

A wooden canoe is shown from a low-angle perspective, pointing towards the top of the frame. The canoe's interior is visible, showing the wooden ribs and a woven seat. The background is a vibrant blue sky with a yellow butterfly on the left and a large green leaf with prominent veins on the right. The text is overlaid on the right side of the image.

Plexor™ Real-Time Quantitative PCR Systems: Multiplexed Assays Made Easy

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Genomics Product Manager
qPCR 2005

Plexor™ Systems Real-Time qPCR Systems: Multiplexed Assays Made Easy

- Plexor Technology
- Plexor Reagents
- Applications of Plexor Technology
 - Gene Expression Analysis
 - SNP Genotyping
- Conclusions



Plexor™ Technology

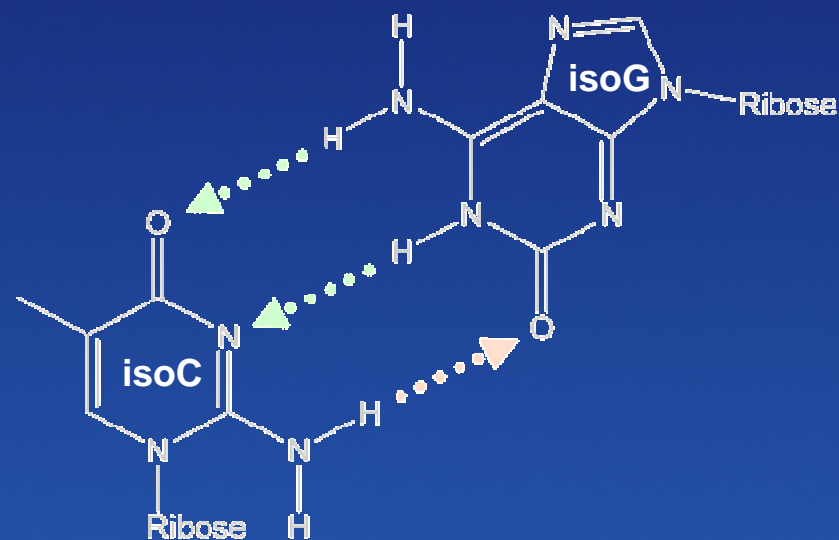
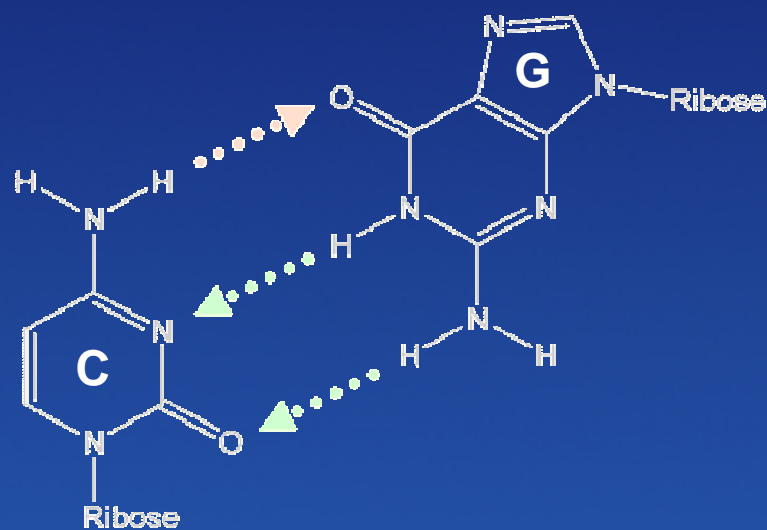
**Specificity from novel
base-pairing**



Promega

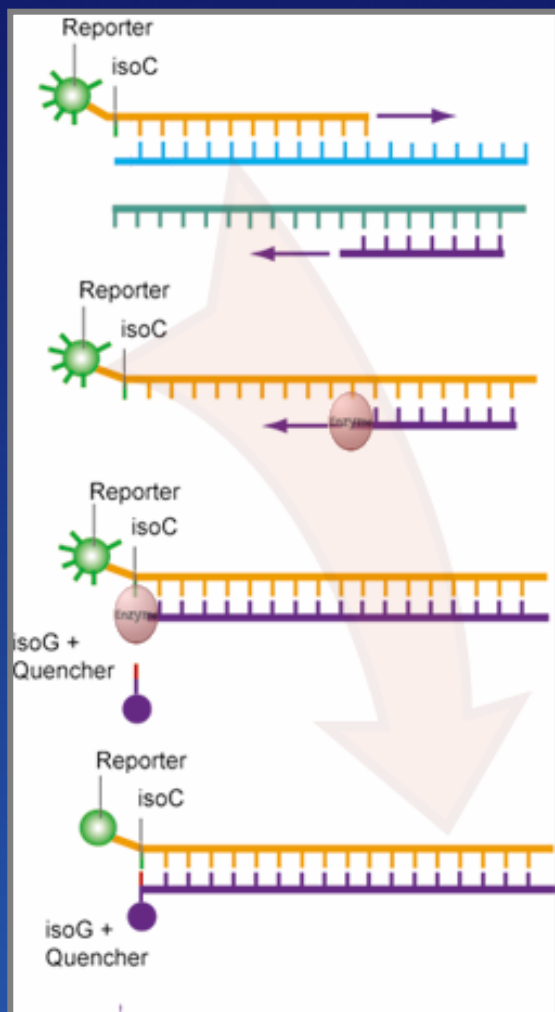
Novel Base-Pairing Drives Plexor™ Technology

- Designed and licensed from EraGen Biosciences
- IsoC & IsoG dNTPs incorporated by DNA Polymerase
- IsoC & IsoG bases do not base pair with ACGTU



Johnson, S.C., et al. (2004) *Nucleic Acids Res.* **32**, 1937-41.

