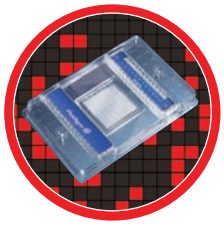
Enabling Loss of Heterozygosity Studies Using Fluidigm Digital Arrays

Loss of heterozygosity (LOH) refers to a change from a heterozygous state in a normal genome to a homozygous state in a paired tumor genome. Research shows that the loss of an entire X chromosome is involved in numerous cancers[1]. For example, 40 percent of ovarian cancers are associated with LOH for regions of the X chromosome[2]. Also, the gain of an X chromosome has been shown to be relatively common in leukemias and lymphomas[3]. In this application note, we demonstrate that the 12.765 Digital Array enables a new level of sensitivity and flexibility in detecting LOH, through experiments on abnormal X chromosome copy number, or aneuploidy. This condition is remarkably common and, therefore, a good model system for other LOH experiments.



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

The 12.765 Digital Array

The 12.765 Digital Array (Figure 1) is an integrated fluidic circuit (IFC), which partitions a single sample into 765 individual 6nL reactions. The ratio of any two sequences in a DNA sample can be calculated using real-time qPCR curves or end point images of positive chambers for one assay versus another assay. Compared to traditional technologies for LOH studies, such as comparative genome hybridization (CGH)[4] and microarray based molecular inversion probe technology (MIP)[5], the digital array offers greatly improved linearity, sensitivity, and ease of use.

Experiments

DNA from cell lines containing 1, 2, 3, 4 or 5 copies of the X chromosome (Coriell Institute for Medical Research) were obtained. Digital arrays were used to test each sample against three separate X chromosome TaqMan® primer-probe sets — FAM-labeled 123B, SMS, and YY2 (BioSearch Technologies) — which were co-amplified in the presence of a single-copy-targeting, VIC-labeled "reference" sequence. Each test was run in duplicate panels within digital arrays. (See Fig. 1.)

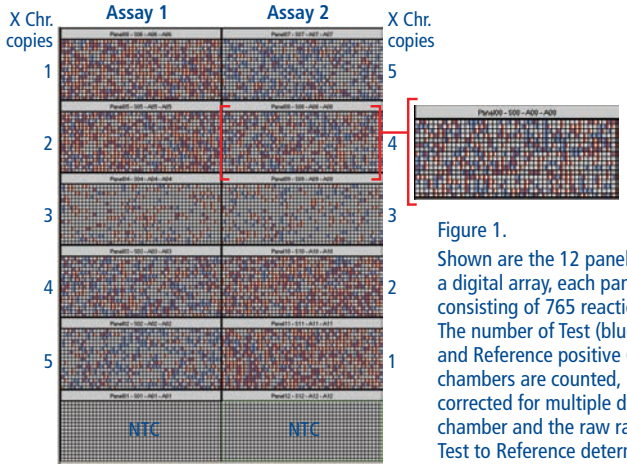


Figure 1.
Shown are the 12 panels of a digital array, each panel consisting of 765 reactions. The number of Test (blue) and Reference positive (red) chambers are counted, corrected for multiple dyes per chamber and the raw ratio of Test to Reference determined.

Simple Linear Fitting to Obtain Copy Numbers

Figure 2 shows the average of three separate assay ratios (Y axis) plotted against known X chromosome copy number (X axis), including error bars that show the standard error of the mean. The ratios produce slopes for DNA samples known to contain 1, 2, 3, 4 or 5 copies of the X chromosome. The individual raw ratio measurements were multiplied by 2 and averaged to obtain copy number per genome. The average response for all assays, over 1-to-5 copy number variants, produced an r^2 value of 0.994, indicating extremely high linear assay performance.

