SCIENCEFORECAST
A digital science library

Research Article
Published: 13 Feb, 2018

SF Journal of Nanochemistry and Nanotechnology

High-Specific Electrochemical Detection of BRCA1 Gene Sequence using Methylene Blue Hybridization Indicator

*Xiaofan Sun, Yiping Zhang, Yaping Tian and Nandi Zhou**

The Key Laboratory of Carbohydrate Chemistry and Biotechnology, Ministry of Education, School of Biotechnology, Jiangnan University, Wuxi 214122, China

Abstract

A high-specific and high-sensitive electrochemical DNA biosensor for human breast cancer susceptibility gene 1 (BRCA1) was developed by using methylene blue (MB) as hybridization indicator. A specially designed capture probe with a thiol group at its 5'-end was firstly self-assembled on gold electrode through Au-S bond. In the solution with high ionic strength, MB combined with the capture probe through its interaction with guanine. Then an intensive electrochemical signal was detected through differential pulse voltammetry (DPV). In the presence of BRCA1 gene sequence, double-stranded DNA (dsDNA) was formed through the hybridization between the target DNA and capture probe. The interaction between MB and dsDNA was intercalative. The monitored peak current in DPV was greatly decreased due to the weaken affinity of MB towards ds-DNA. The decrease of the electrochemical signal was related with the concentration of the target DNA. Under the optimized conditions, the fabricated DNA sensor showed a very wide dynamic response range over six orders of magnitude. The change of current in DPV was linearly related with the logarithm of the concentration of BRCA1 gene sequence in the range from 1nM to 1.5μM. The observable current change appeared even at the concentration of 1pM. The detection was also carried out in the presence of single-base mismatched and three-base mismatched sequences of the target DNA. The results showed that the established DNA biosensor can easily identify those mismatched sequences from the target and therefore has high specificity.

Keywords: BRCA1; DNA biosensor; Methylene blue; Differential pulse voltammetry

OPEN ACCESS

*Correspondence:
Nandi Zhou, The Key Laboratory of Carbohydrate Chemistry and Biotechnology, Ministry of Education, School of Biotechnology, Jiangnan University, Wuxi 214122, China.
Tel: +86-510-85197831
E-mail: zhounandi@jiangnan.edu.cn

Received Date: 20 Dec 2017
Accepted Date: 06 Feb 2018
Published Date: 13 Feb 2018

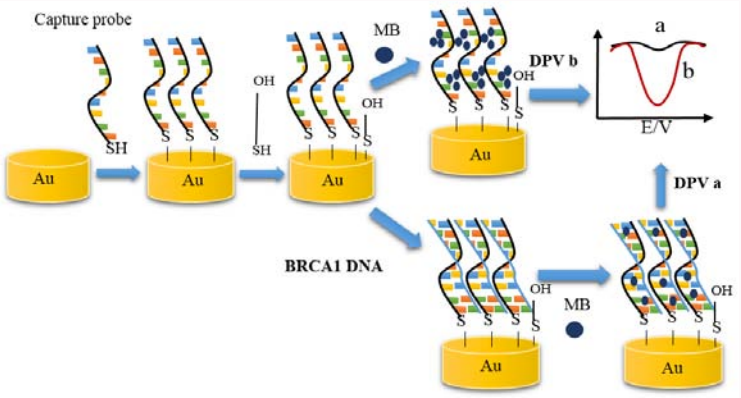
Citation: Sun X, Zhang Y, Tian Y, Zhou N. High-Specific Electrochemical Detection of BRCA1 Gene Sequence using Methylene Blue Hybridization Indicator. SF J Nanochem Nanotechnol. 2018; 1(1): 1002.

ISSN 2643-8135

Introduction

In the last decade, electrochemical DNA sensors with appropriately designed capture probes have been widely used in many fields to realize the detection of gene sequences, bacteria, viruses, trace amounts of heavy metal ions [1-5], *etc.* Electrochemical DNA sensors can be utilized to carry out independent tests, realize the purpose of on-line monitoring, food analysis, environmental monitoring and clinic diagnosis [6-9]. Compared with those traditional DNA testing methods, DNA sensors usually take the advantages of high sensitivity, minimized equipment, low cost, compatibility with micro-fabrication technology and time-saving [10-12]. Moreover, high specificity of DNA sensors offers accurate detection of the target DNA by easy identification of single-base changes in the sequences [13,14].

