Supplementary figures

to the paper "Characterization of the N-ATPase, a distinct, laterally transferred Na^+-translocating form of the bacterial F-type membrane ATPase" by D. V. Dibrova, M. Y. Galperin, and A. Y. Mulkidjanian, published in *Bioinformatics* 26(12):1473-1476 (2010). Abstract. Full text (HTML). Full text (PDF).

A.

Cyanobacteria

N-ATPase c-subunits

```
B1XRK4_SYNP2   1 MSDLALIGSVAMVTAGITIAIGSIGPALGEGMAVARALGAIAQQPDKANMITRTLFVGLAMVESTAIYCLVVSMILLFVNPFWNYFLNQGG---- 91
A8ZNS2_ACAM1   1 MDNIALVGMASIVISGLTIAIGSIGPALGEGRALSQALSALAQQPDEANTITRVLFVGMALVESTAIYCFVITLILIFANPFWTYVVEQASTNGG 95
B1WXB3_CYAA5   1 MDNLGLTGIVSIITAGLTIAIGSVAPAIGEGLALANALRALAQQPDKANTITRTLFVGLALVESTAIYCFVVSMILIFANPFWNYFVQNAGG--- 92
A0ZFR6_NODSP   3 IDNLGLIGMVSIIMAGLTIAIGSIGPAIAEGWAVARALGAMAQQPDQANTITRTLFVGLAIIESTAIYCFVVSMILIFANPFWDYFTSS------ 91
B4WTA0_9SYNE   1 MDNVVLIGIASIVMGGLTIAVGSIAPALGEGRALAQALTALAQQPDEANTITRTLFVGMALVESTAIYCFVITLILLFANPFWAYVTATAGG--- 92
                                            *                                   ***  *
```

F-ATPase c-subunits

```
ATPL_SYNP2     1   MDSLTAAASVIAAALAVGLAAIGPGIGQQGNAAGSAAEGIARQPEAEGKIRGTLLLSLAFMEALTIYGLVVALVLLFANPFA------------ 81
B0BZK8_ACAM1   1   MDPLVAAASVLAAALGIGISTIGPALAQGNAAGKAVEGIARQPEAEGKIRGTLLLCLAFMESLTIYGLVIALVLLFANPFA------------ 81
B1WUH8_CYAA5   1   MNPTVAAASVIAAALAVGLGSIGPGLGQGNASGQAVSGIARQPEAEGKIRGTLLLTLAFMESLTIYGLVIALVLLFANPFA------------ 81
A0ZEZ9_NODSP   1   MDPLVAAASVLAAALAVGLAAIGPGIGQGNAAGQAVEGIARQPEAEGKIRGTLLLSLAFMEALTIYGLVVALVLLFANPFA------------ 81
B4WI82_9SYNE   1   MNPTIASASVIAAALAIGLAAIGPGIGQGNAAGQAVEGIARQPEAEGKIRGTLLLSLAFMEALTIYGLVVALVLLFANPFA------------ 81
                     <--------------MEMBRANE--------------> CYTOPLASM <----------MEMBRANE---------->
```

B.

N-ATPase c-subunits

Archaea

```
Q8TN57_METAC   4 DTYTTIIAVASIATSGLTIGIGVLGPAIGEGRAVATALSSLAQQPDASATITRTLFVGLAMIESLSIYCFVVSMILIFANPFWNTATV------- 91
Q467F4_METBF   4 DTYITTIAVASIATAGITIGIGVIGPAIGEGRAVATALSSLAQQPDASATITRTLFVGLAMIESLAIYCFVVSMILIFANPFWNRALT------- 91
```

Aquificae

```
C0QS18_PERMH   1 MDSLTIIAAVSIFTAGLTIAIGGMMPSRGEGEALTKAIESVARQPEQANNIIRLFFIGAAVVESVAIYSLVIALIILFANPFIHLFTK------- 88
```

Chlorobi

```
Q3B401_PELLD   1 MDILTIVAAVSIFTAGITIAVGSIGPALGEGRAVASALEALAQQPDASSSITRTLFVGLAMIESVAIYCFVTSMILIFANPFWTMALSKAGGH-- 93
B3QNH4_CHLP8   1 MDTLTIIAIVSIATAGLSIGIGVIGPALGQGRAVSSALTALAQQPDAASTITRTLFVGLAMIESIAIYCFVISIILIFANPFWNHAIAQVGGH-- 93
```

Planctomycetes

```
Q7UH07_RHOBA   1 MDSTTSIAVASIIMAGLTTAIGSIGPAFAEGRAVAQALNSIAQQPDSSNTITRTLFVGLAMIESTAIYCFVVSMILLFANPFWNQLTQ------- 88
A6C217_9PLAN   1 MDSNTVIAAVSIFTAGITIAIGSIGPALGEGRALAQALSAIAQQPDEASTITRTLFVGLAMVESTAIYCFVISMILIFANPFWNHFMQAATR--- 92
Q1Q5S1_9BACT   1 MDNVGLIGMVSIIVAGFTIAVGSIGPALGEARAAAQALSSIAQQPDEANTITRTLFVSMAMIESTAIYCFVVAMIVIFANPFWNYVITKAGGQ-- 93
```

Proteobacteria

```
B7RJ30_9RHOB   1 MTDLAIIAAISILTAGLTVCFGAIGPALGEGRTASAALTAIAQQPDAASSISRTLFVSLAMIESTAIYCFVVAMILLFANPFWNAAIAAAKATGS 95
D0CPG0_9RHOB   1 MTDLAIIAAVSIFTAGLTVSFGAIGPALGEGRAAAQALASIAQQPDAAPTLSRTLFVSLAMIESTAIYCFVVAMILIFANPFWDTALELVRTSGQ 95
C6BWK9_DESAD   1 METLGWIAFGSIIAAGLCMGIGAIGPAIGEGMALSRALSSIAQQPDETNTIVKFLFVGMAMVESTAIYCFVLAMILLFANPFWSYFLEKAGG--- 92
A3IYM7_9CHRO   1 MDNLGLTGIVSIITAGLTIAIGSVAPAIGEGLALANALRALAQQPDKANTITRTLFVGLALVESTAIYCFVVSMILIFANPFWNYFVQNAGG--- 92
B4S799_PROA2   3 TTIIAVVAVASIVTAGLTTAIGCIGPALGEGRAVSSALTSLAQQPDAAATITRTLFIGLAMVESVAIYCFVISMILIFANPFWNQVIVQAGGK-- 95
A8LN44_DINSH   1 MTDLAIIAAVSIFTAGLTIAIGAIGPALGEGRAASTALSAIAQQPDAASTLSRTLFVSLAMIESTAIYCFVVAMILIFANPFWEAALEAATVGAG 95
Q0AEJ1_NITEC   1 MTDLALIAAISIFTAGLTIAIGSLGPALGEGRAAAAAIAAIAQQPDAAPTLSRTLFVSLAMIESTAIYCFVVAMILIFANPFWNAMQAVAGG--- 92
Q62EB2_BURMA   1 --MNNLIEVVSIAAAALAVSFGAIGPALAEGRAVGAAMDAIARQPDASGTVSRTLFVGLAMIETMAIYCLVVALLLLFANPFVK----------- 82
Q63IW8_BURPS   1 --MNNLIEVVSIAAAALAVSFGAIGPALAEGRAVGAAMDAIARQPDASGTVSRTLFVGLAMIETMAIYCLVVALLLLFANPFVK----------- 82
Q3A078_PELCD   1 MDYLAWVAVASILSAGLCIGIGAIGPALGEGRALAQALAALAQQPDEANTITRTLFVGLAMVESTAIYCFVVSMILIFANPFWRFFLVRAGEGG- 94
Q1K2D6_DESAC   1 MEAQTWIAMISIFTAGITIAIGSIGSALGEGRAVASALTSLAQQPDSASTITRTLFVGLAMVESTAIYCFVVSMIVLFANPFWNHFLEAAGG--- 92
C7LQ36_DESBD   1 MDSMTIIAVASIIIAGITTGFGTMGPALAEGKAVATALTSLAQQPDASATITRTLFVGLAMIESTAIYCFVVSMILIFANPFWNYAIAQMAGK-- 93
B3QG15_RHOPT   1 ---MNWLAFISIVSAALAVSFGAIGPALAEGRAVAAAMDAIARQPEAANTISRTLFVGLAMIETMAIYCLVIALLLLFANPLLK----------- 81
Q2Y8G7_NITMU   1 MDSITLIAIISILTAGLTISIGVIGPALGEGKAVATALTSLAQQPDVAGTIARTLFVGLAIIESLAIYCFVVSMILIFANPFWDHVIAHGVGK-- 93
Q2LY41_SYNAS   1 MDSMTLIAMVSIVTAGLCMAVGSIGPALGEGNAVKQALTAIAQQPDERNSITRTLFVGLAMIESIAIYCFVISMILIFANPFWSHAIARAGG--- 92
A1SS60_PSYIN   1 MDSMTYIAMISIIIAGLTTGFGTMGPALAEGRAVAAAMASLAQQPDASSTITRTLFVGLAMIESTAIYCFVVSMIILFANPFWDFAVTQAAGK-- 93
A1VPR0_POLNA   1 MDSMTIIAVASIVIAGLTTGFGCMGPAFAEGRAVATALTALAQQPDASATITRTLFVGLAMIESTAIYCFVVSMILIFANPFWNFAIAQAAGK-- 93
Q21ZA1_RHOFD   1 MDSLTIIAVASIVIAGITTGFGCMGPALAEGRAVATALTALSQQPDASATITRTLFVGLAMIESTAIYCFVVSMILIFANPFWNHVIAQTAGK-- 93
A6VWQ4_MARMS   1 MDSMTMIAMVSIITAGLTIAIGVLGPSLGEGKAVATALTSLAQQPDASATITRTLFVGLAMIESTAIYCFVVSMILLFANPFWNYGIAQAAGQ-- 93
Q07YM2_SHEFN   1 MDSITIIAMVSIITAGFTITIGVIGPSLGEGKAVATALSSLAQQPDASATITRTLFVGLAMIESTAIYCFVVTMILLFANPFWNYVIDQAAGI-- 93
A3JAV4_9ALTE   1 MTDLAIIAAISIFTAGFTIAIGCIGPSLAEGRAAAAAIAAIAQQPDAAPTLSRTLFVSLAMIESTAIYCFVVAMILIFANPFWDAAVQAATVTAG 95
C1DEJ6_AZOVD   1 --MDNPIEIVSILGAALAVSFGALGPALAEGRAVAAAMDAIARQPEAAGTLSRTLFVGLAMIETMAIYCLVVAVLLLFANPFVH----------- 82
                     <--------------MEMBRANE--------------> CYTOPLASM <----------MEMBRANE---------->
```

