Multiplexed, Targeted Gene Expression Profiling and Genetic Analysis on Electronic Microarrays

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Background: Electronic microarrays comprise independent microelectrode test sites that can be electronically biased positive or negative, or left neutral, to move and concentrate charged molecules such as DNA and RNA to one or more test sites. We developed a protocol for multiplexed gene expression profiling of mRNA targets that uses electronic field-facilitated hybridization on electronic microarrays.

Methods: A multiplexed, T7 RNA polymerase-mediated amplification method was used for expression profiling of target mRNAs from total cellular RNA; targets were detected by hybridization to sequence-specific capture oligonucleotides on electronic microarrays. Activation of individual test sites on the electronic microarray was used to target hybridization to designated subsets of sites and allow comparisons of target concentrations in different samples. We used multiplexed amplification and electronic field-facilitated hybridization to analyze expression of a model set of 10 target genes in the U937 cell line during lipopolysaccharide-mediated differentiation. Performance of multiple genetic analyses (single-nucleotide polymorphism detection, gene expression profiling, and splicing isoform detection) on a single electronic microarray was demonstrated using the ApoE and ApoER2 genes as a model system.

Results: Targets were detected after a 2-min hybridization reaction. With noncomplementary capture probes, no signal was detectable. Twofold changes in target concentration were detectable throughout the (~64-fold) range of concentrations tested. Levels of 10 targets were analyzed side by side across seven time points. By confining electronic activation to subsets of test sites, polymorphism detection, expression profiling, and splicing isoform analysis were performed on a single electronic microarray.

Conclusions: Microelectronic array technology provides specific target detection and quantification with advantages over currently available methodologies for targeted gene expression profiling and combinatorial genomics testing.

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Technologies allowing analysis of global messenger RNA concentrations have greatly increased our ability to identify molecular changes that correlate with altered physiologic states. Expression profiling has led to identification of sets of gene markers for detection of epithelial ovarian cancer (1), for differentiating acute myeloid leukemia and acute lymphoblastic leukemia (2), and for discriminating between discrete forms of diffuse large B-cell lymphomas (3). Results from these and other studies show that changes in expression of a defined subset of genes can be indicative of specific disease type or stage [for a review, see Ref. (4)].

Although high-density microarray technology has been extremely valuable for global gene expression profiling of cells under a condition of interest, data from such experiments typically lead to identification of a subset of targets that correlates with a particular cellular response. Subsequent validation of these targets and use of a panel of the validated genes as a diagnostic tool require a platform that will enable rapid analysis of specific targets of interest. Conventional microarrays, although well suited for global profiling studies, require large amounts of starting material and are limited to analysis of two sample sources, typically one control and one experimental population (5–7). Northern blots (8), real-time PCR analysis (9–11), serial analysis of gene expression (12), and differential display-PCR (13) have all been used to perform quantitative or semiquantitative expression profiling; however, each of these methods has
limitations with respect to sensitivity and multiplexing capabilities.

Electronic microarrays offer a unique platform that is particularly well suited to expression profiling of a select set of target genes from multiple sample sources. These arrays, comprising 100 microelectrode test sites, use electronic field control to drive the transport and concentration of nucleic acids in the bulk sample solution to specific test sites within the array, facilitating hybridization of targets with complementary probes (14) (Fig. 1; video clip available at http://www.clinchem.org/content/vol48/issue11/). Current and voltage are applied to the test sites via individual wire interconnects; auxiliary outer electrodes can be countercharged to provide an electric field (15). Because conditions favorable for hybridization occur only at those array site(s) that are electronically activated (16), the same set of target genes from different sample sources can be monitored on a single microarray, using sequential electronic hybridizations. Such control represents a particular strength of electronic microarray hybridization for gene expression monitoring, allowing great flexibility in assay configuration while reducing experimental variation arising from between-chip comparisons. In addition, hybridization assays can be accomplished in minutes, rather than the hours required for passive hybridization (14).

We present here the use of electronic microarray hybridization to perform multiplex quantitative gene expression profiling and demonstrate use of this application to monitor concentrations of selected targets in a model system. We also demonstrate the ability to perform multiple genetic analyses on a single microarray by confining different assays to specific subsets of array sites. Electronic microarray technology offers major advantages over currently available methodologies, combining the specificity of targeted expression analysis with the flexibility of examining targets from multiple sample sources on a single array.

