Protocol 10: RT-PCR

1. Material

- RNA
- RT Buffer
- dNTPs (2.5mM each)
- dNTPs (10 mM each)
- oligo dT 18mer (100µM)
- DEPC-H2O
- RevertAid H Minus RT, Fermentas
- RNasin (Ribolock RI, Fermentas)
- Taq-Pol
- Primers (10µM)
- PCR Tubes

2. Instruments

- Thermocycler

3. Experimental procedure

-Use a PCR tube for sample preparation.

-Prepare a master mix

Final volume 25µl

Xµl	1µg RNA
5µl	5xRT Buffer
2.5µl	dNTPs (2.5mM each)
0.5µl	oligo dT 18mer (100µM)
Add to 23µl	DEPC-H_2O (bidest, sterile)

Set up program:

-incubate 5min at 70°C (denaturing)

-incubate for 5min at 37°C (primer annealing)

-Pause

-Add 1µl RevertAid H Minus RT, Fermentas 200U/µl and 1µl RNasin (Ribolock RI, Fermentas 40 U/µl

-incubate 1 hour at 42°C

-incubate 15 min at 70°C (inactivating the enzymes, thus stopping the reaction

-store cDNA at -20°C or start directly a PCR

PCR reaction

Sample preparation on ice

cDNA	1µl
10xPCR buffer	5µl
Primer1 (10µM)	0.5µl
Primer2 (10µM)	0.5µl
dNTPs (10mM each)	1µl
Taq-Pol (xU/µl)	0.25µl
H2O	41µl

Set up your PCR program, eg:

1. 94°C 5min
2. 94°C 30sec
3. 58°C 30sec (temperature depending on your particular primer pair)
4. 72°C 90sec, repeat step 2 to 4 30 times
5. 72°C 10min (extension)
6. 4°C pause