Quantitative Analysis of Gene Expression Using Chemiluminescent Detection on Microarrays

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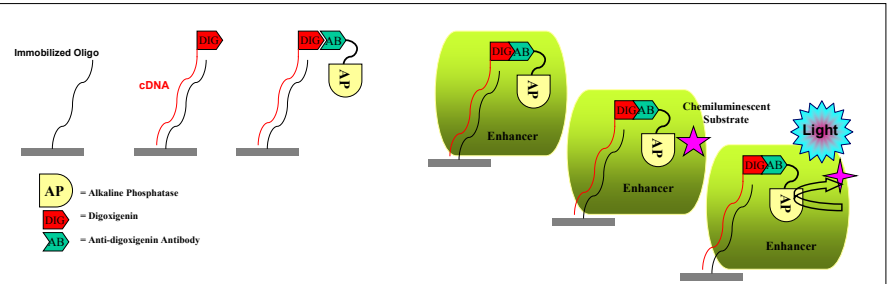
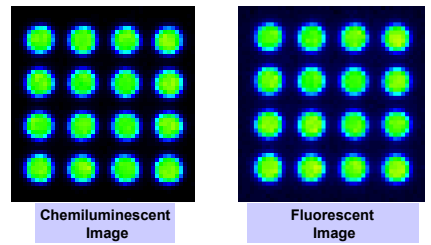
ABSTRACT

Applied Biosystems is developing novel chemiluminescent assay chemistries that are capable of detecting gene targets on microarrays. In this poster we present data that demonstrates the sensitivity, dynamic range, kinetic stability and reproducibility of the chemiluminescent detection assay on the array surface. We will show how the assay chemistry allows for the detection of very low levels of gene expression using microarrays.



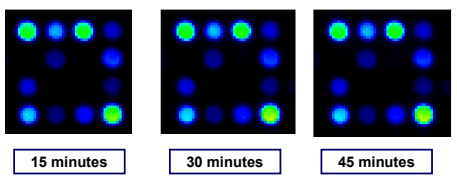
Overview of Chemiluminescent Detection Assay

Figure 1: Overview of the chemiluminescent detection assay. The first step in the assay is the hybridization of digoxigenin-labeled cDNA targets to specific probes immobilized on the microarray surface. Then the anti-DIG-Antibody-Alkaline Phosphatase protein conjugate is added to the array and binds to all hybridized targets. The array surface is then bathed in a chemiluminescent enhancing polymer. Light is generated after the addition of the chemiluminescent substrate, and the cleavage of the substrate by alkaline phosphatase. The assay is performed at 35ºC and is imaged in a specifically developed instrument reader.



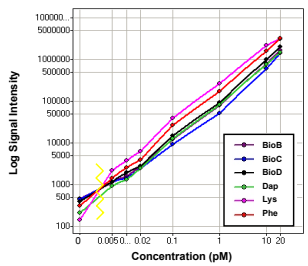
Spatial Stability of Chemiluminescent Signals

Figure 2: Spatial stability of chemiluminescent signals on the microarray surface. A single oligonucleotide probe was spotted on the microarray in a regular pattern with a spot size of about 180 µm and center-to-center spacing of 225 µm. The probe used in this study also had a covalently attached fluorescent LIZ™ dye. A complementary DIG-labeled cDNA was then hybridized to the microarray and the assay described in Figure 1 was performed. The images shown above are both the fluorescent (FL) and chemiluminescent (CL) signals coming from each spot on the array surface. Since the fluorescent label is attached to the probe, the FL image gives a good indication of the spot location, size, and morphology. The CL image shows that light generated by the assay is highly correlated with the fluorescent signals, even though the chemiluminescent substrate is freely diffusible.



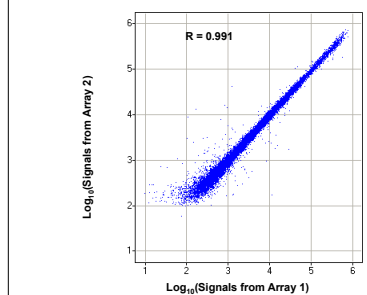
Kinetic Stability of Chemiluminescent Signals

Figure 3: Kinetic stability of chemiluminescent signals on the microarray surface. An array was made by spotting various oligonucleotide probes, each capable of specifically binding to different human cDNA targets. After hybridization with a complex biological sample of cDNA, spots of varying intensity are observed in the CL image of the microarray. This experiment shows three different CL images collected from the same array over time. The images are 25 second snapshots taken at 15, 30, and 45 minutes following the addition of the enzymatic substrate to the assay. Once the microarray reaches 35ºC inside the instrument, the chemistry reaction comes to a steady state that will produce quantitative chemiluminescent signals in the assay for more than an hour.



Sensitivity and Dynamic Range of Assay

Figure 4: Sensitivity and dynamic range of chemiluminescent detection of cDNA targets in a gene expression assay. A series of spike-in experiments were performed to demonstrate the sensitivity of the hybridization assay. Pre-labeled cDNA from six bacterial genes (*BioB*, *BioC*, *BioD*, *Dap*, *Lys*, and *Phe*) was spiked into 1 µgram of human liver cDNA and hybridized on to a series of microarrays. All targets were labeled with digoxigenin, and detected using the standard assay shown in Figure 1. The concentrations of the spikes were 20, 10, 1, 0.1, 0.02, 0.01, 0.005, and 0 picomolar, and each concentration was applied to a separate microarray. The graph shows plots of the chemiluminescent signals quantified for the six spiked-in genes on each array. The signals at the 0.005 pM level are clearly detectable above the no target control and the signals remain linear up to 20 pM.



Reproducibility of Assay

Figure 5: Reproducibility of chemiluminescent detection of gene expression using microarrays. This plot shows the reproducibility of chemiluminescent detection on a pair of microarrays containing unique oligonucleotide probes designed to target human genes. Each microarray was hybridized with the same labeled cRNA target (made from labeling the Universal Human Reference RNA standard available from Stratagene). This correlation plot shows that the assay chemistry is very reproducible and quantitative across microarrays. The plot shows a total of 20,653 data points (probes) across two replicate arrays.

Summary

- A microarray gene expression assay has been developed using proprietary chemiluminescent detection technologies.
- The assay can detect labeled cDNA targets at levels below 10 femtomolar in a hybridization reaction.
- The assay has a linear dynamic range of more than 3 logs: from below 10 fM to above 10 pM.
- The assay can be used to quantify gene expression on microarrays without any diffusion of chemiluminescent signals.
- The kinetics of the alkaline phosphatase reaction come to a steady-state quickly and the reaction is stable for more than an hour.
- The assay generates signals that are reproducible from array-to-array.