

Supplementary Material (online)

Mutations in the Second Alternative Oxidase Gene: A New Approach to Group *Aspergillus niger* Strains

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Figure S1: Schematic overview of branched respiratory electron transport chain in the mitochondrial inner membrane in filamentous fungi to illustrate the metabolic function of alternative oxidase (Aox) and type-II NADH dehydrogenase (And).

Figure S2: The intron-exon structures of the two paralogous genes for alternative oxidase and for alternative NADH dehydrogenase (oxidizing cytosolic NADH) extant in *Aspergillus niger* strain ATCC 1015.

Figure S3: The taxonomically aberrant position of the Onygenales AoxA clade is not a consequence of joint analysis of (orthologous) AoxA proteins with AoxB paralogs.

Figure S4: Alignment of opposite LTR's of the non-autonomous and autonomous copies of ANiTA2 found amongst sixteen *A. niger sensu stricto* isolates with *aoxB* disrupted by retrotransposon insertion.

Figure S5: Ty1/copia retrotransposon ANiTA2, autonomous copy 5129 nt.

Figure S6: DNA similarity between the coding region of *A. awamori aoxB* and the *aoxB* pseudo gene in *Aspergillus niger (sensu lato)* strain An76.

Table S1: The intron-exon structure of *aoxB* and divergently orientated *andB* genes.

Table S2: NCBI-lodged RNA sequence reads that cover exon-exon fusions in *aoxB* and *andB* in *A. calidoustus*, *A. implicatus* and *A. niger* strains (with full-length *aoxB* and *andB* genes).

Table S3: Oligonucleotide primers used for PCR amplification of the *aoxB* and *andB* genes and cDNA prior to sequence verification of mutant alleles.

