Supplementary Table and Figures for Johnson & Edlind, "Mutational Analysis of *Saccharomyces cerevisiae* Fks1"

TABLE S1. Primers used in this study.[a]

pJK90F	5'-TGGCATGTGAGATTCTCCGA-3'
pJK90R	5'-CGTCATATGGATAGGAGCCT-3'
ScFks1-1TAGF	5'-GTGGTTTGTTACGCATGCAAGCTTGATATCGAAATGAACACTGATCAACAACCTT-3'
ScFks1-1204TAGF	5'-GTGGTTTGTTACGCATGCAAGCTTGATATCGAAATGACCACTAGAGGTGGTGT-3'
ScFks1-300TAGR	5'-TGGTCTAGAGGTGTAACCACTTGAGTTCTTAGGTTCCAGGGGAGACAACTGG-3'
ScFks1-445TAGR	5'-TGGTCTAGAGGTGTAACCACTTGAGTTCTTAGGCCAAGTACGGGTCTCTTTATA-3'
ScFks1-475TAGR	5'-TGGTCTAGAGGTGTAACCACTTGAGTTCTTAGGGTAAAATGTTGGTGAATTATATGC-3'
ScFks1-531TAGR	5'-TGGTCTAGAGGTGTAACCACTTGAGTTCTTAGGTCTACGAGATAGATGTTGAG-3'
ScFks1-561TAGR	5'-TGGTCTAGAGGTGTAACCACTTGAGTTCTTAGGTGCAGCAGTGGAGTAGAC-3'
ScFks1-610TAGR	5'-TGGTCTAGAGGTGTAACCACTTGAGTTCTTAGGAAATGCAGCAGTGAATGTTTGA-3'
ScFks1-637TAGR	5'-TGGTCTAGAGGTGTAACCACTTGAGTTCTTAGGGTACGATTCTGAATATTTGGCA-3'
ScFks1-650TAGR	5'-TGGTCTAGAGGTGTAACCACTTGAGTTCTTAGGAATTCTAATTGGATCTCTCAAAG-3'
ScFks1-669TAGR	5'-TGGTCTAGAGGTGTAACCACTTGAGTTCTTAGGACAAAGTACCGCACCCCAC-3'
ScFks1-675TAGR	5'-TGGTCTAGAGGTGTAACCACTTGAGTTCTTAGGAATCTTGGGTTGCACTTTACAA-3'
ScFks1-680TAGR	5'-TGGTCTAGAGGTGTAACCACTTGAGTTCTTAGGAACCAAACCTAAGACAATCTTG-3'
ScFks1-685TAGR	5'-TGGTCTAGAGGTGTAACCACTTGAGTTCTTAGGGAAGTCGGTAGCGATAACCA-3'
ScFks1-715TAGR	5'-TGGTCTAGAGGTGTAACCACTTGAGTTCTTAGGGATAGAAATACCTAAATAGAAAGA-3'
ScFks1-726TAGR	5'-TGGTCTAGAGGTGTAACCACTTGAGTTCTTAGGCAATCTTGTGAAGATATTTCT-3'
ScFks1-742TAGR	5'-TGGTCTAGAGGTGTAACCACTTGAGTTCTTAGGAATTTCCATATCAGTAGTAGCC-3'
ScFks1-1008TAGR	5'-TGGTCTAGAGGTGTAACCACTTGAGTTCTTAGGAGCCAATCTCTGCATAGAGAC-3'
ScFks1-1294TAGR	5'-TGGTCTAGAGGTGTAACCACTTGAGTTCTTAGGGGCATAATAGAATGTTAGGAA-3'
ScFks1-1354TAGR	5'-TGGTCTAGAGGTGTAACCACTTGAGTTCTTAGGCCAATCAACCGCAGGTTGG-3'
ScFks1-1380TAGR	5'-TGGTCTAGAGGTGTAACCACTTGAGTTCTTAGGTTCAATTAGTTCTTGAACAACA-3'
ScFks1-1410TAGR	5'-TGGTCTAGAGGTGTAACCACTTGAGTTCTTAGGAGAGTAGATTTGGCCCGCAA-3'
ScFks1-1425TAGR	5'-TGGTCTAGAGGTGTAACCACTTGAGTTCTTAGGTATATAACGAGCACCACCAATT-3'
ScFks1-1470TAGR	5'-TGGTCTAGAGGTGTAACCACTTGAGTTCTTAGGAGCTTGCCAATGTGCGACAG-3'
ScFks1-1493TAGR	5'-TGGTCTAGAGGTGTAACCACTTGAGTTCTTAGGCTGATGTGGATTGAAAACGAAA-3'
ScFks1-1550TAGR	5'-TGGTCTAGAGGTGTAACCACTTGAGTTCTTAGGACCAGCAGCTTTCTCAGATTC-3'
ScFks1-1590TAGR	5'-TGGTCTAGAGGTGTAACCACTTGAGTTCTTAGGAGTCTTGACACCGGTTTGAG-3'
ScFks1-1630TAGR	5'-TGGTCTAGAGGTGTAACCACTTGAGTTCTTAGGTAAGGGACCAGAGCAGCATG-3'
ScFks1-1666TAGR	5'-TGGTCTAGAGGTGTAACCACTTGAGTTCTTAGGGCTCTCCAAAACCCACATGA-3'
ScFks1-1707TAGR	5'-TGGTCTAGAGGTGTAACCACTTGAGTTCTTAGGGGCTGTATTGGCATGATCGT-3'
ScFks1-1738TAGR	5'-TGGTCTAGAGGTGTAACCACTTGAGTTCTTAGGTGAAAGCTCAATTACCTTGGC-3'
ScFks1-1775TAGR	5'-TGGTCTAGAGGTGTAACCACTTGAGTTCTTAGGCAGTTGACAGATTAAAATCACAT-3'
ScFks1-1821TAGR	5'-TGGTCTAGAGGTGTAACCACTTGAGTTCTTAGGGGCTACAGCAGGACCAATAA-3'
ScFks1-1876TAGR	5'-TGGTCTAGAGGTGTAACCACTTGAGTTCTTAGGTTTTATAGTTGACCAGGTCTTTA-3'
ScFks1-1830*TAGR	5'-TGGTCTAGAGGTGTAACCACTTGAGTTCTTAGGCTATCCAATGTGTTTGTGGATCTTA-3'
ScFks1-1840*TAGR	5'-TGGTCTAGAGGTGTAACCACTTGAGTTCTTAGGCTATAGATTGTGAACAACGCCATC-3'
ScFks1-1850*TAGR	5'-TGGTCTAGAGGTGTAACCACTTGAGTTCTTAGGCTAGTCATTATTGGTTGTATTTATTGG-3'
ScFks1-1860*TAGR	5'-TGGTCTAGAGGTGTAACCACTTGAGTTCTTAGGCTAACTTTGATAAGTTGACATTTGGG-3'
ScFks1-1870*TAGR	5'-TGGTCTAGAGGTGTAACCACTTGAGTTCTTAGGCTACTTTAATGATGGCGTATGAGTA-3'
ScFks1-1876*TAGR	5'-TGGTCTAGAGGTGTAACCACTTGAGTTCTTAGGTTATTTTATAGTTGACCAGGTCTTTA-3'
ScFks1-301F	5'-AGAGTTCGTCATATCGCCTTA-3'
ScFks1-R319AR	5'-CCAAGTAGTCAAGAGCACACTTGTAGATAAAACATAAACATTCAGCAGTGAATGCGACTTGATTAGCTTC-3'
ScFks1-D1102AF	5'-TACATTCAATTAATTGCTGCCAACCAAGATAACTACTTGGAAGAATGTCTGAAGATTAGA-3'
ScFks1-D1102AR	5'-GTTATCTTGGTTGGCAGCAATTAATTGAATGTATTCACCTCTGTAAAAAATCAAAGCATG-3'
ScFks1-D1197AF	5'-CATTATGGTCATCCGGCTTTCATTAATGCTACGTTTATGACCACTAGAGGTGGTGTTTCC-3'
ScFks1-D1197AR	5'-CGTAGCATTAATGAAAGCCGGATGACCATAATGCAATTTACCACCAATTTGAGATAAAGT-3'

