

|      |                                                         |             |              |                               |
|------|---------------------------------------------------------|-------------|--------------|-------------------------------|
|      | (19)<br>(12)                                            | (KR)<br>(A) | (11)<br>(43) | 10-2010-0041560<br>2010 04 22 |
| (51) | Int. Cl.                                                | (71)        | 100          |                               |
|      | <i>C12N 15/82</i> (2006.01) <i>C12N 15/29</i> (2006.01) |             |              |                               |
|      | <i>A01H 1/00</i> (2006.01) <i>A01H 1/06</i> (2006.01)   |             |              |                               |
| (21) | 10-2008-0100802                                         |             |              |                               |
| (22) | 2008 10 14                                              | (72)        | 900          |                               |
|      | 2008 10 14                                              |             |              |                               |
|      |                                                         |             |              | 119 801                       |
|      |                                                         |             |              | 102 1106                      |
|      |                                                         | ( )         |              |                               |
|      |                                                         | (74)        |              |                               |
|      | : 8                                                     |             |              |                               |

(54)

(57)

, *bar* *CP4-EPSPS*, - (marker free)  
; *bar* *CP4-EPSPS*,  
; - (marker free)  
(glufosinate); (glyphosate)

- 6

A. 바스타 처리



비형질전환체

형질전환체-25

B. 글리포제이트 처리



비형질전환체

형질전환체-25

(72)

110-14

111 1401

1 25-27

20050301-034-466-007-01-00

21

2004 04 01 ~2008 12 31

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1

*bar* *CP4-EPSPS* ,  
;  
;

, - (marker free) .

2

1 , (*Festuca arundinacea*) .

3

1 , 1 p35S-EPSPS-B .

4

*bar* *CP4-EPSPS* ,  
, - (marker free) .

5

4 , (*Festuca arundinacea*) .

6

4 , 1 p35S-EPSPS-B .

7

4 .

8

4 - (glufosinate)  
(glyphosate) .

[0001]

*bar* *CP4-EPSPS* ,  
- (marker free)  
- (marker free)

.

[0002]

(*Festuca arundinacea* Schreb.) 15 21

,  
(Buckner et al, 1979, Agronomy 20:1-8).

[0003]

( , 2000, , 20(3):159-173).

[0004]

(Monsanto) 1996  
(roundup ready)  
( , ( )  
(Duke S.O., 2005, *Pest Manag. Sci.*, 61:211-2118),  
(selection marker)  
(  
).

[0005]

"  
"  
(Owed & Zelaya, 2005, *Pest Manag. Sci.*, 61:301-311; Arregui et al.,  
2003, *Pest manag. Sci.*, 60: 163-166; Sandermann H., 2006, *Trends in Plant Sci.*, 11: 324-328).

[0006]

[0007]

[0008]

*bar CP4-EPSPS*  
(phosphinothricin, PPT)  
5  
(Tachibana et al., 1986, *J. Pesticide Sci.*, 11:297-304),

( 1kg/ha ),

enolpyruvylshikimate-3-phosphate synthase) 10 EPSPS (5-  
(Dill G.M., 2005, *Pest Manag. Sci.*, 61:211-218).  
(Sandemann  
H., 2006, *Trends in Plant Science* 11:324-328).

- [0009] *bar* *CP4-EPSPS* (marker free)
- [0010] *bar* *CP4-EPSPS* (marker free)
- [0011]
- [0012] (glufosinate) (glyphosate)
- [0013]
- [0014]
- (Sandermann H. 2006, *Trends in Plant Sci.*, 11: 324-328),
- [0015] *bar* *CP4-EPSPS* (marker free)
- [0016] *bar* *CP4-EPSPS* *bar* pCAMBIA3300(Center for Application of Molecular Biology to International Agriculture, Australia) *bar* *CP4-EPSPS* 1 1 70%

80% , 90% , 95% %"  
 ( , )

[0017]

CP4-EPSPS

bar

[0018]

(*Festuca arundinacea*) , (*Festuca arundinacea*)  
 (*Festuca longifolia*)  
 (*Festuca arundinacea*) -31 ,

[0019]

CaMV 35S, , pEMU,  
 Clp , Clp " "  
 " DNA RNA  
 DNA " "  
 (constitutive) " "

