

Fig. 1

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C.glutamicum      1 -----MNSTQTRAKPARISFLDKYIPLWIIILAMAFGLFLGRSVSGISGLIG-
A.metalloreg     1 -----MGNENVVHEGKG---IGFFERYLTWVAACHIVGVALGQLPAVPETLS--
M.vannielii      1 -----MAKN-----IGFFERYLSFWVFLCIIIFGIIILGKFLPDPMPYLS--
S.medicae        1 -----MRPAQPTIRLSFLDRYLTVVWIFETAMALGLVGLVTFVTCGLPAALD--
B.subtilis       1 -----MK-----RLSFLDRYLTVWIFELAMALGIGLGFIFFSFVGLN--
Synechococcus    1 -----MIMALTASDPKALPSGGSLSEFFERYLTWVVALCQVAGIILGRFPFVABALD-
Pichia           1 -----MTLAQD-----VN-----KKVSLVDKLLPLIILHAIIGVIVSVAPNAQSSF--
S.cerevisiae     1 -----MSEDQKSENSVPSKVMVNRDITLTKLSLWLDLMLPFTIILSHIIVAVISVYVPSRRTFDAE
A.fumigatus      1 MSQARLGSIPDIAKPSPAQAADMEKQGEPLDGPDPGPKQSAFKNLGLDRFLALWIFLAMAGIILGNFVPTGPAIQ-
P.vittata        1 MENS---SAERKQQLALDIALGNDPDSDAKNPDGRTRKLOGLFKQLSLLDRYLTVVWIFIVMAVSIIEGYVVKGVKKAFQ--
D.terio         1 -----MSAGAQAQVAAPRQTPMSGFFERYLTWVVALCQVAGVALGQGLPDPFOALG--

C.glutamicum     48 -AMEVGGISLPIALGLIVMMYPPAKVRYDKTKQIATDKH---LVGVSLLNWWVGPALMPALAWLFP-----
A.metalloreg     47 -EWEYAQVSIPIVAITLWLMYPMMLKIDTSVEATKRP---KGLIVTCVNNWLIKPFIMYLAFAFFKIVFQN-----
M.vannielii      39 -NITISGVNIPITAVLWFMVYPMVVKIDSAVKNLGKSLF-KGSIATWLNWLIKPFISMEILIAFFVTLFSGYLE---
S.medicae        44 -GLSVGTTNVPITAGLILMMYPPAKVRYEBLHOVFADR---ITLSLVQNNWIKGPIMLFGLAVLFLR---
B.subtilis       38 -KIQVGTTSIPITAGLIVMMYPPAKVRYEELGRVFDIK---VILSLVQNNWIKGPIMLFGLAVLFLR---
Synechococcus    53 -ALSIVQVSLPIAVGLFFEMYPMLVKIDEGQAYALKSP---KPVLLILAVNWLKPFIMVVAIOFFLGLWLFAPWITGTE
Pichia           44 GGVTVVGVSVPLAVGMILMMIPPFKVRVEFLNOMFTLRFARS-MISLVLNWIKCPLEMLGLAWLTF-
S.cerevisiae     66 GEPNLMGVSIPLTVGMIVMMIPPIQKVSIESLHKYFYRSYIRKQALSIFLNWVIGPIMLMTALAWMAFP-----
A.fumigatus      79 -KGFVGVSVPIAIGLIVMMYPIILCKVRYEGLHHVFRTRQIWIQAFSIFVNWIIAPFEMILAWAFLP-----
P.vittata        76 -VAETSIVSLPIAIGLIVMMYPIVLCVQVEMLGGVLRQAGSLETLSLSVNLNWWVGPALMTGLAWATLP-----
D.terio         51 -RMEVAQVNLPIVGLIIVWMLVPEMLVKIDGALHOVKEHW---RGLVTLFVNWAKPFSMALLGNLFRQVFPAP-----

