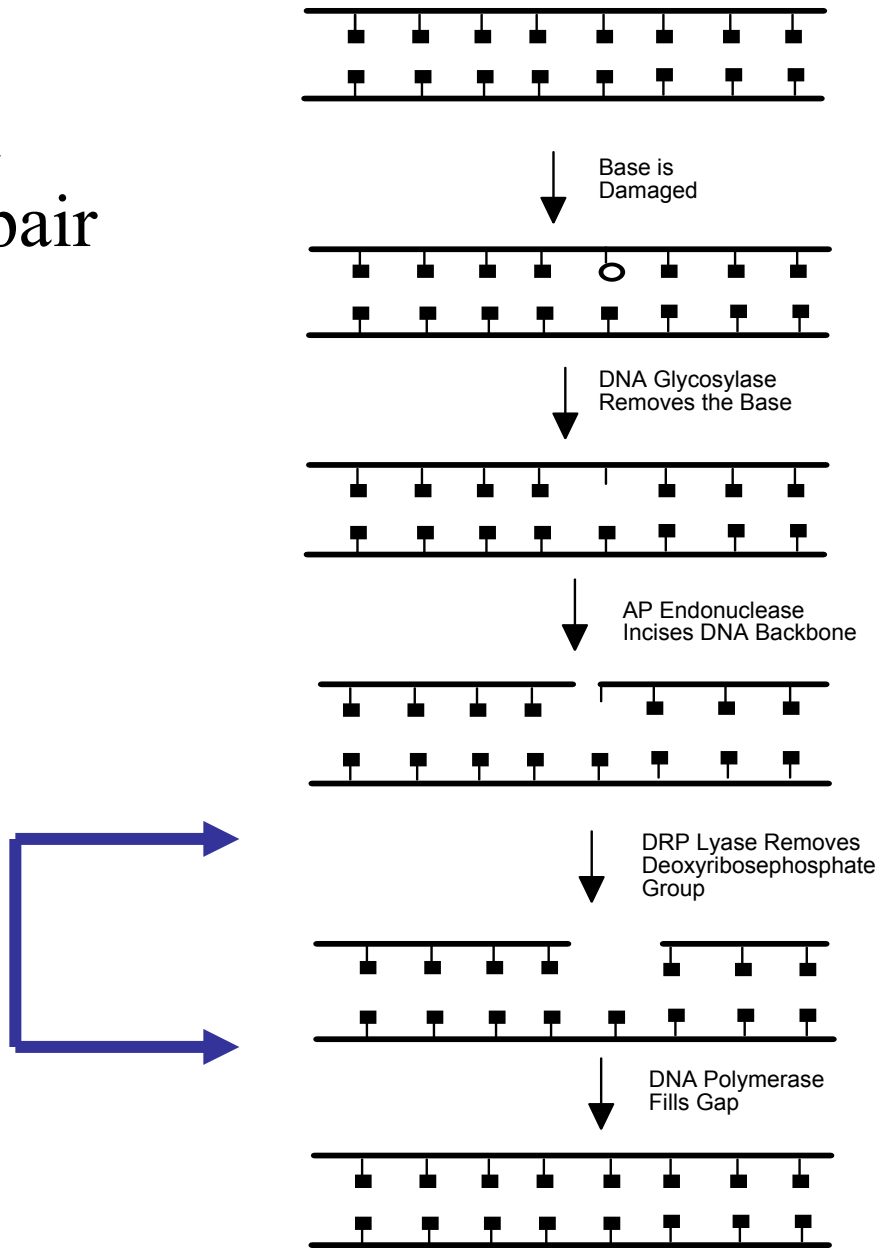
Cancer-Associated DNA Polymerase Beta Variants

Joann B. Sweasy, Ph.D.

Departments of Therapeutic Radiology and Genetics

Yale University School of Medicine

Pol ß Functions in Base Excision Repair



Pol ß is Altered in 30% of Tumors Studied

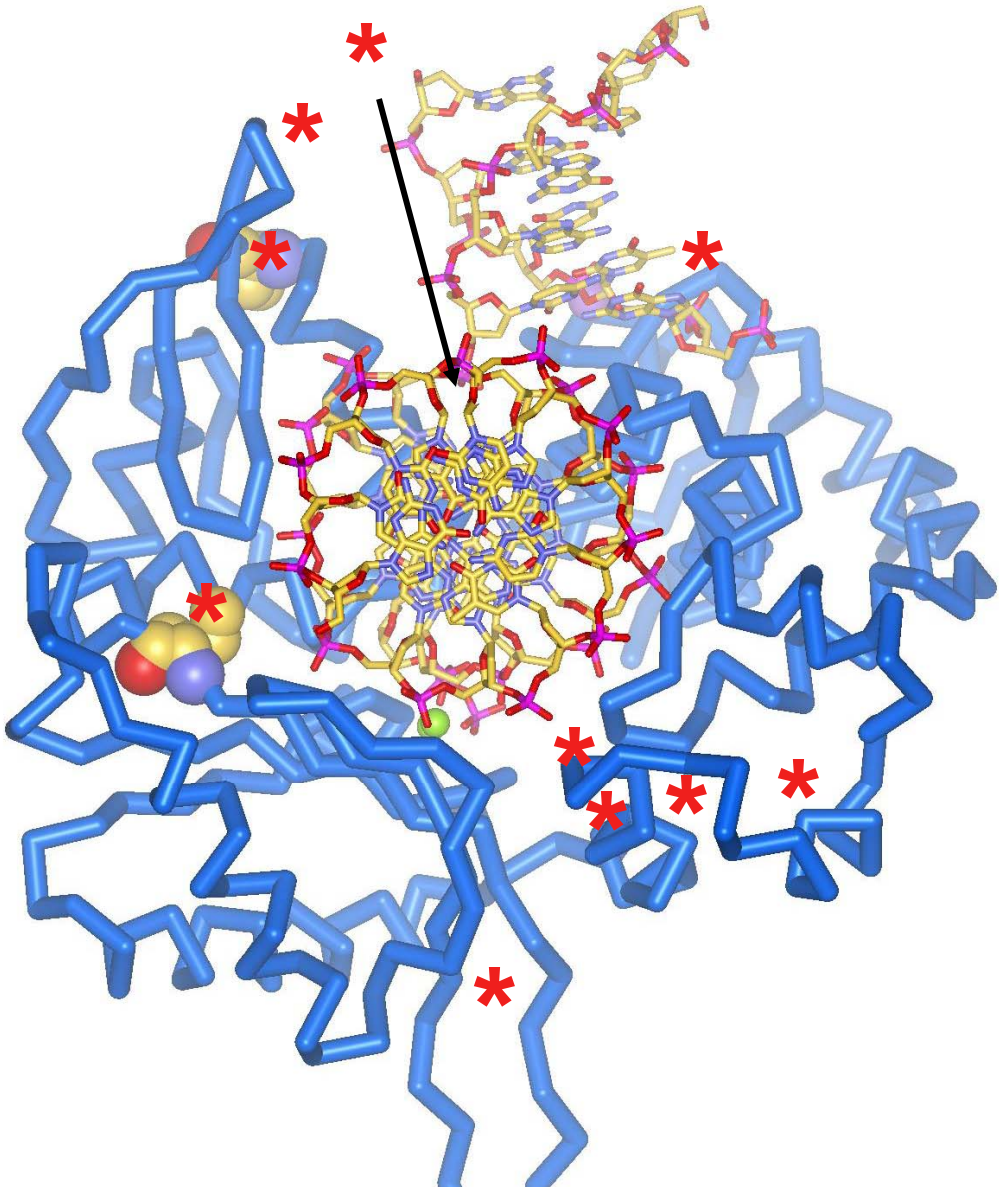
Cancer Type	Number Characterized	Number With Pol ß Variant (%)
Gastric	40	20 (50)
Colorectal	8	6 (75)
Prostate	12	2 (17)
Lung	11	3 (27)
Breast	42	11 (26)
Bladder	26	4 (15)
Esophageal	30	12 (40)
Total	**189**	**58 (30)**

These are not common polymorphisms found in normal individuals.
Mutations are only present in the tumor.

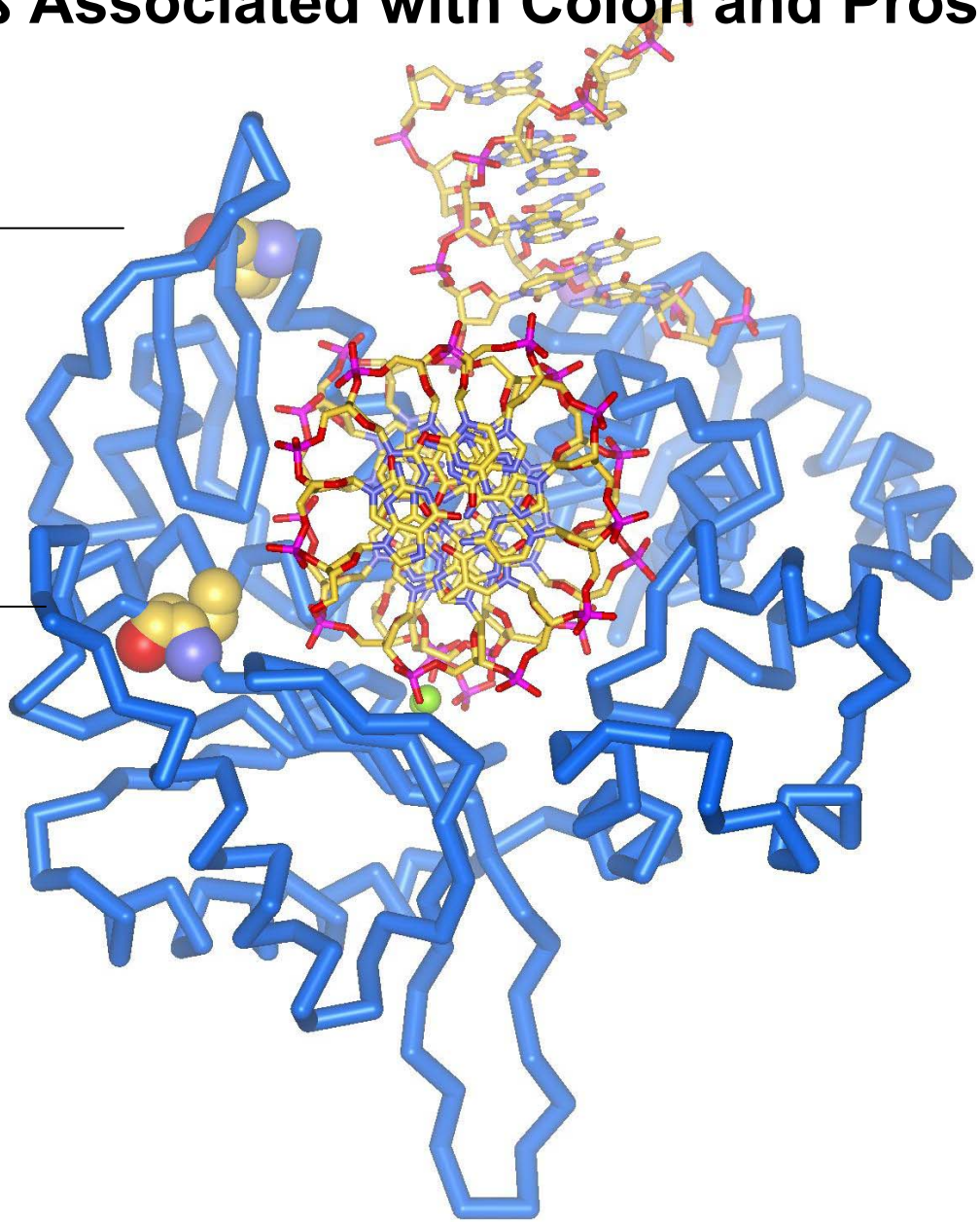
Types of Pol ß Variants Found in Tumors

Type of Variant	Percent
Single Amino Acid Substitution	48
Truncations	12
Deletion of Exon 11	25
Multiple Variants in Tumor	14

