Molecular approaches to assess repair of chromosome breaks

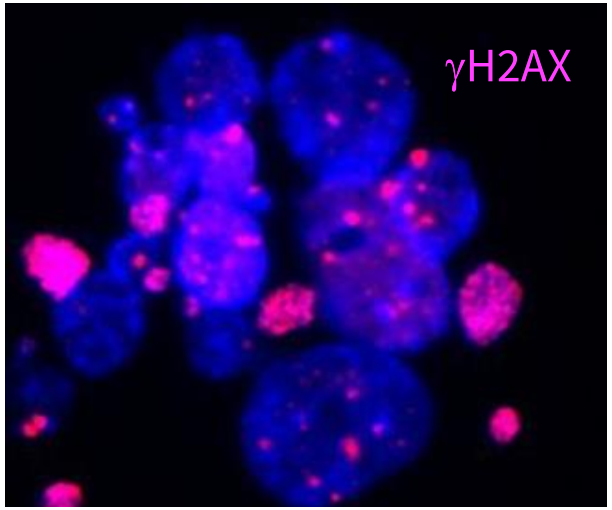
Molecular assays for DSB repair

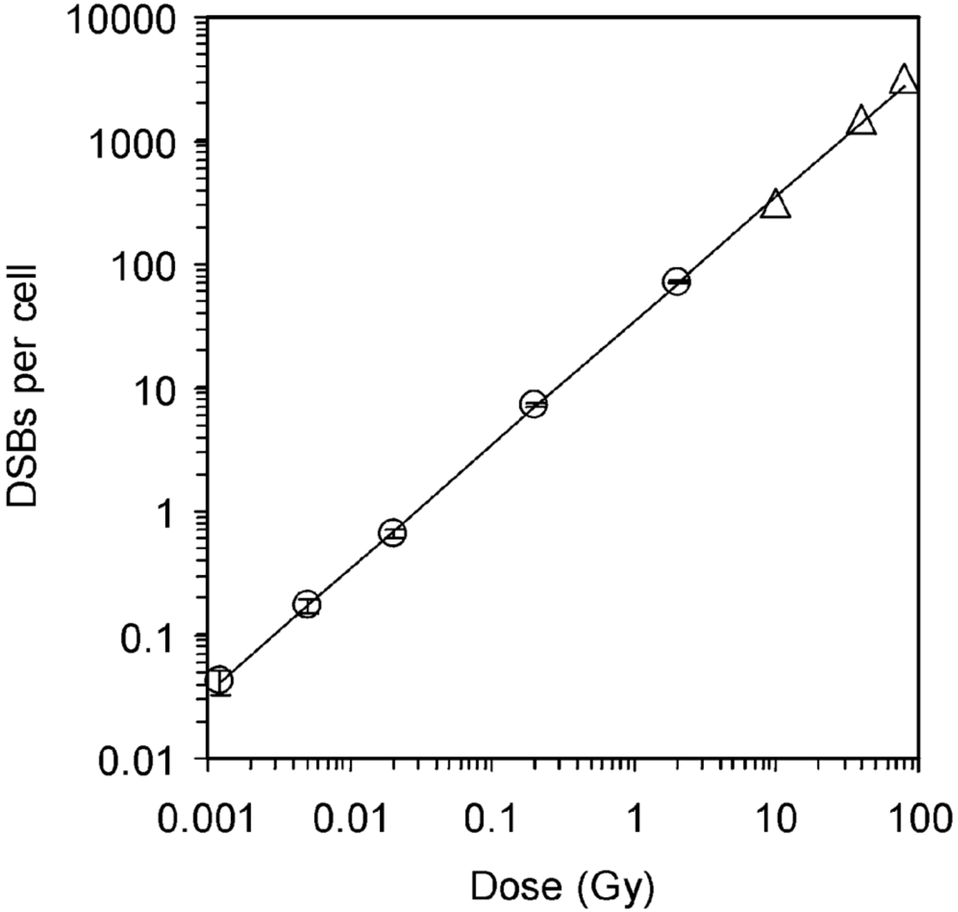
1. Imaging of double strand break surrogates
    - γH2AX
2. Imaging of repair factors
    - Molecules required for repair (e.g. Rad51)
3. Repair
    - Pulse field, COMET; detects>100s of DSBs
    - typically not suitable for low dose IR
    - Repair products
        - Aberrations; cytogenetics, micronuclei
        - Targeted DSBs and reporter assays
        - Sequencing; signatures

- Static picture?
    - not measuring repair unless tracked over time after dose, in living samples

1) DSB surrogates

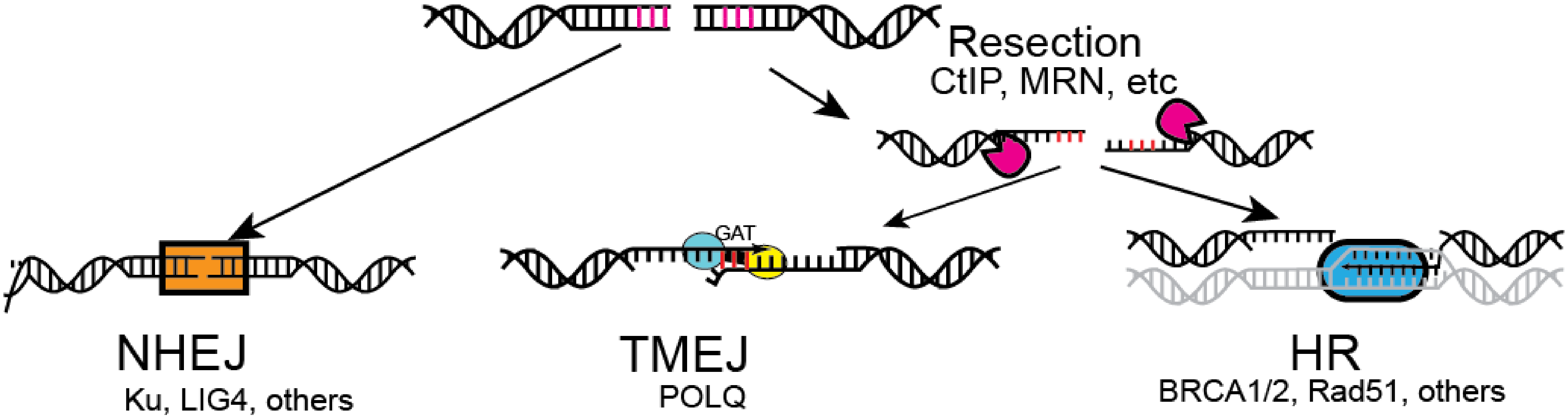
- γH2AX
- 53BP1
- TUNEL





Proc Natl Acad Sci U S A. 2003 Apr 29;100(9):5057-62.

