

sciFLEXARRAYER Application Note
No. 08015

Advanced applications with DNA microarray using sciPOLY3D

sciPOLY3D opens the door to immobilize DNA probes without a functional group directly to an unmodified polymer support. sciPOLY3D acts as a surface-attached hydrogel, where probe oligonucleotides are embedded covalently. Thus anchorage of the probes to the surface is on the one hand very robust and withstands very stringent washing procedures or even elevated temperatures up to 100 °C. This feature renders it suitable for chip-based PCR and melting curve applications. On the other hand, it is a requirement for some applications, that surface-attached probe oligonucleotides can interact with enzymes, e.g. for specific and template-dependent elongation in a multiparametric fashion. Immobilization of probes with sciPOLY3D enables this kind of assay.

A) Enzyme activity at surface-attached DNA oligonucleotides

Performance of enzymatic reactions at solid phase bound biomolecules, such as DNA oligonucleotides in DNA microarrays, offer interesting possibilities for advanced applications. In molecular biology there are many assays where enzyme activity is the key, e.g. specific digestion with nucleases, or amplification reactions. The spatial resolution of microarrays offers straightforward multiplexing of these assays. An important prerequisite is an immobilization chemistry that minimizes steric hindrance and makes biomolecules at the surface accessible for enzymes.

For DNA microarrays fabricated with sciPOLY3D this feature is demonstrated with a digestion assay and an elongation assay.

Materials and Methods

Various concentrations of DNA oligonucleotides with a 3'-conjugated Cy5 were mixed with 1 mg/ml sciPOLY3D and sciPOLY3D SOL2D1 and printed in microarray format using a sciFLEXARRAYER on PMMA (polymethyl methacrylate) slides. Subsequent irradiation with UV light (1.25 J/cm²) leads to covalent attachment of the DNA to the surface-attached sciPOLY3D hydrogel patches.

For the digestion experiment, the slides were incubated with 0.15 U/μl of the enzyme DNase I at 37 °C for 30 minutes. The fluorescence intensity was measured before the incubation step and thereafter.

The elongation experiment was done by performing a labeling NASBA assay¹ on top of a microarray containing various unlabeled probes. A GeneFrame was applied as an incubation chamber and the reaction was performed for 60 minutes at 41 °C. Human total RNA was used as template and primers specific for RPS18 mRNA were used. The microarray contained among others a probe specific for the same RNA sequence.

Results

Incubation of the sciPOLY3D DNA microarrays with the enzyme DNase I leads to digestion of the surface-attached oligonucleotides. These oligonucleotides were labeled with a fluorophore (Cy5) and the signal decrease indicates their digestion. In Fig. 1 it is shown that after 30 min incubation with DNase I, approx. 10 % of the initial fluorescence intensity is left.

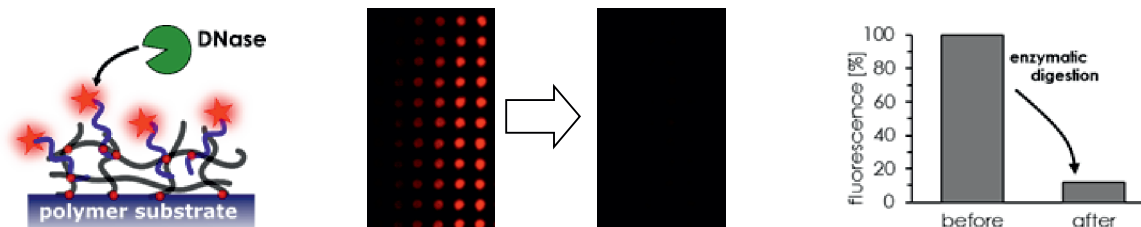


Fig. 1 Signal changes of surface-attached DNA oligonucleotides with fluorescent label due to incubation with enzyme DNase I. This proves accessibility for enzymes of DNA immobilized with sciPOLY3D.

