Near Patient Testing

Paul Debenham
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DNA analysis promises unrivalled benefits of genetics for all

But must it stay as a laboratory science; could it not come to the point of care?

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Genetic diagnostics at point-of-care: An added value vision

- Technology platform designed to empower health professional - not DIY genetics
- DNA-based definition means the most specific advice and treatment can be achieved at primary care
- Potential to revolutionise treatment of infectious agents - from subjective to objective
- Predict and avoid adverse reactions to medications
- Simple non-invasive sampling

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The patient pathway

sampling

consultation

20 minutes
for result

treatment

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The sample pathway

Sample devices

Transfer to reagents cassette

Result

Process for 20 minutes

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Typical Laboratory Process

- Sample Preparation
- PCR Amplification
- PCR products detected and identified
- Results analysis

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Making DNA analysis where the patient is - progress

- Sample preparation — Eliminate
- PCR amplification — Accelerate
- PCR product detection and identification — Simplify
- Results analysis — Automate

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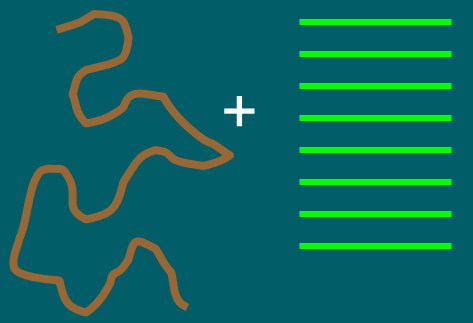
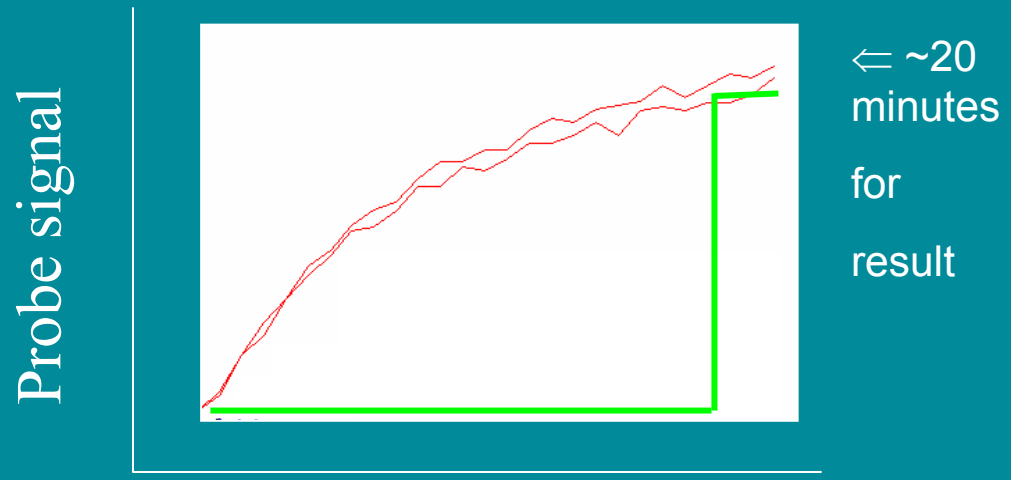
Probes working after faster PCR amplification

DNA in sample + Homogeneous Probe + Rapid PCR =





⇐ ~20 minutes for result

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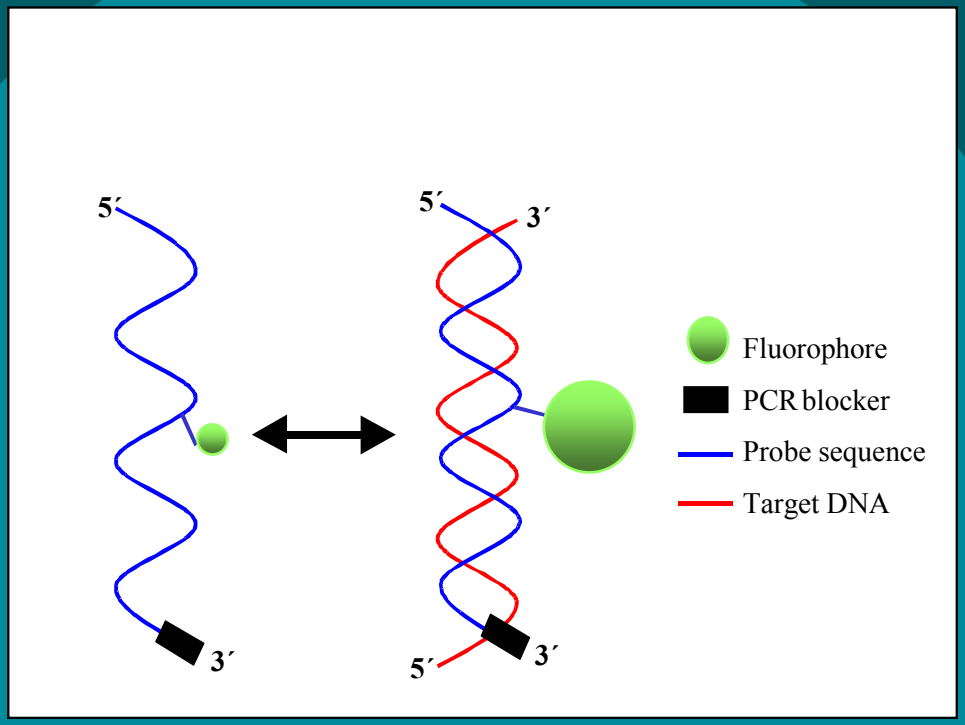
HyBeacon™ probes

