Supplementary Information

MRP3 is a sex determining gene in the diatom Pseudo-nitzschia multistriata

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Supplementary Table 1. List of genes differentially expressed between MT+ and MTsamples in the RNA-seq study. In bold the candidate genes tested in qPCR; the five mating type related genes are underlined and marked with (^). The gene ID, logFC (logarithmic Fold Change) in RNA-seq and qPCR, p-Value, FDR (False Discovery Rate), Description of the predicted protein and the CPM (counts per million) for each RNA-seq library are reported. For the RNA-seq libraries (last nine columns), the strain name is reported above the library code (see also Supplementary Table 5). The table continues on the following page.

								MT+				Ν	1T-	
		qPCR FC				B938	B856	B856	Sy373	Sy373	B857	B857	B939	Sy379
Gene ID	logFC	± variance	p-Value	FDR	Description	Lib41	Lib74	Lib HCUH	Lib CIIO1	Lib CIIO2	Lib45	Lib HCUN	Lib77	Lib CIIP2
0122210	8.65		2.86E-06	3.28E-03	-	0.00	0.81	2.78	1.79	0.90	0.00	0.00	0.00	0.00
<u>0020770^</u>	8.53	7.5 ± 5	8.46E-22	1.00E-17		7.80	14.22	8.68	10.32	16.16	0.09	0.00	0.04	0.00
0109190	7.22		6.64E-14	2.63E-10		2.30	1.47	2.36	1.98	3.08	0.02	0.00	0.03	0.00
0060210	7.11		3.55E-05	1.76E-02	-	0.00	0.10	0.22	0.40	0.61	0.00	0.00	0.00	0.00
0022790	6.49		1.15E-05	6.86E-03	116 kDa U5 small nuclear ribonucleoprotein component	0.12	0.04	0.28	1.11	0.51	0.00	0.01	0.00	0.00
0046260	6.27		1.04E-05	6.47E-03		1.09	0.67	0.04	5.98	2.21	0.05	0.01	0.00	0.05
0104260	6.11		1.04E-04	4E-02	-	0.03	3.65	6.95	0.76	0.87	0.00	0.06	0.00	0.08
0042070	5.36		1.98E-06	2.61E-03	-	3.76	1.70	2.69	4.87	3.93	0.00	0.32	0.00	0.00
0106620	4.93		2.12E-05	1.20E-02	-	5.45	1.62	0.16	4.82	7.85	0.18	0.01	0.27	0.06
0060410	4.57		2.00E-07	3.40E-04	Phosphatidylinositol 4- phosphate 5-kinase 9	0.56	0.63	0.30	1.36	1.16	0.03	0.02	0.04	0.05
0036200	4.40		3.95E-06	3.71E-03	Delta(12) fatty acid desaturase fat-2	8.64	3.56	3.13	41.55	58.31	1.91	0.77	1.20	0.59
0122240^	4.33	3.7 ± 2.6	4.88E-13	1.45E-09	LRR receptor-like serine/threonine-protein kinase GSO1	54.12	166.19	105.89	113.59	143.65	4.97	11.03	4.83	3.82
0108790	3.84		4.33E-06	3.71E-03	-	6.91	3.05	1.57	5.84	2.35	0.20	0.39	0.47	0.07
<u>0024820^</u>	3.06	9.8 ± 3	1.10E-04	4.06E-02		173.97	306.15	214.05	181.47	225.29	25.26	4.72	65.51	10.19
0068000	2.40		7.79E-06	5.61E-03	Elongation of very long chain fatty acids protein 5	19.39	25.93	19.36	22.20	28.41	3.45	7.52	5.69	1.59
0113990	2.39		9.98E-05	4E-02	-	11.87	5.32	12.44	4.00	6.78	1.21	2.68	1.84	0.65
0025090	2.07		4.88E-05	2.23E-02	-	17.00	26.45	20.64	39.55	27.55	8.35	9.32	6.86	2.17
0091290	1.91		3.39E-05	1.75E-02	-	31.09	21.97	24.83	27.49	42.03	13.60	7.36	9.16	3.41

								MT+				M	1T-	
		qPCR FC				B938	B856	B856	Sy373	Sy373	B857	B857	B939	Sy379
Gene ID	logFC	± variance	p-Value	FDR	Description	Lib41	Lib74	Lib HCUH	Lib CIIO1	Lib CIIO2	Lib45	Lib HCUN	Lib77	Lib CIIP2
<u>0085380^/</u> 0041130	*	-6.6 ± 0.8		121	Heat shock factor protein 3	0	0	0	0	0	0	0	0	0
0006960^	-8.17	-9.7 ± 4.6	1.76E-16	1.04E-12	Probable leucine-rich repeat receptor-like protein kinase At1g35710	0.58	1.21	0.20	0.09	0.18	101.66	179.60	232.77	59.40
0081520	-8.04		4.44E-10	8.79E-07	Myb-related protein Zm38	0.00	0.00	0.00	0.00	0.00	2.22	0.65	0.40	1.08
0052370	-6.21		1.04E-04	4E-02	Phosphoenolpyruvate carboxykinase ATP	0.46	1.06	1.11	3.50	6.95	4.34	0.80	8.63	689.14
0020420	-5.11		8.45E-07	1.25E-03	-	3.18	2.86	0.68	0.74	1.13	4.33	186.77	56.10	10.26
0103000	-4.72		7.36E-06	5.61E-03	Cathepsin D	1.37	1.75	0.06	0.97	1.50	4.76	63.40	52.07	2.92
0045400	-4.60	0.2 ± 1.4	1.01E-05	6.47E-03	Putative oxidoreductase YteT	2.04	0.74	6.14	5.04	8.54	31.63	17.37	13.04	342.54
0045410	-4.57		6.59E-05	2.90E-02	Probable NAD(P)H- dependent D-xylose reductase xyl1	1.23	0.39	5.18	6.90	8.90	30.75	9.37	13.73	342.07
0021670	-4.20	0 ± 1.4	3.04E-06	3.28E-03	-	3.18	2.06	2.26	3.48	5.26	29.99	3.23	23.23	174.72
0093550	-4.01	0 ± 1	8.04E-06	5.61E-03		36.61	1.00	5.36	11.26	12.39	223.97	39.82	279.09	320.40
0084970	-3.05	0 ± 43	1.84E-10	4.37E-07	-	1.64	1.05	2.52	2.61	1.71	11.81	21.78	10.58	20.34
0076430	-2.96		1.41E-04	4.82E-02	Glutamate synthase large subunit-like protein YerD	14.83	1.24	6.34	8.94	13.21	53.01	9.63	75.93	130.69
0057230	-2.81		4.66E-05	2.21E-02	-	0.92	1.60	1.45	3.33	2.06	25.46	8.90	2.83	20.12
0004160	-2.51		3.06E-05	1.65E-02	-	7.24	3.43	8.79	4.84	7.38	19.65	9.73	44.16	65.34
0038950	-2.41		1.30E-04	4.69E-02	-	4.40	1.67	12.32	2.32	3.49	25.58	21.24	43.10	12.16
0072100	-2.27		1.42E-04	4.82E-02	Tetracycline resistance protein class A	6.30	3.41	2.75	7.18	5.25	26.61	5.47	43.26	21.20
0078000	-2.19		7.61E-05	3.23E-02	-	1.95	1.99	5.21	1.47	2.20	10.47	10.75	18.06	7.90
0113630	-2.03		4.37E-06	3.71E-03	Protein cereblon	2.76	1.64	2.32	2.92	2.08	15.44	12.96	6.17	7.22

* This gene (two identical gene models) was not present in the output of the differential expression analysis because of a filter on multimapping reads. It has been tested based on its expression profile during the early stages of *P. multistriata* sexual reproduction¹.

Supplementary Table 2. *P. multistriata* strains used in this study. The strain name, mating type defined in experimental crosses, isolation date, origin (environment or lab crosses) and the bands obtained in the genotyping PCR are given, along with the information on whether a given strain has been used for the qPCR analyses, for RNA-seq and/or to clone and sequence the alleles. >SST or < SST = above or below the sexualization size threshold.

Strain name	Mating Type	Isolation date	Origin/Parental strains	Genotyping PCR result	qPCR	RNA- seq	Alleles cloned and sequenced
B935	+	24/05/2012	isolated from water sample		Х		
SH18	+	07/04/2013	Sy776-*SP2+		Х		
MVR1041.6	+	05/02/2013	isolated from water sample		Х		
MVR171.8	+	07/06/2013	isolated from water sample		Х		
B936	-	24/05/2012	isolated from water sample	MB	Х		
SH20	-	07/04/2013	Sy776-*SP2+		Х		
MVR1041.4	-	05/02/2013	isolated from water sample	М	Х		
MVR171.1	-	07/06/2013	isolated from water sample		Х		
1119-15	+	02/09/2014	isolated from water sample	AM			Х
1120-32	-	09/09/2014	isolated from water sample	М			Х
1078-30	+	28/10/2013	isolated from water sample	AB			Х
1120-47	-	02/09/2014	isolated from water sample	MB			
1120-48	-	09/09/2014	isolated from water sample	MB			Х
1075-25	+	08/10/2013	isolated from water sample	AB			
1120-5	+	02/09/2014	isolated from water sample	А			
1120-25	+	02/09/2014	isolated from water sample	AB			
1120-7	-	02/09/2014	isolated from water sample	В			
1120 14	-	02/09/2014	isolated from water sample	В			
Sy373	+	07/072009	isolated from water sample	AM		Х	
Sy379	-	07/07/2009	isolated from water sample			Х	
B854 (A13)	-	13/10/2010	Sy373xSy379	М			
B855 (A1.6)	+	13/10/2010	Sy373xSy379	AB			
VF2.2.9/11	-	02/08/2011	B855 x B854	MB			
B857 (VF2.5.11)	-	02/08/2011	B855 x B854	MB		Х	Х
VF2.1.9	+	02/08/2011	B855 x B854	AM			
B856 (VF2.3.5)	+	02/08/2011	B855 x B854	AM		Х	Х
PmF3.2	+	2012	B856 x ?	А			
LV 77	-	2015	B855 x MVR1041.4	В			
LV 80	+	2015	B855 x MVR1041.4	AM			
LV 84	-	2015	B855 x MVR1041.4	В			
LV 88		2015	B855 x MVR1041.4	В			
LV 89	+	2015	B855 x MVR1041.4	А			
LV 91	+	2015	B855 x MVR1041.4	AM			
LV 92A	-	2015	B855 x MVR1041.4	MB			

