

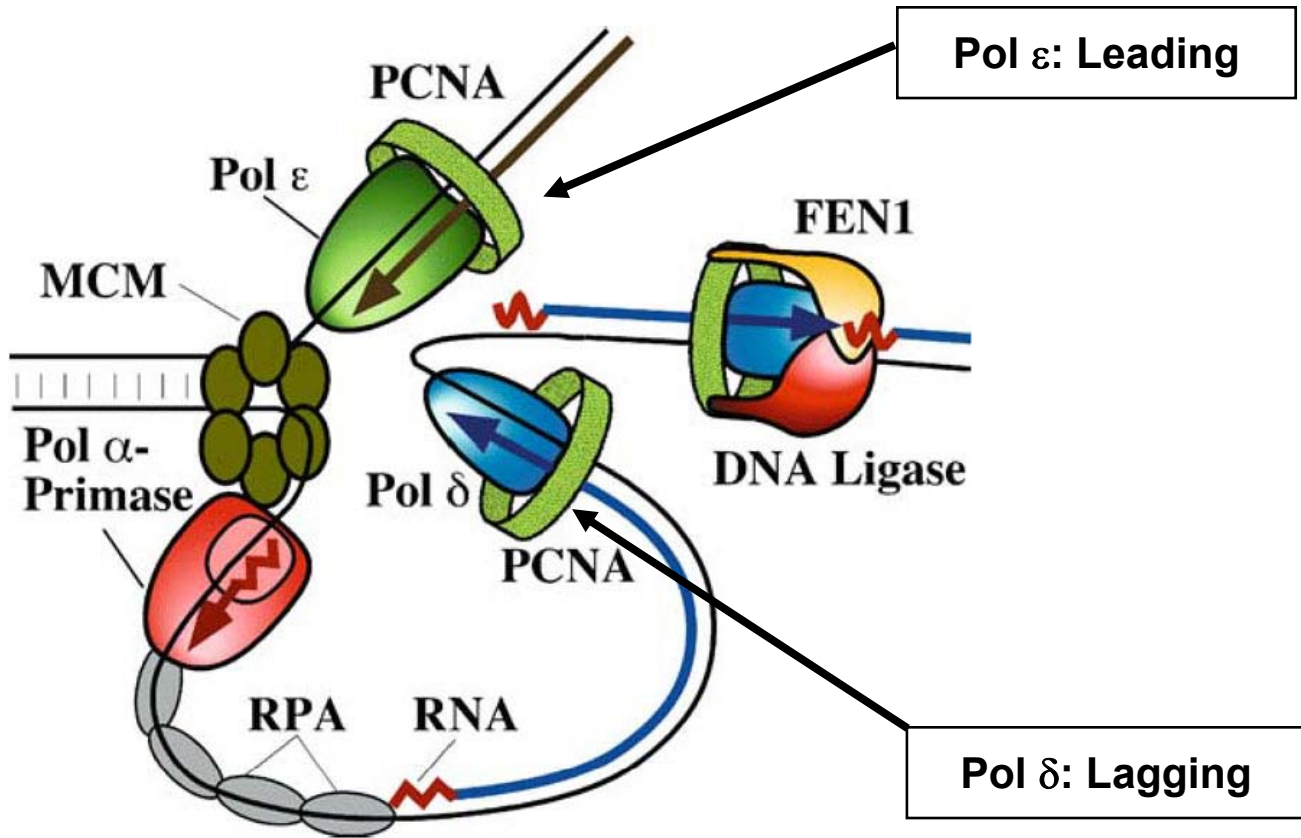
Evidence that Yeast DNA Polymerase ϵ Participates in Leading Strand Replication

Zachary Pursell

Laboratory of Molecular Genetics and Laboratory of Structural Biology
National Institute of Environmental Health Sciences, NIH

