

Achieve High Fidelity, High Sensitivity, and High Yield RNA Amplification That Produces High Quality Array Results

In previous issues of the *EPICENTRE Forum* newsletter,^{1,2} we reported the high quality microarray data obtained by using antisense RNA (aRNA; also called cRNA), or aminoallyl-aRNA (AA-aRNA) produced by the **TargetAmp™ aRNA** or **TargetAmp™ Aminoallyl-aRNA Amplification Kits**. This report summarizes the key benefits that these kits offer the user for their microarray applications.

High fidelity RNA amplification

The TargetAmp® Kits utilize an improved “Eberwine” linear RNA amplification process that faithfully preserves the gene expression profile of the sample. The relative abundance of 20 transcripts in Universal Human Reference RNA (Stratagene), and in adult skeletal muscle RNA, was compared by quantitative real-time RT-PCR (qRT-PCR) before and after RNA amplification, using both the TargetAmp™ 1-Round aRNA Amplification Kit 103, and the TargetAmp™ 2-Round aRNA Amplification Kit 2.0.² The TargetAmp 1-Round (see FIG 1) and 2-Round amplification reactions yielded correlation coefficients of 0.961 and 0.955, respectively. These high correlation coefficients confirm the aRNA (or AA-aRNA) produced by a TargetAmp 1-Round or 2-Round amplification reaction is biologically comparable to the nonamplified RNA. The aRNA can therefore give high quality, biologically valid microarray results; indeed, the high fidelity of the TargetAmp 2-Round aRNA Amplification Kit reaction has now been independently established.³

High sensitivity. . . high yields of aRNA and Aminoallyl-aRNA with virtually no nonspecific amplification product

TargetAmp Kits are optimized to produce high yields of aRNA (or AA-aRNA) from very small amounts of total RNA. A TargetAmp 1-Round Amplification reaction produces microgram amounts of aRNA (or AA-aRNA) from as little as 25 ng of total RNA (Table 1). This corresponds to >5,000-fold amplification of the poly(A) RNA in the total RNA sample.

TargetAmp 2-Round reactions produce microgram amounts of aRNA (or AA-aRNA) from as little as 10 pg of total RNA (about the amount of total RNA in one

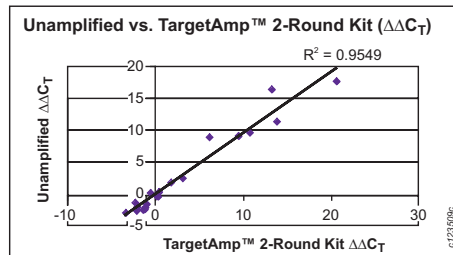


FIG 1. TargetAmp™ 2-Round aRNA Amplification reactions accurately preserve the relative transcript abundance of a sample. The differences in expression levels between sample types are represented as the normalized ΔC_T in qPCR cycles ($\Delta\Delta C_T$).

cell; Table 2), corresponding to >5,000,000-fold amplification of the poly(A) RNA in a total RNA sample.

Most Eberwine T7-based RNA amplification procedures, including other commercial kits, produce a significant amount of template-independent reaction product (nonspecific amplification product). The TargetAmp Amplification Kits utilize a proprietary chemistry that virtually eliminates nonspecific amplification products.

HeLa Total RNA	Aminoallyl-aRNA Yield	Fold-Amplification of Poly(A) RNA
25 ng	2.9 µg	5800
100 ng	11.4 µg	5700
500 ng	69.9 µg	6990

Amount of Total RNA Amplified	aRNA Yield from Total Rat Brain RNA Poly(A) RNA (5% of total rat brain RNA)	aRNA Yield from Total HeLa RNA Poly(A) RNA (2% of total HeLa RNA)	aRNA Yield from Total Rat Kidney RNA Poly(A) RNA (1.5%-2% of total rat kidney RNA)
10 pg (1 cell)	3 µg (6 x 10 ⁶)	1.9 µg (9.5 x 10 ⁶)	1.4 µg (9.3 x 10 ⁶)
100 pg (10 cells)	54 µg (10.8 x 10 ⁶)	19 µg (9.5 x 10 ⁶)	17 µg (11 x 10 ⁶)
500 pg (50 cells)	143 µg (5.7 x 10 ⁶)	74 µg (7.4 x 10 ⁶)	71 µg (9.4 x 10 ⁶)

