Miniaturizing PCR with the Nanodrop™

Cost Reduction with Conservation of Sample

www.innovadyne.com

leading the way in high-precision dispensing

PCR has become a widely used methodology for the analysis of gene expression and genotyping. Key factors that influence use of this technique include high reagent costs and the availability of limited sample.

To address these concerns, the performance of the Nanodrop Dispenser from Innovadyne Technologies, Inc. was evaluated using a TaqMan® assay in a 384-well format. To demonstrate the application of non-contact, nanoliter dispensing to real-time PCR, amplification of the single copy RNase P gene from human genomic DNA, a common verification reference, was chosen as the target for the assay.

384 replicate real-time PCR reactions were set up using traditional liquid handling robots in a 10µL total volume while the Nanodrop was used to assemble 1.25µL total volume reactions using the same components.

Analysis of the data shows that the Nanodrop delivers comparable results while significantly reducing reagent costs and consumption of sample.

TaqMan® Assay Assembly

Dispenser	Conventional	Nanodrop™
Real Time Cycler	ABI Prism™ 7900HT with 384 Well Plate Module	
TaqMan® Universal Master Mix, No AmpErase®UNG	5µL	625nL
Nuclease Free Water	1µL	125nL
5X Primer-Probe Mix*	2µL	250nL
Human Genomic DNA at 0.5ng/uL	2µL	250nL
Total Volume	**10µL**	**1.25uL**

Reactions were cycled using identical 40 cycle amplification profiles in ABI Prism™ 384-Well Clear Optical Reaction Plates.

*Probe consisted of a 5' FAM™ reporter with a 3' TAMRA™ quencher.

Reagent Cost Savings

	10µL Assay	1.25µL Assay
Cost per well	$0.54	$0.07
Cost per plate	$207	$26
Savings/plate	**$181**	
Savings/500 plates*	**$90,500**	

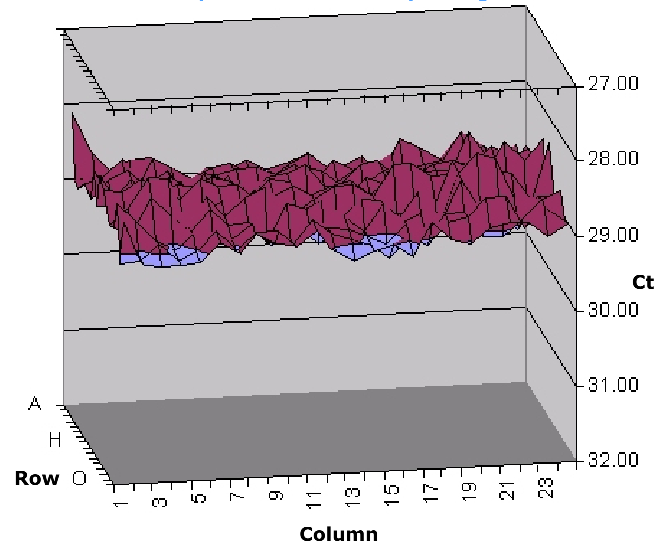
*Potential savings

Summary of Advantages

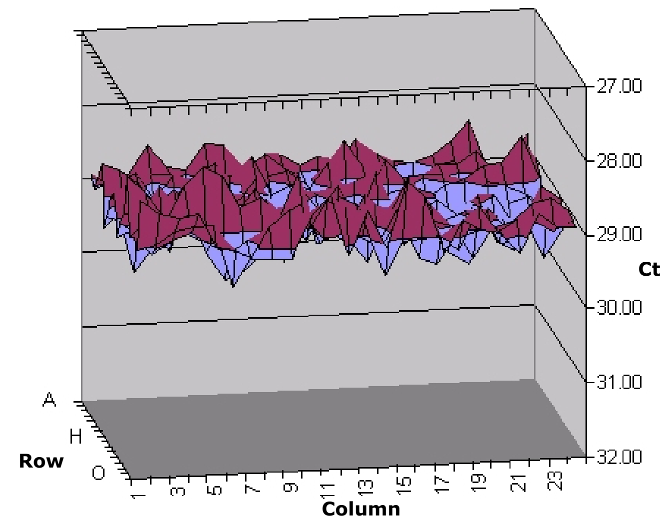
- Minimizes consumption of samples
- Drastically reduces reagent costs
- Produces robust, low-volume assays
- Supports the 96-, 384- and 1536-well formats
- Flexible assay designs

Cycle Threshold (Ct) Values for 384 Replicates

10µL Conventional Dispensing



1.25µL Nanodrop Dispensing



Cycle Threshold (Ct) Metrics

Total Volume	10µL	1.25µL
Ct Mean	28.82	29.03
Ct St. Dev	0.22	0.30
Ct %CV	0.77	1.02
Ct Minimum	28.09	28.13
Ct Maximum	29.29	29.99
Ct Range	1.20	1.86

Ph: 707-547-2500 Fax: 707-547-2501 Web: www.innovadyne.com Email: info@innovadyne.com
Innovadyne Technologies, Inc., 2835 Duke Court, PO Box 7329, Santa Rosa, CA 95407-7329

Miniaturizing PCR with the Nanodrop™

Avoiding Carryover During PCR Assembly

www.innovadyne.com

Prevention of sample cross contamination is a major concern for laboratories utilizing liquid handling robotics to set up PCR reactions. To address the issue when using the Nanodrop Dispenser, several tip wash routines were evaluated for the capacity to eliminate sample carryover.

