

# Application of a miniature biochip using the molecular beacon probe in breast cancer gene *BRCA1* detection

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## Abstract

We report for the first time the application of a biochip using the molecular beacon (MB) detection scheme. The usability of this biochip novel detection system for the analysis of the breast cancer gene *BRCA1* is demonstrated using molecular beacon probes. The MB is designed for the *BRCA1* gene and a miniature biochip system is used for detection. The performance of the biochip-MB detection system is evaluated. The optimum conditions for the MB system for highest fluorescence detection sensitivity are investigated for the detection system. The detection of *BRCA1* gene is successfully demonstrated in solution and the limit of detection (LOD) is estimated as 70 nM.

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**Keywords:** Biochip; Molecular beacon; *BRCA1*; Detection; Fluorescence

## 1. Introduction

Today, breast cancer remains a worldwide public health concern and about 180,000 women are diagnosed with the disease yearly in the US (Kelsey, 1993). *BRCA1*, a breast cancer susceptibility gene, was first identified in 1994. People carrying a mutation (abnormality) in this gene are at an increased risk of breast or ovarian cancer. At least 10% of observed breast cancer cases in the general population are related to the genetic predisposition. The detection of *BRCA1* offers an opportunity to characterize the function of genetic features in breast and ovarian cancer and to screen breast or ovarian cancer patients for the presence of germline mutations. Discovery of a mutation in patients can greatly effect the prediction of cancer risk and help the doctors and patient to take the appropriate steps for treatments.

One of the most unambiguous and well-known molecular recognition events is the hybridization of a nucleic acid to its complementary target. A molecular beacon (MB), a short oligonucleotide with a loop and stem structure, uses this recognition feature. The stem part contains five to seven base pairs, which are complementary to each other but unrelated

to the target oligonucleotide. The loop section of a MB is complementary to its target oligonucleotide. A fluorescing and quenching chemical moiety is covalently attached to the end of each stem. Because the stem keeps these two moieties together in close proximity, the fluorogenic probe is unable to fluoresce. This is due to fluorescence quenching caused by the proximity between the quencher and acceptor (Marras et al., 2002). When a MB is hybridized with its complementary target, the stem is forced apart, thus resulting in the restoration of fluorescence.

Since Tyagi and Kramer introduced molecular beacons in 1996 (Tyagi and Kramer, 1996), they have been used in numerous applications: quantitative PCR (Vogelstein and Kinzler, 1999; Chen et al., 2000), in single nucleotide polymorphism (SNP) and mutation detection (Piatek et al., 1998; Kostrikis et al., 1998), pathogenic detection and RNA detection in a single cell (Chen et al., 2000; Sokol et al., 1998).

In this study, we investigate the use of MB probes along with a miniaturized detection biochip system for the detection of the *BRCA1* gene in solution. Previously, we have developed an integrated circuit (IC) chip, known as the multi-functional biochip (MFB), that has demonstrated great potential for field use. The MFB has a number of distinct advantages over alternate biosensing technologies (Vo-Dinh, 1988; Vo-Dinh et al., 1999; Vo-Dinh and Cullum, 2000; Stokes et al., 2001). These advantages include a

