Workflow for creating a CRISPR model

An undergraduate project

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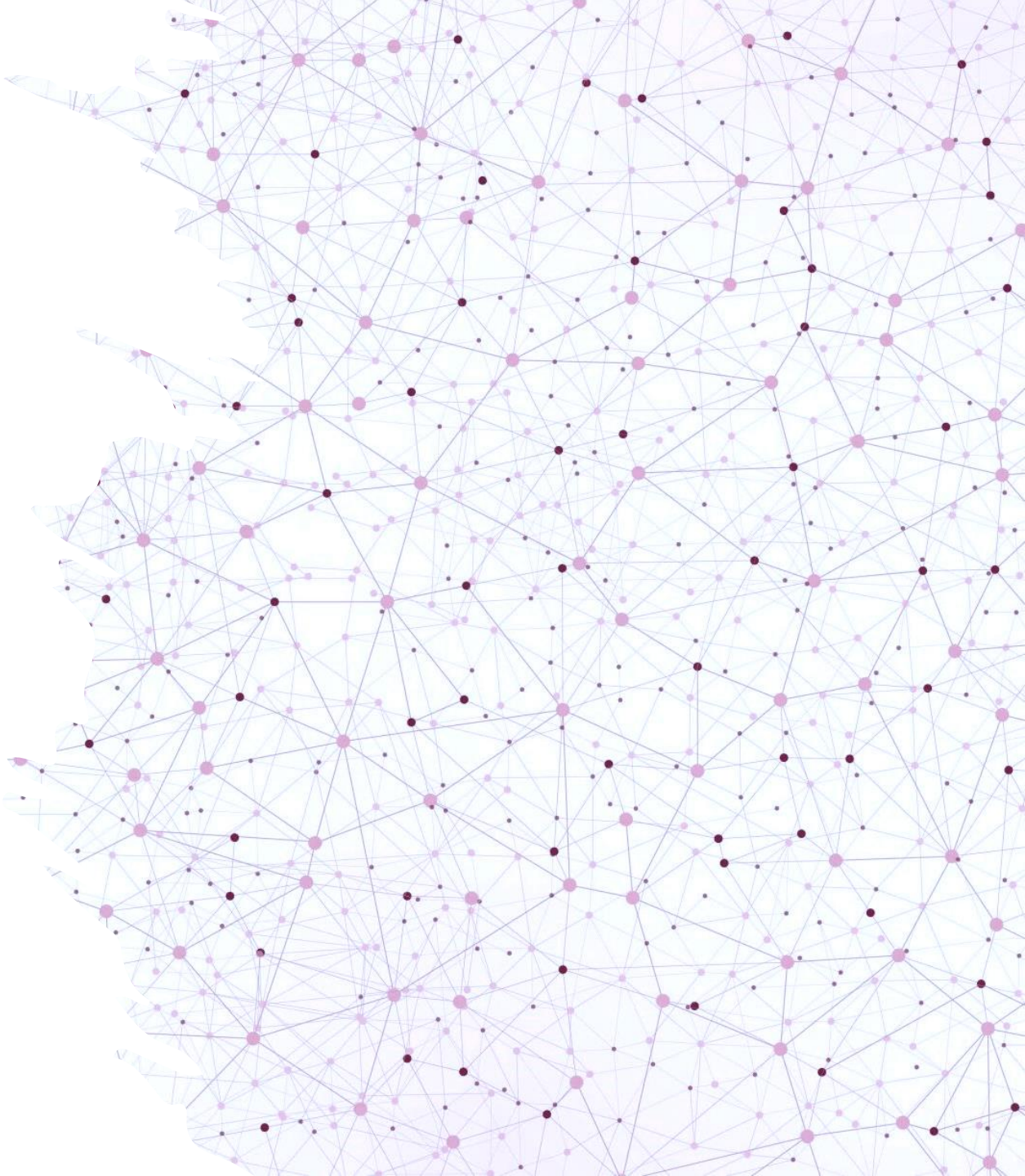
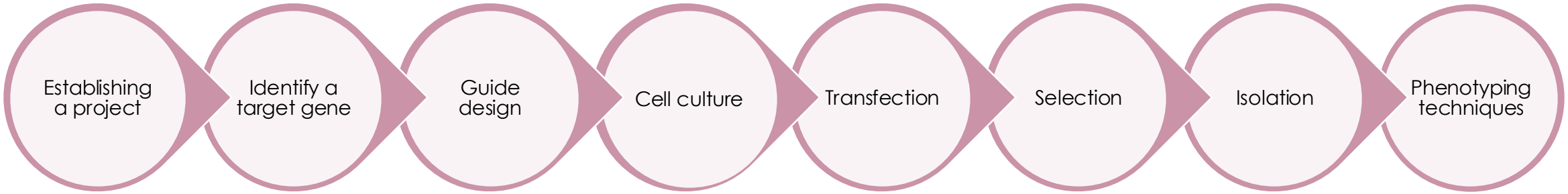
Oxford University

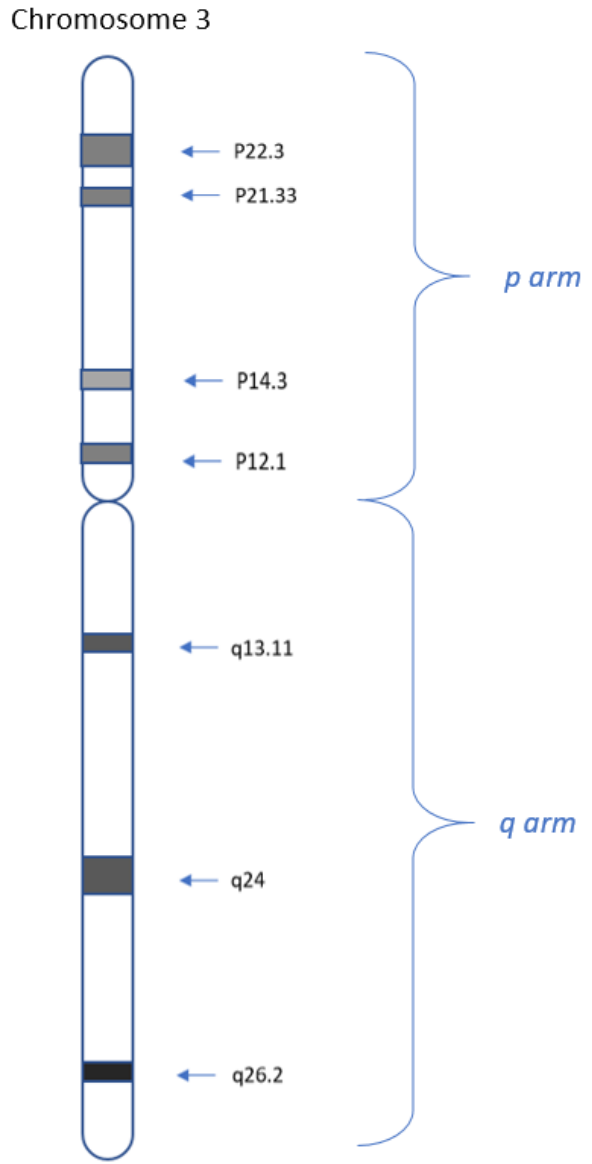


CRISPR Workflow



Establishing a project and target gene

- Targeted the MMR gene, *mlh1* of human chromosome 3
- Defective gene can result in hereditary non-polyposis colon cancer and lynch syndrome
- Cellular model used was a childhood bone cancer cell line tagged with a green fluorescent protein.

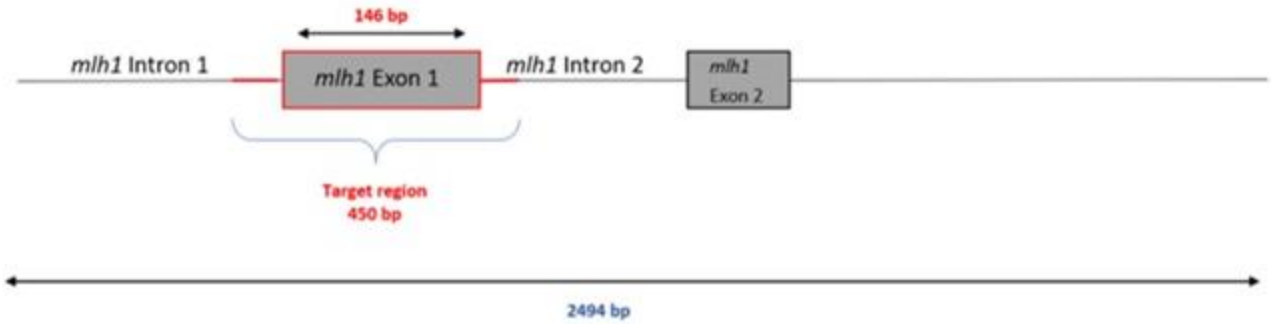


Guide design

- Access the genetic sequence of your target location.

