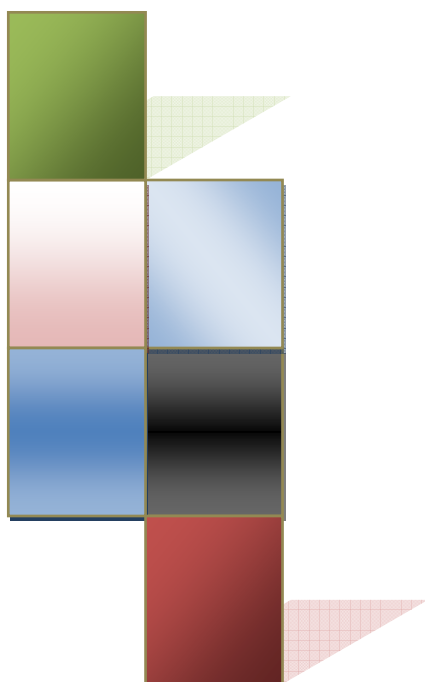


# NanoCinna

Pharmacogenomics Center

## **cDNA synthesis** **Troubleshooting guide**



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## Troubleshooting

Problem	Possible cause	Solution
<b>No bands after electrophoretic analysis of amplified products</b>	Procedural error in first-strand cDNA synthesis	Use the Control RNA to verify the efficiency of the first-strand reaction
	RNase contamination	Add Control RNA to sample to determine if RNase is present in the first-strand reaction.  Maintain aseptic conditions to prevent RNase contamination.  Use RNase Inhibitor in the first-strand reaction.
	Polysaccharide coprecipitation of RNA	Precipitate RNA with lithium chloride to remove polysaccharides.
	Target mRNA contains strong transcriptional pauses	Use random hexamers instead of oligo(dT) in the first-strand reaction.  Maintain an elevated temperature after the annealing step.  Increase the temperature of first-strand reaction (up to 50°C).  Use PCR primers closer to the 3' terminus of the target cDNA.
	Too much first strand product was used in PCR	Use no more than 1/10 of the first-strand reaction.
	Inhibitors of RT present	Remove inhibitors by ethanol precipitation of the mRNA preparation before the first-strand

<p><b>No bands after electrophoretic analysis of amplified products</b></p>		<p>reaction. Include a 70% (v/v) ethanol wash of the mRNA pellet.</p> <p>Test for the presence of inhibitors by mixing 1 µg of control RNA with 1 µg of sample RNA and comparing yields of first-strand cDNA.</p>
<p><b>Poor reverse transcription performance</b></p>	<p>The input amount of RNA is not optimal for the size of RT reaction.</p> <p>Quality of the starting template is poor. Trace amounts of agents used in RNA purification protocols may remain in solution and inhibit first strand synthesis.</p>	<p>It is fundamental to accurately quantify the starting template (RNA) to be reverse transcribed. Use a dilution series of RNA for converting to cDNA using individual RT reactions, before performing real time PCR. All reactions must be performed in triplicate.</p> <p>To remove trace contaminants, re-precipitate the RNA with 96% ethanol and wash the pellet with 80% ethanol.</p>
<p><b>Unsuitable primer</b></p>	<p>Perform synthesis with other primer and analyze the products.</p>	<p>Sequence specific primer should be complementary to the 3'- end of RNA. If the RNA template contains transcriptional pauses, perform synthesis with a random hexamer primer.</p>
<p><b>RevertAid™ M-MuLV reverse transcriptase inhibitors</b></p>	<p>Perform control synthesis with RNA sample mixed up with 1µg of control RNA.</p>	<p>Precipitate RNA sample with 96% ethanol and wash with 80% ethanol. Do not add inhibitors to the reactions mixture.</p> <p>Dilute the sample (along with the inhibitor) to prevent the inhibition.</p>

<b>RNase contamination in the reaction mixture</b>	Perform the control reaction and analyze the products obtained.	Prepare a reaction mixture under aseptic condition, wear gloves at all the time, treat all plastic ware in contact with samples with DEPC. Be careful not to contaminate the solutions.
<b>Degraded RNA template</b>	Check RNA integrity in denaturing Agarose gel electrophoresis or Agilent 2100 bioanalyzer. Sharp 18S and 28S RNA bands should be visible after denaturing agarose gel electrophoresis of total eukaryotic RNA.	<p>Store samples carefully prior to RNA isolation.</p> <p>Disrupt samples completely to prevent RNA degradation and to increase RNA yield.</p> <p>Choose an appropriate RNA isolation kit.</p> <p>Store RNA in RNase-free solution.</p> <p>Accurately quantities RNA and confirm that RNA is high integrity and purity.</p> <p>Maintain your laboratory workspace free of RNase contamination.</p>
<b>Multiple PCR products or smearing</b>	<p>The chosen primers are inappropriate for amplification or that the cycling parameters are not optimal.</p> <p>Contamination by genomic DNA</p>	<p>Adjust the annealing temperature of the reaction or choose a new pair of primers. Additionally, a second pair of “nested” primers may be useful in a secondary amplification of the target cDNA.</p> <p>Pretreat RNA with DNase I. Design primers that anneal to sequence in exons on both sides of an intron or exon/exon boundary of the mRNA to of amplified allow differentiation between amplification of cDNA and products potential contaminating genomic DNA.</p>

<b>Multiple PCR products or smearing</b>	Primers formed dimers	To test if products were derived from DNA, do the No RT control.  Design primers without complementary sequences at the 3' ends.
<b>RT-PCR product in negative control</b>	RNA template is contaminated with DNA.	PCR product in the negative control (RT-) indicates the reaction is contaminated with DNA. Perform DNase I digestion prior reverse transcription
<b>Procedural error in the first strand cDNA synthesis</b>		Repeat cDNA synthesis

## References

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2. [www.fermentas.com](http://www.fermentas.com)
3. <https://commerce.invitrogen.com>

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