Complex Molecular Tests at the Point of Care

Current: 1-hour sample to answer

Future: <1 min PCR

Carl Wittwer, MD, PhD, Department of Pathology, University of Utah

AACC Critical and Point of Care Testing, San Diego, Sept 18, 2014

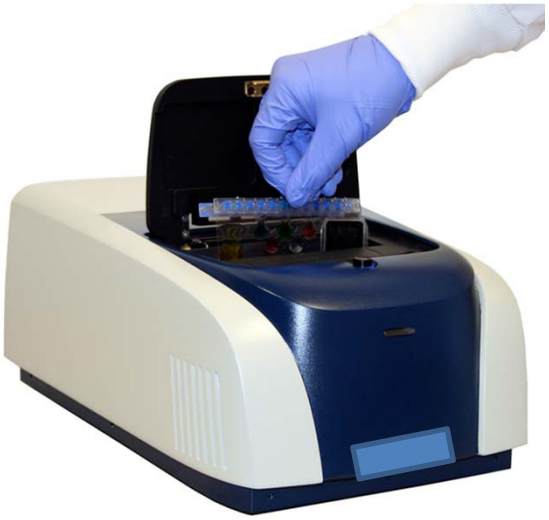
Conflicts of Interest

- BioFire/Idaho Technology
  - CoFounder and Chairman
    - Royalties, Grants, Board
- Canon US Life Science
    - Grants
- ARUP
    - Grants
- NEB
    - Scientific Advisor
- Clinical Chemistry
    - Associate Editor

Integrated Near to the Patient Diagnostics

- Sytem #1
  - Sonication
  - Syringe
  - 6 real-time PCR channels

- System #2
  - Mechanical Disruption
  - Pneumatics
  - 102 high resolution melting wells



1 hour sample-to-answer molecular testing

- Microbe disruption
- Nucleic acid isolation
- Reverse transcription
- First PCR
- Nested PCR
- Analysis
  - Real time PCR
  - High resolution melting
- Multiplexing

Number of Organisms Tested

(comprehensive syndromic panels)

	Respiratory	Blood Culture	Gastrointestinal
Viruses	17		5
Bacteria	3	19	13
Fungi		5	
Parasites			4
Total	20	24	22

Sensitivity/Specificity

	Respiratory	Blood Culture	Gastrointestinal
Viruses	95.9/99.3	98.3/99.8	98.0/99.2
Bacteria	100/100	98.3/99.8	99.2/98.9
Fungi		99.3/99.9	
Parasites			98.5/99.8

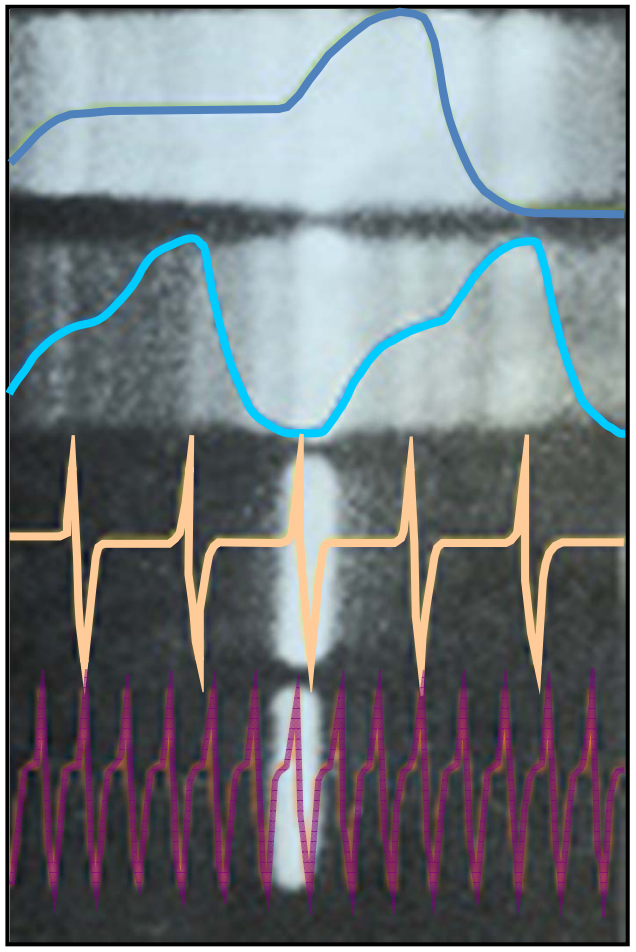
Advantages

- Simple
  - Class 2 (potential CLIA waiver)
- Fast
  - About 1 hour
- Available now
- Comprehensive
  - Clinical syndrome-based diagnostic

Rapid-Cycle PCR

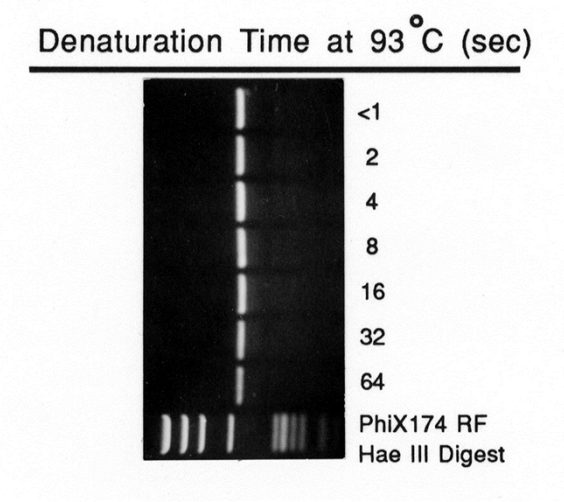
(20-60 second cycles)

- 30 cycles in 10-30 min
- Improved specificity



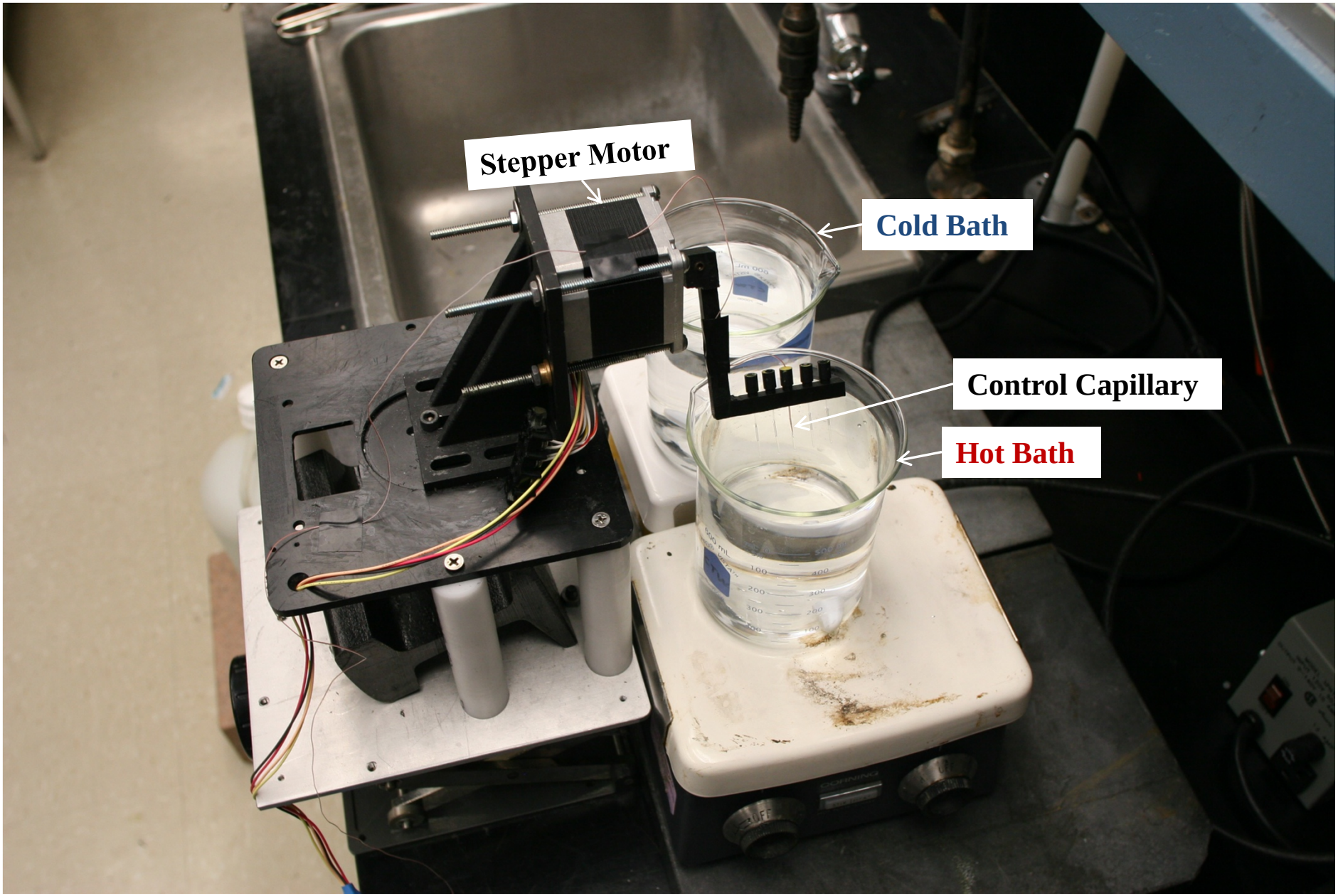
BioTechniques cover: Jan. 1991

How long does it take to….

