

Digital PCR with the qdPCR 37K IFC Using Gene-Specific Assays

For more information, see the Digital PCR Analysis User Guide (PN 68000100) and the Juno System User Guide (PN 100-7070).

Choose a Juno/IFC Controller MX Workflow

Prime	Load and thermal-cycle (PCR)		Image
Juno™	Juno one-step loading and PCR		Biomark™ HD or Biomark or EP1™

Prime	Load	Thermal-cycle (PCR)	Image
Juno or MX	Juno or MX	Juno or FC1™ cycler or Biomark HD/Biomark	Biomark HD or Biomark or EP1

Prime the qdPCR 37K IFC

! IMPORTANT

- Use the qdPCR 37K™ integrated fluidic circuit (IFC) within 24 hours of opening package.
- Due to different accumulator volumes, only use 48.48 syringes with 300 µL of control line fluid.
- Control line fluid on IFC or in the inlets makes IFC unusable.
- Load the IFC within 60 minutes of priming.

- Inject control line fluid into each accumulator on the IFC.
- Remove and discard the blue protective film from bottom of IFC.
- Place the IFC into Juno or MX.
- Choose the instrument and run the script:
 - Juno: **Prime qdPCR 37K**
 - MX: **Prime (167x)**

Prepare Sample Pre-Mix and Samples

- Combine the components in the following table to make the sample pre-mix and the final sample mixture. Scale up appropriately for multiple runs.

Component	Vol./inlet (µL)	Vol./inlet with overage (µL)	Vol./IFC* (µL)
SAMPLE PRE-MIX			
TaqMan® Gene Expression Master Mix (Life Technologies PN 4369016)†	2.0	3.0	180
20X GE Sample Loading Reagent (Fluidigm PN 85000746) ●	0.4	0.6	36
20X gene-specific assays	0.2	0.3	18‡
DNA-free water	0.2	0.3	18
DNA	1.2	1.8	—
Total	4.0	6.0	252

*Enough for 60 reactions

† TaqMan Universal PCR Master Mix (Life Technologies PN 4304437) may be substituted. Fluidigm recommends using TaqMan Gene Expression Master Mix for the qdPCR 37K IFC.

‡ The 20X assay can be removed from the sample pre-mix and added separately if different assays are to be used on the same IFC.

- In a DNA-free hood, combine the TaqMan Gene Expression Master Mix, GE Sample Loading Reagent, DNA-free water, and 20X assay(s) in a sterile tube—enough volume to fill the entire IFC. 4.2 µL of this sample pre-mix can then be aliquoted for each sample (48 total).
- Remove these aliquots from the DNA-free hood and add 1.8 µL of DNA to each, making a total volume of 6 µL in each aliquot.

NOTE For a copy number variation application, substitute RNase P for the DNA-free water.

Load the IFC

! IMPORTANT

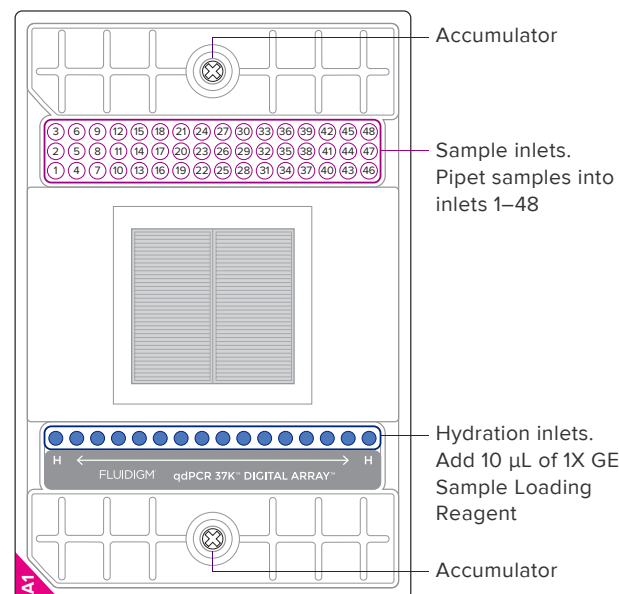
- Vortex thoroughly and centrifuge all sample solutions before pipetting into the IFC inlets. Failure to do so may result in a decrease in data quality.
- While pipetting, do not go past the first stop on the pipette. Doing so may introduce air bubbles into inlets.

