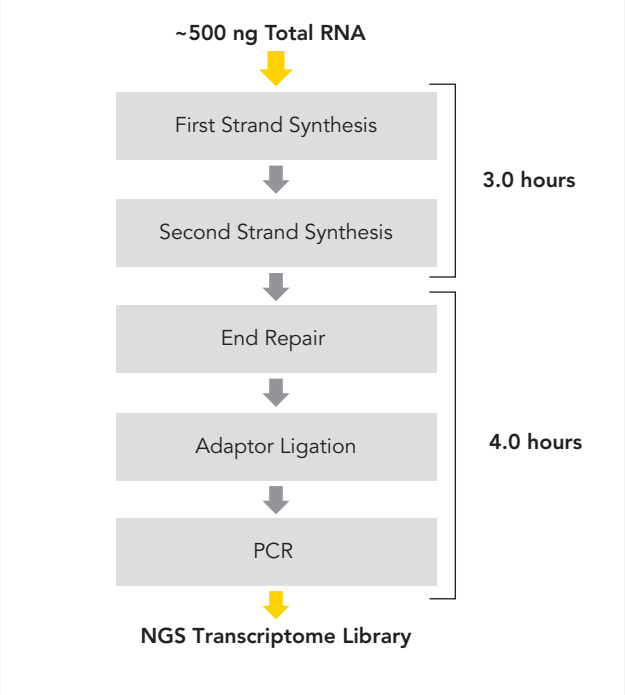
Ovation® Prokaryotic RNA-Seq System

Whole transcriptome profiling in bacteria

Highlights of the Ovation Prokaryotic RNA-Seq System

- **Robust prokaryotic transcriptome profiling —** Enables profiling of all bacterial species, regardless of genome GC content, and is compatible with next-generation sequencing (NGS) technologies
- **Quick, simple workflow —** Provides a workflow, starting with 500 ng total RNA and producing sequencing-ready libraries, that can be completed in about 8 hours when used in concert with the Ovation Ultralow Library Systems
- **Effective reduction of rRNA sequence reads —** Specific positive selection of non-rRNA transcripts to increase the useful data content of each sequencing run

FIGURE 1 **Construction of NGS Transcriptome Libraries**



First and second strand cDNA synthesis using selective primers, enriches for non-rRNA transcripts from bacterial and archaeal total RNA inputs. Following end repair, the double-stranded cDNA is compatible with NuGEN's Ovation® Ultralow Library System, which allows easy sample multiplexing within a single end transcriptome sequencing format.

Introduction

The Ovation® Prokaryotic RNA-Seq System provides a method for the analysis of microbial species and microbiome samples by enabling whole transcriptome profiling. The core technology used in this product enriches for mRNA in NGS libraries and can be applied to transcriptomes extracted from pure and mixed microbial samples in an easy end-to-end workflow, from total RNA to sequencing (see **Figure 1**).

The first and second strand cDNA syntheses are carried out using proprietarily designed primers to create double-stranded cDNA. The resulting cDNA is compatible with NuGEN's Ovation Ultralow Library Systems as well as other workflows using double-stranded cDNA as input for creating sequencing libraries.

The Ovation Prokaryotic RNA-Seq System provides reproducible transcriptome profiles across all prokaryotic species—both Eubacteria and Archaebacteria. To date, we have validated the system's performance across six bacterial species covering a broad range of GC contents.

During construction of NGS transcriptome libraries, first and second strand cDNA syntheses are performed in about three hours, with selective priming to enrich for non-rRNA transcripts from total RNA inputs. The entire workflow, from total RNA to sequencing, using the Ovation Prokaryotic RNA-Seq System in combination with the Ovation Ultralow Library Systems, can be completed in about eight

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hours. Use of the Ovation Ultralow Library Systems also enables multiplex sequencing for greater cost savings.

