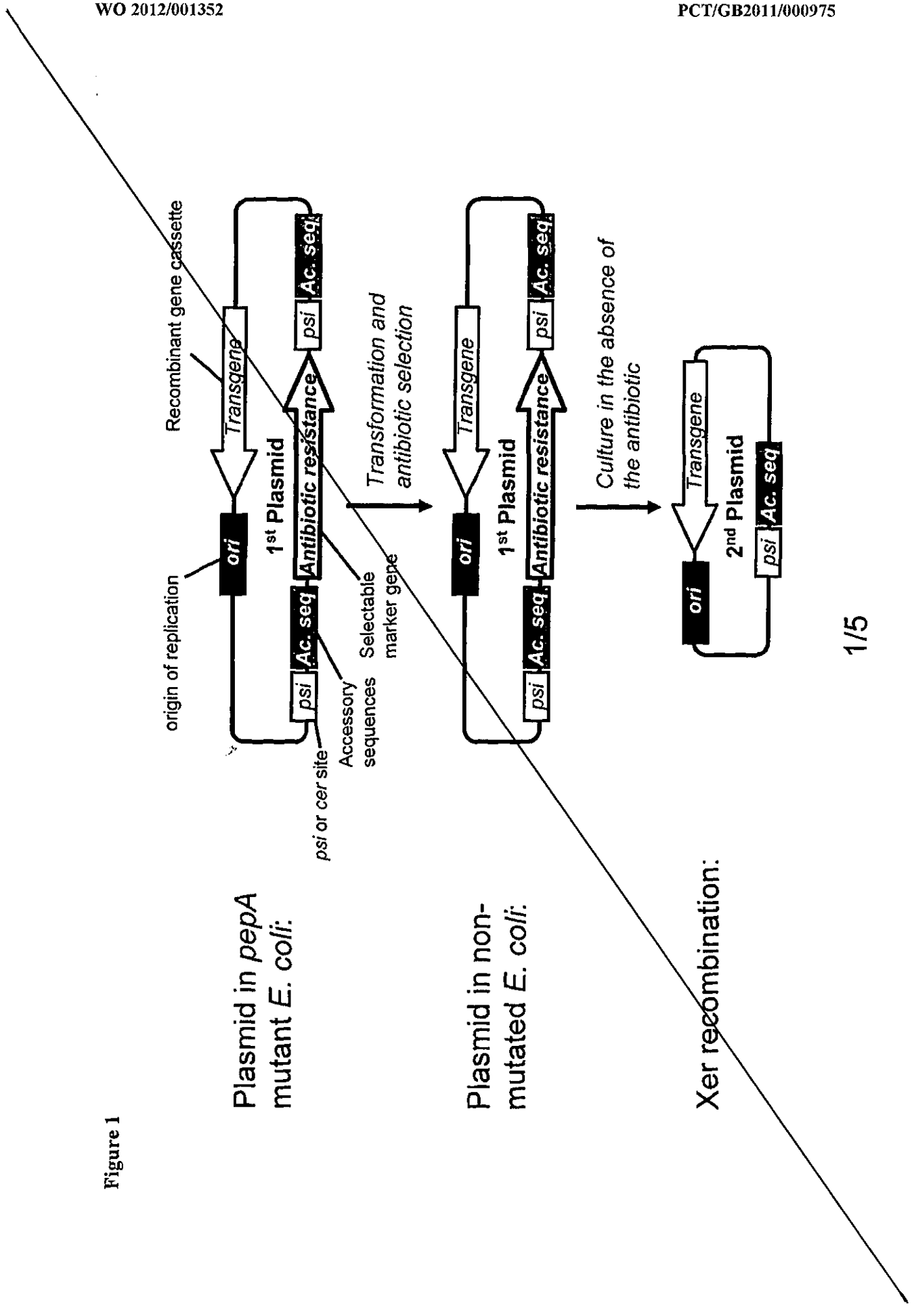

Recombinant gene cassette
Transgene
ori
origin of replication
1st Plasmid
Antibiotic resistance
psi
Ac. seq
Selectable marker gene
Accessory sequences
psi or cer site
Plasmid in pepA mutant E. coli:
Transformation and antibiotic selection
Plasmid in non-mutated E. coli:
Culture in the absence of the antibiotic
2nd Plasmid
Xer recombination:

