

Automation of the ONETest™ PathoGenome Assay

on the Hamilton STAR Liquid Handling System

Targeted Whole Metagenomic Sequencing • 254 Respiratory Pathogens • BAL Specimens

Fusion Genomics Corp., Richmond, BC, Canada

University of Florida Rocky Point Medical Laboratory

1 End-to-End Workflow Overview

1. BAL Sample Collection

Min. volume: 500 μ L
Store 4°C same day, or
-20°C (\leq 1 wk) / -80°C (\leq 12 mo)
Homogenize turbid samples

2. DNA Extraction

MagMAX Microbiome Ultra Kit
Lysis Proteinase K digest
Bead-based capture & wash
Elute in 50 μ L • QC: \geq 5 ng/ μ L

3. Hamilton STAR Automation

Library Construction & Enrichment
Fragmentation End-Repair Ligation
PCR Hybrid Capture Elution
Runtime: ~18 hours fully automated
Final sample in ODTc at 10°C

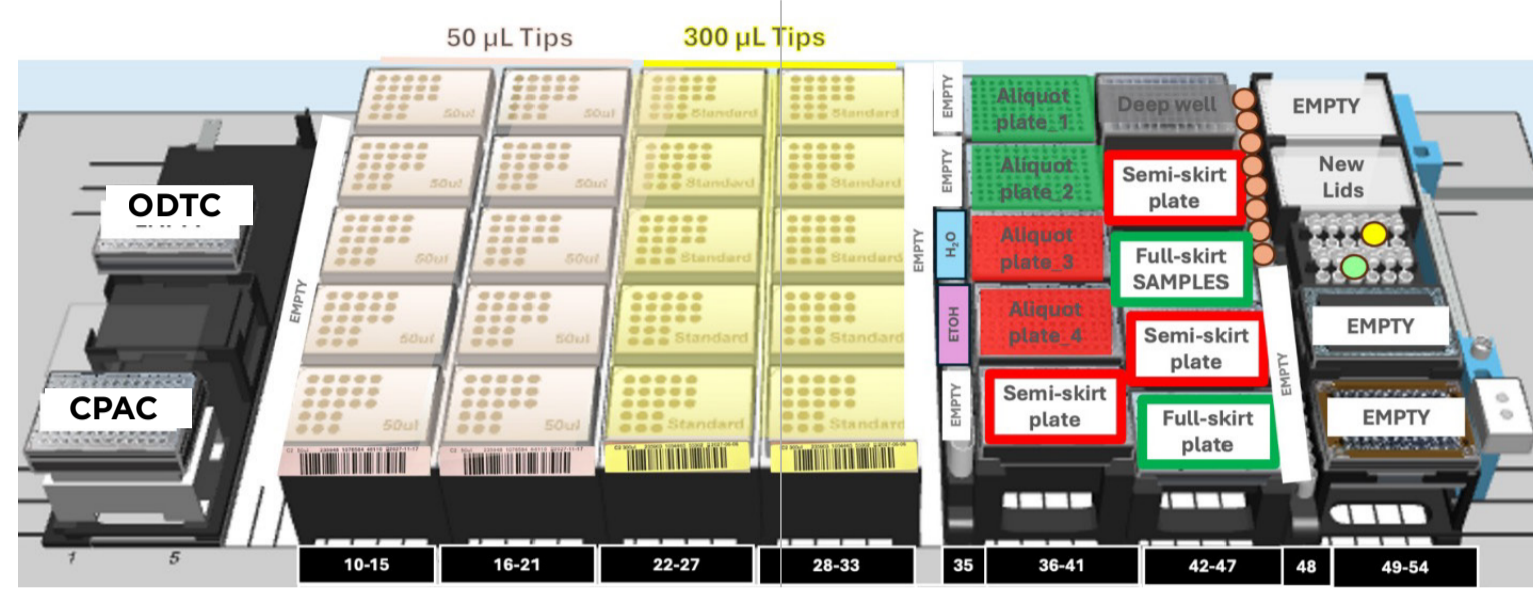
4. Next Gen Sequencing

SP Reagent Kit v1.5, 300 cycles
2x150 bp paired-end sequencing
PhiX control spike 1-2%
Library qPCR (KAPA/NEB) before load

