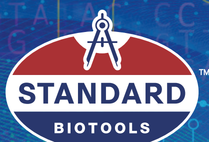

Gene Expression Using Delta Gene Assays on the X9 System

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Introduction

Real-time PCR quantitation with Standard BioTools™ Delta Gene™ assays provides a low-cost alternative to probe-based detection methods. Delta Gene assays use intercalating dye-based detection to perform quantitative PCR analysis of gene expression. This application note demonstrates the compatibility of the X9™ High-Throughput Genomics System with Delta Gene assays and intercalating dyes. Also included is a detailed description of how to perform Delta Gene based real-time PCR on the X9 System using the 48.48, 96.96, and 192.24 integrated fluidic circuit (IFC) formats.

The Standard BioTools X9 System is a high-capacity genomics system that enables cost-effective sample profiling with up to 9,216 individual nanoliter-scale real-time PCR reactions performed concurrently in a single run with minimal operator interaction. Intercalating dye quantitation on the X9 System can offer a cost-effective way to screen large numbers of targets or to optimize panel design and characterization for gene expression applications.

Materials and Methods

A step-by-step description of the materials and methods can be found in Appendix A.

A 16-assay Delta Gene panel targeting ACTB, B2M, CA9, CD19, CD40, COL1A1, CSF2, CSNK2B, GAPDH, MYH2, MYH4, MHY9, MYH15, PROC, RPLP0, and TFRC were designed using the Standard BioTools D3™ assay design service. Assays were pooled and used to preamplify targets in human cDNA derived from brain, heart, and liver along with a universal human cDNA. 18 cycles of preamplification were performed using Preamp Master Mix (Standard BioTools) and the resulting preamplified samples were diluted 1:10 before use. Each preamplified sample was used to create an 8-point, 2-fold dilution series. The 4 resulting dilution series were each run in triplicate on a 96.96 IFC against the 16-assay panel using the 96.96 Express loading method. Real-time PCR was performed in the 96.96 IFC using SsoFast™ EvaGreen® Supermix with Low ROX™ (Bio-Rad).

A custom preset file for real-time PCR based on intercalating dye was created with the Standard BioTools Protocol Editor (v1.0.1) and was run on the X9 System using Standard BioTools Real-Time PCR System Software (v1.0.2). Data were analyzed using Standard BioTools Real-Time PCR Analysis Software (v1.0.2). Average Ct values were calculated from the triplicate datapoints for each sample dilution and assay combination. Detection of expression was determined using the first point of each dilution series. R^2 values were calculated using all datapoints with an average Ct < 20.

Results and Discussion

The X9 System uses IFCs for automated assembly and processing of real-time PCR reactions. IFCs and the X9 System are compatible with a wide range of real-time PCR detection methods, including Standard BioTools Delta Gene assays, optimized for gene expression with the EvaGreen intercalating dye.

Sample	ACTB	B2M	CSNK2B
Universal	0.999	0.999	0.997
Heart	0.995	0.998	0.991
Liver	0.996	0.998	0.992
Brain	0.992	0.998	0.986

Table 1. R^2 values for selected targets

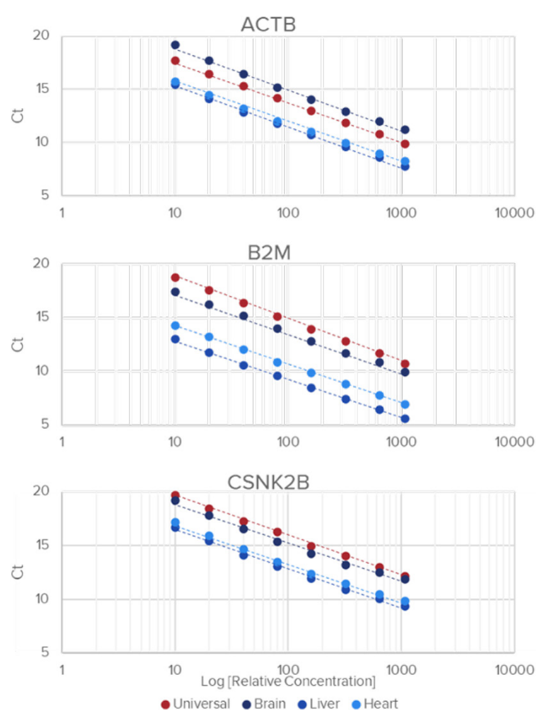


Figure 1. Plots of Ct values vs. Log relative concentration of representative targets show a strong linear relationship.

To demonstrate the compatibility of the X9 System with Delta Gene assays and intercalating dyes, 8-point serial dilutions of cDNA from 4 unique human tissues were run against a panel of Delta Gene assays on the 96.96 IFC. Each point from each of the 4 sample-specific dilution series was run in triplicate, resulting in a total of 96 individual samples on the IFC. All analysis was performed with the average Ct value from the 3 sample replicates. The samples were run against a panel of 16 Delta Gene assays. Each assay was run in 6 replicates for a total of 96 individual assay inputs on the IFC. Each assay replicate was analyzed independently.

