

A photograph of a large building under construction. The building's steel frame is visible, with several floors completed. A yellow crane is positioned on the right side of the building. In the foreground, there is a road with a yellow arrow pointing towards the building. The sky is overcast.

## **Nonhomologous end-joining (NHEJ)**

### **Lessons from yeast**

**Thomas E. Wilson, MD PhD**  
**Department of Pathology**  
**University of Michigan**

## Contributors

NHEJ screens/  
Two-hybrid

### Tom Wilson laboratory

Elissa Karathanasis  
Phil Palmbos  
Leana Topper

3' phosphatase

John Vance  
Anandi Karumbati  
Rajashree Deshpande  
Aswathi Nair

### University of Montreal

Dindial Ramotar  
Arshad Jilani

Pol4/  
Processed NHEJ

Jim Daley  
Renee Vander Laan

### University of Sussex

Aidan Doherty  
Marina Della  
Louise Tonkin  
Robert Pitcher

Bacterial NHEJ

Phil Palmbos  
Jim Daley

### University of Maryland

Alan Tomkinson  
Hui-Min Tseng

# Cancer: a disease of DNA

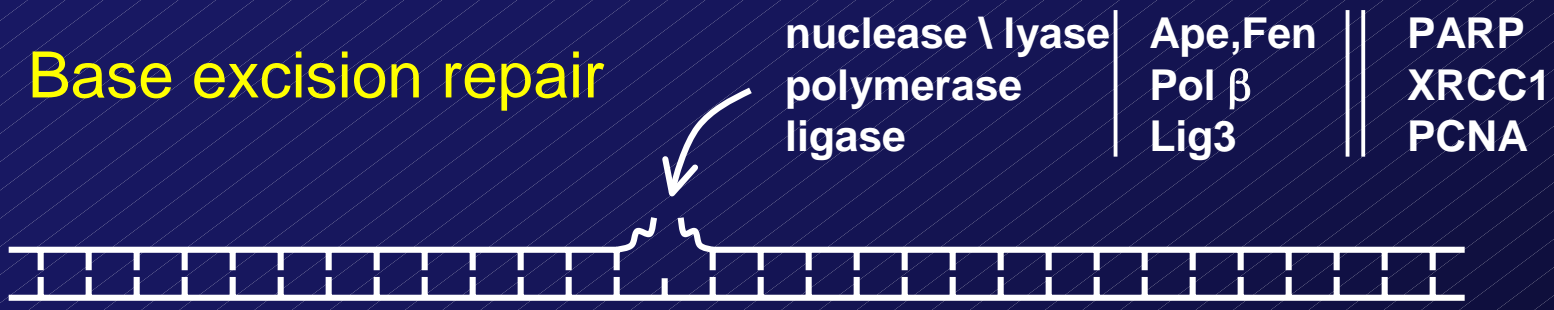


D. Ferguson and F. Alt

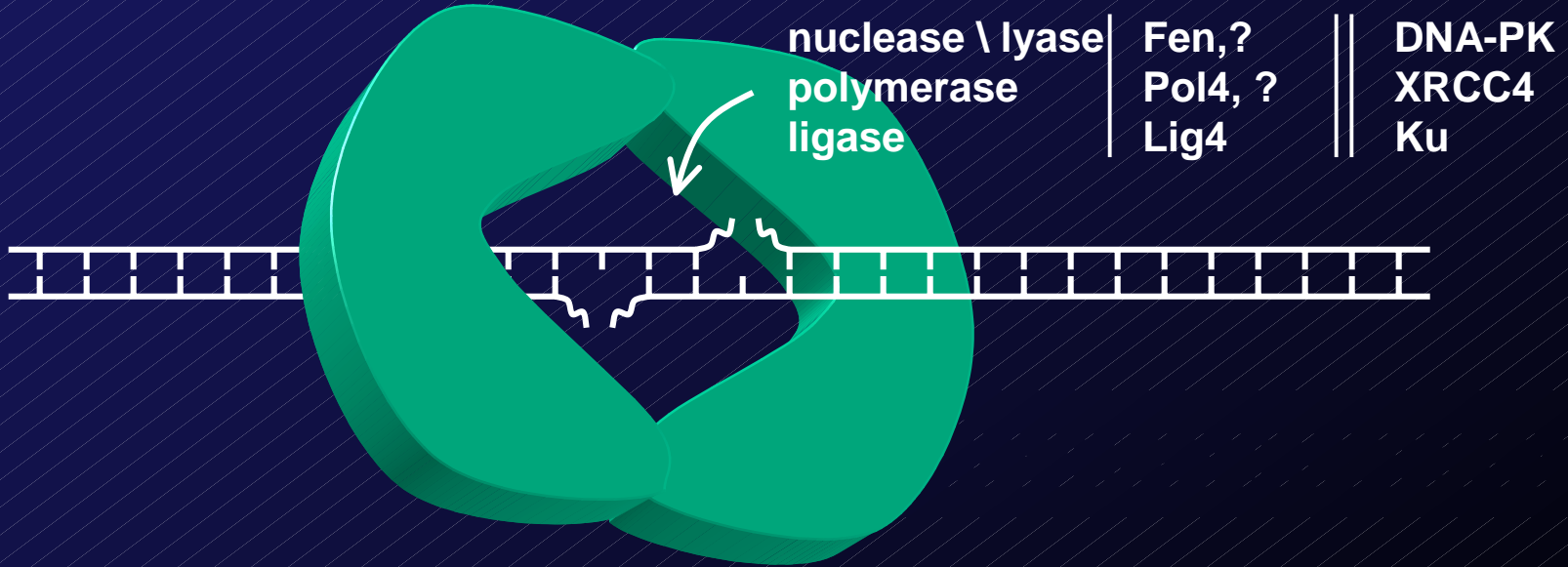
# Homologous recombination



## Base excision repair



## Nonhomologous end-joining





## Themes / conclusions

NHEJ evolved to repair DSBs when homologous recombination is disadvantageous.

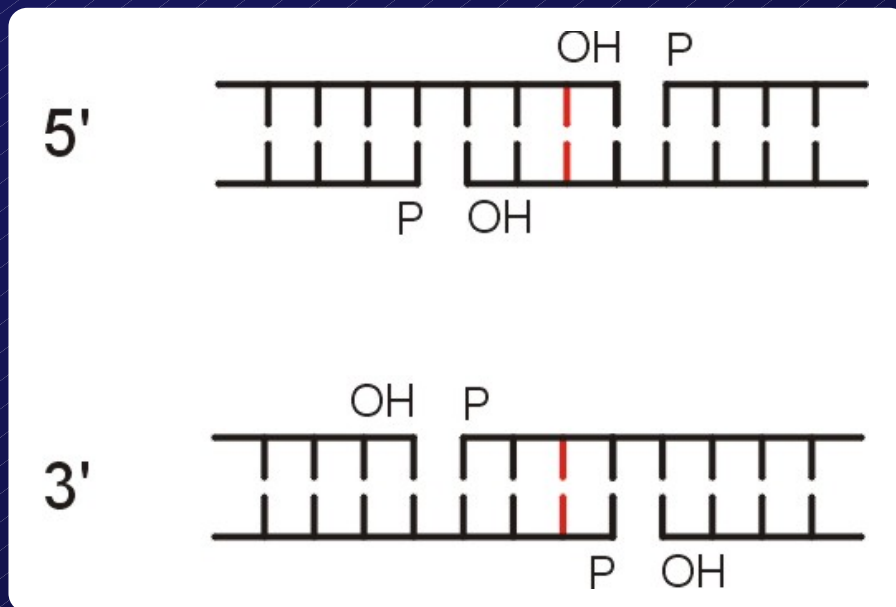
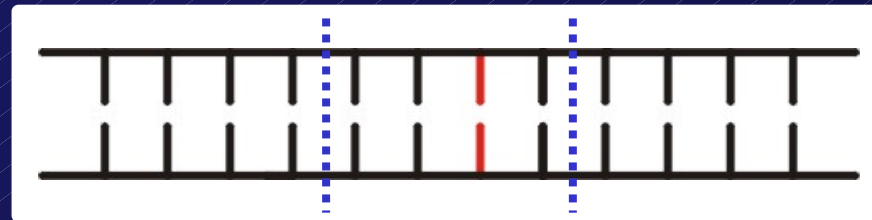
NHEJ proteins act by re-annealing and joining short overhangs.

NHEJ yields efficient and accurate repair of DSBs.

End processing activities exist to deal with terminal damage  
- incompatible ends are a secondary phenomenon.

There is an inherent order to the NHEJ process.

# What is a DSB? Simple overhangs

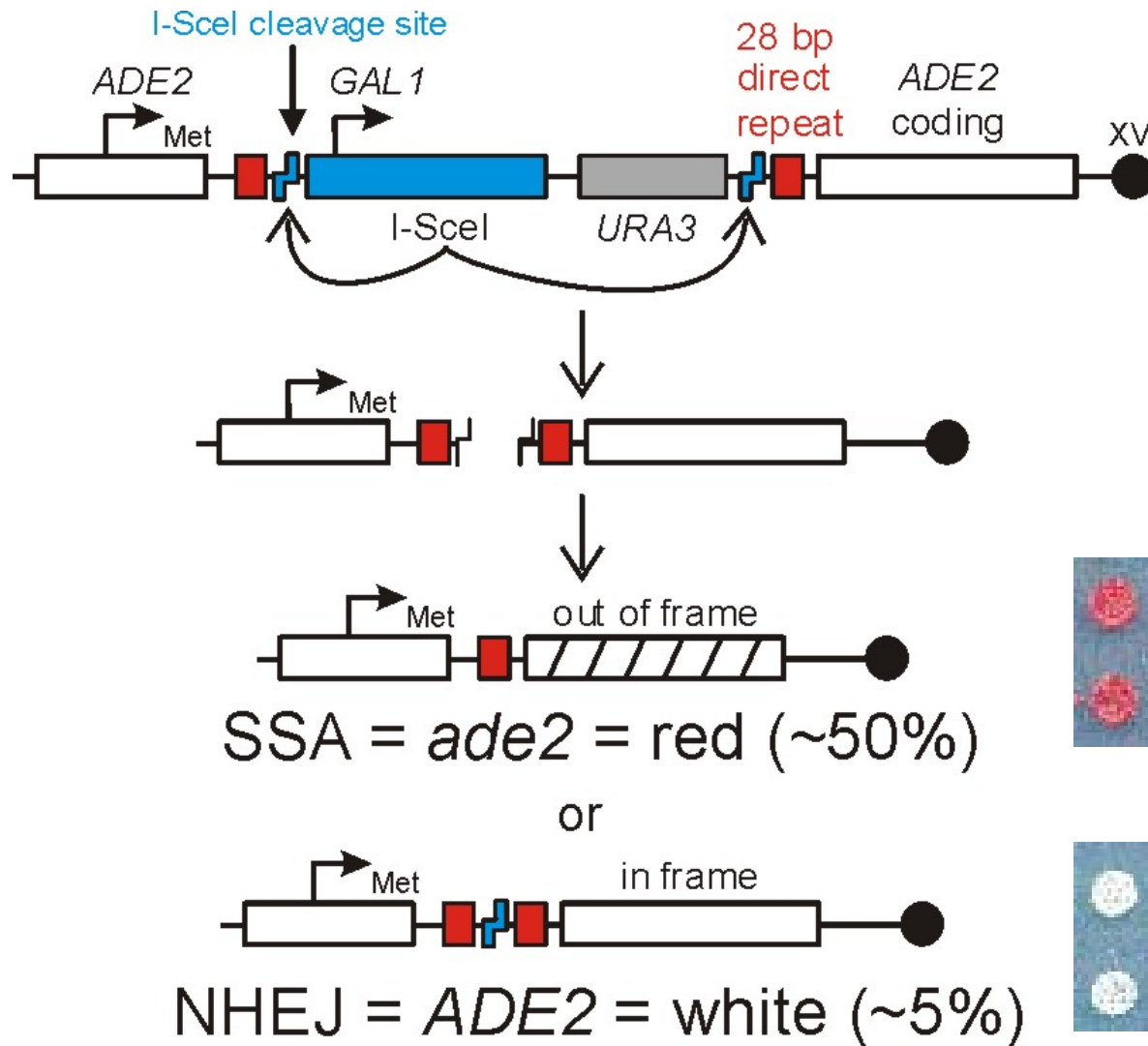


Restriction enzymes  
Rare damage-induced DSBs

“Simple Religation”  
“Precise NHEJ” (a.k.a. “accurate”)

