LESSON FEEDBACK 2

CRISPR-CAS9 TECHNOLOGY APPLICATION
For one target or multiple targets

- GENOME EDITING
- RNA EDITING
- GENE REGULATION

Lesson4

1 lesson4_introCRISPRCas

Administration

- Book administration
  - Edit settings
  - Locally assigned roles
  - Permissions
  - Check permissions
  - Filters
  - Logs
  - Backup
  - Restore
  - Import chapter

Question 1 Incorrect Mark 0.00 out of 1.00

When you want insert a DNA sequence in a specific genomic region and orientation using CRISPR-Cas9 system, what is the approach?

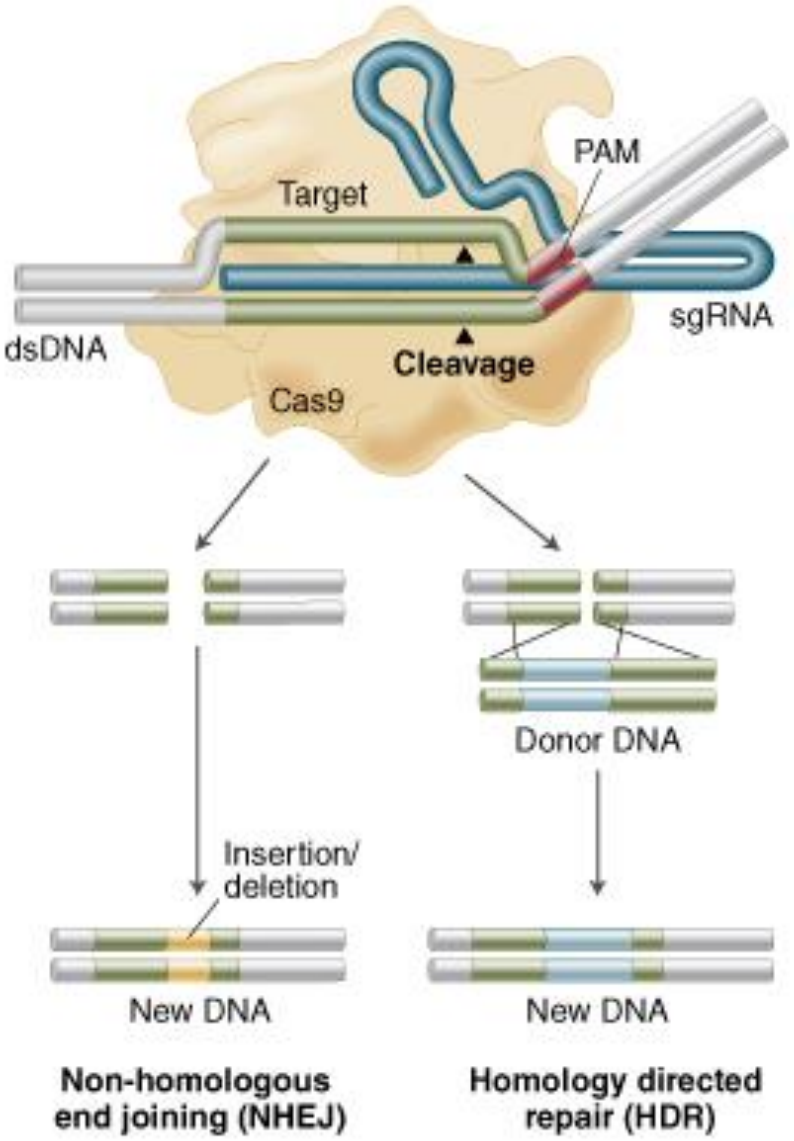
Select one:

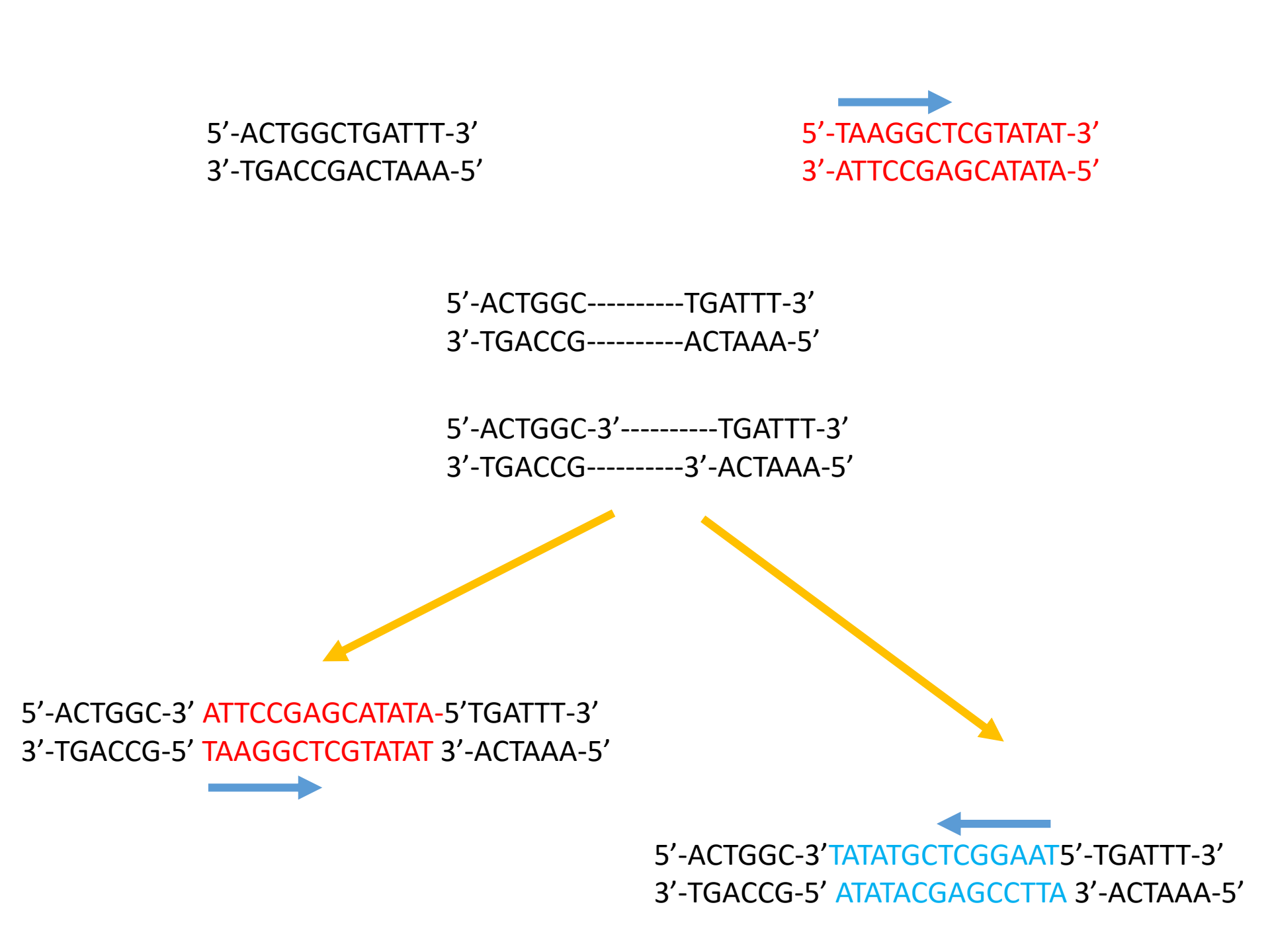
- a. Two DNA guides cut RNA sequence and RNA fragment of interest is insertted by homology-directed repair
- b. Two RNA guides cut the genome and DNA fragment of interest is insertted by homology-directed repair
- c. Two RNA guides cut the genome and DNA fragment of interest is inserted by NON-homologous-end joint repair
- d. One RNA guide cuts the genome and DNA fragment of interest is inserted by homology-directed repair ✗