Supplementary Table 2 continued

LV 106		2015	B855 x MVR1041.4	В			
LV 112		2015	B855 x MVR1041.4	В			
LV 113	-	2015	B855 x MVR1041.4	В			Х
LV 117	-	2015	B855 x MVR1041.4	MB			
LV 121	+	2015	B855 x MVR1041.4	AM			Х
LV 122	-	2015	B855 x MVR1041.4	MB			
LV 123	-	2015	B855 x MVR1041.4	В			
LV 125	+	2015	B855 x MVR1041.4	AM			
LV 127	+	2015	B855 x MVR1041.4	AM			
LV 129	-	2015	B855 x MVR1041.4	В			
LV 130	+	2015	B855 x MVR1041.4	А			Х
LV 136	-	2015	B855 x MVR1041.4	В	Х	Х	
LV 141	+	2015	B855 x MVR1041.4	А			
LV 142	-	2015	B855 x MVR1041.4	MB			
LV 148	-	2015	B855 x MVR1041.4	В			
LV 149	+	2015	B855 x MVR1041.4	AM			
LV 162	+	2015	B855 x MVR1041.4	AM			
LV 164	-	2015	B855 x MVR1041.4	MB			
LV 168	+	2015	B855 x MVR1041.4	А			
LV 169	-	2015	B855 x MVR1041.4	MB			
LV 175	+	2015	B855 x MVR1041.4	А			
LV 177	+	2015	B855 x MVR1041.4	AM			Х
LV 179	+	2015	B855 x MVR1041.4	А			
LV 180	+	2015	B855 x MVR1041.4	А			
LV 181	-	2015	B855 x MVR1041.4	В			
LV 182	+	2015	B855 x MVR1041.4	А			
LV 186	-	2015	B855 x MVR1041.4	MB			
LV 193	-	2015	B855 x MVR1041.4	В			
LV 195	+	2015	B855 x MVR1041.4	AM			
MC 1217	+	13/09/2016	isolated from water sample	-			
MC 1217	-	13/09/2016	isolated from water sample	-			
(13)			r				
MC 1217 (17)	-	13/09/2016	isolated from water sample	M/B			
F4 B1 >SST	NC	06/12/2016	PmF3.2 x MC 1217 13	М			
	when <sst< td=""><td></td><td></td><td></td><td></td><td></td><td></td></sst<>						
F4 B2 >SST	NC	06/12/2016	PmF3.2 x MC 1217 13	M			
	<sst< td=""><td></td><td></td><td></td><td></td><td></td><td></td></sst<>						
F4 B3 >SST	NC. +	06/12/2016	PmF3.2 x MC 1217 13	AM			
	when <sst< td=""><td></td><td></td><td></td><td></td><td></td><td></td></sst<>						
F4 B4 >SST	NC	06/12/2016	PmF3.2 x MC 1217 13	AM		1	

Suppleme	mary re	ible 2 contin	lucu			
F4 B5 >SST	NC	06/12/2016	PmF3.2 x MC 1217 13	М		
B6>SST	NC	06/12/2016	MC 1217 11 x MC 1217 13	AM		
B7 >SST	NC	06/12/2016	MC 1217 11 x MC 1217 13	MB		
B8>SST	NC	06/12/2016	MC 1217 11 x MC 1217 13	AM		
B9>SST	NC when <sst< td=""><td>06/12/2016</td><td>MC 1217 11 x MC 1217 13</td><td>MB</td><td></td><td></td></sst<>	06/12/2016	MC 1217 11 x MC 1217 13	MB		
B10>SST	NC. + when <sst< td=""><td>06/12/2016</td><td>MC 1217 11 x MC 1217 13</td><td>AM</td><td></td><td></td></sst<>	06/12/2016	MC 1217 11 x MC 1217 13	AM		
Sy682	-	07/09/2010	isolated from water sample	MB		
Sy799	-	21/09/2010	isolated from water sample	В		
Sy800	+	21/09/2010	isolated from water sample	В		
Sy798	+	21/09/2010	isolated from water sample	AM		
MM1A1	NT	2011	isolated from water sample	М		
ES814	+	2011	isolated from water sample	AB		
1068 13	-	20/08/2013	isolated from water sample	В		
1068 14	-	20/08/2013	isolated from water sample	В		
1068 33	+	20/08/2013	isolated from water sample	AB		
1068 81	-	20/08/2013	isolated from water sample	MB		
1070 14	+	03/09/2013	isolated from water sample	AB		
1070 13	+	03/09/2013	isolated from water sample	AB		
1068 57	+	20/08/2013	isolated from water sample	AB		
1264 B3a	+	29/08/2017	isolated from water sample	AM		
1264 B4	+	29/08/2017	isolated from water sample	AB		
1266 B3a	-	13/09/2017	isolated from water sample	В		
1266 B4b	+	13/09/2017	isolated from water sample	AB		
KA105 >SST	NC	28/06/2017	MC1217-17 x MC1217-5	AM		
KA204 >SST	NC	28/06/2017	MC1217-17 x LV168	AB		
MR3 >SST	NC	21/06/2017	LV92A5 x PMF3.2	MB		
MR5 >SST	NC	21/06/2017	LV92A5 x PMF3.3	AB		

Supplementary Table 2 continued

NC= non-competent for sex because cell size was above sexualization threshold. NT = not tested, dead, only gDNA available.

Strain name and MT	TUB B	TUB B	20760	20760
		average CT		average CT
SP4 MT-	21.43401		27.72913	
	21.3896	21.397765	27.54986	27.575966
	21.36968		27.4489	
SH20 MT-	22.10721		29.3734	
	22.29175	22.367543	30.14509	29.55899
	22.70367		29.15848	
41.4 MT-	19.07514		24.45341	
	19.3061	19.224321	24.44812	24.456882
	19.29172		24.46911	
171.1 MT-	21.69194		33.27479	
	21.77249	21.717037	33.12741	33.123839
	21.68668		32.96932	
SP2 MT+	22.96304		36.44473	
	22.96545	22.933743	37.78543	37.115078
	22.87274		ND	
SH18 MT+	21.73822		27.16025	
	21.91142	21.712673	27.05754	27.188158
	21.48838		27.34668	
41.6 MT+	20.25243		32.44352	
	20.33863	20.28755	32.15172	32.619483
	20.27159		33.26321	
171.8 MT+	23.14835		29.08989	
	23.03572	23.120984	29.07984	29.242554
	23.17888	1	29.55793	

Supplementary Table 3. Cycle Threshold (CT) values (technical triplicates and average) from qPCR amplification of the control gene tubulin- β (TUB B) and of the gene 0020760.

ND, not determined

Gene ID	logFC	logCPM	PValue	FDR	Gene name
0020770.1	10.84	4.77	5.763E-21	5.397E-17	MRP3
0024810.1	10.40	9.18	1.316E-11	3.081E-08	MRP1
0122240.1	4.57	6.65	1.161E-08	2.174E-05	MRP2
0070330.1	-2.62	5.17	2.313E-05	2.708E-02	MRX1
0058000.1 *	-3.27	3.53	3.516E-05	3.297E-02	MRX2
0036960.1 *	-3.27	3.53	3.520E-05	3.297E-02	MRX2
0041130.1 °	-5.88	3.72	3.889E-08	5.203E-05	MRM1
0085380.1 °	-5.88	3.72	3.868E-08	5.203E-05	MRM1
0006960.1 ^	-6.37	5.77	6.725E-13	2.099E-09	MRM2
0006960.2 ^	-6.37	5.77	6.705E-13	2.099E-09	MRM2

Supplementary Table 4. List of genes differentially expressed in the transgenic strain LV136T3 compared to the wild type strain LV136. Two new putative MR genes are indicated in bold.

Gene models marked with identical symbols are either isoforms (^) or are identical but map on two different scaffolds (* and °).

Supplementary Table 5. Libraries selected for the differential expression analysis with corresponding strain code, strain Mating Type (MT), accession number, reference, sequencing strategy, number of reads before and after the quality check (QC).

Library	Strain	MT	Accession number	Reference	Strategy	Reads	Reads
Name	code					before QC	after QC
41	B938	+	E-MTAB-5469	1	Single-end	28.458.025	28.341.225
45	B857	-	E-MTAB-5469	1	Single-end	34.070.601	33.766.714
74	B856	+	E-MTAB-5469	1	Single-end	19.270.248	19.194.720
77	B939	-	E-MTAB-5469	1	Single-end	27.925.811	27.652.379
CIIO1	Sy373	+	SRX059292	Unpublished	Paired-end	42.736.393	35.243.577
CIIO2	Sy373	+	SRX115126	2	Paired-end	26.423.748	22.914.921
CIIP2	Sy379	-	SRX115125	2	Paired-end	30.856.367	20.823.225
HCUH	B856	+	SRX1070747	Unpublished	Paired-end	54.545.126	50.297.563
HCUN	B857	-	SRX1070749	Unpublished	Paired-end	52.851.954	48.670.573

Primer name	Location	Primer sequence 5'-3'
F147prom	MRP3 promoter	GGCAACAAGTGCTTGCTAATG
R147prom	MRP3 promoter	GATGATGTACAACAATCGGCG
R147p2	MRP3 promoter	GGGTCTACGTACGGTATGGTG
F147p2	MRP3 promoter	CAATCCTACATAGGCCCAATATC
SC432promFW	<i>MRM1</i> promoter	GAGTTCTCTTGCCGGATGATAC
SC432promRV	<i>MRM1</i> promoter	CCCTCATTCACCACATGTGAC
0020770+ F	MRP3 coding region	GCGCAAGCAATCTAAGGTG
0020770+ R	MRP3 coding region	GACGTCGACGGCTATTTTG
F147p3	MRP3 promoter	GGATCCTTTGAGCAACACAG
R147p3	MRP3 promoter	CCAAGTGATGCTGCATACAAG
0.00+F	MRP1 coding region	GTATGGCGCTCACCACTTC
0.00+R	MRP1 coding region	CGTCTTCGACTGCGTCTTC
127.15 F3	MRP2 coding region	CCTCCGAATATGGATACATG
127.15 R3	MRP2 coding region	GAGCTAAACATCGTGACACC
47507F	MRM1 coding region	CCCCTACAAGCTCTTTGATTTG
47507R	MRM1 coding region	GAAATTGTGGTGCCCAAAG
46228F	MRM2 coding region	CCACCGAACTAGGCAACTGTC
46228R	MRM2 coding region	GGCACAGAACCCGTCAAC
FMrp3Eco	MRP3 expressing vector	GGCGGCCGAATTCATGAATGACGAATCGAAT
RMrp3Sma	MRP3 expressing vector	GGCGGCCCCCGGGCTACATCTGCTGCATTAG
FPIH4	NAT expressing vector	AAAGCTACTAGTGGCCATTTTGGAATTTGTCG
RPIH4	NAT expressing vector	TCGACTACCTCAGTCTTCGGCTCTAGAATGACC
FPIIH4	NAT expressing vector	TCGGCTCTAGAATGACCACTCTTGACGACACG
RPIIH4	NAT expressing vector	TCGACTACCTCAGTCTTCGGCTCTAGAATGACC
H4up1	MRP3 and NAT expressing vectors	GTGGGAATGAACAGAACGAGA
NATfor	NAT expressing vector	TGACCACTCTTGACGACACG
NATrev	NAT expressing vector	GTTGACGTTGGTGACCTCC
tArev	MRP3 and NAT expressing vectors	CCCAGCCAAAGTCGAGGTAG

Supplementary Table 6. List of primers used in this study and corresponding sequences.



