



MACQUARIE
University
SYDNEY · AUSTRALIA

BIOL3120: Human genetics and evolutionary medicine

LECTURE 22: CRISPR IN THE 21ST CENTURY



Learning objectives



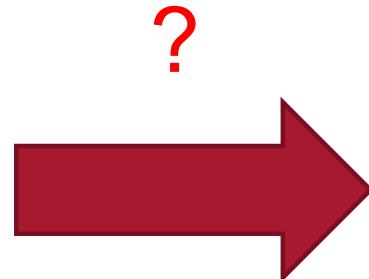
1. Understanding the basics of CRISPR-Cas9
2. What led to the discovery of CRISPR-Cas9 for molecular biology
3. How can CRISPR-Cas9 be used to genetically modify organisms
 1. Knock-out (NHEJ)
 2. Knock-in (HDR)
 3. Base editing
 4. Challenges
4. Uses of CRISPR



MACQUARIE
University
SYDNEY · AUSTRALIA

Understanding the basics of CRISPR-Cas9

CRISPR-Cas9



Current clinical trials using CRISPR



NIH U.S. National Library of Medicine

ClinicalTrials.gov

Find Studies ▾ About Studies ▾ Submit Studies ▾ Resources ▾ About Site ▾ PRS Login

Home > Search Results

Modify Search Start Over +

4 Studies found for: CRISPR | (Map: Australia)

Not enough studies found? Try these search suggestions: +

Search Details Download Subscribe to RSS Show/Hide Columns

Row	Saved	Status	Study Title	Conditions	Interventions	Locations
1	<input type="checkbox"/>	Recruiting	A Safety and Efficacy Study Evaluating CTX120 in Subjects With Relapsed or Refractory Multiple Myeloma	<ul style="list-style-type: none">Multiple Myeloma	<ul style="list-style-type: none">Biological: CTX120	<ul style="list-style-type: none">University of Chicago Chicago, Illinois, United StatesOregon Health and Science University Portland, Oregon, United StatesUniversity of Pennsylvania Philadelphia, Pennsylvania, United States(and 7 more...)
2	<input type="checkbox"/>	Recruiting	A Safety and Efficacy Study Evaluating CTX110 in Subjects With Relapsed or Refractory B-Cell Malignancies (CARBON)	<ul style="list-style-type: none">B-cell MalignancyNon-Hodgkin LymphomaB-cell LymphomaAdult B Cell ALL	<ul style="list-style-type: none">Biological: CTX110	<ul style="list-style-type: none">UCSF Medical Center San Francisco, California, United StatesMayo Clinic Jacksonville, Florida, United StatesEmory University Winship Cancer Institute Atlanta, Georgia, United States(and 14 more...)
3	<input type="checkbox"/>	Recruiting	A Safety and Efficacy Study Evaluating CTX130 in Subjects With Relapsed or Refractory Renal Cell Carcinoma	<ul style="list-style-type: none">Renal Cell Carcinoma	<ul style="list-style-type: none">Biological: CTX130	<ul style="list-style-type: none">Research Site 2 Duarte, California, United StatesResearch Site 5 Hartford, Connecticut, United StatesResearch Site 4 Houston, Texas, United States(and 4 more...)



MACQUARIE
University
SYDNEY · AUSTRALIA

Where does CRISPR-Cas9 come from?

Discovery and use of CRISPR in molecular biology?

