Hepatitis B virus (HBV) detection through end-point PCR.

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This protocol describes the PCR components and conditions used in the molecular screening for Hepatitis B virus (HBV) sequences through end-point PCR. 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.
HBV-FO	1	5'-CAC-CAT-gCA-ACT-TTT-TCA-CCT-CTg-C-3'	25	48	58	1810-1835	561	1
HBV-RO		5'-TCT-gCg-Agg-CgA-ggg-AgT-TCT-3'	21	62	58	2375-2396		2
HBV-FI	2	5'-AAg-CCT-CCA-AgC-TgT-gCC-TTg-g-3'	21	57	56	1866-1887	426	3
HBV-RI		5'-gCA-ggA-ggA-gTg-CgA-ATC-CAC-AC-3'	23	61	61	2266-2289		

***** 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.

PCR components and conditions

Using Vivantis Taq DNA Polymerase (Thermus aquaticus) enzyme (Cat: PL1202 or PL1204).

1st Polymerase Chain Reaction (PCR)

	cf	1x
dH_2O	C*f*	5.19
10x Buffer PCR	1X	1.25
$MgCl_2$ 50 mM	3.0 mM	0.75
dNTPs 10 mM	200 µM	0.25
Oligos 10 µM	800 nM	1
Taq (Vivantis) 5 UI/µL	0.02 UI/µL	0.063
DNA	-	4.00
		vf: 12.5 µl

➔

Run HBV1 in Axygen cycler		
Total time: 1:30 hrs		
94 ºC	2 min	
94 ºC	15 sec	30 cycles
57 ºC	15 sec	
72 ºC	15 sec	
72 ºC	2 min	

2nd Polymerase Chain Reaction (PCR) both fragments

	cf	1x
dH_2O	C*f*	8.44
10x Buffer PCR	1X	1.25
$MgCl_2$ 50 mM	2.0mM	0.5
dNTPs 10 mM	200 µM	0.25
Oligos 10 µM	800 nM	1
Taq (Vivantis) 5 UI/µL	0.02 UI/µL	0.063
1st PCR product	-	1.00
		vf: 12.5 µl

➔

Run HBV2 in Axygen cycler		
Total time: 1:30 hrs		
94 ºC	2 min	
94 ºC	15 sec	30 cycles
60 ºC	15 sec	
72 ºC	15 sec	
72 ºC	2 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.
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. Aslam MA, Identification of Hepatitis B virus core mutants by PCR-RFLP in chronic Hepatitis B patients from Punjab, Pakistan. *Arch Virol* (2007).

2. Dia Sorin, Saluggia, Italia (comunicación personal).

3. CA García & SE Guerra, Laboratorio de Biologia Molecular, Facultad de Medicina UASLP México, 2008.

Revision history

1.0 Original document.

2.0 Thermal cycling conditions modified.

3.0 Optimized protocol.

4.0 Optimized components.

5.0 Changes to document format only.