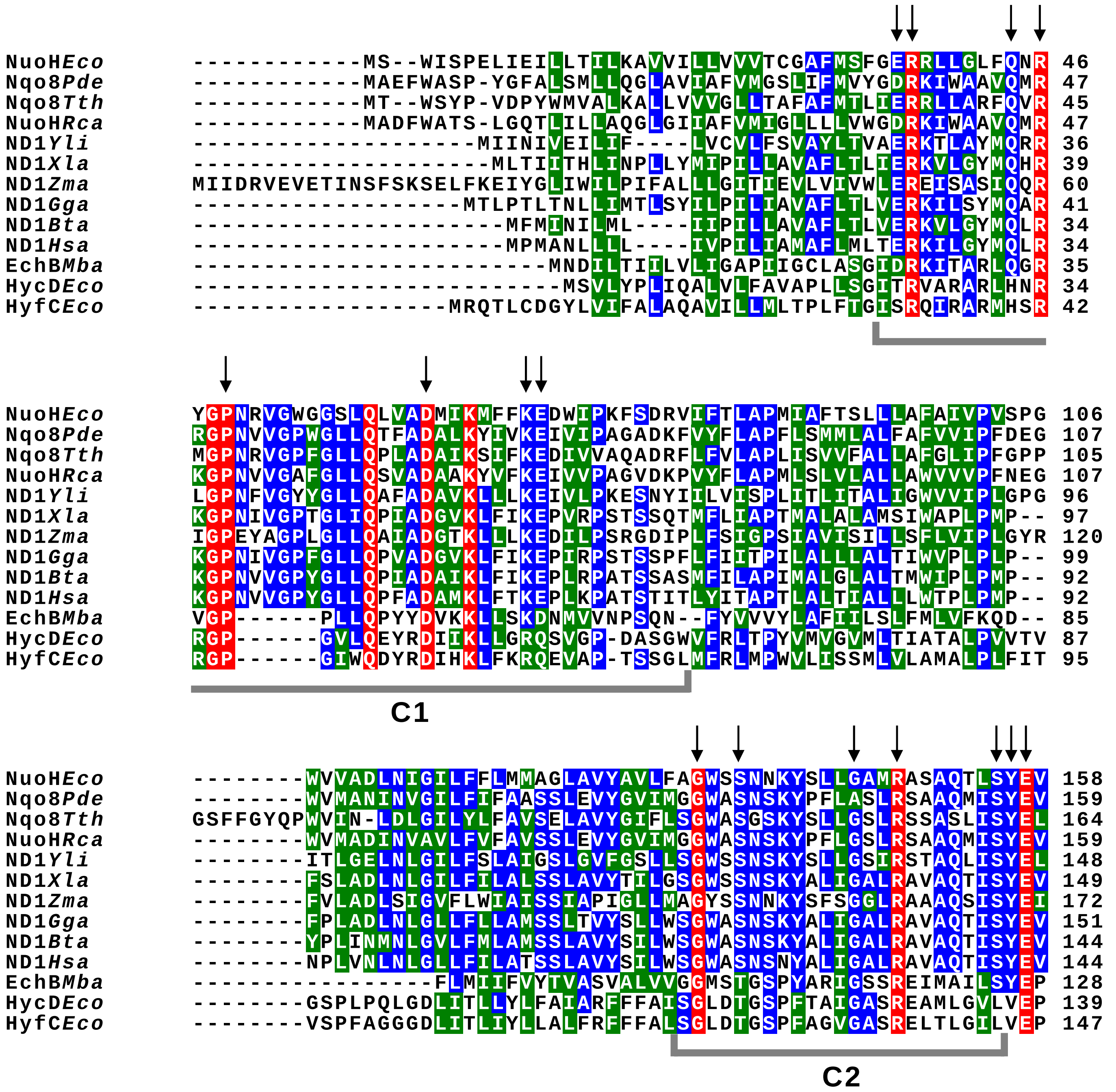

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NuoHEco   ------------MS--WISPELIEILLTILKAVVILLVVVTCGAFMSFGERRLLGLFQNR 46
Nqo8Pde   ------------MAEFWASP-YGFALSMLLQGLAVIAFVMGSLIFMVYGDRKIWAAVQMR 47
Nqo8Tth   ------------MT--WSYP-VDPYWMVALKALLVVVGLLTAFAFMTLIERRLLARFQVR 45
NuoHRca   ------------MADFWATS-LGQTLILLAQGLGIIAFVMIGLLLLVWGDRKIWAAVQMR 47
ND1Yli    --------------------MIINIVEILIF----LVCVLFSVAYLTVAERKTLAYMQRR 36
ND1Xla    ---------------------MLTIITHLINPLLYMIPILLAVAFLTLIERKVLGYMQHR 39
ND1Zma    MIIDRVEVETINSFSKSELFKEIYGLIWILPIFALLLGITIEVLVIVWLEREISASIQQR 60
ND1Gga    -------------------MTLPTLTNLLIMTLSYILPILIAVAFLTLVERKILSYMQAR 41
ND1Bta    ----------------------MFMINILML----IIPILLAVAFLTLVERKVLGYMQLR 34
ND1Hsa    ----------------------MPMANLLLL----IVPILIAMAFLMLTERKILGYMQLR 34
EchBMba   -------------------------MNDILTIILVLIGAPIIGCLASGIDRKITARLQGR 35
HycDEco   --------------------------MSVLYPLIQALVLFAVAPLLSGITRVARARLHNR 34
HyfCEco   ------------------MRQTLCDGYLVIFALAQAVILLMLTPLFTGISRQIRARMHSR 42

NuoHEco   YGPNRVGWGGSLQLVADMIKMFFKEDWIPKFSDRVIFTLAPMIAFTSLLLAFAIVPVSPG 106
Nqo8Pde   RGPNVVGPWGLLQTFADALKYIVKEIVIPAGADKFVYFLAPFLSMMLALFAFVVIPFDEG 107
Nqo8Tth   MGPNRVGPFGLLQPLADAIKSIFKEDIVVAQADRFLFVLAPLISVVFALLAFGLIPFGPP 105
NuoHRca   KGPNVVGAFGLLQSVADAAKYVFKEIVVPAGVDKPVYFLAPMLSLVLALLAWVVVPFNEG 107
ND1Yli    LGPNFVGYYGLLQAFADAVKLLLKEIVLPKESNYIILVISPLITLITALIGWVVIPLGPG 96
ND1Xla    KGPNIVGPTGLIQPIADGVKLFIKEPVRPSTSSQTMFLIAPTMALALAMSIWAPLPMP-- 97
ND1Zma    IGPEYAGPLGLLQAIADGTKLLLKEDILPSRGDIPLFSIGPSIAVISILLSFLVIPLGYR 120
ND1Gga    KGPNIVGPFGLLQPVADGVKLFIKEPIRPSTSSPFLFIITPILALLLALTIWVPLPLP-- 99
ND1Bta    KGPNVVGPYGLLQPIADAIKLFIKEPLRPATSSASMFILAPIMALGLALTMWIPLPMP-- 92
