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Combinational synthesis of oligonucleotides and assembly fabrication of oligonucleotide array

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Abstract

In this paper, a simple, reliable and flexible method, which integrated in situ synthesis with the spotting technique, was reported to fabricate oligonucleotide array. Different oligonucleotide sequences are synthesized on their relative code glass slides through combinational chemistry, thus the slides are broken into smaller pieces, in which the same code pieces have the same probe sequences. An oligonucleotide array is fabricated by arbitrarily assembling these different code pieces onto another solid substrate. In principle experimentation, four different sequences of P16 gene were synthesized and a 5×5 array including these four sequences and the control black was fabricated. The analysis results indicated that the hybridization fluorescence intensity of the same sequences locating different sets on the array gave the approximate values, and the fluorescence intensity ratio of matched sequence to one middle location base mismatched, two base mismatched, three middle base mismatched is $(1.000 \pm 0.080):(0.4991 \pm 0.0671):(0.2360 \pm 0.0044):(0.0493 \pm 0.0033)$. Their relative accuracies were from 6.64 to 10.2%. This result might be used to rapidly screen single-nucleotide polymorphisms (SNPs).

Keywords: Oliogonucleotide array; Combinational synthesis; Assembly fabrication

1. Introduction

DNA microarray anchors tens of thousands of closely arrayed oligonucleotide probes on the surface of solid substrates, on which the process of molecular recognition of DNA can be highly parallel. It has great applications in environmental genomic and pharmacogentic researches. At present, the DNA microarray manufacturing technologies fall into two main categories: on-chip or off-chip synthesis of oligonucleotide probes. The former ones such as photolithography directly in situ synthesize oligonucleotide probes on the chip surface [1,2] and the latter such as mechanical microspotting spot oligonucleotide probes synthesized in advance on the chip surface. One disadvantage of the former approach is the need for photomasks, which are expensive and time-consuming to design and build. While the latter method must synthesize, purify, and store each oligonucleotide probes that are extremely expensive due to the necessary modification of the probes with the attachment of some chemically active group such as the amino group. Therefore, reduction of the cost will be one of the key factors in promoting its public applications of oligonucleotide macroarray technology.

In this paper, a simple, reliable and flexible method for fabricating DNA array was described. Firstly, oligonucleotide sequences were in situ synthesized on the different coded substrate surfaces using combinational chemistry, and the slides were broken into small pieces. Secondly, oligonucleotide arrays could be fabricated by assembly of those different pieces with different oligonucleotide sequences onto another substrate. By integrating in situ synthesis with the spotting technique, this method can obtain many different probes, which has high hybridization efficiency than that of off-chip, and the probe do not need another active group to immobilize the substrate in constructing oligonucleotide arrays. Moreover, the fabrication method of oligonucleotide arrays does not need any expensive models and especially synthesis reagents.

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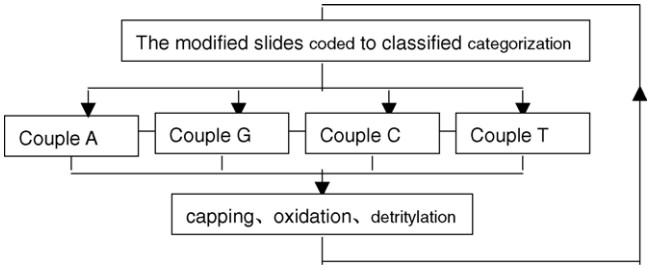

Fig. 1. Schematic illustration of oligonucleotide combinational synthesis.

2. Experimental

2.1. Principle of combinational synthesis of oligonucleotides and assembly fabrication of their array

The solid synthesis of oligonucleotide accords with the standard phosphoramidites chemistry protocol and the combinational synthesis of oligonucleotides are illustrated in Fig. 1. The modification substrates are coded and then classified into four categorizations. Substrates of the first one are immersed into the mixed acetonitrile solution with tetrazole (as catalyst) and dAdp nucleotide (whose 5′-OH has been protected by dimethoxytrity (DMT)). The activation groups of substrate surfaces are coupled with activated phosphoramidite. And then the substrate surfaces of the first categorization are lengthened by a base A. To follow suit, substrates of the second, third, and fourth one can be lengthened by base G, base C and base T only by means of substituting dGdp, dCdp and dTdp for dAdp, respectively. After the couplings are completed, all four categorizations substrates are merged and then placed in a sealed reactor to conduct successively the capping, oxidation and detritylation. According to the above-described method, the merged substrates are reclassified into the other four categorizations in order to couple the second base on these substrate surfaces and then the capping, oxidation and detritylation are conducted. Thus, different oligonucleotide sequences immobilized on the different coded substrates are achieved through above-described classification and reactions, respectively.

