



Application on QuantStudio[™] 12K Flex Real-Time PCR System





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Genomic Assays Portfolio – Core Content



Genomic Assays Portfolio – Core Content



Gene Expression Studies

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Comparison of Expression Profiles between different samples



TaqMan[®] Gene Expression Assays More than just primers and a probe

- Ready to use
- Work every time
- Universal reaction conditions
- Spliceform specific
- Bar-coded
- SNPs/repeats/pseudogenes masked
- Specificity (white paper)
- 3 unique hybridisation events
- 3' Minor Groove Binder & Non-Fluorescent Quencher
- We define the position of the probe
- Detailed annotation & GeneAssistTM Software visualisation





Gene expression assay coverage





TaqMan[®] Gene Expression Family

Same TaqMan[®] Assays, same results, same confidence



What is an OpenArray[®] Plate?

Each through-hole is filled with 33 nl of reagents



OpenArray Plate Layout								
Subarrays	48 (12 x 4)							
Through-holes	64 per subarray (8 x 8)							

	Da	ata Points	per O	penArray P	late						
	(Genotyping 3,072									
	Re	eal Time P	CR	2,688							
ems®	Gibco®	Molecular Probes®	Novex [®]	TagMan®	Ambion®						

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Novex[®]

Ambion

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Assay Loading System

 Assays loaded in
 OpenArray[®] plates using a proprietary loading system







TaqMan[®] OpenArray[®] GEx Pathway Panels

Overview

- Six fixed-content GEx OpenArray[®] Pathway Panels containing 597 to 828 pre-selected TagMan[®] assays
- High throughput analysis of commonly studied gene families involved in disease states, processes, and cellular regulation
- Endogenous control genes for normalization

Applications

- Drug discovery and biomarker validation
- Pharma research
- Clinical disease research

Throughput & Ordering

- Up to 64 samples/day
- 43,000 reactions/day
- Minimum order size 1 panel
- Inventoried
- 2-3 days shipping
- Please contact your local Sales Representative for pricing and to place an order



Research

- Which genes are turned on or off?
- Regulation relative to known genes
- Hypothesis-driven discovery
- Validation
- Screening



TaqMan[®] OpenArray[®] GEx Pathway Panels

- Human Kinome Panel QuantStudio[™] 12K Flex System
 - P/N 4472326, Format 896, 3 samples/panel
- Human Cancer Panel QuantStudio[™] 12K Flex System
 - P/N 4472329, Format 672, 4 samples/panel
- Human Inflammation Panel QuantStudio[™] 12K Flex System
 - P/N 4472327, Format 672, 4 samples/panel
- Human Stem Cell Panel QuantStudio[™] 12K Flex System
 - P/N 4472328, Format 672, 4 samples/panel
- Human Signal Transduction Panel QuantStudio[™] 12K Flex System

Gibco

Molecular Probest

Novex[®]

TagMan[®]

- P/N 4472330, Format 672, 4 samples/panel
- Mouse Inflammation Panel QuantStudio[™] 12K Flex System



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Applied Biosystems¹



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Ion Torrent

Ambion[®]

TaqMan[®] OpenArray[®] Custom Gene Expression

• <u>Choose :</u>

- Over 1.3 million TaqMan Gene Expression assays
- Over 61,000 inventoried assays
- Custom assays



18 (3x) Assays, 48 Samples 56 Assays, 48 Samples



112 Assays, 24 Samples



224 Assays, 12 Samples



Ambion®

168 Assays, 16 Samples

TaqMan[®]

Novex*



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Genomic Assays Portfolio – Core Content



TaqMan[®] Assays



miRNA Functions

- Development (cell fate)
- Cell death (apoptosis)
- Epigenetics

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miRNA in Human Disease

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- Diagnostic markers
- Therapeutic targets
- Therapeutic agents

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Assay Objective:

 Develop a Real-Time PCR TaqMan[®] Assay to quantify mature* microRNAs

*Mature miRNAs are the biologically active form



Precursor miRNA



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TaqMan[®] MicroRNA Assays Design



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Individual Assays Summary

Total of >11500 predesigned Individual Assays

- 1500 Human
- 1050 Mouse
- 650 Rat
- 46 Endogenous controls



RNU24, RNU66, RNU19, RNU38B, RNU49, RNU48, Z30, RNU6B, RNU44, RNU43, U18, RNU58B, RNU58A, RPL21, U54, HY3, U75, U47, snoRNA135, snoRNA142, snoRNA202, snoRNA234, snoRNA251,...

