

Human Immunodeficiency Virus (HIV) integrase region (pol gene) amplification & sequencing.

Created: Oct 26, 2010; Last modified: Mar 23, 2021, Version: 3.0

This protocol describes the nested RT-PCR approach to integrase region (pol gene) amplification and sequencing using integrated proviral DNA or viral RNA as template. This protocol was created to allow for the characterisation of anti-retroviral drug resistance-mutations. The nested strategy allows for better success at sequencing from samples having either low quality, concentration of template or viral load (in the case of RT-PCR). The fragment thus generated partially overlaps on the 5' end with the 3' end of the second reverse transcriptase (RTb) encoding fragment, allowing together with that of the first reverse transcriptase (RTa) and protease encoding fragments for the assembly of full-pol region contigs.

Oligonucleotides

Name	PCR	Sequence*	bp	%GC	Tm	Position †	Amplicon	Ref.
Int-FO	1 a4	5'-CAC-ACA-ARg-gRA-TTg-gRg-gRA-ATg-3'	24	50	58.4	4177-4200	1046 bp	
Int-RO	1st	5'-TAR-Ygg-gAT-gTg-TAC-TTC-TgA-AC-3'	23	43.5	54.3	5210-5223	1040 bp	1
Int-FI	2nd	5'-AAC-AAg-TAg-ATA-ART-TAg-TMA-gT-3'	23	26.1	46.9	4201-4223	994 bp	1
Int-RI	2nd	5'-ATA-CAT-ATg-RTR-YTT-TAC-TAR-RCT-3'	24	27.1	48.2	5122-5195	99 4 0p	

^{*} 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.



RT-PCR components and conditions

Using Vivantis M-MuLV RT enzyme (Cat: ME2305) and Vivantis Taq DNA Pol (Cat: PL1202).

First strand synthesis (RT)

	,	
	cf	1x
dH_2O		2.5 μL
10 μM Forward oligo	1.125 μM	2.3 μL
10 μM Reverse oligo	1.125 μM	2.3 μL
10 mM dNTPs 10 mM	250 μΜ	0.5 μL
RNA		10 μL
		vf: 17.6 μl

RT-1 program in Axygen TC-1				
Total time: 6 min				
95 ℃	2 min	1 avala		
4 °C	2 min	1 cycle		

		<u> </u>
RT Buffer	1x cf	2 μL
RT Enzyme 250 IU/µl	5 IU/μL	0.4 μL
		vf: 20 μl
		J

	Ψ
Run RT-2 program is	n Axygen TC-1

RT-2 program in Axygen TC-1				
Total time: 1:12 hrs				
38 °C	60 min			
95 ℃	5 min	1 cycle		
4 °C	5 min			

1st Polymerase Chain Reaction (PCR)

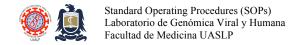
	cf	1x
dH_2O	$\mathbf{C}f$	6.02
10x Buffer PCR	1x	1.25
MgCl ₂ 50 mM	1.50 mM	0.38
dNTPs 10 mM	200 μΜ	0.25
Oligos 10 μM	400 nM	0.50
Taq (Vivantis) 5 UI/μL	0.04 UI/μL	0.10
DNA	-	4.00
		vf: 12.5 μl

Run 1 st PCR in Axygen cycler				
Total time: 1:30 hrs				
95 ℃	3 min			
95 °C	30 sec			
56 °C	30 sec	30 cycles		
72 °C	1 min			
72 °C	5 min			

2nd Polymerase Chain Reaction (PCR)

	cf	1x
dH_2O	$\mathbf{C}f$	9.02
10x Buffer PCR	1x	1.25
MgCl ₂ 50 mM	1.50 mM	0.38
dNTPs 10 mM	200 μΜ	0.25
Oligos 10 μM	400 nM	0.50
Taq (Vivantis) 5 UI/μL	0.04 UI/μL	0.10
1 st PCR product	-	1.00
		vf: 12.5 µl

Run 2 nd PCR in Axygen cycler				
Total time: 1:30 hrs				
95 ℃	1 min			
95 ℃	30 sec			
50 °C	30 sec	30 cycles		
72 °C	1 min			
72 °C	5 min			





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.

References

1. Swanson P, Devare SG, Hackett J Jr. Molecular characterization of 39 HIV isolates representing group M (subtypes A-G) and group O: sequence analysis of gag p24, pol integrase, and env gp41. AIDS Res Hum Retroviruses. 2003 Jul;19(7):625-9. PubMed PMID: 12921095.

Revision history

- 1.0 Original document.
- 2.0 Formatting changes to oligonucleotide sequence.
- 3.0 Changes to document format only.