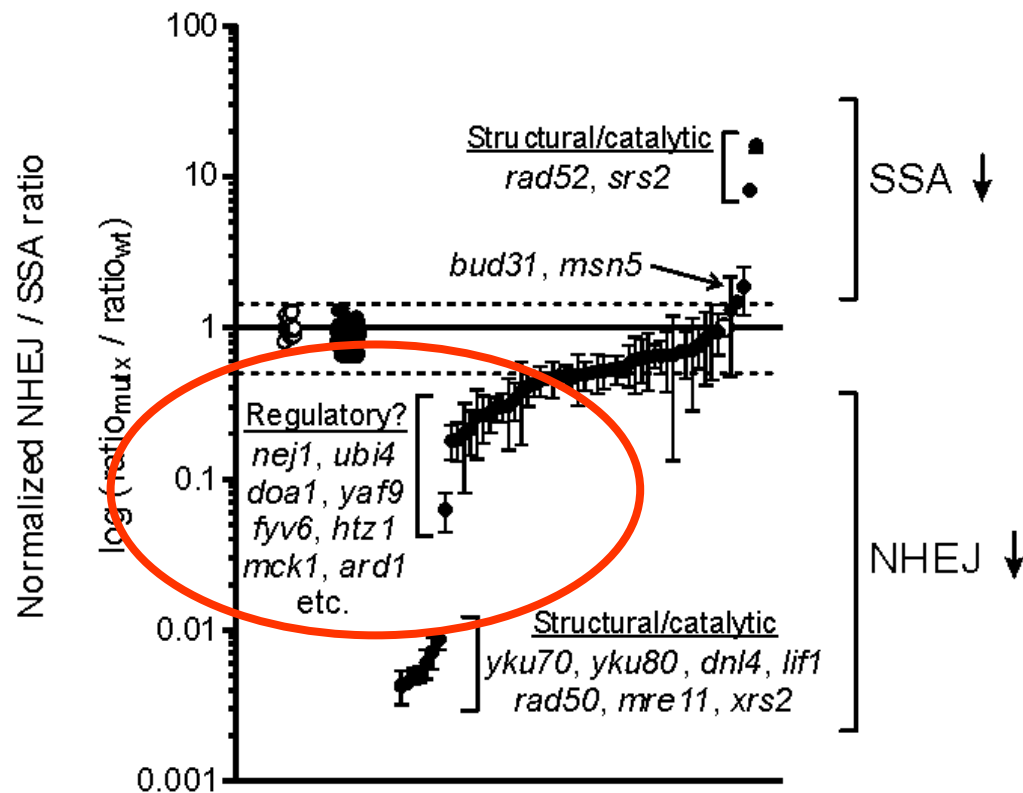
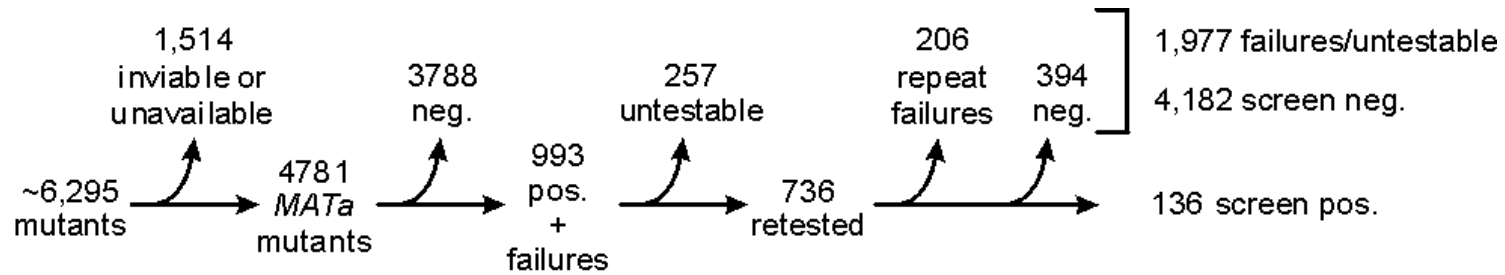
Result = genome restoration

# The "suicide deletion" assay

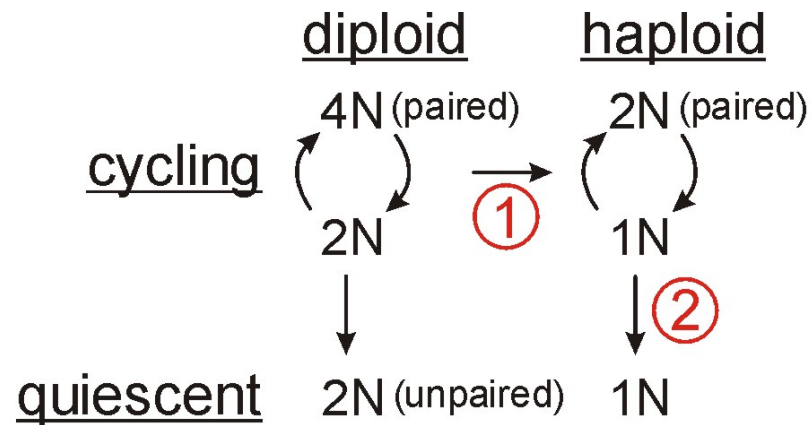




# Suicide deletion: genome-wide screen



# Cell cycle-dependent stimulators of NHEJ efficiency

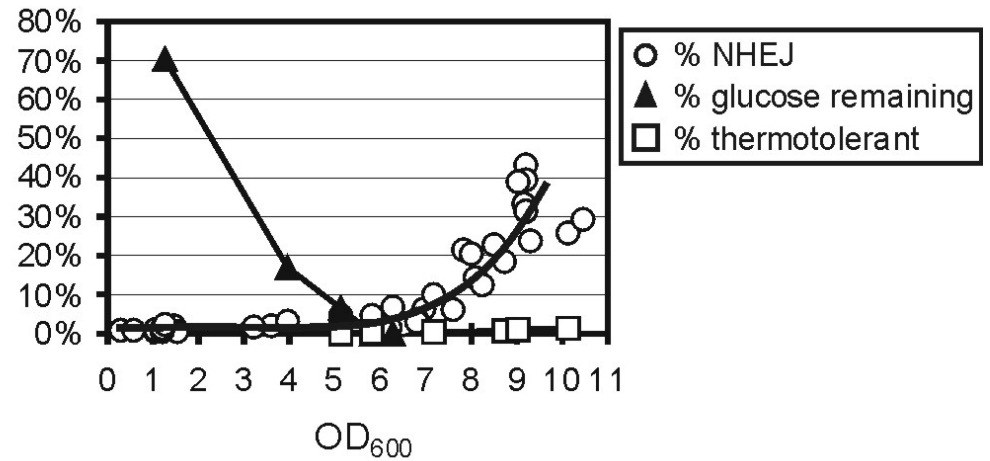
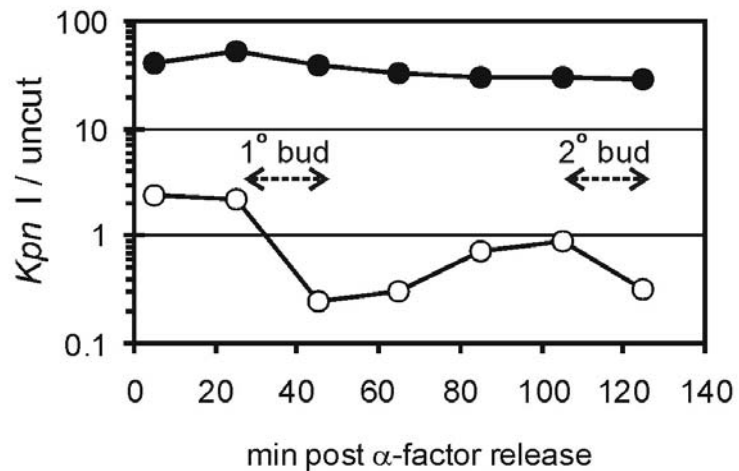


- ① NHEJ is stimulated in haploid cells.
- ② NHEJ is stimulated in nutritionally deprived cells.

