

cDNA Synthesis with Oligo dT Primers: First strand synthesis

Background: This may be done with total RNA or poly-A RNA prepped from cell. Here one copy of cDNA will be made with a poly-T primer. (May isolate total RNA and then prep mRNA by selection with oligoT beads to bind poly-A tail and then make cDNA from this. However, poly-T primer should extend only the mRNA from the total RNA prep).

Notes:

- Be sure to label tubes w/ **cDNA poly-A primer** (vs. RNA or some other primer)
- Always wear gloves, use barrier tips, and autoclaved tubes when working with RNA. Spray down work surface with EtOH. Clean pipettman and wash out inside of barrels.

Reagents: MMLV 5 X Buffer (in -20° Promega kit) -can thaw in 37° heat box
dNTPs -can thaw in 37° heat box
RNasin or RNase out (RNase inhibitor) -keep enzymes in stcooler
Promega MMLV RT
dH2O
AD poly T anchored primer conc. 1µg/µl

Procedure:

- Pipette the following reagents in a microfuge tube: Begin by calculating 2 µg of RNA. Subtract this amount from 12µl of H2O. Add this amount of H2O to tube first, followed by RNA and primer.

1X

2 µg RNA

1 µg Primer (AD poly (T) anchored)

X µl dH2O

Adjust final vol to 13 λ w H2O

- Flick tube to mix

- Heat in 70° water bath for 5 min. (This will disrupt secondary structures formed in the RNA to make it rather linear for the primer to pair to it, etc.) No harm if runs longer than 5 min.
- Remove from water bath directly unto ice. Chill for a few minutes. This will anneal primer to complementary RNA as rxn mixture cools.
- Add 12 λ of the following pre-mix:
1X:

5 λ MMLV 5 X Buffer

5 λ dNTPs

1 λ RNasin (also called RNase Out) RNase inhibitor

1 λ Promega MMLV RT (200u?)

12 λ

- Flick tube. Pulse in microcentrifuge to collect rxn at base of tube.
- Incubate at 42° for 60 min. (Extension of primer occurs via RT).
- Heat-inactivate reverse transcriptase @ 70° for 15 min.
- Use @ 2 λ cDNA in RT-PCR rxns.