Reverse transcription PCR (RT-PCR)

RT-PCR was developed to amplify RNA. The principle is to convert RNA into its complementary DNA (cDNA) by reverse transcriptase which can be amplified by PCR in the normal way (figure 1). RT-PCR provides opportunities to study the original RNA species and detection of expressed genes.

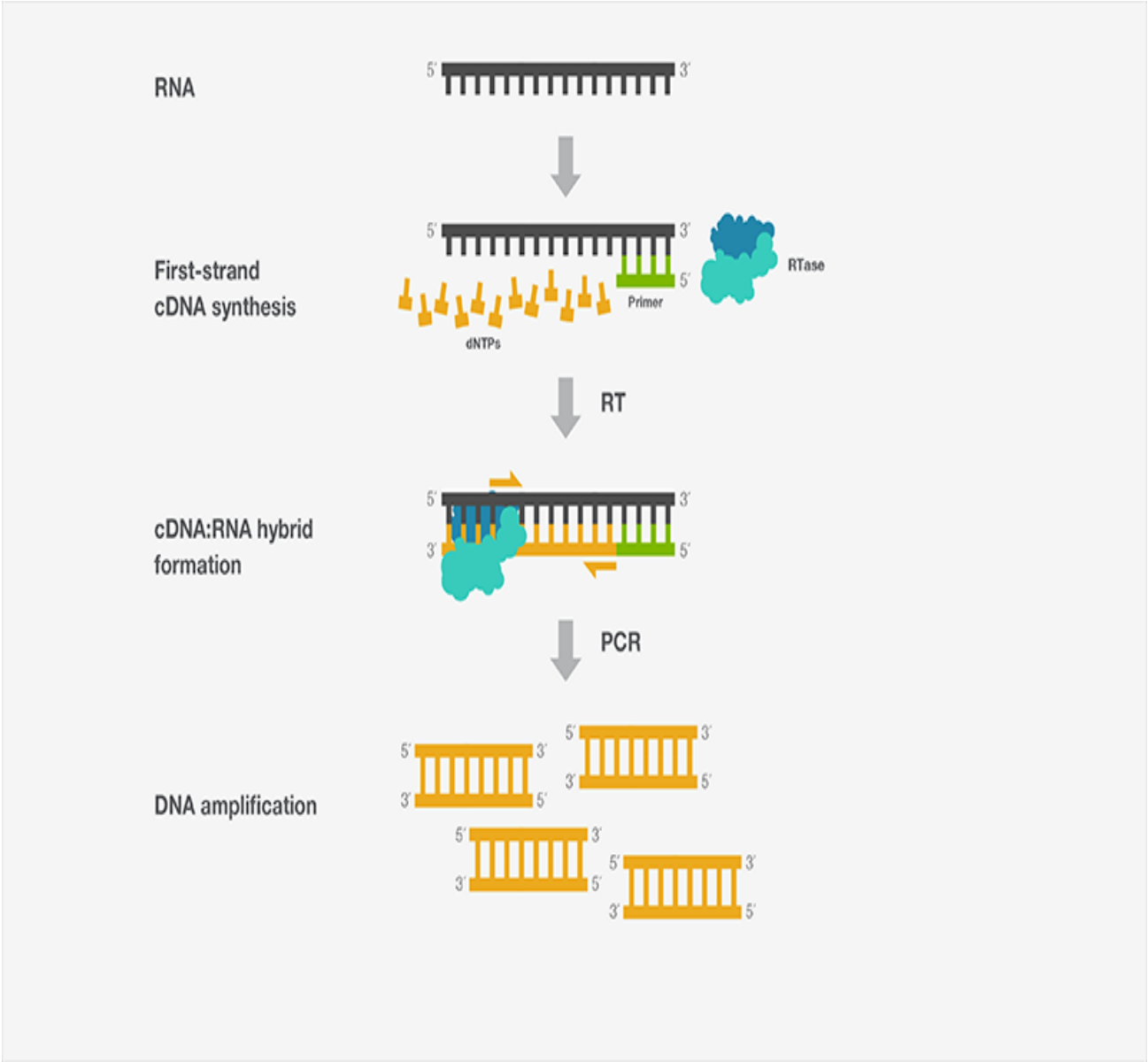

Figure 1 RT-PCR

Gene expression

Expression of most genes results in their transcription to give RNA followed by translation of the RNA to give the final gene product, protein. The mRNA is much more convenient for gene engineering and expression due to the following reasons:

1- The coding sequence of most eukaryotic genes is interrupted by intervening sequences, or introns therefore the original version of a eukaryotic gene is very large and difficult to manipulate. Since mRNA has had the introns removed naturally that is much more convenient for gene engineering and expression (figure 2).

