Resonance Light Scattering (RLS) Technology and Microarrays

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The Two-Color Nucleic Acid Microarray Toolkit™ uses Resonance Light Scattering (RLS) technology for the detection of biotin- and fluorescein-labelled cDNA using anti-biotin and anti-fluorescein coated RLS Particles™ on microarrays. This detection system is designed to provide greater sensitivity and to improve the lower limit of detection compared with fluorescent technologies.

This product was developed by Genicon Sciences and is available through Invitrogen (GeniconRLS™ System) and Qiagen (HiLight™ Array Detection System)[1,2].

What is RLS Technology?

Resonance Light Scattering (RLS) technology is an alternative signal generation and detection method based on nanometer-sized metal particles (RLS Particles™). The metal colloidal particles radiate energy in the form of scattered light when illuminated by a white light source.

The electromagnetic theory states that an oscillating electron emits light coincident with its oscillating frequency. This secondary radiation is referred to as scattered light and the wavelength of the scattered light is determined by the size, shape, and composition of the particle. The monochromatic light signal generated by a single RLS Particle™ is greater than the signal obtained from the most sensitive fluorophore.

The Two-Color Nucleic Acid Microarray Toolkit™ uses 80 nm diameter gold (Au) particles and 60 nm diameter silver (Ag) particles. The Au particles have anti-biotin antibodies attached and the Ag particles have anti-fluorescein antibodies attached.

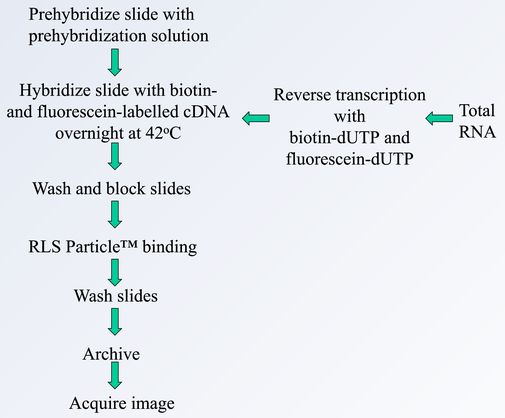

Figure 1. An outline of the RLS procedure.[1]

Signal Detection

White light from the GSD-501™ RLS Detection and Imaging Instrument strikes the RLS Particles™ on the microarray and a CCD camera detects the scattered light generated by the particles.

The RLS Particles™ are exposed to three different wavelengths. The 450 nm wavelength is most sensitive to Ag particles; the 565 nm wavelength is equally sensitive to Au and Ag particles; and the 600 nm wavelength is most sensitive to Au particles. The user must select the optimal exposure time for each wavelength. The exposure time should maximize the dynamic range while minimizing the number of saturated spots. For most microarrays, the image generated by this instrument is actually composed of a multiple smaller images or "tiles" that are put together to generate the final image. The software then "unmixes" the images, in essence determining the spot intensity for both the Au and Ag RLS Particle™ channels. The arrays are quantified using ArrayVision™ RLS software.

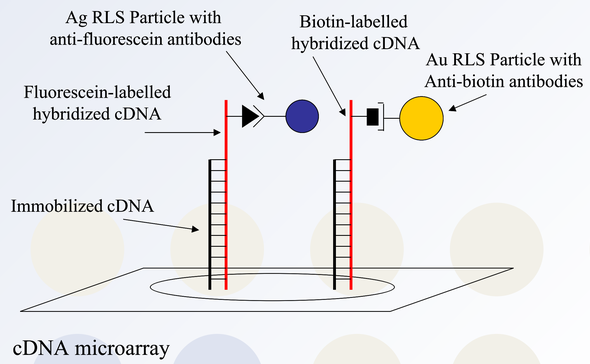

Figure 2. A schematic of the RLS Particle™ hybridization. cDNA labelled with either biotin or fluorescein hybridize to the cDNA on the microarray and a series of washes removes unhybridized labelled-cDNA. A second hybridization with the anti-biotin (Au) and anti-fluorescein (Ag) RLS Particles™ is performed. Another series of washes removes unbound RLS Particles™ prior to archiving and scanning the slide.[1,2]

