

背包问题 DNA 算法的反应设计及其生物实现

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摘 要 有关背包问题的 DNA 算法近年来得到重视,文中实现了求解背包问题的并行搜索解的实验,通过最优的方法完成有限容量背包的物品选择.展示了面向反应的 DNA 片段设计,计算过程为溶液 DNA 高效连接反应,反应结果分别用定量(PCR)和定性(测序)两种方法检测.文中的方法适用于多重约束条件的优化问题.

关键词 背包问题;DNA 计算;引物设计;高效连接;克隆测序

中图法分类号 TP384

DNA Ligation Design and Biological Realization of Knapsack Problem

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Abstract More attention has been paid to DNA computing for Knapsack Problem (KP). The authors realized the biological experiment of parallel searching to solve KP, and used the most optimized method to select items within a limited knapsack. The DNA fragments designing is based on reactions and the computational procedure is high-efficiency DNA ligation. Two methods were used to detect the results. They are PCR (quantitative analysis) and sequencing (qualitative analysis). It is suitable to the optimization of multiple restriction conditions.

Keywords knapsack problem; DNA computing; primers design; high-efficiency ligation; clone sequencing

1 Introduction

DNA fragments have the large storage capabilities and amazing efficiency of molecular self-organization. These two characteristics inspire researchers with designing of DNA computers^[1-2]. In previous work, more attention has been put in the sigma completeness of mathematical theories.

Thus, DNA computers are focused on paradigm or theoretical model. Therefore, DNA computing is limited to the design of “cut-ligase” theoretical system, without full consideration of the DNA molecular ligation efficiency. Sometimes, there is possibility to design the ligation reactions up to decades of fragments to meet the criteria of the problem, regardless of the reaction efficiency or full usage of

收稿日期:2008-04-20;最终修改稿收到日期:2008-10-27. 本课题得到国家自然科学基金(60775052,70701009)、上海市科学技术委员会重点基础研究项目(08JC1400100)、上海市人才发展资金(001)及上海市领军人才后备人选专项资金资助. 朱莹,女,1980年生,博士研究生,从事DNA计算和生物信息学等研究. 任立红,女,1966年生,博士,副教授,从事智能系统、网络智能、DNA计算和生物网络结构等研究. 丁永生(通信作者),男,1967年生,博士,教授,博士生导师,从事智能系统、网络智能、DNA计算、人工免疫系统、生物网络结构、生物信息学、数字化纺织服装、智能决策与分析等研究. E-mail: ysding@dhu.edu.cn, Kongsuwan Kritaya,女,博士,研究员,主要从事动物和人类健康的基因和蛋白质定位研究.

DNA parallel computing energy which is brought by replicates and separates of DNA molecules. In order to illustrate the features of DNA ligation, we designed the DNA computing reaction steps and solutions of Knapsack Problem (KP).

KP is one of the classical optimization problems in operational research^[3-4]. It has important applications in budget control, project selection, material cutting and cargo loading, etc. In solving large problem of multiple operational research algorithms, KP is also regarded as a sub-problem to deal with. For example, satellite communications, designing of shared computer system, in which each progress will lead to additional expenditure of CPU, and layout of shopping centre, in which the type of vendor should be balanced with its scale^[5]. With the continuous development of network technology, Knapsack public key plays an important role in the e-commerce public key designing. It is beneficial to complicated algorithm of multiple operational research if we make development of KP.

KP is a NP difficult problem, which is studied by current researchers in conventional computers^[6-8]. DNA computing which has direct coding and algorithm is the most suitable algorithm to solve this kind of problem. The size and weight of each item are directly mapped into physical characteristics of DNA fragments. Furthermore, these problems are multiple conditions optimization. The length and mass of DNA fragments can be regarded as computing and detecting methods. Currently, a DNA algorithm was designed to solve a standard KP^[9]. In this paper, we will attempt different DNA fragments to solve all kinds of KPs.