Application of Novel IsoG & IsoC Bases

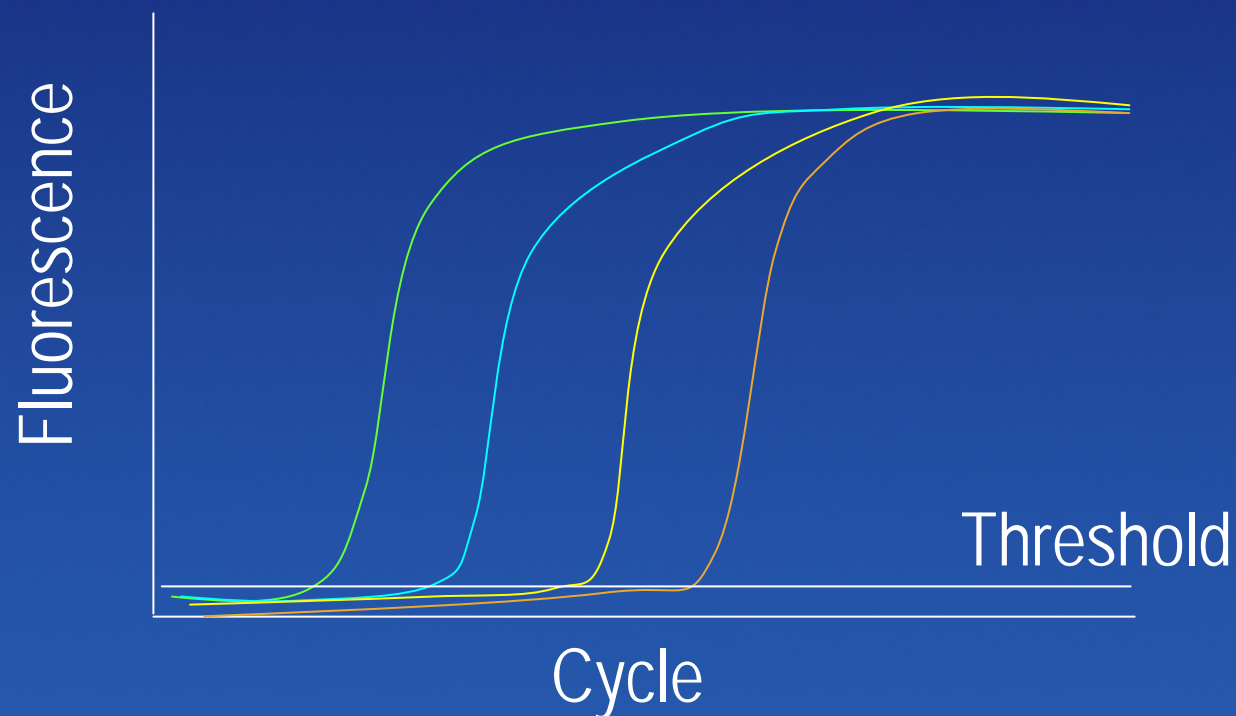


- Two primer method
- Oligo primer synthesized with
 - 5' novel base (isoC)
 - Fluor: FAM, JOE, HEX, ROX, whatever is compatible with your instrument
- Amplification master mix
 - Standard dNTPs
 - Dabcyl-labeled complementary iso-dGTP

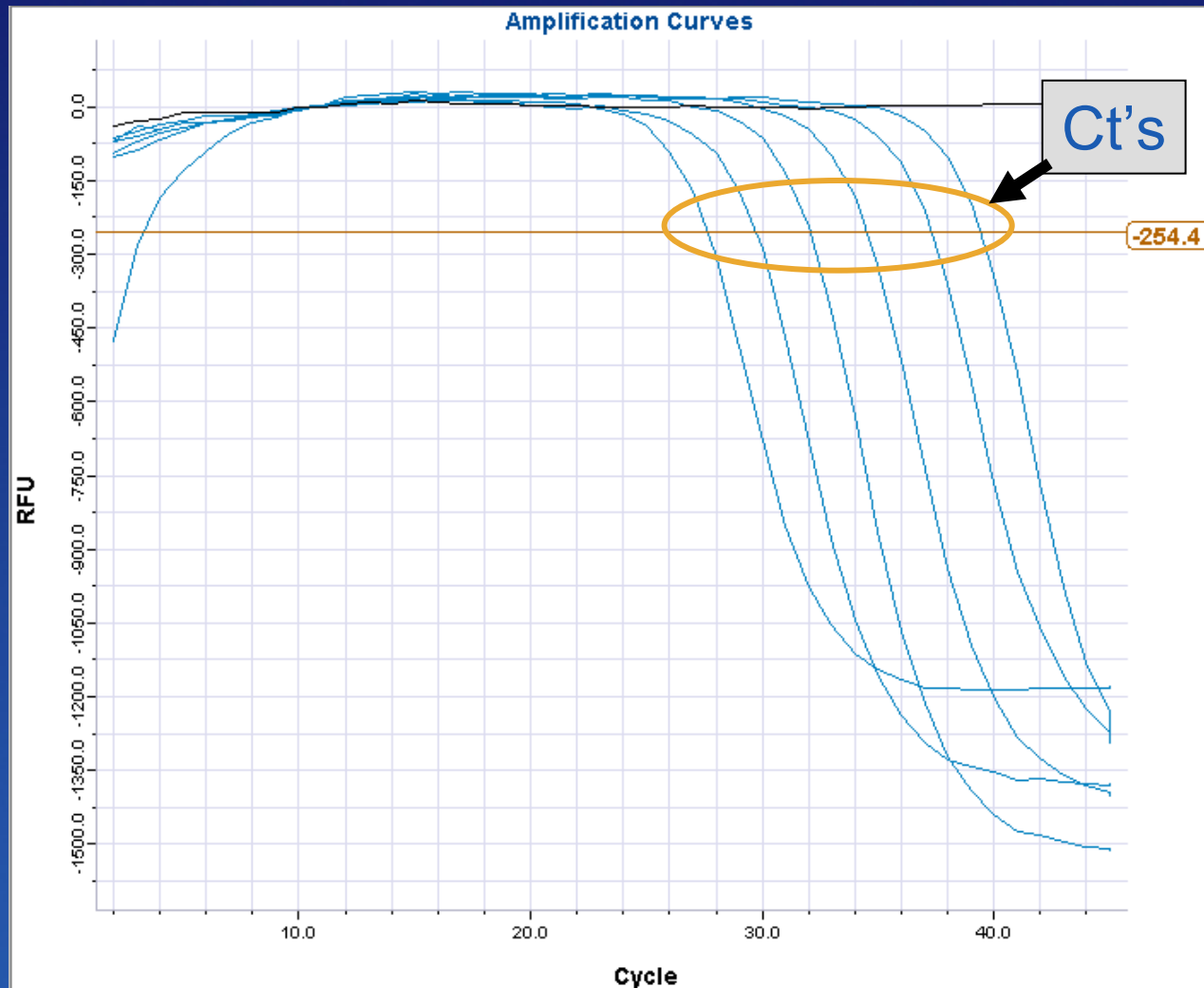
Dabcyl-iso-dGTP incorporated opposite isoC, quenching fluorescence!

Yes, It's Different...

