Reference Panel of Cloned HIV-2 Plasmid DNA for Nucleic Acid Assay Development, Evaluation, and Quality Monitoring

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Abstract

- **Background:** Although human immunodeficiency virus type 2 (HIV-2) infections occur mostly in West Africa, cases have spread to other parts of the world such as Europe, India, and the United States making accurate diagnosis of HIV-2 infection essential. Currently in the US, no FDA-approved commercial nucleic acid assays for HIV-2 are available and several laboratories have developed in-house assays to confirm HIV-2 infections. However, the HIV-2 samples needed to develop, optimize, evaluate and monitor the performance of HIV-2 nucleic acid assays are also severely limited. Thus, there is an acute need for the development and characterization of specialized reagents that could be used for these activities.
- **Objectives:** To develop a panel of plasmid HIV-2 DNA standards containing cloned sequences covering two distinct gene regions known to be useful for various nucleic acid amplification assays. The plasmids represent multiple HIV-2 subtypes from well- characterized isolates obtained from HIV-2 endemic West African countries including the Ivory Coast, Senegal, and Guinea-Bissau.
- **Methods:** Viral stocks from previously described HIV-2 isolates, which included HIV-2 subtype A (n=9), subtype B (n=2), and subtype AB (n=1) were used as the source material for clone generation. RNAs were extracted from all viral stocks using the QIAamp Viral RNA Mini Kit (Qiagen) and were amplified using HIV-2 *LTR* and *pol* specific primers by reverse transcriptase-PCR. Amplicons were inserted into TOPO TA plasmids (Life Technologies) using standard cloning techniques and were transformed into *E. coli* (TOP10 Chemically Competent). Additional sub-cloning modifications such as digesting the insert with EcoR I and reinsertion into the same vector, to prevent insert expression were performed to stabilize clones. Plasmids were purified from each clone using the QuickLyse Miniprep Kit (Qiagen). Plasmids were sequenced and phylogenetic trees (neighbor-joining method) were done to confirm the HIV-2 subtype in each gene region.
- **Results/Conclusions:** The entire *LTR* (~849 bp) and *pol* (~2995 bp) regions were successfully cloned and sequenced; and subtype designation was confirmed for all 12 isolates. This reference panel of HIV-2 plasmid clones provides an easily quantifiable, renewable, and non-infectious set of reagents which will be valuable for the development and evaluation of HIV-2 molecular diagnostic assays as well as reagents for Quality Assurance and Quality Control.

Background

- Inconclusive serological results should be followed by a NAAT for HIV and HIV-2 as needed
- There is no FDA-approved commercial NAAT for the diagnosis of HIV-2 in the United States
- HIV-2 specimens are not readily available for assay development and validation
- Cloned HIV-2 reference panels would be useful for the development and validation of new assays as well as reagents for quality assurance and quality control

Objective

- To generate a novel panel of HIV-2 DNA plasmids that can be used as standards for new assay development and validation

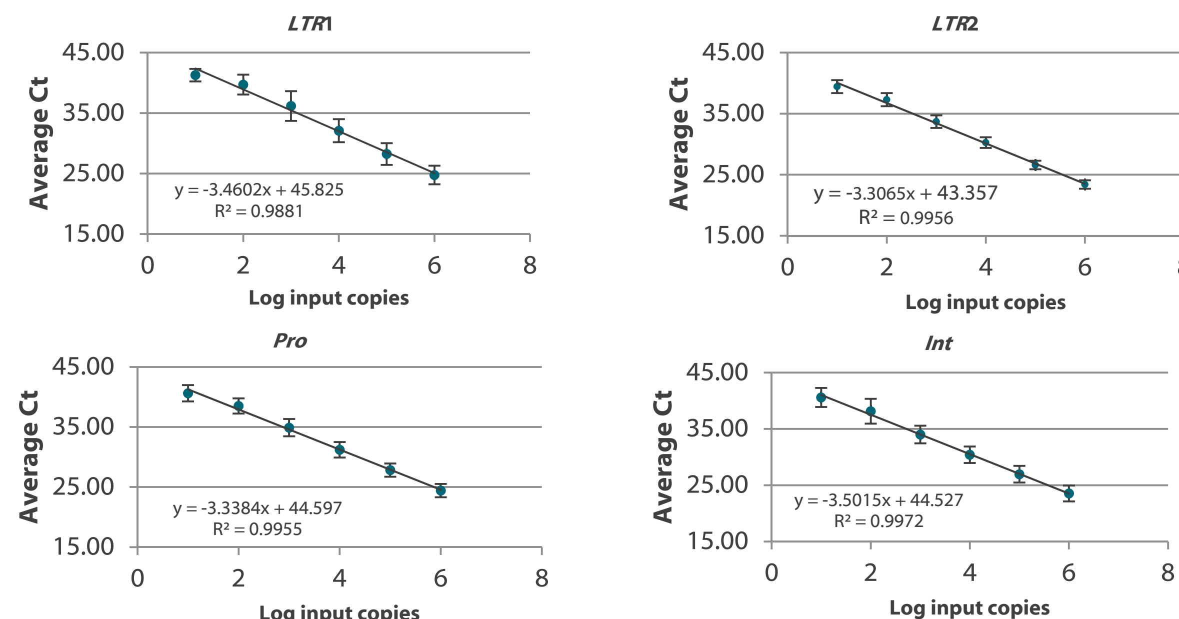
Materials and Methods

- Twelve HIV-2 isolates (9 subtype A, 2 subtype B, and 1 subtype AB) from various West African countries were used as the starting material
- RT-PCR amplification of the HIV-2 RNA *LTR* and *Pol* regions was performed by using six primer sets
- Amplified DNA sequences were inserted into the vector (TOPO) and transform into E. coli
- Three clones (3100319*pol*, 310072*pol*, GB87*pol*) required additional sub-cloning modifications; clones were digested with *EcoR* I and reinserted into the same vector with a ratio of 4:1, cultured at 32˚C overnight
- Plasmid sequences were confirmed by dideoxy sequencing and phylogenetic analysis
- Taqman Real-time PCR using 4 sets of primers (2 LTR, 1 protease and 1 integrase) was used to detect the HIV-2 clones

