

Rapid and Multiplexed NGS Workflow for Infectious Disease Genotyping: Evaluation of SeqWell ExpressPlex™ 2.0 Integrated with ABL’s DeepChek® Platform

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Background

Next-generation sequencing (NGS) is rapidly becoming a cornerstone of infectious disease diagnostics, enabling high-resolution genotyping, drug resistance profiling, and pathogen surveillance. A key challenge remains simplifying workflows to support pooled applications, such as HIV and tuberculosis (TB) in routine or decentralized settings. We evaluated the SeqWell ExpressPlex™ 2.0 system for its speed, robustness, and suitability for multiplexed NGS workflows integrated with ABL’s DeepChek® assays and software.

Materials & Methods

Nineteen samples (HIV and Mycobacterium tuberculosis) were prepared using SeqWell ExpressPlex™ 2.0 and benchmarked against standard NGS protocol (116C/124D, ABL). Libraries were normalized to 5 ng/μL and sequenced on Illumina iSeq100 (2x150bp). Resistance interpretation was performed using DeepChek® software, leveraging the latest Stanford HIVdb algorithm for HIV and the WHO 2021 mutation catalogue for TB.

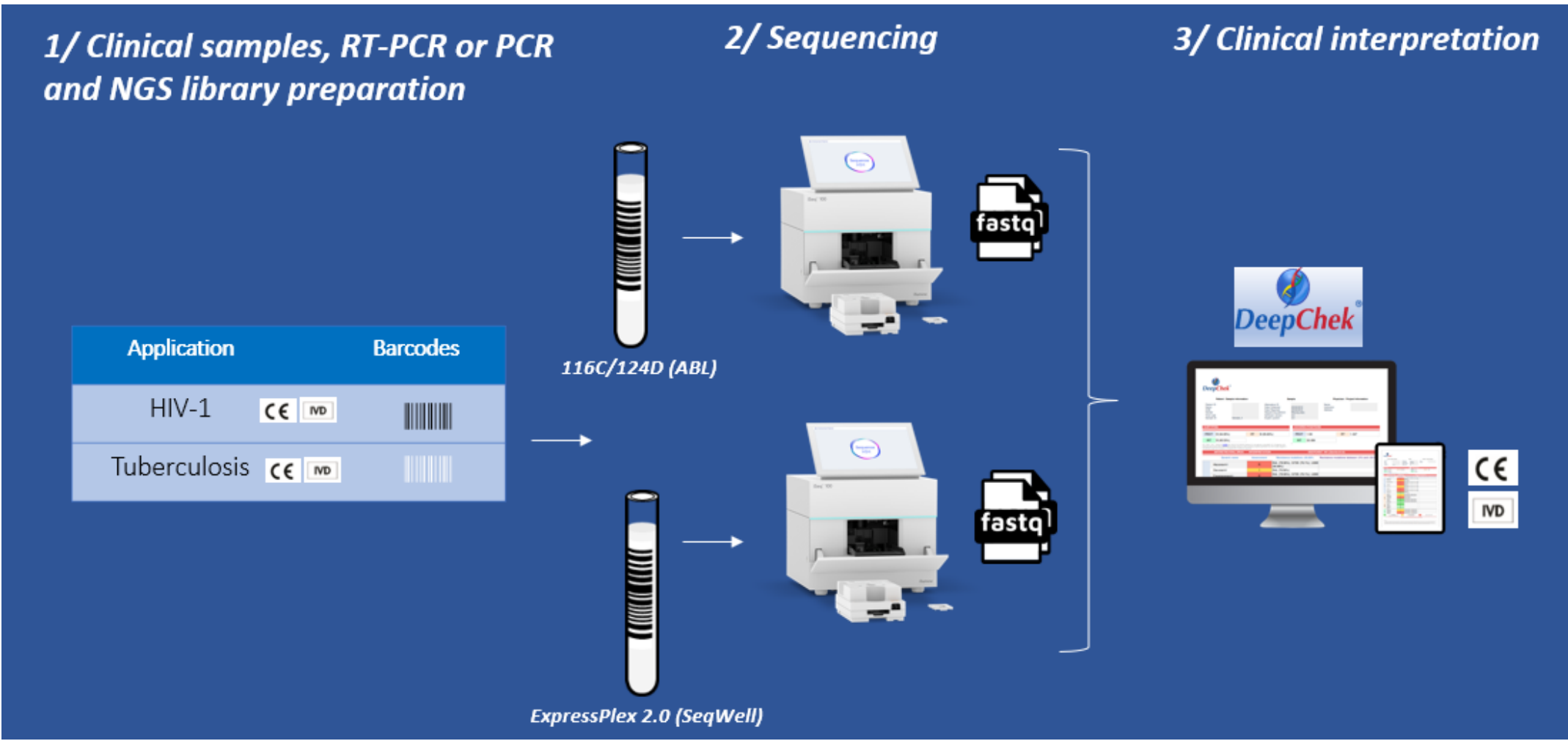


Figure 2. Integrated Workflow for HIV-1 and Tuberculosis Genomic Analysis: From Clinical Samples to NGS-Based Interpretation

Results

SeqWell ExpressPlex™ 2.0 reduced hands-on time by over 50% compared to traditional methods, while maintaining high-quality library metrics (mean: 20.36 ng/μL). A total of 7.69 million reads were generated, with 5.37 million passing filter (PF) and 87.5% successfully assigned to indexes. Read distribution across 19 indexes shows moderate variability (CV = 0.355), ranging from 2.6% to 6.9% per index.

