HIV drug resistance test(In-house)

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Abstract

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Protocol

Primer sequence

Step 1.

Table 1. Primers for detecting drug resistance

Primer name	Sequence	Location (HXB2)	Direction
MAW-26	5′-TGGAAATGTGGA AAGGAAGGA C-3′	2027—2050	Upstream of flanking region
RT-21	5′-CTGTATTTCTGCTATTAAGTCTTTTGA -3′	3509—3539	Downstream of flanking region
PRO-1*	5’-CAGAGCCAACAGCCCCACCA-3’	2147—2166	Upstream of sequence (forward)
RT-20*	5′-CTGCCAGTTCTAGCTCTGCTTC -3′	3441—3462	Downstream of sequence (reverse)
RT4R (backup)*	5’-CTTCTGTATATCATTGACAGTCCAGCT-3’	3300—3326	Downstream of sequence (reverse)
RT1*	5′-CCAAAAGTTAAACAATGGCCATTGACAGA-3′	2604—2632	Forward
PROC1*	5’-GCTGGGTGTGGTATTCC-3’	2826—2842	Reverse

*indicates sequencing primers.

The first round reaction system of reverse transcription and nested amplification

Step 2.

10×One Step RNA PCR Buffer 2.5μl, MgCl2 (25mM) 5μl, dNTP Mixture (10mM) 2.5μl, RNaseInhibitor (40U/μl) 0.5μl, AMVRTaseXL (5U/μl) 0.5μl, AMV-Optimized Taq (5U/μl) 0.5μl, RT-21 (20μM)0.5μl, MAW-26 (20μM) 0.5μl, RNA template according to the load value add 1 to 10μl, added RNase Free dH2O to 25μl.

Amplification conditions of the first round

Step 3.

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Prereact PCR reaction tube added the RNA template 94℃ 2 min, and 50℃ 5min, the configuration of the reaction solution quickly joined in the process, and then reverse transcription 30min 50℃, then 94℃ 2 min to inactivated reverse transcriptase. Then 94℃ 30s, 55℃ 30s, 72℃ 150s ,30 cycles, and extend 10 min at 72℃ to complete the first round reaction.

The nested second round amplification reaction system

Step 4.

In the first round of PCR products as template for the nested second round amplification reaction system are as follows: 10xPCR Buffer 2.5μL, dNTP Mixture (10mM) 0.5μl, PRO-1 (20μM) 0.5μl, RT-20 (20μM) 0.5μl, Taq enzyme (5U/μl) 0.25μl, the template 3μl, add RNase Free dH2O to 25μl.

Amplification conditions of the second round

Step 5.

The reaction procedure is 94℃, pre denatured 5min, 94℃30s, 63℃30s, 72℃ 150s, 30 cycles, and 72℃10min. Two rounds of amplification are expected to have a target size of about 1300bp.

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