Attomoles Quantitative Chemiluminescence for Molecular Diagnostics

by Aaron Ho and Jian Tajbakhsh

The study and use of bioluminescence and chemiluminescence has increased dramatically in the recent years. The applications have extended from ATP-luciferase assay and cell viability tests, to current immunoassay, DNA, genomic, and proteomic analysis. Unlike a fluorescence system, there is no need for an exogenous light source; luminescence is generated from chemical reactions.

Utilizing photoncounting detectors, which count the number of photons generated from a chemical reaction, luminescence assay has become one of the most sensitive and quantitative optical detection methods. During the past 15 years, nucleic acid (NA) hybridization techniques have become increasingly important as discovery tools and for the clinical diagnosis of genetic disorders and infectious diseases [1-3]. The application of these methodologies has tremendously profited from the development of nucleic acid amplification techniques that have enormously improved the sensitivity of detection of nucleic acids such as DNA and RNA their chimeric derivatives. This detection method is suited for low abundant gene transcripts and genotyping of rare allele mutations, as well as in the measurement of low copy number RNA and DNA pathogens (bacteria, viruses) in diverse biological sources, especially in applications which depend on extremely limited nucleic acid samples, such as cancer diagnositics, pharmacogenomics, toxicogenomics, forensics, food industry, and environmental detection.

Molecular diagnostics and target identification

The following section describes a sandwich hybridization assay for quantitative oligonucleotide target detection. The assay combines 1) magnetic bead-based capture probe, 2) target DNA hybridization, and 3) chemiluminescent target detection. For the assay, 5' end biotinylated single-stranded capture oligonucleotides (**MWG BIOtech,** Highpoint, NC), representing a 30 bp sequence within the coding region of human glyceraldehyde-3-phosphate dehydrogenase (GAPDH), was attached to streptavidin coated paramagnetic beads (Dynal MyOne™, **Invitrogen** Corp., Carlsbad, CA) via the biotin-streptavidin bonding. Magnetic beads (20 µg) were incubated with 20 pg of capture oligonucleotide (5' biotin-GCT CTT TGC ACT GGT AGA CAG AGA TCT CAT -3') in 10mM Tris-HCl (pH 7.5)/1mM EDTA/2M NaCl for 20 minutes at room temperature. Unbound material was washed-off, and beads were resuspended in 20 µl of the same buffer (can be stored at 4ºC for several months). The assembled capture oligo-beads were washed and resuspended in phosphate saline buffer (PBS) prior to hybridization with a 30-mer target oligonucleotide with the exact complementary sequence plus carrying a digoxigenin molecule at its 5' end (5'digoxigenin-ATG AGA TCT CTG TCT ACC ABT GCA AAG AGC -3') (Figure 1). Hybridization was performed in MWG Hybridization Buffer (**MWG**

Biotech) for 90 minutes at 42 ºC using a thermal cycler. The hybridized product was subsequently blocked with 2% bovine serum albumin/0.1% Tween 20/PBS for 30 minutes at room temperature before adding 3 mU of a sheep anti-digoxigenin polyclonal antibody conjugated to HRP (**Roche Applied Sciences,** Indianapolis, IN) to a final concentration of 0.15 mU/µl. The immunodetected beads were washed four times with 1 ml of 1% Tween 20/PBS and finally with PBS before pelleting and resuspending in 20 µl of PBS for transfer to a black clear bottom microplate (Cat. no. 265301, **Nalge Nunc International Corp.,** Rochester, NY). Chemiluminescent signal enhancement was achieved by adding HRP LuminMax Super™ substrate (**Maxwell Sensors Inc.,** Santa Fe Springs, CA) and recorded (Figure 2) with a LuminMax-C™ reader (Figure 3).

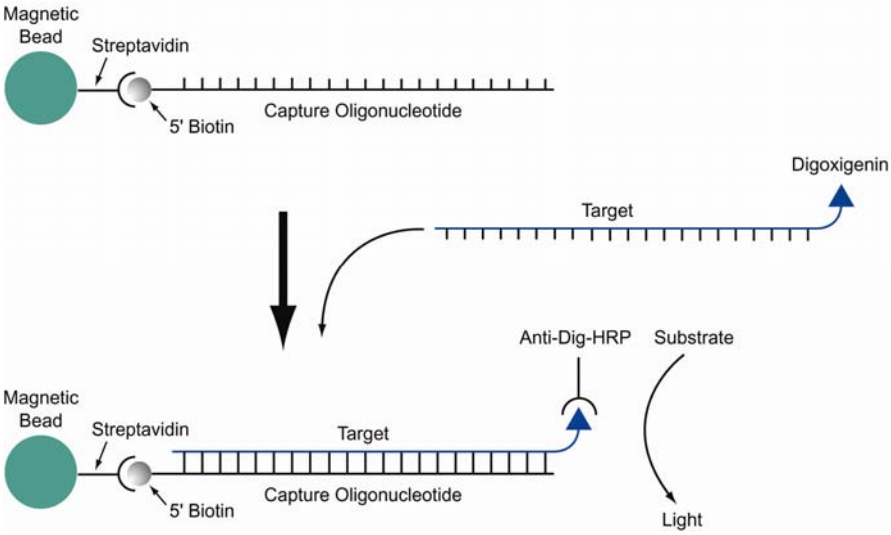

Figure 1: Magnetic bead-based DNA hybridization assay with a label HRP for chemiluminescence detection. Once the substrate is added, the reaction produces luminescence.

