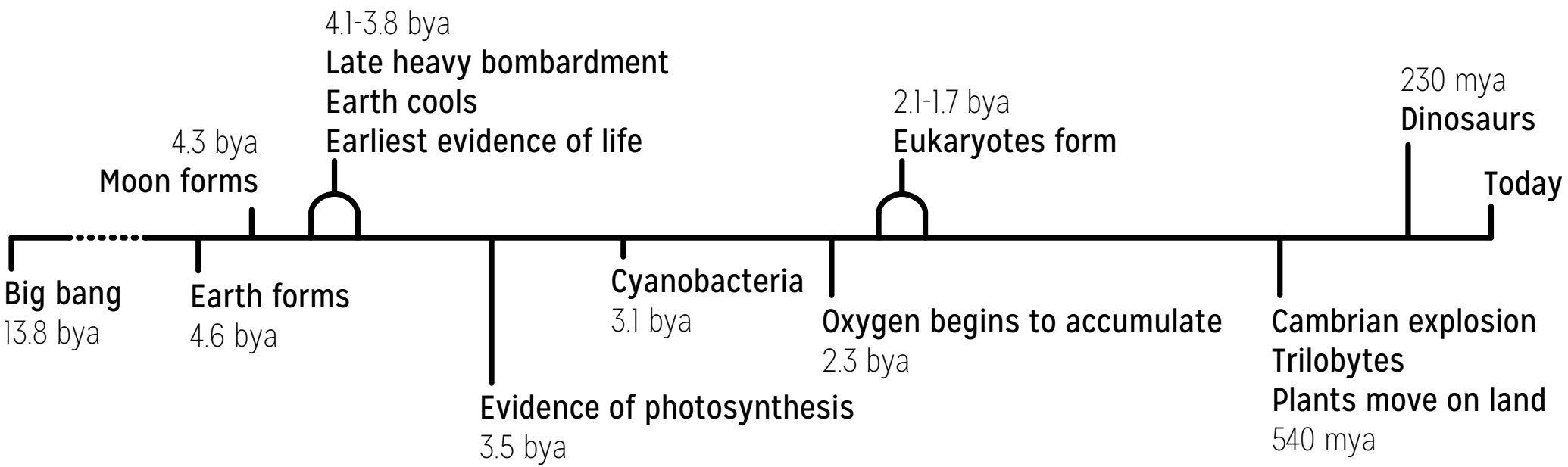
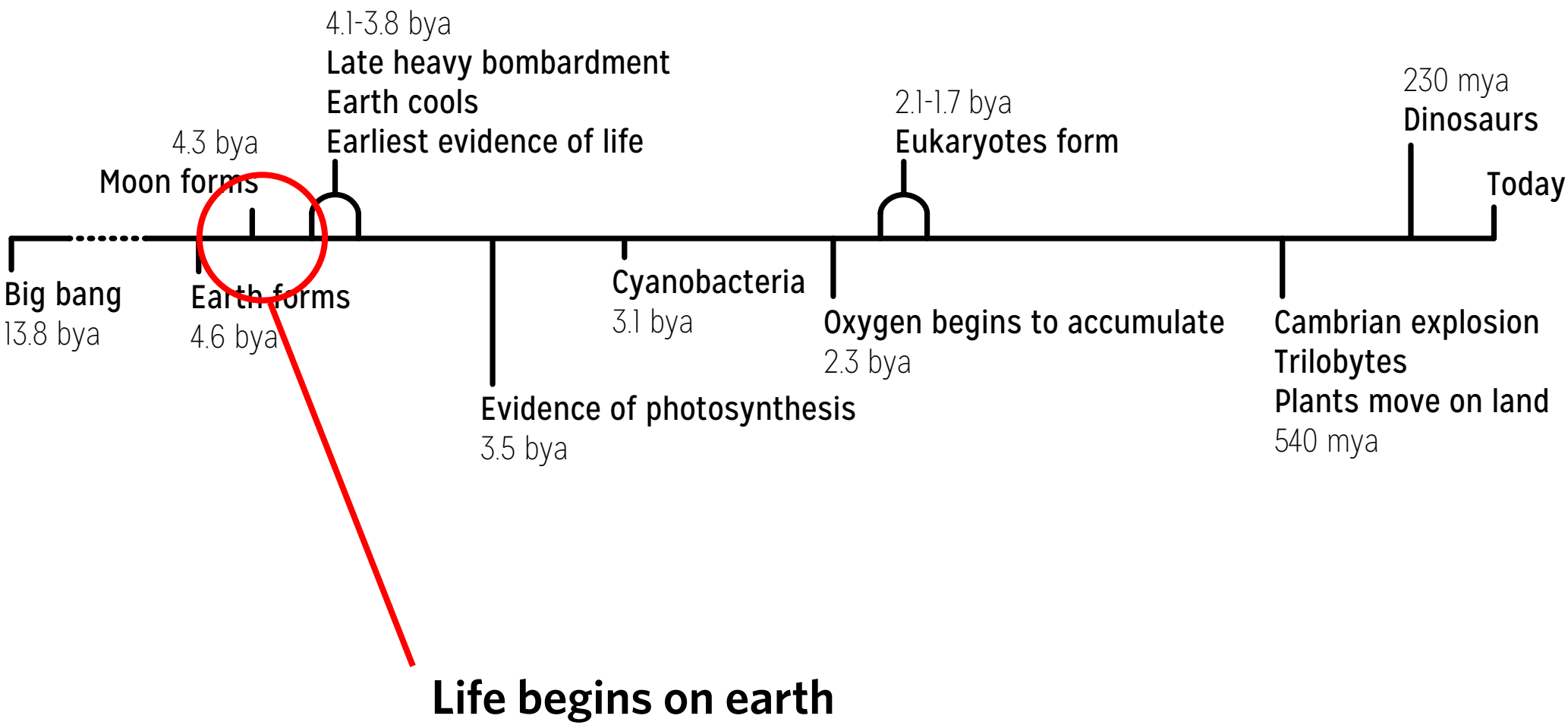
Non-enzymatic replication of sequences containing 4 letters

Noam Prywes
ILASOL
April 11th, 2018

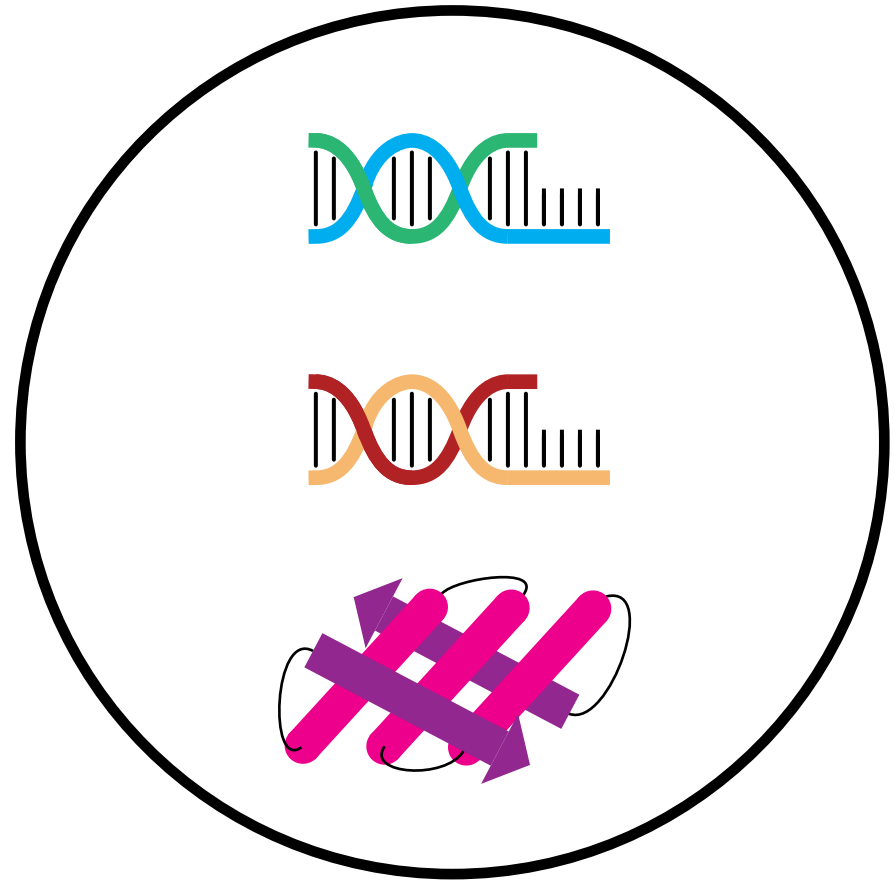
History of the World



History of the World

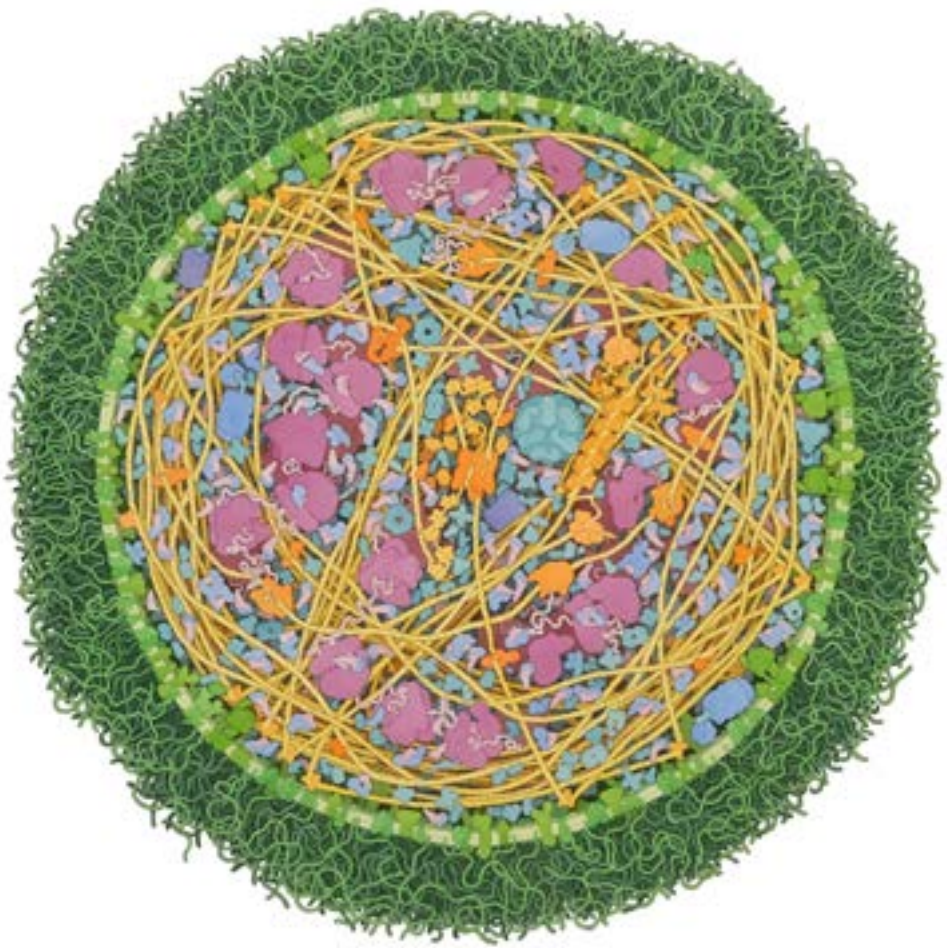


“Modern” life

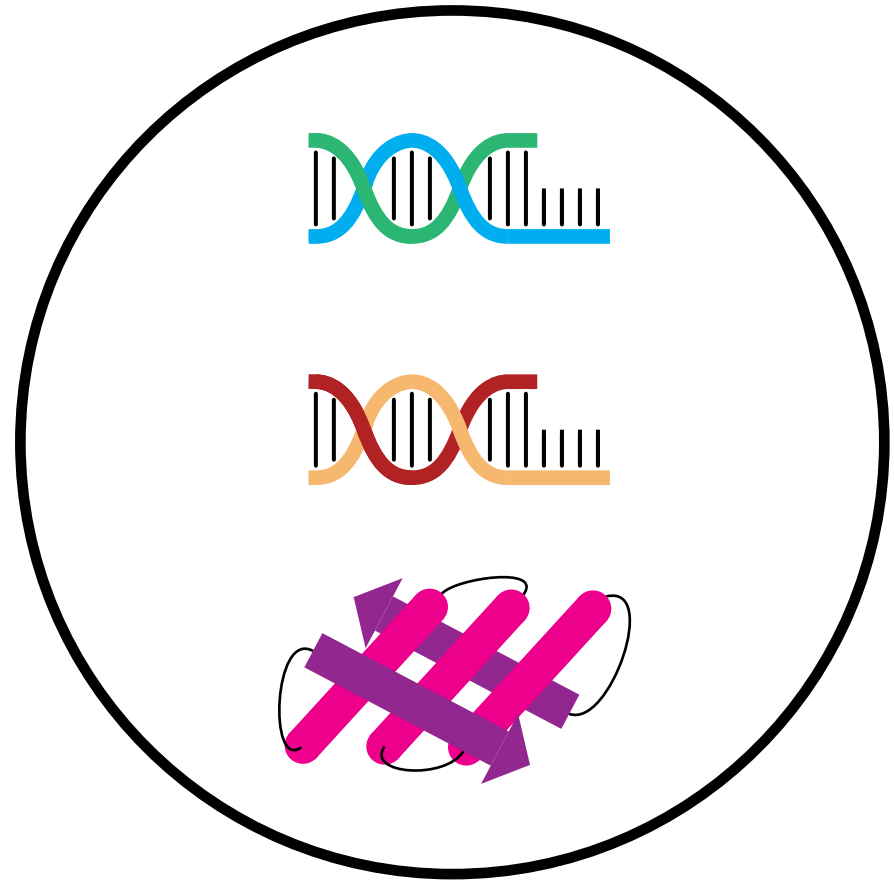


Crick, F. Central dogma of molecular biology. Nature 227, 561–563 (1970).

“Modern” life

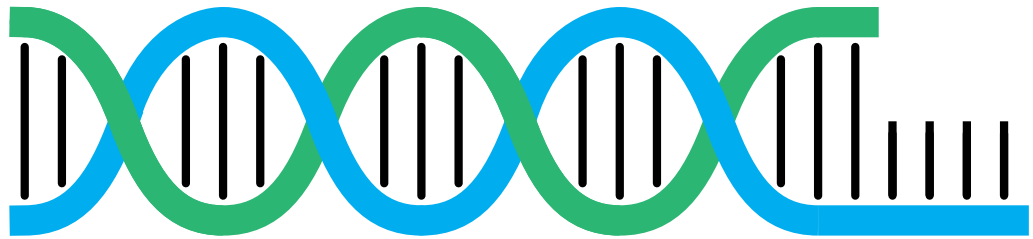


Mycoplasma mycoides



David Goodsell, 2011

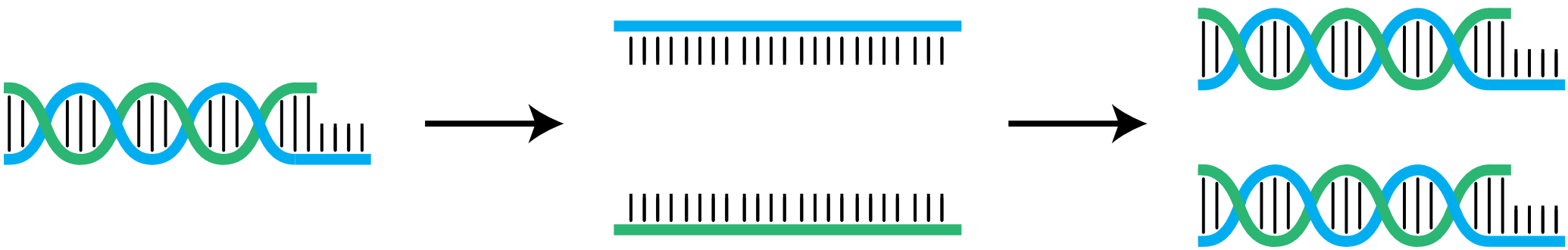
Life is based on information



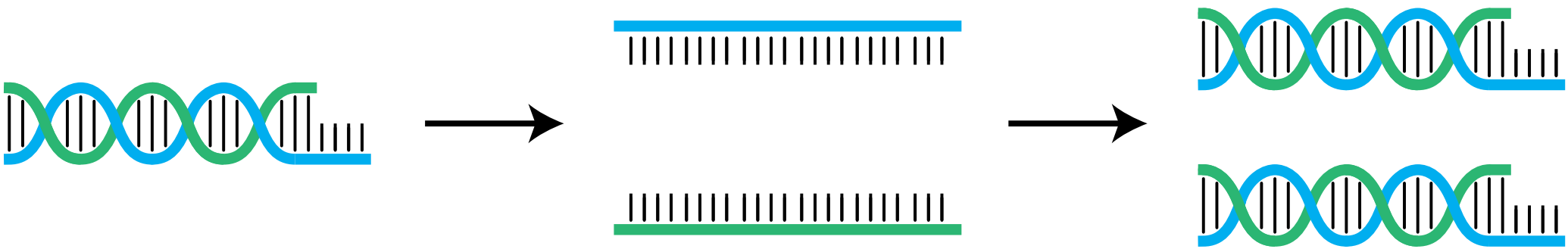
ATGTCCACATTG...

100100011010...

...information that must be copied

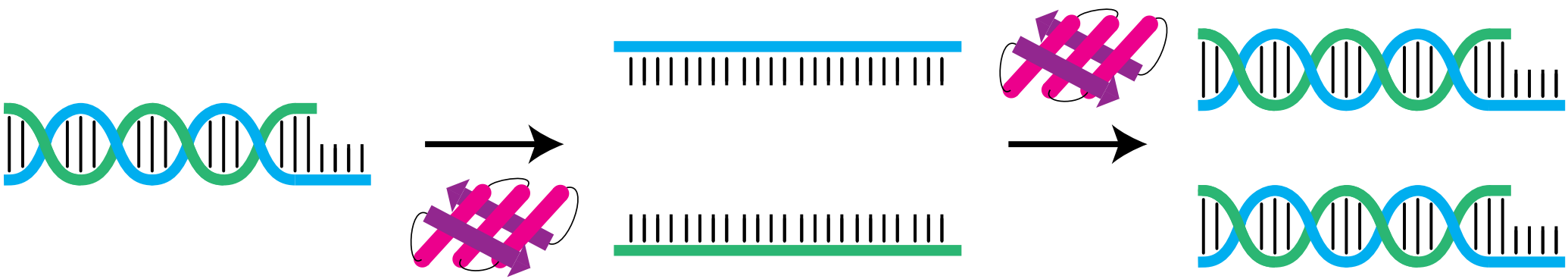


DNA replication



SCRIPTORIUM MONK AT WORK. (From *Lacroix.*)

DNA replication



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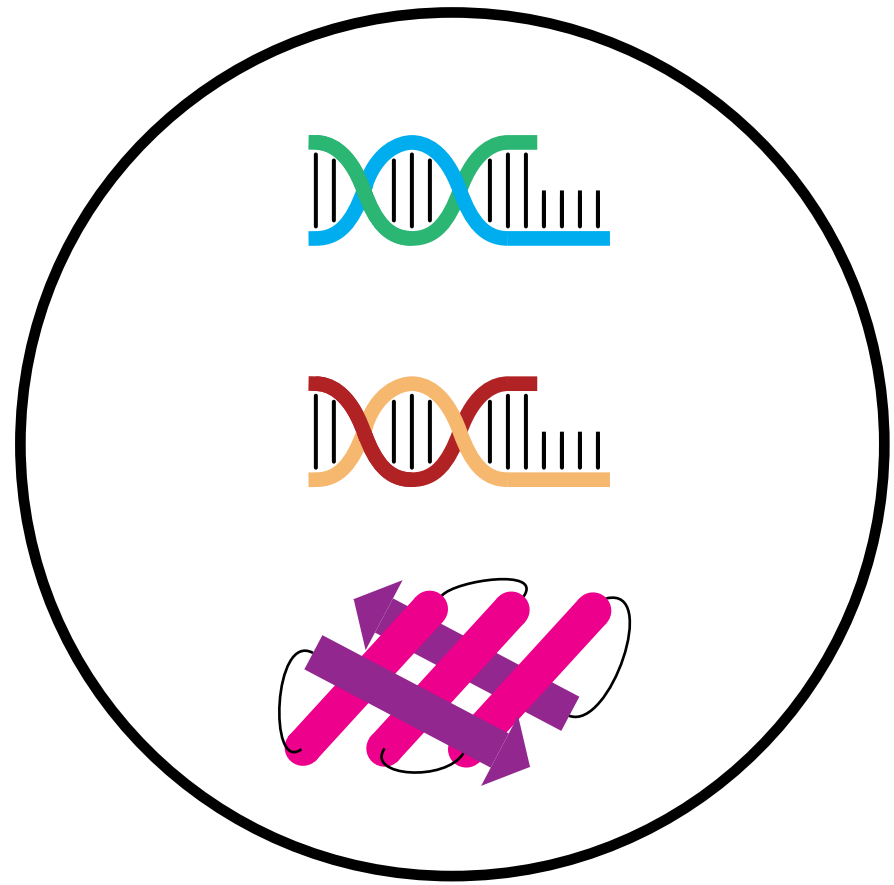
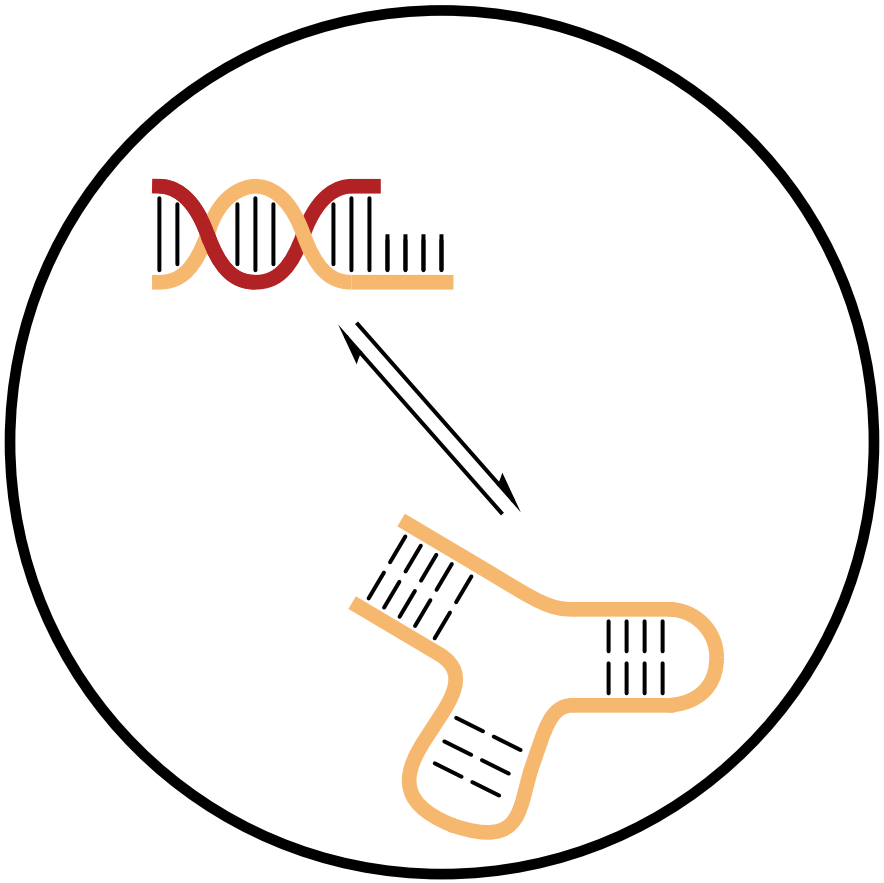
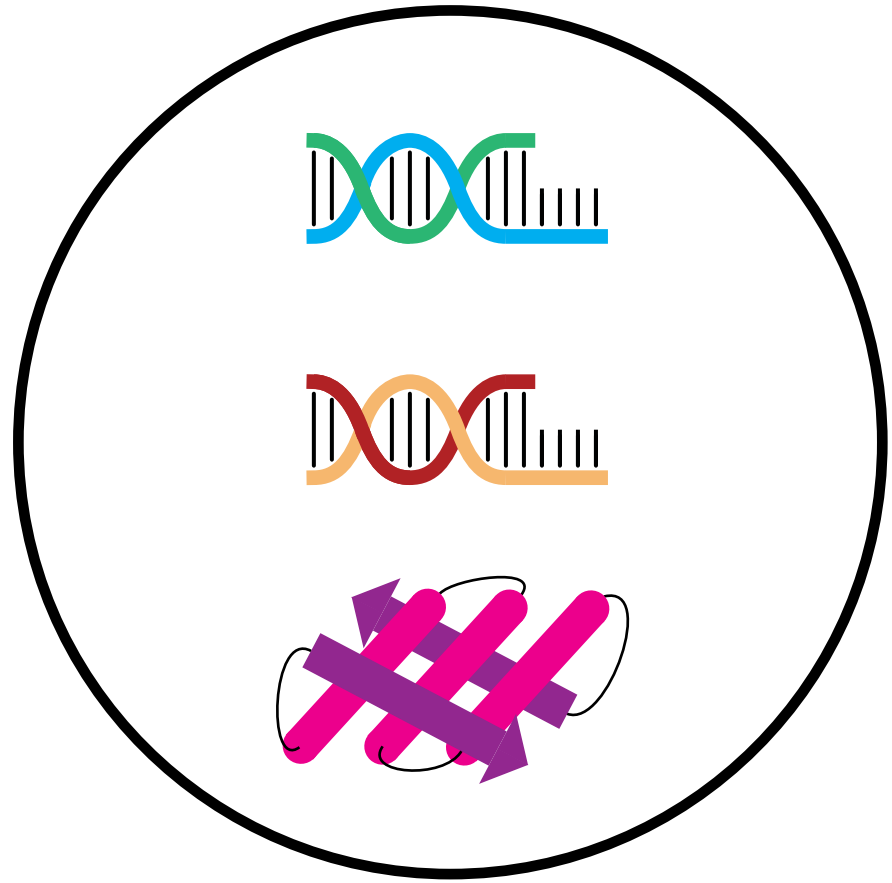
“Modern” life