Figure 1

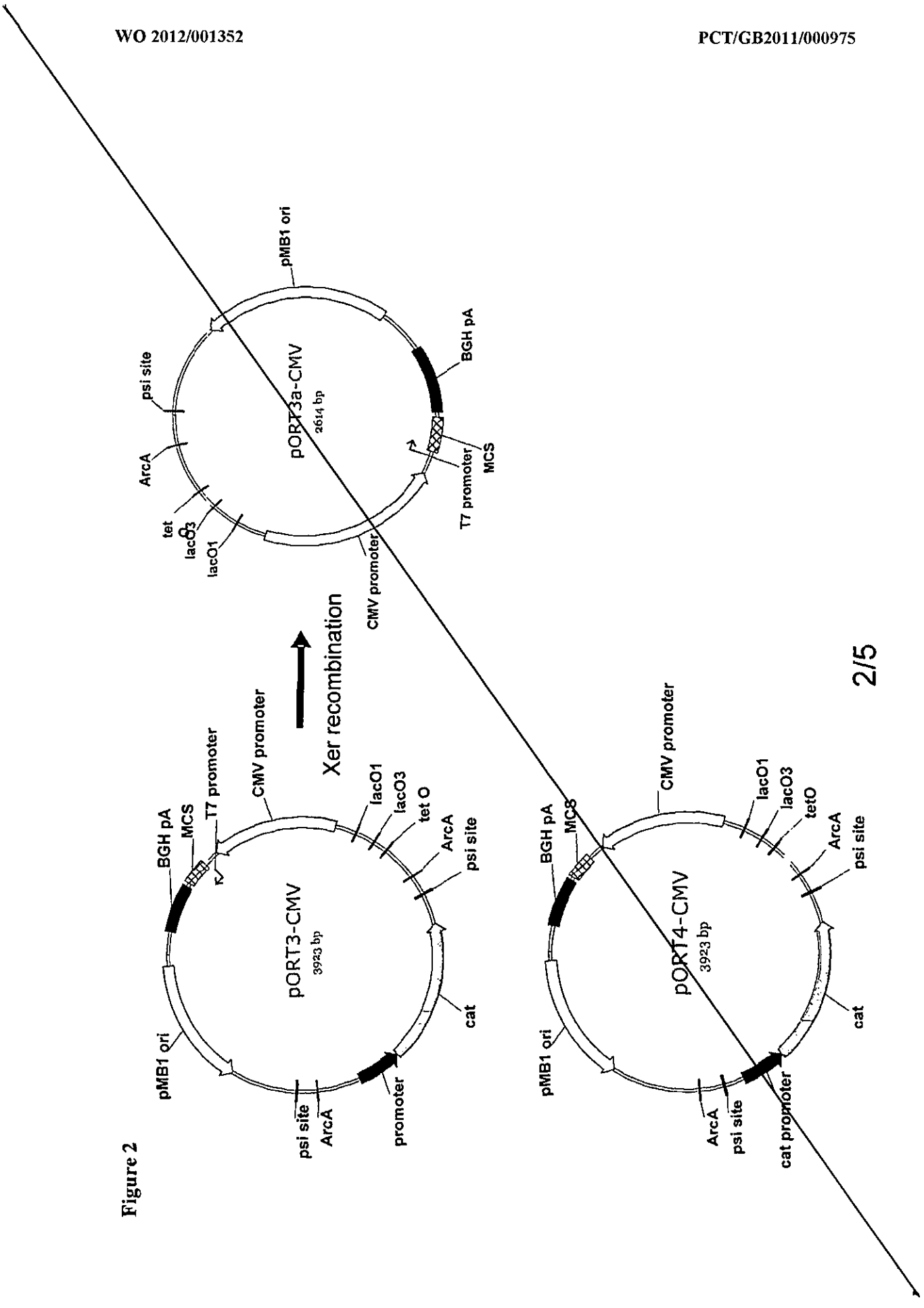

pMB1 ori
BGH pA
psi site
ArcA
tet
lacO3
lacO1
pORT3a-CMV
2614 bp
T7 promoter
MCS
CMV promoter
Xer recombination
BGH pA
MCS
T7 promoter
CMV promoter
lacO1
lacO3
tet O
ArcA
psi site
pORT3-CMV
3923 bp
pMB1 ori
psi site
ArcA
promoter
cat
BGH pA
MCS
CMV promoter
lacO1
lacO3
tetO
ArcA
psi site
pORT4-CMV
3923 bp
pMB1 ori
ArcA
psi site
cat promoter
cat

