

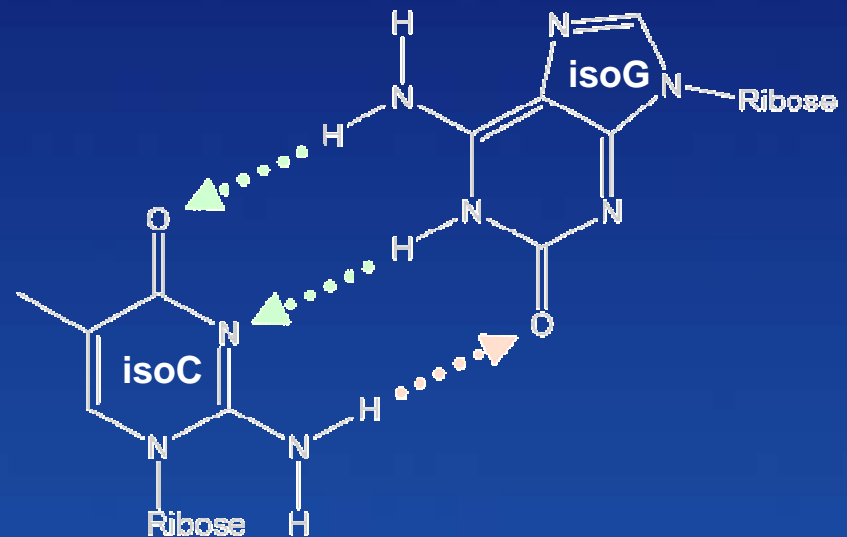
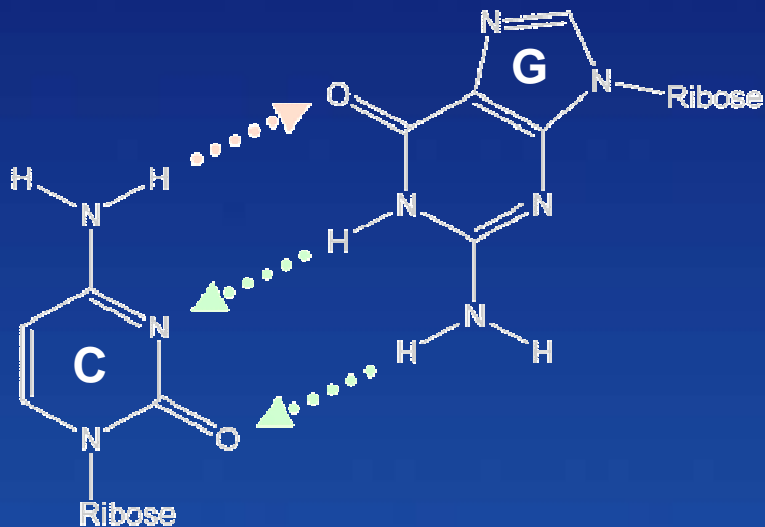
# Quantitative, multiplexed amplification with the Plexor™ qPCR Systems

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Douglas R. Storts, Ph.D.  
Director – R&D

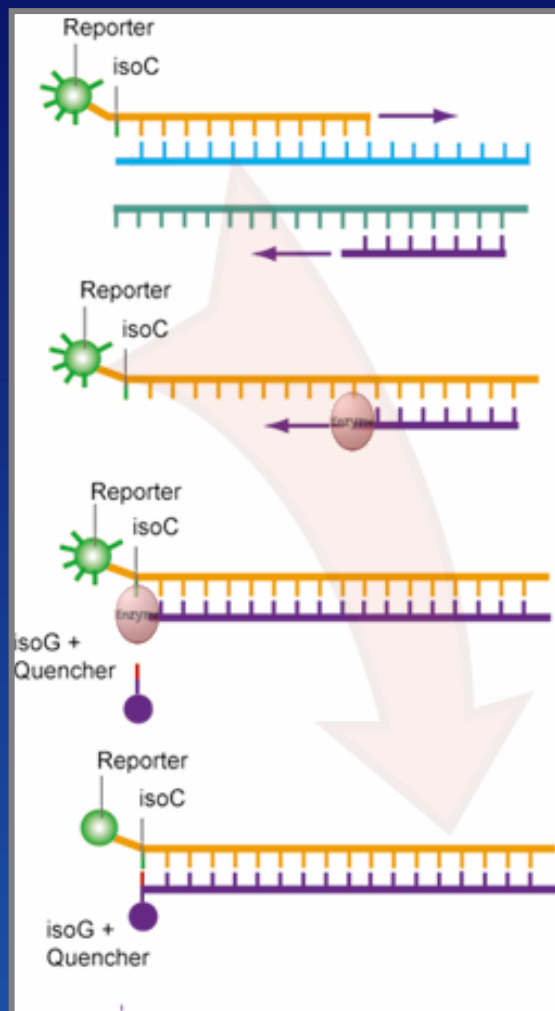
# Novel Base-Pairing

- IsoC & IsoG dNTPs
  - Recognized by DNA Polymerase
  - Do not base pair with ACGTU
  - Licensed from EraGen Biosciences



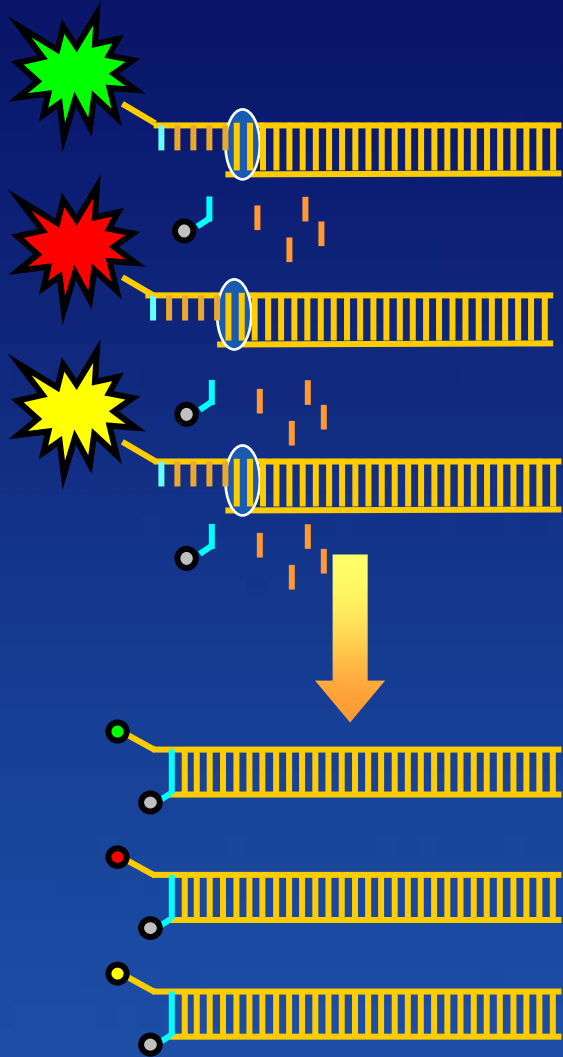
Johnson, S.C., et al. (2004) *Nucleic Acids Res.* **32**, 1937-41.

# Use of IsoG and IsoC in Real-Time PCR



- Two primer method
  - One standard primer
  - One primer modified with iso-dC base and a fluorophore at 5' end
- Amplification master mix contains
  - Standard dNTPs
  - Dabcyl -labeled iso-dGTP

# Dabcyl-iso-dGTP Contact Quenching



- Primers targeting different genes are labeled with iso-dC and different fluorophores
- Dabcyl-iso-dGTP incorporates opposite any iso-dC, resulting in contact quenching

