QUESTIONS

Q: Total number of genes in a human genome
Q: Total number of different cell types in a human body
Q: Which class does EcoRI, BamHI, HindIII, etc. belong to?
Q: Which of the below is true for forward genetics? phenotype → genotype OR genotype → phenotype
Q: FokI is naturally found in_____.
Q2: What are chimeric restriction endonuclease?
Q: Which of the below is protein-guided genome editing technology? Zinc finger nucleases or TALENs or CRISPR-Cas.
Q: What are Zinc finger nucleases?
Q: Which proteins secreted by Xanthomonas bacteria via their type III secretion system when they infect various plant species?
Q: What is TALENs?
Q: What does CRISPR stand for?
Q: What are the three stages in CRISPR-Cas system?
Q: What is guide RNA?
Q: How does bacteria identify foreign vs self DNA?
Q: What is PAM?
Q: Which two Cas proteins are generally present in all the 6 Types (except for IV)?
Q: Name the two lobes present in Cas9 protein?
Q: What are the two nuclease domains in Cas9 protein?
Q: What does "d" stand for in dCas9?
Q: What does "a" and "i" stand in CRISPRa and CRISPRi, respectively?
Q: Name the two DNA repair mechanisms when a DSB takes place?
Q: What is one major problem with genome editing technology?

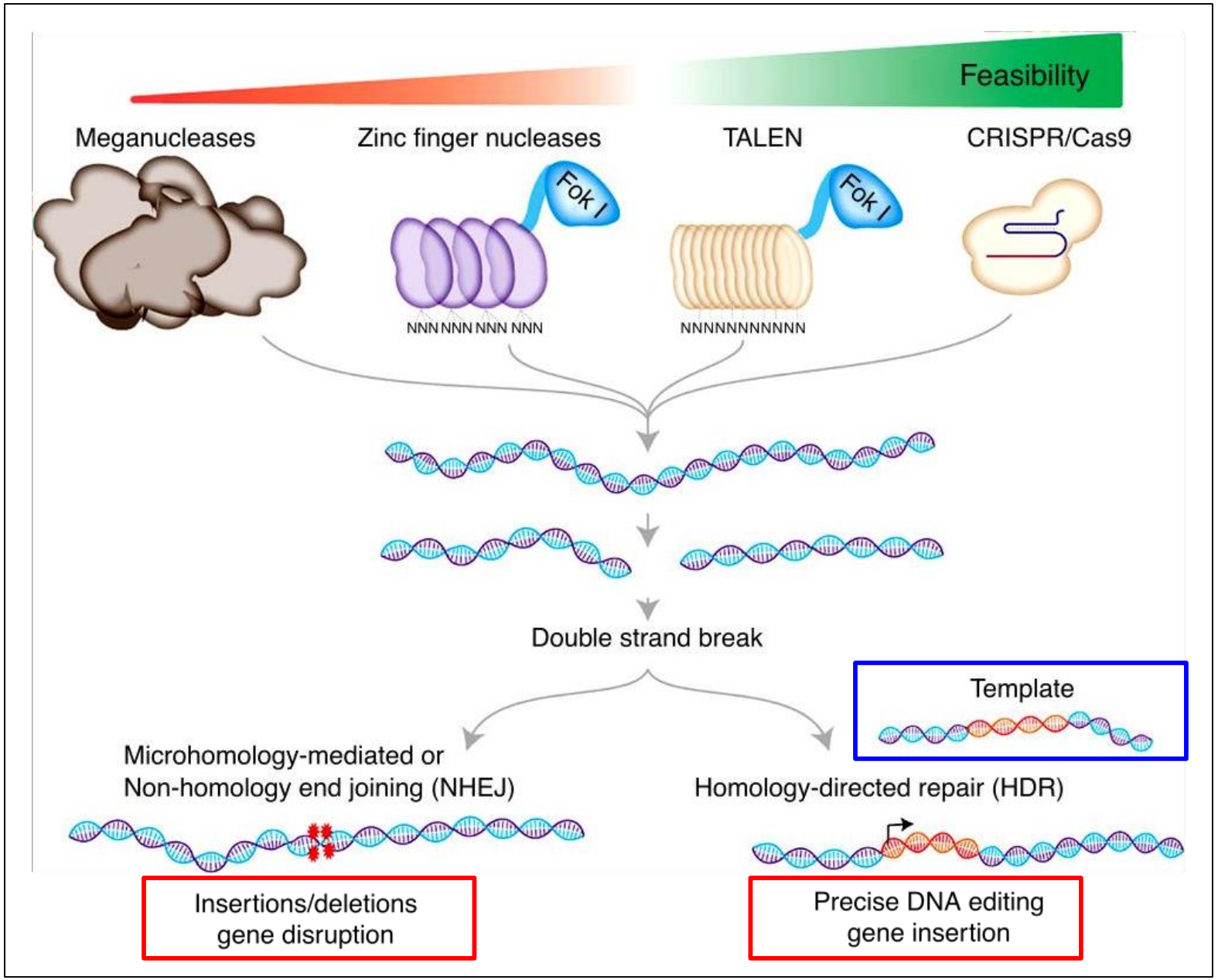
What is Genome Editing?

- Genome editing, or genome editing with engineered nucleases (GEEN) is a type of genetic engineering in which DNA is either
  - **inserted** or
  - **deleted** or
  - **replaced** in the genome of a living organism using **engineered nucleases**, or "**molecular scissors**."

- These nucleases create site-specific **double-strand breaks** (DSBs) at desired locations in the genome.

- The induced double-strand breaks are repaired through **nonhomologous end-joining (NHEJ) or also known as Homology Directed Repair (HDR) – homologous recombination**, resulting in targeted mutations ('edits').

Genome editing technologies



The different generations of nucleases used for genome editing and the DNA repair pathways used to modify target DNA.

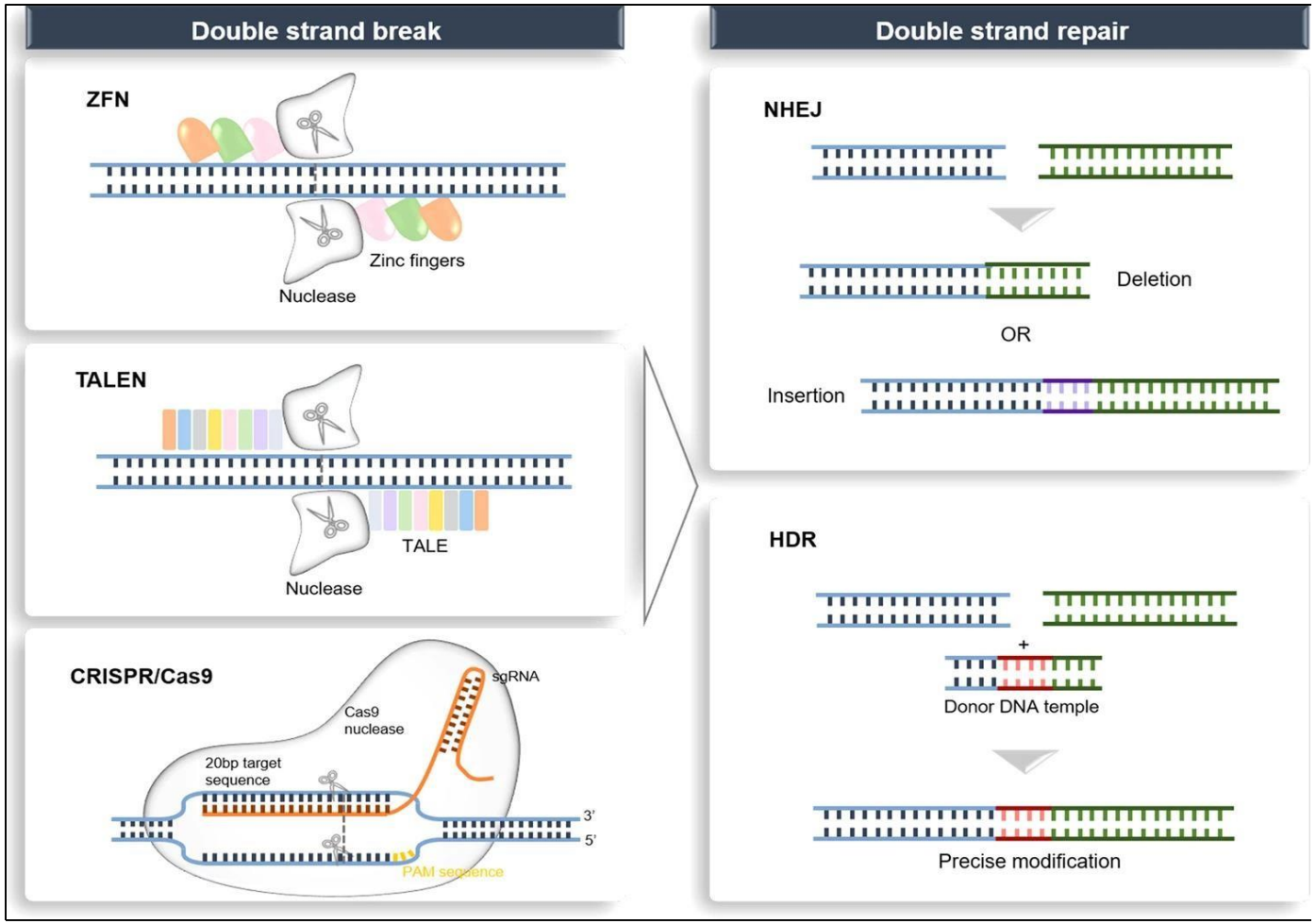
REVIEW ARTICLE OPEN

Applications of genome editing technology in the targeted therapy of human diseases: mechanisms, advances and prospects

Hongyi Li[1], Yang Yang[2], Weiqi Hong[2], Mengyuan Huang[2], Min Wu[3] and Xia Zhao[1]

Signal Transduction and Targeted Therapy (2020)5:1

- Genome editing platforms and mechanisms for DSB repair with endogenous DNA. Genome editing nucleases (ZFNs, TALENs and CRISPR/Cas9) induce DSBs at targeted sites.
- DSBs can be repaired by NHEJ or, in the presence of donor template, by HDR.
- Gene disruption by targeting the locus with NHEJ leads to the formation of indels. When two DSBs target both sides of a pathogenic amplification or insertion, a therapeutic deletion of the intervening sequences can be created, leading to NHEJ gene correction.
- In the presence of a donor-corrected HDR template, HDR gene correction or gene addition induces a DSB at the desired locus.



DSB double-stranded break, ZFN zinc-finger nuclease, TALEN transcription activator-like effector nuclease, CRISPR/Cas9 clustered regularly interspaced short palindromic repeat associated 9 nuclease, NHEJ nonhomologous end-joining, HDR homology-directed repair.

DNA repair mechanisms

- A common form of Genome editing relies on the concept of DNA double stranded break (DSB) repair mechanisms.

- There are **two** major pathways that repair DSB;
  - **Non-homologous end joining (NHEJ) and**
  - **Homology directed repair (HDR).**

- NHEJ uses a variety of enzymes to directly join the DNA ends while the more accurate HDR uses a homologous sequence as a template for regeneration of missing DNA sequences at the break point.

- This can be exploited by creating a vector with the desired genetic elements within a sequence that is homologous to the flanking sequences of a DSB.

- This will result in the desired change being inserted at the site of the DSB.

CRISPR-Cas technology

- "CRISPR" stands for **Clustered Regularly Interspaced Short Palindromic Repeats**.
- Bacterial defense system used to protect themselves against attacks from viruses.
- Bacteria use these genetic sequences to "remember" each specific virus that attacks them.
- They do this by incorporating the virus' DNA into their own bacterial genome. This viral DNA ends up as the spacers in the CRISPR sequence. This method then gives the bacteria protection or immunity when a specific virus tries to attack again.
- Since its initial discovery in 2012, scientists have marveled at the applications of CRISPR (also known as Cas9 or CRISPR-Cas9).

In this process:

- **Guide RNA** ("GPS coordinates") locates the error or the mutation: the word "**No**".
- **Cas9 enzyme** (molecular scissor) makes the break before and after the word "**No**".
- A bioengineered vehicle inserts the **correct piece of DNA**, in this case, the word "**Yes**".


Emamnuelle Charpentier
Jennifer Doudna

The Nobel Prize in Chemistry 2020 was awarded jointly to Emmanuelle Charpentier and Jennifer A. Doudna "for the development of a method for genome editing."

Applications of genome editing technologies

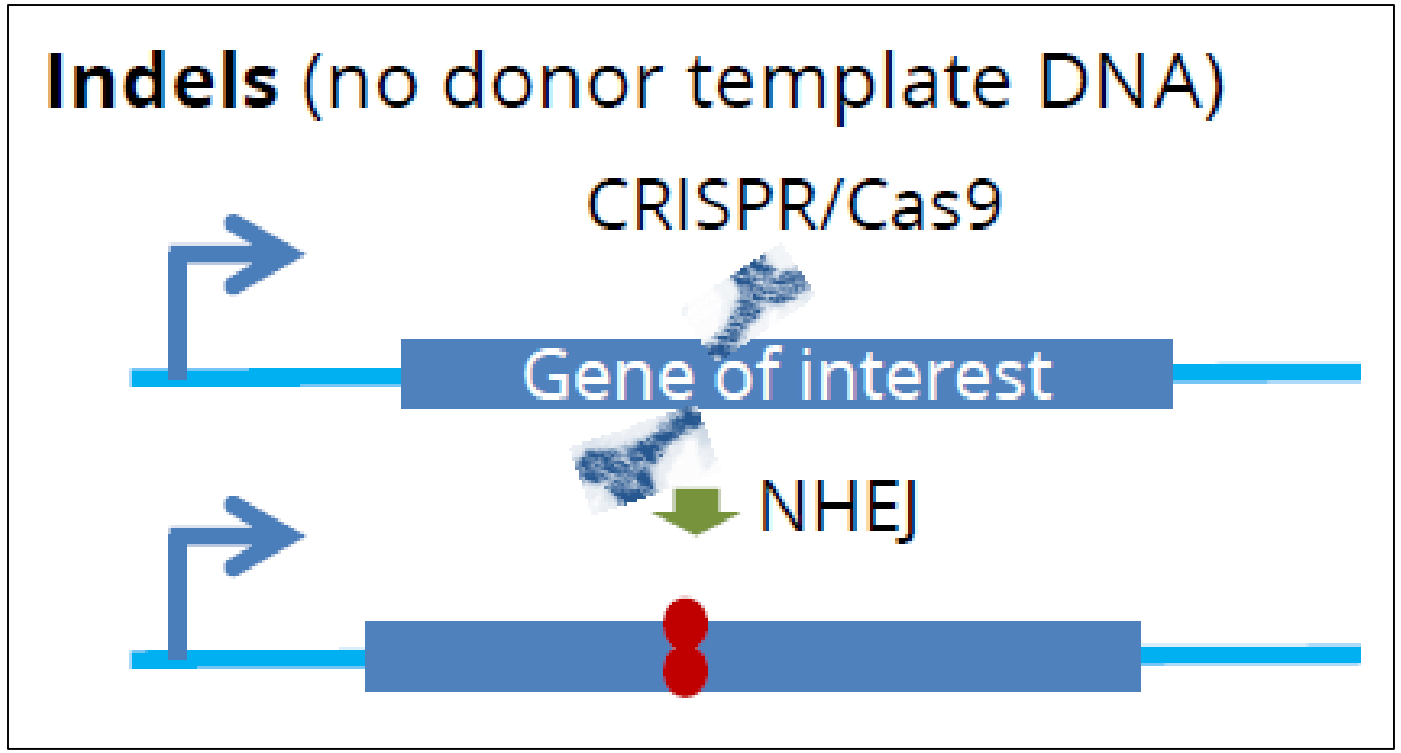
❑ Applications that permanently edit the DNA

He Jiankui; born 1984) is a Chinese biophysicist. He was named as the inaugural director of the Institute of Genetic Medicine at Wuchang Technical College, a private undergraduate college in Wuhan, in September 2023.[2] Before January 2019, He served as associate professor at the Department of Biology of the Southern University of Science and Technology (SUSTech) in Shenzhen, Guangdong, China

Earning a PhD from Rice University in Texas on protein evolution, including that of CRISPR, He learned gene-editing techniques (CRISPR/Cas9) as a postdoctoral researcher at Stanford University in California.[6]
In November 2018, He announced that he had created the first human genetically edited babies,[7][8] twin girls who were born in mid-October 2018 and known by their pseudonyms, Lulu and Nana.[9][10][6][1] The announcement was initially praised in the press as a major scientific advancement.[11] But following scrutiny on how the experiment was executed, He received widespread condemnation.[6][12][13] His research activities were suspended by the Chinese authorities on 29 November 2018,[14] and he was fired by SUSTech on 21 January 2019.[15] On 30 December 2019, a Chinese court found He Jiankui guilty of illegal practice of medicine and sentenced him to three years in prison and a fine of 3 million yuan.[16] He was released from prison in April 2022.[17]
He was listed as one of *Time*'s 100 most influential people of 2019, in the section "Pioneers".[18] At the same time he was variously referred to as a "rogue scientist",[19] "China's Dr. Frankenstein",[20] and a "mad genius".[21]

Applications of genome editing technologies

❑ 1. Gene knockout (delete a gene of interest)

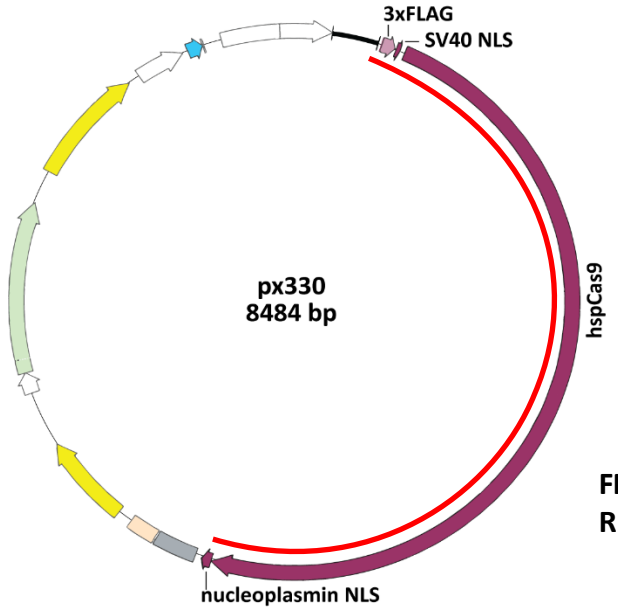


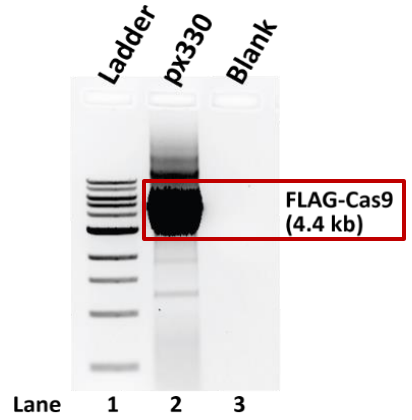
Indels are insertion or deletion of bases in the genome of an organism.

- This will create site-specific **double-strand breaks** (DSBs) at desired locations in the genome.
- The induced double-strand breaks are repaired through **nonhomologous end-joining (NHEJ)**, resulting in targeted mutations ('edits').