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TagMan[®]

- Additional Assays for more than 100 species
- Additional assays released regularly

Custom TaqMan[®] Small RNA Assays



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Profiling with TaqMan[®] Array miRNA cards

Megaplex[™] Pools and TaqMan[®] MicroRNA Arrays



- Benefits
 - Gold standard TaqMan[®] Assay quality data
 - Streamlined workflow data within single working day
 - Significant sample savings as low as 1ng input



MicroRNA QuantStudio[™] 12K Flex Panels

- Broad coverage
 - Run three samples per OpenArray[®] plate
 - 818 assays per sample (754 unique assays)
 - > 3 positive and 1 negative/process control per subarray
- High-throughput
 - 12 samples per ~2.5 hr instrument run
 - 48 samples per 8hr working day
- Cost effective
 - Rapid sample screening with minimal hands-on time
- Minimal sample requirement
 - 100ng of total RNA recommended for a full profile





Novex

Ambion

OpenArray[®] miRNA Profiling Panels

OpenArray[®] Human MicroRNA Panel QuantStudio[™] 12K Flex

- 754 assays/3 samples per panel
- Minimum order size 1 panel
- Inventoried
- 2-3 days shipping
- P/N 4470187

Sample	Sample	Sample
1	2	3
•	-	

OpenArray[®] Rodent MicroRNA Panel QuantStudio[™] 12K Flex

- 748 assays/3 samples per panel
- Minimum order size 1 panel
- Inventoried
- 2-3 days shipping
- P/N 4470188





OpenArray[®] miRNA Panel Profiling Workflow



Genomic Assays Portfolio – Core Content



How can SNPs be used in research?

SNPs with biological effect

- Pharmacogenetics
 - Drug metabolism e.g. Cytochrome p450
- Disease research:
 - e.g. Factor V Leiden, sickle-cell anemia



SNPs used as genetic markers

- Population genetics
 - Disease association
 - > "Association Study"

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TaqMan[®] SNP Genotyping Assays

4.5 million pre-designed human SNP assays for high-density, genome-wide coverage

- 3.5 million HapMap SNPs providing 70% HapMap coverage
- 70,000 coding SNP assays for the detection of SNPs within coding regions, including many putative functional SNPs
- >2,600 Drug Metabolism assays for 220 drug metabolism and transporter genes





Dynamics of Allelic Discrimination

Competition between the two probes

Tm of the mismatched probe < Tm of matched probe



Annealing/extension temperature of 60°C allows:

- binding and cleavage of correct probe
- destabilization of incorrect probe



Real Time Genotyping



life

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TaqMan[®] SNP Genotyping Workflow



TaqMan[®] OpenArray[®] Custom Genotyping **Plates**

Custom Genotyping Plates:

- Inventoried DME assays
- •Over 4.5 million Pre-Designed assays
- •Custom assays

256 Assays x 12 Samples

TaqMan® OpenArray® Off-the-Shelf Panels

Genetic Barcode Panels:

- Genotyping Genetic Barcode Panel 32A
- Genotyping Genetic Barcode Panel 32B
- Genotyping Genetic Barcode Panel 64

Pharmacogenetics Panel

Digital PCR with QuantStudio[™] 12K Flex Real-Time PCR System

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PCR Evolution/Revolution

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Introduction – Digital PCR as a complement to qPCR

- Digital PCR is a new approach to PCR quantification which provides greater precision and sensitivity relative to real-time PCR
- Samples are partitioned across hundreds to thousands of reactions
- Digital PCR is ideal for <u>rare events</u> detection
- Digital PCR enables <u>direct absolute quantification</u> of nucleic acids in a sample

Digital PCR Overview

Digital PCR is an analytical technique for absolute quantification of nucleic acid samples based on PCR amplification of single template molecules

Use the ratio of positive (red) to negative (Black) PCR reactions to count the number of target molecules

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Molecule Counting Requires A "Correction" Factor

- Problem: Due to random assortment, we cannot be assured that each positive reaction received only a single molecule
 - Must have at least one negative reaction (reaction with no molecule)
 - Probability of a reaction receiving zero, one, two, three etc. copies is described by the **Poisson model**
 - Poisson statistics "corrects" for reactions containing multiple molecules and provides a "probability" that our answer is correct

Optimal concentration ("sweet spot") for Digital PCR on OpenArray[®]plate is ~ 30 copies/uL

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Digital PCR: Benefits *Digital Enrichment*

- Allows detection of single target molecules, even at low concentrations in a heterogeneous sample.
 - Per-well signal-to-noise ratio improves dramatically with dilution.

Digital PCR: Benefits *Absolute quantification*

Can obtain absolute quantities (copies per reaction) without using a standard curve.

