Genetic Tools and Reagents

Universal mRNA amplification, sense strand amplification, antisense amplification, cDNA synthesis, micro arrays, gene expression, human, mouse, rat, guinea pig, cloning

Omni-Array™ Sense Strand

mRNA Amplification Kit, 2 ng to 100 ng Version

Catalog No.: 08-0011-02 10 Reactions

Product Manual

Important Storage Instructions

Kit components require different storage conditions.
Upon receipt immediately store components as labeled.

Material Supplied

Omni-Array™ mRNA Amplification Kit

Omni-Array™ mRNA Amplification Kit		
Product	**Catalog No.**	**Size**
Omni-mRNA™ Sense strand mRNA amplification kit, 2 ng Version	08-0011-02	10 rxns

Important Storage Instructions

Kit components require different storage conditions
Upon receipt immediately store components as labeled

Omni-Array™ mRNA Amplification Kit Component Information

Individual components of the kit can be ordered using the appropriate catalog number

Omni-mRNA™ mRNA amplification kit components			
Product	**Catalog No.**	**Size**	**Storage**
Omni-mRNA™ First Strand Synthesis Components	40-5204-10	10 rxns	Store at -20°C
Omni-mRNA™ Second Strand Synthesis Components	40-5208-10	10 rxns	Store at -20°C
Omni-mRNA™ Sense Strand Synthesis Primers	40-9001-10	10 rxns	Store at -20°C
Omni-mRNA™ Antisense Strand Synthesis Primers	49-9002-10	10 rxns	Store at -20°C
Omni-mRNA™ mRNA Amplification Components	40-5212-10	10 rxns	Store at -20°C
Omni-mRNA™ cDNA Purification Components	40-4201-10	10 rxns	Store at Room Temperature
Omni-mRNA™ mRNA Purification Components	40-5036-01	1 ml	Store at Room Temperature
RT-PCRmer™ β-actin RT-PCRmer™	40-1003-10	10 nmols	Store at -20°C
Control Template for T7 RNA Polymerase	40-5081-01	12 µl	Store at -20°C

Kit Components

Reagents and Supplies in Kit for Sense Strand RNA Amplification

Components for cDNA synthesis

A. First Strand Synthesis

Catalog No.	Description	Quantity
40-5204-10	**First Strand Synthesis Kit**	**10 Reactions**
	Kit Components	
40-5104-10	1st Strand Enzyme	12.5 µl
40-5104-B1	5 X 1st Strand Enzyme Buffer	50 µl
40-5190-10	RNase Inhibitor (10 U/µl)	15 µl
40-5055-01	dNTP, 2.5 mM	40 µl

B1. Second Strand Synthesis

Catalog No.	Description	Quantity
40-5208-10	**Second Strand Synthesis**	**10 Reactions**
	Kit Components	
40-5108-10	2nd Strand Enzyme	10 µl
40-5108-B1	5 X 2nd Strand Buffer	500 µl
40-5111-10	RNase H	10 µl

B2. Second Strand Synthesis Primers & Amplification

Catalog No.	Description	Quantity
40-9002-05	**Primers for Sense Strand Synthesis**	**10 Reactions**
	Kit Components	
40-9011-05	Primer S1 (10 µM)	50 µl
40-9021-05	Primer S2 (10 µM)	50 µl
40-9031-05	Primer S3 (10 µM)	50 µl
40-9041-05	Primer S4 (10 µM)	50 µl

C. Purification of Double Stranded cDNA

Catalog No.	Description	Quantity
40-4201-10	**Components for cDNA Purification**	
	Kit Components	
40-4001-05	DNA Purification Buffer G1	5 ml
40-4002-10	DNA Purification Buffer G2	5 ml
40-4003-15	DNA Purification Buffer G3	15 ml
40-4107-15	DNA Spin Columns with collection tubes	15 ea
40-4113-50	Linear Acrylamide	50 µl

D. Antisense Strand RNA Synthesis

Catalog No.	Description	Quantity
40-5212-10	**Components for mRNA Amplification**	**10 Reactions**
	Kit Components	
40-5112-10	T7 Polymerase (200 U/μl)	20 μl
40-5112-B1	T7 Polymerase Buffer	25 μl
40-5061-05	ATP,100 mM	25 μl
40-5062-05	CTP, 100 mM	25 μl
40-5063-05	GTP, 100 mM	25 μl
40-5064-05	UTP, 100 mM	25 μl
40-5042-50	100 mM DTT	25 μl

E. RNA Purification

Catalog No.	Description	Quantity
	Components for RNA Purification	
	Kit Components	
40-3001-02	RNase-free, deionized water	1.5 ml
40-5036-01	5 M Ammonium Acetate	1 ml
40-3604-10	Spin Columns	10 ea

F. Optional: Amplification of Control cDNA Fragment

Catalog No.	Description	Quantity
	Components for Analysis of cDNA by RT-PCR	
	Kit Components	
40-1003-10	β-actin RT-PCRmer™	10 nmols
Primer pair for specific amplification of a 349 bp β-actin cDNA fragment		

G. Control RNA Synthesis Template

Catalog No.	Description	Quantity
40-5081-01	Control Template for T7 RNA Polymerase	12 μl
Restriction enzyme digested plasmid DNA containing the T7 promoter (0.5 μg/μl)		

Strategies and Protocols Involved in Amplification of Sense RNA

Summary
When the availability of total RNA becomes the limiting factor in performing certain experimental procedures, the Omni-RNA Amplification Kit provides a rapid and simple procedure for the generation of usable amounts of high quality sense or antisense strand RNA. The amplified RNA is suitable for microarrays, RT-PCR, cloning, *in vitro* transcription, and a multitude of other applications. Using this amplification protocol, microgram quantities of sense or anti sense RNA can be produced from 100 ng of total RNA in a single round of amplification or as little as 2 ng of total RNA using a 2 round amplification protocol.

The strategy for first strand cDNA synthesis involves template switching and takes advantage of the inherent terminal transferase activity present in reverse transcriptase to add a short poly dC tail at the end of the cDNA transcript. The template switching technique has been shown to be as efficient as conventional cDNA synthesis in terms of recognition of differentially expressed genes (1) and it enriches the cDNA population for full-length transcripts.

