AllGlo™ – A Novel Nucleic Acid Detection Method That Does Not Require a Fluorescence Quencher

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- Applications

- **Introduction**
  1. Fluorogenic technologies for qPCR
  2. The principles of TaqMan probes
  3. The principles of AllGlo probes
- **Advantages**
- **Applications**

Fluorogenic Technologies for Real Time qPCR

♦ Fluorogenic Probes

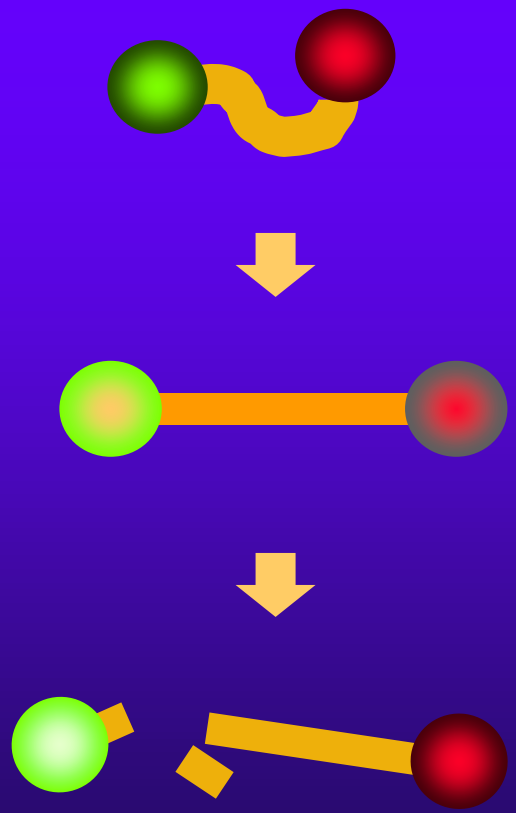
1. TaqMan™ (double labeled) Probe
   Livak, 1996 US Pat 5,538,848.
2. Molecular Beacon
   Tyagi and Kramer, 1996, Nature Biotechnol. 14: 303-306.
3. FRET Probes (hybridization probes)
   Wittwer et al., 2001, US Pat 6,174,670.
4. G-quencher probe
   Wittwer et al., 2003, US Pat 6,635,427.

♦ Fluorogenic Primers

1. Ampliphore™
   Nazarenko et al., 1999, US Patent 5,866,336.
2. LUX ™primer
   Nazarenko et al., Nucleic Acid Research 2002, Vol. 30, No.9.

TaqMan™ (double labeled) probes

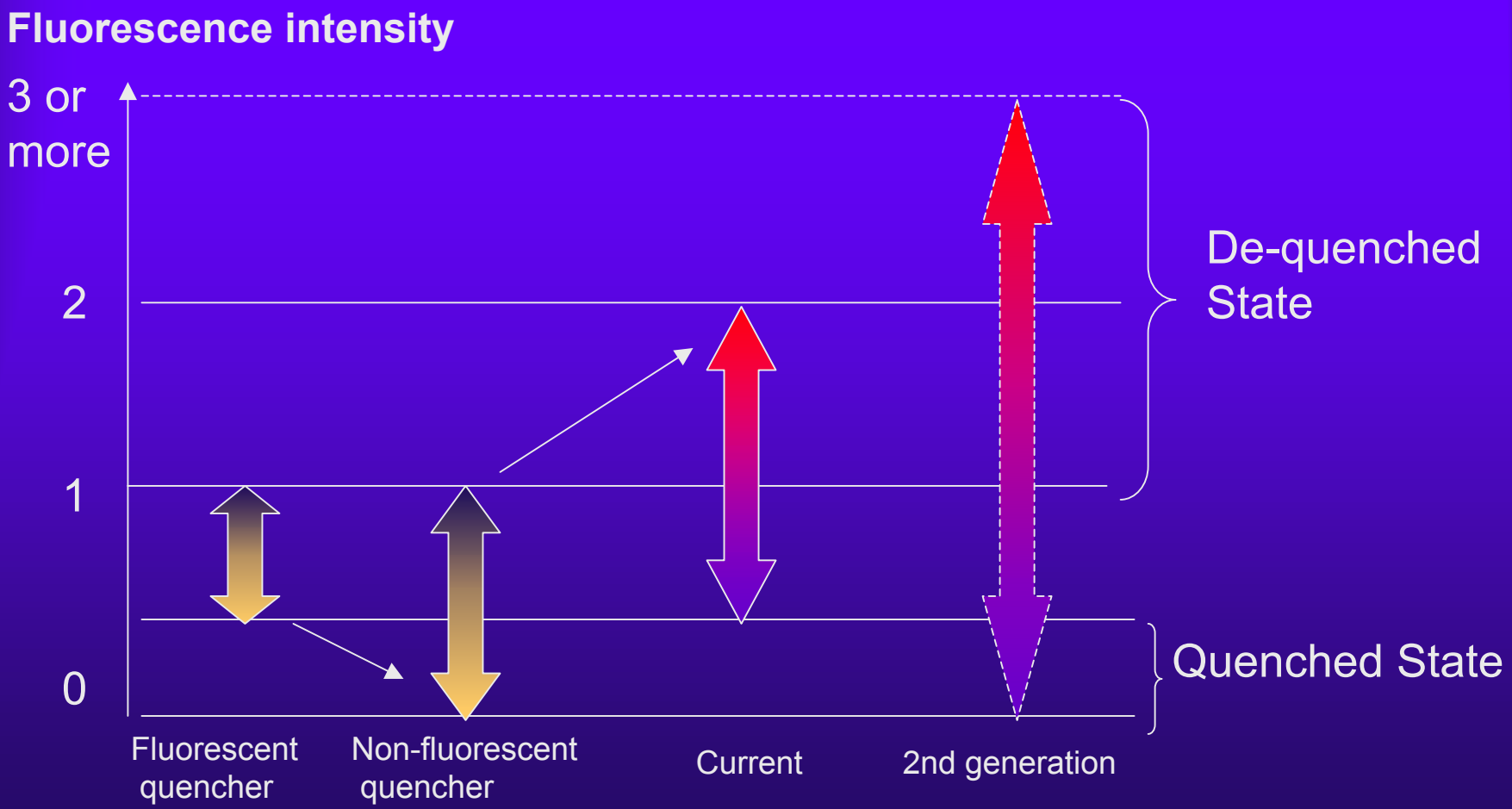
1. Linear oligo
2. One quencher
3. One fluorophore



Challenges

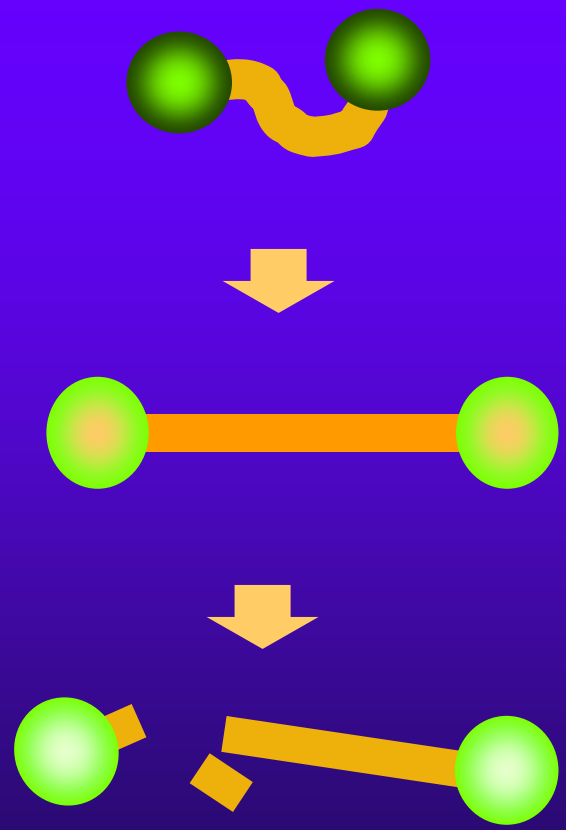
1. Cost
2. Sensitivity

AllGlo increases signal strength by increasing the number of fluorescing molecules



