

Real-Time PCR: the basics

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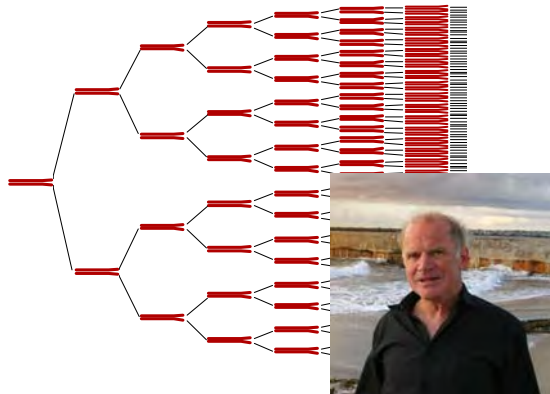
ESCMID 2016
Jan 20, 2016



Disclosures

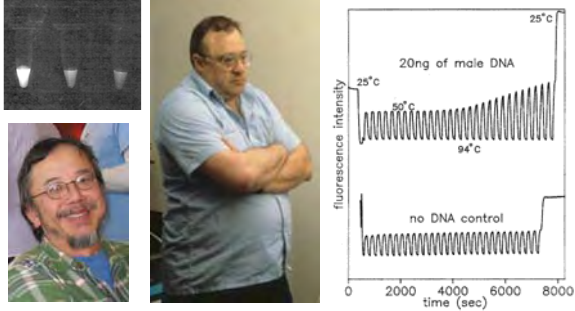
<u>Commercial Interest</u>	<u>What was Received</u>	<u>For What Role</u>
University of Utah/BioFire	Royalties	Patents/Licenses
	SYBR Green I in PCR Melting with Real Time PCR Probe designs Rapid and Extreme PCR High resolution melting	
BioFire Diagnostics, Canon US Life Science	Grants/Support	Research
ARUP, NEB, Inspirata, Clinical Chemistry	Fees	Consultant

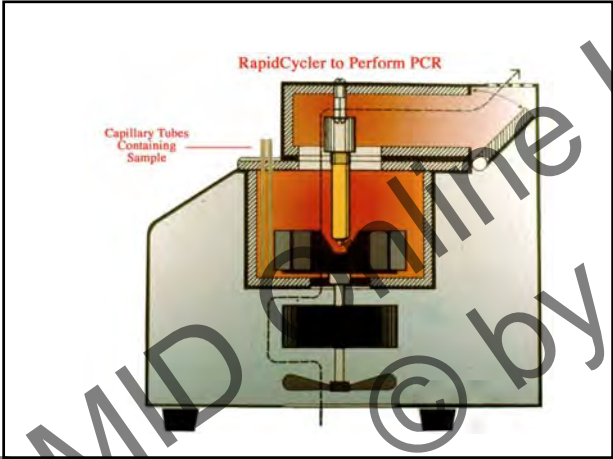
1985-1988: DNA replication in a test tube



The first real-time PCR

(Ethidium Bromide)





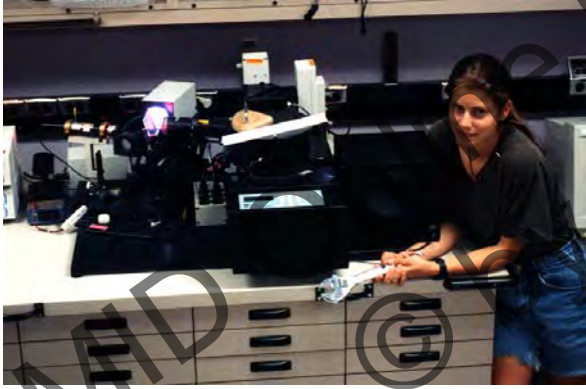
1993: Monitoring Fluorescence during Amplification



