Chapter 2

RNA-Directed Recombination of RNA In Vitro

Niles Lehman, Nilesh Vaidya, and Jessica A.M. Yeates

Abstract

Construction of long RNAs can be achieved in vitro by using ribozymes to recombine shorter RNAs. This can be a useful technique to prepare RNAs when the final product is either very long or contains chemical modifications that are difficult to incorporate using standard in vitro transcription techniques. Here, we describe the use of the *Azoarcus* group I intron ribozyme to recombine shorter RNAs into longer ones. This ribozyme is a generalized RNA recombinase ribozyme that operates rapidly and with high efficiency.

Key words Electrophoresis, Polymerase chain reaction, Recombination, Ribozyme, Group I intron, Azoarcus ribozyme, Transcription

1 Introduction

Ribonucleic acid (RNA) molecules range in size from dinucleotides up to messenger (mRNA) and ribosomal RNA (rRNA) species that can be thousands of nucleotides (nt) long. Preparation of RNAs in the laboratory for in vitro assays or for introduction in vivo can occur by a number of means. In some cases, it is possible to isolate the RNA of interest directly from a cellular source. Many commercially available kits exist to do this, but such procedures rarely result in the purification of a single RNA sequence. To obtain a unique RNA species, typically one will either synthesize (or buy) an oligomer made on a solid support via phosphoramidite chemistry or one will prepare the corresponding DNA sequence and perform an in vitro transcription reaction using RNA polymerase [1]. Many companies sell synthetic RNA oligomers, and lengths up to 100 or more nucleotides are possible with μmol yields. Transcription in vitro is now a very well established protocol with excellent published technical resources [2]. In many cases mg yields of RNA can be made in this fashion with relative ease.

However, there are situations in which biological isolation, chemical synthesis, or in vitro transcription are not viable options. In particular, longer RNAs, chemically modified RNAs, and RNAs

Frank J. Schmidt (ed.), *RNA-RNA Interactions: Methods and Protocols*, Methods in Molecular Biology, vol. 1240, DOI 10.1007/978-1-4939-1896-6_2,

to be derived from shorter existing RNAs may render these traditional means of synthesis either too costly or impractical. In some of these situations an alternative may be to recombine two (or more) shorter RNAs into longer ones. Recombination is distinct from ligation in that it does not require a specific chemical moiety to be on the 5′ or 3′ ends of either short RNA substrate, and we have developed a means by which RNA can be recombined by another catalytic RNA [3], obviating the need for protein enzymes to perform the reaction. RNA-directed recombination of RNAs could be particularly useful to create a new RNA species from highly chemically modified RNA precursors, to create a library of RNA species derived from shorter blocks for in vitro selection [4], or to synthesize a particularly long RNA from manageable shorter pieces. Here, we describe the use of the very efficient and thermostable *Azoarcus* ribozyme as a catalyst to recombine RNAs into other RNAs with high yield. The *Azoarcus* ribozyme [5] can be adopted for generalized RNA-directed recombination of other RNAs [3, 6]. While in principle any group I intron ribozyme can be used, the *Azoarcus* ribozyme has the advantages of being relatively short (ca. 200 nt), very thermostable (active up to 60 °C), very efficient (rapid product release leads to high turnover), and can be engineered to utilize a short 3-nt guide sequence. The only restriction to its use is the engineering of corresponding 3-nt recombination target sequences at the desired splice site(s) (*see* **Note 1**).

2 Materials

Prepare all solutions using ultrapure water. We typically use house de-ionized water that has been polished to >18 MΩ by the use of a Millipore (or equivalent) device. This helps to ensure RNase-free water for gel buffers, extraction solutions, and rinses or washes. For water used in the actual RNA-catalyzed reactions described below, we purchase RNase-free water from an RNA-specialty company such as Ambion/Invitrogen. For RNA storage at −20 °C, this latter water will be spiked with 0.1 mM EDTA to chelate any trace amounts of Mg^{2+} ions that would speed spontaneous RNA hydrolysis. Materials that are hazardous in the following procedures include unpolymerized acrylamide (a neurotoxin; avoid direct skin contact and inhalation), phenol (an irritant; avoid skin contact), and ^{32}P radionuclides (follow all appropriate procedures for use, storage, and disposal of radioactive materials). Most solid chemicals are purchased from Sigma Chemical Company, St. Louis, MO, USA.