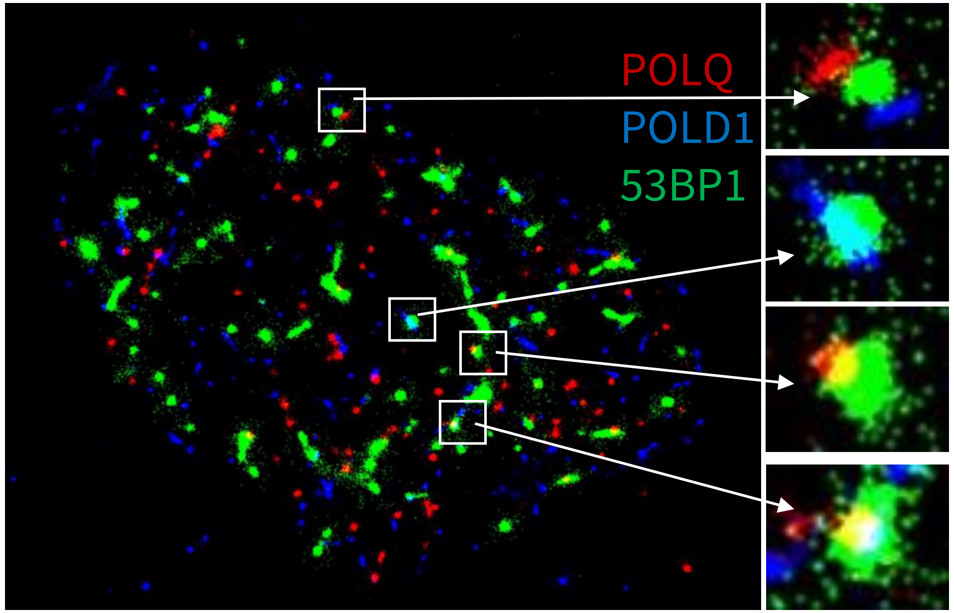
Double strand break repair pathways



Nearly equivalent to…
Microhomology mediated end joining (MMEJ)
Alternative end joining (a-EJ, Alt-NHEJ)

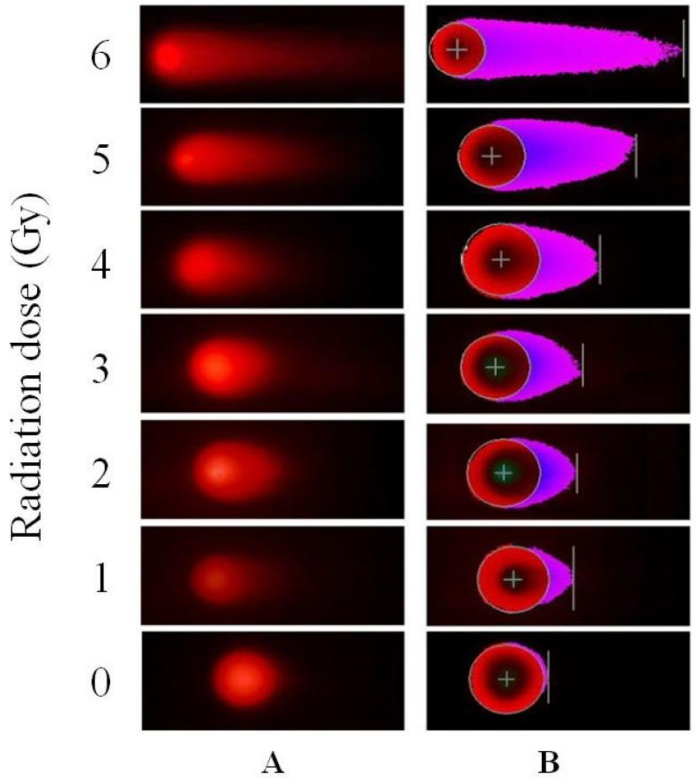
2) Imaging of repair factors

- Immunofluorescence
  - Repair factors aggregate at damage (“colocalize”, form ”focus”)
  - Rarely quantitative; repair typically needs only a handful of active molecules
    - Below detection limits
    - Obscured by passive molecules
    - Exception; Rad51, a marker that accurately reflects active HR
- STORM
  - Stochastic Optical Reconstruction Microscopy
  - more sensitive, quantitative
  - better spatial resolution
    - can identify functional associations



3) Repair

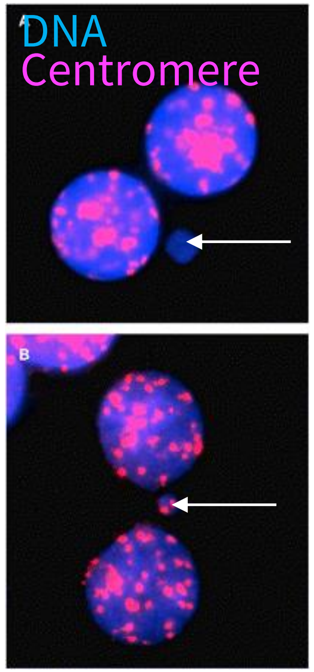
- COMET (and pulse field)
- Embed nucleii in agarose, electrophorese
- Fragmented DNA appears as tail on comet
- Best at 100s of DSBs or more
    - typically not suitable for low dose IR
    - Descriptive; little insight into pathway, consequenc

