Fig. 1

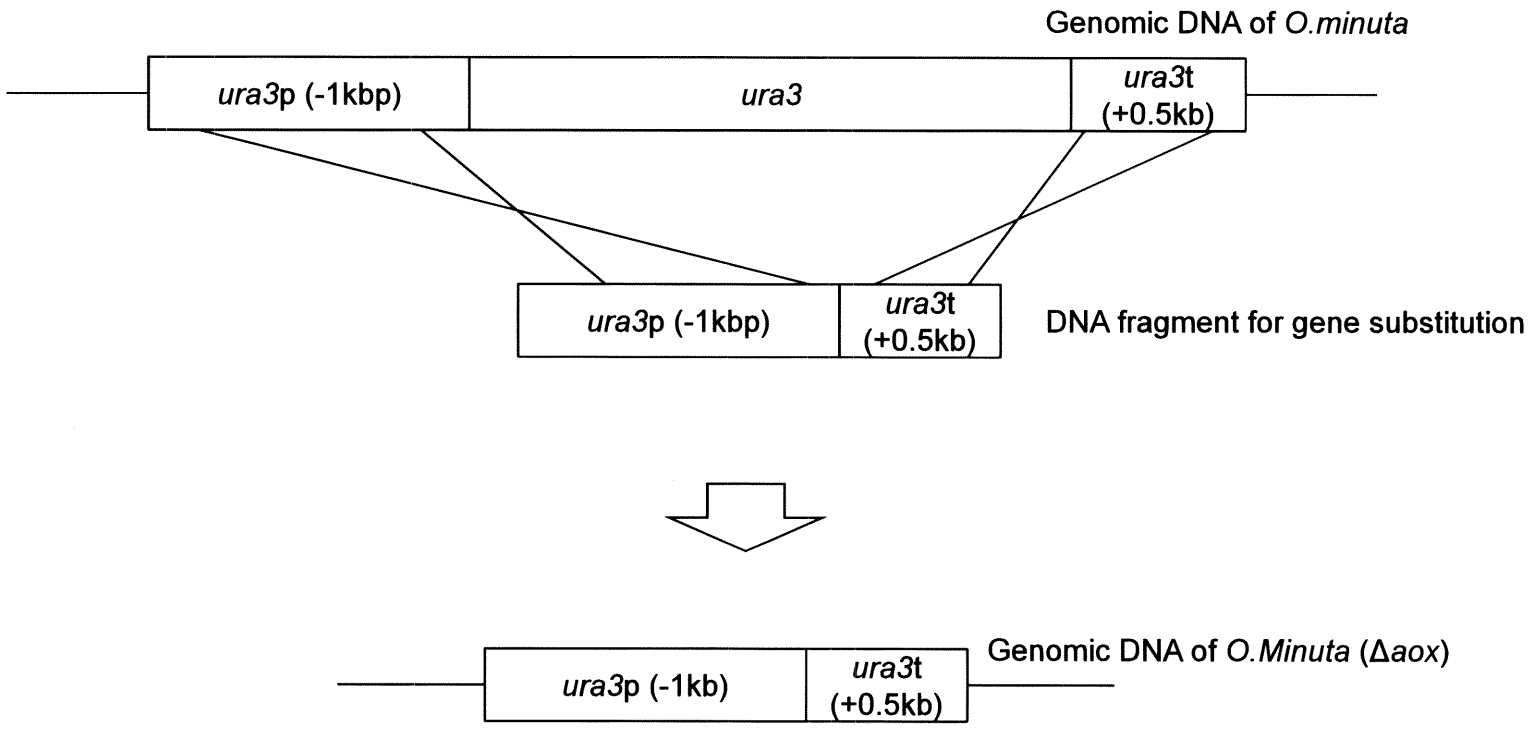

Genomic DNA of O.minuta
ura3p (-1kbp)
ura3
ura3t (+0.5kb)
ura3p (-1kbp)
ura3t (+0.5kb)
DNA fragment for gene substitution
ura3p (-1kb)
ura3t (+0.5kb)
Genomic DNA of O.Minuta (Δaox)

