Ovation® 3´-DGE System

Accurate Eukaryotic Expression Profiling – Offering the advantages of NGS with the convenience of microarrays

Highlights of the Ovation 3´-DGE System

- **Accurate expression profiling:** Suitable for DGE analysis of any eukaryotic transcriptome without the need for prior sequence knowledge for array design
- **Simple, fast, automatable workflow:** Coupled with the optional indexing capability of the Encore™ NGS Library System I offering a nine-hour total workflow from total RNA to libraries for improved sample throughput and reduced price, thereby approaching the convenience of microarrays
- **10 ng total RNA input:** Enabling studies previously not possible
- **Flexible solution:** Compatible with all leading NGS platforms

Introduction

With the rapid advancement of Next Generation Sequencing (NGS) technology, new enabling applications are also emerging quickly, taking advantage of the massively parallel potential of NGS. Whole transcriptome expression profiling has been performed routinely on microarrays in the last decade; although, Digital Gene Expression (DGE, or Tag Profiling) on NGS is challenging the current array-based approach by providing higher sensitivity, larger dynamic range and removing the need to have prior sequence knowledge of the transcriptome (1).

Even with the superior data obtained on NGS platforms, the adoption of DGE to date as a standard laboratory practice has been limiting compared to expression microarrays. Among the major bottlenecks for researchers is the tedious sample preparation method for DGE, long lead time associated with low throughput, as well as the relatively higher cost.

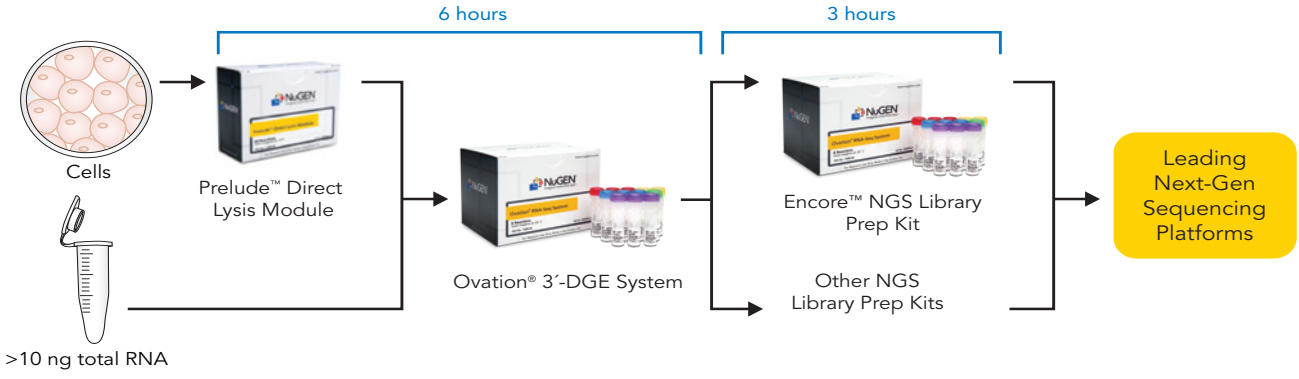
The Ovation 3´-DGE System by NuGEN® is designed to make sample preparation as easy as microarrays. Coupled with the Encore NGS Library System I, now you can unleash the power and potential of NGS, obtaining the highest level of expression data quality combined with the convenience, throughput and price of microarrays.

Simple, fast workflow compatible with all leading NGS platforms

The input for the Ovation 3´-DGE System is flexible and you can choose one of two options that best fits with your experimental design (**Figure 1**):

- 10 ng of purified total RNA
- Direct cell lysate using the Prelude™ Direct Lysis Module from NuGEN

FIGURE 1 **Simple and Fast Ovation 3´-DGE Workflow for Integrated NGS Analysis**



NuGEN® imagine more from less™

Based on the Ribo-SPIA® Single Primer Isothermal Amplification technology, the Ovation 3′-DGE System takes only six hours and generates micrograms of double-stranded cDNA focusing on the most 3′ 150–500 bases proximal to the poly(A) tail (**Figure 2**).

The resulting double-stranded cDNA can then be integrated into standard NGS library preparation procedures. As shown in Figure 1, for users choosing the Illumina NGS platforms, the integrated solution with Encore NGS Library System I produces DGE libraries in just nine hours, greatly simplifying the sample preparation procedure.