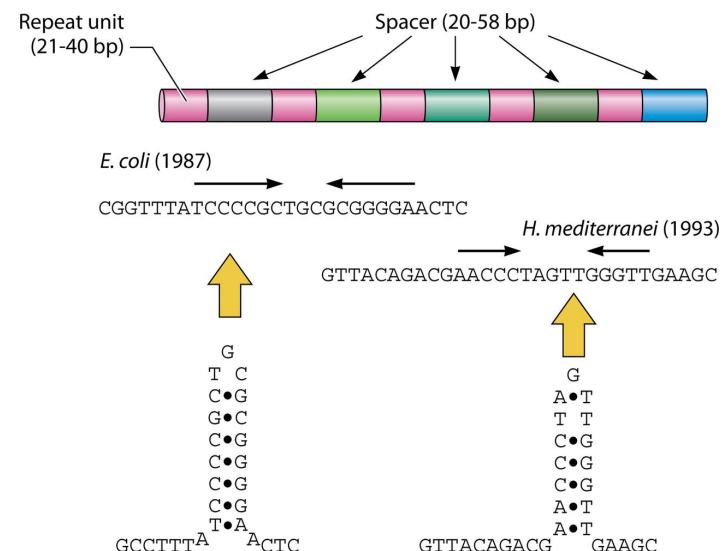
- In 2020 the Nobel Prize for Chemistry was awarded to Prof. Emmanuelle Charpentier and Prof. Jennifer Doudna for Pioneering CRISPR Technology
- Their research provided the first explanation for CRISPR sequences in bacterial genomes AND showed how useful they could be for molecular biology.



Where did CRISPR-Cas9 come from?

CRISPR - clustered regularly interspaced short palindromic repeats

- First discovered in *E. coli* in 1987
- The CRISPR array consists of a repeat unit, which is a constant DNA sequence for a given bacterial strain, and a spacer unit, which is variable between each repeat unit
- The repeat unit consists of a palindromic sequences, which forms a loop when the sequence is transcribed
- PAM sequence



Where did CRISPR-Cas9 come from?

CRISPR is a primitive bacterial immune system

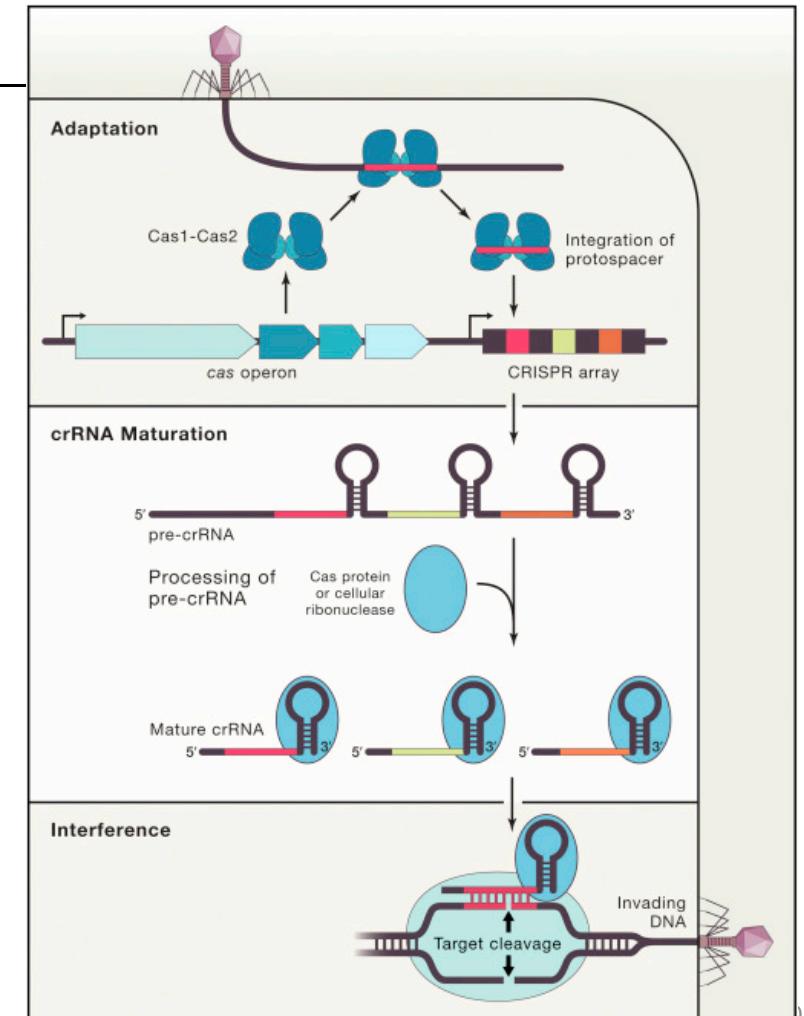
- Bacteria must respond to viral attack from bacteriophages
- Bacteria that survive keep a piece of the viral DNA as a memory, so they can fight off the virus more quickly next time
- When transcribed this nucleic acid memory can be used to quickly identify invading viral DNA



Where did CRISPR-Cas9 come from?