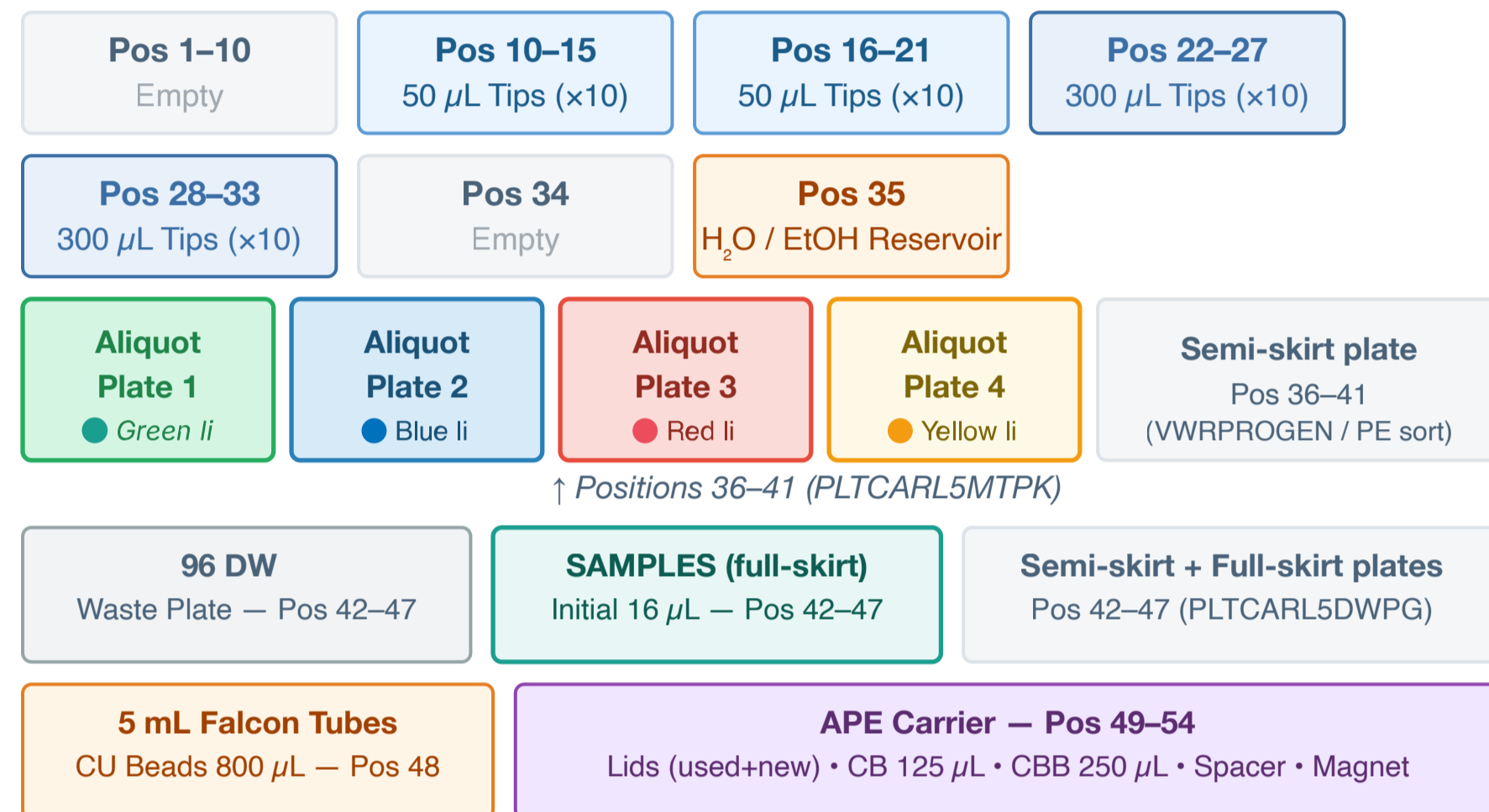
5. FusionCloud™ Analysis

Upload FASTQ Host depletion
Dual taxonomic screening
Genome assembly & metric calc.
CSV + HTML report download

