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Evaluation of the N^6-methyladenosine RNA modification pathway as a driver of tumor proliferation via high-throughput CRISPR screening

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Alterations of N^6-methyladenosine (m^{6}A)

pathway effectors (writers, erasers and readers) characterise different tumors. For example, **METTL3** (writer) **promotes proliferation** in colorectal cancer (**CRC**)[1] and acute myeloid leukaemia (**AML**)[2]. However, there still are open questions on the **mechanisms** by which these proteins contribute to the proliferative behaviour of tumors.

Writers (METTL3, METTL14…)

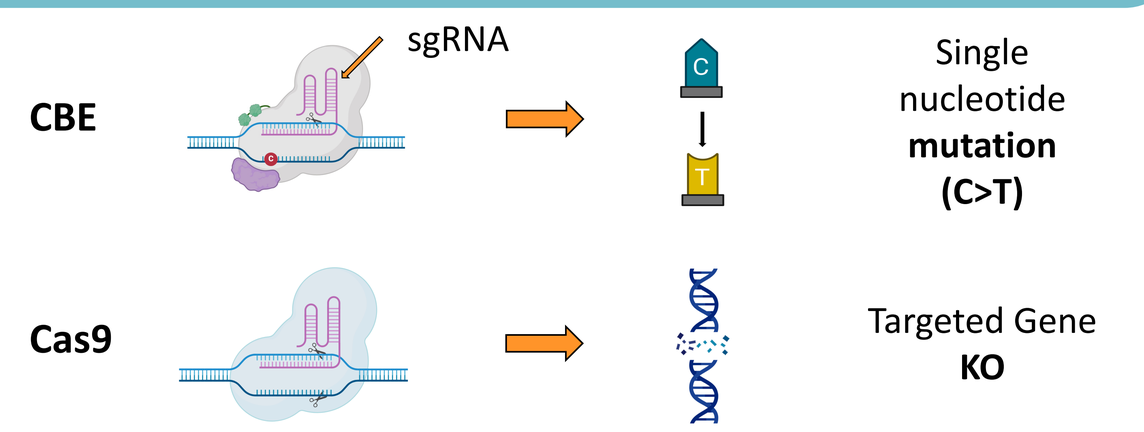
A ⇄ m^{6}A

Erasers (FTO, ALKBH5)

→ **Readers** (YTHFC1, YTHDF1…) → **Cancer cell Proliferation**

Cytosine base editing and CRISPR KO allow

programmable nucleotide changes (C->T) and **gene loss** thanks to the presence of a single guide RNA (**sgRNA)**



The aim is to study m^{6}A in CRC and AML cell proliferation by means of a pooled CRISPR- base editing and Knock-out (KO) screening.

1. Derivation of CBE+ and Cas9+ cell lines

We produced the lentivirus (LV) for stable Cas9 and CBE expression (fig. 1).

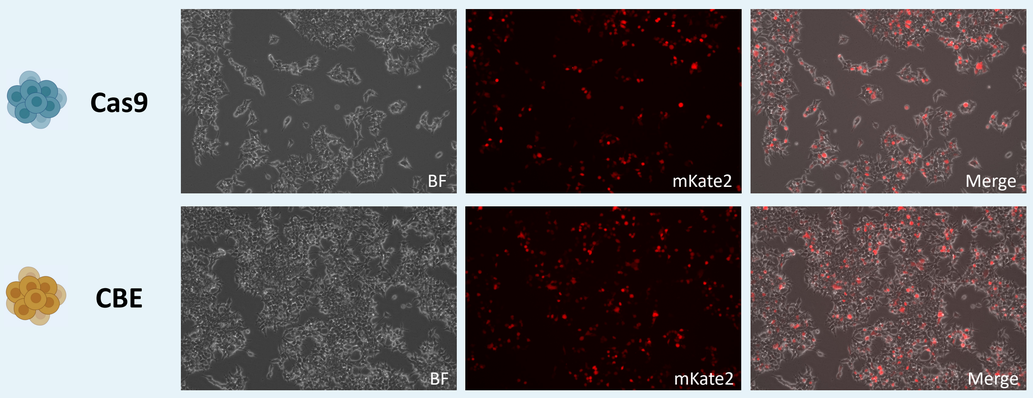


Fig. 1 HEK293TN cells expressing CBE and Cas9, 24 hours post-transfection

2. Preparation of the *in-silico* sgRNA library

We designed a **library targeting m^{6}A genes** and controls and purchased it as **plasmid** sgRNA library.

