

(19)  
(12)(KR)  
(A)(51) 。 Int. Cl.<sup>7</sup>  
C12N 5/10(11)  
(43)10-2004-0093571  
2004 11 06(21) 10-2003-0027492  
(22) 2003 04 30

(71) 134

(72) 5 904 4 414 201

108 1404

(74)

:

(54) B

B (packaging cell line) , B B ,  
 B , , X .  
 B 가 , B 가 B .

3

1 B partial duplex circular  
 DNA , 4 RN  
 A . 3.5 k bp  
 DNA covalently closed circular DNA (cccDNA)  
 가 (reverse transcription) mRNA (template) . RNA  
 DNA B 가 . DNA , -  
 2 . R015 B RNA  
 , , , X . R063 R015 nt.18  
 20 nt. 1902 . R056

nt.2144 nt.2459 ORF가

3 B , X 3 R063 ,

4 R056 RC(relaxed circular DNA), DL(duplex-linear DNA)  
 , SS(single-stranded DNA) HBV HH01, HH10, HH12

5 B (immunostaining) , HH01  
 . DAPI .

B B , , , X ,

가 (Mulligan, 1993), , (Marsh  
 all, E. , Gene therapy on trial. Science 288:951-957, 2000).  
 (tissue tropism) . , B

가 . , B : 10-2001-19645 B  
 : 2

001-19645 B 가 .

(Ganem and Schneider, 2001'Hepadnaviridae: the viruses and their replication,' in *Fundam  
 ental Virology* , 4td edition vol. 2, Fields, B. N., et al., eds, Lippincott-Raven Press, Philadelphia)

가 가 가  
 10 B  
 가

B 가 ( 가  
 , , , X )  
 Huh7 , , X 가  
 , B 가 B 가

HBV

가 , HBV B

X B

SV40 가 ,

가 가 .

G2 , Chang , 293 293T 3(RO63) 가 , Huh7 7

2003 4 19 KCLRf-BP-00078가 가 .

HBV HBV

HBV HBV ( :10-2001-19645 ) (cis-acting element) ,

가 가 HBV HBV

B (packaging cell line) : 2001-19645 B : 2001-19645 B ORF(open reading frame)가 가

가 B 가 , 가 . (cell culture) : 2001-19645 (prototype) B

B , 가 (f

amilial hypercholesterolemia), VIII, IV가 (hemophilia) (he

patocyte-targeting) , B

I. B (Hepatitis B virus)

B (Hepatitis B virus, HBV) (hepadnavirus family) (Ganem amp; Schneider, 2001). (woodchuck hepatitis virus), (duck

hepatitis B virus) . HBV DNA RNA HBV DNA 가 가 (reverse transcriptase activity) DNA

가 DNA (Ryu, W.-S. 2003). HBV 3.2 k bp -DNA 가 (gap)  
(C), (P), (S), X (P) B  
(Nassal and Schaller, 1996).

## II. (Hepadnavirus)

1 (Ganem and Schneider, 2001).  
(receptor) (transcription) (template) covalently closed circular DNA (ccc DNA) HBV cccDNA  
가 3.5 K bp RNA 2.4 k bp 2.1 k bp R  
NA mRNA , 0.7 k bp RNA X RNA DNA (C)  
, (P)가

## III. (reverse transcription)

B (Nassal et al., 1996). RNA가 (core protein) DNA RNA  
(Jeong et al, 2000). RNA 5'  
85 (base pairs) 2 (stem-loop) -  
(Junker-Niepmann et al., 1990). 2 (stem-loop structure)  
가 , RNA RNA가 DNA가

## IV. B

B (replication competent) 가 ( (Friedmann, 1999). (helper) (packaging cell line) B  
( , , ) 가

(Sambrook et al., in *Molecular Cloning: A Laboratory Manual* , Cold Spring Harbor Laboratory, 3rd Ed., N. Y. 2001)

(epsilon), M (molar), mM (millimolar),  $\mu$ l (microliters), ml (milliliters),  $\mu$ g (micrograms), mg (milligrams), K bp (kilo base pairs), ORF(open reading frame), PCR (polymerase chain reaction), PEG (polyethylene glycol), CMV(cytomegalovirus), HBV(hepatitis B virus), FBS(fetal bovine serum), DMEM(Dulbecco's modified eagle media), HEPES(N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid).

