Microarray and sequencing platform

GenomEast Platform
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PRODUCT SHEET: Q-PCR ANALYSIS ON SINGLE CELLS USING THE FLUIDIGM 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 plastic ware (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).

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Be aware that the Platform maintains limited stock of Dynamics™ and C1™ 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 single cells

4.1 Workflow

1. Priming the C1™ IFC array
2. Preparing and loading the cells on the C1™ IFC array.
3. Imaging the cells with a microscope.
4. Cell lysis, reverse transcription and specific target pre-amplification on the C1™.
5. Harvest of the pre-amplified amplicons.
6. Dilution and loading of pre-amplified amplicons and primer pairs on the Dynamic Array using the appropriate IFC controller.
7. Real Time PCR on the Biomark™ HD.

4.2 Cells and assays requirements

Researchers must determine the exact size of their cells as this will determine the type of IFC array that will be used for cell capture (5-10 µm, 10-17 µm or 17-25 µm). The evaluation of cell concentration is also essential for optimal capture rate. A Scepter™ 2.0 Cell Counter from Merck Millipore is available on the Platform.
Special care must be taken to avoid cell debris as they may plug the channels in the IFC array. It may be required to filter your cell suspension in a 20-30 micron filter/gauze such as the PARTEK CellTrics® (not provided by the Platform).

The optimal number of cells to load on the IFC array is 1,000, the minimal number is 200. The volume of cell suspension loaded on the array may vary from 5 to 20 µl. However, the final volume retained on the array is only 5 µl. The final cell suspension is composed of a mixture of “C1 Cell Suspension Reagent” and your cells in their native media, according to a 4:6 ratio (Reagent:Cell). This ratio must always be respected regardless the final volume of cells loaded on the array.

Useful recommendations may be found on “Fluidigm single-cell preparation guide”, Fluidigm PN 100-7697 C1.

Initial Cell Supension in native media	Mixture C1 Cell Suspension Reagent : Cells (40µl:60µl)	Final quantity of cells loaded on the IFC array (5µl)
333 K cells/ml	20,000 cells/100 µl	1,000 cells
250 K cells/ml	15,000 cells/100 µl	750 cells
166 K cells/ml	9,960 cells/100 µl	498 cells
66 K cells/ml	3,960 cells/100 µl	198 cells

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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 concentration of 100 µM.

4.3 Arrays and reagents provided by the Platform

The Platform provides the C1™ IFC Array adapted to the size of the cells to capture: 5-10µm, 10-17 µm or 17-25 µm.

It also distributes all reagents for processing cells on the C1™ as a reconditioned kit containing the appropriate volume of solutions CT1 to CT14 (see below) for one array.

Finally, it furnishes all reagents for Q-PCR analysis on the Biomark™ HD using EvaGreen or TaqMan chemistry.

If needed, Ambion RNA spikes and primers as recommended in Fluidigm protocol using EvaGreen assays are also available.

Reagents	Part Number	Arrays	PCR chemistry
C1 Single-Cell Auto Prep Reagent Kit: • C1 DNA Dilution Reagent (CT1) • C1 Lysis Plus Reagent (CT3) • C1 Loading Reagent (CT7) • C1 PreAmp Dilution Reagent (CT9) • C1 Harvest reagent (CT10) • C1 PreLoading Reagent (CT11) • C1 Blocking Reagent (CT12) • C1 Cell Wash Buffer (CT13) • C1 Cell Suspension Reagent (CT14)	Fluidigm, PN 100-5319	C1™ IFC arrays	-
Single Cell-to-CT kit: • Single Cell Lysis Solution (CT2) • Single Cell Stop Solution (CT4) • Single Cell Vilo (CT5) • Single cell SuperScript RT (CT6) • Single Cell PreAmp Mix (CT8)	Ambion, PN 4458237	C1™ IFC arrays	-
Arraycontrols RNA spikes (optional): • Premix of RNA Spikes (#1, #4, #7) ready to use	Ambion, PN AM1780	C1™ IFC arrays	EvaGreen
RNA spike assays kit (optional)	Fluidigm, PN 100-5582	C1™ IFC and Dynamic™ arrays	EvaGreen
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	Dynamic™ arrays	EvaGreen and TaqMan
20X DNA Binding Dye Sample Loading Reagent	Fluidigm, PN 100-3738	Dynamic™ arrays	EvaGreen

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20X GE Sample Loading Reagent	Fluidigm, PN 85000735 = PN 85000746	Dynamic™ arrays	TaqMan
SsoFast™ EvaGreen® Supermix with Low ROX	Bio-Rad Laboratories, PN 172-5211	Dynamic™ arrays	EvaGreen
TaqMan Gene Expression PCR Master Mix (2X)	Life Technologies, PN 4369016	Dynamic™ arrays	TaqMan

4.4 Reference protocols

- Using C1 to Capture Cells from Cell Culture and Perform Preamplification Using Delta Gene Assays (Fluidigm, PN 100-4904).
- Using C1 to Capture Cells from Cell Culture and Perform Preamplification Using TaqMan Assays (Fluidigm, PN 100-6117).
- Real-time PCR analysis (Fluidigm, PN 68000088 J1).