2- mRNA molecule is only made when the gene for that protein is turned on and expressed. If the gene was expressed under the specific conditions, a PCR product will be produced, whereas, if the gene was switched off, none of this particular mRNA will be present and no band will be generated. Carrying out RT-PCR on an organism under different conditions reveals when the gene was switched on. This allows analysis of which environmental factors bring about expression of any chosen gene. In this example, the gene of interest is expressed in condition 1 but not in condition 2, therefore in condition 1 mRNA from the gene of interest is present and reverse transcriptase generates the cDNA. The PCR primers specific for this gene can now bind to the cDNA and PCR will amplify a DNA band corresponding to the original mRNA. In condition 2 the mRNA is absent and so the RT-PCR procedure does not generate the corresponding DNA band (Figure 3).

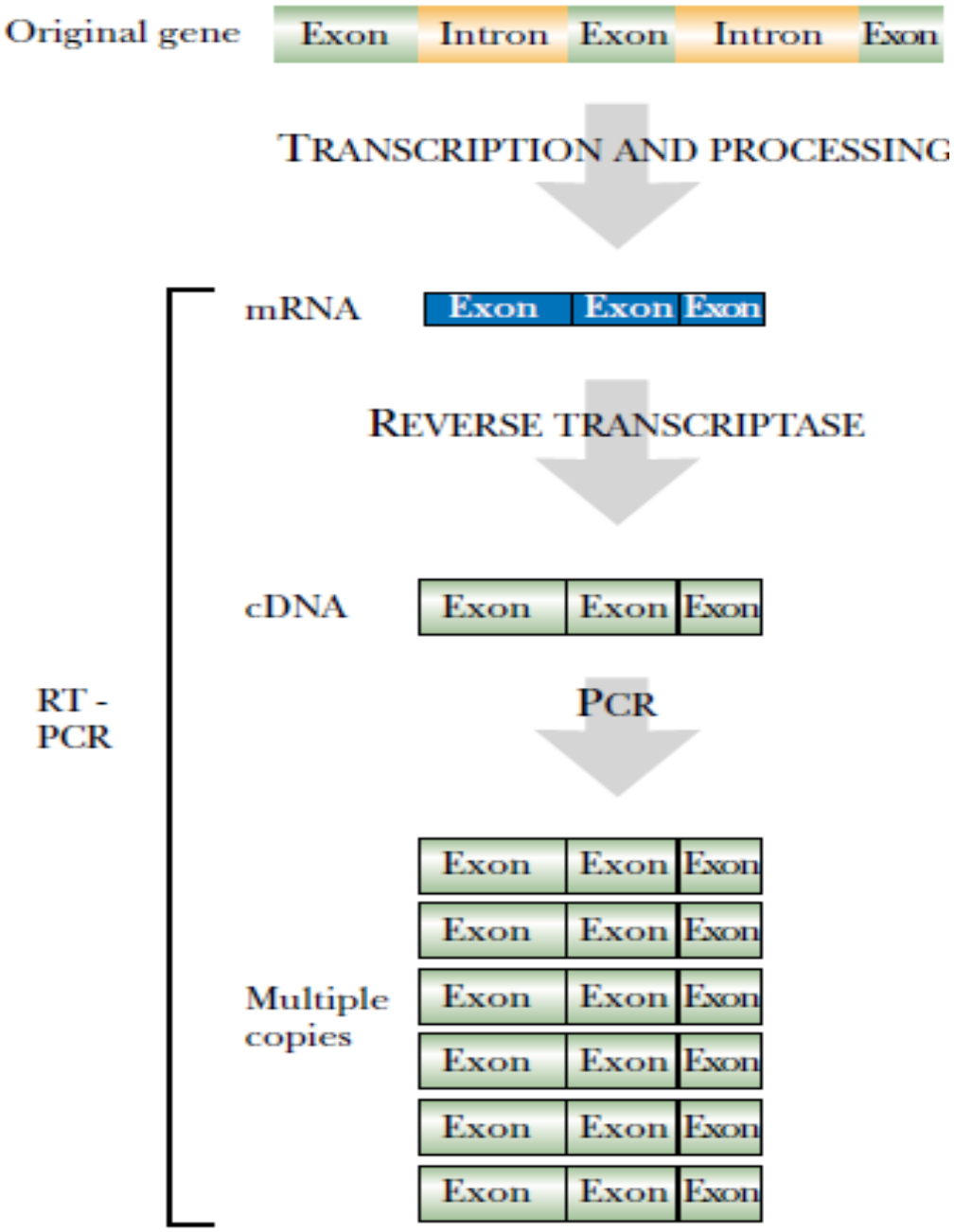

Figure 2 RT-PCR is making a cDNA copy of the mRNA, then using PCR to amplify the cDNA. First, a sample of mRNA is isolated. Reverse transcriptase is used to make a cDNA copy of the mRNA. The cDNA sample then amplified by PCR.