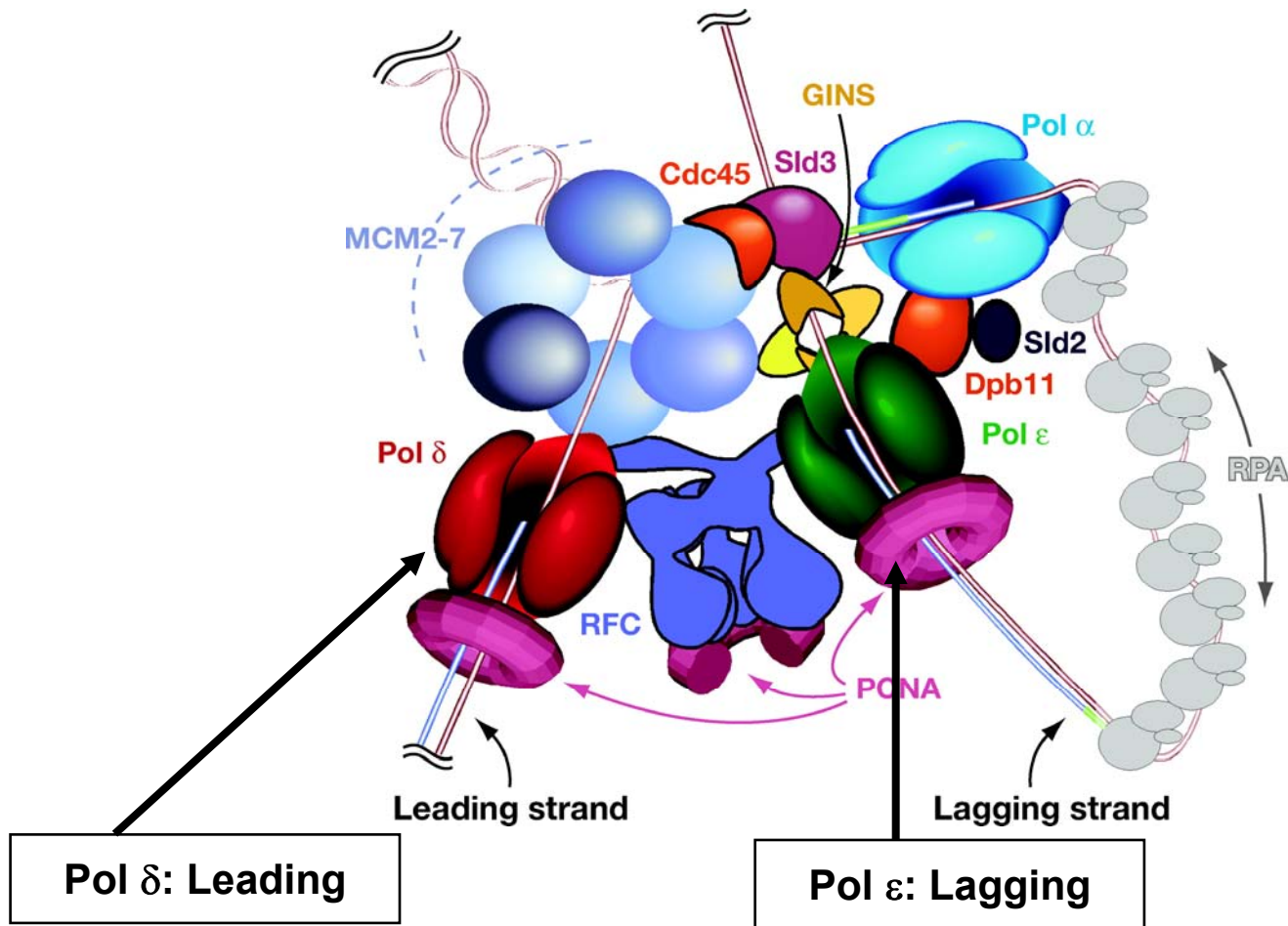
Which Polymerase Replicates Which Strand?