- Simple probe structure, linked only to a single internal ‘reporter’ fluorescent molecule
- Discovered light emitted increases as probe finds matching DNA
- Can work in PCR thermocycling reaction
- Diagnosis built around a temperature defined increase in light emission linked to binding to matched DNA



t°c

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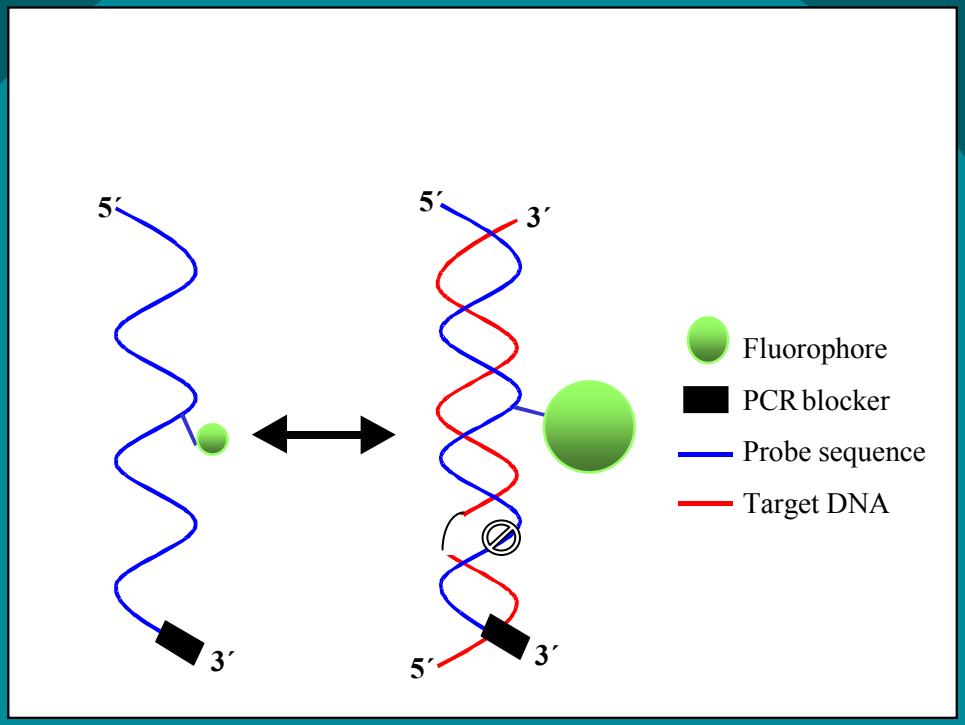
HyBeacon™ probes

- Simple probe structure, linked only to a single internal ‘reporter’ fluorescent molecule
- Discovered light emitted increases as probe finds matching DNA
- Can work in PCR thermocycling reaction
- Diagnosis built around a temperature defined increase in light emission linked to binding to matched and unmatched DNA



Tºc -10ºc

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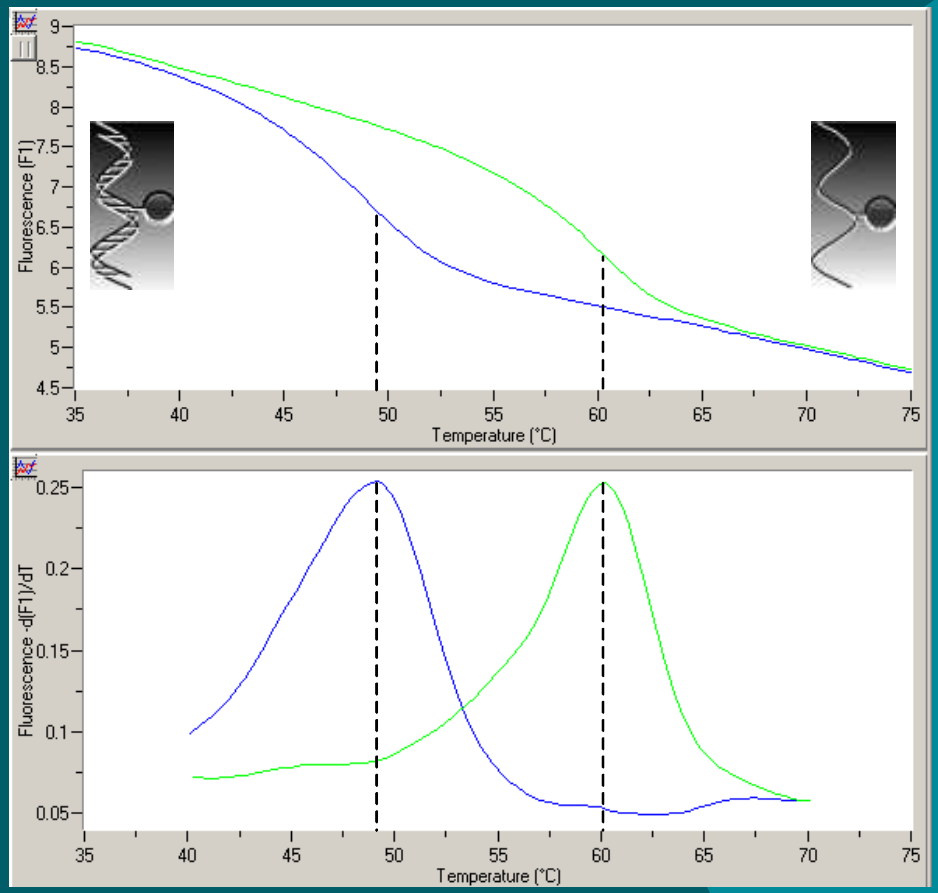
Melt Peak Analysis



