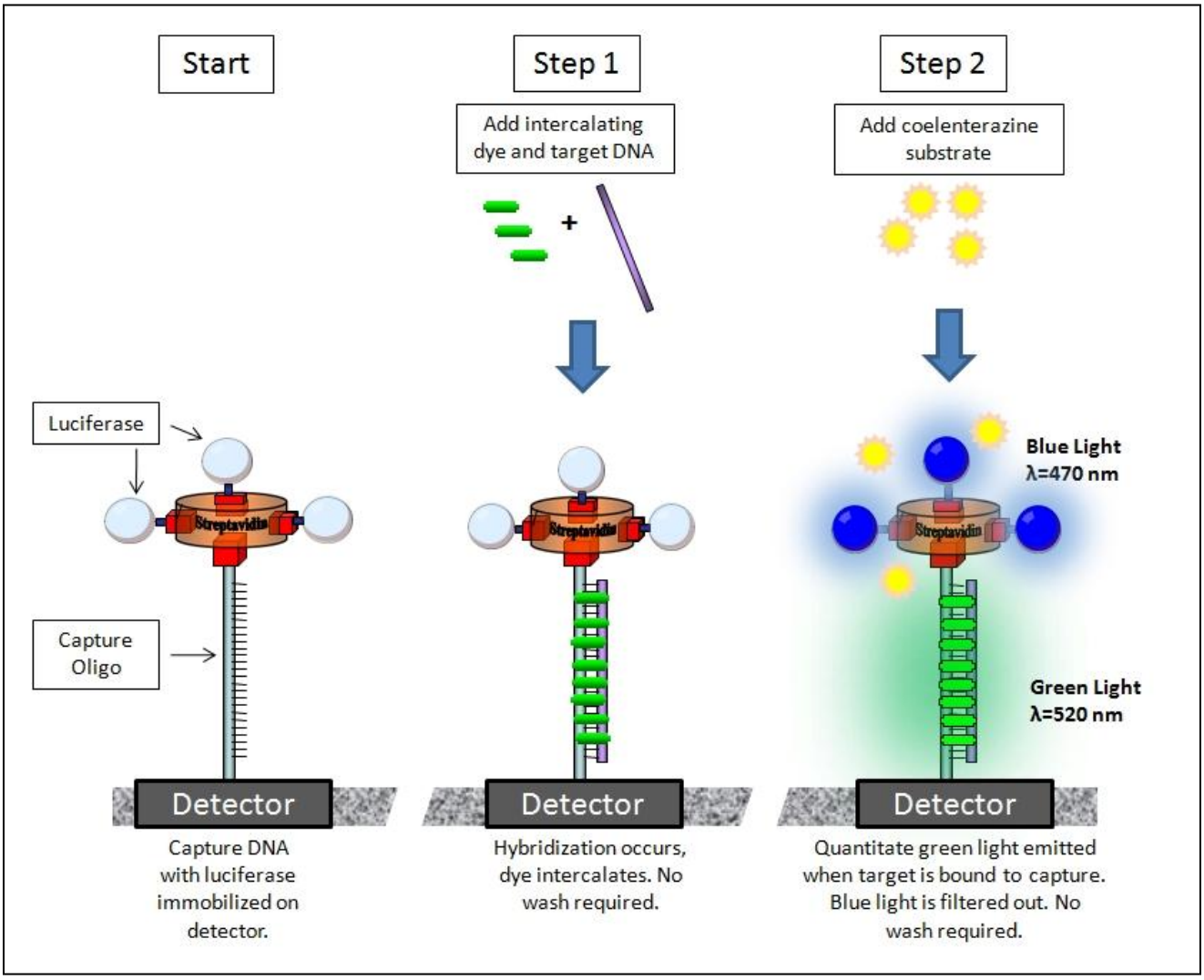
Beacon Biotechnology, LLC

Nucleic Acid Proximity Assay™ (NAPA™)