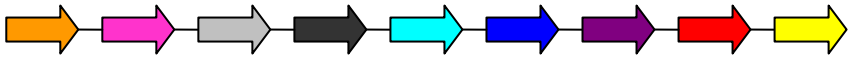
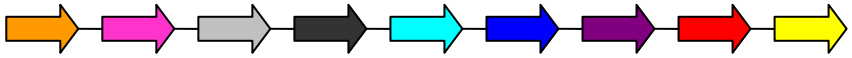
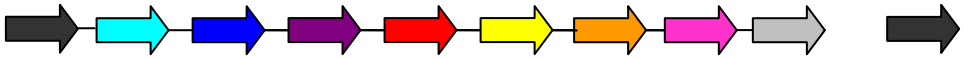
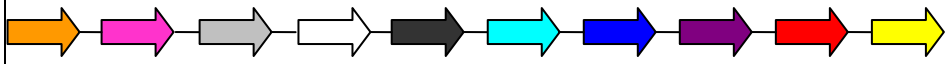
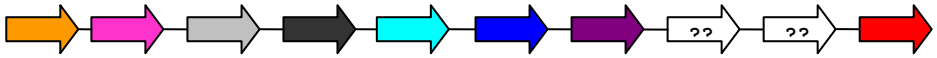
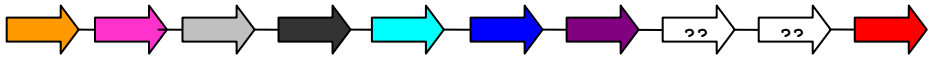
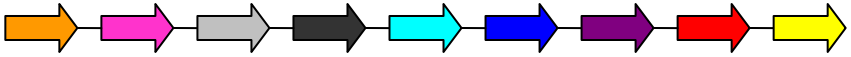
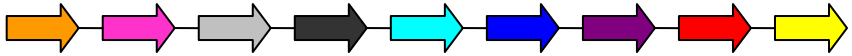
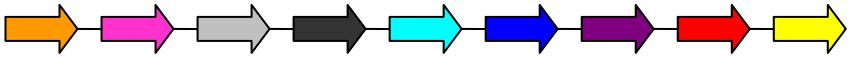
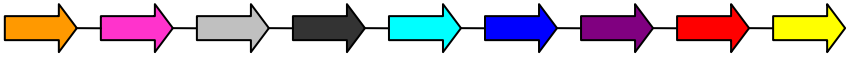
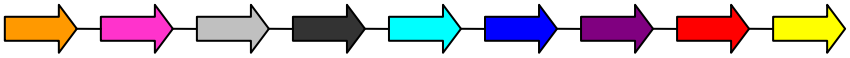
F-ATPase c-subunits

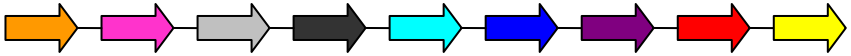
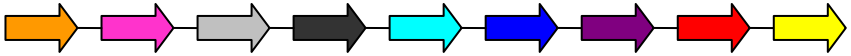
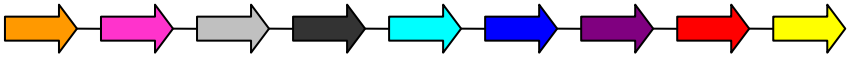
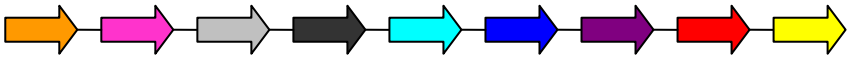
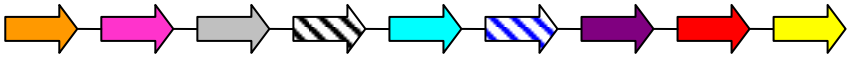
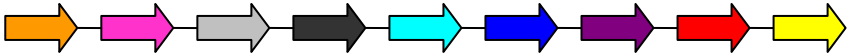
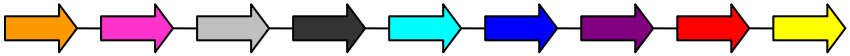
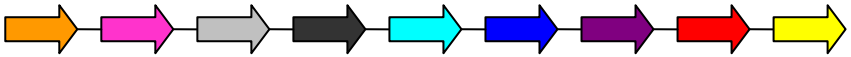
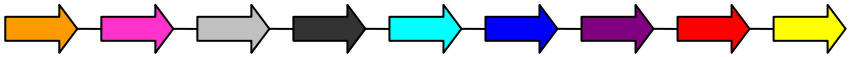
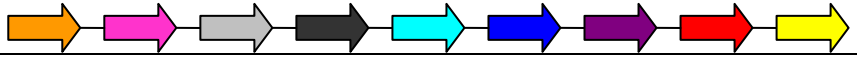
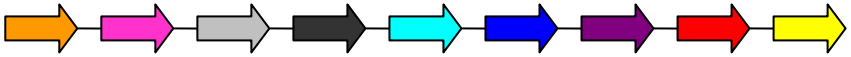
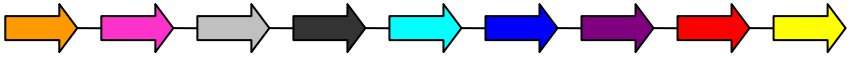
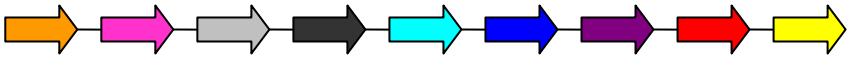
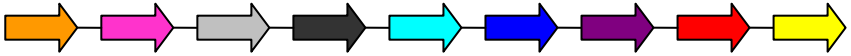
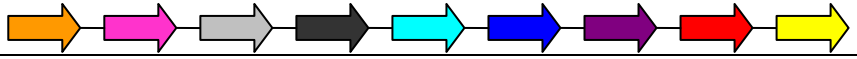
```
ATPL_ILYTA     3 MLFAKTVVLAASAVGAGTAMIAGIGPGVGQGYAAGKAVESVARQPEAKGDIISTMVLGQAVAESTGIYSLVIALILLYANPFVGLLG-------- 89
ATPL_PROMO     3 MVLAKTVVLAASAVGAGAAMIAGIGPGVGQGYAAGKAVESVARQPEAKGDIISTMVLGQAIAESTGIYSLVIALILLYANPFVGLLG-------- 89
                                            *                                   ***  *
Q223D1_RHOFD   1 ---MENILGLVALACGIIVGLGALGASIGIALMGGKFLESSARQPELMNELQTKMFILAGLIDAAFLIGVAIALLFAFAKPFAI----------- 81
A6W3T3_MARMS   1 ---METVVGLTAIAVALLIGLGALGTAIGFGILGGKFLEGAARQPEMVPMLQVKMFIVAGLLDAVTMIGVGIALFFTFANPFVALVAG------- 85
Q07VT9_SHEFN   1 ---METVLGMTAIAVALLIGMGALGTAIGFGILGGKFLEGAARQPEMAPMLQVKMFIVAGLLDAVTMIGVGIALFMLFTNPLGAML--------- 83
A3JGY5_9ALTE   1 ---------MTAIAVALLIGLGALGTAIGFGILGGKFLEGAARQPEMIPMLQVKMFIVAGLLDAVTMIGVGIALFFTFANPFVGQLAG------- 79
C1DND8_AZOVD   1 -------MELVIIAASIMIGLGALSTGIGFALLGGKLLESTARQPELAPQLQTKTFLMAGLLDAVPMIGVGIAMYLIFVVAPSAA---------- 78
```

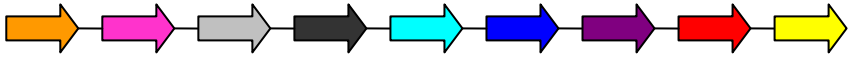
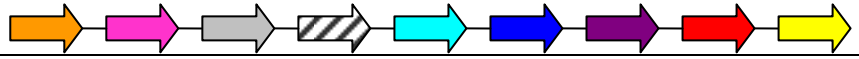
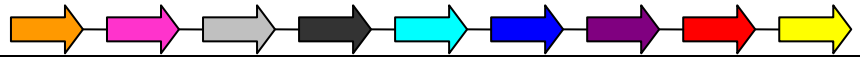
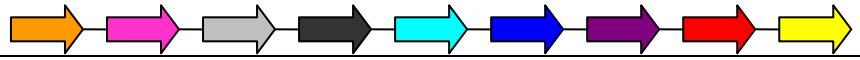
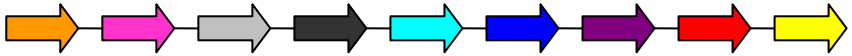
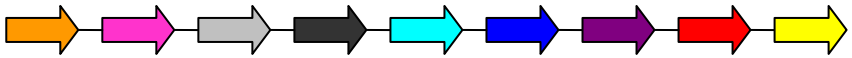
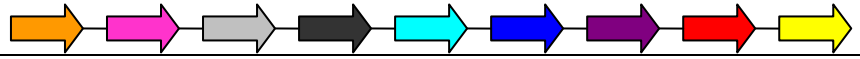
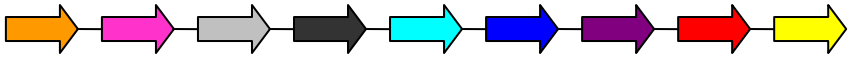
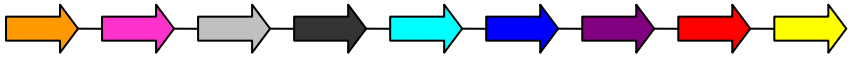
Supplementary Figure S1. **Multiple alignment of *c* subunits of N-ATPases and F_1F_O-ATP synthases.**

A. Comparison of *c* subunits of N- and F-type ATPases encoded in the same cyanobacterial genomes. Amino acid residues that form the Na^+-binding site of N-ATPases (top) and the H^+-binding site of F_1F_O-ATP synthases (bottom) are colored and marked with asterisks. Uncharged residues in the membrane hydrophobic core are shaded yellow. The proteins are listed under their UniProt identifiers, transmembrane segments predicted with TMHMM (Krogh *et al.*, 2001). The organism names are: SYNP2, *Synechococcus* sp. PCC 7002; ACAM1, *Acaryochloris marina*; CYAA5, *Cyanothece* sp. ATCC 51142; NODSP, *Nodularia spumigena*; 9SYNE, *Synechococcus* sp. PCC 7335.