Your answer is incorrect.

The correct answer is: Two RNA guides cut the genome and DNA fragment of interest is insertted by homology-directed repair

Homology directed repair allows correct orientation sequence




5’-ACTGGCTGATTT-3’
3’-TGACCGACTAAA-5’
5’-TAAGGCTCGTATAT-3’
3’-ATTCCGAGCATATA-5’
5’-ACTGGC----------TGATTT-3’
3’-TGACCG----------ACTAAA-5’
5’-ACTGGC-3’----------TGATTT-3’
3’-TGACCG----------3’-ACTAAA-5’
5’-ACTGGC-3’ ATTCCGAGCATATA-5’TGATTT-3’
3’-TGACCG-5’ TAAGGCTCGTATAT 3’-ACTAAA-5’
5’-ACTGGC-3’TATATGCTCGGAAT5’-TGATTT-3’
3’-TGACCG-5’ ATATACGAGCCTTA 3’-ACTAAA-5’

Question 4

Incorrect Mark 0.00 out of 1.00

What is the effect of catalytically deficient Cas13?

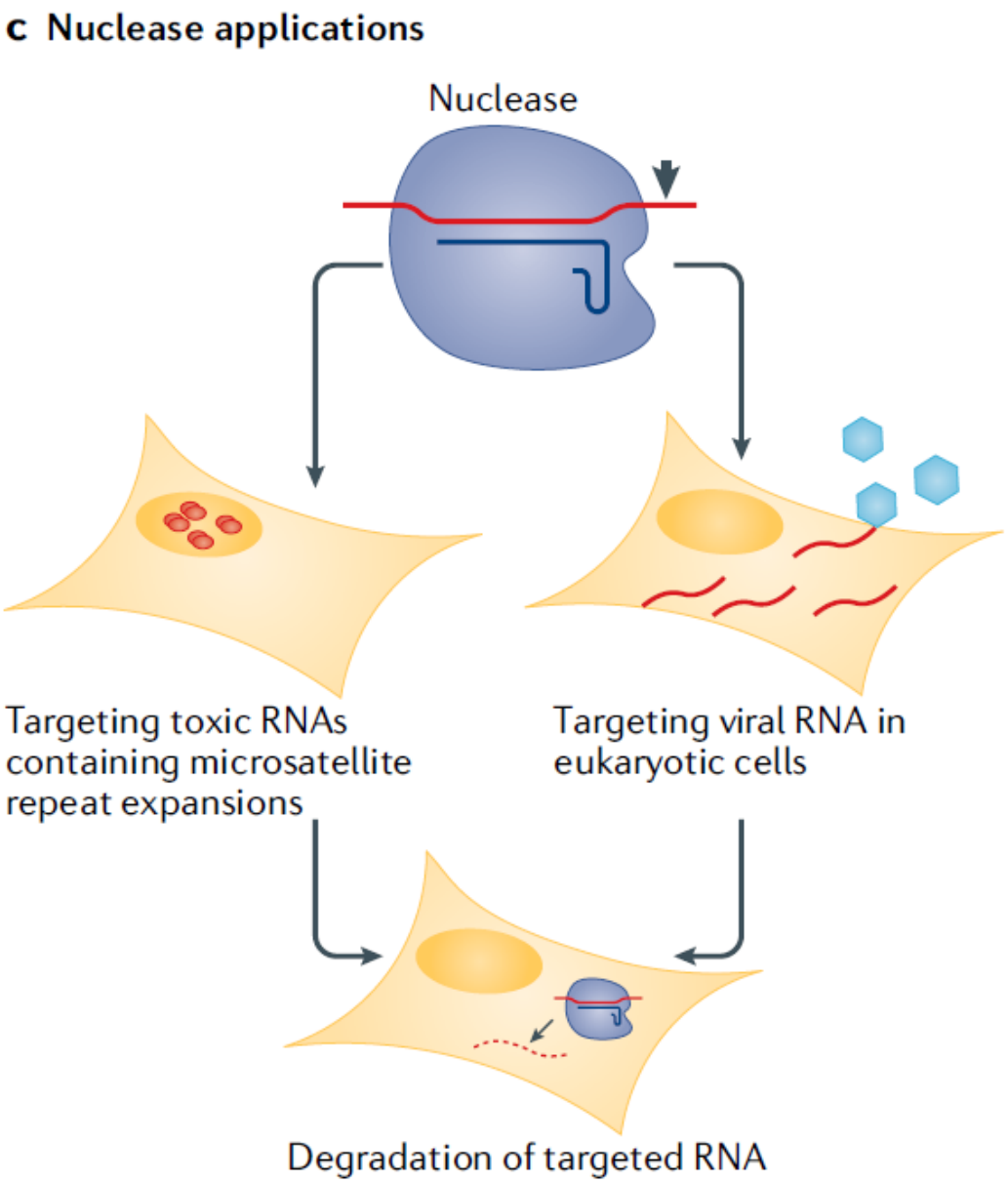
Select one:

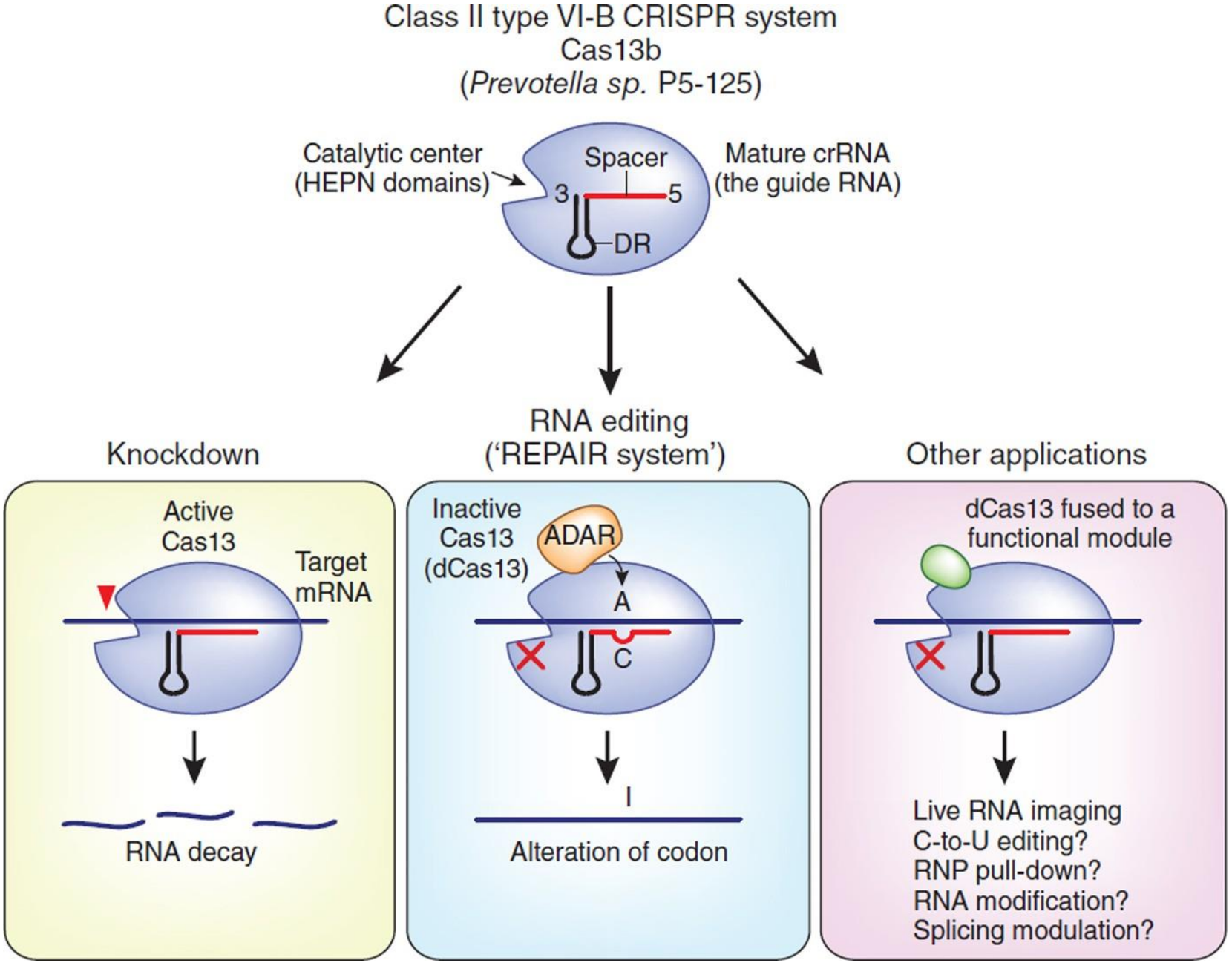
- a. transcription activation
- b. splincing inhibition
- c. mutation introduction ✗
- d. transcription inhibition
- e. splicing activation

