Primer Design and PCR Optimization for High-Multiplex Long-Range Amplification

I have been working with strands of DNA that contain 5000 base pairs and designing primers to amplify regions of this long-stranded DNA. I am working with growth and longevity genes and am looking through regions that have previously been associated with an avian disease called Wooden Breast disease.

Wooden Breast disease is found in the fast-growing meat-type chicken, or broilers. It affects the pectoral muscle, significantly degrading the quality of the breast, leaving the texture hardened or rubbery. I used PCR procedures to identify the gene mutations associated with this disease.

The first step was using NCBI technology to design primers. I was able to design over 100 primer pairs in about 2 weeks. Our experiment required so many primer pairs because of the disease's polygenic tendencies. After some troubleshooting, we discovered that altering the primers' range, PCR product size, max target amplicon size, and primer GC content (%) would give us the results we wanted

Next in our experiment, I prepared our primer pairs into DNA samples and ran them through our thermocycler. The samples are put through different temperature levels, which break up the DNA strands, allowing the primers to bind to separated strands and giving DNA polymerase a starting point to amplify the target sequences.

Afterwards, we use electrophoresis to compare the PCR product to a ladder that offers us a visual result. The process of electrophoresis uses an electrical current sent through a gel to move the PCR product from a negative charge to a positive charge. We use a ladder, which will expand and give a scale for where the successful products should end up in the gel.

The result we are looking for will be displayed as a solid single line. The alternative will be expressed as multiple lines under a well. We call this a non-specific product, and it means our primer has amplified multiple sequences, which is not likely our target sequence. We can rule these genes unhelpful in our experiment.

From the primers that work, we can use them to amplify genes from chicken blood samples, and then sequence them. The desired regions in this experiment are the large chromosomal regions that have been previously known to be associated with the disease, and the primer should target the genes within that region.