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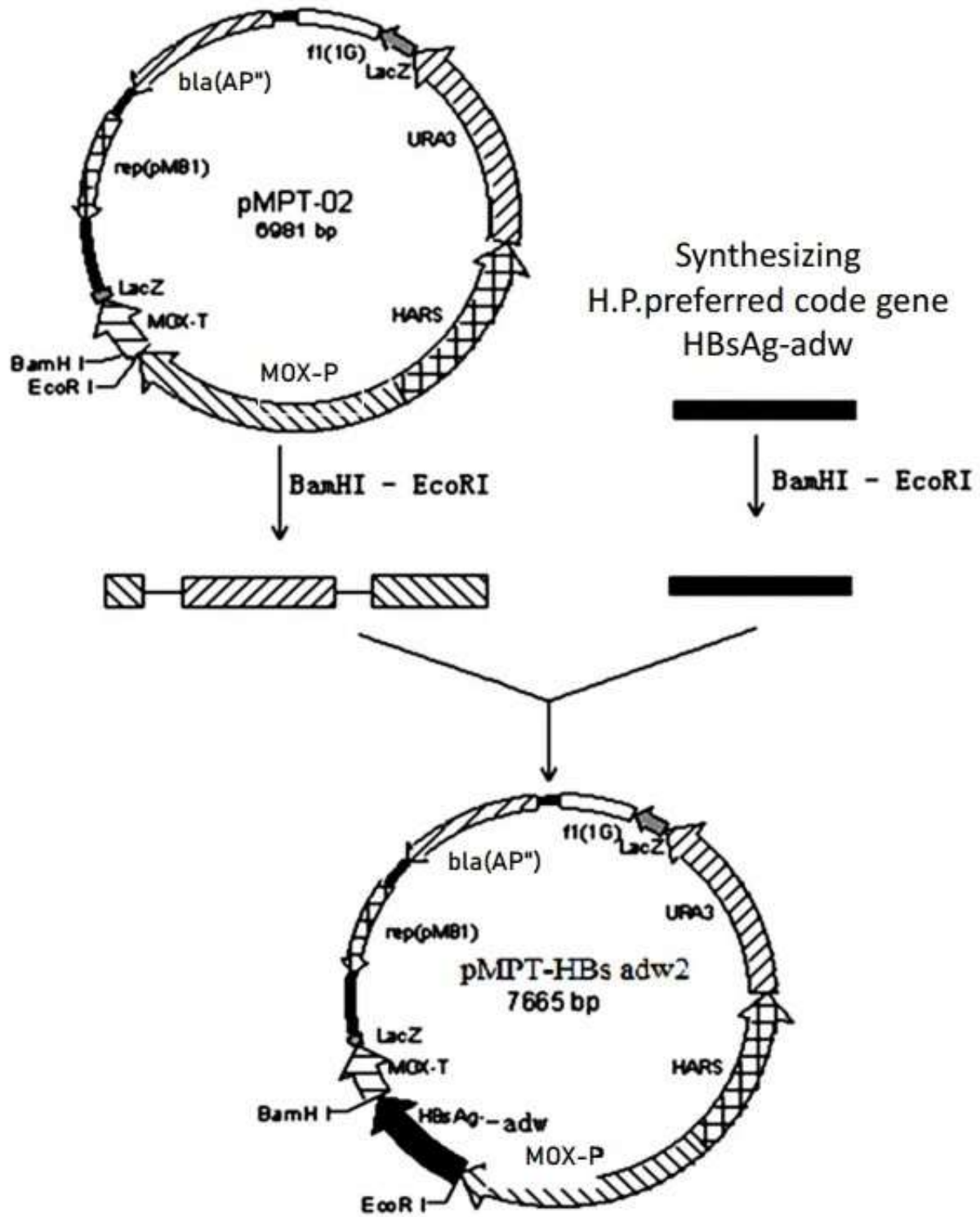