In the sense strand RNA protocol, cDNA synthesis is initiated by a primer (Primer S1) that consists of a non-specific DNA sequence followed by a sequence of poly dT. In addition, a second primer (Primer S2) is included in the first strand reaction, which contains the T7 promoter sequence followed by a poly dG tail. The poly dG tail allows Primer S2 to anneal to the poly dC sequence which reverse transcriptase adds to the 3' end of the newly synthesized cDNA chain. After annealing of Primer S2, reverse transcriptase switches from the RNA template and copies the complementary sequence of the T7 primer to the 3' end of the nascent cDNA chain. Single stranded cDNA is converted to double stranded cDNA using a primer (Primer S3) which contains the T7 promoter sequence and performing a single cycle of PCR. The result is a double stranded DNA template with the T7 promoter oriented at 5' end of the cDNA strand that corresponds to the sense RNA sequence. This is followed by a few additional rounds of PCR using Primer S3 and Primer S4 in order to maximize the efficiency of second strand synthesis.

The final step utilizes T7 RNA polymerase to amplify sense strand RNA. Amplification with T7 polymerase is very rapid and proceeds with a high level of fidelity (2). Furthermore, the incubation time used in the Omni RNA protocol is relatively short (1 hour), thereby minimizing the amount of RNA degradation that occurs with longer incubation protocols (3).

The Omni-Array system offers the user two protocols for amplification of sense strand RNA depending on the initial amount of total RNA present. A single round protocol is sufficient to generate > 10 μg of sense strand RNA from 100 ng of total RNA. When the initial amount of total RNA is less than 100 ng, a two round amplification protocol is recommended. Using two rounds of amplification, > 10 μg of sense strand RNA can be generated from as little as 2 ng of total RNA. The single round protocol can easily be performed in less than 1 day while the 2 round protocol requires approximately 1 ½ days.

Avoiding Contamination with RNase

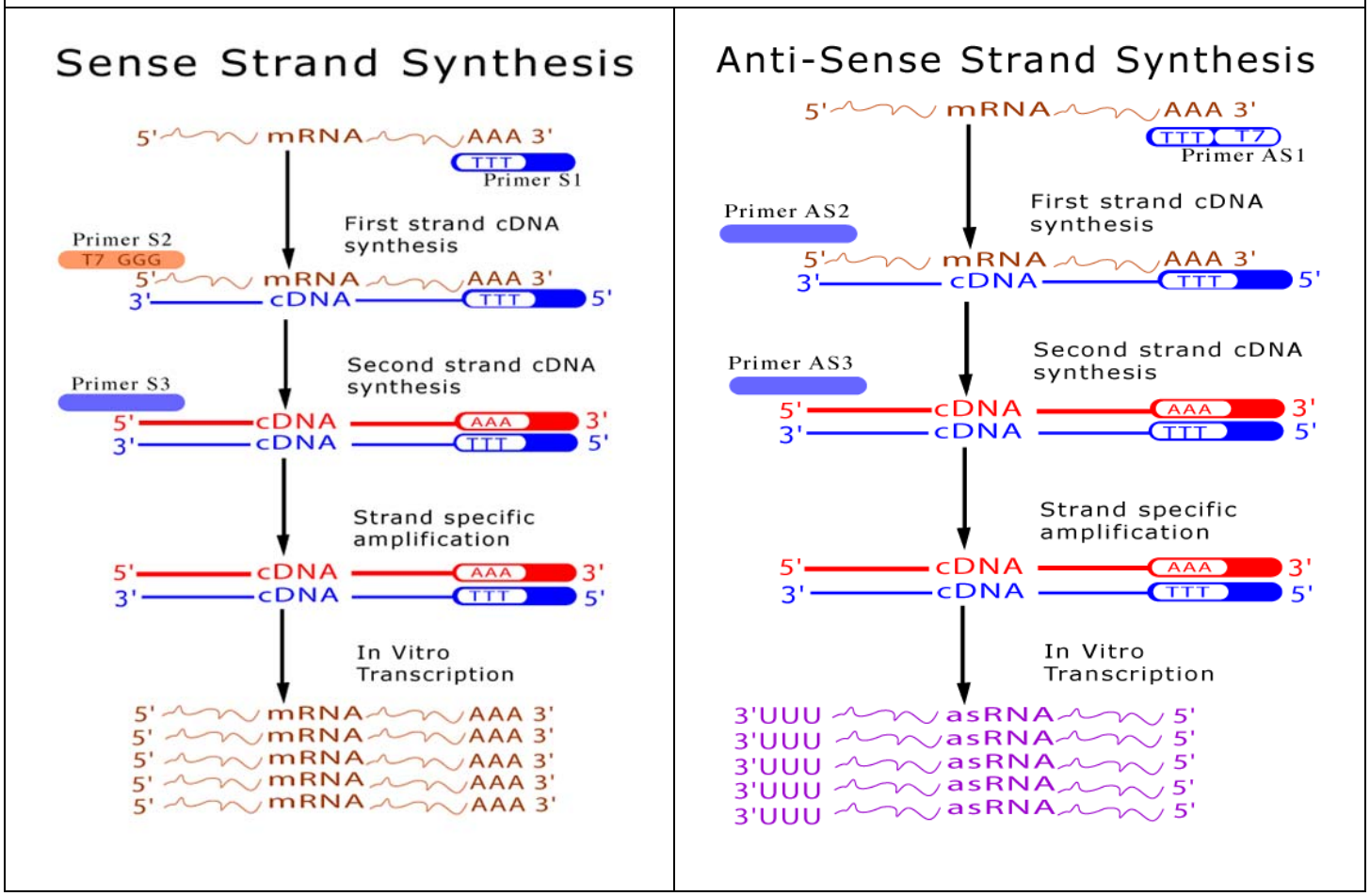
One of the primary causes of experimental failure is due to contamination with RNase. The following suggestions are intended to minimize RNase contamination throughout your experiment:

- Designate a special set of pipettors for use with RNA only.
- Store solution buffers in small aliquots and discard each aliquot after use. Avoid materials or stock solutions that have been used for any other purposes in the laboratory.
- Store separately items of glassware, batches of plasticware, and buffers that are to be used only for experiments with RNA.
- Prepare all solutions and buffers with RNase free glassware and DEPC (diethylpyrocarbonate) treated water. Glassware may be made RNase free by baking at 300°C for 4 hours. RNase contamination in water can be eliminated by incubating with 0.1% DEPC for at least 1 hour at 37°C and then autoclaving for 15 minutes at 15 psi on liquid cycle.
- Chemicals reserved for work with RNA should be dispensed either with disposable spatulas or by tapping the bottle rather than using a spatula.
- Use disposable tips and microcentrifuge tubes certified by the manufacturer to be free of RNase.
- Because skin is a major source of RNase contamination, disposable gloves should be worn at all times and changed frequently.
- Use inhibitors to suppress RNases during the isolation and amplification of RNA. The most commonly used RNase inhibitors are proteins that can be obtained commercially (e.g., RNasin, Promega; Anti-RNase, Ambion).

