Hybridization Probe Chemistries for the LightCycler™ Instrument

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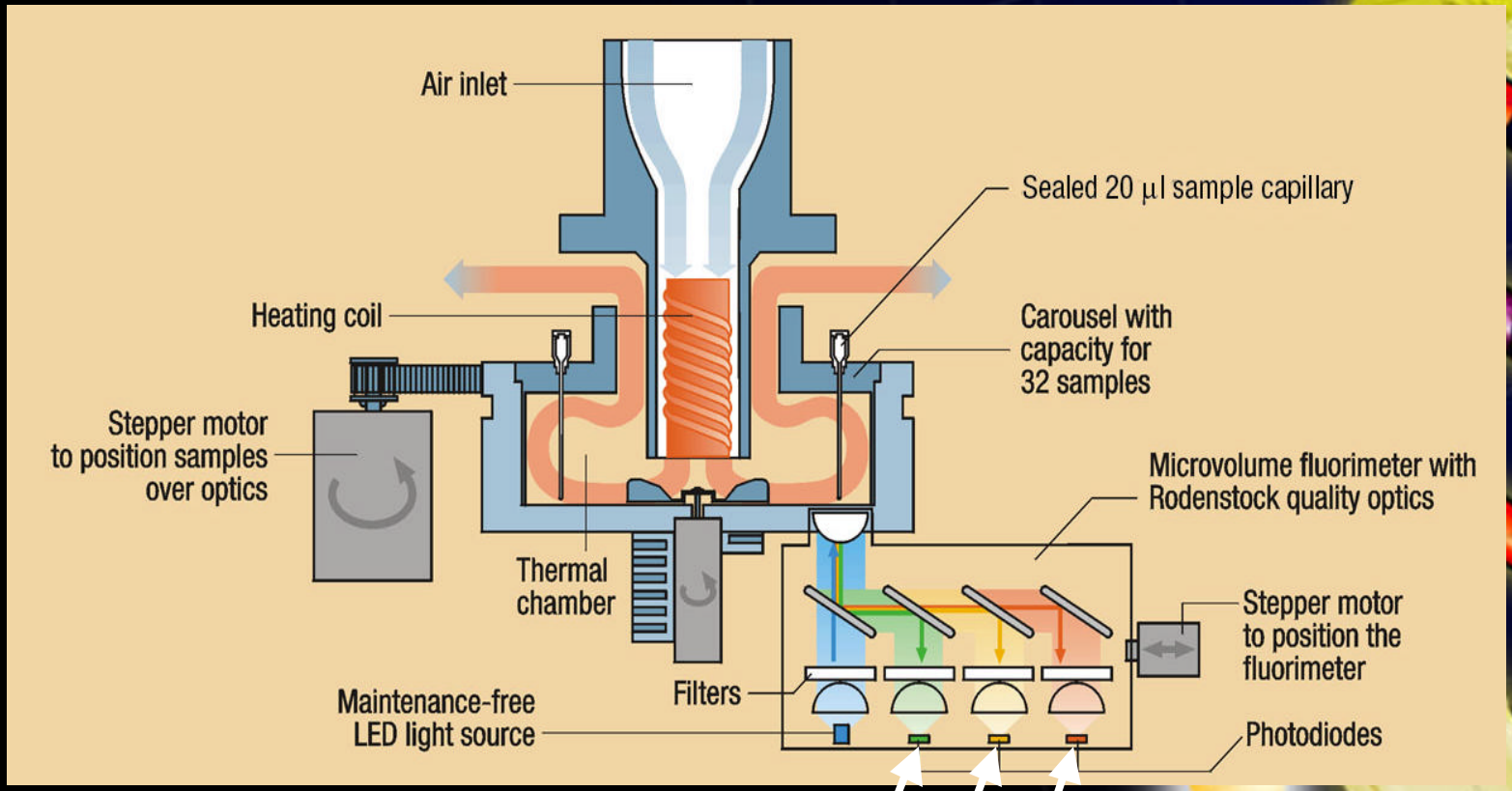
Three Channels for Detection:



Fluorescein and Sybr™ Green

LC Red 640

LC Red 705

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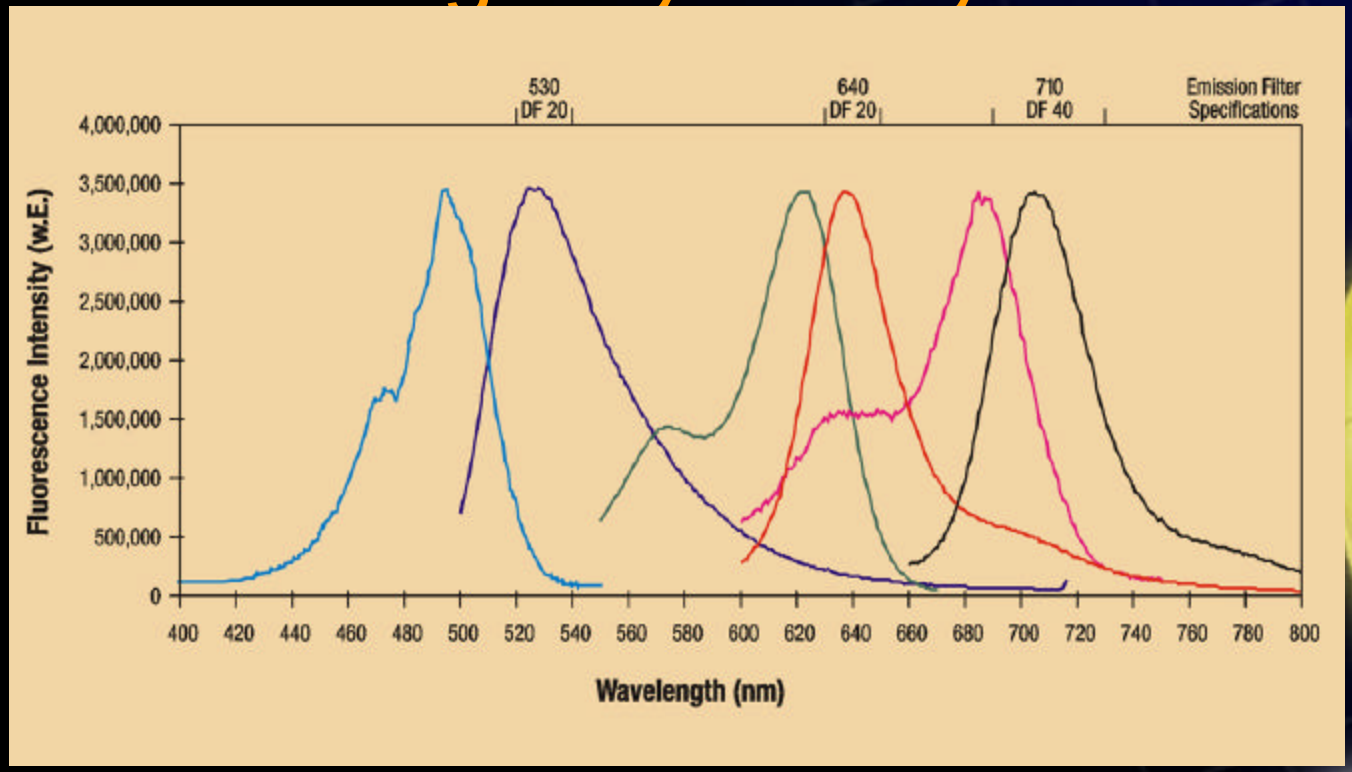
Excitation and Emission Spectra of LightCycler Dyes



