

Product Overview

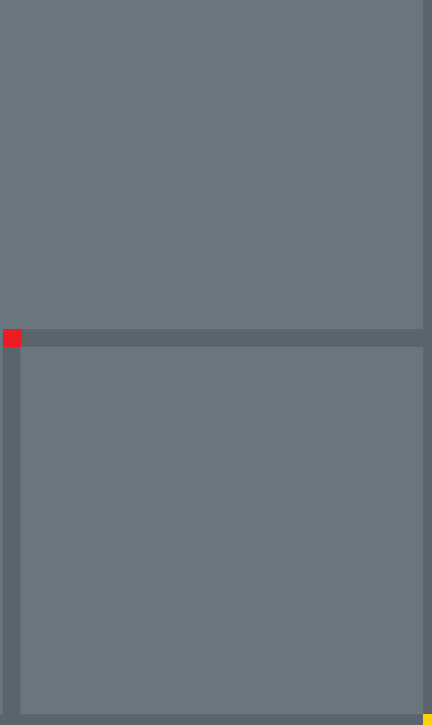
February 2010

INNOVATIVE SAMPLE PREPARATION FOR: RNA | DNA | AUTOMATION

Imagine More from Less™



www.nugeninc.com



“Our group is working toward the discovery and development of biomarkers for the detection of lung cancer and chronic obstructive pulmonary disease before the onset of clinical symptoms. In non-invasive collection from the airway epithelium, the quantity of RNA obtained is limiting. I was pleased to see our NGS results generated from the NuGEN Ovation RNA-Seq protocol strongly correlated with previous findings on microarrays, indicating the Ovation RNA-Seq sample preparation method preserved the transcriptome profile from a very small amount of starting material. We are excited about the outcome and plan to use the Ovation RNA-Seq System for larger-scale biomarker discovery projects in the future.”

Dr. Avi Spira,
*Boston University School
of Medicine*

Revolutionary RNA and DNA Sample Preparation Technologies at Your Fingertips.

You can now prepare samples of any size, from almost any source for analysis on the latest genomic analysis platforms, including the leading next-generation sequencing and microarray systems. By putting innovation at the forefront of all products engineered by NuGEN®, we are committed to helping you stay at the cutting edge of science while ensuring robust and reproducible results. Achieve continued success in your research by making NuGEN your partner in advancing groundbreaking discoveries with the latest in sample preparation reagents.

NuGEN's Ovation®, Applause™, and Encore™ Systems offer a full range of nucleic acid sample preparation solutions: Ovation is used for amplification of difficult small samples; Applause can be used cost effectively for everyday amplifications; and Encore is for post-amplification modification preparing the samples for specific downstream genetic or genomic analysis.

NuGEN System Highlights:

- Linear, isothermal amplification for uniform representation of the whole transcriptome or genome
- Versatile and simple workflows that are compatible with leading genomic analysis platforms, including next-generation sequencing
- Applications for a range of samples, from formalin-fixed, paraffin-embedded, to high quality purified nucleic acids, down to a single cell quantity
- Automation scripts and reagents for diverse robotic platforms



RNA Analysis

Whether samples are small and degraded, or ample and well preserved, NuGEN's RNA amplification systems provide solutions of superior sensitivity for rapid analysis of the transcriptome. Whether the focus is 3' or whole transcript, or the platform requires single-stranded or double-stranded product, NuGEN offers a system to meet your needs.

