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An integrated and sensitive detection platform for biosensing application based on Fe@Au magnetic nanoparticles as bead array carries

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ABSTRACT

A sensitive and selective biosensor platform suited for SNP type using Fe@Au magnetic nanoparticles (GMNPs) to fabricate bead array is described. This new platform integrates the rapid binding kinetics of magnetic nanoparticles carriers, the multiplexing and encoding capabilities of chips, and tagged array. As a DNA sensor, the biotinylated single-stranded DNA was obtained by asymmetry PCR amplification, and then captured by GMNPs modified with streptavidin to form GMNP–ssDNA complexes without further purification. The complexes were immobilized on the slide to fabricate bead array through magnetic field. The bead array was hybridized with the corresponding allele-specific tag probes for each locus, and a pair of given universal detectors were applied to these markers analysis. Using bead array, all samples can be analyzed in one hybridization chamber which lowers the cost of the assay. Using universal tags, only a pair of universal dual-color probes labeled fluorophores was used for multiplex genotyping. Without the need of laborious and time-consuming elution, the experiment process was simple, reproducible and easy to handle. Two SNPs loci from 12 individual samples were discriminated using this platform and the results demonstrated that the expected scores and good discrimination were obtained between the two alleles from the two SNP loci. In summary, the integrated sensitive platform is adaptable and versatile, while offering a high-throughput capability needed for genome research and clinical applications.

1. Introduction

Over the past several years, research in single nucleotide polymorphisms (SNPs) detection has catalyzed important developments in the field of genomics (Sachidanandam et al., 2010; Pennisi, 1998). Clinical investigators have analyzed SNPs alleles in population-based studies to identify loci that are statistically associated with certain diseases and phenotypes (Gresham et al., 2006; Ohnishi et al., 2001). In these studies, the number of samples available for analysis is important for data reliability. To obtain a result with a high degree of confidence, investigators require a method for analyzing multiple SNP loci from thousands of samples. To fulfill this need, the development of a new platform that shortens assay times, simplifies protocols, reduces costs, and is capable of high-throughput, automated SNPs detection is warranted.

Recent advances in microarray and magnetic nanoparticles (MNPs) have allowed high-throughput DNA analysis in multiple samples (Smith et al., 2006; Fan et al., 2006). As one of the most promising tools for nucleic acid detection, microarrays produce a high-throughput "readout", which is especially suitable for high-throughput detection. To take advantages of microarrays' high parallel read-out and multiplexing sample preparation (Gharizadeh et al., 2003), and simultaneously avoid the complex procedure including purification and concentration, a suitable molecular carrier is required. Due to the unique dispersion properties in aqueous solutions and high separation efficiency in magnetic fields, MNPs have attracted a great deal of interest in various fields of advanced biological and medical applications, such as RNA and DNA purification, immunoassays, cell isolation et al. (Katz and Willner, 2004; Akutsu et al., 2004; Qiu et al., 2009; Ye et al., 2009). Currently, several MNPs-based genotyping methods have been reported (Kojima et al., 2005; Matsunaga et al., 2007; Li et al., 2006; Liu et al., 2007). In these methods, PCR products containing a SNP locus were immobilized onto the MNPs and examined by allele-specific hybridization. Because of their accuracy and easy operation, these methods can be used for high-throughput, automated SNP genotyping. However, to remove high fluorescent background a denaturation step must be performed before determining the fluorescence, which makes the assay procedure complex. Furthermore, different samples must be examined in separate tubes, increasing reagent use and subsequent genotyping costs.

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To fully exhibit the powerful capabilities of microarrays and MNPs, a new MNPs array based bioassay using gold-coated, magnetic nanoparticles (GMNPs) as a DNA carrier for high-throughput SNPs genotyping was studied. The biotinylated single-stranded DNA containing a SNP site were obtained directly by asymmetry PCR amplification. And then ssDNA were captured using streptavidin-coated, gold, magnetic nanoparticles (SA-GMNPs). After washing, the single strand DNA–GMNPs (ssDNA–GMNPs) complexes were used to fabricate the GMNPs array. A glass slide with a permanent magnet on the back was used as the substrate, and the ssDNA–GMNPs complexes were immobilized on the slide using a magnetic field. The genotype of each sample was determined by hybridizing the ssDNA on the GMNPs with allele-specific tag probes and universal detectors in a hybridization chamber. Using a GMNPs array-based method, very small amounts of fluorescent probes can be used for genotyping an unlimited number of samples, thereby reducing cost and increasing efficiency. In short, using the GMNPs as DNA carriers, the GMNPs array-based method with tagged array dual-color hybridization could be applied as an efficient and high-throughput tool for SNPs genotyping in a large number of individuals.

