

(19) (KR)  
(12) (B1)

(51) 。 Int. Cl.<sup>7</sup>  
C12N 15/66

(45)  
(11)  
(24)

2004 10 15  
10-0453279  
2004 10 07

(21) 10-2000-0022477  
(22) 2000 04 27

(65)  
(43)

10-2001-0097953  
2001 11 08

(73) 1 452-2

(72) 1015 806

5 504 1301

8 1517 901

423-1 2 403

2 68-475

3가1-871

(74)

:

(54) 3'  
E c l H K I

, E c l H K I

c/HKI 3' , E c l H K I E  
4 E c l H K I T-  
(Gel Elution) pSL - T, pSL - G, pSL - C, pSL - A  
가, T-  
PCR PCR (blue colony) 가 ,

EcIHKI\* \*

1 , T-  
 1a pGEM<sup>R</sup>-T 1b pGEM<sup>R</sup>-T (Easy cloning Vector)  
 2 , T- PCR 가 DNA 2 2  
 3 , *EcI*HKI (restriction site)  
 , ( ) *EcI*HKI  
 4 , *EcI*HKI  
 5a , *EcI*HKI 3' (dTMP) 가 *EcI*HKI  
 5b , *EcI*HKI (T) *EcI*HKI  
 6 , *EcI*HKI 3' (dGMP) 가 *EcI*HKI  
 7 , *EcI*HKI 3' (dCMP) 가 *EcI*HKI  
 8 , *EcI*HKI 3' (dAMP) 가 *EcI*HKI  
 9 , 가 DNA 3' (dTMP), (dAMP), (dGMP), (d  
 CTP) 가 4 (dTMP), (dAMP), N (dTMP), (d  
 dAMP), (dGMP), (dCTP)  
 10 , 가 DNA 3' (dTMP), (dAMP), (dGMP),  
 (dCTP) 가 4 pSL-T, pSL-G, pSL-C, pSL-A  
 10a pSL-T , 10b pSL-G , 10c pSL-C , 10d pSL-A

*cI*/HKI 3' , *EcI*/HKI *E*  
 (primer; 'PCR ' )  
 (polymerase  
 chain reaction; 'PCR' )  
 가, PCR PCR ,  
 PCR DNA PCR  
 , PCR (Taq. DNA polymerase)  
 (dTMP) 3' (dAMP)  
 (dAMP) 3' 가  
 T- DNA 가 PCR 3' (dAM  
 P) 가 (Hu, DNA Cell Biol. 12, 763 (199

3); Magnuson et al., BioTechniques 21, 700, (1996)). , PCR 3' (dAMP)  
 , DNA 가 (dAMP)  
 (dGMP), (dCMP), (dTMP) , PCR  
 (primer) 5' ( , 5'-TCAG..) (dAMP) ( , 5'-GTTC..)  
 (dCMP), (dGMP), (dTMP) (blunt end) 가, (dAMP) (primer)  
 PCR (dAMP) 가 3' , PCR  
 (dTMP) , (dCMP), (d  
 T- , PCR (ligation) (dTMP) 가  
 , PCR PCR  
 3' , T- , 3' (dTMP)  
 , T- DNA Eco RV 3'  
 (blunt end) (terminal deoxynucleotidyl transferase)  
 (dTMP)  
 가 , (dTMP)  
 (dTMP) , T- , PCR  
 (translation reading frame shift) (self ligation) PCR (white colon  
 y)가

PCR  
 , 3' (dTMP)  
 PCR (dAMP), (dGMP), (dCTP) 가  
 4 (nucleotide residue) , PCR 4  
 (ligation) , (A, T, G, C) 가  
 Ecl/HKI , 4

3' (unpaired single overhang) 가  
 가 DNA (linearized double strand vector) , Ecl/HKI  
 , DNA  
 , (restriction site)  
 3' 가 Ecl/HKI , Ecl/HKI

