Rolling circle amplification with electrochemical detection assay for SARS-CoV-2

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Method Article

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Abstract

This protocol describes the rolling circle amplification (RCA) and electrochemical detection of SARS-CoV-2. The procedure consists of 3 parts, which are the amplification, hybridization and detection steps. In the presence of RNA template, the circular DNA template will be ligated to produce a Padlock DNA, which serves as a template for amplification by phi29 DNA polymerase to produce RCA amplicons. The RCA amplicon is a concatemer containing multiple repeats of sequences that are complementary to the circular template. The RCA amplicons are hybridized with redox active probes and detected by electrochemical biosensor using differential pulse voltammetry (DPV). Due to its isothermal nature, RCA can be performed using a simple water bath or heating block. Overall, the whole assay takes approximately 45 min. The assay enables rapid, quantitative results to be obtained for detection of SARS-CoV-2, either in the laboratory or more importantly, in a field setting.

Introduction

The global outbreak of coronavirus disease (COVID-19) is caused by the rapid spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[1]. A person infected with SARS-CoV-2 can either remain asymptomatic or show non-specific clinical symptoms such as fever, cough, or shortness of breath. Even during the incubation period, an infected person is highly contagious and can spread the virus to a non-infected person[2]. Thus, rapid diagnostic testing for SARS-CoV-2 at a large scale is crucial for virus detection, surveillance and swift management of outbreaks[3]. Rolling circle amplification (RCA) is an isothermal amplification method, which been widely used for nucleic acid testing[4]. The assay consists of two parts of amplification step, including ligation and amplification steps. The RCA assay involves the amplification of DNA or ribonucleic acid (RNA) primers annealed to a circular DNA template and generated a concatemer containing multiple repeats of sequences complementary to the circular template using DNA or RNA polymerases[5]. A significant advantage of RCA is that can be performed using a simple water bath or heating block with minimal reagents in a short time[6]. This allows for rapid, widespread deployment of testing kits to outbreak areas or remote regions where laboratory facilities do not exist or are difficult to access. In this protocol, we describe an assay based on electrochemical biosensor with RCA for the rapid detection of SARS-CoV-2. The assay involves sandwich hybridization of RCA amplicons with probes that are functionalized with redox active labels, which are subsequently detected by differential pulse voltammetry (DPV).

Reagents

· 5U/mL T4 DNA ligase and 10X DNA ligase buffer [Thermo scientific, Cat. No. EL0011]

· deoxyribonucleotide triphosphate (dNTPs Set) [Thermo scientific, Cat. No. R0181]

Important note: The set consists of 100 mM aqueous solutions of dATP, dCTP, dGTP and dTTP (Cat. No. R0182) each supplied in a separate vial, preparation 10mM dNTP mixture according to the manufacturer's protocol and mixed well each dNTP solution prior to use.

· phi29 DNA polymerase (10U/µL) and 10X phi29 polymerase buffer [Thermo scientific, Cat. No. EP0092]

· Dynabeads™ MyOne™ Streptavidin T1 [Thermo Fisher Scientific, USA, Cat. No 65601]

· Capture Probe (for *N* gene): CGCAACTGAACTACTTGTCG - Biotin (Integrated DNA Technologies, Singapore)

· Reporter probe (for *N* gene): CAAGATATCGCGTCCTAC – Biotin (Integrated DNA Technologies, Singapore)

· Circular DNA template (for *N* gene): TACGTGATGACGCAACTGAACTACTTGTCGCTGTAGTTCAAGATATCGCGTCCTACCTGTTGCGAC (Integrated DNA Technologies, Singapore)

· Linear target (for *N* gene): GTTCCTCATCACGTAGTCGCAACAGTTCAA (Integrated DNA Technologies, Singapore)

· Capture Probe (for *S* gene): CGCAACTGAACTACTTGTCG - Biotin (Integrated DNA Technologies, Singapore)

· Reporter probe (for *S* gene): ATTCTGTCATGCGCTCAC – Biotin (Integrated DNA Technologies, Singapore)

· Circular DNA template (for *S* gene): ACCAATGGGTCGCAACTGAACTACTTGTCGCTGTAGTTATTCTGTCATGCGCTCACATATACCTGC (Integrated DNA Technologies, Singapore)

· Linear target (for *S* gene): GACATACCCATTGGTGCAGGTATATGCGCT (Integrated DNA Technologies, Singapore)

Equipment

· Digital water-bath (Labtech, model: LWB-106D)

· Hotplate (IKA, model: C-MAG HS7)

· Thermo Scientific™ NanoDrop™ One Microvolume UV-Vis Spectrophotometer (Thermo scientific, Cat. No. 13-400-518)

· 2-electrode screen printed carbon electrodes (Quasense Thailand, Cat. No. BI1701OR)

· PalmSens4 potentiostat with PSTrace software (Palmsens, The Netherlands)

Procedure

RCA assay

1. Prepare ligation solution by mixing 4 mL of 10X DNA ligase buffer, 1 mL of 5U/mL T4 DNA ligase, and 32.6 mL sterile water. **Tip:** Briefly centrifuge to eliminate the air bubbles.

2. Add 0.6 mL of 1 mM circular DNA template and 1.8 mL of target DNA/cDNA/RNA in the tube.

3. Incubate the reaction tube at room temperature for 10 min. Then, heat it at 65°C for 5 min to deactivate the enzyme. The ligation product is called as Padlock DNA. **Tip:** The ligation product can be stored at 4°C until use.

4. In a new reaction tube, prepare the mixture of 1 mL dNTPs, 1 mL of 10X phi29 polymerase buffer, 0.1 mL of phi29 DNA polymerase (10U/µL), and 4.9 mL sterile water.

5. Add 3 mL of the Padlock DNA into the reaction tube in step 4.

6. Incubate the tube at 30°C for 30 min. Then, perform heat inactivation at 95°C for 5 min. The reaction product is the RCA amplicons.

One-step hybridization

1. Add 2 mL of capture probe-conjugated magnetic bead particles, 20 mL of reporter probe modified with redox label and 18 mL of RCA amplicons into a tube.

2. Mix well and incubate at 50°C for 30 min.

3. Then, apply a magnet to the side of the tube for 1.5 min to separate the solid-solution phase.

4. Pipette out the supernatant and add 400 mL of 20 mM PBS-Tween to wash the pellet. **Tip:** Keep the magnet in place while performing the washing step.

5. Pipette out the supernatant and add 400 mL of 20 mM PBS to wash the pellet. Repeat this step twice. **Tip:** Keep the magnet in place while performing the washing step.

6. Resuspended the washed pellet with 150 mL of 0.1 M PB/KCl (pH 7.0).

Electrochemical detection

1. Pipette 30 mL of the hybridization reaction onto the screen-printed carbon electrode.

2. Perform differential pulse voltammetry (DPV) by scanning from -0.8 to -0.05 V, with a step potential of 0.01 V, modulation amplitude of 0.1 V with an interval time of 0.01 sec, and 0.1V/s scan rate.

Troubleshooting

The RCA reaction can be confirmed by resolving the reaction product using 0.8% agarose gel in Tris-acetate-EDTA (TAE) buffer and visualizing the band under UV light. Successful amplification will produce a high molecular weight band near the well of the gel.

Time Taken

Take about 45 min for the whole process.

Anticipated Results

In the presence of the target, a current signal that is proportional to the target concentration would be obtained.

References

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