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Reverse transcription polymerase chain reaction

By

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• Introduction

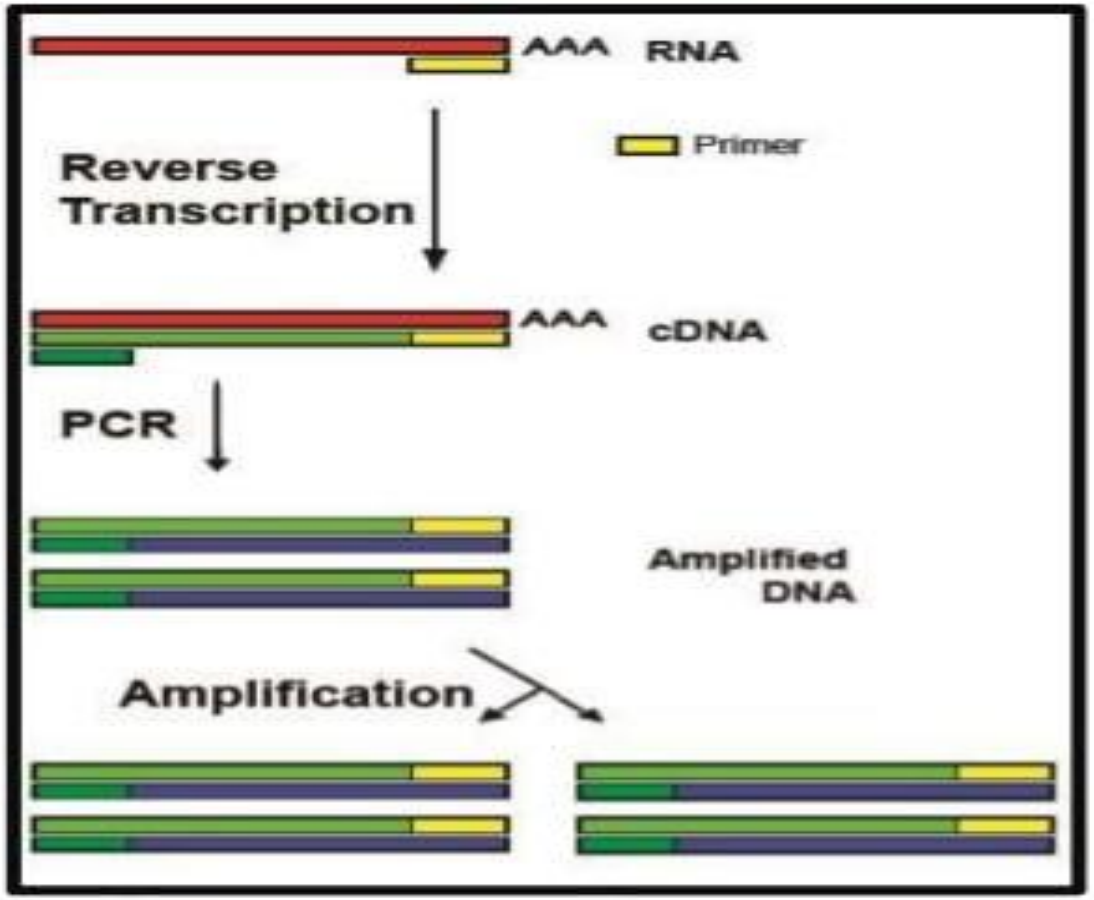
• Reverse transcription polymerase chain reaction (RT-PCR) is one of many variants of polymerase chain reaction (PCR).