Garg & Burgers (2005)
Crit. Rev. Biochem. Molec. Biol. 40:1-14



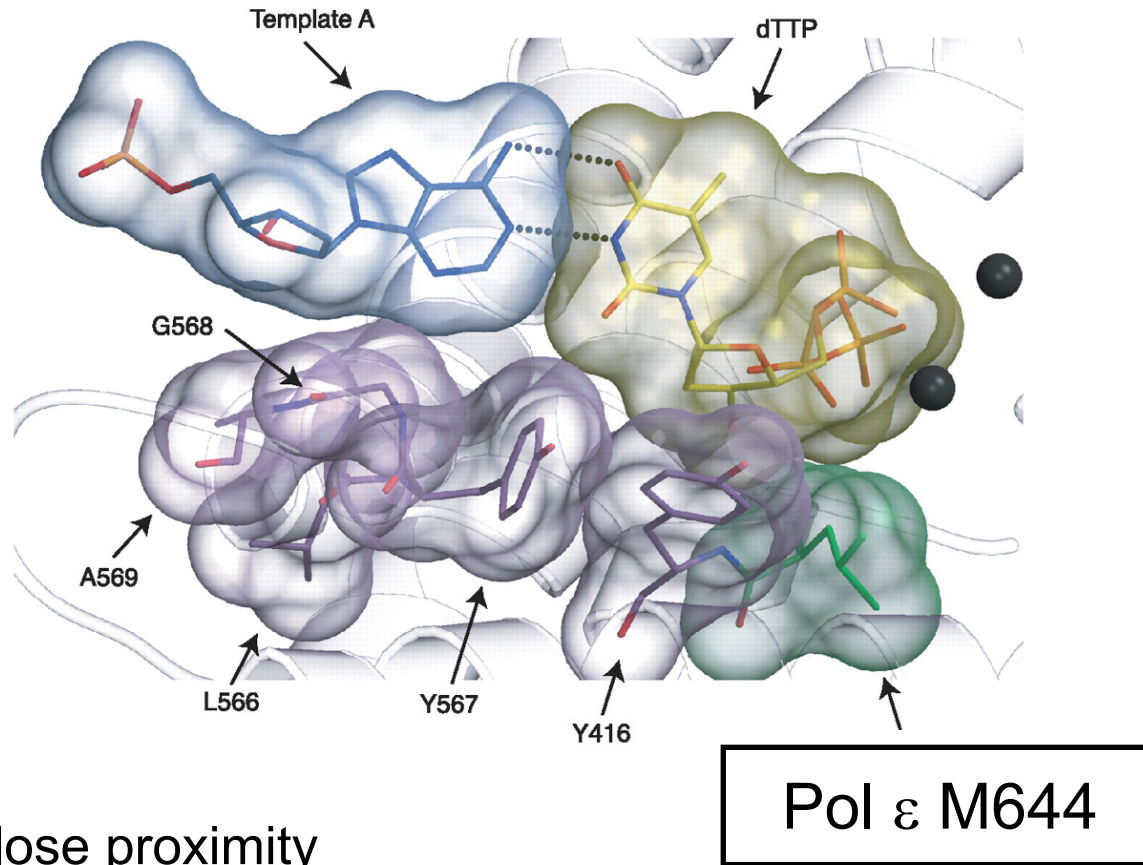
Which Polymerase Replicates Which Strand?

Johnson & O'Donnell (2005)
Ann. Rev. Biochem. 74:283-315



Of course other models are possible.

