Rapid Chemical Quench Flow

Technical Specifications

2 ms minimum reaction time
10,000 s maximum reaction time

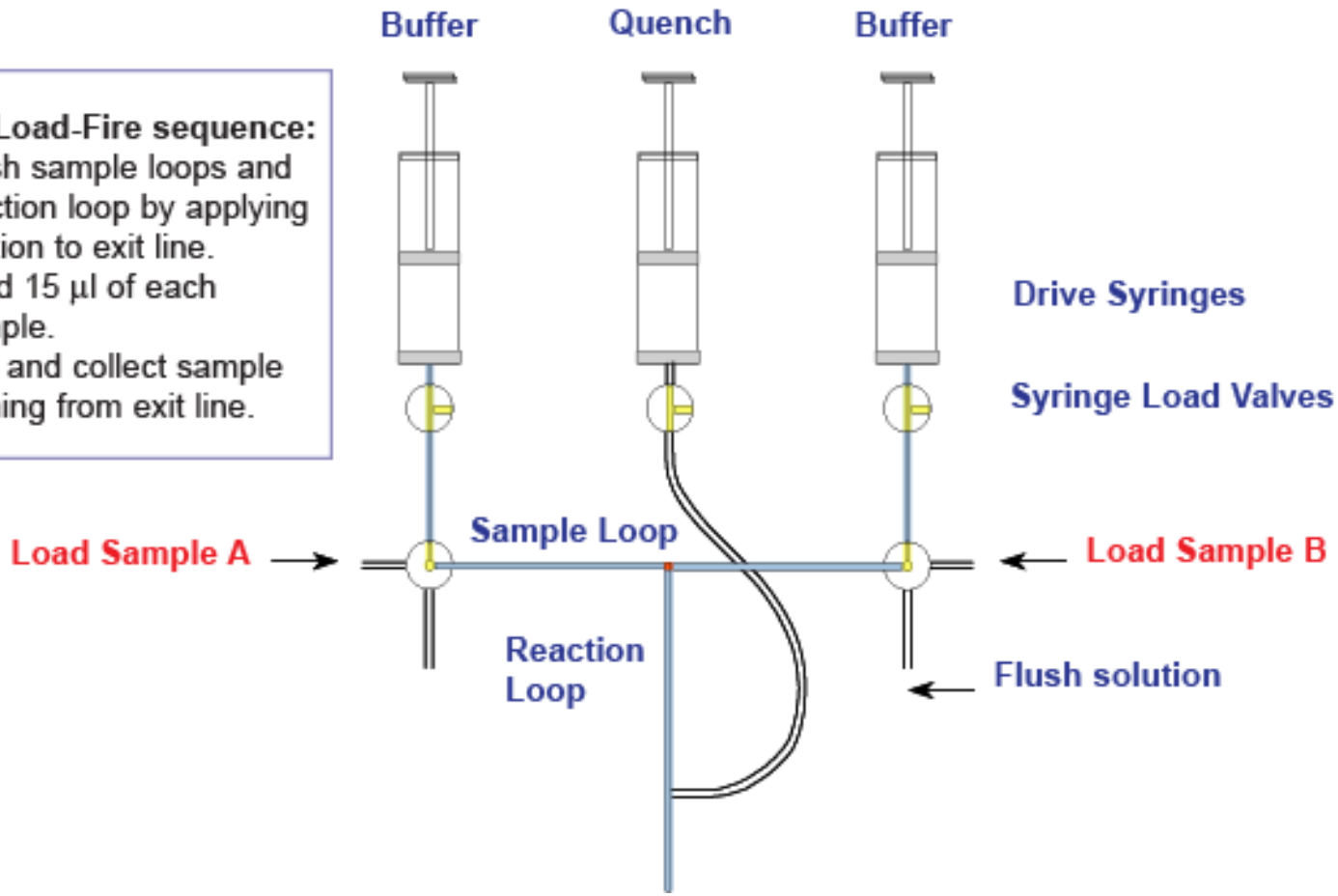
20 μL sample volume (per reaction)

Anaerobic operation possible (just need to put it in glove box).

