

GUEST LECTURE

CRISPR-Cas9

From a bacterial immune system to approved medicine—explained step by step
Editing the Code of Life

Jain (Deemed-to-be University) | 26.09.2026

Department of Chemistry and Biochemistry

Presented by : Avijit Maity | Senior Scientist – Protein Design Pvt. Ltd.



What We'll Cover

- 1 Why CRISPR matters
- 2 CRISPR biology & how it works
- 3 Newer tools: base & prime editing
- 4 Real-world applications
- 5 Ethics & regulation
- 6 Hands-on: design a guide RNA
- 7 Key takeaways & Q&A

BY THE END, YOU'LL BE ABLE TO

- ✓ Explain how bacteria use CRISPR as an immune system
- ✓ Describe how Cas9 finds and cuts DNA
- ✓ Compare NHEJ vs HDR repair and newer editors
- ✓ Design a guide RNA using a free online tool

From Lab Discovery to Approved Medicine in ~10 Years



2012

Doudna&Charpentier show Cas9 can be programmed to cut any DNA we choose.



2020

Doudna & Charpentier win the Nobel Prize in Chemistry for CRISPR-Cas9.



2023

First CRISPR medicine

Casgevy is approved to treat sickle cell disease & β -thalassemia.



IN SIMPLE WORDS

CRISPR works like “Find & Replace” in MS Word —but for DNA. We type the sequence to find, and Cas9 goes there and cuts it.

Why CRISPR Beat the Older Gene-Editing Tools

ZFNs & TALENs

Protein-based (before 2012)

CRISPR-Cas9

RNA-guided(2012 –present)

How it finds DNA

A custom-built protein

A short guide RNA (~20 letters)

New target?

Engineer a whole new protein

Just rewrite the guide RNA

Design time

Weeks to months

Hours to days

Cost & skill

Expensive; needs specialists

Cheap, off-the-shelf; many targets at once



IN SIMPLE WORDS

Old tools = forging a brand-new key for every lock. CRISPR = one master key (Cas9) —you only change the address tag (guide RNA).

Discovery Timeline: 1987 → 2020

1987

Strangerepeating DNA sequences spotted in *E. coli*

Ishino et al.

2012

Cas9reprogrammed into a simple 2-component editing tool

Doudna & Charpentier

2020

NobelPrizein Chemistry

Doudna & Charpentier

2005–07

Spacers found to be viral DNA → CRISPR is a bacterial defence

Mojica, Barrangou

2013

First CRISPR editing in human & mouse cells

Zhang, Church labs

PART 1 • THE BIOLOGY

How Bacteria Invented Gene Editing

Before it was a lab tool, CRISPR was an immune system — protecting bacteria from viruses for millions of years.



CRISPR in Nature: A Bacterial Immune System



1. Invasion

A virus (bacteriophage) injects its DNA into the bacterium.



2. Memory

The bacterium cuts out a small piece of viral DNA and saves it as a new “spacer” in its CRISPR array.



3. Immunity

If the same virus returns, RNA copied from that spacer guides Cas proteins to the viral DNA — and cuts it.



IN SIMPLE WORDS

It's like a police “most-wanted” photo album: bacteria keep a mugshot of every virus that attacked them —and recognise it instantly next time.

Inside the CRISPR Locus: A Genetic Memory Bank



R Repeats

The same short sequence, again and again. They act like separators between memories.

S Spacers

Unique snippets of DNA from past viruses —each one is a stored “mugshot”.

cas cas genes

Code for the Cas proteins that capture new spacers and cut invading DNA.



KEY IDEA

Scientists copied this idea: keep the Cas9 “scissors”, but replace the viral spacer with a guide RNA of OUR choosing —so it cuts wherever we want.

8 Key Terms You'll Hear Today

1

Cas9

The enzyme that cuts DNA —the “scissors”.

2

Guide RNA (sgRNA)

~20-letter RNA that leads Cas9 to its target.

3

PAM

Short signal (e.g. NGG) next to the target that Cas9 must see first.

4

Spacer

A stored piece of viral DNA in the CRISPR array.