Simple, fast workflow integrates seamlessly with library construction protocols

As illustrated in **Figure 1**, the Ovation Prokaryotic RNA-Seq System protocol yields double-stranded cDNA ready for the construction of RNA-Seq libraries. The protocol can be completed in approximately three hours. The amplified cDNA product is ideal for use with NuGEN's Ovation Ultralow Library Systems and can be directly integrated into other NGS library construction methods amenable to low input amounts of double-stranded cDNA.

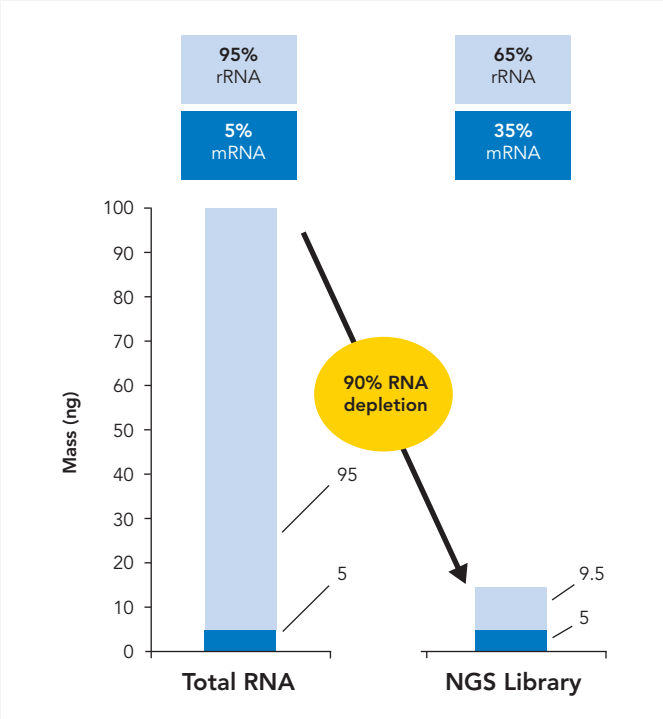
Industry-leading rRNA depletion

Bacterial total RNA typically contains 95% – 99% ribosomal RNA (rRNA). The remaining 1% – 5% of RNA is comprised of the unique RNA species used to create and monitor biologically informative expression profiles (mRNA). Reducing the proportion of rRNA in a sample reduces the necessary number of sequencing reads required to cover these unique RNA species, saving money. As shown in **Figure 2**, the typical performance of the Ovation Prokaryotic RNA-Seq System elevates the proportion of uniquely mapping RNA reads to 10–35% of the total read count.

Currently, the most popular methods for preparing prokaryotic transcriptome libraries involve physical removal of ribosomal RNA by hybridization or nuclease digestion, methods that can also deplete desired non-rRNA transcripts. The ability of the Ovation Prokaryotic RNA-Seq System to enrich for unique RNA species in *E. coli* was compared to that of MICROBExpress™ Bacterial mRNA Enrichment Kit from Ambion®. As shown in **Figure 3**, NuGEN's selective priming methodology was highly effective at depleting rRNA (depleted by 88%) in the sample, as was a single round of MICROBExpress (depleted by 75%). Most impressive, however, were the results obtained when selective priming was combined with the hybridization-based rRNA depletion of MICROBExpress. With just a single round of hybridization-based depletion, rRNA was depleted by 97% in the Ovation Prokaryotic RNA-Seq System sample. As shown in the graph on the right in **Figure 3**, correlation was high between two independent samples exposed to either one or two rounds of MICROBExpress depletion followed by selective priming.

Figure 4 illustrates the overlap in annotated genes between those in a library treated with MICROBExpress and a library constructed using NuGEN's selective priming methodology. As shown in the graph on the right, few genes differed in RPKM values as much as five- or ten-fold between the two libraries.

FIGURE 2 **Ribosomal RNA Depletion from an NGS Transcriptome Library**



In a total RNA sample that is 95% rRNA and 5% uniquely mapping mRNA, elimination of 90% of the rRNA results in an NGS library that is 65% rRNA and 35% unique RNAs. The key feature of this library is a seven-fold enrichment of unique RNA species.