- Melting temperature (Tm) of probe determined by the sequence of the target
- Tm = 50% hybridised probe
- Target detection & identification
- Magnitude of ΔTm depends on nature of mismatch
  - Stable - G/G, G/A, G/T
  - Intermediate - T/T, A/A,
  - Destabilsing - C/A, C/T, C/C

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Development of a CYP2D6*4 assay

Tm = 60.4ºC ± 0.1 ºC

Tm = 49.3ºC ± 0.1 ºC

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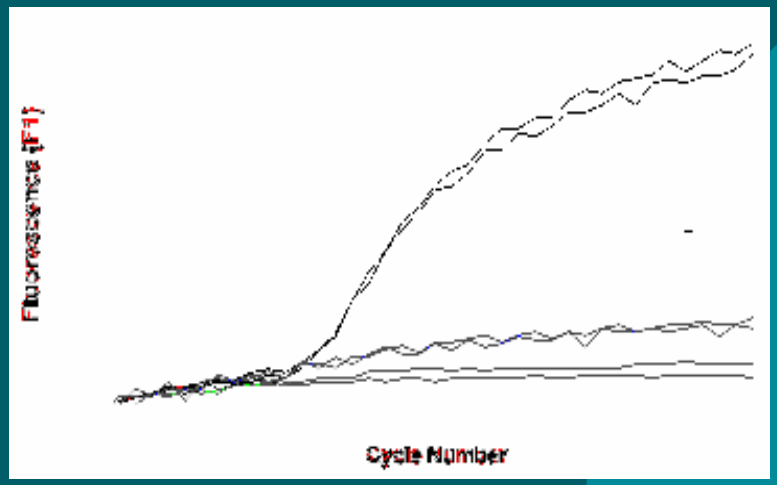
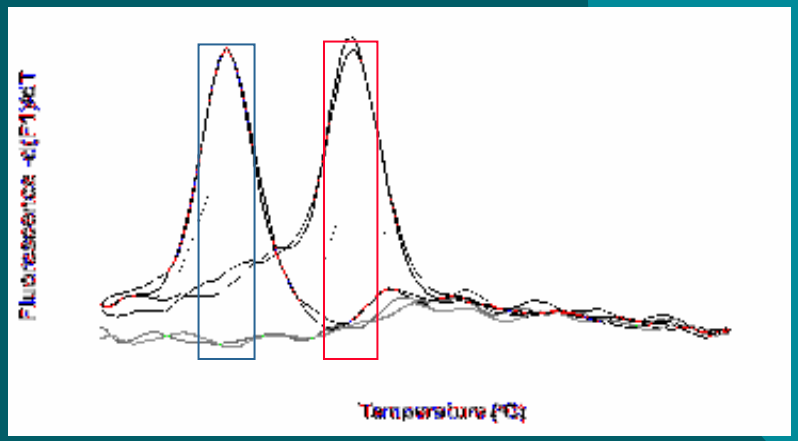
Detection and discrimination of CYP2D6*4 SNP by HyBeacons



Red - probe matches patient DNA
Blue - probe mismatches patient DNA
Black - matched and mismatched DNA present
Green - no DNA



Confirmation test looks at quality of match with temperature

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Development of a Factor V Leiden assay (a key indicator for deep vein thrombosis)

Tm = 55.4ºC

Tm = 45.5ºC

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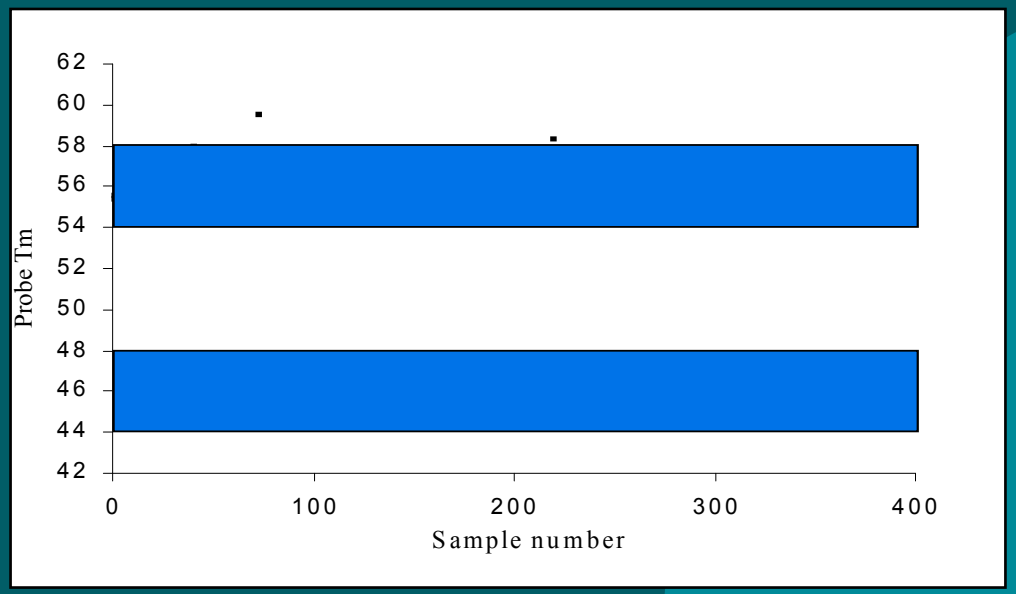
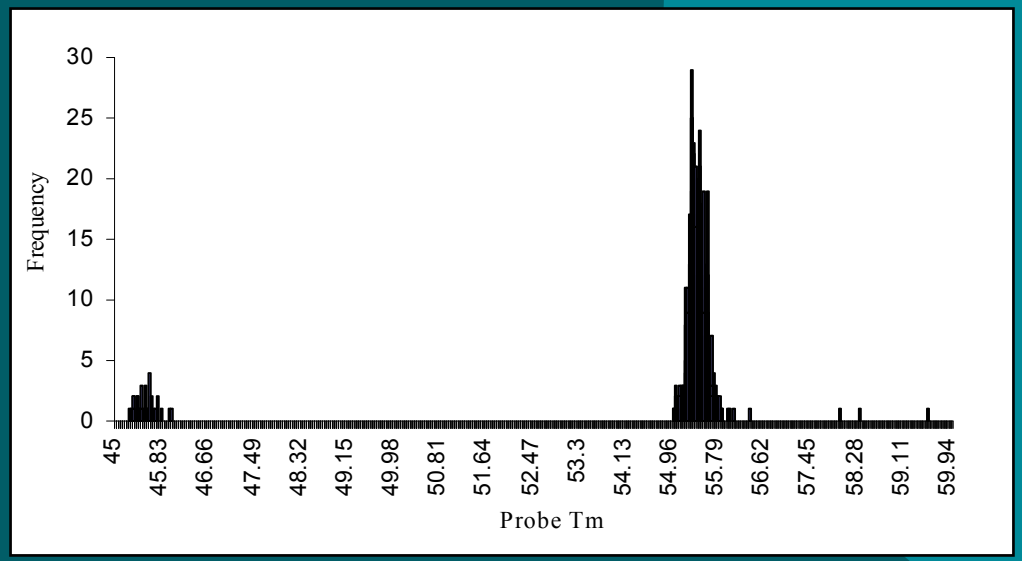
Factor V Lieden, confidence of genotypes