- Excitation Fluorescein
- Emission Fluorescein
- Excitation Red 640
- Emission Red 640
- Excitation Red 705
- Emission Red 705

Channel 1: 510-550 nm
Channel 2: 620-660 nm
Channel 3: 670-750 nm

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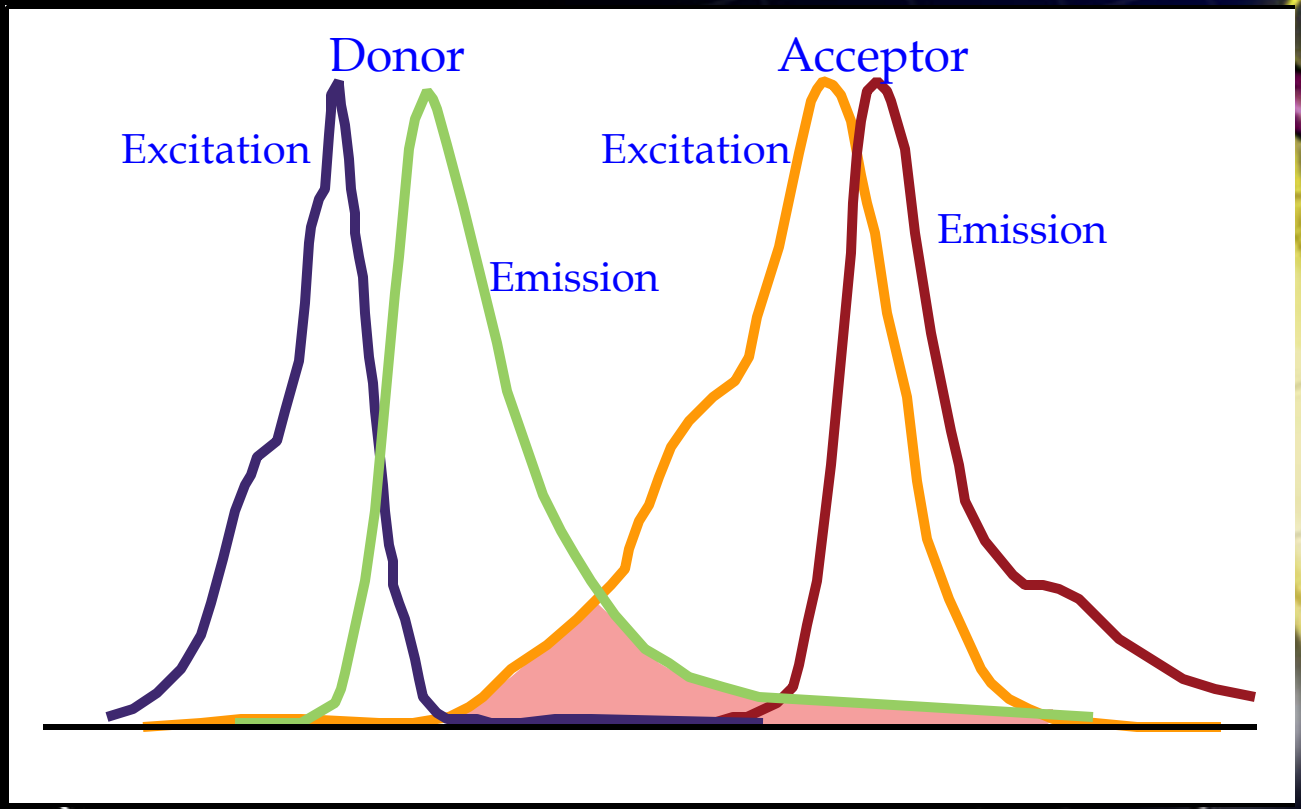
Physical Properties of FRET/Fluorescence



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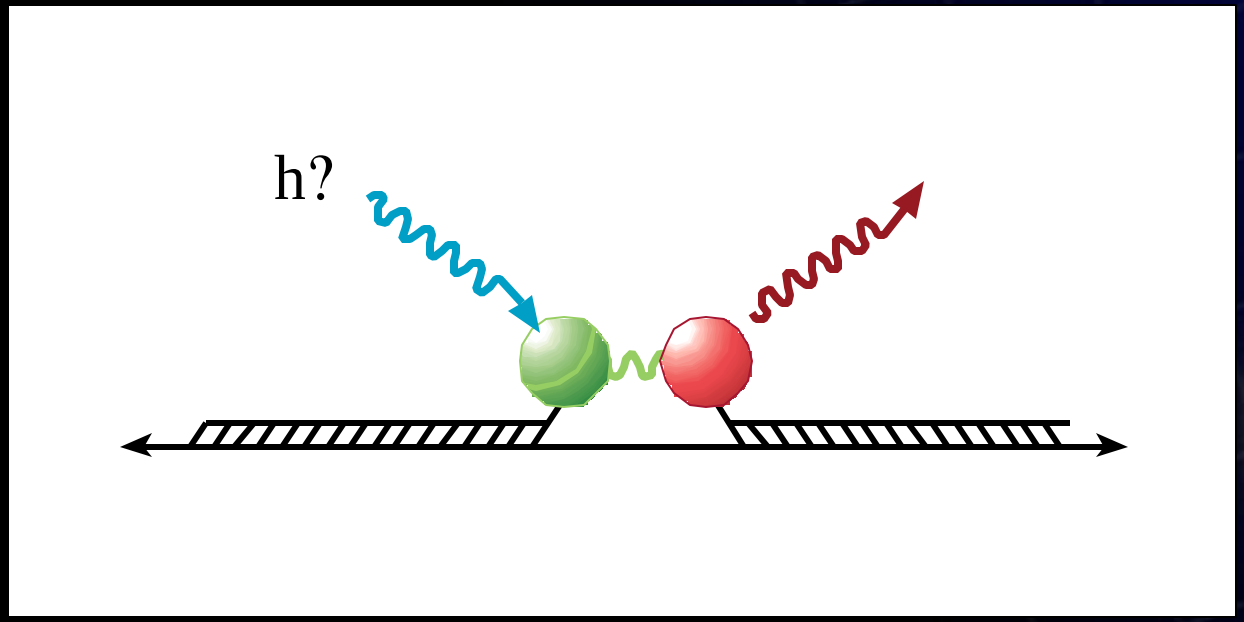
Fluorescence Resonance Energy Transfer (FRET) Concept