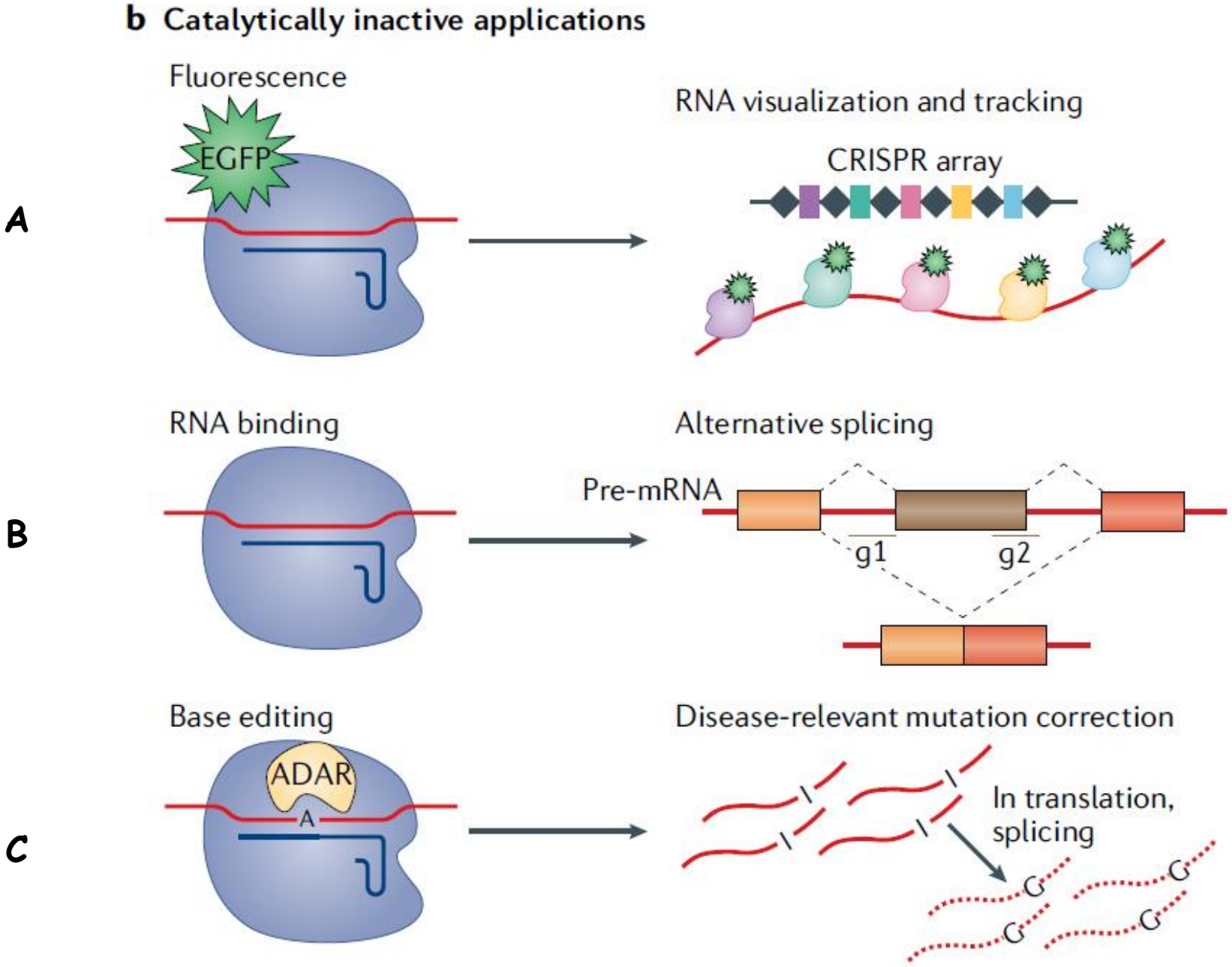
Your answer is incorrect.

The correct answer is: splincing inhibition

Cas13 can be used for targeted RNA degradation in eukaryotic cells for applications such as targeting viral RNA or toxic RNAs




Class II type VI-B CRISPR system
Cas13b
(*Prevotella sp.* P5-125)
Catalytic center
(HEPN domains)
Spacer
3
5
DR
Mature crRNA
(the guide RNA)
Knockdown
Active
Cas13
Target
mRNA
RNA decay
RNA editing
('REPAIR system')
Inactive
Cas13
(dCas13)
ADAR
A
C
I
Alteration of codon
Other applications
dCas13 fused to a
functional module
Live RNA imaging
C-to-U editing?
RNP pull-down?
RNA modification?
Splicing modulation?


b Catalytically inactive applications
A
Fluorescence
EGFP
RNA visualization and tracking
CRISPR array
B
RNA binding
Alternative splicing
Pre-mRNA
g1
g2
C
Base editing
ADAR
A
Disease-relevant mutation correction
In translation, splicing
G
G
G
G

Catalytically deficient Cas13 maintains the capacity to bind to the targeted RNA

A For RNA visualization and tracking purposes, a fluorescent protein can be fused to the catalytically deficient Cas and colocalize with an array of CRISPR RNAs (crRNAs) or gRNAs.

B To promote alternative splicing, catalytically deficient Cas13 can be targeted with gRNAs (g1 and g2) to bind splicing regulating *cis* elements

C Adenosine deaminase acting on RNA (ADAR) can be fused to catalytically deficient Cas for RNA A→I base editing to correct disease-relevant mutations.

Question 3 Incorrect Mark 0.00 out of 1.00

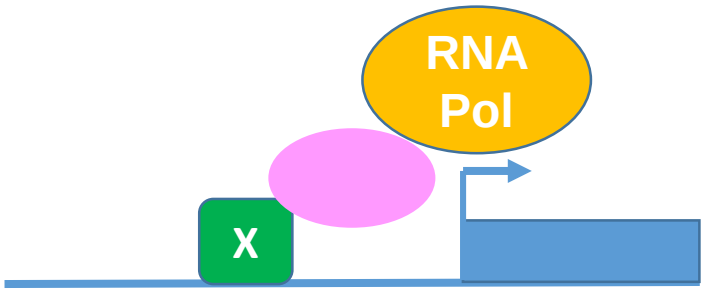
What can you design to demonstrate whether a domain bound enhancer next to GENE A induces gene expression?

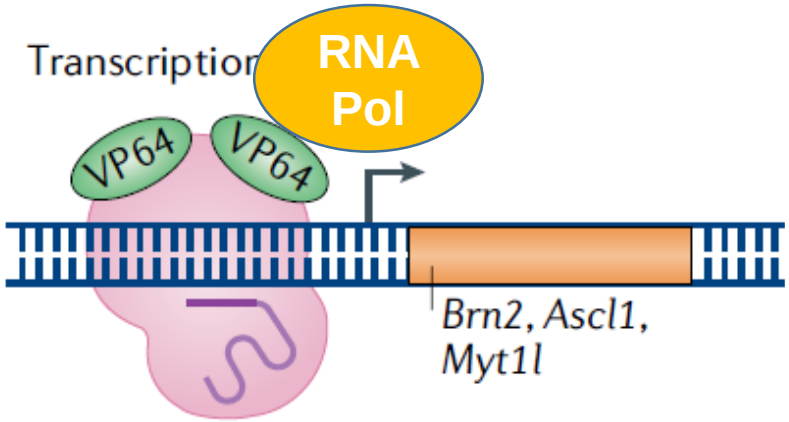
Select one:

- a. Catalytic inactive Cas9 fused to TRANSACTIVATION DOMAIN, GFP, and domain of your interest ✗
- b. Catalytic inactive Cas9 fused to TRANSACTIVATION DOMAIN, TET1, and domain of your interest
- c. Catalytic inactive Cas9 fused to TRANSACTIVATION DOMAIN, VP4, and domain of your interest
- d. Catalytic inactive Cas9 fused to TRANSACTIVATION DOMAIN, KRAB, and domain of your interest

Your answer is incorrect.

