

NuoLEcoli ---MNMLALTIILPLIGFVLLAFSRGRWSENVSAIVGVGSVGLAALVTAFIGVDFFANGE 57
 Nqo12Thermus ---MALLG-TILLPLLLGFALLLGLFGKRMREPLPGVLASGLVLAFLGAGLLLSGG---- 52
 Nqo12Paracoccus -----MEKFVLFAPLIASLIAGLGWRAIGEKAQYLTGGVLFSLISWYLFSLFDGVP- 54
 NuoLRhodobacter -----MTTIIILLAPLLGALIGGGFWRLITEKGAIVVTTGLLFLSCILSWVFLTLPAET- 54
 NuoLPseudomonas ---MNLLPLTFLFPLVGFLLLSFSRGRWSENLVGVGSVGLSALSAAWAIFSFHSSPP 57
 NuoLStreptomyces --MENLIALLVAAPLLGAVVL-LCGRRLLDRVGHWIGTLLAAVSFVLGVVLFADLLGSGA 57
 ND5Neurospora --MY---LSIIILPLLSIVAGFFGRKVGVSQAQLITCLSVIITGLAILAFFEVGFNNI 55
 ND5Yarrowia --MYNAISLIIILPCISWLFPLFFGRQLGYVVFVTRMTSTLIIITTLITYYYFYQLLGN 58
 ND5Bovine MNMFSLSLVTLTLLTTPIMMMSFNTYKPSNYPLYVKT-AISYAFITSMIPTMMFIHSGQ 59
 ND5Human MTMHTTMTTLTTLTSLIPPILTTLVNPKNKNSYPHYVKS-IVASTFIISLFPPTTMFCLDQ 59

NuoLEcoli Q--TYSQPLWTWMSVGFNIGFNLVLDGLSLTMLS SVTVGVGFLIHMYASWYMRGEEG--- 112
 Nqo12Thermus -----ARFQAEWLPG----IPFSLLLDNLSGFMLLIVTVGVGFLIHVYAIGYMGDDPG--- 100
 Nqo12Paracoccus ----RHIPVLDWVVTGDFHAEWAIRLDRDLTAI MLIVVTVSALVHMYS LGYMAHDDNWTH 110
 NuoLRhodobacter ----QHIIHLLDWIRSGALDTSWGI RLDRDLTAI MLIVVTVSALVHLYSWGYMAHDENWTH 110
 NuoLPseudomonas EGGAYSLVLWQWMAAGDFSTNFTLYLDGLSVTMLGVVTVGVGFLIHLFASWYMRGETG--- 114
 NuoLStreptomyces EDRTLTLQHLSWIPVEGFQADVAFRDLQLSMTFVLLITGVGSLIHLYSVAYMEHDERR-- 115
 ND5Neurospora P---VTINLFRWIDSEWYNILWGFQFDSLTVAMLIPVLIISLVHIYSISYMSHDPHN-- 110
 ND5Yarrowia P---INLELFNYLDINIDYLDINYNFEIDALTITMLLAITTISSMVHIYSISYMETDPH--- 112
 ND5Bovine E---LIISNWHWLTIQTLKLSL SFKMDYFSMMFIPVALFVTWSIMEFSMWYMYSDPNIN- 115
 ND5Human E---VIISNWHWATTQTTQLSLSFKLDYFSMMFIPVALFVTWSIMEFSLWYMNSDPNIN- 115