Fig. 2

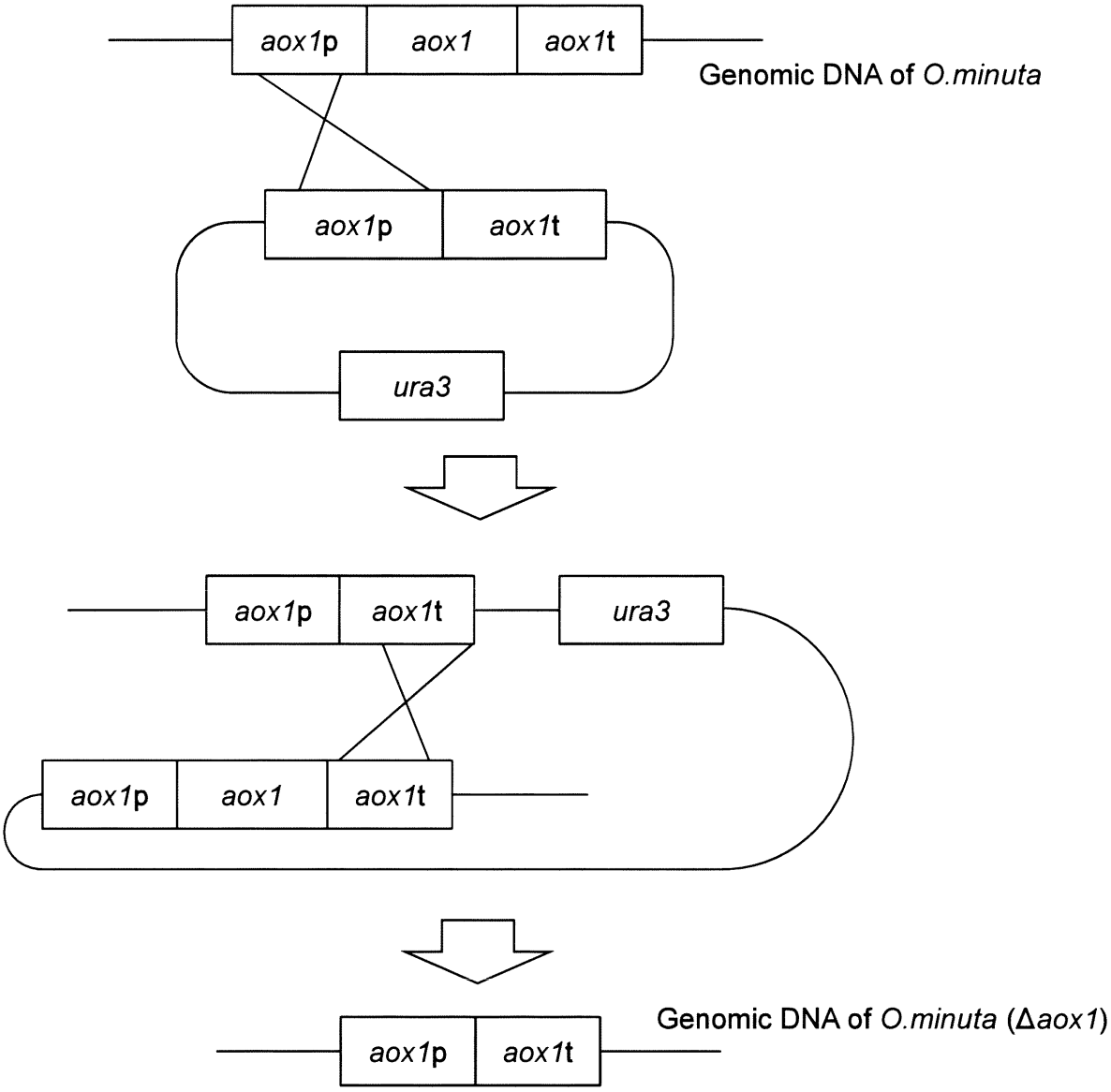

aox1p
aox1
aox1t
Genomic DNA of O.minuta
aox1p
aox1t
ura3
aox1p
aox1t
ura3
aox1p
aox1
aox1t
aox1p
aox1t
Genomic DNA of O.minuta (Δaox1)

