

TargetAmp™ aRNA Amplification Kits: High Fidelity RNA Amplification for High Quality Microarray Results

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In a previous issue of the EPICENTRE Forum newsletter¹ we introduced the TargetAmp™ 1-Round and TargetAmp™ 2-Round aRNA Amplification Kits and documented the high yields of antisense RNA (aRNA; also called cRNA) that they produce. In this report we demonstrate that TargetAmp aRNA amplification reactions preserve the relative transcript abundance of the original samples and that the aRNA produced by both 1-round and 2-round TargetAmp reactions yield high quality microarray results.

Preservation of relative transcript abundance Quantitative Real-Time PCR

The relative abundance of 20 transcripts in Universal Human Reference RNA (Stratagene) and in adult skeletal muscle RNA were compared by quantitative real-time RT-PCR (qPCR) before and after RNA amplification. RNA was amplified independently using both the TargetAmp™ 1-Round aRNA Amplification Kit 103 and the TargetAmp™ 2-Round aRNA Amplification Kit 2.0. The aRNA produced was reverse transcribed using random hexamer primers to produce cDNA. qPCR (SYBR® Green I dye detection) was performed using EPICENTRE Biotechnologies' TAQurate™ GREEN Real-Time PCR MasterMix. Beta-2-microglobulin (B2M) was used for expression level normalization and each cDNA was diluted so that the C_T value for each B2M amplification was as similar as possible. Four data points were averaged and normalized for each primer pair tested.

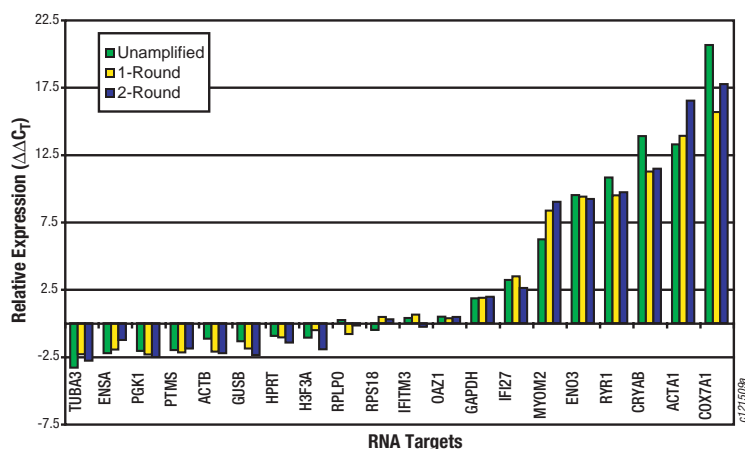


FIG 1. Amplification maintains gene expression levels. The relative abundance of 20 selected transcripts was compared before and after TargetAmp™ Amplification reactions. The differences in expression levels between Universal Human Reference RNA and adult skeletal muscle RNA samples are represented as the normalized ΔC_T in qPCR cycles ($\Delta\Delta C_T$). The results demonstrate that both the TargetAmp 1-Round and 2-Round aRNA amplification reactions preserve the relative gene expression levels.

Results

The results presented in FIG 1 and re-plotted in FIG 2(A and B) demonstrate that the TargetAmp 1-Round and TargetAmp 2-Round aRNA amplification reactions preserve the relative gene expression levels of the sample. The TargetAmp 1-Round Kit yields a correlation coefficient (R^2) = 0.961 and the TargetAmp 2-Round Kit a correlation coefficient (R^2) = 0.955, which is considered excellent preservation of relative transcript abundance after two rounds of amplification.

Performance on Affymetrix® GeneChip® arrays RNA amplification, target labeling, and hybridization

Total RNA was isolated from 6-day and 12-day post-treatment cultured mouse neural cells. The performance of TargetAmp amplified and labeled RNA was evaluated as follows:

1) Four-hundred picograms of total RNA from both the 6-day and 12-day cultures were amplified using the TargetAmp 2-Round Aminoallyl-aRNA Amplification Kit 1.0. The aminoallyl-aRNA (AA-aRNA) produced was then conjugated to Biotin-X-X-NHS (EPICENTRE) according to the product literature.

2) Two-hundred nanograms of total RNA from the 6-day and 12-day cultures were amplified using the TargetAmp 1-Round Aminoallyl-aRNA Amplification Kit 101. The AA-aRNA produced was then conjugated to Biotin-X-X-NHS (EPICENTRE) according to the product literature.

