Figure 1

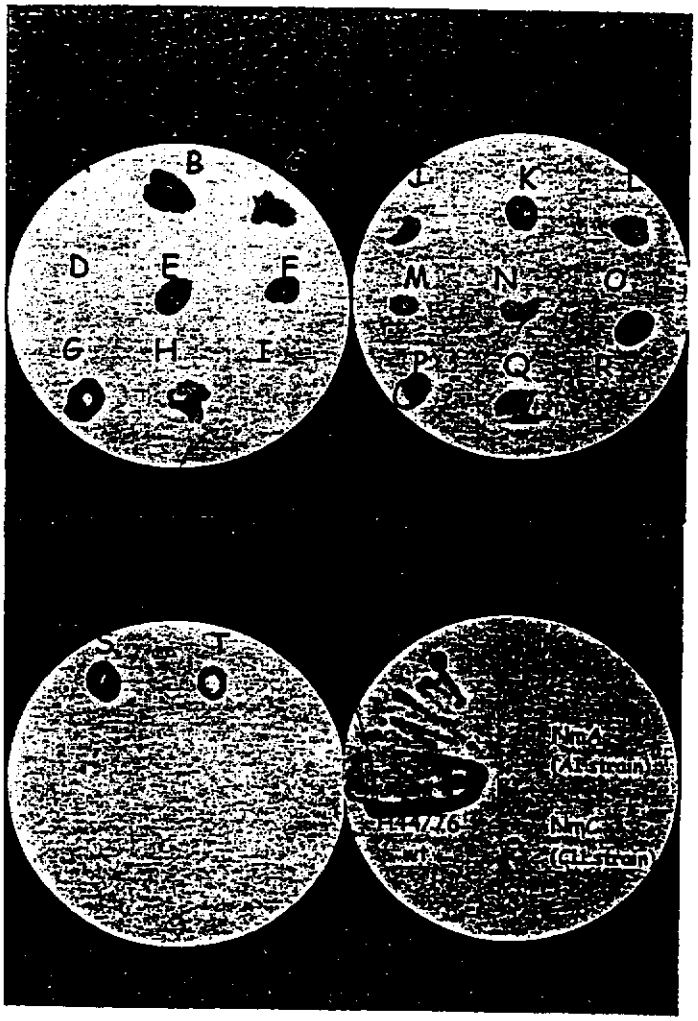


Figure 2

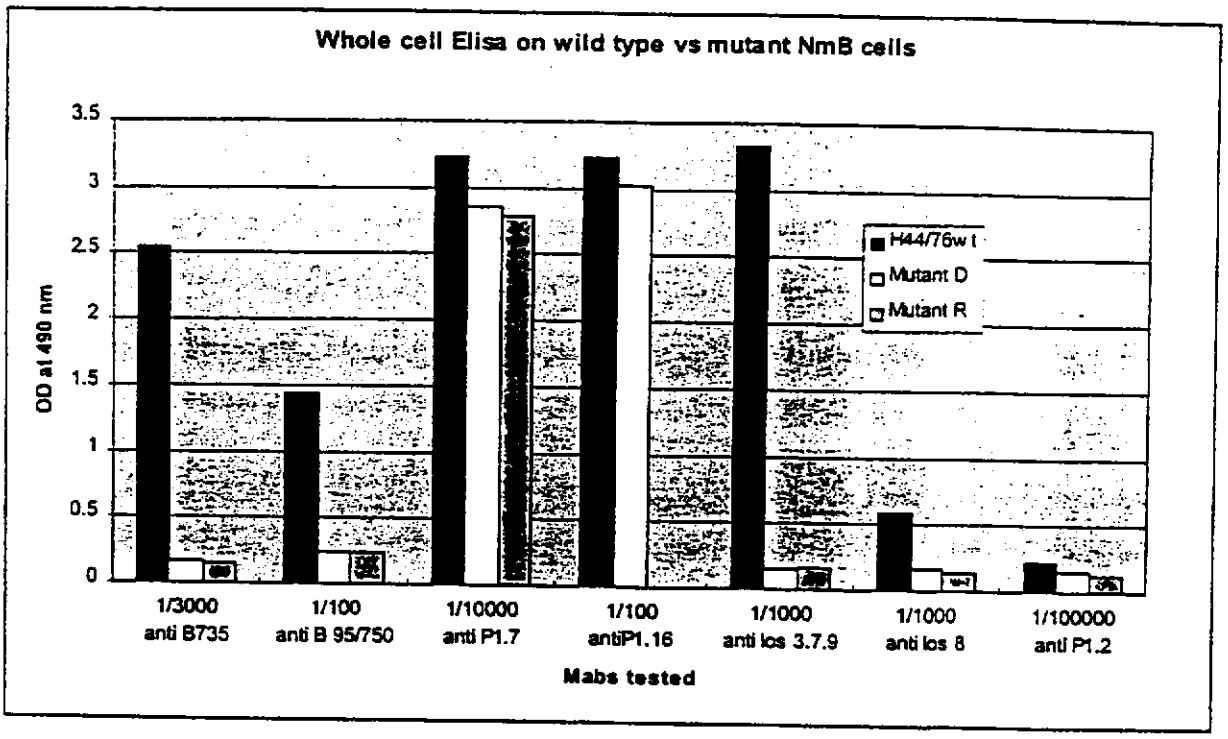

Whole cell Elisa on wild type vs mutant NmB cells
OD at 490 nm
3.5
3
2.5
2
1.5
1
0.5
0
H44/76wt
Mutant D
Mutant R
1/3000 anti B735
1/100 anti B 95/750
1/10000 anti P1.7
1/100 antiP1.16
1/1000 anti los 3.7.9
1/1000 anti los 8
1/100000 anti P1.2
Mabs tested