Ecl/HKI ( 3):  
 5' GACNN N NNGTC 3'  
 3' CTGNN N NNCAG 5'  
 N A, C, G, T , N  
 , Ecl/HKI Ecl/HKI ( ) 3' ( )  
 , Ecl/HKI N)가 , Ecl/HKI  
 , Ecl/HKI Ecl/HKI Ecl/HKI Ecl/HKI  
 /HKI (dAMP), (dCMP), (dGMP), (dTMP) N , Ec  
 Ecl/HKI 3'

, *Ecl* HKI 2 , 2 *Ecl* HKI  
*Ecl* HKI N *Ecl* HKI N , GC  
 가 , A T, G C , *Ecl* HKI N , GC  
 , *Ecl* HKI 3' T, G, C, A 가  
*Ecl* HKI ( 4 ).  
*Ecl* HKI *Ecl* HKI *Ecl* HKI *Ecl* HKI *Ecl* HKI  
 가 , pGEM , pPUC , pBluescript  
*Ecl* HKI *Ecl* HKI *Ecl* HKI *Ecl* HKI *Ecl* HKI  
 , *Ecl* HKI , *Sac* II *Pst* I *Sac* II *Pst* I *Ecl* HKI  
*Hind* III, *Sph* I, *Apa* I, *Nd*  
*Ecl* HKI  
 , *Ecl* HKI 2 *Ecl* HKI  
 (spacer) 가 가 가 가 가  
 가 가 가 가 가  
 , (spacer) , 가  
 가, 3' 가 , *Ecl* HKI  
 , *Ecl* HKI PCR PCR  
 , *Ecl* HKI (LacZ) 가 (LacZ)  
 (open reading frame) , (LacZ) 가 (LacZ)  
 (LB) (plate) (Lac Z) (IPTG) (X-gal) , *Ecl* HKI 가  
 (Lac Z) (Lac Z) (Lac Z) (Lac Z) ,  
 (IPTG) (X-gal) PCR (Lac Z) (LB)  
 가, *Ecl* HKI PCR  
 , PCR  
 PCR (colony) PCR 가  
 , *Ecl* HKI  
 PCR (re  
 porter gene) , GFP(green fluorescence protein) , luc(luciferase) , (Lambd  
 a lethal gene)  
*Ecl* HKI (spacer) , *Ecl* HKI 3'  
 (LacZ)  
*Sac* II *Pst* I *Ecl* HKI 1 8 ST-  
 1 ST-2, ST-3 ST-4, ST-5 ST-6, ST-7 ST-8 (' )  
 , *Ecl* HKI  
 1 2 ST-1 ST-2 100  
 (Hybridization reaction) *Ecl* HKI  
 [ 1; ST-1 ]  
 5'-GGACCGTGAGTCTTTGACAGAGAGTCTGCA-3'  
 [ 2; ST-2 ]  
 5'-GACTCTCTGTCAAAGACTCACGGTCCGC-3'  
 [ *Ecl* HKI (T) ] ( 5a )  
 5'-GGACCG I GAGTCTTTGACAGAGAGTCTGCA-3' (ST-1 )  
 3'-CGCCTGGCACTCAGAAACTGTC I CTCAG-5' (ST-2 )  
 -----  
*Sac* II *Ecl* HKI(spacer) *Ecl* HKI *Pst* I  
 ST-1 ST-2 *Ecl* HKI (T)( 5a ) *Ecl* HKI  
*Ecl* HKI 3' (dTMP)가 ( 5b ).  
 5'-GGACCG I (3') GAGTCTTTGACAGA GAGTCTGCA-3'  
 3'-CGCCTGGC ACTCAGAAACTGTC (3') I CTCAG-5'  
 , *Ecl* HKI *Ecl* HKI , 가  
 , 3' (T)가  
*Ecl* HKI (dGMP) 3 4 SG-1  
 SG-2 *Ecl* HKI (T) *Ecl* HKI (G) (

