

Globin RNA Reduction in Blood Samples

This Technical Note reports a study that compares hybridization results from whole blood samples isolated with PAXgene™ Blood RNA Isolation System and processed with or without Globin RNA Reduction PNA Oligomers.

Gene Expression Assays with Blood Samples

Peripheral blood has been an attractive tissue type for biomedical and clinical research because of its critical role in immune response and metabolism in humans and animal studies, as well as the simplicity and ease of specimen collection. Blood is used in biomarker discovery and development of diagnostics in hematological diseases and is also being explored to discover surrogate markers in a wide range of non-hematological disorders.

A major challenge to the utilization of blood in expression profiling is its mixed population of cell types. Most studies are interested in the peripheral blood mononuclear cells (PBMCs), which make up less than 0.1% of the whole blood sample. A significant fraction of blood cells are reticulocytes, which contain a high proportion of mRNAs encoding the globin polypeptides. This globin RNA tends to reduce the sensitivity of microarray hybridization results, which could potentially obscure clinically relevant information (*Affymetrix blood technote*).

In the past, investigators separated the desired PBMCs from red blood cells prior to RNA isolation. Now, several labs are collecting whole blood samples in PAXgene™ (*PreAnalytiX*) or Tempus™ tubes (*Applied Biosystems*), in order to stabilize expression patterns prior to microarray analysis. Affymetrix and PreAnalytiX released a protocol that pretreats the whole blood samples with globin oligonucleotides and RNaseH to remove globin RNA before processing (*Affymetrix GRP technote*). However, some investigators are hesitant to introduce nucleases into RNA samples. These companies now offer an alternative protocol that uses peptide nucleic acid (PNA) oligomers to address the globin RNA issue (*GeneChip® Blood RNA Concentration Kit*).

PNA Oligomer Mediated Globin RNA Reduction

The latest Affymetrix and PreAnalytiX protocol for globin RNA reduction uses four PNA oligomers whose sequences are complementary to the 3' portions of the alpha and beta hemoglobin RNA transcripts (Figure 1). The PNA oligomers form stable duplex structures with the globin mRNA and block the progression of reverse transcriptase. The inhibition of globin cDNA synthesis dramatically reduces the relative amount of anti-sense, biotin-labeled cRNA corresponding to the hemoglobin transcripts.

TAACGGTATTTGGAG						GTAGTTGGACTTAGG, GCCCTTCATAATATC, ATCCAGATGCTCAAG					
Homo sapiens hemoglobin, alpha 2 (HBA2), mRNA						Homo sapiens hemoglobin, beta (HBB), mRNA					
1	actcttctgg	tcccacaga	ctcagagaga	accaccatg	gtgctgtctc	1	acatttgctt	ctgacacaac	tgtgttctact	agcaacctca	aacagacacc
61	gaccaacgtc	aaggccgctt	gggtaaggt	cggcgcgca	gctggcgagt	61	tgactectga	ggagaagtct	gccgttactg	cctctgtggg	caagtggaac
121	ggccttgag	aggatgttcc	tgtccttccc	caccaccaag	acctacttcc	121	ttggtggtga	ggccctgggc	aggctgctgg	tggtctaccc	ttggaccag
181	ctgagccac	ggctctgccc	aggttaaggg	ccacggcaag	aaggtggccg	181	agtcctttgg	ggatctgtcc	actcctgatg	ctggtatggg	caacctaaag
241	caacccgctg	gcgcacgtgg	acgacatgcc	caacgcgctg	tcgcgccctga	241	atggcaagaa	agtgtctcgt	gcctttagtg	atggcctggc	taacctggac
301	cgcgacaaag	cttcgggtgg	accoggtcaa	cttcaagctc	ctaagccact	301	gcacctttgc	cacactgagt	gagctgcact	gtgacaagct	gcacgtggat
361	gacctgtggc	gcccacttcc	cgccgagtt	caccctcgcg	gtgcacgctc	361	tcaggctcct	gggcaacgtg	ctggctctgtg	tgctggccca	tcactttggc
421	gttctgtgct	tctgtgagca	cggtctgac	ctccaaatac	cgtttaagctg	421	ccccaccagt	gcaggctgcc	tatcagaaag	tggtggctgg	tgtggctaata
481	agccgttctc	cctgcccctc	ggcctccca	acgggcccctc	ctcccctctc	481	acaagtatca	ctaagctcgc	ttctctgctg	tccaatttct	attaaggttt
541	cttctgtgct	ttgataaaa	gtctgagttg	gcggc		541	ctaagtccaa	ctactaaact	gggggatatt	atgaaggccc	ttgagcactc
						601	taataaaaaa	catttatttt	cattgc		

Figure 1: Nucleotide Sequences of the Blocking PNA Oligomers. Human alpha- and beta-globin gene transcripts are shown with the regions complementary to the PNA oligomers are highlighted in red.



The Experiment

Whole blood samples from healthy donors were collected in PAXgene tubes and pooled. Three 5 ug aliquots of the whole blood RNA were mixed with the four globin PNAs (referred to as “PAX+PNA” samples), while three aliquots were not pretreated (referred to as “PAX” samples). Biotinylated cRNA targets were generated for all six samples

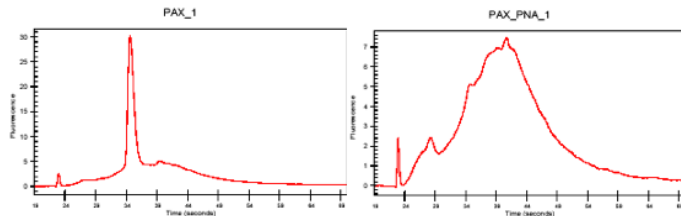


Figure 2: Agilent Bioanalyzer Traces of Biotin-Labeled Targets. cRNA was generated in the absence (*left panel*) or presence (*right panel*) of the blocking PNA oligomers.

