TECHNICAL REPORT

Ovation® Whole Blood Solution Performance

Introduction

Gene expression studies with small and difficult samples have long presented a challenge to research and discovery efforts. Whole blood samples provide one of the more challenging clinical sources of RNA for gene expression analysis.

Peripheral blood is a readily available clinical tissue source for gene expression profiling that is rich in cells that are highly informative as to the state of disease, injury and the effects of drug treatment. These advantages make whole blood a very attractive tissue source for clinical research.

When using whole blood RNA samples for gene expression analysis, the main challenge lies in achieving consistent and robust array results. Standard IVT approaches often result in a considerable portion of samples failing to meet standard array quality metrics due to low present call rates and high scaling factors. This failure is partially attributed to poor and inconsistent amplification.

Another challenge to using whole blood total RNA preparations is the predominance of globin mRNA transcripts. The presence of globin message has been shown to adversely impact gene expression profiling on Affymetrix GeneChip® arrays, significantly decreasing present calls and increasing variability. These globin mRNA effects make it challenging to conduct direct whole blood gene expression profiling on GeneChip arrays without first employing a globin reduction method. Globin reduction requires adding a cumbersome sample handling step, increasing preparation time, costs, RNA input amount requirements and potential for errors and bias while reducing reproducibility.

In some studies, limiting amounts of whole blood specimens present yet another challenge, as is the case for samples obtained from infant patients or animal models. Whole blood obtained from these sources may not provide sufficient quantities for array studies, RT-PCR validation of array results, or archiving for future studies.

The Solution

NuGEN's Ovation Whole Blood Solution meets the challenges presented by whole blood RNA analysis and delivers a sensitive, reproducible, and robust process for total RNA amplification and preparation of cDNA target for analysis on GeneChip arrays.

The Solution comprises three NuGEN products: Ovation Whole Blood Reagent (Part No. 1300), Ovation RNA Amplification System V2 (Part No. 3100) and the Encore® Biotin Module (Part No. 4200). NuGEN's modular products employed in the Whole Blood Solution offer flexibility while providing a simple, fast and automation-friendly approach that lends itself to the high throughput target preparation demands of today's discovery projects.

In this report we present data from various studies demonstrating basic performance of the Ovation Whole Blood Solution performed both at NuGEN and at collaborator sites.

Materials and Methods

RNA sources: UHR total RNA, (Stratagene, Cat.# 740000). Human whole blood RNA prepared using PAXgene Blood RNA kit (QIAGEN, Cat. #762164).

All RNAs were amplified, fragmented and labeled using either the Ovation Biotin System (Part No. 2300, discontinued) using the standard protocol or the Ovation RNA Amplification System V2 (Part No. 3100) and Ovation Whole Blood Reagent (Part No. 1300) following the procedure outlined in the Ovation Whole Blood Reagent user guides.

cDNA was fragmented and labeled using either the standard fragmentation and labeling procedure (Ovation Biotin System) or Encore Biotin Module (Part No. 4200). In the latter case, 4.4 µg cDNA in 25 µL volume was used per fragmentation and labeling reaction.

Array analysis was performed using HG U133 Plus 2.0 GeneChip arrays (Affymetrix, Cat. #900467) with a final cDNA target concentration of 20 ng/µL. Array data was analyzed by Affymetrix GCOS software (GeneChip Operating System, 1.4.0.036) and GeneSpring.

The quantity of total RNA was measured using the Nanodrop ND-1000 spectrophotometer (Wilmington, DE)

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and quality was assessed using the RNA Nano LabChip® (Agilent Cat. #5065-4476) on the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). The Agilent 2100 Bioanalyzer was also used to obtain traces for the amplified cDNA using the Eukaryotic Total RNA Nano program, according to the manufacturer's instructions.

Results

The Ovation Whole Blood Solution amplification reproducibly provides higher cDNA yields from whole blood RNA samples than standard Ovation amplification. **Table 1** lists the average cDNA yields from three sets of whole blood total RNA samples amplified both at NuGEN and at collaborator sites, including one set from rat. In each case, the yields are greater when using the Ovation Whole Blood Solution and easily sufficient for the recommended amount of target to be fragmented and labeled for GeneChip array hybridization.

Table 2 shows the GeneChip global array metrics from a large set of human whole blood samples processed by Collaborator A. The Ovation Whole Blood Solution produced array data with a high degree of sensitivity (Avg. %P = 59.2%) and good reproducibility (%P SD = 1.6%).

Table 3 shows the high degree of signal correlation between the two methods, indicating that the amplification method employed in Ovation Whole Blood Solution does not alter the array expression data or introduce any significant bias when compared with the standard amplification method.