6 ).  
 [ 3; SG-1 ]  
 5'-GGACCGGGAGTCTTTGACAGCGAGTCTGCA-3'  
 [ 4; SG-2 ]  
 5'-GACTCGCTGTCAAAGACTCCCGGTCCGC-3'  
 , *Ecl* HKI (dCMP) 5 6 SC-1  
 SC-2 *Ecl* HKI (T) *Ecl* HKI (C)

( 7 ).  
 [ 5; SC-1 ]  
 5'-GGACCGCGAGTCTTTGACAGGGAGTCTGCA-3'  
 [ 6; SC-2 ]  
 5'-GACTCCCTGTCAAAGACTCGCGGTCCGC-3'  
 , *Ecl* HKI (dAMP) 7 8 SA-1  
 SA-2 *Ecl* HKI (T) *Ecl* HKI(A)

( 8 ).  
 [ 7; SA-1 ]  
 5'-GGACCGAGAGTCTTTGACAGTGAGTCTGCA-3'  
 [ 8; SA-2 ]  
 5'-GACTCACTGTCAAAGACTCTCGGTCCGC-3'  
 1 8 4 *Ecl* HKI *Sac*  
 II *Pst* I 가 *Sac* II *Pst* I *Sac*  
 가 , *Ecl* HKI 가 pGEM (Promega ), pPUC (New England Biolabs  
 ), pBluescript (New England Biolabs ) , ori  
 (aphicilin), (tetracycline) ,  
 multi cloning site ; , *Apa* I, *Hind* I, *Nco* I, *Eco* RI, *Sac* I, *Bam* HI, *Pst* I, *Nde* I; 1 : pGEM-T  
 ) , DNA , *Ecl* HKI 가 PCR  
 , tac (promoter) T7 (promoter)  
 (promoter)  
*Ecl* HKI *Sac* II *Pst* I pGEM-5zf(P  
 romega ) 4 *Ecl* HKI  
 (Sambrook et al., Cold Spring Harbor Lab. Press, New York, 1989)  
 pSL - Te, pSL - Ae, pSL - Ce, pSL - Ge  
 pSL - Te, pSL - Ae, pSL - Ce, pSL - Ge *Ecl* HKI(Promega )  
 (gel elution) ( 9). (gel  
 elution) , *Ecl* HKI 1% 가 (agarose gel) (electrophoresis)  
 (Promega ,cat.# A7170) 가 (purity)가  
 (high activity)  
 3' 3'-T, 3'-A, 3'-G, 3'-C 가  
 pSL - T, pSL - A, pSL - G, pSL - C ( 10).  
 pSL - T, pSL - A, pSL - G, pSL - C 가  
 PCR ,  
 가 , DNA  
 , *Ecl* HKI *Ecl* HKI N  
 , *Ecl* HKI 3'

[ 1; *Ecl* HKI ]  
 100 pmol ST-1 ST-2( 1 2) 4μl , 10X (oligonucl  
 eotide buffer; 0.1M NaH<sub>2</sub> PO<sub>4</sub> ) 10μl, 3 82μl 가 100μl 90 10  
 , MgCl<sub>2</sub> , 14000g 20 가 10mM 100%  
 3 10μl , ST-1 ST-2가 *Ecl* HKI  
 (T)  
 ST-1 ST-2 , SG-1 SG-2( 3 4), SC-1 SC-2( 5 6), SA-1  
 SA-2( 7 8) , *Ecl* HKI (T) *Ecl* HKI (G),  
*Ecl* HKI (C), *Ecl* HKI (A)  
 [ 2; pSL - Te, pSL - Ae, pSL - Ce, pSL - Ge ]

