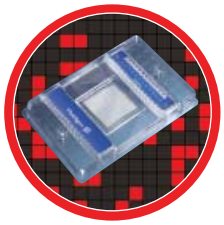
The Study Of Copy Number Variations (CNVs) Using the 12.765 Digital Array™ IFC

Copy number variation (CNV) is a polymorphism studied extensively because of its close association with drug metabolism, cancer, immune diseases, and neurological disorders. In addition, CNV has been used to understand the full spectrum of human genetic variation and also to assess the significance of such variation in disease-association studies. The current method of analyzing CNVs mainly depends on SNP-based microarrays and comparative genomic hybridization and is therefore subject to low sensitivity and low resolution. Current systems based on microwell plates are also limited in that they do not reliably distinguish less than a two-fold difference in copy number of a particular gene in DNA samples.



The 12.765 DIGITAL ARRAY IFC accepts 12 samples and partitions each into a panel of 765 replicate reactions.

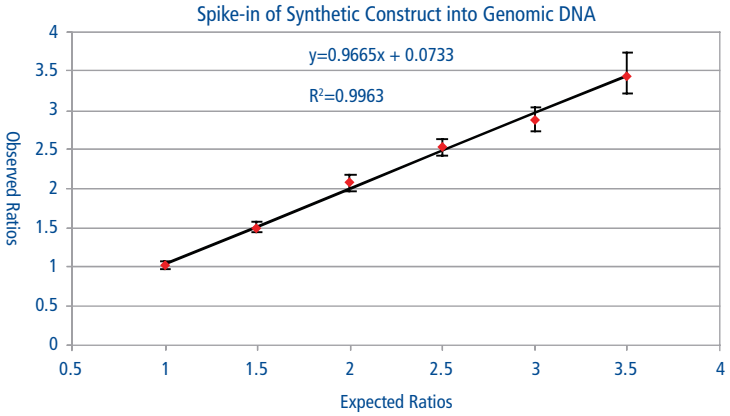
The 12.765 Digital Array IFC

The 12.765 Digital Array IFC enables a new level of sensitivity and flexibility in detecting copy number variations. The Digital Array IFC works by partitioning a single sample into 765 individual 6 nL real-time qPCR reactions. The concentration of any sequence in a DNA sample (copies/µL) can be calculated using the number of positive chambers, that contain at least one copy of that sequence.

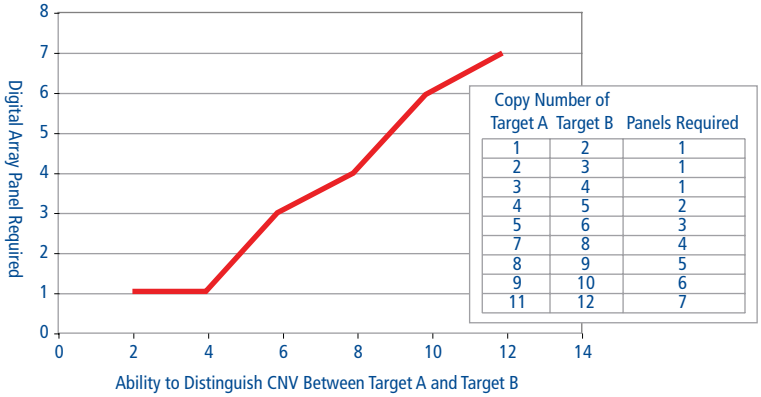
CNV Calculation

The CNV calculation is based on "relative copy number" so that apparent differences in gene copy numbers in different samples are not distorted by differences in sample amounts. The relative copy number of a gene (per genome) can be easily expressed as the ratio of the copy number of a target gene to the copy number of a single copy reference gene in a DNA sample, which is always 1. By using two assays for the two genes (the gene of interest and the reference gene) with two fluorescent dyes on the same Digital Array IFC, we are able to simultaneously quantitate both genes in the same DNA sample. The ratio of these two genes is the relative copy number of the gene of interest in a DNA sample.

Sensitivity



Our data shows that the Digital Array IFC is able to reliably discriminate between as few as four and five copies of a gene (a 0.2-fold change). This platform provides a 10-fold greater differentiation power than conventional real-time qPCR.

