



Scorpions

For Genotyping and Real Time
PCR

Acknowledgements

- Jane Theaker
- Jannine Mann
- Tom Brown
- Rupert Gaut
- Antony Halsall
- Paul Ravetto
- Nicola Thelwell

Outline of Talk

- Introduction to Scorpions
- Some Mechanism and Comparative Data
- Genotyping
- Conclusions

Scorpions Background

- Scorpions was invented in response to a need at AstraZeneca Diagnostics to have a technology for nucleic acid based diagnostics which was:
 - PCR based
 - homogeneous
 - reliable
 - gave freedom to operate

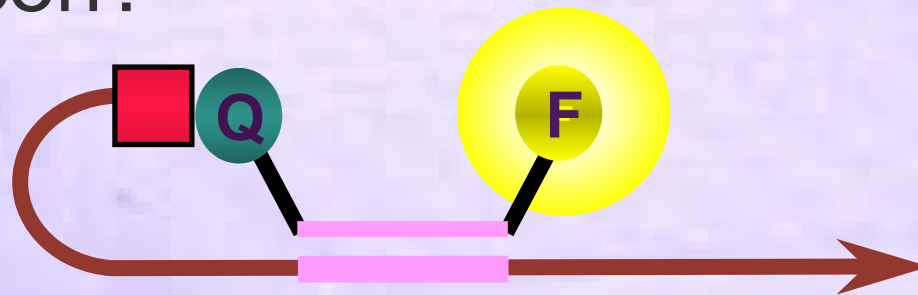
Issues with two probe systems

- Kinetics are slow
 - Energy cost to open stem/loop of probe
 - Bimolecular collision
- Competition for Molecular Beacon Target site from:
 - PCR product synthesis
 - Target/complement reannealing
 - **Target strand folding**
- For Beacons, some signal generation is via 5'-3' nuclease cleavage

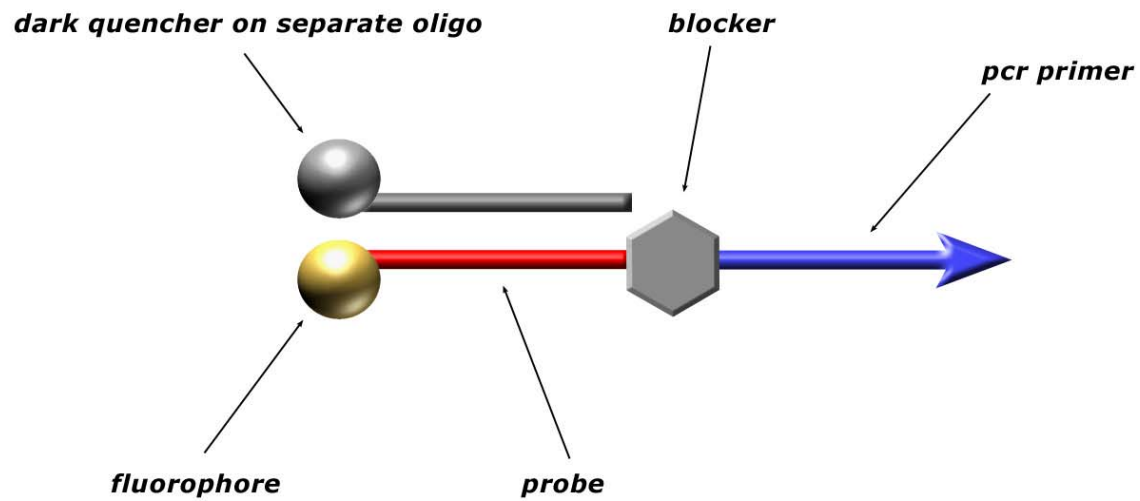
These problems also apply to other bimolecular (Eclipse) and tri-molecular systems (LightCycler Probes)

If the Target Molecule Folds Efficiently...

Why not stick the probe to the amplicon?



Elements of a Scorpions primer

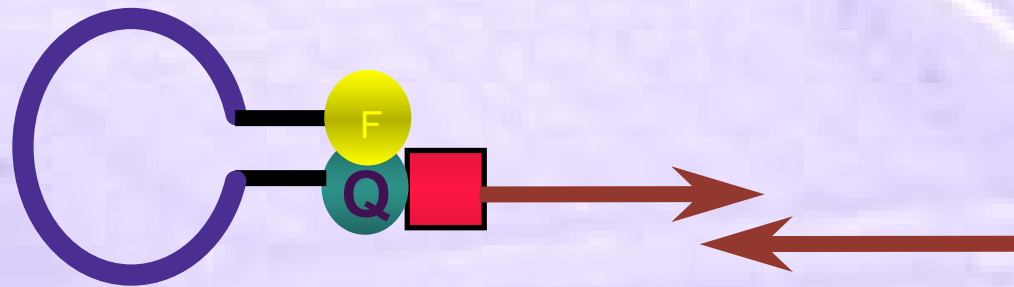
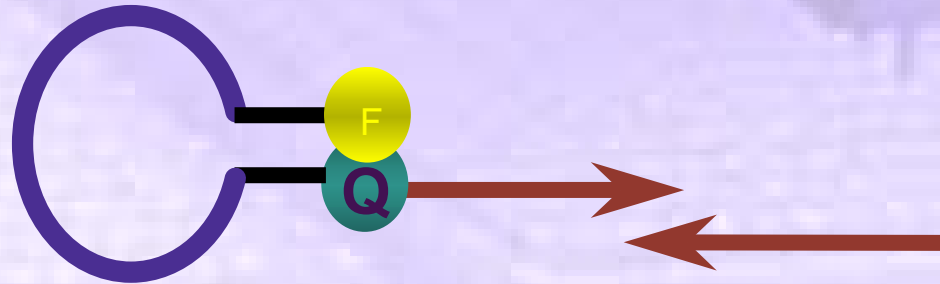


Elements of the Scorpions primer

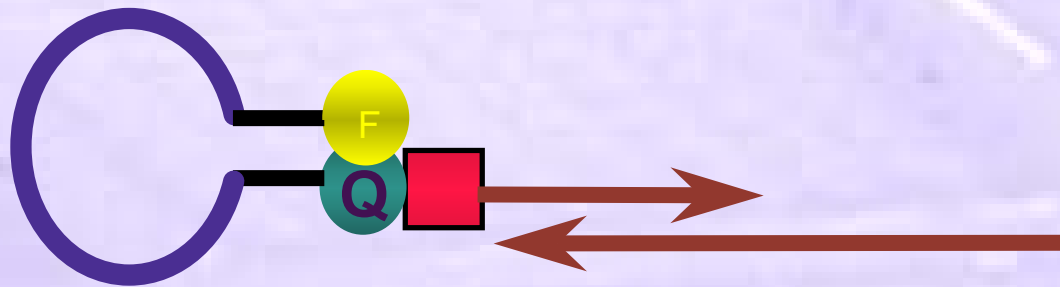
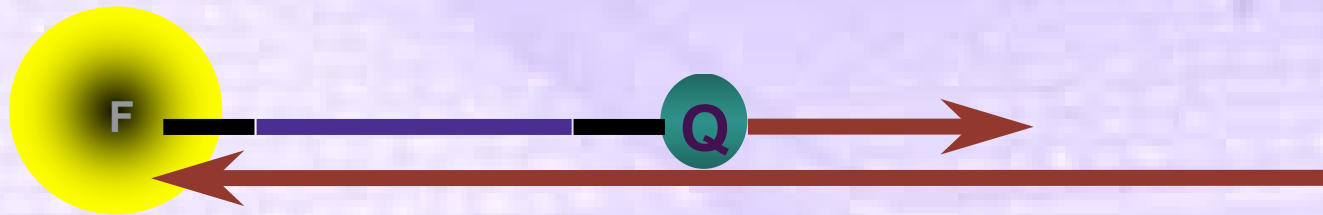


Scorpions is a trademark of DxS Ltd.

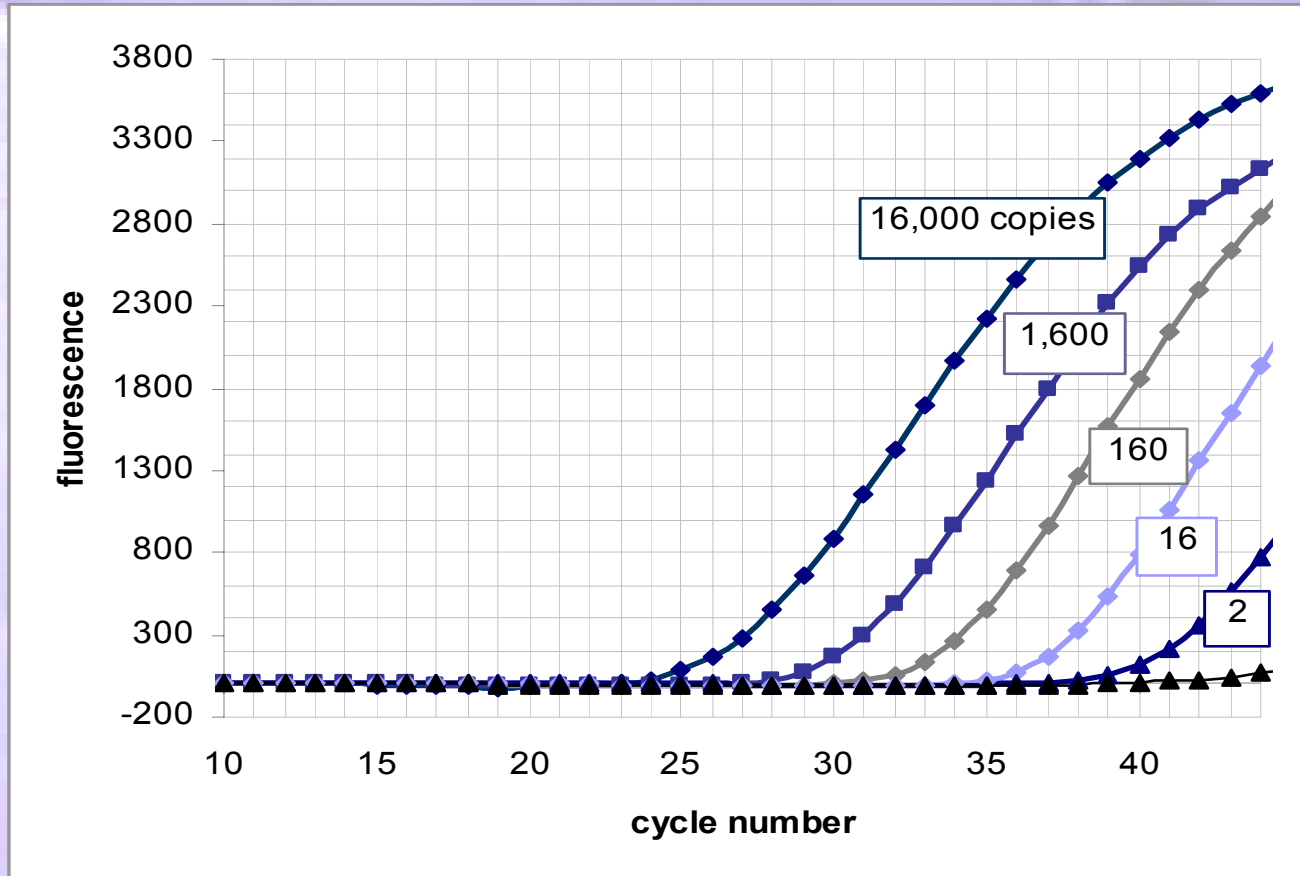
What's the "Blocker" for?