Reproducible transcription profiles regardless of GC content

In a study of six different microbial genomes with widely varying GC content, the Ovation Prokaryotic RNA-Seq System was shown to offer reproducible overall enrichment for mapping reads within a given sample. Gene expression levels were also highly reproducible and covered a dynamic range spanning four orders of magnitude (see **Figure 5**).

Figure 6 illustrates the uniform read depth and sharply delimited 3′ and 5′ boundaries provided by the Ovation Prokaryotic RNA-Seq System. At least 90% of known genes were detected at a coverage depth of 200,000–400,000 uniquely mapping reads.

FIGURE 3 Comparison of Ovation Prokaryotic RNA-Seq System Selective Priming (SP) Approach to MICROBExpress™

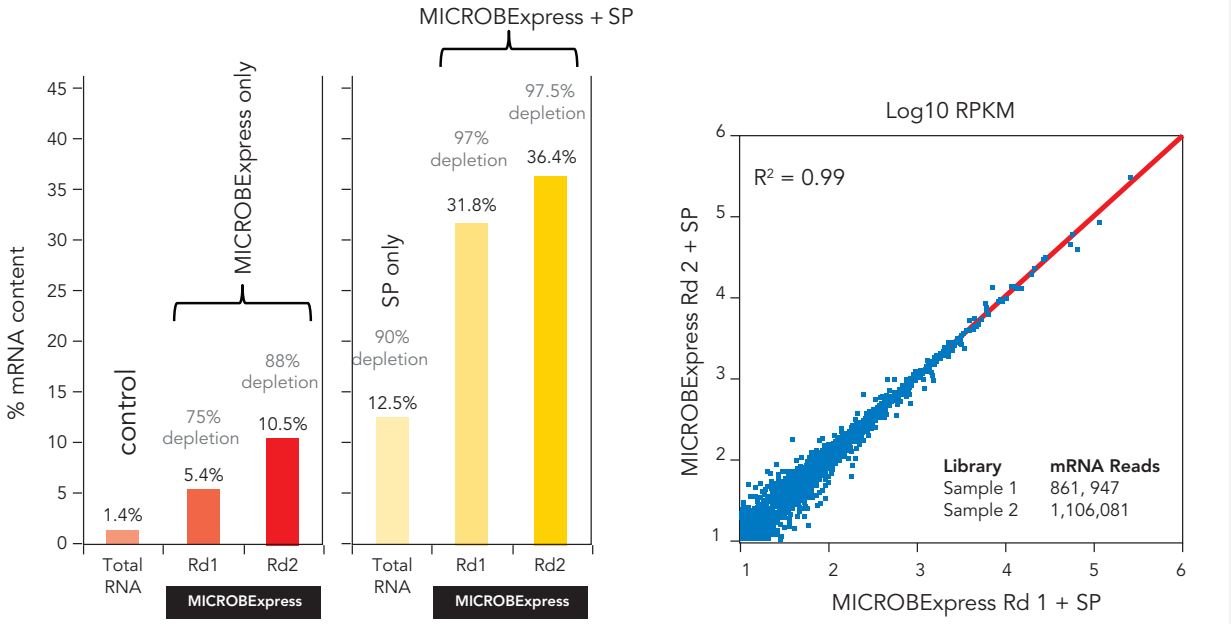
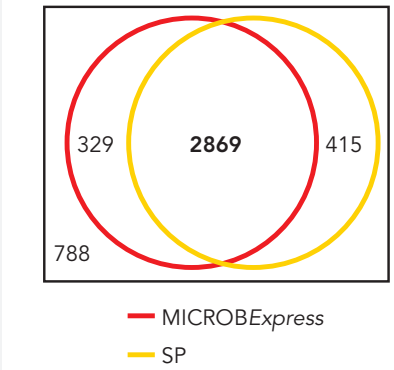
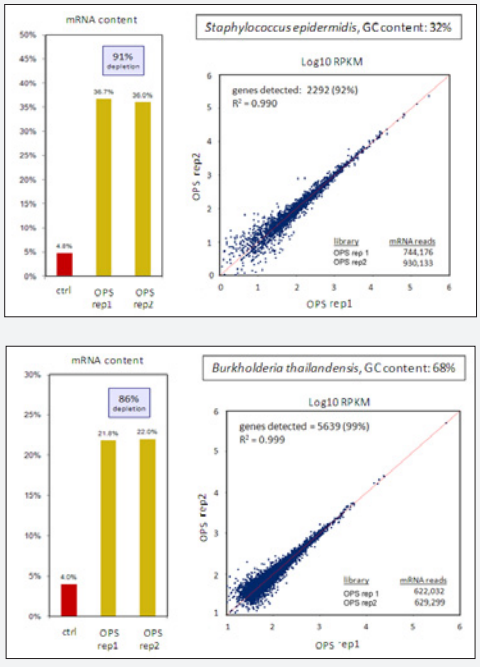