- When the prime script has finished, remove primed IFC from the instrument.
- Pipet 10 µL of 1X GE Sample Loading Reagent into all hydration inlets.
- Pipet 4 µL sample mix into the sample inlets on the IFC.
- Return IFC to Juno or MX, then run script according to operation:

Instrument	Operation	Run script	Continue to
Juno	One-step loading and thermal cycling	One Step qdPCR 37K	“Collect End-Point Data”
Juno	Loading only	Load qdPCR 37K	“Thermal-Cycle the qdPCR 37K IFC”
MX	Loading only	Load (167x)	“Thermal-Cycle the qdPCR 37K IFC”

! IMPORTANT Start IFC run within 1 hour of loading samples.

qdPCR 37K IFC Pipetting Map



Thermal-Cycle the qdPCR 37K IFC

Choose the instrument and run the script:

Instrument	Operation	Run script
Juno	One-step loading and PCR	—
Juno	PCR only	PCR qdPCR 37K
Biomark HD or Biomark	PCR and imaging	Continue to “Collect End-Point Data” and select dPCR Standard v1.pcl

To run this protocol as an end-point read using the FC1 cycler or the Fluidigm stand-alone thermal cycler, see the FC1 Cycler Usage Quick Reference (PN 100-1250) or the Stand-alone Thermal Cycler Usage Quick Reference (PN 6800011), respectively.

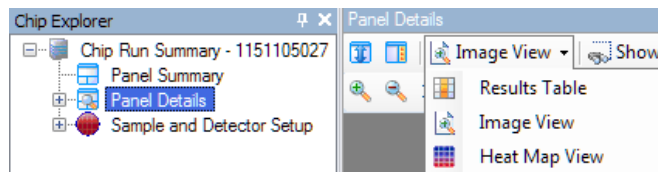
Collect End-Point Data

- 1 Remove any dust particles or debris from the IFC surface.
- 2 Double-click the **Data Collection** icon on the desktop to launch the software.
- 3 Click **Start a New Run**.
- 4 Ensure that the status indicators for the lamp (Biomark and EP1 only) and the camera are green.
- 5 Place the loaded IFC into the instrument. Click **Load**.
- 6 Verify IFC barcode and IFC type.
- 7 Choose project settings (if applicable). Click **Next**.
- 8 Provide a name and select a file storage location for a new IFC run, or browse to select a predefined run file. Click **Next**.
- 9 Choose the application, reference, and probes:
 - a Application type: **Digital PCR**.
 - b Passive reference: **ROX**.
 - c Assay: **Single probe**, **Two probes**, or **More than two probes**.
 - d Select probe types. Click **Next**.
- 10 Browse to and choose the thermal protocol:
dPCR Standard v1.pcl
- 11 Confirm **Auto Exposure** is selected. Click **Next**.
- 12 Verify the IFC run information.
- 13 Click **Start Run**.

Use the Digital PCR Analysis Software

Be sure to click **Analyze** each time you change a parameter in the software.

- 1 Double-click the **Digital PCR Analysis** icon on the desktop to launch the software.
- 2 Click **Open a Chip Run**.
- 3 Double-click a **chiprun.bml** file to open it in the software.
- 4 Click **Sample and Detector Setup** in the Chip Explorer pane.
- 5 Click **New** or **Import**.
- 6 Highlight the wells and then annotate them.
- 7 Click **Editor** in the Sample and Detector Setup pane.
- 8 Choose **Sample Type** from the drop-down menu.
- 9 Enter a name for the sample.
- 10 Choose **Detector Type** from the drop-down menu.
- 11 Enter a name for the detector.
- 12 Click **Update** to see the changes reflected in the highlighted wells.
- 13 Click **Panel Summary** in the Chip Explorer pane.
- 14 Click **Analyze** in the Task pane.
- 15 Click **Panel Summary** or **Panel Details**.
- 16 Choose a view from the drop-down menu:
 - Results Table
 - Image View
 - Heat Map View



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