Assembly fabrication of oligonucleotide array is illustrated in Fig. 2. Oligonucleotides synthesized on the coded substrates are broken into smaller pieces. Assembling and fixing of these elements on another solid substrate to form an oligonucleotide array is shown in Fig. 2.

2.2. Materials and methods

Oligonucleotide synthesis reagents and solvents were purchased from PE Biosystems. Fluorescence tag sequence was purchased from Shanghai Biological Produce Corporation; fluorescence group is Cy3.

2.2.1. Oligonucleotide synthesis

Oligonucleotide automation synthesis was carried out in Model 391 DNA synthesizer (Applied Biosystems). Oligonucleotide automation synthesis on the modified slide was conducted as following: the polydimethylsiloxane film was chiseled a predesigned groove and placed onto the modified slide, and then the synthetic region on the slide was joined with the groove. Teflon plates connecting the in- and out-plastic tube was covered onto the polydimethylsiloxane film and the bases of in- and out-plastic tube were fixed at the superior and the lower of the groove, respectively. The sandwich was fixed with iron clamps and then the slide could be used for oligonucleotide synthesis. The coupling of manual synthesis was carried out in the glove box (MECAPLEX, Switzerland). The concentrations (by volume) of water and oxygen in the glove box were below 0.0005 and 0.0015%, respectively. Teflon plate and polydimethylsiloxane film were regarded as a whole material and then was chiseled four predesigned grooves, then its film surface was placed onto the modified slide and to form four wells. The coupling reagents were directly added into these four wells and coupled with active groups on the modified glass slide, which located in four wells, and then the capping, oxidation and detritylation

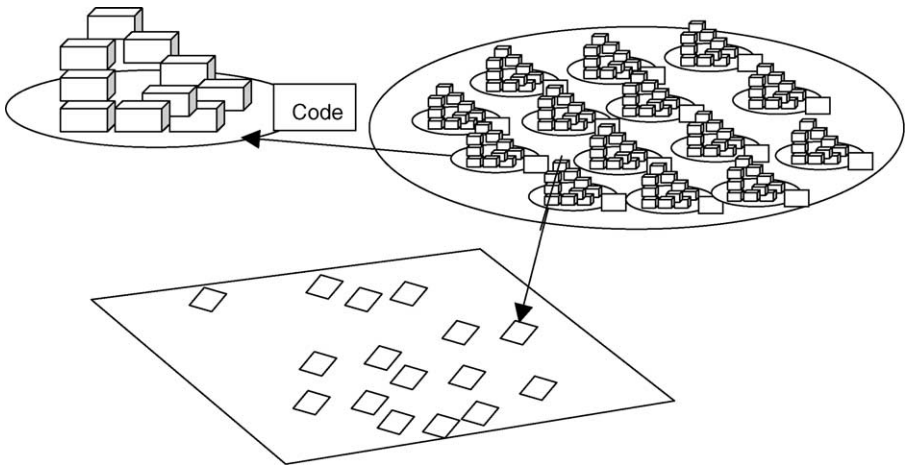

Fig. 2. Schematic illustration of assembly fabrication oligonucleotide array.

were conducted successively in those four wells. The synthetic sequence was 5′-AAC CAC CAA ACA CAC-3′ (P_0), 5′-AAC CAC CGA ACA CAC-3′ (P_1), 5′-AAC CGC CAA ACG CAC-3′ (P_2), and 5′-AAC CGC CGA ACG CAC-3′ (P_3). After the synthesis completion, the slide was shaken in a mixed solution of ethanol and ethanol amine (vol/vol = 1) in a sealed box at 75 °C for 2 h for deprotection. The slide were hybridized in 200 nmol/L Cy3-GTG TGT TTG GTG GTT-3′ solution at 46 °C for 1.5 h in the hybridization chamber, and then rinsed with 0.1% sodium dodecyl sulfate (SDS) in 2× SSC (sodium chloride/sodium citrate buffer), 0.1% SDS in 0.1× SSC, respectively. Scanner imaged the fluorescence signal and its fluorescence intensity was analyzed with software in microarray analysis system (Packard Biochip Technologies).