Conserved Motif A Residue Is A Key Determinant of Polymerase Fidelity

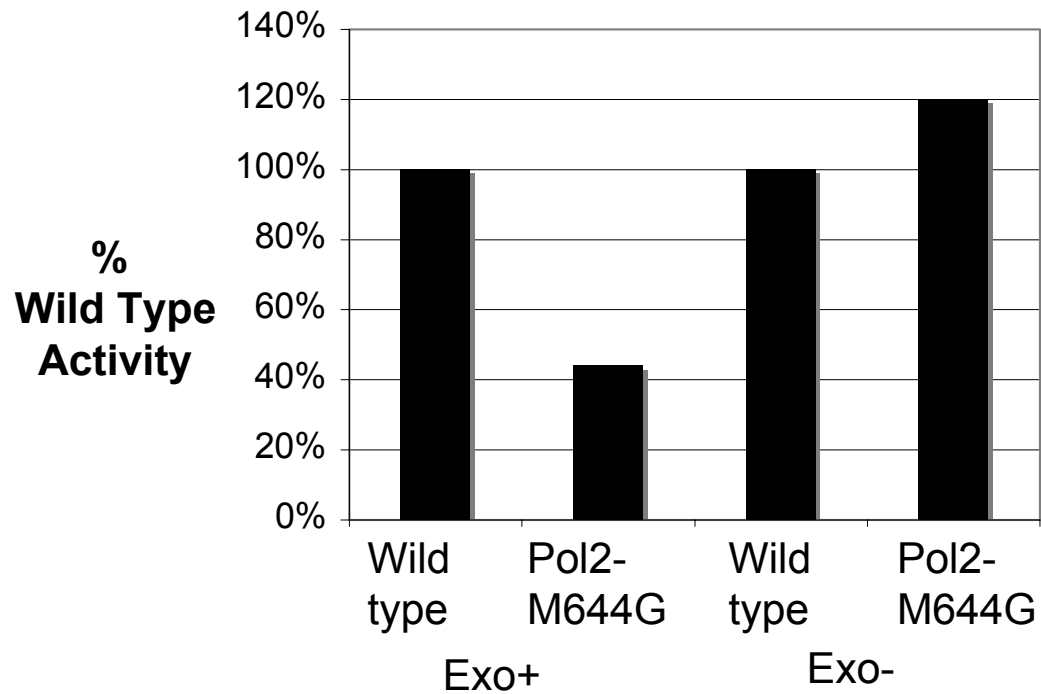


- Active site, close proximity to incoming dNTP
- Substitutions reduce dNTP discrimination

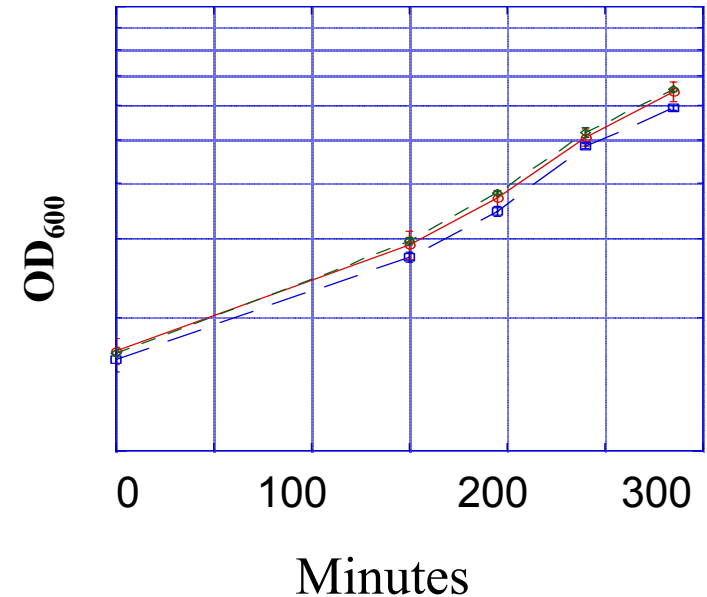
Nick McElhinny et al. *J Biol Chem* **282** (4) 2324-2332
Pursell et al. *NAR* in press