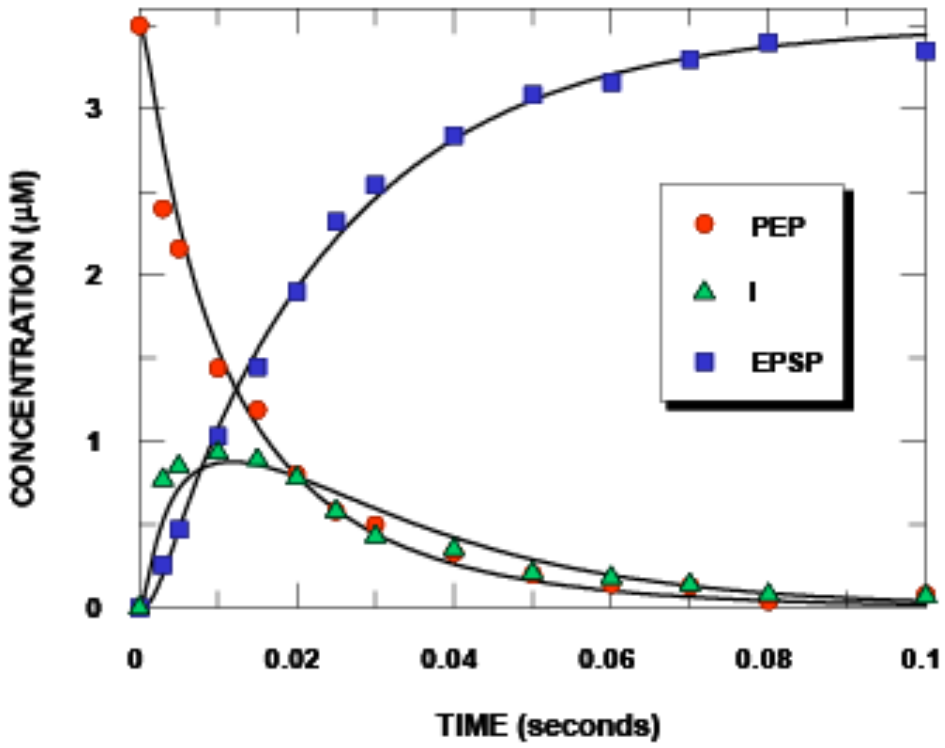
Relatively straightforward to use.

Flush-Load-Fire sequence:

1. Flush sample loops and reaction loop by applying suction to exit line.
2. Load 15 µl of each sample.
3. Fire and collect sample coming from exit line.



Sample Experiment



The chemical quench-flow provides unique information to define the reaction sequence and identify enzyme intermediates. The data on the right shows the time course of a single enzyme turnover obtained using a KinTek RQF-3 for the reaction catalyzed by the enzyme EPSP synthase:

PEP + S3P ⇆ I ⇆ EPSP + Pi

The reaction proceeds by an addition-elimination mechanism through a transiently formed tetrahedral intermediate (**I**). The intermediate is formed in the first 10 milliseconds and decays with the formation of the product, EPSP over the next 80 msec.

This study which led to the identification and isolation of this intermediate serves as an example for the detection of enzyme intermediates [Anderson, K. S., Sikorski, J. A. and Johnson, K. A. (1988) A Tetrahedral Intermediate in the EPSP Synthase Reaction Observed by Rapid Quench Kinetics. *Biochemistry* 27, 7395-7406.] For more information on how to design and interpret single turnover experiments see: Johnson, K. A. (1992) Transient State Kinetic Analysis of Enzyme Reaction Pathways. *The Enzymes* XX, 1-61.

Current Status

The instrument currently resides in 6000A Malott (Chris Fischer Lab).