RNA Storage

It is recommended that the RNA that is to be amplified should be used immediately or stored at -80°C. To avoid repeated freeze/thaw cycles, store in aliquots that are sufficient for each experiment. Likewise, it is advisable to use amplified RNA immediately for its intended purpose. Otherwise freeze in aliquots at -80°C.

Omni-Array™ Amplification Strategy



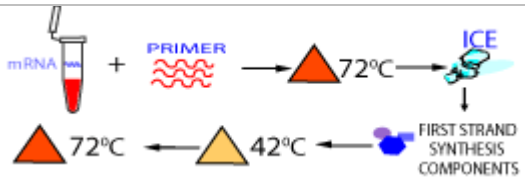
OmniArray™ RNA Amplification Kit

Quick Protocol

See manual for detailed protocol

A. First Strand Synthesis

- Add RNA and primer. Heat denature and chill to anneal
- Add first strand synthesis components
- Incubate at 42^{0}C for 1 hr, then at 68^{0}C for 10 minutes
- Total time required ~ 1.5 hrs.

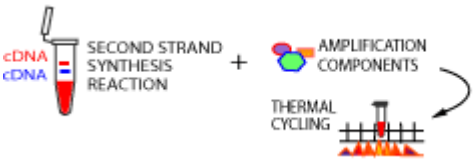


B1. Second Strand Synthesis

- To the first strand synthesis reaction add second strand synthesis components
- Incubate at 37^{0}C for 15 minutes
- Perform single cycle of thermal amplification
- Total time required ~ 30 minutes

B2. Amplification of Double Stranded cDNA

- To the second strand synthesis reaction add amplification components
- Perform 8 cycle of thermal amplification
- Total time required ~ 1 hr.



C. Purification of Amplified Double Stranded cDNA

- Perform spin column purification
- Ethanol precipitate
- Total time required ~ 1 hr

D. Synthesis of Sense Strand RNA

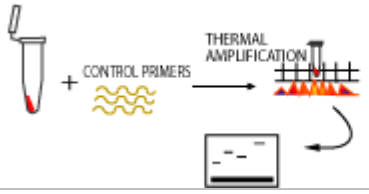
- Add sense strand synthesis components to purified ds cDNA
- Incubate at 37^{0}C for 30 minutes
- Total time required ~ 1 hr.

E. Purification of RNA

- Purify RNA by selective precipitation or spin column
- Total time required ~ 1 hr.

Optional: Amplification of Control PCR product

- Perform PCR with the supplied control primers for β-actin or GAPDH
- Visualize product by agarose electrophoresis
- Total time required ~ 4 hrs.



PROCEDURE FOR AMPLIFICATION OF SENSE RNA

(*Recommended when the initial amount of total RNA is 100 ng or more*)

A. Synthesis of First Strand cDNA

1. In a sterile 0.5 ml reaction tube, combine the following reagents:

Synthesis of First Strand cDNA	
Component	**Volume**
RNA (0.1-3 μg)	X μl
Primer S1	2 μl
Deionized water	X μl
Total Volume	**4 μl**

2. Mix contents by gently pipetting up and down several times.
3. Incubate the tube at 72°C for 2 min., and then place the tube immediately on ice.
4. While the RNA sample is incubating at 72°C, prepare a master mix for all reaction tubes, plus one additional tube. For example, if you plan to run 5 reactions, prepare a master mix for 6 reactions. The extra volume compensates for losses that occur during repetitive pipetting procedures. The Master Mix contains the following components per reaction:

Master Mix Preparation	
Component	**Volume**
5 X First-Strand Buffer	2 μl
RNase Inhibitor	0.5 μl
DTT	0.5 μl
Primer S2	1 μl
dNTP mix (10mM each dATP, dGTP, dCTP, and	1 μl
Reverse Transcriptase	1 μl
Total Volume	**6 μl**

Mix well by pipetting up and down several times.

5. Add 6 μl of the Master Mix to each RNA reaction tube that had been heated and placed on ice. As before, mix the contents thoroughly by gently pipetting up and down several times.
6. Incubate all reaction tubes for 60 minutes at 42°C. The most convenient way to carry out the incubation is in a thermal cycler.
7. Stop the reaction by heating at 68°C for 10 min. This step inactivates the reverse transcriptase.

B. Synthesis of Double-Stranded cDNA

1. For each reaction, combine the following reagents:

Synthesis of Double-Stranded cDNA	
Component	**Volume**
Deionized water	26 µl
5 X second strand synthesis buffer	10 µl
dNTP Mix (10mM each dATP, dGTP,	1 µl
Primer S3 (10 mM)	2 µl
RNase H	0.5 µl
second strand enzyme mix	0.5 µl
Total Volume	**40 µl**

2. Combine the 40 µl second strand synthesis Mix with the 10 µl reaction from the first strand synthesis (A.7).

   Place the tube in a thermal cycler and initiate the following program for 1 cycle:

1 Cycle	
Time	**Temp**
15 minutes	37°C
30 seconds	98°C
30 seconds	60°C
4 minutes	72°C

3. The cDNA is now double-stranded and can be amplified as follows:

   Add 2 µl of **Primer S4** to each tube and return the tubes to the thermal cycler. Initiate the following program:

Time	Temp
2 minutes	98°C
8 cycles	
15 seconds	98°C
30 seconds	65°C
3 minutes	72°C
7 minutes	72°C

4. The double-stranded cDNA must be purified before amplifying with T7 RNA polymerase.

C. Spin-Column Purification of Double-Stranded cDNA

The kit provides you with spin columns, collection tubes, and reagents required for the purification of your double stranded cDNA. The purification procedure will remove all proteins, RNA, primers, and dNTPs from your DNA sample.