Figure 2

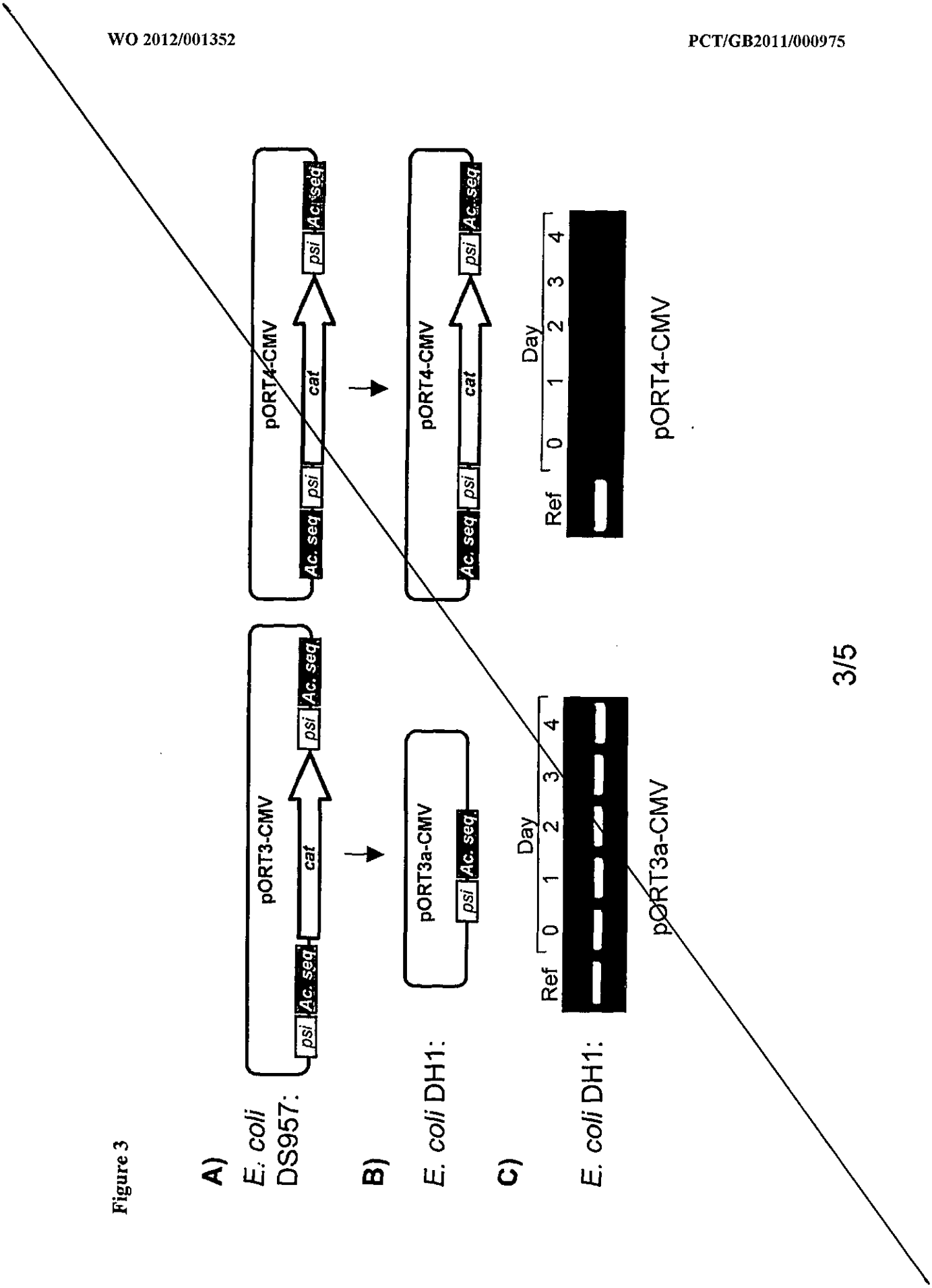

A)
E. coli DS957:
pORT3-CMV
psi
Ac. seq
cat
psi
Ac. seq
B)
E. coli DH1:
pORT3a-CMV
psi
Ac. seq
pORT4-CMV
psi
Ac. seq
cat
psi
Ac. seq
C)
E. coli DH1:
Day
Ref
0
1
2
3
4
pORT3a-CMV
pORT4-CMV