C.glutamicum     113 -----DPELRTGLIIVGLARCIAMVIVNSDMSGDRLEATAVLVAINSVFEQVAMFCALGWYFLOVPSWLGLPTTTA
A.metalloreg     117 ---LIPESLANDYLACAVLLCAAPCTAMVFWNSHLSKGDPAAYTLVQAVNWHILFAFPTVVAILLGTD-----V
M.vannielii      114 ---LLPLELAKEYVAGAILLCAAPCTAMVFWNSHLSKGDPLYTLVQVSTNNHILFAFAPVGLMIGSN-----I
S.medicae        109 -----DYPEYMTGLIIVGLARCIAMVIVNQLARGDNQYVAGLVAFNSIFQLDFESIYAWFFLFLPLFLEGSVI
B.subtilis       103 -----DKPEYMIGLIIVGLARCIAMVIVNNDLSKGDTEYAAQLVAFNSIFQLDFESSVYAYLFTVTPQWLMEGAVV
Synechococcus    129 IIRGTEISLSSSYIACAILLCAAPCTAMVFWNSYLYSGNOGHTLVVVAVNSLTMFLVAPLGRWLLAMSD-----L
Pichia           113 -----DPEERTGILIMIGTARCIAMVIVNNDLACGSTLQCALIVVNSLLQVLLVAPYQLLCKVYISG--DPIPS--
S.cerevisiae     135 -----DYKEYRQGLIIMIGVARCIAMVIVNNDLACGDNLCVVLVITNSLLQVLLVAPYQLIIFYCYVYISH--DHLNLSV
A.fumigatus      147 -----DPELROGLIIVGLARCIAMVIVNNDLACGSDGGEYCALVAVNSILQVLLVAPYQVFFLOVTS-----GDSV
P.vittata        144 -----DIPDFRTGVLVIGIPRCTAMVIVNNDLAKGDADYCALVAVNSILQVLLVTPVALYLLVYVSR-----GKGF
D.terio         121 ---FLPADQLDSYIAGLILLCAAPCTAMVFWNSRITNGDPLFTLSQVALNDALMLVAFAPVGLMIGSN-----I

C.glutamicum     185 QFSEWSIVTSLVFLGIPPLAGVSRITIGBKIKGREWYEQKFLPAISPFALIGLLYTIIVLFLSLOGDQIVSOPWAVVRLA
A.metalloreg     185 IVPDITFLSVVFLTVIPLVGGYLSRKYIVQSKGIEKVENVFLKFFDNVTLVGLLLTLLIIFTQAAVILSNPLHVLILLA
M.vannielii      182 VVPDITFLSVVFLTVIPLVGGYSSRIYFLKNGKEWFEKRFEPKLNNSVFGLLLTLLILFSFOGDLINNPYILLIA
S.medicae        181 DVSWTIAEAVLYLGIPLAGYLSRRILTRAKGEDVYENAFPKISHTLAALELTVIAMFSLKGGDVVRLPFDVLLIA
B.subtilis       175 NITMAEVAKSVFIVLGPVETAGMTRIVLIVKVKKEWYKVFEPKISHTLALLETIVVFSKGLVIVVSLPDDVVRVA
Synechococcus    200 TVPQTHLLSVAVVYGLPLVAGASRIVLTKGREWFEKAFRLYINPIAAALLVTLVLEAFKGDIVENPFHILLIA
Pichia           181 MYSVSLVAKTIVFLGIPITVAGIVLRF---LAKISIGVELGTILSPPFAPLGLLYTVIIFIEKKGDFLETGPAPRCF
S.cerevisiae     205 RVLVEEVAKSVGVLGIPPLGIGIILRLGSLTAEKSNVYKYLRFISPPWALIGFHYTIVFVIFSRGQFIFIEIGSALICF
A.fumigatus      213 AFEVSTAAKSVAVFLGIPLCAILLTRFTLRVWASPKNYDEVELWASEFSLIGLLETIVLVEASQCRQVHOIVSVVRVA
P.vittata        211 HVSWTVAKSVLFLGIPVLAGVLRLLILNFAFCRKYESKFLRFTEGVALIGLLYTFVVFSTQAHQIVDNIHGVVRVA
D.terio         189 TVPDDITLISVVLYIVVPLVLAQVRRHLK-RCQAAFD-RAMQRTGEPWSMAALLLTLLVLLFAFQGEAITROPVITAMLA