Prevention of Non-Specific Signals

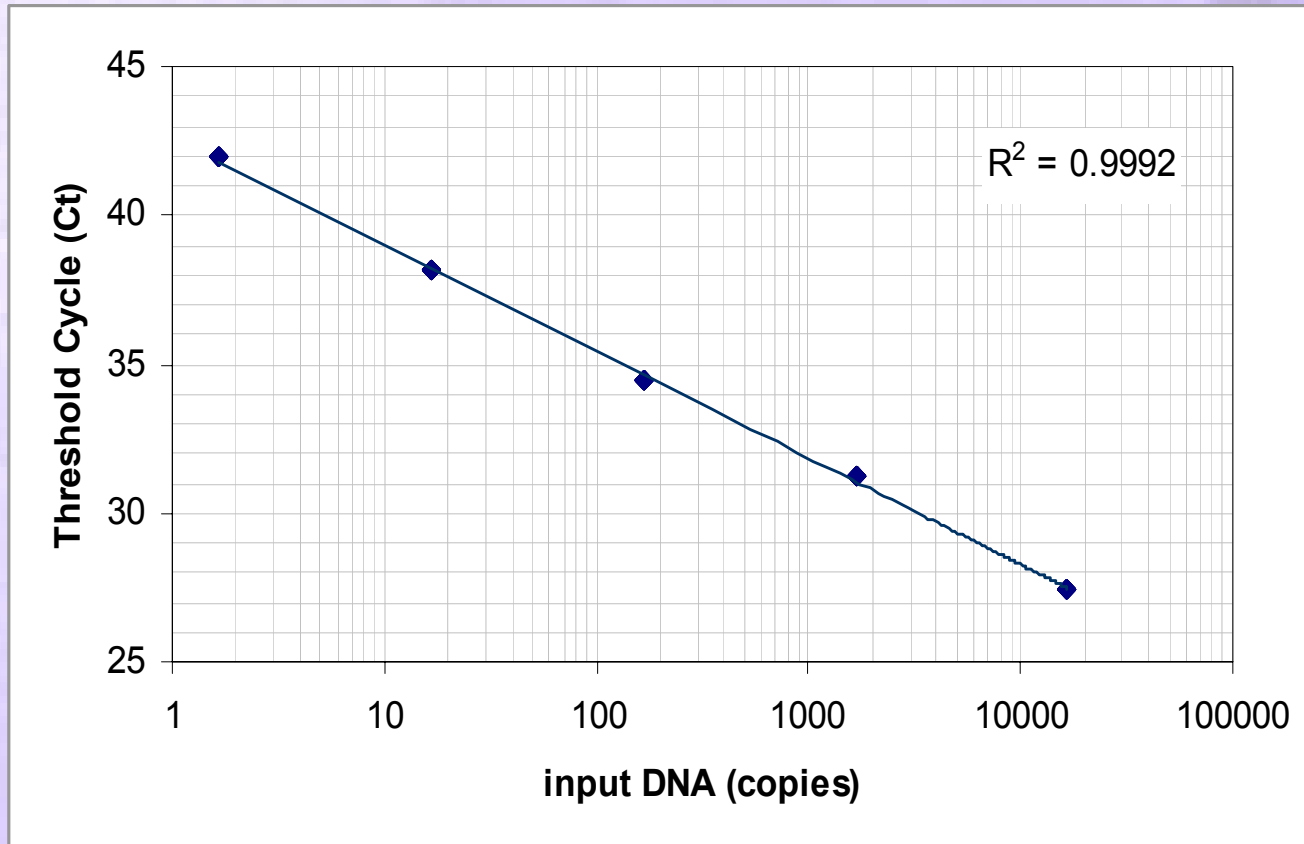


Sensitivity



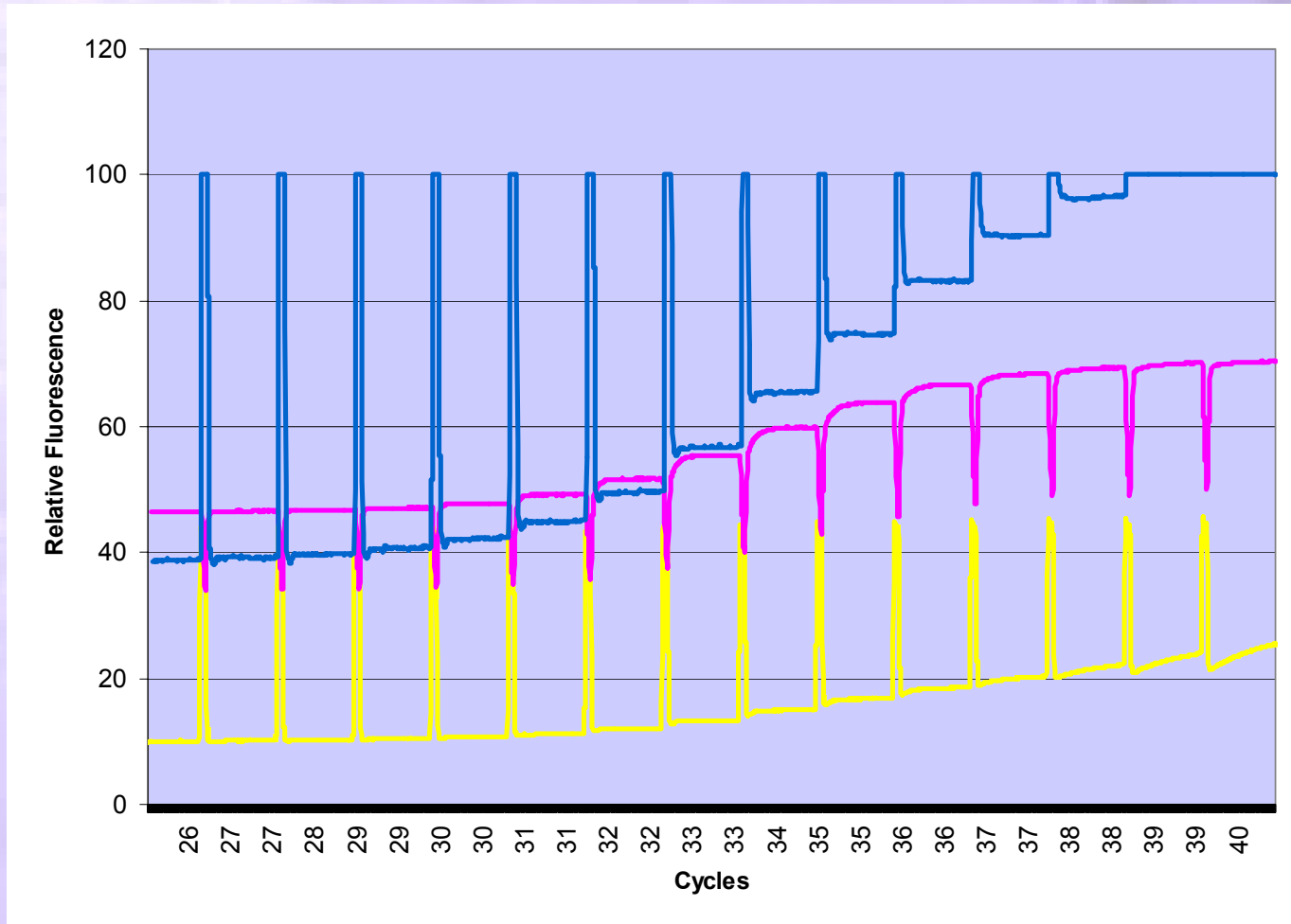
β -actin Scorpions assay –
input dilution series 16,000 –
2 copies

