PCRBIOSYSTEMS
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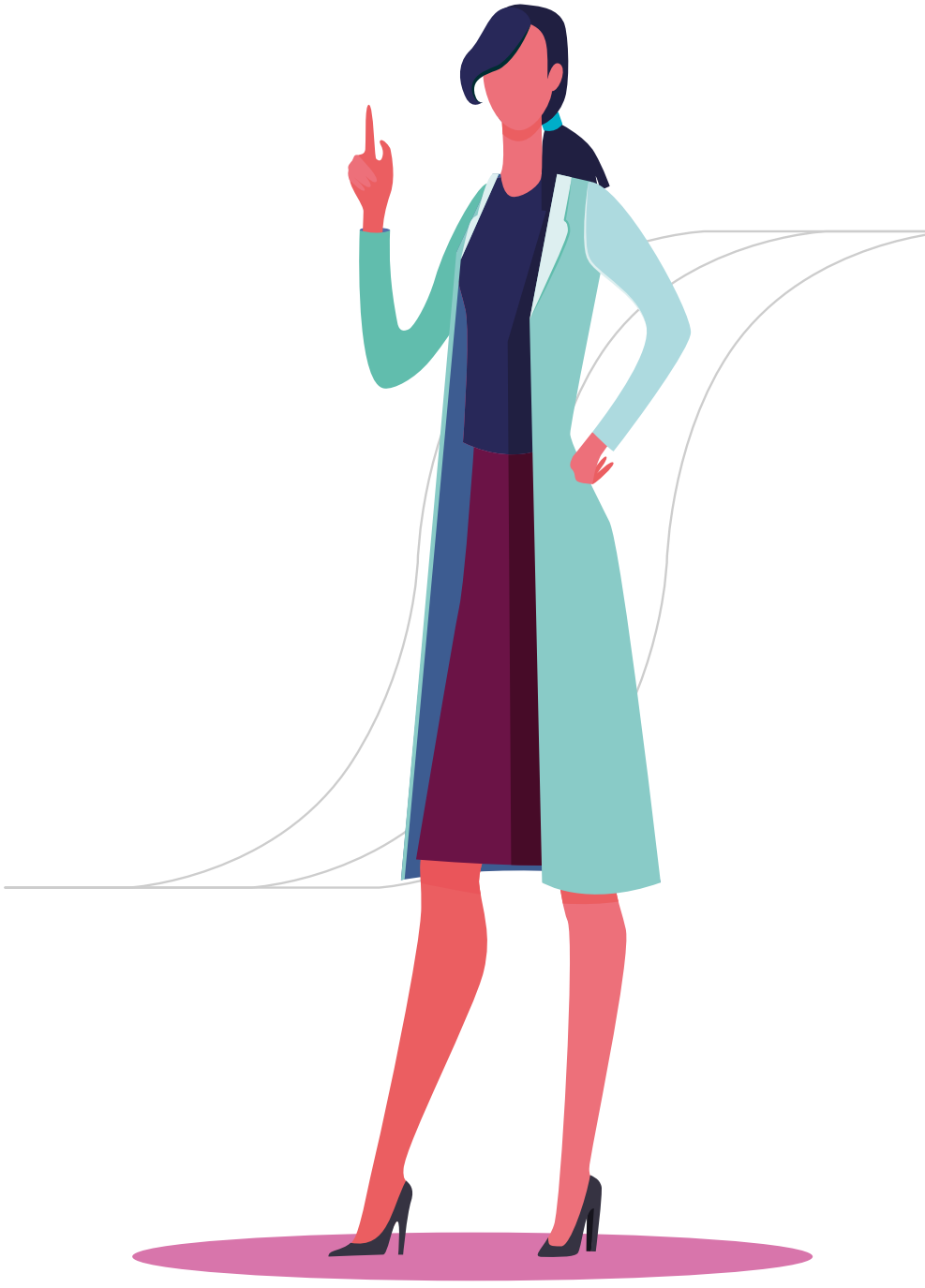
Tips & Tricks

Choosing 1-step or 2-step RT-qPCR



When does it make sense to go for 1-step instead of 2-step RT-PCR? Why would you opt for 2-step if 1-step is faster and saves you both time and effort? Choosing the right one can make a big difference in the ease, time, and cost of a project. This list can help users assess which method is more practical for desired project outcomes.

1. What is 2-step RT-qPCR?

Traditionally, reverse transcription-quantitative PCR (RT-qPCR) and reverse transcription PCR (RT-PCR) are carried out in a 2-step manner. RNA is extracted from the sample of interest and firstly converted to cDNA using a reverse transcriptase in an appropriate reaction buffer. The resulting single-stranded cDNA can then be used as a template for a separate qPCR reaction, or can be stored, as required.

