Designing a qPCR-based assay to detect *Salmonella* species in various sample types

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Introduction

Quantitative real-time polymerase chain reaction (qPCR) is the enzymatic amplification of genetic material which generates millions of copies of DNA. This DNA can be quantified and used for the detection of various microbial agents. qPCR is a key aspect in food microbiology because it identifies specific types of bacterial pathogens in foods. This allows commercialized food products to be tested for consumer safety. (Kralik & Ricchi, 2017).

The process of qPCR is not only able to identify specific types of pathogens, but it quantifies the number of bacteria present in real-time, giving results faster than normal PCR. This makes it an effective method to use when attempting to detect highly infectious bacterial pathogens such as *Salmonella*. *Salmonella* is a major world-wide public health concern due to its prevalence of over 2,000 strains and ability to thrive in major sources of protein like chicken and eggs (Cheng et al., 2008).

The purpose of this study was to evaluate a custom-designed qPCR assay's ability to detect the presence of Salmonella withing different samples. The level of success in the assay is determined by efficiency, which measures the ability to detect and amplify the correct *Salmonella* gene sequence set dilutions.

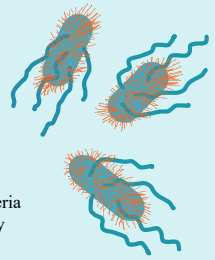


Figure 1 (right): A visual representation of *Salmonella* bacteria amplified from its original size ranging 2–5 microns long by 0.5–1.5 microns wide.

Materials and Methods

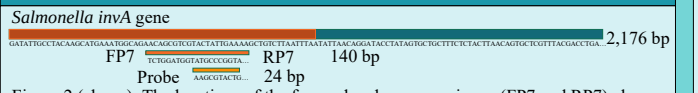


Figure 2 (above): The locations of the forward and reverse primers (FP7 and RP7) along with the probe on the *invA* gene are shown. Their genome sequences and the length of the base pairs (bp) are pictured as well. All 2,176 bp from the *invA* gene are not included due to size. The dark orange region of the gene was used to create the primers and probe.

A gene that would be expressed in almost all *Salmonella* strains is necessary for a versatile assay. The *invA* was chosen due to its presence in 99.4% of *Salmonella* strains, including one of the most prevalent strains, *Salmonella typhimurium*. The bioinformatics program BLAST (Basic Local Alignment Search Tool) was used to find regions of local similarity in the *invA* gene. This search yielded 10 potential primer pairs. Those primer pairs were then processed through gel electrophoresis in order to determine which primer pair would be most efficient based on the clearness of the band (Figure 4). Once Primer Pair 7 was determined to be the optimal primer pair, that gene sequence was imported back into BLAST to generate a compatible probe. The probe and primers were

Materials and Methods (continued)

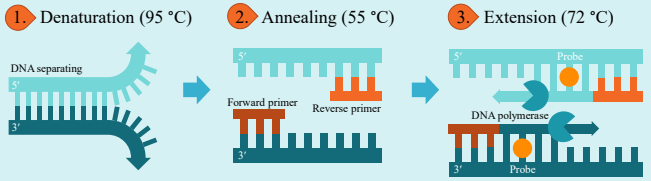


Figure 3 (above): The steps in the process of qPCR including molecular components and temperatures needed to facilitate each step.

added to a qPCR master mix consisting of DNA polymerase, dNTPs, a *Salmonella* G-block, and qPCR buffer. The process of qPCR (Figure 3) was then performed on two dilutions of *Salmonella* G-blocks (a positive control), two dilutions of the entire *Salmonella typhimurium* genome, two lab tap water samples, and one negative control (TE buffer). The results from the qPCR were reported as an amplification and standard curve graph. The efficiency was calculated from the equation indicated in Graph 2.