**Nej1:** Lif1 binding protein

**Doa1:** ubiquitin degradation  
**Mck1:** GSK-3 family kinase  
**Fyv6:** uncharacterized

# Yeast NHEJ varies as a function of the cell cycle

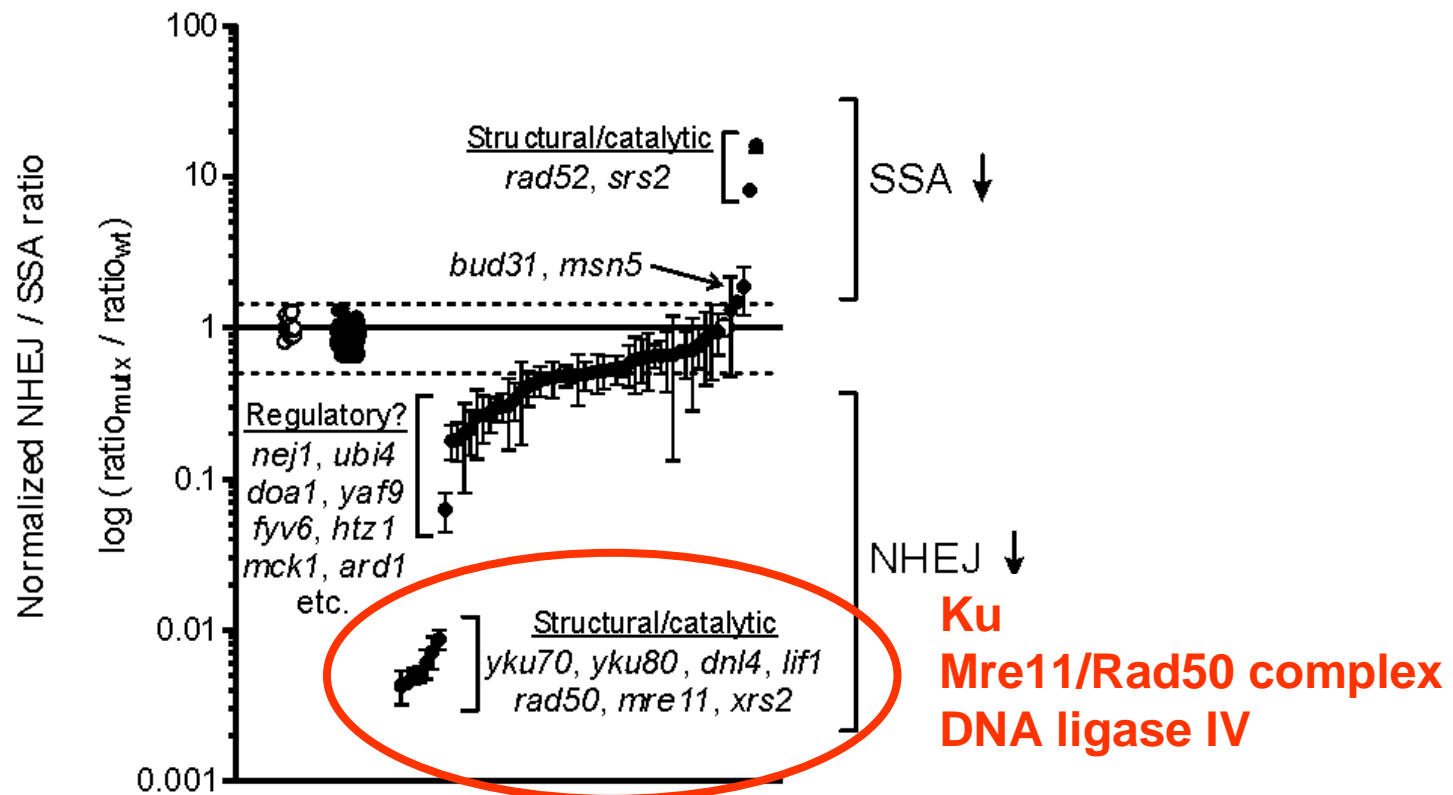
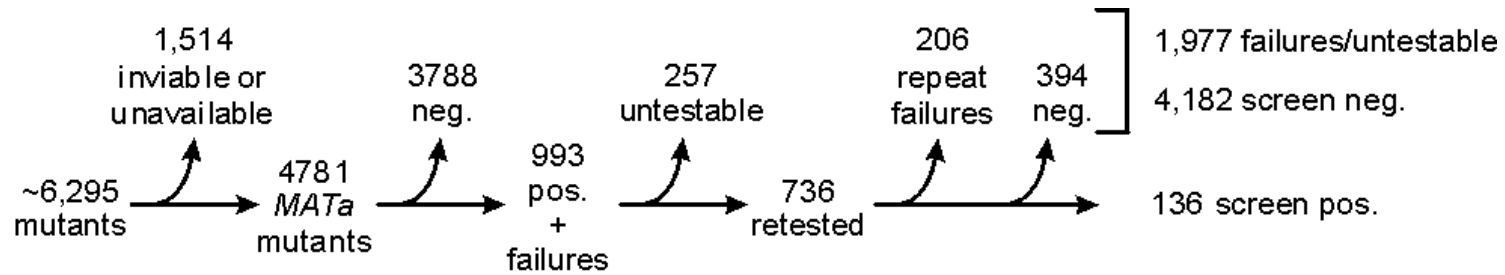


