

# Detect More Transcripts and Use Less RNA in Your Microarray Studies

## **New!** TargetAmp™ 1-Round Biotin-aRNA Amplification Kit 104

Biotin-aRNA (biotin-cRNA) is the labeled target used with the popular Affymetrix® GeneChip® and Illumina® BeadChip microarray platforms. EPICENTRE Biotechnologies' new TargetAmp™ 1-Round Biotin-aRNA Amplification Kit 104 (TargetAmp Kit 104) performs optimally on these platforms and features:

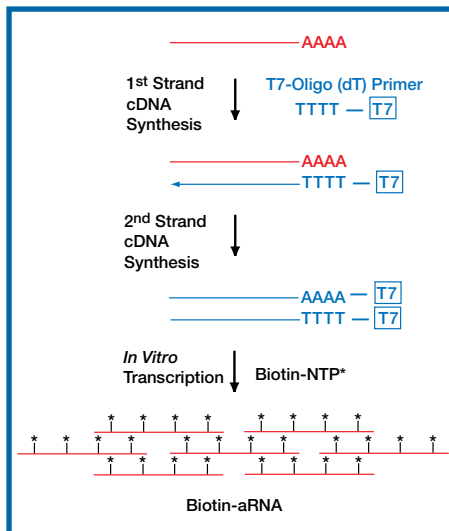
- A linear RNA amplification process that produces microgram amounts of biotin-aRNA from as little as 25 ng of input total RNA.
- A premixed solution of UTP/Biotin-UTP optimized for biotin-aRNA yield, length and signal intensity.
- A fast 6-hour, one-tube reaction.

In this report, we demonstrate that the biotin-aRNA generated by a TargetAmp Kit 104 reaction yields reliable, high quality microarray results on the Illumina platform, detects more transcripts using less input RNA than biotin-aRNA produced by a competitor's kit (Company A), and demonstrates a high degree of concordance with biotin-aRNA produced by biotin-labeling of aminoallyl-aRNA.

### Methods and Results

Biotin-aRNA targets for microarrays were prepared using three commercially available RNA amplification/labeling kits:

1. TargetAmp 1-Round Biotin-aRNA Amplification Kit 104 (FIG 1).
2. Conjugation of Biotin-X-X-NHS (EPICENTRE) to aminoallyl-aRNA



**FIG 1. A TargetAmp™ 1-Round Biotin-aRNA Amplification Kit 104 reaction can be completed in 6 hours.** Biotin-UTP, included in a UTP/Biotin-UTP solution in the kit, is incorporated into aRNA during the *in vitro* transcription step. The reaction is optimized for biotin-aRNA yield, length, and signal intensity.

produced by the TargetAmp 1-Round Aminoallyl-aRNA Amplification Kit 101 (TargetAmp Kit 101, EPICENTRE).

3. Another commercially available 1-round biotin-aRNA synthesis kit (Company A).

One-hundred nanograms of mouse liver total RNA or mouse skeletal muscle total RNA were used in each TargetAmp Kit 104 and TargetAmp Kit 101 reaction, and 400 ng of each RNA were used for Company A

kit reactions. Samples consisting of 850 ng of biotin-aRNA were taken from each of the three amplification/labeling reactions, and then hybridized in duplicate to Sentrix™ MouseRef-8 BeadChip arrays (Illumina) containing 24,049 probes. Data analysis was performed using BeadStudio software (Illumina).

### A TargetAmp 1-Round Biotin-aRNA Amplification Kit 104 reaction generates high yields of biotin-aRNA

The amount of biotin-aRNA produced from different amounts of HeLa cell total RNA by a TargetAmp Kit 104 reaction was quantified by absorbance at 260nm ( $A_{260}$ ), and is shown in Table 1. The TargetAmp Kit 104 reaction produces microgram amounts of biotin-aRNA from as little as 25 ng of input total RNA.

### Detect more transcripts using less input RNA

As shown in Table 2, biotin-aRNA produced by a TargetAmp Kit 104 reaction using 100 ng of input total RNA detected more transcripts than biotin-aRNA produced by Company A's kit using 400 ng of input total RNA.