NuoLEcoli ----YSRFFAYTNLFIASMVVVLVADNLLMYLWGEVGLCSYLLIGFYITDPKNGAAM 168
 Nqo12Thermus ----YSRFFAYFNLFIAMMLTLV LADSYPVMFIGWEGVGLASFLIGFWYKPNQYADSAR 156
 Nqo12Paracoccus DEHYKARFFAYLSFFTFAMLMVLTADNLLQMFFGWEGVGVASYLLIGFYKKASANAAM 170
 NuoLRhodobacter HEAYKARFFAYLSFFTFAMLMVLTSDNLVQMFFGWEGVGVASYLLIGFYKKPSANAAAI 170
 NuoLPseudomonas ----YSRFFAYTNLFIASMLFLVLGDNLLFLYFGWEGVGLCSYLLIGFYYSNRNNGNAAL 170
 NuoLStreptomyces -----RRFFGYLNLFLAAMLILVLADNYLLLYVWEGVGLAS YLLIGFWQHKPSAATAAK 170
 ND5Neurospora -----QRFFSYLSLFTFMMIILV TANNYLLMFVWEGVGVCSYLLVSFWFTRIAANQSSM 165
 ND5Yarrowia ----QVRFFSLLSMFTFMIIILV TGSNYFVLVFWGVEFIGVTSYLLISFWVTRLQAMKSAL 168
 ND5Bovine -----KFFKYLLFLITMLILV TANNL FQLFIGWEGV GIMSFLLIGWYGRADANTAAL 169
 ND5Human -----QFFKYLLIFLITMLILV TANNL FQLFIGWEGV GIMSFLLISWYARADANTAAI 169

NuoLEcoli KAFVVTRVGDVFLAFALFILIYNELGTLNFREMV-ELAPAHFADGN-----NMLMWATLM 221
 Nqo12Thermus KAFIVNRIGDLGFMLGMAILWALYGTLSISELK-EAMEGPKLKNPD-----LLALAGLL 208
 Nqo12Paracoccus KAFIVNRVGDGFLLGIFGIYWL TGSVQFDEIFRQVPQLAQTEIDFLWRDWNANLLGFL 230
 NuoLRhodobacter KAFVVNRVGDGFALGIMGLFFLTDSIDMDVIFASAPELAKTELHFLAWEFNAANLLAVL 230
 NuoLPseudomonas KAFIVTRVGDVFMAFGFLFILFQQFGTLNIQELL-VLAPQKFPEGN-----LWLTLATLA 223
 NuoLStreptomyces KAFIVNRVGD MGLSIGIMLMFLWFGTFAFGPVLGGHGEAGLAAGADEG---KLTAIALM 226
 ND5Neurospora SAFLTNRVGD CFLTIGMFVVLWTLGNLDYATVFS-----LAPYINSDIATIIGIC 215
 ND5Yarrowia SAVLMNRFGDAFFVLGLCVIAYVFGTLNYSTIFATAYLIN-----TDLLVLIMLA 218
 ND5Bovine QAILYNRIGDIGFILAMAWFLTNLNTWDLQQIFMLNPSDS-----NMPLIG 215
 ND5Human QAILYNRIGDIGFILALAWFILHSNSWDPQQMALLNANPS-----LTPLLG 215

NuoLEcoli LLGGAVGKSAQLPLQ~~TW~~LADAMAGPTPV~~SAL~~IHAATMVTAGVYLIAR~~THGL~~FLMTPEVLH 281
 Nqo12Thermus LFLGAVGKSAQIPLMVWLPDAMAGPTPV~~SAL~~IHAATMVTAGVYLIAR~~SSFL~~YSVLPDVS~~Y~~ 268
 Nqo12Paracoccus L~~FV~~GAMGKSAQL~~LLHT~~WLPDAMEGPTPV~~SAL~~IHAATMVTAGVFLVCRMSPL~~YEF~~APDAKN 290
 NuoLRhodobacter L~~FI~~GAMGKSAQL~~FLHT~~WLPDAMEGPTPV~~SAL~~IHAATMVTAGVFLVCRMSPL~~FEY~~APEAKM 290
 NuoLPseudomonas LLGGAVGKSAQLPLQ~~TW~~LADAMAGPTPV~~SAL~~IHAATMVTAGVYLIAR~~CHGL~~F~~TL~~APDILE 283
 NuoLStreptomyces LLLAA~~CG~~KSAQVPLQ~~SW~~LGDAMEGPTPV~~SAL~~IHAATMVTAGVYLI~~VR~~SGAIFNGAPDAQL 286
 ND5Neurospora LLIGAMAKSSQVGLHVWLP~~MAME~~GPTPV~~SAL~~IHAATMVTAGVYLLMRSSPLIEYSSTVLL 275
 ND5Yarrowia LFIAAMAKSAQFGLHNWLT~~LAME~~GPTPV~~SLL~~HAATLV~~TAGI~~YLLLR~~SAN~~ILEYTP~~TV~~LF 278
 ND5Bovine LALAATGKSAQFGLHPWLP~~SAME~~GPTPV~~SALL~~HSSTMV~~VAGIF~~LLIRFYPL~~TENN~~KYIQS 275
 ND5Human LLLAAAGKSAQLGLHPWLP~~SAME~~GPTPV~~SALL~~HSSTMV~~VAGIF~~LLIR~~FHPL~~AENSPLIQ~~T~~ 275