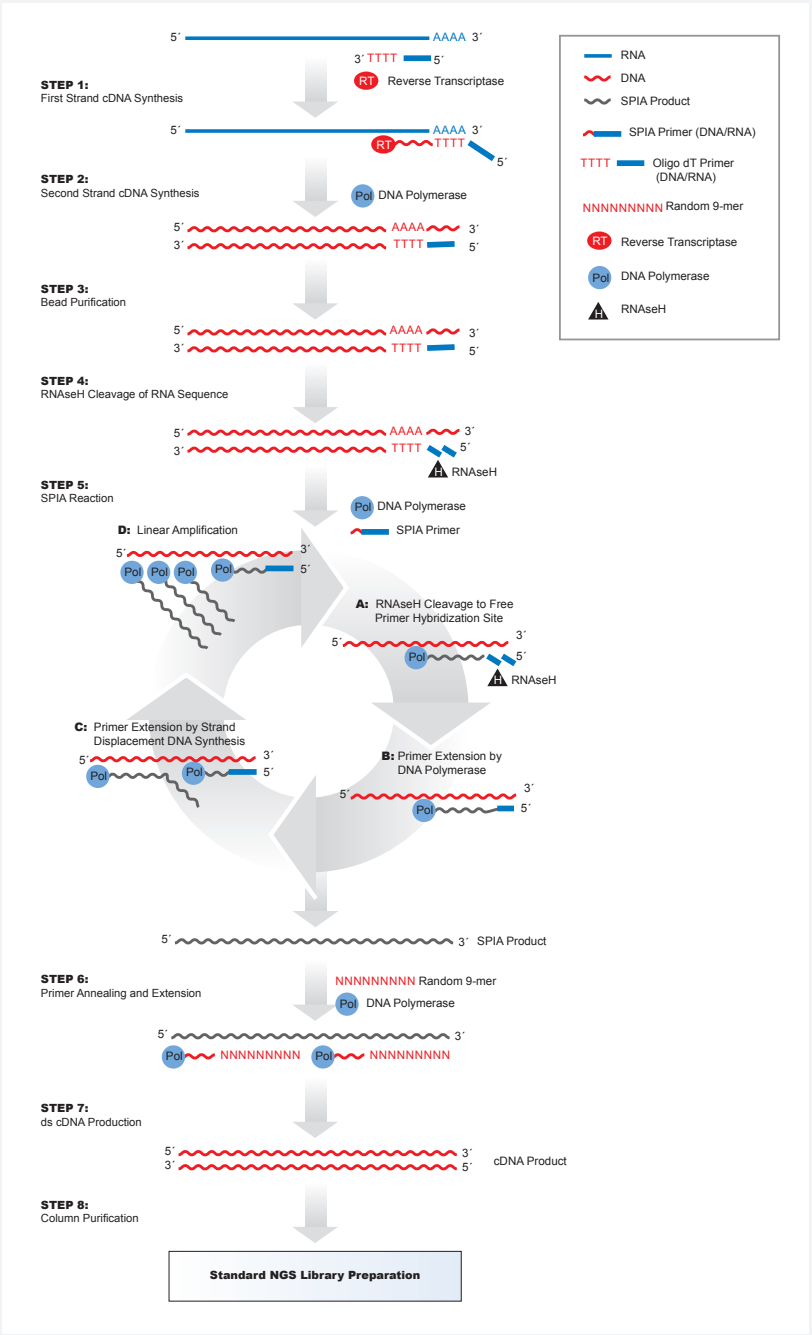
In combination with the streamlined Encore NGS library preparation procedure, the entire process is easily automatable on major robotic platforms, approaching the protocol simplicity of microarrays, enhancing sample throughput and ease of use.

Optional multiplexing making routine DGE studies economically feasible

One of the major obstacles for broader adoption of DGE-based expression profiling today is the high cost associated with running NGS systems. Because of the nature of DGE that focuses on quantitation of the 3′-end of each transcript, the total number of reads per sample can be vastly reduced compared with full-length RNA-Seq experiments. It has been estimated that 4–5 million short reads for each sample is sufficient to provide in-depth coverage and sensitivity even for the low expressing genes.