# Can Dabcyl Quench Red-Shifted Dyes?

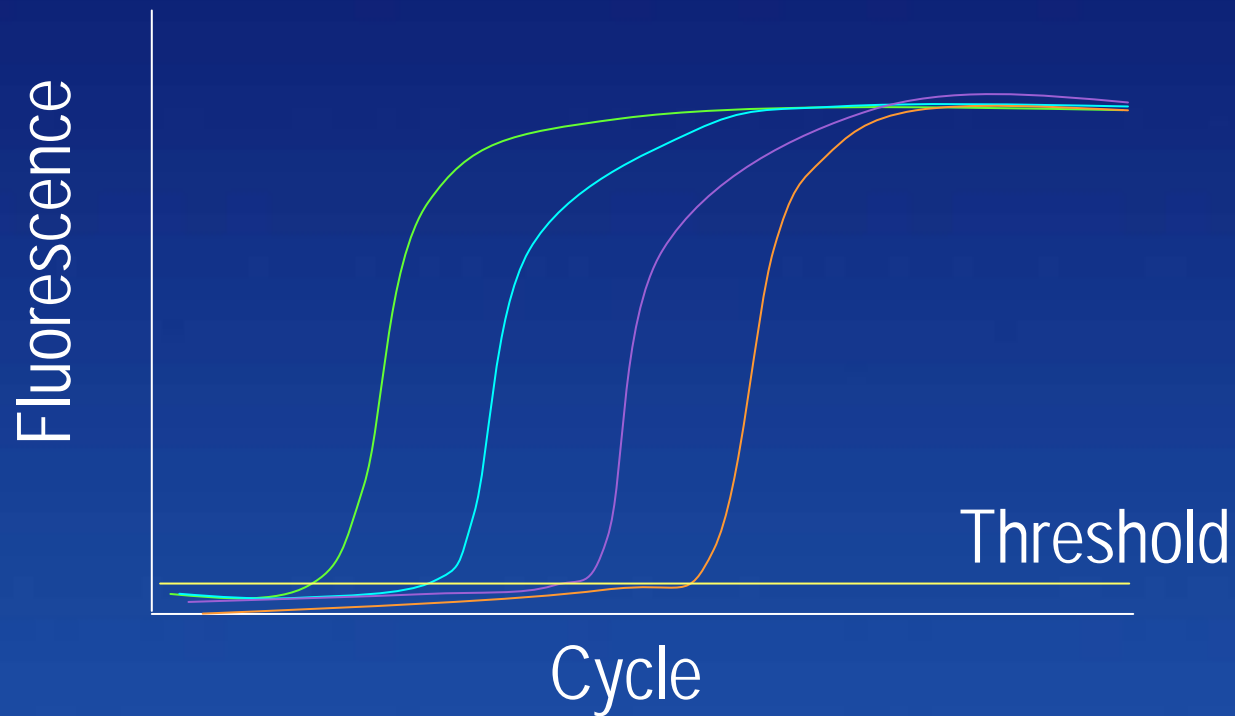
Dye	% Quenching	Emission Peak
FAM	62%	518nm
TET	53%	536nm
HEX	71%	554nm
ROX	64%	606nm
LC <sup>TM</sup> 640	48%	640nm
Cy <sup>TM</sup> 5	35%	667nm

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

# Typical Amplification Curves

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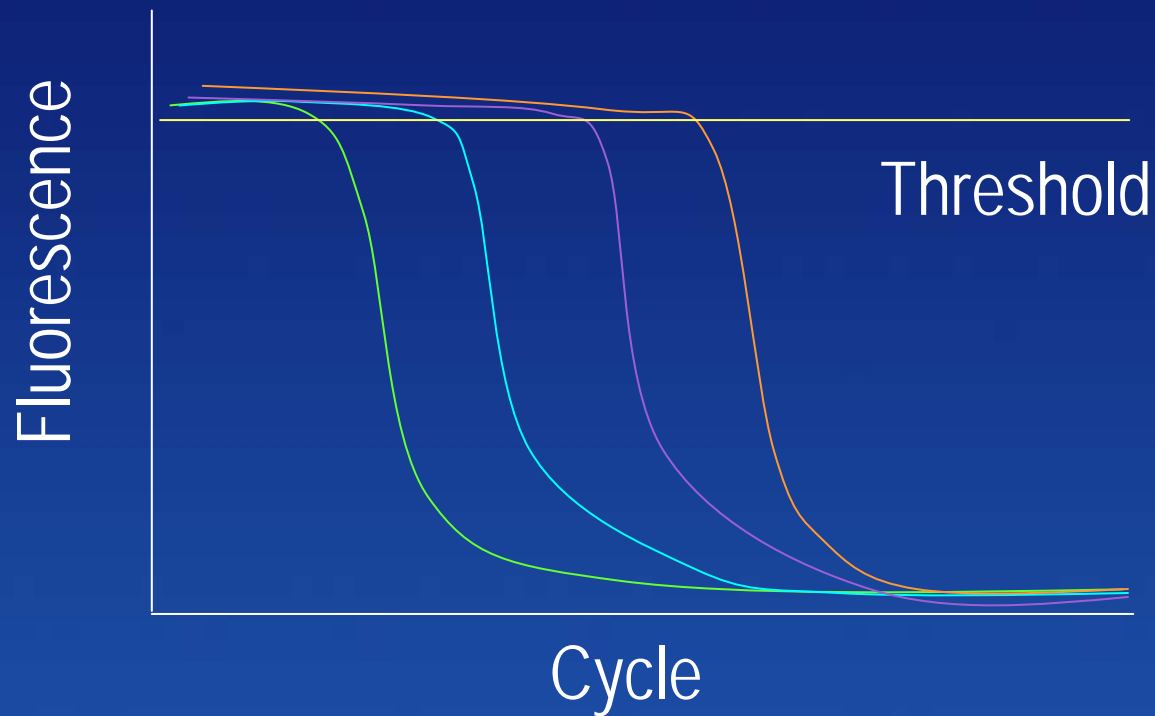
- Gain of fluorescence



# Plexor™ Amplification Curves

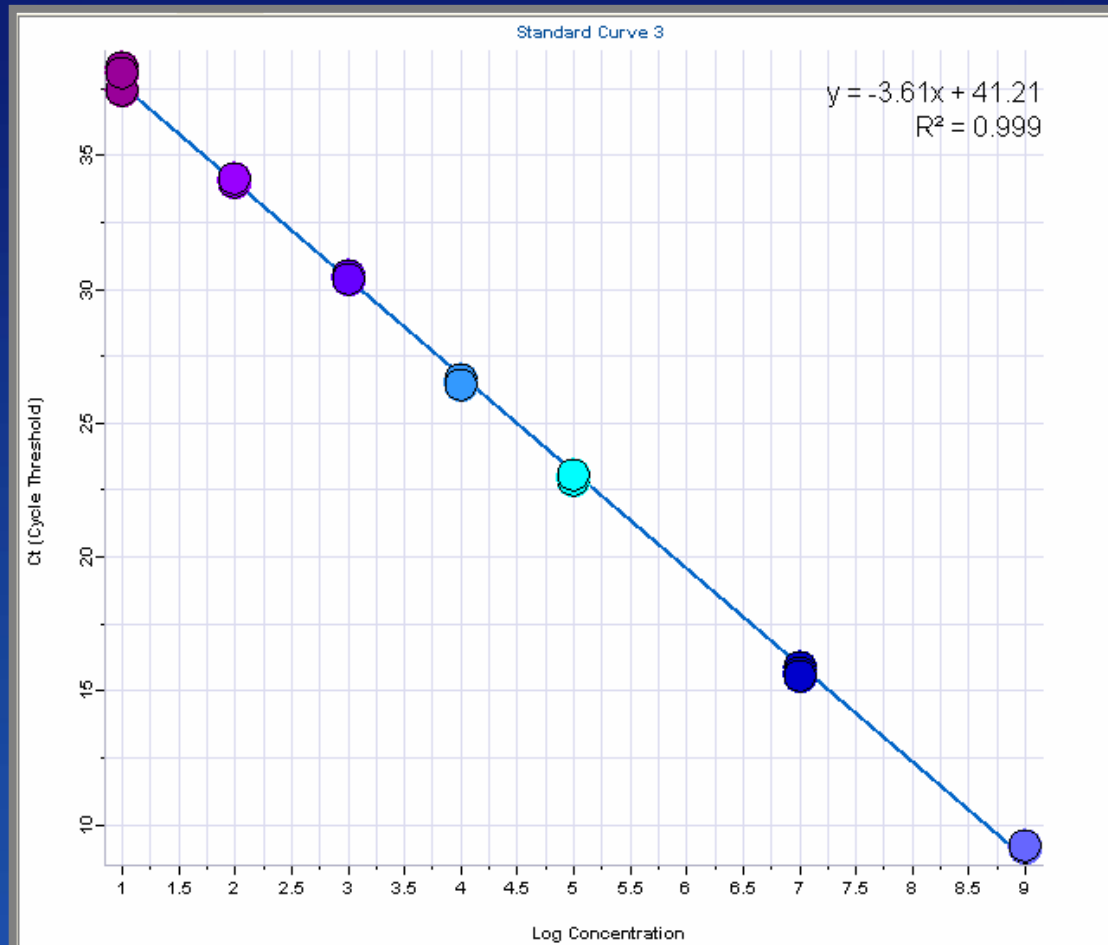
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- Quenching of fluorescence