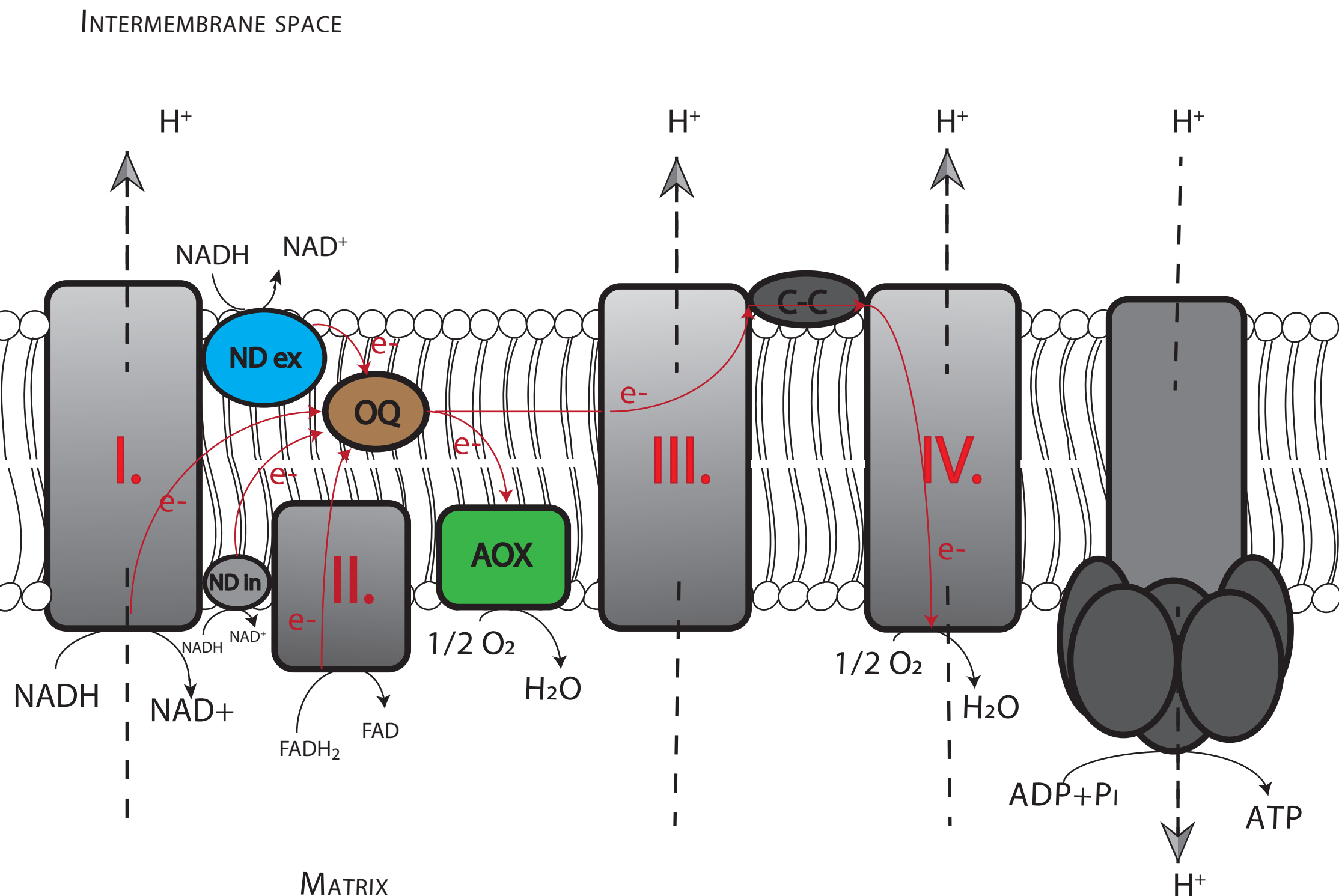


Figure S1. Schematic overview of branched respiratory electron transport chain in the mitochondrial inner membrane in filamentous fungi to illustrate the metabolic function of alternative oxidase (Aox) and type-II NADH dehydrogenase (And). The alternative electron fluxes are indicated in red. Associated translocation of protons over the inner membrane are shown. In the canonical chain, Type-I NADH:ubiquinone oxidoreductase (complex I), Ubiquinol:cytochrome C oxidoreductase (complex III) and Cytochrome C oxidase (complex IV), respectively, couple electron transport down the chain to proton translocation and the buildup of proton motive force that can be used to generate ATP by oxidative phosphorylation (at the right of the scheme). Complex II (succinate dehydrogenase) re-oxidizes mitochondrial FADH_2 formed by the prior conversion of succinate to fumarate in the tricarboxylic acid cycle without pumping protons. Alternative oxidase (Aox; green) accepts electrons directly from the intermediate carrier ubiquinol (brown) and transfers them straight to molecular oxygen, bypassing the electrogenic, proton-translocating Complexes III and IV. NADH/ NAD^+ cannot pass the mitochondrial inner membrane. Alternative NADH dehydrogenases (And) or Type-II NADH:ubiquinone oxidoreductases oxidize either cytosolic (blue) or mitochondrial NADH (grey) and transfer the high-energy electrons to the ubiquinol intermediate (brown) without concomitantly pumping protons. Type-II alternative NADH dehydrogenases thus bypass the proton-translocating (Type-I) Complex I

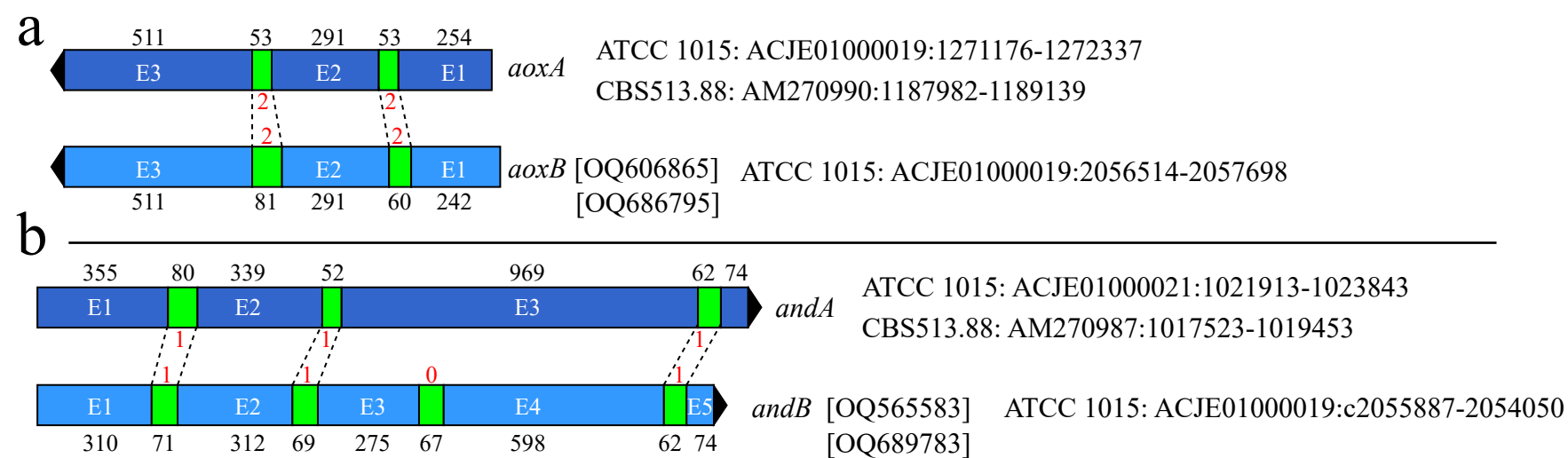
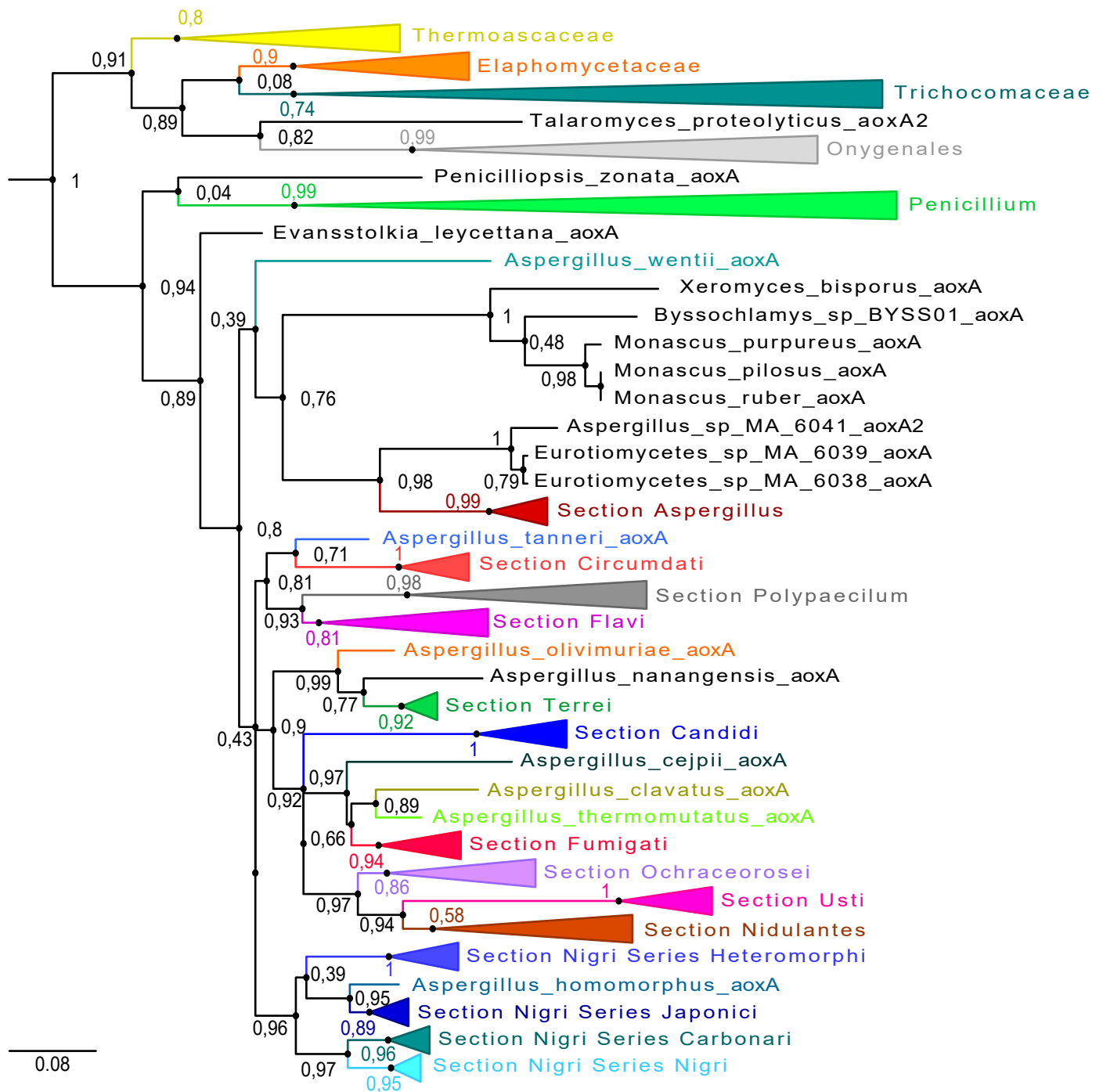