CRISPR is a primitive bacterial immune system

- After infection, bacteriophages release their genome into a host bacteria
- A small piece of DNA is cut out of the virus's genome with Cas1 and Cas2 endonucleases
- This piece of DNA can then be incorporated into the **CRISPR** array of the bacterial genome as a form of memory
- The **CRISPR** array is transcribed and processed to form guide RNA that can be used by Cas9 endonuclease to cut the genome of invading viruses



Components of CRISPR-Cas9

- Cas9 enzyme
- guide RNA
- Delivery system:

Non-Viral

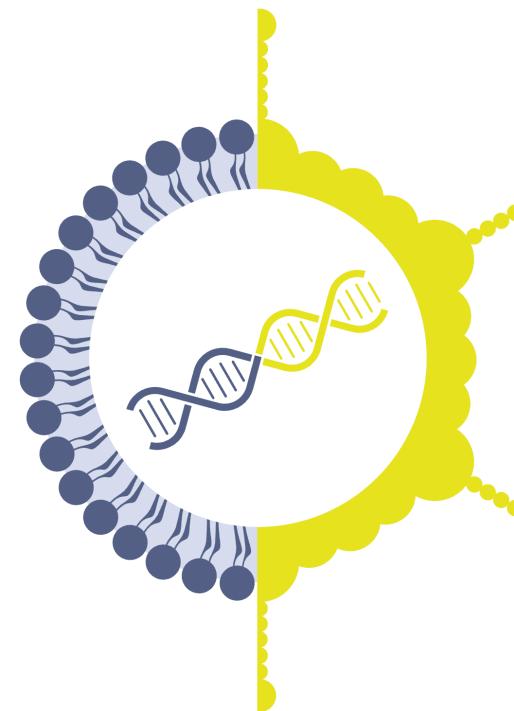
Lipid Nanoparticles (LNPs)

- Increased potency
- Expansion beyond liver delivery
- Improved tolerability



Messenger RNA (mRNA)

- Controlled duration of expression
- Tissue specificity
- Increased potency



Viral

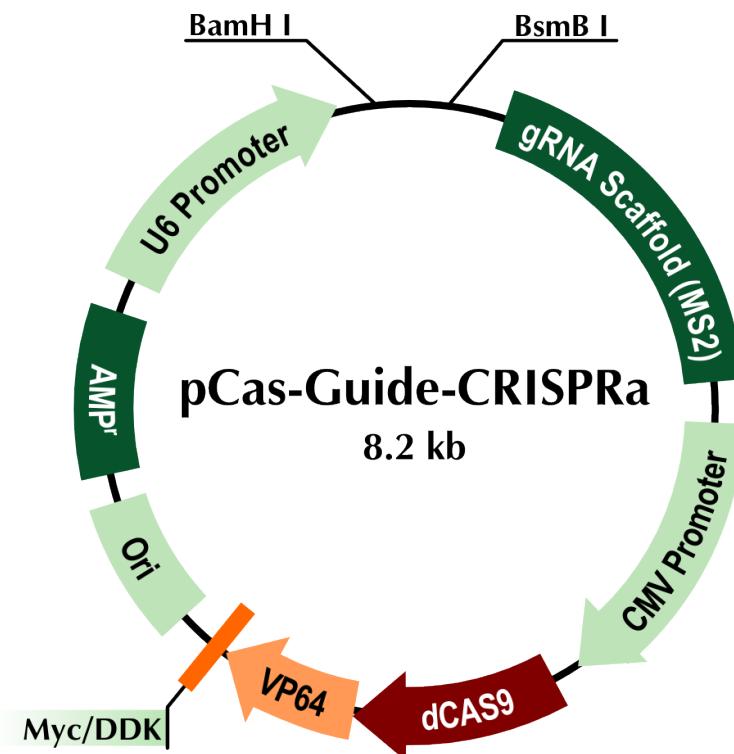
Adeno-Associated Virus (AAV)

