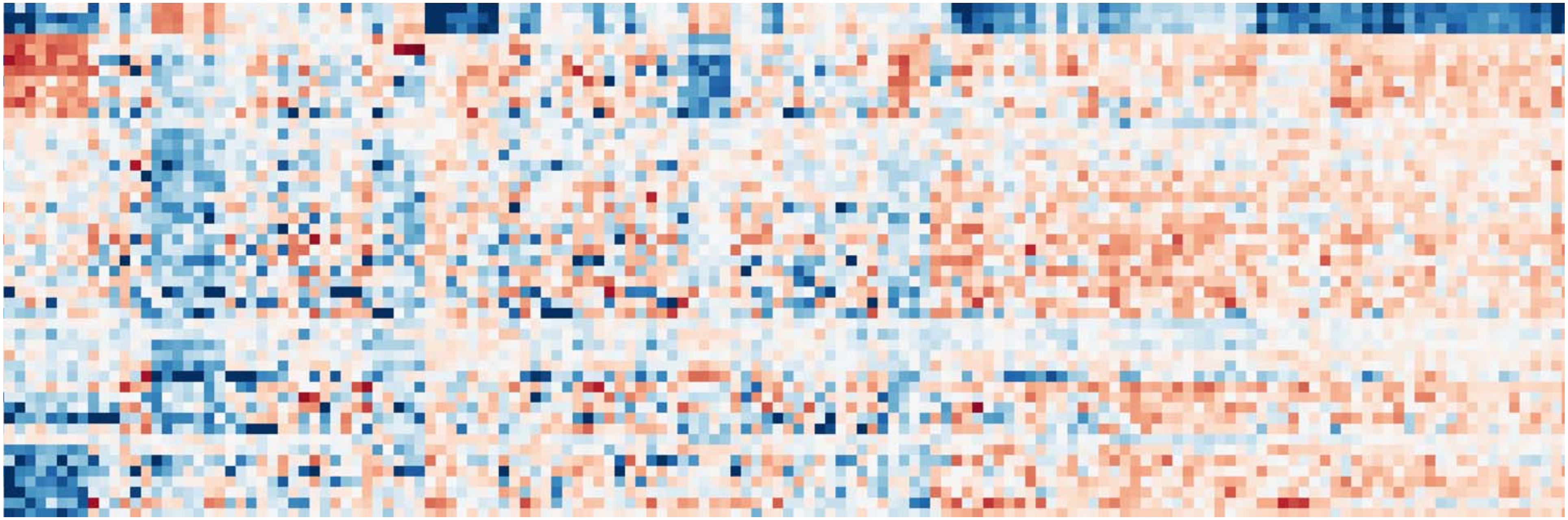
Mapping the processes of genome editing with high-resolution functional genomics



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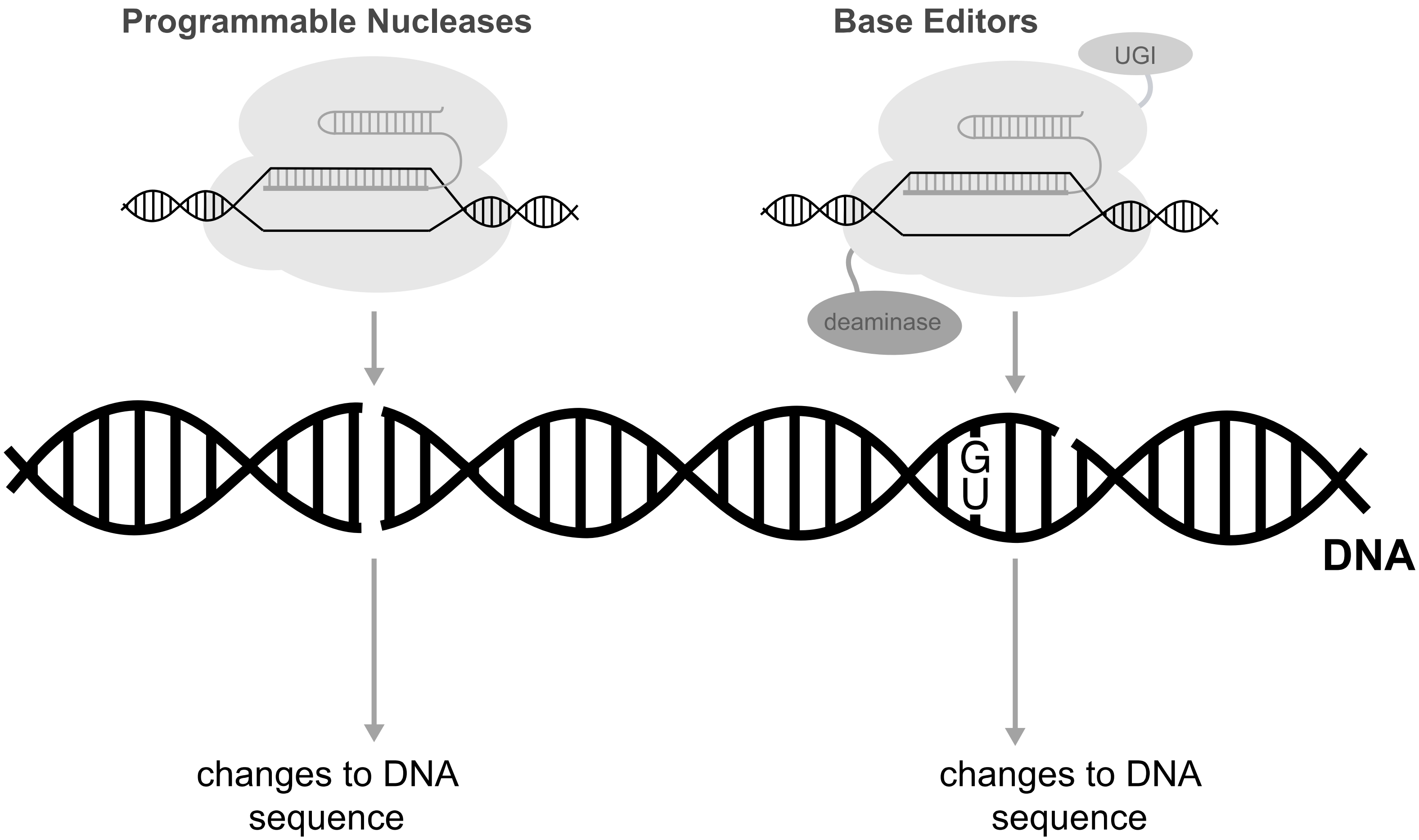
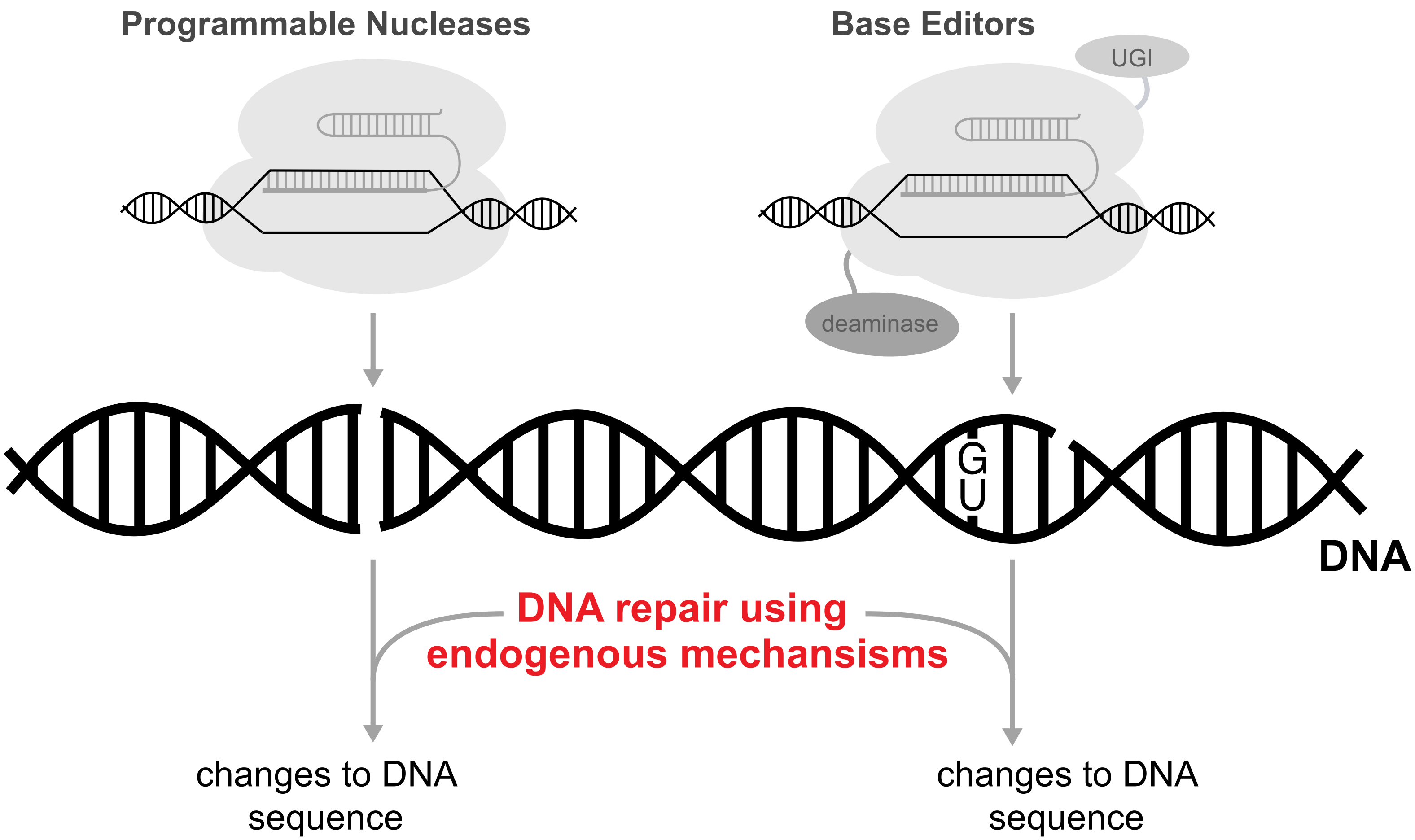
Genome editing technologies make precision damage to alter DNA sequences