- Donor fluorophore is excited by appropriate wavelength
- Donor energy is transferred non-radiatively to the acceptor fluorophore
- Excited acceptor emits at longer wavelength

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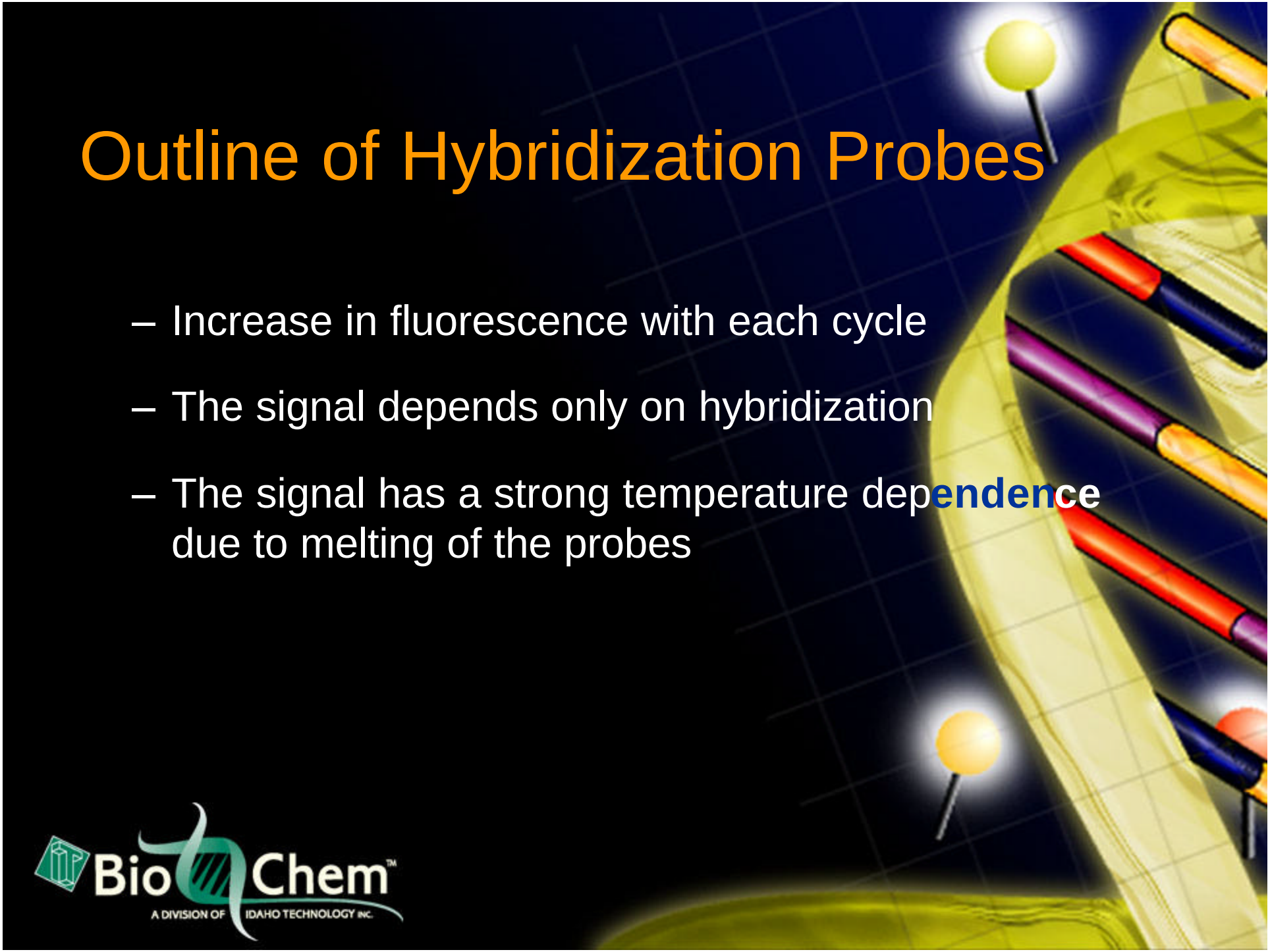
Outline of Hybridization Probes

- Increase in fluorescence with each cycle
- The signal depends only on hybridization
- The signal has a strong temperature dependence due to melting of the probes



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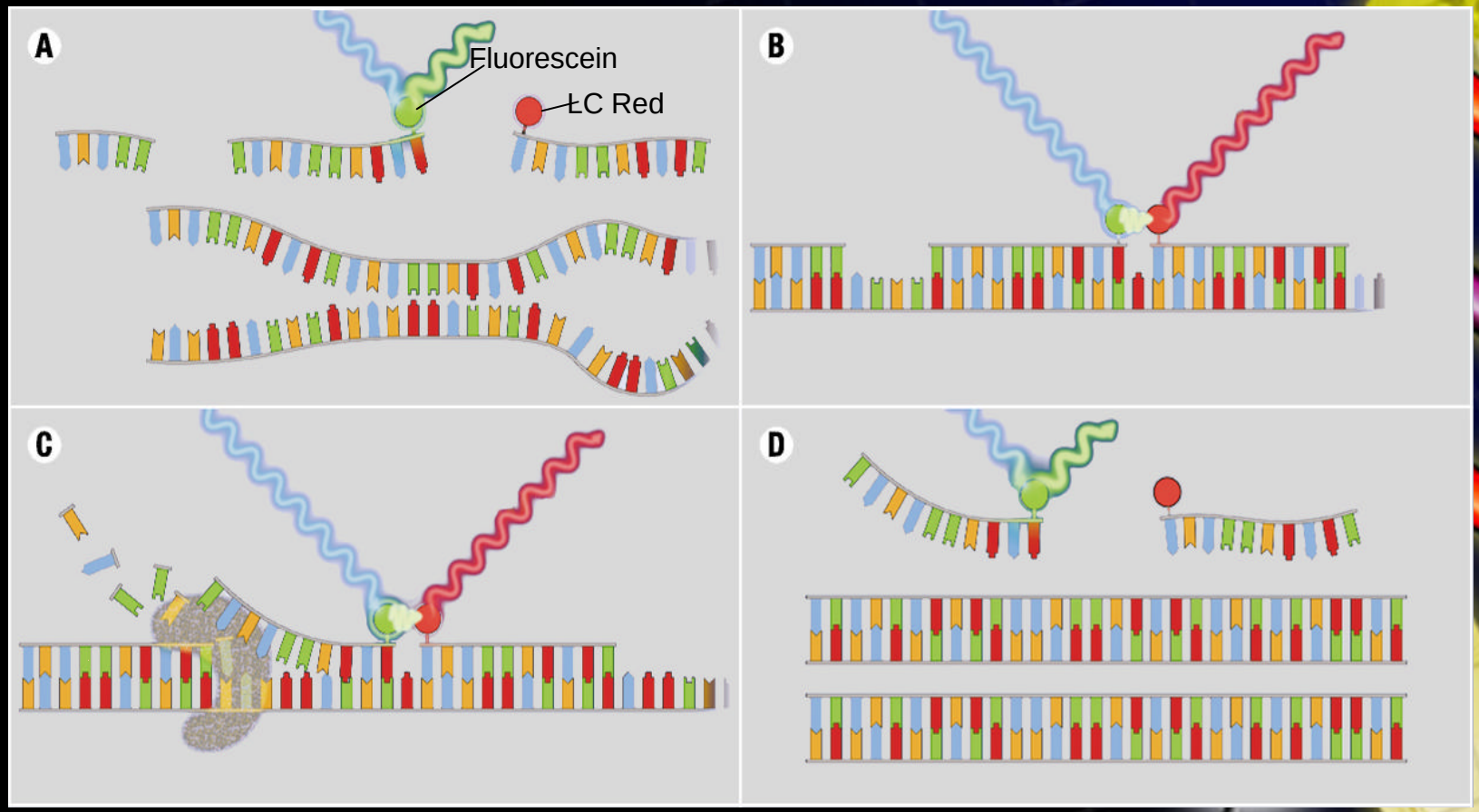
Hybridization Probes Format Overview