[0020]

Ramy1 A , (phaseoline) , (NOS),  $\alpha$  -  
 tumefaciens) (Octopine) (agrobacterium

[0021]

(*Agrobacterium*) ,  
 , bar pCAMBIA 3300(Center for  
 Application of Molecular Biology to International Agriculture, Australia) CaMV 35S  
 CP4-EPSPS

[0022]

DNA ,  
 ( ) , DNA  
 /  
 (Krens, F.A. et al., 1982, Nature 296, 72-74; Negrutiu I. et al., June 1987, Plant Mol. Biol. 8, 363-373),  
 (Shillito R.D. et al., 1985 Bio/Technol. 3, 1099-1102),  
 (Crossway A. et al., 1986, Mol. Gen. Genet. 202, 179-185), (DNA RNA- )  
 (Klein T.M. et al., 1987, Nature 327, 70),  
 ( ) (EP 0 301 316  
 DNA  
 EP A 120 516 4,940,838

[0023]

(*Agrobacterium tumefaciens*), EHA  
 105 (*A. tumefaciens* EHA 105)  
 EHA 105 ( EHA 105/p35E-Bar) 2006 10 25  
 KCTC 11008BP

[0024]

, , MS (Murashige & Skoog, *Physiol. Plant* 15:473-497, 1962)  
 , 0.1 15 mg/L, 5 10 mg/L

[0025]

, (PCR) (Northern hybridization)  
 (shikimate)

[0026]

*bar* *CP4-EPSPS*  
 , - (marker free)

[0027]

, , ( )

[0028]

*bar*  
*CP4-EPSPS*

[0029]

(*Festuca arundinacea*) , (*Festuca arundinacea*)  
 (*Festuca longifolia*)  
 (*Festuca arundinacea*) -31 ,

[0030]

1 p35S-EPSPS-B ,

[0031]

, - (marker free)

[0032]

, -  
 (glufosinate) (glyphosate)

[0033]

[0034]

( )

[0035]

(Sandermann H. 2006, *Trends in Plant Sci.*, 11: 324-328),  
 (Duke et al., 2005, Outlooks on Pest Management -

October: 208-211).

[0036] , , ( ) .

[0037] , . , .

[0038] 1.

[0039]

[0040] ( -31 ) .  
70% 30 , 30%  
(sodium hypochlorite) 30 MS  
(6 mg/L 2,4-D(2,4-dichlorophenoxyacetic acid), 0.1 mg/L BA((6-benzyladenine), 500 mg/L  
L- , 100 mg/L - , 30 g/L , 5 g/L ) 6 .

[0041]

[0042] CaMV 35S  
*CP4-EPSPS* *bar*  
pCAMBIA3300 p35S-EPSPS::Bar ( 1 ).

[0043] EHA 105 , 100 mg/L  
(Km) YEP 28 , 2 3,500 rpm 10  
(MS , 200 uM (AS), 2 mg/L 2,4-D, 500 mg/L L- , 30 g/L ) OD<sub>600</sub>=1.0 .  
1 .  
(MS , 2 mg/L 2,4-D, 200 uM AS, 500 mg/L L- , 30 g/L , 2 g/L  
) 26 5 .

[0044]

[0045] 250 mg/L  
(post-culture) (MS , 500 mg/L (cefotaxime), 3 mg/L 2,4-D, 1mg/L BA, 1g/L  
(casein hydrolysate), 500 mg/L L- , 1 mg/L -HCl, 30 g/L , 3 g/L ) 7  
 . (N6 , 250 mg/L , 5 mg/L  
PPT(phosphinothricin), 1 mg/L 2,4-D, 3 mg/L BA, 1 g/L , 500 mg/L L- , 1 mg/L  
-HCl, 30 g/L , 5 g/L ) 3 (PPT)  
10 mg/L PPT 6  
 . 10 mg/L PPT 1/2MS 4  
 .  
2 .

[0046] 2:

[0047] (Cetyltrimethylammoniumbromide, CTAB) genomic DNA . PCR  
*bar* *CP4-EPSPS* (*bar* : 2 5'-  
CGGTCTGCACCATCGTCAACC-3' 3 5'-GTCCAGCTGCCAGAAACCCAC-3', *CP4-EPSPS* :  
4 5'-ACGATTTTCGACAGCACCTTC-3' 5 5'-

GTGACAGGGTTTTCCGACAC-3') .

