Prelude™ Direct Lysis Module

Enables rapid, accurate and reproducible whole transcriptome analysis from direct cell lysates without RNA isolation

Highlights of the Prelude Direct Lysis Module

- **Simplified workflow:** Enables transcriptome analysis of a large number of samples, with no RNA isolation required
- **Versatile:** Integrates with a range of NuGEN RNA Amplification Systems for analysis by RNA-Seq, 3´-DGE, microarray expression profiling or qPCR
- **Flexible:** Can be used to prepare lysates from a few to thousands of cells, and can be easily automated

The Prelude Direct Lysis Module bypasses the traditionally tedious and time-consuming RNA purification steps to simplify the transcriptome analysis workflow, and continues directly from cell lysis into RNA amplification systems by NuGEN®. Cell washing and lysis take less than five minutes, and nucleic acids are stabilized in the same lysis buffer since this buffer inactivates cellular nucleases and other enzymes. DNA contamination is minimal, as the lysis protocol does not release nuclear contents.

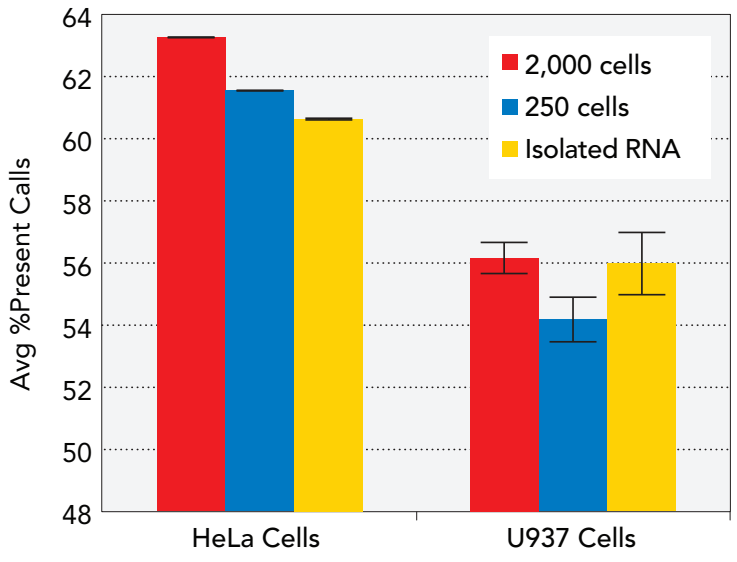
Cell lysates produced with the Prelude Direct Lysis Module are stable for six months at -80˚C and suitable for later processing with the pertinent Ovation® RNA Amplification System. The long-term stability of cell lysates offers the potential to perform multiple experiments using the same sample and to archive samples of interest for future analysis.

Introduction

The protocols used for RNA isolation prior to gene expression studies can be tedious, costly and difficult to implement with a large number of samples, or with a limited number of cells. The Prelude Direct Lysis Module addresses these problems by offering an efficient means of obtaining stabilized RNA ready for downstream processing on a range of transcriptome analysis platforms.

The kit contains a wash and a lysis buffer for preparing cell lysates that can either be stored for later analysis or added directly to the first strand synthesis reaction of the Ovation Pico WTA, PicoSL WTA, RNA-Seq or 3´-DGE Systems. A short incubation of the washed cell pellet in lysis buffer

FIGURE 1 **Percent Present Call Results from Direct Cell Lysates.**



The graph illustrates data from Affymetrix GeneChip Human U133A 2.0 Arrays from triplicate determinations. Cell lysates were prepared using the Prelude Direct Lysis Module, or total RNA was isolated from each cell line using the QIAGEN RNeasy Mini Kit. RNA amplification was conducted using lysates from the indicated number of cells or 2.0 ng isolated total RNA with the Ovation Pico WTA System. The cDNA product was fragmented and labeled using the Encore Biotin Module. A high degree of sensitivity was achieved for all RNA types as evidenced by the high percent present calls in the range of 54.2% to 63.3%.