Establishment of stable Cas9-inducible expression system

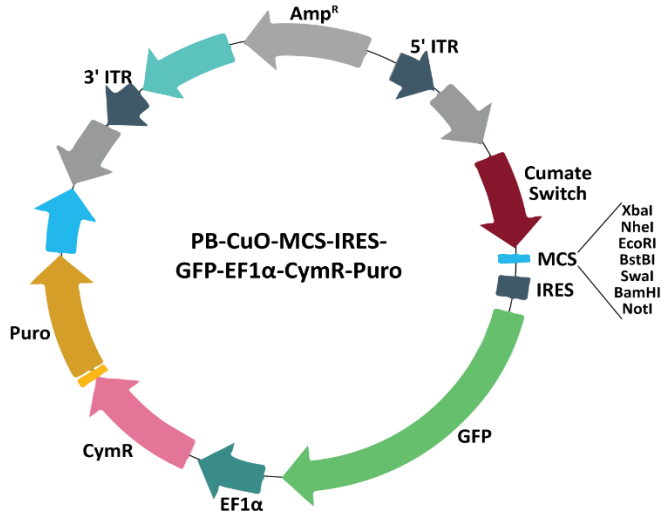
Cloning of *hSpCas9* gene in PiggyBac plasmid





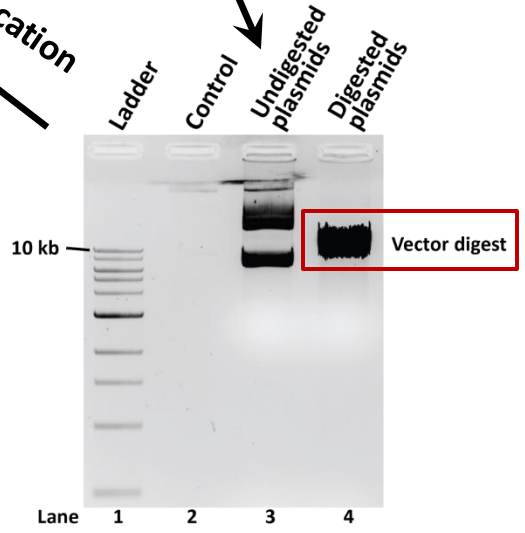
FP: NheI-FLAG
RP: NotI-nucleoplasmin NLS

Double digestion with NheI-NotI

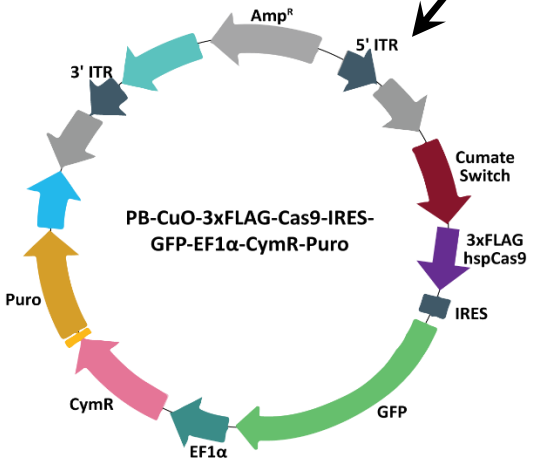


Double digestion with NheI-NotI

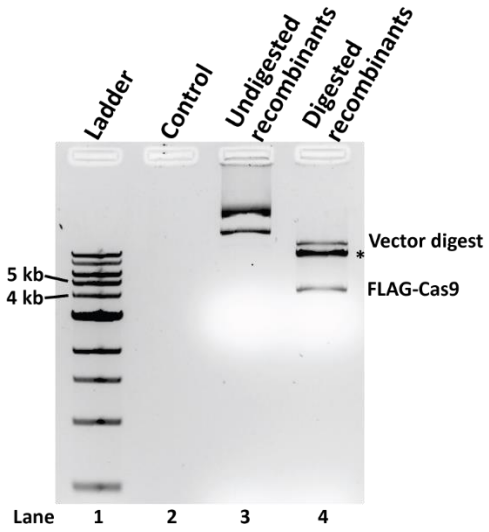
Gel purification



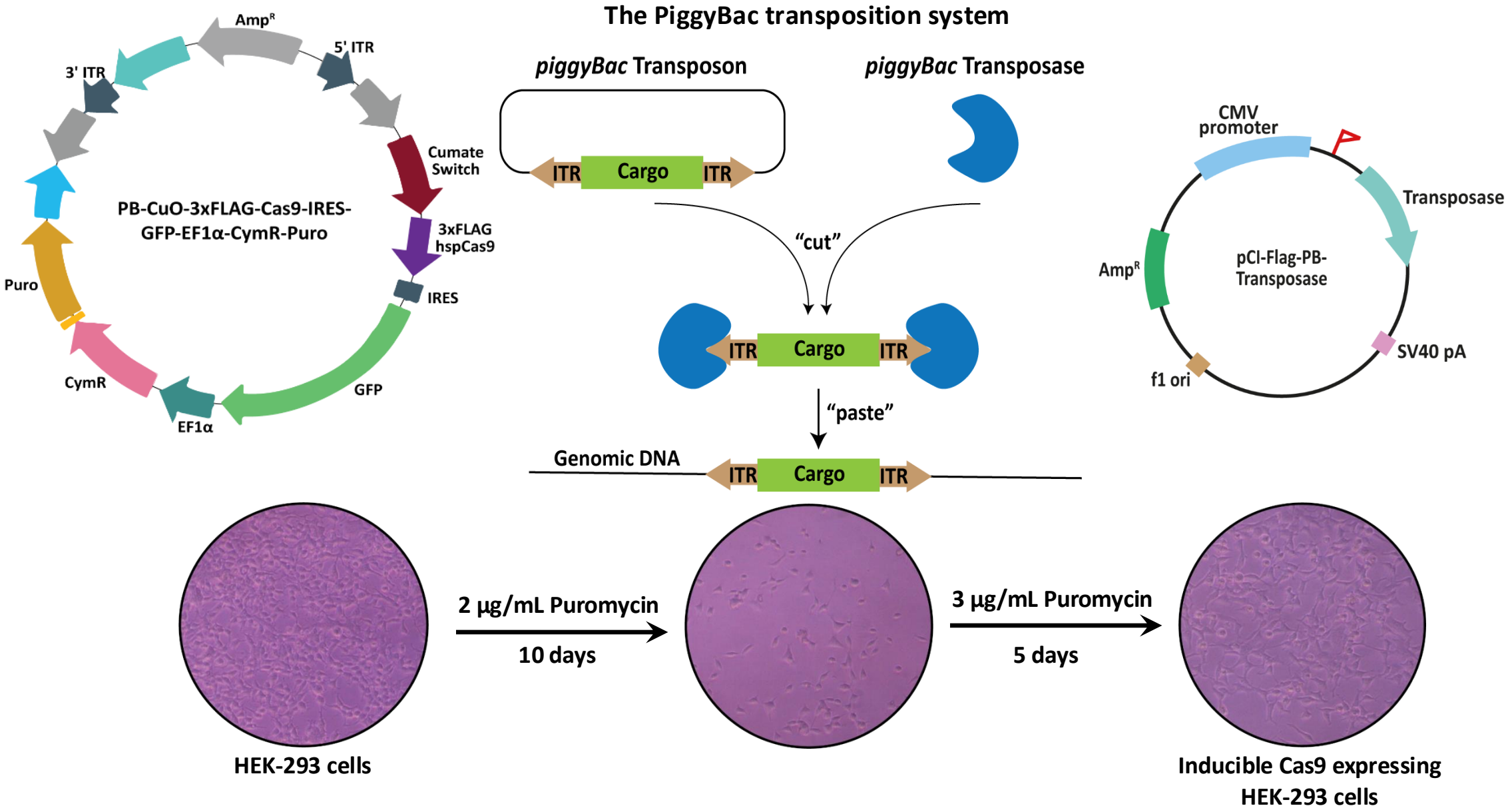
Ligation using T7 Ligase



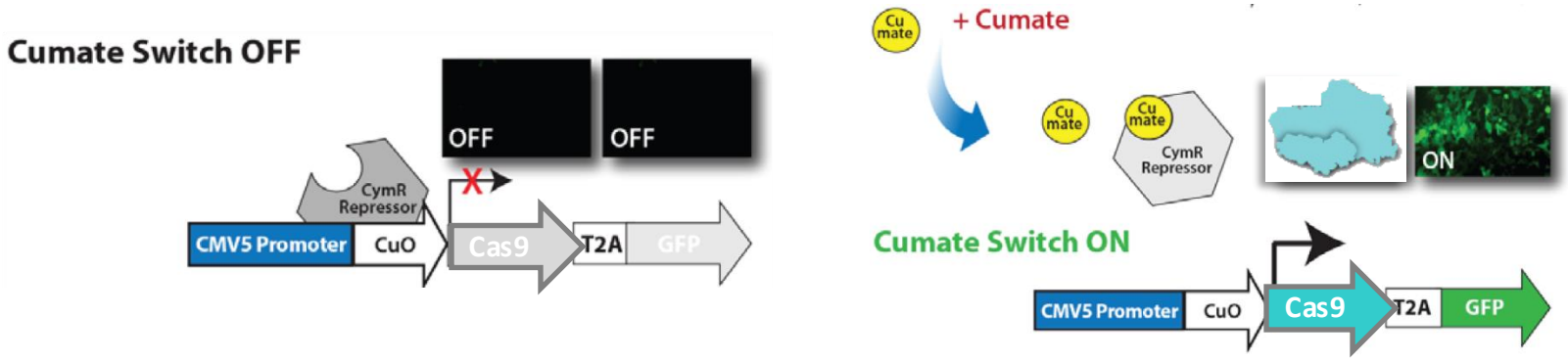
Double digestion with NheI-NotI



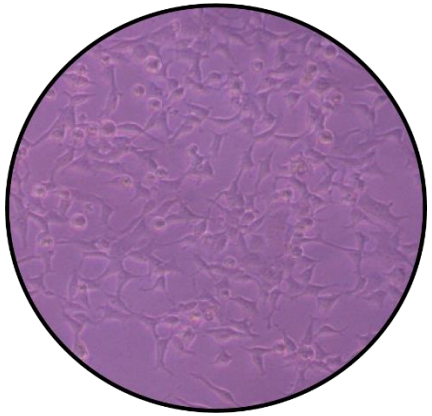
Establishment of stable Cas9-inducible expression system



Establishment of stable Cas9-inducible expression system



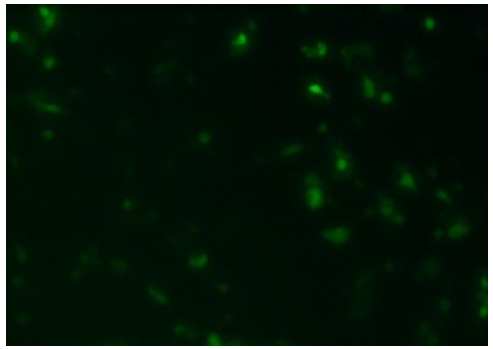
Confirmation of stable integration of *hSpCas9*



Inducible Cas9 expressing HEK-293 cells

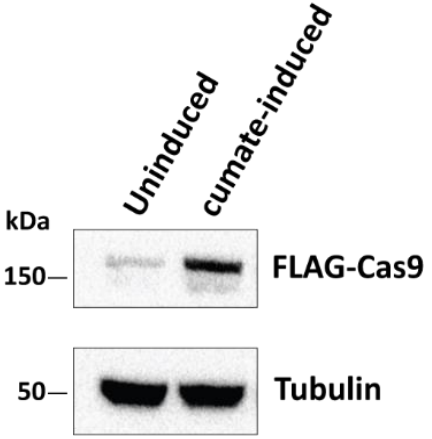
300 µg Cumate

Uninduced

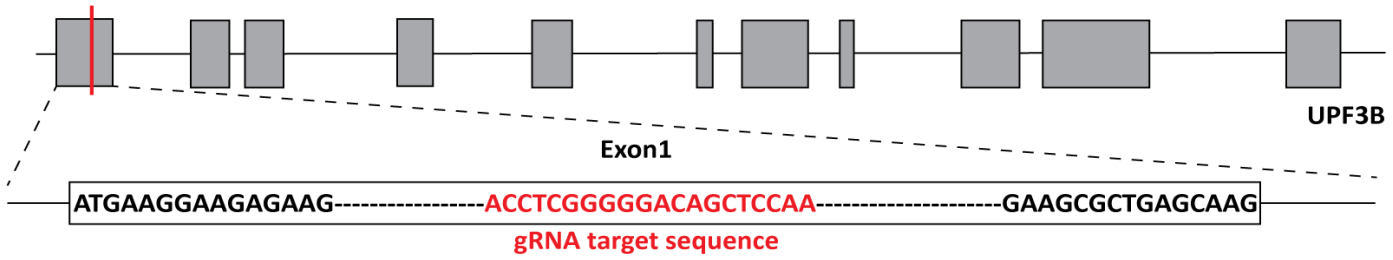


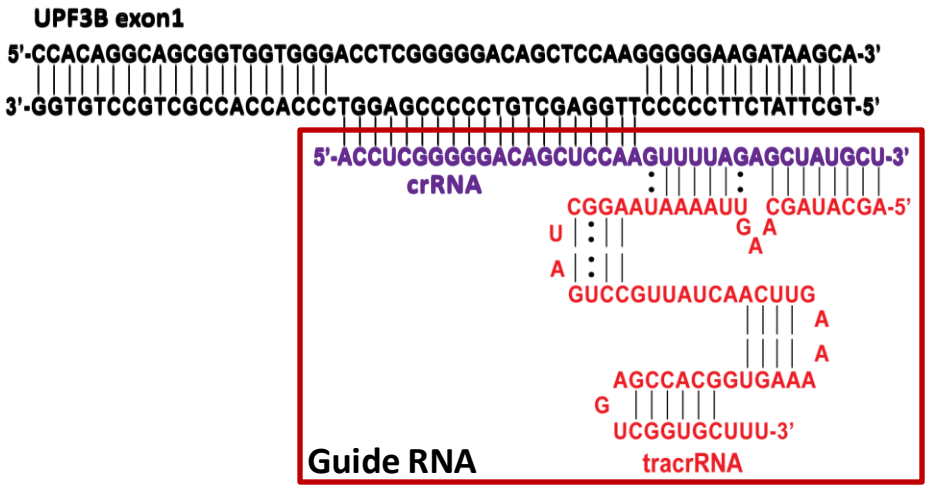
Cumate-induced

Western blotting



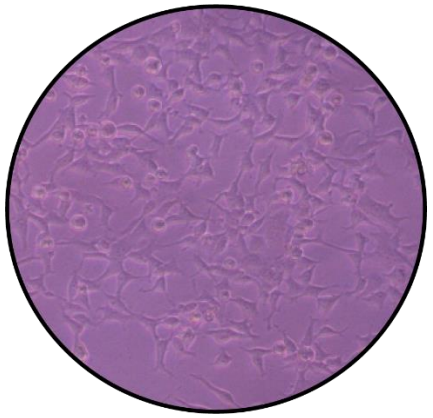
Single gRNA selection and transfection to Cas9





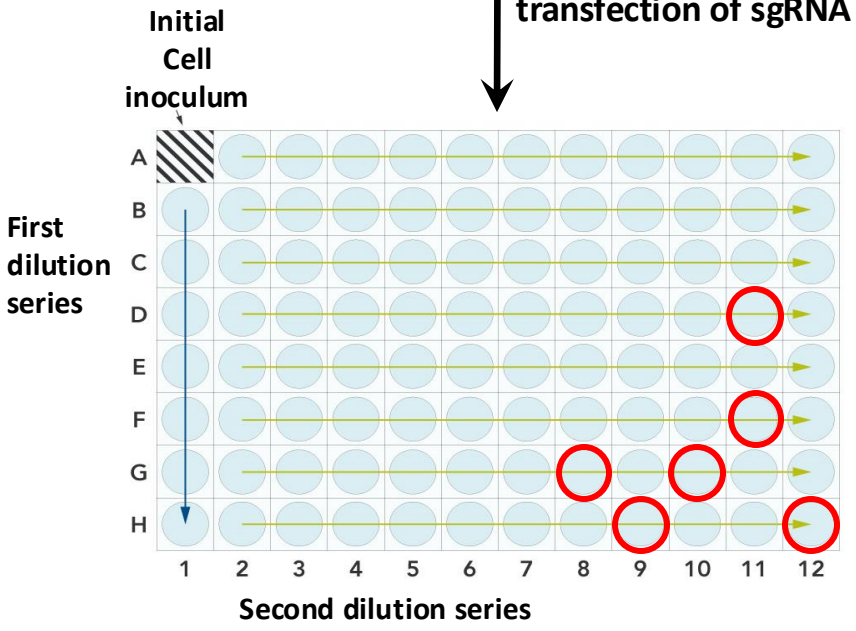
Transfection of 30 nM single-guide RNA

24 hours post-induction

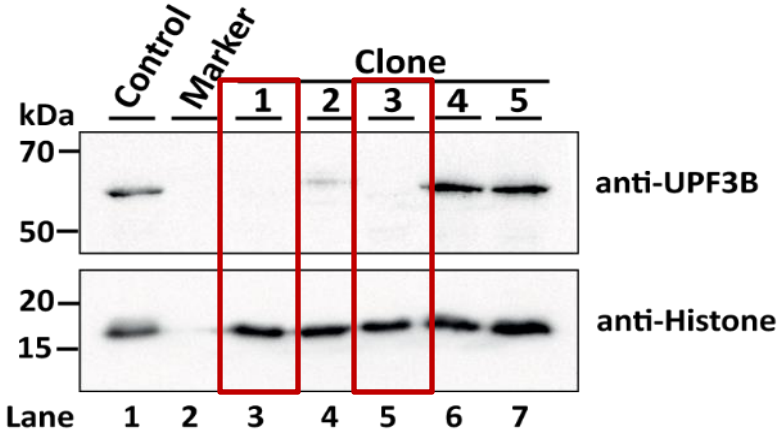


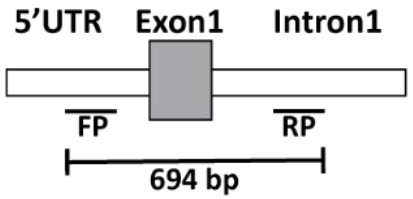
Induction of Cas9 expression using Cumate

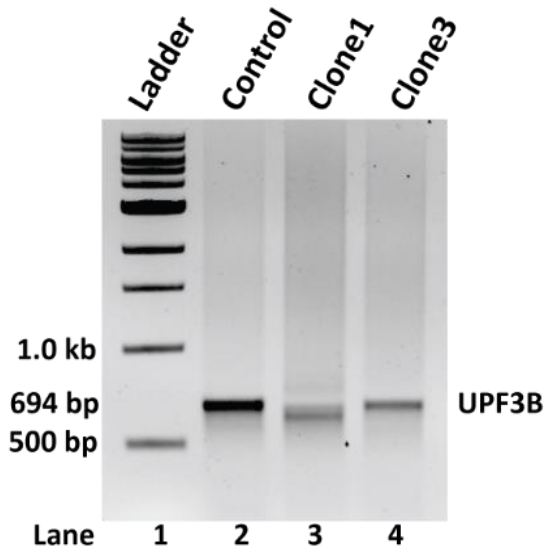
72 hours post-transfection of sgRNA

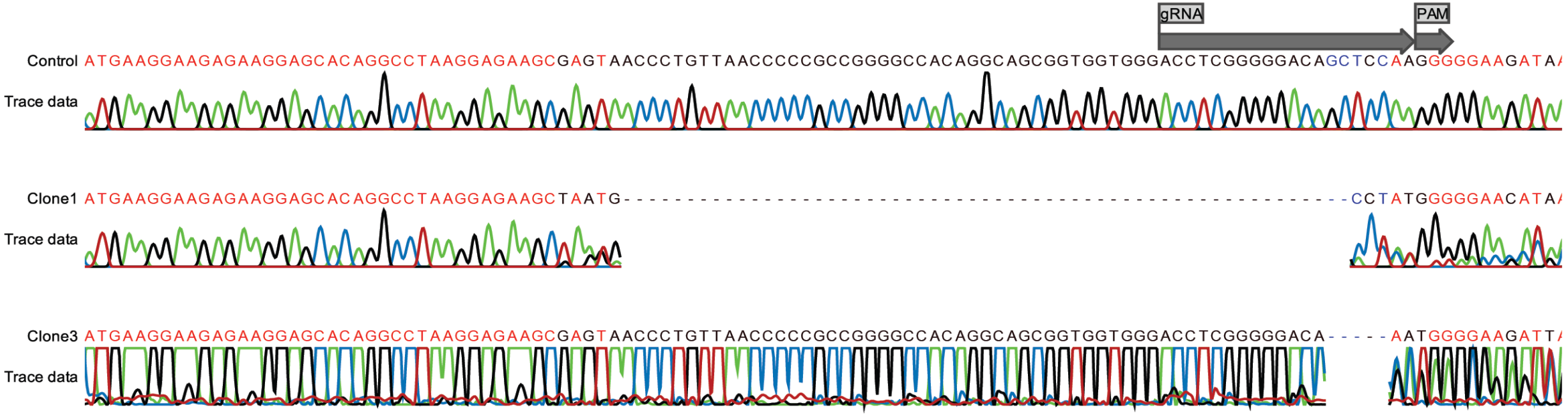


Confirmation of UPF3B-KO





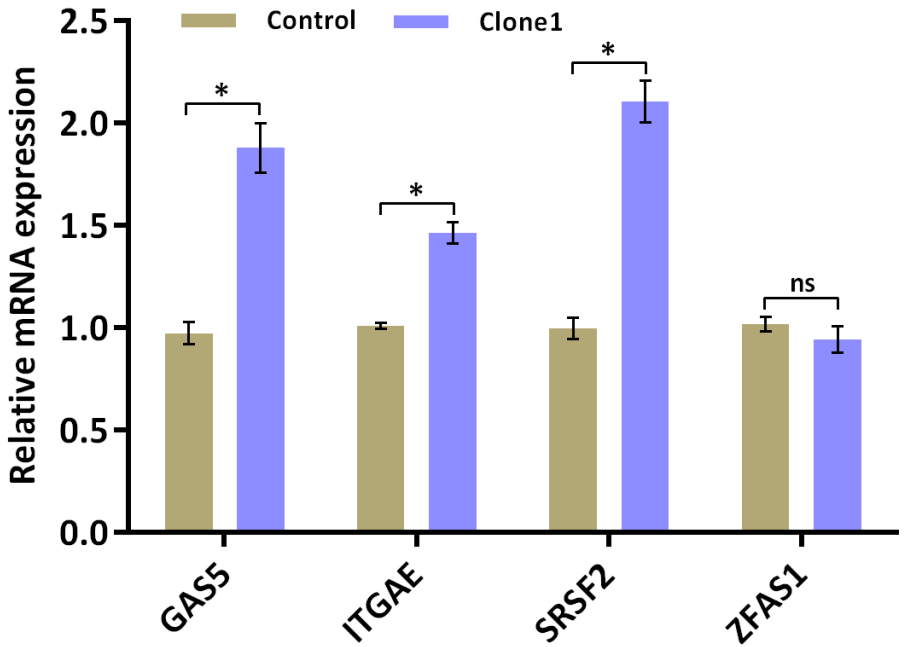


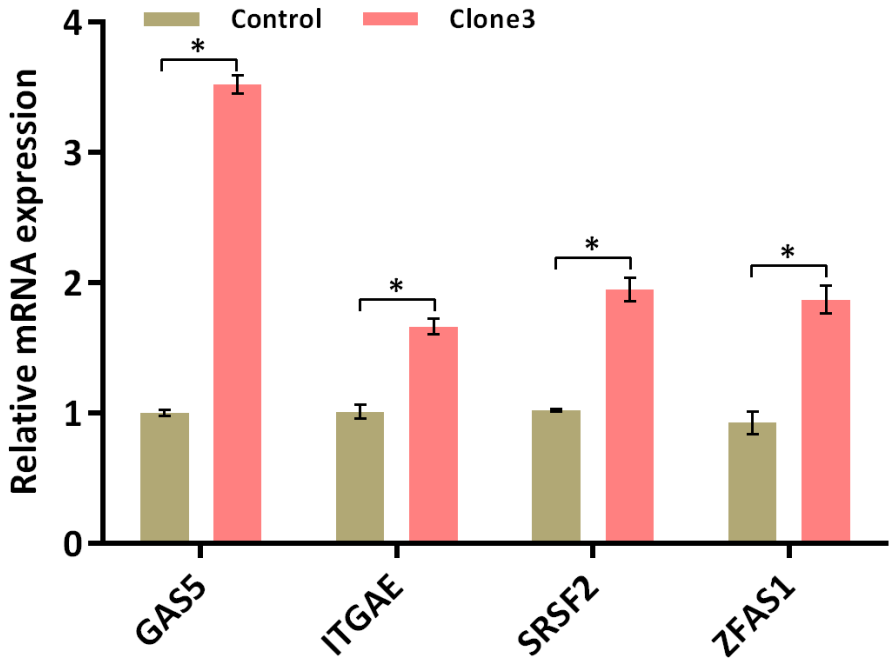


Impact of UPF3B-KO on NMD impairment

Canonical NMD substrates

- GAS5 – Long non-coding RNA, NMD Target
- ITGAE – Integrin subunit alpha E, Retained intron, NMD Target
- SRSF2 – Serine and Arginine rich Splicing Factor 2
- ZFAS1 – Long non-coding RNA, NMD Target





