applied biosystems
by life technologies

HID Real-Time PCR Analysis Software v1.1

Enhanced Quantifiler® Kit Data Analysis

- Streamlines the DNA quantitation workflow with specially designed Quantifiler® assay templates and an intuitive, easy-to-navigate user interface
- Enables rapid DNA sample quality assessment with Human Identification (HID) specific quality flags and analysis summary
- Guides selection of optimal downstream AmpFℓSTR® PCR Amplification Kit with Male:Female Ratio and IPC C_T flags
- Speeds up STR analysis workflow with Dilution Calculation and AmpFℓSTR® Kit Reaction Setup Tools
- Provides customizable reporting features that allow users to select desired information for inclusion in exportable or printable formats
- Simplifies the calibration process and eliminates need for time consuming manual calibration analysis
- New features and enhancements include streamlined reporting, simplified sample input, and additional data analysis functionality

Introduction

The Applied Biosystems Quantifiler® DNA Quantification Kits, including the Quantifiler® Duo kit, have been widely used by human identification (HID) laboratories to aid forensic analysts in the processing of challenging biological samples. The Quantifiler® Duo kit is capable of efficiently determining the relative quantities of male and human DNA in a single reaction while detecting the presence of PCR inhibitors. Quantifiler® Duo quantification results can guide selection of the most appropriate AmpFℓSTR® PCR Amplification Kit in order to maximize the chances of obtaining optimal STR results in the first attempt.

To take full advantage of the data and capabilities offered by the Quantifiler® kits, especially the Quantifiler® Duo kit, HID users require software designed specifically for the human identification application.

The HID Real-Time PCR Analysis Software v1.1 has been developed to complete the Applied Biosystems integrated HID solution for DNA quantification, which includes the 7500 Real-Time PCR System and the Quantifiler® kits. By automatically assessing DNA sample quality and quantity, the HID Real-Time PCR Analysis Software helps guide processing decisions and facilitates selection of the appropriate AmpFℓSTR® Kit and DNA input amount for optimal results. The software provides a streamlined HID specific quantitation workflow, enhanced data review functionality, and the ability to improve STR analysis efficiency, making it an essential component of the premier DNA sample quantification and assessment system for human identification.

Streamlined HID Specific Workflow

The HID Real-Time PCR Analysis Software provides four HID specific templates: Quantifiler® Duo, Quantifiler® Human, Quantifiler® Male, and Quantifiler Human and Male (hybrid plate). Users can easily access each Quantifiler® assay template by clicking one of the icons on the home screen (Figure 1).

In each template, experimental properties, targets, sample types, and standard concentrations are all set as defaults. To start the run, users only need to enter the sample names and well positions for unknown samples. Thus, no set up of multiple templates is required. This feature simplifies and shortens the plate set up process.

The intuitive, easy-to-navigate user interface further streamlines the workflow (Figure 2). Plate Setup, Run, and Analysis function menus are listed in the Experiment Menu on the left navigation pane. The detailed experiment settings and the operations of each function are displayed conveniently on the right pane. Users can perform sample run operation, view plate layout, and obtain the run status in real time. The sample analysis results can be displayed in multiple ways, including Amplification Plot, Standard Curve, Multicomponent Plot, Analysis Summary, Raw Data, and Multiple Plots View. These different analysis views enable users to quickly obtain the run results and perform further analysis based on the analysis settings.

Rapid Sample Quality Assessment

The HID Real-Time PCR Analysis Software employs an HID specific Quality Control Flag system to assist the analyst with data analysis and evaluation of critical information obtained from the Quantifiler® assays. Such information includes the detection of PCR inhibition, reagent contamination, and mixtures of male and female DNA. Quality flags evaluate the slope, y-intercept, and R^2 values of the standard curve(s) as well as the internal PCR control (IPC) C_T value and high or low quantity samples. For example, the IPC C_T flag can help users to determine whether re-extraction may be required or whether using the MiniFiler™ kit for downstream analysis may be a more effective option to obtain meaningful results. In addition, a male to female (M:F) ratio flag specific to the Quantifiler® Duo assay indicates samples containing a mixture of male DNA combined with excess female DNA. If the M:F ratio flag is greater than a user defined threshold, it can guide the analyst to consider use of the AmpFℓSTR® Yfiler® PCR Amplification Kit as the best means to obtain optimal results.

