

The Plexor™ Analysis Software

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INTRODUCTION

In the previous issue of *Profiles in DNA*, a new approach for quantitative PCR was introduced (1). This technology, the Plexor™ System^(e,f), uses a modified nucleotide in the nucleotide mix (dabcyl-iso-dGTP) and within the fluorescently labeled primer (iso-dC). By incorporating the quencher dabcyl linked to iso-dGTP, fluorescence is decreased with every round of amplification (2–4). Consequently, this approach presents distinct amplification curves that decrease in signal as product accumulates. This second installment in the series profiles data analysis using the Plexor™ Analysis Software.

INSTRUMENTATION AND CREATION OF MULTICOLOR DATA

As previously described, the Plexor™ technology employs fluorescently labeled amplification primers for multicolor multiplexing. Simultaneous quantitation of autosomal, Y-chromosomal and internal positive control targets necessitates detection of at least three colors, with one target per color. Development of multicolor chemistry dictates the use of compatible real-time PCR instruments. Although the Plexor™ System offered for general research will be compatible with a variety of instruments and applications, we have focused on a subset of instrument platforms during the development of the Plexor™ System for human DNA quantitation. To best integrate this chemistry with the needs of forensic laboratories, we have limited analysis to instruments that are four-color-capable, use a 96-well plate format and produce quality data, such as those from Applied Biosystems and Stratagene. Data from these instruments can be exported for analysis in the Plexor™ Analysis Software.

PLEXOR™ ANALYSIS SOFTWARE

A key aspect of the Plexor™ System is the dedicated analysis software package, which allows users of different real-time PCR instruments to analyze, produce and share data in a common format. This software is available at no charge to customers using the Plexor™ System.

Data are exported from the instrument software as a *.txt or *.tab file or a file with a similar format. The Plexor™ Analysis Software includes a simple Importer, which allows the user to define the run and analysis details, then select the raw data file(s) to be imported. The Importer includes optional template functions for customizing the plate layout for routine assays and plate layouts.

The Analysis Desktop is a multi-tiered graphical interface for displaying, analyzing, exporting and printing qPCR data (Figure 1). The amplification curves, melt curves, well selector and a tabular report can be viewed from this screen (viewable panels can be selected by the user). Many analysis parameters, sample coloring, sample identifiers, graph axes and other functions are graphically represented and user-definable. In addition to the previously mentioned plate setup template within the Importer, hot keys and a function to define standard serial dilutions are included to

qPCR

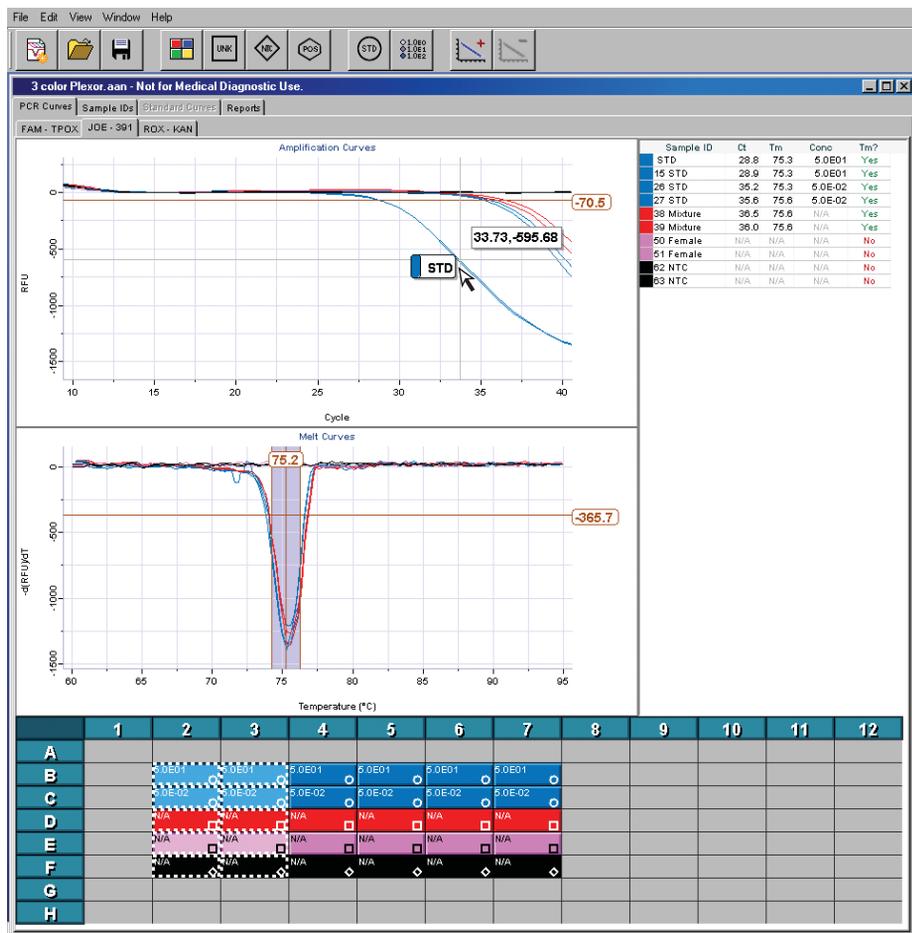


Figure 1. The Analysis Desktop provides access to all analysis, reporting, exporting and printing functions. The PCR Curves view includes the Amplification Curves (top panel), Melt Curves (middle panel), Well Selector (bottom panel) and an abbreviated table report (upper right corner). To help distinguish and select samples, the cursor can be positioned over a line in the Amplification Curves or Melt Curves window, and the sample details will appear in the Amplification Curves panel.

speed analysis. Tabs allow access to views of the standard curve (multiple curves can be defined in each color, if desired), editable sample identifiers and reports that include analysis parameters and concentration of each sample. The report can be exported for further analysis in other software packages, such as Microsoft® Excel.

THE MELT CURVE INCREASES QUALITY CONTROL

When amplified, the close proximity of dabcyl on the 3' end of the unlabeled strand and the fluorophore on the 5' end of the opposite strand of the double-stranded DNA effectively quenches the signal. The "loss of fluorescence" that results from product accumulation is a reversible process. When denatured, these amplicons freely fluoresce. This allows a melt protocol or dissociation function to be performed following amplification by slowly increasing the temperature

from 60°C to 95°C and measuring the signal constantly during this ramp. Product melting produces a significant change in signal as quenched products are denatured, and the resulting change in fluorescence is used to empirically determine the melting temperature (T_m). The T_m is a function of product length and sequence and can be used to confirm homogeneity and, therefore, the specificity of the assay. Suspected false positives can be sorted as "likely user contamination" (product has the expected T_m), "late cycling instrument or assay noise" (no significant melt peak) or "nonspecific amplification" (incorrect T_m and/or broad melt peak). These issues can be occasional concerns with real-time PCR technologies in general. The ability to perform a melt function provides an additional level of verification of products visualized in the amplification curves.

CONCLUSION

With easy-to-use importing functions, flexible analysis tools and a useable report output, the Plexor™ Analysis Software is a highly functional analysis platform for the Plexor™ technology.

The Plexor™ technology is licensed from EraGen Biosciences.

REFERENCES

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