

Simple, low cost assay for detection of HIV-1 antiretroviral resistance

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Abstract

Pre-existing drug resistance before initiation and acquired drug resistance after failure of antiretroviral therapy are serious clinical problems that limit our ability to adequately treat HIV-1 infection. Currently, there are no solutions to these problems in resource-limited settings of the world where the standard genotypic assays for detecting resistance are unaffordable. Thus, a low-cost, low-complexity assay to detect resistance is urgently needed. We have exploited a novel variant of *Taq* polymerase with an enhanced ability to discriminate between a matched versus mismatched primer:template duplex to develop an assay in which amplification only occurs when a diagnostic primer matches a resistance mutation in a target template. The assay uses standard PCR and the presence of amplified DNA can be determined visually at PCR end-point. The simplicity and low cost of the assay would make it feasible to initiate wide-spread resistance testing in resource-limited settings.

Background

Over the last two decades, significant advances have been made in the management of HIV-1 infection. Most of these advances have been due to the rollout of progressively more effective, less toxic, and easier to use antiretroviral therapy (ART). All regions of the world have benefited from this, but the World Health Organization (WHO) estimates that most cases of HIV-1 infection have occurred in resource-limited settings where antiretroviral coverage continues to lag behind that of resource-rich settings. Nonetheless, as many as 10 million individuals in resource-limited settings may be receiving ART.¹

Despite the promise of ART, pre-existing drug resistance before, and acquired drug resistance after initiation of ART play a significant role as both a cause and a consequence, respectively, of ART failure.²⁻⁴ In resource-rich countries, such as the United States, treatment guidelines recommend screening for resistance before initiating and after failing ART.⁵ However, these assays are expensive and beyond the financial realities of healthcare systems in most resource-limited locations.

Thus, there is an unmet clinical need for an affordable, low-complexity assay to detect antiretroviral resistance mutations in resource-limited settings. The objective of this project is to determine whether an approach based on modified allele-specific PCR can be utilized to detect resistance mutations within the HIV reverse transcriptase gene.

Methods

Enhanced allele-specific PCR (AS-PCR) was used to detect polymorphisms in the reverse transcriptase gene that confer resistance to first line antiretroviral drugs. DNA templates harboring resistance mutations were synthesized and used to evaluate the sensitivity and specificity of AS-PCR for six of the most clinically relevant mutations conferring resistance. Positive amplification reactions were visually detected by adding sequence-specific, fluorescently labelled probes to the reactions.

Results

Figure 1. Strategy to selectively amplify reverse transcriptase gene segments containing antiretroviral resistance mutations. Primer binding sites and orientation (blue arrows) define the target amplicons (orange lines).

