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An evolutionary Monte Carlo algorithm for predicting DNA hybridization

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Abstract

Many DNA-based technologies, such as DNA computing, DNA nanoassembly and DNA biochips, rely on DNA hybridization reactions. Previous hybridization models have focused on macroscopic reactions between two DNA strands at the sequence level. Here, we propose a novel population-based Monte Carlo algorithm that simulates a microscopic model of reacting DNA molecules. The algorithm uses two essential thermodynamic quantities of DNA molecules: the binding energy of bound DNA strands and the entropy of unbound strands. Using this evolutionary Monte Carlo method, we obtain a minimum free energy configuration in the equilibrium state. We applied this method to a logical reasoning problem and compared the simulation results with the experimental results of the wet-lab DNA experiments performed subsequently. Our simulation predicted the experimental results quantitatively.

Keywords: DNA computing; Evolutionary algorithm; Monte Carlo method; DNA theorem proving

1. Introduction

DNA is a molecule that carries genetic information, thus building the basis of life. Many researchers have discovered its usefulness as a material for building nanostructures and nanodevices. For example, the Seeman group showed that DNA strands can be used in building many geometrical structures, such as DNA tiles (Winfree et al., 1998). The Mirkin group used DNA to make gold nanoparticles aggregate so that their combined structures had different colors corresponding to their structures (Elghanian et al., 1997). This direction of research has evolved into DNA nanotechnology, such as the synthesis of DNA ratchets (Yan et al., 2002).

The information contained in the base sequence of DNA molecules also opened the possibility of using them as computational building blocks. Adleman demonstrated experimentally that DNA can be used in finding a solution to a directed Hamiltonian path problem by standard laboratory techniques (Adleman, 1994). Braich

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et al. attacked a 20-variable satisfiability (SAT) problem by devising a tool that used glass tubes containing DNA molecules under an electric field at an appropriate temperature (Braich et al., 2002). A "knight problem" with the condition that the knights must not attack each other on a 3×3 chessboard was interpreted as an SAT problem (Faulhammer et al., 2000). The tic-tac-toe game (Stojanovic and Stefanovic, 2003) and a seven-city traveling salesman problem (Lee et al., 2004) were also interesting. DNA computing has also been used in proving theorems. Guo et al. proved Cook's theorem to be correct with DNA-based computing (Guo et al., 2005). Cook's theorem is that if one algorithm for a non-deterministic-in-polynomial-time (NP) complete or an NP-hard problem can be developed, then other problems will be solved by means of reduction to that problem. Others have explored the potential of DNA computing in biotechnology and medicine. For example, the Shapiro group showed that DNA computing can be used in medical diagnosis and drug delivery by synthesizing DNA-computing drugs *in vitro* with restriction enzymes (Benenson et al., 2004).

At the core of all the DNA experiments above lies the sequence-specific DNA hybridization process. Thus, modeling this has been so important that numerous design tools for DNA hybridization have been suggested. For example, the SantaLucia group extensively studied DNA melting phenomena at the nucleotide level providing thermodynamic parameters for the nearest-neighbor (NN) model (SantaLucia and Hicks, 2004; Blake et al., 1999). The Garzon group's electronic analogs of DNA (EDNA) system is another tool for DNA hybridization, also based on the NN model (Garzon and Deaton, 2004). The EDNA system provides a way of performing biomolecular computing with silicon-based computers. Shin et al. developed a multiobjective evolutionary optimization technique to design optimal sequences (Shin et al., 2005). They obtained these sequences by the optimization of six fitness measures: similarity, H-measure, secondary structure, continuity, melting temperature and GC content. Most of these studies aimed at helping in DNA code design and focused on the base sequences within an NN-model framework.

