04. The expression of a gene, Green, is regulated by another protein, Gargoyle. Gargoyle normally inhibits the expression of Green. Gargoyle mRNA in turn, is a target of miR-2232. In cells that lack miR-2232, what would you predict, most likely, is the expression level of Green, compared to wild type cells (higher/lower/same/zero)? Explain your answer.

miR-2232 inhibits Gargoyle, which inhibits Green. Therefore, no miR-2232 = no inhibition of Gargoyle. High levels of Gargoyle = low levels of Green (since Gargoyle inhibits Green).

05. I compare the expression levels of the gene Dracula in cells treated with a control miR, or miR-3090. Compared to control-treated cells, Dracula levels are decreased 50-fold in miR-3090 cells. Since all my controls (-RT, endogenous, positive) worked as predicted, I conclude that Dracula is a target of miR-3090. However, subsequent experiments show that Dracula does not contain the miR-3090 target sequence, and that miR-3090 does not bind Dracula mRNA at all! What is the most likely explanation for these results?

miR-3090 inhibits the expression of another gene that is required for expression of Dracula. So, high levels of miR-3090 = low levels of this other protein. Low levels of this protein = low levels of Dracula, since this other protein is required for Dracula expression.

07. I use a certain fluorescence intensity value as the threshold of detection for qPCR analysis of gene expression. Theoretically, what effect will raising this threshold value have on the measured Ct (increase/decrease/same/zero/undetermined) for a given sample? Explain your answer using a clearly labeled graph (don’t forget to label the axes!)

Since the amount of DNA required to reach the new threshold is higher, we will need to go through more rounds of amplification to reach the new threshold value. Therefore, Ct will increase.

08. I have 93 nmoles of a primer. How many µL of water should I dissolve this in to get a final primer concentration of 100µM? [2 points]

100µM = 100umoles in 1000x1000uL.
Therefore, 93 nmoles in 930 uL (note that the question asks for your final answer in uL!)
09. You perform qPCR to determine expression levels of miR-102 in human breast cancer cells (BRC). After looking at the data (shown), you decide to look for targets of miR-102, and identify the following candidates based on sequence comparisons. Based on your data, and the information provided about each gene, write down if each gene is most likely a miR-102 target, and explain each answer. (2x5 points)

a) Gene: Sithlord
   Involved in cell cycle. Known to be overexpressed in BRC cells
   If Sithlord were a target of miR-102, you would expect that it would be decrease in cancer cells, since BRC cells have higher levels of miR compared to normal cells. Since Sithlord shows the opposite phenotype, it is not likely to be a target.

b) Gene: Beiberkiller
   Normally function is to inhibit cell division
   If Bk is a target of miR-102, we would expect low levels of Bk in BRC cells. Since Bk normally inhibits cell division, low levels of Bk should increase cell division, which is likely in cancer cells. So, Bk is a likely target.

c) Gene: Plutoisaplanet
   Known that deletions in this gene have no phenotypes in any human cells
   Cancer cells should have low levels of Piap, if Piap is a miR-102 target. However, deletions in this gene have no phenotype, suggesting that it does not contribute to the cancer phenotype in any way. Unlikely target.

d) Gene: Whobuysaminivan
   Is an oncogene - increased expression in cancers
   Again, a gene that is target of the miR-102 should be lower in cancer cells. Since this gene is higher, unlikely to be a target.

e) Gene: Killmenow
   Is a transcription factor required for expression of Whobuysaminivan
   High levels of miR-102 = low levels of Kmn. Since Kmn is required for expression of Wbam, we would expect to also see low levels of Wbam in cancer cells. But the previous part says that Wbam is increased in cancer cells. Therefore, Kmn is unlikely to be a miR-102 target.

10. I make 1mL of Solution A by dissolving 100µg of DNA in water. How much of Solution A do I need to add to a reaction that requires 2µg of DNA? Clearly show your logic. (2 points)

100ug in 1000mL of water. Therefore, 2ug in 20uL.