B. Comparison of N-ATPase *c*-subunits (top) with *c*-subunits of the Na^+-translocating F-type ATP synthases from *Ilyobacter tartaricus* (ATPL_ILYTA) and *Propionigenium modestum* (ATPL_PROMO) and H^+-translocating F-type ATP synthases from several N-ATPase-encoding bacteria (bottom).

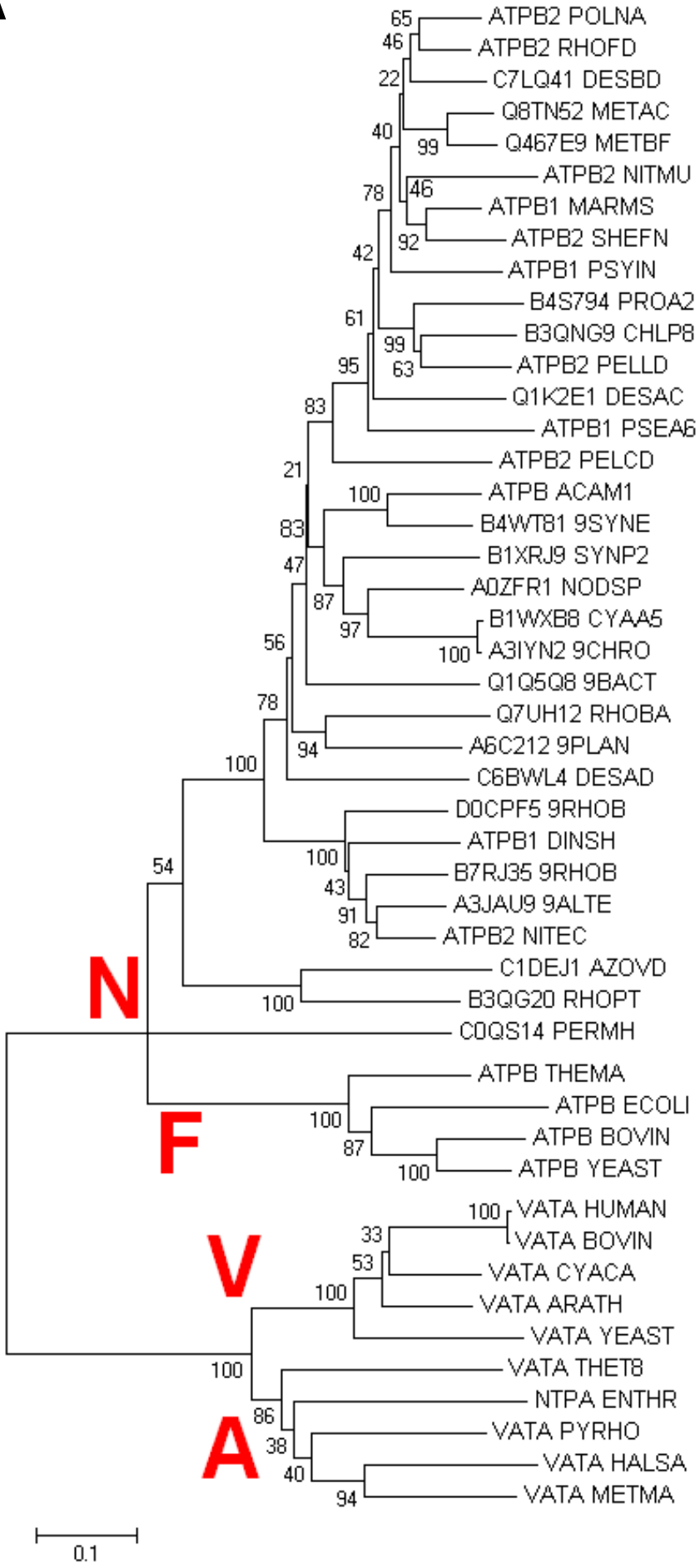
Organism, N-ATPase genes	N-ATPase operon organization	Other ATPase
Archaea	β ε Q R a c b α γ	
Methanosarcina acetivorans C2A: MA2441 → MA2433		A-ATPase: MA4152 → MA4160
Methanosarcina barkeri str. Fusaro: Mbar_A3106 → Mbar_A3098		A-ATPase: Mbar_A0392 → Mbar_A0384
Bacteria		
Aquificae		
Persephonella marina EX-H1 PERMA_1702 → PERMA_1694, 1934		F-ATPase: PERMA0179 → 0184, 0191→0193, 0456, 1050; A-ATPase: 0032 → 0040
Cyanobacteria		
Acaryochloris marina, plasmid pREB4: AM1_D0157 → AM1_D0167		AM1_0890 → AM1_0897, AM1_5328 → AM1_5327
Cyanothece sp. ATCC 51142: cce_1512 → cce1503		cce_2813 → cce_2812; cce_4482 → cce_4489
Cyanothece sp. CCY0110: CY0110_06449 → CY0110_06404		CY0110_17837-CY0110_17957 (unfinished)
Nodularia spumigena CCY9414: N9414_04280 → N9414_04320		N9414_05384 → N9414_05354 (unfinished)
Synechococcus sp. PCC 7002 plasmid pAQ7: SYNPCC7002_G0144 → SYNPCC7002_G0152		SYNPCC7002_A0740 → SYNPCC7002_A0733; A0749 → A0750
Chlorobi		
Chlorobaculum parvum NCIB 8327: Cpar_1064 → Cpar1072		Cpar_0045 → Cpar_0044, 1909 → 1908, 2052 → 2049
Chlorobium luteolum DSM 273: Plut_1063 → Plut_1071		Plut_0021 → Plut_0020; 1985 → 1984; 2098 → 2095
Prosthecochloris aestuarii DSM 271: Paes_0888 → Paes_0896		Paes_0050 → Paes_0049; 0262 → 0263; 2247 → 2244

Planctomycetes		
Kuenenia stuttgartiensis: kuste4592 → kuste4600		kustc0572 → kustc0579 (unfinished)
Planctomyces maris DSM 8797: PM8797T_02714 → PM8797T_02754		PM8797T_28864 → 28829 (unfinished)
Rhodopirellula baltica SH 1: RB4905 → RB4917		RB10206 → RB10219
Proteobacteria		
Alpha-proteobacteria		
Dinoroseobacter shibae DFL 12: Dshi_0435 → Dshi_0443		Dshi_2937 → Dshi_2933; 3031 → 3027
Rhodobacter sphaeroides 2.4.1 plasmid A: RSP_3929 → RSP_3936		RSP_2296 → RSP_2300; 1039 → 1035
Rhodopseudomonas palustris TIE-1: Rpal_1057 → Rpal_1049		Rpal_0174 → Rpal_0170; 0915 → 0911
Silicibacter lacuscaerulensis ITI-1157: SL1157_0484 → SL1157_0493		SL1157_1847 → SL1157_1851; 1970 → 1974
Beta-proteobacteria		
Burkholderia mallei ATCC 23344: BMAA0123 → BMAA0131		BMA2950 → BMA2958
Burkholderia pseudomallei K96243: BPSS1953 → BPSS1945		BPSL3403 → BPSL3395
Nitrosomonas eutropha C91: Neut_2013 → Neut_2021		Neut_0270 → Neut_0278
Nitrosospira multiformis ATCC 25196: Nmul_A1661 → Nmul_A1653		Nmul_A0303 → Nmul_A0311
Polaromonas naphthalenivorans CJ2: Pnap_2336 → Pnap_2328		Pnap_0248 → Pnap0256
Rhodoferax ferrireducens T118: Rfer_1161 → Rfer_1169		Rfer_0113 → Rfer_0105
Gamma-proteobacteria		
Azotobacter vinelandii DJ: Avin19670 → Avin19750		Avin_52230 - Avin_52150
Marinobacter sp. ELB17: MELB17_22670 → MELB17_22715		MELB17_00155 → 00195 (unfinished)

Marinomonas sp. MWYL1: Mmwyl1_1954 → Mmwyl1_1962		Mmwyl1_4469 → Mmwyl1_4461
Pseudoalteromonas atlantica T6c: Patl_2668 → Patl_2676		Patl_4302 → Patl_4294
Psychromonas ingrahamii 37: Ping_0463 → Ping_0471		Ping_3737 → Ping_3729
Shewanella frigidimarina NCIMB 400: Sfri_3049 → Sfri_3057		Sfri_4052 → Sfri_4044
Delta-proteobacteria		
Desulfomicrobium baculatum DSM 4028: Dbac_0952 → Dbac_0944		Dbac_3361 → Dbac_3367; 2797 → 2794
Desulfovibrio salexigens DSM 2638: Desal_2238 → Desal_2230		Desal_3459 → Desal_3453; 3727 → 3724
Desulfuromonas acetoxidans DSM 684: Dace_2593 → Dace_2601		Dace_1466 → Dace_1460; 1446 → 1443 (unfinished)
Pelobacter carbinolicus DSM 2380: Pcar_2989 → Pcar_2997		Pcar_0944 → Pcar_0952; 0013 → 0016; 3136 → 3130;
Syntrophus aciditrophicus SB: SYN_02096 → SYN_02105		SYN_00549 → SYN_00543; 00586 → 00588; 02965

Supplementary Figure S2. **Operon organization of the N-ATPase genes and the F-ATPase genes encoded in the same genomes** (A-ATPases in *Methanosarcina acetivorans*, *M. barkeri*, and *Persephonella marina*). White arrows in the operons from *Acaryochloris marina* and *Cyanothece* spp. indicate uncharacterized genes. The striped arrows for *Rhodobacter sphaeroides* RSP_3932 and RSP_3934 and for *Pseudoalteromonas atlantica* Patl_2671 indicate untranslated ORFs. Gene assignments for unfinished genomes (indicated in the 3[d] column) are tentative and could change upon genome completion.

