

**Table S1.** Sensitivity and specificity of SL $\alpha$  covariance model in detection of HBV.<sup>a</sup>

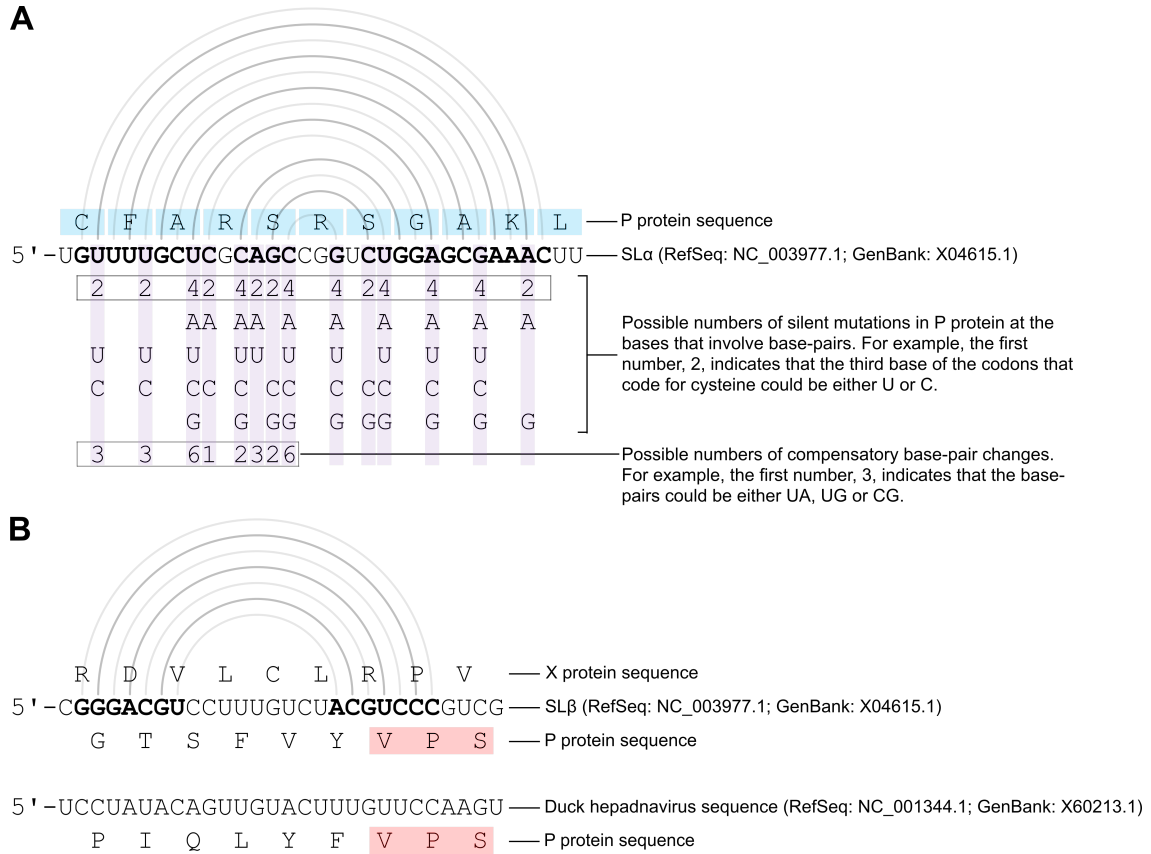
		True positive	True negative	False positive	False negative	Sensitivity	Specificity	Accuracy	Truncated hit	MER score <sup>b</sup>	Score threshold
Training (5-fold cross-validation)	1	1034	5009	0	4	99.6	100	99.9	0	4	28.7
	2	1034	5622	0	4	99.6	100	99.9	0	4	28.7
	3	1033	6405	0	6	99.4	100	99.9	0	6	29.0
	4	1031	5724	0	9	99.1	100	99.9	0	9	28.7
	5	1035	5192	0	5	99.5	100	99.9	0	5	28.7
Testing		1290	6969	0	10	99.2	100	99.9	0	10	28.7