Linear amplification is a key parameter of real-time PCR performance. Linearity of Delta Gene assays on the X9 System was examined by calculating an R^2 value for each dilution series and assay with at least 3 points within a Ct range of 5 to 20. The average R^2 value across all detected targets was >0.99. Results of representative targets are summarized in Table 1 and plots are displayed in Figure 1. These results demonstrate the excellent linear performance of Delta Gene assays on the X9 System.

Precision was examined by calculating the Ct standard deviation of the 3 technical replicates for each datapoint used to calculate the R^2 values. The average Ct standard deviation was 0.04, emphasizing the high precision and reproducibility of the X9 System.

To highlight the utility of Delta Gene assays and the X9 System in gene expression profiling, a heat map of average Ct values was generated for each sample across all 16 assays tested (Figure 2A). The results show clear expression differences between the 4 cDNA samples, highlighting the ability of Delta Gene assays to produce gene expression profiles.

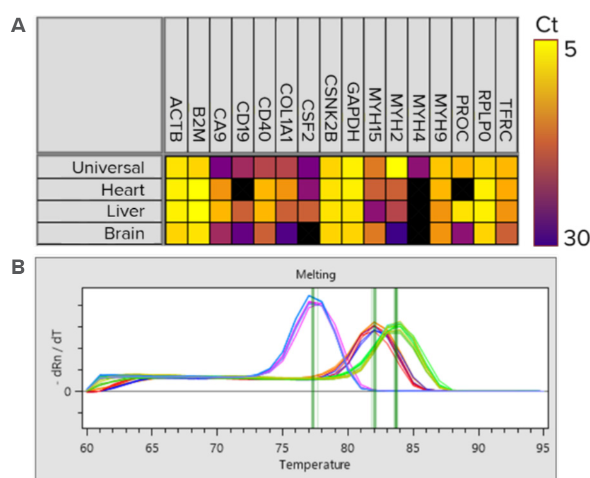


Figure 2. A: heat map of Ct values for all targets examined demonstrating unique expression profiles for all 4 tissue types used in this study. B: Melting curves for, from left to right, RPLPO, CSF2, and CA9.

The X9 System includes the ability to perform melting curve analysis after real-time PCR. This allows the user to monitor the specificity of each assay in each individual reaction using the Standard BioTools Real-Time PCR Analysis Software. As shown in Figure 2B, each assay generates a single peak by melting curve analysis, demonstrating the high specificity of Delta Gene assays.

Summary

Using custom presets created with the Standard BioTools Protocol Editor, the X9 System and associated IFCs are compatible with Delta Gene assays and use of intercalating dye for detection. This allows a cost-effective method to utilize the high-throughput capabilities of the X9 High-Throughput Genomics System.

Appendix A: Protocol for Gene Expression Using EvaGreen Dye on the X9 High-Throughput Genomics System

This appendix describes how to use the X9™ High-Throughput Genomics System or other Standard BioTools™ instrument (Biomark X™) running Standard BioTools Real-Time PCR System Software v1.0.2 or later. When preparing IFCs, follow the guidelines from the Best Practices chapter of the X9 High-Throughput Genomics System Gene Expression and Genotyping User Guide (FLDM-01040).

This application note supports the use of cDNA prepared from RNA samples. Success of a given sample for relative quantification using qPCR depends on both the abundance and the quality of expressed mRNA transcripts within the sample. Lower amounts of total RNA or RNA of lower quality may reduce detection of low-expressed mRNA transcripts. See the X9 System User Guide for recommendations on preparing cDNA from purified RNA samples.

Workflow

1. Prepare the preset file using the Standard BioTools Protocol Editor and import it on the X9 System.
2. Preamplify the cDNA and clean up the preamplification reactions with Exonuclease I.
3. Prepare the final assay and sample mixes.

   NOTE: If you are using a 48.48 IFC-X or a 96.96 IFC with a separate prime step, prime the IFC while preparing the assay and sample mixes. See Appendix B.
4. Inject control line fluid into the IFC.

   NOTE: If you are using a 48.48 IFC-X or a 96.96 IFC with a separate prime step, skip this step.
5. Pipet assay and sample mixes into the IFC. For the 192.24 IFC, pipet Pressure Fluid and Actuation Fluid into the IFC.
6. Run the IFC on the X9 System and collect data.

   Export the run data using a USB drive or to a server on the network, then analyze data using the Standard BioTools Real-Time PCR Analysis Software.

Prepare the Preset File Using the Standard BioTools Protocol Editor

Refer to the Standard BioTools Protocol Editor User Guide (FLDM-01073) for details about creating and using preset files.