A


ATPB2 POLNA
ATPB2 RHOFD
C7LQ41 DESBD
Q8TN52 METAC
Q467E9 METBF
ATPB2 NITMU
ATPB1 MARMS
ATPB2 SHEFN
ATPB1 PSYIN
B4S794 PROA2
B3QNG9 CHLP8
ATPB2 PELLD
Q1K2E1 DESAC
ATPB1 PSEA6
ATPB2 PELCD
ATPB ACAM1
B4WT81 9SYNE
B1XRJ9 SYNP2
A0ZFR1 NODSP
B1WXB8 CYAA5
A3IYN2 9CHRO
Q1Q5Q8 9BACT
Q7UH12 RHOBA
A6C212 9PLAN
C6BWL4 DESAD
D0CPF5 9RHOB
ATPB1 DINSH
B7RJ35 9RHOB
A3JAU9 9ALTE
ATPB2 NITEC
C1DEJ1 AZOVD
B3QG20 RHOPT
C0QS14 PERMH
ATPB THEMA
ATPB ECOLI
ATPB BOVIN
ATPB YEAST
VATA HUMAN
VATA BOVIN
VATA CYACA
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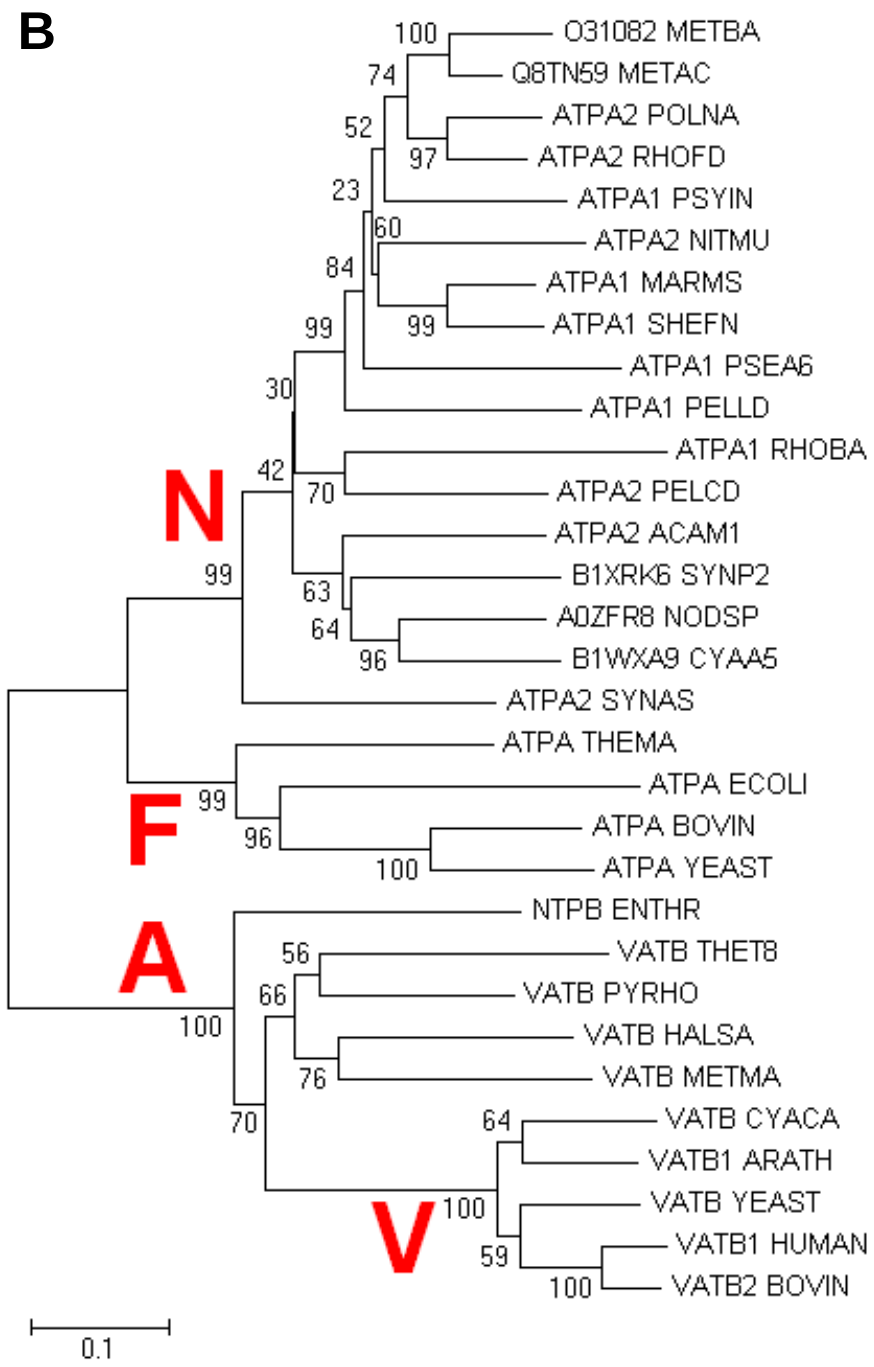
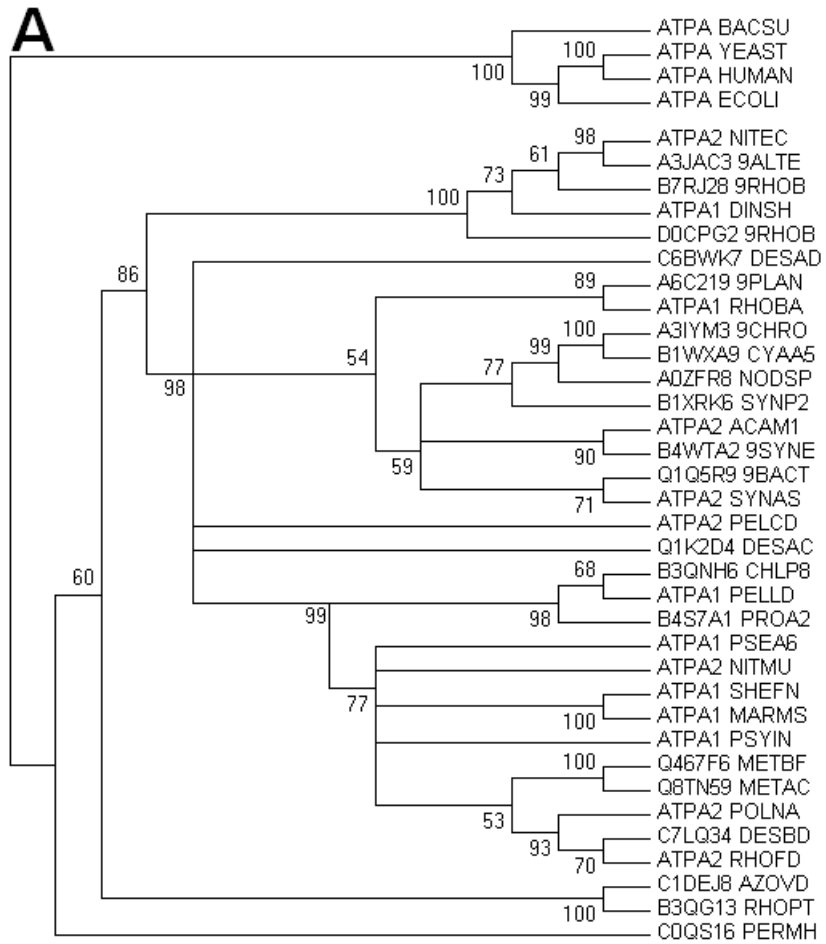
VATA METMA
N
F
V
A
0.1



Supplementary Figure S3. Phylogenetic trees of α- and β-subunits of N- ATPases.

A. Phylogenetic tree of β-subunits of N- and F-type ATPases compared with A subunits of A- and V-type ATPases. B. Phylogenetic tree of α-subunits of N- and F-type ATPases and B subunits of A- and V-type ATPases. Proteins are listed under their UniProt identifiers. Phylogenetic trees were constructed from BLAST-based multiple alignments using neighbor-joining algorithm in MEGA program (Kumar *et al*., 2008). Bootstrap values were calculated from 100 samples. The N-ATPase-encoding organisms are as follows: POLNA, *Polaromonas naphthalenivorans*; RHOFD, *Rhodoferax ferrireducens*; DESBD, *Desulfomicrobium baculatum*; METAC, *Methanosarcina acetivorans*; METBF, *Methanosarcina barkeri*; NITMU, *Nitrosospira multiformis*; MARMS, *Marinomonas* sp. strain MWYL1; SHEFN, *Shewanella frigidimarina*; PSYIN, *Psychromonas ingrahamii*; PROA2, *Prosthecochloris aestuarii*; CHLP8, *Chlorobaculum parvum*; PELLD, *Pelodictyon luteolum*; DESAC, *Desulfuromonas acetoxidans*; PSEA6, *Pseudoalteromonas atlantica*; PELCD, *Pelobacter carbinolicus*; ACAM1, *Acaryochloris marina*; 9SYNE *Synechococcus* sp. PCC 7335; SYNP2, *Synechococcus* sp. PCC 7002; NODSP, *Nodularia spumigena*; CYAA5, *Cyanothece* sp. ATCC 51142; 9CHRO, *Cyanothece* sp. CCY0110; 9BACT, *Kuenenia stuttgartiensis*; RHOBA, *Rhodopirellula baltica*; 9PLAN, *Planctomyces maris*; DESAD, *Desulfovibrio salexigens*; 9RHOB (D0CPF5), *Silicibacter lacuscaerulensis*; DINSH, *Dinoroseobacter shibae*; 9RHOB (B7RJ35), *Roseobacter* sp. GAI101; 9ALTE, *Marinobacter* sp. ELB17**;** NITEC, *Nitrosomonas eutropha*; AZOVD, *Azotobacter vinelandii*; RHOPT, *Rhodopseudomonas palustris* strain TIE-1; PERMH, *Persephonella marina*. SYNAS, *Syntrophus aciditrophicus*.