The construction of electrochemical DNA biosensors mainly consists of three steps [15,16]: (1) modification of single-stranded DNA probes; (2) hybridization between the target sequences and the probes; (3) determination of electrochemical signals using specific indicators. It is very important to choose a proper indicator which can characterize the presence of the targets [17,18]. Generally, electrochemical hybrid indicators are electroactive compounds which can convert the hybridization events into detectable electrochemical signals [1,19]. The proper selection of indicators can effectively improve the sensitivity and specificity of the sensors. Some electrochemical indicators, such as methylene blue (MB), differ in their binding to single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA), resulting in different response current [20,21]. MB is a phenothiazine dye which can be used as a redox indicator [22]. Existing studies show that MB has a specific binding effect to guanine of ssDNA, while has much lower affinity for dsDNA because dsDNA hinders the binding of MB to guanine, resulting in reduced electrochemical signal [1,2,23-26]. Meanwhile, in the solution with low concentration of ions, except the combination with guanine, MB also can combine with the negatively charged phosphate groups of DNA through electrostatic interaction [1]. Under high concentration of ions, the phosphate groups of DNA skeleton form an electron layer. Overall, MB can insert into dsDNA through the DNA groove, and combine with ssDNA



Scheme 1: Principle of the electrochemical detection of BRCA1 gene sequence.

through the interaction with guanine.

With the increased incidence of breast cancer [27], it has become a serious threat to women's health in the new century [28]. The greatest risk factor for breast is inheritance of a mutation in breast cancer susceptibility genes [29]. One of the biomarkers of breast cancer that has received a great attention is breast cancer susceptibility gene-1 (BRCA1) [30]. BRCA1 is one of the high penetrance of breast cancer susceptibility genes as known. One defective copy of BRCA1 in the germline is enough to cause cancer predisposition [31]. Early breast cancer does not have the typical signs and symptoms, so the detection of biomarkers is particularly important in the early diagnosis of breast cancer. Electrochemical DNA biosensor has great potential in such gene-based diagnosis.

Herein, an electrochemical DNA biosensor for BRCA1 gene sequence was fabricated by using MB as hybridization indicator. The biosensor showed high specificity to identify single-base mismatched sequence of the target DNA and satisfactory sensitivity.

Experimental

Materials and reagents

6-mercaptohexanol (6-MCH) and hemin were purchased from Sigma-Aldrich. Human serum was purchased from Beijing Solarbio Science&Technology Co., Ltd. 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid sodium salt (HEPES) was obtained from Sangon Biotech Co., Ltd. Other chemicals were of analytical grade. All solutions were prepared with ultrapure water (18.2 MΩ cm) obtained from a Millipore water purification system.

All oligonucleotides were synthesized and HPLC-purified by Sangon Biotech Co., Ltd. They were diluted to 100μM with 10 mM Tris-HCl buffer (pH7.4) containing 2mM $MgCl_2$, 20mM KCl and 0.2M NaCl. The sequences of the oligonucleotides were as below.

Capture probe: 5'-HS-$(CH_2)_6$- GAACAAAAGGAAGAAAATC -3'

BRCA1 sequence: 5'- GATTTTCTTCCTTTTGTTC -3'

Single-base mismatched sequence: 5'-GATTTTCTTCCTTTTGTT**G** -3'

Three-base mismatched sequence: 5'-GATTTTCTTCCTTTTG**AAG** -3'

Preparation and modification of Au electrode

Au electrode (2mm in diameter) was polished carefully with alumina slurry (0.3 and 0.05μm) and then sonicated in ultrapure water and ethanol for 3min, respectively. Then the polished electrode was electrochemically activated by cyclic scanning in the potential range from -0.2 to +1.5V in 0.5M H_2SO_4. Thereafter the electrode was dried at nitrogen atmosphere.