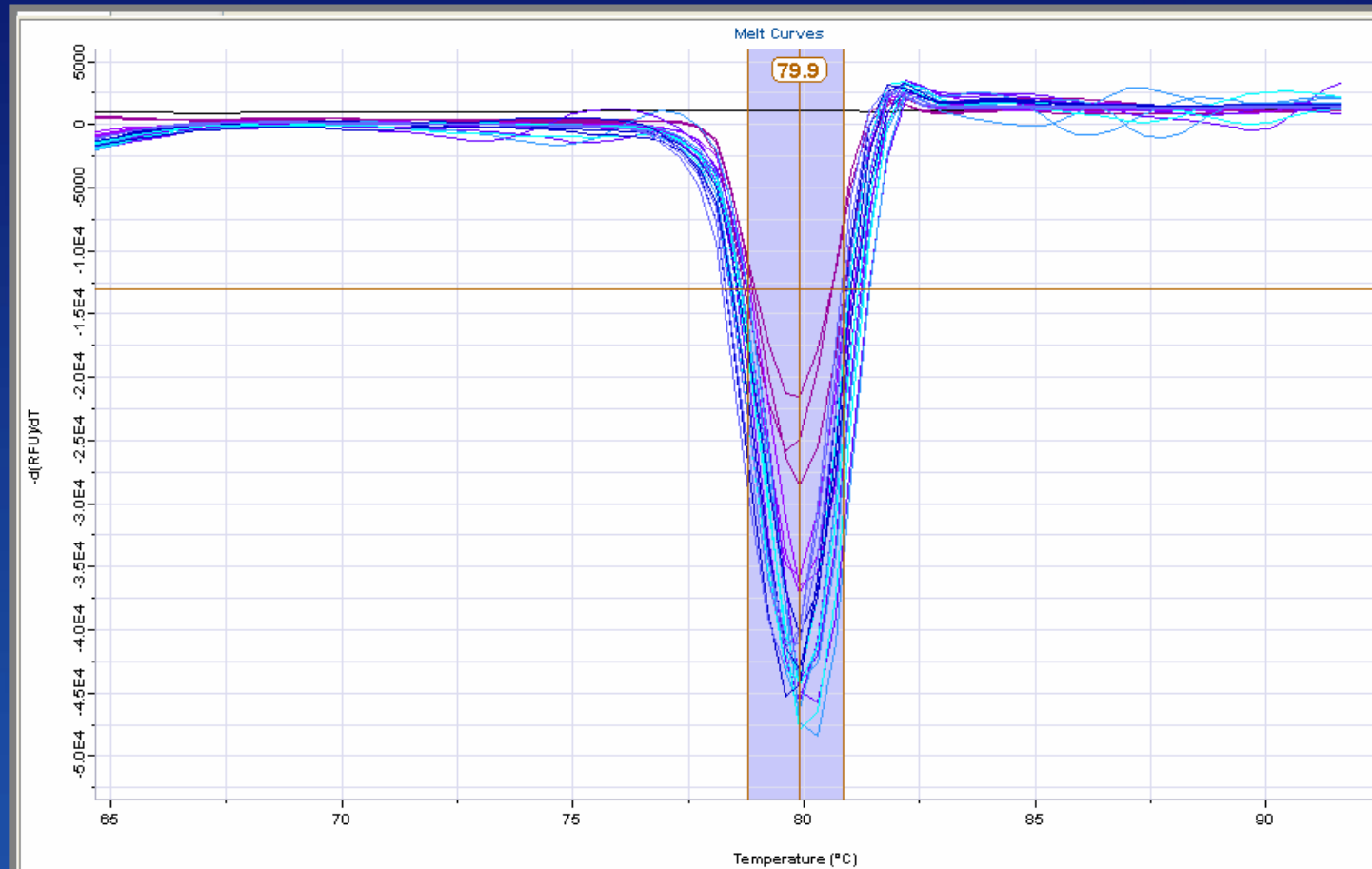
# Plexor™ Method...Same Data

- $C_t$  vs. concentration



# Plexor™ Method...Includes Melt Curves

- Confirmation of specificity





# Multiplexing Applications

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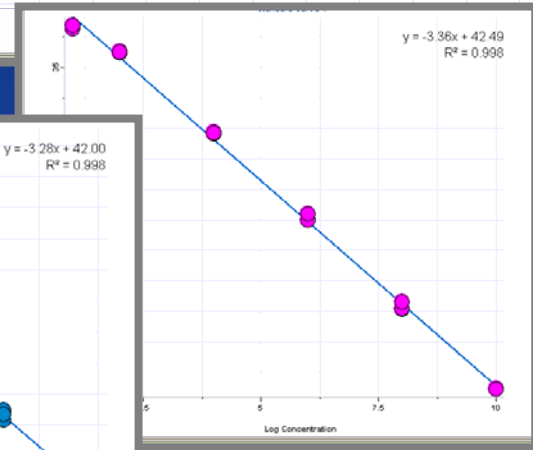
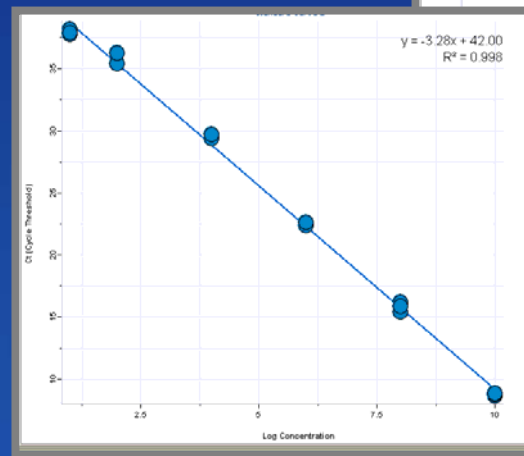
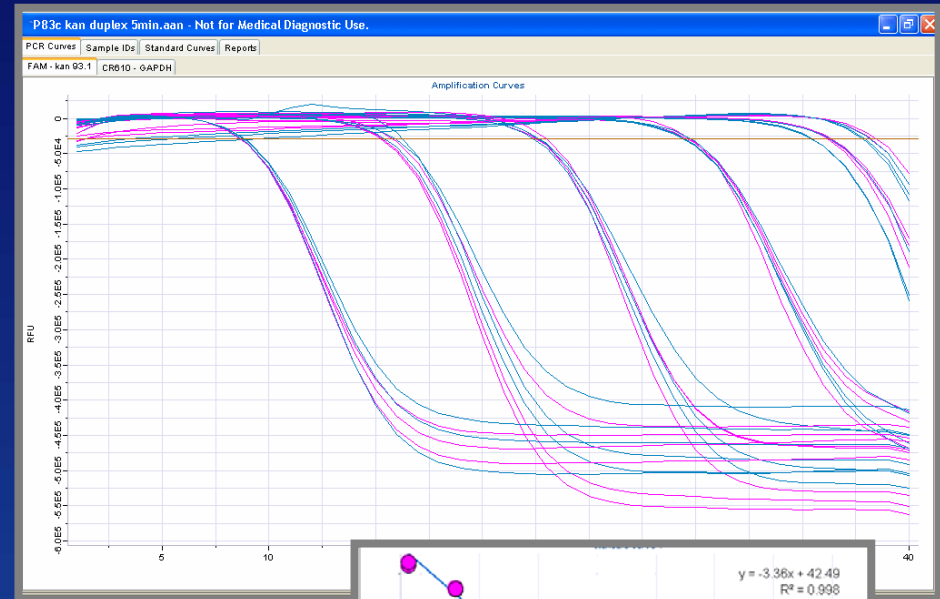
Gene Expression Analysis

# Large Dynamic Range

- Detection of various concentrations of a synthetic transcript in a constant background of 10ng human total RNA using one-step qRT-PCR

- Monoplex reaction
- Duplex reaction (also amplifying GAPDH)