2. Materials and methods

2.1. Materials and reagents

3-Mercaptopropyltriethoxysilane (MPTS) and 11-mercaptoundecanoic acid (MUA) ($HOOCC_{11}SH$) were obtained from Aldrich–Sigma. EDC (1-Ethyl-3-(3-dimethyllaminopropyl) carbodiimide hydrochloride), MES (2-N-morpholino ethanesulfonic acid) and streptavidin were obtained from Shanghai Sangon Biologic Engineering Technology and Service Co. Ltd (Shanghai, China). Taq DNA polymerase (5 U/μL) was purchased from Shanghai Shenergy Biocolor Bioscience & Technology Company. Hybridization solution was purchased from Invitrogen Corporation. Hybridization chamber was obtained from Thermo Fisher Scientific Inc.

The methylenetetrahydrofolate reductase (MTHFR) gene C677T and A1298C polymorphisms (C677T rs1801133, A1298C rs1801131) from 12 different samples were assessed using this method. The sequences of all oligonucleotides including PCR primers, allele-specific tag probes and dual-color universal detectors used for this experiment were shown in Table 1 (see supplementary material). All oligonucleotides used in this research were synthesized by Shanghai Sangon Biologic Engineering Technology and Service Co. Ltd (Shanghai, China). Tag sequences were selected from a randomly generated sequence then edited to remove palindromes and hairpins of four or more bases. All tags were cross-compared against each other to avoid cross-homology.

2.2. Preparation of streptavidin-immobilized Fe@Au magnetic nanoparticles (SA-GMNPs)

γ-Fe_2O_3 magnetic nanoparticles (MNPs) were prepared as described by Katz and Willner (2004). Thiol-modified MNPs were obtained after modification with 3-mercaptopropyltriethoxysilane (MPTS). A layer of colloid gold was formed around MNPs using Au–S bond on the surface of the functionalized MNPs by reduction of Au^{3+} to form GMNPs, which allowed the MNPs more suitable for "bead array" carries in SNP types. The GMNPs were functionalized with carboxylic acid groups by reacting 20 mg of GMNPs with 200 mM 11-mercaptoundecanoic acid (MUA) in ethanol at room temperature. Then the GMNPs were activated by EDC for 30 min at 4 °C with slow tilt rotation. After removing the supernatant magnetically, the activated GMNPs were washed once with 500 μL cold and de-ionized water and once with 500 μL 25 mM MES (pH 6) as quickly as possible to avoid hydrolysis of the activated carboxylic acid groups. Then the streptavidin was immobilized on the GMNPs covalently by the linkage between the amine groups of streptavidin and the carboxylic acid groups on the GMNPs. The streptavidin-coated GMNPs (SA-GMNPs) were finally dispersed in PBS buffer with the concentration of 4 mg/mL and stored at 4 °C.

2.3. Fabrication of bead array

Biotinylated single-stranded PCR products were captured on the GMNPs by directly placing 100 μg of SA-GMNPs in the PCR mixture and incubating at room temperature for 15 min. After magnetic separation, the ssDNA–GMNPs complexes were washed with ddH_2O buffer twice, then the complexes were diluted in 1× PBS buffer to 20 mg/mL for GMNPs array fabrication. In brief, a clean stand standard glass slides (75 mm × 25 mm × 1 mm) were used as the substrate for the GMNPs array and a neodymium–boron (Nd–B) magnet was placed on the backside to provide magnetic field for GMNPs immobilization. The ssDNA–GMNPs complexes were resuspended before printing, by using uncontact microarray printer, 0.1 μL complexes of each sample was deposited onto the slide forming spots of approximately 600 μm in diameter with a 600 μm distance to adjacent spots. After printing, the GMNP array was framed with a 65 μL hybridization chamber, and air dried.

2.4. Immobility assay of bead array

In order to detect the immobility of bead array on the surface of slides, SA-GMNPs combined with biotinylated fluorescent probes were used to fabricate bead array. 0.5 μL 10 μM biotinylated fluorescent probes (5′-Biotin-TGAAGGAGAAGGTGTCTGCGGGA-Cy3, 5′-Biotin-TGAAGGAGAAGGTGTCTGCGGGA-Cy5) were added in 100 μg SA-GMNPs, reacted for 15 min at room temperature, respectively. After washing twice using ddH_2O, the GMNPs–DNA mixture was resuspended in 5 μL 3× SSC (saline-sodium citrate, 45 mM sodium chloride and 0.45 M sodium chloride, pH 7.0), about 0.05 μL GMNPs–DNA was spotted onto the slide conjoined with a permanent magnet on the backside, the bead array was snap-dried for two seconds on the hot plate (100 °C). The GMNPs arrays detached from the magnet were scanned to determine the fluorescent intensities of the bead array. After scanning, the back of the slides were conjoined with magnet again, and the bead array was washed with 3× SSC and ddH_2O twice, each solution for 3 min. Then the bead array was scanned again to determine whether their fluorescent intensities were decreased or not.

