

On-chip selection of DNA ligands using a method for generating point mutations

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1 Introduction

A novel selection method for the acquisition of DNA aptamers that selectively recognize resorufin using on-chip selection in combination with method for point mutations has been developed. This method proved efficient for selection for DNA aptamers of single-stranded oligo-DNAs. A genetic algorithm was applied to produce oligonucleotides for the combinatorial library. A fluorescent molecule, resorufin, was applied for the ligand selection as a target. The binding affinity of the library was analyzed by the DNA chip. This selection method of DNA ligands includes on-chip selection and point-mutated sequence, which the highest affinity was selected. The fluorescence intensity of the library on the DNA chip increased after three repetitions of the selection round. The average of response for the affinity test increased with each generation.

2 Method and Results

2.1 Library construction using a method of generating point mutations

For the initial selection, a stem-loop structure of 20-mer oligonucleotides as a motif with a random region of 10 nucleotides, 5'-GGGGG-N10-CCCCC-3', was generated and chosen randomly from 300 different sequences by computer for the detection of the first selection as a zero generation of resorufin-binding aptamers. The obtained random oligonucleotide library was synthesized on a Combimatrix chip. The sequence of the obtained highest affinity was chosen, called the “mother” sequence. For the next generation, its single point mutant sequences and double point mutant sequences were synthesized. The number of all conceivable sequences for single point mutants and that for double point mutants are $20 \times 3=60$ and $(20 \times 19/2) \times 32=1710$, respectively. Then, we prepared all conceivable sequences for single point mutants and 70 randomly chosen sequences for

double point mutants. The single point mutants were put in the left side on the chip, while the double point mutants were located on the rest region.

2.2 Results

After affinity test using microarray, the next-generation chip was produced with the same computational procedure as the mother sequence in generation by generation. Even when compared to the photograph of the affinity test for the second-generation chip, more spots were detected compare to first-generation chip. Furthermore, the analysis of response, which is the subtraction for the fluorescence intensity of the spot from its surrounding background, showed the higher affinity of ligands obtained in each generation. Considering these results, it is reasonable to assume that this method using a method of generating point mutations is more effective in obtaining aptamers.

We used the method for generating point mutations based on simple GA because we could not obtain more than two sequences of high-affinity spots in this experiment. However, longer bases of oligonucleotide would be screened using complicated mutation and crossover. Besides, it has been reported that linear static analysis would be very useful for the selection of biomolecules [1, 2]. Thus, this on-chip selection is a useful tool for aptamers. Although the oligonucleotide microarray was developed originally for the expression analysis of genes, DNA aptamers can be screened easily and rapidly by using a microarray due to ligand affinity without sequence analysis. Therefore, our method is quite useable, even in the absence of the knowledge of molecular biology. We hope that the selection of aptamers should be much easier in the near future, when the cost of oligonucleotide chips decreases.

3 Discussions

We succeeded in obtaining DNA aptamers that recognize resorufin by on-chip selection using a method for generation point mutations. The results suggest that this method could be a powerful tool to obtain specific aptamers by high-throughput screening for various targets, such as polysacalide, proteins, and aromatic compounds with identical libraries. The data accumulated by systematic on-chip selection with computer-assisted technology of identical libraries for different targets should be useful for the practical prediction of sequences for various targets.

References

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