The pretreated Au electrode was immersed in 100μL of 1.5μM capture probe for 5h, allowing the capture probe to be attached to the surface of Au electrode via Au-S bond. Then the capture probe-modified electrode was washed with 20mM Tris-HCl buffer, dried with nitrogen, and then immersed in 200μL of 2mM 6-MCH for 4h to block the nonspecific adsorption sites on electrode surface. Finally, the electrode was thoroughly washed with 20mM Tris-HCl buffer and dried with nitrogen.

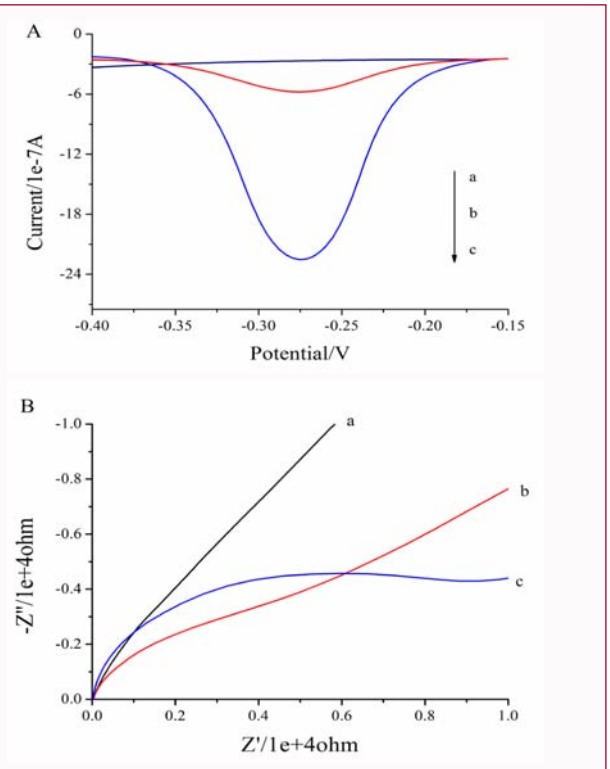


Figure 1: (A) DPV curves obtained in the presence of BRCA1 sequence (a); BRCA1 sequence and MB (b); and MB alone (c). (B) Characterization of the modification of the electrode by EIS: bare Au electrode (a); capture probe-modified Au electrode (b); 6-MCH-blocked capture probe-modified Au electrode (c).

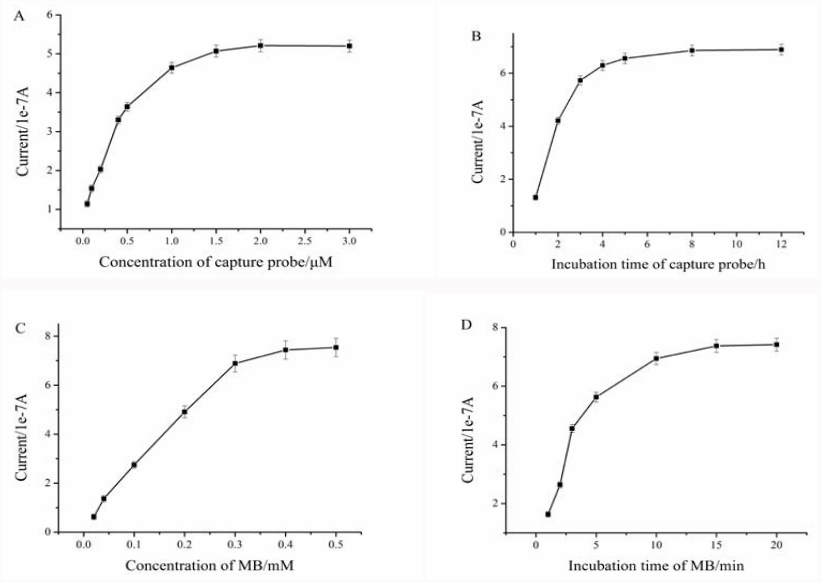


Figure 2: The influence of (A) the concentration of the capture probe, (B) incubation time of the capture probe, (C) the concentration of MB, and (D) incubation time of MB on the peak current in DPV.

