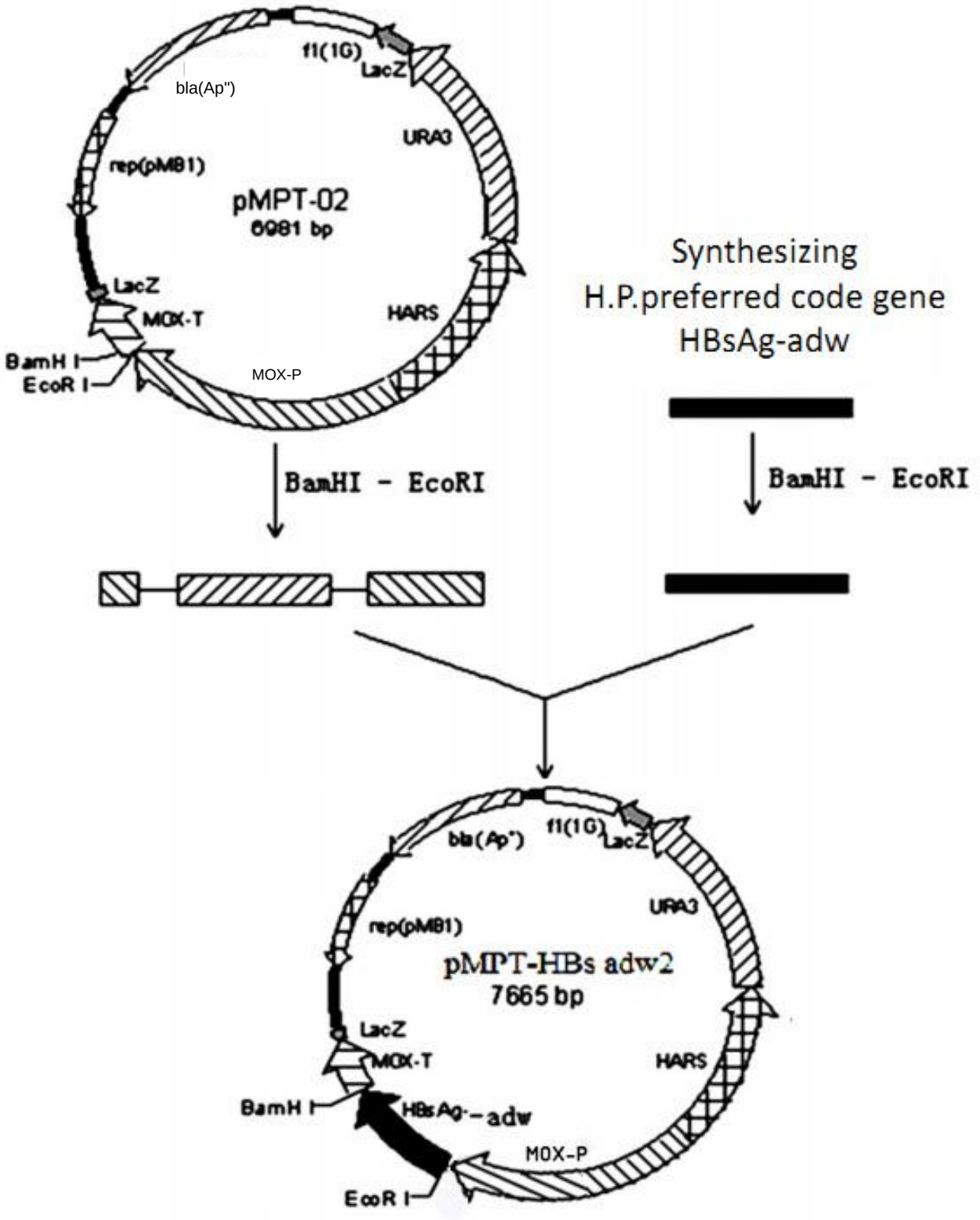

f1(1G)
LacZ
bla(Ap")
URA3
rep(pMB1)
pMPT-02
6981 bp
LacZ
MOX-T
HARS
BamH I
EcoR I
MOX-P
Synthesizing
H.P.preferred code gene
HBsAg-adw
BamHI - EcoRI
BamHI - EcoRI
bla(Ap')
f1(1G)
LacZ
URA3
rep(pMB1)
pMPT-HBs adw2
7665 bp
LacZ
MOX-T
HARS
BamH I
HBsAg-adw
MOX-P
EcoR I