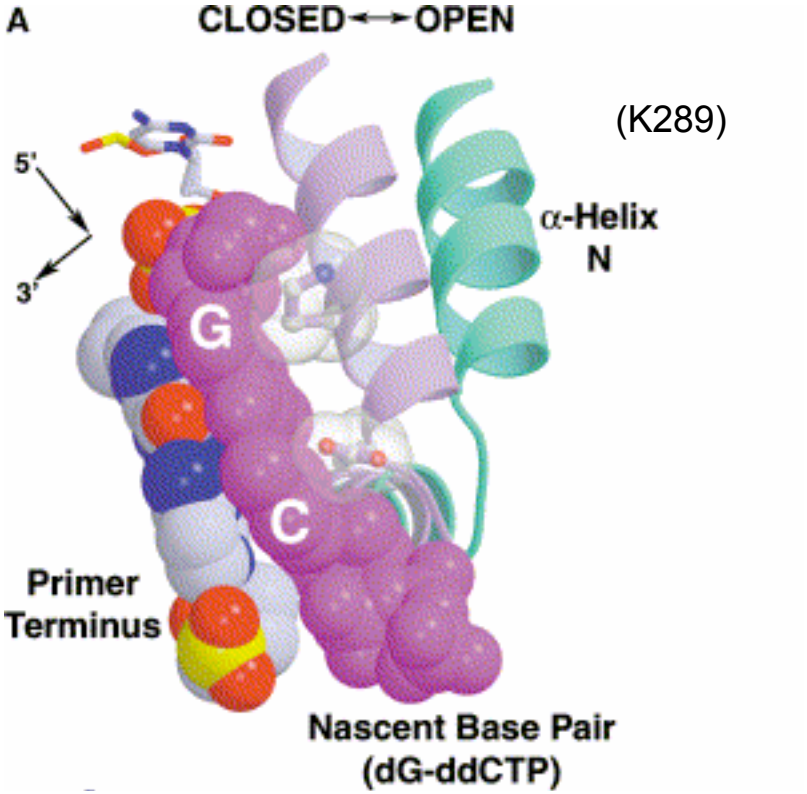
Mutations Map To Different Subdomains of Pol ß



Mutants of Pol ß Associated with Colon and Prostate Cancer

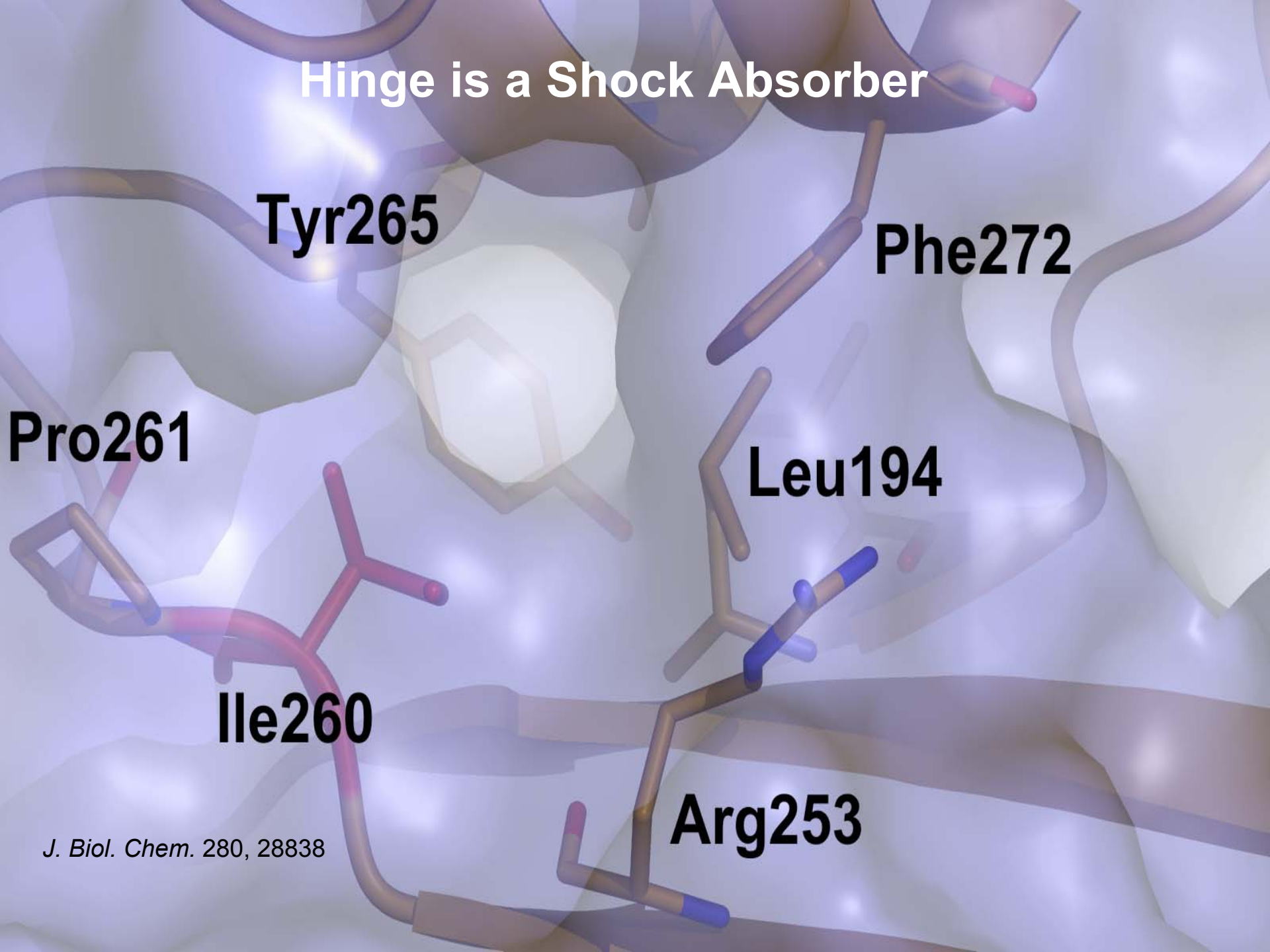


Helix N Stabilizes Nascent Base Pair



Beard *et al., Structure* vol. 11 page 489.

Hinge is a Shock Absorber



J. Biol. Chem. 280, 28838

Are Pol ß Variants Linked to Cancer?

- Does expression of the Pol ß cancer-associated variants confer a transformed phenotype to cells?

- Can the Pol ß cancer-associated proteins induce mutations that could lead to cellular transformation?

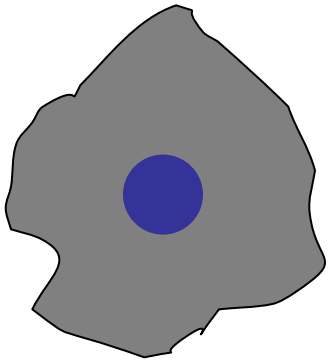
Isolation of Stable Clones Expressing Cancer-derived Mutants