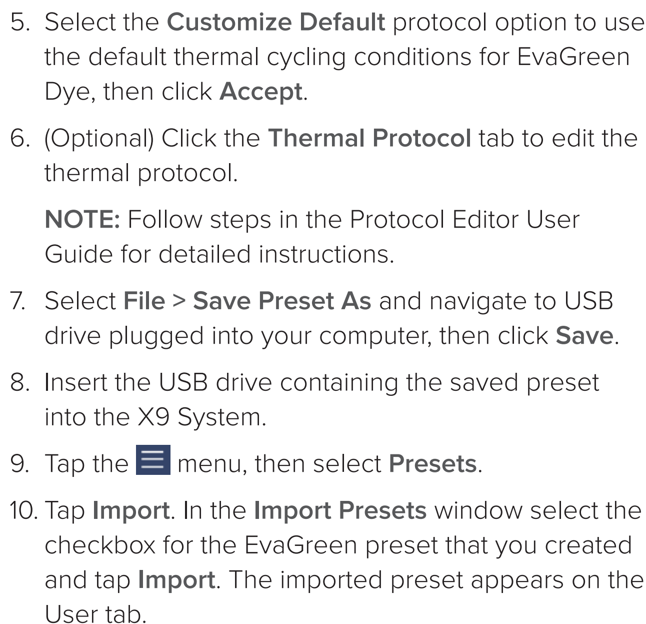
1. Double-click the SBI Protocol Editor icon to open the Protocol Editor.
2. Select **File** > **New Custom Preset** to create a new preset.
3. Under **Source Preset**, select from the drop-down lists:

Application Type	Gene Expression
Source Preset	GE Evagreen or Express GE Evagreen*
IFC	Select IFC type specific to your experiment

* For 96.96 IFCs only. This preset primes, loads, and runs the IFC on the X9 System in 1 step.

4. Enter a name for the preset. This is the name displayed on the X9 System. The description is optional.

5. Select the **Customize Default** protocol option to use the default thermal cycling conditions for EvaGreen Dye, then click **Accept**.
6. (Optional) Click the **Thermal Protocol** tab to edit the thermal protocol.

   NOTE: Follow steps in the Protocol Editor User Guide for detailed instructions.
7. Select **File** > **Save Preset As** and navigate to USB drive plugged into your computer, then click **Save**.
8. Insert the USB drive containing the saved preset into the X9 System.
9. Tap the  menu, then select **Presets**.
10. Tap **Import**. In the **Import Presets** window select the checkbox for the EvaGreen preset that you created and tap **Import**. The imported preset appears on the User tab.

Preamplify cDNA

For detailed information about preparing cDNA, see the cDNA Preparation with Reverse Transcription Master Mix Quick Reference (100-6472).

Pool the Delta Gene Assays

1. In a microcentrifuge tube, combine 1 µL of each 100 µM stock assay. Up to 96 assays can be combined in a single pool.
2. Add DNA Suspension Buffer (Teknova, T0221) to make the final volume 200 µL.

The final concentration of each assay is 500 nM. The total volume can be adjusted proportionally based on the number of samples to be preamplified.

Prepare Preamplification Reactions

1. Thaw the reagents and keep them on ice or in a cold block. Briefly vortex and centrifuge the reagents before use.
2. Prepare the preamplification pre-mix. In a DNA/template-free hood, combine the components in a new, labeled 1.5 mL tube as shown in Table 2.
3. In a DNA/template-free hood, distribute the preamplification pre-mix into each well of a new 8-well strip (for 48.48 IFC-X: 24 µL/well; for 96.96 IFC: 48 µL/well; for 192.24 IFC: 96 µL/well).
4. Using an 8-channel pipette, transfer 3.75 µL of preamplification pre-mix into each well of a new, labeled 96-well plate. Fill 1 well for each sample to be preamplified.
5. Remove plate from the DNA/template-free hood.
6. In a DNA sample hood, add 1.25 µL of cDNA to the appropriate wells of the preamplification plate, making a total volume of 5 µL per well.
7. Tightly seal the plate with clear adhesive film, vortex it for 5 sec, and then centrifuge it at 1,000 × *g* for 1 min.

Component	Vol for 48 Samples (µL)	Vol for 96 Samples (µL)	Vol for 192 Samples (µL)
Preamp Master Mix (100-5744)	52.8	105.6	211.2
Pooled Delta Gene™ assay mix (500 nM)	26.4	52.8	105.6
DNase-free water	118.8	237.6	475.2
Total	198	396	792

Table 2. Preamplification pre-mix

Thermal-Cycle the Preamplification Reactions

Place the preamplification reaction plate in a thermal cycler and cycle using the program in Table 3.

Ten cycles are recommended as a starting point, but this number can be increased to up to 20 cycles if necessary. Determine the appropriate number of cycles empirically. A longer denaturation time could be used if the targets were not adequately denatured, but is not necessary to activate the enzyme.

Clean Up the Preamplification Reactions With Exo I

1. Dilute Exonuclease I (Exo I) to 4 U/µL as shown in Table 4.
2. Evenly distribute the diluted Exo I into each well of a new 8-well strip (for 48.48 IFC-X: 14 µL/well; for 96.96 IFC: 28 µL/well; for 192.24 IFC: 56 µL/well).
3. Using an 8-channel pipette, add 2 µL of diluted Exo I from the 8-well strip to each preamplification reaction for a total of 7 µL per well.
4. Tightly seal the plate with clear adhesive film, vortex it for 5 sec, and then centrifuge it at 1,000 × *g* for 10 sec.

