Plate Preparation for the CFX384™ Real-Time PCR Detection System Using the IDEX Health & Science Innovadyne Dispenser

Carl Fisher[1] and Mary Cornett[2]
[1]Bio-Rad Laboratories, Inc., Hercules, CA 94547; [2]IDEX Health & Science LLC, Rohnert Park, CA 94928

Introduction

High-throughput real-time PCR data collection is becoming increasingly important because a greater number of laboratories are processing more samples for routine screening and for studies in which data from a large number of subjects must be collected to obtain meaningful results. Additionally, to reduce the overall cost of assays, the use of smaller reaction volumes is being explored. Changing from the standard 96-well format to a 384-well format increases capacity considerably; however, the transition can be challenging because of the smaller well size and reduced volumes. To pipet reaction mixtures manually, and still obtain consistent results, requires a considerable amount of skill and can be quite tedious. An alternative to manual pipetting is to aliquot reaction mixes using an automated liquid handling system. This tech note focuses on the Innovadyne Nanodrop II liquid handling system from IDEX Health & Science LLC that, when combined with the CFX384 real-time PCR detection system, is ideally suited for low-volume qPCR.

To demonstrate the ability of the Innovadyne Nanodrop II liquid handling system to reliably aliquot reaction mixes for the generation of qPCR data on Bio-Rad's CFX384 real-time PCR detection system, uniformity and linearity assays were performed. In the uniformity assay, the same reaction mix was dispensed into all wells. An acceptable result is one in which amplification occurs at approximately the same cycle for all wells (±0.2 quantification cycle [Cq] standard deviation). In the linearity assay, template was serially diluted tenfold from 1×10^7 to 1×10^2 to produce a standard curve, which would, ideally, have a reaction efficiency of 95–105%.

The results of the uniformity and the linearity assays (low standard deviation and optimal reaction efficiencies, respectively) demonstrate that the combination of the Innovadyne Nanodrop II liquid handling system and the CFX384 real-time PCR detection system generates consistent and reproducible real-time PCR data.

Methods

Real-Time PCR System and Reagents Used

The CFX384 real-time PCR detection system, iQ™ supermix, iQ multiplex powermix, Hard-Shell® 384-well skirted PCR plates, and Microseal® 'B' adhesive seals were from Bio-Rad Laboratories, Inc. The following template, primers, and probe were used:

- IL-1β plasmid (I.M.A.G.E. Consortium clone ID 324655, American Type Culture Collection): 10^5 copies/µl for the uniformity studies and 10^7 copies/µl starting concentration for the linearity studies
- IL-1β forward primer: 5' TGC TCC TTC CAG GAC CT 3' (Integrated DNA Technologies, Inc.)
- IL-1β reverse primer: 5' GTG GTG GTC GGA GAT TC 3' (Integrated DNA Technologies, Inc.)
- IL-1β FAM probe: 5' FAM-CTCTGCCCTCTGGATGGCG-Black Hole Quencher 1 (BHQ-1) 3' (TriLink BioTechnologies, Inc.)

Real-Time PCR Protocol

The protocol used for amplification was 95°C for 3 min, followed by 40 cycles of 95°C for 10 sec and 55°C for 30 sec, then a plate read.

General Considerations for a Liquid Handling Robotics Setup

- The deck layout should be the most logical to reduce time and avoid transferring samples directly above open stock tubes
- The liquid handling system should be initialized and primed, making sure to clear all previous faults
- Supermix should be gently mixed by inverting, reagents vortexed, and all tubes briefly centrifuged

Liquid Handling Robotics System Hardware

- Innovadyne Nanodrop II 8-channel fixed-tip system
- An Eppendorf centrifuge tube holder was used on the deck of the stage to aspirate the 7 DNA concentrations
- Optional equipment that could be added, but was not used in this case, included an active wash system and a high-profile wash station for faster and more thorough washing procedures. Additionally, a 16-channel Nanodrop Express system is available for higher throughput

Liquid Handling Robotics System Protocols

- The complete protocols used can be obtained by contacting the authors
- A master mix solution was manually prepared that included iQ supermix or iQ multiplex powermix and the appropriate probe and primer sets
- DNA dilutions were made and manually mixed
- Less than 10% reagent overfill was aspirated in all cases
- All plates were sealed and centrifuged prior to running them in the CFX384 system

Uniformity assay at 3 µl per well:

- The master mix for the 3 µl uniformity assay plate was prepared by combining 750 µl 2x iQ supermix, 238 µl nuclease-free water, 4.5 µl each of 100 µM IL-1β forward and reverse primers, and 3 µl of 100 µM IL-1β probe. 500 µl of 10^5 copies/µl plasmid DNA was added to the master mix just prior to plate dispensing. The reagent was mixed and added to the reagent holder on the deck of the Innovadyne Nanodrop II liquid handling system
- The reagent was then aspirated and dispensed with 8 tips into all wells of the 384-well PCR plate

Linearity assay at 5 or 10 µl per well:

- DNA concentration gradients were prepared by adding 90 µl TE buffer to 10 µl of the initial DNA concentration (10^7 copies/µl) and mixed well. 10 µl of this solution was then added to 90 µl TE buffer and serially diluted until DNA concentrations between 10^6 and 10^2 were generated
- Master mix for the 5 µl linearity assay plate was generated by combining 375 µl 2x iQ multiplex powermix, 201 µl nuclease-free water, 2.25 µl each of 100 µM IL-1β forward and reverse primers, and 1.5 µl of 100 µM IL-1β probe. Volumes of all components were doubled for the 10 µl linearity assay plate
- 11 µl of each DNA concentration and the blank were combined with 50 µl of the master mix solution and mixed well in a strip of centrifuge tubes
- Each final solution was aspirated from the centrifuge tubes on the deck of the Innovadyne Nanodrop II liquid handling system (all 7 solutions were aspirated concurrently)
- 1 column of each solution was dispensed with a single tip into 8 rows each

Results

Uniformity Assay

FAM-labeled hydrolysis probe reaction mix (3 µl) was aliquoted into all the wells of a 384-well plate. As shown in Figure 1, even at a low volume, which can make it challenging to obtain reliable data, all wells exhibited a Cq value of 18.7 ± 0.161, which is within acceptable limits for the standard deviation (<0.2).

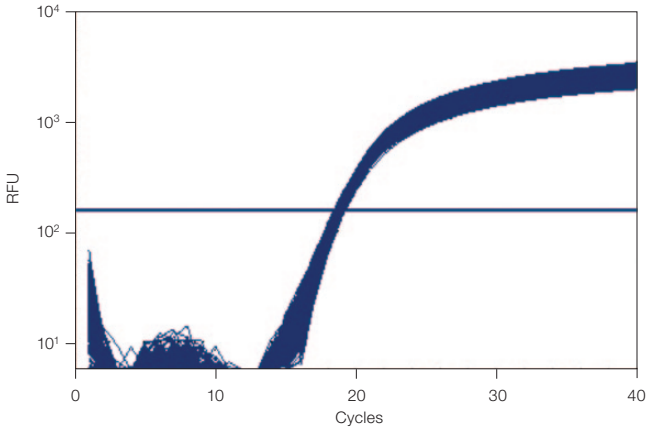

Fig. 1. Excellent low volume (3 µl) uniformity. The same reaction mix (containing a FAM-labeled hydrolysis probe) was dispensed into all wells. Cq standard deviation = 0.161. RFU, relative fluorescence units.

Linearity Assay

For both 5 µl (Figure 2) and 10 µl (Figure 3) reactions, when template was serially diluted tenfold, the resultant standard curves were in the ideal range (95–105%), as were the coefficients of determination (R^2 >0.980).

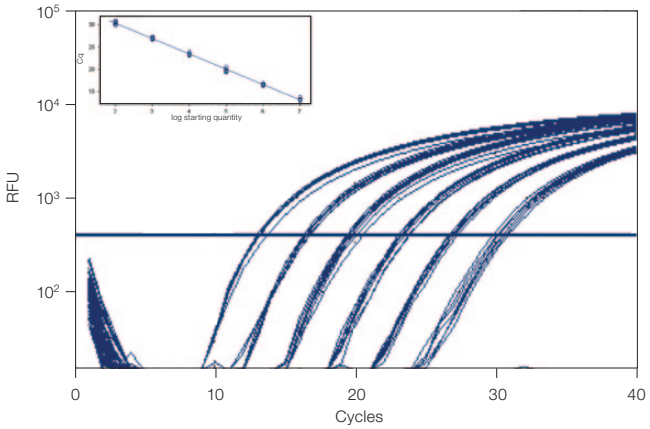

Fig. 2. Broad linear dynamic range using 5 µl reaction volume. Template was serially diluted tenfold with eight replicates each. Inset shows the standard curve. Efficiency = 95.2%, R^2 = 0.998. Cq, quantification cycle; RFU, relative fluorescence units.

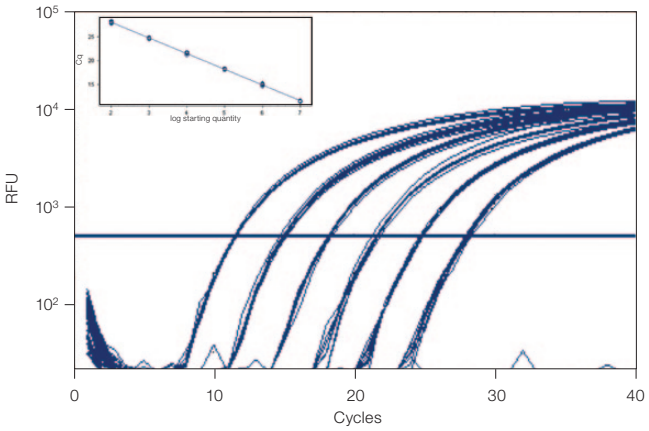

Fig. 3. Broad linear dynamic range using 10 µl reaction volume. Template was serially diluted tenfold with eight replicates each. Inset shows the standard curve. Efficiency = 101.0%, R^2 = 0.999. Cq, quantification cycle; RFU, relative fluorescence units.

Conclusions

As reaction volumes and well sizes decrease and the number of samples that laboratories process increases, it can become a challenge to manually dispense reaction mixes and obtain consistent, reliable data. The results presented in this tech note demonstrate that the Innovadyne Nanodrop II liquid handling robotics system can be easily integrated with the CFX384 real-time PCR detection system to produce 384-well plates containing low-volume reaction mixes that yield high-quality, uniform qPCR data.

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Bulletin 6149 Rev A US/EG *11-1229 0911 Sig 0211*