

# Genome editing

- **Editing the genome with engineered nucleases**
- **A type of gene engineering : DNA is inserted, replaced, or removed from a genome using artificially engineered nucleases, or "molecular scissors."**
- **Nucleases create specific double-stranded break (DSBs) at desired locations in the genome, and harness the cell's endogenous mechanisms to repair the induced break by natural processes.**
- **Fundamental to the use of nucleases in genome editing: concept of DNA double stranded break (DSB) repair mechanics.**
  - Nonhomologous end-joining (NHEJ):uses a variety of enzymes to directly join the DNA ends in a double-strand break.
  - Homology directed repair (HDR): A homologous sequence is utilized as a template for regeneration of missing DNA sequence at the break point.
- **Four families of engineered nucleases**
  - **Meganucleases:** Endodeoxyribonucleases with a large recognition site (double-stranded DNA sequences of 12 to 40 base pairs)
  - **Zinc finger nucleases (ZFNs)**
  - **Transcription Activator-Like Effector Nucleases (TALENs)**
  - **CRISPR (Clustered regularly interspaced short palindromic repeats)/Cas system**

# Hybrid Meganuclease



ZFN



Zinc finger domains

TALEN

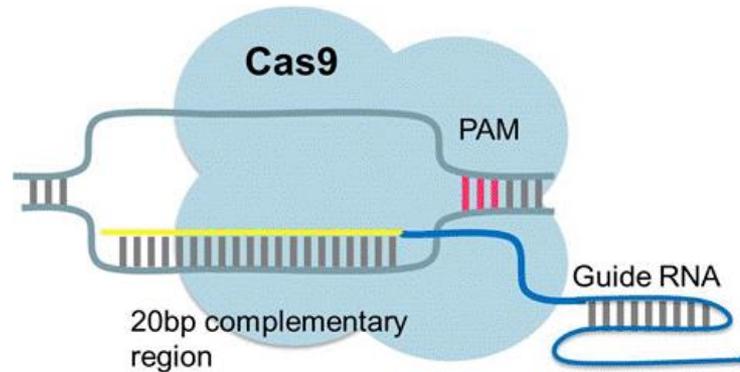