3) Five micrograms of total RNA from the 6-day and 12-day cultures were processed using the biotin-aRNA labeling procedure (essentially a 1-round RNA amplification process) recommended by Affymetrix (the "Affymetrix Protocol").

The resulting biotin-labeled aRNA was then fragmented and hybridized under identical conditions, in triplicate, to Affymetrix MG430 2.0 GeneChip arrays.

Results

Quality metrics

The overall quality of the biotin-labeled targets prepared using the TargetAmp 1-Round and TargetAmp 2-Round Aminoallyl-aRNA Amplification Kits was assessed using standard quality metrics defined by Affymetrix. Table 1 shows average values of selected quality metrics.

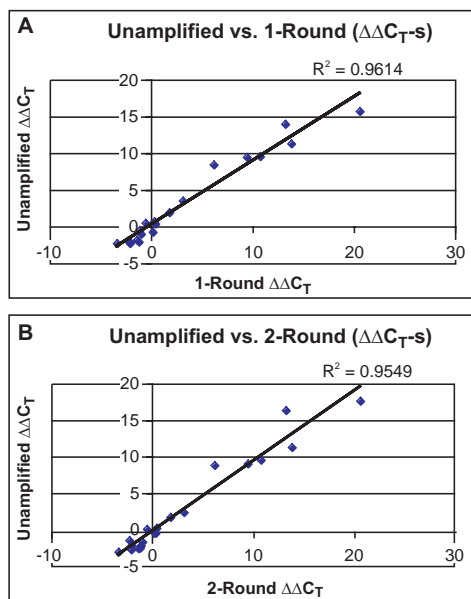


FIG 2. The data of FIG 1 were re-plotted for both the TargetAmp™ 1-Round aRNA Amplification Kit 103 (FIG 2A) and the TargetAmp™ 2-Round aRNA Amplification Kit 2.0 (FIG 2B). Correlation coefficients >0.95 were obtained demonstrating preservation of relative transcript abundance after the TargetAmp aRNA amplification reactions.

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 1. Evaluation by Affymetrix performance metrics of biotin-aRNA produced using the TargetAmp™ 1-Round and TargetAmp™ 2-Round Aminoallyl-aRNA Amplification Kits and Biotin-X-X-NHS.

The Signal, Noise, Background and 3'/5' ratio values are in the expected range for typical experiments. Significantly, the 3'/5' GAPDH and 3'/5' β-actin signal ratios obtained using 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 1 ng of total RNA. The high percentage of Present Calls is indicative of good data representation.

Spike-in Poly-A Controls

Prior to amplification, the GeneChip Poly-A RNA Control (Affymetrix) was added to the mouse total RNA. The Poly-A RNA Control is a mixture of five polyadenylated bacterial transcripts, at final complexity ratios of 1:100,000, 1:50,000, 1:25,000, and 1:7,500. All controls were called "Present" in the aRNA produced by TargetAmp 1-Round and TargetAmp 2-Round reactions and showed signal intensities proportional to their input concentrations (FIG 3).

Call Concordance between TargetAmp™ and Affymetrix protocol

MAS 5.0 software was used to assign a "Present", "Absent" or "Marginal" call to each probe set. "Present" and "Absent" calls were compared between arrays hybridized with the biotin-labeled aRNA produced by the TargetAmp 1-Round Kit 101, the TargetAmp 2-Round Kit 1.0, and

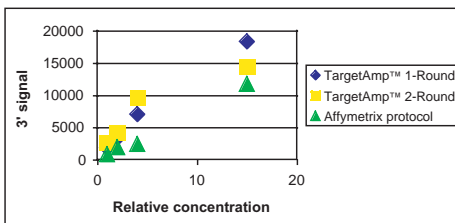


FIG 3. TargetAmp™ 1-Round and TargetAmp™ 2-Round aRNA amplification reactions yield linear RNA amplification of spiked-in poly(A) RNA controls. Data were analyzed using MAS 5.0 software.

using the Affymetrix Protocol. FIG 4 shows the overlap between the probe sets called "Present" for genes expressed in the 12-day old cultures, and Table 2 shows the proportion of probe sets with common or discordant detection calls. The results demonstrate high concordance between calls obtained using biotin-labeled aRNA

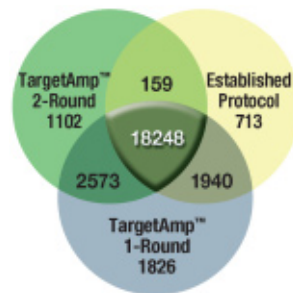


FIG 4. Overlap of present calls using biotin-aRNA produced using the TargetAmp™ 1-Round and TargetAmp™ 2-Round Aminoallyl-aRNA Amplification Kits and the Affymetrix Protocol.