Figure 3

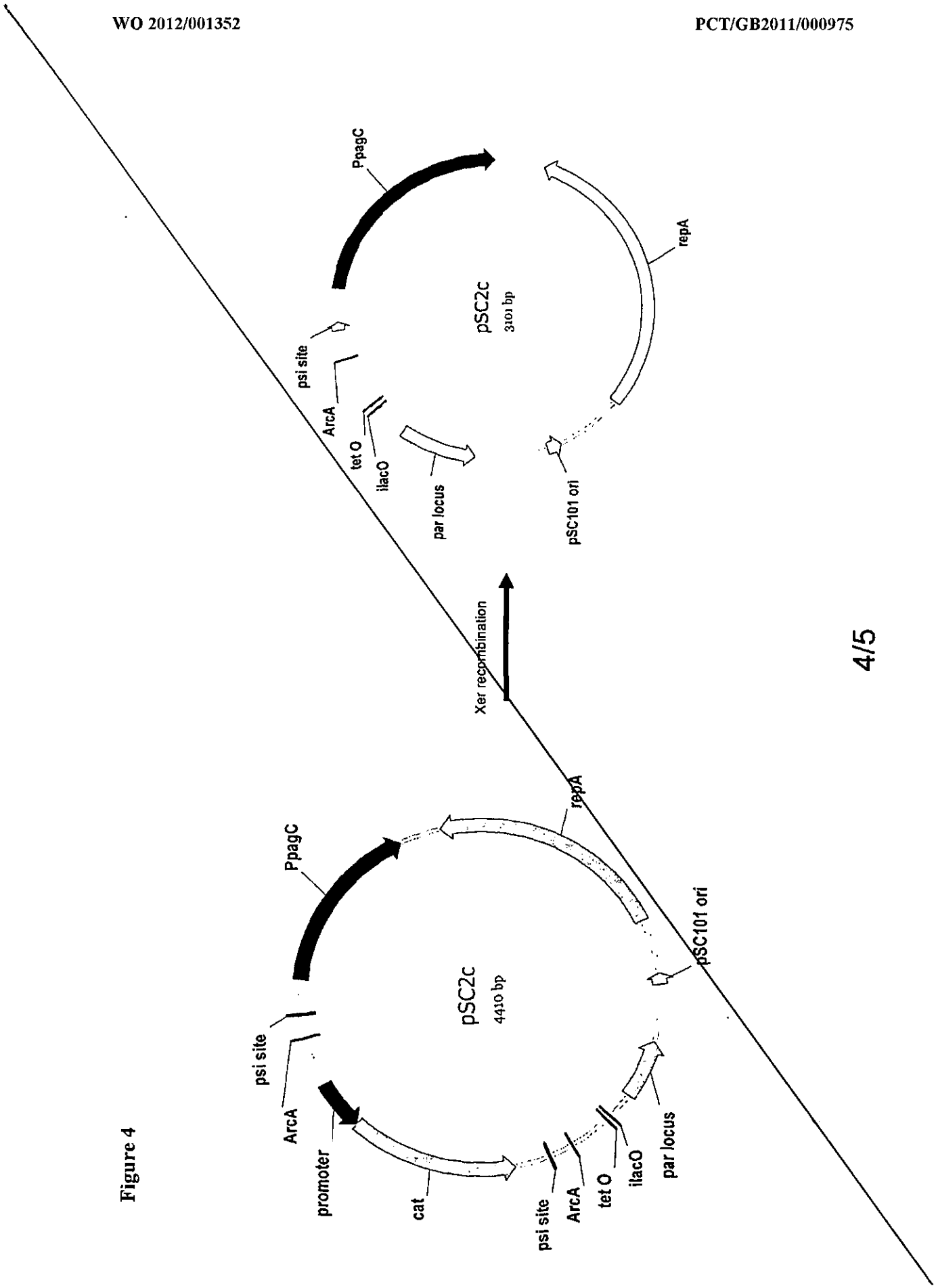

PpagC
psi site
ArcA
tet O
ilacO
par locus
pSC2c
3101 bp
repA
pSC101 ori
Xer recombination
PpagC
psi site
ArcA
promoter
cat
pSC2c
4410 bp
repA
pSC101 ori
psi site
ArcA
tet O
ilacO
par locus