TALE subunits



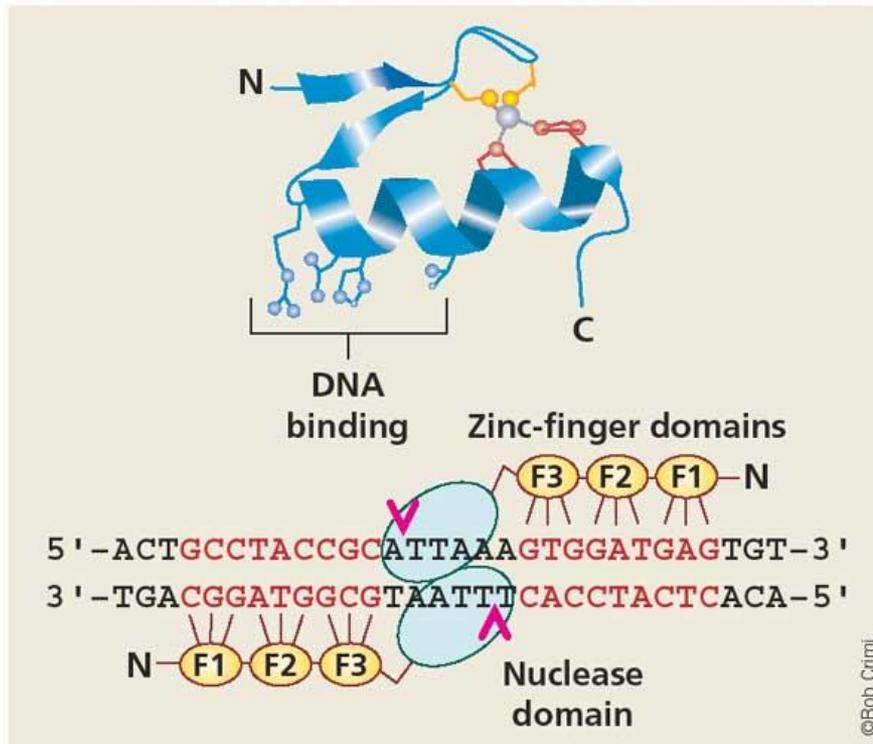
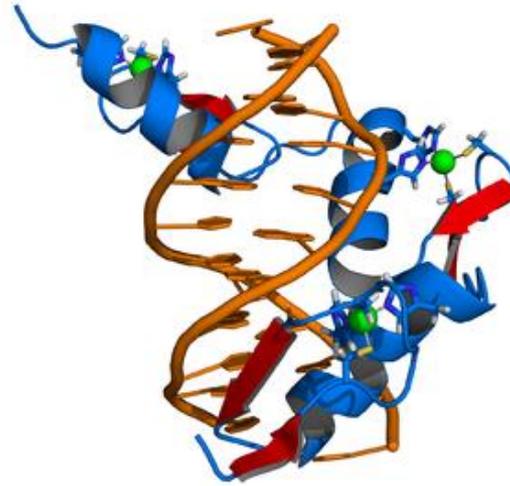
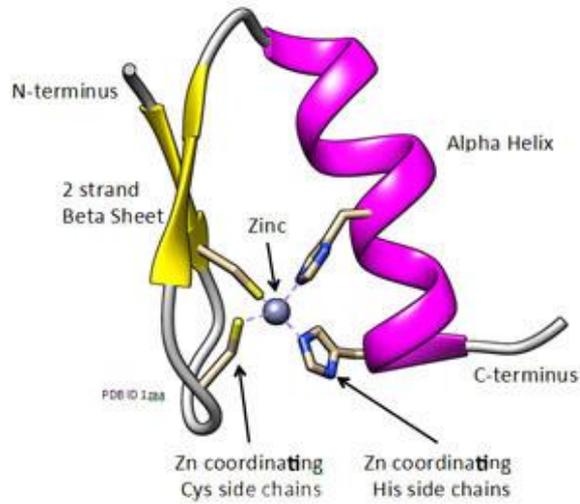
active FokI catalytic subunit heterodimer

Cas9



# Zinc finger nuclease

- Engineered restriction enzymes generated by fusing a zinc finger DNA-binding domain to a DNA-cleavage domain.
- Zinc finger domains can be engineered to target specific desired DNA sequences and this enables zinc-finger nucleases to target unique sequences within complex genomes
- DNA-binding domains of individual ZFNs: typically three and six individual zinc finger repeats and can each recognize between 9 and 18 basepairs
  - Each zinc finger domain perfectly recognize a 3 basepair DNA sequence
- Non-specific cleavage domain : Type II restriction endonuclease FokI
- Used for genome editing of many plants and animals including arabidopsis, tobacco, soybean, corn, *Drosophila melanogaster*, *C. elegans*, *Platynereis dumerilii*, sea urchin, silkworm, zebrafish, frogs, mice, rats, rabbits, pigs, cattle
  - Ex) editing of host cellular co-receptors for HIV
- Potential problems
  - Off-target cleavage
  - Immunogenicity
  - Challenge of generating zinc finger domains that target the desired sequence with sufficient specificity