SAMPLE "A"		
	Affymetrix 1-Round	
TargetAmp™ 1-Round	P	A
P	0.4570	0.0727
A	0.0072	0.3856
TargetAmp™ 2-Round	P	A
P	0.3661	0.0511
A	0.0293	0.4031

SAMPLE "B"		
	Affymetrix 1-Round	
TargetAmp™ 1-Round	P	A
P	0.4476	0.0747
A	0.0138	0.4019
TargetAmp™ 2-Round	P	A
P	0.4081	0.0641
A	0.0489	0.4174

Table 2. Biotin-aRNA produced using the TargetAmp™ 1-Round and TargetAmp™ 2-Round Aminoallyl-aRNA Amplification Kits yield high call concordance with biotin-aRNA produced by the Affymetrix Protocol.

produced by the TargetAmp 1-Round and TargetAmp 2-Round reactions and the Affymetrix Protocol.

Detection of differentially expressed genes

Genes differentially expressed in the 6-day and 12-day cultures were identified using the EBarrays algorithm²⁻³ after robust multi-array average (RMA) normalization.⁴ All 45,000 probe sets were included in the analysis. Expression ratios were calculated from the hybridizations performed using biotin-aRNA produced using the TargetAmp 1-Round Kit (FIG 5A) or the TargetAmp 2-Round Kit (FIG 5B) and biotin-aRNA produced by the Affymetrix Protocol. The differentially expressed genes were ranked by expression ratio, with the highest rank assigned to the highest Day6/Day12 ratio, and lowest rank to the lowest Day6/Day12 ratio. FIGS 5A and B show the correlation of the ranking order between the Affymetrix Protocol and the TargetAmp 1-Round protocol, and between the TargetAmp 1-Round Kit and TargetAmp 2-Round Kit protocols, respectively.

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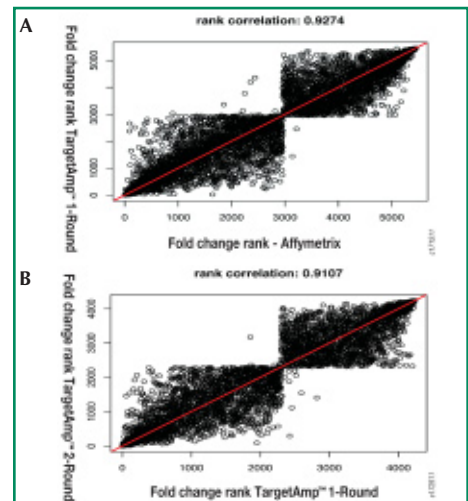


FIG 5. The ranking of the correlation of differentially expressed genes between the TargetAmp™ 1-Round (5A) and TargetAmp™ 2-Round (5B) aRNA amplification reactions and the Affymetrix Protocol.

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Rapid, High Yield Purification of Plasmids Free of RNA and Genomic DNA

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EPICENTRE Biotechnologies' PlasmidMAX™ DNA Isolation Kit produces high quality plasmid DNA free of RNA and genomic DNA, which is pure enough for sequencing and uses only two 1.5 ml tubes, thus avoiding excess plastic waste. Compared to a leading 5-minute spin column-based plasmid purification kit, PlasmidMAX-purified samples gave: higher DNA yields, less residual RNA, and greater or equal sequence read lengths.

The PlasmidMAX procedure is scalable to larger or smaller starting numbers of cells, without having to buy separate size-specific (mini-, midi-, maxi-) kits. The Kit purifies plasmid DNA (size range tested 2.7 to 40 kb) without the need for toxic organic solvents. From 1.5 ml of an overnight *E. coli* culture, PlasmidMAX routinely yields 2 µg or more of high-copy plasmid DNA that is free of RNA, unlike a leading 5-minute plasmid purification kit (See FIG 1).



FIG 1. Comparison of plasmid DNA purified using the PlasmidMAX™ DNA Isolation Kit and a leading 5-minute method. From 400 µl of an overnight *E. coli* K12 culture, plasmids were purified by each method. **Lane 1**, Supercoiled DNA ladder; **Lane 2**, Leading 5-minute purification method; **Lane 3**, PlasmidMAX DNA. The size of the plasmid is 4.5 kb. Arrow indicates RNA contamination in lane 2.

