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Tips & Tricks

Improve your **cDNA synthesis**

SIMPLIFYING 10 YEARS RESEARCH

Follow our tips below and don't let inefficient cDNA synthesis become the stumbling block to your research success. Good quality cDNA is key to reliable qPCR experiments, that's why we've put together a set of tips to help you get the most from your reverse transcription reactions and give your experiments the best start for reliable and reproducible results.

1. Template preparation

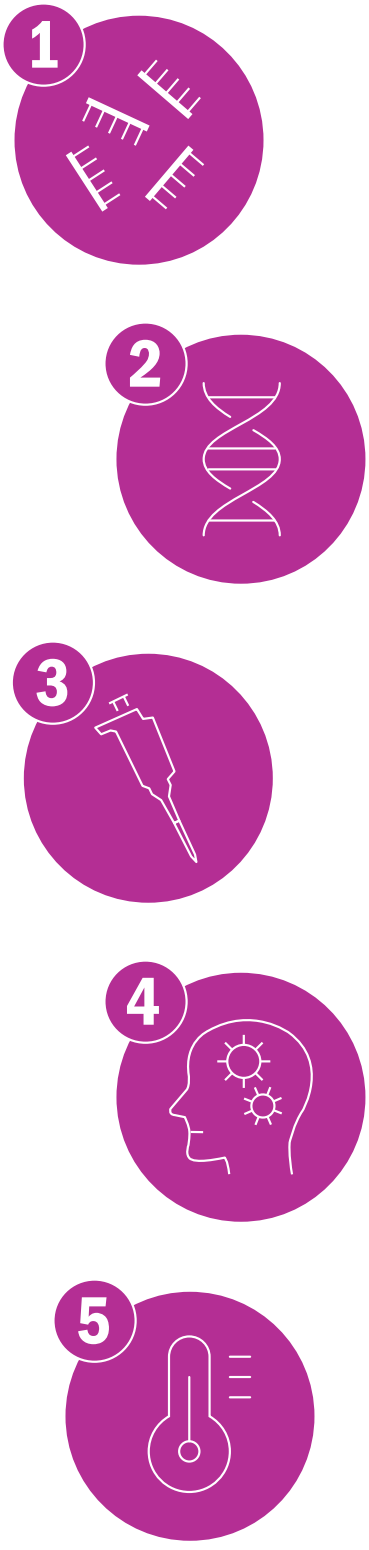
- Prepare good quality template RNA (high purity A260/A280 >1.8 and A260/A230 > 2.0, using your preferred method (column purification, Trizol, magnetic beads. If poly-A enrichment is desired, use appropriate magnetic bead purification.
- Optionally, you can ensure RNA is not degraded using agarose gel electrophoresis. However, this is not a standard requirement for most cDNA applications).

2. Eliminate genomic DNA

- Remove contaminating genomic DNA by DNase digestion (usually DNase I). Ensure DNase is deactivated or removed from the sample, as it will degrade cDNA in downstream steps if traces remain in the sample. This treatment should always be carried out on RNA prepared via column purification. Trizol and similar reagents can completely remove DNA from RNA preparations. However, you can still conduct DNase treatment if you're concerned about DNA contamination.
- If all the above steps have been carried out and you still suspect DNA contamination of your samples, validate total gDNA removal by running a PCR or qPCR on a genomic DNA target. Include a positive gDNA sample as a control and make sure your RNA samples do not have detectable levels of contaminating gDNA.

3. Choose the right reverse transcriptase

- Depending on the application you have in mind for the resulting cDNA, you may require an enzyme with specific characteristics. For instance, tagging for library construction and cloning of unknown sequences (5'RACE) benefit from RTases with terminal transferase activity and/or template switching capability for second strand synthesis, such as MMLV RTase. If shorter transcripts are required, for instance when preparing cDNA for qPCRs, both MMLV and AMV RTases are appropriate.
- Several engineered RTases exist on the market. You can select any one of these based on their optimal reaction temperature, the complexity of the RNA template in used (secondary structures formed), or for their different sensitivity to low abundance templates. Check the manufacture's specifications to find the appropriate RTase to for your application.
- If your RTase mix does not include an RNase inhibitor add an exogenous one to your cDNA synthesis reaction mix, to ensure RNA integrity is protected.



4. Pick the best priming strategy

- If you want to use cDNA for quantification of multiple transcript targets via qPCR or for cDNA library construction, getting a representative number of first strand cDNAs for every transcript is paramount. Thus, a mixed priming approach using random hexamers and oligo-dT primers is recommended (wherever appropriate, for instance using oligo-dT in prokaryotic RNA preparations is pointless, as bacterial transcripts are not polyadenylated).
- If cloning or quantification of only a specific transcript is desired, then acquiring full length transcript coverage is more important than representation. Choose oligo-dT, or gene-specific primers (GSPs), to ensure your target transcript is reverse transcribed in full.
- In rare instances where you desire 3' enrichment of cDNAs, use oligo-dT primers for eukaryotic and archaeal transcripts.

5. Optimise reaction conditions

- Ensure your template RNA is completely denatured before first strand synthesis begins. This is critical when targets include difficult templates (GC-rich, hairpins or other secondary structures). To ensure full template denaturation, incubate RNA and primers in the reaction buffer at 70 ˚C for 5min and then cool rapidly by placing directly on ice prior to adding RTase (and RNase inhibitor if this is separate).
- Identify the optimal incubation time for RTase to synthesise full length first strand cDNA. This can include increasing the incubation time beyond that recommended by the enzyme manufacturer.

Optional: Check the quality of your cDNA

- Validate the quality of your cDNA for the downstream application. If using in qPCRs run a normalising set of reactions to establish the Cq values of a standard reference gene in all samples. You can compare this value to previous batches of cDNA and dilute your samples appropriately to ensure all samples contain comparable concentrations of cDNA if required.
- This approach may not be suitable to confirm successful reverse transcription of long transcripts, because qPCR targets are kept < 0.2 kb. Thus, if you need to ensure presence of long transcripts >0.5 kb, run 2nd strand DNA synthesis (usually standard PCR with appropriate primers) and run part of your cDNA sample on an agarose gel. You should see a smear ranging from about 500 bp to 3-4 kb, verifying that full length transcripts have been reverse transcribed.
- Proceed with your downstream application.

Want to learn more?

Visit our website to find out about our solutions for cDNA synthesis.

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