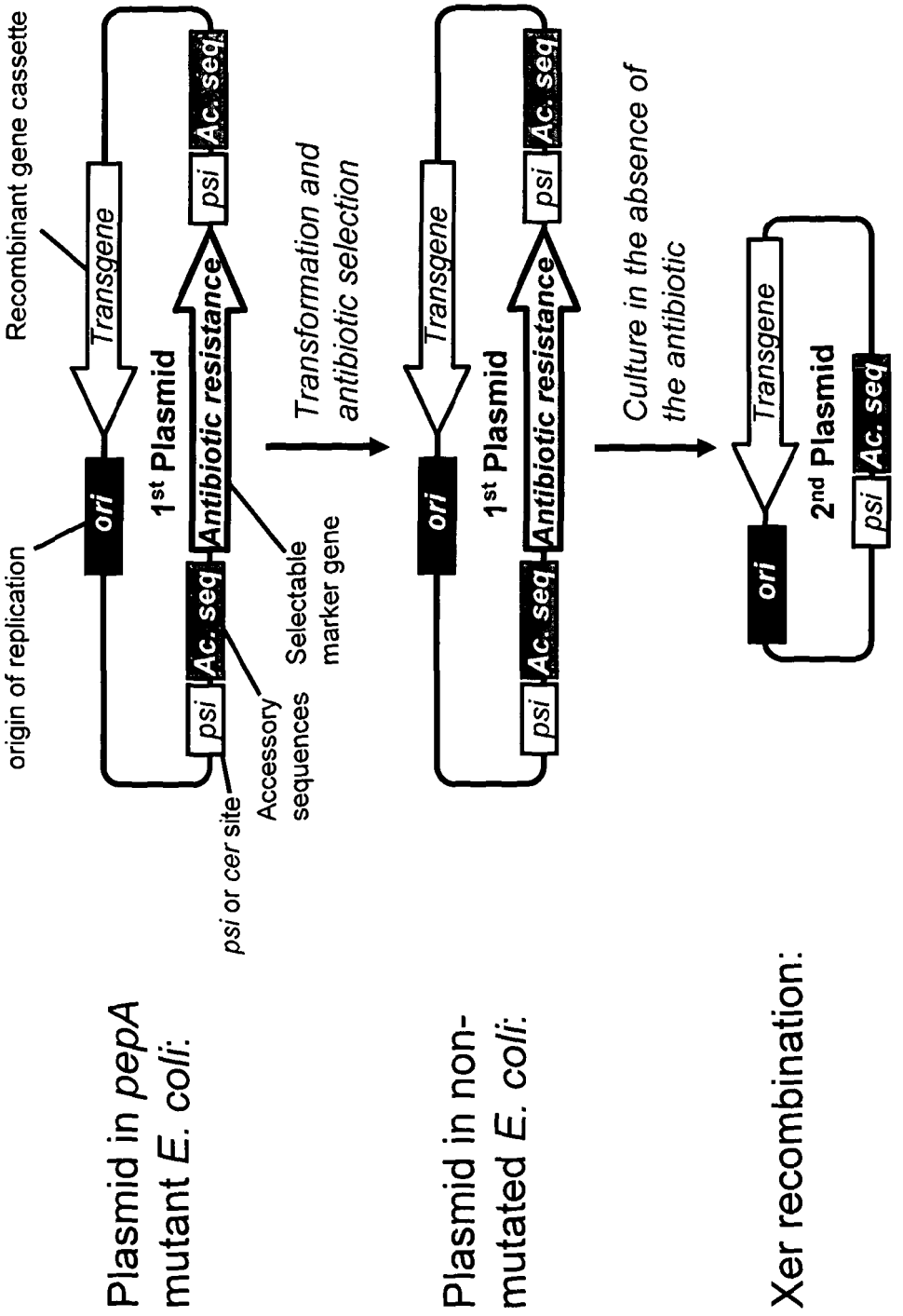

Recombinant gene cassette
Transgene
origin of replication
ori
1st Plasmid
Antibiotic resistance
psi
Ac. seq
psi or cer site
Accessory sequences
Selectable marker gene
Transformation and antibiotic selection
Plasmid in pepA mutant E. coli:
Plasmid in non-mutated E. coli:
Culture in the absence of the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