Empowered by the unique barcode design strategy in the Encore NGS Library Systems, now users can easily multiplex 2–8 samples in each sequencing lane obtaining sufficient read density for DGE applications while greatly reducing the total cost of each sample to the range of the cost of a microarray experiment. This makes DGE an ideal choice for all expression profiling studies given that NGS offers superior sensitivity and dynamic range compared to microarray applications.

FIGURE 2 Schematic of the Ovation 3′-DGE Process



Accurate expression profiling analysis of any eukaryotic transcriptome

Since the Ovation 3′-DGE System initiates the reverse transcription reaction by priming off the poly(A) tail and the NGS technology offers the unique advantage of not requiring any prior sequence knowledge, a single product and workflow is suitable for expression profiling of any eukaryotic transcriptome.

To demonstrate the technical repro-

ducibility of the Ovation 3´-DGE System, libraries were constructed using total RNA from Human Brain Reference and Universal Human Reference (UHR) MAQC samples and sequenced by 40-base pair single-read sequencing on the Illumina Genome Analyzer IIx System.

As shown in **Table 1**, total RNA inputs of 10 ng for each sample generated an average of 27 to 29 million total reads per flow cell lane, with approximately 50–58% unique reads (mapped only once in the reference genomic assembly), and 94% mapped reads (all reads that are mappable to the reference genome). Each of these sequencing metrics was similar across replicate determinations, and generally the same between brain and UHR samples. These parameters do not vary significantly from previously published DGE results following a much longer procedure from higher amounts of starting total RNA (1).

To demonstrate the use of the Ovation 3´-DGE system on non-human transcriptomes, total RNA isolated from C.elegans was utilized and sequencing metrics of the model organism are also included in **Table 1**. The % Unique Reads, % Mapped Reads and % rRNA mirror those obtained from the human samples.

The high degree of reproducibility of the Ovation 3´-DGE System is further illustrated in **Figure 3**, in which a high correlation was observed from two independent technical replicates with R above 0.95.

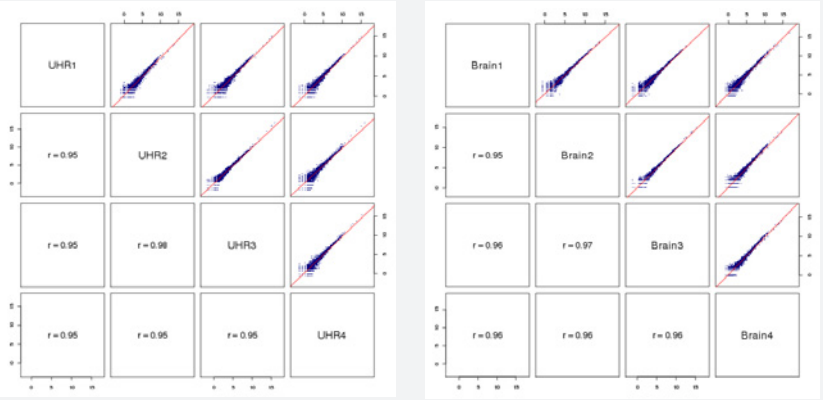
To utilize DGE routinely for expression profiling also requires a high level of accuracy in detecting differential expression levels. The ability of the Ovation 3´-DGE System to provide highly concordant differential expression results compared with qPCR is shown in **Figure 4**. MAQC Human Brain Reference and UHR samples processed with the Ovation 3´-DGE System were analyzed on NGS and compared with the differential expression data generated by qPCR. Differential expression fold changes are concordant with this

TABLE 1 **Sequencing Performance Metrics from Ovation 3´-DGE System for *C.elegans* and Human MAQC Samples**

Parameter	UHR (MAQC A)	Human Brain Reference (MAQC B)
Total Reads	24,121,590	19,986,392
% Unique Reads	49.19	58.44
% Reads Mapped	94.10	94.04
% Reads Mapped to RefSeq	51.01	52.69
% Reads Mapped to rRNA	0.08	0.05