FIG. 1

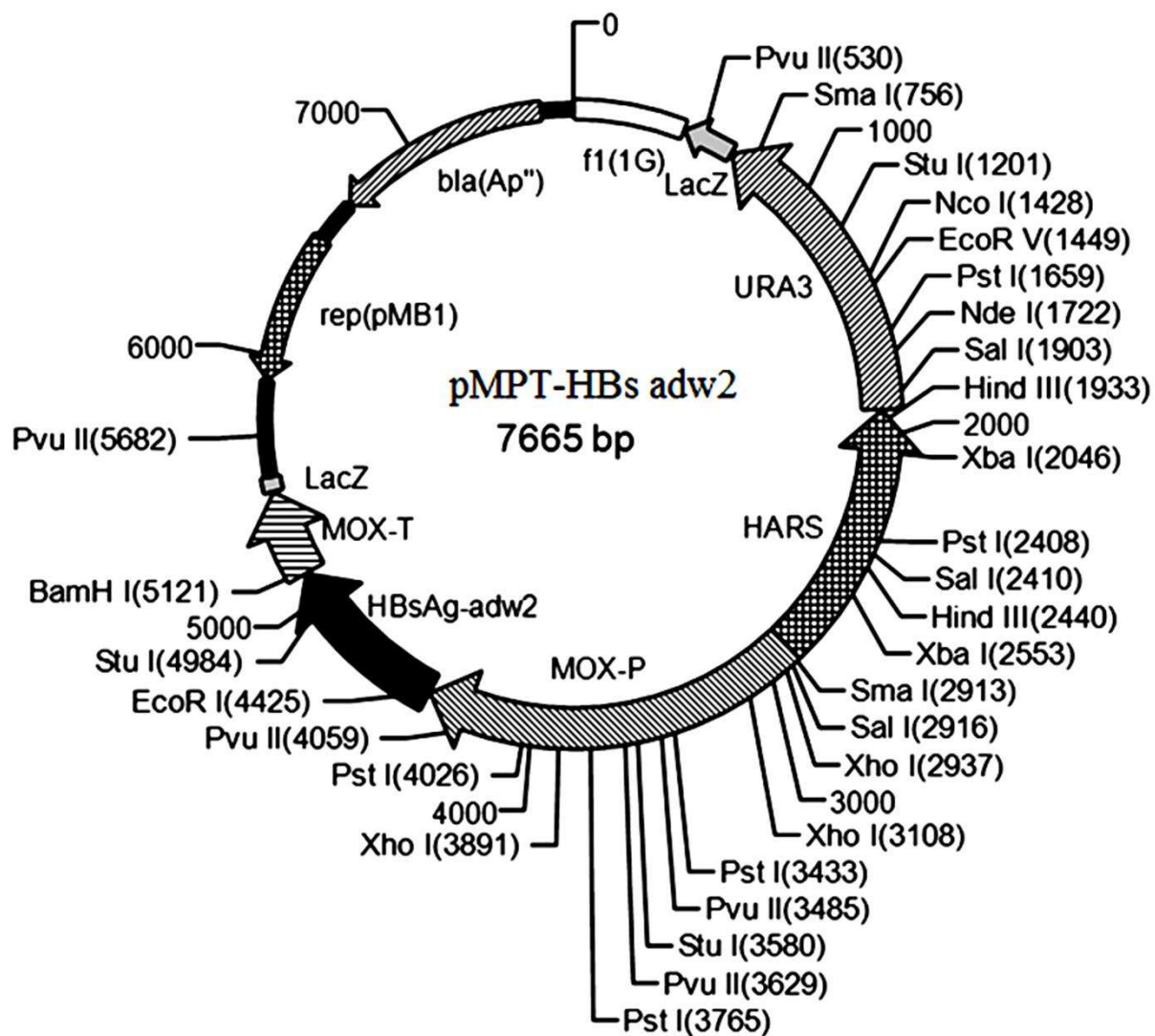


FIG. 2

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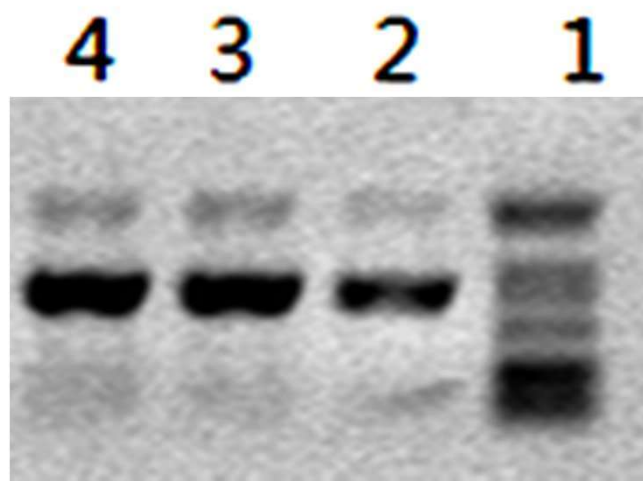


