Technical Note

Integrating the X9 Real-Time PCR System Into an Automated Workstream

Introduction

The capability to efficiently, effectively obtain deep insights from a large number of samples is crucial in many industries and research areas, including agricultural biotechnology, clinical research, and personalized genomics.

Standard BioTools™ proprietary integrated fluidic circuit (IFC) technology delivers high-capacity analytical capabilities that support genomic facilities on the path to discovery, routine quality control, and qualification of genetic materials.

The X9™ Real-Time PCR System is the latest generation of Standard BioTools real-time PCR platforms to support high-capacity genomic analysis, with the power to generate more than 46,000 actual results in an 8 hr shift without need of any dedicated ancillary instrument.

Empowered by Dynamic Array™ IFC consumables, X9 supports commercial and academic laboratories with cost-effective nanoliter-scale reactions, instantaneous implementation or substitution of assays with singleplex setup simplicity, and effortless user experience.

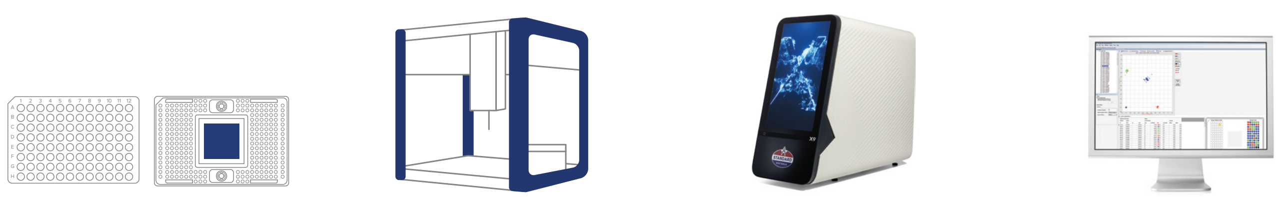
Many laboratories want to integrate analytical workflow steps, such as real-time PCR processing, into automated setups. This is particularly the case with high-throughput genomic facilities.

Here we demonstrate the compatibility and excellent integration of Standard BioTools real-time PCR processes into an automated workstream that does not require any custom redesigning of commercially available automated liquid handler (ALH) systems or configurations.

To prove this, we designed and evaluated a workflow that combines X9 with the OT-2, a benchtop liquid handler from Opentrons (Figure 1).

Our evaluation and results showed the following:

- Easy implementation and setup of an automated workflow that integrates the X9 Real-Time PCR System
- Robust compatibility of conventional ALH pipetting setups with Standard BioTools Dynamic Array IFC products
- Simplification of protocols via autonomous loading of IFC products with minimal operator contribution



1
Prepare
Sample and assay plates or IFC

2
Load robot and start
Let robot run

3
Transfer IFC to X9
Let X9 run

4
Get results

Figure 1. An overview of the automated process demonstrating key steps and user touchpoints.

Results and Discussion

The OT-2 enabled autonomous loading of 3 Standard BioTools IFC formats, the 48.48 Dynamic Array IFC-X, the 96.96 Dynamic Array IFC, and the 192.24 Dynamic Array IFC for Gene Expression (see Table 1 for OT-2 protocol names) (Figure 2A). Automation, via integration with the OT-2, simplified the workflow by eliminating manual pipetting steps, which reduced hands-on time (Figure 2B). Furthermore, reliability of the automated workflows was assessed by real-time PCR looking at cross-contamination, sample validity, and variation across runs of alternating positive and negative (for example, no template control) samples on each IFC (Figure 2C). Cross-contamination (for example, negative samples with positive results) was not observed, and all positive reactions were reported valid. The standard deviation in Ct was <0.08 cycles, demonstrating excellent uniformity across the IFC. These results highlight the compatibility of each IFC with integration in an automated workflow.

Conclusion

Automation simplifies sample loading and reduces hands-on time by eliminating manual pipetting steps. Using an off-the-shelf ALH, the OT-2, the complete automation of IFC loading was demonstrated across the 48.48, 96.96, and 192.24 IFC formats. The results of this study demonstrate the capability to obtain highly uniform data across all IFCs via real-time PCR, 100% validity, and no cross-contamination.

The OT-2 protocols – available from the Opentrons Protocol Library – can be downloaded and adapted to any workflow involving the 48.48, 96.96, or 192.24 IFC formats to support your automation needs.

	A – IFC			B – Automation		C – Results		
	Samples	Assays	Datapoints	Transfers eliminated	Run time (min)	False positives (%)	Valid reactions (%)	Standard deviation (Ct)
48.48	48	48	2,304	12	9	0	100	0.07
96.96	96	96	9,216	24	17	0	100	0.08
192.24	192	24	4,608	32	15	0	100	0.07

Figure 2. Details and results by IFC type. (A) For each IFC, datapoints are generated by interpolating and mixing each sample and assay. (B) Automated OT-2 protocols eliminate manual pipetting steps and reduce hands-on time. (C) Results from each automated OT-2 protocol.