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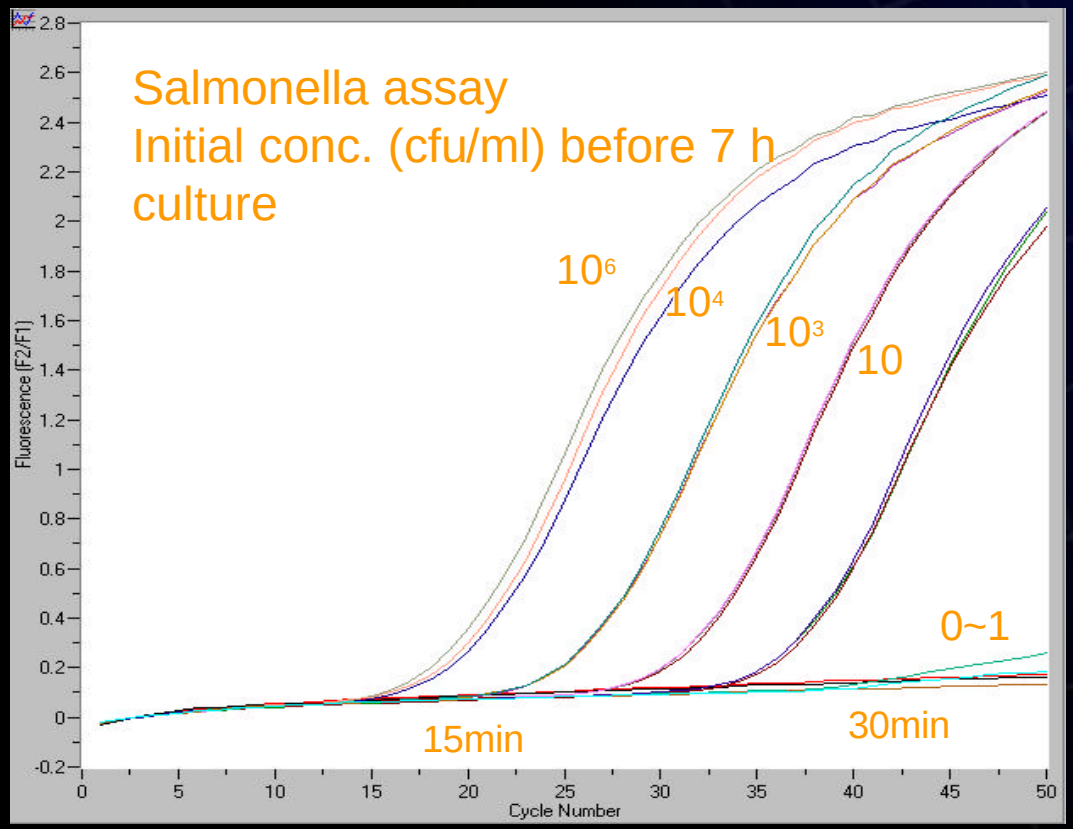
PCR Monitored With Hybridization Probes



Fluorescence Ratio

Cycle

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Advantages of Hybridization Probes:

Ready-to-Go Chemistry for:

-Quantification

-Mutation Detection

-Tm Multiplexing

-Color Multiplexing

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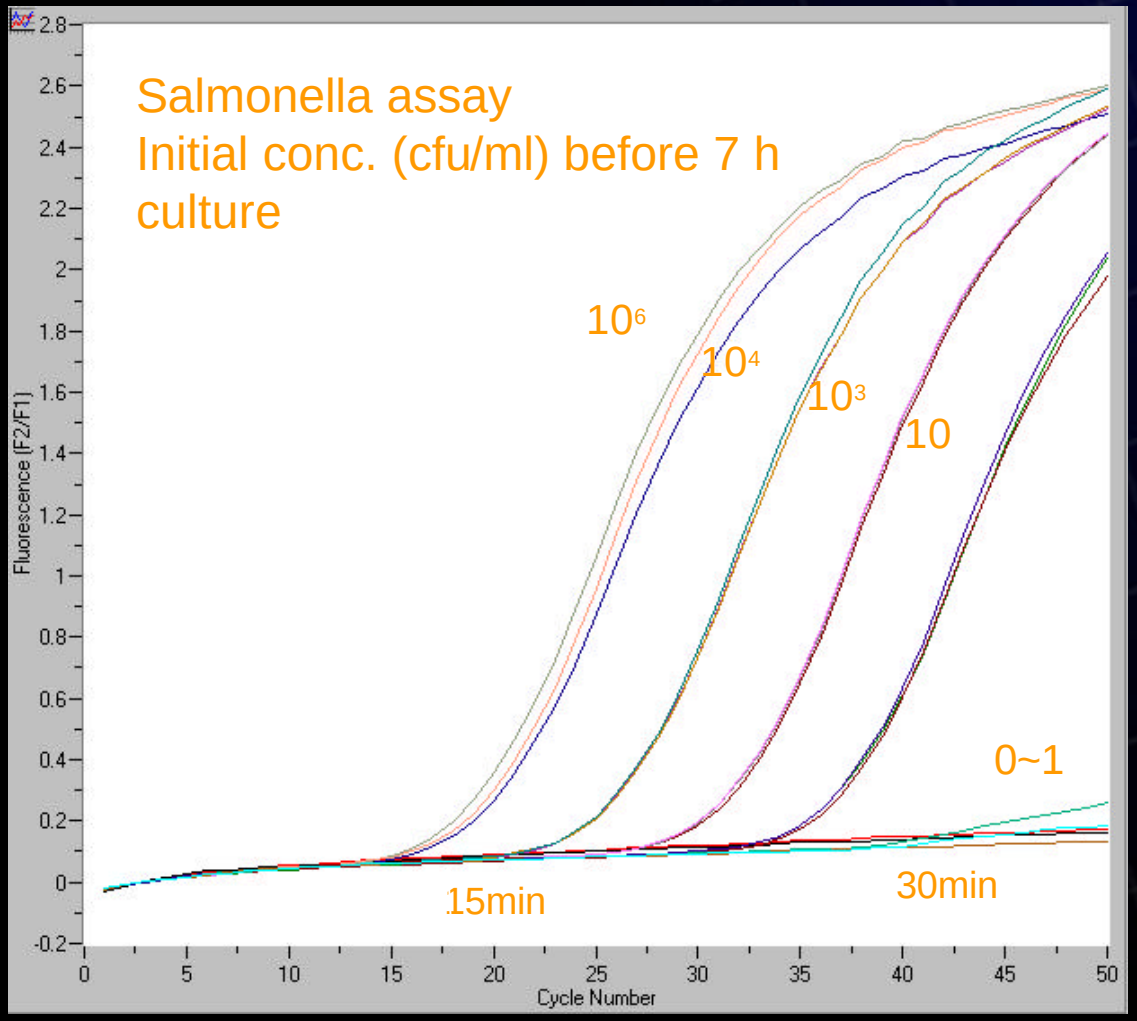
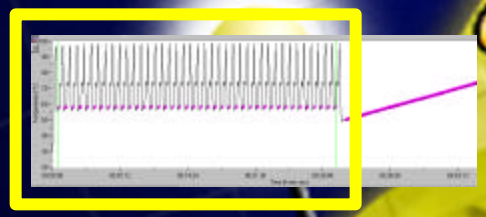
Hybridization Probes





