High Resolution DNA Melting Analysis for Clinical Diagnostics

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Maastricht
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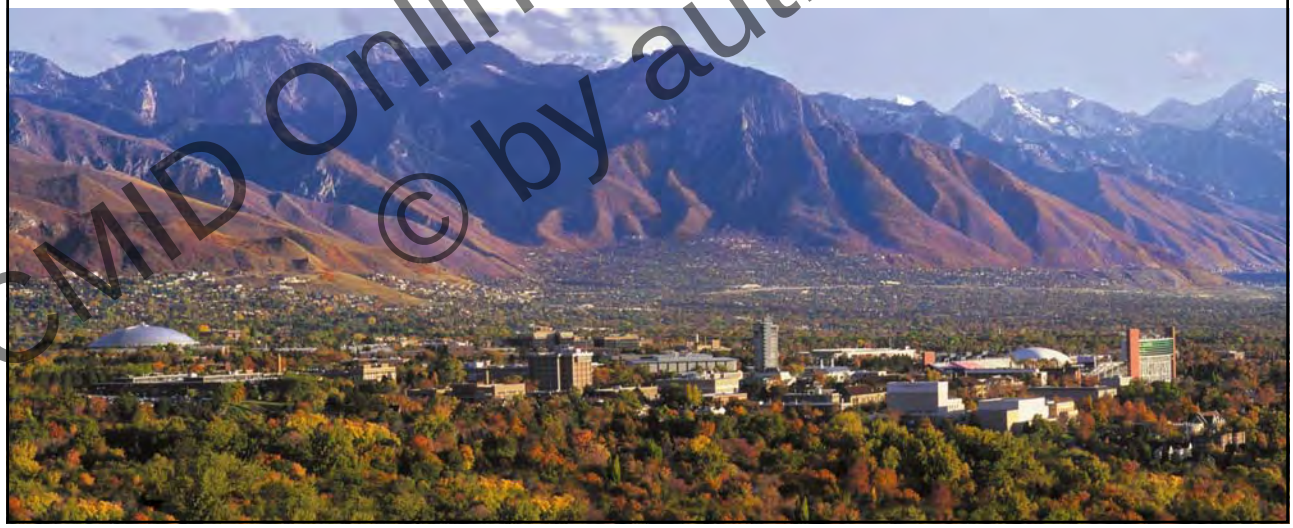
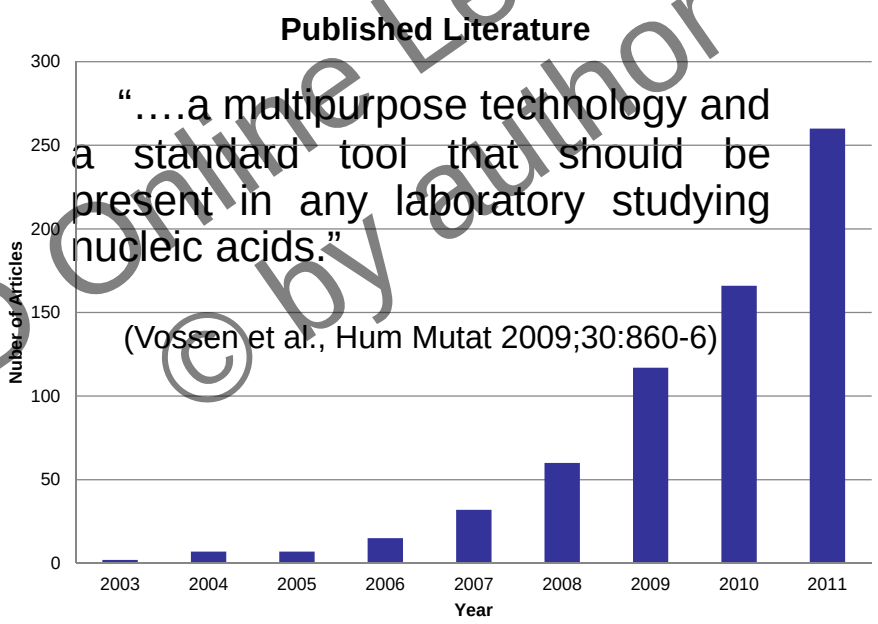


High Resolution Melting

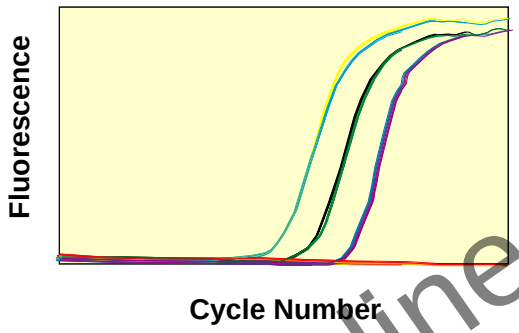
(aka, Hi-Res Melting, HRM, HRMA)



"….a multipurpose technology and a standard tool that should be present in any laboratory studying nucleic acids."

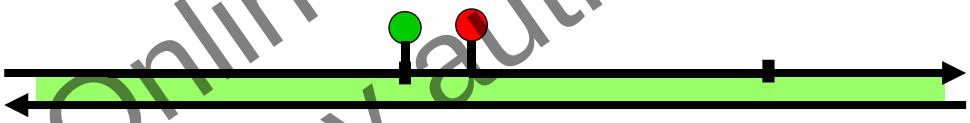
(Vossen et al., Hum Mutat 2009;30:860-6)

Modern melting analysis is performed after PCR



- Advances
  - Fluorescence instead of Absorbance
  - Dyes or Probes
  - Speed

Genotyping by Melting



Unlabeled Probe (No dye)