Temperature	Time	Condition
95 °C	2 min	Hold
95 °C	15 sec	10 cycles
60 °C	4 min	
4 °C	∞	Hold

Table 3. Thermal cycler program for preamplification reactions using Preamp Master Mix (100-5744)

5. Place the preamplification and Exo I plate in a thermal cycler and incubate using the program in Table 5.
6. Dilute the final products to an appropriate concentration for testing using DNA Suspension Buffer (Teknova, T0221). The minimum amount of dilution that should be used is 5-fold, but if Ct values are consistently below 6 for some of the assays the dilution factor may be increased to 10-fold or 20-fold.

   STOPPING POINT: You can store diluted reactions at –20 °C for at least 1 week or use within 60 min for real-time thermal cycling.

Component	Vol for 48 Samples (µL)	Vol for 96 Samples (µL)	Vol for 192 Samples (µL)
DNase-free water	84.0	168.0	336.0
Exonuclease I Reaction Buffer (New England Biolabs®, B0293S)	12.0	24.0	48.0
Exonuclease I (New England Biolabs, M0293S or M0293L)	24.0	48.0	96.0
Total	120.0	240.0	480.0

Table 4. Diluted Exonuclease I

Temperature	Time	Condition
37 °C	30 min	Digest
80 °C	15 min	Inactivate
4 °C	∞	Hold

Table 5. Thermal cycler program for preamplification reactions with Exo I

Vol of Preamplification Reaction and Exo I Dilution (µL)	Vol of DNA Suspension Buffer to Add (µL)		
	5-Fold Dilution	10-Fold Dilution	20-Fold Dilution
7	18	43	93

Table 6. Dilution of Exo I-treated preamplified samples

Prime the 48.48 IFC-X or 96.96 IFC

A separate prime step is required before pipetting the assay and sample mixes into the IFC if you are using the:

- 48.48 Real-Time PCR IFC-X, or
- 96.96 IFC and you selected **GE Eva Green** when you prepared the preset file.

Go to Appendix B on page 10 for instructions about priming the IFC.

If you are using the 96.96 IFC with an **Express GE Eva Green** preset file or the 192.24 IFC, a separate prime step is not required. Continue to Prepare Final Assay and Sample Mixes.

Prepare Final Assay and Sample Mixes

Prepare the Assay Mixes

1. Briefly vortex and centrifuge reagents before use.
2. Dilute the 100 µM combined Delta Gene assays in a new, labeled 96-well plate as shown in Table 7. The final concentration of each primer is 5 µM in the inlet and 500 nM in the final reaction. For IFC inlets that do not correspond to a primer, add DNA-free water in place of the primer.
3. Tightly seal the plate with clear adhesive film, vortex it for a minimum of 20 sec, and then centrifuge it at 1,000 × *g* for a minimum of 30 sec to mix and bring down the contents.

Component	Vol for 10 96.96 IFCs or 48.48 IFC-Xs (µL)*	Vol for 10 192.24 IFCs (µL)*
2X Assay Loading Reagent (85000736)	30.0	20
DNA Suspension Buffer (Teknova, T0221)	27.0	18.0
100 µM combined forward and reverse primers	3.0	2.0
Total	60.0	40.0

* Includes overage

Table 7. 10X assay mixes

Prepare the Sample Mixes

IMPORTANT: If using a 192.24 IFC:

- Pipet with care. The 192.24 Delta Gene Sample Reagent (100-6653) is extremely viscous. Do not vortex the 192.24 Delta Gene Sample Reagent by itself at its stock concentration.
- Vortex thoroughly and centrifuge all assay and sample solutions except the 192.24 Delta Gene Sample Reagent before pipetting into IFC inlets. You can thaw the 192.24 Delta Gene Sample Reagent up to 2 times only.

1. Thaw the reagents and keep them on ice or in a cold block. Briefly vortex and centrifuge the reagents before use.
2. Prepare the sample pre-mix. In a DNA/template-free hood, combine the components in a new 1.5 mL tube as shown in Table 8.
3. Vortex the sample pre-mix from Table 8 and centrifuge it at 1,000 × *g* for 10 sec to bring down contents.
4. Prepare the sample mixes as follows:
   a. In a DNA/template-free hood, evenly distribute the sample pre-mix into each well of a new 8-well strip (for 48.48 IFC-X: 24 µL/well; for 96.96 IFC: 48 µL/well; for 192.24 IFC: 80 µL/well).