- Design your guide on sites such as Benchling.

Target region



Snippet from Ensembl

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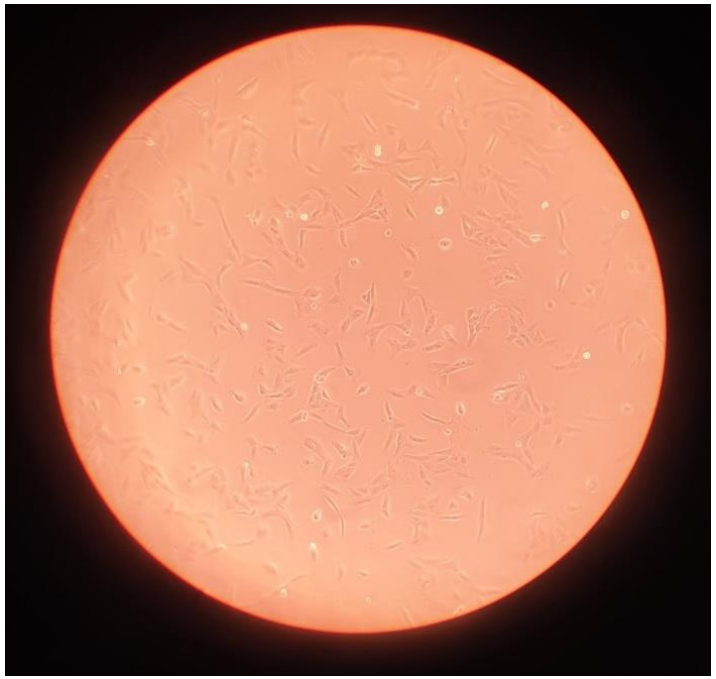
```

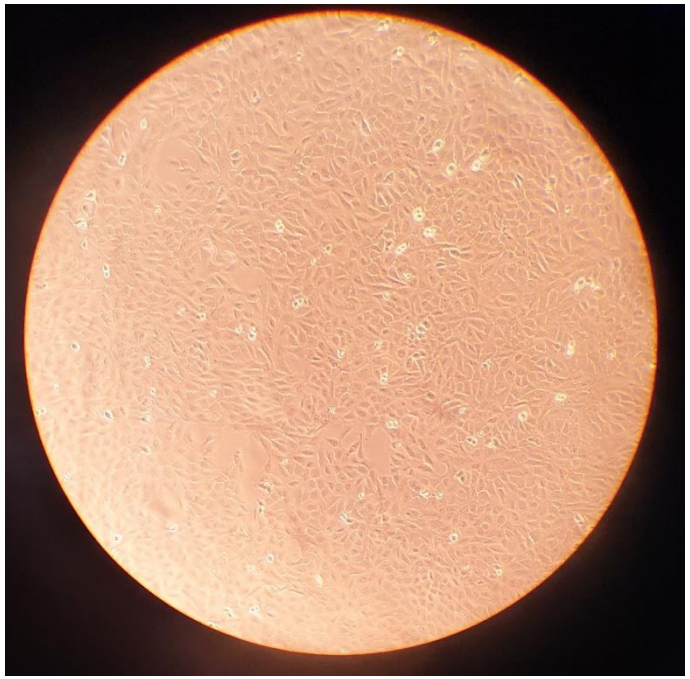
Cell culture

- Growth of chosen cell line

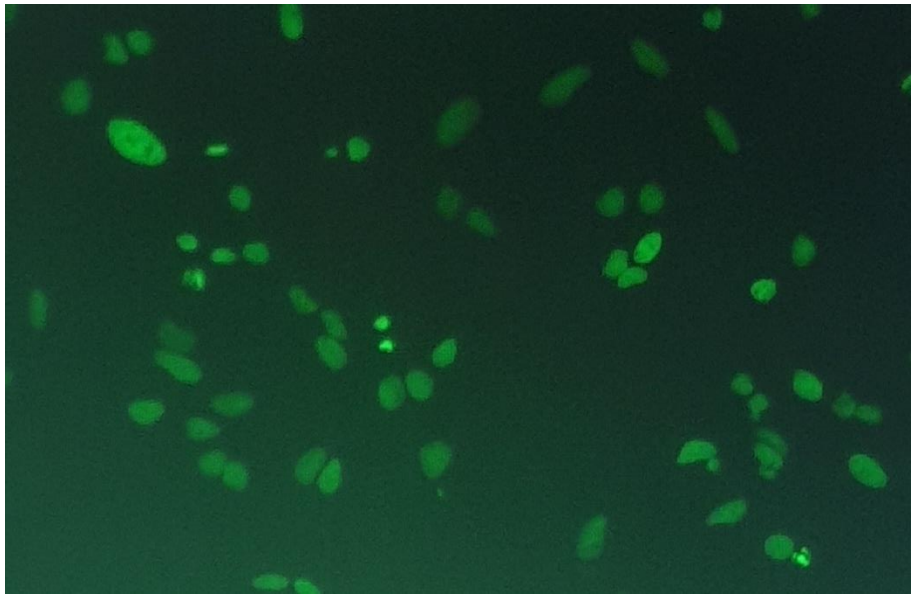
Low confluence



High confluence