ND1Hsa    KGPNVVGPYGLLQPFADAMKLFTKEPLKPATSTITLYITAPTLALTIALLLWTPLPMP-- 92
EchBMba   VGP------PLLQPYYDVKKLLSKDNMVVNPSQN--FYVVVYLAFIILSLFMLVFKQD-- 85
HycDEco   RGP------GVLQEYRDIIKLLGRQSVGP-DASGWVFRLTPYVMVGVMLTIATALPVVTV 87
HyfCEco   RGP------GIWQDYRDIHKLFKRQEVAP-TSSGLMFRLMPWVLISSMLVLAMALPLFIT 95

NuoHEco   --------WVVADLNIGILFFLMMAGLAVYAVLFAGWSSNNKYSLLGAMRASAQTLSYEV 158
Nqo8Pde   --------WVMANINVGILFIFAASSLEVYGVIMGGWASNSKYPFLASLRSAAQMISYEV 159
Nqo8Tth   GSFFGYQPWVIN-LDLGILYLFAVSELAVYGIFLSGWASGSKYSLLGSLRSSASLISYEL 164
NuoHRca   --------WVMADINVAVLFVFAVSSLEVYGVIMGGWASNSKYPFLGSLRSAAQMISYEV 159
ND1Yli    --------ITLGELNLGILFSLAIGSLGVFGSLLSGWSSNSKYSLLGSIRSTAQLISYEL 148
ND1Xla    --------FSLADLNLGILFILALSSLAVYTILGSGWSSNSKYALIGALRAVAQTISYEV 149
ND1Zma    --------FVLADLSIGVFLWIAISSIAPIGLLMAGYSSNNKYSFSGGLRAAAQSISYEI 172
ND1Gga    --------FPLADLNLGLLFLLAMSSLTVYSLLWSGWASNSKYALIGALRAVAQTISYEV 151
ND1Bta    --------YPLINMNLGVLFMLAMSSLAVYSILWSGWASNSKYALIGALRAVAQTISYEV 144
ND1Hsa    --------NPLVNLNLGLLFILATSSLAVYSILWSGWASNSNYALIGALRAVAQTISYEV 144
EchBMba   -----------------FLMIIFVYTVASVALVVGGMSTGSPYARIGSSREIMAILSYEP 128
HycDEco   --------GSPLPQLGDLITLLYLFAIARFFFAISGLDTGSPFTAIGASREAMLGVLVEP 139
HyfCEco   --------VSPFAGGGDLITLIYLLALFRFFFALSGLDTGSPFAGVGASRELTLGILVEP 147
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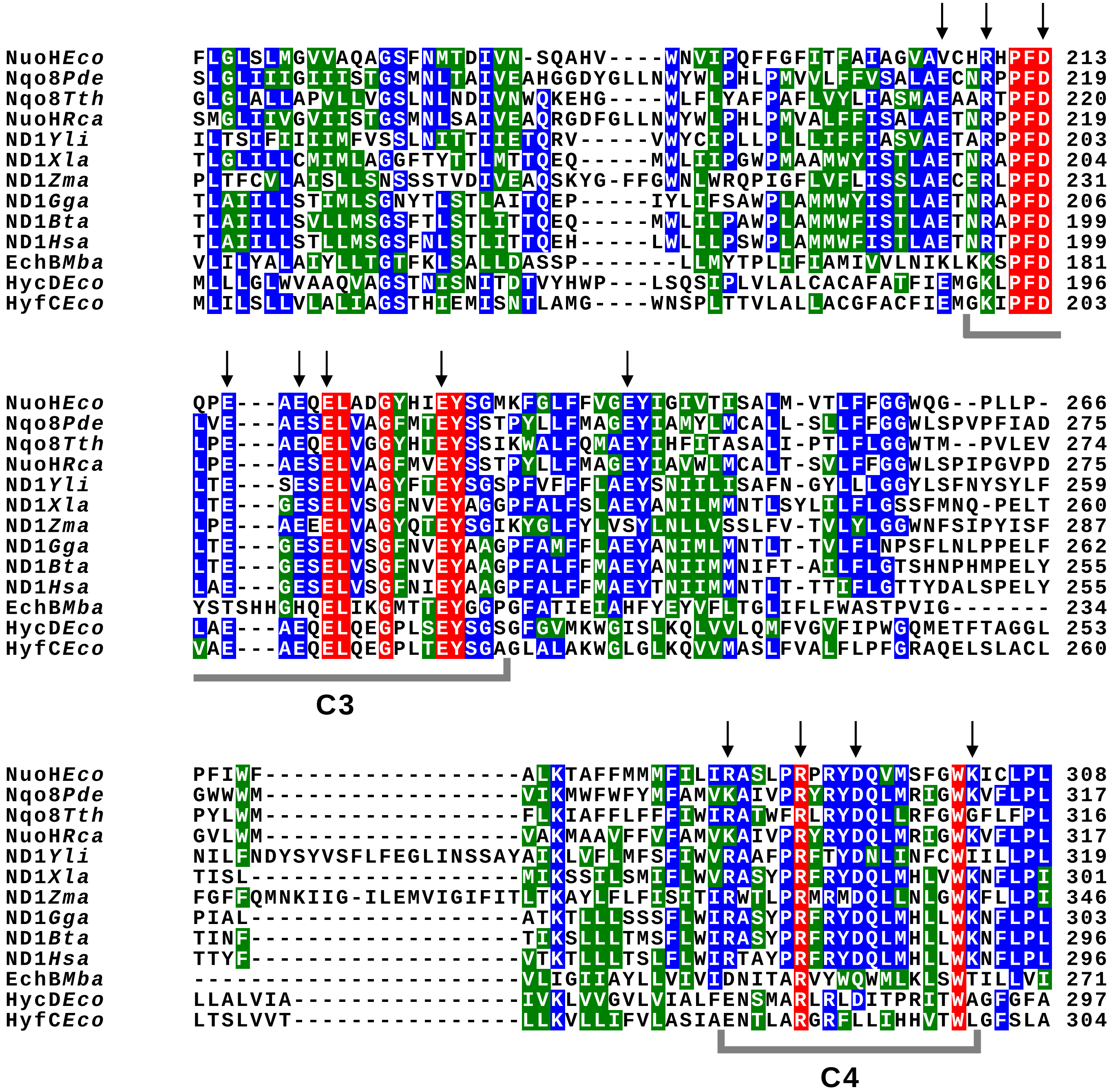

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NuoHEco    FLGLSLMGVVAQAGSFNMTDIVN-SQAHV----WNVIPQFFGFITFAIAGVAVCHRHPFD 213
Nqo8Pde    SLGLIIIGIIISTGSMNLTAIVEAHGGDYGLLNWYWLPHLPMVVLFFVSALAECNRPPFD 219
Nqo8Tth    GLGLALLAPVLLVGSLNLNDIVNWQKEHG----WLFLYAFPAFLVYLIASMAEAARTPFD 220
NuoHRca    SMGLIIVGVIISTGSMNLSAIVEAQRGDFGLLNWYWLPHLPMVALFFISALAETNRPPFD 219
