General Protocol for Running the Universal TaqMan qPCR Analysis

(Hao Wang @ 08/01/2021; edited by TCH)

PART 1: Reverse Transcription

Step 1: Prepare the first step reaction (**RT Mix1**) as described in the following table:

	Volume
Total RNA	5~10 µl (usually 1~5µg total RNA)
TMP-RT primer mix	2 µl
H_2O	Varied vol. to make total vol. of 12µl
Total	12 µl

Run the first step reaction (**RT Mix1**) (**program RT1**) as the following: 70℃, 5min, place **RT Mix1** on ice

Step 2: Prepare **RT Mix2** as described in the following table:

	Volume
10x RT Buffer	2.5 µl
dNTPs (5mM)	1 µl
H_2O	4.5 µl
RT enzyme (M-MuLV)	0.2 µl
Total	8.2 µl

Step 3: Mix reactants in Steps #1 and #2 as described in the following table:

	Volume
RT mix1-Step 1	12 µl
RT mix2-Step 2	8.2 µl
Total	~20 µl

Run the reaction (**program RT**): 37℃, 60min; Then heat the reaction mix at 92℃, 1min; 12℃, ∞.
Add 80ul ddH_2O after RT reaction, store the sample at -80℃ (=cDNA stock solution).
Template for qPCR: 10 µl cDNA/RT product + 490 µl ddH_2O (=1:500 dilution; higher dilutions may be needed)
NOTE: It is important to keep RT products at -80C; Aliquot if possible to avoid repeated thaws.

PART 2: Universal TaqMan qPCR Reaction

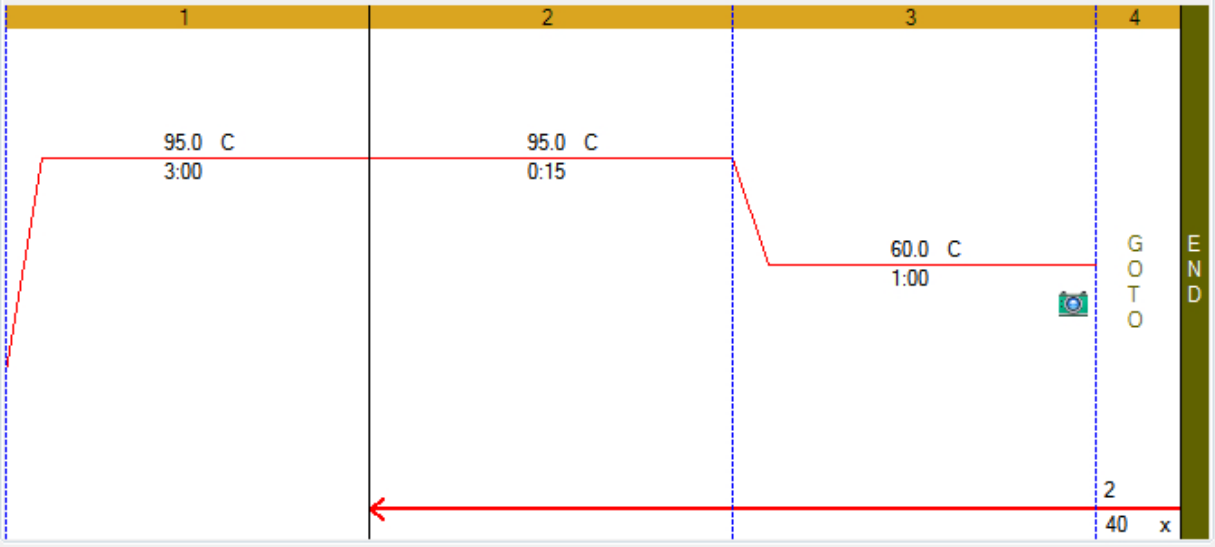
Set up each reaction tube as described in the following on a cooler (in triplicated in general):

	Volume
2x UQE2 Master Mix	5 µl
Forward Primer (10ng/µl)	2 µl
Template	2 µl
TPRP Mix	1 µl
Total	10 µl

Launch the qPCR program (**TaqMan-2021** on the desktop) as described in the following:

Step 1: 95℃，3min
Step 2: 95℃，15sec
Step 3: 60℃, 1min, go to Step 2, 39x

Protocol: Taqman-2021.prcl



	1	95.0 C for 3:00
→	2	95.0 C for 0:15
	3	60.0 C for 1:00
		+ Plate Read
└	4	GOTO 2 , 40 more times
		END