1. Retain Wild Type DNA Polymerase Activity & Growth

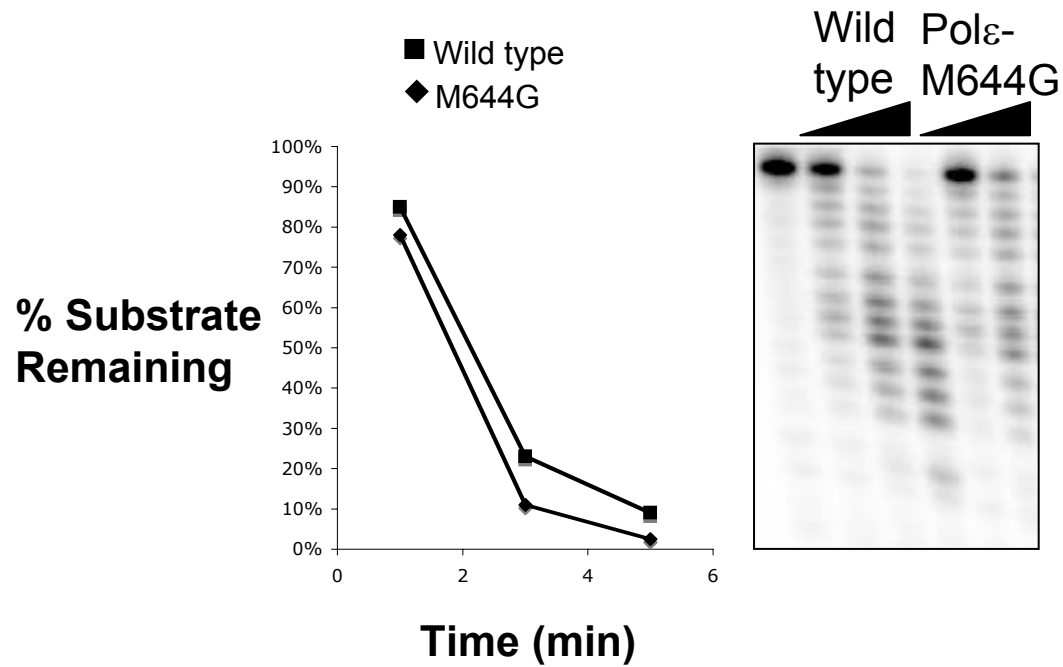
Specific Activity, Activated DNA



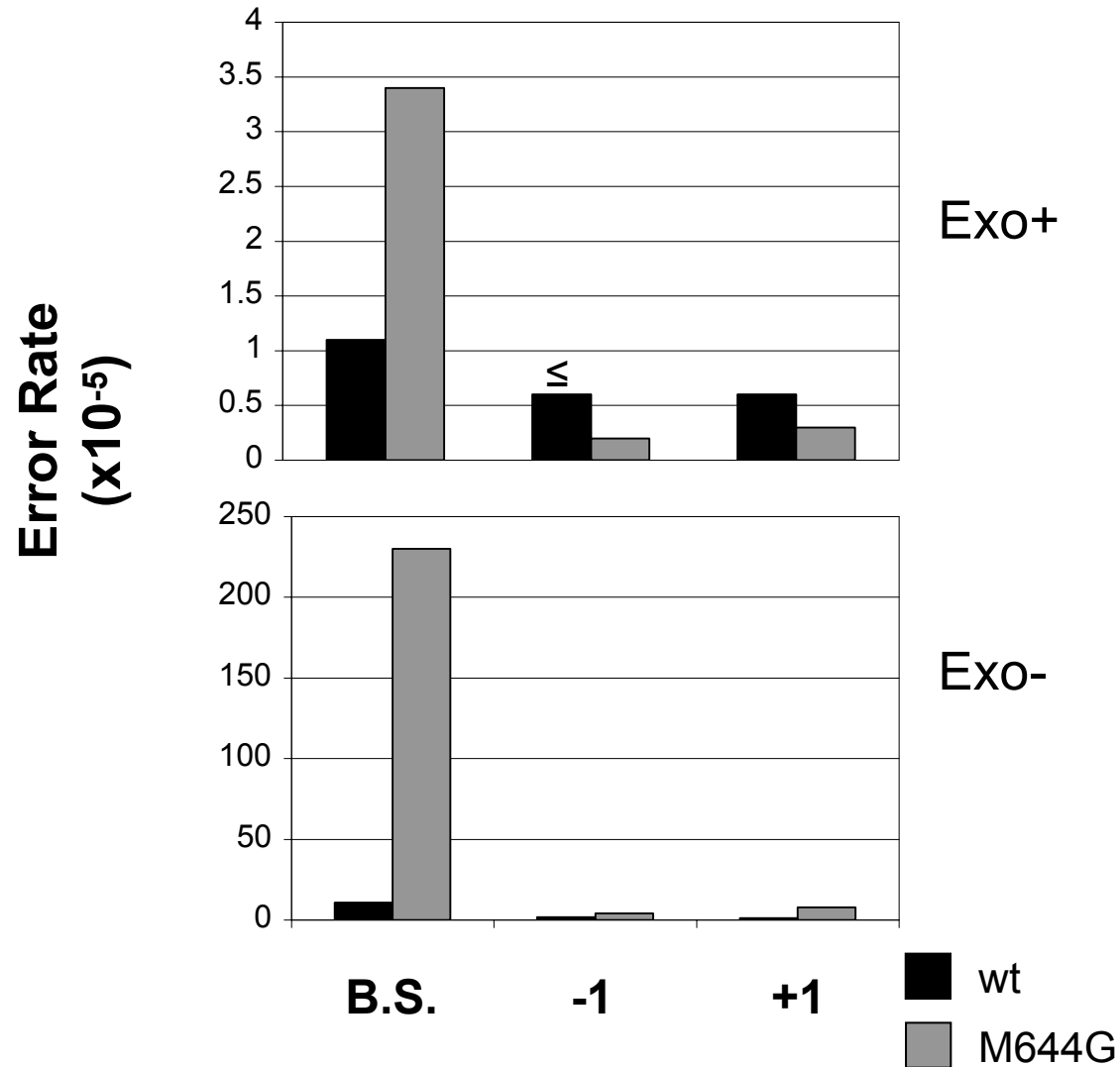
Growth Rate



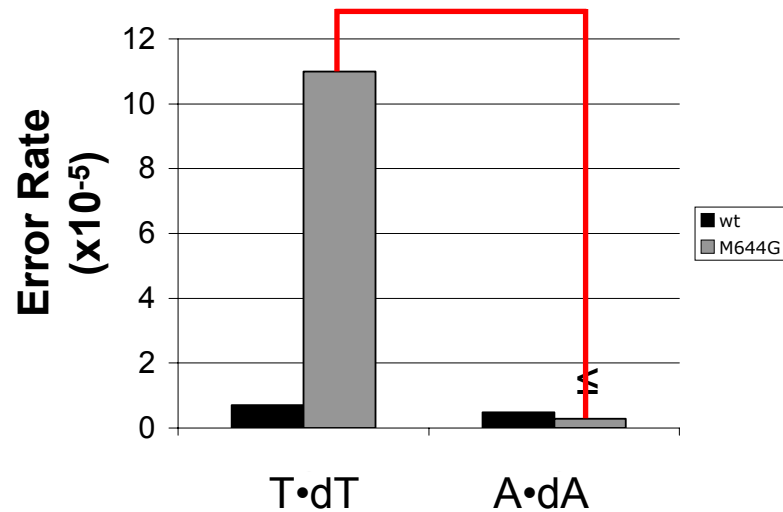
2. Wild Type Exonuclease Activity



3. Reduced *in vitro* Base Substitution Fidelity