pRVYTET

TET

WT or Mutant cDNA

Infected



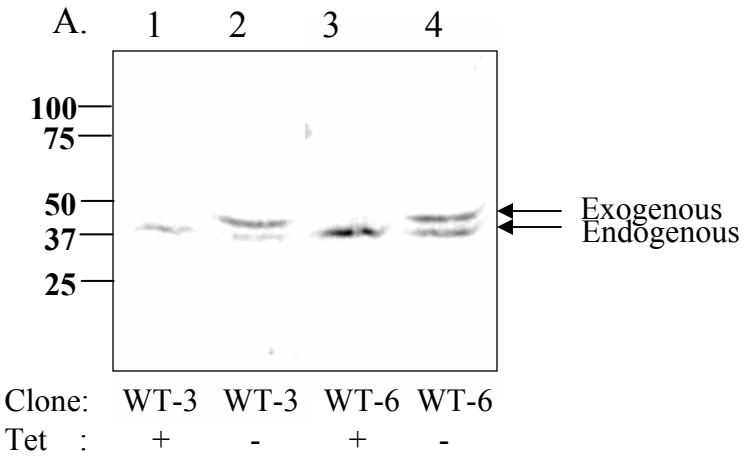
C127λ cells

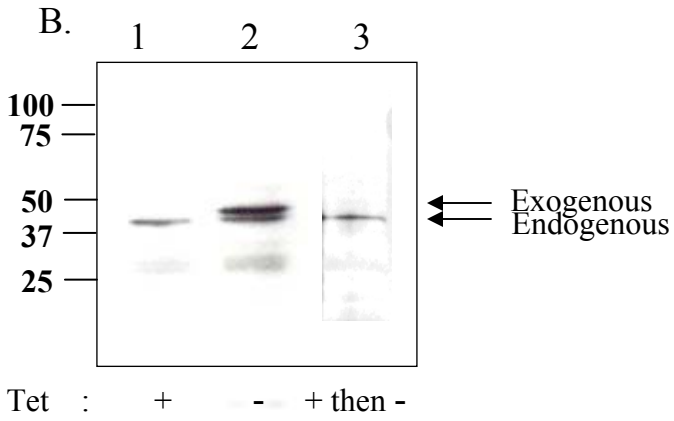
Stable Clones

Passage
+Tet= Pol ß OFF
-Tet= Pol ß ON

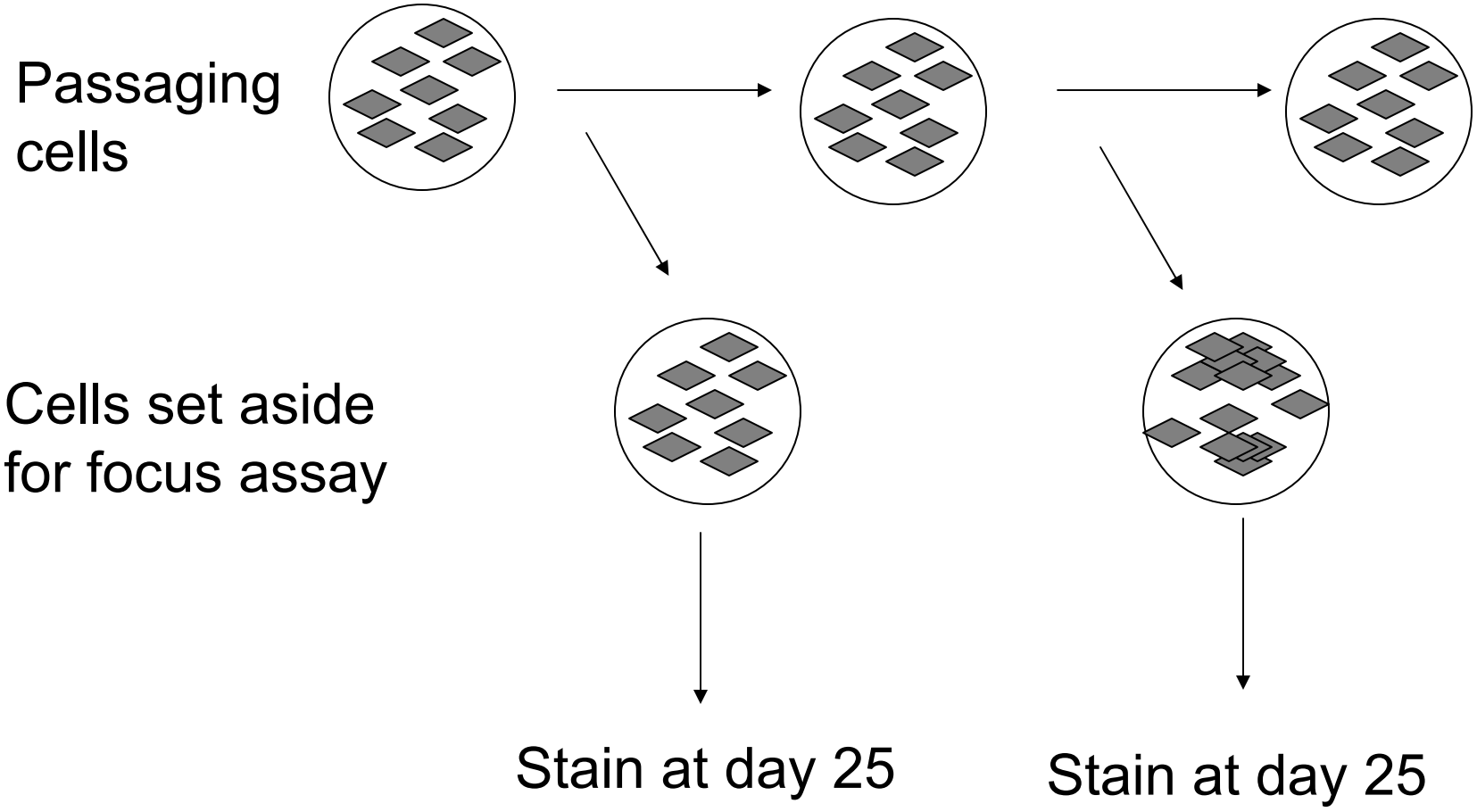
Focus Formation

Variants Are Expressed In C127 Cells



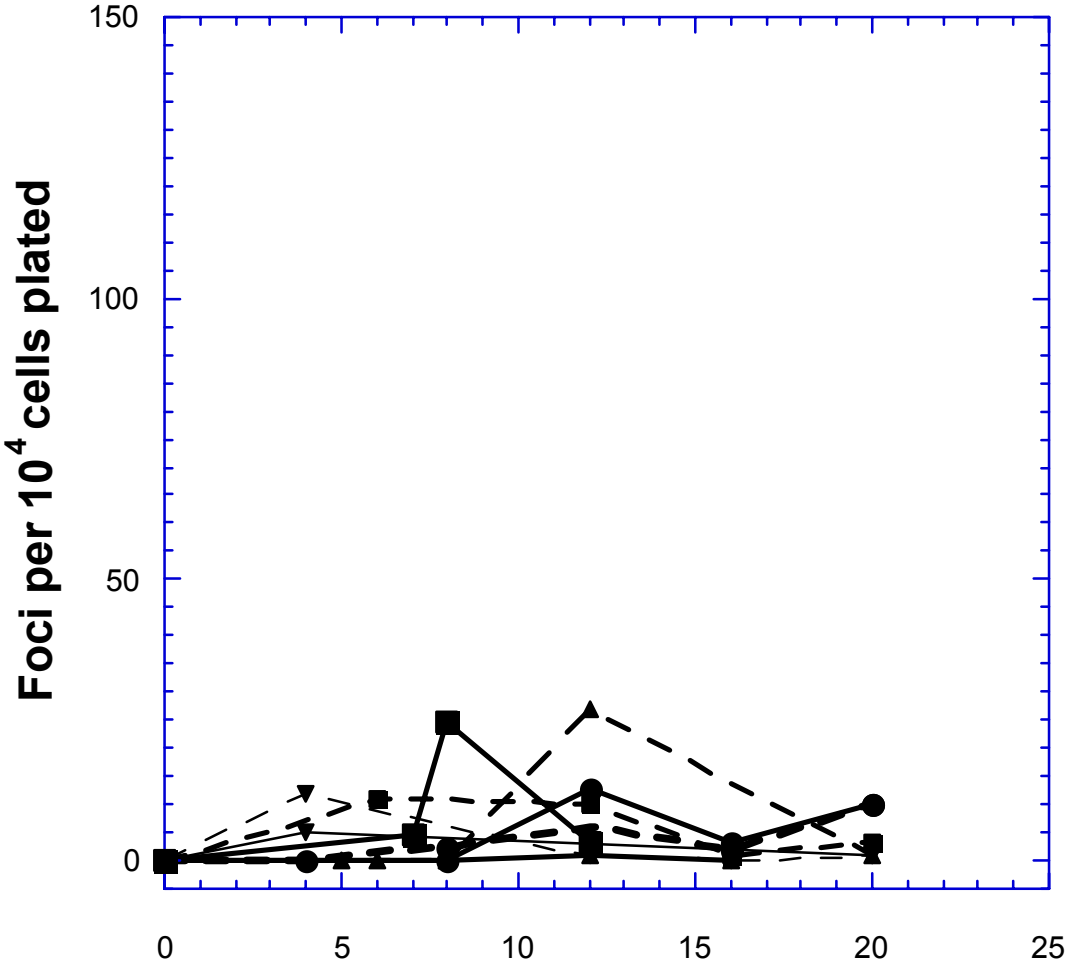


Focus Formation as a Function of Passage



Expression of WT and variants is at most 2-fold over endogenous Pol ß

WT Does Not Induce Focus Formation

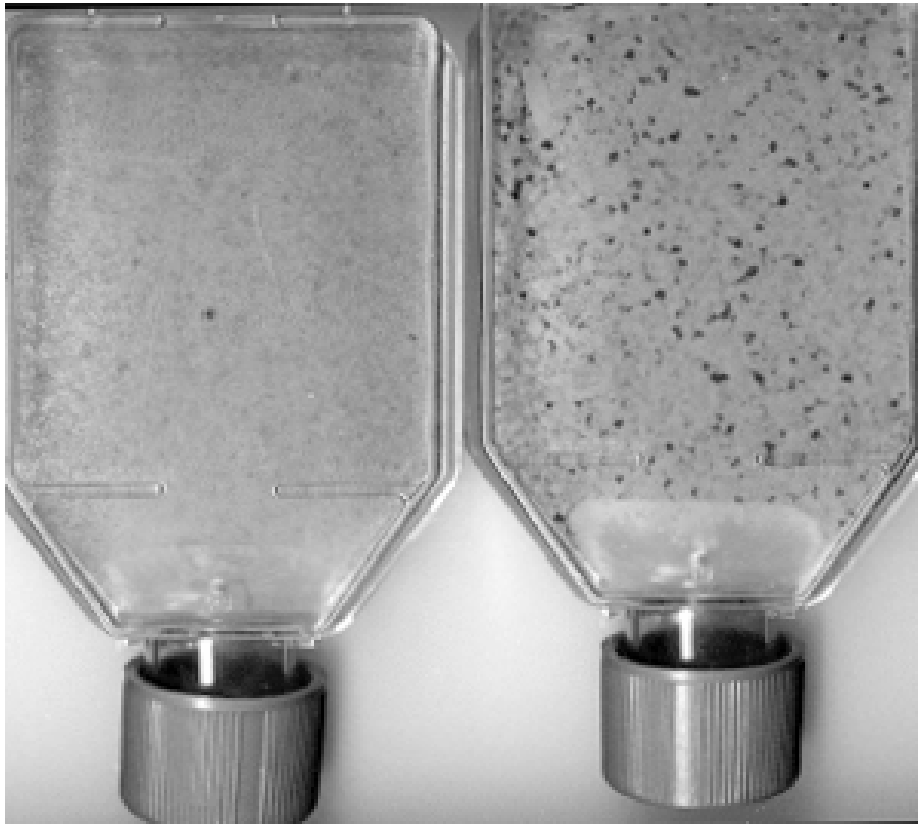


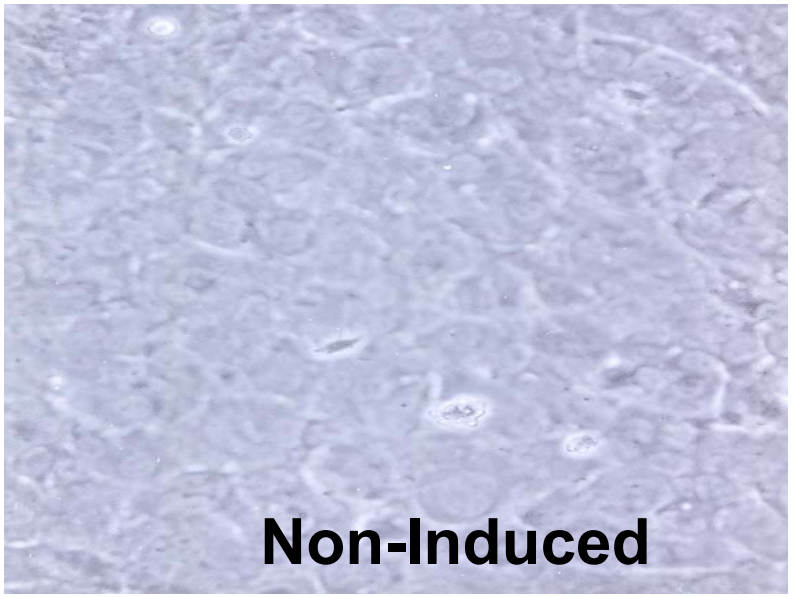
Induced ——————

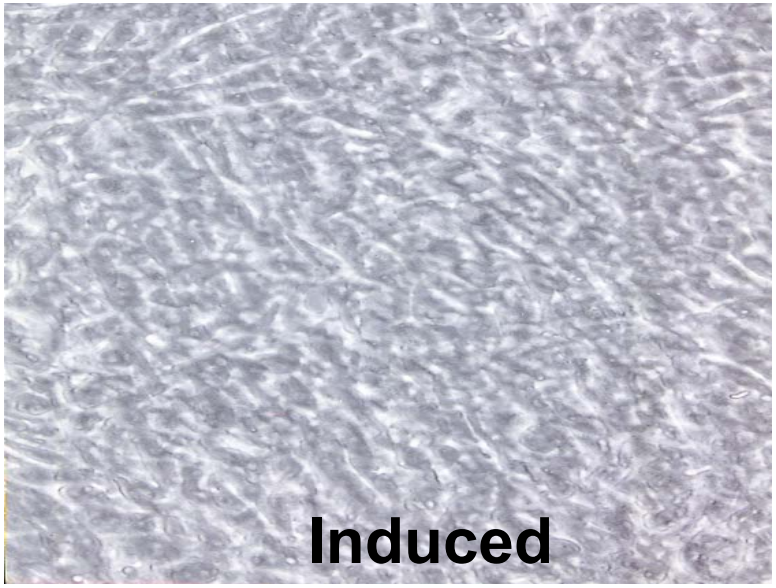