- Existing methods
 - gain of fluorescence

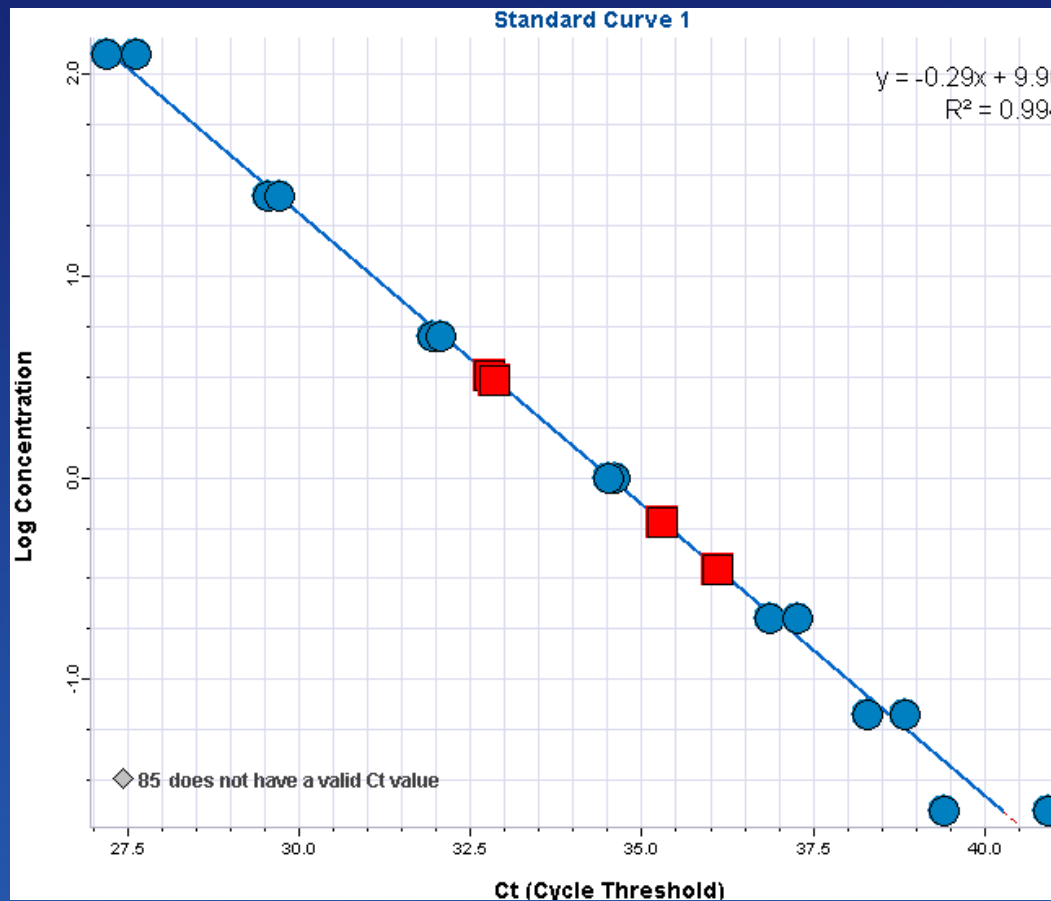


Different... Yet Familiar



C_t vs. Concentration

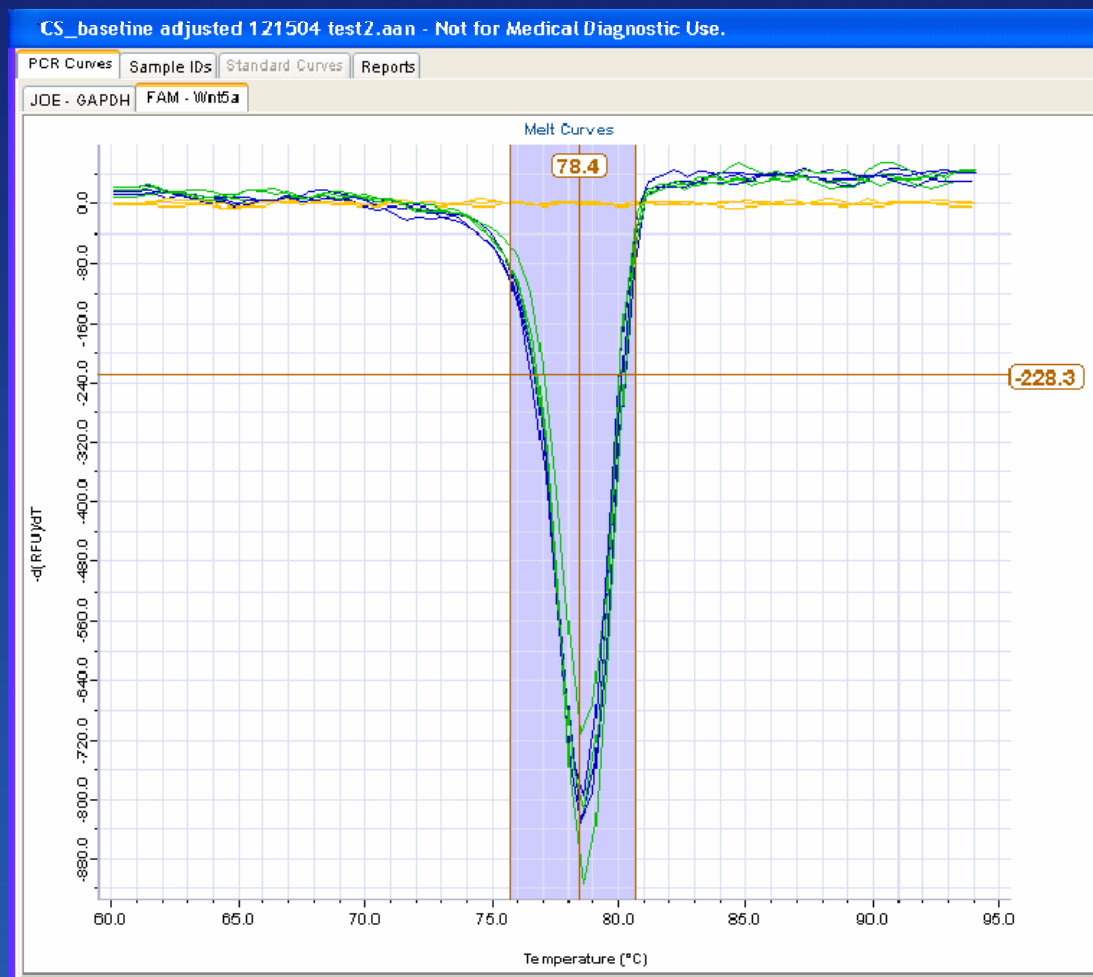
- You get the same data as gain of fluorescence methods



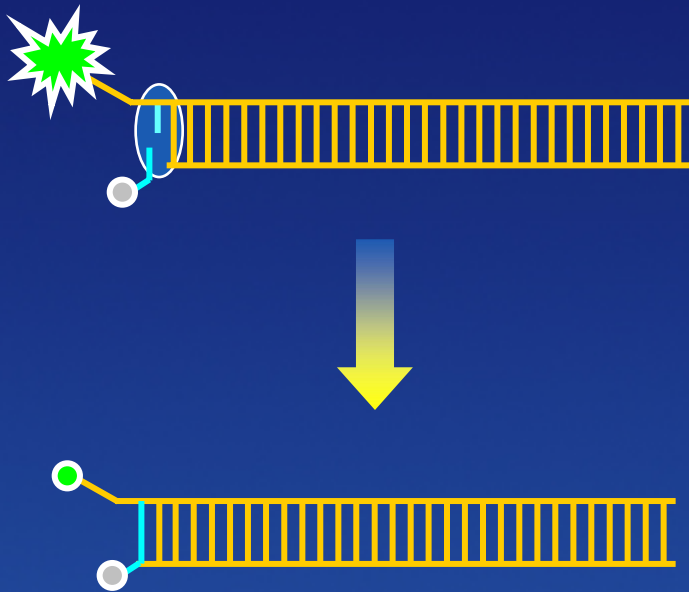
Sample ID	Ct	Tm	Conc
1	27.2	78.3	1.2E02
2	27.6	78.7	1.2E02
3	36.1	80.1	3.5E-01
13	29.5	78.7	2.5E01
14	29.7	78.5	2.5E01
15	32.8	80.5	3.2E00
25	31.9	79.2	5.0E00
26	32.1	79.2	5.0E00
27	35.3	80.5	6.0E-01
37	34.6	79.2	1.0E00
38	34.5	79.2	1.0E00
39	32.9	80.5	3.0E00
49	36.9	79.0	2.0E-01
50	37.3	80.2	2.0E-01
61	38.3	79.5	6.7E-02
62	38.8	79.3	6.7E-02
73	40.9	80.2	2.2E-02
74	39.4	80.2	2.2E-02

Melt Curves

- Confirmation of specificity



Dabcyl-iso-dGTP quenches by proximity



- Interaction governed by iso-dC in primer
- Dabcyl-iso-dGTP incorporated opposite any iso-dC

Dabcyl iso-dGTP makes multiplexing possible!

Can Dabcyl Quench Red Shifted Dyes?

