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# connecting people with science

## CRISPR Informatics Course

07-Oct-2024 to 11-Oct 2024

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# CRISPR Informatics Course

## Determining Indels generated by CRISPR-Cas9

## and

## Validation of knock-out / knock-ins

Sandeep Rajan , Technical Specialist

09-Oct-2024

# What to expect in this session

The session will cover the following :

- Basics of gene editing using CRISPR-Cas9
- Brief overview on methods to determine Cas9 induced breaks
- Focus on using freeware tool – TIDE to determine editing efficiencies and outcomes
  - Practical demonstration
- Brief overview on using Cas9 to facilitate- gene knock-in / tagging
  - Practical demonstration

After this session you should be able to :

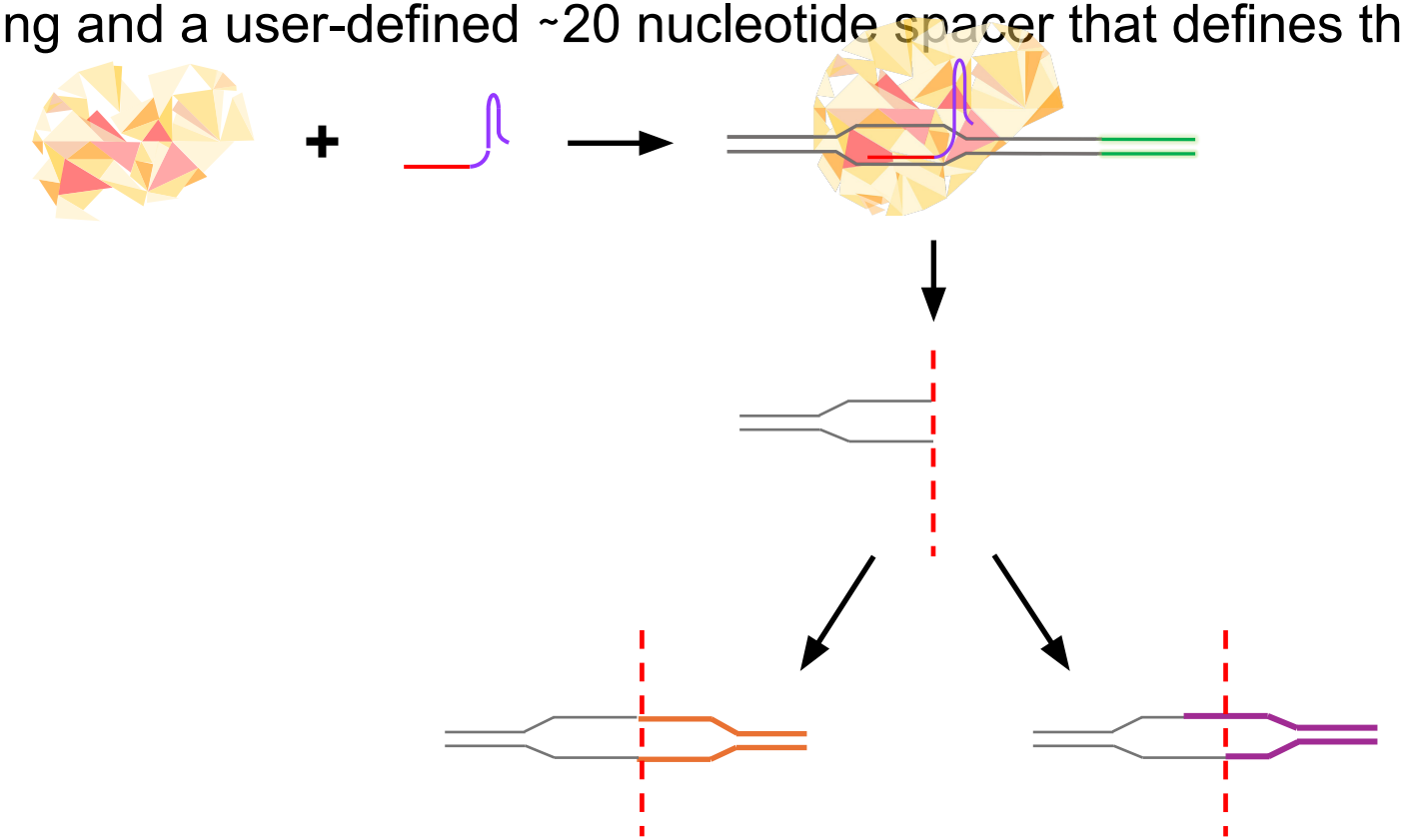
- Use TIDE to determine gRNA efficiency in your cell lines / samples
- Understand information contained on sanger sequencing trace files
- Determine knock-out/ knock-in/ SNV variations occurring with genomic region of interest

# CRISPR-Cas9 editing

Clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR associated proteins (Cas) derived from microbial adaptive immune system to protect against viruses and plasmids.