[0048] , T-DNA genome ( 3 ).

[0049] PCR

20 ug RNA 10 ul DEPC-water , 20 ul RNA .

65 15 , 1.2% (formaldehyde agarose gel) 50V 40

10 × SSC 20 (capillary transfer)

RNA UV-crosslinker (crosslinking) 2 × SSC 5

, high stringency . ,

( 4 ). CP4-EPSPS, bar

CaMV35S .

[0050] , CP4-EPSPS (ELISA) Agdia PSP 74000/0096

PathoScreen Kit ELISA-DAS (Clark and Adams 1997, Journal of General Virology 34:475-483)

(0.168) 25 (2.480~2.675) ,

(1.084) CP4-EPSPS

( 5 ).

[0051] 3:

[0052] CP4-EPSPS

( 2006, 33:57-63 ).

( 1 ).

[0053] 1.

| 블페스큐 식물체     | 쉬키메이트 측정 (380nm 흡광도 증가) |                |                |
|--------------|-------------------------|----------------|----------------|
|              | 3 µg/ml 글리포제이트          | 10µg/ml 글리포제이트 | 30µg/ml 글리포제이트 |
| 비형질전환 블페스큐   | 27.40±5.49              | 29.03±2.87     | 46.87±4.53     |
| 형질전환 블페스큐-13 | 4.59±2.06               | 7.82±1.52      | 10.22±6.07     |
| 형질전환 블페스큐-16 | 4.32±1.52               | 4.80±1.71      | 4.70±0.29      |
| 형질전환 블페스큐-18 | 1.97±0.62               | 9.44±6.11      | 7.83±6.74      |
| 형질전환 블페스큐-21 | 2.35±0.97               | 5.10±0.97      | 8.42±4.84      |
| 형질전환 블페스큐-25 | 2.61±0.95               | 4.89±2.95      | 3.11±2.69      |

[0054]

[0055] , 8

(25 ) (18% ),

(95%, ) 1000 ( µ g/mL) 20ml 14

[0056] , 5 .

14 .

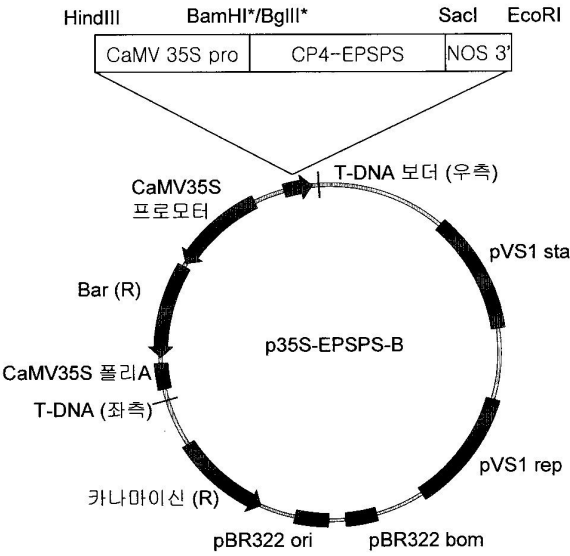
( 6 ).

[0057] 1 p35S-EPSPS-B .