Table 2. Yields of aRNA obtained using the TargetAmp™ 2-Round aRNA Amplification Kit 2.0. Results are the average of multiple experiments using total RNA from three sources, each with a different poly(A) RNA content. The fold-amplification of the poly(A) RNA contained in each is shown in parentheses. Yields of AA-aRNA produced by the TargetAmp™ 2-Round Aminoallyl-aRNA Amplification Kit 1.0 are essentially the same.

Reproducible RNA amplification results

Two samples of total cellular RNA, each isolated from a single laser captured cell from a rat dorsal root ganglion section, were amplified independently using the TargetAmp 2-Round Aminoallyl-aRNA Amplification Kit 1.0. The resulting AA-aRNAs were labeled with the dye CyTM3-NHS, and hybridized in parallel to rat arrays. A scatter plot of the fluorescence intensities of the transcripts detected on the two arrays is shown in FIG 2. The correlation coefficient of 0.993 indicates a highly reproducible amplification reaction even when amplifying RNA from a single cell.

Microarray target labeling options

AA-aRNA produced by the TargetAmp 1-Round Aminoallyl-aRNA Amplification Kit 101, and TargetAmp 2-Round Aminoallyl-aRNA Amplification Kit 1.0, can be readily labeled with biotin (using Biotin-X-X-NHS; EPICENTRE Biotechnologies), or a fluorescent dye such as Cy-NHS in a simple one hour reaction. Alternately, the final *in vitro* transcription step of the TargetAmp

Table 1. Yields of aminoallyl-aRNA obtained using the TargetAmp™ 1-Round Aminoallyl-aRNA Amplification Kit 101. Results are the average of multiple experiments using the HeLa total RNA. Yields of unlabeled aRNA using the TargetAmp™ 1-Round aRNA Amplification Kit 103 are essentially the same.

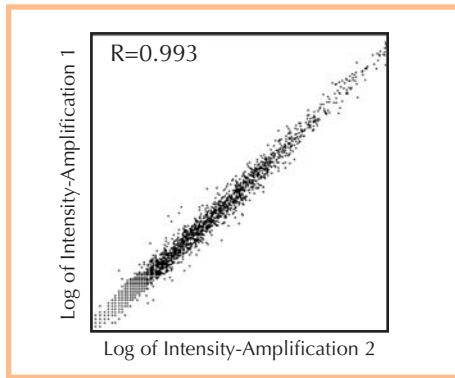


FIG 2. TargetAmp™ aRNA amplification reactions are highly reproducible.

1-Round and 2-Round aRNA Amplification Kits can be readily modified for incorporation of a biotin-NTP (provided by the user), or a fluorescent-NTP (provided by the user).

High quality microarray results

The aRNA and AA-aRNA produced by a TargetAmp 1-Round or 2-Round reaction can be readily labeled with biotin or a fluorescent dye for high quality microarray results using a variety of array platforms, including Affymetrix® GeneChip® or other commercial or spotted arrays. The high quality of biotin-aRNA prepared by conjugating Biotin-X-X-NHS to AA-aRNA produced by the TargetAmp 1-Round and 2-Round Kits is shown in Table 3. Significantly,

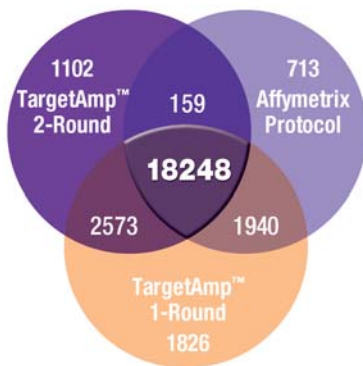


Fig 3. Overlap of present calls using biotin-aRNA produced with the TargetAmp™ 1-Round and TargetAmp™ 2-Round Aminoallyl-aRNA Amplification Kits, and the Affymetrix Protocol.²