1  
 -5zf (Promega )  
 se buffer) 1μℓ, T4 DNA  
 SL - Te, pSL - Ae, pSL - Ce, pSL - Ge  
 [ 3; pSL - Te, pSL - Ae, pSL - Ce, pSL - Ge  
 CaCl<sub>2</sub> (Sambrook et al., Cold Spring Harbor Lab. Press, New York, 1989, p.1.82 - 1.84)  
 JM109 (competent cell)  
 200μℓ  
 (IPTG)  
 . *Ecl* HKI 가  
 (blue colony)  
 1ml (LB)  
 ard plus mini-prep. kit  
*Ecl* HKI  
 [ 4; pSL - T, pSL - G, pSL - C, pSL - A  
 pSL - Ae, pSL - Te, pSL - Ce, pSL - Ge 20μℓ, *Ecl* HKI (Promega ) 1μℓ, E (buffer E)  
 3μℓ, 3 6μℓ 37 4 , *Ecl* HKI *Ecl* HKI  
 , 2 *Ecl* HKI 가 , *Ecl* HKI  
 , pSL - C, pSL - A , Promega DNA Clean-up , pSL - T, pSL - G,  
 1Mℓ 37 5 DNA 1.5Mℓ  
 , 80% (isopropanol) 2Mℓ  
 3 50μℓ 5  
 pSL - T, pSL - G, pSL - C, pSL - A (WPA )  
 < 1>  
 omega pGEM - T (*Ecl* HKI (cat # A3600; 3' pSL - T, pSL - G, pSL - C, pSL - A Pr  
 (Spectrometer, WPA ) 50 ng , 300 bp  
 PCR (Ligation reaction) , (Sam  
 brook et al., Cold Spring Harbor Lab. Press, New York, 1989) (IPTG)  
 (X - Gal) (LB) 37 12 (LB)

[ 1 ]

백터	1회			2회			3회			4회		
	white colony	Blue colony	clone /white colony	white colony	Blue colony	clone /white colony	white colony	Blue colony	clone /white colony	white colony	Blue colony	clone /white colony
pGEM-T vector	71	32	10/10	67	35	9/10	83	41	10/10	75	34	10/10
pSL-T vector	65	7	9/10	75	5	10/10	79	11	10/10	69	9	10/10
pSL-G vector	43	3	10/10	39	7	9/10	38	6	9/10	41	8	10/10
pSL-C vector	44	5	9/10	43	10	10/10	41	5	10/10	39	7	10/10
pSL-A vector	25	2	9/10	31	3	10/10	29	4	9/10	24	6	10/10

PCR (white colony)  
 300bp DNA 가 , 가  
*Sac* II *Pst* I PCR

1 PCR pSL-T, pSL-G, pSL-C, pSL-A (Blue colony) 가 , *Ecl*/HKI pGEM-T  
 (PCR) pGEM-T pSL-T 가  
 7:4:4:3(pSL-T;-G;-C;-A) PCR PCR  
 G : pSL-C : pSL-A = 7 : 4 : 4 : 3 PCR PCR , pSL-T : pSL-PCR  
 pSL-T, pSL-G, pSL-C, pSL-A T- 가  
 3'-C, 3'-G, 3'-A PCR  
 PCR (primer) 3'  
 PCR (sampling) pSL-T, pSL-G, pSL-C,  
 pSL-T, pSL-G, pSL-C, pSL-A  
 PCR , *Ecl*/HKI 4- (four-base vector)

4 *Ecl*/HKI T-  
 (Gel Elution) pSL-T, pSL-G, pSL-C, pSL-A  
 가, T-  
 PCR 가  
 , PCR

(57)

1. DNA PCR  
 DNA 가 3' (end) (dAMP), (dGMP), (dCMP)  
 (dTMP) 가 DNA (linearized double strand vector) 3'  
 (unpaired single overhang) 가 ,  
 가 DNA 3' PCR  
 4 , 4

2.

3.

4.

5.

6.