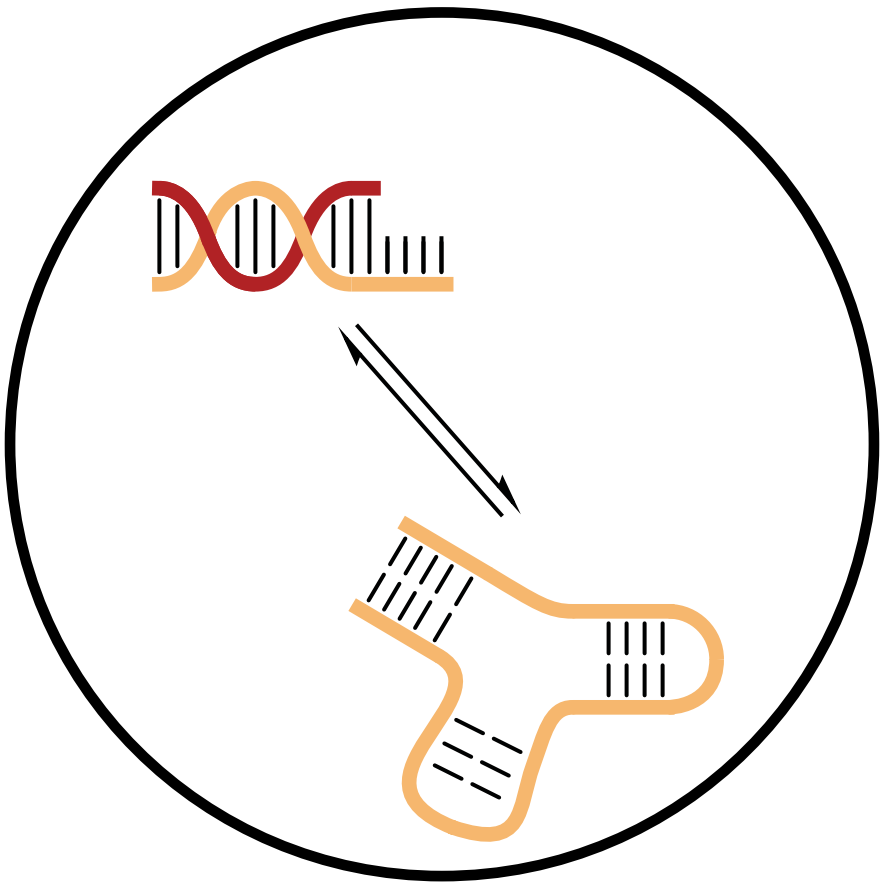
The RNA World

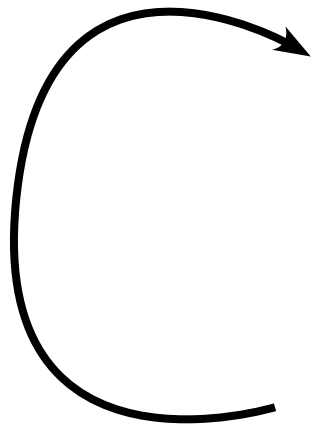


“Modern” life



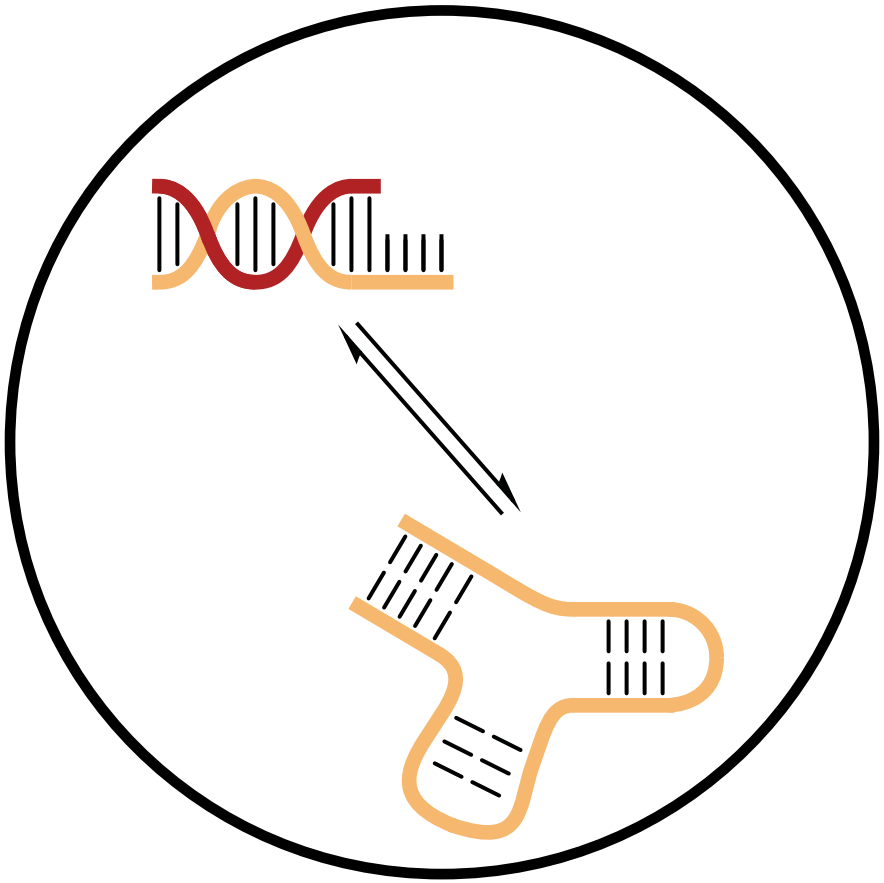
The RNA World

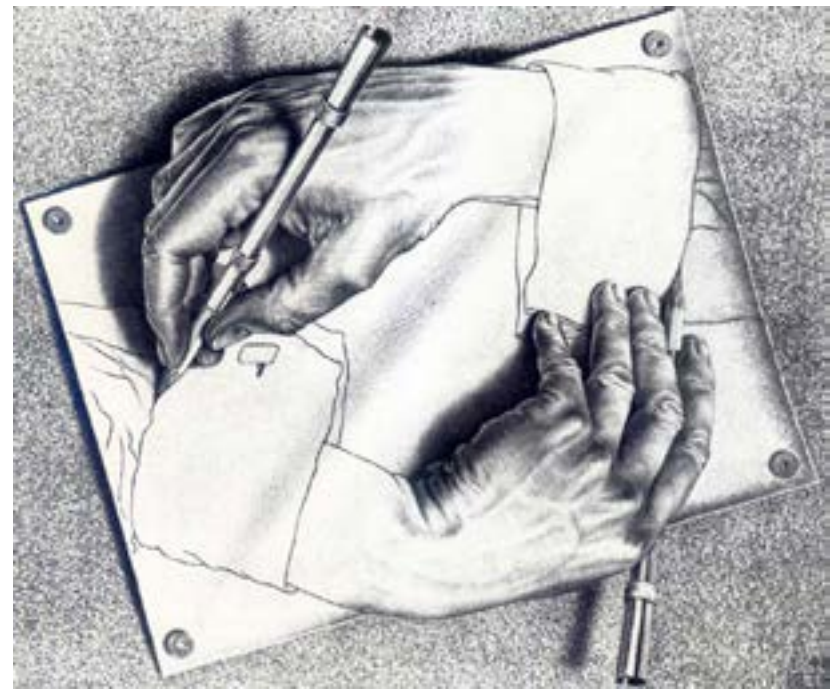




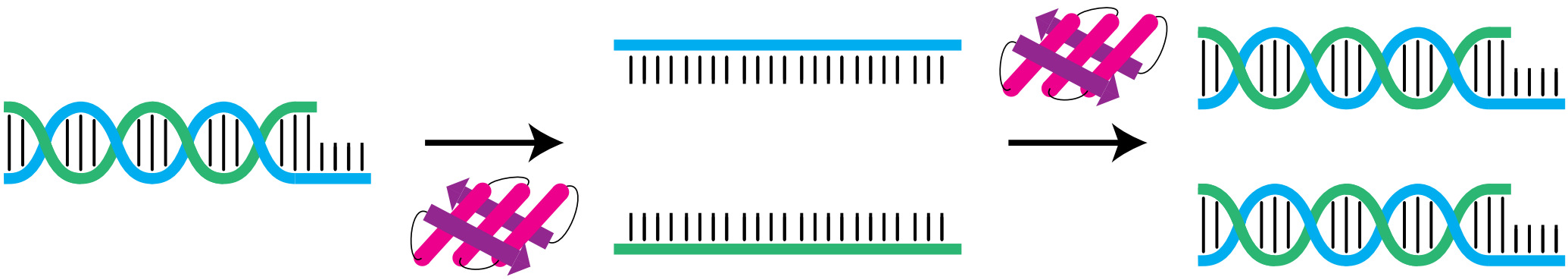
SCRIPTORIUM MONK AT WORK. (From *Lacroix.*)

The RNA World

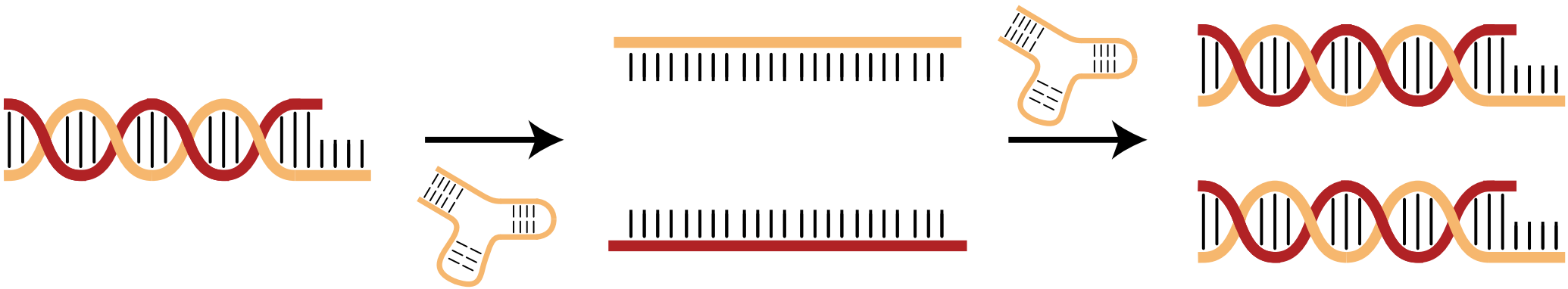




How was information replicated in the RNA world?



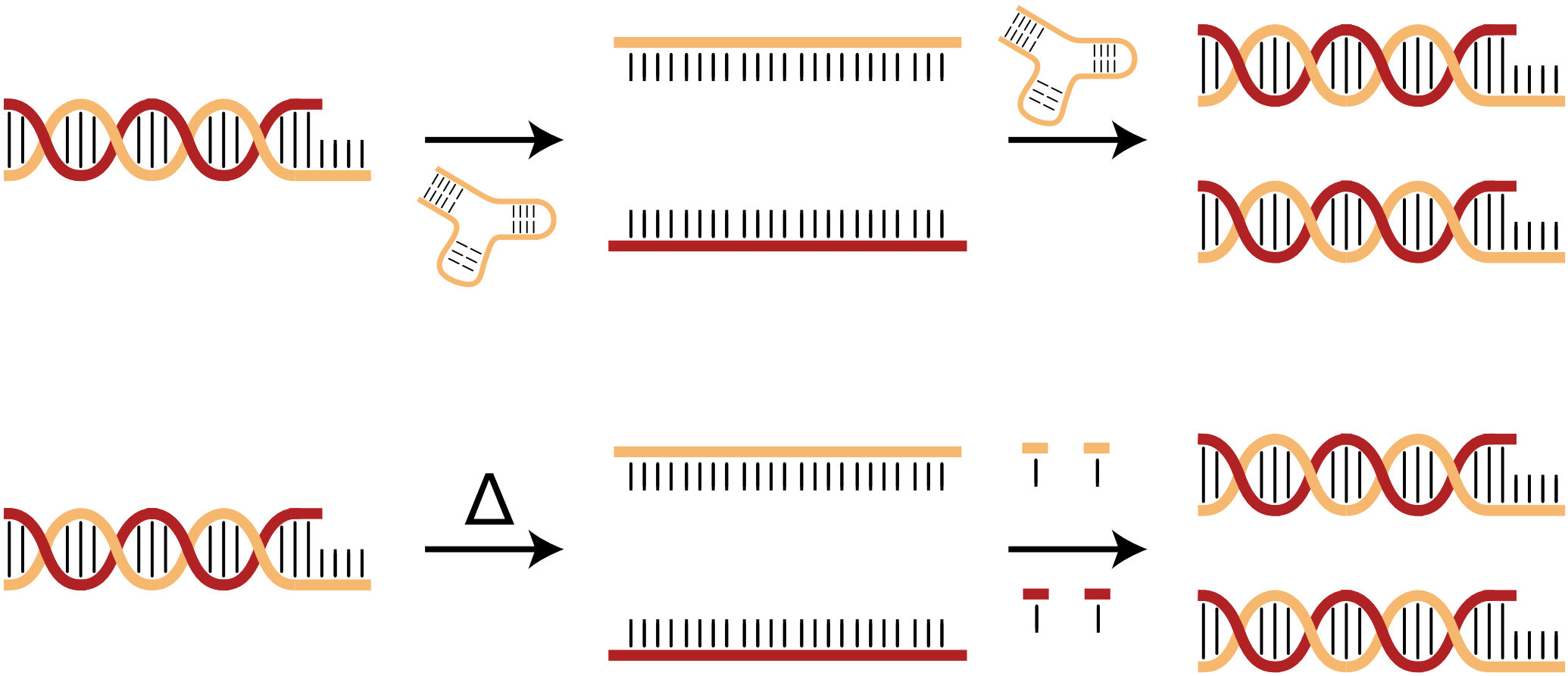
How was information replicated in the RNA world?



How do we get ribozymes in the first place?

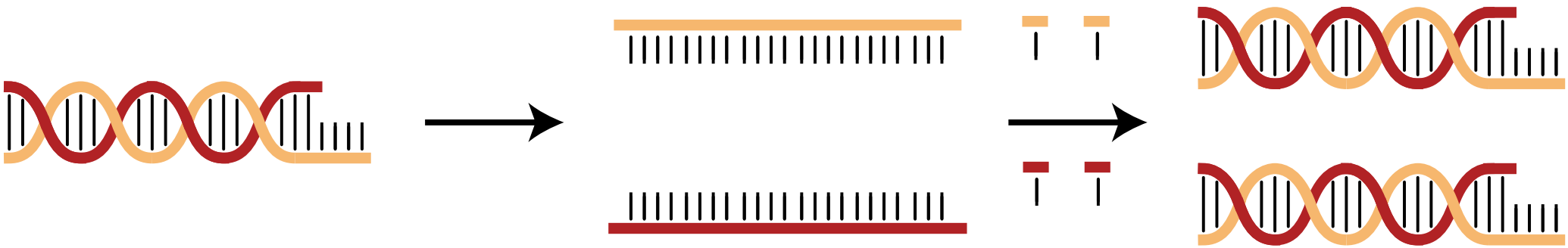
Can some other copying mechanism produce the first ribozymes?

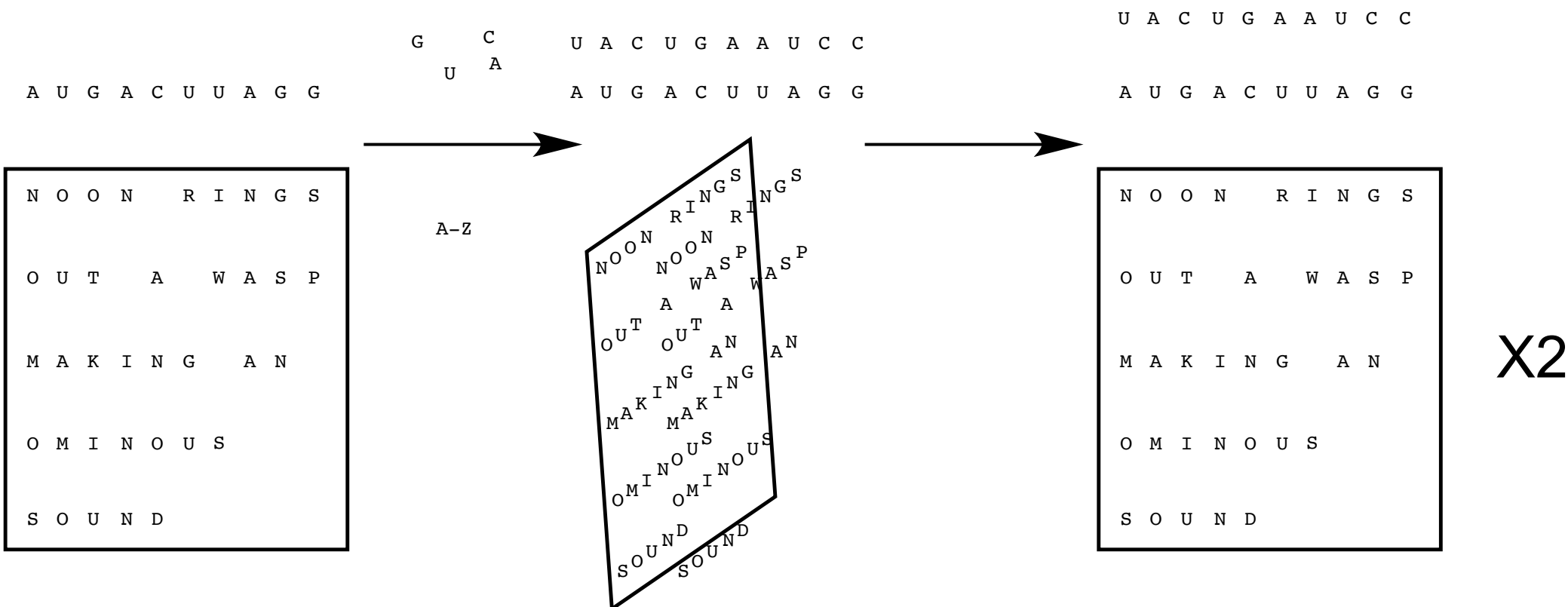
Enzymatic vs. nonenzymatic replication



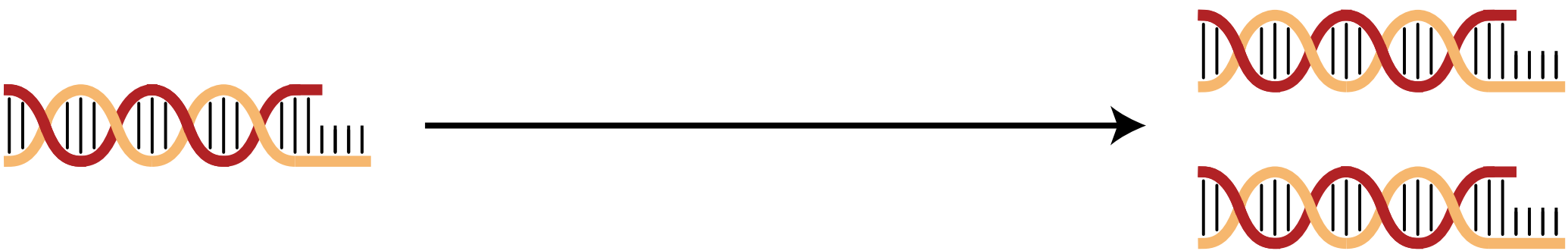
Schramm, G., Grötsch, H. & Pollmann, W. Nicht-enzymatische Synthese von Polysacchariden, Nucleosiden und Nucleinsäuren. Angew. Chem. 73, 619–619 (1961).