Component	Vol for 1 48.48 IFC (µL)	Vol for 1 96.96 IFC (µL)	Vol for 1 192.24 IFC (µL)
2X SsoFast EvaGreen Supermix with Low ROX (Bio-Rad, 172-5211, 172-5212, or 172-5213)	180.0	360.0	613
20X DNA Binding Dye (100-7609)	18.0	36.0	—
192.24 Delta Gene Sample Loading Reagent (100-6653)	—	—	61.3
Total	198.0	396.0	674.3

Table 8. Sample pre-mix

Component	Vol for 48 Samples (µL)	Vol for 96 Samples (µL)	Vol for 192 Samples (µL)
Sample pre-mix (see Table 8)	3.3	3.3	2.9
Preamplified and Exo I-treated sample	2.7	2.7	2.3
Total volume	6	6	5.2

Table 9. Final sample mixes

   b. Using an 8-channel pipette, transfer sample pre-mix into each well of 1 or 2 new, labeled 96-well plates as shown in Table 9. Fill 1 well for each sample inlet on the IFC.
   c. Remove the plate from the DNA/template-free hood, and then add preamplified and Exo I-treated sample to the appropriate wells of the sample plate as shown in Table 9. For IFC inlets that do not correspond to a sample, add DNA-free water in place of the sample.
   d. Tightly seal the plate with clear adhesive film, vortex it for a minimum of 20 sec, and then centrifuge it at 1,000 × *g* for a minimum of 30 sec to bring down the contents.

Inject Control Line Fluid Into the 96.96 or 192.24 IFC

Inject control line fluid into the IFC if you are using the:

- 192.24 IFC, or
- 96.96 IFC and you selected **Express GE Evagreen** when you prepared the preset file

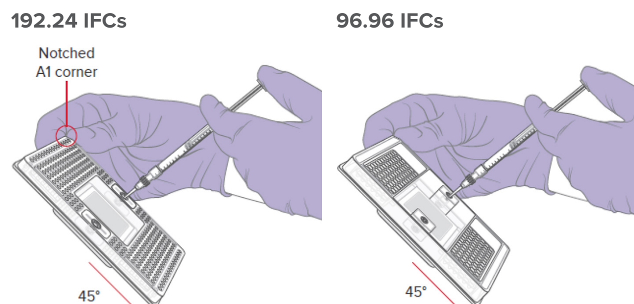
If you are using the 48.48 IFC-X or the 96.96 IFC with a GE EvaGreen preset file, the IFC was primed in a separate step. Skip this section and go to Prepare Final Assay and Sample Mixes on page 5.

IMPORTANT: When injecting control line fluid:

- Follow detailed instructions and best practices for handling IFCs and control line fluid in the X9 System User Guide.
- Only use syringes prefilled with the appropriate volume of control line fluid for the IFC you are using.
- Avoid getting control line fluid on exterior of IFC or in inlets because this makes IFC unusable. If this occurs, use a new IFC.

1. Remove the syringe (for 192.24 IFCs) or syringes (for 96.96 FCs) from the packaging and remove the IFC from the box and foil envelope.

   IMPORTANT: Do not evacuate air from syringe prior to injecting control line fluid (Step 4).



Note the orientation of the A1 corner for the 192.24 IFCs.

Figure 3. Inserting a syringe into an accumulator

2. Place the IFC on a flat surface and actuate the check valve in the top accumulator (for 192.24 IFCs) or each accumulator (for 96.96 IFCs) by pressing gently with the syringe cap.
3. Holding the IFC at a 45° angle, insert the syringe tip into an accumulator (Figure 3).
4. Inject control line fluid into the top accumulator (for 192.24 IFCs) or each accumulator (for 96.96 IFCs) on the IFC. Use the entire contents of the syringe.
5. For 96.96 IFCs only: Inject control line fluid into the other accumulator.

Pipet Assay and Sample Mixes Into the IFC

Refer to the appropriate pipetting map when pipetting final assay and sample mixes into the IFC. Note the orientation of the notched A1 corner. The barcoded edge is on the left side.

1. If using the **48.48-X IFC** (Figure 4) or **96.96 IFC** (Figure 5), pipet assay and sample mixes into the IFC:
    a. Pipet 5 µL of each final 10X assay mix into the respective assay inlets on the IFC.
    b. Pipet 5 µL of each sample mix into the respective sample inlets on the IFC.

If using the **192.24 IFC** (Figure 6), pipet assay and sample mixes and other fluids into the IFC:

a. Pipet 3 µL of each final 10X assay mix into the respective assay inlets on the 192.24 IFC.
b. Pipet 3 µL of each sample mix into the respective sample inlets on the 192.24 IFC.
c. Pipet 150 µL of Actuation Fluid (100-6250) into the P1 reservoir (▬) on the IFC.
d. Pipet 150 µL of Pressure Fluid (100-6249) into each of the P2 and P3 reservoirs (▭) on the IFC.
e. Pipet 20 µL of Pressure Fluid into each of the P4 and P5 inlets (○) on the IFC.

2. Use clear tape to remove any dust particles or debris from the surface of the center of the IFC if necessary.
3. Pull the protective film down and away from the bottom of the IFC. Discard the film.
4. Check the interface plate (XA for the 192.24 IFC, XB for the 96.96 IFC, XC for the 48.48 IFC-X) for dirt or debris and clean it if necessary.
5. Place the interface plate over the IFC, aligning the barcoded edges.