- Diagnostic zones applied to Tm data
- 99.5% of data points within zones
- No data points between zones.
- Genotypes obtained with at least 99.9% confidence
- Demonstrated kit efficiency, reproducibility, accuracy and robustness

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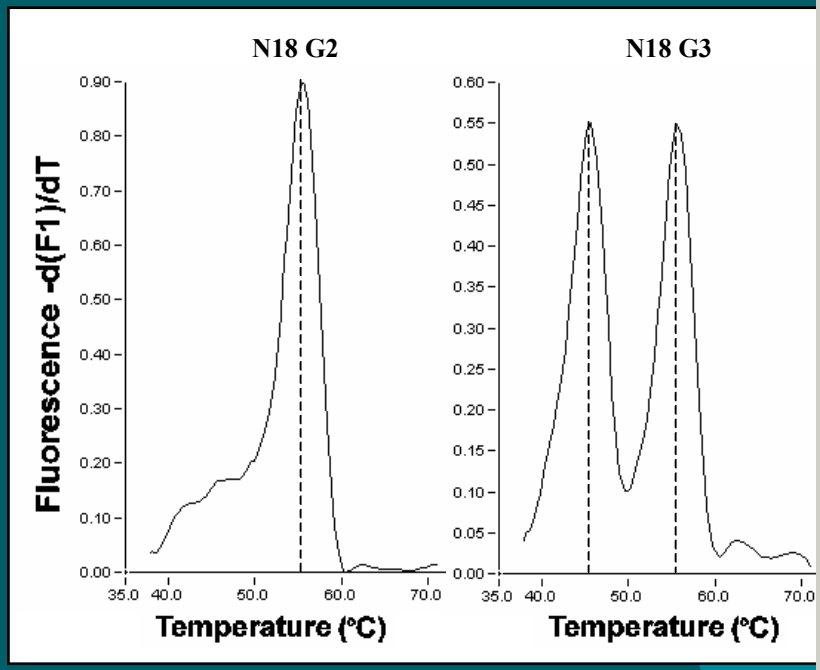
Factor V Leiden Assay



Step 1: Melting Peaks | Step 2: Peak Areas | Extra: Manual Tm

Number of Peaks: Zero / One / Two / Three

Clear All

Weighted Fit

Position	Name	Tm 1	Area 1	SD 1	Tm 2	Area 2	SD 2
1	n18 g2	55.34	4.706	2.093			
2	Repli. of n18 g2	55.34	4.626	2.070			
3	n18 g3	45.32	3.050	2.272	55.52	2.782	2.046
4	Repli. of n18 g3	45.31	2.798	2.167	55.57	2.543	1.930
5	n18 g4	55.36	4.436	2.108			
6	Repli. of n18 g4	55.34	4.215	2.133			
7	n18 g5	55.29	5.129	2.222			
8	Repli. of n18 g5	55.47	3.256	2.223			
9	n18 g6	55.31	4.971	2.180			
10	Repli. of n18 g6	55.35	4.569	1.974			
11	n18 g7	55.28	3.935	2.166			
12	Repli. of n18 g7	55.40	4.258	2.177			
13	n18 g8	55.42	5.129	2.195			
14	Repli. of n18 g8	55.37	4.787	2.191			
15	n18 g9	55.39	2.748	2.178			
16	Repli. of n18 g9	55.30	4.905	2.173			
17	n18 g10	55.33	5.051	2.134			
18	Repli. of n18 g10	55.38	4.656	2.105			
19	n18 g11	55.35	4.673	2.171			
20	Repli. of n18 g11	55.56	3.022	2.176			
21	n18 g12	55.30	4.993	2.226			
22	Repli. of n18 g12	55.46	4.818	2.080			
23	n18 h1	55.47	4.477	2.105			
24	Repli. of n18 h1	55.46	5.146	2.150			
25	n18 h2	55.44	4.767	2.055			
26	Repli. of n18 h2	55.49	4.081	1.850			
27	n18 h3	55.49	4.447	1.883			
28	Repli. of n18 h3	55.44	5.002	2.108			
29	n18 h4	55.40	3.943	2.011			
30	Repli. of n18 h4	55.41	3.949	2.116			
31	neg						
32	neg						