Non-Induced - - - - - -

The I260M Prostate Carcinoma Pol ß Variant Induces Focus Formation

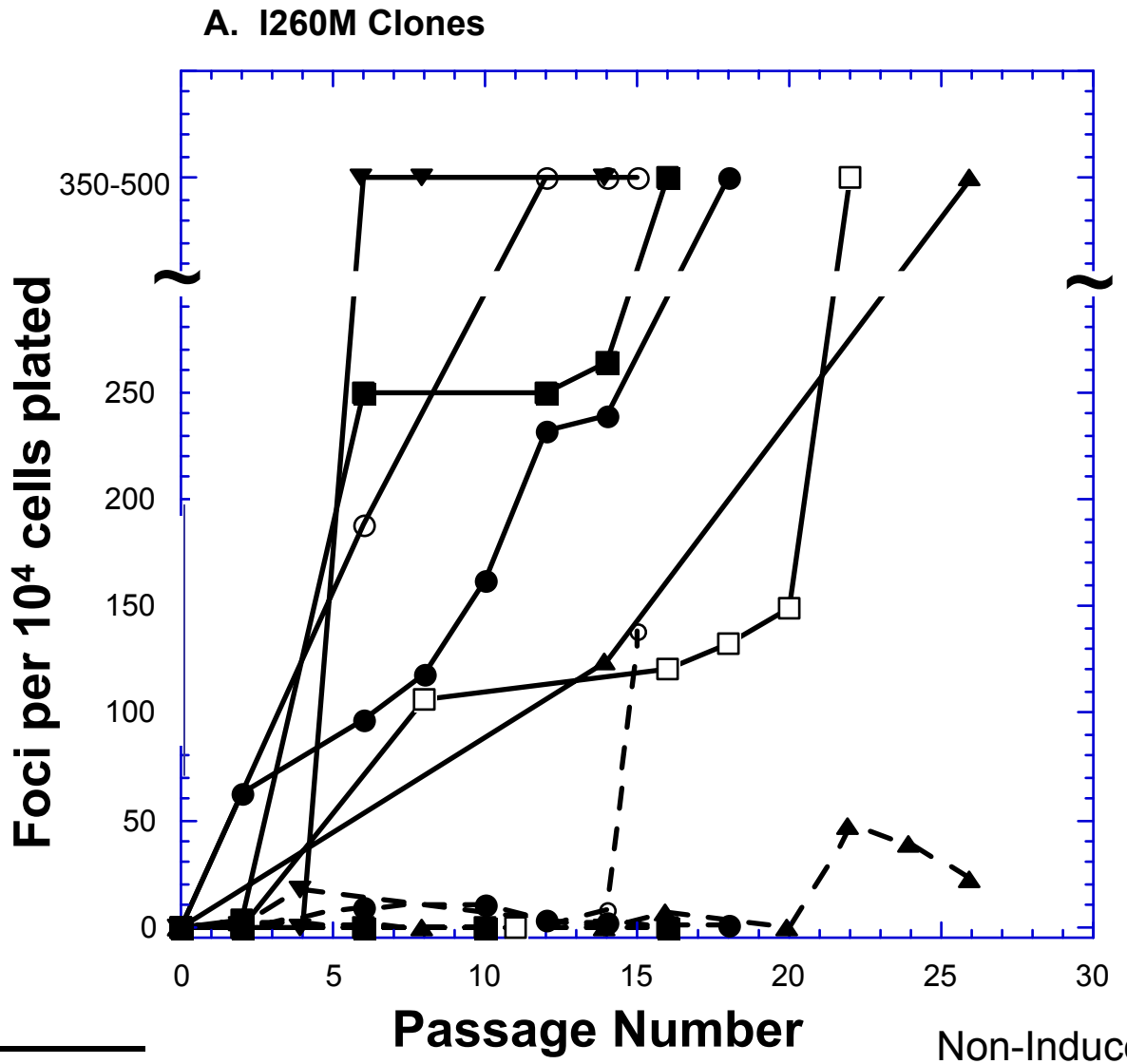
Non-Induced Induced



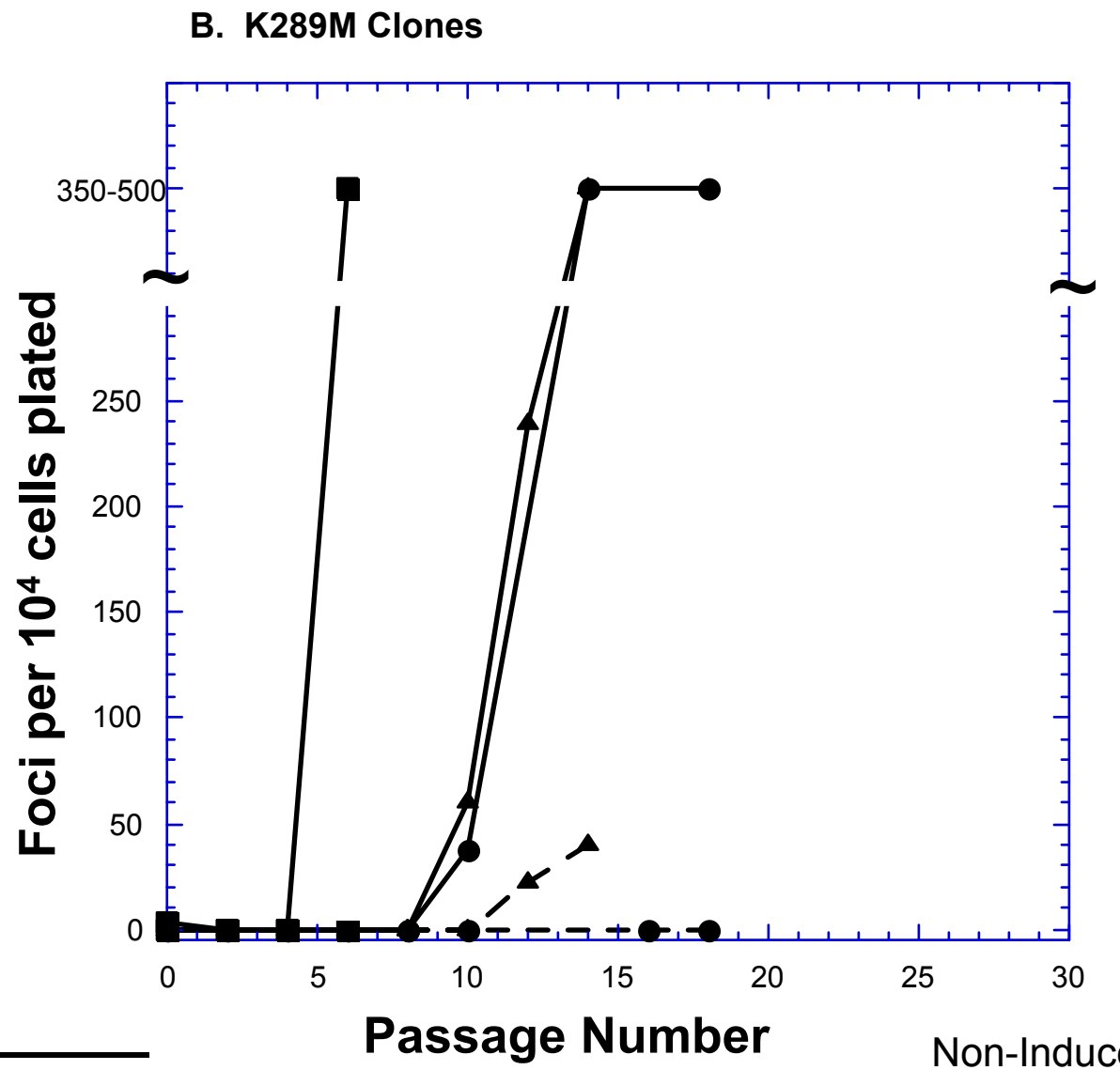




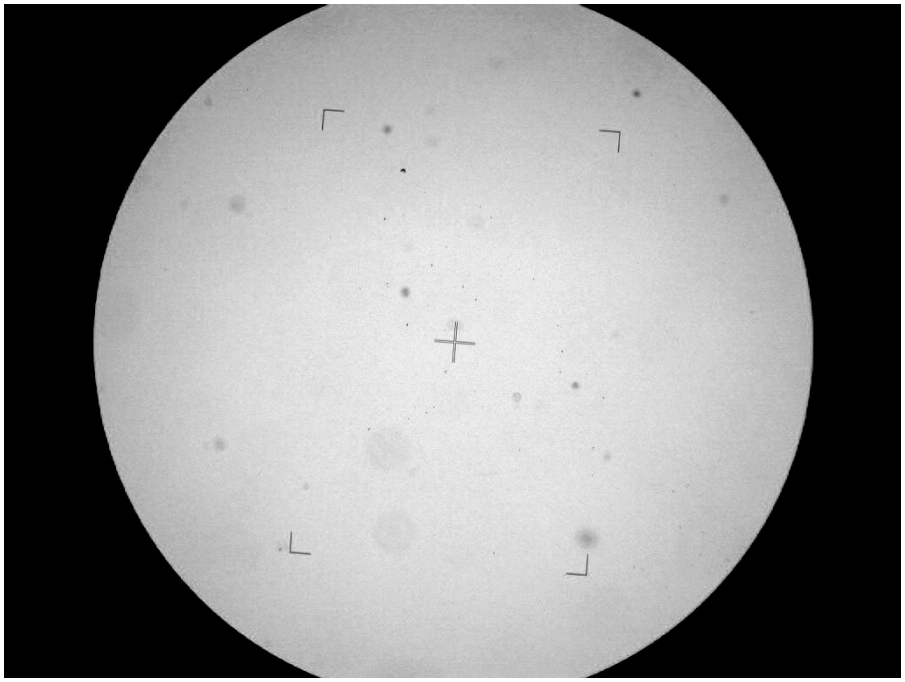
I260M Induces Focus Formation

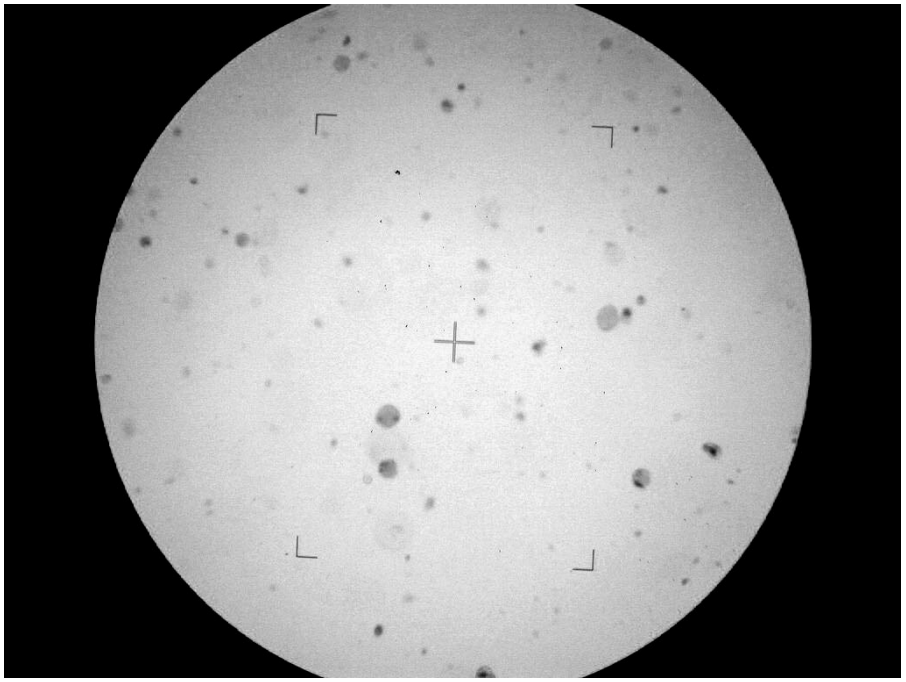


K289M Induces Focus Formation



Variants Induce Anchorage Independent Growth





3±1

Non-induced

27±5

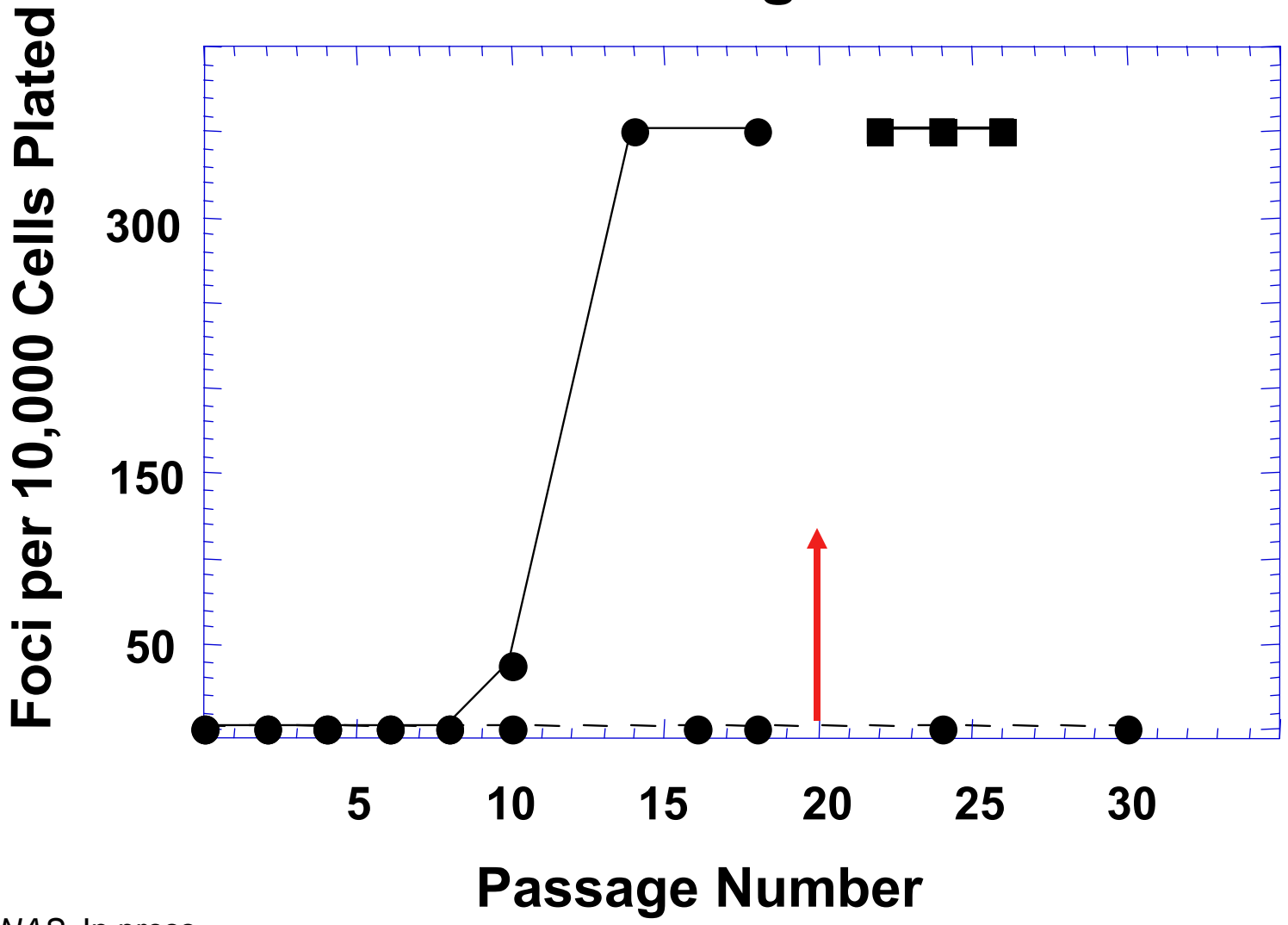
Induced

Do Foci Form When Expression is Extinguished?

No: Suggests that expression of the variant is required for focus formation.

