FIG. 1

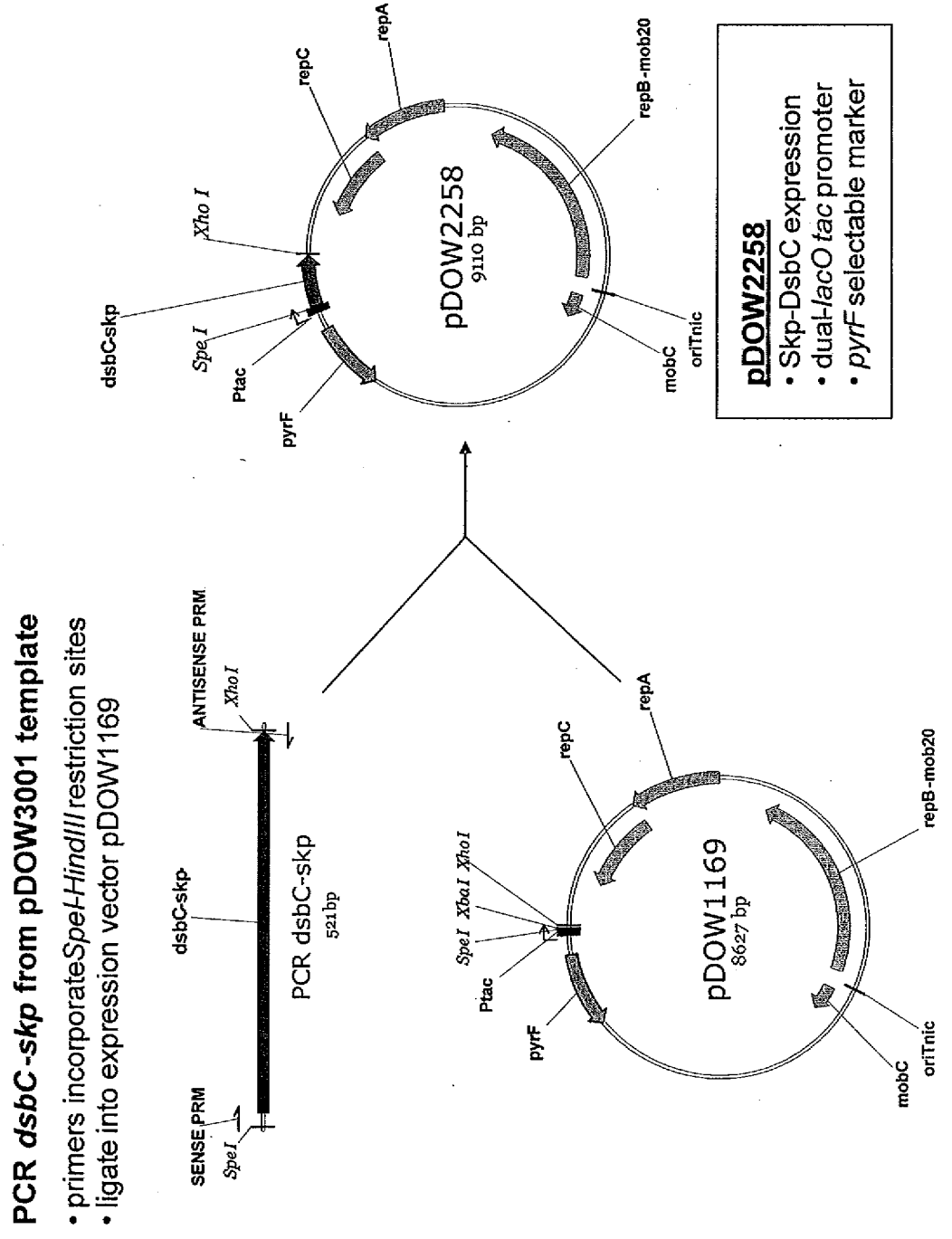

PCR dsbC-skp from pDOW3001 template
• primers incorporate SpeI-HindIII restriction sites
• ligate into expression vector pDOW1169
SENSE PRM
SpeI
dsbC-skp
ANTISENSE PRM
XhoI
PCR dsbC-skp
521 bp
pDOW1169
8627 bp
SpeI XbaI XhoI
Ptac
pyrF
repC
repA
repB-mob20
mobC
oriTnic
pDOW2258
9110 bp
dsbC-skp
XhoI
SpeI
Ptac
pyrF
repC
repA
repB-mob20
mobC
oriTnic
pDOW2258
• Skp-DsbC expression
• dual-lacO tac promoter
• pyrF selectable marker