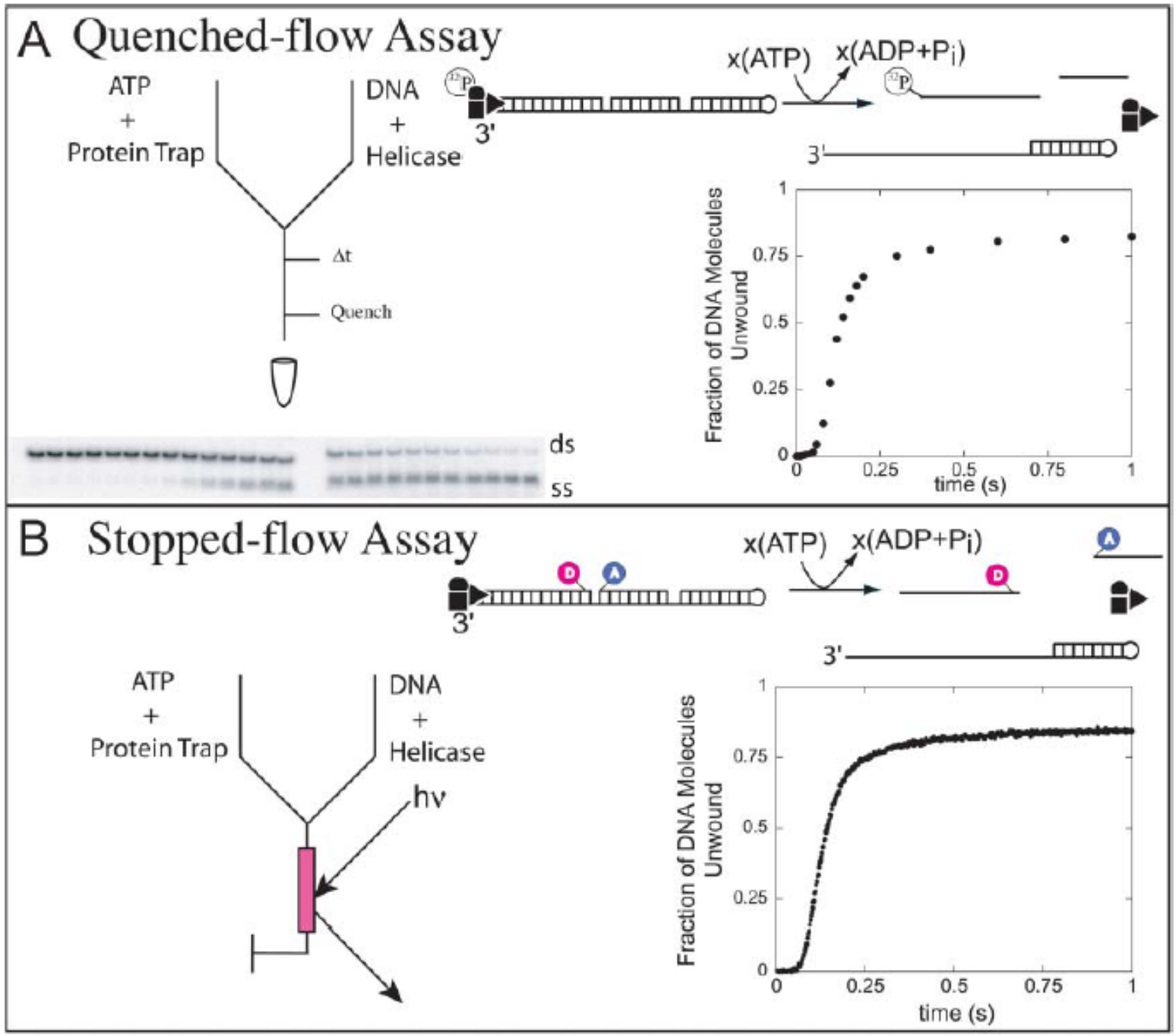
Using the instrument requires EHS training.

All are welcome to use the instrument!

Additional Plug

An excellent complimentary technique for chemical quench flow is fluorescence stopped-flow.

This we also have in the lab – all are welcome to use it, too!


A Quenched-flow Assay
ATP
+
Protein Trap
DNA
+
Helicase
Δt
Quench
ds
ss
x(ATP)
x(ADP+Pi)
3'
Fraction of DNA Molecules Unwound
time (s)
B Stopped-flow Assay
ATP
+
Protein Trap
DNA
+
Helicase
hν
x(ATP)
x(ADP+Pi)
3'
Fraction of DNA Molecules Unwound
time (s)