2 Reaction Design and DNA Coding

2.1 Mathematical Mode of Knapsack Problem

The mathematical mode of a KP is:

max $f(x_1, x_2, \dots, x_n) = \sum_{j=1}^n c_j x_j, j=1, 2, \dots, n$ (1)

s. t. $\sum_{j=1}^n a_{ij} x_j \leq b_j, i=1, 2, \dots, m; x_j \in \{0, 1\}$ (2)

In the equation, n is the number of each item. m is the number of resource. c_j is the profit of item j . b_i is the budget of resource i . a_{ij} is the quantity of resource i occupied by item j . x_j is the 0-1 decision-making parameter (when item j is selected, $x_j=1$; else, $x_j=0$).

KP can be described as the following: Now we have $j(j=1, 2, \dots, n)$ items, each item will consume m kind of resources $a_{ij}(i=1, 2, \dots, m)$. We

will get profit c_j by putting item j into the knapsack. Meanwhile, the total consumed resources I of all the put-in items should not exceed b_i . That is to say, we shall find the best way to put items with different sized and weights into a limited volume knapsack to meet the maximum total value.

2.2 DNA Ligation Fragments Design

According to the mathematical model of KP in the previous section, we provide a DNA computing algorithm and detecting method in solution to solve this Binary KP (BKP). The following is an example of BKP to illustrate it.

Given a set of items S , there are $n=5$ items, among which item i weights w_i and values v_i . We take some items from S to put in the knapsack, maximizing the total value, but within the limitation of total weight, 80kg. The item features is shown in Table 1.

Table 1 Weight and Price of Each Item in Knapsack		
i	w_i (kg)	v_i (dollar)
1	15	33
2	20	24
3	17	36
4	8	37
5	31	12

We design an oligonucleotide fragment D_i to represent item i . The fragment's length is in according with item i 's weight w_i . In order to make high-efficiency of DNA fragments ligation in solution, we set the DNA code for each item as shown in Fig. 1. w_i is twice the item's weight minus 10. It is a double stranded DNA fragment which has 5bp sticky end at both sides. The sticky ends are to make difference among each items. Theoretically, 5 bp can represent 4^5 items. If there are lots of items, we can make more longer sticky ends.

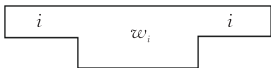


Fig. 1 One DNA strand for item i

The linker is the complementary oligonucleotide DNA strand, partly from D_i and partly from D_j . Thus we give the linker a name as $\overline{D}_{i \rightarrow j}$. With the ligation enzymes in the solutions, item D_i and linker $\overline{D}_{i \rightarrow j}$ will combine each other randomly. To make sure that each item can be put in the knapsack only once, $\overline{D}_{i \rightarrow j}$ should meet the criteria $\{i, j \in [1, 2, 3, 4, 5]\}$, whereas $i < j$. More specifically, we design D_1, D_2, D_3, D_4, D_5 as the followings which is shown in Fig. 2.