Fig. 3

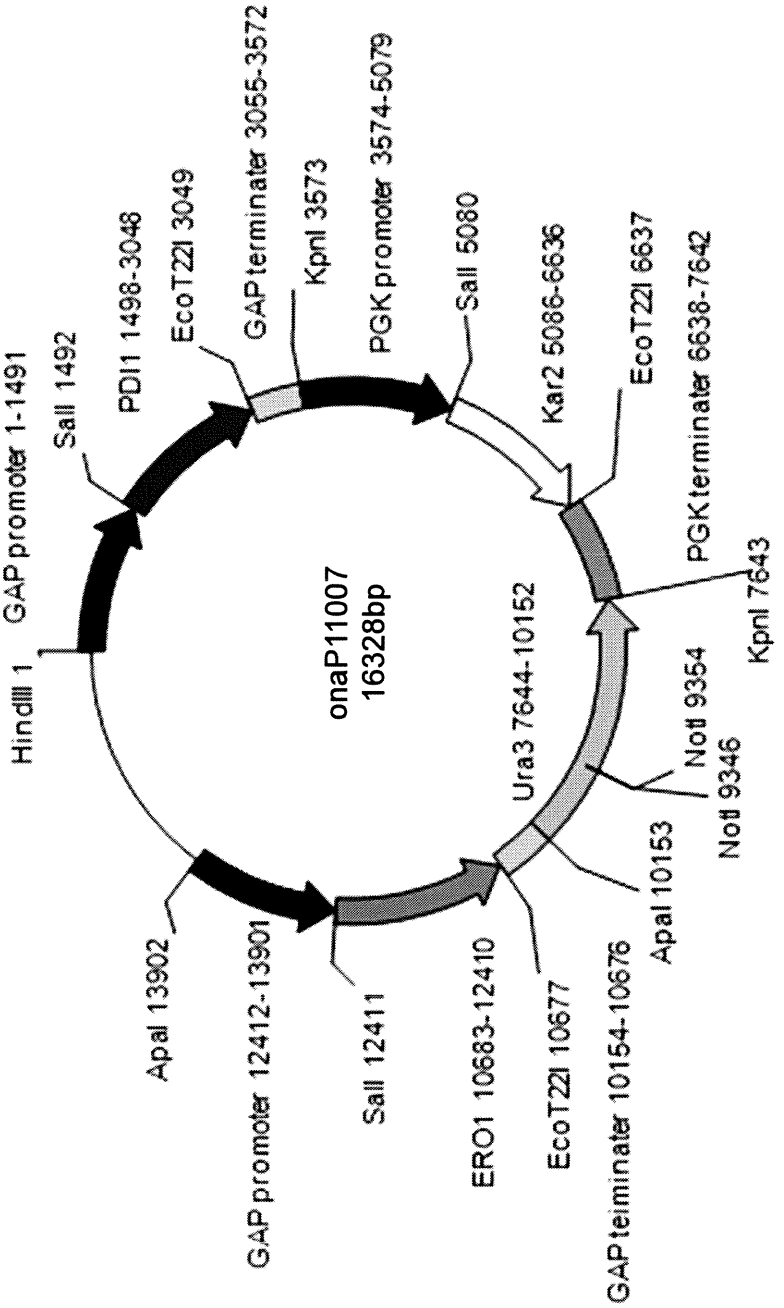

onaP11007
16328bp
HindIII 1
GAP promoter 1-1491
SalI 1492
PDI1 1498-3048
EcoT22I 3049
GAP terminater 3055-3572
KpnI 3573
PGK promoter 3574-5079
SalI 5080
Kar2 5086-6636
EcoT22I 6637
PGK terminater 6638-7642
KpnI 7643
Ura3 7644-10152
NotI 9354
NotI 9346
ApaI 10153
EcoT22I 10677
ERO1 10683-12410
SalI 12411
GAP promoter 12412-13901
ApaI 13902
GAP teiminater 10154-10676

