Downloaded from http://cshprotocols.cshlp.org/ at Anhui Hefei China Science and Technology University on May 19, 2025 -
Published by
Cold Spring Harbor Laboratory Press

Protocol

Reverse Transcription–Polymerase Chain Reaction

Donald C. Rio

Reverse transcription coupled to the polymerase chain reaction (RT–PCR) is commonly used to detect the presence of mRNAs, pre-mRNAs, or other types of RNA such as noncoding RNAs. The method involves using a primer annealed to the RNA of interest. For mRNA, the primer is usually a synthetic oligo$(dT)_{15–18}$, a random hexamer mixture $(dN)_6$, or a synthetic DNA oligonucleotide that is complementary to a specific transcript (a gene-specific primer). This DNA:RNA hybrid serves as a template during reverse transcription, in which the enzyme reverse transcriptase (RT) generates a single-stranded cDNA copy of a portion of the target RNA molecule. Using random hexamer priming, it is possible to obtain representative cDNA copies of sequences from the entire length of the mRNAs and pre-mRNAs in a population. This cDNA can then be used as a template for PCR. On addition of gene-specific primers, a specific DNA fragment corresponding to a portion of the RNA of interest is generated.

Cold Spring Harbor Protocols
www.cshprotocols.org

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.

RECIPE: Please see the end of this protocol for recipes indicated by <R>. Additional recipes can be found online at http://cshprotocols.cshlp.org/site/recipes.

Reagents

[α-^{32}P]dCTP (3000 mCi/mmol, 20 µCi/mL) (for PCR using random hexamer-primed cDNA or oligo [dT]-primed cDNA only; see Step 17)
AmpliTaq DNA polymerase (5 units/µL) and 10× buffer (Applied Biosystems)
Dithiothreitol (DTT) (0.1 M)
dNTPs (each 10 mM)
First-strand buffer for RT–PCR (5×) <R>
Gel (1.8% agarose or 5%–7.5% polyacrylamide [0.8:30 bis-acrylamide: acrylamide]) and electrophoresis buffer (e.g., 1× TBE)
H_2O (RNase-free)
Methanol (10%)/acetic acid (10%) (optional; see Step 20)
Mineral oil (for gene-specific priming method only; see Steps 12 and 23)
Primers

For reverse transcription, use random hexamers ([dN]$_6$) (5 mg/mL), oligo(dT)$_{15}$ or oligo(dT)$_{18}$ (100 pmol/µL), or one gene-specific primer (the 3′ primer is used). For PCR, use two gene-specific primers (one 5′ and one 3′).

Adapted from *RNA: A Laboratory Manual* by Donald C. Rio, Manuel Ares Jr, Gregory J. Hannon, and Timothy W. Nilsen. CSHL Press, Cold Spring Harbor, NY, USA, 2011.

© 2014 Cold Spring Harbor Laboratory Press
Cite this protocol as *Cold Spring Harb Protoc*; doi:10.1101/pdb.prot080887

Downloaded from http://cshprotocols.cshlp.org/ at Anhui Hefei China Science and Technology University on May 19, 2025 - Published by
Cold Spring Harbor Laboratory Press

RNasin (40 units/μL) (Promega)

SuperScript II reverse transcriptase (20 units/μL) (Invitrogen)

SuperScript II is an engineered version of a Moloney murine leukemia virus (M-MuLV) reverse transcriptase. The recombinant enzyme is purified to near homogeneity from E. coli. *It has reduced RNase H activity and increased thermal stability. The enzyme can be used to synthesize first-strand cDNA at higher temperatures than conventional M-MuLV reverse transcriptase, providing increased specificity, higher yields of cDNA, and more full-length cDNA product. It can generate cDNAs up to 12.3 kb in length.*

Total RNA from sample of interest

The RNA must be treated with DNase to remove contaminating genomic DNA in total RNA samples. Genomic DNA can interfere with RT–PCR assays by producing PCR products in the absence of reverse transcriptase. DNase treatment should be performed and then the RNA is purified and quantitated, depending on the method. Before using any PCR-based method, it is critical to keep in mind that the quality of the data obtained is only as good as the quality of the RNA sample analyzed.