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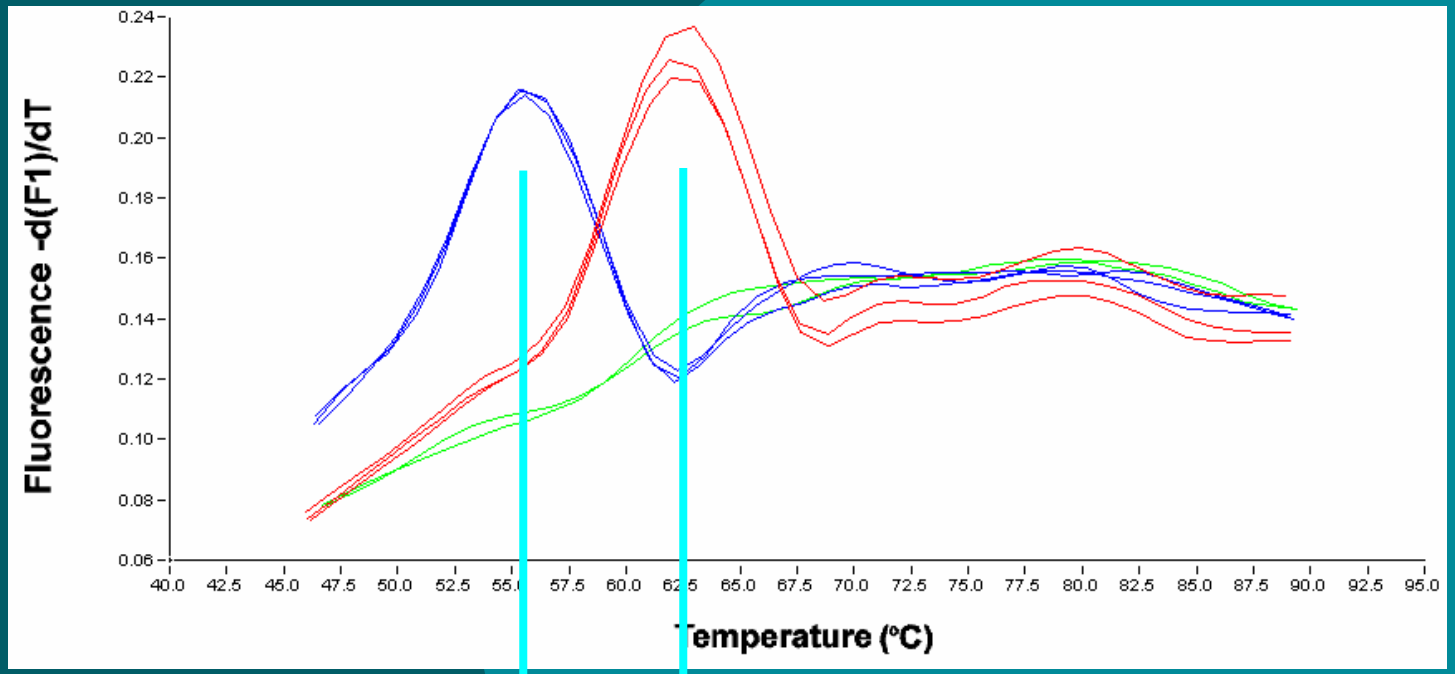
Automated diagnosis



Control

Positive

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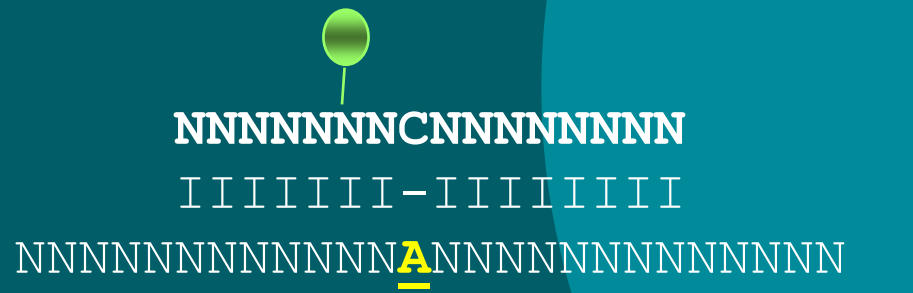
Development of a Chlamydia trachomatis assay