A
ATPA BACSU
ATPA YEAST
ATPA HUMAN
ATPA ECOLI
ATPA2 NITEC
A3JAC3 9ALTE
B7RJ28 9RHOB
ATPA1 DINSH
D0CPG2 9RHOB
C6BWK7 DESAD
A6C219 9PLAN
ATPA1 RHOBA
A3IYM3 9CHRO
B1WXA9 CYAA5
A0ZFR8 NODSP
B1XRK6 SYNP2
ATPA2 ACAM1
B4WTA2 9SYNE
Q1Q5R9 9BACT
ATPA2 SYNAS
ATPA2 PELCD
Q1K2D4 DESAC
B3QNH6 CHLP8
ATPA1 PELLD
B4S7A1 PROA2
ATPA1 PSEA6
ATPA2 NITMU
ATPA1 SHEFN
ATPA1 MARMS
ATPA1 PSYIN
Q467F6 METBF
Q8TN59 METAC
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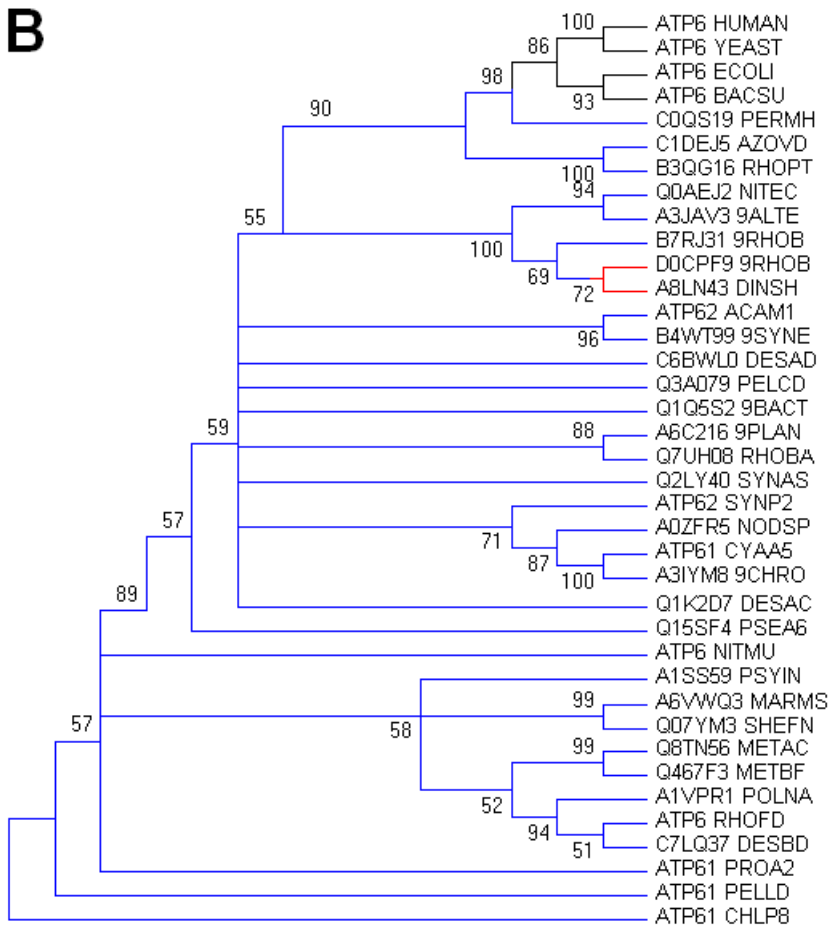
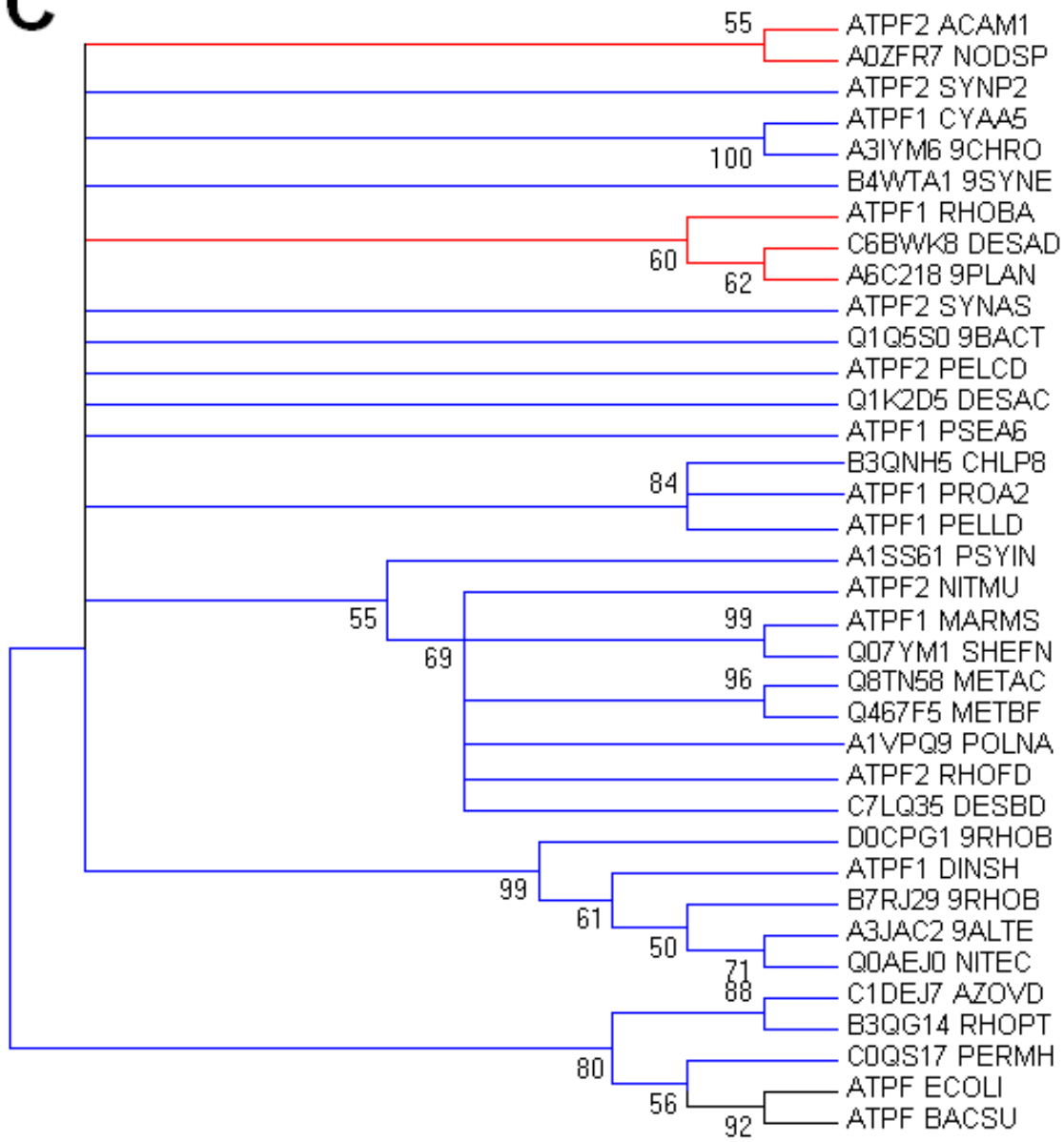
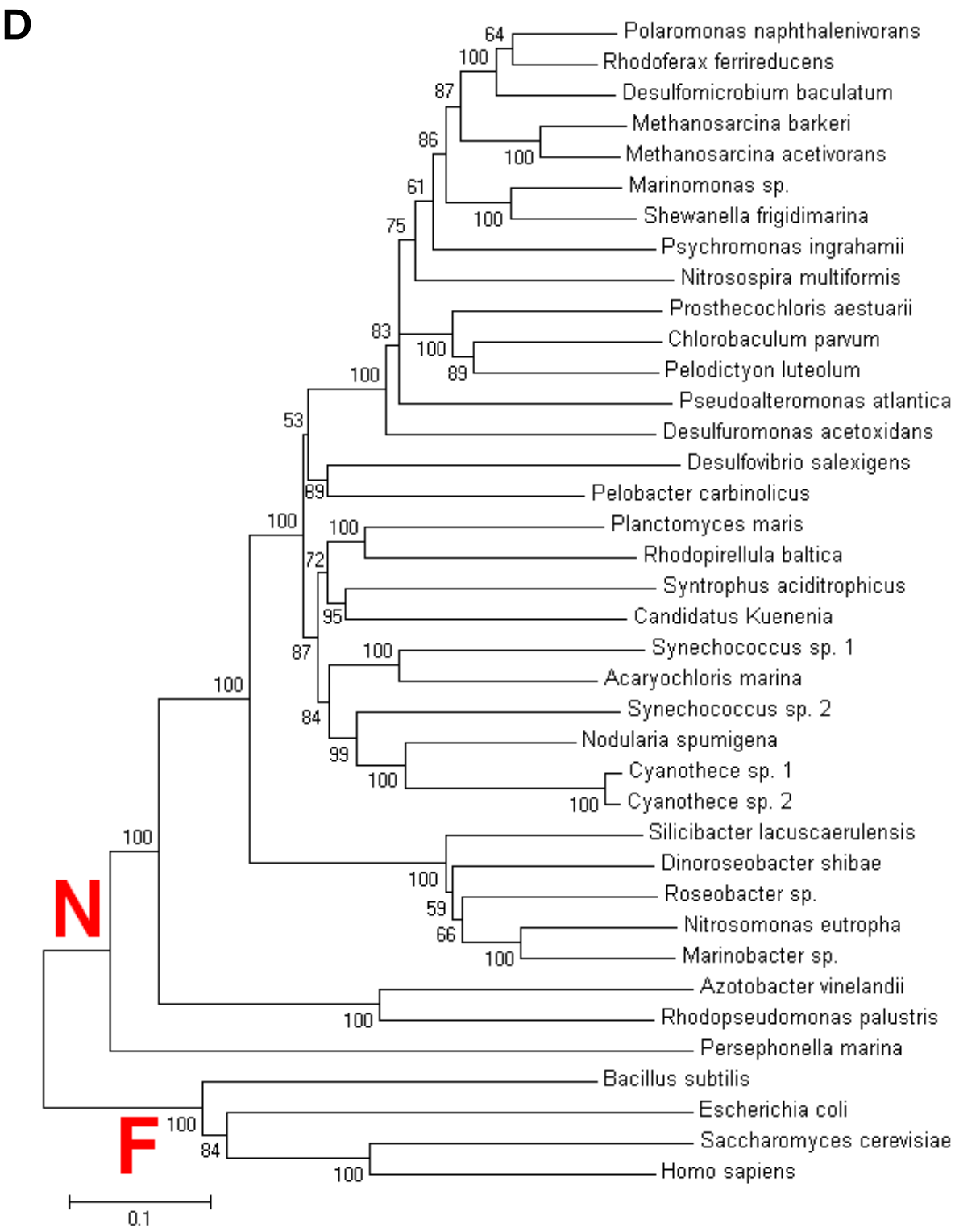
C7LQ34 DESBD
ATPA2 RHOFD
C1DEJ8 AZOVD
B3QG13 RHOPT
C0QS16 PERMH


