

**COMPARISON OF THE BIO-RAD ICYCLER IQ™ 5 AND THE STRATAGENE MX3005P REAL-TIME PCR SYSTEMS IN CONJUNCTION WITH PROMEGA'S PLEXOR™ GI PROTOTYPE SYSTEM FOR THE SIMULTANEOUS QUANTITATION OF AUTOSOMAL AND Y-CHROMOSOME DNA**

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Real-time PCR is a valuable tool in forensic DNA analysis, since it provides a large dynamic range, is automated and can be multiplexed for greater functionality. Moreover, real-time PCR has the ability to detect the amount of amplifiable human DNA in the sample. This provides a measure of DNA quality, providing the examiner with an idea of how well a sample may amplify during short tandem repeat (STR) typing.

Individualization of the male and female fractions of sexual assault evidence is an important step in the successful analysis of forensic casework. Unfortunately, differential extractions do not always succeed at completely separating the sperm and non-sperm fractions and single source STR profiles are not always attainable. Situations arise where the sperm cell DNA is retained in the non-sperm fraction, but the STR primers are overwhelmed by the excess of non-sperm DNA and the sperm DNA in the mixture goes undetected. Situations such as these lend themselves to the use of Y-STR profiling which may be used to isolate and amplify the male DNA, even in the presence of high quantities of female DNA. Knowing that male DNA is present in the sample allows the examiner to make an informed decision to go forward with Y-STR profiling.

Commercial real-time PCR assays can be used to specifically quantify the autosomal and male DNA in a sample. The products currently available require a separate PCR amplification for the autosomal and male DNA quantitations. The necessity of performing two separate reactions for quantitation of autosomal and male DNA is time consuming, costly and requires backtracking unless both quantitations are performed on all casework samples. The Promega Corporation has recently developed a system which could help increase the efficiency of quantifying and triaging these sexual assault case samples. This system, Plexor™ GI Prototype, is a real-time PCR based assay which is able to quantify the autosomal and Y-chromosome DNA in a single PCR reaction.

The utility of the Bio-Rad iCycler IQ™5 and the Stratagene Mx3005P™ were tested using an *Alu*-based SYBR green assay, a multiplex Plexor™ GI Prototype assay (Promega), and a duplex real-time PCR assay developed by the Vermont Forensic Laboratory. DNA was extracted from blood cards, buccal swabs, tissue samples, and cigarette butts using the DNA IQ™ System and/or an organic extraction method. These samples were quantified using a SYBR Green assay on both instruments. The results showed, on average, a less than two-fold difference between the two instruments. When these results were compared to those obtained using AluQuant (Promega), both of the real-time instruments gave results that were an average

of 2-fold higher than the AluQuant system. This comparison is consistent with the results shown by the 2004 NIST DNA quantitation study.

A variety of both male and female single source samples, as well as male/female mixture samples were analyzed using the Plexor™ GI Prototype kit and the Vermont Forensic Laboratory's duplex kit on both real-time instruments. The two duplex kits gave comparable results. A variation of the cycling parameters for Plexor™ GI Prototype was examined. The results suggest that Plexor™ GI Prototype hold times were adequate for maximum amplification efficiency. Both instruments provided comparable quantitation results with Plexor™ GI Prototype.

The comparison of the Bio-Rad iCycler iQ™5 with the Stratagene Mx3005P shows that the two instruments are very similar in their ability to accurately quantify the amount of DNA in a sample. A number of other factors were also examined to compare the two instruments, including ease of use, cost, and compatibility with other systems used in the laboratory. The evaluation of the Plexor™ GI Prototype kit is ongoing. However, the results are promising that this kit could help to increase laboratory efficiency when examining sexual assault cases.