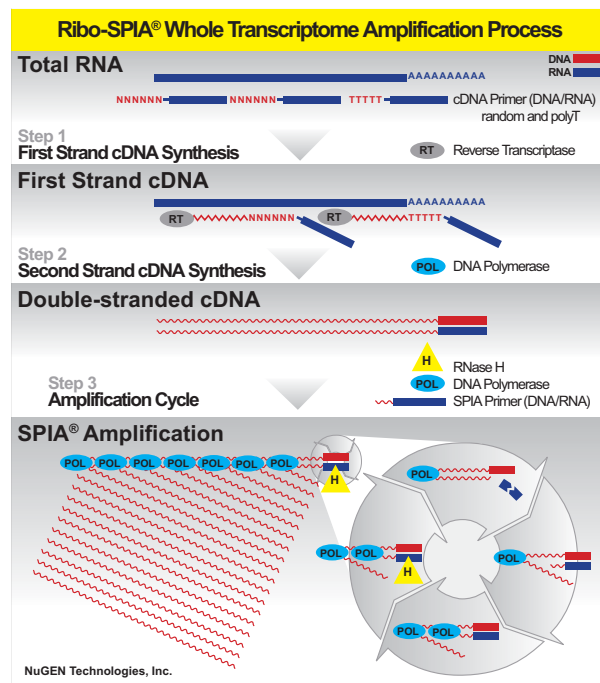
Ribo-SPIA® Technology – Uniform Representation of the Whole Transcriptome

At the core of NuGEN's RNA sample preparation portfolio is our patented and proven Ribo-SPIA® (Single Primer Isothermal Amplification) technology, which produces amplified cDNA from total RNA.

The initial reverse transcription reaction relies on optimized mixtures of oligo (dT) and random DNA/RNA chimeric primers (**Figure 1**), depending on the downstream analytical platform requirements. The approach allows you to choose between a 3'- focused analysis or a whole-transcript analysis.

Following synthesis of the double-stranded cDNA, which incorporates a unique RNA/DNA heteroduplex tag segment at one end, the SPIA amplification step uses an additional chimeric primer, DNA polymerase and RNase H in an isothermal reaction, leading to the rapid accumulation of amplified cDNA. When the analytical platform requires double-stranded cDNA, the single-stranded SPIA® products can be easily converted. Amplified SPIA product typically ranges in size between 50 bases and 1,500 bases and can be optimized to meet downstream analysis requirements.

FIGURE 1 Ribo-SPIA amplification



Ovation® RNA Amplification Systems for Challenging Samples

Ovation RNA Amplification Systems make sample preparation simple, delivering uncompromised results from compromised samples. Very small, degraded and hard-to-replace specimens are no longer a barrier to expression profiling.

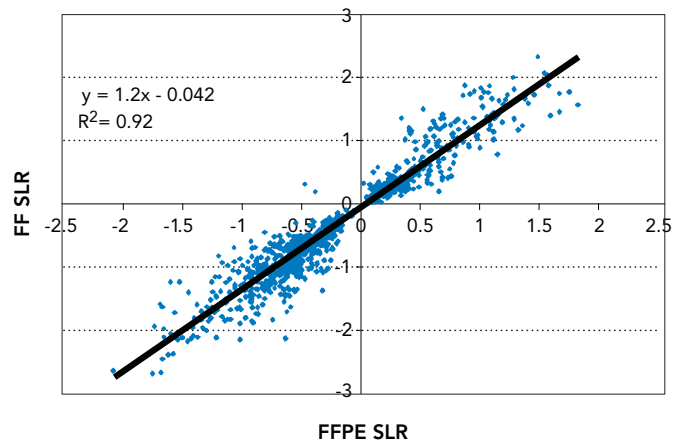
The Ovation family of products allows you to work with a range of samples:

- Samples as small as 10 picograms total RNA
- Degraded samples with RIN scores as low as 2.0
- Formalin-fixed, paraffin-embedded (FFPE) samples (**Figure 2**)
- Whole blood
- Direct cell lysate from the equivalent of one or a few cells (**Figure 3**)

For next-generation sequencing, the first Ovation RNA-Seq System from NuGEN extends the power and flexibility of this new technology to sample preparation directly from as little as 500 picograms of total RNA compared to the standard recommendation of 1 µg and above (**Figures 4 and 5**). In addition, you can now conduct RNA-Seq analysis of your precious samples with a more comprehensive representation of the transcriptome by eliminating the potential bias introduced by poly(A)+ RNA selection and ribosomal RNA reduction.

For post-amplification needs, you can choose the appropriate Encore System to tailor the SPIA product to meet specific requirements of the downstream analytical platforms.