B
ATP6 HUMAN
ATP6 YEAST
ATP6 ECOLI
ATP6 BACSU
C0QS19 PERMH
C1DEJ5 AZOVD
B3QG16 RHOPT
Q0AEJ2 NITEC
A3JAV3 9ALTE
B7RJ31 9RHOB
D0CPF9 9RHOB
A8LN43 DINSH
ATP62 ACAM1
B4WT99 9SYNE
C6BWL0 DESAD
Q3A079 PELCD
Q1Q5S2 9BACT
A6C216 9PLAN
Q7UH08 RHOBA
Q2LY40 SYNAS
ATP62 SYNP2
A0ZFR5 NODSP
ATP61 CYAA5
A3IYM8 9CHRO
Q1K2D7 DESAC
Q15SF4 PSEA6
ATP6 NITMU
A1SS59 PSYIN
A6VWQ3 MARMS
Q07YM3 SHEFN
Q8TN56 METAC
Q467F3 METBF
A1VPR1 POLNA
ATP6 RHOFD
C7LQ37 DESBD
ATP61 PROA2
ATP61 PELLD
ATP61 CHLP8

C


55
ATPF2 ACAM1
A0ZFR7 NODSP
ATPF2 SYNP2
ATPF1 CYAA5
100
A3IYM6 9CHRO
B4WTA1 9SYNE
ATPF1 RHOBA
60
C6BWK8 DESAD
62
A6C218 9PLAN
ATPF2 SYNAS
Q1Q5S0 9BACT
ATPF2 PELCD
Q1K2D5 DESAC
ATPF1 PSEA6
84
B3QNH5 CHLP8
ATPF1 PROA2
ATPF1 PELLD
A1SS61 PSYIN
55
ATPF2 NITMU
99
ATPF1 MARMS
69
Q07YM1 SHEFN
96
Q8TN58 METAC
Q467F5 METBF
A1VPQ9 POLNA
ATPF2 RHOFD
C7LQ35 DESBD
D0CPG1 9RHOB
99
ATPF1 DINSH
61
B7RJ29 9RHOB
50
A3JAC2 9ALTE
71
Q0AEJ0 NITEC
88
C1DEJ7 AZOVD
B3QG14 RHOPT
80
C0QS17 PERMH
56
ATPF ECOLI
92
ATPF BACSU



Supplementary Figure S4. **Topology comparison of the phylogenetic trees for α-subunit (A), *a*-subunit (B) and *b*-subunit (C) of the N-ATPases from different organisms and (D) a consensus tree built from concatenated α-, β-, ε-, *c*- and *b*-subunits.**
Phylogenetic trees were constructed from BLAST-based multiple alignments using neighbor-joining algorithm in MEGA program (Kumar *et al*., 2008). Bootstrap values were calculated from 100 samples, branches with bootstrap value less than 50 were collapsed. Orthologous subunits of F-type ATPases from *Bacillus subtilis*, *Escherichia coli*, yeast and human were used as an outgroup. In panels B and C, branches with the same topology as in A are shown in blue, whereas branches with a different topology are in red.

```
Cyanobacteria
A8ZNS0_ACAM1    4  HSPALFTSLILGSSLGLLYFGGLWITILRFNRTTQPILLMWSSFLLRLGTVLGVFHLILQNGMSDHLLAPLLLTFLGFLGARNLLISSIIPLPKKI  98
B1WXB5_CYAA5    5  FILENLIPFIFGIILGTFYFSSLWITVRQLPTTVYPVRLFIGSFLGRIFVTLFGFYLIMDG-----QWQRILICLGGFILARIVLTNVLKPELSNS  95
A0ZFR4_NODSP    3  NIIYLLIALPLGFTLGIFYFVNLWITVRQIPTTQWPFRLIVGSFVGRTSITILGLYLIMDG-----NWKRMLISLFGFVLARSILINYWQPRQEYN  93
B1XRK2_SYNP2    7  KVTPNLFVFPLGFGLGLFYFTALWFTVKQLTRSQHPVLLIITSGVVRLILAMTALYFMIGG-----HWERVLIALAGFLLARTILINRWRPQTSLS  97
B4WT84_9SYNE    1  -MISVIVLWLVGLGLGGLYFGGLWMTLRRLPRWHHPFLAMGLSLLCRLVVLLGMGGLFLRYPIA-SPFQTVLTISFGVWLSRMMLISAALSISKRS  94
Archaea
Q8TN55_METAC    3  EVFNLISALVAGFLLGAFFFGGLWWTVRKGLLSGQPALWFFGSLLLRTGIAVAGFYFASGG-----HWDRLLMCLLGFFIMRRIVTRLTRAPEEES  93
Q467F2_METBF    3  EILNLFLSLIAGFLLGAVFFGGLWWTVQKGLSSRKPAFWFLGSLLIRISIVIAGFYFVSDG-----HWERLLICLFGFFVMRHIIVRLTRLPEEDQ  93
Aquificae
C0QS20_PERMH    2  KIIVYALLFFLGYLCGILFFNHLFKSSKEAI----LKKKRSTGFFRRFIPFSVVAVAVAYF-----FKIGILFFLLGFYLSRLTFTRLLTDLK---  85
Chlorobi
Q3B403_PELLD    5  MLMGLLPDLLGGLLLGSLFFGGLWLTVRRGLASPRPWLWFGLSSLLRTMLVLAGFWWLAGN-----DWRGFLAALLGFTAAKVLAAVLSRKSPPYE  95
B3QNH2_CHLP8    1  -MTTNLLAILGGFALGLFYFGGLWLTVRKGLFSPHPALLFLTSSLLRTAGVIGGFLLISAG-----DPVRLLLAVGGFVAAKVTSIVLGRRDGASE  90
Planctomycetes
Q7UH09_RHOBA    5  EWVAMTGGFLAGAAAAVFFVASLWWTTVRLPASRQPWLLLCGSALLRMAVVLGVLAMLARS----GDWRILVAAAMGFALIRTVGVCTIAQRDTSF  96
A6C215_9PLAN    5  LLLQLLASLVCGLVLGTLFFGGLWLTIRNLQKVSHPALLFLASALTRTILTISGFWCIGVWLDESSRWQRLTVCLAGFILARMICTRIIRTDQPTF 100
Q1Q5S3_9BACT    4  DIFTLATALAVGMAVGLLNFAGMWSTIHYLPIIKYPSALIMLSYIIRMAIVLATFYFIMDG-----RWERLLVCLVGFFIVRRVLVKRFLPEGSKM  94
Proteobacteria
D0CPF8_9RHOB    5  DWTGFLMGFVSGGVVGAVYFAGLALSIRLALRAARPTALLLTSSALRITMVLGLGWLAAQG-----GPANLAGFAMAFLLARVAAIAMARPERGAA  95
B2IHN9_BEII9   14  QALVLGAHLFGGFLLGVIYFRALWWNTRLLAQG-RRPLLAISIALARFVLLGGLLILASRE-----GVAPLLATVFGLFVGRFLVLRGVWAKIS-- 101
C1DEJ4_AZOVD    9  SALPLGLGLLVGLLAGWAHFSSLAANLRLFTAGR--IAAALALQSLRLGLTVAVLAGLIRL-----DLGALLAGACGLLLARRRLLRREGACR---  94
B3QG17_RHOPT   14  EVVSLVAYLGAGIVLGMLYFRGLWWSVRRFSDGG-KLAATIALMIGRFVVLGGLMTLASLQ-----GALPLLVLALGVLAARAAILRRVREVTP-- 101
A8LN42_DINSH    5  DWTLTGLGAFLGVLAGAAFFAGLAWGMRIALRRARPAPVLLLSAGLRIAALLAVGYWVAGE----GGTSMLLGFAGGFLVARVCILMLARRPVPEE  96
A3JAV2_9ALTE    4  DWSAVLLGLAVGVAVSSLFFVGLAWGMKRALRSRQPEALLLLSAIVRIAMLLGVGFWLATSS---ATAWPMAGFALAFLLVRMVALLWARKRDTTS  96
Q0AEJ3_NITEC   18  DGSAILLGFVVGLPVSALFFMGLNWGIRLALASAYPGRLLMLSFFCRLVVLLAVGFGLIALI---QTLWSLAGYMLAFVLMRVLAVVRAQLTHSVT 110
C6BWL1_DESAD    5  SIMITVAAFGIGLLLSAIHFGGLWLTVRMLPRCERPRMFFWSSYLGRYGITLWGFAQVMSY-----GGGPFVSSFLGFYLLRTYLLANQCGMGMLD  95
A3IYM9_9CHRO    4  FLLENLIPFIFGIMLGAFYFSSLWITVRQLPTTVFPVRLFIGSFLGRILVTLFGFYLIMDG-----QWQRILICLGGFILARIILTNVLKPKLSNS  94
Q62EB4_BURMA   13  DGIAAAIGLALGFAAGACHFVSLGWNSRLFVAG--RAGVALALQFARVALAVAVLVVLARA-----GFGMLVAGSAGFFAARAIAVRRAAALTGAR 101
Q63IW6_BURPS   10  DGIAAAIGLALGFAAGACHFVSLGWNSRLFVAG--RAGVALALQFARVALAVAVLVVLARA-----GFGMLVAGSAGFFAARAIAVRRAAALTGAR  98
Q3A080_PELCD    5  ELGRMALALLTGLLLGAGYFGGLYLTVKSLKRCASPRLLLTASFLIRLLVVLGVFYLLSDW-----GALVMLTAMGGFLIARLGWLRSRYGRGA--  93
Q2LY39_SYNAS    4  YGLSLVGVFAVGMVLGIVYFAILWQTVRRLPVASSPARLLMTSLLIRVGIVLPAFYLIMAG-----RWERIVVALGGFVVVREILIRFWGKGEPHF  94
B4S797_PROA2    3  DLMVFAALFFVGMFLGIFFFGGLWLTVQKSTFSRRPWLWFSISLALRTGVIFVGFYLIAPG-----GLVWLVSSLTGFVAARGLVIVVTRSFPVHQ  93
A6VWQ2_MARMS    9  FWGGVAYAILVGMLLGVFYFAGLWWTVRRLGSSRVVAPLFLFSMLFRISVVMVSFYVFLGN-----DWQKLLLALLGFMVLRLFATRLIRLKDNPV  99
Q07YM4_SHEFN    9  IFVELTLALLAGALLGVFFFAGLWWTVRQLKSTQHVALLFIASLLFRSAIVIAGFYFIAGD-----NGYHLLAGLLGFMLVRLMATRYTLQVEQAQ  99
A1SS58_PSYIN   11  QILPLLPLLLAGAVLGVIFFAGLCWTVQRGIKSAHPGRWFFGSFLLRTAVALSGIYLLTDG-----QWPKLLACMLGFIGARIVVITITGSATNIA 110
Q2Y8G5_NITMU    3  EIMSLLLALVGGVLLGAFFFGGLWWTVRRSIASPRPGTWFVGSMLLRTCIVTAGFYFLLELA--APGKKILFAAVVGFIIARVVATQFLPTPLSPS  96
Q1K2D8_DESAC    4  DWLGGALAVSVGFILGLIFFAGLWWTVKRAVTSPHPALWFIGSMLIRVAITLVGFYWVSAD-----QWWRLLLCTAGFIVARPVVSRTVNQPDTGR  94
A1VPR2_POLNA    5  ETPALVLACIAGGALGAIFFGGLWWTVRKSLVSSRPALWVFASLLLRMGLTMAGFYVVSGG-----DWRRLLACLAGFVMARQVVTRLTRPLDARQ  95
Q21ZA3_RHOFD    3  ETLTLALAWVAGGVLGTIFFGGLWWTVRKGVSSKQPALWLFSSLLLRIGIALAGFYFVSGG-----HWERLLACLLGFVVARLVVTWMTRSSGKNQ  93
C7LQ38_DESBD    3  ETLTLVLALAAGVLLGGLFFGGLWWTVRKGVSSKRPAFWFFASLLLRMGITLTGFHFIAGG-----HLGRLLMCLLGFFIARLVVTRLTRTVEKPA  93
B2TG37_BURPP    8  FAAQFAMGLSVGMLAGAWQFLSLRWNWPLFAQG--RAAAALVLQLARFALTGALLLMLAHA-----GALALLAGMAGILLMRTIAIRCQRGRP---  93
                    <--------MEMBRANE--------> CYTOPLASM <------MEMBRANE----->  OUT   <-----MEMBRANE----> CYTOPLASM
```