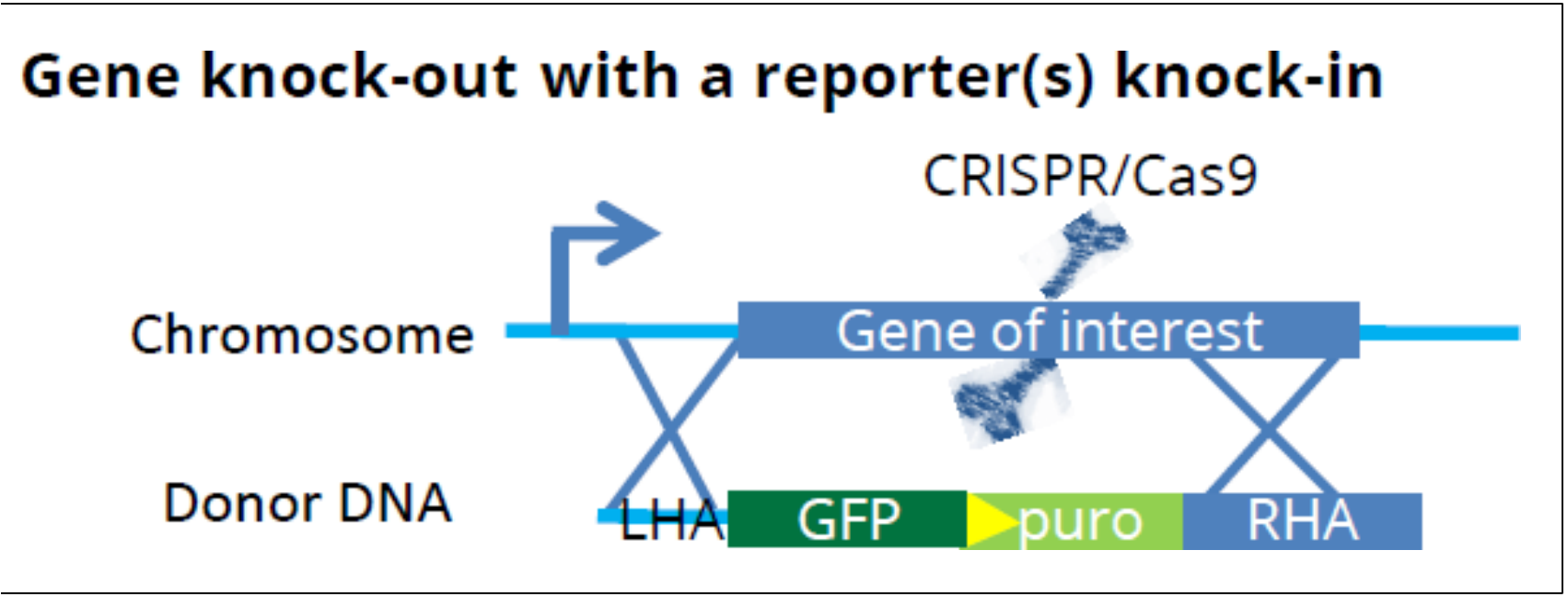
Scientists in China say they are the first to use gene editing to produce customized dogs. They created a beagle with double the amount of muscle mass by **deleting a gene called myostatin**.

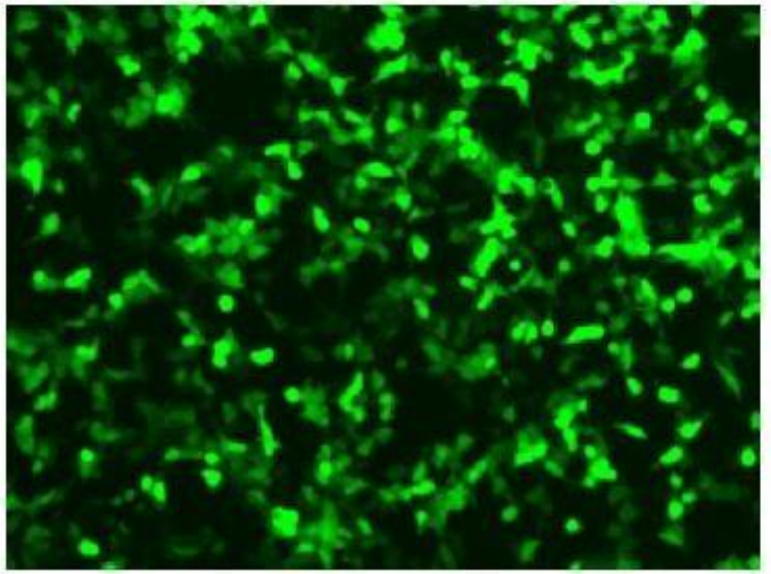
The dogs have "**more muscles and are expected to have stronger running ability, which is good for hunting, police (military) applications**," Liangxue Lai, a researcher with the Key Laboratory of Regenerative Biology at the Guangzhou Institutes of Biomedicine and Health.

Myostatin is a protein produced and released by myocytes that acts on muscle cells to inhibit myogenesis: muscle cell growth and differentiation.

Applications of genome editing technologies

❑ 2. Gene knock-out (with a reporter knock-in)

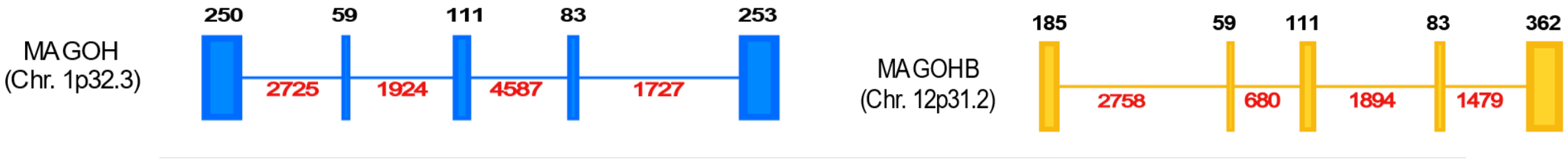


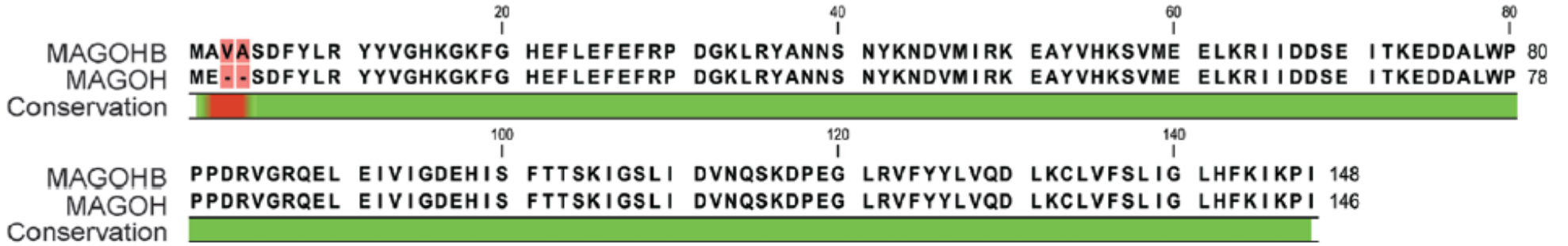


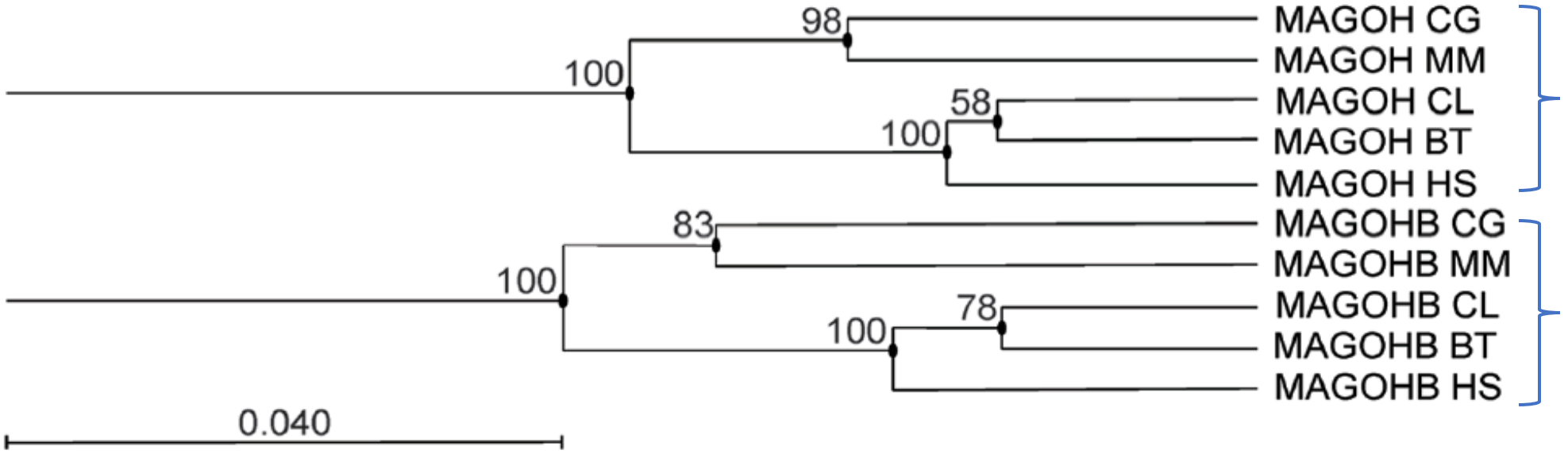
pCas-Guide-EF1a-GFP was transfected into HEK293 cells. The fluorescent picture shows the expression of GFP.

- These nucleases create site-specific **double-strand breaks** (DSBs) at desired locations in the genome.
- The induced double-strand breaks are repaired through **Homology Directed Repair (HDR) – homologous recombination**, resulting in targeted mutations ('edits').