FIG. 1

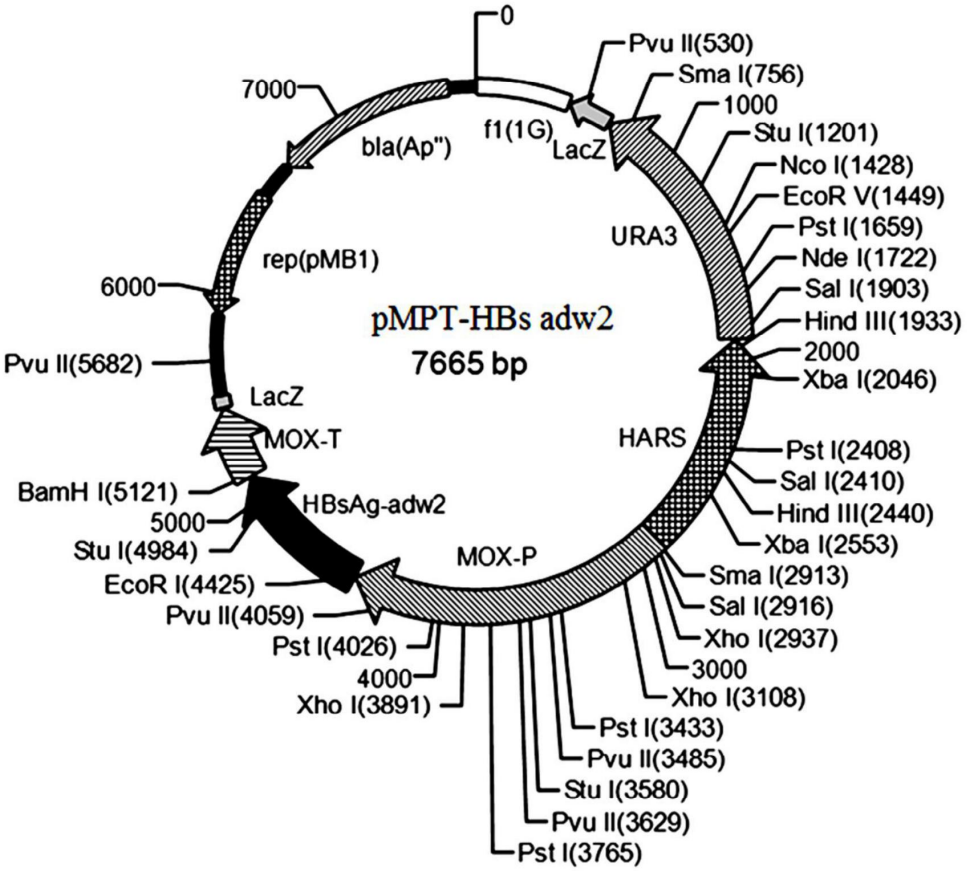

0
Pvu II(530)
Sma I(756)
1000
Stu I(1201)
Nco I(1428)
EcoR V(1449)
Pst I(1659)
Nde I(1722)
Sal I(1903)
Hind III(1933)
2000
Xba I(2046)
Pst I(2408)
Sal I(2410)
Hind III(2440)
Xba I(2553)
Sma I(2913)
Sal I(2916)
Xho I(2937)
3000
Xho I(3108)
Pst I(3433)
Pvu II(3485)
Stu I(3580)
Pvu II(3629)
Pst I(3765)
4000
Xho I(3891)
Pst I(4026)
Pvu II(4059)
EcoR I(4425)
Stu I(4984)
5000
BamH I(5121)
LacZ
MOX-T
HBsAg-adw2
MOX-P
HARS
URA3
LacZ
f1(1G)
bla(Ap")
7000
rep(pMB1)
6000
Pvu II(5682)
pMPT-HBs adw2
7665 bp