Quantitative PCR



β -actin Scorpions assay –
input dilution series 16,000 –
2 copies

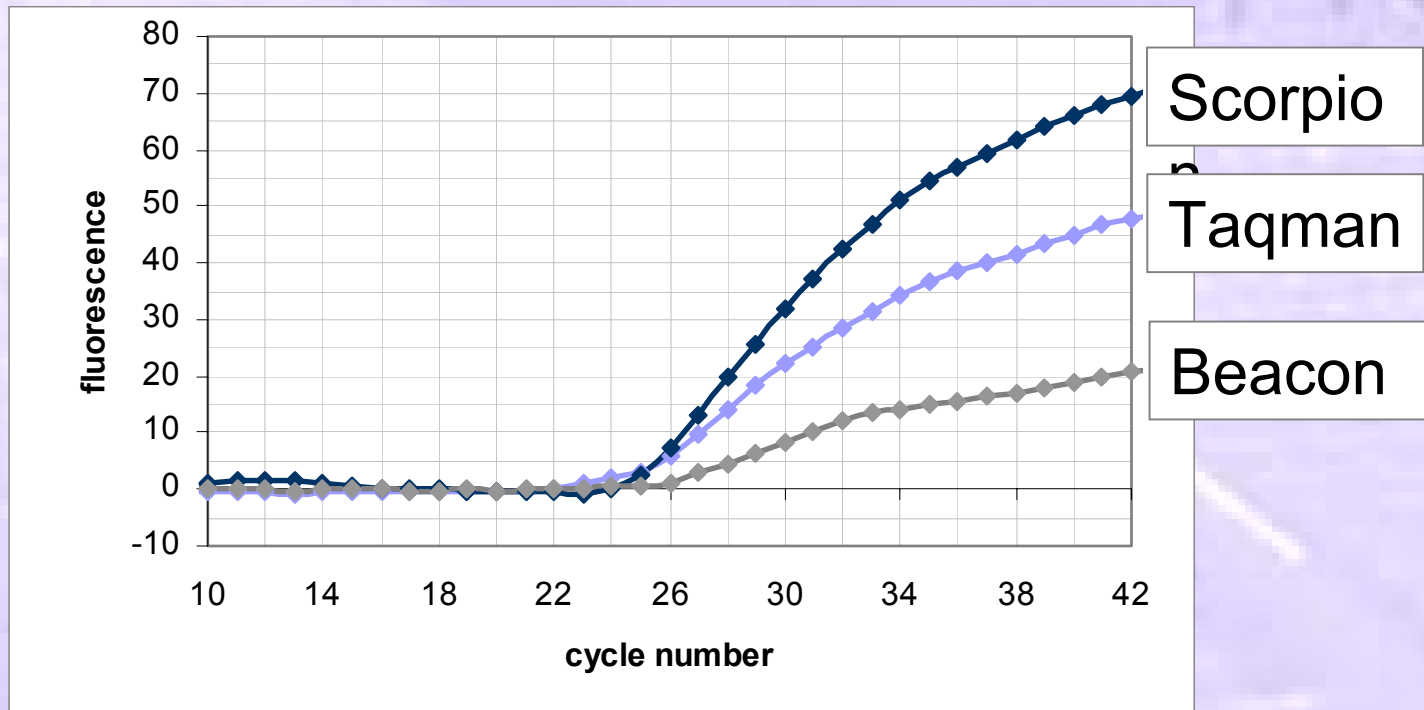
Signal Generation



Summary

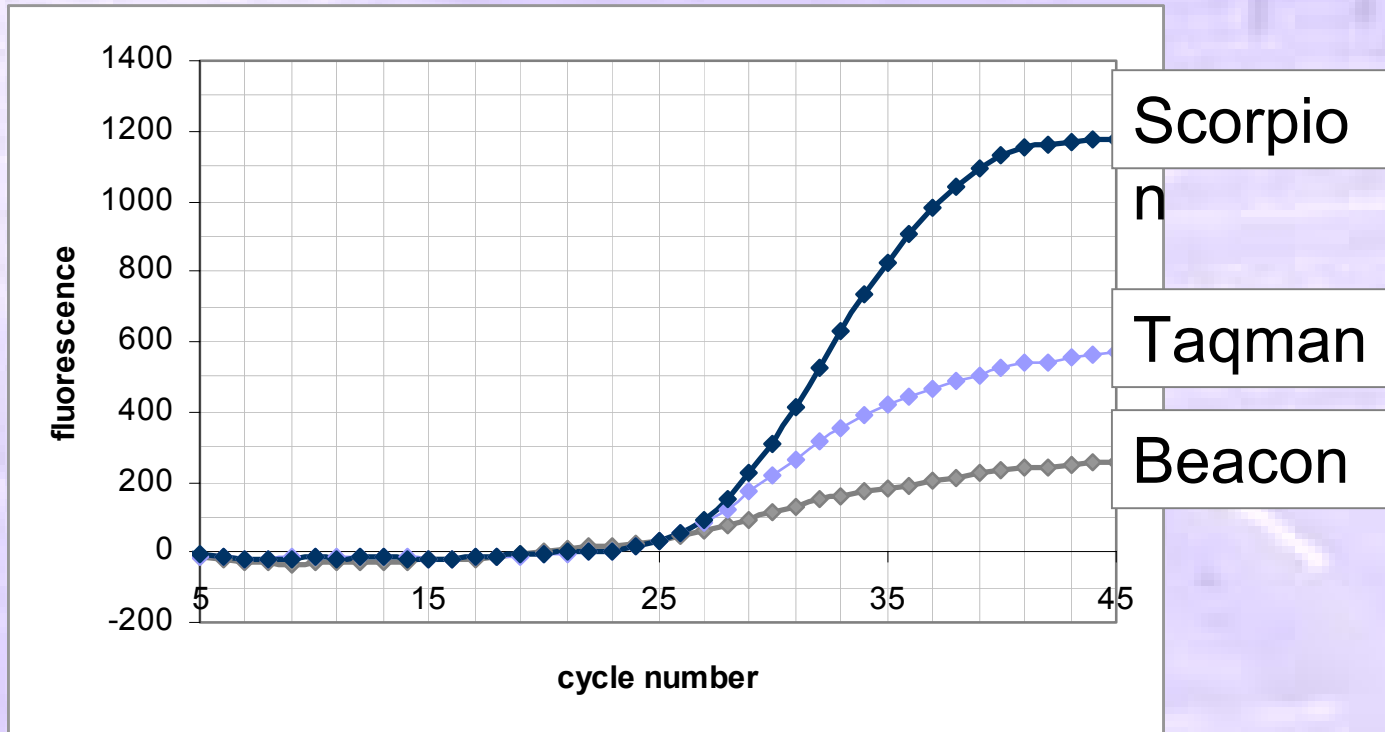
- Homogeneous and Specific fluorescent PCR detection with unique uni-molecular mode of action
- Significant Performance Benefits
 - High Sensitivity
 - Excellent Specificity
 - Support Rapid PCR
 - Reproducible reactions and reliable design
 - Compatible with many detection instruments