changes to DNA sequence

changes to DNA sequence

Endogenous DNA repair pathways actually make the edits



Programmable Nucleases

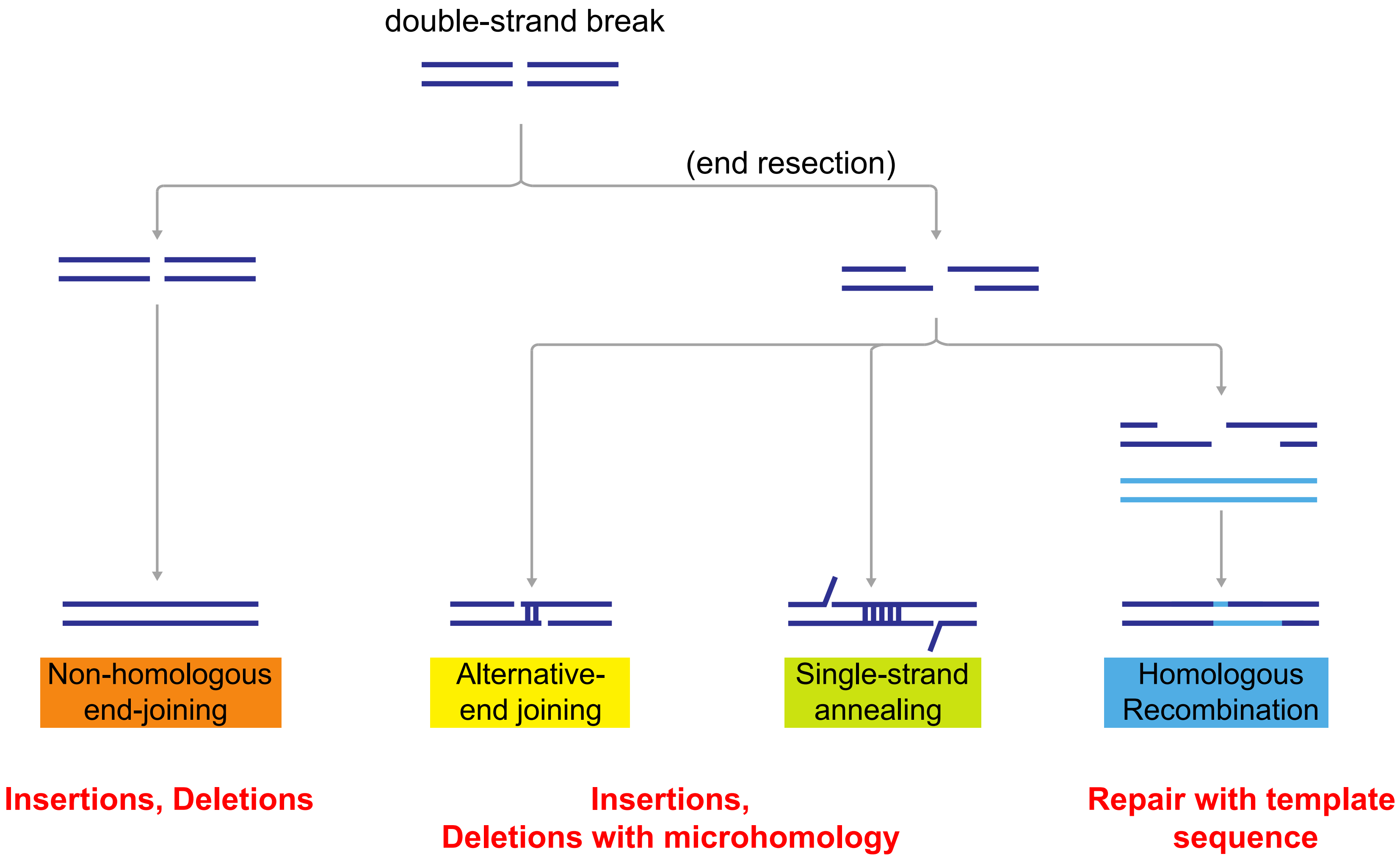
Base Editors

DNA repair using endogenous mechansisms

changes to DNA sequence

changes to DNA sequence

A network of DNA repair pathways has evolved to protect cells (not to edit genomes)



double-strand break

(end resection)