Figure 3

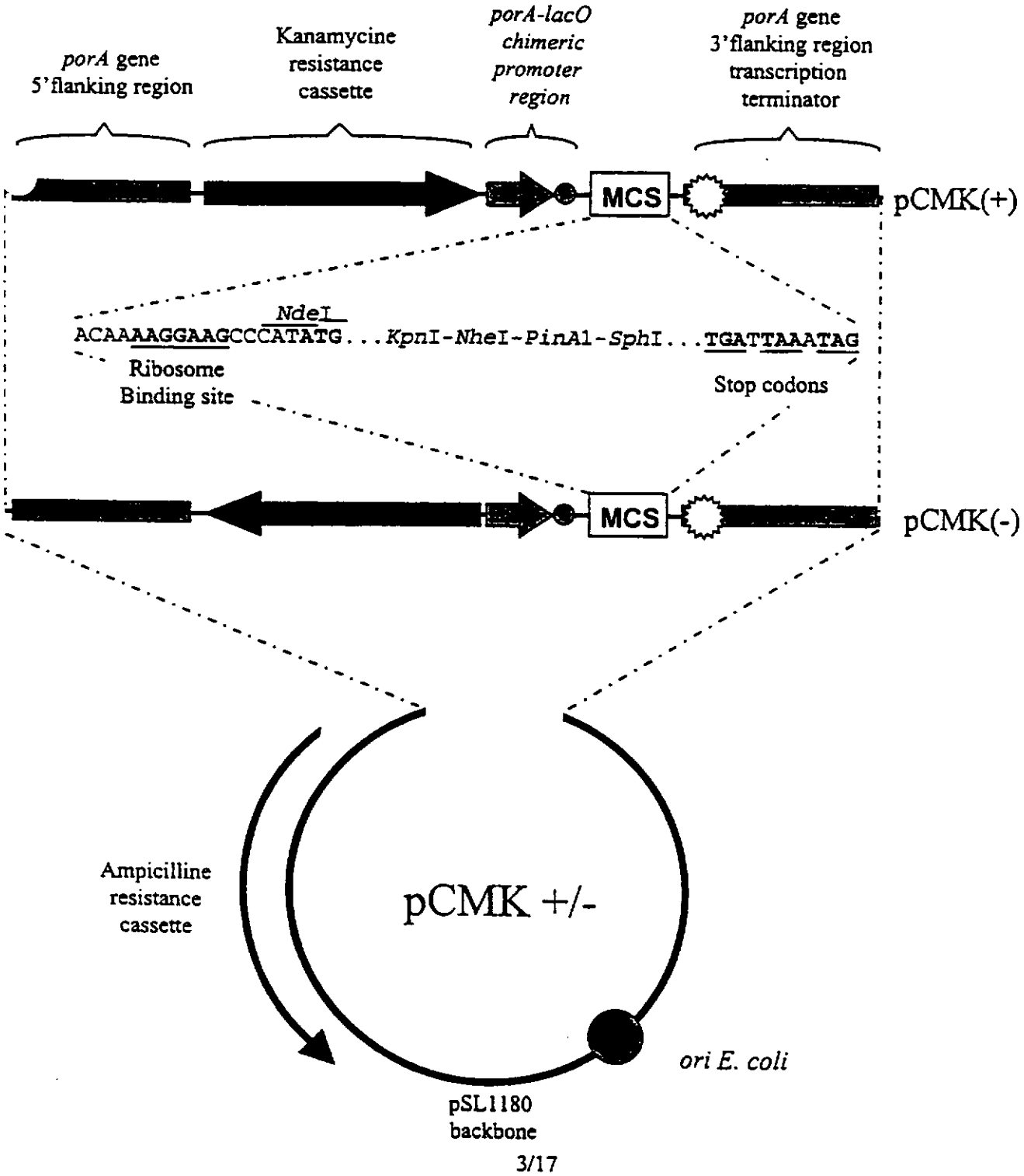

porA gene 5'flanking region
Kanamycine resistance cassette
porA-lacO chimeric promoter region
porA gene 3'flanking region transcription terminator
MCS
pCMK(+)
NdeI
ACAAAAGGAAGCCCATATG...KpnI-NheI-PinA1-SphI...TGATTAAATAG
Ribosome Binding site
Stop codons
MCS
pCMK(-)
Ampicilline resistance cassette
pCMK +/-
ori E. coli
pSL1180 backbone

Figure 4

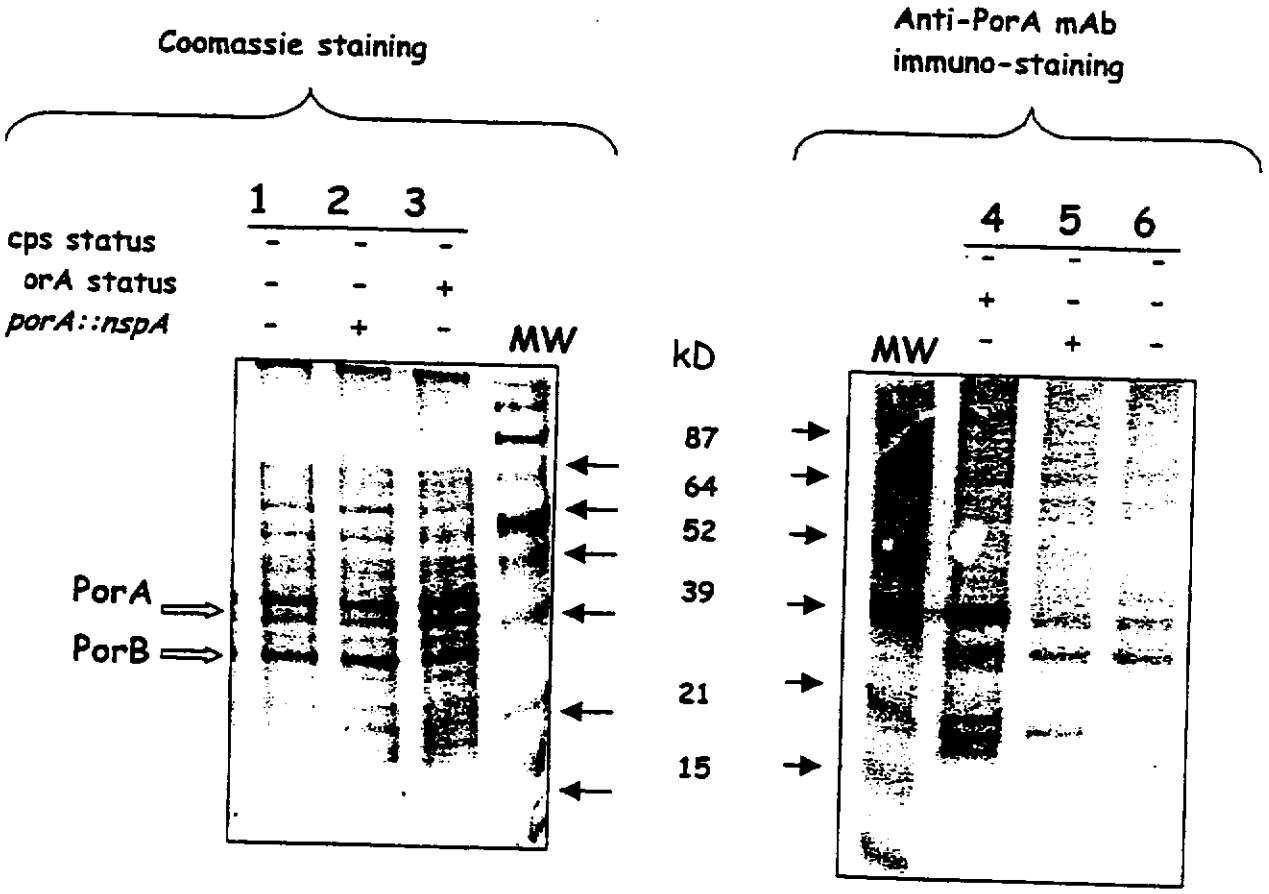

Coomassie staining
Anti-PorA mAb
immuno-staining
1 2 3
4 5 6
cps status
orA status
porA::nspA
MW
kD
87
64
52
39
21
15
PorA
PorB