In terms of suitability for DNA sequencing, PlasmidMAX supported a sequence read length equivalent to that obtained from DNA purified by a leading spin column-based plasmid kit. Additionally, PlasmidMAX caused less linearization than a column-based kit (See FIG 2). Plasmids prepared with both kits gave comparable transfection efficiencies with HeLa cells and had similar insignificant endotoxin levels, exogenous contaminants that can reduce transfection efficiencies of sensitive eukaryotic cells. Table 1 summarizes these comparisons.

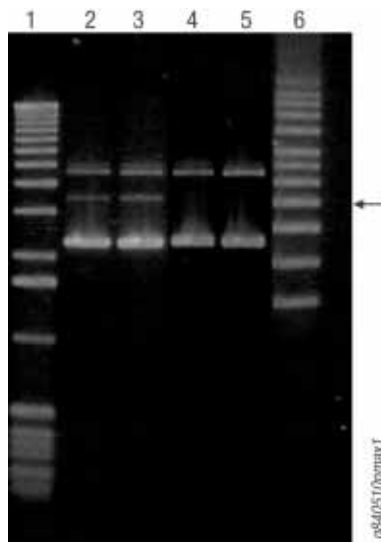


FIG 2. PlasmidMAX™ DNA Isolation Kit causes less linearization than a leading spin column-based method. Lane 1, kb DNA ladder; Lanes 2 and 3, Column-based method; Lanes 4 and 5, PlasmidMAX DNA; Lane 6 Supercoiled kb ladder. The size of the plasmid is 3.5 kb. Arrow indicates linearized plasmid.

Benefits	Plasmid Kit	
	PlasmidMAX™ Kit	Column-based kit
DNA yield from 400 µl of culture	2 µg	1 µg
Transfection	+++	+++
Endotoxin Level	Trace	Trace
Scalable	Yes	No

Table 1. Plasmid transfection efficiencies with HeLa cells and associated endotoxin levels.

www.EpiBio.com/plasmidmax.asp

PlasmidMAX™ DNA Isolation Kit
PMX51050 50 Minipreps

TargetAmp™ Kits—Cont'd from Page 5

Conclusion

In this report, we have demonstrated that the TargetAmp™ 1-Round and TargetAmp 2-Round aRNA amplification reactions maintain the relative transcript abundance in a total RNA sample. This ensures that the aRNA (or aminoallyl-aRNA) produced by the TargetAmp 1-Round and TargetAmp 2-Round reactions yield high quality, biologically valid microarray data, which were confirmed by microarray results obtained with an Affymetrix MG430 2.0 GeneChip as judged by Affymetrix quality metrics, concordance of Present and Absent calls with the Affymetrix Protocol, and detection of differentially expressed genes.

References

1. Khanna, A. Radek, A. and Pease, J. (2005) EPICENTRE Forum 12(1), 4.
2. Newton, M.A., et al. (2001) J. Comput. Biol. 8(1), 37.
3. Kendzioriski, C.M., et al. (2003) Stat. Med. 22(24), 3899.
4. Irizarry, R.A., et al. (2003) Biostatistics 4(2), 249.

www.EpiBio.com/targetamp.asp

TargetAmp™ 2-Round Aminoallyl-aRNA Amplification Kit 1.0

Produce microgram amounts of aminoallyl-labeled aRNA from 1 to 50 cells (10 pg to 500 pg of total cellular RNA) for greater than 5,000,000-fold amplification.

TAA2R4910	10 Reactions
TAA2R4924	24 Reactions

TargetAmp™ 2-Round aRNA Amplification Kit 2.0

Produce microgram amounts of unlabeled-aRNA from 1 to 50 cells (10 pg to 500 pg of total cellular RNA) for greater than 5,000,000-fold amplification.

TAU2R5110	10 Reactions
TAU2R51224	24 Reactions

TargetAmp™ 1-Round Aminoallyl-aRNA Amplification Kit 101

Produce microgram amounts of aminoallyl-aRNA from as little as 25 ng of total cellular RNA.

TAA1R4910	10 Reactions
TAA1R4924	24 Reactions

TargetAmp™ 1-Round aRNA Amplification Kit 103

Produce microgram amounts of unlabeled-aRNA from as little as 25 ng of total cellular RNA.

TAU1R5110	10 Reactions
TAU1R5124	24 Reactions

Biotin-X-X-NHS

Convenient 2.5 mg vials of biotin-X-X-NHS for fast and efficient conjugation of biotin to aminoallyl-aRNA.

BXX51005	5 X 2.5 mg/vial
BXX51010	10 X 2.5 mg/vial