

A Primer on Melting

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Basic Question: What is Hi-Res Melting (HRM)?

A post-PCR set of techniques utilizing high data density and quality, high temperature control, next generation PCR dyes, and specially tuned data analysis techniques to achieve:

- 1) Mutation Scanning
- 2) Genotyping

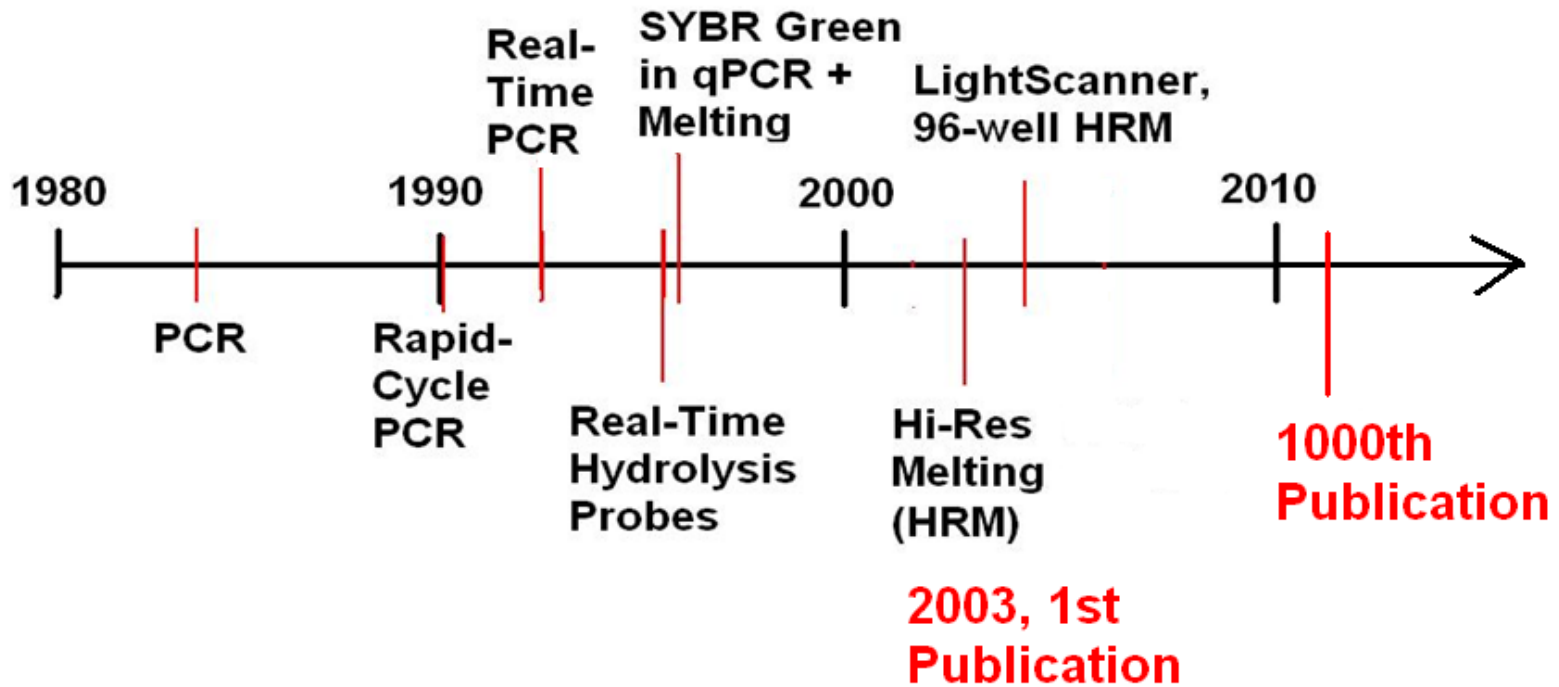
Synonyms??

- HRM
- HRMA
- HRMCA
- Melting Analysis
- High Resolution Melting
- Dye-Binding Post PCR High Resolution Melting Analysis

Where did Melting come from?