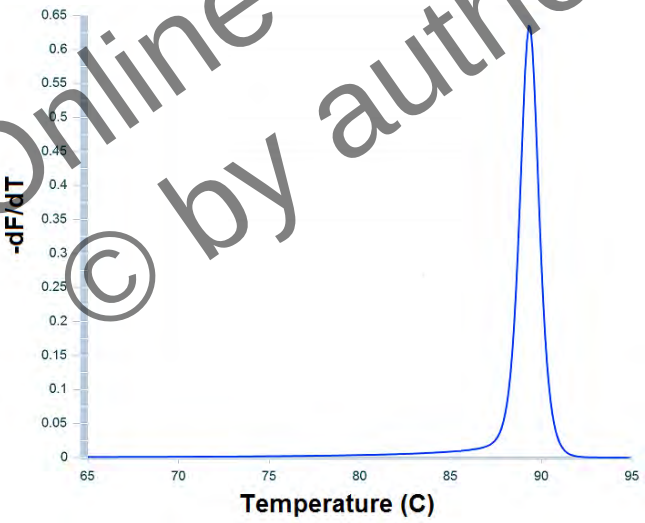
Anal. Biochem. 1997;245, 154-60 (SYBR Green I)
Clin Chem. 2003;49:732-9 (LCGreen)