Tier Two Assay (Hybridization probes

Negative controls remain at base-level, even after 50 cycles

Negative Controls

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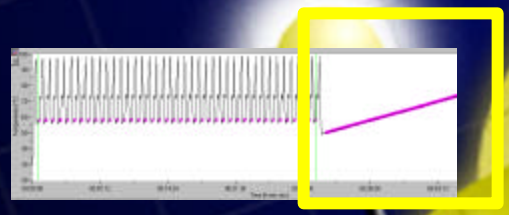
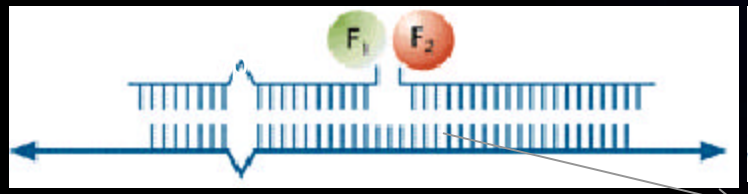
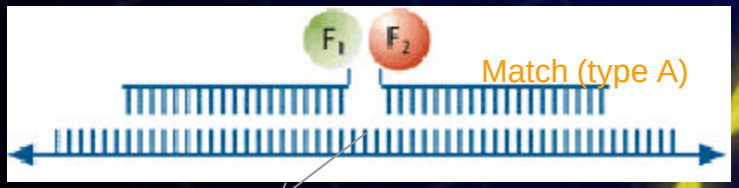
Genotyping with Hybridization Probes