2.5. Sensitivity assay of bead array

To test the sensitivity of the GMNPs array, we prepared gradient GMNPs arrays using purified PCR products. The ssDNA–GMNPs complexes printed on the slides were prepared by mixing 25 μL wild type or mutant single-stranded PCR (ss-PCR) products of different concentrations (varying from 0.1 to 100 nM) with 50 μg SA-GMNPs. The ssDNA–GMNPs complexes were printed onto slide to fabricate bead array and hybridized with allele-specific tag probes and universal detectors. After hybridization, the bead array with fluorophore was scanned to determine the sensitivity of bead array.

2.6. Asymmetry PCR amplification

Single-stranded DNA containing SNP sites were amplified directly using asymmetry PCR amplification. 30 μL mixture containing 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 2.0 mM $MgCl_2$, 200 μM each of the dNTP, 100 ng template DNA, 1.25 U of Taq

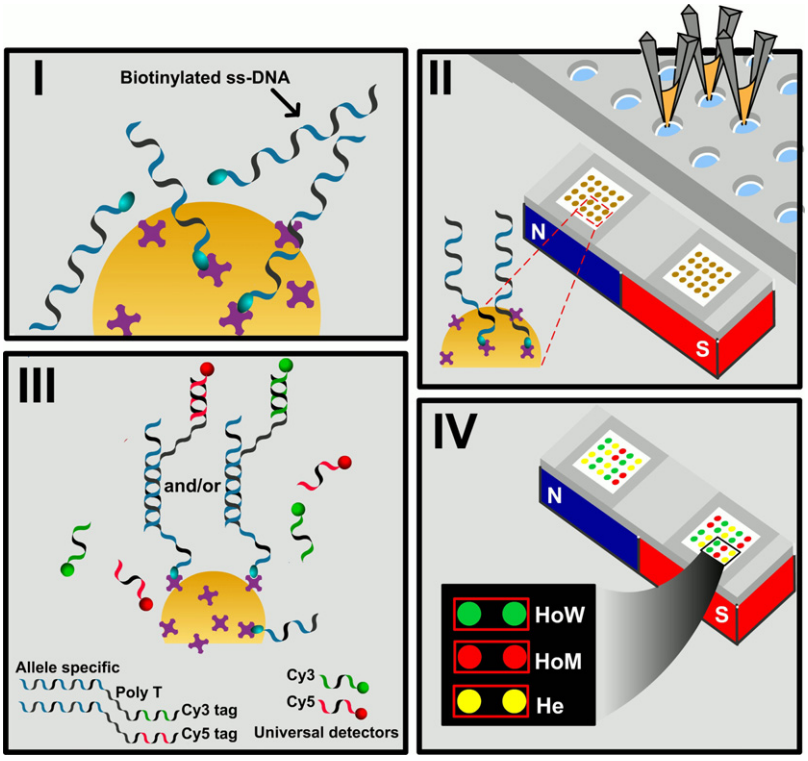

Fig. 1. Schematic presentation of the biosensor platform for gene/protein detection based on Fe@Au magnetic nanoparticles.

DNA polymerase (TaKaRa), 1 μL forward primer (10 μmol/L) and biotinylated reverse primer (40 μmol/L) in MJ PTC-220 Cycler (Bio-Rad Laboratories). The fragment containing C677T locus was amplified with the profile consisting of an initial melting step of 2 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 62 °C, and 30 s at 72 °C; and a final elongation step of 7 min at 94 °C. The fragments containing A1298 locus was amplified with the profile consisting of an initial melting step of 2 min at 94 °C followed by 35 cycles of 1 min at 94 °C, 1 min at 62 °C, and 30 s at 72 °C; and a final elongation step of 7 min at 94 °C.