NuoLEcoli LVGIVGAVTLLLAGFAALVQ~~TD~~IKRVLAYSTMSQIGYMFLALGVQ--AWDA~~AI~~FHLMTHA 339
 Nqo12Thermus AIAVVGLLTAAYGALS~~AF~~GQ~~TD~~IKKIVAYSTISQLGYMFLAAGVG--AYWV~~AL~~FHV~~F~~THA 326
 Nqo12Paracoccus FIVII~~GAT~~TAF~~FAAT~~VGLVQ~~ND~~IKRVIAYSTCSQLGYMFVAAGVG--VYS~~AM~~FHLLTHA 348
 NuoLRhodobacter MVVYVGAVTAF~~FAAT~~VGLVQ~~ND~~IKRVIAYSTCSQLGYMFVAAGSG--VYSV~~AM~~FHLLTHA 348
 NuoLPseudomonas LVGIVGAVTLVL~~LAG~~FAALVQ~~TD~~IKRILAYSTMSQIGYMFLALGVQ--AWDA~~AI~~FHLMTHA 341
 NuoLStreptomyces VVTIVGAVTLL~~FGA~~IVGCAKDDIKKALAGSTMSQIGY~~VLA~~AGLGP~~IGY~~VFAIMHLVTHG 346
 ND5Neurospora LCLWLGAITTVFSSLIGL~~FQ~~DIKKVIAYSTMSQLGMMVIAIGLS--SYNV~~AL~~FHLINHA 333
 ND5Yarrowia IILWIGALT~~TL~~SAGLIAICSN~~DL~~KRIIALSTMSQLGMMTIAIGLS--AYNL~~AL~~FHLLGHA 336
 ND5Bovine ITLCLGAIT~~TL~~FTAMCALTQ~~ND~~IKKIIAFSTSSQLGLMMVTIGIN--QPYL~~AF~~LHIC~~THA~~ 333
 ND5Human LTLCLGAIT~~TL~~FAAVCAL~~TQ~~NDIKKIVAFSTSSQLGLMMVTIGIN--QPHL~~AF~~LHIC~~THA~~ 333