Comparison to other technologies -1



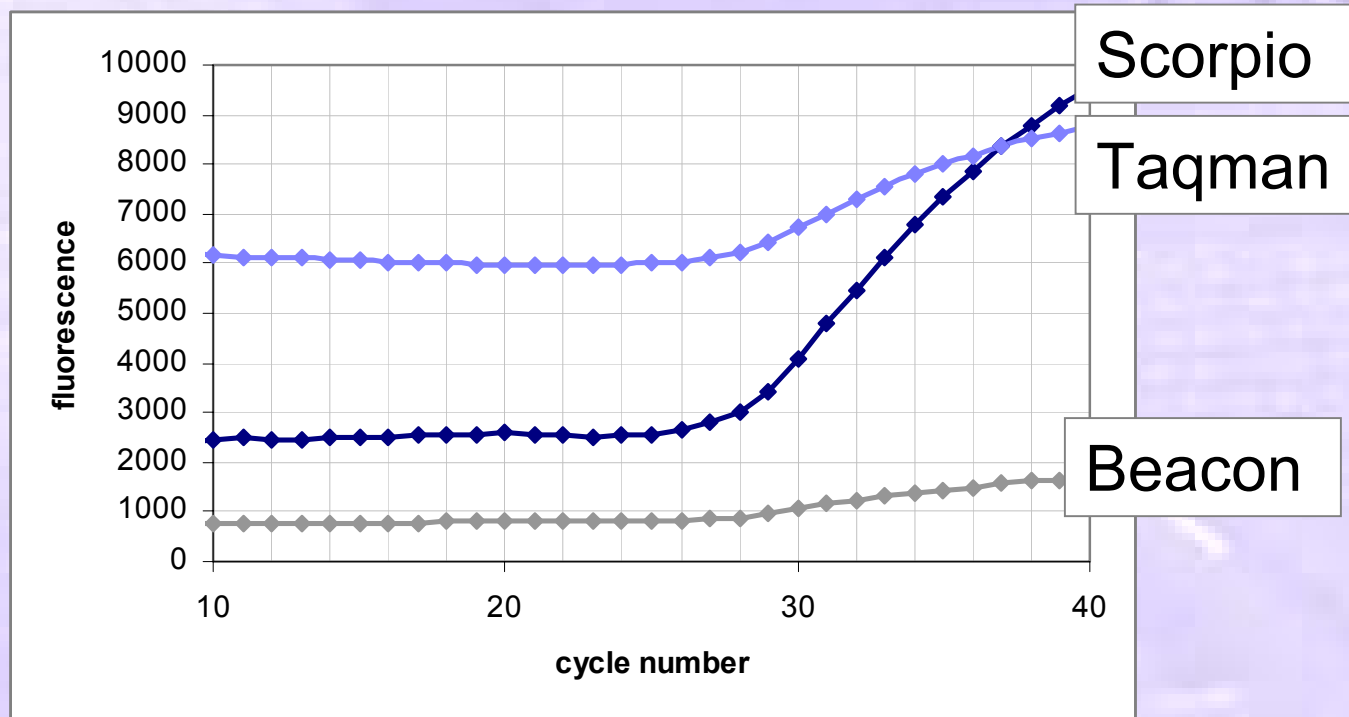
MTHFR assay –probe sequences identical

Comparison to other technologies - II



CYP gene assay –probe sequences identical

Comparison to other technologies - III

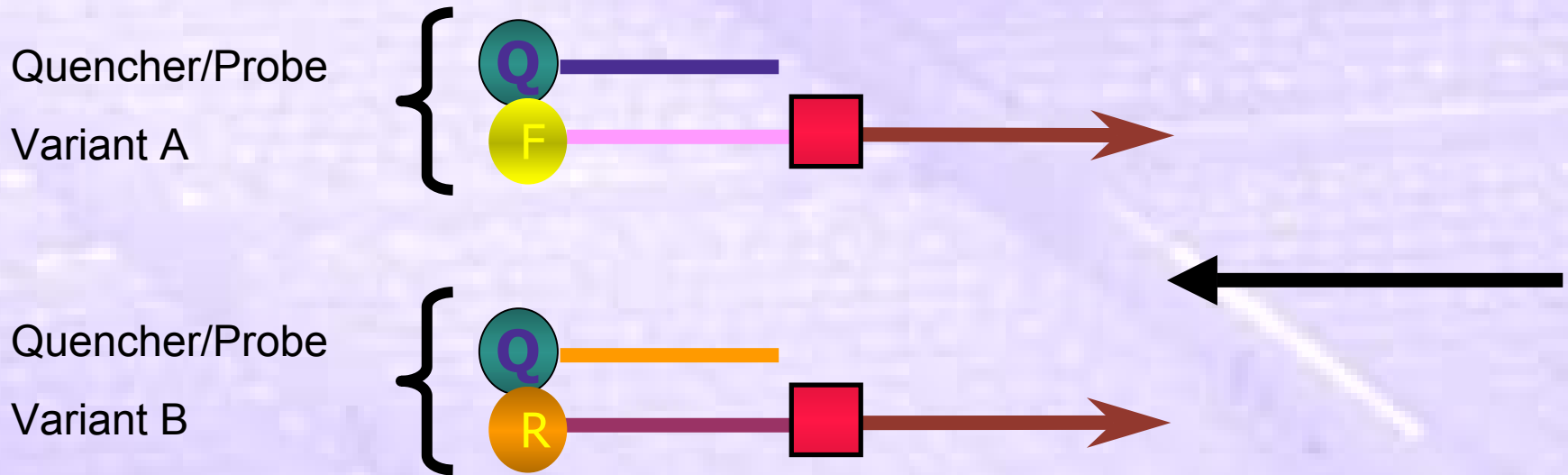


BRCA1gene assay –probe sequences identical
no background correction

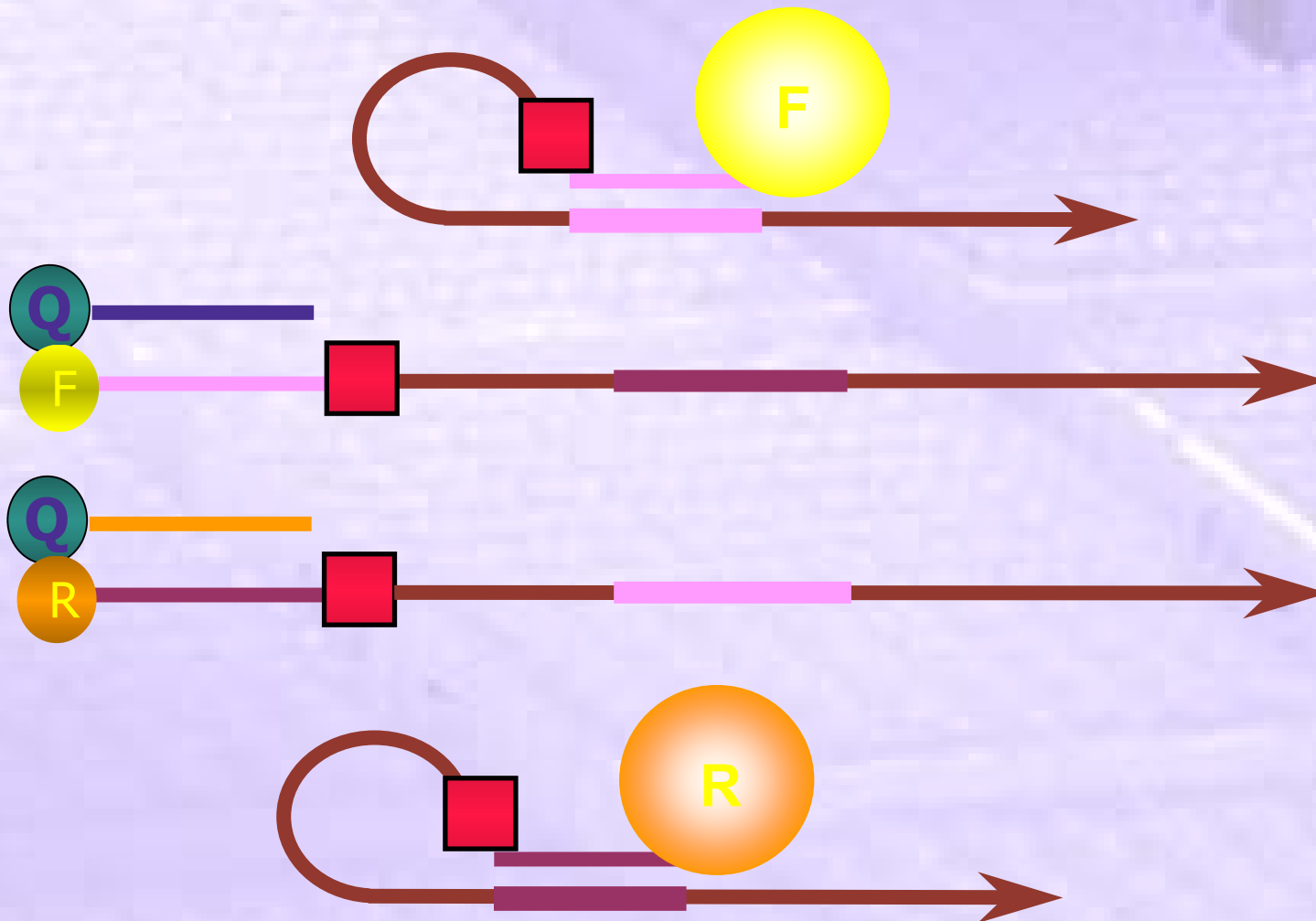
Genotyping Formats

- Allele Specific Hybridisation
 - Probe regions are specific to each allele, primer element is common
- Allele Specific PCR
 - Discrimination through extension/non-extension of matched/mismatched 3' base
 - Probe regions identical but carry different labels

Genotyping Reaction A



Genotyping Products A



Specificity of ASO Approach

Specificity is due to

- “T_m bonus” of unimolecular mode of action allows short probes
- Presence of matched quencher or stable stem/loop provides competition against mismatched hybrids

