

Rapid Viral Load Monitoring of HIV-1 Using CRISPR Technology



A joint venture between The University of Melbourne and The Royal Melbourne Hospital

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1. Introduction

- Over 32 million people receiving antiretroviral therapy require viral load monitoring every 3–6 months to ensure viral suppression, maintain health outcomes, and prevent sexual transmission risk.
- However, access to viral load monitoring remains limited or unavailable due to current diagnostic methods being heavily reliant on lab-based testing.
- A point-of-care (PoC) test is essential in addressing the existing gaps in access to viral load monitoring of HIV-1.
- The aim of this study was to develop a rapid, highly sensitive and specific PoC test that can distinguish unsuppressed from suppressed viral loads using emerging CRISPR-Cas technology.

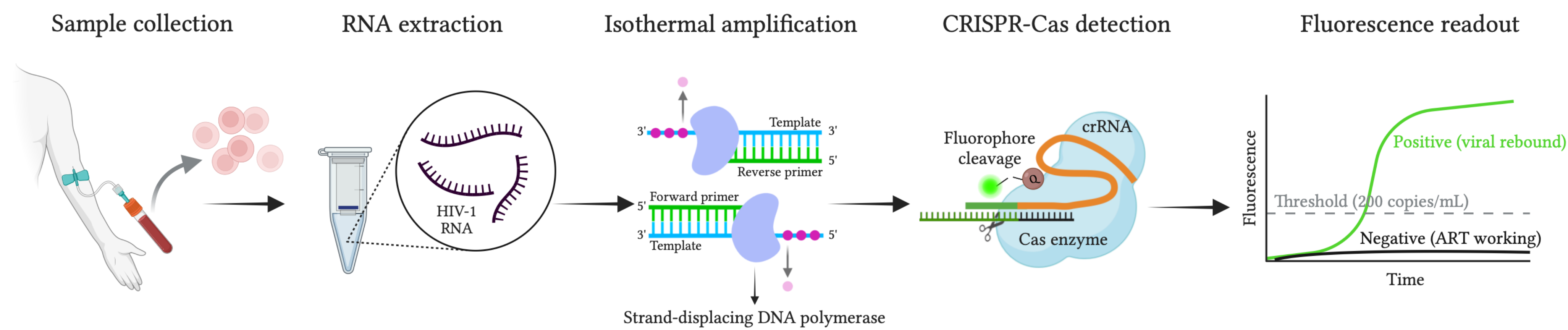
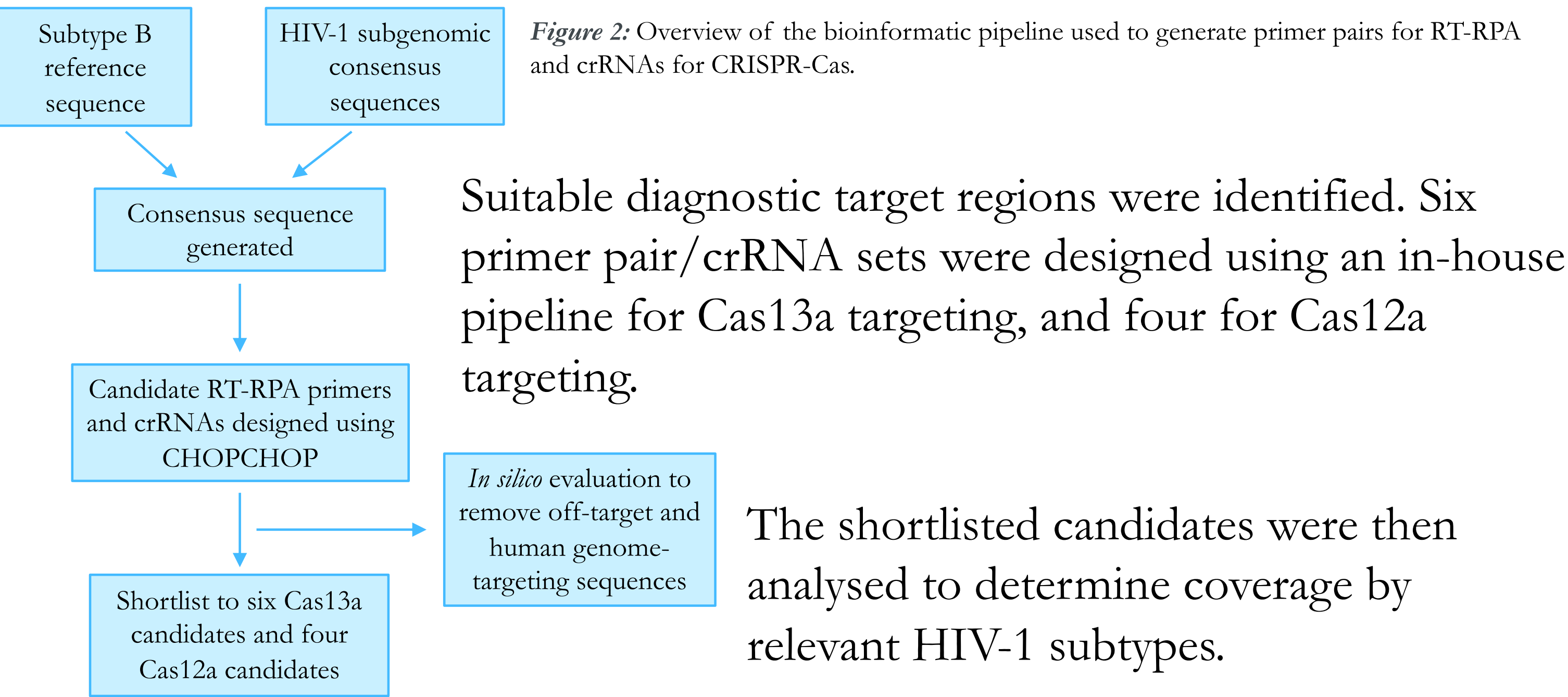