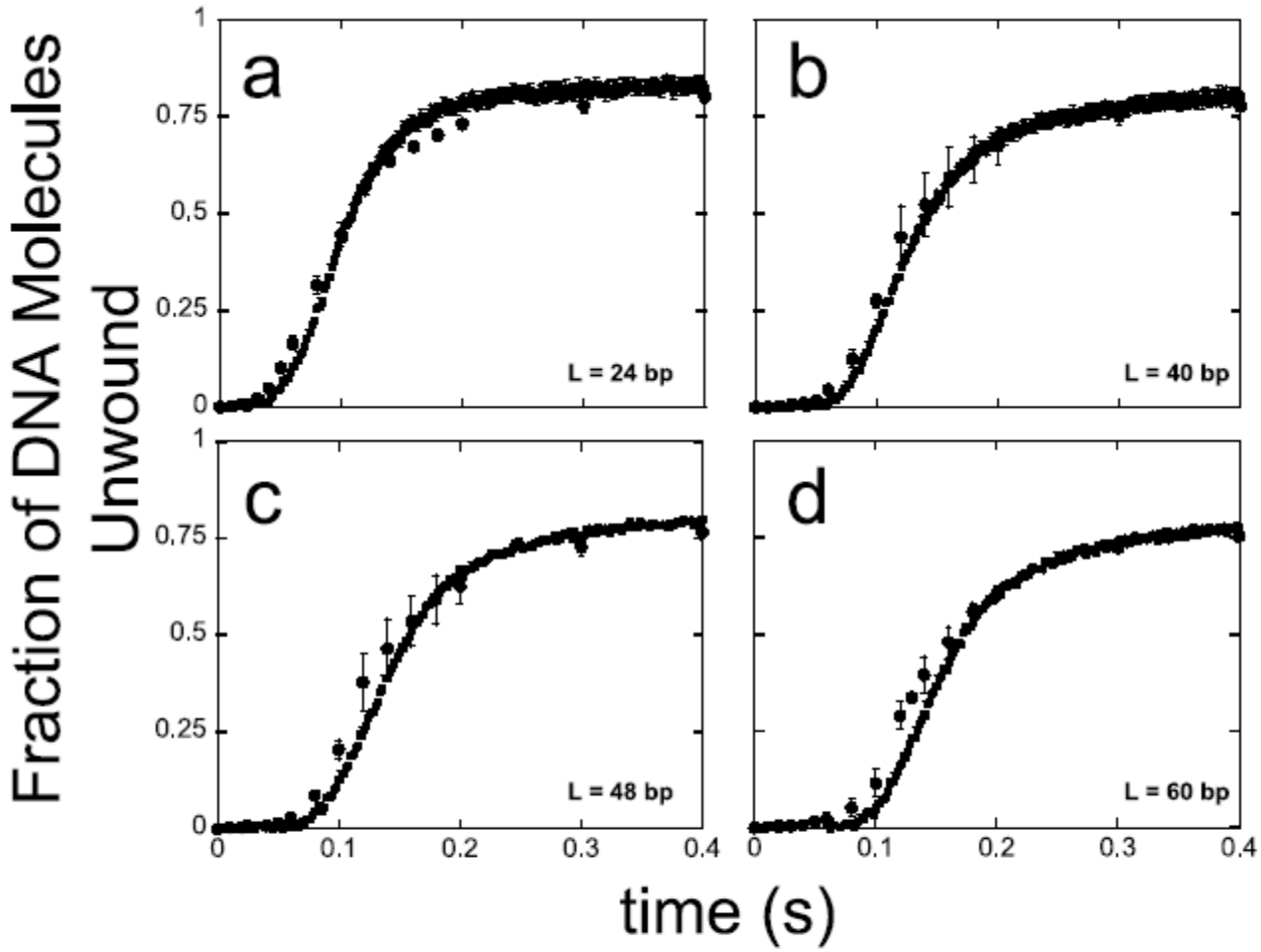


Figure 6. Comparison of single turnover RecBCD-catalyzed DNA unwinding time-courses obtained by chemical quenched-flow *versus* stopped-flow methods. The quenched-flow data (●) in each panel are taken from our study,[23] and represent averages from five independent experiments; the error bars represent the standard deviation of the average. The stopped-flow data for each duplex length were acquired in buffer M at 25 °C as described in Materials and Methods. The Cy3 fluorophore was excited with an excitation wavelength, $\lambda_{ex} = 515$ nm, and the Cy3 fluorescence was monitored at 570 nm using an interference filter. The Cy3 fluorescence time-courses (■) were normalized to the fraction of DNA molecules unwound at 2 s from the chemical quenched-flow experiments. a, $L = 24$ bp, b, $L = 40$ bp, c, $L = 48$ bp, and d, $L = 60$ bp.