2. Benefits of 2-step RT-qPCR

- Enhanced sample stability: cDNA is more stable than RNA and therefore is preferable for long term storage at -20 ˚C (rather than -80 ˚C as required for RNA).
- CDNA produced in this manner can be used for transcript cloning and library construction in addition to qPCR.
- Usually, only a small amount of an extracted RNA sample is used for cDNA synthesis. Multiple batches of cDNA can be generated from one RNA sample, resulting in a greater volume of available cDNA template for downstream qPCRs than the original RNA sample.
- The cDNA synthesis reaction may be thoroughly optimised to reduce bias, increase yield and ensure accurate representation of most or all transcripts or RNA molecules in a sample. Indeed, based on what short oligos are chosen, the total pool of RNAs (including rRNA and tRNA) can be transcribed, not only the mRNA population. A 2-step reverse transcription approach is the only method that allows transcription of total RNA population.
- Both cDNA synthesis and qPCR reactions can be separately optimised and the reaction buffers will be ideal for the separate enzyme.
- Quality of resulting cDNA can be analysed via electrophoresis or bioanalyser. Issues with reverse transcription can be identified and resolved.

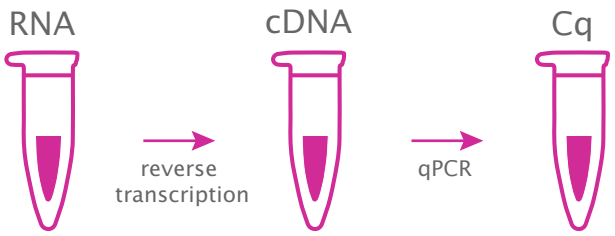
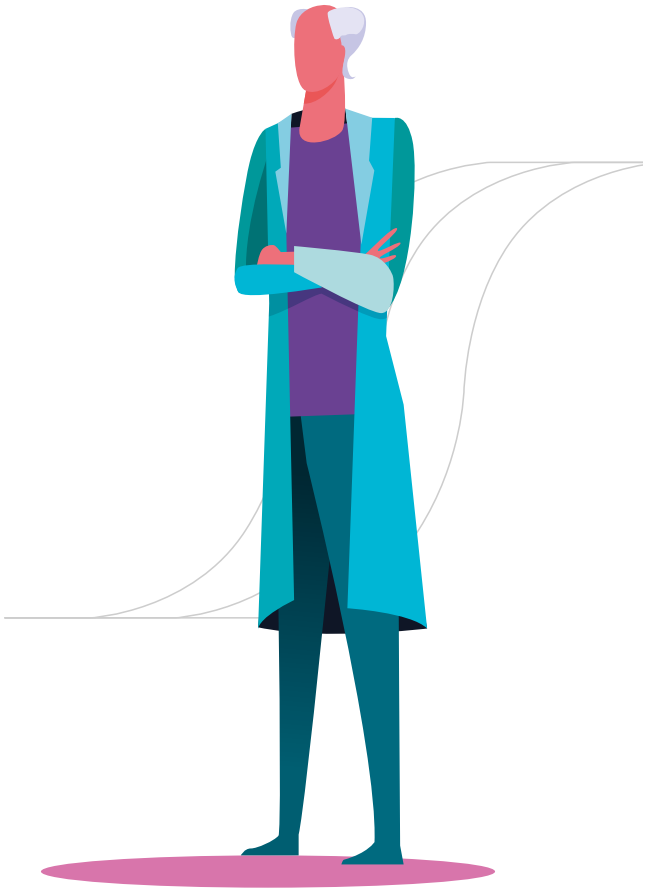


Fig 1. Schematic of a 2-step RT-qPCR workflow. RNA is converted to cDNA in a dedicated reverse transcription reaction. Resulting cDNA can be stored for later use, or used directly in a second qPCR reaction to generate quantitative information (Cq).

3. Trade-offs of 2-step RT-qPCR

- Conducting a dedicated reverse transcription reaction requires more time and requires additional sample handling and consumables.
- A 2-step workflow can be more costly than an equivalent 1-step workflow.
- There is greater potential for user error.
- Additional sample handling increases the potential for sample contamination.
- When only one or a limited number of targets are going to be absolutely quantified (using a reference curve), using non-specific primers may lead to less efficient reverse transcription, or lower cDNA yields than using target-specific primers. Besides, if RNA molecule of interest has low abundancy, transcription may result unbalanced and later on detection may fail.



4. What is 1-step RT-qPCR?

One step, or 1-step, RT-qPCR combines cDNA synthesis and subsequent qPCR in a single tube. 1-Step reactions are driven by an enzyme mix that includes both a reverse transcriptase and a Taq DNA polymerase. A standard qPCR thermocycling programme is extended to include an initial incubation at 42-55 ˚C for cDNA synthesis (RT step) prior to qPCR cycling. Thus, while cDNA synthesis and qPCR are carried out sequentially, the whole process takes place in one tube and in a single thermocycling program, or “in one step”.

5. Benefits of 1-step RT-qPCR

- Faster than a 2-step process.
- Can be cheaper than the corresponding 2-step process.
- Reduced sample handling: workflows go directly from RNA to quantitative data.
- Reduced potential for contamination, due to less sample handling.
- Each reverse transcription reaction is carried out using target-specific primers, which can often lead to more specific products and more sensitive detection.
- Ideal when working with a limited number of targets.