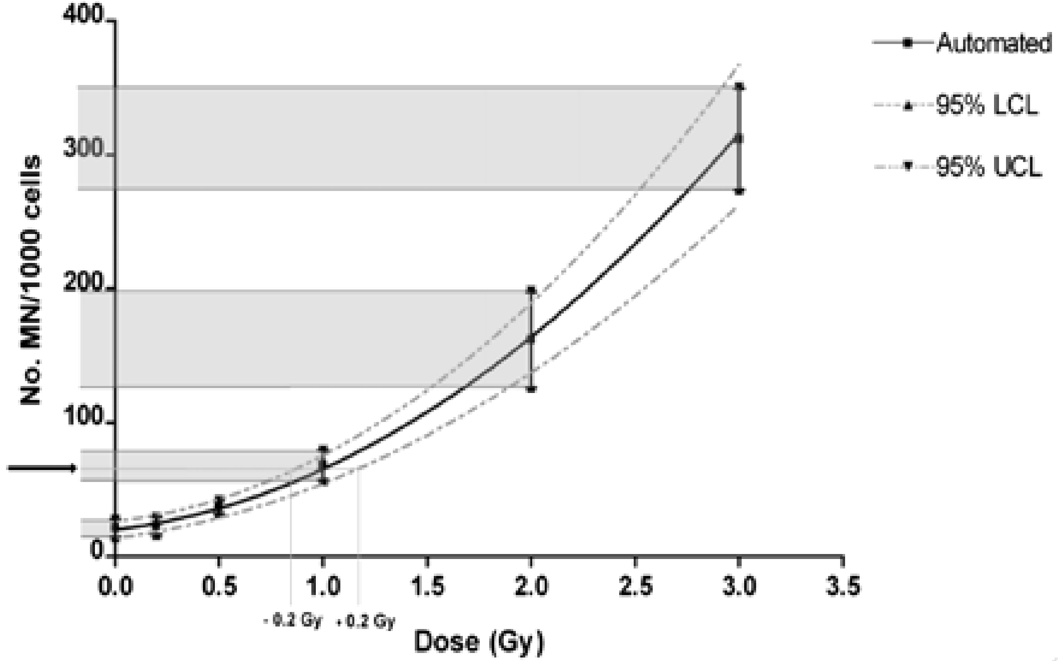


Int J Mol Sci. 2013;14(11):22449-22461.

3) Repair

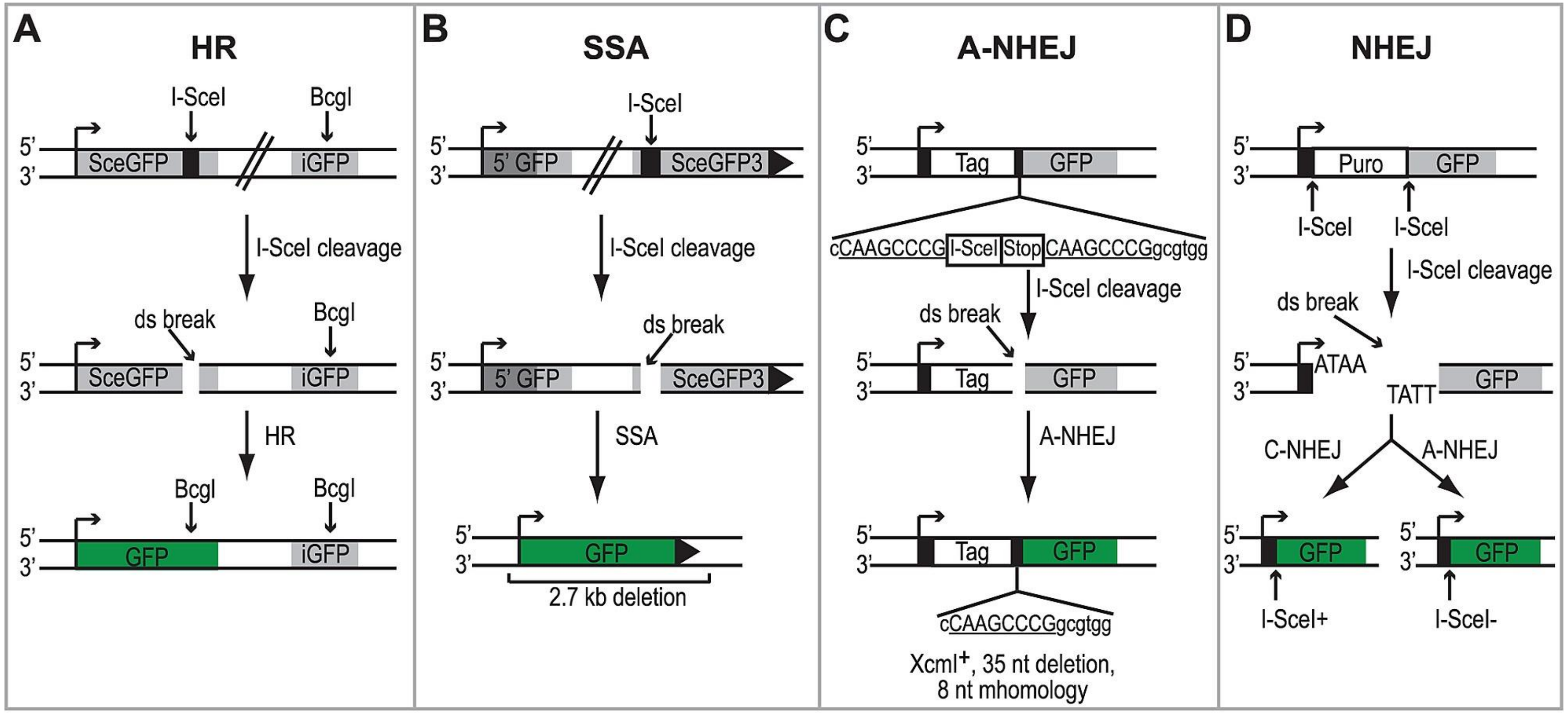
- Aberrant repair
  - Micronucleii; fragmented chromosomes
  - Cytogenetic detection of aberrations
  - Like COMET assay, no insight into mechanism

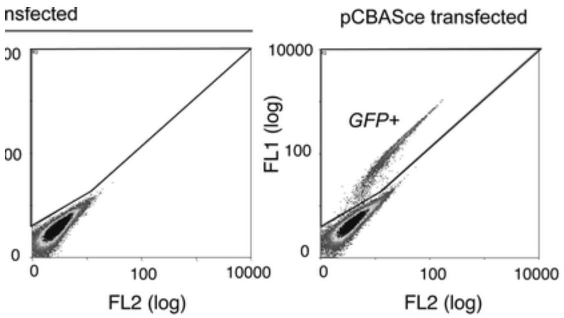




Mutagenesis, Volume 26, Issue 1, January 2011, Pages 11–17.