Dynamic Range:  
 $10^1$  to  $10^{10}$  copies



# Quantitation in Multiplex

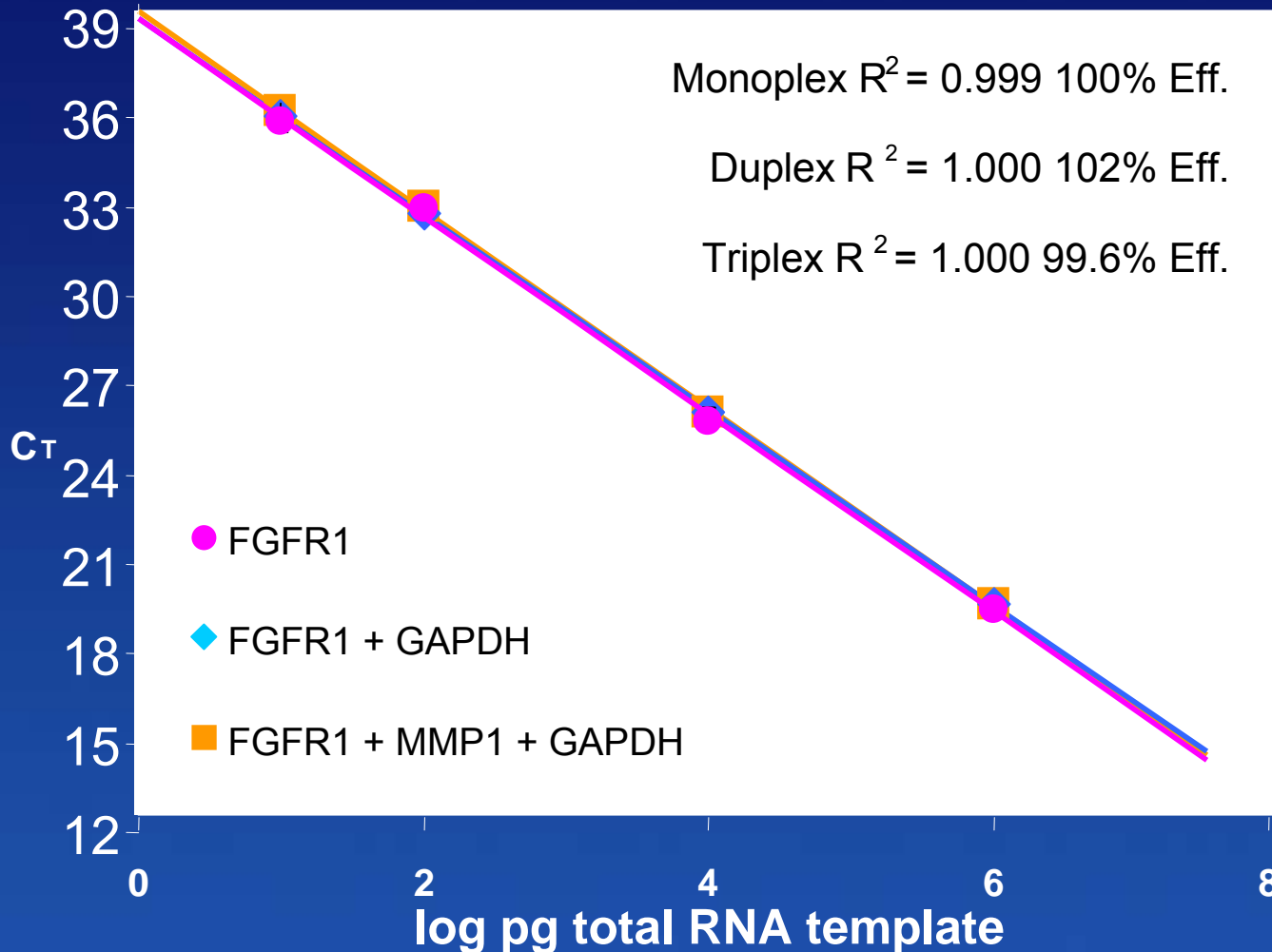
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## Experimental details

- 3 primer sets designed with Plexor™ Primer Design Software
- Biosearch Technologies dye sets
- All targets are human total RNA
- Plexor™ One-Step qRT-PCR System
- Thermal cycling performed on the Applied Biosystems 7500 Real Time PCR System