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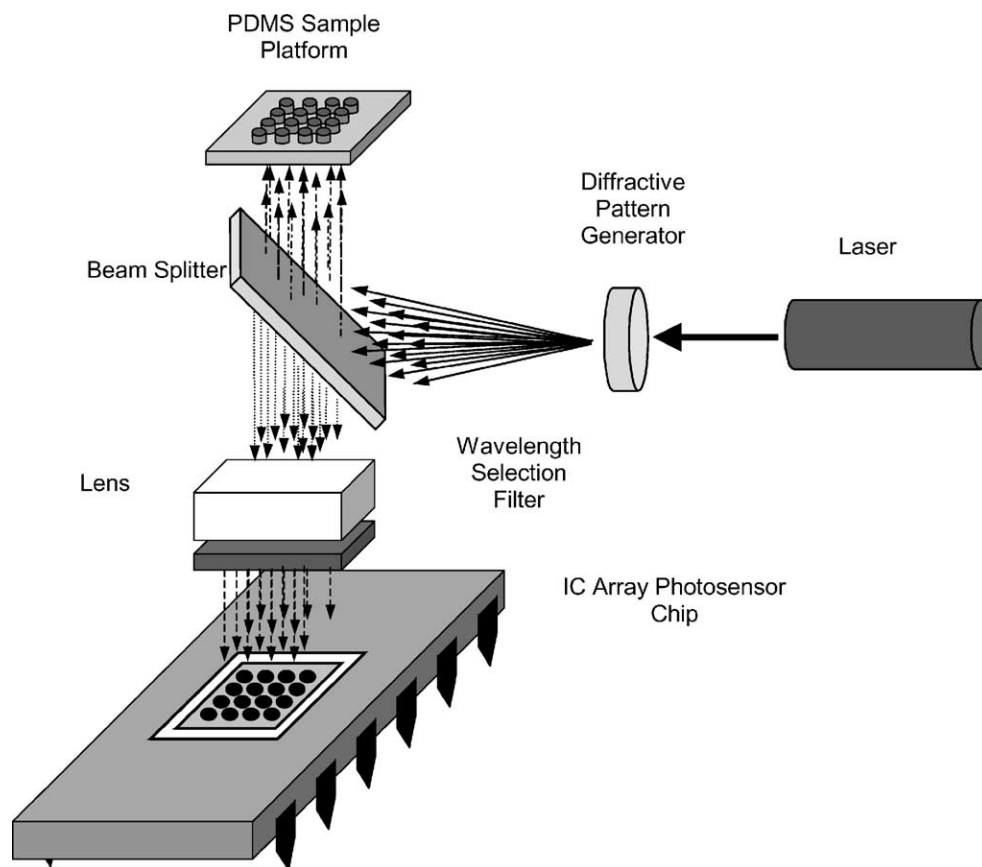


Fig. 2. Schematic diagram of the miniature biochip detection system.

Cullum, 2000). This detection system features an integrated circuit-based  $4 \times 4$  array detector, in which each photodiode operates independently. The individual photodiodes of the  $4 \times 4$  array are sensors with  $900 \mu\text{m} \times 900 \mu\text{m}$  dimensions, and each of them is arranged with 1 mm center-to-center spacing. They are integrated along with amplifiers, discriminators, and logic circuitry on a single solid-state circuit.

The detection system consists of an excitation source, excitation and collection optics, and IC biochip. A diode laser with 5 mW output power and 635 nm wavelength (Model VHK 4.9 mW, Edmund Scientific) is selected for excitation of the Cy5 labels. The laser beam is launched through a diffractive pattern generator, which produced a  $4 \times 4$  array of laser beamlets of equal intensity. The intensity of one single laser spot is estimated as  $\sim 0.2$  mW. A molded micro well  $4 \times 4$  plate of PDMS is visually aligned with the focused laser excitation spots. The image of the laser spot array is projected from the molded PDMS  $4 \times 4$  microwell plate onto the corresponding  $4 \times 4$  array of photo-sensors of the IC detector via a 2.5 cm. diameter,  $f/2$  lens and an emission band-pass filter (HQ 700/75 nm, Chroma Technology Corp.). The output from the IC biochip is recorded as a voltage signal by means of a digital multi-meter (Model 506, Protek). A depiction of the biochip detection system is seen in Fig. 2.

### 3. Results and discussion

Tsourkas et al. (2003) demonstrated that the performance of a MB could be very sensitive to its structural characteristics such as probe and stem lengths. They reported that a stem of at least four bases was required for lowering background noise, and the shorter probe domains (22–25 bases) were required for higher selectivity. In addition, Marras et al. (2002) studied several dyes and molecules as fluorophores and quenchers. These two studies were taken as a reference point for designing the MB used in this study. The stem was composed of seven base pairs and the probe was composed of 22 bases. In order to achieve a full hybridization with the MB, a *BRCA1* gene fragment composed of 123 bases was used. The MB probe was complementary to the 22 bases in the middle of the *BRCA1* gene. When designing the MB probe, the requirements for instrumentation were also taken into account. Because a diode laser with 635 nm wavelength was used, a Cy5 label, which absorbs at the laser excitation wavelength, was chosen.