Materials and Methods

OLIGONUCLEOTIDES

Amplification primers and biotinylated capture probes were synthesized by Synthetic Genetics, Biosource International, and Integrated DNA Technologies, Inc. CyTMS- and Cy3-labeled reporter probes were synthesized by Integrated DNA Technologies. Chimeric primers contained the T7 RNA polymerase consensus sequence (TAATACGACTCCTAGG) at the 5′ end of the primer. Primer sequences are available on request.

CELL CULTURE, TREATMENT, AND TOTAL RNA ISOLATION

The U937 cell line was obtained from American Type Culture Collection. Cells were grown in Gibco™ RPMI (Invitrogen Corp.) supplemented with 100 mL/L Gibco Fetal Bovine Serum and containing 200 units/g penicillin and 200 units/mL streptomycin (Invitrogen). Cells were differentiated with 10 μg/L phorbol 12-myristate 13-acetate (Invitrogen) for 72 h and induced with 500 μg/L lipopoly-
Saccharide (LPS; Sigma-Aldrich). Total RNA was isolated under contract with San Diego State University, with the RNAqueous-Midi Kit (Ambion, Inc.).

**First-strand cDNA synthesis**

Total RNA (1–5 μg) was reverse transcribed with Gibco Superscript™ II RNase H- Reverse Transcriptase (Invitrogen). The first-strand cDNA synthesis was primed with either 500 ng of Gibco oligo(dT)_{12-18} (Invitrogen) or with 1.65 ng of gene-specific primers, according to the manufacturer’s instructions. cDNA synthesis was performed at 42 °C for 30–60 min.

**Target amplification**

*Reverse transcription-PCR.* For reverse transcription-PCR experiments, one-tenth (2 μL) of the cDNA synthesis reaction was used per amplification reaction. PCR primers were used at a final concentration of 200 nM each. Reactions also included 0.05 U/μL PCR reaction AmpliTaq Gold® DNA polymerase (Applied Biosystems), 1x PCR buffer, and 200 μM each deoxynucleotide triphosphate (dNTP; Amersham Biosciences Corp.). Samples were denatured at 95 °C for 10 min, then cycled 40 times at 95 °C for 30 s, 55 °C for 60 s, and 72 °C for 60 s, followed by a final extension at 72 °C for 7 min. Amplification reactions were performed in a Perkin-Elmer 9700 thermocycler.

**Linear amplification.** A total of 5 μL of cDNA was used per reaction. 1x Thermopuff buffer (New England Biolabs), 200 μM each dNTP, and 0.08 U/μL reaction VentR® (exo-) DNA polymerase (New England Biolabs) were used. For each reaction, 50 nM each chimeric T7-target-specific oligonucleotide was added. The final Mg²⁺ concentration was generally adjusted to 4.5 mM. Samples were denatured at 95 °C for 5 min, then cycled 30 times at 95 °C for 30 s, 55 °C for 60 s, and 72 °C for 60 s. We then added 100 nM each antisense gene-specific primer, and samples were incubated at 42 °C for 10 min to allow primers to anneal to single-strand templates. Extension was performed at 70 °C for 10 min. Amplification reactions were performed in a Perkin-Elmer 9700 thermocycler.

**Limited exponential amplification.** First-strand cDNA synthesis was performed in a final volume of 10 μL, using gene-specific primers. Amplification was performed in the same reaction tube as cDNA synthesis, using 1x Thermopol buffer (New England Biolabs), 250 μM each dNTP, 125 mM each chimeric T7/gene-specific primer, and 0.1 U/μL reaction VentT (exo-) DNA polymerase (New England Biolabs). The final Mg²⁺ concentration was generally adjusted to 4.5 mM. Samples were denatured at 95 °C for 2 min, then cycled 15 times at 95 °C for 5 s, 55 °C for 15 s, and 72 °C for 15 s, followed by a final extension at 72 °C for 2 min. Amplification reactions were performed in a Perkin-Elmer 9700 thermocycler.

**Real-time PCR.** For real-time PCR experiments, 10–200 ng of cDNA at 250 ng/μL was added per reaction and PCR primers were added at 50 nM. Reactions also included 0.025 U/μL AmpliTaq Gold DNA polymerase; 1x SYBR Green PCR buffer; 0.01 U/L AmpErase® UNG (1 U/L uracil N-glycosylase); 3 mM MgCl₂; 200 μM each of dATP, dCTP, and dGTP; and 400 μM dUTP (all reagents from Applied Biosystems). Samples were incubated at 55 °C for 2 min for AmpErase activation, denatured at 95 °C for 10 min, and then cycled 40 times at 95 °C for 15 s and 55 °C for 60 s, with a final hold at 4 °C. Amplification reactions were performed in a Perkin-Elmer GeneAmp 5700 Sequence Detection System.