FIG. 2

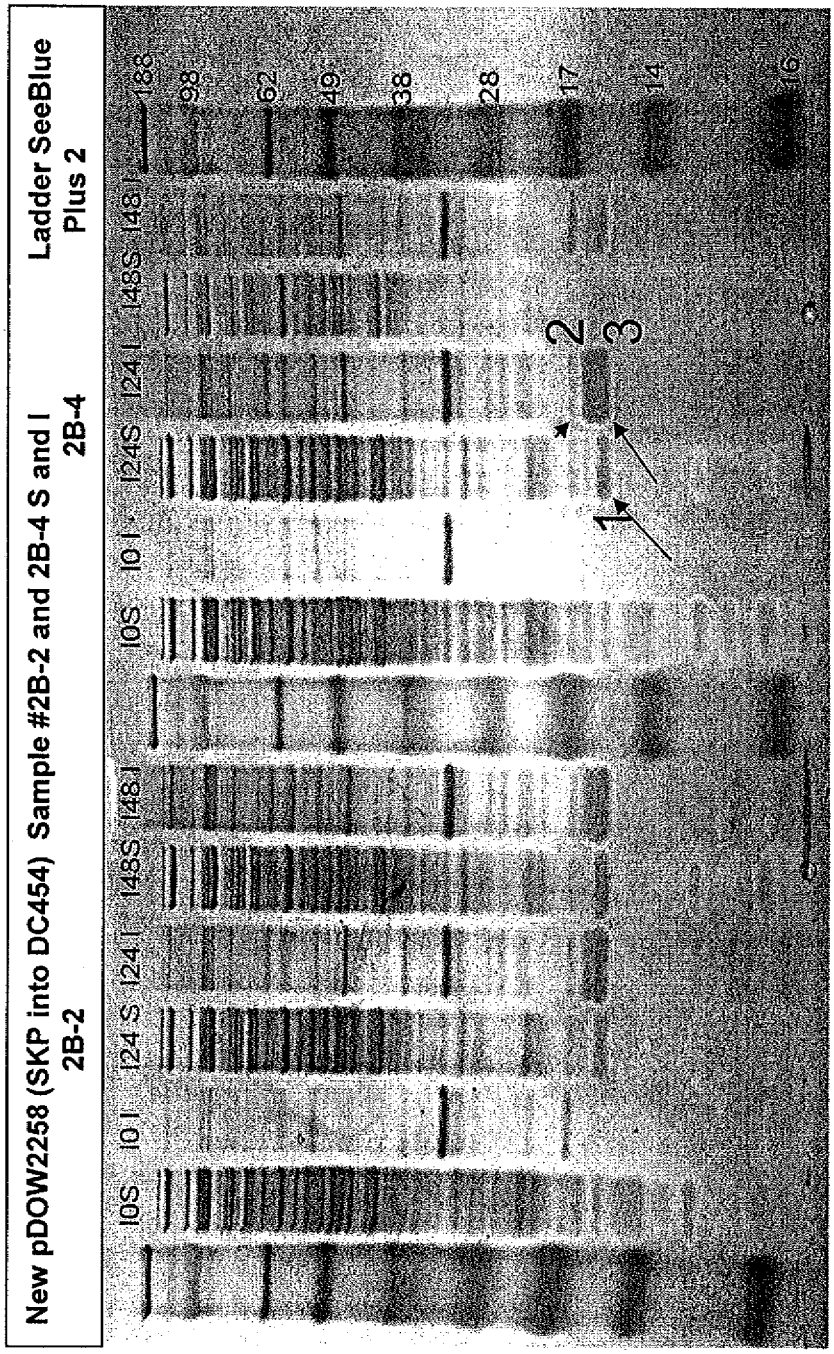

New pDOW2258 (SKP into DC454) Sample #2B-2 and 2B-4 S and I
2B-2
2B-4
Ladder SeeBlue Plus 2
10S
10 I
12 4 S
12 4 I
14 8S
14 8 I
10S
10 I
12 4S
12 4 I
14 8S
14 8 I
188
98
62
49
38
28
17
14
16
1
2
3