MAGOH PARALOGS – MAGOH and MAGOHB

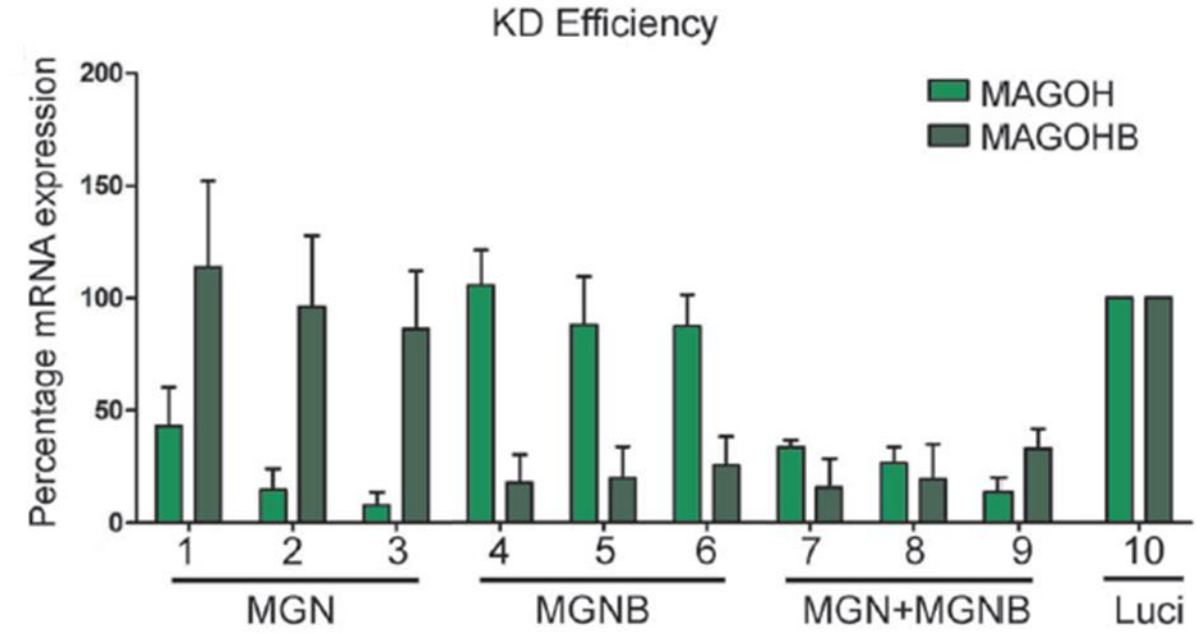


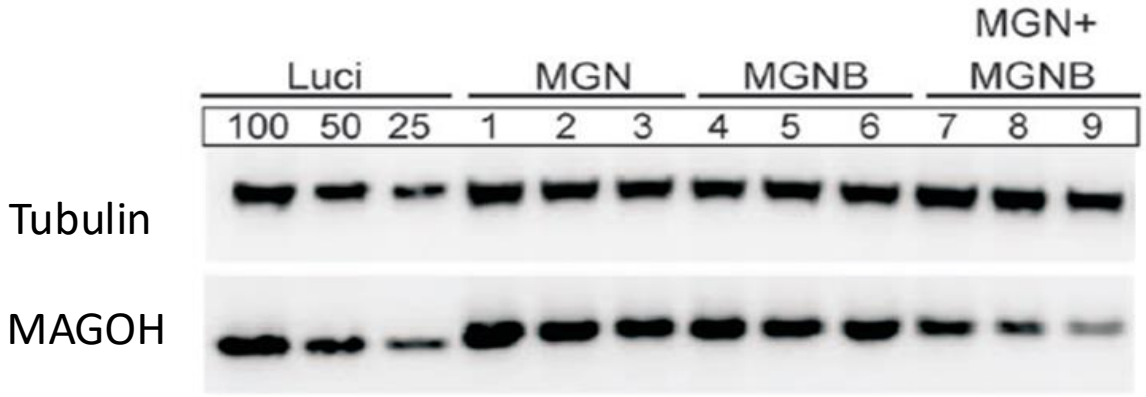




Singh et al. 2013

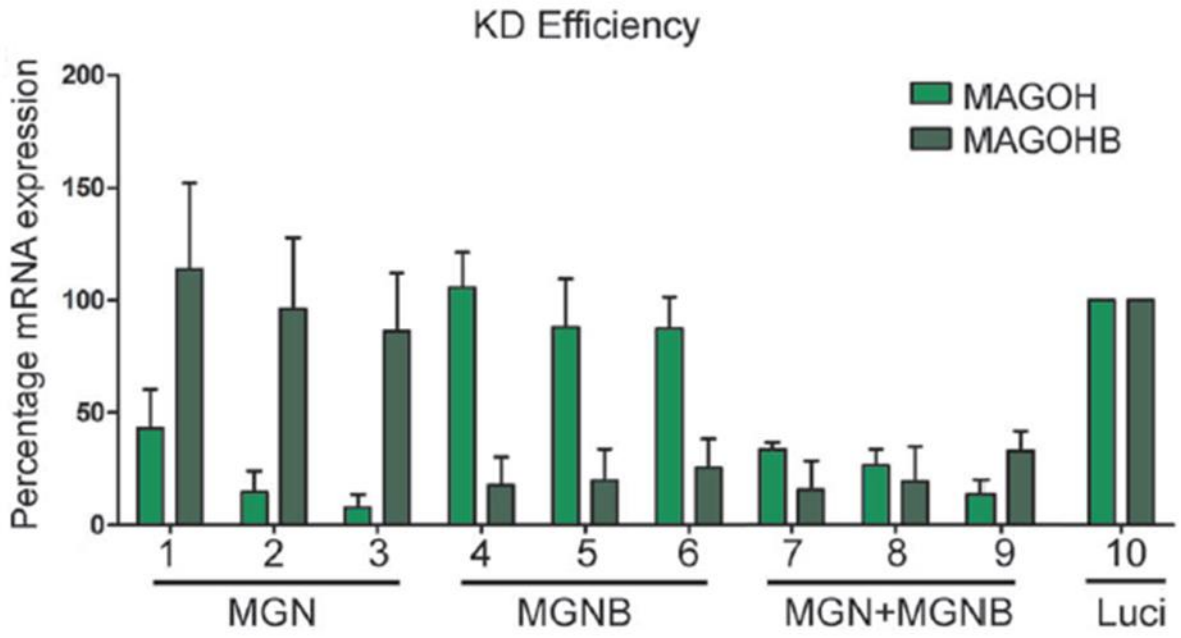
MAGOH PARALOGS – MAGOH and MAGOHB



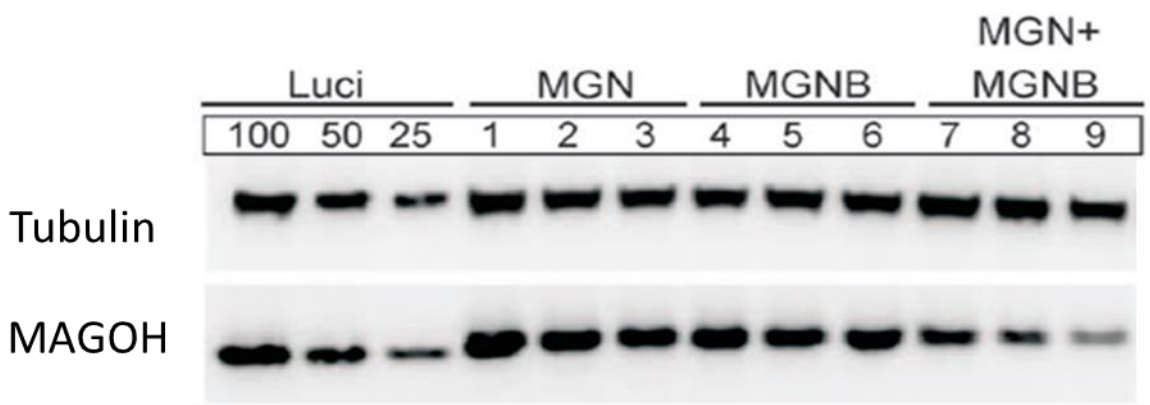


Singh et al. 2013

MAGOH PARALOGS – MAGOH and MAGOHB

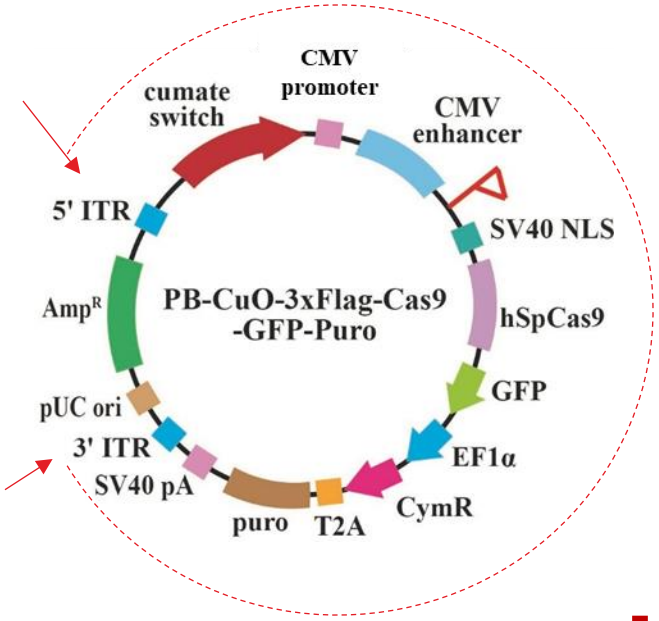


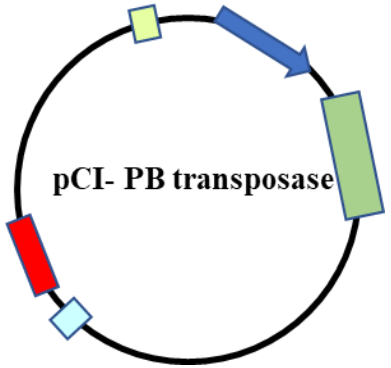
ence hinders
ralogs



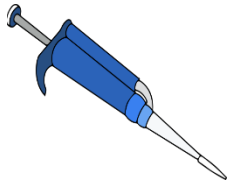
Singh et al. 2013

Generation of stable Cas9 expressing cells

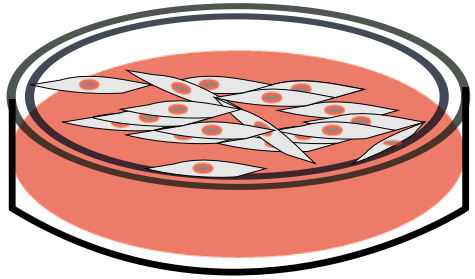




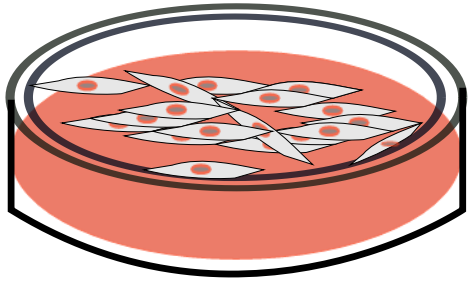
Genomic Integration



Plasmid transfection



Puromycin selection
for 2-3 weeks

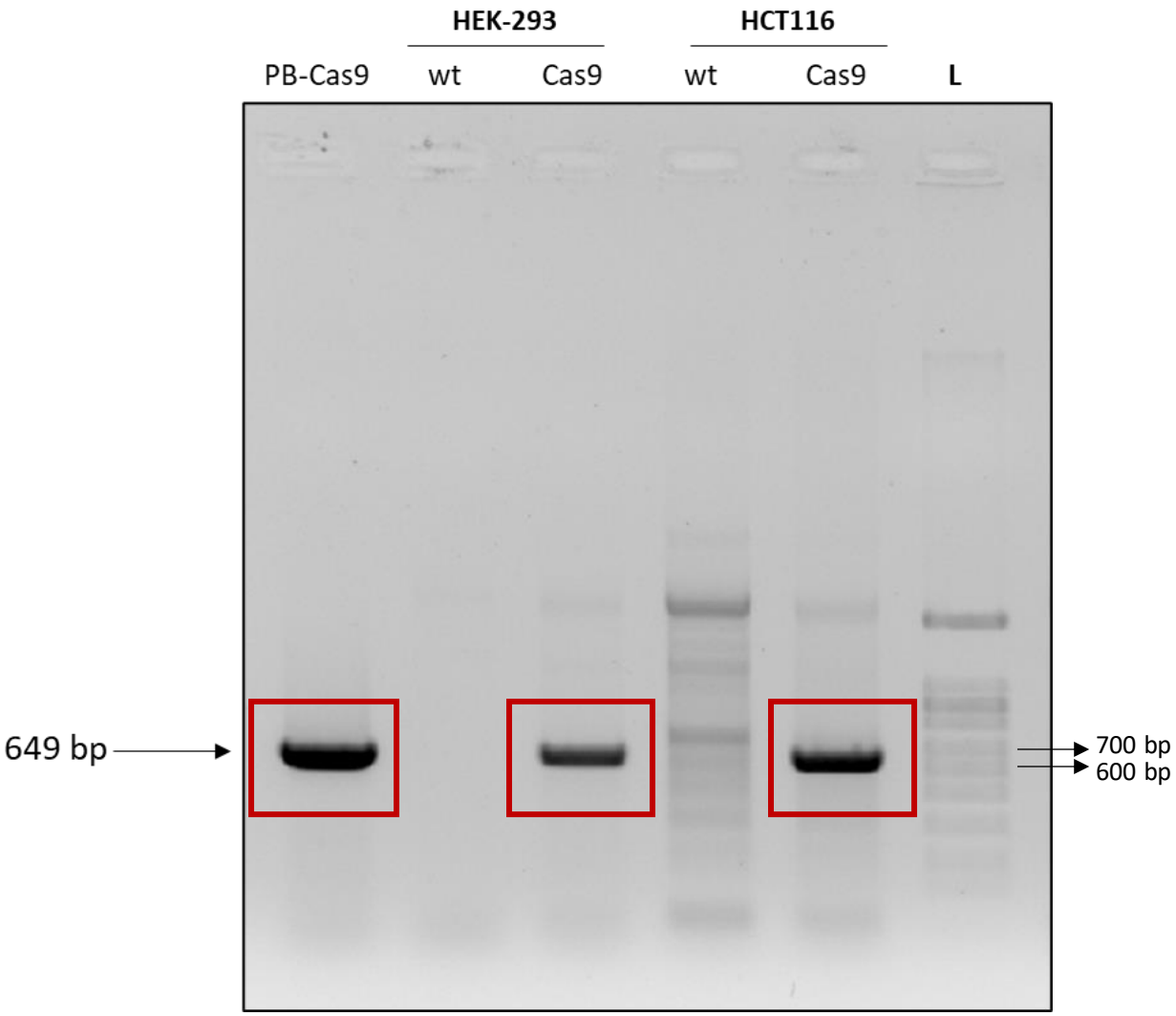


Confirmation of stable cells

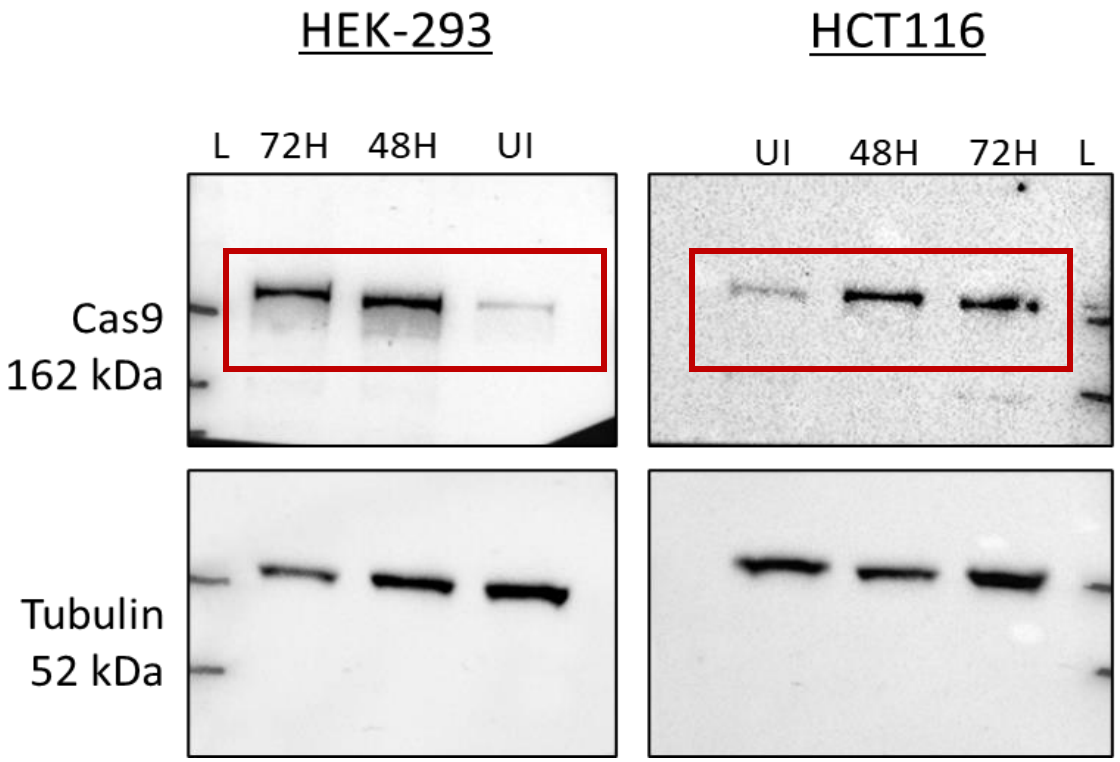
DNA isolation

Western Blotting

Validation of stably integrated Cas9 cells

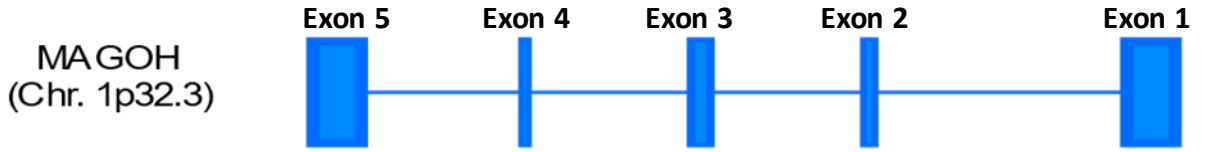


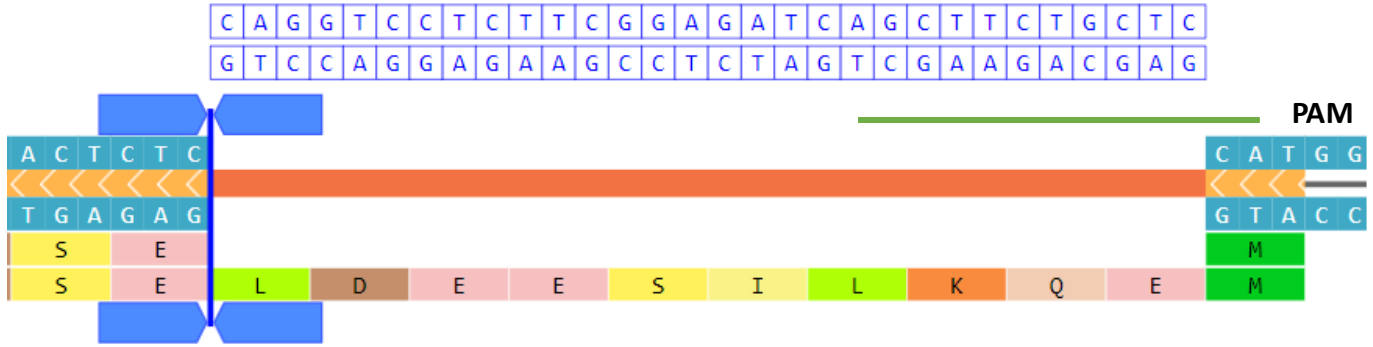
Genomic integration of Cas9



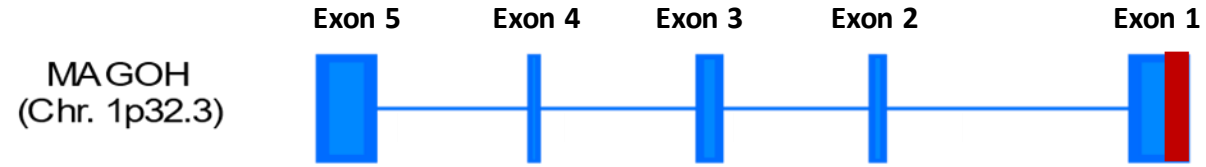
Inducible expression of Cas9 protein in stable cells

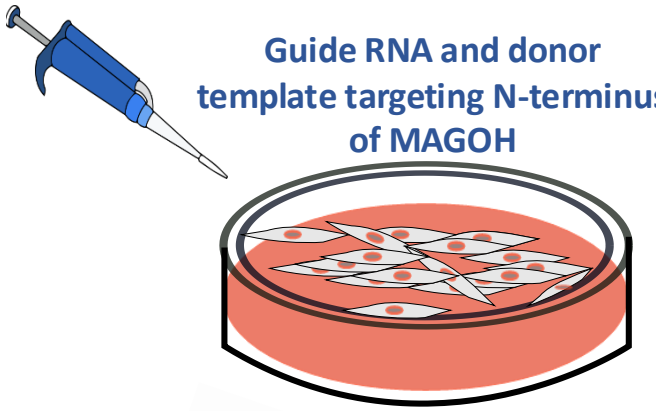
Objective 1 CRISPR mediated endogenous tagging of MAGOH and MAGOHB in cell lines





Myc-tag

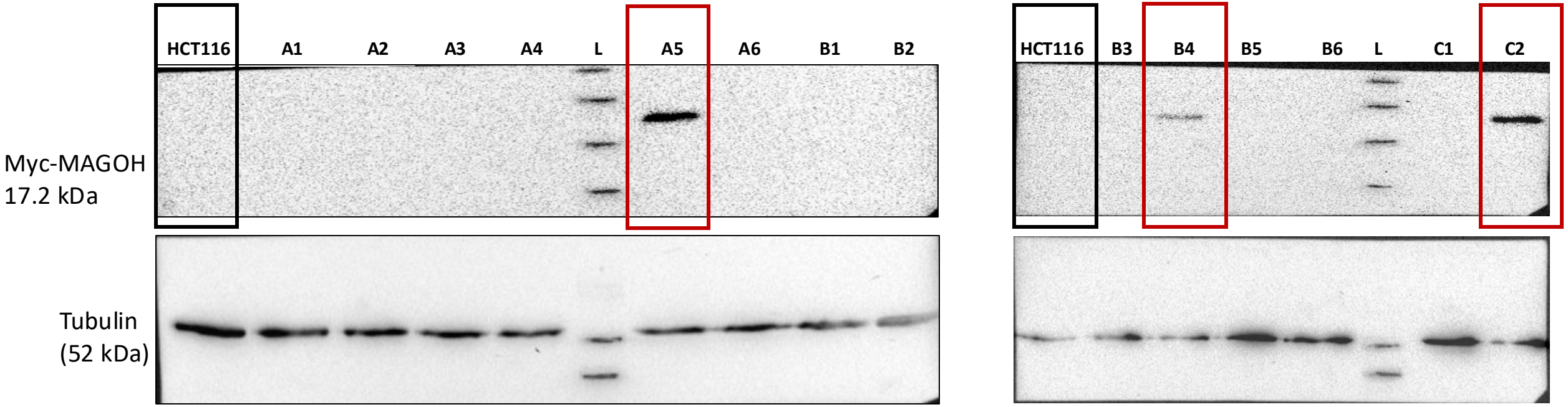


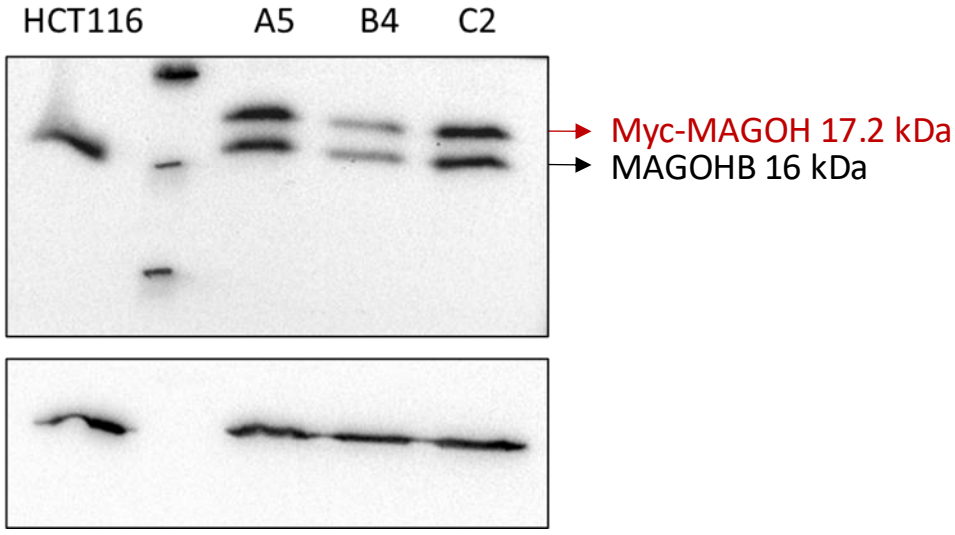


72 h ↓ Single cell dilution

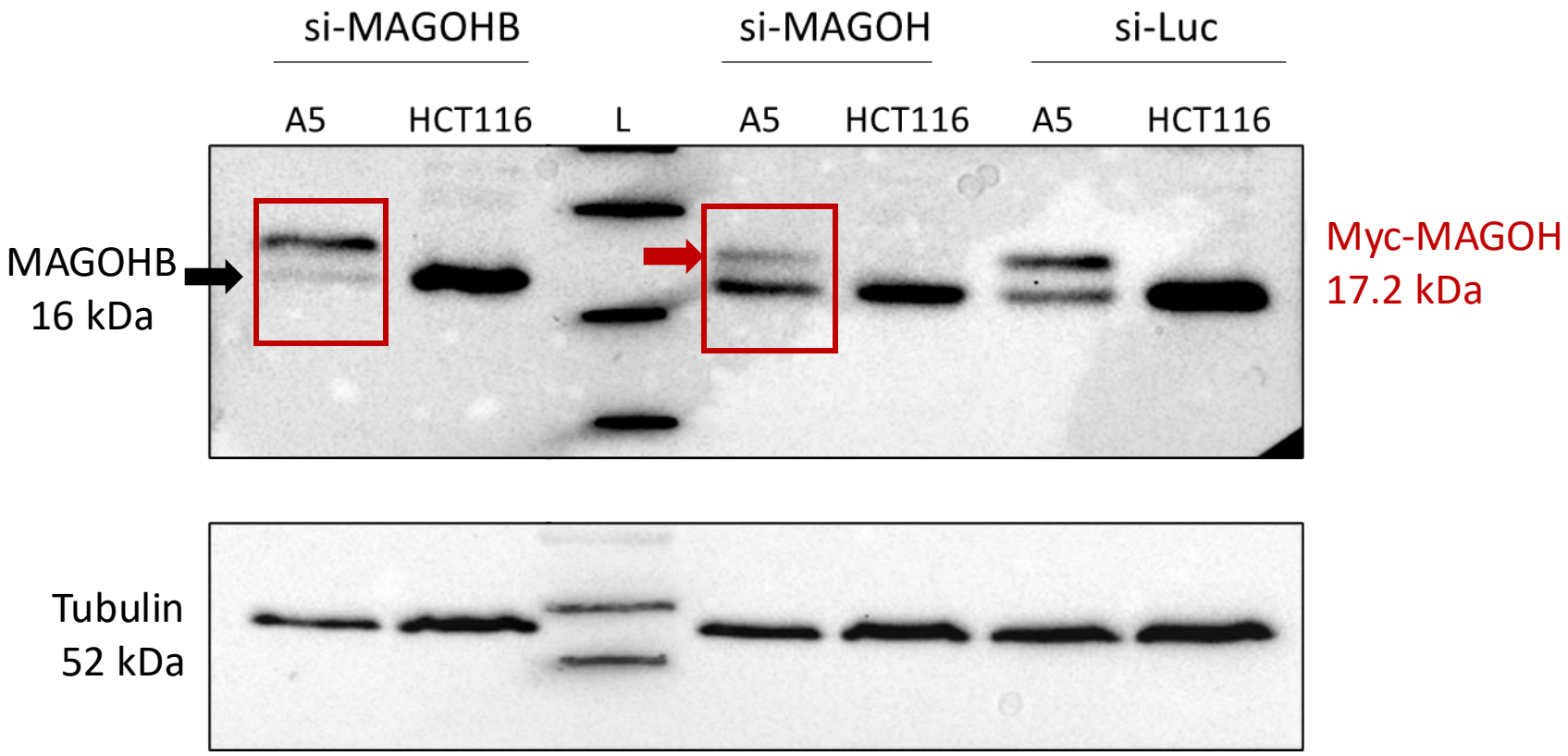
14 single cell clones ↓ Clonal expansion

Expression of Myc-MAGOH





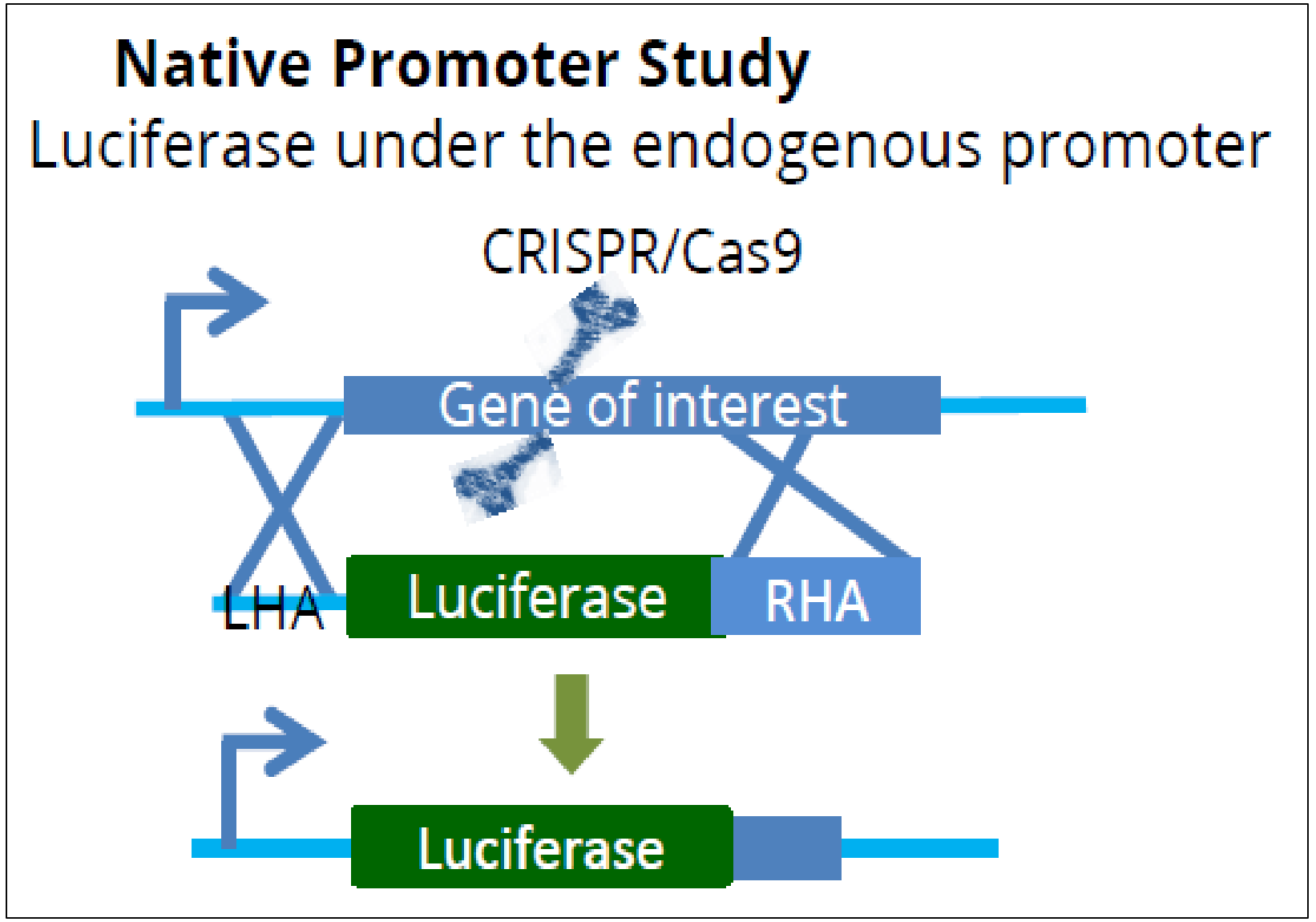
Confirmation of MAGOH specific tagging in HCT116 cells



Endogenously tagging MAGOH with Myc enables us to distinguish MAGOH and MAGOHB proteins