The correct answer is: Catalytic inactive Cas9 fused to TRANSACTIVATION DOMAIN, VP4, and domain of your interest





10.5. CRISPRi and CRISPRa targeting experiments.

gRNAs targeting transcriptional start site regions were retrieved from the Human Genome-wide CRISPRi-v2 and CRISPRa-v2 top5 libraries, respectively [40], and previously validated negative control gRNAs targeting sequences not present in the human genome were retrieved from Addgene (https://www.addgene.org/crispr/reference/grna-sequence/). To repress (CRISPRi) or further activate (CRISPRa) enhancer regions, 20nt-long gRNAs against the core of the enhancer (summit of ATAC-seq/Mediator ChIP-seq signal) were designed using Cas-Designer (http://www.rgenome.net/cas-designer/). gRNA oligonucleotides were cloned into the Lenti-(BB)-hPGK-KRAB-dCas9-2A-BlastR and SAMv2 [41] vectors, respectively, following a previously described protocol [42]. Briefly, oligonucleotides (Thermo Fisher) containing gRNA sequences flanked by BsmBI-compatible overhangs were phosphorylated with T7 polynucleotide kinase (NEB) and annealed. Fragments were ligated into BsmBI-digested destination vector. Ligated constructs were transformed into Stbl3 chemically competent *E. coli* and clones were checked by Sanger sequencing. gRNAs together with average efficiencies of target gene repression/activation and associated references can be found in Supplementary Table 15. New backbones, and CRISPRi and CRISPRa plasmids containing guides that yielded significant changes in gene expression in EndoC βH3 cells have been made available in Addgene (plasmid IDs in Supplementary Table 15). In addition to the 10 enhancers presented here, we targeted 5 enhancers at *MIR4686, MTNR1B, ZMIZ1, ISL1, KCNQ1* loci that did not elicit any significant gene expression changes, or showed changes that were not consistent across independent experiments.

Select one:

- a. RNA guide library to genome-wide screening
- b. RNA plasmid to genome-wide screening
- c. RNA guide library to one genomic region ✗
- d. DNA library to genome-wide screening
- e. RNA interferance oligonucleotide to genome-wide screening

Your answer is incorrect.

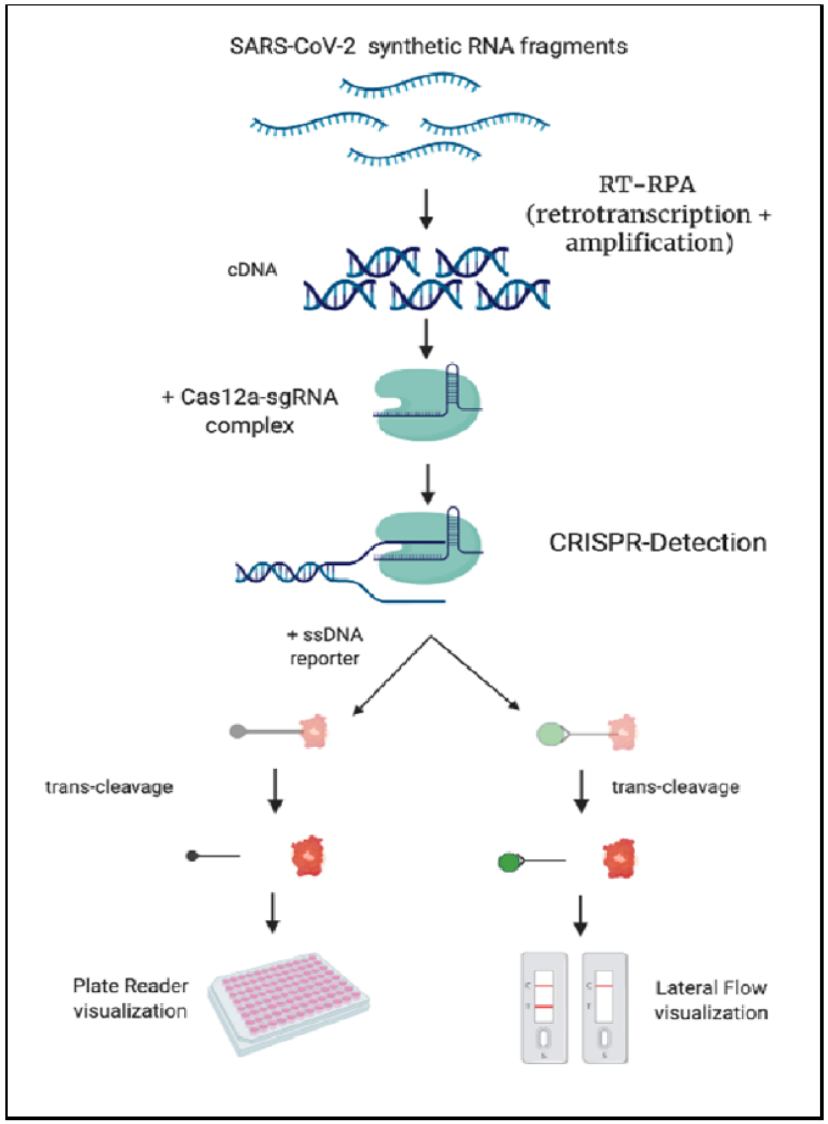
The correct answer is: RNA guide library to genome-wide screening

An ultrasensitive, rapid, and portable coronavirus SARS-CoV-2 sequence detection method based on CRISPR-Cas12

Curti Lucia[1], Pereyra-Bonnet Federico[1,2] and Gimenez Carla Alejandra[2,*]

[1]INPA-National Scientific and Technical Research Council (CONICET)- Argentina-University of Buenos Aires, Argentina

[2]CASPR Biotech, San Francisco California, USA



100

101 **Figure 1**. CRISPR-based detection method for novel coronavirus. General scheme of

102 CRISPR detection procedure. In the two strategies all the process takes less than 60 min.

CRISPR-Cas12 based diagnostic tool to detect synthetic SARS-CoV-2 RNA sequences in a proof-of-principle evaluation. Cas12 is a RNA-guided DNase belonging to the class II type V-A system that induces indiscriminate single-stranded DNA (ssDNA) collateral cleavage after target recognition. This leads to the degradation of ssDNA reporters that, emit a fluorescence signal on cleavage or alternatively, could be detected by lateral flow in a portable manner.