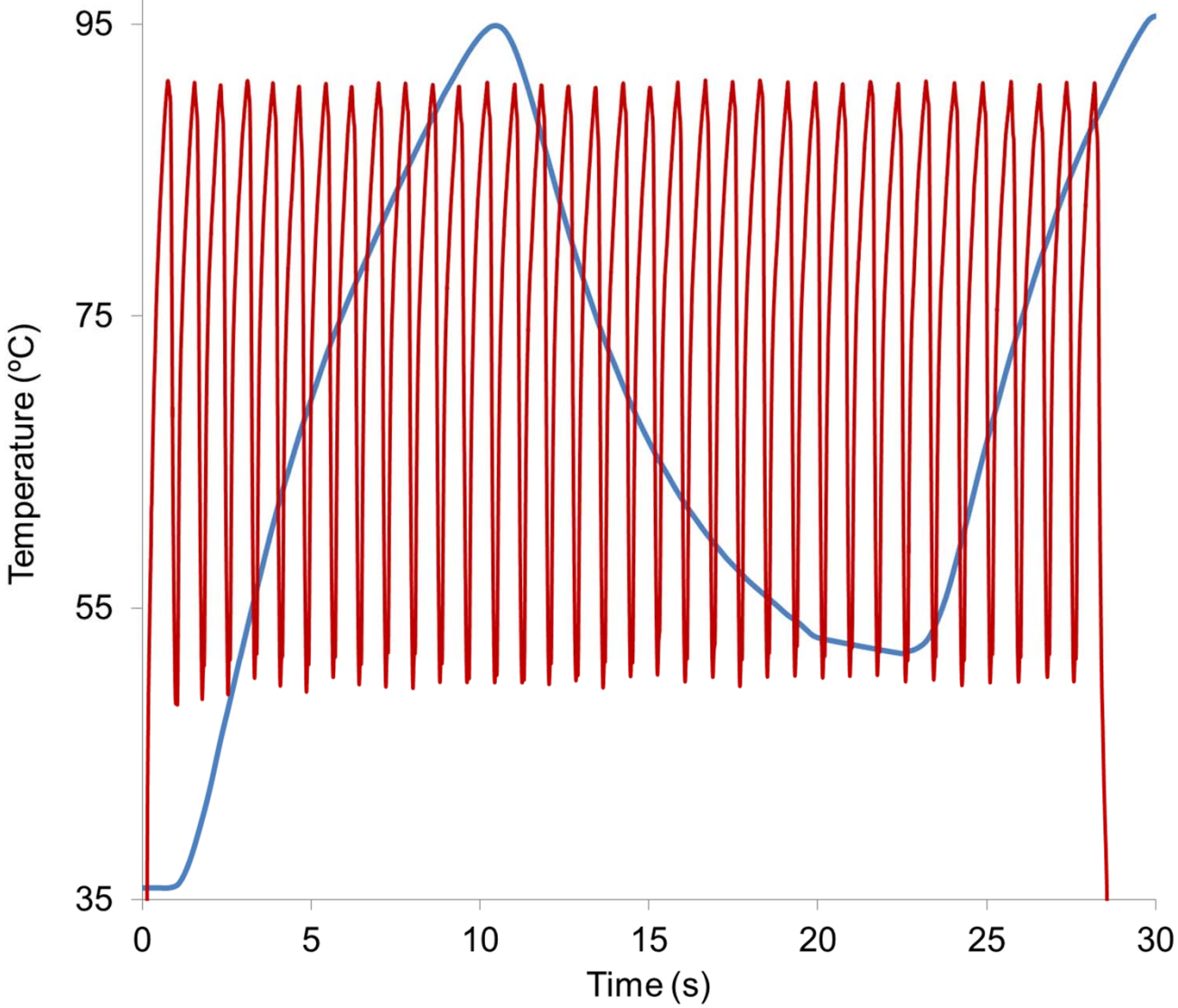


- **Denature**
  - Fast! (<1 sec)
- **Anneal**
  - Depends on [Primers], not [Product]
- **Extend**
  - Complex
  - Depends on [Polymerase]

Back to Water Baths….

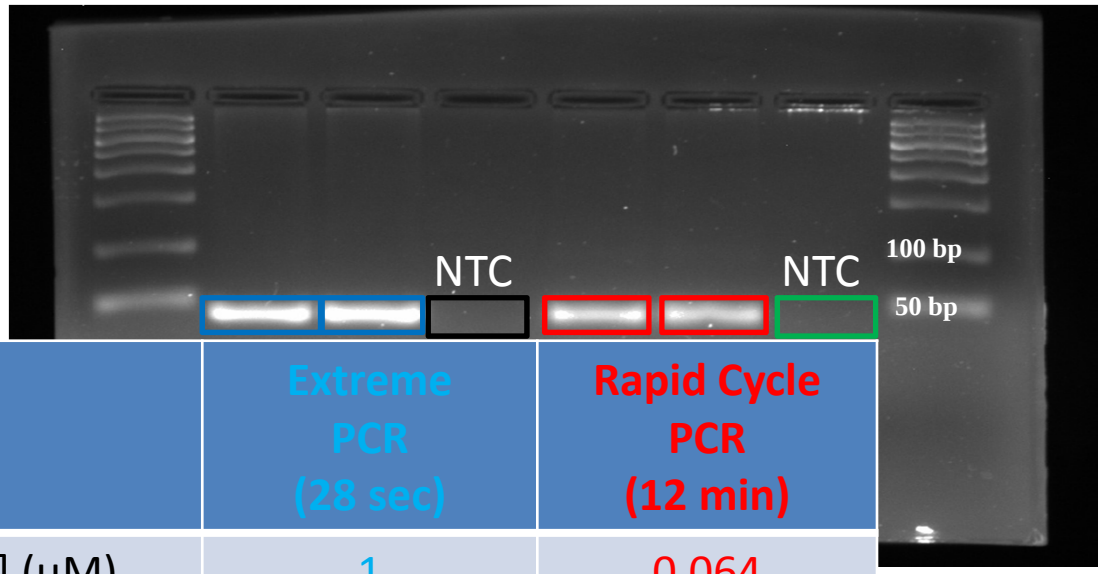


Extreme vs Rapid Cycle PCR

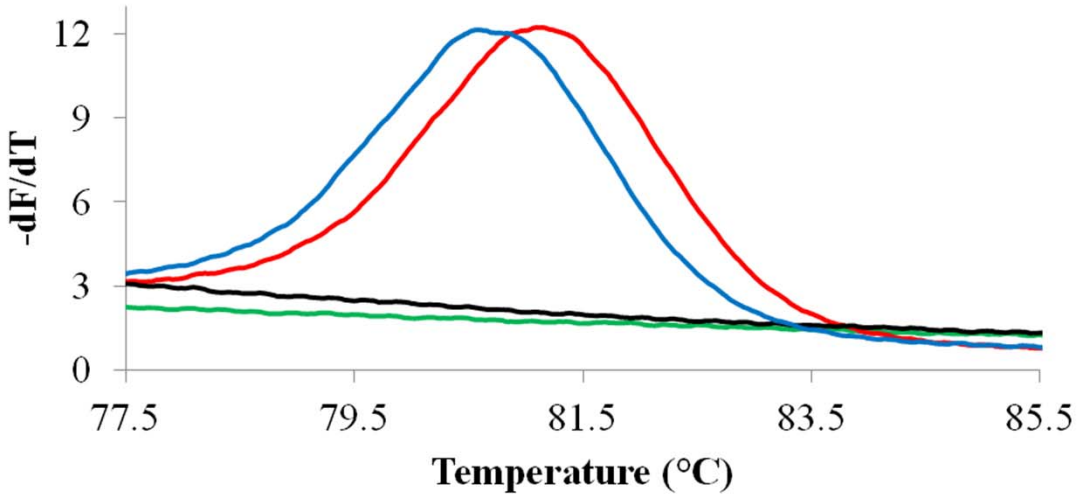


Extreme PCR compared to Rapid Cycle PCR

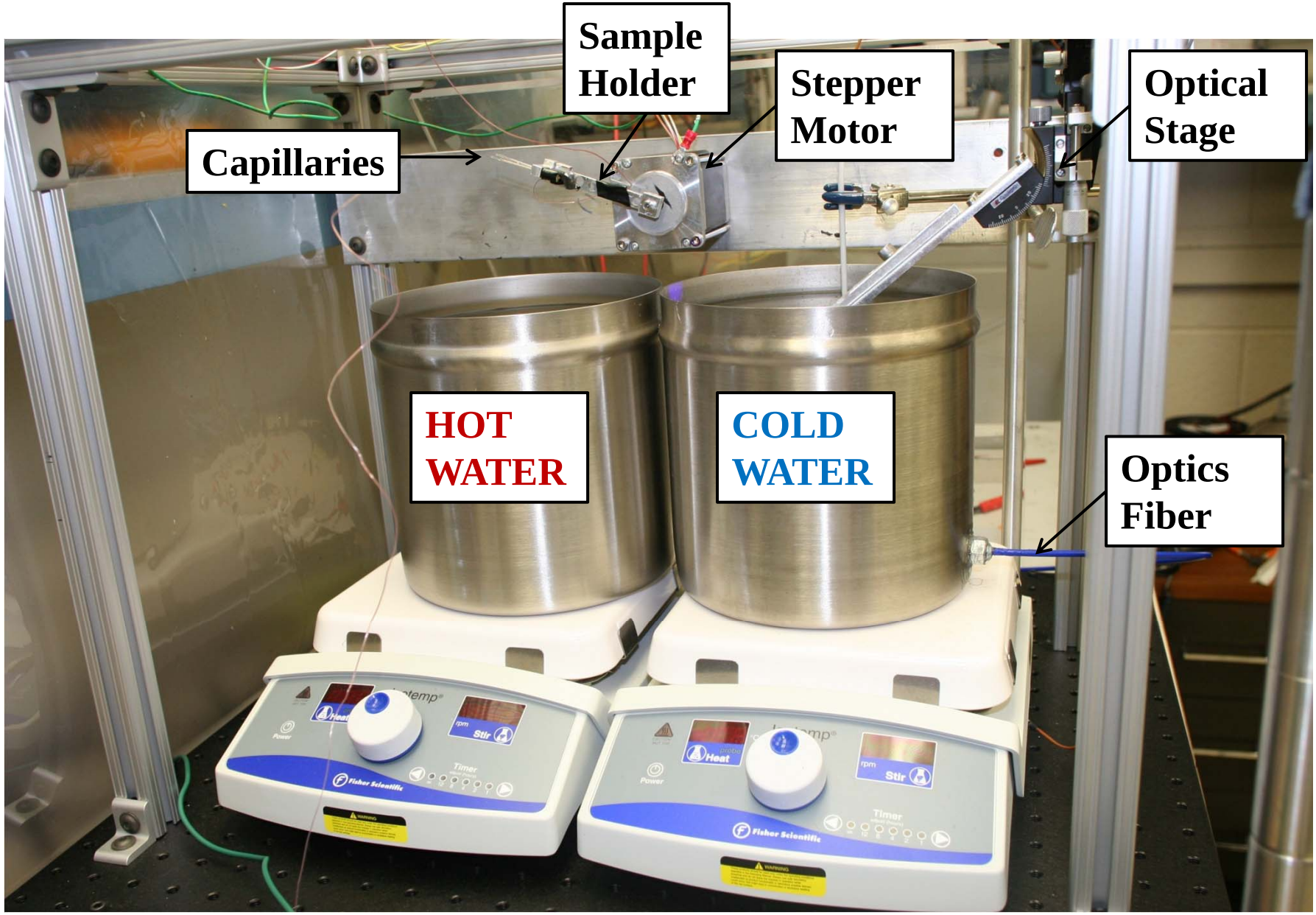
(45 bp human genomic target *KCNE1*)



