

## pGAPZ $\alpha$ C MCS

361 GATTATTGGA AACCAACCAGA ATCGAATATA AAAGGCGAAC ACCTTTCCCA ATTTTGGTTT  
 421 CTCCTGACCC AAAGACTTTA AATTTAATTT ATTTGTCCCT ATTTCAATCA ATTGAACAAC  
 481 TATTTGAAA CG ATG AGA TTT CCT TCA ATT TTT ACT GCT GTT TTA TTC GCA  
 Met Arg Phe Pro Ser Ile Phe Thr Ala Val Leu Phe Ala

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532 GCA TCC TCC GCA TTA GCT GCT CCA GTC AAC ACT ACA ACA GAA GAT GAA ACG  
 Ala Ser Ser Ala Leu Ala Ala Pro Val Asn Thr Thr Thr Glu Asp Glu Thr  
 $\alpha$ -factor signal sequence

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583 GCA CAA ATT CCG GCT GAA GCT GTC ATC GGT TAC TCA GAT TTA GAA GGG GAT  
 Ala Gln Ile Pro Ala Glu Ala Val Ile Gly Tyr Ser Asp Leu Glu Gly Asp

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634 TTC GAT GTT GCT GTT TTG CCA TTT TCC AAC AGC ACA AAT AAC GGG TTA TTG  
 Phe Asp Val Ala Val Leu Pro Phe Ser Asn Ser Thr Asn Asn Gly Leu Leu

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685 TTT ATA AAT ACT ACT ATT GCC AGC ATT GCT GCT AAA GAA GAA GGG GTA TCT  
 Phe Ile Asn Thr Thr Ile Ala Ser Ile Ala Ala Lys Glu Glu Gly Val Ser

*Xho*I\*      Kex2 signal cleavage      *Cla*I    *Eco*R I      *Pml*I      *Sfi*I

736 CTC GAG AAG AGA GAG GCT GAA GCA TCGAT GAATTCACGT GGCCAGCCG GCCGTCT  
 Leu Glu Lys Arg Glu Ala Glu Ala  
 Ste13 signal cleavage

Asp718 I    *Kpn*I *Xho*I      *Sac*II *Not*I      *Xba*I      *myc* epitope

792 CGGATCGGTA CCTCGAGCCG CGGCGGCCGC CAGCTTTCTA GAA CAA AAA CTC ATC TCA  
 Glu Gln Lys Leu Ile Ser

polyhistidine tag

850 GAA GAG GAT CTG AAT AGC GCC GTC GAC CAT CAT CAT CAT CAT CAT TGA GTTT  
 Glu Glu Asp Leu Asn Ser Ala Val Asp His His His His His His \*\*\*

902 TAGCCTTAGA CATGACTGTT CCTCAGTTCA AGTTGGGCAC TTACGAGAAG ACCGGTCTTG

3' AOX1 priming site

962 CTAGATTCTA ATCAAGAGGA TGTCAGAATG CCATTTGCCT GAGAGATGCA GGCTTCATTT

1022 TTGATACTTT TTTATTTGTA ACCTATATAG TATAGGATTT TTTTGTGCAT TTTGTTTCTT

\* The *Xho* I site upstream of the Kex2 cleavage site is used to clone the gene of interest flush with the Kex2 cleavage site (see page 14).