C.glutamicum     265 IPLVIYFVGMFFISLIASKLSG-----MNAKASVSFTAAGNNFELATAVS
A.metalloreg     265 VPLTIQTFEFFEFAVGSWKAWK-----LPHNVASPAQMIKASNFFELAVAVA
M.vannielii      262 IPLTIQTYFIEFGISMMWGFKWK-----LPHNVCAPASFIKASNFFELAVAVA
S.medicae        261 IPLTIYFVIMFTVSPFFAKLID-----TDMPTAVAFATAAGNNFELATAVA
B.subtilis       255 IPLVIYFVLMFFVSPFLGKIKG-----ANMAVITLTAFTAGSNFFELATAVA
Synechococcus    280 VPLFVQTNLIFGITYVAAQKLG-----LCYEDAAPALI KASNFFEVATATA
Pichia           257 APLRVIYFVVMVNAFFGLRWLGRITVLVQENNPLLCGCEKEKESNPKAWKMWCSAPVQEVATHTAFTASNNFELSLSVA
S.cerevisiae     285 VPLVIYFETIAFLTFALMRYLS-----ISRSDTQRECSQDQELLKRVWGRKSCEASESTIMTQCFTMASNNFELSLSIA
A.fumigatus      293 APLVIYFVVPFVTLFISYKMG-----FCMKLSATQSEFTASNNFELATAVA
P.vittata        291 VPLLLYLGIFFFGLGCRGRK-----VPMPTRVITQCFRAGSNFFELATAVA
D.terio         267 VPIILIQVFFNSGLAVWLNKRAG-----EKHSVACPSALIKASNFFELAVAAA

C.glutamicum     312 IGTFGATSAQAACTIGPLIEIPVVLGLVYAMLVLPNLPNDPTLPSSARSTSQIINS
A.metalloreg     312 ITLFGLNSGATLATVVGVLVEVPVMLTLVKISNRTLHWPEVAREN-----
M.vannielii      309 ISLFGIHSCAALATVGVVLEVPVIMLSLVKIANLTKRREKIV-----
S.medicae        308 IAAFGLASPVAFAAVIGPLVEVPVILILVQALVLRGRYFAETAPAAVASEA-----
B.subtilis       302 VGVFGIHSCAFAAAVIGPLVEVPVIMLKVVALVFORCYFGSHSM-----
Synechococcus    327 VVLFGLNSCAALATVGVVLEVPVIMLVEVCRRTAFYPRQPEKASLPDRCLKISP-
Pichia           337 ISLVGANSKQATAATFGPVLIEPLMLLFCVSLFRRTFLWLDVRSEAEPEVTSDS---
S.cerevisiae     360 ISLVGNNSKQATAATFGPVLIEVPVILILDAVARILKPYIWNRRN-----
A.fumigatus      340 VATFGANSNOALASVVGPLIEVPVILGLVYAVKALAKRLAWKD-----
P.vittata        338 VGSFGIDSTOALAAATIGPVLIEVPGRLILVYVGVFORVGPVSV-----
D.terio         314 ISLFGIHSCAALATVVGVLVEVPVIMLVVVRVNRSGWVERG-----
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## Supplementary Materials

**Fig. S1. Multiple alignment of Acr3 homologues.** Representative Acr3 homologues (accession numbers in parentheses) are from *C. glutamicum* ATCC 13032 (YP\_225795), *A. metallire-digens* QYMF (YP\_001319657), *Methanococcus vanniellii* (YP\_001324187), *Sinorhizobium medicae* WSM419 (YP\_001313766), *B. subtilis* (NP\_390456), *Synechococcus* (YP\_475135), *Pichia guilliermondii* (XP\_001481996), *S. cerevisiae* (NP\_015527), *Aspergillus fumigatus* Af293 (XP\_753156), *Danio rerio* (XP\_001921075) and *Pteris vittata* (ACN65413). Sequences were aligned with Clustal W (1). The conserved cysteine residue is indicated (\*).