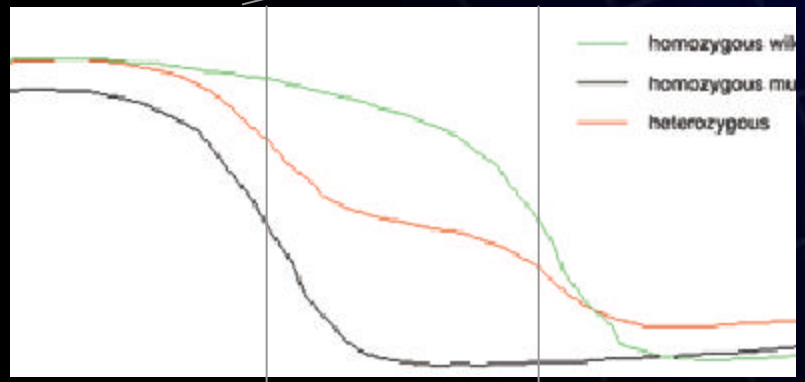


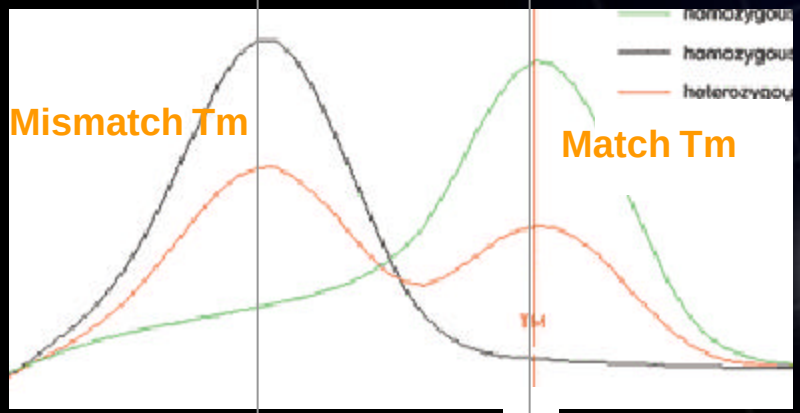




Tier Two Assay Hybridization probes

F





-dF/dT

T

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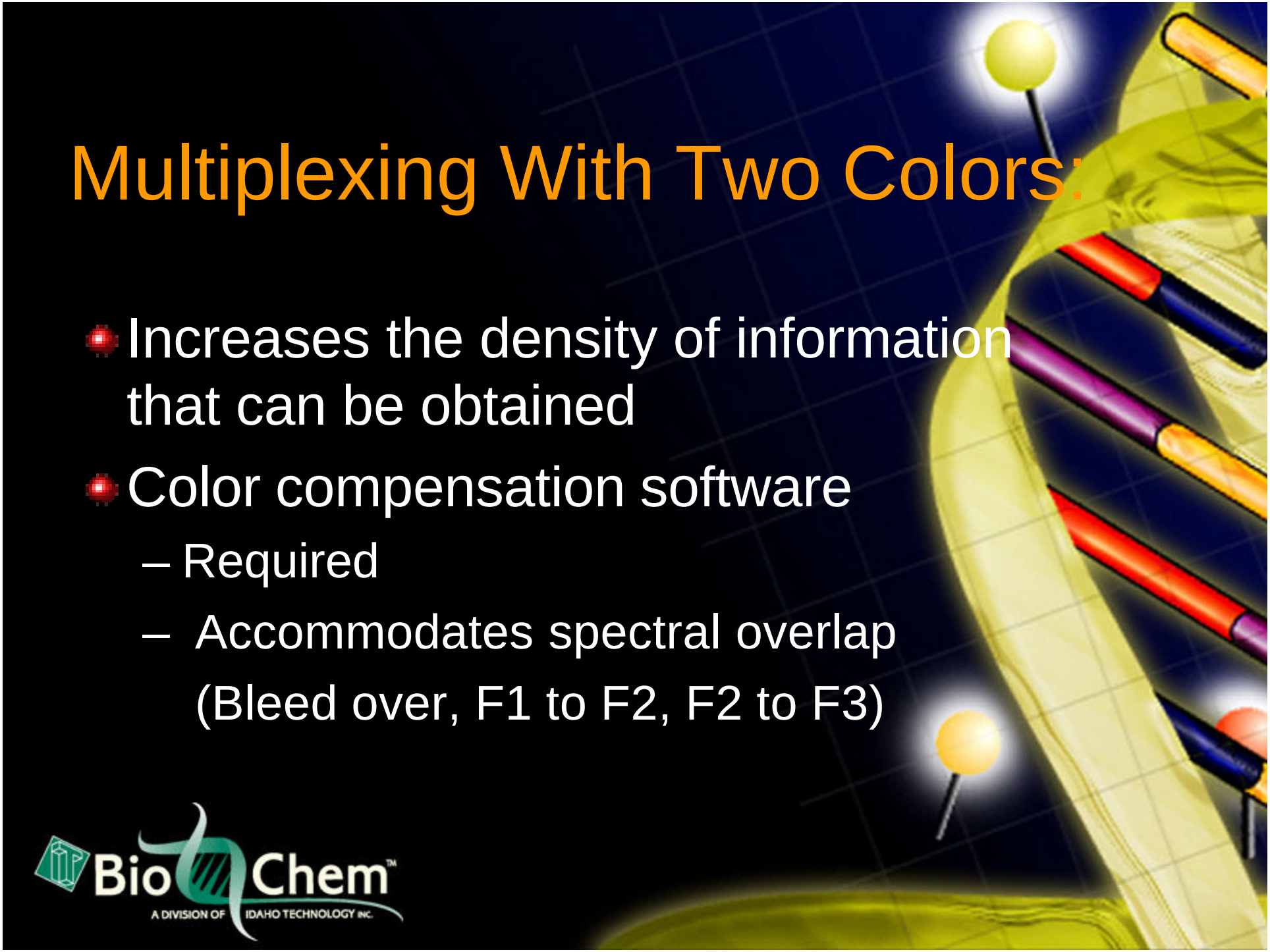
Multiplexing With Two Colors:

- Increases the density of information that can be obtained
- Color compensation software
  - Required
  - Accommodates spectral overlap (Bleed over, F1 to F2, F2 to F3)



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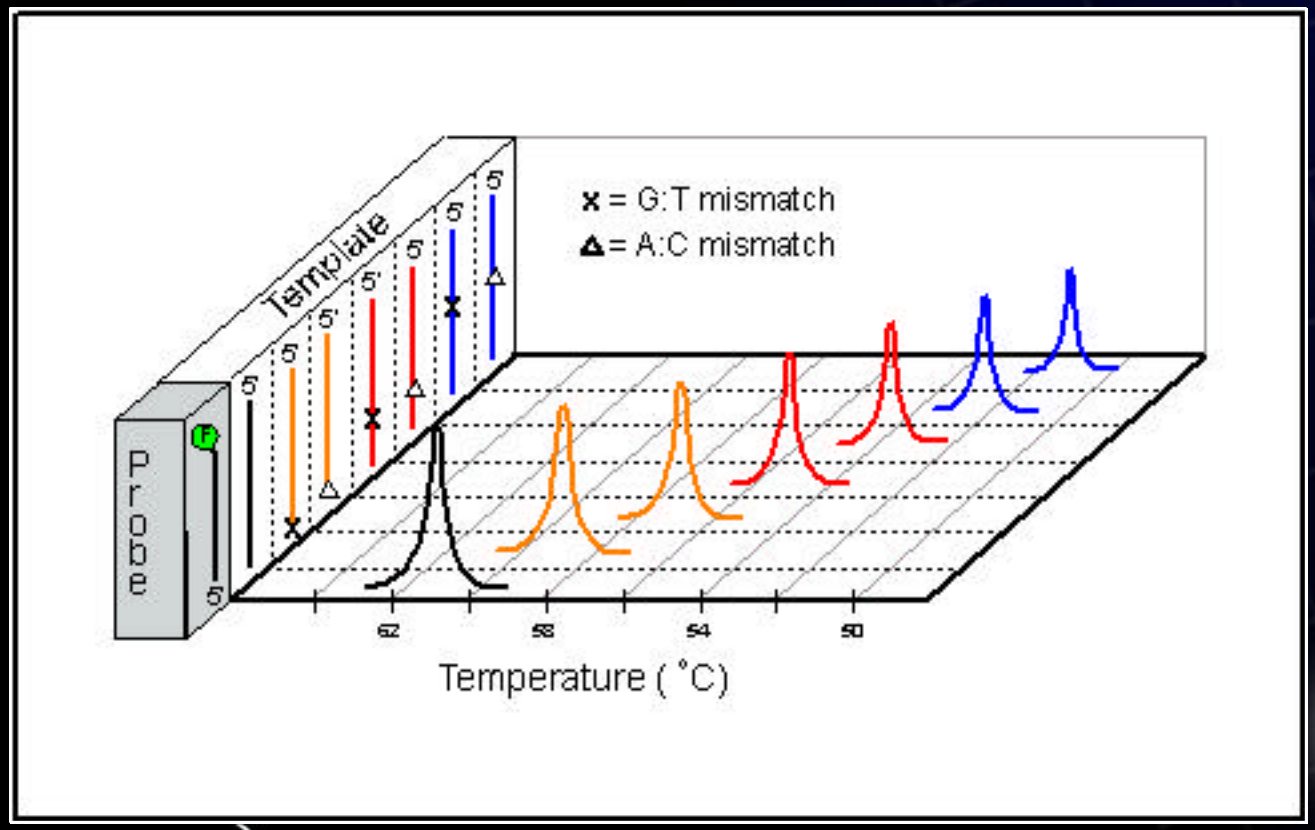
Distinguishing Templates Under a Single Probe by Type and Position of Mismatch