2.7. Hybridization

In hybridization-based genotyping method, hybridization temperature is an important factor for accurate discrimination. Based upon the T_m values of the allele-specific detection probes, two pairs of mutant and wild simulated biotinylated target oligonucleotides containing SNPs loci (as shown in Table 1 in supplementary material) were designed instead of the biotinylated ss-PCR products to hybridize with the allele-specific tag probes and dual-color universal detectors at different temperature, respectively. 80 μg SA-GMNPs contained 15 pmol wild or mutant simulated target oligonucleotides were hybridized with the allele-specific tag probes and dual-color universal detector at various temperatures. The target hybridization mixture (20 μL) contained 6 μL of hybridization solution, 20 pmol of allele-specific tag probes and dual-color universal detectors. Then the hybridization was conducted in MJ PTC-220 Cycler for 1 h. After hybridization, hybrid-GMNPs complexes were washed with 2× SSC containing 1 g L^{-1} SDS and 0.1× SSC containing 1 g L^{-1} SDS subsequently, where each wash takes 5 min for each wash, followed by a second wash in 3× SSC, and then resuspended in 5 μL 3× SSC buffer. The dsDNA–MNPs mixtures were printed directly onto a clean glass slide to fabricate a microarray. After printing, the microarray was dried at room temperature, and the magnet was peeled off from the backside of the slide. The microarray was scanned with a 4100 A Microarray Analysis System (Axon), which was fitted with filters for Cy3 and Cy5. The images acquired by the scanner were analyzed with software Genepix Pro 6.0. For each fluorescent image, the average pixel intensity within each circle was determined and a local background using mean pixel intensity was computed for each spot. The net signal was determined by subtraction of this local background from the mean average intensity for each spot.

After determining the optimal temperature for the hybridization process, the bead array fabricated above was hybridized with allele-specific tag probes and dual-color universal detector in the hybridization chamber and sealed with the cover slide as shown in Fig. 1. The 65 μL hybridization mixture contained 22 μL of hybridization solution, and 20 pmol allele-specific tag probes and dual-color universal detectors, respectively. After incubating at their optimal hybridization temperature, the hybrid-GMNPs array was washed with 2× SSC containing 1 g L^{-1} SDS, 0.1× SSC containing 1 g L^{-1} SDS, and de-ionized water, respectively. Finally, the microarrays were dried at room temperature and scanned to determine the genotype of different samples.

2.8. DNA detection

In principle, the homozygous wild type samples completely matched with wild tag probe, and then hybridized with the Cy3 labeled universal detector, yielded strongly fluorescent Cy3 spots (green fluorescence). The homozygous mutant type samples completely matched with mutant tag probe, and then hybridized with the Cy5 labeled universal detector, and strongly fluorescent Cy5 spots (red fluorescence) were shown. The heterozygote type samples can simultaneously matched with wild and mutant tag probes, and then hybridized with both of the dual-color universal detectors, yielded both Cy3 and Cy5 spots and, therefore, after overlapping, presented the strong "yellow" fluorescence. In this study, we applied this method to analyze C677T and A1298C SNPs of the MTHFR gene from 12 samples. The obtained fluorescence signals from all representative samples were quantified, allelic-fractions

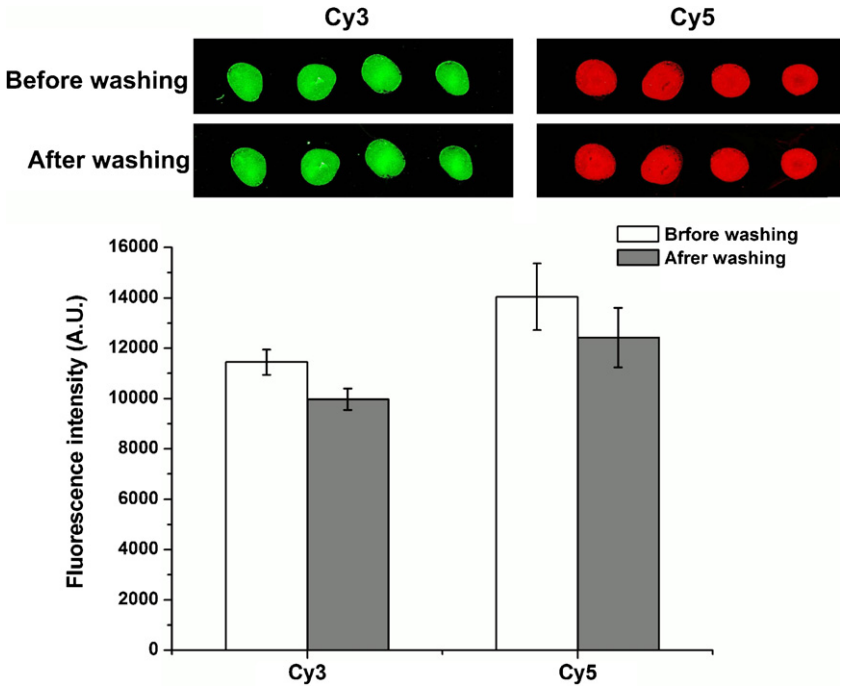

Fig. 2. Immobility assay of magnetic bead array. (A) The fluorescence images before and after washing. (B) Relative fluorescence intensities before and after washing.

were calculated as fluorescence intensity Cy3/(Cy3 + Cy5) to represent the hybridization signals from matched and mismatched probes. Using this formula, we determined that heterozygous samples have allelic-fractions between 0.35 and 0.65, homozygous wild type and mutants have allelic-fractions >0.8 and <0.2, respectively. If samples fell outside of these boundaries, they were classified as ambiguous.