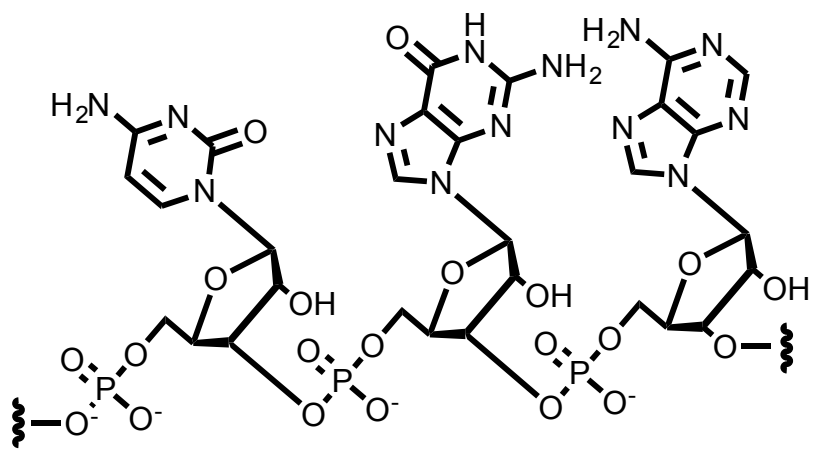
Nonenzymatic replication



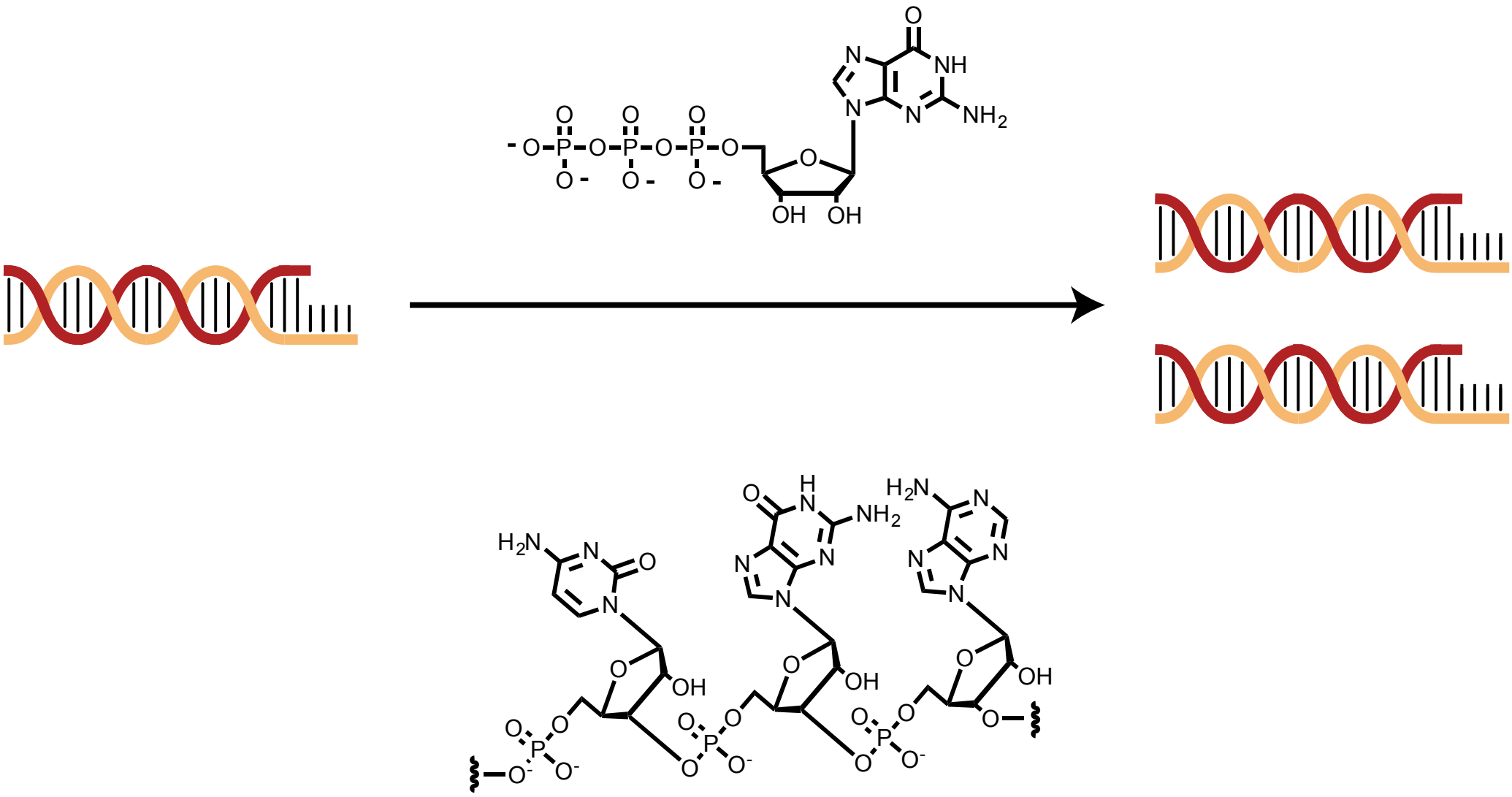


NTPs

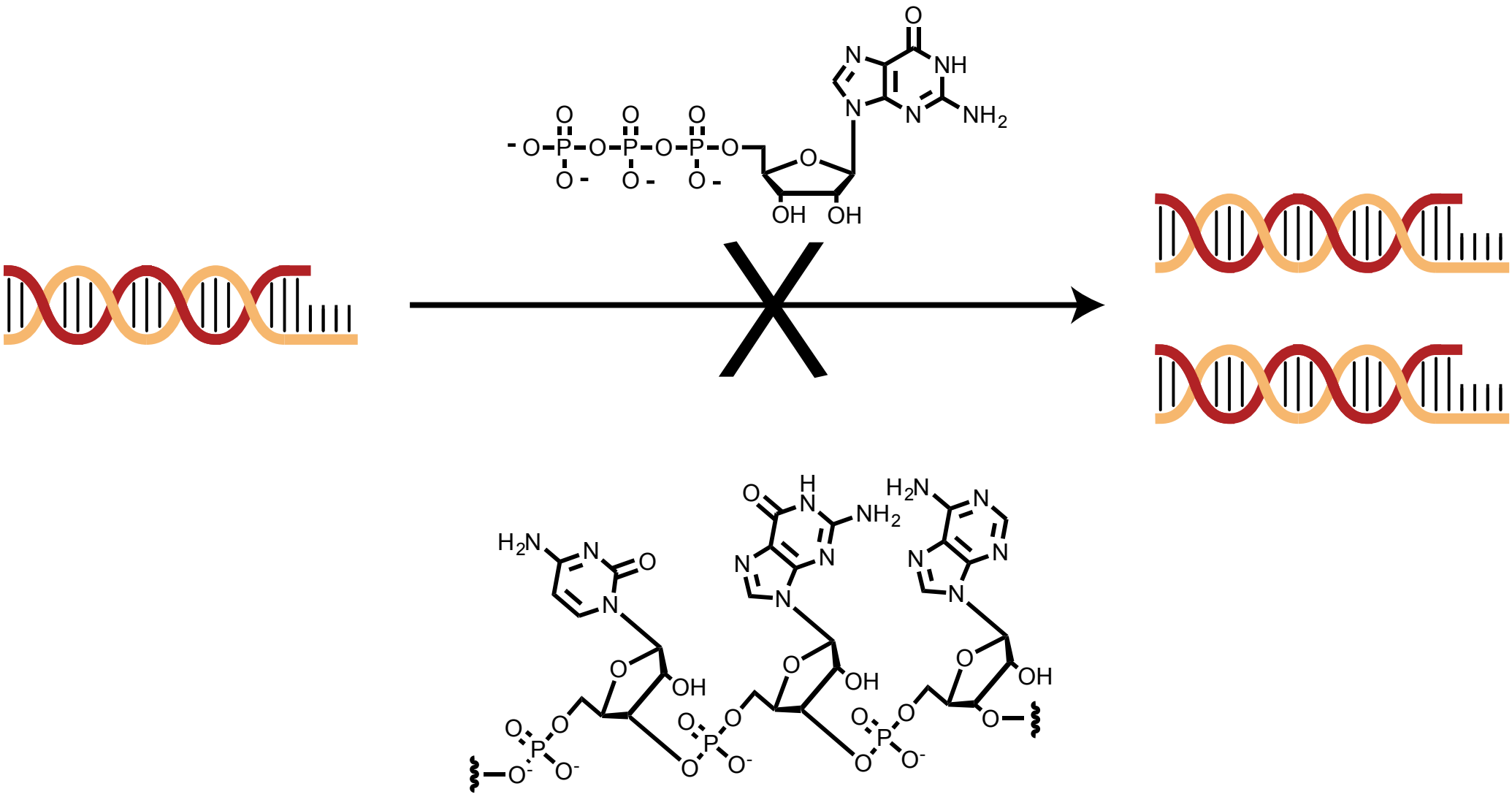




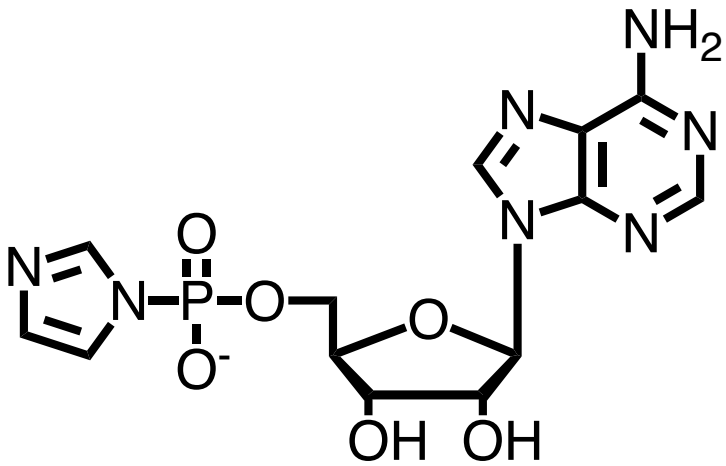
NTPs

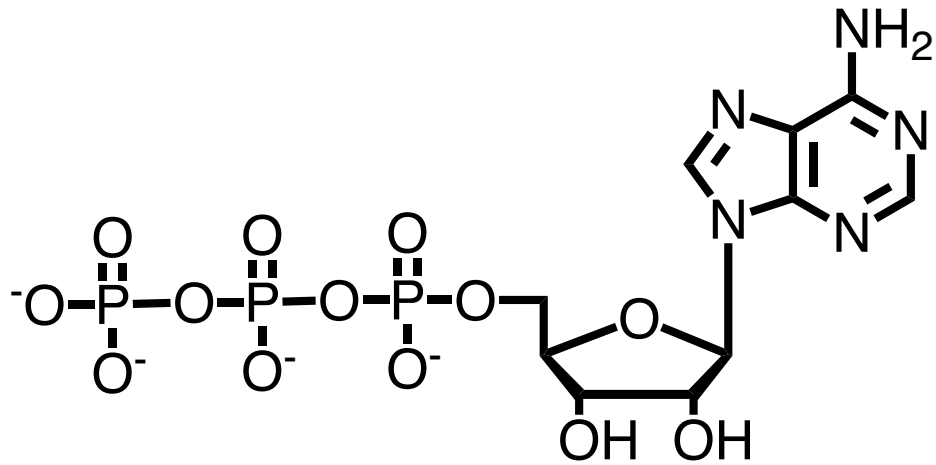


NTPs don't work



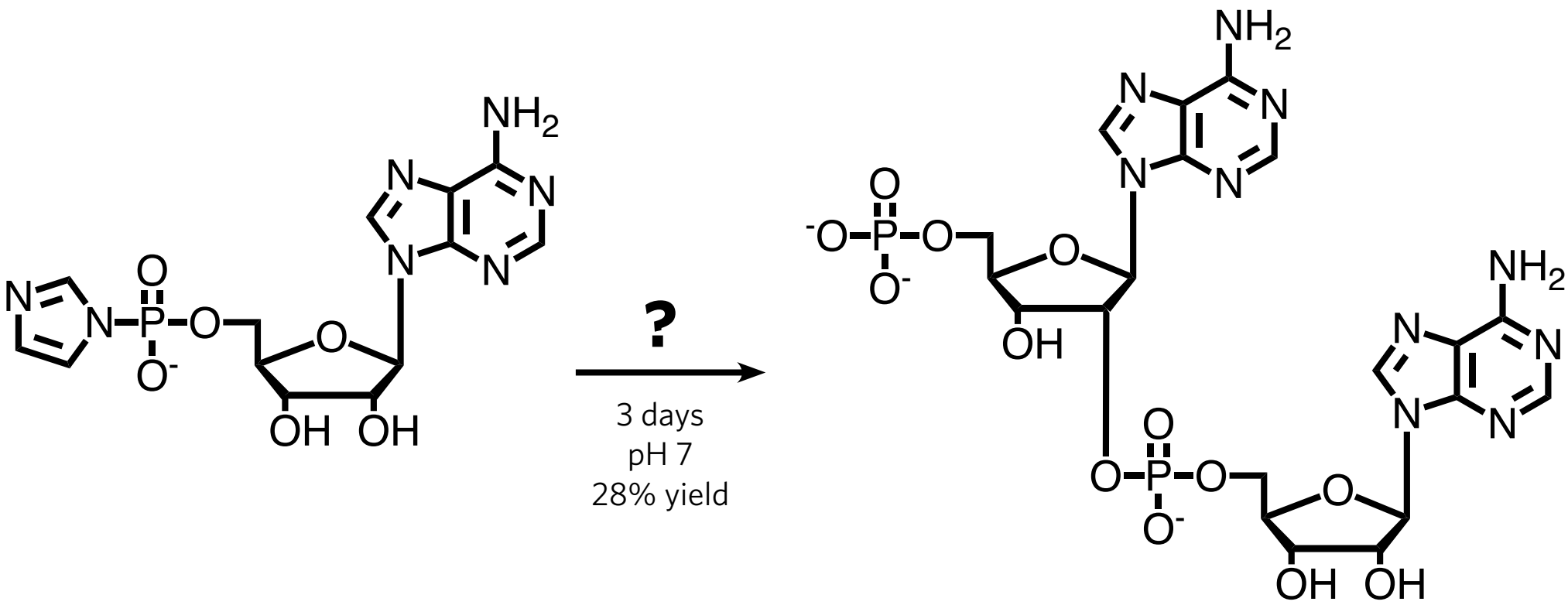
Heterocycle activation





Weimann, B. J., Lohrmann, R., Orgel, L. E., Schneider-Bernloehr, H. & Sulston, J. E. Template-directed synthesis with adenosine-5’-phosphorimidazolide. Science 161, 387 (1968).

Heterocycle activation



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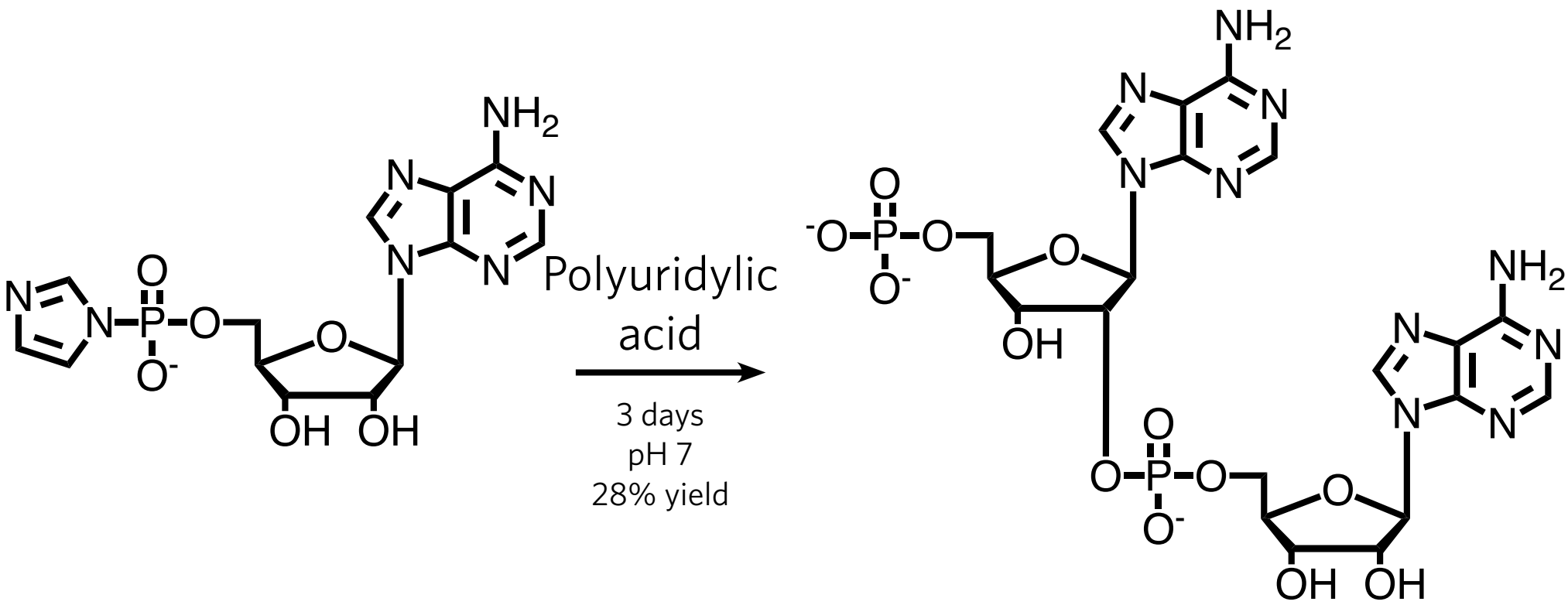

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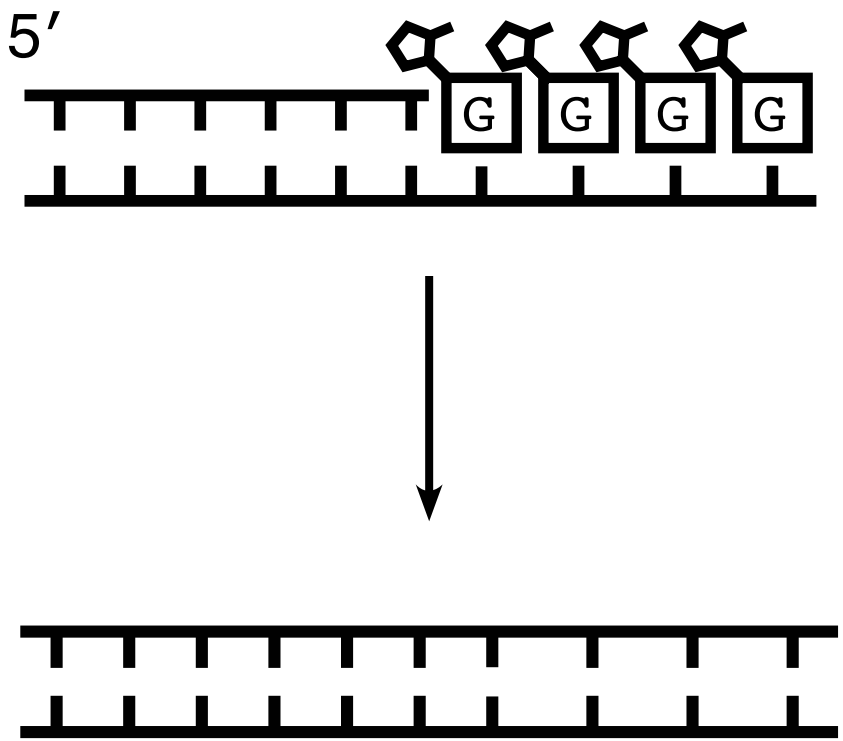
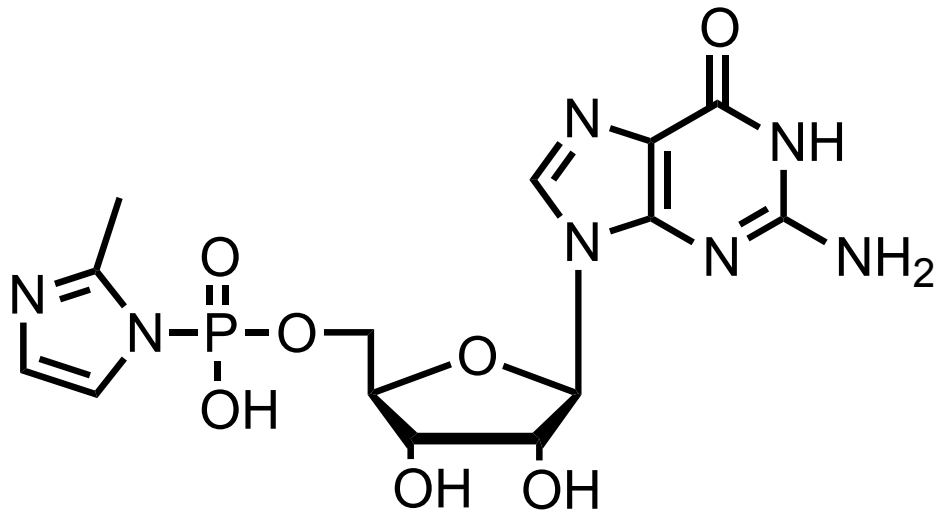
July 1968, almost exactly 50 years ago

Heterocycle activation





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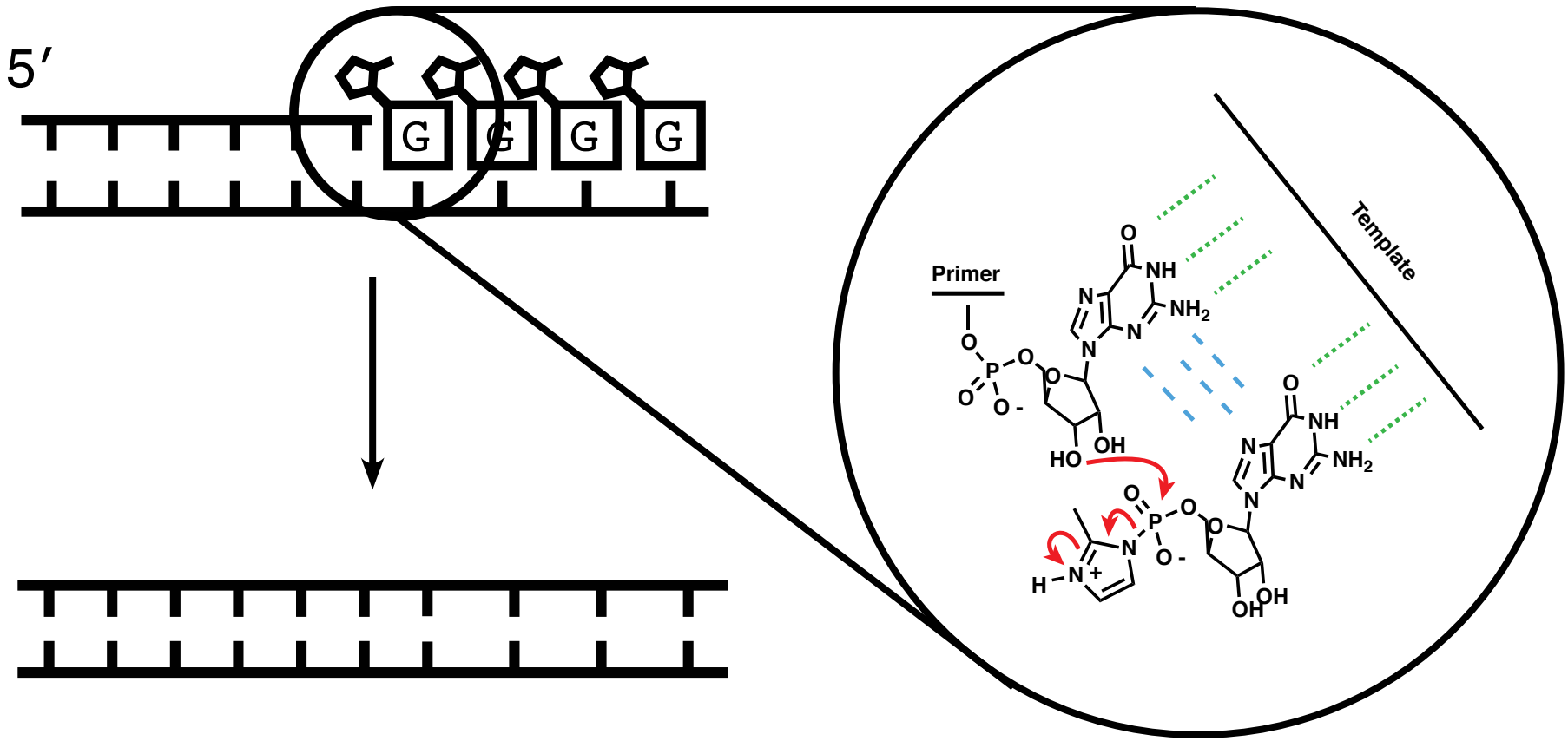
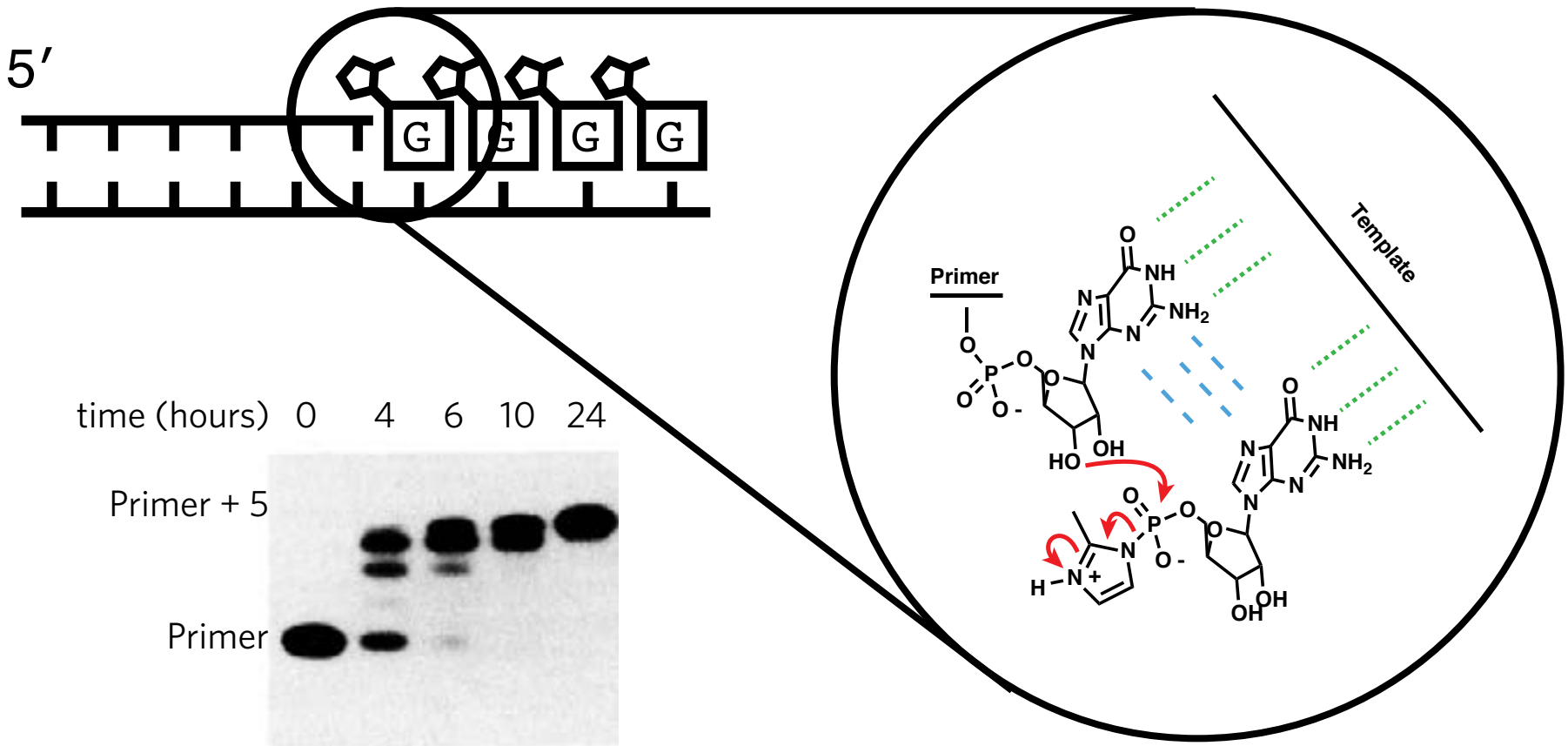
Wu, T., Orgel, L.E. Nonenzymic template-directed synthesis on oligodeoxycytidylate sequences in hairpin oligonucleotides. J Am Chem Soc 114, 317–322 (1992).

Phosphoryl transfer



Wu, T., Orgel, L.E., 1992. Nonenzymic template-directed synthesis on oligodeoxycytidylate sequences in hairpin oligonucleotides. J Am Chem Soc 114, 317–322.

Primer extension



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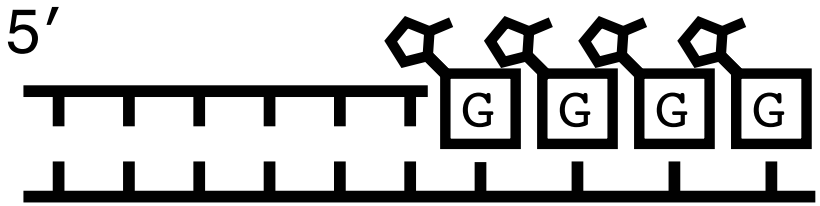
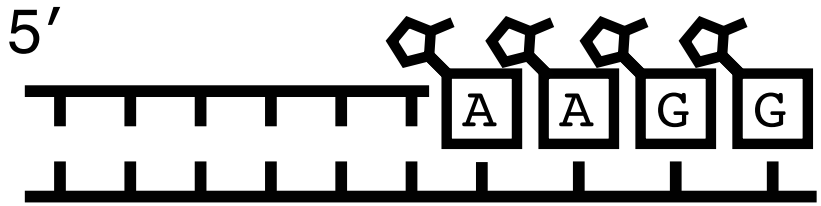
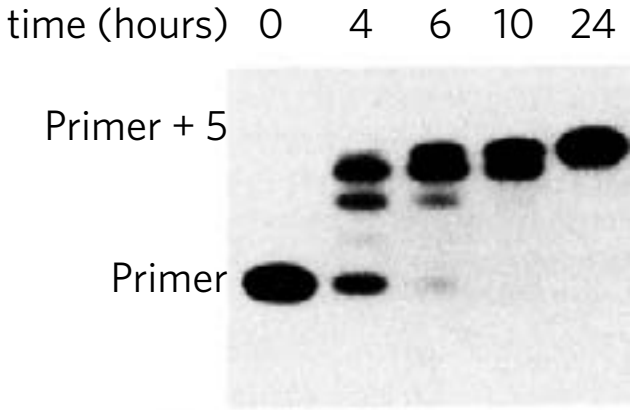
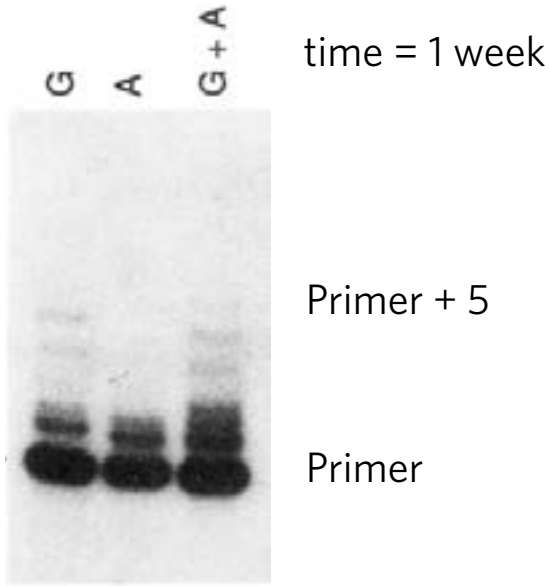
Key challenge: A/U









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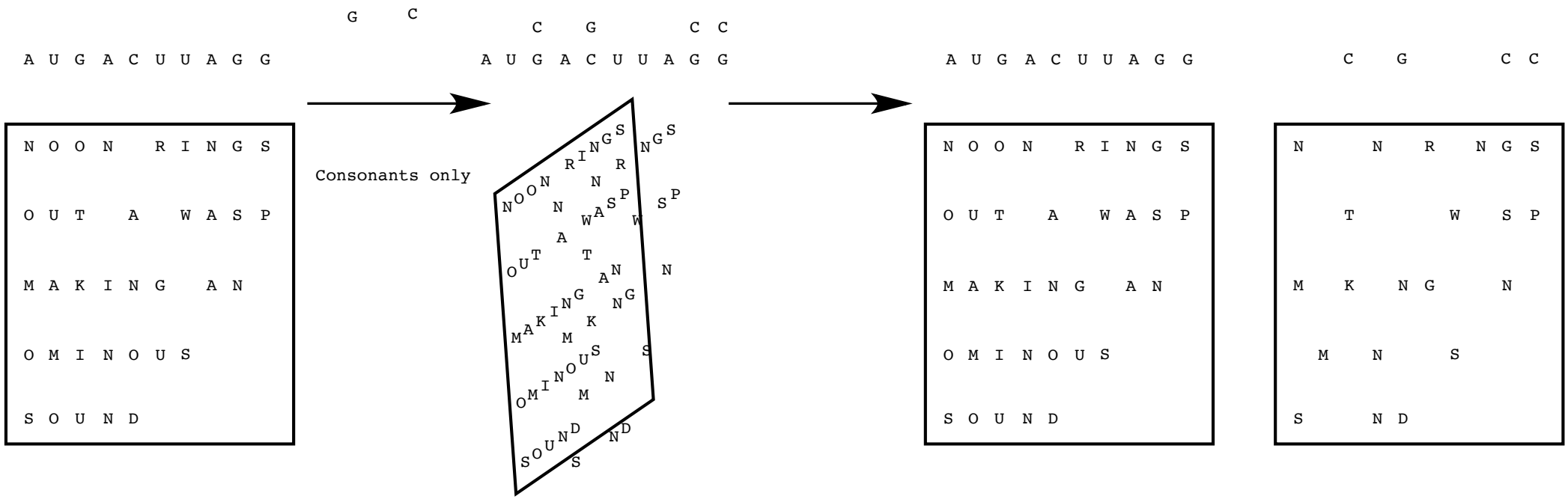
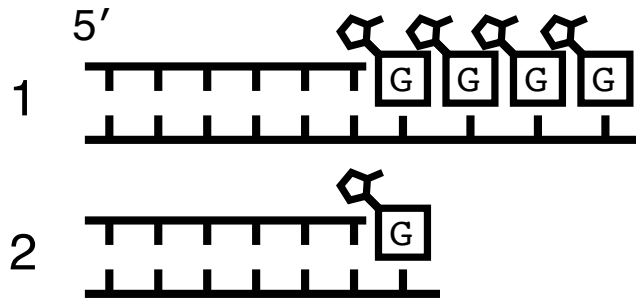
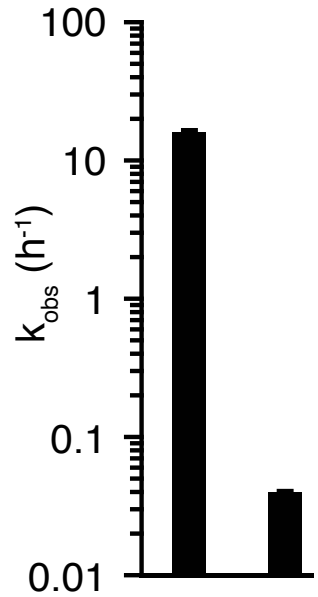
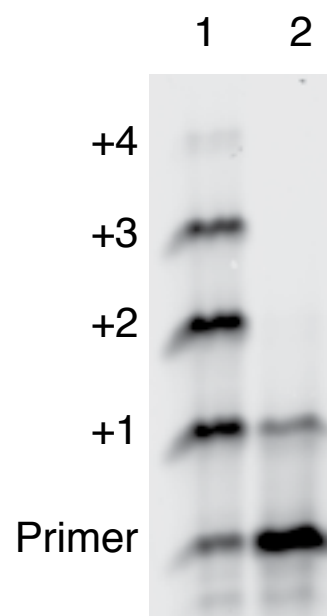


