

Chapter 3

Synthesis of RNA by In Vitro Transcription

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Abstract

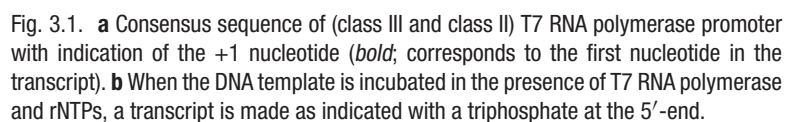
In vitro transcription is a simple procedure that allows for template-directed synthesis of RNA molecules of any sequence from short oligonucleotides to those of several kilobases in μg to mg quantities. It is based on the engineering of a template that includes a bacteriophage promoter sequence (e.g. from the T7 coliphage) upstream of the sequence of interest followed by transcription using the corresponding RNA polymerase. In vitro transcripts are used in analytical techniques (e.g. hybridization analysis), structural studies (for NMR and X-ray crystallography), in biochemical and genetic studies (e.g. as antisense reagents), and as functional molecules (ribozymes and aptamers).

Key words: T7 RNA polymerase, in vitro transcription, template purification.

1. Introduction

RNA is conveniently synthesized by in vitro transcription using the components of bacteriophage systems. The RNA polymerase (RNAP) is a single subunit of about 100 kDa that is highly specific for its 23-bp promoter sequence. With these two simple components, it is possible to make transcripts ranging in size from less than 30 nt to well over 10^4 nt in scales from μg to mg amounts. The most frequently used systems are the T3, T7, and SP6 systems. Here, in vitro transcription is exemplified by the T7 system derived from the T7 phage of *E. coli* established many years ago (1). In vitro transcripts can be used as hybridization probes, in RNase protection or interference experiments, as antisense reagents, for analysis of RNA-binding proteins, to elucidate RNA structure by structure probing, NMR or X-ray crystallography, or as functional molecules (e.g. aptamers and ribozymes). The

The basic strategy is to place the sequence of interest downstream from the T7 promoter. The promoter covers the sequence ranging from -17 to +6 with +1 being the first nucleotide of the transcribed region (*see Fig. 3.1*). Thus, there is not complete freedom in the choice of the sequence at the very 5'-end of the in vitro transcript. Most T7 promoters, like class III promoters (3), have G's at +1, +2, and +3, and the first two G's are critical for transcriptional yield. The alternative class II promoters initiate with an A and have a similar preference for G's at +2 (4). The template for transcription can be (1) a plasmid that typically has the promoter for in vitro transcription immediately upstream from a polylinker for cloning the sequence to be transcribed, (2) a PCR product that has the T7 promoter as part of the 5'-oligonucleotide used in the PCR reaction, and (3) two annealed oligonucleotides that carries the T7 promoter sequence and the template to be transcribed (in this case, only the T7 promoter part of the template needs to be double-stranded) (*see Fig. 3.2*). Most plasmid cloning vectors have one or more promoters for in vitro transcription upstream of multiple cloning sites (MCS) (e.g. the pBluescript (Stratagene) and pGEM (Promega) series). An alternative strategy consists in cloning a DNA fragment including a T7 promoter immediately 5' of the sequence to be transcribed in order to avoid the presence of nucleotides derived from the MCS in the transcript. In this case plasmids like pUC18 and pUC19 are