- Improved tissue specificity
- Reduced immunogenicity
- Self-inactivation



Components of CRISPR-Cas9

- Cas9 enzyme
- guide RNA
- Delivery system:





MACQUARIE
University
SYDNEY · AUSTRALIA



**How can CRISPR-Cas9 be used
to genetically modify
organisms?**

How can CRISPR-Cas9 be used to genetically modify organisms?



- Knock out genes
 - Non-homologous end joining (NHEJ)
- Knock in DNA sequences
 - Homology directed repair (HDR)
- Base editing

How can CRISPR-Cas9 be used to genetically modify organisms?

Gene knockouts

- Potential uses
 - Remove a disease-causing gene
 - Knockout cancerous genes in tumours
 - Remove viral DNA from cells
- Non-homologous end joining (NHEJ)
 - CRISPR-Cas9 used to make a targeted double stranded break in the gene
 - Normal DNA repair machinery comes in to repair broken double stranded DNA
 - 5% of the time this repair creates in-del(insertion deletion) mutations that can make the protein untranslatable from the mRNA



Pax6 gene knockout

How can CRISPR-Cas9 be used to genetically modify organisms?

Gene knockouts

- Non-homologous end joining

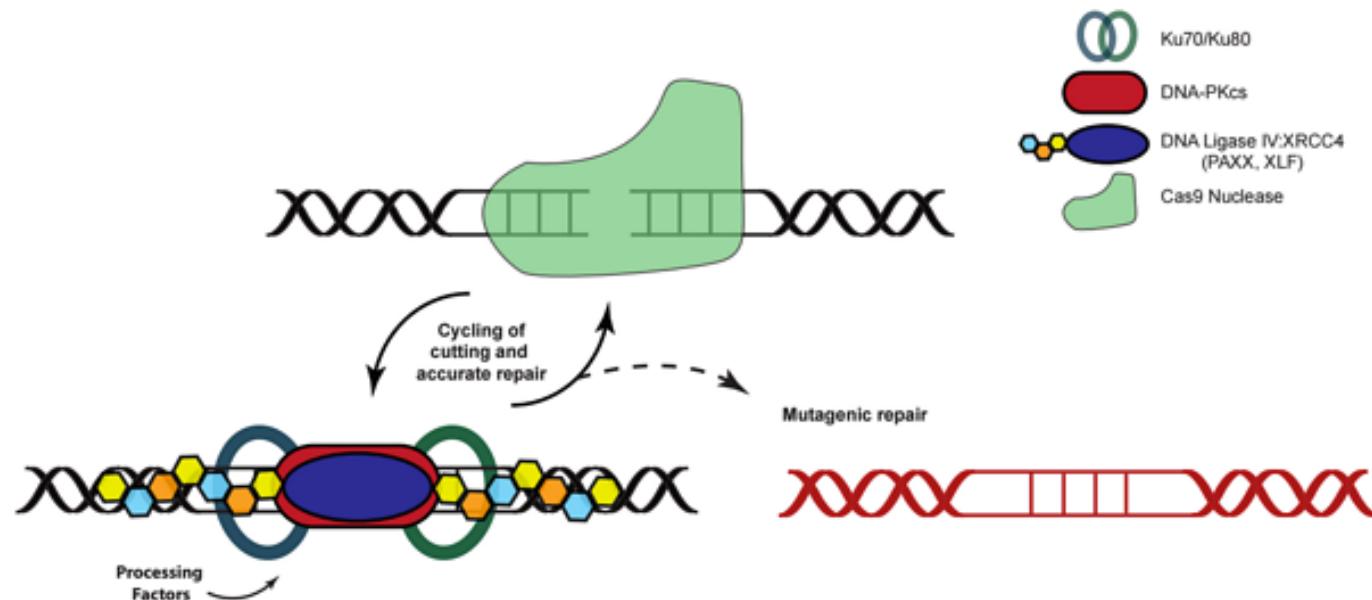


Image courtesy of D. Wyatt and D. Ramsden, UNC at Chapel Hill

How can CRISPR-Cas9 be used to genetically modify organisms?