2.1 Ribozyme Design

The 198-nt "wild-type" L-9 variant of the *Azoarcus* ribozyme is prepared by in vitro run-off transcription from a double-stranded DNA template. At its very 5′ end, this ribozyme has the internal

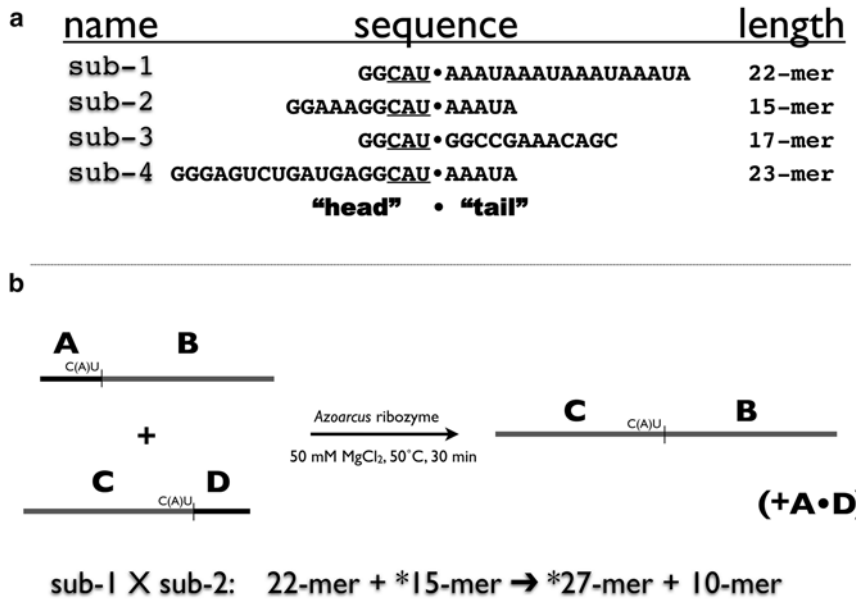

Fig. 1 General recombination scheme. In panel (**a**) are examples of short RNA oligonucleotides that can be recombined with the wild-type *Azoarcus* ribozyme because they contain the target sequence CAU immediately before the splice site (*bullet*). In practice much longer oligomers can be recombined. Panel (**b**) shows a schematic of a recombination between two oligomers to produce a longer RNA. Here the target sequence is generalized in that the middle nucleotide (*A*) can be varied so long as the middle nucleotide in the IGS of the ribozyme is adjusted to its Watson-Crick complement. The *bottom* of panel (**b**) shows an example of how the product can be tracked by the 5′ radiolabeling of one of the substrate oligomers with ^{32}P (*asterisk*) (*see* Fig. 2).

guide sequence (IGS) of 5′-GUG-3′, meaning that it will target recombination sites of the trinucleotide 5′-CAU-3′. A G-U wobble is required at the site immediately preceding the *transesterification* site. The substrate RNAs to be recombined must both possess this target triplet at the recombination site; however, one can vary the middle nucleotide of the target so long as the corresponding change is made in the IGS of the ribozyme [7]. *See* Fig. 1 for a general recombination scheme. Oligomers of any length can be recombined so long as their IGS target is not tightly buried in a highly structured region. The intended product can be tracked during electrophoresis if the 5′ end of one substrate is radiolabeled with ^{32}P (Fig. 1). Multiple successive recombinations can be performed in series if desired.

2.2 DNA Templates

1. Single-stranded DNA oligomers: purchase the following eight oligomers commercially to prepare the wild-type *Azoarcus* ribozyme by recursive gene synthesis, PCR, and in vitro transcription:

   ONL-10 (45-mer): CCGGTTTGTGTGACTTTCGCCA CTCCCTGGACTATGCCTTCACCA.