Dye	% Quenching	Emission Peak
FAM	62%	518nm
TET	53%	536nm
HEX	71%	554nm
ROX	64%	606nm
LC640	48%	640nm
Cy5	35%	667nm

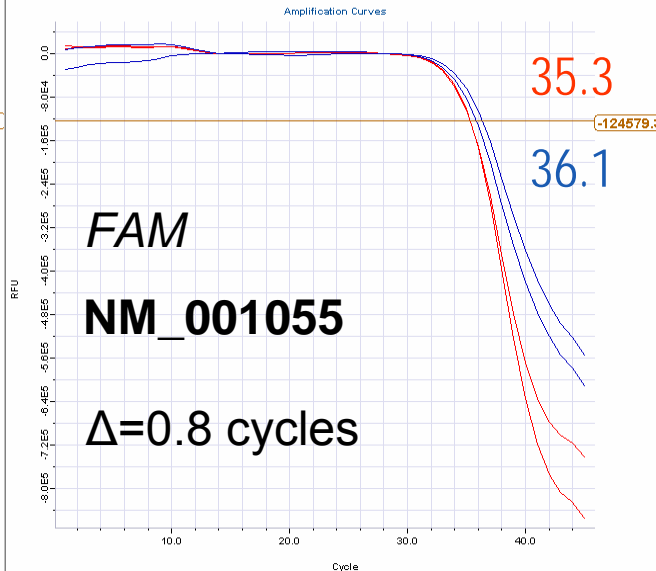
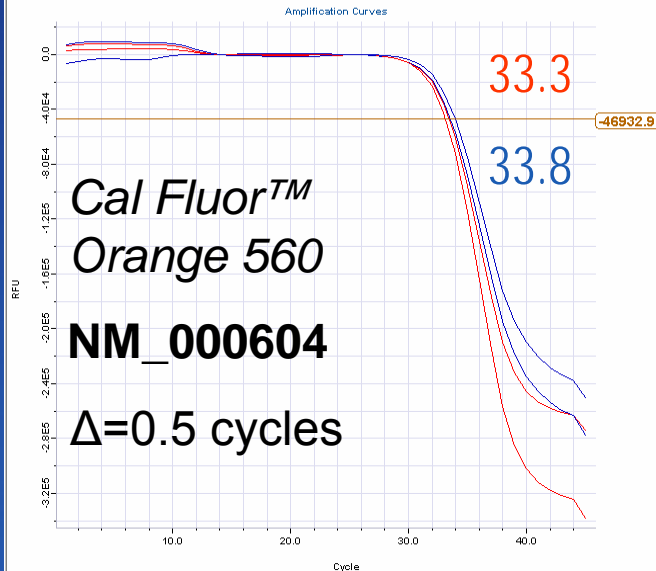
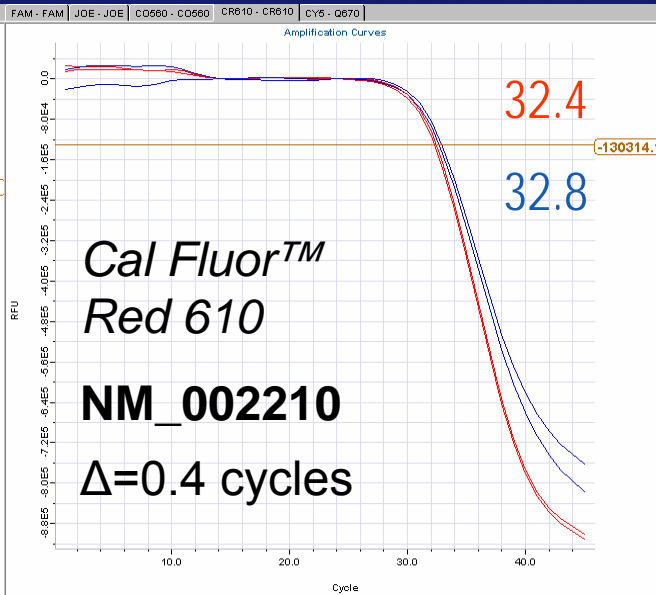
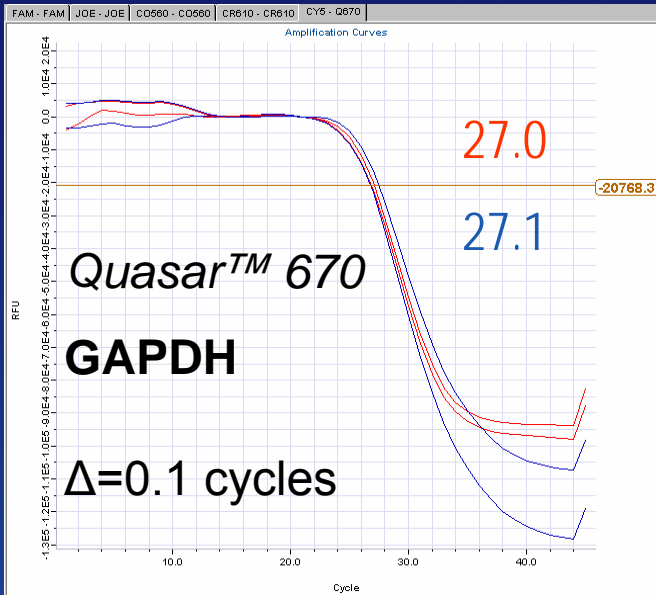
Table 1 in: Sherrill, C.B., *et al.* (2004) *J. Amer. Chem. Soc.* 126, 4550-6.

4-Color Multiplexing

Experimental details

- Four primer sets
- Biosearch Technologies dye sets
- All targets are human cDNA
 - Plexor™ Two-Step qRT-PCR System
- Performed on the ABI® 7500

2-Step qRT-PCR — 4-Color Multiplexing



Red = Monoplex
Blue = 4-plex

ABI® 7500

The background of the slide features a wooden canoe in the foreground, viewed from the front. The canoe is light brown with a woven mat in the center. Behind it is a large, vibrant green leaf with prominent veins, set against a blue sky with soft, colorful clouds. A small butterfly is visible on the left side of the image.

The Plexor™ Reagents

qPCR

Two-Step qRT-PCR

One-Step qRT-PCR



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Plexor™ Systems

- **qPCR System**

- For real-time quantitation from genomic DNA, SNP genotyping
- For 2-step qRT-PCR methods with your cDNA

- **2-step qRT-PCR System**

- ImProm-II™ RT Reagents for cDNA synthesis
- Plexor Master Mix for qPCR from cDNA template

- **1-step qRT-PCR System**

- Combines ImProm-II™ RT with Plexor Master Mix for qRT-PCR directly from RNA template

Plexor™ Reactions

- 25µl standard reaction
- 2X Plexor Master Mix contains:
 - Enzyme (no 5' exo activity)
 - High performance buffer
 - Proprietary primer-dimer inhibitor
 - dATP, dCTP, dGTP, dTTP and Dabcyl-iso-dGTP

For amplification of the
DNA sequence of
interest

For incorporation
opposite the iso-dC in
the Plexor Primers

What you need...