Figure S2. The intron-exon structures of the two paralogous genes for alternative oxidase and for alternative NADH dehydrogenase (oxidizing cytosolic NADH) extant in *Aspergillus niger* strain ATCC 1015. **(a)** Alternative oxidase genes, *aoxA* and *aoxB*. **(b)** Alternative NADH dehydrogenase genes, *andA* and *andB*. The exact location of the four genes in *A. niger* strain ATCC 1015 (sequence contig and coordinates) is mentioned to the right of the gene models. Relevant GenBank accession numbers are included. For the two ubiquitous “A” genes, the exact localisation in *A. niger* strain CBS 513.88 is also given. The exons of the coding regions (i.e., between ATG and STOP) are colored darker blue for the ubiquitous paralogous genes “A” and lighter blue for the rare paralogous genes “B”. Introns are indicated by the green color and vertically connected with the dashed lines to emphasize intron position conservation. The arrowhead gives the orientation of the genes. The intron phase is indicated by the red digit (0; 1; 2). The phase-zero third intron is lost from the ubiquitous gene for the alternative NADH dehydrogenase, *andA*.



Supplementary Figure S3. The taxonomically aberrant position of the Onygenales AoxA clade in maximum likelihood estimations of interrelations of Aox paralogs is not a consequence of joint analysis of (orthologous) AoxA proteins with AoxB paralogs. Essentially the same clade clustering was evident from control phylogenies of exclusively the AoxA input (i.e., with the AoxB paralogs omitted). The Figure shows such a “AoxA-only” control tree constructed using exactly the same methodology for multiple sequence alignment (MAFFT), its trimming (BMGE) and the subsequent maximum likelihood inference (PhyML) (cf. legend to Figure 4b). The rooting outgroup of Lecanoromycetes AoxA was eliminated from view by subtree selection in FigTree. The clades specific to Thermoascaceae, Elaphomycetaceae, Trichocomaceae, Onygenales, the *Penicillium* genus, as well as the various *Aspergillus* sections were collapsed for a more convenient overview. However, section Nigri species were collapsed at the series level. Note the considerable evolutionary distance between the section Nigri series Nigri AoxA clade (at the bottom of the tree), the section Usti (includes *A. calidoustus*) and the estimated origin of paralogous AoxB as a sister clade of the Onygenales AoxA, near the top of the tree.