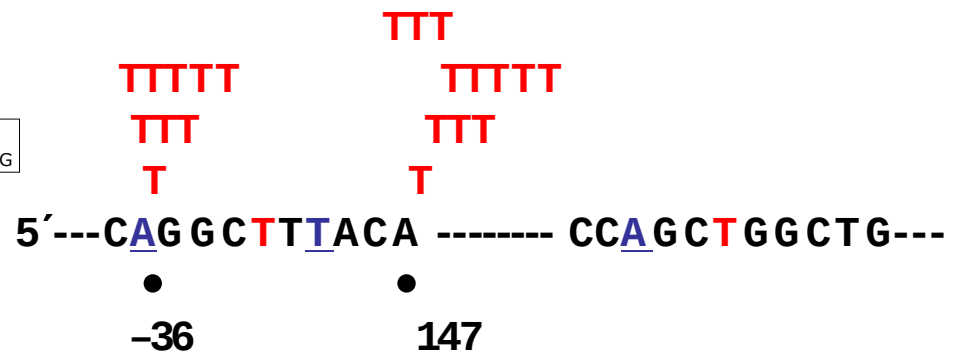


4. Strong *in vitro* Error Rate Bias



≥ 39 -fold

Hot Spots



5. *pol2-M644G* is a Mutator *In Vivo*

	Mutation Rate (x10 ⁻⁷)			
	<i>CAN1</i>		<i>URA3</i>	
<i>wild type</i>	2.3		0.59	
<i>pol2-M644G</i>	8.9	3.9x	4.5	7.6x
$\Delta msh6$	□ □		10	
$\Delta msh6$ <i>pol2-M644G</i>	□ □	58x	610	61x