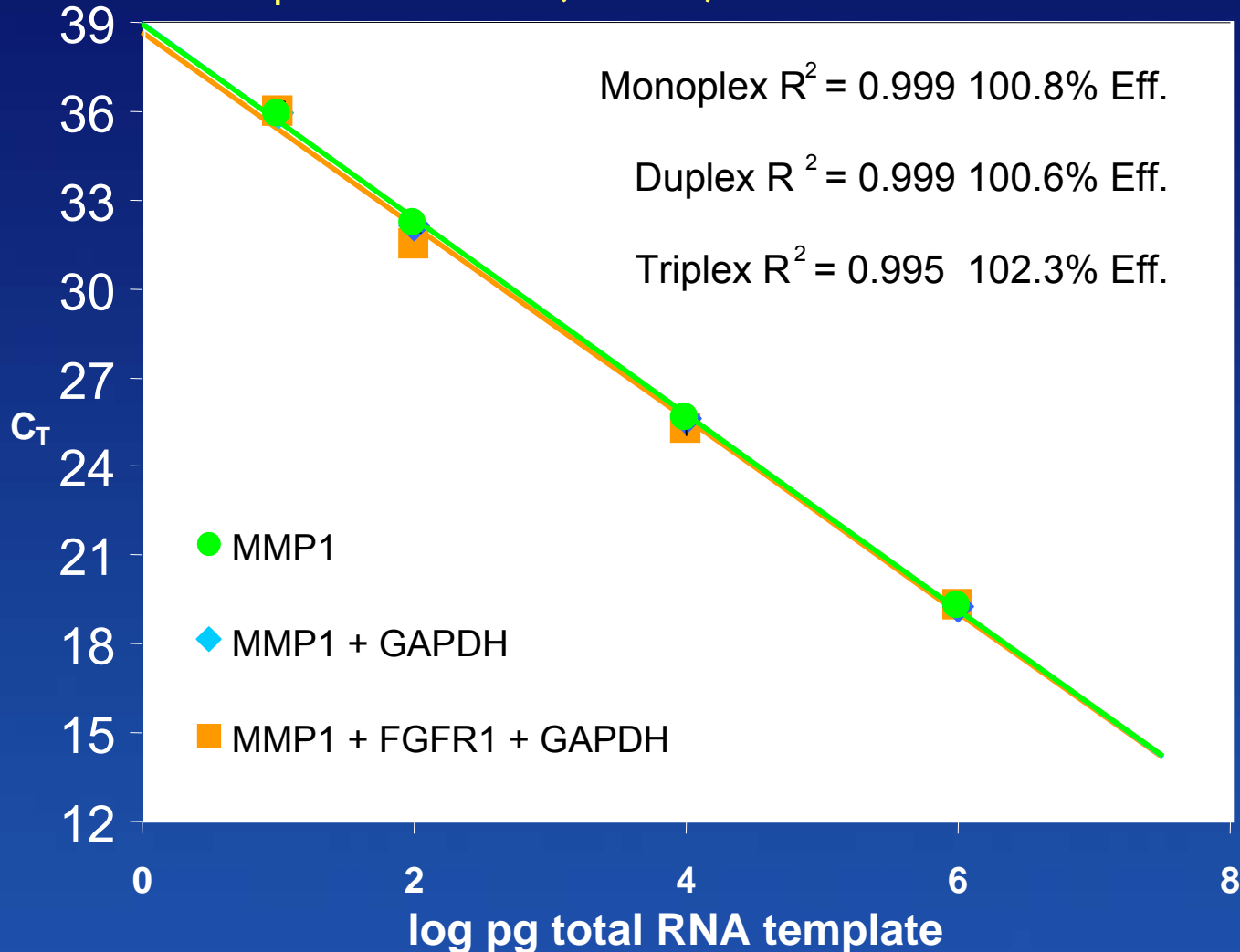
# Consistent Quantitation in Multiplex

## Fibroblast Growth Factor Receptor 1 (FGFR1) - FAM Channel



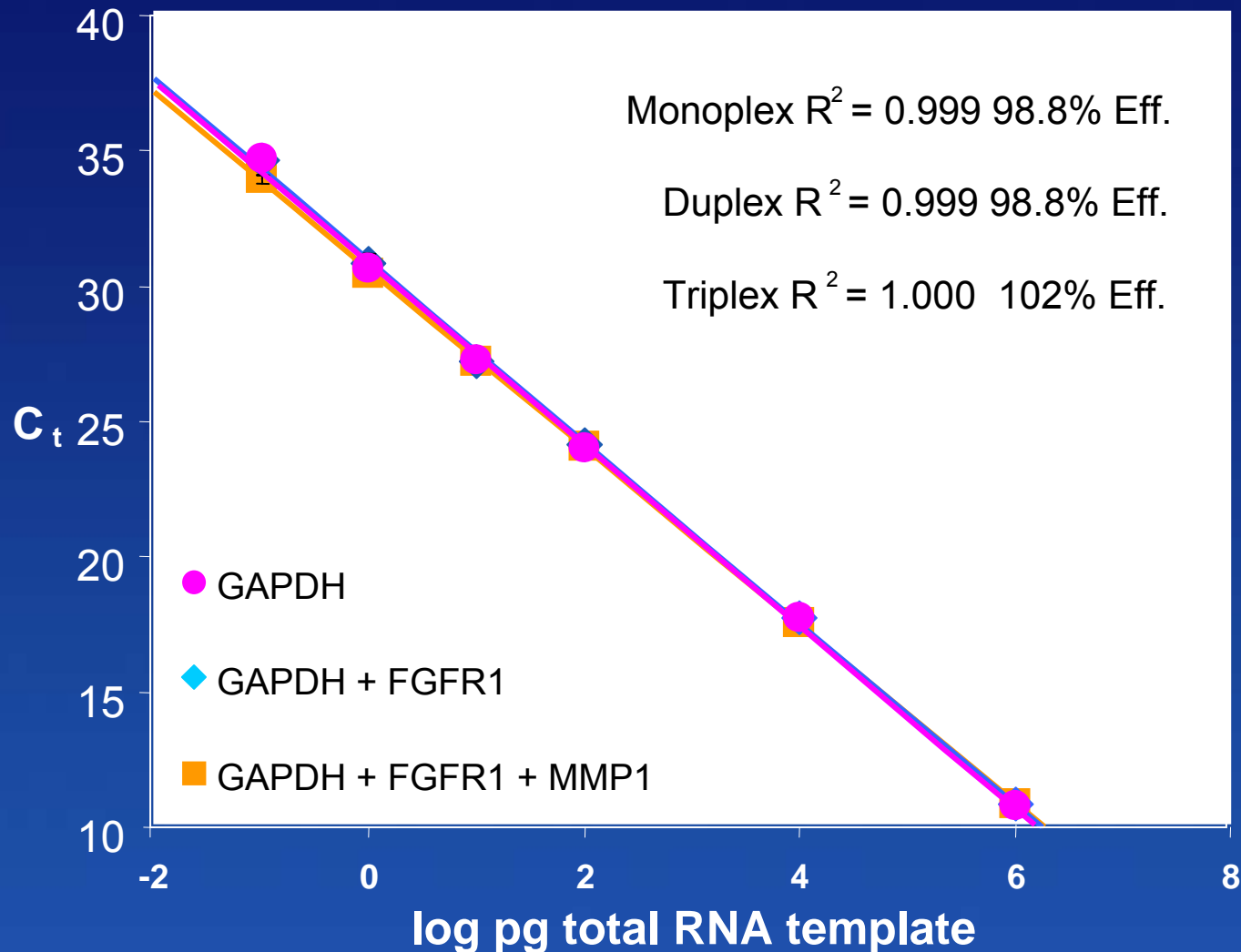
# Consistent Quantitation in Multiplex

## Matrix Metalloproteinase 1 (MMP1) - CalFluor™ Red 610 Channel



# Consistent Quantitation in Multiplex

## Glyceraldehyde-3-phosphotransferase (GAPDH) - JOE Channel



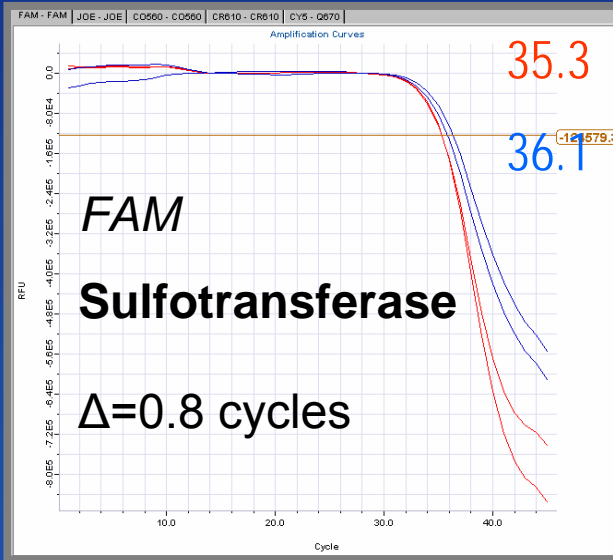
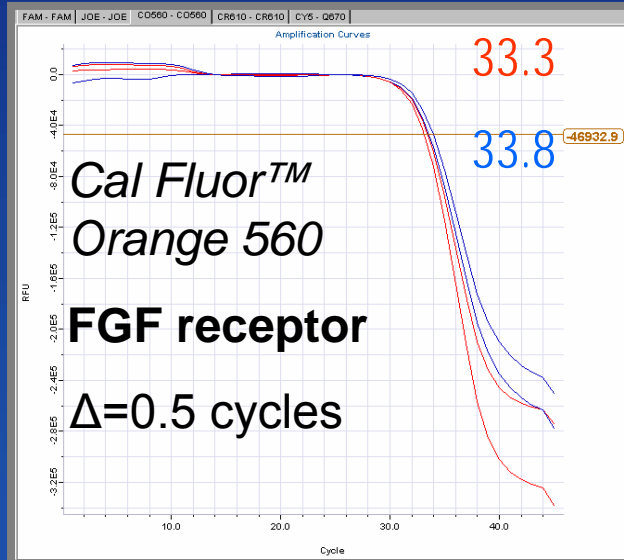
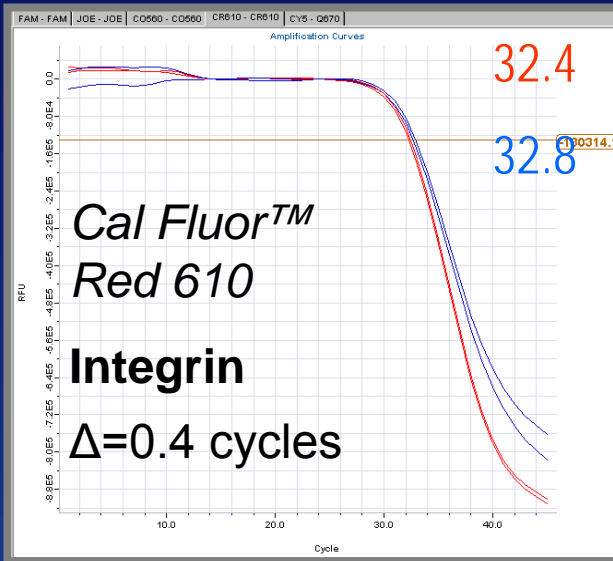
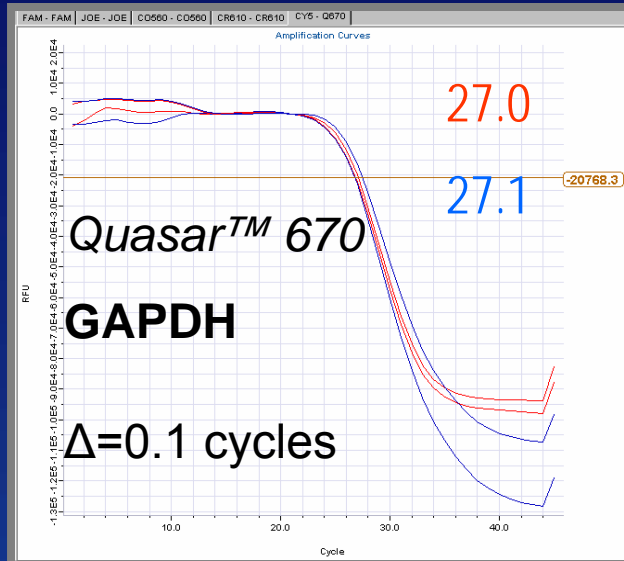
# 4-Color Multiplexing

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## Experimental Details

- 4 primer sets designed with Plexor™ Primer Design Software
- Biosearch Technologies dye sets
- All targets are human cDNA
- Plexor™ Two-Step qRT-PCR System
- Thermal cycling performed on the Applied Biosystems 7500 Real Time PCR System