**In vitro transcription reactions.** For reverse transcription-PCR product or linearly amplified cDNA, 5 μL was used as template for in vitro transcription (IVT) in the T7 polymerase Megashortscript™ Kit (Ambion), according to the manufacturer’s instructions. Reactions were performed at 37 °C for 3–4 h. Two units of DNasel were added, and incubation at 37 °C was continued for 15 min. After incubation, 25 μL of nuclease-free water was added, and RNA products were purified through Bio-Spin 6 columns (Bio-Rad Laboratories) pre-equilibrated with nuclease-free water. For exponentially amplified cDNA, IVT reagents were added directly to the tubes in which amplification was performed; final concentrations of reagents were as recommended by the manufacturer’s instructions, with the addition of 400 U of high-concentration T7 RNA polymerase (Ambion). The final reaction volume was 50 μL. Reactions were performed at 37 °C for 1 h in a Perkin-Elmer 9700 thermocycler. Two units of DNasel were added, and incubation at 37 °C was continued for 15 min. RNA products were purified through Bio-Spin 6 columns, as above. RNA was stored at −80 °C.

For synthesis of cDNA from the RNA products, Gibco Superscript II RNase H- Reverse Transcriptase was used, according to the manufacturer’s instructions, with 125 mM gene-specific primers. cDNA synthesis was performed at 42 °C for 60 min. RNA was hydrolyzed at 65 °C for 15 min by addition of NaOH (final concentration, 0.5 mol/L). cDNA was purified over Bio-Spin 6 columns.

**Electronic addressing of capture probes**

Biotinylated oligonucleotide capture probe(s) (0.5 μmol/L in 50 mmol/L histidine) were electronically addressed to specific sites on the NanoChip® cartridge (Nanogen, Inc.). For endogenous or exogenous control genes, 50 nmol/L capture probe was used. Probes were transported to one or more pads at 400 nA/pad for 60 s.
on the NanoChip WorkStation (Nanogen), according to the manufacturer’s instructions.

**Electronic Hybridization of Targets**

In vitro-transcribed RNA was diluted in 50 mmol/L histidine, heat-denatured at 70 °C for 5 min, and quick-chilled on ice. cDNA was heat-denatured at 95 °C for 5 min. Targets were electronically transported to one or more pads at 400 nA/pad for 2 min on the NanoChip WorkStation, according to the manufacturer’s instructions.

**On-Chip Reporting**

Hybridization of fluorescently labeled oligonucleotide reporter probes. Reporters (0.5 μmol/L) were hybridized to the array in 50 mmol/L sodium phosphate–500 mmol/L sodium chloride for 5 min at 23 °C. Unbound reporters were removed by washing with 50 mmol/L sodium phosphate buffer.

Enzymatic incorporation of fluorescently labeled dCTP. For RNA targets, enzymatic reporting was mediated by Gibco Superscript II RNase H⁻ Reverse Transcriptase in 1× Superscript buffer, 10 mmol/L dithiothreitol, 3.3 μmol/L dATP/dGTP/dTTP, and 3.3 μmol/L Cy5-dCTP (Amer sham Biosciences). For cDNA targets, enzymatic reporting was mediated by Sequenase® (USB Corp.) in 1× Sequenase buffer; remaining reagents were as above. Reactions were performed at 37 °C for 10 min. NanoChip cartridges were washed with 50 mmol/L sodium phosphate buffer; fluorescent signal was detected on the NanoChip WorkStation.

**Results and Discussion**

Quantitative Gene Expression Profiling Using Electronic Microarray Hybridization

To ensure maximum efficiency of electronic field-facilitated transport and hybridization when multiple targets are analyzed, we developed a sample preparation method to reduce complexity and create homogeneously sized targets (Fig. 2). Targets generated were typically 60–150 bp in length. Analysis of RNA targets was more efficient when shorter fragments were used (data not shown); consequently, primers were designed to generate RNA.