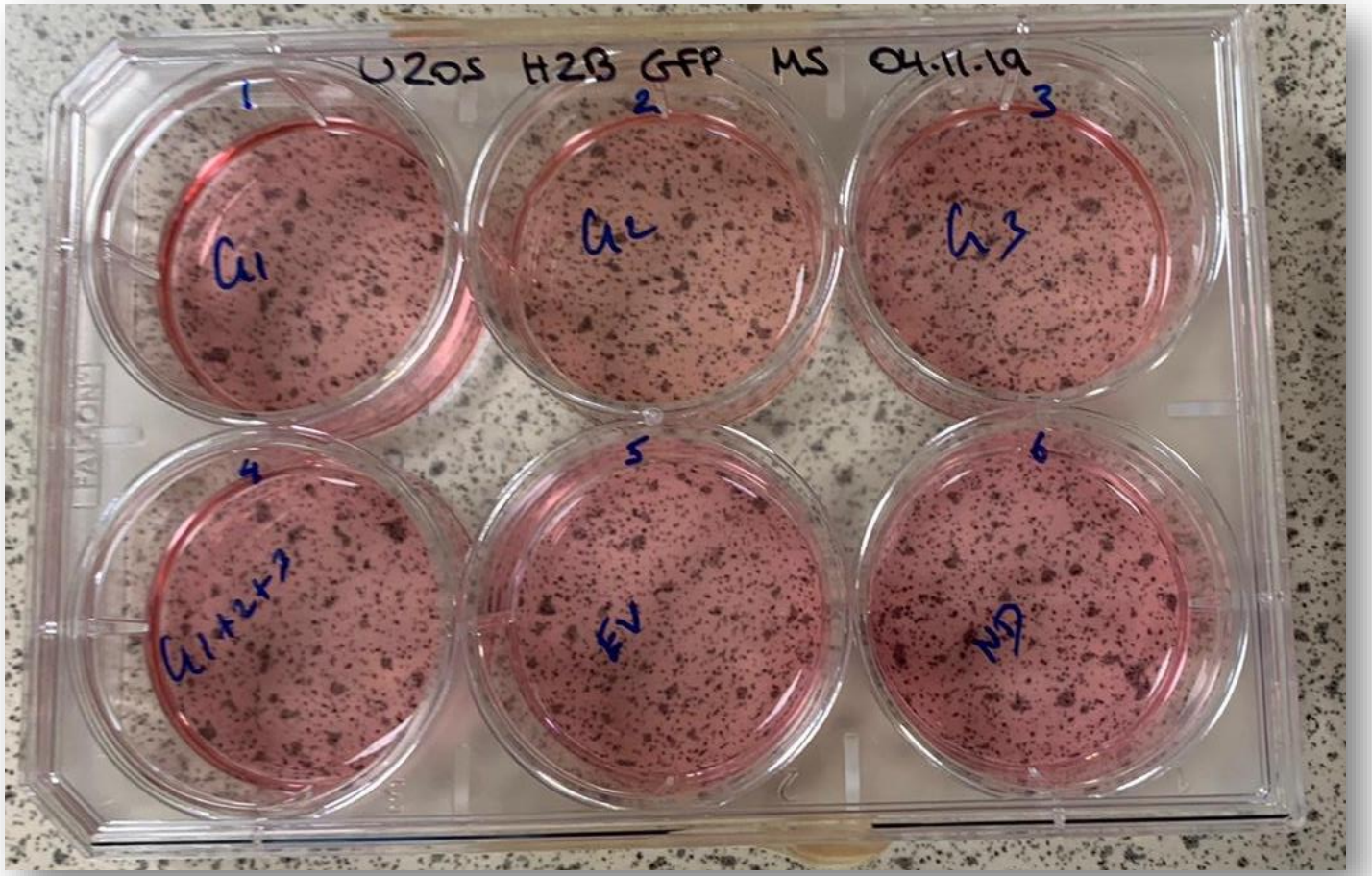
Fluorescent microscopic image



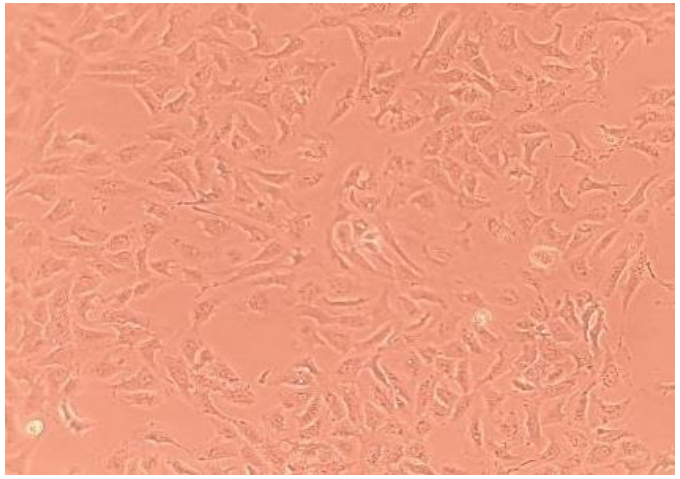
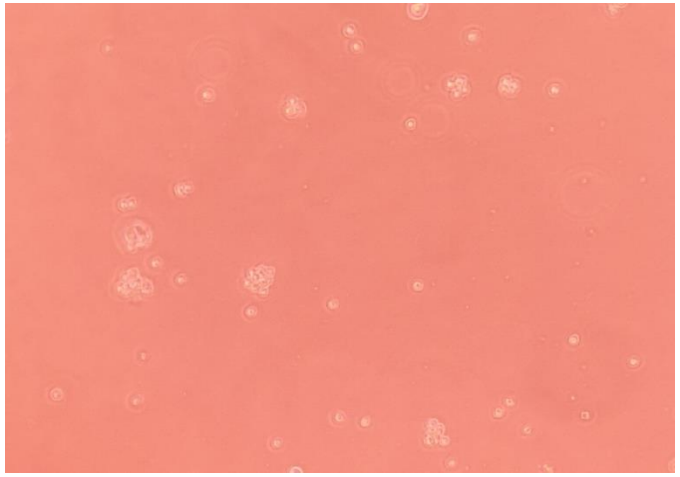
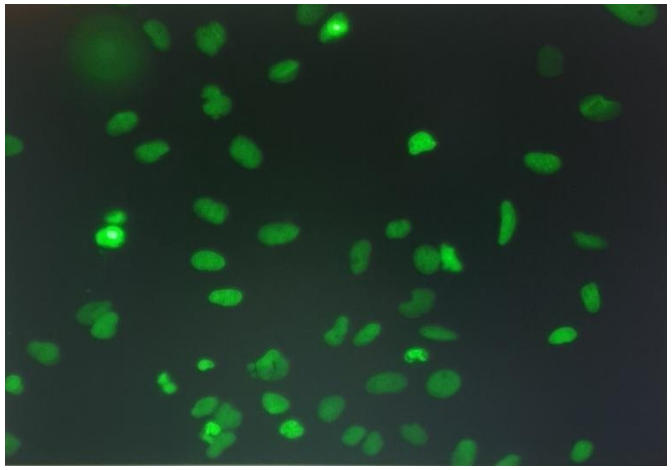
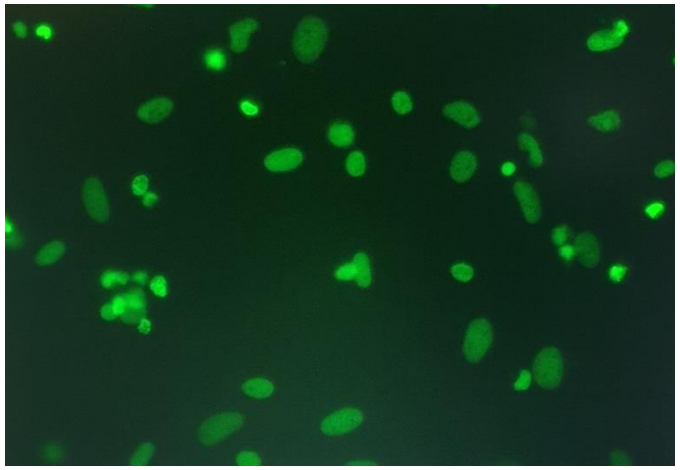
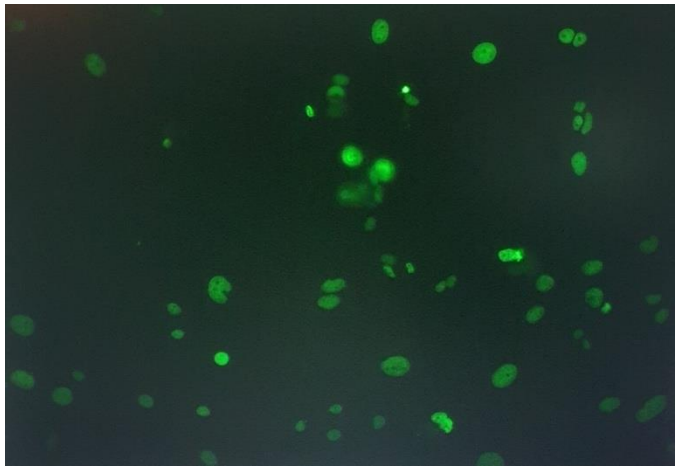
Transfection

- Introducing CRISPR complex into cultured cells

6 well plate