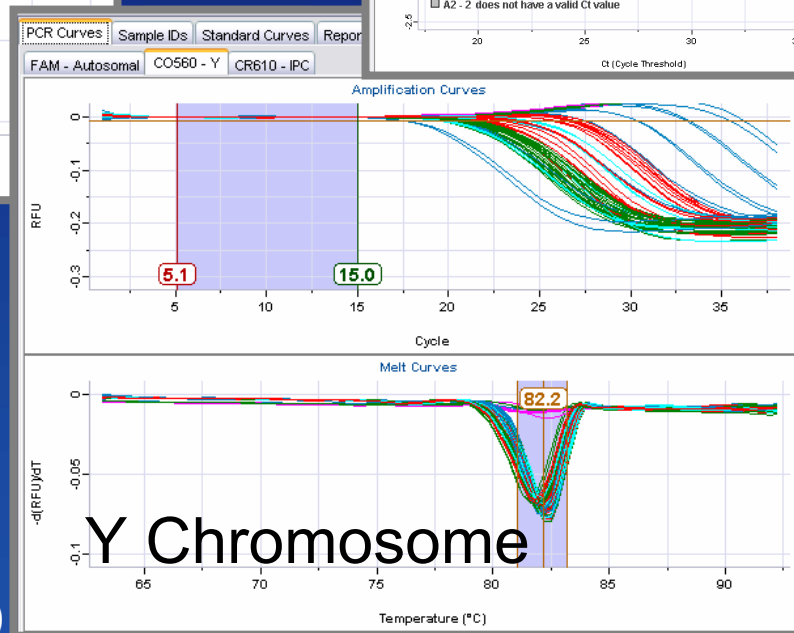
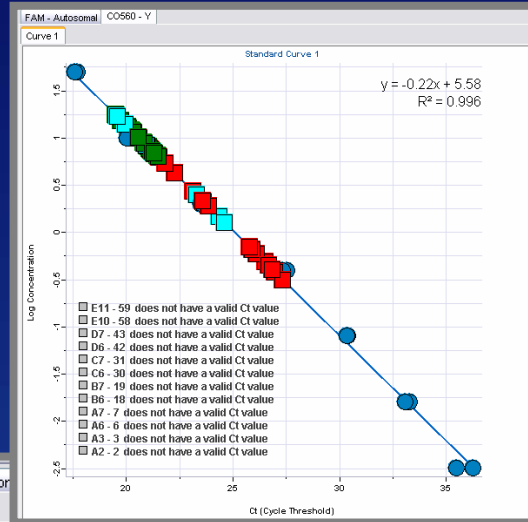
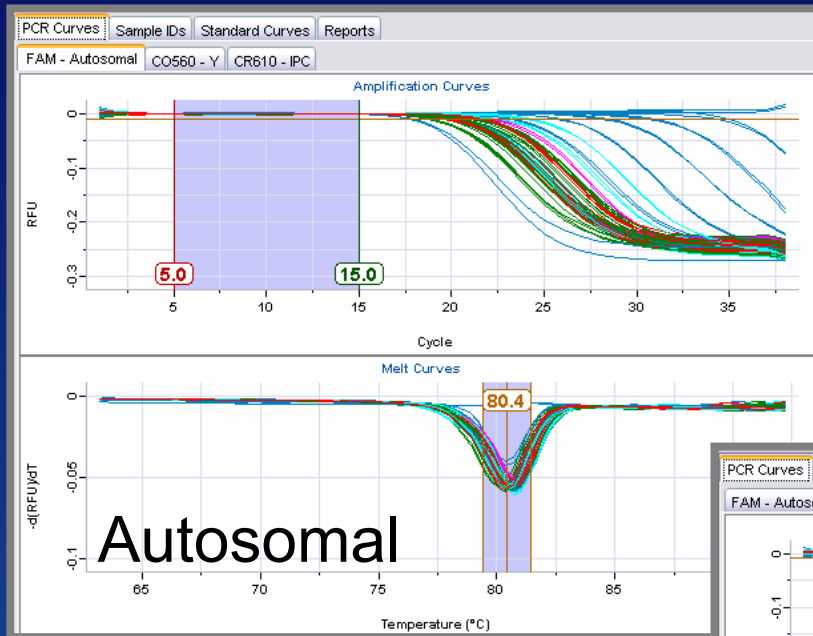
# Quantitate High- & Low-Copy Together



Red = Monoplex  
Blue = 4-plex

AB 7500

# Plexor™ HY Assay



Titration series  
 (3pg-50ng/μl)

Data provided by J. Butler (NIST)

# Summary

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- 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



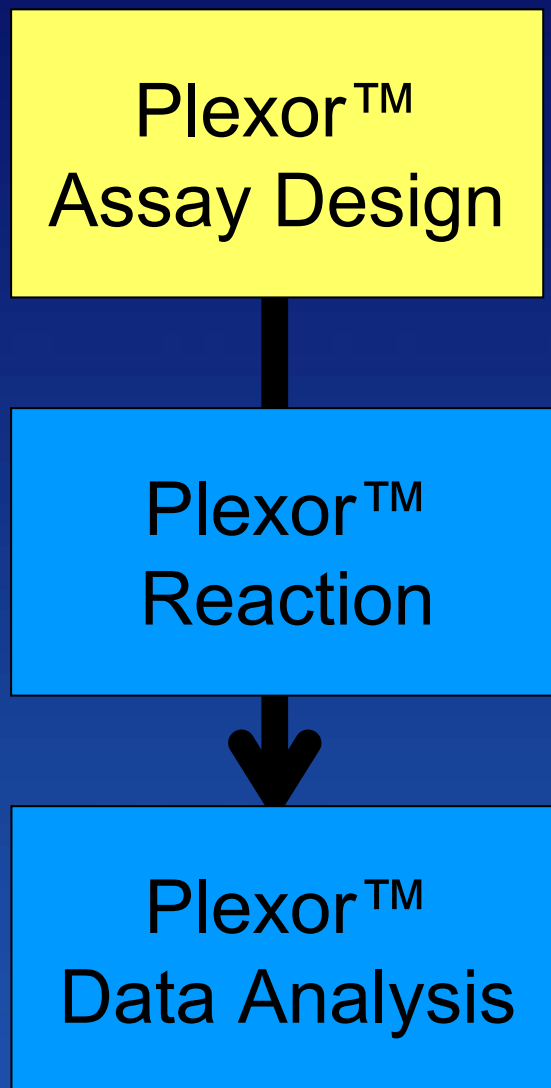
# Assay Design and Analysis

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Plexor™ Primer Design  
System and Analysis  
Software

# Plexor™ Technology in Research

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# Plexor™ Primer Design Website

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- Free access (registration required)



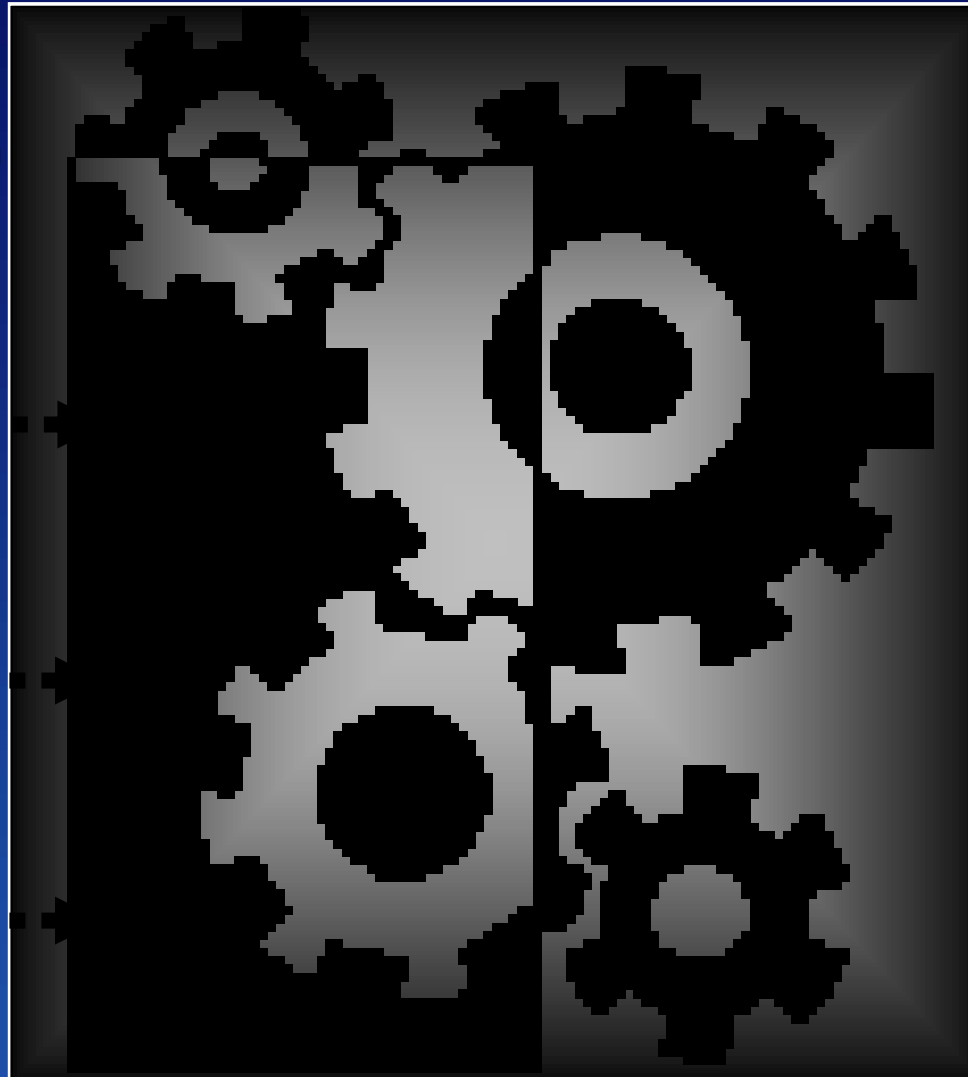
# Plexor™ Primer Design Web Site

Sequence  
#1

Sequence  
#2

Sequence  
#3

Sequence  
#4



Suggested  
Primers  
With  
BLAST  
links

# BLAST Parser

Plexor Blast Parser Results - Microsoft Internet Explorer

File Edit View Favorites Tools Help

Address <http://www.promega.com/techserv/tools/plexor/blast/BlastParserResults.aspx?> Go Links >> NewsStand Preferences

