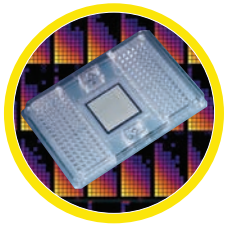
Determining Copy Number Variation (CNV) Using Dynamic Arrays on the BioMark™ System

The Fluidigm 96.96 Dynamic Array provides significant advantages for CNV determination in studies involving large numbers of samples or CNV loci or for those requiring much higher CNV resolution than is achievable using traditional qPCR. With the 96.96 Dynamic Array, only 192 pipetting steps (96 for samples, 96 for primer-probe sets) are required to assemble 9,216 qPCR reactions. This pipetting efficiency reduces the potential for liquid-handling errors while providing the flexibility and ease-of-use to run sufficient technical replicates for a required resolution.



The **Fluidigm 96.96 Dynamic Array** is an integrated fluidic circuit that accepts 96 samples and 96 primer-probe sets and assembles them into 9,216 qPCR reactions.

Experiments

A statistical model was developed to predict the number of replicates required to achieve the desired resolution for CNV discrimination. Subsequently, experiments were performed, using the 96.96 Dynamic Array, to test the modeled predictions.* Analysis was done on subsets of the same dataset, varying the number of sample and/or assay replicates (n = no. of sample replicates * no. of assay replicates). All experiments were performed on the 96.96 Dynamic Arrays and with Fluidigm instrumentation — the IFC Controller HX for loading and the BioMark™ Real-Time PCR System for thermal cycling and fluorescence detection. Prior to CNV analysis, the primer-probe sets were tested for assay efficiency using a standard curve to prevent bias in the $\Delta\Delta$ C_t calculations.

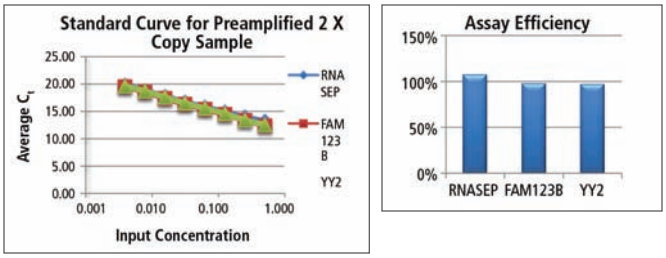


Figure 1. Determining assay efficiency. A serial dilution of the pre-amplified sample was used to determine the reference and target assay efficiencies. Assay efficiency must be uniform to assure bias-free $\Delta\Delta$ C_t calculation.

Results

Determining the relative copy number (RCN) of a target sample relative to a reference requires the calculation of the average C_t for all samples in the target gene(s) and reference gene. The standard deviation of the sample C_t and the number of measurements that determine the mean are used to determine the standard error of the mean (SEM). In general, the 95% confidence limit is defined as follows: 1.96 * SEM or 1.96 *σ/sqrt(n), for n measurements, and standard deviation σ; the factor of 1.96 is the 95% z value for a normal distribution. Properties of a normal distribution are assumed because n is large (>30). The RCN for a target sample is calculated as follows: RCN = $2^{-\Delta\Delta C_t + 1.96 * SEM_{\Delta\Delta C_t}}$ if we assume that the efficiencies of our target and reference assays are similar to and close to "1". As n increases, the SEM decreases, and the estimate of the RCN is improved as shown in the figures.

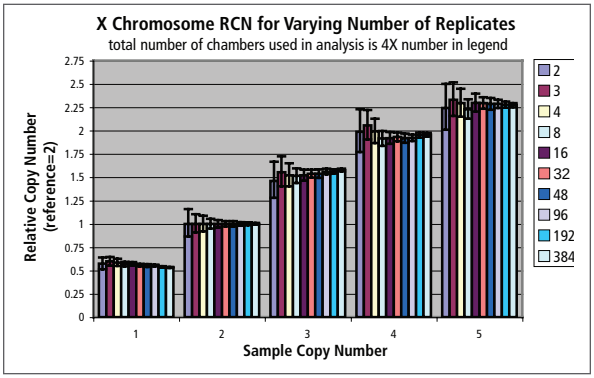


Figure 2. *RCN and confidence limits for varying number of replicates.* Confidence limits may underestimate error when n < 8 (n = no. of sample replicates * no. of assay replicates per sample per assay).