Constructs

5' FAM-AGTACAGTC

3' DAB-TCATGTCAG

3' DAB-TCATCTCAG

(T)₅

+/-

(T)₁₅

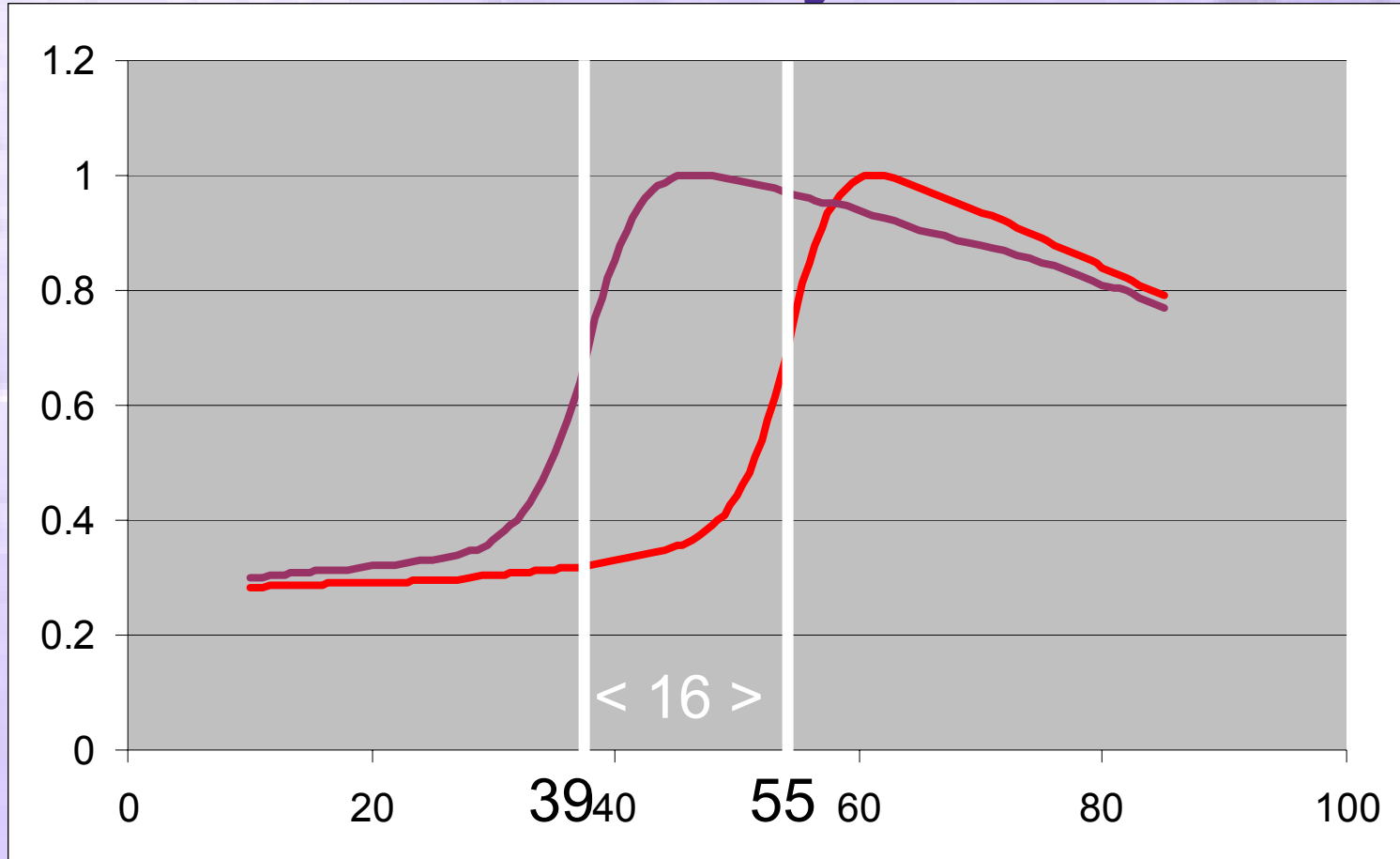
5' FAM-CATAGTACAGTCCAT

3' DAB-GTATCATGTCAGGTA

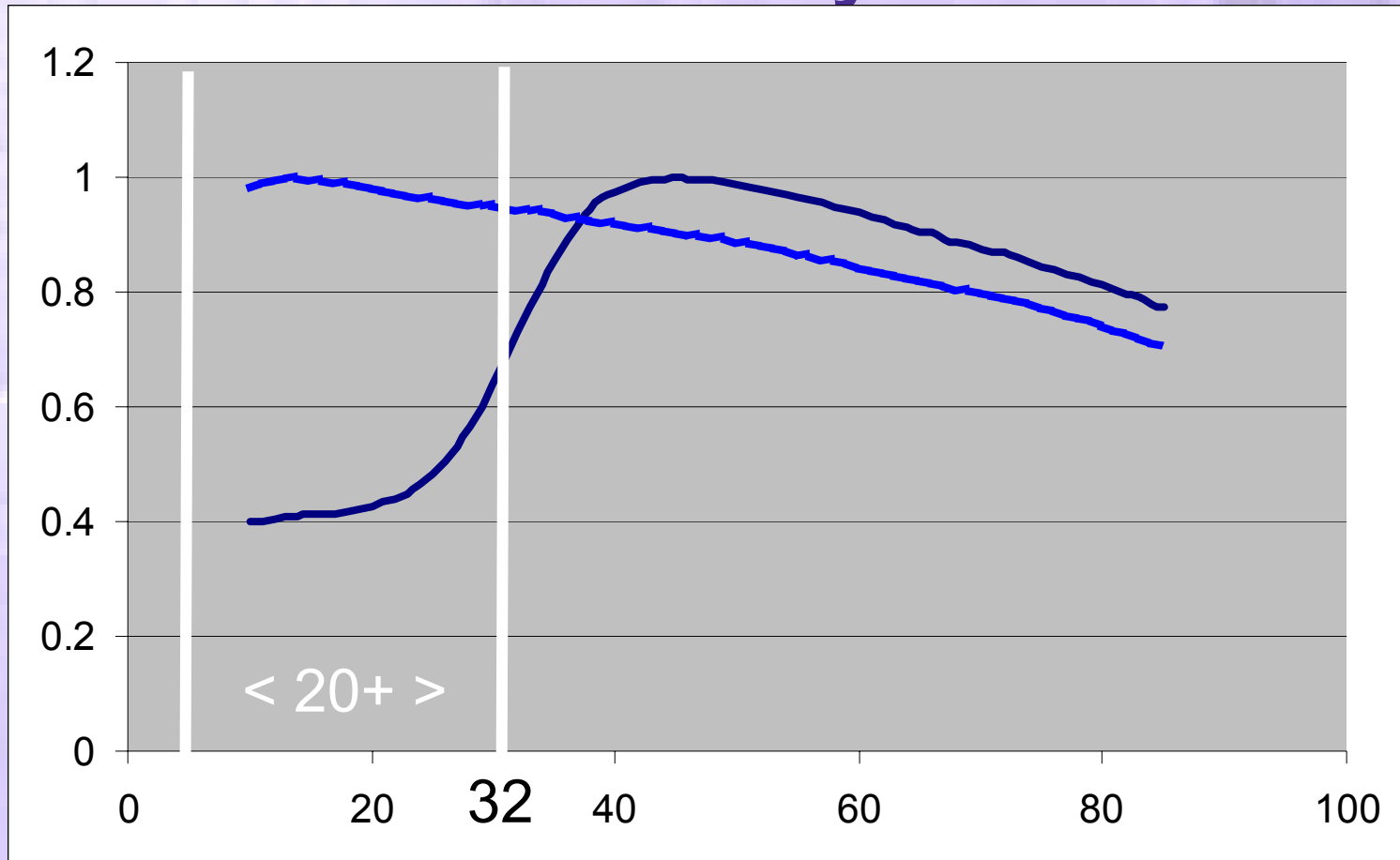
3' DAB-GTATCATCTCAGGTA

(T)₄₅

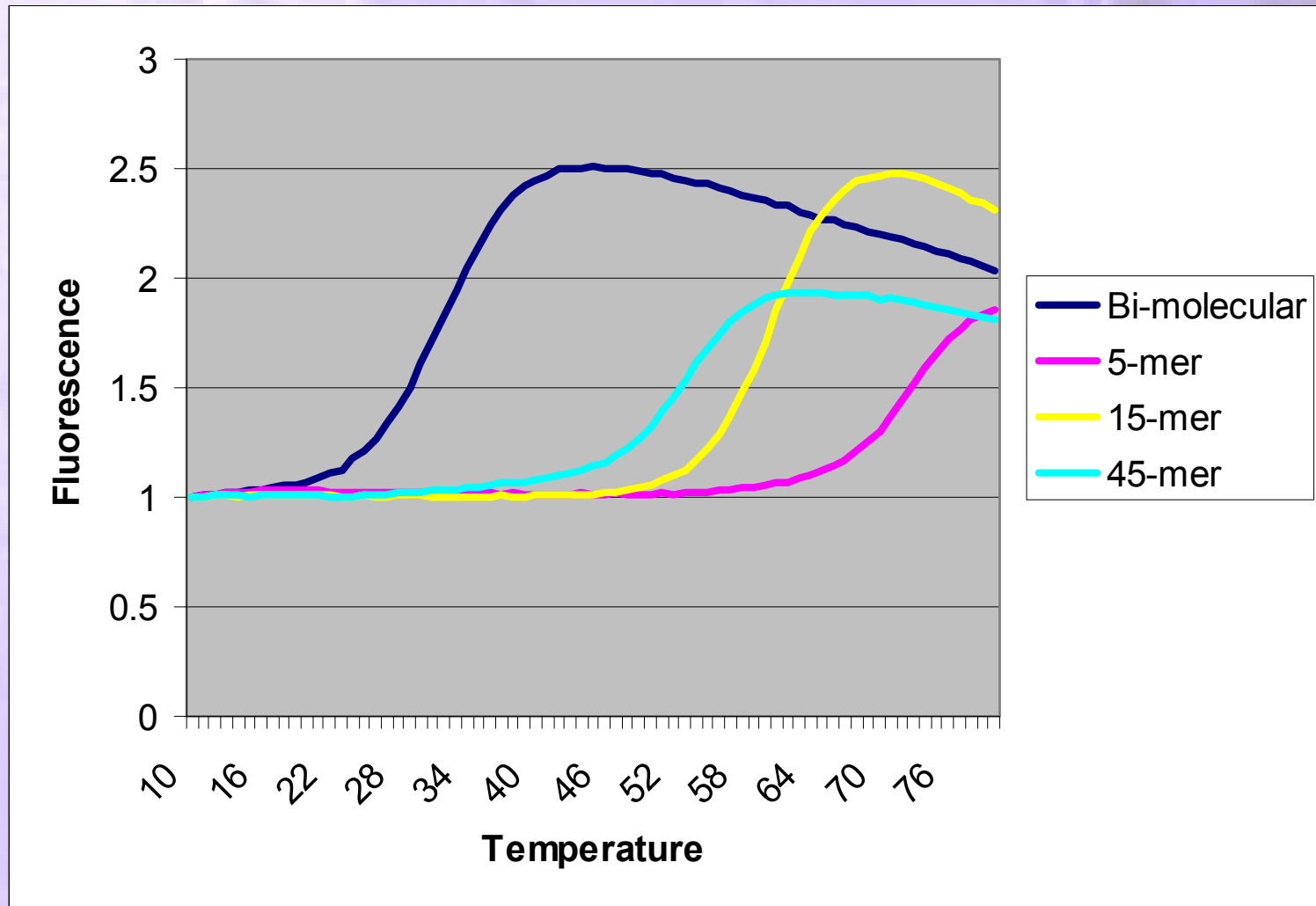
Short probes are more discriminatory: 15-mers



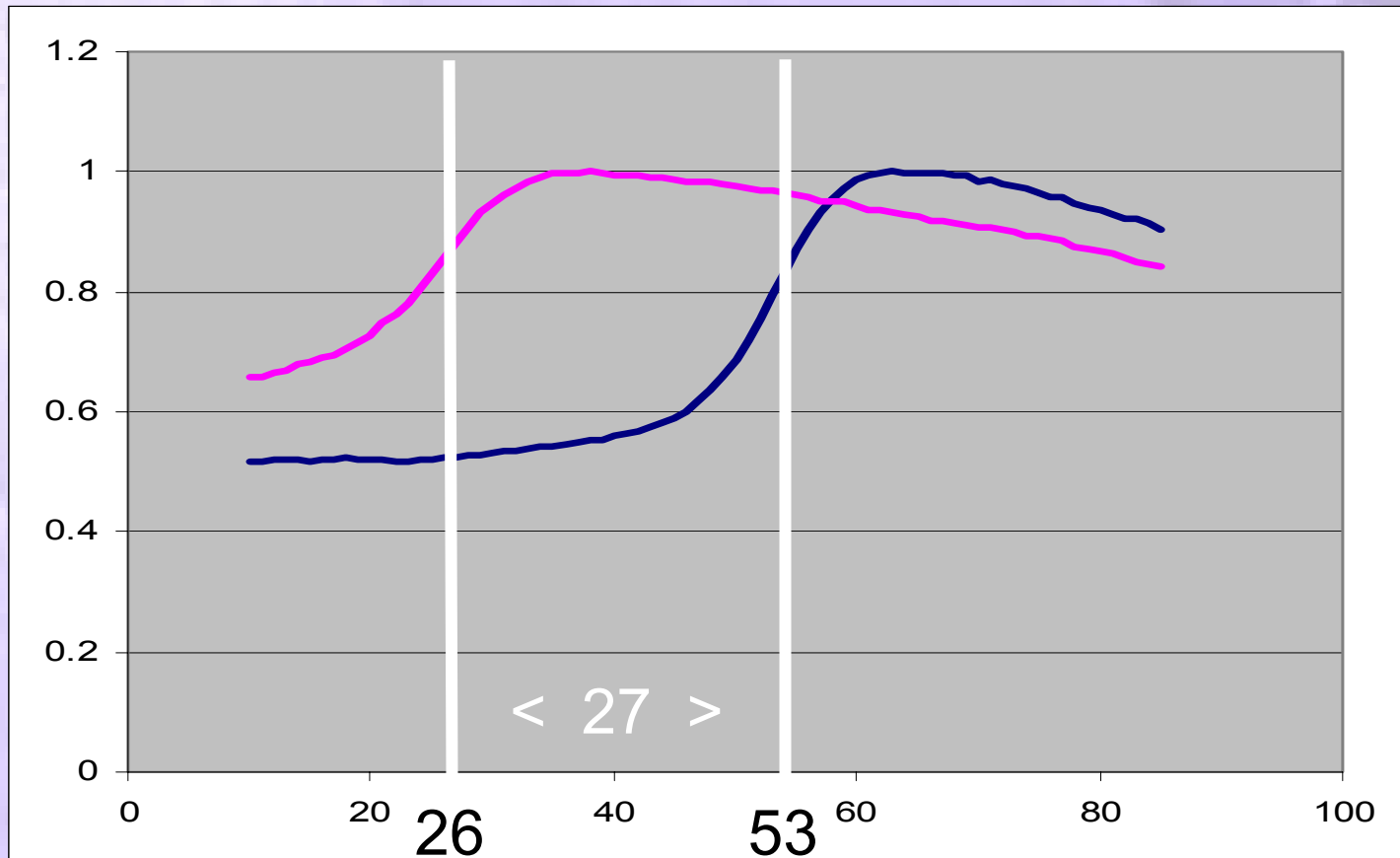
Short probes are more discriminatory: 9-mers