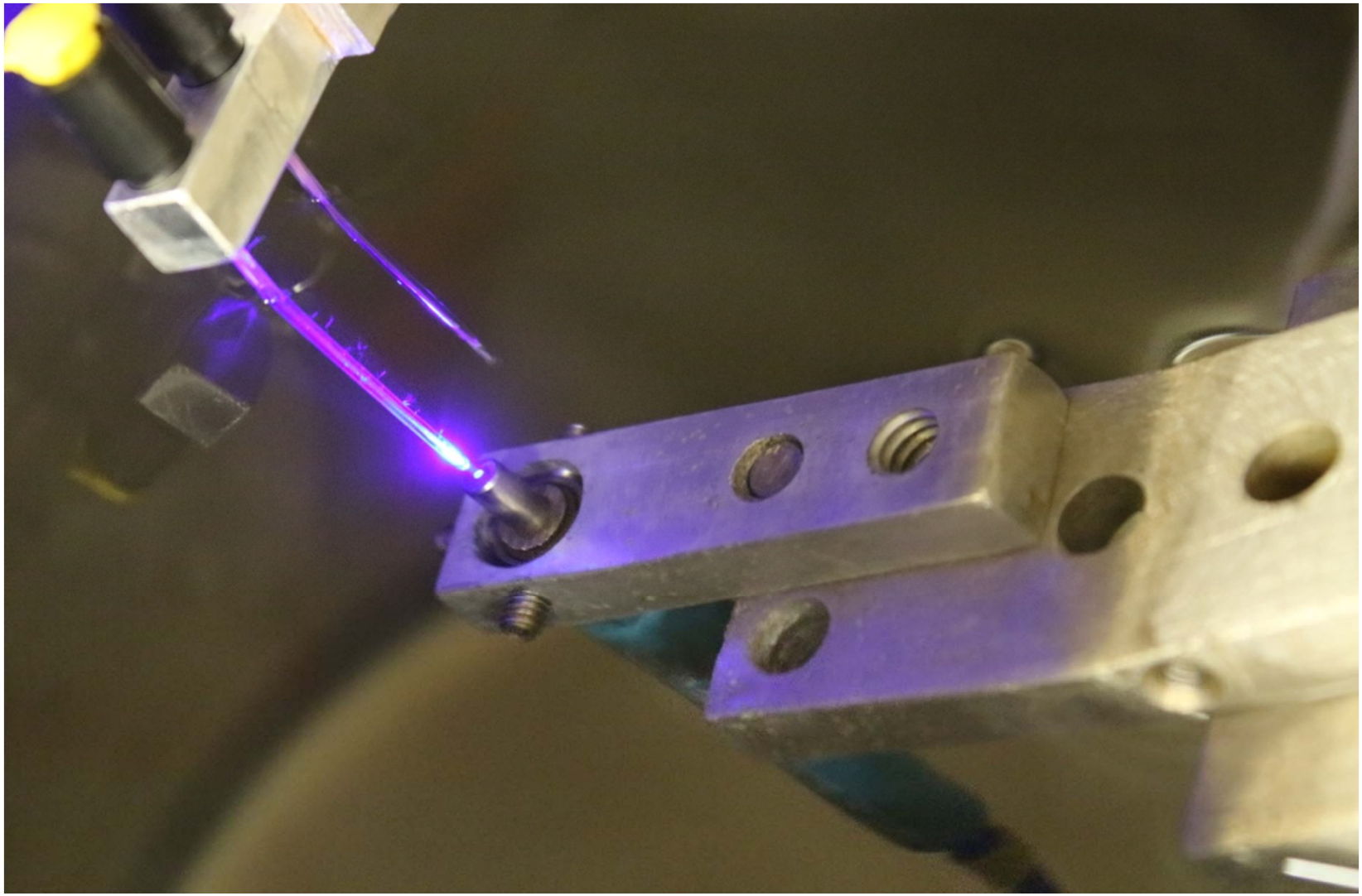
	Extreme PCR (28 sec)	Rapid Cycle PCR (12 min)
[Polymerase] (µM)	1	0.064
[Primers] (µM)	10	0.5