3. Results and discussion

3.1. Development of the method

There is an imperative need for cost-effective, high-throughput and extremely accurate genotyping technologies for adequate monitoring of whole-genome association studies of complex diseases. Many of today's available genotyping technologies fulfilled one or two of these genotyping criteria, but at the expense of other factor(s). In this work, we present a technology that does not compromise on accuracy, low-price and high-throughput which is performed in a bead array format. Our objective was to develop a cheap robust method for screening thousands of genomic DNAs in parallel for multiplex molecular markers. Fig. 1 showed all procedures for automated SNP detection based on bead array. The approach chosen was based upon a tagged array method for scoring SNP markers, involving direct binding of unpurified biotinylated ss-PCR products onto SA-GMNPs, printing the ssDNA–GMNPs complexes onto a clean slide to fabricate bead array, followed by hybridization with the allele-specific tag probes and dual-color universal detectors at an optimal temperature, and finally scanning bead array directly to determine their types. All samples can be analyzed in one hybridization chamber which lowers the cost of the assay. Direct scanning bead array with fluorescent detectors can produced visually discernible scores, and the fluorescence signal intensities were high enough for reliable automated scoring.

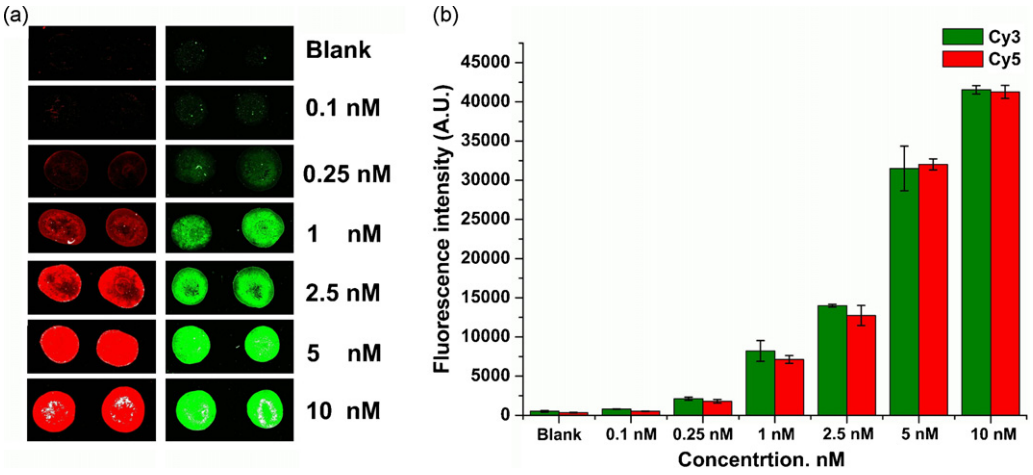

Fig. 3. Sensitivity assay of bead array. (a) The fluorescence images of two-fold GMNPs array for mutant and wild type PCR products, the range of PCR product concentrations were between 0 (control) and 10 nM. (b) The column plot is relative fluorescence intensities for the samples with different concentrations.