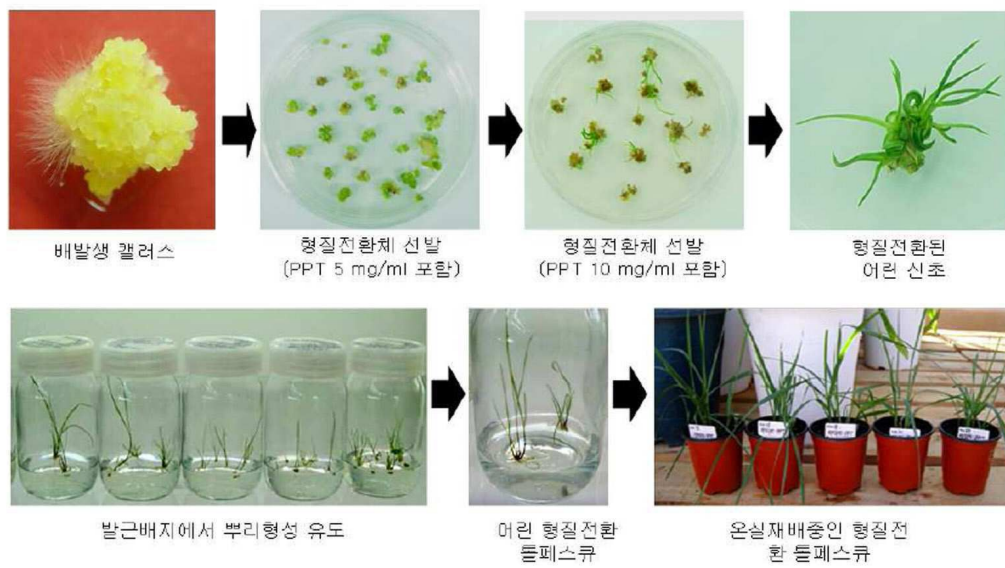
[0058] 2 , ,

|        |    |                          |           |                 |
|--------|----|--------------------------|-----------|-----------------|
| [0059] | 3  | PCR                      | (a) bar   | (bar CP4-EPSPS) |
|        |    |                          | NT        | , (b) CP4-EPSPS |
| [0060] | 4  | (Northern hybridization) | (a) bar   | (bar CP4-EPSPS) |
|        |    |                          | NT        |                 |
| [0061] | 5  | (ELISA)                  | CP4-EPSPS |                 |
|        |    |                          | CP4-EPSPS |                 |
| [0062] | 6  |                          |           |                 |
|        |    | A,                       |           |                 |
|        |    | ( : 18% )                | 3%(v/v)   | 14              |
|        |    |                          | 1000 /mL  |                 |
|        | 14 |                          |           |                 |