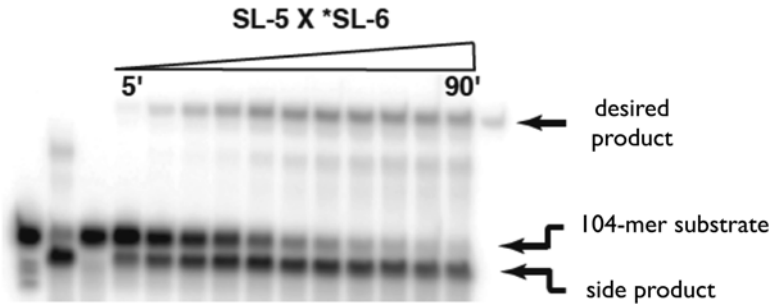

Fig. 2 Tracking of a reaction by the use of ^{32}P. In this case a 152-nt product was recombined using the *Azoarcus* ribozyme [6]. The two substrates for recombination were 58 and 104 nt, and the 5′ end of the 104-mer was 5′-end-labeled with γ-^{32}P•ATP prior to recombination. This substrate is the one that contains the recombination target CAU that ends up in the final product, and this is the substrate that should be radiolabeled. The product can be isolated from the gel using the Dip-N-Dot technique [8]

ONL-11 (54-mer): GAGACTAGACGGCACCCACCTA AGGCAAACGCTATGGTGAAGGCATAGTCCAGG.

ONL-12 (49-mer): GGGTGCCGTCTAGTCTCTACAC CTTCATCGGCGCAGGCGCCGAAGCTTG.

ONL-13 (49-mer): CTAAGCGCCCGCCCGGGCGTA TGGCAACGCCGAGCCAAGCTTCGGCGCC.

ONL-14 (52-mer): GGGCGGGCGCTTAGGTTTCGCC GAATTTGACACCATCCCTTGCGTGGTTTCC.

ONL-15 (49-mer): TAATACGACTCACTATAG(T)GC CTTGCGCCGGGAAACCACGCAAGGGATG.

TAS 2.1a (43-mer): CTGCAGAATTCTAATACGACTCA CTATAGTGCCTTGCGCCGGG.

T20a (21-mer): CCGGTTTGTGTGACTTTCGCC.

All sequences are written 5′-to-3′. The underlined region of oligomer ONL-15 refers to the promoter sequence for T7 RNA polymerase, and the nucleotide in parenthesis can be changed from a T to any other to alter the middle nucleotide of the IGS (boldface type) to create variants of the ribozyme that can utilize other recombination targets. Rehydrate the oligomers to a concentration of 10 μM in 1× TE (pH 8.0).

2. To reconfigure these oligonucleotides for other ribozymes or substrates, use the Assembly PCR Oligo Maker website from the Philip Johnson lab at the University of York: http://www.yorku.ca/pjohnson/AssemblyPCRoligomaker.html.

   This site will guide you in constructing overlapping DNA oligomers for the construction of double-stranded PCR products from which most any RNA can be transcribed.

2.3 Buffers and Solutions

1. Buffer for recursive gene synthesis (1×): 20 mM Tris–HCl (pH 8.8 at 25 °C), 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 2 mM $MgSO_4$, 0.1 % Triton X-100. Make up as a 10× buffer in RNase-free water and store at –20 °C.
2. Buffer for in vitro transcription (1×): 15 mM $MgCl_2$, 50 mM Tris–HCl (pH 7.5), 5 mM dithiothreitol, 2 mM spermidine (add last) (*see* **Note 2**). Make up as a 10× buffer in RNase-free water and store at –20 °C.
3. Polyacrylamide gel running buffer (1× TBE): To make a 10× solution, mix 108 g Trizma base (Sigma T-6066), 55 g boric acid, and 7 g Na_2EDTA in a total volume of 1.0 L of water. Heat gently with stirring to dissolve (*see* **Note 3**). Dilute tenfold with water prior to use as running buffer in an electrophoresis apparatus.
4. Eight percent acrylamide/bis-acrylamide solution (19:1:: acrylamide:Bis): add 161 mL water to 100 g acrylamide:bisacrylamide dry mixture (Fisher BP1364-100), heat gently with stirring until dissolved; this makes a 40 % solution. Add 200 mL of this solution to about 100 mL of water in a 1.5 L beaker on a stirring hotplate with a large stir bar. Add 100 mL of 10× TBE, turn on heat to low and with vigorous stirring add 467 g of dry urea. When all the urea has gone into solution, add water to a total volume of 1.0 L; the final urea concentration will be 8 M. Store in a 1 L Wheaton bottle at room temperature until use.
5. Ammonium persulfate: 10 % solution in water (*see* **Note 4**).
6. *N*,*N*,*N*′,*N*′-tetramethyl-ethylenediamine (TEMED): Purchase commercially (United States Biochemical 76320) (*see* **Note 5**).
7. 3-[4-(2-Hydroxyethyl)-1-piperazinyl]propanesulfonic acid (EPPS) buffer: Dissolve 25.3 g solid EPPS (Aldrich 16,374-0 or Sigma E-1894) in about 75 mL water. Add approximately 30 pellets of KOH (Sigma P-5958), check pH. Continue to add KOH pellets until pH stabilizes at 8.3. Add water to exactly 100 mL.
8. Crush-and-soak solution: 200 mM $NaCl_2$, 10 mM Tris (pH 7.5), 1.0 mM EDTA.
9. Ribozyme *trans*-splicing buffer (1×): 30 mM EPPS (pH 7.5), 50 mM $MgCl_2$. Make up as a 5× buffer in RNase-free water and store at –20 °C.
10. Deoxyribonucleotide triphosphate mixture (1×): 0.2 mM each dATP, dGTP, dCTP, and dTTP in water. Make as a 10× solution (2 mM each) and store at –20 °C.
11. Ribonucleotide triphosphate mixture (1×): 10 mM each ATP, GTP, CTP, and UTP in water. Make as a 5× solution (2 mM each) and store at –20 °C.