The enzymatic elongation of surface-attached oligonucleotides is shown by the performance of an amplification assay on top of a microarray. Here we used the isothermal RNA amplification method NASBA, where among others a Reverse Transcriptase is involved and is able to elongate the sciPOLY3D embedded oligonucleotides in a template dependent manner. Fig. 2 shows the elongation of the RPS18 specific probe; nucleotides with a fluorescent label (DY647) were incorporated.

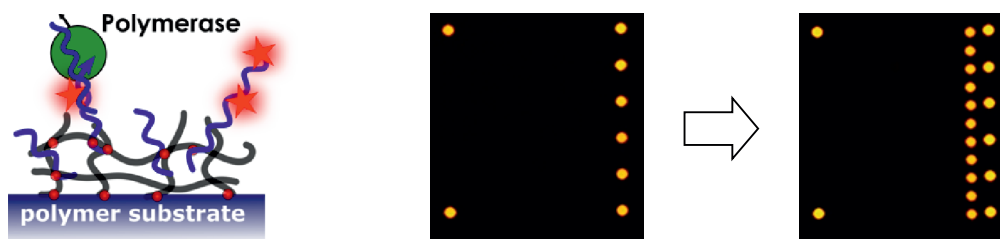


Fig. 2 Enzymatic elongation of immobilized probes. In this example, a labeling amplification reaction (NASBA assay) was performed on top of a sciPOLY3D microarray. The white arrow indicates the column of spots which contained the specific probe.

B) Melting curve analysis on DNA microarrays

In nucleic acid tests it is often necessary to differentiate between highly similar analytes, which differ in only one nucleotide (e.g. SNP genotyping or microRNA). The specificity of the probe:analyte hybridization is strongly temperature dependent. The performance of a melting curve analysis can help to clearly discriminate between genotypes.

Materials and Methods

Microarrays containing various probes specific for a panel of sequences with cancer related SNPs were produced with sciPOLY3D as described above. The labeled RNA analytes were prepared with a NASBA assay from human total RNA and CASP8 specific primers and incubated on the microarray at room temperature for 30 minutes. The subsequent melting curve and simultaneous fluorescence detection was performed in a TIRF setup.

Results

Due to the rising temperature during a melting curve analysis, the difference between wildtype and mutant sequences can be visualized. The mismatch probe hybrid is less stable and dissociates at a lower temperature than the perfect match probe hybrid. In Fig. 3 an example is shown, where three possible cases are shown with RNA from a patient with wildtype RNA, a heterozygous sample and a patient carrying homozygous mutation in the CASP8 gene.

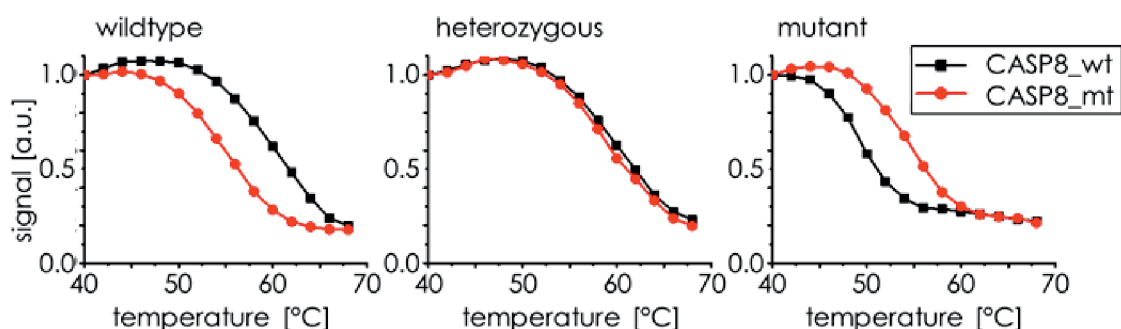


Fig. 3 Signal changes of probe:analyte hybrids in the course of rising temperature allows SNP (single nucleotide polymorphism) discrimination. Probe oligonucleotides are immobilized with sciPOLY3D on native PMMA. Continuous fluorescence detection is realized with a TIRF read-out system.