Genotyping with Dyes

- Small Amplicon
- Unlabeled Probe
- Snapback Primer

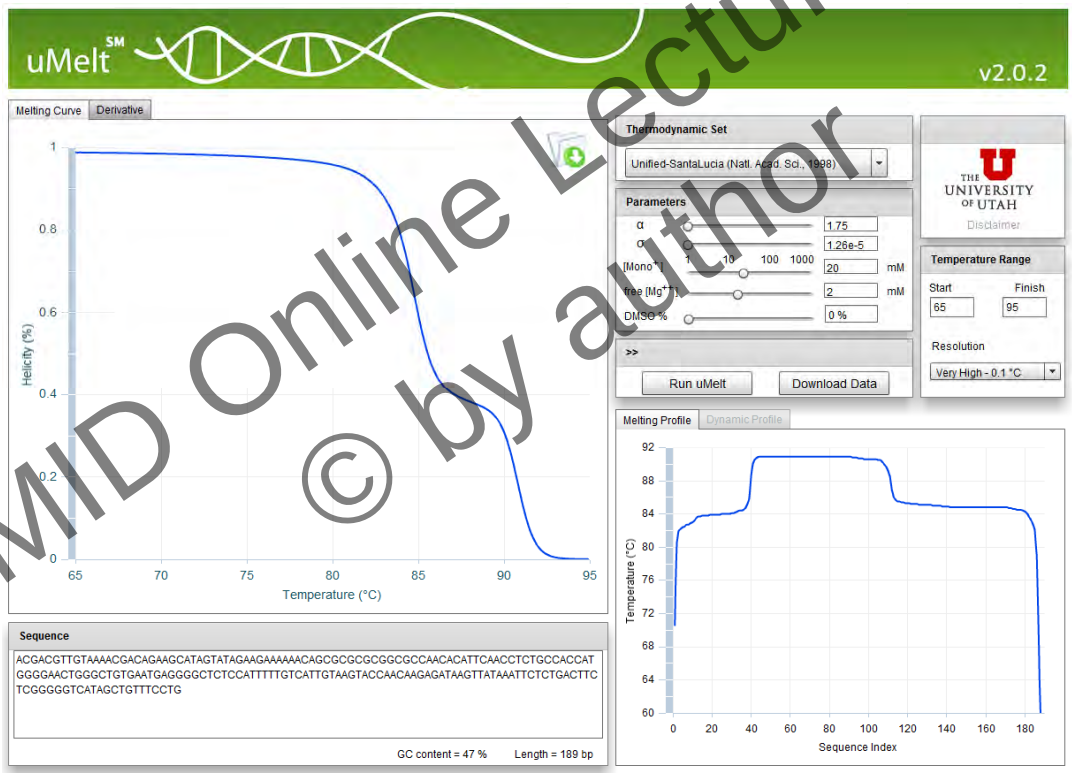
Amplicon Melting as PCR Quality Control

- Expect a single transition



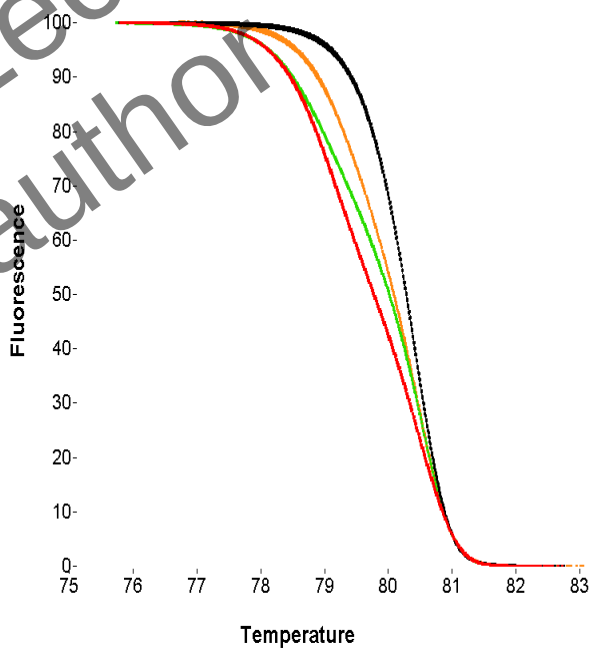
Melting Curve Prediction

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

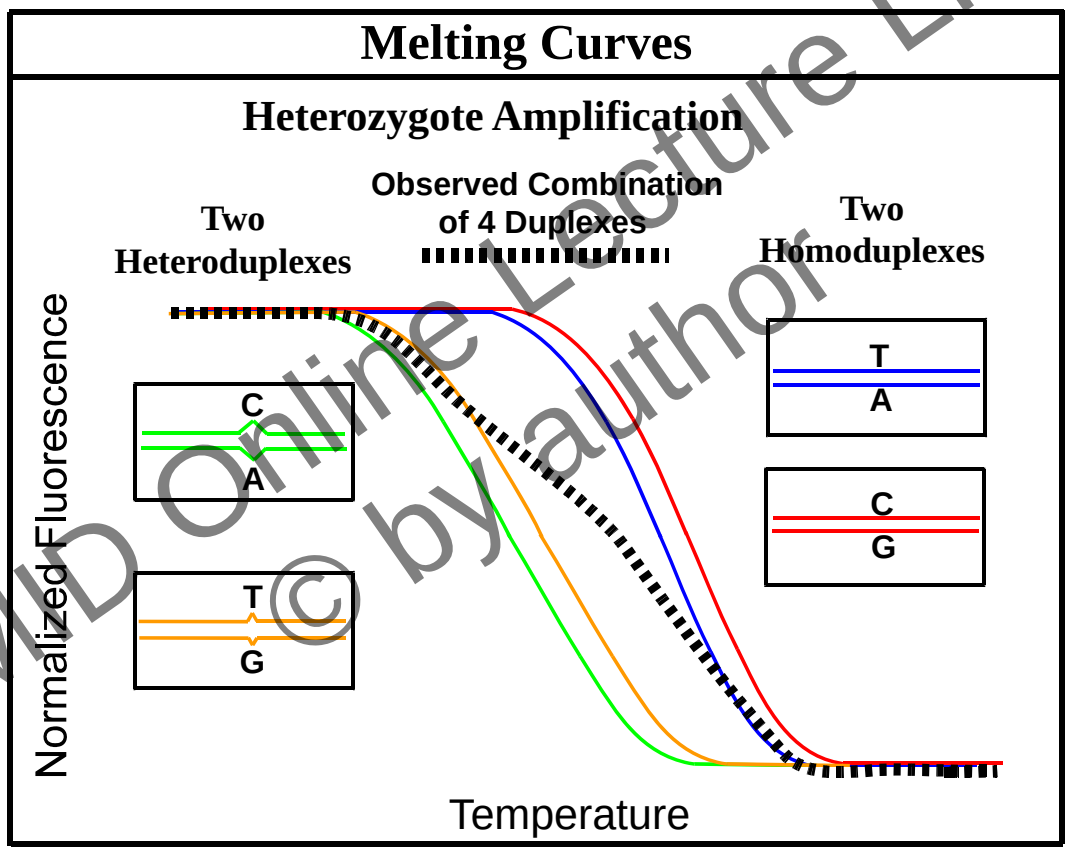


100 bp Product

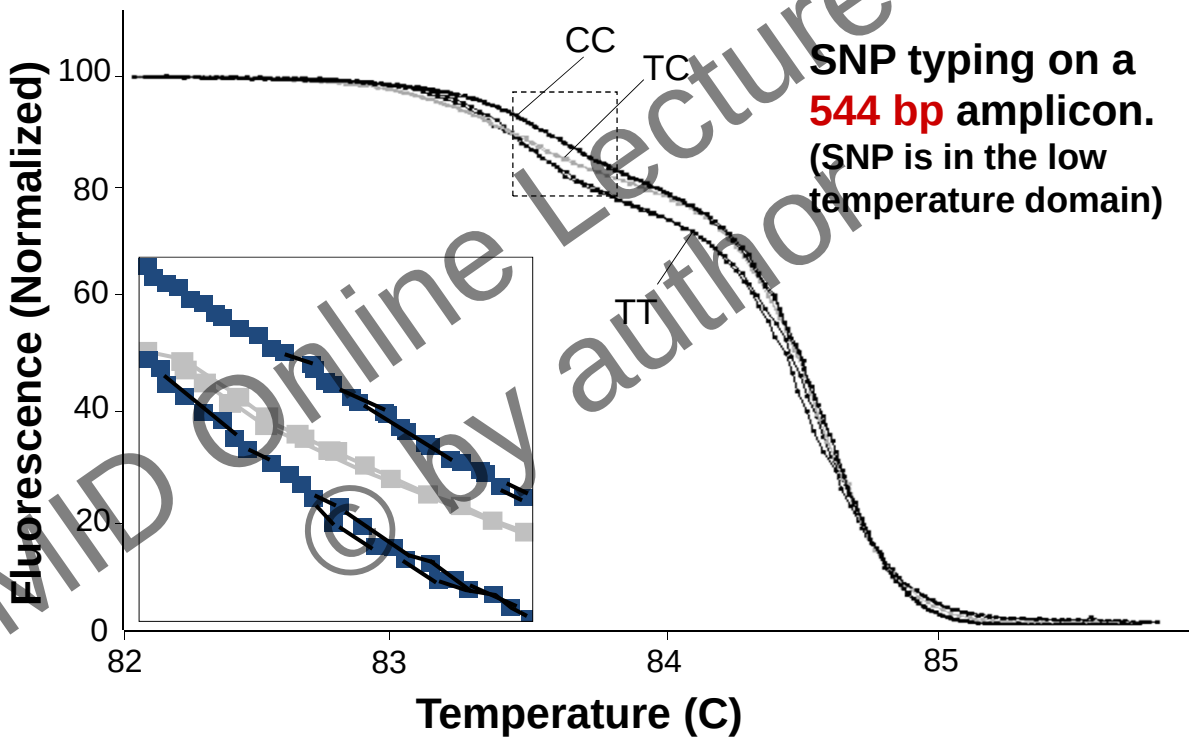
C/C Homozygote
C/G Heterozygote
C/T Heterozygote
C/A Heterozygote



- Homozygotes are easily distinguished from heterozygotes
- Different heterozygotes are easily distinguishable



