MS2 PCR (Oligos IDMO04-05)

**1 primer-set**

**5 template concentrations**

Chart, line chart

Description automatically generated A PCR run was done using MS2 cDNA (synthesized using a kit from iScript) at 5 different concentrations with the same primer-set (IDMO04-05) at \*0.2 uM. The different template concentrations are as follows: 1x, 10x, 100x, 1000x, and 0x (negative control). The Template was diluted into TE. The experiment was run in triplicate. The data is represented in the plots below:

Chart, histogram

Description automatically generated

These first three graphs include the data for all 15 samples (5 concentrations in 3 sets).

Chart, histogram

Description automatically generated

|  |  |
| --- | --- |
| Concentration/Set | Tm Value |
| 1x/1 | 82.67 |
| 1x/2 | 82.38 |
| 1x/3 | 82.24 |
| 10x/1 | 83.14 |
| 10x/2 | 82.44 |
| 10x/3 | X |
| 100x/1 | X |
| 100x/2 | 83.4 |
| 100x/3 | 83.35 |
| 1000x/1 | X |
| 1000x/2 | X |
| 1000x/3 | X |

Chart, line chart

Description automatically generated To better analyze the data, the next several plots represent the relationship of the different concentrations in each individual set.

Amplification signal of the first set.

The 1x and 10x concentrations appear to amplify as expected but the 100x concentration does not amplify. The 1000x concentration appears to be faulty data, possibly due to a cracked/dirty capillary.

Chart, line chart

Description automatically generatedChart, histogram

Description automatically generated

Amplification signal of the third set.

Only the 1x conc. has a strong signal.

Amplification signal of the second set.

The 1x concentration has a similar signal to that of the first set, but the other concentrations differ dramatically. The 10x conc. only starts to amplify around cycle 40 but in the first set it had a strong signal.

The opposite occurs for the 100x conc. while it did not seem to amplify in the first set, the sample in the second set shows a strong signal.

The 1000x conc. did not produce a signal.

1x: All three amplify.

10x: Only the first set amplifies.

100x: Only the second set amplifies.

1000x: None amplify, possibly bad capillary for the first set.

Neg: The negative control in the first set starts to amplify around cycle 37 but the other two sets do not amplify.

Chart, line chart

Description automatically generated

To make the standard curve, the average of the 1x, 10x, and 100x cp values were taken and plotted. 1000x was excluded because there was no significant signal displayed by the PCR exp. Since not all concentrations showed a signal in each set, the standard curve deviates significantly from the data points.