Reverse Transcription

1 PRECAUTION AND SET UP

When doing experiments always wear a lab coat and gloves.

We will compare BiP gene expression between sample A (control) and sample B (drug-treated). Beta-actin (ACTB) is an internal control.

Use filtered tips and discard them immediately after use to minimize any contamination.

Label two 0.2mL PCR tubes to distinguish samples and your group.

2 MAKING REACTION MIX

Reverse transcription (RT) reagents used in this course:

ReverTra Ace qPCR RT Master Mix (TOYOBO)

This master mix allows making reverse-transcribed cDNA from 1pg-1000ng total RNA.

In this training course, 100ng of total RNA will be used.

Procedure:

Take 8μL of total RNA in 0.2mL PCR tubes. Make two tubes, one for sample A and the other for sample B.

Total RNA	8μL (100ng)

Denature the RNA at 65℃ for 5 minutes in a thermal cycler.

Directly chill on ice.

（Do not chill RNA by thermal cycler. Take out the tubes when the block is still hot and transfer them on ice box.）

Add 2μL of 5x Master Mix and mix by pipetting (avoid to make too much bubbles), then spin down the tubes.

Make no-RT control if necessary. (In this seminar, our stuff will prepare the no-RT controls for comparison.)

Apply the tube in a thermal cycler and reaction them with following temperature and time.

37°C	15min.	･･･････････ (RT reaction with low temp.)
50°C	5min.	･･･････････ (RT reaction with high temp.)
98°C	5min.	･･･････････ （Denaturing the RT enzyme.）
4°C	hold	

Bring them on ice.

(You can store them at 4℃ for short-term or -20℃ for long-term storage.)

Proceed to 1st PCR.