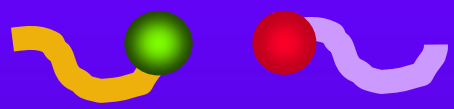
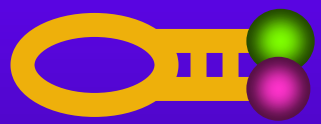
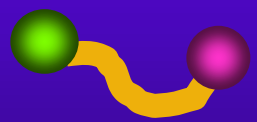
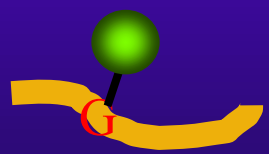
AllGlo probes

1. No quencher.
2. Two identical reporting fluorophores.

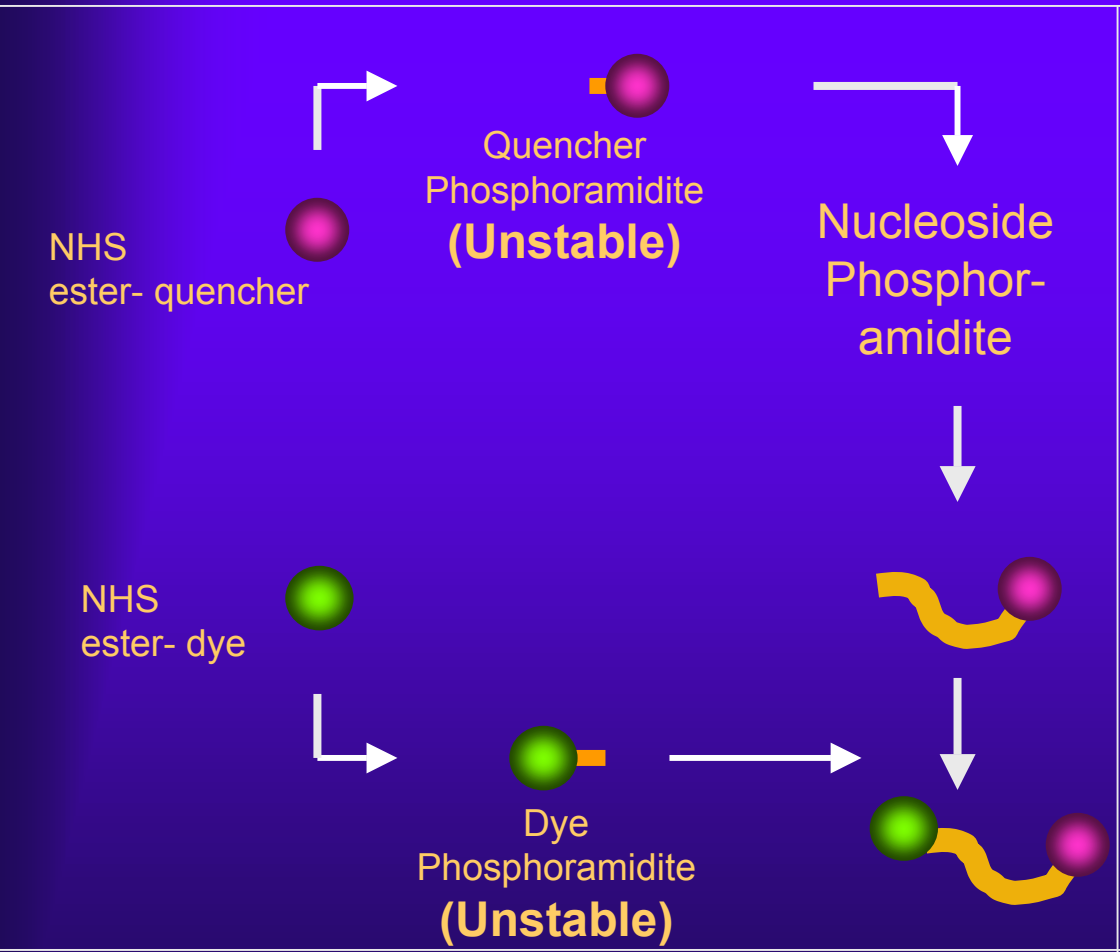


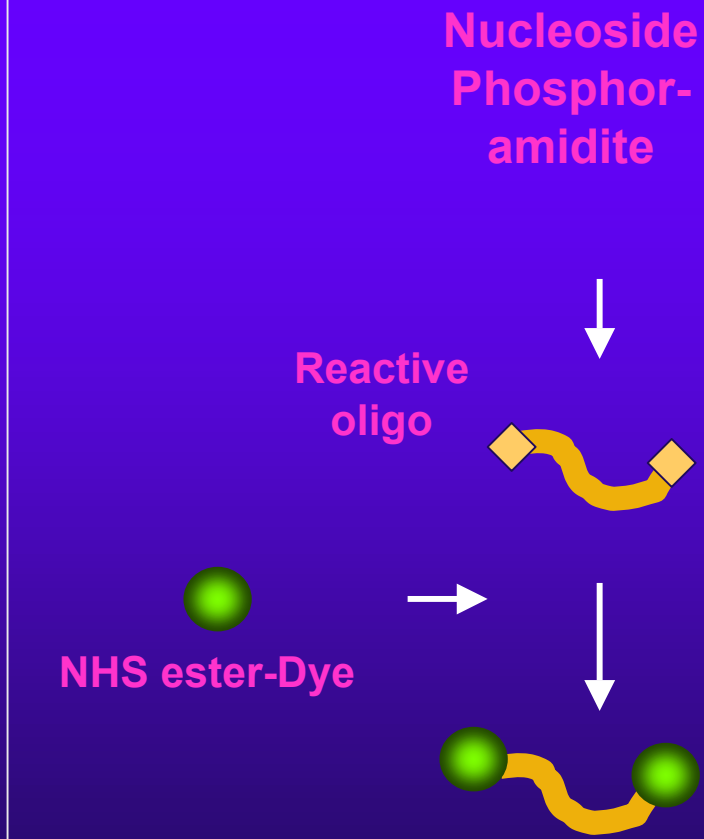
- Introduction
- **Advantages**
- Applications

1. Simple in Composition

Number of components in fluorogenic probes		
FRET Probes		4
Molecular Beacon		3+ secondary structure
TaqMan™ Probe		3
G-quencher probes		2
AllGlo		2