### Call Concordance

Biotin-aRNA targets for microarray studies are often produced by conjugating a biotin-NHS ester, for example Biotin-X-X-NHS, to aminoallyl-aRNA produced by kits such as EPICENTRE's TargetAmp Kit 101. We determined the concordance between transcript sets detected by biotin-aRNA produced using

Amount of input HeLa total RNA	Yield of biotin-aRNA
25 ng	4.1 µg
100 ng	13.7 µg
500 ng	64.9 µg

**Table 1. A TargetAmp™ 1-Round Biotin-aRNA Amplification reaction produces microgram amounts of biotin-aRNA (biotin-cRNA) from as little as 25 ng of input total RNA, representing a greater than 5000-fold amplification of the poly(A) RNA contained in the total RNA sample.**

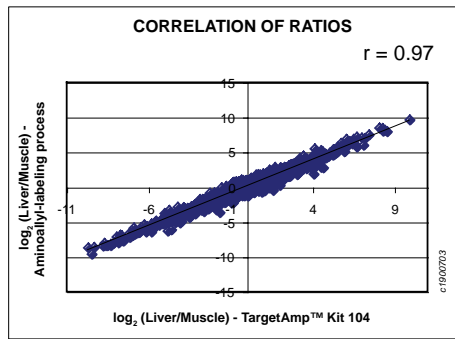
Transcripts detected	Mouse Skeletal Muscle RNA		Mouse Liver RNA	
	TargetAmp™ Kit 104	Company A Kit	TargetAmp™ Kit 104	Company A Kit
Source	TargetAmp™ Kit 104	Company A Kit	TargetAmp™ Kit 104	Company A Kit
Amount of input RNA	100 ng	400 ng	100 ng	400 ng
Transcripts detected	9373	8056	8652	8164

**Table 2. Biotin-aRNA produced by a TargetAmp™ 1-Round Biotin-aRNA Amplification Kit 104 reaction detects more transcripts than biotin-aRNA produced by Company A's kit.** Hybridization and data analysis were as described in the text.



**FIG 2. Biotin-aRNA produced by the TargetAmp™ 1-Round Biotin-aRNA Amplification Kit 104 displays high concordance with biotin-aRNA produced by the TargetAmp™ 1-Round Aminoallyl-aRNA Amplification Kit 101 plus Biotin-X-X-NHS.** Biotin-aRNA was produced from 100 ng of mouse skeletal muscle total RNA by the two methods. Hybridization and data analysis were as described in the text.

the aminoallyl-labeling process and using biotin-aRNA produced by the TargetAmp Kit 104. The overlap of detected ( $p < 0.05$ ) and not-detected ( $p > 0.05$ ) transcript-sets obtained upon hybridizing biotin-aRNA produced by the two methods is illustrated in FIG 2. Biotin-aRNA produced by the TargetAmp Kit 104 demonstrates a high concordance with biotin-aRNA produced by the aminoallyl-labeling method.



**FIG 3.** Biotin-aRNA produced by the TargetAmp™ 1-Round Biotin-aRNA Amplification Kit 104 demonstrates high degree of correlation with biotin-aRNA produced by the TargetAmp™ 1-Round Aminoallyl-aRNA Amplification Kit 101 plus Biotin-X-X-NHS.

**Consistent relative gene expression data**

The  $\log_2$  values of liver/skeletal muscle expression ratios were calculated and compared for two biotin-aRNA synthesis methods and are shown in FIG 3. The data demonstrates a very high correlation ( $r \geq 0.97$ ) between biotin-aRNA produced by the TargetAmp Kit 104 and biotin-aRNA produced by the aminoallyl-labeling method of the TargetAmp Kit 101 plus Biotin-X-X-NHS.