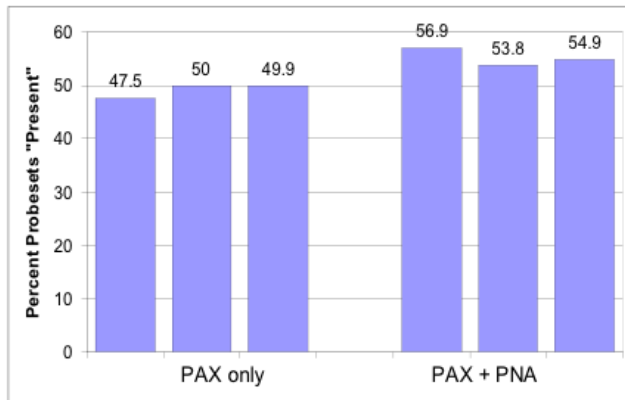


Figure 3: The Effect of PNA Treatment on the Percentage of Probesets called “Present” by MAS 5.0 software.

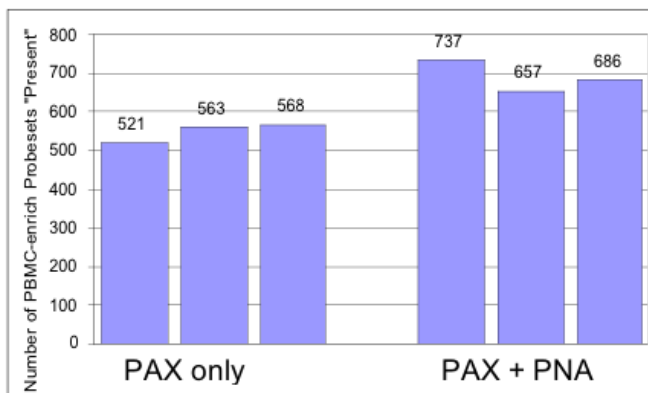


Figure 4: The Effect of PNA Treatment on the Number of BMC-Related Probesets called “Present” by MAS 5.0 software.

Reduced Globin cRNA

As expected, electrophoretic traces of the PAX cRNA targets show a significant peak corresponding to the globin transcripts (Figure 2). This band is reduced, but not completely eliminated, in the PAX+PNA samples, which show a diffuse distribution of cRNA sizes.

Increased Detection of Transcripts

The targets were fragmented and hybridized to HG_U133A_2.0 GeneChips. Expression data were extracted with MAS 5.0 software. The hybridization results demonstrate that globin RNA reduction resulted in improved sensitivity, as demonstrated by an increase in transcripts called “Present” in the PAX+PNA samples. As shown in Figure 3, approximately 50% of the probesets were detected in the three PAX samples. This value was increased to approximately 55% in the three PAX+PNA samples.

Increased Detection of BMC-Related Transcripts

The increase in sensitivity is even more dramatic for those transcripts whose expression is enriched in PBMCs. An average of 551 BMC-related probesets was detected in the PAX samples, while more than 650 BMC-related probesets were detected in the PAX+PNA samples. These figures represent a 26% increase in sensitivity when globin RNA was reduced using the PNA oligomers.



Decreased Signal Variation

One method of evaluating the reproducibility of microarray results is to examine the distribution of coefficient of variation (CV) results for each probeset on the array. As shown in Figure 5, the median CV for the PAX samples is 23%. When the signal variation for all transcripts on the array is examined, a statistically significant reduction to 19% is observed for the globin RNA reduced replicates. A corresponding significant decrease in signal variation is also observed across the PBMC-related probesets.

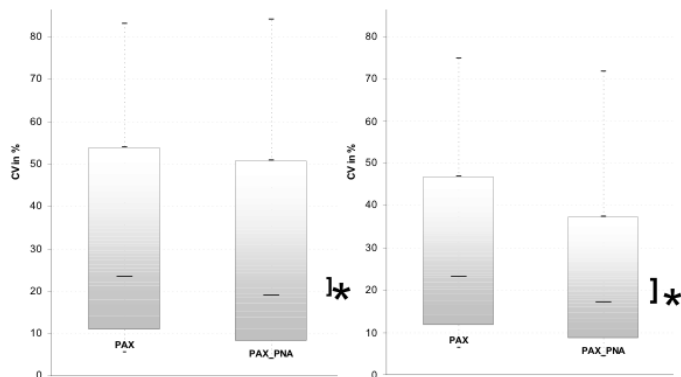


Figure 5: The Effect of PNA Treatment on MAS 5.0 Signal Variation. Targets for three replicate samples were hybridized to human U133A_2.0 GeneChips®. CV values are presented either for all probesets on the array (*left panel*) or for probesets that are primarily expressed in PBMCs (*right panel*). Statistically significant ($p < 0.001$) shifts in the median CV are noted by asterisks.

Summary

Collection of whole blood specimens in clinical setting for expression profiling represents a significant advantage. The near instantaneous stabilization of both the expression profile and the RNA itself serves to reduce artifacts and greatly increases convenience. The recently developed globin RNA reduction procedure using PNA oligomers significantly improves the data quality of expression profiles by both increasing the number of transcripts detected and decreasing the variation in signal values between replicates.