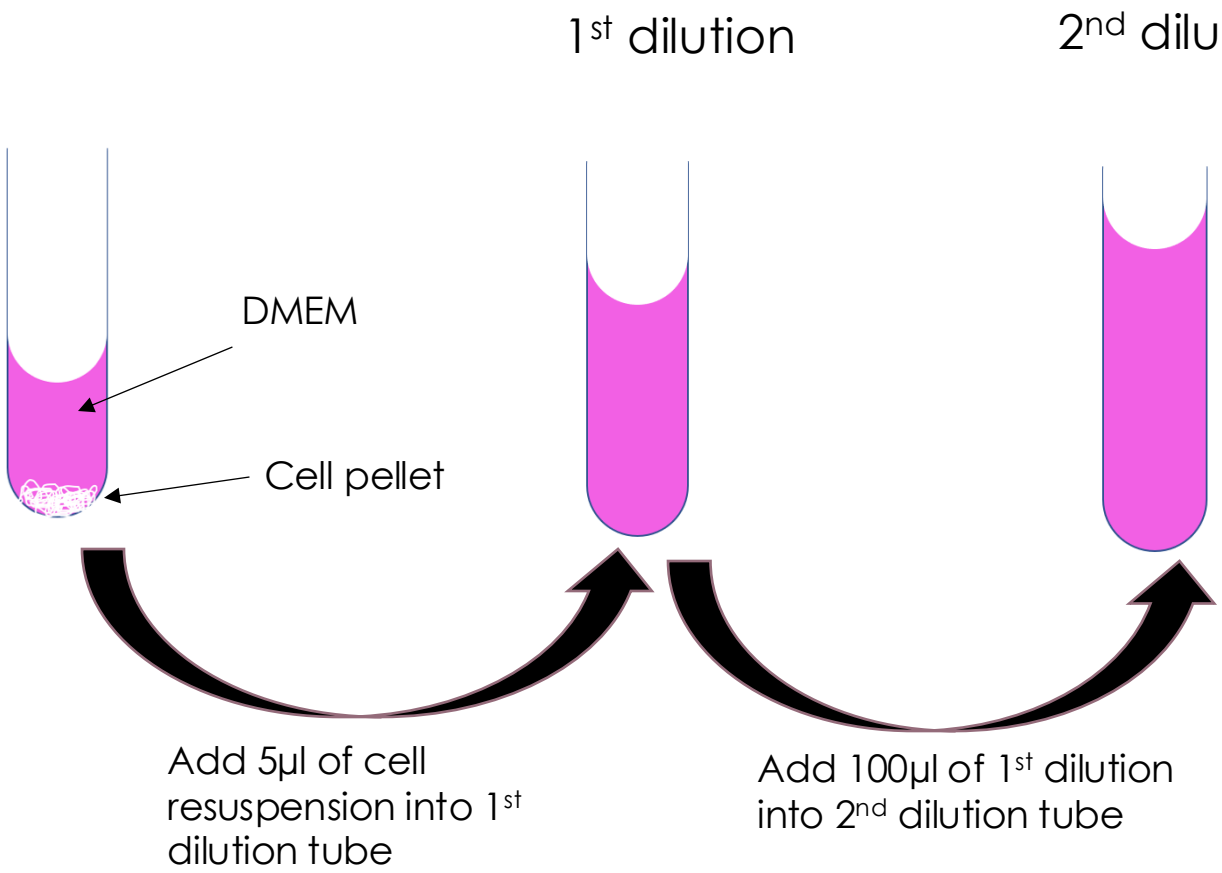


Selection

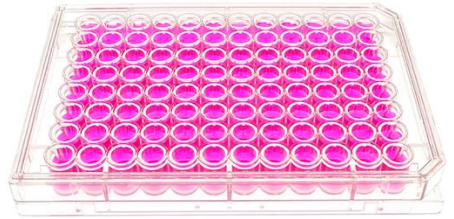
	Before Transfection	Post Transfection	72h post selection release
Brightfield images			
Fluorescent images			

- Selection of cells which contain the CRISPR complex

Isolation



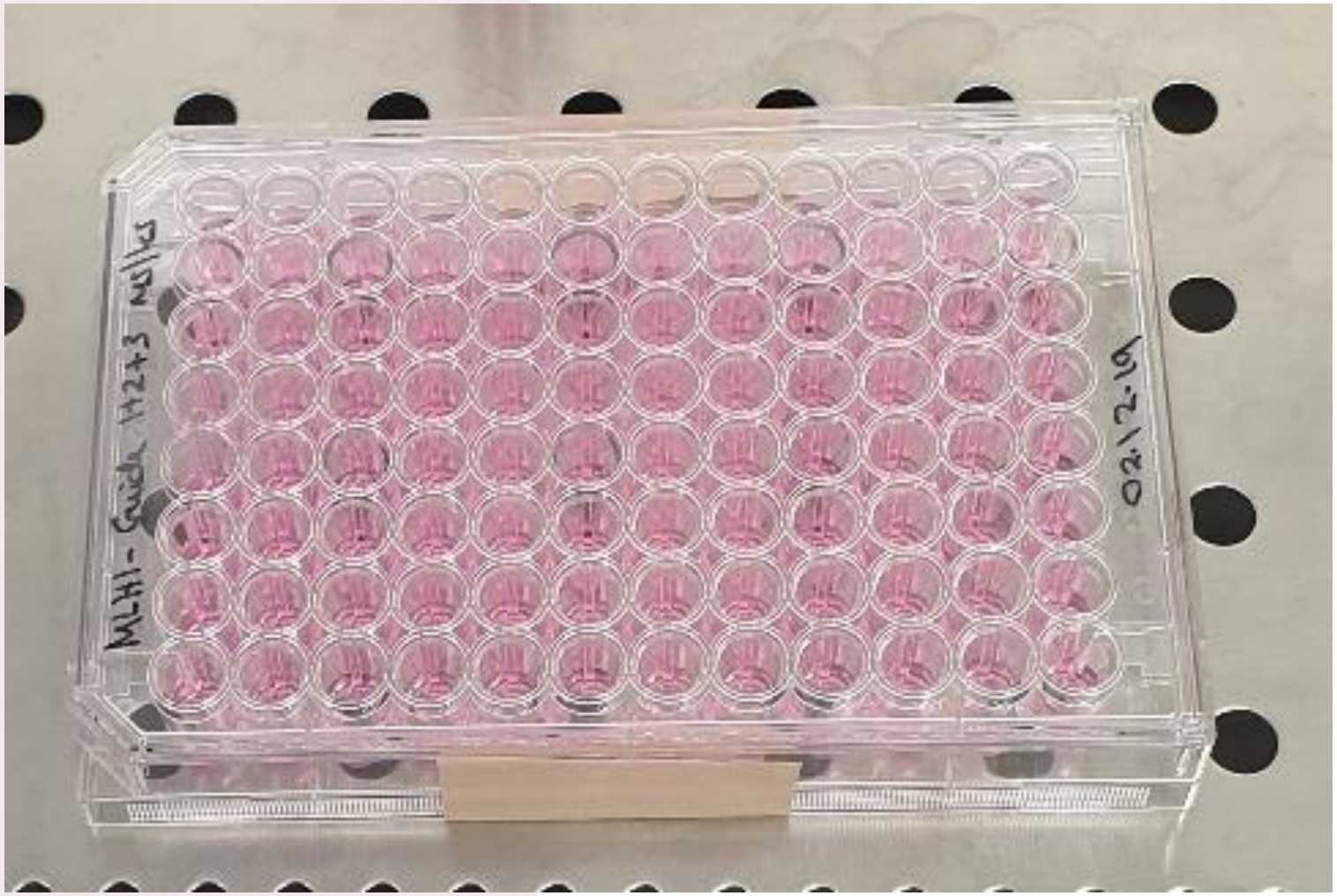
Load 100µl of 2nd dilution into each well of a 96 - well plate.