Materials and Methods

Overview and Configuration

To demonstrate IFC compatibility with automation, workflows were developed for the most common Standard BioTools IFC formats: 48.48, 96.96, and 192.24. All pipetting was carried out by an OT-2 pipetting robot (999-00111, Opentrons) equipped with GEN2 pipettes (Single-Channel P300 and 8-Channel P20, Opentrons). Workflows that detail the OT-2 deck setup and protocol files that control the OT-2 are available for download from the Opentrons Protocol Library (protocols.opentrons.com). Each OT-2 protocol file was tested with 2 IFCs, and sample plates, assay plates, and IFCs were freshly prepared as described below.

Standard BioTools Dynamic Array 48.48: Load 4 µL
Standard BioTools Dynamic Array 96.96: Load 4 µL
Standard BioTools Dynamic Array 192.24: Load 4 µL

Table 1. OT-2 protocol names.

Sample Plate Preparation

Sample plates were prepared manually for use in automated IFC loading on the OT-2. Volumes listed are for 96 samples for use on the 96.96 IFC; sample preparation for 48 or 192 samples was scaled accordingly. 348 µL of TaqMan® Fast Advanced Master Mix (4444557, Thermo Fisher Scientific™), 35 µL of 20X GE Sample Loading Reagent (85000735, Standard BioTools), and 87 µL of DNA Dilution Reagent (100-5361, Standard BioTools). The positive sample mix was generated by mixing 225 µL of sample pre-mix with 75 µL of Universal cDNA (C4234565-R, BioChain®). The negative sample mix (for example, no template control) was generated by mixing 225 µL of sample pre-mix with 75 µL of DNA Dilution Reagent. Independent sample mixes were prepared for each IFC. To generate the final sample plate, 6 µL of positive and negative sample mix were transferred to each well of a 96-well plate (89218-290, VWR®) in an alternating pattern. After distribution of the samples, the plate was sealed, centrifuged to ensure the contents were at the bottom of the wells, and transferred to the OT-2.

Assay Plate Preparation

An assay against GAPDH was used in all assay inlets of the IFC. The assay consists of forward and reverse primers and a FAM™ labeled probe. Equal volumes of stock assay and 2X Assay Loading Reagent (100-7611, Standard BioTools) were mixed to create the final assay mix. Final assay mix was prepared in bulk for all IFCs and stored at −20 °C in aliquots until use. A new assay plate was prepared for each IFC run. Assay plates were prepared by distributing 6 µL of final assay mix to each well of a 96-well plate for the 96.96 IFC. For the 48.48 IFCs, 48 wells were filled, and for the 192.24, 24 wells were filled. After distribution of the assays, the plate was sealed, centrifuged to ensure the contents were at the bottom of the wells, and transferred to the OT-2.

IFC Preparation

The 48.48 Dynamic Array IFC-X (102-1950), the 96.96 Dynamic Array IFC (BMK-M-96.96), and the 192.24 Dynamic Array IFC for Gene Expression (100-6266) were used in this study. To prepare the IFCs, the accumulators were manually filled with Control Line Fluid (48.48: 89000020; 96.96: 89000021; 192.24: 100-4058) and the protective film was removed from the bottom of the IFC. The prepared IFC was then transferred to the appropriate location on the OT-2. The 192.24 IFC requires additional reagents for operation. For this IFC, 370 µL of Pressure Fluid (100-6473) and 170 µL of Actuation Fluid (100-6473) were aliquoted into separate 1.5 mL tubes (76332-068, VWR) and placed in the appropriate positions of the OT-2 tube rack.

Analysis

Real-time PCR was performed on X9 (X9-X9). The number of datapoints produced varied by IFC depending on the format. The Ct was calculated for each datapoint. A datapoint was determined to be negative if the Ct was >12 cycles higher than the average Ct for that IFC.

Ordering information

Description	Catalog Number
48.48 Dynamic Array IFC-X – Real-Time PCR	102-1950
48.48 Dynamic Array IFC-X – Real-Time PCR – 10 IFCs	102-1969
96.96 Dynamic Array IFC for Gene Expression	BMK-M-96.96
GE 96.96 Dynamic Array Sample & Assay Loading Reagent Kit – 10 IFCs	85000802-R
192.24 Dynamic Array IFC for Gene Expression	100-6266
GE 192.24 Dynamic Array Reagent Kit – 10 IFCs	100-6267
Control Line Fluid Kit – 48.48	89000020
Control Line Fluid Kit – 96.96	89000021
Control Line Fluid Kit – 192.24	100-4058
GE 192.24 Dynamic Array Reagent Kit – 1 IFC	100-6473
GE 192.24 Dynamic Array Reagent Kit – 10 IFCs	100-6267

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CORPORATE HEADQUARTERS
2 Tower Place, Suite 2000
South San Francisco, CA 94080 USA
Toll-free: 866 359 4354 in the US and Canada
Fax: 650 871 7152
standardbio.com

TECHNICAL SUPPORT
North America | +1 650 266 6100 | Toll-free (US/CAN): 866 358 4354 | techsupport@standardbio.com
Latin America | +1 650 266 6100 | techsupportlatam@standardbio.com
Europe/Middle East/Africa/Russia | +33 1 60 92 42 40 | eu.support@standardbio.com
Japan | +81 3 3662 2150 | techsupportjapan@standardbio.com
China (excluding Hong Kong/Macau) | +86 21 3255 8368 | techsupportchina@standardbio.com
All other Asia-Pacific countries/India/Australia | +1 650 266 6100 | techsupportasia@standardbio.com

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