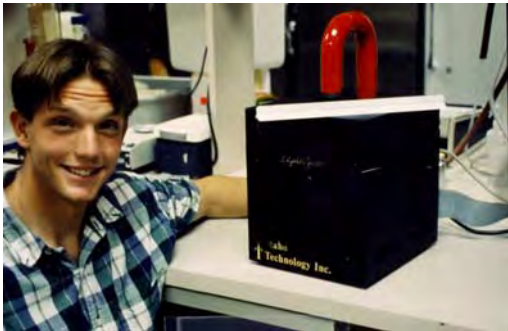
1994: RapidCycler + Fluorimeter



1995: LightCycler Prototype



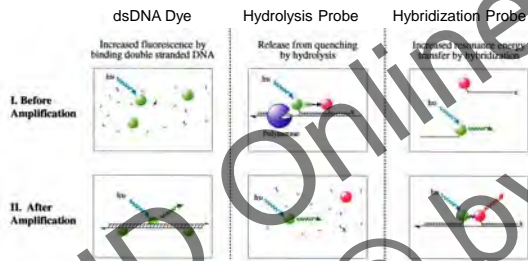
1996: LightCycler 24



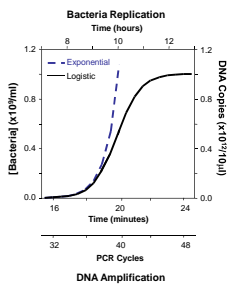
1998 – Instrument Contrast

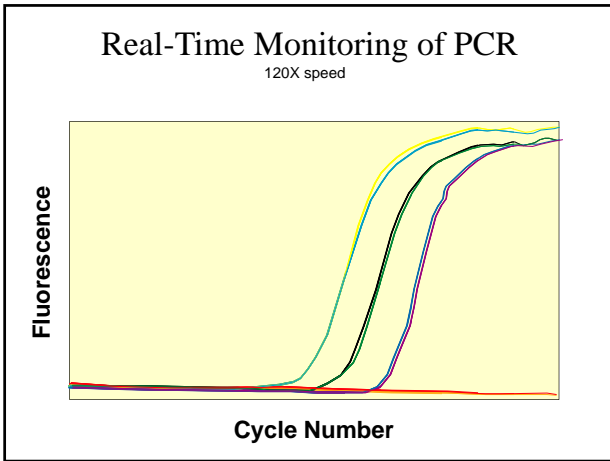


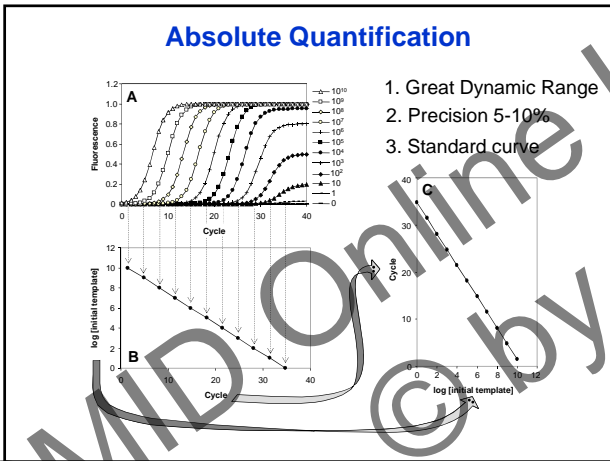
Three Fluorescence Monitoring Schemes for PCR