1. In a 1.5 ml microcentrifuge tube, combine the 50 μl of ds cDNA obtained from the amplification reaction in section B with 150 μl of deionized water.
2. Add 200 μl of DNA Purification Solution G1 and mix thoroughly by pipetting up and down.
3. For each reaction, place a spin column into its corresponding collection tube.
4. Pipet the 400 μl of solution obtained in step 2 into the spin column.
5. Place the spin column and its collection tube in a microcentrifuge and spin for 1 minute at 2000 rpm.
6. Discard the column flow through.
7. Replace spin column in the collection tube and add 400 μl of solution G2.
8. Centrifuge for 1 minute at 2000 rpm and discard the flow through.
9. Replace spin column in collection tube and add 500 μl of solution G3.
10. Centrifuge for 1 minute at 2000 rpm and discard the flow through.
11. Replace the spin column in the collection tube and centrifuge for 1 minute at 10,000 rpm to completely dry the spin column. *This step is very important in order to get maximum recovery of the DNA in step 12.*
12. Place the spin column in a clean 1.5 ml microcentrifuge tube and add 50 μl of deionized water that has been warmed to 60°C. Allow it to sit for 1 minute and centrifuge for 1 minute at 10,000 rpm.
13. Repeat step 12 one time.
14. To the eluted cDNA, add 3 μl of linear acrylamide, 10 μl of 3M NaOAc, pH 5.2, and 300 μl of absolute ethanol. Mix thoroughly by vortexing and place the tube at -20° C for two hours, or at -80°C for 15 minutes.
15. Centrifuge at maximum speed for 5 minutes. Carefully remove the supernatant with a pipet and add 200 μl of 70% ethanol.
16. Centrifuge at maximum speed for 1 minute and remove the supernatant.
17. Recentrifuge the tube briefly and, using a disposable pipet tip, remove the last traces of ethanol. Allow the tube to stand open for about 15 minutes.

D. Synthesis of Sense Strand RNA with T7 Polymerase

1. Dissolve the purified cDNA in 7 μl of deionized water and transfer to a 0.5 ml reaction tube.
2. Add the following reagents in the order listed: (Reagents should be added to the reaction tubes at room temperature because the presence of spermidine in the reaction buffer may cause the DNA template to precipitate if the tubes are placed on ice.)

Synthesis of Sense Strand RNA w/ T7	
Component	**Volume**
10X Reaction Buffer	2 μl
ATP (100 mM)	1.8 μl
GTP (100 mM)	1.8 μl
CTP (100 mM)	1.8 μl
UTP (100 mM)	1.8 μl
DTT (100 mM)	2 μl
T7 polymerase	1.8 μl
Total volume	**20 μl**

Incubate for 1 hour at 37°C.

E. Purification of RNA

1. In a 1.5 ml microcentrifuge tube, combine the 20 μl of amplified RNA obtained in **Step D** with 300 μl of RNA Binding Buffer and mix thoroughly.
2. For each reaction, place an RNA spin column into its corresponding collection tube.
3. Pipet the 320 μl of solution obtained in step 1 into the spin column.
4. Place the spin column and its collection tube in a microcentrifuge and spin for 1 minute at maximum speed.
5. Discard the contents of the collection tube and add 350 μl of RNA wash buffer.
6. Centrifuge at maximum speed for 1 minute.
7. Repeat steps 5 and 6 once.
8. Place the spin column in a clean 1.5 ml microcentrifuge tube, add 50 μl of RNase free water, and allow to stand for 1 minute.
9. Centrifuge for 1 minute at maximum speed.
10. Ethanol precipitate the RNA by the addition of 5 μl of 3M sodium acetate, pH 5.2, 3 μl of linear acrylamide, and 150 μl of absolute ethanol.
11. Place at -20°C for 2 hours or at -80°C for 15 minutes.
12. Centrifuge at maximum speed for 10 minutes and remove the supernatant with a pipet tip, being careful not to dislodge the pellet.
13. Recentrifuge the tube briefly and using a disposable pipet tip, remove the last traces of ethanol. Allow the tube to stand open for about 15 minutes.

Alternatively, you can use the following procedure:

Ammonium Acetate Precipitation

1. Transfer the 20 μl of RNA reaction mixture from step D to a 1.5 ml microcentrifuge tube and add 20 μl of 5 M ammonium acetate.
2. Place the tube on ice for 10 minutes and centrifuge at maximum speed for 10 minutes.
3. Remove the supernatant and add 200 μl of ice cold 70% ethanol.
4. Centrifuge again at maximum speed for 1 minute and remove the supernatant.
5. Recentrifuge the tube briefly and using a disposable pipet tip, remove the last traces of ethanol. Allow the tube to stand open for about 15 minutes.
6. RNA can be dissolved in deionized, RNase-free water for assessment of quality and quantity.

PROCEDURE FOR TWO ROUND AMPLIFICATION OF SENSE STRAND RNA

(*Recommended when the initial amount of total RNA is less than 100 ng*)

First Round

A. Synthesis of First Strand cDNA

1. In a sterile 0.5 ml reaction tube, combine the following reagents:

Synthesis of First Strand cDNA	
Component	**Volume**
RNA (2-50 ng)	X μl
Primer S1	1 μl
Sterile deionized water	X μl
Total Volume	**4 μl**

2. Mix contents by gently pipetting up and down several times.
3. Incubate the tube at 72°C for 2 min., and then place the tube immediately on ice.
3. While the RNA sample is incubating at 72°C, prepare a master mix for all reaction tubes, plus one additional tube. For example, if you plan to run 5 reactions, prepare a master mix for 6 reactions. The extra volume compensates for losses that occur during repetitive pipetting procedures. The Master Mix contains the following components per reaction:

Master Mix Preparation	
Component	**Volume**
5 X First-Strand Buffer	2 μl
RNase Inhibitor	0.5 μl
DTT	0.5 μl
Primer S2	1 μl
dNTP mix (10 mM each dATP, dGTP, dCTP, and	1 μl
Reverse Transcriptase	1 μl
Total Volume	**6 μl**

Mix well by pipetting up and down several times.