[a] Underline indicates homology with pJK90 plasmid

* Primer incorporates STOP codon following indicated Fks1 residue

FIG. S1. ClustalW2 alignment of Fks1 sequences from fungi representing all four fungal phyla. TMHMM-predicted TMH are shown in red; PRO-TMHMM-predicted TMH are underlined. Hotspots 1, 2, and 3 are indicated.

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S. cerevisiae   -------------------------------------------MNTDQQPYQG------- 10
C. albicans     -----------------------------------------MSYNDNNNHYYDPN----Q 15
A. fumigatus    ----------------------------------------MSGYQQGGGHYNDGYGHQEH 20
C. neoformans   -----------------------------------------MSYPNPPPPPKG------- 12
R. oryzae       -------------------------------MADTNSKETTVDHHDEDEDFIDCAPNFPS 29
A. macrogynus   MGQPAGLNDGFTRYPGSHAGHKPQQACHRTVDRSRLSSNRLSKQLSVQQLQLQLPPDGLS 60


S. cerevisiae   QTDYTQGPGNGQSQEQDY--------DQYGQPLYPSQADG-YYDPNVAAG---------- 51
C. albicans     QGGMPPHQGGEGYYQQQY--------DDMGQ--QPHQQD--YYDPN-------------- 49
A. fumigatus    GDSFYQDEHGQAYYDHDYGDGYYDRSGYYGPDSNHNQQEGGYYDAG-------------- 66
C. neoformans   SASFSSSSSDPFNQTNQLP--------YDSNAQFPPQHAFAHPSAP-------------- 50
R. oryzae       NDNVENEDVKPQKGLEEIRAYYTNTVEGSSHDTNPAPHGQIYEDDLKLPSPTLSM----- 84
A. macrogynus   HDHAALGERNSVKVLNVAGGMLGAGAAPSAQWTSPHPNAIRQHDALRASTKRPIHGDDLT 120
                               .             .             .

S. cerevisiae   -----TEADMYGQQPPNESYDQDYTNGEYYGQPPNMAAQ--DGENFSDFSSYGP-PGTPG 103
C. albicans     --------AQYQQQPYDMDGYQDQAN--YGGQPMNAQGYNADPEAFSDFSYGGQTPGTPG 99
A. fumigatus    --------QPHDDYYGDHYYDQGNGQQGYDNRGRRRGDSEEDSETFSDFTMRSETARAAD 118
C. neoformans   --------NPGAGGAGVAPPGQGGQYAPYYDNEPEMGGR-WEGGGMGRETWASESGWSQN 101
R. oryzae       ---NVPLHSTYNENAIKDDQQAKKQNASPFSAKKVGNPYKQTQGNLYAFSANSSTTYHPN 141
A. macrogynus   TSRGTALVSSASTVASDFDDDEDDDDDEYDGKARVQQHGPATWSPNNRPTHQRLNQGNLG 180
                                              .                  :         .

S. cerevisiae   YDSYG-----------------------------------------------------GQ 110
C. albicans     YDQYG-----------------------------------------------------TQ 106
A. fumigatus    MDYYGRGDERYNSYAD------------------------------------------SQ 136
C. neoformans   EPNYP------------------------------------------------------- 106
R. oryzae       QDTFDVDILASLADGDDG--------------------------------------RYAK 163
A. macrogynus   FDDFDNVSVMSGRSIISSRVANSARGSYTGPGFMSSLRRLTEGSVFGAANTASPASVIST 240
                   :

S. cerevisiae   Y---------TASQMSYG-EPNSSGTSTPIYG----NYDPNAIAMALPNEPYPAWTADSQ 156
C. albicans     Y---------TPSQMSYGGDPRSSGASTPIYGGQGQGYDPTQFNMSS-NLPYPAWSADPQ 156
A. fumigatus    YGGRGYGYRPPSSQISYG-ANRSSGASTPVYG---MDYGNALPAGQRSREPYPAWASDGQ 192
C. neoformans   -----------PSDYHGGPGYLPSRASTPTFE--------GSNAGHRPRDPYPAWTVDAN 147
R. oryzae       YASSADSMLALLANTKYKGSQMLGHNESQSSVGSAHPMTSSIQGYATQEYPYPSWGPEGN 223
A. macrogynus   LVDTKVGPEELLDEFSVVDEQREPKGHTRFVSMQEQLLHPESQDSRTSLPLPSRFAPSDP 300
                             :             :                        . :  .

S. cerevisiae   SPVSIEQIEDIFIDLTNRLGFQRDSMRNMFDHFMVLLDSRSSRMSPDQALLSLHADYIGG 216
C. albicans     APIKIEHIEDIFIDLTNKFGFQRDSMRNMFDYFMTLLDSRSSRMSPAQALLSLHADYIGG 216
A. fumigatus    VPVSKEEIEDIFLDLVNKFGFQRDSMRNMYDHLMTMLDSRASRMTPNQALLSLHADYIGG 252
C. neoformans   IPLSKEEIEDVLIDLANKFGFQKDSSRNVYDFLMIQLDSRASRMSPNQALLTLHADYIGG 207
R. oryzae       VPISFRELQKIFDNLAKKFGFQKDSVKNMSQHLMAMLDSRASRLIPQVALDSLHADYIGG 283
A. macrogynus   RPVTRELIEDMFIDLQRRFGFQADSVRNQLEYMLTLIDSRVARMSLDLAIASLHADIIGG 360
                 *:. . ::.:: :* .::*** ** :*  :.::  :*** :*:    *: :**** ***

S. cerevisiae   DTANYKKWYFAAQLDMDDEIG-FRNMSLGKLSRKARKAKKKNKKAMEEANPE--DTEETL 273
C. albicans     DNANYRKWYFSSQQDLDDSLG-FANMTLGKIGRKARKASKKSKKARKAAEEHGQDVDALA 275
A. fumigatus    DNANYRRWYFAAHLDLDDAVG-FANMKLGKADRKTRKARKAAKKAAQQNPEN---VEETL 308
C. neoformans   EHANYRKWYFAAQLDLDDAIGAVQNPGLNRVRSVARRGGK-TKNPLATAQEK-------- 258
R. oryzae       DNANYKKWYFASQMDIYDQTE----------------EEKNVAKDIGDEHEL-------- 319
A. macrogynus   ENANYRQWYVSTFGPHELGPD-------------------PSADLMGTLTKK-------- 393
                : ***::**.::                               .