Figure 1: Schematic representation of diagnostic workflow: RNA is extracted from HIV-1 positive blood samples. Isothermal amplification (RT-RPA) is performed on target RNA. The amplicons are utilised in a CRISPR-Cas reaction, where target-specific recognition by a guide RNA (crRNA)-Cas12/13 complex results in fluorophore cleavage. Fluorescence output is read across 30 minutes.

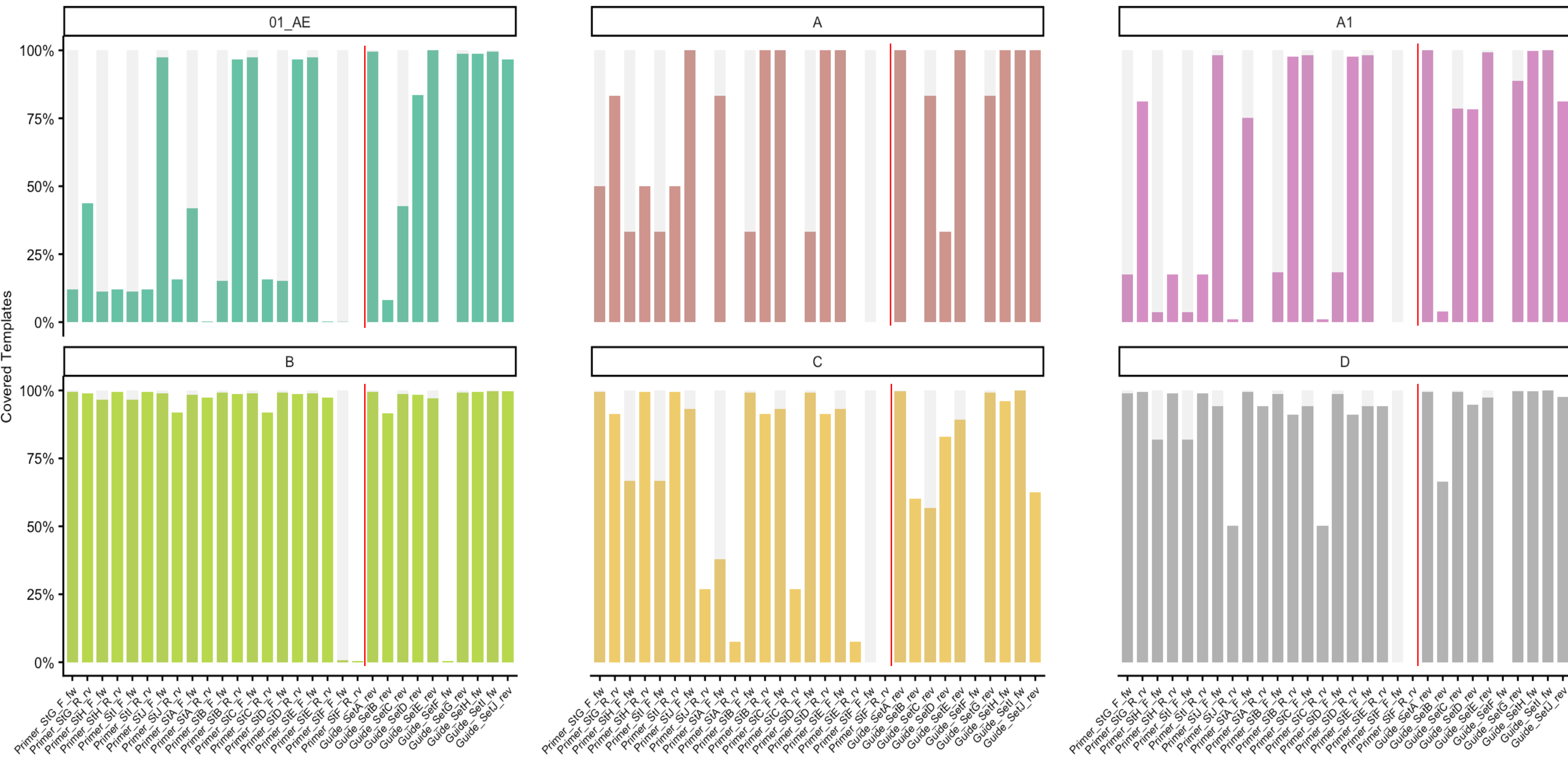
2. Assay design



Suitable diagnostic target regions were identified. Six primer pair/crRNA sets were designed using an in-house pipeline for Cas13a targeting, and four for Cas12a targeting.

The shortlisted candidates were then analysed to determine coverage by relevant HIV-1 subtypes.

Figure 3: Subtype alignment reflecting % templates covered by candidate primers and crRNAs (allowed mismatches = 3).



3. Screening candidate primer/crRNA sets

- An immortalised human CD4⁺ T-cell line with an integrated HIV-1 provirus (J-Lat 10.6) was used to screen candidate Cas13a and Cas12a sets *in vitro*, and sensitivity (fluorescence output) was assessed.
- Sets demonstrating highest sensitivity and/or earliest time to sensitivity were selected for further analysis.