5. Add 6μl of the Master Mix to each RNA reaction tube that had been heated and placed on ice. As before, mix the contents thoroughly by gently pipetting up and down several times.
6. Incubate all reaction tubes for 60 minutes at 42°C. The most convenient way to carry out the incubation is in a thermal cycler.
7. Stop the reaction by heating at 68°C for 10 min. This step inactivates the reverse transcriptase.

B. Synthesis of Double-Stranded cDNA

1. For each reaction, combine the following reagents:

Synthesis of Double-Stranded cDNA	
Component	**Volume**
Deionized water	26 µl
5 X second strand synthesis buffer	10 µl
dNTP Mix (10mM each dATP, dGTP,	1 µl
Primer S3 (10 mM)	2 µl
RNase H	0.5 µl
second strand enzyme mix	0.5 µl
Total Volume	**40 µl**

2. Combine the 40 µl second strand synthesis Mix with the 10 µl reaction from the first strand synthesis (A.7).

   Place the tube in a thermal cycler and initiate the following program for 1 cycle:

1 Cycle	
Time	**Temp**
15 minutes	37°C
30 seconds	98°C
30 seconds	60°C
4 minutes	72°C

3. The cDNA is now double-stranded and can be amplified as follows:

   Add 2 µl of **Primer S4** to each tube and return the tubes to the thermal cycler. Initiate the following program:

Time	Temp
2 minutes	98°C
3 cycles	
15 seconds	98°C
30 seconds	65°C
3 minutes	72°C
7 minutes	72°C

4. The double-stranded cDNA must be purified before amplifying with T7 RNA polymerase.

C. Spin-Column Purification of Double-Stranded cDNA

The kit provides you with spin columns, collection tubes, and reagents required for the purification of your double stranded cDNA. The purification procedure will remove all proteins, RNA, primers, and dNTPs from your DNA sample.

1. In a 1.5 ml microcentrifuge tube, combine the 50 μl of ds cDNA obtained from the amplification reaction in section B with 150 μl of deionized water.
2. Add 200 μl of DNA Purification Solution G1 and mix thoroughly by pipetting up and down.
3. For each reaction, place a spin column into its corresponding collection tube.
4. Pipet the 400 μl of solution obtained in step 2 into the spin column.
5. Place the spin column and its collection tube in a microcentrifuge and spin for 1 minute at 2000 rpm.
6. Discard the column flow through.
7. Replace spin column in the collection tube and add 400 μl of solution G2.
8. Centrifuge for 1 minute at 2000 rpm and discard the flow through.
9. Replace spin column in collection tube and add 500 μl of solution G3.
10. Centrifuge for 1 minute at 2000 rpm and discard the flow through.
11. Replace the spin column in the collection tube and centrifuge for 1 minute at 10,000 rpm to completely dry the spin column. *This step is very important in order to get maximum recovery of the DNA in step 12.*
12. Place the spin column in a clean 1.5 ml microcentrifuge tube and add 50 μl of deionized water that has been warmed to 60°C. Allow it to sit for 1 minute and centrifuge for 1 minute at 10,000 rpm.
13. Repeat step 12 one time.
14. To the eluted cDNA, add 3 μl of linear acrylamide, 10 μl of 3M NaOAc, pH 5.2, and 300 μl of absolute ethanol. Mix thoroughly by vortexing and place the tube at -20°C for two hours, or at -80°C for 15 minutes.
15. Centrifuge at maximum speed for 5 minutes. Carefully remove the supernatant with a pipet and add 200 μl of 70% ethanol.
16. Centrifuge at maximum speed for 1 minute and remove the supernatant.
17. Recentrifuge the tube briefly and, using a disposable pipet tip, remove the last traces of ethanol. Allow the tube to stand open for about 15 minutes.

D. Synthesis of Sense Strand RNA with T7 Polymerase

1. Dissolve the purified cDNA in 7 μl of deionized water and transfer to a 0.5 ml reaction tube.
2. Add the following reagents in the order listed: (Reagents should be added to the reaction tubes at room temperature because the presence of spermidine in the reaction buffer may cause the DNA template to precipitate if the tubes are placed on ice.)

Synthesis of Sense Strand RNA w/ T7	
Component	**Volume**
10X Reaction Buffer	2 μl
ATP (100 mM)	1.8 μl
GTP (100 mM)	1.8 μl
CTP (100 mM)	1.8 μl
UTP (100 mM)	1.8 μl
DTT (100 mM)	2 μl
T7 polymerase	1.8 μl
Total volume	**20 μl**

Incubate for 1 hour at 37°C.

E. Purification of RNA

1. In a 1.5 ml microcentrifuge tube, combine the 20 μl of amplified RNA obtained in **Step D** with 300 μl of RNA Binding Buffer and mix thoroughly.
2. For each reaction, place an RNA spin column into its corresponding collection tube.
3. Pipet the 320 μl of solution obtained in step 1 into the spin column.
4. Place the spin column and its collection tube in a microcentrifuge and spin for 1 minute at maximum speed.
5. Discard the contents of the collection tube and add 350 μl of RNA wash buffer.
6. Centrifuge at maximum speed for 1 minute.
7. Repeat steps 5 and 6 once.
8. Place the spin column in a clean 1.5 ml microcentrifuge tube, add 50 μl of RNase free water, and allow to stand for 1 minute.
9. Centrifuge for 1 minute at maximum speed.
10. Ethanol precipitate the RNA by the addition of 5 μl of 3M sodium acetate, pH 5.2, 3 μl of linear acrylamide, and 150 μl of absolute ethanol.
11. Place at -20°C for 2 hours or at -80°C for 15 minutes.
12. Centrifuge at maximum speed for 10 minutes and remove the supernatant with a pipet tip, being careful not to dislodge the pellet.
13. Recentrifuge the tube briefly and using a disposable pipet tip, remove the last traces of ethanol. Allow the tube to stand open for about 15 minutes.

II. Second Round

II. A. Synthesis of First Strand cDNA

1. Dissolve the sense strand RNA obtained from the previous step in 3 μl of RNase free water.
2. Add 1 μl of Primer S1 and transfer the mixture to a sterile 0.5 ml reaction tube.
3. Incubate the tube at 72°C for 2 min., then place the tube immediately on ice.
4. While the RNA sample is incubating at 72°C, prepare a master mix for all reaction tubes, plus one additional tube.

