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Improvement of hybridization signals of gold label silver stain gene detection

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Abstract: The factors that influence the colorimetric gene detection of gold label silver stain and improve the detection signals are studied. The influence of amino DNA probes and thiol DNA modified gold nanoparticles is investigated based on a sandwich hybridization system. An increase in amino probe concentration brings about an increase in hybridization signal which reaches a threshold corresponding to the saturated concentration of amino probes bounded onto a glass slide surface. Since the steric hindrance effect of nanoparticles is dominant over the influence of a surface area, the bigger gold nanoparticles lead to weaker hybridization signals. The hybridization efficiency enhances significantly with the increase of the thiol DNA modified nanoparticle concentrations. Experimental results show that 125 μmol/L of the amino DNA probe concentration, 15 nm of the gold nanoparticle diameter, and 4.07 nmol/L of the thiol DNA modified gold nanoparticle concentration are optimal for the detection system. The hybridization signals can be improved remarkably by choosing optimal hybridization conditions.

Key words: gold label silver stain; gold nanoparticle; hybridization signal

Sequence-specific gene detection is of significant interest as a method of diagnosis of genetic and pathogenic disease. Colorimetric gene detection has been studied systematically with the advantages of non-radioactivity, non-quenching effect of fluorescence and simple analytical equipment[1-3]. The reported systems involve DNA linked nanoparticle network[2], DNA-mediated supramolecular mono- and multilayered nanoparticle structures[4], size-selective scattering of nanoparticle probes[5], and silver amplified detection with nanoparticle probes[6]. These researches principally focus on the selectivity and sensitivity of the method, and pay little attention to the hybridization conditions.

It has been reported that the gold label silver stain detection of a DNA array is of a selectivity over three times greater and a sensitivity in excess of two orders of magnitude of those observed for analogous fluorophore system, respectively[6]. This detection system, therefore, is believed to possess great potential. However, the influence of the concentration of nanoparticle labeled DNA probe and the diameter of nanoparticle Au and the optimal concentration of amino probe are less well addressed. Can the detective signals be further improved through controlling the hybridization conditions? Here, the above parameters are studied to determine the optimal hybridization conditions and improve the hybridization signals.

1 Experiment

1.1 General methods

Colliod Au solutions (15, 30, 60 nm) were custom-ordered from Structure Probe, Inc. Amino- and thiol-capped oligonucleotides were purchased from Shanghai Shenyou Biotechnology Company, with the following sequences (based on the severe acute respiratory syndrome (SARS) sequence):

S1: 5'-ACG TGA CGA ATA GCT TCT-NH_2-3'

S2: 5'-AGA AGC TAT TCG TCA CGT TCG TGC GTG GAT TGG CTT-3'

S3: 5'-SH-AAG CCA ATC CAC GCA CGA-3'

A Beckman J-30I centrifuge was used for the centrifugation of Au nanoparticle solution. PS-I ICP/AES (Leeman) was used to determine the concentrations of atomic gold in the DNA-modified gold nanoparticle solution.

The hybridization procedure was performed in Ref. [7]. In brief, a solution of the complementary target (S2) in MicroHyb buffer was hybridized to amino probe (S1) array spotted on the slide for 4 h at

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26 ℃, washed with a 2 × SSC/0.1% SDS solution for 10 min, then with a 0.1 × SSC/0.1% SDS solution for 10 min. Next, a solution of oligonucleotide-functionalized gold nanoparticles was hybridized to the array for 4 h at 26 ℃, washed with 2 × SSC/0.1% SDS and 0.1 × SSC/0.1% SDS solutions, respectively, and then washed with 2 × PBN[0.3 mol/L $NaNO_3$ and 10 mmol/L Na_2HPO_4/NaH_2PO_4 buffer (pH 7)]. The hybridized slide was submerged in a silver enhancer solution (solutions of 0.5 g $AgNO_3$/2 mL H_2O, 1.7 g hydroquinone/30 mL H_2O and 2.55 g citric acid/2.35 g trisodium citrate/10 mL H_2O, mixed simultaneously) for 8 min at room temperature, and then washed with water. The greyscale values were taken from scanner images and analysis.