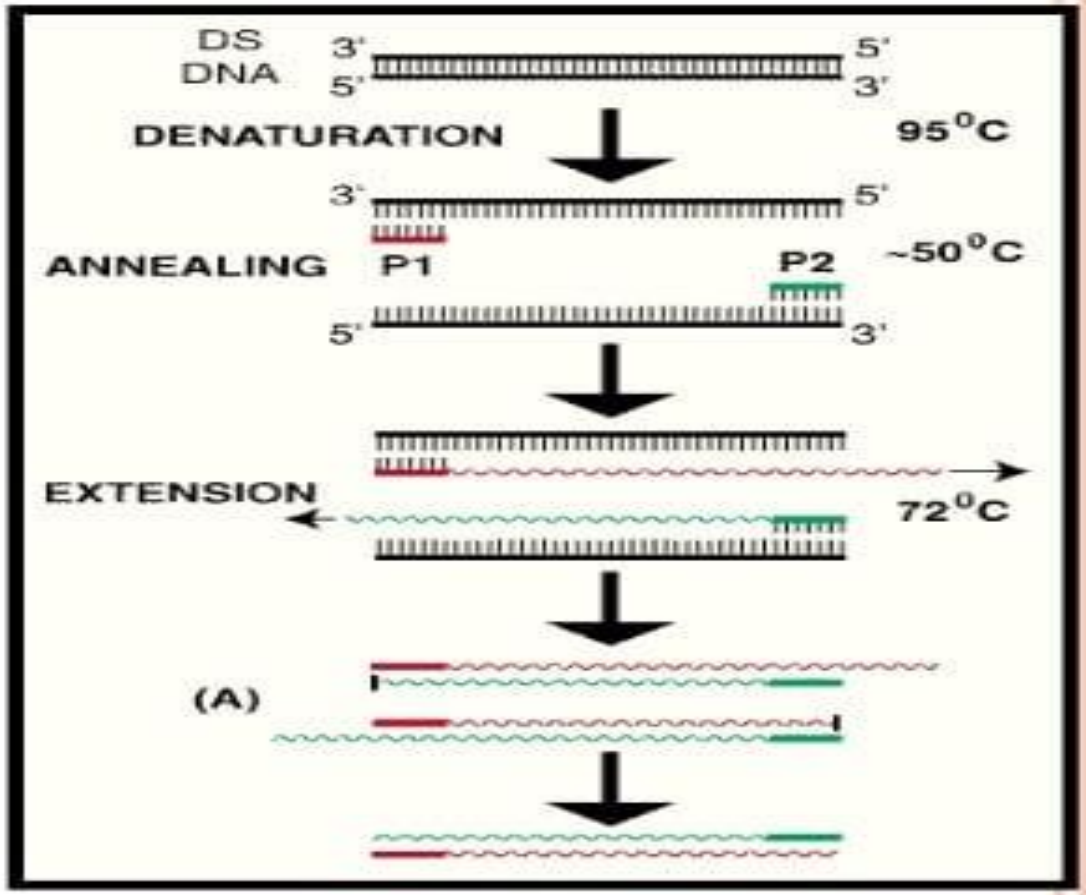
•This technique is commonly used in molecular biology to detect RNA expression.

•RT-PCR is often confused with real-time polymerase chain reaction (qPCR). However, they are separate and distinct techniques.

•RT-PCR is used to detect gene expression through creation of complementary DNA (cDNA) transcripts from RNA.

RT-PCR V/S PCR


AAA
RNA
Primer
Reverse Transcription
AAA
cDNA
PCR
Amplified DNA
Amplification


DS DNA
3'
5'
5'
3'
DENATURATION
95°C
3'
5'
ANNEALING
P1
P2
~50°C
5'
3'
EXTENSION
72°C
(A)

DIFFERENCE BETWEEN RT-PCR AND TRADITIONAL PCR:

- Although RT-PCR and the traditional PCR both produce multiple copies of particular DNA isolates through amplification, the applications of the two techniques are fundamentally different.
- Traditional PCR is used to exponentially amplify target DNA sequences.
- RT-PCR is used to reverse transcribe mRNA to cDNA and then amplify this result using traditional PCR.

