

(19)
(12)

(KR)
(A)

(51) 。 Int. Cl. ⁷
C12Q 1/68

(11)
(43)

2002 - 0007331
2002 01 26

(21)	10 - 2001 - 7011764
(22)	2001 09 15
	2001 09 15
(86)	PCT/DK2000/00127
(86)	2000 03 17

(87)	WO 2000/56916
(87)	2000 09 28

(81)

:

- 가

가 가

가

가

AP ARIPO : 가

EA :

EP :

OA OAPI : 가

(30) PA199900383 1999 03 18 (DK)

(71) / - 2950 9

(72)	- 3060			55
	- 2100	Ø	가	가 26
	- 2720	1		225

(74)

:

(54) L N A

DNA . LNA DNA , (locked) (LNA) DNA
 . " - " LNA - (, LNA
 () 3') 가 ()
) ,

(variant)

가 (point mutation)
 (defective protein)
 ; - (6 A → T)
 , LDL (Atherosclerosis(1992), 96:91 - 107) - B - ()
 3500 CGG → CAG (Proc. Natl. Acad. Sci. U.S.A (1989) 86:587 - 591)
 IX VIII (Factor IX and Factor VIII deficiency),
 V (Factor V Leiden), X(Fragil X),
 A, B, I,
 , - 1 ,
 ,

, (oncogene), 30 가 . ras 가 , ras 3 ras (activ e)" ras " " " "ras .ras . 90% , 50% , 50% , 10 20% ras (H - ras, Ki - ras, N - ras) . TP35 . / (missense mutation) 250 , TP53 (nonsense mutation) (Rb1) p16 . TP53 . TP53 가 가 T P53 . 4 8 DNA TP53 4 (domain) . (hot spot) 175, 213, 245, 248, 249, 273, 282 . (higher le vel of relationship) 가 , () (fingerprint)" , HIV , HPV, EBV, HSV, 215 A → T : Science (1998)246:1155 - 1158) . B C CM V . , , rRNA . (difference) . (alternat ive variant) .

noise)

159

PCR
and Nucl.Acids Res. 17(1989), 8093 - 8099] . [Oncogene Research 1 (1989), 235 - 241
(area) PCR
(restriction fragment length polymorphisms(RFLP)

EP - 1 - 0 332 435 US 5,605,794

3' - (mismatch) 가 C:A (Kwok et al., (1990). Nucleic Acids Research, 18:999 - 1005). 가 , EP - A - 0 332 435 가

가

, PCR

: 1 PCR

, PCR

2

DNA -

PCR

PCR

PCR

PCR

[Biochem. Biophys. Res. Commun.(1989), 160:441 - 447] dNTP 가
 . 가 , (batch)

가 (WO 89/09835) , 가
 .
 .
 . 가
 가 가 . ,
 6
 (Pro. Natl. Acad. Sci. U. S. A.(1991), 88:189 - 193).

[Pro. Natl. Acad. Sci. U.S.A.(1985), 82:1585 - 1588] [New England Journal of Medicine (1987), 317:9
 85] " - " (ASO)
 . 20bP
 (match)

DNA PCR DNA
 가

[Nucleic Acids Research(1989), 17:2437 - 2448] EP - A - 0 333 465 ((priming) =COP) 가 PCR
 - DNA , ASO - PCR
 . ASO - ,
 5% 가 , 가 -
 PCR , 가 , 12
 12% , DNA
 PCR 10 ,
 가 -
 - 12 16
 -
 DNA ,
 ,
 - , COP , .

NA DNA D

LNA . LNA RNA - 2 (I). LNA DNA RNA
 3630(1998)). , LNA DNA RNA (Tetrahedron, 54, 3607 -
 LNA(s) , PCR , LNA
 가 가 . ()

a) A :
 LNA - A A , 가
 3' - ;

b) , , .
 , 가 , .
 " " 가 (Q
 Q') , Q' Q " A"
 A A A
 (Q'₁, Q'₂, ..., Q'_e) A₁, A₂, ..., A_e
 , RNA DNA rRNA
 " " A (Q, Q'₁, Q'₂, ..., Q'_e)
 A A
 " " (original)
 " " ,
 " " .
 - - (: G - C, A - T, A - U).
 (: 7 - - dGTP, dIPT, LNA -)

e₁) c₁) d₁) 가 ;

f₁) .

가 PCR

"

"

-

가

A

Q

Q'

:

a)

Q'

A

Q'

A

A

LNA

;

b)

;

c)

;

d)

c)

Q

A

Q'

A

A

,

LNA

;

e)

c)

d)

가

;

f)

.

, LNA -

(s)

,

3'

,

A,

,

,

,

-

.

3

TP5

LNA -

/DNA -

,

가

:

가

A

a)

A

LNA

;

b)

;

c)

b)

.

1
c) RNA
가
A
;
c₁)
d₁) c₁)
가 A
;
e₁) c₁) d₁) 가
f₁)
가 PCR
A
;
a) A LNA
;
b) ;
c) ;
d) c)
가 A
;
e) c) d) 가
f)
3' - LNA - (A)
DNA , RNA DNA , DNA , Taq, Pfu, Pwo T
th
) A PCR Q() A) (Q'(
3' - 가 Q' A ,

LCR PCR
LNA -
(: 8bp)
()
(join)
가
가
DNA
DNA
(join)/
" "
2
가
()

a) :

a) 5'-Nu_a(Nu_bLNA_c)_mNu_dA_eNu_f-3'

, A A LNA ; LNA LNA ; Nu
; a,b,c,d f 0 30 , m 1 8 , e 1 6 , , a,b,c,d,e f
5 .

A A
A LNA .

가
가 .

