MS2 PCR (Multiple Template Concentrations)

**1 primer set**

**5 template concentrations**

A PCR run was done using MS2 cDNA (synthesized using a kit from iScript) at 5 different concentrations with the same primer-set (IDMO02-03) at \*0.2 uM. The different concentrations are as follows: 1x, 10x, 100x, 1000x, 0x (negative control). The Template was diluted into TE.

Errors:

* When diluting the template, instead of mixing 1 uL of 1x template with 9 uL TE, 1 uL of 1x template was mixed with 10 uL TE to create the 10x dilution so the actual dilutions are slightly more diluted than a legitimate 10x dilution.
  + This error applies to the 10x, 100x, and 1000x dilutions.

Chart

Description automatically generated

The curves look as expected with the Ct value proportional to template concentration with no amplification signal from the negative control.

\*Our stock primers are 0.2 uM forward and 0.2 uM reverse primers diluted into TE making a 0.4 uM primer-set. The dilution to 0.2 uM for the primer-set refers to the dilution of the 0.4 uM stock into TE at a ratio of 1:10.

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| --- | --- |
| Concentration | Cp Value |
| 1x | 25.97 |
| 10x | 29.79 |
| 100x | 32.82 |
| 1000x | 35.00 |

Chart, line chart

Description automatically generated

When the Cp values are plotted as a function of Concentration, the relationship is linear with a slope of 3.01.

Chart, line chart

Description automatically generatedChart, line chart

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The Melting curves also look as expected and have similar Tm values due to the same template being used.

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| --- | --- |
| Template Dilution | Tm Value |
| 1x | 83.16 |
| 10x | 82.76 |
| 100x | 83.03 |
| 1000x | 83.00 |