1 WELL = 1 CELL

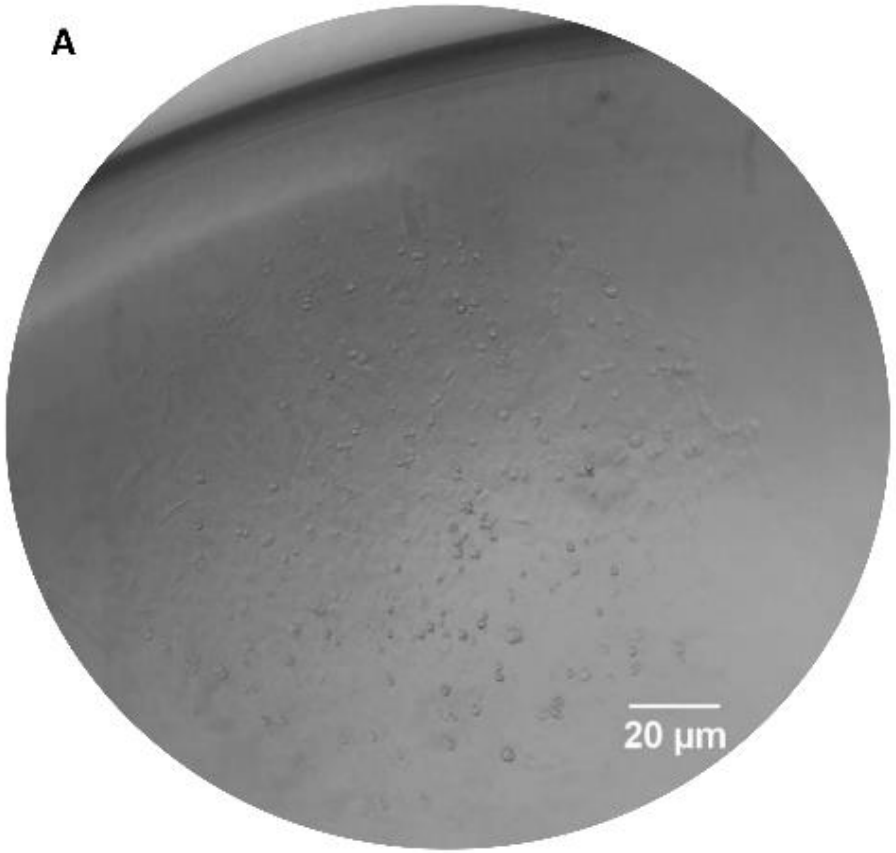
- Isolating edited cells into single colonies

96 well monoclonal isolation plate

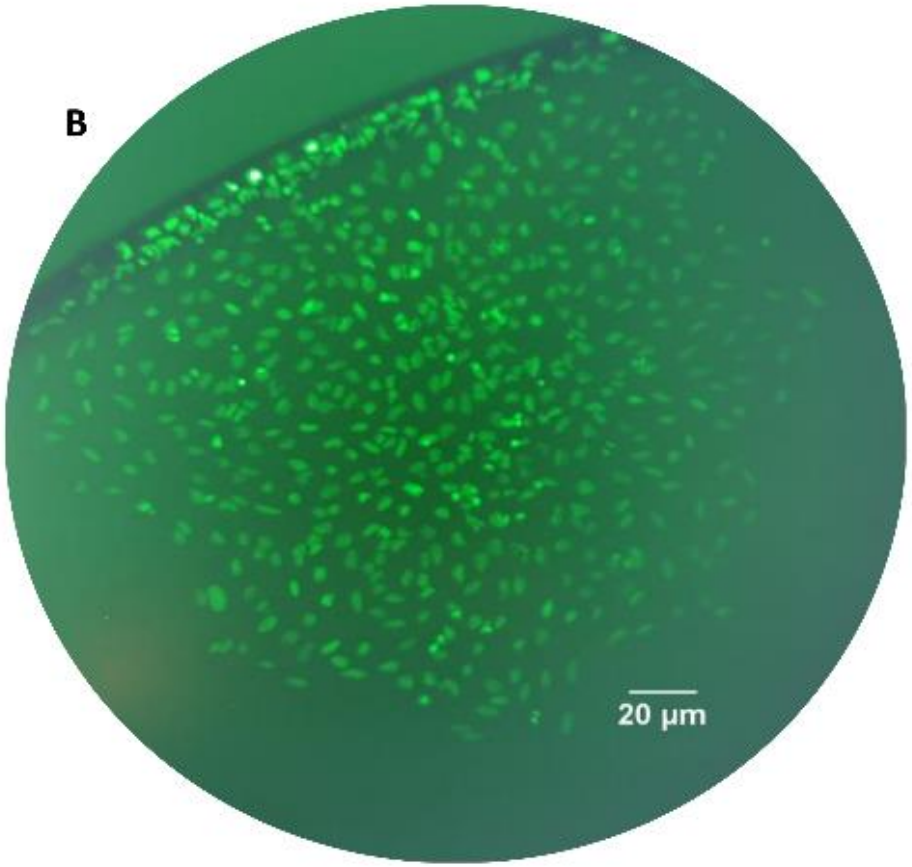


Monoclonal culture

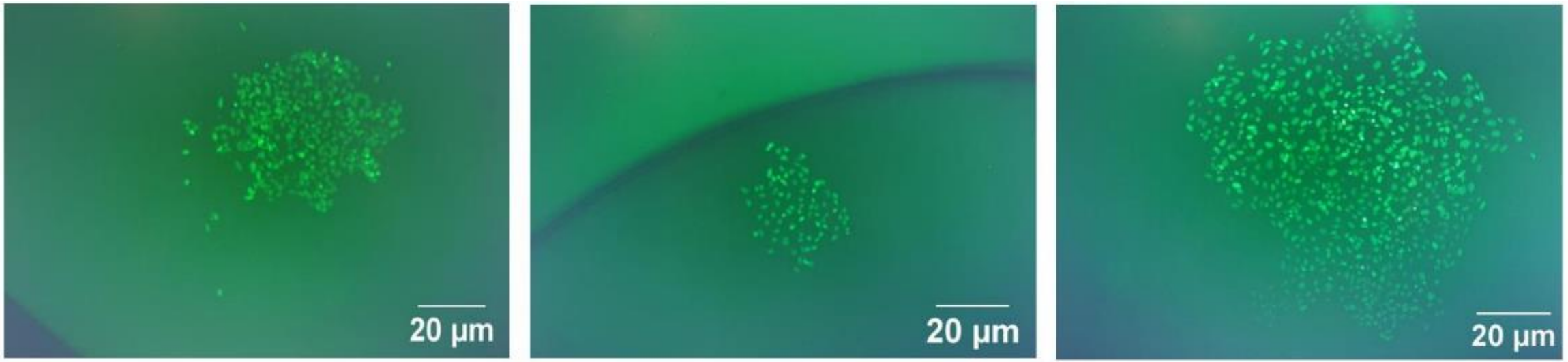
Brightfield images



Fluorescent images

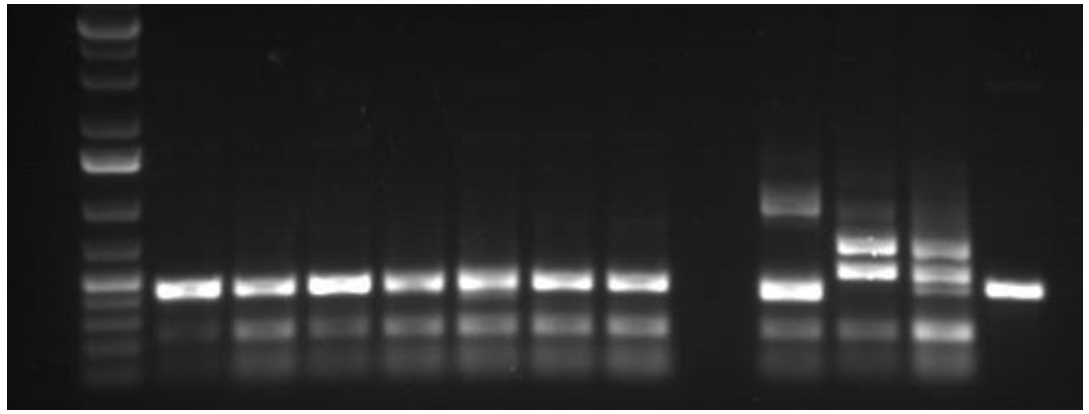


Fluorescent images

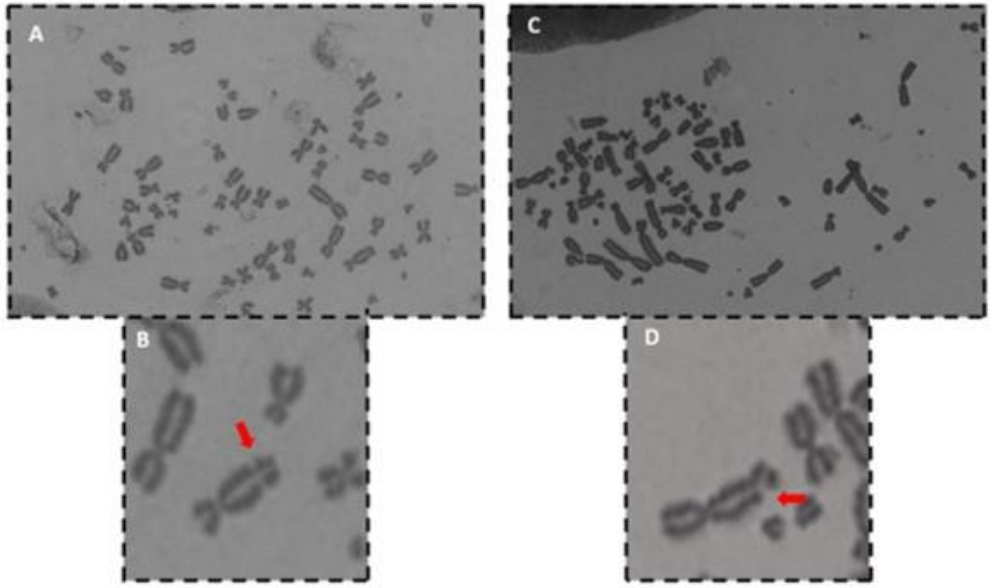


Phenotyping techniques

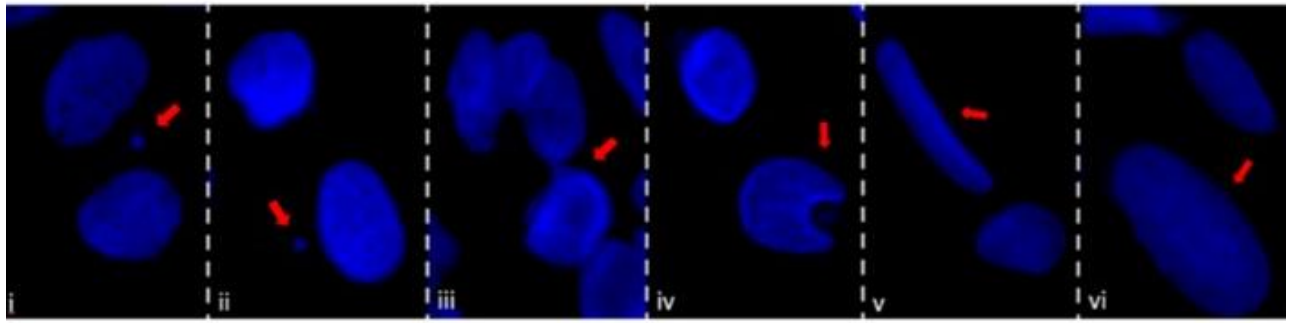
Gel electrophoresis

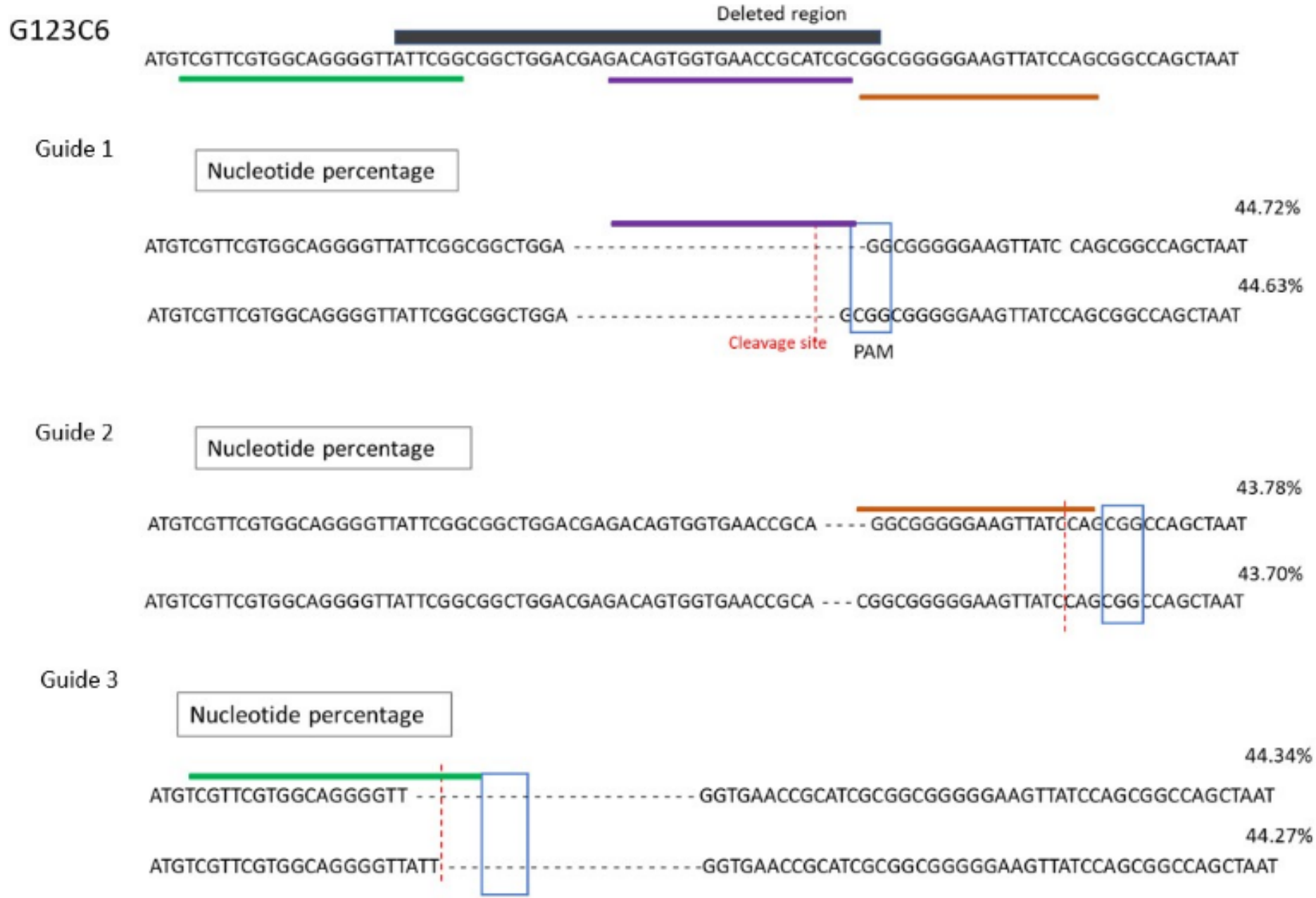


Metaphase spread



Nuclear morphology




G123C6
Deleted region
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Guide 1
Nucleotide percentage
44.72%
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44.63%
