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An extension-quenching-extension sequencing on a microarray

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ABSTRACT

Inherent problems exist with sequencing-by-synthesis (SBS) methods which use fluorescein-labeled nucleotide incorporation into a target template based on a polymerase chain reaction (PCR). These problems include lowering the cost of sequencing and the removal of fluorescence in DNA sequencing for further reading. How can these sequencing problems be resolved? We present a sequencing strategy which we call an extension-quenching-extension sequencing on a microarray based on a two-primer hyperbranched rolling circle amplification (HRCA). The microarray is a high throughput and low-cost tool.

The template on a microarray for SBS was prepared by HRCA. The Cy 5-labeled deoxyribonucleoside triphosphate (dNTP) species were incorporated in the extension reactions. We discovered that copper ($CuSO_4$) can quench the fluorescence in DNA sequencing because it exhibits an energy-transfer mechanism of quenching from the fluorescein to the bound Cu^{2+} ion. The fluorescein label needs to be destroyed after the readout by $CuSO_4$ before further reading is possible.

This paper describes the process used which discovered a successful combination of temperature, concentration, and duration of use for $CuSO_4$ which successfully quenched the fluorescence. In this experiment, we used a known sequence as a template in order to provide a strategy for sequencing.

1. Introduction

Over the past decade DNA sequencing methods advanced rapidly. Sequencing-by-synthesis (SBS) [1] is one of the several promising sequencing methods. Other methods include solid phase mini-sequencing [2], genetic bit analysis [3], multiplexed DNA sequencing-by-synthesis [4], arrayed primer extension [5] and so on. These new technologies aim to achieve a better price to performance ratio, aiming for the coveted goal of $1000 per genome [6].

Currently, templates for most sequencing use mainly double-strand DNA produced by PCR [7]. Therefore, the preparation of a sequencing template is too expensive, labor intensive, and time consuming [8]. Lu et al. introduced a novel way to prepare the DNA templates for the DNA sequencing-by-synthesis based on RCA for DNA fragment libraries of the whole genome. The products of RCA were single DNA molecules with more than 1000 copies of the genomic DNA fragment. RCA reaction is an isothermal in vitro method for the hybridization-triggered enzymatic synthesis of hundreds to billions of linear copies of small, single stranded, circular DNA probes [9,10]. Pihlak et al. used the RCA in situ on the glass as a template for DNA sequencing in 2008 [11]. This paper reports using HRCA in solution as a template for DNA sequencing.

We can immobilize the product of RCA on acryl-modified slide surface after exploring a few methods for immobilizing RCA. Fig. 1 shows that the sequence of DNA template could be read with therminator enzyme at the 3′ terminus of the primer strand; the primer was extended with fluorescein label which was complementary to the template base. The fluorescein label incorporated by the polymerase could then be identified so that the sequence could then be read. The fluorescence collected in the incorporation reaction in the SBS needed to be destroyed before further reading could be completed [4]. Odedra et al. studied the chemistry of enzyme-labile, Pd-cleavable and photocleavable linkers [12–26]. Ju et al. reported a viable sequencing scheme involving the use of 3′-O-allyl Pd-cleavable fluorescent nucleotide analogs [27] and Turcatti et al. reported the cleavable disulfide fluorescent nucleotides as reversible terminators [28]. However, the specially modified dNTP with fluorescein is highly modified and difficult to incorporate nucleotides [29]. And UV is used in some methods in the removal of fluorescence in DNA sequencing. We describe our scientific method

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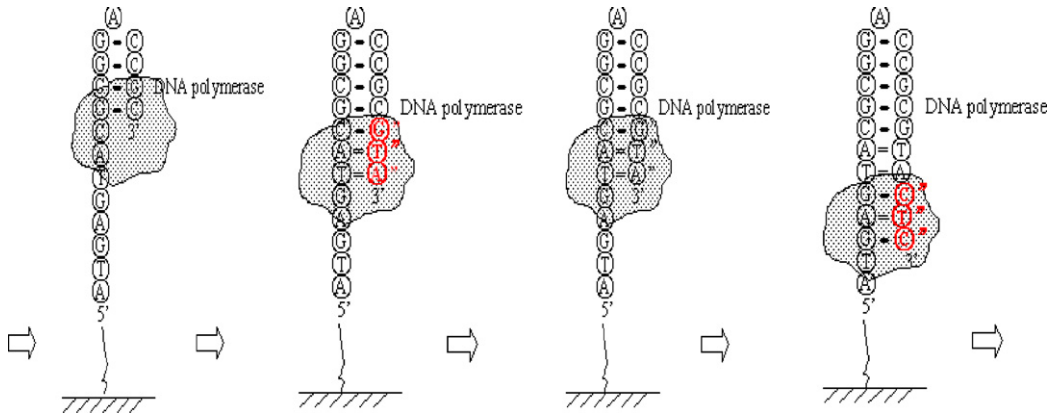