가

, " "
(: X-)
(5-) - 1 -
, DOXYL(N - 4,4 -), PROXYL(N - 2,2,5,5 -), TEMP
O(N - 2,2,6,6 -), Cy3 Cy5
(NBD), , 7 -
- 2 - - 1 - (spin) (:
(parama
gnetic) (: Cu+2, Mg+2)), (:
, β -

A, D, E,), (: , (), (EGF), (PDGF) , , , Cy3, Cy5 .

가

EP - A - 0 324 474

(:) (capture)

(WO 96/31557).

().

가) (

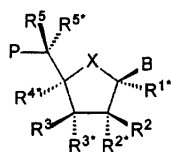
(life style)

()

(archae),

(pool - screening) , 가 , apo B 3500 - 64 () 가 , AIDS 가 , E.Coli A - T ,E.ColiK 12(가) (array format) (Nature Genetics, suppl. vol.21, Jan 1999, (WO 96/31557) LNA LNA PCR 가 PCR (in situ) PCR (thermocyclers) . PCR 2 LNA DNA:DNA , 3' LNA(s) 가 PCR () 가 가

가 가 :


$$, X - O -, -S -, -N(R^{N*}) -, -CR^6(R^{6*}) -$$

B ;

P (succeeding) 5' - 5' - R⁵ ;

$$R^3 \quad R^{3*} \quad (\text{preceding}) \quad 3' - \quad P^* \quad ;$$
$$\begin{array}{l} R^{4*} \quad R^{2*} \\ > C=Z \end{array} \quad -C(R^a R^b)-, -C(R^a)=C(R^a)-, -C(R^a)=N-, -O-, -Si(R^a)_2-, -S-, -SO_2-, -N(R^a)-, \\ 1-4 \quad / \quad ,$$
[illegible]

P P* R^{1*}, R², R³, R^{3*}, R⁵, R^{5*}, R⁶ R^{6*},
C₁₋₁₂ -, C₂₋₁₂ -, C₂₋₁₂ -, C₁₋₁₂ -,
, C₂₋₁₂ -, , C₁₋₁₂ -, C₁₋₁₂ -, , , ,
, , , - , - (C₁₋₆ -)
-6 -) , - (C₁₋₆ -) - , -C₁₋₆ - - ,
- (C₁₋₆ -) -C₁₋₆ - - , C₁₋₆ - - , C₁₋₆ -
, C₁₋₆ - , , , C₁₋₆ - , DNA ,
(

B),
- (NR^N) -
(
C₁₋₄
C₁₋₄
가
R^N
C₁₋₄
1-5
-O-, -S-,
()
),
; R^{N*}

" LNA " (Locked Nucleoside Analogues) 가 .

가

Benner, U.S. 5,432,27

2

DNA

DNA RNA , 2 3

(4,7 - 1,10 -)

6 - -1,4 - 1,4 -

1

2 3 1 2 3

(EDTA),

가 (: , ,),

1 2 3 /

C_{1-20} - β - /

:

DNA 가

" / " DNA ,
 / , " K " / " 5 - 6 - 가 M - K - (M ")
 . , B가 DNA , , , ,
 , B M - K - (M DNA , , , ,
 , " / " , K 5 - 6 - " / ")
 1 - 50 , 1 - 30 , 1 - 15 가 .

, " - " -
 (: Hermanson , " Immobilized Affinity Ligand Techniq
 ues" , Academic Press, San Diego, California(1992), p.137 - ff). , 400
 100 , /
 가 , K
 , , , , / , /
 , - β - / , , ,
 , /
 5 - 6 - " / "
 . 가 가 ,
 가 가 .

, K " / " 5 - 6 -
 .
 , B , , , ,
 .
 () , P , 5' -
 . 가 LNA가 5' - " " 가 , 가 " LNA"
 가 5' - " " 5' - R⁵ (R⁵ (5'
 가 : R^{5*}) P) (5' - 5'
 5' - 가).
 , 3' - (P*) R³ R^{3*}
 , R^{3*} (3' -
 3').

R^{3*} (" ") R^3 () P^* 2 가
 . " " ($R^{3*} = P^*$) " " LNA (2 -
 /)가 가 DNA, RNA LNA (가)
 . - LNA LNA($R^3 = P^*$) ,
 (/ 2 -)
 . " " ($R^{3*} = P^*$) DNA, RNA LNA
 (가) LNA -
 ($R^3 = P^*$) 가 DNA, RNA LNA

가

LNAs, " " " " 5' - " , PNAs " " 3' - . LNA 가 LNA . (), " " LNAs

$$\text{R}^{2*} - \text{O} - \text{CH}_2 - \text{R}^{4*}$$

$$\begin{aligned}
 & \text{LNA(s)} \quad (R^{2*} - R^{4*}) \quad \text{가} \\
 & - (CR^*R^*)_r - Y - (CR^*R^*)_s - , - (CR^*R^*)_r - Y - (CR^*R^*)_s - Y - , - Y - (CR^*R^*)_{r+s} - Y - , - Y - (\\
 & CR^*R^*)_r - Y - (CR^*R^*)_s - , - (CR^*R^*)_{r+s} - , - Y - , - Y - Y - , \quad Y - O - , - S - , - S \\
 & i(R^*)_2 - , - N(R^*) - , > C=O, - C(=O) - N(R^*) - , \quad - N(R^*) - C(=O) - , \quad R^* \\
 & , \quad , \quad , \quad , \quad , \quad , \quad - \quad (C_{1-6} -) \quad , \\
 & C_{1-6} - , \quad C_{1-6} - , \text{DNA} \quad , \quad , \quad , \quad , \\
 & , \quad , \quad / \quad , \quad (\quad) R^* \\
 & ; \quad r \quad s \quad 0-4 \quad r+s \quad 1-4 \quad . \quad - Y - , (CR^*R^*)_{r+s} - , - (\\
 & CR^*R^*)_r - Y - (CR^*R^*)_s - , \quad - Y - (CR^*R^*)_{r+s} - Y - , \quad r \quad s \quad 0-3 \quad , \quad r+s \\
 & 1-4 \quad .
 \end{aligned}$$

