Confirmation of Differentially Expressed Genes in Diabetic Muscle Found Using Atlas™ Plastic Microarrays

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We used the Atlas™ Plastic Human 8K Microarray to compare gene expression between normal and diabetic skeletal muscles. We detected more than 300 up-regulated genes and more than 200 down-regulated genes in diabetic tissue. To validate this differential expression, we performed semi-quantitative RT-PCR on 50 randomly selected genes and confirmed the differential expression for 90 percent of the genes with 3-fold differences.

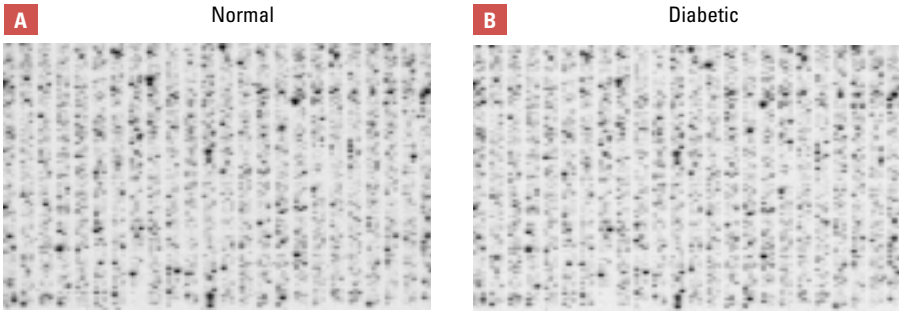

Figure 1. Expression profiling of normal and diabetic human skeletal muscle using Atlas™ Plastic Human 8K Microarrays. Total RNA (10 µg) from normal (**Panel A**) and diabetic (**Panel B**) human skeletal muscle tissues were isolated and labeled with ^{33}P using the Atlas Pure Total RNA Labeling System (#K1038-1). The probes were hybridized to separate Atlas Plastic Arrays according to the User Manual. The Plastic Arrays were washed using a high salt buffer (2X SSC, 0.1% SDS) then by a low salt buffer (0.1X SSC, 0.1% SDS) at 58°C for 10 minutes, and followed by a room temperature rinse using 0.1X SSC. The hybridized arrays were exposed to Fuji ^{33}P screens for 5 days and scanned on the Storm 860 Phosphorimager from Molecular Dynamics at 50-micron resolution. We analyzed the images using AtlasImage 2.01 and the global sum normalization method.

Previously, nylon membranes and glass slides were the only formats available for gene expression profiling. While radioactive detection combined with nylon membranes is the most sensitive and well-established method of expression profiling, limitations in signal resolution restrict the maximal printing density. As a result, it is not feasible to print more than a few thousand genes on a standard nylon membrane. Glass slides using fluorescent detection provide the resolution necessary to print at a far greater density; however, the use of these slides requires specialized reagents and equipment that are not commonly found in molecular biology laboratories. Atlas™ Plastic Microarrays combine the benefits of conventional hybridization and detection techniques with high gene density on a plastic format.

Like glass slides, the plastic format is nonporous, which decreases nonspecific binding and results in a clean background with little washing. While the plastic format can be stripped and reprobed several times like the nylon membranes, the rigid plastic maintains its original configuration, thus reducing the time required to align the grid for image analysis. AtlasImage™ 2.01, with its auto-alignment features, makes image analysis easy with Plastic Arrays.

Profile 8,300 human genes

We hybridized ^{33}P-labeled total RNA from normal and diabetic muscles to duplicate Atlas Plastic 8K Human Microarrays (Figure 1) and analyzed the phosphorimages using AtlasImage 2.01. Of the 8,300 genes surveyed, we found that more than 500 genes were differentially regulated more than 2 fold between the two samples. Diabetic tissues had 359 up-regulated and 210 down-regulated genes (Table I).

RT-PCR confirmation of differential expression

We used RT-PCR to confirm the profiling results obtained using the plastic format. We randomly selected 50 differentially expressed genes and analyzed their expression using RT-PCR. Of the genes that exhibited signal intensity differences of 3 fold or greater, we confirmed 92 percent by RT-PCR. Limitations of the semi-quantitative RT-PCR method could not allow us to reliably confirm differential expression of genes with signal intensity ratios of 2 or less. We found that 83 percent of the differentially expressed genes with signal intensity ratios ranging from 1–1.5 fold did not demonstrate differential expression using RT-PCR. Table I displays the results for 14 of the 50 genes with expression differences ranging from 1.6–95 fold.