FIG. 3

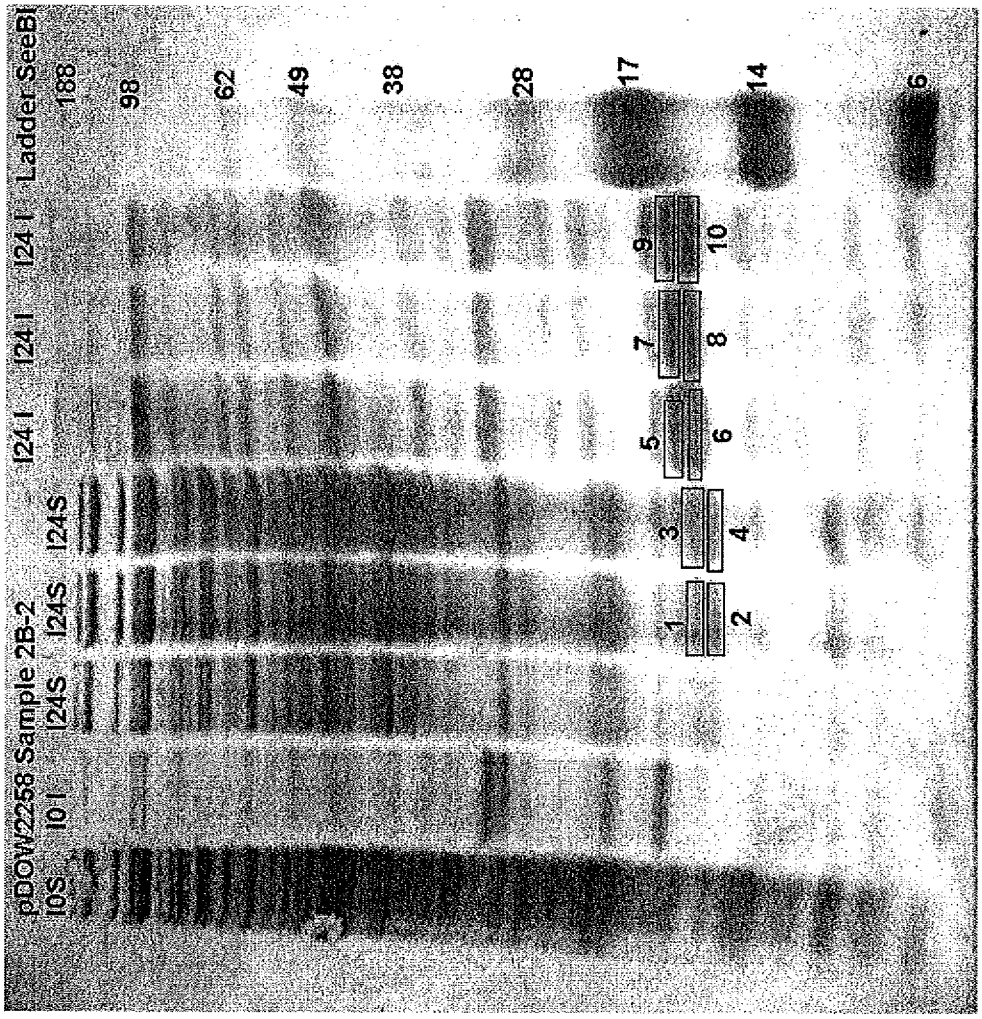
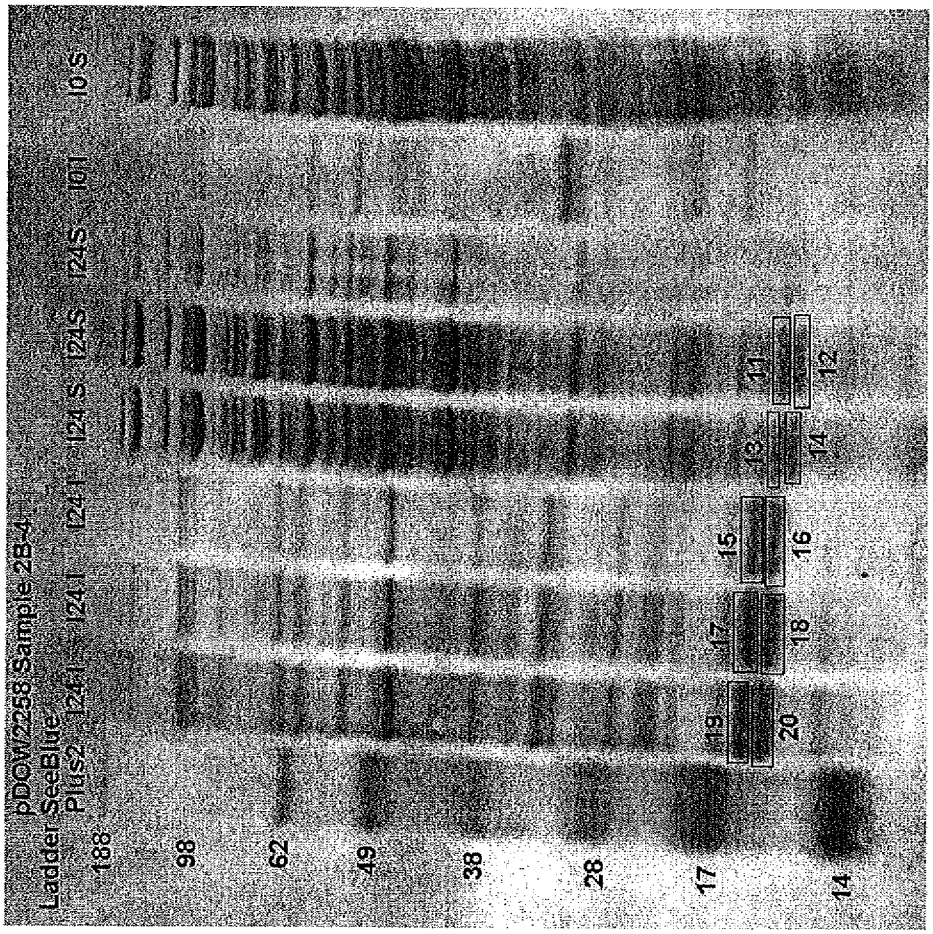
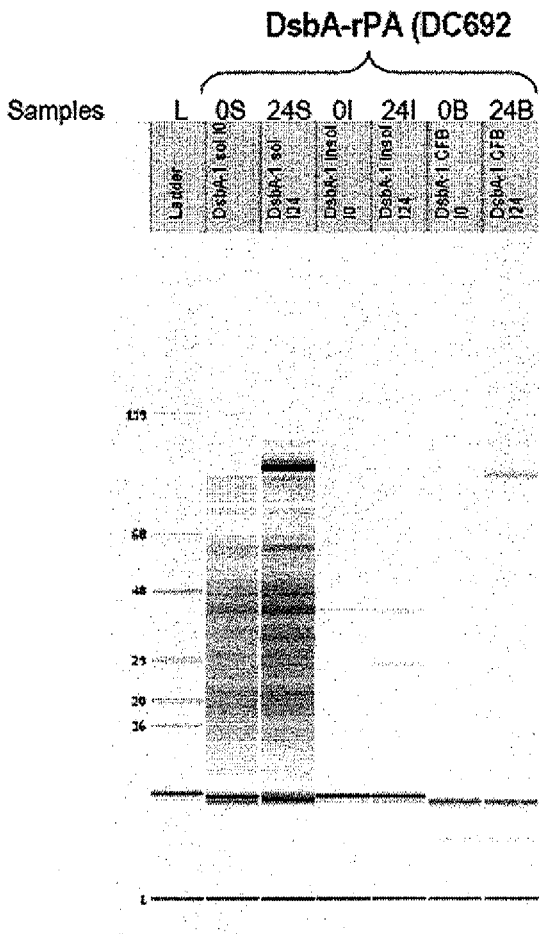

