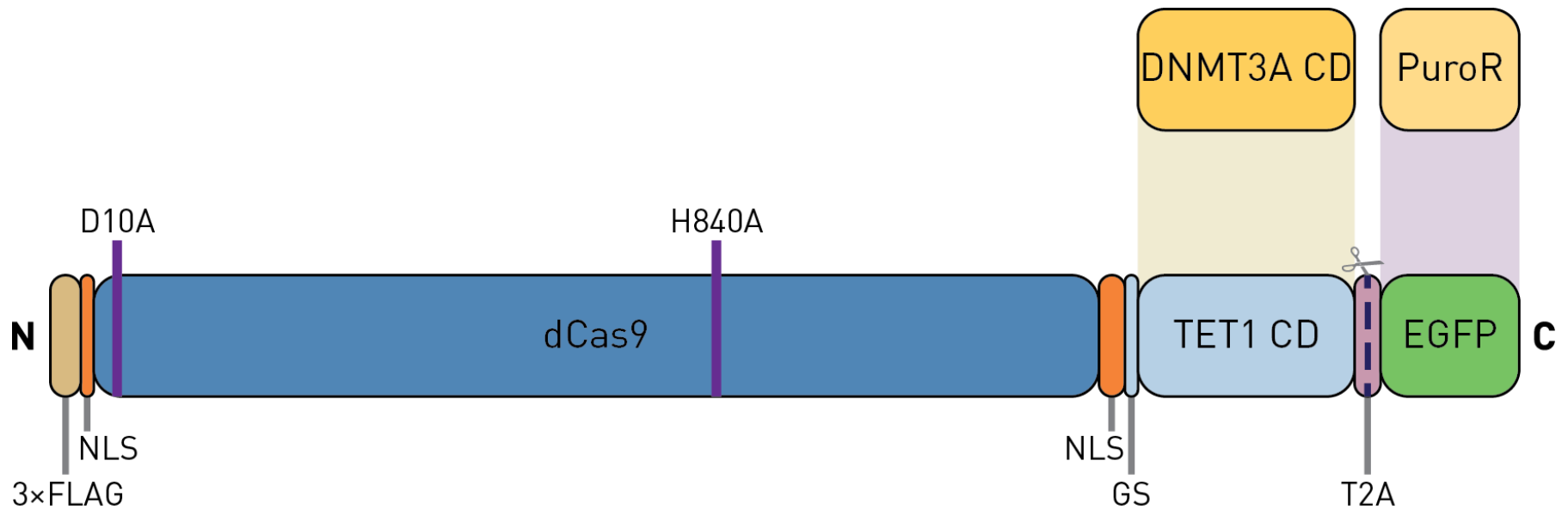


IF:
Cas9

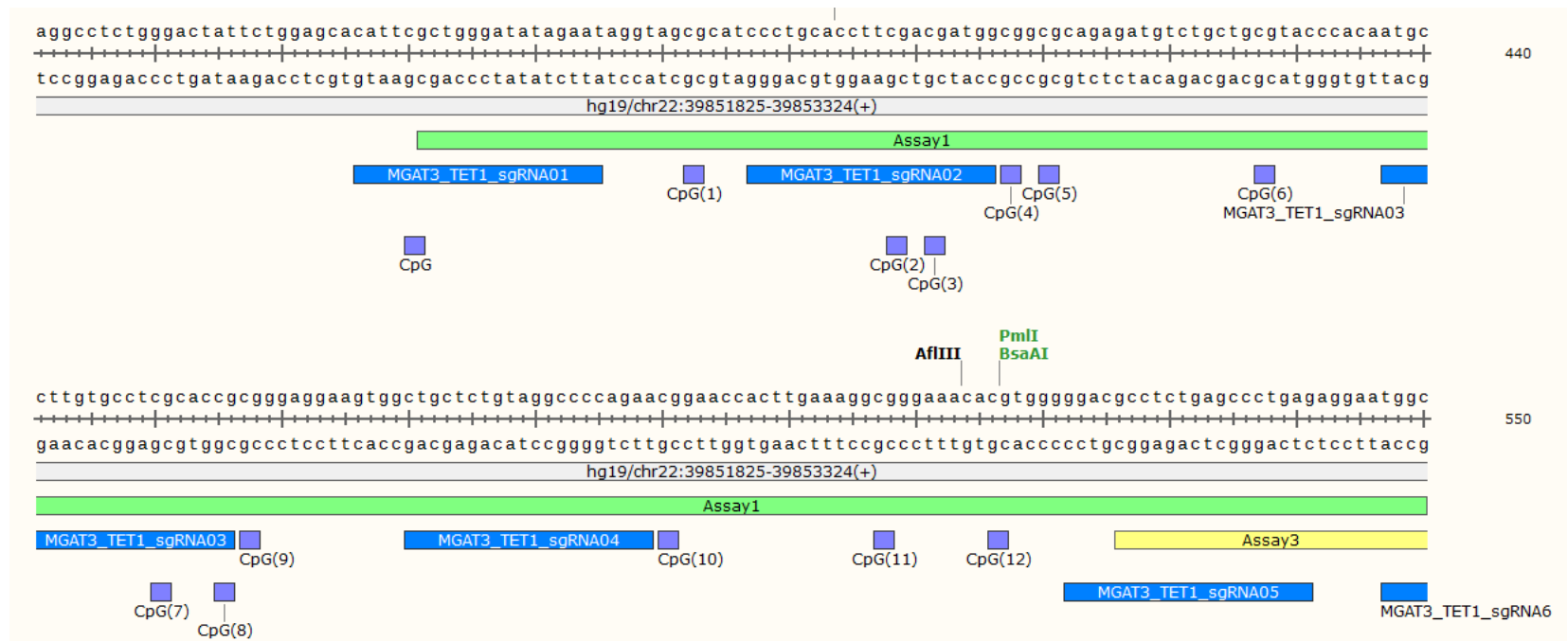
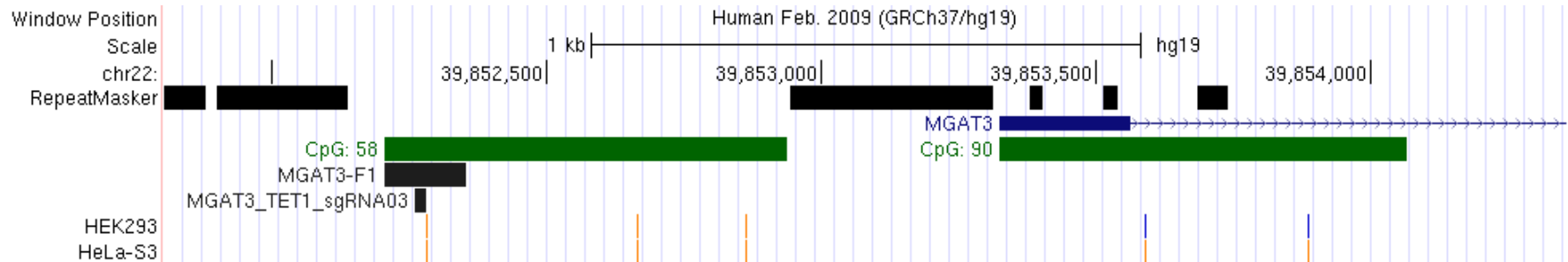
Cas9-TET1 construct