Supplementary Figure S4. Alignment of opposite LTR's of the non-autonomous and autonomous copies of ANiTA2 found amongst sixteen *A. niger sensu stricto* isolates with *aoxB* disrupted by retrotransposon insertion (*aoxB* allele III)

[illegible]

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[118/162] 72.84 % ID [copy disrupting aoxB]
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ATTTAAATGTTGAGGCAGTAACCCCTGTAGGCTCAGTCATGTGGCTGCGCTCAGGCCGCCACCAAGCGCCTCTTCGCGTGGTTGCGCTGCGTGTTTCTAGATAGTAATATAATATACTTTTACTATATTCTTCACTTTTCGTTTCTTCCGCCTGTAGTATTTGAATAGA----

-----TGTGAGATAGTAATTCTATAAACTTAATTATATGACTGTGCTTAGACTGCCTACCAAGCGCCTCTTCGTGCTGGTTGCGCTGCGCGTTCCTAGATAGTAATATAACATACTTTTATTGTATTCTTTGCTTTTCGTTTCTTCCGCCTGTAGCGTTCGAACATTTAAAT

[131/162] 80.86 % ID

[illegible]

[141/164] 85.98 % ID

[illegible]

[125/163] 76.69 % ID

[illegible]

[116/161] 72.05 % ID

-ATCAGATGTTGAGGCAGTAATCCTGTAGGCTTGGTACAGTGGCTGCGCTCAGGCCGCCACCAAGCGTTTCTTCGCGCTGGTTGCGCTGTGCATTCTAGATAGTAATATAATATACTTTTACTACATTCTTCGTTTCGTTTCTTCCGCCTGTAGTGTTTAAATAGA----
 -----ATATTAAGATAGTAATTTTATAGATTTAGTTATGTGGCTGTGCTCAGGCCGCCTACTAAGCGCCTCTTCGTGTTGGTTGTGCTGCGCTTCTAGATAGTAATATAATATACTTTTACTGCATTCTTCGTTTCGTTTCTTCCGCCTGTAGCGTTTCAACATCGAT-----

[134/163] 82.21 % ID

[illegible]

[142/160] 88.75 %

AGAAAC TGTGTGAGGCAGTAACCCGTGTAGGCTCGGTACAGTGGCCGCGCTCAGGCCGCCACCAAGCGCCTCTTCGCGCTGGTTGCGCTGCGCGTTTCTAGATAGCAATACAACACACTTTTGCTGCATTCTTTGCGTTTGTTTCTTCTCCTGTAGTTTGAACAG-----

-----TGTGTGAGGCAGTAACCCGTGTAGGCTCGGTACAGTGGCCGCGCTCAGGCCGCCACCAAGCGCCTCTTCGCGCTGGTTGCGCTGCGCGTTTCTAGATAGCAATACAACACACTTTTGCTGCATTCTTTGCGTTTGTTTCTTCTCCTGTAGTTTGAACAGAAAC

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[159/159] 100 % ID [autonomous copy ANiTA2: Ty1/Copia family]
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AGAAAC TGTGTGAGGCAGTAACCCCTGTAGGCTCGGTACAGTGGCGCGCTCAGGCCGCCACCAAGCGCCTCTTCGCGCTGGTTGCCTGCGCGTTTCTAGATAGCAATACAAACACACTTTTGCTGCATTCTTTGCGTTTGTCTTCTCTCTGTAGTTTCGAACAGAAAC

Recombined LTRs (solo LTR with TSDs)

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[autonomous copy recombined out: solo LTR 100 % identical]
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The target site duplication (TSD) directly up- and downstream of the transposon LTR's is highlighted by the green background.

[illegible]

LTR = 159 nt (purple: 5' & 3' = 100 % identical); TSD = 6 nt 5'-AGAAAC (green).
ORF = 4512 nt (yellow); Integrase-RT-RNaseH polyprotein (Ty1/copia order) = 1503 AA

Supplementary Figure S6. DNA similarity between the coding regions of *A. awamori* *aoxB* and the *aoxB* pseudo gene in *Aspergillus niger* (sensu lato) strain An76