*Ecl*/HKI *Ecl*/HKI *Ecl*/HKI , *Ecl*/HKI

*Ecl*/HKI

5' GACNNN ↓ NNGTC 3'

3' CTGNN ↓ NNNCAG 5'

*Ecl*/HKI N *Ecl*/HKI N

7.

*Ecl*/HKI

6 , *Ecl*/HKI *Ecl*/HKI 가  
 (reporter gene) (open reading frame) *Ecl*/HKI

8.  
 7 , (reporter gene) (LacZ) *Ecl*/HKI

9.  
 6 , *Ecl*/HKI , 1 2 가 가  
 가 , 3 4 5 6 7 8 가 가

10.  
*Ecl*/HKI 2 가 , 1 8

11.  
 6 9 *Ecl*/HKI *Ecl*/HKI *Ecl*/HKI

12.  
*Ecl*/HKI *Ecl*/HKI 가 .

13.  
 11 , (LacZ) , *Ecl*/HK  
 (reporter gene) (open reading frame)

14.

15.  
 3' (3'-end) 가 가 DNA

6 9 *Ecl*/HKI , *Ecl*/HKI *Ecl*/HKI

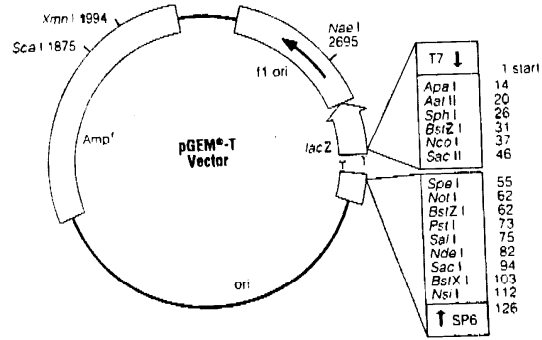
*Ecl*/HKI (ligation reaction) 1 ;  
*Ecl*/HKI *Ecl*/HKI 2 ;  
*Ecl*/HKI 3

16.  
 15 , *Ecl*/HKI 가 (reporter gene)가  
*Ecl*/HKI (open read  
 ing frame) 1 ;  
*Ecl*/HKI 가 , 1 2

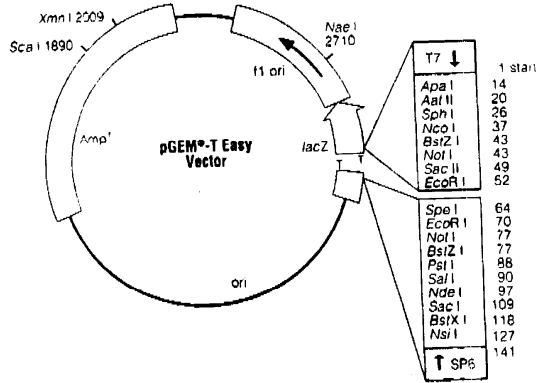
17.



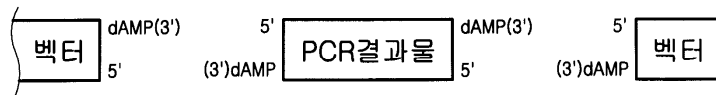
1a



1b



2

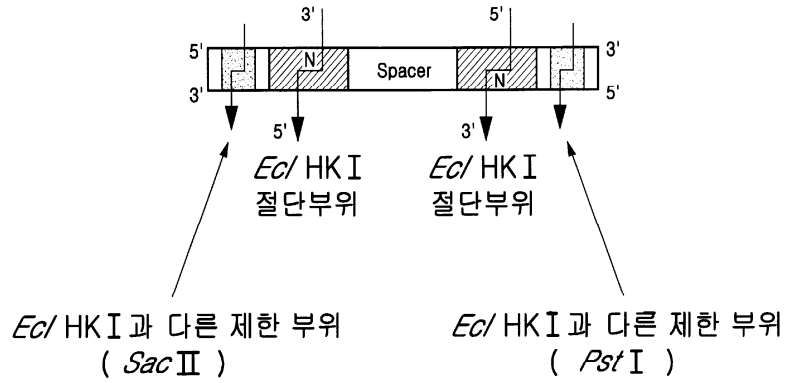


3

5' GACNNN ↓ NNGTC 3'