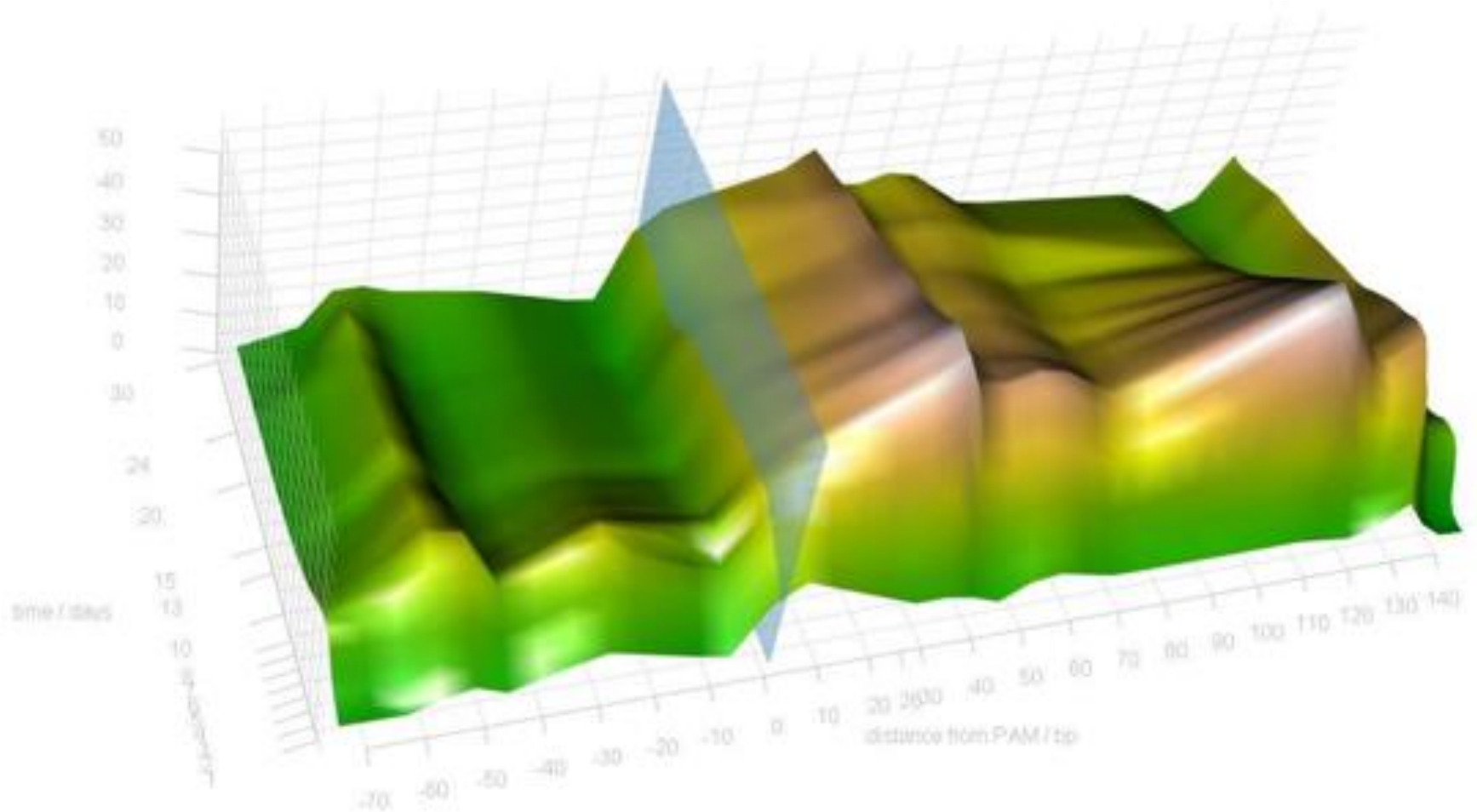
Cas9-TET1 cloning



MGAT3 assay for TET1



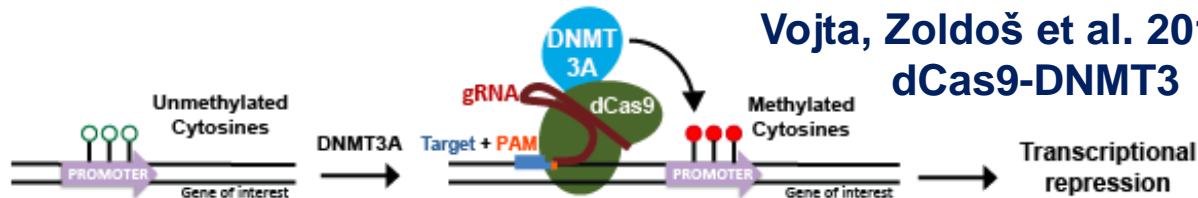
Time course (TET1)



(5.1)

The modular approach

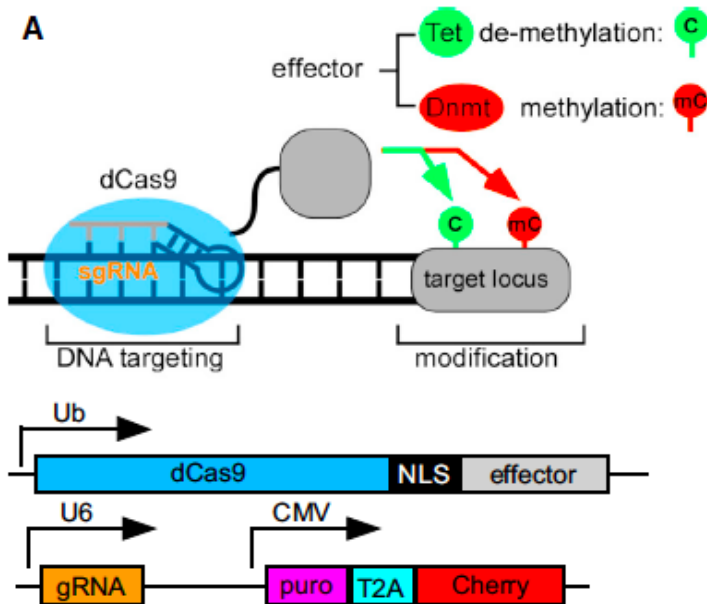
CRISPR/Cas9-based tools



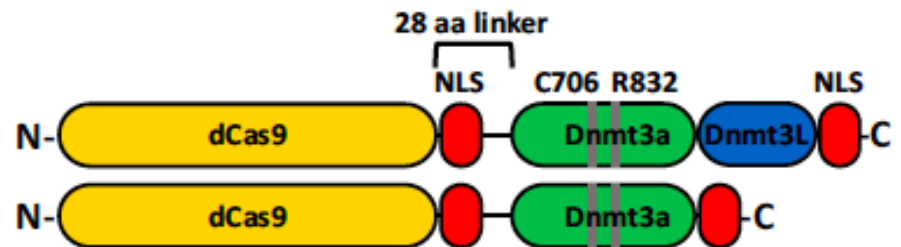
Vojta, Zoldoš et al. 2016
dCas9-DNMT3

Nucleic Acids Research:
DNA methyltransferase)

Figure 3. dCas9-DNMT3A methylates cytosines at promoters and enhancers. This targeted methylation is

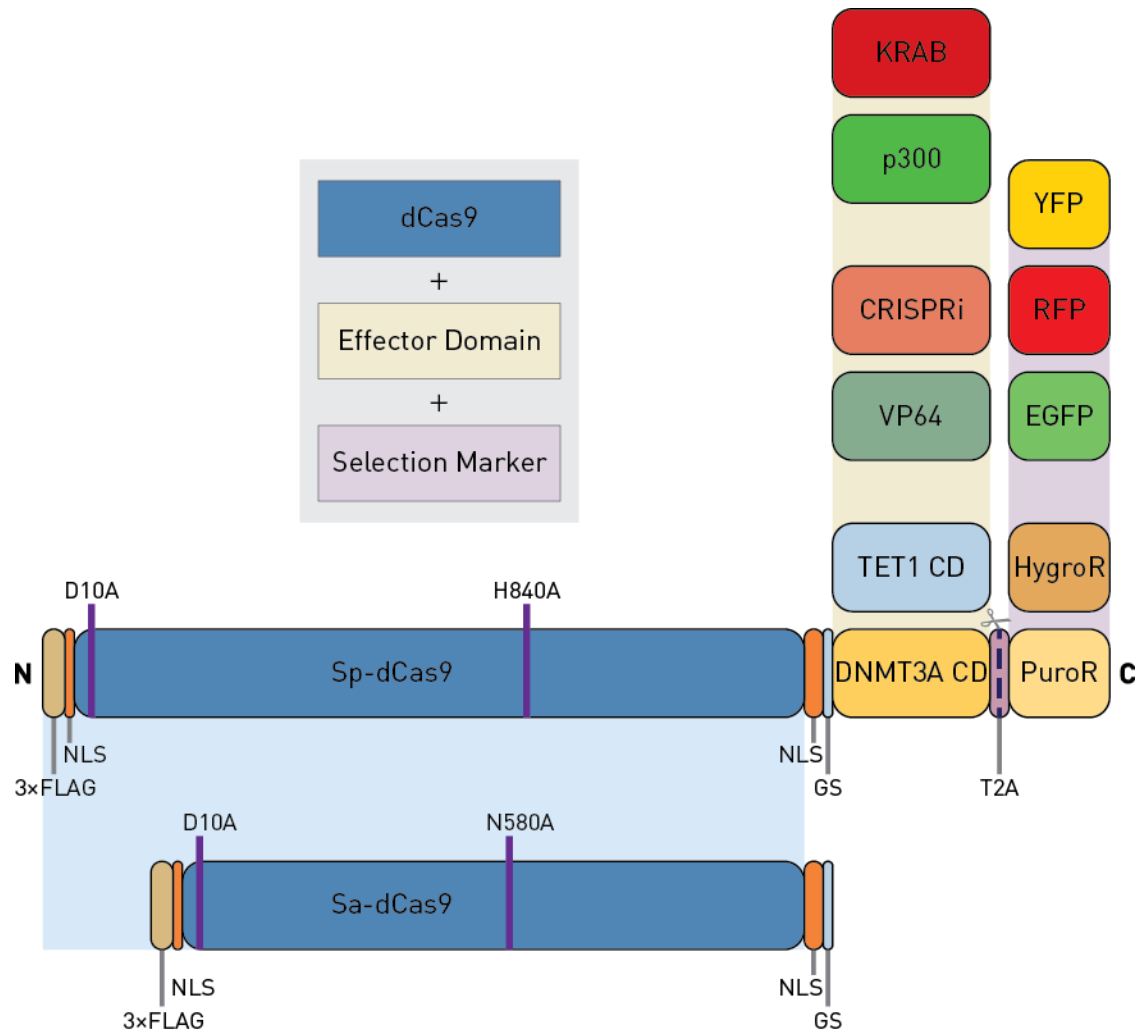


Liu, Jaenisch et al. 2016 Cell
dCas9-DNMT3A & dCas9-TET1



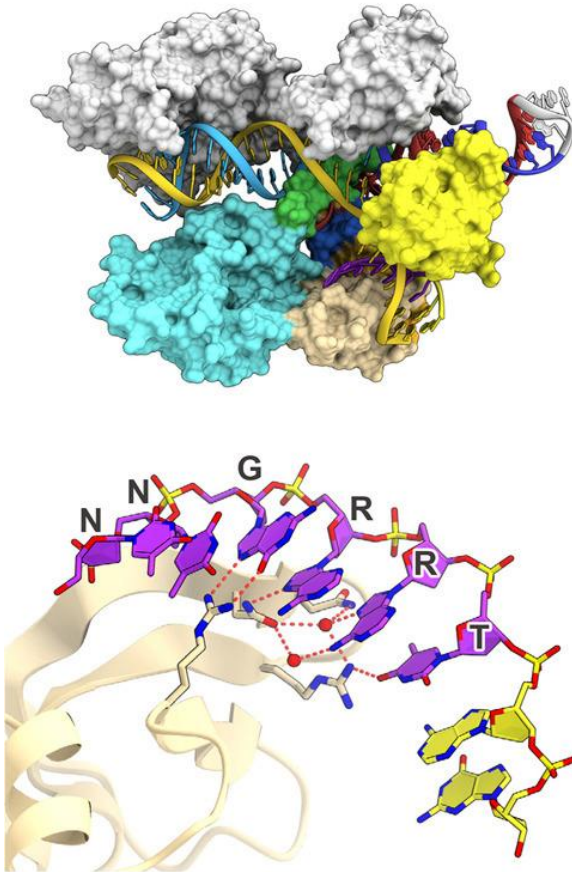
Stepper, Jurkowski et al. 2017 NAR
Cas9-DNMT3A-DNMT3L

