Multiplex PCR screening of HBV, HIV and CMV.

Created: Aug 27, 2010; **Last modified**: Mar 23, 2021, **Version:** 2.0

This protocol describes the PCR components and conditions used for the screening of three blood-borne pathogens through multiplexed nested endpoint PCR. This protocol allows for the identification of human immunodeficiency virus (HIV-1), hepatitis B virus (HBV) and human cytomegalovirus (CMV). It includes a KIR3DL2 framework gene inner control of larger size than target amplicons.

Oligonucleotide primers used

Name	Target	PCR	Sequence	bp	%GC	Tm[b]	Position	Amplicon	Ref
HIV-FO	HIV-1	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		
HBV-FO	HBV	1	5'-CAC-CAT-gCA-ACT-TTT-TCA-CCT-CTg-C-3'	25	48	58	1810-1835	561	2
HBV-RO			5'-TCT-gCg-Agg-CgA-ggg-AgT-TCT-3'	21	62	58	2375-2396		3
HBV-FI		2	5'-AAg-CCT-CCA-AgC-TgT-gCC-TTg-g-3'	21	57	56	1866-1887	426	4
HBV-RI			5'-gCA-ggA-ggA-gTg-CgA-ATC-CAC-AC-3'	23	61	61	2266-2289		
CMV-FO2	CMV	1	5’-gAA-TTC-gCg-CAT-gAT-CTC-3’	18	50	52	912-929	815	5
CMV-RO2			5’-ggA-AAC-gTg-TCC-gTC-TT-3’	17	53	52	1711-1727		
CMV-FI2		2	5’-gCg-AgT-AAA-gTT-CCA-gTA-3’	18	44	49	968-985	719	4
CMV-RI2			5’-gTT-CTg-gCA-Agg-YA-3’	14	57	49	1675-1687		
F3DL2	3DL2	1	5’-TCA-TgC-TgT-ACA-AAg-AAg-ACA-gAA-g-3’	25	40	54	E3(45)	1713	7
R3DL2			5’-ATg-ACT-gTC-TCT-CCT-gAT-TTC-Ag-3’	23	43	53	E4(113)		
3DL2FI		2	5’-TAC-AgA-TgT-Cgg-ggT-TCA-Cg-3’	20	55	57	E3	1500	
3DL2RI			5’-ggA-ggg-Aag-gTT-TTC-TgT-ggT-TTC-3’	24	50	58	E4		

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PCR components

First PCR		1 rx (µl)
dH_2O	C*f*	1.4
10x Buffer PCR	1X	1.25
$MgCl_2$ 50 mM	3.0 mM	0.75
4x dNTPs 10 mM	200 µM	0.25
Oligos HIV-ext. 10 µM	800 nM	1
Oligos HBV-ext 10 uM	400 nM	0.5
Oligos CMV-ext. 10 µM	800 nM	1
Oligos KIR3Dl2-ext. 10 µM	200 nM	0.25
Taq 5 UI/µL	0.4 IU/µL	0.1
cDNA	-	6
	Vf	12.5
	↓	
	Run Multiplex 1	

Second (nested) PCR		1 rx (µl)
dH_2O	C*f*	12.8
10x Buffer	1X	2.5
$MgCl_2$ 50 mM	2.0mM	1.0
4x dNTPs 10 mM	200 µM	0.5
Oligos HIV-int 10 µM	800 nM	2.0
Oligos HBV-int 10 uM	400 nM	1.0
Oligos CMV-int 10 µM	800 nM	2.0
Oligos KIR3Dl2-int 10 µM (1/8)	50 nM	1.0
Taq 5 UI/µL	0.4 IU/µL	0.2
1:10 diluted 1st PCR product	-	2.0
	Vf	25.0
	↓	
	Run Multiplex 2	

PCR conditions

Total time: 1:58 hrs		Denaturing		Annealing	Extension		
Multiplex 1	Temperature	94°	94°	62°	72°	72°	4°
	Time	5 min	30 sec	30 sec	30 sec	5 min	5 min
			x30 cycles				

Total time: 1:58 hrs		Denaturing		Annealing	Extension		
Multiplex 2	Temperature	94°	94°	54°	72°	72°	4°
	Time	5 min	30 sec	30 sec	30 sec	5 min	5 min
			x30 cycles				

Electrophoresis conditions

Add 5 µL of orange loading buffer to 25 µL of 2nd PCR, load 25µL into 1.5% agarose gel with at least 1.5 inch comb separation for 55 minutes at 130 VDC (5.2 V/cm).

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Notes

1. Clean work area with 70% Ethanol before and after preparing PCR master mix.

2. Prepare all PCRs in ice or cold-pack microtube racks to lower static evaporative rate and volume losses.

3. Vortex master mix before aliquoting into individual microtubes.

4. Prepare 1st and 2nd PCR mastermix in PCR enclosures. After cycling 1st PCR open tubes in post-PCR area ONLY and add 125 µL of dH_2O to dilute PCR product 1:10 for use in 2^{nd} (nested) PCR.

References

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Revision history

1.0 Original document.

2.0 Changes to document format only.

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