pDOW2258 Sample 2B-2 Ladder SeeBl
I0S I0 I I24S I24S I24S I24 I I24 I I24 I
188
98
62
49
38
28
17
14
6
1
2
3
4
5
6
7
8
9
10

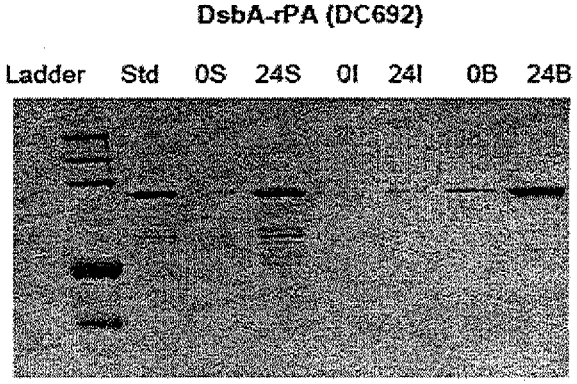
FIG. 3B


Ladder SeeBlue Plus2
pDOW2258 Sample 2B-4
124 I
124 I
124 I
124 S
124 S
124 S
10 I
10 S
188
98
62
49
38
28
17
14
19
20
17
18
15
16
13
14
11
12



Cloned Gene	Expression Target	Host strain
DsbA leader-PA83	**periplasmic**	**WT DC454**

Legend:
S – soluble
I – insoluble
B – cell free broth
L - ladder



Clone	Leader	Soluble (g/L)	Insoluble (g/L)	Cell free broth (g/L)	N-terminus
DC692	DsbA	1.1	0.05	0.23	E V K Q E