The experimental conditions were first optimized to achieve the highest fluorescence signal. Because fluorescence signal is directly related to hybridization efficiency, the first priority was to optimize the hybridization conditions. In solution, single-stranded DNA carries negative

charge. The presence of a cation in the media can accelerate the hybridization process by neutralizing (at least partially) the negative charge on the single-stranded DNA. The addition of divalent cations in the hybridization solution was reported to be the best choice (Li et al., 2001). Thus, the effect of varying concentrations of MgCl<sub>2</sub> solution on the hybridization efficiency was examined. Fig. 3 shows that a higher fluorescence yield was obtained with increasing MgCl<sub>2</sub> concentration up to 100 mM MgCl<sub>2</sub>, at which concentration a plateau in fluorescence signal was attained. A further increase in concentration of MgCl<sub>2</sub> had little effect on the fluorescence yield. A 100 mM MgCl<sub>2</sub> solution was used for the following experiments reported here.

Fig. 4 shows the evaluation of the system using the PDMS-well platform. The final MB concentration was 2.5 μM in all the wells. From left to right, the first four wells of the PDMS platform did not contain any solution (i.e. designated blank). The second row only contained MB

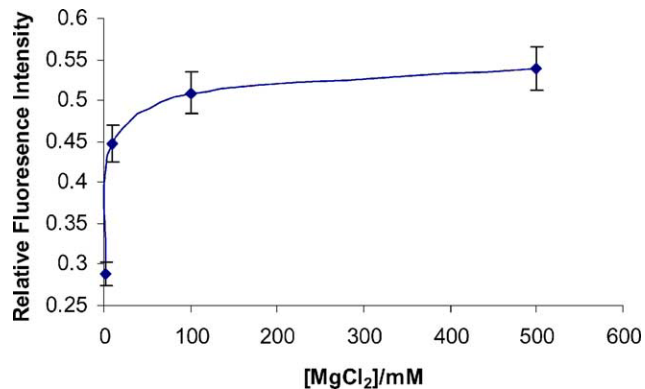
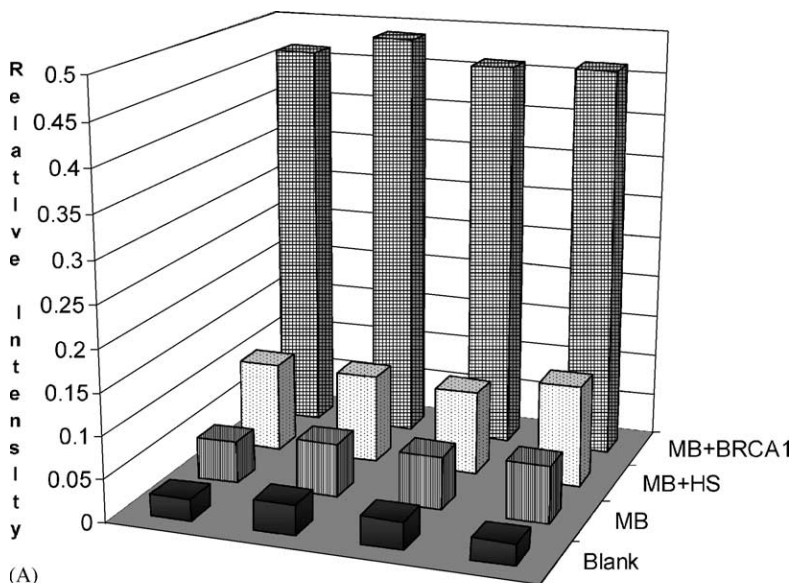
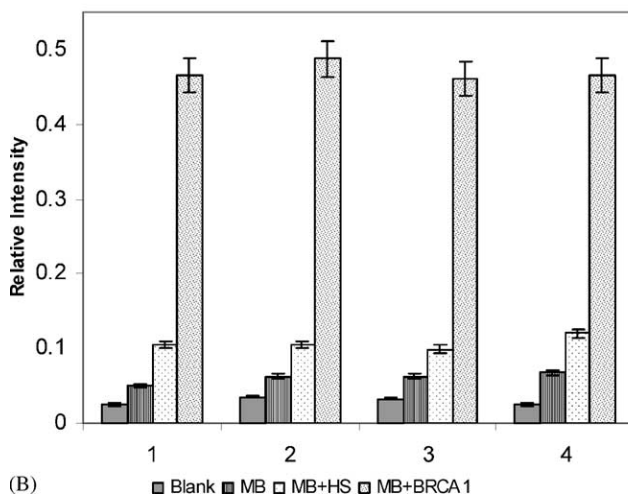


Fig. 3. Effect of MgCl<sub>2</sub> concentration on fluorescence yield.