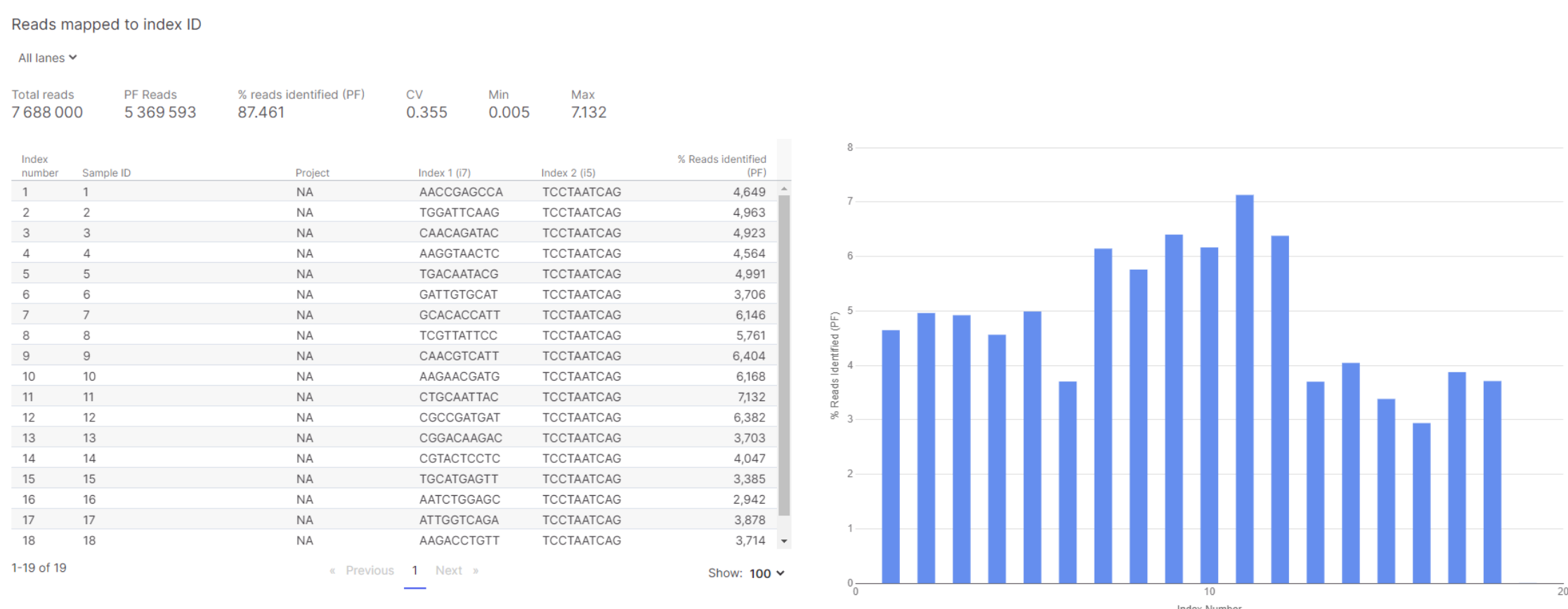


Figure 3. Index Read Distribution and Identification Efficiency in iSeq100 NGS Workflow

HIV and TB genotyping yielded accurate resistance calls in full concordance with expected profiles. The system enabled efficient pooling of multiple infectious disease assays within a single NGS run, demonstrating excellent compatibility with ABL’s DeepChek® software and analysis pipelines.

Sequencing of HIV samples revealed high mapping efficiency (>95%) across libraries. Subtype B was predominant, with tropism mostly dual/mixed (DM). Major protease (PR) mutations included M46I and G73S, while reverse transcriptase (RT) mutations such as K103E, Y188L, and G190A were detected in plasma samples with high viral loads. Integrase (INT) mutations were observed at positions E157Q and I74I, indicating potential resistance pathways.

Table 1. Bioinformatics analysis using MicrobioChek (ABL) to identify HIV-1 drug resistances obtained from Complete Genomics G10-FR versus iSeq100 (Illumina) platforms.

No.	Material	Material	Library	Mapping	Library	Success	RT	INT	PR	Coverage
1	LH001	Plasma	116C/124D (ABL)	116C/124D (ABL)	116C/124D (ABL)	No	100%	100%	100%	100%
			116C/124D (ABL)	116C/124D (ABL)	116C/124D (ABL)	No	100%	100%	100%	100%
2	10100	Plasma	116C/124D (ABL)	116C/124D (ABL)	116C/124D (ABL)	No	100%	100%	100%	100%
			116C/124D (ABL)	116C/124D (ABL)	116C/124D (ABL)	No	100%	100%	100%	100%
3	10100	Plasma	116C/124D (ABL)	116C/124D (ABL)	116C/124D (ABL)	No	100%	100%	100%	100%
			116C/124D (ABL)	116C/124D (ABL)	116C/124D (ABL)	No	100%	100%	100%	100%
4	10100	Plasma	116C/124D (ABL)	116C/124D (ABL)	116C/124D (ABL)	No	100%	100%	100%	100%
			116C/124D (ABL)	116C/124D (ABL)	116C/124D (ABL)	No	100%	100%	100%	100%