In this paper, we propose a novel Monte Carlo algorithm for simulating DNA hybridization and used evolutionary computation to take into account the combinatorial effect of DNA molecules and simplified thermodynamics of DNA hybridization. In our previous study, we proposed an evolutionary algorithm that uses a Markov chain Monte Carlo approach to identify unknown configurations with probabilistic modeling of search space (Zhang and Cho, 2001). In this work, rather than identifying each possible configuration of DNA strands with probabilistic modeling, we combine a population-based evolutionary search and a Metropolis selection. Random selection of two parent strands is in proportion to the numbers of these within the population so that the model actually performs a population-based search. After one of every possible option of DNA hybridization or denaturation is chosen, the Metropolis selection allows or denies that particular process and eventually drives the system into a minimum free energy configuration of the system at the given temperature. After reaching equilibrium, simulated annealing is used to keep the system in thermal equilibrium as it is cooled (Kirkpatrick et al., 1983). The evolutionary nature of the Metropolis algorithm and simulated annealing has been studied as a possible evolutionary mutation operator (Droste et al., 2001). In our simulation, we used four parameters that could be extracted from other experiments (Klump and Ackermann, 1971). In this sense, our algorithm is minimal and powerful in simulating reacting DNA molecules, whereas the NN model uses more than 20 parameters (SantaLucia and Hicks, 2004; Blake et al., 1999; Garzon and Deaton, 2004). This reduced number of parameters will be effective in simulating very long DNA strands.

Our evolutionary Monte Carlo method has been applied to theorem proving by resolution refutation in artificial intelligence (Nilsson, 1998; Lee et al., 2003). This theorem proving is a logical inference process from given statements and logical relations among them. In the simulation, we counted the number of bonds and the number of target double-stranded (ds) DNA strands as a function of temperature. The increasing number of target dsDNA in the simulation corresponds to the completion of theorem proving. To confirm our algorithm, we performed wet-lab experiments with synthetic DNAs, in which we obtained the number of bonds by measuring SYBR Green I fluorescence. We also measured the concentration of the target dsDNA by gel electrophoresis when test samples arrived at a temperature well below the melting temperature, which confirmed the formation of the target dsDNA. The numerical simulation result of the number of bonds matched the experimental data very well.

The paper is organized as follows. In Section 2, we describe the evolutionary Monte Carlo algorithm in detail. In Section 3, our algorithm is applied to theorem proving and simulation results are provided. In Section 4, we report on our wet-lab experiments with synthesized DNA molecules. In Section 5, we summarize our work, discuss relevant issues and draw conclusions.

2. Evolutionary Monte Carlo method

The physical objects in the simulation of reacting DNA molecules are single-stranded DNAs (ssDNAs). Each ssDNA has a base sequence designed for a given purpose, for example, the theorem proving to be described in Section 3. We assume that all the DNAs are in thermal equilibrium with the temperature T.

The important thermodynamic quantity in the following algorithm is Gibbs free energy, which is defined by:

$$G = H_b - TS_u, \tag{1}$$

where H_b is the total binding energy, which is negative, and S_u is the entropy of unbound base pairs, which is positive. Therefore, the Boltzmann probability for the system to be in a particular state C is given by $(1/Z)e^{-G(C)/k_BT}$, where $Z \equiv \sum_C e^{-G(C)/k_BT}$ and k_B is the Boltzmann constant.

Let us consider both the high and the low temperature limits. If the temperature is very high, all ssDNAs will be unbound because the entropy term in Gibbs free energy is very large. If the system is at a low temperature, the ssDNAs will form dsDNAs because the binding energy term in Gibbs free energy becomes large in the negative and the entropy term becomes negligible.

Therefore, assembling ssDNAs into a dsDNA or disassembling a dsDNA into the original ssDNAs will be determined by the thermodynamics of the binding energy term and the entropy term in Gibbs free energy.

The evolutionary Monte Carlo algorithm proceeds as follows:

(i) We start with an initial configuration of N ssDNAs with m types at a high temperature. The total number of ssDNAs is $N \times m$.

(ii) From the configuration, we choose two ssDNAs, which are indexed as the i-th and j-th. The two chosen ssDNAs can be alone or participating in a bound DNA. There are four cases possible when we choose the i-th and j-th ssDNAs.
   (a) Both are unbound ssDNAs → The physical objects are two ssDNAs.
   (b) One is unbound and the other is bound to another ssDNA → The physical objects are an unbound ssDNA and a clustered DNA, which includes the bound ssDNA.
   (c) Both are bound to different DNAs → The physical objects are two clusters, each of which includes each of the bound ssDNAs.
   (d) Both are bound together with (or without) ssDNAs → The physical object is one cluster that includes the i-th and j-th ssDNAs.

(iii) We test all possible M ways of binding the selected physical object(s).

(iv) We then choose one of all the possible M ways of binding. Here, we choose a binding configuration with equal probability. This procedure corresponds to a population-based evolutionary search. If this choice is accepted by the Metropolis selection in the next step, offspring molecules will be produced in the hybridization process, which mimics the evolution of DNAs.