Fig. 1. Illustration of extension-quenching-extension sequencing on microarray [4]. In this paper, the template with modified RCA primer was immobilized on the glass slides. The sequencing primer could hybridize on the template that was immobilized on the glass and form a hairpin structure at the 3′-end. After the hairpin structure formed, the fluorescein label extended with DNA polymerase by sequencing-by-synthesis method.

of testing a solution to this problem in the form of a successful inexpensive method using a commonly available reagent, $CuSO_4$, based on its price and simply used comparing with the above methods. This process is known as an extension-quenching-extension sequencing method on a microarray.

2. Materials and methods

2.1. Preparation of the acryl-modified Slides

Glass slides were prepared by first soaking in 10% aqueous nitric acid for 2 h. The slides were then rinsed with water and acetone and air dried. The cleaned slides were then soaked in 10% 3-methacryloxypropyltrimethoxy saline in acetone for 1 h, followed by washing in acetone and air drying [30].

2.2. RCA reaction

The open circle probe is produced from the fragments of lect-intype oxidized LDL receptor gene (OLR1, GeneBank accession no. AF079167, Table 1). The ligation reaction was carried out in a total 8 μl volume mixture as follows. First the mixture was denatured in 95 °C for 10 min and slowly cooled to room temperature. Next 10× T4 ligase buffer and T4 ligase were added, respectively, for 1 μl to the mixture. Then the mixture was continually incubated at 16 °C for more than 16 h. After ligation, the RCA primer was used for amplification at 40 °C for more than 24 h. Each 25 μl of reagent contained 0.25 μM dNTPs, 4.8 U Bst DNA polymerase, 10× Thermopol reaction buffer 2.5 μl and 0.2 μg/μl BSA (bovine serum albumin) [31].

2.3. Attachment of RCA products to the acryl-modified slide surface and signal detection

After RCA, RCA products were processed by ethanol precipitation. Solutions containing RCA products with the modification involves attachment of acrylamide groups to DNA, 3% acrylamide monomer (29:1 acrylamide:bis-acrylamide), 30% glycerol, 1% APS were prepared, and then were spotted on the prepared glass slides using a microarrayer (Capital Biochip Corporation, China). After spotting, the slide was placed in a humid sealed chamber with a well containing N,N,N′,N′-tetramethyl ethylenediamine (TEMED). The pressure in the sealed chamber was reduced to about 1000 Pa, and this pressure was maintained for 1 h at room temperature. Under this pressure, TEMED was vaporized and diffused into the spots to induce the copolymerization between acrylamide groups and acryl groups. The slide was incubated in 0.10 M NaOH for electrophoresis for 8 min to obtain single stranded DNA (ssDNA) for hybridization analysis [32].

To detect the signal of amplification and form a hairpin structure at the 3′-end for sequencing, hybridization with a Cy 5-labeled probe was performed in a moist chamber at 40 °C for 2 h. Then the slides were rinsed with 2× SSC/0.2 SDS and double distilled water, dried and scanned.

2.4. Fluorescence quenching

The following steps were used to quench the fluorescence. The known sequence with a hairpin structure was immobilized onto the surface of acryl-modified glass slides. If the fluorescein-dNTP was not complementary to the bases in the polymerase extension system, the spots on the array remained

Table 1
The sequence of RCA.

OCP	5′-P-TAGCTAGAATCAAAAATGTTGAGTACGACGAATCTGTATGCTAAT*GCGGCGTGATGTA*TTATGCGTATAGAAATAATACAGA-3′
T	5′-ACCTTTATGTCAACATTTTTGATTCTAGCTATCTGTATTATTTCACCTAGCTT-3′
RCA primer	5′-acryl-$(T)_{10}$ATTAGCATACAGATTCGTCGTACT-3′ 5′-acryl-$(T)_{10}$ATTAGCATACAGATTCGTCGT-3′
Sequencing Primer	5′-Cy5-*GCGGCGTGATGTA*

Underlined segments of open circle probe (OCP) are complementary to the RCA primer. The 5′- and 3′-end segments of OCP probes are complementary with template. The italic and underlining segments of OCP have the same sequences with sequencing primer.

