Reaction Temperature Constraints in DNA Computing

Russell Deaton
The Department of Electrical Engineering
The University of Memphis
Memphis, TN, 38152
rjdeaton@memphis.edu

Abstract

Using the thermodynamics of DNA melting, a technique is proposed to choose a reaction temperature for the DNA computation that minimizes the potential for mishybridizations.

Adleman[Adleman, 1994] showed the computational potential of the hybridization reaction and other molecular biology protocols. A basic framework for a computation based on oligonucleotide template matching reactions, or hybridizations, consists of three steps: 1) encoding of the problem instance in DNA oligonucleotides such that solution is enabled with molecular biology protocols, 2) the basic processing of the pool of oligonucleotides with hybridization and ligation reactions, and 3) extraction of the result with separation techniques, such as polymerase chain reaction (PCR) or hybridization to probe sequences attached to magnetic beads.

Ideally, oligonucleotide hybridizations occur only between Watson-Crick complements. Depending upon the conditions under which the hybridization is done, however, base pairs that are not Watson-Crick complements, or mismatched base pair, can occur[Sambrook et al., 1989]. In addition, olgionucleotides can hybridize in various alignments that are shifted from the designed one. These mishybridizations can produce false positives, or solutions to the DNA computation that appear to be correct, but actually are not, and false negatives, or the failure to produce a solution to a problem with DNA computation when one actually exists. The effect of the reaction conditions is characterized as the hybridization stringency. In general, as the reaction temperature of the hybridization is increased up to a critical point, the stringency increases. The temperature at which half the population of perfectly matched oligonucleotide hybrids will have dissociated into single strands is called the melting temperature, T_m. The melting temperature is determined from curves of UV absorbance versus temperature, and can be interpreted as the fraction of single strands versus temperature[Wetmur, 1997]. Under conditions of low stringency, oligonucleotides can hybridize with more mismatched base pairs and over shorter lengths than under conditions of high stringency. Therefore, under assumptions of perfect Watson-Crick hybridization or perfect Watson-Crick complementation between oligonucleotides, an effect of the hybridization stringency is to introduce a possibility of false positives and negatives through mismatched hybridizations and shifted hybridizations. These mishybridizations can occur either in the basic processing step (step 2 above) or in the extraction process (step 3 above).

In this paper, a method based on the thermodynamics of DNA melting is used to estimate the reaction temperature for a given oligonucleotide encoding of a problem. The nearest-neighbor base stacking model[Borer et al., 1974] for the melting temperature of short oligonucleotides is used. The estimated reaction temperature should minimize the potential for mishybridizations. An upper limit on the reaction temperature is estimated from the melting temperature and half-width of the melting curve of the oligonucleotide with the lowest melting temperature. A lower limit is set by requiring that the oligonucleotide with the highest melting temperature have minimum potential for mishybridizing with its nearest neighbor, as measured by the Hamming distance.

Based on a model of nearest neighbor stacking interactions, a formula for non-self complementary oligonucleotide melting temperature is given by[Borer et al., 1974],

$$T_m = \frac{\Delta H^\circ}{\Delta S^\circ - R \ln(C_t/4)}, \qquad (1)$$

where ΔH° is the enthalpy change of the hybridization, ΔS° is the entropy change of the hybridization, R is the gas constant, and C_t is the concentration of the single-stranded oligonucleotides. Mismatched base pairs reduce the melting temperature, and therefore, the chance of mismatched hybridizations. In the classic estimate[Bonner et al., 1973], T_m decreases approximately 1°C per 1% mismatch between hybridizing oligonucleotides[Bonner et al., 1973].

The reaction temperature for a DNA computation must meet several criteria related to the melting temperatures of the oligonucleotides that represent the problem instance. To enable the computation, the reaction temperature should be chosen so that the pair of W-C complement oligonucleotides with the lowest melting temperature hybridize in sufficient number to allow their participation in the computation. Therefore, the reaction temperature should be less than the lowest melting temperature, T_m^l, plus 1/2 the width of the melting curve, $\Delta T^l_{(T_m)}$,

$$T_r < T_m^l + \frac{1}{2}\Delta T^l_{(T_m)}. \tag{2}$$

By choosing the reaction temperature to allow hybridization of the oligonucleotide with the lowest melting temperature, the possibility of mishybridizations between oligonucleotides with higher melting temperatures is introduced. For example, suppose we choose T_r to allow hybridization of an n-mer composed of all A-T base pairs. Then, approximately, all consecutive G-C base pairs of length $n/2$ can bind, producing potential frameshifted hybridizations. Likewise, hybridizations with mismatches are also possible. To minimize this potential for mishybridization, T_r should be chosen high enough to provide stringent conditions. To estimate the appropriate T_r, the rule of a 1°C decrease in the melting temperature for each 1% mismatch between the oligonucleotides is used. The percent mismatch between two oligonucleotides is the Hamming distance d between them under the operation of reverse Watson-Crick complementation divided by their length n[Deaton et al., 1998]. Therefore, the melting temperature of an oligonucleotide with another oligonucleotide, a Hamming distance d away is approximately

$$T_{mm} = T_m - 100\frac{1^\circ\mathrm{C}}{1\% mismatch}\frac{d}{n}, \tag{3}$$

where T_{mm} is the melting temperature of the mismatched hybridization, and T_m is the melting temperature of perfect W-C complements. To minimize the potential for mismatched hybridizations, the reaction temperature should be

$$T_r > T^h_{mm} + \frac{1}{2}\Delta T^h_{(T_{mm})}, \tag{4}$$

where the h superscript indicates the highest such melting temperature for a set of oligonucleotides.

Therefore, the reaction temperature should be chosen such that

$$T^h_{mm} + \frac{1}{2}\Delta T^h_{(T_{mm})} < T_r < T^l_m + \frac{1}{2}\Delta T^l_{(T_m)}. \tag{5}$$

This choice of reaction temperature should optimize the performance of a DNA computation with respect to mishybridizations involving mismatched base pairs.

In conclusion, the melting curves for oligonucleotide hybridization have been used to estimate a reaction temperature for a DNA computation which minimizes the potential for mishybridizations with mismatched base pairs. To summarize, the reaction temperature should be high enough to melt mishybridizations, and low enough to allow all planned hybridizations to occur in sufficient number to participate in the computation.

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