Fabrication of the electrochemical DNA biosensor

The capture probe-modified Au electrode was then immersed into the hybridization solution with different concentration of BRCA1 DNA and incubated for 2h to allow the complete hybridization between the complementary sequences of capture probe and the target DNA. After hybridization, the electrode was incubated in 1mL of 20mM Tris buffer containing 0.4mM MB and 0.2M NaCl (pH 7.4) for 15min. After that, differential pulse voltammetry (DPV) was conducted in 20mM HEPES buffer containing 20mM KCl (pH 8.0) in the potential range from -0.6 to -0.15V. The electrochemical measurements were conducted on a CHI 660e electrochemical workstation.

Results and Discussion

The principle of the electrochemical DNA biosensor

The principle of the electrochemical DNA sensor is depicted in Scheme 1. A capture probe with a thiol group at 5'-end and complementary to BRCA1 sequence was designed and self-assembled onto the surface of Au electrode via Au-S bond. When the target DNA was introduced into the hybridization system and incubated with capture probe-modified Au electrode, the target DNA can be captured onto the electrode surface. After the introduction of the target DNA, dsDNA forms on the electrode surface, leading to the reduced adsorption of MB and the corresponding peak current in DPV. In the absence of the target DNA, the capture probe modified electrode can combine with a large number of MB, producing an intensive current signal in DPV. The value of peak current is related with the amount of MB on the electrode, and therefore the concentration of the target DNA.

To verify the feasibility of the principle, experiments were performed under different conditions and the obtained DPV responses were compared. As shown in Figure 1A, when the capture probe modified Au electrode was dipped in the solution containing BRCA1 sequence (without MB), only low peak current can be observed in DPV (curve a). The DPV response was significant increased after the addition of MB (curve c). However, in the presence of the target DNA and MB, the current response in DPV was barely increased (curve b). Therefore, the electrochemical responses of MB vary dramatically after the introduction of the target DNA, and the proposed DNA sensor is reliable for sensitive detection of sequence-specific DNA.

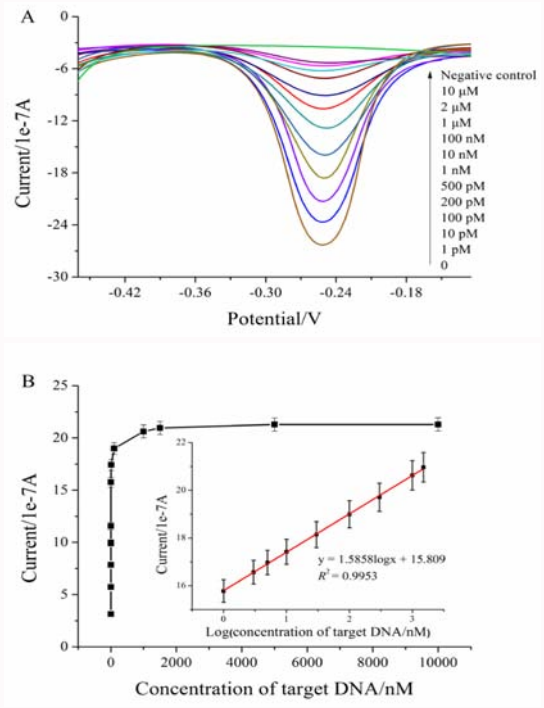


Figure 3: (A) DPV curves obtained in the presence of different concentration of BRCA1 sequence; (B) The relationship between the peak current in DPV and the logarithm of the concentration of BRCA1 sequence; Inset shows the linear relationship.

Electrochemical impedance spectroscopy (EIS) can effectively characterize the electron transfer rate between the electrode and the solution species, and therefore the modification procedure of the electrode. As shown in Figure 1B, compared with bare Au electrode

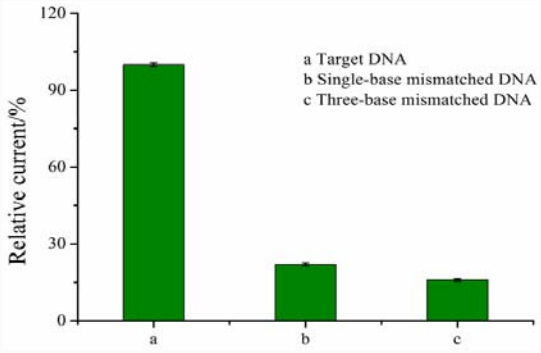


Figure 4: The histogram of the current response in the presence of BRCA1 sequence and mismatched sequences.