2 Hamilton STAR Deck Configuration



Carrier Layout (left → right, positions 1-54)



On-deck Modules



8-channel span • CO-RE grippers • Plate magnet • Multiflex carriers

Start-up Sequence

- Power-cycle: OFF Hamilton → OFF CPAC → Reboot PC
- ON Hamilton → ON ODTc → ON CPAC controller
- Open Method Manager → Select Fusion Genomics → ONETest™ Run
- Select: sample count / stages / P300 tips / P50 tips → OK → Run starts
- On completion: samples in ODTc column 1, full-skirt plate, 20 μ L, 10°C
Tape aliquot plates 1-4 to deck before run. Cut tube caps (CB/CBB) to avoid collision.

Note:

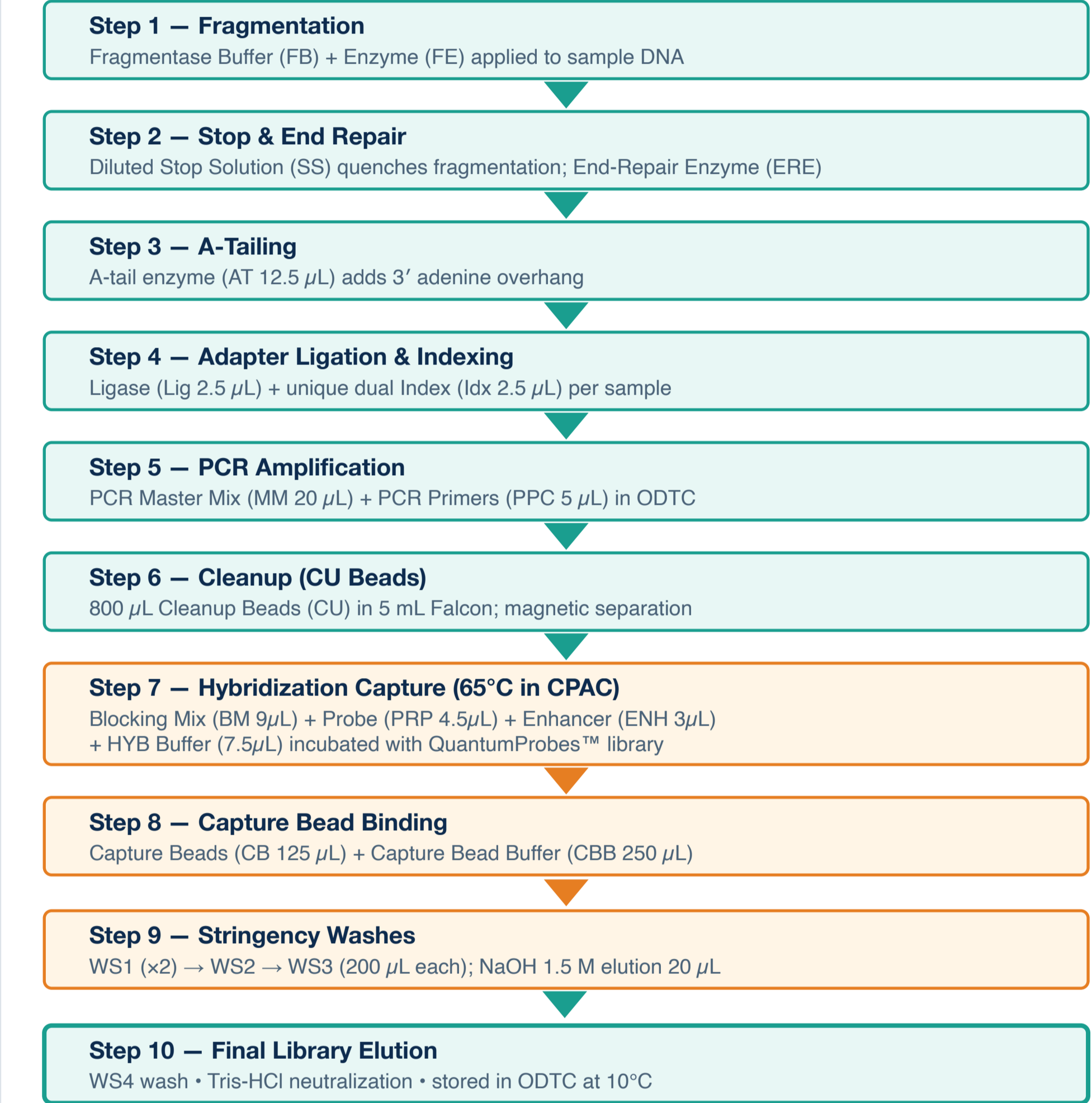
Push all racks fully back. Tape aliquot plates 1-4. No-Template Controls not recommended.