1

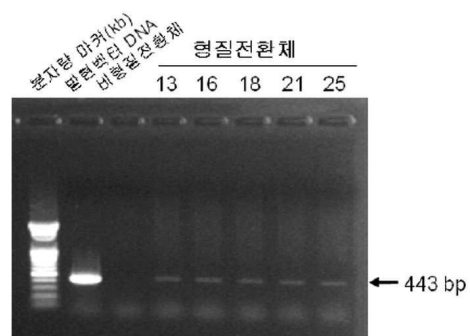


2

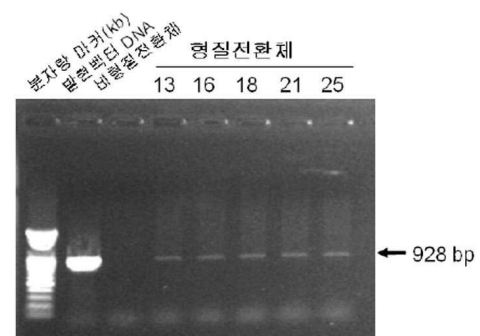


3

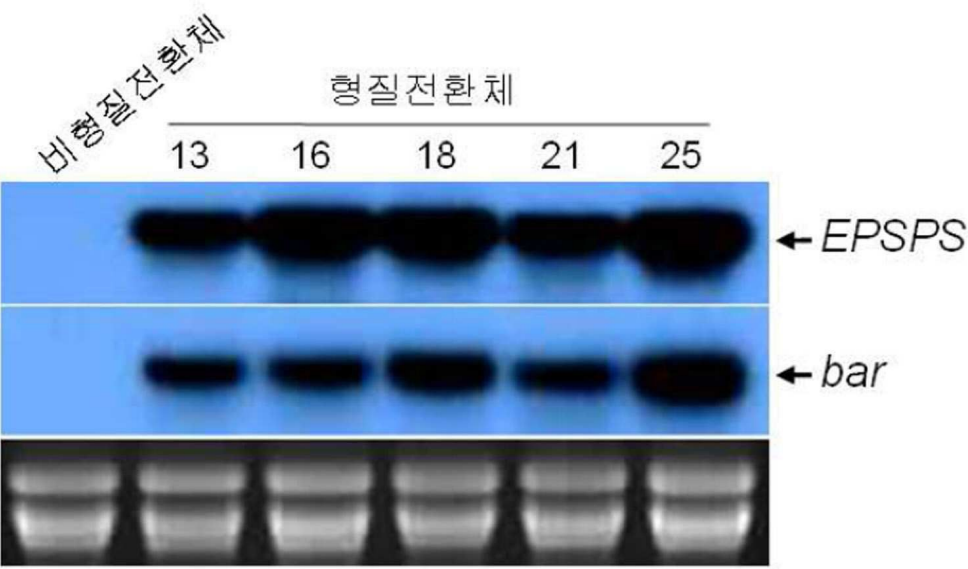
## A. bar 유전자의 PCR 확인



## B. EPSPS 유전자 PCR 확인

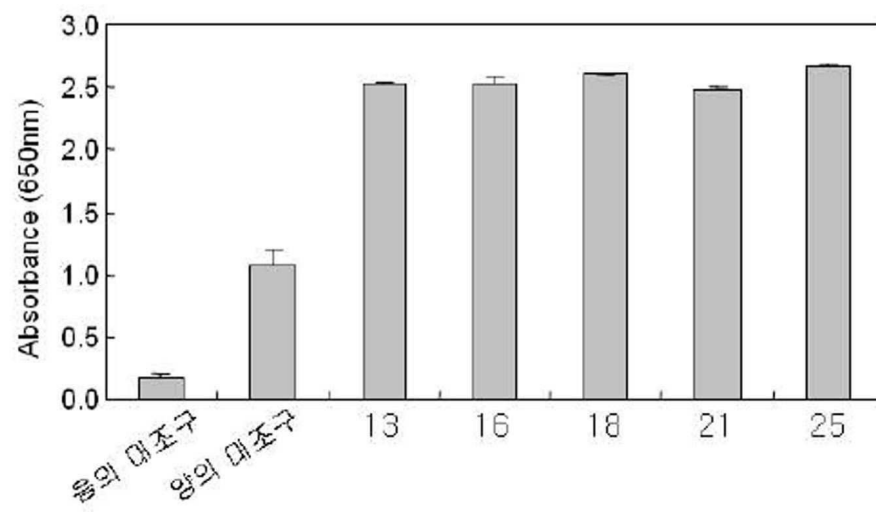


4

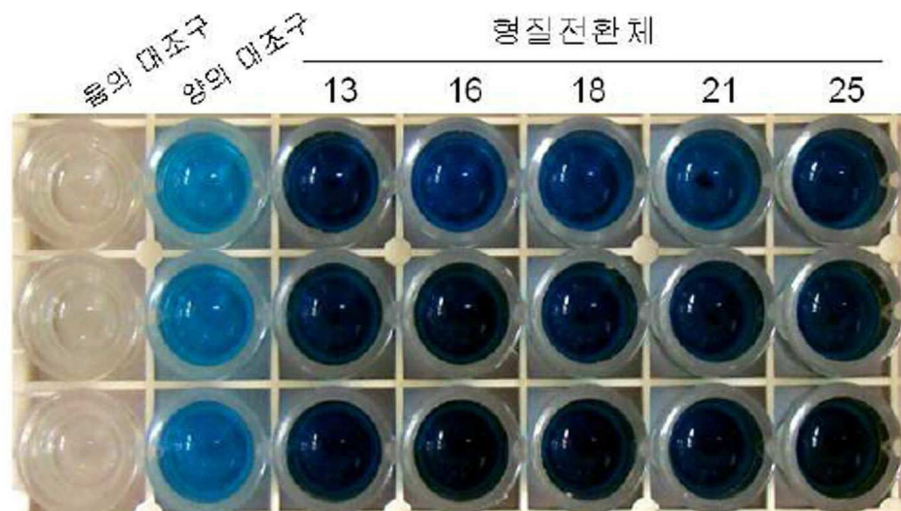


5

A



B



6

A. 바스타 처리

B. 글리포제이트 처리



비형질전환체

형질전환체-25

비형질전환체

형질전환체-25

<110> KOREA RESEARCH INSTITUTE OF CHEMICAL TECHNOLOGY  
INDUSTRY-ACADEMIC COOPERATION FOUNDATION GYEONGSANG NATIONAL UNIVERSITY