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consistently yields >95% lysed cells as monitored by trypan blue exclusion. In comparison to workflows requiring upfront isolation of total RNA prior to amplification, use of the Prelude Direct Lysis Module can reduce the assay time by several hours, in particular with studies using a large number of samples.

High-Quality Array Results from Direct Cell Lysates

An important criterion for evaluating a direct cell lysis approach is performance in comparison to workflows using isolated total RNA. To examine how these two methods compare, HeLa cells (an adherent cell line) and U937 (a suspension cell line) were each processed using the Prelude Direct Lysis Module, or total RNA was isolated from the respective cell type using the QIAGEN RNeasy® Mini Kit. Two input amounts of each cell lysate, corresponding to 250 or 2,000 cells, together with 2.0 ng of isolated total RNA were amplified using the Ovation Pico WTA System, and the cDNA product fragmented and labeled using the Encore™ Biotin Module. The labeled targets were then hybridized to Affymetrix GeneChip® Human U133A 2.0 Arrays, and data analysis performed with the Expression Console v1.1 software package from Affymetrix.

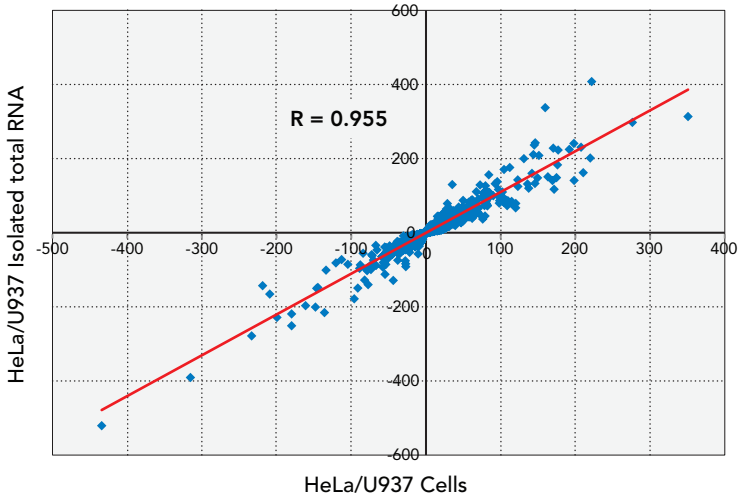
As shown in **Figure 1,** good sensitivity was obtained both from direct cell lysates and isolated total RNA, with average percent present calls in the range of 54.2% to 63.3% across all sample types. In addition, there was strong correlation between the different target preparation methods as measured by the linear signal Pearson correlation coefficient, R, in **Table 1.** These values illustrate that the same high-quality results can be obtained with the Ovation Pico WTA System and Encore labeling method using isolated total RNA or direct cell lysates as the starting material. In addition, linear signal R values for the 250 and 2,000 cell inputs to direct lysis are highly correlated, showing that the Prelude Direct Lysis Module yields consistent results over a wide range of cell numbers. Lastly, as shown in **Figure 2,** differential gene expression between HeLa and U937 cells was reliably measured with a high degree of concordance using either cell lysates prepared with the Prelude Direct Lysis Module, or equivalent amounts of isolated total RNA.

TABLE 1 **Correlation between Cell Lysates and Isolated RNA.**

Source of Total RNA	HeLa Cells	U937 Cells
250 cells and 2,000 cells	0.984	0.985
250 cell and 2.0 ng Isolated RNA	0.969	0.967
2,000 cells and 2.0 ng Isolated RNA	0.951	0.943

Triplicate averaged linear signal R values were obtained between four independent users. The samples were prepared and analyzed as described in Figure 1. The results demonstrate a high level of concordance between varying cell inputs, and between cell lysates and the corresponding isolated total RNA from the same cell type.

FIGURE 2 **Fold-Change Correlation of Differentially Expressed Genes.**



The results show the concordance of fold-change differences for 3,040 differentially expressed genes compared between HeLa and U937 cells (p-value ≤0.001). The samples were prepared and analyzed as described in Figure 1, with differentially expressed genes called at >2-fold absolute change. Data from direct cell lysates are plotted on the X-axis, and data from isolated total RNA on the Y-axis.