Figure 4

Figure 5

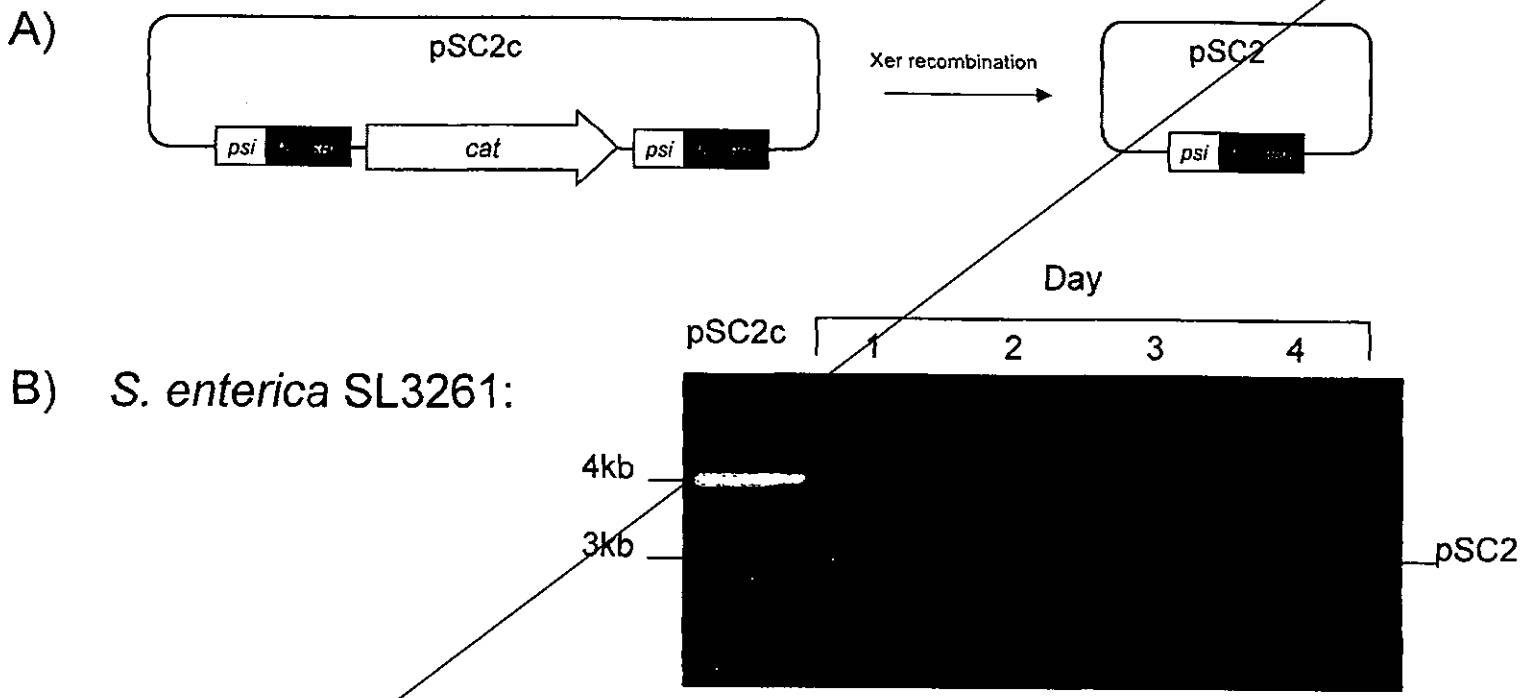
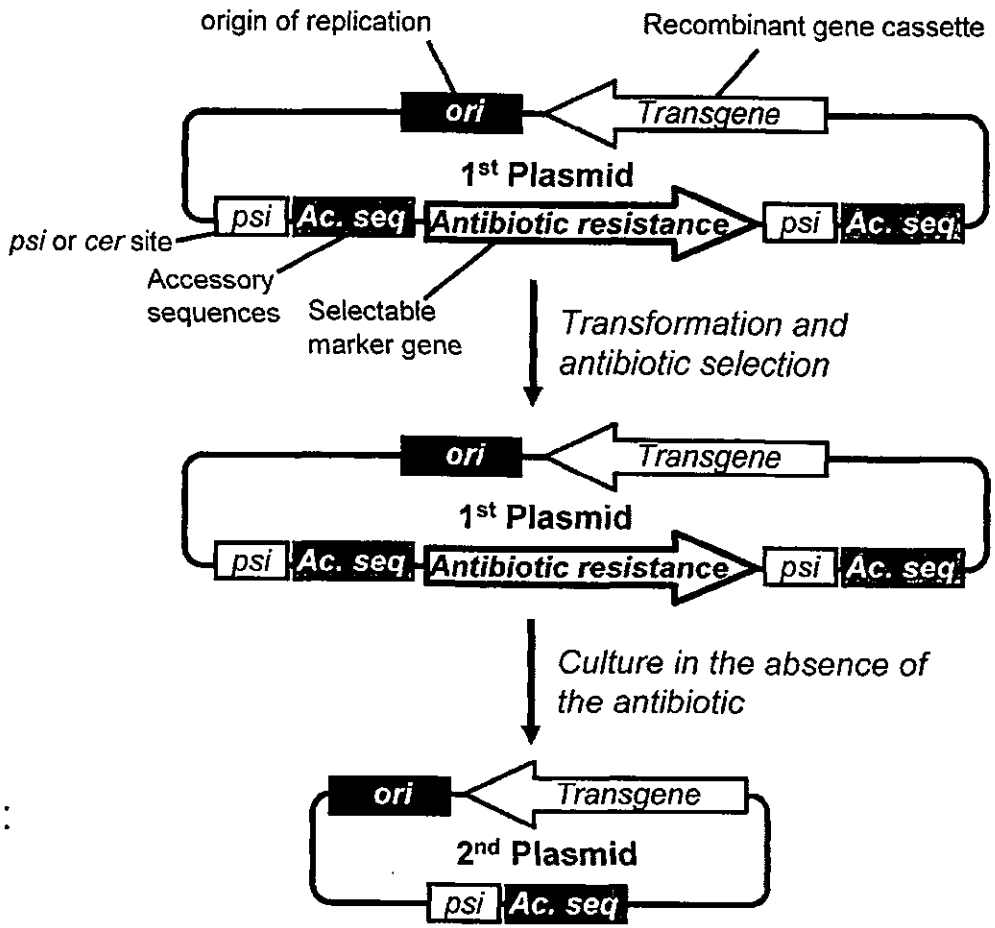

A)
pSC2c
psi
cat
psi
Xer recombination
pSC2
psi
B) S. enterica SL3261:
Day
pSC2c
1
2
3
4
4kb
3kb
pSC2

Figure 1


Plasmid in *pepA* mutant *E. coli*:
origin of replication
Recombinant gene cassette
ori
Transgene
1st Plasmid
psi
Ac. seq
Antibiotic resistance
psi
Ac. seq
psi or cer site
Accessory sequences
Selectable marker gene
Transformation and antibiotic selection
Plasmid in non-mutated *E. coli*:
ori
Transgene
1st Plasmid
psi
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Antibiotic resistance
psi
Ac. seq
Culture in the absence of the antibiotic
Xer recombination:
ori
Transgene
2nd Plasmid
psi
Ac. seq

