Title	RT-PCR using SYBR green technology		
Protocol Number	MOLBIO20005	Written by	Duha A.
Adapted from	MO Lab, SYBR manual & kit protocol	Version	1.0
Date created	17 June 2020	Version update	NA

- cDNA concentration is estimated to be the same as the RNA concentration used during cDNA synthesis (for example, 1µg RNA in 20µL reaction will give 1µg of cDNA).
- cDNA library should be between 100fg- 100ng

Reagents:

- SYBR Green Supermix
- Primers (10µM)

Protocol:

1. Thaw the frozen 2x iQ™ SYBR® Green supermix, template, and primers on ice. Gently mix each tube to ensure thorough resupension of components before use.
2. Briefly spin the tubes in a microcentrifuge to collect contents at the bottom of the tubes
3. In new eppendorfs, dilute all the cDNA strands in 1:10.
4. For each primer prepare primer mix by adding forward primer, reverse primer, and water (1:1:2) beforehand, or in 1 step with the master mix.
5. Reaction mix:

Components	**Volume (Fv = 10µL) for 1 reaction**	**Volume (Fv = 10µL) for 12 reactions**
SYBR Green Supermix	5 µL	60 µL
Forward primer	0.25 µL	3 µL
Reverse primer	0.25 µL	3 µL
Nuclease free water	3.5 µL	42 µL
cDNA	1 µL	1 µL (per reaction)

6. Run the plates using CFX Real-time machine (Bio-Rad).
7. Set the following cycle

1.	2.	3.	4.	
95°C	95°C	57°C	72°C	Cycles
3 mints	15 sec	15 sec	30 sec	39

8. Set the melt curves at 65-95°C, increment 0.5°C, 0:05
9. Collect the measurements in a USB and analyze the data