A Custom Assay for a sub-population of Salmonella Heidelberg

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ABSTRACT
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Salmonella Heidelberg is a serotype that has been responsible for large outbreaks of foodborne illness. Though typically associated with the food industry, recent reports indicate that Salmonella Heidelberg is also being found in fresh produce. This study was conducted so that a qPCR assay could be designed to detect Salmonella Heidelberg within the produce at the time of harvest. We worked with 10 different Salmonella Heidelberg isolates that contained the same PFGE pattern. Using the Ion PGM™ Sequencer we were able to assemble the sequencing reads and create a consensus genome. When comparing the isolates to 116 Heidelberg genomes (8 E. coli specimens + 108 from other species) it was found that the isolates were similar to each other and different when compared to the other genomes. These SNPs were identified as different from the rest of the Heidelberg genome, shown to have enough of a difference that allowed us to design qPCR TaqMan® assays. The SNPs were used to identify the specific Heidelberg strain used as a positive control. The SNPs were also used to exclude the isolates from use for standard typing. When qPCR is performed using the ABI 7300 under Absolute Quantitation the results will be de-facto a phylogenetic tree. The SNP data was performed using the FG methods with no detection on the FAM channel, whereas negative will be detected on the FAM channel with a potential detection on the VIC channel seen at a much higher Cq value.

INTRODUCTION
Life Technologies engaged its Custom Assay Development process at the request of a Salmonella Heidelberg collaborator to develop a qPCR assay to detect Salmonella Heidelberg within their production facility. We looked at 10 different Salmonella Heidelberg isolates to determine if a qPCR assay could be developed to detect Salmonella Heidelberg. After the isolates were received from the collaborator, we were able to assemble the sequencing reads and perform a comparison of the isolates genome.

Figure 1. Custom Assay Development Workflow

Figure 2. Samples Used for This Study

Figure 3. Sequencing Workflow

RESULTS

The above shows results from all sequencing runs. Read length ranged from 192-217 base pairs (bp). Sequencing coverage was greater than 40X for all samples resulting in an average of 107 contigs for each sample after assembly.

Figure 4. Sequencing Data

Figure 5. Phylegenetic tree based on SNPs

470 non-synonymous SNPs were identified in core regions which were common to all 116 Heidelberg genomes. A phylogenetic tree was generated for a subset of these genomes using the neighbor-joining method in MEGA4 (2). The cluster outplot in red includes all the genomes obtained from sequencing collaborator samples as well as closely related genomes available in Genbank.

Figure 6. Assay Characteristics

As qPCR analysis, all 18 confirmed S. Heidelberg samples were detected using the assay (red dataset). The assay also includes an internal positive control (IPC) that is used to help gauge the quality of the qPCR data. B Line of Detection (LOD). The 0. Heidelberg assay was used to determine the LOD using DNA isolated from the strain supplied by the collaborator. The results were displayed as an average of all 18 samples. For the assay, a CT cutoff of 40 would equate to an LOD of ~10 CFUs detected per reaction. We recommend additional experiments be performed to set an appropriate CT cutoff for each sample of interest.

Figure 7. Specificity

The assay was tested against an exclusion panel of various strains of Salmonella and related pathogens and found to be specific for Salmonella Heidelberg.

CONCLUSIONS

- The Custom Assay Development process was used to design a TaqMan®-qPCR assay for S. Heidelberg at the request of a collaborator.
- All 18 samples were sequenced within 3 days of receiving pure cultures from the collaborator on the Ion PGM™ System.
- Data analysis, including genome assembly and design of specific TaqMan® probes and primers, was completed in eight days.
- Assay development and manufacturing was completed in 10 days.
- The selected assay detects less than 10 CFU per reaction of S. Heidelberg genomic DNA.
- The assay is specific for the detection of S. Heidelberg and exclusive of other Salmonella serotypes and non-S. Heidelberg organisms.

REFERENCES

TRADEMARKS/LICENSES
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