Automated Whole Genome Sequencing Platform for Bacterial Strain Typing

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BACKGROUND

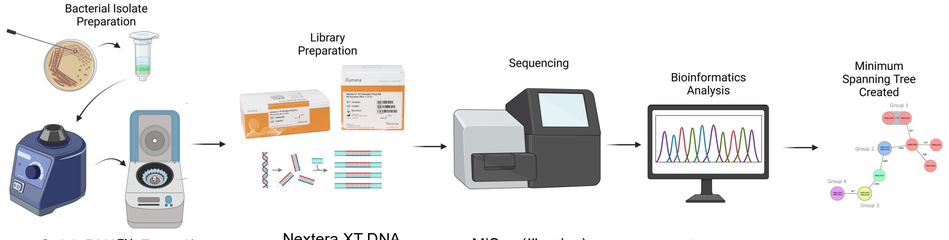
- Bacterial strain typing using whole genome sequencing (WGS) plays a key role in nosocomial outbreak investigations
- While WGS is an improvement over traditional typing methods due to higher resolution, manual WGS workflows can be time-consuming

OBJECTIVES

- Compare a manual WGS method to an automated workflow using the ClearDx™ platform (Clear Labs) for use in bacterial strain typing in terms of comparability of results, cost, and time.

METHODS

FIGURE 1: MANUAL WGS METHOD WORKFLOW



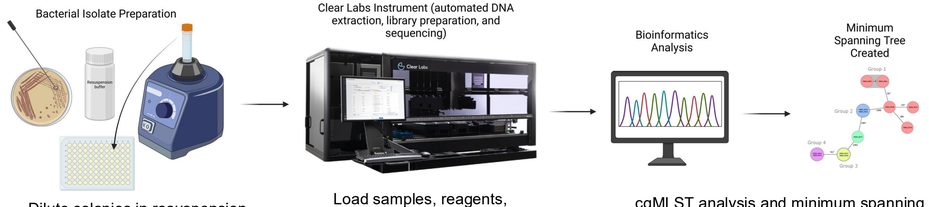
Quick-DNA™ Fungal/ Bacterial MiniPrep Kit (Zymo) & DNA quantification with Quantus™ fluorometer (Promega)

Nextera XT DNA Library Preparation Kit (Illumina)

MiSeq (Illumina)

cgMLST analysis and minimum spanning trees created using SeqSphere (Ridom) and previously validated allelic difference thresholds

FIGURE 2: AUTOMATED WGS METHOD WORKFLOW



Dilute colonies in resuspension buffer, vortex with extraction beads for 30 seconds, pipette 100 µL into 96-well plate

Load samples, reagents, and consumables onto ClearDx™ platform (2 Illumina iSeqs onboard)

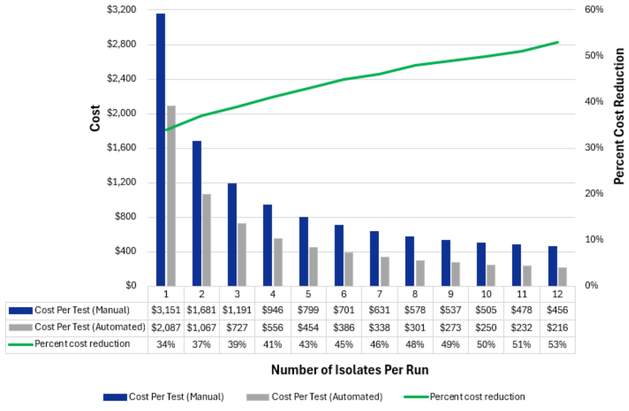
cgMLST analysis and minimum spanning trees created using SeqSphere (Ridom) and previously validated allelic difference thresholds

RESULTS

TABLE 1: BACTERIAL ISOLATES SEQUENCED

Organism	Number of Isolates
Acinetobacter baumannii	8
Campylobacter jejuni/coli	16
Clostridioides difficile	8
Cutibacterium acnes	12
Enterobacter cloacae complex	4
Enterococcus faecalis	7
Enterococcus faecium	12
Escherichia coli	8
Klebsiella pneumoniae	8
Legionella pneumophila	8
Pseudomonas aeruginosa	10
Serratia marcescens	8
Staphylococcus aureus	72
Staphylococcus epidermidis	12
Staphylococcus lugdunensis	12
Streptococcus agalactiae	9
Streptococcus pyogenes	12

FIGURE 3: COST PER TEST BY BATCH SIZE



Cost differences were compared by calculating labor, reagents, consumables and standard overhead for each method. Automation reduced labor costs by 93% and overall costs from 34 to 53% for run sizes of one to 12 isolates, respectively

TABLE 2: AVERAGE TIME PER TESTING STEP

Step	Manual WGS Method		Automated WGS Method	
	Hands-on Time	Additional Automated Analytical Time	Hands-on Time	Additional Automated Analytical Time
Sample Preparation	30 minutes	Not applicable	30 minutes	Not applicable
Instrument Setup	Not applicable	Not applicable	30 minutes	Not applicable
Library Preparation	3 hours	Not applicable	Not applicable	7 hours
Sequencing	30 minutes	39 to 42 hours	Not applicable	19 hours
Bioinformatics Analysis	1 hour	Not applicable	1 hour	Not applicable
Total	5 hours	39 to 42 hours	2 hours	26 hours
Total Time	44 to 47 hours		28 hours	

DISCUSSION

- 222/224 (99%) isolates that generated sequences showed concordant groupings between automated and manual methods
- Mantel Spearman correlation of paired distance matrices showed statistically similar results between automated and manual methods
- Use of Clear Dx™ platform decreased turnaround time from 44 to 47 hours with manual method to average of 28 hours with automation, eliminating 3 hours of manual work
- Cost analysis predicted cost savings of 34 to 53% compared to manual method

CONCLUSIONS

- Integration of automation into a bacterial WGS workflow yielded nearly equivalent results to a more manual workflow with improved turnaround time and projected cost savings

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