Non-homologous end-joining

Alternative-end joining

Single-strand annealing

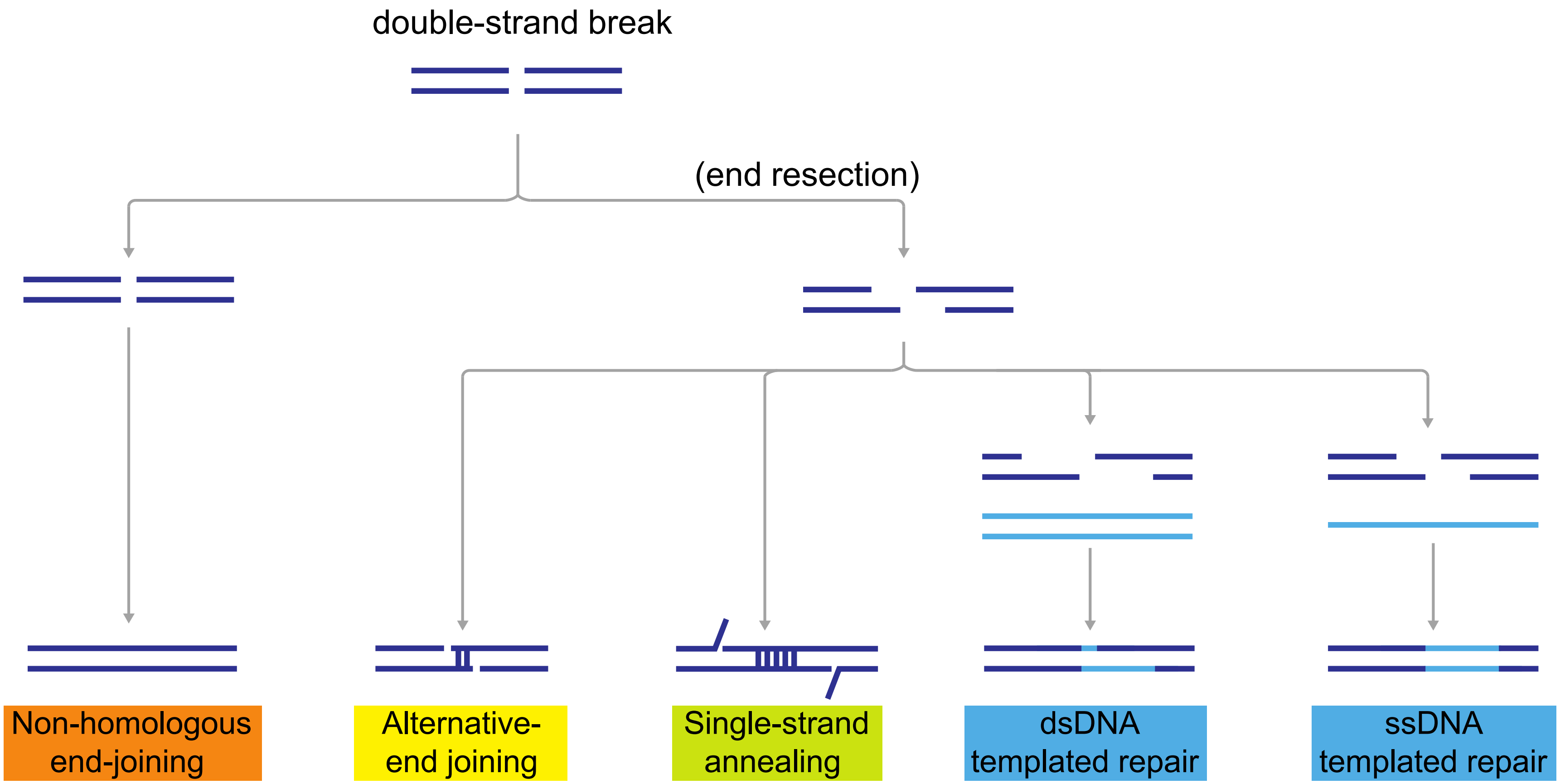
Homologous Recombination

Insertions, Deletions

Insertions,
Deletions with microhomology

Repair with template sequence

Incorporation of sequences from exogenous templates enables genome editing downstream of end resection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Single-strand annealing

dsDNA templated repair

ssDNA templated repair

Insertions, Deletions

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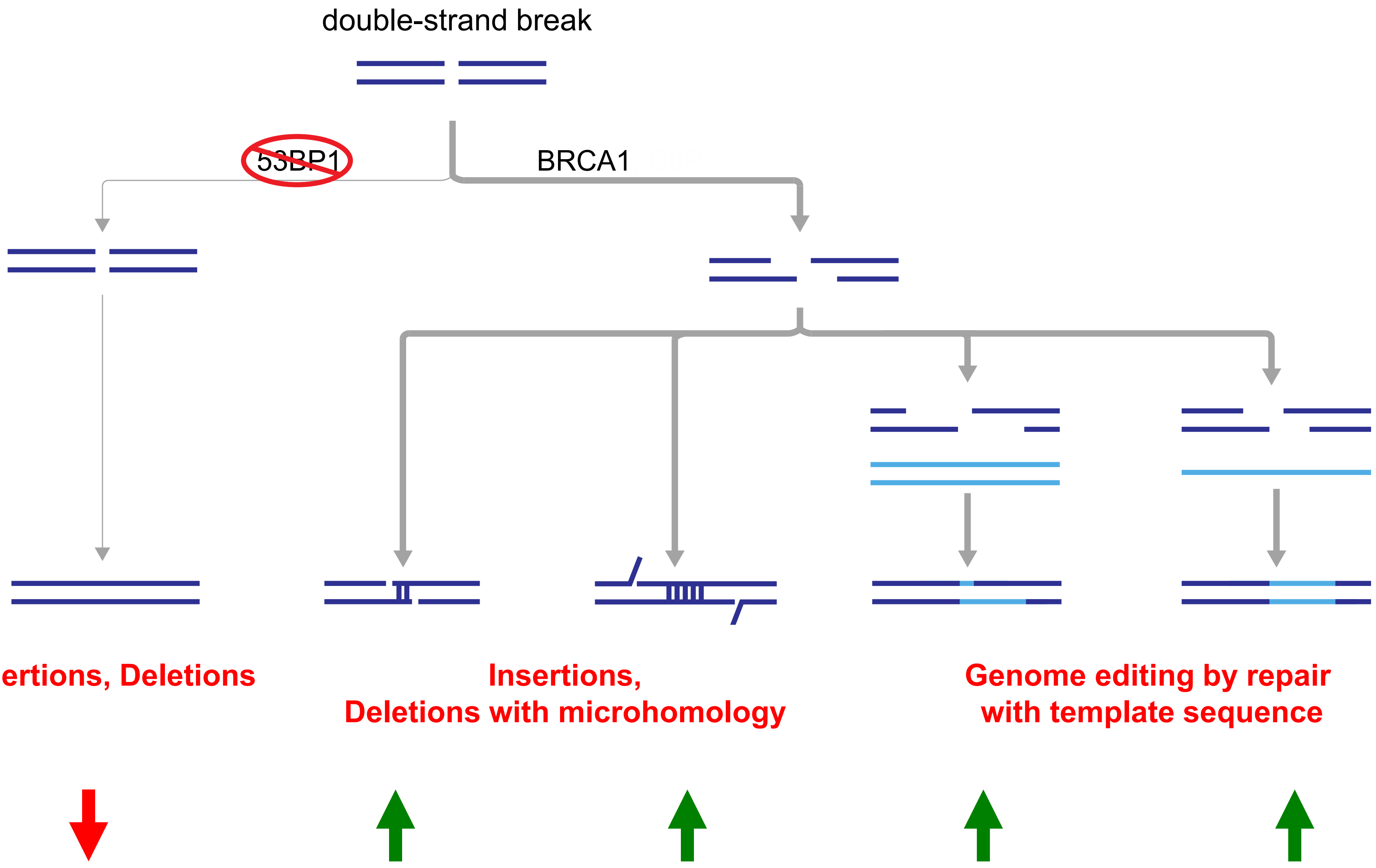
Deletions with microhomology

Genome editing by repair
with template sequence

Inhibition of competitive branches modulates pathway activity and therefore editing outcomes



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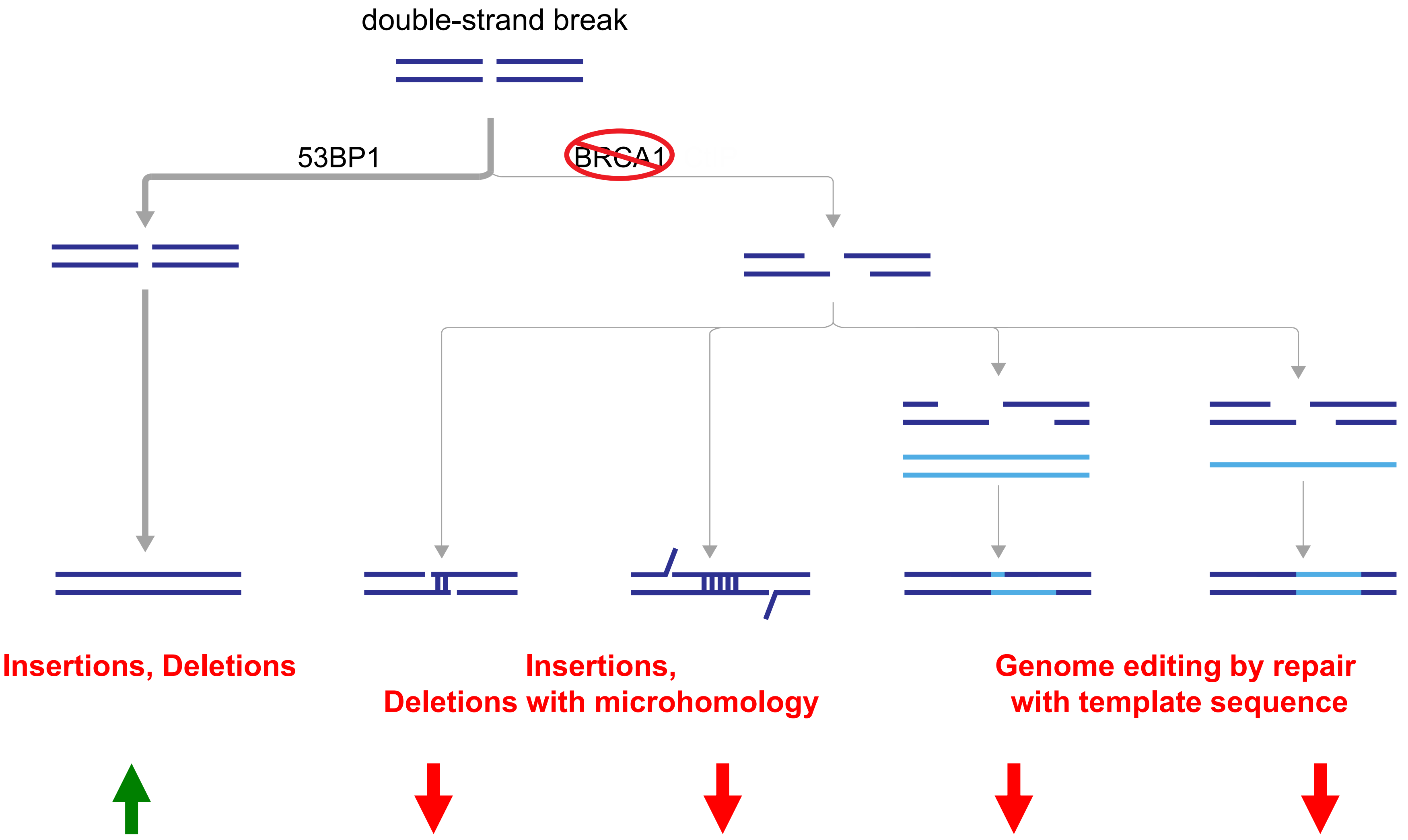