S. cerevisiae   NKIEGDNSLEAADFRWKAKMNQLSPLERVRHIALYLLCWGEANQVRFTAECLCFIYKCAL 333
C. albicans     NELEGDYSLEAAEIRWKAKMNSLTPEERVRDLALYLLIWGEANQVRFTPECLCYIYKSAT 335
A. fumigatus    EALEGDNSLEAAEYRWKTRMNKMSQHDRVRQLALFLLCWGEANQVRFLPECLCFIFKCAD 368
C. neoformans   -------SLESATSRWRTAMNNMSQYDRLRQVALYLLCWGEAAQVRFMPECLCFIFKCAD 311
R. oryzae       --------LLRHEEKWLLRMRNLSNSEKLQDLALYLLLWGEAAPIRYTPEALCFIYKMAS 371
A. macrogynus   ----------VVRSRWRKVMKRLTQADRAMQTALWFCIWGEAANIRFMPECLCFLFKLAC 443
                              :*   *. ::  ::  . **::  ****  :*: .*.**:::* *

S. cerevisiae   DYLDSPLCQQRQEPMPEGDFLNRVITPIYHFIRNQVYEIV-DGRFVKRERDHNKIVGYDD 392
C. albicans     DYLNSPLCQQRQEPVPEGDYLNRVITPLYRFIRSQVYEIY-DGRFVKREKDHNKVIGYDD 394
A. fumigatus    DYYNSPECQNRVEPVEEFTYLNEIITPLYQYCRDQGYEIV-DGKYVRRERDHNQIIGYDD 427
C. neoformans   DYYRSPECQNRQEAVPEGLYLRAVIKPLYRFLRDQGYEVV-DGKFLRRERDHDKVIGYDD 370
R. oryzae       DYYRHHSSTEKPD-VPEGTYLDTIIKPLYQFFRDQTYVLK-ENKYVKRERDHDKVIGYDD 429
A. macrogynus   DHLTLDKAAPSPIAVLDGAFLDKVITPLYRAYREQGYNLSEDGKLVRQRRDHSDIVGYDD 503
                *:     .      : :  :*  :*.*:*:  *.* * :  :.: :::.:**..::****
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S. cerevisiae   LNQLFWYPEGIAKIVLEDGTK---LIELPLEERYLRLGDVVWDDVFFKTYKETRTWLHLV 449
C. albicans     VNQLFWYPEGISRIIFEDGTR---LVDIPQEERFLKLGEVEWKNVFFKTYKEIRTWLHFV 451
A. fumigatus    MNQLFWYPEGIERIALEDKTR---LVDIPPAERWTKLKDVVWKKAFFKTYKETRSWFHMI 484
C. neoformans   VNQLFWYPEGISRITLNDNTR---LVDIPPAQRFMKFDRIDWNKVFFKTYLEKRSFFHLL 427
R. oryzae       VNQFFWHPTFYDQIIVSETDKNSTLGKLPPHERYNALKDVNWAKTFKKTYKEKRTWMHAS 489
A. macrogynus   LNDMFRDWDALAALATKGGQR---LLEMPASERYWMLDQVEYK--LRKTYLEKRTPLHLV 558
                :*::*        :  .   :   * .:*  :*:  :  : :   : *** * *: :*

S. cerevisiae   TNFNRIWVMHISIFWMYFAYNSPTFYTHNYQQLVDNQPLAAYKWASCALGGTVASLIQIV 509
C. albicans     TNFNRIWIIHGTIYWMYTAYNSPTLYTKHYVQTINQQPLASSRWAACAIGGVLASFIQIL 511
A. fumigatus    TNFNRIWVIHLGAFWFFTAFNAQSLYTDNYQQQVNNKPPGYRIWSAVGFGGALVSFIQIA 544
C. neoformans   VNFNRIWVLHISVFWFFTAYNAPSIYAPSGSTTATTP----MAWSMTGLGGFVATLIMIA 483
R. oryzae       VNFSRVWVIHIVTFWYYITANAYSLYLDPDKEIANQEVS--VQISIVALGGMVACLLVLI 547
A. macrogynus   TNFSRIWILHLVGLYYMAMGQADFMFPEAMHR--------ARHWSLVACGSAIGVVVTMV 610
                .**.*:*::*    :     ::  ::                  :  . *. :  .: :

S. cerevisiae   ATLCEWSFVPRKWAGAQHLSRRFWFLCIIFGINLGPIIFVFAYDKDT-VYS---TAAHVV 565
C. albicans     ATLFEWIFVPREWAGAQHLSRRMLFLVLIFLLNLVPPVYTFQITKLV-IYS---KSAYAV 567
A. fumigatus    ATICEWMYVPRRWAGAQHLTKRLMFLILVFVINLAPGVFVFAYSKSMGISK---TIPLIV 601
C. neoformans   ATLAEFSYIPTTWNNTSHLTRRLIFLLIILAITGGPSIYIAFFNQTG-------HVALIL 536
R. oryzae       GSCAELAYLPFSWSRSKHLIRRVTFLLILMAVNTGPSFYCLFLDRIS-------AISKTV 600
A. macrogynus   AKWAEYSFLPITVKRTAYLVWCQSVLFAILIANLAPTAFIYWFTGQDLAQRGAGPVAMGV 670
                ..  *  ::*     : :*     .*  ::  .  *  :                 .  :

S. cerevisiae   AAVMFFVAVATIIFFSIMPLGGLFTSYMKKSTRRYVASQTFTAAFAPLHGLDRWMSYLVW 625
C. albicans     SIVGFFIAVATLVFFAVMPLGGLFTSYMNKRSRRYIASQTFTANYIKLKGLDMWMSYLLW 627
A. fumigatus    GIVHFFVALATFVFFSVMPLGGLFGSYLKKHGRQYVASQTFTASFPRLHGNDMWMSYGLW 661
C. neoformans   GIVQFFCSVVATIAFATLPSGRMFGDRVAGKSRKYLANQTFTASYPALGFYPRVASFLLW 596
R. oryzae       SVIQLLISVGTTLFLAIVPPARLFE-RKSKHTREDLANEVFTANFPPLKRIDRIMSVCLW 659
A. macrogynus   TAAHLVLSVVTSCYLLFVPPAQLFR-RPWLLTRQDLVETGFIAHFVPLTPWDHALSLGTW 729
                    :. :: :   :  :* . :*        *. :..  * * :  *       *   *