<120> ANTIBIOTICS MARKER FREE FESTUCA SPP.PLANTS HAVING RESISTANCE  
AGAINST TWO HERBICIDES

<130> pn08199

<160> 5

<170> KopatentIn 1.71

<210> 1  
<211> 1604  
<212> DNA  
<213> Roundup-Ready soybean

<220>  
<221> gene  
<222> (1)..(1604)  
<223> CP4-EPSPS gene

<400> 1  
agatctttca agaatggcac aaattaacaa catggcacaa gggatacaaa cccttaatcc

|                                                                    |      |
|--------------------------------------------------------------------|------|
| caattccaat ttccataaac cccaagttcc taaatcttca agttttcttg tttttggatc  | 120  |
| taaaaaactg aaaaattcag caaatcttat gttggttttg aaaaaagatt caatttttat  | 180  |
| gcaaaagttt tgttccttta ggatttcagc atcagtggct acagcctgca tgcttcacgg  | 240  |
| tgcaagcagc cgccccgcaa ccgccccgaa atcctctggc ctttccggaa ccgtccgcat  | 300  |
| tcccggcgac aagtcgatct cccaccggtc cttcatgttc ggcggtctcg cgagcggatga | 360  |
| aacgcgcatac accggccttc tggaaggcga ggacgtcatc aatacgggca aggccatgca | 420  |
| ggccatgggc gccaggatcc gtaaggaagg cgacacctgg atcatcgatg gcgtcggcaa  | 480  |
| tggcggcctc ctggcgcttg aggcgccgct cgatttcggc aatgccgcca cgggctgccg  | 540  |
| cctgaccatg ggctctgctg gggcttacga tttcgacagc accttcacg gcgacgcctc   | 600  |
| gctcacaag cgccgatgg gccgcgtgtt gaaccgcgtg cgcgaaatgg gcgtgcaggt    | 660  |
| gaaatcgaa gacggtgacc gtcttcccgt taccttgcgc gggccgaaga cgccgacgcc   | 720  |
| gatcacctac cgctgcccga tggcctccgc acaggtgaag tccgccgtgc tgctcgccgg  | 780  |
| cctcaacacg cccggcatca cgacggtcac cgagccgac atgacgtgcg atcatagga    | 840  |
| aaagatgctg cagggttttg gcgccaacct tacctgcgag acggatgcgg acggcgtgcg  | 900  |
| caccatccgc ctggaaggcc gcggcaagct caccggccaa gtcacgacg tgccgggcga   | 960  |
| cccgtcctcg acggccttcc cgctgggttc ggccttgcgt gttccgggt ccgacgtcac   | 1020 |
| catcctcaac gtgtgatga accccaccg caccggcctc atcctgacgc tgcaggaaat    | 1080 |
| gggcccgcac atcgaagtca tcaacctgcg ccttgccggc ggcgaagacg tggcggacct  | 1140 |
| gcgcgttcgc tcctccacgc tgaagggcgt cacggtgccg gaagaccgcg cgctccgat   | 1200 |

gatcgacgaa tatccgattc tcgctgtcgc cgccgccttc gcggaagggg cgaccgtgat 1260  
gaacggtctg gaagaactcc gcgtcaagga aagcgaccgc ctctcggccg tcgccaatgg 1320  
cctcaagctc aatggcgtag attgcgatga gggcgagacg tcgctcgtcg tgcgtggccg 1380  
ccctgacggc aaggggctcg gcaacgcctc gggcgccgcc gtcgccaccc atctcgatca 1440  
ccgcatcgcc atgagcttcc tcgtcatggg cctcgtgtcg gaaaaccctg tcacggtgga 1500  
cgatgccacg atgatcgcca cgagcttccc ggagttcatg gacctgatgg ccgggctggg 1560  
cgcaagatc gaactctccg atacgaaggc tgcttgatga gctc 1604

<210> 2  
<211> 21  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> primer

<400> 2  
cggctctgcac catcgtcaac c 21

<210> 3  
<211> 21  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> primer

<400> 3  
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<210> 4  
<211> 20  
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<213> Artificial Sequence

<220>

<223> primer

<400> 4

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20

<210> 5

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> primer

<400> 5

gtgacagggt tttccgacac

20