Google  Go  Bookmarks 60 blocked ABC Check AutoLink AutoFill Send to Settings

**Promega**

**Plexor Primer Design BLAST analysis results**  
Wednesday, March 14, 2007

You may select a single species or genus to view

[What does the Blast Parser do?](#)

**NM\_006617- analyzed results from BLAST performed against the RefSeq RNA database.**

Primer 1	Primer 2
TCTGTAGGCCCTGTTTCTCCTG	AGCGTTGGAACAGAGGTTGGA

A BLAST search was performed against the RefSeq database. The primer appears likely to anneal to a single gene from each of the species shown below in green. It is unlikely to misprime other genes from these species. If you are using RNA samples from one of these species and are examining all forms of the gene listed, the primer is unlikely to show non-specific amplification.

<ul style="list-style-type: none"><li>Bos taurus (cattle):<ul style="list-style-type: none"><li>LOC522383( similar to nestin)</li></ul></li><li>Canis familiaris (dog):<ul style="list-style-type: none"><li>LOC490410( similar to nestin)</li></ul></li><li>Homo sapiens (human):<ul style="list-style-type: none"><li>NES( nestin)</li></ul></li><li>Macaca mulatta (rhesus monkey):<ul style="list-style-type: none"><li>LOC718562( similar to nestin)</li></ul></li><li>Pan troglodytes (chimpanzee):<ul style="list-style-type: none"><li>NES( nestin)</li></ul></li></ul>	<ul style="list-style-type: none"><li>Homo sapiens (human):<ul style="list-style-type: none"><li>NES( nestin)</li></ul></li><li>Macaca mulatta (rhesus monkey):<ul style="list-style-type: none"><li>LOC718562( similar to nestin)</li></ul></li><li>Pan troglodytes (chimpanzee):<ul style="list-style-type: none"><li>NES( nestin)</li></ul></li><li>Tetrahymena thermophila SB210:<ul style="list-style-type: none"><li>TTHERM_00532580( hypothetical protein)</li></ul></li><li>Trypanosoma cruzi strain CL Brener:<ul style="list-style-type: none"><li>Tc00.1047053508851.154( hypothetical protein)</li></ul></li></ul>
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The species shown in red have hits from more than one gene in the Refseq RNA Database. If you wish to use this primer for one of these species, you should be cautious.

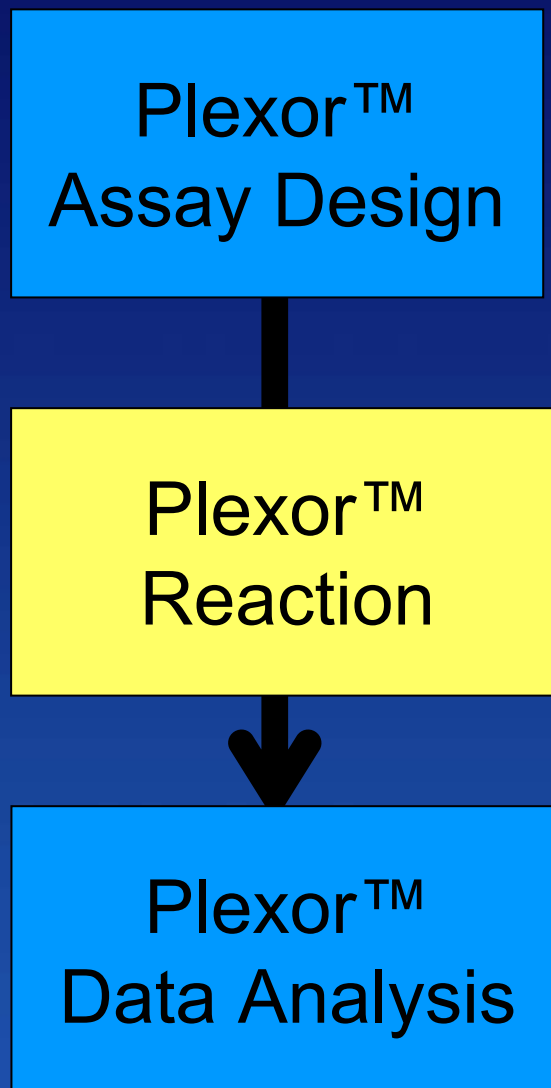
There were no species in the RefSeq database for which this primer looks like it will prime

- Oryza sativa (japonica cultivar-group) (Japanese rice):

start  Inbox ... Project ... Plexor ... Micros... 3:02 PM Promega

# Plexor™ Technology in Research

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# Plexor™ Systems

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- **qPCR System**

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

- **Two-step qRT-PCR System**

- ImProm-II™ Reverse Transcriptase reagents for cDNA synthesis
- Plexor™ Master Mix for qPCR from cDNA template

- **One-step qRT-PCR System**

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

# Plexor™ Reactions

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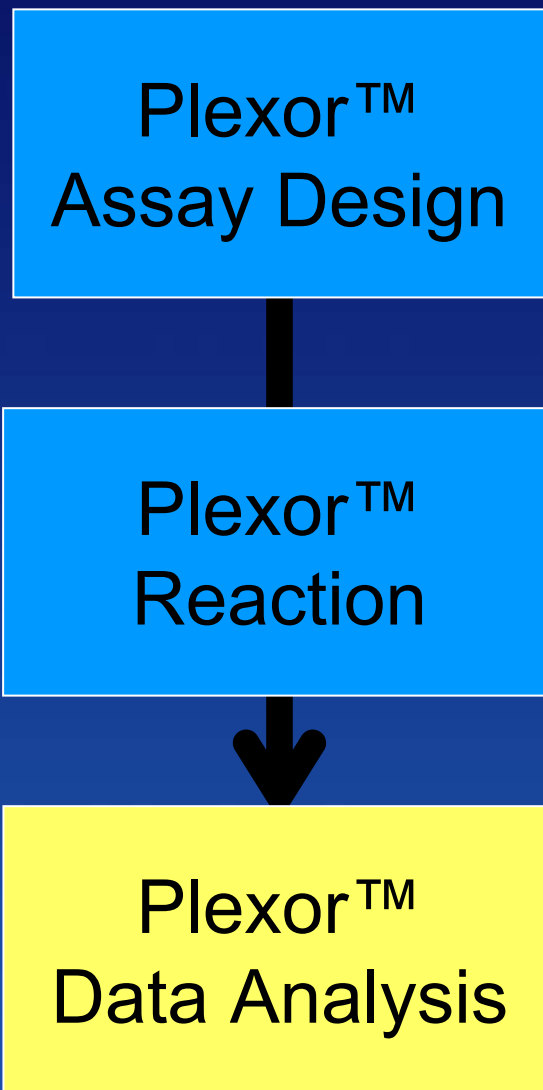
- 25µl standard reaction
- 2X Plexor™ Master Mix contains:
  - Enzyme (devoid of 5' exonuclease activity)
  - High performance buffer
  - Proprietary primer-dimer inhibitor
  - dATP, dCTP, dGTP, dTTP and Dabcyl-iso-dGTP