Modular Toolbox



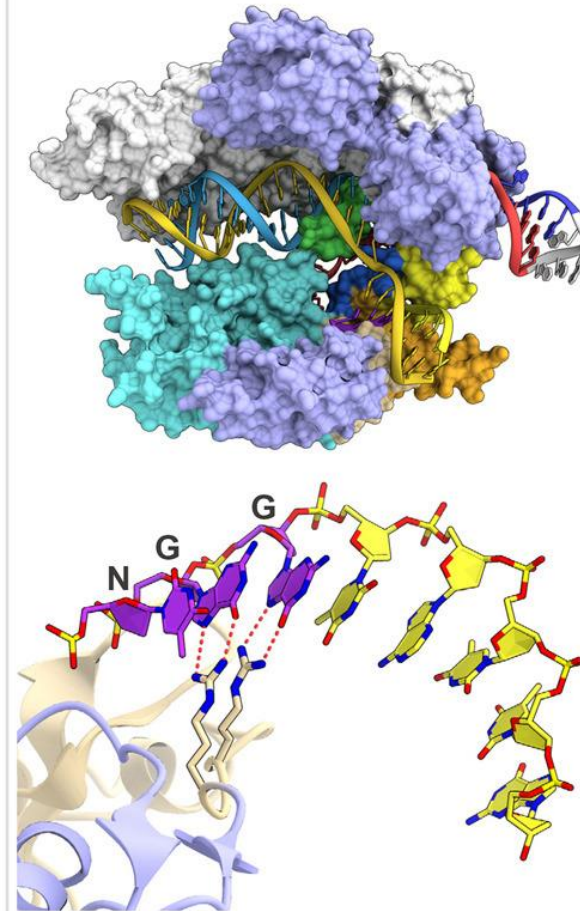
SaCas9

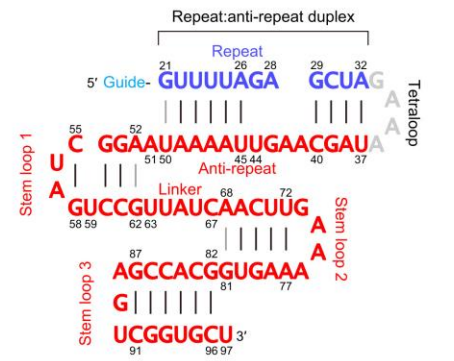
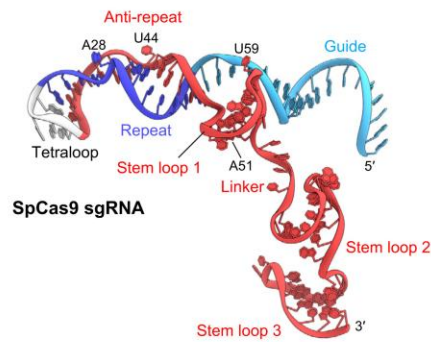
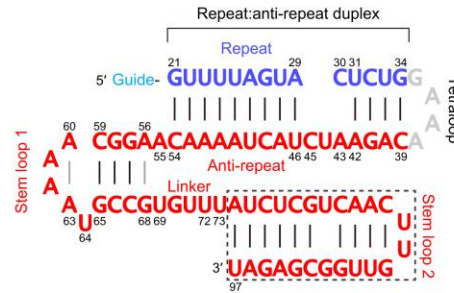
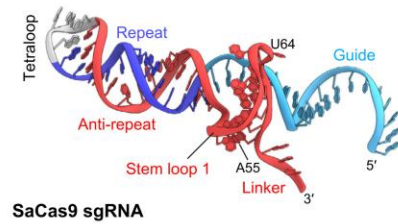
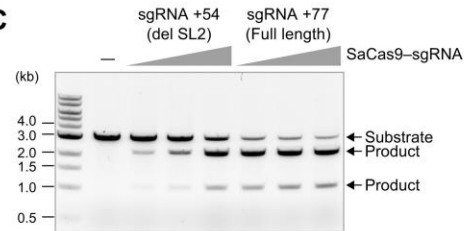
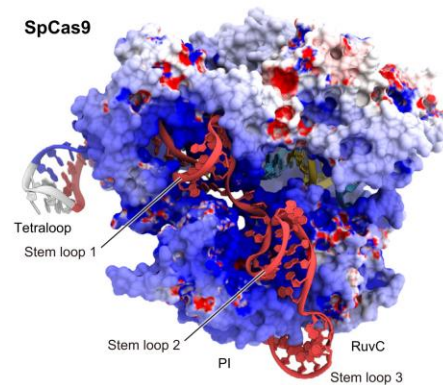
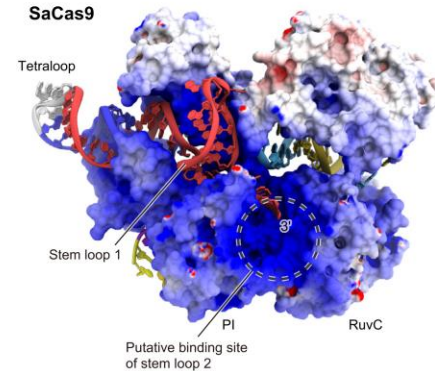
- ▶ 1053 aa
- ▶ NNGRR(T) PAM