**Doa1**: involved in ubiquitin-mediated degradation

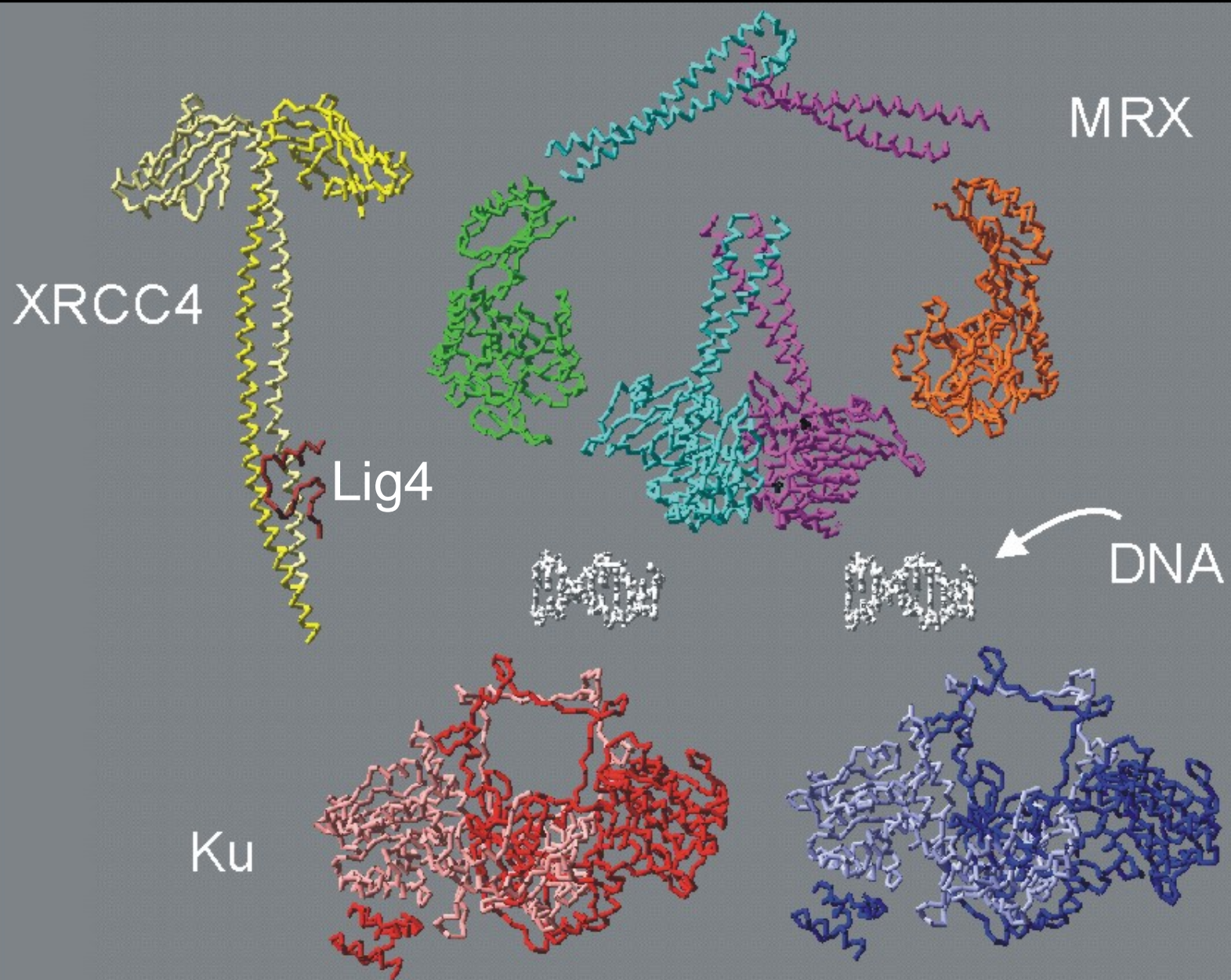
**Mck1**: GSK-3 family protein kinase

**Fyv6**: largely uncharacterized

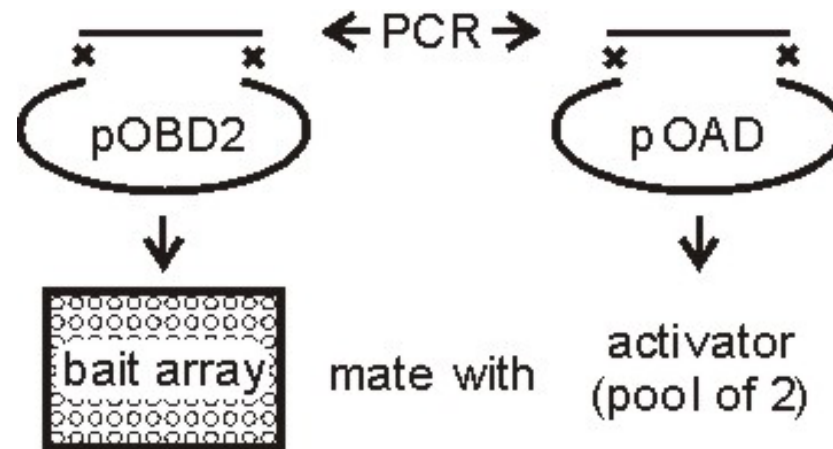
# Suicide deletion: genome-wide screen



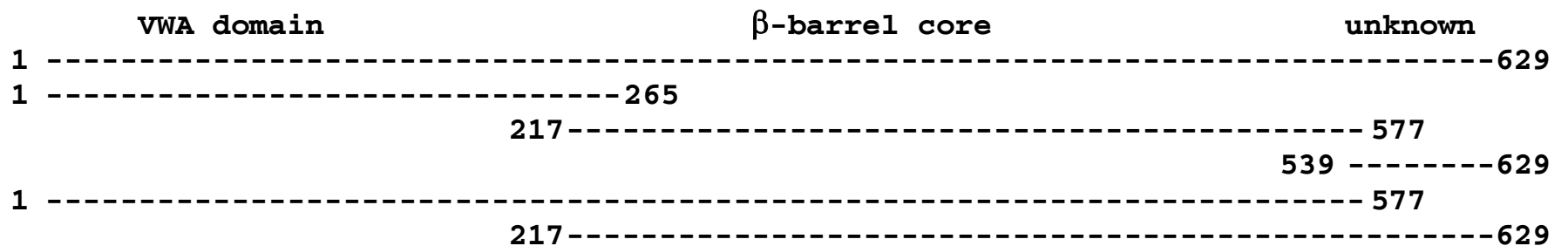
# The NHEJ puzzle



# Systematic NHEJ 2-hybrid analysis



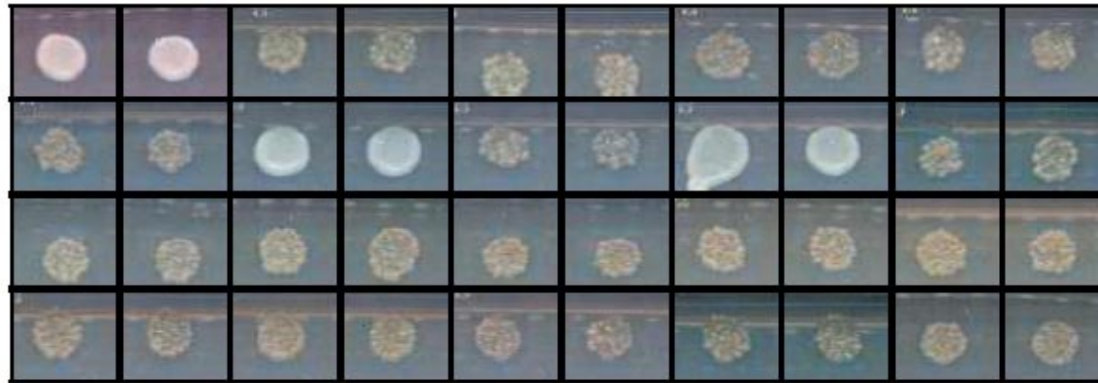
## Yku80



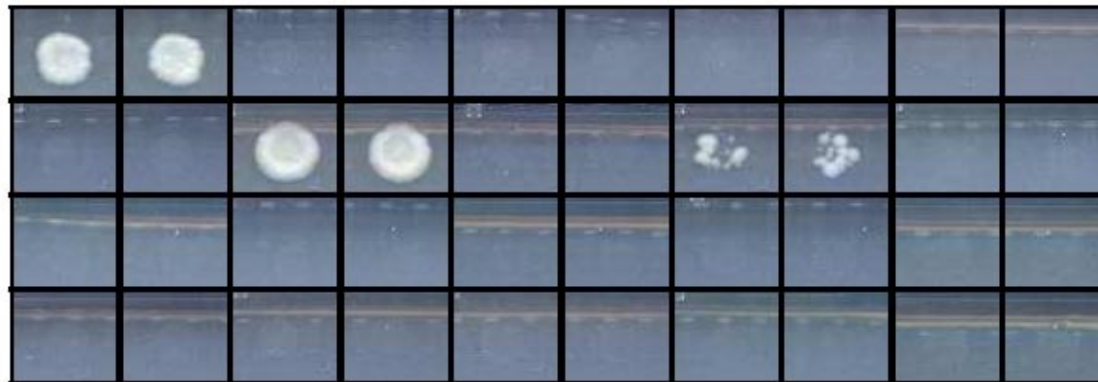


# Systematic NHEJ 2-hybrid analysis

Glu-His, 3 day

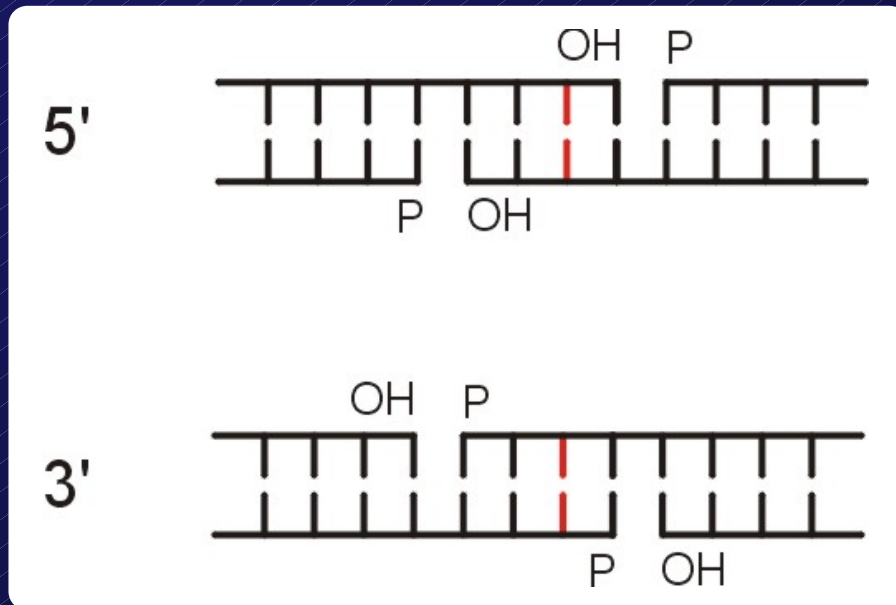
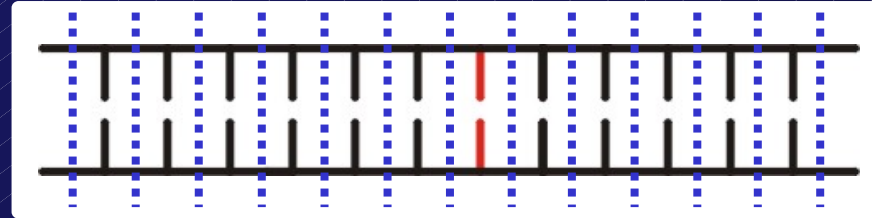


Glu-Ade, 5 day





# What is a DSB? Simple overhangs

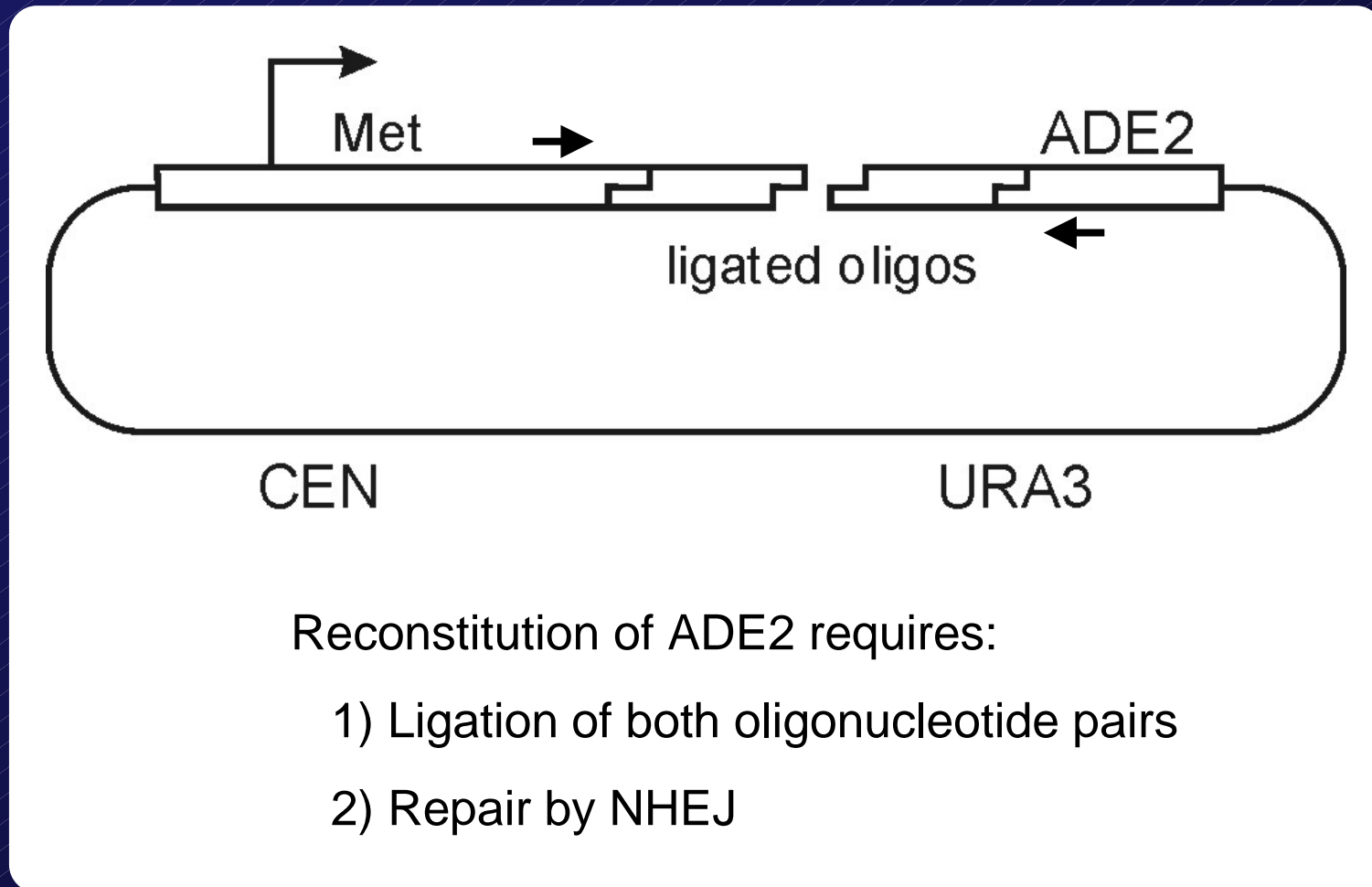


Restriction enzymes  
Rare damage-induced DSBs

“Simple Religation”  
“Precise NHEJ” (a.k.a “accurate”)