Equipment

Bucket with ice

Gel electrophoresis system (for polyacrylamide and agarose gels) and power supply, vacuum gel drier, and detection equipment, as required (see Steps 19, 20, and 25)

PCR tubes (200 μL)

Thermal cycler

Cold Spring Harbor Protocols
www.cshprotocols.org

METHOD

RT–PCR proceeds in two stages. First, oligo(dT)$_{15}$, (dN)$_6$, or a gene-specific primer is annealed to the target mRNA population, and reverse transcriptase and dNTPs are added to allow first-strand cDNA synthesis (reverse transcription, or RT). Second, new primers, fresh dNTPs, and Taq *polymerase are added to generate a PCR to amplify the cDNA of interest. These products are then run on either on an agarose gel or on a native polyacrylamide gel and analyzed.*

Reverse Transcription (RT): cDNA Priming and Synthesis

Three options are presented here; the selection is based on the choice of primer to be used. Steps 1–5 use oligo(dT)$_{15}$ priming, Steps 6–10 use random hexamer priming (dN)$_6$, and Steps 11–15 use gene-specific priming for first-strand cDNA synthesis. It is always necessary to run a parallel RT–PCR without reverse transcriptase to detect genomic DNA contamination of the RNA sample. Any amplified products observed in the absence of the enzyme arise from DNA contamination, and it is necessary to remove the DNA from the RNA sample by DNase I treatment before proceeding further.

Priming with Oligo(dT)$_{15}$

1. Program a thermal cycler with the following program:

Step 1	10 min	70°C
Step 2	at least 2 min	4°C
Step 3	60 min	42°C
Step 4	hold	4°C

2. Mix the following in a 200-μL PCR tube:

Total RNA	5 μg
Oligo(dT_{15}) or oligo(dT_{18}) (100 pmol/μL)	1 μL
dNTPs (each 10 mM)	1 μL
H_2O (RNase-free)	to 12 μL

3. Run the first two steps of the thermal cycler program from Step 1. Let the reaction cool for at least 2 min at 4°C and halt the program. Move the tube to an ice bucket while adding the reverse transcriptase reagents in Step 2.

 Cite this protocol as *Cold Spring Harb Protoc*; doi:10.1101/pdb.prot080887

Downloaded from http://cshprotocols.cshlp.org/ at Anhui Hefei China Science and Technology University on May 19, 2025 - Published by
Cold Spring Harbor Laboratory Press

4. Add the following components to the PCR tube:

First-strand buffer (5×)	4 µL
DTT (0.1 M)	2 µL
RNasin (40 units/µL)	1 µL
SuperScript II reverse transcriptase (20 units/µL)	1 µL

For multiple reactions, these components can be prepared as a master mix and then 8 µL of the master mix can be added to each tube.

5. Return the tube to the thermal cycler and allow the thermal cycler program to proceed through completion.

Proceed immediately to Step 16. Otherwise, store the cDNA; it will keep indefinitely at –20°C.

Priming with Random Hexamers

6. Program a thermal cycler with the following programs.

i. The heat/snap-cool protocol is as follows:

Step 1	5 min	65°C
Step 2	hold	0°C

ii. The RT program is as follows:

Step 1	10 min	4°C
Step 2	10 min	15°C
Step 3	20 min	42°C
Step 4	15 min	70°C
Step 5	hold	4°C

This program maximizes cDNA synthesis by allowing a slow increase in the temperature to 42°C, which allows the random hexamers to be efficiently annealed and extended.

7. Mix the following in a 200-µL PCR tube:

Total RNA	5 µg
$(dN)_6$ (5 mg/mL)	1 µL
dNTPs (each 10 mM)	1 µL
H_2O (RNase-free)	to 12 µL

8. Run the heat/snap-cool protocol on the thermal cycler. Remove the tube and place in an ice bucket.

9. Add the following components to the tube on ice:

First-strand buffer (5×)	4 µL
DTT (0.1 M)	2 µL
RNasin (40 units/µL)	1 µL
SuperScript II reverse transcriptase (20 units/µL)	1 µL

For multiple reactions, these components can be prepared as a master mix and then 8 µL of the master mix can be added to the tube.

10. Return the tubes to the thermal cycler and run the reverse transcription program on the thermal cycler.

Proceed immediately to Step 16. Otherwise, store the cDNA; it will keep indefinitely at –20°C.