12. Polyacrylamide gel-loading dye: To 70 mL water add the following: 20 g sucrose, 50 mg bromphenol blue dye, 1 mL 10 % (w/v) SDS, 10 mL 10× TBE. To 10 mL of this mixture, add 11 g of urea. Heat gently until dissolved and store at 4 °C. This is a 2× solution.

2.4 Denaturing Polyacrylamide Gel Electrophoresis Components

1. Adjustable slab gel system (CBS Scientific ASU-250) (*see* **Note 6**).
2. Notched glass plates (16.5 cm, 28 cm).
3. 1.5 mm thick plastic spacers (28 cm).
4. Gel comb (eight wells, 1.5 cm thick) (CBS Scientific VGC-1508).
5. Aluminum plate (16 cm × 16 cm × 0.5 cm).
6. Electrical gel tape, yellow (3 M, 1 in. wide).
7. Power supply capable of delivering at least 500 V.
8. UV reflective flexible TLC plates (20 cm × 20 cm) (Whatman 4410 222).
9. Hand-held short-wave UV lamp (Spectroline ENF-240C).
10. Razor blades.
11. Vortexer.
12. Large binder clips (2 in.)

3 Methods

Wear gloves and avoid RNase contamination (from skin, sweat, coughing, etc.) at all times.

3.1 RNA Preparation and Purification

1. To perform recursive gene synthesis, mix 1 μL each of DNA oligomers NL-10, NL-11, NL-12, NL-13, NL-14, and NL-15 (10 μM each) in 33 μL of water in a 100 μL thin-walled PCR microcentrifuge tube. NL-15 may be modified as needed to alter the IGS of the resulting ribozyme (*see* Subheading 2.2, **item 1** above). Add 5 μL of the 10× recursive gene synthesis buffer, 5 μL of the 10× dNTP mix, and 1 μL of Vent DNA polymerase (New England Biolabs, 5 U/μL). Incubate tube in a PCR machine with the following cycling parameters: 94 °C for 5 min, (94 °C for 1.5 min, 54 °C for 2 min, 72 °C for 3 min) × 8 cycles, 72 °C for 5 min, 4 °C hold.
2. To PCR amplify the resulting dsDNA template, add 0.1–2 μL of the reaction from the previous step (without any clean-up) to a standard 100 μL PCR reaction using the two primers TAS 2.1a and T20a. Do four such reactions in parallel. Cycling parameters that are efficient for this reaction are: 94 °C for 5 min, (92 °C for 1 min, 57 °C for 1 min, 72 °C for 1 min) × 25 cycles, 72 °C for 5 min, 4 °C hold. Check the products by

agarose gel electrophoresis. One clean band at 226 bp will be needed to proceed to in vitro transcription. Purify the PCR products from each 100 µL PCR reaction by the use of QIAquick PCR purification spin column (Qiagen 28104) and recover the DNA in 30 µL of water. Combine the four 30 µL outputs for the next step (*see* **Note 7**).