(v) We calculate the acceptance probability A with:

$$A = min\{1, e^{-(\Delta G/k_BT)}\}, \tag{2}$$

where $\Delta G \equiv G(C') - G(C)$, C' is the new configuration and C is the current configuration (Metropolis et al., 1953).

When two ssDNAs form a dsDNA, we can calculate ΔGas:

$$\Delta G = \Delta H - T\Delta S. \tag{3}$$

The ΔH can be calculated easily as it sums the binding energies of the A–T and G–C pairs and the binding energies of other pairs (see Table 1). The entropy is reduced when the pairs are formed; therefore, we use the same entropy change for any possible pair because we want to make our model as minimal as possible. In short, we have only four parameters (Table 1 and its caption) in our model.

(vi) If the hybridization process is not chosen, we attempt a denaturation process. We consider a new configuration where i or j is separated from one of its bound strands. The probability of acceptance is given in Eq. (2).

Table 1
Parameters in the Monte Carlo simulation

Base pair	Binding energy (kcal/MBP)
G–C	−9.0
A–T	−7.2
Others	−5.4*

The lower three values are the binding energy of Watson–Crick and other pairs. The values are from a reported experiment (Klump and Ackermann, 1971), except for the binding energy for the other pairs (*). The value for the other pairs is the sum of the enthalpy of a single hydrogen bond (1.8 kcal/MBP, where MBP denotes a mole base pair) and the stacking enthalpy of DNA (3.6 kcal/MBP). The fourth item is the assigned value of 23 cal/(deg MBP) for the entropy change $\Delta S_{\text{base pair}}$ between the hybridized and denatured state of a base pair. This value is from the same report.

(vii) We repeat (ii)–(vi) until the system reaches its thermal equilibrium, and then count the number of target DNAs and the number of base pairs bound together.
(viii) We decrease the temperature by an appropriate amount and repeat (ii)–(vii). The amount of variation in temperature should be small enough to satisfy the annealing condition in a real experiment.

Note that we use simulated annealing in step (viii). This method is designed for optimizing combinatorial problems (Kirkpatrick et al., 1983). If we cool the system slowly enough from a high temperature, it is likely to be in a stable state with minimal global energy.

3. Simulation results for theorem proving

In this section, we describe a theorem-proving problem using DNA molecules and show the simulation results with the algorithm developed in Section 2.

Given a set of axioms, the objective of theorem proving is to show that a statement is true. Basically, our logical inference is made by resolution refutation (Nilsson, 1998; Lee et al., 2003). In our work, five axioms are given as $\neg Q \vee \neg P \vee R$, $Q \vee \neg T \vee \neg S$, S, T, P, which are true, and the sentence to be proved is R.

Because we are interested in the consistency of R with the given axioms, we use the proof by refutation method. First, we negate the R as $\neg R$. Then, we make conjunction of the five axioms and $\neg R$. If we obtain *nil*, which is always false, then we can say that $\neg R$ is false and finally we can prove that R is true.

We use the identity that $(\neg A \vee B) \wedge A \equiv B$ when A is true. Also note that, if A and B are true, then $(A \wedge B)$ is also true trivially. For example, let us consider that the operation of $(Q \vee \neg T \vee \neg S) \wedge S = Q \vee \neg T$. $Q \vee \neg T$ is true because both $Q \vee \neg T \vee \neg S$ and S are true. The statements obtained in each step in Fig. 1 are all true and the last resolution with $\neg R$ produces a contradiction. Thus, we can conclude R is proved.

In molecular theorem proving, we use DNA molecules for representing logical formulae and apply the hybridization of Watson–Crick complementary pairs to the process of resolution refutation. Six sentences are encoded in synthetic DNAs and their sequences are shown in Table 2. In Fig. 2, we show the structure of the target dsDNA, which is *nil* in Fig. 1. If we obtain the target dsDNA, we have proven that the theorem is true. Table 2 and Fig. 2 are adopted from Lee et al. with the permission of the authors (Lee et al., 2003).