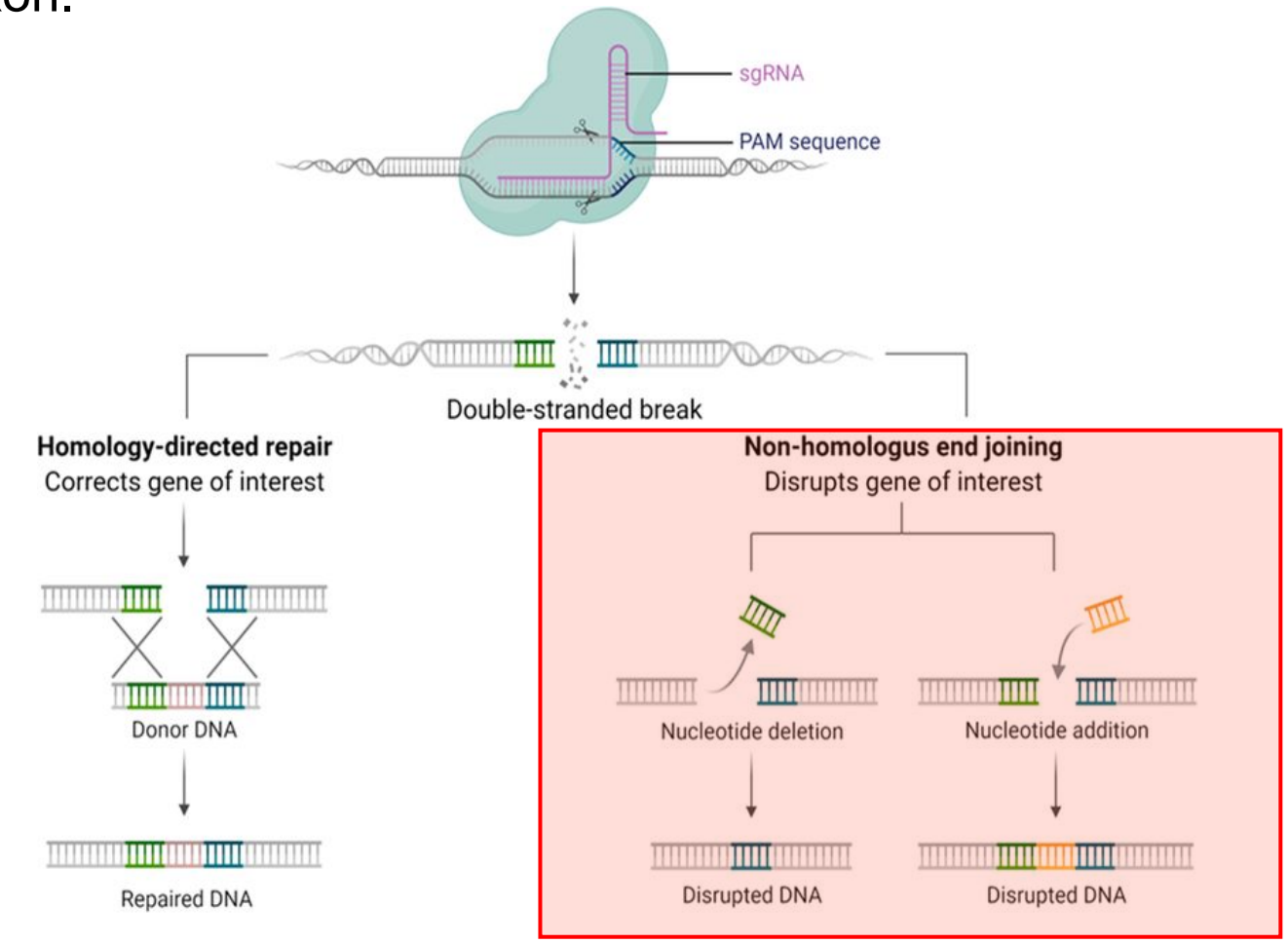
Cas9 is the endonuclease that can generate DSB when guided by the target sequence (gRNA)

gRNA / sgRNA is a short synthetic RNA composed of a scaffold sequence necessary for Cas-binding and a user-defined ~20 nucleotide spacer that defines the genomic target to be modified.



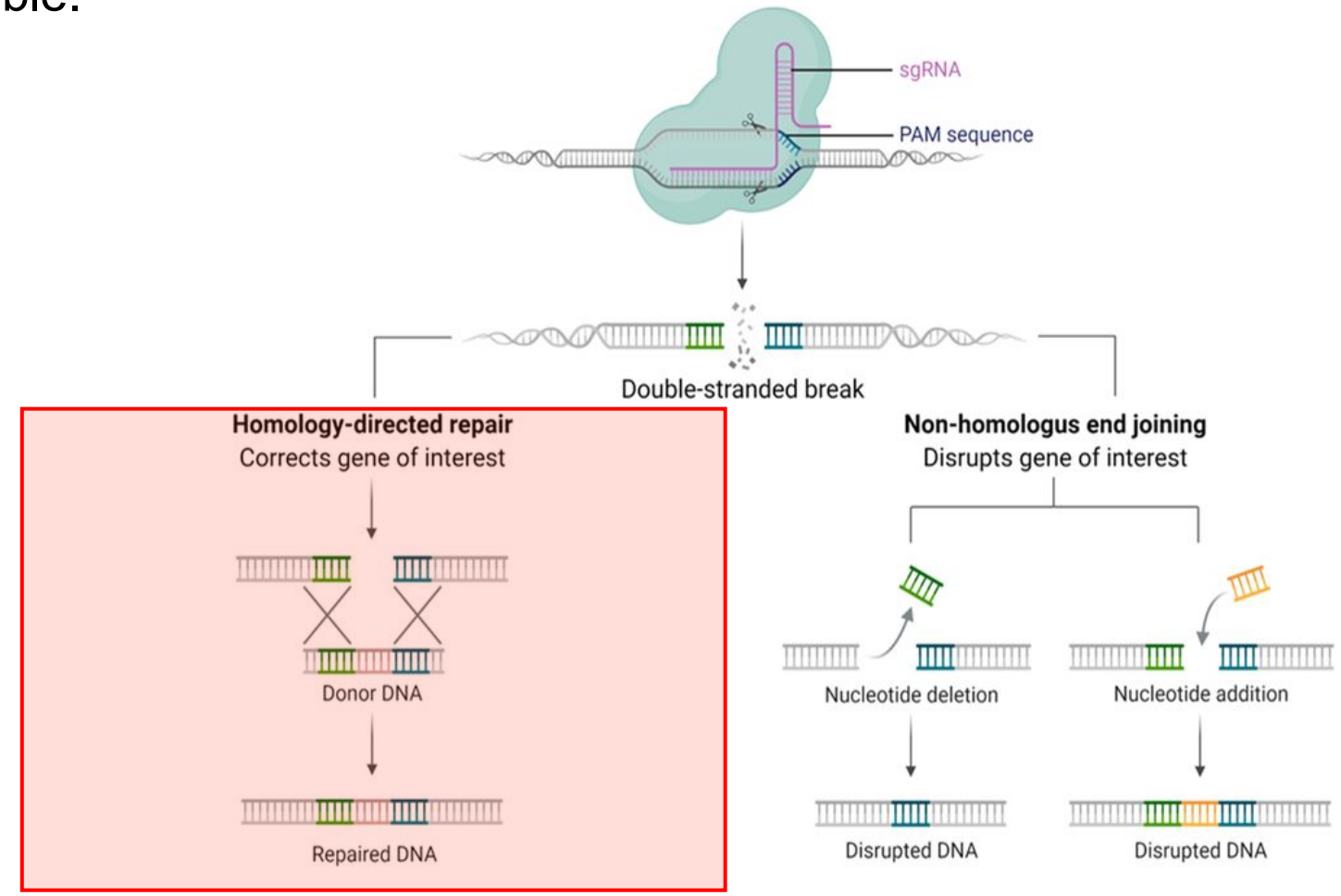
# CRISPR-Cas9 editing – Generating Gene KOs

CRISPR-Cas9 system can be used to generate knock-out cell lines. An insertion or deletion induced by a single guide RNA is often used to generate knock-out cells, however, some cells express the target gene by skipping the disrupted exon, or by using a splicing variant, thus losing the target exon.