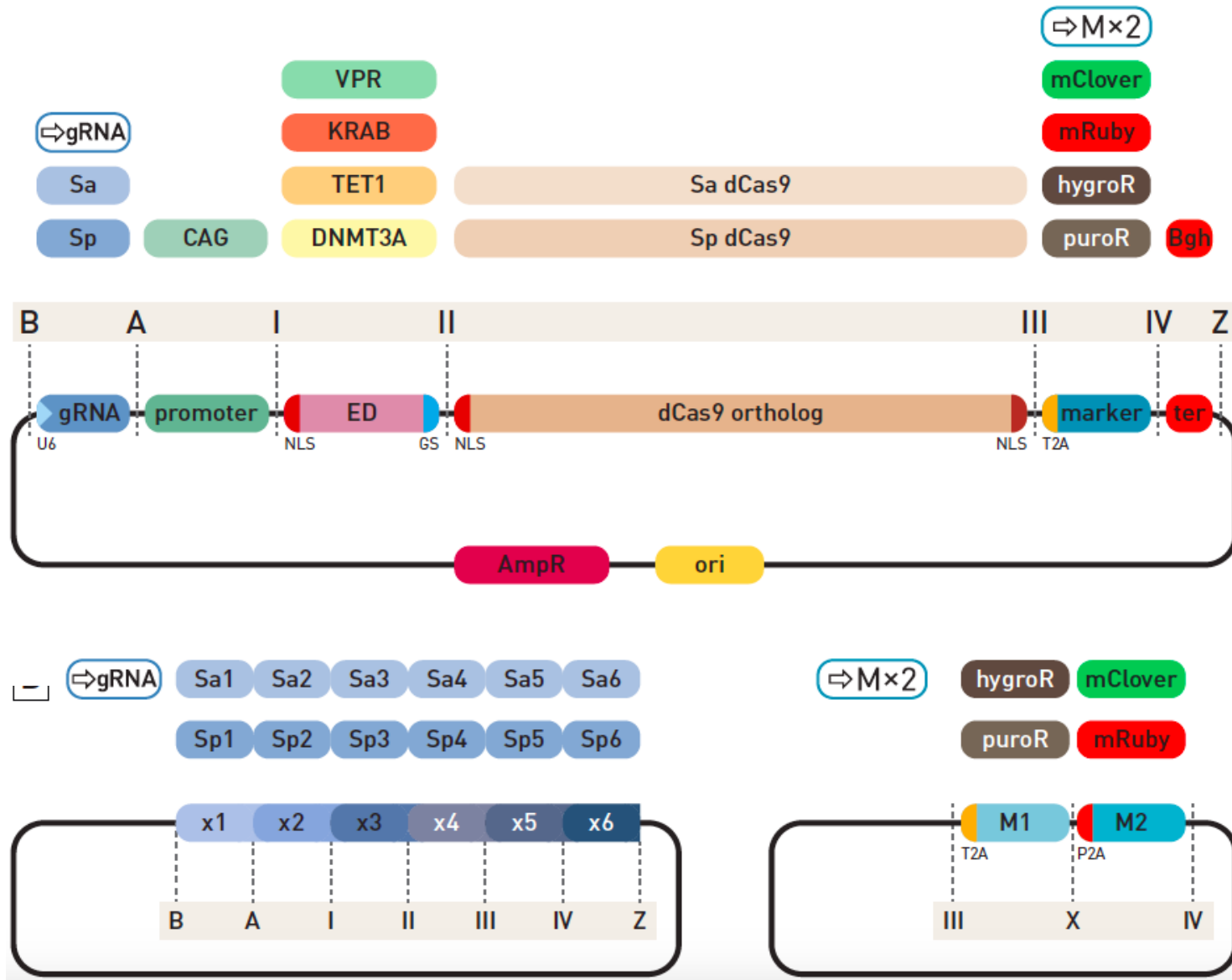
SpCas9

- ▶ 1368 aa
- ▶ NGG PAM

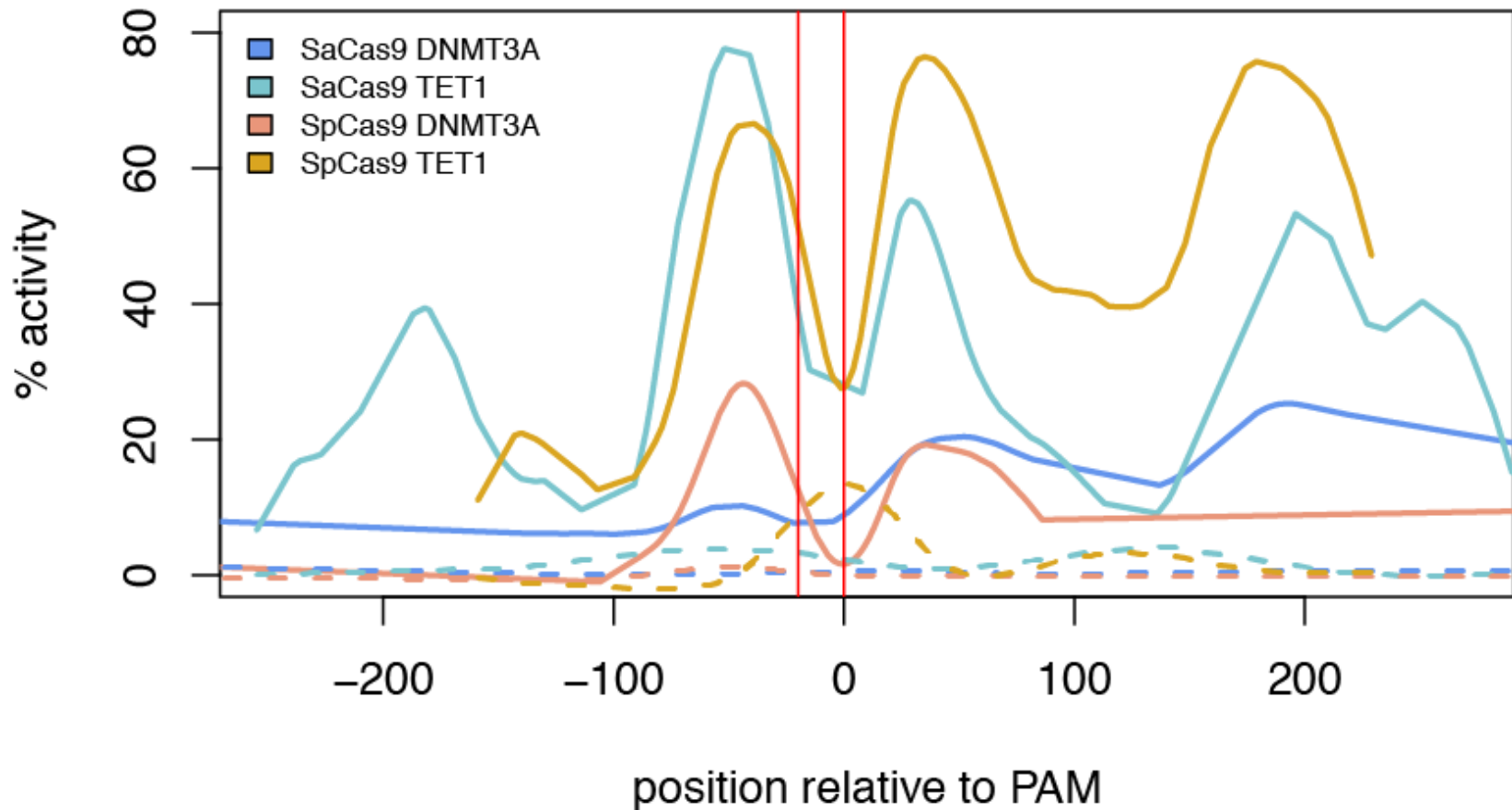


A**B****C****D****SaCas9**

The modular toolbox



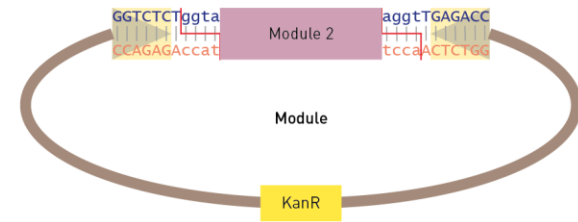
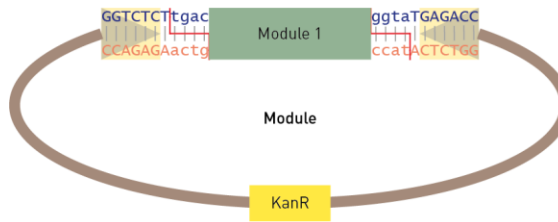
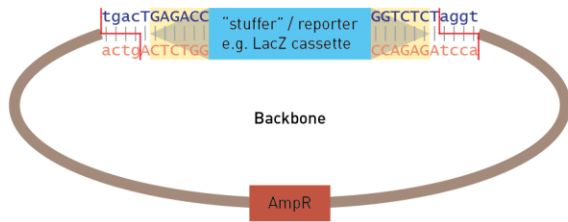
Active fusions have similar profiles



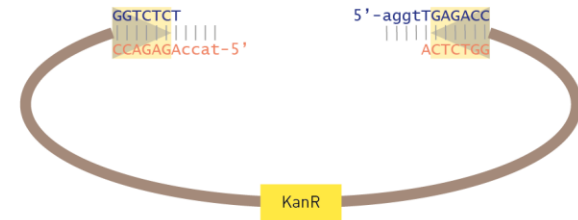
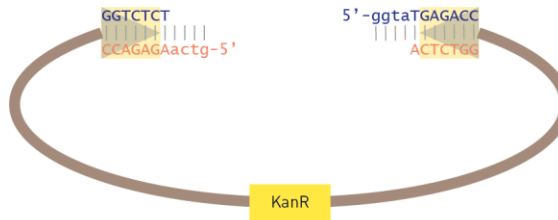
(5.1.1)

Cloning considerations and golden-gate
assembly

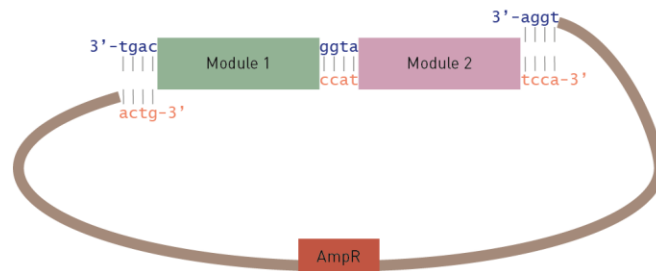
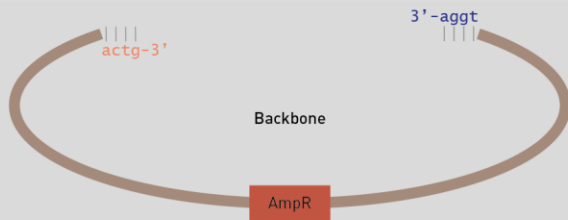
Golden Gate assembly



