Optimization of Annealing Temperature in Polymerase Chain Reaction on Constructing a Arrestin Variant (Arr3T137W)

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Abstract

Polymerase chain reaction (PCR) is one of the most prevalent and effective methods implemented in cloning, sequencing, and DNA profiling. Among the three essential steps in PCR: denaturation (95 °C), annealing, and elongation (72 °C), the annealing temperature could impact the amplification of the targeted DNA fragments. This experiment evaluated the effects of annealing temperature on PCR effectiveness and the optimal temperature for maximum yield and precision. To construct a single tryptophan arrestin3 variant, we intend to replace the Threonine with tryptophan by PCR. The effectiveness of this procedure was examined using agarose gel electrophoresis. This enabled comparison to previously effective PCR analysis, for sample identification and product conformation. The control sample at 46°C yielded an accurate and viable PCR product. A deviation of 10°C above or below the control temperature was conducted and analyzed via the same method. The sample at 36°C yielded results similar to the control. However, the sample conducted at 56°C yielded no product due to the annealing efficacy has been dramatically decreased at elevated temperatures. For the tested PCR reaction, annealing temperatures in the range of 36-46°C are effective, whereas higher temperatures are not effective.

Introduction

Polymerase chain reaction (PCR) is an extremely common tool implemented in cloning, sequencing, and DNA profiling. This experiment used PCR to construct a single tryptophan arrestin3 variant, intended to replace the Threonine with tryptophan. The optimal temperature to conduct PCR and enable the annealing process was studied. PCR involves 3 crucial steps: denaturation, annealing, and elongation. This experiment focused specifically on the process of annealing while at various temperatures. The standard temperature for the PCR process is 46°C. Samples were conducted at the conditions of 36°C, 46°C, and 56°C in order to test the top and bottom limits for their viability in the annealing process.

Experimental

PCR Procedure
All of the following were incorporated into a PCR vial
-82 µL:H_2O
-10 µL:Buffer-(10x)
-1 µL:F. primer-(A3T137Wr)
-1 µL:R. primer-(g3BamH1F)
-1 µL:Template-(g3NN221)
-4 µL:dNTP
-0.5 µL:TAG
-Run PCR at desired temperature 4 hours

Gel Electrophoresis Procedure
-1% agarose gel in mold
-Add 1.5 µL of ethidium bromide
-Buffer added as needed (TAE)
-Mix dye and DNA
  -2µL dye
  -5µL DNA
-Pipet into mold
-Pipet 8µL of standard
-Run at 120V for 40 minutes

Results

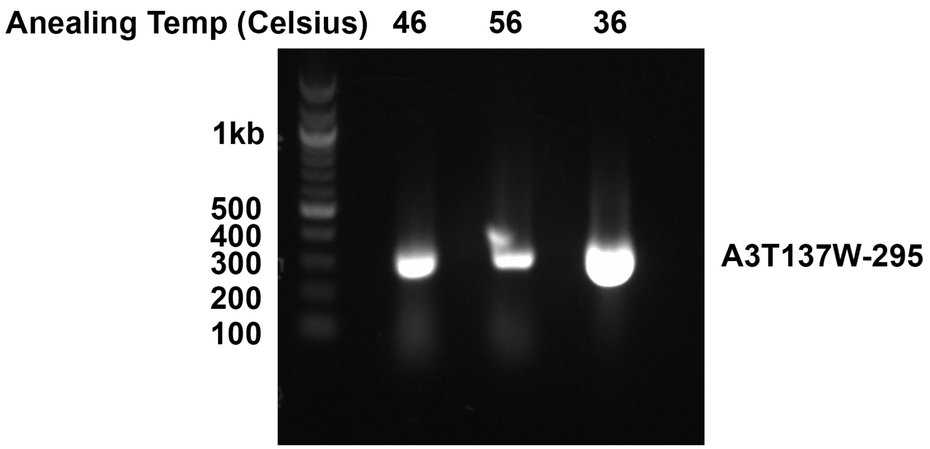
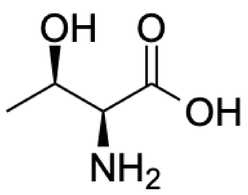
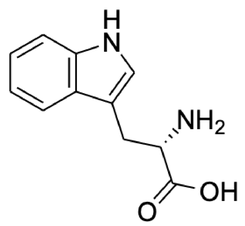


Figure. PCR products (A3T137W-295) generated at various temperature were checked by agrose gel electrophoresis. The results showed only one major DNA band of DNA fragment was observed at the expected size at all three annealing temperatures.



threonine



tryptophan

Discussion

During this experiment we saw the development of two main ideas. First, the PCR ran at 56°C and its corresponding gel electrophoresis had a very poor yield. This demonstrated that the annealing step didn't occur properly at a higher temperature. The reason for this most closely correlates with the protein being denatured due to thermic effects. Secondly, the PCR ran at 36°C had the same, if not better yield than the control at 46°C. Demonstrating that a lower temperature would still allow the annealing step to occur as intended. Overall, we concluded that higher PCR temperatures run a risk of denaturing the protein, while reasonably lower temperatures still allowed PCR to function properly.

Future Work

A possibility of future work on this experiment would include 2 main paths. First, continuing to run PCR on variant T137 at an increasing lower temperature until the annealing process or another segment of the PCR process beings to fail. Second, conducting another round of similar testing, however on a different protein to examine if these results were protein dependent, or function as a general rule for PCR. This would enable a more complete and in-depth understanding of the optimal settings for PCR, and the possible variability introduced by a variety of proteins.

References

Kowalski, Kathiann. "Explainer: How PCR Works." *Science News for Students*, 21 Dec. 2020, https://www.sciencenewsforstudents.org/article/explainer-how-pcr-works.
"Polymerase Chain Reaction (PCR) Fact Sheet." *Genome.gov*, https://www.genome.gov/about-genomics/fact-sheets/Polymerase-Chain-Reaction-Fact-Sheet.

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