Figure 5

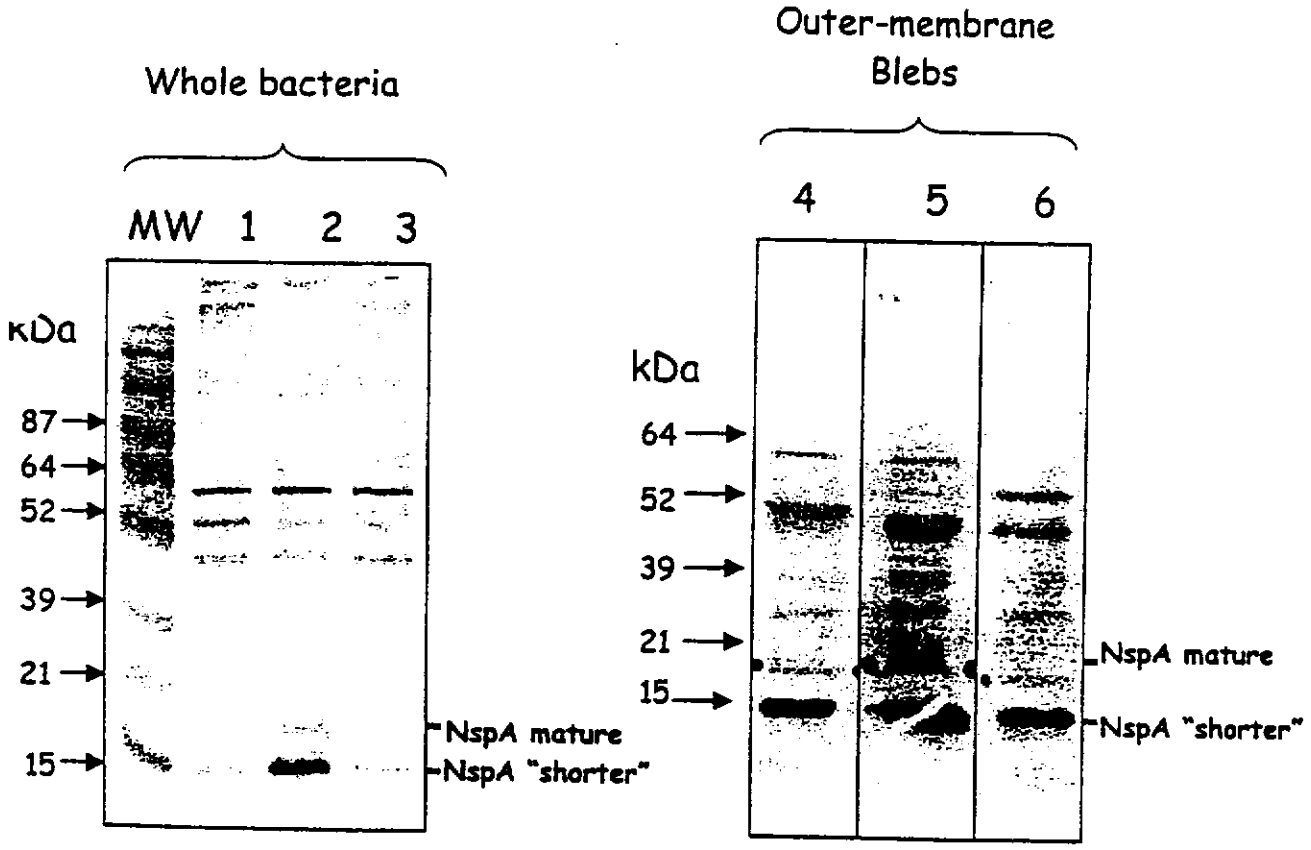

Whole bacteria
MW 1 2 3
kDa
87
64
52
39
21
15
NspA mature
NspA "shorter"
Outer-membrane Blebs
4 5 6
kDa
64
52
39
21
15
NspA mature
NspA "shorter"