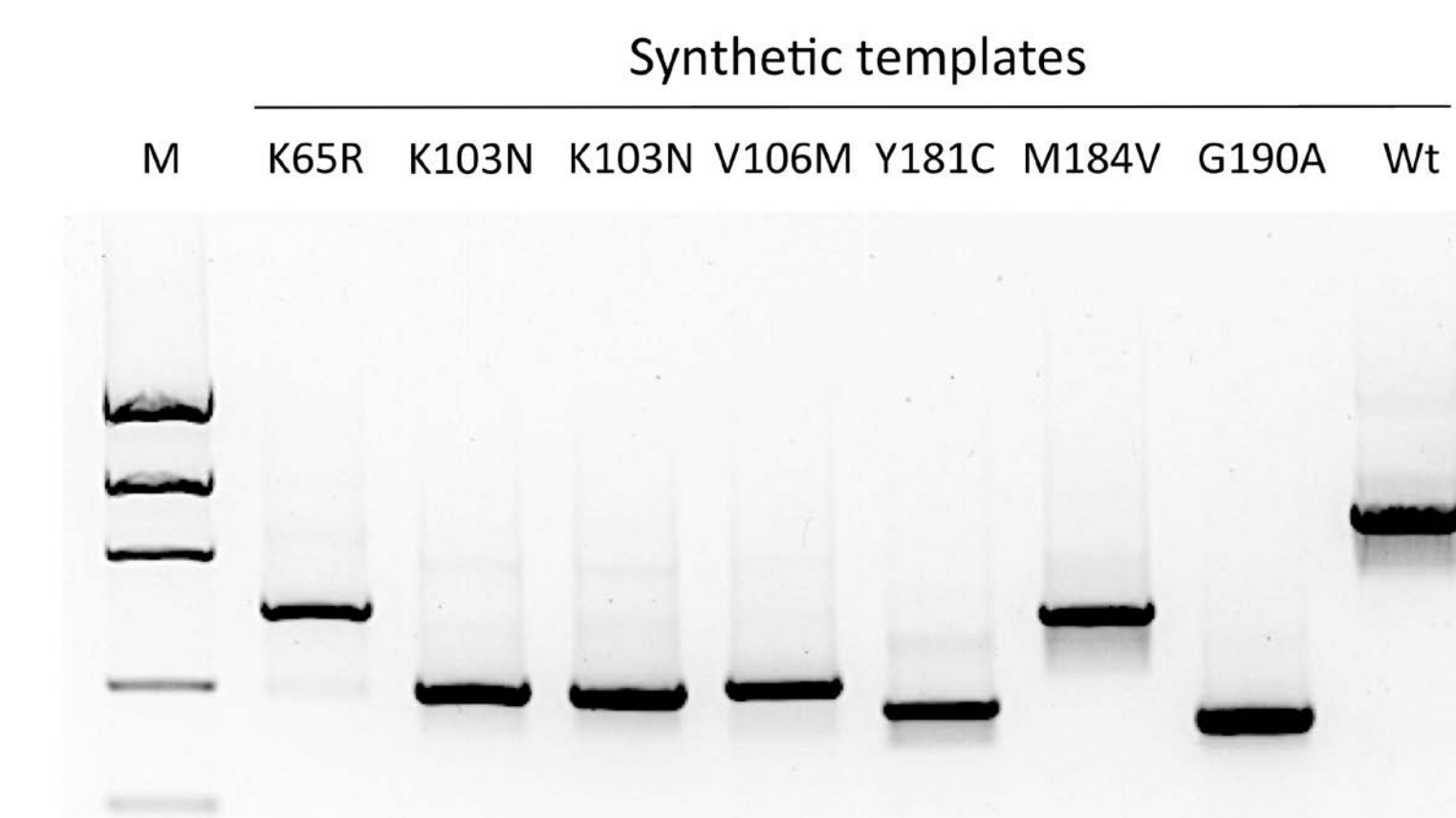
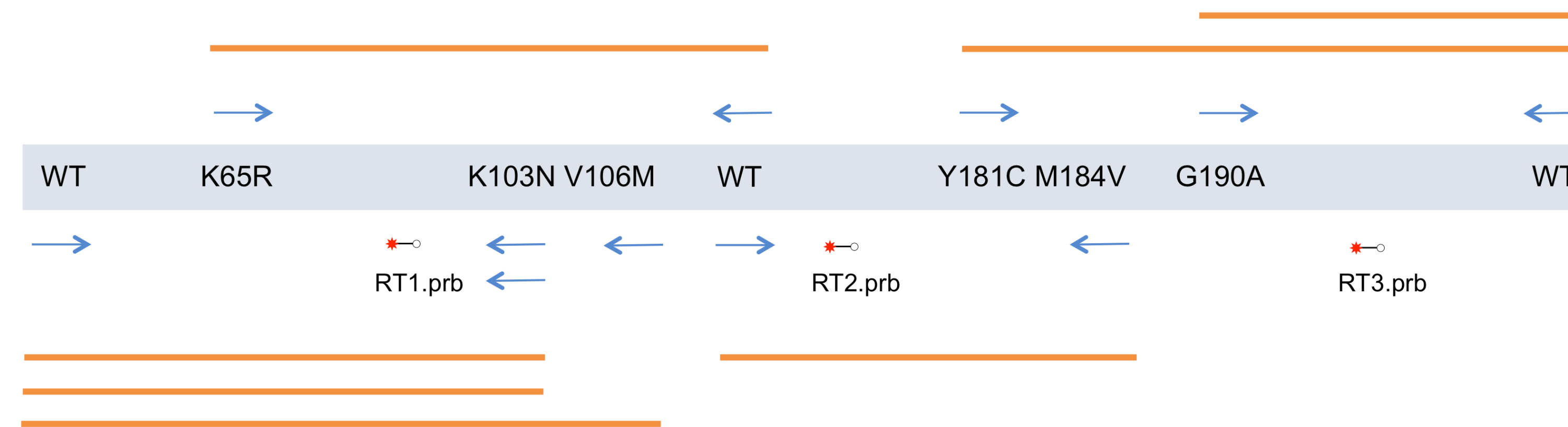
