Preparation of RT-PCR for real-time PCR

1. Make Hexamer-RNA mix:

Hexamer(random primer)	2 ul
RNA solution (about 10pg)	10 ul
Total volume	12 ul

- Manipulate in the PCR tube ,
- incubate 70℃ , 5 min ;
- then keep on ice.

2. Make RT mix:

5 x First strand buffer	5 ul
0.1M DTT	2 ul
10mm dNTPs	1 ul
RNAsin	0.2 ul
Total volume	8.2 ul

3. Prepare reaction mix:

Hexamer-RNA mix	12 ul
RT mix	6.5 ul
RT enzyme	0.3-0.5 ul
Total volume	19 ul

1. The program of RT:

   Incubate 37℃ for 1h; then 95℃ for 1min(for killing RT. RT interferes with Taq), and keep on ice.

2. Then we get 19 ul c DNA, add more 81 ul ddH_2O, total volume 100 ul.

3. when continuing for real-time PCR , you must be careful the dilution fold of the different samples . For minimum of the correct parameter (that's important for good real-time PCR result) , you must dilute the c DNA samples accoding to the RNA concentration , the variance of the samples , and so on .

NOTE:

1) All the step must be operated on ice.
2) In most cases , 0.3 ul RT enzyme is enough.

3) Incubate in the PCR machine .
4) If the cells is only a little when collecting RNA , you can diluted RNA in less water .