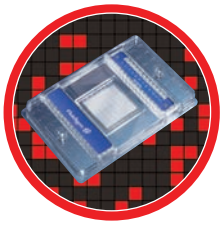
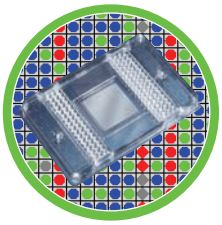
Efficient Drug Metabolism Studies Using the Fluidigm System

The Fluidigm system streamlines drug metabolism studies by allowing accurate copy number and SNP genotyping studies to be performed on a single, easy-to-use system. In this experiment, we use Fluidigm digital arrays and dynamic arrays to determine the copy number of the CYP2D6 gene and the SNP status of the CYP2D6 and CYP2C9 genes.

The CYP2D6 gene is involved in the metabolism of 20–25 percent of clinical drugs[1] and exhibits copy number variations (CNVs) and single nucleic acid polymorphisms (SNPs) linked to 10 percent of adverse drug reactions (ADRs)[2]. Another polymorphism of the CYP450 gene, CYP2C9, causes significantly altered metabolism of certain drugs, such as warfarin, and thus dramatically different therapeutic effects of a given dosage.



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



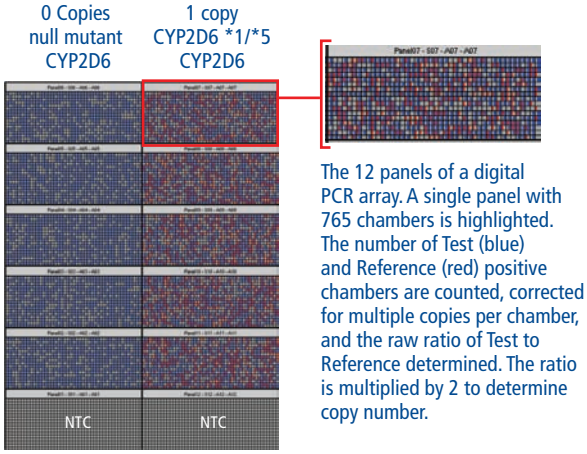
The **48.48 DYNAMIC ARRAY** accepts 48 samples and 48 primer-probe sets and combines them into 2,304 assays.

The 12.765 Digital Array

The 12.765 Digital Array is an integrated fluidic circuit (IFC) that enables a new level of sensitivity and flexibility in detecting copy number variations. The digital array works by partitioning a single DNA sample into 765 individual 6 nL real-time qPCR reactions. Real-time qPCR curves are used to determine the number of chambers positive for a particular sequence — those containing at least one copy of the target sequence — and thus the concentration (copies/µL). By using two primer-probe assays, each with a different wavelength reporter, the ratio of the two targets can be easily calculated.

Quantification of CYP2D6 Copy Number

Fluidigm 12.765 Digital Arrays were used to determine the number of copies of the CYP2D6 gene in CYP2D6 DNA samples (ParagonDx) relative to RNase P, a single copy reference gene. The CYP2D6 DNA samples were loaded into the arrays, without prior optimization, and co-amplified with RNase P. The digital array produces a positive curve in any reaction chamber where a single copy of the CYP2D6 gene is present. The FAM (test) and VIC (reference) positive chambers were then counted and a calculation was performed to determine the ratio of CYP2D6 copies in the sample relative to RNase P. The output ratio is multiplied by 2 to obtain copy number(s) per genome.



The 12 panels of a digital PCR array. A single panel with 765 chambers is highlighted. The number of Test (blue) and Reference (red) positive chambers are counted, corrected for multiple copies per chamber, and the raw ratio of Test to Reference determined. The ratio is multiplied by 2 to determine copy number.