The problem is more than just weaker binding of A and U vs. G and C

Importance of downstream binders

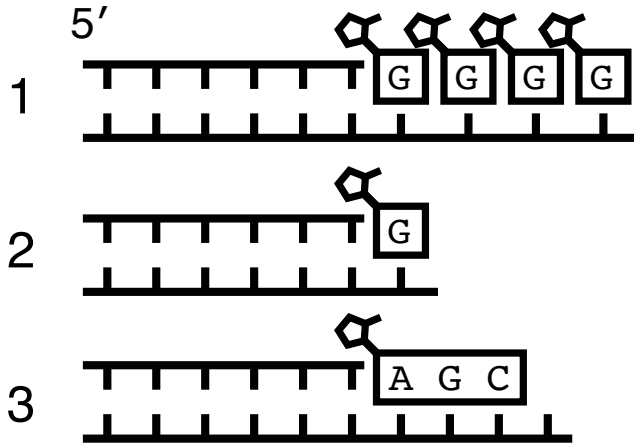


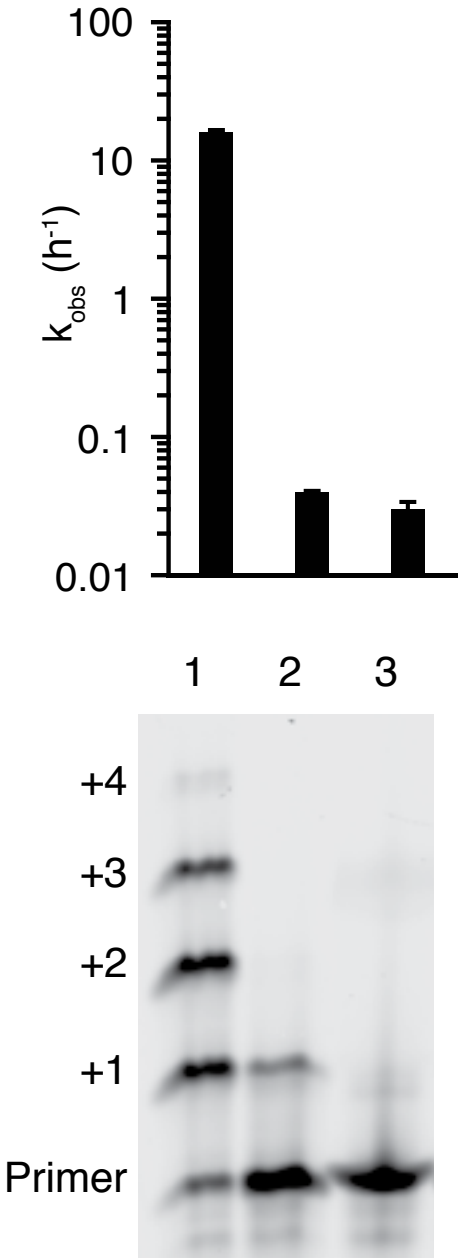




10 minutes

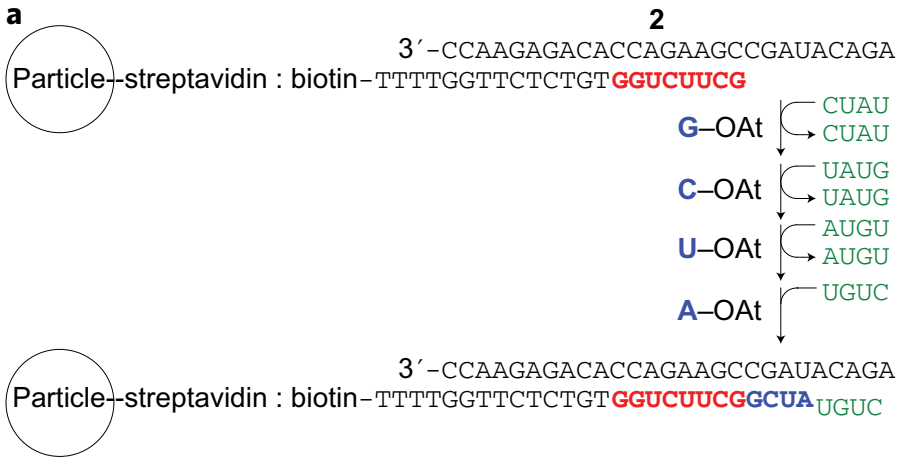
Slow trimer ligation

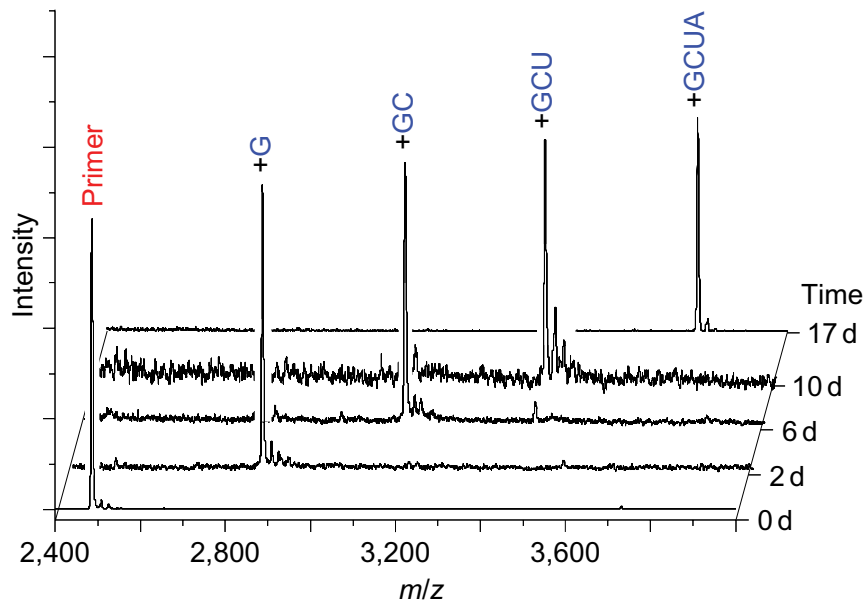




10 minutes

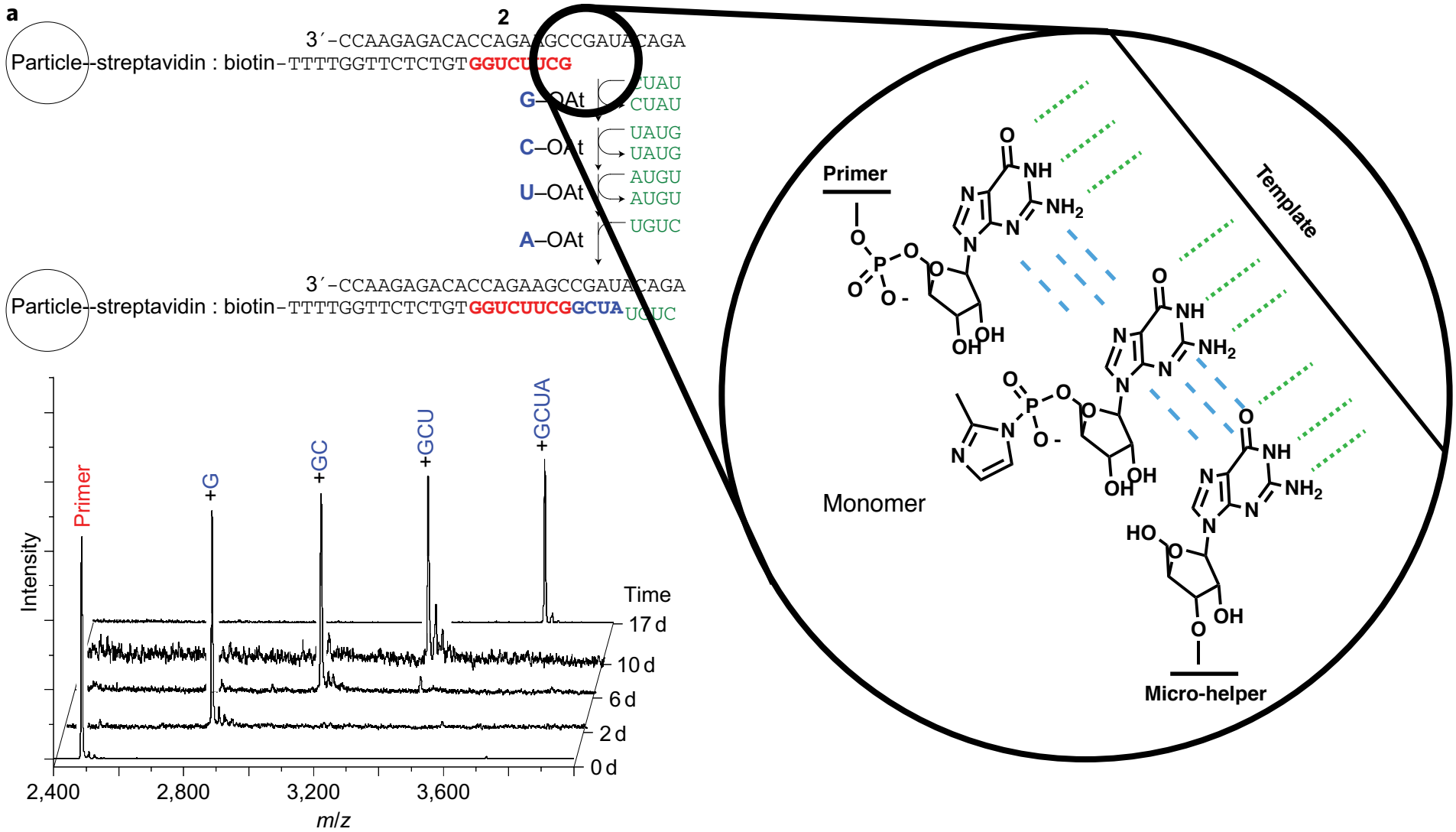
Micro-helpers





Deck, C., Jauker, M., Richert, C., 2011. Efficient enzyme-free copying of all four nucleobases templated by immobilized RNA. Nature Chem 3, 603–608.

Micro-helper mechanism



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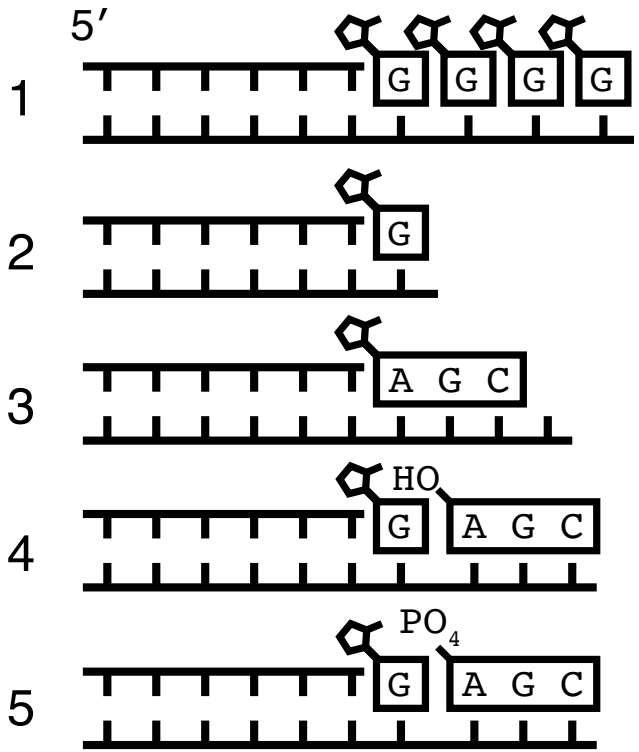
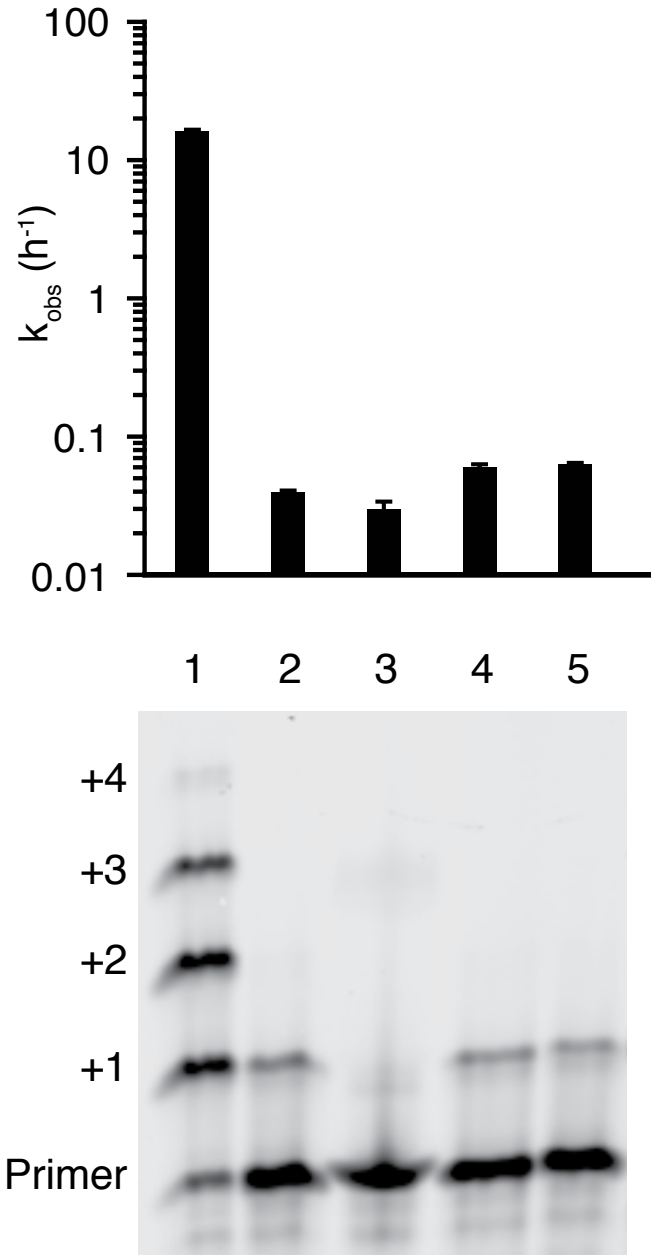
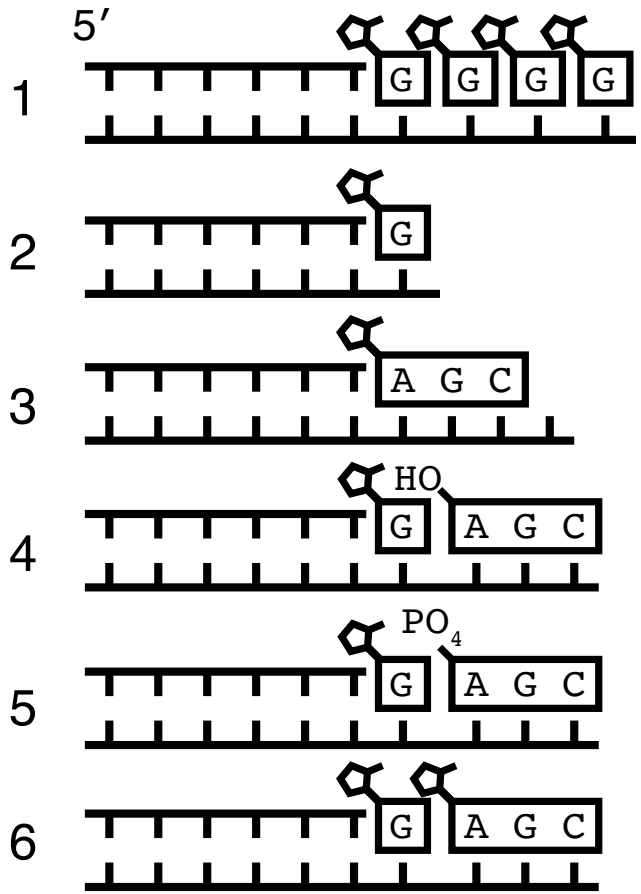
Weak micro-helper assistance