3. Add all 120 µL of the PCR DNA into a 600 µL microcentrifuge tube containing 40 µL of the 5× rNTP mix, 20 µL of the 10× transcription buffer, and 1 µL of commercial high-concentration T7 RNA polymerase enzyme (200 U/µL). Add water to 200 µL. Incubate at 37 °C for 4–16 h (*see* **Note 8**). Add 3 µL of RNase-free DNAse I enzyme (Fermentas EN0521; 1 U/µL) and return to 37 °C for an additional 30 min. Add 6 µL of 0.5 M EDTA. Perform two equal volume (ca. 210 µL) acid-phenol extractions, and 1 chloroform:isoamyl alcohol::24:1 extraction, recovering the (top) aqueous phase each time. Transfer resulting aqueous phase to a 1.5 mL microcentrifuge tube and add 20 µL 3 M sodium acetate and 660 µL 100 % ethanol. At this point the tube may be stored indefinitely at −20 °C or the RNA can be precipitated immediately. Split the solution between two 1.5 mL tubes and spin at full speed (13,200 rpm equates to 16,100 × *g*) on a table-top centrifuge (e.g., Eppendorf 5415D) for 30 min. Remove the supernatant and wash the RNA pellet once with 80 % ethanol and once with 100 % ethanol. Dry the pellet in a speed-vac and rehydrate the RNA well in 20 µL of water and 20 µL of gel-loading dye.
4. To prepare a preparative polyacrylamide gel, place the two glass plates together using a spacer on each long edge. Carefully tape up the three sides that do not contain the notch with gel tape. Wearing goggles, prepare 75 mL of 8 % polyacrylamide gel in a 250 mL Erlenmyer flask. Add 360 µL of 10 % ammonium persulfate and 33 µL of TEMED and swirl gently without introducing bubbles. Hold the plate set at a 45 ° angle and pour the gel solution into the notch until it reaches the top. Lower the plates to vertical and insert the comb into the notch. Clamp the plates together on either side with the binder clips.
5. Once the gel has polymerized (40–60 min), cut a slit in the bottom side with a razor blade, and place the plate set into the gel rig and use the clips to clamp into place via the plate ears. Clamp the aluminum block onto the back of the glass plates. Fill the top and bottom wells of the gel rig with 1× TBE, remove the comb, and rinse out the wells using a disposable syringe equipped with an 18-G needle. Heat the RNA samples to 80 °C for 2–5 min and place back on ice. Quickly load all 40 µL of the RNA-dye solution into 2–4 of the wells and electrophorese at 50 mA (about 500 V) for about 2–4 h. The dye front will be near the bottom of the gel at that point.

6. Turn off the power to the gel, remove the glass plate set and carefully separate the plates; the gel should stick to one of them. Place a piece of plastic wrap onto the gel, flip the plate over, and coax the gel onto the plastic wrap using a spatula. Place the wrap and gel onto the UV-reflective TLC plate, turn out the lights in the laboratory, and visualize the RNA by the use of a hand-held UV lamp at 254 nm. Wear goggles and work rapidly to prevent UV-induced damage to the RNA. Quickly use a clean, flame sterilized razor blade to cut above, below, and along the edges of the RNA, which should appear as a shadow about 3 in. below the top of the gel.
7. Turn off the UV lamp, turn the lights back on, and excise the gel slice(s) with clean and sterilized teflon-coated forceps. Transfer the gel slice(s) to a clean piece of overhead transparency film. Slice the gel into as many small cubes as possible with a clean razor blade, and carefully transfer these cubes into a 2 mL microcentrifuge tube. Tap the cubes to the bottom of the tube and cover them completely with crush-and-soak solution.
8. Insert the tube into a piece of styrofoam (or some other platform) secured to the top of a vortexer in a 4 °C cold room. Slowly shake the gel cubes in the crush-and-soak solution overnight.
9. Briefly centrifuge the tube to get all the materials to the bottom of the tube and remove the supernatant using a P1000 pipetman onto the surface of a Nanosep MF spin column (Pall Corporation). Rinse the cubes with an additional 100–200 μL of crush-and-soak solution and remove that supernatant to add to the solution in the spin column. Spin the Nanosep MF device for 1 min at full speed (13,200 rpm) on a table-top centrifuge. Recover the flow through and either precipitate the RNA by ethanol, or for additional purity, load all of the flow-through onto a Nanosep 10K spin column (Pall Corporation). Spin the Nanosep 10K device for about 5–10 min at low speed (5,000 × *g*) on a table-top centrifuge until the solution on the membrane has dropped to about 20–30 μL.
10. Measure the UV absorbance (A_{260}) of the recovered RNA solution and calculate its concentration by Beers law. For the *Azoarcus* ribozyme we use an extinction coefficient (ε_{260}) of 1,865,400 L/mol-cm. Dilute the RNA to final concentration of 10 pmol/μL (10 μM) using 0.1 mM EDTA as a diluent. The RNA can be made more concentrated if desired. Store RNA at −20 °C until use in a recombination reaction.