FIGURE 2 Highly concordant differential expression analysis results between FF and FFPE samples

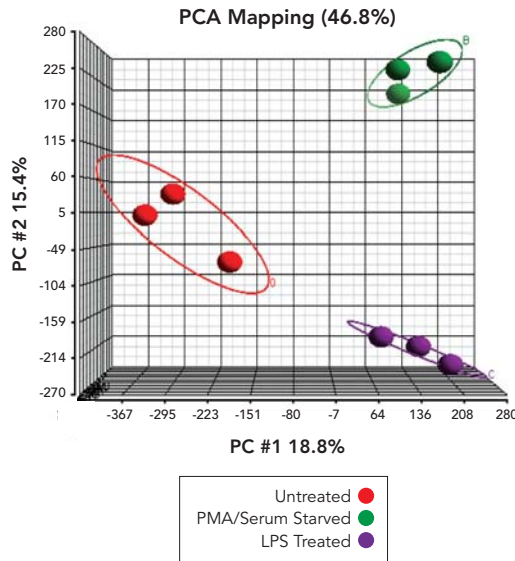


Using the WT-Ovation™ FFPE System, total RNA isolated from lung cancer and normal FFPE samples (50 ng) as well as matching fresh frozen (FF) tissues (2 ng) were amplified, labeled and hybridized to Affymetrix GeneChip® U133A Arrays. 815 genes were detected to be highly differentially expressed (signal log ratio, SLR) between the cancer and normal samples. Highly concordant results are demonstrated between FF and FFPE samples.

“Our clients show increasing interest in linking the molecular mechanism of disease to patient clinical outcomes. Most of the samples they need analyzed to accomplish this are in an FFPE format, which can impact the ability to recover intact RNA suitable for quantitative RT-PCR studies. NuGEN’s Ovation RNA amplification literally rescued a difficult FFPE project we performed for a client in 2008. The laser-capture micro-dissection (LCM) of FFPE tumor yielded very few cells for study. The Ovation kit allowed us to recover and amplify sufficient RNA to complete quantitative RT-PCR successfully.”

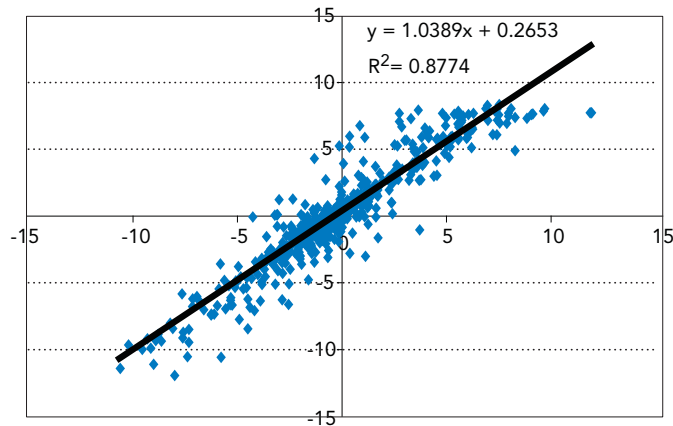
Dr. Samir Alhasan,
Director of Cell and Molecular
Services at Asterand plc

FIGURE 3 Clustering of microarray data obtained with the WT-Ovation One-Direct System from cell lysates of 10 cells



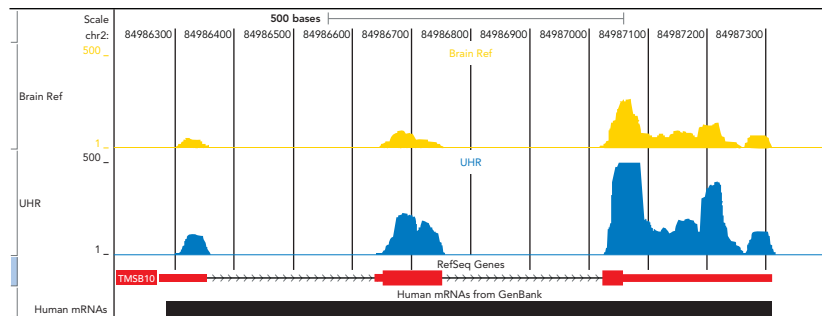
In order to demonstrate array performance with cell lysates, triplicate amplifications were performed representing 10-cell samples of untreated THP-1 cells (red), PMA treated/serum starved cells (green), and PMA treated/serum starved cells after exposure to LPS (purple) using the WT-Ovation One-Direct System. The amplified cDNA was labeled and hybridized to Affymetrix GeneChip® Human Gene 1.0 ST arrays. Principal Components Analysis (PCA) was performed using Partek Genomics Suite software. An analysis of the array data reveals differential expression of genes consistent with published data on gene expression profiles in LPS stimulation models (not shown).