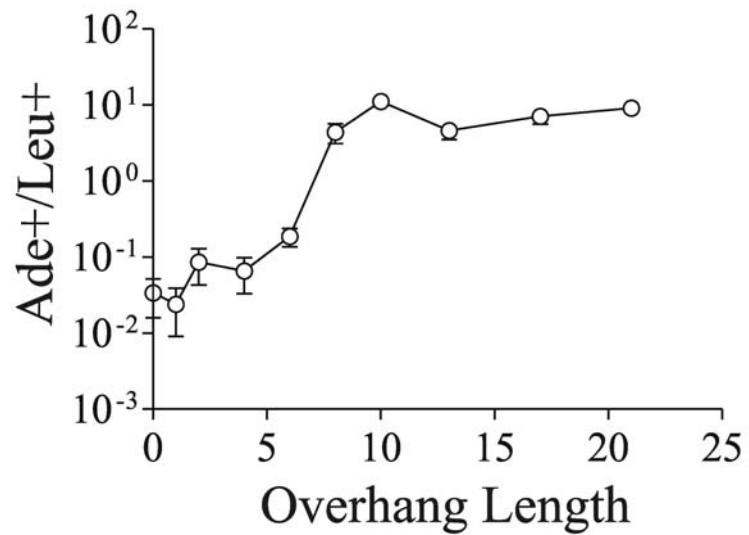
Result = genome restoration

# Oligonucleotide Modified Plasmid Assay (OMP)

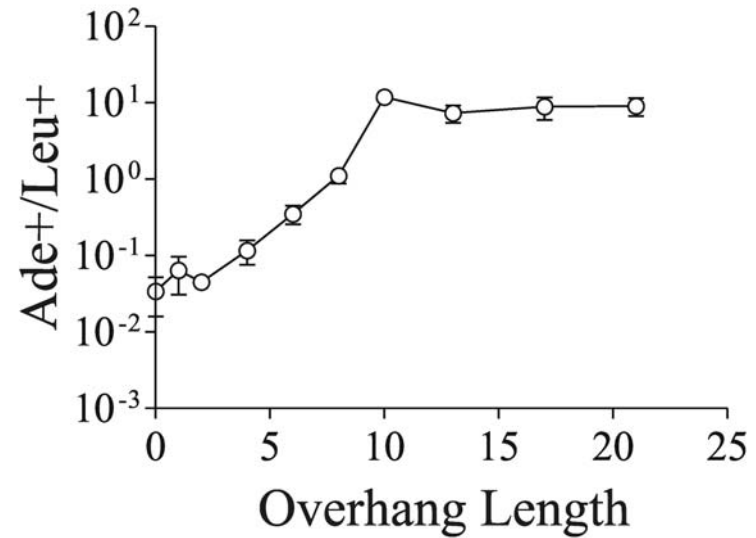


# DSB rejoining as a function of overhang length

## 3' overhang

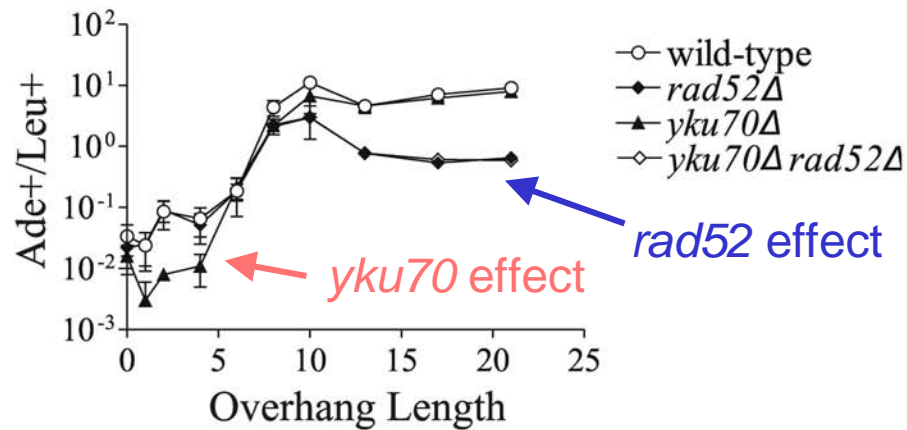


## 5' overhang

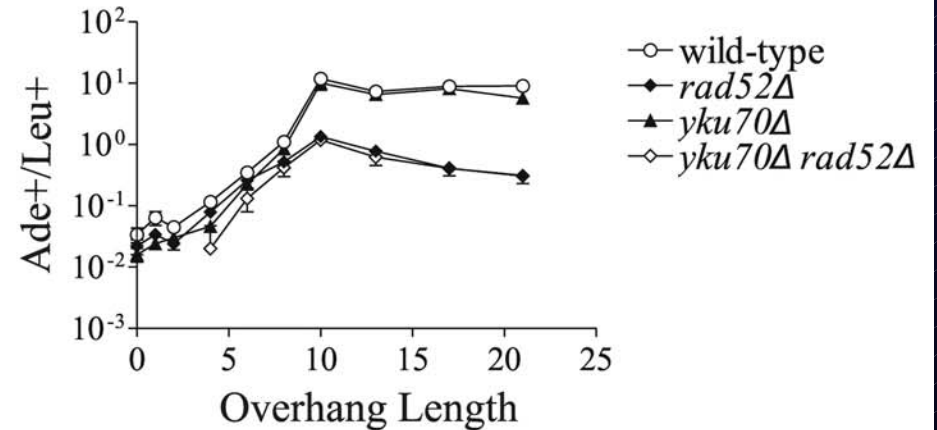


# DSB rejoining at long overhangs is NHEJ independent but Rad52 dependent

## 3' overhang

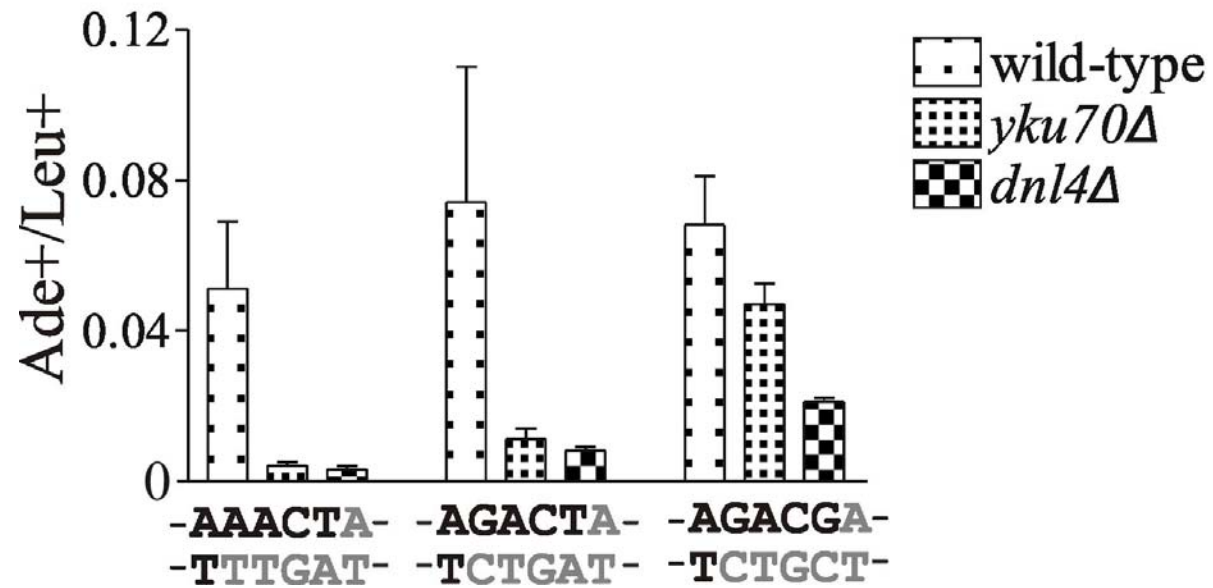


## 5' overhang



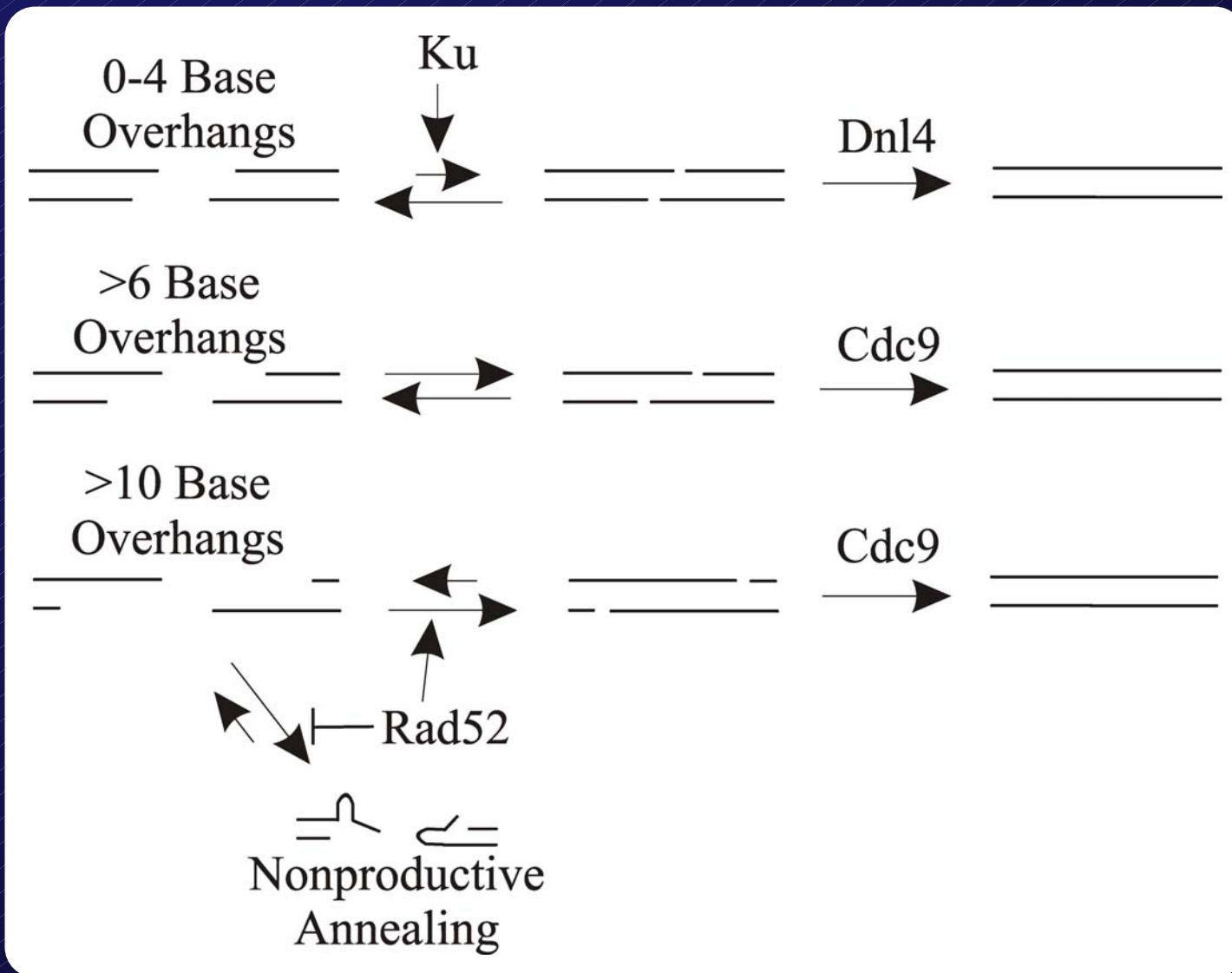


# NHEJ as a function of overhang GC content

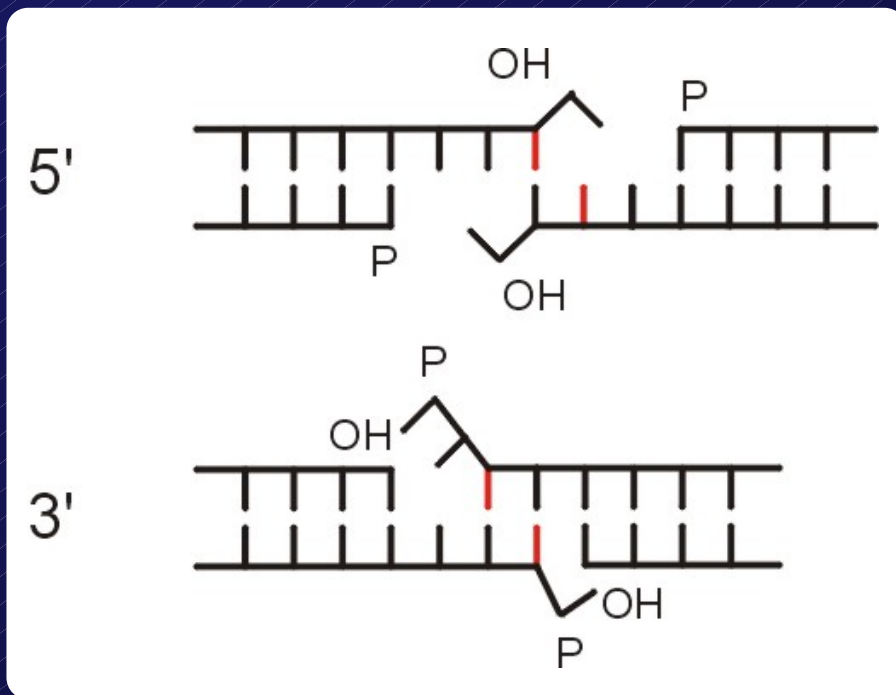
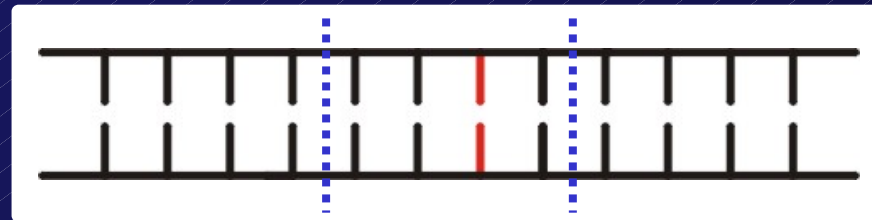


# of GCs:  
1  
2  
3  
in 4 nt  
overhang

# DSB rejoining as a function of overhang length: summary



# What is a DSB? Overhang misalignment



Mispairing of compatible overhangs

“Processed NHEJ”