NuoLEcoli FFKALLFLASGSVILACHH-EQNIFKMGG~~L~~RKSIPLVYL~~CFL~~VGGAALS--ALPLVTA-G 395
 Nqo12Thermus FFKALLFLASGSVIHALGG-EQDVRKMGG~~L~~WKHL~~PQ~~TRW~~HAL~~IGALALG--GLPLLS--G 381
 Nqo12Paracoccus FFKAMLFLGAGSVIHAMHH-EQDMRNYG~~L~~RKKI~~PL~~TFWAMMIGTF~~AIT~~GVGIPL-THLG 406
 NuoLRhodobacter FFKAMLFLGAGSVIHAMHH-EQDMRNYG~~L~~RKKI~~P~~TFAIMMIG~~TL~~AITGVGIPFFSIGG 407
 NuoLPseudomonas FFKALLFLASGAVIVACHH-EQNIFKMGG~~L~~WKKL~~PL~~AYAS~~SV~~VGGAALA--ALPFLTA-G 397
 NuoLStreptomyces FFKAGLFLGAGSVMHGMND-EVDMRRY~~G~~L~~R~~KYMPVT~~FV~~TFGLGYLAI--GFPGLS--G 401
 ND5Neurospora FYKALLFLGAGSVIHAVAD-NQDFRKF~~G~~L~~K~~NYL~~PL~~TYSVMLIASLSLV--AFPYMT--G 388
 ND5Yarrowia FFKALLFMSAGSIIHSILNESQDIRTYG~~L~~LSYL~~P~~TYICITIASLSLM--AMPGLT--G 392
 ND5Bovine FFKAMLFMCSGSIHSLND-EQDIRKMGG~~L~~FKAMP~~FT~~TALIVGSLALT--GMPFLT--G 388
 ND5Human FFKAMLFMCSGSIHNLNN-EQDIRKMGG~~L~~LK~~TM~~PLTSTSLTIGSLALA--GMPFLT--G 388

NuoLEcoli -----FYSKDEILAGAMAN--GHINLMVAG--LVGAFM~~TS~~LYTFRMIFIVFHGKEQI- 443
 Nqo12Thermus -----FWSKDAILAATLTYPFGGVGFYVGA--LLVAVLTAM~~Y~~AMRWFVLVFLGEERG- 431
 Nqo12Paracoccus ----FAGFLSKDAIIESAYA-GSGYAFWLL----VIAACFTSFYSWRLIFLTFY~~G~~KPRG- 456
 NuoLRhodobacter VPVGFAGYLSKDAIIESAFASGN~~G~~FAFYVL----VAAAGM~~TS~~SFYSWRLIFLTFYGEARG- 462
 NuoLPseudomonas -----FYSKDEILWEAFAS--GHRELLIAG--LVGAFL~~TS~~IYTFRLIFVAFHGE~~P~~KT- 445
 NuoLStreptomyces -----FYSKDKIIEAFAKG-GTEGWILGACALLGA~~AIT~~AYMTRV~~ML~~MTFFGEERWR 453
 ND5Neurospora -----FYSKDFILESAYG-QFSFSGVAVYIIATIGAI~~FT~~TLYSVKVL~~YL~~TFLANPN~~GY~~ 440
 ND5Yarrowia -----YYTKDIIIESTYG-SYSISNYVVYWIAYLSAVL~~TC~~VYSMKILY~~LT~~TFYSNPN-- 442
 ND5Bovine -----FYSKDLIEAANTSYTNAWALLMT---LIATSFTAIYSTR~~I~~IFFALLGQPRFP 438
 ND5Human -----FYSKDHIIETANMSYTNAWALSIT---LIATSLTSAYSTRMILL~~TL~~TGQPRFP 438

NuoLEcoli -----HAHAVKGVTHSL-PLIVLLLIL 463
 Nqo12Thermus -----HHHPHEAPPVMLWPNHLLALG 452
 Nqo12Paracoccus -----DHHAHDHAHESPPVMTIPLGVLAIG 481
 NuoLRhodobacter -----DHHKHDHAHESPAVMLAPLALLAVG 487
 NuoLPseudomonas -----EAHAGHGISHWL-PLSVLIVL 465
 NuoLStreptomyces -----NAPTPSPAKPDVEPAEETRGTYEPPHPHESPLMTIPMIVLAVG 497
 ND5Neurospora IHFYRHFILYERLYVYVSYTGKEEFYLPKHMSKEINNLRPSVSGEGGFLLSLPLVILALF 500
 ND5Yarrowia -----NNTITYYNAHESNIYITLPMFILAIF 460
 ND5Bovine -----TLVNINENNPLLINSIKRLLIG 460
 ND5Human -----TLTNINENNPTLLNPIKRLAAG 460