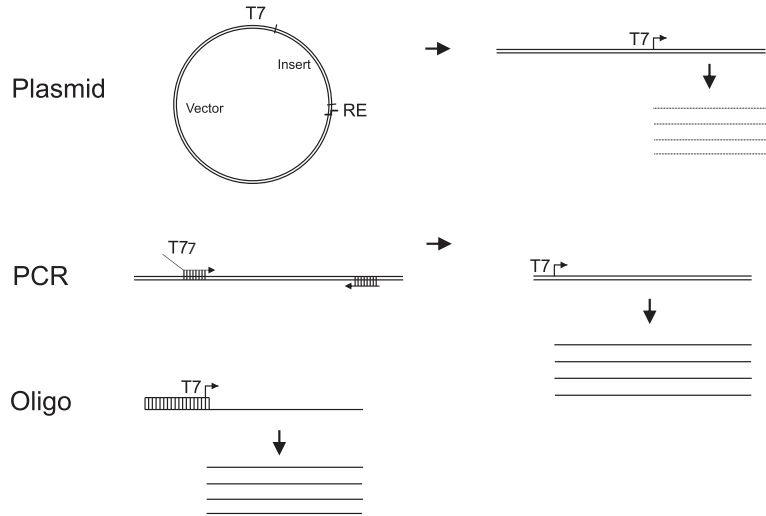


Fig. 3.2. Three different types of DNA templates for in vitro transcription. In the *upper panel*, a circular plasmid with the insert of interest cloned between a T7 promoter and a unique restriction enzyme site is linearized and transcribed from the promoter to yield multiple RNA transcripts terminated by “running-off” the template. In the *middle panel*, a DNA template (genomic DNA, cDNA, or a cloned fragment) acts as a template in PCR with a 5'-primer containing a T7 promoter (with no complementarity to the template) fused to a specific sequence complementary to the sequence of interest and a similarly specific 3'-primer. The resulting PCR-product is transcribed into RNA. In the *lower panel*, a short oligo corresponding to the T7 promoter sequence is annealed to an oligo that has the complementary sequence fused to a template sequence of interest. The partially double-stranded oligos can be transcribed into short RNAs.

preferred due to the absence of a built-in T7 promoter. Cloned templates are used for long transcripts (> 100 nt) and annealed oligo's for very short transcripts. When large amounts of RNA are needed, it is better to use a cloned template in order to generate enough template using simple and economical techniques based on bacterial culture and plasmid extraction. When small amounts are needed, PCR-products are probably the most convenient due to the flexibility in design of the template and the ease of its production.

Transcription termination in the natural setting occurs at specific terminator sites called Rho-independent terminators (5). In this mechanism, the 3' end of the mRNA forms a hairpin structure about 7–20 base pairs in length directly followed by a U-rich stretch (6). The hairpin formation promotes pausing of the RNA polymerase and leads to the disruption of the transcription complex. However, for in vitro transcripts, termination usually intervenes by “run off,” that is when the polymerase falls off at the very end of the template. With the PCR and oligo templates this is defined by the ends of the template products. With cloned templates this is achieved by linearizing the plasmid by restriction enzyme digestion downstream from the sequence of interest.

The average rate of in vitro transcription is 200–260 nt/s and the frequency error about 6×10^{-6} (7). In addition, the use of artificial templates for T7 transcription can result in sequence heterogeneities at the 5' and 3' ends of transcripts. For some applications, like in NMR or X-ray crystallography, homogeneity of the ends is crucial. Some sequences located at the 5' end of DNA templates render the T7 RNAP inaccurate during the initiation of transcription. For example, when the template sequence starts with a stretch of 5–6 G residues, untemplated G residues can be integrated in the transcripts (8). If the 5' end of the sequence does not start with guanine residues but with 5'C₊₁AC/G as in the human mitochondrial lysyl and prolyl-tRNAs, transcription will occur but leads to incorporation of one additional nucleotide (preferentially a purine) or to skipping of the +1 and +2 residues (9). It is likely that other sequences could present similar transcription defects. One solution to problems like these is to fuse a cleavage ribozyme 5' to the RNA of interest (10, 11). In this case, the natural +1 to +6 residues of the natural T7 promoter can be used regardless of the starting sequence of the RNA of interest guaranteeing efficient transcription and efficient control of the 5' sequence content. The 3' end of the transcript can similarly be heterogeneous. During run-off transcription T7 RNAP has a tendency to incorporate one or several non-templated nucleotides at the 3'-end, thus leaving the pool of transcripts with heterogeneous 3'-ends. This problem is addressed by incorporating a sequence that encodes a *cis*-acting cleavage ribozyme like the Hepatitis delta virus (HDV ribozyme) at the 3'-end of the template (*see Fig. 3.3*) (11). By using an optimized HDV ribozyme, homogenous RNA 3'ends can be easily generated even at low Mg²⁺ concentration (12). During transcription, the HDV ribozyme folds into an active conformation and cleaves the transcript (*see Fig. 3.3*). However, the competition between the folding of the RNA of interest and the folding of the HDV ribozyme could lead to reduced cleavage efficiency. This problem normally can be tackled by optimization of temperature, pH and salt conditions (13).