Figure 6

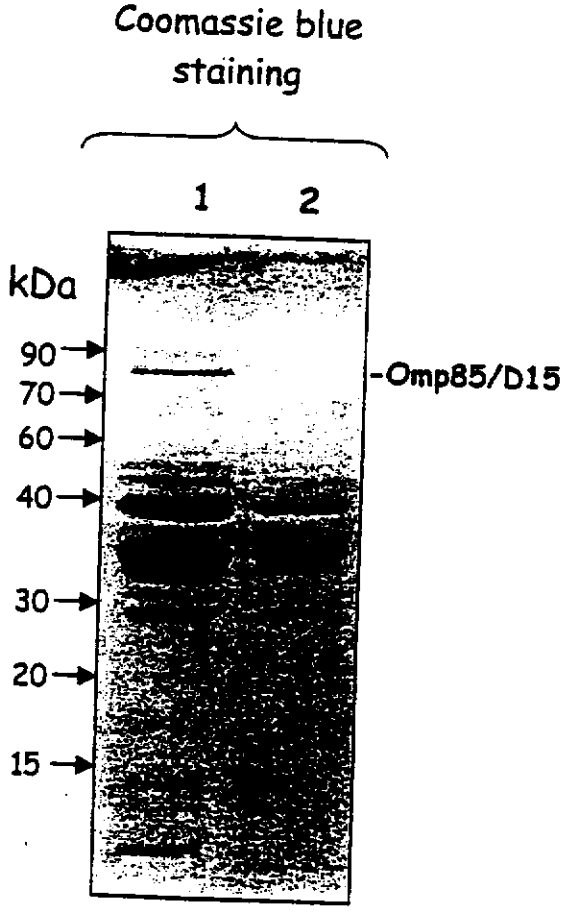


Figure 7

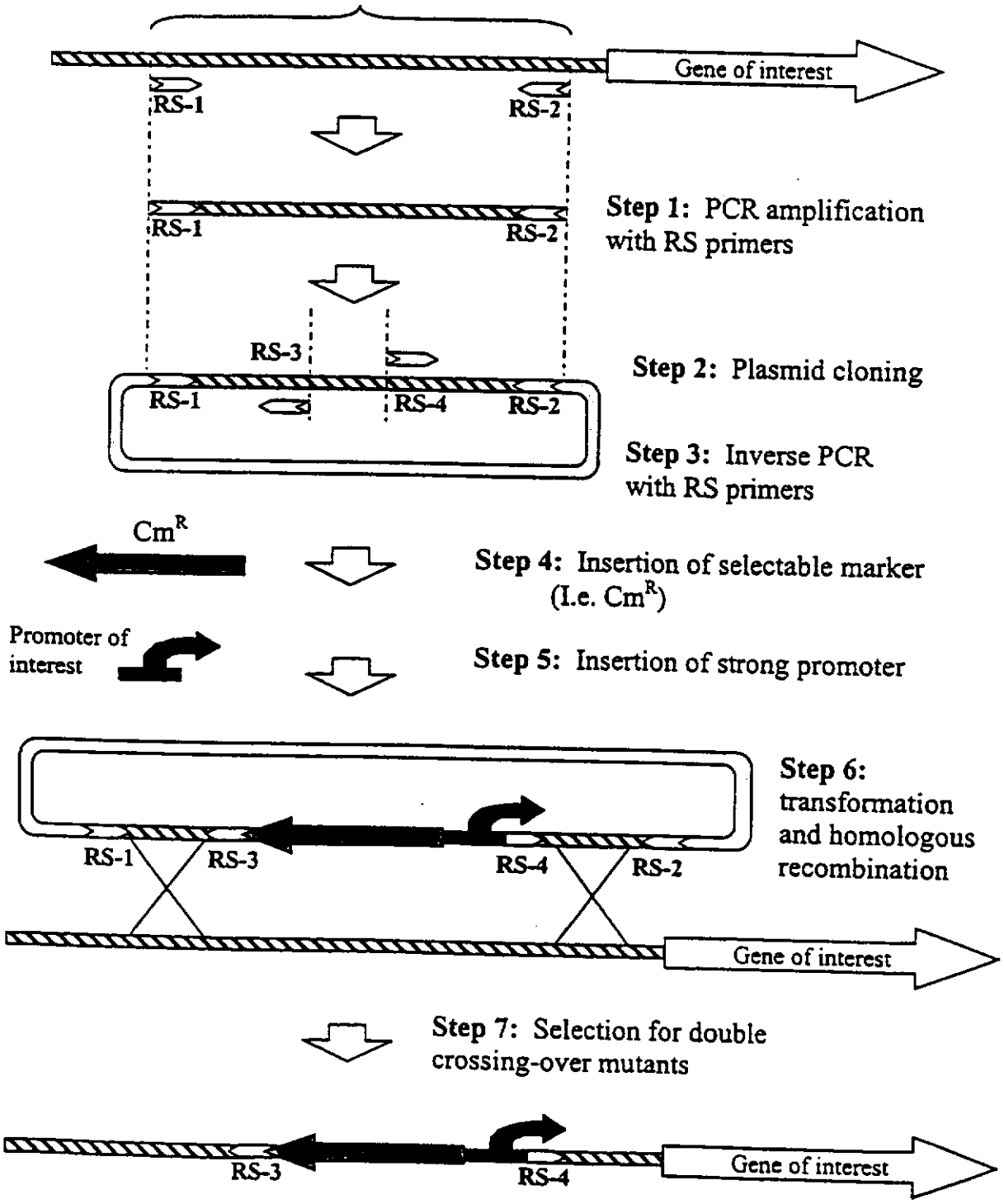

Gene of interest
RS-1
RS-2
RS-1
RS-2
Step 1: PCR amplification with RS primers
RS-3
RS-1
RS-4
RS-2
Step 2: Plasmid cloning
Step 3: Inverse PCR with RS primers
Cm^R
Step 4: Insertion of selectable marker (I.e. Cm^R)
Promoter of interest
Step 5: Insertion of strong promoter
RS-1
RS-3
RS-4
RS-2
Step 6: transformation and homologous recombination
Gene of interest
Step 7: Selection for double crossing-over mutants
RS-3
RS-4
Gene of interest

