



Time and Motion Study of a Fully Automated Isolate Whole Genome Sequencing (iWGS) Platform for Surveillance of Foodborne Pathogens

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Background

PulseNet, developed by the Centers for Disease Control and Prevention, established whole genome sequencing (WGS) as the gold standard for the surveillance of foodborne pathogens. The MiSeqbased sequencing platform currently used for WGS has drawbacks that limit use for rapid epidemiological applications of this technology. Clear Dx[™] Microbial Surveillance WGS (Clear Labs, San Carlos, CA) has developed a fully automated platform that addresses some of the recognized limitations. This study was done to compare the Clear Labs automated sequencing process with the current manual process established by PulseNet.

Methods

A time and motion study to compare the manual Illumina MiSeq workflow currently used in the Nebraska Public Health Lab (NPHL) against a beta prototype of the Clear Dx[™] platform was conducted. Both workflows were independently performed by two technicians. Comparisons included the number of human touch points encountered, the total hands-on time for sample processing including DNA extraction, total run time, and the number of disparate pieces of lab equipment required for the entire workflow.

Manual Illumina MiSeq Workflow

- 1. Resuspend bacteria in buffer
- 2. Load consumables and samples onto EZ2 extractor
- Check concentration on Qubit
- Dilute DNA
- thermocycler

- thermocycler
- 9. Transfer samples to MIDI plate and pellet beads on magnetic plate
- 11. Pellet beads on magnetic plate and transfer supernatant to new wells

- 14. Pellet beads and transfer supernatant to new plate
- 15. Pool supernatant and check concentration on Qubit
- 16. Denature pool and dilute (if needed)
- 17. Heat denatured pool then cool on ice
- 18. Wash flow cell and prep MiSeq
- 19. Load pooled samples into cartridge and run on MiSeq
- 20. Results in ~44 hours

	Manual MiSeq	Automated Clear Dx™ (beta prototype)
Instrument(s) Needed	EZ2 Extractor Qubit Thermocycler MiSeq	Clear Dx™
Kits Needed	Extraction kit Qubit kit Library prep kit	Microbial Surveillance Kit
Number of Samples Per Run	24	12
Human Touch Points	>20	1
Hands-on Time	140 minutes	33 minutes
Total Time to Results	44 hours	27 hours

. Add tagmentation master mix to samples and run 15 minute tagmentation program on

6. Add TSB to samples and run 15 minute post tagmentation program on thermocycler Transfer samples to MIDI plate and wash three times using magnetic plate 8. Resuspend beads in PCR master mix and run 30 minute amplification program on

10. Transfer supernatant to new wells and add dilute beads to samples; incubate for five minutes 12. Add concentrated beads to samples and wash twice on magnetic plate 13. Air dry for three minutes, then resuspend in RSB and incubate another five minutes.



Automated Clear Dx[™] Workflow

- 1. Resuspend bacteria in buffer
- Vortex to mix samples and 2. aliquot into sample plate
- 3. Load flow cell into iSeq cartridge
- 4. Load consumables and samples onto Clear Dx[™]
- 5. Results in ~27 hours

Conclusions

The fully automated Clear Dx[™] platform would improve laboratory efficiency and provide results faster to optimize epidemiological investigating. Additionally, a lower skill threshold for laboratory staff to perform the Clear Dx[™] system would allow for greater flexibility in laboratory operations. Additional studies are needed to define hardware tolerance and to meet the PulseNet criteria for results reporting.