

Rolling circle replication produces multiple copies of a single circular template.

**Rolling circle replication** (RCA) is a process of unidirectional nucleic acid replication that can rapidly synthesize multiple copies of circular molecules of **DNA** or **RNA**, such as **plasmids**, the **genomes** of **bacteriophages**, and the **circular RNA** genome of **viroids**. Some eukaryotic viruses also replicate their **DNA** or **RNA** via the rolling circle mechanism.

As a simplified version of natural rolling circle replication, an isothermal DNA amplification technique, rolling circle amplification was developed. The RCA mechanism is widely used in [molecular biology](#) & [biomedical nanotechnology](#), especially in the field of [biosensing](#) (as a method of signal amplification).<sup>[1]</sup>

## ^ Circular DNA replication

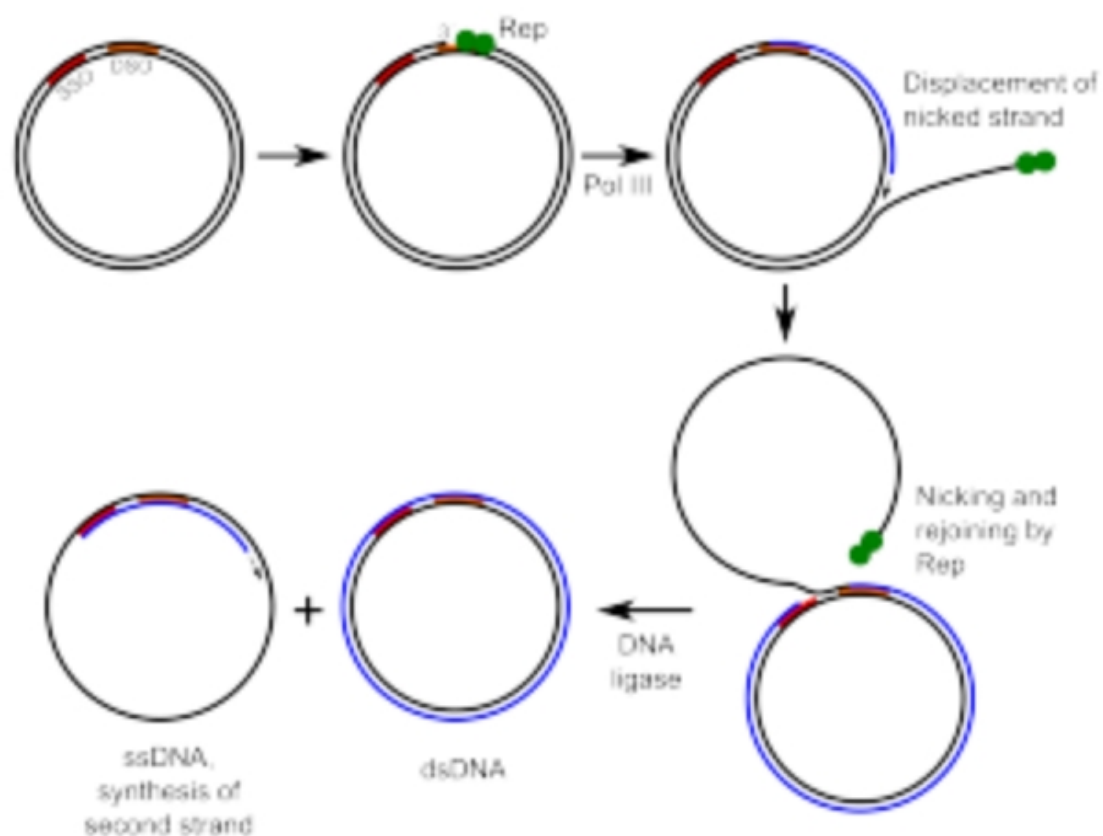


Illustration of rolling circle replication.

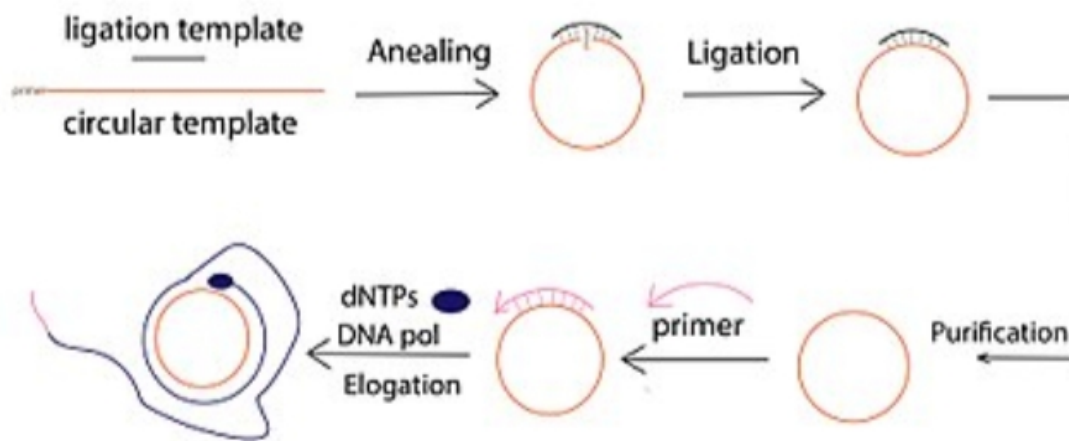
Rolling circle **DNA replication** is initiated by an initiator protein encoded by the plasmid or bacteriophage DNA, which nicks one strand of the double-stranded, circular DNA molecule at a site called the double-strand origin, or DSO. The initiator protein remains bound to the 5' phosphate end of the nicked strand, and the free 3' hydroxyl end is released to serve as a **primer** for DNA synthesis by **DNA polymerase III**. Using the unnicked strand as a template, replication proceeds around the circular DNA molecule, displacing the nicked strand as single-stranded DNA. Displacement of the nicked strand is carried out by a host-encoded helicase called PcrA (the abbreviation standing for plasmid copy reduced) in the presence of the plasmid replication initiation protein.

