First strand cDNA synthesis

Adapted from: https://www.neb.com/protocols/2012/09/12/first-strand-cdna-synthesis-e5310

Aim of the experiment

This experiment can be used to synthesize cDNA with an RNA template using specific primers.

Materials

- OneTaq RT-PCR kit (NEB E5310S)
- Gene-specific primer (IDT)
- template RNA

Procedure

1. To a PCR tube add following reagents:

   Table 1: Reaction mix

Concentration	Chemicals
up to 2 μg	Template RNA
1 μM	Primer
fill up to 8 μl	nuclease free H_2O

   For every sample, pipette a duplicate (same pipetting scheme) as negative control.

2. Incubate, 5 min, 70 °C to denature RNA.
3. Add 10 μl 2x M-MuLV Reaction Mix to both tubes.
4. To one tube, add 2 μl M-MuLV Enzyme Mix.

5. Add 2 μl nuclease free H_2O to the other tube. This is the negative control.
6. Incubate, 1 h, 42 °C.
7. Inactivate the enzyme by incubating, 4 min, 80 °C.
8. Store at -20 °C.