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NuoLEcoli STFVGALIVPPLQG-----VLPQTTELAHG----- 488
 Nqo12Thermus SVLAGYLALPHPL-----PNVLEPFLKPALAEV----- 480
 Nqo12Paracoccus AVFAGMVWYGPFFGDHHKVT--EYFHIAGAHHEAAEGEE--AEHATAEAPVEHAVADTAT 537
 NuoLRhodobacter SVLAGMVWYHSFFGD--KVA--SFFNLPAAAHEAHGTEHATEGHVPEAAMTAEAAHEAA 543
 NuoLPseudomonas STFVGALITP-----PLAGVLPESVGHAGGE----- 491
 NuoLStreptomyces SVGAGAFFSIG-----DRFMHWLEPITGHSHGD----- 525
 ND5Neurospora SIFFGFITKDFIFIGLGSNFFIDNSLFIHPIHEIMIDTEF----- 539
 ND5Yarrowia AMFAGWILKDIYLGVTDFVGT--HILPNNFSYFDTEF----- 504
 ND5Bovine SLFAGYIISN-----NIPPTTIPQMTM----- 482
 ND5Human SLFAGFLITN-----NISPPSPFQTT----- 481

NuoLEcoli -----SML-----TLEITSGVAVVVGILLAAWLWLGK 515
 Nqo12Thermus -----EAHHLSLGA-----EWGLIALSAAVALLGLWAG- 508
 Nqo12Paracoccus AEG---EAAAEAEHAEIAAPVGGAIYMHPDNHIMDEAHHAPAWVKVSPFVAMVLGLITAW 594
 NuoLRhodobacter MAGTMAMAEPAAEHAVAKAPQG-AIFMAETNHVIHDAHGVPDWVKLSPFGAMVTGFFFAW 602
 NuoLPseudomonas -----AKHS-----LELASGAIAGILLAALLFLGQ 518
 NuoLStreptomyces -----S-----PISAGAVTGATVACMVIGVAVAWAQY 552
 ND5Neurospora -----AV-----PTLFKLLPFIFTISFSLIALVLSEKY 567
 ND5Yarrowia -----SITQFYKLLP-----LISAIVSILIVVLENEF 532
 ND5Bovine -----PYYLKTALIVTILGFILALEISN 506
 ND5Human -----IPLYLKLTAVALTFLGLLTALDLNY 506

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NuoLEcoli RTLVTSIANSAPGRLLGTWY----NAWGFWDLYDKVFKPFLGIAWLKRDPLNSMMN- 570
 Nqo12Thermus FVFFQRKVFPAWYLAFEAASR----EAFYVDRAYNALIVNPLKALAEALFYGDRGLLSG- 563
 Nqo12Paracoccus TFYIANPSLPRRLAAHEPALYRFLLNKWFDEIYEFIFVRPAKWLGRVLWKGGDGAVIDG 654
 NuoLRhodobacter LYYIGDKPLPGRTARALPGLYRFLLNKWFDELFDLLFVNPAKSLGRKLWKGGDGAVIDG 662
 NuoLPseudomonas RRFVSALAKSAPGRFFGTWY----HAWGFWDLYDKLFPYLLLCQLLGRDPIDRTL- 573
 NuoLStreptomyces GRRPVPVAVAPR-GSLLTRAAR----RDLLQDDFNHVVLRGGEHLTRSLVYVDHTVV-DG 606
 ND5Neurospora PNLVVHFKLSRLGYNLFGFFNQRFLLVLFYNYKITNLVLDLGGQITKILDKGSIELGPF 627
 ND5Yarrowia A--IVFNLNKYYINTVYSIFNQKLVSQILNHFIIFKGLVTSNGIAHVVDKGSYLRLGPV 590
 ND5Bovine MTKNLKYHYPSNAFKFSTLLG-----YFPTIMHRLAPYMNLSMSQKSASSLLDLIWL 559
 ND5Human LTNKLMKKSPLCTFYFSNMLG-----FYPSITHRTIPYLGLLTSQNLPLLLLDLTWLE 559

