Robust target labeling from small amounts of RNA for Illumina® Expression BeadChip® arrays

The Illumina® Expression BeadChip® arrays are the preferred system for array-based gene expression studies because of their low input requirement, high sensitivity, low cost and high throughput. Two target labeling procedures—the TargetAmp™-Nano and TargetAmp™-Pico Target Labeling Kits for Illumina Expression BeadChip arrays—generate microgram amounts of biotin-antisense RNA (aRNA) from nanogram and picogram amounts of RNA, respectively, and yield high-quality expression analysis results.

Microarray-based transcription profiling studies have provided important advances in our understanding of differential gene expression in heterogeneous cell populations—for example, in cancerous versus normal tissue. The Illumina Expression BeadChips, a popular choice for array-based expression profiling, use biotin-labeled aRNA (sometimes called complementary RNA, or cRNA) as the target. We evaluated BeadChip array results obtained using biotin-aRNA produced by two Epicentre kits: the TargetAmp-Nano and the TargetAmp-Pico Target Labeling Kits for Illumina Expression BeadChip.

High yields of biotin-aRNA

The TargetAmp-Nano and TargetAmp-Pico kits use an improved Eberwine[1] linear RNA amplification and labeling procedure that generates microgram amounts of biotin-aRNA from nanogram and picogram amounts of total RNA, respectively. The kits have been optimized to maximize the yield of biotin-aRNA and to minimize reaction time and the number of pipetting steps. A TargetAmp-Nano Kit reaction is completed in about 6 h, which enables the user to prepare biotin-aRNA target and begin BeadChip hybridization on the same day.
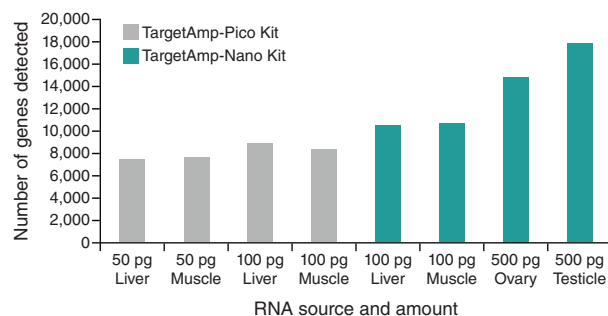
Table 1 reports the yield of biotin-aRNA produced from a TargetAmp-Nano kit reaction and from a TargetAmp-Pico kit reaction using various amounts of input total RNA. The actual yield of biotin-aRNA produced from a sample is dependent on the poly(A) RNA content of the total RNA sample, the amount of input RNA and the integrity of the RNA. Both the TargetAmp-Nano and TargetAmp-Pico kits produce sufficient biotin-aRNA for sample replicates or for archiving.

Genes detected

One of the most important metrics used to evaluate a microarray target labeling method is the number of genes detected by the labeled target. We prepared biotin-aRNA from 100 ng of mouse liver and mouse skeletal muscle RNA with the TargetAmp-Nano kit, and from 50 pg and 100 pg of mouse liver and mouse muscle total RNA with the TargetAmp-Pico kit. The labeled targets were hybridized to the Mouse Ref-8 Expression BeadChip array (Illumina). In a separate experiment, we prepared biotin-aRNA from 500 ng each of RNA extracted from human ovary and testicle using the TargetAmp-Nano kit. The labeled targets were hybridized to the HumanHT-12 v4 Expression BeadChip array (Illumina). The data generated were normalized using BeadStudio (Illumina). The number of genes detected ($P < 0.05$) from each sample is shown in **Figure 1**. The large number of genes detected, even with biotin-aRNA produced from as little as 50 pg of RNA, demonstrates the robust target labeling provided by the TargetAmp kits.

Figure 1 | Genes detected using labeled target prepared by the TargetAmp-Nano and TargetAmp-Pico kits. Target RNA from the indicated source was labeled using either the TargetAmp-Nano or TargetAmp Pico kit. The target RNA was hybridized to either the Mouse Ref-8 Expression BeadChip or the HumanHT-12 v4 Expression BeadChip (Illumina).