(A)



(B)

Fig. 4. (A) Demonstration of detection of BRCA1 gene with MB probe system and comparison of with MB probe, from left to right, with blank, itself, controlled hybridization with HS DNA, and BRCA1 gene. (B) Two-dimensional plot of the results and error bars of the biochip detection system.

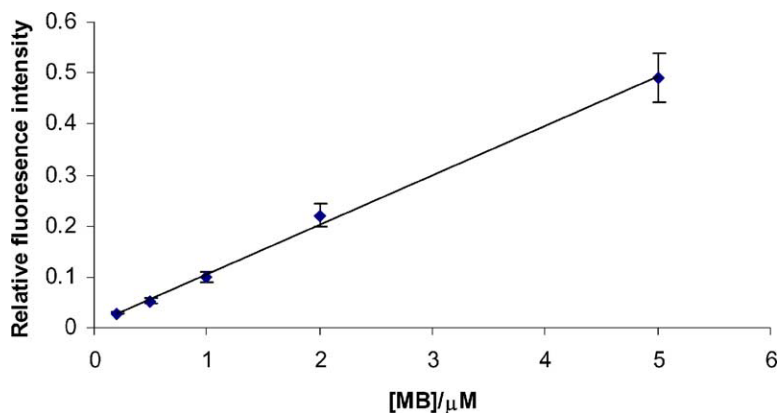


Fig. 5. Calibration curve for determination of LOD. The  $k$  value was taken as 3. The slope and  $R$  values were 0.11 and 0.99, respectively.

at a concentration of 2.5  $\mu\text{M}$ . Even though optimum conditions were used to achieve the best hybridization conditions, and fluorescence efficiency and collection, there was still some background noise due to incomplete quenching. This can be seen easily by comparing the first row to the second row in Fig. 4A. The third row contained a MB and HS DNA to examine nonspecific binding to DNA. The final concentration of HS DNA was 10 mg/ml after mixing with the MB solution. As seen in the figure, there is some increase in the fluorescence intensity due to nonspecific interaction between MB and HS DNA. This could be due to some regions, which have some degree complementary with the MB. The hybridization of the MB probe with 5  $\mu\text{M}$  *BRCA1* gene is shown in the last row on the right. With this *BRCA1* gene concentration, a reasonable fluorescence yield was obtained. Fig. 4B shows the results of the biochip in a two-dimensional format with the relative standard deviations demonstrating the excellent reproducibility of the detection system.

It is important to determine the minimum concentration of *BRCA1* gene that must be added to a MB probe solution to obtain a detectable signal. Thus, the determination of limit of detection (LOD) was attempted. The *BRCA1* gene and MB were prepared in each PDMS-well with concentrations of 0.2, 0.4, 2.0, and 5.0 and 2.5  $\mu\text{M}$ , respectively. The fluorescence intensity was directly proportional to the increased concentration of *BRCA1* gene. Fig. 5 shows the linear relationship between intensity and *BRCA1* concentration, thus indicating the possibility for quantitative analysis. The LOD was estimated as 70 nM by using the  $k$  value of 3 to calculate LOD. The  $R$  value for the calibration curve was 0.99.