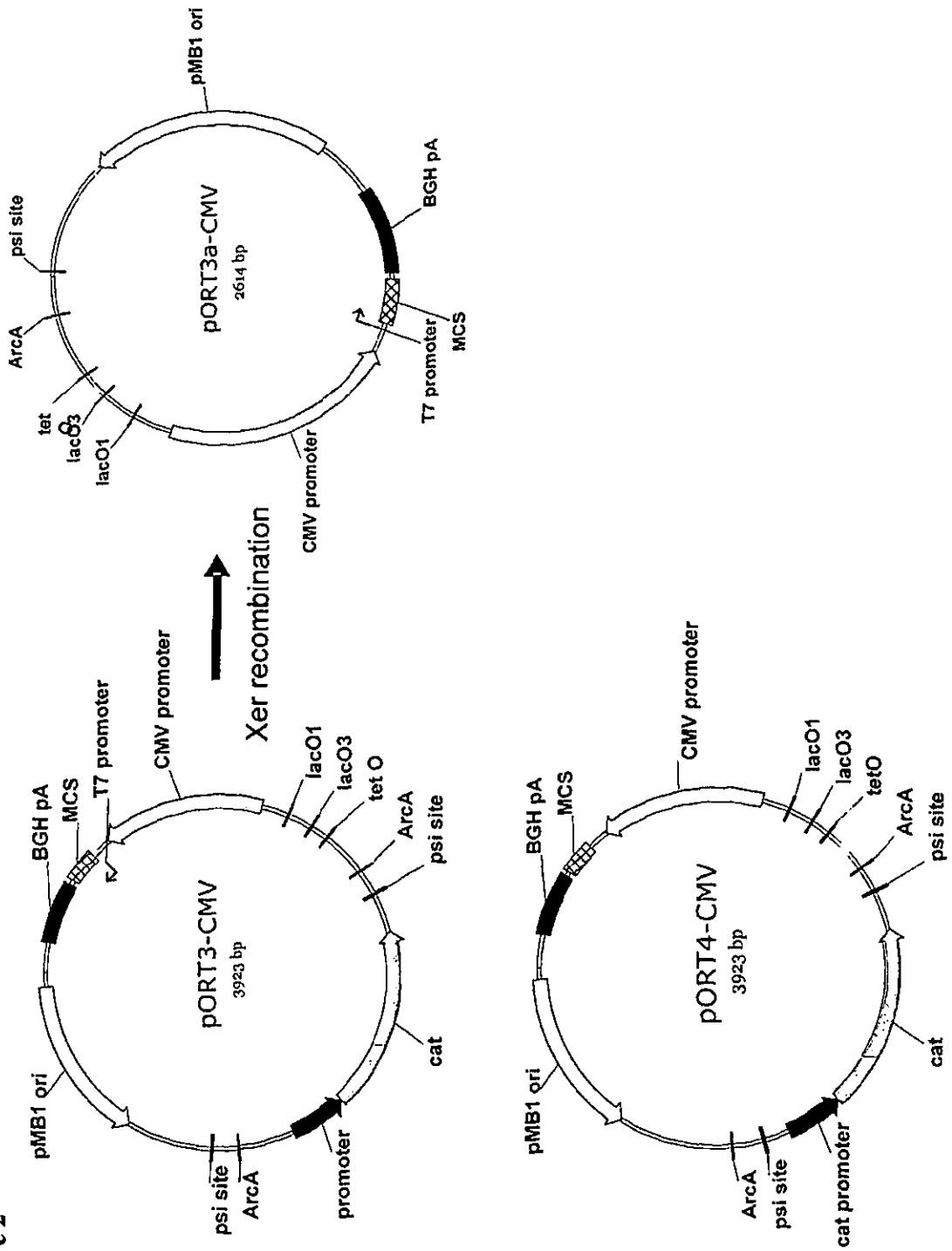
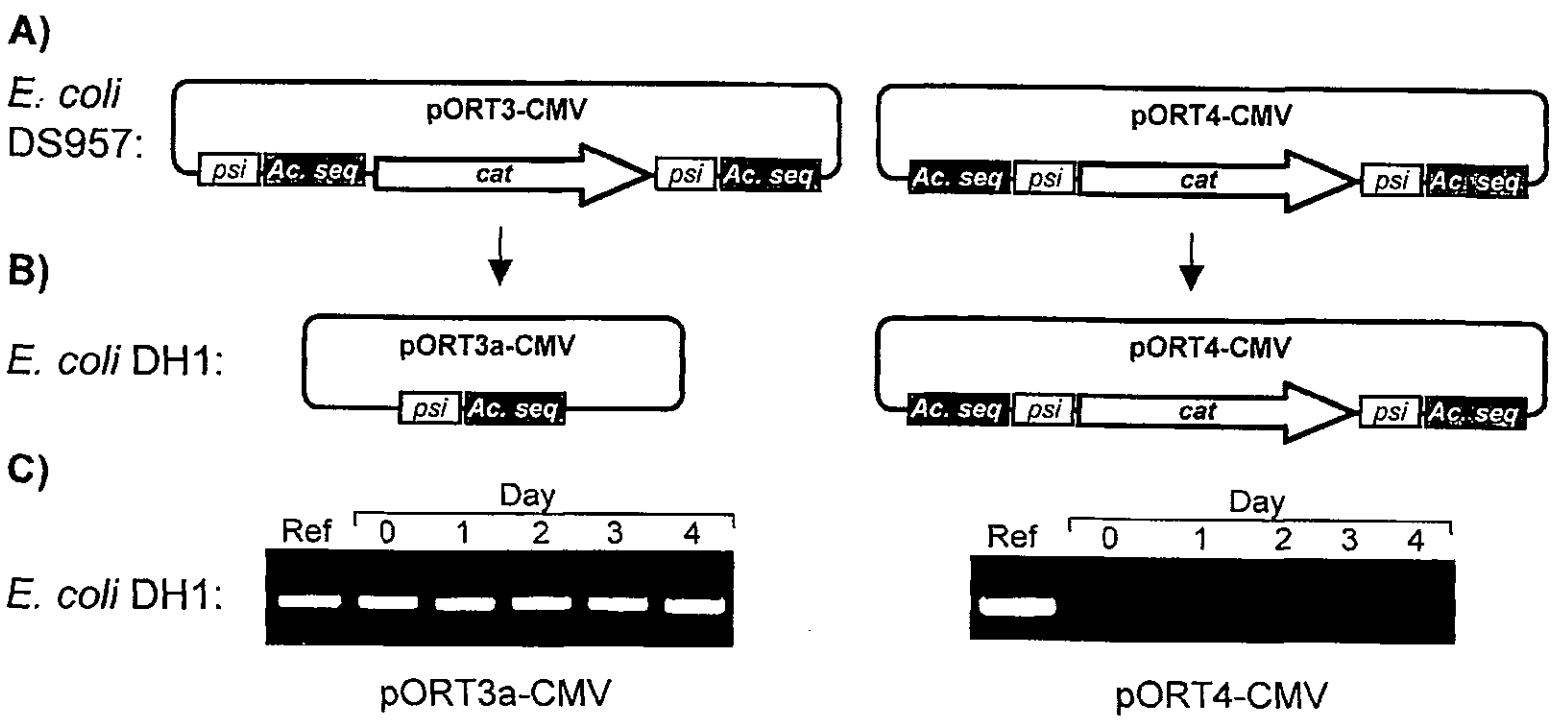