- **Plexor™ Primer Design Software**
 - Engineered for multiplex assays
 - Free web access
- **Plexor™ Primer Sets**
 - BioSearch Technology
 - Eurogentec
 - Intergrated DNA Technologies (IDT)
- **Real-Time Instrument**
 - Existing instruments capable of detecting more than one fluor
 - ABI, Roche, etc.
- **Plexor™ Analysis Software**
 - Analyzes quenching data from a variety of instruments
 - Free download

A wooden canoe is shown from a low-angle perspective, looking down its length towards the bow. The canoe's interior is made of light-colored wood with a central longitudinal rib and several cross-ribs. A woven mat is placed on one of the cross-ribs. The background is a composite image: the top half is a large, vibrant green leaf with prominent veins, and the bottom half is a blue sky with soft, colorful clouds. A small butterfly is visible in the upper left corner of the sky area.

Applications of Plexor™ Technology

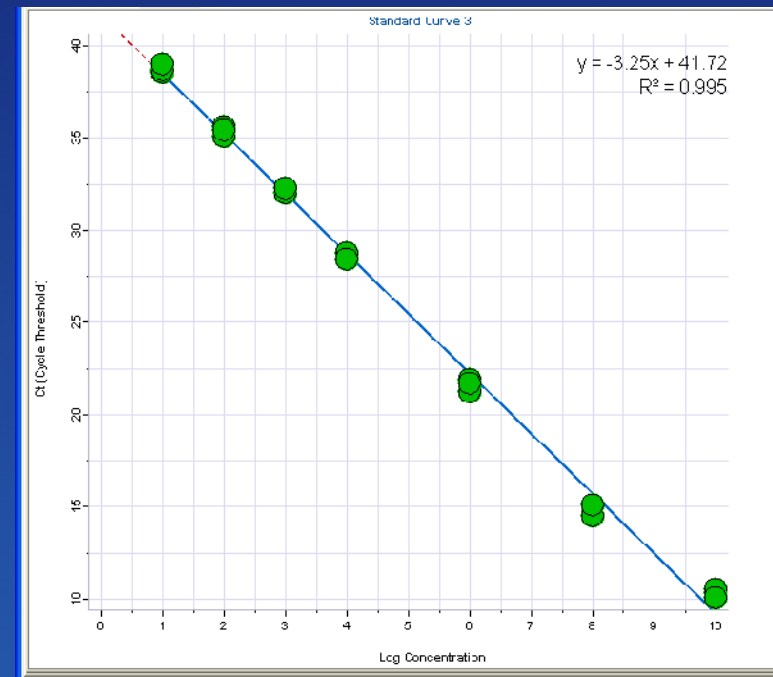
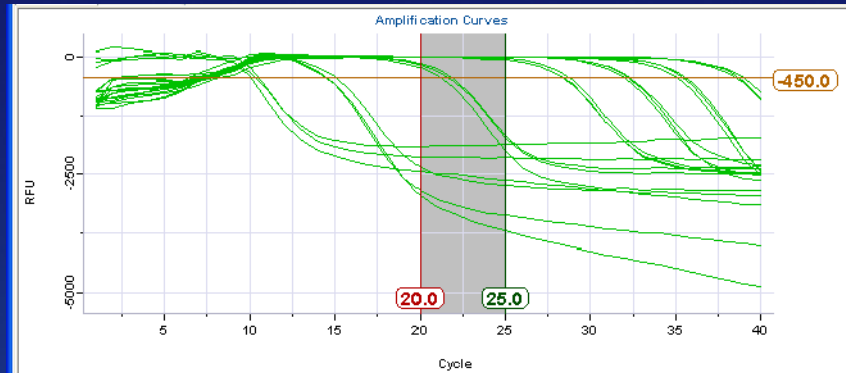
Gene Expression Analysis



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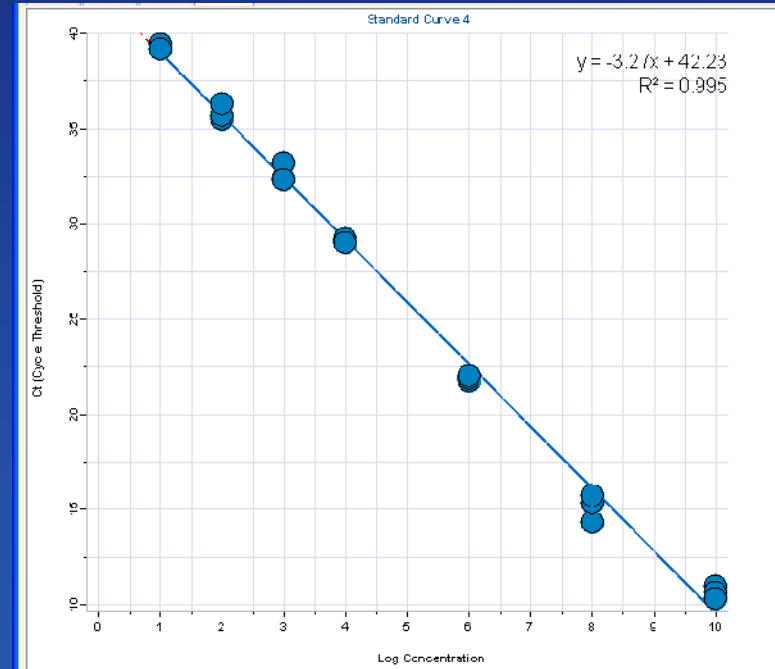
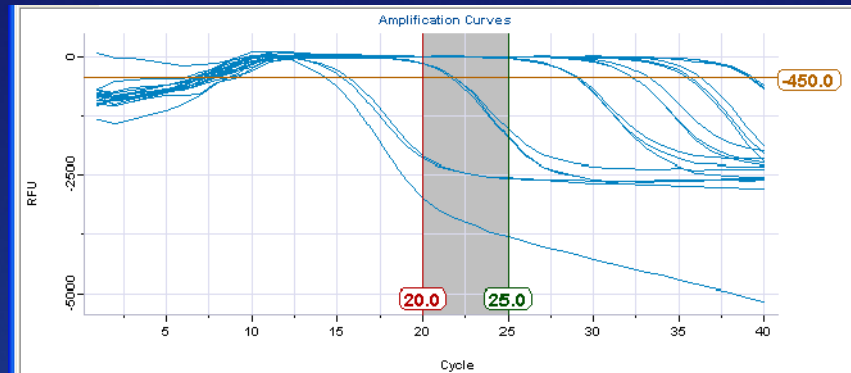
Large Dynamic Range...Monoplex Reactions

- Detection of Kanamycin 1.6kb synthetic transcript RNA (93 bp amplicon)
 - Dilutions in background of 10ng human total RNA
 - Kanamycin (FAM) primers
- **Dynamic Range:**
 10^1 to 10^{10} copies



