MAINTENANCE OF GENE REPRESENTATION AFTER AMPLIFICATION USING THE OVATION™ AMINOALLYL SYSTEM

BACKGROUND

When the amount of RNA obtained for gene expression analysis is limiting, RNA must be amplified before analysis using a process that maintains accurate representation of gene abundance for very rare to commonly expressed sequences. Therefore, amplification must be linear over a wide dynamic range.

The Ovation™ Aminoallyl System uses the simple, isothermal Ribo-SPIA™ RNA Amplification process which generates micrograms of cDNA from nanograms of total RNA in under four hours. This innovative process overcomes the time requirement and complexity limitations of T7-based methods.

Here we assess the linearity and dynamic range of the Ribo-SPIA™ process, both essential to maintaining accurate gene representation in gene expression analysis experiments.

MATERIALS AND METHODS

A plant sequence, without homology to known non-plant sequences currently in public databases, was added to total human RNA, amplified using the Ovation™ Aminoallyl System process, and the relative amounts of plant sequence detected in the amplified cDNA products by TaqMan analysis.

The equivalent of 100 to 1,000,000 copies per sample of plant RNA (Spike 8, A. thaliana NAC1, 457 bp, cat. #252208; Stratagene) was added to 20 ng of total RNA (HeLa, cat. #7354400-41, Stratagene). Duplicates of each sample were amplified using the protocol and reagents provided in the Ovation™ Aminoallyl System (cat. #2101-12, NuGEN). The relative amount of added plant RNA was then determined by duplicate TaqMan assay of the amplified cDNA products using probe primer sets complementary to Spike 8 (contact NuGEN™ for details) and the ABI Prism 7700 Sequence Detector (Applied Biosystems).

RESULTS AND CONCLUSIONS

The Ovation™ Aminoallyl System provided linear amplification for low to high abundance sequences over a dynamic range of four orders of magnitude (Figure 1). Transcripts present from 100 to 1,000,000 copies per sample were amplified to the same extent, thereby maintaining the same relative abundance of each original RNA transcript present in the amplified cDNA.

The linearity and very wide dynamic range of the Ribo-SPIA™ process used in the Ovation™ Aminoallyl System are essential elements in obtaining accurate microarray and Q-PCR results in gene expression studies using small samples.

System Specifications

Cat No.: 2101-12, 12 reactions

Input: 5-100 ng total RNA

Yield: 5-10 µg single stranded cDNA

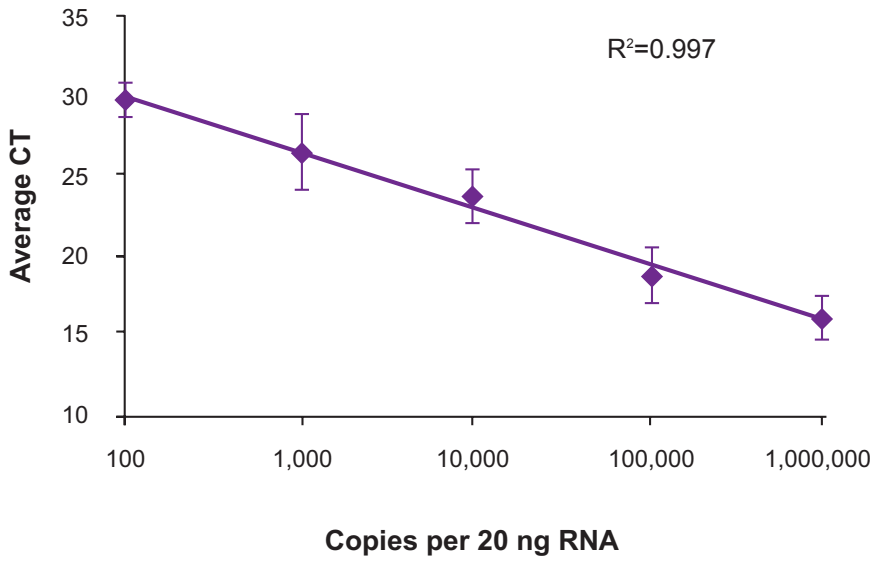

Figure 1. Linearity and Dynamic Range of Ribo-SPIA™ Amplification Used in the Ovation™ Aminoallyl System.
Samples were amplified and added plant sequence detected as described. The Cycle Threshold (CT) is an average of four data points for each amount of plant sequence shown.

NuGEN Technologies, Inc.
Headquarters USA
821 Industrial Road, Unit A • San Carlos, CA 94070 USA • Toll Free Tel: 888.654.6544 • Toll Free Fax: 888.296.6544 • www.nugeninc.com
custserv@nugeninc.com • techserv@nugeninc.com

Canada:
MJS BioLynx Inc.
P.O Bag 1150, 300 Laurier Blvd.
Brockville, ON K6V 5W1
Toll Free: 1-888-593-5969
Tel: (613) 498-2126
Fax: (613) 342-1341
sales@biolynx.ca or tech@biolynx.ca
www.biolynx.ca/contact-biolynx.html

Europe:
NuGEN Technologies, Inc.
P.O. Box 149,
6680 AC Bemmel
The Netherlands
Tel: +31(0)13 5780215
Fax: +31(0)13 5780216
europe@nugeninc.com
www.nugeninc.com

Asia:
MediBIC.
Daido Seimei Kasumigaseki
Building 8F, 1-4-2
Kasumigaseki, Chiyoda-ku,
Tokyo 100-0013, JAPAN
Tel: +81-3-5510-2313
Fax: +81-3-5510-2312
info@medibic.com
www.medibic.com

Australia:
Integrated Sciences Pty. Ltd.
2 McCabe Place
PO Box 731
Willoughby NSW 2068 Australia
Tel: 02 9417 7866 or
1 800 252 204 (Australia only)
Fax: 02 9417 5066
tech@integratedsci.com.au
www.integratedsci.com.au/contactus.asp

Israel:
ZOTAL Biological
& Instrumentation
4 Habarzel Street
Tel Aviv 69710, Israel
Tel:+972.3.6492444
Fax:+972.3.6496664
sales@zotal.co.il
www.zotal.co.il

NuGEN™