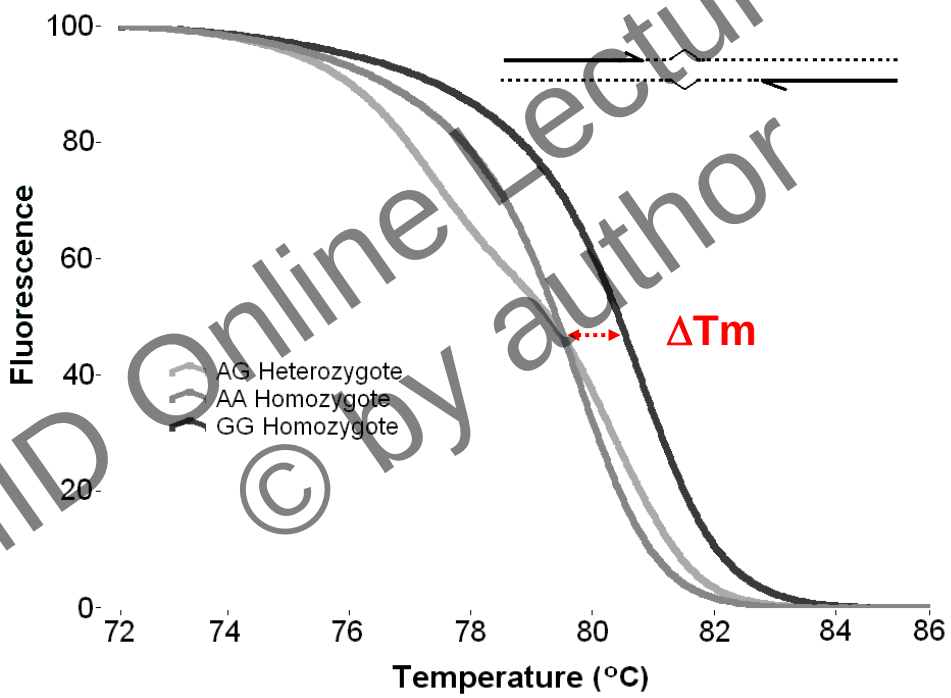
Single Base Genotyping



SNP typing on a 544 bp amplicon.
(SNP is in the low temperature domain)

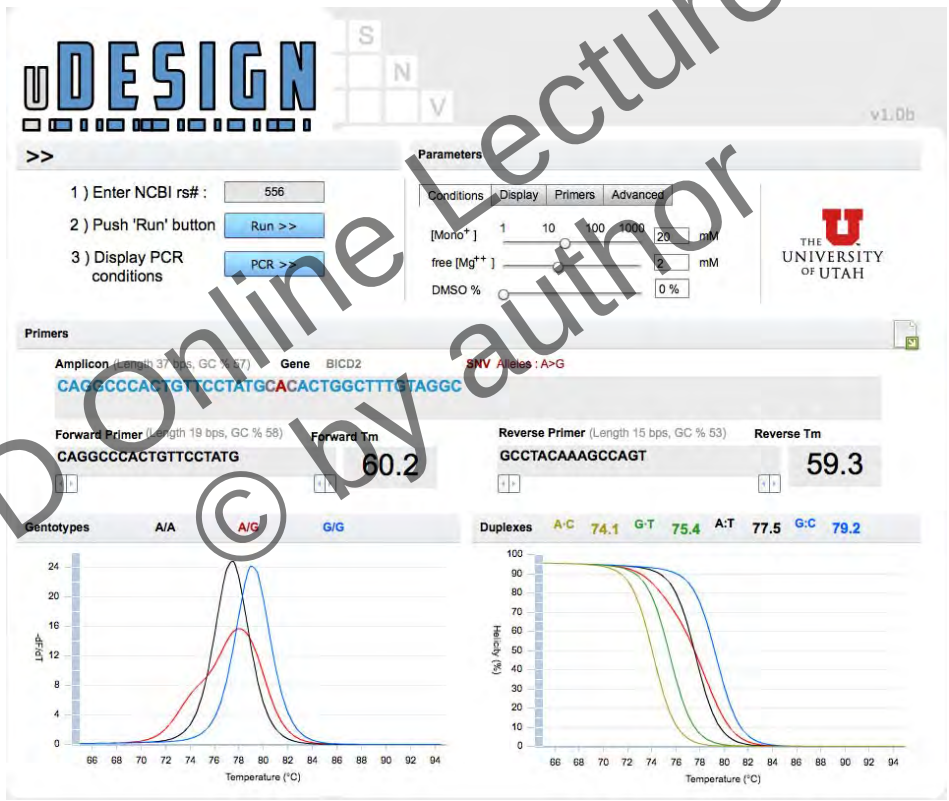
Clin Chem 49:853-60;2003.

Small Amplicon Genotyping



Clin Chem 50: 1156 – 64, 2004

Genotyping by Small Amplicon Melting – Assay Design (dna.utah.edu)



Small Amplicon Genotyping

- Simplest of all genotyping methods!
- Limitation:
  - Some homozygotes cannot be distinguished
    - 4% of SNPs
    - Many small insertions and deletions
- Solutions:
  - If ΔTm< 0.2 C by prediction, use:
    - Internal Temperature Control
    - Quantitative Heteroduplex Analysis
    - Unlabeled probes
    - Snapback primers