Another concern can be the concentration of rNTPs in the course of the transcription reaction. This problem arises when one of the nucleotides is used at limiting concentrations e.g. during synthesis of radioactive body-labelled transcripts. During the initiation process, the RNA polymerase initially produces short, abortive oligoribonucleotides of 9–12 nt in length. At some point, the polymerase switches to processive transcription leading to full-length products. If the first 9–12 nucleotides are rich in a nucleotide that is used at limiting concentrations (e.g. several U's when attempting to make a transcript labelled at high specific activity with [α -³²P]UTP), the switch to processive

transcription is made more difficult and the ratio between full length and abortive transcripts decreases. As a consequence of this phenomenon, [α - 32 P]GTP is frequently avoided as a label because G's are inherently rich at the 5'-end of the transcripts.

In vitro transcription protocols are easily modified to allow for synthesis of modified transcripts. T7 RNAP can initiate transcription with guanosine or GMP to obtain 5'-OH or 5'-monophosphate ends. The latter gets more easily dephosphorylated as compared to a triphosphate 5' end for subsequent 5' end labelling using [γ - 32 P]ATP and T4 polynucleotide kinase. Dinucleotides (e.g. ApG) or various cap analogues, e.g. 7-methylguanosine (to obtain mRNA transcripts with native-like 5'-ends) can also be used for transcription initiation. The cap nucleotide protects the transcript against degradation by 5' exonucleases present in extracts and supports translation of the transcript. T7 RNA polymerase use variety of modified nucleoside 5' triphosphates for internal modification by incorporation. Biotinylated or digoxigenylated nucleotides can be incorporated to make non-radioactive probes for hybridization. Photoreactive nucleotides can be incorporated for synthesis of modified RNAs for various biochemical analyses. The nucleotide analog interference mapping method (NAIM, *see* Suydam and Strobel (14) for review) also relies on the property of the T7 RNA polymerase to incorporate modified nucleotides in transcripts. In this method, 5'-O-(1-thio)-nucleoside triphosphate analogs that are commercially available (GlenResearch, VA, USA) are incorporated at a 5% rate by transcription. After purification of the RNA using an activity assay specific to the studied RNA, iodine cleavage is performed so as to identify residues that are important for activity. The wild-type T7 RNA polymerase or the mutant Y639F (15) (Epicentre, WI, USA), which also allows efficient incorporation of nucleotides with a modified 2' position, such as 2'-deoxy or 2'-fluoro can be used in this case. (*See* Gruegelsiepe (2) for a more detailed discussion of the applications of modified transcripts.)

All the protocols below describe the various procedures for in vitro transcription from plasmid- and PCR-derived templates (*see* **Fig. 3.2**). All these protocols provide simple methods to produce RNA by using a commercial T7 RNA polymerase. However, the commercial T7 RNA polymerase could be easily replaced by an in-house T7 RNA polymerase made by expression and purification of an His-tagged T7 RNA polymerase (plasmid pT7-911Q (16)). Then follow protocols for making unlabelled and 32 P-labelled transcripts. The protocols are for small-scale transcriptions, but they can be scaled up without problems. Similarly, the specific activity of the radioactive transcripts can be altered by adjusting the ratio between UTP and [α - 32 P]UTP. Depending on the use of the transcript, a simple phenol:chloroform extraction directly followed by an ethanol precipitation of the transcript may be

sufficient. Transcripts that are used as hybridization probes are purified by gel-filtration to get rid of the unincorporated nucleotides for reasons of radiation hazards and to allow for a simple evaluation of the probe. A protocol for gel filtration and a simple calculation of the specific activity of the probe is included. In other cases, gel purification of the transcripts is required and a simple protocol for this ends the chapter.