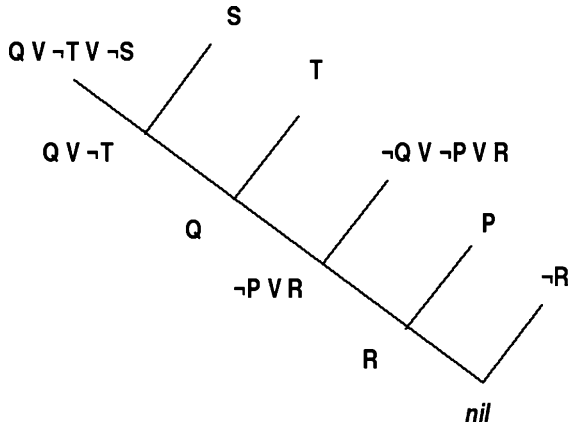

Fig. 1. Proof tree for the resolution refutation in theorem proving. Given five axioms, we need to prove R is true. If we obtain *nil* after resolving $\neg R$ with five axioms, we can conclude R is proved.

Table 2
Sequences for logical formulae used in the theorem-proving problem (in order from 5′ to 3′)

Clause	Sequence (5′→3′)
$\neg Q \vee \neg P \vee R$	CGTACGTACGCTGAA
	CTGCCTTGCGTTGAC
	TGCGTTCATTGTATG
$Q \vee \neg T \vee \neg S$	TTCAGCGTACGTACG
	TCAATTTGCGTCAAT
	TGGTCGCTACTGCTT
S	AAGCAGTAGCGACCA
T	ATTGACGCAAATTGA
P	GTCAACGCAAGGCAG
$\neg R$	CATACAATGAACGCA

We simulated the DNA reaction process for theorem proving on a silicon-based computer with the algorithm explained in Section 2. First, we built the population of the six types of strands shown in Table 2. Simulations were performed for 300, 600 and 900 molecules for each sequence. The temperature was decreased from 95 °C to 1 °C by 1 °C decrement; for each decrease in temperature, 10^8 reaction trials were made.

The simulation results are shown in Figs 3 and 4. In Fig. 3, we show the total number of pairs of *A–T*, *G–C*

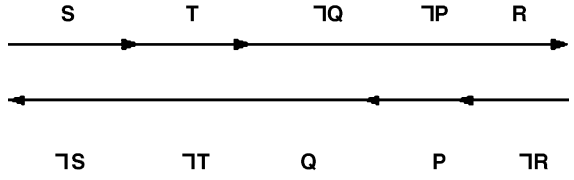

Fig. 2. Target dsDNA whose formation is the outcome of proving the theorem (the arrows are from 5′ to 3′).

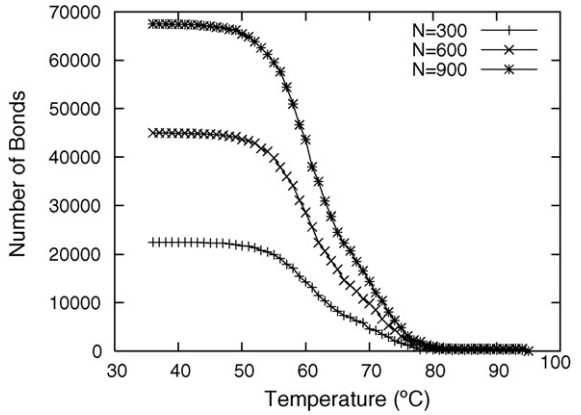

Fig. 3. Simulation result of the number of bonds as a function of temperature for $N = 300$, 600 and 900, where N is the number of ssDNA of each type. The pairing of bonds started to occur around 80 ° C and saturated at around 50 °C.

and other pairs as a function of temperature. Each curve stands for the data for $N = 300$, 600 and 900, where N is the number of ssDNAs for each species. In this figure, we found that the bonds began to form at around 80 ° C and the number of bonds saturated at around 50 °C. This simulation result is consistent with the wet-lab experimental result in the next section.

Fig. 4 shows the number of target dsDNAs as a function of temperature. These data are important because they give the solution to the theorem-proving problem. We find that the target dsDNAs began to form at around 63 ° C and the number saturated at around 45 °C. Note that this result is very hard to obtain in wet-lab experiments, which implies that our estimation predicts the number of target dsDNAs in those situations.