Yes: Suggests that the variant induced a heritable change (mutation) in the cell, resulting in focus formation. The mutation(s) could be in a growth control gene.

Focus Formation Continues After Expression Is Extinguished

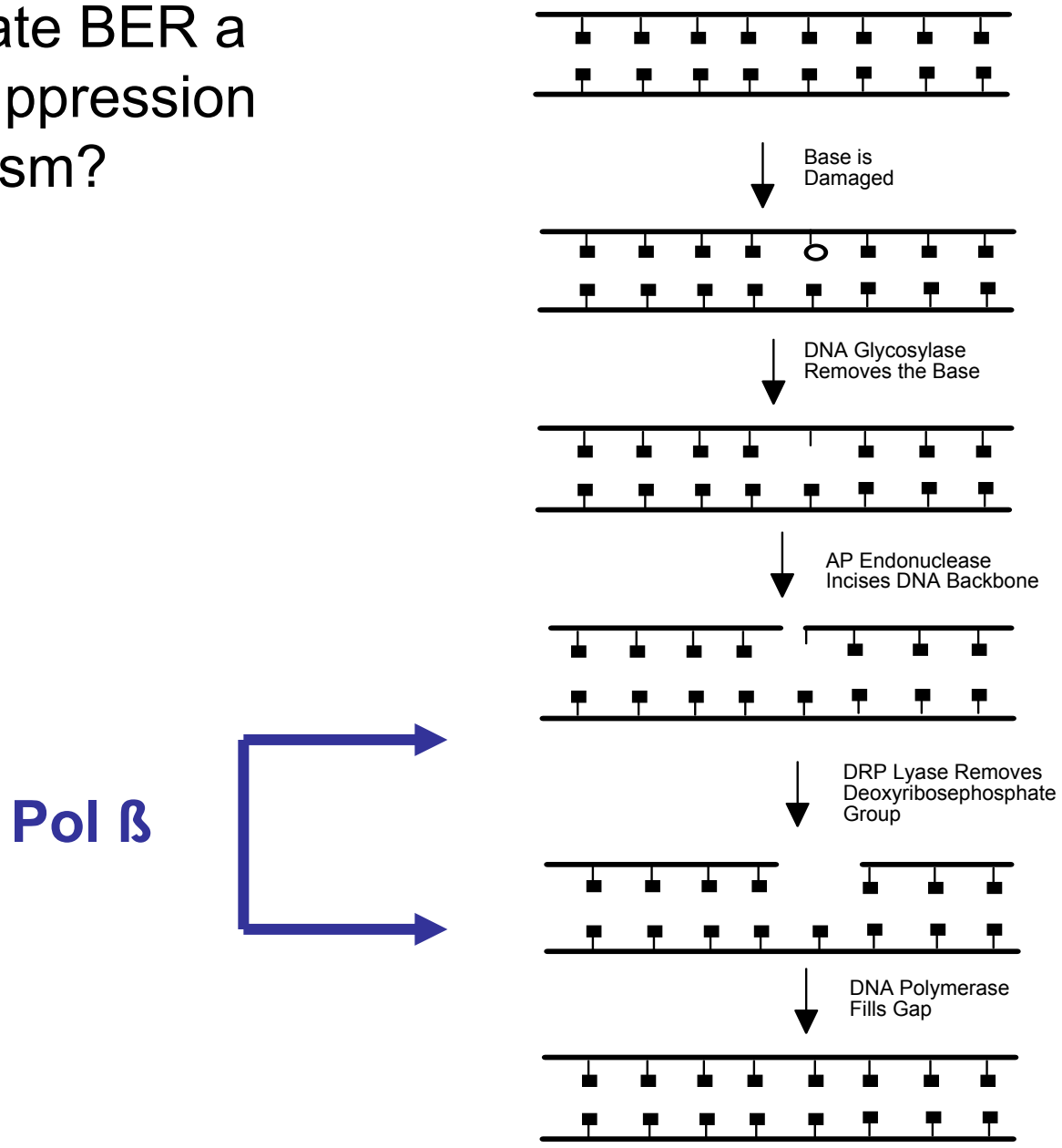


PNAS, In press

Summary

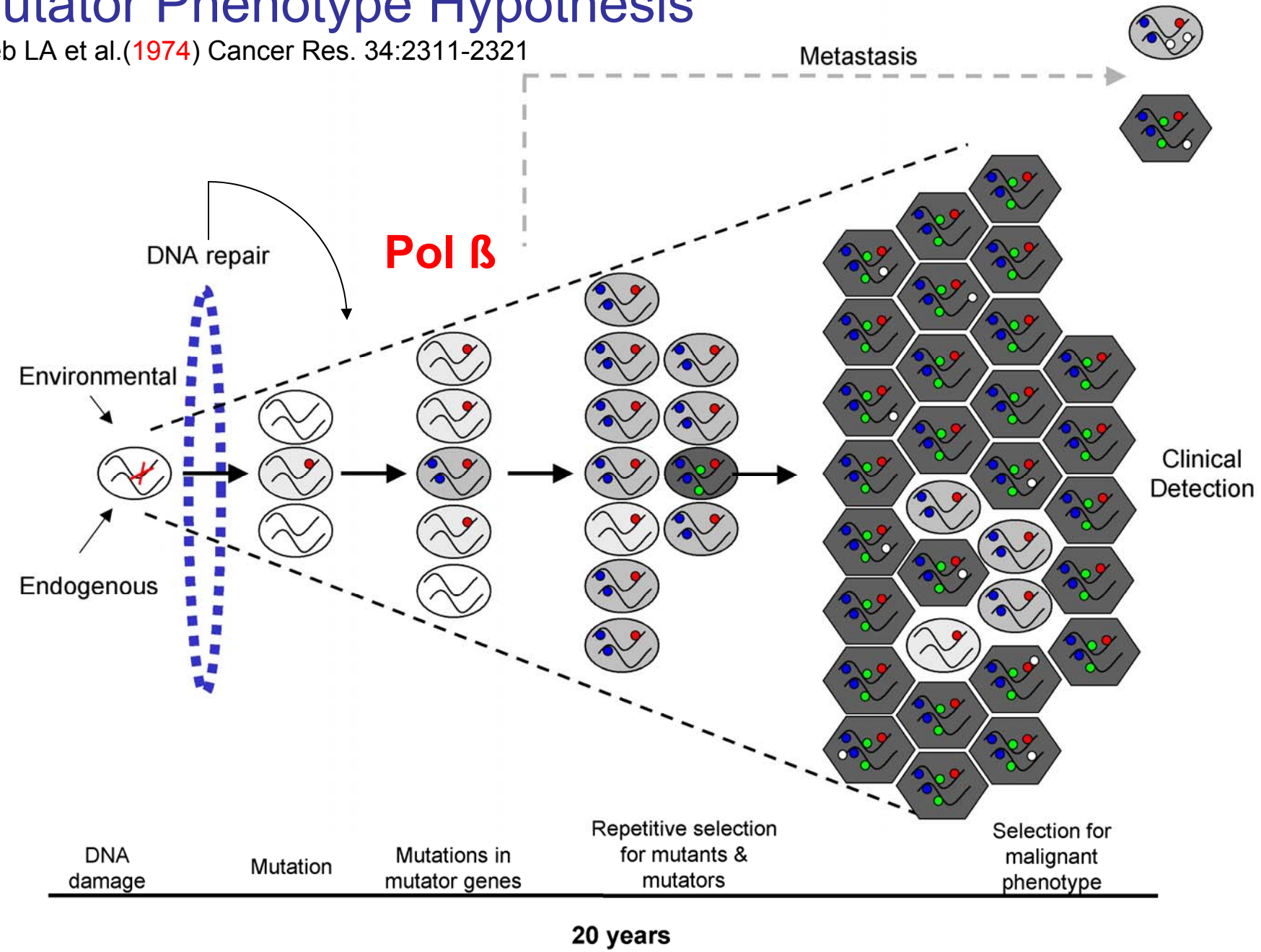
- Expression of I260M, K289M, D160N, and E295K result in cellular transformation.
- Cells maintain the transformed phenotype once expression has been extinguished.
- Results are consistent with transformation resulting from a heritable change (mutations).

Is accurate BER a tumor suppression mechanism?