2. Fewer Steps in Synthesis

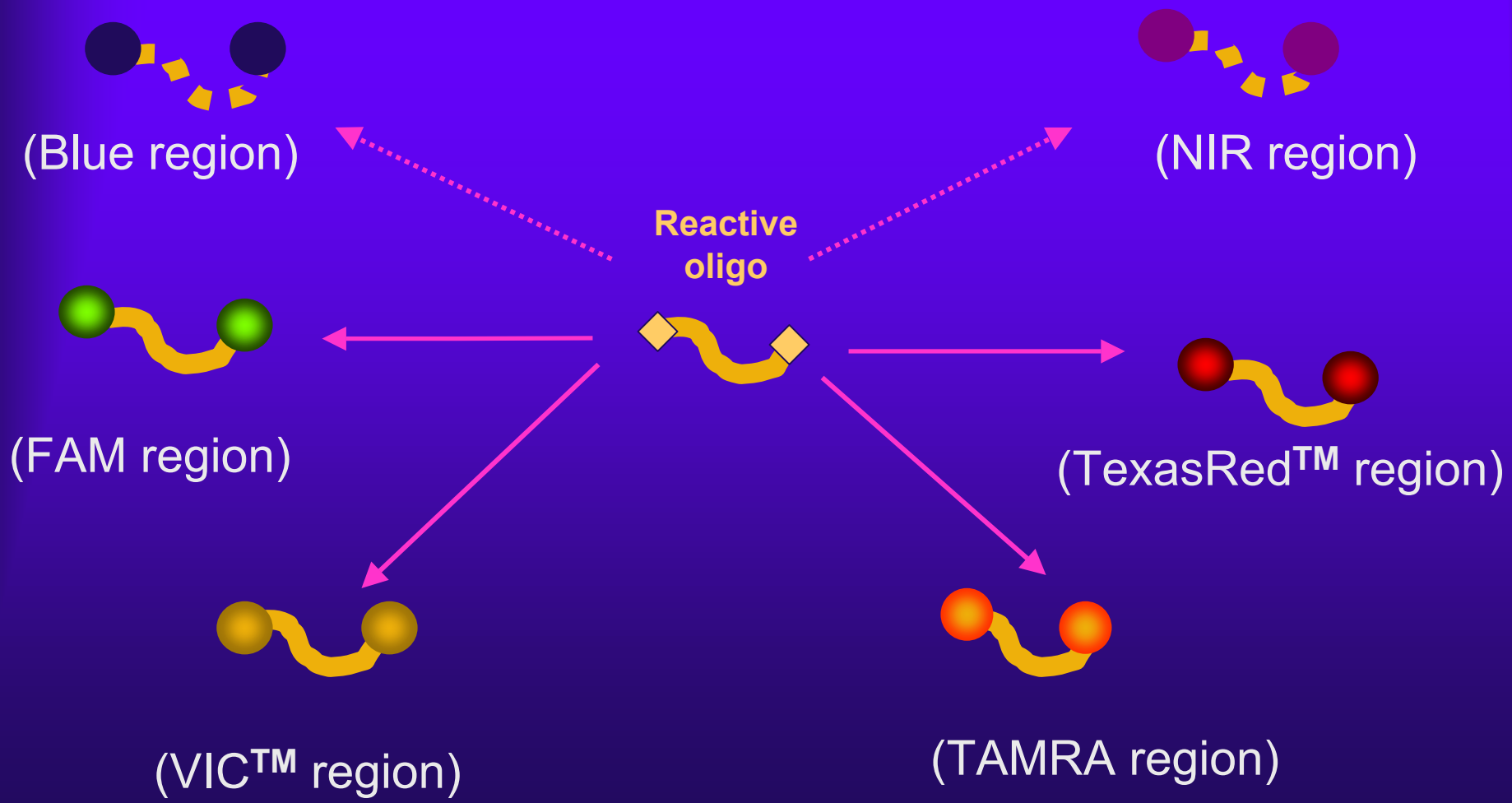




Dual-label Probe

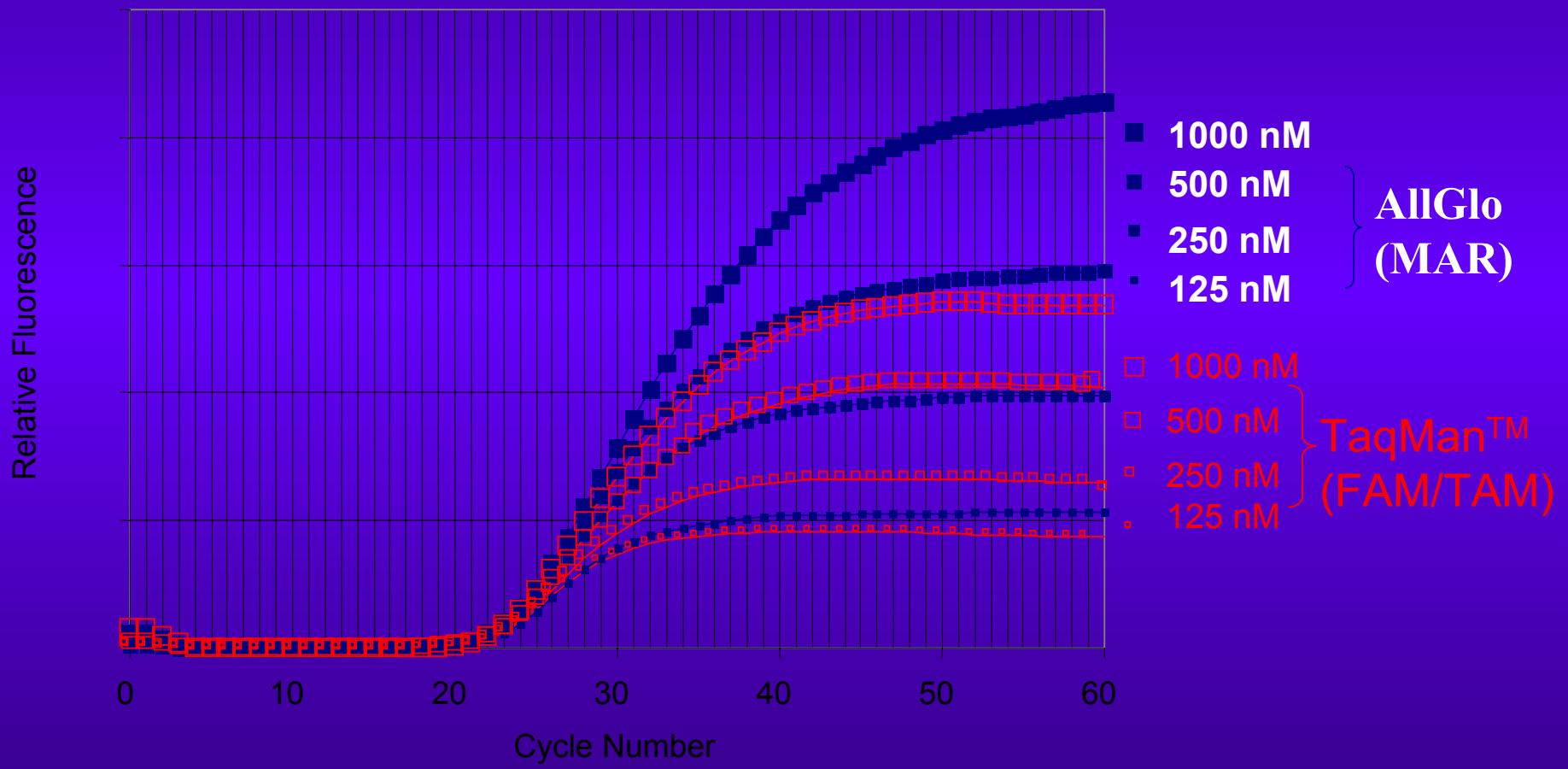
AllGlo

3. One synthesis, multiple colors



4. Stronger Signal

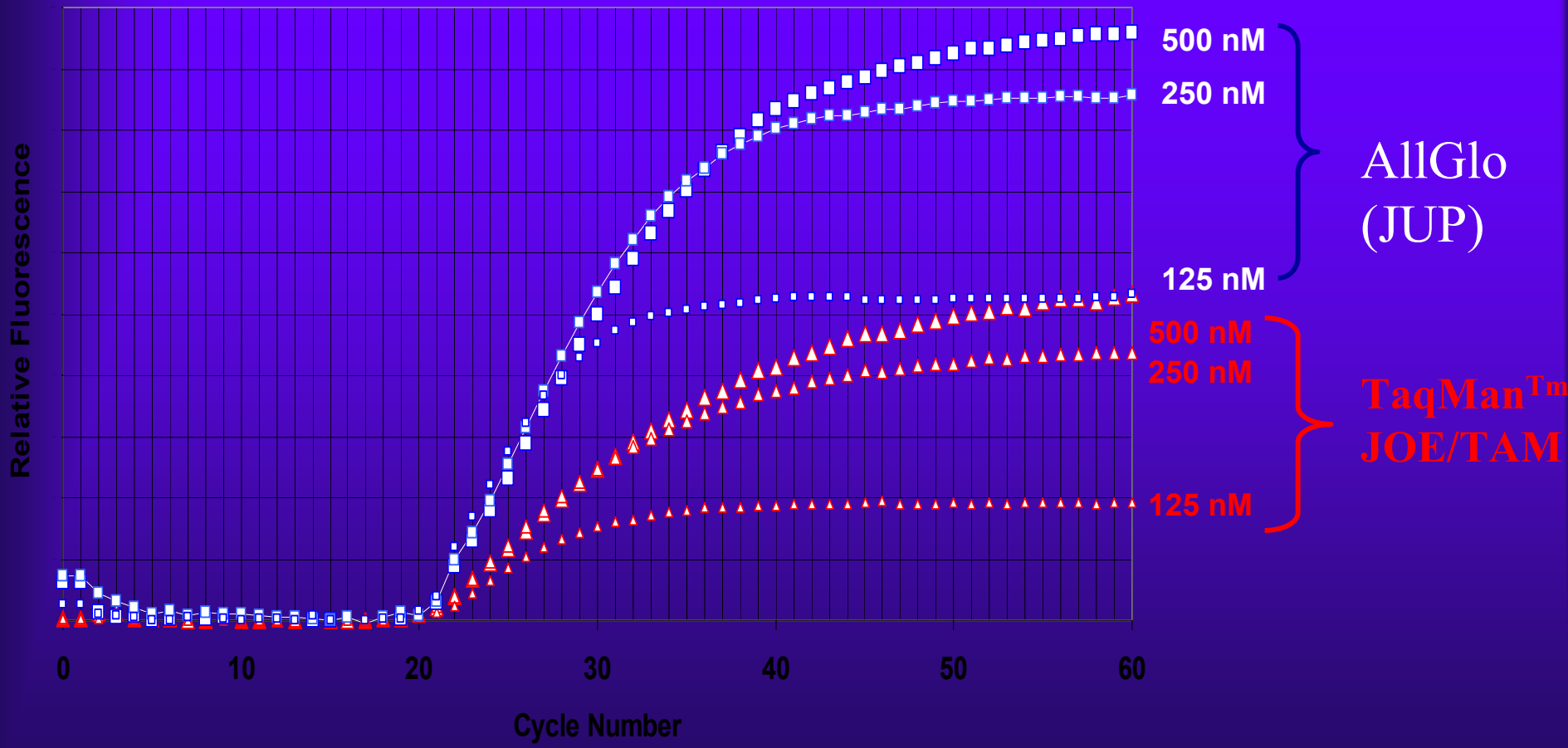
(1) cMyc in FAM region



* Optics optimized for FAM; All conditions are same except labels