BsaI

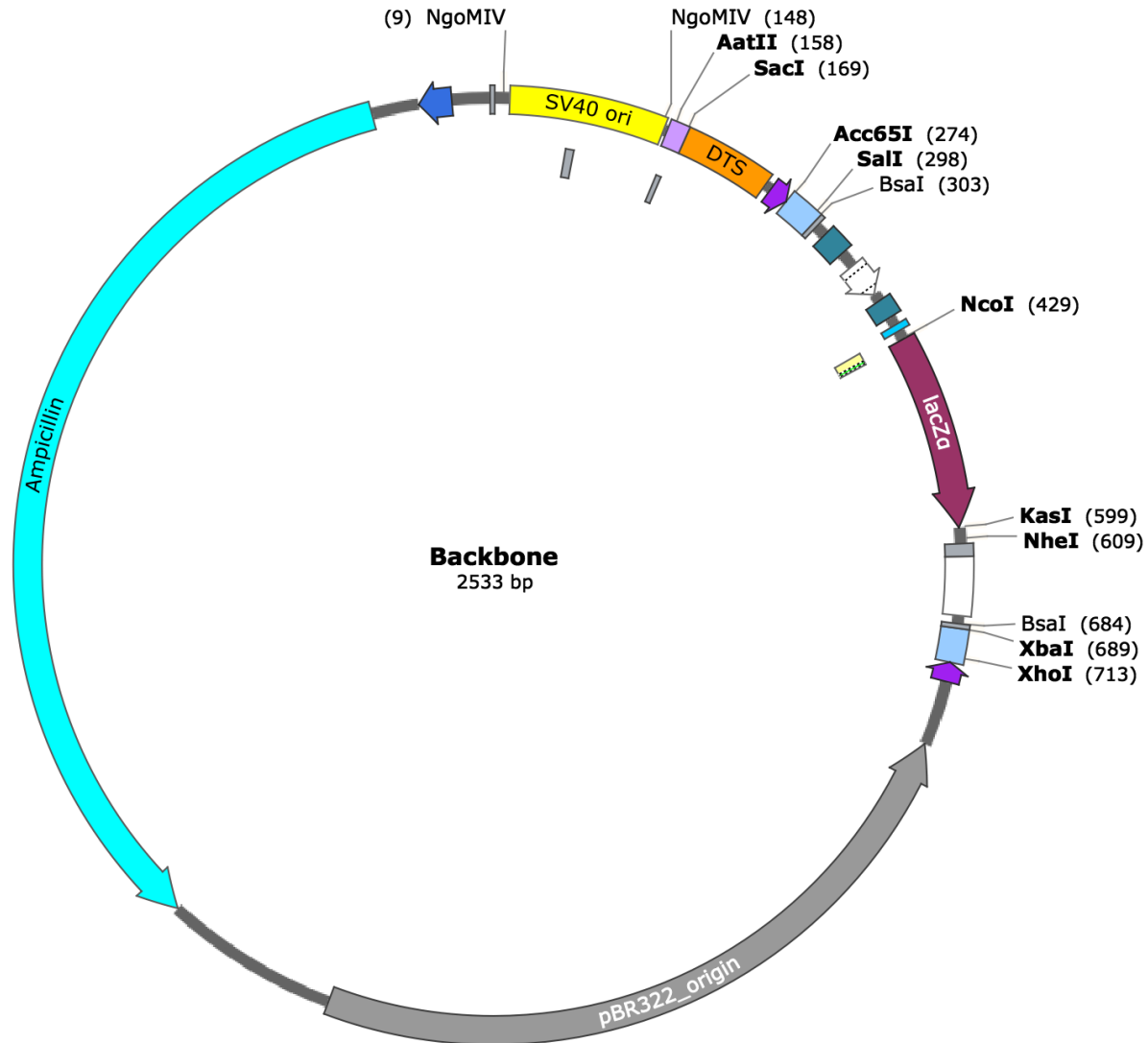


Ligate

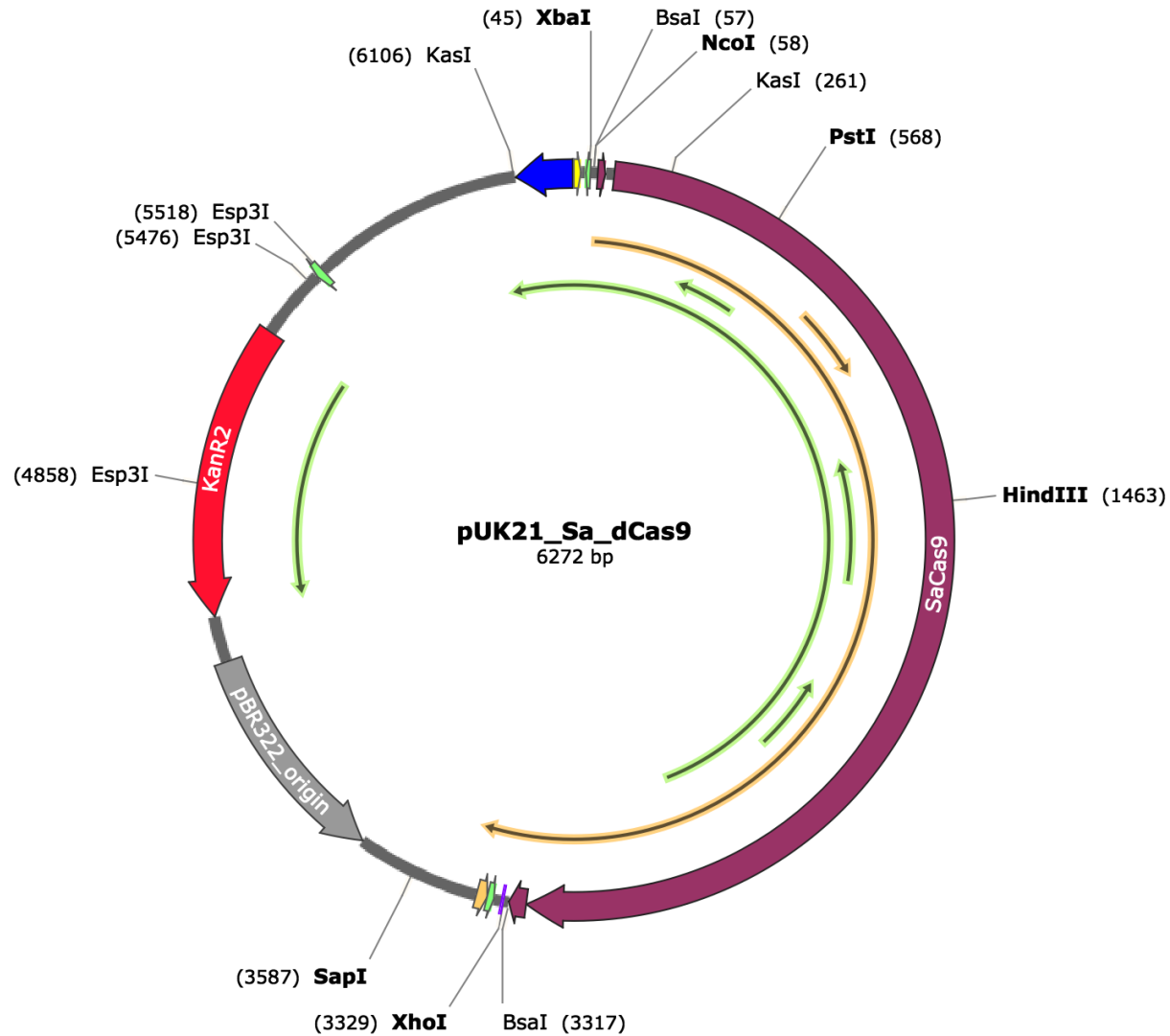


Select for
AmpR

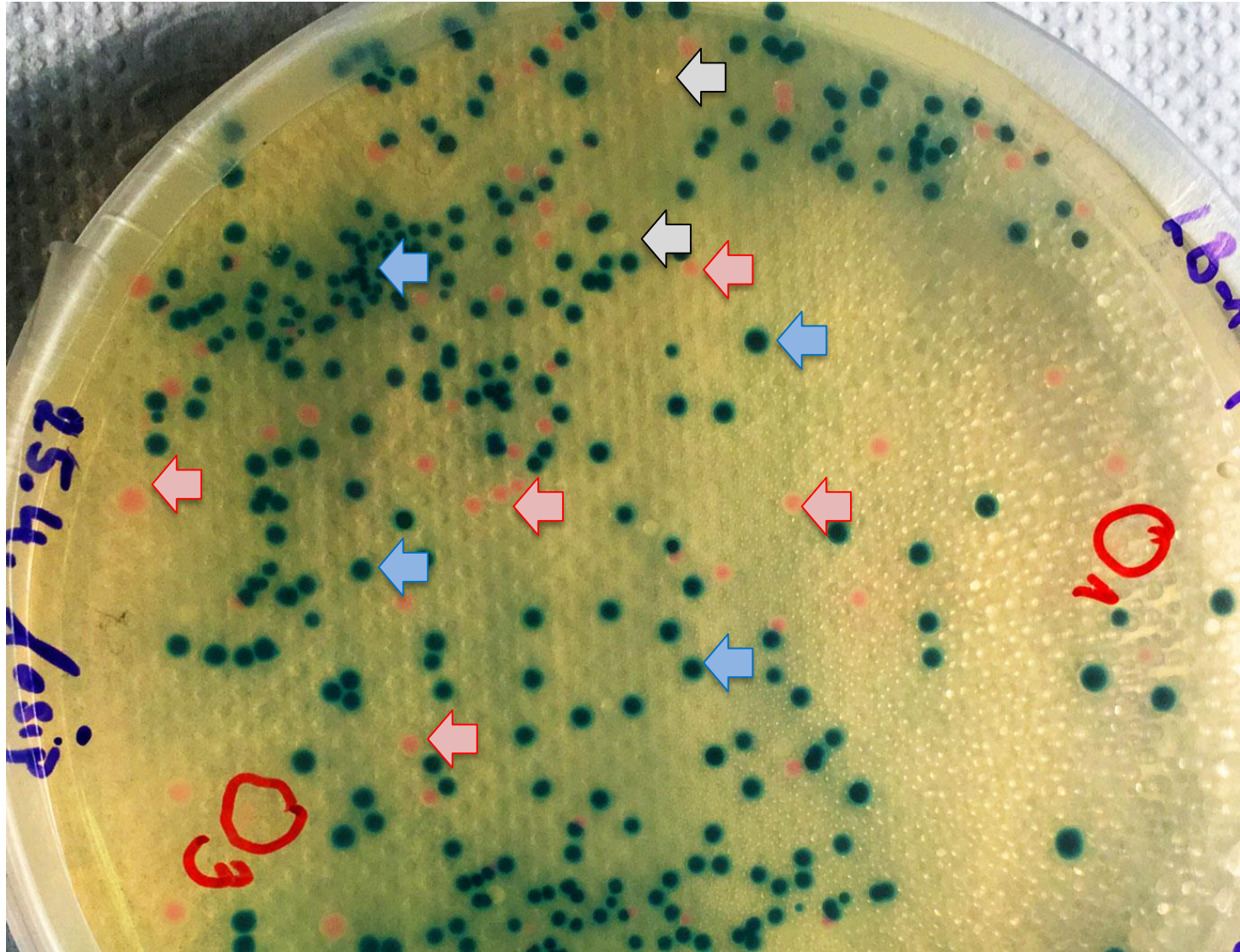
Basic “Backbone”



Basic “Module”



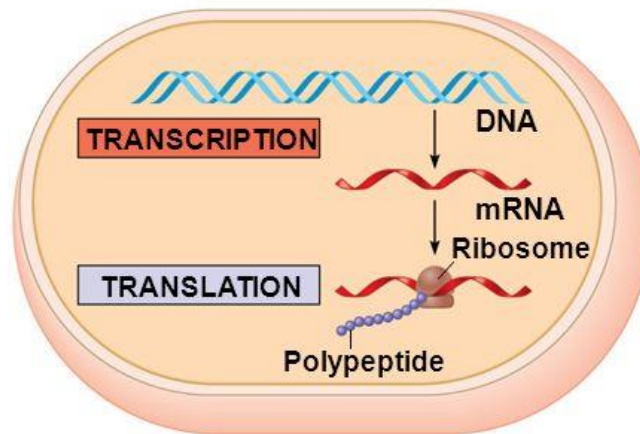
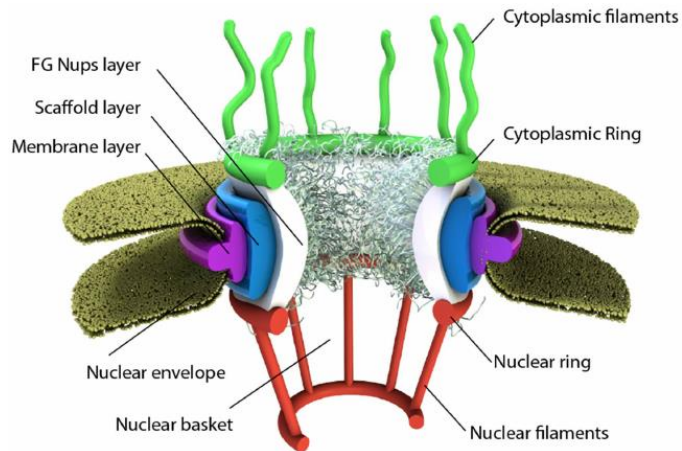
Assembly Results