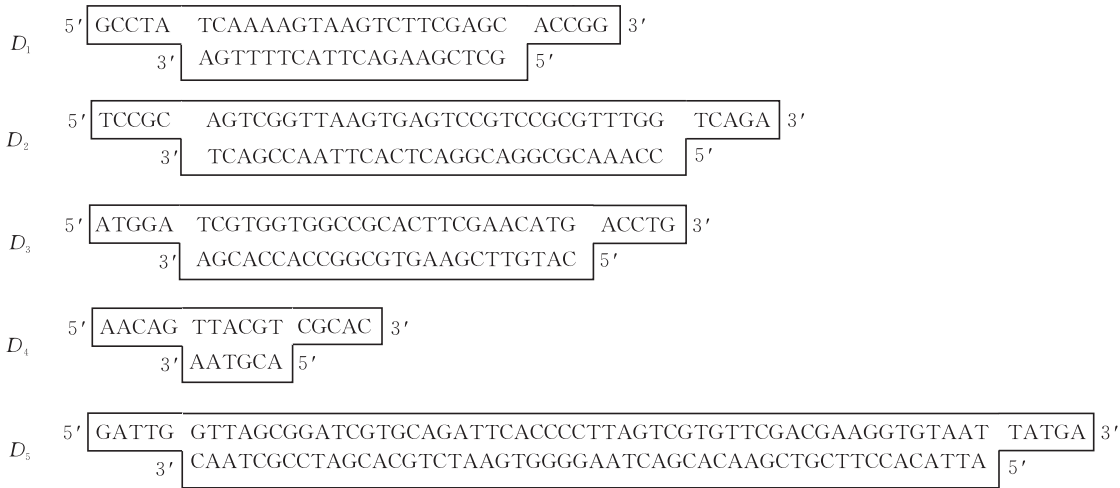


Fig. 2 DNA strands of each item

Meanwhile, we order 10 linker fragments. They are $\bar{D}_{1 \rightarrow 2}$, $\bar{D}_{1 \rightarrow 3}$, $\bar{D}_{1 \rightarrow 4}$, $\bar{D}_{1 \rightarrow 5}$, $\bar{D}_{2 \rightarrow 3}$, $\bar{D}_{2 \rightarrow 4}$, $\bar{D}_{2 \rightarrow 5}$, $\bar{D}_{3 \rightarrow 4}$, $\bar{D}_{3 \rightarrow 5}$, $\bar{D}_{4 \rightarrow 5}$. Note that $i < j$, and the linkers are the exact complementary strands of the 5bp sticky ends, which are combined together.

These oligonucleotide fragments are put into one tube for ligation. The result DNA strands represent a solution set of random item combination. The 10 linkers of single strand DNA fragments are shown in Fig. 3.

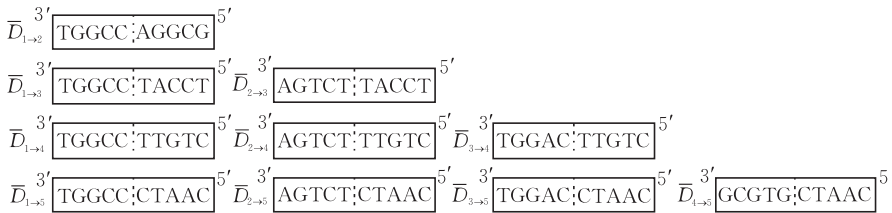


Fig. 3 Linkers between every other items

2.3 Computing and Detecting

(1) Computation

Consider that the ligation between the hydrogen bond of double-stranded items and linkers are not firm. It is better to add a phosphate group at the sticky ends so that the DNA strands can be bonded firmly.

We mix the DNA fragments which represent items and linkers in the knapsack. The reaction results will be DNA strands, whose mobility ratio is in reverse logarithm relationship with its quantities of base pairs in the gel substance. Therefore, we only reclaim all the DNA fragments whose length is no more than 160 bp.

(2) Detection

The feasible solution is from biological detection. Beside taking the double-stranded DNA items and linkers as the reaction materials, which is mentioned in the previous section, it is necessary to put in high-efficient enzymes and corresponding detection methods in order to use DNA solution ligation reaction to realize the algorithm of KP. In this section, we explain the two detection meth-

ods, i. e. PCR for quantitative analysis and sequencing for qualitative analysis.

Cut the gel, which contains a cluster of DNA molecules (near the band of 160bp or so) from the previous step. Put the gel into PCR procedure, if there exists a clear band, it means there is a set of feasible solutions. In according to oligonucleotide fragment D_i , the number i component in the solution set is $x_j = 1$, else $x_j = 0$. As far as all the DNA molecules can be detected in parallel, the computation procedure happened simultaneously.