Large Dynamic Range...Duplex Reactions

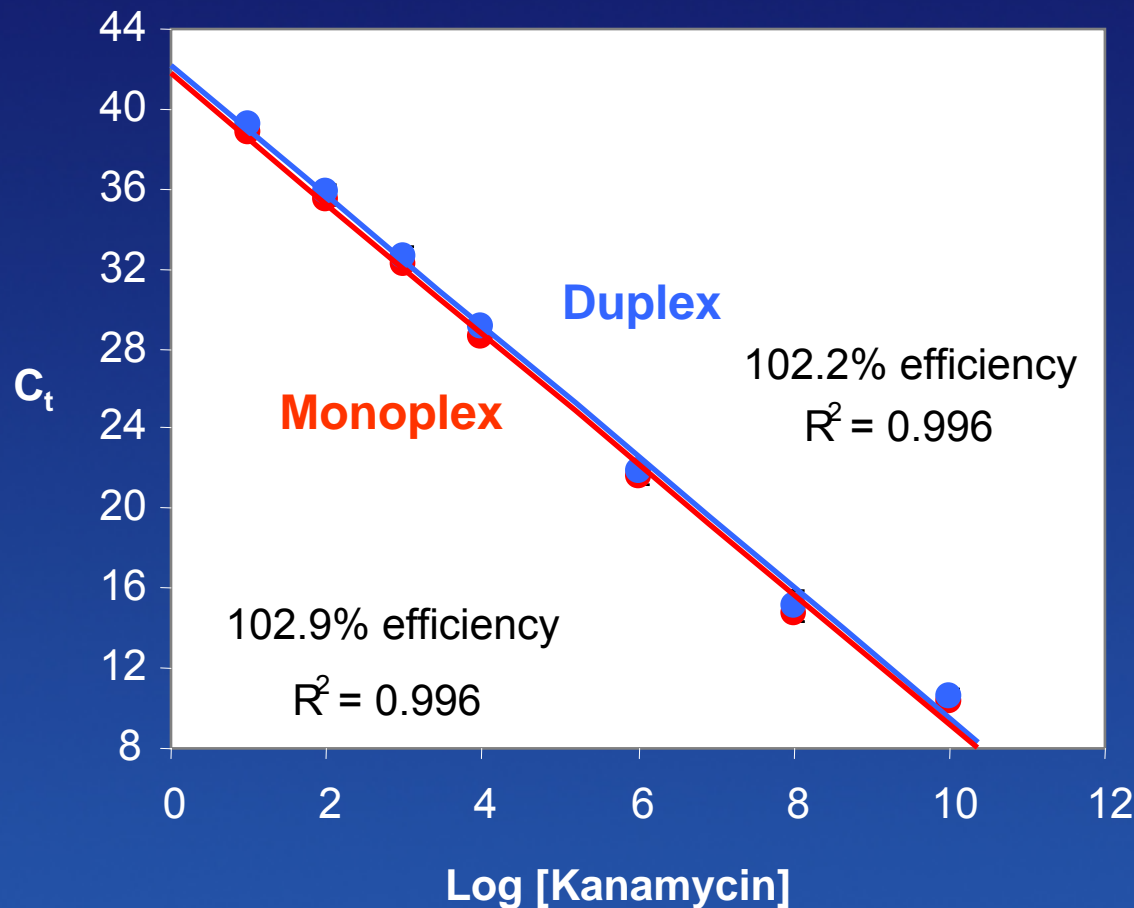
- Detection of Kanamycin 1.6kb synthetic transcript RNA
 - Dilutions in background of 10ng human total RNA
 - Kanamycin (FAM) primers
 - GAPDH (JOE) primers
- **Dynamic Range:**
 10^1 to 10^{10} copies



Plexor™ One-Step qRT-PCR System



Consistent C_t Values in Monoplex & Duplex



- Detection of synthetic target in background of 10ng human total RNA
- Dynamic range from 10^1 to 10^{10}
- C_t Values consistent in monoplex and duplex reactions

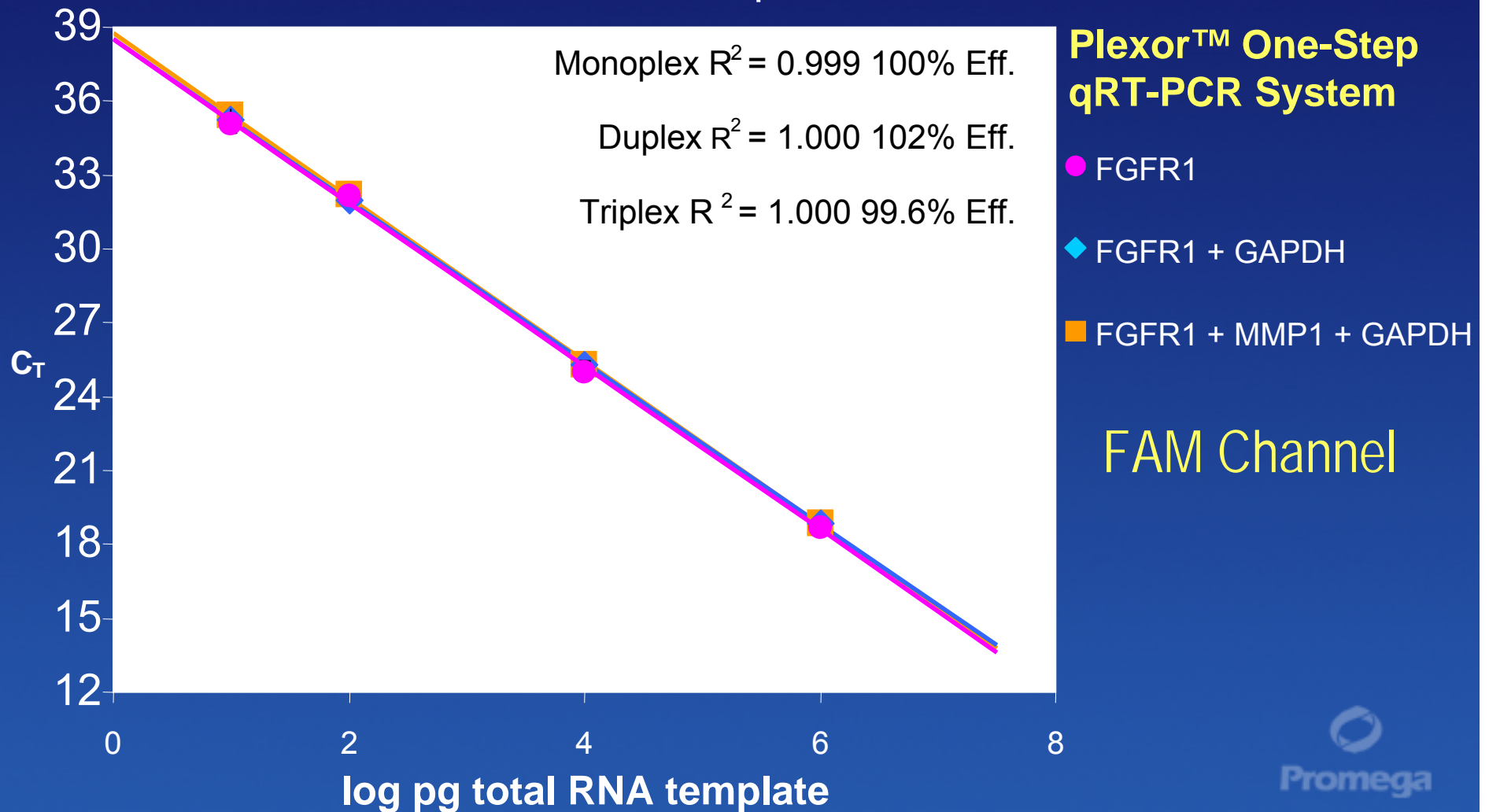
Quantitation in Multiplex

Experimental Details

- Three primer sets designed to multiplex together with Plexor™ Primer Design Software
- Biosearch Technologies dye sets
- All targets are human total RNA
 - Plexor™ One-Step qRT-PCR System
- Performed on the ABI® 7500

