



Eberwine-type Kits Generate High Yields of aRNA from 25 to 500 ng of Total RNA

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EPICENTRE's new TargetAmp™ 1-Round Aminoallyl-aRNA Amplification Kit 101 enables the highest yields of aminoallyl-labeled antisense RNA (aRNA or cRNA), in the shortest time, for gene expression profiling of samples containing 25 to 500 ng of total RNA.

The kit uses an improved RNA amplification process (see insert on page 5) based on the method developed by Professor James Eberwine and colleagues.¹ The amplification protocol was improved by identifying better enzymes, optimizing enzyme reactions and reaction times, eliminating column cleanup of cDNA, and reducing the generation of “background RNA.” The TargetAmp method uses a simple “One Tube” protocol that produces aminoallyl-aRNA (AA-aRNA). The resulting AA-aRNA can be efficiently biotinylated or dye-labeled using Biotin-X-X-NHS (see page 7) or a Cy™-dye NHS ester, respectively, for subsequent gene expression profiling on Affymetrix GeneChip® Arrays, GE CodeLink™ Arrays, or on other platforms, including self-spotted arrays.

The TargetAmp Kit 101 amplifies poly(A) RNA at least 5000-fold. Examples of AA-aRNA yields obtained from different starting amounts of total RNA from HeLa cells are presented in Table 1.

Here we demonstrate the high quality data that can be obtained using GeneChip Arrays and biotin-aRNA produced by biotinylation of AA-aRNA generated using the TargetAmp 1-Round Aminoallyl-aRNA Amplification Kit 101.

HeLa Total RNA	HeLa Poly(A) RNA (assume poly(A) RNA is 2% of total RNA)	Aminoallyl-aRNA Yield	Fold-Amplification of Poly(A) RNA
25 ng	0.5 ng	2.9 µg	5800
50 ng	1.0 ng	5.2 µg	5200
100 ng	2 ng	11.4 µg	5700
500 ng	10 ng	69.9 µg	6990

Table 1. Yields of AA-aRNA obtained using the TargetAmp™ 1-Round Aminoallyl-aRNA Amplification Kit 101. Results are the average of multiple experiments using HeLa Total RNA.

Methods

Total RNA was isolated from mouse neural cells cultured for 6 or 12 days. For each sample, 200 ng were amplified using the TargetAmp 1-Round Aminoallyl-aRNA Amplification Kit 101 and purified by spin column chromatography. The AA-aRNA was biotin-labeled by conjugation with Biotin-X-X-NHS (EPICENTRE) and purified using the protocols supplied with the product. The resulting biotin-aRNA, as well as biotin-aRNA produced by the standard Affymetrix biotin-aRNA labeling procedure, was fragmented and hybridized to GeneChip Mouse Genome 430 2.0 Arrays, in triplicate.

Results

Amplified labeled target RNA generated using the TargetAmp 1-Round Aminoallyl-aRNA Amplification Kit 101 was evaluated for gene expression profiling with Affymetrix GeneChip arrays based on four criteria: the size of AA-aRNA produced, 3'-to-5' ratios, % Present Calls, and the correlation of differential gene expression compared to the standard Affymetrix T7 RNA polymerase amplification protocol.

Size distribution of the AA-aRNA

The size of the AA-aRNA produced by the TargetAmp 1-Round Aminoallyl-aRNA Amplification Kit 101 was analyzed by denaturing agarose gel electrophoresis and with the Agilent Bioanalyzer. The TargetAmp Kit 101 produced AA-aRNA with a size distribution between 250 to 4000 bases, with an average size of about 1200 bases (data not shown).

3'-to-5' ratios and % Present Calls

The average of the 3'-to-5' ratios and % Present Calls obtained using biotin-aRNA produced by the TargetAmp 1-Round Aminoallyl-aRNA Amplification Kit 101 and Biotin-X-X-NHS are shown in Table 2. These values are well within the quality assessment guidelines established by Affymetrix for target biotin-aRNA performance, and demonstrate that the TargetAmp Kit 101 yields high quality expression profile data using GeneChip Arrays.

Fold Amplification	>5000
3'/5' ratio GAPDH	1.05 +/- 0.05
3'/5' ratio β-actin	2.8 +/- 0.01
% Present Calls	56.3 +/- 2 %
Reproducibility (2 amplifications)	0.99

Table 2. AA-aRNA produced using the TargetAmp™ 1-Round Aminoallyl-aRNA Amplification Kit 101, and subsequently biotinylated, produced high quality GeneChip Arrays as determined by Affymetrix quality assessment guidelines.

Correlation of differential gene expression

Chips hybridized to biotin-aRNA produced using the TargetAmp Kit 101 protocol were compared to chips hybridized to biotin-aRNA produced using the standard Affymetrix protocol, with respect to differentially expressed genes in mouse neural cells cultured for 6 or 12 days. Differentially expressed genes were identified and compared using the GeneSpring software package (Silicon Genetics). The 12-day vs. 6-day expression ratios obtained with target RNA prepared using the TargetAmp Kit 101 protocols versus the standard Affymetrix protocol are plotted in Figure 1. The correlation coefficient of 0.90 demonstrates good agreement between differentially expressed genes identified by the TargetAmp Kit 101 protocol compared to the standard Affymetrix T7 RNA polymerase protocol.