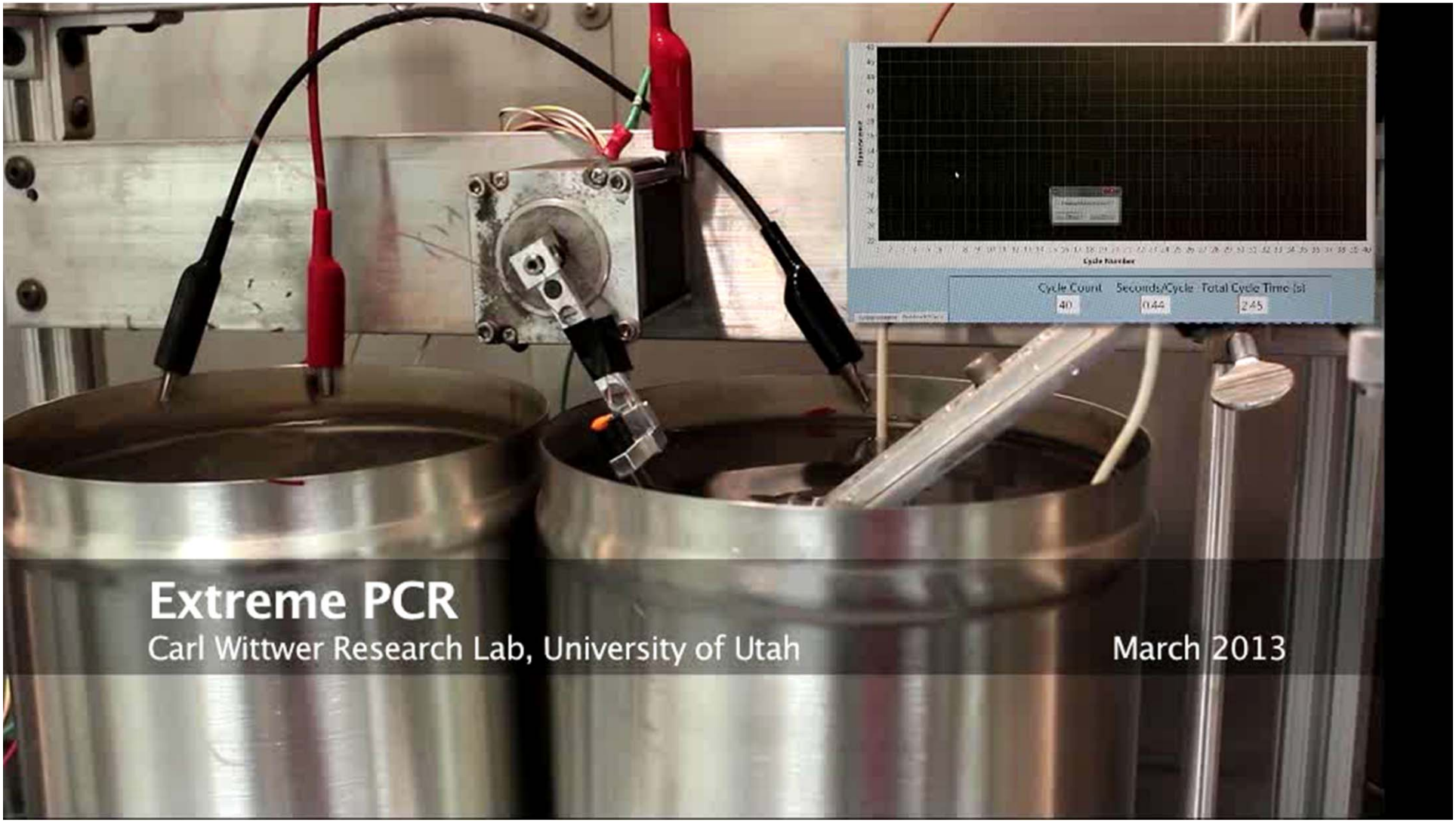


Real time Extreme Instrument



Sample Interrogation



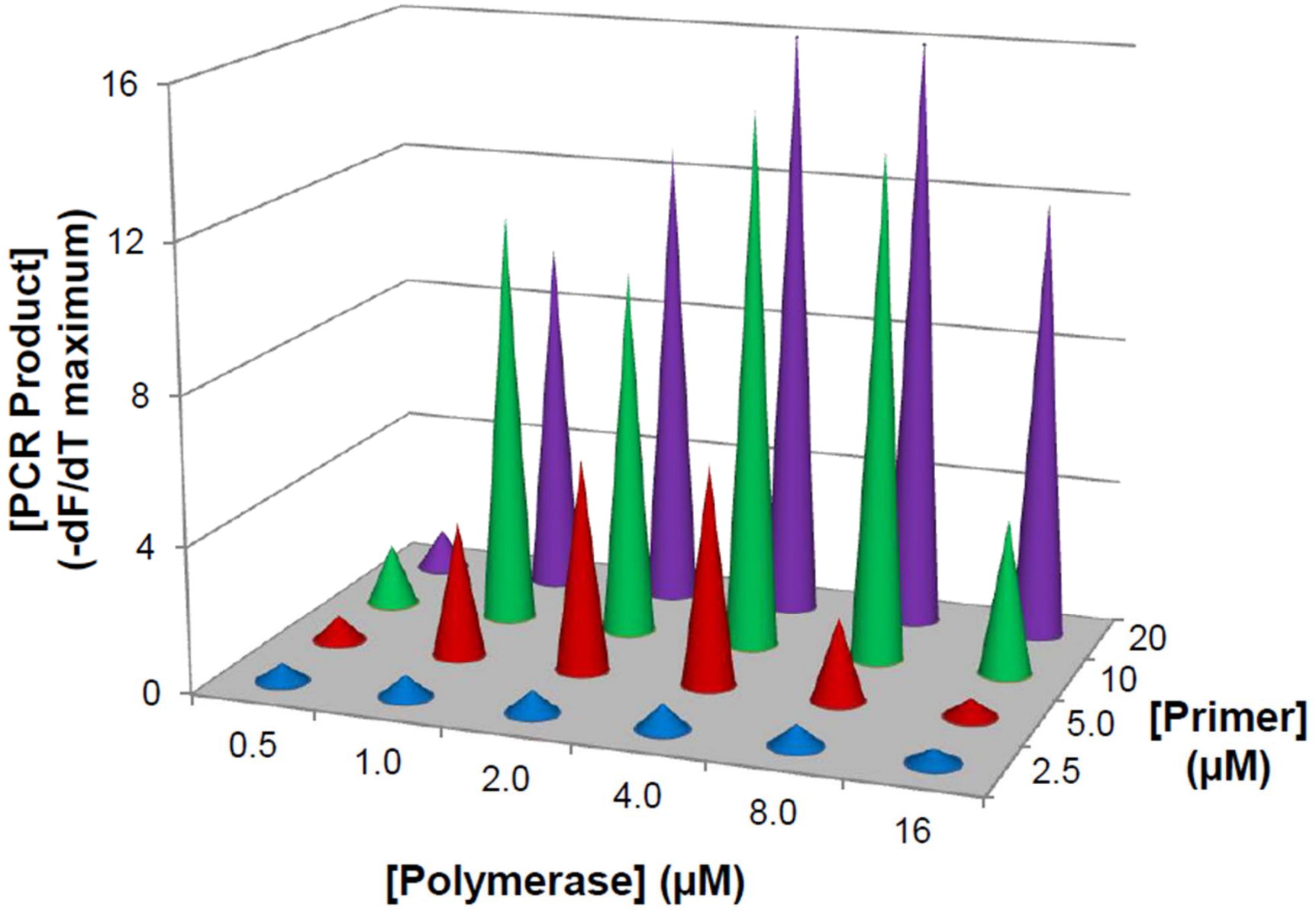


Extreme PCR

Carl Wittwer Research Lab, University of Utah

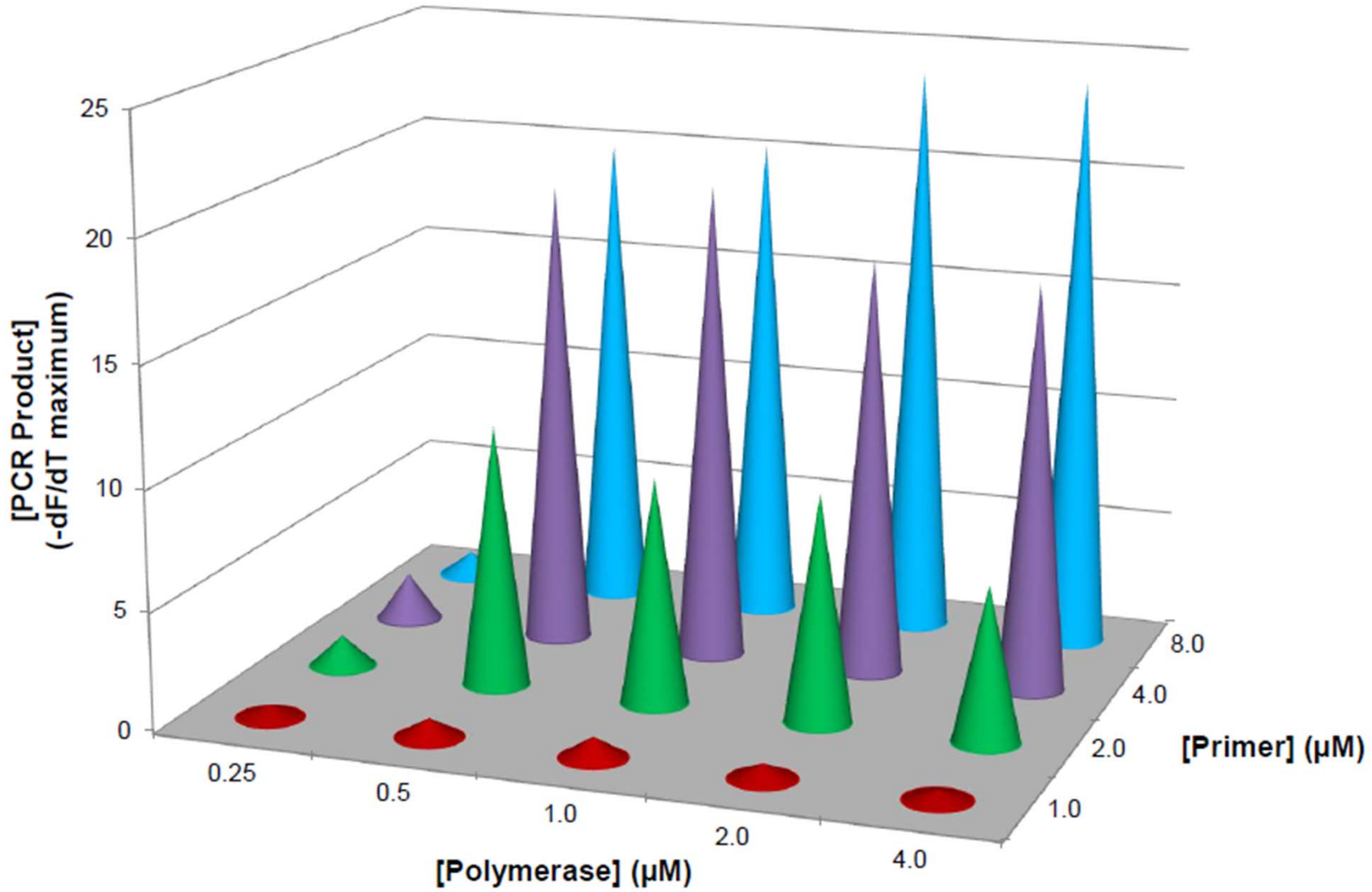
March 2013

Polymerase and Primer Optimization *IRL10RB* (49 bp)



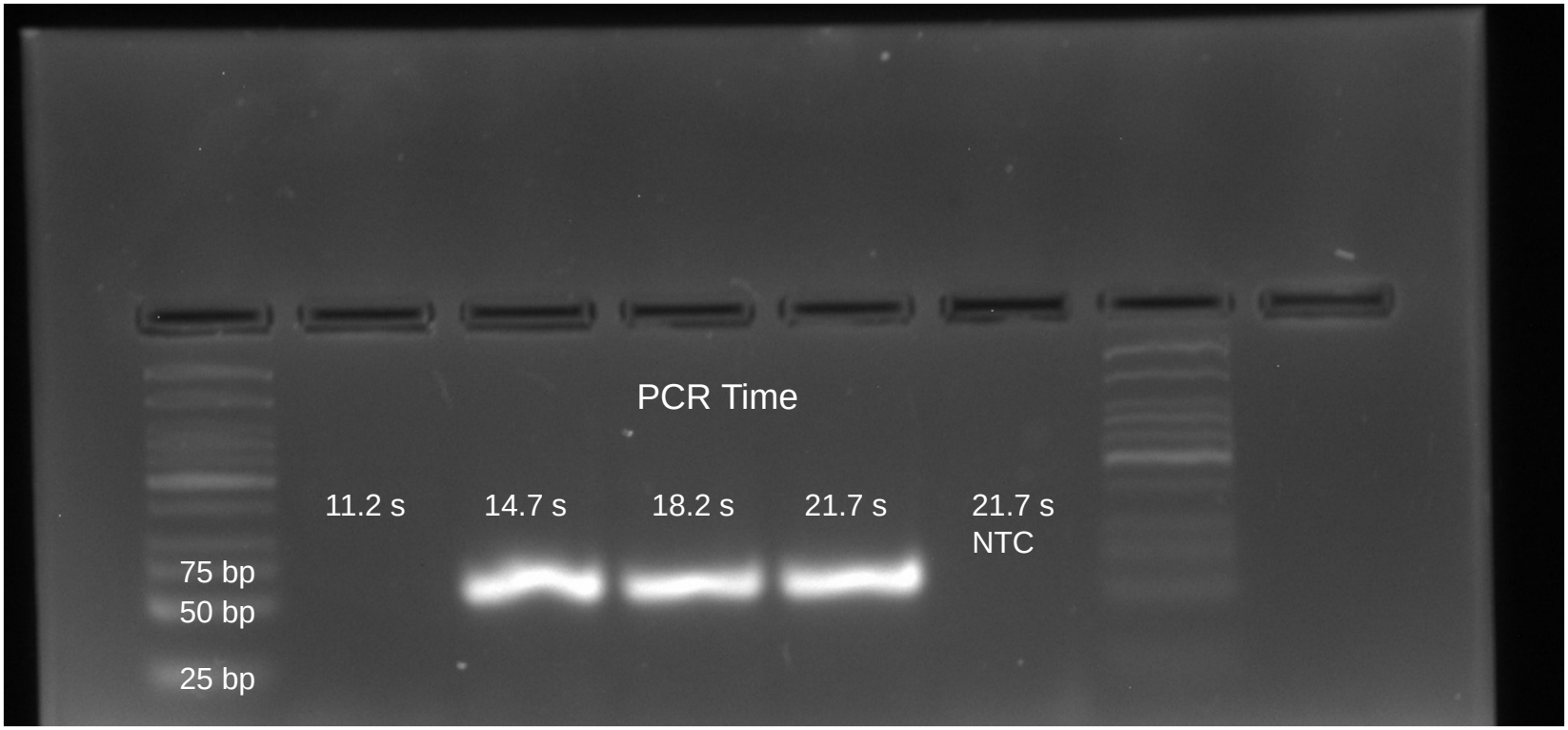
Polymerase and Primer Optimization *NQO1* (102 bp)

