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

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Promega

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® HY DNA Quantitation System.

Methods

Plexor® Technology for Real-Time PCR

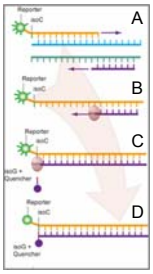
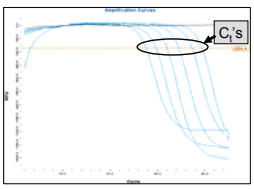


Fig. 1: Plexor® real time technology.

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.

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

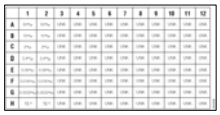
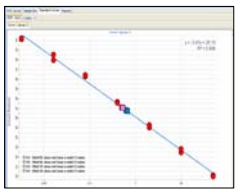


Fig. 3: Plate setup.

Standard reactions are pipetted with 2 µl template DNA (up to 9 µl possible) and a final volume of 20 µl per reaction. Amplification is done with a 38-cycle, two-step 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 mixture of DNA of several male individuals. No cell-line DNA is used.

Fig. 4: Plexor® HY standard curve.

Male/Female Mixtures: Impact on Male DNA Quantitation

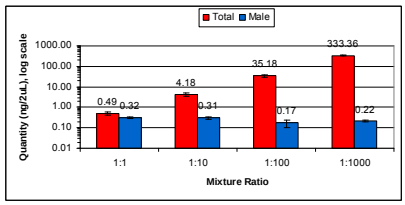


Fig. 5: Detection of minor amounts of male DNA.

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.

Verification of Low Concentration Product

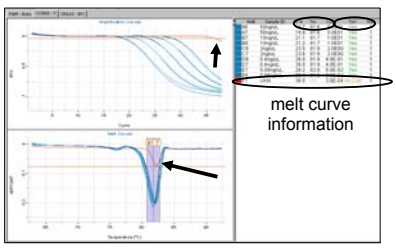


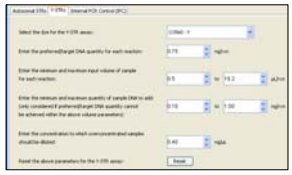
Fig. 6: Melt curve.

Melt curve analysis confirms correct product length.

This is valuable for late C_t amplifications and to reduce false positive results.

Results

Plexor® Analysis Software: STR Normalization Function



- Target (ng/rxn)
- Volume (µl/rxn)
- Input range (ng/rxn)
- Desired dilution for concentrated samples

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® Forensics Report

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

Summary

- The Plexor® HY System includes Promega hot-start technology.
- Simultaneous quantification of autosomal and Y-chromosome DNA means
  → less variability
  → 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.