Applications of genome editing technologies

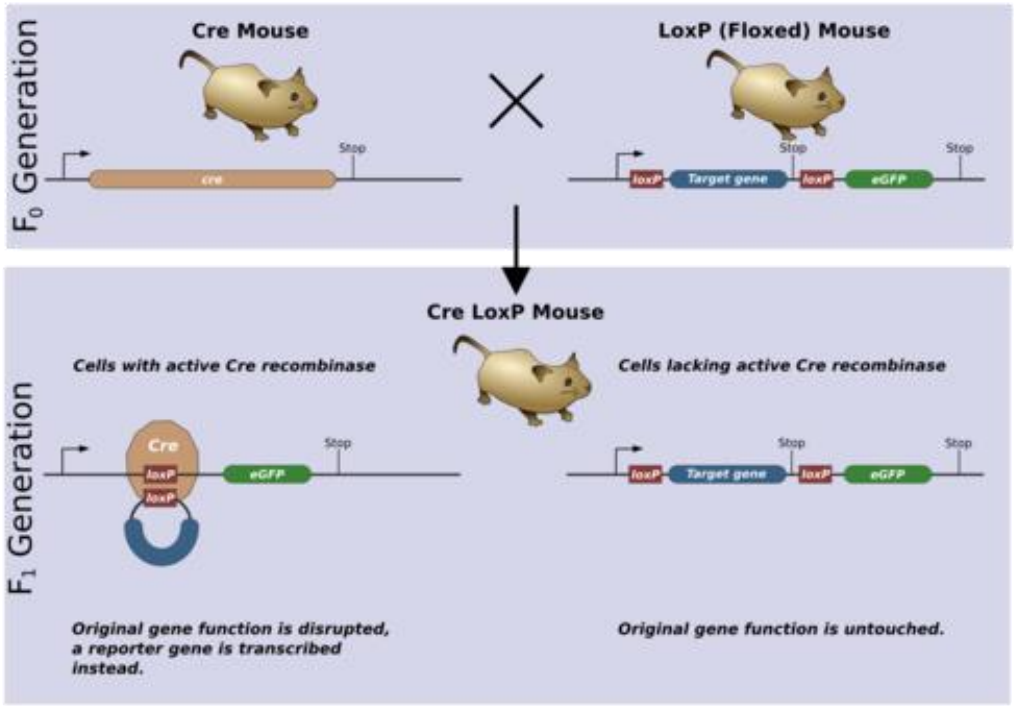
❑ 3. Native promoter study

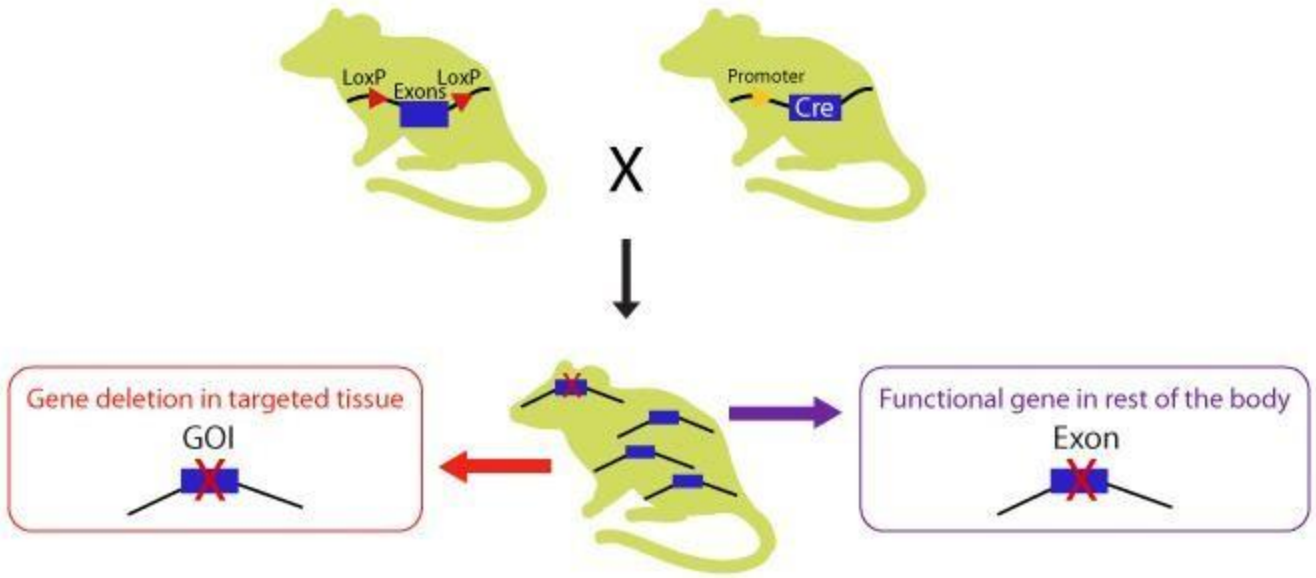


Applications of genome editing technologies

❑ 4. Conditional knockout

(For essential genes or tissue specific study inserting LoxP sites around the exon(s) to be knocked out)



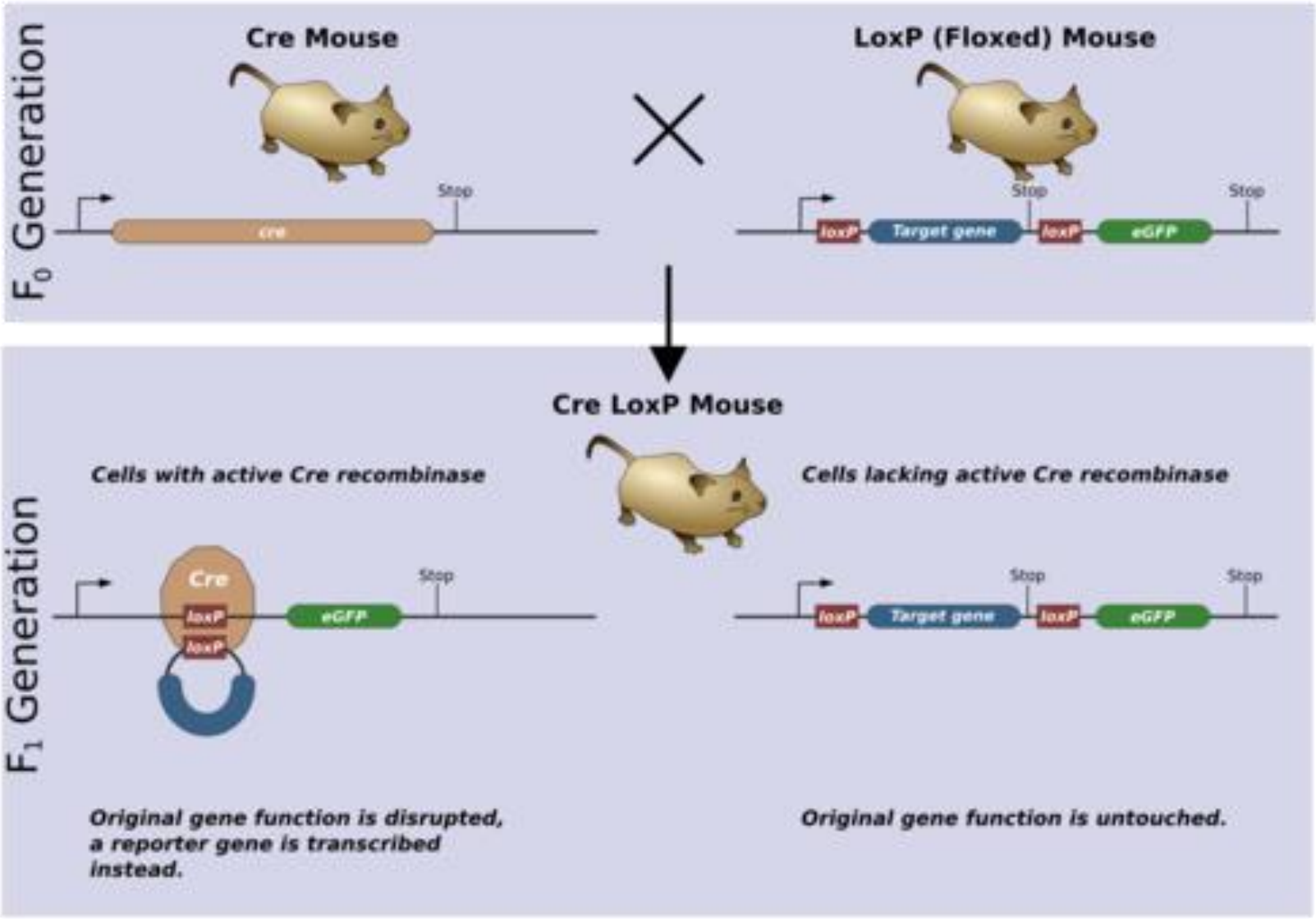


- ❑ Cre Recombinase is a Type I topoisomerase from bacteriophage P1 that catalyzes the site-specific recombination of DNA between loxP sites.
- ❑ Cre-Lox is a powerful tool for genetic manipulation in vivo because it allows for excellent spatial and temporal control of gene expression.
- ❑ This is invaluable when working with animal models, where unchecked gene expression or complete gene knockout may be detrimental or lethal.

Applications of genome editing technologies

❑ 4. Conditional knockout

(For essential genes or tissue specific study inserting LoxP sites around the exon(s) to be knocked out)

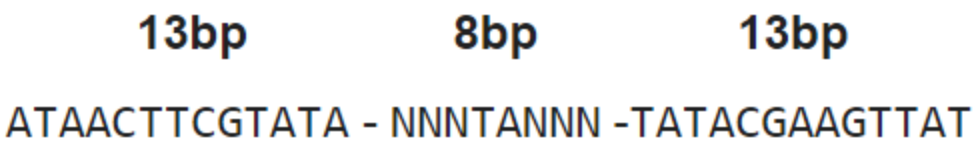


Applications of genome editing technologies

❑ 4. Conditional knockout

(*loxP*)

- ***loxP*** **(locus of X-over P1) is a site on the bacteriophage P1 consisting of 34 bp. The site includes an asymmetric 8 bp sequence, variable except for the middle two bases, in between two sets of symmetric, 13 bp sequences.**



- **The 13 bp sequences are palindromic but the 8 bp spacer is not, thus giving the loxP sequence a certain direction.**
- **If the two loxP sites are in the same orientation, the floxed sequence (sequence flanked by two loxP sites) is excised; however if the two loxP sites are in the opposite orientation, the floxed sequence is inverted. If there exists a floxed donor sequence, the donor sequence can be swapped with the original sequence.**
- **This technique is called recombinase-mediated cassette exchange and is a very convenient and time-saving way for genetic manipulation.**

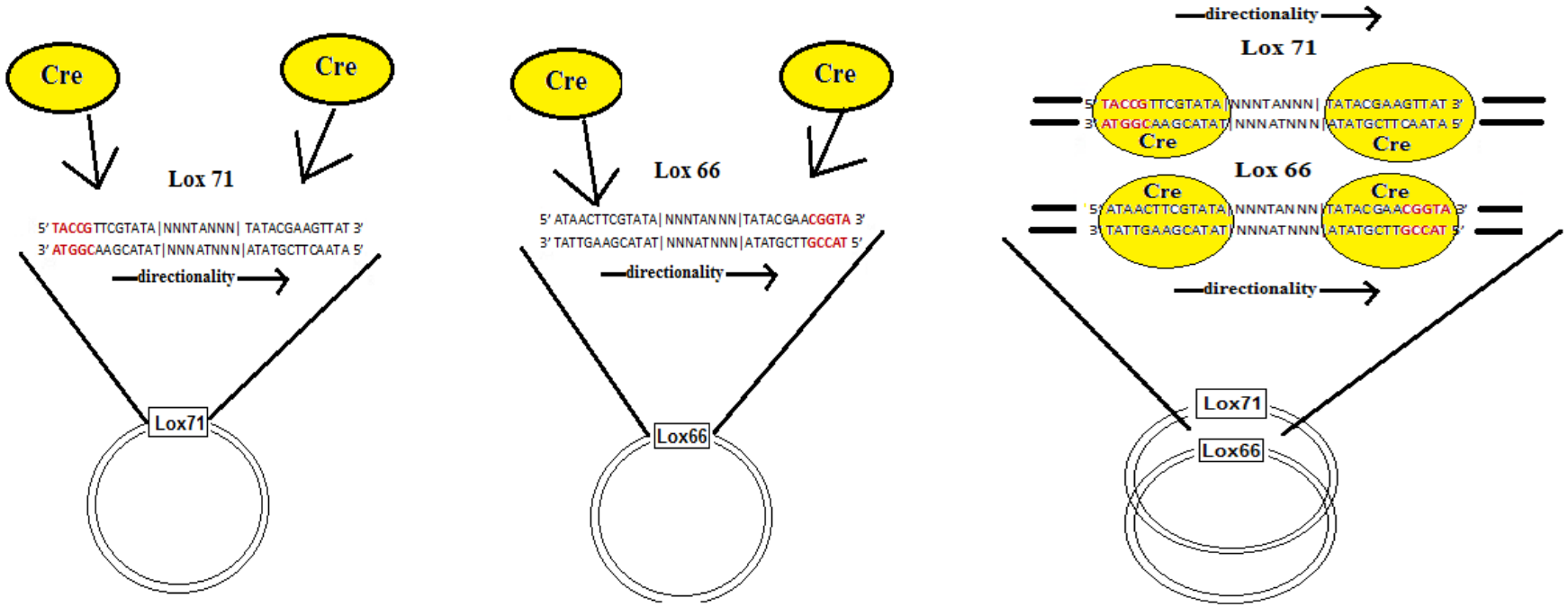
Example Alternate loxP Sites[24][25]

Name	13bp Recognition Region	8bp Spacer Region	13bp Recognition Region
Wild-Type	ATAACTTCGTATA	ATGTATGC	TATACGAAGTTAT
lox 511	ATAACTTCGTATA	ATGTATaC	TATACGAAGTTAT
lox 5171	ATAACTTCGTATA	ATGTgTaC	TATACGAAGTTAT
lox 2272	ATAACTTCGTATA	AaGTATcC	TATACGAAGTTAT
M2	ATAACTTCGTATA	AgaaAcca	TATACGAAGTTAT
M3	ATAACTTCGTATA	taaTACCA	TATACGAAGTTAT
M7	ATAACTTCGTATA	AgaTAGAA	TATACGAAGTTAT
M11	ATAACTTCGTATA	cgaTAcca	TATACGAAGTTAT
lox 71	TACCGTTCGTATA	NNNTANNN	TATACGAAGTTAT
lox 66	ATAACTTCGTATA	NNNTANNN	TATACGAACGGTA
loxPsym	ATAACTTCGTATA	atgtacat	TATACGAAGTTAT

Applications of genome editing technologies

❑ 4. Conditional knockout

- The Cre protein is a site-specific DNA recombinase that can catalyse the recombination of DNA between specific sites in a DNA molecule. These sites, known as *loxP* sequences, contain specific binding sites for Cre that surround a directional core sequence where recombination can occur.

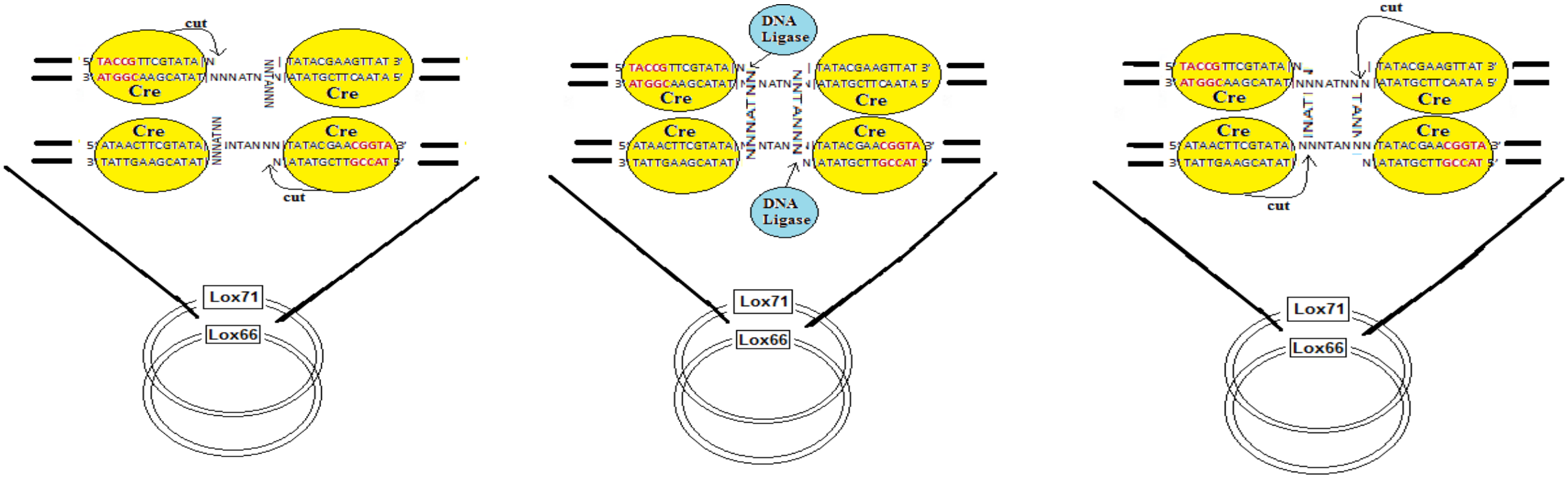


- Cre recombinase proteins bind to the first and last 13 bp regions of a lox site forming a dimer. This dimer then binds to a dimer on another lox site to form a tetramer. Lox sites are directional and the two sites joined by the tetramer are parallel in orientation.

Applications of genome editing technologies

❑ 4. Conditional knockout

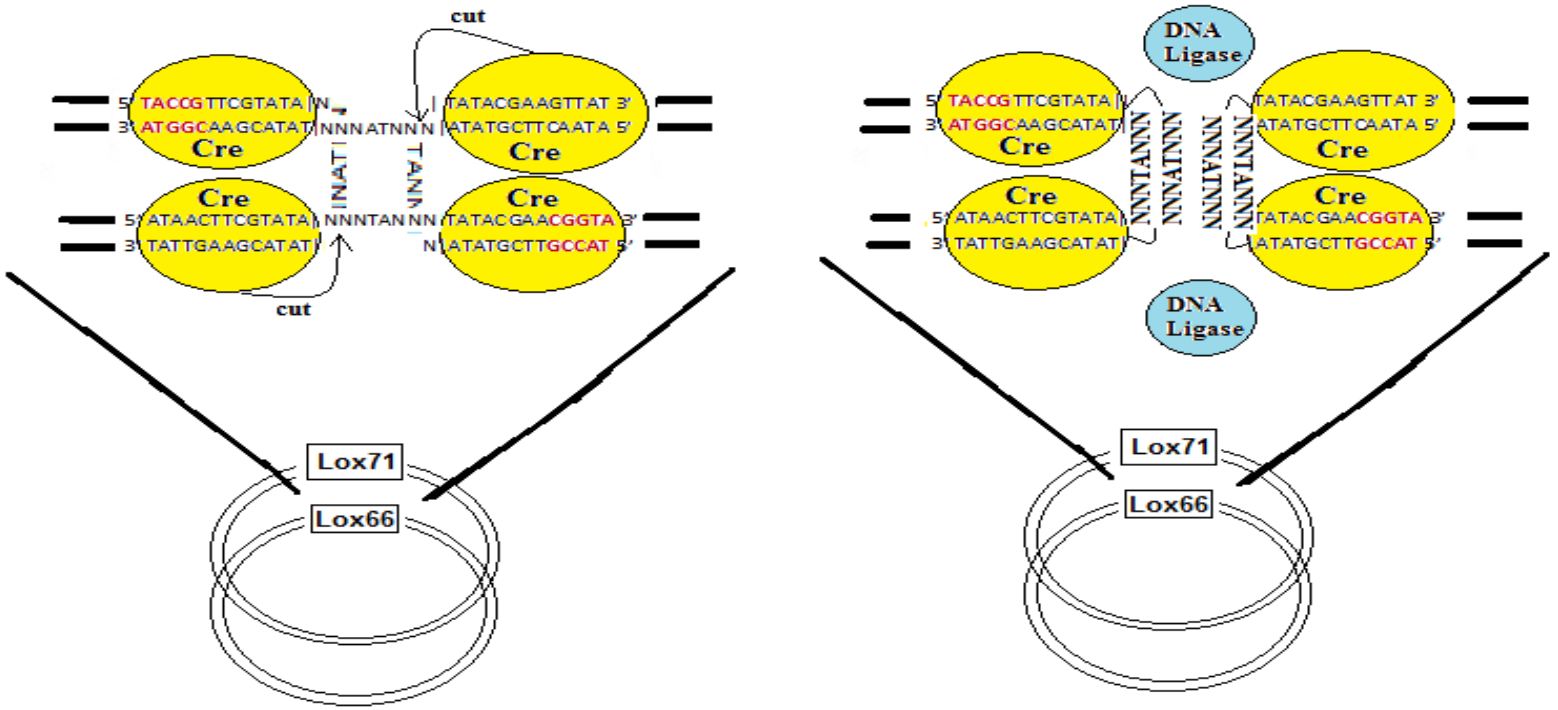
(For essential genes or tissue specific study inserting LoxP sites around the exon(s) to be knocked out)



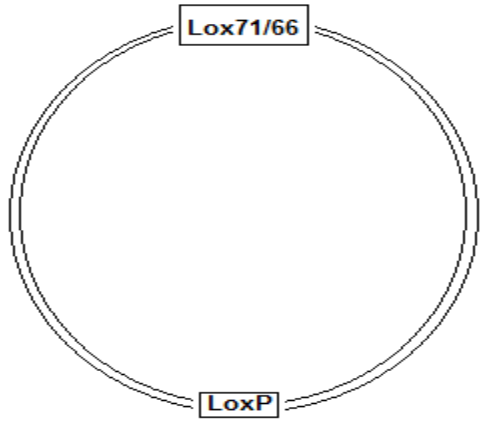
- **The double stranded DNA is cut at both *loxP* sites by the Cre protein. The strands are then rejoined with DNA ligase in a quick and efficient process.**

Applications of genome editing technologies

❑ 4. Conditional knockout



- Two plasmids can be joined using the variant lox sites 71 and 66.