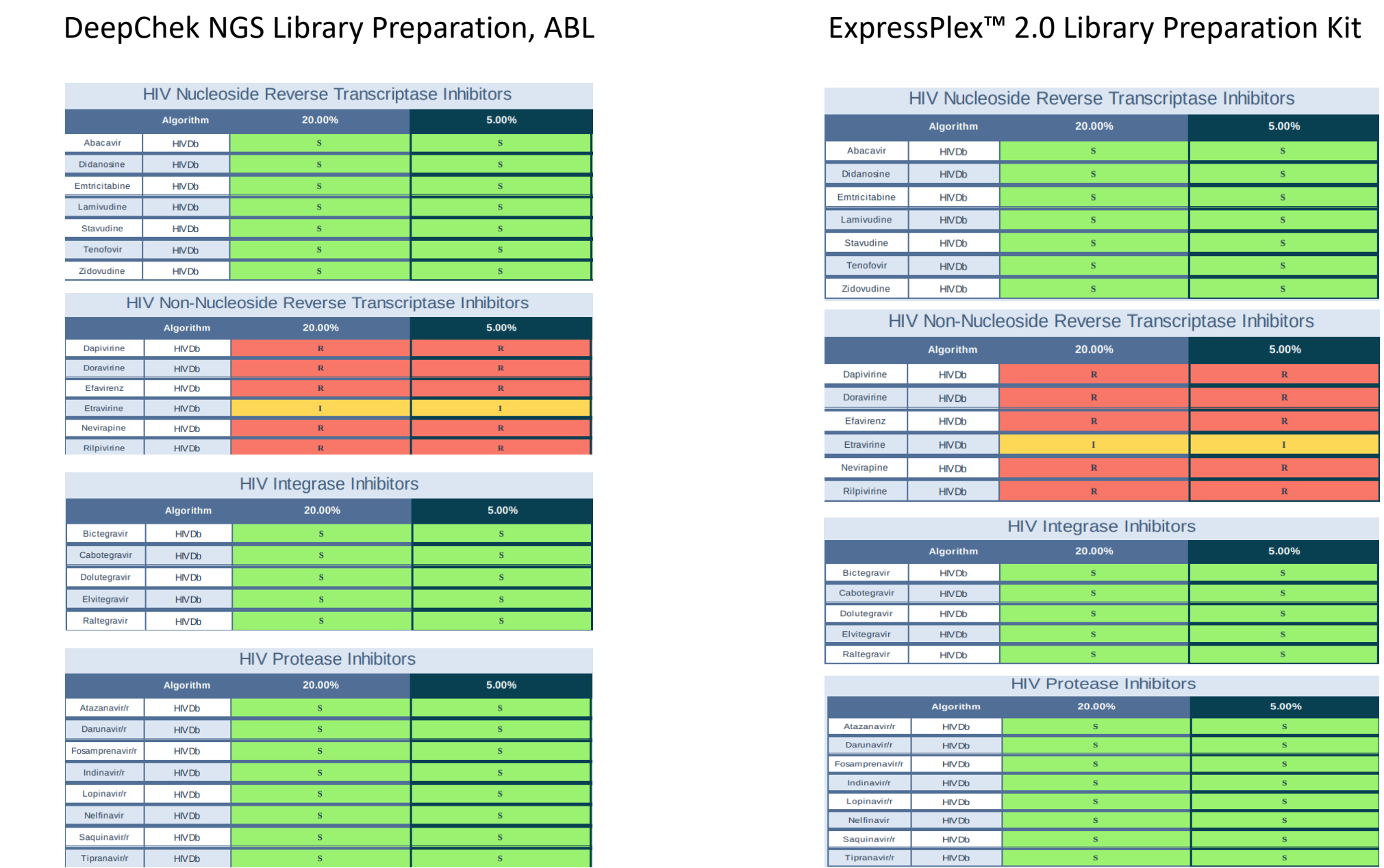


Figure 4. Comparison of NGS Library Preparation Methods for HIV Drug Resistance Analysis.

Analysis based on the WHO mutation catalogue (v2021-06) identified resistance-associated mutations for key drugs: katG and inhA for isoniazid, rpoB for rifampicin, and pncA for pyrazinamide. MDR-TB markers were present at thresholds of 20% and 3%, confirming multidrug resistance. No significant mutations were detected for second-line drugs such as bedaquiline and linezolid.