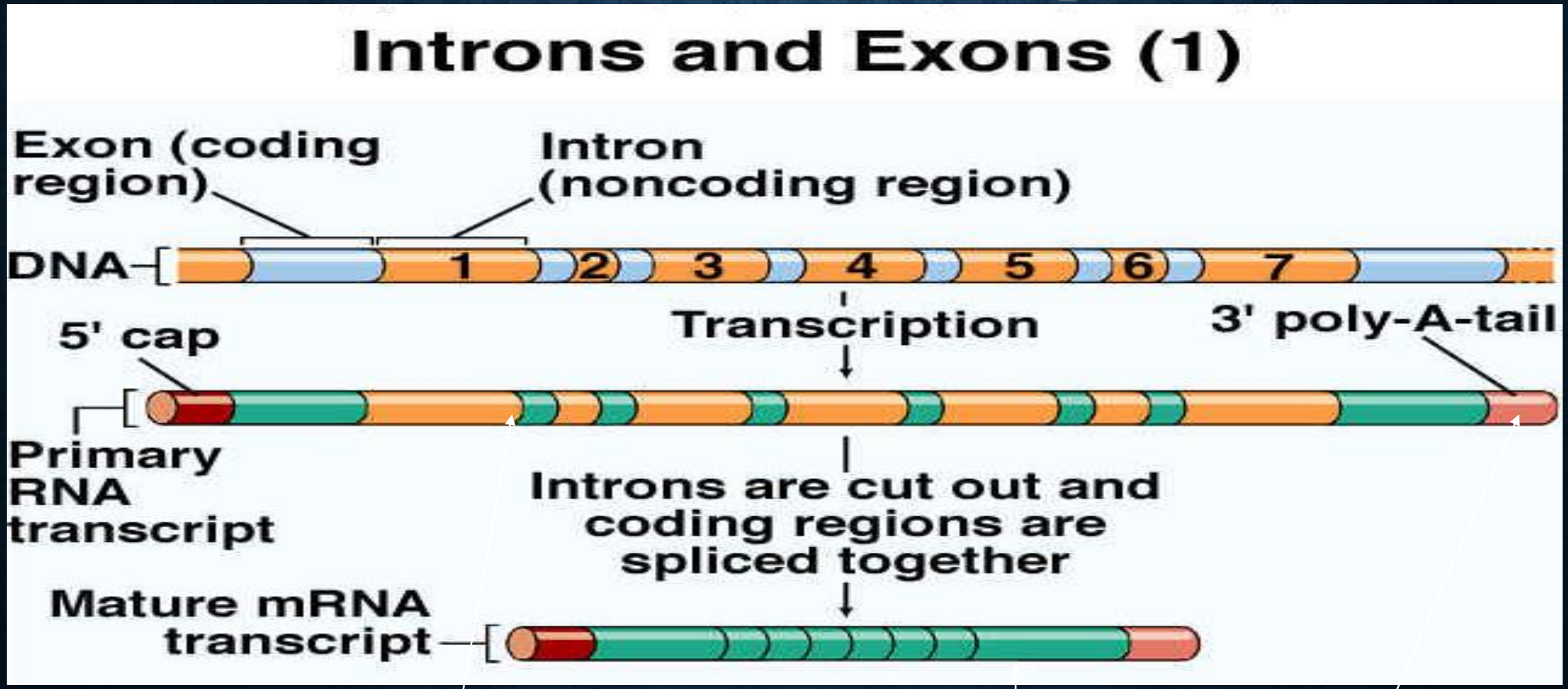
•There are several reasons why researchers would use this opposed to PCR:-

1. Since mRNA is the message that is sent for translation, RT-PCR can give us a measurement of gene expression that PCR cannot.

2. The cDNA produced does not contain the introns that DNA does; therefore RT-PCR can provide us with genes that may be inserted into prokaryotes (which cannot and do not splice the introns out).

•This technique is also used for diagnosing genetic diseases as well as studying certain viruses whose genetic information are exclusively composed of RNA.