1.2 Concentration determination of DNA-modified gold nanoparticles

The coupling of the oligomers (S3) to the 15 nm, 30 nm and 60 nm gold colloids was carried out as described elsewhere[1]. According to the density and atomic weight of gold, the number of atoms per nanoparticle with a given diameter can be determined as follows:

$$N = \frac{\frac{4}{3}\pi R^3 \rho}{M} \times 6.02 \times 10^{23}$$

Assuming spherical particles, the numbers of atoms in 15-, 30- and 60-nm-diameter particles were estimated to be 1.042×10^5, 8.334×10^5 and $6.666\ 8 \times 10^6$, respectively. The total number of gold atoms in the DNA-modified gold nanoparticle solution was determined by atomic emission spectroscopy (AES). So, the molar concentration of nanoparticles was determined by comparing the number of gold atoms in a particle of known diameter to the total number of atoms in the solution[2].

2 Results and Discussions

2.1 Influence of amino probe concentration

To investigate the influence of amino probe (S1) concentration, hybridization was performed under varying concentrations of S1 (see Fig.1). The amino probe-functionalized glass surface was exposed to 100 nmol/L oligonucleotide target (S2) and then 15 nm nanoparticle labeled probe (S3) and immersed in silver amplification solution for 5 min. The silver-staining signals were scanned and analyzed by histogram averages in Adobe Photoshop. In general, the greyscale values increased gradually accompanying the increase of S1 concentration. No signal appeared while the concentrations of amino probe were less than 5 μmol/L. The signal increased significantly as the concentration increased from 10 μmol/L to 125 μmol/L, and reached a threshold while the probe concentration was above 125 μmol/L. This trend may be that the amino probes bounded onto the glass slide surface were saturated at this concentration.

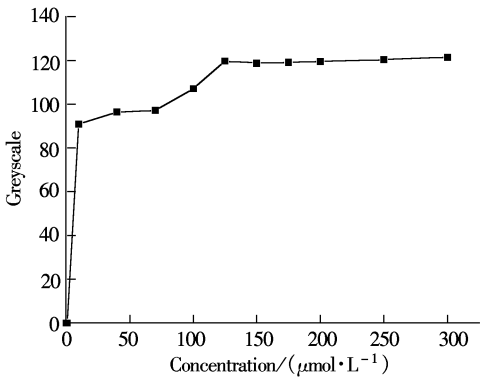

Fig.1 Greyscale values as a function of amino probe concentration of S1

2.2 Influence of gold nanoparticle diameter

The contents of Au in 15, 30 and 60 nm oligonucleotide-modified Au nanoparticle stock solutions were analyzed by AES. The concentrations of nanoparticles in the stock solutions were calculated by the aforementioned method. Then the stock solutions were all diluted to the same nanoparticle gold concentration (55.6 pmol/L) with hybridization buffer. The hybridization was taken under 125 μmol/L amino probe and 100 nmol/L target at 22 ℃. The silver staining signals are shown in Fig.2. The greyscale values obtained from histogram averages in Adobe Photoshop are 25.73 (60 nm), 42.47 (30 nm) and 56.47 (15 nm), respectively, which increased with the decrease of the nanoparticle diameters. There are two factors related to the nanoparticle diameter that influence the hybridization signals. One is the surface area of nanoparticle gold. Under the same hybridization efficiency, bigger nanoparticle diameters, i.e. bigger surface areas of nanoparticle, lead to stronger hybridization signals. The other is the steric hindrance effect of nanoparticles. On one side, the bigger nanoparticles cannot move as freely as the smaller nanoparticles in the course of hybridization. On the other side, the bigger nanoparticle modified DNA that has hybridized to the target DNA hinders more nanoparticle labeled probes to approach the target

DNA. Therefore, the steric hindrance effect results in lower hybridization efficiency and then weaker hybridization signals. In general, the influence of surface area and steric hindrance effect of gold nanoparticles is opposite. In our experiment, it may be explained that the steric hindrance effect of nanoparticles is dominant, which leads to lower hybridization efficiency resulting in weaker signal for the bigger nanoparticles.