Genotyping by Melting with dsDNA dyes

- No covalent labels
- Multiplexing by temperature, not color

Unlabeled Probe Melting

Amplicon Melting

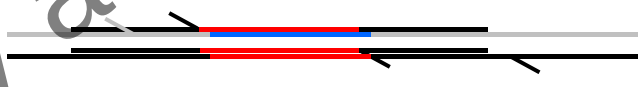
Asymmetric PCR

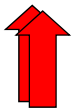
Small amplicons

3'-blocked oligos

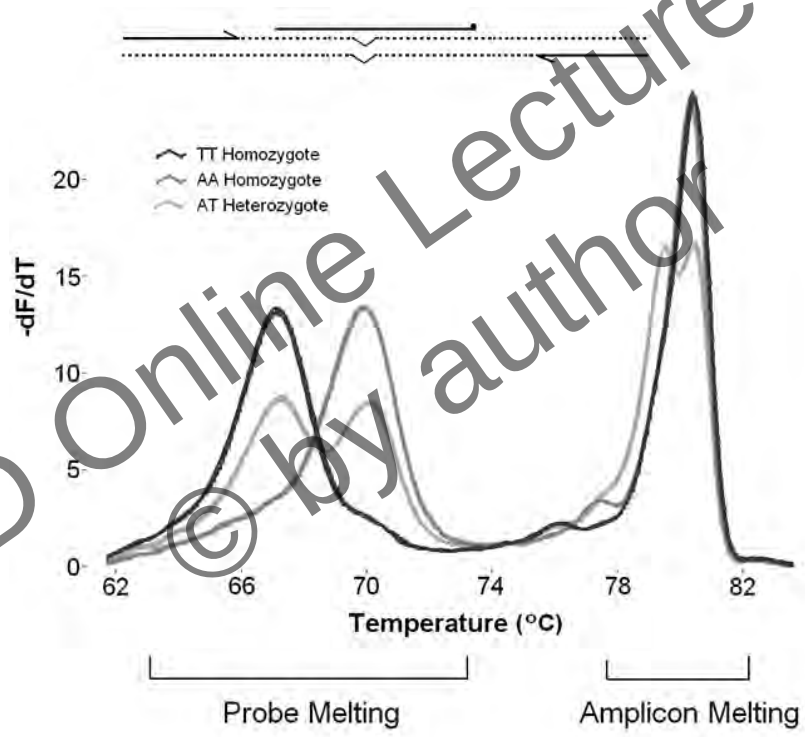
Rapid cycling

High PCR efficiency





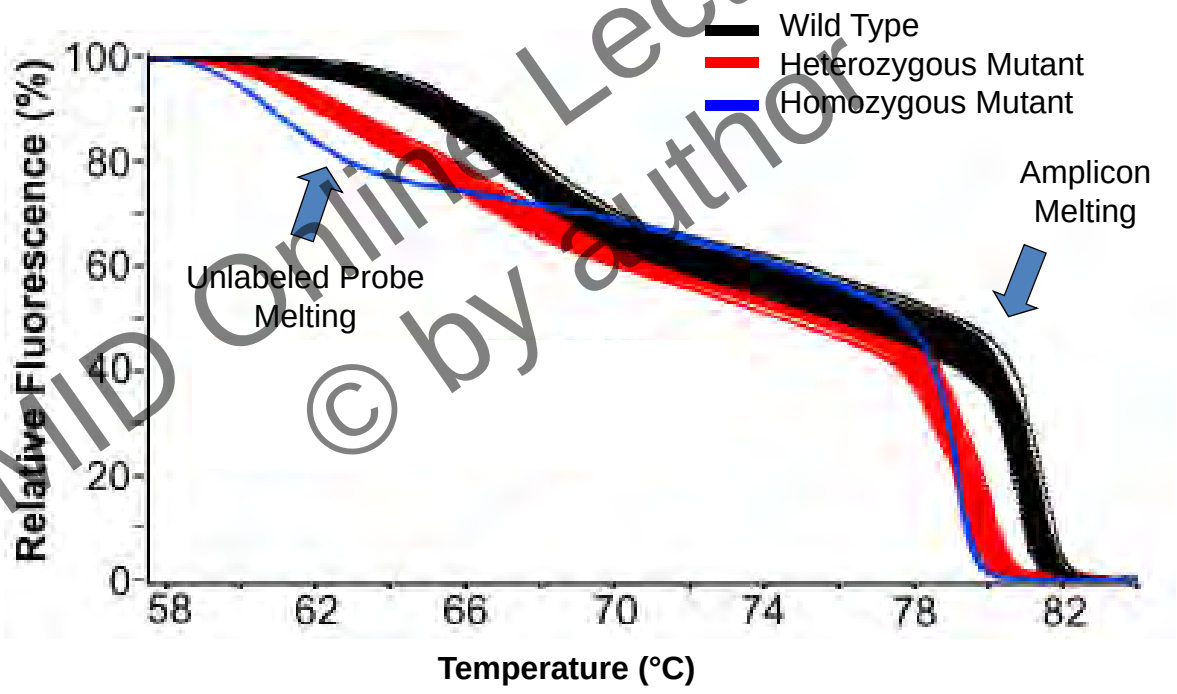
Genotyping with Unlabeled Probes



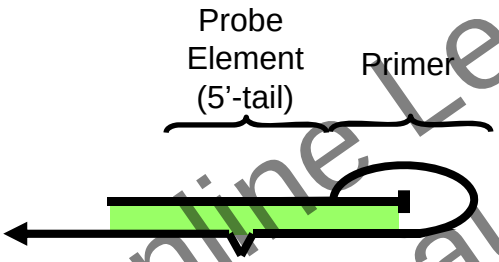
Clin Chem 50: 1328 – 35, 2004.

Simultaneous Unlabeled Probe and Amplicon Melting

(Factor V Leiden)