Knock in DNA sequences

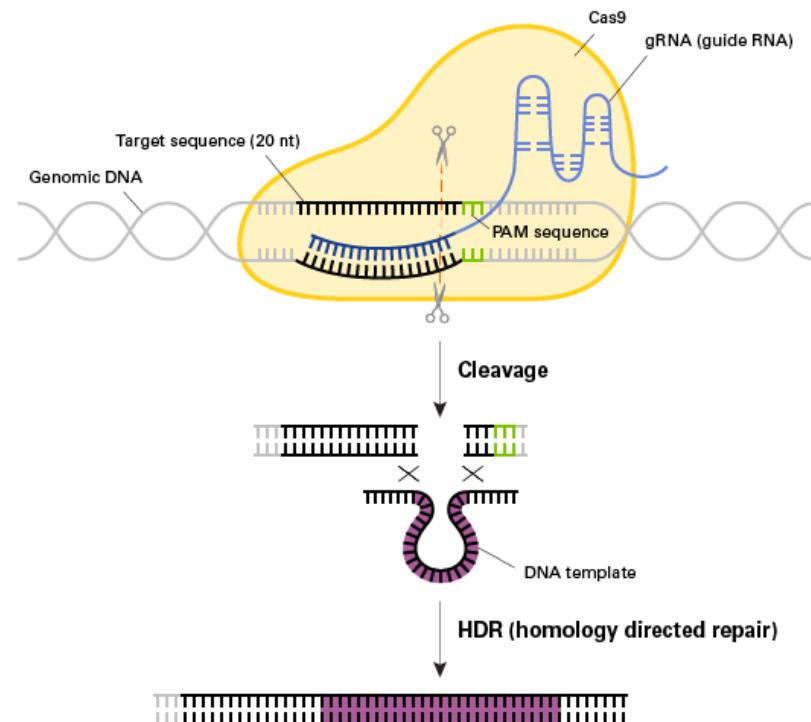
- Can be used to repair disease causing mutations
- Introduce novel pieces of DNA in a specific location
- Any application that requires introducing a new piece of DNA into the genome
- Homology Directed Repair (HDR)
 - CRISPR-Cas9 used to make a targeted double stranded break in the gene
 - New template strand binds homologously to the blunt DNA ends
 - DNA Polymerase fills in missing bases



How can CRISPR-Cas9 be used to genetically modify organisms?

Knock in DNA sequences

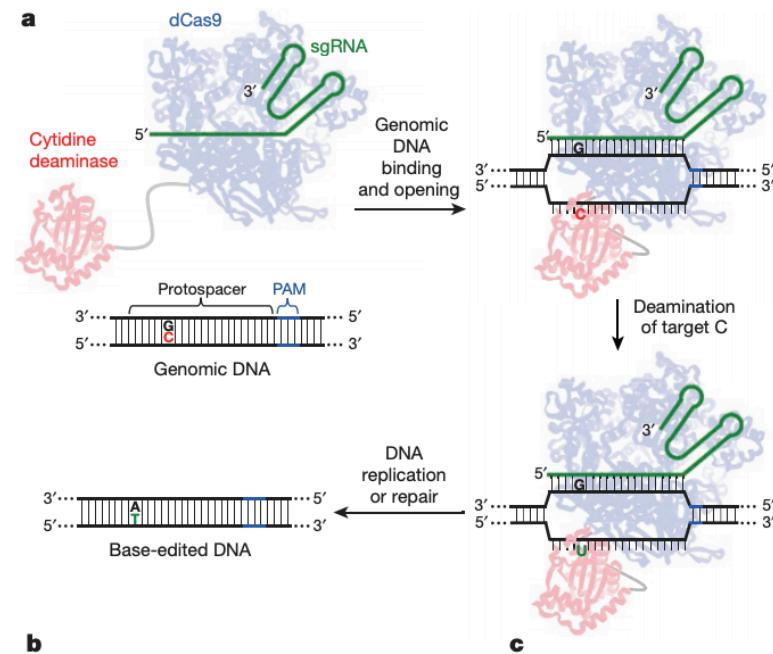
- Homology Directed Repair (HDR)



How can CRISPR-Cas9 be used to genetically modify organisms?