**Conclusion**

We have shown that the biotin-aRNA (biotin-cRNA) produced by a TargetAmp™ 1-Round Biotin-aRNA Amplification Kit 104 reaction yields high quality and reproducible microarray data on the Illumina BeadChip platform. A TargetAmp Kit 104 reaction generates microgram amounts of biotin-aRNA from as little as 25 ng of input total RNA. Biotin-aRNA produced by a TargetAmp Kit 104 reaction detected more genes than biotin-aRNA produced by another commercially available kit (Company A). Additionally, expression ratio data obtained with biotin-aRNA produced by a TargetAmp Kit 104 reaction are fully consistent with data obtained with biotin-aRNA produced by the aminoallyl-aRNA labeling process.

**Acknowledgment**

EPICENTRE Biotechnologies would like to thank Dr. Seth Crosby and Michael Heinz (Genome Sequencing Center, Washington University School of Medicine, St. Louis, MO) for their participation in the generation and analysis of data presented here.

**Produce microgram amounts of target aRNA from picogram amounts of total RNA**

EPICENTRE's TargetAmp™ 2-Round aRNA Amplification Kit 2.0 and TargetAmp 2-Round Aminoallyl-aRNA Amplification Kit 1.0 produce microgram quantities of aRNA and aminoallyl-aRNA respectively from the total RNA of as little as one cell (about 10 pg)!

The TargetAmp 2-Round Amplification Kits feature:

- Fast 2-day reactions.
- Linear RNA amplification that preserves the relative transcript abundance of the sample.
- High quality microarray results using Affymetrix™ GeneChip®, Illumina® and other oligo and cDNA arrays.

Visit [www.EpiBio.com/targetamp.asp](http://www.EpiBio.com/targetamp.asp) for more information, supporting data and recent publications citing use of the TargetAmp Kits.

**Table. Yields of aRNA from a TargetAmp™ 2-Round aRNA Amplification Kit 2.0 reaction.** Yields of aminoallyl-aRNA from EPICENTRE's TargetAmp 2-Round Aminoallyl-aRNA Amplification Kit 1.0 are virtually identical.

Amount of Input Total RNA	aRNA yield from Total Rat Brain RNA	aRNA yield from Total HeLa RNA	aRNA yield from Total Rat Kidney RNA
10 pg (1 cell)	3 µg	1.3 µg	1.4 µg
50 pg (5 cells)	19 µg	8 µg	7 µg
100 pg (10 cells)	53 µg	19 µg	15 µg
500 pg (50 cells)	161 µg	71 µg	72 µg

[www.EpiBio.com/targetamp.asp](http://www.EpiBio.com/targetamp.asp)

**TargetAmp™ 1-Round Biotin-aRNA Amplification Kit 104**

TAB1R6910	10 Reactions
TAB1R6924	24 Reactions

- Produces microgram amounts of biotin-aRNA from 25 ng – 500 ng total RNA.
- Greater than 5000-fold amplification.
- Optimized for use with SuperScript™ III Reverse Transcriptase (provided by the user).

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

TAA1R4910	10 Reactions
TAA1R4924	24 Reactions

- Produces microgram amounts of aminoallyl-aRNA from 25 ng – 500 ng total RNA.
- Greater than 5000-fold amplification.
- Optimized for use with SuperScript™ III Reverse Transcriptase (provided by the user).

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

TAA2R4910	10 Reactions
TAA2R4924	24 Reactions

- Produces microgram amounts of Aminoallyl-aRNA from 10 pg – 500 pg total RNA.
- Greater than 5,000,000-fold amplification.
- Optimized for use with SuperScript™ II & SuperScript™ III Reverse Transcriptase (provided by the user).

**TargetAmp™ 2-Round aRNA Amplification Kit 2.0**

TAU2R5110	10 Reactions
TAU2R51224	24 Reactions

- Produces microgram amounts of unlabeled-aRNA from 10 pg – 500 pg total RNA.
- Greater than 5,000,000-fold amplification.
- Optimized for use with SuperScript™ II & SuperScript™ III Reverse Transcriptase (provided by the user).

[www.EpiBio.com/biotin.asp](http://www.EpiBio.com/biotin.asp)

**Biotin-X-X-NHS**

BXX51005	5 X 2.5 mg
BXX51010	10 X 2.5 mg