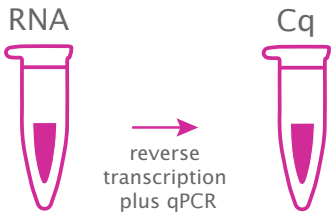
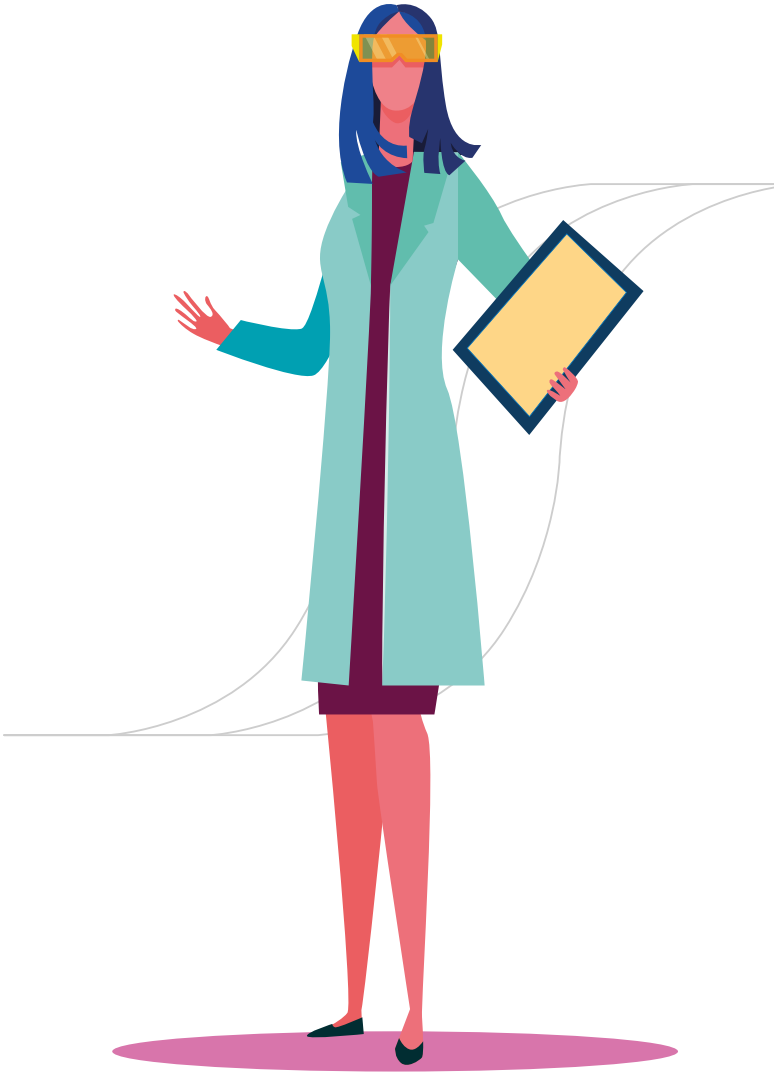


Fig 2. Schematic of a 1-step RT-qPCR workflow. RNA is converted to cDNA by a reverse transcriptase and is immediately used as template in a qPCR reaction in one tube, generating quantitative information (Cq). No discrete cDNA sample remains for later use.

6. Trade-offs of 1-step RT-qPCR

- All of the cDNA generated is consumed in one RT-qPCR reaction, the same sample cannot be used in other downstream processes.
- Target-specific primers may result in different efficiencies during reverse-transcription, resulting in bias when comparing expression levels of different targets.
- Using this approach, troubleshooting or optimisation of the reverse transcription and qPCR reactions cannot be easily conducted.
- Quality of cDNA cannot be separately assessed.
- RNA samples are less stable than cDNA, thus requiring storage at lower temperature (-80 ˚C). Frequent freeze-thaw cycles of the RNA samples can often compromise their quality and hence that of RT-qPCR results.



Four criteria for choosing the optimal RT-qPCR strategy:

1. Experimental Goals

- High Throughput: If speed and throughput are critical, consider 1-step RT-qPCR.
- Detailed Optimization: For experiments requiring fine-tuned reaction conditions, 2-Step RT-qPCR offers greater flexibility.

2. Sample Quality and Quantity

- Low-Quality RNA: Use 2-step RT-qPCR for better handling of low-quality or degraded RNA, because cDNA synthesis can be optimised.
- Limited Sample: 2-step allows multiple analyses from a single cDNA synthesis reaction.

3. Cost and Resource Availability

- Budget Constraints: Consider if cDNA will be needed for anything other than RT-qPCR. If yes, choose a 2-step workflow.
- Reagent Costs: 2-step workflows may be more expensive per reaction and increase labour costs.

4. Technical Expertise

- Simplicity: 1-step RT-qPCR offers a straightforward workflow with fewer steps.
- Expertise Required: 2-step RT-qPCR requires more technical expertise for optimal results.
- Reduced Potential for Error: 1-step RT-qPCR requires less handling and therefore the potential for mistakes is reduced.

Want to learn more?

Visit our website to find out about our solutions for 1-step and 2-step RT-qPCR.

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