58 sec PCR (30 cycles, 1.93 sec/cycle)



14.7 second PCR

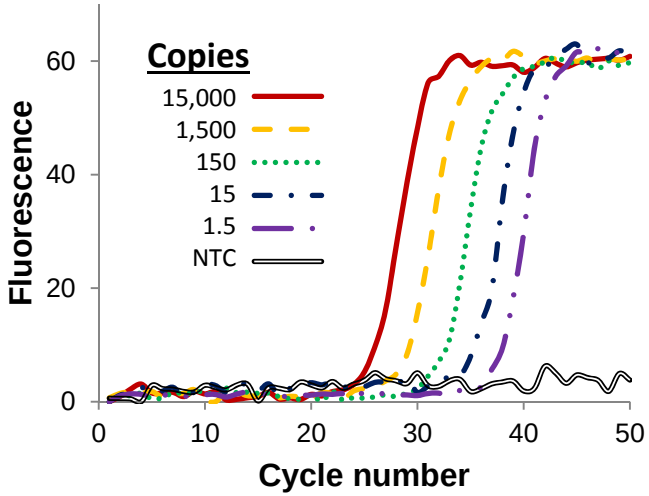
60 bp *AKAP10* (35 cycles, 0.42 sec/cycle)

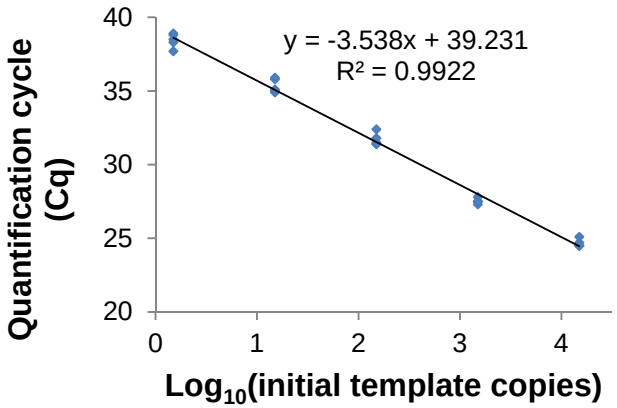


8 µM polymerase, 20 µM Primers, 5 mM $MgCl_2$

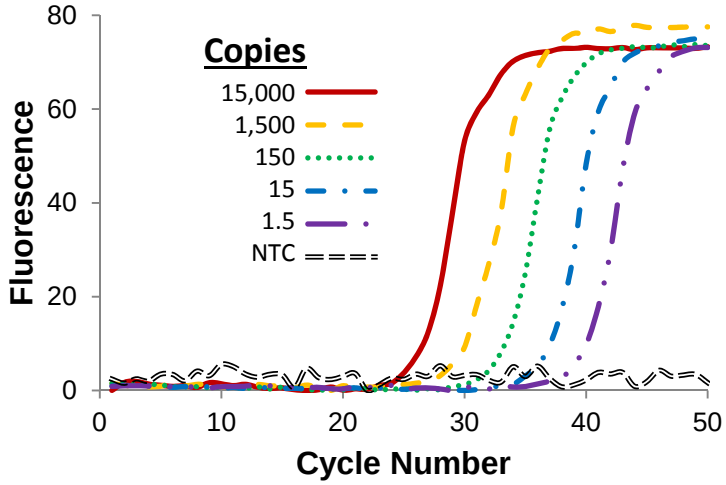
Extreme PCR Efficiency and Sensitivity

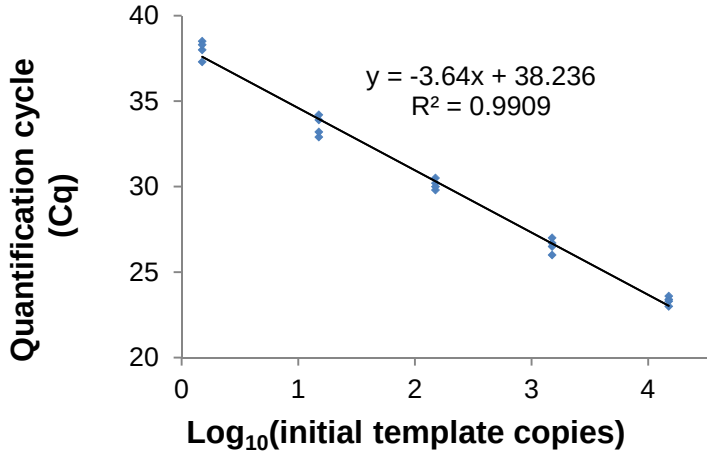
91.7% (45 bp, 28 sec PCR)





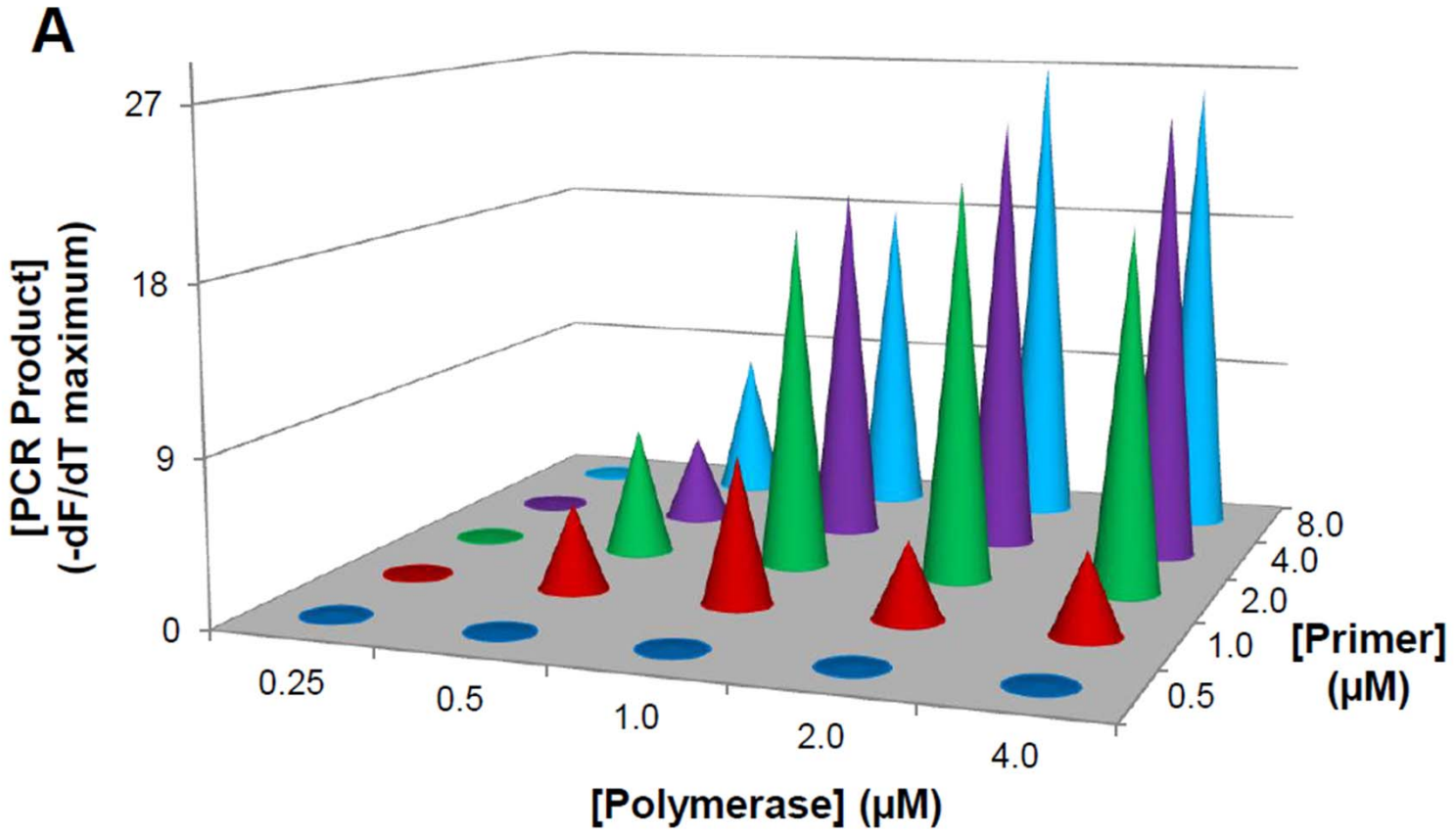
95.8% (102 bp, 58 sec PCR)