The scatter graph in **Figure 1** provides an example of the degree of replicate amplification correlation on GeneChip using the Ovation Whole Blood Solution.

From a study of ~100 samples, 15 clinical whole blood RNA samples were chosen for amplification, fragmentation and labeling using the Ovation Whole Blood Solution. The samples had been previously processed using the standard Ovation Biotin RNA Amplification and Labeling System. The 15 samples were chosen to reflect a broad representation of sample and array quality, with an emphasis on the most challenging samples with the poorest array results.

In the original analysis, using the standard Ovation Biotin System, approximately one half of the 15 samples failed to yield sufficient cDNA to meet the standard array target amount requirement. All 15 samples processed using the Ovation Whole Blood Solution, yielded sufficient cDNA for fragmentation, labeling and hybridization to arrays. Yield results are shown in **Figure 2**.

Of the 15 amplified and labeled targets, 12 were chosen for hybridiza-

TABLE 1. **Human and rat whole blood RNA amplifications using the Ovation Whole Blood Solution. Results show consistent yields and robust amplification in three separate studies.**

Site	Input	N	Avg Yield (µg)/ SD
NuGEN	20 ng HWB RNA	12	9.1/ 0.46
Collaborator site A	20 ng HWB RNA	40	9.5 / 1.3
Collaborator site B	20 ng Rat WB RNA	24	9.0/ 0.29

TABLE 2. **Collaborator A analyzed 30 of 40 human and rat whole blood amplification samples on GeneChip® arrays. Averages and standard deviation (SD) metrics show highly reproducible results.**

Array Metrics	SF	Raw Q	%P	GAPDH 3′/5′	β-actin 3′/5′	Avg. Pearson Correlation of RMA Signal
Average	1.88	1.14	59.2%	1.74	3.92	0.992
SD	0.44	0.16	1.56%	0.12	0.46	0.002

TABLE 3. **Signal correlations between standard Ovation Amplification System and the Ovation Whole Blood Solution. Results show that each approach is extremely reproducible and that the Ovation Whole Blood Solution data remain very consistent when compared with those from standard Ovation amplification.**

Methods	Avg. R^2 / SD
WB vs. WB	0.990 / 0.0013
Std vs. Std	0.988 / 0.0042
WB vs. Std	0.982 / 0.0042

tion on HG U133 Plus 2.0 GeneChip arrays. The resulting array metrics are shown in **Table 4**.

Using the Ovation Whole Blood Solution, the array global metrics improved dramatically over those generated with targets processed using the standard Ovation method. We observed significantly improved sensitivity as measured by percent present calls and decreased variability in scale factors and 3′/5′ ratios. Percent present calls improved from an average of 30% (SD=11%) to an average of 61% (SD=3.7%). Scale factors decreased in magnitude and variability from an average of 25 (SD=17) to an average of 4.2 (SD=5.4). Beta-actin 3′/5′ ratios, a metric often used as a measure of RNA sample quality, decreased from an average value of 94 (SD=94) to an average value of 16 (SD=22).

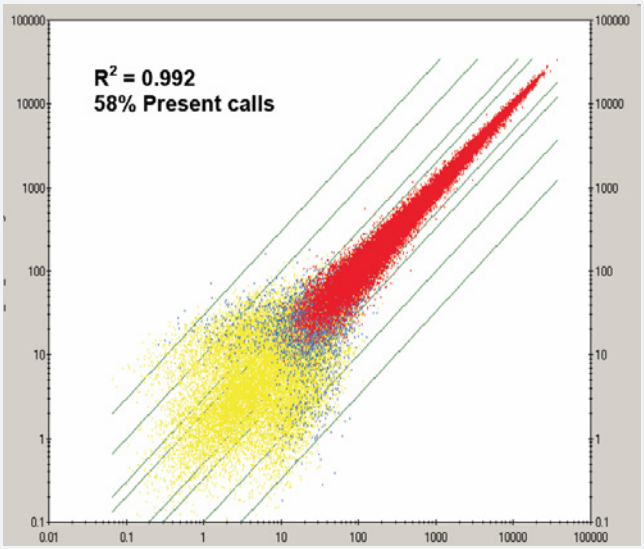
These results demonstrate that the Ovation Whole Blood Solution produces superior GeneChip gene expression data on whole blood samples, without globin reduction, and can rescue challenging whole blood samples that would otherwise fail array QC.

Conclusion

The Ovation Whole Blood Solution yields up to 12 µg of cDNA from as little as 20 ng of whole blood total RNA, without the need for globin reduction. The amplified cDNA can be used directly in qPCR analysis or stored for future studies. Data concordance and reproducibility are improved because the same amplified cDNA product can be processed in a two hour fragmentation and labeling method, yielding biotin-labeled target cDNA that can be hybridized directly to GeneChip arrays in the same day, without additional purifications.