4. Stronger Signal

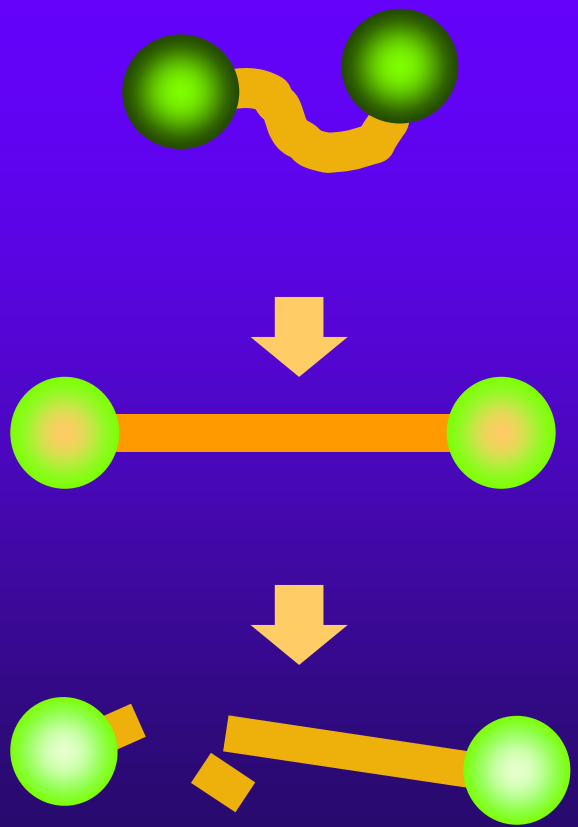
(2) cMyc in VIC region



* Optics optimized for JOE; All conditions are same except labels

5. More Options for Detection

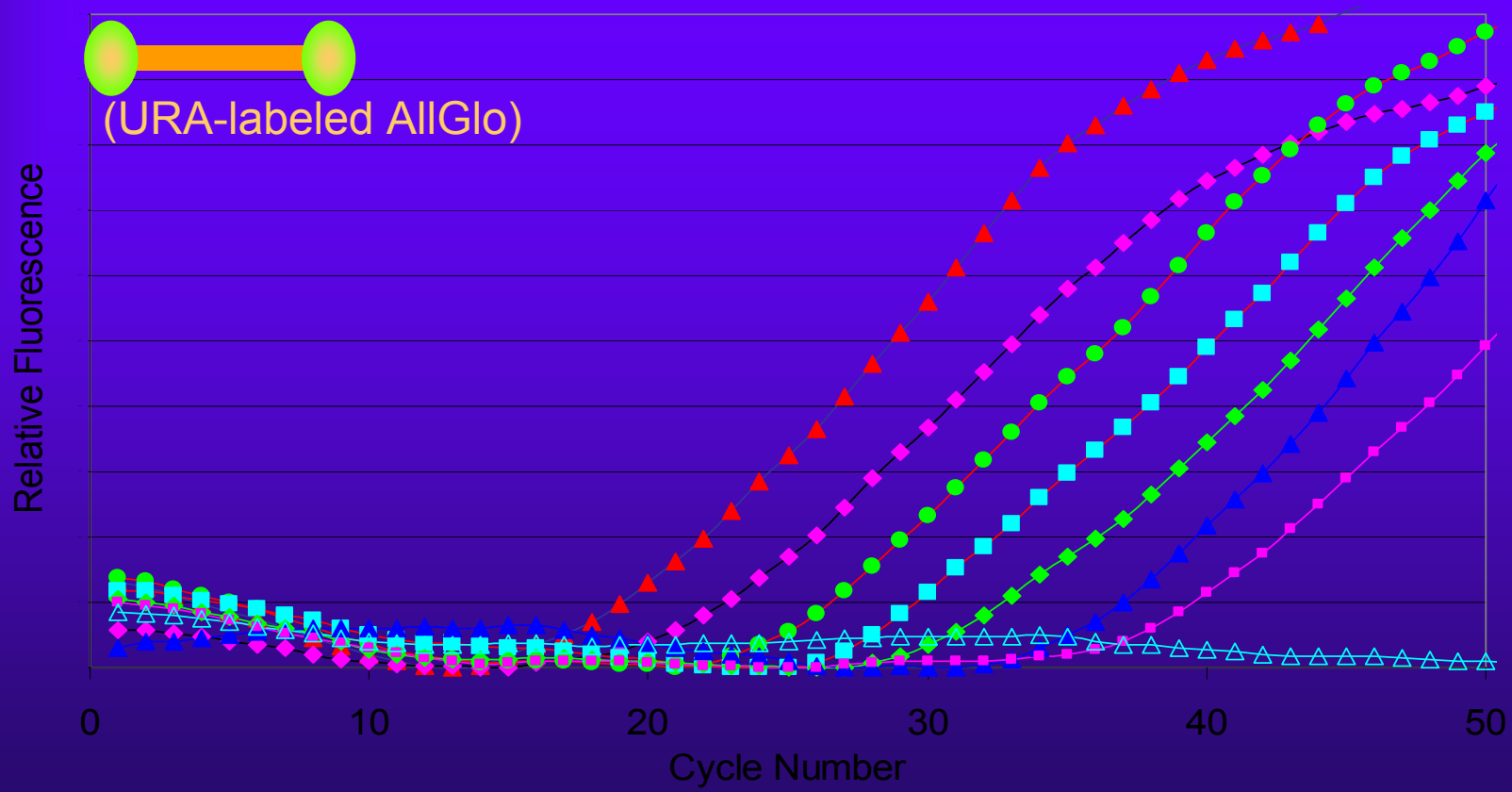
at hybridization or after cleavage



Hybridization