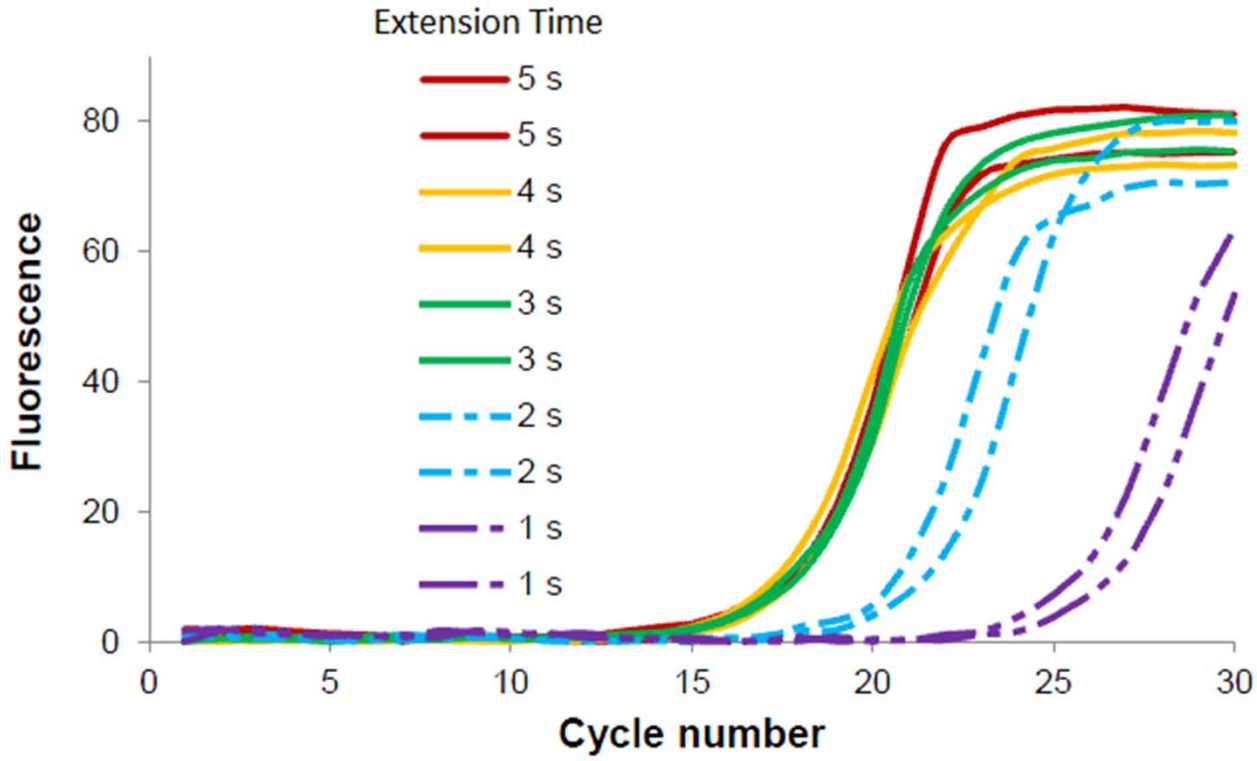
Polymerase and Primer Optimization

(Synthetic Template, 300 bp)



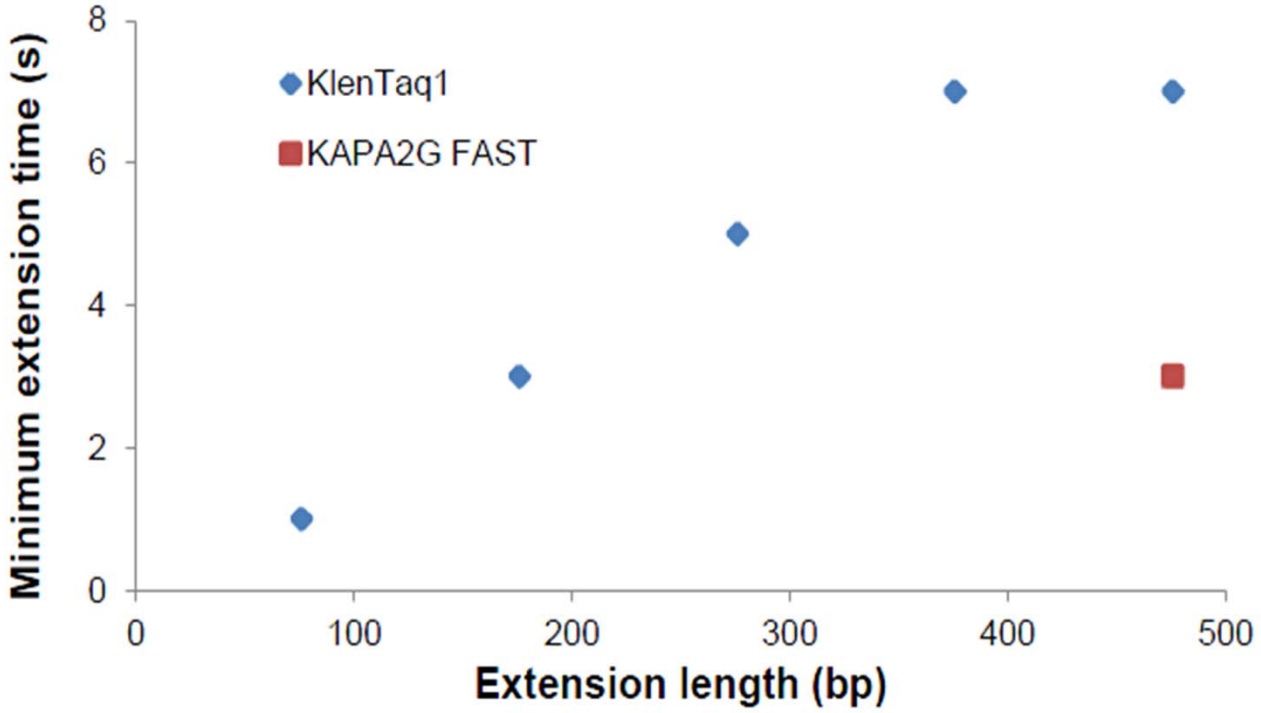
Minimum extension time required for a 500 bp product

(KAPA2G Fast polymerase)



Extreme PCR for >100 bp Targets

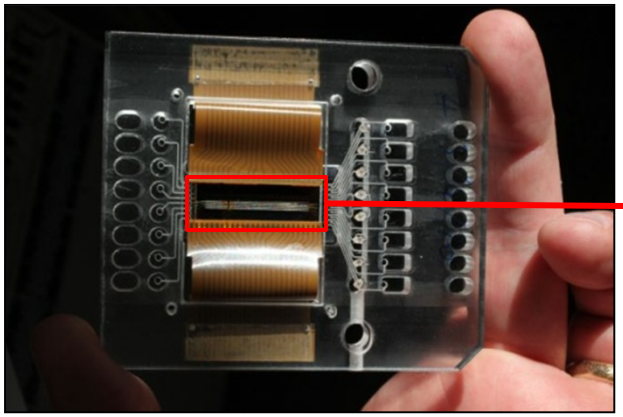
KlenTaq1: One second required for every 60 bps
KAPA2G Fast: One second required for every 160 bps



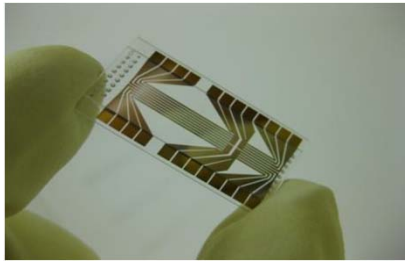
Extreme PCR Challenges

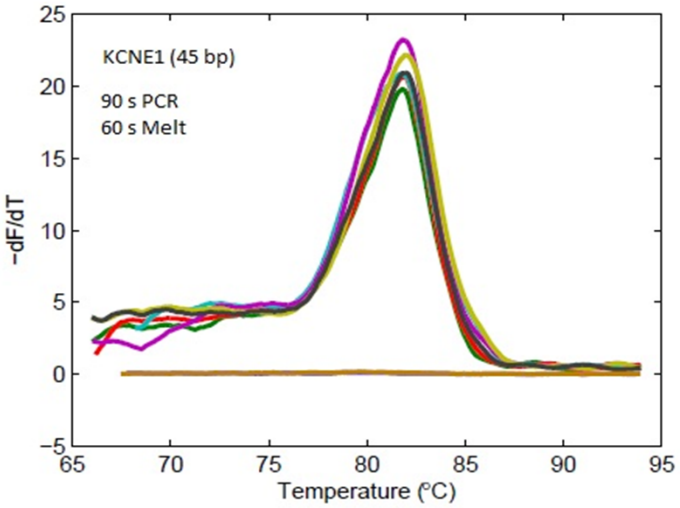
- High [Polymerase] (20X)
  - Patent expirations (KlenTaq™)
  - Bulk sources (<$1/5 µl reaction)
- High [Primers] (20X)
- Hot start method
- Instrumentation

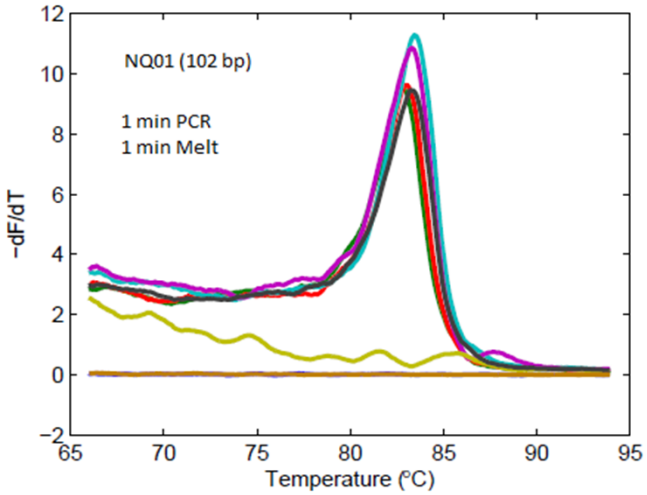
Microfluidics for Extreme PCR and High Resolution Melting (Canon U.S. Life Sciences)