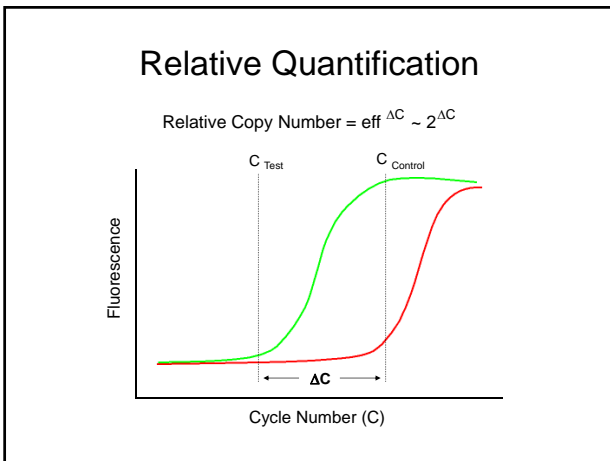


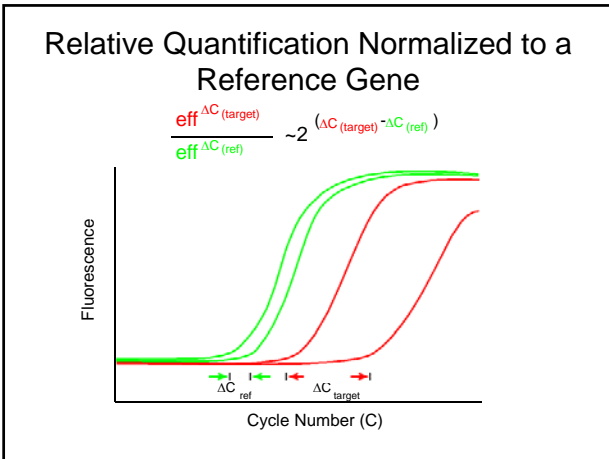
PCR is Like Bacterial Growth



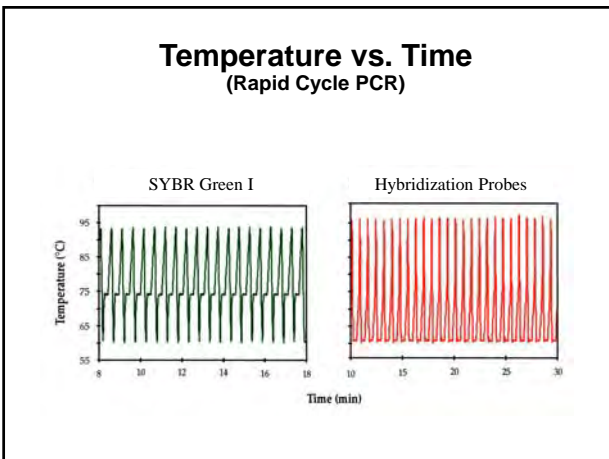


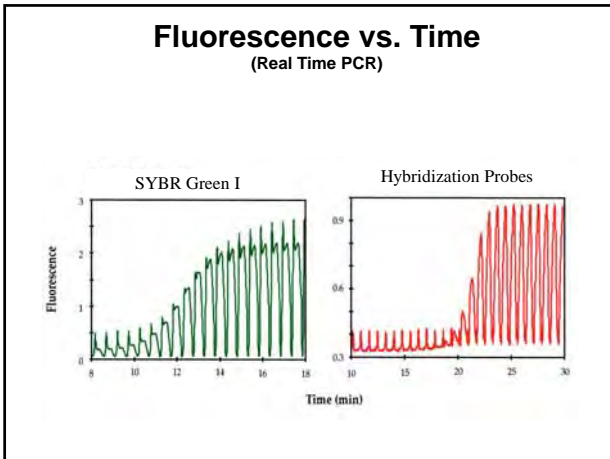


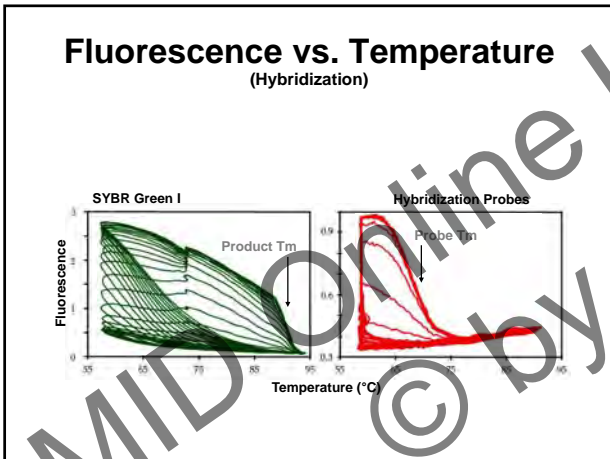


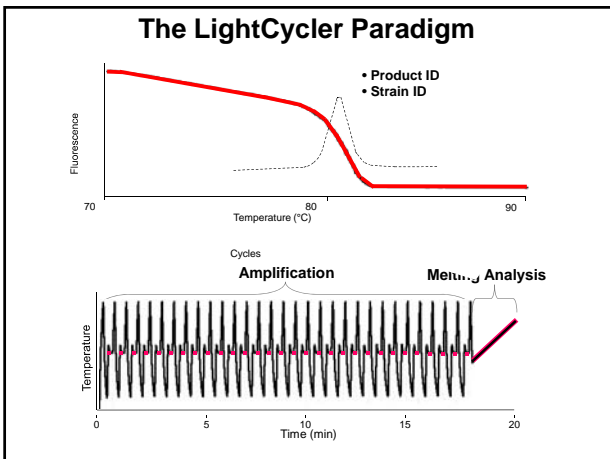


- ### Real-Time Parameters
1. Time
 2. Temperature
 3. Fluorescence
 - Once Each Cycle (Quantification)
 - With Changing Temperature
 - Product melting
 - Probe melting







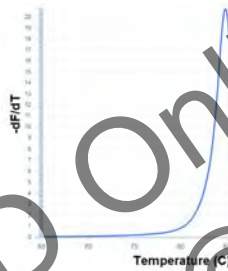


Melting Analysis

- 1997 – Melting Analysis during and after PCR
 - Probes and dyes
- 2003 - High Resolution Melting (dyes)
 - Simplest Genotyping Methods
 - Small Amplicon
 - Unlabeled Probe
 - Snapback Primer
 - Scanning
 - Relative copy number
- 2014 - High Speed Melting
 - <30 seconds