Figure 1

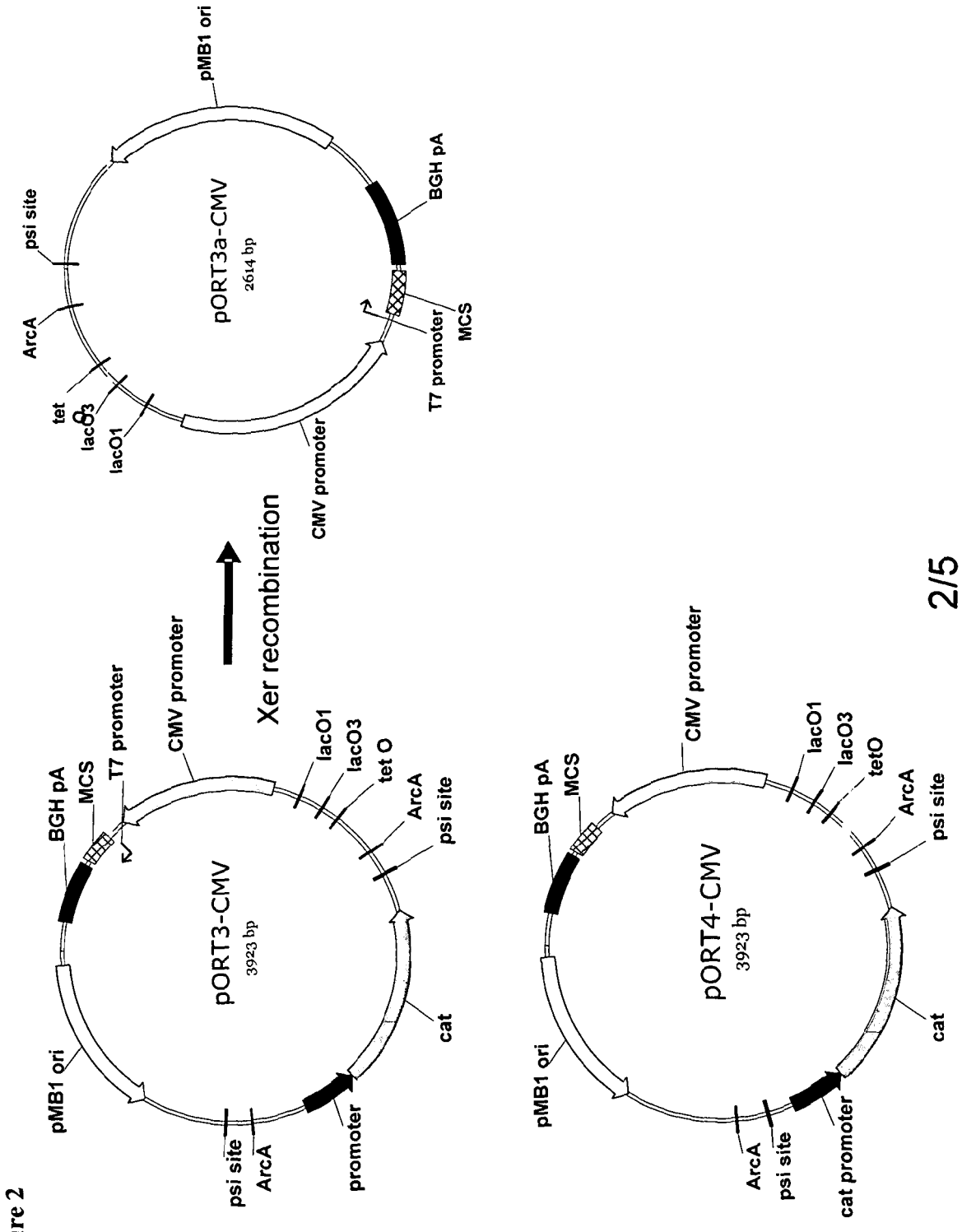
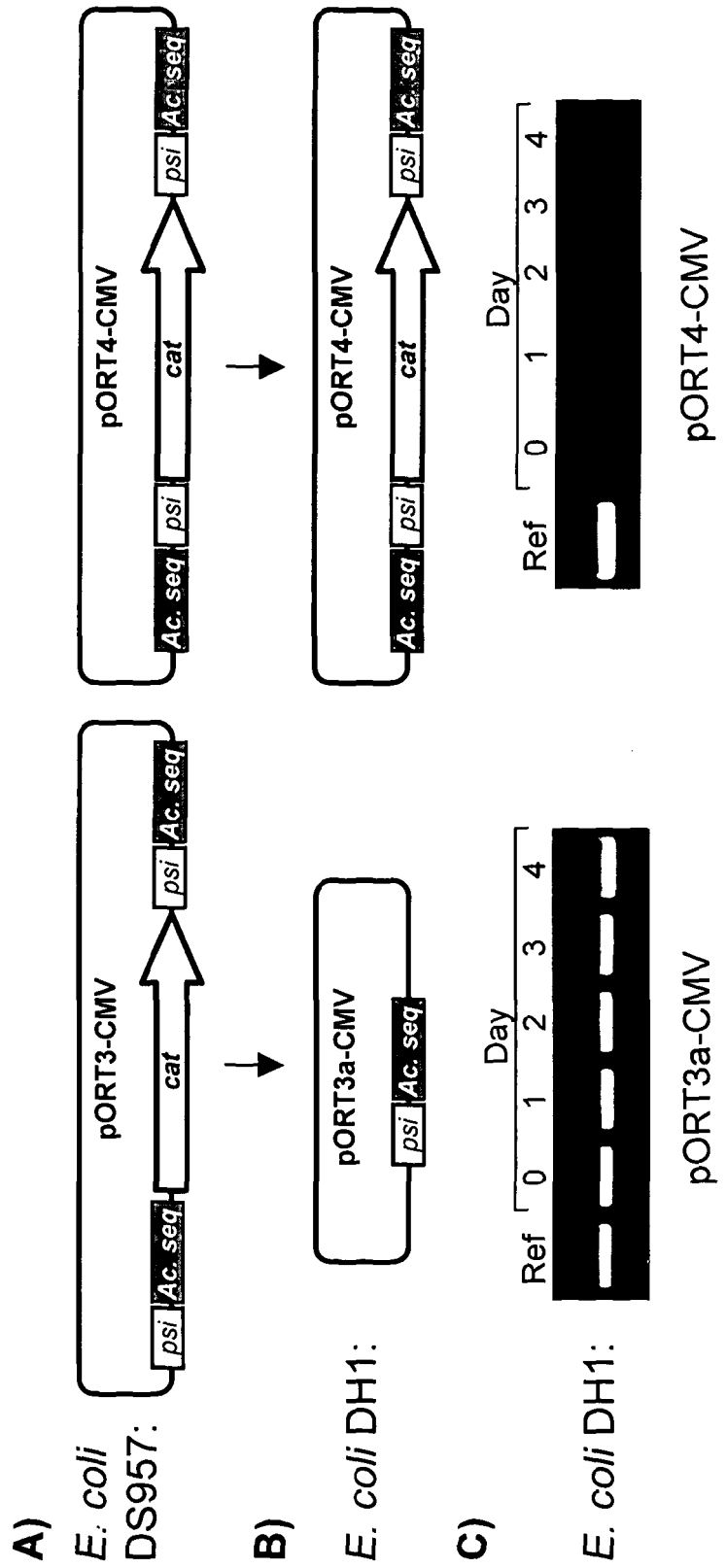

pORT3a-CMV
2614 bp
pMB1 ori
BGH pA
MCS
T7 promoter
CMV promoter
psi site
ArcA
tet
lacO3
lacO1
Xer recombination
pORT3-CMV
3923 bp
lacO1
lacO3
tet O
ArcA
psi site
cat
promoter
pORT4-CMV
3923 bp
tetO
cat promoter

