GEroNIMO and RUMIGEN Joint Final Event

Epigenome editing in animal production A prospective review

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Preprint: hal.science/hal-05410119v2

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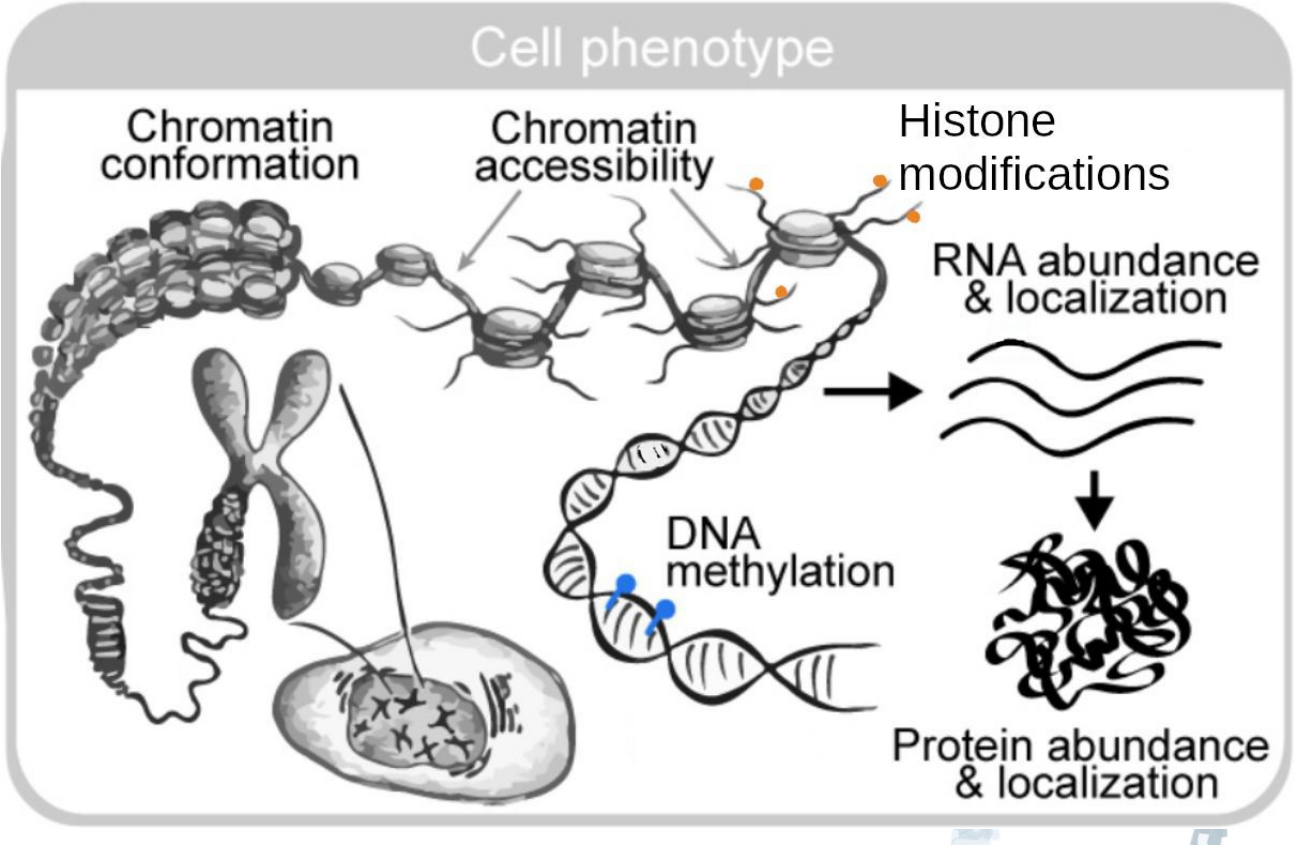
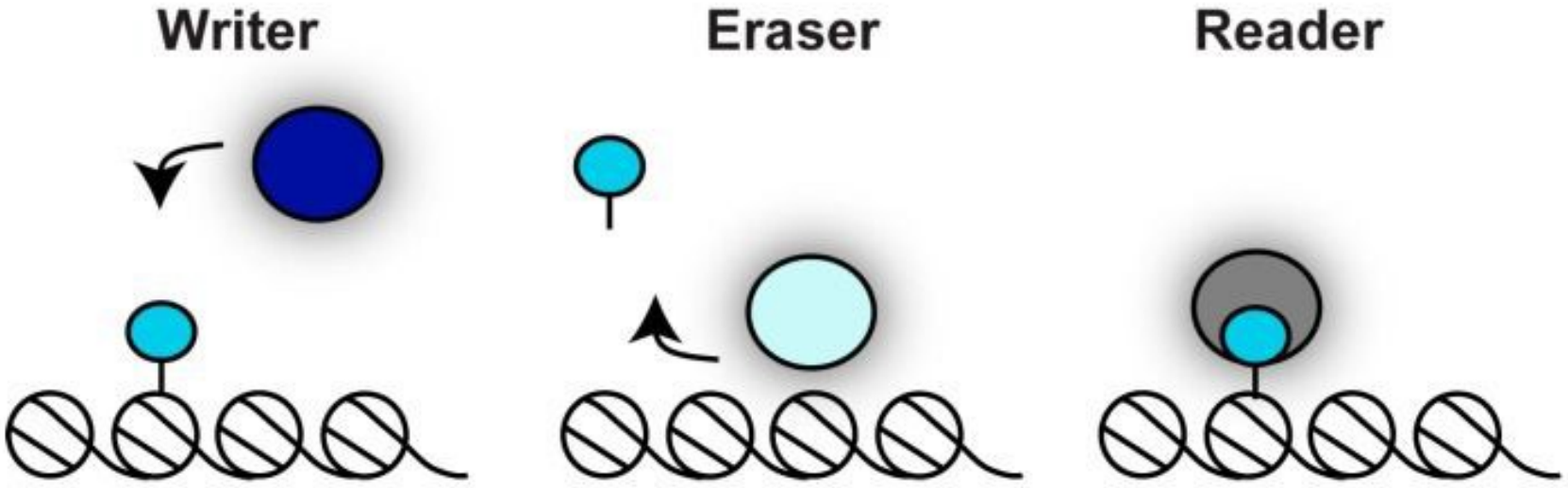
Multi-layered epigenetic control of gene expression