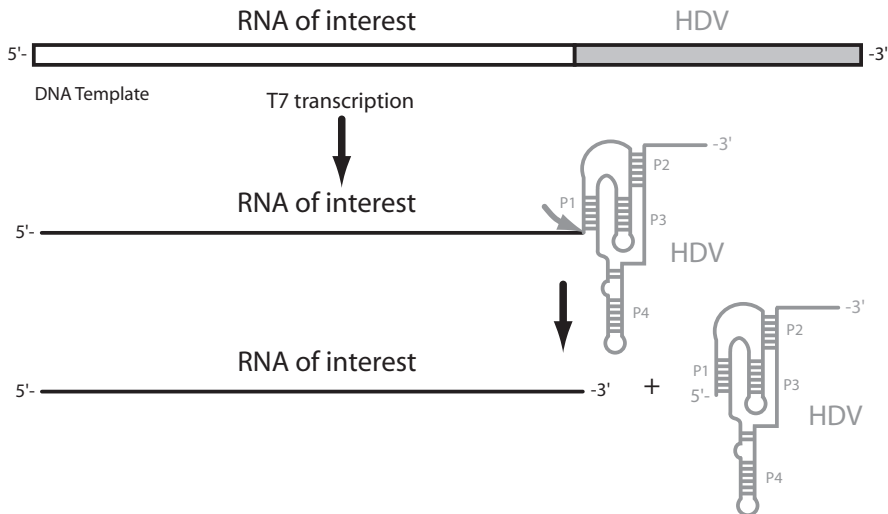


Fig. 3.3. The 3' cassette allowing for obtaining homogeneous RNA 3' ends. The transcribed DNA molecule (linearized plasmid, PCR product) includes an extra cassette downstream from the sequence encoding the RNA of interest. This cassette (grey) is transcribed into a self-splicing ribozyme (the HDV ribozyme). The cleavage activity of the HDV ribozyme leads to the release of the RNA of interest bearing a 2',3'-cyclic phosphate group at the 3' end.

2. Materials

2.1. Templates for In Vitro Transcription

2.1.1. Plasmid DNA Templates for In Vitro Transcription

1. Plasmid including the sequence to be transcribed downstream from a T7 promoter and upstream from a unique restriction enzyme site to be used for linearization (*see Note 1*).
2. Restriction enzyme and corresponding buffer.
3. Proteinase K.
4. Phenol:chloroform:isoamylalcohol (25:24:1).
5. 96% ethanol.
6. 70% ethanol.
7. TE 8.0 (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA).

2.1.2. PCR Templates for In Vitro Transcription

1. Template DNA (genomic DNA, cDNA or a cloned fragment inserted into a vector).
2. Oligonucleotides designed to amplify the sequence of interest (*see Note 2*).
3. Thermostable DNA polymerase with proof-reading activity such as *PfuI*.
4. 10× polymerase buffer (usually provided by the supplier of the polymerase; *see Note 3*).
5. 10× dNTP-mix (2 mM of each dNTP).
6. PCR clean-up kit (e.g. GenElute™ PCR Clean-Up Kit Sigma).

2.2. In Vitro Transcription

2.2.1. In Vitro Transcription of Unlabelled Transcripts

1. Template DNA (*see Section 2.1.2*) at 1 µg/µL of a 3 kb linearized plasmid or 0.2 µg/µL of a 600-bp PCR-product. This will result in a final concentration of T7 promoter in the transcription of ~20 nM.
2. 10× polymerase buffer: 100 mM NaCl, 80 mM MgCl₂, 20 mM spermidine, 800 mM Tris-HCl, pH 8.0.
3. 100 mM DTT.
4. 10× rNTP mix: 10 mM of each rNTP.
5. T7 RNA polymerase (20 U/µL).

2.2.2. In Vitro Transcription of ³²P-Labelled Transcripts

1. Template DNA at 1 µg/µL of a 3 kb linearized plasmid or 0.2 µg/µL of a 600-bp PCR-product. This will result in a final concentration of T7 promoter in the transcription of ~20 nM.
2. 10× polymerase buffer: 100 mM NaCl, 80 mM MgCl₂, 20 mM spermidine, 800 mM Tris-HCl, pH 8.0.
3. 100 mM DTT.
4. 10× rNTP mix “low UTP” for radio-labelled transcripts: 1 mM UTP, 10 mM of each of ATP, CTP, and GTP (*see Note 4*).
5. T7 RNA polymerase (20 U/µL).
6. [α -³²P]UTP (3,000 Ci/mmol; 10 mCi/mL) (this corresponds to ~3 µM in UTP, *see Note 5*).

2.3. Purification

2.3.1. Purification of Transcripts by Gel Filtration

1. Sephacryl S-200 columns (GE Healthcare).

2.3.2. Gel Purification of Transcripts

1. Denaturing polyacrylamide gel.
2. TBE 10× electrophoresis buffer.
3. Ethidium bromide staining solution.
4. Elution buffer: 0.25 M sodium acetate, pH 6.0, 1 mM EDTA.
5. Phenol saturated with elution buffer.
6. Glycogen.
7. 96% ethanol.
8. TE 7.6 (10 mM Tris-HCl, pH 7.6, 0.1 mM EDTA).