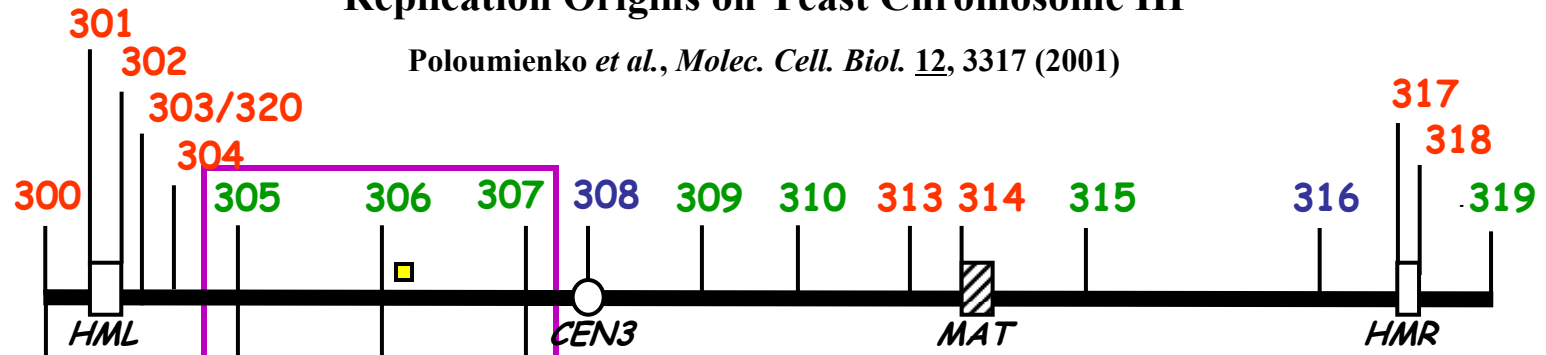
6. Distinctive mutational signature.

45 of 61 *ura3* mutants from the *pol2-M644G* strain contained a T-A to A-T transversion. Most of these were at two positions.

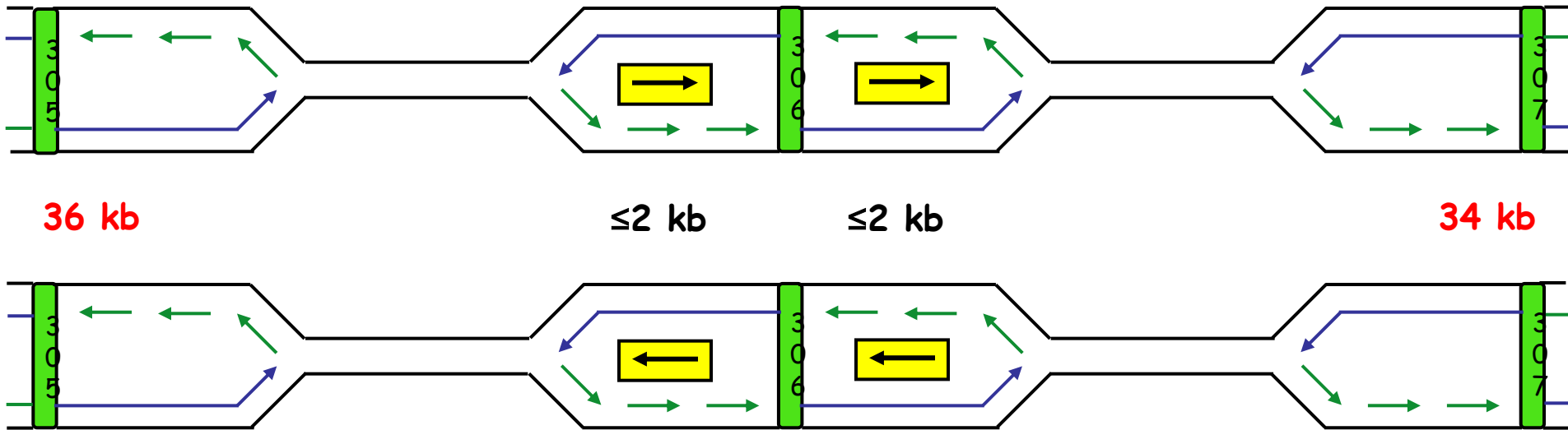
Strategy for Assigning Leading and Lagging Strands