Priming with Gene-Specific Primers

11. Program a thermal cycler with the following programs.

Cold Spring Harbor Protocols
www.cshprotocols.org

Downloaded from http://cshprotocols.cshlp.org/ at Anhui Hefei China Science and Technology University on May 19, 2025 - Published by
Cold Spring Harbor Laboratory Press

i. The following program will anneal the primer to the template DNA:

Step 1	30 min	80°C to 22°C
Step 2	10 min	22°C
Step 3	hold	4°C

ii. The following program will extend the primers:

Step 1	10 min	4°C
Step 2	5 min	4°C to 15°C
Step 3	10 min	15°C
Step 4	5 min	15°C to 22°C
Step 5	10 min	22°C
Step 6	5 min	22°C to 37°C
Step 7	10 min	37°C
Step 8	5 min	37°C to 42°C
Step 9	60 min	42°C
Step 10	5 min	95°C
Step 11	hold	4°C

12. Mix the following in a 200-µL PCR tube:

Total RNA	1–2 µg
First-strand buffer (5×)	2 µL
Gene-specific primer (3′ only)	25 pmol
RNasin (40 units/µL)	0.3 µL
H_2O (RNase-free)	to 10 µL

Adjust the amount of total RNA according to the relative abundance of the transcript. Overlay the reaction with 30 µL of mineral oil if using a thermal cycler without a heated lid.

13. Place the reaction in the thermal cycler and run the annealing program from Step 11.i. After the program is complete, remove the reaction from the thermal cycler and immediately place it on ice.
14. Add the following components to the tube on ice:

First-strand buffer (5×)	3 µL
RNasin (40 units/µL)	0.3 µL
dNTPs (each 10 mM)	1.5 µL
SuperScript II reverse transcriptase (20 units/µL)	1.5 µL
H_2O (RNase-free)	to 15 µL

15. Place the reaction in the thermal cycler and run the cDNA synthesis program from Step 11.ii. After the program is complete, remove the reaction from the PCR machine.

Mineral oil can be removed by extracting with chloroform using standard procedures.

To use the cDNA for PCR, proceed immediately to Step 21. Otherwise, store the cDNA; it will keep indefinitely at –20°C.

Polymerase Chain Reaction (PCR) and Detection of Products

Follow Steps 16–20 if using oligo(dT)-primed or random hexamer-primed cDNA (from Steps 5 or 10, respectively) or follow Steps 21–25 if using cDNA produced with gene-specific primers (from Step 15). In Steps 16–20, [α-^{32}P]dCTP is added to radioactively label the cDNA products, and the products are then analyzed by native acrylamide gel electrophoresis and quantitative phosphorimaging. Steps 21–25 use nonradioactive PCR and agarose gel electrophoresis. For sample results, see Figure 1.

Cold Spring Harbor Protocols
www.cshprotocols.org

Downloaded from http://cshprotocols.cshlp.org/ at Anhui Hefei China Science and Technology University on May 19, 2025 - Published by
Cold Spring Harbor Laboratory Press

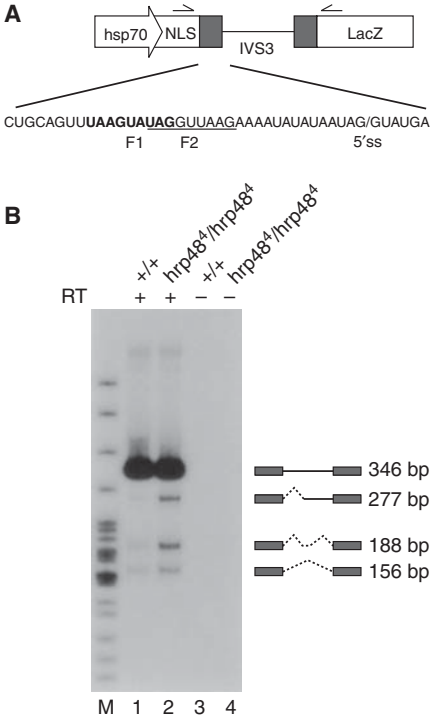

FIGURE 1. RT–PCR assay to detect alternative splicing isoforms. (*A*) Transgenic *Drosophila* strains were used to test the effects of mutations in the splicing factor *hrp48* on P-transposable-element third-intron splicing using an hsp70-P-element splicing reporter (Hammond et al. 1997). (*B*) Wild-type and mutant (lanes *1* and *2*) samples were subjected to RT with gene-specific primers and [^{32}P] dCTP in the PCR step and analyzed by native polyacrylamide gel electrophoresis. Control samples prepared without reverse transcriptase (–RT) were run in parallel (lanes *3* and *4*). This control indicates whether there is genomic DNA contamination. (Reprinted, with permission, from Hammond et al. 1997 [American Society for Microbiology].)