3. Methods

3.1. Templates for In Vitro Transcription

3.1.1. Plasmid Templates for In Vitro Transcription

1. Digest the (RNase-free) plasmid DNA (e.g. 100 µg) with an appropriate restriction enzyme that cleaves downstream of the T7 promoter and the segment to be transcribed.
2. Add proteinase K to a final concentration of 50 µg/mL and incubate for 30 min at 37°C in order to remove the restriction enzyme from the template DNA.
3. Extract twice with one volume of phenol-chloroform (*see Note 6*).
4. Precipitate the template with 2.5 vols of 96% ethanol.
5. Resuspend the DNA to 1 µg/µL in TE 8.0.
6. Run an aliquot (e.g. 0.5 µg) of the DNA on an agarose gel to check the linearization of the plasmid (*see Note 7*).

3.1.2. PCR Templates for In Vitro Transcription

1. Design the oligos for PCR-amplification.
2. Make a standard PCR reaction.
3. Purify the PCR product using a commercial PCR clean-up kit (GenEluteTM PCR Clean-Up Kit Sigma) according to the manufacturer's instructions.

3.2. In Vitro Transcription

3.2.1. In Vitro Transcription of Cold (i.e. Unlabelled) Transcripts

1. Set up the transcription reaction by adding the components in a siliconized or Teflon-coated tube in the following order at room temperature (*see Note 8*):

- 5 μL of $5\times$ transcription buffer
- 4 μL of $10\times$ rNTP mix
- 2.5 μL of 100 mM DTT
- 11.5 μL DEPC-treated dH_2O
- 1 μL of template DNA (linearized plasmid or PCR-product)
- 1 μL 10 U of the appropriate (in this case T7) RNA polymerase (*see Note 9*)
- Incubate for 30–60 min at 37°C .

3.2.2. In Vitro Transcription of ^{32}P -Labelled Transcripts (see **Note 5** for ^{32}P -Handling)

1. Set up the transcription reaction by adding at room temperature the components in a siliconized or Teflon-coated tube in the following order:

- 5 μL of $5\times$ transcription buffer
- 4 μL of $10\times$ rNTP mix “low UTP”
- 2.5 μL of 100 mM DTT
- 6.5 μL DEPC-treated dH_2O
- 1 μL of template DNA (linearized plasmid or PCR-product)
- 5 μL of 3,000 Ci/mmol, 10 mCi/ml [α - ^{32}P]UTP
- 1 μL 10 U of the appropriate (in this case T7) RNA polymerase

2. Incubate for 30–60 min at 37°C .

3.3. Purification

3.3.1. Purification of Transcripts by Gel Filtration

1. Prepare the column according to the manufacturer’s recommendation (usually a brief, low-speed spin to remove storage buffer).
2. Add the transcription reaction on top of the column and spin briefly (typically 2 min) at low speed ($735\times g$).
3. Collect the eluate containing the transcript. Most of the unincorporated nucleotides are retained in the column. If the transcript is radioactive, an aliquot can be removed and used for estimation of the specific activity without further purification (*see Note 10*).

3.3.2. Gel Purification of Transcripts

1. Run the transcription mixture on a denaturing polyacrylamide gel (*see Note 11*; *see Fig. 3.4*). The type of gel depends on the size of the transcript to be purified, but in most cases, a 5% polyacrylamide gel will be appropriate.
2. Visualize the RNA by ethidium bromide staining or UV_{254} -shadowing over Xerox paper. Radioactive transcripts are detected by autoradiography using fluorescent markers to help in alignment of the gel and autoradiogram.

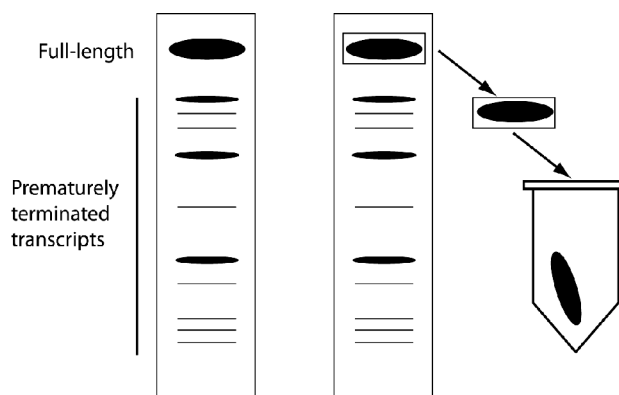


Fig. 3.4. Gelelectrophoretic separation of a transcription reaction. In addition to the full-length transcript, several prematurely terminated transcripts are seen. The full-length transcript can be excised from the gel and eluted into a buffer from which it can be recovered. Premature termination is typical when the concentration of one nucleotide is lowered to favour synthesis of radioactive transcripts of high specific activity. The presence of sequences in the template that resemble terminators or other sequences that are difficult to transcribe will similarly result in short transcripts.