As soon as the feasible solution set S is get, we select those bacteria that contains the right solution by blue-white screening for sequencing. Read each feasible solution in set S , and compare its value of target function. In this example, the optimized solution is (1,1,1,1,0), and the corresponding target function value is 111bp. In reference with the previous designed DNA fragments for each items, the result is that we should select item 1, item 2, item 3, item 4 into the knapsack, and the total weight is 60kg. The result DNA strand is drawn in Fig. 4.

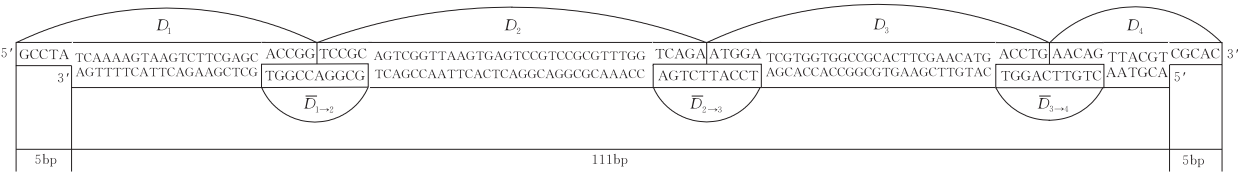


Fig. 4 DNA strand of the optimized solution

3 Materials and Methods

3.1 Preparation of Materials

(1) Markers (ladders) details

The ladders we used in experiment were Fermentas GeneRuler DNA ladder^[10-12]. Ultra Low Range. See Fig. 7 below for details of ladder.

(2) All primers details, including dsDNAs, linkers and PCR primers

Lists of the oligonucleotides that we ordered

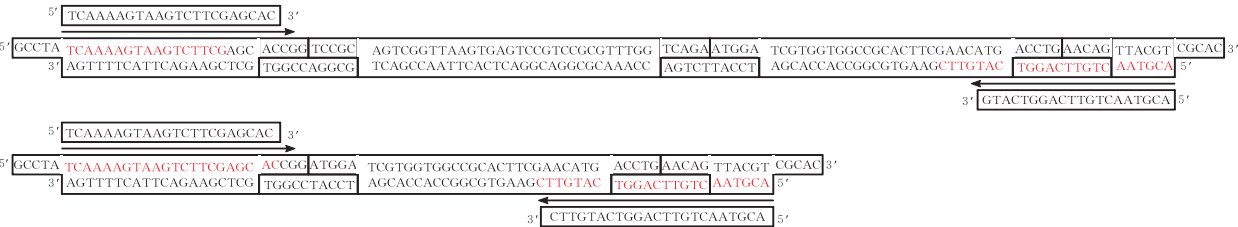


Fig. 5 These primers cannot tell the difference of the two similar potential results

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Fig. 6 Primers designing performances

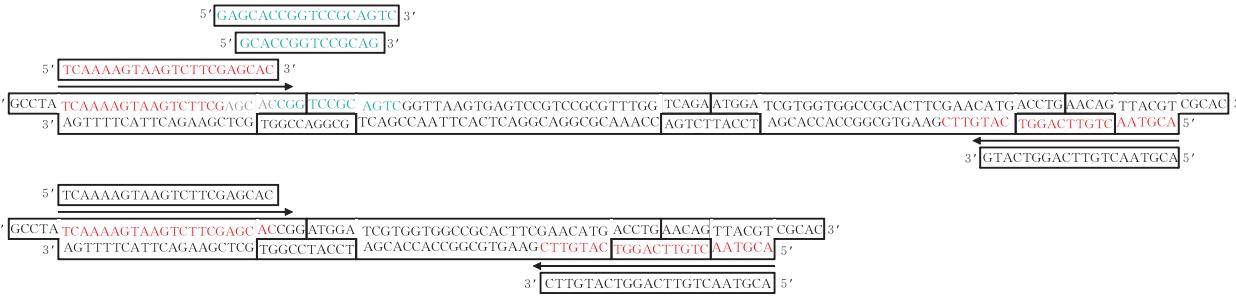


Fig. 7 The primers that can tell the difference of the two similar potential results

From Fig. 5, we can see that if we follow the rule of primer designing, which tells us that all primers need to be 20bp in length. Therefore, in this example, the primers could not span the linkers. Meanwhile, the second solution strand will be amplified by PCR inadvertently. Furthermore, we design multiple primers to span the linkers, after PCR, only the first DNA strand is amplified. Fig. 6 shows the primers parameters we used.