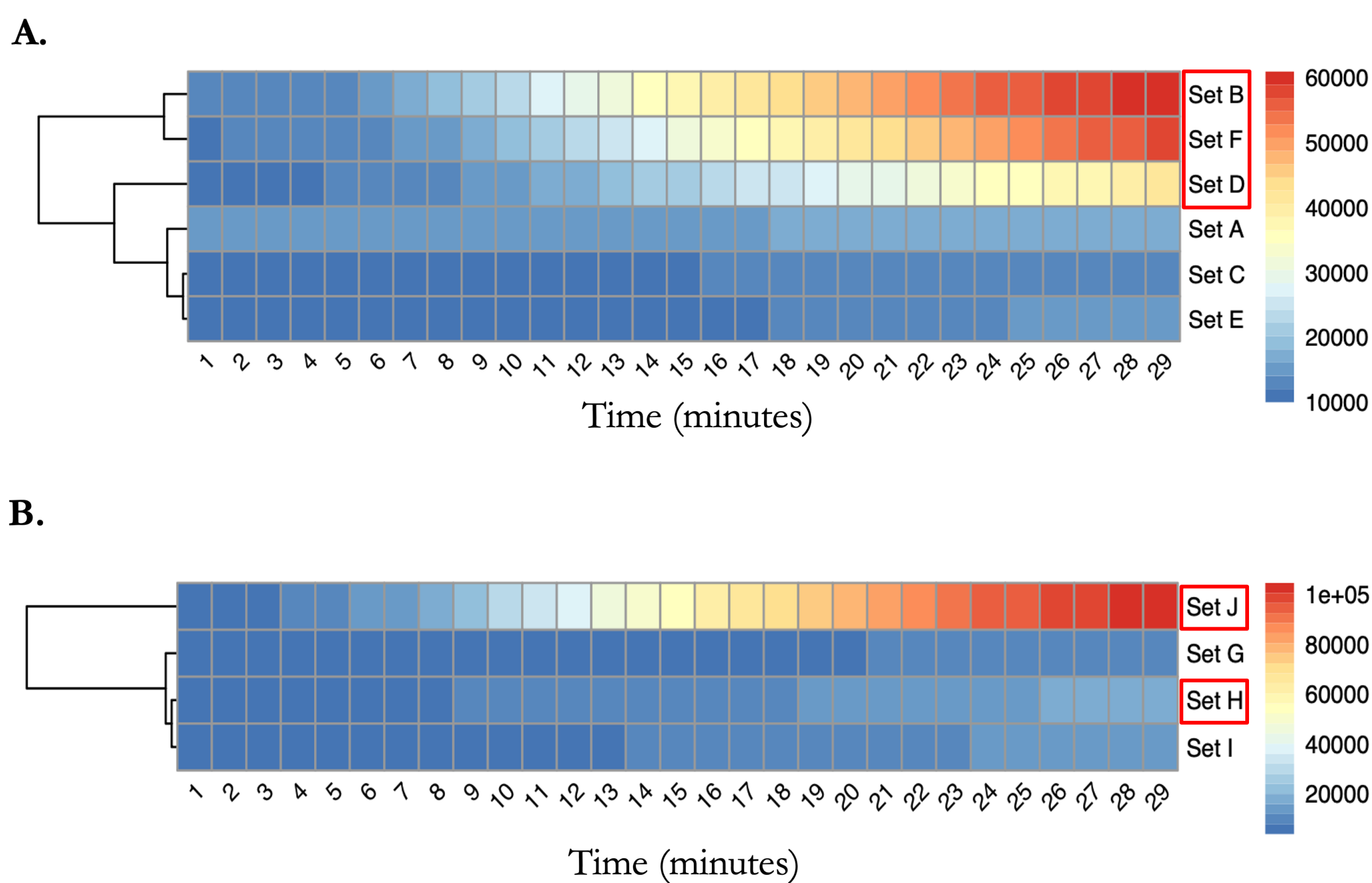


Figure 4: Heatmaps depicting best-performing candidate primer/crRNA sets. A. Sensitivity heatmap of six Cas13a candidate primer/crRNA sets screened across 30 minutes using 100s of copies/μL of J-Lat 10.6 (n=3). B. Sensitivity heatmap of four Cas12a candidate primer/crRNA sets screened across 30 minutes using 100s of copies/μL of J-Lat 10.6 (n=3).