Replication Origins on Yeast Chromosome III

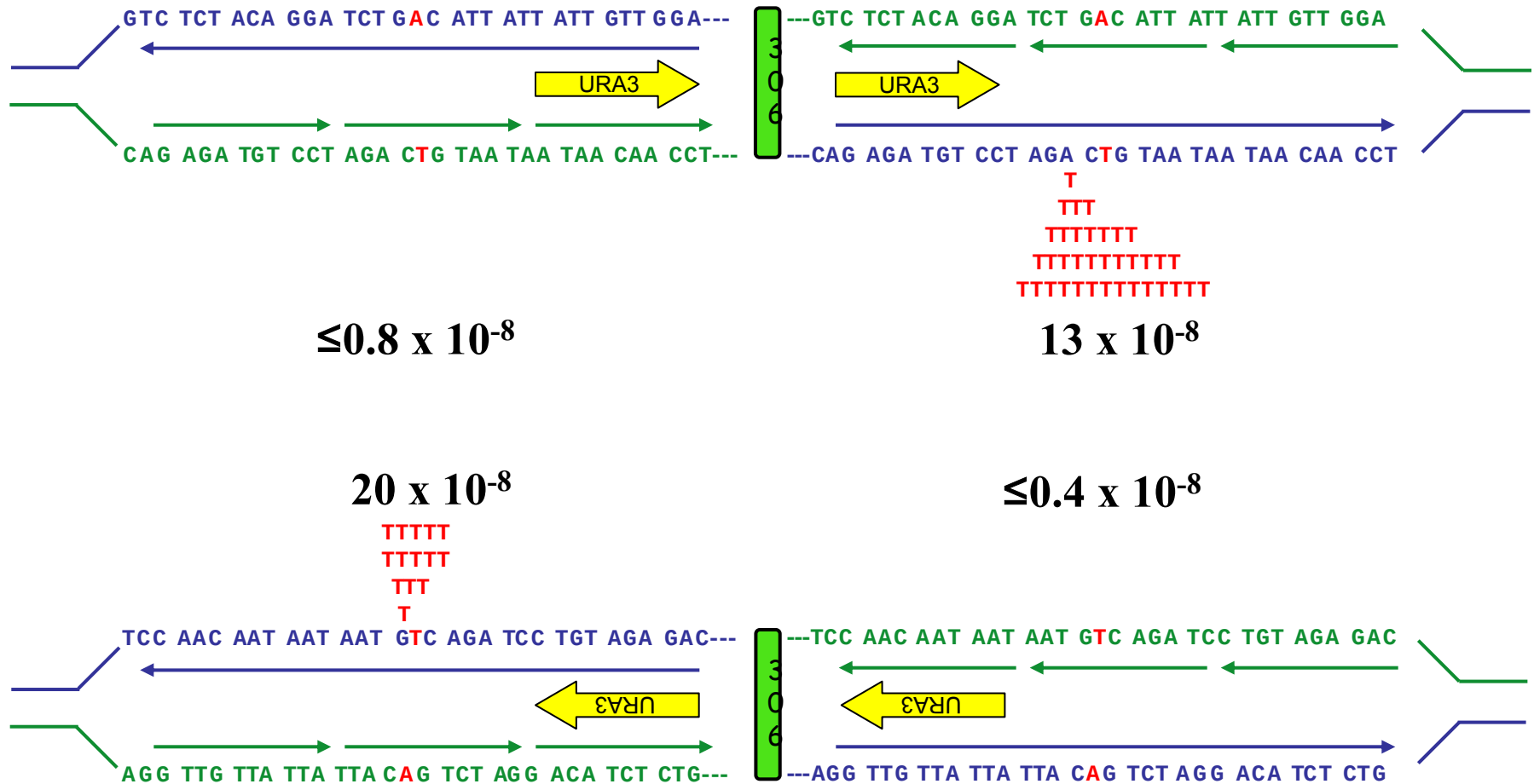
Poloumienko *et al.*, *Molec. Cell. Biol.* 12, 3317 (2001)



Replication forks travel at a similar rate from 305, 306 and 307



Bias in Mutagenesis at Position 686 by Location and Orientation



A similar asymmetric pattern is seen at template position 279.

Inference:

Pol ϵ participates in leading strand replication

Questions: What about Pol δ ?

What about Pol α ?

What about replication timing? (early v. late)

What about chromosomal location?

(telomere, centromere)

What about chromatin?

(euchromatin v. heterochromatin)

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