(curve a), the electron transfer resistance (R_{et}) of the capture probe-modified electrode was apparently increased (curve b), due to the electrostatic repulsion of the negatively charged capture probe against $Fe(CN)_6^{4-/3-}$. When the electrode was further blocked with 6-MCH, R_{et} was significantly increased (curve c). These results confirmed the successful modification of the electrode, which was ready for detection of the target DNA.

Optimization of the detection conditions

In order to obtain satisfactory performance of the electrochemical DNA biosensor, several key factors, including the concentration of the capture probe, the incubation time of the capture probe, the concentration of MB and the incubation time of MB are optimized.

The coverage of the capture probe attached to the surface of Au electrode depends on the concentration of the capture probe used to modify the electrode and the incubation time of modification, and has significant influence on the capturing of the target DNA, and therefore the performance and reproducibility of the detection. The effect of the concentration of the capture probe was firstly investigated. Different concentration of the capture probe ranging from 0.05μM to 3μM were used to modify bare Au electrodes respectively, and then the capture probe-modified electrodes were incubated with BRCA1 sequence, followed by incubation with MB. Finally DPV was performed and the peak current in DPV curves was plotted to the concentration of the capture probe used to modify the electrode. As shown in Figure 2A, the peak current increases with the increased concentration of the capture probe. However, the peak current no longer increases when the concentration reaches 1.5μM. Therefore, 1.5μM capture probe was chosen to modify Au electrode.

The incubation time of the capture probe with Au electrode was then investigated. Different incubation time ranging from 1h to 12h was used to modify Au electrode at the fixed concentration of the capture probe. Then the capture probe-modified electrodes were incubated with BRCA1 sequence, followed by incubation with MB. As shown in Figure 2B, the peak current in recorded DPV curves increases with the extended incubation time. As the incubation time reaches 8h, the increase of peak current levels off, indicating the saturation of the modification. Therefore, the incubation time for modification of the capture probe was fixed at 8h.

The effect of the concentration of MB was then investigated by addition of MB ranging from 0.02μM to 0.5μM to the hybridization solution. Then DPV was performed and the peak current in DPV curves was plotted to the concentration of MB. As shown in Figure 2C, the peak current continuously increases when the concentration of MB is lower than 0.4μM, and then levels off. Thus 0.4μM was the optimal concentration of MB.

Finally, the incubation time of MB was investigated. The capture probe-modified electrodes were incubated with BRCA1 sequence, followed by incubation with MB. Different incubation time ranging from 1min to 20min was used at the fixed concentration of MB. As shown in Figure 2D, the peak current in recorded DPV curves increases with the extended incubation time. As the incubation time reaches 15min, the increase of peak current levels off, indicating the saturation of the modification. Therefore, the incubation time for MB was fixed at 15min.

The performance of the electrochemical DNA biosensor

Under the optimal conditions, the sensitivity and dynamic response range of the electrochemical DNA biosensor were investigated. A series of different concentration of BRCA1 sequence was mixed in hybridization solution. Then the capture probe-modified electrode was immersed into the hybridization solution and transferred into MB solution thereafter. After the introduction of MB, the DPV curves of the electrodes were recorded. As shown in Figure 3A, the peak current in DPV decreases with the increased concentration of BRCA1 sequence in the range from 1pM to 10μM. The relationship between the peak current in DPV and the logarithm of the concentration of target DNA was studied and shown in Figure 3B. A linear relationship can be derived in the range from 1nM to 1.5μM (Figure 3B, inset). The linear regression equation is y=1.5858logx+15.809, where x represents the concentration of BRCA1 DNA (nM), and y represents the peak current.