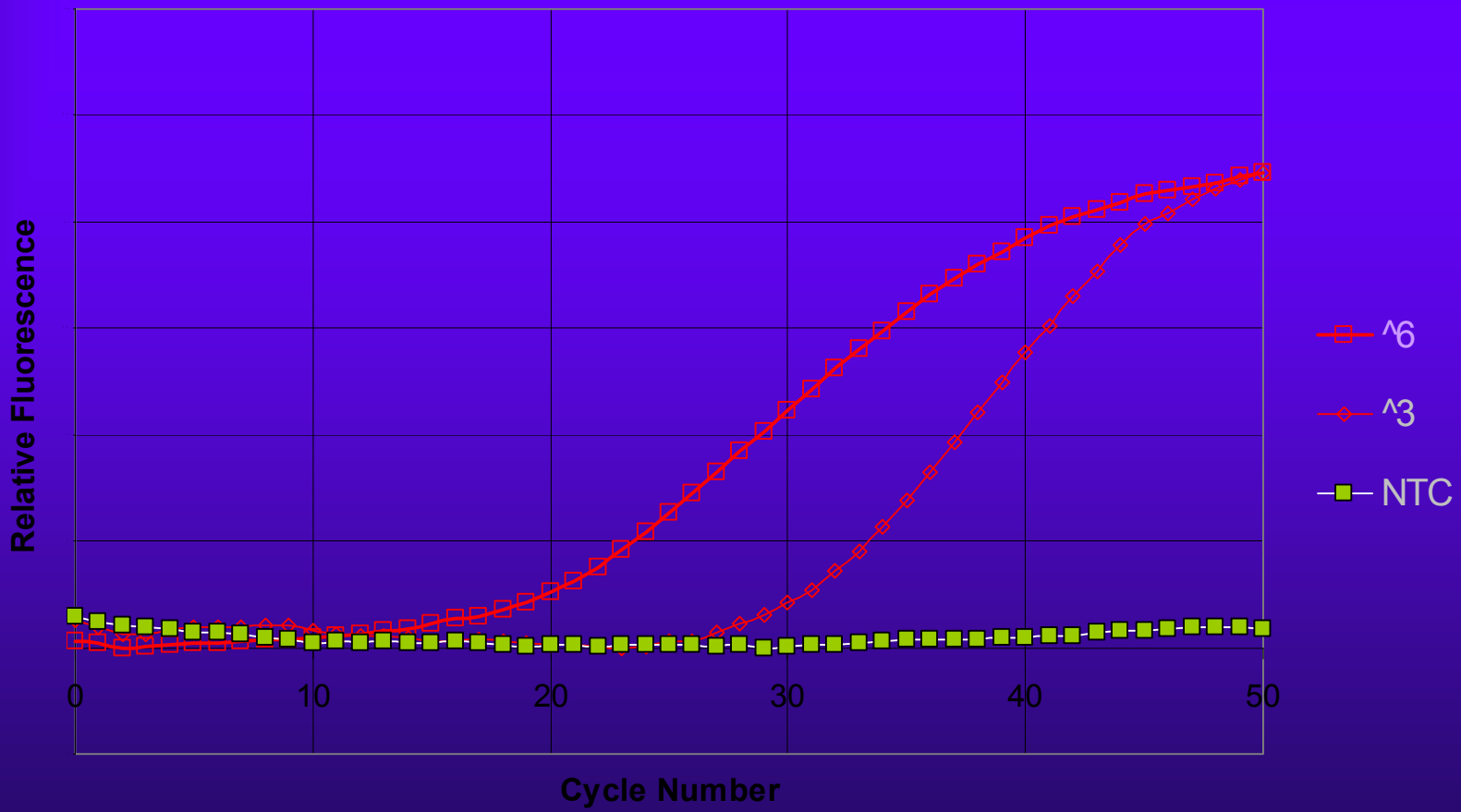
Cleavage

5. Detection at Hybridization Step



HCV templates from 1 copy to 10^6 copies amplified with Titanium Taq

6. AllGlo as primers as well as probes

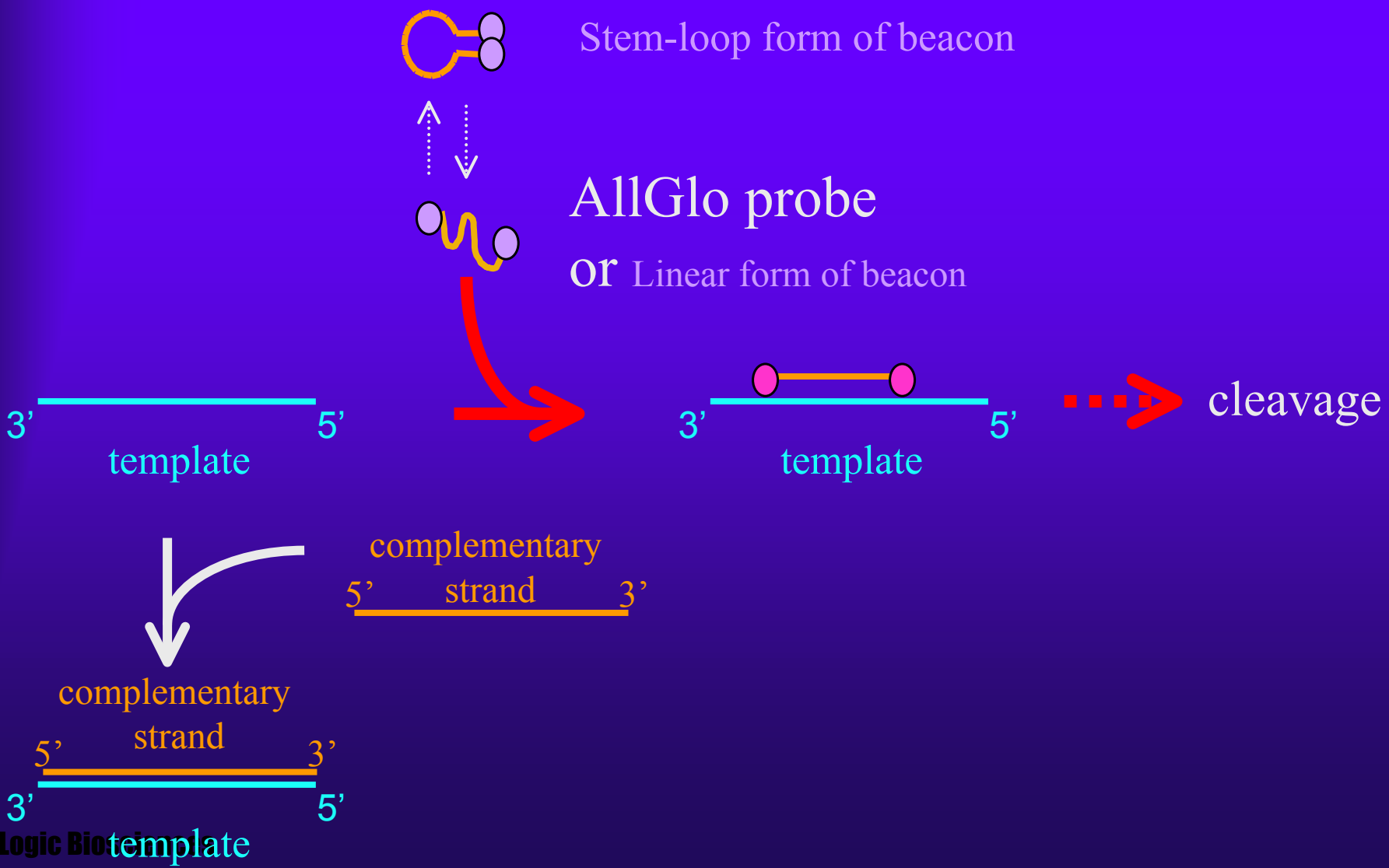


MAR-labeled reverse primer of GAPDH : GAA GAT GGT GAT GGG ATT