3. Excise the full-length transcript using a scalpel. Avoid carrying over excessive amounts of polyacrylamide.
4. Place the gel slice in a tube containing 400 μL of elution buffer and an equal volume of phenol (*see Notes 12 and 13*).
5. Shake the tubes at room temperature for several hours or over night in the cold room (4°C). The time required will depend on the size of the RNA and the acrylamide gel concentration.
6. Spin and transfer all the liquid to a new tube.
7. Spin and transfer the aqueous phase to a new tube. Add 4 μL of glycogen and 1,200 μL of ethanol to precipitate the RNA.
8. Resuspend in dH_2O or TE buffer.

4. Notes

1. A restriction enzyme that leaves 5'-protruding ends is preferred in the linearization of the plasmid because T3 and T7 polymerases can initiate transcription from the ends of DNA fragments. This type of initiation is most prevalent with 3'-protruding termini followed by blunt ends and 5'-protruding termini. Non-specific initiation is suppressed in transcription buffers with increased (100 mM) NaCl

concentration. However, this will also result in a decrease of the total transcription efficiency by approximately 50%.

2. The 5'-oligo should incorporate the class III T7 promoter sequence: 5'-TAATACGACTCACTAT AGG(G) or the class II promoter sequence for ApG transcription starter: 5'-TAATACGACTCACT ATTAG (*see Fig. 3.1*) both of them directly followed by specific target sequence. For this and the 3'-oligo, we typically use 15- to 20-mer sequences with a T_m around 50°C as calculated adding 2°C for each A or T in the sequence and 4°C for each C or G. This simple approach for designing oligos rarely fails. However, it is also possible to use software made to optimize primer design, such as Primer3 found at <http://frodo.wi.mit.edu>.
3. The free $[Mg^{2+}]$ must be adjusted according to the nucleotide concentration. Since each nucleotide chelates one Mg^{2+} ion, the total $[Mg^{2+}]$ should exceed the total nucleotide concentration by approximately 5 mM.
4. Any of the four rNTPs can be used as label. The main concern is to avoid using a nucleotide that is prevalent in the first 10–12 nucleotides of the transcript and this criteria will in many cases argue against GTP because G's are required at +1 and +2 and preferred at +3 positions.
5. ^{32}P is a high energy β -emitter. Avoid exposure to the radiation and radioactive contamination. Wear disposable gloves when handling radioactive solutions. Check your gloves and pipettes frequently for radioactive contamination. Use protective laboratory equipment (protective eyeglasses, Plexiglas shields) to minimize exposure to radiation. Dispose of radioactive waste in accordance with the rules and regulations established at your institution.
6. To increase the recovery in extractions of small volumes it is sometimes advisable to increase the volume of the sample prior to extraction. For DNA samples this can be done by addition of DEPC-water.
7. Incomplete digestion can be due to suboptimal conditions or the possibility that some of the DNA was not exposed to the enzyme. As a result, subsequent transcription will lead to transcripts of the full plasmid including vector sequences. To avoid this, siliconized or Teflon-treated tubes should be used in the restriction enzyme digestion and the sample should be given a brief spin after the addition of the enzyme to collect all of the components in the bottom of the tube. One other possibility is to transfer the sample to a new tube before the next step. In this way, droplets on the side of the tube that were not exposed to the enzyme are not carried over to subsequent steps.