3.2 Recombination Reactions

1. Design, synthesize, and purify the two RNA substrates for recombination as described above in Subheadings 2.1 and 3.1 (*see* **Note 1**). Use the ratio 1:2.5:2.5::*Azoarcus*:substrate 1:substrate 2 to achieve maximal yield. For substrates with

single recombination targets each that are located in regions with little or no secondary structure, you can expect yields of 70 % or greater. For example, to obtain 20 pmol of a desired RNA product, employ 10 pmol *Azoarcus* ribozyme and 25 pmol each of your two substrates.

2. Incubate all RNAs together at 50 °C in a single test tube in 20 μL total volume containing *trans*-splicing buffer at a final concentration of 1× (*see* **Note 9**). Larger or smaller total volumes can be used if needed, but we would recommend doing multiple parallel reactions in 20 μL if more RNA product is needed. The reaction time will depend on the degree of secondary structure in your substrates, but typically 30 min to 1 h will suffice to get maximum yield. Because the yield as a function of time is roughly Gaussian [3], you will need to test a few reaction times to optimize it for your specific substrates.
3. Stop the reaction(s) by adding 2 μL of 0.5 M EDTA and placing the tubes on ice.
4. Add an equal volume of 2× acrylamide gel-loading dye, denature and electrophorese the RNA through an 8 % polyacrylamide gel as described above in Subheading 3.1, **step 5**.
5. Isolate, purify, and quantify the RNA as described above in Subheadings 3.1, **steps 6–10**. Note that you will need to be able to distinguish your desired RNA product from the 198-nt *Azoarcus* ribozyme RNA. Alternatively it is possible to track your RNA product by radiolabeling if the 5′ end of one substrate has been kinased with ^{32}P (*see* Fig. 2). *See* ref. 8 for more details.

4 Notes

1. In principle any two RNAs can be recombined using the *Azoarcus* ribozyme. There must be a CNU sequence (where N can be any nucleotide) immediately prior to the recombination site in both substrates; *see* Fig. 1. If CAU is chosen as the recombination target, then the IGS of the *Azoarcus* ribozyme must be GUG; if CGU is chosen as the recombination target, then the IGS of the *Azoarcus* ribozyme must be GCG, etc. Choose the recombination target sequence in order to minimize the appearance of that triplet at other locations in either substrate. Ideally there would be no other such triplet outside of the recombination target site. However it is possible to tolerate others, especially if they are buried in strong secondary structure regions. If there are more than one recombination targets in either substrate, then the recombination reaction will still proceed as desired, however the yield of full-length product will be lower. One option to ameliorate the existence of

more than one recombination target is to do sequential recombination reaction in series (e.g., recombine two substrates and then the product of that reaction with a third substrate). Alternatively, other group I introns with longer IGS sequences can be used. The *Tetrahymena* ribozyme for example can be engineered to have a 6-nt IGS (5′-GGAGGG-3′, which targets 5′-CCCUCU-3′), although this reaction must be performed at 42 °C for a longer time (ca. 4 h) and is less efficient [3].