   The Master Mix contains the following components per reaction:

Master Mix Preparation	
Component	**Volume**
5 X First-Strand Buffer	2 μl
RNase Inhibitor	0.5 μl
DTT	0.5 μl
Primer S2	1 μl
dNTP mix (10mM each dATP, dGTP, dCTP, and	1 μl
Reverse Transcriptase	1 μl
Total Volume	**6 μl**

   Mix well by pipetting up and down several times.

5. Add 6 μl of the Master Mix to each RNA reaction tube that was heated and placed on ice. As before, mix the contents thoroughly by gently pipetting up and down several times.
6. Incubate all reaction tubes for 60 minutes at 42°C. The most convenient way to carry out the incubation is in a thermal cycler.
7. Stop the reaction by heating at 68°C for 10 min.

II. B. Synthesis of Double-Stranded cDNA

1. For each reaction, combine the following reagents:

Synthesis of Double-Stranded cDNA	
Component	**Volume**
Deionized water	26 µl
5 X second strand synthesis buffer	10 µl
dNTP Mix (10 mM each dATP, dGTP,	1 µl
Primer S3 (10 mM)	2 µl
RNase H	0.5 µl
second strand enzyme mix	0.5 µl
Total Volume	**40 µl**

2. Combine the 40 µl second strand synthesis Mix with the 10 µl reaction from the first strand synthesis (A.7).

   Place the tube in a thermal cycler and initiate the following program for 1 cycle:

1 Cycle	
Time	**Temp**
15 minutes	37°C
30 seconds	98°C
30 seconds	60°C
4 minutes	72°C

3. The cDNA is now double-stranded and can be amplified as follows:

   Add 2 µl of P**rimer S4** to each tube and return the tubes to the thermal cycler. Initiate the following program:

Time	**Temp**
2 minutes	98°C
3 cycles	
15 seconds	98°C
30 seconds	65°C
3 minutes	72°C
7 minutes	72°C

4. The double-stranded cDNA must be purified before amplifying with T7 RNA polymerase.

II. C. Spin-Column Purification of Double-Stranded cDNA

The kit provides you with spin columns, collection tubes, and reagents required for the purification of your double stranded cDNA. The purification procedure will remove all proteins, RNA, primers, and dNTPs from your DNA sample.

1. In a 1.5 ml microcentrifuge tube, combine the 50 μl of ds cDNA obtained from the amplification reaction in section B with 150 μl of deionized water.
2. Add 200 μl of DNA Purification Solution G1 and mix thoroughly by pipetting up and down.
3. For each reaction, place a spin column into its corresponding collection tube.
4. Pipet the 400 μl of solution obtained in step 2 into the spin column.
5. Place the spin column and its collection tube in a microcentrifuge and spin for 1 minute at 2000 rpm.
6. Discard the column flow through.
7. Replace spin column in the collection tube and add 400 μl of solution G2.
8. Centrifuge for 1 minute at 2000 rpm and discard the flow through.
9. Replace spin column in collection tube and add 500 μl of solution G3.
10. Centrifuge for 1 minute at 2000 rpm and discard the flow through.
11. Replace the spin column in the collection tube and centrifuge for 1 minute at 10,000 rpm to completely dry the spin column. *This step is very important in order to get maximum recovery of the DNA in step 12.*
12. Place the spin column in a clean 1.5 ml microcentrifuge tube and add 50 μl of deionized water that has been warmed to 60°C. Allow it to sit for 1 minute and centrifuge for 1 minute at 10,000 rpm.
13. Repeat step 12 one time.
14. To the eluted cDNA, add 3 μl of linear acrylamide, 10 μl of 3M NaOAc, pH 5.2, and 300 μl of absolute ethanol. Mix thoroughly by vortexing and place the tube at -20° C for two hours, or at -80°C for 15 minutes.
15. Centrifuge at maximum speed for 5 minutes. Carefully remove the supernatant with a pipet and add 200 μl of 70% ethanol.
16. Centrifuge at maximum speed for 1 minute and remove the supernatant.
17. Recentrifuge the tube briefly and, using a disposable pipet tip, remove the last traces of ethanol. Allow the tube to stand open for about 15 minutes.

II. D. Synthesis of Sense Strand RNA with T7 Polymerase

1. Dissolve the purified cDNA in 7 μl of deionized water and transfer to a 0.5 ml reaction tube.
2. Add the following reagents in the order listed: (Reagents should be added to the reaction tubes at room temperature because the presence of spermidine in the reaction buffer may cause the DNA template to precipitate if the tubes are placed on ice.)

Synthesis of Sense Strand RNA w/ T7	
Component	**Volume**
10X Reaction Buffer	2 μl
ATP (100 mM)	1.8 μl
GTP (100 mM)	1.8 μl
CTP (100 mM)	1.8 μl
UTP (100 mM)	1.8 μl
DTT (100 mM)	2 μl
T7 polymerase	1.8 μl
Total volume	**20 μl**

Incubate for 1 hour at 37°C.

E. Purification of RNA

1. In a 1.5 ml microcentrifuge tube, combine the 20 μl of amplified RNA obtained in **Step D** with 300 μl of RNA Binding Buffer and mix thoroughly.
2. For each reaction, place an RNA spin column into its corresponding collection tube.
3. Pipet the 320 μl of solution obtained in step 1 into the spin column.
4. Place the spin column and its collection tube in a microcentrifuge and spin for 1 minute at maximum speed.
5. Discard the contents of the collection tube and add 350 μl of RNA wash buffer.
6. Centrifuge at maximum speed for 1 minute.
7. Repeat steps 5 and 6 once.
8. Place the spin column in a clean 1.5 ml microcentrifuge tube, add 50 μl of RNase free water, and allow to stand for 1 minute.
9. Centrifuge for 1 minute at maximum speed.
10. Ethanol precipitate the RNA by the addition of 5 μl of 3M sodium acetate, pH 5.2, 3 μl of linear acrylamide, and 150 μl of absolute ethanol.
11. Place at -20°C for 2 hours or at -80°C for 15 minutes.
12. Centrifuge at maximum speed for 10 minutes and remove the supernatant with a pipet tip, being careful not to dislodge the pellet.
13. Recentrifuge the tube briefly and using a disposable pipet tip, remove the last traces of ethanol. Allow the tube to stand open for about 15 minutes.