- The double stranded DNA is cut at both *loxP* sites by the Cre protein. The strands are then rejoined with DNA ligase in a quick and efficient process.
- The result of recombination depends on the orientation of the *loxP* sites.
- inverted *loxP* sites will cause an inversion of the intervening DNA, while a direct repeat of *loxP* sites will cause a deletion event. If *loxP* sites are on different chromosomes it is possible for translocation events to be catalysed by Cre induced recombination.

Applications of genome editing technologies

❑ 4. Conditional knockout

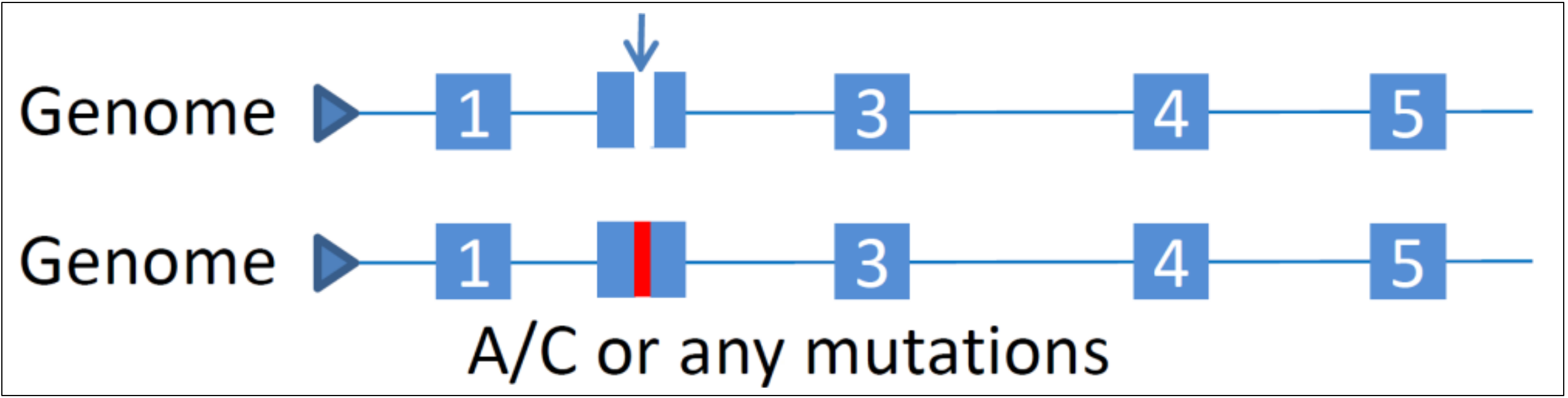
(For essential genes or tissue specific study inserting LoxP sites around the exon(s) to be knocked out)

- ❑ If a vital gene is knocked out it can prove lethal to the organism.
- ❑ In order to study the function of these genes site specific recombinases (SSR) were used.
- ❑ The two most common types are the Cre-LoxP and Flp-FRT systems.
- ❑ Cre recombinase is an enzyme that removes DNA by homologous recombination between binding sequences known as Lox-P sites.
- ❑ The Flip-FRT system operates in a similar way, with the Flp recombinase recognising FRT sequences.
- ❑ By crossing an organism containing the recombinase sites flanking the gene of interest with an organism that express the SSR under control of tissue specific promoters, it is possible to knock out or switch on genes only in certain cells.
- ❑ These techniques were also used to remove marker genes from transgenic animals.
- ❑ Further modifications of these systems allowed researchers to induce recombination only under certain conditions, allowing genes to be knocked out or expressed at desired times or stages of development

Applications of genome editing technologies

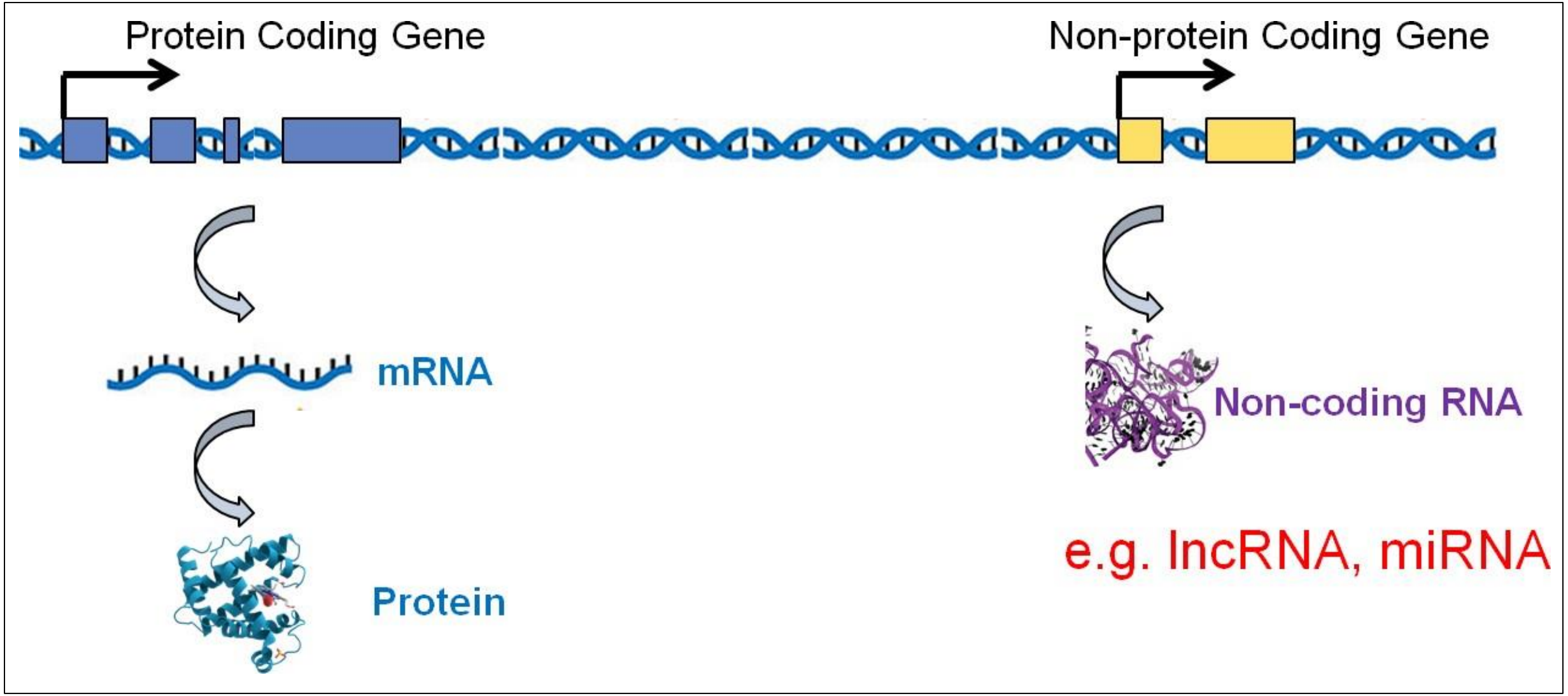
❑ 5. Specific mutations

(1) Desired SNP introduction or correction
(2) Desired insertions/deletions
(3) Tagging the endogenous genes



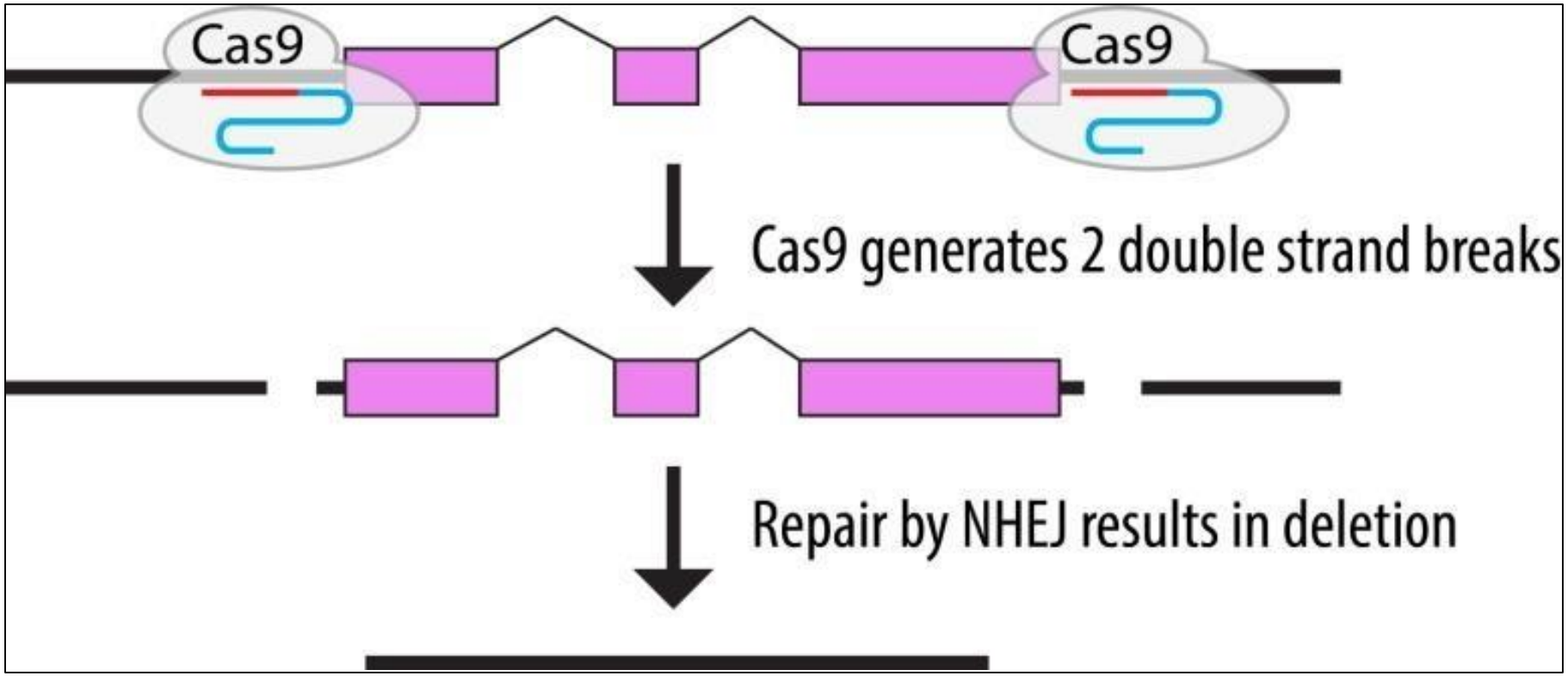
Applications of genome editing technologies

❑ 6. Non-protein Coding Gene disruption



Applications of genome editing technologies

❑ 7. Large chromosomal deletions



CRISPR/Cas9 system has been shown to be very effective in creating large chromosomal deletions, which is useful for genetic analysis of chromosomal fragments, functional study of gene clusters in biological processes, and so on.

Mol Ther. 2017 Aug 2;25(8):1736-1738. doi: 10.1016/j.ymthe.2017.05.021. Epub 2017 Jun 17.

Targeted Deletion of an Entire Chromosome Using CRISPR/Cas9

Fatwa Adikusuma [1], Nicole Williams [2], Frank Grutzner [3], James Hughes [2], Paul Thomas [4]

Affiliations + expand

PMID: 28633863 PMCID: PMC5542798 DOI: 10.1016/j.ymthe.2017.05.021

Free PMC article

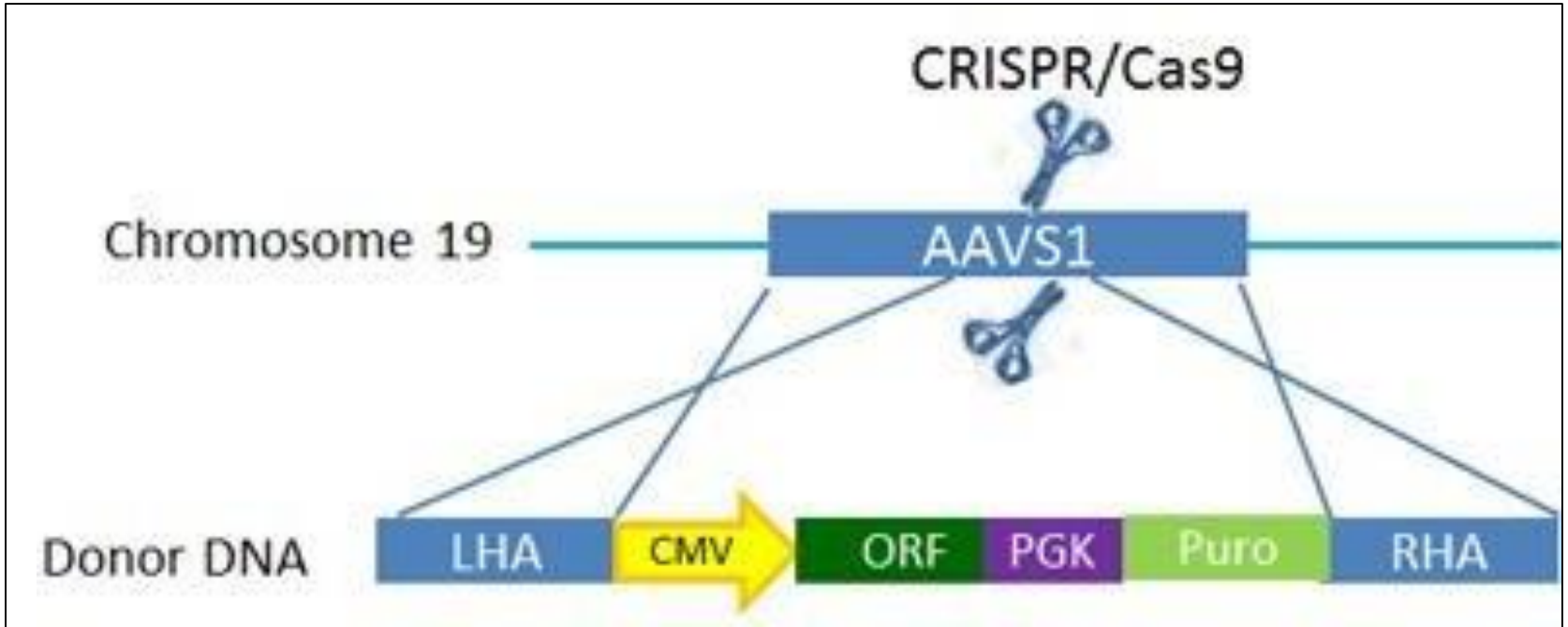
Abstract

CRISPR/Cas9 genome editing can facilitate efficient deletion of genomic region, but it has not been used to delete an entire chromosome. Here, Adikusuma et al. show proof-of-concept for efficient CRISPR-mediated selective chromosome deletion by removing the centromere or shredding the chromosome arm in mouse embryonic stem cells and zygotes.

Applications of genome editing technologies

❑ 8. Exogenous gene Insertion

- ❑ Adeno-associated virus integration site 1 (AAVS1) in human genome is **a safe harbor** for transgene integration.
- ❑ AAVS1 is a viral integration site that in humans is encoded by the AAVS1 gene located on chromosome 19.
- ❑ A controlled Gene Knock-in e.g. controlled copy number and location.



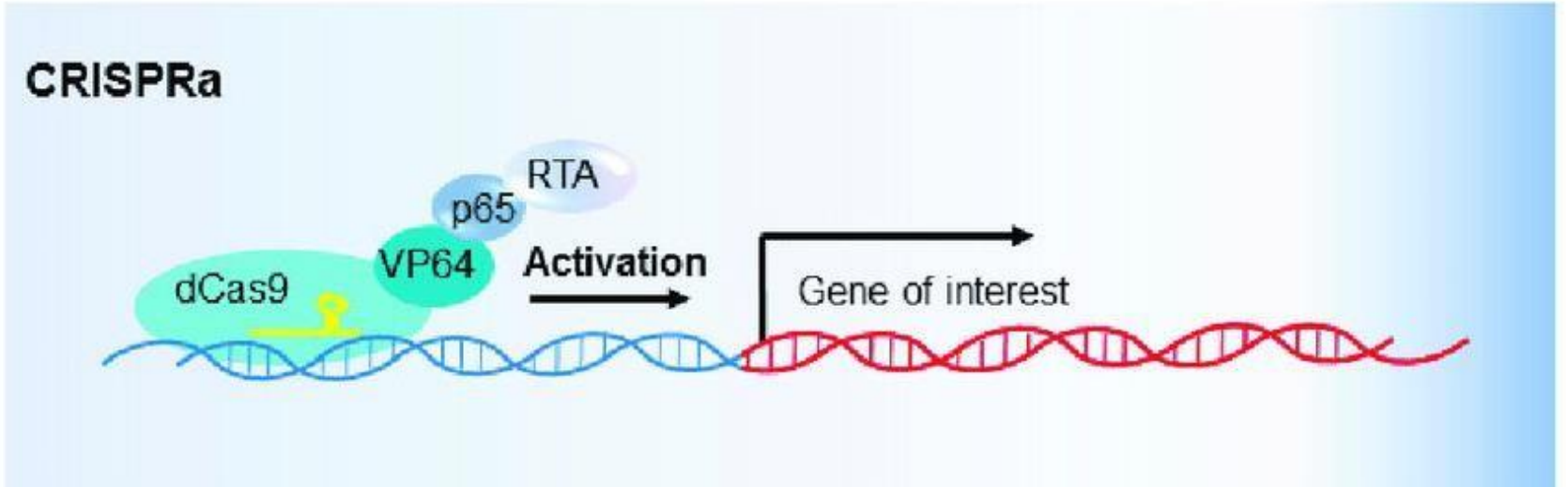
Applications of genome editing technologies

- Gene regulation (transcriptional activation or repression)

Applications of genome editing technologies

❑ 8. CRISPRa (CRISPR activation)

- ❑ The Vp64-p65(RelA)-Rta(regulator of transcription activation), or **VPR**, dCas9 activator was created by modifying an existing dCas9 activator, in which a Vp64 transcriptional activator is joined to the C terminus of dCas9.
- ❑ In the dCas9-VPR protein, the transcription factors p65 and Rta are added to the C terminus of dCas9-Vp64. Therefore, all three transcription factors are targeted to the same gene.
- ❑ The use of three transcription factors, as opposed to solely Vp64, results in increased expression of targeted genes. When different genes were targeted by dCas9, they all showed significantly greater expression with dCas9-VPR than with dCas9-VP64.
- ❑ It has also been demonstrated that dCas9-VPR can be used to increase expression of multiple genes within the same cell by putting multiple sgRNAs into the same cell.



VP64:

- **VP64** is a transcriptional activator composed of four tandem copies of VP16 (a viral protein sequence of 16 amino acids that are used for transcriptional activation) connected with glycine-serine (GS) linkers.
- When fused to another protein domain that can bind near the promoter of a gene, VP64 acts as a strong transcriptional activator.

p65:

- Transcription factor **p65** also known as nuclear factor NF-kappa-B p65 subunit is a protein that in humans is encoded by the RELA gene.
- RELA, also known as p65, is a REL-associated protein involved in NF-κB heterodimer formation, nuclear translocation and activation.
- Phosphorylation and acetylation of RELA are crucial post-translational modifications required for NF-κB activation.
- RELA has also been shown to modulate immune responses, and activation of RELA is positively associated with multiple types of cancer.

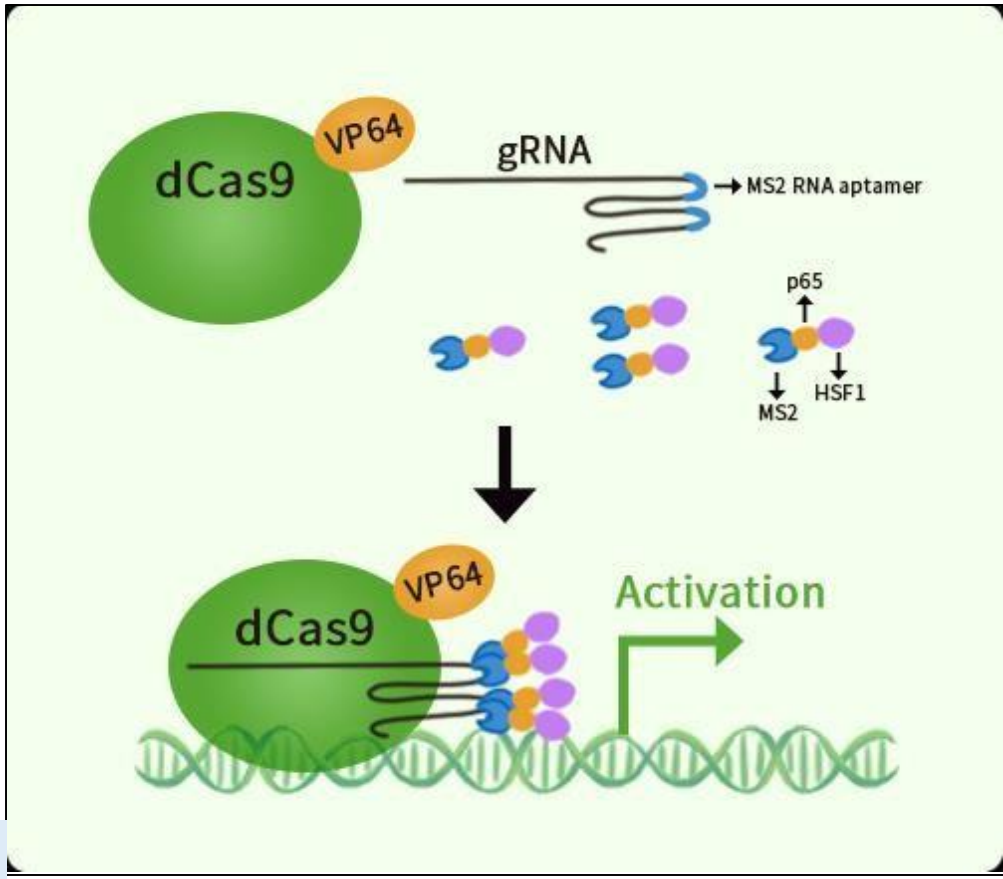
Rta:

- **Rta**, mainly encoded by open reading frame 50 (ORF50), is the product of an immediate-early gene of human herpesvirus-8 (HHV-8)/Kaposi's sarcoma-associated herpesvirus.
- Rta is a transcriptional activator that is both necessary and sufficient to disrupt viral latency and activate the expression of downstream viral lytic genes.