$$\begin{array}{c} \text{LNA} \quad \text{R}^{2*} \quad \text{R}^{4*} \text{가} \quad \text{-O-}, \text{-S-}, \text{-N(R}^*)\text{-}, \text{- (CR}^*\text{R}^* \\ \text{)}_{r+s+1} \text{-}, \text{- (CR}^*\text{R}^*)_{r}\text{-O- (CR}^*\text{R}^*)_{s}\text{-}, \text{- (CR}^*\text{R}^*)_{r}\text{-S- (CR}^*\text{R}^*)_{s}\text{-}, \text{- (CR}^*\text{R}^*)_{r}\text{-N(R}^*)\text{- (CR}^*\text{R}^*)_{s}\text{-}, \text{-O-} \\ \text{(CR}^*\text{R}^*)_{r+s}\text{-O-}, \text{-S- (CR}^*\text{R}^*)_{r+s}\text{-O-}, \text{-O- (CR}^*\text{R}^*)_{r+s}\text{-S-}, \text{-N(R}^*)\text{- (CR}^*\text{R}^*)_{r+s}\text{-O-}, \text{-O- (CR}^*\text{R}^*)_{r+s} \\ \text{-N(R}^*)\text{-}, \text{-S- (CR}^*\text{R}^*)_{r+s}\text{-S-}, \text{-N(R}^*)\text{- (CR}^*\text{R}^*)_{r+s}\text{-N(R}^*)\text{-}, \text{-N(R}^*)\text{- (CR}^*\text{R}^*)_{r+s}\text{-S-}, \text{-S- (} \\ \text{CR}^*\text{R}^*)_{r+s}\text{-N(R}^*)\text{-} \end{array}$$

R* , C₁₋₆ - , C₁₋₆ - , DNA , R*

, LNA, R^{*} DNA, B
(
).
P, P*, LNA(s), R^{1*}, R², R³, R^{3*}, R⁵, R^{5*}, R⁶, R^{6*}, C₁₋₆ -, C₂₋₆ -, C₁₋₆ -, C₁₋₆ -, (C₁₋₆ -), (C₁₋₆ -) -, C₁₋₆ -, C₁₋₆ -, DNA, R^{N*}, C₁₋₄ -.

LNAs, X -O-, -S-, -NR^{N*} -, -O- P, P*, LNA(s), R^{1*}, R², R³, R^{3*}, R⁵, R^{5*}, R⁶, R^{6*}.
X -O-, R², C₁₋₆ -, R³, R^{3*}, P*, R^{1*}, R⁵, R^{5*}, (R^{2*} - R^{4*}) -O-, -(CH₂)₀₋₁ -O-(CH₂)₁₋₃ -, -(CH₂)₀₋₁ -S-(CH₂)₁₋₃ -, -(CH₂)₀₋₁ -N(R^N) -(CH₂)₁₋₃ -, -(CH₂)₂₋₄ -, -O-CH₂ -, -S-CH₂ -, -NR^H -CH₂ -, R^{2*}, R^{4*}, (X=O) 5, LNA, R^{2*}, R^{4*} 가 -O-CH₂ -, -S-CH₂ -, -NR^H -CH₂ -; X가 O, B, R³, R^{3*} 가 P*, R^{1*}, R³, R⁵, R^{5*}.

LNA, LNA, (B), LNA, 가

LNA, LNA, (LNA(s)), 가

R^{3*}, R^{3*}, P*, R³, P*, 3, LNAs, P*, 1 - 10000, LNA(s), 0 - 10000, 3, 5, 7, 2 - 15000, 2 - 100, 3 - 100, 2 - 50, 3 - 50, 5 - 50, 7 - 50.