5

Double-strand break

A cut through BOTH strands of the DNA helix.

6

NHEJ

Quick, messy repair —often switches a gene off.

7

HDR

Precise repair that copies from a template DNA.

8

Indel

A small insertion or deletion of DNA letters.

PART 2 · THE MECHANISM

How CRISPR-Cas9 Works

Three simple steps:

1 FIND

2 CUT

3 REPAIR



Three Parts of the CRISPR-Cas9 System



Cas9 protein

The molecular scissors

An enzyme that opens up the DNA and cuts both strands at one precise spot.



Guide RNA (sgRNA)

TheGPS

A ~20-letter RNA, designed by us, that tells Cas9 exactly where to go.



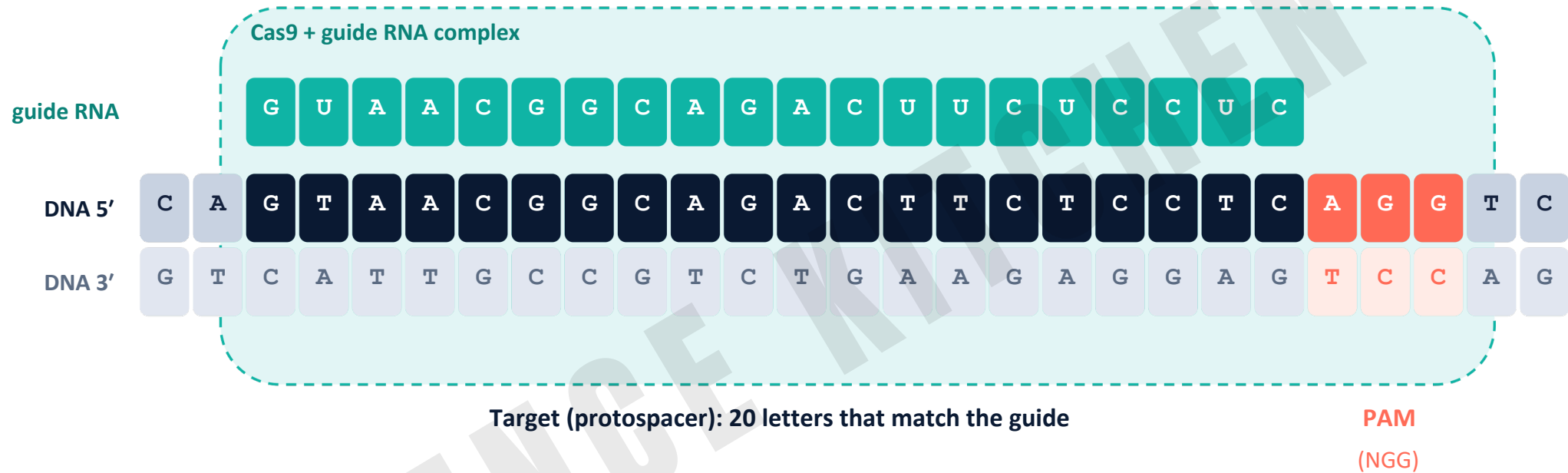
PAM sequence

The docking signal

A short motif (e.g. NGG) beside the target. Cas9 must find it before it can cut.

Cas9 + guide RNA = a programmable “search-and-cut” machine. Change the guide → change the target.

