PCR Reaction Setup Application

Automation of PCR Reaction Setup Application Using the Biomek 4000 Laboratory Automation Workstation

Ruth Zhang, Ph.D., Amy Yoder, and William Godfrey, Ph.D., Beckman Coulter Life Sciences, Indianapolis, IN

Abstract

Polymerase chain reaction (PCR) assays continue to play an important role in research laboratories. The PCR Reaction Setup is offered on the Biomek 4000 Laboratory Automation Workstation. This application note guides users through the process of setting up a PCR reaction for 1 to 192 samples in 96-well plates with any combination of Master Mix, Primers, and Samples (Figure 1). It is the ultimate automation solution for complicated PCR reaction setup processes.

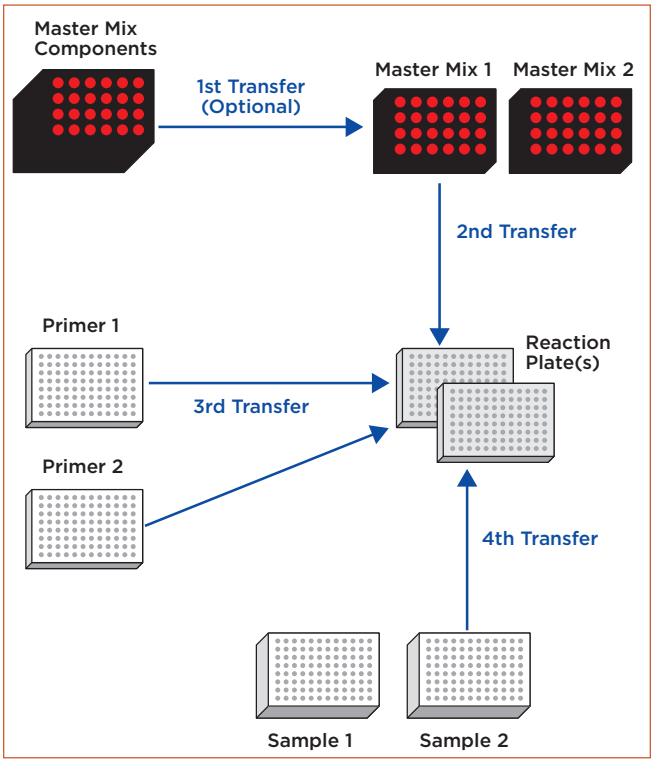

Figure 1. PCR Reaction Setup Process

Instrument Setup Process and Key Features

1. Supports 1 to 192 high throughput configurations with up to two master mixes, two primers and two sample source labware including master mix made from single components (Figure 1).
2. Allows sample tracking by creating a sample ID file at setup (Figure 2).

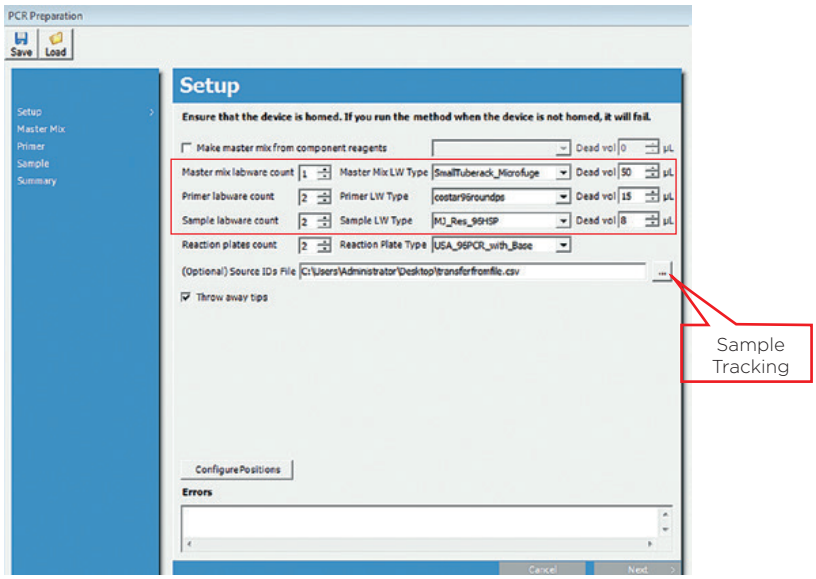

Figure 2. Up to two 96-well PCR reaction plates can be created in one reaction setup process.