                         ___hotspot 1___
S. cerevisiae   VTVFAAKYSESYYFLVLSLRDPIRILSTTAMR-CTGEYWWGAVLCKVQPKIVLGLVIATD 684
C. albicans     FLVFLAKLVESYFFLTLSLRDPIRNLSTMTMR-CVGEVWYKDIVCRNQAKIVLGLMYLVD 686
A. fumigatus    VCVFGAKLAESYFFLTLSFKDPIRILSPMQIHQCAGVKYIGNVLCHKQPQILLGLMFFMD 721
C. neoformans   FLVFGCKFTESYFFLTLSFRDPMKVMNGMKVQ-NCHDKYFGNGLCTNQPAFALAVMFVMD 655
R. oryzae       LCVFTCKLLESYFFLALSFKDPLKVISKMNVT-NCNDKIIGTMLCDQMPRITIVIMLLMD 718
A. macrogynus   GTILFIKFIGGYFYLVRPFSGVLESWWEAAGKCPVDEIMCGPVTYGG-----MALLVALQ 784
                  ::  *   .*::*. .: . :.                            : ::   :

                     _hotspot 3_
S. cerevisiae   FILFFLDTYLWYIIVNTIFSVGKSFYLGISILTPWRNIFTRLPKRIYSKILATTDMEIKY 744
C. albicans     LLLFFLDTYMWYIICNCIFSIGRSFYLGISILTPWRNIFTRLPKRIYSKILATTEMEIKY 746
A. fumigatus    LTLFFLDSYLWYIICNTVFSVARSFYLGVSIWSPWRNIFSRLPKRIYSKVLATTDMEIKY 781
C. neoformans   LTLFFLDTFLWYVIWNTVFSIARSFAIGMSIWTPWKDIFARLPKRIYAKILATDDMEVKY 715
R. oryzae       LVLYFLDTYLWYIIWNTAFSVARSFYLGISIWTPWRNIFCRLPKRIFVKLLATTDIQVNY 778
A. macrogynus   TVLYFLDTYLWYMVVLALVSVGLNMVDGISALSMWRETFYKLPNRIVSKLIAVDG-GVPL 843
                  *:***:::**::    .*:. .:  *:*  : *:: * :**:**  *::*.    :

S. cerevisiae   KPKVLISQVWNAIIISMYREHLLAIDHVQKLLYHQVPSEIEGKRTLRAPTFFVSQDDNNF 804
C. albicans     KPKVLISQIWNAIVISMYREHLLAIDHVQKLLYHQVPSEIEGKRTLRAPTFFVSQDDNNF 806
A. fumigatus    KPKVLISQVWNAIIISMYREHLLAIDHVQKLLYHQVPSEQEGKRTLRAPTFFVSQEDQSF 841
C. neoformans   KPKVLVSQVWNAVIISMYREHLLSIEHVQKLLYHQIQSDQPGKRTLRAPAFFISQSEKGS 775
R. oryzae       KPKVLCSQIWNAIVLTMYREHLITADHAQRMIYQQEINTMDGQRNLRSPTFFVSQEDTSF 838
A. macrogynus   RMKQASSEIWNSIVLAMYKEHLLPAESLPKMLYQIHPN-AADQDCVIEPRFFLAHEDSKS 902
                : *   *::**:::::**:***:. :   :::*:   .   .:  :  * **:::.:

S. cerevisiae   ETEFFPRDSEAERRISFFAQSLSTPIPEPLPVDNMPTFTVLTPHYAERILLSLREIIRED 864
C. albicans     ETEFFPRNSEAERRISFFAQSLATPMPEPLPVDNMPTFTVFTPHYSEKILLSLREIIRED 866
A. fumigatus    KTEFFPPGSEAERRISFFAQSLSTPMPEPLPVDNMPTFTVLIPHYSEKILLSLREIIRED 901
C. neoformans   KAEFFPKGSEAERRICFFAQSLTTSIPAPIPVDAMPTFTVLVPHYSEKILLSLREIIREE 835
R. oryzae       KTEYFPQGGEAERRIHFFAQSLTTPMPAPHPVQCMPLFTVLTPHYGEKILLSLREIIREE 898
A. macrogynus   KGEFFPTDSEVERRLSYFSQSLCMNVPEPTPVARMPTFSVLIPHYSEKILLSLEDCTERE 962
                : *:** ..*.***: :*:***   :* * **  ** *:*: ***.*:*****.:  ..:

S. cerevisiae   DQFSRVTLLEYLKQLHPVEWECFVKDTKILAE---------------------------- 896
C. albicans     DQFSRVTLLEYLKQLHPVEWDCFVKDTKILAE---------------------------- 898
A. fumigatus    EPYSRVTLLEYLKQLHPHEWDCFVKDTKILAD---------------------------- 933
C. neoformans   DQNTRVTLLEYLKQLHPVEWDNFVRDTKILAE---------------------------- 867
R. oryzae       DNSTRVTLLEYLKQLHPNEWDNFVKDTKILASGDEKSIGT-------------------- 938
A. macrogynus   DKSSRITLLEYLKTLYPDEWDNFVAESKIMEHEQALSDSLGDSLPIPGGAGFDGHGSDRM 1022
                :  :*:******* *:* **: ** ::**:
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S. cerevisiae    --------------------------------ETAAYE-GNENEAEKEDALKSQIDDLPF 923
C. albicans      --------------------------------ETAAYENGDDSEKLSEDGLKSKIDDLPF 926
A. fumigatus     --------------------------------ETSQFN--GEPEKSEKDVAKSKIDDLPF 959
C. neoformans    --------------------------------ESDAFN-GGNPFASDEKEEAKKADDIPF 894
R. oryzae        --------------------------------SSTLNEPTHDSDPDNRTKKLPKNDDLPF 966
A. macrogynus    STASSPSMRPHGGLPRASWCPQQEKAAVHDNGTGEQNDDLLAEDDDEDEEARETAEEKMF 1082
                                                      :        .        ::  *

S. cerevisiae    YCIGFKSAAPEYTLRTRIWASLRSQTLYRTISGFMNYSRAIKLLYRVENPEIVQMFGGNA 983
C. albicans      YCIGFKSAAPEYTLRTRIWASLRSQTLYRTVSGFMNYARAIKLLYRVENPELVQYFGGDP 986
A. fumigatus     YCIGFKSAAPEYTLRTRIWSSLRSQTLYRTVSGFMNYSRAIKLLYRVENPEVVQMFGGNS 1019
C. neoformans    YTIGFKSAAPEYTLRTRIWASLRAQTLYRTVSGFMNYSKAIKLLYRVENPEVVQLFGGNT 954
R. oryzae        YCIGFKTSKPEYTLRTRIWASLRAQTLYRTVSGFMNYKKAIKLLYRVENPDNIRVYQDDK 1026
A. macrogynus    RAVGYTNPSPSFVLRTRIWTSLRSQTLYRTVSGFMNYNKAIQIRHRAENLHYLFQGVCSE 1142
                   :*:... *.:.******:***:******:****** :**:: :*.** . :     .