Fig. 4

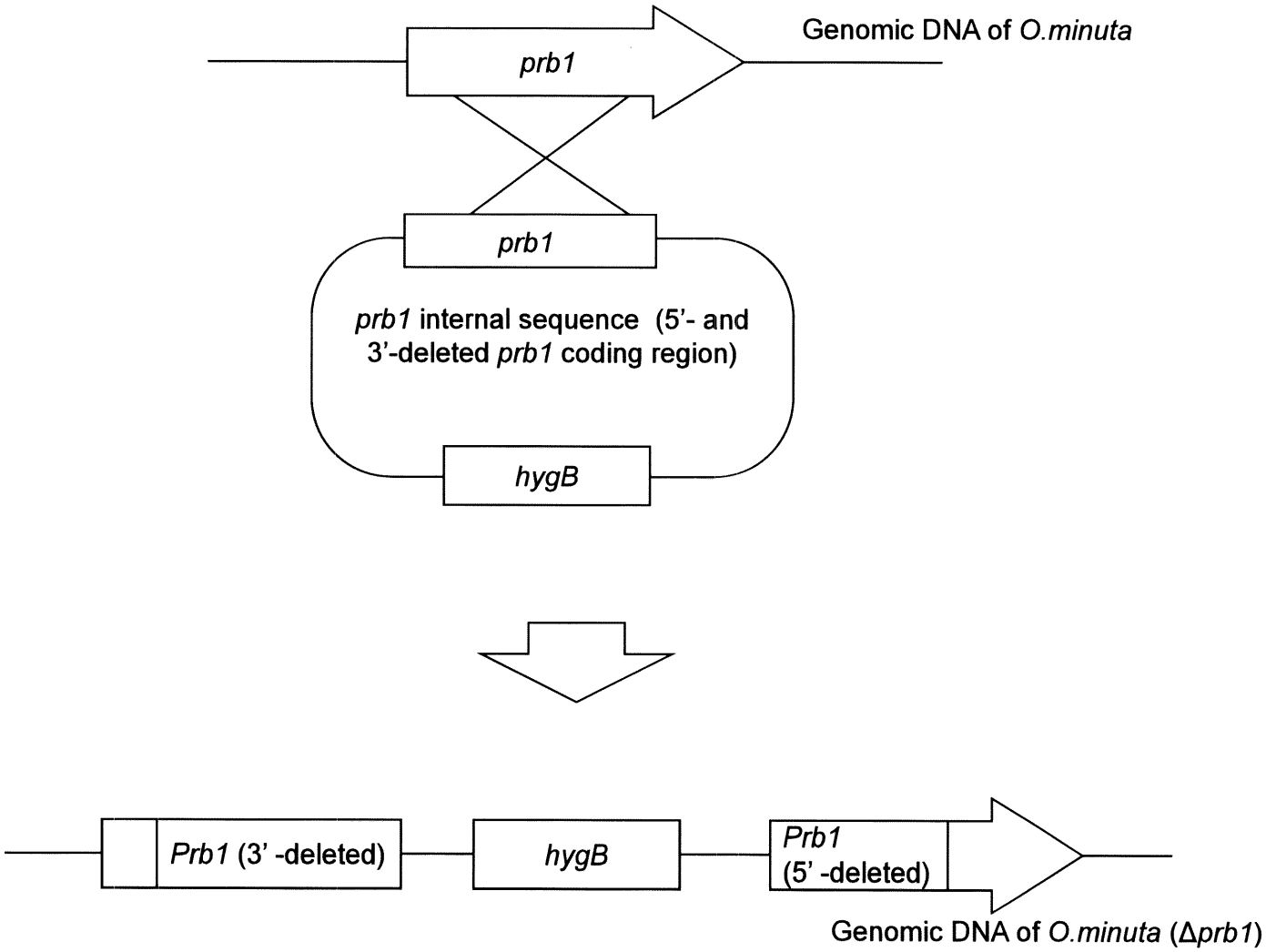
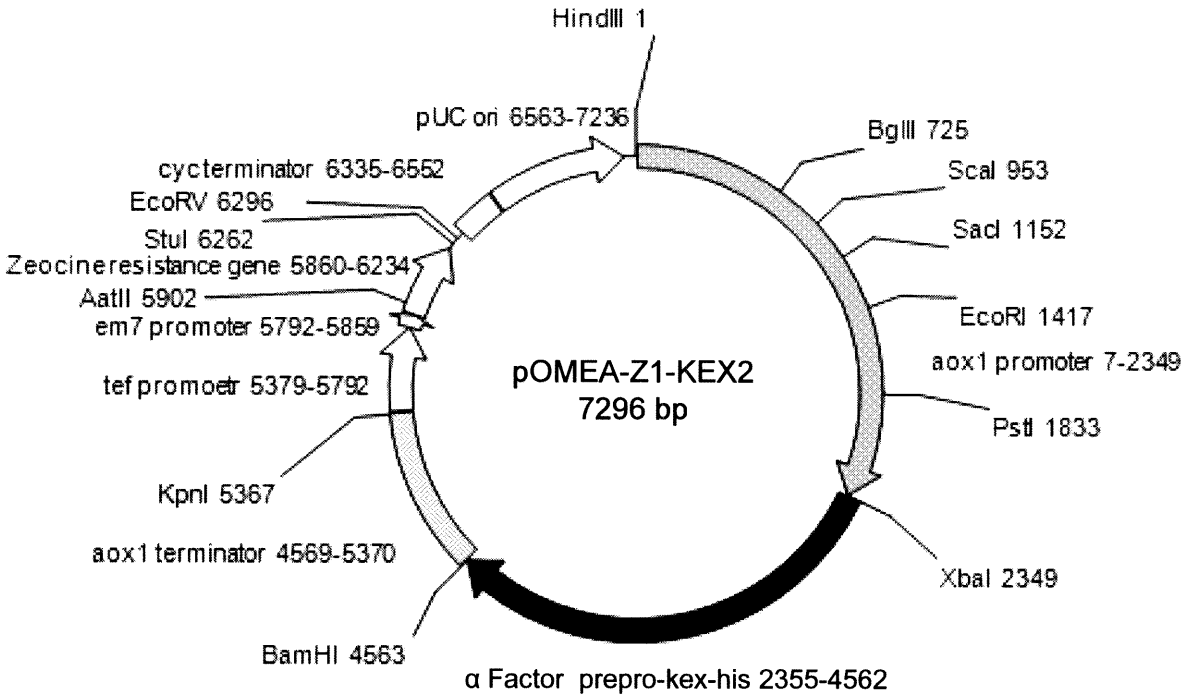

Genomic DNA of O.minuta
prb1
prb1
prb1 internal sequence (5'- and 3'-deleted prb1 coding region)
hygB
Prb1 (3' -deleted)
hygB
Prb1 (5' -deleted)
Genomic DNA of O.minuta (Δprb1)

Fig. 5



pOMEA-Z1-KEX2

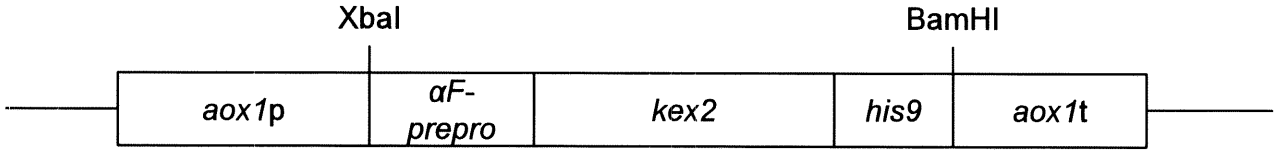
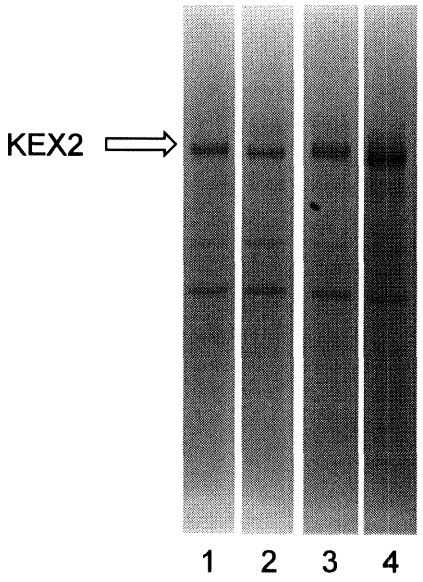