**Table S1: Strains and plasmids**

Strains/Plasmids	Genotype/description	Reference
<u>Strains</u>		
JM109	<i>endA1, recA1, gyrA96, thi, hsdR17</i> (rk-, mk+), <i>relA1, supE44, λ<sup>-</sup>, Δ(lac-proAB)</i> , [F', <i>traD36, proAB, lacI<sup>qZ</sup> ΔM15</i> ]	(2)
AW3110	<i>Δars::cam F<sup>-</sup> IN(rrnD-rrnE)</i>	(3)
<u>Plasmids</u>		
pKK223-3	Cloning and expression vector with <i>tac</i> promoter, Ap <sup>r</sup>	Pfizer/Pharmacia New York, NY
pKMB1	1.3 kilobase fragment of R773 <i>arsB</i> inserted into <i>EcoRI-HindIII</i> digested pKK223-3, Ap <sup>r</sup>	(4)
pTrcHis2A	cloning and expression vector, Ap <sup>r</sup>	Invitrogen
pTrcHis2A-AmAcr3-His	1.1 kb of <i>NcoI-HindIII</i> fragment containing <i>acr3</i> gene from the <i>ars1</i> operon of <i>A. metalliredigens</i> QYMF. Six histidine codons added to the 3' end of the <i>Amacr3</i> gene, Ap <sup>r</sup>	This study
pTrcHis2A-CgAcr3-His	1.1 kb of <i>NcoI-HindIII</i> fragment containing <i>acr3</i> gene from the <i>ars1</i> operon of <i>C. glutamicum</i> ATCC 13032. Six histidine codons added to the 3' end of the <i>Cgacr3</i> gene, Ap <sup>r</sup>	This study
pAmAcr3 <sub>C27S</sub> -His	Site-directed mutagenesis of Cys <sup>27</sup> codon to Ser codon in the <i>Amacr3</i> gene of pTrcHis2A-AmAcr3-His	This study
pAmAcr3 <sub>C91S</sub> -His	Site-directed mutagenesis of Cys <sup>91</sup> codon to Ser codon in the <i>Amacr3</i> gene of pTrcHis2A-AmAcr3-His	This study
pAmAcr3 <sub>C138S</sub> -His	Site-directed mutagenesis of Cys <sup>138</sup> codon to Ser codon in the <i>Amacr3</i> gene of pTrcHis2A-AmAcr3-His	This study
pAmAcr3 <sub>C138A</sub> -His	Site-directed mutagenesis of Cys <sup>138</sup> codon to Ala codon in the <i>Amacr3</i> gene of pTrcHis2A-AmAcr3-His	This study
pAmAcr3 <sub>C27/91S</sub> -His	Site-directed mutagenesis of Cys <sup>27/91</sup> codons to Ser codons in the <i>Amacr3</i> gene of pTrcHis2A-AmAcr3-His	This study
pAmAcr3 <sub>C27/91/138S</sub> -His	Site-directed mutagenesis of Cys <sup>27/91/138</sup> codons to Ser codons in the <i>Amacr3</i> gene of pTrcHis2A-AmAcr3-His	This study
pCgAcr3 <sub>C129S</sub> -His	Site-directed mutagenesis of Cys <sup>129</sup> codon to Ser codon in <i>Cgacr3</i> gene of pTrcHis2A-CgAcr3-His	This study

pCgAcr3 <sub>C141S</sub> -His	Site-directed mutagenesis of Cys <sup>141</sup> codon to Ser codon in <i>Cgacr3</i> gene of pTrcHis2A-CgAcr3-His	This study
pCgAcr3 <sub>C129A</sub> -His	Site-directed mutagenesis of Cys <sup>129</sup> codon to Ala codon in the <i>Cgacr3</i> gene of pTrcHis2A-CgAcr3-His	This study
pCgAcr3 <sub>C129/141S</sub> -His	Site-directed mutagenesis of Cys <sup>129/141</sup> codons to Ser codons in the <i>Cgacr3</i> gene of pTrcHis2A-CgAcr3-His	This study

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**Table S2: Plasmids for SCAM**