(packaging cell line) Huh7 R06  
3 (pCMV-CPS) DNA , G418 sulfate  
가 , Huh7

## 1: HBV

## 1-1. R063 (pCMV-CPS)

pcDNA3(invitrogen, U. S. A) *Eco* R I, *Xho* I HBV-ayw subtype PCR  
 (Galibert, F., E. Mandart., F. Fitoussi, P. Tiollais, and P. Charnay. Nucleotide sequence of the  
 hepatitis B virus genome (subtype ayw) cloned in *E. coli*. Nature 28: 646-650(1979)).  
 (forward primer) 5' *Eco* R I (reverse primer) 5'  
*Xho* I PCR *Eco* R I (nt. 1903)-*Xho* I (nt.2454) R015  
 : 10-2001-19645 R062  
*Bsp* E I(nt.2331), *Apa* I R062 *Bsp* E I, *Apa* I R063

Forward primer( 1): 5-CATG GAATTC ATGGACATCGACCCT-3 ( *Eco*R I site underlined)

Reverse primer( 2): 5-CCG CTCGAG CTAACATTGAGATTCCCGAGA-3' ( *Xho* I site underlined)

## 2:R063 (pCMV-CPS)

## 2-1.

Huh7 10% FBS(fetal bovine serum) 10 mg/M $\ell$  gentamicin DMEM(Dulbecco's modified eagle media, Gibco-BRL) 3 Huh7  
 75 % 100 mm (phosphate buffered saline)  
 10  $\mu$ g 0.25 M CaCl<sub>2</sub> 694 ml  
 2X HEPES [280 mM NaCl, 50 mM HEPES acid, 1.5 mM Na<sub>2</sub> HPO<sub>4</sub> (pH 7.1)]  
 20  
 16 [DMEM, 10% FBS, 10 mg/M $\ell$  gentamicin]  
 , 3

## 2-2. HBV DNA B

3 , PEG HBV DNA (Jeong et al, 2000).  
 (phosphate buffered saline) [10 mM  
 M Tris (pH 7.5), 1 mM EDTA, 50 mM NaCl, 1 % Nonidet P-40]  
 6 mM MgCl<sub>2</sub> DNase I (50 ??g/ml) 37?? 30 , 4X  
 PNE [26 % PEG, 1.4 M NaCl, 40 mM EDTA]  
 (proteinase K, Sigma, USA) 37?? 2  
 (25:24:1)  
 , TE [10 mM Tris (pH 8.0), 1 mM EDTA] DNA

DNA 1.3 % 가 , ( *Current Protocols in Molecular Biology* ,  
 Ausubel, F. et al., eds., Wiley and Sons, New York, 1995)

## 3: (packaging cell line)

## 3-1.

Huh7 (Nakabayashi et al., Cancer Res. 42:3858-63(1982)) (packaging cell line)  
 R063 (pCMV-CPS) DNA G418 sulfate  
 Huh7 10% FBS 10 mg/M $\ell$  gentamicin DMEM (Gibco-BRL)  
 3 Huh7 50 % 60 mm  
 (phosphate buffered saline)  
 10  $\mu$ g 0.25 M CaCl<sub>2</sub> 250 ml , 2X HEPES [280  
 mM NaCl, 50 mM HEPES acid, 1.5 mM Na<sub>2</sub> HPO<sub>4</sub> (pH 7.1)]  
 30  
 16 [DMEM, 10% FBS, 10 mg/M $\ell$  gentamicin] , G418 sulfate 200  $\mu$ g/  
 M $\ell$  , 3 G418 s

ulfate 400  $\mu\text{g}/\text{Ml}$  3 , , 800  $\mu\text{g}/\text{Ml}$  3 , G418 sulfate 1000  
 G418 sulfate 600  $\mu\text{g}/\text{Ml}$  3 , , 800  $\mu\text{g}/\text{Ml}$  3 ,  
 $\mu\text{g}/\text{Ml}$  가 5%  $\text{CO}_2$  40 .

4:

#### 4-1. HBV DNA

, DNA 2-2 . HBV  
 HBV SS(single-stranded DNA), DL(duplex-linear), RC(relax  
 ed circular DNA) RC( DNA가 (virion particle)  
 HBV RC DNA 가 HBV  
 (replication-defective mutant) 가 RC DNA ,

#### 4-2.

HBV HBV (probe)  
 . 2-2 DNA , R063 (??- C+ P+) (re  
 plication-defective deletion mutant) R056 (??+ C- P-) Huh7 (Nakabayash  
 i et al., Growth of human hepatoma cells lines with differentiated functions in chemically defined medium. Ca  
 ncer Res. 42:3858-63(1982) (positive control)  
 RC(relaxed circular) DNA, DL(double-stranded linear) DNA, SS (single- stranded) DNA .( 4  
 1) (negative control) (encapsidation signal) (??) R06  
 3 DNA가 .( 4 2) ,  
 (replication-defective deletion mutant) R056(??+ C- P-)  
 , single-strand DNA, duplex-linear DNA , relaxed-circular DNA  
 ( 4, 3-5). HH01 mL 0.  
 1 ~ 1.0 x 10<sup>6</sup> .