Fig. 6-1



1: NBRC10746+PEK
2: Δ*aox1* + PEK
3: NBRC10746+ PEK d*prb1*
4: Δ*aox1* + PEK d*prb1*

CBB staining
Reduced

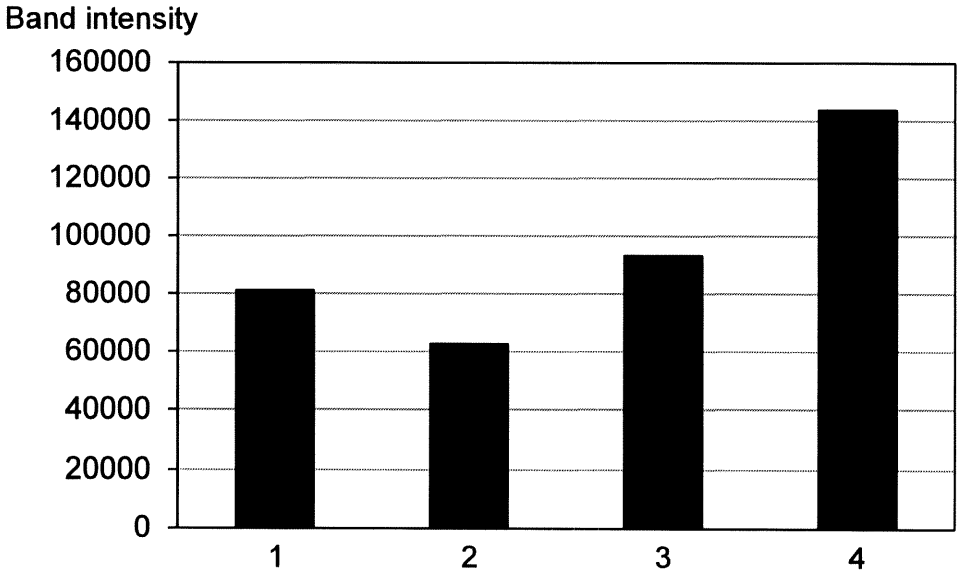


Fig. 6-2

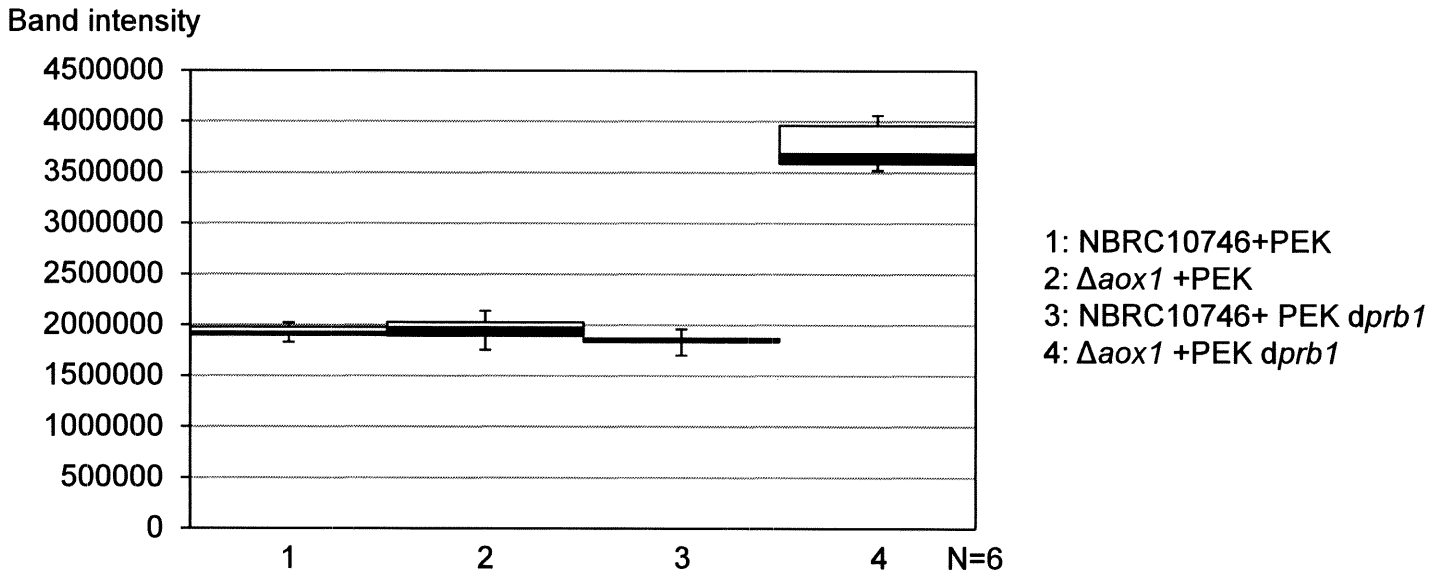
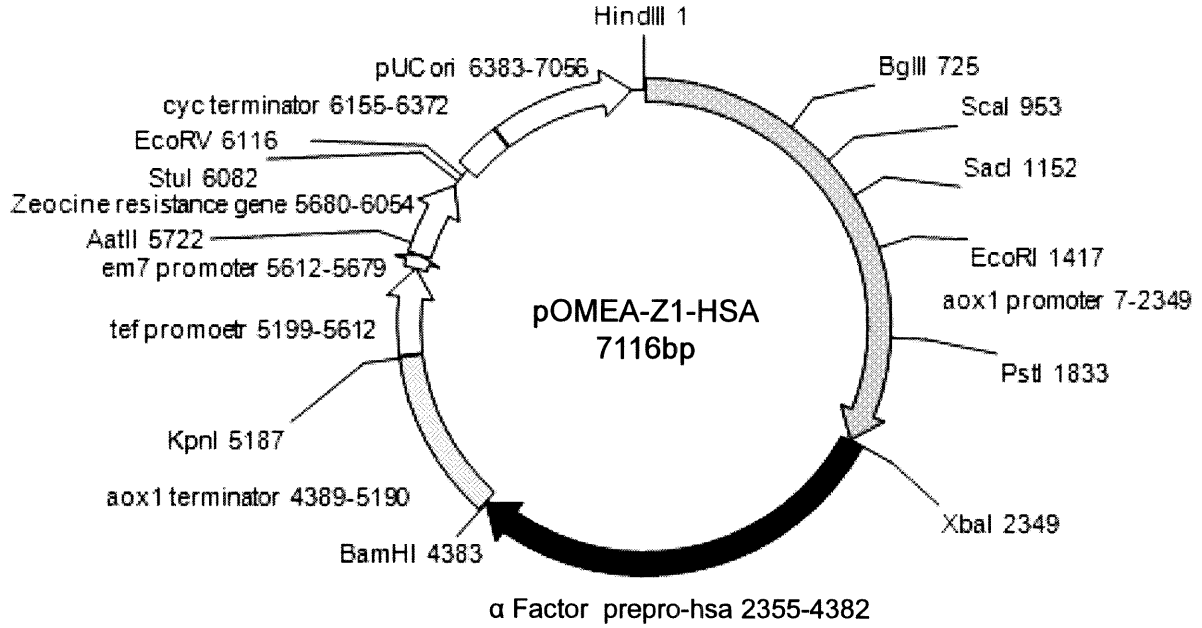