pMB1 ori
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3923 bp
cat
psi site
ArcA
promoter
pMB1 ori
BGH pA
MCS
CMV promoter
lacO1
lacO3
tetO
ArcA
psi site
pORT4-CMV
3923 bp
cat
ArcA
psi site
cat promoter

Figure 2

Figure 3


A)
E. coli DS957:
pORT3-CMV
psi
Ac. seq.
cat
psi
Ac. seq.
pORT4-CMV
Ac. seq.
psi
cat
psi
Ac. seq.
B)
E. coli DH1:
pORT3a-CMV
psi
Ac. seq.
pORT4-CMV
Ac. seq.
psi
cat
psi
Ac. seq.
C)
E. coli DH1:
Day
Ref
0
1
2
3
4
pORT3a-CMV
Day
Ref
0
1
2
3
4
pORT4-CMV

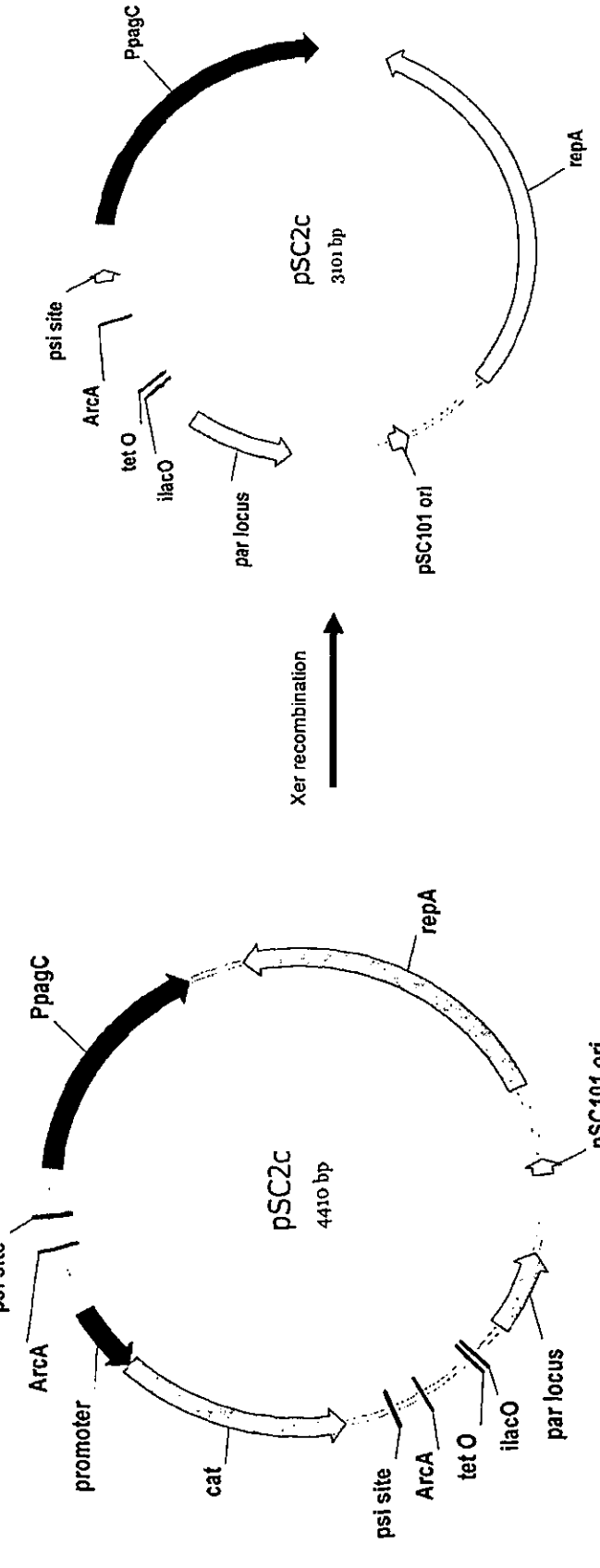
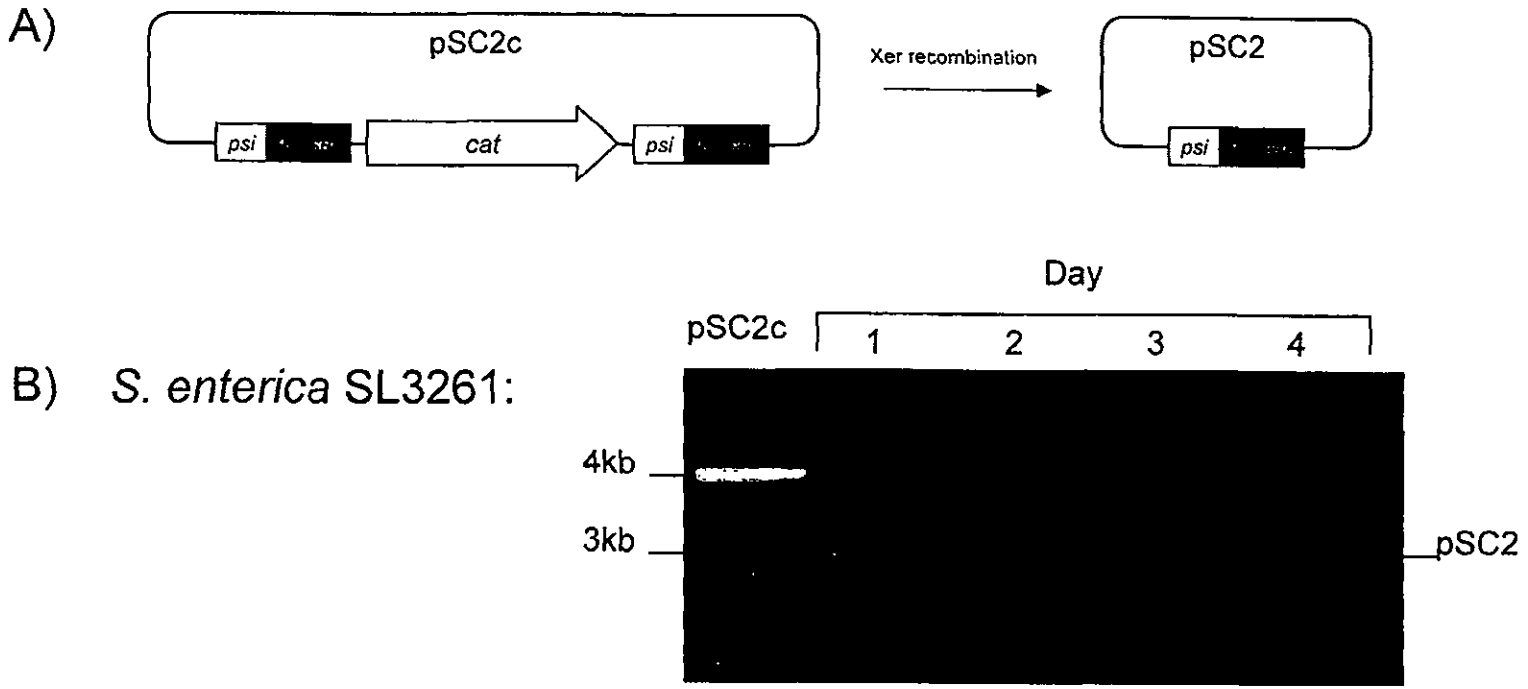

PpagC
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4410 bp
pSC101 ori
psi site
ArcA
tet O
ilacO
par locus

Figure 4

Figure 5


A)
pSC2c
psi
cat
psi
Xer recombination
pSC2
psi
B) S. enterica SL3261:
Day
pSC2c
1
2
3
4
4kb
3kb
pSC2