Figure 8

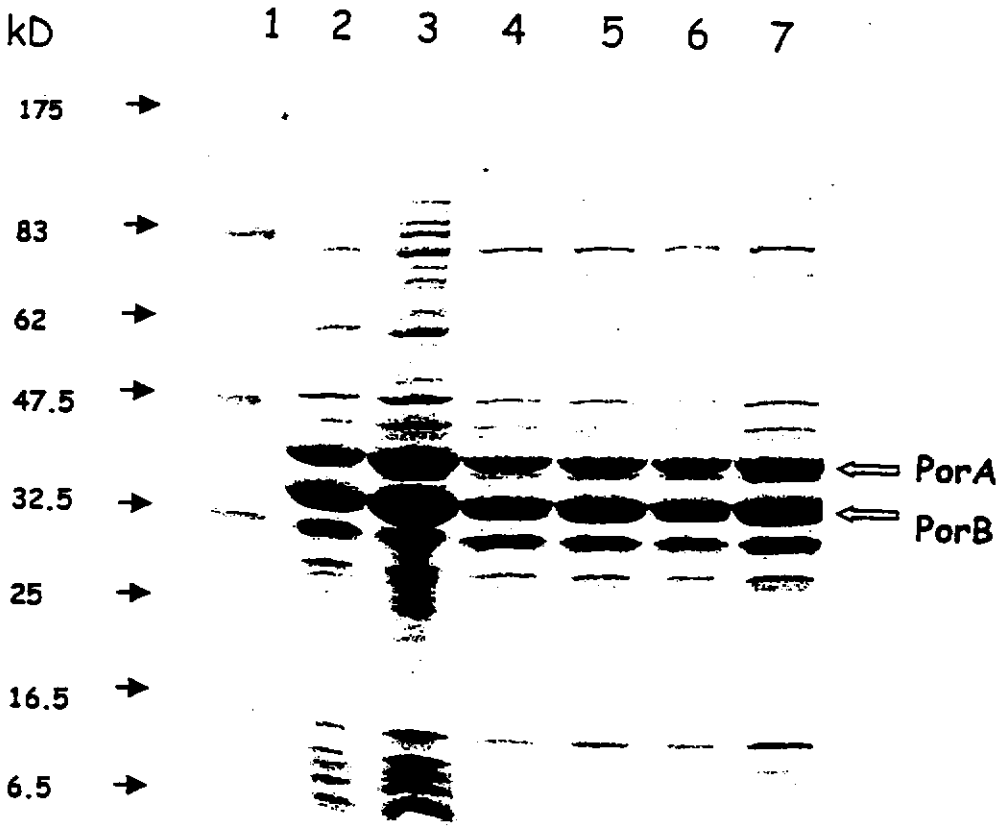


Figure 9

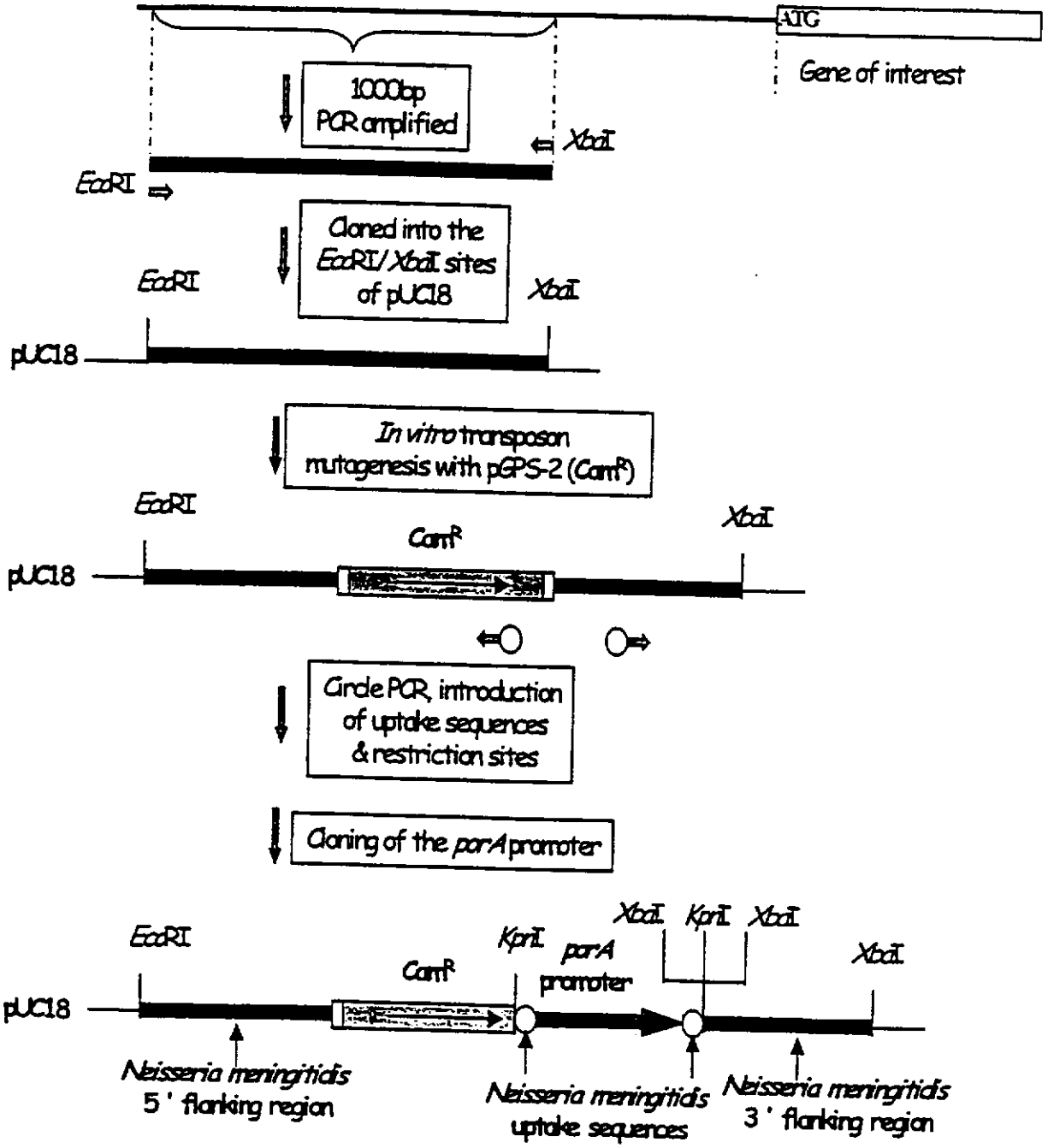

ATG
Gene of interest
1000bp PCR amplified
XbaI
EcoRI
Cloned into the EcoRI/XbaI sites of pUC18
EcoRI
XbaI
pUC18
In vitro transposon mutagenesis with pGPS-2 (CamR)
EcoRI
XbaI
CamR
pUC18
Circle PCR, introduction of uptake sequences & restriction sites
Cloning of the porA promoter
XbaI
KpnI
XbaI
EcoRI
KpnI
porA promoter
XbaI
CamR
pUC18
Neisseria meningitidis 5' flanking region
Neisseria meningitidis uptake sequences
Neisseria meningitidis 3' flanking region

Figure 10

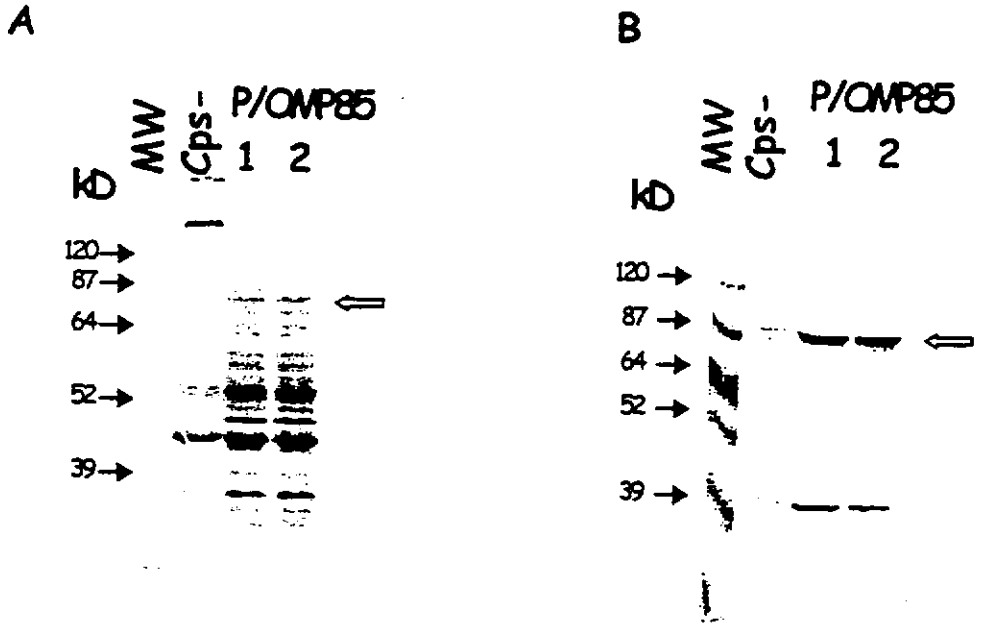

A
MW
Cps-
P/OMP85
1
2
kD
120
87
64
52
39
B
MW
Cps-
P/OMP85
1
2
kD
120
87
64
52
39