FIG. 4

6/11
FIG. 5

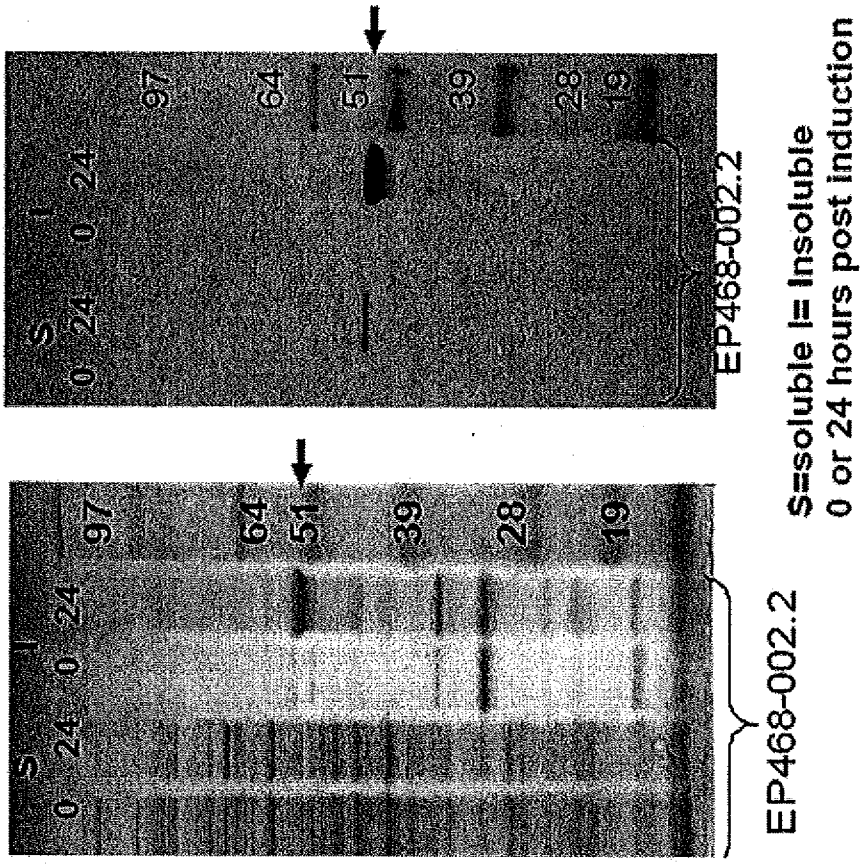

S I S I
0 24 0 24
97
64
51
39
28
19
EP468-002.2
S=soluble I= Insoluble
0 or 24 hours post induction
EP468-002.2