Supplementary Figure 1. Schematic representation of the life cycle in centric (left) and pennate (right) diatoms. Diatoms are diploid (2N) and gametes are the only haploid (N) stage in their life cycle. Centric diatoms are homothallic and anisogamous, while pennate diatoms are mostly heterothallic and isogamous or physiologically anisogamous. Auxospores, not surrounded by the rigid siliceous frustule, can expand, and within them the maximum sized initial cell is produced. This cell will start dividing mitotically.



SignalP-4.1 prediction (euk networks): MRP1

AsaFind output

This is ASAFind version 1.1.5. You used SignalP-4.1. You submitted 1 proteins 0 of your proteins were SignalP negative 1 of your proteins were SignalP positive 0 of these were predicted to go to the plastid 0 of these were predicted with high confidence 0 of these were predicted with with low confidence

Supplementary Figure 2. Outputs of prediction software for transit peptides for the MRP1 protein. Top, output of the SignalP 4.0 software, which can predict the presence of a secretory signal peptide, a ubiquitous protein sorting signal that targets proteins for translocation across the endoplasmic reticulum (ER) membrane. Bottom, output of the AsaFind software, a prediction tool that identifies proteins with a signal peptide for transport to diatom plastids. The MRP1 protein contains a signal peptide but it is not predicted to go to the plastid.



Supplementary Figure 3. PCR amplification of a 728 bp region upstream of the *MRM1* **gene.** Electrophoretic gel with amplicons obtained from two MT+ and three MT- strains.



Supplementary Figure 4. The A allele segregates with the MT+ phenotype. *P. multistriata* pedigree with three generations. Each individual is represented by a square for MT+ or a circle for MT- and is defined by the generation number (in Roman on the left panel) and a progressive Arabic number (in red). Strain names are reported above each square or circle when available. The alleles of each individual are indicated below the symbol. The question mark indicates uncertainty on the second allele. In generation II subscript numbers next to brackets indicate how many individuals of that MT with that genotype were obtained from the cross of I-1 and I-3. Electropherograms show bands obtained from a selection of individuals in the pedigree. For all other genotypes refer to Supplementary Table 2. The strain circled in red was used to produce the reference genome¹.

>A allele

TACTAGGAGTACTAGTAGTAGTGGGCAGTACTACTAGTAGTAGCTACTAATAGTACTAAT AGTACTATTAGTGGTGGTCTTACTACTACTAGTAGTAGTAGCACCAGTCGTAGTACTAGC AAAGTACAGCAGTACTACTAGTAGTATTACTAGTAGTAGTAGTAGTACTAGACAAGTAA TATAGCAGTACTAATAGTGGTAGTGAAATAAGTTTCTGCTATTCATTGTACAATCCTACA TAGGCCCAATATCAATGACAAACATTTAGGAAAATCAAAATAATGAAGATACAATGACAC ATACTATGGGAACAGAGGCAAGTACCACCTAAAGACACTTGTTATTATTGTACTCATAAC AAGTAAACAATTAATGGGGTCAAGTAAGAAACTTGAAAAACTACCAATTACCAACAACCA GAGAAATGGCGTTTATTCTTGTTTTGTTTCTGACGGCTTCTGCACAATGGTAGCAACGT AGATTAAAAGAGGACTGAACCAATTGAATCGCTGATTGGACCCGGGAGCCTTTTTTGTTC AAGACACTGGACAAGGCTTAAACGATTTTTGAGTAGGAGTATGTGCCTTTTTTCTTCGAT CACAGATCATGTTCAAGGAGGAAAGAATAATACTACGAGTAATATTGTGAATAGAACCAC GATAAAAACGAATACAACATCACAGCACAAGGCATGGCATTGTACTCAGCATAGCACAAC ACACCGAACAACACATAAAAGCCATTTCACTACACTtgtaAGCTCCGAAATCAATCAGTA AAAAAGAAAAAAAGATCTTTAGTCAAAATGAATGACGAATCGAATAAAGAATGGACCTAC TTGCAGAAACTGGCTCTCCGCTATAACCAGTGCCATCGAGAAGAGAAAAGACTTTTTGCG AAATGCGAAGTCTATAATATAGTTCTTGAAAAGGGAGGTTCGTTTTTCGAAATAACGAAC AAAATAGCCGTCGACGTCACGGCGGACGAAATGAAATCAACTACAAAGATCATGCAAGTT TTTCGAGATATAAATAAACGTAACAAGAAGAGTAAAGGGTCTGCAAAGGCGAAAAAGAAG CCATCTTTCACTTCTCGAACGAAAAAAAAAAAAACCGTCTTTTTGCACTTTACAAACTACT AGAGCAAGAGAATCACGAACGACCAAAATGAAAATAAAAACTCAACCAAAAACGCAGGTGC GTCAAGCCTAGCATAATTGAAGCGCCAATCATGAATTGTGTGAGGGATGCTTTACTGATG CCGGTCAGGGGTAACGGATCCGAGCTGAAGCGACTTATTGCGGAAAGTAATCATCGCTTC TCAACAAATAATGAAACATTTGATTTAATGGCCGTGGAACCACCTCAGCTAGAGAACTCG TTTAGTGCACTGATCATGGCGGATGAGGTAACAACGGAACTTCCAACTGATTCTCAAATG AGTGTGAACCTACACCTGCACAAAAGAGTACAGCGACTAGAAAATCTTGTTGGGATGCTA ATGCAGCAGATGtaqAGAGTCTAGTGTAGCTAAATACTTAAACATGGAAACAGGAATCAC TCGACGCTACGTGTATCAAAAAATATGCAGCGATCGGATTTGCGCAACAAATCTGCTCT CTGGGCTGGCATCAAATTCCAAGGAAAATGTTCCGCCCGATGGTGACTACTTTGACTAAA AAACTTGCTTGAACTCCTGTTACAGCAAAATCAGATGCGCGTGAAAATATTTGGTCAGCA AGAGCACGCAAAACTATTTGGTTGCAAGGAATAAAATTTGAGCACGTTC >M allele

AGTAGTAGTAGTAGGAGGAGTACTAGTAGTAGTGGGCGGGTACTACTAGTAGTAGCTACTA CTAGTACTAATAGTACTATTAGTGGTGGTCTTACTACTACTAGTACTACTAGTAGCACCA GTCGTAGTACTAGCAAAGTACAGTACTACTAGTTATTACTAGTAGTAGTACTACTACTAG ACAAGTAATATAGCAGTACTAATAGTGGTAGTGAAATAAGTTTCTGCTATTCATTGTACA ATCCTACATAGGCCCAATATCAATGACATACATTTAGGAAAATCAAAATAATGAAGATAC AATGACACATACTATGGGAACAGAGGTAAGTACCACCTAAAGACACTTTTTATTATTGTA CCCACATCAAGTAAACAATTAATGGGGTCAAGTAAGAAACTTGAAAAACTACCATTACCAA CACAACCAGAGAAATGGCGTTTATTCTTGTTTTTGTTTCTGACGGCTTCTGCACAATGGT AGCAACGTAGATTAAAAGAGGACTGAACCAATTGAATCGCTGATTGGACCCGGGAGCCTT TTTTGTTCAAGACACTGGACAAGGCTTAAACGATTTTTGAGTAGGAGTATGTGCCTTTTT TCTTCAATCACAGATCATGTTCAAGGAGGAAAGAATAATACTATGAGTAATATTGTGAAT AGAACCACGATAAAAACGAATACAACATCACAGCACAAGGCATGGCATTGTACTCAGCAT AGCACAACACACAGAACGACACATAAAAGCCATTTCACTACACTtgtaAGCTTCGAAATC AATCAATACAATTCATCGCCGATTGTTGTACATCATCATTTGTCTACTCCATATTACAAG TCGGTTTGCTGCAGAAACTGGCTCTTCGCTATAACCAGTGCCATCGAGAAGAGAAAAGAC TTTTTGCGAAATGCGAAGTCTATAATATAGTTCTTGAAAAGGGAGGTTCATTTTTCGAAA TAACGAACAAAATAGCCGTCGACGTCACGGCGGACGAAATGAAATCAACTACAAAGATCA TGCAAGTTTTTCGAGATATAAATAAACGTAACAAGAAGAGTAAAGGGTCTGCAAAGGCGA AAACTACAAGAGCAAGAGAATCACGAACGACCAAAATGAAAATAAAAACTCAACCAAAAC GCAGGTGCGTCAAGCCTAGCATAATTGAAGCGCCAATCATGAATTGTGTGAGGGATGCTT TGGGTACTCCGGTCAGGGGTAACGGATCCGAGCTGAAGCGACTTATTGCGGAAAGTAATC ATCGCTTCTCAACAAATAATGAAACATTTGATTTAATGGCCGTGGAACCACCTCAGCTAG AGAACTCGTTTAGTGCACTGATCATGGCGGATGAGGTAACAACGGAACTTCCAACTGATT CTCAAATGAGTGTGAACCTACACCTGCACAAAAGAGTACAGCGACTAGAAAATCTTGTTG GGATGCTAATGCAGCAGATGTAGAGAGTCTAGTGTAGCAAAATACTTAAACATGGAAACA GGAATCACTCGACGCTACGTGTACCAAAAAATATGCAGCGATCGGATTTGCGCAACACAA TCTGCTCTCTGGGCTAGCATCAAATTCCAATGAAAATGTTCCGCCCGATGGTGACTACTT TGACTAAAAAACTTGCTTGAACTCCTGTTACAGCAAAATCAGATGCGCGTGAAAATATTT GGTCAGCAAGAGCACGCAAAACTATTTGGTTGCAAGGAATAAAATTTGAGCACGTTC >B allele

