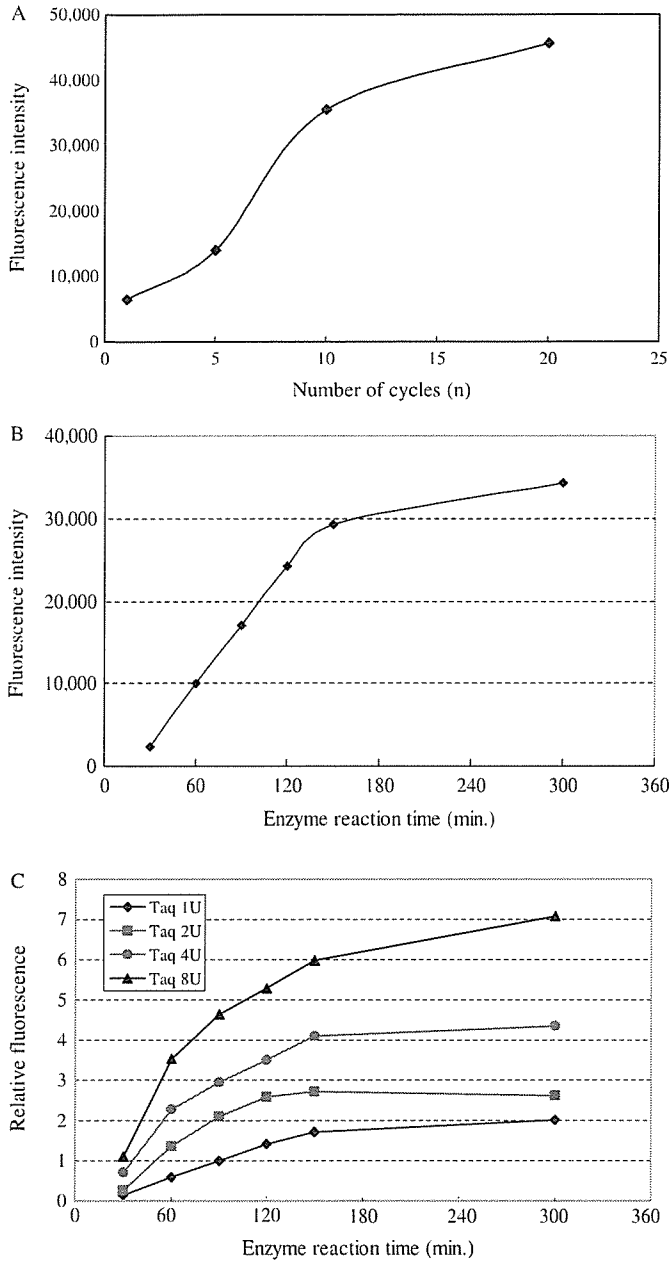

Figure 6. Kinetics and efficiency of the MPEX reaction on S-Bio® PrimeSurface®. (**A**) Thermocycling procedure (a denaturing step at 95°C for 1 min, an annealing step at 50°C for 3 min) in the presence of a buffer solution of 100 pM template DNA, nucleotides (dNTPs) including with Cy3-labeled dUTP and *Taq* DNA polymerase resulted in DNA amplification from 1 to 20 cycles. (**B**) The kinetics time course of the MPEX amplification reaction in the concentration of 500 pM template DNA proceeded linearly on both surfaces during the first 30 to 120 min at a constant temperature of 37°C. (**C**) Effect of varying the *Taq* DNA polymerase concentration from 1U to 8U under the same conditions as (B).

was proportional to the concentration of *EX Taq* DNA polymerase in the source surface, as shown in Figure 6C. The shape of this curve demonstrates that the reaction rates were dependent on the concentration of DNA polymerase in the source plastic plate at a picomolar concentration range of template DNA. This is the first evidence of MPEX amplification occurring on the surface of S-Bio® PrimeSurface® at a constant temperature. In addition, the DNA template, which is present in solution at a picomolar concentration level, can be highly amplified exclusively on a plastic plate grafted with a novel MPC polymer.

Sequencing by synthesis (SBS) via MPEX biosynthesis

Sequencing By Hybridization (SBH) was developed in the late 1980s as an alternative to gel-based sequencing. In general, it uses a universal DNA microarray, which harbors all probe oligonucleotide sets, e.g. 5–10mer on a membrane or a glass chip. These oligonucleotide probes are hybridized to an unknown DNA fragment, the sequence of which remains to be determined (10,44–48).