Base editing

- Uses a modified Cas9 enzyme that does not contain endonuclease activity
- Fusing this to a cytidine deaminase enzyme, which will introduce a C → T substitution
- Importantly, this does not involve any double stranded DNA breaks, so less chance for InDel mutations



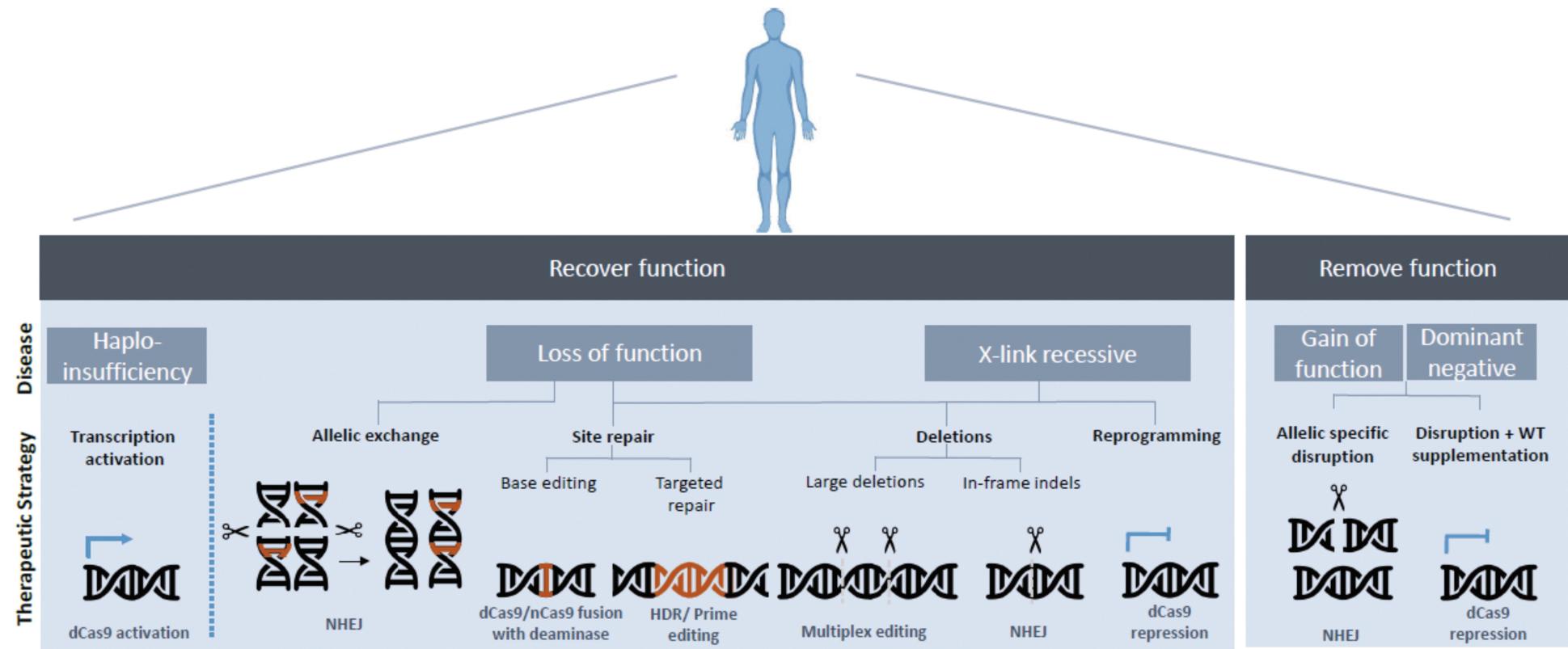


MACQUARIE
University
SYDNEY · AUSTRALIA

Some challenges

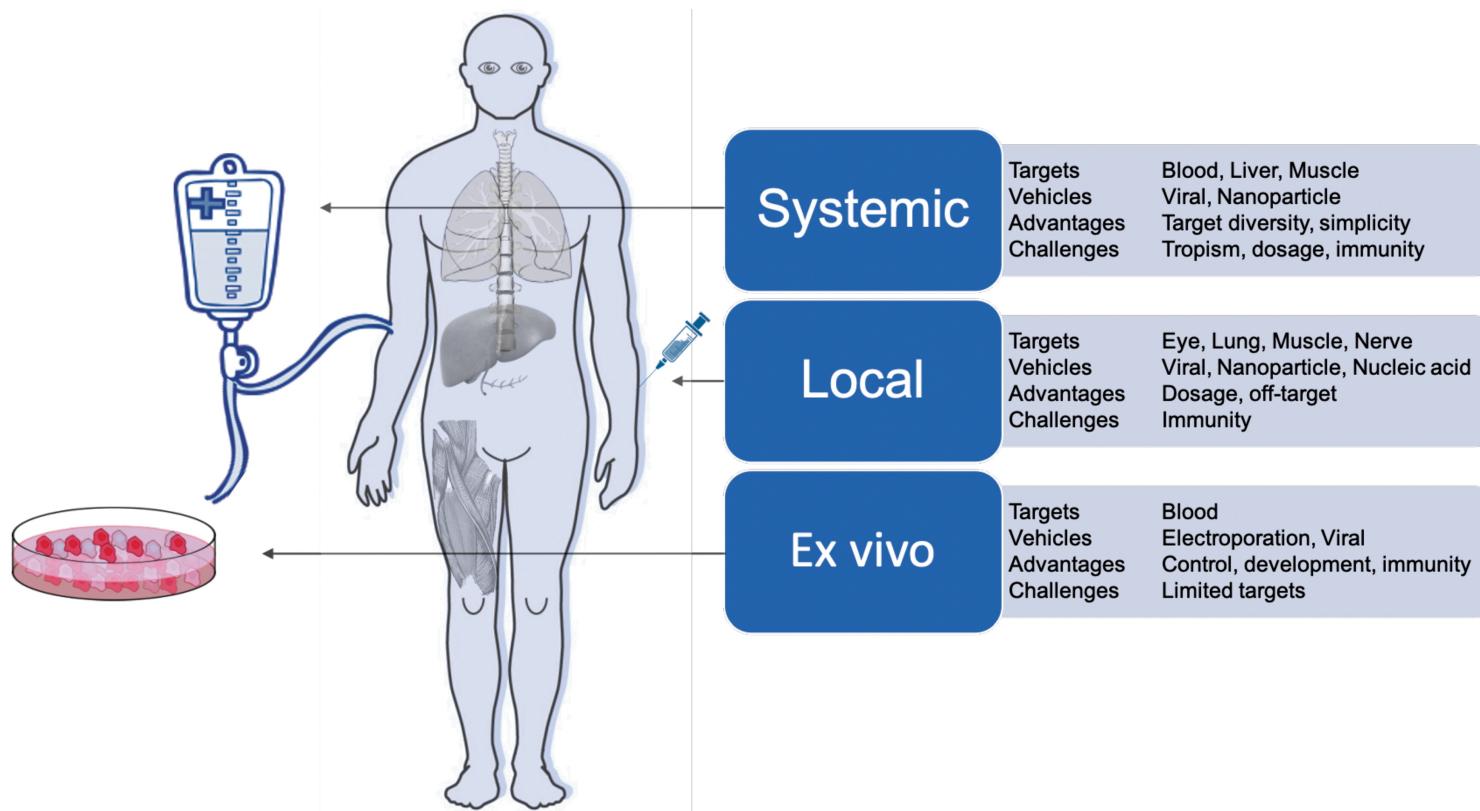
Challenges of CRISPR

PAYOUT – WHAT KIND OF CHANGE IS NECESSARY FOR TREATMENT



Challenges of CRISPR

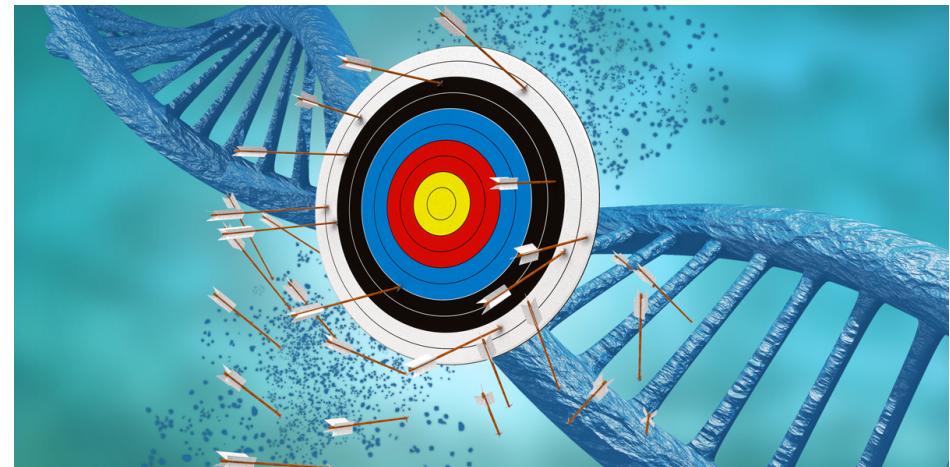
DELIVERY – HOW SHOULD WE INTRODUCE THE MACHINERY?



Challenges of CRISPR