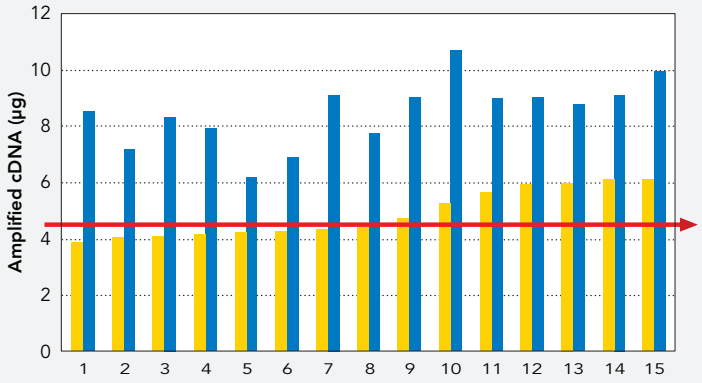
By eliminating the need for globin reduction, NuGEN's Ovation Whole Blood Solution reduces costs and preparation time compared to alterna-

FIGURE 1. **Array reproducibility.**



Duplicate samples were prepared from 20 ng of human whole blood RNA using the Ovation Whole Blood Solution, according to the product user guides. RNA samples were isolated using the Agencourt RNAdvance™ Blood kit on the Beckman ArrayPlex. Targets were hybridized to Affymetrix GeneChip HG U133 Plus 2.0 arrays. Linear regression analysis provides a strong signal correlation (R^2= 0.992) with 58% present calls, demonstrating very high reproducibility across transcript levels starting with only 20 ng of input whole blood total RNA.

FIGURE 2. **Yields generated using the Whole Blood Solution.**



Using the Ovation Whole Blood Solution (blue bars), sufficient cDNA was generated from every sample for array analysis, rescuing those samples that would have previously failed to meet yield requirements and been excluded from the study. Half of the RNA samples shown here did not have sufficient yields for array analysis when using standard Ovation amplification (yellow bars). The horizontal red arrow indicates the minimum yield for GeneChip Arrays (4.4 µg).

tive methods. Those improvements, in turn, reduce errors and bias.

In addition, the low RNA input amount requirements for this approach help preserve precious samples and further reduce costs associated with sample stabilization by reducing the number of PAXgene Blood DNA Tubes required from two or more to just one.

NuGEN's Ovation Whole Blood Solution delivers a sensitive, reproducible, easy-to-automate, robust process for whole blood total RNA amplification and preparation of cDNA targets for analysis on GeneChip arrays, in just one day.

Systems Specifications

Ovation® Whole Blood Reagent
1300-12, 1300-60 (12, 60 reactions)
1300-A01 (for automation)

Ovation® RNA Amplification System V2
3100-12, 3100-60 (12, 60 reactions),
3100-A01 (for automation)

- **Whole blood RNA Input:** 20–50 ng total
- **cDNA yield:** 5–12 µg cDNA

Encore® Biotin Module
4200-12, 4200-60 (12, 60 reactions)
4200-A01 (for automation)

- **Input:** 4.4 µg total whole blood cDNA
- **Yield:** 4.4 µg fragmented and labeled target cDNA

TABLE 4. **Yields and array metrics generated using the Whole Blood Solution. The 12 samples shown in Figure 2 were analyzed on GeneChip® arrays and show excellent array metric results.**

Sample No.	Yield (µg)	SF	Background	%P	GAPDH 3′/5′	β-actin 3′/5′
1	7.7	17.43	35.2	52.4%	3.2	71.6
2	9.9	10.77	35.8	61.8%	2.9	41.6
3	10.7	9.72	34.5	60.5%	2.9	38.5
4	8.7	1.07	41.5	60.6%	1.4	2.7
5	9.0	1.19	37.6	61.5%	1.6	3.2
6	9.0	1.07	37.9	62.5%	1.5	2.7
7	8.3	2.46	37.0	68.7%	1.6	3.6
8	7.1	1.29	37.9	61.7%	2.5	11.2
9	6.9	1.66	37.7	59.5%	1.9	8.2
10	9.1	1.25	38.4	62.7%	1.5	2.4
11	7.9	1.19	40.2	63.3%	1.5	2.6
12	9.1	1.32	40.3	60.6%	1.4	2.7
Average	**8.6**	**4.2**	**37.8**	**61.3%**	**2.0**	**15.9**
SD	**1.1**	**5.4**	**2.1**	**3.7%**	**0.7**	**22.5**

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