

RNA amplification to produce anti-sense strand

Adapted from Marko, N., Frank, B., Quackenbush, J. and Lee, N. A robust method for the amplification of RNA in the sense orientation. 2005. *BioMed Central Genomics* 2005, 6:27 and from the NSF Rice Oligonucleotide Array Project (<http://www.ricearray.org/>)

In this protocol total RNA is amplified by *in vitro* transcription in order to obtain a sufficient quantity of RNA to perform cDNA microarray hybridizations while maintaining the original gene expression profile of the target samples.

Single strand cDNA synthesis

Bring volume of total RNA (4ug) to 17 ul with DEPC water or drying in speed-vac at 42°G as necessary

- Add
 - total RNA 17 ul
 - T7-Oligo(dT) primer* (100 ng/ul) 1.4 ul
 - Superas-In RNase inhibitor* (20 U/ul) 1.4 ul

- Heat at 70°G for 10 min.
- Cool to 4°G for 5 min.

- Add
 - 5X first strand buffer 7 ul
 - 0.1 M DTT 4 ul
 - dNTP mix (50 mM) 1.6
 - SuperScript reverse transcriptase* 2.6 ul

- Incubate at 42°G for 2.5 hours.
- Cool reaction to 4°G for 2 min.

Second strand cDNA synthesis

- Combine sscDNA with 5 ul of nonamer (100 ng/ul).
- Incubate at 95°C for 3 min.
- Keep in ice
- Add:

• 5X second strand buffer	15 ul
• dNTPs mix (25 mM each)	8 ul
• E. coli DNA polymerase I (10 U/ul)	8 ul
• E. coli DNA ligase (10 u/ul)	2 ul
• RNase H (5 u/ul)	2 ul
- Incubate at 16°C for 2 hours

Purify double-stranded cDNA

Follow minelute PCR Clean Up Kit from QIAGEN*.

In vitro transcription

Use T7 Megascript Kit* (Ambion No. 1338)

aRNA purification

Follow RNAeasy MINELUTE Kit from QIAGEN*

RNA quantification

Follow Ribogreen RNA quantification reagent manual* (Invitrogen No. R11491).

*Reagents specification

PicoPure™ RNA Isolation Kit (Arcturus No. KIT0204)

RNA quantification reagent manual (Invitrogen No. R11491).

Supers-In RNase inhibitor (Ambion No. 2692)

SuperScript II reverse transcriptase (Invitrogen No. 18064)

E. coli DNA polimerase (New England Biolabs No. M0209L)

E. coli DNA ligase (New England Biolabs M0205L)

RNase H (New England Biolabs M0297L)

T3 Megascript Kit (Ambion No. 1338)

Ribogreen RNA quantification reagent (Invitrogen No. R11491)

Wizard® SV Gel and PCR Clean-Up system (Promega No. A9281).

Sodium Carbonate Buffer (Na_2CO_3): 1M, pH 9.0

Dissolve 10.8 g Na_2CO_3 in 80 mL of MilliQ water and adjust pH to 9.0 with 12 N HCl; bring volume up to 100 mL with MilliQ water. Check periodically the pH because it might change over time

T7-Oligo(dT) primer (OPERON)

3'–

GGCCAGTGAATTGTAATACGACTCACTATAGGGAGGCGGTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT- 5'

T3n9 (OPERON)

3' –GCGCGAAATTAACCCTCACTAAAGGGAGANNNNNNNNN- 5'

T3n9'

3' – TCTCCCTTTAGTGAGGGTTAATTTGCGGC- 5'