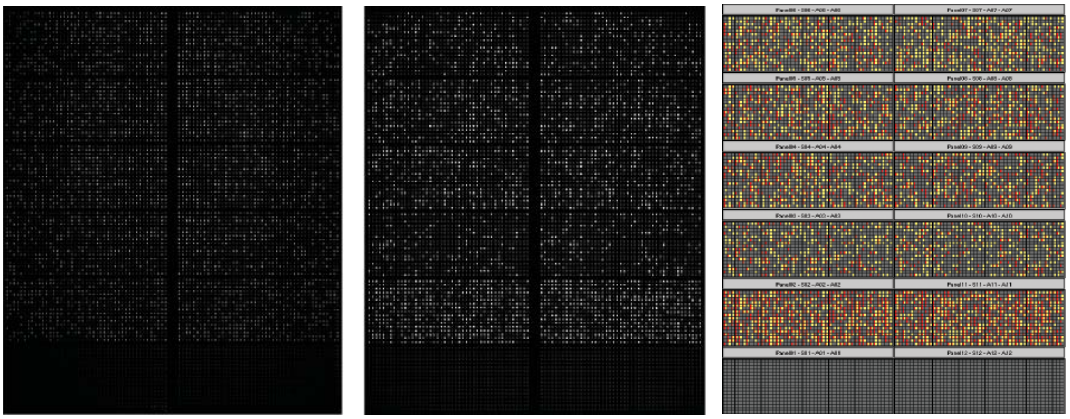


Copy Number of Target A	Target B	Panels Required
1	2	1
2	3	1
3	4	1
4	5	2
5	6	3
7	8	4
8	9	5
9	10	6
11	12	7

As shown above, discriminatory power increases with the use of replicate assay panels. The greater the replicate panels, the more accurate the copy number estimation. Thus, the use of one panel distinguishes a single copy difference between two targets whereas seven panels are necessary to discriminate between 11 and 12 copies.

Experiment

The copy numbers of the CYP2D6 gene in five human DNA samples (Coriell Cell Repositories) were measured using RNase P, a single copy gene, as reference. Reaction mixes containing the TaqMan® reagents (Applied Biosystems) for both genes and DNA samples were loaded into a Digital Array IFC and then thermal cycled on a BioMark Real-Time PCR System.



RNase P (VIC) CYP2D6 (FAM)

Five DNA samples were analyzed in this digital array for two genes (RNase P (VIC TaqMan® assay) and CYP2D6 (FAM TaqMan® assay)). Copy numbers of both genes were used to calculate the ratio of the CYP2D6 gene to the RNase P gene for each sample. The two bottom panels were NTC (no template control).

(1) VIC (RNase P) and FAM (CYP2D6) images of the same Digital Array IFC taken at the end of the PCR reaction.

(2) Software-generated composite heatmap showing the chambers with positive signals for either or both genes, each labeled with a different fluorescent dye (yellow and red in the figure).

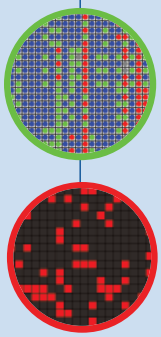
	DIGITAL PCR		
SAMPLES	CYP2D6 /RNASE P	# CYP2D6 PER CELL	ALLELIC STATUS
1	0.53	1	DELETION
2	0.97	2	NORMAL
3	0.98	2	NORMAL
4	0.48	1	DELETION
5	1.51	3	DUPLICATION

Conclusion

Conventional technologies — such as comparative genomic hybridization, high-density SNP genotyping arrays — are limited for CNV analysis for the following reasons: They require large amounts of material; are time-consuming; subject to reagent variability; may produce results that have high rates of false positives, low resolution, and low sensitivity; and often lack a fine degree of quantitative discrimination (e.g. real-time qPCR). In comparison, the Digital Array IFC accurately quantitates copy number while conserving materials and time and allowing flexibility of assay design.

WORK FLOW

1. **Prime**
   Prime the IFC to prepare for samples and assays.
2. **Transfer**
   Transfer samples and assays into separate inlets on the IFC.
3. **Load**
   Place the IFC on the IFC controller to automatically setup reaction chambers.
4. **Thermal Cycle**
   Place the IFC onto the FC1™ Cycler and start the PCR protocol.
5. **Read**
   Place the IFC on the EP1™ Reader for fluorescence detection.
6. **Analyze**
   Use analysis software to view and interact with results for the run or for multiple runs.



Corporate Headquarters
Fluidigm Corporation
7000 Shoreline Court, Suite 100
South San Francisco, CA 94080 USA
Toll-free: 1.866.FLUIDLINE (1.866.358.4354)
Fax: 650.871.7152
www.fluidigm.com

Sales
North America: 650.266.6170 | biomark@fluidigm.com
Europe/EMEA: +33 1 60 92 42 40 | biomarkeurope@fluidigm.com
Japan/Korea: +81 3 3555 2351 | biomarkasia@fluidigm.com
Asia: biomarkasia@fluidigm.com

MRKT00078g