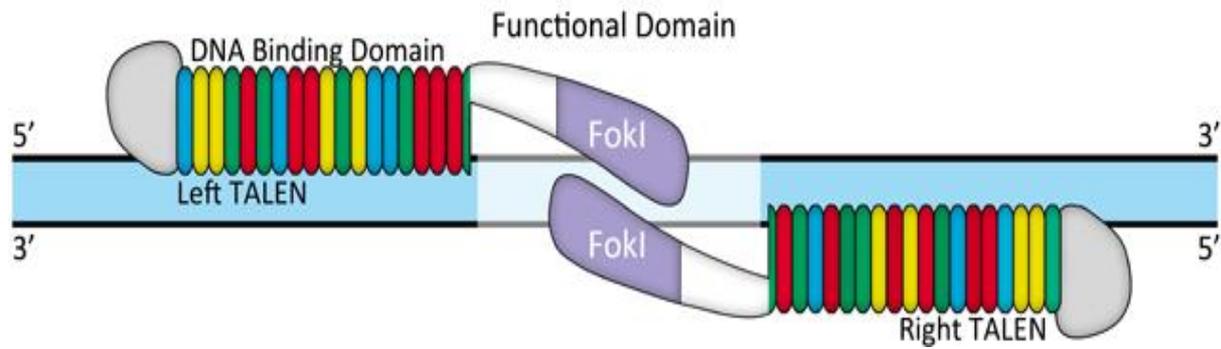
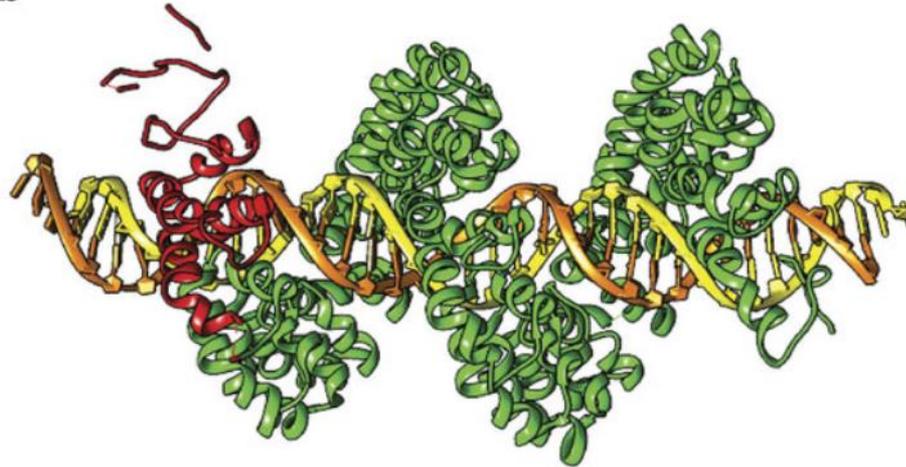
# TALEN (Transcription activator-like effector nuclease)

- **TALE + Nuclease:** Engineered restriction enzymes to cut specific sequences of DNA.
- **TALE DNA-binding domain**
  - Proteins that are secreted by Xanthomonas bacteria via their type III secretion system when they infect plants.
    - bind promoter sequences in the host plant and activate the expression of plant genes that aid bacterial infection
  - Contains a repeated highly conserved 33–34 amino acid sequence with divergent 12<sup>th</sup> and 13<sup>th</sup> amino acids.
    - These two positions, referred to as the Repeat Variable Diresidue (RVD), are highly variable and show a strong correlation with specific nucleotide recognition.
    - This straightforward relationship between amino acid sequence and DNA recognition allows for the engineering of specific DNA-binding domains by selecting a combination of repeat segments containing the appropriate RVDs
- **Non-specific DNA cleavage domain : FokI endonuclease**
- **TALEN: wild-type FokI and its variants**