Figure 11

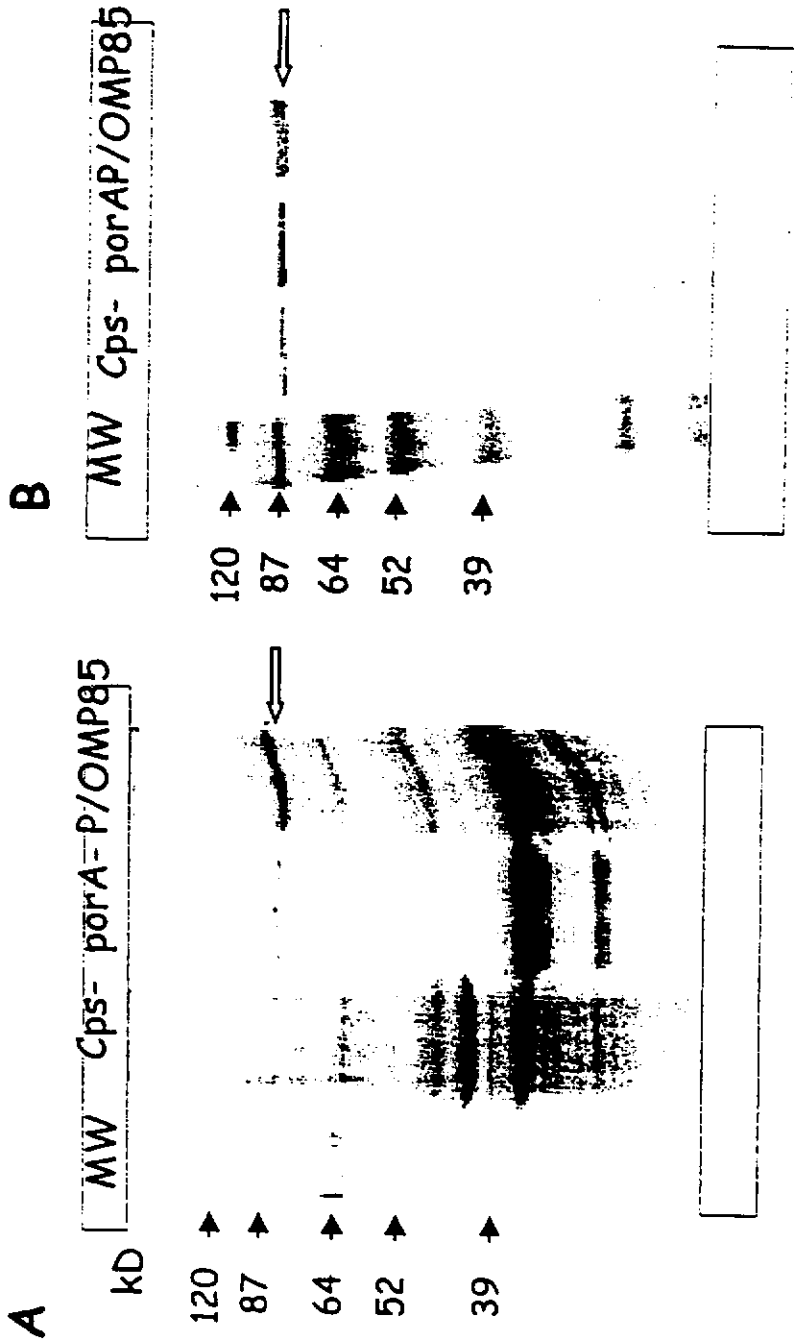

A
kD
MW Cps- porA-P/OMP85
120
87
64
52
39
B
MW Cps- porAP/OMP85
120
87
64
52
39

Figure 12

Schematic representation of the recombinant PCR strategy used to delete the *lacO* in the chimeric *porA/lacO* promoter.