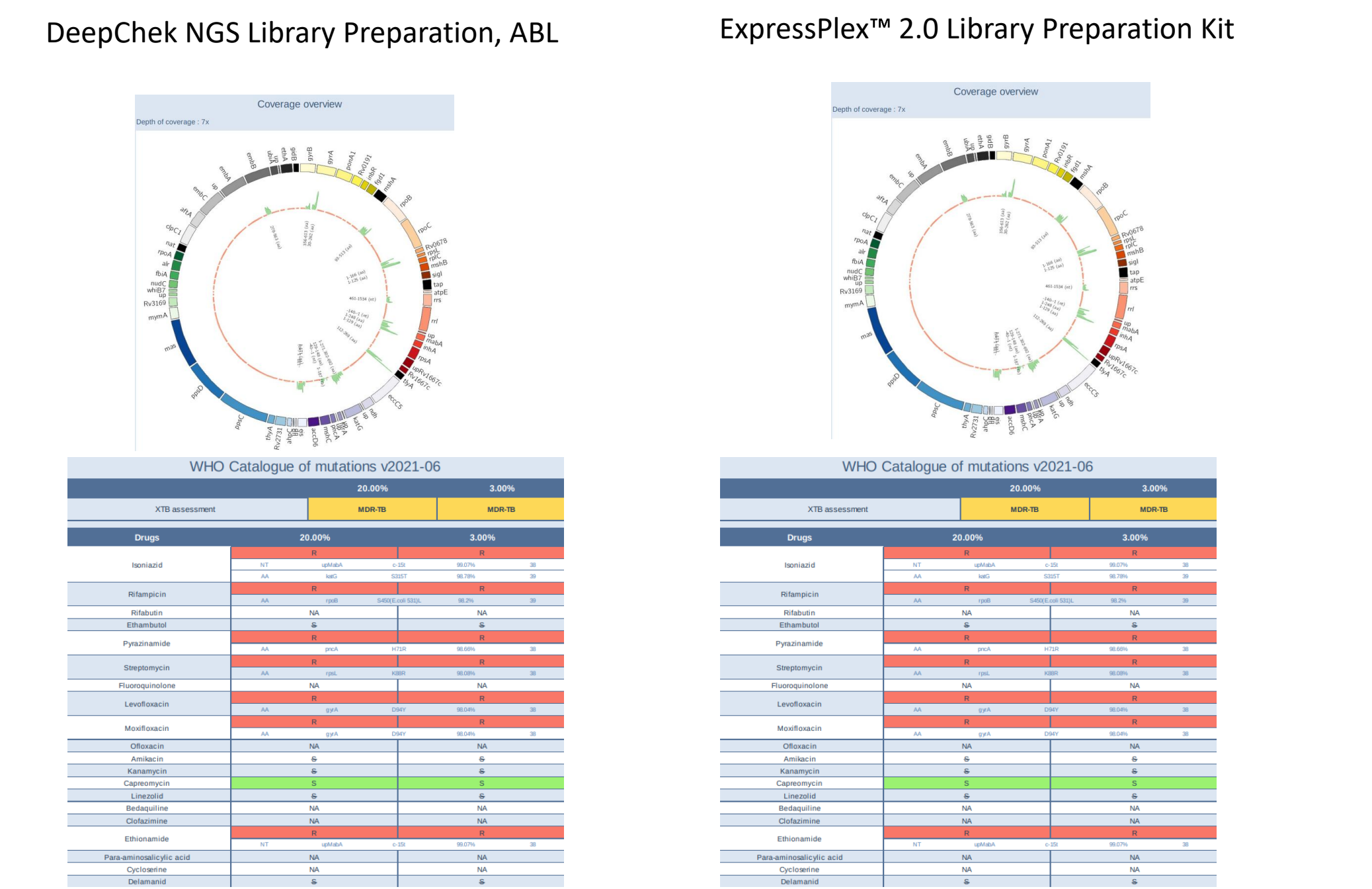


Figure 5. Comparison of NGS Library Preparation Methods for TB Drug Resistance Analysis.

Conclusion

NGS is emerging as a versatile platform for infectious disease diagnostics, and the ability to pool multiple assays in a single run is essential to optimize resources and turnaround time. The SeqWell ExpressPlex™ 2.0 system, combined with ABL’s DeepChek® solution, offers a rapid, integrated, and scalable approach for multi-pathogen genotyping. This supports broader implementation of NGS in clinical and surveillance settings. Additional studies are ongoing to validate scalability and pathogen expansion.

References

- 1: Vellas, et al. Comparison of short-read and long-read next-generation sequencing technologies for determining HIV-1 drug resistance. J Med Virol. 2024.
- 2: Martinez, et al. Advancing Drug Resistance Detection: Comparative Analysis Using Short-Read and Long-Read NGS Technologies. LabMed 2025.
- 3: Mohamed, et al. From Capillary Electrophoresis to Deep Sequencing: An Improved HIV-1 Drug Resistance Assessment Solution Using In Vitro Diagnostic (IVD) Assays and Software. Viruses. 2023.