Band intensity
4500000
4000000
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
1
2
3
4
N=6
1: NBRC10746+PEK
2: Δaox1 +PEK
3: NBRC10746+ PEK dprb1
4: Δaox1 +PEK dprb1

Fig. 7


HindIII 1
BglII 725
ScaI 953
SacI 1152
EcoRI 1417
aox1 promoter 7-2349
PstI 1833
XbaI 2349
α Factor prepro-hsa 2355-4382
BamHI 4383
aox1 terminator 4389-5190
KpnI 5187
tef promoetr 5199-5612
em7 promoter 5612-5679
AatII 5722
Zeocine resistance gene 5680-6054
StuI 6082
EcoRV 6116
cyc terminator 6155-6372
pUC ori 6383-7056
pOMEA-Z1-HSA
7116bp

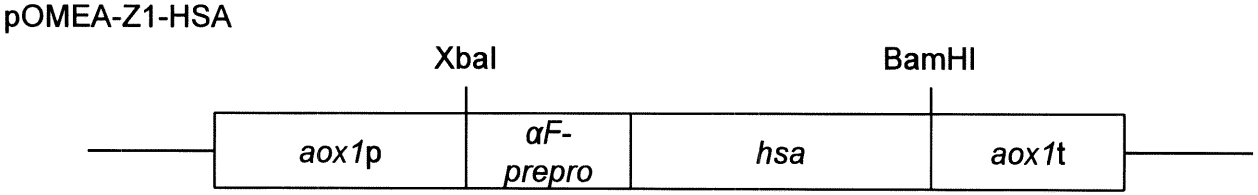

pOMEA-Z1-HSA
XbaI
BamHI
aox1p
αF-
prepro
hsa
aox1t

Fig. 8

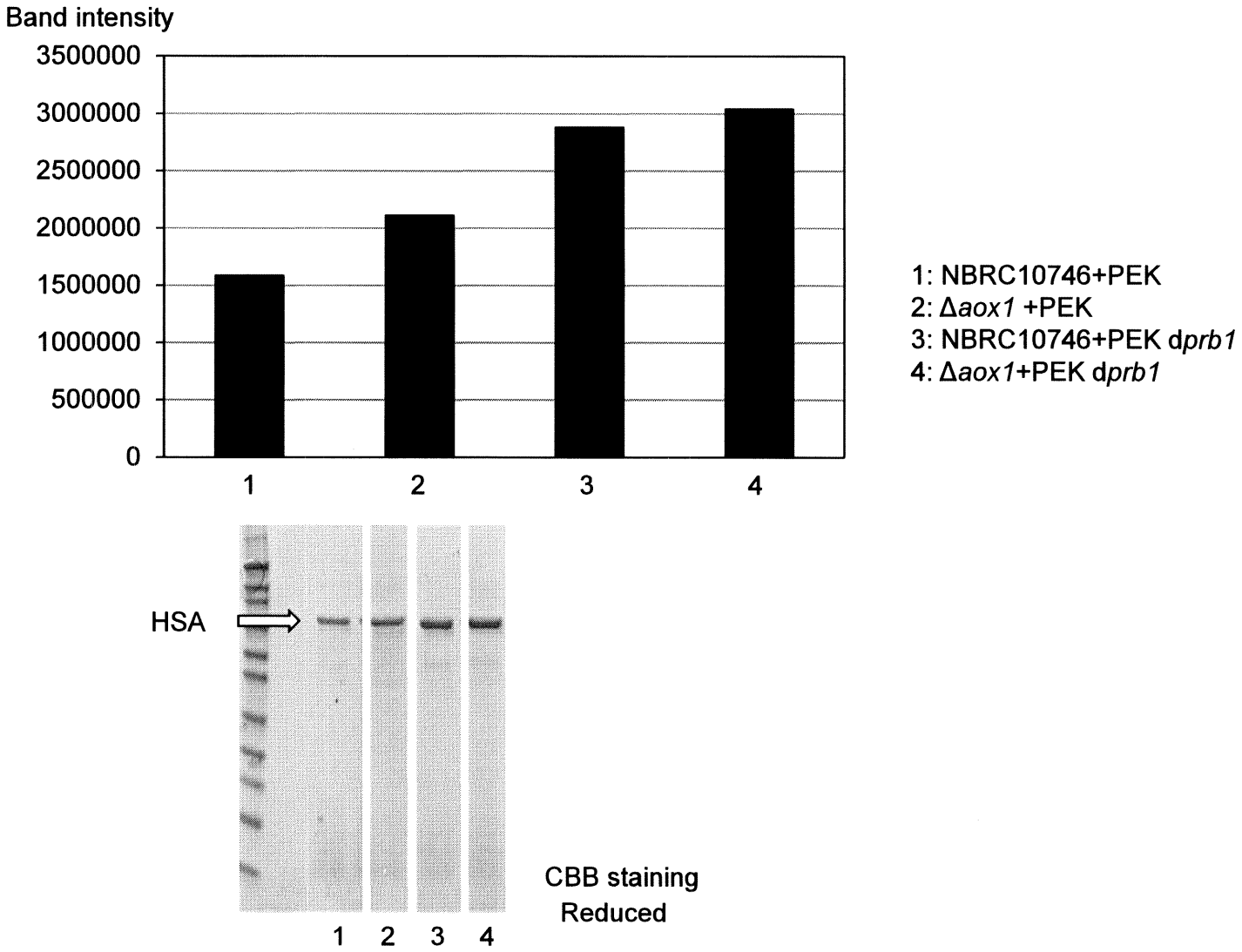
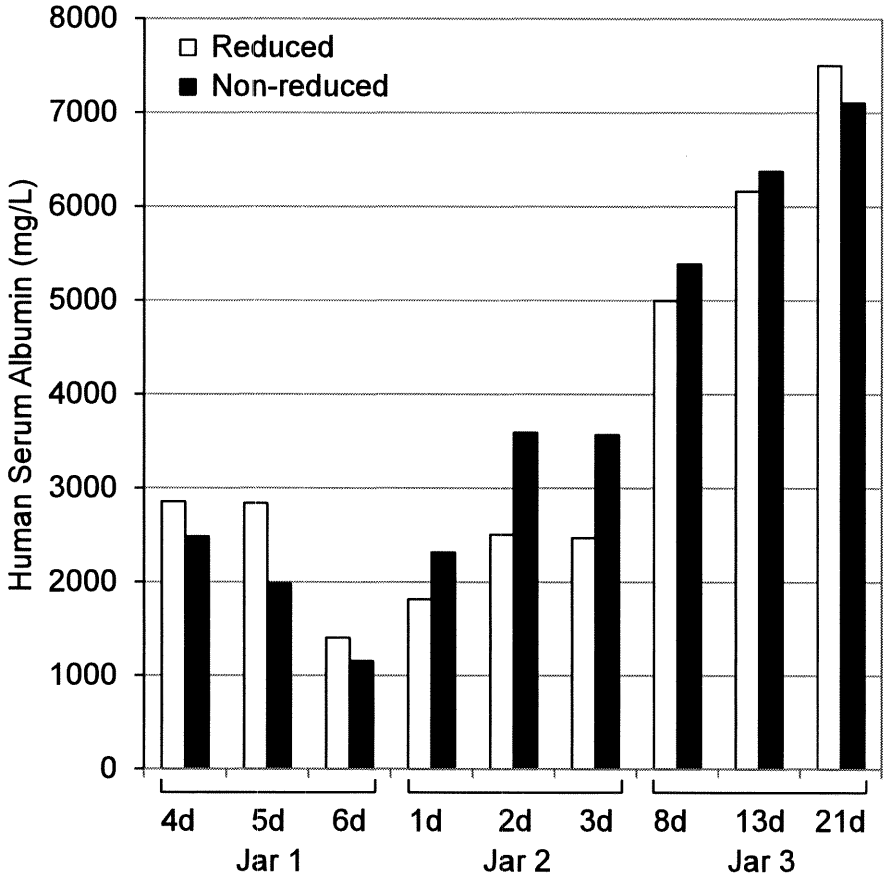
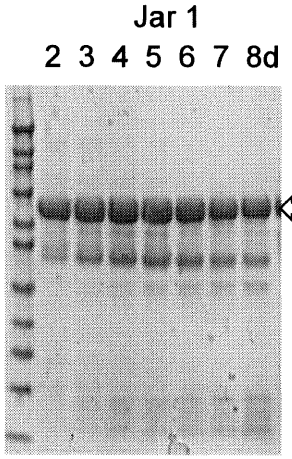
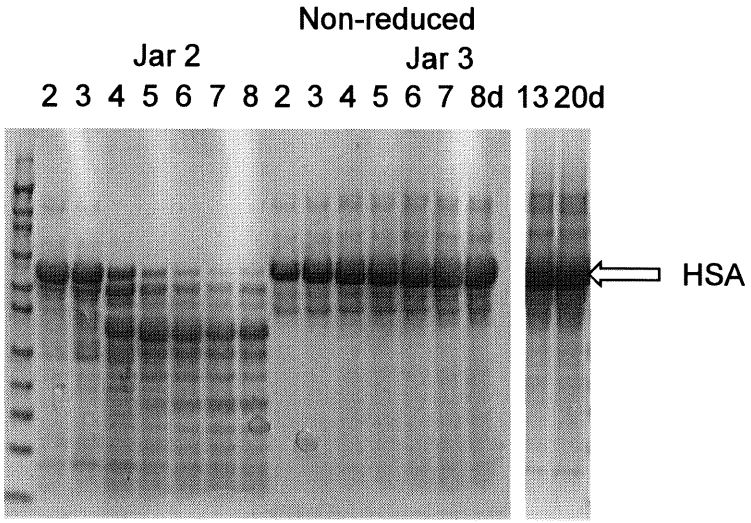