ATGTCGTTCGTGGCAGGGGTTATTCGGCGGCTGGA----------GCGGCGGGGGAAGTTATCCAGCGGCCAGCTAAT
Cleavage site
PAM
Guide 2
Nucleotide percentage
43.78%
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43.70%
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Guide 3
Nucleotide percentage
44.34%
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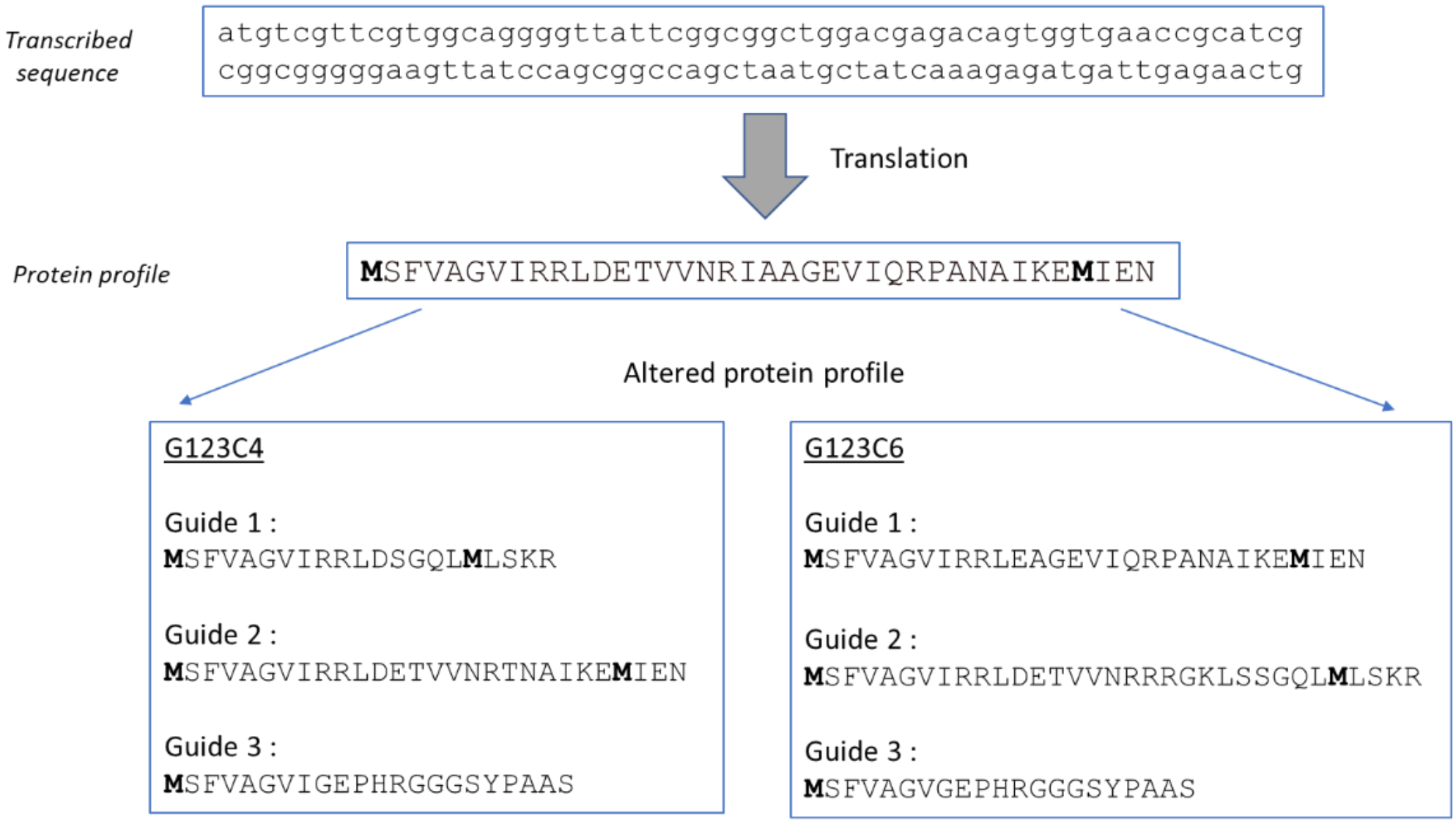
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Translation
Protein profile
MSFVAGVIRRLDETVVNRIAAGEVIQRPANAIKEMIEN
Altered protein profile
G123C4
Guide 1 :
MSFVAGVIRRLDSGQLMLSKR
Guide 2 :
MSFVAGVIRRLDETVVNRTNAIKEMIEN
Guide 3 :
MSFVAGVIGEPHRGGGSYPAAS
G123C6
Guide 1 :
MSFVAGVIRRLEAGEVIQRPANAIKEMIEN
Guide 2 :
MSFVAGVIRRLDETVVNRRRGKLSSGQLMLSKR
Guide 3 :
MSFVAGVGEPHRGGGSYPAAS

Why is this workshop valuable?