**Table S2.** Sensitivity and specificity of SL $\beta$  covariance model in detection of HBV.<sup>a</sup>

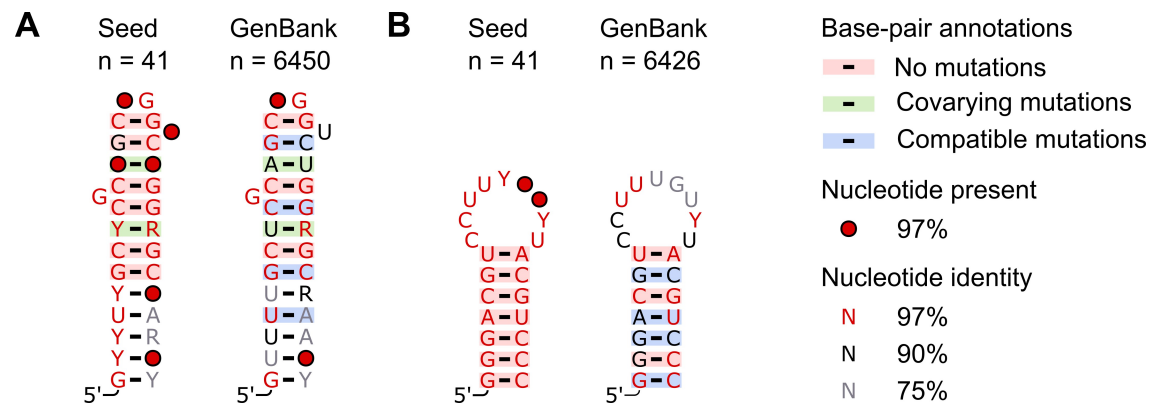
		True positive	True negative	False positive	False negative	Sensitivity	Specificity	Accuracy	Truncated hit	MER score <sup>b</sup>	Score threshold
Training (5-fold cross-validation)	1	1033	5009	0	5	99.5	100	99.9	0	5	33.1
	2	1031	5622	0	7	99.3	100	99.9	0	7	33.1
	3	1028	6405	0	11	98.9	100	99.9	0	11	33.2
	4	1030	5724	0	10	99.0	100	99.9	0	10	33.1
	5	1032	5192	0	8	99.2	100	99.9	0	8	33.1
Testing		1292	6969	0	8	99.4	100	99.9	0	8	33.1

<sup>a</sup> Complete genomes of 6495 *Orthohepadnavirus* and 34921 other viruses (retrieved from GenBank viral division at 24 February 2016).

<sup>b</sup> Minimum error rate (MER) score: total number of false positive and false negative.<sup>1,2</sup>



**Figure S1.** The constraints of P and X reading frames on the base-pairs in SLα and SLβ (RefSeq: NC\_003977.1; GenBank: X04615.1). **(A)** SLα is located within the P reading frame (blue shading). There are a total of 3888 possible sequence variants that allow compensatory base-pair changes while preserving the P amino acid sequence ( $3 \times 3 \times 6 \times 1 \times 2 \times 3 \times 2 \times 6 = 3888$ ; purple shading). **(B)** SLβ is located within the overlapping reading frames of P and X proteins. The stem of SLβ would be expected to lack compensatory base-pair changes. The conserved functional motif of RNase H domain of P proteins of mammalian and duck (RefSeq: NC\_001344.1; GenBank: X60213.1) hepadnaviruses is highlighted in pink.<sup>3</sup> Bases that involve in base-pairs are indicated in boldface. Base-pairs are indicated as arcs. It should be noted that on 23 October 2015, NC\_003977.1 (genotype C; GenBank: X04615.1) was replaced with a different HBV genome, NC\_003977.2 (genotype D; GenBank: V01460.1).



**Figure S2.** RNA consensus diagrams for (A) SL $\alpha$  and (B) SL $\beta$  based on seed sequences (covariance models) or all mammalian hepadnaviruses in GenBank (retrieved at 24 February 2016). Default settings of R2R 1.0.4 were used.

## References

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2. Zhou Y, Lu C, Wu Q-J, Wang Y, Sun Z-T, Deng J-C, Zhang Y. GISSD: Group I Intron Sequence and Structure Database. *Nucl Acids Res* 2008; 36:D31–7; PMID:17942415;  
<http://dx.doi.org/10.1093/nar/gkm766>
3. Chen Y, Marion PL. Amino acids essential for RNase H activity of hepadnaviruses are also required for efficient elongation of minus-strand viral DNA. *J Virol* 1996; 70:6151–6; PMID:8709240