Result of Transcription



Principle of RT PCR

•Traditionally, RT-PCR involves two steps: the RT reaction and PCR amplification.

•RNA is first reverse transcribed into complementary DNA (cDNA) using an enzyme, reverse transcriptase.

•The resulting cDNA is used as templates for subsequent PCR amplification using primers specific for one or more genes.

RT PCR- TYPES

There are two types of RT PCR

1. One step RT PCR

• RT-PCR can be carried out as one-step; in which all reaction components are mixed in one tube prior to initiation of the reaction.

•Although one-step RT-PCR offers simplicity and convenience and minimizes the possibility for contamination, the resulting cDNA cannot be used for detecting multiple messages from a single RNA sample as in two-step RT-PCR.

-in one-step RT-PCR take mRNA targets (up to 6 kb) and subjects them to reverse transcription and then PCR amplification in a single test tube.

Protocol:

1. Select a one-step RT-PCR kit, which should include a mix of reverse transcriptase and the PCR system such as Taq DNA Polymerase and a proofreading polymerase.

2. Prepare a reaction mix, which will include dNTPs, primers, template RNA, necessary enzymes and a buffer solution.

4. Adding the mix to a PCR tube for each reaction. Then add the template RNA.

5. Place PCR tubes in the thermal cycler to begin cycling.

The first cycle is reverse transcription to synthesize cDNA.

The second cycle is initial denaturation. During this cycle reverse transcriptase is inactivated. The next 40 to 50 cycles are the amplification program, which consists of three steps: (1) denaturation.

(2) annealing.

(3) elongation.

6. The RT-PCR products can then be analyzed with gel electrophoresis.

2. Two-step RT-PCR

Two-step RT-PCR, as the name implies, occurs in two steps.

First the reverse transcription and then the PCR.

This method is more sensitive than the one-step method.

Step one

1. Combine template RNA, primer, dNTP mix, and nuclease-free water in a PCR tube.

2. Add RNase inhibitor and reverse transcriptase to the PCR tube.

3. Place PCR tube in thermal cycler for one cycle that includes annealing, extending and then inactivating reverse transcriptase.