Briefly, 2.5 µL TaqMan® assays for the human RNase P gene were assembled using the Nanodrop. DNA samples and corresponding blank controls were dispensed alternately across 8 consecutive columns of a 384-well plate. One of four wash routines was performed subsequent to each DNA addition. Wash effectiveness was then evaluated by assessing real-time amplification Cycle Threshold (Ct) Values in the corresponding blank control wells.

The data show that the Nanodrop is capable of performing PCR assembly without carryover. A wash with 2.5 mL of water alone is sufficient to prevent carryover contamination while additional treatments combining a 2.5 mL water wash with either a 2% Micro90 detergent or a 2% bleach wash is also effective, and importantly, does not inhibit reaction performance.

Experimental Procedure

- Step 1: Dispense 1.25 µL of Taqman® Universal Master Mix (No AmpErase® UNG) to all wells
- Step 2: Dispense 250 nL of Nuclease Free Water to all wells
- Step 3: Dispense 500 nL of 5X Primer-Probe Mix to all wells
- Step 4: Dispense 500 nL of DNA sample at 0.5 ng/µL to alternating wells of a single column
- Step 5: Perform specified wash treatment
- Step 6: Dispense water blank to corresponding wells of the adjacent column
- Step 7: Repeat steps 4-6 for all wash treatments
- Step 8: Cycle and read reactions in an ABI Prism™ 7900HT with a 384-Well Module using a 40 cycle amplification profile

TaqMan® Reaction Assembly

Component	Addition	Volume
TaqMan® Universal Master Mix, No AmpErase®UNG	1st	1.25µL
Nuclease Free Water	2nd	250nL
5X Primer-Probe Mix*	3rd	500nL
DNA Template or Water Blank	4th	500nL
Total Volume		**2.5uL**

* Probe consisted of a 5' FAM™ reporter with a 3' TAMRA™ quencher

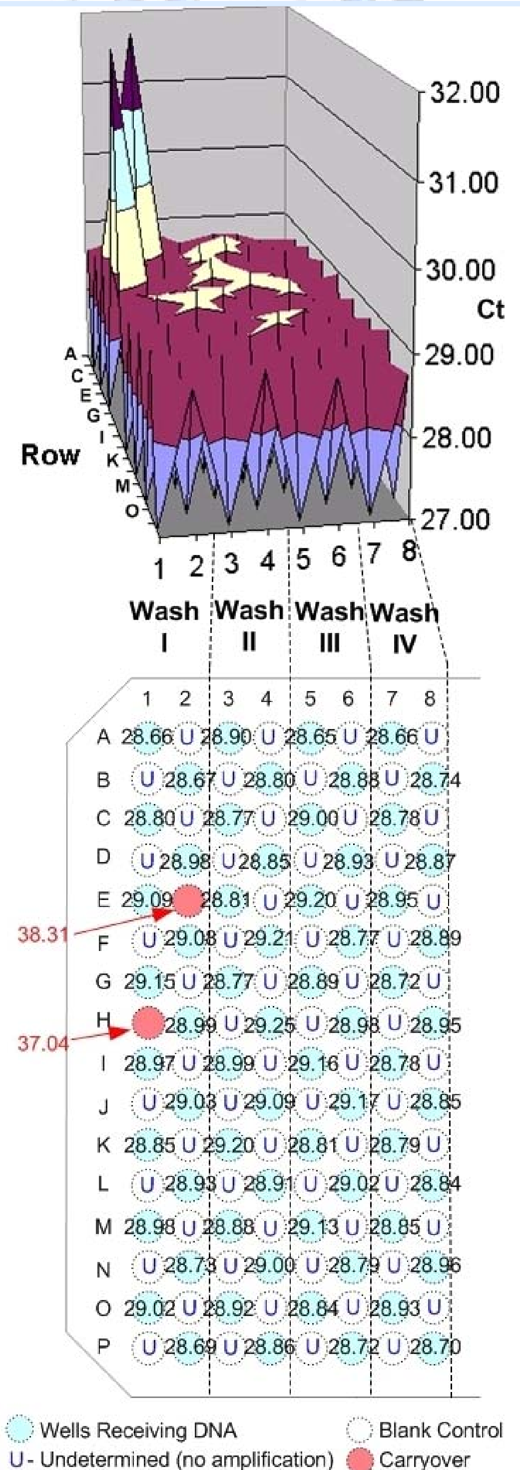
Wash Treatments

Wash I	Wash with 0.6 mL/tip of water
Wash II	Wash with 2.5 mL/tip of water
Wash III	Wash with 0.5 mL/tip of 2% Micro 90 detergent followed by 2.5 mL/tip of water
Wash IV	Wash with 0.5 mL/tip of 2% bleach followed by 2.5 mL/tip of water

Summary of Advantages

- Assembly of PCR based assays without carryover
- Simple wash routines
- Eliminates disposable tip costs
- Reduces plastic waste in the environment

Cycle Threshold Values for DNA Samples and Blank Controls



Innovadyne Technologies, Inc. (USA), 2835 Duke Court, PO Box 7329, Santa Rosa, CA 95407-7329 Ph: 707-547-2500 Fax: 707-547-2501

Innovadyne Technologies, Inc. (Europe), PO Box 360, Chorley, PR6 7WW, UK Ph: 44(0) 1772-698948 Cell: 44(0) 7966-079154

Fax: 44(0) 1772-698948 Web: www.innovadyne.com Email: info@innovadyne.com