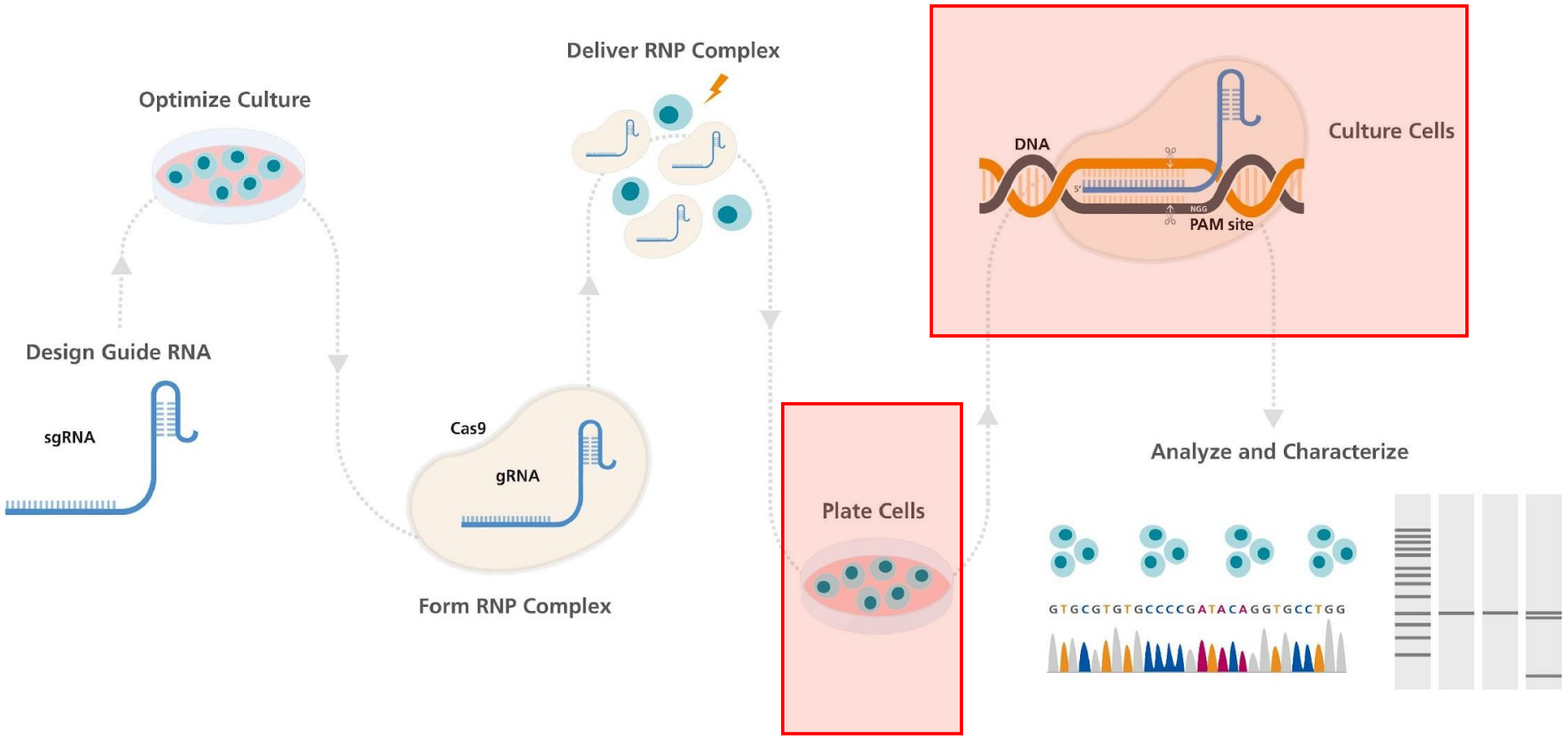
# CRISPR-Cas9 editing – Generating novel lines

CRISPR/Cas9 gene knock-in or gene replacement system is designed to facilitate the insertion, removal, or replacement of a specific gene(s) within a given genome. The site-specific insertion of protein tags, modified promoters, and other regulatory sequences are also possible.



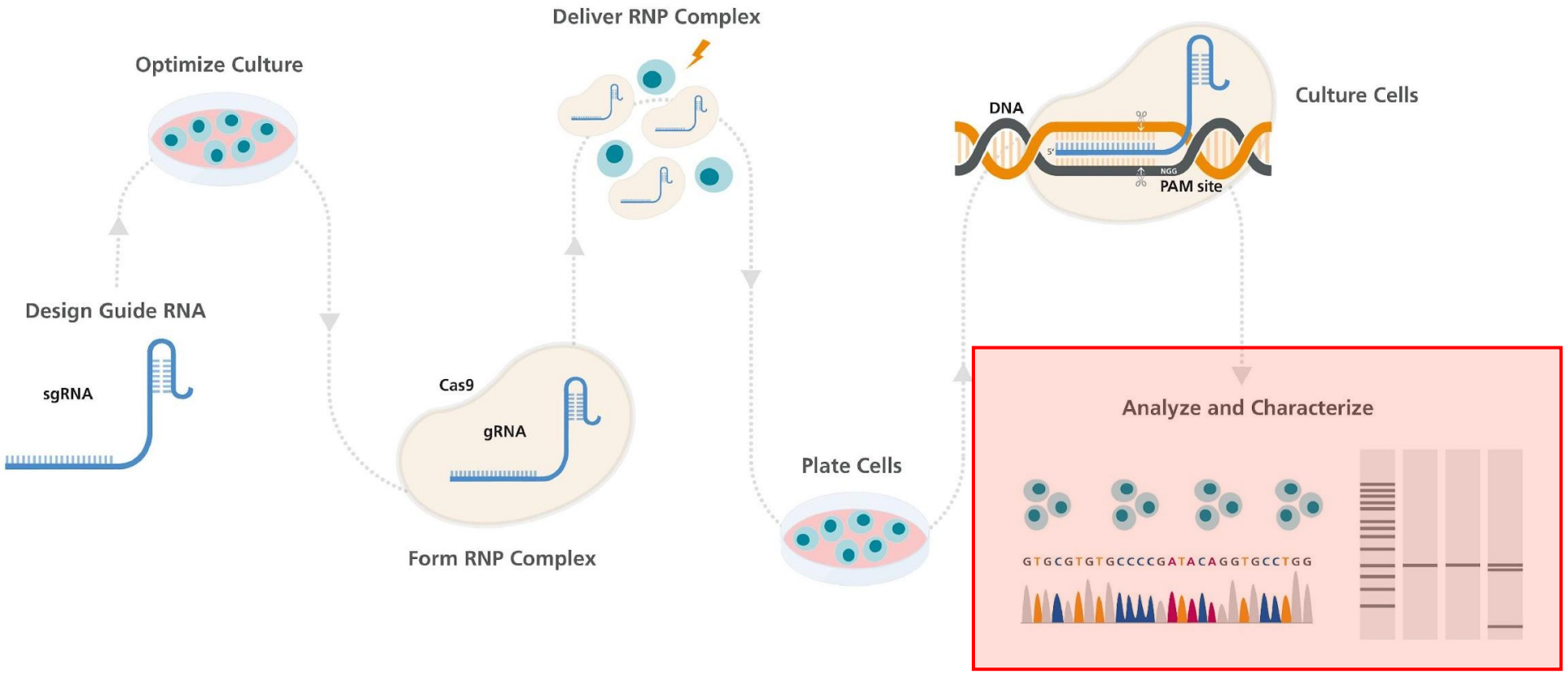
# CRISPR-Cas9 edits - how to validate them