- History of post-PCR melting
 - Carl Wittwer invented post-PCR DNA melting in 1996 with the LightCycler® and SYBR® Green I
 - High resolution melting came together in 2003, with the HR-1 system and LCGreen®

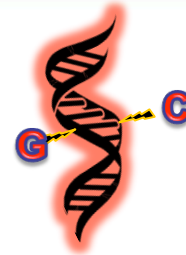
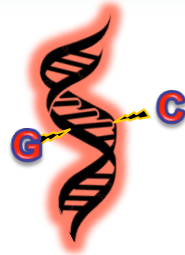
PCR / Melting Analysis Timeline



Homozygous Melt Curve Analysis Concept

Paternal

Maternal

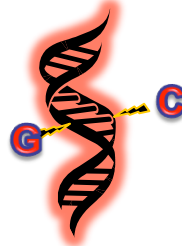


PCR



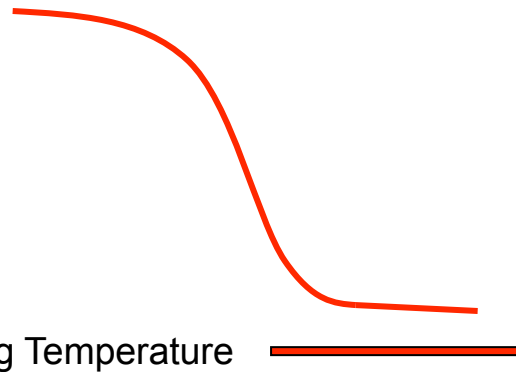
Heteroduplex Formation then Melt

Only 1 allele combination possible



Fluorescence ↑

Increasing Temperature →



Example T_m

78°C

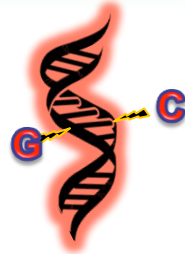
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Heterozygous Melt Curve Analysis Concept

Paternal

Maternal

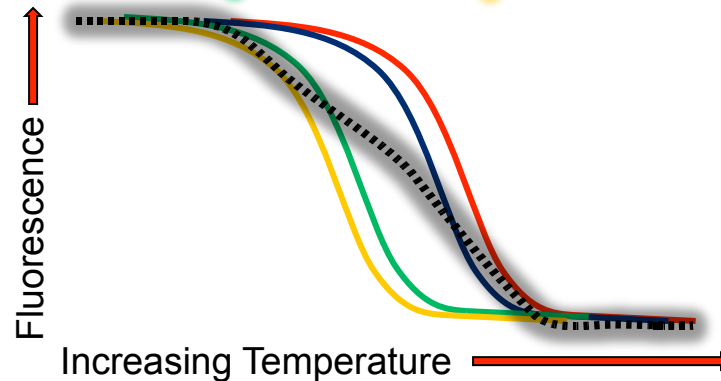
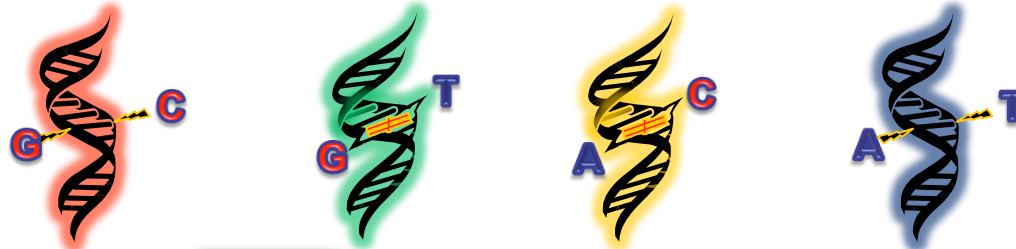


PCR



Heteroduplex Formation then Melt

4 different allele combinations possible



Example Tm

78°C

76°C

70°C

68°C

Observed melt curve represents the combination of all 4 different allele combinations

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Definition of Scanning & Genotyping

- Scanning

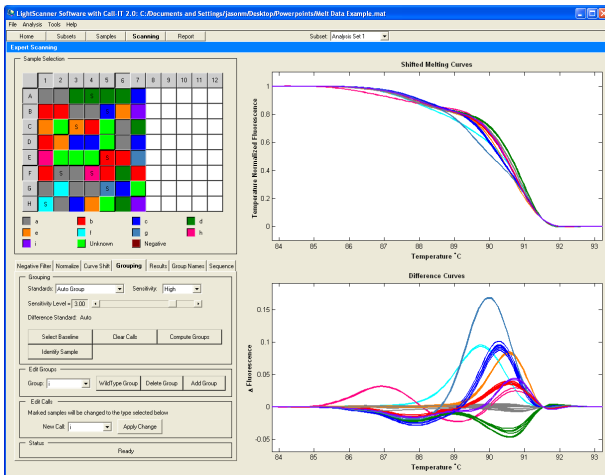
- Looking for NEW or RARE *heterozygous* variants
- Detecting heteroduplexes across entire amplicon, using LCGreen® Plus or other advanced dyes