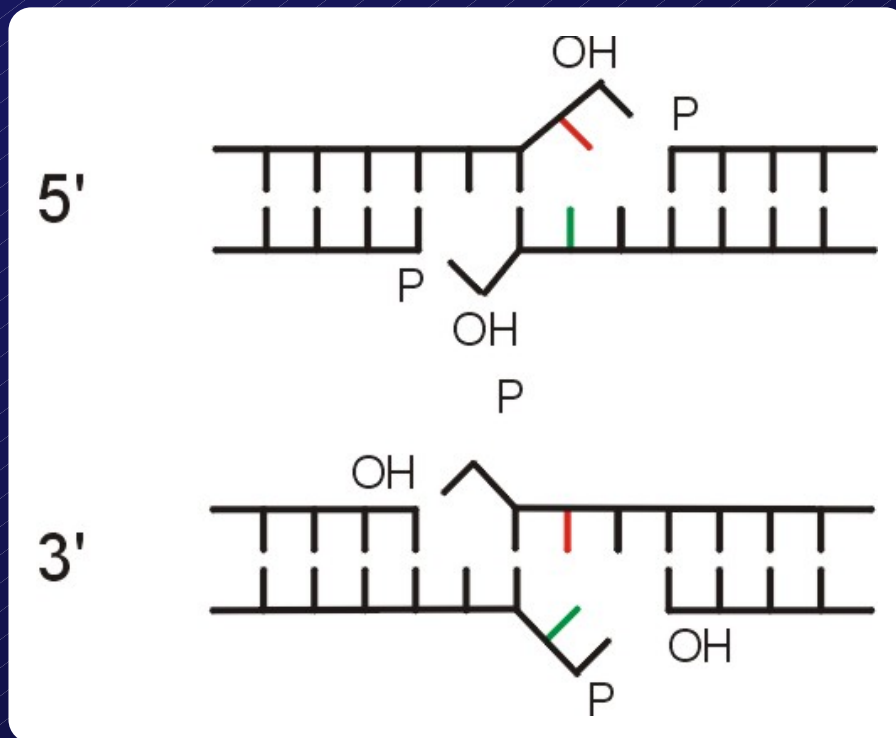
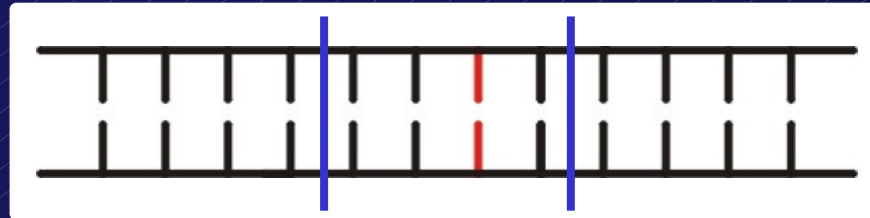
“Imprecise NHEJ”

Result = frameshift mutation

# NHEJ is fundamentally accurate (but not quite) - a “worst case” scenario

join	inferred alignment	wild type	
M(+2)	<u>AA</u> <u>CGCGTT</u> TTGCGC AA	0.19 %	<b>&gt;5-fold bias</b>
M(0)	<u>AACGCGTT</u> TTGCGCAA	1.1 %	

# What is a DSB? Incompatible overhangs

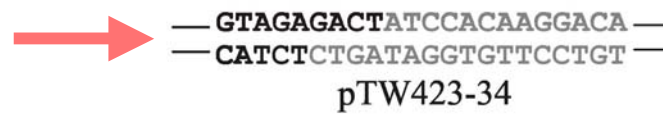
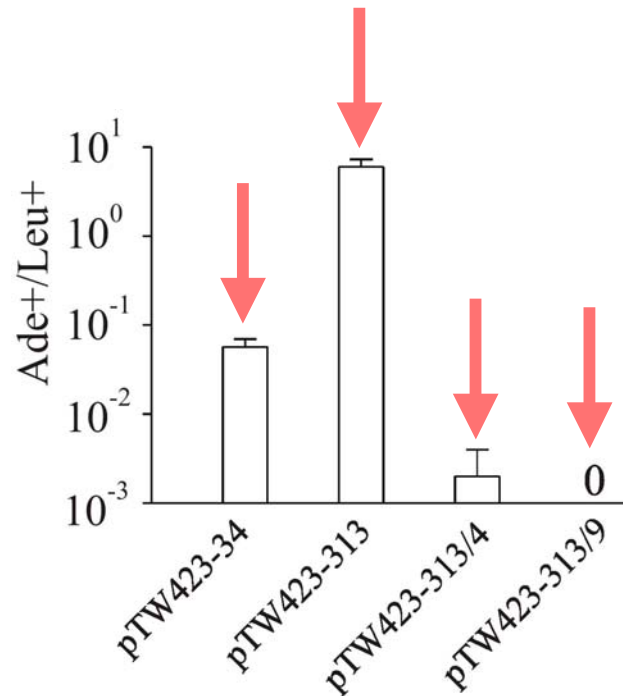


Two incorrectly paired DSBs  
(Single resected DSB)

“Processed NHEJ”  
NOT “Imprecise NHEJ”!

Result = Rearrangement

# Rejoining of long overhangs is very precise

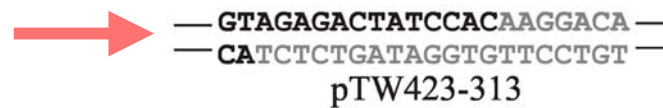


**bp**

**Accuracy**

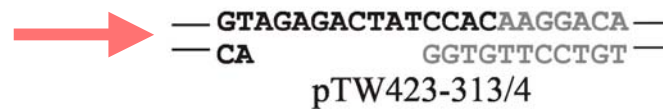
4

0.81



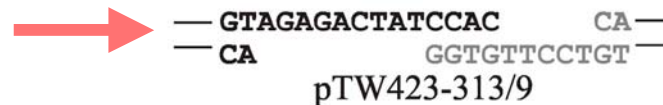
13

1.00



4

0.20



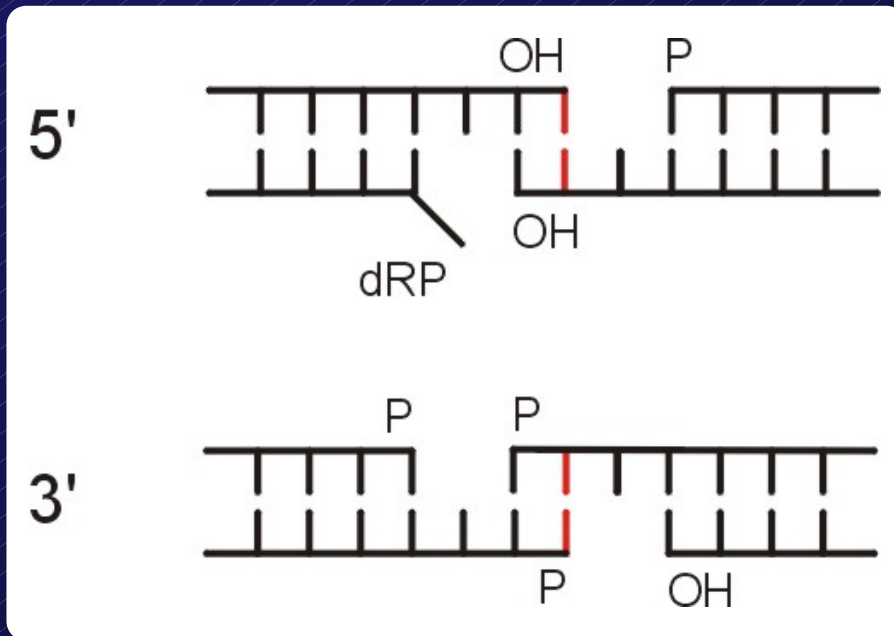
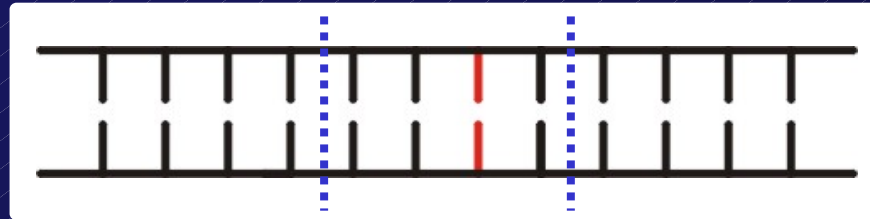
4

0



# What is a DSB?

Missing and damaged terminal nucleotides



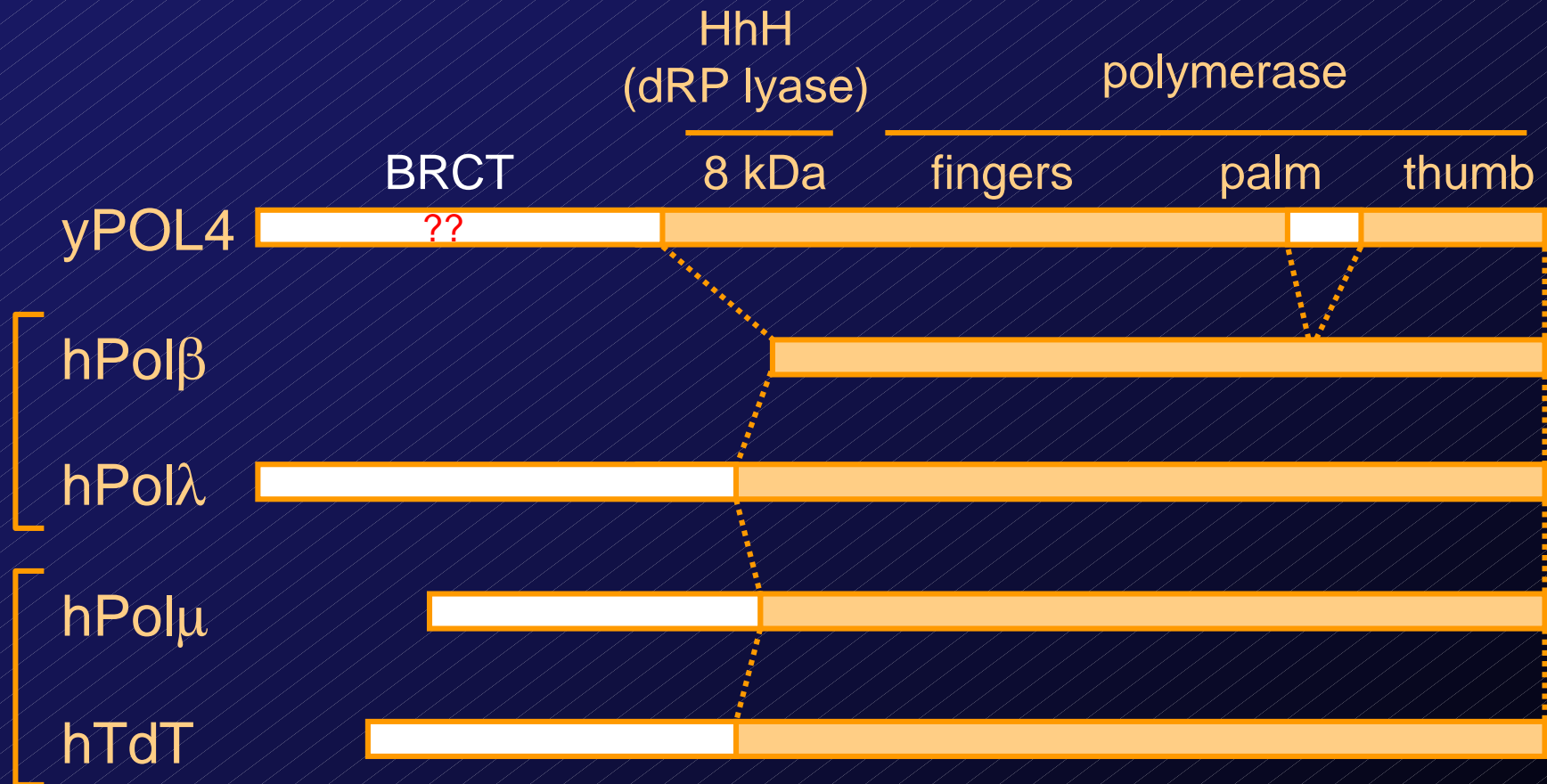
Typical damage-induced DSB  
AP endonucleases, lyase etc.

“Processed NHEJ”  
“Precise NHEJ”

Result = genome restoration

NOT mutagenic!