Patent Pending



DNA-based assays essentially elucidate the sequence of a target DNA or RNA molecule in biological samples based on the rule that guanidine pairs with cytosine (G-C base pair) and adenosine pairs with thymidine (A-T base pairs): A single strand of DNA will bind to a complimentary strand of DNA only when the complimentary pairing is exact (or nearly exact). By attaching a DNA molecule with a specific nucleotide sequence to a surface (capture molecule), that DNA molecule will only bind the complimentary DNA sequence, called the target sequence, in a biological sample. Under the right conditions of temperature and salt, even one nucleotide difference in the target sequence and the complimentary capture sequence will prevent the strands from coming together (annealing). If we construct a gene chip with one location on the chip to recognize the normal (wild type) target sequence, and a different location on the chip that will pair with the mutant DNA, then we can query a patient sample as to what sequences are present by determining what set of sequences form duplexes on the microarray surface.

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Once the strands anneal, it is necessary to detect the annealing event. As discussed above, typically this is done by attaching dye molecules to the target strand. If the target strand anneals to the capture strand, illuminating the annealed strands with a laser allows visual detection because the captured dye-coupled DNA fluoresces. The dye typically shifts the wavelength of the incoming laser light. The laser light wavelength can be filtered out, allowing only the dye shifted light to be seen. Unbound dye-labeled DNA must be washed away thoroughly so that it does not contribute to the background "noise" of the detection and the thoroughness of the washing step is a source of variability in conventional dye-based DNA detection.

Beacon's NAPA™ technology (patent pending) **eliminates the need to wash away unbound dyes,** thus eliminating a major source of variability inherent in existing DNA assays. The BrightSPOT™ System will **work with dirty samples**, because the light signal generated by the NAPA™ assay travels the minimum distance to the detector—the assay is physically coupled to the light-sensing detectors.

In addition to the NAPA™ technology reducing the number of assay steps, the extraordinary sensitivity of the detector may eliminate the need for amplification (PCR) in most biologically relevant clinical samples, thereby **significantly reducing the time to a result** from the current hours (or days) to minutes. The BrightSPOT™ System with NAPA™ technology has demonstrated detection of **0.4 attomoles of dsDNA**.

The BrightSPOT™ System and the NAPA™ technology harmonize beautifully to create a very simple and powerful assay. The BrightSPOT™ System is an extraordinarily sensitive, but simple detector. An orange filter on the BrightSPOT™ System blocks blue light, but is transparent to green light. The BrightSPOT™ System will only detect the green light. The blue light will not be measured.

The NAPA™ technology is designed so that blue light is always on, but because of the filter, the detectors **do not "**see" blue light. When a target DNA in the test sample is captured, the blue light energy is absorbed by the intercalated dye and re-emitted as green light. The filter is transparent to green light and the BrightSPOT™ System "sees" the green light at a detector location corresponding with a specific test. There are 112 individual detectors on the device enabling up to 112 simultaneous tests per sample.

The method for changing the blue light to green light is the Nucleic Acid Proximity Assay™ (NAPA™). The NAPA™ technology generates blue light from a chemical reaction. The reaction uses a light-producing enzyme (luciferase) from a deep sea organism, similar to the way fireflies produce light with their bioluminescent enzyme (firefly luciferase). An important difference is that fireflies produce yellow-green light while most marine organisms produce blue light, which propagates most efficiently through sea-water.

Fluorescent dyes are ubiquitous in biological assays because of their ability to absorb light at a given wavelength (color) and then re-emit the light at a different wavelength. Typically, a fluorescent dye is attached to a target molecule. The target molecule is visualized or detected by shining light at a wavelength known to be absorbed by the dye (the excitation wavelength of the incident light). The light is absorbed by the dye and the dye re-emits the light at a different wavelength (the emission wavelength). The target molecule can be detected by placing a filter between the target molecule and the detector that is opaque to the laser light (incident light), but transparent to light emitted by the dye. The detector "sees" only the dye-emitted green light (emission signal) when a target has been captured.