Finding the Target: PAM + Base-Pairing



The golden rule

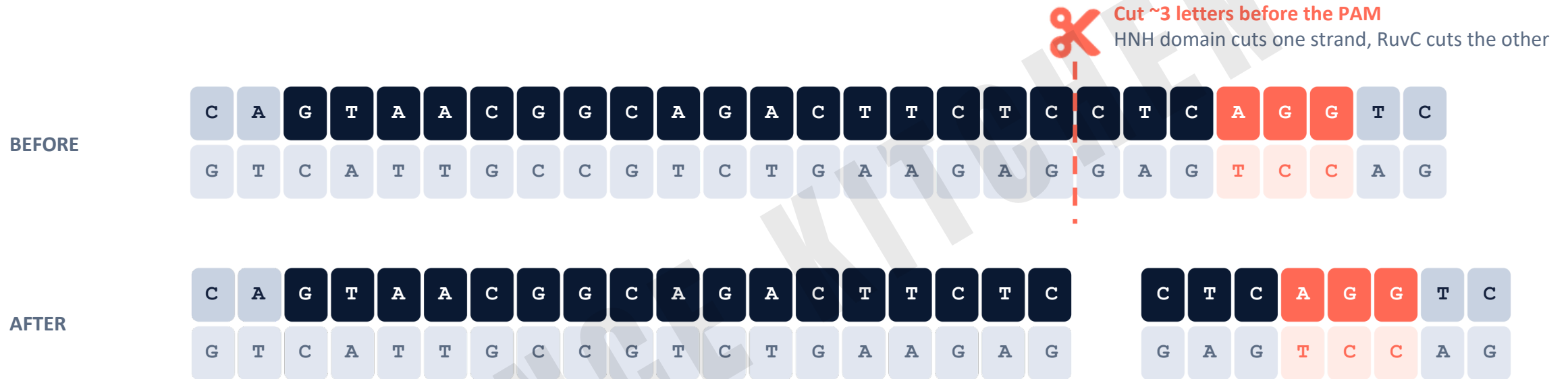
NoPAM → nocut, even if the guide RNA matches perfectly.



IN SIMPLE WORDS

PAM is a parking spot: Cas9 can only stop where there's one, then checks if the address (guide) matches.

Cutting the DNA: The Double-Strand Break



What happens next?

Cas9's job is done. A broken chromosome is an emergency, so the cell's own repair machinery rushes in —and the way it repairs decides the final edit.

The Cell Repairs the Break: Two Paths, Two Results



NHEJ

Non-Homologous End Joining

FAST & DEFAULT

- No template needed —just glues the ends back
- Most common pathway in most cells
- Error-prone: adds or removes a few letters (indels)

Result: Gene KNOCK-OUT (switch off)



HDR

Homology-Directed Repair

PRECISE & SLOWER

- Needs a donor DNA template we supply
- Works mainly in dividing cells
- Copies the template exactly

Result: Gene CORRECTION / KNOCK-IN



IN SIMPLE WORDS

NHEJ = taping a torn page back together (fast, but a few words get lost). HDR = reprinting the page from a master copy (slow, but exact).

PART 3 · BEYOND CAS9

New Tools & the Real World

Base editing, prime editing, CRISPR diagnostics —and where CRISPR is already changing lives.



Base Editing: Changing a Single DNA Letter

Cas9 “nickase” (cut only one strand) + **deaminase enzyme** = chemically changes one base into another — with no double-strand break.

~30%

of known human genetic diseases are caused by a single wrong DNA letter — including sickle cell disease.

BEFORE



wrong letter

AFTER



fixed: A → G



IN SIMPLE WORDS

Like using a pencil eraser to fix one typo — instead of tearing the page and taping it back. Safer, because the DNA is never fully cut.

Prime Editing: “Search & Replace” for DNA

Cas9 nickase + reversetranscriptase + pegRNA

The pegRNA does two jobs: it finds the target AND carries the new sequence to write in. No double-strand break, no donor template.



1. Search

pegRNA finds the target site and Cas9 nicks one DNA strand.



2. Write

Reverse transcriptase copies the new sequence from the pegRNA into DNA.



3. Replace

The cell keeps the newly-written strand and removes the old one.

12 / 12 base swaps possible



IN SIMPLE WORDS

Base editing = a pencil for one letter. Prime editing = full “Find & Replace”: all letter swaps plus small insertions & deletions.

Cas12 & Cas13: From Editing to Diagnostics

Not every Cas protein is used for editing. **Cas12** (finds DNA) and **Cas13** (finds RNA) have a useful “side effect”:



1. Find

Cas12/13 locates the pathogen's DNA or RNA in a sample.



2. Activate

Once switched on, it shreds ANY nearby reporter molecules.



3. Signal

Cut reporters give a colour line on a paper strip — positive!