The simplest but often the least specific method of identifying successful CRISPR genome editing is to observe phenotypes of edited cells. In some experiments, the expected phenotype from the gene editing process is known.



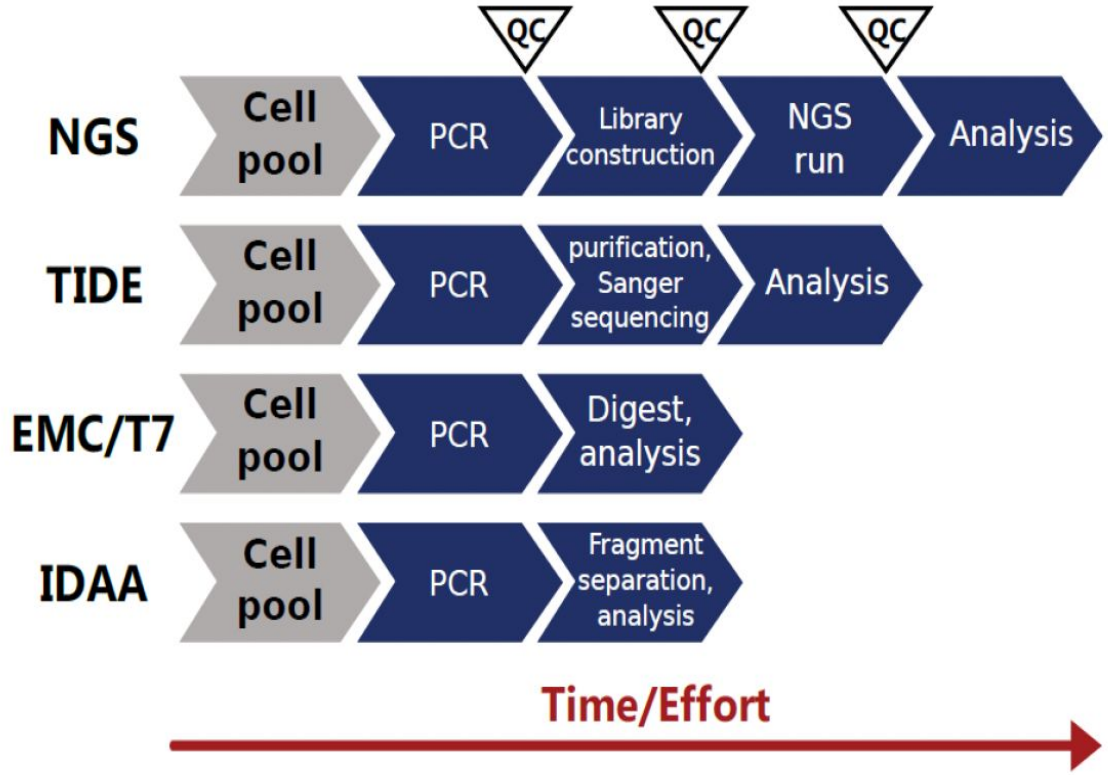
# CRISPR-Cas9 edits - how to validate them

A more in depth view of the genomic edit that translates to the phenotype can be determined by investigating the genetic contact in and around the edited sites.



# CRISPR-Cas9 edits – what are the methods are available and what do they provide

To determine the edits and its subsequent effects a range of techniques are available

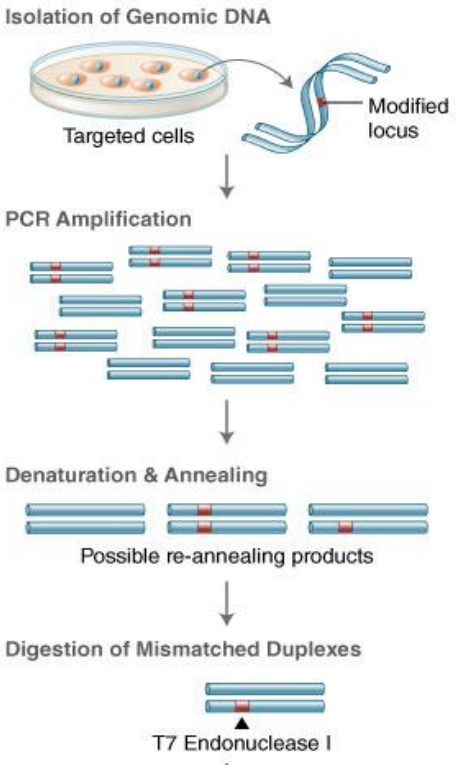



| | Sensitivity | Resolution | Scalability | Cost |
|---|---|---|---|---|
| NGS | **high** | **high** | low | high |
| TIDE | medium | medium | medium | medium |
| EMC/T7 | low | low | **high** | **low** |
| IDAA | **high** | medium | **high** | **low** |