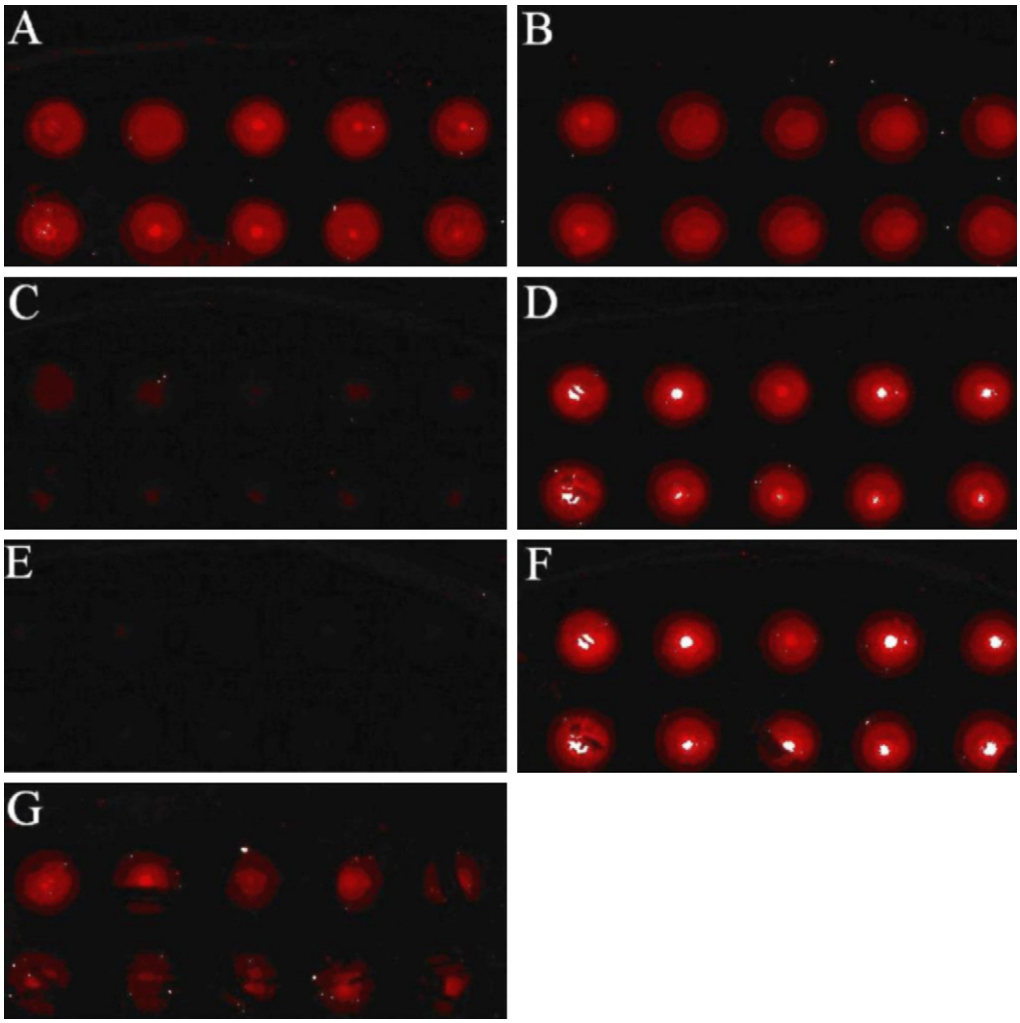

Fig. 2. (A) The hybridization image of the HRCA products; (B) the hybridization image of the RCA products using single primer; (C) the microarray image showing quenching after hybridizing; (D) the microarray image showing a reading of the sequence of TTAT; (E) the microarray image of quenching after reading the sequence of TTAT; (F) the microarray image of reading the sequence of GTGC; and (G) the microarray image after carrying out for 12 times.

dark on the fluorescent image. If the fluorescein-dNTP was complementary to the bases in the polymerase extension system, the spots on the array fluoresced [4]. Initial experimentation with many chemicals led us to the discovery that using $CuSO_4$ would successfully quench florescence. $CuSO_4$ could quench the fluorescence because it had an energy-transfer mechanism of quenching from the fluorescein to the bound Cu^{2+} ion [33]. The extended sequence could be read after the fluorescence quenching.

2.5. Polymerase extension reaction

Next, we described the polymerase extension reaction. The template with modified RCA primer was immobilized on the glass slides. The sequencing primer could hybridize on the template that was immobilized on the glass and form a hairpin structure at the 3′-end. The concentration of hybridization probe with Cy 5-labeled was 1 μM in this paper. After the hairpin structure was formed, the fluorescein label extended with DNA polymerase by sequencing-by-synthesis method.

