Pre-Test 2
Determination of the range of calibration curve (2nd PCR)

0 PRECAUSION

Use filter tips for pipetting. Use longer tip if possible (e.g. use P20 tip for taking 5μL, not P2-short tip.)

Be mindful to avoid any contact of pipetter nose with tube rim.

Dispose a pipette tip when you treat any thick DNA solution with it.

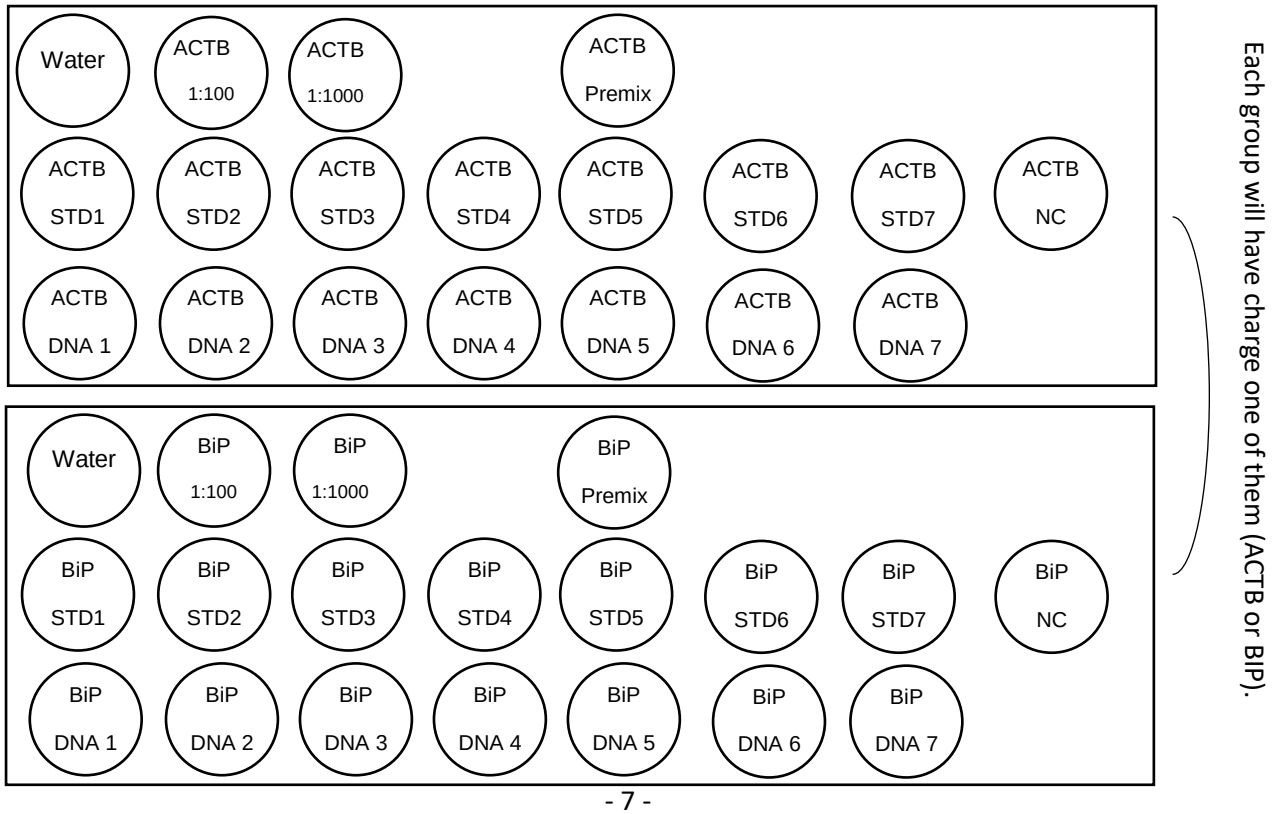
Wash your hands with gloves on or change gloves after handling thick DNA solution.