ND1Yli     ILTSIFIIIIMFVSSLNITTIIETQRV-----VWYCIPLLPLLLIFFIASVAETARPPFD 203
ND1Xla     TLGLILLCMIMLAGGFTYTTLMTTQEQ-----MWLIIPGWPMAAMWYISTLAETNRAPFD 204
ND1Zma     PLTFCVLAISLLSNSSSTVDIVEAQSKYG-FFGWNLWRQPIGFLVFLISSLAECERLPFD 231
ND1Gga     TLAIILLSTIMLSGNYTLSTLAITQEP-----IYLIFSAWPLAMMWYISTLAETNRAPFD 206
ND1Bta     TLAIILLSVLLMSGSFTLSTLITTQEQ-----MWLILPAWPLAMMWFISTLAETNRAPFD 199
ND1Hsa     TLAIILLSTLLMSGSFNLSTLITTQEH-----LWLLLPSWPLAMMWFISTLAETNRTPFD 199
EchBMba    VLILYALAIYLLTGTFKLSALLDASSP-------LLMYTPLIFIAMIVVLNIKLKKSPFD 181
HycDEco    MLLLGLWVAAQVAGSTNISNITDTVYHWP---LSQSIPLVLALCACAFATFIEMGKLPFD 196
HyfCEco    MLILSLLVLALIAGSTHIEMISNTLAMG----WNSPLTTVLALLACGFACFIEMGKIPFD 203

NuoHEco    QPE---AEQELADGYHIEYSGMKFGLFFVGEYIGIVTISALM-VTLFFGGWQG--PLLP- 266
Nqo8Pde    LVE---AESELVAGFMTEYSSTPYLLFMAGEYIAMYLMCALL-SLLFFGGWLSPVPFIAD 275
Nqo8Tth    LPE---AEQELVGGYHTEYSSIKWALFQMAEYIHFITASALI-PTLFLGGWTM--PVLEV 274
NuoHRca    LPE---AESELVAGFMVEYSSTPYLLFMAGEYIAVWLMCALT-SVLFFGGWLSPIPGVPD 275
ND1Yli     LTE---SESELVAGYFTEYSGSPFVFFFLAEYSNIILISAFN-GYLLLGGYLSFNYSYLF 259
ND1Xla     LTE---GESELVSGFNVEYAGGPFALFSLAEYANILMMNTLSYLILFLGSSFMNQ-PELT 260
ND1Zma     LPE---AEEELVAGYQTEYSGIKYGLFYLVSYLNLLVSSLFV-TVLYLGGWNFSIPYISF 287
ND1Gga     LTE---GESELVSGFNVEYAAGPFAMFFLAEYANIMLMNTLT-TVLFLNPSFLNLPPELF 262
ND1Bta     LTE---GESELVSGFNVEYAAGPFALFFMAEYANIIMMNIFT-AILFLGTSHNPHMPELY 255
ND1Hsa     LAE---GESELVSGFNIEYAAGPFALFFMAEYTNIIMMNTLT-TTIFLGTTYDALSPELY 255
EchBMba    YSTSHHGHQELIKGMTTEYGGPGFATIEIAHFYEYVFLTGLIFLFWASTPVIG------- 234
HycDEco    LAE---AEQELQEGPLSEYSGSGFGVMKWGISLKQLVVLQMFVGVFIPWGQMETFTAGGL 253
HyfCEco    VAE---AEQELQEGPLTEYSGAGLALAKWGLGLKQVVMASLFVALFLPFGRAQELSLACL 260
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C3