"", "", "", 가

() , 가 LNA(s)(
)
 " "
 : , () ()
 B

LNA(s)
 " " -CH₂ -, -O -, -S -, -NR^H -, > C=O, > C=NR^H, > C=S, -Si(R["])₂ -, -S
 O -, -S(O)₂ -, -P(O)₂ -, -PO(BH₃) -, -P(O,S) -, -P(S)₂ -, -PO(R["]) -, -PO(OCH₃) -, -PO(NHR^H)
 - (R^H C₁₋₄ -, R["] C₁₋₆ -)
 2 4 , 3 /
 -CH₂-CH₂-CH₂ -, -CH₂-CO-CH₂ -, -CH₂-CHOH-CH₂ -, -O-CH₂-O -, -O-CH₂-CH₂ -, -O-CH₂
 -CH=(R⁵), -CH₂-CH₂-O -, -NR^H-CH₂-CH₂ -, -CH₂-CH₂-
 NR^H -, -CH₂-NR^H-CH₂ -, -O-CH₂-CH₂-NR^H -, -NR^H-CO-O -, -NR^H-CO-NR^H -, -NR^H-CS-NR^H
 -, -NR^H-C(=NR^H)-NR^H -, -NR^H-CO-CH₂-NR^H -, -O-CO-O -, -O-CO-CH₂-O -, -O-CH₂-CO-O
 -, -CH₂-CO-NR^H -, -O-CO-NR^H -, -NR^H-CO-CH₂ -, -O-CH₂-CO-NR^H -, -O-CH₂-CH₂-NR^H -,
 -CH=N-O -, -CH₂-NR^H-O -, -CH₂-O-N=(R⁵), -CH₂-O-N
 R^H -, -CO-NR^H-CH₂ -, -CH₂-NR^H-O -, -CH₂-NR^H-CO -, -O-NR^H-CH₂ -, -O-NR^H -, -O-CH₂-S,
 -S-CH₂-O -, -CH₂-CH₂-S -, -O-CH₂-CH₂-S -, -S-CH₂-CH=(
 R⁵), -S-CH₂-CH₂ -, -S-CH₂-CH₂-O -, -S-CH₂-CH₂-S -, -CH₂-S-CH₂ -, -CH₂-SO-CH₂ -, -
 CH₂-SO₂-CH₂ -, -O-SO-O -, -O-S(O)₂-O -, -O-S(O)₂-CH₂ -, -O-S(O)₂-NR^H -, -NR^H-S(O)₂-
 CH₂ -, -O-S(O)₂-CH₂ -, -O-P(O)₂-O -, -O-P(O,S)-O -, -O-P(S)₂-O -, -S-P(O)₂-O -, -S-P(O,
 S)-O -, -S-P(S)₂-O -, -O-P(O)₂-S -, -O-P(O,S)-S -, -O-P(S)₂-S -, -S-P(O)₂-S -, -S-P(O,S)
 -S -, -S-P(S)₂-S -, -O-PO(R["])-O -, -O-PO(OCH₃)-O -, -O-PO(OCH₂CH₃)-O -, -O-PO(OCH₂CH
 2S-R)-O -, -O-PO(BH₃)-O -, -O-PO(NHR^N)-O -, -O-P(O)₂-NR^H -, -NR^H-P(O)₂-O -, -O-P(O,N
 R^H)-O -, -CH₂-P(O)₂-O -, -O-P(O)₂-CH₂ -, -O-Si(R["])₂-O - ; -CH₂-CO-NR^H -, -CH₂
 -NR^H-O -, -S-CH₂-O -, -O-P(O)₂-O -, -O-P(O,S)-O -, -O-P(S)₂-O -, -NR^H-P(O)₂-O -, -O-P
 (O,NR^H)-O -, -O-PO(R["])-O -, -O-PO(CH₃)-O -, -O-PO(NHR^N)-O -(R^H C₁₋₄ -
 , R["] C₁₋₆ -)가

가 Mesmaeker , Current Opinion in Structural Biology 1995, 5, 343 - 355

5' - , P 5 -

LNA가 5' - P가 5' -
 5' - C₁₋₆ -, C₁₋₆ -, -W-A' - ,
 C₁₋₆ - W -O -, -S- -N(R^H)- , R^H C₁₋₆ -, A' DNA ,
 B).

:" , " , " "

-O-P(O)₂-O⁻ , -O-P(O)₂-O-P(O)₂-O⁻ , -O-P(O)₂-O-P(O)₂-O-P(O)₂-O⁻

가 5' -

가 3' - 3' -
 C_{1-6} - C_{1-6} -
 $-W-A'-$, $W-O-$, $-S-$ - $N(R^H)-$, R^H , C_{1-6} -
 A' DNA ,
 (B) .

LNAs :

$G - [Nu - L]_{n(0)} - \{ [LNA - L]_{m(q)} - [Nu - L]_{n(q)} \}_q - G^*$

$q = 1 - 50$;

$n(0) \dots n(q) = 0 - 10000$;

$m(1) \dots m(q) = 1 - 10000$;

$n(0) \dots n(q) = m(1) \dots m(q) = 2 - 15000$;

$G = 5' -$;

Nu
 ;

LNA ;

$3' - L Nu LNA , L G^*$
 ;

LNA - L

, LNAs , ,

" "

2

, PNA - , LNAs , - LN
 $A(R^3 = P^*) ()$ " " LNA($R^{3*} = P^*$) ()
 가

,
 T_m , 3.5 , 4.0 , 5.0 , DNA 2.5 ,
 $\times N$, $3.5 \times N$, $4.0 \times N$, $5.0 \times N$ T_m 2.5
 N).

RNA , 가
4.0 ,
5.0 , 6.0 , 7.0 , DNA
 T_m , T_m , $4.0 \times N$, $5.0 \times N$,
 $6.0 \times N$, $7.0 \times N$ (N).

" " ()

T_m , a) , (, 1.0 μ M) DNA
:

a) 10 mM Na_2HPO_4 , pH 7.0, 100 mM NaCl, 0.1 mM EDTA;

b) 10 mM Na_2HPO_4 , pH 7.0, 0.1 mM EDTA;

c) 3 M (TMAC), 10 mM Na_2HPO_4 , pH 7.0, 0.1 mM EDTA.

가 B가 ,
가

A , , RNA , DN

T_m , T_m .

1% , 2% , 3%
, 4% , 5% , 6% , 7% , 8% , 9% , 10%
, 11% , 12% , 13% , 14% , 15%
, 20% , 30% , 50% , 70%
100% .

3' -

(LNAs가) LNAs , 가
가
()
($^+ \text{N}(\text{R}^g)_3 \text{R}^h$ (R^g R^h) C_{1-6} - , C_{2-6} - ,
)

Remington's Pharmaceutical Sciences, 17 Ed., Alfonso R. Gennaro (Ed.), Mack Publishing Compa
ny, , PA, U.S.A, 1985 Encyclopedia of Pharmaceutical Technology .

, " 가 " , LNA
S

1 (matching) 3' - LNA 가 PCR .

2 PCR 3 DNA - (directed)
:

A) PCR 3 ,
(mismatching) X ;

B) 2 PCR , 4 - ;

C) 가 PCR , 1 4 PCR .