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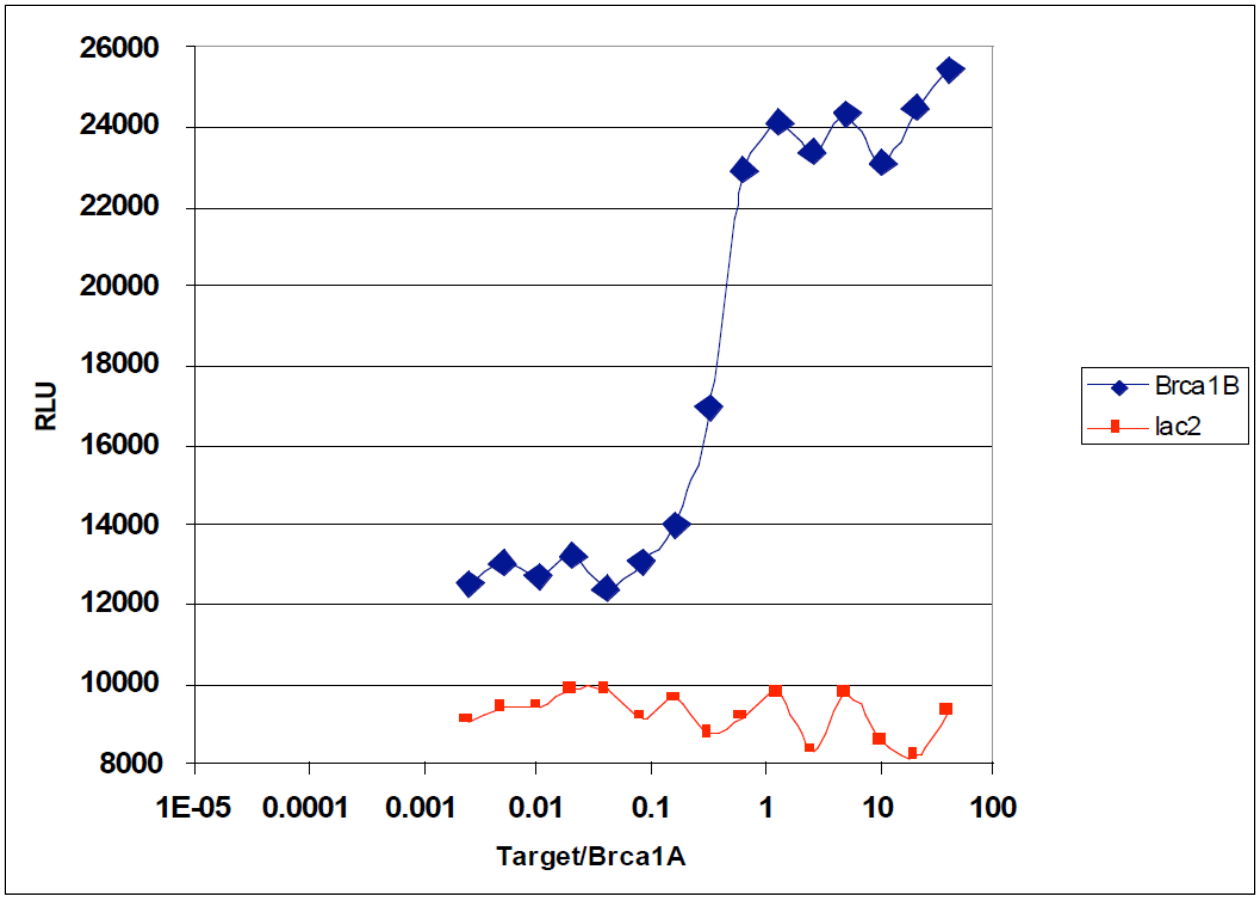

Figure: This figure illustrates the use of NAPA™ technology to detect DNA sequences of clinical interest. The capture nucleotide is capable of annealing to a 20 nucleotide stretch of human gene containing a mutation known to increase an individual's risk of breast cancer, the BRCA1 mutation. A sequence from the *E. coli* lac gene is used as a negative control to show that irrelevant sequences are not detected. This demonstrates that the BrightSPOT™ System could be used to develop a rapid test for cancers and other nucleic acid based tests.

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