Alternatively, you can use the following procedure:

Ammonium Acetate Precipitation

(*This is only an option after the second round of amplification, since there is not a sufficient amount of RNA for efficient precipitation by this method following just one round of amplification*)

1. Transfer the 20 μl of RNA reaction mixture from step D to a 1.5 ml microcentrifuge tube and add 20 μl of 5 M ammonium acetate.
2. Place the tube on ice for 10 minutes and centrifuge at maximum speed for 10 minutes.
3. Remove the supernatant and add 200 μl of ice cold 70% ethanol.
4. Centrifuge again at maximum speed for 1 minute and remove the supernatant.
5. Recentrifuge the tube briefly and using a disposable pipet tip, remove the last traces of ethanol. Allow the tube to stand open for about 15 minutes.
6. RNA can be dissolved in deionized, RNase-free water for assessment of quality and quantity.

Troubleshooting

A. First Strand Synthesis

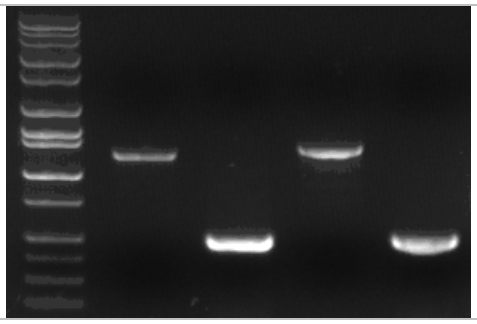
Two problems that can occur during first strand synthesis that will cause the amplification procedure to fail are: 1.)The reverse transcriptase reaction is not working, and 2.) Template switching is not taking place. To determine whether reverse transcriptase successfully generated single stranded cDNA, do the following:
Upon completion of first strand synthesis, remove a 1 μl aliquot of the reaction mixture and in a 0.5 ml reaction tube combine the following components (using appropriate β-actin RT-PCRmer™ available from Gene Link).

First Strand Synthesis	
Component	**Volume**
cDNA	1 μl
10 X Reaction Mix	5 μl
dNTP mix	4 μl
β-actin primers	2 μl
deionized water	37 μl
Enzyme Mix	1 μl
Total volume	**50 μl**

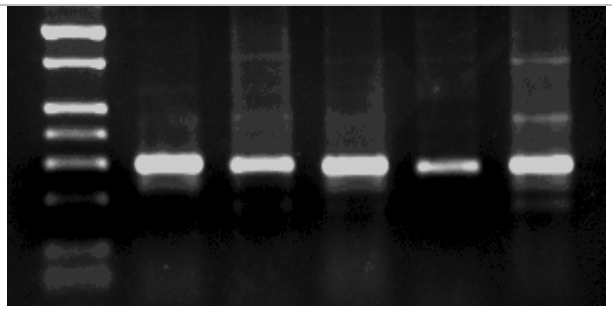
Mix thoroughly, place the tube in a thermal cycler, and initiate the following program:

First Strand Synthesis	
Time	**Temp**
2 minutes	95°C
30 cycles	94°C 1 minute
	55°C 30 seconds
	72°C 1 minute
7 minutes	72°C
Hold	4°C

Prepare a 1.5% agarose in 1 X Tris Acetate buffer (40 mM Tris Acetate, 1 mM EDTA, pH 7.5) and run a 5 μl aliquot of the PCR reaction along with appropriate DNA molecular weight markers. The β-actin PCR product should appear as a 289 base pair band.



p53 cDNA amplification from human Omni-mRNA™ pooled reference mRNA. Lane 1, molecular weight markers; lanes 2 and 4, ~1.3 kb 5' end fragment of p53; lane 3 and 5, ~500 bp of middle portion of p53. Lanes 2-3 and 4-5 represent reproducible different preparations.



Guinea Pig β-actin amplification. An amplified fragment of 289 bp. Lane 1 is molecular weight markers. Lanes 2-6 are β-actin control PCR product from brain, liver, intestine, skeletal muscle and spleen first strand cDNA.

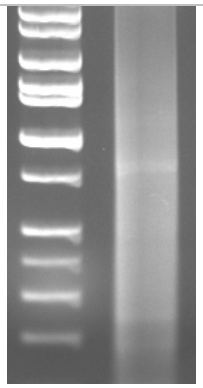
The following protocol can be used to determine whether template switching has occurred during first strand synthesis. In a 0.5 ml reaction tube combine the following components:

First Strand Synthesis	
Component	**Volume**
First Strand cDNA	1 µl
10 X Reaction Mix	5 µl
dNTP mix	4 µl
Primer S3	2 µl
Primer S4	2 µl
deionized water	35 µl
Enzyme Mix	1 µl
	50 µl

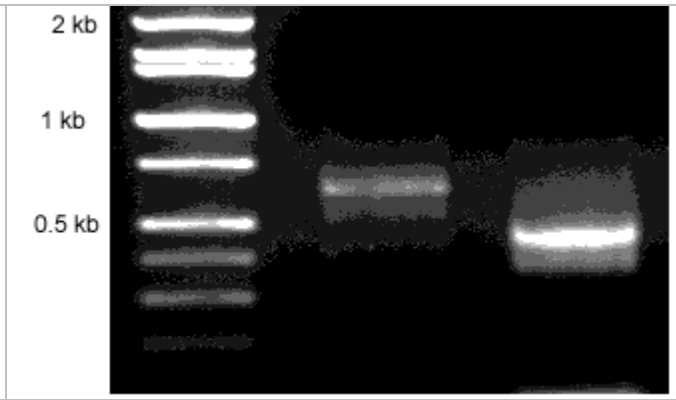
Mix thoroughly, place the tube in a thermal cycler, and initiate the following program:

First Strand Synthesis	
Time	**Temp**
2 minutes	95°C
25 cycles	94°C 30 seconds
	65°C 30 seconds
	68°C 3 minute
7 minutes	72°C
Hold	4°C

Prepare a 1% agarose in 1 X Tris Acetate buffer (40 mM Tris Acetate, 1 mM EDTA, pH 7.5) and run a 5 µl aliquot of the PCR reaction along with appropriate DNA molecular weight markers. The amplified cDNA should appear as a smear that ranges from about 4 Kb to 0.5 Kb.