3. Supports both prepared PCR master mix and master mix made from individual PCR components (Figure 3).

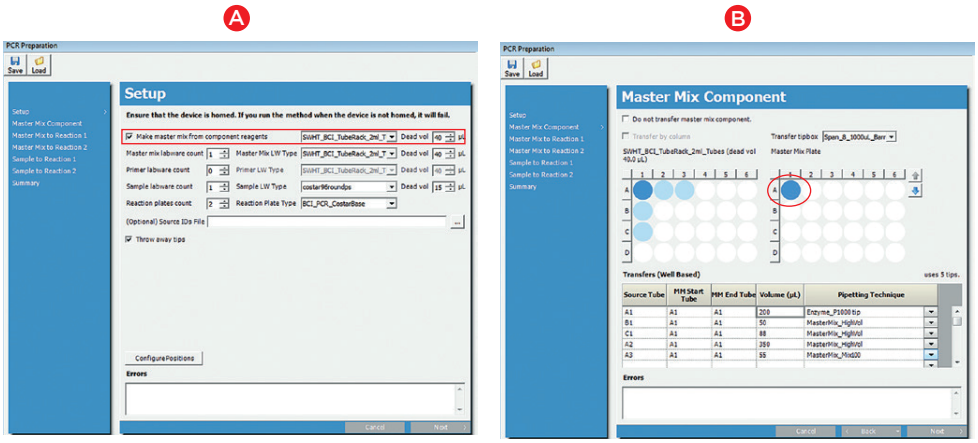

Figure 3. Master Mix can be made from individual PCR components, i.e., AmpliTaq DNA polymerase, dNTP, MgCl2, Triton X-100, Waters and Primers. All PCR components can be transferred from tubes of source labware to the tube of destination labware.

4. Keeps track of tip usage and alerts the user if more tip boxes are needed (Figure 4).

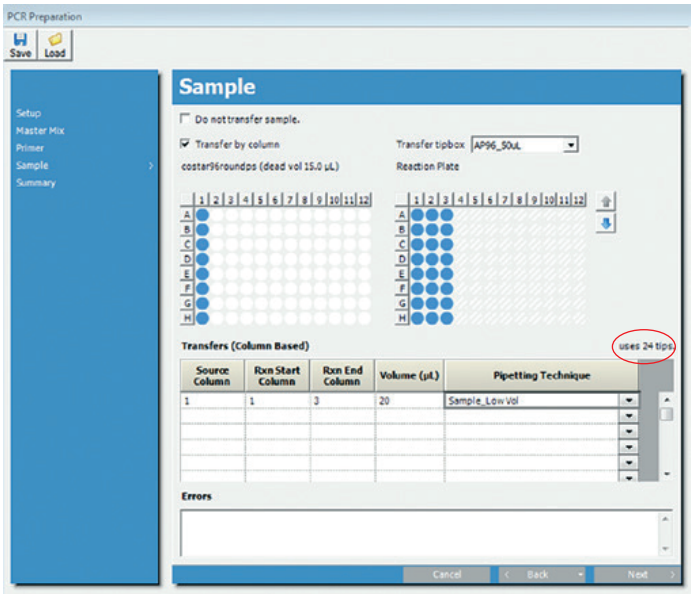

Figure 4. Tip usage is automatically tracked and calculated per number of samples processed.

5. Allows saving and importing of setup conditions to repeat prior setup processes (Figure 5).

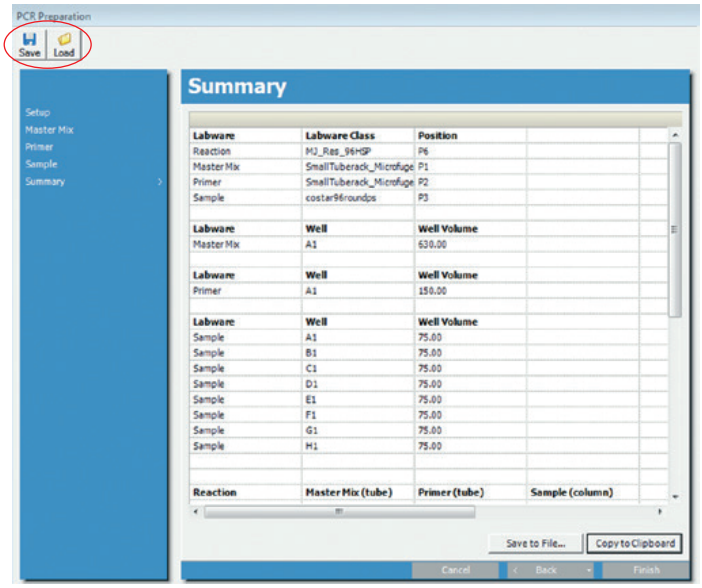

Figure 5. Click the "Save" button to save the setup condition. Click the "Load" button to import a saved file for repeat PCR reaction setup conditions.