PCR Using Oligo(dT)-Primed or Random Hexamer-Primed cDNA

16. Program a thermal cycler with the following program:

No. of cycles	Denaturation	Annealing	Polymerization
1	3 min at 94°C		
15–35	30 sec at 94°C	30 sec at 55°C	30 sec at 72°C
1			5 min at 72°C
Hold at 4°C			

For amplification products longer than 500 nucleotides, increase the polymerization time by 30 sec for each additional 500 nucleotides. The number of cycles will need to be in the linear range of amplification to make this assay "semiquantitative." See Discussion.

17. Mix the following:

H_2O	40.5 µL
AmpliTaq buffer (10×)	5 µL
dNTPs (each 10 mM)	1 µL
Gene-specific 5′ primer (15 pmol/µL)	1 µL
Gene-specific 3′ primer (15 pmol/µL)	1 µL
[α-^{32}P]dCTP (3000 mCi/mmol, 20 µCi/mL)	0.5 µL
AmpliTaq DNA polymerase (5 units/µL)	0.5 µL
cDNA from Step 5 or Step 10	0.5 µL

18. Place the reaction in the thermal cycler and run the program from Step 16.
19. Load 5 µL of each reaction on a 5%–7.5% native polyacrylamide gel (0.8:30 bis-acrylamide: acrylamide) in 1× TBE.
20. Fix the gel in 10% methanol/10% acetic acid and dry on a vacuum gel drier. Subject the gel to quantitation by phosphorimaging.

    If the gel is thin (<0.75 mm), gel fixation is optional.

    See Troubleshooting.

Cold Spring Harbor Protocols
www.cshprotocols.org

Downloaded from http://cshprotocols.cshlp.org/ at Anhui Hefei China Science and Technology University on May 19, 2025 - Published by
Cold Spring Harbor Laboratory Press

PCR Using cDNA Produced with Gene-Specific Primers

21. Program a thermal cycler with the desired parameters for the amplification reaction. Use an annealing temperature that is 5°C less than the melting temperature (T_m) of the primers.

No. of cycles	Denaturation	Annealing	Polymerization
1	3 min at 94°C		
20–30	30 sec at 94°C	1 min at 45°C–58°C	30 sec at 72°C
1			5 min at 72°C
Hold at 4°C			

22. Set up the cDNA amplification reaction as follows:

AmpliTaq buffer (10×)	2 µL
Gene-specific primer (3′) used in Step 12	25 pmol
Other gene-specific primer (5′) than used in Step 12	50 pmol
cDNA from Step 15	5–10 µL
H_2O	to 20 µL

Typically, it is not necessary to add more dNTPs to the PCR because there were sufficient dNTPs in the RT reaction.

23. Place the reaction tube in the thermal cycler with the cap open.

24. Begin the program. Once the thermal cycler has reached ~80°C, add 5 µL of the following mix:

AmpliTaq buffer (10×)	0.5 µL
AmpliTaq DNA polymerase (5 units/µL)	0.25 µL
H_2O	4.25 µL

This step is called a manual "hot start" and helps to reduce nonspecific annealing of the primers. Make sure that the mix has been added to all samples before the annealing/extension cycles begin. See Discussion.

25. Load 5–10 µL of each amplification reaction on a 1.8% agarose gel and detect using standard procedures.

See Troubleshooting.

Cold Spring Harbor Protocols
www.cshprotocols.org

TROUBLESHOOTING

Problem (Steps 20 and 25): The reactions are not visible by acrylamide or agarose gel electrophoresis.
Solution: Consider the following.

- When this procedure does not work, it is often because of poor primer design. It is critical that the two PCR primers be T_m-matched (Sambrook et al. 1989). Also check primers for structure or potential homodimer formation. It is worth spending some effort on primer design before using this protocol (see Discussion).
- To determine whether any product is present, try blotting the gel to a membrane and hybridizing with a specific ^{32}P-labeled probe. Also, try radiolabeling the PCR product(s) during the PCR. If using the labeling method, analyze the results by native polyacrylamide gel electrophoresis as described in Steps 19 and 20.