from:
Camp, Platt & Treutlein,
cell phenotypes, 2019

Epigenetic marks, localised on the chromatin fiber

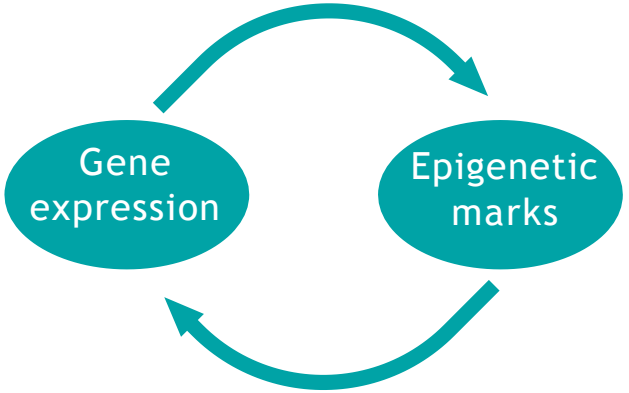


from: Buscaino
Chromatin-mediated regulation, 2019

- DNA methylation
- DNA hydroxy-methylation
- Histone post-translationnal modifications
- Histone variants

Classical approaches and their limits

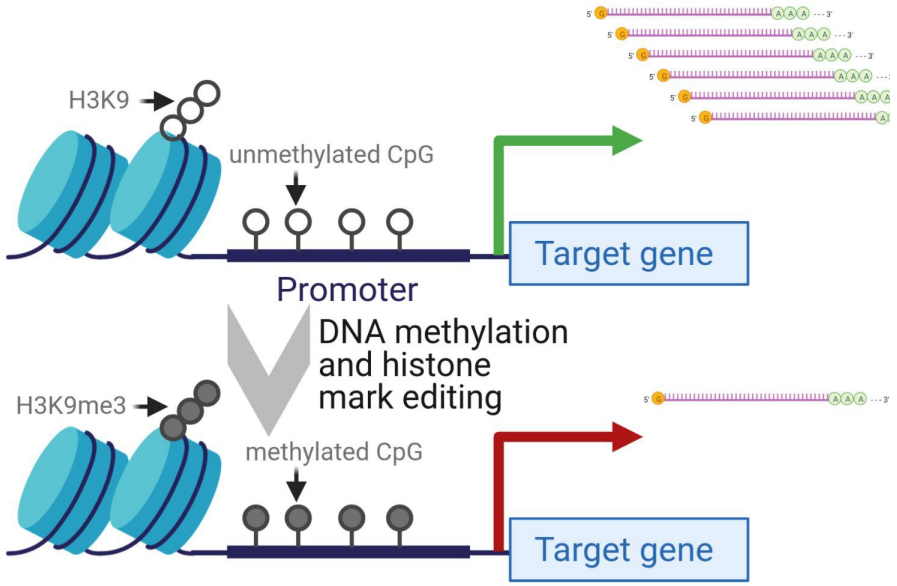
Classical approach	Limitation
Comparative mapping of epigenetic marks and gene expression levels	Correlations, but no causal relationship
Knock-out, knock-down, over-expression of epigenetic writer, eraser, reader	Cannot discriminate between primary effects and secondary / whole genome effects
Chemical inhibition of epigenetic enzyme	Cannot discriminate between primary effects and secondary / whole genome effects



Epigenome editing

Targeted modification of one (or several) epigenetic mark at one (or several) genomic locus

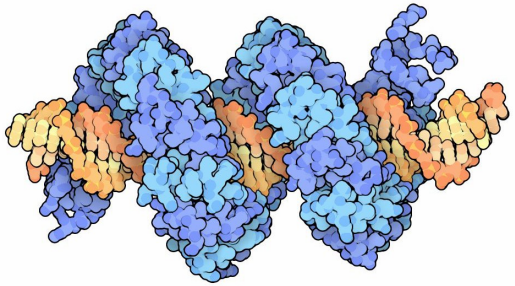
- Investigation of the causal mechanisms linking epigenetic marks and gene expression regulation
- Validation of predicted distal regulatory elements
- Modification of a target gene expression level without modifying the DNA sequence



In vivo epigenome editing

Study	Species	Epi-editing tool	Delivery method	Host genome altered?
Kojima-Kita et al., 2016[30]	mice	ZF-MIWI2	Integration of the transgene into the murine genome	Yes
Lei et al., 2017[31]	mice	dCas9-effector	Microinjection of zygote with plasmid and gRNA	No
Thakore et al., 2018[32]	mice	dSaCas9-effectors	Infection of the liver by AAV8 after injection into the tail vein	No
Zhou et al., 2018[29]	mice	dCas9-SunTag + aGCN4-effectors	Mice transgenic at the Rosa26 locus, and viral infection (AAV) or hydrodynamic injection to express Cre recombinase and guide RNA.	Yes
Wangensteen et al., 2018[33]	mice	dCas9-effectors	Integration of gRNA and dCas9 + effectors in the murine genome	Yes
Williams et al., 2018[26]	chicken embryos (no hatching)	dCas9-effectors	Electroporation of the neural crest	No
Wei et al., 2019[34]	mice	dCas9-effecteurs	Microinjection of mRNA and gRNA into oocytes	No
Peter et al., 2019[25]	mice	dCas9-SunTag + aGCN4-effectors	In utero electroporation of the brain with plasmids	No
Gemberling et al., 2021[28]	mice	dCas9-effector	Transgenic mice at the Rosa26 locus, and viral infection (AAV) to express Cre recombinase and guide RNA	Yes
Takahashi et al., 2023[35]	mice	Insertion then excision of a CpG free ~ 4kb wide DNA fragment	Nucleofection of ES cells and creation of chimeric mice	Yes
Saunderson et al., 2023[36]	mice	dCas9-effectors	Nucleofection of hematopoietic stem cells and injection of edited cells into immunocompromised mice.	No (but using xenograft on immuno-depressed mice)
Cappelluti et al., 2024[37], Tremblay et al., 2025[38]	mice	ZF-effectors, dCas9-effectors	Intravenous injection of mRNA-LNPs	No
Kannan et al., 2025[39]	mice	OMEGA-effectors	Intravenous injection of AAV	No