3 LNA PCR " " - EQ3053
/EQ3213 . " G" ApoB " G - " , " A"
ApoB " A - " " - EQ3157
/EQ3176 . " C"
ApoB " G - " , " T" ApoB " A - "
DNA Mass TM (GibocoBRL cat. no. 10068 - 013, Life Technologies Inc., Rockville, MD, USA)

4 LNA DNA . LNA/DNA - EQ3053/EQ3213
DNA - EQ3647/EQ3213 . PCR
" G" ApoB " G - " , " A" ApoB " A -
" , " H₂O" . DNA Mass TM (GibocoBRL cat. no. 10068 -
013, Life Technologies Inc., Rockville, MD, USA)

5 - - . EQ3053/EQ3213 " A -
" " G - " " G - " (G,), " A
- " (A,) 가 .

6 EQ3053/EQ3213 " A - " (A,) " G - " (G,
) PCR (melting) . (amplicon)
SyBR I . 1 (- dF/dT)

1

LNA

:

LNA (amidites) 2 5 가 , DNA -
 (Pharmacia Gene Assembler Special^R, Pharmacia AB, Uppsala, Sweden)
 (0.2 μmol) (stepwise) 99% 2' -
 CPG
 32% (55 5 10) 5' - O - DMT - ON
 LNAs 0.05M pH 7.0(1.5cm/)
 HPLC(Delta PakC - 18, 300A, 0.4 x 30cm) 5' - O - DMT - ON LNAs
 (30 - 35) 80% (1cm, , 1) (detritylati
 on) , LNAs HPLC ().

DNA (Roche Molecular System cat. no. 1 667 327. Roche Molecular Biochemicals, H
 vidovre, Denmark) DNA 5M
 ApoB3500 (Ludwig et al. (1987) DNA 6: 363 - 372; M19828, ON4)
 ApoB PCR - ApoB " G - " . TOPOTM TA
 (Invitrogen, cat. no. K4500 - 01, Invitrogen Corporation, Carlsbad CA USA) PCR
 pCR^R 2.1 - TOPO . QUIAGEN^R (QUIAGEN GmbH, Hilden, Germany)
 DNA . ALFexpress II DNA (Ame
 rsham Pharmacia Biotech) DNA DNA .

PCR

PCR (Hybaid, Middlesex, UK) 0.5M PCR
 . dNTP (Amersham Pharmaci
 a Biotech AB, Uppsala, Sweden) :

dATP, dGTP, dTTP, dCTP: 200 μM

1.5mM MgCl₂

5μl 10 x GeneAmp PCR (Perkin - Elmer Corporation, Norwalk, CT, USA)

1 μM EQ 3053, 1

1 μM EQ 3054, 2

1 AmpliTaq Gold^R (Perkin - Elmer cat. no. N808 - 0240, Perkin - Elmer corporation, Norw
 alk, CT, USA).

2μl DNA(1).

50μl :

: 95 15

: 35 (94 30 ; 63 30 ; 72 30)

: 72 10

4

10 μ l PCR 2 μ l (40% , 0.25% , 0.25% ,
 0.1M EDTA pH8.0) 가 1 x TAE(50 x TAE = 242g ,
 57.1M ℓ , 100M ℓ 0.5M EDTA pH8.0), 0.5 μ g/M ℓ (Sigma - Aldrich cat. no. E7637, S
 igma - Aldrich Denmark A/S, Vallenbaek Strand, Denmark) 1% SeaKem^R, Agarose(FMC BioProducts, P
 hiladelphia, PA, USA) 5V/cm 0.5 - 1 - VDS
 (Amersham Pharmacia Biotech AB, Uppsala, Sweden) 1

PCR - LNA0 - 3 - ' 가
 EQ3053/ApoBR2 EQ3054/ApoBR2 DNA ApoB
 " G - " (4) - EQ3053/ApoBR2(1,
 3) 667bp PCR , 30.4g ApoB " A - " (5)
 - EQ3053/ApoBR2 PCR 1

EQ3053 (서열번호 1): 5'-CCT ACT TGA ATT CCA AGA GCA CAC G^{LNA}-3'

EQ3054 (서열번호 2): 5'-CCT ACT TGA ATT CCA AGA GCA CAC A^{LNA}-3'

ApoBR2 (서열번호 3): 5'-TTT AGA TCA TTT AGT TTC AGC CC-3'

ApoB " G - " (4):
 5'-CTTACTTGAA TTCCAAGAGC ACAC^GGTCTT CAGTGAAGCT GCAGGGCACT-3'

ApoB " A - " (5):
 5'-CTTACTTGAA TTCCAAGAGC ACAC^AGTCTT CAGTGAAGCT GCAGGGCACT-3'

2

LNA - PCR ApoB R3500Q

B(ApoB) LDL - (LDL) - (fa
 milial) ApoB 100 3500(arg $\rightarrow$ gln) (G $\rightarrow$ A)
 (- ApoB R 3500Q) LDL - LDL
 LDL 가 1:500 ,
 1:1,000,000 (Harrison's Principles of Internal Medicine, 14th edition. by Fauci, A.S.
 et al.,(Eds) McGraw Hill).

LNA - PCR ApoB R3500Q " G - " " A -
 " PCR .

:

1 LNA (DNA Technology, Aarhus, Denmark) DNA HPLC .

1 , ApoB3500 ApoB PCR - A
 poB " G - " " G - " , ApoB3500 (4) ApoB PCR - 3500 (3 DNA , " A - " . PCR

2 . PCR , TOPOTM TA^R (Invitrogen, cat. no. K4500 - 01, Invitrogen Corporation, Carlsbad CA USA) pCR^R 2.1 - TOPO . QUIAGEN^R
 (QUIAGEN GmbH, Hilden, Germany) DNA
 . ALFexpress II DNA (Amersham Pharmacia Biotech)
 DNA DNA .

PCR

(Eppendorf - Netheler - Hinz GmbH, Hamburg, Germany)
 0.5M $\varnothing$ PCR . dNTP
 (Amersham Pharmacia Biotech AB, Uppsala, Sweden) .