Problem (Steps 20 and 25): There is inappropriate or nonspecific amplification.
Solution: Do not assume that sharp bands produced after a PCR are the correct RT–PCR products; make sure that the PCR product is of the anticipated size. It is helpful to perform restriction enzyme digestions or sequencing on the PCR products to confirm that the correct fragment has been amplified. Often, the use of gene-specific primers produces a much lower background and

 Cite this protocol as *Cold Spring Harb Protoc*; doi:10.1101/pdb.prot080887

Downloaded from http://cshprotocols.cshlp.org/ at Anhui Hefei China Science and Technology University on May 19, 2025 - Published by
Cold Spring Harbor Laboratory Press

reduces nonspecific PCR amplification products. The use of a "hot start" (as in Step 24) may also reduce nonspecific amplification (see Discussion).

DISCUSSION

Advice on Primer Selection and Design

The decision of whether to use oligo$(dT)_{15}$ or $(dN)_6$ priming will depend on the goal. If validating or detecting spliced mRNA isoforms and if the intron(s) of interest is not located near the 3′ end of the transcript, then random-primed cDNA synthesis will be essential. For certain applications, it may be preferable to perform RT using a mixture of oligo(dT) and random hexamer primers in the same reaction tube. For instance, it may be useful when performing RT–PCR to detect alternate splicing patterns for a gene with introns near the 3′ end (close to the oligo[dT] primer on the poly [A] tail) and near the 5′ end (close to the random hexamer primers) (e.g., Fig. 1). In the protocol by Castle et al. (2003), random hexamer priming was optimized to equally represent all portions of an mRNA as cDNA, whereas oligo(dT) priming obviously biases the cDNA products toward the 3′ end of the gene. That said, a novel mutant and more processive reverse transcriptase called ArrayScript (Ambion), an engineered version of M-MuLV that improves the yield of full-length cDNA using oligo (dT) mRNA priming of reverse transcription, is often used for cDNA synthesis for microarray analysis.

The success of an RT–PCR (or any PCR) depends critically on primer design. Below are some suggestions for designing RT-PCR primers (adapted from www1.qiagen.com/literature/render.aspx?id=23570).

1. Typical primers for RT–PCR are 18–30 nucleotides long and have a G + C content of 40%–60%.
2. It may be helpful to access web-based tools such as Primer3 (Rosen and Skaletsky 2000) to aid in primer design (http://biotools.umassmed.edu/bioapps/primer3_www.cgi).
3. A simple formula that can be used to estimate the melting temperature (T_m) is

$$T_m = 2^\circ C \times (A + T) + 4^\circ C \times (G + C).$$

4. If at all possible, make an effort to design primer pairs with similar or identical T_m values.
5. Optimal annealing temperatures are empirical, i.e., the optimal annealing temperature for a given primer pair may be either above or below the calculated T_m. As an initial starting point, try an annealing temperature at 5°C below the T_m.
6. Make every attempt to avoid complementarity of the 3′ terminal two or three bases on the primer pairs so as to reduce the formation of primer dimers. Use available software programs for this.
7. Make sure to avoid base sequence mismatches between the very 3′ end of the primer and the template cDNA target sequence.
8. Make sure to avoid putting extended runs of three or more Gs or Cs at the 3′ end of the primer.
9. If possible, avoid a *T* at the extreme 3′ end of the primer because it has been observed that primers with a *T* at the 3′ end have a potential for mispriming caused by mismatch tolerance.
10. Avoid any extended complementary sequences within a single primer and between the two primers of a primer pair.
11. For single-stranded DNA oligonucleotide primers, the spectrophotometric conversion for primers is as follows: 1 A_{260} unit = 33 μg/mL or 20 A_{260} units = 660 μg/mL.