The Analysis Summary is displayed immediately after the analysis and displays the associated quality flags with each sample type (Standard, NTC, and Unknown) and the standard curve metric flags. It summarizes the number of samples that meet or do not meet the thresholds, with hyperlinks to the corresponding samples in the well table and plate layout. Users can simply click the hyperlink to gain more detailed information from flagged sample wells (Figure 3).

This feature allows users to easily perform DNA sample quality assessment by quickly identifying the samples requiring review.

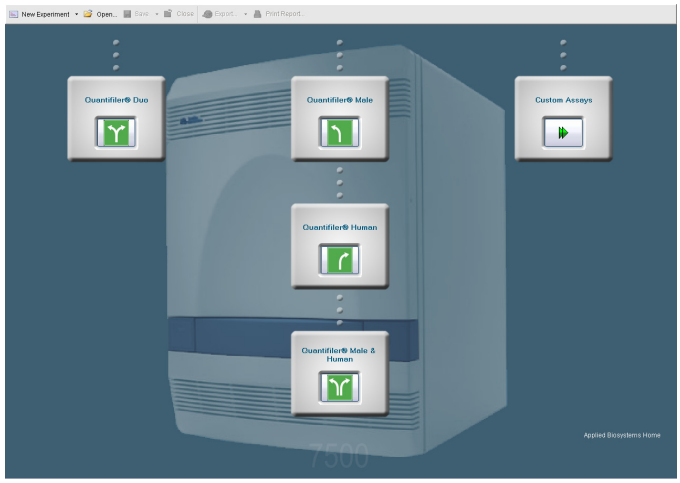

Figure 1. The home screen provides easy access to HID Specific Templates for Quantifiler® Duo, Quantifiler® Human and Quantifiler® Male assays.

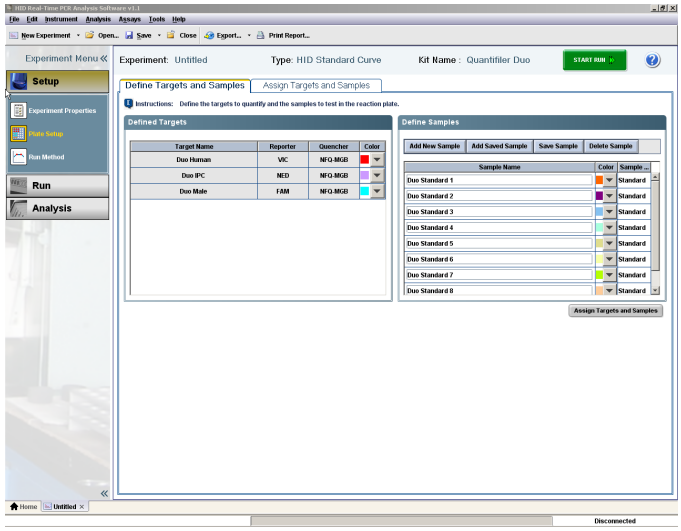

Figure 2. An intuitive user interface streamlines the workflow with easy access to Plate Setup, Run and Analysis function menus in the left navigation pane.