Mutator Phenotype Hypothesis

Loeb LA et al.(1974) Cancer Res. 34:2311-2321

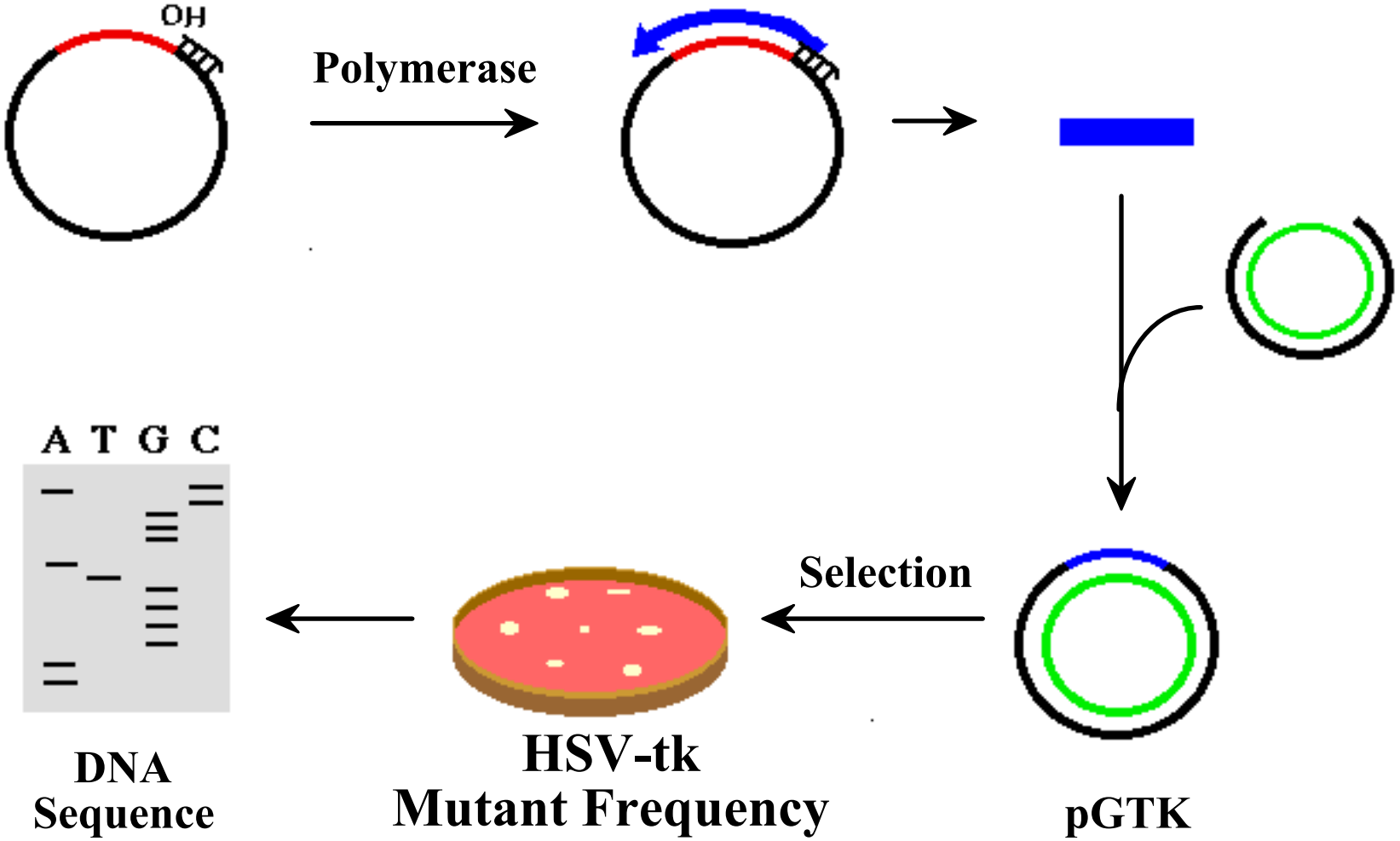


Are Pol ß Variants Linked to Cancer?

- Does expression of the Pol ß cancer-associated mutants confer a transformed phenotype to cells?

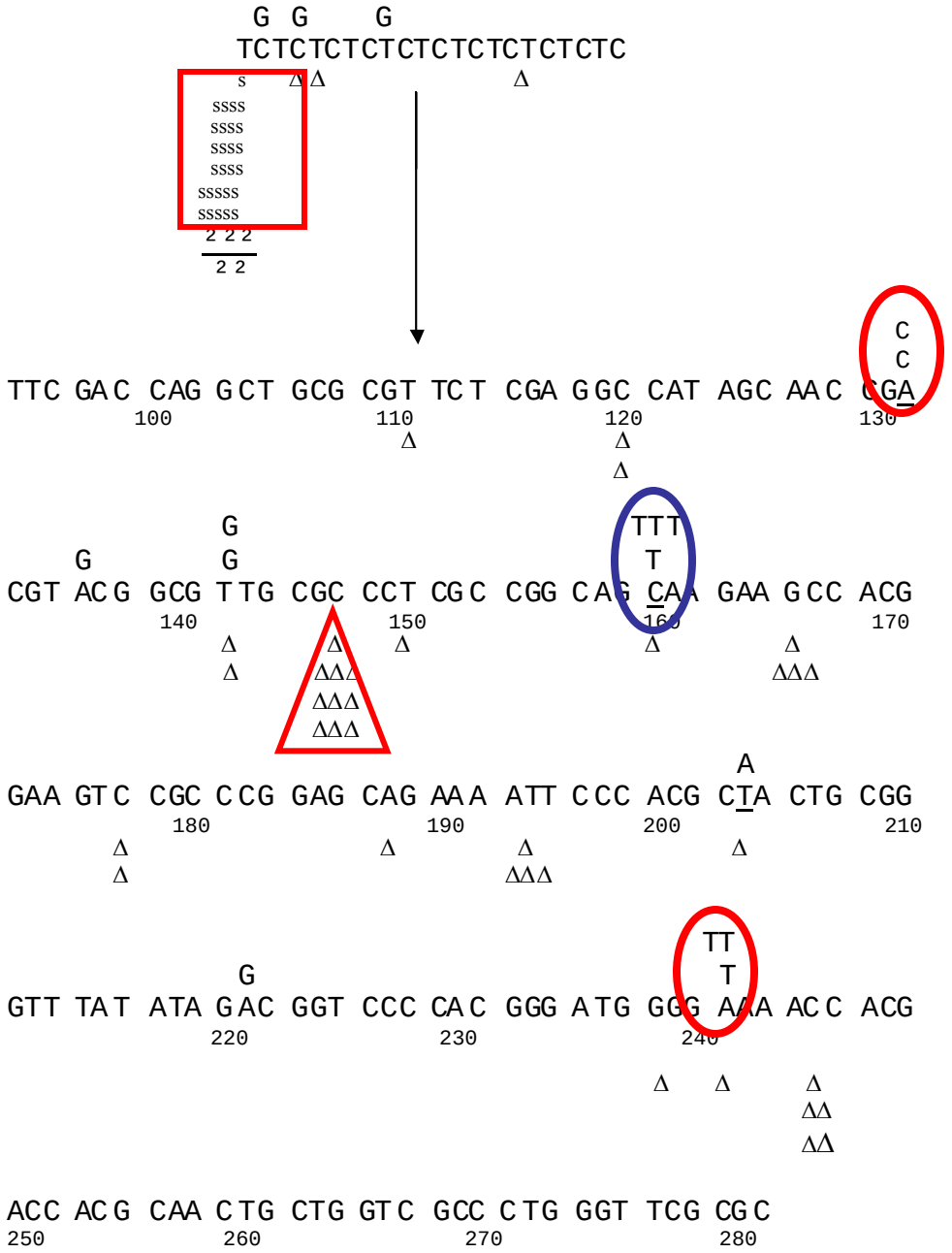
- Can the Pol ß cancer-associated variants induce mutations that could lead to cellular transformation?

The *in vitro* HSV-*tk* Forward Mutation Assay



Eckert et al., 1997

I260M is a Sequence-Specific Mutator



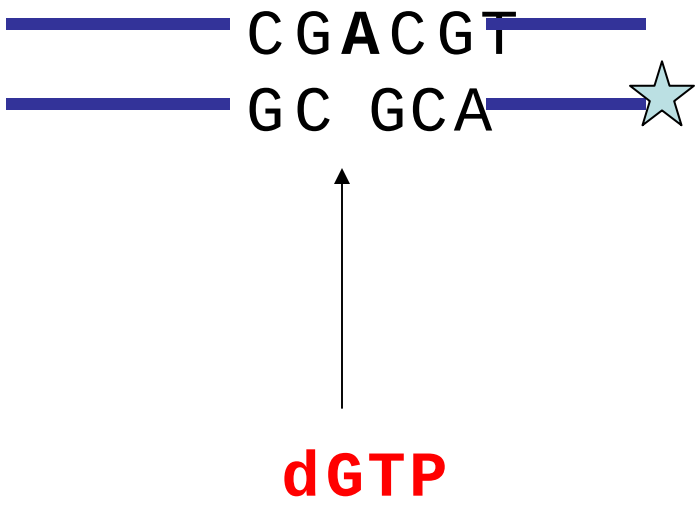
I260M Induces Different Types of Mutations than WT Pol ß

(x 10^{-4})

	WT	**I260M**
Mutation Frequency	**78**	**230**
TC Repeat	**29**	**67**
Expansion	**11**	**61**
Deletion	**7**	**5**
-1 base frameshifts at dipyrimidines	**9**	**52**
Base Sub.	**9**	**21**
Transversions	**2**	**15**
Transitions	**7**	**6**

I260M Misincorporation

For a A>C transversion:



I260M Misincorporates dGTP Opposite A

	FIDELITY
§-WT	
A:T	
A:G	188,462
I260M	
A:T	
A:G	1,286

146X

I260M Fidelity Depends Upon Sequence Context

CGTACG CGGTTG
GCATGC**A**GCCAAC

A,C, and G are misinserted by I260M but not WT.

ACCAAC CAACCT
TGGTTG**A**GTTGGA

I260M and WT have equal fidelity (high).

CGTACG GGGTTG
GCATGC**T**CCCAAC

I260M and WT have equal fidelity(low).