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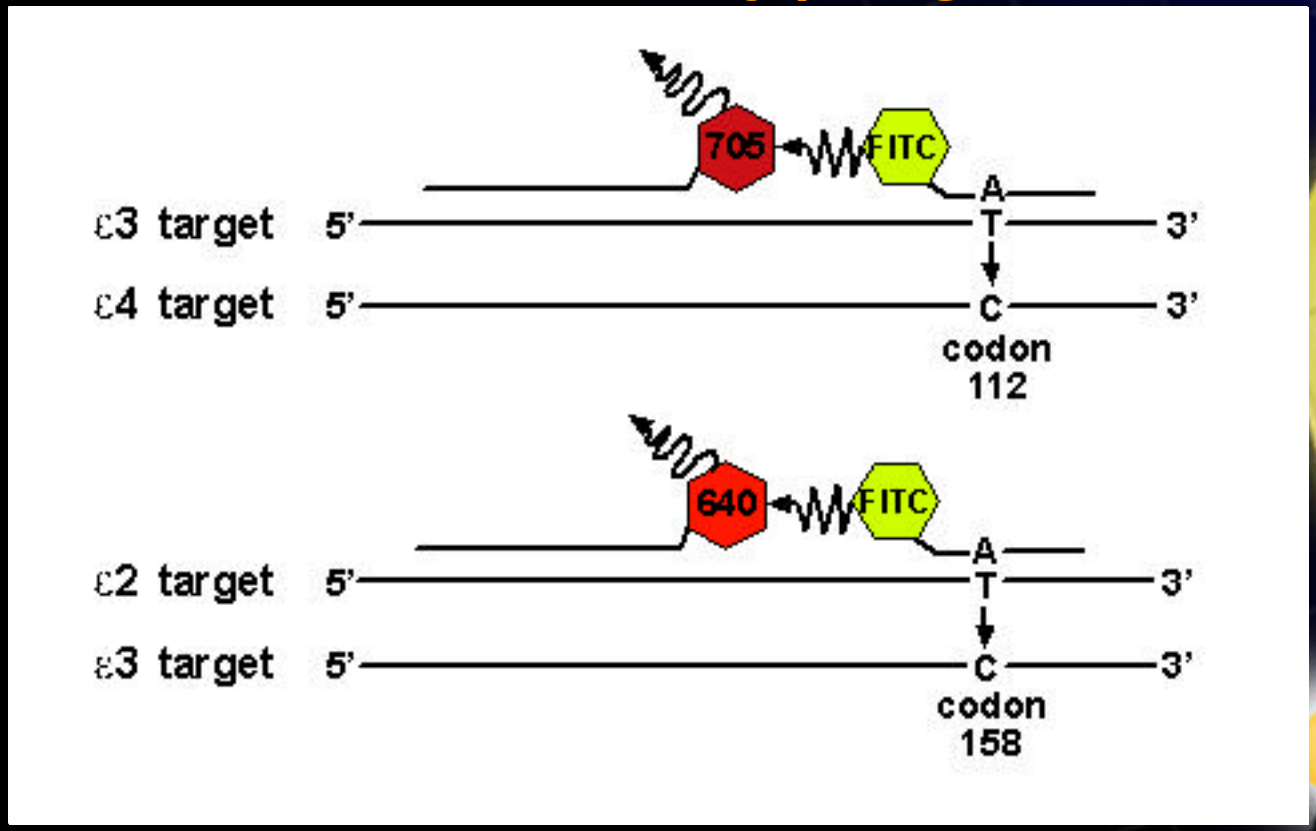
Color Multiplexing Hybridization Probes for Genotyping



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Multiplexing by Color and Tm

Two Color/T_M Multiplex
LCR Dye 640 and 705
Sensors
Fitc Anchor

Channel 1 (F1)	Channel 2 (F2)	Channel 3 (F3)
Fluorescein	LCR 640	LCR 705
Probe	T_M Multiplex	T_M Multiplex
Anchor/Exciter	2 or more peaks	1 or more peaks

Pre-Developed Hybridization probe Kits available through IT BioChem

- Mus musculus BALB/c HPRT exon 3 (ACCESSION K01509)
- Rattus norvegicus HPRT exon 3 (ACCESSION AF009656)
- Homo sapiens HPRT exon 3 (ACCESSION BC000578)
- Human Papilloma Virus Types 16, 18, and 45 Detection (High Risk) and Types 31, 56, and 66 Detection (Medium Risk)

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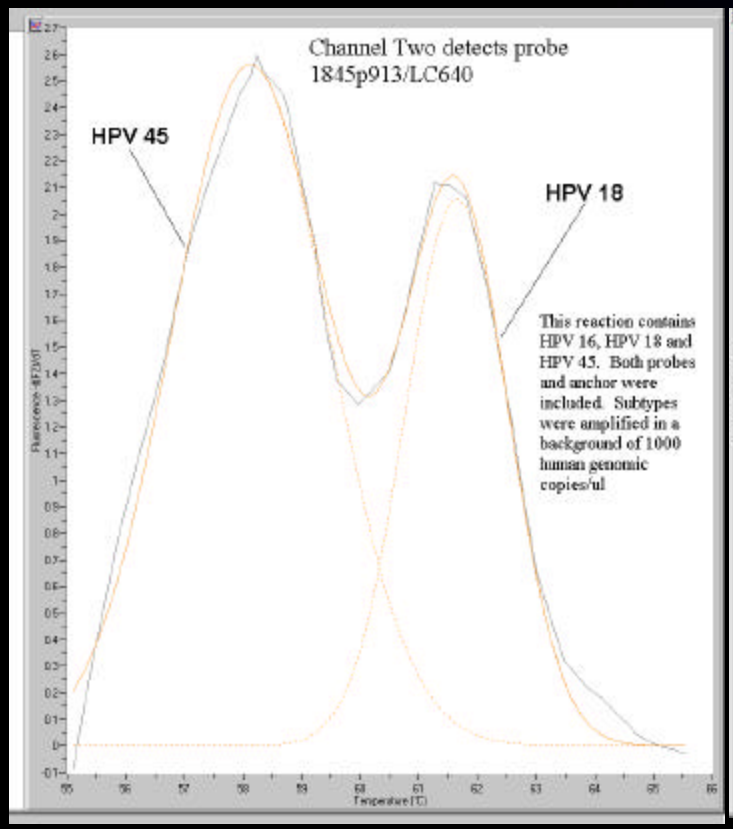
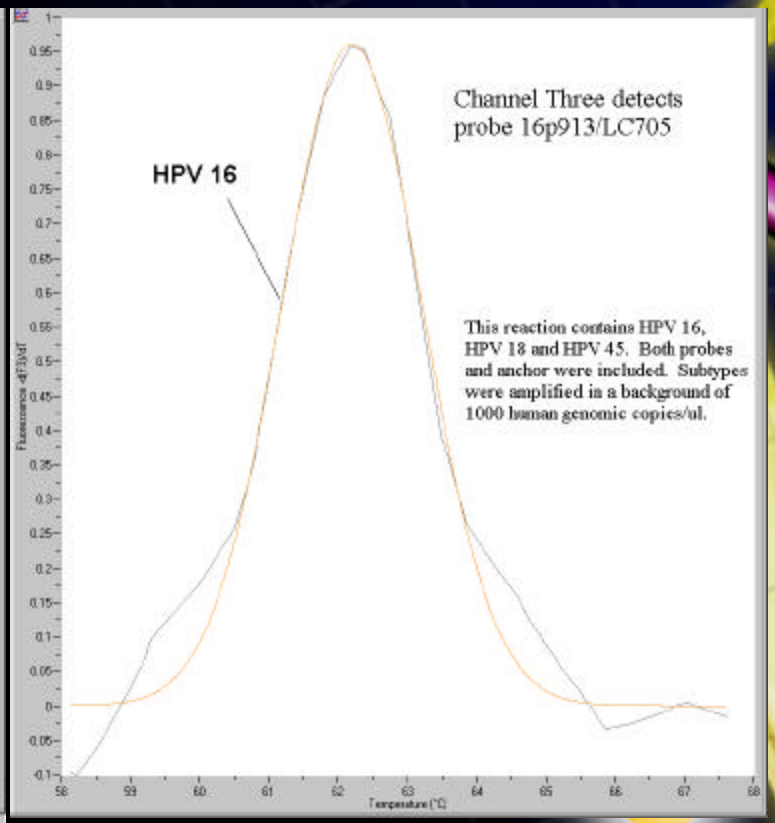
Multiplex PCR: HPV16, HPV 18 and HPV 45