Experimental gene set: m^{6}A effectors and controls

↓

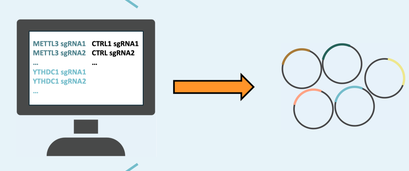
sgRNA library targets all the Cs in the gene set

↓

Filtering by predicted editing efficiency and mutation

↓

15 000 sgRNAs screening library



3. Optimization of the lentivirus preparation for the sgRNA library

We tested different ratios of plasmids (sgRNA library, packaging, psPAX2 and envelope, pMD2.G), and performed the test with sgRNA targeting HEKsite2 as surrogate for the library.
Since the **LV2 protocol** displays higher production of infectant particles (fig. 2), we will use it to generate the sgRNA library lentivirus.

Plasmid	LV1[3]		LV2[4]	
	ug	Ratio	ug	Ratio
HEKsite2	**2,8**	2,1	**5,4**	4,5
psPAX.2	13,4	10,0	5,4	4,5
pMD2.G	1,4	1,0	1,2	1,0

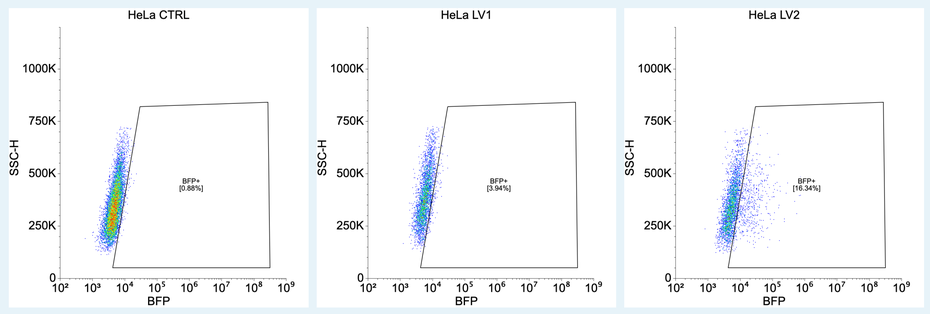
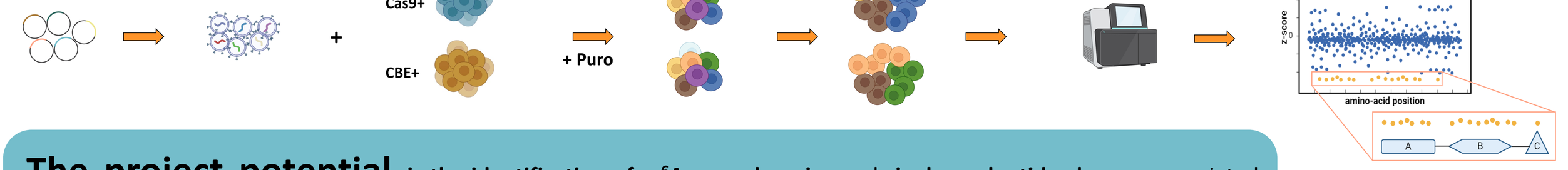


Fig. 2 HeLa cells transduced with 0,05 ul LV1 and LV2 preparations

NEXT steps

Preparation of the sgRNA lentiviral library → **Transduction into CBE+ and Cas9+ cells and selection** (+ Puro) → **Proliferation screening** (T_0 → T_1) → **Next Generation Sequencing of sgRNAs at T_0 and T_1** → **Data analysis to identify significant mutations**



The project potential is the identification of m^{6}A gene domains and single nucleotide changes associated with proliferation phenotype at **high resolution** and provide novel candidate **druggable sites** for anti-cancer therapies.
The sgRNA library will be then tested in a perturbed context, e.g., treatment with METTL3 small molecule inhibitor, to address the impact of single nucleotide changes of m^{6}A effectors in **drug response**.

Which protein domains are involved in proliferation?

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Promega Rising Researcher Innovation Award

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