Surface-based DNA computing operations: DESTROY and READOUT

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Abstract

DNA computing on surfaces is where complex combinatorial mixtures of DNA molecules are immobilized on a substrate and subsets are tagged and enzymatically modified (DESTROY) in repeated cycles of the DNA computation. A restriction enzyme has been chosen for the surface DESTROY operation. For the READOUT operation, both cycle sequencing and PCR amplification followed by addressed array hybridization were studied to determine the DNA sequences after the computations. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

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1. DESTROY

Surface-based DNA computing (Smith et al., 1998) uses the following operations to manipulate DNA strands attached to the surface: MARK (hybridization), DESTROY (enzymatic modification), UNMARK (denaturation), and READOUT (sequence determination). The MARK and UNMARK operations have been intensively studied in our previous work (Frutos et al., 1997; Liu et al., 1998). In the DESTROY operation, enzymes remove every UNMARKED strand, leaving on the surface only the MARKED DNA molecules. Enzymes that can be employed for this step are: (a) single-strand specific exonuclease; and (b) double-strand specific restriction enzyme. *E. coli* exonuclease I (Exo I) was used for the DESTROY operation in single-word DNA computing (Frutos et al., 1997). However, the same enzyme can not be used for the DESTROY operation in DNA computing strategies based on multiple words (Liu et al., 1999). This issue brought up the idea of trying restriction enzymes. A major difference between restriction digestion and the exonuclease digestion is that double stranded instead of single stranded DNA will be destroyed by restriction enzymes. However, by adding a poly-

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merase extension step before restriction enzyme digestion, this new approach would give the same DNA computation results at the end.

Eight 4-cutter restriction enzymes (New England Biolabs) were chosen and screened. Enzyme substrates were fluorescein labeled oligonucleotide duplexes (either a 24mer linear duplex, or a 59mer self-complementary hairpin), that were digested in solution assays. To assess the cutting efficiency and specificity, the final incubation mixture was run on a polyacrylamide denaturing gel.

Among the eight restriction enzymes tested, three of them were found to have high oligonucleotide cutting efficiency (> 95%). These three enzymes were studied in surface experiments. A surface with immobilized DNA probes was prepared. The complementary fluorescein labeled DNA strands were hybridized to the surface-bound DNA probes to form DNA duplexes. The surface was then exposed to a solution of restriction enzyme for 4 h. After enzymatic digestion, fluorescein labeled strands of the duplexes were melted off from the surface, collected and run on a polyacrylamide denaturing gel. The restriction enzyme efficiency was quantified by comparing the fluorescence signal intensity of full length DNA bands and shorter DNA bands (enzyme cleavage product). Of the three restriction enzymes tested, Dpn II was determined to have the highest cutting efficiency (90%) and specificity on the surface.

2. READOUT

In DNA computing, the way to determine the answer to the query is to identify the DNA sequences of the strands remaining on the surface at the end of the computation cycles. Two approaches to this problem are: (1) conventional electrophoresis-based DNA sequencing; or (2) hybridization to word-specific addressed arrays. The strategy required determining the sequence will depend strongly upon the number of molecules in the solution present in the sample. One possibility is direct sequencing or cycle sequencing if there are enough copies (molecules) of a given solution present on the surface; the other is PCR amplification or conventional cloning followed by addressed array readout.

In cycle sequencing (Blakesley, 1993), several variables were tested for obtaining optimal sequencing results such as template to primer ratio, dNTP to ddNTP ratio, and cycle conditions. An important goal was to determine the minimum amount of DNA template needed for cycle sequencing. After careful design of the template, polymerase screening was done and AmpliTaq DNA polymerase CS (Perkin–Elmer) was determined to be the best enzyme for the cycle sequencing reactions. Under the enzyme's optimal conditions, the minimum amount of DNA template needed for cycle sequencing is between 25–50 femtomole. It is estimated that the amount of DNA template left on the surface is just enough to be sequenced by cycle sequencing, if there is only one kind of DNA molecule left on the surface after computations. However, when there is a combinatorial mixture of DNA molecules on the surface, each kind of DNA molecule will be proportionally diluted, and the amount of DNA template might then be too low to be detected by cycle sequencing. Therefore, PCR amplification (which produces far more copies than does cycle sequencing) will be required.

The second strategy for readout is PCR amplification (Erlich et al., 1991) followed by addressed array detection. There are three different states in which the final set of DNA molecules might be found: (a) still attached to the surface; (b) cleaved from the surface but still in solution above the surface; or (c) cleaved from the surface and transferred to an analysis vessel. Since the complementary strand contains the same information, determination of the complement sequences gives the same results. There are three choices to determine the sequences: (1) cycle sequencing (explained above); (2) surface PCR, in which the PCR amplification of the complementary strands is carried on the surface without the melting off step; (3) solution PCR amplification, in which the complementary strands are removed from the surface, collected and PCR amplified in a solution environment. Both methods (2) and (3) will be followed by addressed array READOUT.

Surface PCR was carried out on a gold surface, on which thiol-modified DNA oligonucleotides were immobilized. After hybridization and washing, only the correct DNA solutions were in the double-stranded form on the surface. The DNA duplexes were separated by a 95°C heating step in a PCR cycle. The melted off complementary strand was then used as a template for the same PCR reaction. The results show that the complementary strands were successfully amplified; however, the amount of the DNA was not enough for addressed array READOUT. Therefore, another solution PCR was necessary to amplify the DNA molecules. The PCR reaction conditions were optimized by varying the Mg concentration, primer concentration, dNTP concentration and cycle conditions.

Solution PCR has also been studied, and was found to be the most sensitive method for READOUT. After the MARK and DESTROY operations, the gold surface with double-stranded DNA was put in an in situ PCR instrument (Perkin–Elmer) at 95°C for 10 min to melt off the fluorescently labeled strands. The solution was collected and put in a standard solution-phase PCR instrument (DNA Engine, MJ Research). Solution PCR products were run on a polyacrylamide denaturing gel. The signal intensity of fluorescence DNA bands from the gel showed that a higher yield of solution PCR was achieved than that of surface PCR amplification. The subsequent readout step was successfully conducted by putting the solution PCR products on an addressed array, which was immobilized with known-sequence DNA probes. Only the complementary DNA strands of expected solutions were hybridized to the array. Those DNA probes that were lit up with fluorescence would contain the sequences of the DNA solutions. Therefore, unknown DNA solutions after computations were resolved by observation of fluorescence pixels on an addressed DNA array.

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