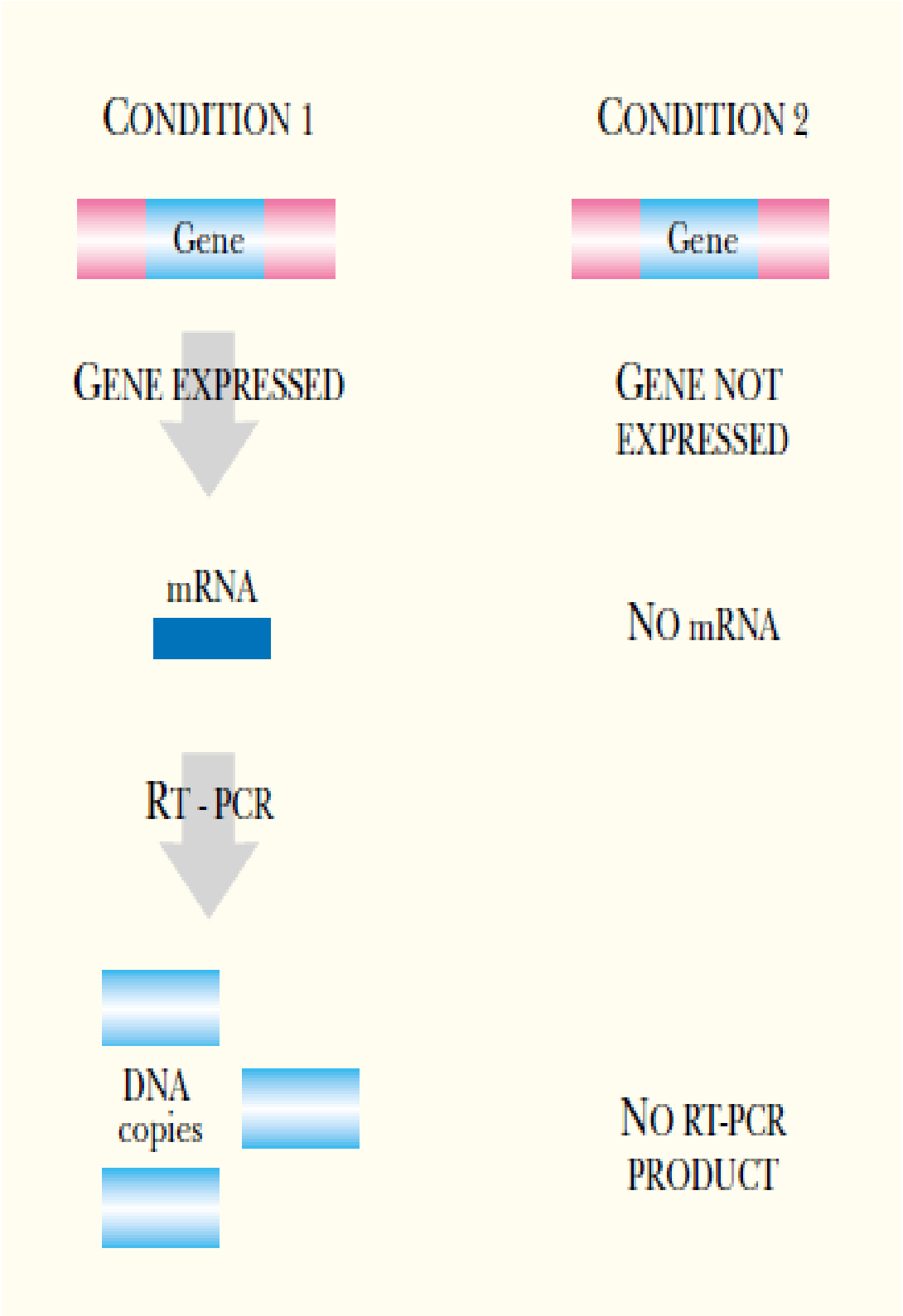

Figure 3. RT-PCR for gene expression in two conditions

The cDNA synthesis

There are three basic strategies for the synthesis of cDNA (figure 4):

1. Specific priming:
2. Oligo-dT priming: with eukaryotic mRNA bearing a poly(A) tail, a complementary oligo (dT) primer may be used.
3. Random priming: random hexamers may be used which randomly anneal to the mRNAs in the complex. Such primers provide a free 3 hydroxyl group which is used as the starting point for the reverse transcriptase.