List of HIV-2 isolates and specific primers used to generate 12 *LTR* and 12 *pol* plasmids

Isolate	Subtype	Gene	Insert Size	Vector	Primers (5'-3') Forward	Primers (5'-3') Reverse
310072	B	LTR	984	pCR™ 4-TOPO® TA	TGGAAGGGATTTACTATAGTGAGAGA	TCCCGCTCCTCACGCTG
		Pol	3043		GGAAAGAAGCCTCGCAACTT	GGCACTACAATCCAATTCTT
A2270	A	LTR	849	pCR2.1®-TOPO® TA	TGGAAGGGATGTTTTACAGTGAG	GGATTTTCCTGCCTTGGTTT
		Pol	2945	pCR™ 4-TOPO® TA	CAACAGCACCCCCAGTAGAT	ATC TTGGCTTTCCTRCTTGG
310319	B	LTR	897	pCR™ 4-TOPO® TA	TGGAAGGGATTTTTTATAGTGAAAGAAGAC	CAGGAAAATCCCTAGCAGGTTG
		Pol	3174		GGAAAGAAGCCTCGCAACTT	TGCAAGTCCACCAAGCCCAT
SLRHC	A	LTR	849	pCR™ 4-TOPO® TA	TGGAAGGGATGTTTTACAGTGAG	GGATTTTCCTGCCTTGGTTT
		Pol	2957		CAACAGCACCCCCAGTAGAT	ATAGTCRRTGATGATCTTYGCRTTCCT
7924A	A	LTR	872	pCR™ 4-TOPO® TA	TGGAAGGGATTTTTTATAGTGAAAGAAGAC	GGATTTTCCTGCCTTGGTTT
		Pol	2995		CAACAGCACCCCCAGTAGAT	CCAAGTGGGAACCACTATCC
A2267	A	LTR	849	pCR2.1®-TOPO® TA	TGGAAGGGATGTTTTACAGTGAG	GGATTTTCCTGCCTTGGTTT
		Pol	2945	pCR™ 4-TOPO® TA	CAACAGCACCCCCAGTAGAT	ATC TTGGCTTTCCTRCTTGG
77618	A	LTR	849	pCR™ 4-TOPO® TA	TGGAAGGGATGTTTTACAGTGAG	GGATTTTCCTGCCTTGGTTT
		Pol	2945		CAACAGCACCCCCAGTAGAT	CCAAGTGGGAACCACTATCC
A1958	A	LTR	862	pCR™ 4-TOPO® TA	TGGAAGGGATTTTTTATAGTGAAAGAAGAC	TGCTAGGGATTTTCCTGCCTCCGTTTC
		Pol	2957		CAACAGCACCCCCAGTAGAT	ATAGTCRRTGATGATCTTYGCRTTCCT
7312A	A/B*	LTR	872	pCR™ 4-TOPO® TA	TGGAAGGGATTTTTTATAGTGAAAGAAGAC	CAACCTGCTAGGGATTTTCCTG
		Pol	3235		AGCCAAGCAATGCAGGGCTCCTAG	GTTGCAATTCTCCTGTTCTATGCTTCAGAT
GB87	A	LTR	849	pCR2.1®-TOPO® TA	TGGAAGGGATGTTTTACAGTGAG	GGATTTTCCTGCCTTGGTTT
		Pol	2945	pCR™ 4-TOPO® TA	CAACAGCACCCCCAGTAGAT	ATC TTGGCTTTCCTRCTTGG
GB122	A	LTR	849	pCR2.1®-TOPO® TA	TGGAAGGGATGTTTTACAGTGAG	GGATTTTCCTGCCTTGGTTT
		Pol	2995		CAACAGCACCCCCAGTAGAT	CCAAGTGGGAACCACTATCC
60415K	A	LTR	849	pCR™ 4-TOPO® TA	TGGAAGGGATGTTTTACAGTGAG	GGATTTTCCTGCCTTGGTTT
		Pol	2995		CAACAGCACCCCCAGTAGAT	CCAAGTGGGAACCACTATCC

Real-time PCR assay for detection of HIV-2 DNA. Data show the average Ct values of all 12 plasmid dilution panels amplified by 4 sets of primers/probes.



Results/Conclusion

- Twelve LTR and 12 Pol DNA plasmids from 12 HIV-2 isolates were generated
- All 24 plasmid sequences were subtype confirmed
- Real-time PCR detected less than 100 copies of target DNA using our in-house HIV-2 assay, except for primer set LTR1 which detected 1000 copies of SLRHC LTR plasmid due to sequence mismatch in the probe region
- This panel of HIV-2 plasmid provides a great resource for HIV-2 assay development
- These reagents are easily quantifiable, renewable, and non-infectious

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