FIGURE 4 Present Calls for 4401 Genes



Venn diagram of all 4401 annotated ORFs in *E. coli* showing genes detected by both methods, one method alone or neither method.

FIGURE 5 The Ovation Prokaryotic RNA-Seq System (OPS) Performance



SP priming performance in bacteria with low GC content (*S. epidermidis*) and high GC content (*B. thailandensis*). Similar performance was observed for *S. aureus* (33% GC), *V. vulnificus* (47% GC), *E. coli* (51% GC) and *P. aeruginosa* (67% GC).

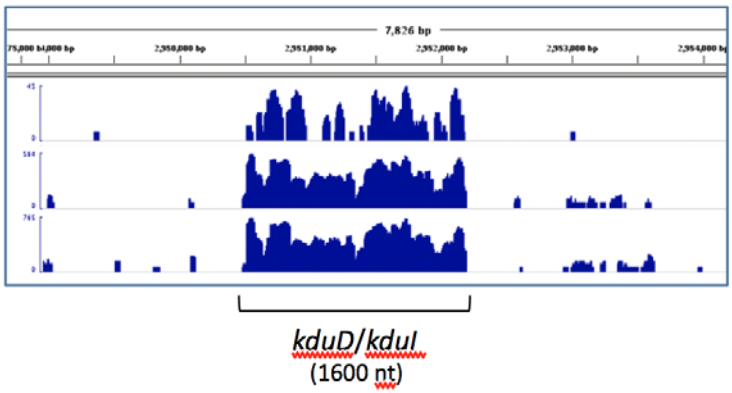
Conclusion

NuGEN's Ovation Prokaryotic RNA-Seq System, in combination with our Ovation Ultralow Library Systems, provides researchers with the capability to generate bacterial transcriptome sequences both efficiently and economically. The Ovation Prokaryotic RNA-Seq System is a complete solution for complementary whole transcriptome profiling studies with industry-leading rRNA reduction across all bacterial species. The low sample input requirement, and a simple end-to-end workflow from total RNA to NGS libraries make this system ideal for a wide variety of microbial samples.

ORDERING INFORMATION

Part No.	Product Name
9030	Ovation® Prokaryotic RNA-Seq System
Related Products	
0303	Ovation Ultralow Library System
0304	Ovation Ultralow IL Multiplex System 1–8
0305	Ovation Ultralow IL Multiplex System 9–16
0330	Ovation Ultralow DR Multiplex System 1–8
0331	Ovation Ultralow DR Multiplex System 9–16
Technical Documents	
Ovation Prokaryotic RNA-Seq System User Guide Ovation Prokaryotic RNA-Seq System White Paper	

FIGURE 6 **Uniformity of Transcript Coverage**



The horizontal axis shows a portion of the *E. coli* genome that includes the *KduD-kdul* bicistronic operon. The vertical axis shows the density of tag reads on a logarithmic scale. The sequencing results of three independent transcriptome libraries are shown; one is the random primer control and the other two are replicates of SP-generated libraries.

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