For the detection assays, the SARS-CoV-2 fragments were synthesize as complementary DNA oligonucleotides and treated with fill-in PCR to generate the DNA templates. These DNA templates were transcribed into RNA using an in vitro transcription Kit under the control of a T7 promoter. A target amplification step was performed using the TwistAmp® Basic recombinase polymerase amplification (RPA) kit and RT was 70 carried out. To generate the CRISPR-detection complex, they mixed the LbCas12a endonuclease with single guide RNA .For plate reader-based assays, ssDNA reporters labeled with FAM were included in the detection mix; while for portable detection with paper strips, ssDNA reporters labeled with biotin were included,fluorescence measurements were acquired at 10 min intervals.

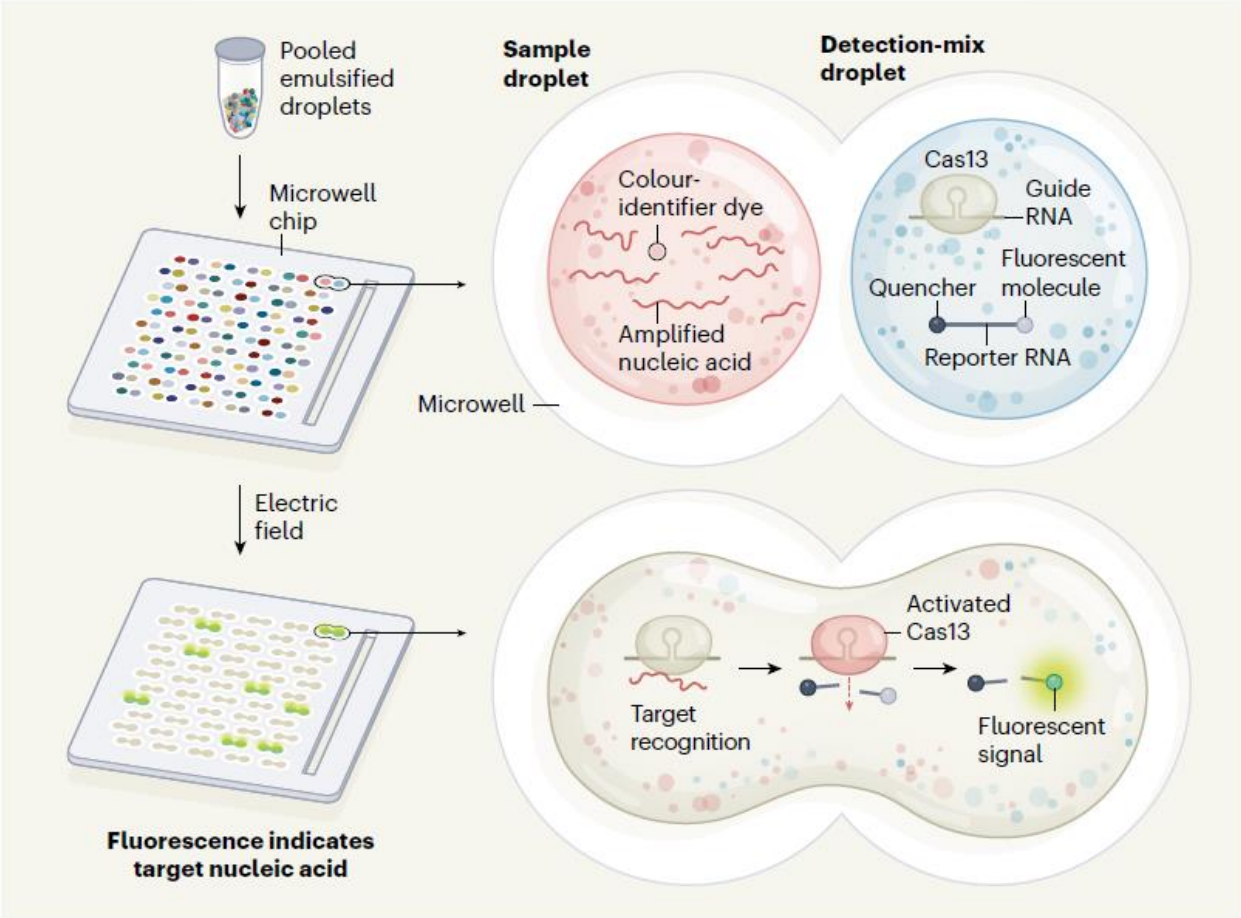
Based on visualization of these results in a fluorescence spectrophotometer, the limit of detection (LOD) for ORF1ab coronavirus sequences was estimated to be up **to 10 copies/µL which is 4 orders of magnitude lower than the viral load**.

A possible criticism for this study is that the method has not been tested using patient samples.

CRISPR tool interrogates a line-up of viral suspects

Gregory A. Storch

Rapid, reliable identification of an unknown viral infection is challenging. Use of CRISPR technology can simultaneously detect nucleic acids of many viruses and pinpoint specific ones, such as the virus that causes COVID-19.