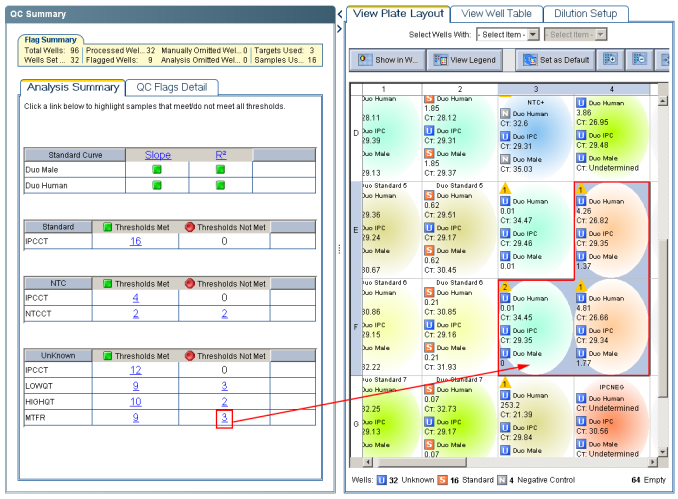

Figure 3. The Analysis Summary provides a quick overview of samples that meet or do not meet thresholds, with hyperlinks to view more detailed information on flagged samples in the well table and plate layout.

Dilution Calculation Tool

The Dilution Calculation Tool provides instructions based on user preferences to normalize samples prior to STR amplification. Users can set Dilution Scheme parameters including Pipetting Overage (%), Minimum Pipetting and Maximum Sample Volumes. Users can also choose either One Step Dilution or System Select Dilution (maximum of a two-step dilution) Method as well as choose a desired Dilution Factor (Figure 4).

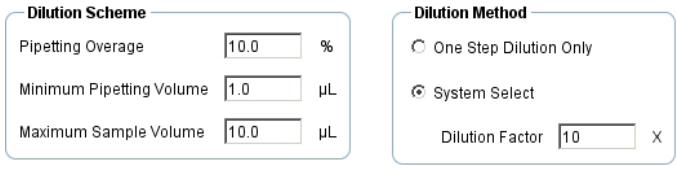

Figure 4. The Dilution Calculation Tool enables users to easily normalize samples.

AmpFℓSTR ® Kit Reaction Setup Tool

The AmpFℓSTR® Kit Reaction Setup Tool contains details for each STR kit utilized for analysis in the STR kit Library (Figure 5). The parameters include the PCR Master Mix Components and Component Volumes, Target Concentration, and additional reactions per amplification setting to account for amplification controls and pipetting overage. Once the analyst selects the AmpFℓSTR® Kit(s) for downstream analysis in the well table, the dilution and STR kit Library preferences are applied to the sample. Users can edit the dilution preferences or target concentration for each sample. This tool guides the user to set up the downstream STR reactions in a simple and easy manner.

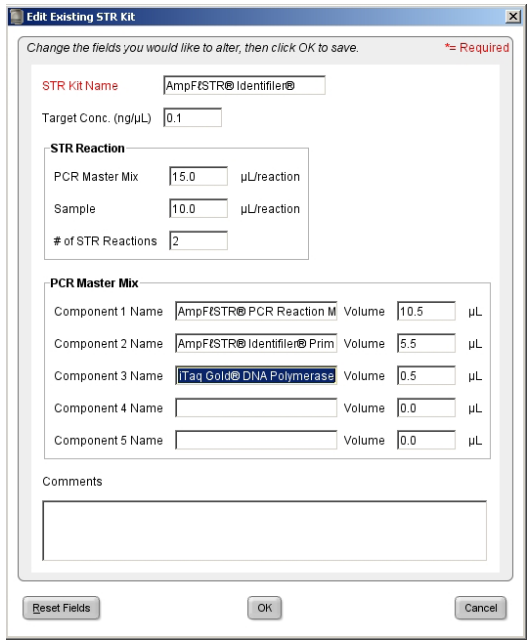

Figure 5. The STR Kit Reaction Setup Tool guides users to set up downstream STR reactions in a simple and easy manner.

Customizable Reporting Features

The software can generate the report in PDF format that can be saved or printed for inclusion in a case file. It can also export the report in Excel spreadsheet format, enabling users to perform additional analysis if needed. Users can select desired information to be included in the report, such as Results, Amplification Plot, Standard Curve(s), etc. (Figure 6).