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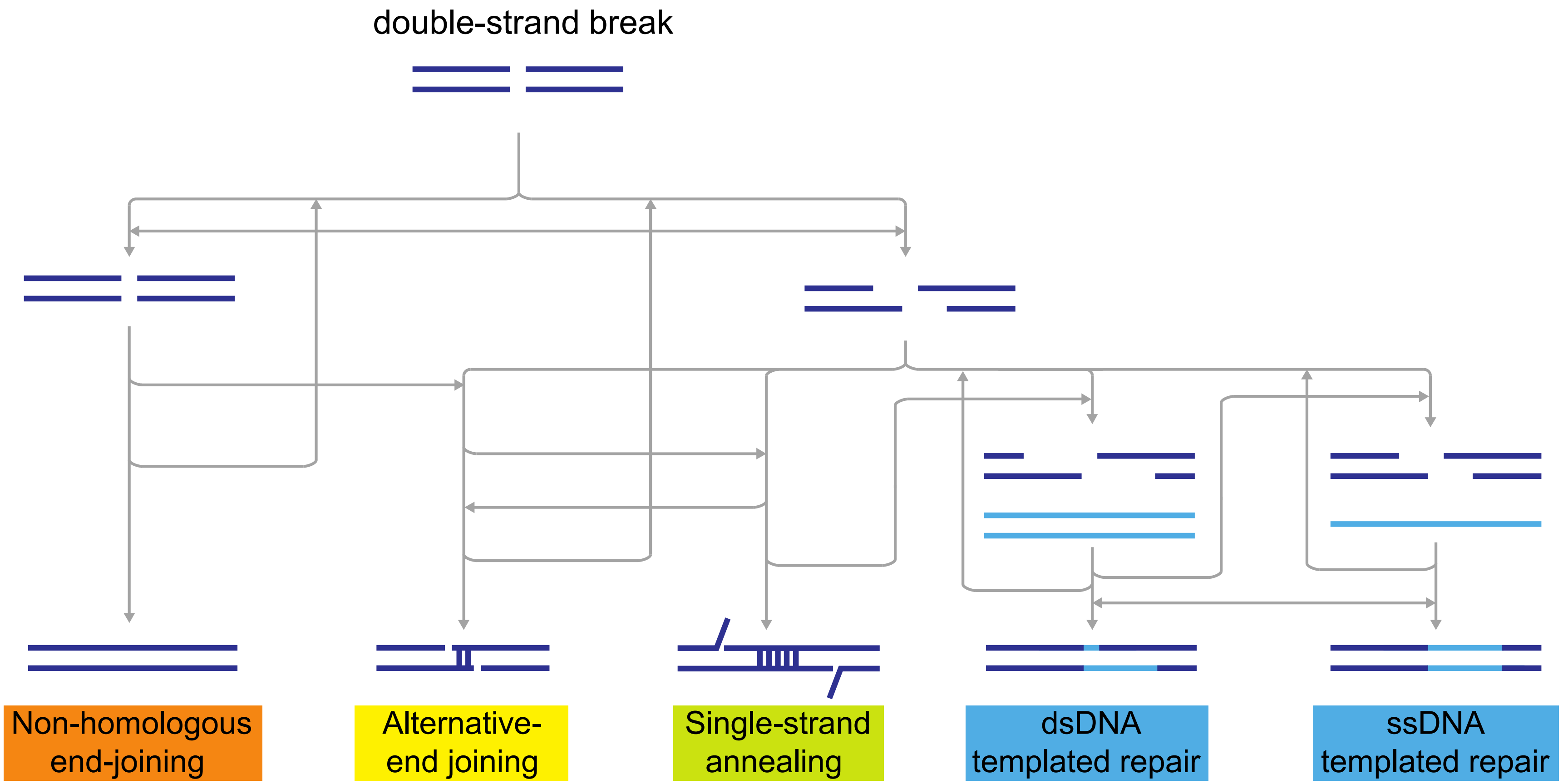
**Genome editing by repair
with template sequence**

Systematic knowledge about the organization of these pathways would aid precision editing efforts



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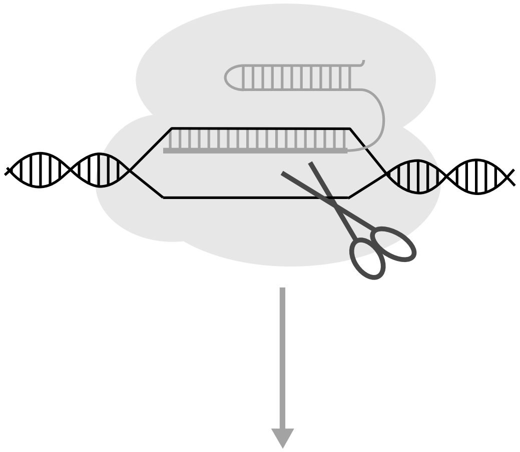
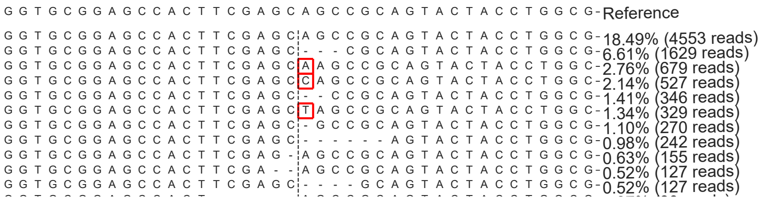
Genome editing by repair
with template sequence

Two CRISPR-based technologies now make systematic interrogation of DNA repair experimentally tractable

1. CRISPR-Cas systems make DNA damage and sequencing-based methods can ‘read out’ genome edits



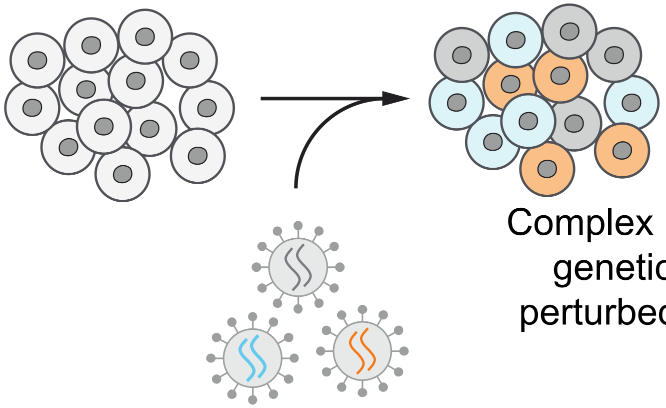


```
GGTGCGGAGCCACTTCGAGCAGCCGCAGTACTACCTGGCG-Reference

GGTGCGGAGCCACTTCGAGCAGCCGCAGTACTACCTGGCG-18.49% (4553 reads)
GGTGCGGAGCCACTTCGAGC---CGCAGTACTACCTGGCG-6.61% (1629 reads)
GGTGCGGAGCCACTTCGAGCAAGCCGCAGTACTACCTGGC-2.76% (679 reads)
GGTGCGGAGCCACTTCGAGCCAGCCGCAGTACTACCTGGC-2.14% (527 reads)
GGTGCGGAGCCACTTCGAGC--CCGCAGTACTACCTGGCG-1.41% (346 reads)
GGTGCGGAGCCACTTCGAGCTAGCCGCAGTACTACCTGGC-1.34% (329 reads)
GGTGCGGAGCCACTTCGAGC-GCCGCAGTACTACCTGGCG-1.10% (270 reads)
GGTGCGGAGCCACTTCGAGC------AGTACTACCTGGCG-0.98% (242 reads)
GGTGCGGAGCCACTTCGAG-AGCCGCAGTACTACCTGGCG-0.63% (155 reads)
GGTGCGGAGCCACTTCGA--AGCCGCAGTACTACCTGGCG-0.52% (127 reads)
GGTGCGGAGCCACTTCGAGC----GCAGTACTACCTGGCG-0.52% (127 reads)
```

