

Table S1. Forward and reverse primers used in PCR amplification of 11 regions of the 60-kb segment

Primer name	Region amplified (see Fig. 1--2)	Primer sequence
Naeg1458F	1458-2384	AAC TGT GAC TTC TTG CGC TTC
Naeg2384R	1458-2384	TCT TCC GTG GTA ATT CTA CCG
Naeg2329F	2329-3459	CAT CCA TTA ATT GAT AAT TGC AAT GAC
Naeg3459R	2329-3459	TGA AAG TAA TTC CTC GAG TTG TTC
Naeg15278F	15278-16455	TTA TGC ATG AGT TGA AAG TGA GCC
Naeg16455R	15278-16455	GGG TTG GGA TCT AAA TAG GC
Naeg16411F	16411-17445	GAC CAC TCT TGA GGA TTG AGC
Naeg17445R	16411-17445	GAC ATC ATC ACC ACG AAA CGG
Naeg25036F	25036-26108	AGA AGC GAG TTA AAT GTT TCC G
Naeg26108R	25036-26108	GCT CAT TGG TTT GGG TTT ACC
Naeg28285F	28285-29371	TGG TCA CAA GAT CTC TCG TGG
Naeg29371R	28285-29371	GTC AAG GAA CGA ACG TTT CC
Naeg35101F	35101-36316	AGC TGT CAA TGC GAG TTT CC
Naeg36316R	35101-36316	CTA CGA CAT TAT TCT TAC TGA TCC
Naeg39275F	39275-40281	TAT CTC GCA ACT CTT TCA ACA G
Naeg40281R	39275-40281	GAT AAA TCC ATG ACA ATT GTT ATA ACC
Naeg42890F	42890-43997	CGA GTA ATG AGC AAT CGC C
Naeg43997R	42890-43997	TGT ATC AAA ATC AAT ATC TCC TCC
Naeg47294F	47294-48323	ATA CAG TGG AAT CAT AGA GTC C
Naeg48323R	47294-48323	CAA AAT ATT GAC AGA CTG AAA ATG C
Naeg52899F	52899-53976	GGA AGA AGA TGT TGT TTA ATG ATG G
Naeg53976R	52899-53976	ACT TGA CAA GAA TTT GAG TTG CC

Table S2. *N. fowleri* mitochondrial genome annotation based on BLAST searching and RNA scanning software

Annotation	Gene name	<i>N. gruberi</i> homologue ¹	Start (<i>fowleri/gruberi</i>)	End (<i>fowleri/gruberi</i>)	<i>N. fowleri</i> length (bp)	<i>N. gruberi</i> length (bp)	<i>Nf/Ng</i> % length	E-value ²	Identities (%) ²	Cove	Score (rRNA)
Rnl	Large subunit rRNA	NC_002573	1/1	2694/2573	2694	2573	121	N/A	85	N/A	741.8
Asn tRNA	Asparagine tRNA	NC_002573	2710/2698	2781/2769	72	71	101	N/A	100	74.86	N/A
Nad1	NADH dehydrogenase subunit 1	NP_066498	2810/2802	3787/3785	978	984	99	0	85	N/A	N/A
Rps7	Ribosomal protein S7	NP_066499	3788/3796	4942/5019	1155	1224	94	1.00E-84	47	N/A	N/A
Phe tRNA	Phenylalanine tRNA	NC_002573	5093/5116	5163/5186	71	71	100	N/A	99	56.34	N/A
ATP1	ATP synthase F1 subunit alpha	NP_066500	5250/5259	6902/6911	1653	1653	100	0.00E+00	94	N/A	N/A
Orf312	Orf312	NP_066501	6963/6973	7697/7911	735	939	78	2.00E-08	60	N/A	N/A
His tRNA	Histidine tRNA	NC_002573	7713/7936	7783/8006	71	71	100	N/A	90	62.61	N/A
Nad4L	NADH dehydrogenase subunit 4L	NP_066502	7811/8038	8071/8301	261	264	99	6.00E-51	92	N/A	N/A
Met tRNA	Methionine tRNA	NC_002573	8090/8319	8162/8390	73	72	101	N/A	93	67.24	N/A
Phe tRNA	Phenylalanine	NC_002573	8182/8406	8252/8476	71	71	100	N/A	100	58.04	N/A