BDHI_Aawamori	ATGGTACGCCGAAATGCCATCCTTTTCAGCCCGATAAGCCGAAAGAGGTCAATGTACCTT	60
BCMY_An76	ATGATACGCCGGAATGCCGTCCTCTTCAGTCCGATAGGGCGAAGTAGGTCAATACATCCT	60
BDHI_Aawamori	ACAAACATGCATCGCATTTTCATTGCACAACAGACCAGAAAAGCCCTTAAATCAACACAG	120
BCMY_An76	CCAAACATGCATCGCATTTTCATTGCACAATAACCAGGCAGACCACGGAATTAACACAA	120
BDHI_Aawamori	CTCATACTTCTTGCTGGACAAGCGGGCTTTGCCACCTCAACTCCTTTATCATTTCAAGTTG	180
BCMY_An76	CCGATACTTCTTGCTGGACAAGCGGGCTTTGCTACCTCCACTCCTTTATCATTTCAAGCTG	180
BDHI_Aawamori	CAATCGCCACCAACAAAATTGACCCGAATCAAGGAGACAGCTATTGTCTGGAACACCCA	240
BCMY_An76	CAATCACCAACCAAAAATCAACCCGAATCAAGAAGACGGCCATTGTCTGGAACACCCA	240
BDHI_Aawamori	ATgtcagcccatatcctcccttttcacTTTTTgtgtctgtctcttctaactc----	295
BCMY_An76	ATatgagttcacctatctcccttttcttTTTTTgtgtctgtctcttctctgtctctcctg	300
BDHI_Aawamori	-----ggaaagCTATACAGAAAAGGAGATTGAAATATCACTGTAGCTCATCGCGA	348
BCMY_An76	acaattaaaaaaaCTATACAGAGAAAAGAGATTCTGATATCACTGTGGCTCATGGCAA	360
BDHI_Aawamori	AGCCAAATGCCTTTCTGACAAGATCGCCTTGGGCTTGGTACGTTGCTTCGATGGTGCAC	408
BCMY_An76	TGCTAAGTCTCTTTCTGATAAGTTCGCCTTGGCTTGGTACGTTGTCTTCGATGGTACAC	420
BDHI_Aawamori	TGATTTCTGACTGGGTATCGAAATCCGAAAGGAAAGAAAGAAATCGCAGACAAGTTTGA	468
BCMY_An76	TGATTTCTGACTGGGTATCGAAATCCTGAAGGGAAAGAAAGTCGTAGACAAGTTTGA	479
BDHI_Aawamori	TATGACTGAGAGAAAATGGCTTATTCGTTTCATTTTCTCGAATCGGTTGCTGGGTGCC	528
BCMY_An76	GATGACTGAGACAAAGCCAATTATTAGTTTCACCATTCTCGAATCGGTTGCAAGGTGCC	538
BDHI_Aawamori	TGGTATGGTGGGCGGCATGCTCCGACATCTGAAGAGTATCAGACGGATGAAGAGGGATCA	588
BCMY_An76	CGGTATGGTGGGCGGCATGCTCCGACATTTAAAGAGTGTAGACAGATGAAGAAGGATCC	598
BDHI_Aawamori	TGGATGgtcagttacaagggaataaaat-aattatctccaagtacttagcagtagctaca	646
BCMY_An76	TGGATGgtcagttgcaggggaataaaataaatatcttcaaatgttaaaagtagctaca	658
BDHI_Aawamori	caagaagctgcagactgacacaacgcagGATCGAGTCCTTGATTGACGAAGCCTACAAC	706
BCMY_An76	caagaagctgctgactgacacaactgcagGATTGAGTCCTTGATTGAAGAAGCCTACAAC	718
BDHI_Aawamori	GAGCGCATGCACCTCTTGACATTCTCGAACTGGCAGATCCAGGTATCTTCATGCGCTTA	766
BCMY_An76	GAGCGCATGCACCTCTTGACATTCTCGAACTGGCAGATCCAGGTATATTTCATGCGCTTC	778
BDHI_Aawamori	GTTGTCTTGGCCGCGCAGGGCTCTTCTTCAATGCCTTCTTGTCTCCTACCTTGTTCG	826
BCMY_An67	ATGGTCTTGGCCGCGCAGAGCTCTTTTAAATGCCTTCTTGTCTCCTATCTCGTTCTA	838
BDHI_Aawamori	CCCAAGACATGTCACCGGTTTGTGGATACCTAGAGGAAGAAGCTGTCATTACCTATACA	886
BCMY_An76	CCCAAAACATGTCACCGGTTGCTCGGATACCTACAGGAAGAAGCTGTCATTACCTTTACA	898
BDHI_Aawamori	CACGCAATCCGTCAACTTCAAGCTGGAAAACTGCCAGCATGGGACAATTTATCAGCACCG	946
BCMY_An76	CCTGCAATCCATGAACCTCAAGCTGGAAAGCTGCACGCATGGGACGATCTACAGCACCG	958
BDHI_Aawamori	GAGATTGCAATCAAATATTGGAGAATGCCAGAAAGGAAAACAGAGATGTTGATTGTGTTG	1006
BCMY_An76	GAGATTGCAATCAAAGATTGCAGAATGCCAAAGGAAAACAGAGATGTTGATTGTGTTG	1018
BDHI_Aawamori	CTTTATGTTCCGGCGGATGAAGCGAAGCATCGCGAGGTTAACCATACTTTGGGAAACTTA	1066
BCMY_An76	CTCTATGTTCCGGATGGATGAAGCGAAGCATCGCGAGGTTAACCATACTTTGGGGA-----	1073
BDHI_Aawamori	GACGAGCATGAAGATCCCAATCCTTATACTGCGCTTTATCACGATGGGAATAATGCTGGT	1126
BCMY_An76	---CAGCAAGAGATCCCAATCCTTATACTGCGCTTTATCACGAAGGGAATAATGTTGGT	1130
BDHI_Aawamori	CCCTCAGCGTCGCCCGATGCAGGGAAGCCTATTGGATGGGAGCGAGATGAACATAATTTAG	1186
BCMY_An76	TCCTCAGCGTCGCCATCGCAGGAAGCCTTTTGGGTTAGAGCGAATGAAGTAATTTAG	1190

Three frameshift mutations are indicated by the hyphen in the red background in the aligned An76 sequence as opposite to the green background in the *A. awamori* sequence. The 5'-donor, lariat branch point- and 3'-acceptor sequences in both introns are given in red lettering. These three sequence elements in the seemingly noncanonical first intron in An76 are in the turquoise background.

