Recommended Protocol for HBV genotyping and for antiviral resistance analysis.

This amplification protocol is based on a nested PCR. The final amplicon is approximately 1kb and will cover the entire HBsAg region as well as domains A to E of the overlapping polymerase region.

All primer sequences shown are 5'-3'.

Outer sense:	HBV Z - AGC CCT CAG GCT CAG GGC ATA
Outer antisense:	HBV 3 – CGT TGC CKD GCA ACS GGG TAA AGG
Inner sense:	HBV P - TCA TCC TCA GGC CAT GCA GT
Inner antisense:	HBV M- GAC ACA CTT TCC AAT CAA TNG G

First Round PCR

PCR Master mix per sample: (All reagents are from Invitrogen)

2.5µl	10XPCR Buffer
0.75µl	50mM Mg Cl_2
0.5µl	10mM dNTPS
0.1µl	Taq Polymerase
0.5µl	HBV Z (20pmol/µl)
0.5 µl	HBV 3 (20pmol/µl)
15.15µl	dH_2O

Add 5µl viral DNA to 20µl PCR Master Mix.

Cycling conditions

95^0C	5 Minutes	
94^0C	30 Seconds	34 Cycles
55^0C	30 Seconds	
72^0C	1 Minute	
72^0C	2 Minutes	

Second Round PCR

PCR Master mix per sample:

5µl	10XPCR Buffer
1.5µl	50mM Mg Cl_2
1µl	10mM dNTPS
0.2µl	Taq Polymerase
1µl	HBV P (20pmol/µl)
1µl	HBV M (20pmol/µl)
39.3µl	dH_2O

8.3 Add 1µl first round product to 49µl PCR Master Mix.

Cycling conditions

95^0C 5 Minutes

94^0C 30 Seconds
50^0C 30 Seconds
72^0C 1 Minute
} 34 Cycles

72^0C 7 Minutes

Sequencing PCR

Sequencing reactions should be set up in accordance with the instructions provided by the manufacturer.

NB – Each sample second round amplicon is analysed using each of the following primers in individual reactions:

HBV P - TCA TCC TCA GGC CAT GCA GT

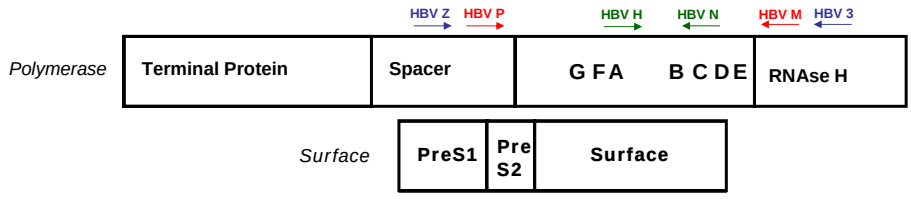
HBV M- GAC ACA CTT TCC AAT CAA TNG G

HBV N- ACTGAGCCAGGAGAAACGGACTGAGGC

HBV H- TATCAAGGAATTCTGCCCGTTTGTCCT

Comment

The use of a nested approach facilitates sequencing of low level HBV DNA such as is found in the anti-HBe seropositive individual. The four sequencing reactions allow secure sequence data for assembly of the small HBs contig.



Primer Name	Position (numbered in accordance with Pugh et al., 1986)
HBV Z	1179-1199
HBV 3	2478-2455
HBV P	1292-1311
HBV M	2306-2287
HBV N	1991-1965
HBV H	1767-1793

Outer primers

Inner primers

Additional sequencing primers