:

(G -) ApoB3500

dATP, dGTP, dTTP, dCTP: 200 μ M

1.5mM MgCl₂

5 μ l 10 x GeneAmp PCR (Perkin - Elmer corporation, Norwalk, CT, USA)

1 μ M EQ 3053, 1

1 μ M EQ 3213, 6

1 AmpliTaq Gold^R (Perkin - Elmer cat. no. N808 - 0240, Perkin - Elmer corporation, Norwalk, CT, USA)

2 μ l DNA (50ng / μ l).

50 μ l

PCR - pCR^R 2.1 - TOPO ApoB " G - " " A

:

: 95 15

: 35 (94 30 ; 55 30 ; 72 30)

: 72 10

4

.

PCR 가 (: 1). 2% 가
 1:30,000 GelStar^R (FMC BioProducts, Rockland, Maine, USA)
 , UV - (Model TM - 20E UV Products, Upland, CA, USA) (Kodak W
 ratten #9 Eastman Kodak Co., Rochester, NY, USA) (Polaroid LTD., St. Alb
 ans, UK)

(A -) ApoB3500

dATP, dGTP, dTTP, dCTP: 200 μ M1.5mM MgCl₂5 μ l 10 x GeneAmp PCR (Perkin - Elmer corporation, Norwalk, CT, USA)1 μ M EQ 3053, 71 μ M EQ 3213, 8

1 AmpliTaq Gold^R (Perkin - Elmer cat. no. N808 - 0240, Perkin - Elmer corporation, Norw
 alk, CT, USA)

2 μ l DNA (50ng / μ l).50 μ l

PCR - pCR^R2.1 - TOPO ApoB " G - " " A
 - "

:

: 95 15

: 35 (94 30 ; 67 30 ; 72 30)

: 72 10

4

.

PCR 가 (: 1). 2% 가
 1:30,000 GelStar^R (FMC BioPorducts, Rockland, Maine, USA)
 , UV - (Model TM - 20E UV Products, Upland, CA, USA) (Kodak W
 ratten #9 Eastman Kadak Co., Rochester, NY, USA) (Polaroid LTD., St. Alb
 ans, UK)

PCR - LNA - 3' - 가
 EQ3053/EQ3213 EQ3157/EQ3176 ApoB " G - " "
 A - " DNA 's .

EQ3053 (서열번호 1): 5'-CCT ACT TGA ATT CCA AGA GCA CAC G^{LNA}-3'

EQ3213 (서열번호 6): 5'-GTT TTT CGT ACT GTG CTC CCA GAG-3'

EQ3157 (서열번호 7): 5'-CCC TGC AGC TTC ACT GAA GAC T^{LNA}-3'

EQ3176 (서열번호 8): 5'- CAC CTC TTA CTT TTC CAT TGA GT-3'

ApoB " G - " (4):
 5'-CTTACTTGAA TTCCAAGAGC ACACGGTCTT CAGTGAAGCT GCAGGGCACT-3'

ApoB " A - " (5):
 5'-CTTACTTGAA TTCCAAGAGC ACACAGTCTT CAGTGAAGCT GCAGGGCACT-3'

ApoB " G - " (4) EQ3053/EQ3213(
 1, 6) 153bp PCR , 30.4g ApoB " A - " (5)
 - E PCR - 3.

ApoB " " " A - " (5) - EQ3157
 /EQ3176(7, 8) 145bp PCR , ApoB " " " G -
 " (4) PCR - 3.
 - EQ3053/EQ3213 EQ3157/EQ3176 .

3

DNA

LNA DNA 가 PCR .
 2 LNA - EQ 3053 ApoB3500 (G -
)

LNA - EQ 3053 EQ 3053 DNA

:

hus, Denmark 1) LNA DNA HPLC (DNA Technology, Aar

PCR

(Eppendorf - Netheler - Hinz GmbH, Hamburg, Germany)

0.5M

PCR

. dNTP

(Amersham Pharmacia Biotech AB, Uppsala, Sweden)

:

LNA - 6) 1 μ M EQ 3053(1) 1 μ M EQ 3213(

PCR - 6) 1 μ M EQ 3647(9) 1 μ M EQ 3213(

LNA DNA :

dATP, dGTP, dTTP, dCTP: 200 μ M1.5mM $MgCl_2$ 5 μ l 10 x GeneAmp PCR (Perkin - Elmer corporation, Norwalk, CT, USA)1 μ M EQ 3053, 71 μ M EQ 3213, 8

1 AmpliTaq Gold^R (Perkin - Elmer cat. no. N808 - 0240, Perkin - Elmer corporation, Norwalk, CT, USA)

2 μ l DNA (50ng / μ l).50 μ l

ApoB " G - " " A - " 2

:

: 95 15

: 35 (94 30 ; 55 30 ; 72 30)

: 72 10

4

PCR 2% 가 가 , 2
GelStar^R (FMC BioPorducts, Rockland, Maine, USA) .

4 , ApoB " G - " (4)
EQ3053/EQ3213(1, 6) 153bp PCR , ApoB " A - " (
5) - PCR - 4.

EQ3053 (서열번호 1): 5'-CCT ACT TGA ATT CCA AGA GCA CAC **G**^{LNA}-3'
EQ3213 (서열번호 6): 5'-GTT TTT CGT ACT GTG CTC CCA GAG-3'

ApoB " G - " (4):
5'-CTTACTTGAA TTCCAAGAGC ACAC**G**GTCTT CAGTGAAGCT GCAGGGCACT-3'

ApoB " A - " (5):
5'-CTTACTTGAA TTCCAAGAGC ACAC**A**GTCTT CAGTGAAGCT GCAGGGCACT-3'

, " G - " " A - " DNA - EQ
3674/EQ3213(9, 6) 153bp PCR - 4.
EQ3647 (서열번호 9): 5'-CCT ACT TGA ATT CCA AGA GCA CAC G-3'
EQ3213 (서열번호 6): 5'-GTT TTT CGT ACT GTG CTC CCA GAG-3'

DNA - LNA - 가 ApoB R 3500Q

4

(Roche) (Lightcycler) LNA

LNA - PCR , (Roche Diagnostics GmbH, Mannhe
im, Germany) PCR 1 Tm
PCR .

