Releasing the promise of HRM
Custom designed real-time PCR assays
Real-time PCR reagents
Custom designed HRM assays

HRM reagents
High Resolution Melting analysis (HRM)

Step 1. Real-time PCR in presence of intercalating dye
High Resolution Melting analysis (HRM)

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Step 1. Real-time PCR in presence of intercalating dye
High Resolution Melting analysis (HRM)

Step 2. Melting analysis
High Resolution Melting analysis (HRM)
Genotyping by amplicon melting analysis
The Promise of HRM

**Simple**
Simple Post PCR melting analysis
Automated software of genoptying

**Low cost**
No expensive probes required

**Reliable**
All types of mutation can be determined

**Explosive range of applications**
Clinical diagnostics
Clinical research
Gene Scanning
The reality of HRM

- Literature is sparse and controversial
- Complex studies are hard to replicate
- No standardisation of the method
Custom designed HRM assays

HRM reagents

- Any variation
- Any species
- Any machine
A MasterMix for HRM

Real-time PCR MasterMix

Priority:
Amplification plot CT

HRM Mastermix

Priority:
Melting Curve
PrimerDesign Precision HRM MasterMix with HRM dye
A dye for HRM

A novel asymmetric Cyanine dye

Minor groove binder with saturating chemistry

Improved fluorescent properties over BeBo

Chromofy®
Comparison with other Saturating dyes

SYBR green®, EVA green®, SYTO9®, Chromoﬁ®, LCgreen®
Custom designed HRM assays

HRM reagents

Any variation
Any species
Any machine
HRM assays
- Simple HRM analysis of the amplicon
**Problem:** Some variations are difficult to type

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![Graph showing the relationship between SNP class and Tm Shift](image-url)
HRM with a raZor probe™

Step 1. Asymmetric real-time PCR in presence of intercalating dye and raZor probe™
HRM with a raZor probe™

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HRM with a raZor probe™

Step 1. Asymmetric real-time PCR in presence of intercalating dye and raZor probe™
HRM with a raZor probe™

Step 2. Melting analysis
**Problem:** Some variations are difficult to type

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![Graph showing Tm shift]
Problem : Some variations are difficult to type
Problem solved : raZor probe™
Normalised data on the Rotorgene 6000

raZor probe™

Amplicon
Optimisation of raZor probe™
Custom designed HRM assays

HRM reagents

Any variation
Any species
Any machine
Roche480 normalisation across the amplicon

TNF$\alpha_{252A \rightarrow G}$

Only Heterozygotes identified
Roche480 normalisation across the probe

TNFα252A→G

Gene Scanning analysis module
Roche480 probe based normalisation

TNFα252A→G

Melt curve genotyping module
Amplicon gene scanning is strong (heterozygote detection)
Amplicon genotyping is weaker
raZor probe™ based genotyping very strong

raZor probe™ genotyping data on the Roche 480
Roche LightCycler 1.5
TNFα252A→G
Bio-Rad iCycler iQ5
TNFα252A→G
raZor probe™ genotyping on different platforms

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TNFα252A→G
## Machine limitations for HRM

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<tr>
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Factor V Leiden

SNP mutation in the factor V gene in exon 10

Type 1 SNP : A to G

5% of Europeans carry the variant form

Overproduction of thrombin leading to excess fibrin generation and excess clotting
Factor V Leiden Factor V Leiden raZor probe™ kit
- raw melting curve data
Factor V Leiden Factor V Leiden raZor probe™ kit
- normalised data (rotorgene 6000)
Factor V Leiden raZor probe™ kit
- difference plots

amplicon

raZor™ probe
Factor V Leiden raZor probe™ kit
- clinical validation

Acknowledgement

World Reference Reagent
Factor V Leiden Human gDNA Reference Panel
NIBSC code: 04/224

National Institute for Biological Standards and Control
Blanche Lane
South Mimms
Potters Bar
Hertfordshire
EN6 3QG
Custom designed HRM assays

HRM reagents

Any variation
Any species
Any machine
razor sharp genotyping

HRM
High Resolution Melting Analysis

Quality Reagents
razor sharp genotyping

HRM