4. Dual-target assay avoids diagnostic escape

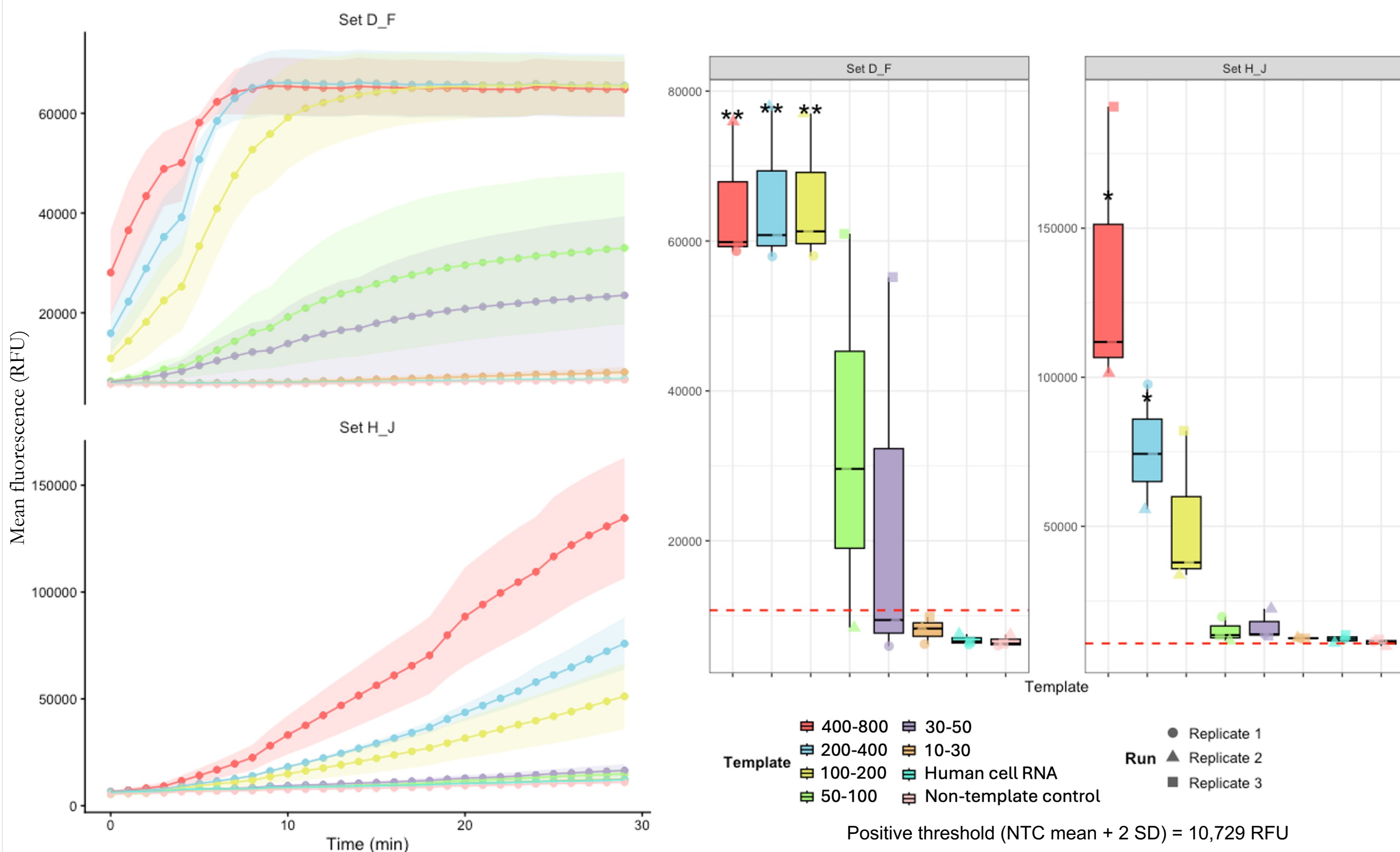


Figure 5: A limit of detection (LoD) dual-target assay. Data are shown as mean fluorescence (RFU) ± SEM (n=3) in line graphs. Mean fluorescence signal (n=3) is plotted at endpoint, 29 minutes in boxplots. One-sided, unpaired t-test performed (*p<0.05, **p<0.01). RFU = Relative Fluorescence Units.

A serial dilution of J-Lat 10.6 was tested to assess analytical sensitivity of the developed assay *in vitro*. The Cas13a and Cas12a sets demonstrated LoDs of <200 and <50 copies/μL, respectively.

5. Varied subtypes correctly identified

HIV-1 positive clinical samples of subtype CRF01_AE and subtype B were correctly identified by the dual-target assay.