<b>Plasmids</b>	<b>Genotype/description</b>	<b>Reference</b>
pAmAcr3 <sub>V6C</sub> -His	Site-directed mutagenesis of Val <sup>6</sup> codon to Cys codon in the <i>Amacr3</i> gene of pAmAcr3 <sub>C27/91S</sub> -His	This study
pAmAcr3 <sub>A40C</sub> -His	Site-directed mutagenesis of Ala <sup>40</sup> codon to Cys codon in the <i>Amacr3</i> gene of pAmAcr3 <sub>C27/91S</sub> -His	This study
pAmAcr3 <sub>S46C</sub> -His	Site-directed mutagenesis of Ser <sup>46</sup> codon to Cys codon in the <i>Amacr3</i> gene of pAmAcr3 <sub>C27/91S</sub> -His	This study
pAmAcr3 <sub>S54C</sub> -His	Site-directed mutagenesis of Ser <sup>54</sup> codon to Cys codon in the <i>Amacr3</i> gene of pAmAcr3 <sub>C27/91S</sub> -His	This study
pAmAcr3 <sub>S76C</sub> -His	Site-directed mutagenesis of Ser <sup>76</sup> codon to Cys codon in the <i>Amacr3</i> gene of pAmAcr3 <sub>C27/91S</sub> -His	This study
pAmAcr3 <sub>T81C</sub> -His	Site-directed mutagenesis of Thr <sup>81</sup> codon to Cys codon in the <i>Amacr3</i> gene of pAmAcr3 <sub>C27/91S</sub> -His	This study
pAmAcr3 <sub>I97C</sub> -His	Site-directed the mutagenesis of Ile <sup>97</sup> codon to Cys codon in <i>Amacr3</i> gene of pAmAcr3 <sub>C27/91S</sub> -His	This study
pAmAcr3 <sub>S121C</sub> -His	Site-directed mutagenesis of Ser <sup>121</sup> codon to Cys codon in the <i>Amacr3</i> gene of pAmAcr3 <sub>C27/91S</sub> -His	This study
pAmAcr3 <sub>L127C</sub> -His	Site-directed mutagenesis of Leu <sup>127</sup> codon to Cys codon in the <i>Amacr3</i> gene of pAmAcr3 <sub>C27/91S</sub> -His	This study
pAmAcr3 <sub>A130C</sub> -His	Site-directed mutagenesis of Ala <sup>130</sup> codon to Cys codon in the <i>Amacr3</i> gene of pAmAcr3 <sub>C27/91S</sub> -His	This study
pAmAcr3 <sub>T149C</sub> -His	Site-directed mutagenesis of Thr <sup>149</sup> codon to Cys codon in the <i>Amacr3</i> gene of pAmAcr3 <sub>C27/91S</sub> -His	This study
pAmAcr3 <sub>G151C</sub> -His	Site-directed mutagenesis of Gly <sup>151</sup> codon to Cys codon in the <i>Amacr3</i> gene of pAmAcr3 <sub>C27/91S</sub> -His	This study
pAmAcr3 <sub>T156C</sub> -His	Site-directed mutagenesis of Thr <sup>156</sup> codon to Cys codon in the <i>Amacr3</i> gene of pAmAcr3 <sub>C27/91S</sub> -His	This study
pAmAcr3 <sub>I174C</sub> -His	Site-directed mutagenesis of Ile <sup>174</sup> codon to Cys codon in the <i>Amacr3</i> gene of pAmAcr3 <sub>C27/91S</sub> -His	This study
pAmAcr3 <sub>T182C</sub> -His	Site-directed mutagenesis of Thr <sup>182</sup> codon to Cys codon in the <i>Amacr3</i> gene of pAmAcr3 <sub>C27/91S</sub> -His	This study
pAmAcr3 <sub>V186C</sub> -His	Site-directed mutagenesis of Val <sup>186</sup> codon to Cys codon in the <i>Amacr3</i> gene of pAmAcr3 <sub>C27/91S</sub> -His	This study
pAmAcr3 <sub>T190C</sub> -His	Site-directed mutagenesis of Thr <sup>190</sup> codon to Cys codon in the <i>Amacr3</i> gene of pAmAcr3 <sub>C27/91S</sub> -His	This study