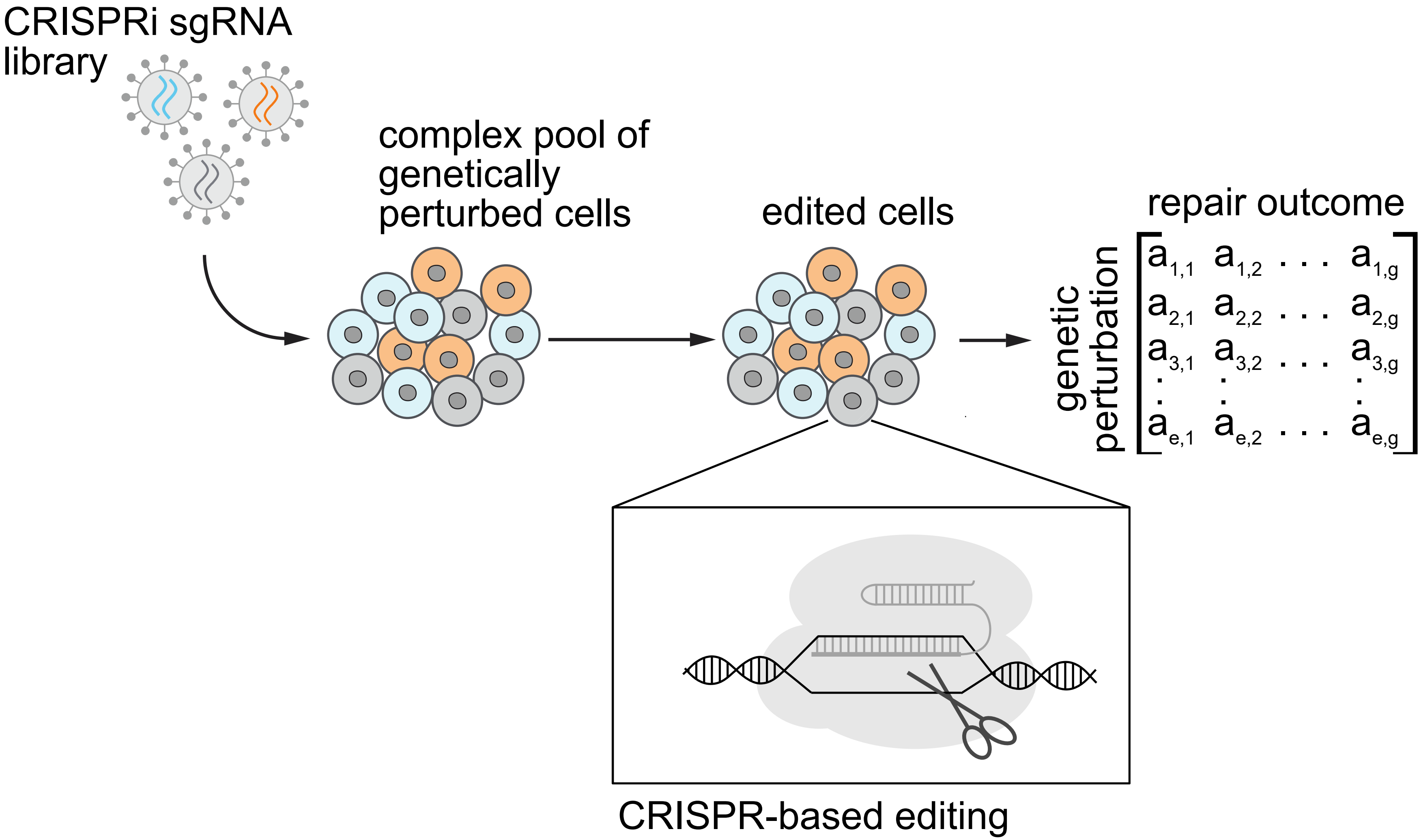
Image courtesy of http://crispresso.pinellolab.partners.org

2. CRISPR-based screens for interrogation of gene function



Complex pool of genetically perturbed cells

Together these technologies can quantify gene effects on single-molecule repair outcomes



CRISPRi sgRNA library

complex pool of genetically perturbed cells

edited cells

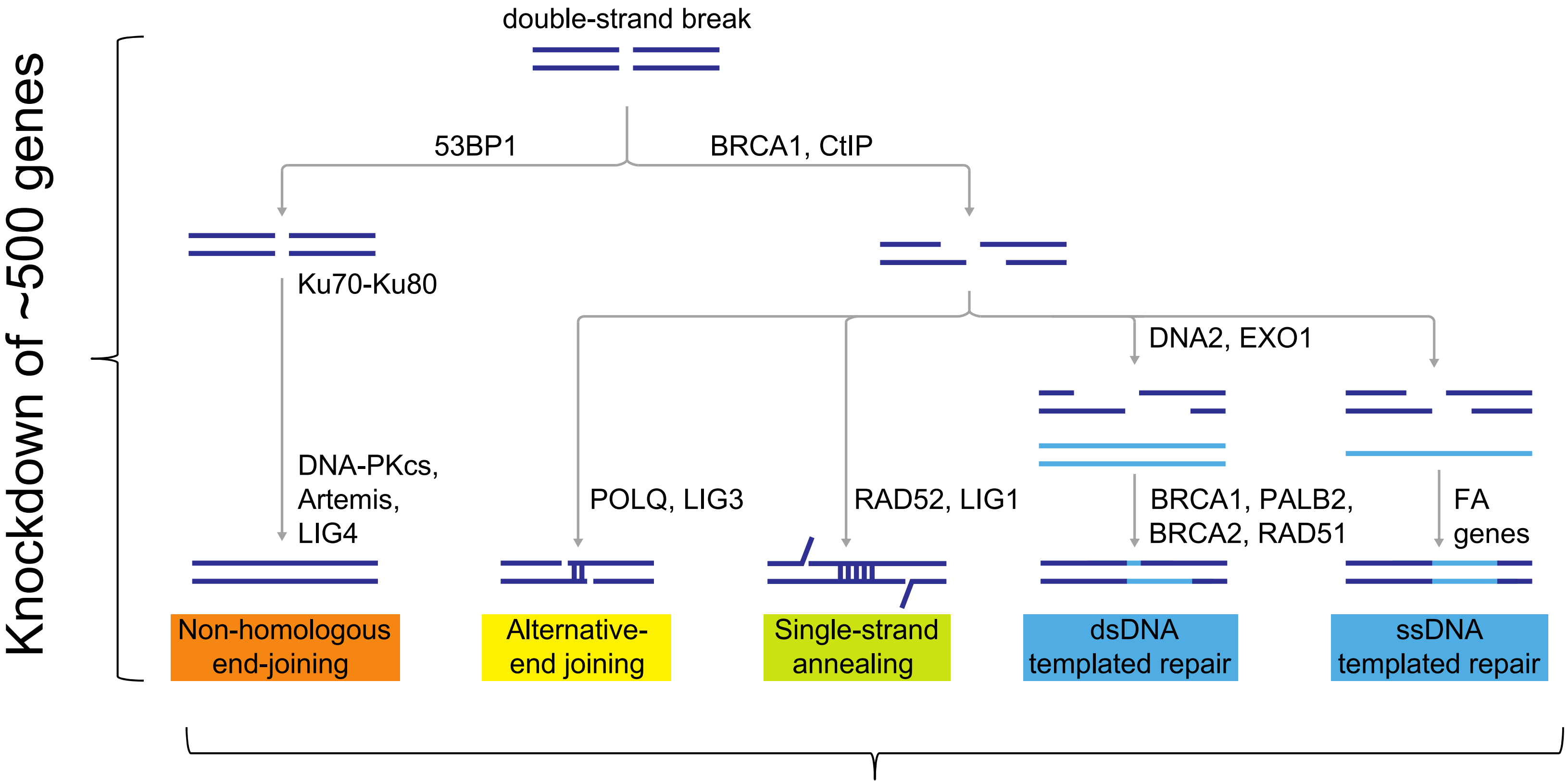
repair outcome

genetic perturbation

$$\begin{bmatrix} a_{1,1} & a_{1,2} & \cdots & a_{1,g} \\ a_{2,1} & a_{2,2} & \cdots & a_{2,g} \\ a_{3,1} & a_{3,2} & \cdots & a_{3,g} \\ \vdots & \vdots & & \vdots \\ a_{e,1} & a_{e,2} & \cdots & a_{e,g} \end{bmatrix}$$