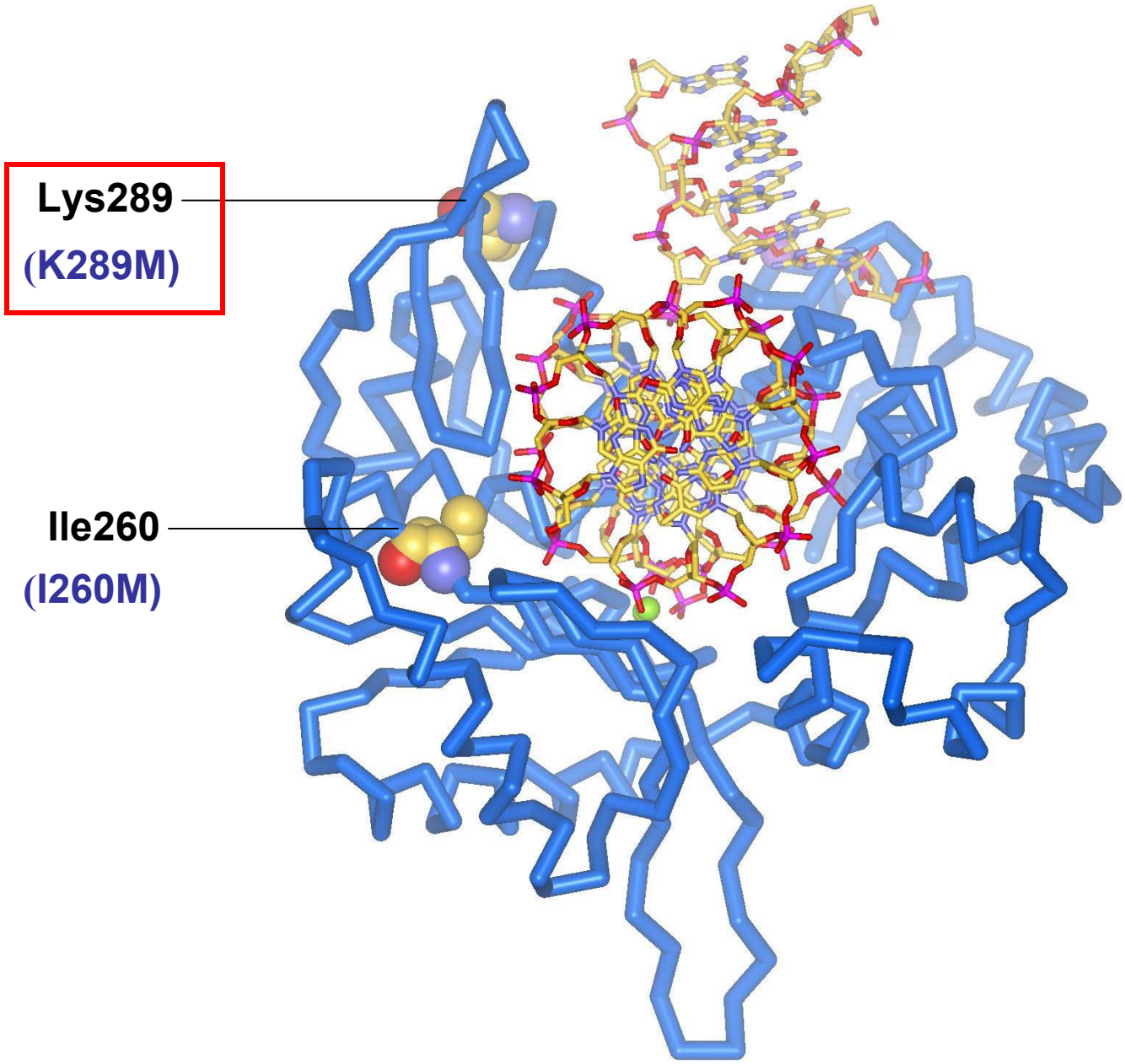
CGCAGT GCGTGG
GCGTCA**T**CGCACC

T is misinserted by I260M but not WT.

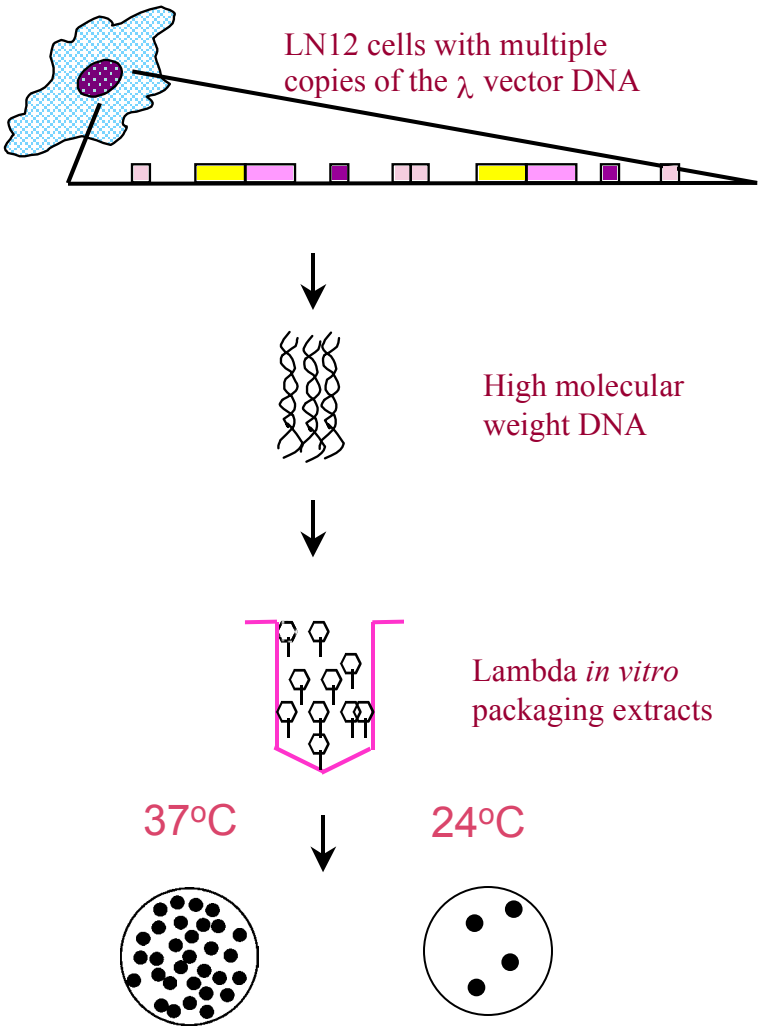
CTTCTT CTGCCG
GAAGAA**C**GACGGC

A is misinserted by I260M but not WT.

Mutants of Pol ß Associated with Colon and Prostate Cancer



Lambda Shuttle Vector System



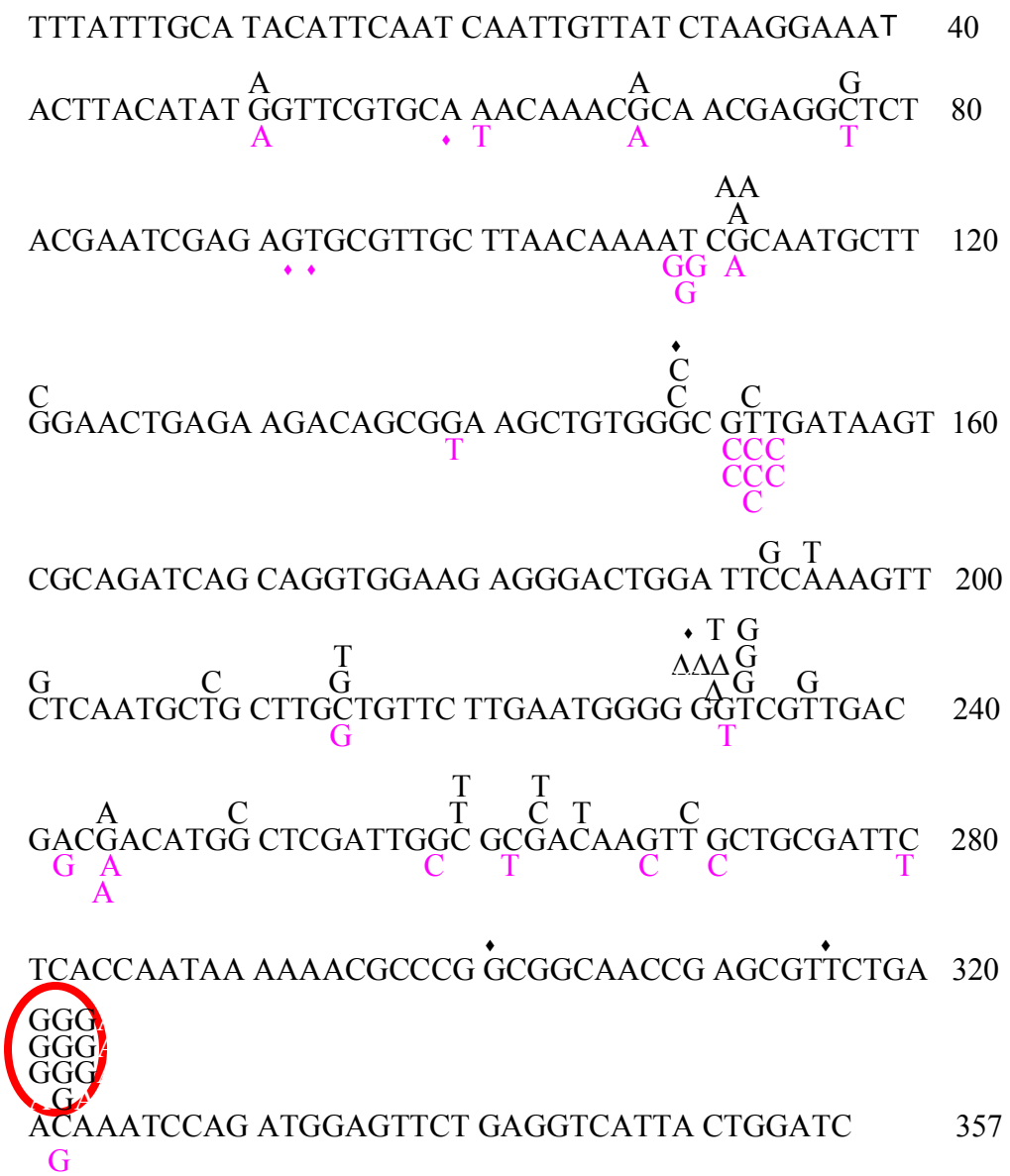
--at 37°C, all phages enter lytic pathway
at 24°C, only mutants lyse the cells

-- selects for point mutations and small deletions and insertions.

-- sensitive with minimal bias.

-- amenable for sequence analysis.

The Mutational Specificity of K289M Is Different From WT



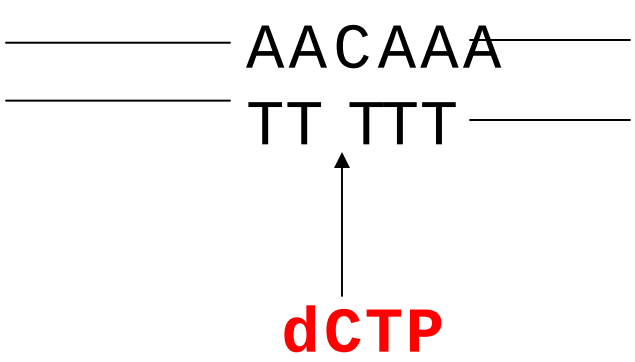
K289M Induces Mutations at Same Sites As WT, But at Higher Frequencies

	Mutation Frequency
WT	2.9×10^{-6}
K289M	4.8×10^{-5}

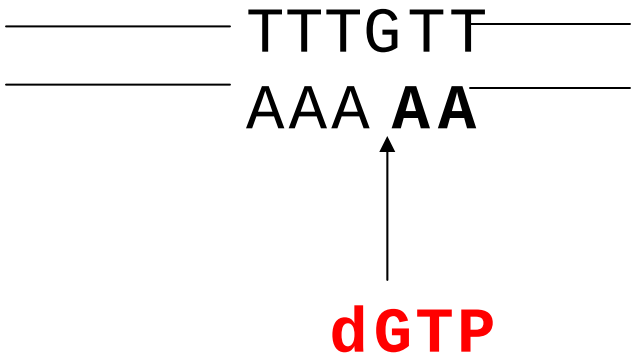
(*in vivo*)

K289M Misincorporation

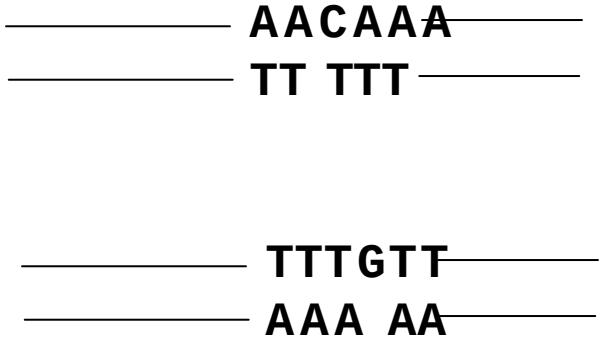
For a C>G transversion:



For a G>C transversion:



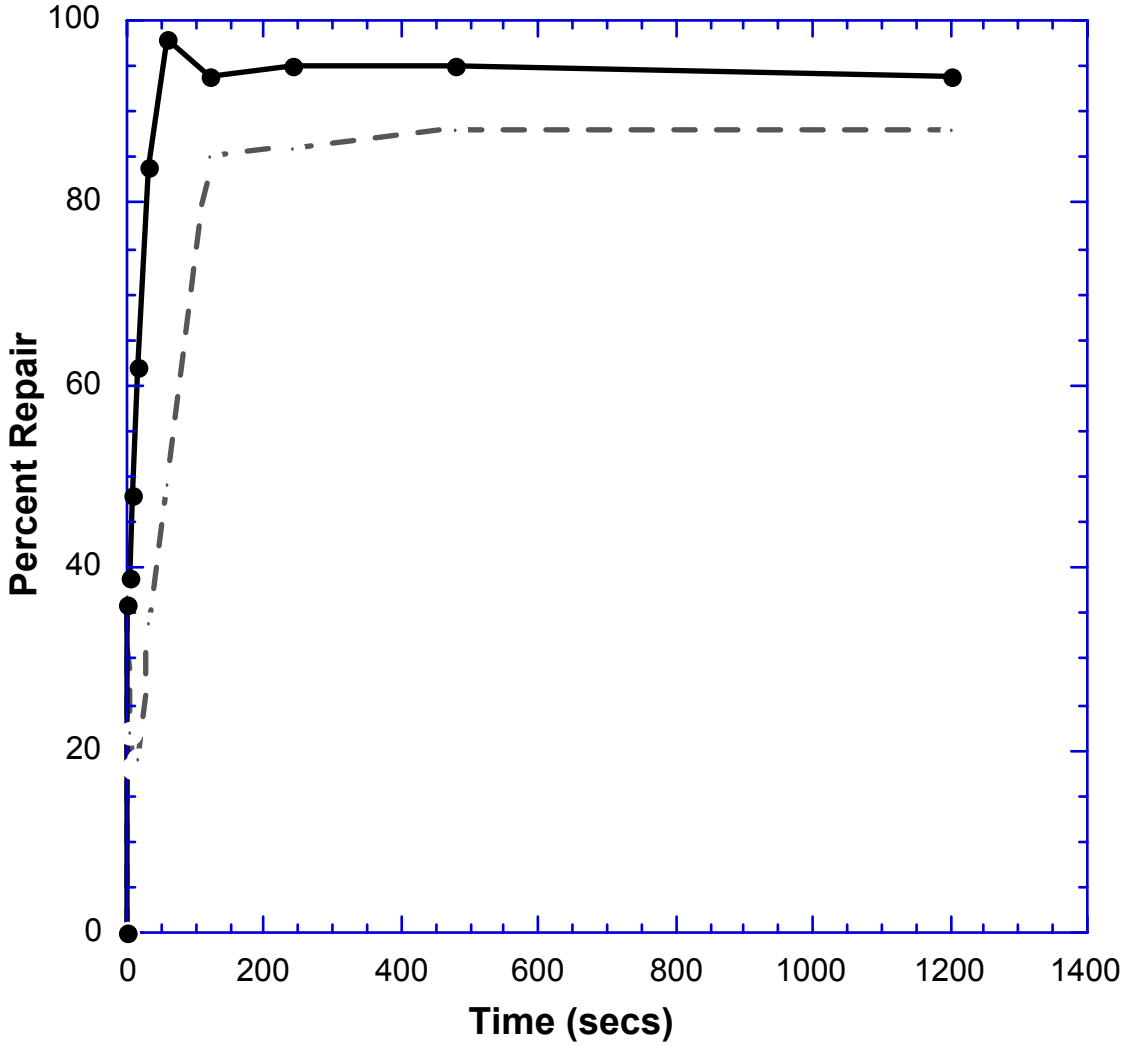
K289M Misincorporates dCTP Opposite C and dGTP Opposite G



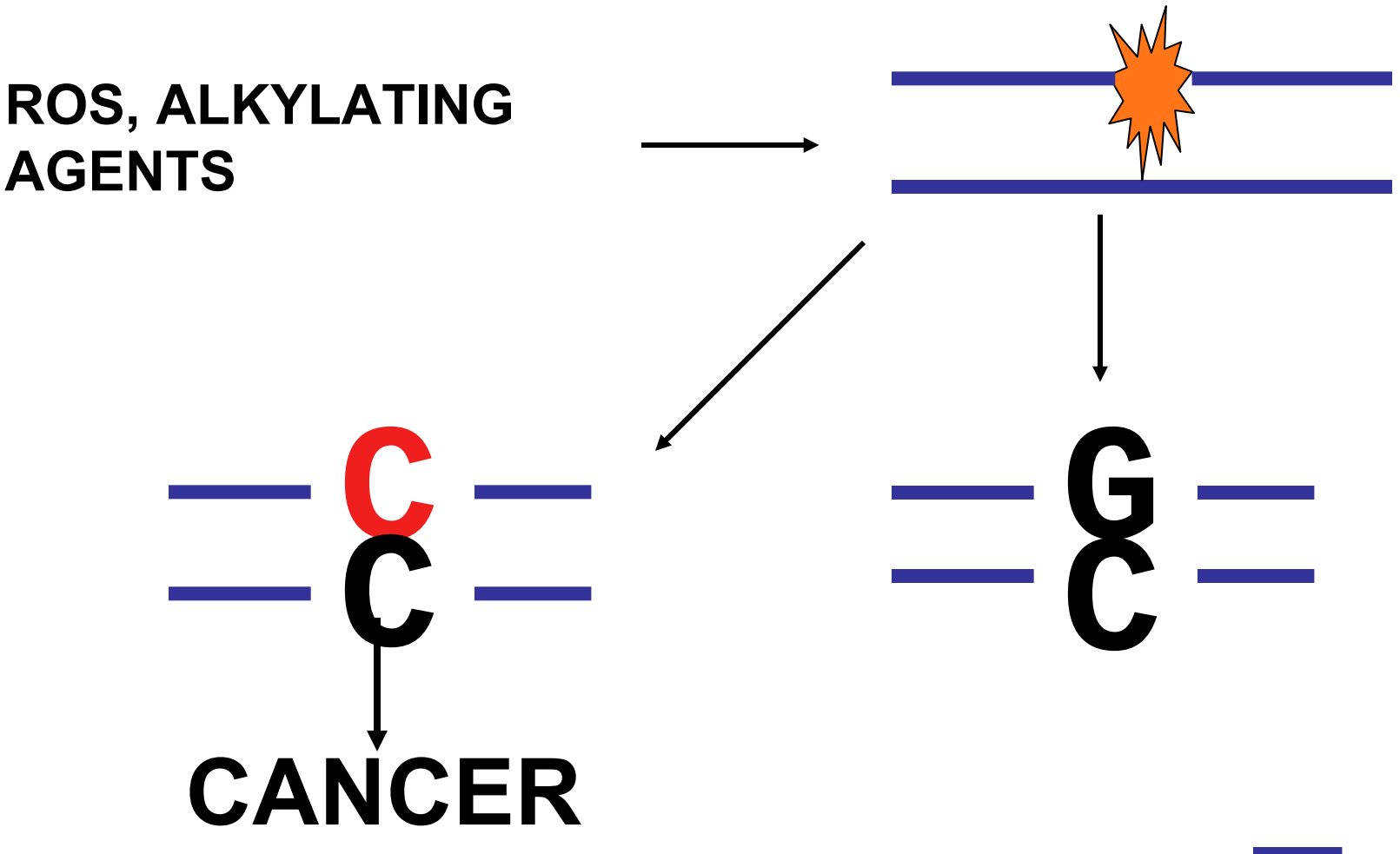
	FIDELITY	
	§-WT	K289M
C:G		
C:C	82,000	3,100
G:C		
G:G	13,400	55

PNAS 101, 6074

K289M and I260M Function in BER

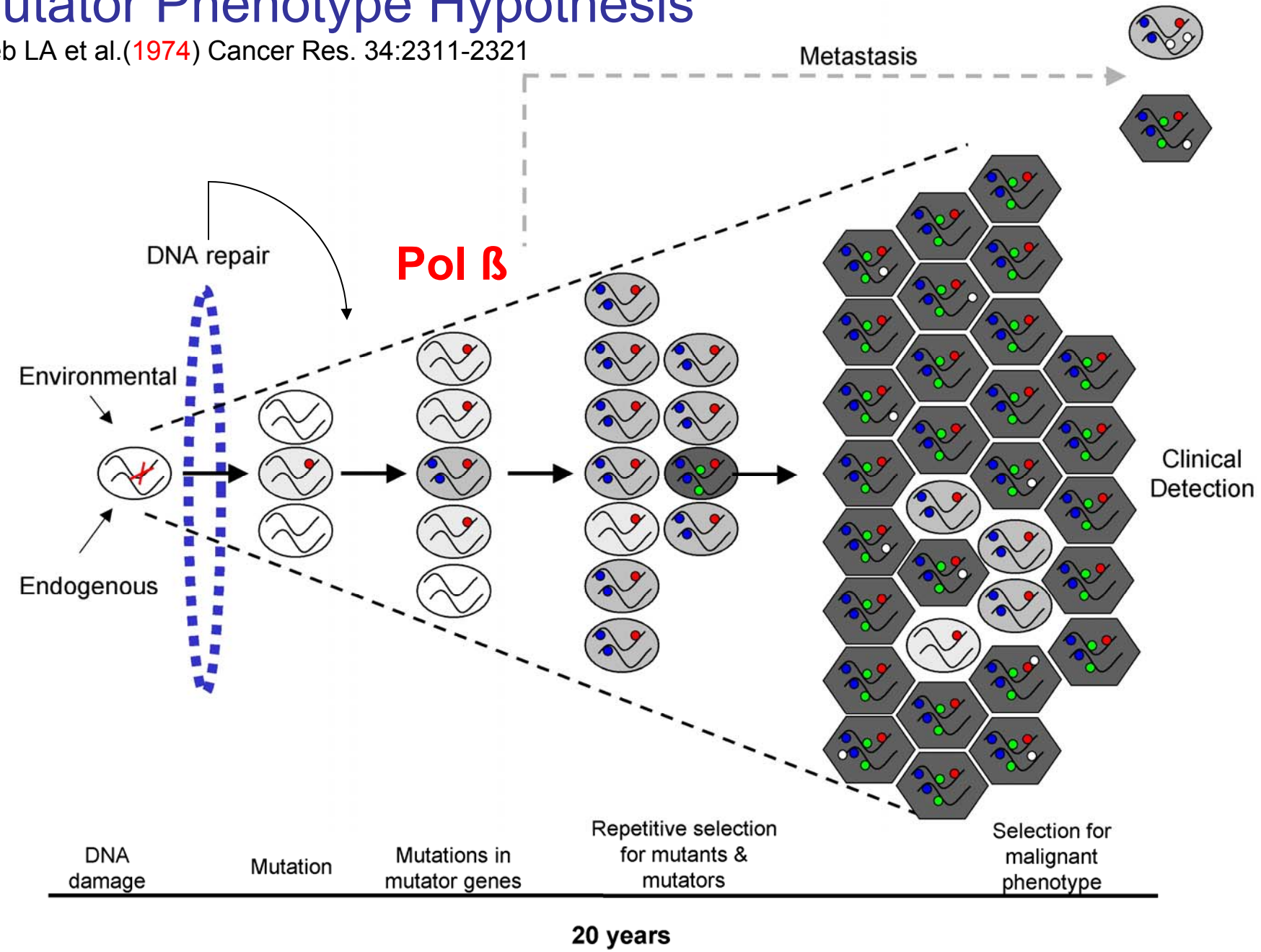


Mutations Are Inserted By Polymerase Beta Variants During Base Excision Repair



Mutator Phenotype Hypothesis

Loeb LA et al.(1974) Cancer Res. 34:2311-2321



Summary

- Tumor-associated variants are sequence-specific mutators that may introduce few deleterious mutations.
- Mutations are induced in key growth control genes or genes that control genome stability.
- Cells with these mutations may be selected, resulting in tumorigenesis or more aggressive disease.

Summary

- Expression of the Pol ß tumor-associated variants results in cellular transformation.
- Cellular transformation appears to result from mutations and selection.
- The Pol ß variants are linked to the etiology of human cancer.


Daniel DiMaio
CA16038
DUNKIN' DONUTS