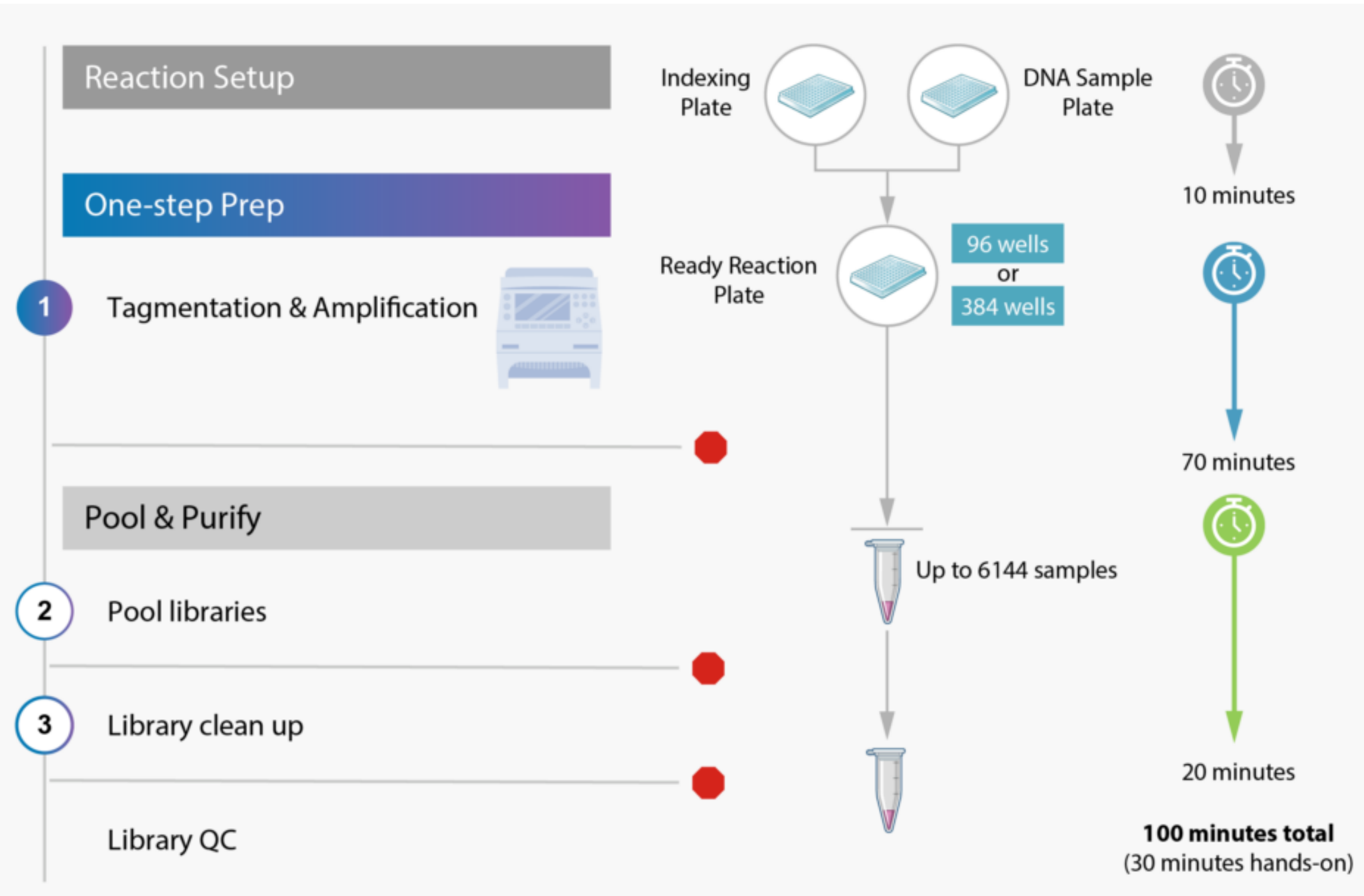


Figure 1. ExpressPlex One-step Library preparation.