6. Provides standard pipetting templates and techniques which can also be customized by users.
7. Provides sample tracking report upon run completion.

Results

The Biomek 4000 Laboratory Automation Workstation automated PCR Reaction Setup application offers flexible sample and reagent test combinations, limited only by the number of available tips and the amount of reagents on deck. This application can be used to prepare PCR and real-time PCR (qPCR). The system was used to prepare 32 replicate qPCR reaction plates from a single sample. This automated qPCR reaction setup data shows consistent amplification, with CVs of 1.74% for the 32 reactions (Figure 6). The PCR Reaction Setup Application was also utilized to prepare several PCR reactions on the Biomek 4000 Laboratory Automation Workstation which demonstrated no well-to-well cross-contamination from this automated PCR reaction setup process (Figure 7).

Automated Real-Time PCR Reaction Setup results in low CVs from 32 sample reactions

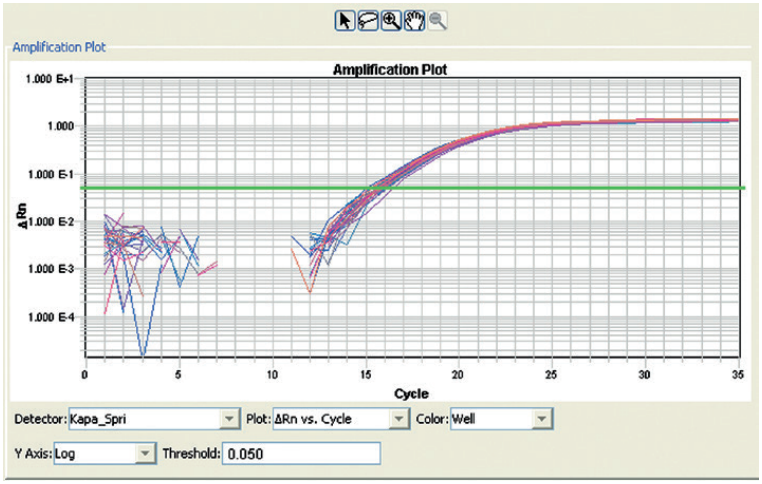

Figure 6. Real-Time PCR amplification (20 µL/reaction): using ABI 7900HT Fast Real-Time PCR System. The above data contains the β-actin real-time PCR reaction results from 32 samples (average Ct = 15.72 with 1.74% CV). Each β-actin qPCR reaction amplified 8 ng human genomic DNA (Promega), β-actin primer pairs (Promega), and KAPA SYBR FAST qPCR Master Mix (KAPABIOSYSTEMS).

The cross contamination tests indicate no well-to-well cross-contamination.

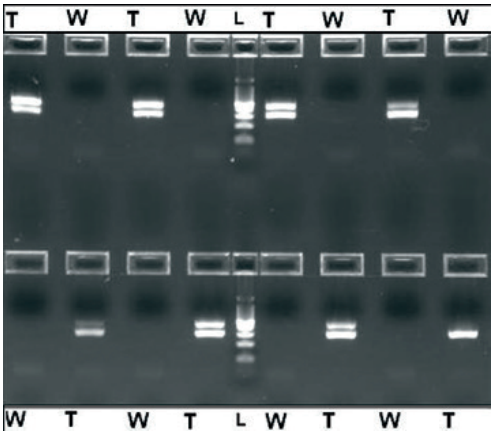

Figure 7. Cross-Contamination Results (25 µL/reaction): Human genomic DNA (24 ng/reaction), β-actin primer pairs and ready-to-use PCR master mix (Promega) were used for automated PCR cross-contamination tests. The test was conducted by amplifying gDNA template (T) and Water (W) in alternating wells. The absence of β-actin amplicons (285 bp) in negative wells indicates an absence of cross contamination. The middle lane is 100 bp DNA Ladder (L) (Promega).

Summary

With the ready-to-use PCR Reaction Setup Application for the Biomek 4000 Laboratory Automation Workstation, the user can set up a variety of PCR reactions, i.e., PCR and qPCR, using either default labware and techniques or user-modified labware and techniques by simply following the self-guided PCR User Interface. The application provides consistent results without the fear of cross contamination. The P1000SL tool allows the creation of large volume master mix from individual PCR components located on the Biomek 4000 Laboratory Automation Workstation deck using the standard Beckman Coulter P1000 tips, that further facilitate the ease of use and flexibility in preparing PCR reactions.

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