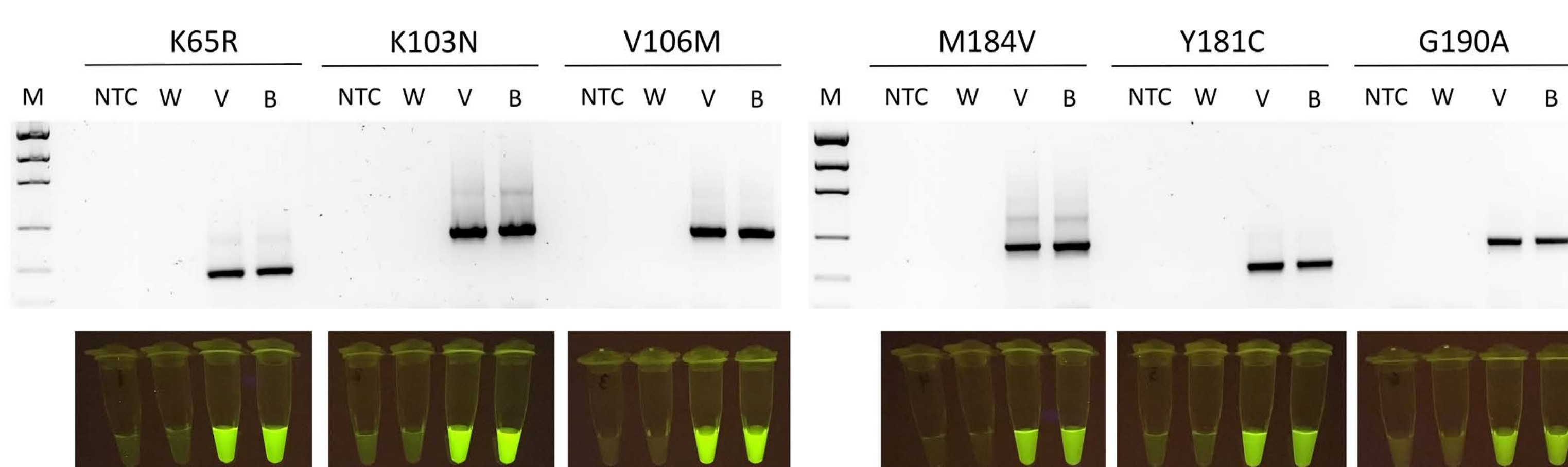