We have developed Sequencing By Synthesis (SBS) via MPEX biosynthesis such as SBH examined the detection limit of oligonucleotide lengths of less than 15mer. The results with 30 cycles of primer extension reaction using relatively shorter length oligonucleotide primers sets were obtained at a concentration of 500 pM DNA template, as shown in Figure 7. Laser scanning image resulting from MPEX reaction led to different lengths of oligonucleotides (8, 10 and 15mer) and DNA sequences to the complementary template DNA. The primer extension signal of DNA sequence No. 7 (10mer) and No. 13 (8mer), which have lower GC% in these sequences as shown in Figure 7, considerably decreased corresponding to other 8 and 10mer primers. These results suggested that the shorter-length oligonucleotides such as 8mer could be discriminated by the template DNA via the MPEX biosynthesis. Further work is necessary to validate this approach with actual data.

CONCLUSIONS AND FUTURE CHALLENGES

We have demonstrated that DNA oligonucleotide probes are covalently immobilized on the PrimeSurface® plastic surface via an amine at their 5′ terminus. This type of surface chemistry offers extraordinarily stable thermal properties because of the absence of a pre-activated glass slide surface. Our results suggest that the plastic platform S-BIO® PrimeSurface® can clear challenging technical hurdles for DNA microarrays in hybridization–based analysis in the near future. In addition, when the oligonucleotide DNA template is in solution on DNA arrays, the sequence-specific primer extension reaction and sequential DNA amplification can be processed on the solid surface by thermal cycling such as used in the PCR method. In this case, oligonucleotide DNA primers on the PrimeSurface® can be highly amplified exclusively at the picomolar concentration range of complementary oligonucleotide templates. The major advantage of the MPEX method over the hybridization method is that a single set of optimal reaction conditions can be used to genotype all sequence variants, enabling simplification and optimization of the assay design. Finally, we have demonstrated that the MPEX reaction condition can be simplified as shown by the kinetics study at a constant temperature. These results suggested that totally different approaches of genome analysis such as SNP analysis, SBS via MPEX biosynthesis and the detection of noncording micro RNA by reverse transcriptase can be developed using our DNA microarray platform PrimeSurface® through MPEX techniques. Further work on device development is needed

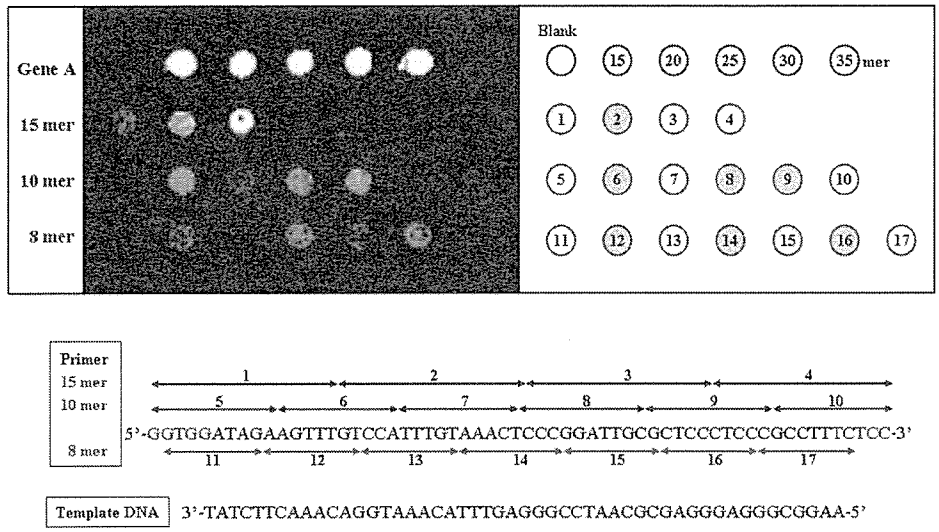

Figure 7. Laser scanning image resulting from MPEX reaction obtained on different short length oligonucleotides (8, 10 and 15mer) and DNA sequences via complementary template (5′-Cy5-AAGGCGGGAGGGAGCGCAATCCGGGAGTTTACAAATGGACAAACTTCTAT-3′). Thermocycling procedure (a denaturing step at 95°C for 1 min, an annealing step at 30°C for 5 min) in the presence of a buffer solution of 500 pM template DNA, nucleotides (dNTPs) including with Cy3-labeled dUTP and *Taq* DNA polymerase resulted on 30 cycles of DNA amplification.

to enable precision control of the temperature because the plastic substrate generally has lower thermoconductivity than a glass slide.

We conclude by stressing that while hybridization–based sequencing assays are still in their infancy, improvement of our primer extension method should offer an even more powerful analytical tool for accurate and high–throughput resequencing strategy and mutational analysis. We will focus on simplifying the analytical procedure, such as of the DNA polymerase reaction conditions and preparation chemistry, to extend the technology for the analysis of nucleic acids (DNA and RNA) extracted from clinical and environmental samples. Our method using a PMBN-coated plastic substrate has the potential to become a widely applicable tool for laboratories performing large-scale analyses and for use as a DNA microarray platform.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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