3 Automated Library Preparation Steps

Reagent Pre-Plating Summary

Plate	Lid	Key Reagents (col → vol/well)
Plate 1	Green	FB 2 μ L FE 2 μ L Diluted SS 30 μ L ERE 40 μ L AT 12.5 μ L Lig 2.5 μ L MM 20 μ L Idx 2.5 μ L
Plate 2	Blue	SS 5 μ L PPC 5 μ L PPC 5 μ L BM 9 μ L
Plate 3	Red	PRP 4.5 μ L ENH 3 μ L HYB 7.5 μ L WS1/2/3 200 μ L NaOH 20 μ L Tris 50 μ L
Plate 4	Yellow	Diluted WS1 100 μ L Diluted WS4 200 μ L (x2)

Automated Protocol Steps (Hamilton Run)

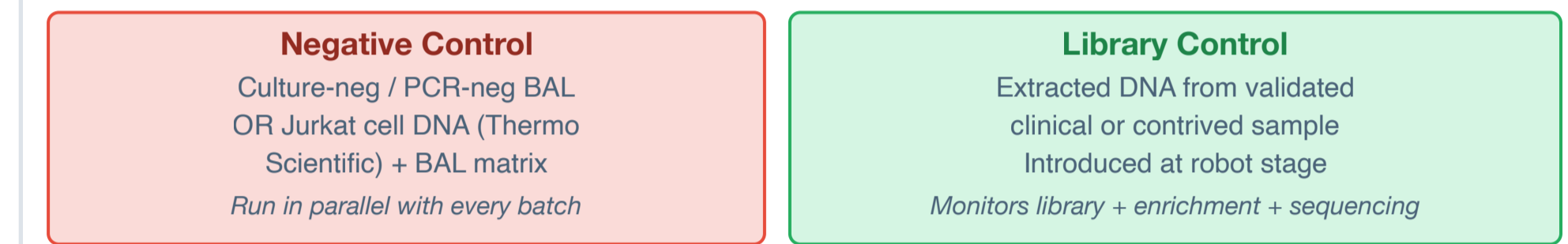


4 Multi-Stage QC Framework

QC Checkpoints by Stage

Stage	Method	Pass Criteria
1. BAL Receipt	Sample volume check	\geq 500 μ L; no clots/excess blood
2. Post-Extraction	DNA yield	\geq 5 ng/ μ L (Qubit HS DNA)
3. Post-Library	Library yield	\geq 10 ng/ μ L; \geq 5 nM (qPCR)
4. Library Sizing	Fragment distribution	~330 bp avg; \geq 50% in 280-380 bp
5. Post-Sequencing	Read quality	Q20 \geq 70% • Q30 \geq 50% • reads \geq 5M
6. Bioinformatics	Signal qualification	FPKM • BoC • Relative Abundance

Control Strategy



NTC not recommended for ONETest™

Pathogen Signal Categorization

Category	Outlier Criteria Met	Clinical Implication
STRONG	All 3: FPKM + Assembly Length + Relative Abundance	Highly significant
MODERATE	Any 2 of 3 criteria	Significant
WEAK	Only 1 of 3 criteria	Low significance