# The PolX Family of DNA Polymerases

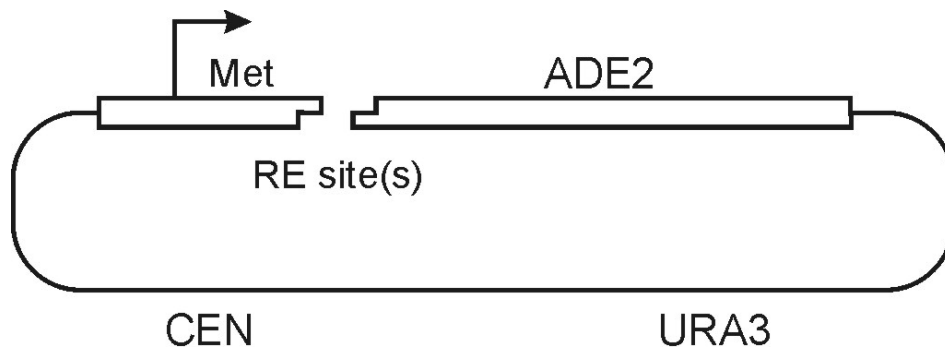


# Pol4 mediates a subset of processed NHEJ events

Pol4:


PolX family polymerase


homology to hPol  $\lambda > \mu > \beta$



join	inferred alignment	polarity	<i>pol4</i> fold reduction
SK(-1)	$\begin{array}{c} \text{AGCA} \text{CT} \\ \text{TC} \text{TTGGA} \\ \text{CA} \end{array}$	3'	>95
HO(+2)	$\begin{array}{c} \text{GCAACA} \text{GT} \\ \text{CG} \text{TTGTCA} \\ \text{T} \end{array}$	3'	>70
MB(+1)	$\begin{array}{c} \text{TA} \text{GATCCT} \\ \text{ATGC} \text{GA} \\ \text{GC} \end{array}$	5'	28
SK(+1)	$\begin{array}{c} \text{AGCAT} \text{CT} \\ \text{TC} \text{ATGGA} \\ \text{C} \end{array}$	3'	>24
HO(-3)	$\begin{array}{c} \text{GCAGT} \\ \text{CGTCA} \\ \text{TTG} \\ \text{ACA} \end{array}$	3'	8
MB(+3)	$\begin{array}{c} \text{TA} \text{GATCCT} \\ \text{ATGCGC} \text{GA} \end{array}$	5'	2.3
SK(-3)	$\begin{array}{c} \text{AGCCT} \\ \text{TCGGA} \\ \text{CA} \\ \text{ATG} \end{array}$	3'	1.7
M(+2)	$\begin{array}{c} \text{AA} \text{CGCGTT} \\ \text{TTGCGC} \text{AA} \end{array}$	5'	1.7
XB(+2)	$\begin{array}{c} \text{AC} \text{GATCCT} \\ \text{TGAGCT} \text{GA} \end{array}$	5'	1.4

## Modular Evolution of Polynucleotide Kinase Domains

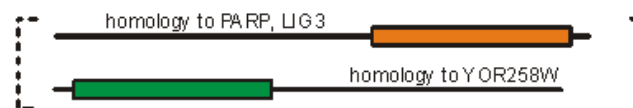
 = 3' phosphatase

 = 5' kinase

Human, *S. pombe*, etc.



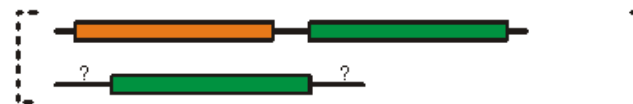
*Arabidopsis*



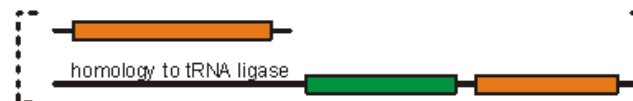
*S. cerevisiae*



*Dictyostelium*



AcNPV



T4

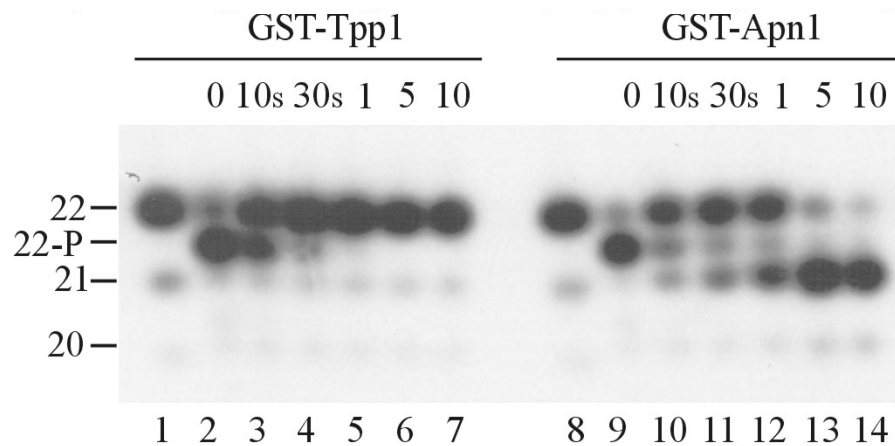


# Tpp1 is a robust and specific 3' phosphatase

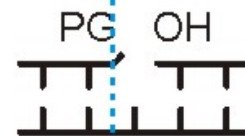
**Yeast (and most organisms)  
have 2 AP endos:**