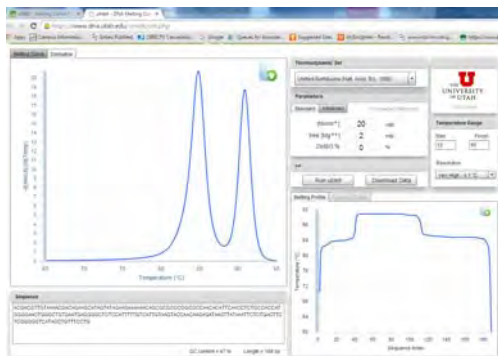
Amplicon Melting with Dyes

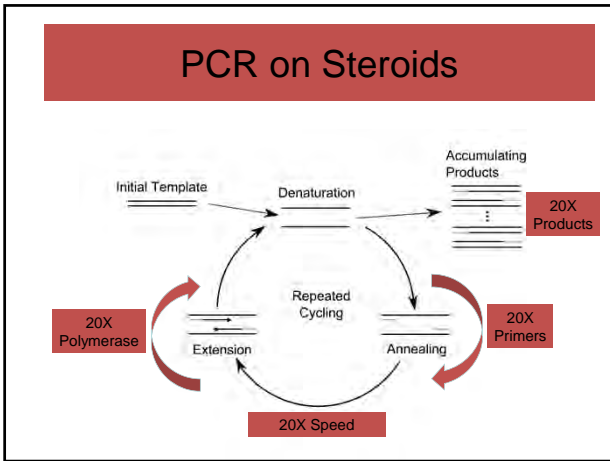
- Expect PCR product

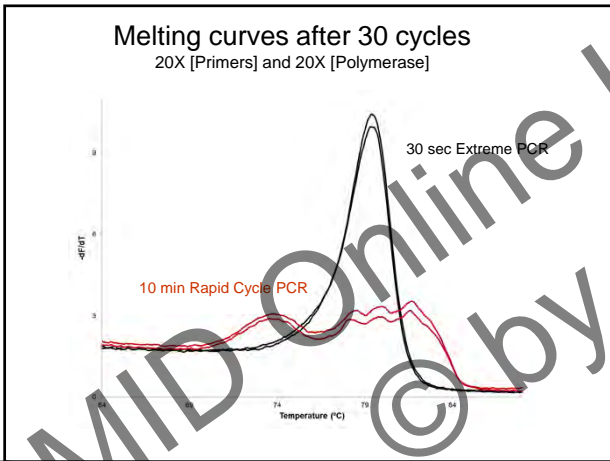


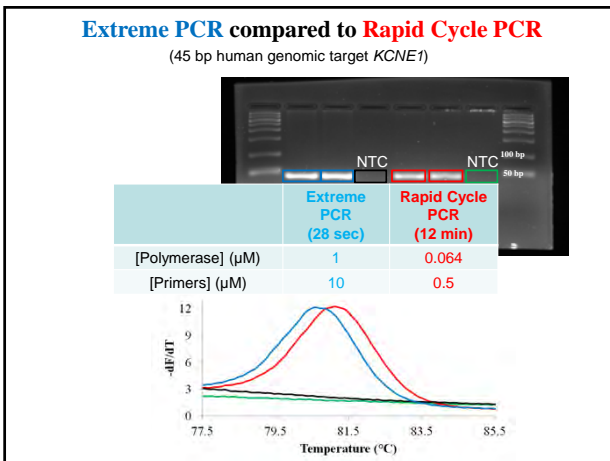
Melting Curve Prediction

(dna.utah.edu, Bioinformatics. 2011 Apr 1;27(7):1019-20)

