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Analytical Validation of the Thermo Fisher ABI HIV-1 Genotyping Kit for Illumina Sequencing, Benchmarked Against Sanger, Using the Exatype Platform

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Executive Summary

Hyrax Biosciences, through its Exatype platform, delivers scalable **HIV drug-resistance** analysis to global programmes - processing over **150,000 HIV sequences** for **1,146 users across 70+ organisations** in **103 countries**. More than half of these programmes operate in Global Health Equity settings, positioning Exatype as one of the most **widely adopted** and **trusted platforms** for routine Sanger-based **HIV genotyping worldwide**.

Building on this foundation, Hyrax Biosciences **validated** whether Illumina NGS data generated using the Thermo Fisher HIV-1 Genotyping Kit with Integrase, when analysed on Exatype, delivers drug-resistance results equivalent to the Sanger (capillary electrophoresis sequencing) gold standard. This analysis shows **strong cross-platform concordance**, demonstrating that the kit performs consistently whether sequenced on Illumina or Sanger. Exatype was used as the **common analytical pipeline**, ensuring **standardised processing** and **fully comparable outputs**.

Key Outcomes:

- **Specificity**: >98.7% concordance between Illumina and Sanger consensus sequences.
- **Repeatability**: ≥99.0% intra-sample concordance across replicates.
- **Sensitivity**: Equivalent detection of mutations at ≥20% prevalence (Sanger gold standard); Illumina additionally resolves variants below this threshold.
- **Throughput**: Illumina generated ~340,000 reads per replicate, delivering high coverage across PR, RT and IN targets.

Background and Rationale

At the end of 2022, **29.8 million people** were on antiretroviral therapy (World Health Organisation, HIV Statistics, 2025)[1], yet **HIV drug resistance** remains a major global health threat. Global HIV drug-resistance monitoring requires **validated workflows** that are **accurate, scalable, and compatible across sequencing technologies**. While Sanger sequencing

remains widely used, laboratories are increasingly shifting toward NGS platforms such as Illumina to improve throughput and sensitivity.

Hyrax Biosciences undertook this **analytical validation** to assess whether Illumina sequencing data generated using the Thermo Fisher ABI HIV-1 Genotyping Kit with Integrase, analysed on the Exatype platform, provides results consistent with Sanger sequencing while enabling deeper variant profiling. Both the Sanger sequencing and Illumina NGS data were processed using Exatype ensuring directly comparable outputs.

Validation Results

Study design: included 5 samples × 4 replicates for PR (protease) and RT (reverse transcriptase) - sequenced as a single amplicon - and IN (integrase). All samples and replicates were amplified with the Thermo Fisher ABI HIV-1 Genotyping Kit with Integrase, sequenced on Illumina and Sanger, and analysed on Exatype.

Quantitative Outcomes

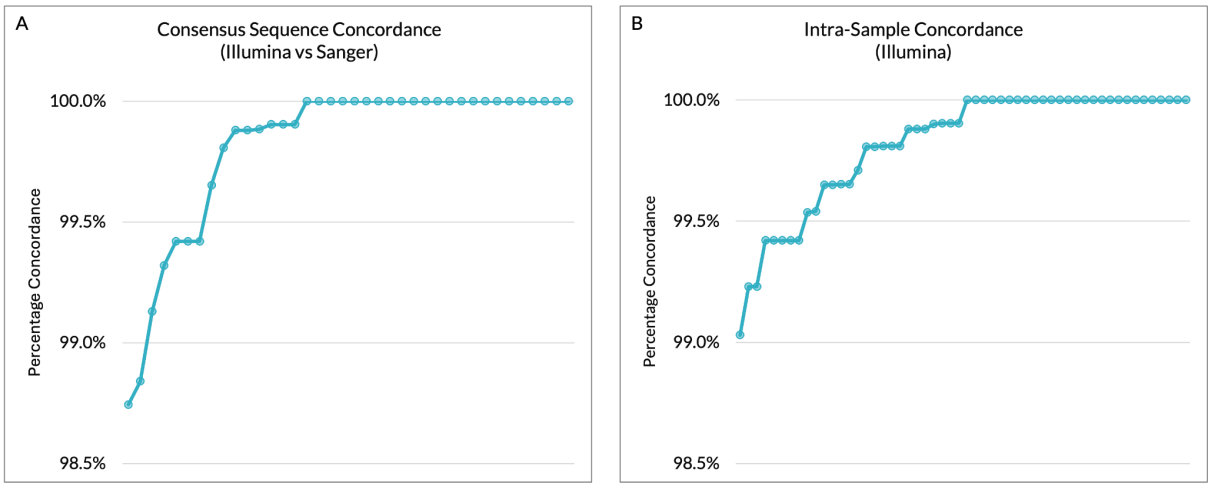

Figure 1: Intra- and Between-Sample Consensus Sequence Concordance

Metric	Result	Benchmark / Notes
Specificity	>98.7% Illumina-Sanger consensus concordance (Fig. A)	Confirms platform equivalency
Repeatability	>99.0% concordance across replicates (Fig. B)	Demonstrates high robustness
Sensitivity	Matching detection of major variants (≥20% Sanger standard)	Equivalent performance to Sanger
Sensitivity threshold	Sanger threshold 15 - 20%[2]; Illumina threshold ≥1%	Enhanced minor-variant resolution with Illumina

Read depth	~340,000 reads per replicate; ≥89% mapped	Supports high-confidence variant calls
Coverage	>15,000× average across PR, RT, IN	Exceeds minimum required (≥14x) for DR calling
Drug-resistance calls	Consistent across platforms	Reliable HIV-DR detection

Key observations:

- **Reproducibility** across Illumina and Sanger sequences was ≥98%, confirming **assay performance.**
- **Minor differences** between Illumina and Sanger (and among replicates) were primarily attributable to shifts in variant prevalence at **mixture sites**.

Analytical and Applied Implications enabled by Hyrax Biosciences' Exatype Platform

This **validation** confirms that programmes can continue using the same Thermo Fisher ABI HIV-1 Genotyping Kit they have relied on for years, while seamlessly expanding from Sanger to Illumina without changing their **analytical workflow**. By processing both Sanger and Illumina data through the same **Exatype** pipeline, laboratories retain identical interpretation rules, mixture thresholds, reporting formats, and drug-resistance outputs - **ensuring continuity across longitudinal datasets**.

Illumina's high read depth, combined with **Exatype's** automated QC and unified variant-calling framework, offers improved minor-variant detection without altering established Sanger-based thresholds. This **cross-platform consistency** allows laboratories to increase throughput and sensitivity using familiar reagents and a familiar analysis environment, **removing the need for new bioinformatics** tools or retraining. In practice, it reduces operational burden, supports scalable national surveillance, and keeps historical Sanger data comparable as programmes transition to NGS.

Conclusion

Illumina sequencing, generated using the Thermo Fisher ABI HIV-1 Genotyping Kit with Integrase and analysed through **Hyrax Biosciences' Exatype platform**, provides **HIV-1 drug-resistance** results equivalent to Sanger while adding the benefits of high throughput and improved minor-variant resolution. This **analytical validation** confirms **Exatype** as a **robust, cross-platform solution** capable of supporting **large-scale HIV-DR surveillance and laboratory implementation** across **diverse sequencing environments**.

References

1. World Health Organisation (WHO). *HIV statistics, globally and by WHO region*, Information Sheet, 2025
2. Steegan *et al.*, 2023, *Advancing HIV Drug Resistance Technologies and Strategies: Insights from South Africa's Experience and Future Directions for Resource-Limited Settings*, Diagnostics, 13(13):2209