Table I: Partial list of differentially expressed genes

Down-regulated genes in diabetic skeletal muscle	GenBank Acc.#	Fold Difference	Confirmed
carbonic anhydrase III, muscle specific	NM_005181	23	+
ATPase, Ca^{++} transporting, plasma membrane 2	L20977	2	+
calpain 3, (p94)	NM_000070	2	+
Up-regulated genes in diabetic skeletal muscle	**GenBank Acc.#**	**Fold Difference**	**Confirmed**
colipase, pancreatic	NM_001832	95	+
DnaJ (Hsp40) homolog, subfamily B, member 1	D49547	93	+
unactivated progesterone receptor, 23 kD	NM_006601	21.5	+
heat shock 70 kD protein 1A	M11717	19.5	+
heat shock 90 kD protein 1, alpha	X07270	15.2	+
dynein, cytoplasmic, light polypeptide	NM_003746	11.4	+
stress-induced-phosphoprotein 1 (Hsp70/Hsp90-organizin)	NM_006819	7.8	+
X-box binding protein 1	NM_005080	5.8	+
prothymosin, alpha (gene sequence 28)	NM26708	3.67	+
pM5 protein	NM_014287	2.84	+
proteasome (prosome, macropain) subunit, beta type, 7	NM_002799	1.6	+

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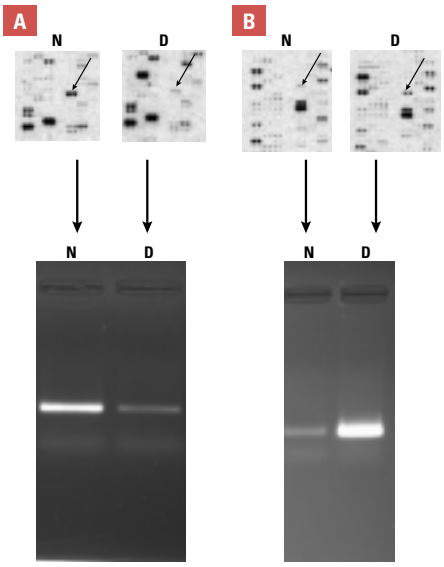

Figure 2. RT-PCR analysis and the corresponding array picture for two genes listed in Table I. We performed RT-PCR using the Advantage™ RT-for-PCR Kit (#K1402-1) and Advantage™ 2 PCR Kit (#K1910-y) on 50 randomly selected genes that showed differential expression using the Atlas Plastic Human 8K Microarray. We used the RT-PCR protocol in the Custom Atlas Primers User Manual (#PT3270-1), but substituted random N15 primers for the oligo(dT) primers in the first strand synthesis steps. We removed aliquots of PCR products after every 2 cycles and electrophoresed on an agarose gel. **Panel A.** The carbonic anhydrase III (CAIII) gene is down regulated in diabetic muscle. **Panel B.** The heat shock protein *hsp86* gene is up regulated in diabetic muscle. D=Diabetic, N=Normal.

Figure 2 shows RT-PCR results and expression array data for two of these up-regulated genes, carbonic anhydrase and the heat shock protein *hsp86*. In diabetic muscle tissue, carbonic anhydrase III (CAIII) was down regulated. Carbonic anhydrases are a class of metalloenzymes that catalyze the reversible hydration of carbon dioxide. The expression of the CAIII gene is strictly tissue specific, CAIII is present at high levels in skeletal muscle and at much lower levels in cardiac and smooth muscle. The *hsp86* gene was observed to be up regulated in the diabetic muscle tissue. Currently, the biological significance of these differentially expressed genes and the other genes listed in Table I is unknown.

In conclusion, RT-PCR validated the expression results we obtained using Atlas Plastic Human 8K Microarray and demonstrated that the plastic microarray format provides accurate, semi-quantitative expression results. The Plastic Arrays combine affordable hybridization techniques with high gene densities for reliable gene expression profiling.

Product	Size	Cat. #
Advantage 2 PCR Kit	30 rxns	K1910-y
AtlasImage 2.01	CD-ROM	V1213-1
Atlas Plastic Human 8K Microarray	2 arrays	7905-1
Atlas Pure Total RNA Labeling System	each	K1038-1
Advantage RT-for PCR Kit	25 rxns	K1402-1

Print you own Plastic Arrays!

Now you can print onto the same plastic that we use for our Atlas™ Plastic Human and Mouse Microarrays. The Atlas Plastic Printing Kit (#K1846-1) contains 10 plastic films (8 x 12 cm), 2 ml of 8X printing buffer, and a User Manual. Plastic is the ideal surface for printing long oligonucleotides or RNA when the detection method is radioactivity or chemiluminescence. BD Biosciences Clontech tested dozens of plastics and buffers before assembling this kit—now you can benefit from our experience.