Supplementary Table S1: The intron-exon structure of axoB and divergently orientated andB genes from the start to the stop codon. The sequence contig and the coordinates precisely localise (the coding parts of) the exons for each gene in each fungus. The phase and size of the position-conserved introns separating consecutive exons are given. For *Aspergillus implicatus* and *Penicillium swiecicki*, the data refer to the sequence assemblies accessible at the MycoCosm website of the Joint Genome Institute (US Department of Energy). We did not use the results from autoannotation: such predictions prove incorrect in *Aspergillus niger* ATCC 1015, NRRL3 and CBS 513.88 for both axoB and andB.

		exon1	intron1	exon2	intron2	exon3	intron3	exon4	intron4	exon5
A. niger ATCC1015 andA	NA	ACJE01000021 1021913-1022267 355 bp	phase 1 80 bp	ACJE01000021 1022348-1022686 339 bp	phase 1 52 bp	ACJE01000021 1022739-1023707 969 bp	phase 1 62 bp	ACJE01000021 1023770-1023843 74 bp		
A. niger ATCC1015 axoA		ACJE01000019 1271176-1271429 254 bp	phase 2 53 bp	ACJE01000019 1271483-1271773 291 bp	phase 2 53 bp	ACJE01000019 1271827-1272337 511 bp				
	ATG- CAT*	exon1	intron1	exon2	intron2	exon3	intron3	exon4	intron4	exon5
A. niger ATCC1015 andB	626bp	ACJE01000019 c2055887-2055578 310 bp	phase 1 71 bp	ACJE01000019 c2055506-2055195 312 bp	phase 1 69 bp	ACJE01000019 c2055125-2054851 275 bp	phase 0 67 bp	ACJE01000019 c2054783-2054186 598 bp	phase 1 62 bp	ACJE01000019 c2054123-2054050 74 bp
A. niger ATCC1015 axoB		ACJE01000019 2056514-2056755 242 bp	phase 2 60 bp	ACJE01000019 2056816-2057106 291 bp	phase 2 81 bp	ACJE01000019 2057188-2057698 511 bp				
A. awamori IFM 58123 andB	621bp	BDHI01000007 c2238650-2238341 310 bp	phase 1 71 bp	BDHI01000007 c2238269-2237958 312 bp	phase 1 69 bp	BDHI01000007 c2237888-2237614 275 bp	phase 0 67 bp	BDHI01000007 c2237546-2236949 598 bp	phase 0 66 bp	BDHI01000007 c2236882-2236809 74 bp
A. awamori IFM 58123 axoB		BDHI01000007 2239272-2239513 242 bp	phase 2 61 bp	BDHI01000007 2239575-2239865 291 bp	phase 2 81 bp	BDHI01000007 2239947-2240457 511 bp				
A. welwitschiae CCMB 674 andB	625bp	PUWS01000026 128688-128997 310 bp	phase 1 71 bp	PUWS01000026 129069-129380 312 bp	phase 1 69 bp	PUWS01000026 129450-129724 275 bp	phase 0 67 bp	PUWS01000026 129792-130389 598 bp	phase 1 62 bp	PUWS01000026 130452-130525 74 bp
A. welwitschiae CCMB 674 axoB		PUWS01000026 c128062-127821 242 bp	phase 2 59 bp	PUWS01000026 c127761-127471 291 bp	phase 2 81 bp	PUWS01000026 c127389-126879 511 bp				
A. calidoustus SF006504 andB	551bp	CDMC01000004 c622233-621924 310 bp	phase 1 49 bp	CDMC01000004 c621874-621536 339 bp	phase 1 67 bp	CDMC01000004 c621468-621194 275 bp	phase 0 73 bp	CDMC01000004 c621120-620526 595 bp	phase 1 73 bp	CDMC01000004 c620452-620379 74 bp
A. calidoustus SF006504 axoB		CDMC01000004 622785-623020 236 bp	phase 2 52 bp	CDMC01000004 623072-623362 291 bp	phase 2 70 bp	CDMC01000004 623433-623943 511 bp				
A. implicatus CBS 484.95 axoB	544bp	Aspimp1 scaffold_11 206645-206877 (- strand) 233 bp	phase 2 49 bp	Aspimp1 scaffold_11 206305-206595 (- strand) 291 bp	phase 2 61 bp	Aspimp1 scaffold_11 205733-206243 (- strand) 511 bp				
A. implicatus CBS 484.95 andB		Aspimp1 scaffold_11 207421-207718 298 bp	phase 1 74 bp	Aspimp1 scaffold_11 207793-208131 339 bp	phase 1 64 bp	Aspimp1 scaffold_11 208196-208473 278 bp	phase 0 58 bp	Aspimp1 scaffold_11 208532-209144 613 bp	phase 1 60 bp	Aspimp1 scaffold_11 209205-209278 74 bp
A. implicatus CBS 484.95 axoB2 [paralog]	NA	Aspimp1 scaffold_2 49588-49847 (- strand) 260 bp	phase 2 57 bp	Aspimp1 scaffold_2 49255-49530 (- strand) 276 bp	phase 2 64 bp	Aspimp1 scaffold_2 48680-49190 (- strand) 511 bp				
P. swiecickii 182 6C1 axoB	613bp	Penswil scaffold_5 470967-471232 266 bp	phase 1 82 bp	Penswil scaffold_5 471315-471605 291 bp	phase 0 60 bp	Penswil scaffold_5 471666-472176 511 bp				
P. swiecickii 182 6C1 andB		Penswil scaffold_5 469712-470354 (- strand) 643 bp	phase 1 62 bp	Penswil scaffold_5 469372-469649 (- strand) 278 bp	phase 0 57 bp	Penswil scaffold_5 468684-469314 (- strand) 631 bp	phase 1 59 bp	Penswil scaffold_5 468551-468624 (- strand) 74 bp		

