

Supplementary Information

A novel and stress adaptive alternative oxidase derived from alternative splicing of duplicated exon in oyster *Crassostrea virginica*

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Figure S1 cDNA and protein sequences of two variants of CvAOX with primers highlighted. Primer sequences are provided in Table 1.

CvAOXA

CATGTGCGCTTGCCTTTGGGGTTATAAGGGGATTACGTGCATTCAAGGATATTTAAGTGGGAAACATTTGAATAAGTAAAAATATTACATGTCTAGTGAGGAAATCTGTGTAATAAGTAAAACTACAGTGGATCGTAGCAAAT
 ATGGGAAGTTTACGACAAATACGAGATTAAGTAAAAATGTGTCCAGGATTTTTGTAAACAAATGAAGGATGCGGAAAAATGCTTCAATCTACTGAGAATAAATGGAATTCGCACTGGTAGGGTTGCGCACCCTGCAACAAAGGCT
 M G S L R Q I T R L S E N C V R I F C K Q M K D A E N A S I L L R I N G I R T G S G L R T A A T K A
 GATGTTGATGAAAAATTAAGAAATCAAGAAGGAAATTCGAAAAATCCAGACCCAAATCACCTGGATCATTTTAGAAAGTCGACAGACACAGAGACTTGTGGAGTCGCTCAAGGACCCCTCCTCTATGGGCACACACCCCTC
 D V D E N I K K F K E G N F E K I P D P N H L D H F R K S Q S T E T L V E S L K D P P P M G T H T L
 CCACATCCGATATGGTCAGAGGAAGAACTCCATAATGAAAAATCACCACAAGCCTCCAGAGGGCTTTGTTGACAAGCTTGCTCCGATCTGTGAAGCTGTTGAGATCAACGTTTGAATTTGTTAACAGGATCAACTGGGGTGAAGA
 P H P I W S E E E L H N V K V T H K P P E G F V D K L A F R S V K L L R S T F D L L T G F N W G E R
 TCAAAGAAAAATGGTCTTACGAATCTGTTCTCGGAGACTGTTGCTGGGGTACCCGGAATGGTTGCAGCGATGACGCGACATTTACTACTGCGCAGGCTCAAACGAGACCAGGATGGATACACACTCCTAGAGGAAGCAGAA
 S K K K W V L R I C F L E T V A G V P G M V A A M T R H L H S L R R L K R D H G W I H T L L E E A E
 AACGAGCGGATGCATTAATGACGGCCTTACAGCTGCGTCAGCCATCTGGTTATTGAGAATGGCGTCATCGTGTCTCAAGGGGCTTTGTTGACAATGTTGAGTGGCGTACCTGCTTAGCCCCGTTTCTGCCATCGCTTTGTCGGG
 N E R M H L M T A L Q L R Q P S W L F R M G V I V S Q G A F V T M F S G A Y L L S P R F C H R F V G
 TACCTAGAGGAGGAAGCAGCTTTTACCTCAAGTGTGAAAGGATAGATAAGATCCGGTCCCTTAAACACTGGCAGACTCAGAAGCTCCGGACGTGGCTATCCGCTACTGGAACCTCCGAACTGCATCCATGAAAGGATGAGT
 Y L E E E A V F T Y S K C L K D I E S G P L K H W Q T Q K A P D V A I R Y W K L P E T A S M K D V V
 Ind F → Del F →
 ← Ind R ← Del R
 TTGGCAATACCGCAGATGAGGCGCACACAGGGTCTGCAATCACACACTGGCTTCCATGAAAGAGCAATATAATCCCTACGACCCGGGAAGTGAACAGCGGAACTAAGGACACTGAAGGGTCATTAACGCTATAGTTTATGCT
 L A I R A D E A H R V V N H T L A S M K E D E Y N P Y E P G K *
 Exon 10
 TGTGTACCAATGTTGGCTTACGTTCTTTCAGAATTTGCATACCAGACATTGTGTACTGTTTGTACACGTTGTAATGTTAAATACCCGACATGACGTAAACATCAAAACATCAATCAATCTTACAGCTTTTAAAGTATGCAAT
 GATATTAACGTGATTTCCGCCGAATTTTGAACATTTCAATTTGAATTTGTTACGCTAGAAGATCATTGAAACCCGTGGCTTGCATTTATAGACTGAATCAGTAAATGTCATTTGTGATGCCATGATTTCTATACAATTTGTAG
 TAAACGTGAAAGAAAAATCATACCGGATATGATGATTGAGTTATTGTGATGTTGTTGAATTTGATGACTCCTGGATGAAATATGATTGTAAGTTTGTGTTTACAAAATCTCTGTTTAAAAACATGAATTTTATTGTAATAA
 GTTT