#### 4. Conclusions

Rapid, simple, and cost-effective medical devices for screening multiple medical diseases and infectious pathogens are essential for early diagnosis and improved treatments of many illnesses. An important factor in medical diagnostics is rapid, selective, and sensitive detection of gene mutations. In this work, detection of the *BRCA1* cancer susceptibility gene using MB probes and a miniature

biochip detection system was demonstrated. The MB was designed for use with the biochip system. HS DNA was used as a control to evaluate the degree of nonspecific binding. The detection system and hybridization conditions were optimized for the LOD determination. It was found that the  $\text{MgCl}_2$  concentration of 100 mM was adequate to achieve the optimum hybridization conditions. The LOD was estimated to be 70 nM. Although 70 nM are reasonably a low amount, this detection limit needs to be improved further. Background noise due to incomplete quenching of the fluorescing component of the MB and nonspecific interaction with noncomplementary DNA sequences is a fundamental problem with MB detection systems. These problems are serious challenges not easy to overcome for experiments in solutions. Therefore, the goal of our future studies will focus on the improvement of the LOD by using improved collection optics and better MB system design. Although further studies are required to fully assess the capability of the MB detection scheme for gene detection, the results presented in this study illustrate the potential of integrated biochip systems as diagnostic tools at the physician's office.

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#### References

- Chen, W., Mulchandani, A., 2000. Molecular beacons: a real-time polymerase chain reaction assay for detecting *Salmonella*. *Anal. Biochem.* 280 (1), 166–172.
- Kelsey, J.L., 1993. Breast cancer epidemiology—summary and future directions. *Epidemiol. Rev.* 15 (1), 256–263.

- Kostrikis, L.G., Tyagi, S., Mhlanga, M.M., Ho, D.D., Kramer, F.R., 1998. Molecular beacons—spectral genotyping of human alleles. *Science* 279 (5354), 1228–1229.
- Li, J., Tan, W.H., Wang, K., Xiao, D., He, X., Tang, Z., 2001. Ultrasensitive optical DNA biosensor based on surface immobilization of molecular beacon by a bridge structure. *Anal. Sci.* 17 (10), 1149–1153.
- Marras, S.E.A., Kramer, F.R., Tyagi, S., 2002. Efficiencies of fluorescence resonance energy transfer and contact-mediated quenching in oligonucleotide probes. *Nucleic Acids Res.* 30 (21), e122.
- Piatek, A.S., Tyagi, S., Pol, A.C., Telenti, A., Miller, L.P., Kramer, F.R., Alland, D., 1998. Molecular beacon sequence analysis for detecting drug resistance in *Mycobacterium tuberculosis*. *Nat. Biotechnol.* 16 (4), 359–363.
- Sokol, D.L., Zhang, X.L., Lu, P.Z., Gewitz, A.M., 1998. Real time detection of DNA RNA hybridization in living cells. *Proc. Natl. Acad. Sci. U.S.A.* 95 (20), 11538–11543.
- Stratis-Cullum, D., Griffin, G.D., Mobley, J., Vass, A., Vo-Dinh, T., 2003. A miniature biochip system for detection of aerosolized *Bacillus globigii* spores. *Anal. Chem.* 75 (2), 275–280.
- Stokes, D.L., Griffin, G.D., Vo-Dinh, T., 2001. Detection of *E. coli* using a microfluidics-based antibody biochip detection system. *Fresenius J. Anal. Chem.* 369 (3–4), 295–301.
- Tsourkas, A., Behlke, M.A., Rose, S.D., Bao, G., 2003. Hybridization kinetics and thermodynamics of molecular beacons. *Nucleic Acids Res.* 31 (4), 1319–1330.
- Tyagi, S., Kramer, F.R., 1996. Molecular beacons: probes that fluoresce upon hybridization. *Nat. Biotechnol.* 14 (3), 303–308.
- Vo-Dinh, T., 1988. Development of a DNA biochip: principle and applications. *Sens. Actuators B Chem.* 51 (1–3), 52–59.
- Vo-Dinh, T., Cullum, B., 2000. Biosensors and biochips: advances in biological and medical diagnostics. *Fresenius J. Anal. Chem.* 366 (6–7), 540–551.
- Vo-Dinh, T., Alarie, J.P., Isola, N., Landis, D., Wintenberg, A.L., Ericson, M.N., 1999. DNA biochip using a phototransistor integrated circuit. *Anal. Chem.* 71 (2), 358–363.
- Vogelstein, B., Kinzler, K.W., 1999. Digital PCR. *Proc. Natl. Acad. Sci. U.S.A.* 96 (16), 9236–9241.