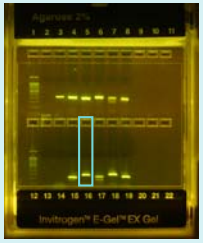
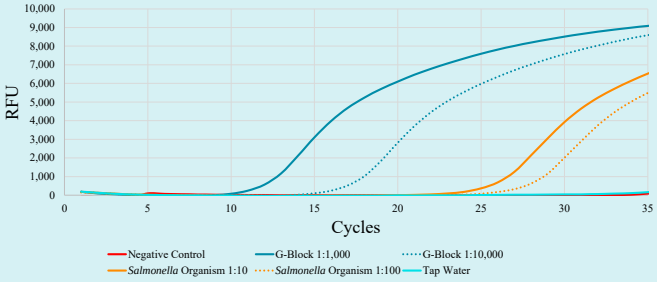


Figure 4 (right): Running gel electrophoresis determined that Primer Pair 7 (the well outlined with a blue perimeter) resulted in the clearest and most aligned band at 140 bp and therefore were the optimal primers.

Results

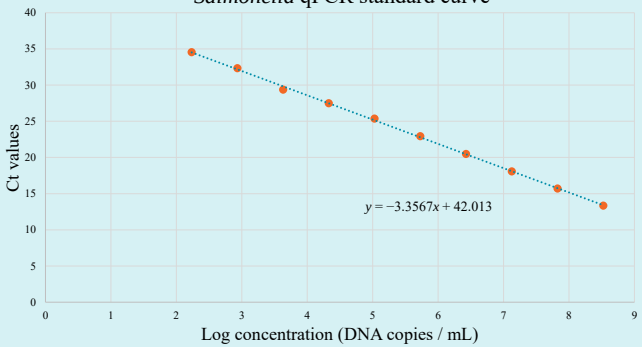
Salmonella qPCR amplification



Graph 1 (above): As DNA is amplified, relative fluorescence units (RFU) of the probe are detected after each cycle and a logistic curve is formed as a sign that DNA is being amplified. The positive control samples containing *Salmonella* DNA fragments (G-blocks) were almost immediately detected and amplified. The samples containing a real *Salmonella* organism's DNA (*Salmonella typhimurium*) were detected after more cycles due to the lesser concentration of DNA as compared to the positive control. It is known that the negative control contained no *Salmonella*, therefore creating a RFU of 0. The lab sampled tap water also remained a constant RFU of 0, indicating no *Salmonella* was present.

Results (continued)

Salmonella qPCR standard curve



Graph 2 (above): The equation $E = (10^{-1/\text{slope}} - 1) \times 100$ calculated an efficiency of 98% for the standard curve meaning that as the amount of initial *Salmonella* DNA increases, the number of cycles it takes to amplify that DNA consistently decreases. The Ct value that is the threshold for when DNA becomes detectable. The higher the Ct value, the less *Salmonella* DNA is present and therefore the Ct values decrease as more initial DNA is copied. The slope of –3.3567 falls within the range that indicates an acceptable reaction efficiency that is between 90% and 110%, supporting the success of this assay.

Conclusion

The purpose of this project was to create and evaluate the accuracy of a qPCR assay's ability to detect *Salmonella*. The assay was able to detect and copy *Salmonella* DNA in samples that contained *Salmonella*, and no DNA was copied in the negative controls. The assay ran at an efficiency level of 98%, indicating consistent and accurate amplification when measuring the relationship between cycles run and DNA concentration.

A modification for further analysis of this assay can include the use of multiple target genes from a variety of *Salmonella* organisms. This would result in an assay that is able to identify multiple strains of *Salmonella* in each sample tested. The process done to create and analyze this assay can be adjusted to target genes found in other common foodborne pathogens.

References

Cheng, C.-M., Lin, W., Van, K. T., Phan, L., Tran, N. N., & Farmer, D. (2008). Rapid detection of Salmonella in foods using real-time PCR. *Journal of Food Protection, 71*(12), 2436–2441. https://doi.org/10.4315/0362-028x-71.12.2436

Kralik, P., & Ricchi, M. (2017). A basic guide to real time PCR in microbial diagnostics: definitions, parameters, and everything. *Frontiers in Microbiology, 8*(108), 1–9. https://doi.org/10.3389/fmicb.2017.00108