CvAOXB

CATGTGCGCTTGCCTTTGGGGTTATAAGGGGATTACGTGCATTCAAGGATATTTAAGTGGGAAACATTTGAATAAGTAAAAATATTACATGTCTAGTGAGGAAATCTGTGTAATAAGTAAAACTACAGTGGATCGTAGCAAAT
 ATGGGAAGTTTACGACAAATACGAGATTAAGTAAAAATGTGTCCAGGATTTTTGTAAACAAATGAAGGATGCGGAAAAATGCTTCAATCTACTGAGAATAAATGGAATTCGCACTGGTAGGGTTGCGCACCCTGCAACAAAGGCT
 M G S L R Q I T R L S E N C V R I F C K Q M K D A E N A S I L L R I N G I R T G S G L R T A A T K A
 GATGTTGATGAAAAATTAAGAAATCAAGAAGGAAATTCGAAAAATCCAGACCCAAATCACCTGGATCATTTTAGAAAGTCGACAGACACAGAGACTTGTGGAGTCGCTCAAGGACCCCTCCTCTATGGGCACACACCCCTC
 D V D E N I K K F K E G N F E K I P D P N H L D H F R K S Q S T E T L V E S L K D P P P M G T H T L
 CCACATCCGATATGGTCAGAGGAAGAACTCCATAATGAAAAATCACCACAAGCCTCCAGAGGGCTTTGTTGACAAGCTTGCTCCGATCTGTGAAGCTGTTGAGATCAACGTTTGAATTTGTTAACAGGATCAACTGGGGTGAAGA
 P H P I W S E E E L H N V K V T H K P P E G F V D K L A F R S V K L L R S T F D L L T G F N W G E R
 TCAGAGAAAAATGGTCTTACGAATCTGTTCTCGGAGACTGTTGCTGGGGTACCCGGAATGGTTGCAGCGATGACGCGACATTTACTACTGCGCAGGCTCAAACGAGACCAGGATGGATACACACTACTAGAGGAAGCAGAA
 S E K K W V L R I C F L E T V A G V P G M V A A M T R H L H S L R R L K R D H G W I H T L L E E A E
 AACGAGCGGATGCATTTGATGACGGCCTTACAGCTGCGTCAGCCAACTGGTTATTGAGAATGGCGTCATCGTGTCTCAAGGGGCTTTGTTGACAATGTTGAGTGGCGTACCTGCTTAGCCCCGTTTCTGCCATCGCTTTGTCGGG
 N E R M H L M T A L Q L R Q P T W L F R M G V I V S Q G A F V T M F S V A Y L L S P R F C H R F V G
 TACCTAGAGGAGGAAGCAGCTTTTACCTCAAGTGTGAAAGGATAGATAAGATCCGGTCCCTTAAACACTGGCAGACTCAGAAGCTCCGGACGTGGCTATCCGCTACTGGAACCTCCGAACTGCATCCATGAAAGGATGAGT
 Y L E E E A V F T Y S K C L K D I E S G P L K H W Q T Q K A P D V A I R Y W K L P G P A T M K D V I
 Ind F → Ind F →
 ← Ind R ← Ind R
 TTTAACAATCGGGCGGACGAGGCCAACCCGGAAAGTGAACACGTTCTGCGCTCCCTACCAACCACCAAAATAAACCCCTTCCCTACCTGGTCAATAAAGAACACACAGACCCCTAAATGTGCTCATCTGTCTTTTCAGGAAACTG
 F N I R A D E A N H R K V N H V L A S L P T D K Y N P F L P G Q *
 Exon 10
 CATCCATGAAGGATGACTGTTGGCAATACCGCAGATGAGGCGCACACAGGGTCTGTAATCACACACTGGCTTCCATGAAAGAGGCAAGTAATAATCCCTACGACCCGGGAAGTGAACAGCCGGAACCTAAGGACACTGAAGGGTCA
 TTAACGCTATAGTTTATGCTGTTGACCAATGTTGGTCTTACGTTCTTTCAGAATTTGCATACCAGACATTGTGTACTGTTTGTACACGTTGTAATGTTAAATACCCGACATGACGTAAACATCAAAACATCAATCAATCTT
 CAGCTTTTAAAGTATGATGATTAATAACTGATTTCCGCCGAATTTTGAACATTTCAATTTGAATTTGTTACGCTAGAAGATCATTGAAACCCGTGGCTTGCATTTATAGACTGAATCAGTAAATGTCATTTGTGATGCCATG
 ATTTCTATACAATTTGTAGTAAACGTGAAAGAAAAATCATACCGGATATGATGATTGAGTTATTGTGATGTTGTTGAATTTGATGACTCCTGGATGAAATATGATTGTAAGTTTGTGTTT

Figure S2 The uncropped images of electrophoretic gels of all the PCR products. Both genomic DNA and cDNA of *C. virginica* were amplified using Primer Ind F and Ind R to detect the indel. Genomic DNA templates are from four wild populations: Rhode Island (RI), Delaware Bay (DB), Florida (FL) and Texas (TX). Nine cDNA templates are from the oyster samples used in air exposure challenging (Time 0, gill). The expected size of the amplicon in cDNA is 267 bp for the long variant and 97 bp for the short variant.