<b>Plasmids</b>	<b>Genotype/description</b>	<b>Reference</b>
pAmAcr3 <sub>L203C</sub> -His	Site-directed mutagenesis of Leu <sup>203</sup> codon to Cys codon in the <i>Amacr3</i> gene of pAmAcr3 <sub>C27/91S</sub> -His	This study
pAmAcr3 <sub>I219C</sub> -His	Site-directed mutagenesis of Ile <sup>219</sup> codon to Cys codon in the <i>Amacr3</i> gene of pAmAcr3 <sub>C27/91S</sub> -His	This study
pAmAcr3 <sub>V236C</sub> -His	Site-directed mutagenesis of Val <sup>236</sup> codon to Cys codon in the <i>Amacr3</i> gene of pAmAcr3 <sub>C27/91S</sub> -His	This study
pAmAcr3 <sub>V252C</sub> -His	Site-directed mutagenesis of Val <sup>252</sup> codon to Cys codon in the <i>Amacr3</i> gene of pAmAcr3 <sub>C27/91S</sub> -His	This study
pAmAcr3 <sub>S255C</sub> -His	Site-directed mutagenesis of Ser <sup>255</sup> codon to Cys codon in the <i>Amacr3</i> gene of pAmAcr3 <sub>C27/91S</sub> -His	This study
pAmAcr3 <sub>L258C</sub> -His	Site-directed mutagenesis of Leu <sup>258</sup> codon to Cys codon in the <i>Amacr3</i> gene of pAmAcr3 <sub>C27/91S</sub> -His	This study
pAmAcr3 <sub>I263C</sub> -His	Site-directed mutagenesis of Ile <sup>263</sup> codon to Cys codon in the <i>Amacr3</i> gene of pAmAcr3 <sub>C27/91S</sub> -His	This study
pAmAcr3 <sub>S282C</sub> -His	Site-directed mutagenesis of Ser <sup>282</sup> codon to Cys codon in the <i>Amacr3</i> gene of pAmAcr3 <sub>C27/91S</sub> -His	This study
pAmAcr3 <sub>L287C</sub> -His	Site-directed mutagenesis of Leu <sup>287</sup> codon to Cys codon in the <i>Amacr3</i> gene of pAmAcr3 <sub>C27/91S</sub> -His	This study
pAmAcr3 <sub>S293C</sub> -His	Site-directed mutagenesis of Ser <sup>293</sup> codon to Cys codon in the <i>Amacr3</i> gene of pAmAcr3 <sub>C27/91S</sub> -His	This study
pAmAcr3 <sub>A307C</sub> -His	Site-directed mutagenesis of Ala <sup>307</sup> codon to Cys codon in the <i>Amacr3</i> gene of pAmAcr3 <sub>C27/91S</sub> -His	This study
pAmAcr3 <sub>T313C</sub> -His	Site-directed mutagenesis of Thr313 codon to Cys codon in the <i>Amacr3</i> gene of pAmAcr3 <sub>C27/91S</sub> -His	This study
pAmAcr3 <sub>S319C</sub> -His	Site-directed mutagenesis of Ser319 codon to Cys codon in the <i>Amacr3</i> gene of pAmAcr3 <sub>C27/91S</sub> -His	This study
pAmAcr3 <sub>T325C</sub> -His	Site-directed mutagenesis of Thr325 codon to Cys codon in the <i>Amacr3</i> gene of pAmAcr3 <sub>C27/91S</sub> -His	This study
pAmAcr3 <sub>S343C</sub> -His	Site-directed mutagenesis of Ser343 codon to Cys codon in <i>Amacr3</i> gene of pAmAcr3 <sub>C27/91S</sub> -His	This study
pAmAcr3 <sub>T346C</sub> -His	Site-directed mutagenesis of Thr346 codon to Cys codon in the <i>Amacr3</i> gene of pAmAcr3 <sub>C27/91S</sub> -His	This study