GCTGCCAAAGCTCAGACAACAGCACCACTAGCAACAATAATTAGCTATACTACTAGTAGTA GTAGTGGGCggtactactagtagtaGCTACTACTAGTACTAATAGTACTATTAGTGGTGG TCTTACTACTAGTAGTAGTAGCACCAGTCGTAGTACTAGCAAAGTACAGTACTACTA GTTATTACTAGTAGTAGTACTACTACTAGACAAGTAATATAGCAGTACTAATGGTGGTAG TGAAATAAGTTTCTGCTATTCATTGTACAATCCTACATAGGCCCCAATATCAATGACATAC ATTTAGGAAAATCAAAATAATGAAGATACAATGACACATACTATGGGAACAGAGGTAAGT TTCCTATCTACCACCATACCGTACGTAGACCCACATCAAGTAAACAATTAATGGGGTCAA GTAAGAAACTTGAAAACTACCATTACCAACAACAACCAGAGAAATGGCGTTTATCCTTGTT TTTGTTTCTGACGGCTTCTGCACAATGGTAGCAACGTAGATTAAAAGAGGACTGAACCAA TTGAATCGCTGATTGGACCCGGGAGCCTTTTTTGTTCAAGACACTGGACAAGGCTTAAAC AATTTTTGAGTAGGAGTATGTGCCTTTTTTCTTCGATCACAGATCATGTTCAAGGAGGAA AGAATAATACTACGAGTAATATTGTGAATAGAACCACAATAAAAACGAATACAACATCAC AGCACAAGGCATGGCATTGTACTCAGCATAGCACAACACACAACAACAACAACAACAACAACACA AtttcactacacttgtaAGCTTCGAAATCAATCAATACAATTCATCGCCGATTGTTGTAC CAAAATGAATGACGAATCGAATAAAGAATGGACCTACTACTTTGGTCGAGGAGGCGCTTG CAACCAACAGCGCAAGCAATCTAAGGTGGTCGGTTTGTTGCAGAAACTGGCTCTTCGCTA TAACCAGTGCCATCGAGAAGAGAGAAAAGACTTTTTGCGAAATGCGAAGTCTATAATATAGT TCTTGAAAAGGGAGGTTCGTTTTTCGAAATAACGAACAAAATAGCCGTCGACGTCACGGC CAAAATGAAAATAAAAACTCAACCAAAACGCAGGTGCGTCAAGCCTAGCATAATTGAAGC GCCAATCATGAATTGTGTGAGGGATGCTTTACTGATGGCCAGCGCGAAAAGTCAATCGCT TCCTGACAAAGGACAAACAAGCCGGGGTTGGGTACTCCGGTCAGGGGTAACGGATCCGA GCTGAAGCGACTTATTGCGGAAAGTAATCATCGCTTCTCAACAAATAATGAAACATTTGA TTTAATGGCCGTGGAACCACCTCAGCTAGAGAACTCGTTTAGTGCACTGATCATGGCGGA TGAGGTAACAACGGAACTTCCCAACTGATTCTCAAATGAGTGTGAACCTACACCTGCACAA AAGAGTACAGCGACTAGAAAATCTTGTTGGGATGCTAATGCAGCAGATGTAGAGAGTCTA GTGTAGCTAAATACTTAAACATGGAAACAGGAATCACTCGACGCTACGTGTACCAAAAAA TATGCAGCGATCGGATTTGCGCAACACAATCTGCTCTCTGGGCTAGCATCAAATTCCAAT AGCAAAAATCAGATGCGCGTGAAAATATTTGGTCAGCAAGAGCACGCAAAACTATTTGGTT GCAAGGAATAAAATTTGAGCACGTTC

>N allele

ATGACACATACTATGGGAACAGAGGtAAGTACCACCTAAAGACACTTtTTATTATTGTAC CCACATCAAGTAAACAATTAATGGGGTCAAGTAAGAAACTTGAAAAACTACCATTACCAAC ACAACCAGAGAAATGGCGTTTATTCTTGTTTTGTTTCTGACGGCTTCTGCACAATGGTA GCAACGTAGATTAAAAGAGGACTGAACCAATTGAATCGCTGATTGGACCCGGGAGCCTTT TTTGTTCAAGACACTGGACAAGGCTTAAACGATTTTTGAGTAGGAGTATGTGCCTTTTT CTTCaATCACAGATCATGTTCAAGGAGGAAAGAATAATACTACGAGTAATATTGTGAATA GAACCACGATAAAAACGAATACAACATCACAGCACAAGGCATGGCATTGTACTCAGCATA GCACAACACAGAACAACACATAAAAGCCATTTCACTACACTTGTAAGCTtCGAAATCA ATCAaTACAATTCATCGCCGATTGTTGTACATCATCATTTGTCTACTCCATATTACAAqC CGGTTTGTTGCAGAAACTGGCTCTTTGCTATAACCAGTGCCATCGAGAAGAGAAAAGACT TTTTGCGAAATGCGAAGTCTATAATATAGTTCTTGAAAAGGGAGGTTCATTTTTCGAAAT AACGAACAAAATAGCCGTCGACGTCACGGCGGACGAAATGAAATCAACTACAAAGATCAT GCAAGTTTTTCGAGATATAAATAAACGTAACAAGAAGAGTAAAGGGTCTGCAAAGGCGAA AACTACAAGAGCAAGAGAATCACGAACGACCAAAATGAAAATAAAAACTCAACCAAAACG CAGGTGCGTCAAGCCTAGCATAATTGAAGCGCCAATCATGAATTGTGTGAGGGATGCTTT GGGTACTCCGGTCAGGGGTAACGGATCCGAGCTGAAGCGACTTATTGCGGAAAGTAATCA TCGCTTCTCAACAAATAATGAAACATTTGATTTAATGGCCGTGGAACCACCTCAGCTAGA GAACTCGTTTAGTGCACTGATCATGGCGGATGAGGTAACAACGGAACTTCCAACTGATTC TCAAATGAGTGTGAACCTACACCTGCACAAAAGAGTACAGCGACTAGAAAATCTTGTTGG GATGCTAATGCAGCAGATGTAGAGAGTCTAGTGTAGCAAAATACTTAAACATGGAAACAG GAATCACTCGACGCTACGTGTACCAAAAAATATGCAGCGATCGGATTTGCGCAACACAAT CTGCTCTCTGGGCTAGCATCAAATTCCAATGAAAATGTTCCGCCCGATGGTGACTACTTT GACTAAAAAACTTGCTTGAACTCCTGTTACAGCAAAATCAGATGCGCGTGAAAATATTTG GTCAGCAAGAGCACGCAAAACTATTTGGTTGCAAGGAATAAAATTTGAGCACGTTC

Supplementary Figure 5. Sequences of the *MRP3* **alleles.** Nucleotide sequences of the A, M, B and N alleles.

A_allele	AATAATTAGCTATACTACT 22							
M_allele B_allele N_allele	GTGAATTGTAATACGACTCACTATAGGGCGAATTGGGCCCTCTAGATGCATGC	0 60 0						
A_allele M_allele B_allele N_allele	ACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTACTA	76 33 120 0						
A_allele M_allele B_allele N_allele	ACTACTACTACTACTACTACTACTACTACTACTACTACTA	136 87 179 0						
A_allele M_allele B_allele N_allele	ACTACTACTACTACTACTACTACTACTACTACTACTACTA	196 147 239 0						
A_allele M_allele B_allele N_allele	AGTAGTGGGCAGTACTACTAGTAGTAGCTACTAATAGTACTAATAGTACTATTAGTGGTG AGTAGTGGGCGGTACTACTAGTAGTAGCTACTACTAGTACTAATAGTACTATTAGTGGTG AGTAGTGGGCggtactactagtagtaGCTACTACTAGTACTAATAGTACTATTAGTGGTG	256 207 299 0						
A_allele M_allele B_allele N_allele	GTCTTACTACTAGTAGTAGTAGCACCAGTCGTAGTACTAGCAAAGTACAGCAGT GTCTTACTACTACTAGTACTAGTAGCAGCAGTCGTAGTACTAGCAAAGTAC GTCTTACTACTACTAGTACTAGTAGCACCAGTCGTAGTACTAGCAAAGTAC	313 261 350 0						
A_allele M_allele B_allele N_allele	ACTACTACTAGTTATTACTAGTAGTACTAGTAGTACTAGACAAGTAATATAGCAGTACTA AGTACTACTAGTTATTACTAGTAGTAGTACTACTACTAGACAAGTAATATAGCAGTACTA AGTACTACTAGTTATTACTAGTAGTAGTACTACTACTAGACAAGTAATATAGCAGTACTA	373 321 410 0						
A_allele M_allele B_allele N_allele	ATAGTGGTAGTGAAATAAGTTTCTGCTATTCATTGTACAATCCTACATAGGCCCAATATC ATAGTGGTAGTGAAATAAGTTTCTGCTATTCATTGTACAATCCTACATAGGCCCAATATC ATGGTGGTAGTGAAATAAGTTTCTGCTATTCATTGTACAATCCTACATAGGCCCAATATC	433 381 470 0						
A_allele M_allele B_allele N_allele	ААТGACAAACATTTAGGAAAATCAAAATAATGAAGATACAATGACACATACTATGGGAAC AATGACATACATTTAGGAAAATCAAAATAATGAAGATACAATGACACATACTATGGGAAC AATGACATACATTTAGGAAAATCAAAATAATGAAGATACAATGACACATACTATGGGAAC ATGACACATACTATGGGAAC ***************	493 441 530 20						
A_allele M_allele B_allele N_allele	AGAGGCAAGTACCACCTAAAGACACTTGTTATTATTGTACTCATAACCGATTCCAGATTC AGAGGTAAGTACCACCTAAAGACACTTTTTATTATTGTACTCATAACCGATTCCAGATTC AGAGGTAAGTACCACCTAAAGACACTTTTTATTATTGTACTCATAACCGATTCCAGATTC AGAGGtAAGTACCACCTAAAGACACTTtTTATTATTGTACTCATAACtGATTCCAGATTC *****	553 501 590 80						