	tRNA										
Orf164	Orf 164	NP_066503	8414/8637	8899/9131	486	495	98	2.00E -27	37	N/A	N/A
Rps12	Ribosomal protein S12	NP_066504	8902/9135	9288/9521	387	387	100	5.00E -75	88	N/A	N/A
Gln tRNA	Glutamine tRNA	NC_002573	9289/9537	9359/9607	71	71	100	N/A	89	75.47	N/A
Cox1	Cytochrome c oxidase subunit 1	NP_066505	9612/9939	11513/11840	1902	1902	100	0.00E +00	93	N/A	N/A
Cox11	Haem biosynthesis protein	NP_066506	11612/11968	12286/12636	675	669	101	6.00E -74	52	N/A	N/A
Sdh2	Succinate:cytochrome c oxidoreductase	NP_066507	12290/12672	13120/13484	831	813	102	1.00E -146	74	N/A	N/A
Nad11	NADH dehydrogenase subunit 11	NP_066508	13223/13573	15295/15660	2073	2088	99	0	63	N/A	N/A
Cox3	Cytochrome c oxidase subunit 3	NP_066509	15390/15739	16283/16632	894	894	100	0	87	N/A	N/A
Trp tRNA	Tryptophan tRNA	NC_002573	16282/16648	16352/16718	71	71	100	N/A	100	72.62	N/A
Pro tRNA	Proline tRNA	NC_002573	16407/16754	16479/16826	73	73	100	N/A	95	63.59	N/A
Nad8	NADH dehydrogenase subunit 8	NP_066510	16513/16863	16992/17342	480	480	100	3.00E -110	91	N/A	N/A

Nad5	NADH dehydrogenase subunit 5	NP_066511	17004/17351	19007/19351	2004	2001	100	0	75	N/A	N/A
Cob	Apocytochrome b	NP_066512	19074/19432	20525/20925	1452	1494	97	0	93	N/A	N/A
ATP3	ATP synthase F1 subunit gamma	NP_066513	21018/20997	21881/21863	864	867	100	1.00E-171	79	N/A	N/A
Orf145	Orf 145	NP_066514	21947/21927	22405/22364	459	438	105	3.00E-36	69	N/A	N/A
Nad6	NADH dehydrogenase subunit 6	NP_066515	22454/22416	23059/23021	606	606	100	9.00E-98	75	N/A	N/A
Rps2	Ribosomal protein S2	NP_066516	23059/23021	23847/23815	789	795	99	3.00E-119	66	N/A	N/A
ATP8	ATP synthase F0 subunit 8	NP_066517	23923/23888	24255/24220	333	333	100	2.00E-39	78	N/A	N/A
Rps10	Ribosomal protein S10	NP_066518	<24670/24233	24912/24895	243	663	37	2.00E-26	63	N/A	N/A
Rpl11	Ribosomal protein L11	NP_066519	24909/24900	25346/25346	438	447	98	4.00E-55	56	N/A	N/A
Rpl2	Ribosomal protein L2	NP_066520	25386/25370	26186/26176	801	807	99	8.00E-133	69	N/A	N/A
Rps19	Ribosomal protein S19	NP_066521	26188/26176	26451/26442	264	267	99	6.00E-17	43	N/A	N/A
Rps3	Ribosomal protein S3	NP_066522	26457/26443	28004/28014	1548	1572	98	1.00E-150	53	N/A	N/A
Rpl16	Ribosomal	NP_066523	28006/28014	28431/28439	426	426	100	2.00E	75	N/A	N/A

