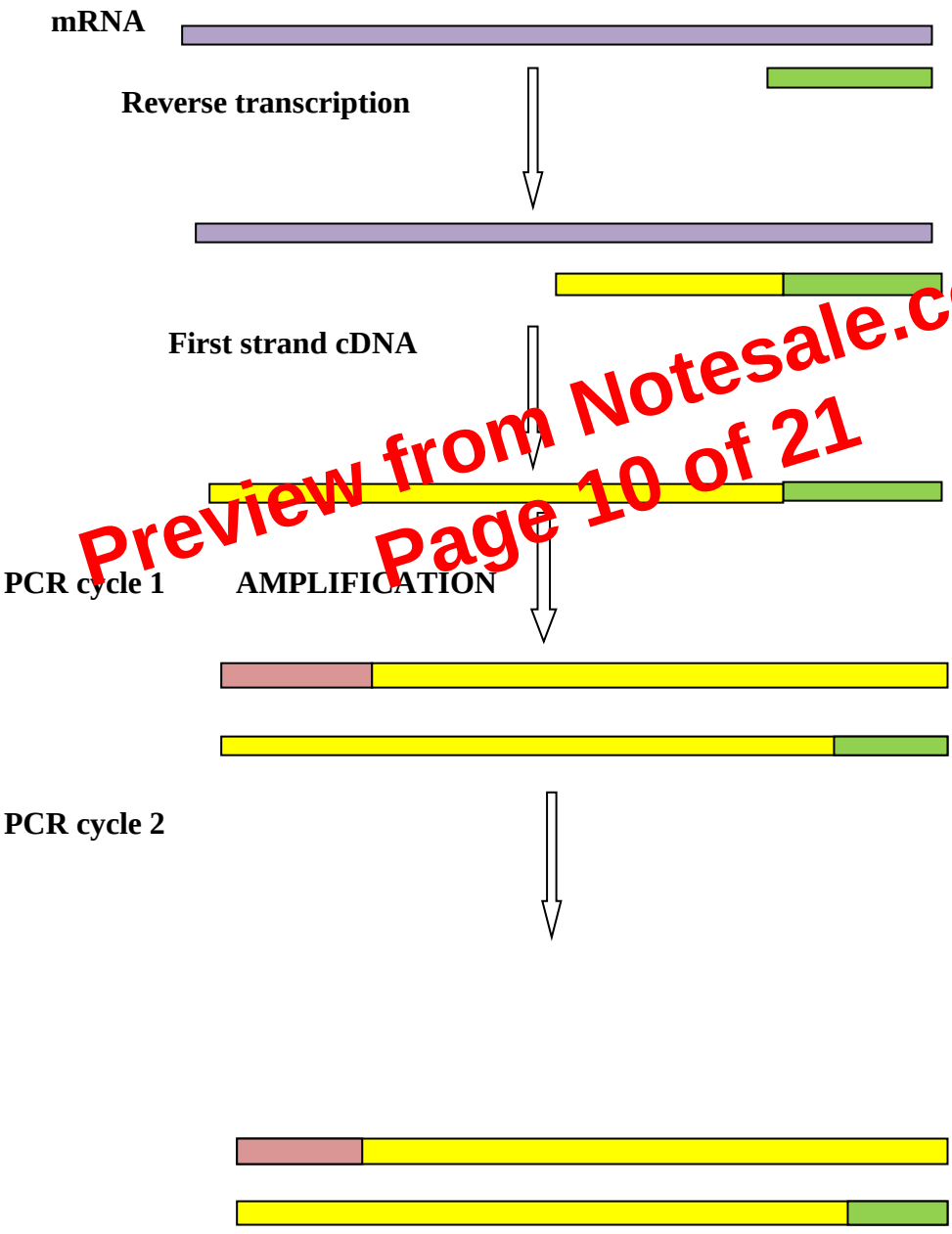
Reverse transcriptase enzyme lacks proof reading capability and hence it has high error rates during DNA synthesis. But this high error rate of viral reverse transcriptase enzyme provides selective advantage for their survival in the host system.

Synthesis of DNA from an RNA template via reverse transcriptase produces cDNA. Reverse transcriptase use an RNA template and a short primer complementary to 3'end of RNA to direct the synthesis of first strand of cDNA, which can be used directly as a template for the PCR.

Reverse transcription PCR is carried out in two ways:

i. ONE STEP RT-PCR:



Applications:

- Gene detection analysis
- Template quantification
- RNA detection
- Forensic studies
- Pathogen identification
- Mutation analysis
- Gene deletion analysis
- Linkage analysis

4. **Nested PCR:**

In this PCR type, two sets of primers are used against same target and two successive PCR reactions occur for the same. This technique was designed to increase the sensitivity and specificity of the assay reaction.

- The first set of primer is used for annealing the sequence upstream from the second set of the primers. It is also referred as outer primers that are used to amplify a large fragment of the gene which is used as a template in the second round of PCR.
- The second set of primer is nested with respect to the first set of the primer. It is also referred as inner primer or nested primer that is used to target a small region of the amplicon (PCR products).

The traditional approach to nested PCR to perform a number of PCR cycles using first set of primers and then open the reaction vessel and add the second, nested set of primers to run the second PCR cycle.

With this technique the major problem is amplicon contamination and consequential loss of specificity of assay. To address this issue, single-tube nested PCR (STN PCR) reactions have been developed wherein both sets of primers are added to the initial reaction vessel and an extended PCR is performed.

Cycle 1 DNA melting and primer biding

FIRST PCR run

- Restriction digestion for identification of the insert DNA is not required.
- It is time effective technique.

Disadvantages:

- Although the technique is cost effective, fast and reliable, any mutation cannot be detected.
- The sequence information cannot be obtained by the colony PCR. Sequencing is required to be done for the confirmation of the DNA transformation.
- The chance of false positive results is high.

Applications:

- This technique is widely used for screening of recombinants.
- It is used for the assay of number of colonies simultaneously and there is no need of storing large number of transformed clones for long period.
- It is widely used for cDNA screening.

7. Hotstart PCR:

In conventional PCR we mix all the components like DNA polymerase, $MgCl_2$, PCR buffer, template DNA, dNTPs, forward and reverse primers in a PCR tube in ice. In this the DNA polymerase has some residual activities, which results in primer dimer formation and non specific primer annealing. In order to prevent this, we use another technique Hotsart PCR.

In this technique we withheld the key components of PCR reaction like $MgCl_2$ and DNA polymerase from the reaction mixture until specific temperature is reached.

There are three types of hotstart PCR:

- **Manual hotstart PCR**: In this PCR we add the reaction components in the reaction tube as in traditional PCR but we do not add DNA polymerase and $MgCl_2$ in the reaction mixture. Then it is subjected to PCR and when the temperature reaches about 65°C to 70°C, we manually add the $MgCl_2$ and DNA polymerase to the reaction tube. Then the reaction proceeds and amplification is performed without any error.