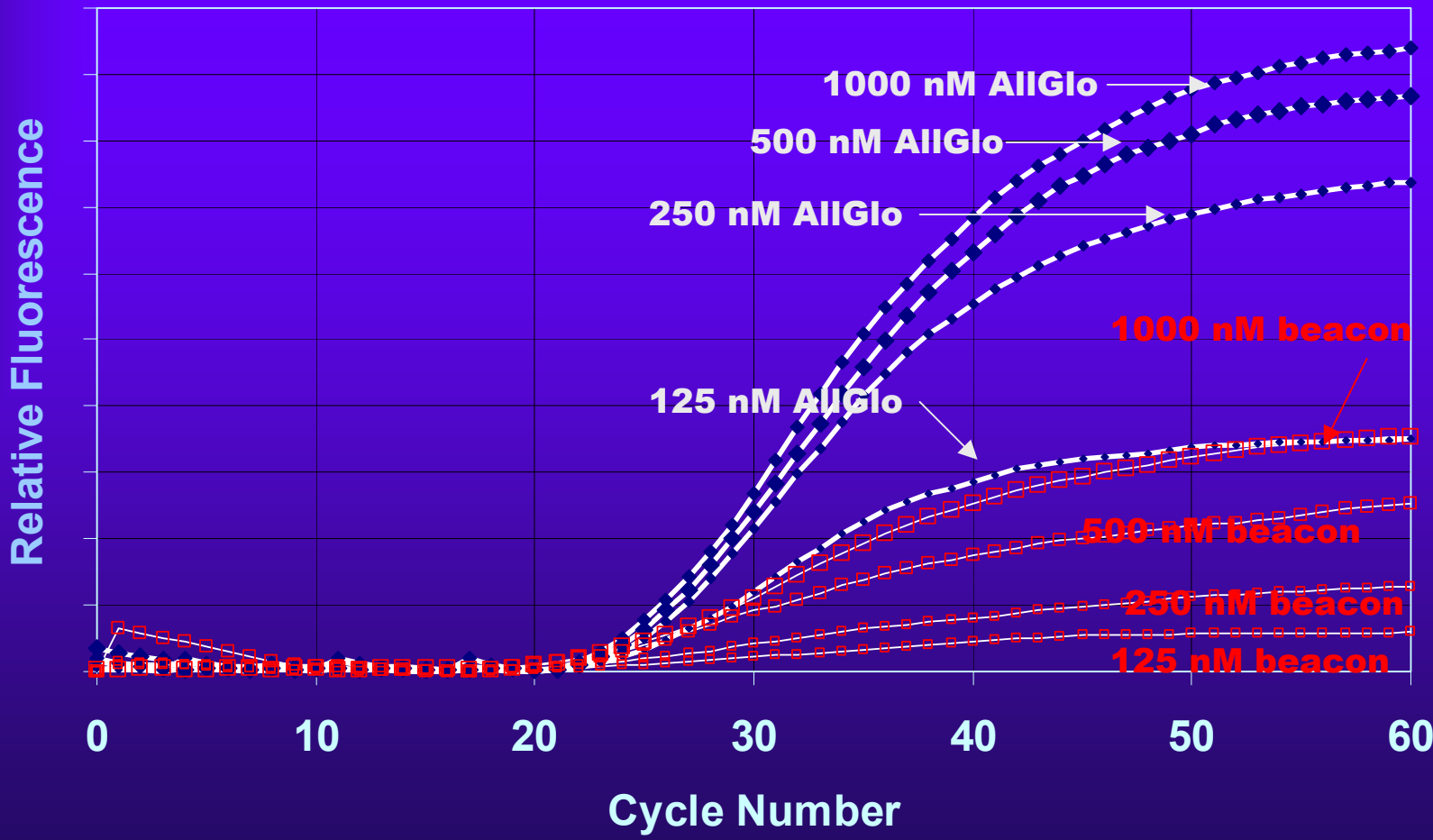
Summary of advantages

1. Simple composition
2. Fewer synthesis steps
3. One synthesis, multiple colors
4. Stronger signals
5. Detect via hybridization or after cleavage
6. Use as probes or primers
7. Whole range of colors
8. Compatible with existing instruments and enzyme systems