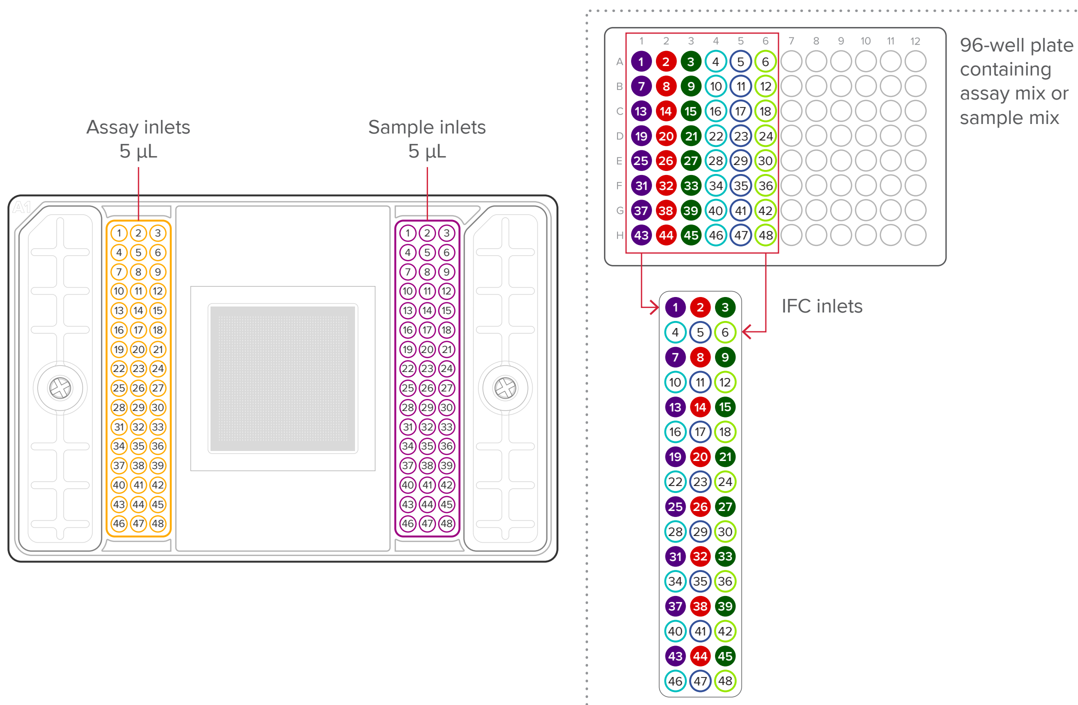


Figure 4. Pipetting map for 48.48 IFC-X

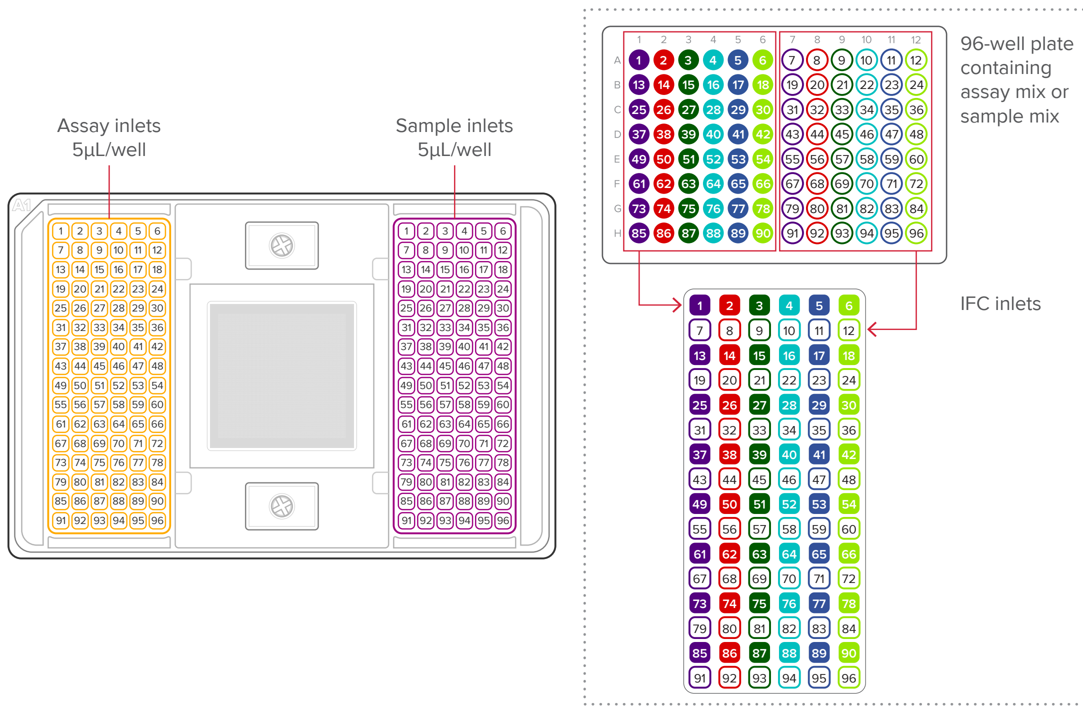


Figure 5. Pipetting map for 96.96 IFCs

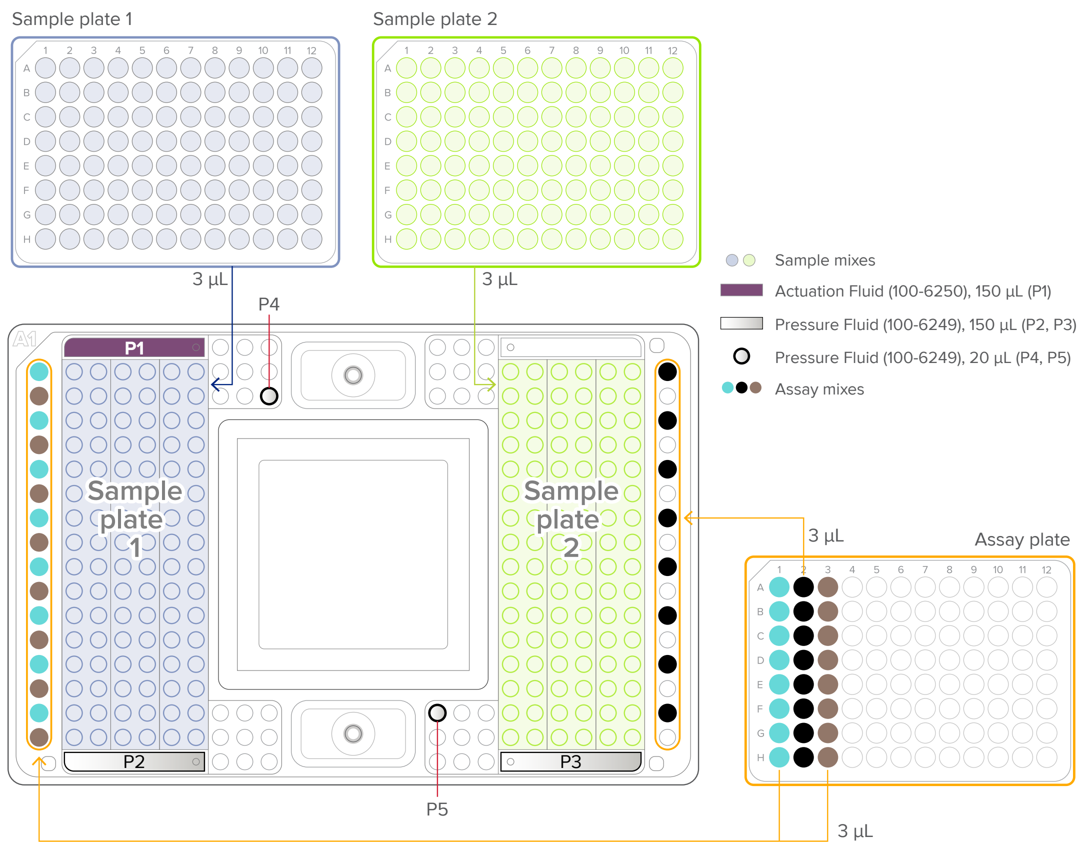


Figure 6. Pipetting map for 192.24 IFCs

Run the IFC on X9

1. Tap the X9 touchscreen to unlock it, and, if required, log in to the instrument.
2. Tap **Open** to eject the instrument tray. Place the IFC (containing control line fluid and assay and sample mixes) and interface plate (XA for the 192.24 IFC, XB for the 96.96 IFC) on the tray. Face the barcoded edges of the IFC and interface plate forward and align the notched corner of the IFC to the notch on the tray.
3. Tap **Next**. The system scans the IFC and interface plate barcodes, and then closes the tray.

   NOTE

   - If Customize Run Name is enabled in Settings, after the tray closes you can enter or change the run name, add notes, and import sample plate and assay plate files (*.plt or *.csv).
   - If using the 48.48 IFC-X or the 96.96 IFC with the **GE Evagreen** preset, the system starts the run.
4. Select the EvaGreen preset file that you imported on the X9 System, then tap **Start**. The system starts the run.
5. When the run is complete, export your data to a USB drive or network location.
6. Tap **Open** to eject the tray, remove the IFC and interface plate from the instrument, and then tap **Close**.
7. Annotate and analyze the exported data using the Standard BioTools Real-Time PCR Analysis Software. For more information about using the software, see the Standard BioTools Real-Time PCR Analysis Software User Guide (FLDM-01051).

Appendix B: Prime the 48.48 IFC-X or 96.96 IFC

Prime the IFC only if you are using the:

- 48.48 Real-Time PCR IFC-X, or
- 96.96 IFC and you selected **GE Evagreen** when you prepared the preset file.

IMPORTANT: When injecting control line fluid:

- Follow detailed instructions and best practices for handling IFCs and control line fluid in the Control Line Fluid Loading Procedure Quick Reference (68000132).
- Only use syringes prefilled with the appropriate volume of control line fluid for the IFC you are using.
- Avoid getting control line fluid on exterior of IFC or in inlets because this makes IFC unusable. If this occurs, use a new IFC.