The specificity of the DNA sensor reflects the ability to discriminate the target DNA from other sequences with slight differences. Thus the specificity of the fabricated DNA biosensor was checked by comparing the current response towards BRCA1 DNA to those of mismatched sequences, including single-base and three-base mismatched sequences. As shown in Figure 4, the peak current of BRCA1 DNA is much higher than those of mismatched sequences. About 20% of the peak current remained for the single-base mismatched sequence, and only 16% of the peak current remained for the three-base mismatched sequence. Therefore, the constructed electrochemical DNA biosensor has excellent specificity.

Conclusion

In summary, a sensitive electrochemical DNA biosensor was fabricated to detect sequence-specific DNA. Taking BRCA1 as an example, with rationally designed capture probe, the electrochemical signal of MB was utilized to characterize the hybridization of the target DNA with the capture probe, and quantify the concentration of the target DNA. The fabricated DNA biosensor exhibited high sensitivity, which can show observable current change in the presence of 1pM BRCA1 sequence. And the wide dynamic response range of the DNA sensor extend its application area. Meanwhile, the discrimination of single-base and three-base mismatched sequences verified high specificity of the sensor, which is critical in the application in clinic diagnosis.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (no. 31271860) and the Fundamental Research Funds for the Central Universities (JUSRP51402A).