Consistent Results with Frozen Samples

Use of the Prelude Direct Lysis Module allows researchers to conveniently lyse cells and stabilize RNA for later amplification and gene expression analysis. This workflow flexibility is particularly important for studies using a large number of samples, for time course studies requiring a precise endpoint or in cases where cell lysis is performed in 96- or 384-well plate formats. As

shown in **Table 2,** similar array results were obtained for freshly processed HeLa and U937 cells, or with cell lyates stored at -80˚C for four weeks. The added flexibility of working with stored cell lysates allows researchers to process a larger number of cell samples while performing downstream amplification and transcriptome analysis at a later time.

High Concordance with qPCR Assays

Similar to the results obtained with microarrays, the preparation of samples with the Prelude Direct Lysis Module yields comparable results to qPCR assays performed with isolated total RNA. The results in **Figure 3** show triplicate raw C_T values for three housekeeping genes (ACTB, GAPDH and PGK1) in HeLa and U937 cells with four different inputs of the source RNA. RNA amplification was performed with the Ovation Pico WTA System, and qPCR conducted using 2.0 ng of the resulting purified SPIA® product (cDNA). The results are highly concordant, with an R value of 0.991, indicating consistent and reproducible performance with qPCR using direct cell lysates or the respective isolated total RNA.

RNA-Seq Performance Metrics

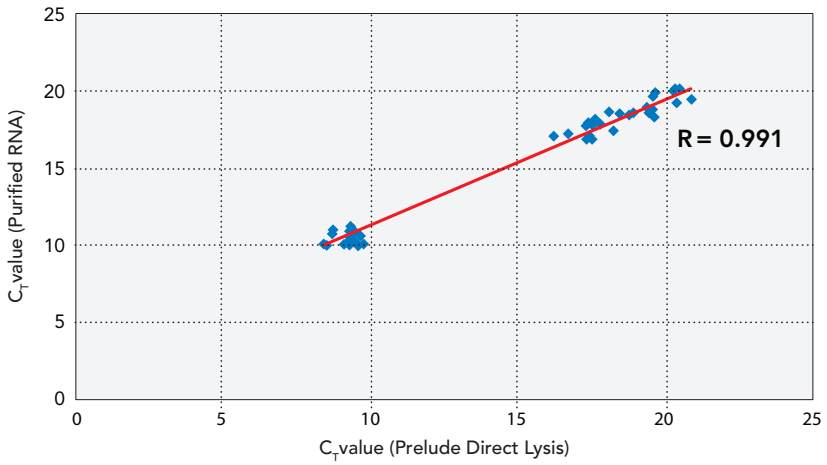
In addition to transcriptome analysis by arrays and qPCR, direct cell lysis provides similar advantages in preparing samples for Next-Generation Sequencing. To examine the utility of the direct lysis method for RNA-Seq, HeLa cells were processed using the Prelude Direct Lysis Module, or total RNA isolated from HeLa cells using QIAGEN RNeasy® Mini Kit. Cell lysates or isolated total RNA was amplified using the Ovation RNA-Seq System, and the resulting double-stranded cDNA used to construct libraries using the Encore NGS Library System I for sequencing on the Illumina Genome Analyzer IIx platform. The sequencing metrics obtained from each sample were high quality and very similar, with each sequencing run exceeding 25 million total reads per flowcell lane, and mapped reads >90%. Importantly, the percentage of rRNA reads was <5% for both samples, demonstrating that this feature of the Ovation RNA-Seq System is maintained when used in conjunction with the Prelude Direct Lysis Module. Lastly, as shown in **Figure 4,** there was a high degree of correlation between the Log_2 RPKM values for each sample type (linear R = 0.92) with an N=21,300 transcripts, again showing that comparable RNA-Seq results are obtained with cell lysates and isolated RNA.

