Lambda PCR (Trial 1)

2 Annealing Temperatures

2 Primer-Sets

2 Primer Concentrations

Chart, line chart

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Description automatically generated PCR was done with Lambda assays IDMO12-13 and IDMO14-15. Each reaction had ~10,000 copies of Lambda template. The primer-sets were diluted to 0.4 and 0.04 uM. The two annealing temperatures were 60°C and 63°C. The results from both temperatures are similar so only Temp. 2 (63°C) will be presented.

Chart, line chart

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As shown in the graphs above, the only solution that showed a strong signal was the one with the IDMO12-13 primer set at 0.4 uM.

The intention while setting up this experiment was to test the primer-sets at 0.2 uM and 0.1 uM and not 0.4 uM and 0.04 uM. Unfortunately, the dilutions were not calculated correctly, resulting in different concentrations. After reviewing the steps taken during set-up, it was realized that the target concentrations were not achieved.

Only one sample showed a strong signal most likely because it had the highest concentration of primers.

The Tm value for 12-13 0.4uM+ is 89.03.

A second trial was done to correct the dilution error.

Lambda Trial 2

For the second trial, the two annealing temperatures were set to 60°C and 65°C. The primer-sets were diluted to 0.5 and 0.2 uM.

Temp 1 (60°C)

Chart

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When the annealing temperature is set to 60°C, the solutions with primer-sets 12-13 and 14-15 both at 0.5 uM provide a signal, but 12-13 does not provide a very strong signal.

Amplification of the negative controls is also seen which could indicate the presence of primer-dimers. The 0.2uM negative controls don’t amplify as much as the 0.5uM.

Chart

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|  |  |
| --- | --- |
| Sample | Tm Value |
| 12-13 0.5uM | 87.71 |
| 14-15 0.5uM | 82.58 |

Chart, histogram

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When the annealing temperature was set to 65°C, each of the positive controls seemed to have amplified but the 0.2 uM primer-sets did not produce a strong signal.

The negative controls for the 0.5 uM primer-sets started to amplify but for the 0.2 uM, little to no amplification was observed.

|  |  |
| --- | --- |
| Sample | Tm Value |
| 12-13 0.5uM | 88.14 |
| 12-13 0.2uM | 88.60 |
| 14-15 0.5uM | 82.33 |
| 14-15 0.2uM | 83.48 |

Based on the data received from the two trials, 65°C seems to be the much better option for the annealing temperature, producing a much stronger signal than the samples whose annealing temperature was 60°C. The optimum

The optimum primer concentration is harder to determine from the two options because while the 0.5uM primer-sets produced a much stronger signal, there is also more opportunity for primer-dimers to form.