1 SET UP

From this step in this course, two groups make up a team and do experiments together. Each group has charge of BiP or ACTB.

Label 1.5mL tubes for this experiment as below in advance of any reagent handling.

Take 1mL of water in the tube labelled “water”.



Aliquot 1mL of pure water in the labelled tube from provided 50mL tube. Use this water exclusively during the entire experiment in order to prevent contamination of the stock water.

Take 45μL of water in 1.5mL tubes labelled as “DNA1” ~”DNA7”.

Take 198μL of water in the tube labelled as “*** 1:100”

Take 90μ L of water in the tube labelled as “*** 1:1000”

2 MAKING PREMIX SOLUTION

Add following reagents in the labelled (xxx Premix) 1.5mL tube.

ddH_2O	136	
2x Master Mix	170	
50x ROX	6.8	
10μM Primer mix.	10.2	(BiP or ACTB)
Total	323	(μL)

Vortex and spin down.

Aliquot 38μL each in the 1.5mL tubes labelled as”～STD” and ”～NC”.

Add 2μL of water in the NC（negative control）tube.

Mix by vortexing and spin it down.

Keep above 1.5mL tubes (8 tubes) containing the premix solution in a drawer.

3 MAKING DNA SOLUTION SERIES FOR STANDARD CURVE.

（The followings are already done in the previous step）

Take 198μL of water to the tube labelled "*** 1:100".

Take 90μL of water to the tube labelled "*** 1:1000".

Take 45μL of water in tubes labelled as "xxx DNA1" through "xxx DNA5".

The PCR product from Pre-Test 1 will be the "standard DNA concentrate". For this time, use the PCR product of sample A.

（IMPORTANT）Bring the standard DNA concentrate at this point for first time to avoid any contamination to happen.

Make standard DNA solution by diluting the standard DNA concentrate.

Take 2μL of the standard DNA concentrate in the 1:100 tube, vortex and spin down.

Take 10μL of the 1:100 solution in the 1:1000 tube, vortex and spin down.

If you have thick DNA solution on your gloves, wash them by water or change the gloves.

Add 5μL of 1:1000 solution to DNA1 tube. Use P20 tip (not P2-short tip). If you use P2-short tip, the pipette nose would be contaminated with thick DNA.

Mix by vortexing. Spin down if the solution is attached on the behind the lid.

Take 5μL of DNA1 solution to DNA2 tube, mix, and spin down as above.

Take and mix DNA2 solution to DNA3 tube in the same manner.

Repeat the same thing for DNA4 through DNA7.

4 MAKING REACTION MIXTURE

Start from thinner DNA7 solution for following procedures.

Add 2μL of DNA7 solution to STD7 tube. Mix by vortexing and spin down. Hereafter the solution is called "**reaction mix"**.

Do the same thing for STD6 through STD1.

Wash your hands with gloves on or change gloves.

5 ALIQUOT IN PCR TUBES

(See Supplementary Table 1 for aliquot pattern.)

Set two 8-well PCR strips on a tube rack.

> Set it on vertically as its one end come close to you and the other come away from you. The NC (water) reaction mix will be put in the far end and the STD1 in near end. Make a small label to distinguish which is which if necessary.

Apply 20μL each of NC reaction mix in the far end of 8-well strips.

Apply 20μL each of STD7 reaction mix in the second far end of the 8-well strips.

When making aliquots, try not to move around your pipette above the already-aliquoted wells having thinner DNA in order to minimize any possible contamination from your tip that might contain thicker DNA. This is the frequent cause of contamination in NC and thin DNA reaction mix.

Aliquot STD6 through STD1 in the same way (20μL each).

Cap the tubes by a lid strip.

Spin them down and apply to the real-time PCR system (StepOne Plus).

Start the reaction.

(If you do not start a reaction immediately, keep the tube strips in dark and at 4℃ in a refrigerator.)