2.2.2. Combinational synthesis of oligonucleotides and assembly fabrication of their array

Oligonucleotide sequences were synthesized according to Section 2.1 and four probes of P16 were synthesized [3]. Then the glass slides having sequences were cut into smaller substrates manually so that their areas were about 10 mm^2 (3.2 mm × 3.1 mm). The smaller substrates were split, joined and fixed on a solid substrate to form an oligonucleotide array. In this paper, four probes of P16 and the control formed an oligonucleotide array according to special designing. Oligonucleotide array were hybridized in 200 nmol/L Cy3-GTG TGT TTG GTG GTT-3′ solution at 46 °C for 1.5 h in the hybridization chamber, and then rinsed with 0.1% SDS in 2× SSC at 46 °C, and 0.1% SDS in 0.1× SSC, respectively. Scanner imaged the fluorescence signal and its fluorescence intensity was analyzed with software in microarray analysis system (the scan plain was the confocal plain which had the maxiture signal).

3. Results and discussion

3.1. Comparison of oligonucleotide arrays fabricated by different methods

Fig. 3(a)–(c) shows the correspondent hybridization results of the oligonucleotide arrays from the different methods with Cy3-GTG TGT TTG GTG GTT-3′. The array of Fig. 3(a) was manual in situ synthesized, and (b) was automatic, but (c) was off-slide. These hybridization results were

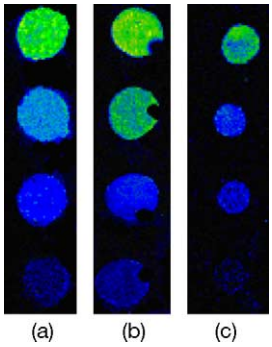

Fig. 3. Hybridization fluorescence images of oligonucleotide array fabricated by different methods: (a) automatic in situ synthesis; (b) manual in situ synthesis; (c) off-chip synthesis.

all satisfactory and clearly distinguish different probes by fluorescence intensities (Table 1). Comparing their results, one could find that the fluorescence intensities of the same sequences gave approximately equal values in Fig. 3(a) and (b), indicating that under the conditions described here both manual in situ synthesis and automatic could give rise to high coupling efficiency. However, the fluorescence intensity in Fig. 3(c) was much weaker than those of the relative sequences in Fig. 3(a) or (b), which was in accordance with the nature of different fabrication methods. In Fig. 3(c), probes were immobilized on the glass slide via common chemistry and the immobilization efficiency was not perfect. Whereas, bases of oligonucleotides in Fig. 3(a) or (b) were added by the standard phosphoramidites chemistry protocol and had the high coupling efficiency (generally 99%) without additional probe immobilization and the fragment of the synthesized probes were short, the accuracy of the purposed sequence was definitive. So, amounts of probe synthesized in situ were greater than that of off-chip.

3.2. Oligonucleotide sequences combinatorial synthesis and assembly fabrication of oligonucleotide array

Oligonucleotides are synthesized only from four different nucleotides (A, G, C, T), and, therefore, a lot of different oligonucleotide sequences can be synthesized by combinatorial chemistry at the same time. The solid synthesis of the standard phosphoramidites chemistry protocol is extraordinary maturation. The classifications of substrates were not complicate. And at the same time, owing to tetrazole and nucleoside monomer in acetonitrile-mixed solution

Table 1
The fluorescence intensities of different oligonucleotide arrays

Synthesis method	The fluorescence intensity			
	P_0 (P_0/P_0)	P_1 (P_1/P_0)	P_2 (P_2/P_0)	P_3 (P_3/P_0)
Automatic synthesis in situ	28347 (1.000)	14079 (0.4966)	7443 (0.2625)	3043 (0.1073)
Manual synthesis in situ	26703 (1.000)	13238 (0.4957)	6245 (0.2342)	1377 (0.0516)
Off-chip synthesis	10645 (1.000)	3959 (0.3717)	1778 (0.1671)	542 (0.0509)

P_n/P_0: the ratio value of the fluorescence intensity of P_n to that of P_0 ($n = 0$–3).