	protein L16							-73			
Rpl14	Ribosomal protein L14	NP_066524	28440/28445	28811/28816	372	372	100	2.00E-65	82	N/A	N/A
Rpl5	Ribosomal protein L5	NP_066525	28822/28817	29397/29395	576	579	99	3.00E-73	63	N/A	N/A
Rps14	Ribosomal protein S14	NP_066526	29397/29395	29693/29691	297	297	100	7.00E-31	56	N/A	N/A
Rps8	Ribosomal protein S8	NP_066527	29698/29704	30108/30129	411	426	96	1.00E-43	50	N/A	N/A
Rpl6	Ribosomal protein L6	NP_066528	30118/30134	30603/30634	486	501	94	4.00E-54	57	N/A	N/A
Rps11	Ribosomal protein S11	NP_066529	30604/30636	32274/32417	1671	1782	94	3.00E-150	46	N/A	N/A
Rps13	Ribosomal protein S13	NP_066530	32264/32380	32728/32871	465	492	95	1.00E-56	65	N/A	N/A
Nad4	NADH dehydrogenase subunit 4	NP_066531	32748/32889	34136/34313	1389	1425	97	0	77	N/A	N/A
YejU	ABC transporter subunit	NP_066532	34137/34318	34784/34950	648	633	102	5.00E-10	45	N/A	N/A
Nad2	NADH dehydrogenase subunit 2	NP_066533	34785/34969	36263/36447	1479	1479	100	9.00E-178	60	N/A	N/A
Ser tRNA	Serine tRNA	NC_002573	36303/36559	36377/36633	75	75	100	N/A	93	42.59	N/A
ATP6	ATP synthase FO subunit 6	NP_066534	36420/36666	37163/37418	744	753	99	5.00E-131	80	N/A	N/A

Nad3	NADH dehydrogenase subunit 3	NP_066535	37187/37443	37549/37805	363	323	100	7.00E-73	87	N/A	N/A
Cox2	Cytochrome c oxidase subunit 2	NP_066536	37689/37967	38546/38812	858	846	101	0	89	N/A	N/A
Orf504	Orf 504	NP_066537	38636/38895	40219/40409	1584	1515	105	9.00E-177	50	N/A	N/A
Ser tRNA	Serine tRNA	NC_002573	40243/40428	40316/40501	74	74	100	N/A	86	64.91	N/A
Nad7	NADH dehydrogenase subunit 7	NP_066538	40370/40546	41557/41733	1188	1188	100	0	91	N/A	N/A
Ile tRNA	Isoleucine tRNA	NC_002573	41557/41981	41629/42053	73	73	100	N/A	89	76.42	N/A
yejR	Haem lyase	NP_066539	41636/42054	43024/43478	1389	1425	97	3.00E-46	41	N/A	N/A
Tyr tRNA	Tyrosine tRNA	NC_002573	43056/43506	43138/43588	83	83	100	N/A	90	73.27	N/A
Ymf16	SecY-independent transporter protein	NP_066540	43404/43856	44192/44650	789	795	99	4.00E-24	39	N/A	N/A
Ser tRNA	Serine tRNA	NC_002573	44210/44662	44283/44736	74	75	99	N/A	91	55.66	N/A
Leu tRNA	Leucine tRNA	NC_002573	44289/44740	44370/44821	82	82	100	N/A	87	57.85	N/A
Asp tRNA	Aspartic acid tRNA	NC_002573	44374/44824	44447/44896	74	73	101	N/A	96	74.55	N/A
Leu tRNA	Leucine tRNA	NC_002573	44555/44910	44535/44992	81	83	98	N/A	95	61.14	N/A
Rps4	Ribosomal	NP_066541	44566/45022	45897/46470	1332	1449	92	1.00E	45	N/A	N/A

	protein S4							-78			
Met tRNA	Methionine tRNA	NC_002573	45905/46495	45976/46566	72	72	100	N/A	93	69.23	N/A
ATP9	ATP synthase F0 subunit 9	NP_066542	46047/46636	46265/46854	219	219	100	4.00E-45	99	N/A	N/A
Lys tRNA	Lysine tRNA	NC_002573	46288/46947	46360/47019	73	73	100	N/A	90	82.19	N/A
Met tRNA	Methionine tRNA	NC_002573	46367/47022	46438/47093	72	72	100	N/A	89	71.01	N/A
Arg tRNA	Arginine tRNA	NC_002573	46970/47161	47042/47233	73	73	100	N/A	96	66.09	N/A
Nad9	NADH dehydrogenase subunit 9	NP_066543	47070/47267	47627/47827	588	561	105	5.00E-103	75	N/A	N/A
Glu tRNA	Glutamic acid tRNA	NC_002573	47662/47858	47734/47930	73	73	100	N/A	93	54.27	N/A
Rns	Small subunit rRNA	NC_002573	47889/48138	49451/49716	1563	1579	99	N/A	87	N/A	589.4

¹The accession given for homologues of RNAs is that of the entire *N. gruberi* mitochondrial genome (NC_002573). Percent identity was calculated with respect to the sequence feature with a corresponding annotation. For protein-coding genes, the accession numbers of the *N. gruberi* homologues are given.