Tm = 55ºC ± 0.1 ºC



Tm = 47. ºC ± 0.1 ºC

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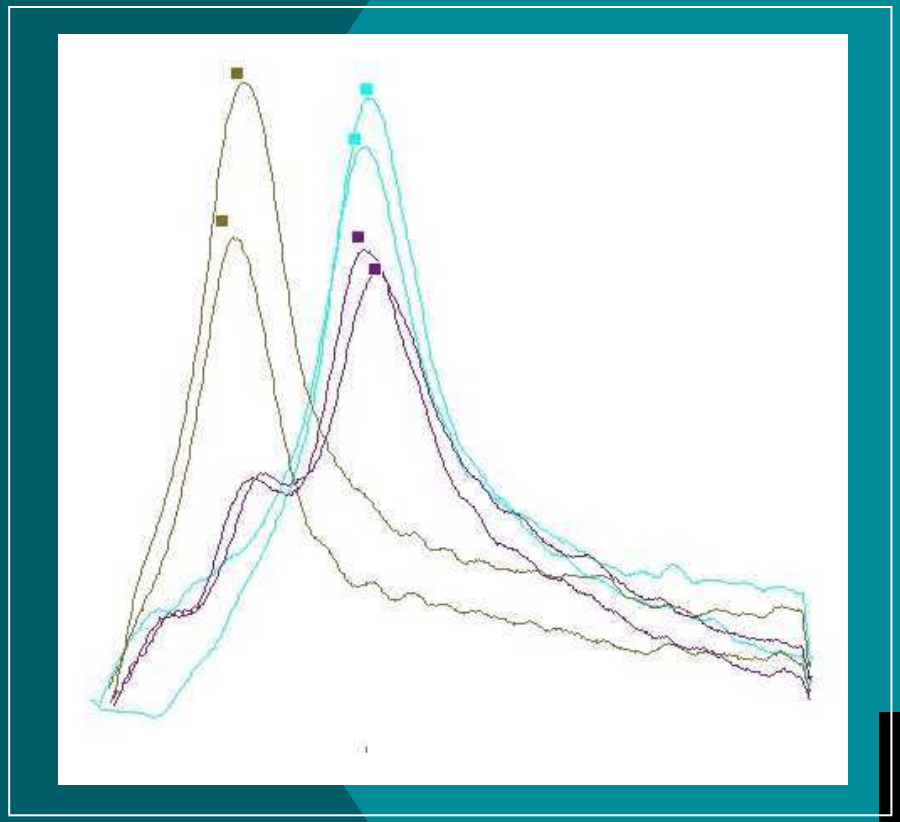
Chlamydia diagnostic with internal positive control



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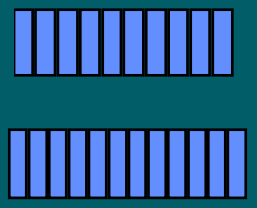
STR profiling

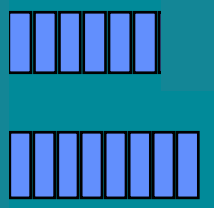
..GTATGCCATTG ATTG ATTG ATTG ATTGCCGGCAT..

..GTATGCCATTG ATTG ATTGCCGGCAT..

Each person has 2 copies of each STR Locus



Person A
Type 10,12



Person B
Type 6,8

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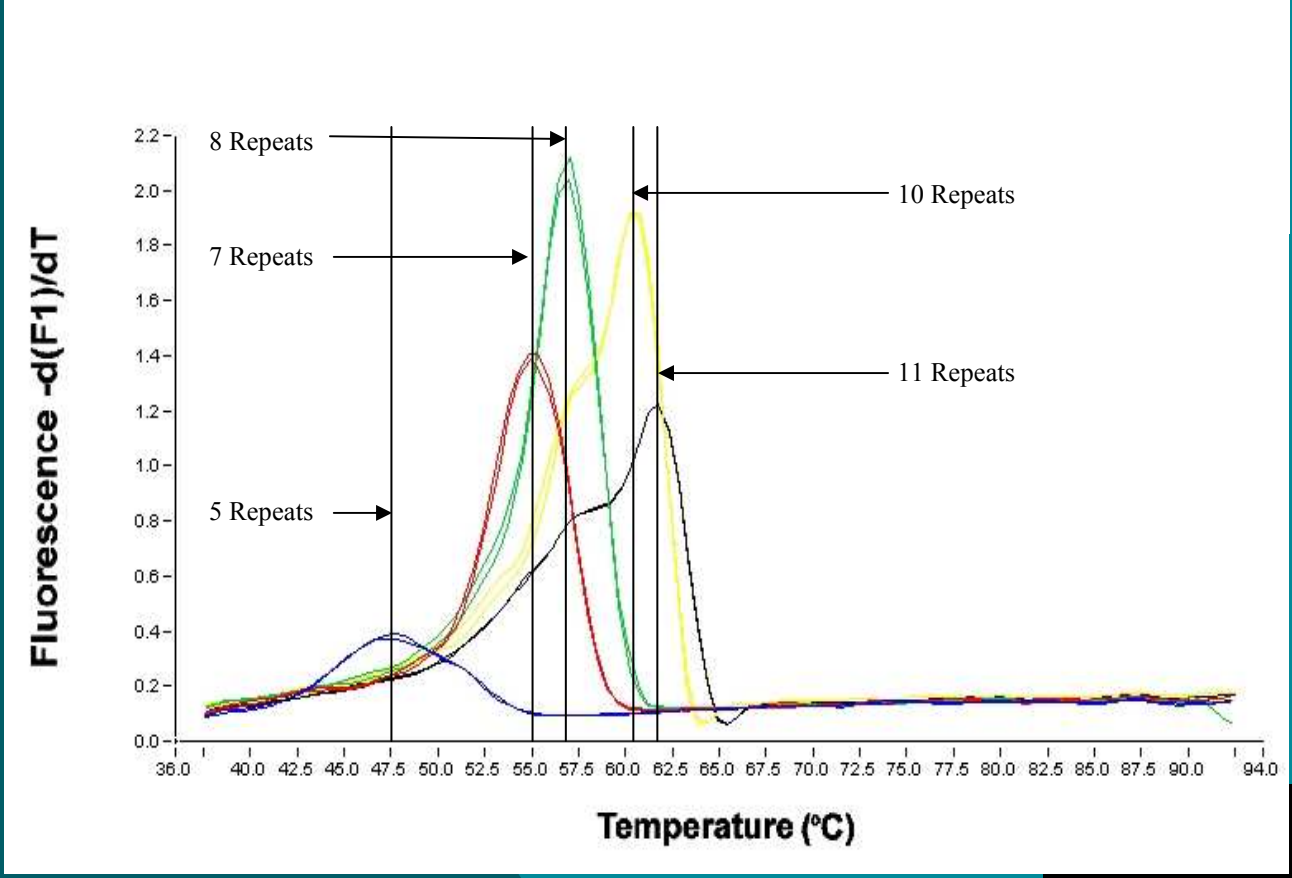
Potential of HyBeacons to discriminate STRs of different repeat numbers