Simplified Instrument Calibration

In addition to simplifying sample analysis, the software also simplifies the 7500 Real-Time PCR System calibration procedure. Step-by-step wizard-based instructions guide users through each calibration process (ROI, Background, Optical, Pure Dye, RNase-P Verification Plate). At the end of each calibration, the software automatically analyzes the calibration and displays the result as Pass or Fail. Time-consuming manual calibration analysis is no longer required. The software automatically records the date and time of each calibration, and the user can conveniently check when the calibration is due at any time. All of these functions can be easily accessed via the Instrument Maintenance Manager.

Easy Switch to Custom Assay Mode

For users who need to perform custom assays, they can easily switch to the Custom Assay Mode by simply clicking Assays on the top menu bar and selecting Custom Assays. This provides flexibility for users who may wish to use the software for different applications or configurations.

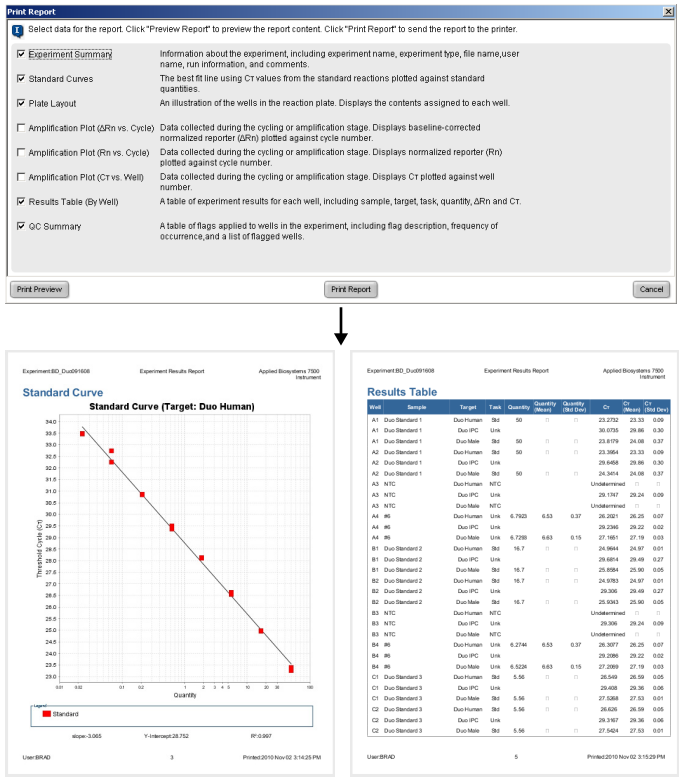

Figure 6. Customizable reporting features provide flexibility to select desired information for reporting in pdf format.

SYSTEM REQUIREMENTS

Component	Recommended Requirements	Minimum Requirements
Computer	• Pentium 4 or compatible processor, 2.0 GHz • 1 GB RAM • One hard drive with 10 GB available • 20/48X IDE CD-ROM drive • USB v2.0 • Ethernet network interface adapter (10BASE-T) • UL listed • CE marked • FCC labeled	• Pentium 4 or compatible processor, 1.2 GHz • 1 GB RAM • One hard drive with 10 GB available • 20/48X IDE CD-ROM drive • USB v1.1 • Ethernet network interface adapter (10BASE-T) • UL listed • CE marked • FCC labeled
Monitor	• 1280 x 1024 pixel resolution for full screen display • 16-inch • 32-bit color • UL listed	• 1280 x 1024 pixel resolution for full screen display • 16-inch • 32-bit color • UL listed
Operating System	• Microsoft Windows® XP, Service Pack 3	• Microsoft Windows® XP, Service Pack 3

ORDERING INFORMATION

Description	P/N
HID Real-Time PCR Analysis Software v1.1	4413973
7500 Real-Time PCR System for Human Identification with Dell Notebook	4366605
7500 Real-Time PCR System for Human Identification with Dell Desktop	4366604

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