- Join a revolutionary field
- Enhance laboratory skills
- Open several opportunities
- Networking opportunities
- Assist in further education

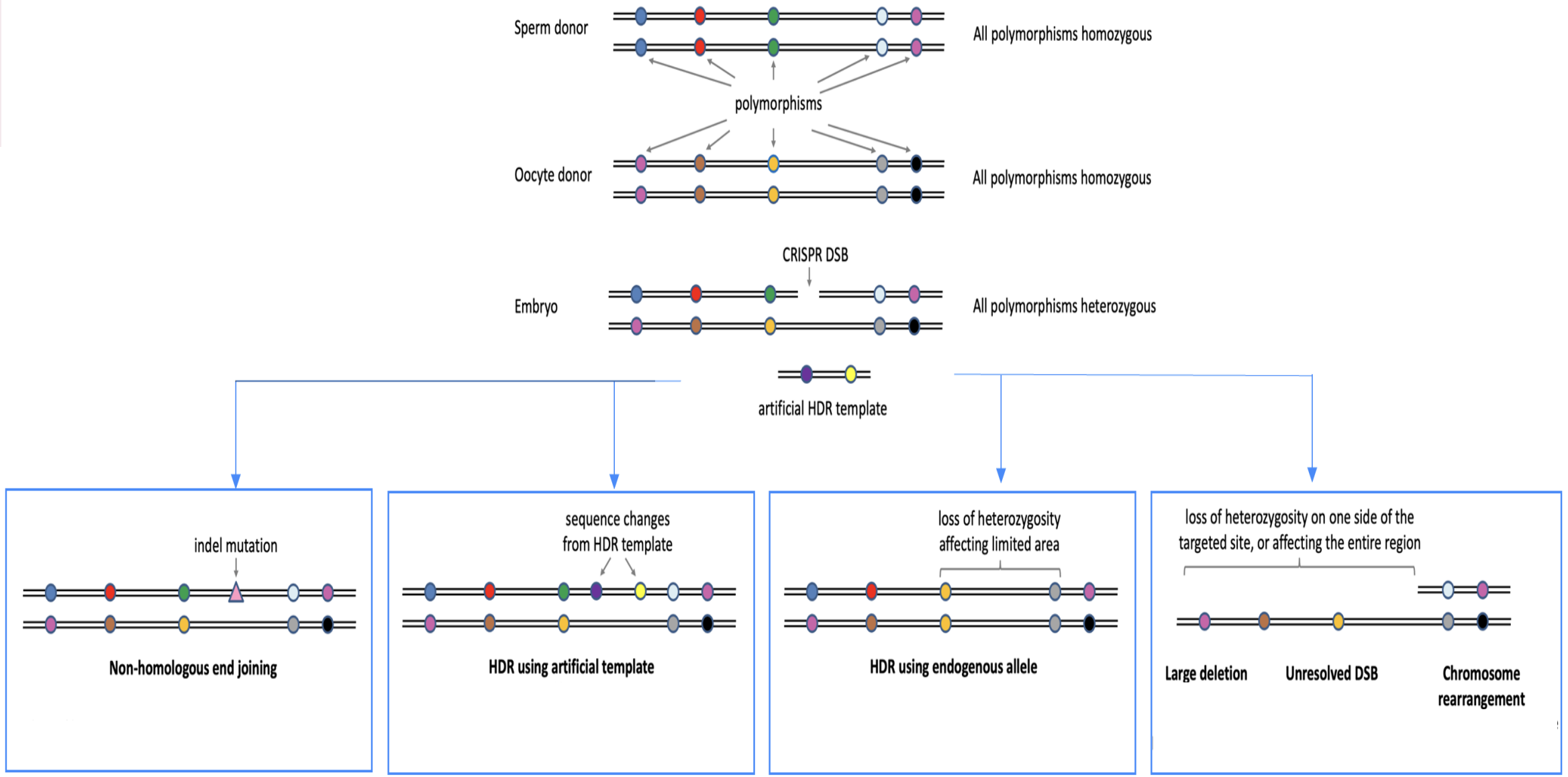
Most current research

A research fellow at Oxford University designed a project to introduce CRISPR into human embryos at two different stages of development.