Single-step extension and multiple-step extension are two types of extension in DNA sequencing. Single-step extension is a base-to-base extension method. Multiple-step extension is more than a base for an extension in sequencing. In this experiment, we used multiple-step extension method. In the first step, the mixture of 0.3 μM dTTP and 0.1 μM dATP labeled with Cy 5 and New England Biolabs (NEB)'s Therminator DNA polymerase were used based on the sequencing primer and template sequence. The microarrays were incubated with the mixture above for 10 min at 65 °C. After incubation with the mixture, the microarrays were rinsed with 2× SSC/0.2 SDS and double distilled water, dried and scanned. After a few bases mixture extended, it showed the extension was successful if next bases mixture can extend. In the subsequent step, the mixture of dCTP, dGTP and dTTP was used after quenching. The incorporation reactions were then repeated cyclically with the various base mixtures.

2.6. Image scanning and data processing

The slides were scanned by ScanMicroarray® Lite. The images were analyzed with GenePix Pro3.0 software. The average pixel intensity within each circle was determined for each spot. Signal intensities of individual spots were exported to Excel spreadsheets and were analyzed by standard methods.

2.7. The damage of the quenchers to the surface of the slides and DNA

The difference between the surfaces of the slides that the quenchers were not used and used was shown by AFM. AFM was

from Molecular Imaging Inc., USA. It could show the damage of the surfaces on the slides. This could also show the damage of DNA on the surfaces of the slides.

2.8. The quenching difference of the quencher to different fluorescein-labeled dNTPs

Fluorescence spectrofluorometer from USA measured the difference of fluorescent intensity between before and after adding the quencher to fluorescein-labeled dNTPs. The fluorescein-labeled dNTPs were FITC-labeled dNTP, Cy 3-labeled dNTP, Cy 5-labeled dNTP and TAMAR-labeled dNTP. The efficiencies of quenching were compared.

3. Results and discussion

3.1. Immobilization of RCA products and signal detection

Rehman et al. described the use of acrylamide-modified nucleic acids polymerized with acrylamide monomers to fabricate gel-based microarrays [34]. The slide was incubated in 0.10 M NaOH for electrophoresis for 8 min because this can obtain single stranded DNA (ssDNA) for hybridization analysis and the fluorescence background of the gel-based microarray can be lowered [32]. Pan et al. reported using a low-frequency ultrasound of 40 kHz to lower the fluorescence background [35].

As shown in Fig. 2A and B, the hybridization image of the HRCA products using both double primers (5′-acryl-$(T)_{10}$ATTAGCATACAGATTCGTCGTACT-3′; 5′-acryl-$(T)_{10}$ATTAGCATACAGATTCGTCGT-3′) was stronger than the hybridization image of the RCA products using single primer (5′-acryl-$(T)_{10}$ATTAGCATACAGATTCGTCGT-3′) because the average fluorescent intensity of HRCA products is 8000 and the average fluorescent intensity of RCA is 6800. This demonstrates that the RCA product using double primers was more efficient than using the single primer. Rehman et al. reported 50 μM acrylamide probe captured −10 pmol oligonucleotide and the capture probe density is estimated to be 260 fmol/mm^2. Therefore, we used the RCA products using both double primers to sequence DNA.

3.2. Fluorescence quenching

We tested fluorescent quenching by detecting 20 spots at a time and repeating 5 times. The fluorescence of the hybridization was quenched by $CuSO_4$.

Fig. 3A demonstrated how the quenching efficiency increased with different $CuSO_4$ concentrations. The difference of quenching efficiency at different $CuSO_4$ concentrations is less. It showed the concentration of $CuSO_4$ had a less effect on the quenching efficiency. When the concentrate was more than 0.02 mol/l, the increasing of quenching efficiency becomes slow. So the concentrate was set at 0.02 mol/l. With a concentration of 0.02 mol/l, the fluorescence quenching efficiency fluorescence was about 97%. Most of the fluorescence could be quenched at this concentration.

The quenching time affected the fluorescent intensity. Fig. 3B shows the concentration of $CuSO_4$ was at 0.02 mol/l. A quenching time of 5 min resulted in successful quenching of the most of the fluorescence. Before 5 min, the fluorescence continued to disappear fast. After 5 min the fluorescence continued to disappear slowly and completely disappeared in the 20 min because the reaction needs a limited time and it becomes slowly after the limited time. Therefore, the quenching time was set at 5 min.