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**Fig. 2. Scheme for target generation.**

Cellular RNA is used as template for generation of single-stranded cDNA; cDNA synthesis is directed by a nonreplicable oligo(dT)₂₀ or by one or more gene-specific primers. The single-stranded cDNA is then used in an amplification reaction mediated by chimeric T7/target-specific primers; this amplification reaction is either linear or exponential, as determined by the presence or absence of the 3′ primer for each target of interest. A second, linear amplification is achieved via T7 RNA polymerase-mediated IVT. The amplified RNA, or single-stranded cDNA targets generated from the RNA, are loaded onto NanoChip cartridges by use of the NanoChip Molecular Biology Workstation and detected by electronic hybridization to target-specific capture probes followed by enzymatic extension of the capture probes in the presence of dye-labeled dCTP.
targets 60–90 bases in length. Total cellular RNA was converted to cDNA; this single-stranded cDNA served as a template for amplification. When multiple targets from a single sample source were to be analyzed, all primers were included in a single reaction, such that all targets were amplified simultaneously. Amplification could be either linear or limited exponential, based on the amount of starting material. The cDNA synthesis and amplification steps can be performed sequentially in one tube, minimizing sample handling and reducing potential contamination issues.

To determine the expression of the targets, the processed sample was electronically hybridized to biotinylated, target-specific capture oligonucleotides arrayed on a semiconductor chip. The surface of these microarrays was coated with a hydrogel layer containing streptavidin (15, 16) (Fig. 1). Biotinylated capture probes were transported and concentrated to one or more specific array sites via electronic activation; interaction with the streptavidin contained in the permeation layer led to retention of the capture oligonucleotides at the discreet test sites. Amplified targets were then electronically hybridized to the immobilized complementary capture probes. Sequential hybridization of amplified targets from distinct sample sources thus enabled side-by-side analysis of those targets from different cell populations. To detect hybridized targets, an enzymatic reporting method was developed, by which all capture probes hybridized to targets were simultaneously extended in the presence of a fluorescently labeled dNTP. The intensity of the fluorescent signal thus correlated with the amount of target hybridized on the microarray.

SPECIFICITY OF ELECTRONIC MICROARRAY HYBRIDIZATION

To demonstrate the specificity conferred by electronic microarray hybridization, a matrix analysis was performed with six different targets and seven distinct capture probes. For this experiment, biotinylated capture oligonucleotides were electronically addressed to columns of a microarray. Target genes were converted to cDNA, amplified in monoplex PCR reactions, and electronically hybridized across rows of the same array, as indicated in Fig. 3. Only sites containing target and the complementary capture probe generated a fluorescent signal; no cross-hybridization was detected with nonspecific capture probes (Fig. 3). In addition, the electronic activation confined the hybridization reaction to those array sites that were positively biased; the IL1 target, for example, hybridized to the complementary capture only in row 1, although the same capture probe was present in the remaining array pads in the first column. These data confirm that the electronically mediated hybridization reaction is specific and is confined to those array sites that are selected by electronic activation of those sites.

DETECTION OF TWOFOOLD DIFFERENCES IN TARGET CONCENTRATION

To assess sensitivity to changes in expression, a synthetic RNA target was generated as a model system. The prokaryotic β-lactamase gene was amplified using a chimeric 5’ amplification primer containing the T7 consensus promoter sequence upstream of the β-lactamase-specific region; the 3’ primer included a poly(dT) sequence downstream of the target-specific region. Use of the amplification product as template in an IVT reaction thus generated β-lactamase RNA that contains a poly(A⁺) tail. A titration series was created by adding dilutions of this synthetic mRNA into eukaryotic total RNA; after amplification, target was hybridized to complementary capture probes on an electronic microarray. As shown in Fig. 4, twofold dilutions of β-lactamase mRNA could be readily detected after electronic hybridization to a β-lactamase-specific capture probe. Further experimentation indicated that the dynamic range of detection is three to four logs (data not shown).

To determine whether twofold changes in target concentration could be detected in a complex system, multiplex amplification of nine genes was performed. After IVT, twofold serial dilutions of the sample were generated. Hybridization of the dilutions to capture probes specific for each target demonstrated that twofold changes in target concentration could be detected after
multiplex amplification and electronic hybridization (Fig. 4C).