3' CTGNN ↓ NNNCAG 5'

4



5a

5'-GGACCGTGAGTCTTTGACAGAGAGTCTGCA-3' (ST-1 primer)  
 3'-CGCCTGGCACTCAGAACTGTCTCTCAG-5' (ST-2 primer)  
 -----  
 SacII *Ec*HKI(spacer)*Ec*HKI PstI

5b

5'-GGACCGT(3') GAGTCTTTGACAGA GAGTCTGCA-3'  
 3'-CGCCTGGC ACTCAGAACTGTC (3')TCTCAG-5'

6

5'-GGACCGGGAGTCTTTGACAGCGAGTCTGCA-3'  
 3'-CGCCTGGCCCTCAGAACTGTCGCTCAG-5'

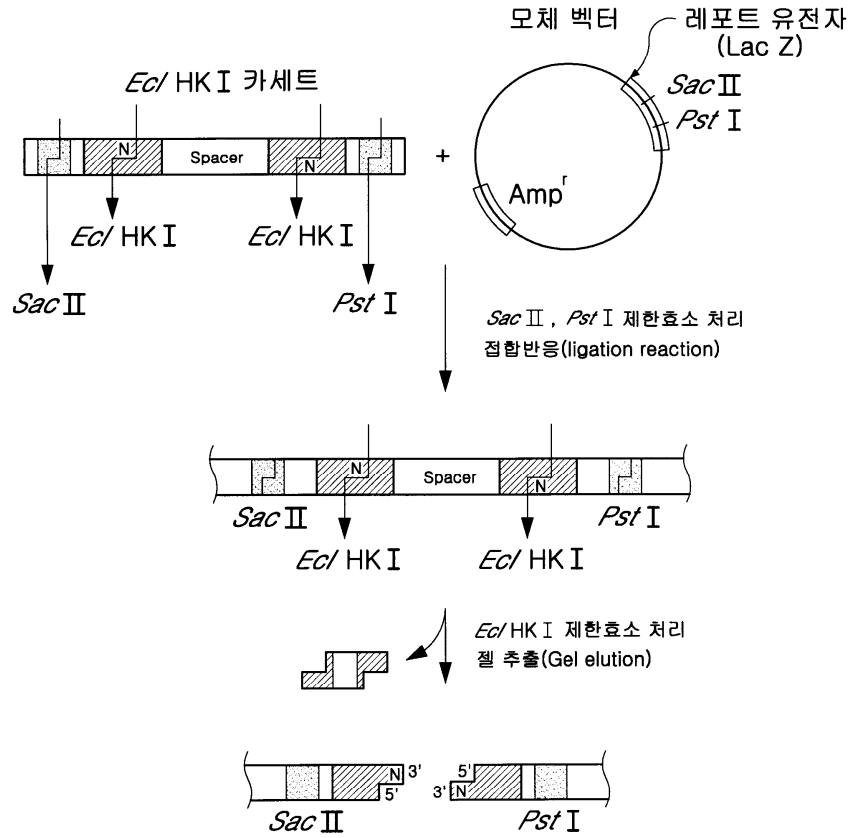
7

5'-GGACCGGAGTCTTTGACAGGGAGTCTGCA-3'  
 3'-CGCCTGGCGCTCAGAACTGTCCTCAG-5'