**a**

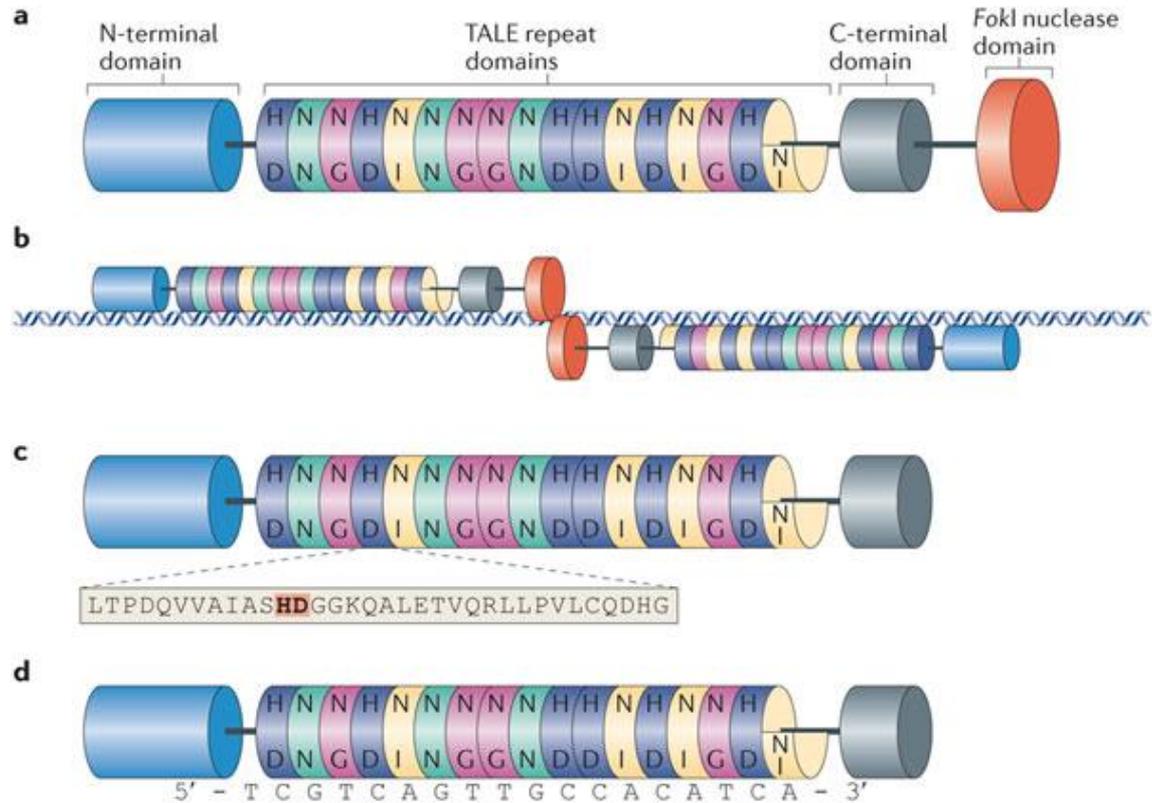


**b**



## Engineering of TALE repeat domains

Repeat domains bind to single bases in the target sequence according to the TALE code.



# Applications

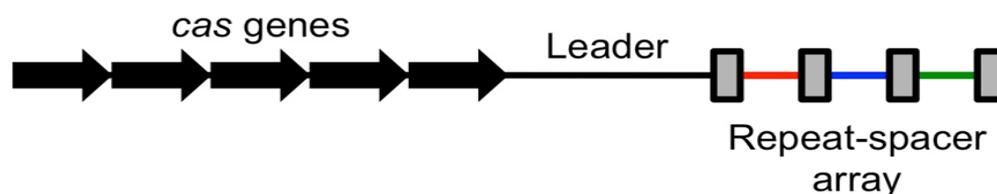
- Highest precision of the currently available technologies
- Editing plant genomes to creating economically important food crops with favorable nutritional qualities.
- Engineer stably modified human embryonic stem cell and induced pluripotent stem cell (iPSCs) clones and human erythroid cell lines,
- Generate knockout *C. elegans*, knockout rats, knockout mice, and knockout zebrafish.
- Generate knock-in organisms: Sp110 knock-in cattle to induce increased resistance of tuberculosis.
- Correct the genetic errors: in vitro correction of the genetic defects that cause disorders such as sickle cell disease, xeroderma pigmentosum, and epidermolysis bullosa