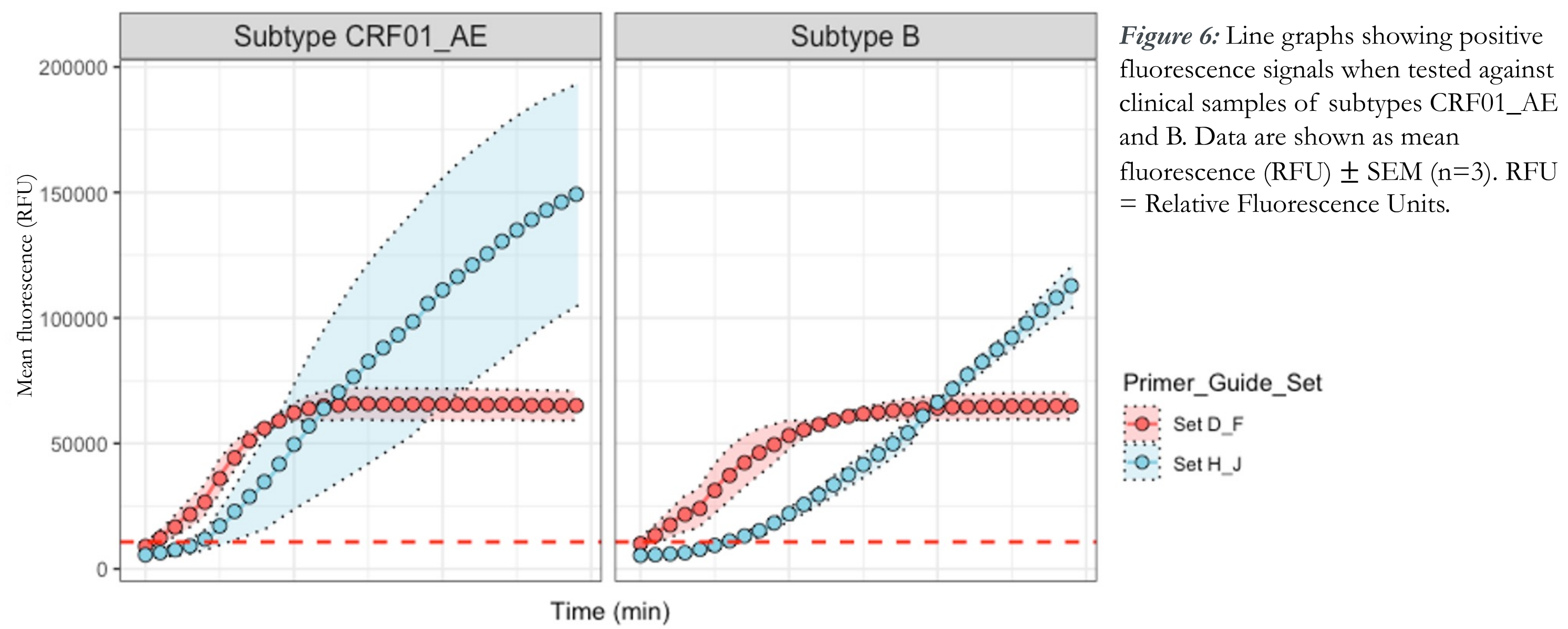


Figure 6: Line graphs showing positive fluorescence signals when tested against clinical samples of subtypes CRF01_AE and B. Data are shown as mean fluorescence (RFU) ± SEM (n=3). RFU = Relative Fluorescence Units.

6. Conclusions and future directions

We demonstrate the feasibility of a novel CRISPR-based, rapid viral load monitoring assay.

Future work

- Test on clinical samples across global subtypes.
- Test with a panel of similar pathogens to assess specificity.
- Compare sensitivity to current standard, RT-qPCR.
- Transfer to a portable device for broader PoC implementation.

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- The best-performers of Cas13a and the best-performers of Cas12a were multiplexed for a dual-target assay to limit diagnostic escape.