S. cerevisiae    EGLERELEKMARRKFKFLVSMQRLAKFKPHELENAEFLLRAYPDLQIAYLDEEPPLTEGE 1043
C. albicans      EGLELALERMARRKFRFLVSMQRLSKFKDDEMENAEFLLRAYPDLQIAYLDEEPALNEDE 1046
A. fumigatus     EKLERELERMARRKFKIVVSMQRYAKFNKEERENTEFLLRAYPDLQIAYLDEEPPVNEGE 1079
C. neoformans    DQLERELERMARRKFKFVVSMQRYSKFNKEEHENAEFLLRAYPDLQIAYLDEEPPRKDGG 1014
R. oryzae        DRLENELDVLTRSKFKFIVAMQRYAKFNKAENEDAEFLFKAFPDLQVAYIDEEPSAEEGG 1086
A. macrogynus    EEIEDEADYVSSRKFRLVVAMQRYQHFDKNDLESVELLFSVFADLAVSYIEEVPSAVEGE 1202
                 : :*   : ::  **:::*:***  :*.  : *..*:*: .:.** ::*::* *.  :.

S. cerevisiae    EPRIYSALIDGHCEILDNGRRRPKFRVQLSGNPILGDGKSDNQNHALIFYRGEYIQLIDA 1103
C. albicans      EPRVYSALIDGHCEMLENGRRRPKFRVQLSGNPILGDGKSDNQNHAVIFHRGEYIQLIDA 1106
A. fumigatus     EPRLYSALIDGHCELLENGMRKPKFRIQLSGNPILGDGKSDNQNHSIIFYRGEYIQVIDA 1139
C. neoformans    ESRIFSALIDGHSEIMPNGRRRPKFRIELPGNPILGDGKSDNQNHAIVFYRGEYLQLIDA 1074
R. oryzae        EVTYYSALIDGHAPIMENGKRKPYFRVRLPGNPILGDGKSDNQNHAIIFYRGEFLQLVDA 1146
A. macrogynus    PPTYYSCLIDGWCNMTASGARQPRFRIRLPGNPILGDGKGDNQNSAIVFTRGEVLQLIDA 1262
                     :*.**** . :  .* *:* **:.*.*********.**** :::* *** :*::**

S. cerevisiae    NQDNYLEECLKIRSVLAEFEELNVEQVNPYAPGLRYEEQTTN-HPVAIVGAREYIFSENS 1162
C. albicans      NQDNYLEECLKIRSVLAEFEEMNVEHVNPYAPNLKSEDNNTKKDPVAFLGAREYIFSENS 1166
A. fumigatus     NQDNYLEECLKIRSVLAEFEELTTDNVSPYTPGIP----STNTNPVAILGAREYIFSENI 1195
C. neoformans    NQDNYLEECLKIRNVLGEFEEFKVSTQSPYAAQGH---ADFAKFPVAILGAREYIFSENI 1131
R. oryzae        NQDNYLEECLKIRNVLGEFEVLEPIQESPYSPSYQ----KSNSSPVAIVGAREYIFSENI 1202
A. macrogynus    NQDSYLEESLKVANVLSEFELPGDSVHSPYSPRYN-----RGRAPVAIVGTREHIFSERV 1317
                 ***.****.**: .**.***       .**:.            ***::*:**:****.

S. cerevisiae    GVLGDVAAGKEQTFGTLFARTLSQIGGKLHYGHPDFINATFMTTRGGVSKAQKGLHLNED 1222
C. albicans      GVLGDVAAGKEQTFGTLFARTLAQIGGKLHYGHPDFLNATFMLTRGGVSKAQKGLHLNED 1226
A. fumigatus     GVLGDVAAGKEQTFGTLFARTLAQIGGKLHYGHPDFLNGIFMTTRGGISKAQKGLHLNED 1255
C. neoformans    GILGDIAAGKEQTFGTLAARSLSYIGGKLHYGHPDFLNAIYMNTRGGVSKAQKGLHLNED 1191
R. oryzae        GILGDVAAGKEQTFGTLTQRIMAKSGGKLHYGHPDFLNAIYMNTRGGVSKAQKGLHLNED 1262
A. macrogynus    GSLADVAAGKEFAFGTLIQRAMTRLGSRLQYGHPDLLNSLYMITRGGVSKAQKGLHLNED 1377
                 * *.*:***** :****  *  ::  *.:*:*****::*. :* ****:************

S. cerevisiae    IYAGMNAMLRGGRIKHCEYYQCGKGRDLGFGTILNFTTKIGAGMGEQMLSREYYYLGTQL 1282
C. albicans      IYAGMNAMMRGGKIKHCEYYQCGKGRDLGFGSILNFTTKIGAGMGEQMLSREYFYLGTQL 1286
A. fumigatus     IYAGMNAMIRGGRIKHCEYYQCGKGRDLGFGSILNFTTKIGTGMGEQMLSREYYYLGTQL 1315
C. neoformans    IFAGMLAFGRGGRIKHSEYYQCGKGRDLGFGTILNFQTKIGTGMGEQMLSREYYYLGTQL 1251
R. oryzae        IYAGMNSFIRGGRIKHTEYFQCGKGRDLGFGSILNFTTKIGTGMGEQMLSREYYYIGTQL 1322
A. macrogynus    IYAGMNVLSRGGRIKHSEYLQCGKGRDLGFTSVLNFTAKLGSGAGEQVLSRDVWYLGTRL 1437
                 *:***  : ***:*** ** ********** ::*** :*:*:* ***:***: :*:**:*

S. cerevisiae    PVDRFLTFYYAHPGFHLNNLFIQLSLQMFMLTLVNLSSLAHESIMCIYDRNKPKTDVLVP 1342
C. albicans      PLDRFLSFYYGHPGFHINNLFIQLSLQVFILVLANLNSLAHEAIMCSYNKDVPVTDVLYP 1346
A. fumigatus     PLDRFLSFYYAHPGFHINNMFIMLSVQMFMIVLINLGALKHETITCRYNPDLPITDPLRP 1375
C. neoformans    PIDRFLTFYYGHPGFHINNILVMMSVQVFMLALVFLGTLNKQLTVCRYSSGGDILPGQS- 1310
R. oryzae        PLDRFLTFYYAHPGFHLNNIFIMLSVQMFMLAALFISAMGASLTICEYNADAPEDVALTP 1382
A. macrogynus    PLDRLLSYYFAHIGFYINAAFVVASVVLFLWTSAMAAVMQAR------HPGKCTGKDAAS 1491
                 *:**:*::*:.* **::*  ::  *: :*: .      :           .
```

hotspot 2

```
S. cerevisiae    IGCYNFQPAVDWVRRYTLSIFIVFWIAFVPIVVQELIERGLWKATQRFFCHLLSLSPMFE 1402
C. albicans      FGCYNIAPAVDWIRRYTLSIFIVFFISFIPLVVQELIERGVWKAFQRFVRHFISMSPFFE 1406
A. fumigatus     TYCANLTPIVDWVNRCIISIFIVFFISFVPLAVQELTERGVWRMAMRLAKHFGSVSFMFE 1435
C. neoformans    -GCYNLVPVFKWIKRCIISIFIVFWMAFVPLFVQELTERGTGRAILRLCKHFLSLSPVFE 1369
R. oryzae        EGCYNLVPIFDWVKRCILSIVAVFLVAFLPLFLQELTEKGFWRSLTRIGKHFASLSPLFE 1442
A. macrogynus    TCPTDLATLQDWVSLCVSELIFSLLISFLPLLLQMVNEYGVGRALKRILMQFFSLVSLFE 1551
                     :: .  .*:     .:.  : ::*:*: :* : * *  :   *:  :: *:  .**
```