## Drawbacks:

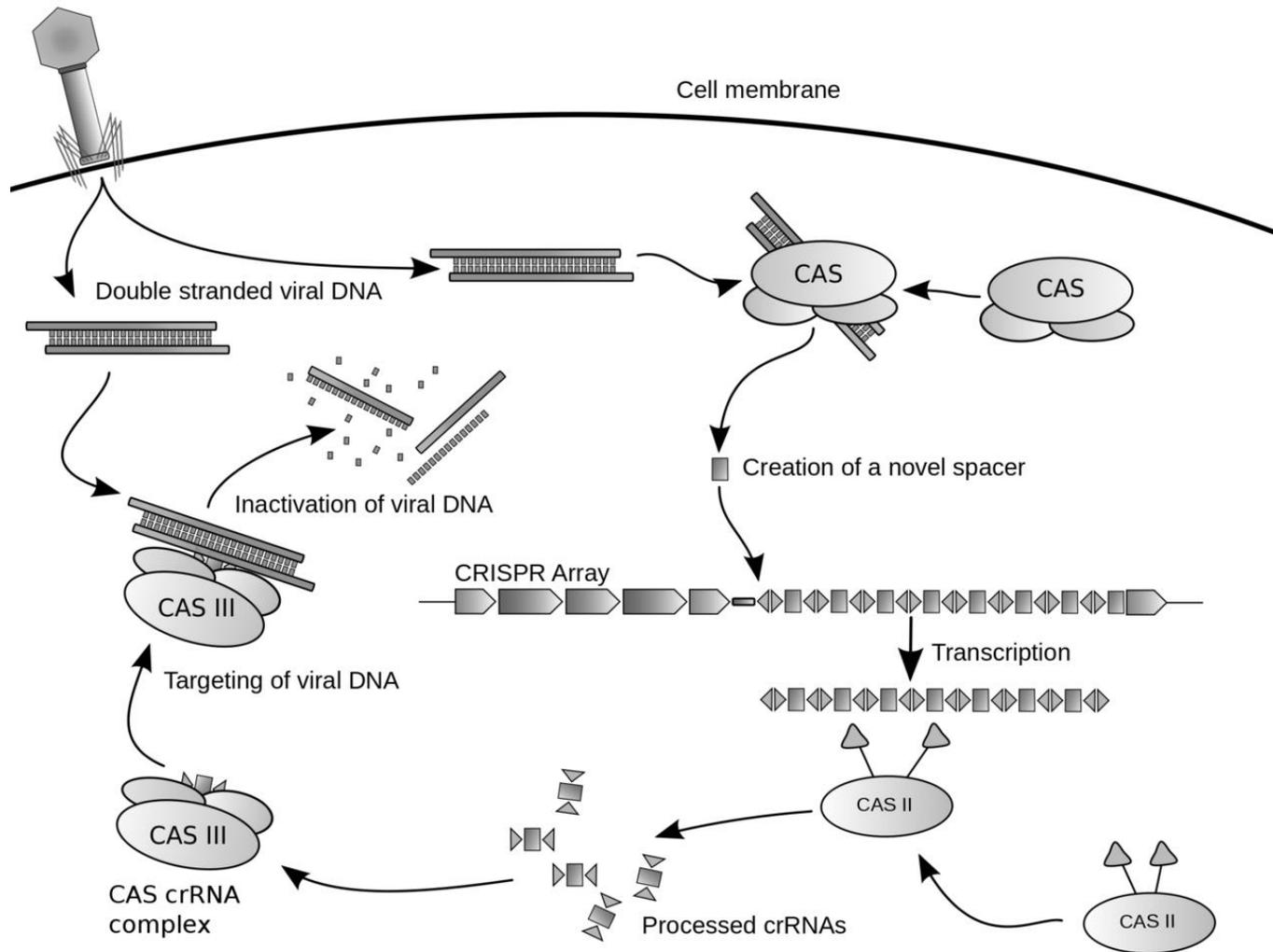
- Unlike ZNFs, TALEN recognizes single nucleotides: far more straightforward to engineer interactions between TALEN DNA binding domains and their target nucleotides
  - ultimately 200 times more expensive than CRISPR and takes several months more