(3) Preparation of PCR Materials

For the PCR we used Invitrogen Taq DNA Polymerase. Taq polymerase is a thermostable DNA polymerase named after the thermophilic bacterium *Thermus aquaticus* from which it was originally isolated^[13]. Taq polymerase was identified^[13] as an enzyme to be able to withstand the protein-denaturing conditions (high temperature) required during PCR^[14]. Therefore it replaced the DNA polymerase from *E.coli* originally used in PCR^[15]. Taq’s temperature optimum for activity is 75°C ~ 80°C, with a halflife of 9 minutes at 97.5°C, and can replicate a 1000 base pair strand of DNA in less than 10 seconds at 72°C^[16].

3.2 Experiment Procedures

(1) Put D1 to D5 and all the linkers in tubes and concentration.

(2) Dilute oligonucleotides, linkers and primers. The lypholised oligonucleotides, linkers and primers were made up to 1mM concentration with 10mMTrisEDTA buffer.

Double Stranded DNA was generated by mixing 10ul each of the 1mM stock solution short and long fragments. The mixture was heated to 90°C and allowed to cool to room temperature over 30 minutes during which time the long and short fragments annealed. From the 1mM stock solution linkers were diluted to 5uM working solutions. For dsDNA the mixture was diluted to a 1uM working solution. These working solutions were made up using 10mMTrisEDTA buffer as well.

Prior to beginning the experiment, the working solutions of the linkers and dsDNA (see point 2 below) were treated with *T*₄ Polynucleotide Kinase to add a Phosphate group to the 5’ end of each oligonucleotide (as we discussed). This step is essential for the successful ligation of synthesised oligonucleotides.

(3) Ligation mixture.

It is shown in Table 2.

Table 2 Ligation Mixture Proportion

Solution Name	Solution Concentration	Solution Dose
Linkers	5microMol/L	0.5microL each
dsDNA	1microM/L	2.5microL each
T4 DNA Ligase	10units/microL	1microL
Buffer 5x		1microL
Total		20microL

Incubate the ligation reaction at 4°C for 16hrs. We did not add 4ul of 5x buffer, because in the *T*₄ Polynucleotide Kinase reaction the buffer for this reaction was *T*₄ DNA ligase buffer, so the linkers, and dsDNA were already buffered with the *T*₄ DNA ligase buffer. The 1ul of 5x buffer was added to the ligation reaction to top up the amount of ATP in the ligation reaction.

3.3 Cloning and Sequencing

(1) Gel Extraction.

(2) Cloning.

The process of cloning is divided into several steps, and takes numerous days to complete. The procedure is illustrated in Fig. 8.

(3) Analyzing Positive Clones.

At this step we did a PCR screen of the colonies to check for the presence of an insert of the approximate size we are after. We do not have an electronic copy of this result. Those colonies that show an insert of the correct size we went ahead with, are as shown in Fig. 9.

Transformation	1) Cloning reaction set-up: 4.5ul gel excised clean up (there will be 5 of these) 1ul salt solution 1ul TOPO vector 2) Mix gently. Incubate at room temperature for 30 min 3) Add 2ul to 50ul of DH5alpha E. coli cells and mix gently 4) Incubate on ice for 30 minutes 5) 'Heat-shock'-incubate at 42°C for 30 secs. Place immediately back on ice
Recovery	6) Add 250ul room temperature S.O.C. medium 7) Cap the tubes and shake at 37°C for 1hr
Plating	8) Spread on pre-warmed LB plates already containing 50mg/L Ampicillin, as well as 40ul of 40mg/ml X-gal and 40ul of 100mM IPTG. The ampicillin-an antibiotic-allows only those bacteria containing the vector you want to grow. The x-gal allows for what is referred to as blue/white screening). 9) Incubate at 37°C for 14hrs