5:

#### 5-1.

3-1 , 10 % FBS 10 mg/Ml gentamicin DMEM (Gibco-BRL)  
 . , 6 (well) (cover glass) 30 %  
 , 가 100 % (MeOH) 1 Ml 5 block  
 ing solution [1X tigen(HBcAg) 2 , 3 % BSA(bovine serum albumin)] 1 rabbit anti-HBV core an  
 -rabbit 30 0.05% -20(tween-20) 2 FITC

#### 5-2.

B FITC  
 (negative control) Huh7 . 5

ORF (open reading frame)가 가 B

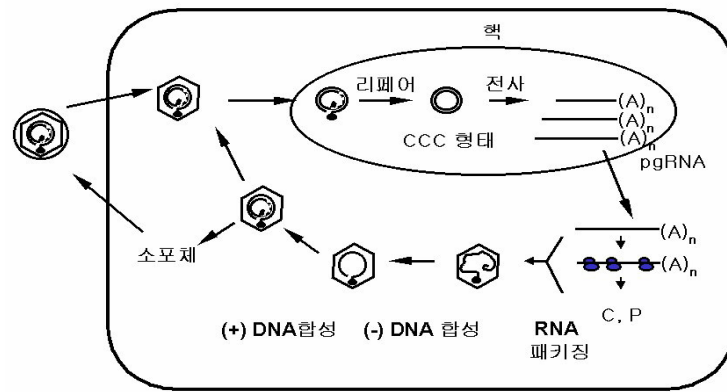
가 가 , 가 . B

가 B 가 가 B 가 . B 가

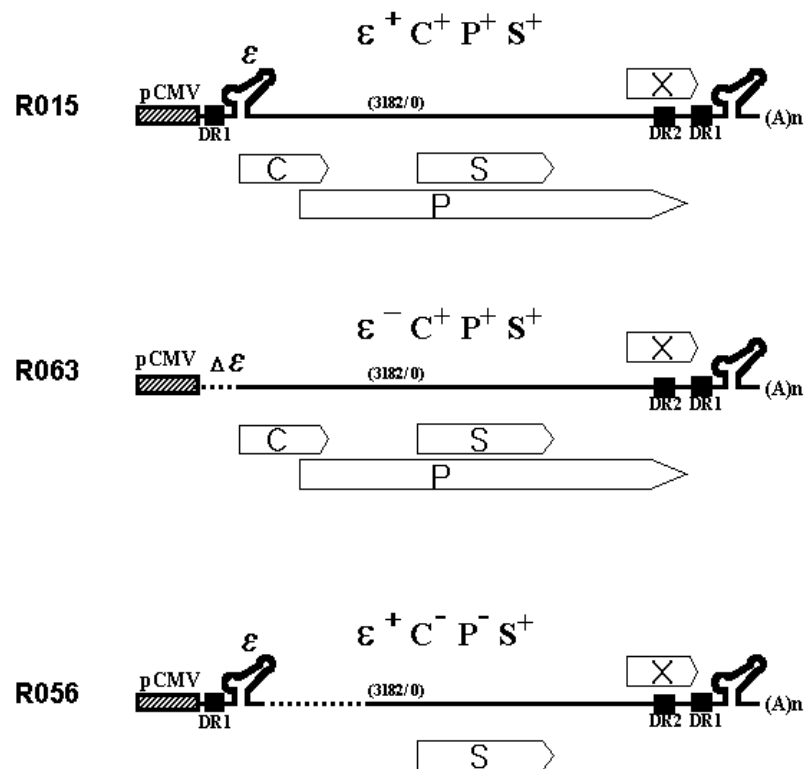
(57)

1. 가 , HBV ,  
 , B X B .
2. 1 ,  
 SV40 , B .
3. 1 ,  
 3(RO63) B .
4. 1 3 ,  
 HepG2 , Huh7 , Chang B , 293 , 293T .
5. 4 ,  
 Huh7 B .
6. 5 ,  
 KCLRF-BP-00078 B .
7. 1 3 HBV HBV .