Applications of genome editing technologies

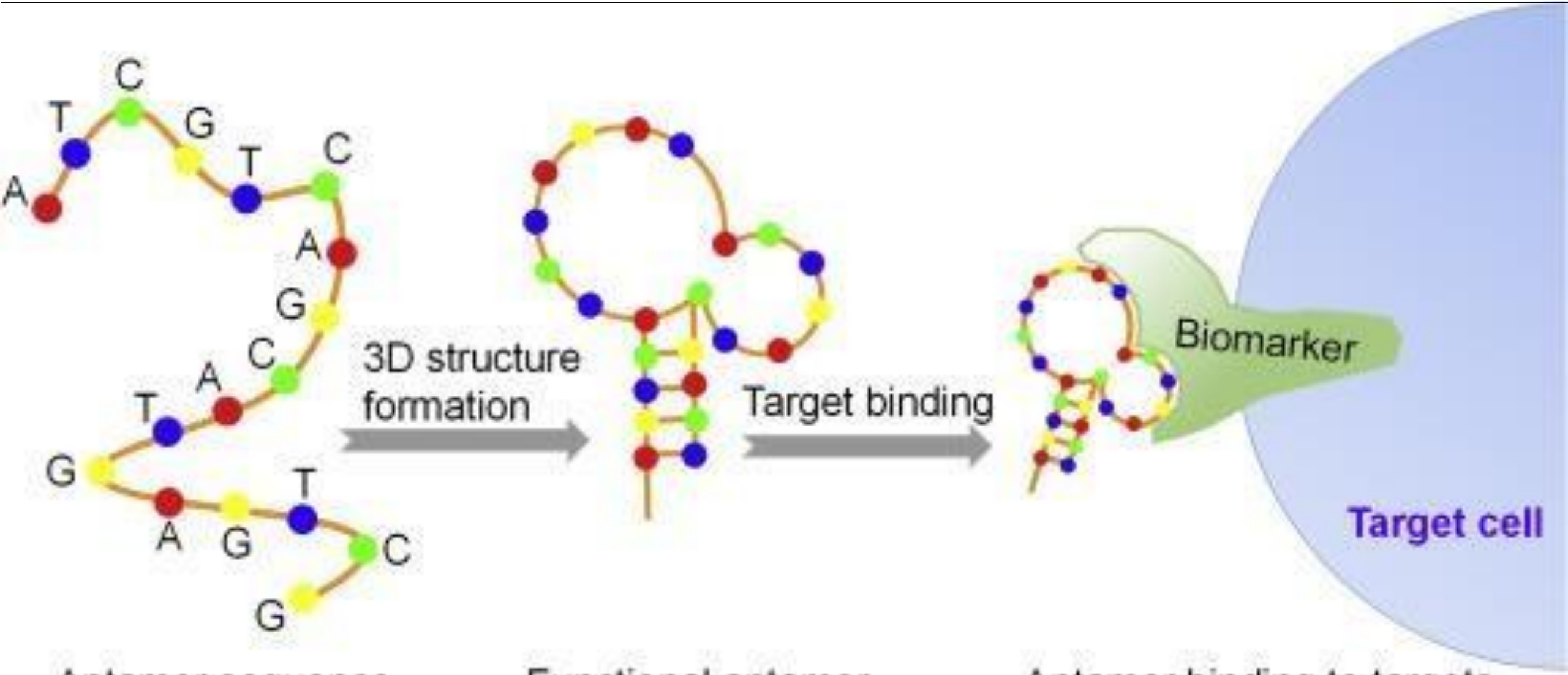
❑ 8. CRISPRa (CRISPR activation) - Synergistic activation mediator (SAM)

- ❑ CRISPRa SAM is a robust CRISPR gene activation system to activate gene expression using CRISPR technology.
- ❑ The CRISPRa SAM consists of dCas9-VP64, a modified gRNA containing MS2 RNA aptamers, and MS2-p65-HSF1 activation domains.
- ❑ VP64 has four copies of VP16, a viral protein that is used for transcriptional activation.
- ❑ P65 (RelA) and HSF1 (heat shock factor 1) are transcription activation domains.
- ❑ When p65 and HSF1 are brought in close proximity to dCas9-VP64 via interaction of MS2 with MS2 RNA aptamers in gRNA, the three transactivators can synergistically upregulate gene expression.
- ❑ CRISPRa SAM system can robustly activate both coding and non-coding RNA (lincRNA).



The transactivation domain or trans-activating domain (TAD) is a transcription factor scaffold domain which contains binding sites for other proteins such as transcription coregulators. These binding sites are frequently referred to as activation functions (AFs).

APTAMERS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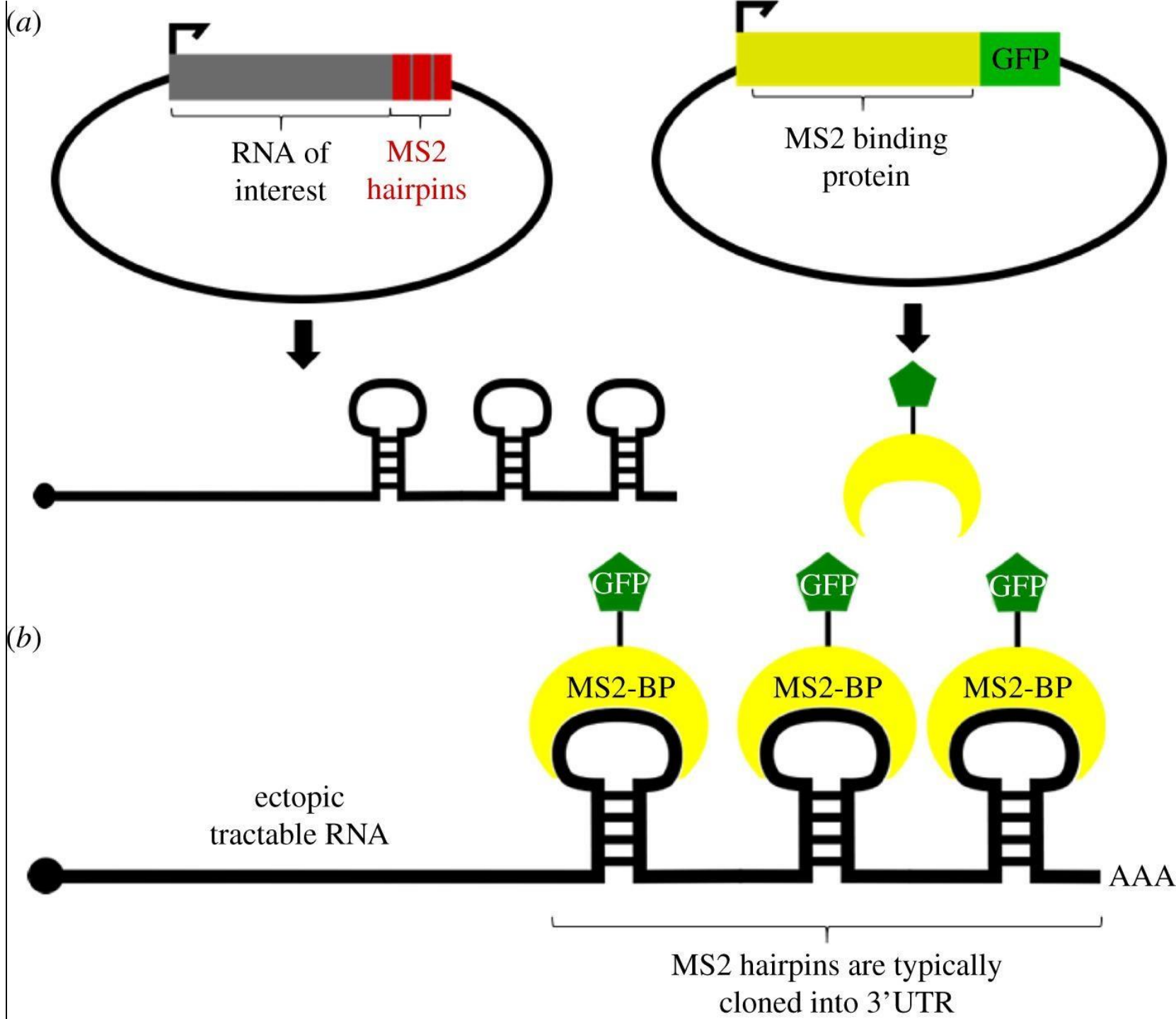
3D structure formation
Target binding
Biomarker
Target cell
Aptamer sequence
Functional aptamer
Aptamer binding to targets

- **MS2 tagging** is a technique based upon the natural interaction of the MS2 bacteriophage coat protein with a stem-loop structure from the phage genome, which is used for biochemical purification of RNA-protein complexes and partnered to GFP for detection of RNA in living cells.



Applications of genome editing technologies

❑ 8. CRISPRa (CRISPR activation)

- ❑ The **SunTag** activator system uses the dCas9 protein, which is modified to be linked with the SunTag.
- ❑ The SunTag is a repeating polypeptide array that can recruit multiple copies of antibodies. Through attaching transcriptional factors on the antibodies, the SunTag dCas9 activating complex amplifies its recruitment of transcriptional factors.
- ❑ SunTag system can recruit multiple copies of an antibody-fusion protein.
- ❑ In order to guide the dCas9 protein to its target gene, the dCas9 SunTag system uses sgRNA.

- ❑ Tanenbaum et al.(2014) are credited for creating the dCas9 SunTag system.
- ❑ In this study, the antibodies were bound to transcriptional factor VP64.
- ❑ In order to transport the antibodies to the nuclei of the cells, they attached NLS tag. To confirm the nuclear localization of the antibodies, sfGFP was used for visualization purpose. Therefore, the Antibody-sfGFP-NLS-VP64 protein was developed to be interact with dCas SunTag system.
- ❑ The antibodies successfully bound to SunTag polypeptides and activated target gene in cells.
- ❑ Comparing with the dCas9-VP64 activation complex, they were able to increase the target gene expression 5-25 times greater in cell lines and also the respective target protein overexpression.
- ❑ Thus, the dCas9-SunTag system can be used to activate genes that are present latently such as virus genes.

A Protein-Tagging System for Signal Amplification in Gene Expression and Fluorescence Imaging

Marvin E. Tanenbaum,[1,2] Luke A. Gilbert,[1,2,3,4] Lei S. Qi,[1,3,4] Jonathan S. Weissman,[1,2,3,4] and Ronald D. Vale[1,2,*]
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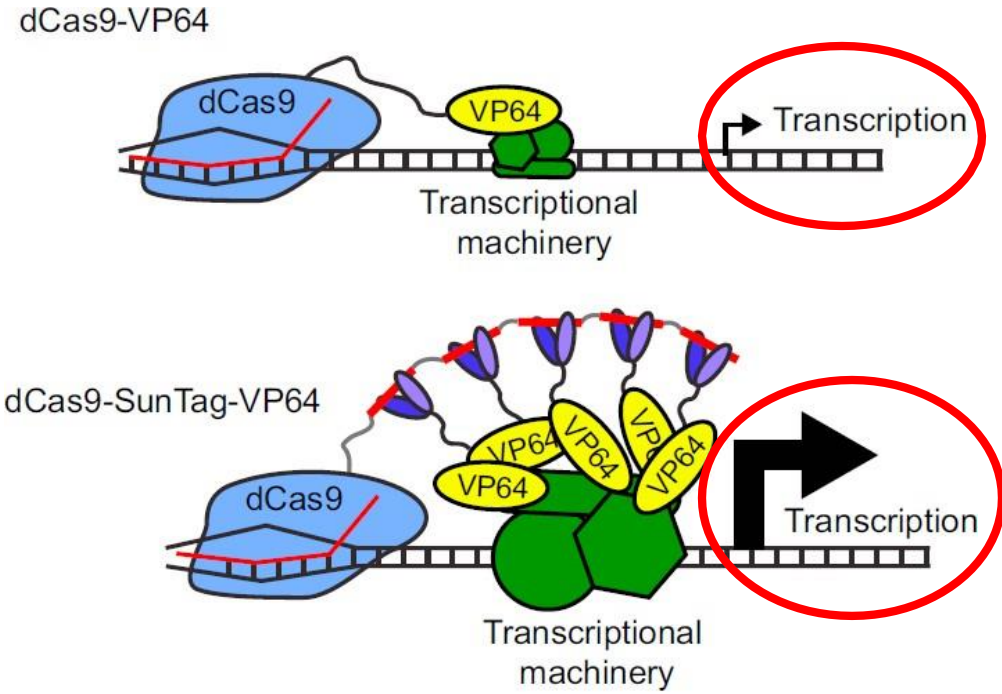
Cell *159*, 635–646, October 23, 2014 ©2014 Elsevier Inc.

Superfolder (sfGFP) is a robustly folded green fluorescent protein which folds when even when fused to poorly folded polypeptides (Pédelacq et al., 2006).



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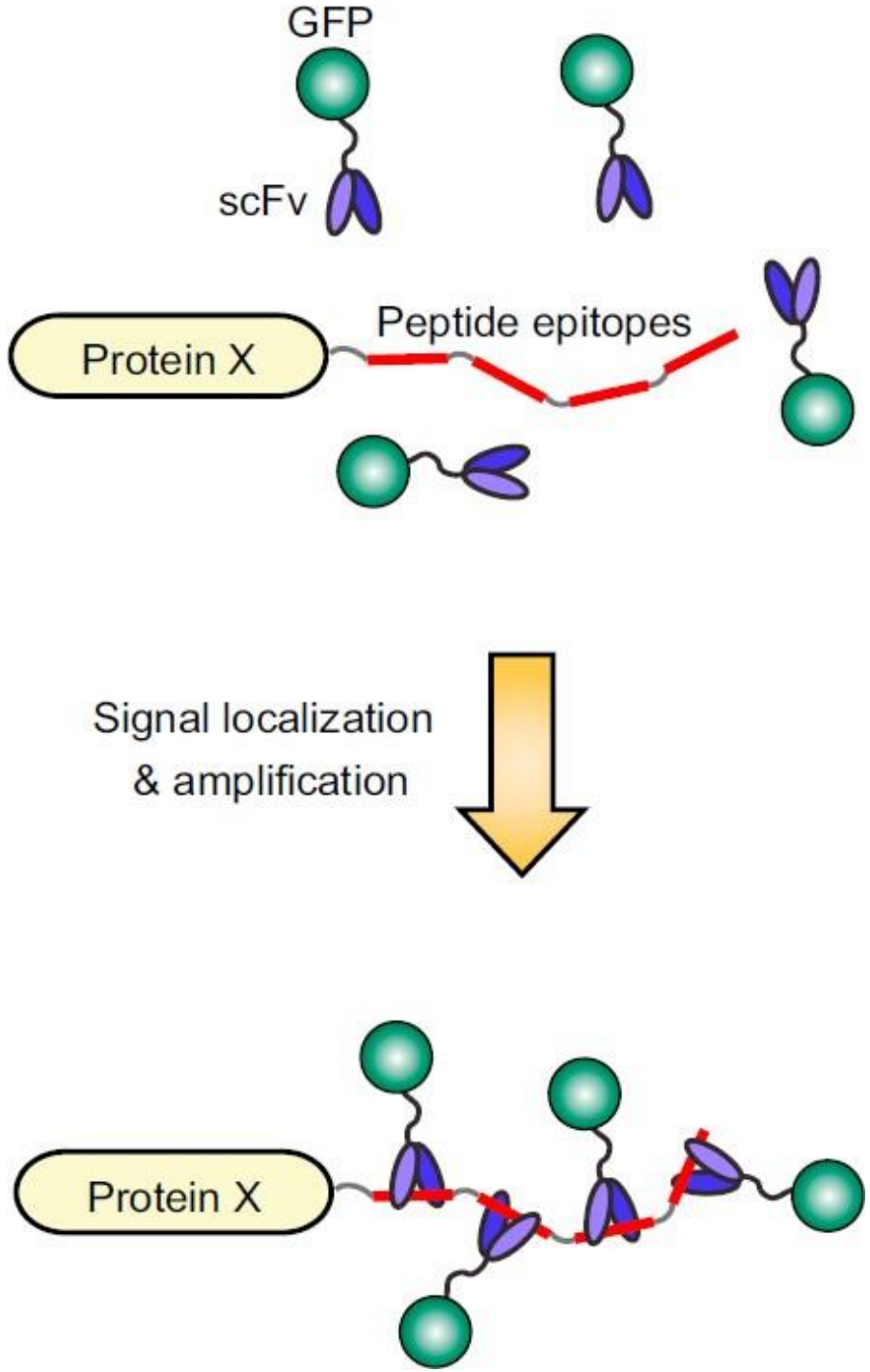
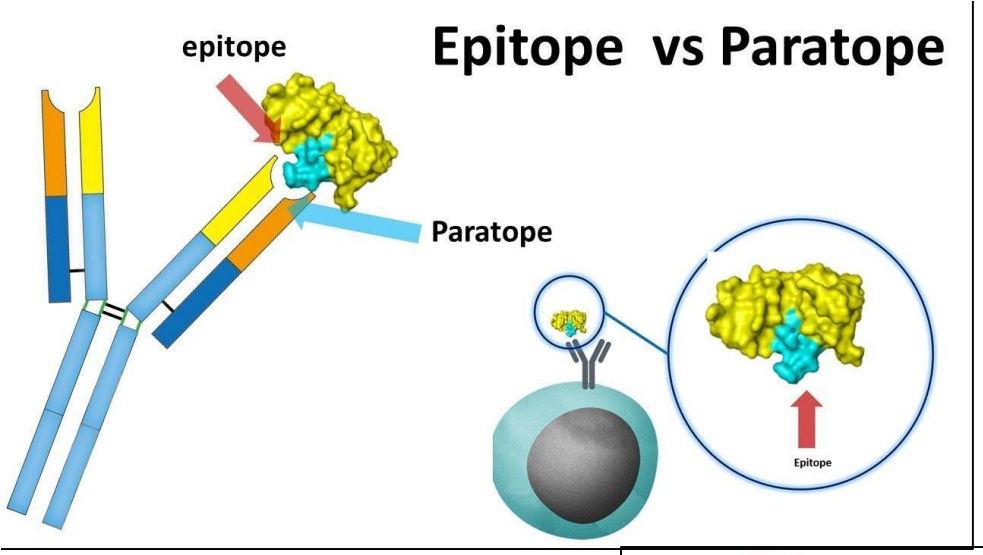
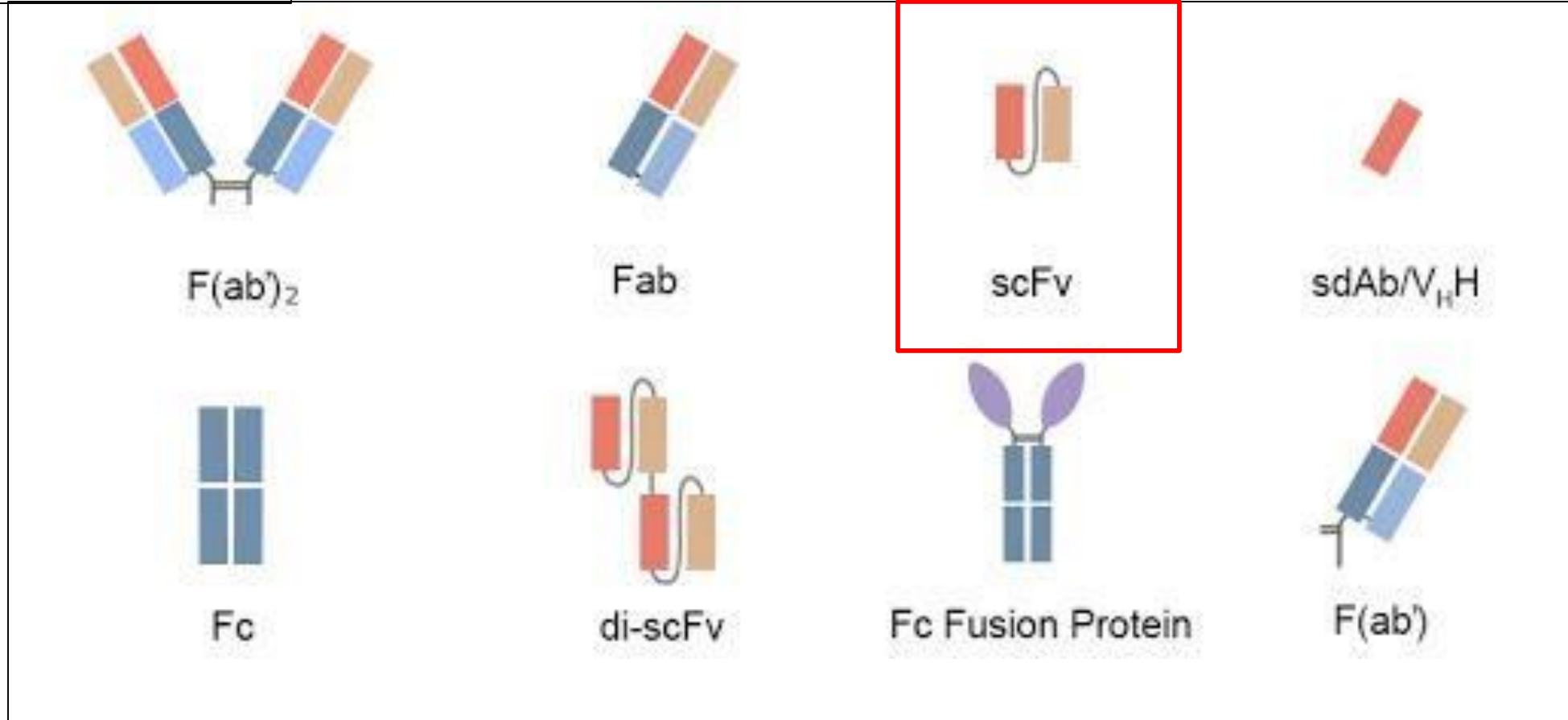
Cell *159*, 635–646, October 23, 2014