Band intensity
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
1
2
3
4
1: NBRC10746+PEK
2: Δaox1 +PEK
3: NBRC10746+PEK dprb1
4: Δaox1+PEK dprb1
HSA
1 2 3 4
CBB staining
Reduced

Fig. 9



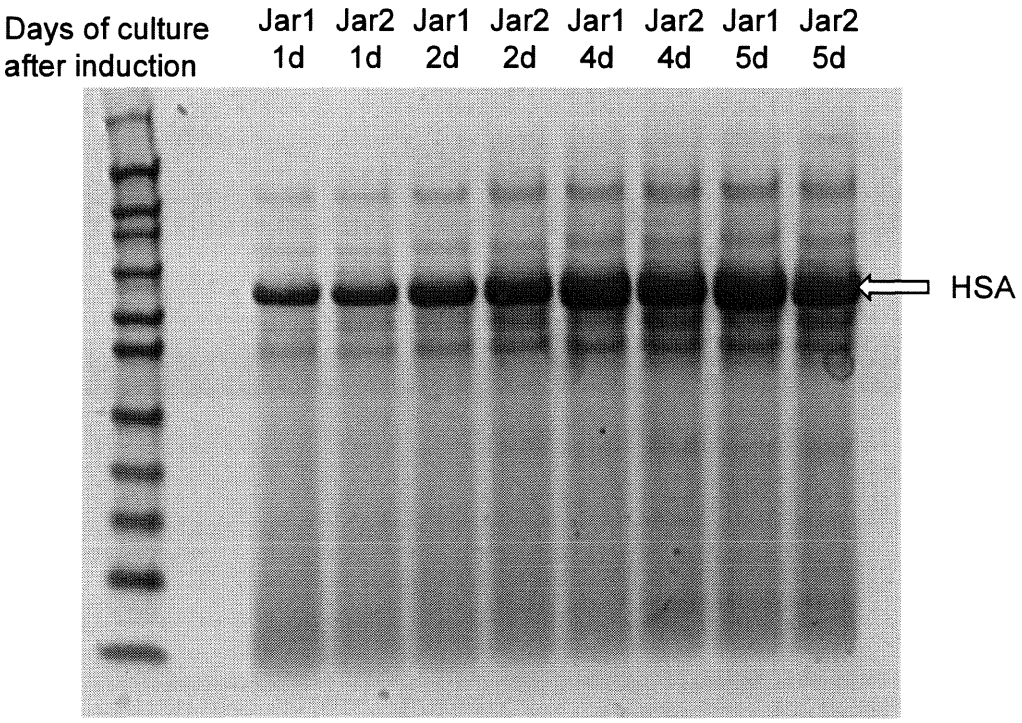




Jar1: NBRC10746+PEK,
Jar2: NBRC10746+PEK, nitrogen source fed-batch culture, regulated to pH 7
Jar3: ⊿*aox1*+PEK d*prb1*, nitrogen source fed-batch culture, regulated to pH 7

CBB staining Non-reduced

Fig. 10



Non-reducing SDS-PAGE/CBB staining

Jar1: NBRC10746⊿*aox*+PEK d*prb1*, low-methanol-induced culture
Jar2: NBRC10746⊿*aox*+PEK d*prb1*, carbon source starvation-induced culture