MULTIPLEXED GENE EXPRESSION PROFILING
The ability to confine hybridization to specific sites on a microarray provides a unique advantage for gene expression profiling, i.e., the ability to analyze targets from multiple sample sources on a single array. To demonstrate this feature, we analyzed 10 target genes from the monocyte-like cell line U937 at various time points after differentiation with phorbol myristate acid and treatment with bacterial LPS, using the sample preparation method outlined in Fig. 2. Polyadenylated \( \beta \)-lactamase RNA was added into each sample of cellular RNA and amplified along with the 10 endogenous targets; the \( \beta \)-lactamase RNA served as an exogenous normalization control. The 11 targets were converted to cDNA by use of gene-specific primers, amplified in a multiplexed reaction using the 11 distinct T7/target-specific primers to mediate limited exponential amplification, and used as templates for IVT. The target pools from each time point were electronically hybridized to specific columns of a microarray to which target-specific capture probes had been addressed across rows. The \( \beta \)-lactamase capture oligonucleotide was mixed with each capture probe and coaddressed to array sites. After hybridization of target pools, the \( \beta \)-lactamase gene was detected by hybridization of a complementary Cy3-labeled reporter probe. The endogenous targets were detected via enzymatic reporting with Cy5-dCTP. The results from this analysis are shown in Fig. 5A (graphically represented in Fig. 5B). The left panel represents the Cy5 fluorescent signals from the 10 endogenous targets, whereas the panel on the right shows the Cy3 signal corresponding to the \( \beta \)-lactamase reference gene. The \( \beta \)-lactamase concentration remained relatively unchanged in the different cDNA populations, as demonstrated in the graphical representation of the average \( \beta \)-lactamase values for each time point. This reference gene was used to ascertain that lack of specific signal (e.g., for COX2 in nondifferentiated cells) reflected an accurate result rather than a failure of amplification. Each endogenous target gene exhibited a distinct expression pattern over the time course of LPS treatment, as demonstrated in Fig. 5.

Expression profiles for some targets were also assessed with real-time PCR; the expression patterns observed with the two methods were quite similar, as illustrated in Fig. 5C (a table of the observed fold-changes obtained with the two methods are available as a data supplement with the online version of this article at http://www.clinchem.org/content/vol48/issue11/). These results indicate that electronic field-facilitated hybridization enables quantitative expression analysis in a novel format that allows simultaneous assessment of target concentrations from multiple sample sources.

PERFORMANCE OF MULTIPLE GENETIC ANALYSES ON A SINGLE MICROARRAY
To draw correlations between genetic content, patterns of gene expression, and the resulting effects on protein synthesis, information from many different types of genetic analyses needs to be combined. As demonstrated with the gene expression profiling experiments described above, electronic hybridization allows different samples to be sequestered to specific locations on the microarray.
Fig. 5. Multiplexed gene expression profiling using electronic hybridization.

(A), total RNA was isolated from the U937 cell line at various times after differentiation with phorbol myristate acid and treatment with LPS. A polyadenylated β-la gene was added into the cellular RNA before cDNA synthesis. Eleven targets (10 test genes plus the β-la gene) were converted to single-stranded cDNA with use of gene-specific primers; the targets were then simultaneously amplified in a multiplexed reaction, using limited exponential amplification followed by IVT as described in Materials and Methods. The RNA targets generated by the IVT reaction were electronically hybridized to target-specific capture probes and to the β-la capture probe, which had been coaddressed across rows. The β-la target was detected by passive hybridization of a Cy3-labeled β-la reporter oligomer (right panel), whereas cellular targets of interest were detected by enzymatic reporting with Cy5-dCTP (left panel). (B), graphical representation of data presented in panel A; β-la values represent an average of the Cy3 signal from the 10 pads at each time point. (C), representative real-time PCR data from selected targets. O/N, overnight; NI, nondifferentiated; Ct, threshold cycle.
We were interested in extending this feature, utilizing a single electronic microarray to perform multiple genetic analyses; the apolipoprotein E (ApoE) and apolipoprotein E receptor (ApoER2) genes were selected as model systems to demonstrate this flexibility of the platform.

Alleles of the ApoE gene have been implicated in susceptibility to cardiovascular disease (17, 18) and sporadic late-onset Alzheimer disease (19–21); some reports have indicated that expression of the ApoE alleles may be differentially regulated (22, 23). The receptor for the ApoE protein, ApoER2, has many different splicing isoforms; although no correlation has been drawn between the expression of specific ApoER2 isoforms and disease state, the preferential expression of one or more isoforms may be relevant (24–26).