At fertilisation

Day 3 embryo

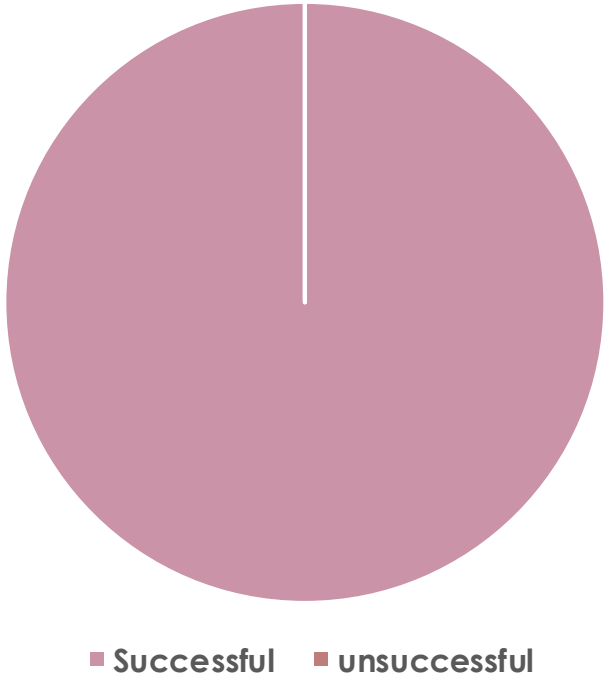
Unpublished data



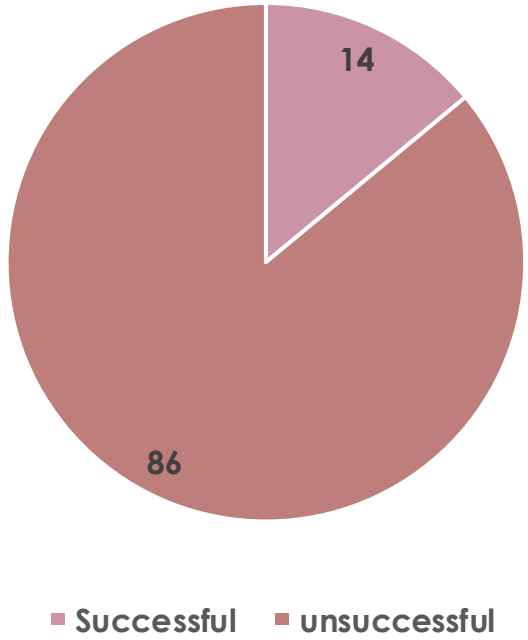
Unpublished data

Results

Editing efficiency of ICSI group

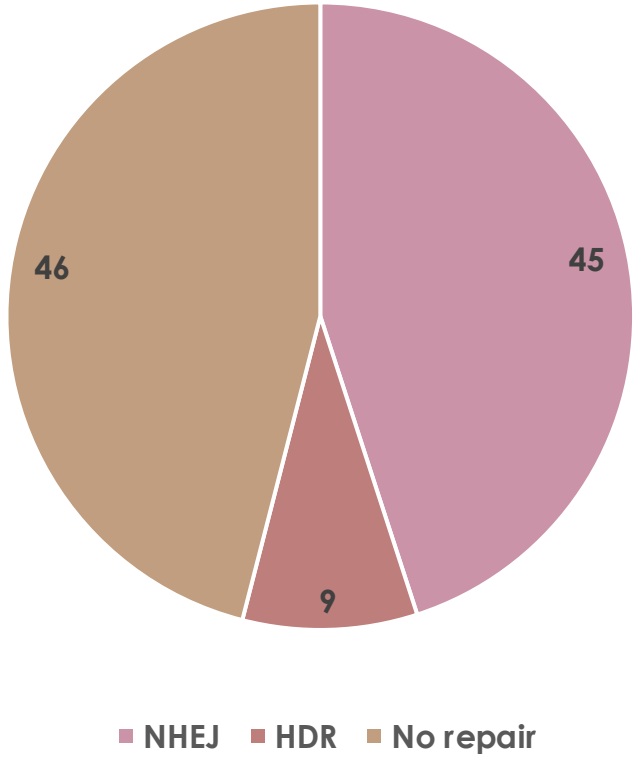


Editing efficiency of cleavage group

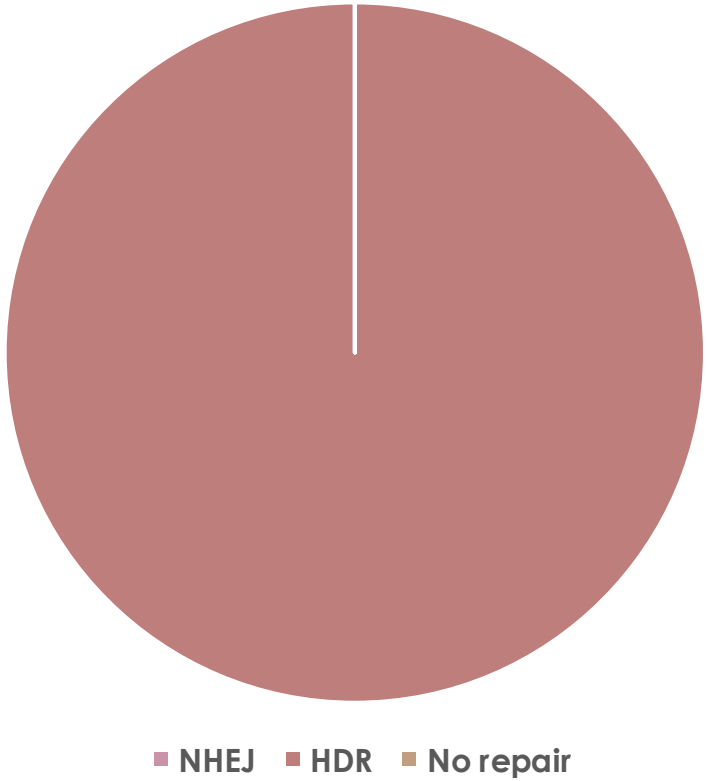


Unpublished data

Repair mechanism of ICIS repair group



Repair mechanism of cleavage group



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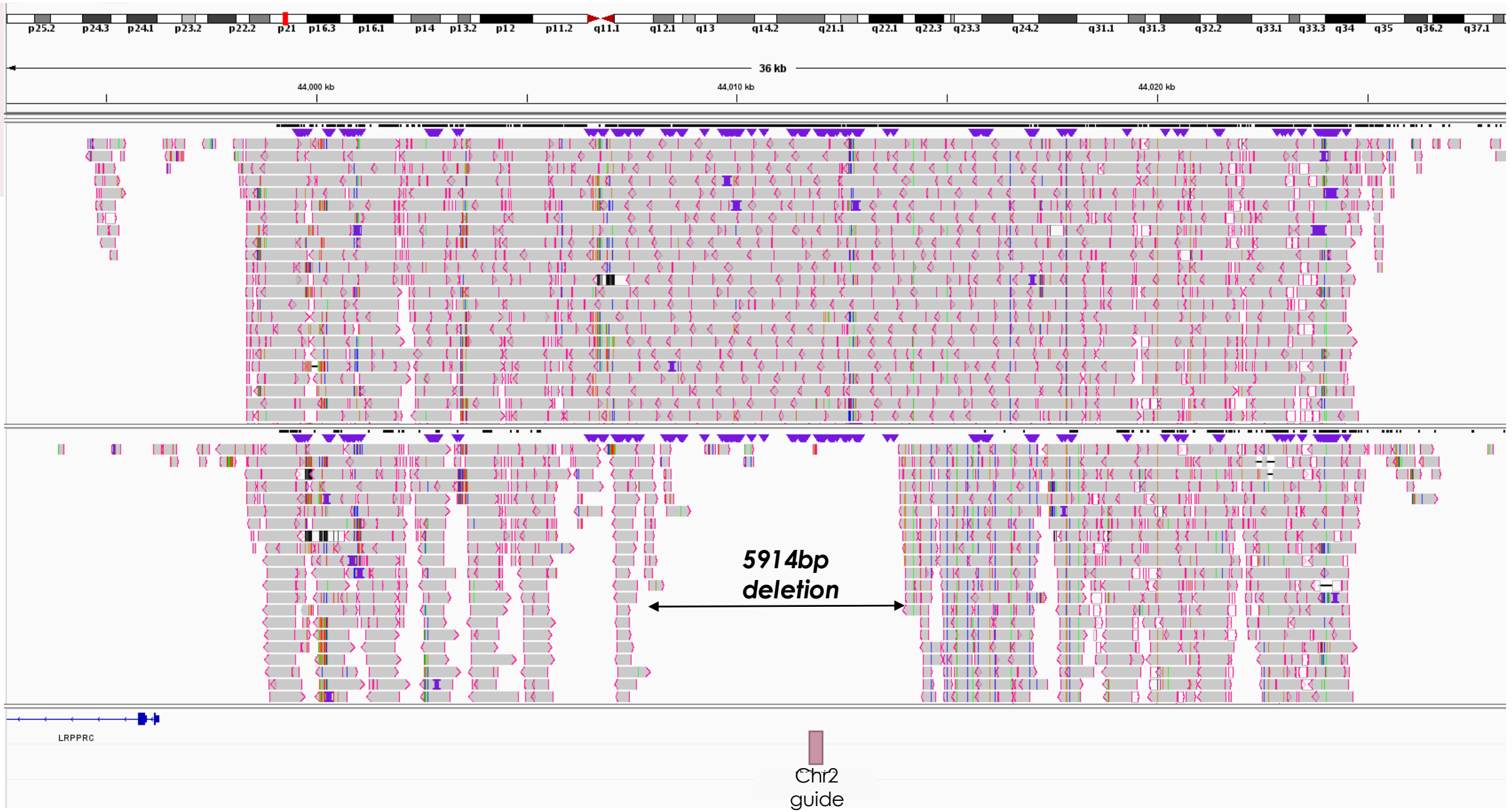
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36 kb
44,000 kb
44,010 kb
44,020 kb
5914bp deletion
LRPPRC
Chr2 guide

Conclusion

- Successfully developed a method to detect large deletions.
- Embryos at an early stage have little capacity for DNA repair.
- Embryonic genome activation is crucial.
- HDR most desired but NHEJ is the dominant repair mechanism.