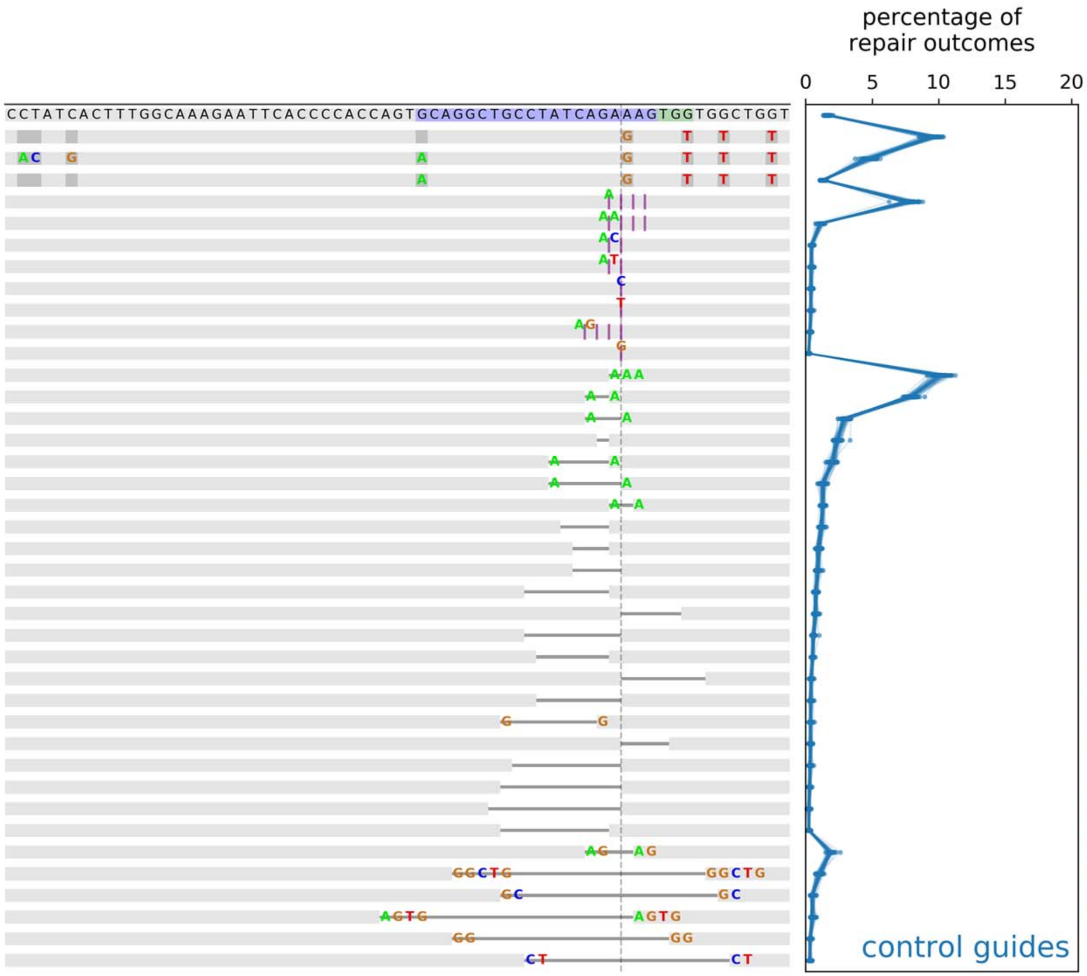
CRISPR-based editing

Systematic knowledge about the organization of these pathways would aid precision editing efforts



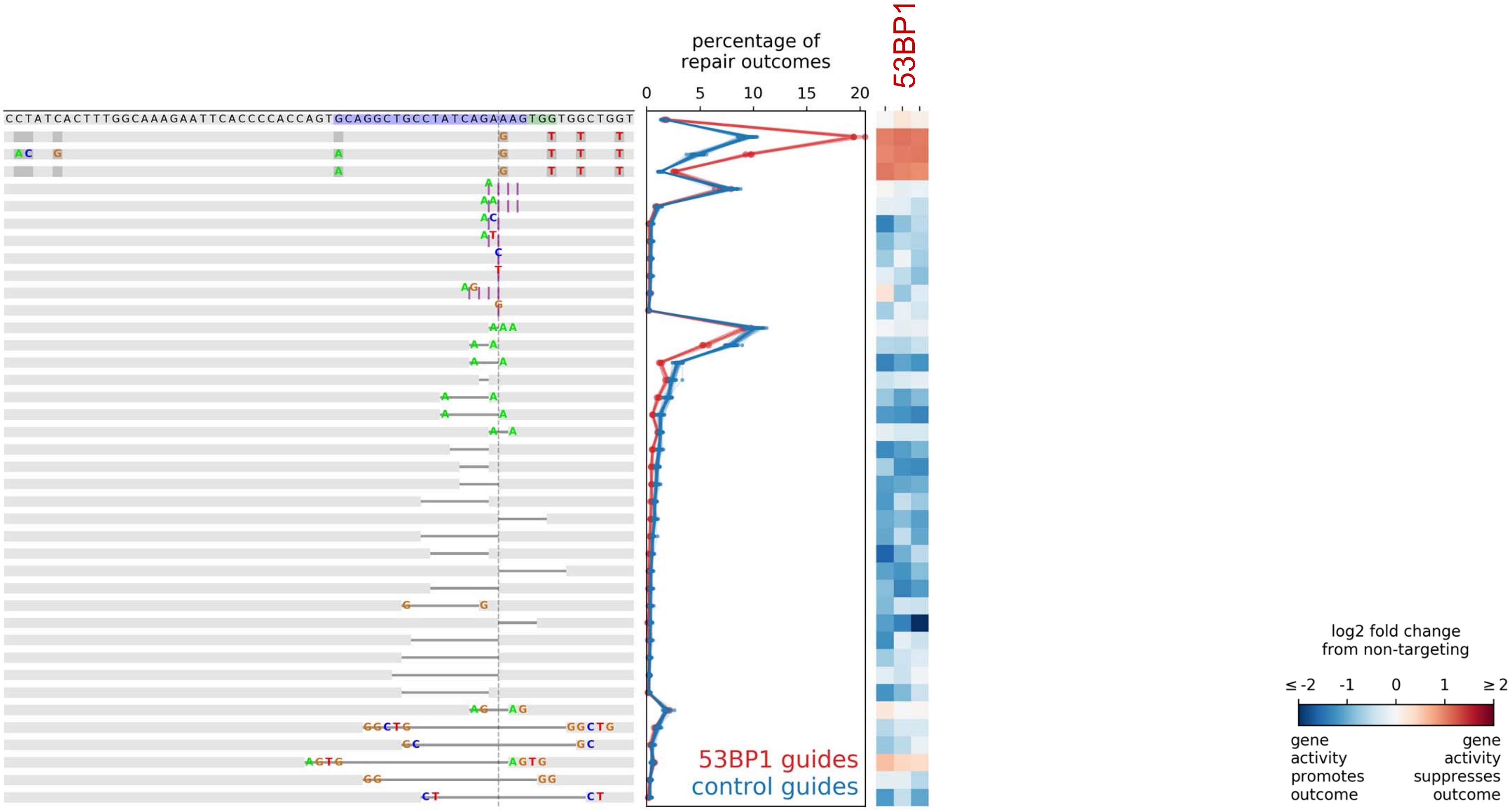
100s to 1,000s of distinguishable repair outcomes

Evaluating editing outcomes as mutational signatures gives a high-resolution view of DNA repair