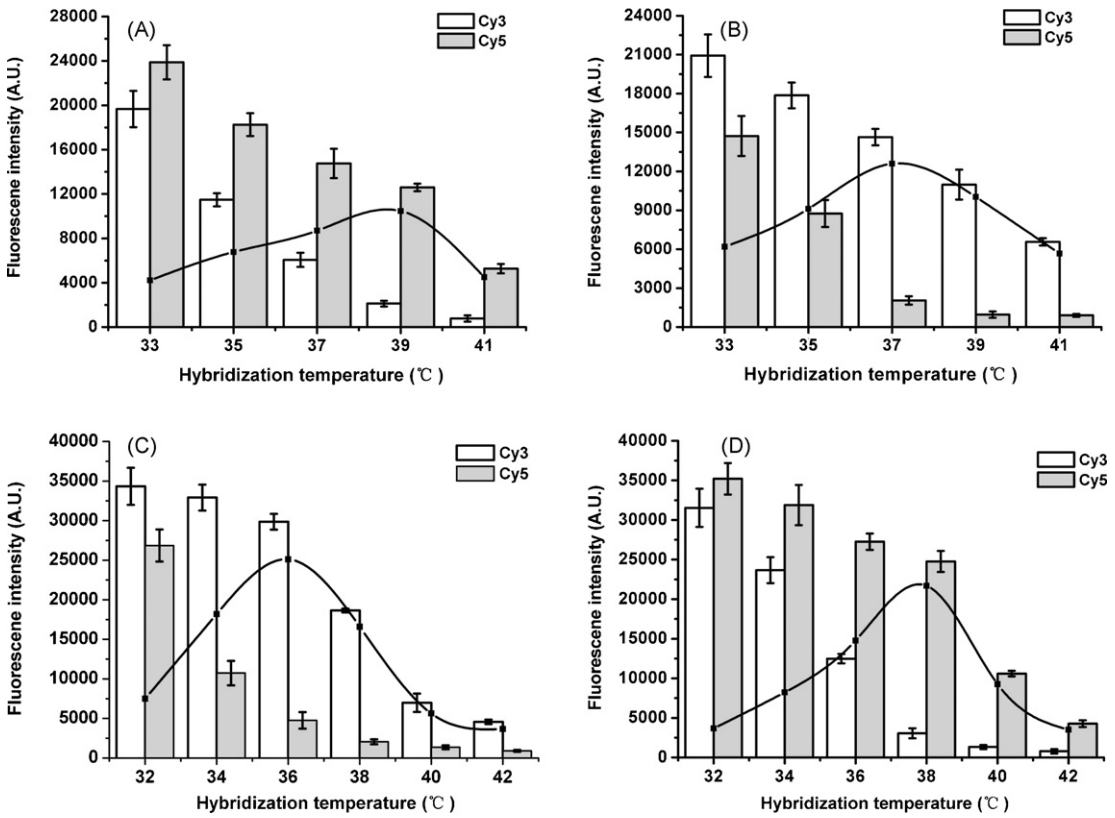

Fig. 4. Effect of hybridization temperature on SNP detection for C677T (A, B), A1298C (C, D), SNP loci. (A, C) Hybridization results of wild simulated targets hybridized with allele-specific tag probes and dual-color universal detectors at various temperatures at two loci. (B, D) Hybridization results of mutant simulated targets hybridized with allele-specific tag probes and dual-color universal detectors at various temperatures at two loci. The signal ratios (match:mismatch) of fluorescence intensities at each temperature were labeled as curve above the columns.

3.2. Immobility assay of bead array

Magnetic beads can be immobilized firmly on the surface of slide through magnetic field, and would not be dropped off during the hybridization and wash processes, this is the key to fabricating bead array. In our experiments, the SA-GMNPs combined with biotinylated fluorescent probes were spotted on the slides, and the fluctuations in the fluorescent intensities of the bead array before and after the wash process, provided us an estimation of the bead array's immobility. Fig. 2A shows the microarray scanning results before and after wash. The fluorescent intensities after wash were decreased a little bit, and the amplitude variation was within 8%, as shown in Fig. 2B. There was no significant difference between the fluorescent intensities before wash and after wash ($t = 10.630$, df = 1, $p = 0.06 > 0.05$). The results indicated that the magnetic beads can be immobilized on the slide surface firmly through magnetic field, and it's feasible for DNA detection using bead array.

3.3. Sensitivity assay of bead array

Fig. 3a shows the fluorescence images of the GMNPs array at various PCR product concentrations, and the blank SA-GMNPs are used for comparison. Satisfactory reproducibility was obtained. Using the formula, homozygous wild type and mutants have allelic-fractions >0.8 and <0.2, the achieved detection limit was 0.1 nM with a saturation concentration of 10 nM. Fig. 3b shows that the wild type and mutant targets give equal fluorescent intensities at the same concentration, while the blank GMNPs produces a very low fluorescent background demonstrating the excellent use of the GMNP array for SNPs detection.

3.4. Hybridization

In our study, biotinylated wild and mutant simulated targets of two loci were used to optimize the hybridization temperature. Fig. 4A and B shows the optimal hybridization temperature obtained for C677T locus. Fluorescent intensities of the denatured probes after hybridization using wild and mutant simulated target oligonucleotides were detected, respectively. The highest signal ratio (match:mismatch) of wild and mutant simulated targets could be obtained when hybridization temperature was performed at 39 °C, 37 °C, respectively. The reason for the wild simulated target's optimum temperature being higher than the mutant one was because the melting temperature of 677CC probe is higher than 677TT probe. Based on the above hybridization results, the further assays for hybridization of SNP genotyping were performed at the optimal temperature of 38 °C. In the same way, the optimal hybridization temperature of A1298C were 37 °C as shown in Fig. 4C and D.