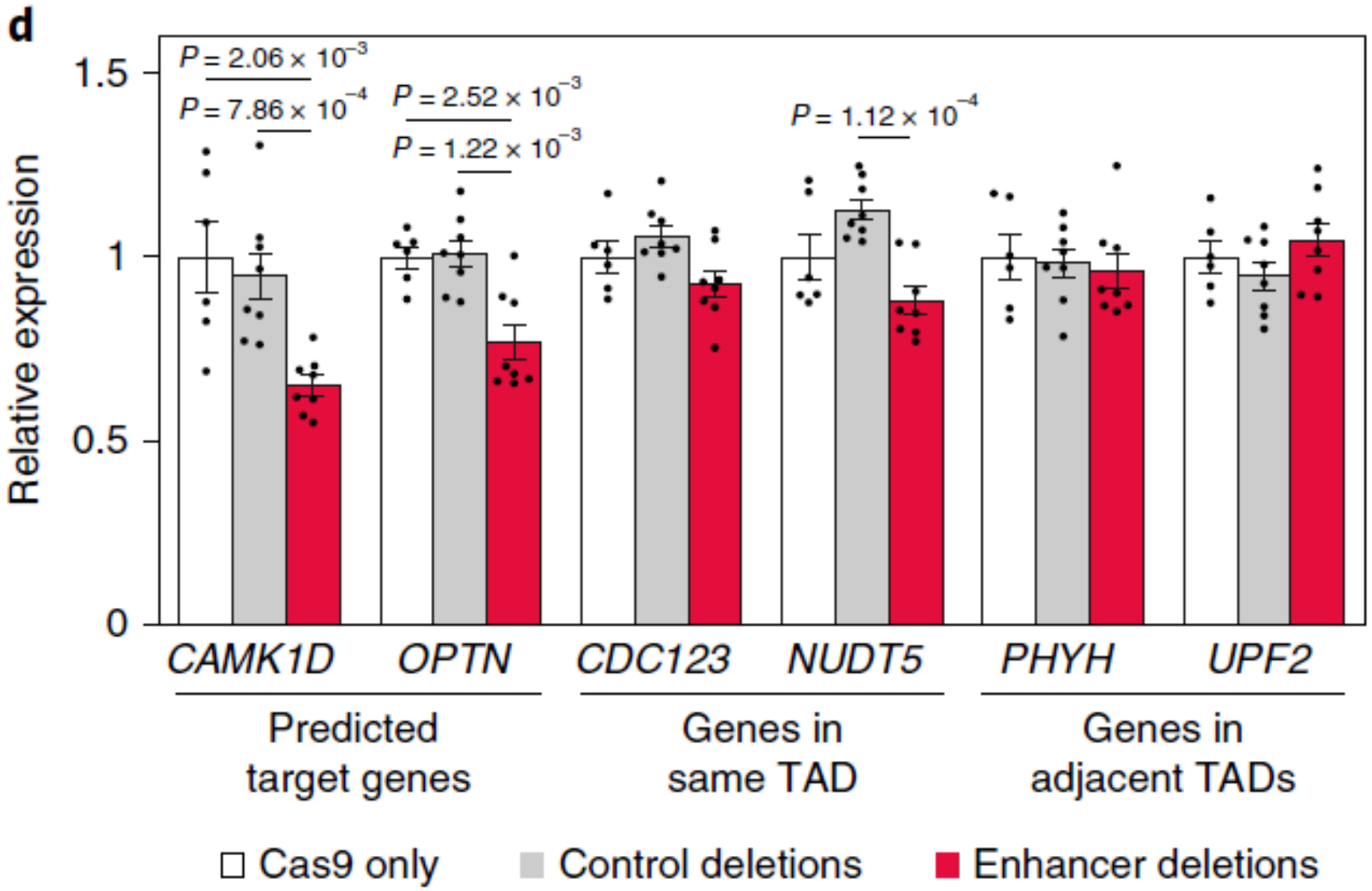


VIRUS DETECTION KIT

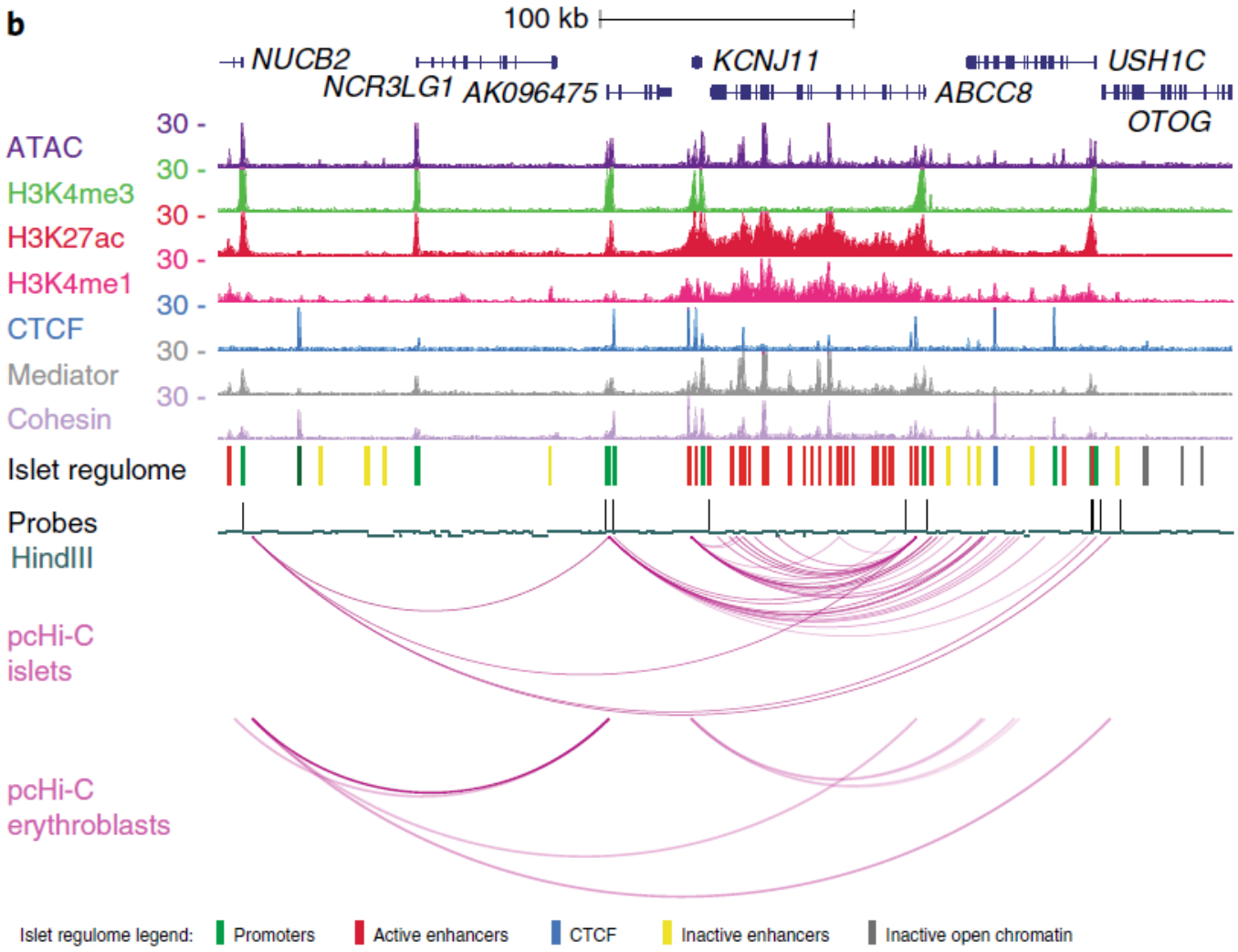
Taking into account:

- Samples
- Detection methods
- Specificity for Covid-19

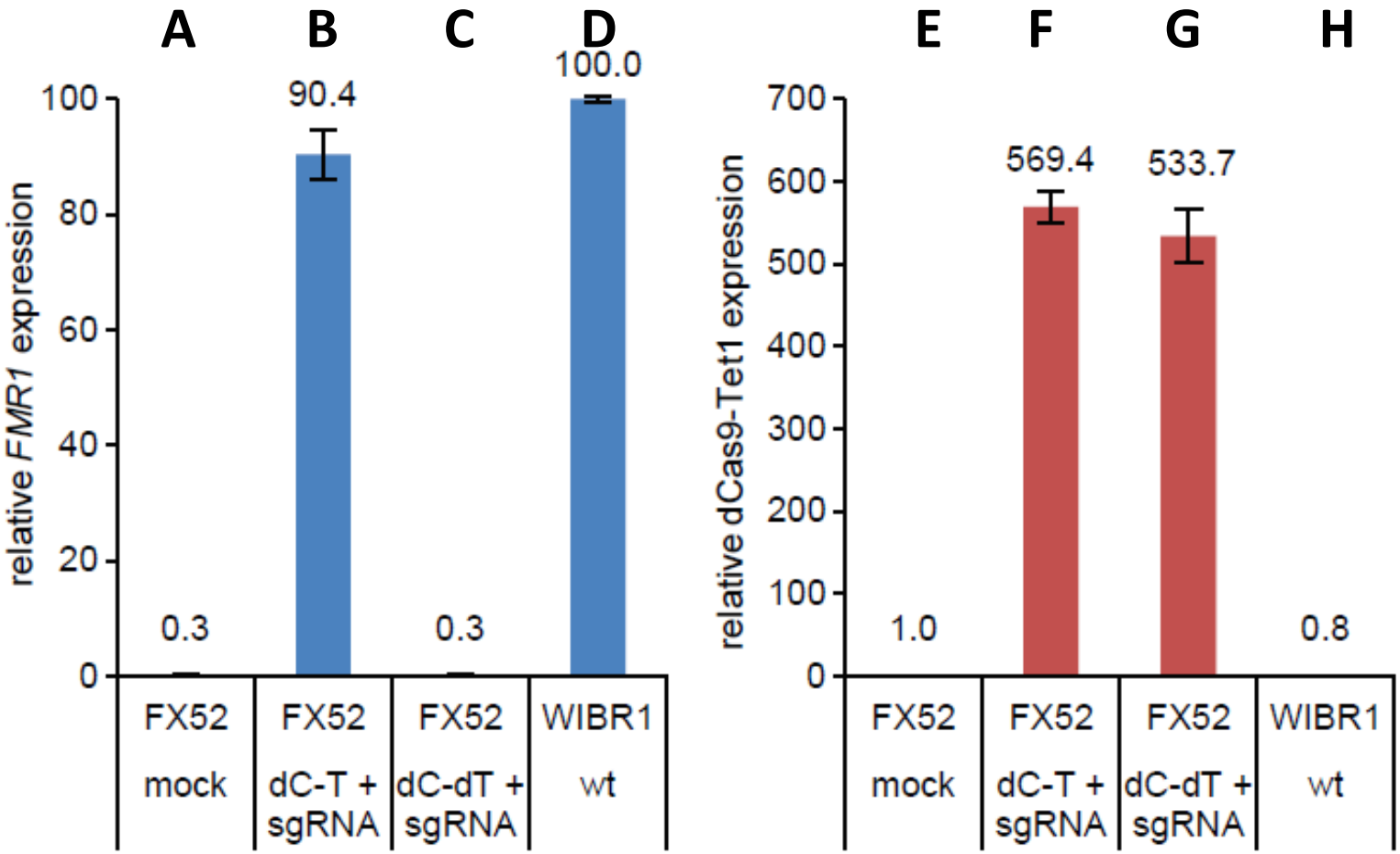
What is the gene regulated in TAD domain?



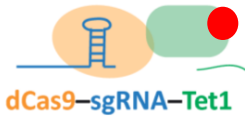
What are the pink arc in the bottom and why are different between islet and erythroblasts?



Which do samples demonstrate that CRISPR-TET1 reactivates FMR1 gene?



dC-T sgRNA



dC-dT sgRNA

To test whether the **reactivation of FMR1 in methylation-edited FXS cells** is sustainable in vivo, FX52 mock- or methylation-edited neuronal precursor cells (NPCs) were labeled with GFP or red fluorescent protein (RFP) lentiviruses, respectively, and then the mixture of these **two types of NPCs was injected into the P1 mouse brain for subsequent analysis 1 or 3 months after transplantation**