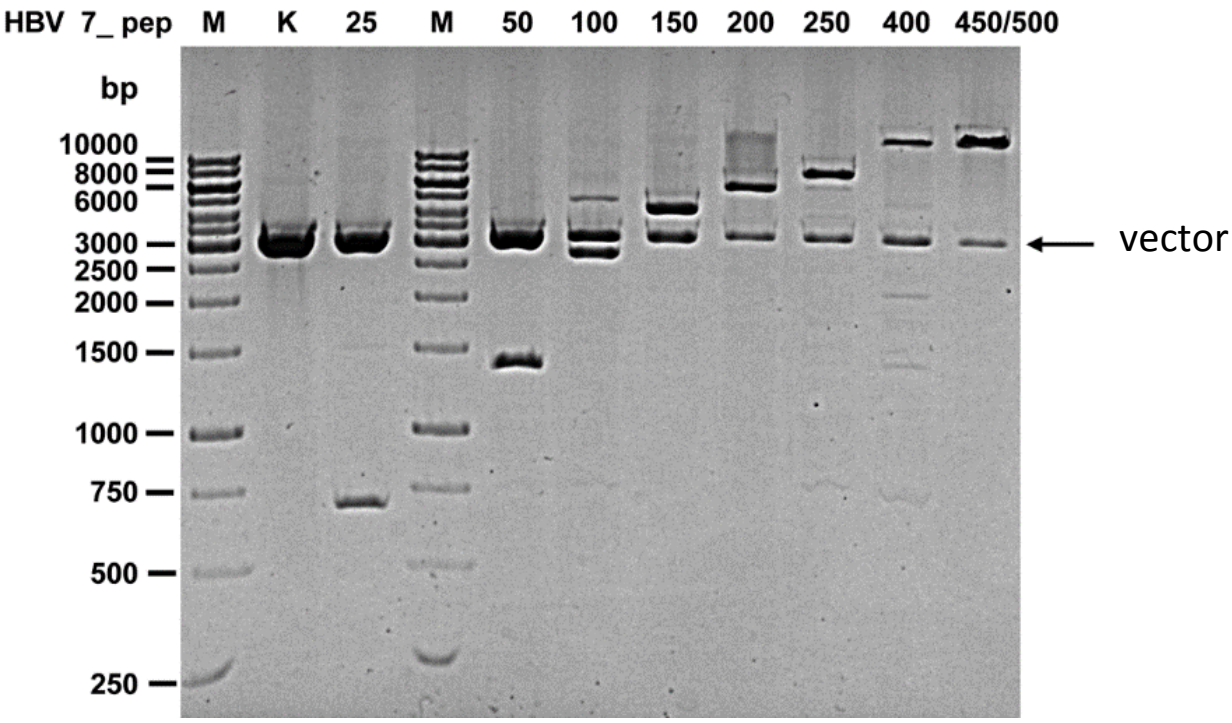
Validation of an epitope-coding DNA segment amplification technology: obtaining 500 copies of the 7-aminoacid HBV within a single ORF.



Second round of the in vitro amplification reaction of 25-mer concatamer of 7-aminoacid model HBV epitope.

The epitope mini-gene amplification reaction can be regulated to yield polyproteins ranging from 2-mer polyepitopic protein to app. 500-mer or more by varying parameters such as: (i) modifying ligation conditions by inclusion of linear polymers, (ii) changing ligase concentration, (iii) changing buffer/salt composition and concentration, (iv) modifying ligation reaction temperature and duration, (v) conducting of pre-ligation of SapI-excised epitope gene prior to addition of amplification vector or (vi) chemical synthesis of an oligonucleotide monomer to be amplified, containing several copies of an epitope gene, among others. Figure shows results of the second round amplification reaction of 25-mer and amplification products cloning, analysed by SapI excision of inserts from separated clones. M, DNA molecular size marker; K, amplification vector without an insert; 25, insert containing 25-mer of HBV epitope; 50, 50-mer; 100, 100-mer; 150, 150-mer; 200, 200-mer; 250, 250-mer; 400, 400-mer; 450/500, 450-500-mer (the precise insert length could not be determined on the agarose gel used). The vector-insert junctions and ORF integridy were verified by DNA sequencing.