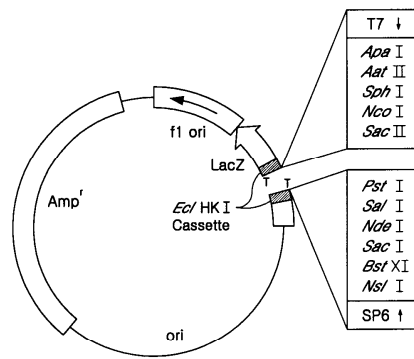
8

5'-GGACCGAGAGTCTTTGACAGTGAGTCTGCA-3'  
 3'-CGCCTGGTCTCAGAACTGTCACTCAG-5'

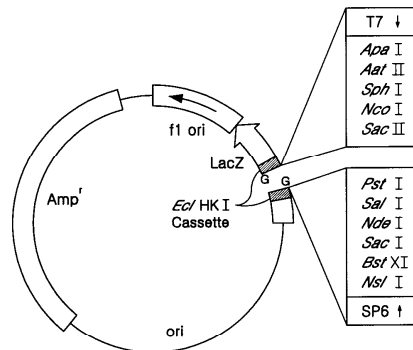
9



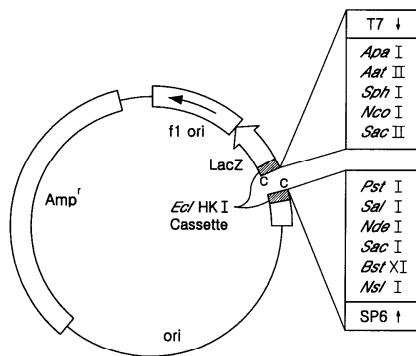
10a



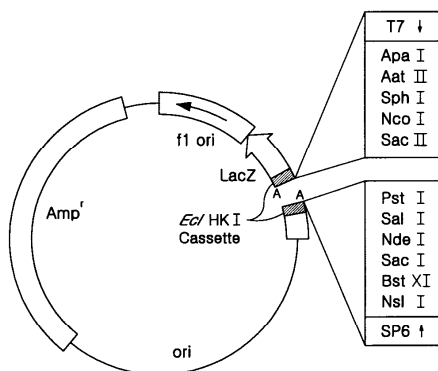
10b



10c



10d



<110> Seoul Scientific Co., Ltd <120> a vector wherein each of 3' end has a projected base same or nonc  
 <130> complementary to each other, EclHKL cassette, and methods for using them <130>  
 <140> pa00048 <160> 8 <170> KOPATIN 1.5 <210> 1 <211> 30 <212> DNA <213> Artificial Sequence <220> <223>  
 a primer for EclHKL cassette <400> 1 ggaccgtgag tctttgacag agagtctgca  
 30 <210> 2 <211> 28 <212> DNA <213> Artificial Sequence <220> <223>  
 a primer for EclHKL cassette <400> 2 gactctctgt caaagactca cgttccgc  
 28 <210> 3 <211> 30 <212> DNA <213> Artificial Sequence <220> <223> a primer for EclHKL  
 cassette <400> 3 ggaccgggag tctttgacag cgagtctgca  
 30 <210> 4 <211> 28 <212> DNA <213> Artificial Sequence <220> <223> a primer for EclHKL  
 cassette <400> 4 gactcgctgt caaagactcc cgttccgc 28 <210>  
 5 <211> 30 <212> DNA <213> Artificial Sequence <220> <223> a primer for EclHKL cassette <400>  
 5 ggaccgagag tctttgacag ggagtctgca 30 <210> 6 <211>  
 28 <212> DNA <213> Artificial Sequence <220> <223> a primer for EclHKL cassette <400> 6 gact  
 ccctgt caaagactcg cgttccgc 28 <210> 7 <211> 30 <212> D  
 NA <213> Artificial Sequence <220> <223> a primer for EclHKL cassette <400> 7 ggaccgagag tctttg  
 acag tgagtctgca 30 <210> 8 <211> 28 <212> DNA <213> A  
 rtificial Sequence <220> <223> a primer for EclHKL cassette <400> 8 gactcactgt caaagactct cgttccgc