FIG. 6

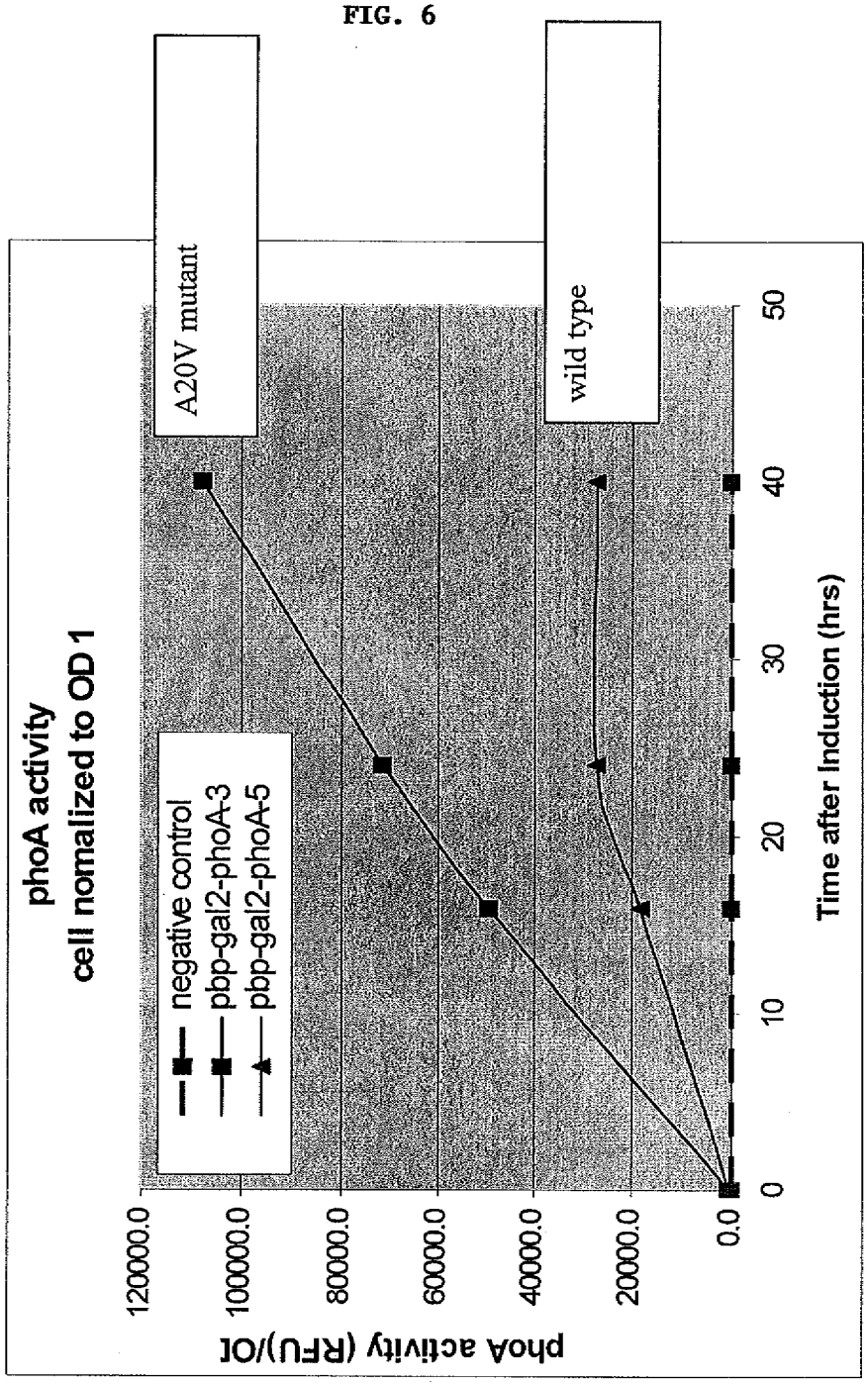

phoA activity
cell nomalized to OD 1
negative control
pbp-gal2-phoA-3
pbp-gal2-phoA-5
A20V mutant
wild type
phoA activity (RFU)/OD
120000.0
100000.0
80000.0
60000.0
40000.0
20000.0
0.0
0
10
20
30
40
50
Time after Induction (hrs)