**Apn1 (Endo IV-like)**

**Apn2 (Exo III-like)**



**AP endo  
substrates**

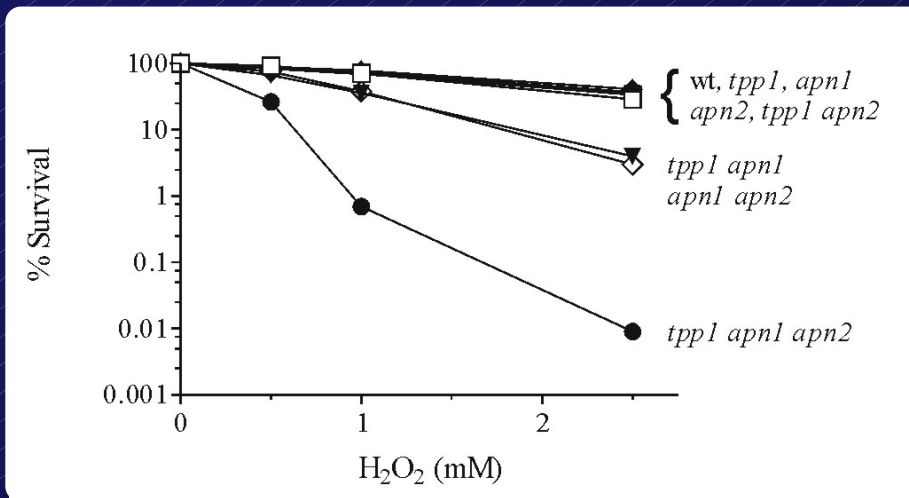


**Tpp1/PNKP  
substrates**

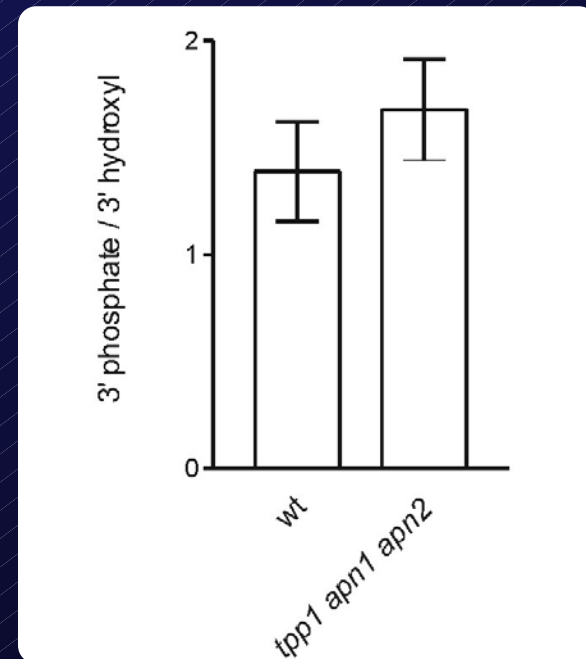


# Tpp1 is not required for NHEJ at 3' phosphates

## BER

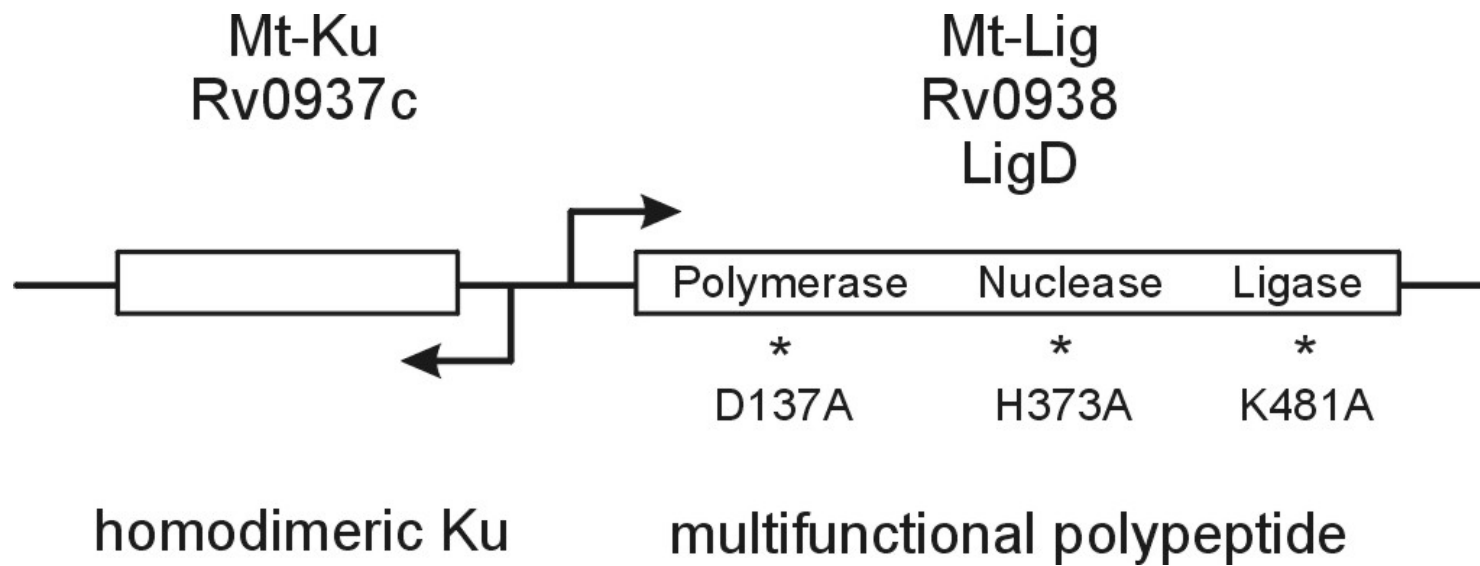


## NHEJ

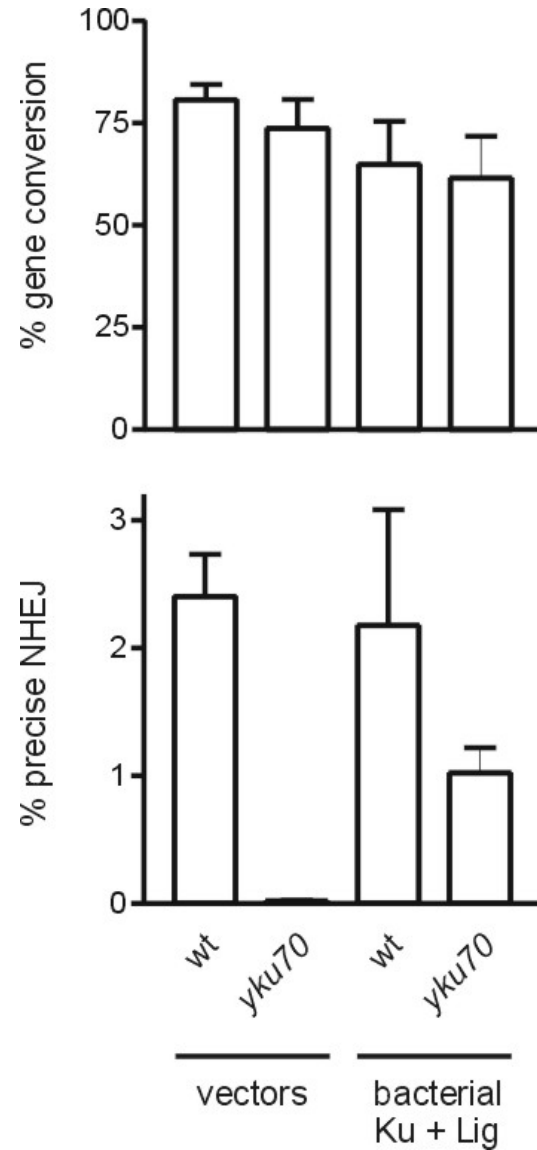
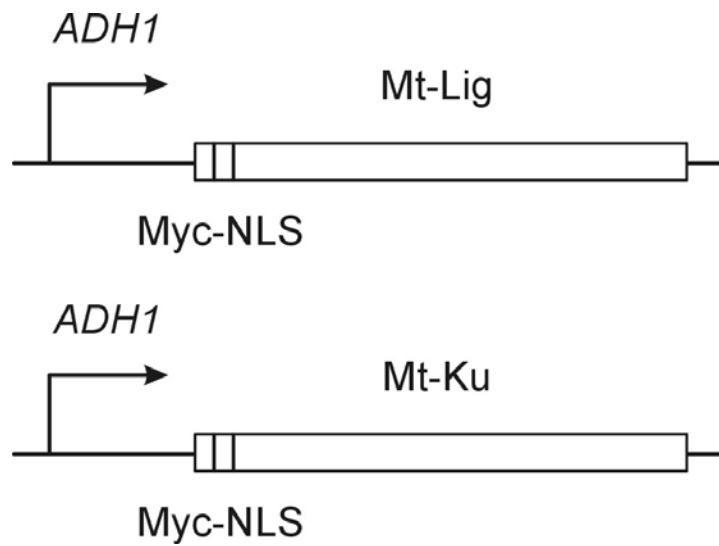




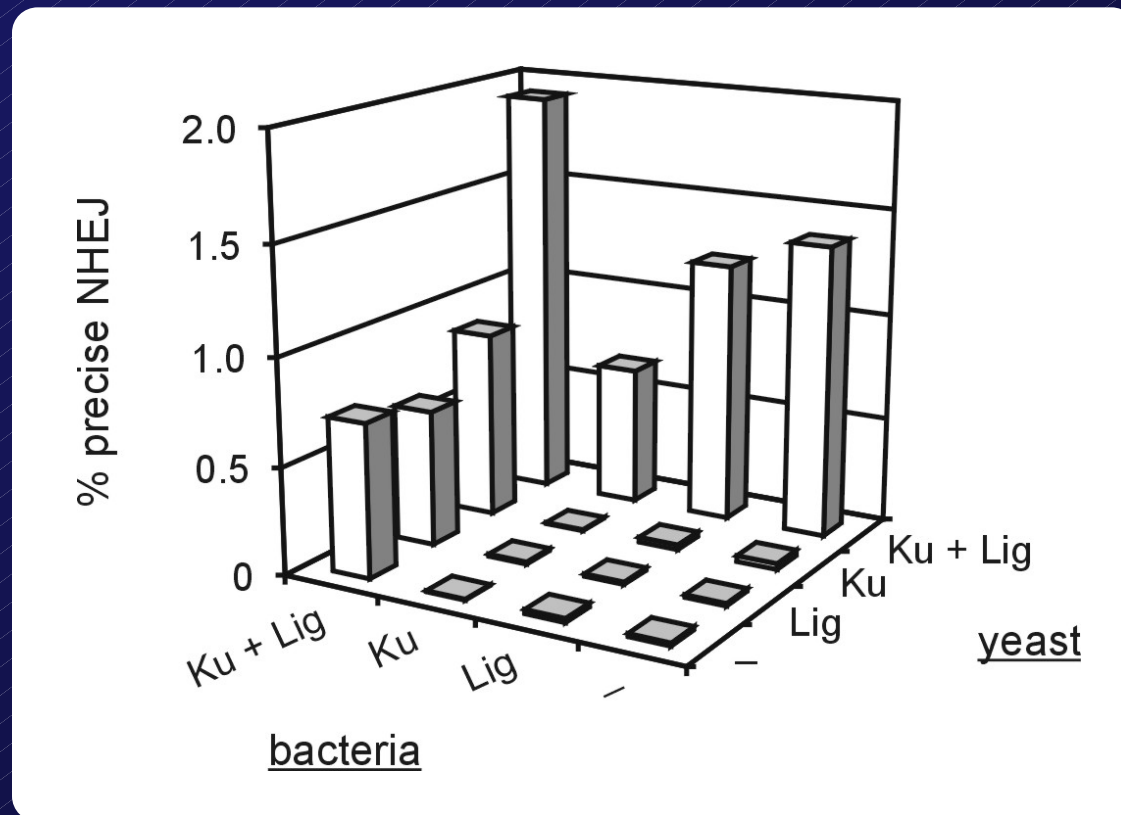
# *M. tuberculosis* NHEJ operon



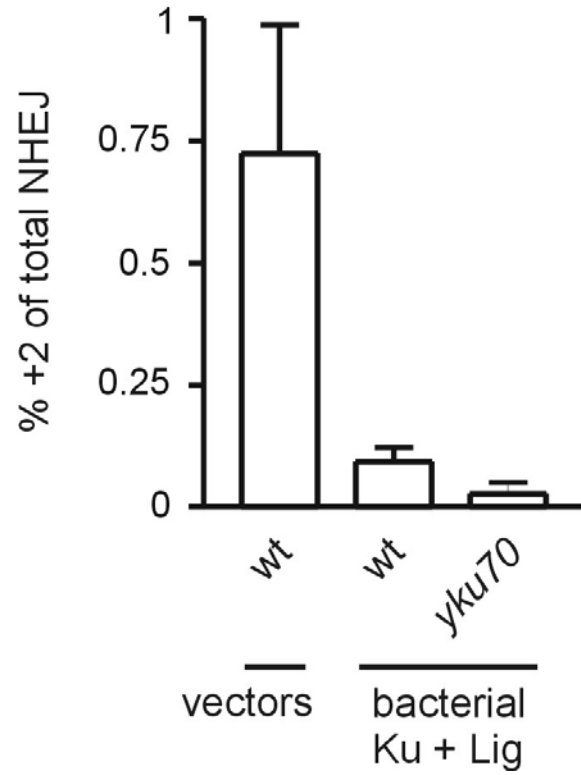
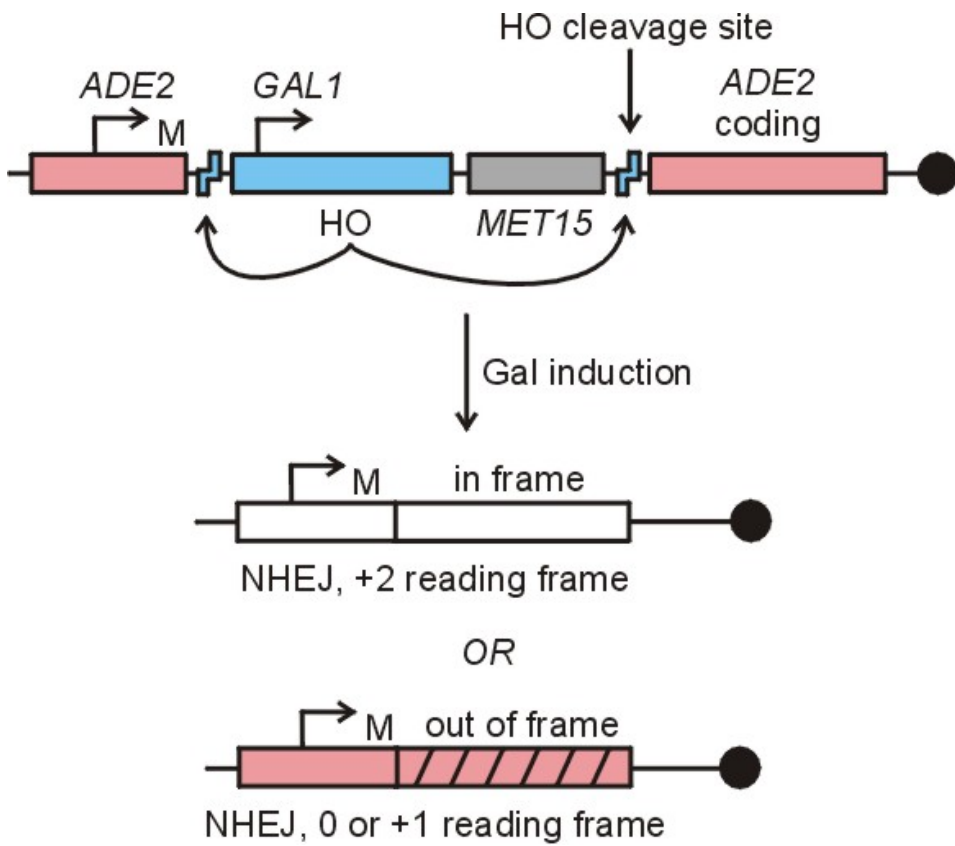
# Reconstitution of simple religation NHEJ



# Ku and ligase are species-specific partners



# Mtb NHEJ is very precise



**HA! WE'RE** THE SUPER EFFECTIVE TEAM AROUND HERE! YkoV & YkoU CAN DO EVERYTHING IT TAKES YOUR WHOLE EUKARYOTIC REPAIR TEAM TO DO!

**RUBBISH!** WE'RE AN INTEGRATED, HIGHLY-SPECIALISED, SKILLED TEAM, EVOLVED AND STREAM-LINED FOR MAXIMAL EFFICIENCY...

**GARBAGE!** I'VE NEVER SEEN SUCH FLAGRANT OVER-MANNING! YOUR SO-CALLED 'TEAM' IS JUST A BUNCH OF LAZY, SOFT LAY-ABOUTS...

**SHUT IT, BEAN-BRAIN!** YOUR SYSTEM IS DANGEROUSLY SHORT-STAFFED! WHERE'S THE COVER IF ANYTHING GOES WRONG?

**DON'T YOU LECTURE ME** ABOUT STAFFING LEVELS, YOU WEASELLING, ARROGANT...

...STUPID...

IGNORANT--

☆!?!&@!

**WELCOME 2003**  
TRADES UNION CONGRESS

**TODAY!**  
NON-HOMOLOGOUS  
END-JOINING  
DOUBLE STRAND  
BREAK REPAIR  
COMPLEXES.  
UNION