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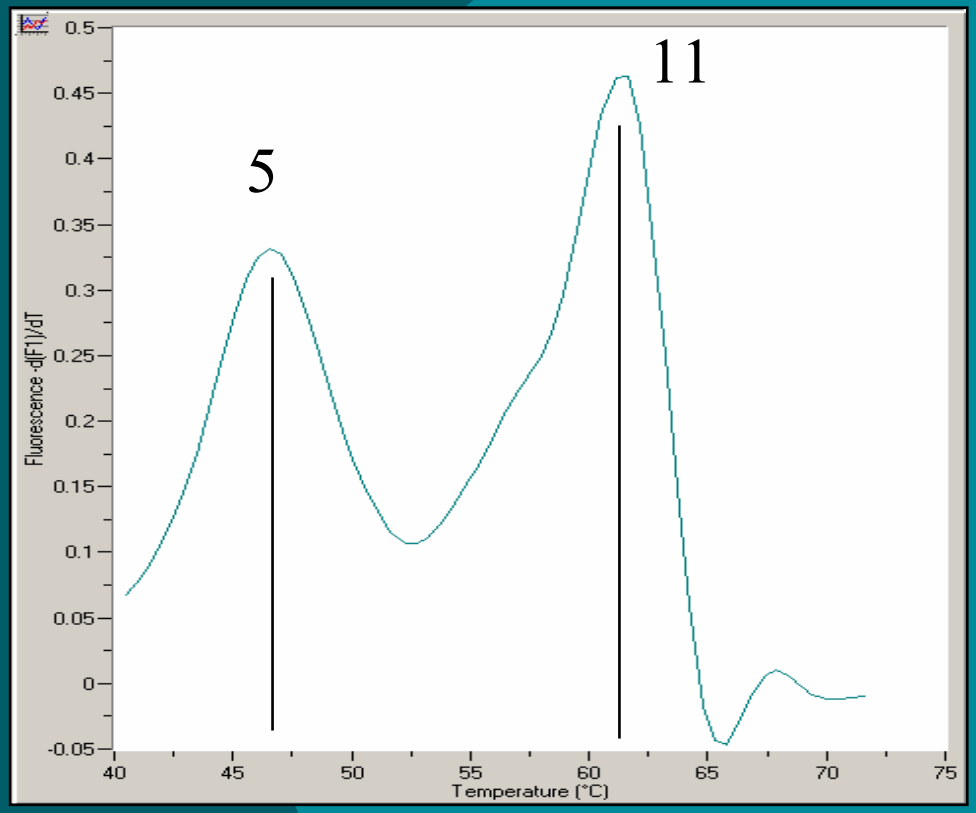
STR profile direct from saliva



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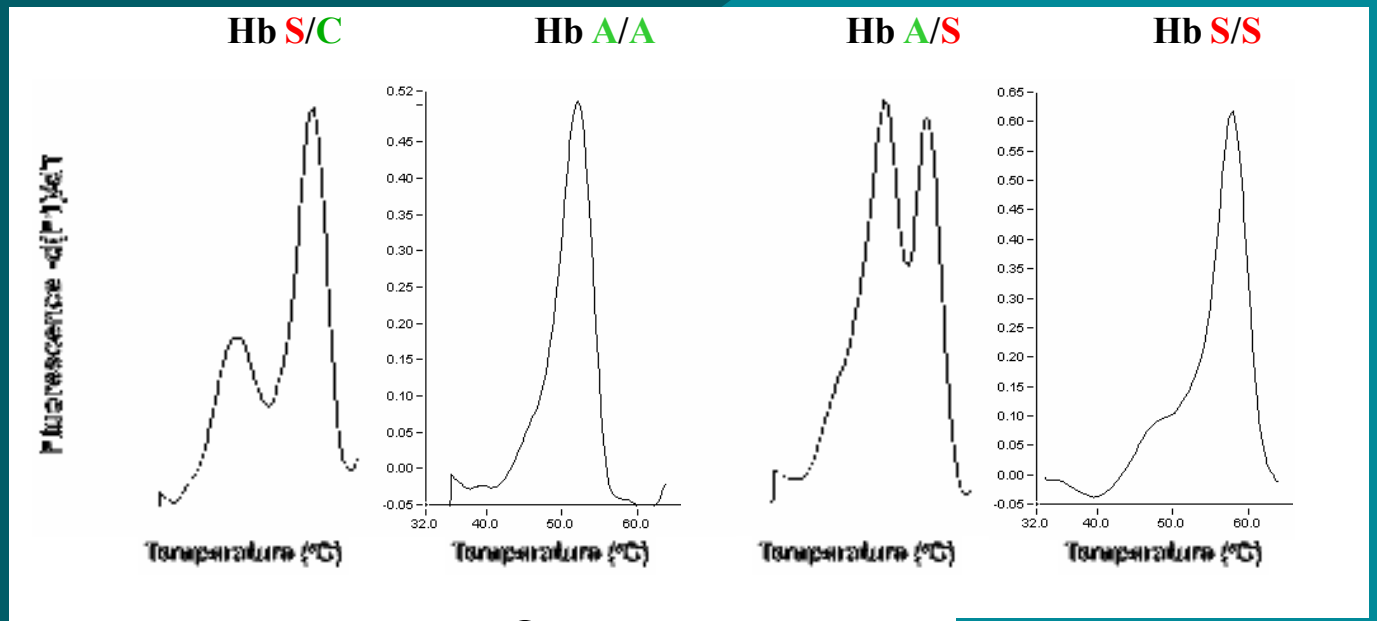
Sickle Cell Anaemia