Fig. 8 Preparation of cloning

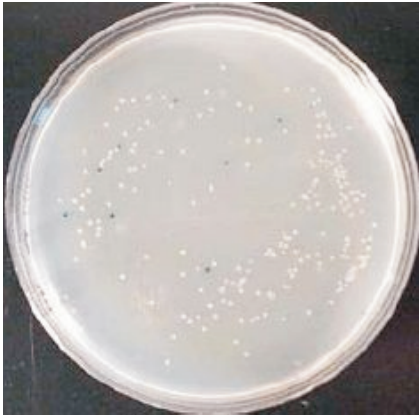
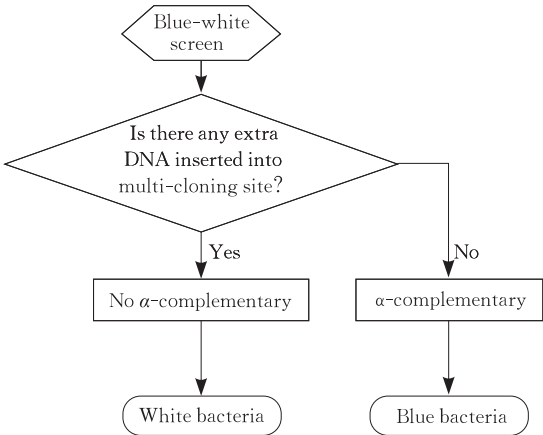


Fig. 9 Flowchart and picture of blue-white screen

Pick five white colonies from the plates. Place these pickings into 10ml LB containing 50mg/L Ampicillin. Incubate for at 37°C shaking for 14hrs. White colonies indicate that an insertion has occurred in the vector — possibly the DNA fragment we want. Blue colonies indicate no insertion in the vector.

(4) Miniprep which is a method of extracting the plasmid (vector) so that you can use this plasmid for further work (eg. verifying clone by sequencing).

(5) Clean up the sequencing sample.

(6) Use the Applied Biosystems Genetic Analyser to sequence.

4 Experiment Results

4.1 PCR Result of Reaction Product

PCR Cycling conditions are set to 94°C × 2 1/2 minutes, 94°C × 15 seconds, Various annealing (57°C ~ 63°C) × 15 seconds, 72°C × 60 seconds (35 cycles), 72°C × 7minutes. We used a Biorad icycler thermal cycler. The PCR gel products are shown in Fig. 10.

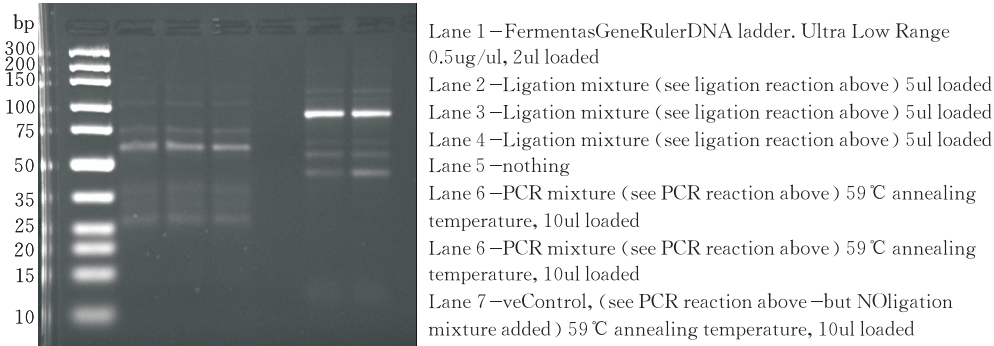


Fig. 10 Gel Picture after PCR and descriptions of each lane

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