cDNA Synthesis
Original directions for 2 ug mRNA
For every additional 1 ug of mRNA the volume of the reaction increases 10 ul

ORIGINAL
1st strand
mRNA 13 ul
Add 2ul biotin labeled oligo dT (100 pmol/ul) – goal 0.5 ug primer/ug mRNA
Heat to 70C 10 min, then place on ice

Add 5 ul 1st strand buffer
Add 40 u Rnasin ribonuclease inhibitor (1 ul)
Heat at 42C for 5 min

Add 2.5 ul 40 mM Na pyrophosphate
Add 30 u AMV RT (1.5 ul)
(TOTAL VOL 25 ul)
Incubate at 42C for 1 hr

2nd strand
1:5 dilution of 1st strand
e.g. total volume 100 ul, add 20 ul 1st strand

Add 20 ul 1st strand
Add 40 ul 2nd strand buffer
Add 5 ul acetylated BSA (1 mg/ml)
Add 23 u DNA polymerase 1 (2.5 ul)
Add 0.8 u Rnase H (0.3 ul)
Add H2O to total volume 100 ul
Incubate at 14C for 2 hr

Heat sample at 70C for 10 min then ziz
Add 2 u T4 DNA polymerase/ug input RNA
Incubate 10 min at 37C

Add 10 ul 200 mM EDTA to stop rxn

Estimate 6 ug mRNA
mRNA 13 ul
Add 12 ul oligo dT (biotinolyated) promega mRNA kit (50 pmol/ul)
Heat 70C 10 min, chill on ice 5 min

Add 15 ul 1st strand buffer
Add 3 ul Rnasin ribonuclease inhibitor
Heat 42C 5 min

Add 7.5 ul Na pyrophosphate
Add 4.5 ul AMV RT
Add 10 ul H2O
Heat 42C 1hr

Place on ice

FINAL VOL 25 + 40 = 65 ul

2^{nd} strand
60 ul 1^{st} strand
120 ul 2^{nd} strand buffer
15 ul BSA
7.5 ul DNA pol 1
0.9 ul RNase H
97 ul H2O
Final Vol 300 ul

Stop with 10 ul 200 mM EDTA

Precipitate cDNA
Add equal vol phenol:chloroform:isoamyl
Mix
Centrifuge 2 min top speed
Aqueous new tube
Add 1/10 vol 2.5 M Na acetate pH 5.2
Add 2.5 vol 100% etoh at –20C
Incubate 10 min at –70C
Centrifuge 5 min top speed
Wash pellet with 80% etoh

Resuspend in 21 ul Low TE

Ran 1 ul of sample on 1% TAE agar gel.
Visualized appropriate product