- Genotyping

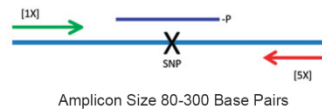
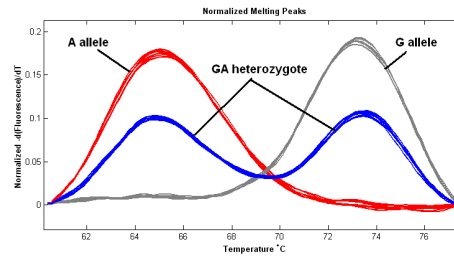
- Looking for KNOWN variants, heterozygous OR homozygous
- Using LunaProbes™ or Small Amplicon melting

Hi-Res Melting Core Applications

Scanning

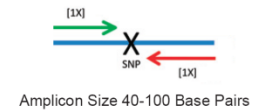
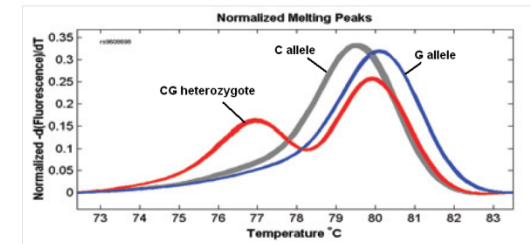


