

# RCA-Based Detection Methods for Resolution Refutation

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**Abstract.** In molecular resolution refutation, the detection of empty clauses is important. We propose a rolling circle amplification-based detection method for resolution refutation. The rolling circle amplification (RCA) technique is known to be able to distinguish and amplify circular DNA. In this paper, we describe the representation of clauses and the RCA-based detection method. Bio-lab experiments show the basic idea for this method is correct.

## 1 Introduction

Many researchers utilized the massive parallel reaction of DNA molecules to perform logical computation [8, 9]. In our previous work, we proposed a DNA computing method for theorem proving with resolution refutation in propositional logic using hybridization, PCR and gel electrophoresis [5]. But, as indicated in that paper, there was some difficulty with the PCR step because of DNA structure.

In this paper, we propose a detection method using the rolling circle amplification (RCA) technique to overcome these difficulties. RCA is known to be able to amplify circular DNA, and can be used to distinguish various topological structures [4]. As will be explained later, the empty clause has circular form. Therefore, RCA can be used to detect and amplify the empty clause.

In the following, the improved method of molecular resolution refutation will be described. We performed a simple experiment to test the feasibility of the proposed method and provided the result. In addition, we applied the method for proving the pigeon hole principle in lab experiment (in progress).

The rest of the paper is organized as follows. A brief introduction to the pigeon hole principle (PHP) and RCA is given in Section 2. Section 3 will describe experimental results and conclusions are drawn in Section 4.

## 2 Methods

### 2.1 Resolution Refutation and the Pigeon Hole Principle

Refutation is a technique which proves the target formula by showing that the negation of the goal results in inconsistency. Resolution refutation is a kind of refutation which uses the resolution when showing the inconsistency. It requires that every formula is expressed in clause form. A clause form in propositional logic is defined as a set of literals connected with disjunctions ( $\vee$ ). A clause which contains no literal is called an empty clause (*nil*). From two clauses  $\mathcal{A}$  and  $\mathcal{B}$ , resolution draws  $(\mathcal{A} - \{v\}) \vee (\mathcal{B} - \{\neg v\})$ , where  $v$  be a literal such that  $v \in \mathcal{A}$  and  $\neg v \in \mathcal{B}$ . We say that we resolved  $\mathcal{A}$  and  $\mathcal{B}$  on  $v$  and the product of resolution is called a *resolvent*. During applying resolution repeatedly, if a *nil* is produced, it is shown that the given clauses are not consistent. For it is proven that the negation of the goal leads to inconsistency, the goal is proven.

As a benchmark problem for the scaled-up version of our previous work [5], we choose the PHP which has been used as a test case for a proof system. Generally speaking, the PHP means a tautology which states that there exists no one-to-one mapping from  $m$  objects (pigeons) to  $n$  objects (holes) where  $m > n$ , and is denoted as  $PHP_n^m$  [1]. To prove  $PHP_n^m$  with resolution refutation, we need to express the negation of  $PHP_n^m$  in clause form as follows:

1.  $P_{i,1} \vee P_{i,2} \vee \dots \vee P_{i,n}$ , for each  $i \leq m$ , and
2.  $\neg P_{i,k} \vee \neg P_{j,k}$ , for each  $i, j \leq m, k \leq n, i \neq j$ ,

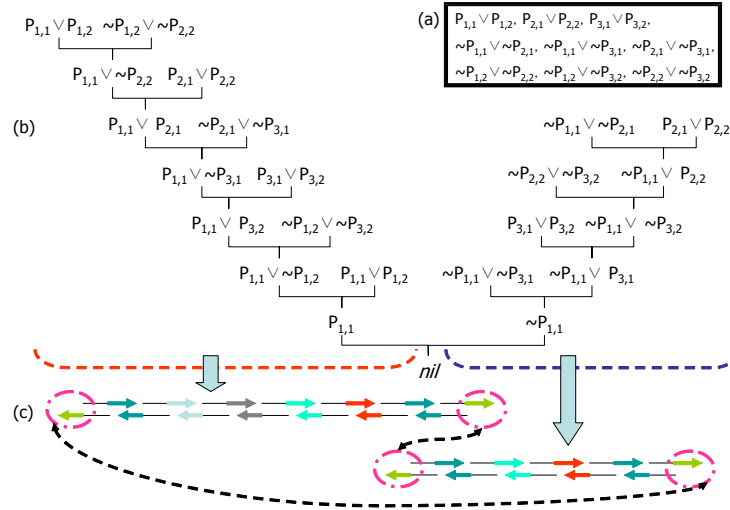
where  $P_{i,j}$  denotes the mapping of pigeon  $i$  to hole  $j$ . In this paper,  $PHP_2^3$  will be considered. The clauses of  $\neg PHP_2^3$  and one of its proof tree are given in Fig. 1-(a,b).

### 2.2 RCA-Based Detection of Empty Clauses

Before explaining the RCA-based detection method for the empty clause, let us explain molecular representation of clause form. As in [8], each variable is encoded with a unique sequence. And the negation of the variable is represented with its complementary sequence. A clause with  $c$  literals is represented with molecules of  $c$  branches. Each branch has a sticky end whose single-stranded region corresponds to each literal in the clause.

With this scheme, the hybridization of complementary sticky ends means the resolution of a literal from the clauses. To prevent inverse resolution, we ligated the hybridization mixture. In our example of  $PHP_2^3$ , an empty clause will have the form of a double-stranded ring as illustrated in Fig. 1-(c). In this way, each DNA ring represents a proof tree.

