First strand cDNA synthesis

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Equipment and reagents

- cDNA synthesis system (Gibco BRL)
- DEPC treated water
- 25 μM $T_{11 \text{ or } 12}$VN oligonucleotide primer

Method

1. Mix the following reagents in a microcentrifuge tube:
   - 1 μl total RNA (2 μg)
   - 5 μl of 5 × reverse transcriptase reaction buffer
   - 2.5 μl of 25 μM $T_{11 \text{ or } 12}$VN primer
   - 2.5 μl of 200 μM dNTP mix
   - 11.5 μl DEPC H_2O
2. Mix by pipetting gently, then heat to 65 °C for 3 min, then let stand at room temperature for 3 min. Repeat this step one more time.
3. Allow the reaction mix to cool to room temperature then add:
   - 1.25 μl of 0.1 M DTT
   - 1.25 μl Superscript (250 U)
4. Mix by pipetting.
5. Incubate at 42 °C for 60 min.
6. Heat inactivate reaction by incubating at 95 °C for 5 min.

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