# CRISPER /Cas

- CRISPER: Clustered regularly interspaced short palindromic repeats
- Microbial adaptive immune system that uses RNA-guided nucleases to cleave foreign genetic elements: prokaryotic immune system that confers resistance to foreign genetic elements such as plasmids and phages
  - Found in about 40% of sequenced bacterial genomes and 90% of sequenced archaea.
  - When invaded by foreign genetic elements, the first stage of the immune response is to capture and insert them into a CRISPR locus in the form of a spacer
  - Each repetition is followed by short segments of a spacer DNA from previous exposures to foreign DNA (a virus or plasmid): Cas 1 and II involved in spacer acquisition
- Small clusters of Cas (CRISPR-associated system) genes :located next to CRISPR sequences
- RNA harboring the spacer sequence helps Cas proteins recognize and cut exogenous DNA
- Protospacer adjacent motif (PAM): a 2-6 base pair DNA sequence following the DNA sequence targeted by the Cas9 nuclease in the CRISPR bacterial immune system.
  - Essential targeting component which distinguishes bacterial self from non-self DNA, thereby preventing the CRISPR locus from being targeted and destroyed by nuclease.



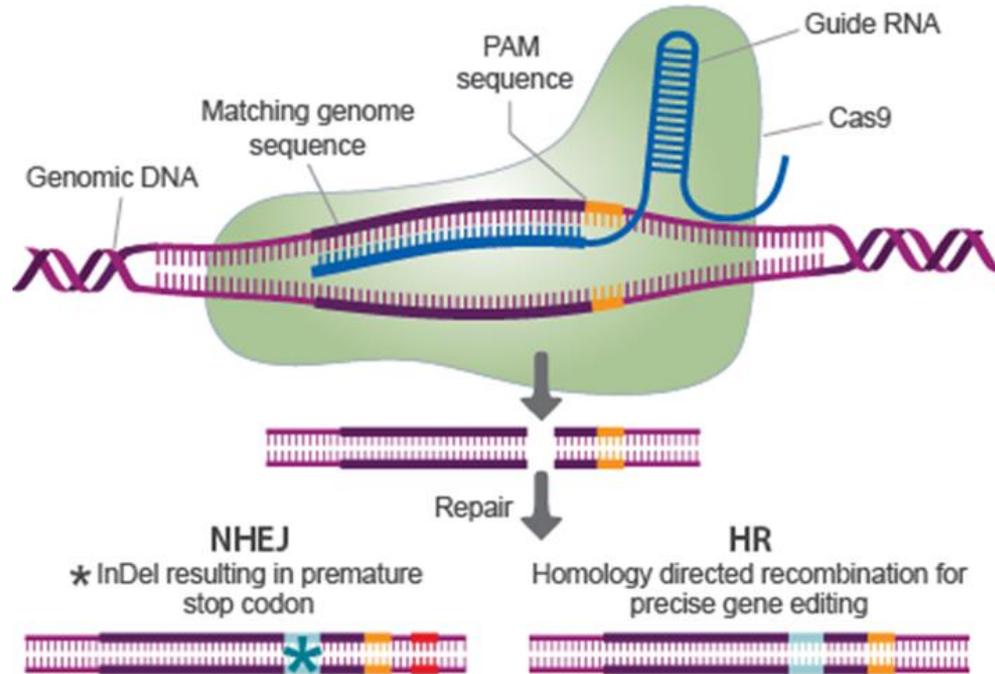
# CRISPR prokaryotic antiviral defense mechanism