FIGURE 4 Highly concordant differential expression results using the Ovation RNA-Seq System on NGS compared with TaqMan data



Differential expression data were generated for MAQC A (Human Universal Reference) and MAQC B (Human Brain Reference) by both Illumina Solexa Genome Analyzer Ix and qPCR. RNA-Seq data from 10 ng of total RNA is plotted on X-axis, qPCR data plotted on Y-axis. 659 TaqMan probes that uniquely map to the RefSeq annotations used in the RPKM calculations are represented. The qPCR results were originally reported in Shi, L. et al. *Nature Biotechnology* (2006) 24(9): 1151-1161.

FIGURE 5 Differential expression detected with the Ovation RNA-Seq System on NGS



Data from UCSC genome browser demonstrating differential expression of the TMSB10 gene in Human Brain Reference vs. Universal Human Reference (UHR) MAQC samples. The average # reads/base is 190 for UHR and 69 for Brain ref, indicating 2.75-fold difference in expression levels.

“NuGEN Ovation® labeling chemistry is the cornerstone of our blood biomarker and micro-sample labeling efforts to meet the increasing demand for robust amplification chemistry compatible with both commercial microarrays and in-house qPCR-based low-density arrays. After an exhaustive evaluation of the most promising technologies, we selected the NuGEN Ovation system based on starting input amount, sensitivity, reproducibility, ability to process whole blood, and automation potential.”

Norm Allaire,
 Scientist, Molecular Profiling
 Group, Biogen Idec

Applause™ RNA Amplification Systems for Standard RNA Analysis

The Applause™ Amplification Systems deliver single-tube, single-day, add-and-incubate assays for everyday gene expression analysis. Powerful, cost-effective amplification can begin on samples of 50 nanograms or more using NuGEN’s Ribo-SPIA technology. NuGEN’s Applause systems provide a full range of sample preparation approaches for use with a variety of microarray and qPCR platforms.

For modification following Applause amplification, choose the appropriate Encore System to tailor the SPIA product to meet specific requirements of the downstream analytical platforms.

Encore™ Post-Amplification Systems

Compatible with Ovation or Applause, NuGEN’s Encore Systems provide rapid and simple post-amplification fragmentation and/or labeling of SPIA products. The integrated complete solution for sample preparation is tailored for specific microarray platforms such as Affymetrix, Agilent or Illumina.

DNA Analysis

NuGEN brings a fresh solution to DNA amplification with straightforward and simple whole genome amplification, offering an out-of-the-box solution with broad downstream applications.

Ovation WGA System for DNA Analysis

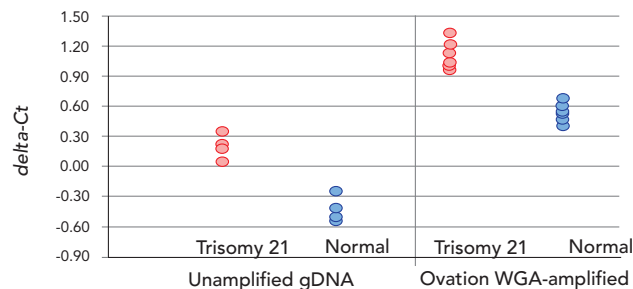
The Ovation WGA System relies on NuGEN's proprietary SPIA technology to power the only amplification system capable of uniform representation for the whole genome. NuGEN's linear whole genome amplification enables efficient and accurate genetic analysis, including copy number variation studies on even the smallest samples — particularly important to research in cancer, stem cells, neurobiology and immunology.