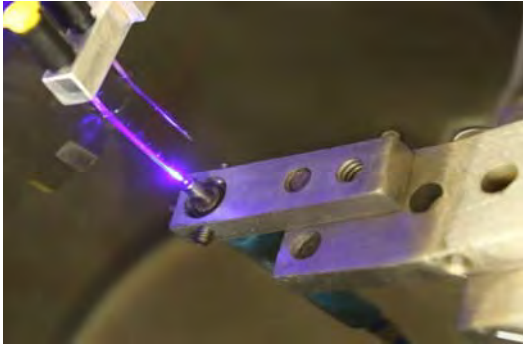








Sample Interrogation

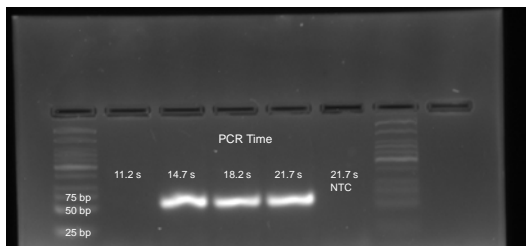


Video

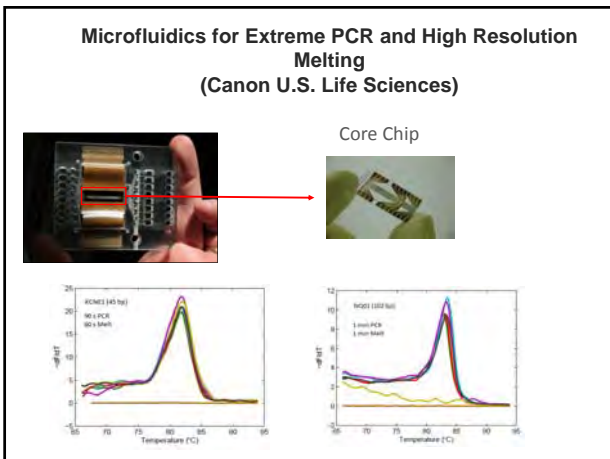
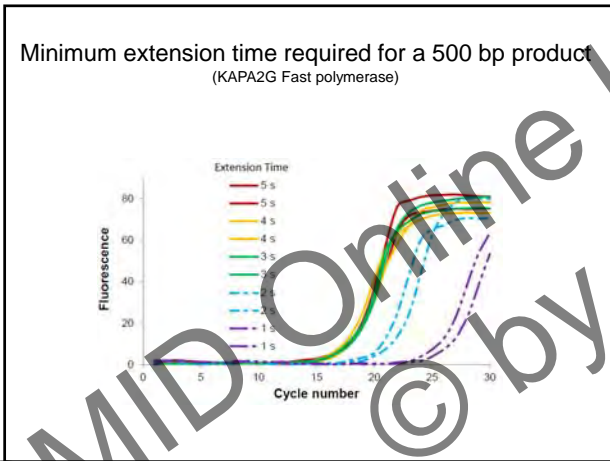
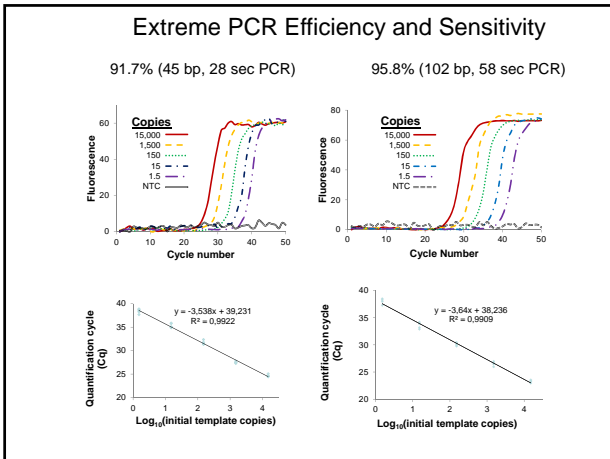


14.7 second PCR

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

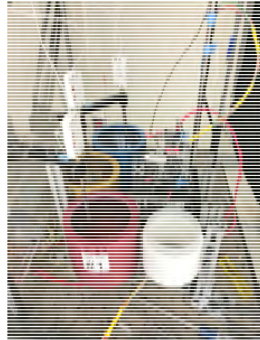


8 μ M polymerase, 20 μ M Primers, 5 mM MgCl₂



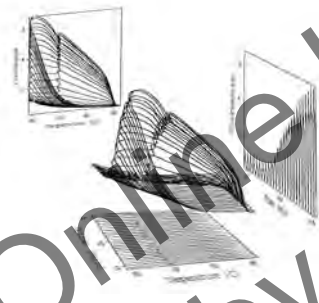
Next.....

- Four water baths
- Two stepper motors
- Ice start
- RT- PCR
- Multiplexing
- Integrating
 - Melting Analysis
 - Sample Prep



Thanks!

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



More information:

- Publication: Clin Chem. 2015 Jan;61(1):145-53
- Editorial: Clin Chem. 2015 Jan;61(1):4-5.
- Podcast: http://media.aacc.org/CCJPodcasts/ClinChem_201501_Wittwer_Mackay.m
- Blog: <http://www.dnainfo.com/nz/extremePCR>
- Website: <https://www.dna.utah.edu>