The quenching temperature also affected the fluorescent intensity with the $CuSO_4$ concentration maintained at 0.02 mol/l. Fig. 3C shows how a higher temperature resulted in a larger

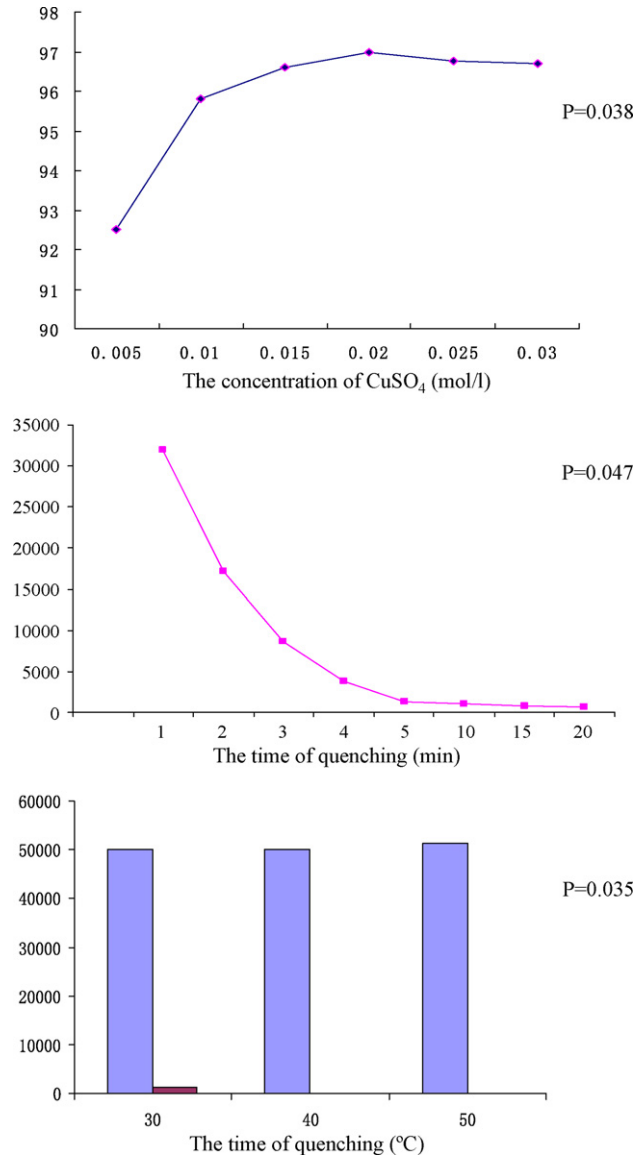

Fig. 3. (A) The change of fluorescent intensity with quenching time. (B) The change of fluorescent intensity in relation to quenching temperatures. Blue: before the quenching process; red: after the quenching process. Quenching time: 5 min. (C) The change of quenching efficiency with the $CuSO_4$ concentrations. The process was done by detecting 20 spots at a time and repeating 5 times. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

level of destruction of the fluorescence. The 30 °C temperature was easily achieved and resulted in almost complete quenching of the fluorescence. Therefore, a quenching temperature of 30 °C was selected in this experiment. As noted above, experimentation resulted in the selection of a quenching temperature of 30 °C with a quenching time of 5 min. The equation of quenching efficiency: $F(\%) = (F1 - F2)/F1 \times 100$, where $F1$ and $F2$ are the observed fluorescence before quenching and the fluorescence after quenching, respectively. The equation of extension efficiency: $G(\%) = (G2 - G1)/(G0 - G1) \times 100$, where $G0$, $G1$ and $G2$ are initial fluorescence before quenching, the fluorescence after quenching and the observed fluorescence after an extension, respectively. The extension efficiency was about 95% and as high as 97% in the single step after the fluorescence was quenched. We could efficiently quench the accumulated fluorescence in the extensions with a concentration of $CuSO_4$ at 0.02 mol/l and a reaction time of 5 min and successfully produce the desired incorporation reactions. The reac-

tion could successfully continue to replicate the sequence without mismatching after quenching.

3.3. Polymerase extension reaction

Fig. 2D shows a typical microarray image reading and shows how it looks with the sequence TTAT. The incorporation reactions were repeated cyclically with the base mixtures for the known sequence with a hairpin structure immobilized on acryl-modified chips. The extensions could be carried out at the same time in each array spot. Fig. 2E and F shows the sequences TTAT and GTGC, respectively, as they were read in the microarrays. The fluorescence was quenched. Then the incorporation reactions were repeated cyclically with the various base mixtures. In this experiment, the HRCA product dissipated slowly after each repetition of incorporation reaction followed by fluorescence quenching (Fig. 2G). That is, the fluorescence was successfully quenched 10 times allowing 11 extensions. By using these methods, a length of 42 bases was successfully read.