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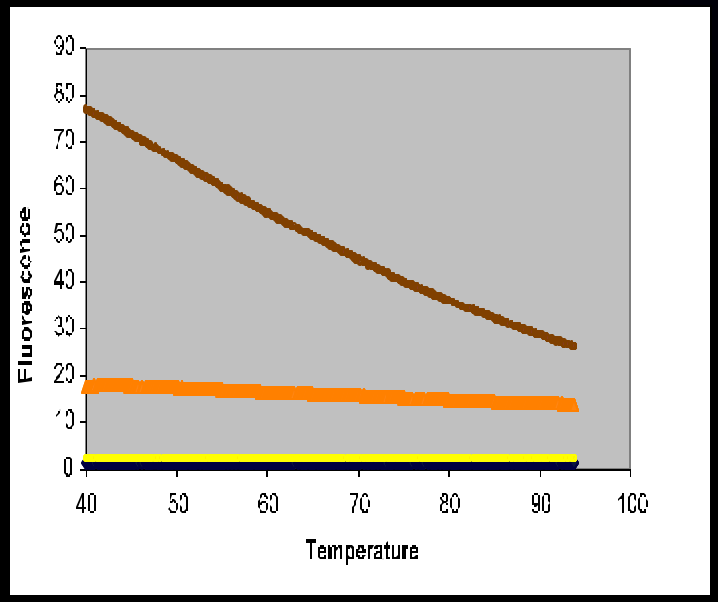
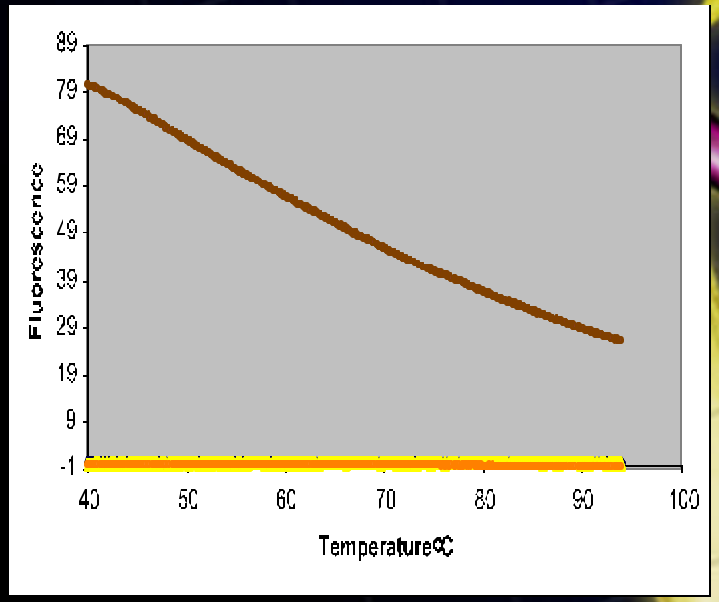
Signal Bleed Over From Channel 2 Into Channel 3





Before compensation

After compensation

This is the only extra work that has to be done to get Hybridization Probes to do all of these things

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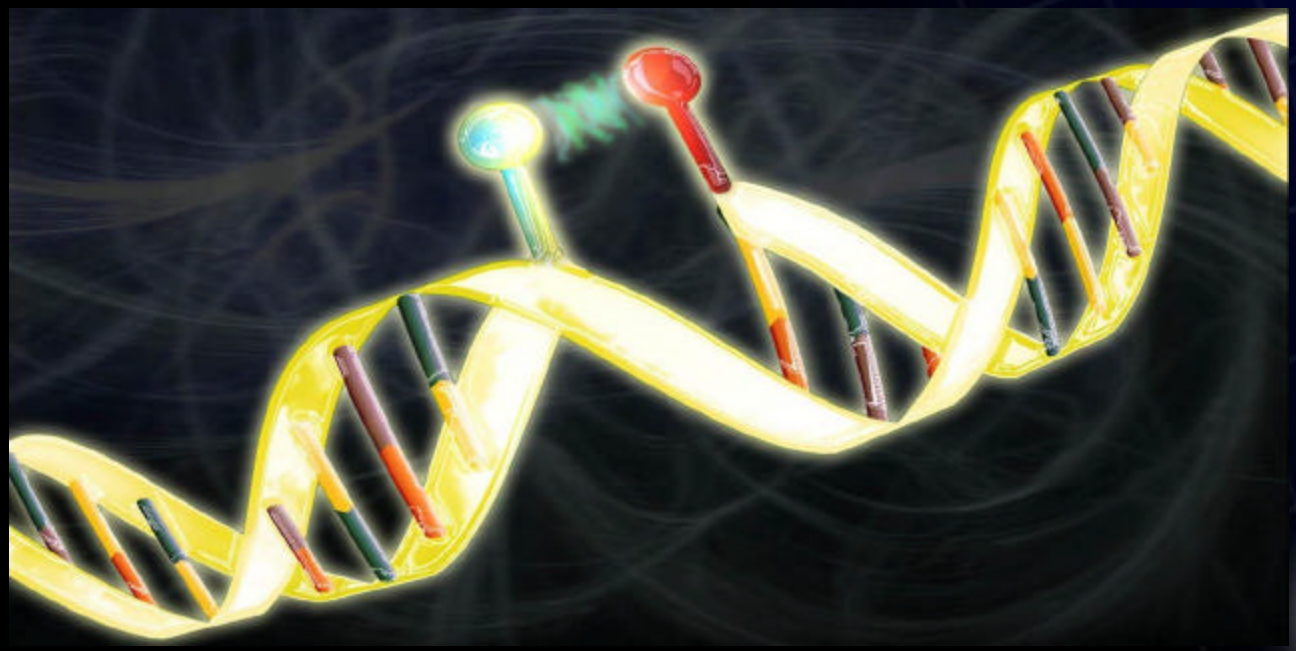
For help with probe design please give us a call, 800-735-6544, extension 8.



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