Probe Melting

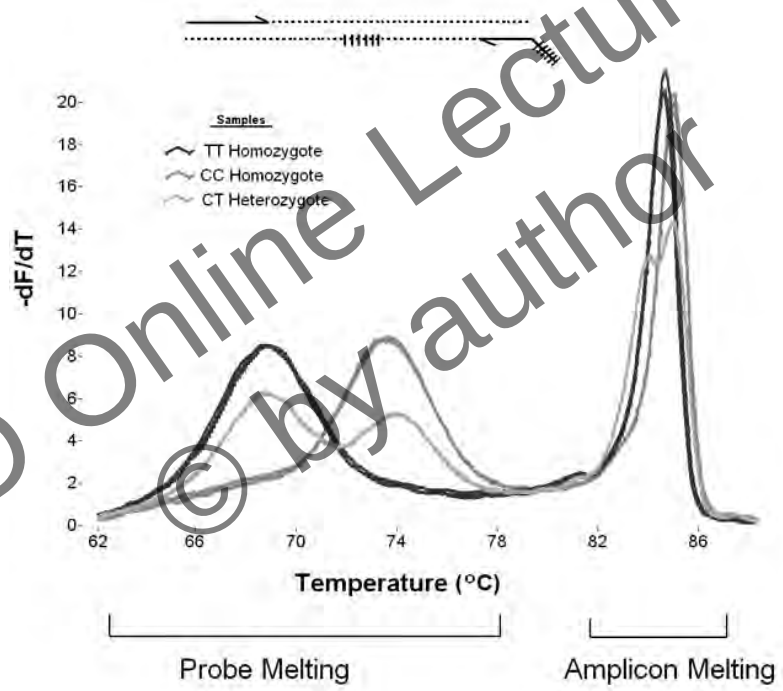


Unlabeled Probe

Hairpin Primer

Clin Chem. 2004;50:1328-35

Genotyping with Snapback Primers



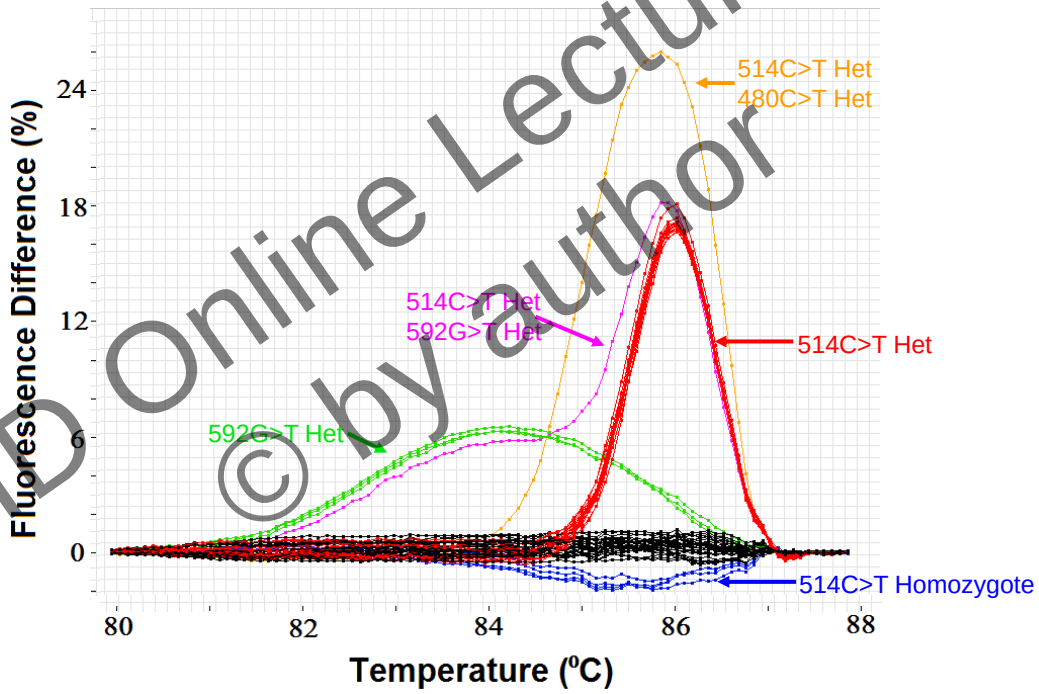
Clin Chem 54: 1648 – 56, 2008

Genotyping vs Scanning

- Genotyping analyzes one locus for different variants
- Scanning determines whether any variants are present in a PCR product.

Amplicon Scanning

(Visualization of Genetic Variation on Difference Plots)



Meksem, K., Kahl, G. (Eds.), The handbook of plant mutagenesis and mutant screening., Wiley-VCH, 2010, pp. 149-165.

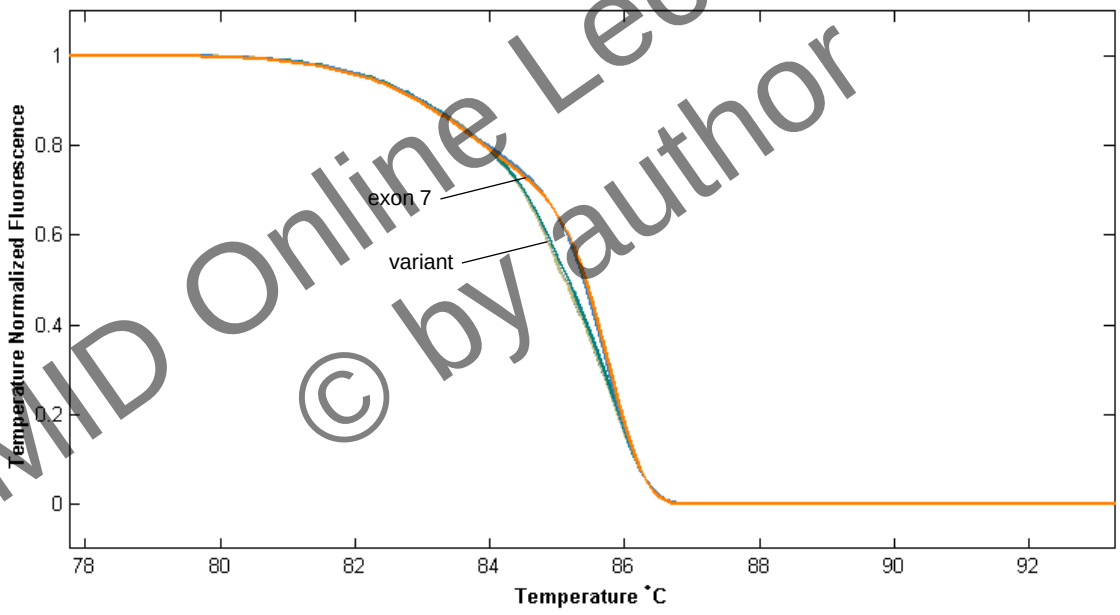
Rapid Genetic Analysis

(Chronic Granulomatous Disease)

- X-linked gene (CYBB)
- High resolution amplicon melting analysis
  - Scanning 13 exons
  - Tail primers with common sequences
  - Primer plates
- Sequencing rare variants