3.4. Image scanning and data processing

Fig. 4 shows the pixel intensity with sequenced bases. The pixel intensity changed less slowly in the total reaction because the rate of loss of the RCA product slowed. The number of read bases could be rapidly distinguished automatically using Gene Pix Pro3.0 software.

Fig. 4 and Table 2 show a representative experiment in which fluorescence was totally removed 11 times and the extensions were carried out 11 times. By using this sequencing method, 42 bases can be successfully read with a single slide preparation (Table 2) for the known sequence in the experiment. It may become more difficult to sequence DNA for an unknown DNA sequence because each base tries to be used, respectively, in order to match the template for each extension step. This method may stimulate new advances in quenching technologies.

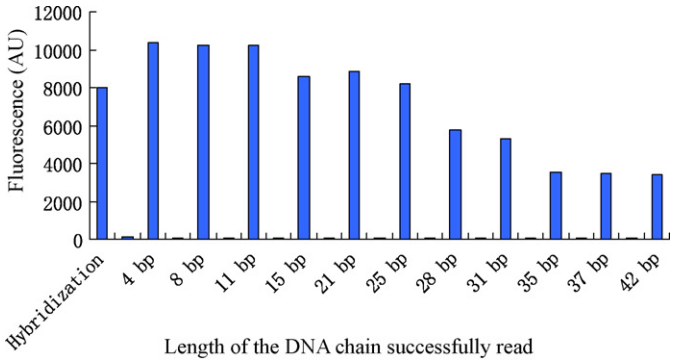

Fig. 4. The analysis of DNA sequencing.

3.5. The damage of the quenchers to the slides and DNA

We can find that $CuSO_4$ caused less damage to the acryl-modified slides and DNA on their surfaces by comparing Fig. 5A with B. H_2O_2 was ever reported to quench the fluorescence [36].