Epigenome editing: **Guidance** **system** + effectors + delivery method



By David Goodsell - RCSB PDB Molecule of the Month, CC BY 3.0,

ZF arrays, TALE(N)s

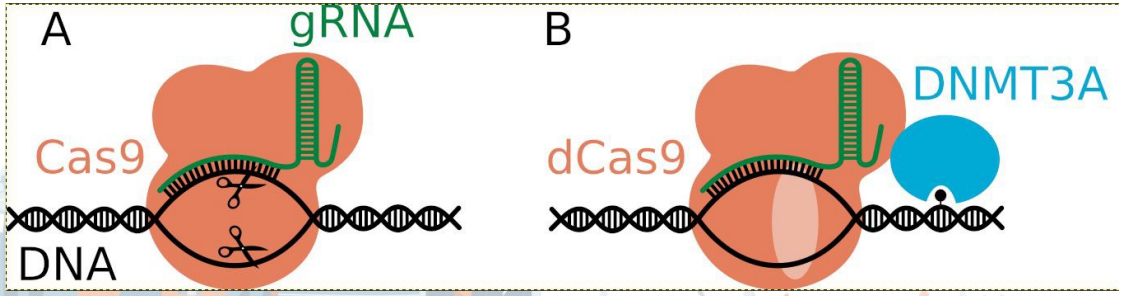
- Historical approach
- Need to re-assemble TF domains for each target region

Inactivated (dead) CRISPR Cas9

- Targeting through guide RNA, commercially available in a few days
- Large construct size (> 4kb)

Alternative Cas:

- Staphylococcus aureus Cas9 (~3 kb), Cas12a (~3 kb), EbCas12a, and Cas12f (1.5 kb)
- PAM and tracRNA might be different than for canonical Cas9
- Commercial availability of sgRNA might be limited



Epigenome editing: Guidance system + **effectors** + delivery method

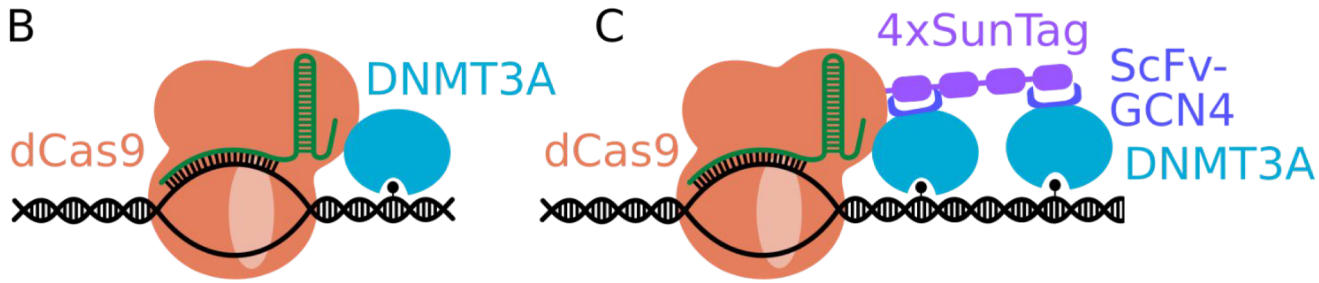
Repressive marks (gene repression)

- CpG methylation: DNMT3A ± DNMT3L, MQ1
- Repressive domain: KRAB, MECP2

Active marks (gene activation)

- DNA demethylation: TET1
- Activation domain: VP64, p65, Rta

Direct fusion or modular design



Epigenome editing: Guidance system + effectors + **delivery** **method**

Delivery method	**Limits**
DNA Lipofection	Limited transfection efficiency in some contexts
RNA Lipofection	Limited transfection efficiency in some contexts
RNP (ribonucleoproteins) Lipofection	RNP non commercially available for epigenome editing
Electroporation / Nucleofection	Mostly *in vitro*, *in vivo* : chicken neural crest, chimeric embryos, other tissues through surgery
Genome integration	Host genome modification
Lentivirus / Adenovirus / AAV	Sanitary measure, might induce host genome modification
Hydrodynamic delivery	Putatively traumatic, might require complex surgery

