

## RT-PCR Protocol

### RT

<u>Components for cDNA synthesis</u>	<b>Final volume</b> <b>8.7 µL</b>	<b>3X RXN.</b> <b>Final volume</b> <b>26.1 µL</b>	<b>Final amount</b>
RNA (250ng/ul)	2 µL of dilution	6 µL of dilution	500 ng
Exogenous RNA Control (Cab)	1 µL (10 ng/µl)	1 µL (10 ng/µl)	10 ng
Random Hexamers (50µM)	1 µL	3 µL	2.5 µM
RNase free water	4.7 µL	16.1 µL	-----

Run the above samples on the **Thermocycler** using program **CMT**

	<b>Temperature</b>	<b>Time</b>	<b>Reaction</b>
<b>CMT:</b>	70°C	10 minutes	Denaturation of template RNA
	4°C	5 minutes	<b>PAUSE: Add Master Mix!!!!</b>
	23°C	10 minutes	Annealing of primer to RNA
	42°C	2 hour	Extension of cDNA chain
	4°C	hold	

<u>Final Components</u>	<b>Final volume</b> <b>20 µL</b>	<b>Master Mix =</b> <b>10 rxns.</b>	<b>Master Mix =</b> <b>40 rxns.</b>	<b>Final concentration</b>
10X TaqMan RT Buffer	2 µL	20 µL	80 µL	1X
25 mM MgCl <sub>2</sub>	4.4 µL	44 µL	176 µL	5.5 mM
deoxyNTPs Mixture (2.5 mM)	4 µL	40 µL	160 µL	500 µM per dNTP
RNase Inhibitor (20 U/L)	0.4 µL	4 µL	16 µL	0.4 U/µL
MultiScribe Reverse Transcriptase (50 U/µL)	0.5 µL	5 µL	20 µL	1.25 U/µL

**\*Add 11.3 µL of Master Mix to each reaction at the 4°C step.**

**\*For 3X RXN. add 33.9 µL of Master Mix to each reaction at the 4°C step.**

Read the amount of cDNA (nucleic acid / W.C.) on the spectrophotometer, once program "CMT" is completed.

(1:50) dilution: (2µL of cDNA + 98µL 10mM Tris HCl pH 7.5)

The Applied Biosciences protocol for SybrGreen RT-PCR suggests 1-10ng of cDNA template and 3  $\mu\text{L}$  of 5 $\mu\text{M}$  [] primers be used per reaction.

I typically make three times the following amounts in one tube, and then aliquot into three wells for triplicate reactions.

### PCR

Components	For 25 $\mu\text{L}$ total vol.	No Template 25 $\mu\text{L}$
2X SYBR green PCR master mix	12.5 $\mu\text{L}$	12.5 $\mu\text{L}$
Forward & Reverse Primers together (5 $\mu\text{M}$ each)	3.0 $\mu\text{L}$	3.0 $\mu\text{L}$
cDNA template (1-10 ng/ $\mu\text{L}$ )	1 $\mu\text{L}$	None
DEPC-H <sub>2</sub> O	8.5 $\mu\text{L}$	9.5 $\mu\text{L}$

### For multiple reactions:

Components	For 100 $\mu\text{L}$ total vol. (x 4 wells)	For 250 $\mu\text{L}$ total vol. (x 10 wells)	For 750 $\mu\text{L}$ total vol. (x 30 wells)
2X SYBR green PCR master mix	50 $\mu\text{L}$	125 $\mu\text{L}$	375 $\mu\text{L}$
Forward & Reverse Primers together (5 $\mu\text{M}$ each)	12 $\mu\text{L}$	30 $\mu\text{L}$	90 $\mu\text{L}$
cDNA template (1-10 ng/ $\mu\text{L}$ )	2 $\mu\text{L}$	10 $\mu\text{L}$	30 $\mu\text{L}$
DEPC-H <sub>2</sub> O	36 $\mu\text{L}$	85 $\mu\text{L}$	255 $\mu\text{L}$