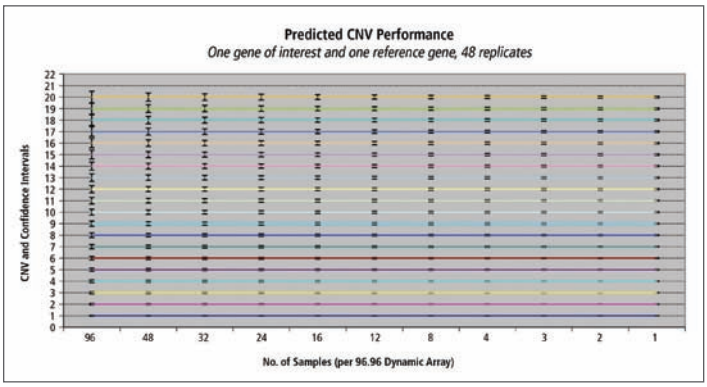


Figure 3. Maximum resolution. With only 2 targets (target of interest and a reference target), the confidence interval is sufficiently low to detect the difference between copy numbers as long as the error bars do not touch.

		Predicted Result					Experimental Result Gene 1					Experimental Result Gene 2				
		CNV Resolution Required					CNV Resolution Required					CNV Resolution Required				
# of Samples	# of Targets	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
96	6	0.13	0.21	0.30	0.38	0.47	0.08	0.17	0.44	0.39	0.40	0.08	0.17	0.39	0.35	0.41
48	6	0.09	0.15	0.21	0.27	0.33	0.05	0.12	0.28	0.28	0.29	0.05	0.12	0.25	0.25	0.29
24	6	0.06	0.11	0.15	0.19	0.23	0.05	0.08	0.18	0.20	0.20	0.05	0.08	0.17	0.19	0.20
12	6	0.05	0.08	0.11	0.14	0.17	0.04	0.06	0.13	0.14	0.13	0.04	0.05	0.12	0.13	0.13
6	6	0.03	0.05	0.07	0.10	0.12	0.03	0.04	0.09	0.09	0.10	0.03	0.04	0.09	0.09	0.10
4	6	0.03	0.04	0.06	0.08	0.10	0.01	0.02	0.03	0.04	0.04	0.01	0.02	0.03	0.04	0.05

Table 1. Measured confidence interval range for two target genes. The range is compared to the predicted estimates. Results for both genes closely match the expected results, validating the model as a tool in determining the number of replicates required for a given sensitivity.

Conclusion

Scientists who study CNVs benefit significantly from a statistical model as a guide to determine quickly and easily the optimal number of samples and target replicates to obtain the desired resolution. Results of experiments on the 96.96 Dynamic Array closely match the expected values in the model. Moreover, the 96.96 Dynamic Array provides an ideal format for CNV studies using the $\Delta\Delta C_t$ method because the pipetting efficiency and throughput minimize pipetting errors and allow calibrators and reference samples to be run with the assays in parallel.

References

[1] Taylor, John R., An Introduction to Error Analysis, University Science Books, 1982, p.56

[2] Livak, K., Schmittgen, T., Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the $2^{-\Delta\Delta C_t}$ Method, Methods. 2001 Dec;25(4):402-8

[3] S. Dube, et al, "Resolution of a Nanofluidic Biochip for Copy Number Variation and Application to X Chromosome Aneuploidy," Proceedings of IASTED International Symposium on Computational Biology and Bioinformatics (CBB 2008), Orlando, Florida, Nov 2008. ArXiv e-preprint, arXiv:0809.1460v2, Oct 2008, URL http://arxiv.org/abs/0809.1460v2

*The results are for example only and may differ depending on specific experimental conditions.

Contributing Scientists

Suzanne Weaver, Alain Mir, Simant Dube, Jian Qin, and Ramesh Ramakrishnan, of Fluidigm Corporation.

WORK FLOW

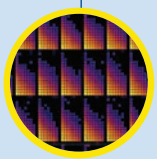
1 Prime
Prime the dynamic array to close the interface valves, preventing premature mixing of samples and assays.

2 Transfer
Pipette samples, premixed with master mix, into separate sample inlets and the primer-probe sets into separate primer-probe inlets on the frame of the chip.

3 Load
Place the dynamic array on the IFC controller, and use the software interface to pressure load the assay components into reaction chambers. Assay components are automatically combined on-chip.

4 Run
Place the dynamic array on the BioMark Real-Time PCR System for thermal cycling and fluorescence detection.

5 Analyze
Use real-time qPCR Analysis software to view and to interact with amplification curves, color-coded heat maps, and C_t data for the run.



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

MRKT00116