FIG. 2

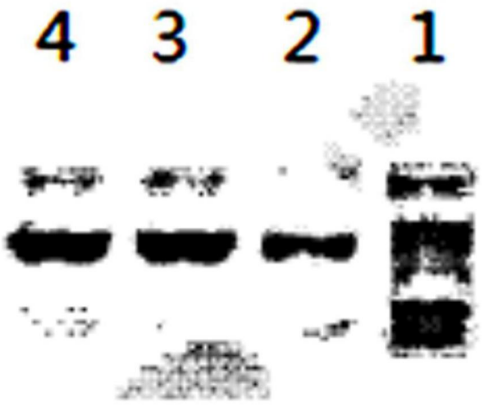


FIG. 3

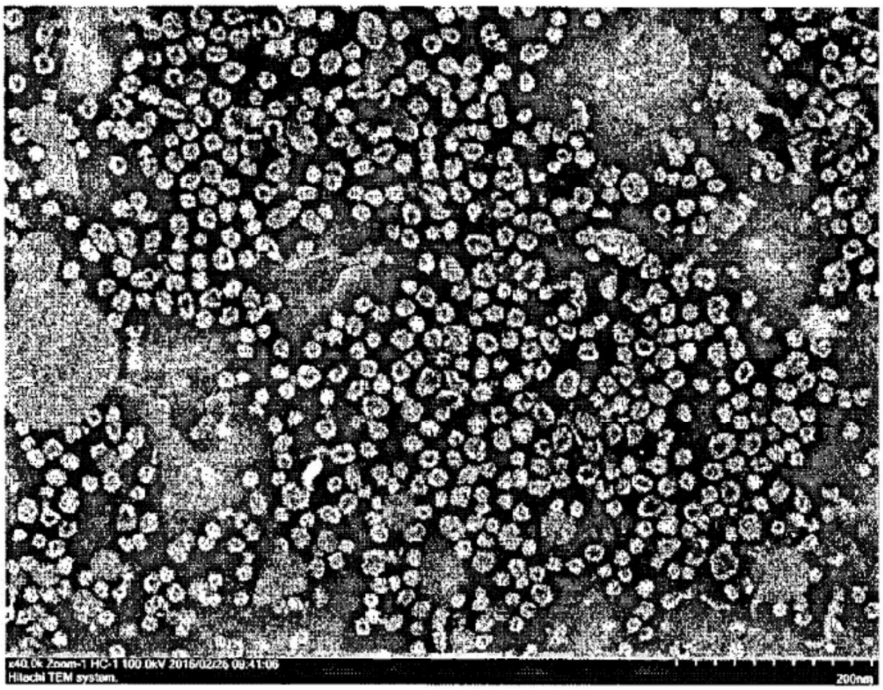
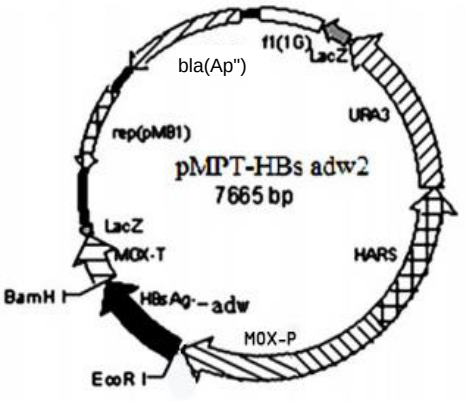

x40.0k Zoom-1 HC-1 100.0kV 2018/02/26 09:41:09
Hitachi TEM system.
200nm

FIG. 4



Uracil-deficient Hansenula Genetically engineering host strain HU-11

cell electroporation transformation

selection medium for plate culture

Selecting a single colony of uracil prototrophic transformants

The growth rate of the bacteria is obviously accelerated, and PCR detection of HBsAg gene band length

Selection medium shake flask culture Continuous subculture for 20 to 400 generations

Screening multiple copies of heterologous integrated transformed clones

RIA detects HBsAg expression levels

Methanol induction culture for 72 hours

Screening for HBsAg high expression level transformed clones

Quantitative PCR for detection of HBsAg gene copy number
RIA detects HBsAg expression levels

YPD complete medium shake flask culture for 48 hours
Transformation selection medium plate cloning culture

Screening out high-copy, high-expression clones of free plasmids

Enzymatic cleavage map analysis of HBsAg gene
DNA sequencing analysis of HBsAg gene

Selection medium shake flask culture

Selection of genetically stable primary recombinant strains of the recombinant Hansenula HBsAg engineering strain

FIG. 5