A_allele	GTGCCACACGTTCCTATCTACCACCATACCGTACGTAGACCCACATCAAGTAAACAATTA	613 561
M_allele		650
N_allele	GTGCCACACGTTCCTATCTACCACCATACCGTACGTAGACCACATCAAGTAAACAATTA	140
~ .]] .] .		(7)
A_allele		6/3
M_allele		021 710
B_allele N_allele		200
N_differe	*****	200
A_allele	TATTCTTGTTTTTGTTTCTGACGGCTTCTGCACAATGGTAGCAACGTAGATTAAAAGAGG	733
M_allele	TATTCTTGTTTTGTTTCTGACGGCTTCTGCACAATGGTAGCAACGTAGATTAAAAGAGG	681
B_allele	TATCCTTGTTTTTGTTTCTGACGGCTTCTGCACAATGGTAGCAACGTAGATTAAAAGAGG	770
N_allele	TATTCTTGTTTTTGTTTCTGACGGCTTCTGCACAATGGTAGCAACGTAGATTAAAAGAGG *** ***************************	260
A allele	ACTGAACCAATTGAATCGCTGATTGGACCCGGGAGCCTTTTTTGTTCAAGACACTGGACA	793
Mallele	ACTGAACCAATTGAATCGCTGATTGGACCCGGGAGCCTTTTTTGTTCAAGACACTGGACA	741
B_allele	ACTGAACCAATTGAATCGCTGATTGGACCCGGGAGCCTTTTTTGTTCAAGACACTGGACA	830
N_allele	ACTGAACCAATTGAATCGCTGATTGGACCCGGGAGCCTTTTTTGTTCAAGACACTGGACA **********************************	320
A allele	AGGCTTAAACGATTTTTGAGTAGGAGTATGTGCCTTTTTTCTTCGATCACAGATCATGTT	853
M allele	AGGCTTAAACGATTTTTGAGTAGGAGTATGTGCCTTTTTTCTTCAATCACAGATCATGTT	801
B allele	AGGCTTAAACAATTTTTGAGTAGGAGTATGTGCCTTTTTTCTTCGATCACAGATCATGTT	890
N_allele	AGGCTTAAACGATTTTTGAGTAGGAGTATGTGCCTTTTTTCTTCaATCACAGATCATGTT ********* *************************	380
A allele	CAAGGAGGAAAGAATAATACTACGAGTAATATTGTGAATAGAACCACGATAAAAACGAAT	913
Mallele	CAAGGAGGAAAGAATAATACTATGAGTAATATTGTGAATAGAACCACGATAAAAACGAAT	861
B_allele	CAAGGAGGAAAGAATAATACTACGAGTAATATTGTGAATAGAACCACAATAAAAACGAAT	950
N_allele	CAAGGAGGAAAGAATAATACTACGAGTAATATTGTGAATAGAACCACGATAAAAACGAAT ***********************************	440
A allele	ACAACATCACAGCACAAGGCATGGCATTGTACTCAGCATAGCACAACACCGAACAACA	973
Mallele	ACAACATCACAGCACAAGGCATGGCATTGTACTCAGCATAGCACAACACACAGAACGACA	921
Ballele	ACAACATCACAGCACAAGGCATGGCATTGTACTCAGCATAGCACAACACACATAACAACA	1010
N_allele	ACAACATCACAGCACAAGGCATGGCATTGTACTCAGCATAGCACAACAACAACAACAACA ******************	500
A allele	CATAAAAGCCATTTCACTACACTtgta A GCT C CGAAATCAATCA G TACAATTCATCGCCG	1033
M_allele	CATAAAAGCCATTTCACTACACTtgta A GCT T CGAAATCAATCA A TACAATTCATCGCCG	981
B ^{allele}	CATAAAAGCCAtttcactacacttgtaAGCTTCGAAATCAATCAATACAATTCATCGCCG	1070
N_allele	CATAAAAGCCATTTCACTACACTTGTA A GCT T CGAAATCAATCA A TACAATTCATCGCCG	560
A allele	ATTGTTGTACATCATCATTTGTCTACTCCATATTACAAACAA	1093
M_allele	ATTGTTGTACATCATCATTTGTCTACTCCATATTACAA G CAAGTGACAAAAAGAAAAAA	1041
B allele	ATTGTTGTACATCATCATTTGTCTACTCCATATTACAA G CAAGTGACAAAAAGAAAAAAA	1130
N_allele	ATTGTTGTACATCATCATTTGTCTACTCCATATTACAA G CAAGTGACAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	620
A allele	GATCTTTAGTCAAAATGAATGACGAATC G AATAAAGAATGGACC T ACTACTTCGGTCGAG	1153
M allele	GATCTTTAGTCAAAATGAATGACGAATC G AATAAAGAATGGACC T ACTACTT C GGTCGAG	1101
Ballele	GATCTTTAGTCAAAATGAATGACGAATC G AATAAAGAATGGACC T ACTACTT T GGTCGAG	1190
N_allele	GATCTTTAGTCAAAATGAATGACGAATCAAATAAAGAATGGACCCACTACTTCGGTCGAG	680
A_allele	GAGGCGCTTGCAACCAACAGCGCAAGCAATCTAAGGTGG T CGGTTTGTTGCAGAAACTGG	1213
M_allele	GAGGCGCTTGCAACCAACAGCGCAAGCAATCTAAGGTGG T CGGTTTGTTGCAGAAACTGG	1050
B_allele	gagguguttgcaacuaacagugcaagcaatctaAggtgg t cggtttgttgCAGAAACTGG	1250

N_allele	GAGGCGCTTGCAACCAACAGCGCAAGCAATCTAAGGTGGCCGGTTTGTTGCAGAAACTGG ***********************************	740
A_allele	CTCTCCGCTATAACCAGTGCCATCGAGAAGAGAAAAGACTTTTTGCGAAATGCGAAGTCT	1273
M_allele	CTCT TC GCTATAACCAGTGCCATCGAGAAGAGAAAAGACTTTTTGCGAAATGCGAAGTCT	1221
B_allele	CTCT TC GCTATAACCAGTGCCATCGAGAAGAGAAAAGACTTTTTGCGAAATGCGAAGTCT	1310
N_allele	CTCT TT GCTATAACCAGTGCCATCGAGAAGAGAAAAGACTTTTTGCGAAATGCGAAGTCT	800
	**** **********************************	
A_allele	ATAATATAGTTCTTGAAAAGGGAGGTTC G TTTTTCGAAATAACGAACAAAATAGCCGTCG	1333
M_allele	ATAATATAGTTCTTGAAAAGGGAGGTTC A TTTTTCGAAATAACGAACAAAATAGCCGTCG	1281
B_allele	ATAATATAGTTCTTGAAAAGGGAGGTTC G TTTTTCGAAATAACGAACAAAATAGCCGTCG	1370
N_allele	ATAATATAGTTCTTGAAAAGGGAGGTTC A TTTTTCGAAATAACGAACAAAATAGCCGTCG ********************************	860
A allele	ACGTCACGGCGGACGAAATGAAATCAACTACAAAGATCATGCAAGTTTTTCGAGATATAA	1393
M_allele	ACGTCACGGCGGACGAAATGAAATCAACTACAAAGATCATGCAAGTTTTTCGAGATATAA	1341
B allele	ACGTCACGGCGGACGAAATGAAATCAACTACAAAGATCATGCAAGTTTTTCGAGATATAA	1430
N_allele	ACGTCACGGCGGACGAAATGAAATCAACTACAAAGATCATGCAAGTTTTTCGAGATATAA ******************************	920
A_allele	ATAAACGTAACAAGAAGAGTAAAGGGTCTGCAAAGGCGAAAAAGAAGCCATCTTTCACTT	1453
M_allele	ATAAA <mark>C</mark> GTAACAAGAAGAGTAAAGGGTCTGCAAAGGCGAAAAAGAAGCCATCTTTCACTT	1401
B_allele	ATAAA T GTAACAAGAAGAGTAAAGGGTCTGCAAAGGCGAAAAAGAAGCCATCTTTCACTT	1490
N_allele	ATAAA C GTAACAAGAAGAAGAAGAAGGGTCTGCAAAAGGCGAAAAAGAAGCCATCTTTCACTT ***** ******************************	980
A_allele	CTCGAACGAAAAAAAAAAAGC <mark>C</mark> GTCTTTTTGCACTTTACAAACTAC T AGAGCAAGAGAAT	1513
M_allele	CTCGAACGAAAAAAAAAAAGC C GTCTTTTTGCACTTTACAAACTAC A AGAGCAAGAGAAT	1461
B_allele	CTCGAACGAAAAAAAAAAGC <mark>T</mark> GTCTTTTTGCACTTTACAAACTAC A AGAGCAAGAGAAT	1550
N_allele	CTCGAACGAAAAAAAATAAGC C GTCTTTTTGCACTTTACAAACTAC A AGAGCAAGAGAAT *******************************	1040
A_allele	CACGAACGACCAAAATGAAAATAAAAACTCAACCAAAACGCAGGTGCGTCAAGCCTAGCA	1573
M_allele	CACGAACGACCAAAATGAAAATAAAAACTCAACCAAAACGCAGGTGCGTCAAGCCTAGCA	1521
B_allele	CACGAACGACCAAAATGAAAATAAAAACTCAACCAAAACGCAGGTGCGTCAAGCCTAGCA	1610
N_allele	CACGAACGACCAAAATGAAAATAAAAACTCAACCAAAACGCAGGTGCGTCAAGCCTAGCA ***********************************	1100
A_allele	TAATTGAAGCGCCAATCATGAATTGTGTGAGGGATGCTTTACTGATGGCCAGCGCGAAAA	1633
M_allele	TAATTGAAGCGCCAATCATGAATTGTGTGAGGGATGCTTTACTGATGGCCAGCGCGAAAA	1581
B_allele	TAATTGAAGCGCCAATCATGAATTGTGTGAGGGATGCTTTACTGATGGCCAGCGCGAAAA	1670
N_allele	TAATTGAAGCGCCAATCATGAATTGTGTGAGGGATGCTTTACTGATGGCCAGCGCGAAAA *************************	1160
A allele	GTCAATCGCTTCCTGACACAAGGACAAACAAGCCGGGGTTGGGTACTCCGGTCAGGGGTA	1693
M allele	GTCAATCGCTTCCTGACAAAGGACAAACAAGC T GGGGTTGGGTACTCCGGTCAGGGGTA	1641
Ballele	GTCAATCGCTTCCTGACAAGGACAAACAAGCCGGGGTTGGGTACTCCGGTCAGGGGTA	1730
N_allele	GTCAATCGCTTCCTGACACAAGGACAAACAAGC T GGGGTTGGGTACTCCGGTCAGGGGTA ********************************	1220
A_allele	ACGGATCCGAGCTGAAGCGACTTATTGCGGAAAGTAATCATCGCTTCTCAACAAATAATG	1753
Mallele	ACGGATCCGAGCTGAAGCGACTTATTGCGGAAAGTAATCATCGCTTCTCAACAAATAATG	1701
Ballele	ACGGATCCGAGCTGAAGCGACTTATTGCGGAAAGTAATCATCGCTTCTCAACAAATAATG	1790
N_allele	ACGGATCCGAGCTGAAGCGACTTATTGCGGAAAGTAATCATCGCTTCTCAACAAATAATG *******************************	1280
A_allele	AAACATTTGATTTAATGGCCGTGGAACCACCTCAGCTAGAGAACTCGTTTAGTGCACTGA	1813
M_allele	AAACATTTGATTTAATGGCCGTGGAACCACCTCAGCTAGAGAACTCGTTTAGTGCACTGA	1761
B_allele	AAACATTTGATTTAATGGCCGTGGAACCACCTCAGCTAGAGAACTCGTTTAGTGCACTGA	1850
N_allele	AAACATTTGATTTAATGGCCGTGGAACCACCTCAGCTAGAGAACTCGTTTAGTGCACTGA	1340
-	* * * * * * * * * * * * * * * * * * * *	