4. Proceed directly to PCR or store on ice until PCR can be performed.

Step two

1. Add a master mix (containing buffer, dNTP mix, MgCl2, Taq polymerase and nuclease-free water) to each PCR tube.

2. Add appropriate primer.

3. Place PCR tubes in thermal cycler for 30 cycles of the amplification program, which includes three steps:

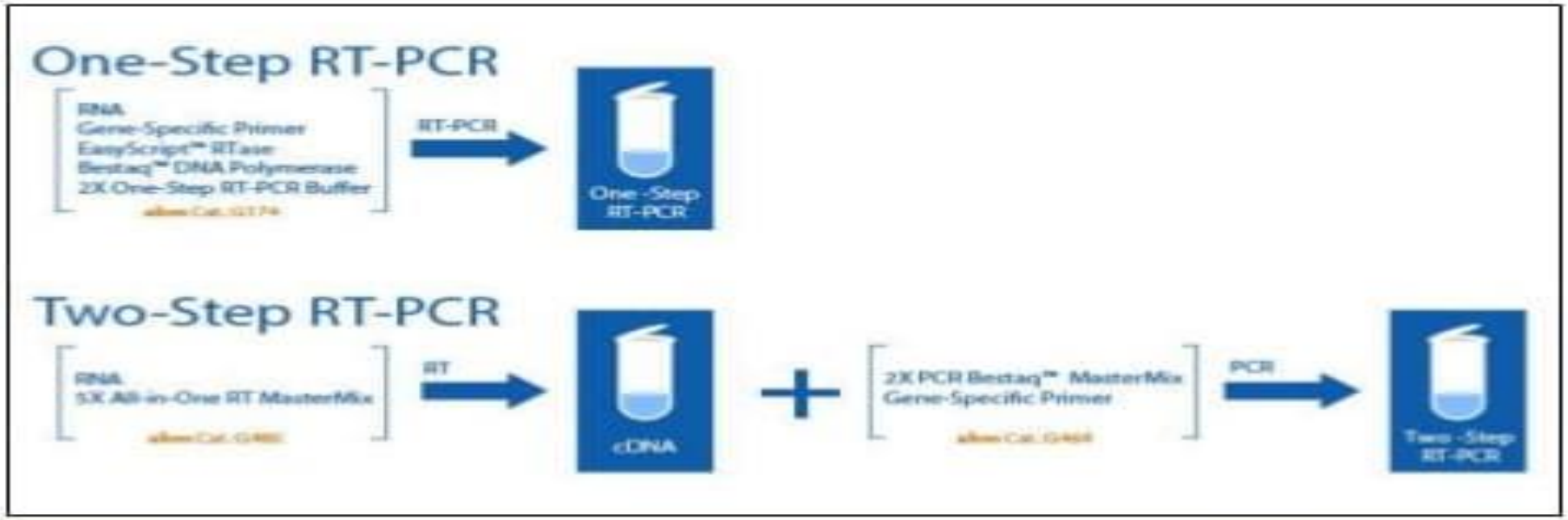
(1) Denaturation

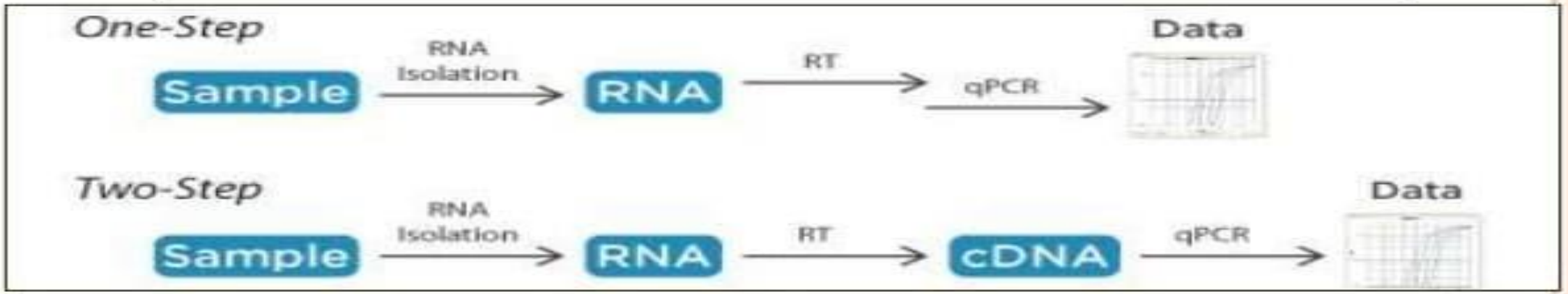
(2) annealing

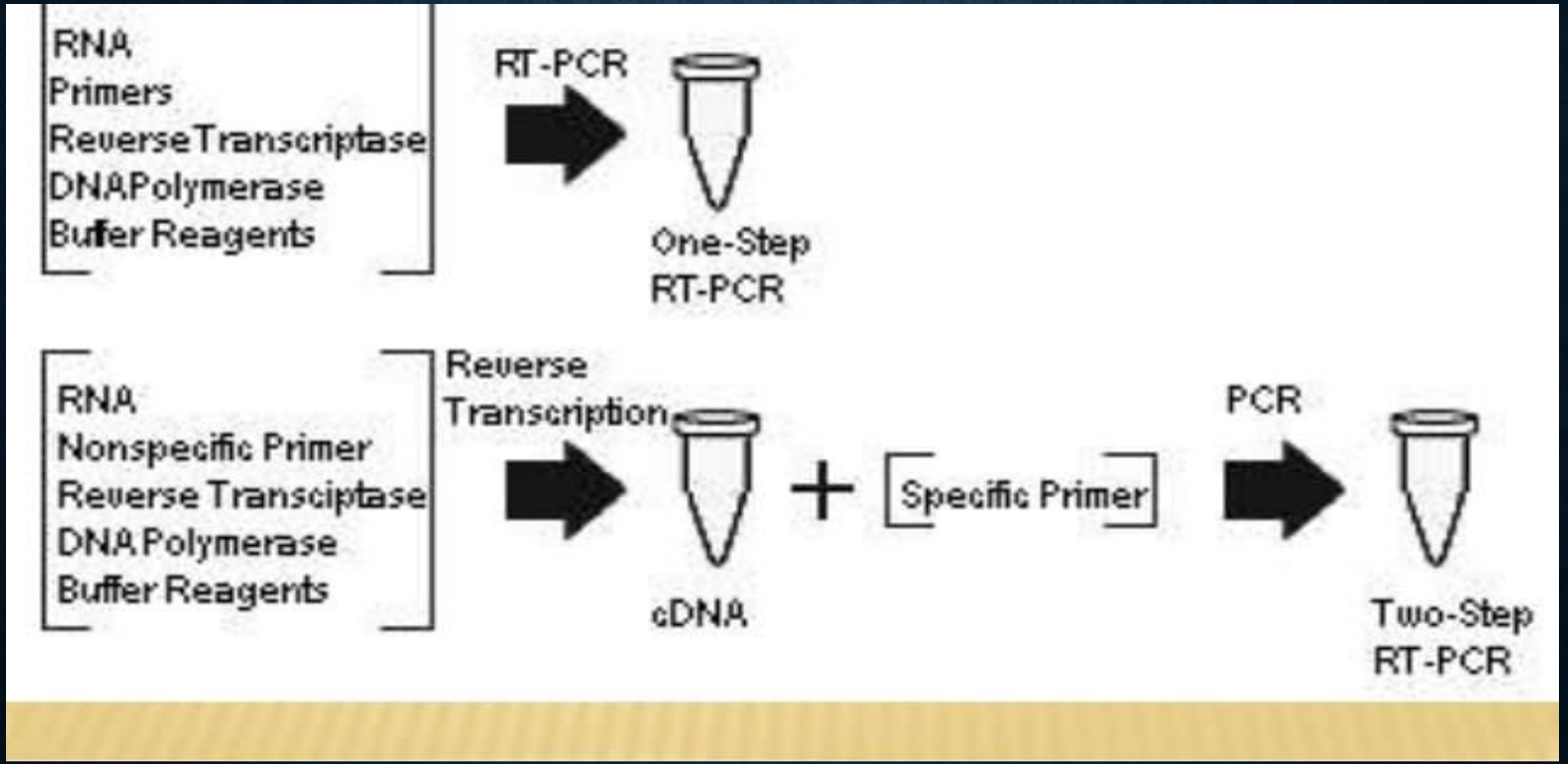
(3) elongation.

4. The RT-PCR products can then be analyzed with gel electrophoresis.

ONE-STEP V/S TWO-STEP RT-PCR PROCEDURES






RNA
Primers
Reverse Transcriptase
DNA Polymerase
Buffer Reagents
RT-PCR
One-Step
RT-PCR
RNA
Nonspecific Primer
Reverse Transcriptase
DNA Polymerase
Buffer Reagents
Reverse
Transcription
cDNA
Specific Primer
PCR
Two-Step
RT-PCR

APPLICATION OF RT-PCR

- The exponential amplification via RT-PCR provides for a highly sensitive technique in which a very low copy number of RNA molecules can be detected.

•Gene Insertion- RT-PCR can also be very useful in the insertion of eukaryotic genes into prokaryotes.

•Studying the genomes of viruses whose genomes are composed of RNA, such as Influenzavirus A and COVID-19.

•Genetic Disease Diagnosis- RT-PCR can be used to diagnose genetic disease

•Cancer Detection- Researchers are working on ways to use RT-PCR in cancer detection to help improve prognosis, and monitor response to therapy.

THANK YOU