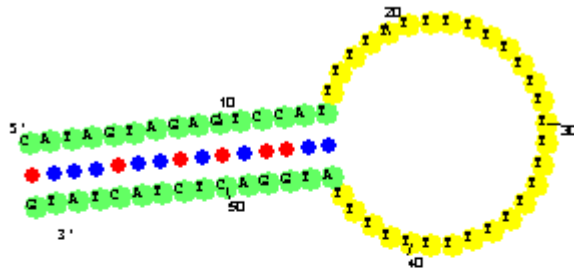
Tm Bonus



Discrimination of 9-mer Unimolecular Probe



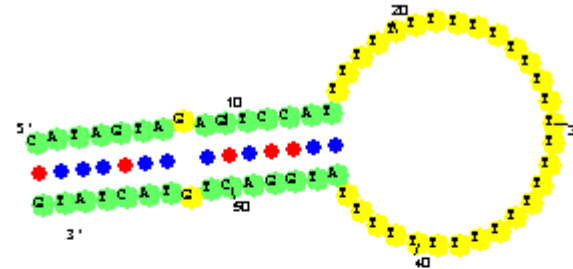
Discrimination With No Stems



stem benefits A MATCH

$dG(60.00^\circ\text{C}) = -0.55$ kcal/mole
 $dH = -110.40$ kcal/mole
 $dS = -329.73$ cal/mole K
 $T_m = 61.67^\circ\text{C}$
[Na⁺] = 0.05 mol/L
[Mg⁺²] = 0.00 mol/L

T_m=61.7

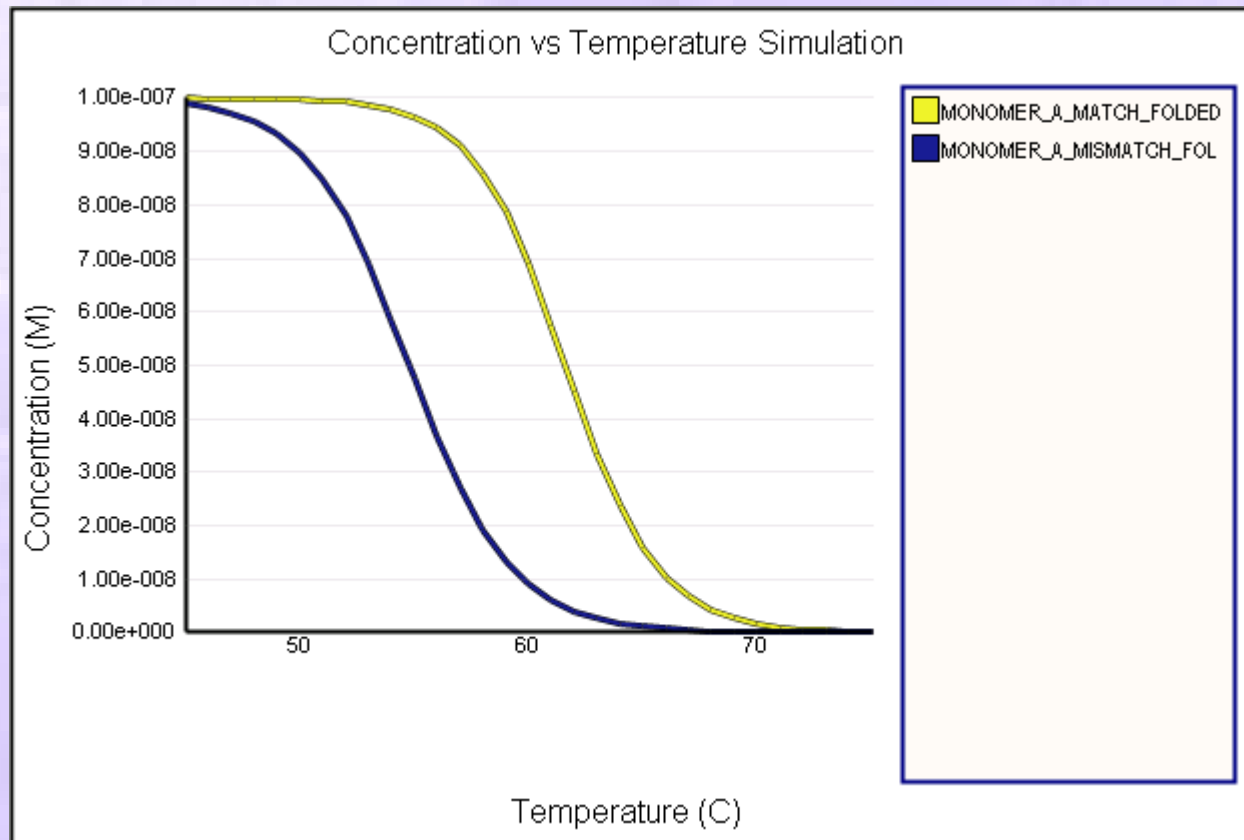


stem benefits A MISMATCH

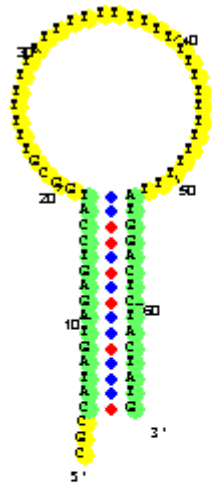
$dG(60.00^\circ\text{C}) = 1.48$ kcal/mole
 $dH = -95.90$ kcal/mole
 $dS = -292.30$ cal/mole K
 $T_m = 54.94^\circ\text{C}$
[Na⁺] = 0.05 mol/L
[Mg⁺²] = 0.00 mol/L

T_m=54.9

Without Stem



Discrimination With Stems



stembenefits As MATCH

$dG(60.00^{\circ}\text{C}) = -0.60 \text{ kcal/mole}$
 $dH = -114.80 \text{ kcal/mole}$
 $dS = -342.79 \text{ cal/mole K}$
 $T_m = 61.75^{\circ}\text{C}$
 $[\text{Na}^+] = 0.05 \text{ mol/L}$
 $[\text{Mg}^{+2}] = 0.00 \text{ mol/L}$

