Human immunodeficiency virus (HIV) proviral DNA detection through end-point PCR.

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This protocol describes the optimized end-point PCR component and conditions for the detection of integrated proviral sequences of the human immunodeficiency virus type 1. This method relies on a nested PCR approach in which the product of an initial PCR is used as a template for a second PCR using different (nested) oligonucleotides. This approach is more sensitive than single-pass PCRs and as such requires greater care and discipline to avoid contamination throughout setup.

Oligonucleotides

Name	PCR	Sequence*	bp	%GC	Tm	Position †	Amplicon	Ref.
HIV-FO	1	5'-TAC-Agg-AgC-AgA-TgA-TAC-Ag-3'	20	45	50	141-161	294	1
HIV-RO		5'-CCT-ggC-TTT-AAT-TTT-ACT-gg-3'	20	40	48	418-438		
HIV-FI	2	5'-ggA-AAC-CAA-AAA-TgA-TAg-gg-3'	20	40	48	221-241	130	
HIV-RI		5'-ATT-ATg-TTg-ACA-ggT-gTA-gg-3'	20	40	48	331-351		

***** Reverse oligonucleotide primer sequences given in this table are the reverse-complement of the sequence present in the alignments and as they should be ordered for synthesis.

† Primer binding sites given on table are based on HXB2 reference sequence.

Components

First PCR		1 rx (µl)
dH_2O	C*f*	5.19
10x Buffer PCR	1X	1.25
$MgCl_2$ 50 mM	3.0 mM	0.75
4x dNTPs 10 mM	200 µM	0.25
2x Oligos 10 µM	800 nM	1.00
Taq 5 UI/µL	0.02 UI/µL	0.063
gDNA	-	4.00
	Vf	12.5
	↓	
	Run HIV1	

Second (nested) PCR		1 rx (µl)
dH_2O	C*f*	8.44
10x Buffer	1X	1.25
$MgCl_2$ 50 mM	2.0mM	0.5
4x dNTPs 10 mM	200 µM	0.25
2x Oligos 10 µM	800 nM	1
Taq 5 UI/µL	0.02 UI/µL	0.063
1^{st} PCR product	-	1.00
	Vf	12.5
	↓	
	Run HIV2	

Conditions (Total estimated time: 3 hrs)

Time: 1:32 hrs		Denaturing		Annealing	Extension		
HIV1	Temperature	94°	94°	55°	72°	72°	4°
	Time	2 min	15 seg	15 seg	15 seg	2 min	5 min
			x30 cycles				

Time: 1:32 hrs		Denaturing		Annealing	Extension		
HIV2	Temperature	94°	94°	50°	72°	72°	4°
	Time	2 min	15 seg	15 seg	15 seg	2 min	5 min
			x30 cycles				

Notes

1. Clean workbench with 0.1% NaOCl 0.1% followed by 70% Ethanol before and after work.
2. Preparation of RT mastermix should only be performed in the RT-PCR room.
3. Preparation of PCR mastermix and addition of sample DNA should only be performed in the pre-PCR enclosure or area.
4. Addition of positive template DNA should be performed on instrument (post-PCR) area.
5. All mastermixes should be prepared on ice to prevent excess evaporation.
6. Vortex and spin all mastermixes before and after aliquoting to PCR tubes.
7. For the first PCR distribute 8.5 µL of the mastermix to each tube and then add 4 µL of genomic DNA (± 100 ng/µL.
8. For the second PCR distribute 11.5 µL of mastermix to each tube and then add 1 µL of undiluted 1st PCR product.

References

1. Albert, J. Simple, sensitive, and specific detection of Human Inmunodeficiency Virus type 1 in clinical specimens by polymerase chain reaction with nested pimers. *Journal or Clinical Microbiology* **28**, 1560 - 1564 (1990).

Revision history

1.0 Original document.

2.0 Optimized protocol.

3.0 Optimized protocol

4.0 Changes to document format only.