NAM Color Compensation Attempt

To attempt a color compensation experiment, four individual Master Mixes were designed using the components from Eikon’s kit. Each Master Mix contained only 1 set of primers instead of all 4 that were in the original Master Mix. The PCR was performed using the same parameters as the previous experiment.

Below are the amplification curves by the EEEV, SLEV, and NAM Master Mixes respectively. The Master Mix containing WNV failed to produce a strong signal.

Chart, line chart

Description automatically generatedChannel 640:

Chart, line chart

Description automatically generated

Chart, line chart

Description automatically generated

Channel 530:

Chart, line chart

Description automatically generatedChart, line chart, histogram

Description automatically generated

The NAM assay did not show a strong signal in channel 530.

Chart, line chart

Description automatically generatedChannel 705:

Chart, line chart

Description automatically generated

Chart, line chart

Description automatically generated

The negative controls all failed to produce a signal so there was negligible contamination if any.

Trial 2

The data represented by the curves in the first trial do not seem to reflect the expected outcome based on the channels in the PCR and the emission spectra of each dye.

Chart, histogram

Description automatically generated

According to the emission spectra, it is expected that WNV would show the strongest signal in channel 530 and little to no signal shown for the other samples. However, in trial 1, WNV did not show a strong signal in any of the three channels. Instead, SLEV showed the strongest signal, which would have been expected to not show signal in channel 530.

A second trial was performed to identify sources of error which may have caused the experimental outcome to deviate from the theory.

Channel 530:

Chart, line chart

Description automatically generatedChart, line chart

Description automatically generated

The outcome of this trial is much closer than the previous trial. WNV shows the strongest signal while SLEV did not show a strong signal in any channel.

Chart, line chart

Description automatically generatedChannel 640:

Chart, line chart

Description automatically generatedChart, line chart

Description automatically generated

Channel 705:

Chart, line chart

Description automatically generated

Chart, line chart

Description automatically generated

Chart, line chart, histogram

Description automatically generated