Supplementary Figure S5. Alignment of the AtpR subunits of the N-ATPase. The last line shows transmembrane topology prediction from TMHMM (Krogh *et al.*, 2001) and Phobius (Käll *et al.* 2007) servers. The characteristic Arg residues located inside of the 2nd and 3d TM segments are colored blue. The proteins are listed under their UniProt identifiers.

```
Q8TN58_METAC MA2435        175 ASSPVLVRTAFDLPQAQRELIKKTIKETL----GIEVQVCFETVPDLVSGIELTANGQKVAWSIAGYLSSLEEGIDELLQEQPRSEARTEP 261
Q467F5_METBF Mbar_A3100    175 SPGQVLIRTAFDLPQAQRDSIKKTIKETL----GIEIQPRFETAPDLVSGIELTTDGQKVAWSIADYLTSMQKSIDELLNEQPENKAKTEP 261
C0QS17_PERMH PERMA_1699    174 ERNTVTVETAYPLSEEEIESIKKSIKDMF----GIDVTIKTEERKDLIAGVKIHIASKMIDASLEGQISVFETLLRDKIETS--------- 251
ATPF2_ACAM1  AM1_D0164     176 RGEELVISSTFPMPEDRKAQILAVLHQYAPA--MESSVYQFVTIPDLICGIELKIPGYKLAWTIEQYLEQLEVKLNQVWDGMEKSWVEQG- 263
ATPF1_CYAA5  cce_1506      175 TDNGLIIRSHFEISPESRNRLLSSLQQTHI---YQGDNVQFMTNSDLICGIELQASDYKIAWNLKDYVEALEI------------------ 244
A0ZFR7_NODSP N9414_04310   172 SATEIIIYSSFDIPQTQRQEIIKILQSQQI---INSKNIKFTTSPDLICGIKLQISNYQISWTFDDYLQTLSEQFSTIIKQTTNKP----- 254
ATPF1_PELLD  Plut_1069     170 SGGTLVLRSGFEMGEEEKELVRKTLADRF----GYKGRLDFMTEESYRGGIALEQGGRSIEWSVNSRLEAIDEASSALLDGPDDEMNEEEG 257
ATPF1_PROA2  Paes_0894     175 KSLNPLVRSTFPLSSSLQERIQAVVRDVF----SIDTQLRFEEGDDMVGGIELSMNGHSVSWSVRSYLDSLEKMTAELLEGAE-------- 253
ATPF1_RHOBA  RB4915        175 SGNPVLVRSAKGLDSSDQNQIRDAIHRVF----ENKVEVRFESEPALIAGIEMDAGGYSLPWNAERTLKTMEANVA--------------- 246
A6C218_9PLAN PM8797T_02744 175 SDPQMLVESAFELTQPERKNITDLIHDYL----SREVTIRFRVNHELICGIDLHLAGYKISWNLQESLEELEEEFVRSLNDVISTDSEPGI 261
ATPF1_DINSH  Dshi_0441     170 DARQAVALTRDPLPEEVQARLRSDLGDVL-----EGVSLRFEVDPGQSPGLNLRLGGAQLGWTVDSYLNGLEATLAEAAGRPARRGGHDAA 255
ATPF2_NITMU  Nmul_A1655    199 GIAVILVRSAFELPAEQQEKISTVIRETL----KEEVRFNFGVEPNLISGIEMIAGGHKVAWSVAGYLASLEEEVSRLLNAKGAVGEGEAK 285
ATPF2_RHOFD  Rfer_1167     175 ASEPALVRSAFELPADQRAAIQNAINETF----SADIPLHFATAPEVVCGIELSTNGQKVGWSITDYLASLEKGVDELLKERDKAEPQSEL 261
C1DEJ7_AZOVD Avin_19730    171 ESAALTLRVPRTPSAGELEACRQALARAL----GREPELKVAVDPGLIAGLELEGSNAVVRNSFRADLARLHRELSHESA----------- 246
ATPF1_PSEA6  Patl_2674     175 SGNQIVVRSAQPLAEAQKKQLLACLQQYL-20-APSIKLSESIVPRLINGIELTMGGWKLAWSTDNYLAELQEDVEAEFIPFTETLLGLPE 281
Q07YM1_SHEFN Sfri_3055     179 AAQPILIRTAFALPTPQCSLIEAAIKKIL----SHDIPIQFSIEPDVISGIELSIKGQKISWSINEYLSSLAKRVDELLQSPMNIEQANDT 265
ATPF2_PELCD  Pcar_2995     173 SERGVLVRSPFTLPPELRDRLTQGVRQAL----GEEIDMQYQDRADMPLGIELTVGGLKLSWGVDSYFEQLERDVATLYDAQAATVSEGSP 259
ATPF2_SYNAS  SYN_02103     175 SEEGAIVQSAFALFPEDRQRLTDTVRDLLG---KPDAVIRYQESSDLIGGIEFLASGHRIAWSISDYLEHLEQDFDRVLHEEVRQTLPKPS 262