SHERLOCK & DETECTR

Paper-strip or tube tests built on this idea — used for rapid, low-cost detection of pathogens, including COVID-19.

Why microbiologists care

Detects a specific bacterial or viral sequence in under an hour — no PCR machine needed. Ideal for field and point-of-care testing.

CRISPR in Medicine



Approved gene therapy

Casgevy (exa-cel) edits a patient's own stem cells to treat sickle cell disease & β -thalassemia —approved in the UK, US & EU (2023–24).



Cancer immunotherapy

CRISPR-enhanced CAR-T cells are engineered to hunt tumour cells better —including “off-the-shelf” donor versions.



In-body (in vivo) editing

Editing tools injected directly to treat liver disease (transthyretin amyloidosis), inherited blindness and more — in trials.



Disease modelling

CRISPR-made cell lines and animal models help study diseases and speed up drug discovery.

CRISPR in Agriculture & Microbiology



Agriculture

- 1 Disease-and drought-resistant crops
- 2 Longer shelf life & better nutrition
- 3 Precision breeding without adding foreign DNA



Microbiology & industrial biotech

- 1 Engineered probiotics & phage-based antimicrobials
- 2 Tools to study antibiotic-resistance genes
- 3 Optimised microbes for biomanufacturing

Ethics & Governance



Somatic vs germline

Somatic edits affect only the patient. Germline edits (sperm, egg, embryo) are passed to future generations — far more restricted.



The He Jiankui case (2018)

A researcher in China created the first gene-edited babies without proper oversight —leading to global condemnation, a prison sentence and stricter rules.



Rules differ by country

In India, genome-editing research follows ICMR & DBT guidelines; germline editing for reproduction is not permitted.



Access & equity

Casgevy costs roughly \$2–2.2 million per patient. Who can actually afford CRISPR medicine?

DEBATE: Should editing human embryos ever be allowed —for example, to prevent a deadly disease?

PART 4 · HANDS-ON

Let's Get Hands-On!

Design a guide RNA using a free bioinformatics tool.

Open your laptop → chopchop.cbu.uib.no



Designing a Guide RNA: 5 Easy Steps

Tool: CHOPCHOP —free, no login

Target gene: HBB (β -globin —the sickle cell gene)

01

Open the tool

Go to chopchop.cbu.uib.no in your browser. No account needed.

02

Set the target

Genome: Homo sapiens (hg38). Target type: Gene. Enter HBB.

03

Choose CRISPR type

Select CRISPR/Cas9, keep default knockout settings, click Submit.

04

Review the guides

A ranked list of guide RNAs appears, each with a genome view.

05

Pick your best guide

Sort by efficiency and note your top 2–3 candidates.

Reading Your Results: What Makes a Good Guide?

High efficiency

+

High specificity

+

40–60% GC

=

A great guide RNA



Efficiency score

How well the guide is predicted to cut here. Higher = more edited cells.



Specificity (off-target) score

Fewer similar matches elsewhere in the genome = a safer guide.



GC content

Aim for roughly 40–60%. Too low or too high can weaken the guide.



Restriction-site overlap

Handy later to screen edited colonies with a simple restriction digest.

Pro tip: paste your top guide into CRISPOR (crispor.tefor.net) for a second opinion before ordering a real guide RNA.

5 Things to Remember

- 1 CRISPR is a borrowed bacterial immune system —not invented from scratch.
- 2 Guide RNA + PAM = cheap, programmable precision.
- 3 The repair pathway (NHEJ vs HDR) decides the final edit.
- 4 Base & prime editors edit more gently —no double-strand break.
- 5 The science is moving faster than the rules —ethics must keep up.

FREE TOOLS & RESOURCES

CHOPCHOP

chopchop.cbu.uib.no —guide design

CRISPOR

crispor.tefor.net —off-target check

Benchling

benchling.com —free academic suite

Addgene CRISPR 101

addgene.org/crispr—plasmids & guides

NEB CRISPR

neb.com—protocols & enzymes

Thank You

Avijit Maity | Senior Scientist

✉ amaityexplorer786@gmail.com