LunaProbes (unlabeled probes) Genotyping

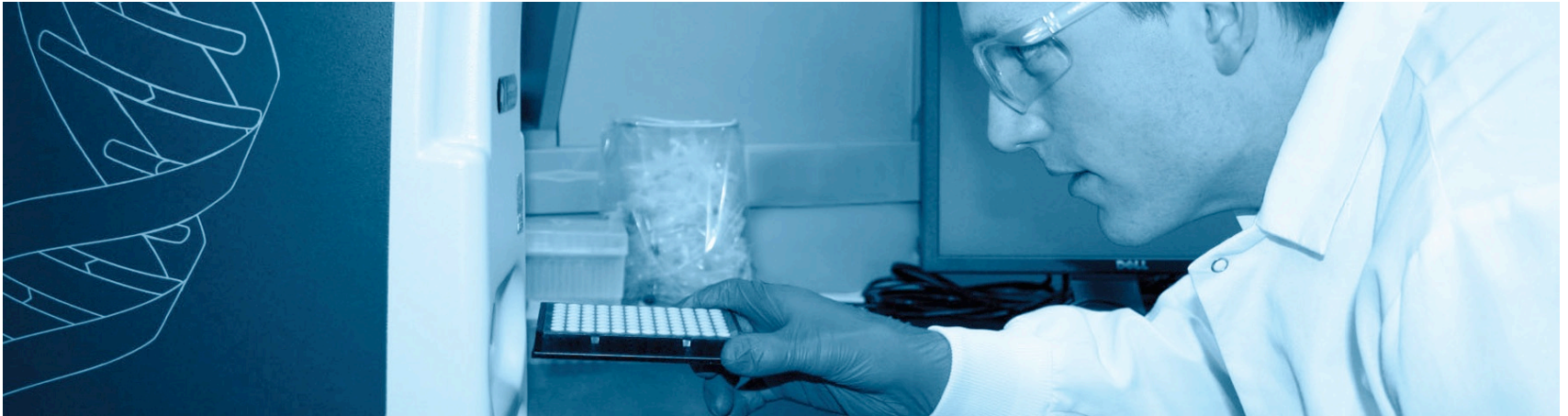


█ = Forward Primer █ = Reverse Primer
█ = Probe Element X = SNP

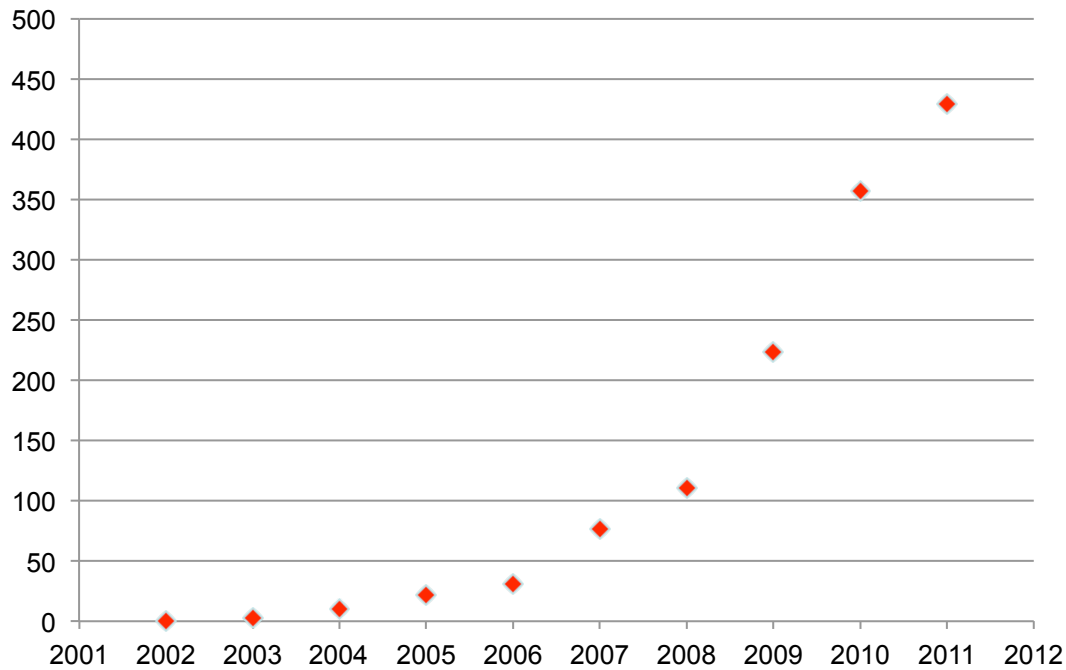
Small Amplicon Genotyping



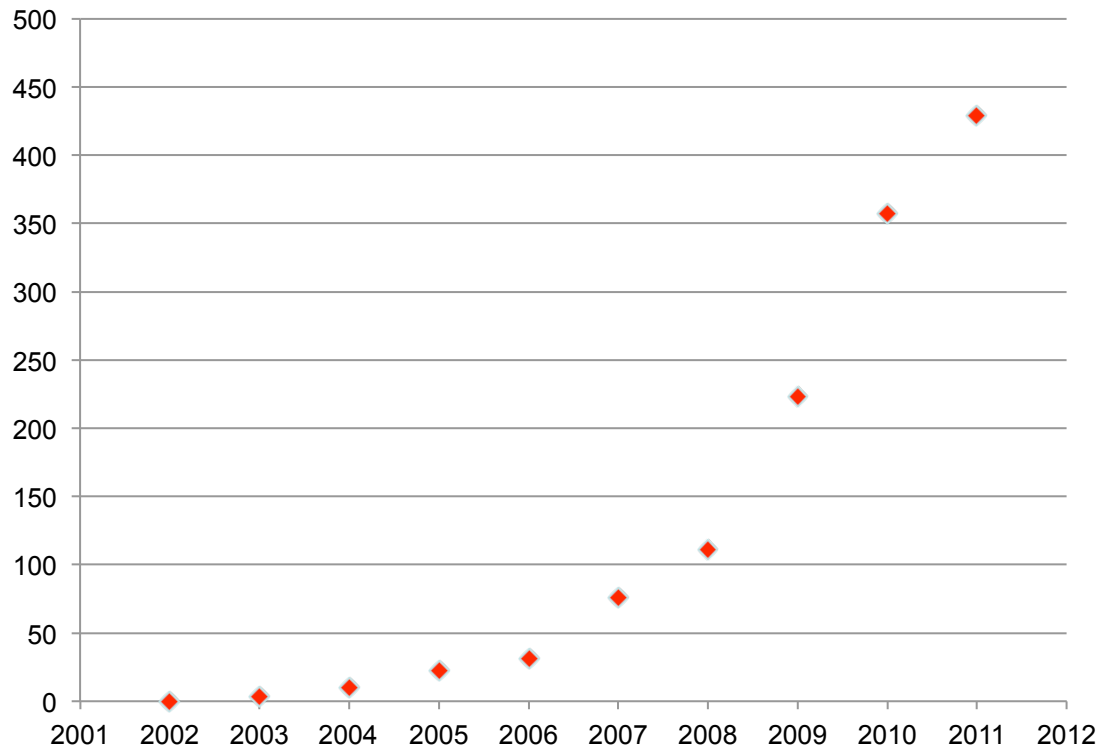
Q: Do the Instruments from any particular company predominate in Hi-Res Melting Publications?