²In relation to the *N. gruberi* orthologue.

Table S3. Annotation of *N. fowleri* 60-kb segment based on BLAST results

Possible Gene	Annotation	Length (aa)	<i>N. gruberi</i> hit	% length of <i>Ng</i> hit	E-value	% Identity	Notes
Contig9_1_165_659	putative	145	N/A	N/A	N/A	N/A	Not supported by Ng/NR BLASTs
Contig9_2_1673_2002	putative	110	N/A	N/A	N/A	N/A	Not supported by Ng/NR BLASTs
Contig9_3_2113_2703	putative	197	N/A	N/A	N/A	N/A	Contains reverse transcriptase domain
Contig9_4_3139_3870	Naegleria-specific	244	XP_002674212	44	2.00E-20	47	Contains RNase H superfamily domain
Contig9_5_6233_4411	Inositol 1,4,5-trisphosphate receptor	606	XP_002675052	22	0	64	Missing most of protein, frameshift, 1 intron
Contig9_6_7742_9509	Unknown	589	XP_002675053	92	4.00E-71	36	Contains LRRs, one intron
Contig9_7_10042_11291	Naegleria-specific	417	XP_002680747	77	1.00E-118	56	Three introns
Contig9_8_12703_14563	Unknown	620	XP_002674978	90	6.00E-157	59	Two introns, contains Vps9 domain
Contig9_9_18345_19483	Naegleria-specific	1138	XP_002675151	79	1.00E-53	27	One intron
Contig9_10_17987_15908	possible cytosolic	693	XP_002675072	113	0	75	None

	carboxypeptidase 6						
Contig9_11_22176_22842	putative	666	N/A	N/A	N/A	N/A	Not supported by Ng/NR BLASTs
Contig9_12_23291_24589	Naegleria-specific	433	XP_002675150	96	3.00E-48	43	None
Contig9_13_24415_24118	putative	100	N/A	N/A	N/A	N/A	Not supported by Ng/NR BLASTs
Contig9_14_26791_27213	putative	141	N/A	N/A	N/A	N/A	Not supported by Ng/NR BLASTs
Contig9_15_27582_29939	Naegleria-specific	734	XP_002675145	92	3.00E-46	35	Four introns
Contig9_16_26041_24938	Cathepsin B	258	XP_002678773	114	2.00E-56	53	Four introns
Contig9_17_30413_30770	putative	136	N/A	N/A	N/A	N/A	Not supported by Ng/NR BLASTs
Contig9_18_37776_33830	Naegleria-specific	1313	XP_002679077	115	0	41	3 introns, Possible ser/thr kinase
Contig9_19_32550_28611	putative	346	N/A	N/A	N/A	N/A	Not supported by Ng/NR BLASTs
Contig9_20_31356_30170	Naegleria-specific	392	XP_002682536	109	2.00E-17	42	None
Contig9_21_37014_37437	putative	141	N/A	N/A	N/A	N/A	Not supported by Ng/NR BLASTs

Contig9_23_40599_40961	unknown	121	N/A	N/A	N/A	N/A	None
Contig9_24_43494_44097	unknown	201	5453 ¹	69	2.50E-60	84	Contains Erv1/Alr domain
Contig9_25_42800_42445	putative	118	N/A	N/A	N/A	N/A	Not supported by Ng/NR BLASTs
Contig9_26_51515_44333	ATM (ataxia telangiectasia mutated)	2394	XP_002682330	109	0	45	Ser/thr kinase; cell cycle checkpoint kinase
Contig9_27_53454_54366	Hsp40	304	XP_002682244	100	9.00E-89	64	DNAJ and RRM domains
Contig9_28_53074_52273	Naegleria-specific	266	XP_002682329	93	5.00E-75	58	Two introns
Contig9_30_59348_60465	Naegleria-specific	358	XP_002675601	116	3.00E-13	25	low similarity
Contig9_31_60061_59660	putative	133	N/A	N/A	N/A	N/A	Not supported by Ng/NR BLASTs
Contig9_32_58903_56581	ADAP	774	XP_002675599	98	0	67	1 intron, ArfGAP domain, two PH domains
Contig9_33_55908_55399	unknown	136	46060 ¹	65	1.20E-44	72	PUB domain

¹ Homologue identified in the Joint Genome Institute *N. gruberi* database only.