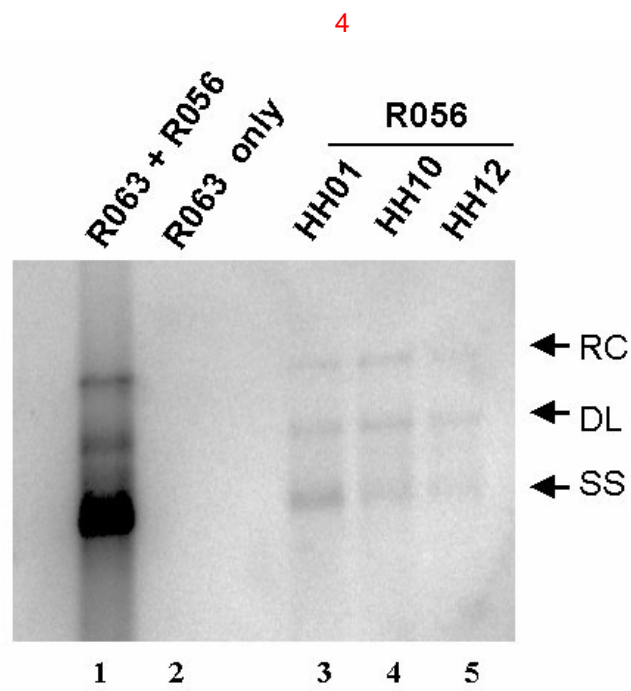
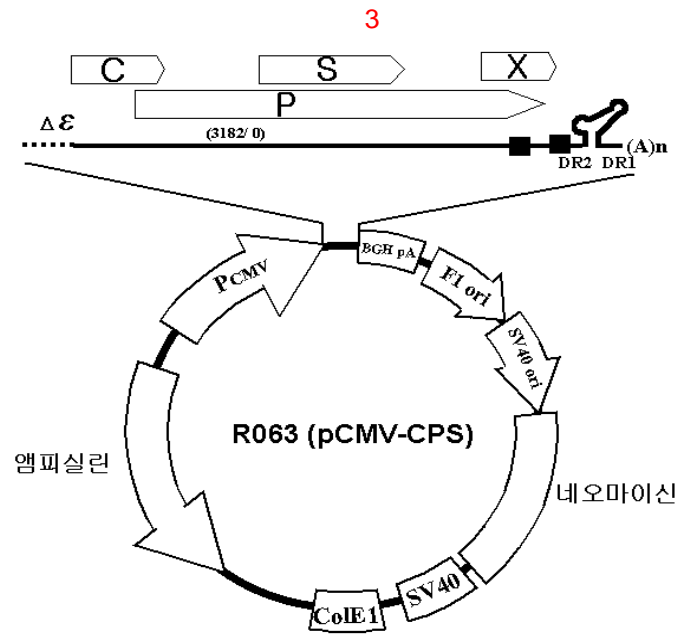
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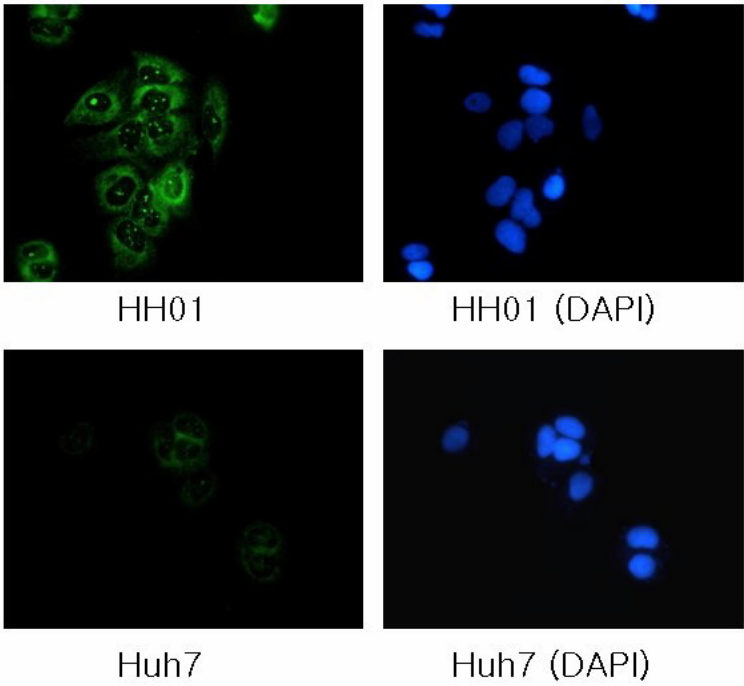






5

HBV 코아 항원의 면역 염색



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<400> 2

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&lt;210&gt; 3

&lt;211&gt; 8678

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; R063 Helper plasmid(pCMV-CPS)

&lt;400&gt; 3

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