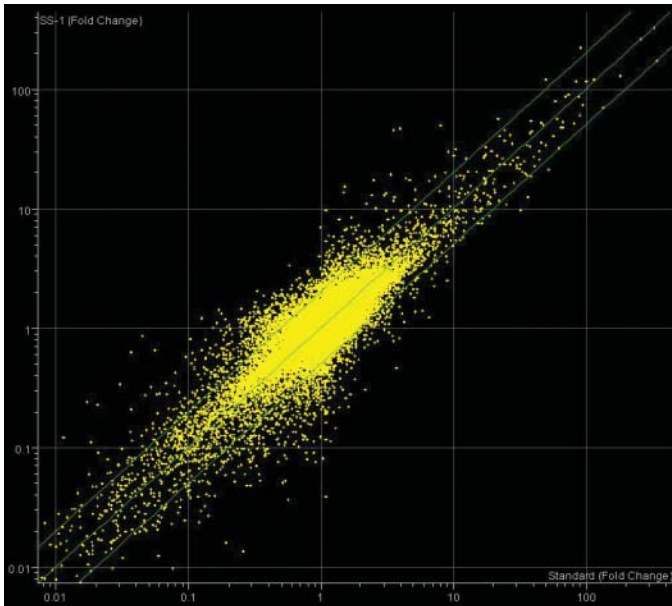


Figure 1. Differentially expressed genes identified using biotin-aRNA produced by the TargetAmp™ 1-Round Aminoallyl-aRNA Amplification Kit 101, and subsequent biotinylation, and biotin-aRNA produced by the standard Affymetrix T7 RNA polymerase protocol gave a correlation coefficient of 0.90. The 'Y' axis shows 12-day vs. 6-day gene expression ratios obtained using biotin-aRNA target produced by the TargetAmp 1-Round Kit 101 protocol. The 'X' axis shows the 12-day vs. 6-day gene expression ratios obtained using biotin-aRNA target produced by the Affymetrix standard procedure.

Conclusion

The TargetAmp 1-Round Aminoallyl-aRNA Amplification Kit 101 amplifies poly(A) RNA at least 5000-fold in 6 hours. This level of amplification results in sufficient AA-aRNA from 25 to 500 ng of total

RNA to produce high quality gene expression profiles using Affymetrix GeneChip or other microarray platforms.

Reference

1. Van Gelder, R.N. et al. (1990) *PNAS USA* 87, 663.

Two TargetAmp 1-Round aRNA Amplification Kits are now available:

Two TargetAmp 2-Round aRNA Amplification Kits are now available:

www.EpiBio.com/targetamp.asp

TargetAmp™ 1-Round Aminoallyl-aRNA Amplification Kit 101*

Produces **aminoallyl-labeled aRNA** from 25 to 500 ng of total cellular RNA.

TAA1R4910	10 Reactions
TAA1R4924	24 Reactions

TargetAmp™ 1-Round aRNA Amplification Kit 103*

Produces **unlabeled-aRNA** from 25 to 500 ng of total cellular RNA.

TAU1R5110	10 Reactions
TAU1R5124	24 Reactions

* These kits have been optimized for use with SuperScript™ III (Invitrogen) Reverse Transcriptase, which is not included in the kits.

www.EpiBio.com/targetamp.asp

TargetAmp™ 2-Round Aminoallyl-aRNA Amplification Kit 1.0*

Produces **aminoallyl-labeled aRNA** from 10 to 500 pg (1 to 50 cells) of total cellular RNA.

TAA2R4910	10 Reactions
TAA2R4924	24 Reactions

TargetAmp™ 2-Round aRNA Amplification Kit 2.0*

Produces **unlabeled-aRNA** from 10 to 500 pg (1 to 50 cells) of total cellular RNA.

TAU2R5110	10 Reactions
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* These kits have been optimized for use with SuperScript™ II & III (Invitrogen) Reverse Transcriptases, which are not included in the kits.

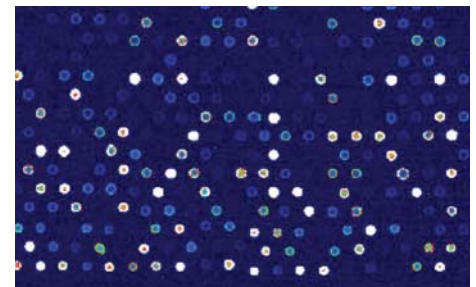
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Biotin-X-X-NHS, for Biotin Labeling of Aminoallyl-RNA

Biotin-X-X-NHS provides an efficient and cost-effective method for labeling aminoallyl-aRNA or aminoallyl-RNA produced using EPICENTRE's TargetAmp™ Aminoallyl-aRNA Amplification Kits (see Page 4) or AmpliScribe™ T7 Aminoallyl-RNA Transcription Kit, respectively.



EPICENTRE's Biotin-X-X-NHS:

- Contains a long spacer arm to maximize binding to avidin or streptavidin conjugates by minimizing steric hindrance between multiple biotinylated sites.
- Is supplied as a convenient 2.5 mg powder in an air-tight vial with a self-sealing septum.
- Is packaged under dry inert gas and is stable for at least one year when stored in powdered form at +4°C.
- Provides more cost-effective labeling than direct incorporation of biotin-UTP.

Biotin-X-X-NHS can also be used for labeling aminoallyl-cDNA and proteins.

www.EpiBio.com/biotin.asp

Biotin-X-X-NHS

BXX51005	5X	2.5 mg vials
BXX51010	10X	2.5 mg vials