Footnote: ATG-CAT* : The column specifies the distance between the start codons of the divergent axoB and andB genes. NA: Not applicable. The ubiquitous axoA and andA genes (top of the Table) are never linked. The A. implicatus axoB2 gene is not linked to a type-II NADH dehydrogenase gene.

Supplementary Table S2. NCBI-lodged RNA sequence reads that cover exon-exon fusions in *aoxB* and *andB* in *A. calidoustus*, *A. implicatus* and *A.niger* (with full-length *aoxB* and *andB* genes).

Species Gene Intron	Database (NCBI)	SRA reads	Species Gene Intron	Database (NCBI)	SRA reads	Species gene Intron	Database (NCBI)	SRA reads
A.calidoustus andB intron 1 TTAGGCACT	ERX631993	ERR677102.16649058.2	A.calidoustus aoxB intron 1 CCAATCTAC	ERX631996	ERR677105.6854148.2			
	ERX631995	ERR677104.15682812.1 ERR677104.15682812.2		ERX631995	ERR677104.2332297.1	A.implicatus andB intron 1 CTGGGCACT	SRX5208515	not covered
	ERX631996	ERR677105.2162584.1 ERR677105.2162584.2		ERX631994	ERR677103.2510794.2 ERR677103.1656912.1 ERR677103.900396.2	A.implicatus andB intron 2 TTTGGAATC	SRX5208515	SRR8399217.21403927.2 SRR8399217.387016.1
A.calidoustus andB intron 2 TTTGGATT	ERX631988	ERR677097.373254.2		ERX631993	ERR677102.2082084.2	A.implicatus andB intron 3 CCCTCGATTTTA	SRX5208515	not covered
	ERX631990	ERR677099.18680577.2 ERR677099.18680577.1 ERR677099.2869718.1		ERX631992	ERR677101.10577410.1 ERR677101.8218975.1 ERR677101.8193533.1	A.implicatus andB intron 4 AGTGTCCAG	SRX5208515	SRR8399217.2264778.1 SRR8399217.25258407.1 SRR8399217.992531.1 SRR8399217.23243498.1
	ERX631993	ERR677102.14622834.2						
	ERX631991	ERR677100.9382643.1						
	ERX631996	ERR677105.17252672.2 ERR677105.17252672.1	A.calidoustus aoxB intron 2 GGATGGATT	ERX631996	ERR677105.2013743.2			
A.calidoustus andB intron 3 CCTTCTGTGCTT	ERX631989	ERR677098.13859357.2 ERR677098.2998495.2 ERR677098.2998495.1 ERR677098.22770266.2 ERR677098.13859357.1		ERX631995	ERR677104.16482873.2 ERR677104.16482873.1	A.implicatus aoxB intron 1 CCAATCTAT	SRX5208515	SRR8399217.6320403.2
A.calidoustus andB intron 4 AGTGC CCGG	ERX631992	ERR677101.1542532.2 ERR677101.1542532.1		ERX631994	ERR677103.1971655.2			
	ERX631994	ERR677103.6474623.1 ERR677103.6474623.2		ERX631993	ERR677102.23350232.2 ERR677102.10276109.2 ERR677102.4764038.2 ERR677102.4764038.1	A.implicatus aoxB intron 2 GGATGGATC	SRX5208515	SRR8399217.23319380.2 SRR8399217.8293776.1 SRR8399217.6320403.1
	ERX631993	ERR677102.17188126.1 ERR677102.17188126.2 ERR677102.12370809.2		ERX631992	ERR677101.7231996.1			
A.niger aoxB intron 1 CCAATCTAC	SRX9722265	SRR13293334.22709813.2	A.niger andB intron 1 AGTGCCTAA	SRX11975055	SRR15678655.579521.2	A.niger andB intron 3 TAGGACAGA	SRX12789281	SRR16587860.3272965.1
A.niger aoxB intron 2 GGATGGATC	SRX11975789	SRR15679403.6264149.1	A.niger andB intron 2 TATCCAAA	SRX11975055	SRR15678655.579521.1	A.niger andB intron 4 CCGAGAGCT	SRX18320010	SRR22348904.213785.1
				SRX12789281	SRR16587860.708123.1			
				SRX15688375	SRR19638148.2825246.1			

Footnote: Per database, a maximum of five SRAs covering the exon-exon junction after excision of the indicated intron are given.