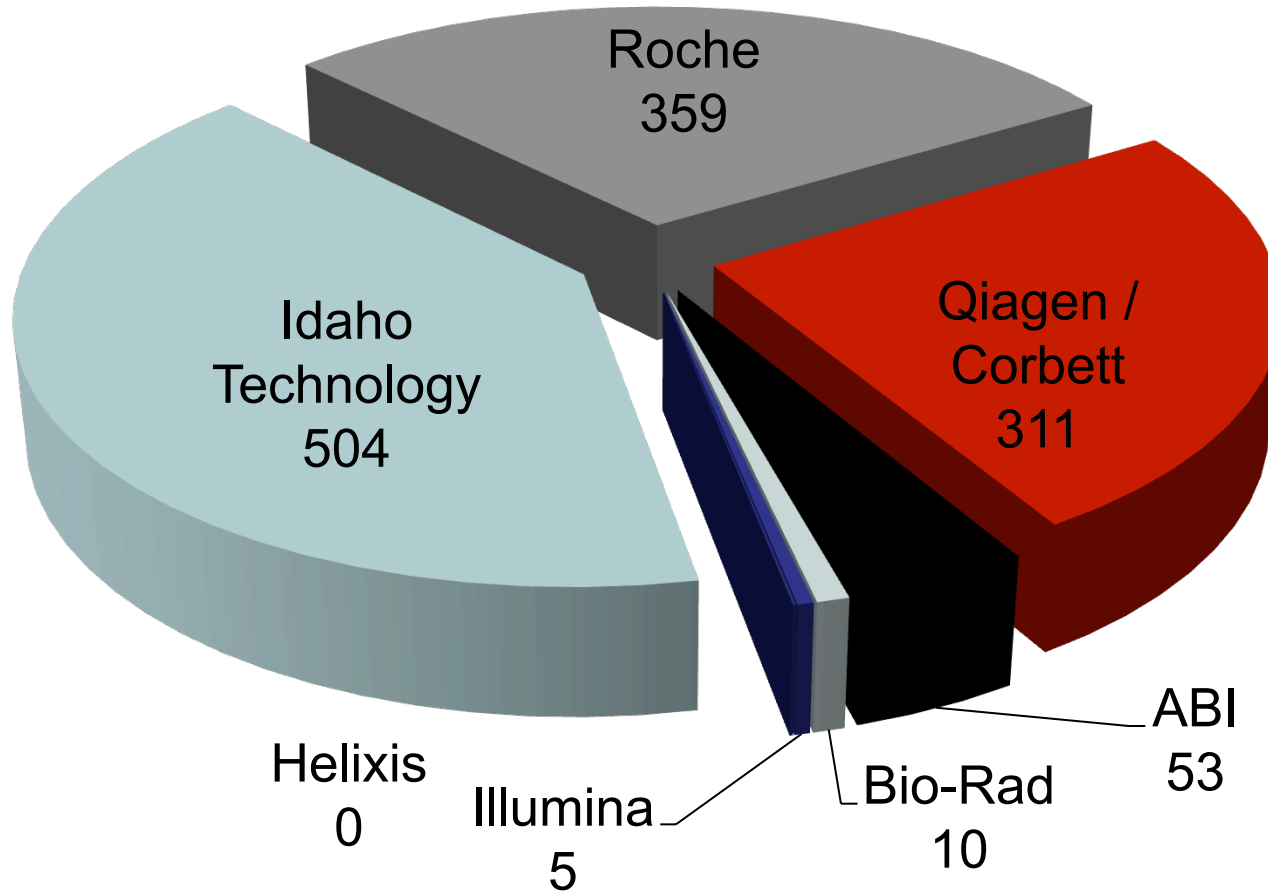
Mystery Graph...