pdb|2WSS_S                     EEEEEE   EEEEEE                                          HHHHHHH
ATPD_ECOLI                 105 ATAEVDVISAAALSEQQLAKISAAMEKRL----SRKVKLNCKIDKSVMAGVIIRAGDMVIDGSVRGRLERLADVLQS-------------- 177
ATPO_BOVIN                 137 GEVPCTVTTASALDETTLTELKTVLKSFLSK--GQVLKLEVKIDPSIMGGMIVRIGEKYVDMSAKTKIQKLSRAMREIL------------ 213
ATPD_ACAM1   AM1_0895      110 NTVLAEVTSTVELTDEQRHAITDKVKHMSQ---AAQVDLETSIDPDLIGGVIIKIGSQVLDASIRGQLRRMNSSITSLS------------ 185
ATPD_NITMU   Nmul_A0307    106 GVLDAKVISAFAMSDAQLKDLVTDLEAKF----KRKIEAKVSVDADLIGGVIVEIGDEVLDASVRGKLEAMAVALKS-------------- 178
ATPD_RHOFD   Rfer_0109     104 GSSDAVVYSAFAIDSTALAELAATLEQRF----ARKLNVSVQLQPELIGGIRVVVGDEVLDSSVKARLEQMKMALTA-------------- 176
ATPD_AZOVD   Avin_52190    106 KTIDVELETAYELSAEQLETLAAALSKRL----DRSVNPRQVVNPALIGGLVIRAGDLVVDGSVRGKLSQLAESLKS-------------- 178
ATPD_PSEA6   Patl_4298     105 KEIEADVTSATELNAKQQADISAALEKRL----ARKVKLNCSVDPTLIAGVKIKAGDTVIDGSVKSKLNRLTDALQA-------------- 177
ATPD_SHEFN   Sfri_4048     105 KEVEADVVSATELSSEQQQQISVSLEKRL----ARKVKLNCSIDASLIGGVIIKSGDLVIDGSVSGKLSRLSEKLQS-------------- 177
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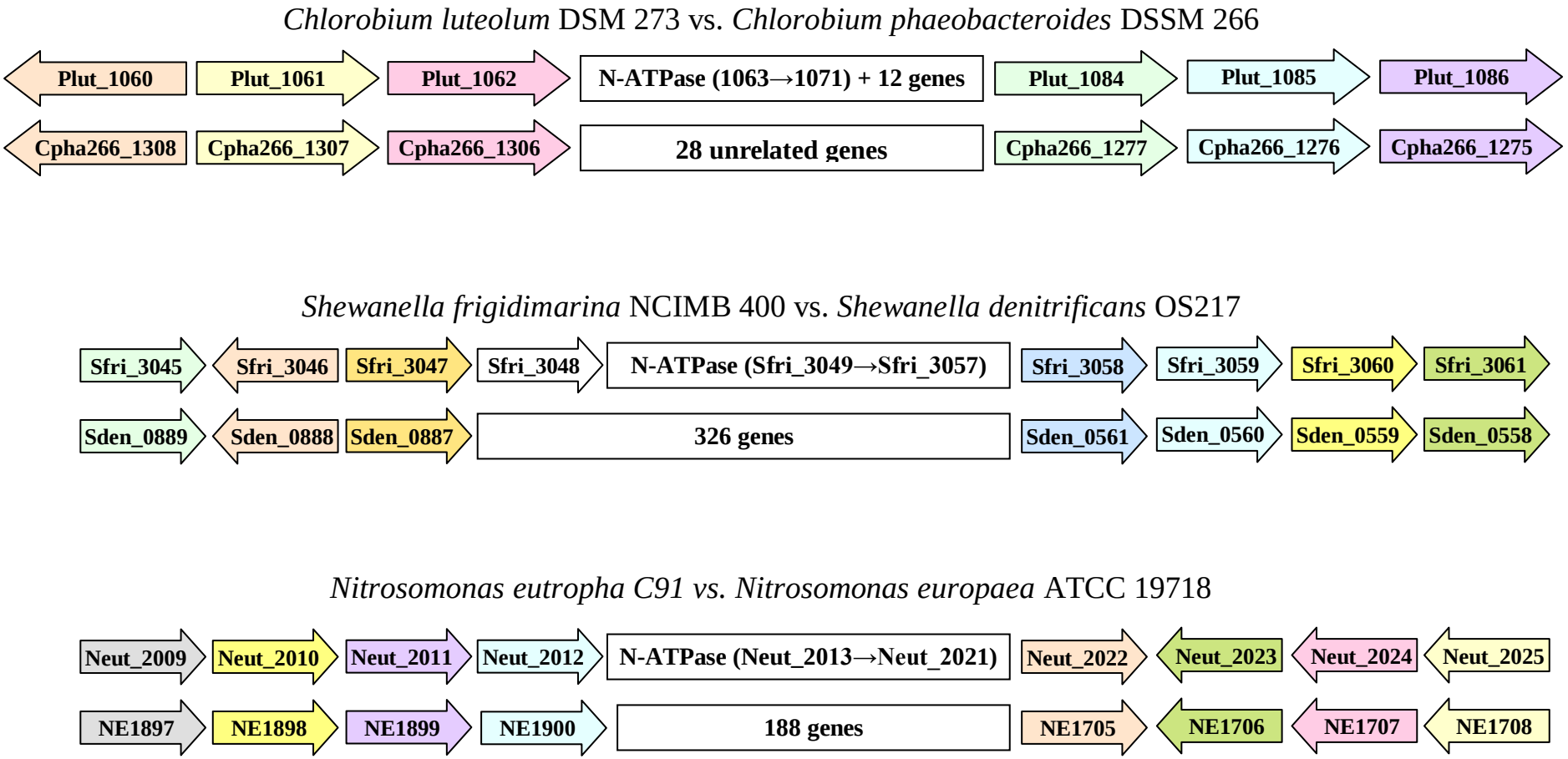
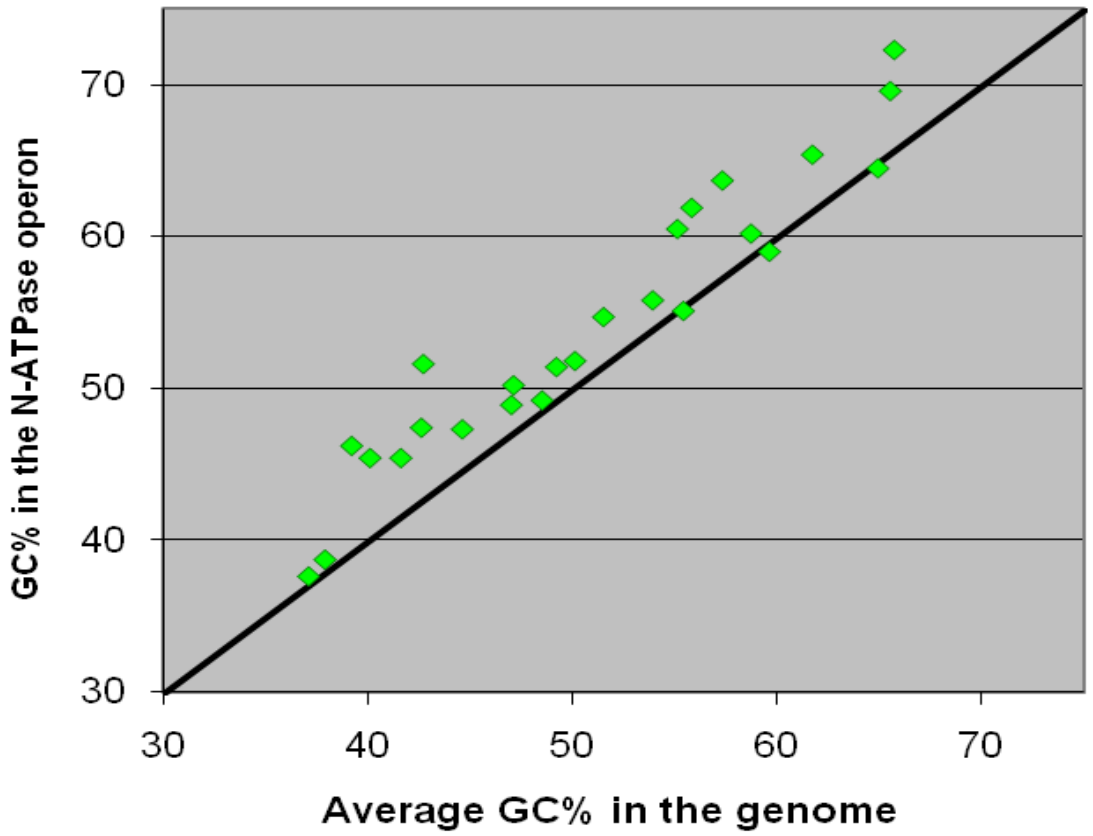
Supplementary Figure S6. **Alignment of the C-terminal fragments of the N-ATPase *b*-subunits (top) with *δ*-subunits of F-ATPases** from *Escherichia coli* (ATPD_ECOLI), bovine mitochondria (ATPO_BOVIN), and of H^+-translocating F-ATPases from the same organisms (bottom). Structural elements from the bovine oligomycin-sensitivity conferring protein (OSCP, *δ*-subunit) structure (PDB entry: 2WSS_S), reported by Rees *et al.* (2009) *Proc. Natl. Acad. Sci. USA*, **106**, 21597-21601, are indicated as follows: E, β-strand; H, α-helix. Conserved Gly and Ser residues are shown in bold typeface, conserved hydrophobic amino acid residues are shaded yellow. The proteins are listed under their UniProt identifiers and genome locus names.



Supplementary Figure S7. Gene neighborhoods of the N-ATPase operons. In each pair of closely related organisms, the top one encodes the N-ATPase whereas the bottom one does not. Orthologous genes within each pair of organisms are indicated with the same colors. The arrow lengths do not reflect the lengths of the respective genes.



Supplementary Figure S8. Correlation between the GC content of the N-ATPase genes and the total genome. GC-content data for the complete genomes were taken from the RefSeq database (Pruitt *et al.*, 2009) and those for the N-ATPase operons were calculated using the online tool at the http://www.genomicsplace.com/gc_calc.html web site.