

# Miniaturizing PCR

## with the Innovadyne™ Nanodrop™

### Cost Reduction with Conservation of Sample

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 Innovadyne Nanodrop Dispenser 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 10 µL total volume while the Nanodrop was used to assemble 1.25 µL 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
TaqMan Universal Master Mix, No AmpErase®UNG	5 µL	625 nL
Nuclease Free Water	1 µL	125 nL
5X Primer-Probe Mix*	2 µL	250 nL
Human Genomic DNA at 0.5 ng/µL	2 µL	250 nL
<b>Total Volume</b>	<b>10 µL</b>	<b>1.25 µL</b>

Reactions were cycled using identical 40 cycle amplification profiles in ABI Prism 384-Well Clear Optical Reaction Plates using an ABI 7900HT configured with a 384 Well Plate Module.

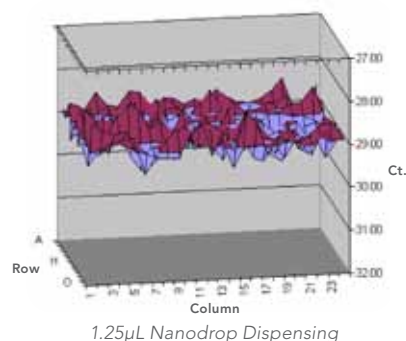
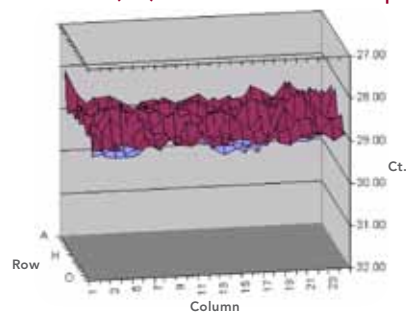
\*Probe consisted of a 5' FAM™ reporter with a 3' TAMRA™ quencher.

#### Reagent Cost Savings

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

\*Potential savings

### Cycle Threshold (Ct) Values for 384 Replicates



### 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) Metrics

Total Volume	10 µL	1.25 µL
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

## Avoiding Carryover During PCR Assembly

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  $\mu$ L TaqMan<sup>®</sup> 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% Micro 90 detergent or a 2% bleach wash is also effective, and importantly, does not inhibit reaction performance.

## Experimental Procedure

- ▶ **Step 1:** Dispense 1.25  $\mu$ L of Taqman<sup>®</sup> Universal Master Mix (No AmpErase<sup>®</sup> 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/ $\mu$ L 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<sup>™</sup> 7900HT with a 384-Well Module using a 40 cycle amplification profile

### TaqMan Reaction Assembly

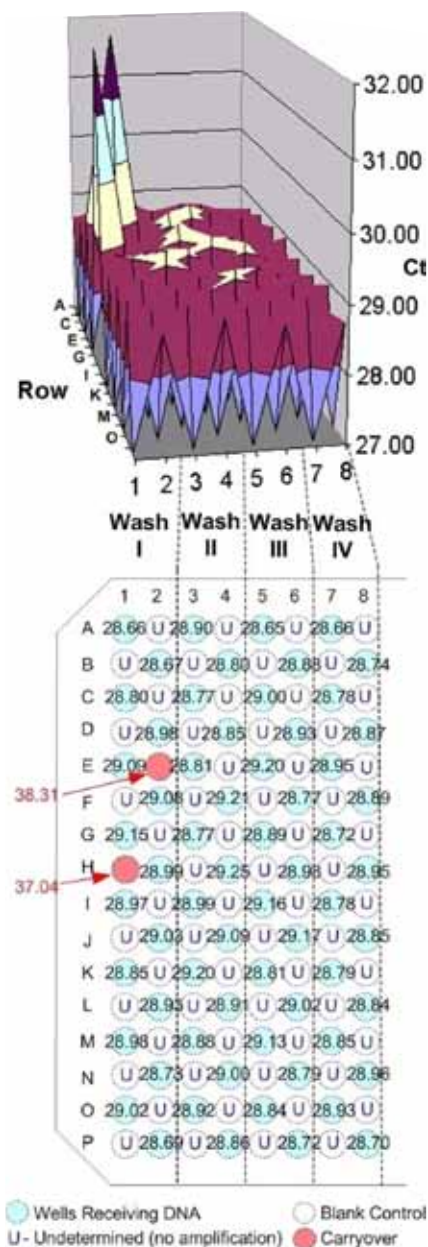
Component	Addition	Volume
TaqMan Universal Master Mix, No AmpErase <sup>®</sup> UNG	1st	1.25 nL
Nuclease Free Water	2nd	250 nL
5X Primer-Probe Mix*	3rd	500 nL
WDNA Template or Water Blank	4th	500 nL
Total Volume		2.5 $\mu$ L

\* Probe consisted of a 5' FAM<sup>™</sup> reporter with a 3' TAMRA<sup>™</sup> quencher

### Wash Treatments

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

## Cycle Threshold Values for DNA Samples and Blank Controls



## Summary of Advantages

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

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