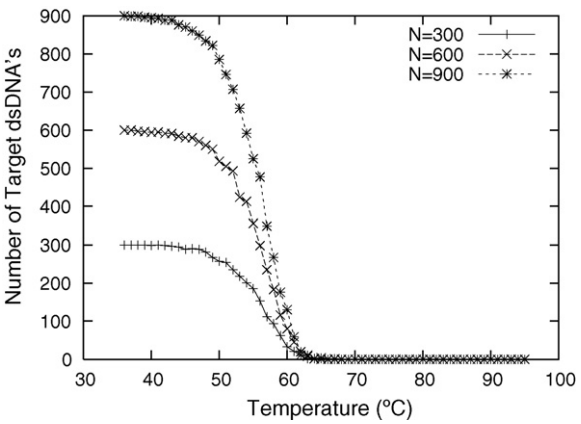

Fig. 4. Simulation result of the number of target dsDNAs as a function of temperature for $N = 300$, 600 and 900, where N is the number of ssDNAs of each type. The formation of the target dsDNAs began at around 63 ° C and saturated at around 45 °C.

4. Theorem-proving *in vitro* experiment

4.1. Design and synthesis of sequences

The sequences in this experiment were designed with the Nucleic Acid Computing Simulation Toolkit (Shin et al., 2002, 2005). The resulting oligomer sequences are shown in Table 2. All oligonucleotides were purchased from Genotech (Bioneer, Daejon, Korea). Each sequence pellet was brought to a stock concentration of 100 pmol/μl in TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8.0) and stored at −20 ° C until use.

4.2. Quantitative annealing of oligonucleotides

All six single strands were adjusted to 15 pmol (2 ×: 30 pmol, 3 ×: 45 pmol) in a final volume of 20 μl. The ratio of DNA to IQ SYBR Green supermix (100 mM KCl, 40 mM Tris–HCl, pH 8.4, 0.4 mM each dNTP, 50 U/ml iTaq DNA polymerase, 6 mM $MgCl_2$, SYBR Green I, 20 nM fluorescein) in the reaction was 1:1 (by volume). The hybridization buffer was 1 M NaCl solution. The sample was maintained at 95 ° C for 5 min and temperature annealing was performed as we cooled the sample slowly to 5 ° C using iCycler (Bio-Rad, Hercules, CA, USA). The cooling rate for annealing was 1 °C/10 min. All reactions were performed in triplicate with independent sample preparation and repeated at least twice for each reaction.

4.3. Visualization of the hybridized mixture by electrophoresis

The hybridized mixture was electrophoresed on 12% polyacrylamide gels. The running buffer consisted of 0.045 M Tris-borate and 0.001 M EDTA (pH 8.0) (TBE). Gels were run on a Mini Polyacrylamide Gel System (Bio-Rad) for 150 min at 80 V with constant voltage. To define the molecular size of the hybridized mixture, 25 bp DNA Step Ladder (Promega, Madison, WI, USA) was loaded.

4.4. Analysis of the experiment results

The SYBR Green I fluorescence intensity is proportional to the number of DNA bonds (Zipper et al., 2004). Therefore, its graph is the experimental confirmation of the simulation of the bond number. In Fig. 5, we show the fluorescence intensity of SYBR Green I as a function of temperature. Total fluorescence intensity of each of eight test tubes was normalized to the mean value. The error bars in Fig. 5 indicate the interval of 50% certainty. We

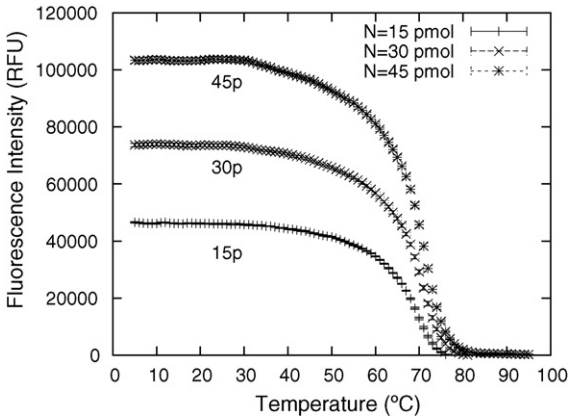

Fig. 5. Fluorescence intensity of SYBR Green I as a function of temperature. *N* is the amount of ssDNA of each type. The intensity started to grow at 80 ° C and saturated near 50 °C, which is consistent with the simulation results. The cooling rate was 1 °C/10 min and proved sufficient for annealing.

found that the fluorescence intensity started to increase at around 80 ° C and saturated at around 50 °C. Remarkably, this experimental result for the number of bonds is similar to our simulation result shown in Fig. 3.