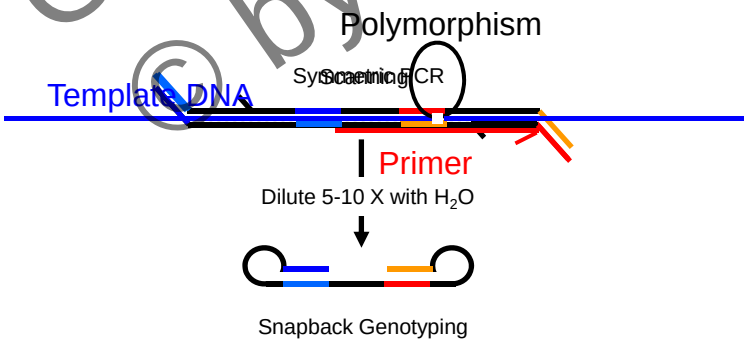
Scanning Analysis

(Normalized & Overlaid Melting Curves)



Increasing the Clinical Specificity of Scanning

Primers	Percentage of amplicons that require sequencing
Sequencing	10-25%
Bulge-inducing	3-10%
Snapback genotyping	0.3-0.5%



Common Polymorphism Analysis

(*CFTR* c.4521G>A)

Amplicon Scanning

Duplication and Retyping

Normalized Fluorescence F (%)

-dF/dT (%/°C)

Temperature (°C)

Difference

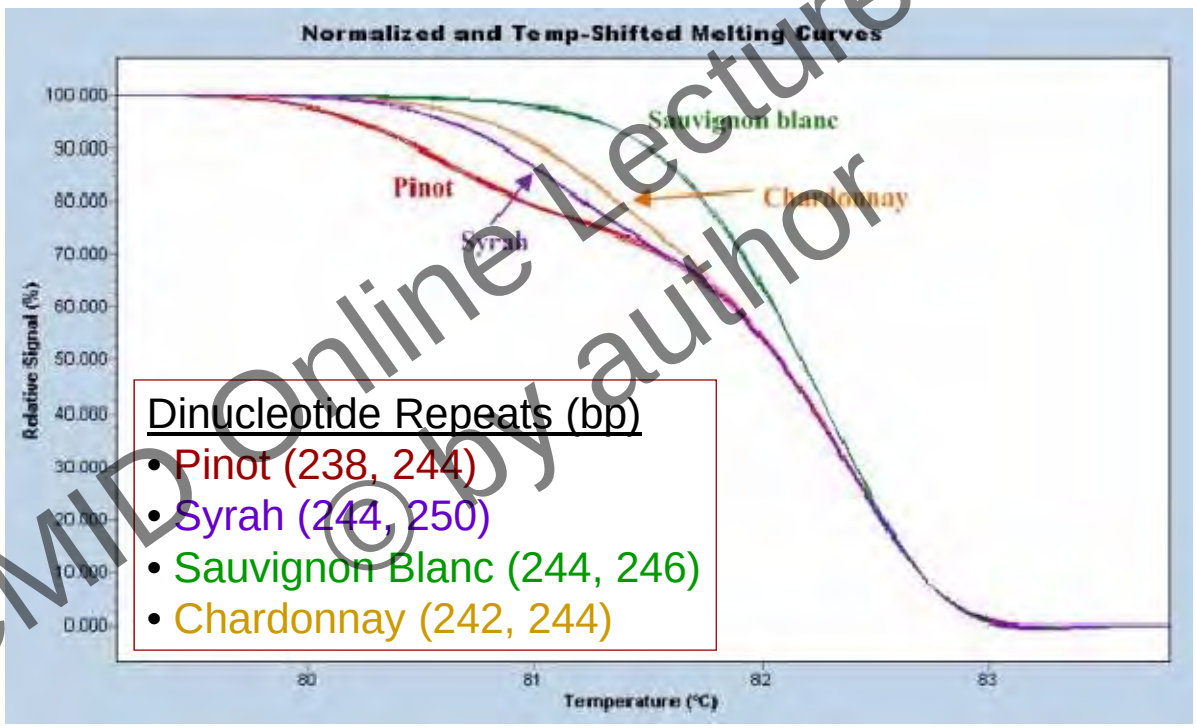
Temperature (°C)

Scanning Summary

- Inexpensive (PCR reagents + dye)
- Rapid (PCR time + melting)
- Detects "all" heterozygous variants
- Most heterozygous variants have unique melting curves (93%)
- Some homozygotes detected without mixing (~75%), easier with mulitple domains.
- Does not detect large insertions or deletions
- Requires good PCR
  - Single PCR product
  - Consistent environment (salt, etc.)

Additional Applications

Variety Identification of Grapevines



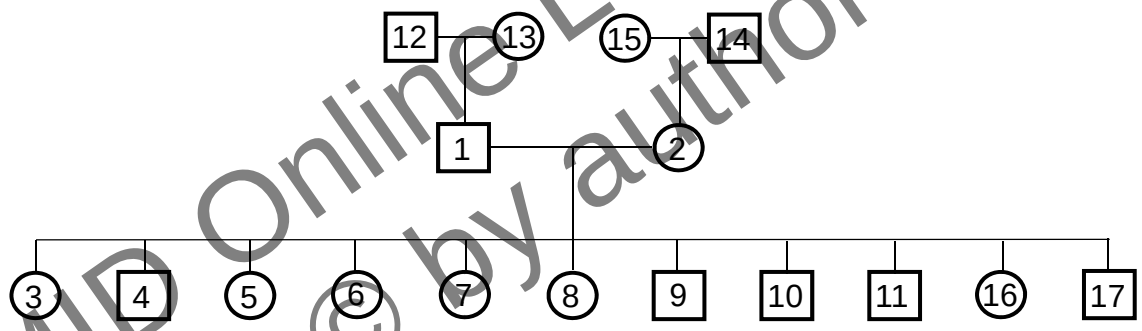
Dinucleotide Repeats (bp)

- Pinot (238, 244)
- Syrah (244, 250)
- Sauvignon Blanc (244, 246)
- Chardonnay (242, 244)