A allele	TCATGGCGGATGAGGTAACAACGGAACTTCCAACTGATTCTCAAATGAGTGTGAACCTAC	1873
Mallele	TCATGGCGGATGAGGTAACAACGGAACTTCCAACTGATTCTCAAATGAGTGTGAACCTAC	1821
B allele	TCATGGCGGATGAGGTAACAACGGAACTTCCAACTGATTCTCAAATGAGTGTGAACCTAC	1910
Nallele	TCATGGCGGATGAGGTAACAACGGAACTTCCAACTGATTCTCAAATGAGTGTGAACCTAC	1400
-	* * * * * * * * * * * * * * * * * * * *	
A_allele	ACCTGCACAAAAGAGTACAGCGACTAGAAAATCTTGTTGGGATGCTAATGCAGCAGATGt	1933
M_allele	ACCTGCACAAAAGAGTACAGCGACTAGAAAATCTTGTTGGGATGCTAATGCAGCAGATGT	1881
B_allele	ACCTGCACAAAAGAGTACAGCGACTAGAAAATCTTGTTGGGATGCTAATGCAGCAGATGT	1970
N_allele	ACCTGCACAAAAGAGTACAGCGACTAGAAAATCTTGTTGGGATGCTAATGCAGCAGATGT *********************************	1460
A_allele	agAGAGTCTAGTGTAGC T AAATACTTAAACATGGAAACAGGAATCACTCGACGCTACGTG	1993
M_allele	AGAGAGTCTAGTGTAGC A AAATACTTAAACATGGAAACAGGAATCACTCGACGCTACGTG	1941
B_allele	AGAGAGTCTAGTGTAGC T AAATACTTAAACATGGAAACAGGAATCACTCGACGCTACGTG	2030
N_allele	AGAGAGTCTAGTGTAGC A AAATACTTAAACATGGAAACAGGAATCACTCGACGCTACGTG ***********************************	1520
A_allele	$\mathtt{TA}^{\mathbf{T}}\mathtt{CAAAAAATATGCAGCGATCGGATTTGCGCAACACAATCTGCTCTCTGGGCT{\mathbf{G}}\mathtt{GCATC}$	2053
M_allele	TACCAAAAAATATGCAGCGATCGGATTTGCGCAACACAATCTGCTCTCTGGGCT A GCATC	2001
B_allele	TA C CAAAAAATATGCAGCGATCGGATTTGCGCAACACAATCTGCTCTCTGGGCT A GCATC	2090
N_allele	TACCAAAAAATATGCAGCGATCGGATTTGCGCAACACAATCTGCTCTCTGGGCTAGCATC ** ************************************	1580
A_allele	AAATTCCAA G GAAAATGTTCCGCCCGATGGTGACTACTTTGACTAAAAAACTTGCTTG	2113
M_allele	AAATTCCAA T GAAAATGTTCCGCCCGATGGTGACTACTTTGACTAAAAAACTTGCTTG	2061
B_allele	AAATTCCAA T GAAAATGTTCCGCCCGATGGTGACTACTTTGACTAAAAAACTTGCTTG	2150
N_allele	AAATTCCAA T GAAAATGTTCCGCCCGATGGTGACTACTTTGACTAAAAAACTTGCTTG	1640
A_allele	CTCCTGTTACAGCAAAATCAGATGCGCGTGAAAATATTTGGTCAGCAAGAGCACGCAAAA	2173
M_allele	CTCCTGTTACAGCAAAATCAGATGCGCGTGAAAATATTTGGTCAGCAAGAGCACGCAAAA	2121
B_allele	CTCCTGTTACAGCAAAATCAGATGCGCGTGAAAATATTTGGTCAGCAAGAGCACGCAAAA	2210
N_allele	CTCCTGTTACAGCAAAATCAGATGCGCGTGAAAATATTTGGTCAGCAAGAGCACGCAAAA *********************	1700
A allele	CTATTTGGTTGCAAGGAATAAAATTTGAGCACGTTC 2209	
M_allele	CTATTTGGTTGCAAGGAATAAAATTTGAGCACGTTC 2157	
B allele	CTATTTGGTTGCAAGGAATAAAATTTGAGCACGTTC 2246	
Nallele	CTATTTGGTTGCAAGGAATAAAATTTGAGCACGTTC 1736	
_	* * * * * * * * * * * * * * * * * * * *	

Supplementary Figure 6. Sequence alignment of the four *MRP3* **alleles.** Alignment of the A, M, B and N *MRP3* alleles. The putative transcription start site is in bold and underlined, in the transcribed portion of the gene nucleotide variants are indicated with colored letters. Asterisks indicate bases identical in all four sequences.



Supplementary Figure 7. *MRP3* genomic landscape. A portion of scaffold 147 is depicted. The genomic regions upstream and downstream of *MRP3*, indicated by a red rectangle, are rich in repetitive sequences (gray arrowheads in the lower track) and no coding genes can be found except for one gene located 4 kb upstream of *MRP3*.



Supplementary Figure 8. Syntenic analysis of the *MRP3* **genomic region.** From the top, schematic representations of *Pseudo-nitzschia multiseries* (Pmse) scaffold 523, *Pseudo-nitzschia multistriata* (Pm) scaffold 147, *Fragilariopsis cylindrus* (Fc) scaffold 13, scaffold 69, scaffold 44 and scaffold 52. Scaffold segments are numbered with starting and ending nucleotide positions. Arrowheads represent genes. Homologous genes share a single color across species. *P. multistriata* gene names are indicated below the arrowheads, only the last five numbers of gene IDs are reported. The *MRP3* gene (ID 0020770) and its homologue in *F. cylindrus* are indicated by a vertical black arrow. No *MRP3* homologs can be found in the *Pseudo-nitzschia multiseries* genome. Synteny can be observed among the three species considered for genes upstream and downstream of *MRP3*, while *MRP3* itself and its neighboring gene 0020760 do not appear to retain synteny.

a

MRP3 A Pm	MNDESNKEWTYYFGRGGACNQQRKQSKVVGLLQKLALRYNQCHREEKRLFAKCEVYNIVL	60
MRP3 M Pm	MNDESNKEWTYYFGRGGACNQQRKQSKVVGLLQKLALRYNQCHREEKRLFAKCEVYNIVL	60
MRP3 B Pm	MNDESNKEWTYYFGRGGACNQQRKQSKVVGLLQKLALRYNQCHREEKRLFAKCEVYNIVL	60
MRP3 N Pm	MNDESNKEWTHYFGRGGACNQQRKQSKVAGLLQKLALCYNQCHREEKRLFAKCEVYNIVL	60

MRP3 A Pm	EKGGSFFEITNKIAVDVTADEMKSTTKIMQVFRDINKRNKKSKGSAKAKKKPSFTSRTKK	120
MRP3 M Pm	EKGGSFFEITNKIAVDVTADEMKSTTKIMQVFRDINKRNKKSKGSAKAKKKPSFTSRTKK	120
MRP3 B Pm	EKGGSFFEITNKIAVDVTADEMKSTTKIMQVFRDINKCNKKSKGSAKAKKKPSFTSRTKK	120
MRP3 N Pm	EKGGSFFEITNKIAVDVTADEMKSTTKIMQVFRDINKRNKKSKGSAKAKKKPSFTSRTKK	120

MRP3 A Pm	NKPSFCTLQTTRARESRTTKMKIKTQPKRRCVKPSIIEAPIMNCVRDALLMASAKSQSLP	180
MRP3 M Pm	NKPSFCTLQTTRARESRTTKMKIKTQPKRRCVKPSIIEAPIMNCVRDALLMASAKSQSLP	180
MRP3 B Pm	NKLSFCTLQTTRARESRTTKMKIKTQPKRRCVKPSIIEAPIMNCVRDALLMASAKSQSLP	180
MRP3 N Pm	NKPSFCTLQTTRARESRTTKMKIKTQPKRRCVKPSIIEAPIMNCVRDALLMASAKSQSLP	180
	** ************************************	
MRP3 A Pm	DTRTNKPGLGTPVRGNGSELKRLIAESNHRFSTNNETFDLMAVEPPQLENSFSALIMADE	240
MRP3 M Pm	DTRTNKLGLGTPVRGNGSELKRLIAESNHRFSTNNETFDLMAVEPPQLENSFSALIMADE	240
MRP3 B Pm	DTRTNKPGLGTPVRGNGSELKRLIAESNHRFSTNNETFDLMAVEPPQLENSFSALIMADE	240
MRP3 N Pm	DTRTNKLGLGTPVRGNGSELKRLIAESNHRFSTNNETFDLMAVEPPQLENSFSALIMADE	240
	***** *********************************	
MRP3 A Pm	VTTELPTDSQMSVNLHLHKRVQRLENLVGMLMQQM 275	
MRP3 M Pm	VTTELPTDSQMSVNLHLHKRVQRLENLVGMLMQQM 275	
MRP3 B Pm	VTTELPTDSQMSVNLHLHKRVQRLENLVGMLMQQM 275	
MRP3 N Pm	VTTELPTDSQMSVNLHLHKRVQRLENLVGMLMQQM 275	

b

CAMNT_0003586753_Pseudo-nitzschia_delicatissima TRINITY_DN7331_c0_g1_i1_Pseudo-nitzschia_arenysensis MRP3_Pseudo-nitzschia_multistriata CAMNT_0008188179_Pseudo-nitzschia_australis CAMNT_0047551615_Pseudo-nitzschia_fraudulenta CAMNT_0013109375_Pseudo-nitzschia_pungens_cingulata CAMNT_0013140303_Pseudo-nitzschia_pungens_pungens jgi_Fracy1|272356_Fragilariopsis_cylindrus CAMNT_0041255159_Fragilariopsis_kerguelensis_L2-C3 CAMNT_0011363179_Fragilariopsis_kerguelensis_L26-C5

CAMNT_0003586753_Pseudo-nitzschia_delicatissima TRINITY_DN7331_c0_g1_i1_Pseudo-nitzschia_arenysensis MRP3_Pseudo-nitzschia_multistriata CAMNT_0008188179_Pseudo-nitzschia_australis CAMNT_0047551615_Pseudo-nitzschia_fraudulenta CAMNT_0013109375_Pseudo-nitzschia_pungens cingulata CAMNT_0013140303_Pseudo-nitzschia_pungens_pungens jgi_Fracy1|272356_Fragilariopsis_cylindrus CAMNT_0041255159_Fragilariopsis_kerguelensis_L2-C3 CAMNT_0011363179_Fragilariopsis_kerguelensis_L26-C5