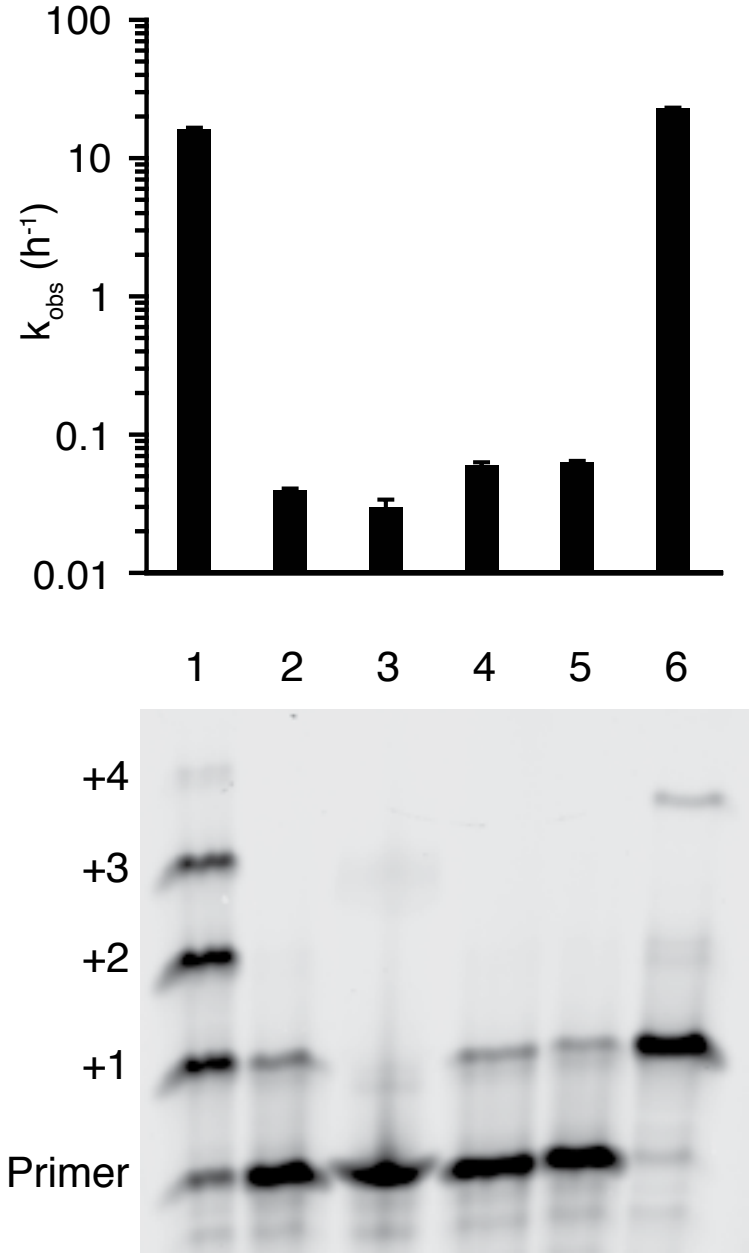




10 minutes

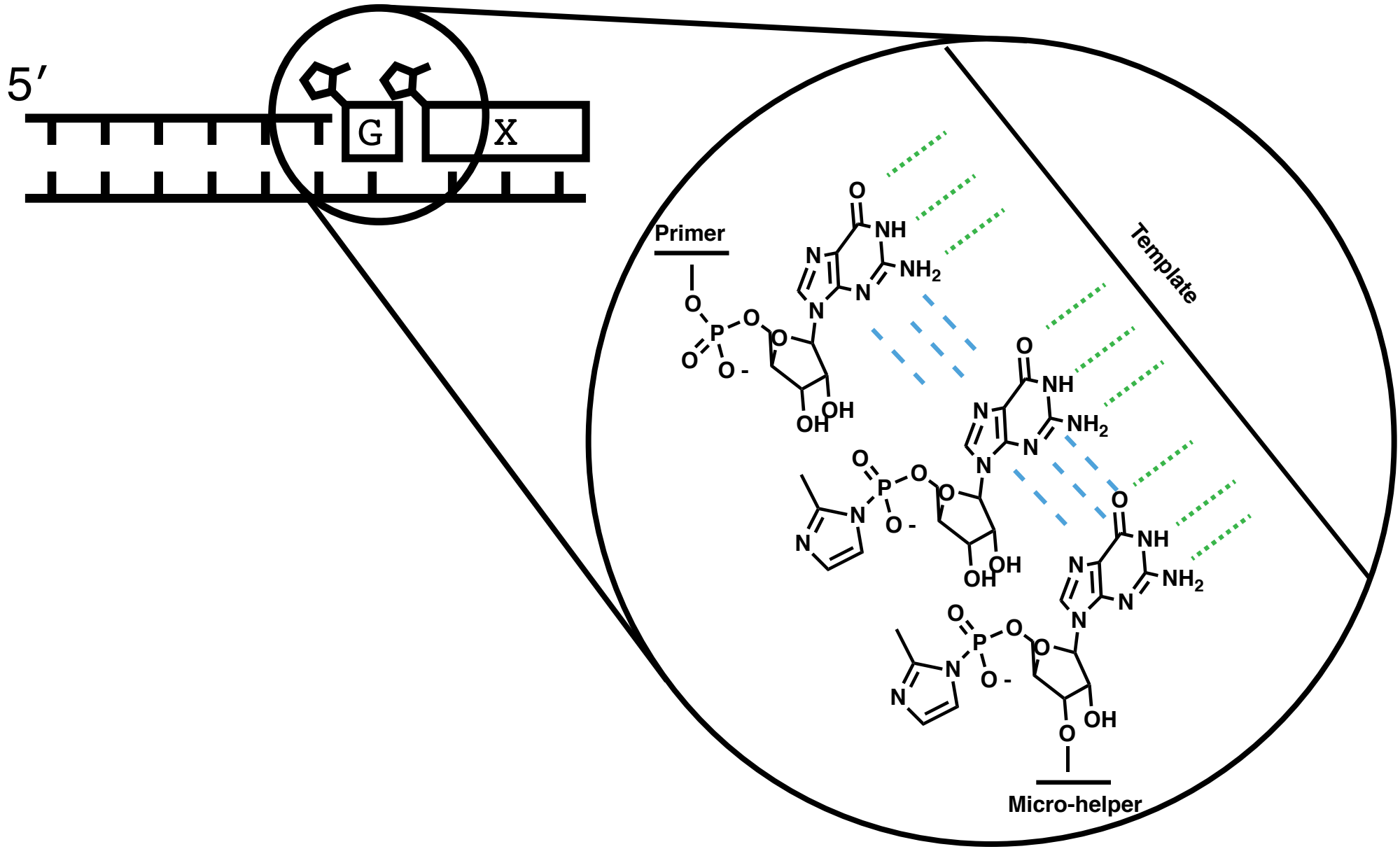
Activated micro-helper



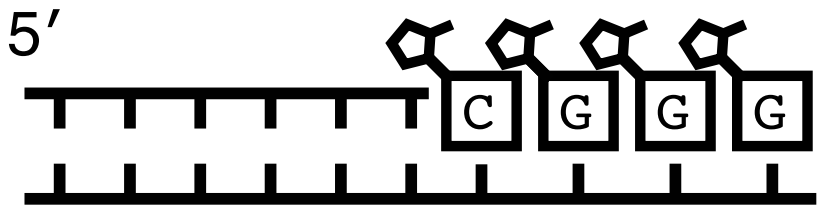


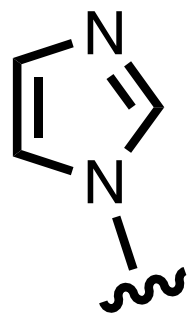
10 minutes

Activated micro-helper

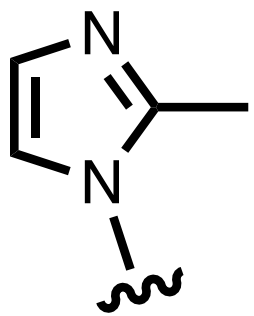


Leaving group effect

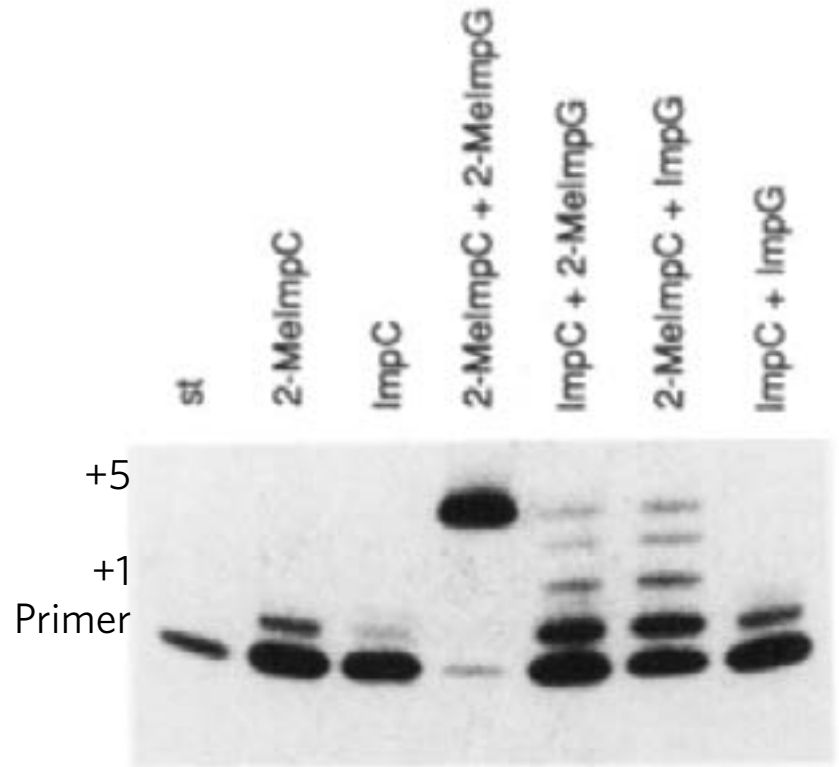




Imidazole

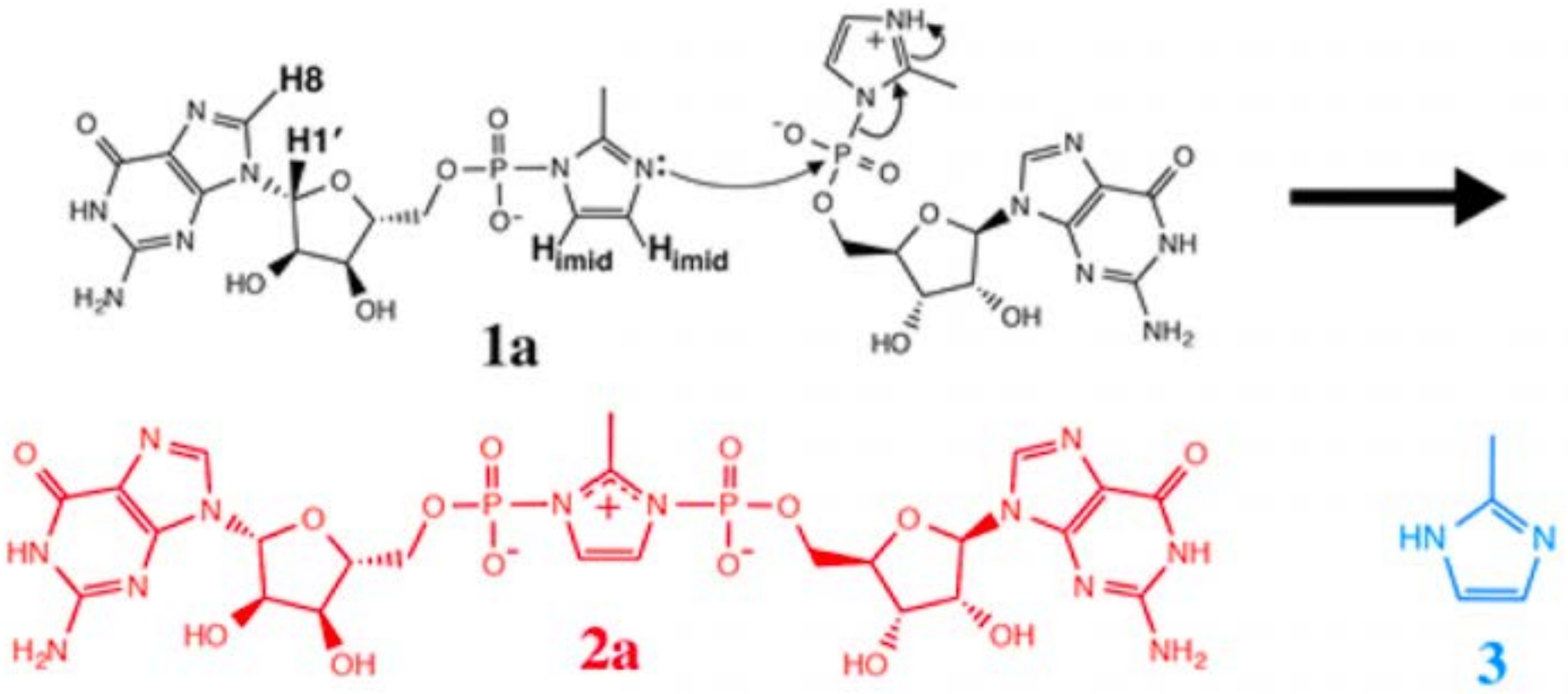


2-Methylimidazole



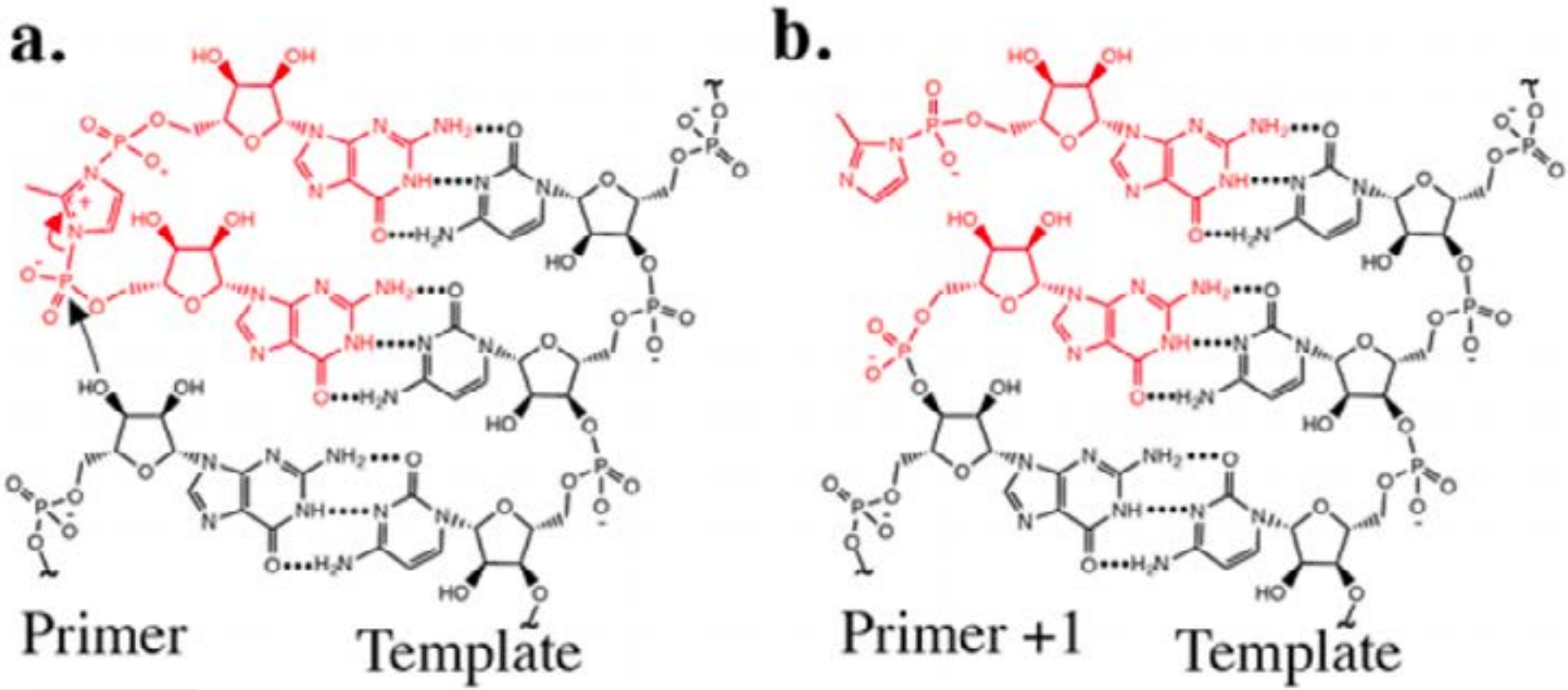
Wu, T., Orgel, L.E., 1992. Nonenzymic template-directed synthesis on oligodeoxycytidylate sequences in hairpin oligonucleotides. J Am Chem Soc 114, 317–322.

Bridged intermediate



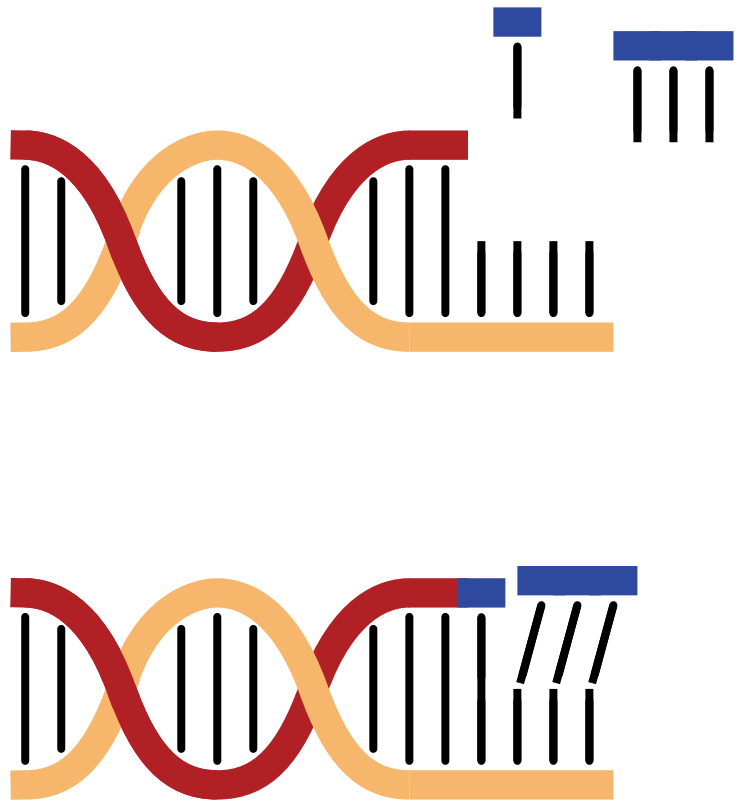
Walton, T, Szostak, JW “A Highly Reactive Imidazolium-Bridged Dinucleotide Intermediate in Nonenzymatic RNA Primer Extension” JACS 2016

Bridged intermediate

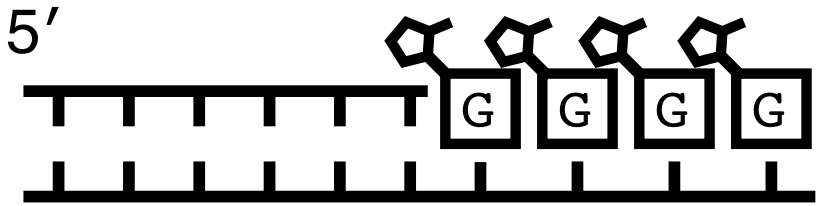


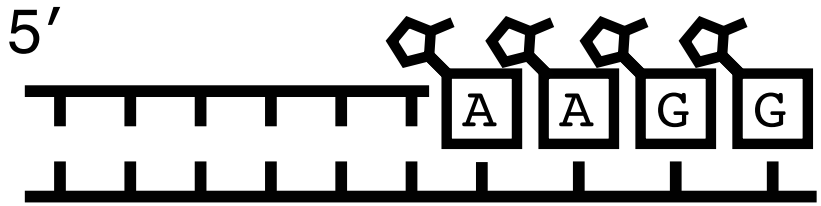
Walton, T, Szostak, JW “A Highly Reactive Imidazolium-Bridged Dinucleotide Intermediate in Nonenzymatic RNA Primer Extension” JACS 2016

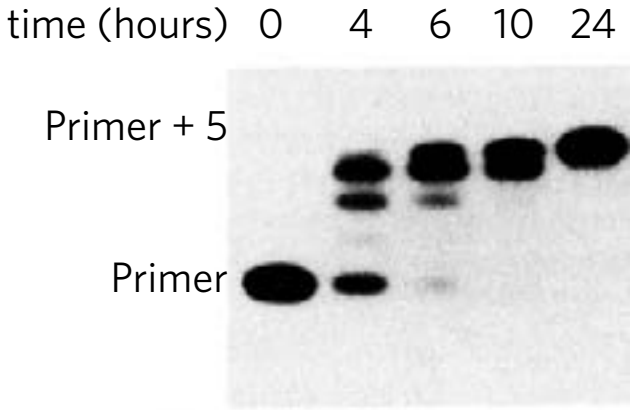
Micro-helpers

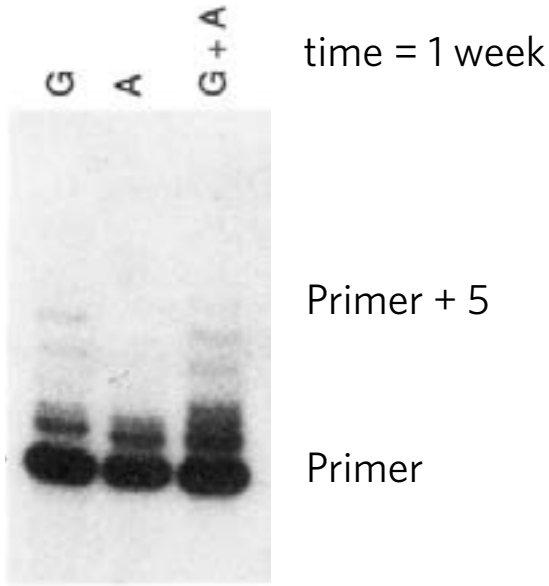


Key challenge: A/U



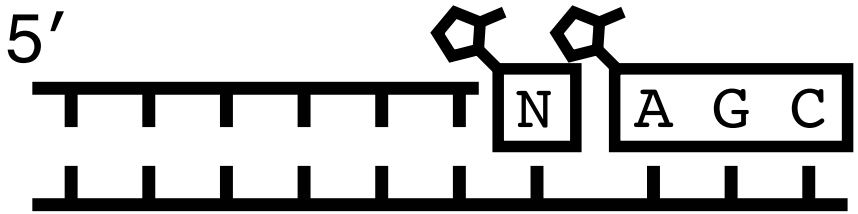


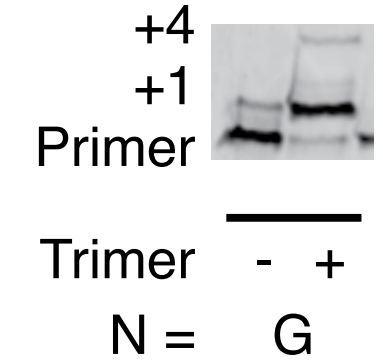


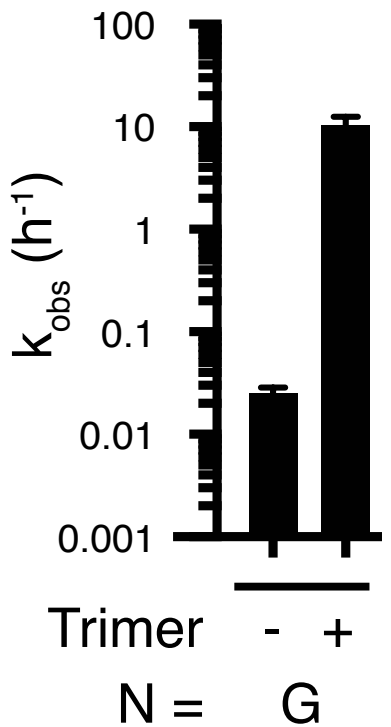


Wu, T., Orgel, L.E., 1992. Nonenzymic template-directed synthesis on oligodeoxycytidylate sequences in hairpin oligonucleotides. J Am Chem Soc 114, 317–322.

Other monomers

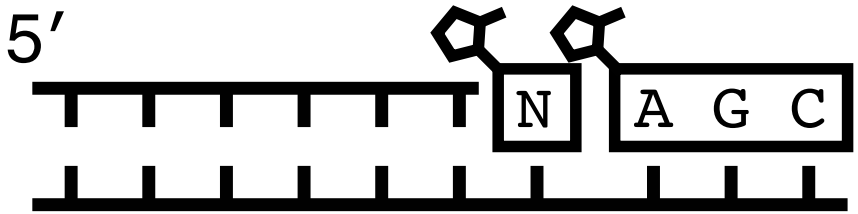


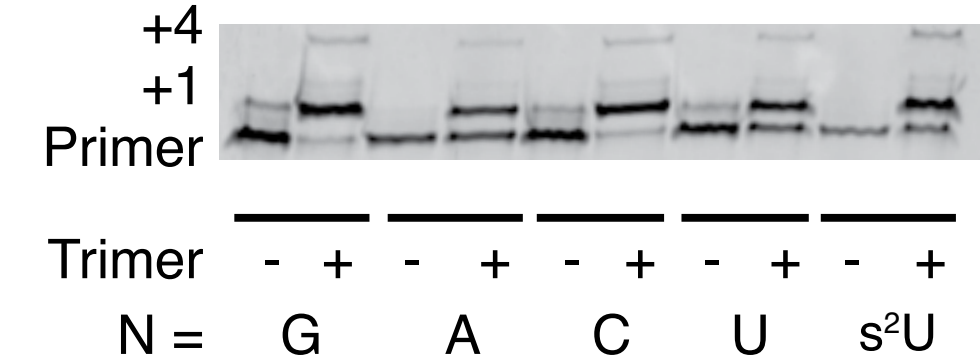


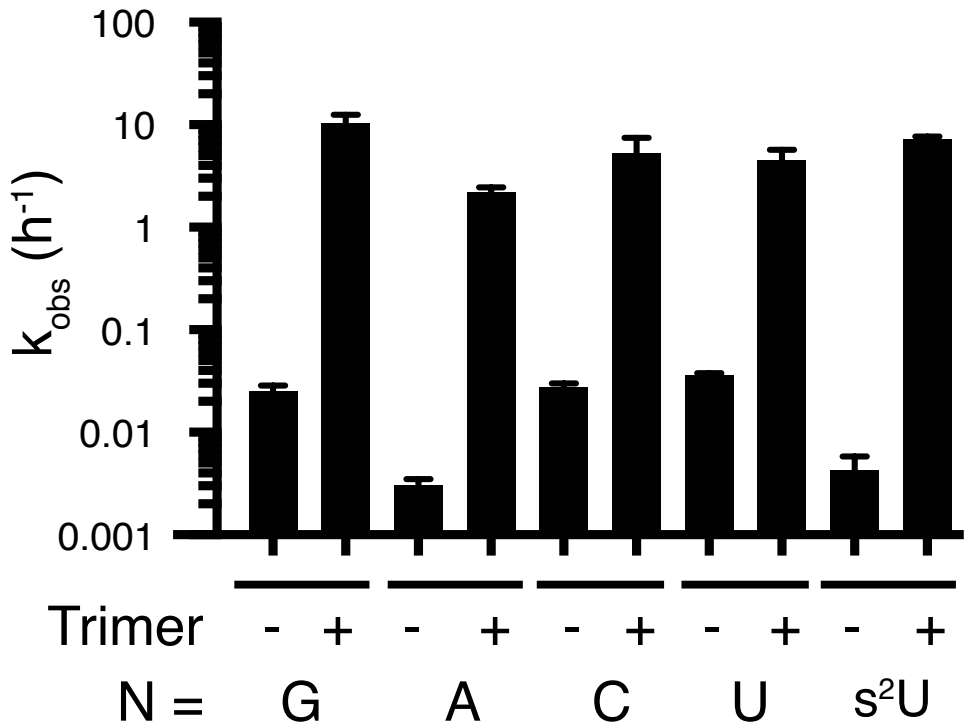


1 hour

Other monomers







Heuberger, B. D., Pal, A., Del Frate, F., Topkar, V. V. & Szostak, J. W. J Am Chem Soc 137, 2769–2775 (2015).

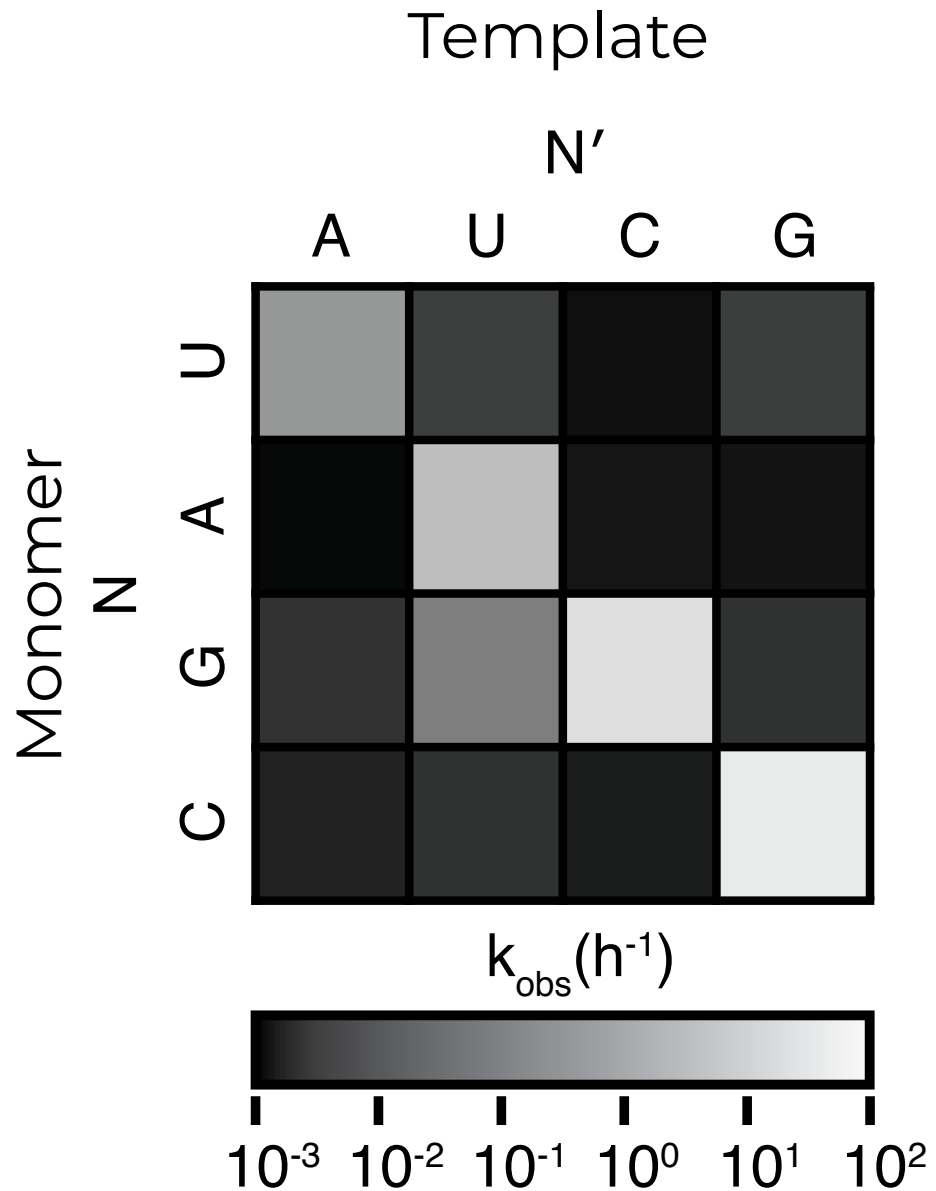
Prywes, N., Michaels, Y. S., Pal, A., Oh, S. S. & Szostak, J. W. Chem. Commun. (Camb.) 52, 6529–6532 (2016).

1 hour

Fidelity

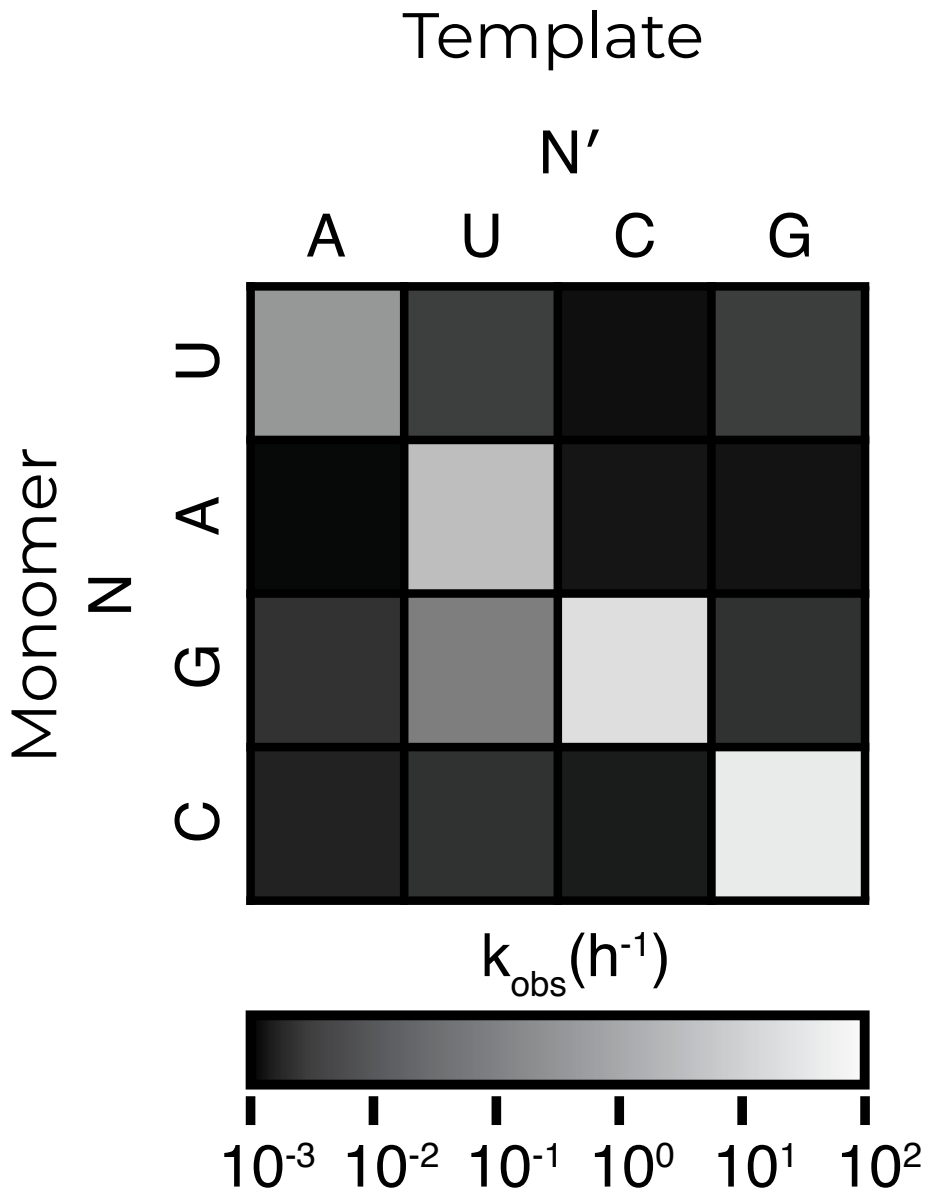
Fidelity


5′
N
A G C
N′


Template
N′
A U C G
Monomer N
U A G C
$k_{obs}(h^{-1})$
10^{-3} 10^{-2} 10^{-1} 10^{0} 10^{1} 10^{2}

Fidelity

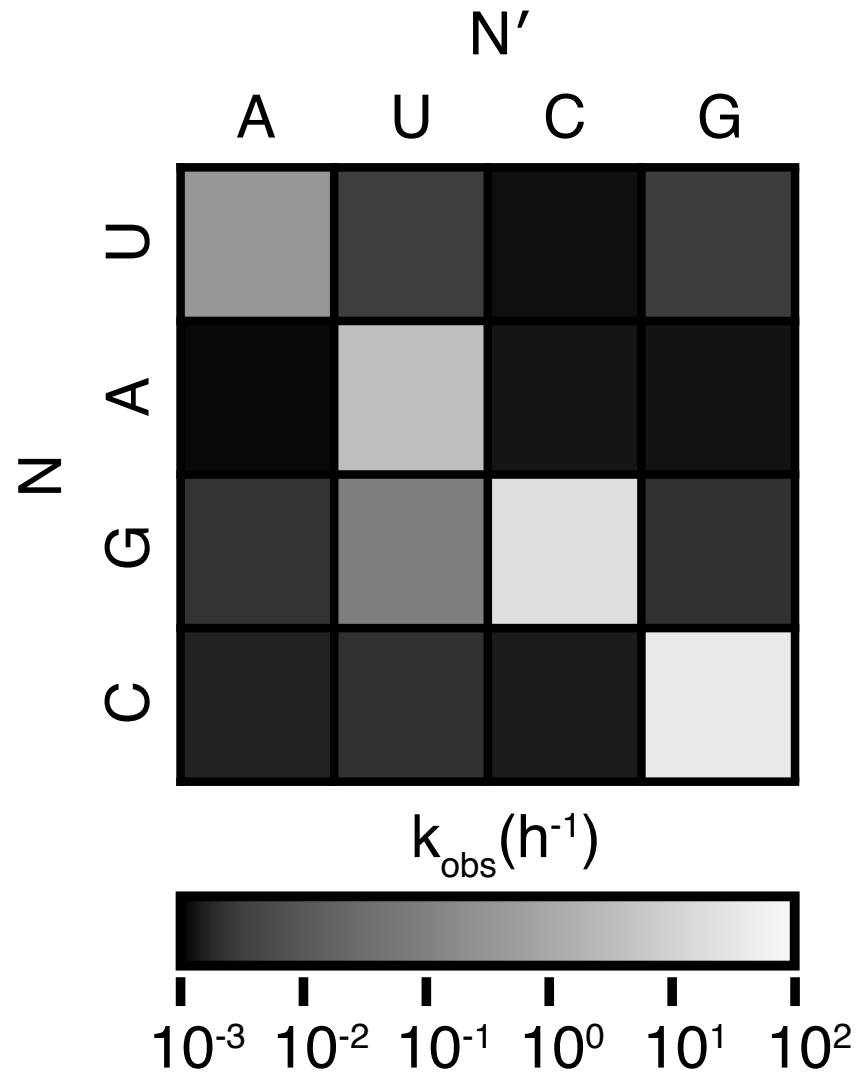
$$\frac{1}{4}\sum_{i\epsilon(\mathrm{A,U,G,C})}\frac{k_{j:i'}}{\sum_{j\epsilon(\mathrm{A,U,G,C})}k_{j:i'}}$$



Fidelity

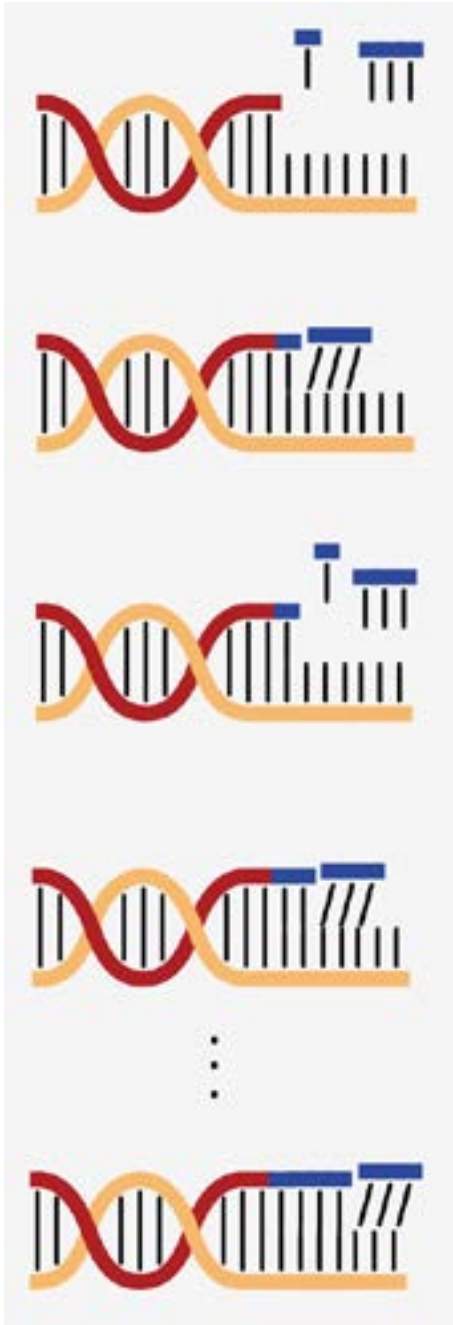
$$\frac{1}{4}\sum_{i\epsilon(\mathrm{A,U,G,C})}\frac{k_{j:i'}}{\sum_{j\epsilon(\mathrm{A,U,G,C})}k_{j:i'}} = 98\%$$

50nt “genome”

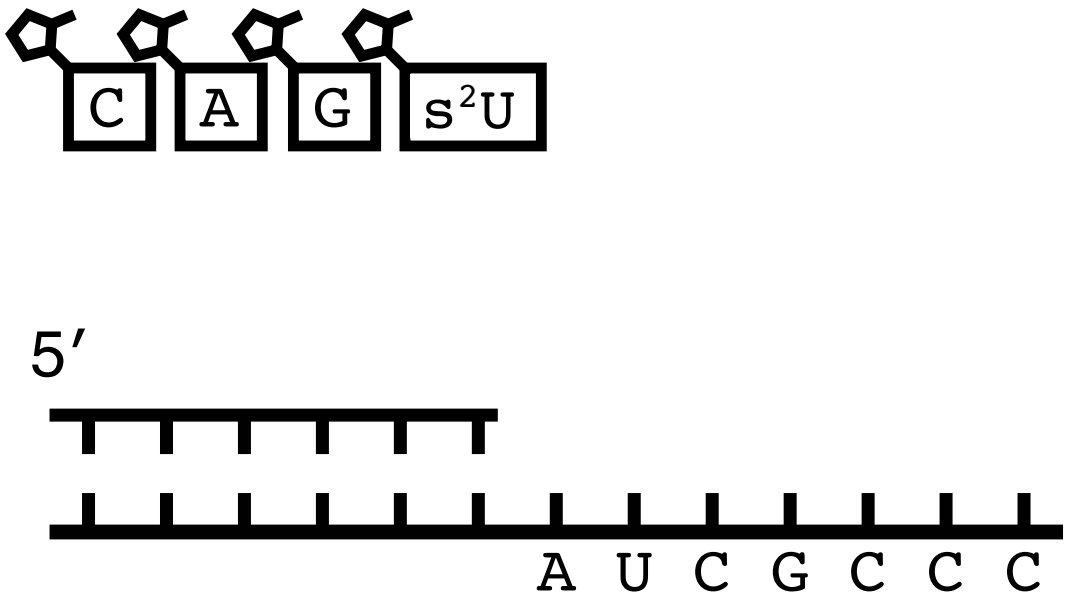


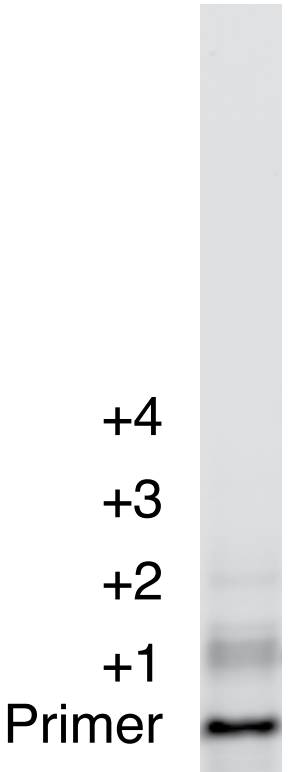
Eigen, M. Selforganization of matter and the evolution of biological macromolecules. Naturwissenschaften 58, 465–523 (1971).

Multiple monomer polymerization



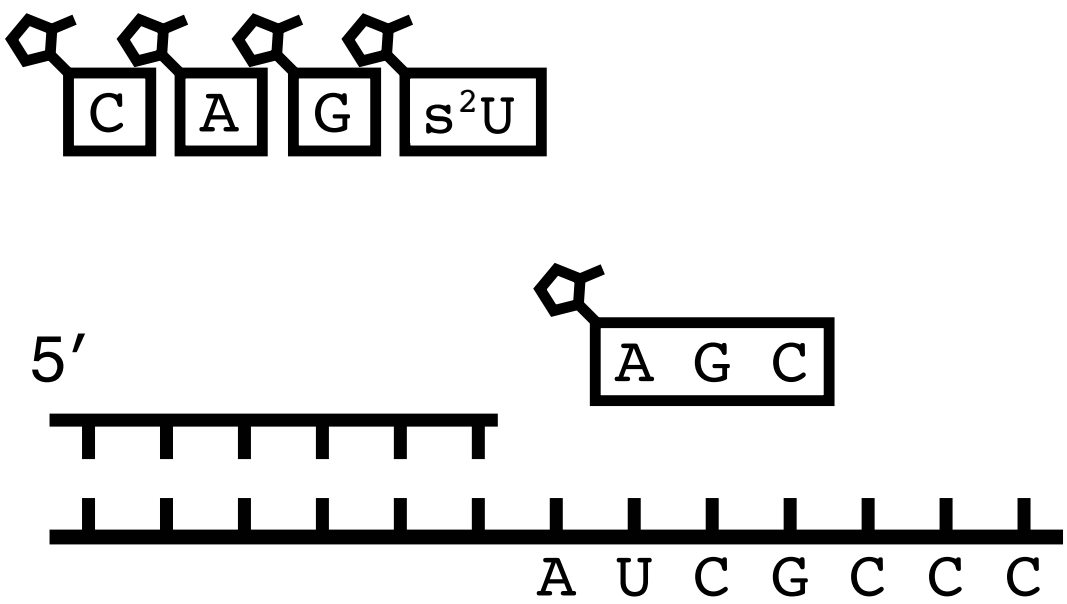
Multiple monomer polymerization

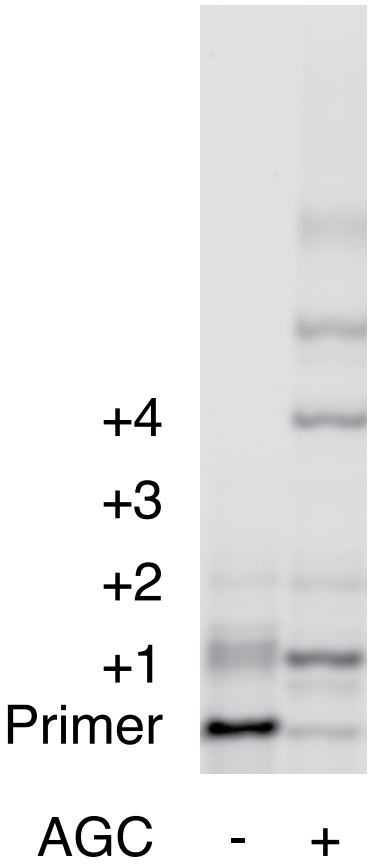




16 hours

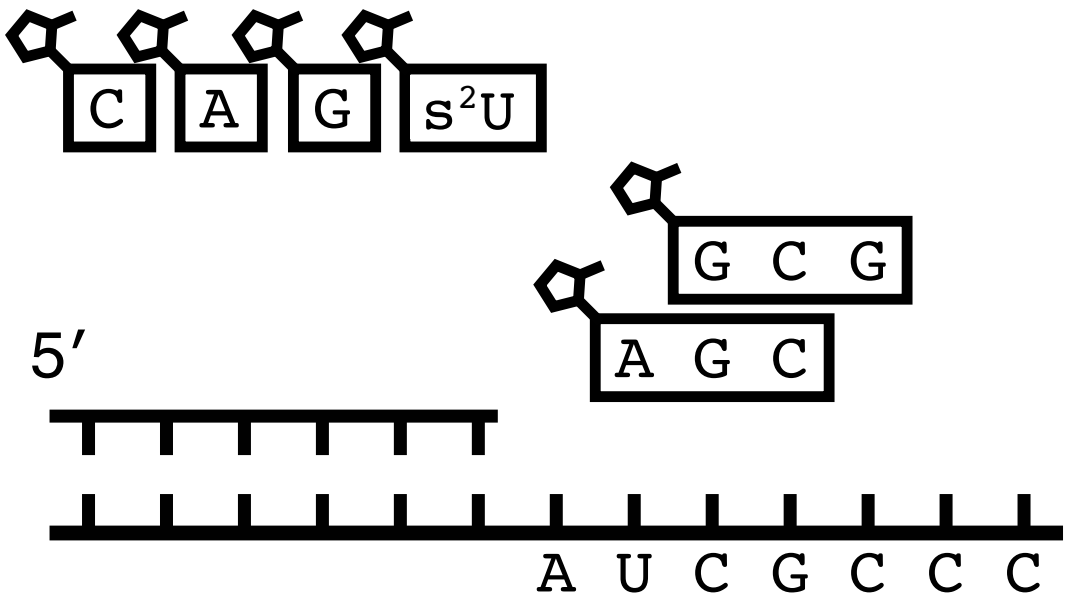
Multiple monomer polymerization

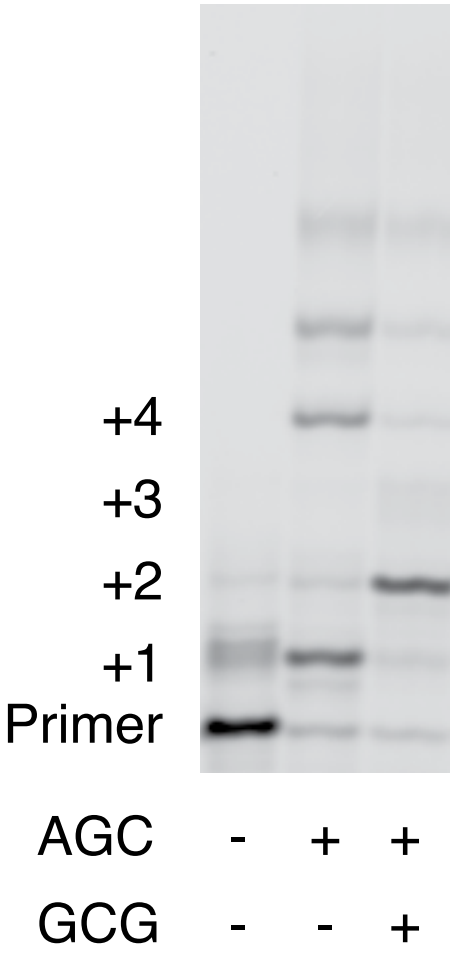




16 hours

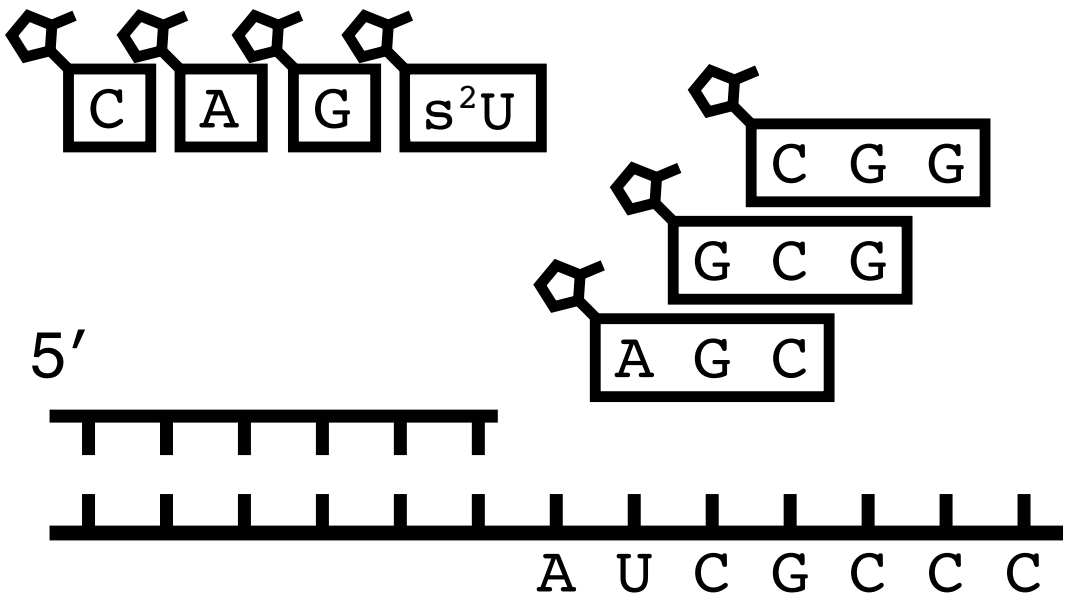
Multiple monomer polymerization

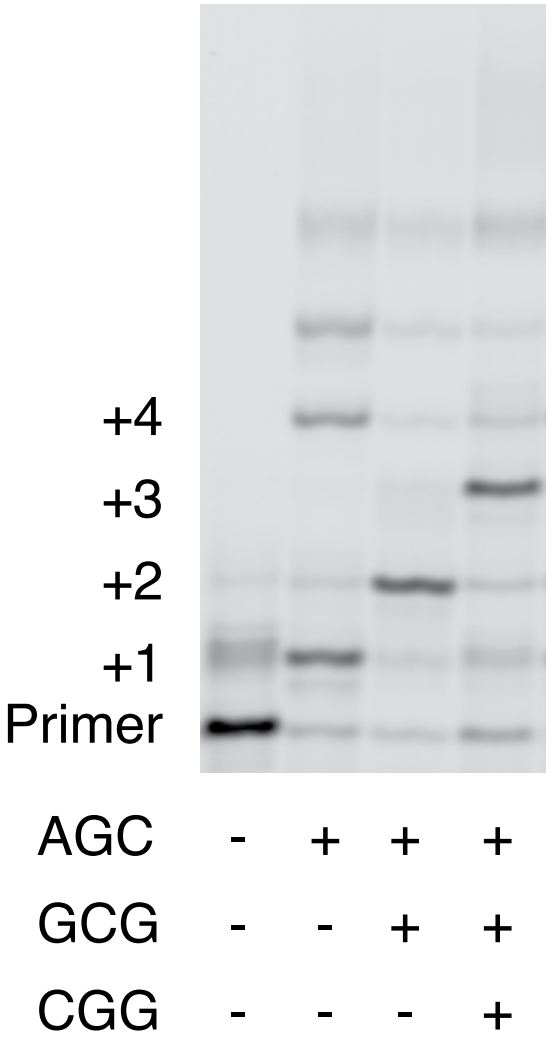




16 hours

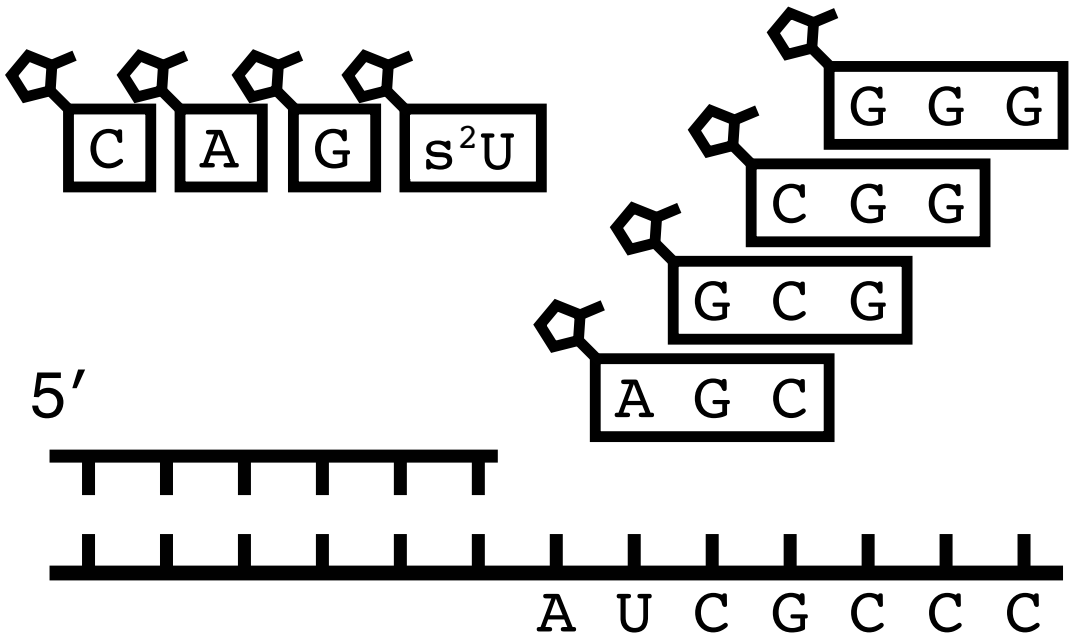
Multiple monomer polymerization

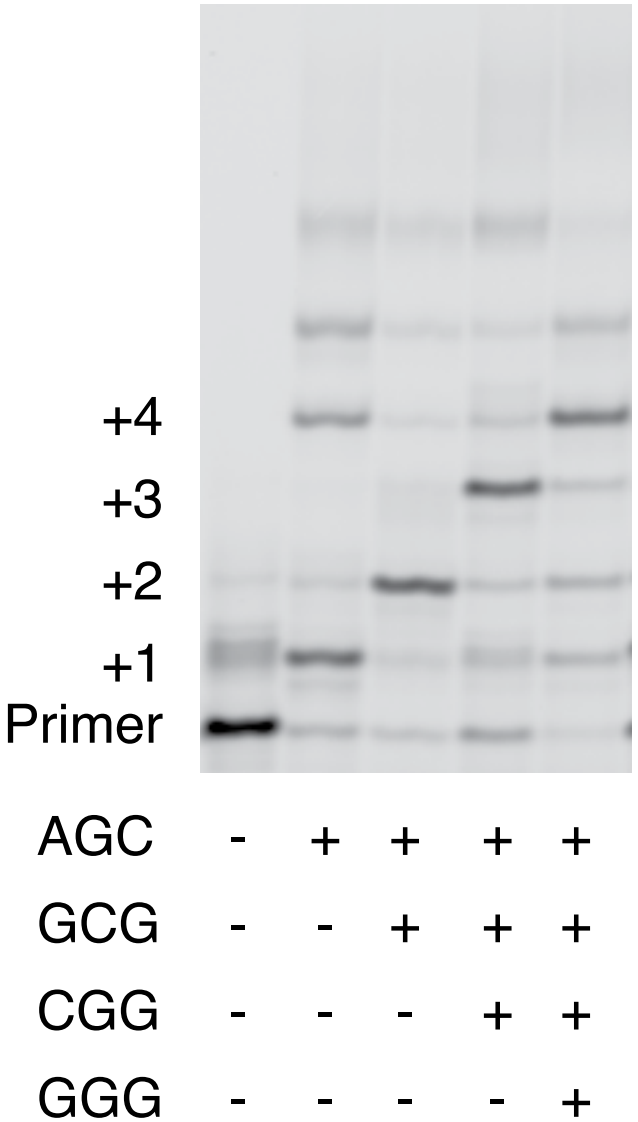




16 hours

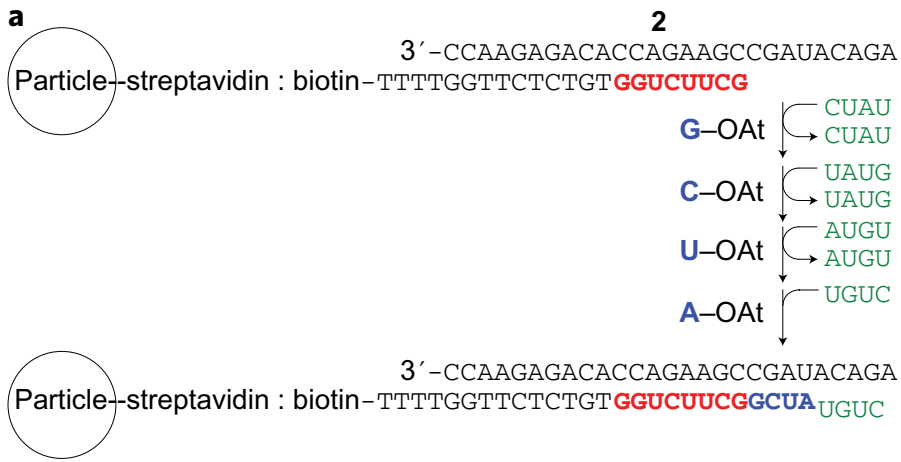
Multiple monomer polymerization

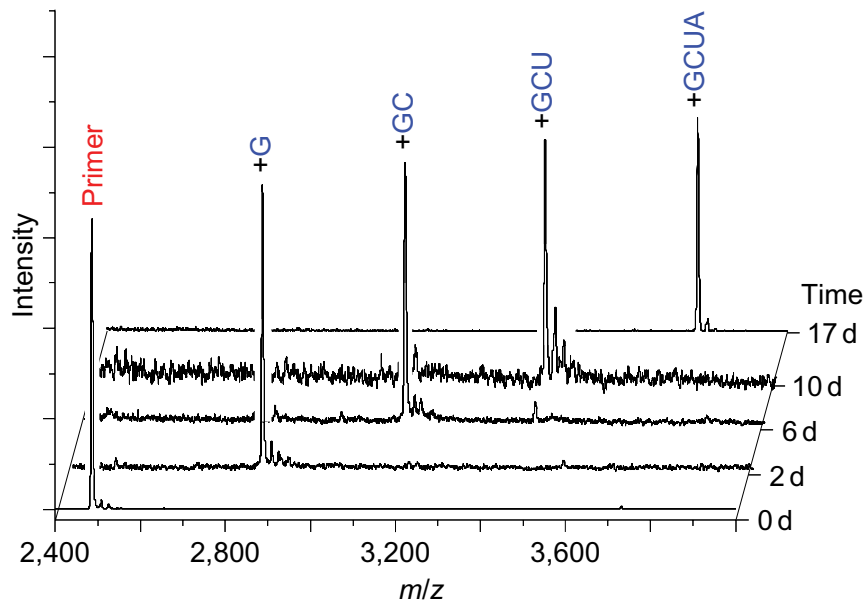




16 hours

Longer synthesis

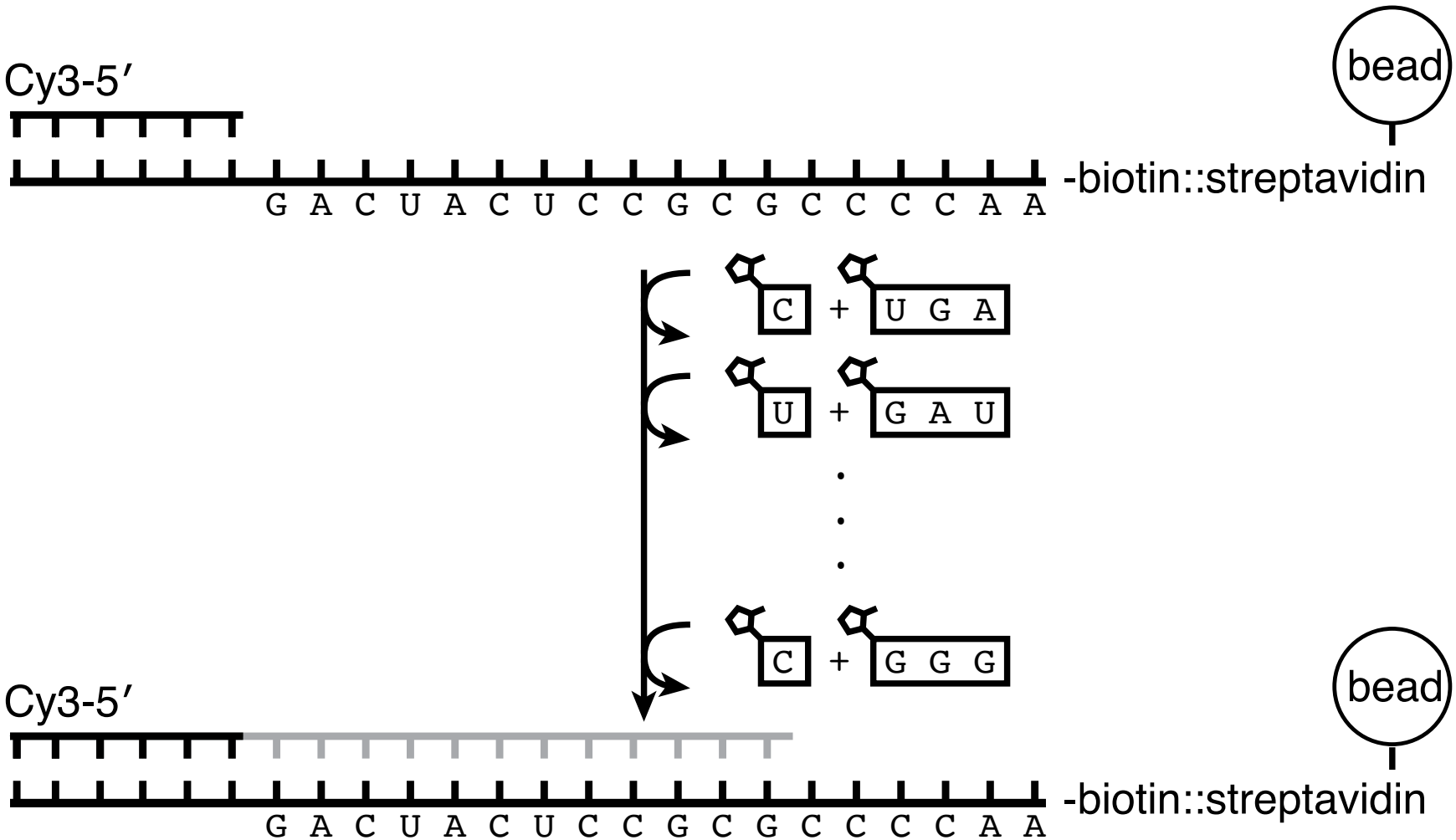




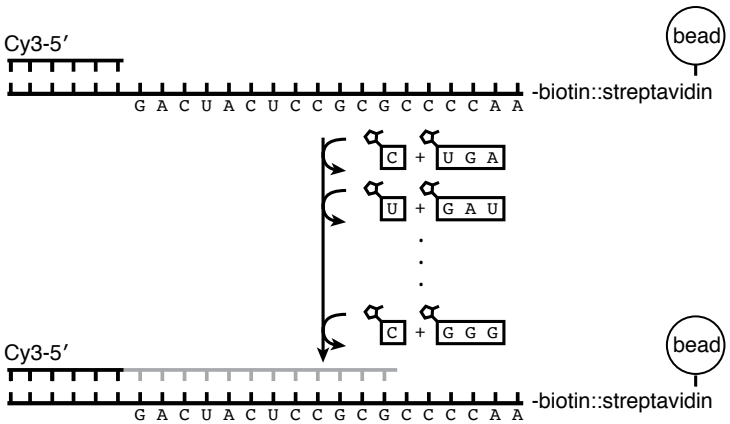
Beads

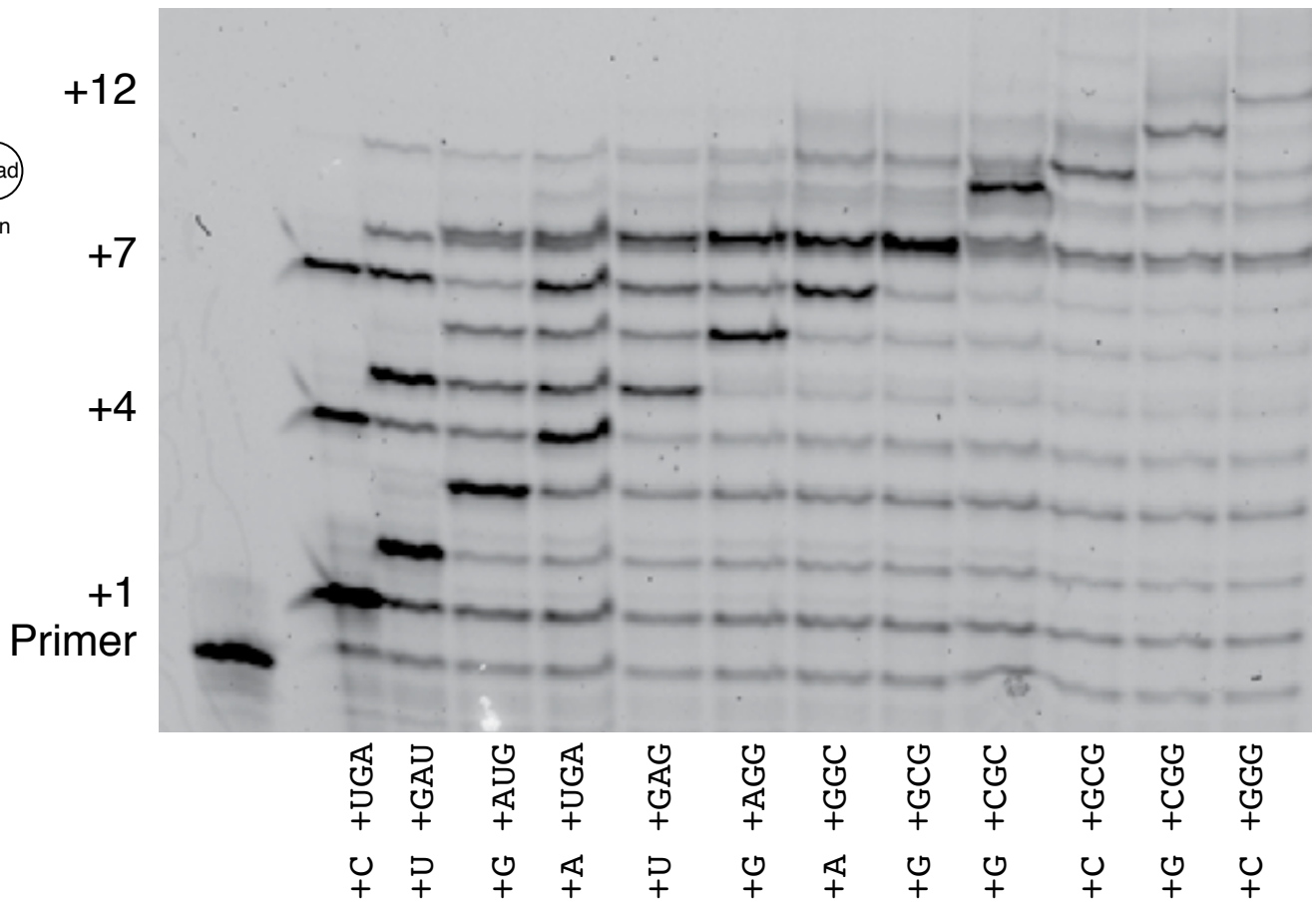
Deck, C., Jauker, M., Richert, C., 2011. Efficient enzyme-free copying of all four nucleobases templated by immobilized RNA. Nature Chem 3, 603–608.

Bead immobilization



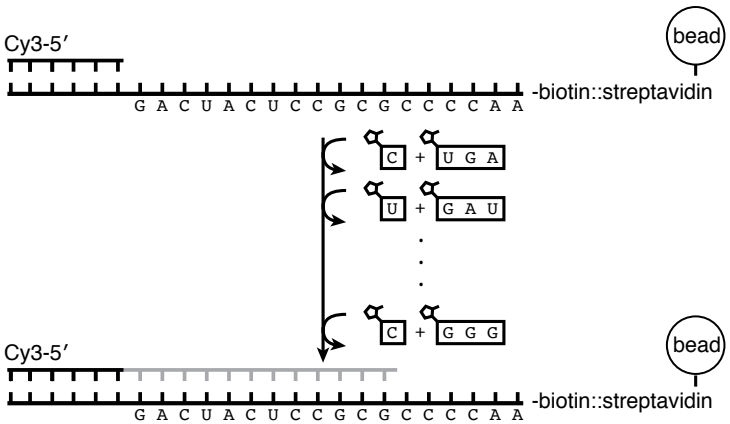
Longer synthesis

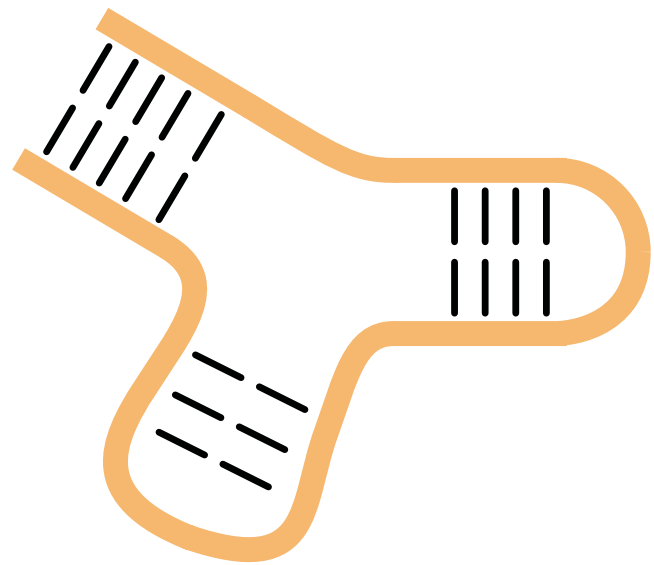


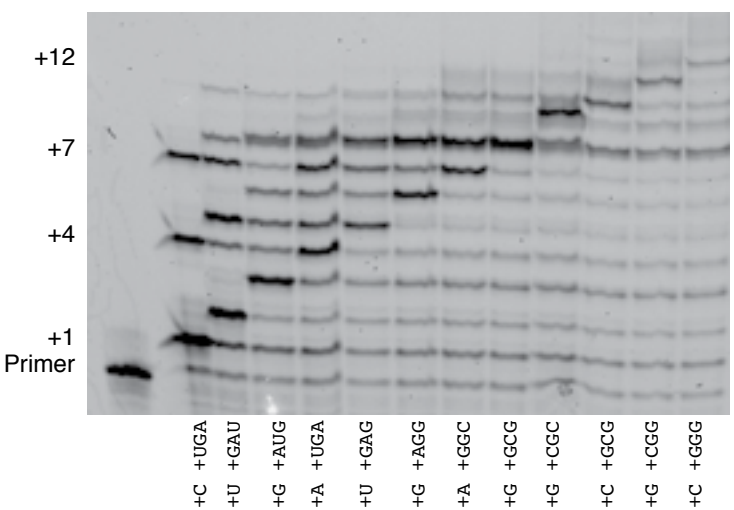


~150 hours total

Longer synthesis







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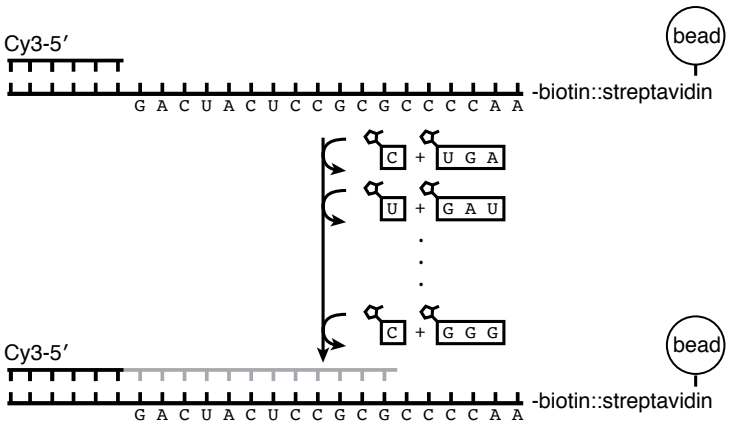
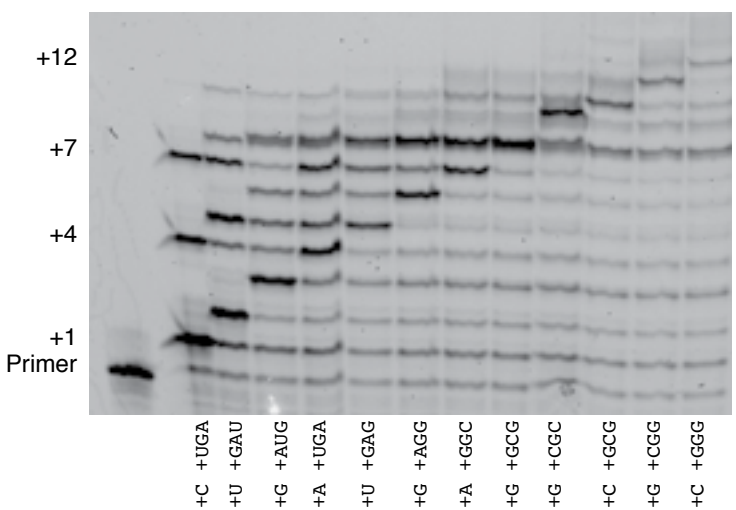
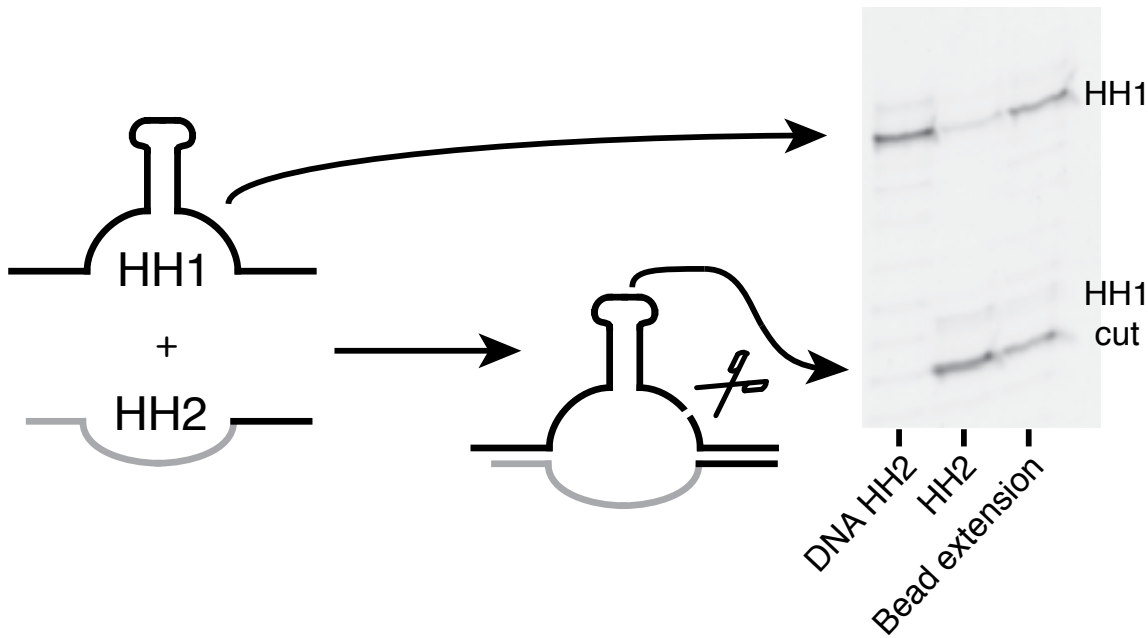
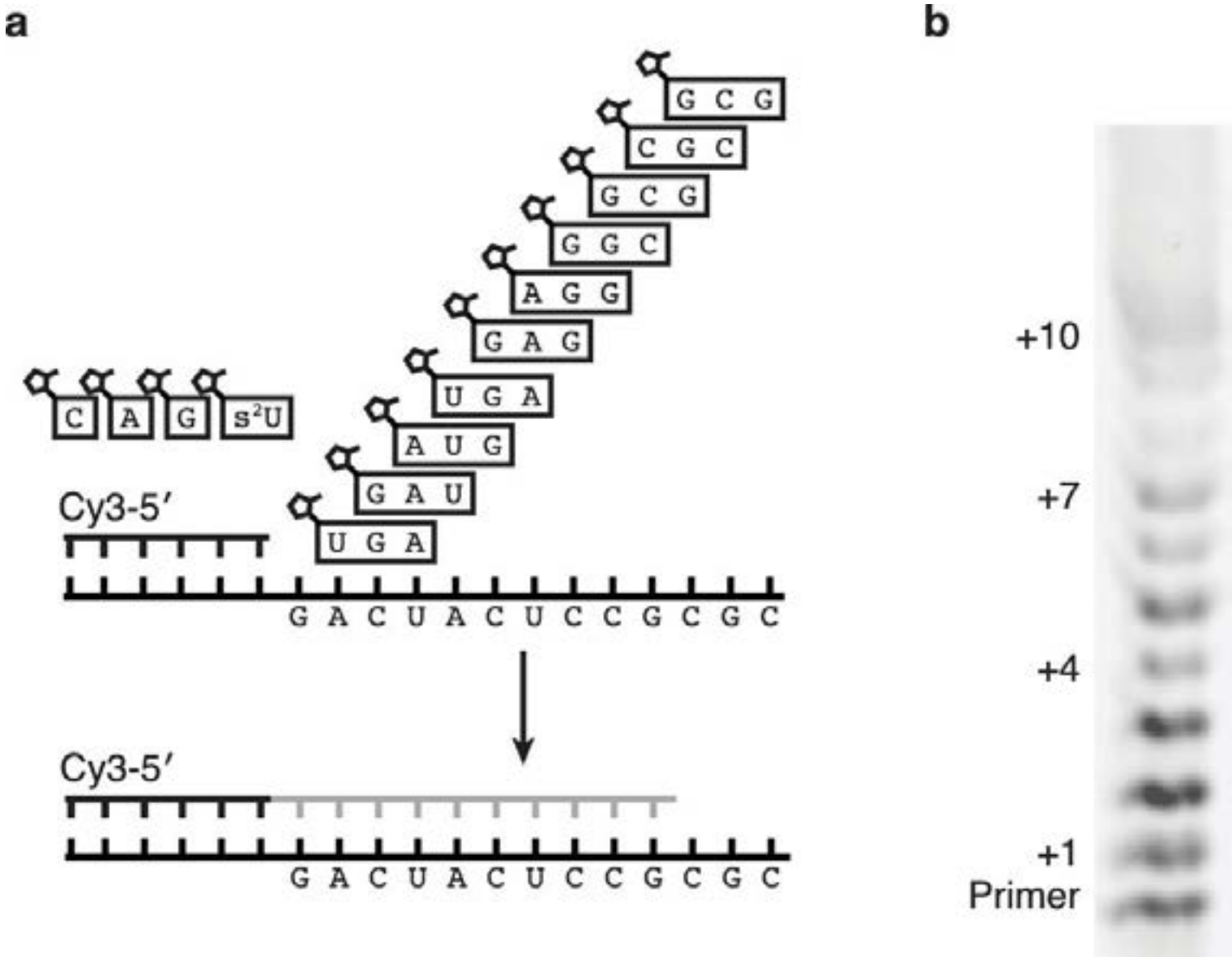


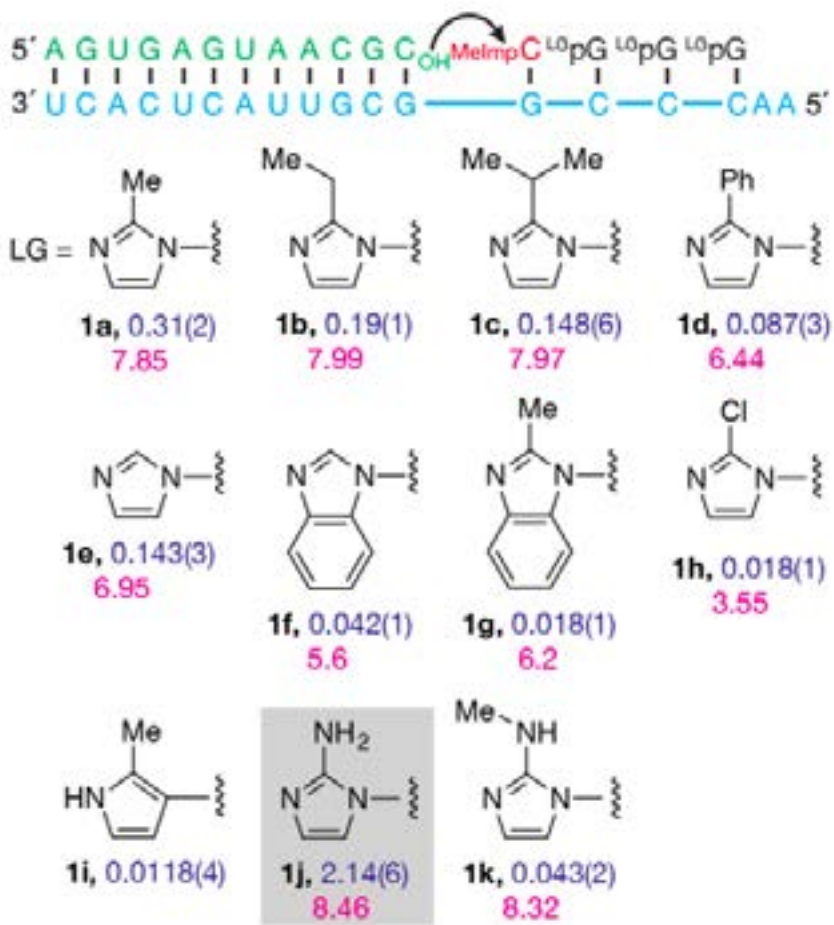


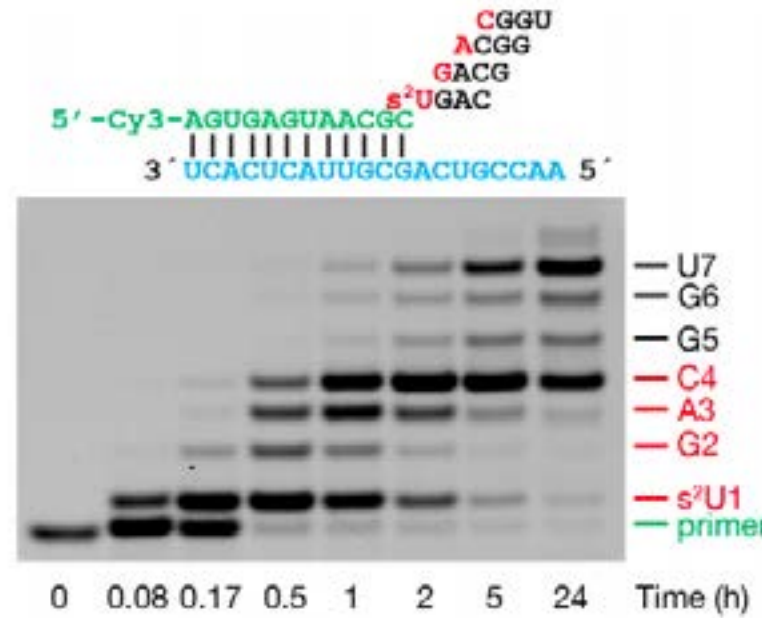


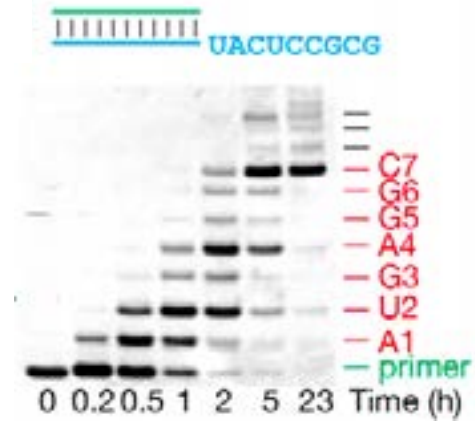
One pot hammerhead



Aminoimidazole leaving group

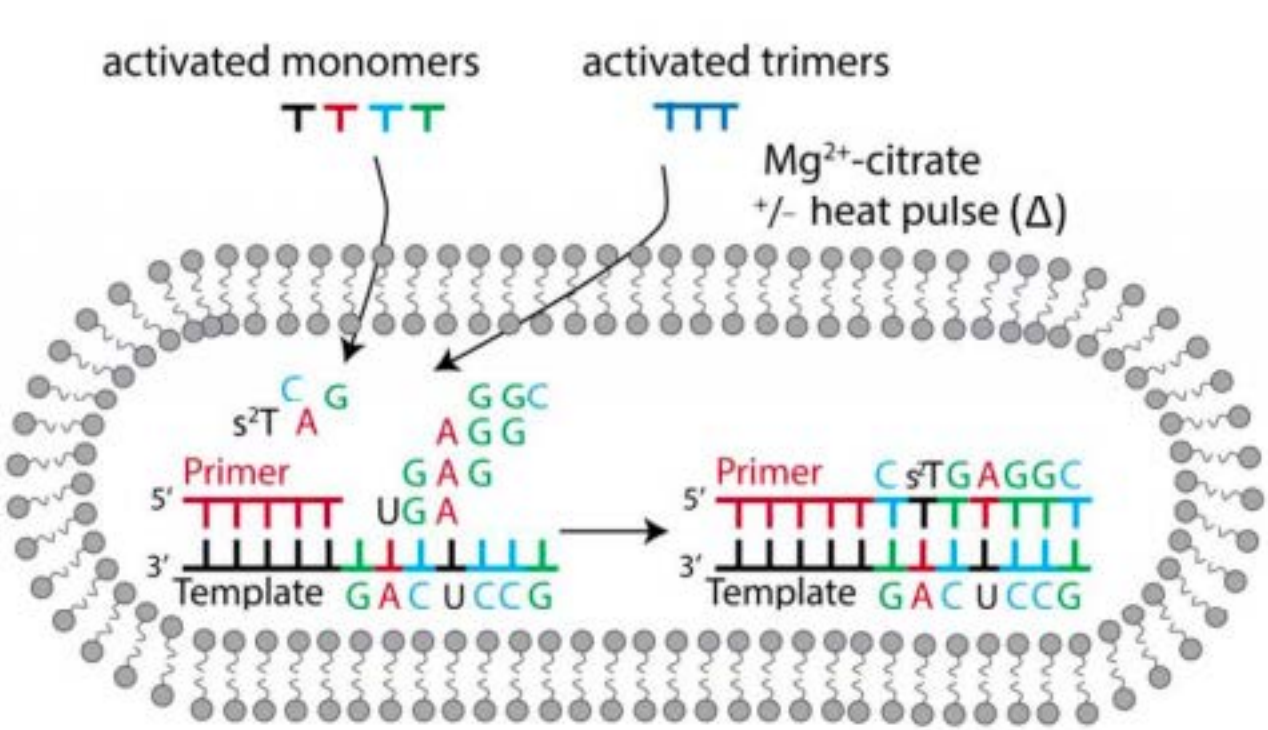


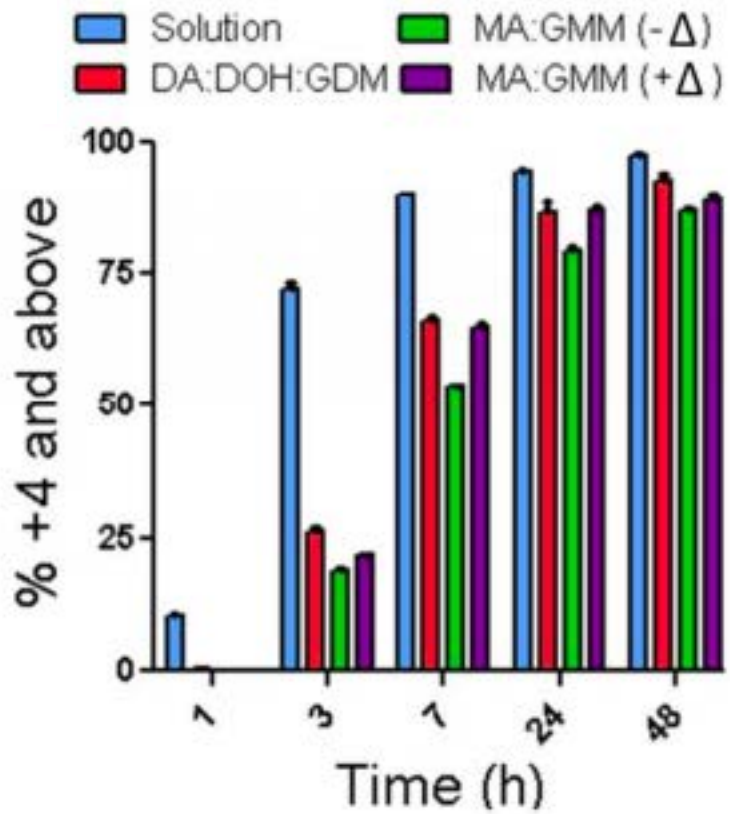




Li Li, Noam Prywes, Chun Pong Tam, Derek K. O'Flaherty, Victor S. Lelyveld, Enver Cagri Izgu, Ayan Pal, and Jack W. Szostak. "Enhanced Nonenzymatic RNA Copying with 2-Aminoimidazole Activated Nucleotides." Journal of the American Chemical Society (2017).

In-vesicle replication





Derek K. O'Flaherty, Neha P. Kamat, Fatima N. Mirza, Li Li, Noam Prywes and Jack W. Szostak. "Copying of mixed sequence RNA templates inside model protocells." Journal of th American Chemical Society (2018).

Acknowledgements

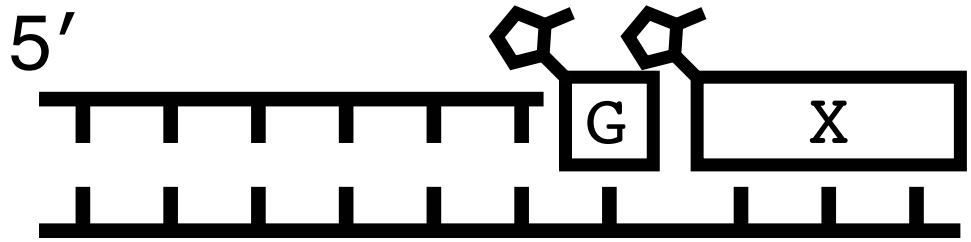
Jack Szostak

Francesca Del Frate
Craig Blain
Li Li
Travis Walton
Neha Kamat
Derek O’Flaherty

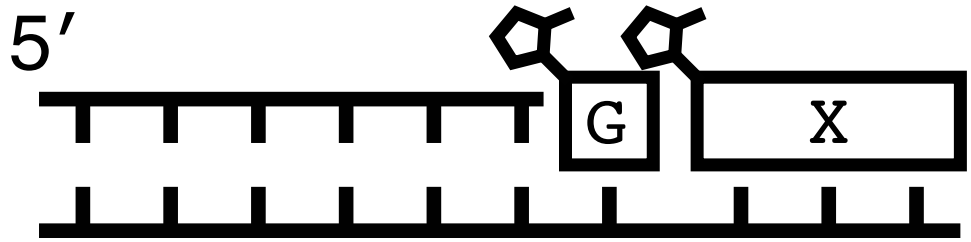
Szostak Lab

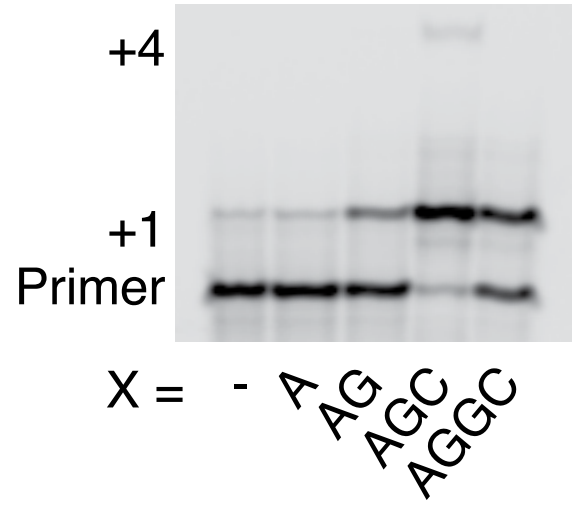
Questions?

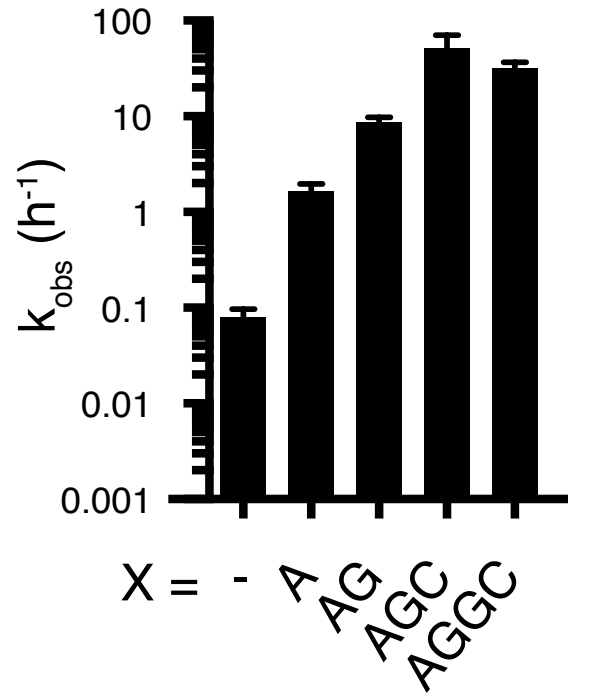
Effect of micro-helper length



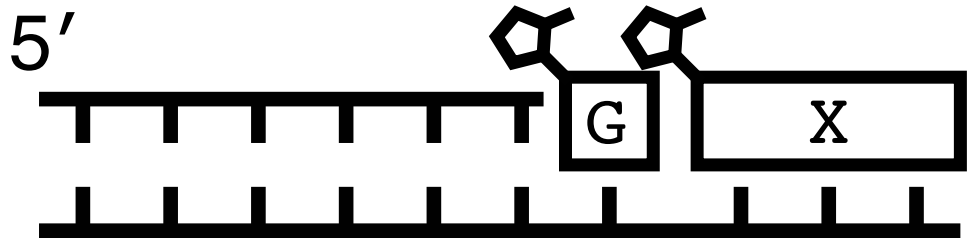
Effect of micro-helper length

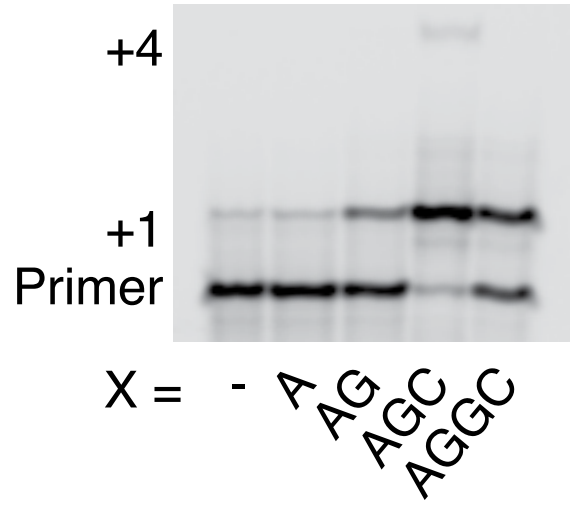


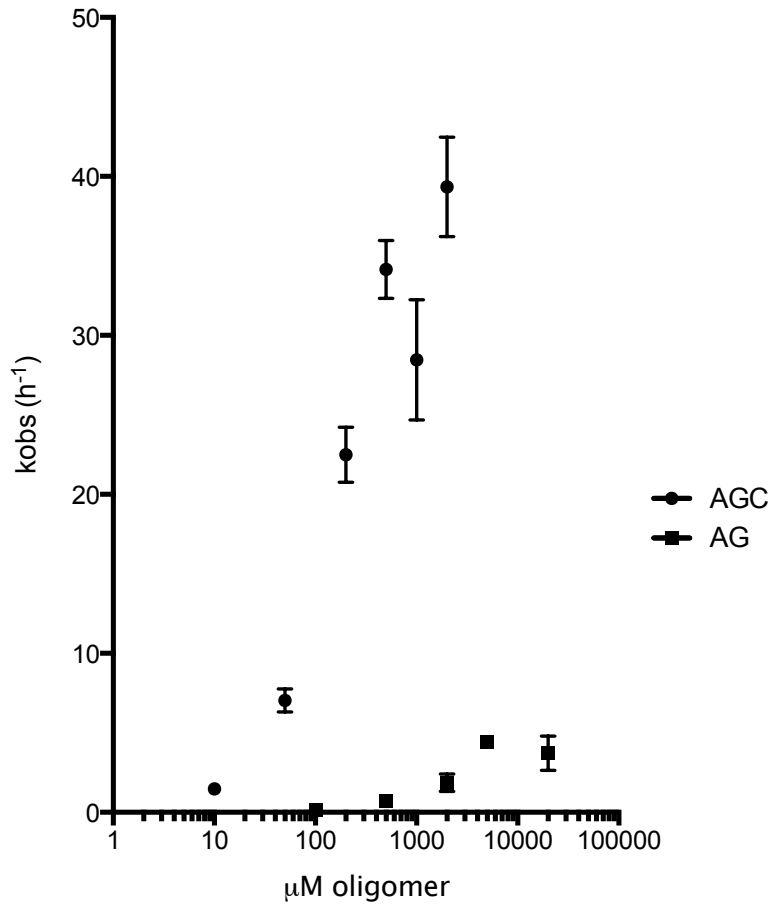


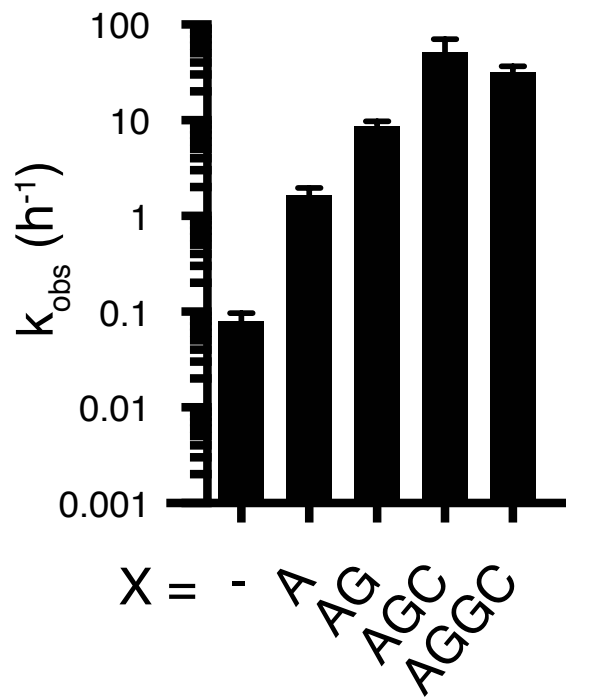


Effect of micro-helper length

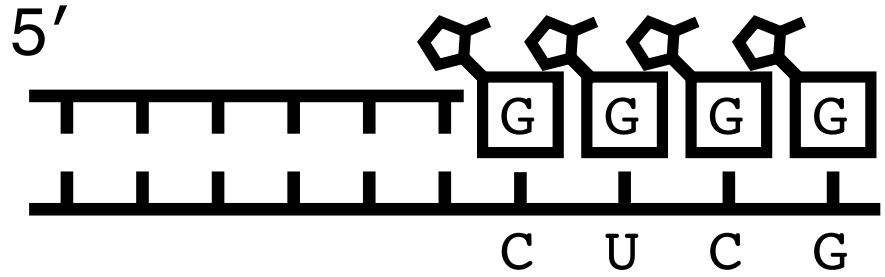


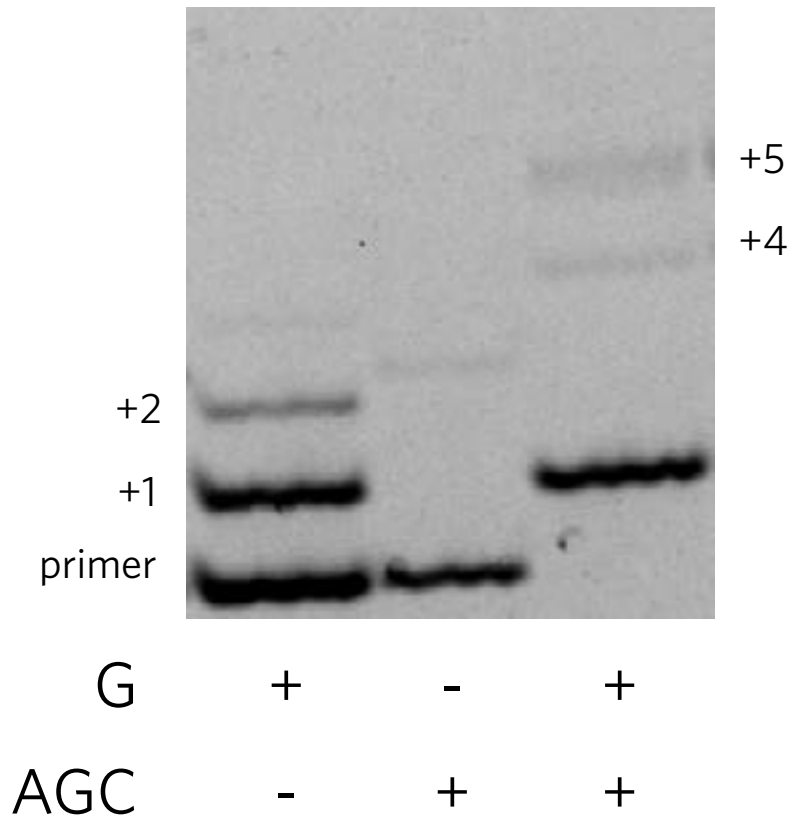






Curious result





Two chickens, two eggs

#1

Proteins make RNA, RNA makes proteins

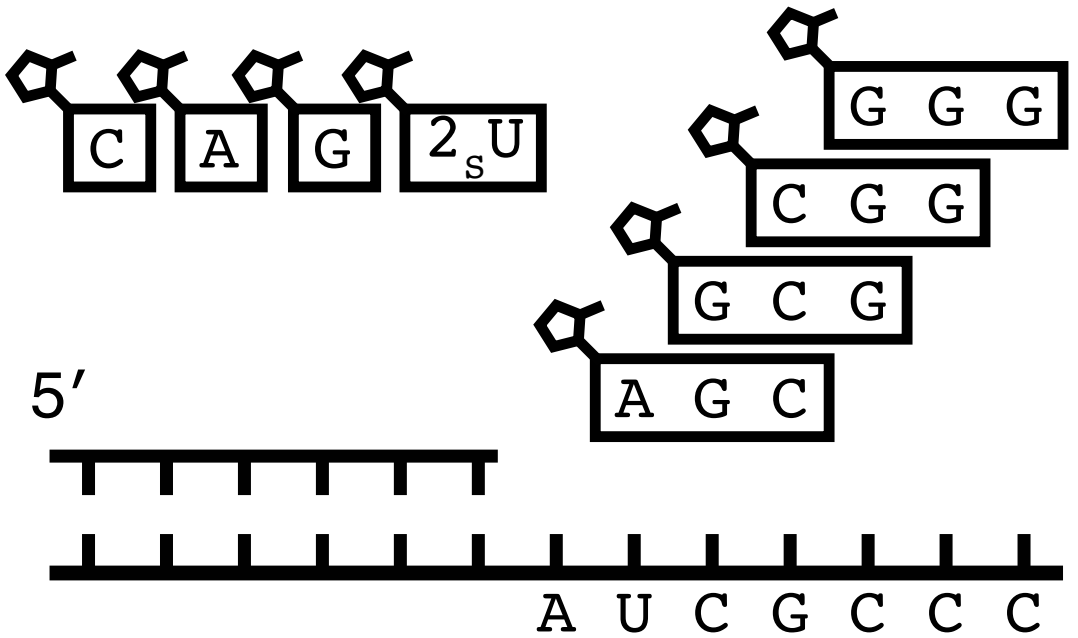
Solution - RNA replicase makes RNA in the RNA world

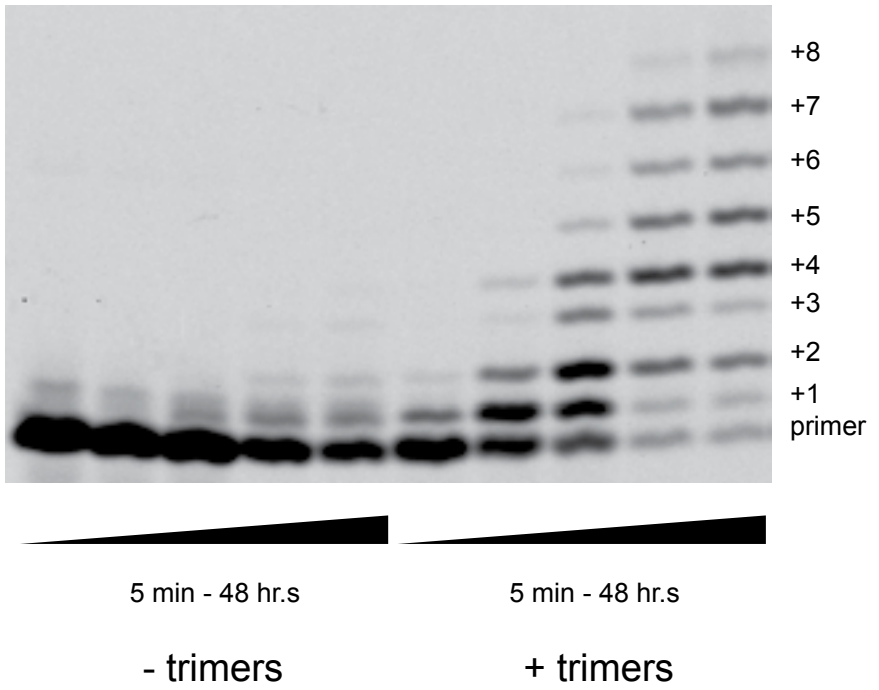
#2

"Without evolution it appears unlikely that a self-replicating ribozyme could arise, but without some form of self-replication there is no way to conduct an evolutionary search for the first, primitive self-replicating ribozyme."

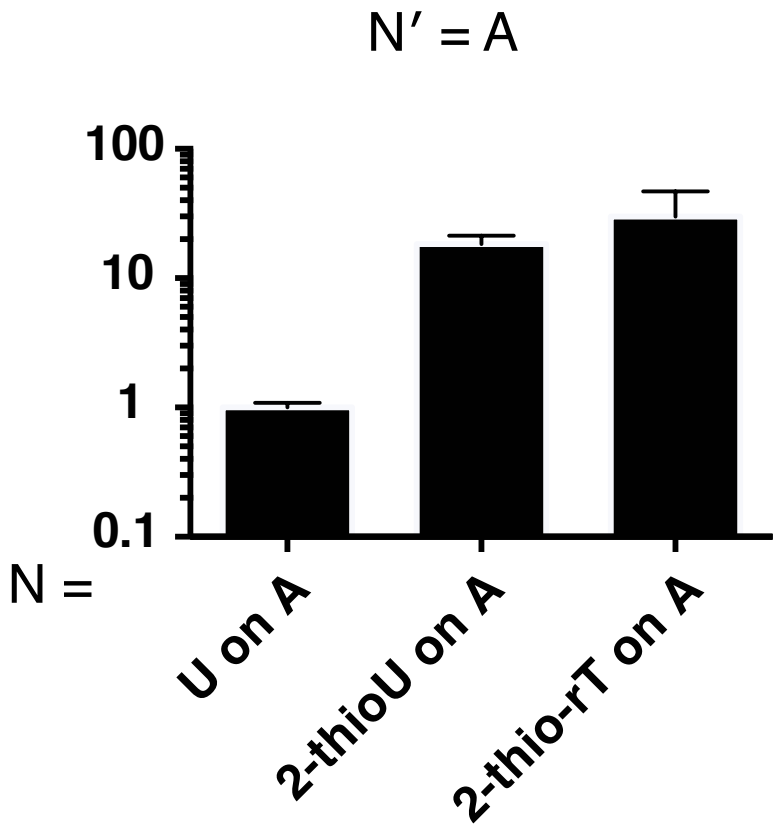
Robertson, M.P., Joyce, G.F., 2012. The origins of the RNA world. Cold Spring Harbor Perspectives in Biology 4.

Multiple monomer polymerization

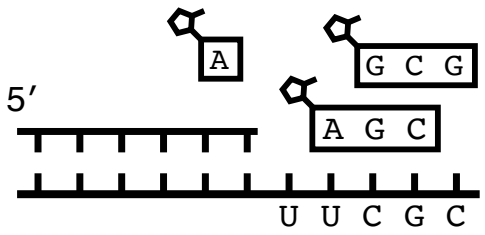


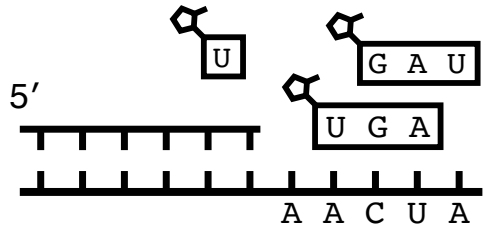


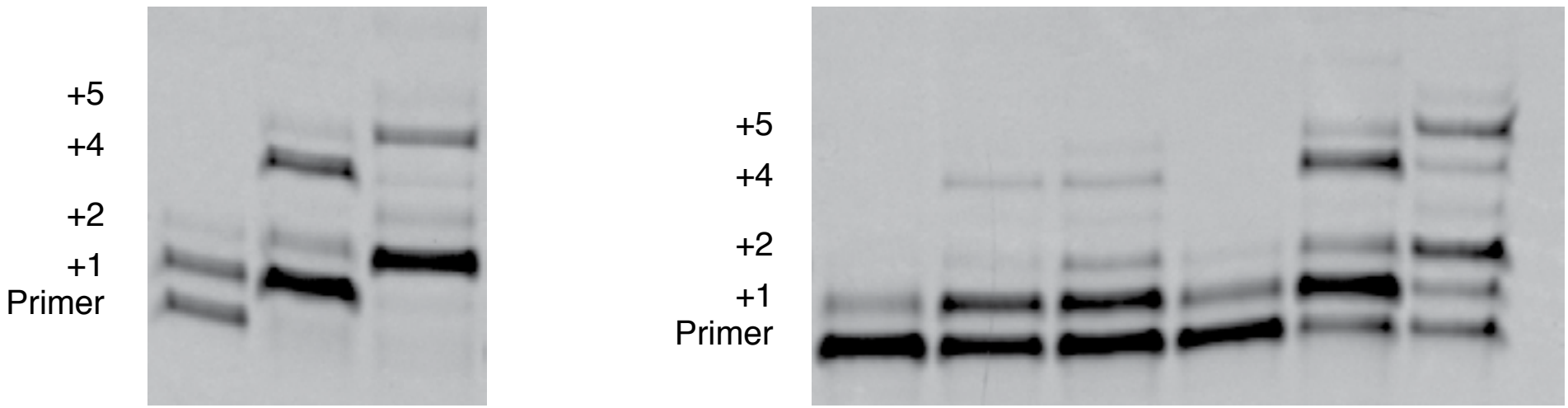
Benefit of thioU and thioT



Two A or U additions

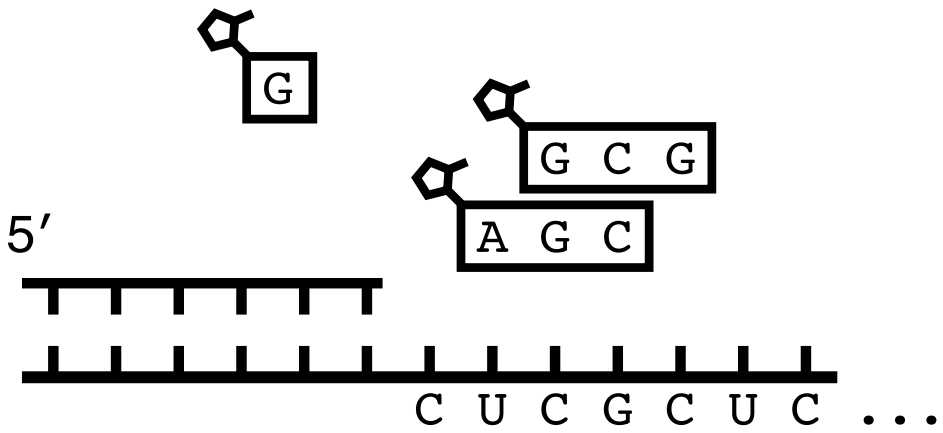


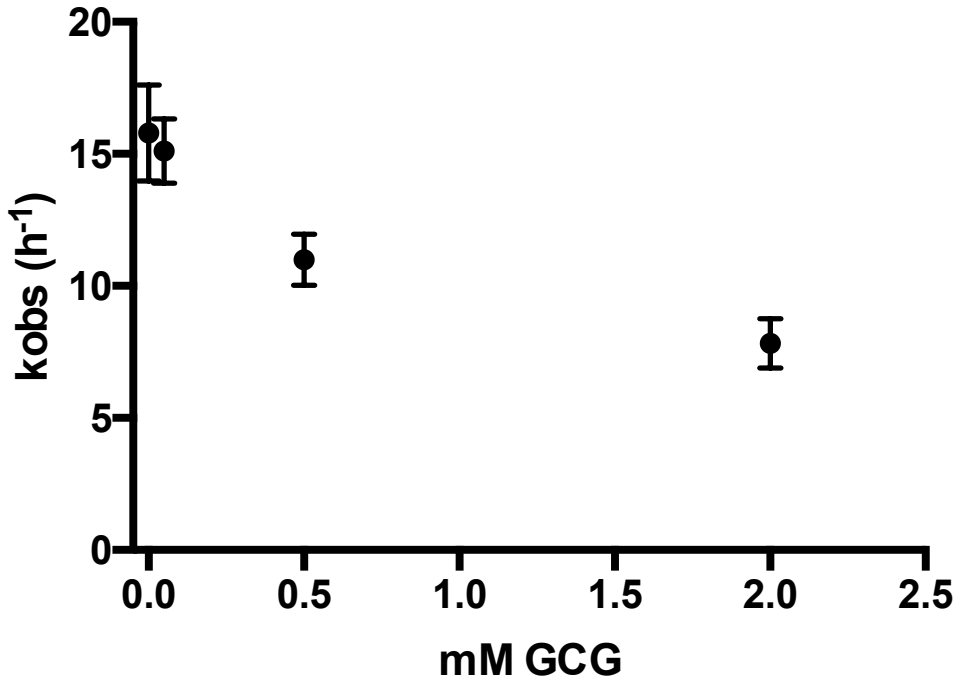




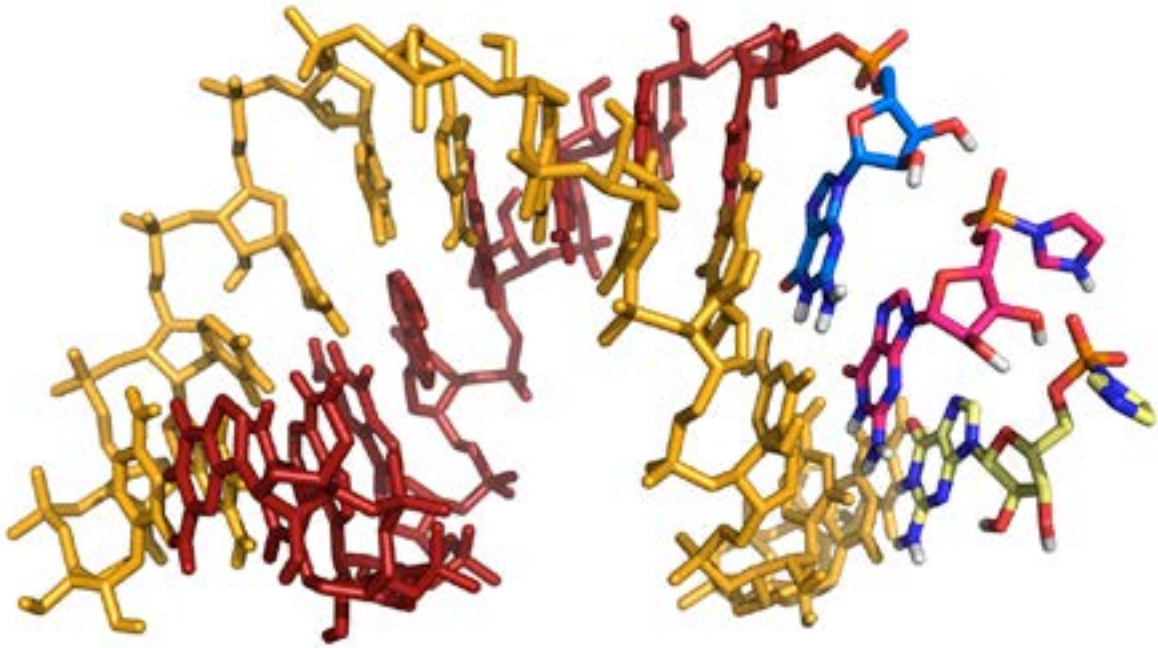
A	+	+	+			
AGC	-	+	+			
GCG	-	-	+			
U	+	+	+	-	-	-
2-thioU	-	-	-	+	+	+
UGA	-	+	+	-	+	+
GAU	-	-	+	-	-	+

Overlapping trimers





Non-enzymatic primer extension



Courtesy of Li

One pot aminoimidazole