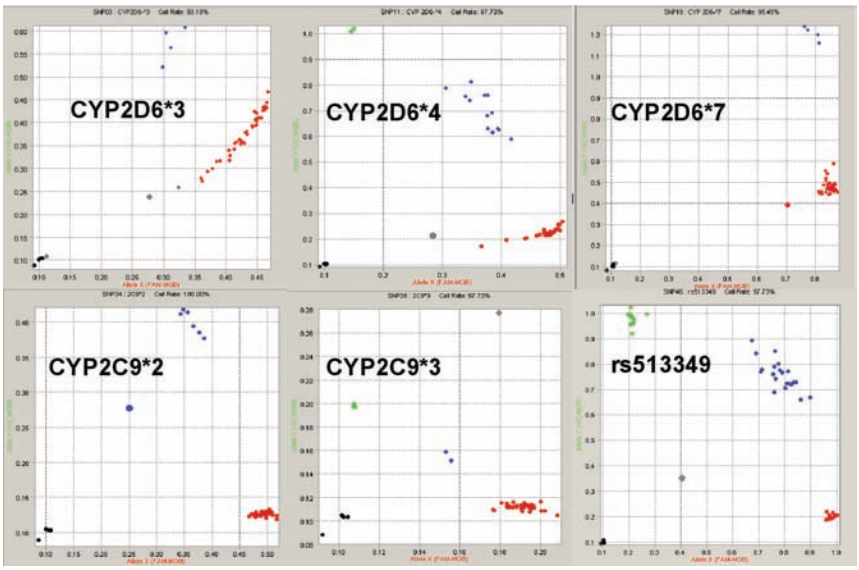
DNA Copy Number

Shown below are the copy number ratios of CYP2D6 to the RNase P reference gene as well as the confidence intervals (CI). The possible results for this application include 0, 1, and 2 copies per genome, although more than 2 copies are also detectable. CYP2D6 copy number calculated as a ratio of CYP2D6 assay and RNase P reference assay. The possible results include a deletion, single copy, and wild-type. (The digital array is also capable of identifying replications.)

SAMPLE NAME	REFERENCE DNA COPY NO. STATUS	95% CI LOW	COPY NO. PER GENOME	95% CI HIGH
CYP2D6 5/5	DOUBLE DELETION	0.0	0.0	0.0
CYP2D6 1/5	1 COPY	0.9	1.0	1.0
CYP2D6 1A/1A	2 COPIES (WILD-TYPE)	1.8	2.0	2.1

The 48.48 Dynamic Array — SNP Genotyping

The 48.48 Dynamic Array is an IFC that allows users to multiplex 48 gDNA samples against 48 primer-probe assays, for a total of 2,304 individual reactions in a single chip. The dynamic array was used to call genotypes for the DNA used in the experiment. Results show 99.9 percent concordance between replicate runs and successful cluster separation, without the need to optimize the assays for the Fluidigm system. Cluster diagrams below point to the generic applicability and robustness of the system using off-the-shelf reagents.



Conclusion

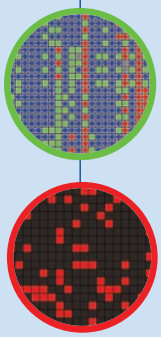
The results demonstrate that the Fluidigm system provides scientists and clinical researchers with a cost effective and practical solution to determine SNP genotypes and copy number variation of genes. The results are highly accurate and provide superior quality CNV data when compared to conventional methods. The system is an ideal platform for conducting clinical research for determining patient's SNP and CNV status that may lead to personalized drug dosing to reduce the risk of ADRs for the relevant pharmaceuticals.

References

1. Ingelman-Sundberg, M. (2004) "Pharmacogenetics of cytochrome P450 and its applications in drug therapy: the past, present, and future." *Trends Pharmacol.* 25:193–200.
2. Lazarou, J., Pomeranz, B.H., and Corey, P.N. (1998) "Incidence of adverse drug reactions in hospitalized patients: a meta-analysis of prospective studies." *JAMA*, 279, 1200–1205.

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.



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