3) Reporter assays



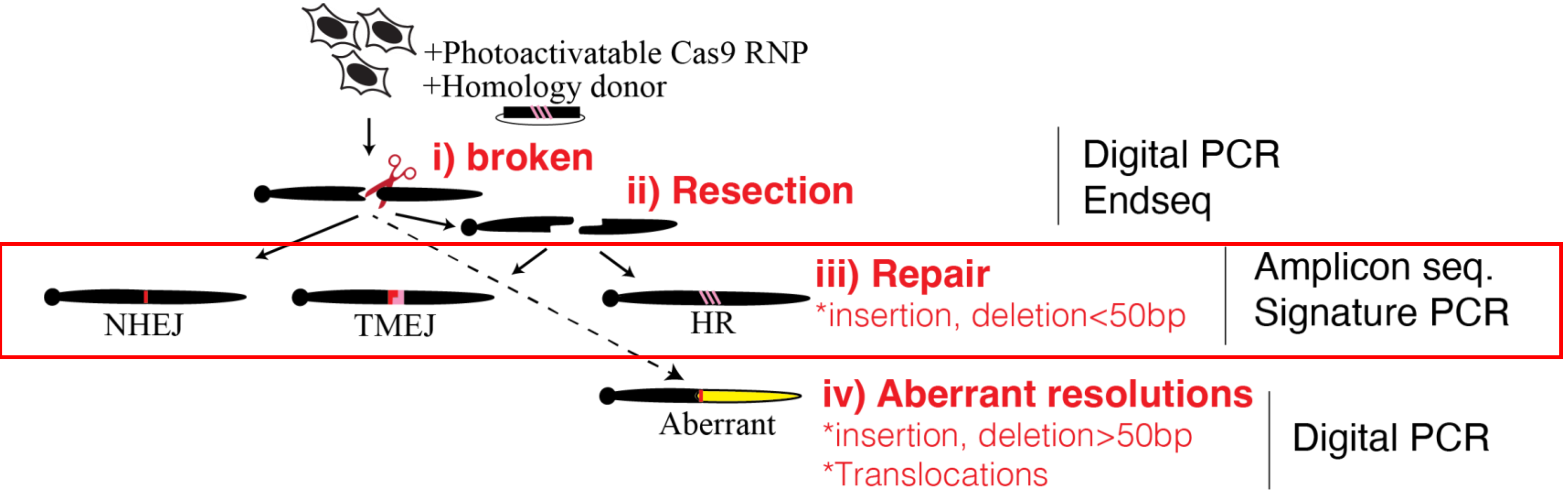


PLoS Pathog. 2012;8(8):e1002862.

Measure efficiency of pathway specific “signature” repair product

3) Sequencing repair products

- Targeted, induced chromosome break
- Track ALL intermediates, products, over time
  - Combination of digital PCR, amplicon sequencing
  - Insertions and deletions (indels) <50bp=accurate repair
  - Deletions>50 bp, translocations=aberrant



+Photoactivatable Cas9 RNP
+Homology donor

i) broken

ii) Resection

Digital PCR
Endseq

NHEJ
TMEJ
HR

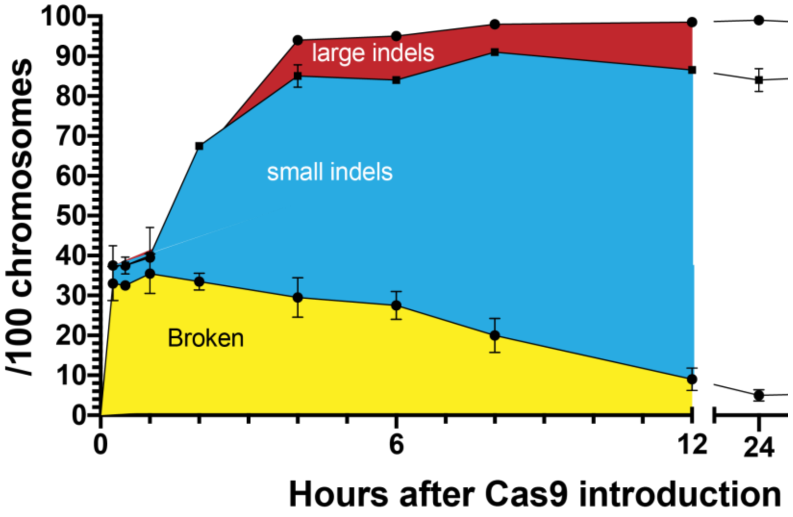
iii) Repair
*insertion, deletion<50bp

Amplicon seq.
Signature PCR

Aberrant

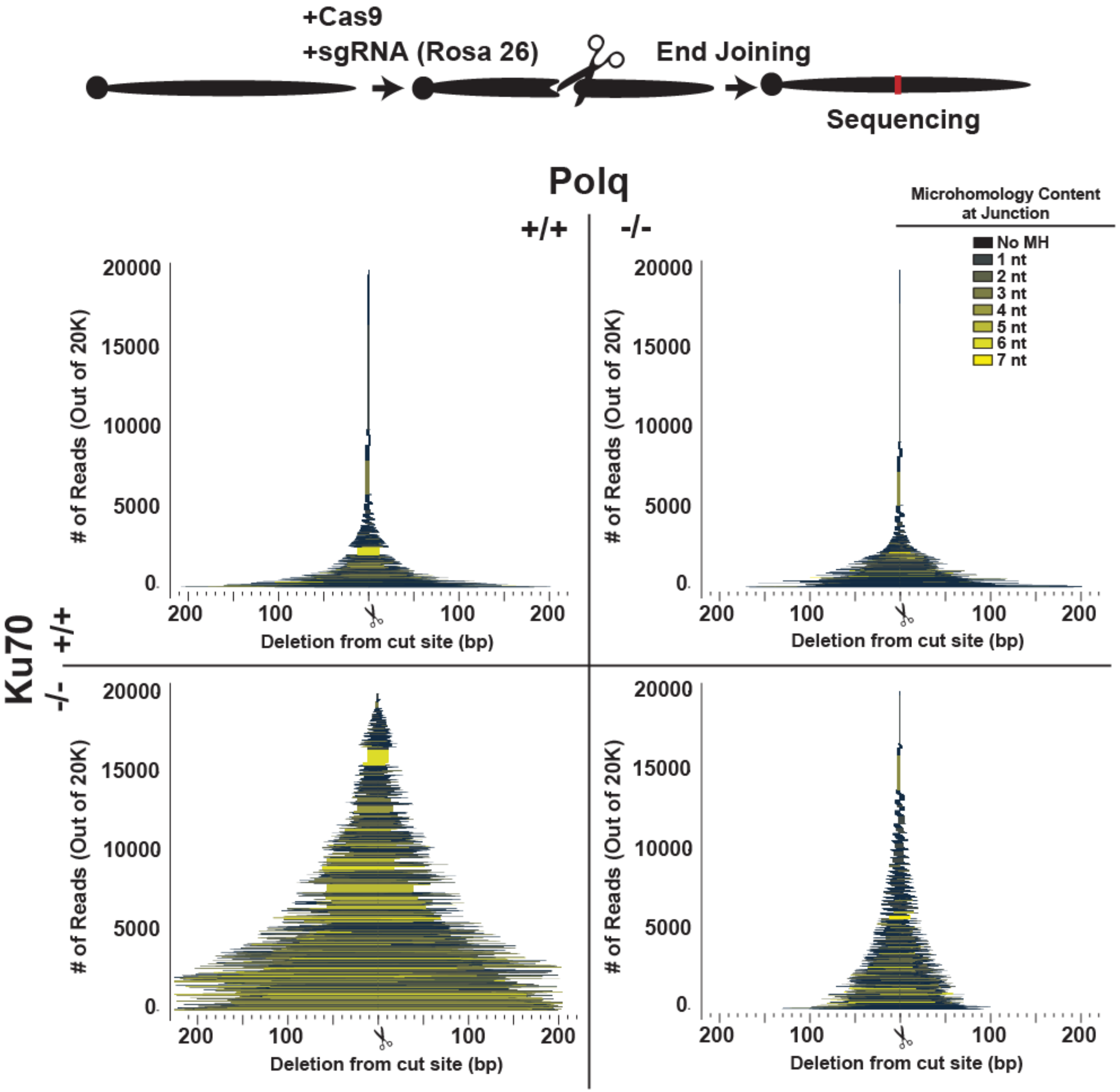
iv) Aberrant resolutions
*insertion, deletion>50bp
*Translocations