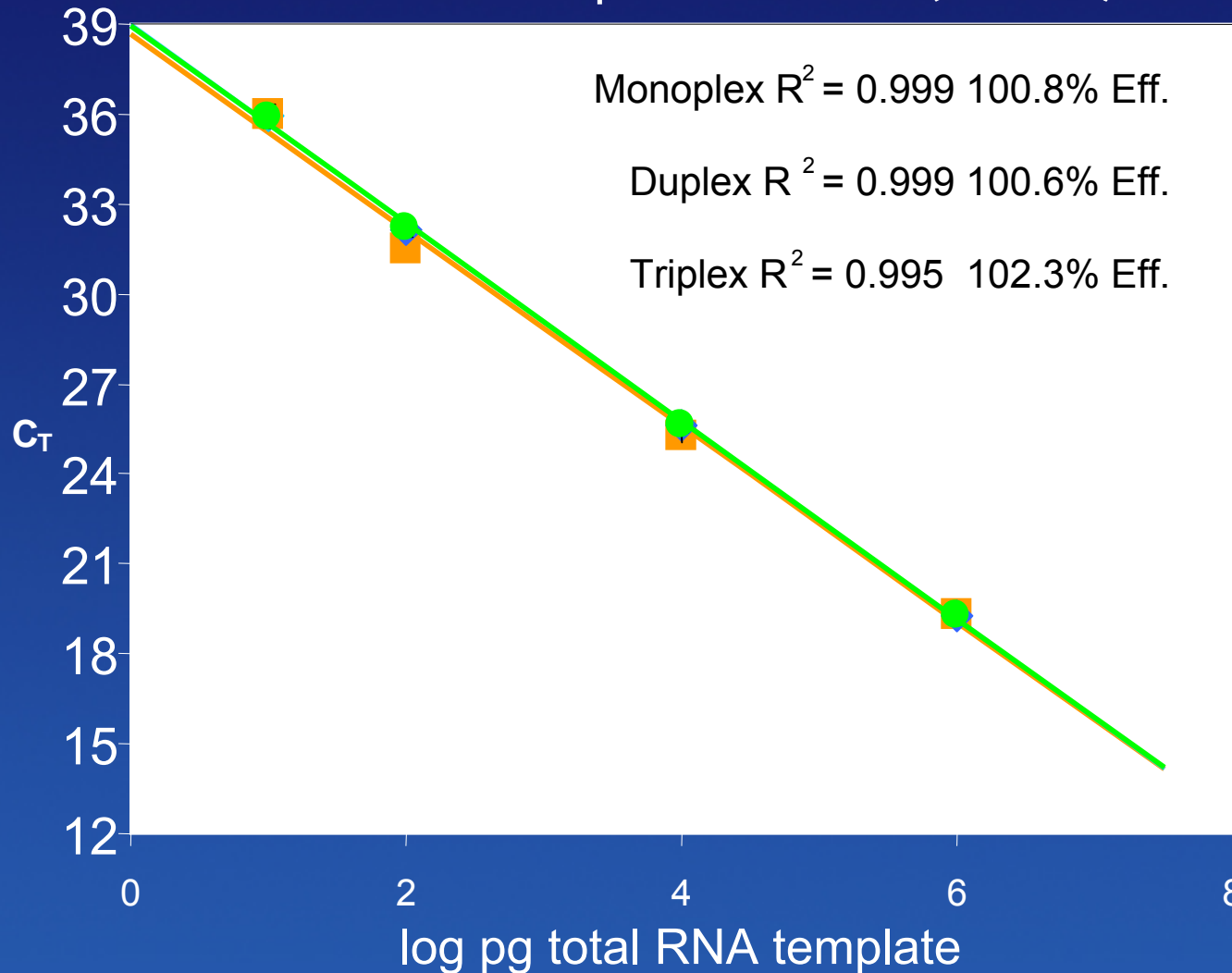
Quantitation in Multiplex

Fibroblast Growth Factor Receptor 1 (FGFR1)



Quantitation in Multiplex

Matrix Metalloproteinase 1 (MMP1)