Productive and unproductive binding events



The effective concentration of beacon is much less than actual concentration

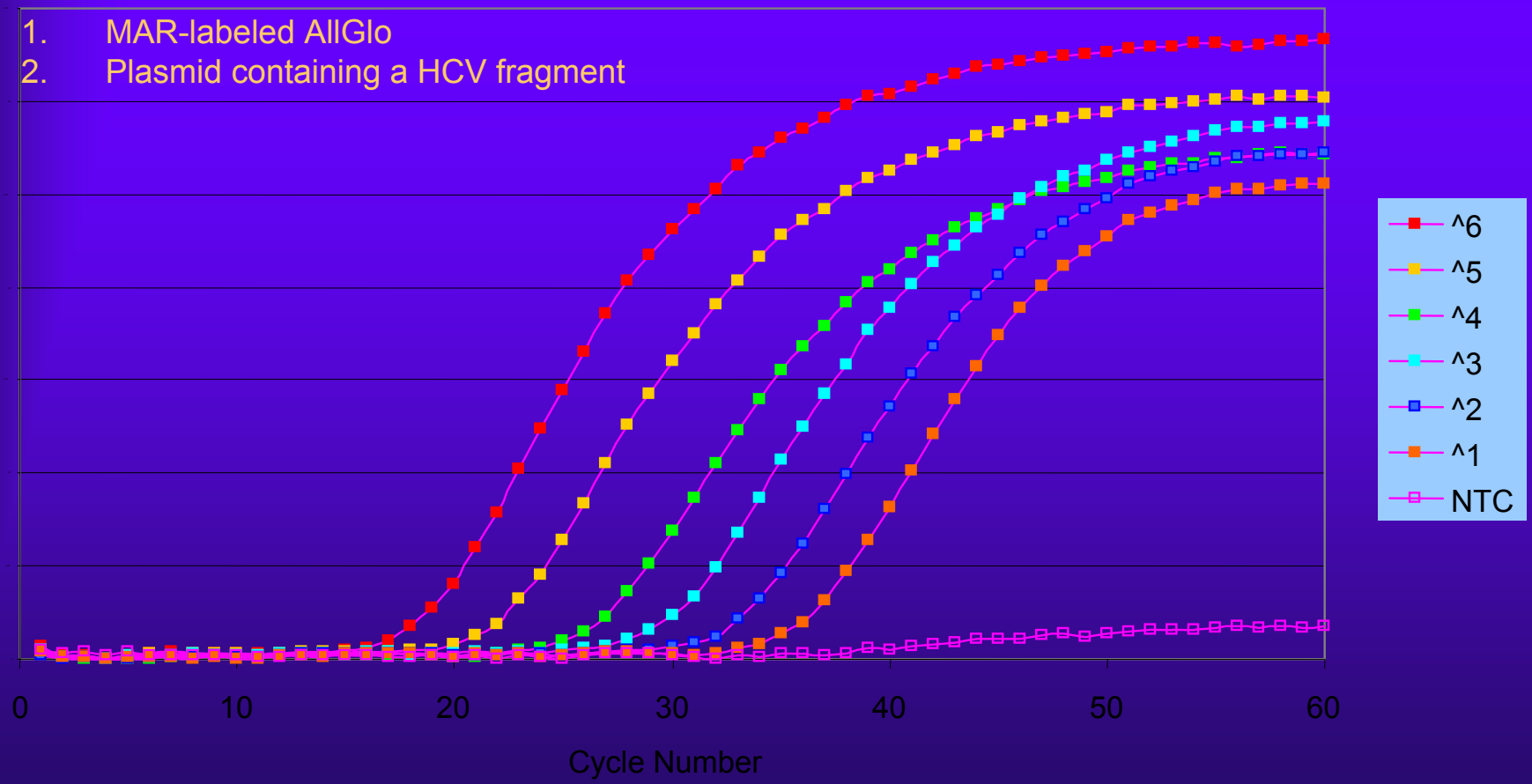


… even in hydrolysis reactions

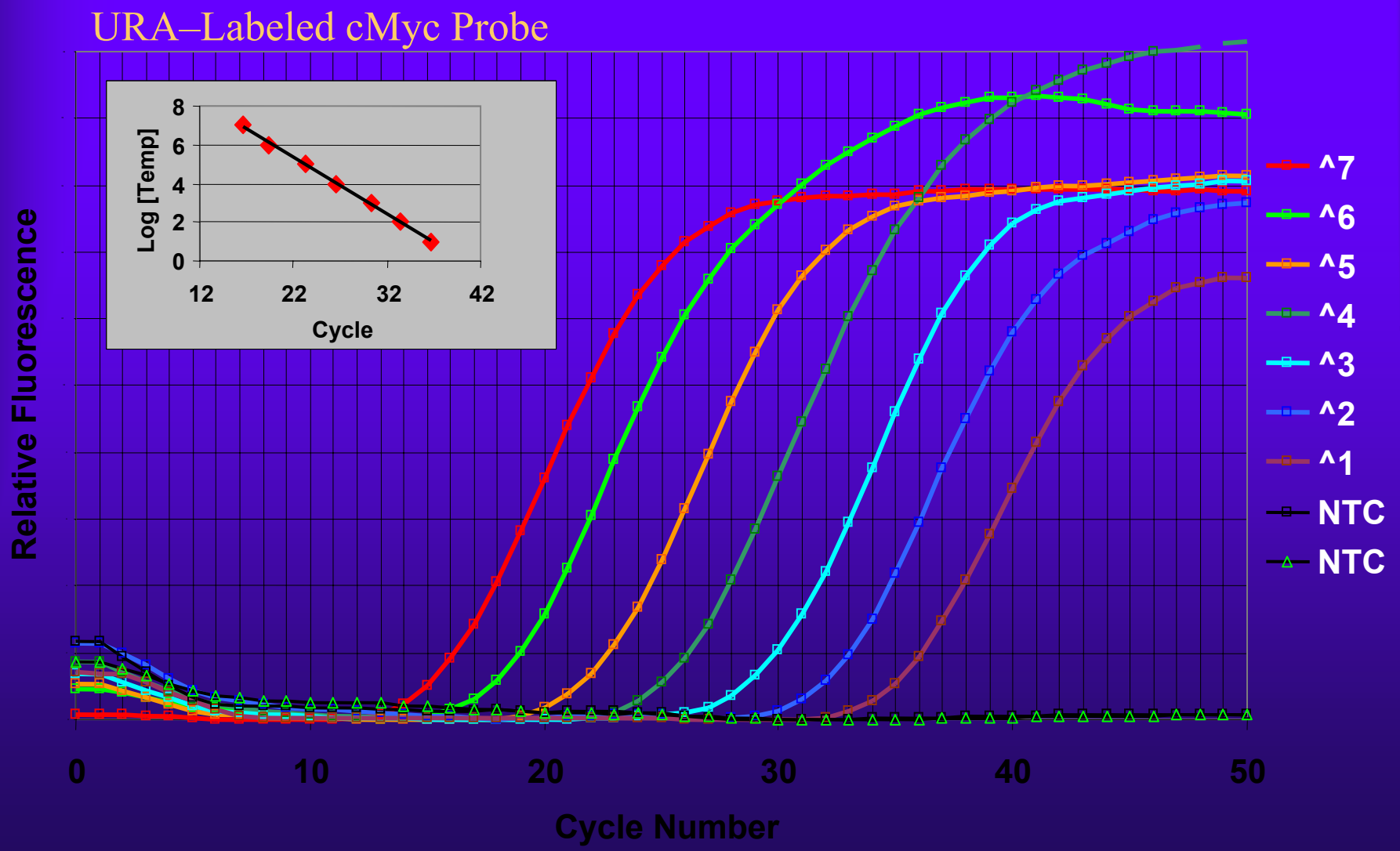
- Introduction
- Advantages
- Application Examples:

1. Pathogen detection
2. Gene quantitation
3. SNP typing

Viral detection-HCV

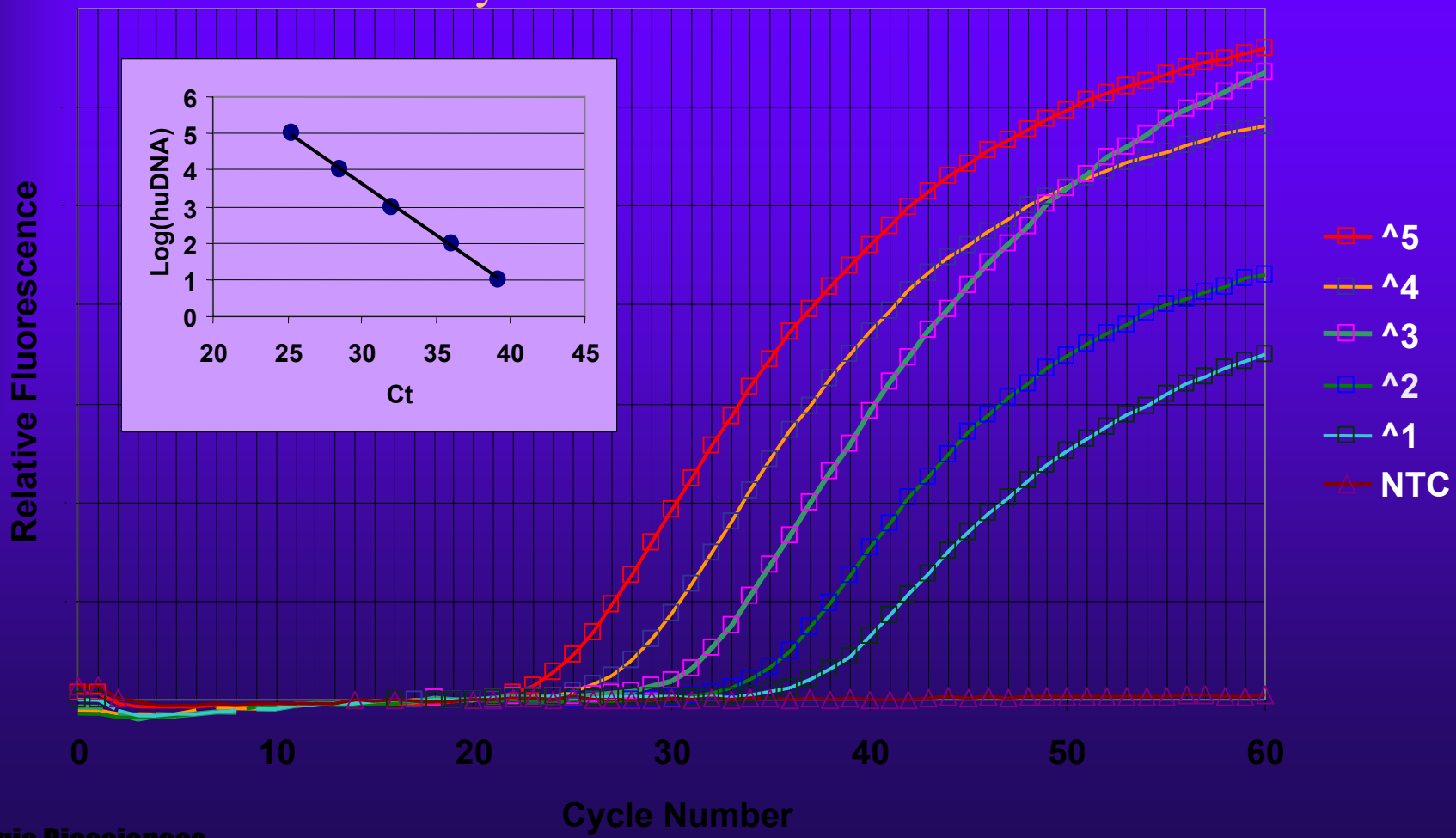


Gene quantitation using plasmid DNA



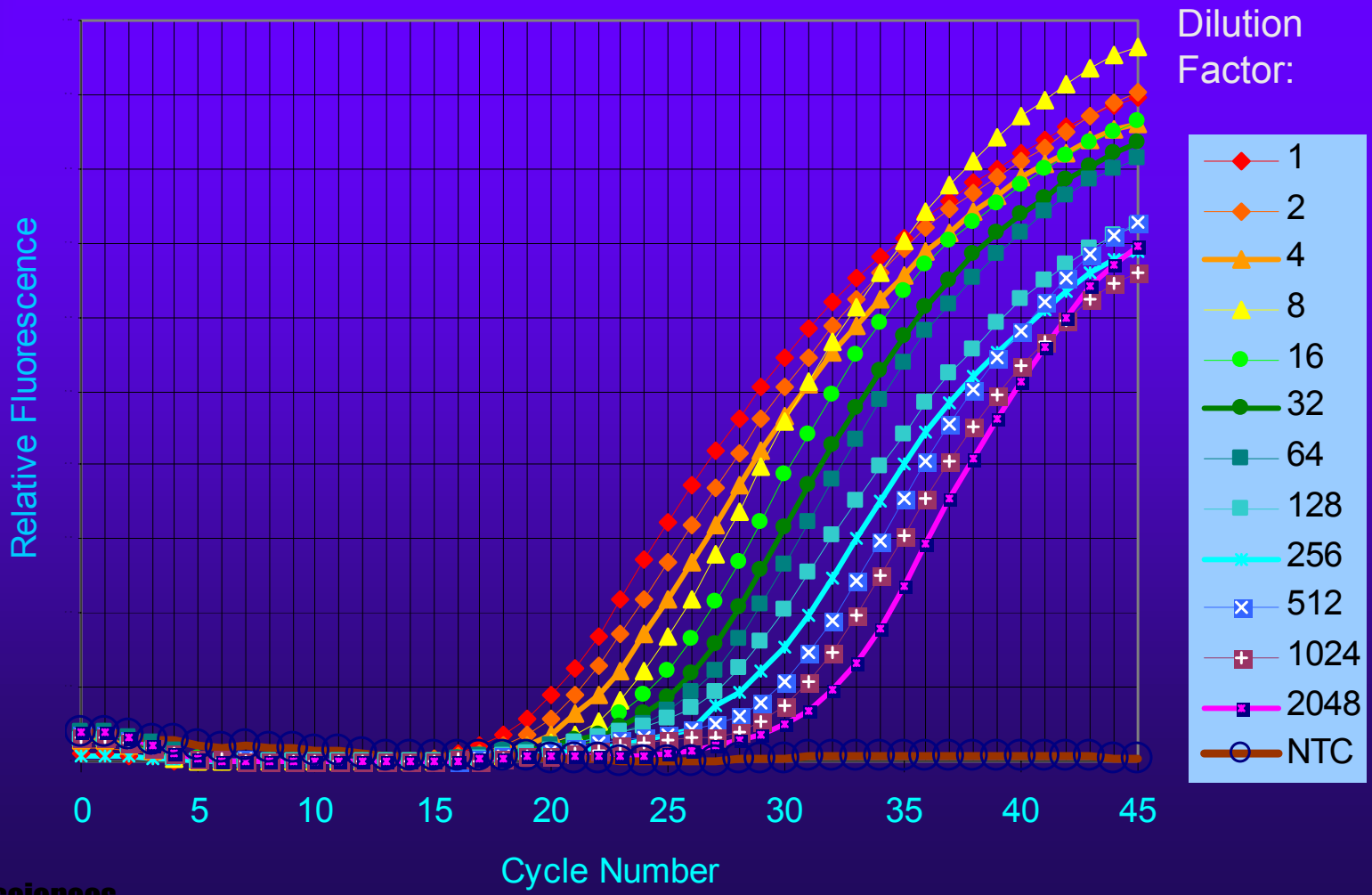
Gene quantitation using human genomic DNA

URA–Labeled cMyc Probe

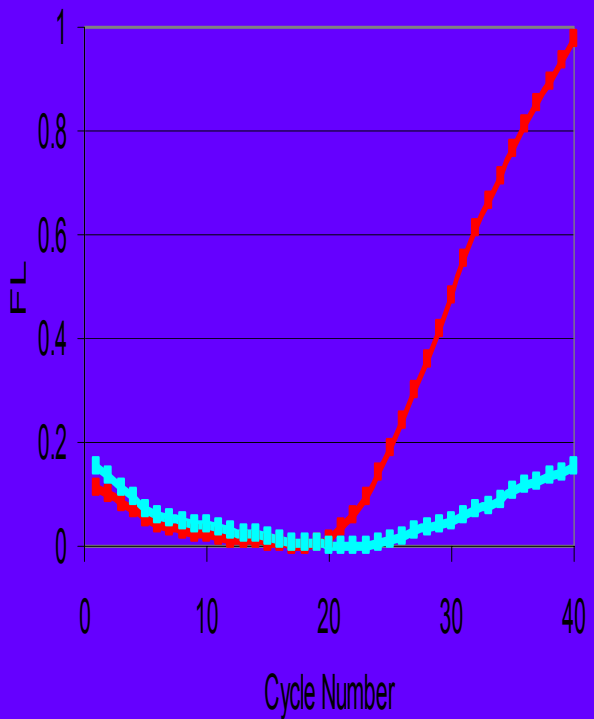


Gene quantitation using human cDNA

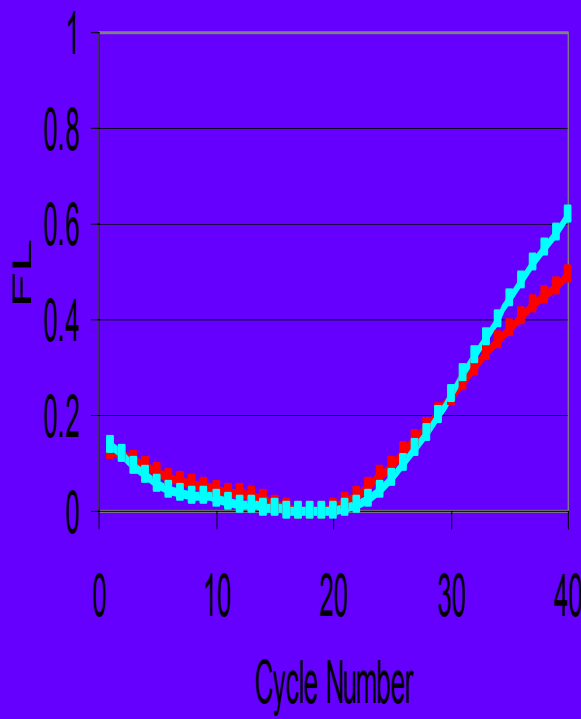
1. MAR-Labeled GAPDH Probe
2. 10 µL reactions



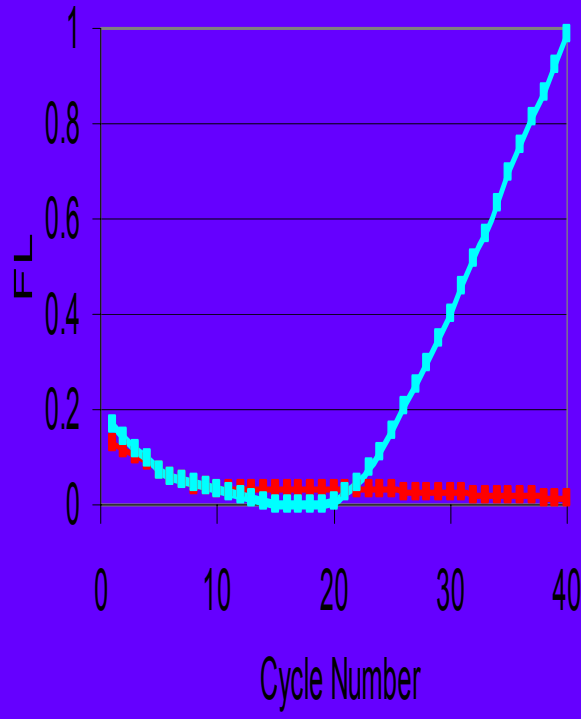
SNP Typing – duplex assay



CC



CT



TT

estrogen receptor gene
Allele C: MAR
Allele T: JUP

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Summary

- Simple
- Bright
- Versatile
- Robust
- Economic

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