FIG. 7

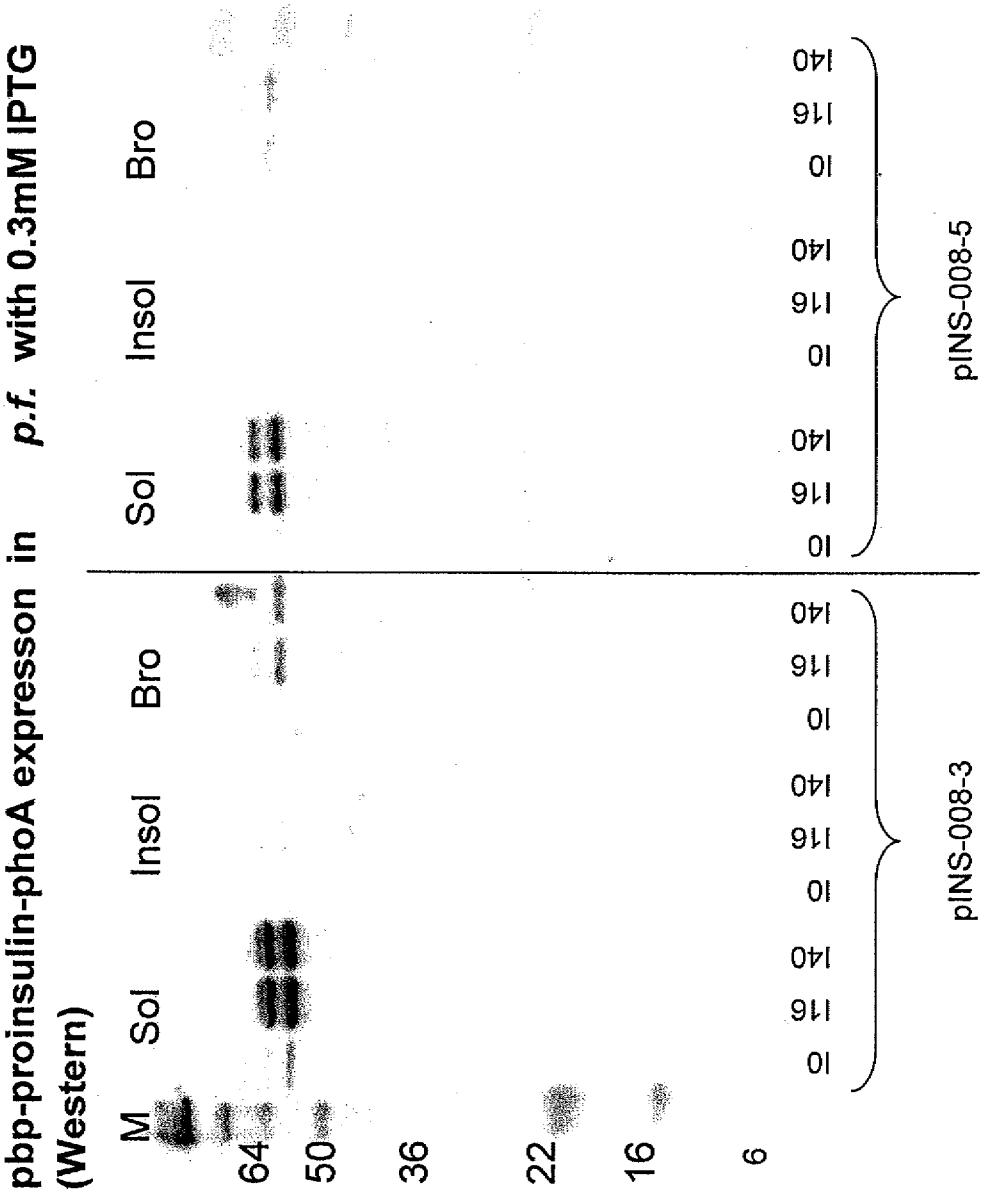

pbp-proinsulin-phoA expresson in p.f. with 0.3mM IPTG
(Western)
M
Sol
Insol
Bro
64
50
36
22
16
6
pINS-008-3
pINS-008-5
10
116
140