# Understanding the outcomes of genome editing

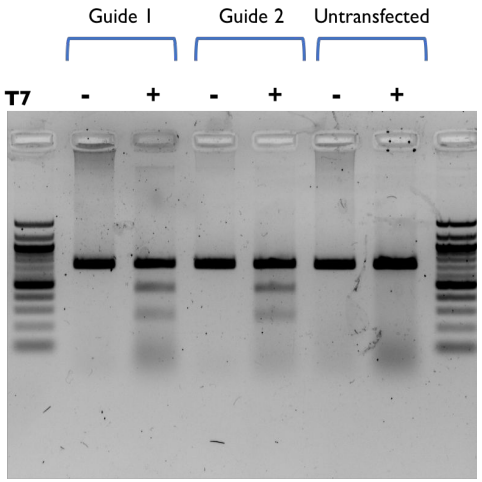
## Surveyor nuclease assay

- **Surveyor** nuclease **assay** is an enzyme mismatch cleavage **assay**
- Can detect single base mismatches or small insertions or deletions (indels).
- **Surveyor** nuclease is part of a family of mismatch-specific endonucleases that were discovered in celery (CEL nucleases).




## T7 endonuclease 1

- T7 Endonuclease I recognizes and cleaves non-perfectly matched DNA.
- PCR products are annealed and digested with T7 Endonuclease I.
- Fragments are analyzed to determine the efficiency of genome targeting.