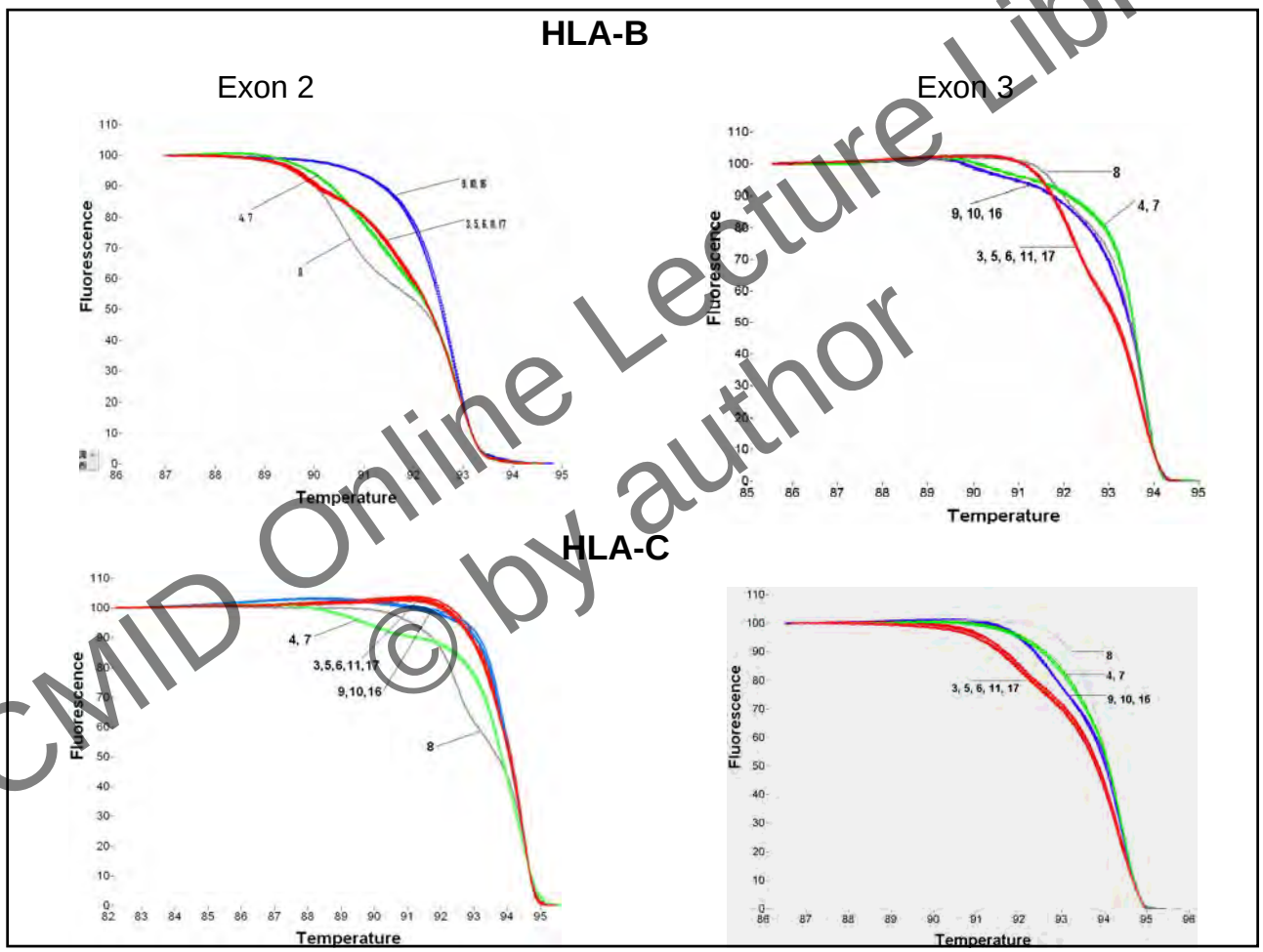
John Mackay et al., Linnaeus Laboratory, Plant Methods 2008, 4:8.

Hi-Res Melting of HLA

Tissue Antigens. 2004 Aug;64(2):156-64

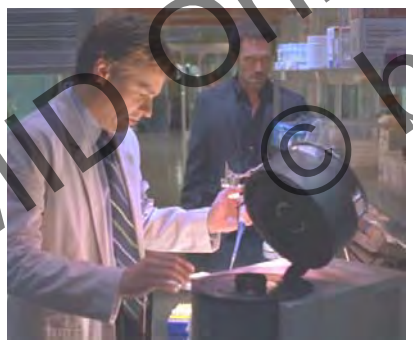


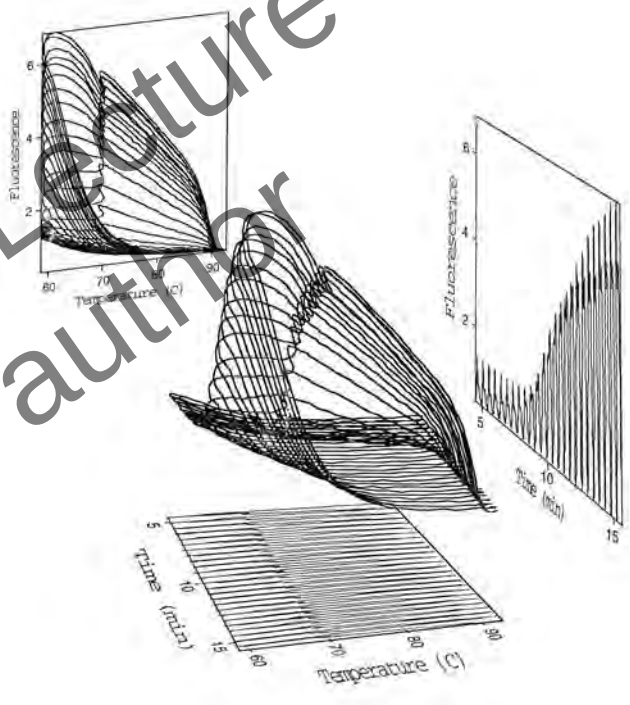
Match instead of genotype



Thanks!

BioFire Dx / BioMerieux
NIH
ARUP
Roche Applied Science
State of Utah
University of Utah





http://dna.utah.edu