```
S. cerevisiae   VFAGQIYSSALLSDLAIGGARYISTGRGFATSRIPFSILYSRFAGSAIYMGARSMLMLLF 1462
C. albicans     VFVAQIYSSSVFTDLTVGGARYISTGRGFATSRIPFSILYSRFADSSIYMGARLMLILLF 1466
A. fumigatus    VFVCQIYANAVHQNLSFGGARYIGTGRGFATARIPFGVLYSRFAGPSIYAGARSLLMLLF 1495
C. neoformans   VFSTQIYMHSILNDLTFGGARYIATGRGFATTRISFSILYSRFAGPSIYLGMRTLVLLLF 1429
R. oryzae       IFVTQIYTNSVLENLVYGGARYIGTGRGFATSRISFATLYSRFTGPSIYVGARNFLIMLF 1502
A. macrogynus   GFAIYVYSSMFWHNLALGGAGYIATGRAVSTARAPFSTLYQRFSRQGISLGVALGMLALL 1611
                 *   :*   .  :*  *** **.***..:*:* .*. **.**:  .*  *    :: *:

S. cerevisiae   GTVAHWQAPLLWFWASLSSLIFAPFVFNPHQFAWEDFFLDYRDYIRWLSRGNNQYHRN-- 1520
C. albicans     GTVSHWQAPLLWFWASLSALMFSPFIFNPHQFAWEDFFLDYRDFIRWLSRGNTKWHRN-- 1524
A. fumigatus    ATSTVWTAALIWFWVSLLALCISPFLFNPHQFAWNDFFIDYRDYLRWLSRGNSRSHAS-- 1553
C. neoformans   ITLTVWVPHLIYFWITVVGLCVAPFLFNPHQFAIADFIIDYREFLRWMSRGNSRTHAN-- 1487
R. oryzae       ASLAYWIPHLIYFWFTVVALIVSPFVFNPNQFAPVDFLVDYREFIRWMSRGNSKPHAN-- 1560
A. macrogynus   VSTQGFQAALVFYWIFVISYALSPFLFNPNQFVLADFLLDYRVTLRWLFAGHQAPAPRNL 1671
                 :   : . *:::*  : .  .:**:***:**.  **::***  :**:  *:


S. cerevisiae   SWIGYVRMSRARITGFKRKLVGDESEKAAGDASRAHRTNLIMAEIIPCAIYAAGCFIAFT 1580
C. albicans     SWIGYVRLSRSRITGFKRKLTGDVSEKAAGDASRAHRSNVLFADFLPTLIYTAGLYVAYT 1584
A. fumigatus    SWIGFCRLSRTRITGYKRKLLGVPSEKGSGDVPRARLTNIFFSEIIAPLVLVAVTLVPYL 1613
C. neoformans   SWVGYCRLSRTRVTGFKRKRLGLPSEKLSSDVPRAPWKAILIGEIIGPICLAILFVICYL 1547
R. oryzae       SWISHTRSARARITGYKRS-KSTEATSGIADMPRASMAAMIISEIIFPLIYAILCTIPYA 1619
A. macrogynus   SWANLVVADRGRLT------------GERGGATRARLRVFIVRNLLADLVVIAFAVGLYG 1719
                ** .     * *:*               .. .**    .:. :::            :

S. cerevisiae   FINAQTGVK--------TTDDDRVNSVLRIIICTLAPIAVNLGVLFFCMGMSCCSGPLFG 1632
C. albicans     FINAQTGVTSYPYEINGSTDPQPVNSTLRLIICALAPVVIDMGCLGVCLAMACCAGPMLG 1644
A. fumigatus    YINSRTGVR---------DNPEPTDAILRLAIVAAGPIAINAGVAGVFFGMACCMGPIFS 1664
C. neoformans   FIKSFAVDG------------QIQPGLVRIAIIALGPIVWNMALLITLFLISVFLGPCLN 1595
R. oryzae       FTKGFDATDP-------TLPSTGPSGLLRIGVIALAPVLMNVAGLAMFFAMSVGIGCVLS 1672
A. macrogynus   YAHPKFSILD---------------SIIRVAAWPAAMLGVCFAMSAVSLALSLFLGQIP- 1763
                : :                      . :*:   . . :    .     : ::   *

S. cerevisiae   MCCKKTGS-VMAGIAHGVAVIVHIAFFIVMWVLESFNFVRMLIGVVTCIQCQRLIFHCMT 1691
C. albicans     LCCKKTGA-VIAGVAHGVAVIVHIIFFIVMWVTEGFNFARLMLGIATMIYVQRLLFKFLT 1703
A. fumigatus    MCCKKFGA-VLAAIAHAIAVIVLLAIFEVMFFLESWSWPRMLIGMIAAAAIQRFIYKLII 1723
C. neoformans   SYTHQFGA-TMAALAHFGAVAGMLVFFELLWFLELWNTSHAVLGIIAVISVQRCIFKFLI 1654
R. oryzae       SVGKQVWARSWAALAHGWSVMNLIIIFEVFFVLEEWNLTNVVLGVVAISSIQRFVLKLLT 1732
A. macrogynus   -----FVARLVPAIGYGLPTLALIAIAWVAWALEGFALPGTLVALFPFAAVLHAIETLAL 1818
                       :   ..:.:  ..   : :  : :  * :     ::.: .     : :

S. cerevisiae   ALMLTREFKNDHANTAFWTGKWYGKGMGYMAWTQPSRELTAKVIELSEFAADFVLGHVIL 1751
C. albicans     LCFLTREFKNDKANTAFWTGKWYNTGMGWMAFTQPSREFVAKIIEMSEFAGDFVLAHIIL 1763
A. fumigatus    ALALTREFKHDQSNIAWWTGKWYN--MGWHSMSQPGREFLCKITELGYFSADFVLGHVLL 1781
C. neoformans   AVFLSREFKHDETNRAWWTGVWFNRGLGSHALSQPAREFVVKTIEMGLYSADFIACHLLL 1714
R. oryzae       VLCLTREFKHDEANRGWWTGKWYG-------------------------RGQFLNQPVSL 1767
A. macrogynus   FFVPPEFDTKGTAHRAFWTGRWHT--LGAFAPVYVVRELLVVPFELAWFALDWATIHVLV 1876
                    ..  .:. :: .:*** *.                            ::    : :