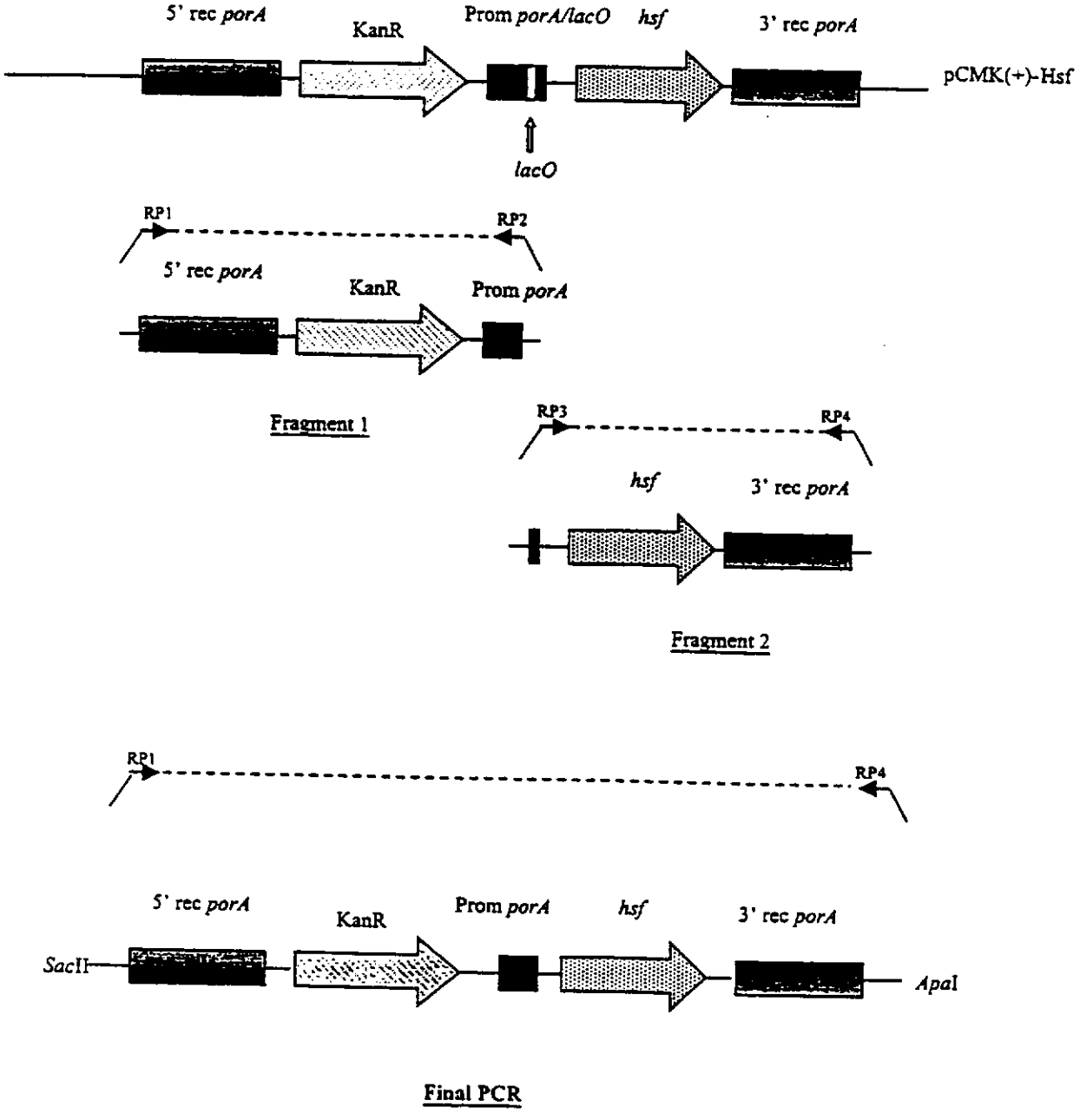


Figure 13

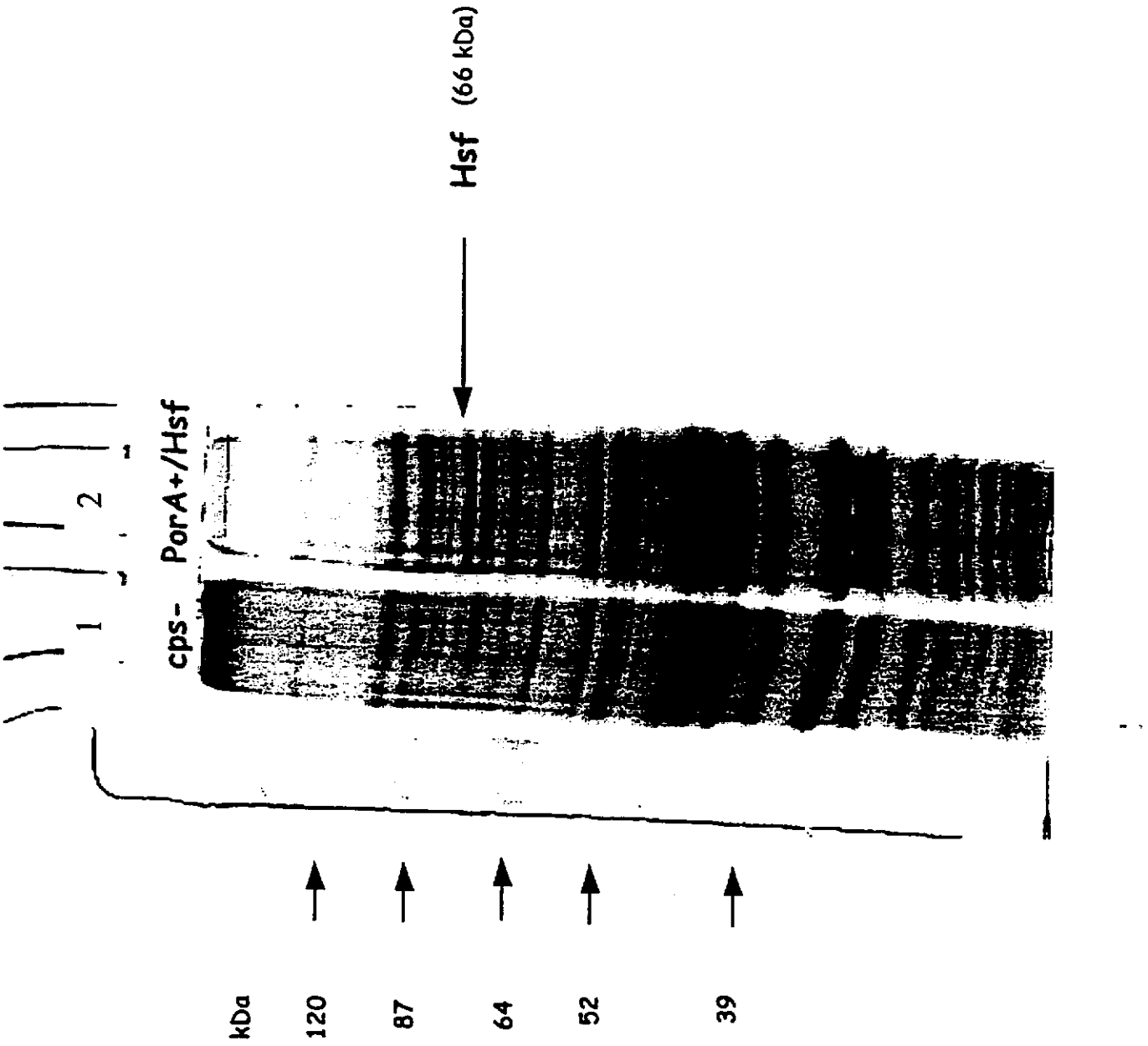


Figure 14

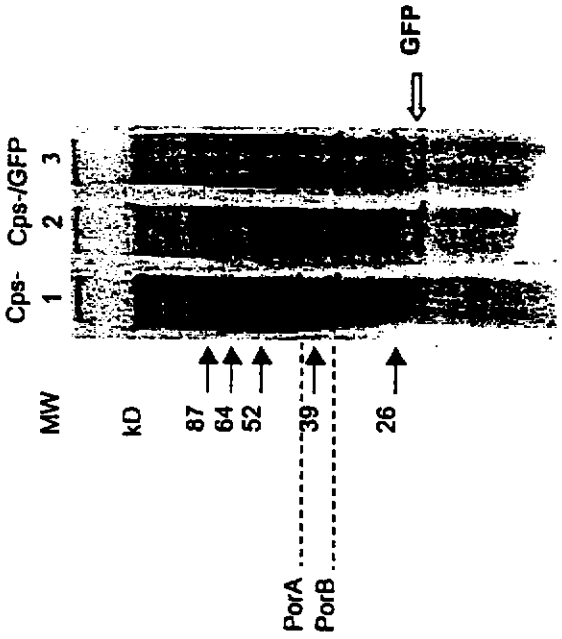


Figure 15

SDS Page 1

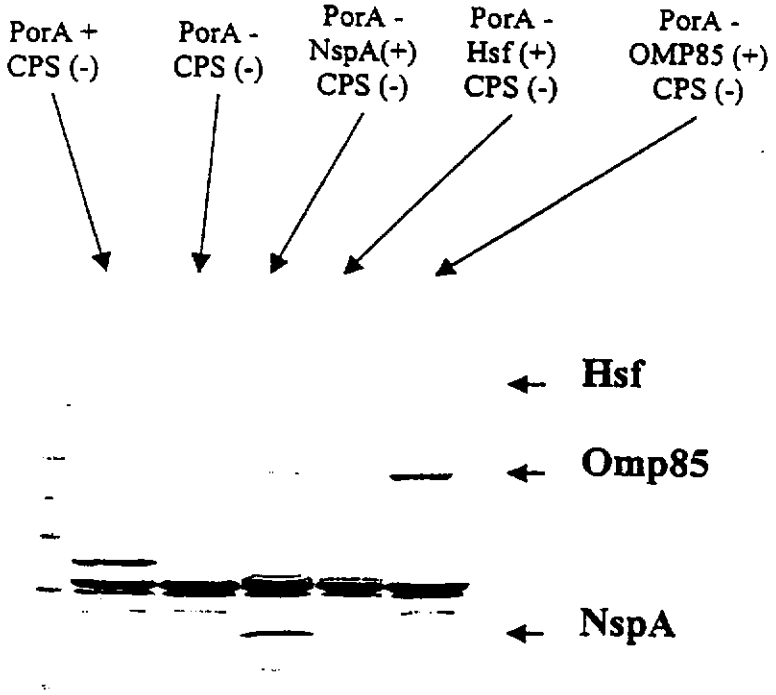


Figure 16

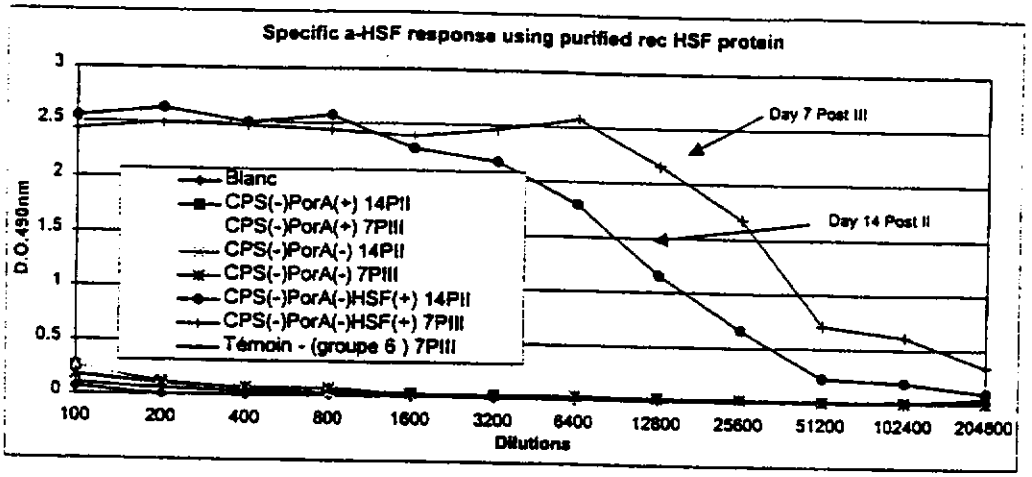
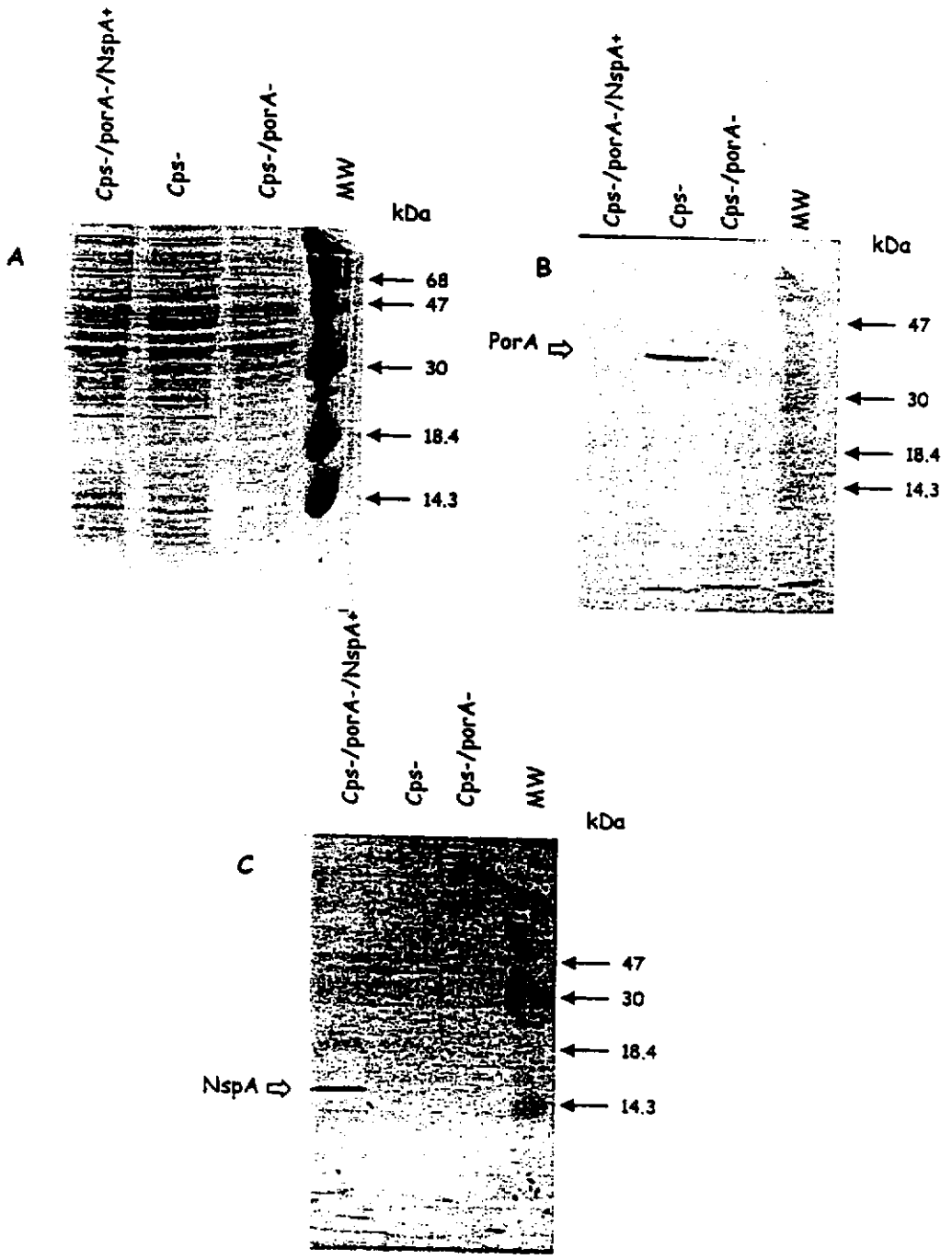

Specific a-HSF response using purified rec HSF protein
D.O.490nm
3
2.5
2
1.5
1
0.5
0
100
200
400
800
1600
3200
6400
12800
25600
51200
102400
204800
Dilutions
Day 7 Post III
Day 14 Post II
Blanc
CPS(-)PorA(+) 14PII
CPS(-)PorA(+) 7PIII
CPS(-)PorA(-) 14PII
CPS(-)PorA(-) 7PIII
CPS(-)PorA(-)HSF(+) 14PII
CPS(-)PorA(-)HSF(+) 7PIII
Témoin - (groupe 6) 7PIII

Figure 17


A
Cps-/porA-/NspA+
Cps-
Cps-/porA-
MW
kDa
68
47
30
18.4
14.3
B
Cps-/porA-/NspA+
Cps-
Cps-/porA-
MW
kDa
PorA
47
30
18.4
14.3
C
Cps-/porA-/NspA+
Cps-
Cps-/porA-
MW
kDa
NspA
47
30
18.4
14.3