Now, all that is left is the detection of the empty clause, if any. Detecting a small amount of DNA is very difficult. Therefore, we used RCA to amplify the empty clause selectively, for the empty clause has a circular structure. To get more clear output, we deleted the non-empty clauses using exonuclease-III. Since the non-empty clauses have at least one sticky end, exonuclease-III can digest them.



**Fig. 1.** (a) The clauses of  $\neg PHP_2^3$ . (b) One of possible resolution proof trees. (c) The molecular proof tree constructed from (b). The pair of ellipses connected to each other by dashed arrows represent complement sticky ends. If these two sticky ends hybridize together, a double-stranded DNA ring will be constructed

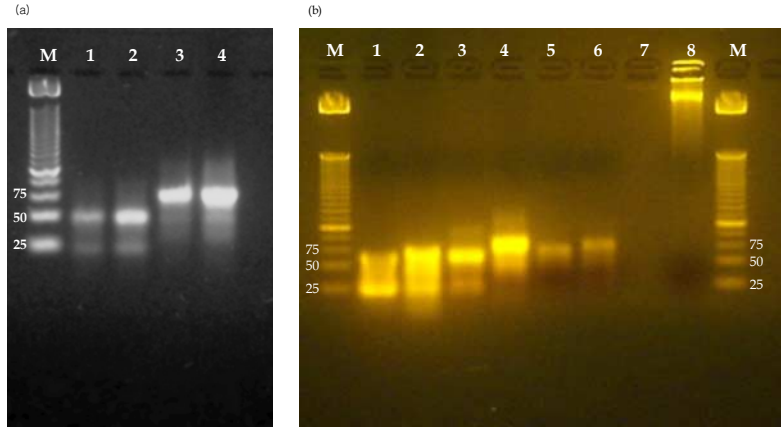
### 3 Experimental Results

We tested the principle of the RCA-based method on a test problem. We test whether the RCA technique can be used to distinguish circular DNA from others. More specifically, we tried to amplify molecules of three different kinds of structure. The three kinds of structure compared are sticky ends, blunt ends, and a circular form. If only circular DNAs are amplified, we can conclude that the empty clause can be detected and amplified by RCA.

The sequences used to make sticky ends and blunt ends were designed by NACST/Seq using evolutionary optimization as described in [7]. For a circular DNA, we used the pUC19 plasmid. All oligonucleotides were synthesized by Genotech Co. The designed sequences are shown in Table 1. To determine the condition for the enzyme reaction, we referenced [2, 3, 6]. The experiment includes hybridization, reaction with exonuclease-III, RCA and gel electrophoresis.

**Table 1.** Sequences designed for the test experiment

Sequences with two sticky ends	
3'-	CGTACGTACGCTGAACTGCCTTGCCTTGACTGCGTTCATTGTATG-5'
5'-TTCGTCATCGCTGGTTAACTGCGTTAACTGCATGCATGCGACTT	-3'
Sequences with blunt ends	
3'-AAGCAGTAGCGACCAATTGACGCAAATTGACGTACGTACGCTGAACTGCCTTGCCTTGACTGCGTTCATTGTATG-5'	
5'-TTCGTCATCGCTGGTTAACTGCGTTAACTGCATGCATGCGACTTGACGGAACGCAACTGACGCAAGTAAACATAC-3'	



**Fig. 2.** (a) The electrophoresis of oligonucleotide mixture of blunt ends and sticky ends on 3% agarose gel. Lanes 1,2: hybridization result of sticky ends. 100 pmol and 200 pmol of the mixture, respectively. Lanes 3,4: hybridization result of blunt ends. 100 pmol and 200 pmol of the mixture, respectively. Lane M: 25 bp ladder. (b) The electrophoresis of RCA results on 3% agarose gel. Lanes 3,4: hybridization result of blunt ends and sticky ends respectively. Lanes 5,6: RCA product of lanes 3 and 4, respectively. Lane 7: Exonuclease III digested product of lanes 3 and 4. Lane 8: RCA product of pUC19. Lane M: 25 bp ladder

Fig. 3 shows the result of hybridization and gel electrophoresis. To confirm the formation of sticky ends and blunt ends, we analyzed the hybridization mixture by gel electrophoresis as shown in Fig. 3 (a). As can be seen in lane 5 of Fig. 3 (b), the molecules with blunt ends or sticky ends were amplified by RCA. But in comparison to lane 8, the positions of bands in each lane are different. Amplifying circular template with RCA produces very long strands. On the contrary, RCA with linear template produces strands of the same length with the original. As a result, with the RCA method, we can distinguish circular DNAs from the others.

The results demonstrate that RCA can amplify the circular DNAs selectively, and thus can be used to detect the empty clauses. Based on this result, we applied the method to proving  $PHP_2^3$ . In this case, the overall procedure is the same as that of the previous experiment. But, the ligation step is included after hybridization. In this ligation step, all clauses in a proof tree get connected and are never separated in the following steps. The experiment for this problem is in progress.

## 4 Conclusions

We presented an RCA-based detection method for solving theorem proving problems. By trying to amplify molecules with various structures, we showed that

RCA can be used to detect a circular DNA. Since the empty clause has a circular form in our representation, this result supports the RCA-based detection method for empty clauses. Because our method uses a constant number of simple lab operations, it is highly extensible. Also, with the help of lab-on-a-chip technique, it may be automated.

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