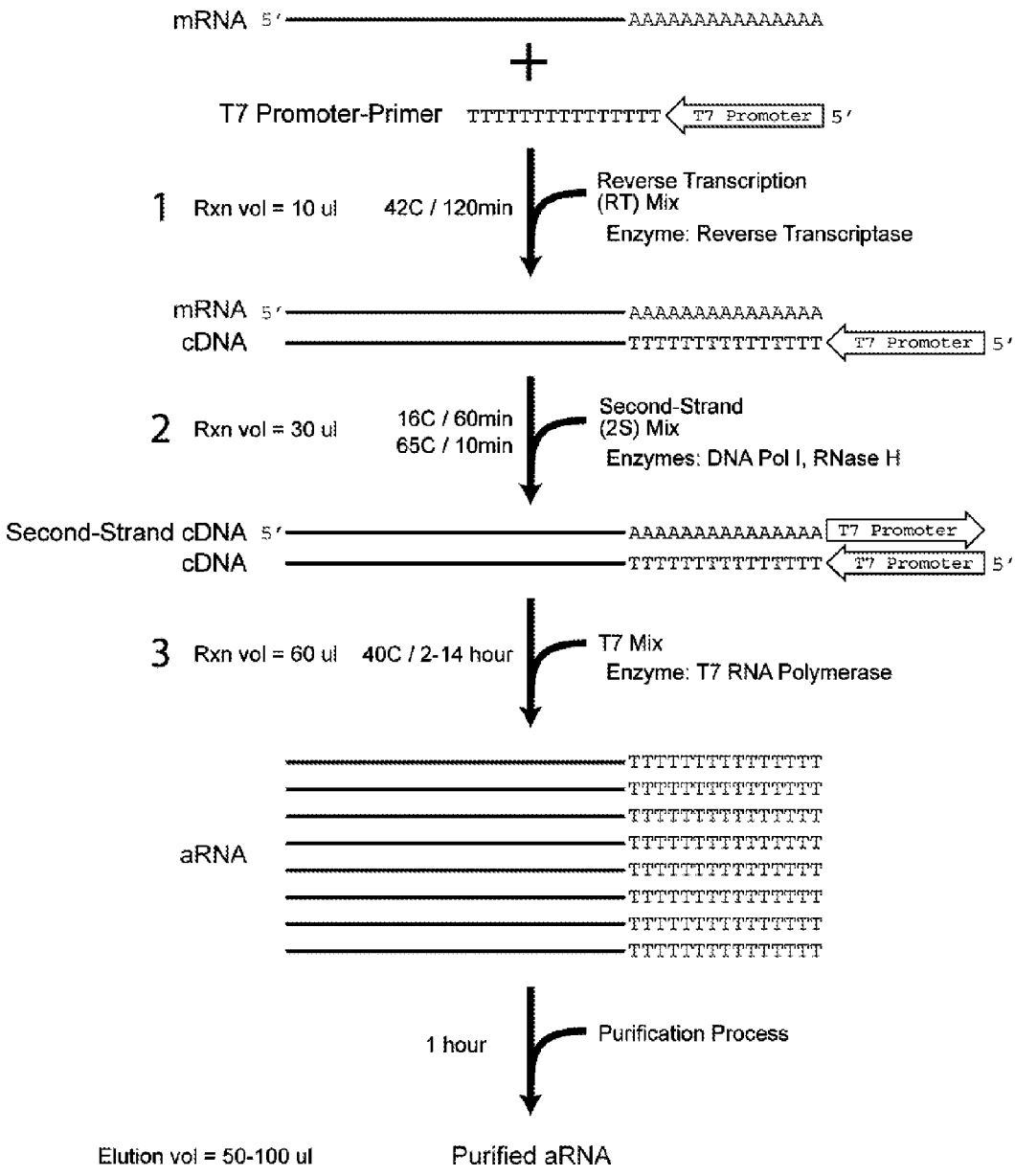
mRNA 5′
AAAAAAAAAAAAAAA
T7 Promoter-Primer
TTTTTTTTTTTTTTT
T7 Promoter
5′
1
Rxn vol = 10 ul
42C / 120min
Reverse Transcription
(RT) Mix
Enzyme: Reverse Transcriptase
mRNA 5′
AAAAAAAAAAAAAAA
cDNA
TTTTTTTTTTTTTTT
T7 Promoter
5′
2
Rxn vol = 30 ul
16C / 60min
65C / 10min
Second-Strand
(2S) Mix
Enzymes: DNA Pol I, RNase H
Second-Strand cDNA 5′
AAAAAAAAAAAAAAA
T7 Promoter
cDNA
TTTTTTTTTTTTTTT
T7 Promoter
5′
3
Rxn vol = 60 ul
40C / 2-14 hour
T7 Mix
Enzyme: T7 RNA Polymerase
aRNA
1 hour
Purification Process
Elution vol = 50-100 ul
Purified aRNA

Figure 25