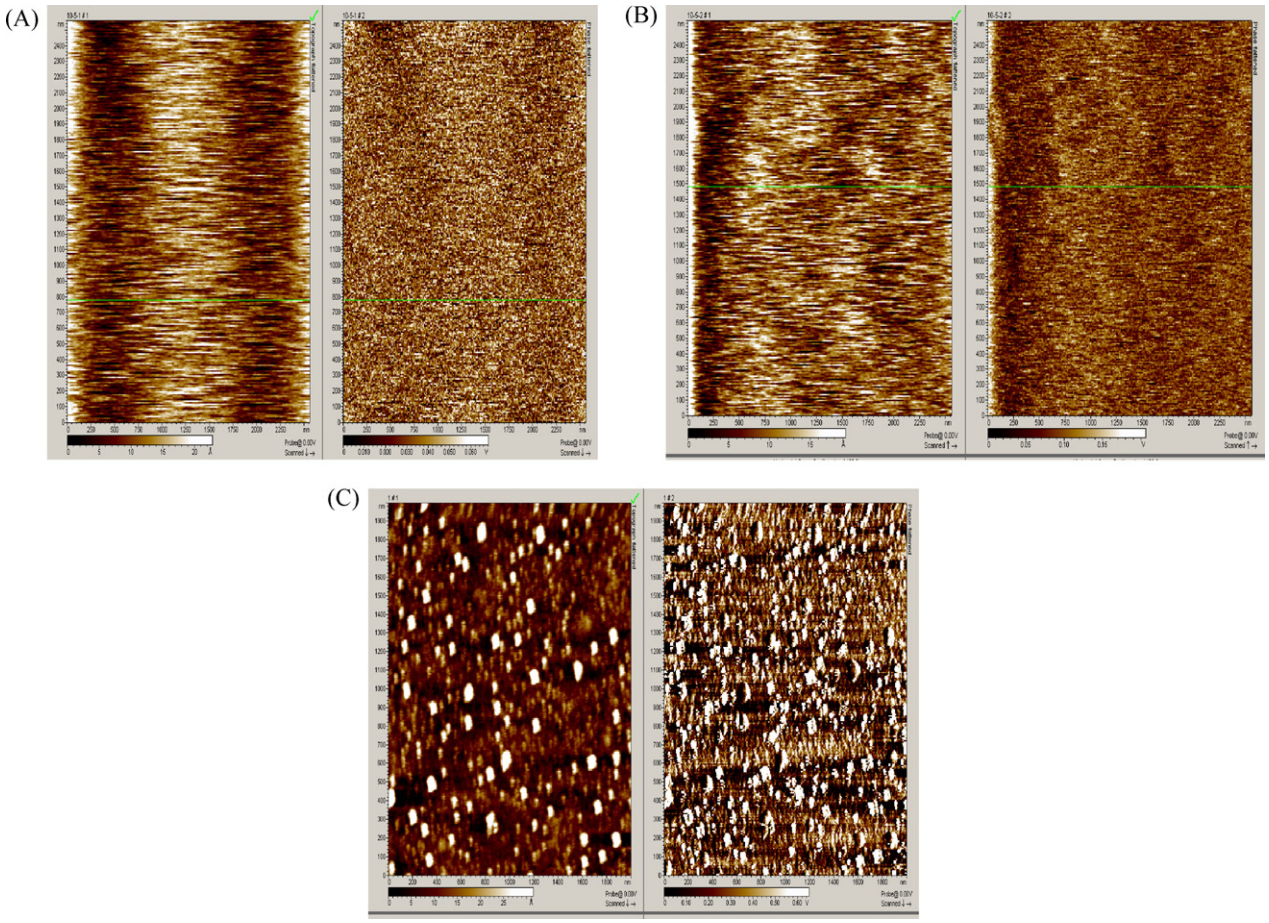

Fig. 5. The AFM images of the surfaces on the acryl-modified slides: (A) the surface on the acryl-modified slides; (B) the surface on the acryl-modified slides that $CuSO_4$ was used; and (C) the surface on the acryl-modified slides that H_2O_2 was used.

Table 2
Results of DNA sequencing.

Step	1	2	3	4	5	6	7	8	9	10	11
Bases	TTAT	GCGT	ATA	GAAA	TAAATA	CAGA	TAG	CTA	GAAA	TC	AAAAA

However, we found that H_2O_2 caused more damage to the acryl-modified slides and DNA on their surfaces by comparing Fig. 5A with C.

3.6. The quenching difference of the quencher to different fluorescein-labeled dNTPs

0.02 mol/l Cu SO_4 was separately used to quench the Cy 3-, Cy 5-, FITC- and TAMAR-labeled dNTP. The reactions were at the same condition. Fluorescence spectrofluorometer measured the intensity of fluorescence.

The intensity of fluorescence after quenching/before quenching was separately Cy 3 (48.74%), Cy 5 (5.7%), FITC (26.84%) and TAMAR (68.23%). From this result we can know that TAMAR > Cy 3 > FITC > Cy 5 based on the difficulty of quenching. Cy 5 was easily to be quenched for $CuSO_4$.

3.7. Comparison with related sequencing methods

Pihlak et al. used the RCA for DNA sequencing. It is RCA in situ on the glass as a template [11]. We ever reported RCA in solution for the template of sequencing [36]. In this experiment, we got more templates for sequencing using HRCA and further improved the method of preparing the template.

Fluorescence removal is an important problem for sequencing. However, the highly modified and difficult to incorporate nucleotides for removing the fluorescence need to be further improved. Some researchers have tried to further solve the problem in sequencing without highly modified nucleotides. Mir and co-workers reported using a method called Cycical Ligation and Cleavage to sequence, They used RNase to blend at 37 °C for 1 h to remove the fluorescence. The number of sequenced bases is only 3. RNase is more expensive than $CuSO_4$ [37].

We ever reported using hydrogen peroxide (H_2O_2) to deactivate the fluorescence as one of the oxidants. However, hydrogen peroxide (H_2O_2) cause damage to the skin of the operator and also to DNA. Furthermore, the removal times in sequencing are four before the extension stops [36]. We used $CuSO_4$ to improve the method of removing the fluorescence and the removal times are 11 before the extension stops.

4. Conclusion

We report a method for using an extension-quenching-extension DNA sequencing process using a microarray. We used HRCA in solution to prepare the templates for SBS and successfully quenched the fluorescence by using $CuSO_4$. In this experiment, the fluorescence was totally removed for 11 times and the extensions were carried out 11 times. And a 42 base section of DNA in the known sequence was successfully read. $CuSO_4$ caused less damage to DNA. The reaction can continue without mismatch after quenching. This process exhibits a novel way to DNA sequencing. The process we have used exhibits positive characteristics which may facilitate the next generation of sequencing.