Cold Spring Harbor Protocols
www.cshprotocols.org

Downloaded from http://cshprotocols.cshlp.org/ at Anhui Hefei China Science and Technology University on May 19, 2025 - Published by
Cold Spring Harbor Laboratory Press

12. Molar conversions:

Primer length (nucleotides)	pmol/mg	20 pmol
18	168	119 ng
20	152	132 ng
25	121	165 ng
30	101	198 ng

Hot-Start PCR

The complexity of cellular cDNA is high and can lead to spurious priming of DNA before or during the denaturation step in the PCR procedure. The formation of "primer dimers" can also occur during the initial phases of a PCR in which the temperature is raised from ambient to 95°C. It is therefore often useful in RT–PCRs to perform a manual "hot-start" reaction in which the *Taq* (or other thermostable) DNA polymerase is added after the reactions reach at least 80°C. Alternatively, it is possible to use a commercial polymerase, available from many companies and provided in an inactive state. These polymerases are a modified form of the recombinant 94-kDa *Taq* DNA polymerase, have no polymerase activity at ambient temperatures, and remain inactive until an extended (2–15 min) incubation at 95°C. HotStarTaq DNA Polymerase, for example, allows for a quick PCR setup and prevents the formation of misprimed products and primer dimers because all reaction components can be combined at room temperature. HotStarTaq DNA polymerase is activated by a 15-min, 95°C incubation step that can easily be incorporated into existing thermal cycling programs. A hot-start protocol usually provides high PCR specificity and often increases the yield of the specific PCR product.

Semiquantitative PCR

Once the RT–PCR conditions for an RNA of interest have been established, it is often desirable to determine the relative abundance of that RNA in one or more samples (e.g., in samples prepared from different tissues, cell lines, or cell fractions). A convenient method for accomplishing this is called semiquantitative PCR. In this approach, it is necessary to determine the number of amplification cycles that are needed both to visualize a product (which is almost always radioactive) and to determine a point (cycle number) where the amount of product is proportional to the amount of input cDNA—the exponential or so-called linear portion of the product accumulation curve (Fig. 2). The exponential phase of amplification occurs during PCR when the PCR products

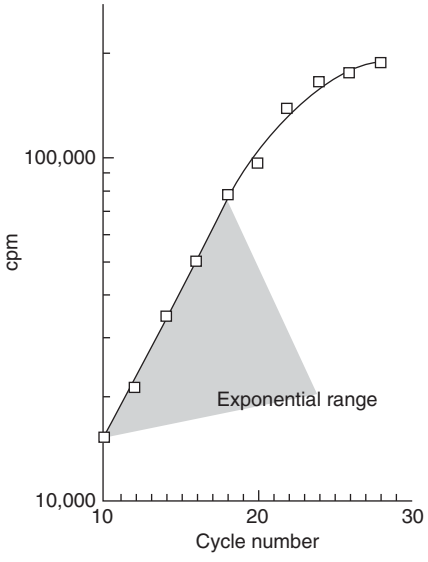

FIGURE 2. Plot of cycle number versus log of the phosphor-imaging signal. The exponential (so-called linear) range of the curve is indicated. (Redrawn from www.ambion.com/[www.ambion.com/techlib/append/pcr_linearrange.html/].)

Cold Spring Harbor Protocols
www.cshprotocols.org

Cite this protocol as *Cold Spring Harb Protoc*; doi:10.1101/pdb.prot080887

Downloaded from http://cshprotocols.cshlp.org/ at Anhui Hefei China Science and Technology University on May 19, 2025 - Published by
Cold Spring Harbor Laboratory Press

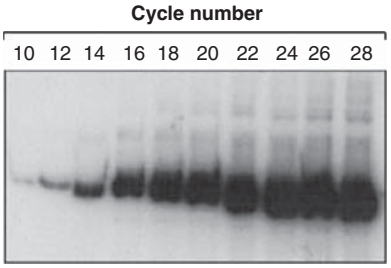

FIGURE 3. Phosphorimaging of PCRs at different cycle numbers. This gel using ^{32}P-labeled RT–PCRs with samples taken at different cycle numbers indicates the increase in product formed and the lack of linearity in the accumulation of cDNA product with increasing cycle number. (Reprinted from www.ambion.com/ [www.ambion.com/techlib/append/pcr_linearrange.html/].)

are accumulating at a constant rate and when the reaction components are still in excess over the product(s).

To empirically determine the exponential range, typically a PCR master mix is prepared that includes 5–10 µL/Ci of [α-^{32}P]dCTP (10 mCi/mL; 400–800 Ci/mmol) in addition to the normal PCR components and cDNA template. The master mix is then split into 10 different aliquots that are then separately subjected to PCR. Samples are removed from the thermal cycler at the indicated cycle numbers and resolved by electrophoresis on a native 5% polyacrylamide gel in TBE buffer (see Fig. 3). The gel is then dried and the radiolabeled cDNA products are quantitated by phosphorimaging. The cycle number (*x*-axis) is plotted versus the log of the ^{32}P signal (*y*-axis) and a straight line is obtained for samples in the exponential (linear) phase of the amplification curve (see Fig. 2).