(5.1.2)

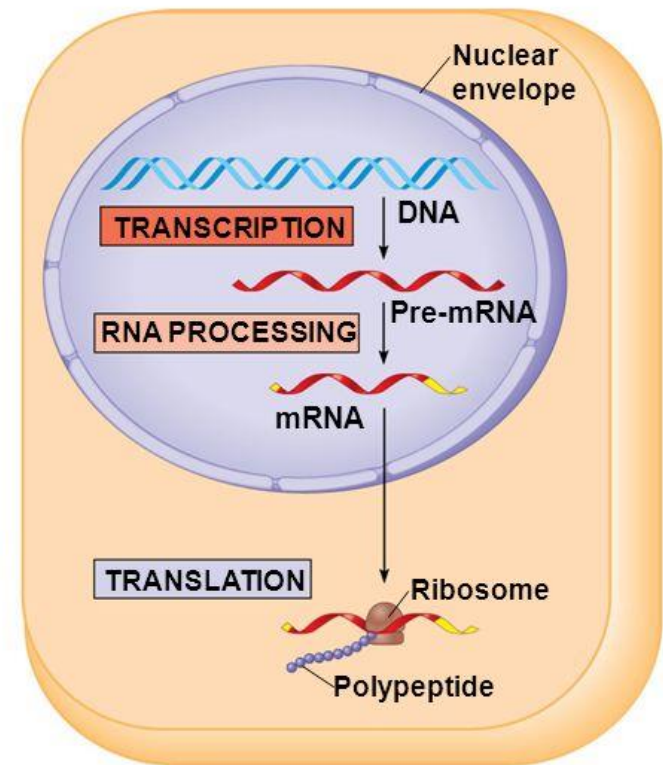
Other considerations

Compartments of a eukaryotic cell



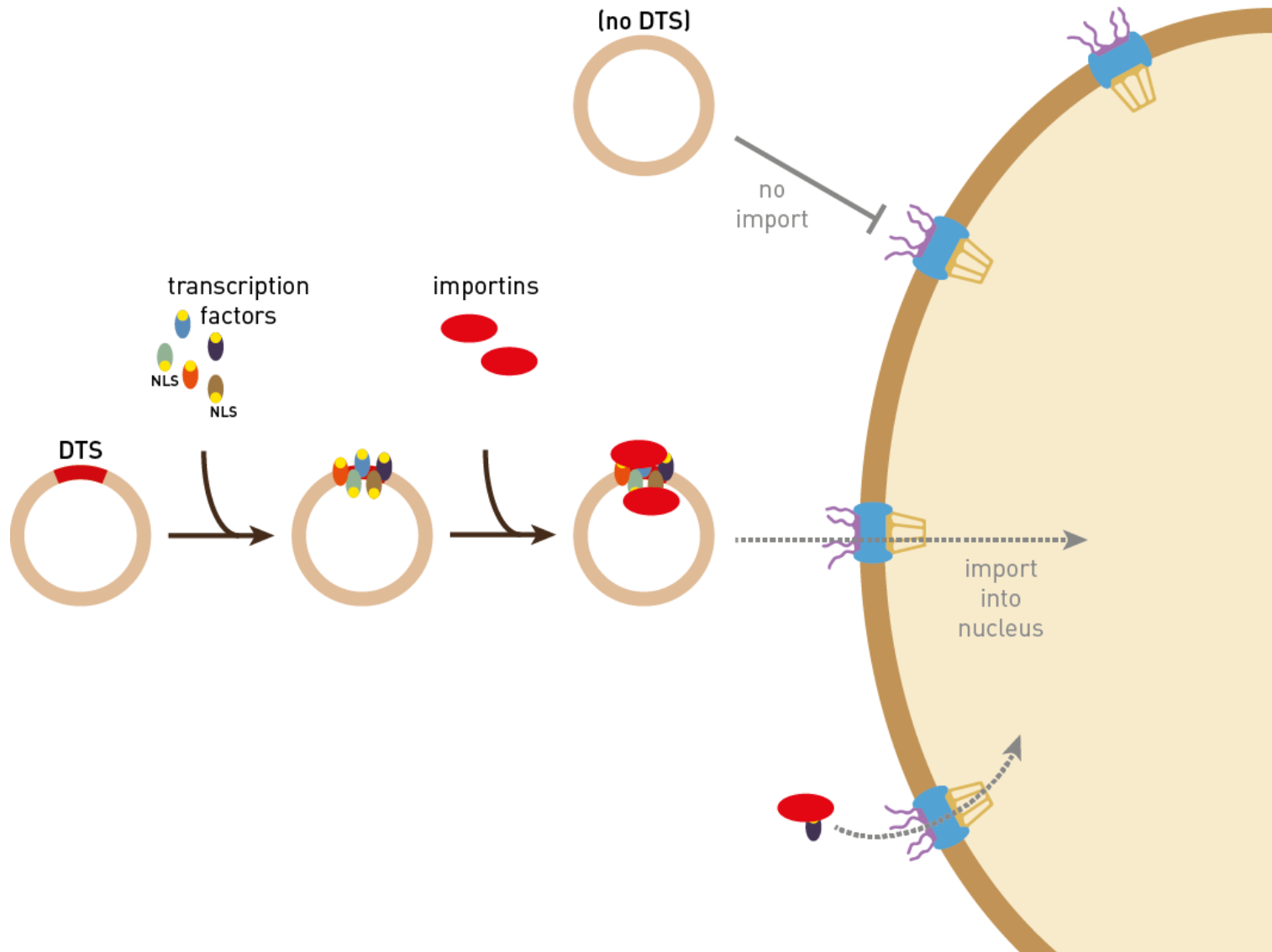
(a) Bacterial cell

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(b) Eukaryotic cell

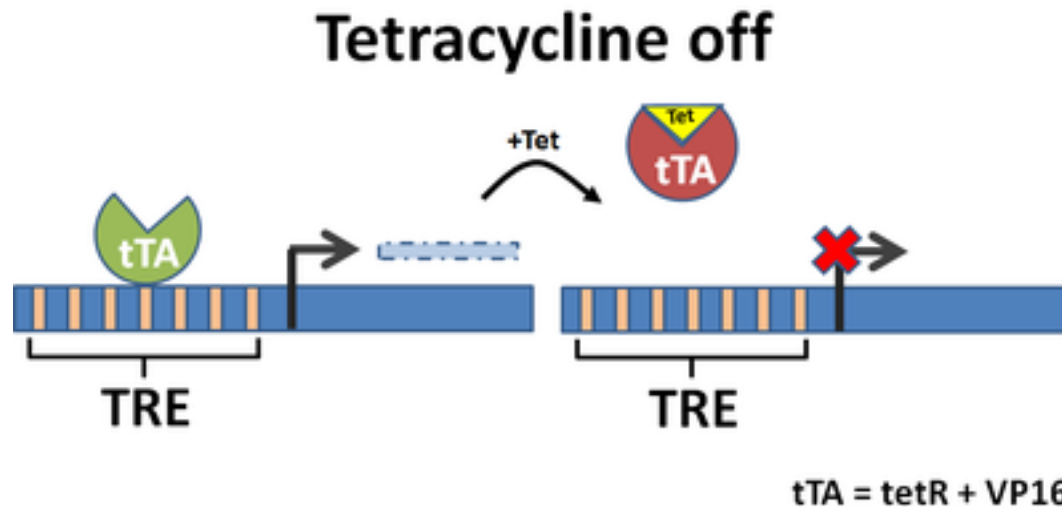
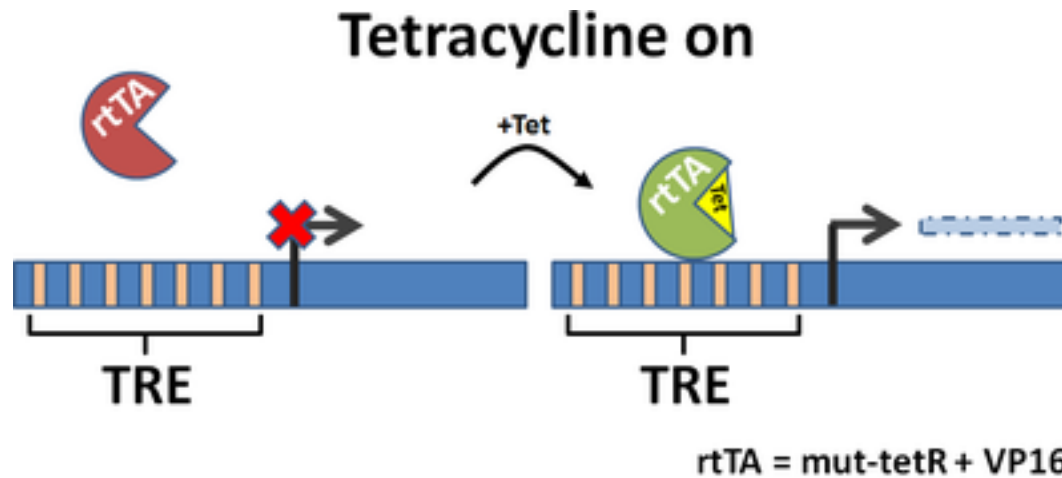
Import into the nucleus