FIG. 3

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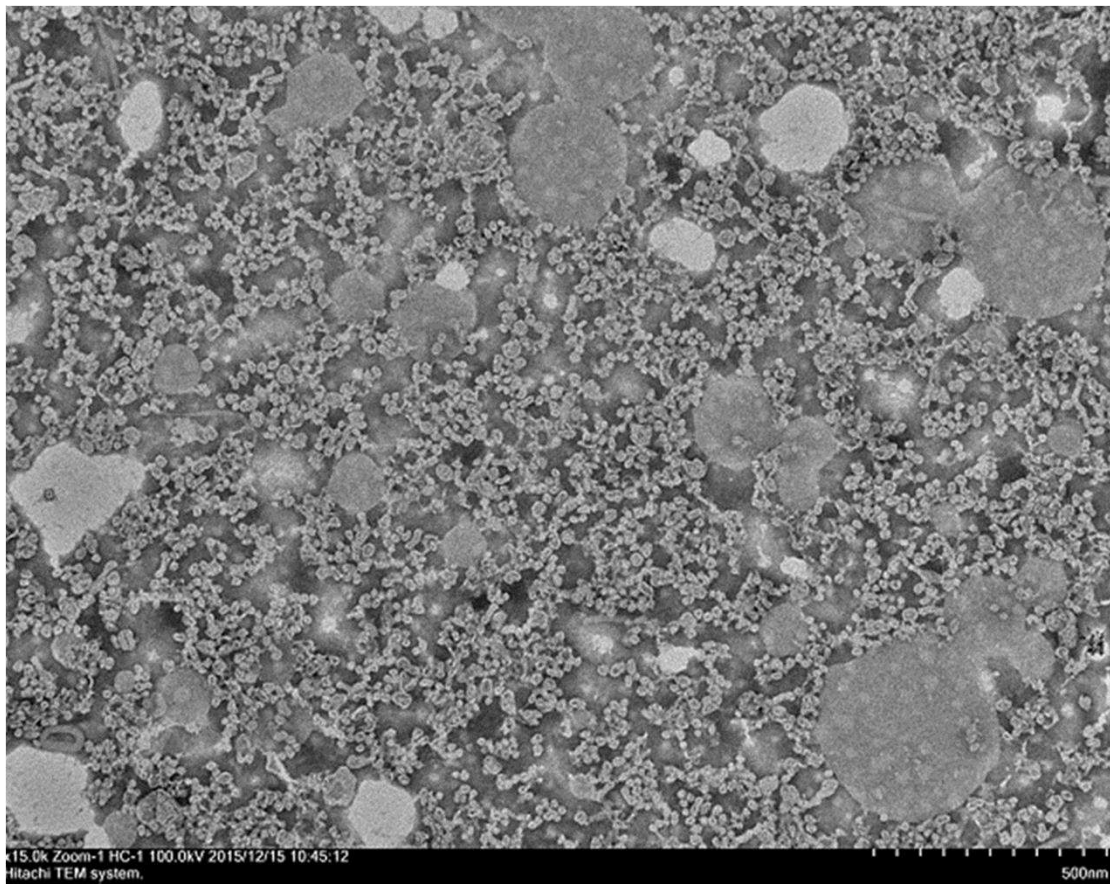
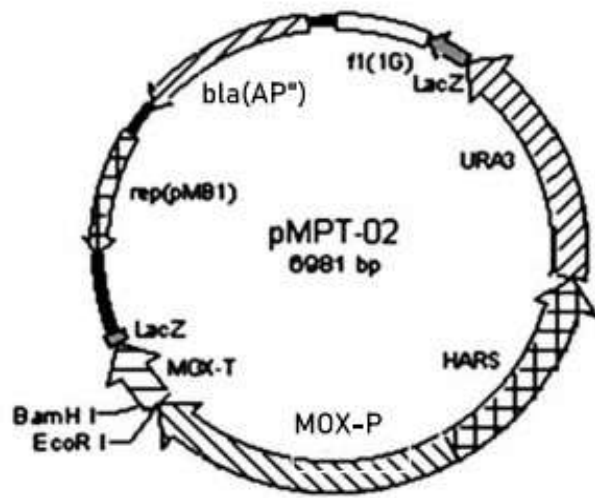


FIG. 4



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