Group	Average Copies Per Reaction	Lower onfidence Level	Upper Confidence Level	Total Replicates
BKVseries, BKV, 24.38650000	6.0425	4.9462	8.9206	767
BKVseries, BKV, 10.97390000	3.7534	3.3236	4.2389	768
BKVseries, BKV, 4.93830000	1.6199	1.4836	1.7687	768
BKVseries, BKV, 2.22220000	0.8031	0.7283	0.8856	768
BKVseries, BKV, 1.00000000	0.3212	0.2805	0.3678	768
BKVseries, BKV, 0.45000000	0.1839	0.1547	0.2186	768
BKVsingle,BKV,1.00000000	0.8477	0.8079	0.8896	3072
Empty,Digital Assay, 1.00000000	NaN	NaN	NaN	0

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QuantStudio[™] Digital PCR Workflow

Digital PCR Applications

Rescue of ambiguous qPCR results

Reference standards

Open Array[®] System for Viral DNA Detection

- DNA extracted from AcroMetrix Primary Standards (EBV, CMV, & BK)
- Cocktail Reactions with OpenArray[®] TaqMan[®] Digital PCR Master Mix
- I dPCR run: 3 OpenArray[®] plates (1 plate per sample)
- 768 replicates per concentration

Relative dilution factors

Reference standards

Results : Heat map

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Actual	
cp/hole	95% Interval
0.039	0.0267 - 0.0554
0.062	0.0463 - 0.0825
0.122	0.0987 - 0.1500
1.210	1.1063 - 1.3236

Actual	
cp/hole	95% Interval
0.059	0.0439 - 0.0793
0.116	0.0935 - 0.1435
0.286	0.2480 - 0.3297
3.060	2.7574 - 3.3964

Actual	
cp/hole	95% Interval
0.097	0.0769- 0.1224
0.165	0.1379- 0.1982
0.362	0.3177- 0.4113
3.508	3.1280- 3.9348

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Number of Replicates Determines Digital PCR Precision

Precision is dependent upon number of replicates

3 plates

 Confidence intervals relate to number of reactions & ratio of negatives to total

1

Depending on goal of experiment, throughput or precision can be
 emphasized

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QuantStudio™ Digital PCR: Workflow

DigitalSuite™ Software

- Maintains existing OpenArray[®] Digital PCR Software functionality AND ...
- Seamless integration into QuantStudio™
 Data Collection and Analysis software
 - One-click launch from QuantStudio[™] software
 - Same architecture as GEx, GT software
- Improved workflow
 - Fewer steps to answer
 - Direct calculation of copies / uL
 - Faster plate set-up, no need to generate template file
 - Real-time amp curves and heat-map views allow user QC of Digital results

- Supports duplex analysis
 - Visualization of FAM[™] and VIC[®] dye digital results
- Fully licensed
 - 30-day free trial or

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Digital PCR: DigitalSuite[™] Software

- Edit sample information independent of primary software
- Auto-filters unfilled wells
- Includes tool to help plan experiments
- Results include amplif curves, copy #/µl, Heat-Maps, Confidence Intervals, Precision data,...
- Exportable Excel[®] Spreadsheet results
- View amplification events graphically
- Provides graphs ready for use in

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Editor Actions	_									_	_		_		_				
Sample	A1	A2	A3	A4	AS	A6	A7	A8	A9	A10	A11	A12		Selected Sub	array med	,	Reject Changes		
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Assay	C1									C10		C12	â	OpenArray SN	ID	Sample	Assay	Dilution	
RNaseP 🏷	D1					D6				D10	D11	D12		EJM89	A1	NA17203	RNaseP	1.000000	
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	<u> </u>	<u></u>	<u> </u>	C4	<u> </u>	0	Ľ	<u> </u>	<u> </u>	C10	CII	C12	per	EJM89	A7	NA17211	RNaseP	1.000000	
Apply to Selected	D1			D4		D6	D7	D8	D9				0	EJM89	A8	NA17211	RNaseP	1.000000	
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Openarray Mate: EUM09 Subarray: C11	B1	B2	B3	B4	BS	B6	B7	B8	B9			B12	rra	EJM89	A12	NA	Digital Assay	1.000000	
Sample: NA	<i>C</i> 1	0	0	C 4	CE.	<i>c</i> (67	<i>c</i> 0	<i>c</i> 0	C10	C11	C12	I A	EJM89	B1	NA17203	RNaseP	1.000000	
Assay: Digital Assay		C2	- C3	C4	- C5	C6	<u> </u>	C8	Lea	C10	CII	C12	per	EJM89	B2	NA17203	RNaseP	1.000000	
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	_									1	1			EJM89	B4	NTC	RNaseP	1.000000	

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Merci pour votre attention

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