The cDNA is then subjected to PCR using a specific primer pair.

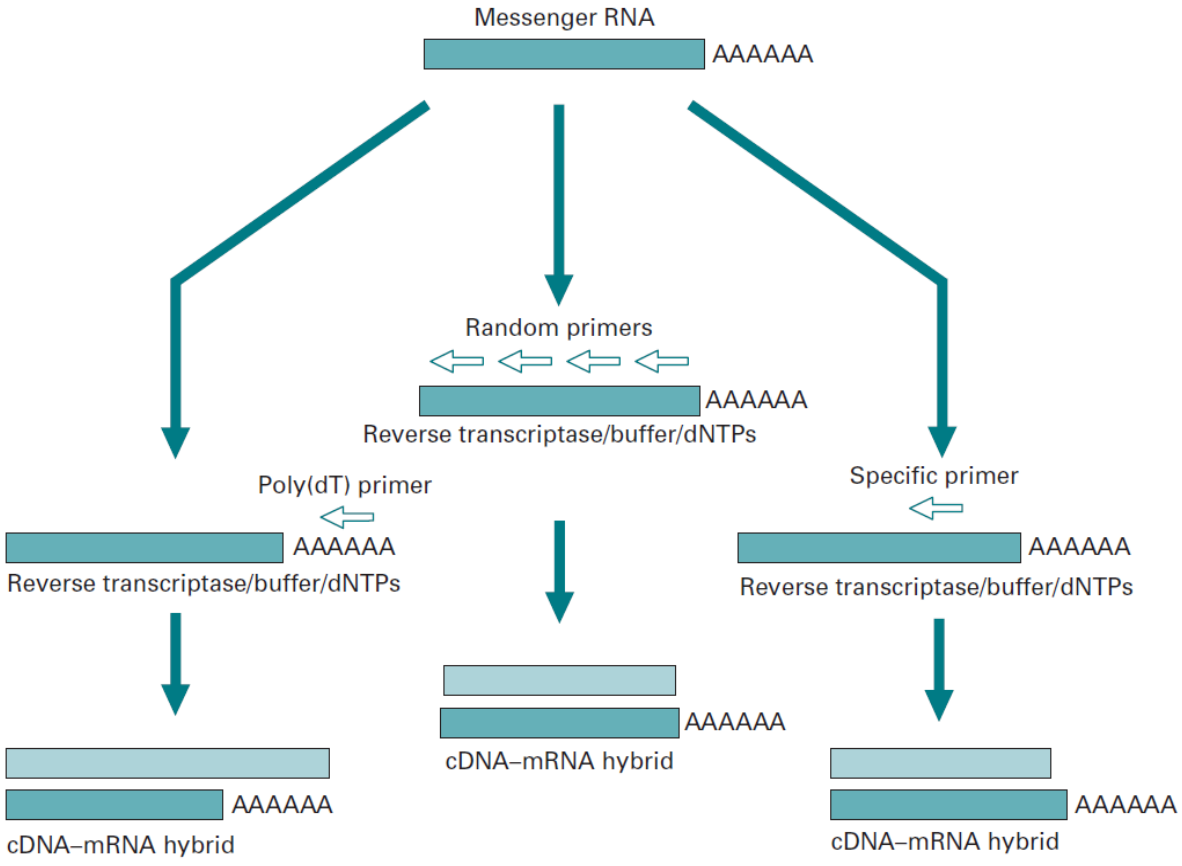

Figure 4. Strategies for producing first-strand cDNA from mRNA

The types of RT-PCR

In performing RT-PCR, one-step and two-step methods are the two common approaches, each with its own advantages and disadvantages (Figure 5).