ApoB R3500Q " G - " :

:

1 LNA (DNA Technology, Aar
hus, Denmark) DNA HPLC .

PCR

PCR

- :
- 2 $\mu\ell$ - DNA SyBR I (Roche Diagnostics GmbH, Mannheim, Germany).
- , PCR , SyBR Taq .
 - 1 $\mu\ell$ DNA (50ng / $\mu\ell$)
 - 1 μ M EQ 3053(1) 1 μ M EQ 3213(6)
 - 3mM MgCl₂

20 $\mu\ell$

" G - " " A - " 가 . ApoB " G -
" " A - " 2 .

:

: 95 2 , 20 /sec

: 44 (95 0 ; 55 5 ; 72 7)

Tm :

1: 95 , 0

2 : 65 . 10

3: 95 , 0 ; 0.1 /sec.

: 4 , 30

" - EQ3053/EQ3213(1, 6) " G - " " A -
" - PCR - 5

6 , - EQ3053/EQ3213 82 Tm PCR .

EQ3053/EQ3213 -

(57)

1.

a) Q' A A , LNA Q ' A ;

b) ;

c) b) A Q Q' Q' .

2.

a) Q' A A , LNA Q ' A ;

b) ;

c) , ;

d) c) Q' A A , LNA Q A ;

e) c) d) 가 ;

f) Q Q' . A

3.

a) A Q' A A , LNA Q ' ;

b) ;

c) , ;

d) c) Q A Q' A A , LNA ;

e) c) d) 가 ;

f) Q Q' A

4.

a) A LNA ;

b) ;

c) b) , A

5.

a) A LNA ;

b) ;

c) , ;

d) c) A 가 ;

e) c) d) 가 ;

f) A

6.

a) A , LNA ;

b) ;

c) , ;

d) c) A 가 ;

e) c) d) 가 ;

f) 가 A

7.

- a) , A
가 LNA :
b) ;
c) b) A
.

8.

- a) , A
가 LNA :
b) ;
c) , ;
d) b) A
c) 가 LNA ;
e) c) d) 가 ;
f) A
.

9.

- 1 8 , A가
A A .

10.

- 1 9 , A 가
LNA .

11.

1 10 , 가 a) :

5'-Nu_a(Nu_bLNA_c)_mNu_dA_eNu_f-3' a)

, A A LNA ; LNA LNA ; Nu
; a,b,c,d f 0 30 , m 1 8 , e 1 6 , , a,b,c,d,e f
5 .

12.

11 , a a 10 , b 0 , c 4 , d 8 , e 1 , f 0 , m 0 .

13.

11 , a a 0 , b 1 , c 1 , d 8 , e 1 , f 0 , m 5 .

14.

11 , a a 10-30 , b 0 , c 0 , d 0 , e 1-4 , f 1-8 , m
0 .

15.

11 , a a 10-30 , b 0 , c 0 , d 0 , e 1-4 , f 1 , m
0 .

16.

11 , a a 10-30 , b 0 , c 0 , d 0 , e 1-4 , f 0 , m
0 .

17.

11 , a a 10-30 , b 0 , c 0 , d 0 , e 1 , f 0 , m 0
.

18.

11 , a a 10 , b 0 , c 0 , d 0 , e 4 , f 8 , m 0 .

19.

11 , a a 24 , b 0 , c 0 , d 0 , e 1 , f 0 , m 0 .

20.

19, 가

21.

20 , 가 (array format)

22.

19, 가

23.

20 22 , .

24.

1 21 , 가

25.

4 21 , 가

26.

25 , 가 .

27.

28.

27, 가 (archae),

29.

27

30.

27

31.

28 30 ,

32.

28 30 , ,

33.

28 30 ,

34.

28 30 , ,

35.

28 30 ,

36.

28 30 , ,

37.

28 30 ,

38.

28 30 , (life style)

39.

28 30 ,

40.

38 , , 가 ,

41.

27 40 , .

42.

1 6 , 가 .

43.

42 , 가 DNA .

44.

42 , 가 DNA .

45.

44 , DNA 가 Taq, Pfu, Pwo Tth .

46.

42 , 가 RNA .

47.

7 8 , 가 .

48.

47 , 가 가 .

49.

a) ;

b) ;

c) Q' A A LNA Q A
Q' , A Q
.

50.

a) ;

b) ;

c) ;

d) Q' A A LNA Q A
 , A Q
 Q' .

51.

a) ;

b) ;

c) A LNA A
 ,
 .

52.

a) ;

b) ;

c) ;

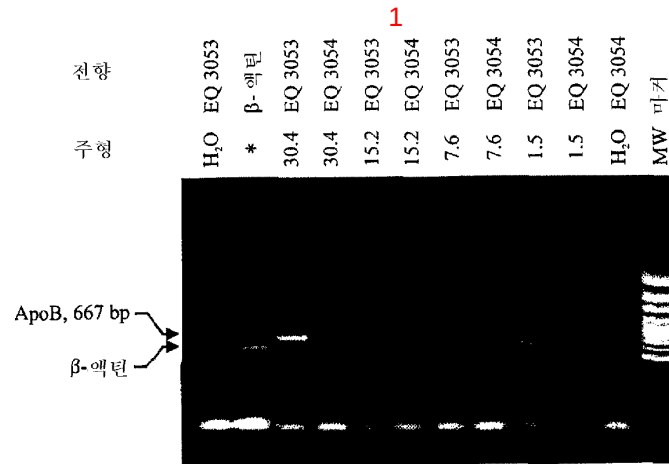
d) A LNA A
 ,
 .