Continued DNA synthesis can produce multiple single-stranded linear copies of the original DNA in a continuous head-to-tail series called a **concatemer**. These linear copies can be converted to double-stranded circular molecules through the following process:

First, the initiator protein makes another nick in the DNA to terminate synthesis of the first (leading) strand. **RNA polymerase** and DNA polymerase III then replicate the single-stranded origin (SSO) DNA to make another double-stranded circle. **DNA polymerase I** removes the primer, replacing it with DNA, and **DNA ligase** joins the ends to make another molecule of double-stranded circular DNA.

As a summary, a typical DNA rolling circle replication has five steps:<sup>[2]</sup>

1. Circular dsDNA will be "nicked".
2. The **3' end** is elongated using "unnicked" DNA as leading strand (template); **5' end** is displaced.
3. Displaced DNA is a lagging strand and is made double stranded via a series of **Okazaki fragments**.
4. Replication of both "unnicked" and displaced ssDNA.
5. Displaced DNA circularizes.

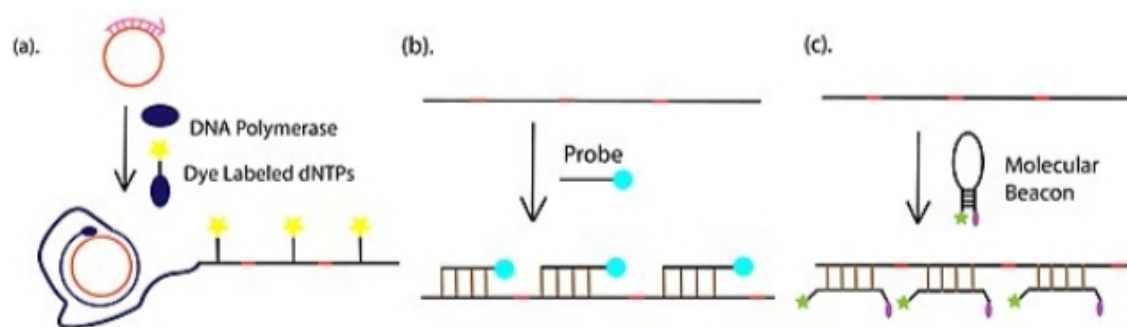


The molecular mechanism of Rolling Circle Amplification (RCA)

The derivative form of rolling circle replication has been successfully used for amplification of **DNA** from very small amounts of starting material.<sup>[1]</sup> This amplification technique is named as Rolling circle amplification (RCA). Different from conventional DNA amplification techniques such as **polymerase chain reaction (PCR)**, RCA is an isothermal **nucleic acid amplification** technique where the polymerase continuously adds single nucleotides to a primer annealed to a circular template which results in a long concatemeric ssDNA that contains tens to hundreds of tandem repeats (complementary to the circular template).<sup>[11]</sup>

There are five important components required for performing a RCA reaction:

1. A DNA polymerase
2. A suitable buffer that is compatible with the polymerase.
3. A short DNA or RNA primer
4. A circular DNA template
5. Deoxynucleotide triphosphates (dNTPs)



The detection methods of RCA product

The polymerases used in RCA are [Phi29](#), Bst, and Vent exo-DNA polymerase for DNA amplification, and T7 [RNA polymerase](#) for RNA amplification. Since Phi29 DNA polymerase has the best processivity and strand displacement ability among all aforementioned polymerases, it has been most frequently used in RCA reactions.

Different from polymerase chain reaction (PCR), RCA can be conducted at a constant temperature (room temperature to 37C) in both free solution and on top of immobilized targets (solid phase amplification).

There are typically three steps involved in a DNA RCA reaction:

1. Circular template ligation, which can be conducted via template mediated enzymatic ligation (e.g., T4 DNA ligase) or template-free ligation using special DNA ligases (i.e., CircLigase).
2. **Primer**-induced single-strand DNA elongation. Multiple primers can be employed to hybridize with the same circle. As a result, multiple amplification events can be initiated, producing multiple RCA products ("Multiprimed RCA"). A linear RCA product can be converted into multiple circles using restriction enzyme digestion followed by template mediated enzymatic ligation.

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3. Amplification product detection and visualization, which is most commonly conducted through fluorescent detection, with fluorophore-conjugated dNTP, **fluorophore**-tethered complementary or fluorescently-labeled **molecular beacons**. In addition to the fluorescent approaches, **gel electrophoresis** is also widely used for the detection of RCA product.