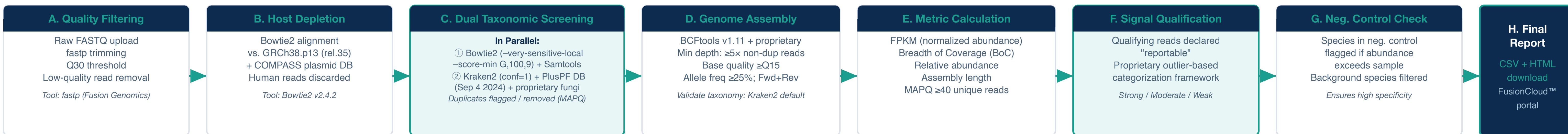
Species in negative controls flagged when control abundance exceeds sample abundance. Categorization thresholds are proprietary (Fusion Genomics).

Validation Highlights



Activity	Hands-on	Elapsed Time
Deck / reagent setup	15 min	15 min
Robot startup / method setup	5 min	5 min
Fragmentation → ligation → PCR	—	300 min
Off-deck drying / lyophilization	10 min	40 min
Hybridization	5 min setup	240 min
Capture, wash, final PCR	—	210 min
Library QC, normalization, quantification	90 min	120 min
Sequencer loading + run	5 min	7 h
FusionCloud™ analysis	—	<15 min
TOTAL	130 min	<24 h

5 FusionCloud™ Bioinformatics Analysis Pipeline



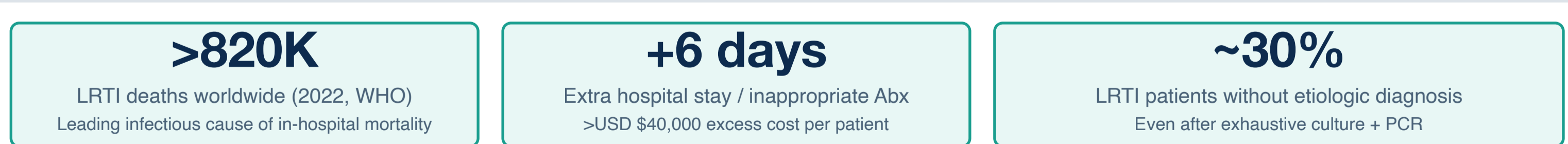
6 Reference Databases (FusionCloud™)

Database	Content	Version / Access Date
NCBI	Bacterial, fungal, and viral genomes	August 12, 2022
ENA	661K high-quality microbial sequences	Latest version
Kraken2 PlusPF	K-mer taxonomic profiling database	September 4, 2024
COMPASS	Plasmid reference sequences	Front. Microbiol. 2020
GRCh38.p13	Human genome (host depletion)	Release 35
Local DB	318,735 bacterial core-genome seqs • 383,472 fungal orthologs • 907 viral orthologs Custom QuantumProbes™ for taxon-specific capture	

Database Update Schedule

- Reference databases: screened semiannually
- Software stack: continuous monitoring; stable releases queued monthly
- Emergency patches: within 48 h of critical security/bug releases

7 Clinical Rationale & Unmet Need



Diagnostic Gap vs. Existing Methods

Method	Strengths	Key Limitations
Culture	Broad taxonomic range; AST	Slow (48h-3 wks); low sensitivity for fastidious organisms
PCR Panels	Rapid (\leq 4h); high sensitivity	Limited to ~20-30 targets; misses DNA viruses & fungi
WmGS	Hypothesis-free; broad scope	High cost; low pathogen:host ratio; heavy compute
ONETest™	254 targets; species-level; 6.5x FPKM	Hybrid capture efficiency; automated; cloud analysis

ONETest™ reagents and software are supplied for research use only and are not intended for diagnostic or therapeutic use. Any clinical use requires independent validation, performance, interpretation, and reporting by an appropriately accredited clinical laboratory under its applicable regulatory, accreditation, and quality framework.