CAMNT 0003586753 Pseudo-nitzschia delicatissima TRINITY_DN7331_C0_g1_i1_Pseudo-nitzschia_arenysensis MRP3_Pseudo-nitzschia_multistriata CAMNT_0008188179_Pseudo-nitzschia_australis CAMNT_0047551615_Pseudo-nitzschia_fraudulenta CAMNT_0013109375_Pseudo-nitzschia_pungens_cingulata CAMNT_0013109375_Pseudo-nitzschia_pungens_pungens jgi_Fracy1|272356_Fragilariopsis_cylindrus CAMNT_0041255159_Fragilariopsis_kerguelensis_L2-C3 CAMNT_0011363179_Fragilariopsis_kerguelensis_L26-C5

CAMNT 0003586753 Pseudo-nitzschia delicatissima TRINITY_DN7331_c0_g1_i1_Pseudo-nitzschia_arenysensis MRP3_Pseudo-nitzschia_multistriata CAMNT_0008188179_Pseudo-nitzschia_australis CAMNT_0047551615 Pseudo-nitzschia fraudulenta CAMNT_0013109375 Pseudo-nitzschia pungens_cingulata CAMNT_0013140303 Pseudo-nitzschia pungens_pungens jgi_Fracy1|272356_Fragilariopsis_cylindrus CAMNT_0041255159_Fragilariopsis_kerguelensis_L2-C3 CAMNT_0011363179_Fragilariopsis_kerguelensis_L26-C5

CAMNT 0003586753_Pseudo-nitzschia_delicatissima MRP3_Pseudo-nitzschia_multistriata CAMNT_0008188179_Pseudo-nitzschia_australis CAMNT_0047551615_Pseudo-nitzschia_fraudulenta CAMNT_0013109375_Pseudo-nitzschia_pungens_cingulata CAMNT_0013140303_Pseudo-nitzschia_pungens_pungens jgi_Fracy1|272356_Fragilariopsis_cylindrus CAMNT_0041255159_Fragilariopsis_kerguelensis_L2-C3 CAMNT_0011363179_Fragilariopsis_kerguelensis_L26-C5

CAMNT 0003586753 Pseudo-nitzschia_delicatissima TRINITY_DN7331_c0_g1_i1_Pseudo-nitzschia_arenysensis MRP3_Pseudo-nitzschia_multistriata CAMNT_0008188179_Pseudo-nitzschia_australis CAMNT_0047551615_Pseudo-nitzschia_fraudulenta CAMNT_0013109375_Pseudo-nitzschia_pungens_cingulata CAMNT 0013140303 Pseudo-nitzschia pungens pungens jgi Fracy1|272356 Fragilariopsis cylindrus CAMNT 0041255159 Fragilariopsis kerguelensis L2-C3 CAMNT_0011363179_Fragilariopsis_kerguelensis_L26-C5

CAMNT_0003586753_Pseudo-nitzschia_delicatissima TRINITY_DN7331_c0_g1_i1_Pseudo-nitzschia_arenysensis MRP3_Pseudo-nitzschia_multistriata CAMNT_0008188179_Pseudo-nitzschia_australis CAMNT_0047551615_Pseudo-nitzschia_fraudulenta CAMNT_0013109375_Pseudo-nitzschia_pungens_cingulata CAMNT_0013140303_Pseudo-nitzschia_pungens_pungens jgi_Fracy1|272356_Fragilariopsis_cylindrus CAMNT_0041255159_Fragilariopsis_kerguelensis_L2-C3

-----MSETSKSEWDFYFGRGGGSNKKRQVSKANILVHDLAFRYSY--CSQTEKRLFAK ------MSDCSNSEWDFYFGRGGGSNQKRQVSKANSLVQDLAVRYSY--CRQTEKRLFAK 52 ------MNDESNKEWTYYFGRGGACNQQRKQSKVVGLLQKLALRYNQ--CHREEKRLFAK 52 -----HPHQT DE EWN FYF GRGRSRNHQRKS SKV SGLLQN LA PRYRT--CD PGHRRL FAK 52 0 -----MT DTARE QWV FYF GRGGIGNKKRKC SKV SELLQKLA PRY ST--CH PTERRL FAK 52 -----0 _____ 0 MMMMNNSLNNNDEWN FYFGRGAENNKARKT SRANNLIGELAPLYIDPRTRPSDKQTFAK 60 0 NEVYDTVVKNGGTFFLVANKEIIDVTKDFDDTISRIMQSFRDINKSRRATSQTLSTNVEA 112 NEVYDTVVNNGGT FFLVTNKEF IDVTKD FDDTINRIMQSFRD INKICKTTSQPVSTRAEA 112 CEVYN IVLEKGGS FFE ITN KIAVDVTADEMKSTT KIMQVFRD INKRNKKSKGSAKAKKK-111 HEVYNAVLKKGGSFFKLQNNMPVDVTADEFESTTKIMQAFRDIKKQCKLASTQSHLGSSH 112 -----MT KIMOA FRD INKOCR TPPRPS PS-LHP 27 NEVYNTVLNNGGAFFQIKDKLPVNVTANEHHSTTKIMQAFRDINKNGKNVPTHSHVGTHS 112 -----MOA FRD INKNGKNVPTHSHVDTHS 24 _____ 0 OKVYDVVVNNGGRFIEKDKNI----TADKVACLKKIMOGLRDCNKVVNKO-----CK 108 -----MQGLRDCNKVVNKQ-----CK 16 KKVT----------NKSMOST 124 KKVPSQKRSSPILMPT-----RKNMQST 140 131 143 PSSPRKK--RPLSTPGAGKKNSPPKKKAMTSRQSRNDSSME-RPSTMVHDVPSRLNRNHP 84 PSP--PR-----P------KTK---TKA-----P------KRKVQ 129

SLNTKRVPSLKKSSRPKRRCIRPSVIEPPEIDCVRDTLRVIDTEKITPYTTQL---HIG 181 SVKTKRVSLIKKSPRPKRRCIRPSIIEPPVIECVRDALRVVDADKIKQNGKTQI---SVG 197 RARESRTTKMKIKTQPKRRCVKPSIIEAPIMNCVRDALLMASAKSQSLPDTRT----NK 186 STKNPSPTMP--SSPPKPRCVKPSIIEPPFIDCVRDARRMVDVNESIPEISAVSSEMKPS 201 SIKRPSTIMHDVISRPKRRCLKPSIIEPPITECVKEALRVVKMDKIKPRGNSIKAKKVVE 144 ST KGP SPTMR--L SPAKRRCVK PSI IEP PIL DCVRKALRM FNADE SIP DVG SDRAER HCE 187 ST KRP SPTMR--LSPAKRRCVKPSI IEP PIMDCVREALRMFNVDE SIP DVG SDRAERHCE 101 -----MTSALK-----VD 8 SLKRTSSIIP--VRCQKRACVKLPIVEAPIIECVRDALNVAKISGENQADTVN----ID 195 SLKRTSSIIP--VRCQKRACVKLPIVEAPIIECVRDALNVAKISGENQADTVN----ID 103

PS PPP PR-----P-----KTN---TKA-----P-----P-----KRKVQ

PLPPSSP-----CLEIINVP PLPPSSP-----CLEIINVP PLPPSSP-----CLEIINVP

ELNILCDDG--ESRNT SINENGLCQLAADDELEKMVVEKLKLDNFFSQVVMTDEIKARHP 239 EIHTT SCDD--GT SKSKFDERGLCQLAADDA FEKMVVEPPKLDNS FSQLIMTDE IKA---252 XGLGT PVRGNG SELKRLIAESN HRFSTNNET FDLMAVEPPQLENS FSALIMADEVTT ---243 SNHKI PVSGGG SAQKPLVKKDT IRNLST DDMFDTMAVEEPKLESS FSALIMADE ITA---258 RNV-LGGDGGERIEKSFDGKYGHHALTYDDPLARMAVEPPKLNDSFSALIMTDEITA---200 NSNOA SDVTCGOTPL---NKDSTRHFLSNDTFETMIVEPPKLENSFSALIMADE ITA---241 NSNQASDVTCGQTPL---NKDSTRHFSSNDAFETMIVEPPKLENSFSALIMADEITA---155 GENSLSGG----GG---ARQELYGSEDNDVLERMAAEPPKLENSFSALIMADEITA---57 EKKAPSGI----RV---AREEKCDNENKDILEIMALEPPQLENSFSALIMTDEITA---244 EKKAPSGI----RV---AREEKCDNENKDILEIMALEPPQLENSFSALIMTDEITA---152 . .: : * * :*:. ** ::*:*::: OOSLNLHLRVORLENLVAMLMOIKSRLPTDAORSANLNLHSRVORLENLVSMVMORKNDE 299

 	 HLPTDSQQS	VNLNL	ISRVQRLE	NLVAMLMQQKN	DE 287
 	 -ELPTDSQMS	VNLHL	IKRVQRLE	NLVGMLMQQM-	275
 	 -DLPTDSQKS	INQNL	IERVQRLE	NLVGMLMQQQN	VD 293
 	 -VLPSDSQKS	INENLE	ISRIQRLE	NLVAMLMQQKN	DE 235
 	 -DLPTDSQKS	VNQNLE	IKRVQRLE	NLVSMLMQQQN	ED 276
 	 -DLPTDSQKS	VNQNLI	IKRVQRLE	NLVSMLMQQQN	ED 190
 	 -DLPTDSQKN	INQSL	IARVQRLE	NLVAMLMQQKN	ED 92
 	 -DLPTGSLEE	YKQSVI	IARVQRLE	NLVAMLMQQKN	EE 279
 	 -DH	VLI	LARVORLE	NLVTMLMQQKN	EE 177
		:	*:***	*** * ***	

KELKSAARIISNF	312
MELLLK	293
	275
LERLL	298
MELLLK	241
LERLL	281
LERLL	195
LERLL	97
FERLL	284

212

52

43

142

50

0

Supplementary Figure 9. MRP3 protein sequence alignments. A. Alignment of the predicted proteins translated from the four *MRP3* alleles. **B.** Alignment of the predicted proteins translated from the *MRP3* homologues identified in public databases. * indicates positions which have a fully conserved residue, : indicates conservation between groups of strongly similar properties, . indicates conservation between groups of weakly similar properties.



Supplementary Figure 10. *MRP3* **expression in strains** >**SST. A.** PCR amplification of an *MRP3* fragment from the cDNA of two strains >SST (lanes 1-2) and of two strains <SST (lanes 3-4). gDNA is used as a control for the reaction (lane 5). **B.** PCR amplification of an intron-containing fragment of the control gene *CDK-A* from the cDNA of the same strains >SST shown in a. + indicates MT+, - indicates MT-.



Supplementary Figure 11. Sex reversal in the transgenic strain F4B2T7. A. PCR showing integration of full length NAT resistance gene and of the *MRP3* transgene in the genomic DNA of the LV136T3 transformant (lane 2), and of the *Sh-ble* resistance gene and *MRP3* transgene in the genomic DNA of the F4B2T7 transformant (lane 3). Note that the plasmids used for transformation all have the same H4 promoter and the same FcpA terminator, amplification with a primer in the promoter and a primer in the terminator yields a 1.2 kb band for the *MRP3* transgene. **B.** Images of crosses of transformant strain F4B2T7 and of wild-type MT- strain F4B2 with the MT- strain LV113 and the MT+ strain LV94. F4B2T7 cells mated with the MT- strain producing gametes, whereas they did not mate with the MT+ strain. The opposite occurred for the wild type strain F4B2. Scale bar= 20 μm.