What makes a good expression cassette

- Main considerations for efficient expression
 - Codon usage
 - 5' UTR secondary structure
 - uORFs
 - Introns
 - Nonsense-mediated degradation (RNA)
 - 3' UTR and miRNAs; WPRE

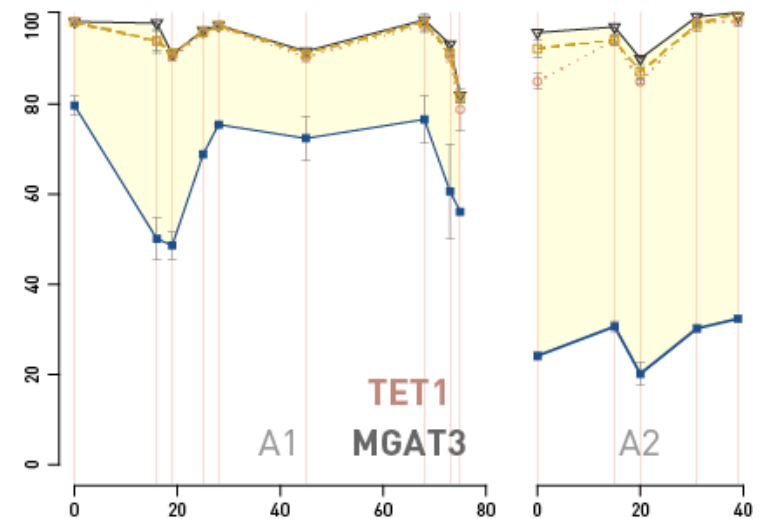
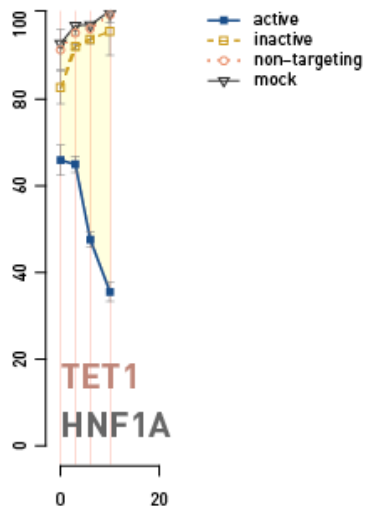
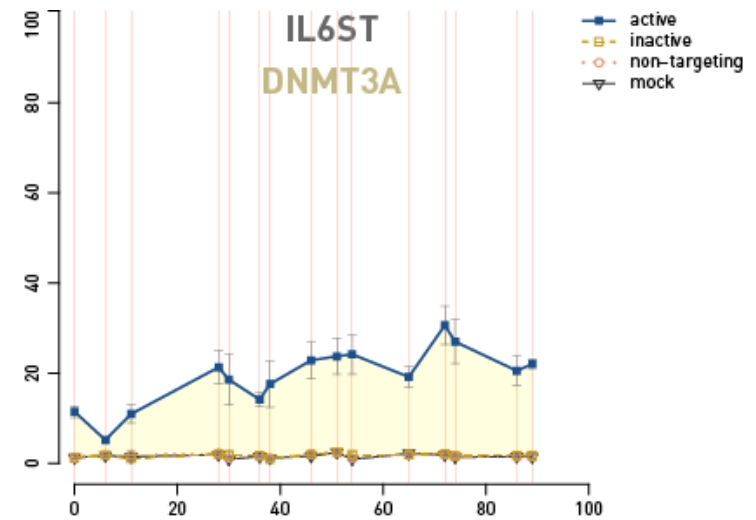
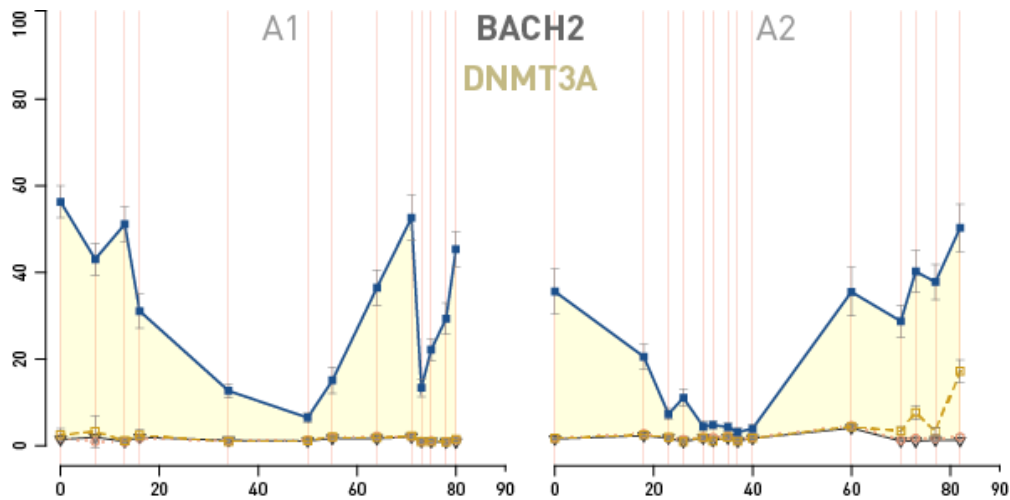
Inducible promoters



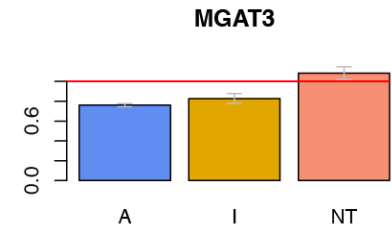
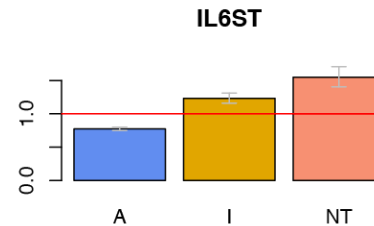
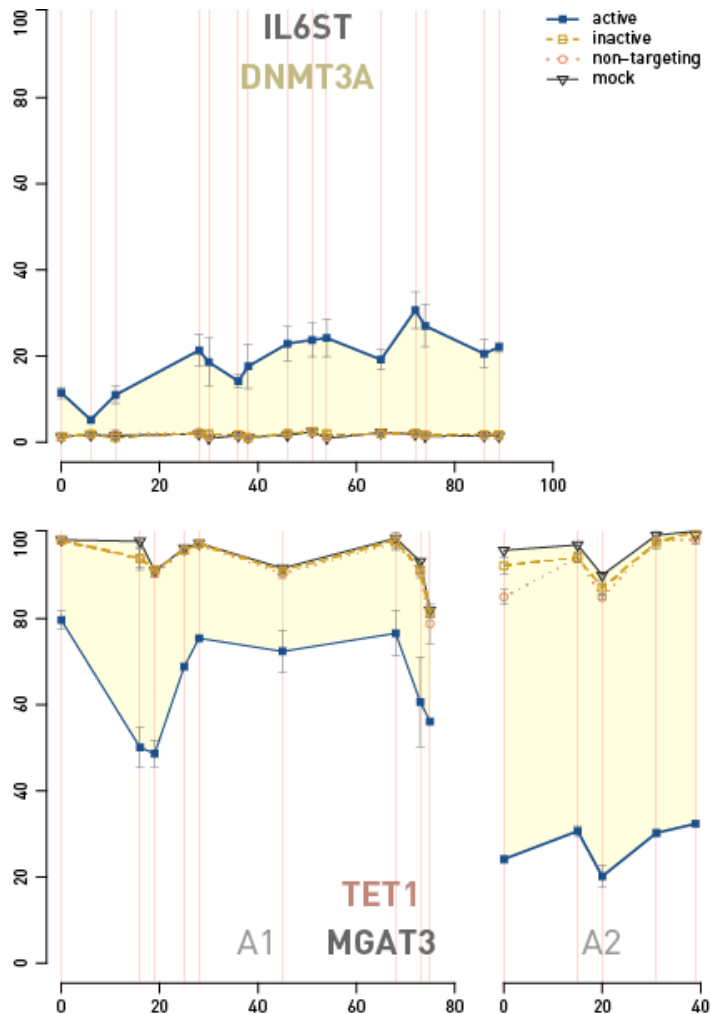
(5. 2)

Antagonistic methylation and direct regulation

Antagonistic methylation

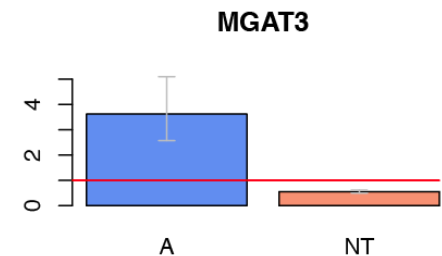
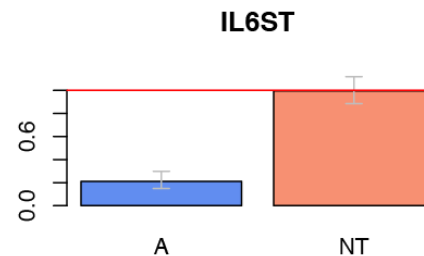
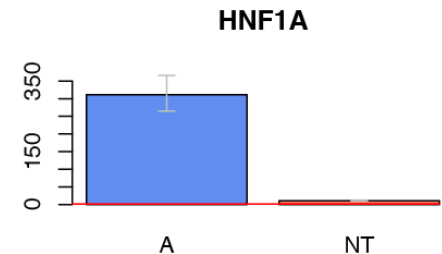
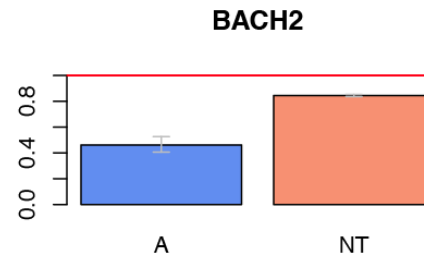