40 most common outcomes shown

Depletion of known pathway regulators changes the distribution of edits



40 most common outcomes shown

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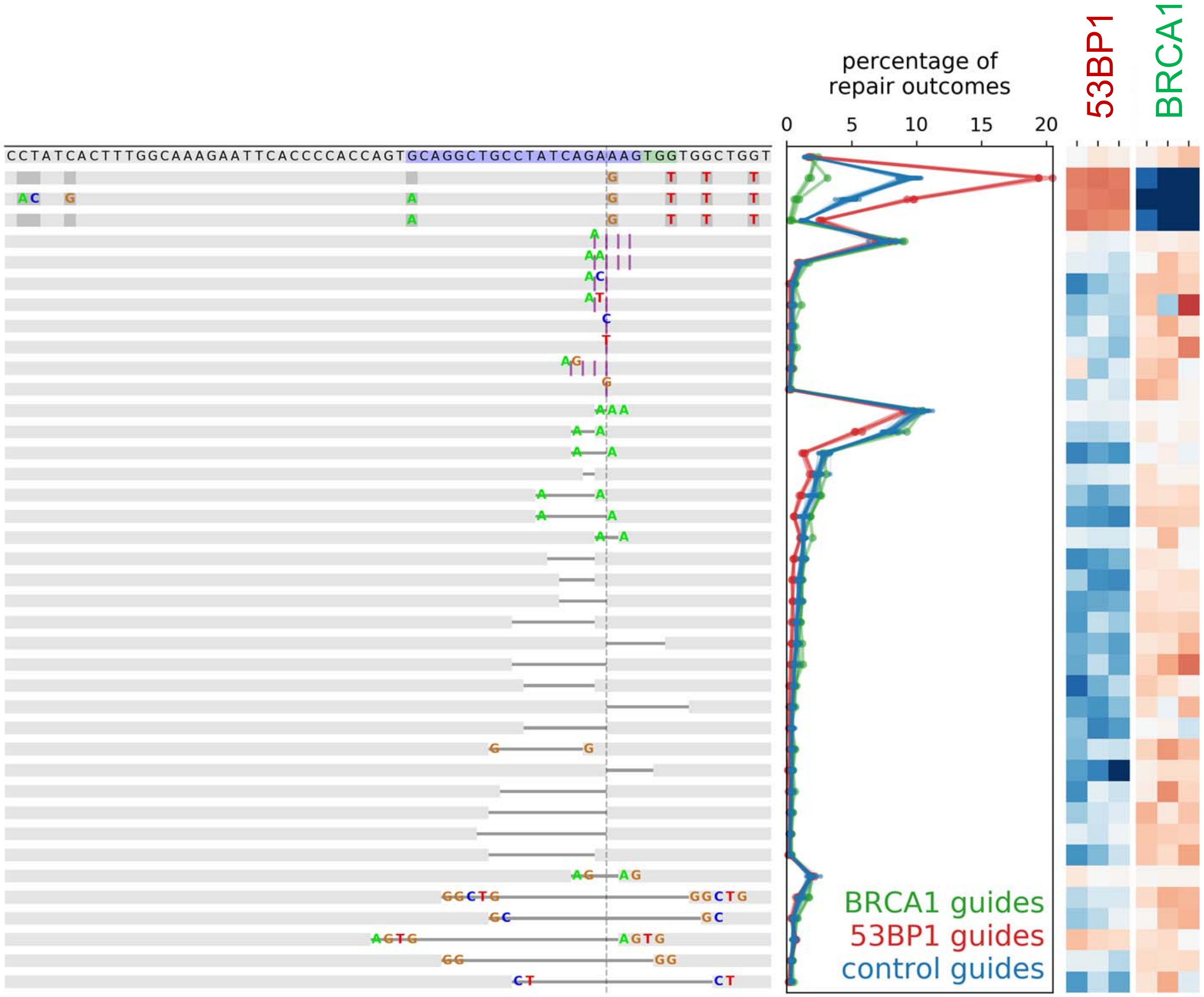
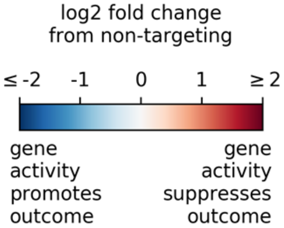
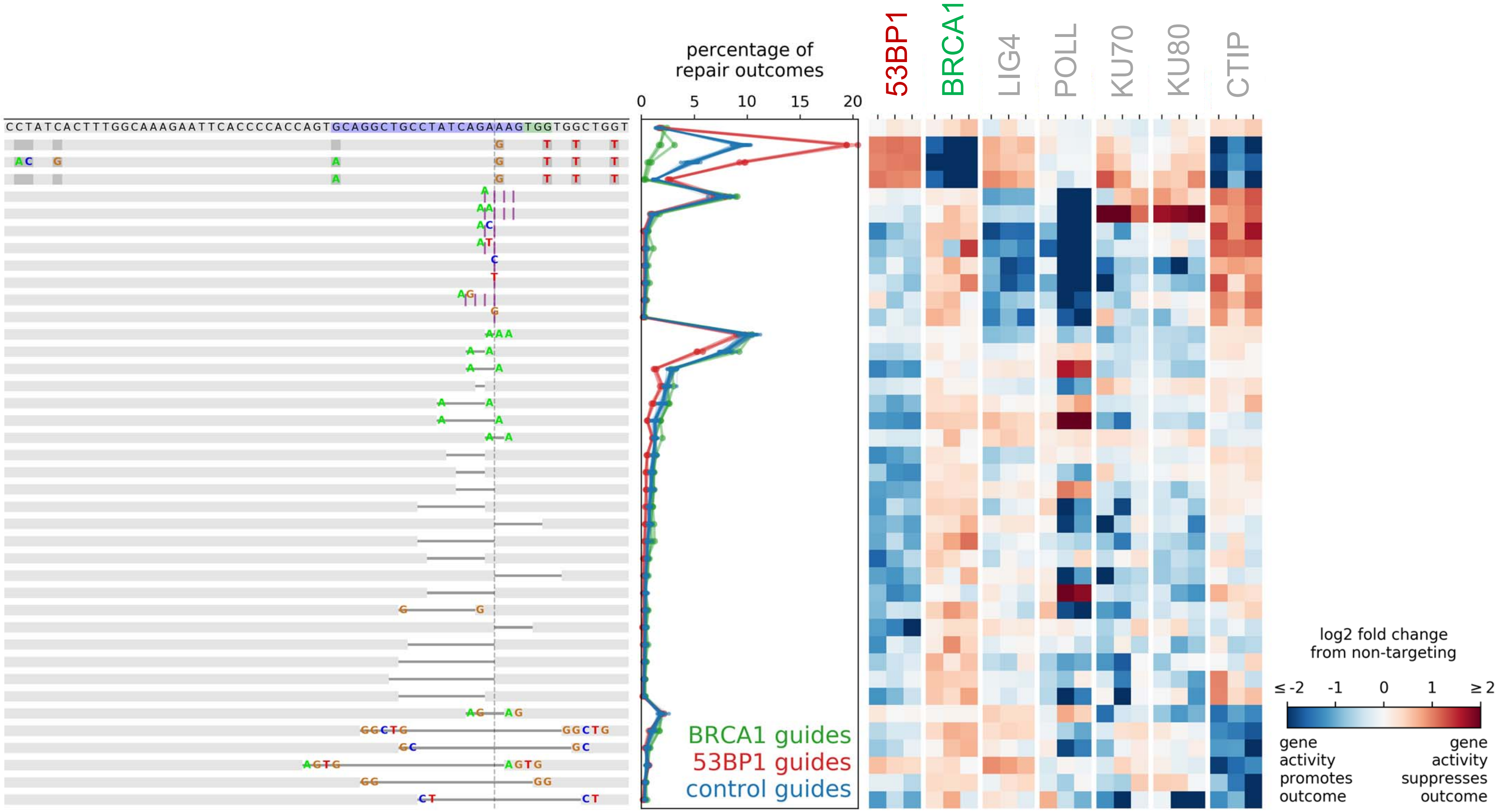


40 most common outcomes shown

BRCA1 and 53BP1 have antagonistic roles in DNA repair

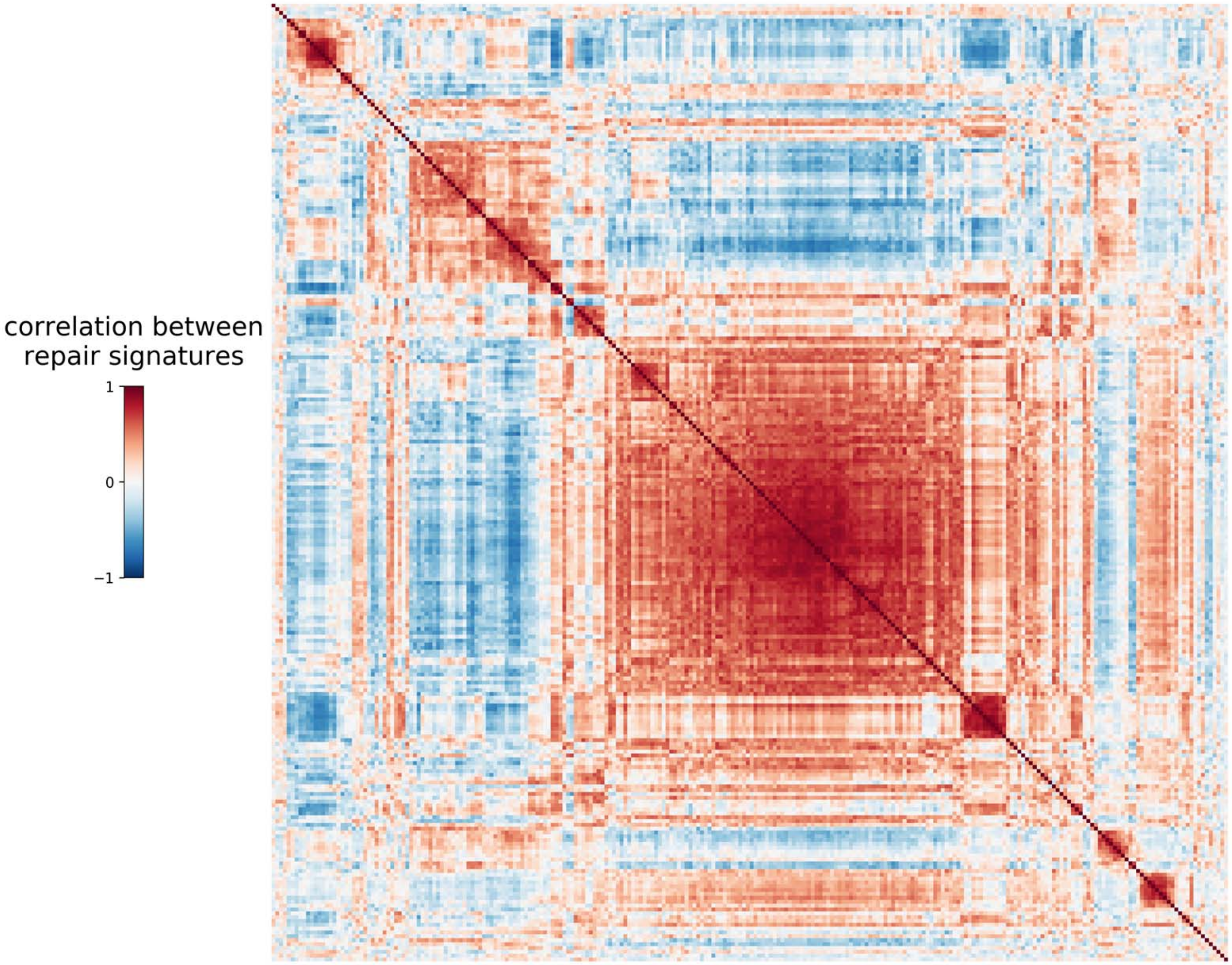


Depletion of known pathway regulators changes the distribution of edits

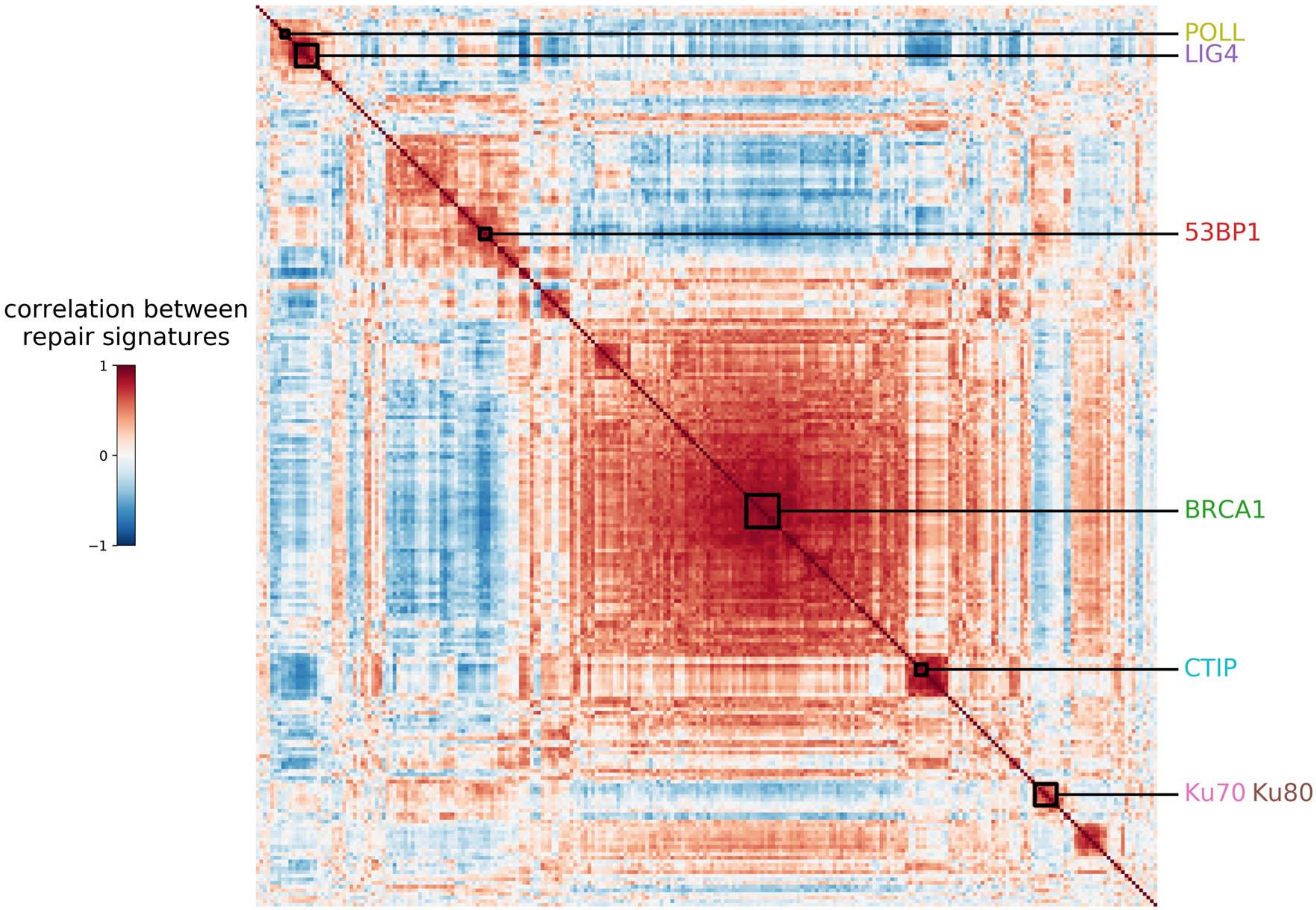


40 most common outcomes shown

Correlation between edit signatures delineates the genetic pathways of DNA repair in an unbiased way



Correlation between edit signatures delineates the genetic pathways of DNA repair in an unbiased way



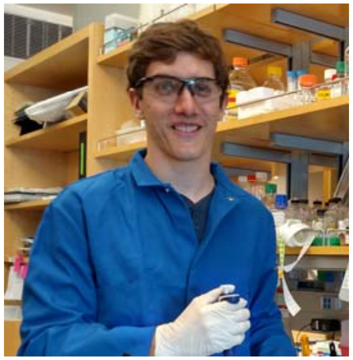
The value of assaying DNA repair pathway activity in a quantitative, unbiased manner

- Has practical implications for genome editing strategies that aim to enrich for particular events (not just alter pathway balance)

- Allows us to isolate mechanisms that cells use to maintain genome integrity and study how they work

- Gives us a powerful way to deduce gene-gene functional relationships and understand how repair mechanisms interact

Acknowledgements


Jeff Hussmann


Jonathan Weissman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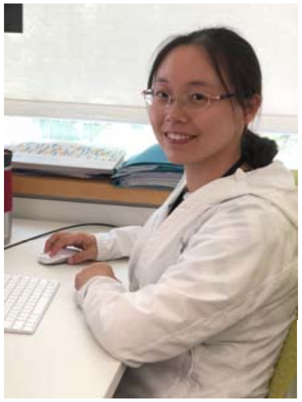
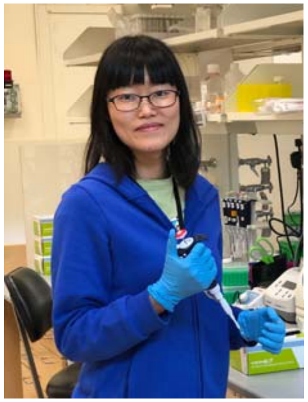
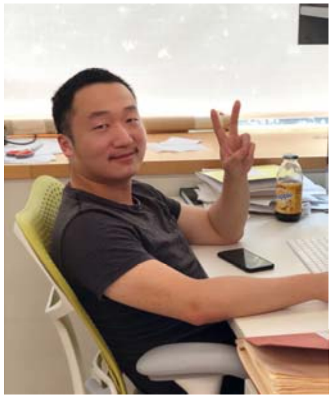
Albert Xu


Purnima Ravisankar


Sherry Liu


Jia Ling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Britt Adamson is a member of a ThinkLab Advisory Board for Celsius Therapeutics.