Jim Pease

Jim Pease is at Epicentre (an Illumina company), Madison, Wisconsin, USA. Correspondence should be addressed to J.P. (jim.pease@epicentre.com).

APPLICATION NOTES

Table 1 | Yield of biotin-aRNA from the TargetAmp-Nano and TargetAmp-Pico kits.

Input HeLa cell total RNA	50 pg	100 pg	500 pg	25 ng	100 ng	500 ng
Biotin-aRNA yield (TargetAmp-Nano Kit)				3.1 µg	10.8 µg	75.6 µg
Biotin-aRNA yield (TargetAmp-Pico Kit)	8 µg	19 µg	71 µg			

Correlation of expression ratios

We also examined the correlation of gene expression ratios using biotin-aRNA produced by the TargetAmp-Nano and TargetAmp-Pico kits. Biotin-aRNA was generated from 100 pg (TargetAmp-Pico kit) and 100 ng (TargetAmp-Nano kit) of RNA extracted from mouse liver and skeletal muscle tissue. There was a strong correlation ($R = 0.93$) between results obtained by the two procedures (**Fig. 2**), even though there was a 1,000-fold difference in the amount of RNA used for target preparation.
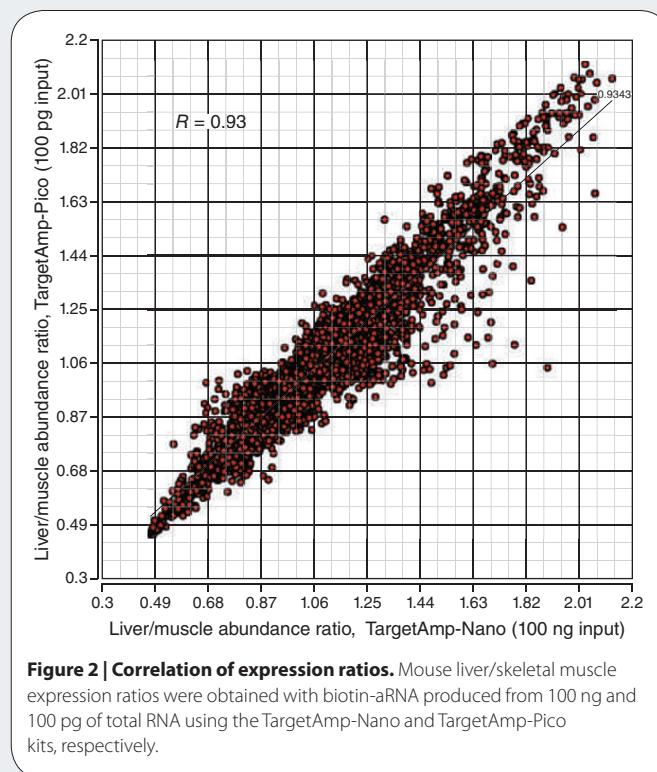
Conclusions

The TargetAmp-Nano Target Labeling Kit for Illumina Expression BeadChip and the TargetAmp-Pico Target Labeling Kit for Illumina Expression BeadChip kits generate microgram amounts of biotin-aRNA from as little as 50 pg of total cellular RNA. The biotin-aRNA produced detects a large number of genes on the Illumina Expression BeadChip arrays. Expression ratios determined with biotin-aRNA prepared with the TargetAmp-Nano and TargetAmp-Pico kits show high correlation with each other.

1. Van Gelder, R.N. *et al.* Amplified RNA synthesis from limited quantities of heterogeneous cDNA. *Proc. Natl. Acad. Sci. USA* **87**, 1663–1667 (1990).

Figure 2 | Correlation of expression ratios. Mouse liver/skeletal muscle expression ratios were obtained with biotin-aRNA produced from 100 ng and 100 pg of total RNA using the TargetAmp-Nano and TargetAmp-Pico kits, respectively.

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