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NuoHEco    PFIWF------------------ALKTAFFMMMFILIRASLPRPRYDQVMSFGWKICLPL 308
Nqo8Pde    GWWWM------------------VIKMWFWFYMFAMVKAIVPRYRYDQLMRIGWKVFLPL 317
Nqo8Tth    PYLWM------------------FLKIAFFLFFFIWIRATWFRLRYDQLLRFGWGFLFPL 316
NuoHRca    GVLWM------------------VAKMAAVFFVFAMVKAIVPRYRYDQLMRIGWKVFLPL 317
ND1Yli     NILFNDYSYVSFLFEGLINSSAYAIKLVFLMFSFIWVRAAFPRFTYDNLINFCWIILLPL 319
ND1Xla     TISL-------------------MIKSSILSMIFLWVRASYPRFRYDQLMHLVWKNFLPI 301
ND1Zma     FGFFQMNKIIG-ILEMVIGIFITLTKAYLFLFISITIRWTLPRMRMDQLLNLGWKFLLPI 346
ND1Gga     PIAL-------------------ATKTLLLSSSFLWIRASYPRFRYDQLMHLLWKNFLPL 303
ND1Bta     TINF-------------------TIKSLLLTMSFLWIRASYPRFRYDQLMHLLWKNFLPL 296
ND1Hsa     TTYF-------------------VTKTLLLTSLFLWIRTAYPRFRYDQLMHLLWKNFLPL 296
EchBMba    -----------------------VLIGIIAYLLVIVIDNITARVYWQWMLKLSWTILLVI 271
HycDEco    LLALVIA----------------IVKLVVGVLVIALFENSMARLRLDITPRITWAGFGFA 297
HyfCEco    LTSLVVT----------------LLKVLLIFVLASIAENTLARGRFLLIHHVTWLGFSLA 304
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C4

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NuoHEco      TLINLLVTAAVILWQAQ-------------------------------- 325
Nqo8Pde      SLGWVVLVAILARYEILGGFWARFAVGG--------------------- 345
Nqo8Tth      ALLWFLVTALVVALDLPRTYLLYLSALSFLVLLGAVLYTPKPARKGGGA 365
NuoHRca      SLAWVVVVAFLAKFEVLGGFWARWSIGA--------------------- 345
ND1Yli       LFGIFLIIPSTLYIFDSFPTLI--------------------------- 341
ND1Xla       TLAMTLWHISLPISMLGLPSQT--------------------------- 323
ND1Zma       SLGNLLLTTSSQLVSL--------------------------------- 362
ND1Gga       TLALCLWHTSMPISYAGLPPI---------------------------- 324