3.5. DNA detection

In this study, the correct identification of three genotypes, C677T, A1298C genes, from 12 samples was demonstrated. The assay achieved the expected scores and showed good discrimination between the two alleles for the two loci. The entire array was spotted in three replicates, and the fluorescence pattern was highly reproducible between replicates. For C677T polymorphism, the fluorescent signals were shown in Fig. 5A, and the three genotypes gave clearly different profiles. The fluorescence signals of these samples were quantified and the clear ratio differences were illustrated between the three clusters (Fig. 5B). This allowed

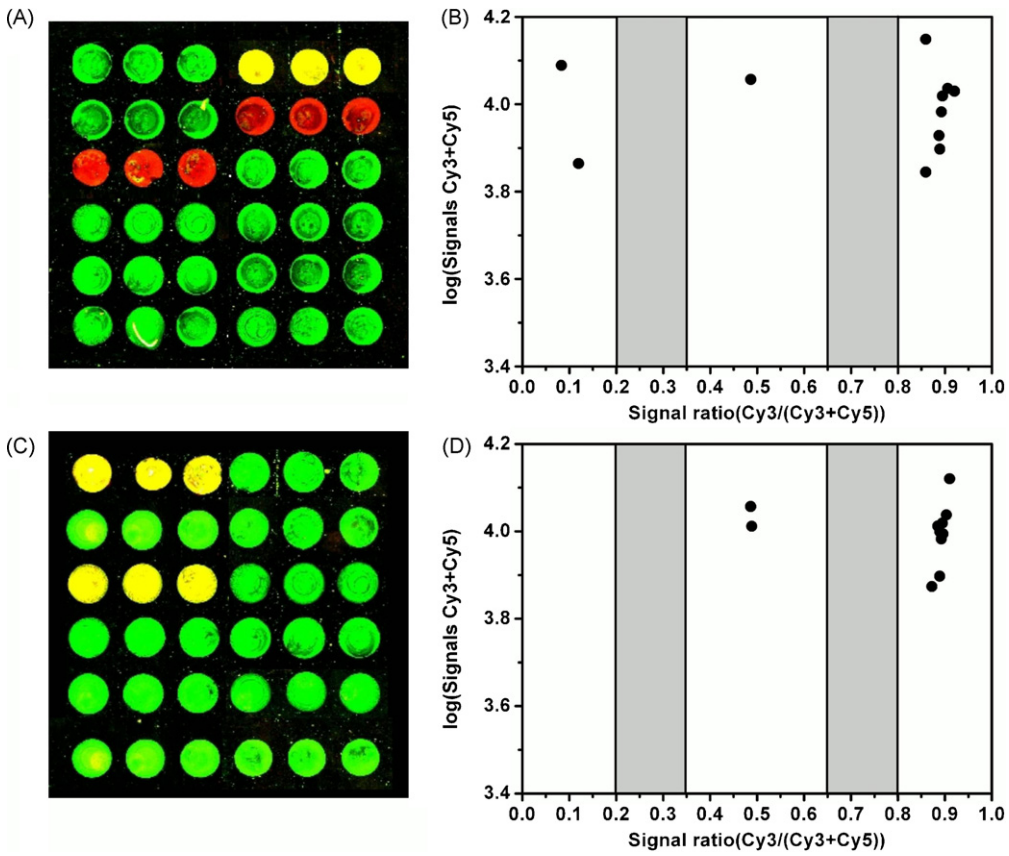

Fig. 5. Results of SNP detection of 12 samples based on SA-GMNPs assayed for two SNP loci including C677T, A1298C, respectively. (a) The fluorescence images of microarray assayed for the two loci. Each sample was spotted three replicates in a column. (b) All spots were measured for the Cy3 and Cy5 fluorescence intensities to generate a scatter plot.

unequivocal genotype assignment for the three loci. From left to right in Fig. 5B, we observed that the allelic-fractions 0.08 and 0.12 for the mutant samples, and from 0.85 to 0.91 for wild type samples. Allelic-fraction values for homozygous wild (HoW) and mutant (HoM) samples should be as close as possible to 1.0 and 0, respectively. Thus, the nearer testing allelic-fractions value close to the expected value, the higher match/mismatch ratio. The allelic-fractions value of 0.48 was classified as a heterozygote (He) sample.

The same samples for A1298C locus gave clearly different profiles as shown in Fig. 5C, and D showed the cluster analysis obtained when 12 individual samples were genotyped. Strong spots (green) yielding Cy3 signals when the genotype factor G ranged from 0.87 to 0.92 indicated they were 1298AA homozygous wild. The spots (yellow) yielding both Cy3 and Cy5 signals when the G value was 0.48 and 0.49, these indicated that they were 1298AC heterozygote.