$T_m=61.7$

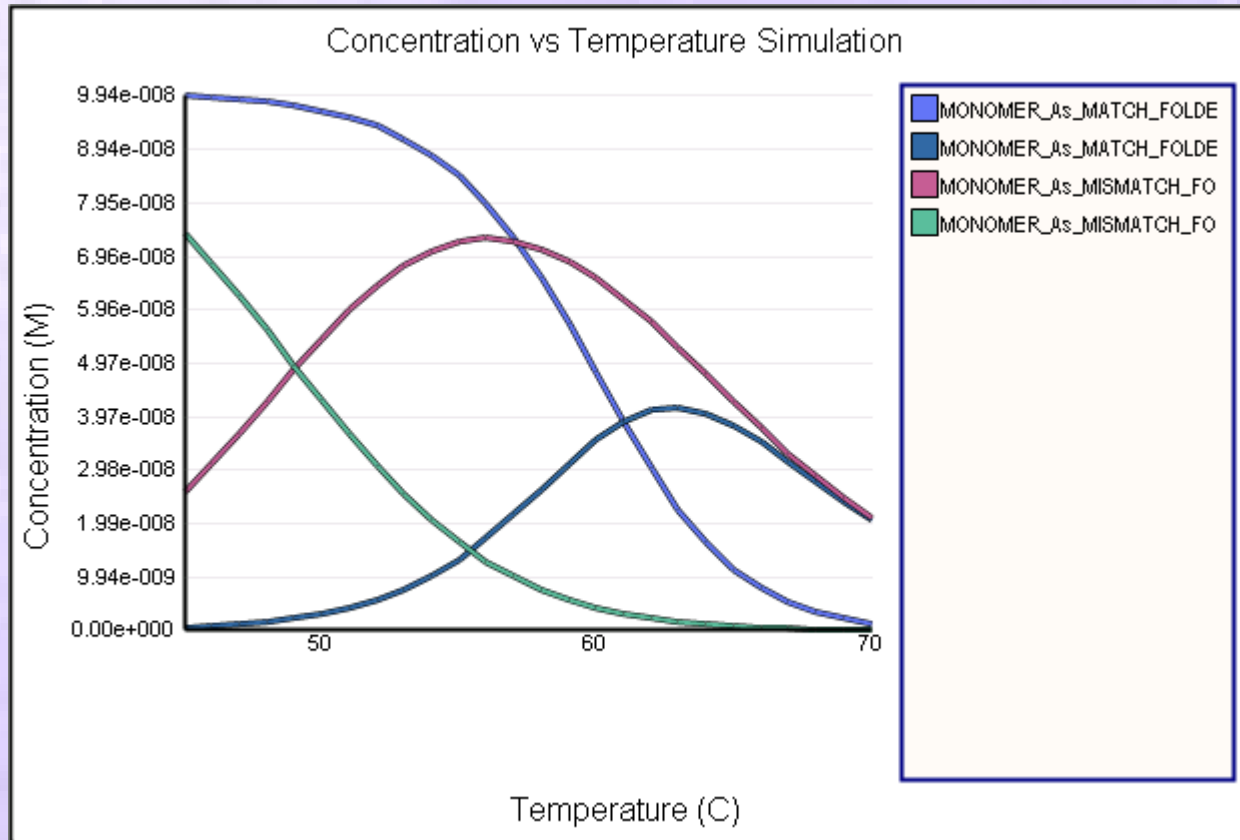


stembenefits As MISMATCH

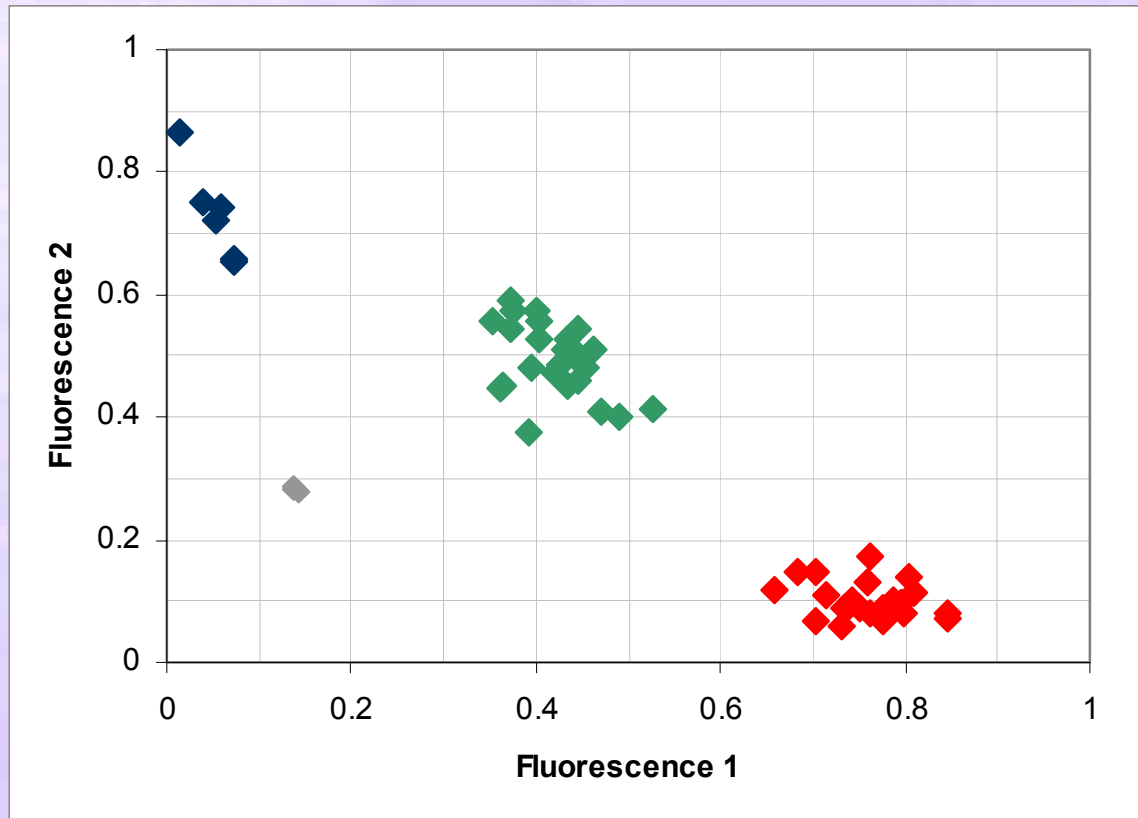
$dG(60.00^{\circ}\text{C}) = -0.56 \text{ kcal/mole}$
 $dH = -48.50 \text{ kcal/mole}$
 $dS = -143.90 \text{ cal/mole K}$
 $T_m = 63.89^{\circ}\text{C}$
 $[\text{Na}^+] = 0.05 \text{ mol/L}$
 $[\text{Mg}^{+2}] = 0.00 \text{ mol/L}$

$T_m=63.9$

With Stem



Allele Specific Hybridisation End-point PCR



How ARMS Works

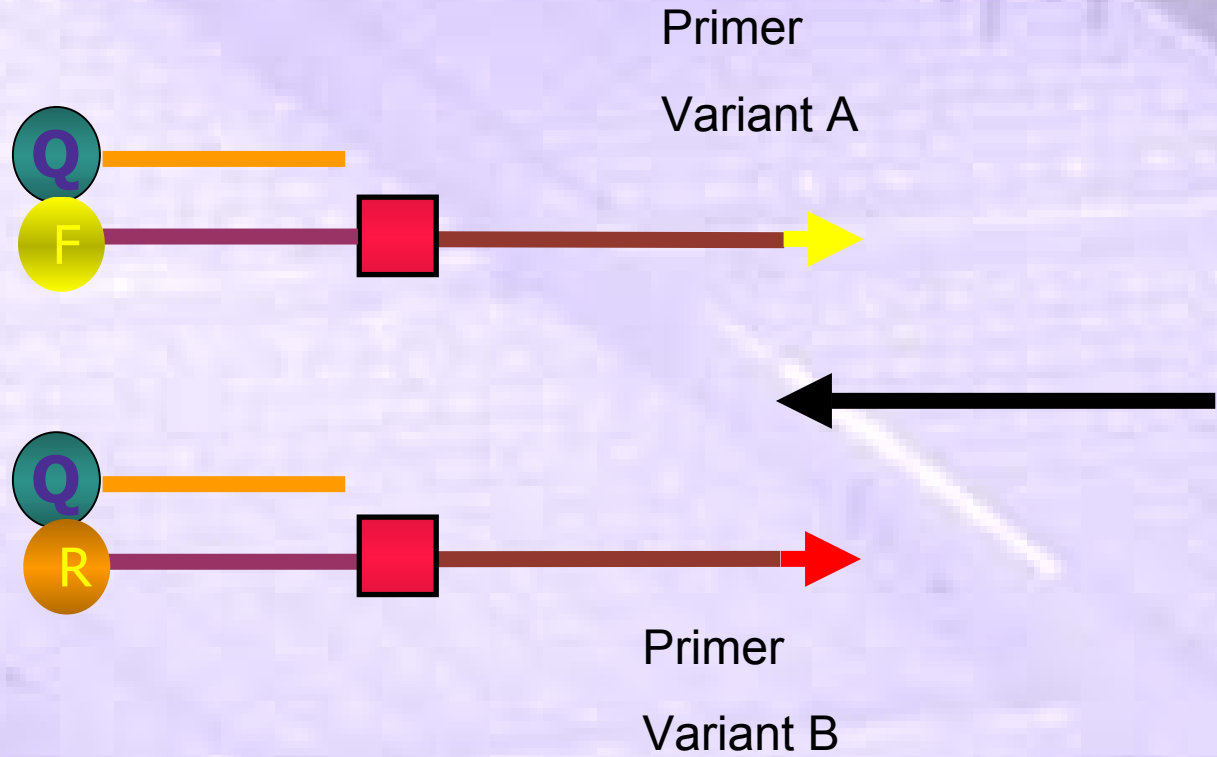
A



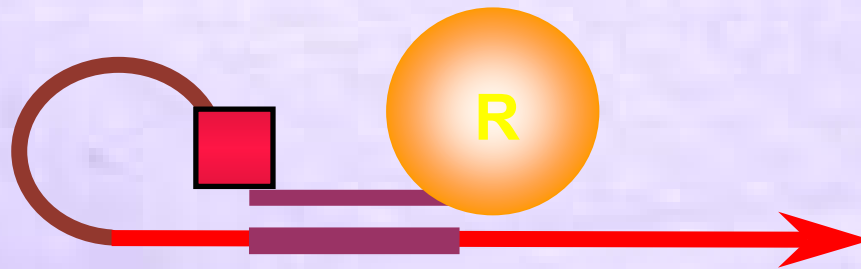
B



Genotyping Reaction B



Genotyping Products B



Specificity

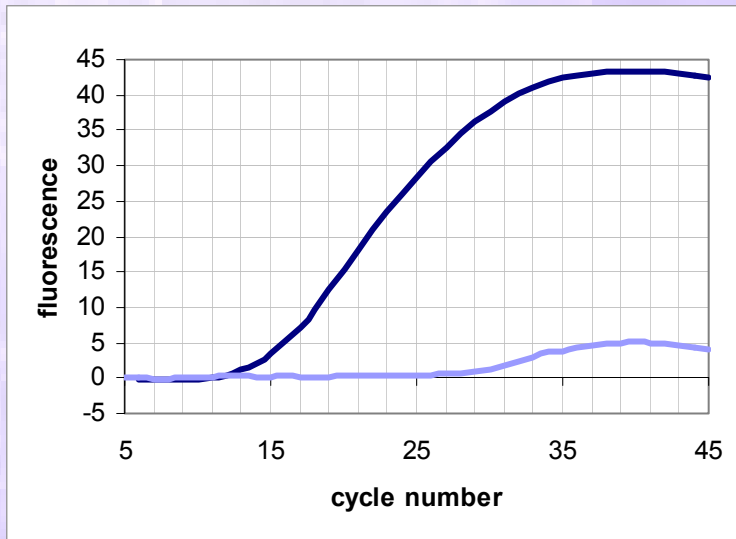
- Sequence specific primer preferentially amplifies matched target and not mismatched target
- Allows detection of rare alleles even in a high background of mismatched target.

Allele specific hybridisation and extension can be combined

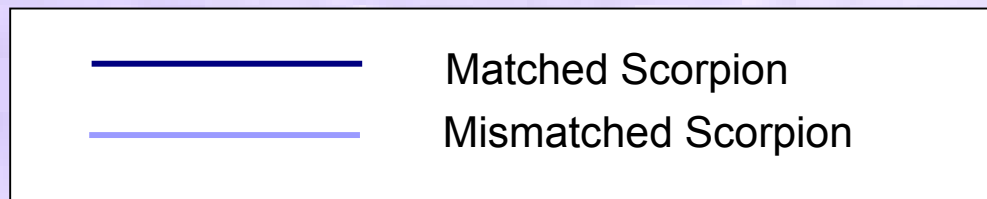
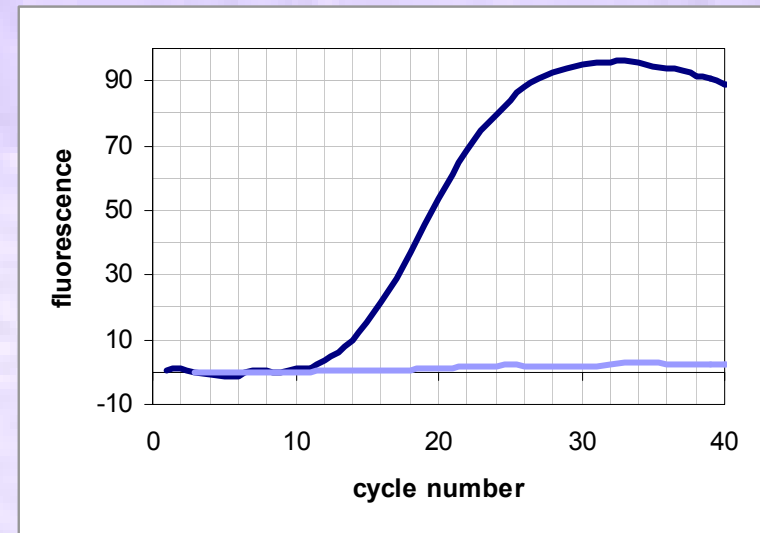
Name	Sequence
HSV1PB2	ROX-CGCCCAGAAC C ACGGGCG-MR- HEG-CCACCTGAACGAGCTGGC GG 3'
HSV2PB2	FAM-CGCCCAG G AC G ACGGGCG-MR- HEG-CCACCTCAGCGATCTCGC CT 3'
HSV PB Rev2	GTCACGAACTCGGGGCCGTAC