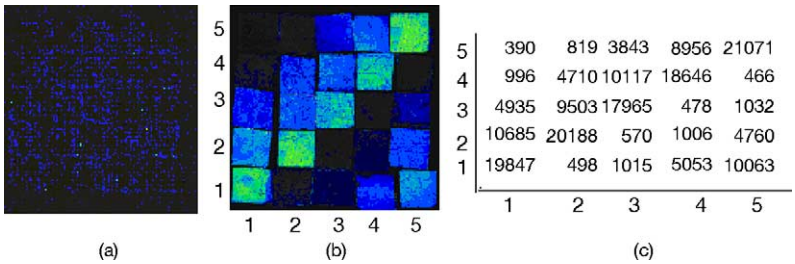

Fig. 4. Hybridization fluorescence images and fluorescence intensities of 5 × 5 probes array: (a) before hybridization; (b) after hybridization; (c) fluorescence intensities of 5 × 5 probes array of (b).

Table 2
Location sets of 5 × 5 probes array

Probes	Sequences	Location sets of probes
P_0	5′-AAC CAC CAA ACA CAC	(1, 1), (2, 2), (3, 3), (4, 4), (5, 5)
P_1	5′-AAC CAC CGA ACA CAC	(1, 5), (2, 1), (3, 2), (4, 3), (5, 4)
P_2	5′-AAC CGC CAA ACG CAC	(1, 4), (2, 5), (3, 1), (4, 2), (5, 3)
P_3	5′-AAC CGC CGA ACG CAC	(1, 3), (2, 4) (3, 5), (4, 1), (5, 2)
P	Control (no)	(1, 2), (2, 3), (3, 4), (4, 5), (5, 1)

Table 3
Fluorescence intensity data analysis of 5 × 5 probes array

	P_0	P_1	P_2	P_3	P
Average	19543	9853	4660	973	480
S.E. (σ_0)	1581	654	477	87	64
Relative accuracy (%)	8.01	6.64	10.2	8.97	13.3

P_0:P_1:P_2:P_3 = (1.000 ± 0.080):(0.4991 ± 0.0671):(0.2360 ± 0.0044):(0.0493 ± 0.0033).

could maintain good reactive activity within 10 h; nucleoside reagents might be sufficiently used in combinatorial chemistry method for synthesis oligonucleotide sequences [4]. In situ synthesis oligonucleotide sequences as probes used to fabricate the oligonucleotide array has an important advantage that these oligonucleotides need no another immobilization process to bond them on the substrate.

In principle experimentation, the parallel result of oligonucleotide array fabricated by assembly method was investigated. If oligonucleotide array fabricated by this method can be used in practical application, the parallel results of oligonucleotide array may be reached. This means that the hybridization fluorescence signals of the some sequences located in different sets should have approximately equal values. In this paper, 5 × 5 probes array which consist of four different oligonucleotide sequences and the control glass sample was fabricated to identify those arrays which had whether or not good parallel results. Table 2 shows those five samples location sets on the array. And Fig. 4(a) and (b) were the hybridization fluorescence images of the array before and after hybridization, respectively. The background of the pieces' edges show no significant fluorescence signal, meaning that the pieces may be very small, and the number of pieces or probes per area assembled on the substrate could be large. Fig. 4(c) was the fluorescence intensities of 5 × 5 probes array of (b). The results indicated that the best parallel results of oligonucleotide array had reached and the relative accuracies were within 10.2% (Table 3). The four different probes fluorescence signals hybridized with Cy3-GTG TGT TTG GTG GTT-3′ had clear differentiation (Fig. 4(b) and (c)) and P_0:P_1:P_2:P_3 = (1.000 ± 0.080):(4991 ± 0.0671):(0.2360 ± 0.0044):(0.0493 ± 0.0033) (Table 3).

4. Conclusion

Using combinational chemistry to synthesize oligonucleotides can obtain many different probe sequences in short time and thus fabricate reliably and flexibly oligonucleotide array by assembling those synthesized probes was reported in this paper. In this method, the solid synthesis of oligonucleotides is extraordinary maturation and nucleoside reagents might be fully used in combinational chemistry protocol. The classification of substrates were not complicated. This oligonucleotide array fabrication has an important advantage that these oligonucleotides do not need another immobilization process to bond them on the substrate.

Acknowledgements

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