# Delivery

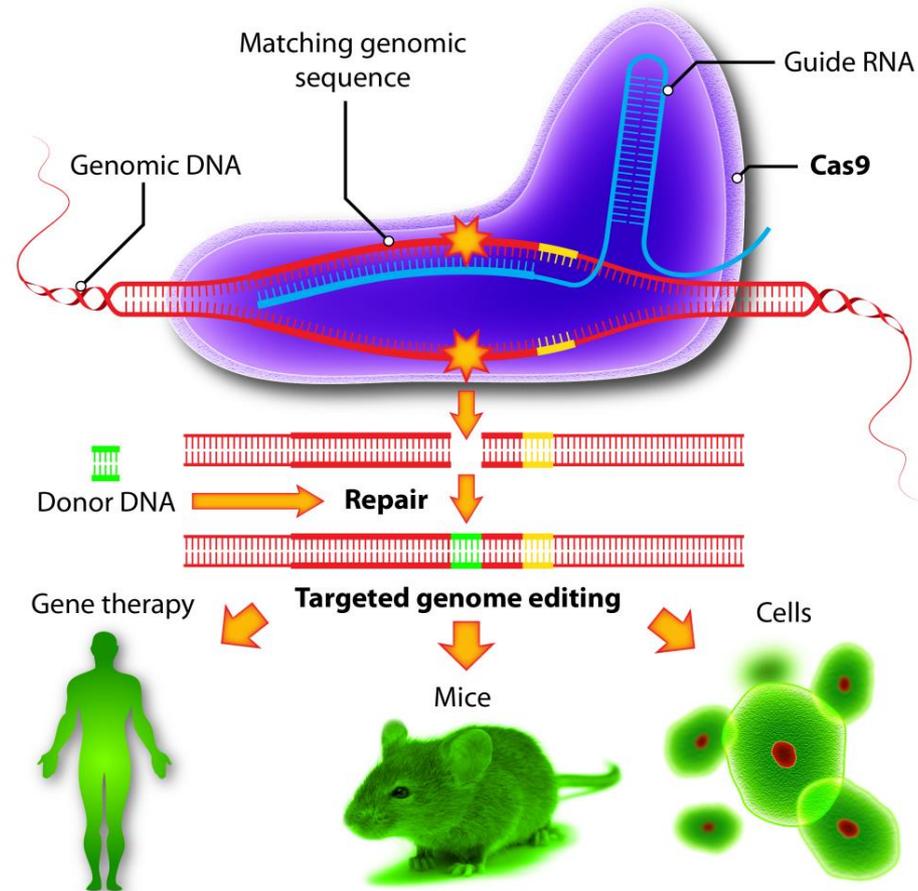
- Viral or non-viral systems for delivery of the Cas9 and sgRNA into target cells.
- Electroporation of DNA, RNA or ribonucleocomplexes: most common and cheapest system.
  - Deep sequencing of a target site: up to ~20% efficiency
  - Hard-to-transfect cells: stem cells, neurons, hematopoietic cells, etc.
  - Require more efficient delivery systems based on lentivirus (LVs), adenovirus (AdV) and adeno-associated virus (AAV).

# Genome editing using CRISPER/Cas 9



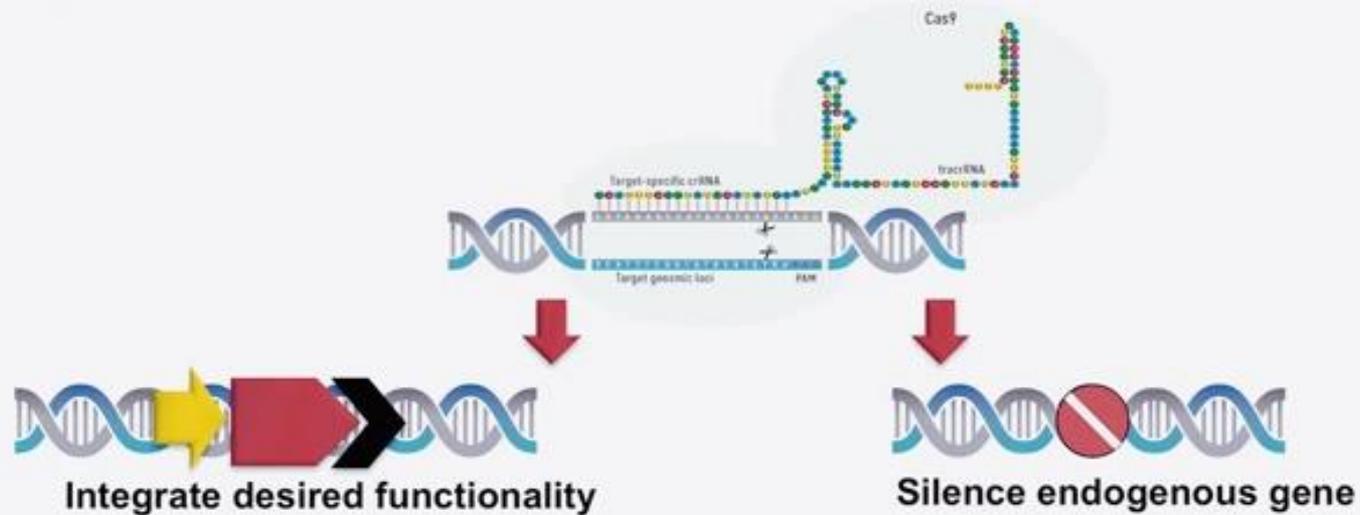
- Use ribonucleotide complex formation instead of protein/DNA recognition.
  - gRNAs can target nearly any sequence in the genome and they can be cheaply produced
- Much more efficient and less expensive than both TALEN and ZNF
  - More than 600 papers regarding CRISPER/Cas