53.

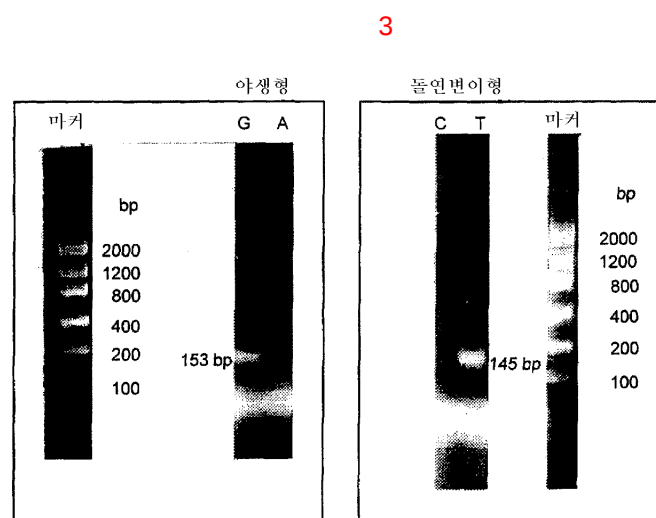
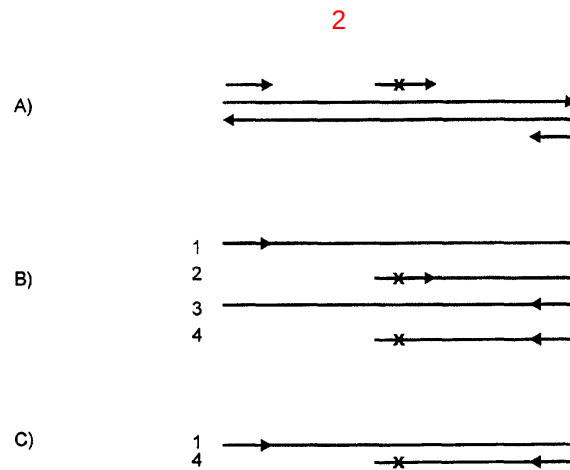
a) ;

b) ;

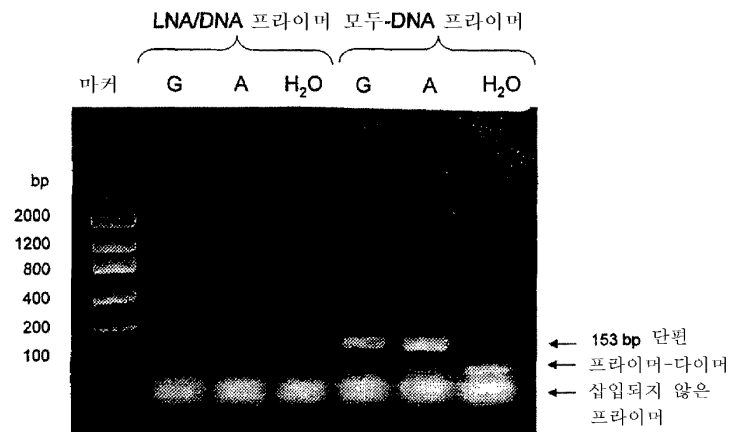
c) 가 A A
 A LNA A
 ,
 .



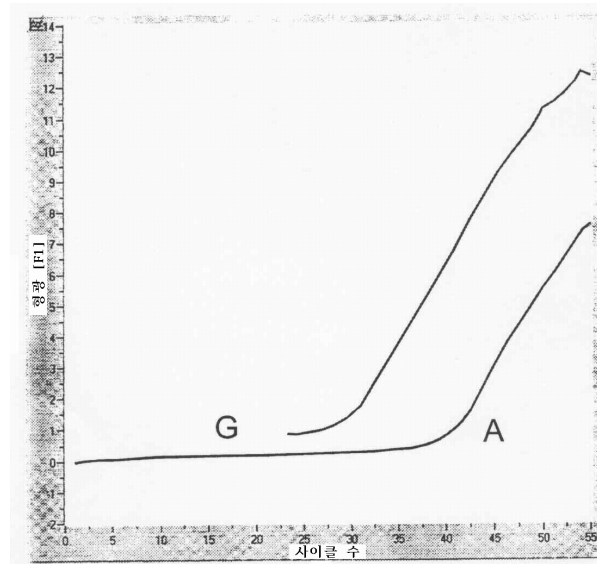
* 계놈 DNA



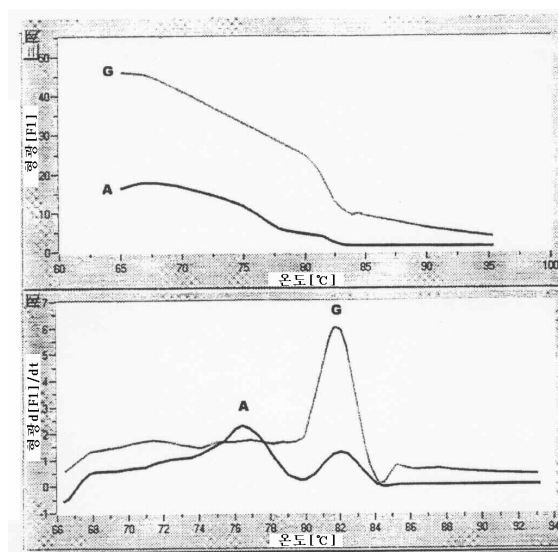
4



5



6



<110> Exiqon
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 <221> modified_base
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 <223> G LNA
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