OFF TARGET EFFECTS – WHAT UNINTENDED EDITING MIGHT WE DO

- It's always important to consider the possible off-target effects
- Typically, the greatest concern with CRISPR therapy as consequences can be serious
- Optimise guide RNA
- Optimise Cas enzyme for desired outcome
- Optimise delivery strategy



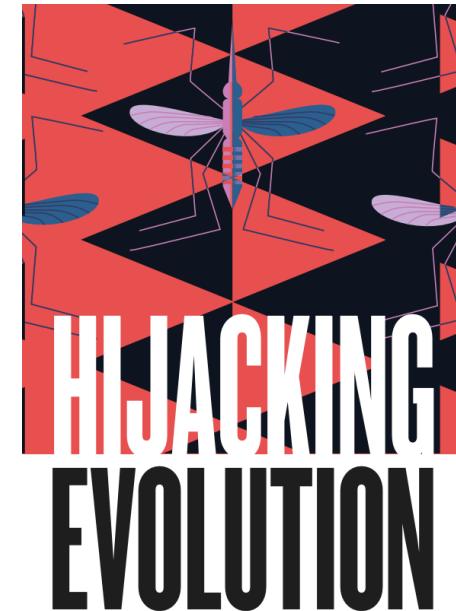


MACQUARIE
University
SYDNEY · AUSTRALIA

Uses of CRISPR

Uses of CRISPR

- Treat genetic diseases: knock outs, knock ins, base editing
- Treat infectious diseases:
 - Remove viral genome from cells: HIV, HPV, Herpes viruses, Epstein Barr virus, etc.
 - Manage bacterial antibiotic resistance
- Cancer therapies
 - Train the immune system to fight cancer
 - Delete cancer causing mutations in tumors
- Diagnostic tools
- Suppress mosquitos to control malaria, only-sons gene drive



Gene-drive technology could alter the genome of an entire species. Researchers need to answer these key questions before deploying it in the wild.

BY MEGAN SCUDELLARI

Learning objectives



1. Understanding the basics of CRISPR-Cas9
2. What led to the discovery of CRISPR-Cas9 for molecular biology
3. How can CRISPR-Cas9 be used to genetically modify organisms
 1. Knock-out (NHEJ)
 2. Knock-in (HDR)
 3. Base editing
 4. Challenges
4. Uses of CRISPR