# CRISPR/Cas 9 system



- Ethical issues: Editing of human embryos
  - First approval in UK for human healthy embryos to alter genes active after fertilization
- Off-target effect : non-specific mutations

# Genome Editing Made Easier



Disease-resistant transgenic plants



Stem cell engineering & gene therapy



Tissue & Animal disease models

# Applications

- **Biomedicine**

- Target virulence factors, genes encoding antibiotic resistance and other medically relevant sequences of interest: Muscle differentiation, cancer, inflammation, fetal hemoglobin, sickle-cell anemia
- Eradicate viral DNA in the case of Epstein-Barr virus (EBV)
  - removal of cancer-causing EBV from tumor cells
- Transplantation of animal organs into people:
  - Retroviruses present in animal genomes could harm transplant recipients.
  - Elimination of 62 copies of a retrovirus's DNA from the pig genome in 2015.
- Use in tissue engineering and regenerative medicine: creation of human blood vessels that lack expression of MHC class II proteins, which often cause transplant rejection

# Disease models

- Creation of animals for research that mimic disease and test drugs
  - Show what happens when a gene is knocked down or mutated.
- Editing at the germline level to create animals where the gene is changed everywhere
- Human cellular models of disease.
  - Human pluripotent stem cells with mutations in genes relevant to polycystic kidney disease (PKD) and focal segmental glomerulosclerosis (FSG).
  - Modified pluripotent stem cells are subsequently grown into human kidney organoids that exhibited disease-specific phenotypes.

# Agriculture

- Improvement of food production: Benefit farmers, consumers, agribusiness, sustainable farming systems, and industrial agriculture
- Possible avoidance of “GMO” issue
  - U.S. policy is product-based, and the product will not include foreign genetic material. In cases where editing introduces sequences from close crop wild relatives, the product might even be genetically indistinguishable from the results of conventional crossbreeding
- Crop resistance to pests, drought and frost: wheat, rice, soybeans, potatoes, sorghum, oranges and tomatoes
  - Creating a strain of wheat that is resistant to powdery mildew and a destructive fungal disease.
  - DuPont is developing corn and wheat strains with drought resistance
  - Oilseed rape with herbicide resistance
  - Mushroom and potato with less browning.

# Challenging Issues

- Improving safety and specificity of the nucleases.
  - Off-target effects
- Known nucleases do not cover all possible target sequences :  
Construction of sequence specific enzymes for all possible sequences is costly and time consuming
- Requirement of better understanding of the basic recombination and repair machinery of DNA.

# Perspectives of genome editing

- **Genomic editing will bring positive and negative consequences, and should be evaluated on a full range of social and environmental effects.**
- **It is needed to treat CRISPR not as a single technology, but as a toolbox full of technologies, each of which is specific to the mutation, organism and ecosystems in question.**
- **Understanding gene functions in plants and animals**
- **Gene therapy in humans**
  - **Targeted gene mutation**
  - **Creating chromosome rearrangement**
  - **Study gene function with stem cells**
  - **Transgenic animals**
  - **Endogenous gene labeling**
  - **Targeted transgene addition**