Single stranded cDNA generated from 100 ng total RNA was amplified by PCR for 24 cycles as described in the above text. A 5 µl aliquot was subjected to electrophoresis at 6 mA/cm for 45 minutes



PCR amplification of ss cDNA generated from amplified mRNA using primers specific for androgen receptor cDNA (lane 1) and interleukin-8 cDNA (lane 2). The primers were directed toward the 5′ ends. The full length androgen receptor cDNA is 3.7 kb and the PCR product spans the region from 311 to 995. The full length interleukin-8 cDNA is 1.6 kb and the PCR product spans the region from 181 to 662. PCR amplification was performed essentially the same as described in the text for PCR amplification of β-actin cDNA.

B. T7 RNA Polymerase Amplification

Problem	Potential Causes	Suggestions
Low yields of RNA synthesized using the standard transcription protocol.	cDNA template may be precipitated by the presence of spermidine in the transcription buffer.	Make sure the components of the reaction are at room temperature and added in the order listed.
	NaCl concentration is greater than 30 mM.	Residual NaCl may be left over after ethanol precipitation step. Be sure the pellet was washed with 70% ethanol. Wash twice with 70% ethanol if necessary.
	RNase contamination.	Any solutions not provided should be made up in water that has been treated with 0.1% DEPC.
	Inactive RNA polymerase.	The activity of the RNA polymerase can be determined by in vitro transcription with the control DNA template provided.
Presence of a high proportion of short transcripts.	Premature termination of RNA synthesis.	Lower the incubation temperature from 37ºC to 30ºC, which has been shown to increase the proportion of full-length transcripts.

Additional Methods

Quantification of Amplified RNA and Analysis by Gel Electrophoresis

Following amplification, the RNA can be quantified by ultraviolet light absorbance at 260 nanometers (A_{260}). One absorbance unit at A_{260} is equal to approximately 42 μg of RNA. A concentration of 1 μg/ml is necessary to achieve reasonable accuracy with this method. More sensitive quantitative methods, such as Molecular Probes' RiboGreen® Assay, that can detect RNA down to low nanogram levels are also available.

Qualitative assessment of the amplified RNA can be achieved by gel electrophoresis on 1.5% agarose gel in 1 X TAE containing 0.5 μg/ml ethidium bromide. Approximately 500 ng of RNA is sufficient for analysis. Due to the disparity in length of RNA transcripts, the sample should appear as an elongated smear on the gel.

Although denaturing gels (containing 2.2 M formaldehyde, glyoxal, or 8 M urea) provide the best separation of denatured RNA, it is possible to obtain satisfactory results utilizing non-denaturing gels if the RNA has been heated in a denaturing buffer prior to loading. Combine 1 μl of RNA, 1 μl of RNA loading buffer, and 8 μl of RNA denaturing buffer and heat to 70°C for 5 minutes.

RNA loading buffer	**RNA denaturing buffer**
1 mM EDTA	10 ml deionized formamide
0.4% bromphenol blue	3.5 ml 37% formaldehyde
1 mg/ml ethidium bromide	1.5 ml 10 X MOPS buffer
50% Glycerol. Use a high grade of glycerol. Lower grades contain ribonuclease activity. Store the loading buffer in aliquots at -20°C.	Store in aliquots at -20°C and discard after 2 freeze-thaw cycles.
10 X MOPS buffer	
0.2 M MOPS (pH 7.0)	
20 mM sodium acetate	
10 mM EDTA	
To prepare 100 ml	
Dissolve 4.18 g of MOPS (3-[N-morpholino] propane sulfonic acid) in 70 ml DEPC-treated H_2O and adjust pH to 7.0 with 2 N NaOH. Add 2 ml of DEPC-treated 1 M sodium acetate and 2 ml DEPC-treated 0.5 M EDTA. Adjust volume to 100 ml with DEPC-treated H_2O	

References

1. *Wang J, Hu L, Hamilton SR, Coombes KR, and Zhang W (2003) RNA Amplification Strategies for cDNA Microarray Experiments Biotechniques, 34:394.*
2. *Zhao H, Hastie T, Whitfield ML, Borresen-Dale A-L, Jeffery SS (2002) Optimization and evaluation of T7 based RNA linear amplification protocols for cDNA microarray analysis BMC Genomics 3:31.*
3. *Spiess A-N, Mueller N, Ivell R (2003) Amplified RNA degradation in T7-amplification methods results in biased microarray hybridizations BMC Genomics 4:44*

Ordering Information

Omni-Array™ mRNA amplification kits			
Product	**Catalog No.**	**Size**	**Price $**
Omni-Array ™ Sense strand mRNA amplification kit, 2 ng to 100 ng Version	08-0011-02	10 rxns	495.00
Omni-Array ™ Antisense strand mRNA amplification kit, 2 ng to 100 ng Version	08-0021-02	10 rxns	495.00

Ordering Information Related Products

Omni-cDNA™ First Strand pooled cDNA			
Product	**Catalog No.**	**Size**	**Price $**
Human Omni-cDNA™ first strand pooled cDNA	10-0100-05	5 μg	425.00
Mouse Omni-cDNA™ first strand pooled cDNA	10-0200-05	5 μg	425.00
Rat Omni-cDNA™ Rat first strand pooled cDNA	10-0300-05	5 μg	425.00
Guinea Pig Omni-cDNA™ first strand pooled cDNA	10-2100-05	5 μg	425.00

Omni-mRNA™ amplified pooled reference mRNA Quantity supplied of 25 μg is sufficient for direct hybridization of 20 microarrays			
Product	**Catalog No.**	**Size**	**Price $**
Human Omni-mRNA™ amplified pooled reference mRNA	08-0100-25	25 μg	395.00
Mouse Omni-mRNA™ amplified pooled reference mRNA	08-0200-25	25 μg	395.00
Rat Omni-mRNA™ amplified pooled reference mRNA	08-0300-25	25 μg	395.00
Guinea Pig Omni-mRNA™ amplified pooled reference mRNA	08-2100-25	25 μg	395.00

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