For amplification of the  
DNA sequence of  
interest

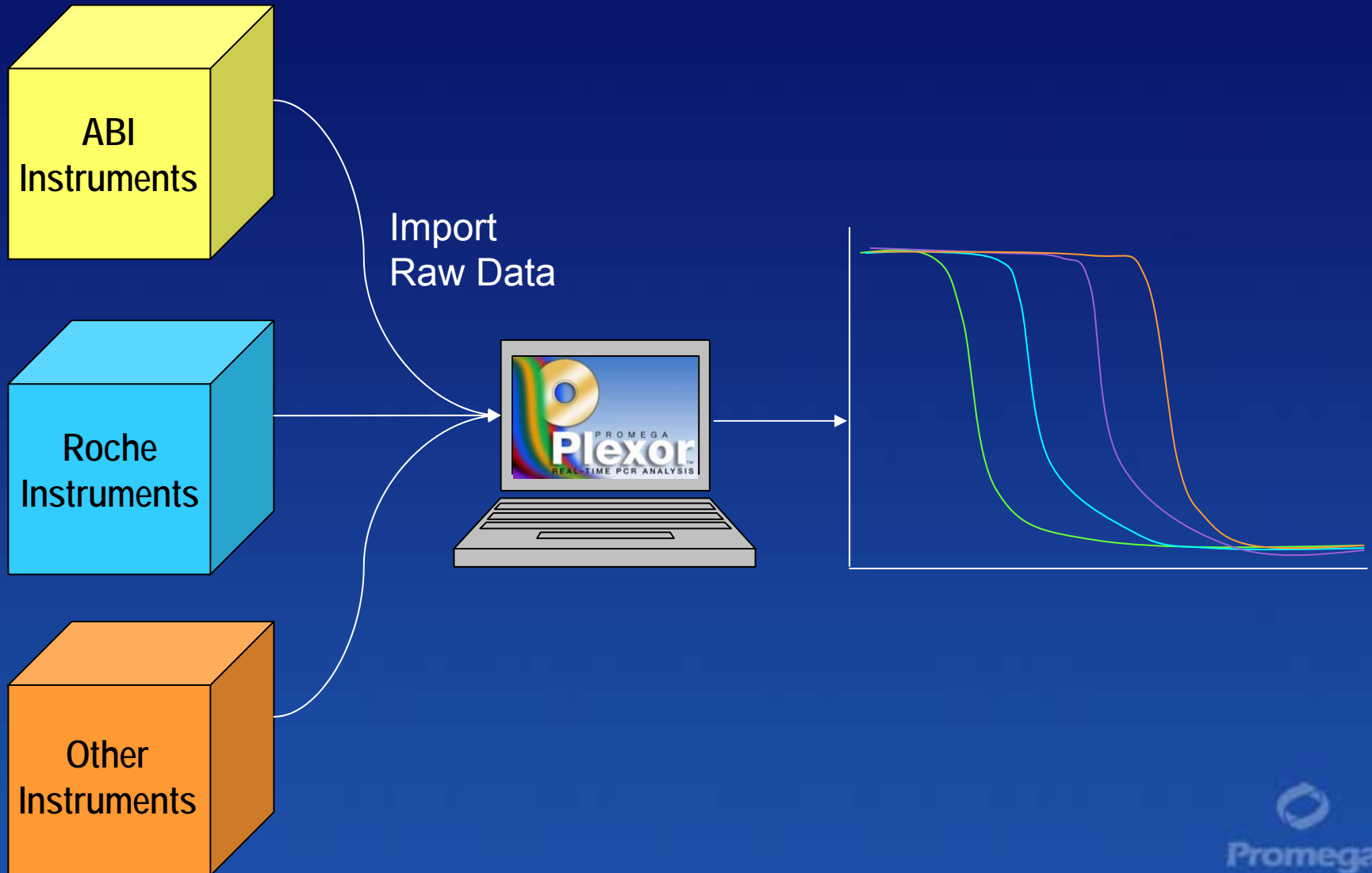
For incorporation  
opposite the iso-dC in  
the Plexor™ primers

# Plexor™ Technology in Research

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# Raw Data to Analyzed Data



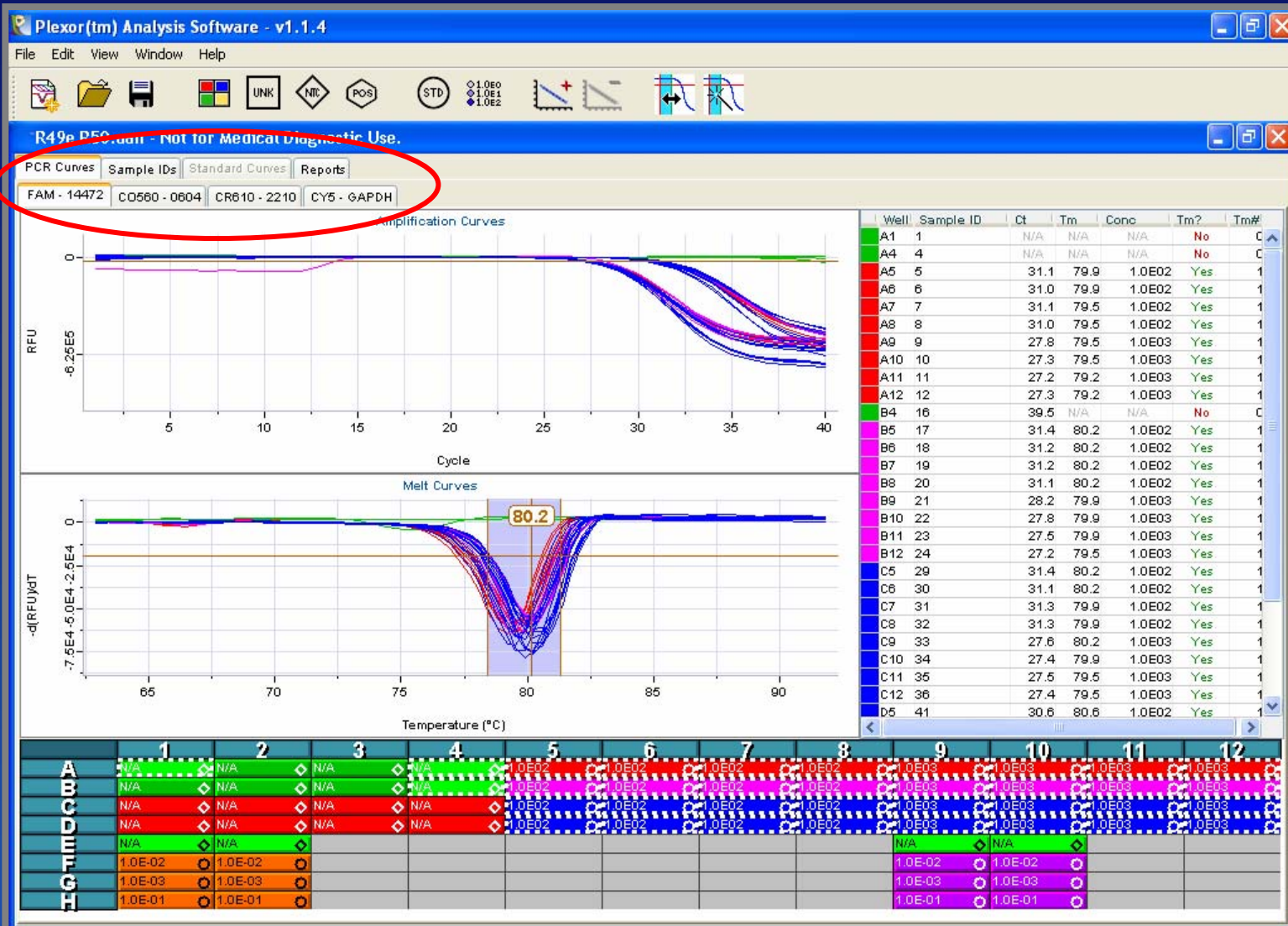
# Supported Instruments

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Plexor™ reagents  
and Data Analysis  
Software for:

- ABI PRISM® 7000
- ABI PRISM® 7700
- AB 7300, 7500, 7900HT
- Corbett Rotor-Gene™
- Roche LightCycler® 1 & 2
- Roche LightCycler® 480
- BioRad iCycler™ (2 & 4 color)
- MJ Opticon® Instruments
- Cepheid Instruments
- Stratagene Instruments
- *et al.*

# Plexor™ Analysis Software Desktop



# Summary

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- **Specificity** from novel base-pairing
- **Simple** assay design
- **Easy** multiplex reactions
  - Increased productivity
  - Accurate quantitation
- Tools and support to employ Plexor™ technology freely available

# Acknowledgements

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## Research & Development

Cheryl Bailey      Gary Madsen  
Steve Ekenberg      Nadine Nassif  
Katharine Hoffman      Cynthia Sprecher  
Susan Frackman      Douglas Storts  
Benjamin Krenke

## Software

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Jennifer McTaggart  
Lou Mezei  
Ethan Strauss  
Rick Smith

## Nucleic Acid Chemistry

Amanda Glebs      Jen Romanin  
Julie Heger      Michael Ma  
Dave Leland      Myra Schink  
Kristina Pearson      Katie Zurbuchen



Michael Ho  
Jim Prudent  
Mike Moser  
Scott Johnson