Parameter	*C. elegans* 1	*C. elegans* 2	*C. elegans* 3	Average
Total Reads	27,479,905	29,041,863	29,263,708	28,595,159
% Unique Reads	77.5	78.8	78.5	78.3
% Reads Mapped to WS200 Assembly	90.6	90.1	89.5	90.1
% Reads Mapped to RefSeq	62.8	60.0	58.5	60.5
% Reads Mapped to rRNA	10.6	8.4	7.8	8.9

FIGURE 3 **High Technical Reproducibility of the Ovation 3´-DGE System**



Three independent amplification replicates of 10 ng of MAQC A and B RNA samples were amplified using the Ovation 3´-DGE System and sequenced on 2 lanes of Illumina GAIIx employing the Encore library prep kit muliplex barcodes (four samples in each lane). Normalized log^2 transformed DGE counts for reads mapping to the 500 most-3´ bases of mRNA RefSeq sequences and corresponding Pearson correlation values are shown on the scatterplots. Excellent technical reproducibility (R=0.95-0.98) and dynamic range greater than 10^5 can be observed on the plots.

reference expression fold change (R = 0.88) without significant data compression, as evidenced by the slope (0.94).

To ensure that the Ovation 3′-DGE System provides broad coverage detecting the expressed transcripts, the genes "detected" by DGE were compared with the gene list detected on microarrays for both MAQC samples (data not shown). There is significant overlap between the genes detected by the two technology platforms. Genes identified by DGE, but not by arrays, are predominantly low in abundance, further illustrating the high sensitivity of the NGS platform for analyzing low-expression transcripts, which would have been missed using the microarrays.

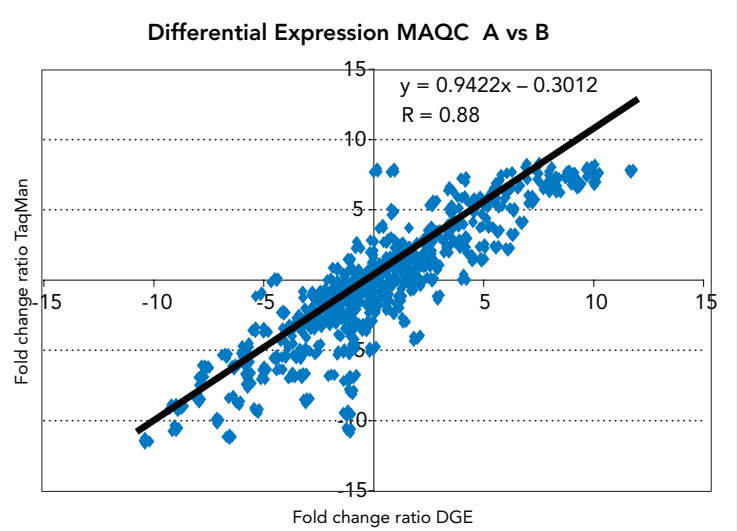
Conclusion

The Ovation 3′-DGE System offers a simple, quick and robust solution for DGE analysis on all leading NGS platforms with the convenience of microarrays. With increased sample throughput, improved protocol ease of use, faster turnaround and reduced cost, DGE can now be used for routine gene expression analysis on any eukaryotic organism, from samples as low as 10 ng of total RNA or cell lysates. Enjoy the advantages of NGS with the convenience of microarrays.

References

(1) Asmann et al., 3′ tag gene expression profiling on human brain and universal reference RNA using Illumina Genome Analyzer. *BMC Genomics* (2009), 10:531

FIGURE 4 **Highly Concordant Differential Expression Results Obtained Comparing DGE and qPCR**



Differential gene expression data from normalized DGE reads (total of ~20M MAQC A and ~24M MAQC B) mapping to the 500 most-3′ bases of the RefSeq mRNAs were compared with the TaqMan QPCR data (N=541).

ORDERING INFORMATION

Catalog No.	No. of Reactions
Ovation 3′-DGE System	
7200-08	8 reactions
Complete DGE Solution without Indexing (including Ovation 3′-DGE System (1x) and Encore NGS Library System (1x))	
360	8 reactions
Complete DGE Solution with Indexing (including Ovation 3′-DGE System (4x) and Encore NGS Multiplex System (1x))	
361	32 reactions

Technical Documents
Ovation 3′-DGE System User Guide

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