An example of in vivo Epigenome Editing (EE) in mice

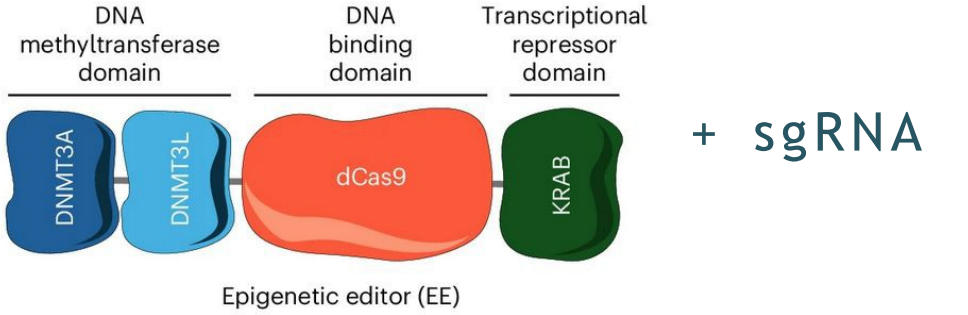
Article | Open access | Published: 10 February 2025

A potent epigenetic editor targeting human *PCSK9* for durable reduction of low-density lipoprotein cholesterol levels

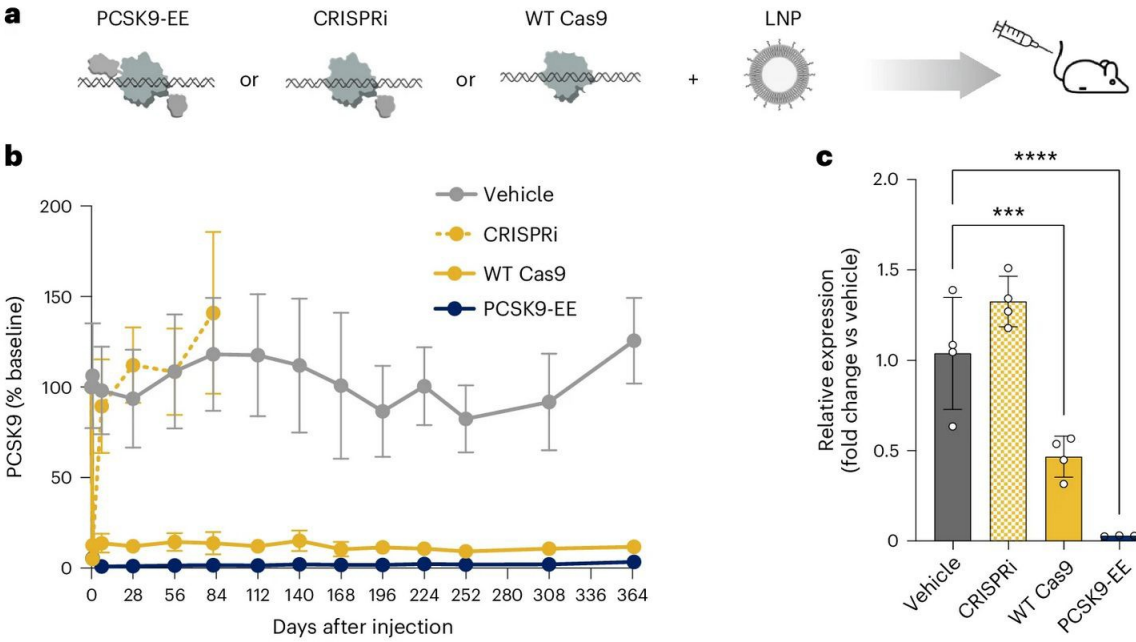
Frederic Tremblay ✉, Qiang Xiong, Shrijal S. Shah, Chih-Wei Ko, Kenneth Kelly, Mary S. Morrison, Cristiana Giancarlo, Ricardo N. Ramirez, Erica M. Hildebrand, Sarah B. Voytek, Gabriel K. El Sebae, Shane H. Wright, Liam Lofgren, Scott Clarkson, Christine Waters, Samantha J. Linder, Songlei Liu, Taesun Eom, Shefal Parikh, Yuki Weber, Salette Martinez, Padma Malyala, Sahar Abubucker, Ari E. Friedland, … Aron B. Jaffe