4. Conclusion

In this study, a novel strategy for high-throughput and low cost SNPs genotyping by using magnetic nanoparticles array and dual-color universal tag hybridization has been reported. Previously, we have reported a SNP detection method based on magnetic nanoparticles. This conformation significantly improved the efficiency of the genotype identification through immobilization of PCR products directly onto the magnetic nanoparticles without purification and concentration. To avoid the high fluorescent background of GMNPs, the fluorescence intensity analysis was obtained by denaturing matched fluorescence probes hybridized with the targets. However, a different pair of fluorescent probes was still necessary when analysis each SNP locus, so the cost will be very high when dealing with thousands of DNA samples for multiplex SNP loci. To avoid this problem, allele-specific tag probes were adopted, with a part as allele-specific probe hybridizing with ssDNA–GMNPs and the other part as universal tags hybridizing with universal fluorescent detectors, respectively. Simultaneously, different samples had to be detected in separate tubes, which caused excessive use of fluorescent probes, and increased the genotyping cost, especially when dealing with large amounts of samples. In this study, we address the application of SA-GMNPs to specifically detect SNPs based on GMNPs array. The main advantage of the novel SA-GMNPs array used in SNP detection lies in two facts: (1) the reaction system can be easily controlled by using an external magnetic field which allows it to be easily automated, and (2) all samples can be analyzed in one hybridization chamber which lowers the cost of the assay. In hybridization-based genotyping methods, the major cost arises from the expensive fluorescent probes. Using a GMNPs array-based method, very small amounts of fluorescent probes can be used for genotyping an unlimited number of samples, thereby saving time, and increasing efficiency. Overall, using the GMNPs as DNA carriers, the GMNPs array-based method with tagged array dual-color hybridization could be applied as an efficient and high-throughput tool for SNPs genotyping in a large number of individuals. Simultaneously, the sensitive and selective biosensor platform also can be used in immunoassay.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2010.07.078.

References

Akutsu, J., Tojo, Y., Segawa, O., Obata, K., Okochi, M., Tajima, H., Yohda, M., 2004. Biotechnol. Bioeng. 86, 667–671.
Fan, J.B., Chee, M.S., Gunderson, K.L., 2006. Nat. Rev. Genet. 7, 632–644.
Gresham, D., Ruderfer, D.M., Pratt, S.C., Schacherer, J., Dunham, M.J., Botstein, D., Kruglyak, L., 2006. Science 311, 1932–1936.
Gharizadeh, B., Kaller, M., Nyren, P., Andersson, A., Uhlen, M., Lundeberg, J., Ahmadian, A., 2003. Nucleic Acids Res. 31, e146.
Katz, E., Willner, I., 2004. Angew. Chem. Int. Ed. 43, 6042–6108.
Kojima, T., Takei, Y., Ohtsuka, M., Kawarasaki, Y., Yamane, T., Nakano, H., 2005. Nucleic Acids Res. 33, e150.
Li, S., Liu, H.N., Wang, Z.F., Hou, P., Guo, Y.F., He, Q.G., He, N.Y., 2006. Anal. Biochem. 359, 277–279.
Liu, H.N., Li, S., Wang, Z.F., Ji, M.J., Nie, L.B., He, N.Y., 2007. J. Biotechnol. 131, 217–222.
Matsunaga, T., Maruyama, K., Takeyama, H., Katoh, T., 2007. Biosens. Bioelectron. 22, 2315–2321.
Ohnishi, J., Yamamoto, S., Tsuchiya, N., Hatta, Y., Komata, T., Tokunaga, K., 2001. Ann. Hum. Genet. 65, 197–206.
Pennisi, E., 1998. Science 281, 1787–1789.
Qiu, J.D., Xiong, M., Liang, R.P., Peng, H.P., Liu, F., 2009. Biosens. Bioelectron. 24, 2649–2653.
Sachidanandam, R., Weissman, D., Schmidt, S.C., Kakol, J.M., Stein, L.D., Marth, G., Sherry, S., Mullikin, J.C., Mortimore, B.J., Willey, D.L., 2010. Nature 409, 928–933.
Smith, J.E., Wang, L., Tan, W., 2006. Trends Anal. Chem. 25, 848–855.
Ye, M., Zhang, Y.Y., Li, H.T., Zhang, Y.Q., Tan, P., Tang, H., Yao, S.Z., 2009. Biosens. Bioelectron. 24, 2339–2345.