The Ovation WGA System's faithful and reproducible whole-genome representation requires minimal assay optimization (**Figure 6**). From as little as 10 nanograms of genomic DNA, the Ovation System produces micrograms of amplified SPIA product with low dropout rates and minimal operator variation. You can confidently adopt the simple Ovation workflow regardless of analytical platform, allele of interest or sample source.

"In our studies, only the Ovation WGA System can be used for important quantitative measurements from amplified gDNA. We often receive very little starting material. Right out of the box, Ovation WGA System provided amplified material from our limited gDNA and performed as well as unamplified gDNA in several quantitative PCR assays. We have tried other commercial WGA systems and have never been able to make accurate quantification measurement following amplification. We are switching to the Ovation WGA System for all our clinical gDNA applications."

Dr. Andrew Brooks,
Director of the Biomics
Research and Technology Center
at the University of Medicine and
Dentistry of New Jersey

FIGURE 6 Robust and reproducible detection of Trisomy 21 with the Ovation WGA System



Blood samples were collected from six patients with Trisomy 21 as well as six normal donors, and genomic DNA was isolated. Amplifications were performed in triplicate using 10 ng of isolated gDNA. TaqMan assays were carried out using 40 ng of unamplified gDNA or 40 ng of amplified SPIA product for each replicate. Delta-Ct values were derived from the relative comparison of a Chromosome 21 locus to a Chromosome 12 locus (GAPDH). In order to distinguish the additional Chr21 marker, the $\Delta\Delta C_t$ method was employed, where the expected value from a 3:2 chromosomal imbalance should be 0.6. $\Delta\Delta C_t$ values of 0.6 were consistently obtained from the SPIA amplified material and clearly distinguished the affected samples from the control samples, performing in a very similar way to the unamplified material.

Automation and Supporting Partners

"WT-Ovation technology is very helpful for developing standardized IVD [In Vitro diagnostics] solutions for oncology that can be used nimbly in a clinical setting. Ultimately, this new system will reduce the time between sample acquisition and critical diagnosis, allowing physicians to make important and personalized decisions about a patient's therapy."

DX Peter Kaspar,
Vice President of Research and Development, bioMérieux Corporate

Automation

NuGEN's Automation Solutions accelerate high-throughput nucleic acid amplification, fragmentation and labeling, thereby significantly streamlining sample preparation.

NuGEN's team of automation scientists works closely with each customer and produces automation control software scripts to meet their individual needs. Automation solutions currently exist for Beckman Biomek FX, Beckman Biomek ArrayPLEX, Hamilton STARlet and Affymetrix GeneChip Array Station (GCAS).

Supporting Partners

Genomic Diagnostic Testing Partners

Several leading diagnostic companies chose NuGEN to partner in the development of genomic diagnostic and prognostic tests. In support of its partners in the development and commercialization of assays, NuGEN established rigorous quality standards, which led to ISO 13485:2003 certification in 2008. NuGEN will be compliant with FDA-promulgated Good Manufacturing Practices (cGMP) regulations in early 2010, making it one of the first reagent companies to meet these exacting qualifications.

Service Provider Partners

A wide selection of commercial service providers can help you tackle programs of any size. Our authorized service providers undergo special training from NuGEN scientists to ensure their proficiency with NuGEN technology and their adherence to the proven protocols that generate high-quality data with your precious samples.

Visit our website at www.nugeninc.com for the most current list of our service providers.