Fig. 6 shows the gel electrophoresis image of our DNA solution. The clear band at the 75 bp region confirms that many target dsDNAs were formed after annealing, as predicted by simulation in Fig. 4. Note

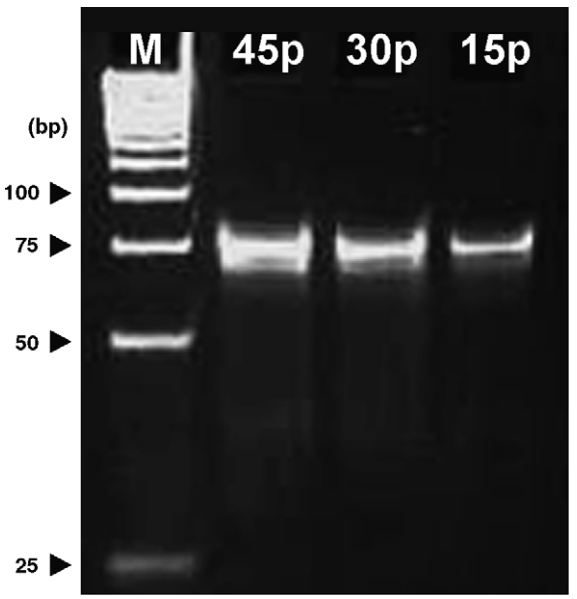

Fig. 6. Gel electrophoresis images of theorem-proving DNA solutions after annealing for 15, 30 and 45 pmol. Lane M shows the size markers, lane 1 shows 45 pmol, lane 2 shows 30 pmol and lane 3 shows 15 pmol ssDNA of each type. The brightness at 75 base pairs (bp) is proportional to the number of target DNAs.

that the number of target dsDNAs as a function of temperature could not be measured in this experiment.

5. Conclusion

We have presented an evolutionary Monte Carlo method that simulates DNA hybridization. This algorithm uses population-based evolutionary search with Metropolis selection. These two processes simulate DNA reactions in an evolutionary manner. The acceptance of a given reaction process is determined by the Metropolis algorithm considering only four parameters of enthalpy and entropy changes. Simulated annealing was used to keep the system in the state of minimum Gibbs free energy as we lowered the temperature slowly. With our evolutionary Monte Carlo algorithm, we obtained the numbers of bonds as a function of temperature. The simulation results were consistent with subsequent experiments using synthesized DNA molecules designed for solving the theorem-proving problem. The number of target dsDNAs was obtained as a function of temperature in the simulation, which is very hard to obtain in real DNA experiments. We obtained many target dsDNAs after annealing in this simulation, which was confirmed by a clear band at the 75 bp region in the gel electrophoresis image. The presence of this band showed that the theorem was proved.

Of course, there will be issues in the practical use of our algorithm. First, it does not address general mathematical problems such as Fermat's last theorem. The theorem proving in this work is a problem in artificial intelligence adopting the resolution refutation (Nilsson, 1998; Lee et al., 2003). We emphasize here that we perform this kind of theorem proving by enumerating the number of target dsDNAs, i.e. proofs, during the annealing process by taking possible combinations of statements into account. Second, there may be DNA mismatches in the annealing process. We point out that we used DNA sequences of 15-mer for each symbol, which were designed to match correctly to avoid those possible mismatches. For example, $\neg Q \vee \neg P \vee R$ is a single statement with three symbols. Note that the length of 15-mer is longer than that of 10-mer of Adleman's Hamiltonian path problem (Adleman, 1994) and is equal to that of 15-mer of the knight problem (Faulhammer et al., 2000). Third, theorem proving with five axioms and one clause seems to be easy when the number of statements is small. However, other experiments have used around 10 statements. For example, Yang et al. applied a theorem-proving approach to medical diagnosis using microRNAs, DNAs and gold nanoparticles (Yang et al., 2007). They used microRNAs as input data,

DNAs as logic elements and the gold nanoparticles as display devices. They used 10 statements. The Winfree group implemented a 12 microRNA logic-gate circuit (Seelig et al., 2006). This was used for detecting gene expression patterns. They used 12 statements. Although, we used about half the number of statements of those reported, we believe we can apply our algorithm to more statements in future work.

In conclusion, we have shown that an evolutionary Monte Carlo can simulate the DNA hybridization process. The reliability of our algorithm was confirmed by a molecular biology experiment. This algorithm could be used in modeling other DNA hybridization experiments with silicon-based computers.

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