

Determining the Cleavage Rate of Gen Endonuclease using CE



THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL

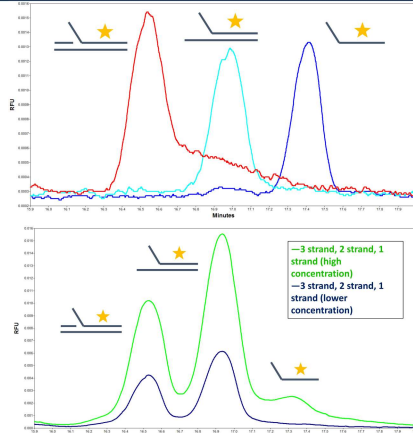
Genevieve Holliday with Kristen Irons and Shunsen Ren in the Erie Lab

Background

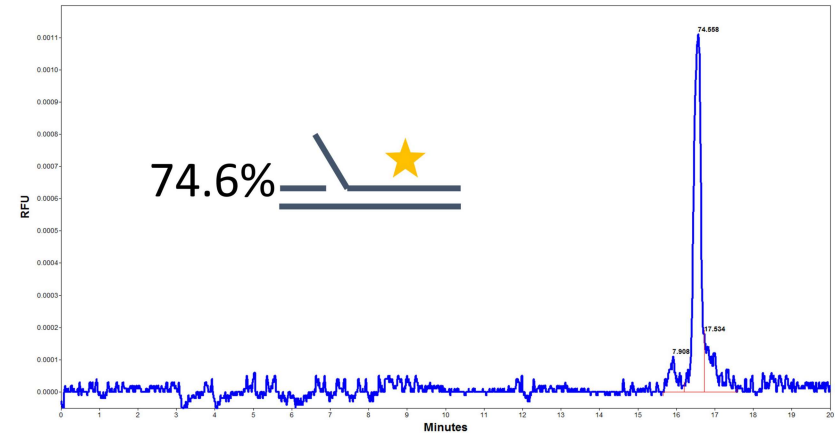


Human flap endonuclease¹ (3Q8K), similar to *Drosophila melanogaster* Gen endonuclease, cleaves 5'-flap DNA

Modifying Capillary Conditions Allows the Separation of Longer DNA in CE

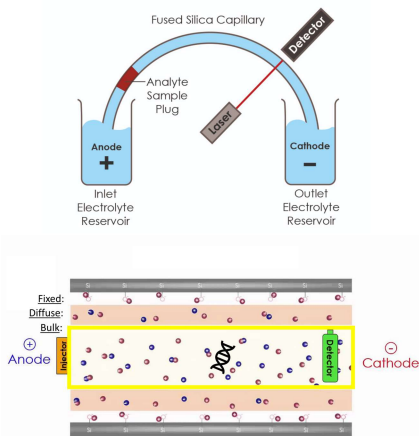


Two zoomed-in electropherograms obtained with an mPEG-silane-coated capillary, with reversed direction for the separation of poly-DT flap (~50 base pairs)

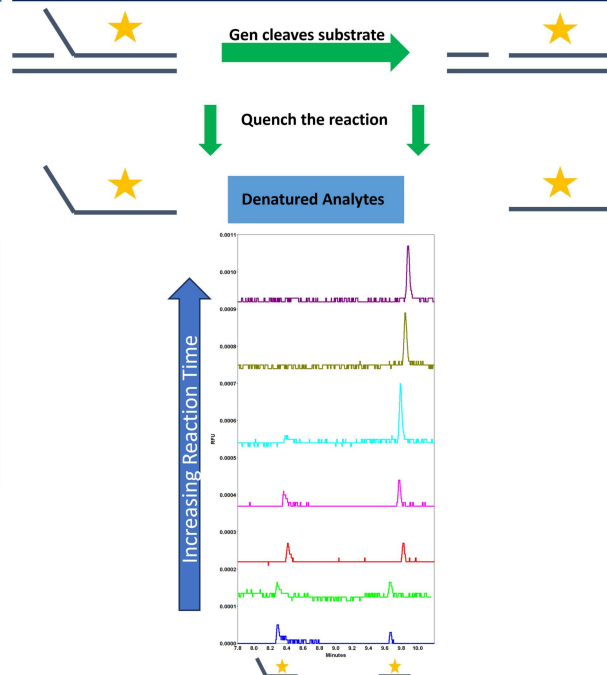


A full electropherogram for 20 nM annealed substrate with 74.6% annealing efficiency

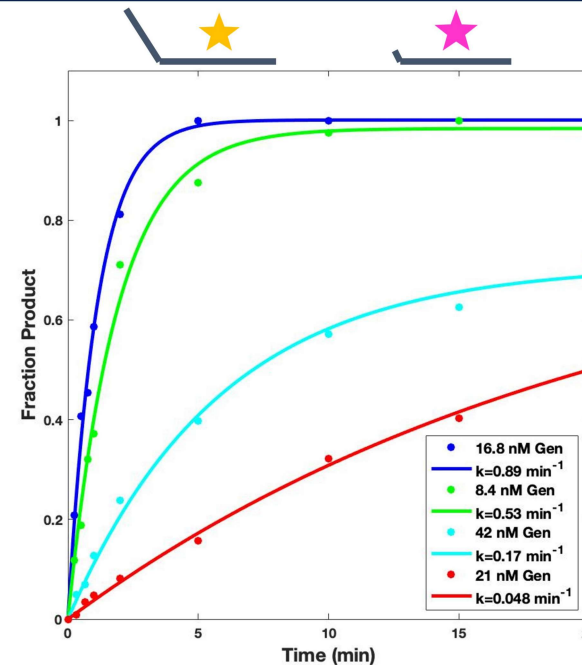
Capillary Electrophoresis



Separation of Gen and Substrate Reaction Products by CE Reveals Cleavage Rates



Seven overlaid electropherograms, each for sequential time points in the Gen cleavage reaction with 16.8 nM Gen



The overlaid Fraction Product vs. Time curves of 8.4 nM and 16.8 nM Gen cleaving a poly-DT flap and 42 nM and 21 nM cleaving a single DT flap

Conclusion

1. Tweaking capillary conditions (short end vs. long end, coating, etc.) can be used to increase separation efficiency of longer DNA substrates
2. The rate of 16.8 nM Gen was faster than that of 8.4 nM Gen
3. The cleavage rate for DNA with a relatively longer flap was faster than the rate for DNA with a shorter flap

References

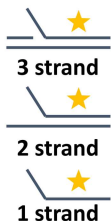
¹RCSB Protein Data Bank, <https://doi.org/10.2210/pdb3Q8K/pdb>.

²Atik Cameras, <https://www.atik-cameras.com/capillary-electrophoresis/>.



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Two purposes:
1. Determine the annealing efficiency of the flap substrate. CE² separates the products shown to the right:



2. Determine the cleavage rate of a flap substrate in complex with Gen (like the one pictured in the background section)