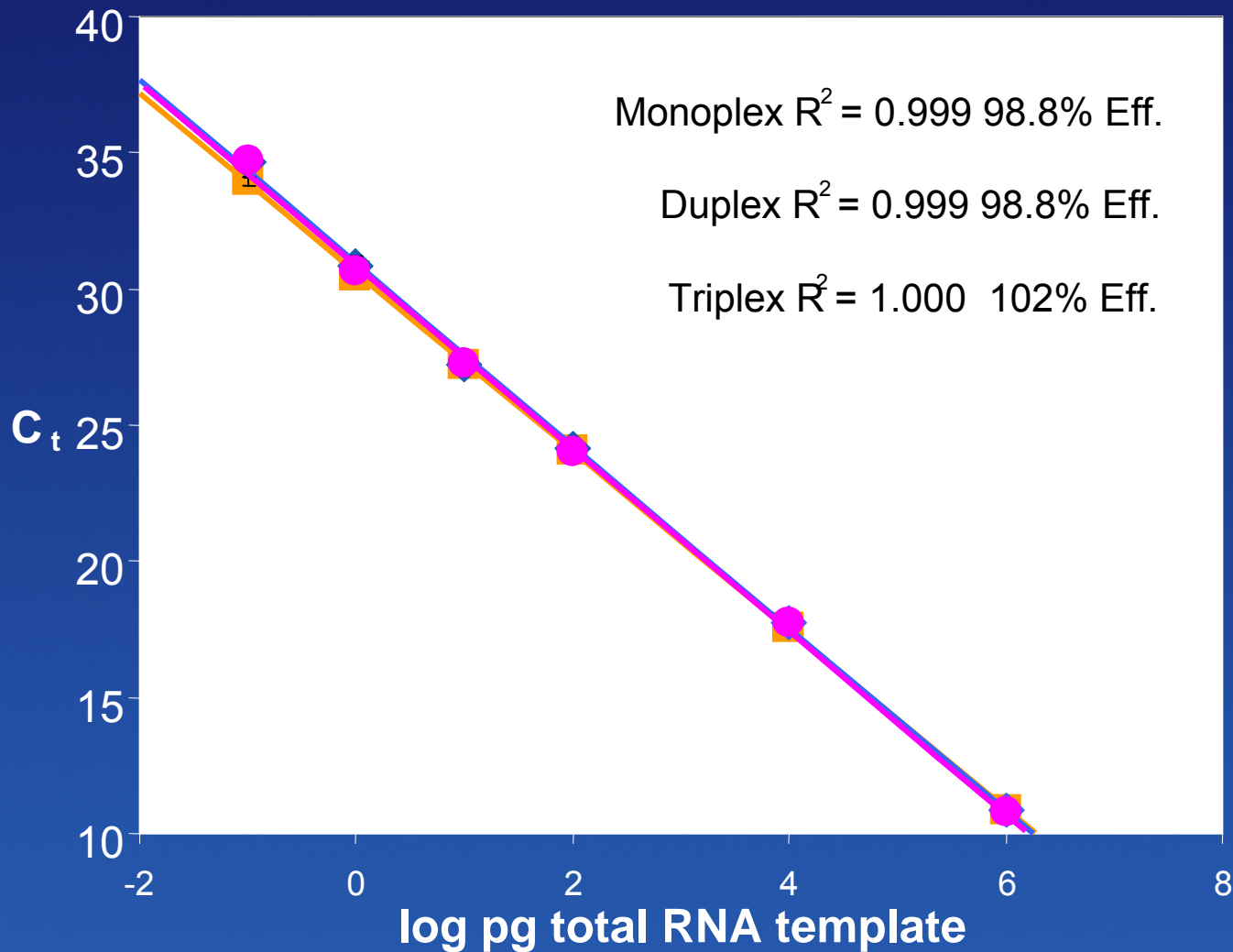
Plexor™ One-Step qRT-PCR System

- MMP1
- ◆ MMP1 + GAPDH
- MMP1 + FGFR1 + GAPDH

CalFluor™
Red 610
Channel

Quantitation in Multiplex

GAPDH



Plexor™ One-Step qRT-PCR System

- GAPDH
- ◆ GAPDH + FGFR1
- GAPDH + FGFR1 + MMP1

JOE
Channel



Applications of Plexor™ Technology

SNP Genotyping

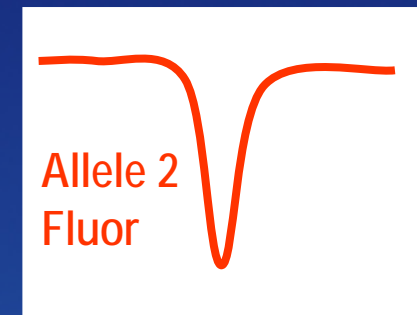
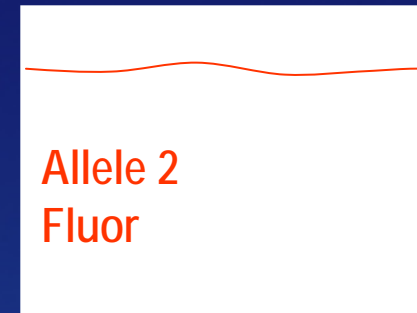
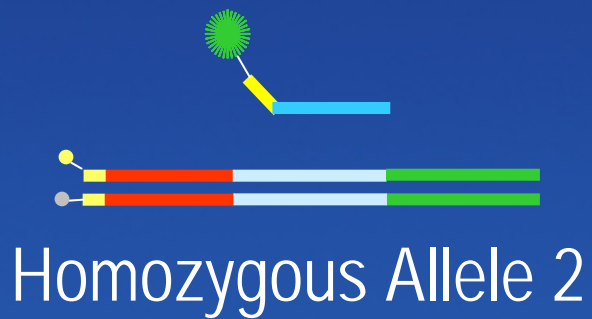


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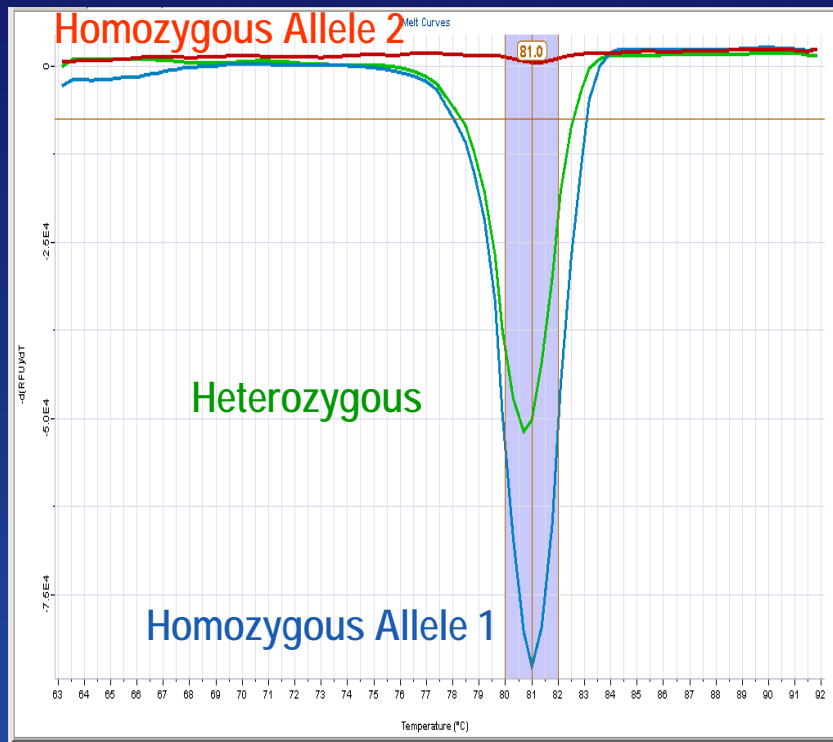
SNP Genotyping with Plexor™ Technology

- Based on 3 primer allele specific PCR
- Each allele specific primer labeled with a different fluor
- Genotype determination is by endpoint melt curve analysis

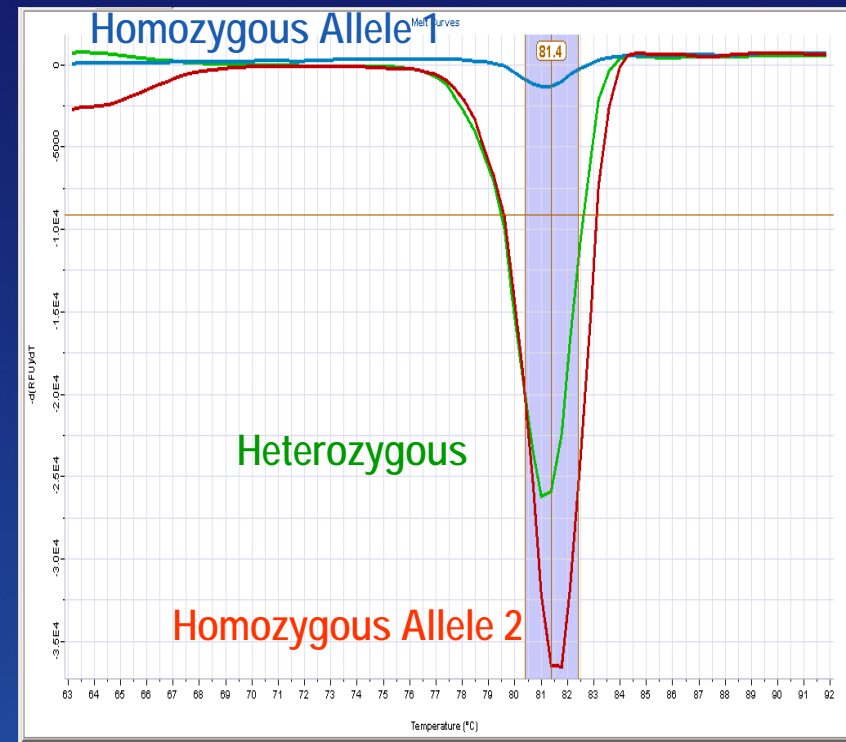
Genotyping by Melt Curve Analysis



Genotyping- Hemochromatosis HFE H63D



Allele 1 Primer
FAM Channel



Allele 2 Primer
CalFluor™ Orange 560
Channel

Summary

- Two primer method allows easy multiplexing
- High correlation of C_t values between monoplex and multiplex (less than 1 C_t change)
- Accurate quantitation of lower copy messages in multiplex with high copy messages
- Three Primer method for closed tube genotyping (thermal melt)

Acknowledgements

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EraGen Biosciences

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Jim Prudent
Mike Moser

More Questions?

For more information,
please contact:
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Legal disclaimer

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*The above primary European Pat. Nos. 201,184 and 200,362 will expire on March 28, 2006. In the U.S., the patents covering the foundational PCR process expired on March 29, 2005.