HbC -48° C

HbA -52° C

HbS -58° C

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Freeze-dried reagents

- Sample added directly to Genedrive cartridge
- Sample to result in ~20 minutes

molecular SENSING plc

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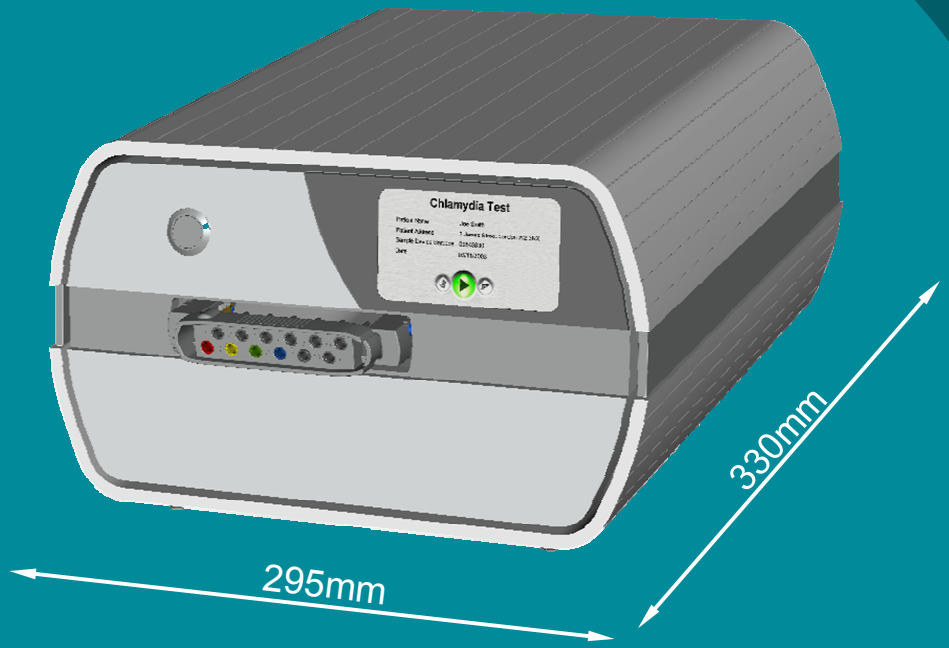
Genedrive® - key features

- 24 samples (2x12)
- Single use plastic disposable cartridge
- 50 cycles and melt analysis within 30 minutes
- Compatible with SYBR Green and Fluorescein (495/520nm)
- Highly sensitive optics



molecular SENSING plc

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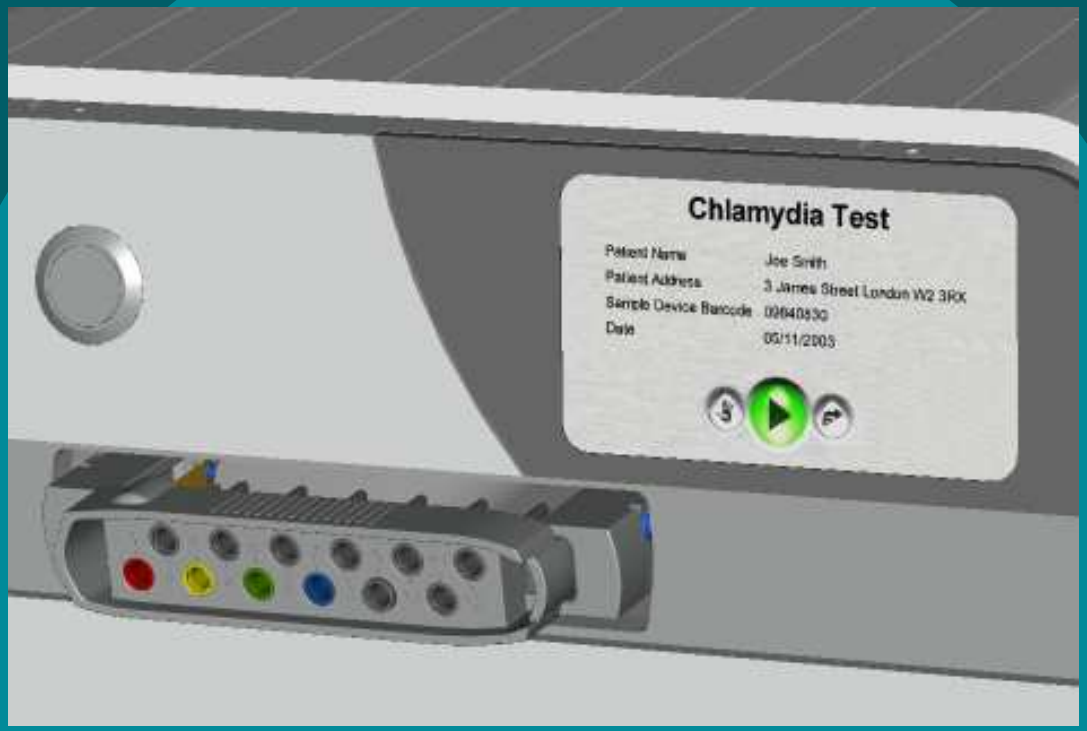
End-user Mode

- User interface on instrument
- Scan barcode on cartridge to instruct instrument which assay conditions to perform
- Automated data analysis and characterisation of samples as positive, negative or fail



molecular SENSING plc

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HyBeacon assays

Target Example	Category
NAT2	Human - DNA
Influenza	Virus - RNA
Adenovirus	Virus - DNA
Streptococcus pneumoniae	Bacterium (Gram +ve) - DNA
Chlamydia trachomatis	Intracellular parasite - DNA