**Table S3: Oligonucleotide primers for PCR cloning of *acr3* genes<sup>1</sup>**

Primer	Sequence (5'→3')	Target / Direction
Amacr3-NcoI Fw	GAATT <u>CCATGGG</u> TAAATGAAAATGTTGTCC	For cloning <i>Amacr3</i> to pTrcHisA2, forward primer
Amacr3-HindIII Rv	CCCTTA <u>AAGCTTT</u> TAAATGATGATGATGATGATG ATTTTCTCTTGCTACC	For cloning <i>Amacr3</i> to pTrcHisA2, reverse primer
Cgacr3a-5'-EcoRI	CCGGA <u>AATTC</u> ATGACAAACTCAACTCAGACG CGGGCC	For cloning <i>Cgacr3</i> to pGEM®-T Easy, forward primer
Cgacr3a-3'-KpnI	CGGTCAGG <u>TACCT</u> GAGTTGATGATTTGGCT GGTAGAACGAGCTGATGG	For cloning <i>Cgacr3</i> to pGEM®-T Easy, forward primer
Cgacr3-NcoI Fw	GAATT <u>CCATGG</u> CAAACACTCAACTCAG	For cloning <i>Cgacr3</i> to pTrcHisA2, forward primer
Cgacr3-HindIII Rv	CCCTTA <u>AAGCTTT</u> TAAATGATGATGATGATGATG TGAGTTGATGATTTGGCT	For cloning <i>Cgacr3</i> to pTrcHisA2, reverse primer

<sup>1</sup>Restriction sites underlined

**Table S4: Oligonucleotide primers for cysteine mutagenesis**

<b>Primer</b>	<b>Sequence (5'→3')</b>	<b>Target / Direction</b>
Amacr3(C27S) Fw	GTGGGTAGCTGCATCTATTATCGTAGGG	AmAcr3 Cys <sup>27</sup> to Ser, Forward
Amacr3(C91S) Fw	GTATCATTGTCACCTCTGTTACCAATTGG	AmAcr3 Cys <sup>91</sup> to Ser, Forward
Amacr3(C138S) Fw	GGAGCAGCTCCCTCTACTGCAATGGTATTTG	AmAcr3 Cys <sup>138</sup> to Ser, Forward
Amacr3(C138A) Fw	GGAGCAGCTCCCGCTACTGCAATGGTATTTG	AmAcr3 Cys <sup>138</sup> to Ala, Forward
Cgacr3(C129S) Fw	GGACTCGCACGAAGTATTGCGATGGTC	CgAcr3 Cys <sup>129</sup> to Ser, Forward
Cgacr3(C141S) Fw	GTCTGATATGTCCAGTGGAGACCGCGAG	CgAcr3 Cys <sup>141</sup> to Ser, Forward
Cgacr3(C129A) Fw	GGACTCGCACGAGCTATTGCGATGGTC	CgAcr3 Cys <sup>129</sup> to Ala, Forward
Amacr3(V6C) Fw	GTGGGTAGCTGCATCTATTATCGTAGGG	AmAcr3 Val <sup>6</sup> to Cys, Forward
Amacr3(C27S) Fw	GTGGGTAGCTGCATCTATTATCGTAGGG	AmAcr3 Cys <sup>27</sup> to Ser, Forward
Amacr3(A40C) Rv	CGACTTAATGTTTCTGGAACACATGGTAACAA TTGACCG	AmAcr3 Ala <sup>40</sup> to Cys, Reverse
Amacr3(S46C) Rv	GTTCCAGAAACATTATGTCGATGGGAATATGC	AmAcr3 Ser <sup>46</sup> to Cys, Reverse
Amacr3(S54C) Rv	GGAATATGCCCAAGTGTGTATTCCTGTAGCAA TTTTAATTTG	AmAcr3 Ser <sup>54</sup> to Cys, Reverse
Amacr3(S76C) Rv	GCTTAAAATTGATTTTACCTGTATTGTAGAAGC GAC	AmAcr3 Ser <sup>76</sup> to Cys, Reverse
Amacr3(T81C) Rv	CTTGGGCTTTTTACACGCTTCTACAATAC	AmAcr3 Thr <sup>81</sup> to Cys, Reverse
Amacr3(I97C) Rv	CATTGTAAATGGTTTGCATAACCAATTGGTAAC AC	AmAcr3 Ile <sup>97</sup> to Cys, Reverse
Amacr3(S121C) Rv	GTAATCATTGGCTAAACACTCTGGAATCAAATT TTG	AmAcr3 Ser <sup>121</sup> to Cys, Reverse
Amacr3(L127C) Rv	CACTGCACCTGCGCAGTAATCATTGGC	AmAcr3 Leu <sup>127</sup> to Cys, Reverse
Amacr3(A130C) Rv	GATTACTTGGCAGGTTGCGTGTTATTGGGAG	AmAcr3 Ala <sup>130</sup> to Cys, Reverse