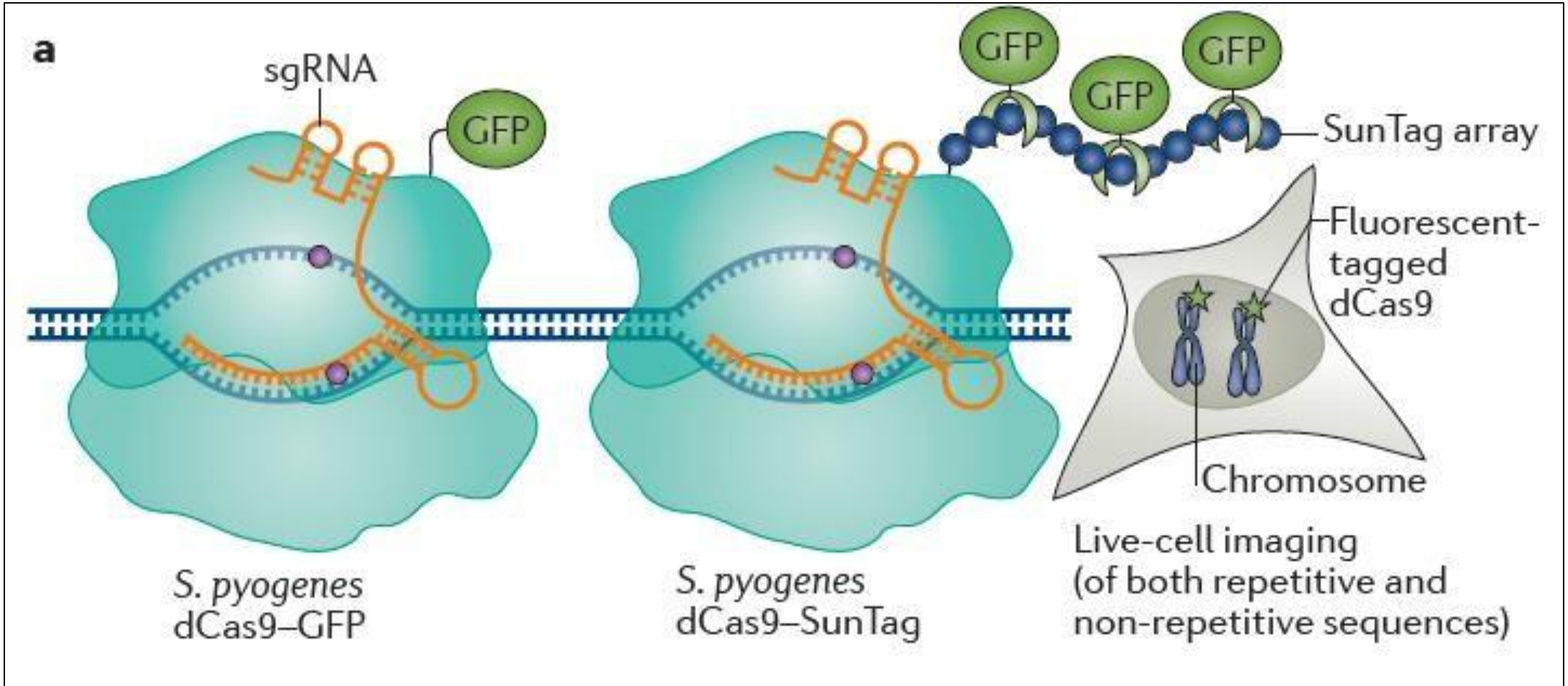


Single-chain variable fragment (scFv)
A single-chain variable fragment (scFv) is not actually a fragment of an antibody, but instead is a fusion protein of the variable regions of the heavy (VH) and light chains (VL) of immunoglobulins, connected with a short linker peptide of ten to about 25 amino acids.



Beyond editing: repurposing CRISPR–Cas9 for precision genome regulation and interrogation

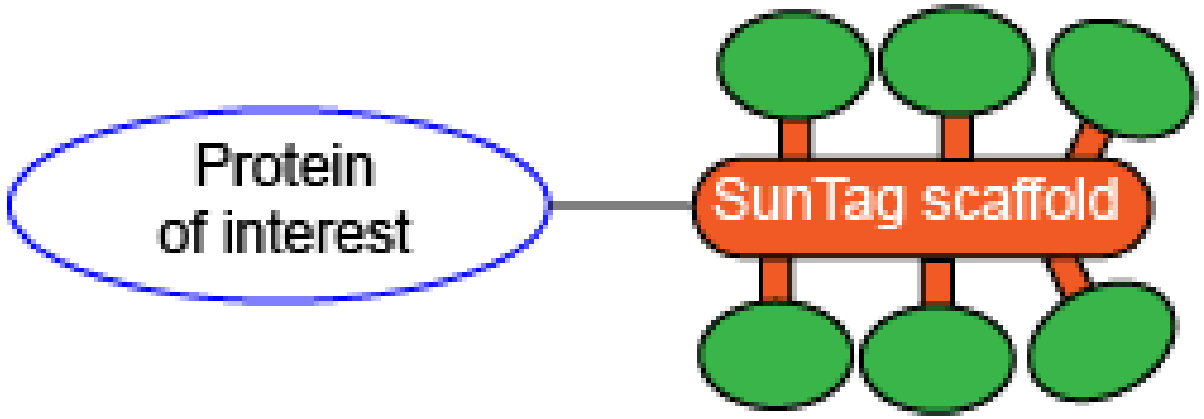
Antonia A. Dominguez[1–3], Wendell A. Lim[4–7] and Lei S. Qi[1–3]



Traditional FP fusion protein

Low signal:
Only one copy of GFP is directly fused to the protein of interest

SunTag fusion protein



High signal:
The SunTag scaffold recruits up to 24 copies of scFV GFP

Comparing traditional GFP fusion proteins to SunTag fusion proteins. A traditional GFP fusion fuses one copy of GFP to a protein of interest. Rather than fuse GFP to a protein, SunTag fusions contain a synthetic scaffold that recruits GFP fused to the scFV antibody.

Beyond editing: repurposing CRISPR–Cas9 for precision genome regulation and interrogation

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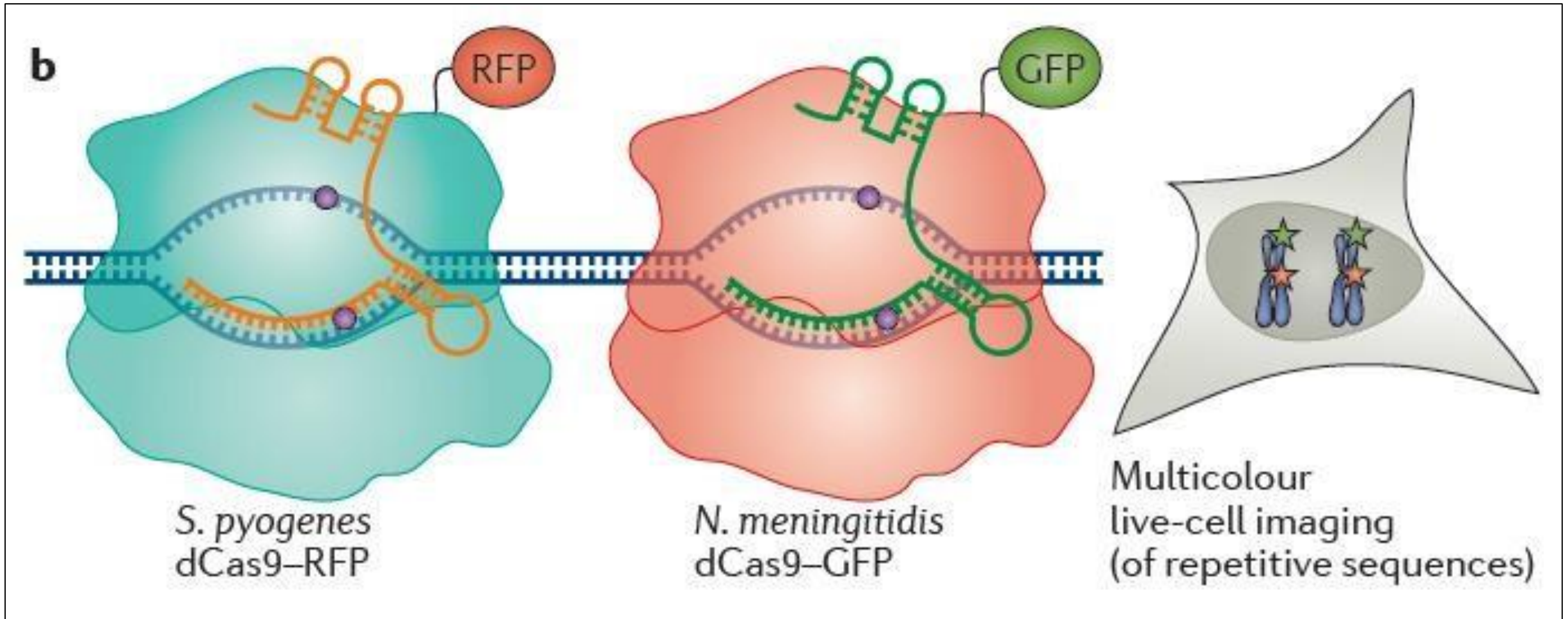


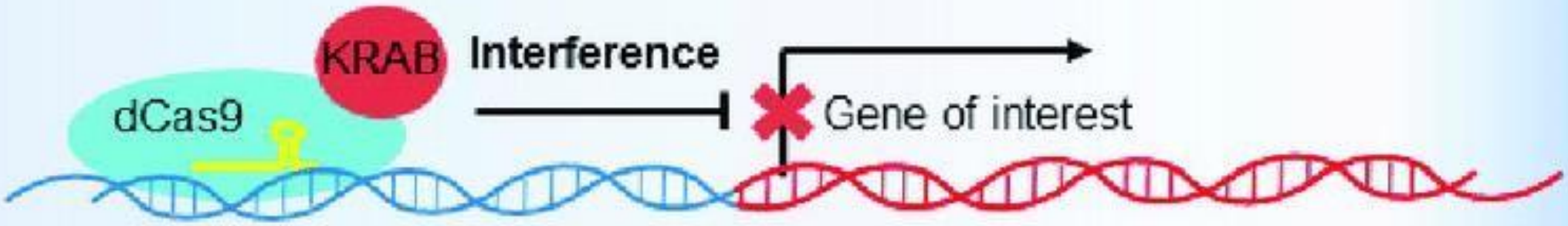
Applications of genome editing technologies

❑ 9. CRISPRi (CRISPR interference)

- ❑ CRISPRi can also repress transcription via an effector domain.
- ❑ Fusing a repressor domain to dCas9 allows transcription to be further repressed by inducing heterochromatinization.
- ❑ For example, the well-studied Krüppel associated box (KRAB) domain can be fused to dCas9 to repress transcription of the target gene up to 99% in human cells.
- ❑ The Krueppel-associated box (KRAB) is a domain of around 75 amino acids that is found in the N-terminal part of about one third of eukaryotic Krueppel-type C2H2 zinc finger proteins (ZFPs).
- ❑ The functions currently known for members of the KRAB-containing protein family include transcriptional repression of RNA polymerase I, II and III promoters, binding and splicing of RNA, and control of nucleolus function.
- ❑ The KRAB domain functions as a transcriptional repressor when tethered to the template DNA by a DNA-binding domain.
- ❑ A sequence of 45 amino acids in the KRAB A subdomain has been shown to be necessary and sufficient for transcriptional repression. The B box does not repress by itself but does potentiate the repression exerted by the KRAB A subdomain

CRISPRi

- ❑ The KRAB domain is a category of transcriptional repression domains present in approximately 400 human zinc finger protein-based transcription factors (KRAB zinc finger proteins).
- ❑ The KRAB domain presents one of the strongest repressors in the human genome.
- ❑ KRAB domains can be fused with dCas9 CRISPR tools to form even stronger repressors.



Applications of genome editing technologies

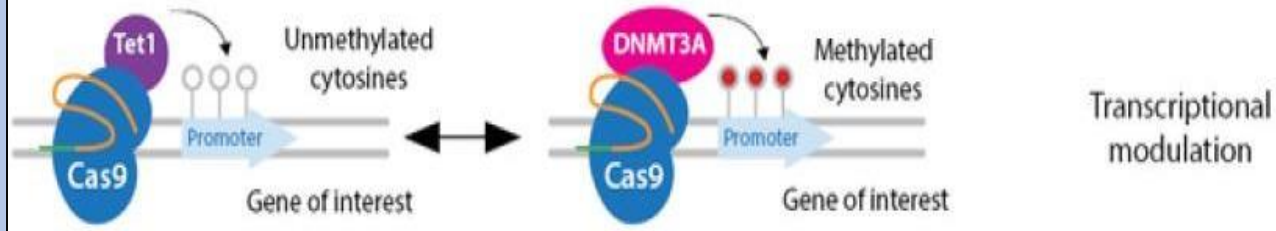
❑ 10. Epigenetic modifications using CRISPR

- ❑ Inactive Cas enzymes can be fused to epigenetic modifiers like p300, TET1, DNMT3A to create programmable epigenome-engineering tools.
- ❑ These tools alter gene expression without inducing double-strand breaks by modifying the methylation state of cytosines in a gene's promoter or by inducing histone acetylation or demethylation.
- ❑ CRISPR epigenetic tools are specific for particular chromatin and DNA modifications, allowing researchers to isolate the effects of a single epigenetic mark.
- ❑ Another potential advantage of CRISPR epigenetic tools is their persistence and inheritance. CRISPR activators and repressors are thought to be reversible once the effector is inactivated or removed from the system.
- ❑ In contrast, epigenetic marks left by targeted epigenetic modifiers may be more frequently inherited by daughter cells. In certain cases, epigenetic modifiers may work better than activators/repressors in modulating transcription.
- ❑ However, since the effects of these tools are likely cell type- and context-dependent, it might be beneficial to try multiple CRISPR strategies when setting up your experimental system.

Histone acetyltransferase p300 regulates transcription of genes via chromatin remodeling by allowing histone proteins to wrap DNA less tightly and functions as a transcriptional co-activator protein

Ten-eleven translocation methylcytosine dioxygenase 1 (TET1) is a member of the TET family of enzymes, which is involved in DNA demethylation and therefore gene regulation.

DNMT3A is a member of the family of DNMTs, which catalyze the addition of methyl groups to cytosine residues of CpG dinucleotides. These dinucleotides tend to cluster in 5 promoter regions upstream of genes, and increased methylation of these CpG islands is associated with decreased expression of the associated downstream gene. The observation that many cancers often display aberrant methylation relative to healthy tissue has led to the hypothesis that hypermethylation, particularly in the promoters of tumor suppressor genes, plays a role in cancer pathogenesis.

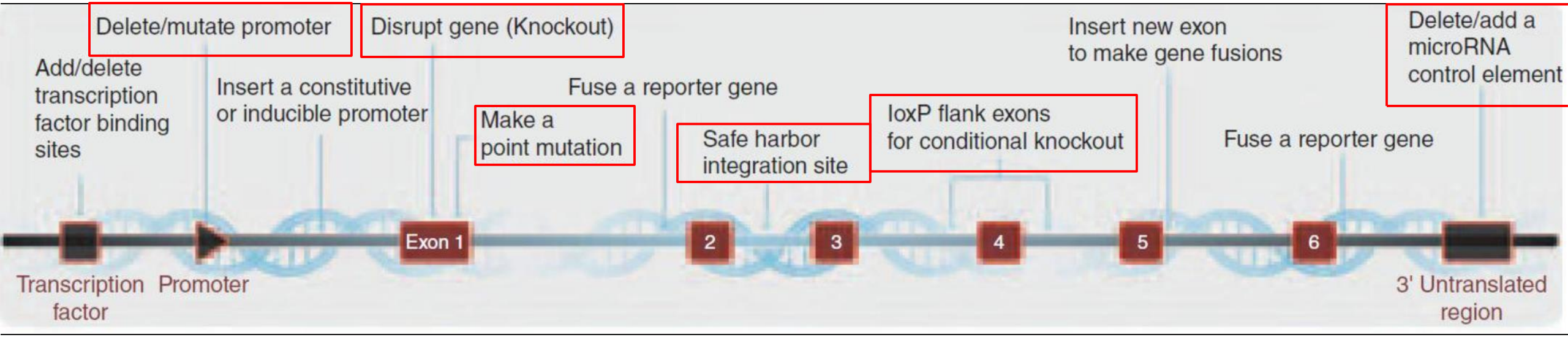


Applications of genome editing technologies

❑ 10. Genome-Wide Screens Using CRISPR

- ❑ The ease of gRNA design and synthesis, as well as the ability to target almost any genomic locus, make CRISPR the ideal genome engineering system for large-scale forward genetic screening.
- ❑ Forward genetic screens are particularly useful for studying diseases or phenotypes for which the underlying genetic cause is not known.
- ❑ In general, the goal of a genetic screen is to generate a large population of cells with mutations in, or activation/repression of, a wide variety of genes and then use these cells to identify the genetic perturbations that result in a desired phenotype.
- ❑ Before CRISPR, genetic screens relied heavily on shRNA technology, which is prone to off-target effects and false negatives due to incomplete knockdown of target genes. In contrast, CRISPR is capable of making highly specific, permanent genetic modifications that are more likely to ablate target gene function.
- ❑ CRISPR has already been used extensively to screen for novel genes that regulate known phenotypes, including resistance to chemotherapy drugs, resistance to toxins, cell viability, and tumor metastasis.
- ❑ Currently, the most popular method for conducting genome-wide screens using CRISPR involves the use of pooled lentiviral CRISPR libraries.

Other applications



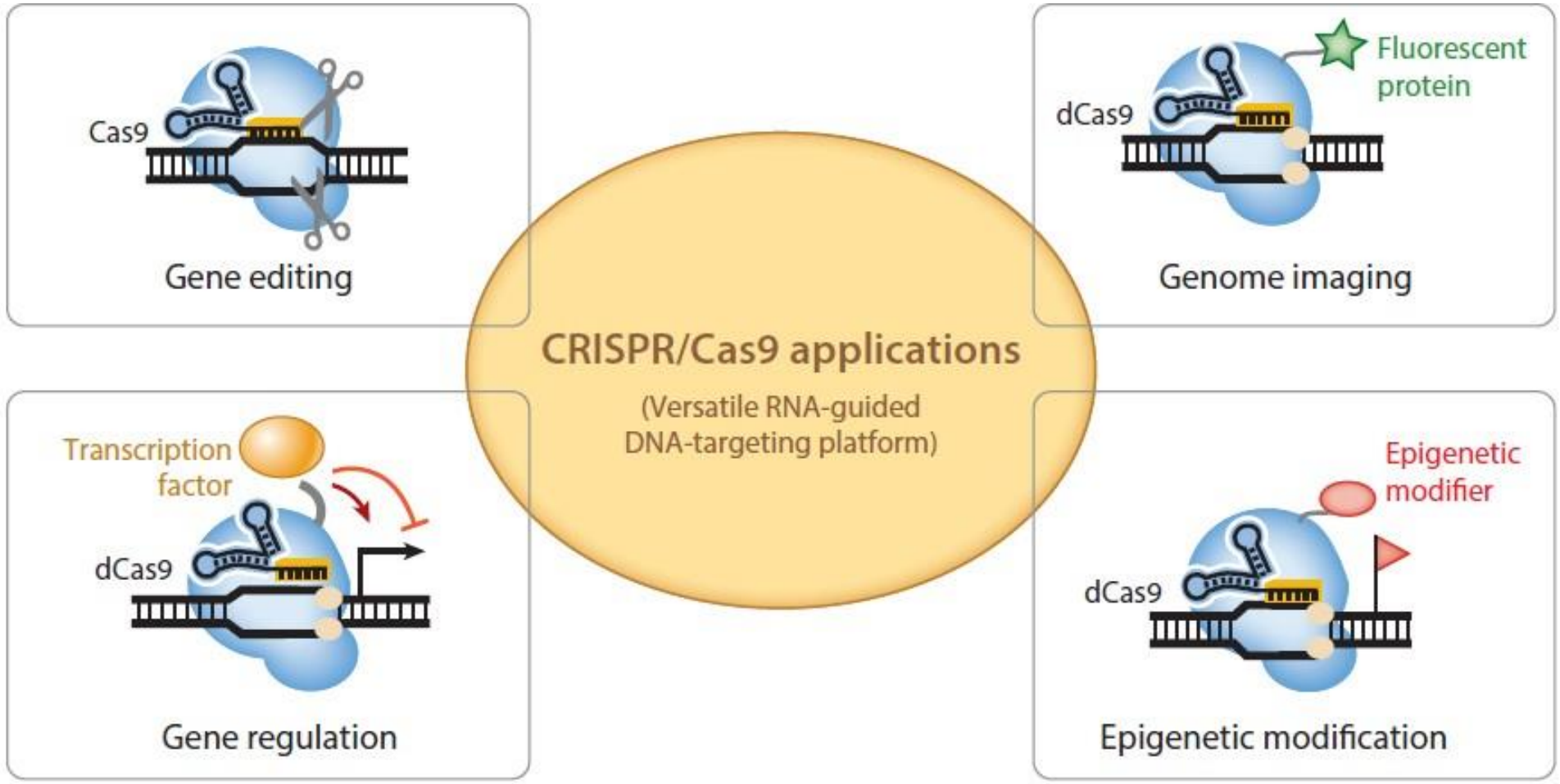


Figure 1

Overview of CRISPR/Cas9 applications. This system has been adapted and developed for gene editing, transcription regulation, chromosome imaging, and epigenetic modification. Gene editing is based on the nuclease activity of Cas9, whereas the three other applications use the catalytic, nuclease-deactivated form of Cas9 (dCas9). Fusing dCas9 to various effector domains enables the sequence-specific recruitment of transcription regulators for gene regulation, fluorescent proteins for genome imaging, and epigenetic modifiers for epigenetic modification.