NuoLEcoli	-IPAV--LSRFAGKG-LLLS <u>ENGYLRWYVASMSIGAVVVLALLMVL</u> R-----	613
Nqo12Thermus	-YFGLGGAARSLGQG-LARL <u>QTGYLRVYALLFVLGALLLLGVM</u> RW-----	606
Nqo12Paracoccus	T <u>INGVAMGLIPRLTRA</u> AVRV <u>QSGYLFHYAFAMVLGIVGLLIWVM</u> MARGAH-----	703
NuoLRhodobacter	A <u>INGLALGWIPFFTR</u> VAGRI <u>QSGYLFHYAFAMVLGIVALMFV</u> VVRTGGMN-----	712
NuoLPseudomonas	- <u>VVPF</u> --SVRGGHNL-LSLT <u>ENGRLRWYAASLV</u> GGAA <u>ILLGALL</u> LA-----	615
NuoLStreptomyces	- <u>VVNGTAASVGG</u> LSGRMRRL <u>QNGFARSYAVSMF</u> GGAA <u>LLVAATLLM</u> RAV-----	654
ND5Neurospora	<u>GLEKVLIKWSKDIAS</u> LSSTS <u>IVTNYALFILVGFILYVFTFIS</u> LLEGGLDLNLSL <u>FILL</u> LSL	687
ND5Yarrowia	<u>G</u> INRLLNKASYNVINLSSNTR <u>SS</u> ----LSMNSML <u>LILITIVS</u> LLLLVLVMNVNFI <u>IIVIPVL</u>	646
ND5Bovine	A <u>ILP</u> KTISLAQMKA <u>STLVTN</u> <u>QKGLIKLYFLSFLITILIS</u> MILFN <u>FHE</u> -----	606
ND5Human	<u>KLLP</u> KTISQHQISTSIITST <u>QKGMIKLYFLSFFFPLILIT</u> LLLLIT-----	603
NuoLEcoli	-----	
Nqo12Thermus	-----	
Nqo12Paracoccus	-----	
NuoLRhodobacter	-----	
NuoLPseudomonas	-----	
NuoLStreptomyces	-----	
ND5Neurospora	TSSTSSSDSKEGKMIKKAVVSTKNKNIR	715
ND5Yarrowia	<u>IS</u> ----- <u>ILYILFS</u> -----	655
ND5Bovine	-----	
ND5Human	-----	

Supplemental Figure 1. Comparison of the amino acid sequence of *E. coli* NuoL subunit with its homologs. The totally conserved residues are shown in red letters, while blue letters indicate residues conserved in almost 60% of the cases. Amino acids that are conserved in almost 50% of the cases or the residues having similar characteristics in about 80% of the cases are shown in green letters. Yellow shadows indicate the predicted transmembrane helices. Potential transmembrane helices are underlined. Red arrows indicate residues mutated for a STOP codon. Green arrows point at the location where the HL helix was truncated in the Δ V519-K581 mutant. Sequence sources and their accession numbers are: NuoLEcoli: *Escherichia coli* K-12 [P33607], Nqo12Thermus: *Thermus thermophilus* [Q56227], Nqo12Paracoccus: *Paracoccus denitrificans* [P29924], NuoLRhodobacter: *Rhodobacter capsulatus* [P50939] NuoLPseudomonas: *Pseudomonas aeruginosa* [15597843], NuoLStreptomyces: *Streptomyces coelicolor* A3 [21222956], ND5Neurospora: *Neurospora crassa* [1171827], ND5Yarrowia: *Yarrowia lipolytica* [Q9B6D3], ND5Bovine: *Bovis taurus* [28381381], ND5Human: *Homo sapiens* [P03915].