This is the number of High Resolution Melting Pubs / year



Distribution of Hi-Res Melting Publications



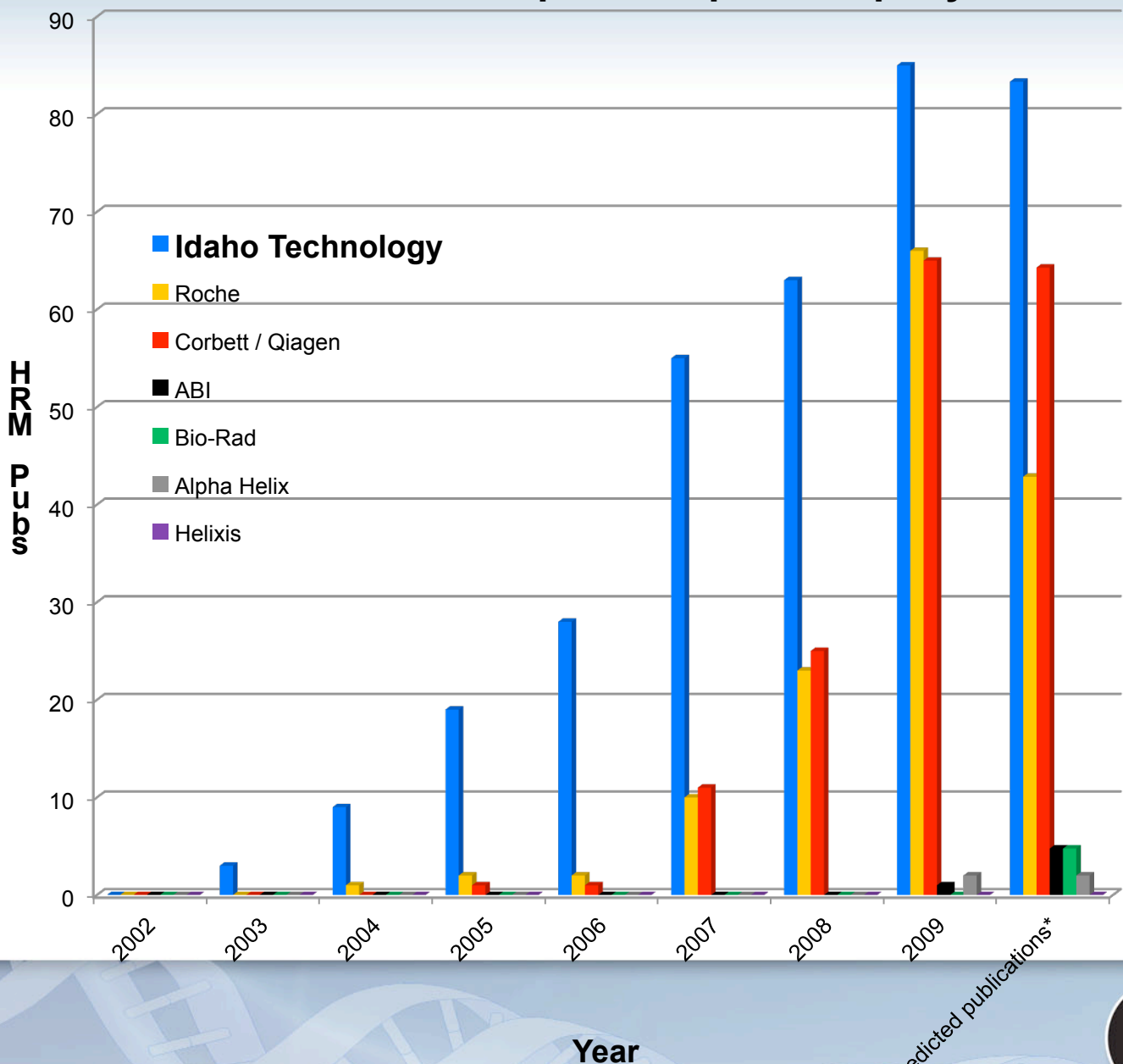
* January 1st 2003 through June 2010
N = 553 publications

End

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HRM Publications per Year per Company



Which Systems can do* HRM?

- HR-1
- LightScanner
- LightScanner 32
- ABI 7500 FAST
- Qiagen Rotor-Gene
- LightCycler 480
- CFX 96
- eco PCR

*Data quality, depth of customer support, melting time, chemistry, and software all vary considerably.



Melting Time & Data Density in Plate Systems

- Bio-Rad CFX96
- LightCycler 480
- ABI 7500 FAST
- LightScanner 96
- 10 points
- 50 points
- 50 points
- 80-120* points
- > 45 min
- > 45 min
- > 45 min
- 5 min

*Raw data collected from LightScanner. Exact number of points dependent on Exposure setting