HSV specificity

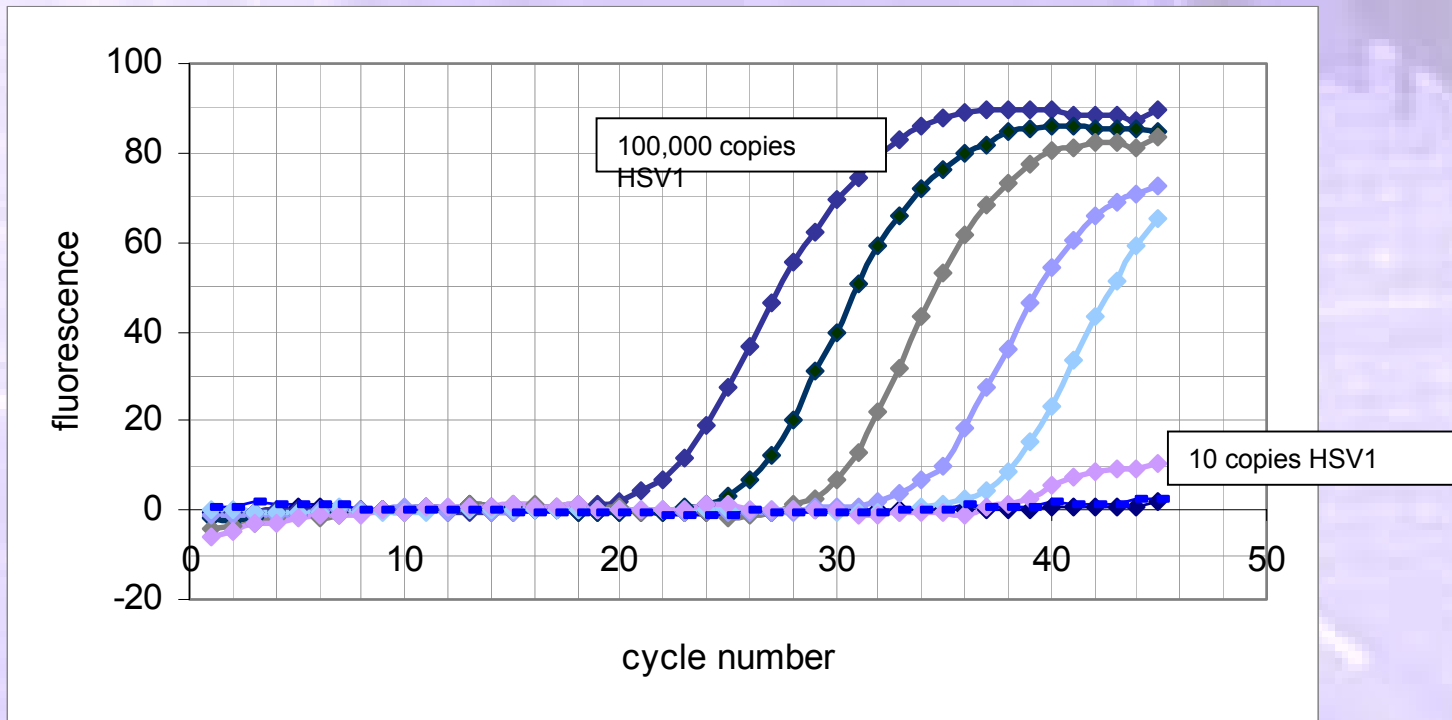
10^8 copies of
HSV2 target



10^8 copies of
HSV1 target

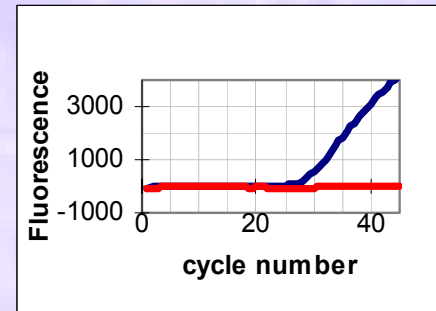
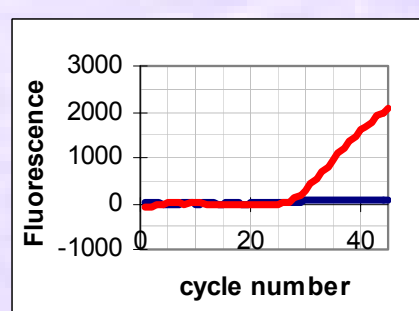
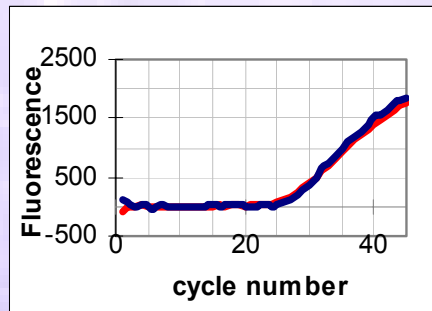
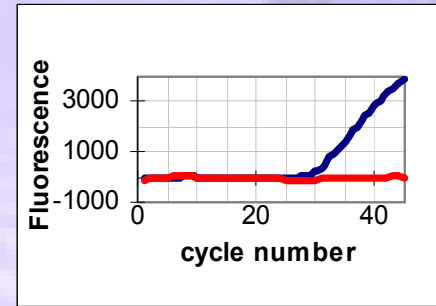
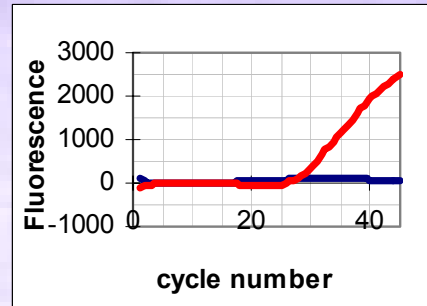
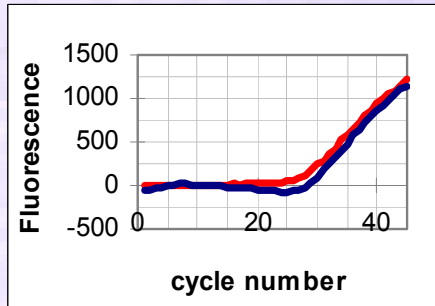


HSV specificity - Admixtures



detection of HSV1 sequence in a background of 100,000 copies of HSV2

Allele specific amplification Real-time PCR

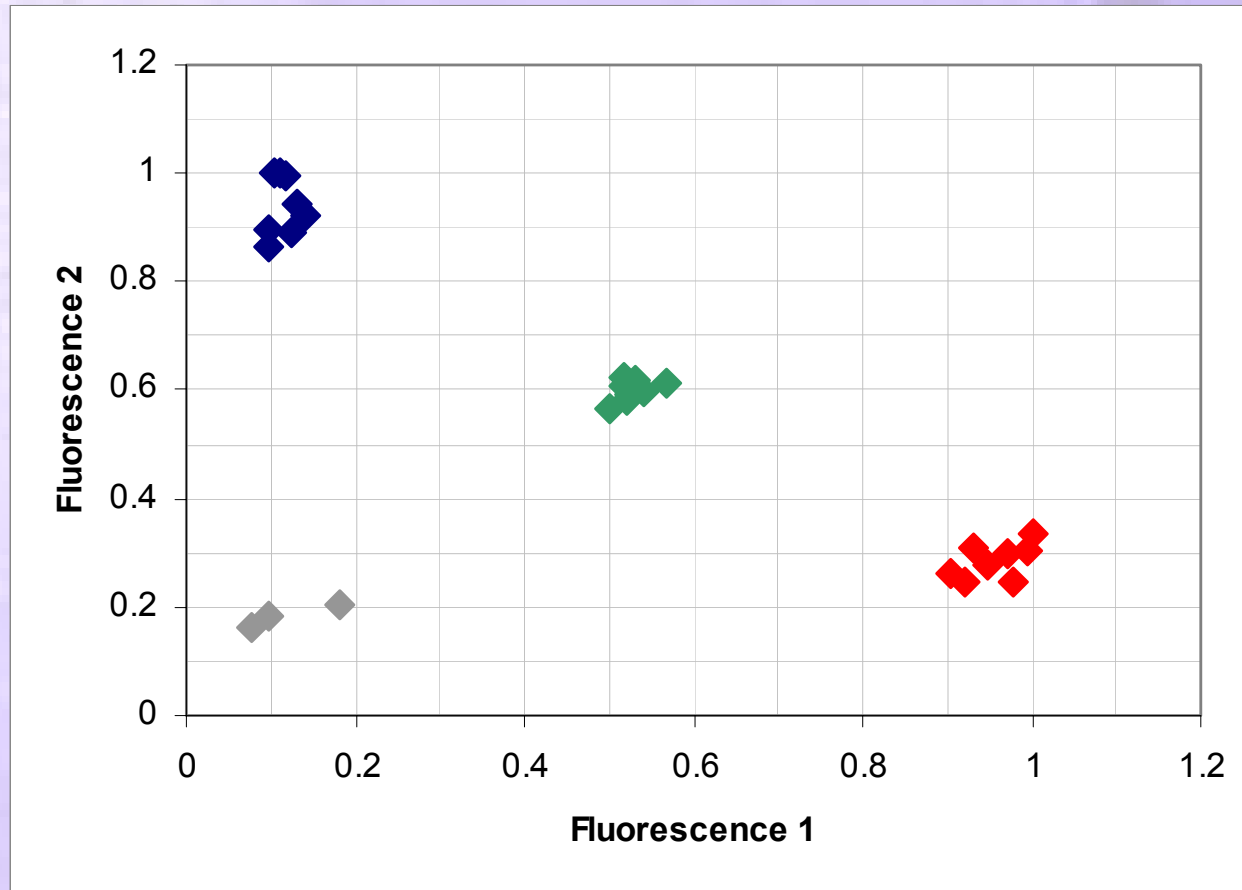


AB heterozygotes – both reactions positive

AA homozygotes – only the red labelled A scorpion is positive

BB homozygotes – only the blue labelled B Scorpion is positive

Allele Specific Amplification End-point PCR



Scorpions Compatible with a wide range of platforms

- ABI 7700, 7000
- Roche LightCycler
- Cepheid Smart Cycler
- Stratagene MX4000, MX3000
- BioRad i-cycler
- Corbett Rotorgene
- Standard Laboratory Fluorimeters

Scorpions

- Unique, specific, homogeneous detection method
- Two probe/quencher configurations
- Two highly specific ways to analyse SNPs
- Suitable for Q-PCR and Endpoint Methods
- Quenched system so multiplexing “straightforward”
- Fits all instruments