8. The order of assembling the reaction is to avoid spermidine precipitation of the template DNA, especially at low temperatures.
9. Alkaline pyrophosphatase can be added to the transcription reaction at 2 ng/ μ L. The phosphatase we use is purified from *E. coli* and commercially available at Sigma-Aldrich. This hydrolase cleaves the insoluble pyrophosphate into phosphate. Hence, the RNA pellet obtained by ethanol precipitation of the transcription reaction is free of pyrophosphate, which greatly facilitates further solubilization in an appropriate buffer. Furthermore, the hydrolysis of pyrophosphate drives the chemical equilibrium towards the formation of pyrophosphate, which means enhancing the polymerization of the RNA by the T7 RNAP and improving the transcription yield.
10. RNA labelled to a high specific activity is unstable and should be used within a couple of weeks if full-length RNA is required.
11. As an alternative to elution by diffusion, the RNA can be electro-eluted from the gel slice placed in a dialysis bag in an electrophoresis chamber (1 h at 10 V/cm in TBE) or using dedicated commercial equipment.
12. In some protocols the gel slice is crushed or freeze-thawed. In our experience this will give rise to difficulties with small pieces of polyacrylamide in downstream steps. We prefer to avoid this and have not experienced less recovery of transcript from this.
13. Break the hinge of the tube by pressing it against the table and wrap in parafilm. This will prevent leakage from the tube during shaking.

References

1. Milligan, J. F., Uhlenbeck, O. C. (1989) Synthesis of small RNAs using T7 RNA polymerase. *Methods Enzymol* 180, 51–62.
2. Gruegelsiepe, H., Schön, A., Kirsebom, L. A., Hartmann, R. K. (2005) Enzymatic RNA synthesis using bacteriophage T7 RNA polymerase, in: (Hartmann, R. K., Bindereif, A., Schön A., Westhof E., eds.), *Handbook of RNA Biochemistry*. WILEY-VCH Verlag GmbH & Co. KGaA, Germany, pp. 3–21.
3. Milligan, J. F., Groebe, D. R., Witherell, G. W., Uhlenbeck, O. C. (1987) Oligoribonucleotide synthesis using T7 RNA polymerase and synthetic DNA templates. *Nucleic Acids Res* 15, 8783–8798.
4. Huang, F., Yarus, M. (1997) 5'-RNA self-capping from guanosine diphosphate. *Biochemistry* 36, 6557–6563.
5. Jeng, S. T., Gardner, J. F., Gumpert, R. I. (1990) Transcription termination by bacteriophage T7 RNA polymerase at rho-independent terminators. *J Biol Chem* 265, 3823–3830.
6. Dunn, J. J., Studier, F. W. (1983) Complete nucleotide sequence of bacteriophage T7 DNA and the locations of T7 genetic elements. *J Mol Biol* 166, 477–535.

7. Brakmann, S., Grzeszik, S. (2001) An error-prone T7 RNA polymerase mutant generated by directed evolution. *ChemBiochem* 2, 212–219.
8. Pleiss, J. A., Derrick, M. L., Uhlenbeck, O. C. (1998) T7 RNA polymerase produces 5' end heterogeneity during in vitro transcription from certain templates. *RNA* 4, 1313–1317.
9. Helm, M., Brule, H., Giege, R., Florentz, C. (1999) More mistakes by T7 RNA polymerase at the 5' ends of in vitro-transcribed RNAs. *RNA* 5, 618–621.
10. Fechter, P., Rudinger, J., Giege, R., Theobald-Dietrich, A. (1998) Ribozyme processed tRNA transcripts with unfriendly internal promoter for T7 RNA polymerase: production and activity. *FEBS Lett* 436, 99–103.
11. Price, S. R., Ito, N., Oubridge, C., Avis, J. M., Nagai, K. (1995) Crystallization of RNA-protein complexes I. Methods for the large-scale preparation of RNA suitable for crystallographic studies. *J Mol Biol* 249, 398–408.
12. Schurer, H., Lang, K., Schuster, J., Morl, M. (2002) A universal method to produce in vitro transcripts with homogeneous 3' ends. *Nucleic Acids Res* 30, e56.
13. Bevilacqua, P. C., Brown, T. S., Nakano, S., Yajima, R. (2004) Catalytic roles for proton transfer and protonation in ribozymes. *Biopolymers* 73, 90–109.
14. Suydam, I. T., Strobel, S. A., Daniel, H. (2009) Nucleotide analog interference mapping. *Methods Enzymol* 468, 3–30.
15. Sousa, R., Padilla, R. (1995) A mutant T7 RNA polymerase as a DNA polymerase. *EMBO J* 14, 4609–4621.
16. Ichetovkin, I. E., Abramochkin, G., Shrader, T. E. (1997) Substrate recognition by the leucyl/phenylalanyl-tRNA-protein transferase. Conservation within the enzyme family and localization to the trypsin-resistant domain. *J Biol Chem* 272, 33009–33014.

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