ND1Bta       TLALCMWHVSLPILTSGIPPQT--------------------------- 318
ND1Hsa       TLALLMWYVSMPITISSIPPQT--------------------------- 318
EchBMba      SLVNIDTCTLVESN----------------------------------- 285
HycDEco      FLAFVSLLAA--------------------------------------- 307
HyfCEco      ALAWVFWLTGL-------------------------------------- 315
```

Supplemental Figure 1. Similarity of the deduced amino acid sequences among the *E. coli* NuoH subunit and its homologues from various organisms. The alignment was carried out using the ClustalW program (1). Red boxes with white letters show perfectly conserved residues among the listed organisms. Blue boxes with white letters illustrate conserved residues among at least seven organisms. Green boxes with white letters are residues that are similar among at least seven organisms. Amino acids mutated in this study are marked by arrows. Sequence sources and their Swiss-Prot accession numbers are: NuoH*Eco, E. coli* K-12 NuoH subunit [P0AFD4]; Nqo8*Pde, P. denitrificans* Nqo8 subunit [P29920]; Nqo8*Tth, T. thermophilus* Nqo8 subunit [Q60019]; NuoH*Rca, R. capsulatus* NuoH subunit [P42032]; ND1*Yli, Y. lipolytica* ND1 subunit [Q9B6E8]; ND1*Xla, X. laevis* ND1 subunit [P03890]; ND1*Zma, Z. mays* chloroplast ND1 subunit [P25706]; ND1*Gga, Gallus gallus* ND1 subunit [P18936]; ND1*Bta, B. taurus* ND1 subunit [P03887]; ND1*Hsa, H. sapiens* ND1 subunit [P03886]; EchB*Mba, Methanosarcina barkeri* EchB subunit [O59653]; HycD*Eco, E. coli* K-12 HycD subunit [P16430]; HyfC*Eco, E. coli* K-12 HyfC subunit [P77858].

Reference List

1. Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A., Lopez, R., Thompson, J. D., Gibson, T. J., and Higgins, D. G. (2007) *Bioinformatics*. **23,** 2947-2948