Figure 2. Synthesis of DNA templates harboring resistance mutations. DNA templates with resistance mutations were generated by PCR amplification using standard *Thermus aquaticus* DNA polymerase (*Taq*) that tolerates mismatched primer:template duplexes.

Figure 3. Mutant templates are specifically amplified using highly selective AS-PCR. Equivalent amounts (1×10^4 copies) were subjected to 40 cycles of AS-PCR using either wild-type template, mutant template or a combination of both. Positive reactions are identified by ethidium bromide staining of agarose gels or by fluorescent signal.



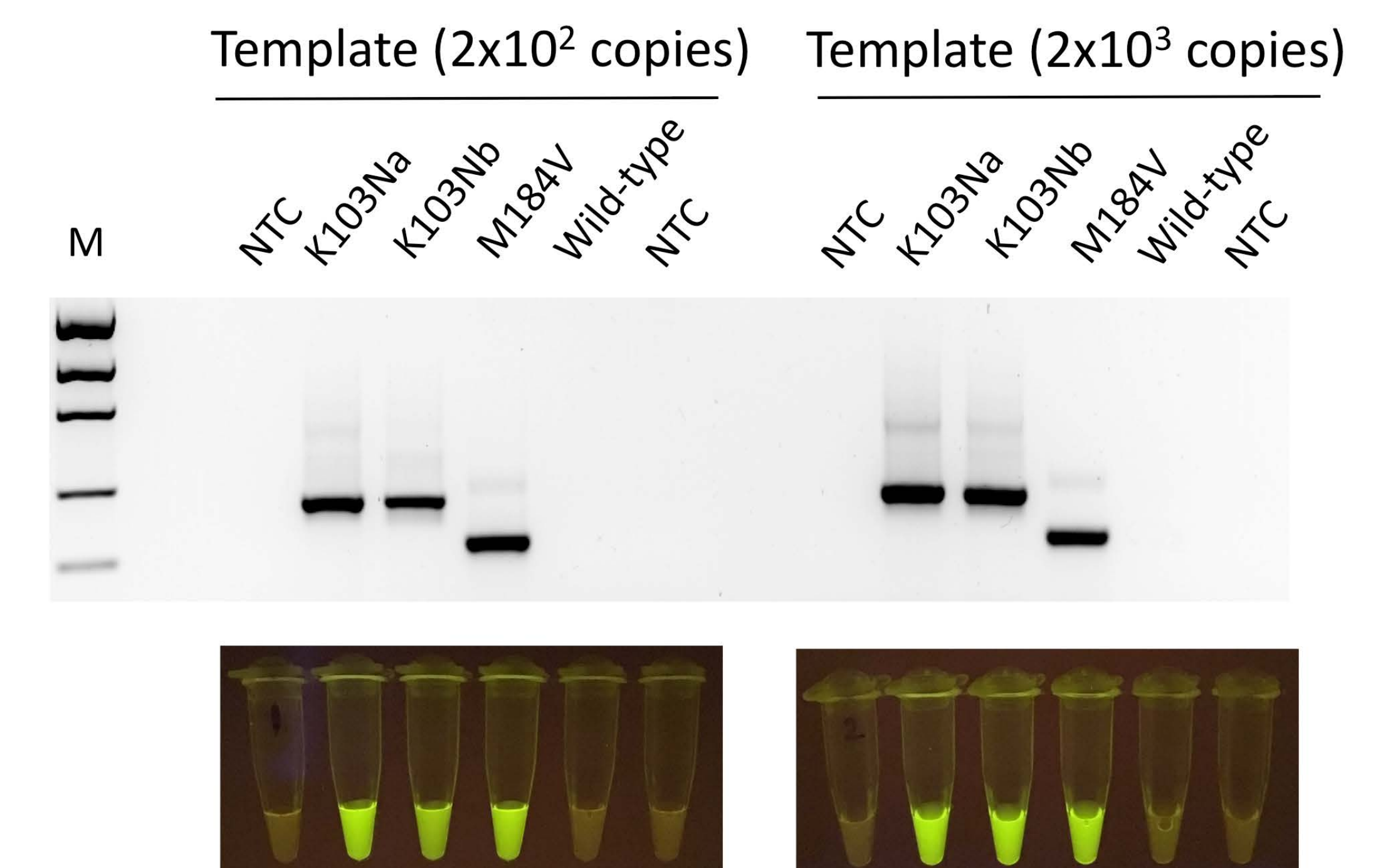
M, size marker; NTC, no template control; W, wild-type template; V, variant template; B, both templates

Figure 4. Enhanced AS-PCR for HIV-1 resistance mutations is highly sensitive. Representative results using M184V template dilutions demonstrating detection of low copy number target. Fluorescence intensity can be quantified using a portable, battery-powered fluorimeter.



Results

Figure 5. AS-PCR multiplexing simultaneously and efficiently detects K103N and M184V polymorphisms.



Conclusions

- AS-PCR for each target is highly selective.
- Amplification of mutant template is not inhibited by the presence of an equivalent amount of wild-type template.
- Sensitivity is excellent with a limit of detection ≤ 10 copies
- Detection of K103N and M184V mutations by multiplex PCR is similar to detection via singleplex reactions.
- Closed system reduces post-PCR handling that could lead to sample misidentification or carry over DNA contamination.
- Estimated cost is 1-2 US dollars per reaction.

References

- Joint United Nations Programme on HIV/AIDS (UNAIDS), Global Report: UNAIDS report on the global AIDS epidemic 2013. http://www.unaids.org/en/media/unaids/contentassets/documents/epidemiology/2013/gr2013/UNAIDS_Global_Report_2013_en.pdf. Accessed on August 3, 2015.
- Wainberg MA, Friedland G. Public health implications of antiretroviral therapy and HIV drug resistance. *JAMA* 1998;279:1977-83.
- Wittkop L, Gunthard HF, de Wolf F, et al. Effect of transmitted drug resistance on virological and immunological response to initial combination antiretroviral therapy for HIV (EuroCoord-CHAIN joint project): a European multicohort study. *Lancet Infect Dis* 2011;11:363-71.
- Bertagnolio S, Perno CF, Vella S, Pillay D. The impact of HIV drug resistance on the selection of first- and second-line ART in resource-limited settings. *J Infect Dis* 2013;207 Suppl 2:S45-8.
- Gunthard HF, Aberg JA, Eron JJ, et al. Antiretroviral treatment of adult HIV infection: 2014 recommendations of the International Antiviral Society-USA Panel. *JAMA* 2014;312:410-25.