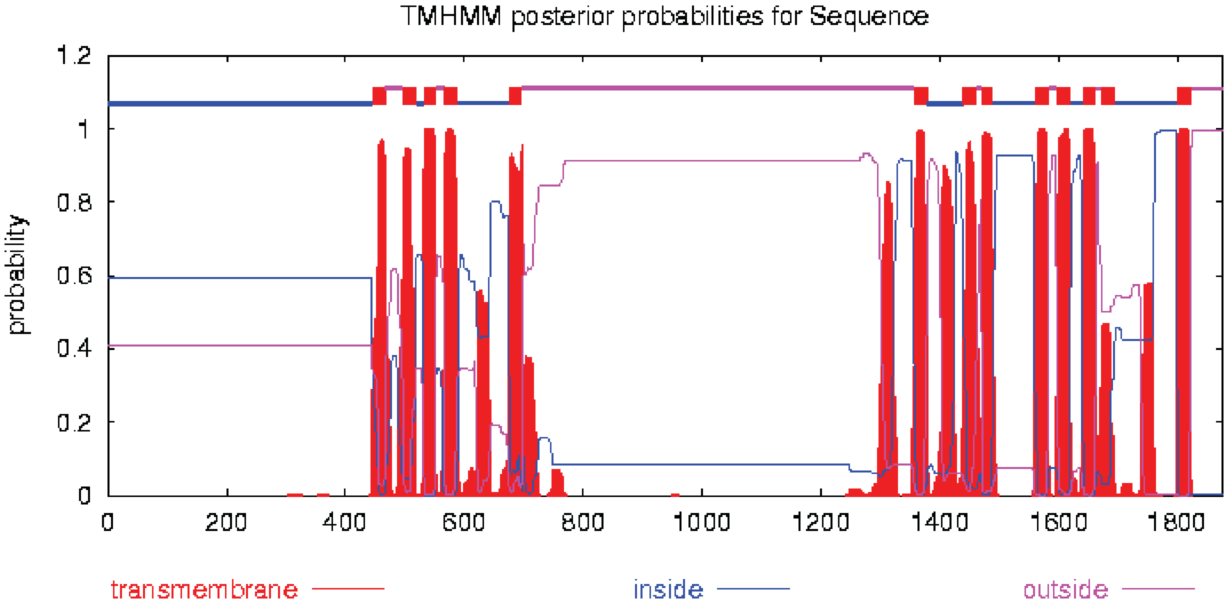
S. cerevisiae   ICQLPLIIIPKIDKFHSIMLFWLKPSRQIR------------PPIYSLKQTRLRKRMVKK 1799
C. albicans     FCQLPFLFIPLVDRWHSMMLFWLKPSRLIR------------PPIYSLKQARLRKRMVRK 1811
A. fumigatus    FAMLPALCVPFIDKFHSVMLFWLRPSRQIR------------PPIYSLKQSKLRKRRVIR 1829
C. neoformans   ALLTIPMFIPYFDRVHATMLFWLAPNQQIR------------PPIYSFRQRSQRRKIVFK 1762
R. oryzae       FVKLSK------------------------------------------------------ 1773
A. macrogynus   AALFPVCLVPFVSDVHTLMLLWVRKPKKSDNGAPANGSGGAVADATAKPKHRRRRLRIAG 1936

S. cerevisiae   YCSLYFLVLAIFAGCIIGPAVASAKIHKHIGDSLDG---VVHNLFQPINTTNNDTG---- 1852
C. albicans     YCVLYFAVLILFIVIIVAPAVASGQIAVDQFANIGGSGSIADGLFQPRNVSNNDTGNHRP 1871
A. fumigatus    FAILYFGMLILFLVLLIAPLVVRSMGLVKTPN-------LPFNLLQPLDKDNNDTMV--- 1879
C. neoformans   YGLLYLIIQGIFIALIVVPIIFKDVAG-----------------LTPKSVPFNGII---- 1801
R. oryzae       ------------------------------------------------------------
A. macrogynus   AALMLAGTLLVLAALVVVPAIVPGPLAKIVAG--------IAGHLPLHALKAIPAAP--- 1985