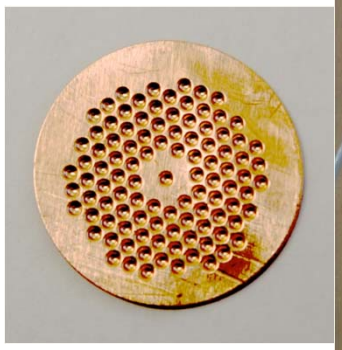
Core Chip

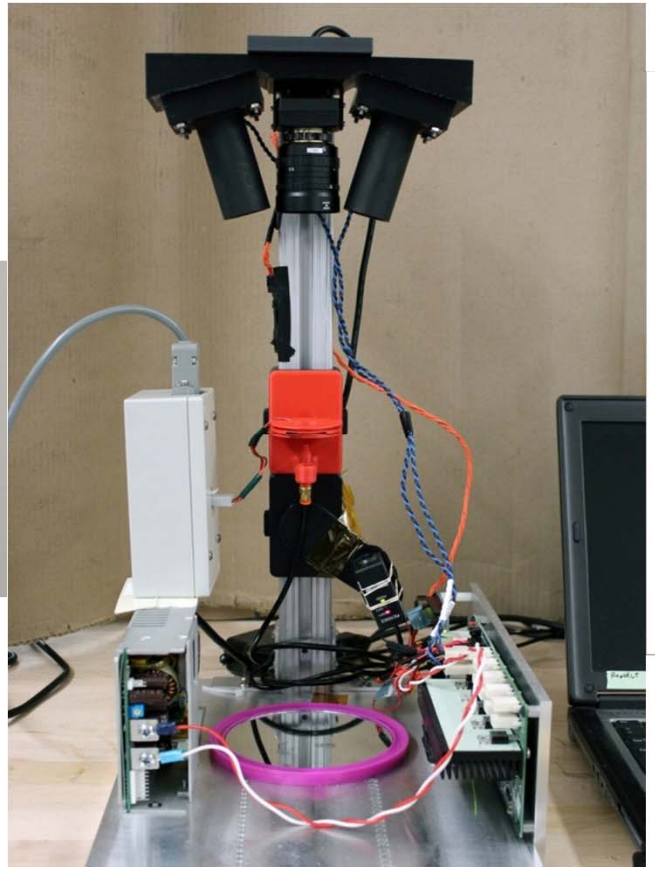


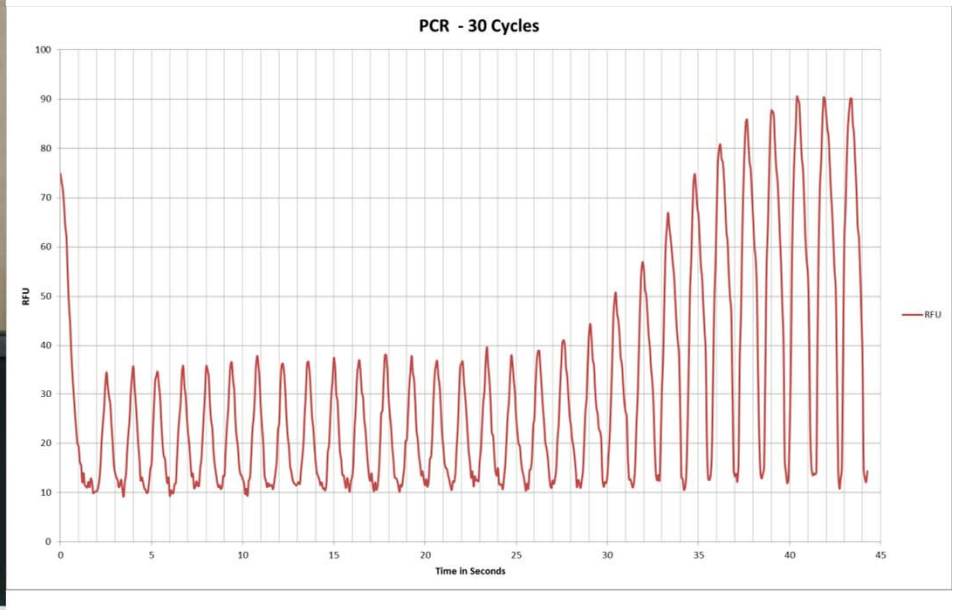




Inductive Heating / Forced Air Cooling
(BioFire Diagnostics/bioMerieux)



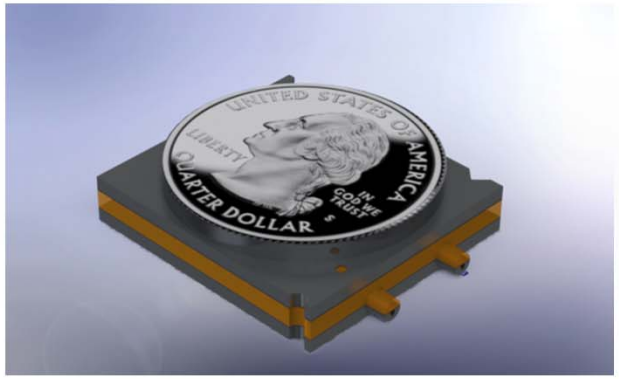




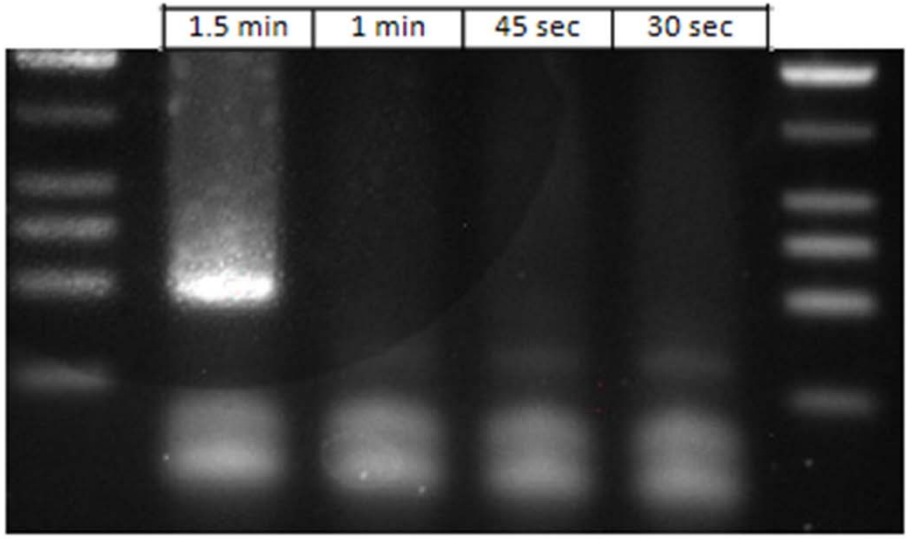
30 cycles in 45 seconds

Continuous Flow PCR - 45 bp genomic

(Thermal Gradient Device)

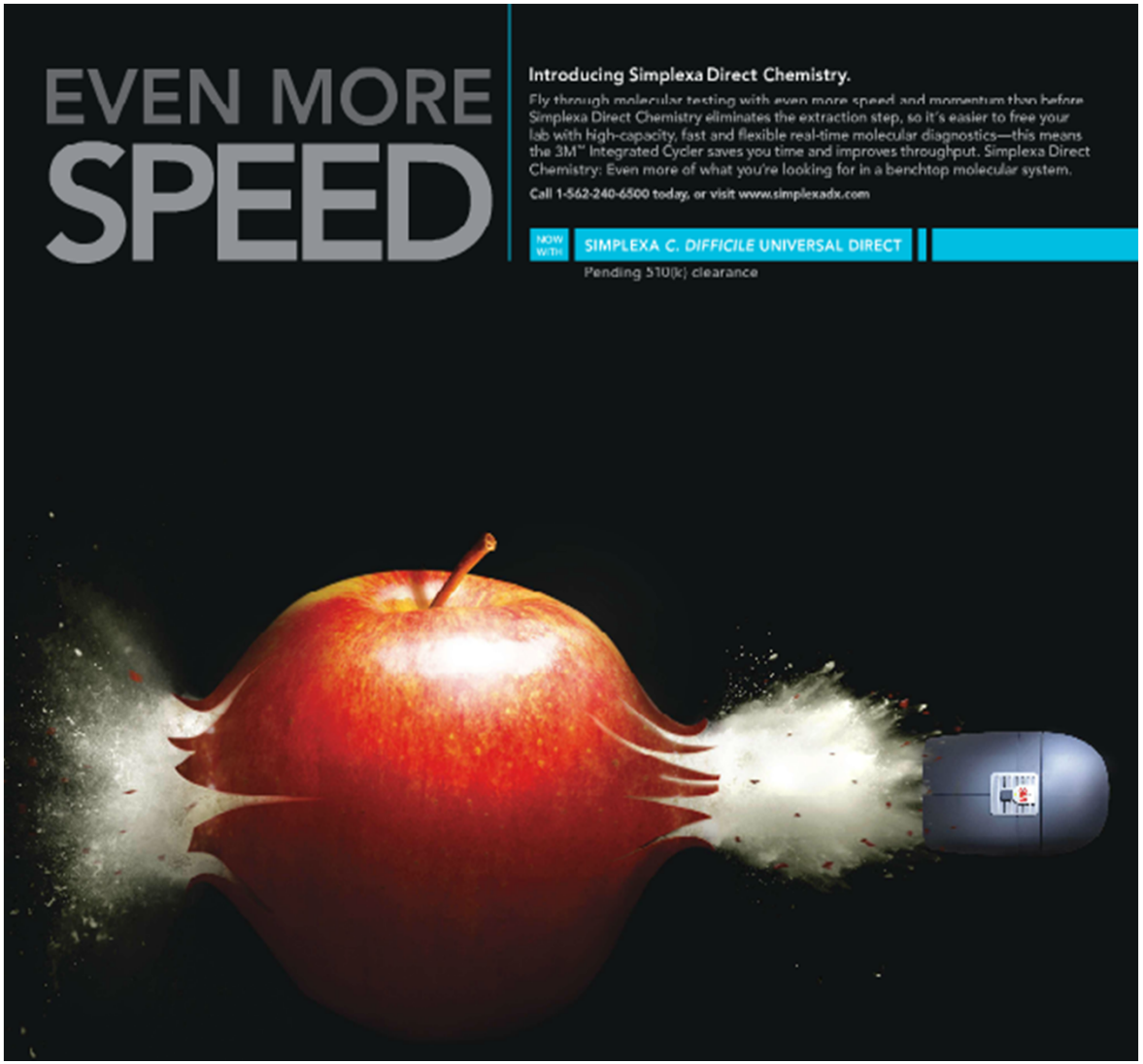


PCR Time (min)