Detection of single-nucleotide polymorphisms (SNPs) by use of electronic microarrays has been described previously (27). To demonstrate detection of different splicing isoforms, we designed an assay to discriminate between two splice variants of the ApoER2 gene; one isoform contained exon 17 spliced to exon 18, whereas the other contained exon 17 spliced directly to exon 19. A 5’ T7/exon 17-specific primer was used to amplify the ApoER2 gene; after amplification, 3’ primers specific for either exon 18 or exon 19 were used to fill in the single-stranded amplification products. After IVT, the RNA was hybridized to capture probes specific for either the exon 17/18 isoform or the exon 17/19 isoform and enzymatically reported. The SNP and splicing isoform detection assays were performed along with allele-specific gene expression profiling on nucleic acids derived from brain tissue samples (Fig. 6).

Four human brain tissue samples were used for these analyses. For SNP detection, the ApoE gene was amplified from genomic DNA using one biotinylated and one nonbiotinylated primer. The biotinylated ApoE targets

from each sample were electronically addressed to duplicate array sites. Stabilizer oligonucleotides were then electronically hybridized to these amplicons, and ApoE variants were detected with allele-specific reporter probes. For gene expression profiling and splicing isoform detection, total RNA was isolated and amplified using T7/target-specific primers, as described above. Capture probes for the gene expression profiling and splicing isoform detection assays were electronically addressed, and the amplified ApoE and ApoER2 RNAs were hybridized to complementary capture oligonucleotides.

After enzymatic reporting (ApoER2 splice variants) or allele-specific reporter hybridization (ApoE gene expression), the Cy3 and Cy5 fluorescent signals were imaged.

For genotyping analysis at amino acid 112, the Cy3-labeled reporter corresponds to the ApoE4 variant and the Cy5-labeled reporter corresponds to the ApoE3 allele. Thus, the ApoE4 homozygote (Fig. 6, rows 1 and 7) hybridized stably to the Cy3 reporter probe, whereas the ApoE3 homozygote (Fig. 6, rows 4 and 10) retained only the Cy5 reporter. The two heterozygous samples (Fig. 6, rows 2 and 8 and 3 and 9) stably hybridized to both reporters. The gene expression analysis showed corresponding results; for the sample containing only the ApoE4 variant, only ApoE4 mRNA was detected (Fig. 6, rows 1 and 7); the two heterozygous samples expressed mRNA from both alleles at similar amounts (Fig. 6, rows 2 and 8 and 3 and 9). Similarly, the ApoE2 variant at amino acid 158, indicated by the Cy5-labeled reporter probe, was present in only one of the samples (Fig. 6, rows 2 and 8), and only ApoE2 mRNA was detected in this sample. For splicing isoform detection, both exon 17/18 and 17/19 splice variants were detected in all samples; this result is expected because previous reports indicated that both isoforms are present in brain tissue (28). These data demonstrate that electronic microarrays provide a novel format for performing multiple genetic analyses on a single platform by enabling the user to sequester reactions to discrete subsets of array sites.

The introduction of high-density microarrays, serial analysis of gene expression, differential display-PCR, and other methods has enabled genome-wide transcription profiling; application of these technologies has been invaluable in identifying subsets of genes that appear to have different degrees of expression in various disease states. More targeted gene expression profiling will become increasingly relevant to clinical diagnostics as correlations between expression patterns and cell states are established. Currently available methodologies are not, however, well suited to analysis of small subsets of (5–100) targets. Methods that enable analysis of very large numbers of genes require large amounts of starting material and may not represent the most cost-effective option for evaluating a small percentage of the total number of expressed genes across many different patient samples. Methods such as real-time PCR offer tremendous sensitivity and are ideal for quantitative analysis of a few targets, but such technologies are not easily multiplexed for panels of candidate genes. The sample preparation and electronic hybridization method described here enables quantitative, multiplexed gene expression analysis and can be performed in as little as 5 h. The protocol requires relatively small quantities of total RNA, consistent with monitoring expression patterns in limited samples. Furthermore, the open architecture of the electronic microarray enables flexibility in assay design; as additional molecular markers are correlated with a medically relevant cell state, those targets can be easily incorporated into the expression profile analysis. The ability to quickly and accurately monitor expression patterns of defined gene sets will be increasingly critical in diagnostic settings as more molecular markers indicative of cell state are defined.

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