

## **Task 1 Template Resource**

### **1. Curve Alpha**

- a) No amplification makes the curve look abnormal
- b) The potential reason for the abnormality is no detectable PCR product
- c) The steps to take when repeating the experiment to avoid no amplification are as follows:
  - i) Use the correct RNA isolation methodology
  - ii) Make sure the isolation method retains the miRNA fraction
  - iii) Confirm the miRNA sequence information is the latest

### **Curve Beta**

- a) Delayed amplification makes the curve look abnormal
- b) The potential reasons for the abnormality are:
  - i) the RNA sample may be of poor quality,
  - ii) not enough template sequence,
  - iii) reverse transcription may not be optimal,
  - iv) and/or assay design may not detect all of the splice variants for the gene of interest.
- c) The steps to take when repeating the experiment to avoid delayed amplification are as follows:
  - i) perform RNA purification on a sample using a new purification method,
  - ii) choose the RNA extraction kit based on sample type to assure a high yield, purity of nucleic acid, and a complete removal of PCR inhibitors,
  - iii) and/or check the alignment of the assay with the target sequence.

### **Curve Gamma**

- a) Sigmoidal amplification curves makes the curve look abnormal
- b) The potential reasons for the abnormality are:
  - i) baseline setting in the instrument's data analysis software may be too low
  - ii) and/or high level of fluorescent noise during the early cycles of PCR
- c) The steps to take when repeating the experiment to avoid delayed amplification are as follows:
  - i) analyze and adjust the Auto Baseline setting to ensure the end cycle is not set too low,
  - ii) and/or make sure to mix the aqueous sample fully with the master mix prior to cycling.

## **2. Cycle threshold question**

- a) The approximate Ct of the pink gene is 21
- b) The approximate Ct of the red gene is 12
- c) The lime green gene has the highest number of copies because a low Ct indicates a high concentration of genes.

- d) The aqua gene has the lowest number of copies because a high Ct indicates low concentration of genes.

### 3. Specificity Hypothetical

- a) The number of true positives is 505
- b) The number of true negatives is 450
- c) The number of false negatives is 20
- d) The number of false positives is 25
- e) The sensitivity of the test is 96.19
  - i)  $100 \times 505 / (505 + 20) = 50,500/525 = 96.19$
- f) The specificity of the test is 94.74
  - i)  $100 \times 450 / (25+450) = 45,000/475 = 94.74$