TABLE 2 **Stability of Frozen Cell Lysates.**

Source of Total RNA	HeLa Cells	U937 Cells
250 cells – Fresh vs Frozen	0.976	0.989
2,000 cells – Fresh vs Frozen	0.978	0.991

Washed cell pellets were lysed using the Prelude Direct Lysis Module and amplified directly using the Ovation Pico WTA System, or stored at -80˚C for four weeks prior to amplification. Triplicate averaged linear signal R values are shown. The results demonstrate a high level of concordance between fresh and frozen cell lysates, further adding to the workflow flexibility afforded by the direct lysis approach.

FIGURE 3 **qPCR using Direct Cell Lysates.**



Plotted are raw C_T values for three housekeeping genes (ACTB, GAPDH and PGK1) in HeLa and U937 cells at four inputs of the source RNA (2,000 cells, 500 cells, 16 ng RNA, 4 ng RNA) to the Ovation Pico WTA System. The qPCR reactions were performed using 2.0 ng of the resulting purified SPIA product (cDNA). The R value of 0.991 indicates high concordance between qPCR using direct cell lysates, or the respective isolated total RNA.

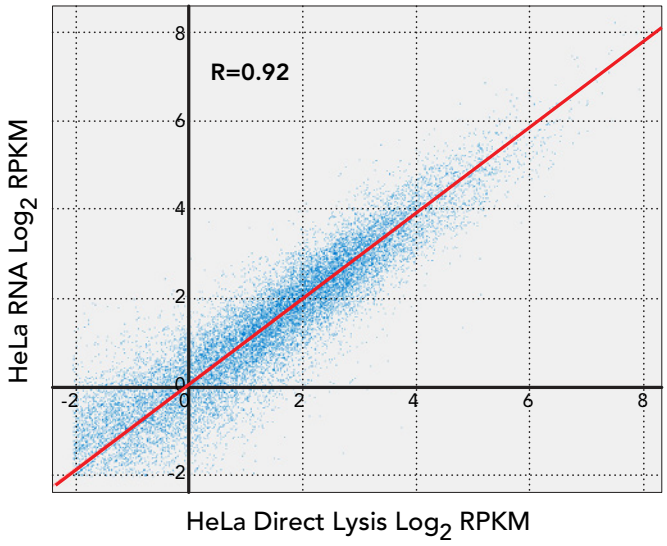
Conclusion

The Prelude Direct Lysis Module is the first commercial reagent kit to enable transcriptome analysis from direct cell lysates using the range of analytical platforms–microarray profiling, RNA-Seq, 3´-DGE and qPCR. The kit eliminates the need for RNA isolation using a fast and simple protocol for cell lysis and integrates directly with the Ovation RNA amplification systems. Adding to the ease of use and versatility of this protocol, cell lysates may be assayed immediately with no further purification steps or stored frozen for later analysis. This new kit extends the value of NuGEN's sample preparation solutions and further expands researchers' capabilities for larger, more efficient gene expression studies.

ORDERING INFORMATION

Part No.	No. of Reactions
Prelude™ Direct Lysis Module	
1400-24	24 reactions
1400-A01	96 reactions
Related Products:	
Ovation® Pico WTA System Ovation® PicoSL WTA System Ovation® RNA-Seq System Ovation® 3´-DGE System Encore™ NGS Library System I Encore™ NGS Multiplex System I Encore™ Biotin Module Encore™ BiotinIL Module	
Technical Documents	
Prelude Direct Lysis Module User Guide	

FIGURE 4 **Highly Concordant Results by RNA-Seq using Cell Lysates or Isolated RNA.**



2,000 HeLa cells were processed using the Prelude Direct Lysis Module, or total RNA was isolated from HeLa cells using the QIAGEN RNeasy Mini Kit. Cell lysates or 20 ng of isolated total RNA was amplified using the Ovation RNA-Seq System, and the resulting double-stranded cDNA used to construct libraries using the Encore NGS Library System I for sequencing on the Illumina Genome Analyzer IIx platform. Single-read sequencing was done with 40 base-pair reads. Fastq reads were processed with Tophat v1.0.11 and Bowtie 0.12.3 to generate RPKM values (Reads Per Kilobase per Million mapped reads) for 21,300 transcripts. Log_2 RPKM values are plotted.

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