2. Dithothreitol (DTT) and spermidine can be purchased commercially as 1 M stocks. However we typically make these solutions in the laboratory as follows. For DTT, dissolve 3.09 g solid into 20 mL of 0.01 mM NaOAc (pH 5.2). Filter sterilize with a syringe filter and simultaneously aliquot into ca. 1 mL fractions in 1.7 mL microcentrifuge tubes. Store at –20 °C. For spermidine, add about 10 mL of RNase-free water into a 5 mL stock bottle of spermidine (Sigma 85558). Let dissolve completely and pour into a 50 mL conical Falcon plastic centrifuge tube. Rinse the stock bottle with about 10 mL more water and add to Falcon tube. Repeat with one more rinse with about 10 mL water. Add additional water to Falcon tube to 34.4 mL (add exactly this amount of water to a companion tube for comparison to estimate liquid height). Filter sterilize with a syringe filter and simultaneously aliquot into ca. 1 mL fractions in 1.7 mL microcentrifuge tubes. Store at –20 °C.
3. This solution may be autoclaved prior to use, but if care is used to avoid RNase contamination (wearing gloves at all times, using clean and RNase-free water rinsed glassware, using sterile and/or RNase free pipets, stirbars, spatulas, etc., using barrier pipet tips, and in general being cautious of where solutions and items have been and what they have come in contact with) then this is not necessary.
4. We typically make up 4 mL of a 10 % solution by adding 0.4 g of dry ammonium persulfate to 4 mL of water in a plastic 10 mL Falcon tube and store it at 4 °C for up to a week. If it is more than a week old, it is best to make a fresh solution.
5. Store at 4 °C in manufacturer's bottle. Use stock bottle for up to 6 months if stored properly.
6. Many vertical slab gel electrophoresis rigs will suffice for RNA isolation and testing. We prefer the units from CBS Scientific (available through VWR) for their durability and versatility.
7. It is also possible to precipitate the DNA in ethanol at this stage rather than use a spin column for purification. Rehydrate the DNA in 1/8th of the total volume of the PCR reaction(s).
8. Transcription reactions can proceed for as few as 4 h and as long as overnight without significant differences in yield or

quality. Shorter reaction times will not yield as much RNA, while longer times will lead to more spontaneous RNA degradation.

9. It is often not necessary to prefold RNAs either separately or together prior to the reaction, but if yields are not sufficient, you can pre-heat all three RNAs to 80 °C for 2–5 min in pure water, then add the 5× *trans*-splicing buffer, and allow the reaction to cool to 50 °C and start the incubation time.

Acknowledgements

We would like to thank A. Burton and B. Larson for help during the preparation of this manuscript. This work was supported by the NASA grant NNX10AR15G to N.L.

References

1. Milligan JF, Groebe DR, Witherell GW, Uhlenbeck OC (1987) Oligoribonucletide synthesis using T7 RNA polymerase and synthetic DNA templates. Nucleic Acids Res 15:8783–8798
2. Rio DC, Ares M Jr, Hannon GJ, Nilsen TW (2011) RNA: a laboratory manual. Cold Spring Harbor Press, Cold Spring Harbor, NY
3. Riley CA, Lehman N (2003) Generalized RNA-directed recombination of RNA. Chem Biol 10: 1233–1243
4. Lehman N, Unrau PJ (2005) Recombination during in vitro evolution. J Mol Evol 61:245–252
5. Reinhold-Hurek B, Shub DA (1992) Self-splicing introns in tRNA genes of widely divergent bacteria. Nature 357:173–176
6. Hayden EJ, Riley CA, Burton AS, Lehman N (2005) RNA-directed construction of structurally complex and active ligase ribozymes through recombination. RNA 11:1678–1687
7. Draper WE, Hayden EJ, Lehman N (2008) Mechanisms of covalent self-assembly of the *Azoarcus* ribozyme from four fragment oligonucleotides. Nucleic Acids Res 36: 520–531
8. Burton AS, Madix RA, Vaidya N, Riley CA, Hayden EJ, Chepetan A, Arenas CD, Larson BC, Lehman N (2009) Gel purification of radiolabeled nucleic acids via phosphorimaging: Dip-N-Dot. Anal Biochem 388:351–352

http://www.springer.com/978-1-4939-1895-9

RNA-RNA Interactions
Methods and Protocols
Schmidt, F.J. (Ed.)
2015, X, 219 p. 37 illus., 6 illus. in color., Hardcover
ISBN: 978-1-4939-1895-9
A product of Humana Press