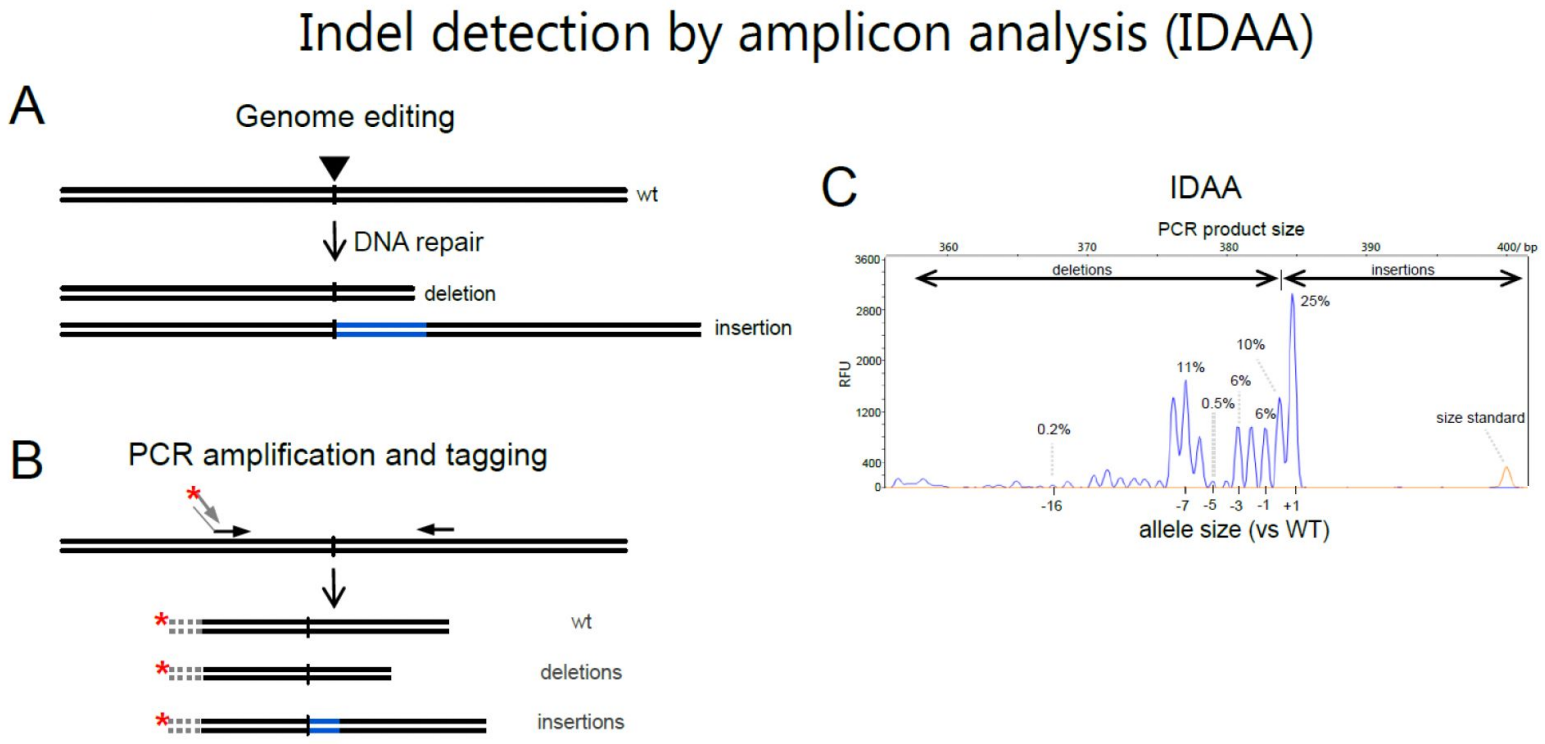

HEK293 cells

# Understanding the outcomes of genome editing

**IDAA (Indel detection by Amplicon Analysis)**

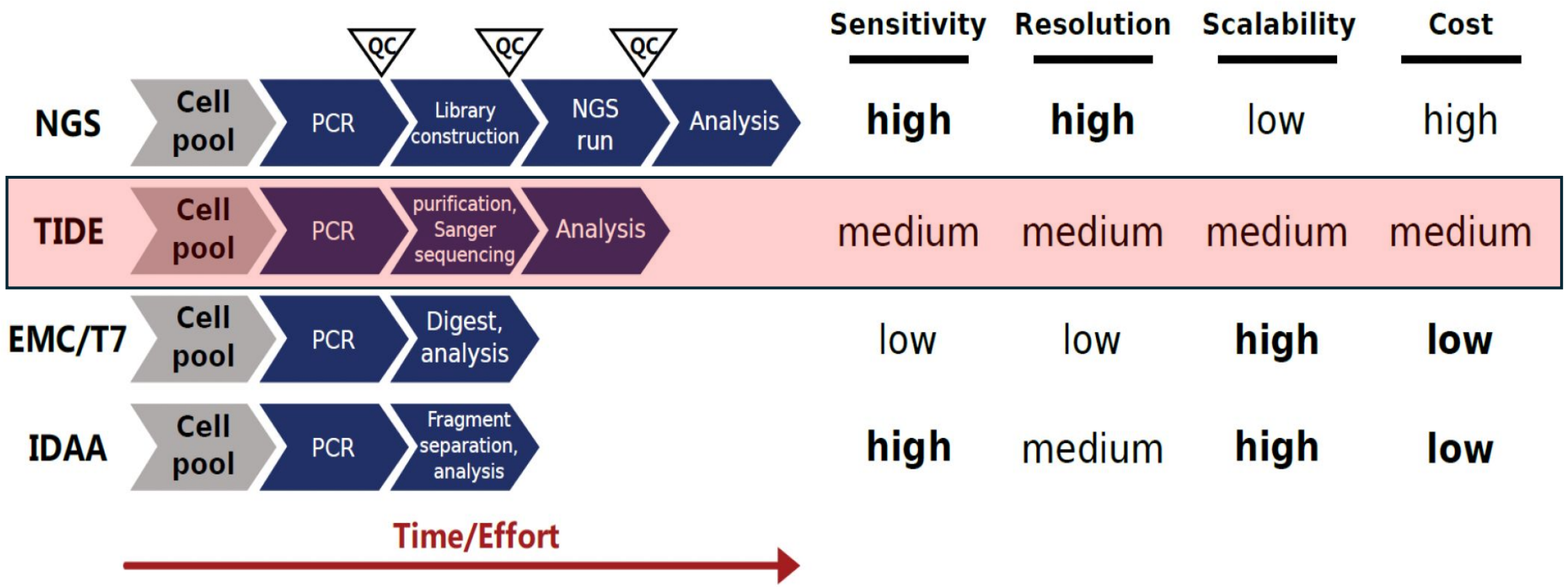
- An alternative non-sequencing based proprietary method that enables sensitive and quantitative detection of InDels.

- The methodology is based on a novel tri-primer PCR amplification method and capillary electrophoretic fragment analysis using a standard DNA sequenator instrument.



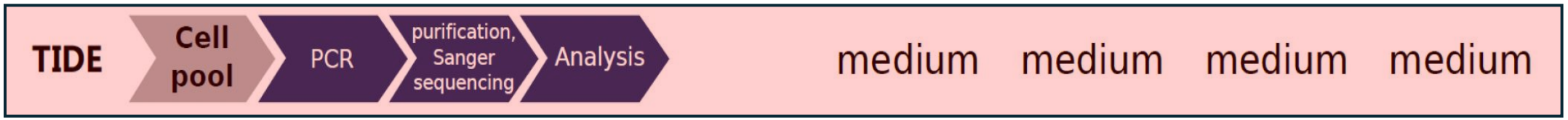
# Understanding the outcomes of genome editing

**TIDE – Tracking of Indels by Decomposition**



# Understanding the outcomes of genome editing

**TIDE – Tracking of Indels by Decomposition**

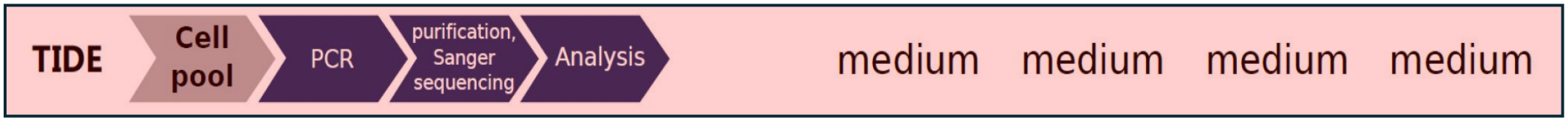


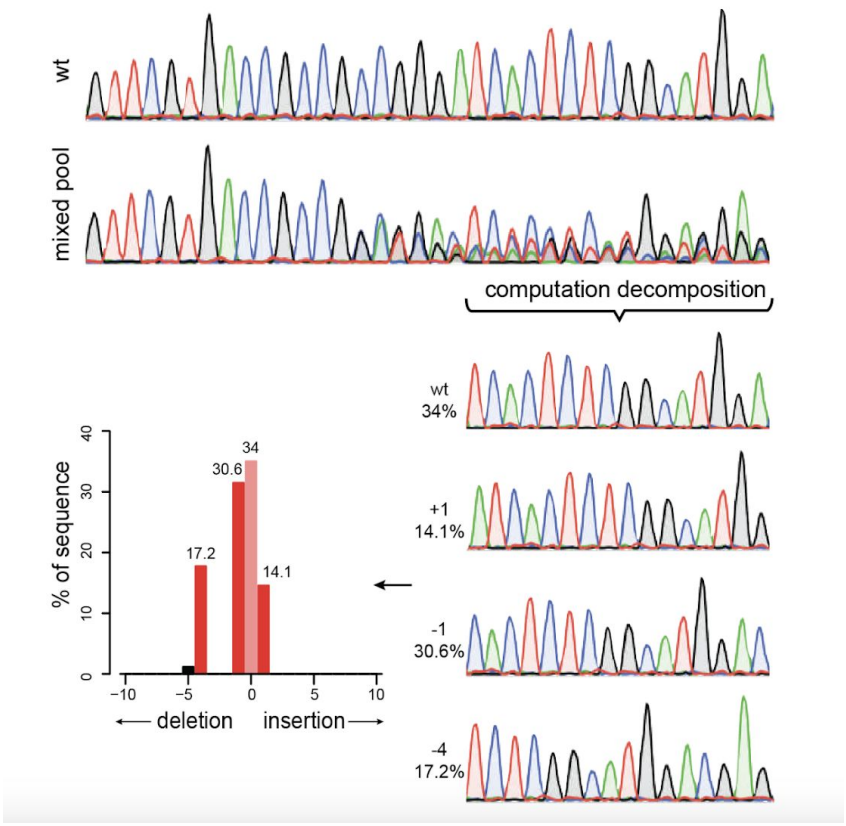

- TIDE is a simple and accurate assay to precisely determine the spectrum and frequency of targeted mutations generated in a pool of cells by genome editing tools such as CRISPR/Cas9, TALENs and ZFNs.