FIG. 8

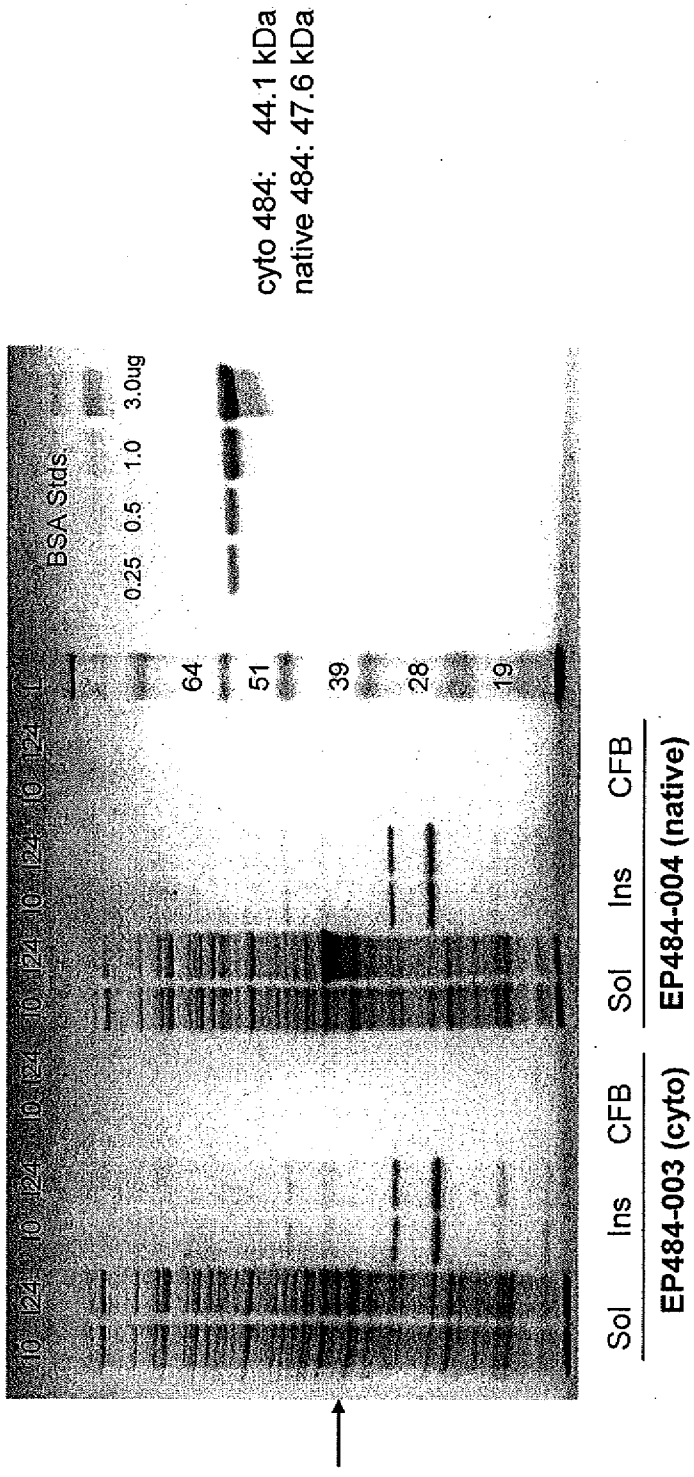

cyto 484: 44.1 kDa
native 484: 47.6 kDa
BSA Stds.
0.25
0.5
1.0
3.0ug
64
51
39
28
19
Sol
Ins
CFB
EP484-003 (cyto)
Sol
Ins
CFB
EP484-004 (native)

FIG. 9

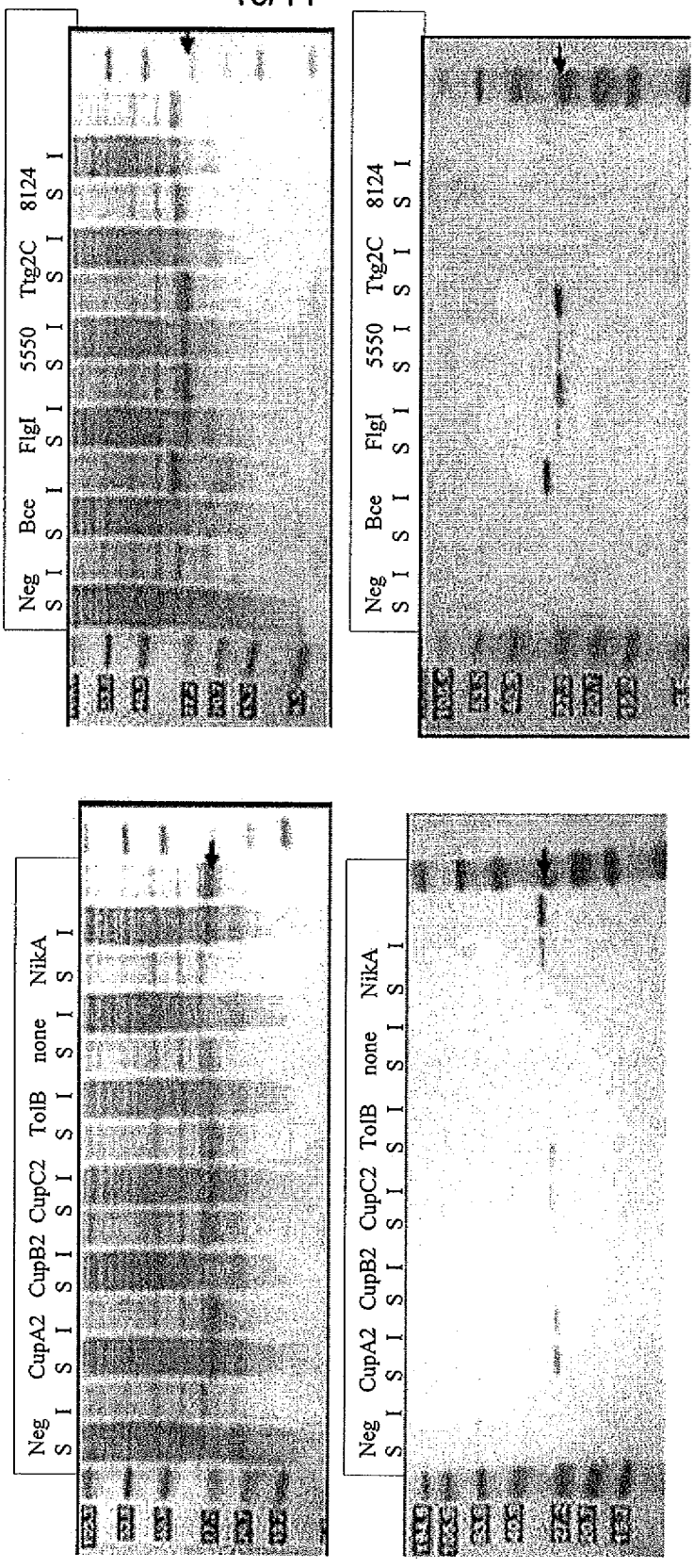

Neg S
Bce I
FlgI S
5550 I
Ttg2C S
8124 I
CupA2 S
CupB2 I
CupC2 S
TolB I
none S
NikA I

FIG. 10

