16 Feb 2021

NAM. 18S (Mosquito) PCR

Using a kit provided by “Eikon”, a PCR run was performed following Eikon’s protocol. Three reactions were created by pipetting 5 uL of their Master Mix into three Eppendorf tubes followed by 5 uL of the Positive Control, Negative Control (water), and the Sample (NAM. 18S) into the three respective tubes. The samples were held at 45°C for 15 minutes (reverse transcription) then at 95°C for 2 minutes (initial denaturation) before inserting samples into PCR. The PCR settings are as follows: 95°C for 3 seconds (denature) and 55°C for 32 seconds (anneal) run for 50 cycles.

Data was recorded for three wavelengths: 530 nm, 640 nm, and 705 nm.

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The Negative Control Starts to amplify around Cycle 40, possibly due to contamination during pipetting.

NAM. 18S (Mosquito) PCR (Trial 2)

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Description automatically generated To ensure accuracy, the experiment was executed again following the same instructions as the first trial. Shown below is the data:

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The data from both trials look similar and as expected with the largest amplification curve being the Positive Control and the Sample (NAM. 18S) amplifying less than the Positive Control.

The results for the Negative Control, however, differ slightly. In Trial 1, the Negative Control started to amplify around Cycle 40 but in Trial 2, the Negative Control did not amplify. This could be due to possible contamination in the first trial and fixed by taking greater care in the second trial.

Color Compensation

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Description automatically generated The dyes needed to create a color compensation file for our PCR machine were not readily available, so we used the individual components of the kit’s Master Mix to make 4 individual master mixes, each one containing only one primer-set with its corresponding dye with a balance of TE. The color comp. experiment was then applied to the data presented above and the adjusted amplification curve is presented below.

This result should be a more accurate representation of the relative fluorescence of each reaction, but it is unsure if the color compensation file was made or applied correctly since the protocol in the user manual was not followed.