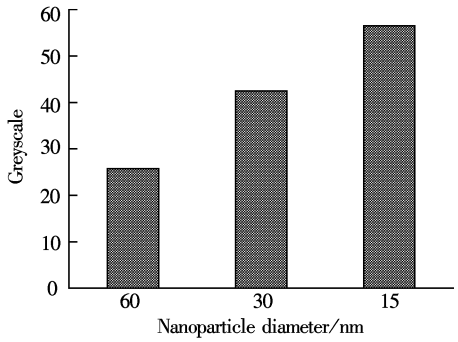

Fig.2 Hybridization signals of varying nanoparticle diameters with the same nanoparticle concentration

2.3 Influence of gold nanoparticle concentration

The 15 nm thiol modified nanoparticle gold stock solution was diluted to different concentrations. The hybridization was taken under 125 μmol/L amino probe, 100 nmol/L target DNA. Hybridization temperature was 22 ℃. The greyscale values are 192.25, 130.00 and 67.88, corresponding to 4.070 nmol/L, 2.350 nmol/L and 0.407 nmol/L nanoparticle concentrations, respectively (see Fig.3). The signals faded along with reducing the nanoparticle concentration. This attributed to the higher hybridization efficiency from higher concentrations of thiol DNA modified nanoparticle gold.

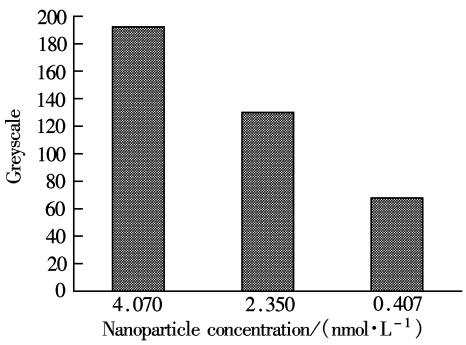

Fig.3 Hybridization signals of varying nanoparticle concentrations with the same nanoparticle diameter

3 Conclusion

The concentration of amino probe, the diameter and concentration of thoil DNA modified gold nanoparticles are studied based on colorimetric detection by a sandwich hybridization system. The hybridization signals increase with the increase of amino probe concentration. The increase reaches a threshold at a certain concentration because of the saturated bound of amino probe onto the glass slide surface. A bigger nanoparticle diameter leads to lower greyscale values resulting in the steric hindrance effect of nanoparticles being dominant over the influence of surface area. The hybridization efficiency enhances with the increase of the thiol DNA modified nanoparticle concentration. Experimental results show that 125 μmol/L of the amino DNA probe concentration, 15 nm of the gold nanoparticle diameter, 4.07 nmol/L of the thiol DNA modified gold nanoparticle concentration are optimal for the detection system. The hybridization signals of colorimetric gene detection can be improved significantly by choosing the optimal hybridization conditions.

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金标银染基因检测方法中杂交信号的提高

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摘要: 研究了金标银染基因检测方法中的影响因素以及提高 DNA 杂交信号的途径. 在“三明治”杂交系统中, 着重研究了氨基 DNA 探针和纳米金标记巯基 DNA 探针对杂交的影响. 随着氨基探针浓度的增加, 杂交信号增加, 探针达到一定浓度后信号不再增加, 形成一个平台, 说明连接于玻片表面的探针已达到饱和. 由于纳米金颗粒空间位阻效应的影响大于纳米颗粒表面积的影响, 标记的纳米金颗粒越大, 杂交信号越弱. 而随着金标巯基 DNA 探针浓度的增加, 杂交效率显著增加, 导致杂交信号提高. 优化后的杂交条件为: 氨基 DNA 探针浓度为 125 μmol/L, 纳米金粒径为 15 nm, DNA 标记后的纳米金颗粒浓度为 4.07 nmol/L. 通过上述杂交条件优化, 可提高杂交银染的信号.

关键词: 金标银染; 纳米金; 杂交信号

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