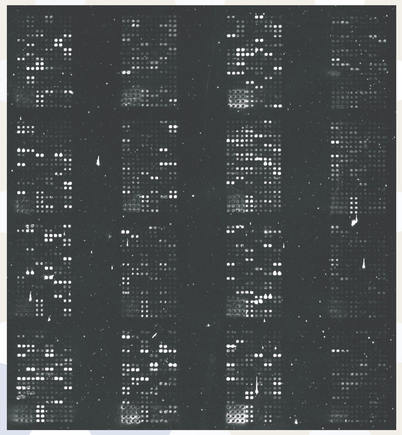

Figure 3. A Hum1.7k4 array hybridized with biotin-labelled cDNA made from 2 µg of Human Universal Reference RNA (Stratagene). The exposure time for this image is 1 second. The image obtained from the Au RLS Particles™ is shown.

Figure 4. The GSD-501™ RLS Detection and Imaging Instrument.

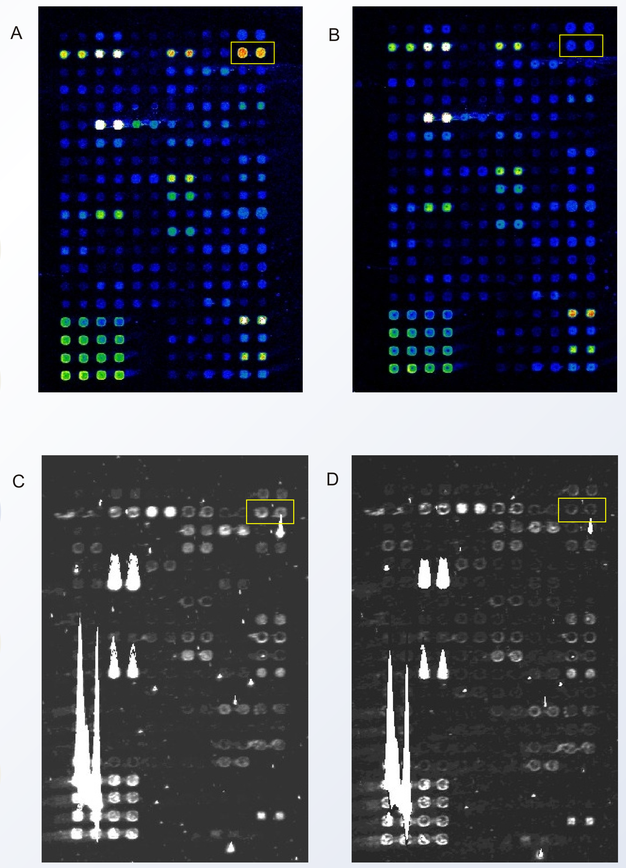

Figure 5. A comparison of Cy5/Cy3 fluorescence and RLS Particle™ detection on a Human1.7k4 cDNA microarray. Images of a Human1.7k4 subarray hybridized with treated (HeLa cells treated with 2.5 mM azetidine for 22 hours to induce a heat shock response; 10 µg total RNA sample labelled with Cy3; image A) and control (HeLa; 10 µg sample labelled with Cy5; image B) samples. Below, the RLS Particle™ images with the treated (also HeLa treated with 2.5 mM azetidine for 22 hours; 5 µg total RNA labelled with fluorescein/detected with Ag RLS Particles™; image C) and control (HeLa; 5 µg total RNA labelled with biotin/detected with Au RLS Particles™; image D) samples. The microarray elements representing the gene for the 70kDa Heat Shock protein is outlined in yellow. In fluorescent and RLS Particle™ images, the expression of the 70kDa heat shock gene is upregulated in the treated HeLa samples (images A and C).

Summary

Sensitive - Able to use 1-2 µg total RNA for cDNA microarray experiments without amplification.

Archiveable, stable signal - No photobleaching or quenching due to the metallic nature of the RLS Particles™ allows the slide to be archived.

Time - The RLS protocol takes an extra few hours compared to direct labelling with fluorescent molecules.

References

[1]Invitrogen Website. http://www.invitrogen.com/content.cfm?pageid=9912

[2]Qiagen Website. http://www1.qiagen.com/Products/MicroarrayAnalysis/MicroarrayAnalysisSystems/HiLightArraySystem/HiLightDual-ColorKit.aspx?

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