cDNA Synthesis Protocol NEB MuLV-Reverse transcriptase

1- Step 1:

1. Mix per reaction:
    a. 1 µl dNTP (10 mM)
    b. 0.4 µl Oligo (dT) 500 ng/ µl (=100µM)
2. Transfer 1.4 µl into each PCR tube.

3. Add 1 µg RNA (volume to be calculated based on concentration of each sample) and adjust total volume to 16µl with RNase-free H_2O
4. 65 ^{0}C 5 min (temperature can be increased to max. 80°C)
5. Put samples on ice, temperature of PCR block can be adjusted to 42°C.

2- Step 2:

1. adding 4 µl of the following master mix to samples kept on ice:
   Reverse transcriptase buffer: 2.00 µl
   Reverse transcriptase (MULV): 0.25 µl
   RNase inhibitor: 0.50 µl
   RNA free water: 1.25 µl

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2. Continue PCR:
   42 ^{0}C 60 min
   90 ^{0}C 10 min
   12 ^{0}C forever