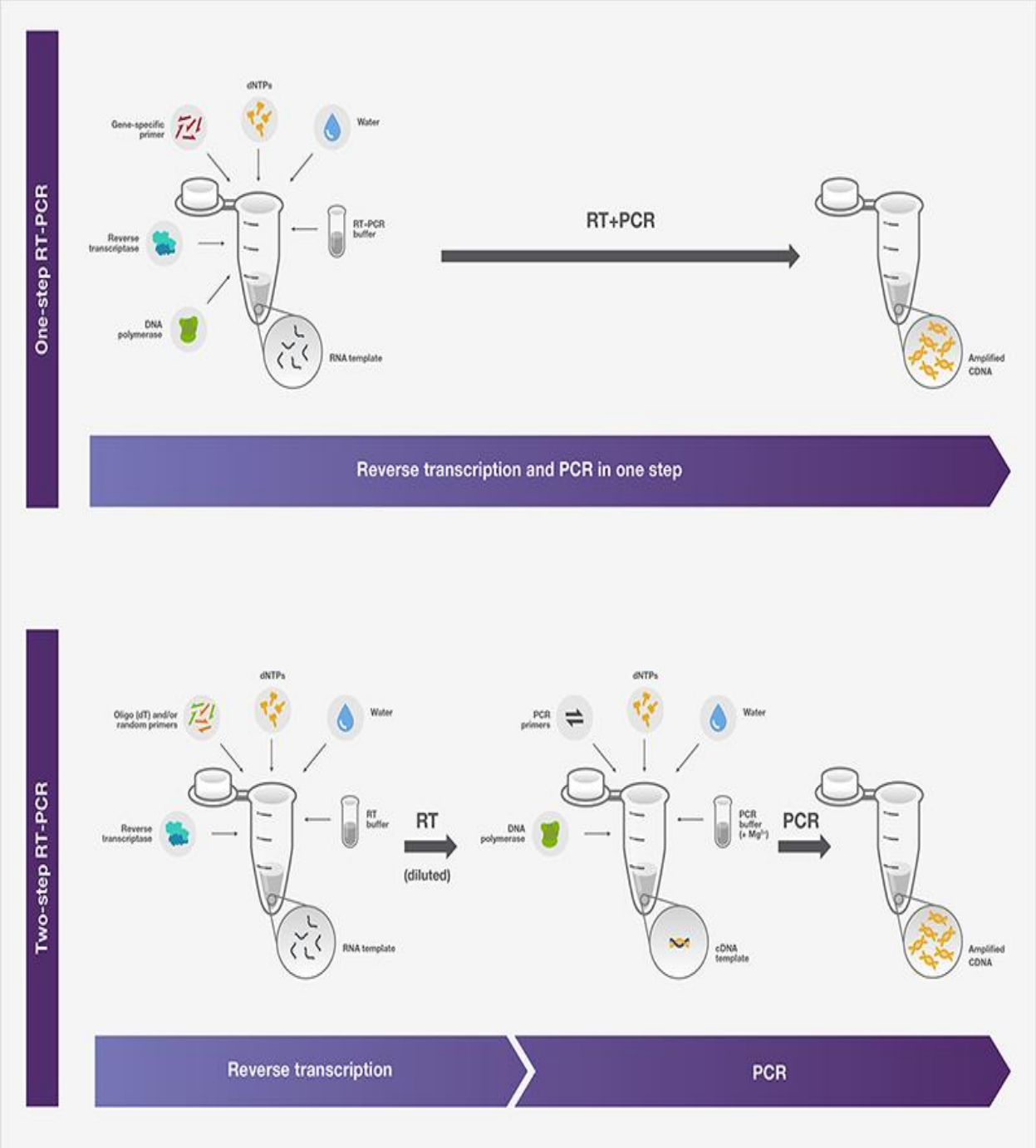

Figure 5 one and two steps RT-PCR

1- One-step RT-PCR

One-step RT-PCR combines first-strand cDNA synthesis (RT) and subsequent PCR in a single reaction tube.

Advantage:

1. Simple
2. Minimize possible contamination
3. Processing of large numbers of samples

Disadvantage: One-step RT-PCR uses gene-specific primers for amplification, limiting the analysis to one gene per RNA sample.

2- Two-step RT-PCR

Two-step RT-PCR entails two separate reactions, beginning with first-strand cDNA synthesis (RT), followed by amplification of a portion of the resulting cDNA by PCR in a separate tube.

Advantage:

1. Useful for detecting multiple genes in a single RNA sample.
2. The separation of RT and PCR reactions allows for optimization of reaction conditions for each step
3. Flexibility with reverse transcription priming (oligo-dT primers, random hexamers, or gene-specific primers)

Disadvantage:

1. Include multiple steps for an extended workflow
2. Increasing the chance of contamination.