Effects of methylation on expression



Direct regulation (KRAB/VPR)

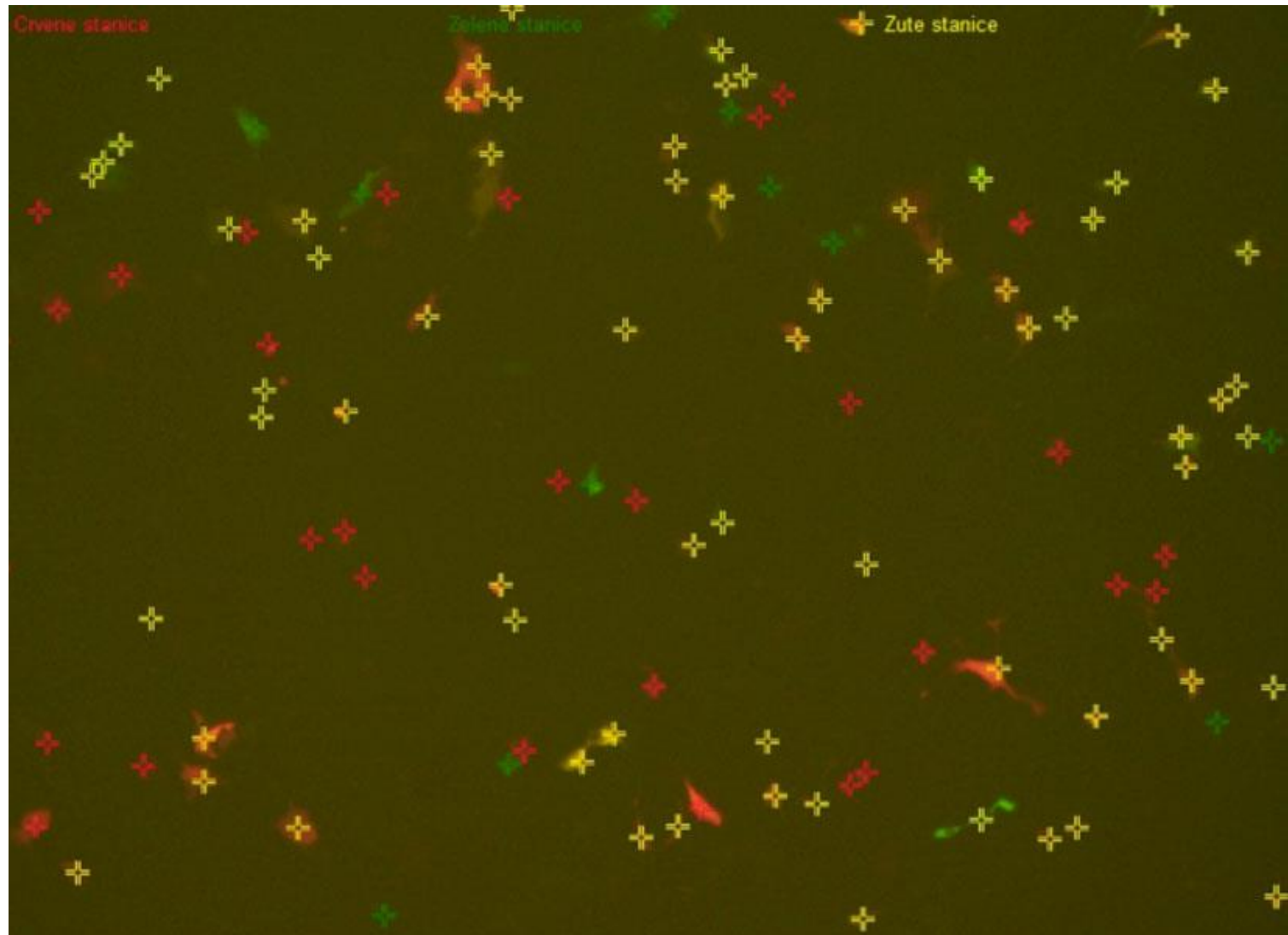
- KRAB - recruits HP1, HKMT (H3K9me3) – local condensation hindering positioning of RNA PolII
- VPR – recruits chromatin modifiers which cause chromatin decondensation, Accumulation of H3K27ac, H3K4me3, binding of PolII



dSpCas9-KRAB

dSaCas9-VPR

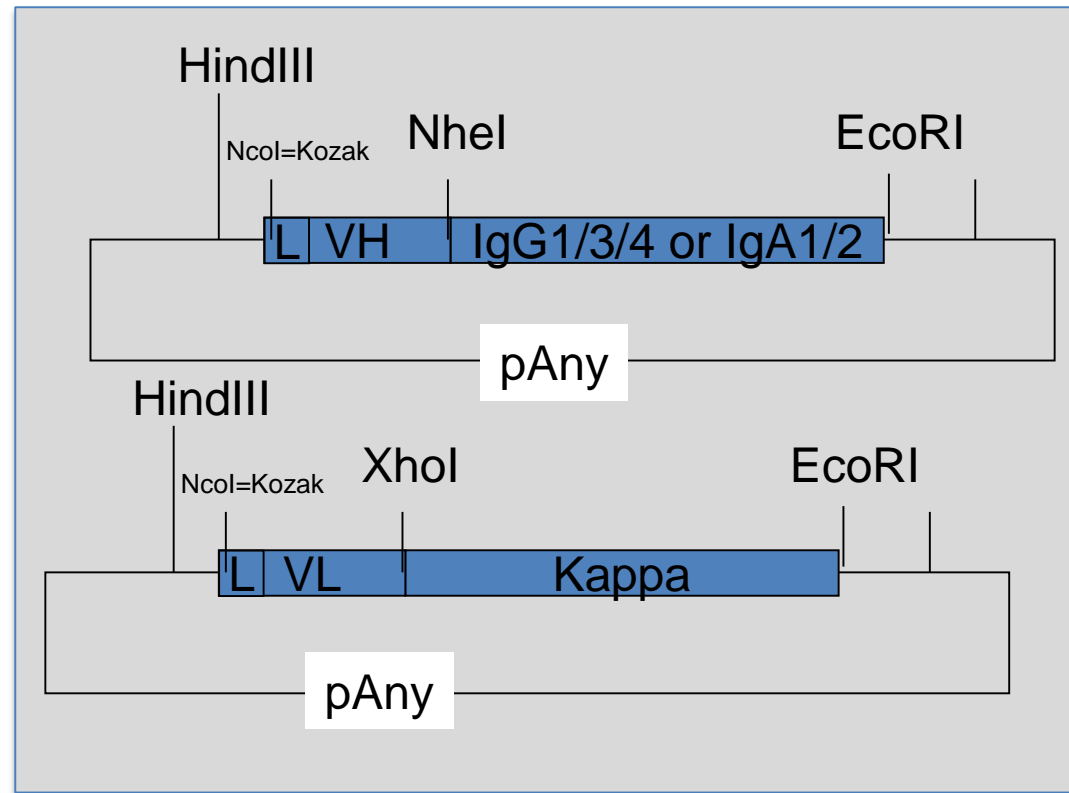
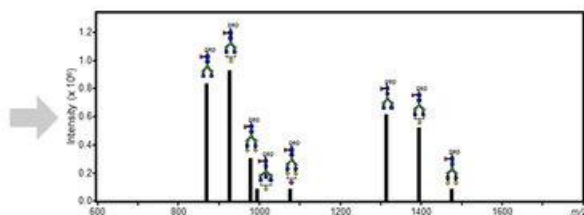
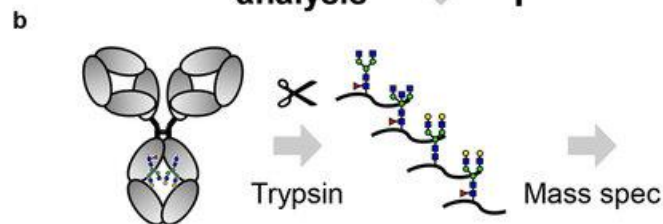
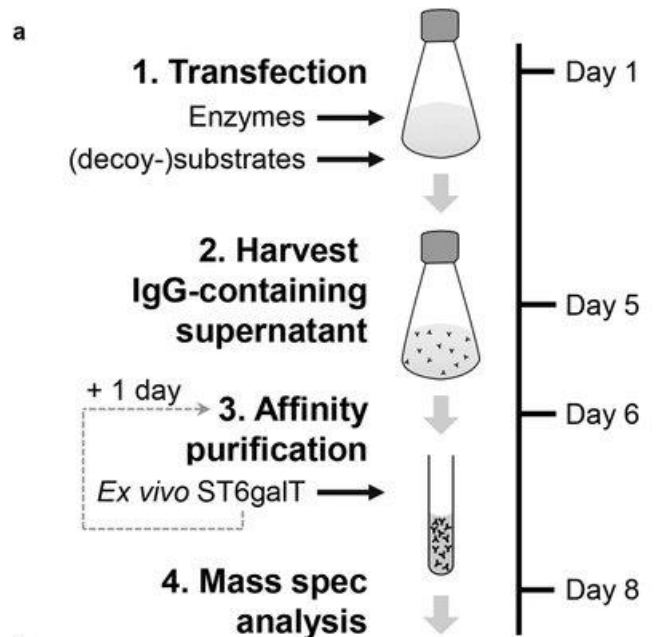
Dual transfection: challenges



(5. 3)

IgG glycosylation: case study

HEK Freestyle model



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