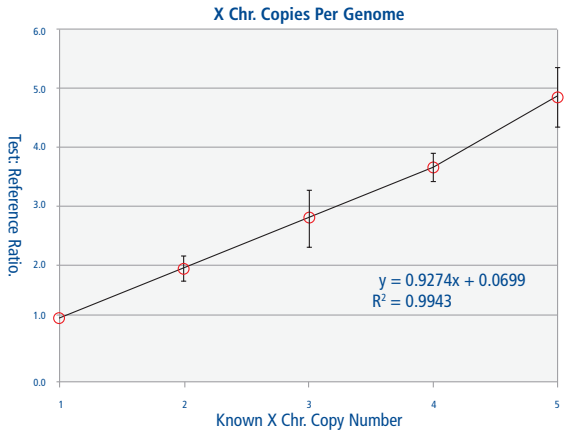


Figure 2.

Experiment Results

The table below provides the raw ratios for individual X chromosome tests. The X chromosome mean copies per genome is determined by multiplying the mean ratio by 2. The last column on the right shows the standard error of the mean (SEM).

KNOWN X CHR. COPY NUMBER	RAW FAM123 RATIO	RAW SMS RATIO	RAW YY2 RATIO	MEAN COPIES PER GENOME	SEM
1X Chr.	0.51	0.49	0.61	1.0	0.07
2X Chr.	0.77	1.15	0.96	1.9	0.22
3X Chr.	1.10	1.19	1.86	2.8	0.48
4X Chr.	1.63	2.05	1.79	3.6	0.24
5X Chr.	2.03	2.34	2.90	4.8	0.51

The samples selected for these tests are similar or identical to those examined in CGH assays[4] and MIP-based microarrays studies[5]. Results from this experiment using digital arrays produced copy number estimations at least as discriminating as CGH and MIP determined methods while significantly reducing hands-on technical manipulation. Moreover, the ability to run multiple TaqMan® assays in a digital PCR format provides both biological robustness and assay redundancy, compensating for assay-to-assay amplification differences. If multiple loci are targeted simultaneously, overall assay results are valid even if there are single mutations or deletions at localized primer–probe binding sites.

Conclusions

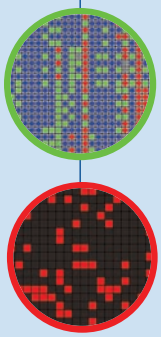
Using the Fluidigm 12.765 Digital Array allows researchers to distinguish small, yet biologically relevant, differences in gene copy number within highly complex genomic DNA samples.

References

1. Moertel CA, Dahl RJ, Stalboerger PG, Kimmel DW, Scheithauer BW, Jenkins RB. “Gliosis specimens contain clonal cytogenetic abnormalities.” *Cancer Genet Cytogenet* 1993:67:21–7.
2. Osbome RJ, Leech V. “Polymerase chain reaction allelotyping of human ovarian cancer.” *Br J Cancer* 1994;69:429–38.
3. Sandberg AA. “The X chromosome in human neoplasia, including sex chromatin and congenital conditions with X-chromosome anomalies. In: Sandberg AA, editor. Cytogenetics of the mammalian X chromosome, part B: X chromosome anomalies and their clinical manifestations. New York: Alan R. Liss, 1983:459–98.
4. Visakorpi T, Hyytinen E, Kallioniemi A, Isola J, Kallioniemi OP. ”Sensitive detection of chromosome copy number aberrations in prostate cancer by fluorescence in situ hybridization.” *Am J Pathol* 1994; 145:624–30.
5. Pinkel D, Segraves R, Sudar D, Clark S, Poole I. Kowbel D, Collins C, Kuo,W, Chen C, Zhai Y, et al. (1998) “High resolution analysis of DNA copy number variation.”

WORK FLOW

1. **Prime**
   Prime the IFC to prepare for samples and assays.
2. **Transfer**
   Transfer samples and assays into separate inlets on the chip.
3. **Load**
   Place the IFC on the IFC controller to automatically setup reaction chambers.
4. **Thermal Cycle**
   Place the IFC onto the Stand-Alone Thermal Cycler and start the PCR protocol.
5. **Read**
   Place the IFC on the EP1 Reader for fluorescence detection.



Fluidigm®

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: +31 20 578 8853 | biomarkeurope@fluidigm.com
Japan/Korea: +81 3 3555 2351 | biomarkasia@fluidigm.com
Asia: +65 9431 3790 | biomarkasia@fluidigm.com

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