<b>Primer</b>	<b>Sequence (5'→3')</b>	<b>Target / Direction</b>
Amacr3(T149C) Rv	GTTTGGAGTCATTTATGCAAGGGTGACCCAG	AmAcr3 Thr <sup>149</sup> to Cys, Reverse
Amacr3(G151C) Rv	GTATAGGCTGGGTCACACTTTGTAAATG	AmAcr3 Gly <sup>151</sup> to Cys, Reverse
Amacr3(T156C) Rv	CACCTGTACCAAGCAATAGGCTGGGTC	AmAcr3 Thr <sup>156</sup> to Cys, Reverse
Amacr3(I174C) Rv	GTAAAATTGCAACACAGGGTGTAAGGC	AmAcr3 Ile <sup>174</sup> to Cys, Reverse
Amacr3(T182C) Rv	GGGAACAATGACATCGCATATTCCTAGTAAAA TTGCAAC	AmAcr3 Thr <sup>182</sup> to Cys, Reverse
Amacr3(V186C) Rv	GGTATCATAGGGACAAATGACATCGGTTATTC	AmAcr3 Val <sup>186</sup> to Cys, Reverse
Amacr3(T190C) Rv	CAGATAAGAATAGGCAATCATAGGGAAC	AmAcr3 Thr <sup>190</sup> to Cys, Reverse
Amacr3(L203C) Rv	GATAAATATCCTCCAACGCAAGGAATAACAAT AAATAACAC	AmAcr3 Leu <sup>203</sup> to Cys, Reverse
Amacr3(I219C) Rv	CATTTTCAAATACTCGCACCCCTTACTCTG	AmAcr3 Ile <sup>219</sup> to Cys, Reverse
Amacr3(V236C) Rv	GTTAATAATAACCCGCAGATTGTTACATTATC	AmAcr3 Val <sup>236</sup> to Cys, Reverse
Amacr3(V252C) Rv	GATTACTTAAAATACACTCTGCTTGGAATG	AmAcr3 Val <sup>252</sup> to Cys, Reverse
Amacr3(S255C) Rv	CCAAGCAGAGGTTATTTTATGTAATCCATTACA CGTGTTG	AmAcr3 Ser <sup>255</sup> to Cys, Reverse
Amacr3(L258C) Rv	CAACAACACGTGGCATGGATTACTTAAAATAA CC	AmAcr3 Leu <sup>258</sup> to Cys, Reverse
Amacr3(I263C) Rv	CACGTGTTGTTGTGCGCTGTACCACTTAC	AmAcr3 Ile <sup>263</sup> to Cys, Reverse
Amacr3(S282C) Rv	CAATTTCCATGCCTTGCACCATCCATAAGC	AmAcr3 Ser <sup>282</sup> to Cys, Reverse
Amacr3(L287C) Rv	CAACGTTATGAGGGCATTTCATGCCTTTG	AmAcr3 Leu <sup>287</sup> to Cys, Reverse
Amacr3(S293C) Rv	CATTCCAGCTGGGCAAGCAACGTTATG	AmAcr3 Ser <sup>293</sup> to Cys, Reverse
Amacr3(A307C) Rv	GTAATTGCTACGGCAACGCACAATTCAAAAAA G	AmAcr3 Ala <sup>307</sup> to Cys, Reverse

Primer	Sequence (5'→3')	Target / Direction
Amacr3(T313C) Rv	GCCGTAGCAATTTGCCTATTCGGCTTG	AmAcr3 Thr <sup>313</sup> to Cys, Reverse
Amacr3(S319C) Rv	CTATTCGGCTTGAAGTGC GGAGCCACACTAG	AmAcr3 Ser <sup>319</sup> to Cys, Reverse
Amacr3(T325C) Rv	GCCACACTAGCATGTGTAGTCGGTGT TTTAG	AmAcr3 Thr <sup>325</sup> to Cys, Reverse
Amacr3(S343C) Rv	CATTAGTCAAAATCTGTAATAGAACAAAGAC	AmAcr3 Ser <sup>343</sup> to Cys, Reverse
Amacr3(T346C) Rv	CAAAATCTCTAATAGATGCAGACATTGGTTTC	AmAcr3 Thr <sup>346</sup> to Cys, Reverse

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