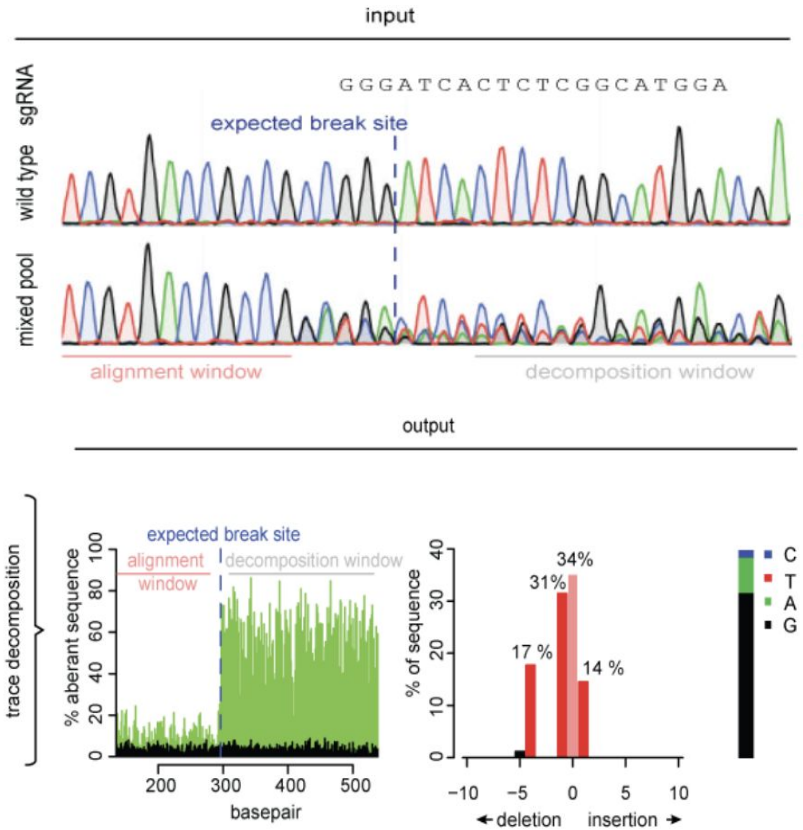
- TIDE requires only standard molecular biology reagents and involves three simple steps:
  - One pair of standard PCR reactions.
  - One pair of standard capillary ("Sanger") sequencing reactions.
  - Analysis of the two resulting raw sequencing files using the TIDE web tool.