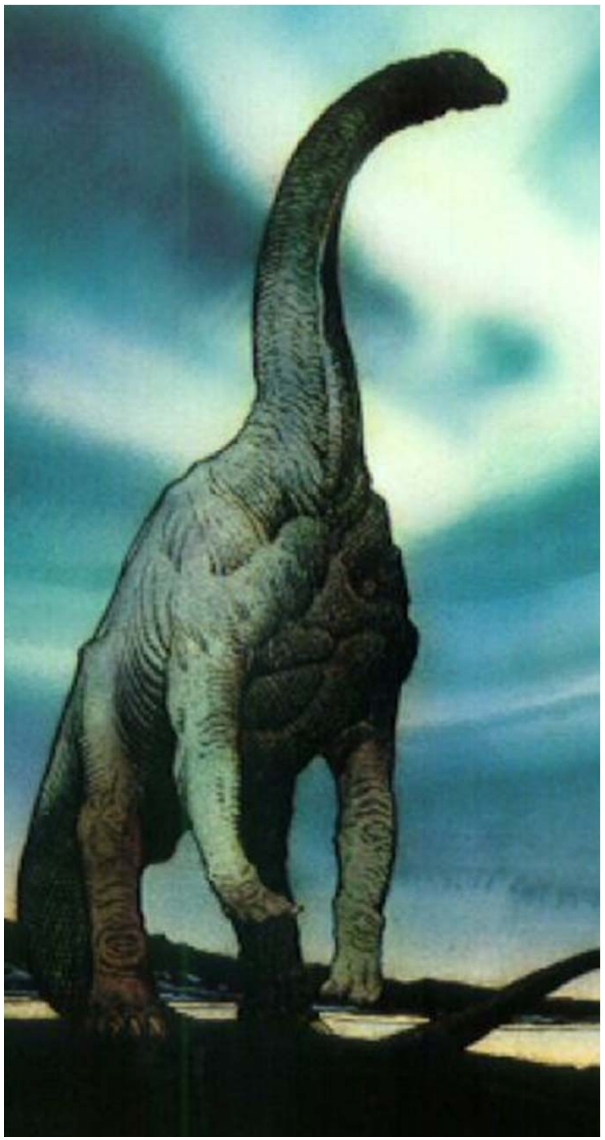


Is speed important?

30 min PCR



Extreme PCR Applications



- Infectious Disease
  - Critical Dx
  - Epidemic Control/Triage
  - BioThreat
  - Point of Care/Impact
  - Point of Consumption (Food/Water Testing)
  - Intimate Encounters
  - Drones
- Genetics
  - Field Forensics
  - In-vitro fertilization
  - Clinical Trials
- Oncology
  - Intra-operative

But….

- Who cares if you do PCR in 15 seconds if sample preparation takes 30 min?

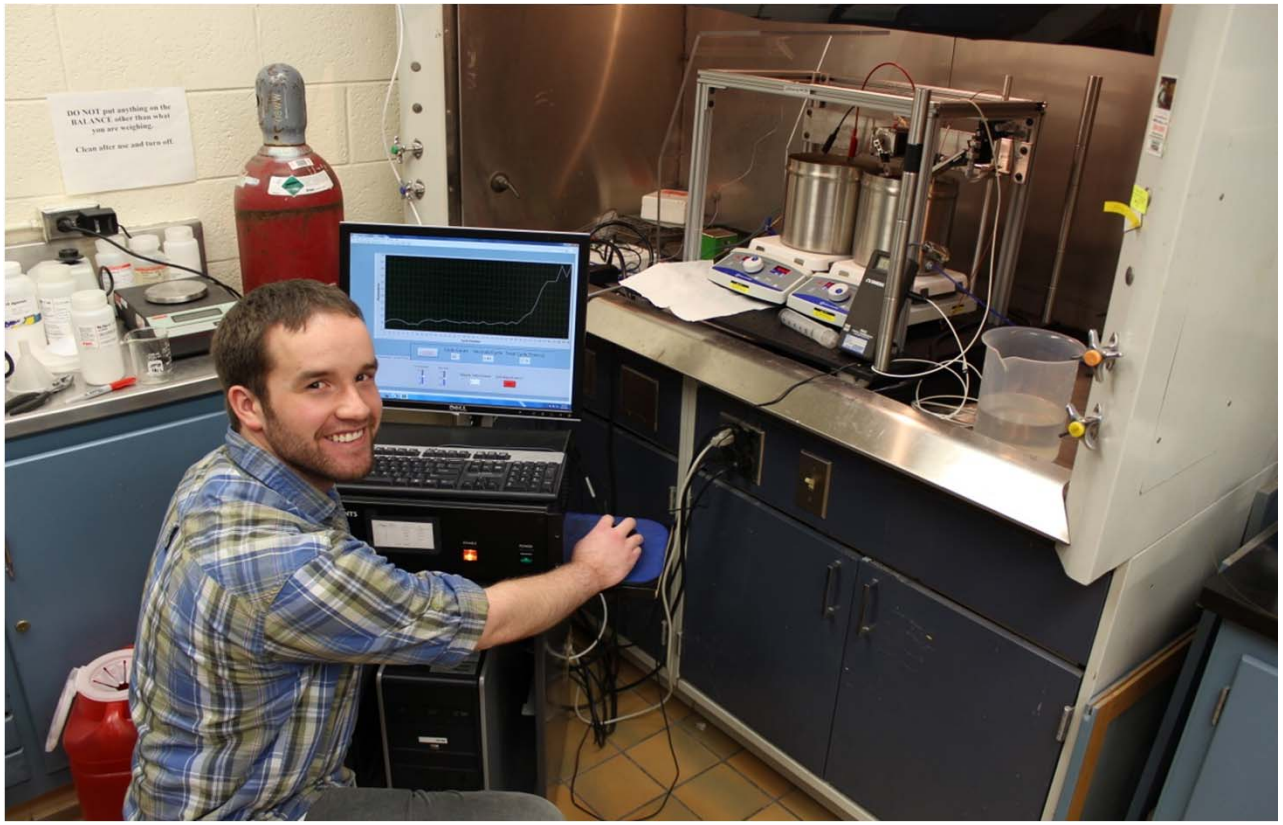
- Molecular diagnostics requires:
  - 1) sample preparation
  - 2) amplification
  - 3) analysis

Need for rapid sample preparation (<1 min)!

Summary

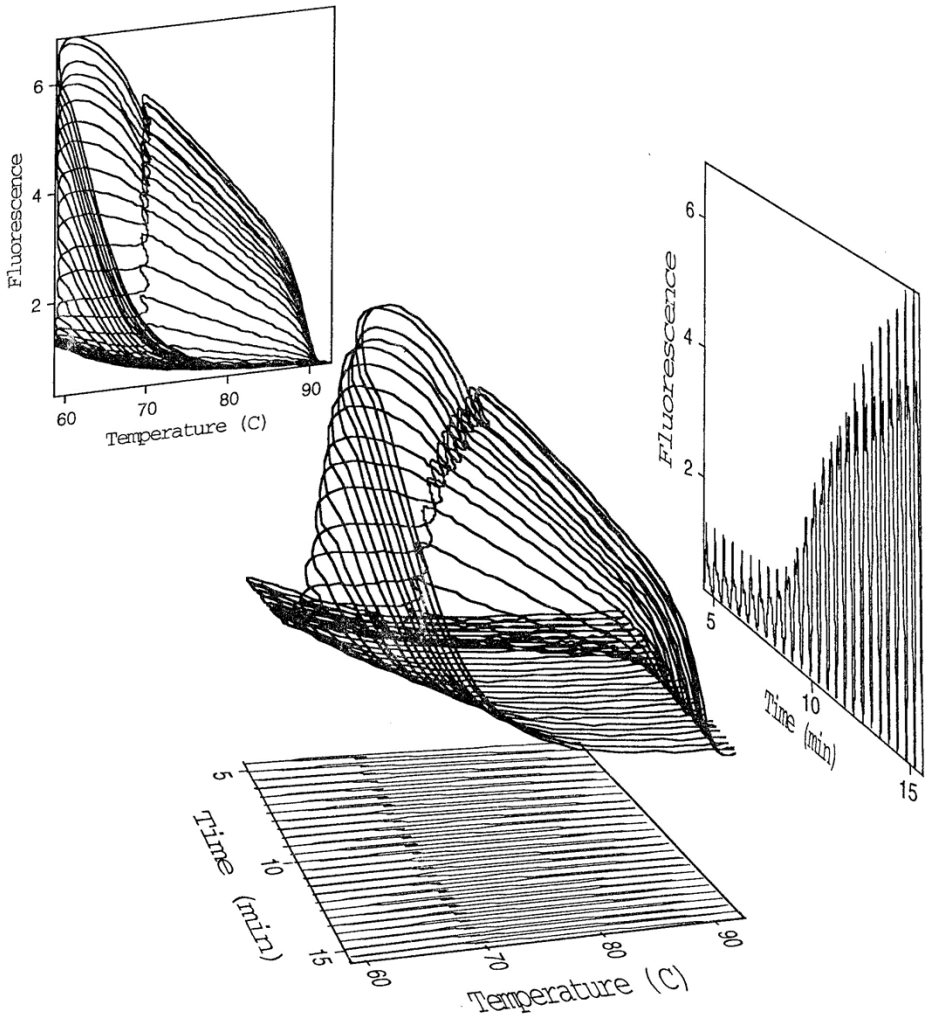
- Increase PCR speed:
  - 20X over rapid-cycle PCR (10 min)
  - 200X over regular PCR (2.5 hr)
- Without sacrificing:
    - Efficiency
    - Sensitivity (single copy human genomic DNA)
    - Specificity
- By altering chemistry:
    - Increase [polymerase] 20X
    - Increase [primers] by 20X
- Obtaining:
    - 20X [product]

Jared Farrar



Thanks!

BioFire Dx / bioMerieux
NIH
ARUP
Roche Applied Science
Canon U.S. Life Sciences
State of Utah
University of Utah



http://dna.utah.edu