References

1. P Kara, K Kerman, D Ozkan, B Meric, A Erdem, Z Ozkan, M. Ozsoz Electrochemical genosensor for the detection of interaction between methylene blue and DNA. Electrochemistry Communications. 2002; 4 : 705-709.
2. B Meric, K Kerman, D Ozkan, P Kara, S Erensoy, US Akarca, et al. Electrochemical DNA biosensor for the detection of TT and Hepatitis B virus from PCR amplified real samples by using methylene blue. Talanta. 2002; 56: 837-846.
3. NJ Ronkainen, HB Halsall, WR Heineman. Electrochemical biosensors. Chemical Society reviews. 2010; 39: 1747-1763.
4. D Ivnitski, I Abdel-Hamid, P Atanasov, E Wilkins. Biosensors for detection of pathogenic bacteria. Biosensors and Bioelectronics. 1999; 14: 599-624.
5. S Han, X Zhou, Y Tang, M He, X Zhang, H Shi, Y Xiang. Practical, highly sensitive, and regenerable evanescent-wave biosensor for detection of Hg(2+) and Pb(2+) in water. Biosensors and Bioelectronics. 2016; 80: 265-272.
6. AA Lubin, RY Lai, BR Baker, AJ Heeger, KW Plaxco, A Chem. Sequence-specific, electronic detection of oligonucleotides in blood, soil, and foodstuffs with the reagentless. reusable E-DNA sensor, Analytical Chemistry. 2006; 78: 5671-5677.
7. AP Silverman, ET Kool. Detecting RNA and DNA with templated chemical reactions. Chemical Reviews. 2006; 106: 3775-3789.
8. F Lucarelli, S Tombelli, M Minunni, G Marrazza, M Mascini. Electrochemical and piezoelectric DNA biosensors for hybridisation detection. Analytica Chimica Act. 2008; 609: 139-159.
9. K Hu, D Lan, X Li, S Zhang. Electrochemical DNA biosensor based on nanoporous gold electrode and multifunctional encoded DNA-Au bio bar codes. Analytical Chemistry. 2008; 80: 9124.
10. J Wang. Electrochemical biosensors: Towards point-of-care cancer diagnostics. Biosensors and Bioelectronics. 2006; 21: 1887-1892.
11. H Yin, Y Zhou, Z Xu, L Chen, D Zhang, S Ai. An electrochemical assay for DNA methylation, methyltransferase activity and inhibitor screening based on methyl binding domain protein. Biosensors and Bioelectronics. 2013; 41: 492-497.
12. T Wang, L Zhou, S Bai, Z Zhang, J Li, X Jing, G Xie. Ultraspecific electrochemical DNA biosensor by coupling spontaneous cascade DNA branch migration and dual-signaling sensing strategy. Biosensors and Bioelectronics. 2016; 78: 464-470.
13. SO Kelley, EM Boon, JK Barton, NM Jackson, MG Hill. Single-base mismatch detection based on charge transduction through DNA. Nucleic acids research. 1999; 27: 4830.
14. Z Zhang, JL Li, J Yao, T Wang, D Yin, Y Xiang, et al. Energy driven cascade recognition for selective detection of nucleic acids with high discrimination factor at room temperature. Biosensors and Bioelectronics. 2016; 79: 488-494.
15. Sassolas, LB And, LJ Blum. DNA Biosensors and Microarrays. Chemical Reviews. 2008; 108: 109.
16. Y Xiao, RY Lai, KW Plaxco. Preparation of electrode-immobilized, redox-modified oligonucleotides for electrochemical DNA and aptamer-based sensing. Nature protocols. 2007; 2: 2875-2880.
17. E Hamidi-Asl, JB Raoof, AS Meibodi, ZH Bazgir. Celestine blue as a new indicator in electrochemical DNA biosensors. Science China Chemistry. 2015; 59: 128-134.
18. TG Drummond, MG Hill, JK Barton. Electrochemical DNA sensors. Nature biotechnology. 2003; 21: 1192-1199.
19. EE Ferapontova. Electrochemical Indicators for DNA Electroanalysis. Current Analytical Chemistry. 2011; 7: 51-62.
20. Erdem, K Kerman, B Meric, D Ozkan, P Kara, M Ozsoz. DNA biosensor for Microcystis spp sequence detection by using methylene blue and ruthenium complex as electrochemical hybridization labels. Turkish Journal of Chemistry. 2002; 26: 851-862.
21. L Cui, M Lu, Y Li, B Tang, CY Zhang. A reusable ratiometric electrochemical biosensor on the basis of the binding of methylene blue to DNA with alternating AT base sequence for sensitive detection of adenosine. Biosensors and Bioelectronics. 2017; 102: 87-93.
22. Remo Rohs, Heinz Sklenar, A Richard Lavery, B Röder. Methylene Blue Binding to DNA with Alternating GC Base Sequence: A Modeling Study. Journal of the American Chemical Society. 2000; 122: 2860-2866.
23. Tani, AJ Thomson, JN Butt. Methylene blue as an electrochemical discriminator of single- and double-stranded oligonucleotides immobilised on gold substrates. Analyst. 2001; 126: 1756-1759.
24. Y Jin, X Yao, Q Liu, J Li. Hairpin DNA probe based electrochemical biosensor using methylene blue as hybridization indicator. Biosensors and Bioelectronics. 2007; 22: 1126-1130.
25. W Yang, M Ozsoz, DB Hibbert, JJ Gooding. Evidence for the Direct Interaction Between Methylene Blue and Guanine Bases Using DNA-Modified Carbon Paste Electrodes. Electroanalysis. 2015; 14: 1299-1302.
26. Erdem, K Kerman, B Meric, M Ozsoz. Methylene Blue as a Novel Electrochemical Hybridization Indicator. Electroanalysis. 2015; 13: 219-223.
27. WF Li, Z Hu, NY Rao, CG Song, B Zhang, MZ Cao, et al. The prevalence of BRCA1 and BRCA2 germline mutations in high-risk breast cancer patients of Chinese Han nationality: two recurrent mutations were identified. Breast Cancer Research and Treatment. 2008; 110: 99.
28. J Zhang, R Pei, Z Pang, T Ouyang, J Li, T Wang, et al. Prevalence and characterization of BRCA1 and BRCA2 germline mutations in Chinese women with familial breast cancer. Breast Cancer Research and Treatment. 2012; 132: 421-428.
29. R Roy, J Chun, SN Powell. BRCA1 and BRCA2: different roles in a common pathway of genome protection. Nature Reviews Cancer. 2011; 12: 68.
30. Mangia, A Malfettone, C Saponaro, S Tommasi, G Simone, A Paradiso. Human epidermal growth factor receptor 2, Na/H exchanger regulatory factor 1, and breast cancer susceptibility gene-1 as new biomarkers for familial breast cancers. Human Pathology. 2011; 42: 1589-1595.
31. AR Venkitaraman. Cancer susceptibility and the functions of BRCA1 and BRCA2. Cell. 2002; 108: 171-182.