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References

[1] E.D. Hyman, Anal. Biochem. 174 (1988) 423–436.
[2] A.C. Syvanen, Hum. Mutat. 13 (1999) 1–10.
[3] T.T. Nikiforov, R.B. Rendie, P. Goelet, et al., Nucleic Acids Res. 22 (1994) 4167–4175.
[4] S.A. Aksyonov, M. Bittner, L.B. Bloom, et al., Anal. Biochem. 348 (2006) 127–138.
[5] N. Tonisson, A. Kurg, K. Kaasik, E. Lohmussaar, Clin. Chem. Lab. Med. 38 (2000) 165–170.
[6] K. Marusina, Genet. Eng. Biotechnol. News 26 (2006) 20.
[7] M. Margulies, M. Egholm, W.E. Altman, et al., Nature (2005 July) 31.
[8] M.L. Metzker, Genome Res. 15 (2005) 1767–1776.
[9] V. Vadim, Demidov Encyclopedia of Diagnostic Genomics and Proteomics, 2005, pp.1175–1179.
[10] F.B. Dean, J.R. Nelson, T.L. Giesler, R.S. Lasken, Genome Res. 11 (2001) 1095–1099.
[11] A. Pihlak, G. Bauren, E. Hersoug, et al., Nat. Biotechnol. 26 (2008) 676–684.
[12] R.S. Sarfati, T. Berthod, C. Guerreiro, B. Canard, J. Chem. Soc. [Perkin Trans. 1]: Org. Bioorg. Chem. (1995) 1163–1171.
[13] M.L. Metzker, R. Raghavachari, S. Richards, Nucleic Acids Res. 22 (1994) 4259–4267.
[14] I. Rasolonjatovo, S.R. Sarfati, Nucleosides Nucleotides 17 (1998) 2021–2025.
[15] T.S. Seo, X. Bai, H. Ruparel, et al., Proc. Natl. Acad. Sci. U.S.A 101 (2004) 5488–5493.
[16] H. Ruparel, L. Bi, Z. Li, et al., Proc. Natl. Acad. Sci. U.S.A 102 (2005) 5932–5937.
[17] L. Bi, D.H. Kim, J. Ju, J. Am. Chem. Soc. 128 (2006) 2542–2543.
[18] M.B. Welch, K. Burgess, Nucleosides Nucleotides 18 (1999) 197–201.
[19] I. Rasolonjatovo, S.R. Sarfati, Nucleosides Nucleotides 18 (1999) 1021–1022.
[20] Z. Li, X. Bai, H. Ruparel, et al., Proc. Natl. Acad. Sci. USA 100 (2003) 414–419.
[21] T.S. Seo, X. Bai, D.H. Kim, et al., Proc. Natl. Acad. Sci. USA 102 (2005) 5926–5931.
[22] G. Lu, K. Burgess, Bioorg. Med. Chem. Lett. 16 (2006) 3902–3905.
[23] J. Shi, M.T. Boyce-Jacino, P. Goelet, WO Patent 9964437 (1999), to Orchid Biocomputer, Inc., USA.
[24] J.W. Parce, T.T. Nikiforov, A.B. Mehta, A.W. Chow, M.R. Knapp, WO Patent 2000050642 (2000), to Caliper Technologies Corp., USA.
[25] Q. Meng, D.H. Kim, X. Bai, et al., J. Org. Chem. 71 (2006) 3248–3252.
[26] R. Odedra, A. Simmonds, L. Pickering, WO Patent 2001092284 (2001), to Amersham Pharmacia Biotech UK Ltd., UK.
[27] J. Ju, D.H. Kim, L. Bi, et al., Proc. Natl. Acad. Sci. USA 103 (2006) 19635–19640.
[28] G. Turcatti, A. Romieu, M. Fedurco, P. Tairi, Nucleic Acids Res. 36 (4) (2008) e25.
[29] K.U. Mir, H. Qi, O. Salata, G. Scozzafava, Nucleic Acids Res. 37 (1) (2009) e5.
[30] P. Xiao, L. Cheng, Y. Wan, et al., Electrophoresis 27 (2006) 3904–3915.
[31] D. Zhou, R. Zhang, R. Fang, et al., Electrophoresis 29 (2008) 626–633.
[32] P. Xiao, H. Huang, G. Zhou, Z. Lu, Electrophoresis 28 (2007) 1903–1912.
[33] F. Gouanvé1, T. Schuster, E. Allard, R. Méallet-Renault, C. Larpent, Adv. Funct. Mater. 17 (15) (2007) 2746–2756.
[34] F.N. Rehman, M. Audeh, E.S. Abrams, P.W. Hammond, M. Kenney, T.C. Boles, Nucleic Acids Res. 27 (1999) 649–655.
[35] Z. Pan, Y. Li, D. Zhou, et al., Anal. Biochem. 142 (2007) 605–611.
[36] L. Gao, H. Lu, L. Xu, Z. Lu, J. Nanosci. Nanotechnol., 2010, in press.
[37] U. Mir Kalim, H. Qi, S. Oleg, S. Giuseppe, Nucleic Acids Res. 37 (1) (2009) e5.