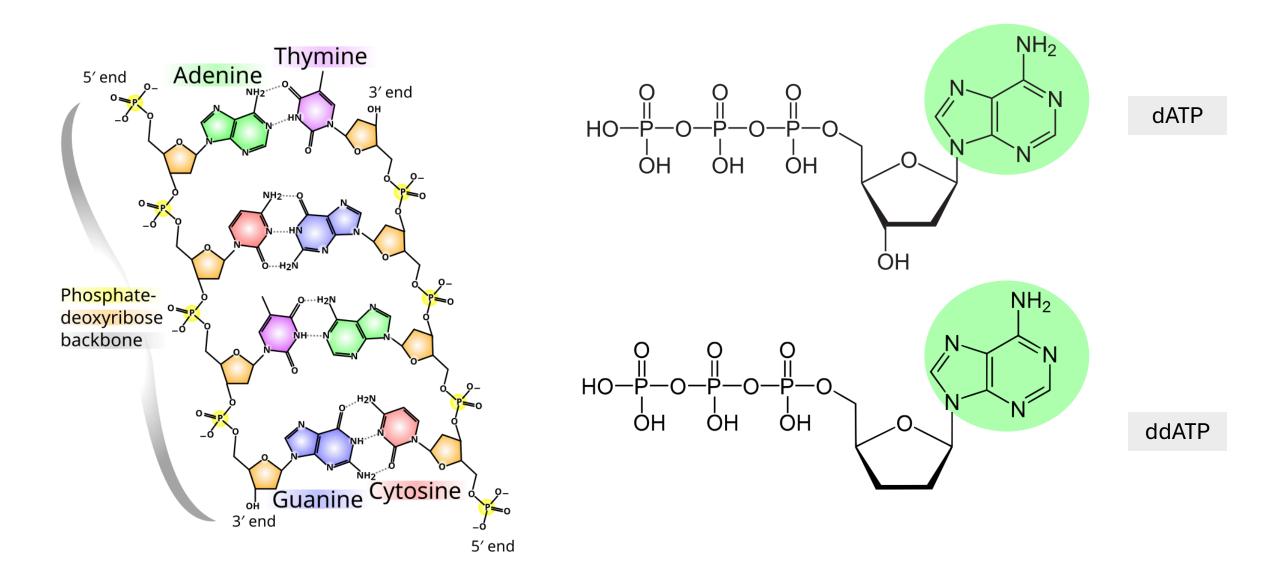
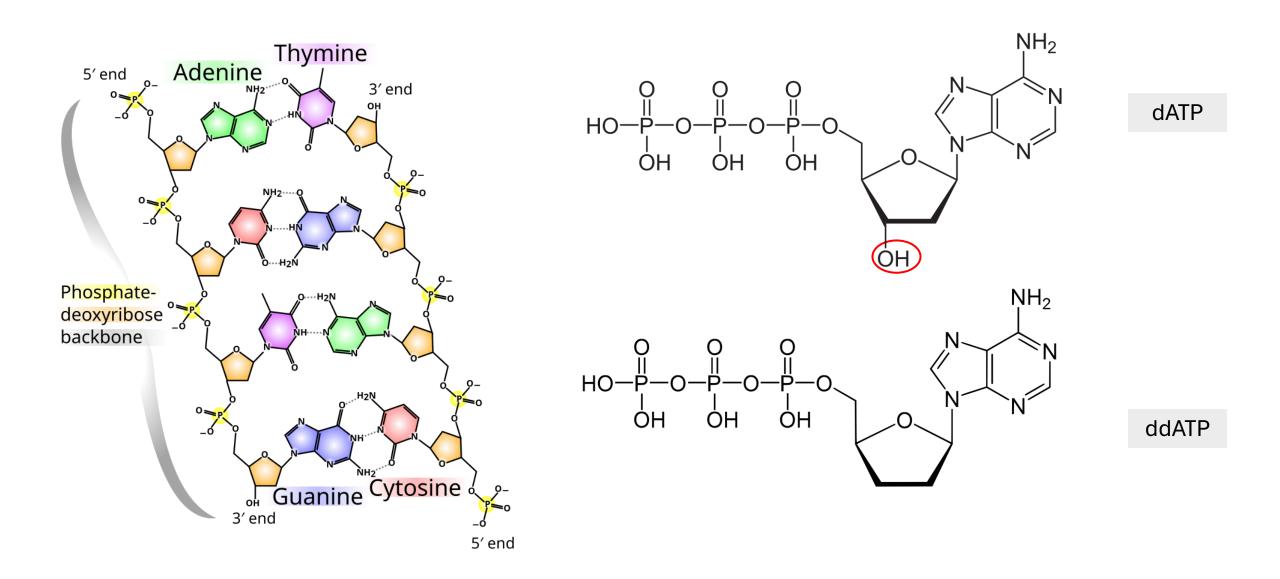
## Real-Time DNA Sequencing

Lena Eiwanger

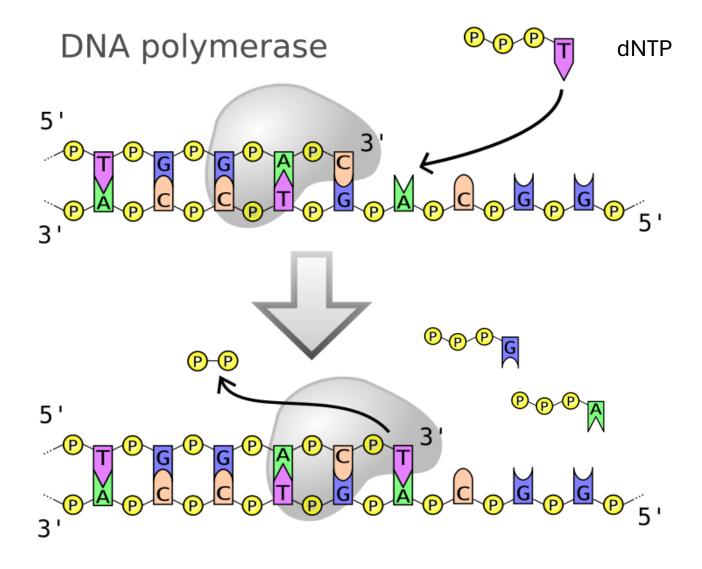
- Chemical Basics
- Sanger Sequencing Method
- Real-Time DNA Sequencing from Single Polymerase Molecules
- Summary



Wikipedia: DNA; Wikipedia: Nukleosidtriphosphate; Wikipedia: Didesoxyribonukleosid-Triphosphate

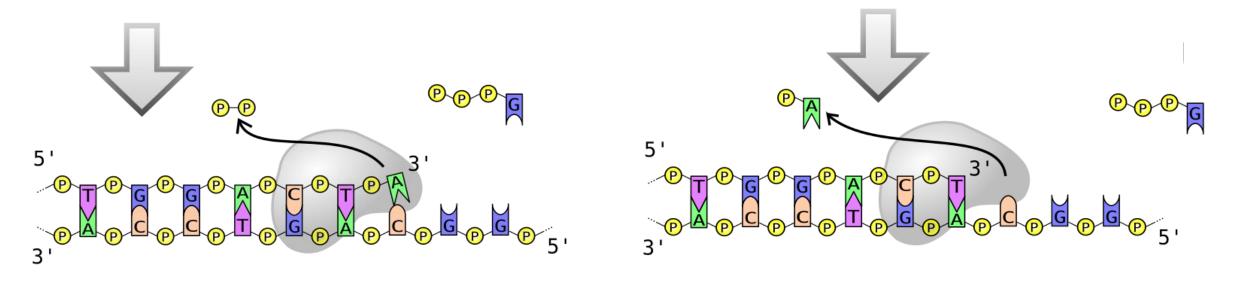


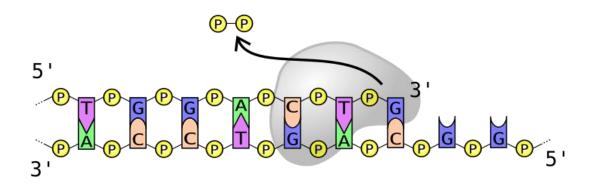
Wikipedia: DNA; Wikipedia: Nukleosidtriphosphate; Wikipedia: Didesoxyribonukleosid-Triphosphate



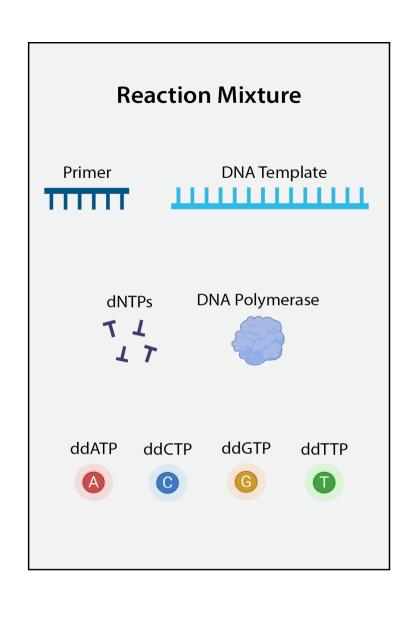
Wikipedia: DNA replication

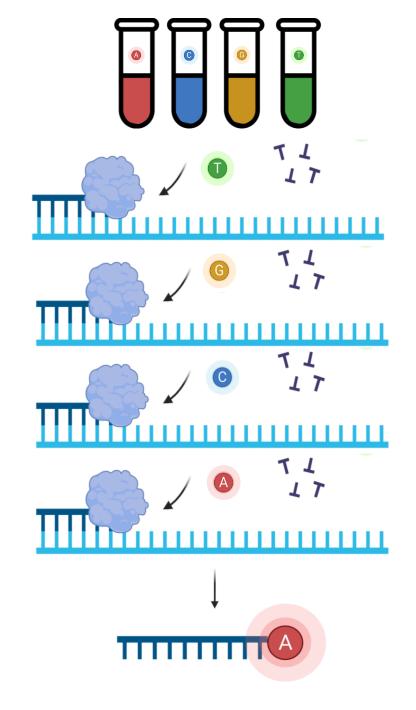
## **Chemical Basics**

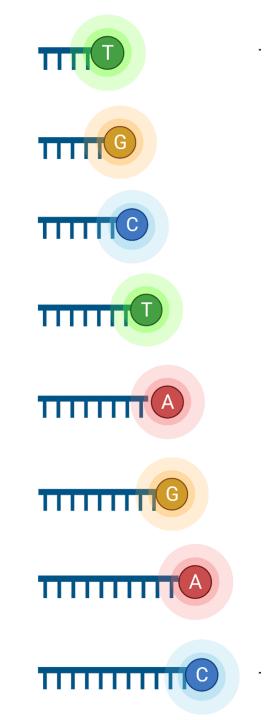




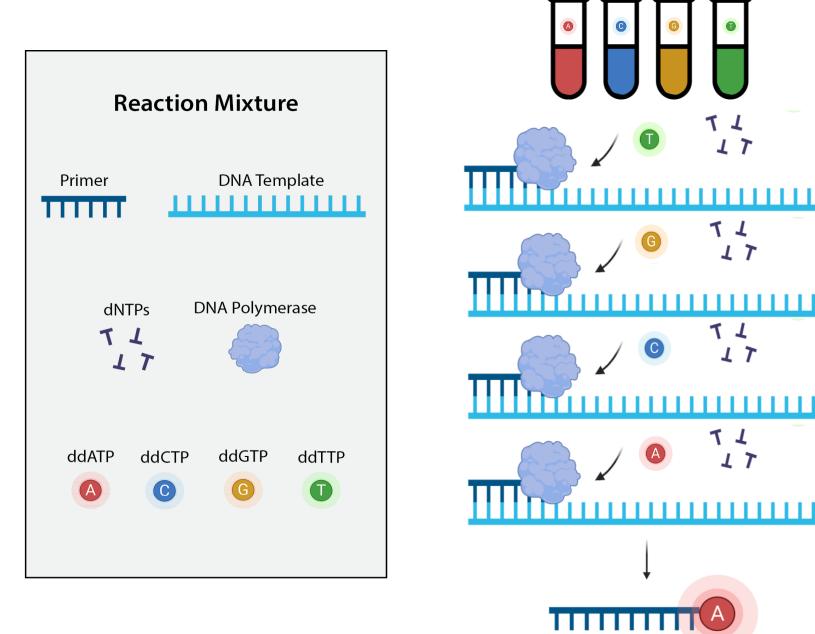
Wikipedia: DNA replication

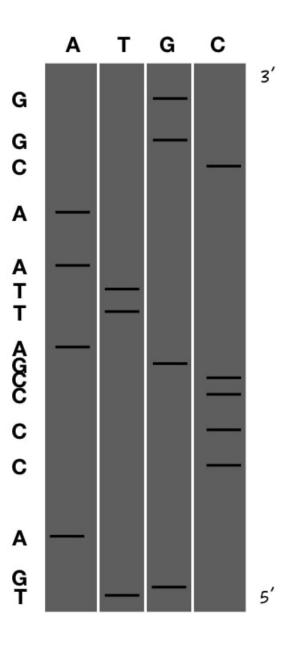




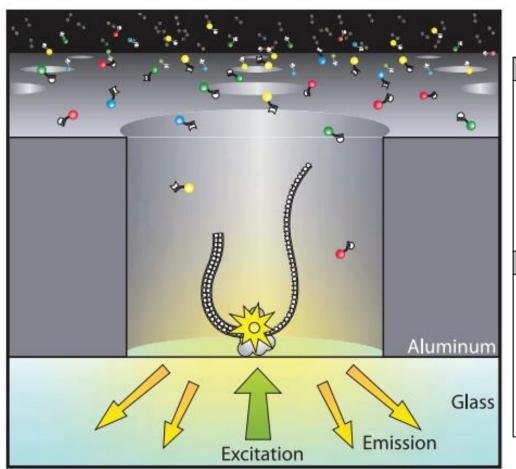


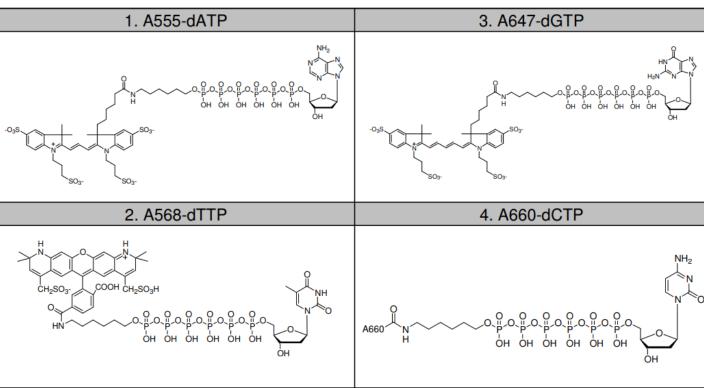
**AAT Bioquest: Sanger Sequencing** 

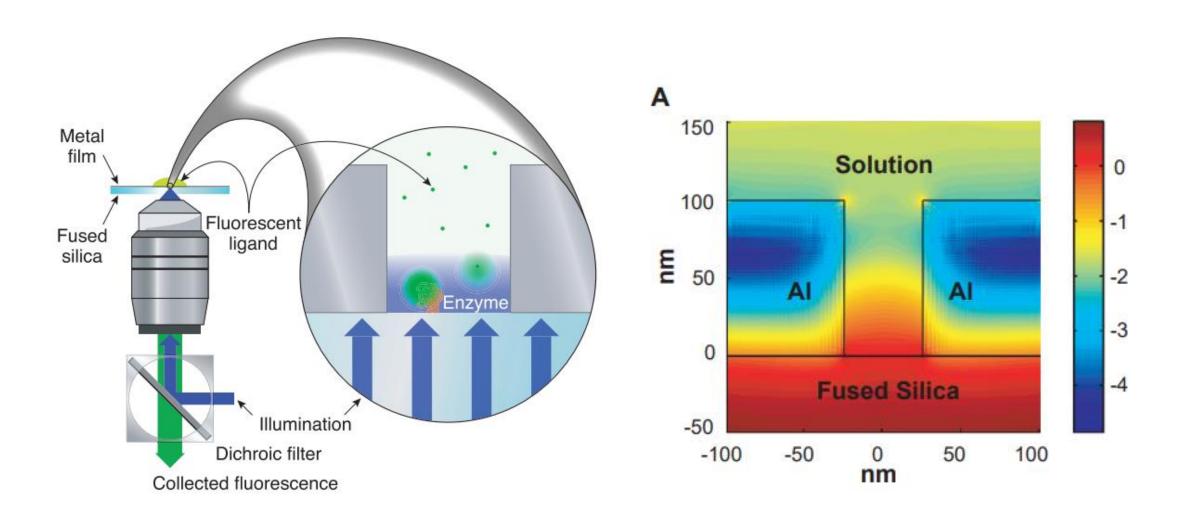




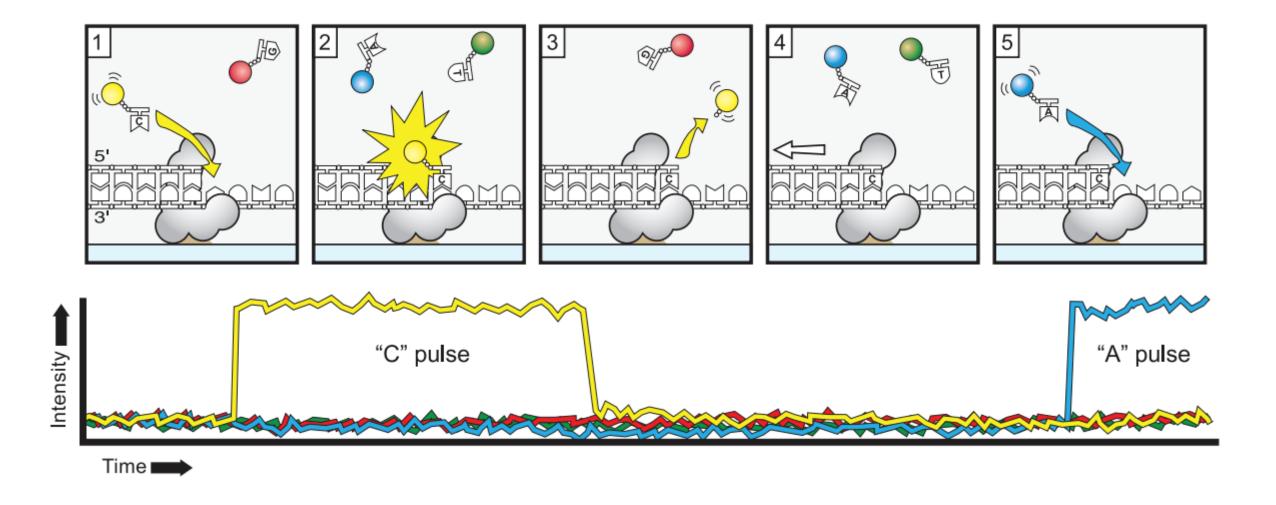
AAT Bioquest: Sanger Sequencing; Genetic Education: A Step-by-Step Process on How to Read Sanger Sequencing Gel?



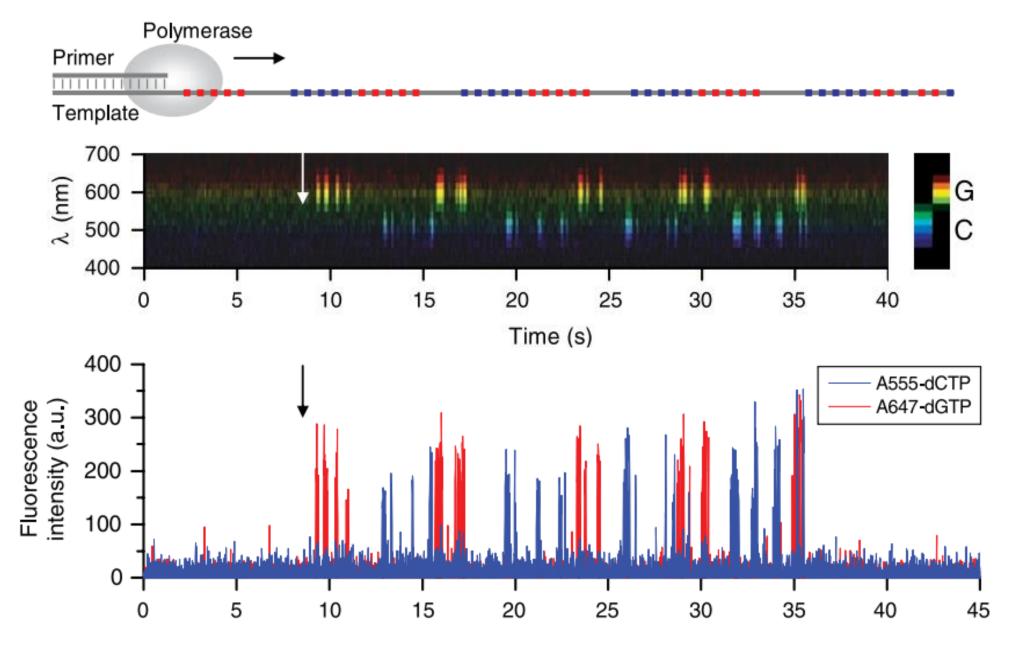




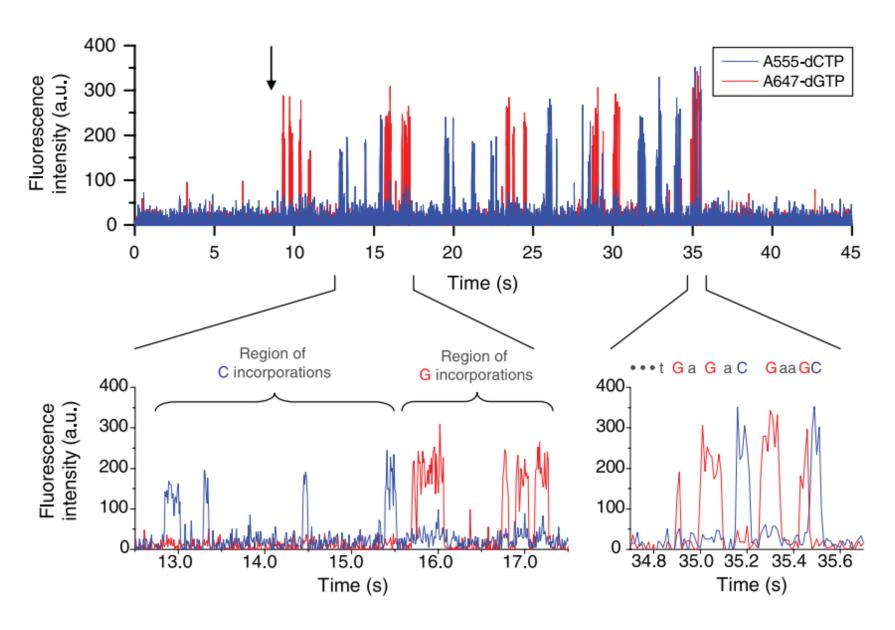
Zero-Mode Waveguides for Single-Molecule Analysis at High Concentrations, M. J. Levene et al.



Real-Time DNA Sequencing from Single Polymerase Molecules, John Eid et al.



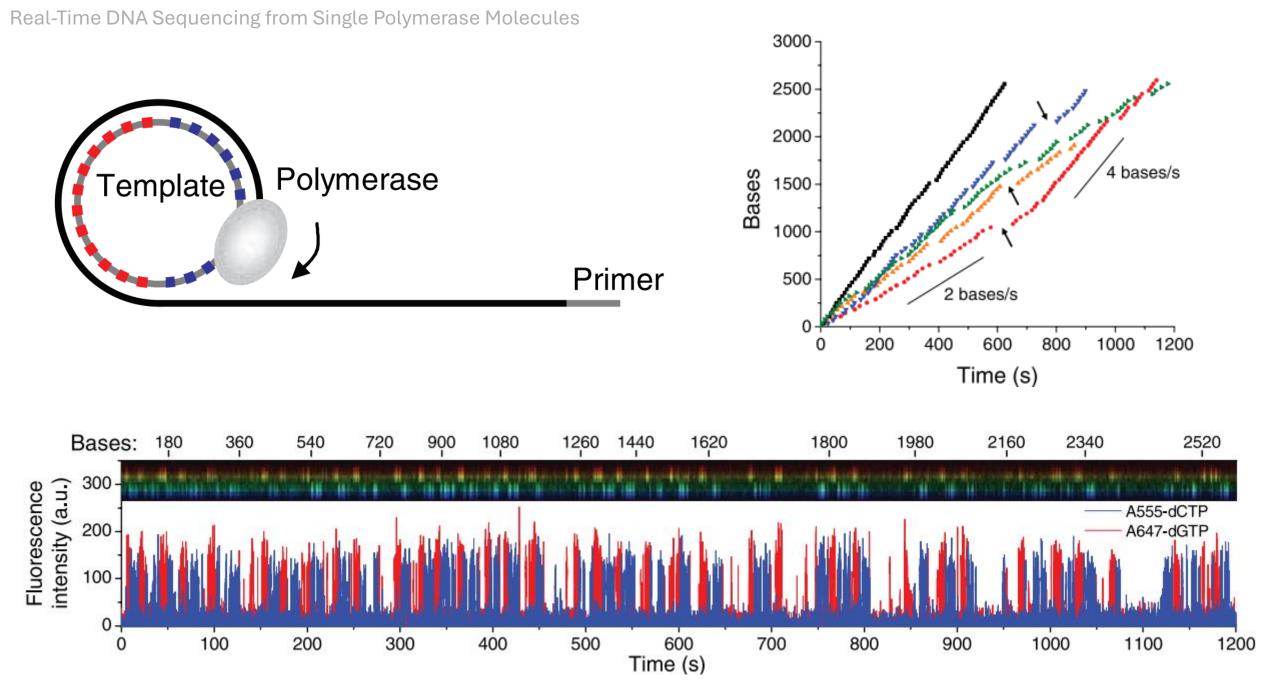
Real-Time DNA Sequencing from Single Polymerase Molecules, John Eid et al.



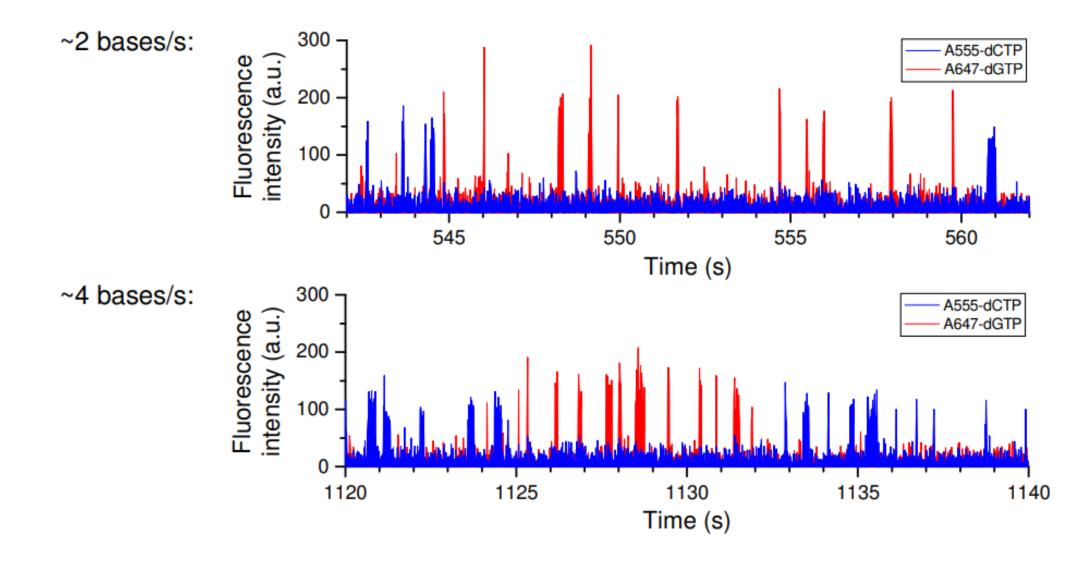
Real-Time DNA Sequencing from Single Polymerase Molecules, John Eid et al.

	A555-dCTP			A647-dGTP			Errors			Synthesis
ZMW	PW (ms)	Brightness (photons/s)	SNR	PW (ms)	Brightness (photons/s)	SNR	Mismatches	Insertions	Deletions	rate (bases/s)
1	60	4457	16	80	4274	14	2	2	1	2.9
2	60	8016	26	40	13361	30	2	3	0	4.7
3	65	8435	24	50	4937	14	1	2	2	4.5
4	80	9091	28	40	5678	12	2	3	0	2.5
5	65	6014	21	50	4176	12	1	3	0	3.4
6	35	9167	22	50	6042	17	0	1	3	5.1
7	55	4073	13	50	11258	24	3	2	0	4.0
8	50	6068	16	55	5445	16	2	2	2	3.0
9	40	7349	19	60	5728	17	0	0	4	5.7
10	90	7331	26	45	8512	21	2	1	0	5.5
All (n=740)	77 ± 30	7353 ± 2970	24 ± 10	70 ± 27	8408 ± 3381	25 ± 10	$0.6 \pm 0.5$	1.3 ± 0.8	2.1 ± 1.0	4.7 ± 1.7

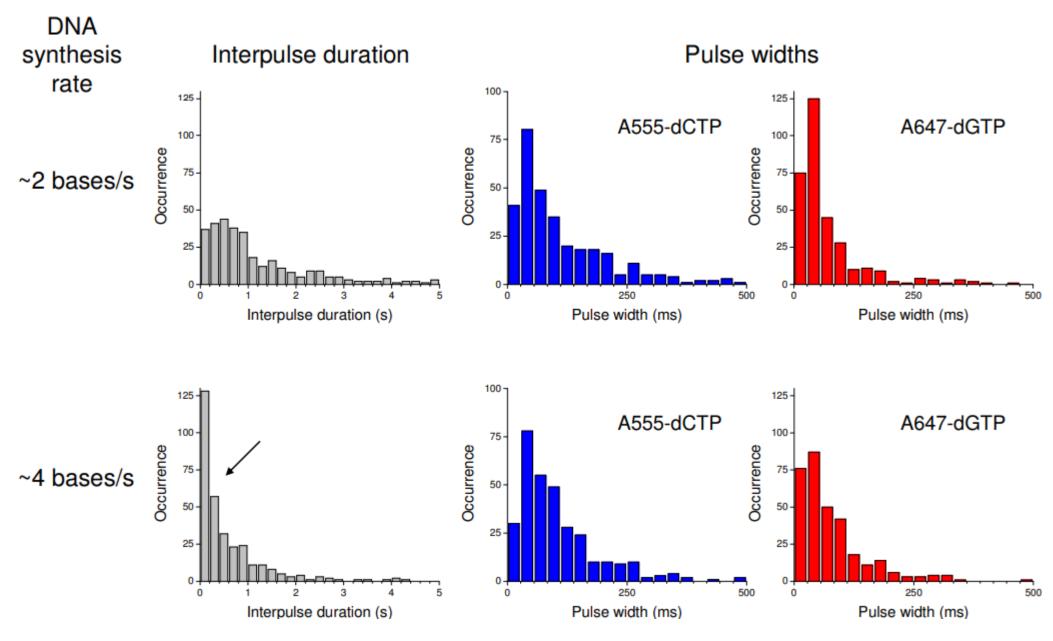
Real-Time DNA Sequencing from Single Polymerase Molecules, John Eid et al.



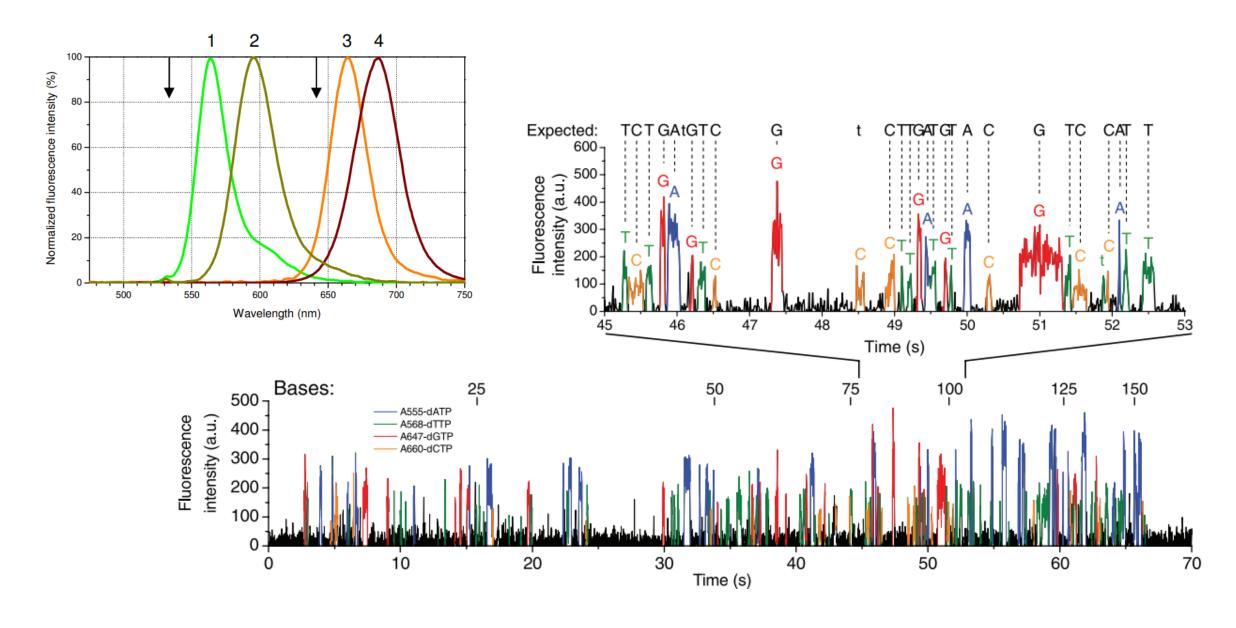
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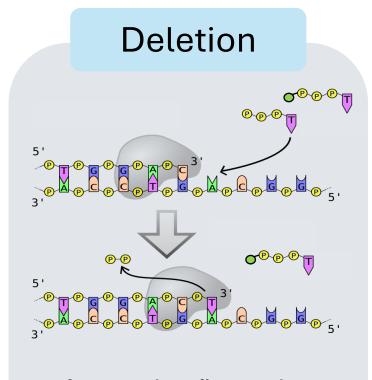
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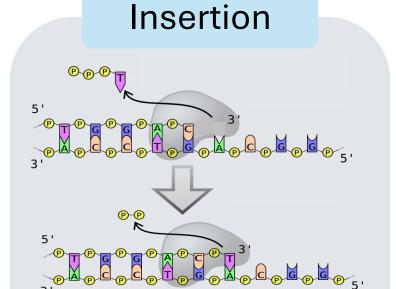
Real-Time DNA Sequencing from Single Polymerase Molecules, John Eid et al.



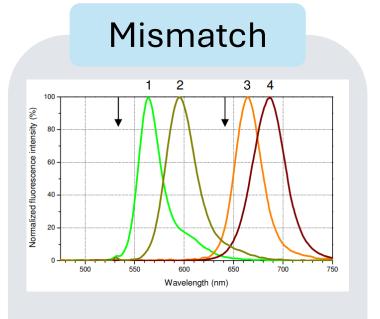
Real-Time DNA Sequencing from Single Polymerase Molecules, John Eid et al.



- Increasing fluorophore brightness
- Improving efficiency of light collection



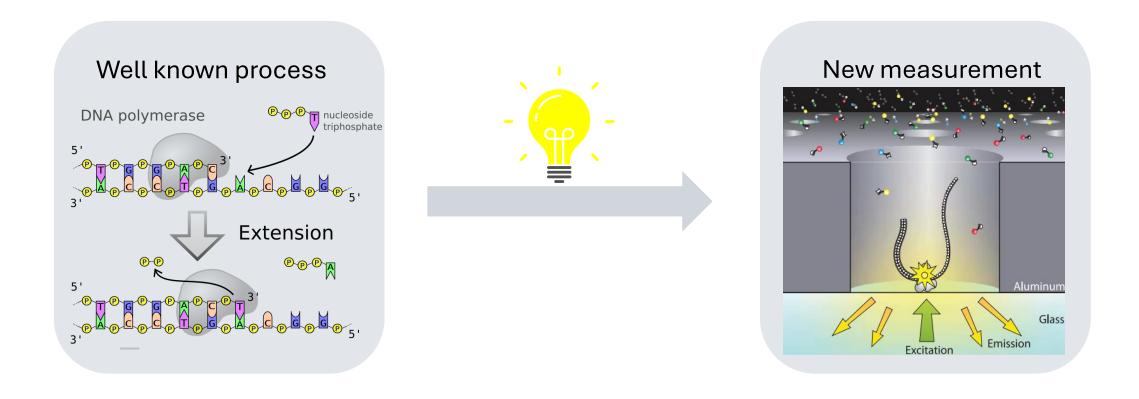
Modifying the enzyme



 Finding dye sets with larger spectral saparations

## 99,3% median accuracy

Real-Time DNA Sequencing from Single Polymerase Molecules, John Eid et al.



Meaningful research does not always have to be complicated!

Wikipedia: DNA replication; Real-Time DNA Sequencing from Single Polymerase Molecules, John Eid et al.