	TargetAmp™ 1-Round Aminoallyl-aRNA Amplification Kit 101	TargetAmp™ 1-Round aRNA Amplification Kit 103	TargetAmp™ 2-Round Aminoallyl-aRNA Amplification Kit 1.0	TargetAmp™ 2-Round aRNA Amplification Kit 2.0
Amount of input HeLa total RNA	≥25ng	≥25ng	≥10pg	≥10pg
aRNA yield (see Tables 1 & 2 for more information)	3 – 70 µg	3 – 70 µg	1.9 – 74 µg	1.9 – 74 µg
Fold amplification of poly(A) RNA	>5000	>5000	>5,000,000	>5,000,000
Time required	6 hours	6 hours	2 Days	2 Days
End product	Aminoallyl-aRNA	Unlabeled-aRNA	Aminoallyl-aRNA	Unlabeled-aRNA

Table 4. Which TargetAmp™ Kit is best for you?

the 3'/5' GAPDH, and 3'/5' β-actin signal ratios obtained using AA-aRNA produced by the TargetAmp 2-Round protocol are similar to, or better than, those obtained with the GeneChip Eukaryotic Small Sample Target Labeling Version II protocol (Affymetrix) when starting with one nanogram of total RNA. The overlap of Present Calls from the same experiment is shown in FIG 3, and is indicative of good data representation.

Choose the TargetAmp Kit that is right for you

Unlike other commercial RNA amplification kits, each of the four TargetAmp Kits contains specific enzymes and concentrations of primers, nucleotides, and buffers to yield optimized results for each particular application. Table 4 is designed to help you identify which of the TargetAmp Kits best meets your needs.

References

1. Khanna, A. et al., (2005) EPICENTRE Forum 12(1), 4.
2. Khanna, A. et al., (2006) EPICENTRE Forum 13(1), 4.
3. Yang, F. et al., (2006) Oncogene 25(9), 1413.

www.EpiBio.com/targetamp.asp

TargetAmp™ 1-Round Aminoallyl-aRNA Amplification Kit 101
 TAA1R4910 10 Reactions
 TAA1R4924 24 Reactions

TargetAmp™ 1-Round aRNA Amplification Kit 103
 TAU1R5110 10 Reactions
 TAU1R5124 24 Reactions

TargetAmp™ 2-Round Aminoallyl-aRNA Amplification Kit 1.0
 TAA2R4910 10 Reactions
 TAA2R4924 24 Reactions

TargetAmp™ 2-Round aRNA Amplification Kit 2.0
 TAU2R5110 10 Reactions
 TAU2R51224 24 Reactions

Biotin-X-X-NHS
 BXX51005 5 X 2.5 mg vials
 BXX51010 10 X 2.5 mg vials

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Amplification and labeling protocol	Mouse neural cell RNA post-treatment	% Present Calls	Signal	Noise	Background	3'/5' GAPDH	3'/5' β-actin
TargetAmp™ 1-Round Kit 101	6-day	55.0%	1401	1.99	58.5	1.01	2.72
TargetAmp™ 1-Round Kit 101	12-day	56.4%	1411	2	60.07	1.07	2.7
TargetAmp™ 2-Round Kit 1.0	6-day	48.1%	1588	1.7	50.13	4.07	10.3
TargetAmp™ 2-Round Kit 1.0	12-day	50.7%	1434	1.7	51.3	6.6	10.9

Table 3. Evaluation of biotin-aRNA produced by the TargetAmp™ 1-Round Aminoallyl-aRNA Amplification Kit 101, TargetAmp™ 2-Round Aminoallyl-aRNA Amplification Kit 1.0, and Biotin-X-X-NHS using Affymetrix MG430 2.0 GeneChip arrays.²