# Understanding the outcomes of genome editing

## TIDE – Tracking of Indels by Decomposition

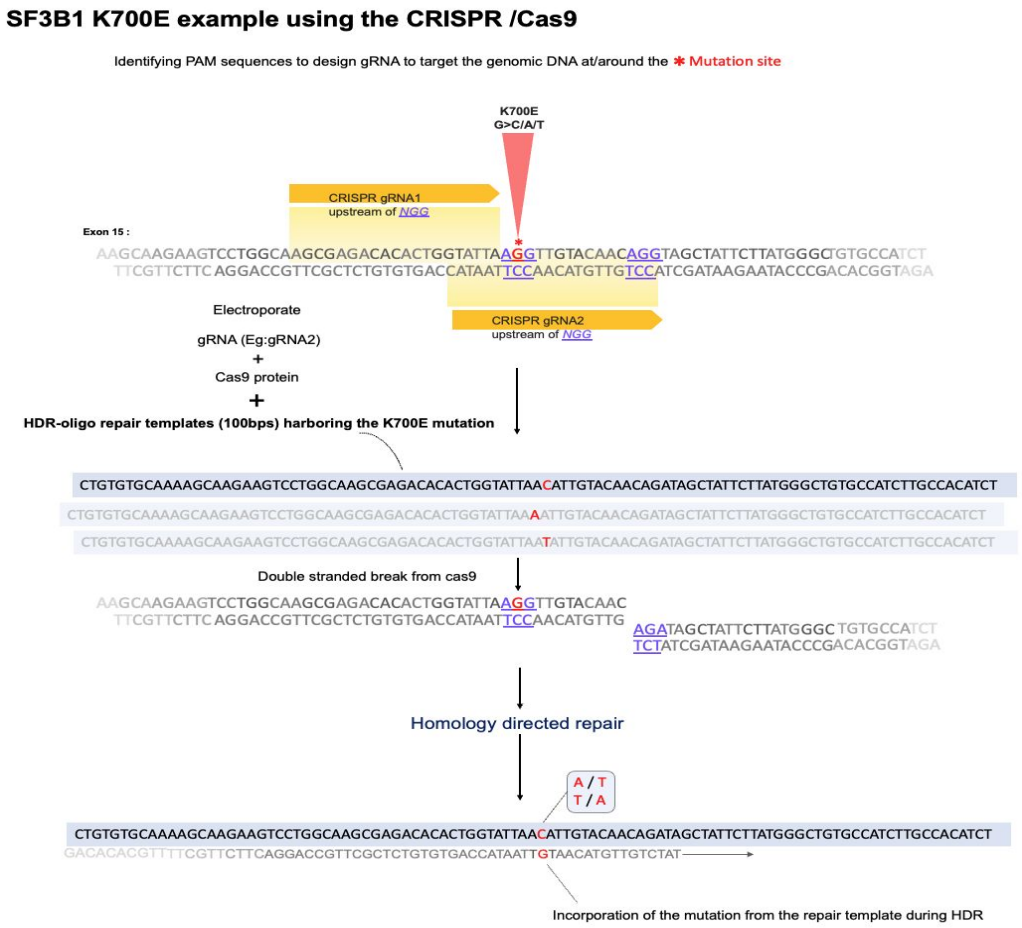


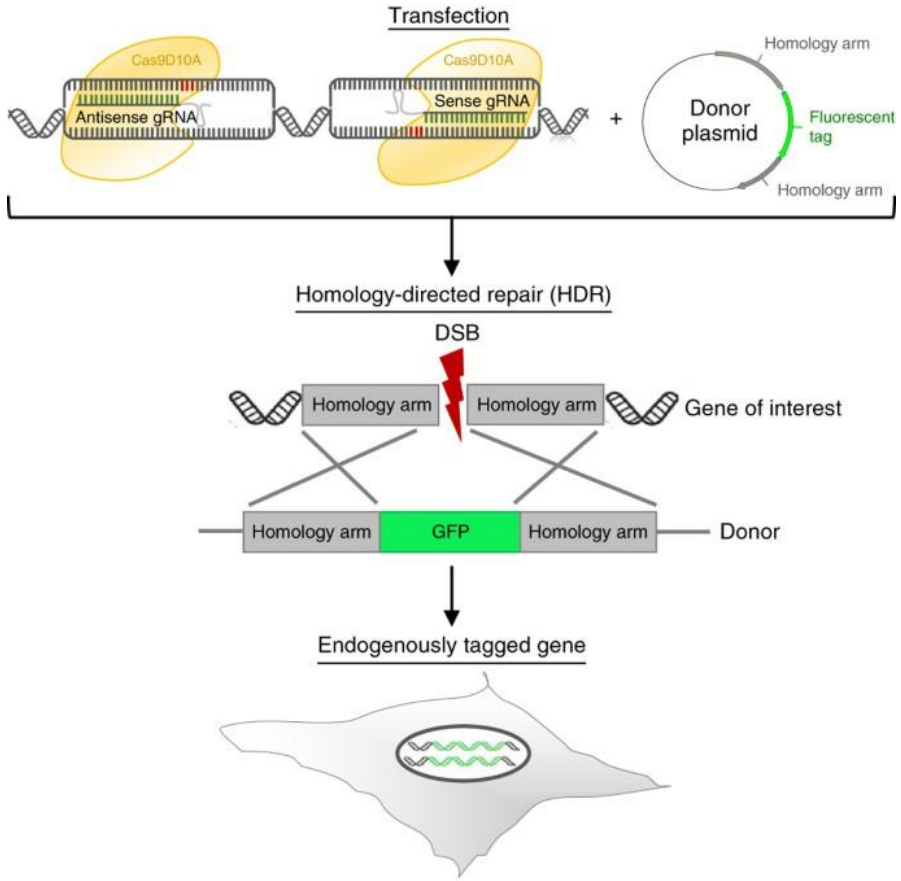



# Determining knock-in/ nucleotide variations

## Gene knock-in thorough HDR (Homology Directed “Repair)

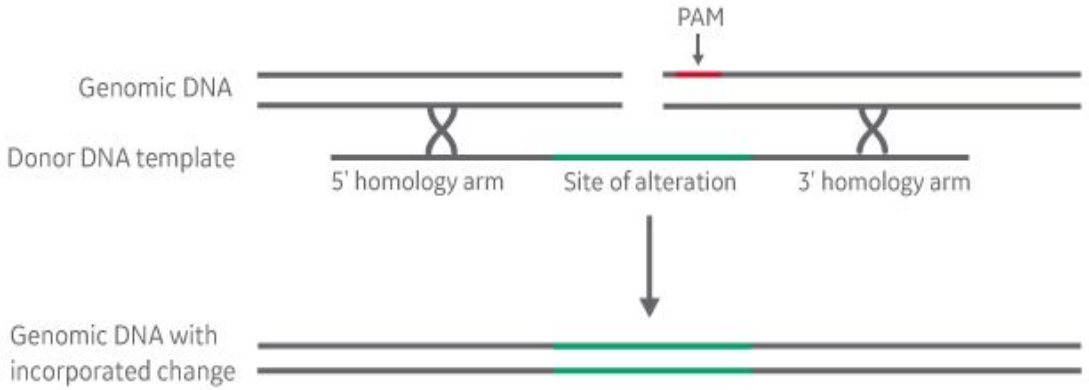


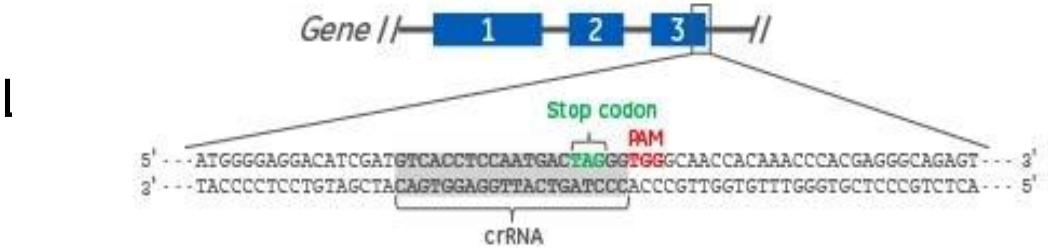



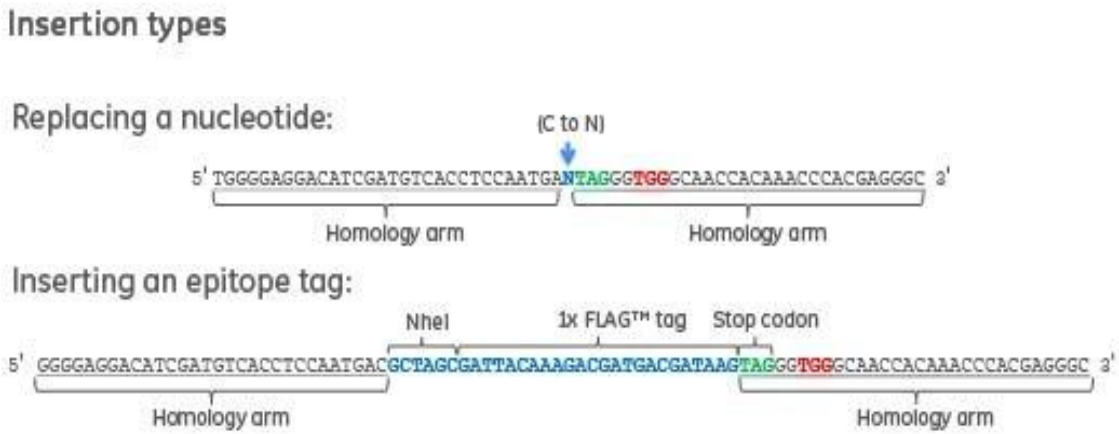
# Determining knock-in/ nucleotide variations

**Gene knock-in thorough HDR (Homology Directed  Repair)**

- Example  : Tagging in a fluorescent reporter to DI




- We perform the editing in cells and pick single clones of these edited cells to scrutinize for knock-in
- Multiple rounds of preliminary QCs
- Process takes from anywhere from about 15 to 30 days to obtain an inserted/  knock-in line

# In Summary

**We looked into**

- Understanding effects of double strand break created by Cas9
- How can we
  - Analyse them
  - Quantify them
  - Use information to choose efficient guides
  - Use information to determine success rate of desired outcome

- We also briefly discussed on
  - Understanding data coming out of sanger sequencing
  - Using sanger sequencing data to determine knock-in
  - Various QC methods used at various stages of the process

- Q and A – quiz format

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# questions?

Please contact wellcomeconnectingscience.org for more information.