## Development of a New Real-Time PCR Quantitation System for Human and Y DNA Analysis and Normalization Software

Matthias Lindner, Benjamin Krenke, Cindy Sprecher, Nadine Nassif, Curtis Knox and Doug Storts Promega Corporation, Madison, Wisconsin, USA



## Introduction

Promega has developed a new technology for real-time quantitative PCR. This technology offers an advantage over currently available systems by simultaneously quantitating both the total human DNA and male-specific DNA within a sample, in addition to an internal PCR control. This technology is known as the Plexor<sup>®</sup> HY DNA Quantitation System.

## Methods

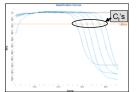
#### Plexor® Technology for Real-Time PCR

Fig. 1: Plexor® real time technology.

A 5'-labeled primer with iso-dC adjacent to a fluorescent label starts the amplification reaction (A). The reverse primer is unlabeled (B). During elongation, dabcyl-iso-dGTP is incorporated opposite iso-dC (C), which leads to quenching of fluorescence (D).

Proximity of dabcyl and the reporter quenches fluorescence.

#### Signal Decreases as Product Accumulates



The number of cycles required to cross an amplification threshold, known as the cycle threshold ( $C_t$ ), is related to input quantity.

 $\ensuremath{\mathsf{C}}_t$  values for samples are compared.

Fig. 2: Plexor® HY amplification curves

## Multicopy Targets

- Increased sensitivity and reduced impact of primer site mutation
- Autosomal and Y targets have similar copy number
- Degraded DNA is less likely to be amplified as products are longer than those in competitor kits
- Target lengths: autosomal target: 99 bp Y-chromosomal target: 133 bp Internal PCR control (IPC) target: 150 bp

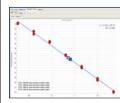
## Methods/Results

#### Plate and Reaction Setup

	1	2	3	4	5	6	7			18	11	12	Fig. 3: Plate setup.
٨	100	$(1)^{-1} e^{i \theta}$	1,996	1.04	1.04	1.895	1.84	1,056	1,756	1.04	1.0.0.	-10.00	Tig. 5. Thate setup.
8	$1/m_{H}$	$\gamma(m_{0})$	1,846	104	1,146.	1,840	1,846	0.000	1,796	1,746	1,846	10.00	
¢	10	$\sim$	UP IK.	17.8	1.04	UNK.	1745	UNK	UNK.	17.81	1.046	-10.00	Standard reactions are
0		104	1996	17.0	10.00	1,840	1745	1796	1796	17.00	17.00	10.00	
t	$0.017 m_{\rm P}$	1:00%	UPH.	1.04	1.0.0	UNK.	1.145	1,000	1766	1.04	1.14	10.0	pipetted with 2 µl templ
۶			UPP.	17.8	1.04	1.741	1745	UNK.	1.000	1.7.8.	1.7.8.	10.00	
6	1.00		1.946	1.04	10.0	1.846	1.846	1,048	1/14	1.046	1,54	10.0	DNA (up to 9 µl possibl
н	10-	10.4	1,896	10.0	1.04	1.84	1.04	1,000	1706	1.04	1.04	10.0	and a final volume of 2

per reaction. Amplification is done with a 38-cycle, twostep protocol.

#### Standard Curve 3.2 pg/µl – 50 ng/µl



The system reproducibly detects DNA amounts as low as 6.4 pg in 2µl of extract. The DNA standard is a

I he DNA standard is a mixture of DNA of several male individuals. No cell-line DNA is used.

Fig. 4: Plexor® HY standard curve.

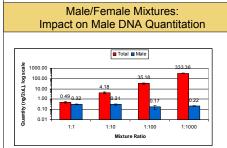
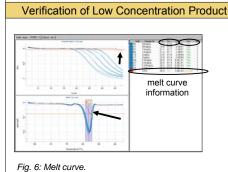


Fig. 5: Detection of minor amounts of male DNA.

A constant amount of male DNA was amplified in presence of increasing quantities of female DNA. Quantitation of 0.3 ng male DNA was minimally affected by addition of significant amounts of female DNA.



rig. 6. Men curve.

Melt curve analysis confirms correct product length. This is valuable for late  $C_t$  amplifications and to reduce false positive results.

# Results

#### Plexor<sup>®</sup> Analysis Software: STR Normalization Function

lated the day for the YUR assess	0.066.1							
Even the preferred to get OAL quartity for each machine	10	ż	-4-		- Target (ng/rxn)			
Etter fra internet and traceton read values of paralle for and-suchas:	-		w (112	<b>1</b> abu	- Volume (µl/rxn)			
Effer for sensor and sources quetty of parals (200 to all) Links condered if potential and 200 quetty cared to achieve of the for discuss class constants)	jus:	1	au (1.50	E	- Input range (ng/rxn)			
Enter für opnantit aller to vitalt som opnantit aller angelen einselfen Bahat	1.41		ngia		- Desired dilution for concentrated samples			
Dies fes conseil also is obei concernit del angle desatte dans:	int Text	-	ngta					

#### Fig. 6: STR normalization function.

Normalization is based on laboratory-defined parameters. Data are used to calculate sample input volumes. Separate normalization for autosomal and male DNA is possible.

#### Plexor<sup>®</sup> Forensics Report

															18 0
	-	1000 540		-	-	1	-	-	-	-	12		-	2	and a
-		100	-		-				-				_	-	-
	11														
- 21		1.2													
ALC: N			- 6464					1.18							
				1000											- 2 -
			1100	1000		-				billion of	- 14	1000		100	1000
frances -			0.00		- 10										
10.0	- 24	- Indiana	- 6. ALM -	- 44	-									1.0	- 2 -
	A HOLE		11101			(Friday)	1	CORN		10.046	-	1000		1941	14
They 110-	- 2		-	1.24											
1010		Contract of	1.00	1.46											
	Arrise:		1.946	1.5.460	54	( <b>68</b> )						1.00		191	1.14
2410		hended		1.000											
2419		Renderi	194	1980											180
INDE	Areas.		7.744	1000		C. 64 C.	1.18	000.000	112.00		- 10	100		190	16
194.75	. 81	<b>Animi</b>	1.00	1.000											
106.75		in a cipi	1.00	1.86											
	3948		1.1440	1.1994	111				100	1.44		1000			10 M
174 00		for the second		1.14											- 5 -
		hered	1.04	1000											
(MM)	Treas.		1.000	1000		008600	- 10		1.1					CHC	1.14
1.00415		Burdled	0.000	6.001											- 5 -
110,10		haund 1	4.64												
Alternative Contention	-		1.000	1	54										100

Fig. 8: Plexor® HY Forensics report. The Forensics report function of the Plexor® HY Analysis Software.

## Summary

• The Plexor<sup>®</sup> HY System includes Promega hot-start technology.

• Simultaneous quantification of autosomal and Y-chromosome DNA means

- $\rightarrow$  less variability
- $\rightarrow$  less time
- → more valuable data

• Multicopy targets ensure increased sensitivity and reduce the impact of primer site mutation.

• Consistently and reproducibly detect 6.4 pg of DNA with a volume up to 9 µl of template DNA.

• Internal PCR control and melt-curve analysis guard against false negative and false positive results, allowing you to be confident in your data.

• Long amplification products result in less amplification of degraded DNA.

• Analysis and normalization software is designed for forensic community.