1. Remove the syringes from the packaging and remove the IFC from the box and foil envelope.

   IMPORTANT: Do not evacuate air from the syringes prior to injecting control line fluid (Step 4).

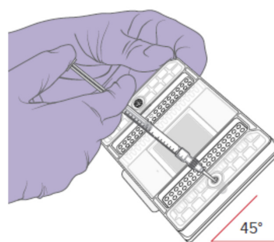
2. Place the IFC on a flat surface and actuate the check valve in each accumulator by pressing gently with the syringe cap.
3. Holding the IFC at a 45° angle, insert the syringe tip into an accumulator (Figure 7).
4. Inject control line fluid into each accumulator on the IFC. Use the entire contents of the syringe.
5. Remove and discard the protective film from bottom of IFC.
6. Place the appropriate interface plate (XC for the 48.48 IFC-X, XB for the 96.96 IFC) over the IFC. Align barcoded edges.
7. On the X9™ High-Throughput Genomics System, tap Open to eject the tray, and then place the IFC (containing only control line fluid) and the interface plate on the tray. Tap **Start**.
8. Select the GE EvaGreen preset file that you imported on the X9 System, and then tap **Start**.
9. When priming is complete, tap **Next**, tap **Open**, then remove the IFC and interface plate from the instrument. Tap **Close**.

IMPORTANT

- Do not leave tray open while pipetting samples and assays into IFC.
- Run the IFC on the X9 System within 60 min of completing IFC priming.

10. Pipet the assay and sample mixes into the primed IFC as instructed in Pipet Assay and Sample Mixes Into the IFC on page 7.

48.48 IFCs



96.96 IFCs

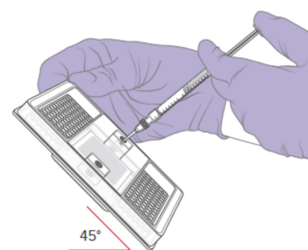


Figure 7. Inserting a syringe into an accumulator

Appendix C: Software Requirements and Ordering Information

Refer to the X9™ System User Guide for information on required equipment and consumables for each GE IFC type.

Required Software

The following software is required for this protocol. For software updates, go to standardbio.com/software.

- Standard BioTools™ Real-Time PCR System Software v1.0.2 or later
- Standard BioTools Real-Time PCR Analysis Software v1.0 or later
- Standard BioTools Protocol Editor v1.0 or later

Reagents From Standard BioTools

To order reagents and IFCs, go to store.standardbio.com.

Delta Gene Assays

Delta Gene™ assays (100 µM each forward and reverse primer stock mixture for each assay of interest) can be designed and ordered through the D3™ Assay Design Group on the Standard BioTools website. For more information, see the D3 Assay Design User Guide (100-6812) or go to d3.standardbio.com.

Reagents and Consumables for the 48.48 IFC-X

Component	Quantity
GE 48.48 Dynamic Array™ DNA Binding Dye Sample & Assay Loading Reagent Kit—10 IFCs (100-3400-R)	1 kit
Preamp Master Mix – 5 Tubes (100-5581)	1 kit
RT Master Mix, 5 Tube Kit (100-6299)	1 kit
Control Line Fluid Kit—48.48 (89000020)	20 syringes
48.48 Dynamic Array IFC-X – Real Time PCR (102-1950)	1 IFC

Reagents and Consumables for the 96.96 IFC

Bundle	Bundle	Quantity
Biomark™ 96.96 Complete Bundle—Delta Gene—10 IFCs (101-0350)	GE 96.96 Dynamic Array DNA Binding Dye Sample & Assay Loading Reagent Kit—10 IFCs (100-3415-R)	1 kit
	Preamp Master Mix – 5 Tubes (100-5581)	1 kit
	RT Master Mix, 5 Tube Kit (100-6299)	1 kit
	Control Line Fluid Kit—96.96 (89000021)	20 syringes
	96.96 Dynamic Array IFC for Gene Expression (BMK-M-96.96)	10 IFCs

Reagents and Consumables for the 192.24 IFC

Bundle	Bundle	Quantity
Biomark 96.96 Complete Bundle—Delta Gene—10 IFCs (101-0351)	192.24 GE Delta Gene Sample and Assay Reagent Kit—10 IFCs (100-6654)	1 kit
	Preamp Master Mix – 5 Tubes (100-5581)	1 kit
	RT Master Mix, 5 Tube Kit (100-6299)	1 kit
	Control Line Fluid Kit—192.24 (100-4058)	10 syringes
	192.24 Dynamic Array IFC for Gene Expression (100-6266)	10 IFCs

Additional Reagents

Product Name	Source	Part Number
SsoFast EvaGreen Supermix with Low ROX	Bio-Rad	172-5211 (5 mL) 172-5212 (10 mL) 172-5213 (20 mL)
Exonuclease I Reaction Buffer	New England Biolabs	B0293S
Exonuclease I, 20 U/µL	New England Biolabs	M0293S (3,000 units) M0293L (15,000 units)
DNA Suspension Buffer	Teknova	T0221
DNase-free water	Major laboratory supplier	—

Learn more at **standardbio.com/x9**

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