Strain gene Intron	Database (NCBI)	SRA reads
A. implicatus aoxB2 intron1 CCTGTATAC	SRX5208515	SRR8399217.31224079.1 SRR8399217.30796192.2 SRR8399217.30796192.1 SRR8399217.29964246.2 SRR8399217.29933532.2 SRR8399217.29533741.1
A. implicatus aoxB2 intron2 GGGTGGATC	SRX5208515	SRR8399217.31331017.2 SRR8399217.31167892.1 SRR8399217.30842627.2 SRR8399217.29836882.2 SRR8399217.29657382.2 SRR8399217.29442532.1

Table S3. Oligonucleotide primers used for PCR amplification of the *aoxB* and *andB* genes and cDNA prior to sequence verification of mutant alleles.

Strain	Primer	Sequence (5'-)
<i>Aspergillus niger</i> ATCC 1015 <i>aoxB</i> and cDNA	1015- <i>aoxB</i> -5prime-Fw1	CGTTCGATCTTCTCGCTAAG
	1015- <i>aoxB</i> -intern-Rv1	GTGTATAGGTAATGACAGCT
	1015- <i>aoxB</i> -intern-Fw2	GTGCACTGATTTCGTGACTG
	1015- <i>aoxB</i> -3prime-Rv2	CCATAAAGAATAACACCTGA
	1015- <i>aoxB</i> -5prime-cDNA-Fw1	ATCCCTGCCGAGGTGTCCAA
	1015- <i>aoxB</i> -5prime-cDNA2-Fw1	GAGACAGCTATTGTCTGGAA
	1015- <i>aoxB</i> -intern-cDNA-Rv1	TGACAGCTTCTTCCCTCCAGG
<i>Aspergillus niger</i> ATCC 1015 <i>andB</i> gene and cDNA	1015- <i>andB</i> -3prime-Fw1	GCTCGGTACCGAGGAAAGT
	1015- <i>andB</i> -intern-Rv1	GAGCAGAATGACACTGAGCT
	1015- <i>andB</i> -intern-Fw2	GGAAACAGTCTCTGTACATC
	1015- <i>andB</i> -5prime-Rv2	GAACCAGGCCAGATATACAGC
	1015- <i>andB</i> -5prime-cDNA1-Rv2	AGTAGAGGCAGATCCGTCAA
	1015- <i>andB</i> -5prime-cDNA2-Rv2	CCAGGCCAGATATACAGCCTA
<i>Aspergillus niger</i> CBS 554.65 <i>aoxB</i> and <i>andB</i>	1015- <i>andB</i> -3prime-Fw1	GCTCGGTACCGAGGAAAGT
	1015- <i>aoxB</i> -intern-Rv1	GTGTATAGGTAATGACAGCT
	554-65_ <i>andB</i> -special-Fw1	GCAAGAGATCCTTGGTGAGT
	554-65_ <i>andB</i> -special-Fw2	AGTTGCACTGTATCTCGTTG
<i>Aspergillus niger</i> CBS 147343 <i>aoxB</i>	1015- <i>aoxB</i> -5prime-Fw1	CGTTCGATCTTCTCGCTAAG
	554-65_ <i>aoxB</i> -intern-Rv1	GTGTATAGGTGATGACAGCT
	1015- <i>aoxB</i> -intern-Fw2	GTGCACTGATTTCGTGACTG
	124-48_ transposon-Rv1	CTACAGGGTTACTACCTCAA
	124-48_ transposon-Fw1	CCGCCTGTAGCGTTCGAACA
	1015- <i>aoxB</i> -3prime-Rv2	CCATAAAGAATAACACCTGA
<i>Aspergillus niger</i> CBS 630.78 <i>aoxB</i>	630-78- <i>aoxB</i> -5prime-Fw1	CGTTTGATCTTCTCGCTAAG
	1015- <i>aoxB</i> -intern-Rv1	GTGTATAGGTAATGACAGCT
	1015- <i>aoxB</i> -intern-Fw2	GTGCACTGATTTCGTGACTG
	630-78- <i>aoxB</i> -3prime-Rw2	CCATAAGAATAACACCTGAA
<i>Aspergillus niger</i> CBS 769.97 <i>aoxB</i>	630-78- <i>aoxB</i> -5prime-Fw1	CGTTTGATCTTCTCGCTAAG
	1015- <i>aoxB</i> -intern-Rv1	GTGTATAGGTAATGACAGCT
	1015- <i>aoxB</i> -intern-Fw2	GTGCACTGATTTCGTGACTG
	630-78- <i>aoxB</i> -3prime-Rw2	CCATAAGAATAACACCTGAA