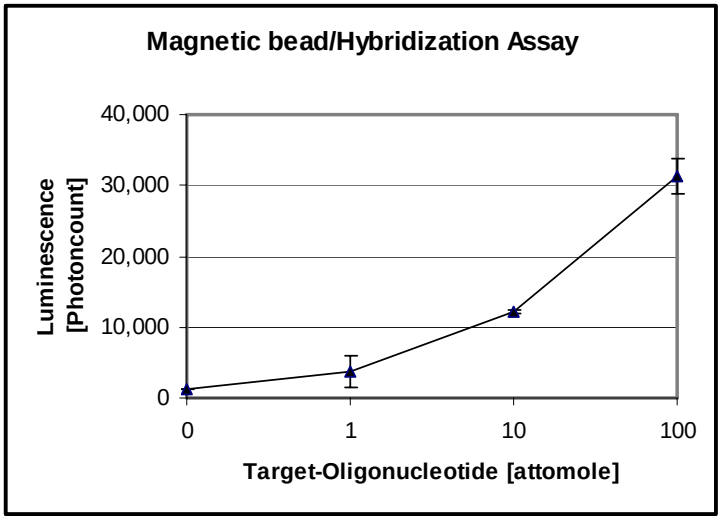

Figure 2: The magnetic bead-based sandwich hybridization assay has a detection limit between 1 and 5 attomole of target oligonucleotide and a dynamic range of three to four orders of magnitude.

Figure 3: LuminMax-C™ offers attomoles sensitivity by quantitative photoncounting.

The LuminMax-C™ reader offers microplate instruments for cell biology, molecular biology and immunology applications in the field of cancer research, drug development, proteomics and genomics. It is also suitable for testing antibody activity prior to their use in enzyme-linked immunosorbent assays (ELISA) such as modularly utilized for the hybridization sandwich assay described above. We have tested the sensitivity of the anti-digoxigenin antibody in a titration assay using different antibody concentrations, while keeping the amount of chemiluminescent substrate constant (Figures 4). The system offers excellent sensitivity, accuracy, ease of use, compactness, and affordability. The system measures the luminescence intensity in a 96-well microplate (black or white). The microplate has a clear bottom; therefore, the luminescence can be accurately detected from the bottom of the well. Because it uses state-of-the-art photoncounting multiplier tube as the detector, the system has the ability to count a single photon generated from the reaction, meaning it can detect very small amounts of analyte in the samples. A CD, with user-friendly software, is provided for easy installation. LuminMax-C utilizes PC or notebook as its microprocessor. The system is interfaced to a computer by a simple plug-in (USB or serial port) connection. After click on the "Go!" button, the system automatically and quickly scans all of the selected microwells and displays the results. The resulting data is displayed as a spreadsheet in Microsoft® Excel™ (**Microsoft**, Redmond, WA) format.

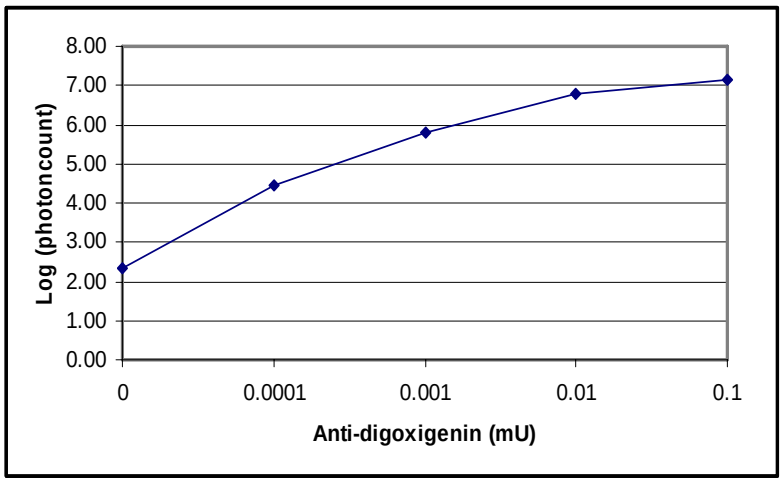

Figure 4: The dynamic range of the polyclonal antibody reaches over seven orders of magnitude from 30 mU to 10^{-5} mU using the same amount of LuminMax Super HRP substrate.

References

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