LV136 gDNA

b



Supplementary Figure 12. Exogenous expression of MRP3 in the transgenic strain

LV136T3. A. The *MRP3* transcript expressed in the LV136T3 strain (bottom track) has the sequence of the transgene inserted and not of the endogenous gene (top track). The transgene was obtained by cloning the LV149 strain transcript (see Results). Note that LV149 and LV136 have different alleles with two consecutive SNPs (indicated by the two arrows). LV149 has the A/M genotype T**YCG** (see Figure 3B), while LV136 has the B/N genotype T**TYG**. Also note that the sequenced fragment of LV136 gDNA has been amplified with forward primer F147p2 which is outside of the *MRP3* CDS. Therefore, it only amplifies the endogenous locus and not the transgene which is inserted randomly in the genome.

B. IGV visualization of the RNA-seq reads obtained from strains LV136, LV136T3, B938 and B856 mapped to the scaffold 147 region where the *MRP3* gene lies. Note that no reads are present for the MT- strain LV136 as the gene is not expressed, and that the reads present in the transgenic strain LV136T3 correspond to the CDS of the gene only, in contrast to the wild type MT+ strains B938 and B856 in which reads can be found also for the 5' and 3' UTRs. Vertical black arrows indicate the start and end of the transcript in each strain. Gray peaks indicate the number of reads (indicative of the levels of expression), colored vertical bars indicate nucleotides that differ with respect to the reference genome sequence, gray arrowheads are single reads mapped to the genome. A schematic representation of the transcript with its 5' UTR, CDS and 3' UTR in their approximate position in the genome is shown below.



Supplementary Figure 13. *MRP3* in the LV136T3 F1 progeny. A. PCR screen to detect the presence of transgenes in the genomic DNA of the LV136T3 transformant F1 progeny. Amplification with a primer in the H4 promoter and a primer in the FcpA terminator yield a 1.2 kb band for the *MRP3* transgene and a 1 kb band for the NAT transgene. Note that the two plasmids used for transformation have the same promoter and the same terminator. In the top gel, lanes 1-8 contain F1 strains, lane 9-10 contain two wild type strains, lane 11 contains the LV136T3 transformant. As a quality control for the DNA, in the bottom gel the amplification of a control gene, *CDK-A*, is shown for the F1 strains that were negative for the presence of transgenes in the top gel. **B.** *MRP3* expression in the F1 progeny. Top gel, PCR amplification on the cDNAs obtained from the F1 progeny (lanes 1-8), from the LV136T3 transformant (lane 9), from the non-transformed LV136, and from an MT+ strain used as control. Lanes 12-13 contain gDNAs used as control *CDK-A* gene is shown for the same samples.

>TRINITY DN16811 c0 g1 i1 ATTCATCGATCACAAATATTCACGACAAAATCACTTTCAAATCAGAATTCATACAGATTC ACAATAACGAAAATTGAAAACATTCAAAAGGCCATTGAAAGCAAAATCGAGAGCCAAAAAC ATATTGCAAACGATTTTCGTTTATTCATCATGTCACGATACAATCCAAAAGCCACCATCC GTGAAGACGAAGAGTATCTCGATGCCGAGGTGGCTCCAAGAACTCCGGAAAATTCTGTAG TGACGGCCGGAGTCGTCCTATCTTCGCCTTCTACGACCATTGGCCGCGAACAACATCCTG ACCAAACCAATCAAGTCCTCGAATCCCAGTTTGCCAGCAAAGACTTTGATGACGACTTTA ACAAGAAGAAGAAACTTTAGTTTTGACTGGTTCATTTGGACCATGTTGGGAATGACCATTG TGCTCTTGACCTTGGTTGGATATGGATTCGGTTCGGGCCTTTATCTTCCCAACAAGACAA CTGTTGGCGGTGATGCTATTGCCAACTCCAACGATGCCGCAAGTCTTGCTGCGAGTGATG AAGATTACAAGTACAGCATCATGACCCCCTTGGGCTTGCCCATTGTCATGGAAGGTGACT CTGCCCAAGCTCAGGCGATTGATTGGCTGGCCTTTGACGACGAACCCCTCTTTGACCCAA ATGCGATGGAAACCCAGGAAGACCAACAAAAGAGCGATTGACACAGCGCTATGCCTTGG TGGTGTGGTATTTTGACCAGGGTGGCCCAGCCATGTGGACAACCTTGAATCGGGAAGAAT CTGCCGGATGGATTGTGAACGGTGCCGGGGTTCACGAGTGCAACTGGAGGGGAATCGATT GCGACTACAGCGAAATCAGTGATGGGAAAGTCATTGGACTTCGGCTATCGCCTATTGGCG GGCTCTTGTTGACGGGTTCCTCCGTGTCTTCCGAGCTAGGGTTGCTGACAGGCCTCCAAC GCATCGATTTTGCCGATCAGCGCATTCAGGGGGAAGATTCCAAATTCATGGGCCCTCCTGA CCAATCTTGAAACGGTGGTTCTTTCTGACAACAAGTTGCAATCGACGATTCCCGATTGGA TTCCCTCGTCCTTTGCCACTCTCCAAAAATTGGAAAGACTAGAGCTACAACAGAATCCCC AGCTCCGGGGTCCATTTGAAGTATTGTTTTCCCACAACAATATGACTTCCGGCCCTCTAA AGACCCTCGAGCATTTAGATCTTTCCAACACTGATCTGGAGGGTGAGCTCCCCAGTACCA CTCTCCCATCCCTCAAGTTTCTGCGAATGTGGAATCTCAATGGATTGGTAGGGACAATCC CAACACAAATCGGGAGCTGGTCCAACCTGGAATACTTGAGCCTAATGGAAAACCAAAACA TCATGGGGAGCATCCCCACCGAGTTGGGCCTACTGACAAATCTCGAAGCCATCGAGATCC TCGACAGCTTCCTCATGTCTGGAACTCTACCAACCGAGCTGGGCAATCTCAACCTCAAGA GACTCAACGTTCGTTACATAAACCAAACTGGAATCCTTCCCGTTGAGTGGTCCAATATTA GTAGCCTACATGTTTTGGATGTCAGCAACAACCATCTGGAAGGGACGATCCCCCCACAAT ACTCTCAACTCAGCCAGCTCGAGTTCTTTTTTTCTTCCAGTACAACAAGTTGACTGGAGAAG TTCCCGGCGGAGTTTGTTCCCTCGAAAATCGTCAGGAATTTCTCGTTGATTGTGATAAGG GAGTTGGAAGAGGCAATATTACTTGTCTCTGCTGTTCGTGCATTTGATCAGCAATGCTTC TACAAGAGTAAATGCGGGTTCAAACTATAATGATGGAAGAATTAATGATACTAATAGCAC TAAATTTCATATTTCCTCCATCGTTTTAAAATAAAGATAGCAGATTTCTTGAAATCAAAT CGTGGAAATATCAGTT

>TRINITY_DN7331_c0_g1_i1

CACTGCGAACACAACGTCTCATGAGCACGAAAACTGAAATGCAATTTGAAAAGTAATGTT CTAGCTACAATCGAAACGGCATTATTTTCTTCGCTACGTGTGATTAGACTCAATTTAAAG TATGTCAGACTGTTCGAATAGTGAATGGGATTTCTATTTCGGACGGGGTGGTGGCTCGAA CCAGAAGCGACAAGTTTCAAAAGCCAATAGTTTGGTGCAAGATTTGGCTGTTCGTTATAG TTACTGTAGACAAACTGAGAAAAGGCTCTTTGCAAAGAACGAAGTTTACGATACTGTTGT TAACAATGGCGGCACTTTCTTTCTTGTTACCAATAAAGAATTCATTGACGTCACGAAGGA CTTTGACGATACTATAAATAGGATCATGCAAAGCTTTCGAGACATCAATAAAATTTGCAA AACAACATCCCAACCAGTATCAACAAGGGCTGAAGCGAAAAAAGTGCCGTCGCAGAAAAG ATCATCTCCGATTCTAATGCCAACAGCTCGAGACGCGAACAGAAAAAACATGCAATCCAC GAGTGTCAAAAACCAAAAGAGTGTCATTAATCAAGAAATCACCTCGTCCAAAGCGTAGATG TATACGGCCCAGTATAATCGAACCGCCTGTTATTGAATGCGTGAGGGATGCCCTCAGAGT TGTCGACGCCGACAAAATAAAGCAAAATGGGAAAACACAGATCAGTGTCGGGGGAGATACA TACTACTTCATGCGATGATGGAACATCCAAGAGCAAGTTCGATGAAAGGGGTCTTTGTCA ACTGGCAGCAGATGATGCATTTGAGAAAATGGTCGTGGAACCACCAAAGCTAGATAATTC TTTCAGTCAATTGATCATGACGGATGAAATCAAAGCGCATCTTCCAACTGACTCACAACA GTCGGTCAATCTCAATCTTCATTCAAGAGTACAACGTCTAGAGAACCTGGTAGCGATGCT AATGCAACAAAAGAACGATGAAATGGAACTACTGTTAAAATAGGTTCTTTTCTTTGCTA ATCTGAGGAAGATTGTGTGTGTGTGGCTGTGTTGATACTGGTGCTGGTGGTACAAGTAGCA GTTTTGTTGCCATTCTTCAGTTTACGCTATGTCTATCGTTTAAATCGCGCCGCGTTTTTC AAGACCCGGGCATGCATCATTCAGTCGACATATCATTTCTGAGGTATTTGAAACAGAGAC ATGAGGATTTGGGTTAGTAAATGTCGTCTAACGGGCAGACAAATTTGGAGCGATGCTTAA GGATTCTAATCACAATAGAGTACAATCGCATTTAGCAAAGATTTATATCATCGCAAAAAA AAAAA

Supplementary Figure 14. *MRP2* and *MRP3* nucleotide sequences in *Pseudo-nitzschia* arenysensis.

Supplementary Note 1: Uncropped gel images



Uncropped gel Fig. 2b



Uncropped gel Fig. 2c-d







Uncropped gels Fig. 4a



Uncropped gel Fig. 4c



Uncropped gels Fig. 4d

Supplementary References

- 1. Basu, S. *et al.* Finding a partner in the ocean: molecular and evolutionary bases of the response to sexual cues in a planktonic diatom. *New Phytol.* **215**, 140–156 (2017).
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