

PRODUCT SHEET: Q-PCR ANALYSIS ON TOTAL RNA USING THE FLUIDIGM MICROFLUIDIC SYSTEM

1 Fluidigm technology

The BioMark™ system is a real-time PCR equipment developed by Fluidigm. It is compatible with multiple PCR chemistries (Taqman, SybrGreen, EvaGreen, UPL, etc.) on all sample types. It is especially adapted for the simultaneous quantitative expression analysis of hundred genes in a large number of samples. When used in combination with the Fluidigm C1™ Single-Cell Auto Prep System, it offers the opportunity to quantify up to 96 genes in 96 single cells.

PCR reactions are conducted in dedicated arrays, the Dynamic™ Arrays, some integrated fluidic circuits (IFC). For expression analysis, these arrays exist in several formats: 48x48, 96x96 or FlexSix containing 6 independent partitions of 12x12. The loading of primers, DNA samples and reagents is automated using an IFC Controller.

Because of the small final volume of PCR reactions (9 to 6 nl), it is recommended to increase the concentration of target genes in each sample before using the BioMark™ HD. This specific pre-amplification (STA: Specific Target Amplification) consists in a multiplex PCR using a pool of all primers that will be further used in the BioMark™ HD.

When analyses are conducted on single cells, cell capture and lysis, cDNA synthesis by reverse transcription and specific pre-amplification take place in the C1™ Single-Cell Auto Prep System using dedicated microfluidic devices. Several types of C1™ IFC arrays are available depending on the size of the cells to capture (5-10 µm, 10-17 µm and 17-25 µm), but also depending on the final application chosen, Q-PCR on a set of target genes or mRNA Seq.

2 Equipment and materials available

The complete Fluidigm system housed in the GenomEast Platform includes:

- One Biomark™ HD.
- Two IFC Controllers MX and HX.
- One C1™ Single-cell Autoprep system.

In addition to this equipment, the Platform gives access to:

- A dedicated workbench and a DNA-free hood.
- A GeneAmp® PCR System 9700.
- A refrigerate centrifuge for 96-wells plates.
- Two multichannel pipettes with adapted tips (1-10 µl and 5-50 µl).
- A complete set of pipettes with adapted tips (1-10 µl, 5-20 µl, 20-200µl, 200-1000 µl).
- Regular plasticware (8-tubes strips, PCR plates, etc.) and lab equipments (vortex, microcentrifuge, etc.).

3 Conditions for equipment use

The Fluidigm system is open-access, only upon reservation from 8 AM Monday until 17 PM Friday. IGBMC members may access our booking calendar via the intranet from the Platform website. For external users, please contact the Platform by mail (thibault@igbmc.fr).

Be aware that the Platform maintains limited stock of Dynamic™ and IFC arrays as well as reagent kits. It is thus necessary to plan your experiment well in advance and to contact the Platform to evaluate your specific needs. Any demand of reagents and consumables must be accompanied by a submission form available on the Platform website on the page “Project submission”.

At first use, researcher must always be assisted by a qualified user. The use of the Fluidigm system undertakes you to respect the general conditions of equipment use from the GenomEast Platform (DOC05) accessible on its website. Any damage to the equipment owed to carelessness or an inappropriate use will automatically be charged to the user team.

4 Q-PCR analysis on total RNA

4.1 Workflow

1. Reverse transcription of total RNA into cDNA.
2. Pre-amplification of selected targets using a pool of all primer pairs to be further used on the Biomark™ HD.
3. If necessary, treatment with exonuclease to eliminate unused primers.
4. Priming the Dynamic™ array.
5. Dilution and loading of pre-amplified amplicons and primer pairs on the Dynamic™ Array using the appropriate IFC controller.
6. Real Time PCR on the Biomark™ HD.

4.2 Samples and assays requirements

Researchers must prepare their total RNA samples. Upon request, quantification by fluorimetry and validation by capillary electrophoresis of input RNAs may be performed by Platform members at extra cost. Using Fluidigm Master Mix, reverse transcription reactions are carried out in 5 µl with 1 µl of total RNA samples. Up to 1.25 µL of this reaction can be used in a 5-µL pre-amplification reaction.

Requirements for total RNA input	
Quantity per reaction*	2 ng to 250 ng, provide for 10-14 cycles of pre-amplification. 2.5 pg to 2 ng, provide for 15-20 cycles of pre-amplification.
Quality	DO260/DO280 ≥ 1.8. RIN ≥ 7 and absence of genomic contamination on an Agilent Bioanalyzer profile.

* It may be necessary to run a preliminary experiment to determine the optimal conditions for pre-amplification. We recommend to use 100 ng of total RNA with 14 cycles of pre-amplification as a starting point.

Researchers must choose their preferred PCR chemistry then, design and order assays for their selected genes. Primers need to be designed to reduce the potential for primer-dimer formation.

For Taqman chemistry, Applied TaqMan® Gene Expression Assays should consist of a 20X mix of unlabeled PCR primers and TaqMan® MGB probe (FAM™ dye-labeled). For EvaGreen chemistry, assays should come as a forward and reverse primer mix with each primer at a final concentration of 100 µM.

4.3 Arrays and reagents provided by the Platform

The Platform provides, as needed, the appropriate Gene Expression Dynamic™ Arrays (96x96, 48x48 or FlexSix) as well as reagents for all steps from reverse transcription of total RNA up to Q-PCR on the Biomark™ HD:

Reagents	Part Number	Dynamic™ arrays	PCR chemistry
Reverse Transcription Master Mix	Fluidigm, PN 100-6298, 100-6299	-	-
PreAmp Master Mix	Fluidigm, PN 100-5580, PN 100-5581	-	-
Exonuclease I	New England BioLabs, PN M0293L	-	-
DNA Suspension Buffer (10mM Tris-HCl, 0.1mM EDTA, pH 8.0)	InVitrogen P/N 12090-015	-	-
2X Assay Loading Reagent	Fluidigm, PN 100-5359 = PN 85000736	FlexSix, 96x96, 48x48	EvaGreen and TaqMan
FLEXsix DELTAgene Sample Reagent	Fluidigm, PN 100-7673	FlexSix	EvaGreen
20X DNA Binding Dye Sample Loading Reagent	Fluidigm, PN 100-3738	96x96, 48x48	EvaGreen
20X GE Sample Loading Reagent	Fluidigm, PN 85000735 = PN 85000746	96x96, 48x48	TaqMan
SsoFast™ EvaGreen® Supermix with Low ROX	Bio-Rad Laboratories, PN 172-5211	FlexSix, 96x96, 48x48	EvaGreen
TaqMan Gene Expression PCR Master Mix (2X)	Life Technologies, PN 4369016	FlexSix, 96x96, 48x48	TaqMan

4.4 Reference protocols

The following user guide, accessible on Fluidigm website, provides information about the analysis software and all protocols for Real-Time PCR on the BioMark™ HD:

- Real-time PCR analysis (Fluidigm, PN 68000088 J1)