Figure 2

Figure 3


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

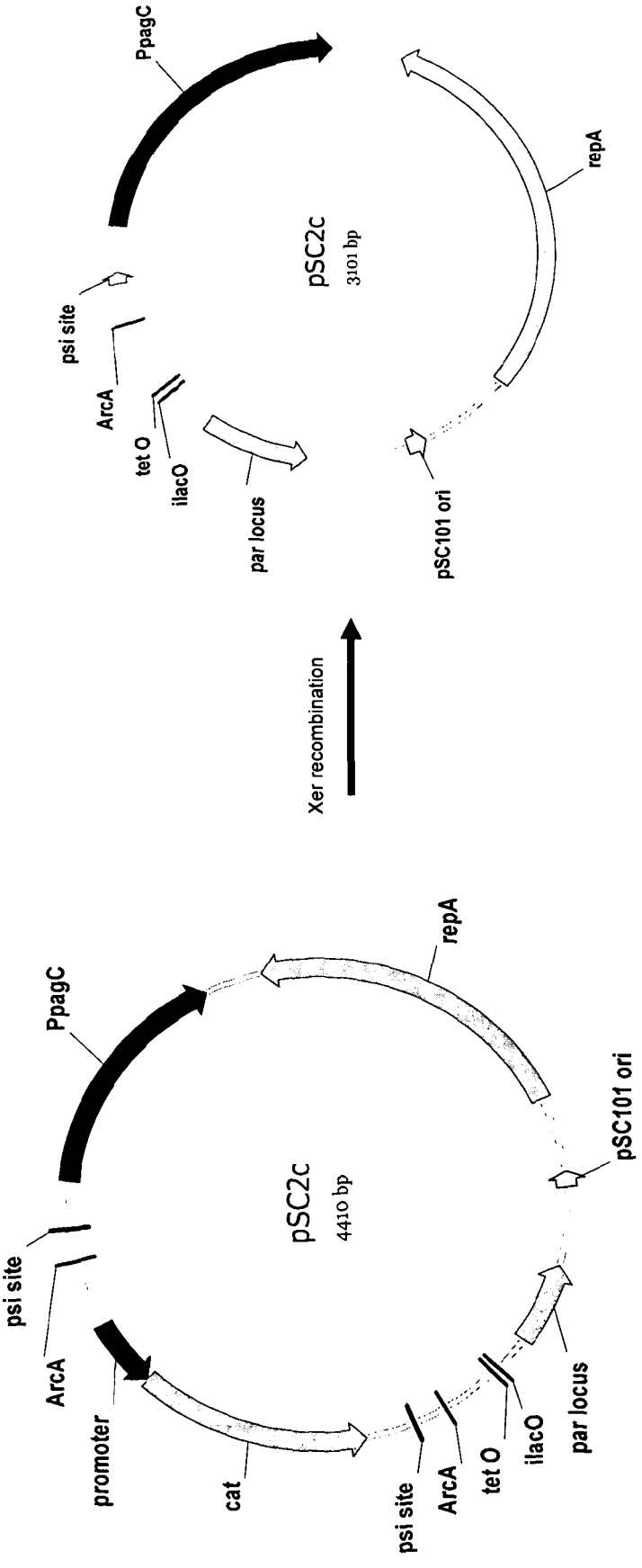

PpagC
psi site
ArcA
tet O
ilacO
par locus
pSC2c
3101 bp
repA
pSC101 ori
Xer recombination
PpagC
repA
psi site
ArcA
promoter
cat
pSC2c
4410 bp
pSC101 ori
psi site
ArcA
tet O
ilacO
par locus