+ Show authors

Nature Medicine **31**, 1329–1338 (2025) | Cite this article



- Single injection of mRNA +sgRNA in the tail
- Effects on gene expression in the liver stable over a year



Key differences between epigenome and genome editing

With epigenome editing:

- no cleavage, no changes in the DNA sequence (*dead* Cas9)
- target a broader regions (~200 bp for DNA methylation, a few consecutive histones for histone tail modifications)
- probably not stable in most context (spontaneous reversion in days? weeks?)
  - in vitro
  - adult somatic tissues
  - genomic insertion of epigenetic editing tools
  - Transient changes of gene expression can have useful / long lasting effects

Putative application in farm animals

- The tools (targeting systems, effectors, delivery) are getting better and better every year.
- Main application at the moment: academic use for functional genomics, mostly in vitro.
- Applications will be highly dependent on the target gene(s) and the delivery method (i.e. somatic tissue vs germ cells).
- In vivo commercial applications in farm animal production are highly dependent on **economic feasibility**, **public acceptance**, and **intellectual property concerns**.
- The ethical aspects of epigenome editing in farm animals are not easily dissociable from the current ethical concern in highly specialized farm animal production systems. Each potential application will require its own ethical assessment.

COVID19 mRNA vaccine as a point of comparison

- Injection of a foreign mRNA with medium to long term consequence
- Part of our society is ready to accept biotechnological innovations **if the benefits are sufficiently obvious**
- ~15 € per dose

Putative fields of application?

Veterinary applications:

- Pathogene treatment, including preventive treatment
- Pain management

Production system applications:

- Hunger management: breeding performance, specialty food
- Alternative to hormonal treatments (i.e. for artificial insemination)
- Egg or sperm sexing

Conclusion

Epigenome editing (EE): targeted deposition of epigenetic marks.

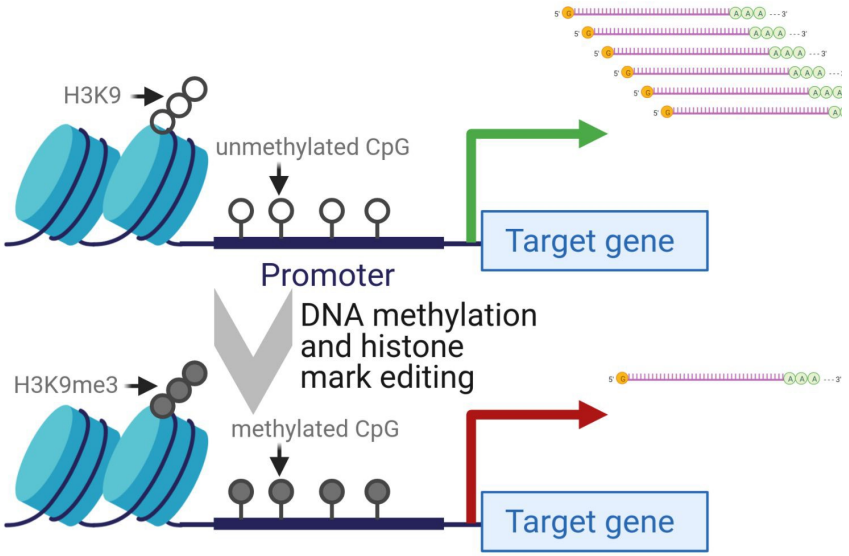
EE is a fantastic tool to understand gene expression regulation.

EE can be use to alter a target gene expression level, in vitro and in vivo.

Fast evolving field and tools.

First commercial applications in livestock will probably look more like RNA vaccines or drugs than like GMO or genome editing.

Preprint: hal.science/hal-05410119v2



GEroNIMO and RUMIGEN Joint Final Event

Breeding the Future

Genomics, Epigenomics & Societal Acceptability for Sustainability in Livestock

THANK YOU

TOWARDS IMPROVEMENT OF **RUMI**NANT BREEDING THROUGH **GEN**OMIC AND EPIGENOMIC APPROACHES

GENOME AND EPIGENOME ENABLED BREEDING IN MONOGASTRICS

Funded by the European Union