RNA Sample Preparation						
	Starting materials and sample sizes					
Platform	Good Quality >50 ng	Good Quality 5-50 ng	Good Quality & Degraded 500 pg - 50 ng	Whole Blood 5-50 ng	Total RNA or Cell Lysate 10-500 pg	FFPE 50 ng
Next Generation Sequencing (Illumina Solexa, Life Technologies SOLiD and Roche 454)	NEW! Ovation RNA-Seq System Part No. 7100	NEW! Ovation RNA-Seq System Part No. 7100	NEW! Ovation RNA-Seq System Part No. 7100	NEW! Ovation RNA-Seq System Part No. 7100		
Affymetrix GeneChip® 3' Arrays	NEW! Applause 3'- Amp System Part No. 5100 Encore Biotin Module Part No. 4200	Ovation Amp v2 Part No. 3100 Encore Biotin Module Part No. 4200	Ovation Pico WTA System Part No. 3300 Encore Biotin Module Part No. 4200	Ovation Amp v2 Part No. 3100 Ovation Whole Blood Reagent Part No. 1300 Encore Biotin Module Part No. 4200	WT-Ovation One-Direct System Part No. 3500 Encore Biotin Module Part No. 4200	WT-Ovation FFPE System V2 Part No. 3400 Encore Biotin Module Part No. 4200
Affymetrix GeneChip® Exon/Gene ST Arrays	Applause WT-Amp ST and WT-Amp Plus ST Part No. 5500 / 5510 Encore Biotin Module Part No. 4200	Ovation Pico WTA System Part No. 3300 WT-Ovation Exon Module Part No. 2000 Encore Biotin Module Part No. 4200	Ovation Pico WTA System Part No. 3300 WT-Ovation Exon Module Part No. 2000 Encore Biotin Module Part No. 4200	Ovation Pico WTA System Part No. 3300 WT-Ovation Exon Module Part No. 2000 Encore Biotin Module Part No. 4200	WT-Ovation One-Direct System Part No. 3500 Encore Biotin Module Part No. 4200	WT-Ovation FFPE System V2 Part No. 3400 WT-Ovation Exon Module Part No. 2000 Encore Biotin Module Part No. 4200
Agilent Dual-Mode Gene Expression Arrays		Ovation Amp v2 Part No. 3100 NuGEN's Agilent Solution Application Note	NEW! Ovation PicoSL WTA System Part No. 3310 NuGEN's Agilent Solution Application Note	Ovation Amp v2 Part No. 3100 NuGEN's Agilent Solution Application Note	WT-Ovation One-Direct System Part No. 3500 NuGEN's Agilent Solution Application Note	WT-Ovation FFPE System V2 Part No. 3400 NuGEN's Agilent Solution Application Note
Illumina Whole Genome Expression BeadChips		Ovation Amp v2 Part No. 3100 NEW! Encore BiotinIL Module Part No. 4210	NEW! Ovation PicoSL WTA System Part No. 3310 NEW! Encore BiotinIL Module Part No. 4210	Ovation Amp v2 Part No. 3100 NEW! Encore BiotinIL Module Part No. 4210	WT-Ovation One-Direct System Part No. 3500 NEW! Encore BiotinIL Module Part No. 4210	WT-Ovation FFPE System V2 Part No. 3400 NEW! Encore BiotinIL Module Part No. 4210
Quantitative PCR		WT-Ovation Amp Part No. 2210	NEW! Ovation PicoSL WTA System Part No. 3310 Ovation Pico WTA System Part No. 3300	WT-Ovation Amp Part No. 2210	WT-Ovation One-Direct System Part No. 3500	WT-Ovation FFPE System V2 Part No. 3400

New! DNA Sample Preparation

Ovation WGA System Part No. 6100

The Ovation WGA System is used to generate micrograms of amplified product from 10 ng of genomic DNA for analysis on a range of analytical platforms including q-PCR and aCGH.

Product Selection Guide

Automation					
	NuGEN System				
Platform	Ovation RNA Amplification (includes whole blood)	Encore Biotin Module	WT-Ovation FFPE RNA Amplification	Ovation Pico WTA System	WT-Ovation Exon Module for ST Array Analysis
Beckman Biomek® FX	√	√		√	
Beckman Biomek® Array Plex	√	√	√		
Hamilton STARlet	√	√			
Affymetrix GeneChip® Array Station (GCAS)	√	√		√	√

Highlighted customer publications. For a complete list, please visit our website: www.nugeninc.com

Chiu IM, Chen A, Zheng Y, Kosaras B, Tsiftoglou SA, Vartanian TK, Brown RH Jr, Carroll MC., "T lymphocytes potentiate endogenous neuroprotective inflammation in a mouse model of ALS," *Proc Natl Acad Sci USA*, 2008 Nov 18;105(46):17913-8. Epub 2008 Nov 7.