When comparing RNA samples, it is always necessary to normalize them based on the level of an RNA that is known to be constant (e.g., an abundant housekeeping mRNA such as glyceraldehyde phosphate dehydrogenase [GAPDH], rp49, actin, or tubulin). Once the linear range for this control transcript is established in each sample, the most concentrated sample should be diluted to match the concentration of the least concentrated. It is often desirable to "prenormalize" the RNA concentrations of the samples using spectrophotometry, if possible, so that each sample gives the same signal at the same cycle number. Once these conditions have been met, one can proceed to quantitate the transcript of interest, keeping in mind that the number of cycles required to reach the linear range for a less abundant transcript is going to be significantly greater (e.g., by 5–15 cycles) than the number needed for the abundant housekeeping gene.

In summary, semiquantitative PCR is an accepted and powerful method for quantifying the relative abundance of specific RNAs in different preparations. It is important to realize and remember, however, that this approach is not useful for absolute quantitation. For quantitative PCR, see **Basic Quantitative PCR Using Real-Time Fluorescence Measurements** (Ares 2014).

RECIPE

First-Strand Buffer for RT–PCR (5×)

Reagent	Quantity (for 1 mL)	Final concentration
Tris–HCl (1 M, pH 8.3)	250 µL	0.25 M
KCl (1 M)	375 µL	375 mM
$MgCl_2$ (1 M)	15 µL	15 mM
DTT (0.5 M)	200 µL	0.1 M
H_2O	160 µL	

Store for up to 6–12 mo at –20°C.

REFERENCES

Ares M. 2014. Basic quantitative PCR using real-time fluorescence measurements. *Cold Spring Harb Protoc* doi:10.1101/pdb.prot080903.

Castle J, Garrett-Engele P, Armour CD, Duenwald SJ, Loerch PM, Meyer MR, Schadt EE, Stoughton R, Parrish ML, Shoemaker DD, et al. 2003.

Cold Spring Harbor Protocols
www.cshprotocols.org

Downloaded from http://cshprotocols.cshlp.org/ at Anhui Hefei China Science and Technology University on May 19, 2025 - Published by
Cold Spring Harbor Laboratory Press

Optimization of oligonucleotide arrays and RNA amplification protocols for analysis of transcript structure and alternative splicing. *Genome Biol* **4:** R66.

Hammond L, Rudner DZ, Kanaar R, Rio DC. 1997. Mutations in the hrp48 gene, which encodes a *Drosophila* heterogeneous nuclear ribonucleoprotein particle protein, cause lethality, developmental defects and affect P-element third-intron splicing in vivo. *Mol Cell Biol* **17:** 7260–7267.

Rozen S, Skaletsky H. 2000. Primer3 on the WWW for general users and for biologist programmers. *Methods Mol Biol* **132:** 365–386.

Sambrook J, Fritsch EF, Maniatis T. 1989. *Molecular cloning: A laboratory manual*, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Cold Spring Harbor Protocols
www.cshprotocols.org

Cite this protocol as *Cold Spring Harb Protoc*; doi:10.1101/pdb.prot080887

Downloaded from http://cshprotocols.cshlp.org/ at Anhui Hefei China Science and Technology University on May 19, 2025 - Published by
Cold Spring Harbor Laboratory Press

Reverse Transcription–Polymerase Chain Reaction

Donald C. Rio

Cold Spring Harb Protoc; doi: 10.1101/pdb.prot080887

Email Alerting Service Receive free email alerts when new articles cite this article - **click here.**

Subject Categories Browse articles on similar topics from *Cold Spring Harbor Protocols.*

Amplification of DNA by PCR (86 articles)
cDNA (62 articles)
Detection of PCR Products (24 articles)
Molecular Biology, general (1314 articles)
mRNA (132 articles)
Polymerase Chain Reaction (PCR) (145 articles)
Polymerase Chain Reaction (PCR), general (185 articles)
RNA (317 articles)
RNA, general (269 articles)
RT-PCR (49 articles)

To subscribe to *Cold Spring Harbor Protocols* go to:
http://cshprotocols.cshlp.org/subscriptions

© 2014 Cold Spring Harbor Laboratory Press