Figure 4

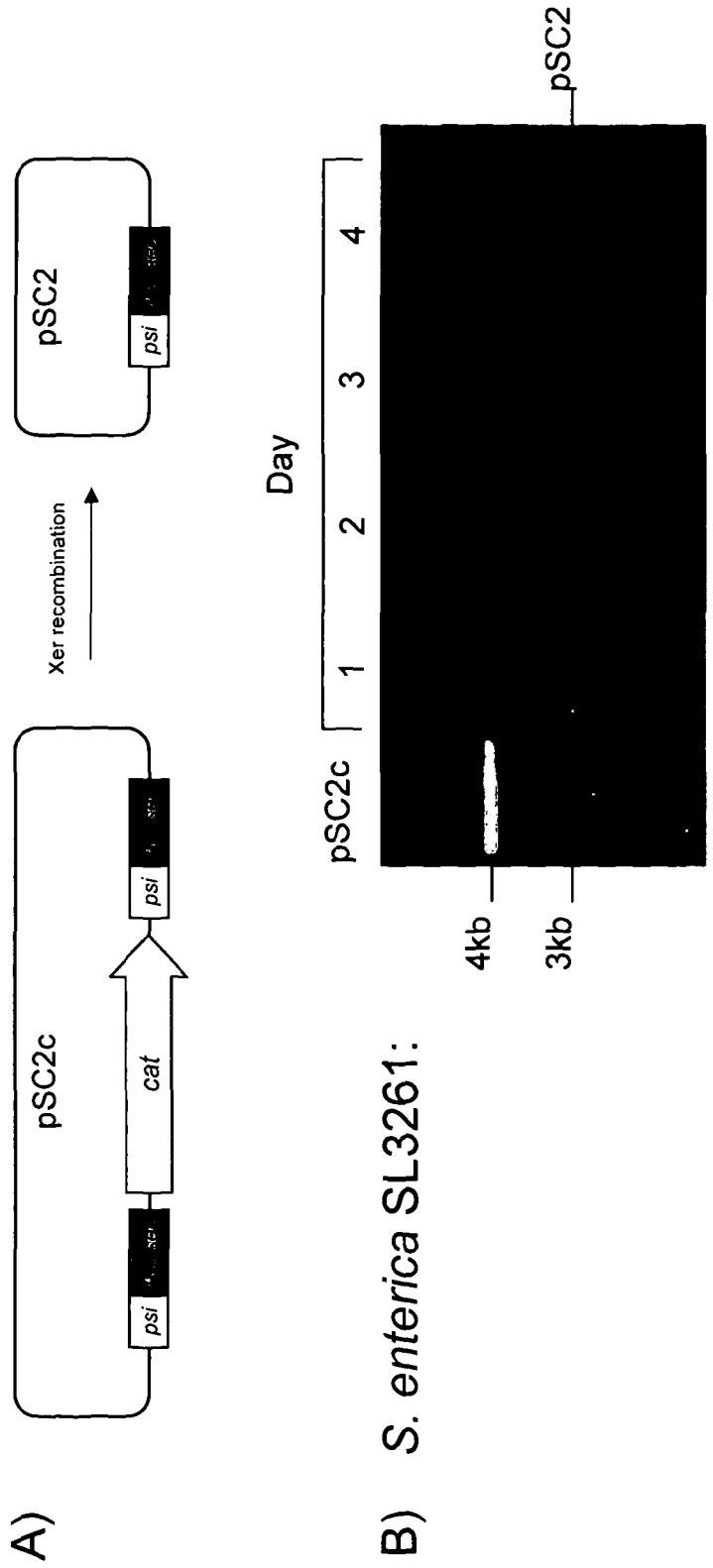

A)
pSC2c
psi
cat
psi
Xer recombination
pSC2
psi
B) S. enterica SL3261:
pSC2c
Day
1
2
3
4
pSC2
4kb
3kb

Figure 5