Digital PCR

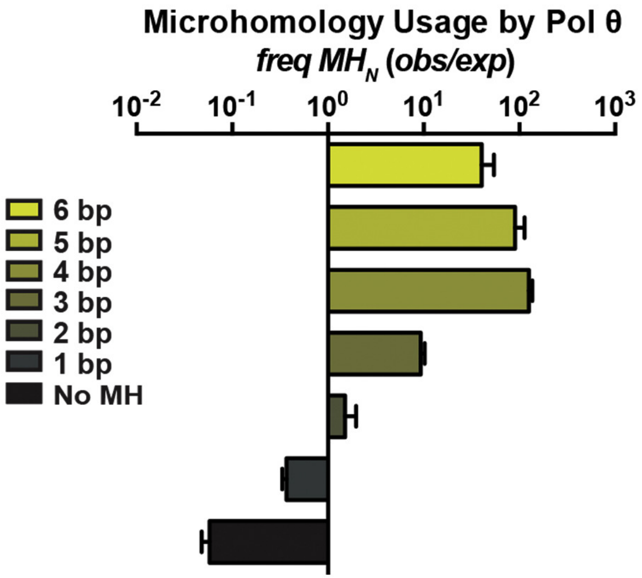


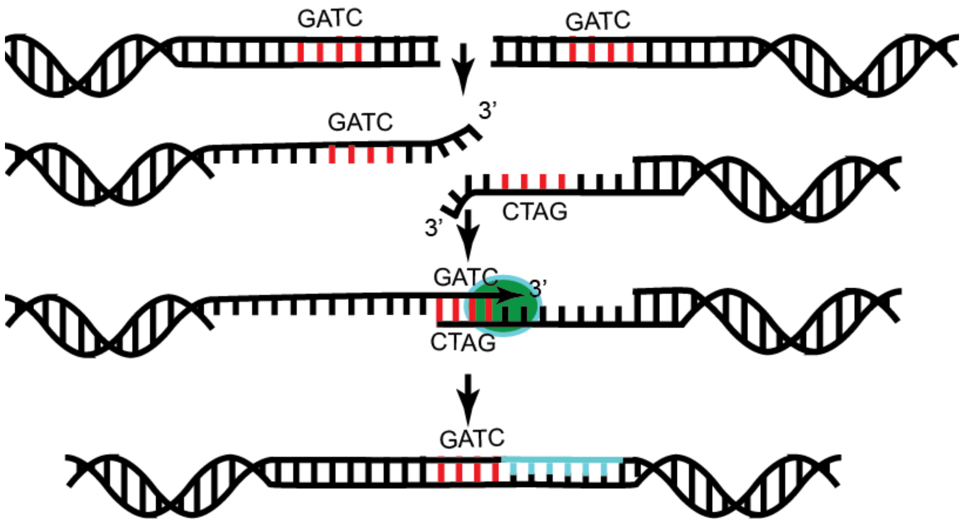
3) Repair

- “Repairseq”
- Ku (NHEJ) required for
  - short (<5bp) insertion and deletion
  - Little role for microhomology
- POLQ required for deletions at microhomologies (yellow)

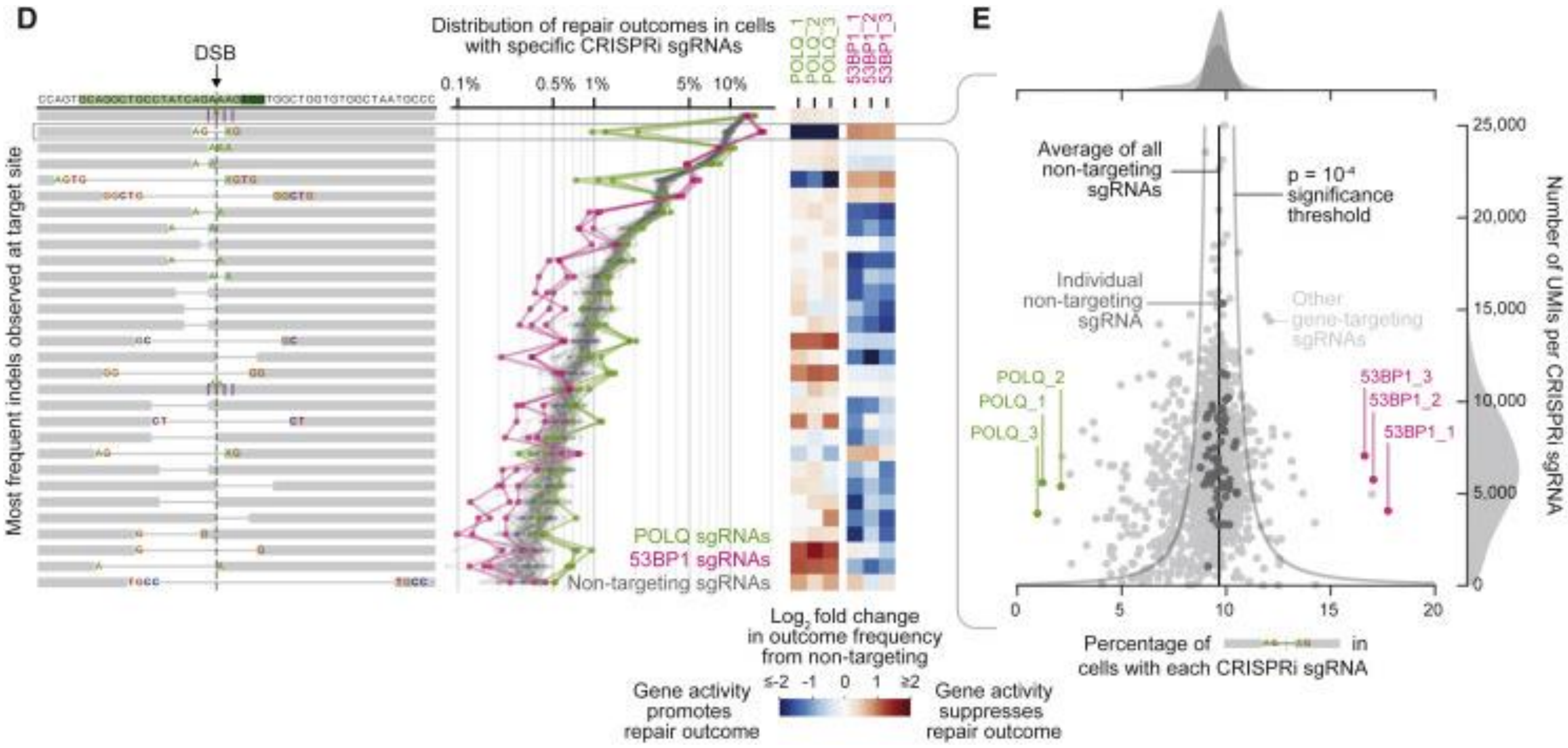


TMEJ and microhomologies





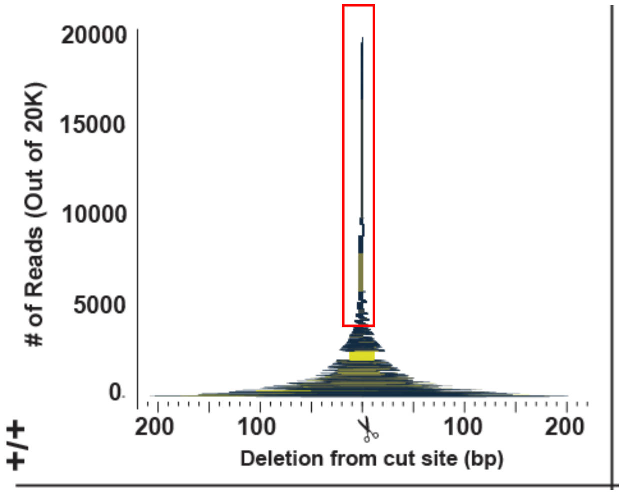
3) Repairseq



Cell. 2021 Oct 28;184(22):5653-5669.e25. 2021 Oct 20.

3) Repair signatures

- Whole genome sequencing
  - Statistics driven ”clustering” of somatic mutation types, according to e.g. sequence context, tumor type
  - Accurate repair is “invisible”
- DSB repair: focus on insertion and deletions (“indels”)
  - most, but not all, of NHEJ…even TMEJ=small indels; 0-5 bp, but…
  - This is quickly buried underneath more frequent replication associated errors
    - For anything short of very high dose
  - longer insertion and deletions…



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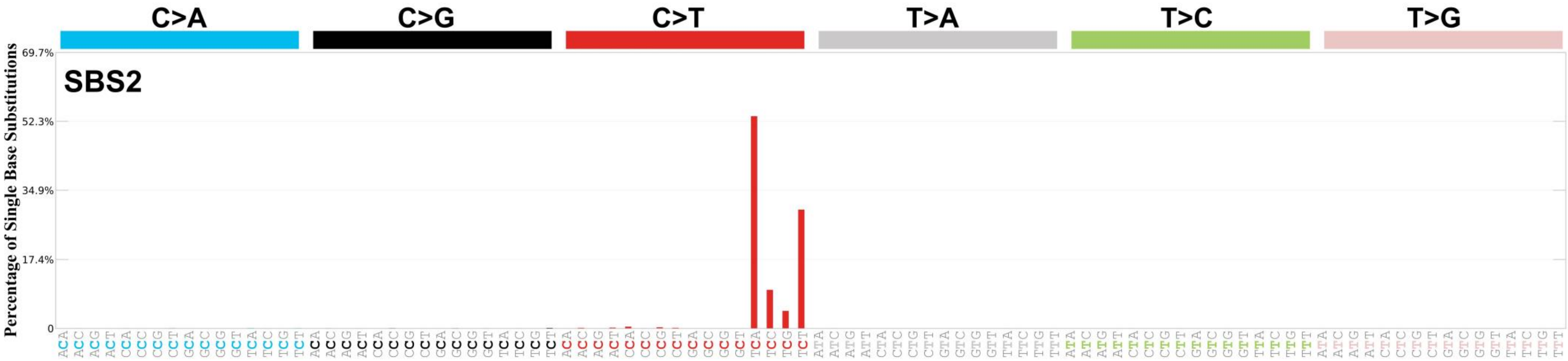
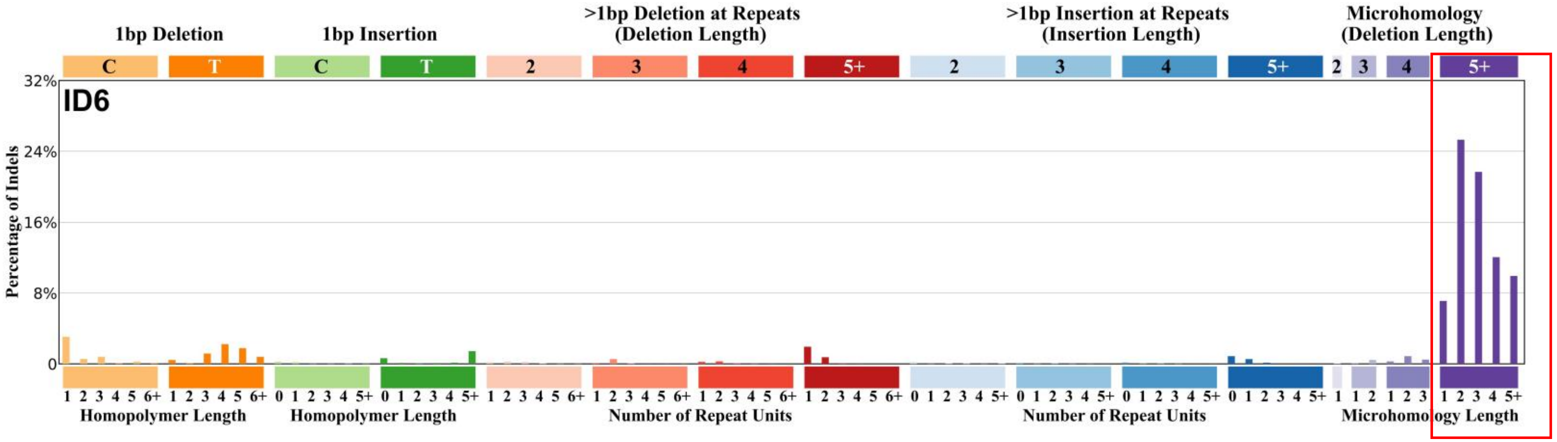


Nature. 2020 Feb;578(7793):94-101.

3) DSB Repair signatures

- Repair by TMEJ (mostly)



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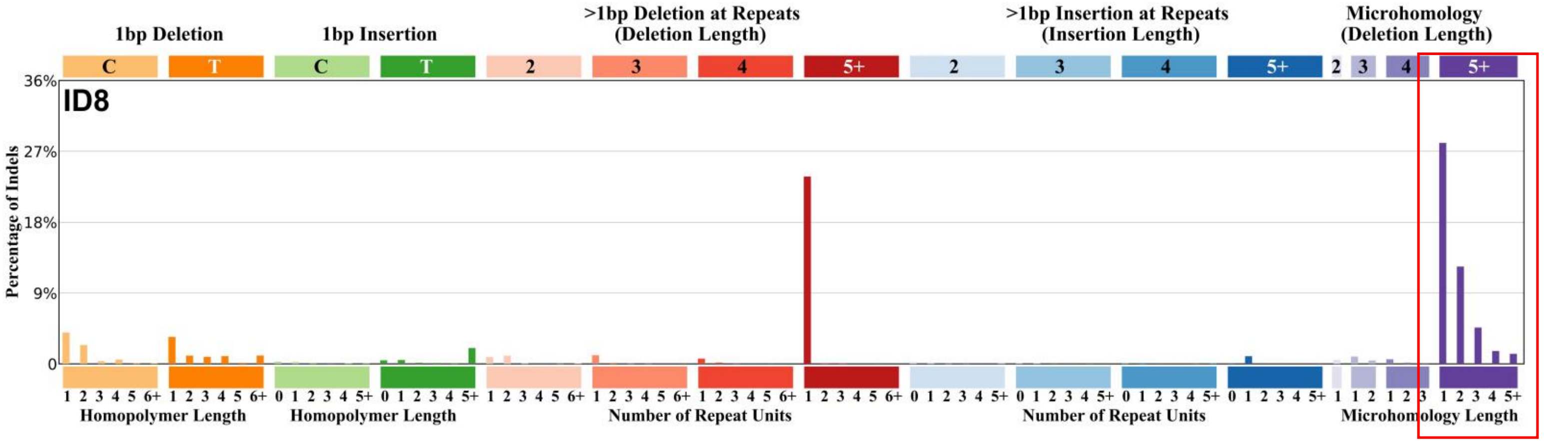
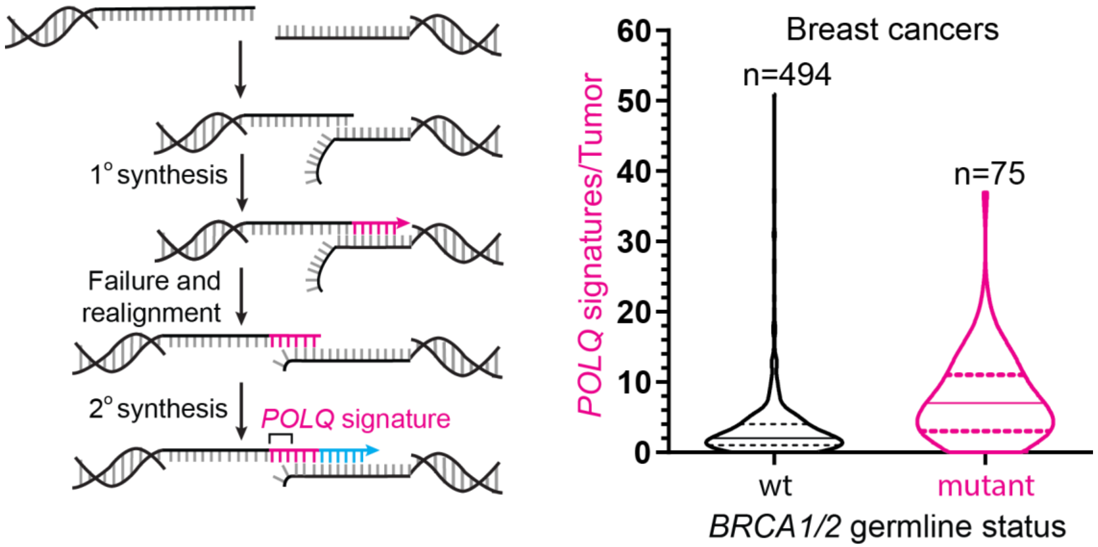


Nature. 2020 Feb;578(7793):94-101.

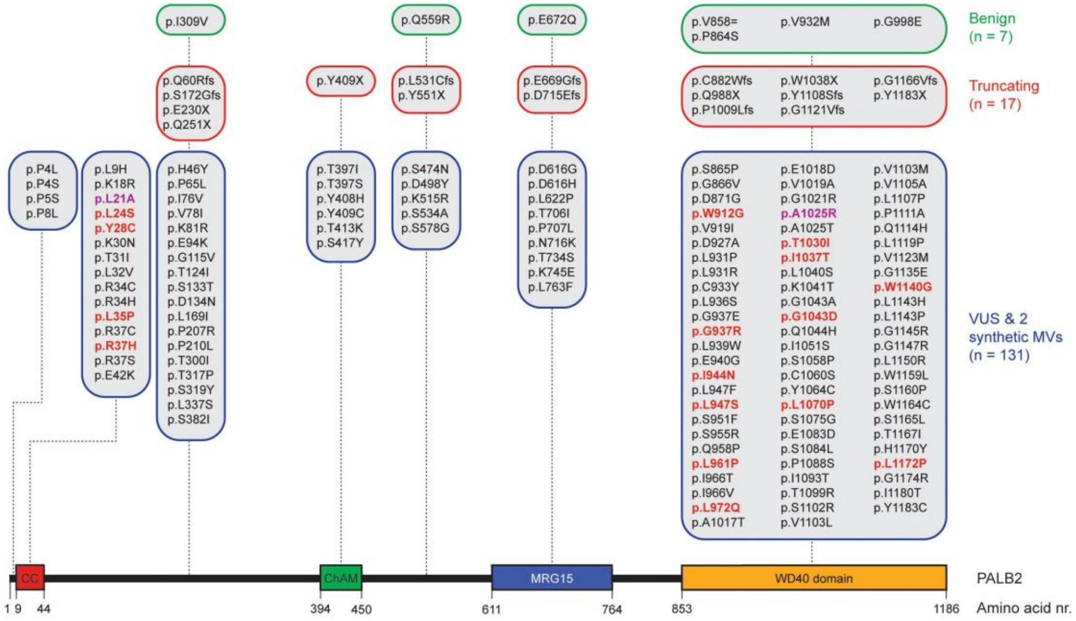
More exotic indels

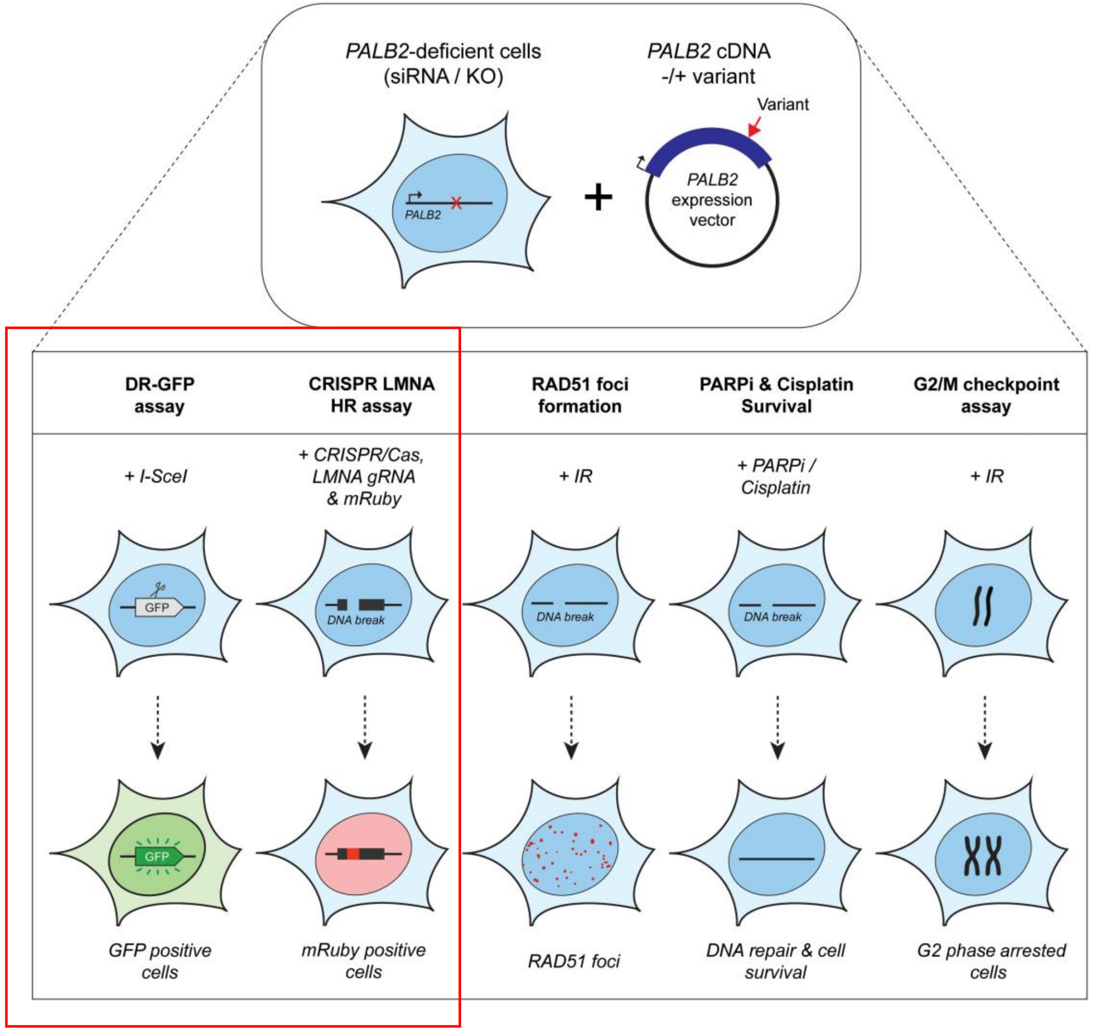
- TMEJ
  - Templated insertions
  - <15 bp repeat of nearby sequence(within 30 bp)
  - Interrupted repeat, NOT tandem
- NHEJ
  - short (10-50 bp) tandem repeat



Templated insertions in BRCA mutant cancer

3) Functional assays to predict effects of variants of uncertain significance





Front Mol Biosci. 2020 Sep 16;7:169.

4) Future directions

- Need more robust, quantitative, and sensitive methods to image damage, repair factors
- Signature detection
  - Currently stats only- blind to aetiology
- Employ newly integrated functional assays and genetic models (like Repairseq) to
  - Provide aetiology for signatures
  - Evaluate factors (environmental, genetic) that impact repair efficiency
  - Including effects of variants of uncertain significance on DSB repair genes