Ebert BL, Pretz J, Bosco J, Chang CY, Tamayo P, Galili N, Raza A, Root DE, Attar E, Ellis SR, Golub TR., "Identification of RPS14 as a 5q- syndrome gene by RNA interference screen," *Nature*, 2008 Jan 17;451(7176):335-9, Comment in: *Nature*, 2008 Jan 17;451(7176):252-3.

Essers MA, Offner S, Blanco-Bose WE, Waibler Z, Kalinke U, Duchosal MA, Trumpp A., "IFNalpha activates dormant haematopoietic stem cells in vivo," *Nature*, 2009 Apr 16;458(7240):904-8. Epub 2009 Feb 11.

Lassmann S, Kreutz C, Schoepflin A, Hopt U, Timmer J, Werner M., "A novel approach for reliable microarray analysis of microdissected tumor cells from formalin-fixed and paraffin-embedded colorectal cancer resection specimens," *Mol Med*, 2009 Feb;87(2):211-24, Epub 2008 Dec 6.

Linton K, Hey Y, Dibben S, Miller C, Freemont A, Radford J, Pepper S., "Methods comparison for high-resolution transcriptional analysis of archival material on Affymetrix Plus 2.0 and Exon 1.0 microarrays," *Biotechniques*, 2009 Jul;47(1):587-96.

Koziczak-Holbro M, Joyce C, Glück A, Kinzel B, Müller M, Tschopp C, Mathison JC, Davis CN, Gram H., "IRAK-4 kinase activity is required for interleukin-1 (IL-1) receptor- and toll-like receptor 7-mediated signaling and gene expression," *J Biol Chem*, 2007 May 4;282(18):13552-60, Epub 2007 Mar 2.

Talasz AH, Powell AA, Huber DE, Berbee JG, Roh KH, Yu W, Xiao W, Davis MM, Pease RF, Mindrinos MN, Jeffrey SS, Davis RW., "Isolating highly enriched populations of circulating epithelial cells and other rare cells from blood using a magnetic sweeper device," *Proc Natl Acad Sci USA*, 2009 Mar 10;106(10):3970-5, Epub 2009 Feb 20.

Imagine More From Less™

NuGEN's innovative RNA and DNA sample preparation systems meet your growing demand for quantitative, high-fidelity and high-throughput transcriptomic and genomic analysis. You can overcome the limitations of other amplification systems with NuGEN's reagents, achieving reliable, reproducible results no matter the quality or size of the starting sample.

Make NuGEN your partner in genomic analysis.

ORDERING INFORMATION

Product Name	Catalog No.
RNA Sample Preparation	
Ovation RNA Amplification Systems	
Ovation® RNA-Seq	7100
Ovation® PicoSL WTA	3310
Ovation® Pico WTA	3300
Ovation® Amp V2	3100
Ovation® WB Reagent	1300
WT-Ovation™ One-Direct	3500
WT-Ovation™ FFPE V2	3400
WT-Ovation™ Amp	2210

Product Name	Catalog No.
RNA Sample Preparation	
Applause RNA Amplification Systems	
Applause™ WT-Amp Plus ST	5510
Applause™ WT-Amp ST	5500
Applause™ 3' Amp	5100
Post-Amplification Systems	
Encore™ Biotin IL Module	4210
Encore™ Biotin Module	4200
WT-Ovation™ Exon	2000
Agilent Solution	*
Illumina Protocol	*

* View NuGEN Application Notes at www.nugeninc.com for more details.

Product Name	Catalog No.
DNA Sample Preparation	
Ovation® WGA System	6100
Automation Solutions	
Contact NuGEN for more details	

NuGEN products are available in a variety of reaction sizes. For more details, visit www.nugeninc.com.

NuGEN Technologies, Inc.

Headquarters USA

201 Industrial Road, Suite 310
San Carlos, CA 94070 USA
Toll Free Tel: 888.654.6544
Toll Free Fax: 888.296.6544
custserv@nugeninc.com
techserv@nugeninc.com

Europe

P.O. Box 149,
6680 AC Bommel,
The Netherlands
Tel: +31-13-5780215
Fax: +31-13-5780216
europa@nugeninc.com

For our international distributors contact information, visit our website

www.nugeninc.com



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