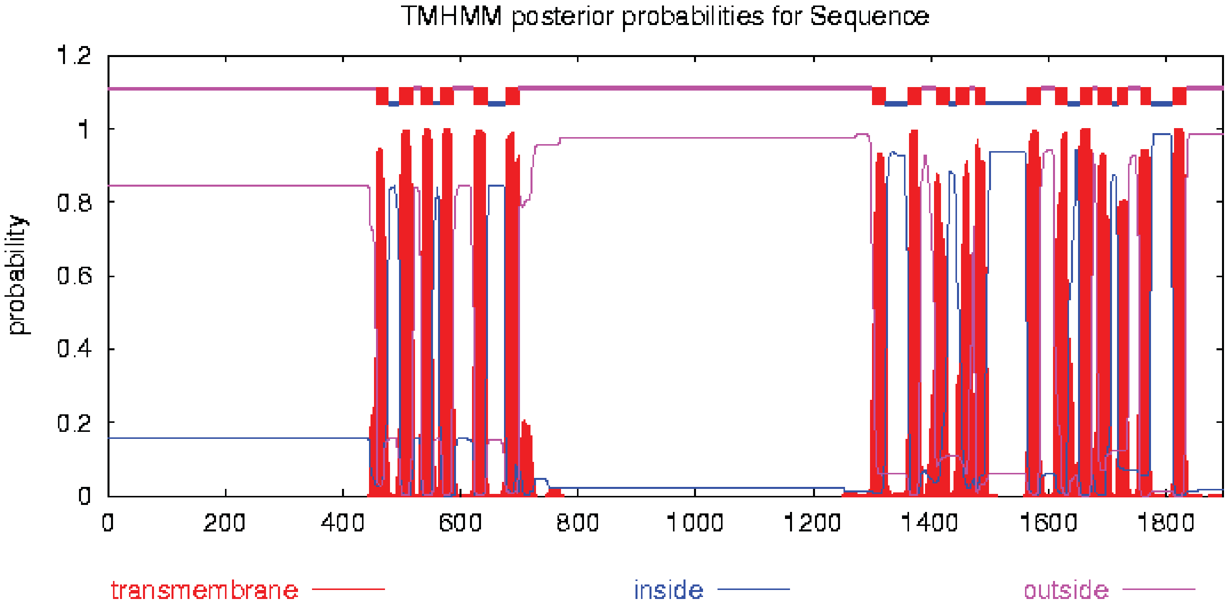
S. cerevisiae   -SQMSTYQSHYYTHTPSLKTWSTIK- 1876
C. albicans     KTYTWSYLSTRFTGSTTPYSTNPFRV 1897
A. fumigatus    -TYTGNNIPAGFEPVESASSVATATS 1904
C. neoformans   --------------------------
R. oryzae       --------------------------
A. macrogynus   --------------------------
```

FIG. S2. TMH predictions for fungal Fks1 sequences generated using TMHMM.

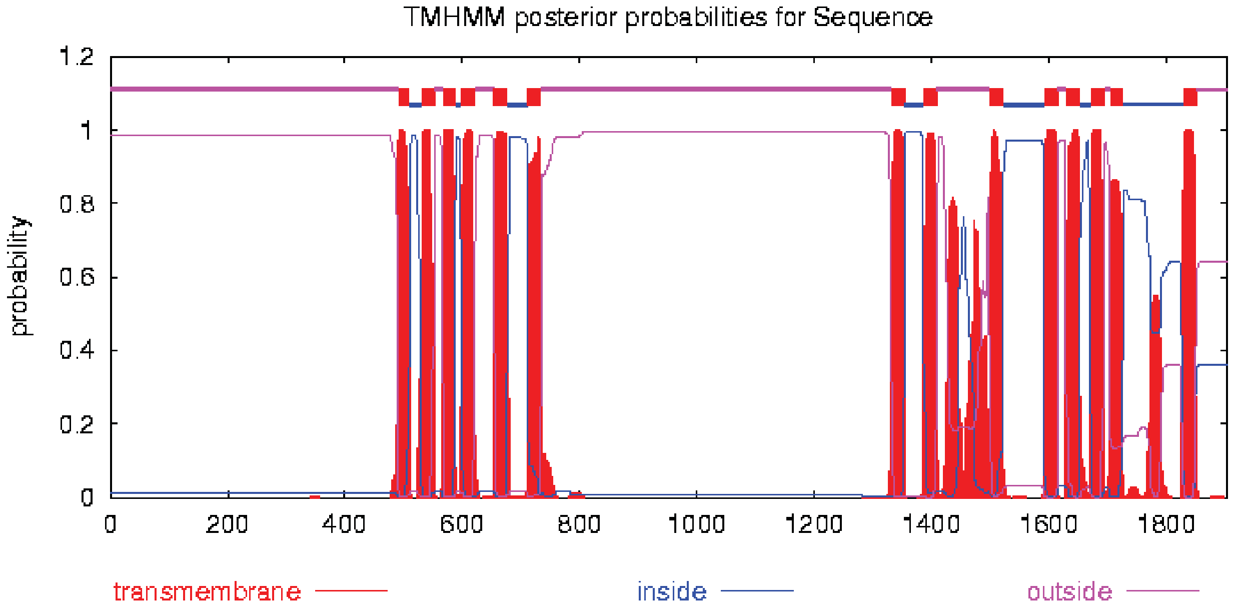
Saccharomyces cerevisiae Fks1:



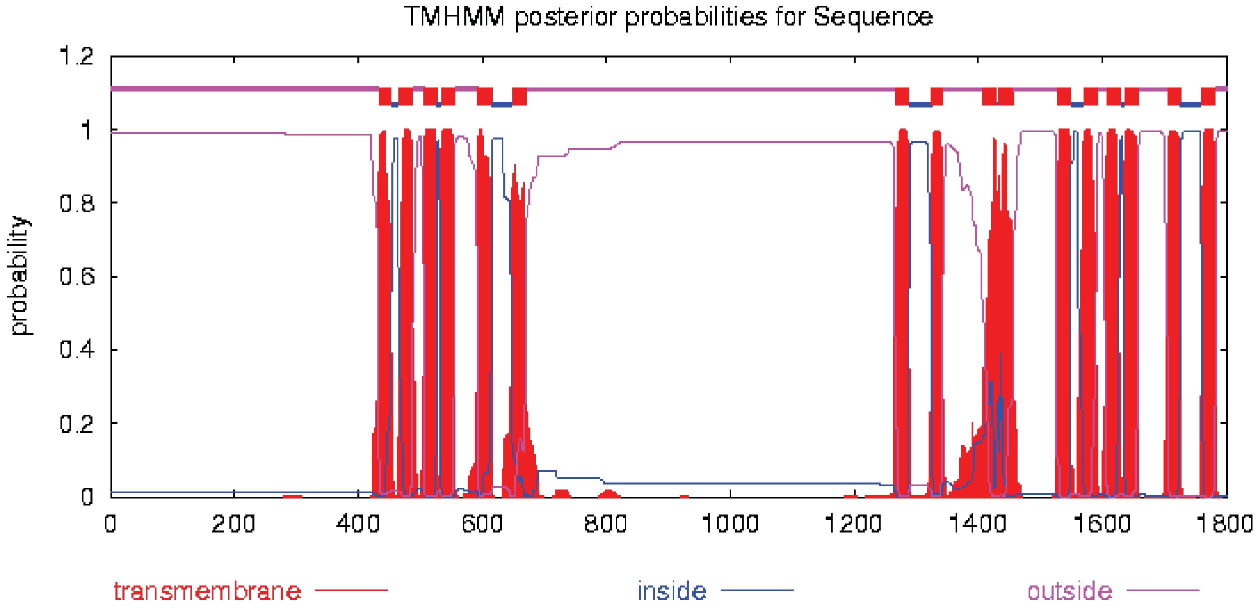
Candida albicans Fks1/Gsc1:



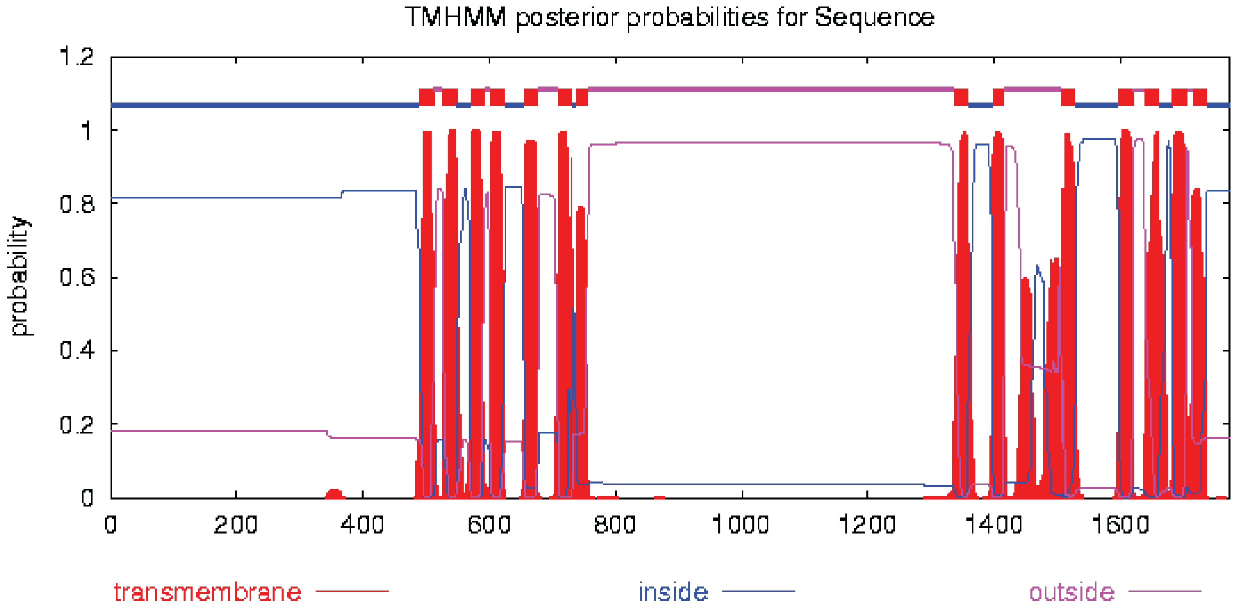
Aspergillus fumigatus Fks1:



Cryptococcus neoformans var. *grubii* Fks1:



Rhizopus oryzae Fks1:



Allomyces macrogynus Fks1: