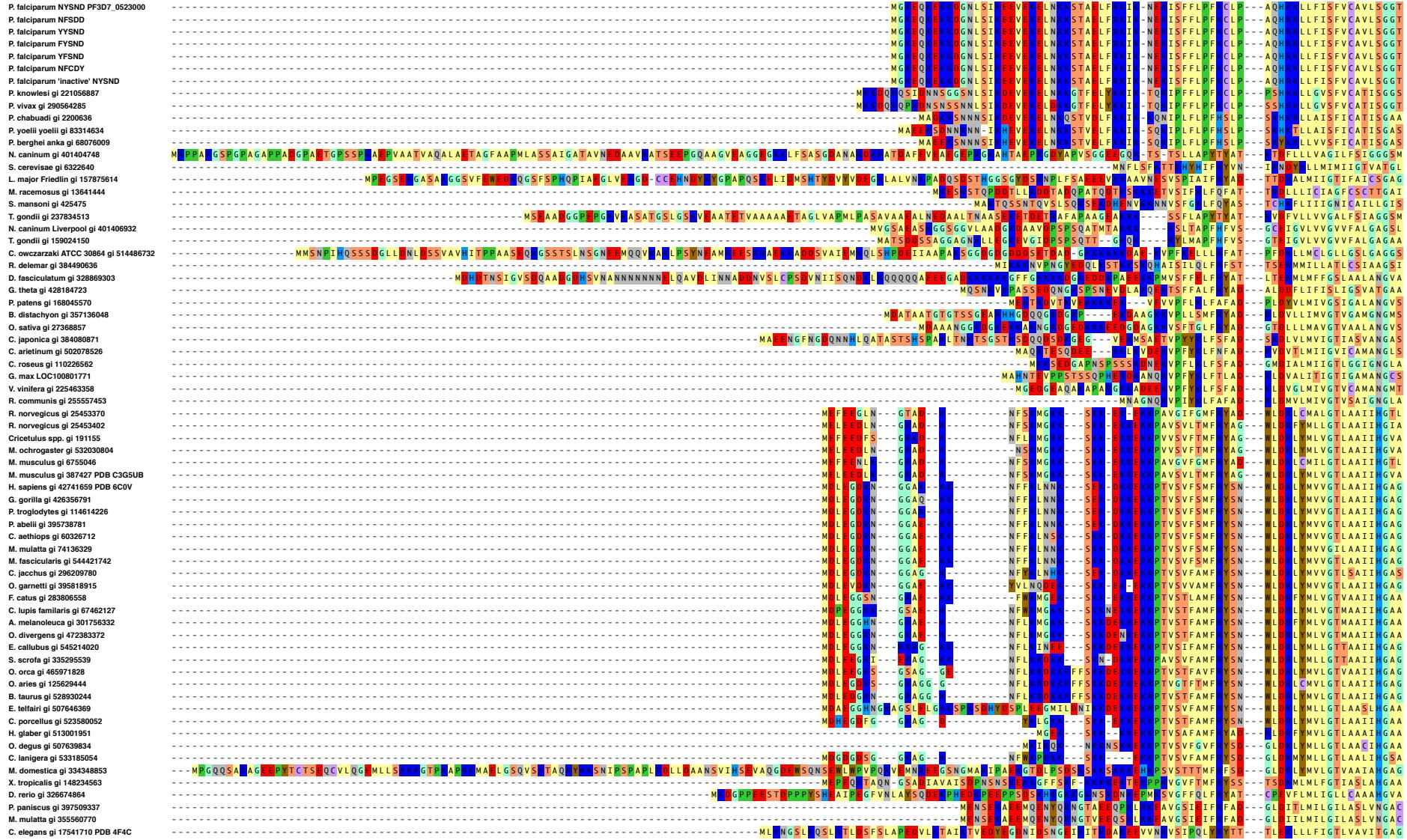


## N-terminus

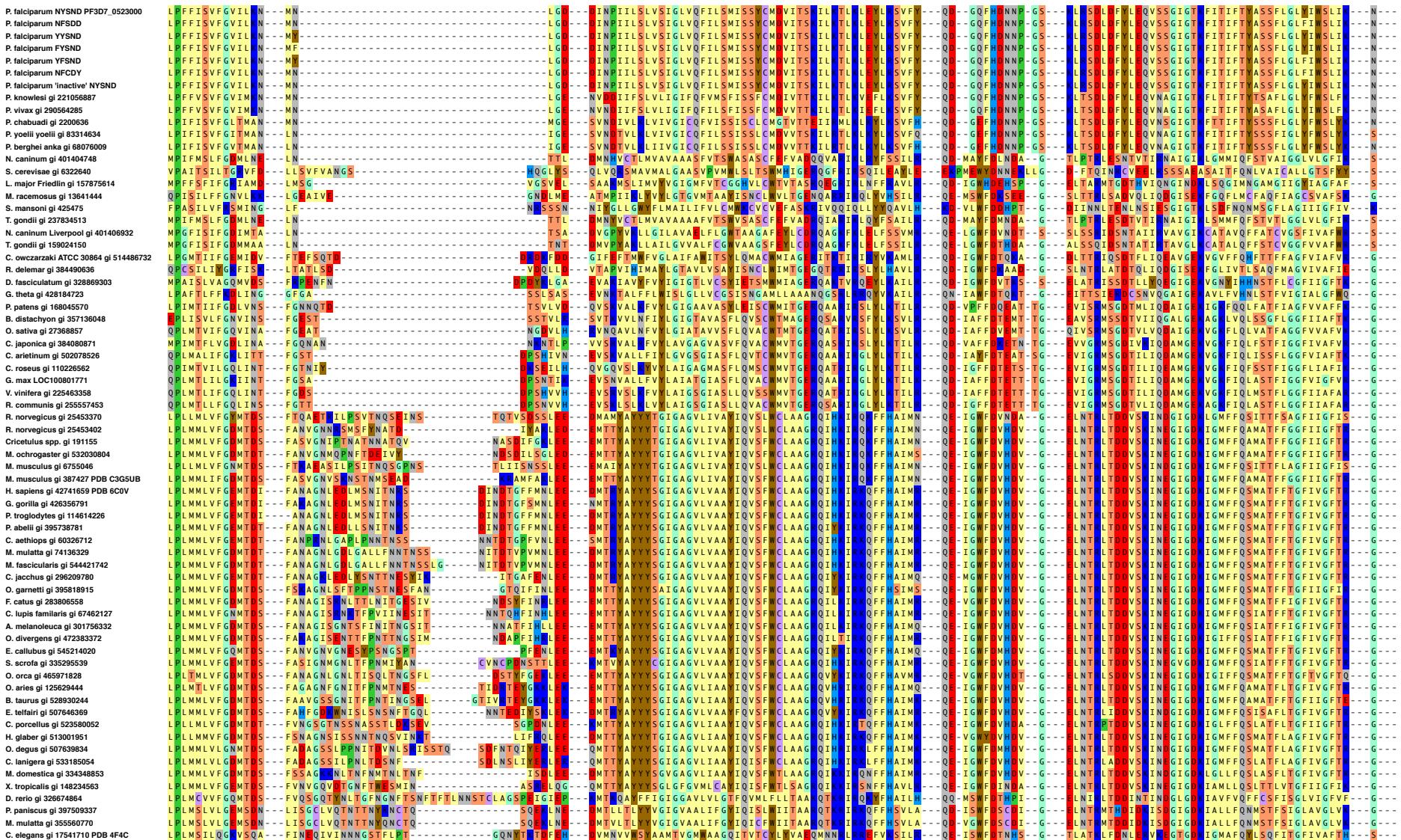
## TMD 1...



...TMD 1

TMD 2

TMD 3



TMD 4

TMD 5

TMD 6...

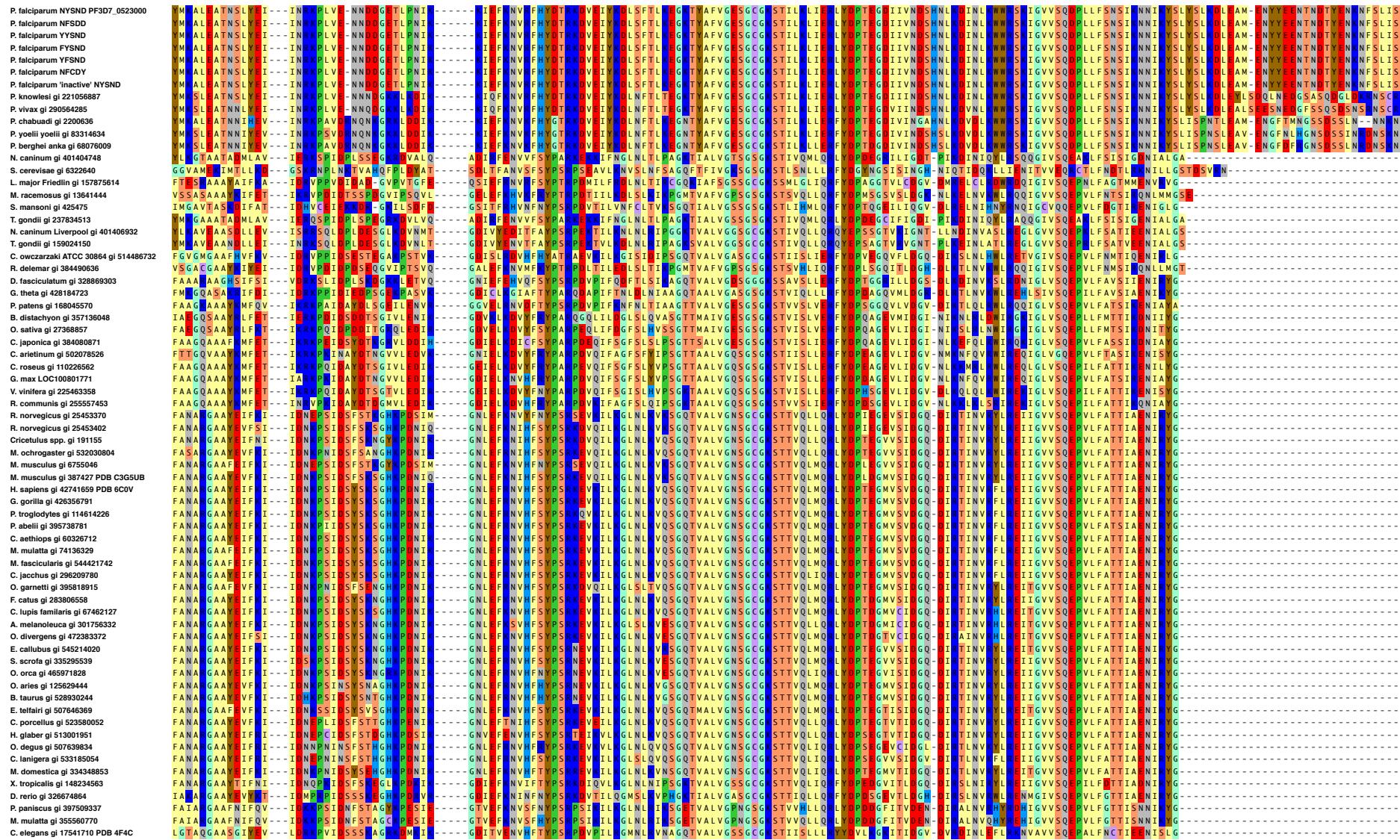
*P. falciparum* NYSND PF3D7\_0523000*P. falciparum* NFSDD*P. falciparum* YNSND*P. falciparum* FVSND*P. falciparum* YFSND*P. falciparum* NFCDY*P. falciparum* 'inactive' NYSND*P. knowlesi* gi 221056887*P. vivax* gi 290564285*P. chabaudi* gi 2200636*P. yoelii* yoelii gi 83314634*P. berghei* anka gi 68076009*N. caninum* gi 401407474*S. cerevisiae* gi 6322640*L. major* fischeri gi 157875614*M. ramosus* gi 13641444*S. mansoni* gi 425475*T. gondii* gi 237834513*N. caninum* Liverpool gi 401406932*T. gondii* gi 15924150*C. oswarzakai* ATCC 30864 gi 51446732*R. dellemar* gi 38449036*D. fasciculatum* gi 328689303*G. thetis* gi 426184723*P. patens* gi 168045570*B. distachyon* gi 357136048*O. sativa* gi 27368857*C. japonica* gi 384080871*C. arelinum* gi 502078526*C. roseus* gi 110226562*M. max* LOC100801771*V. vinifera* gi 225463358*F. communis* gi 255557453*R. norvegicus* gi 25453370*R. norvegicus* gi 25453402*Cricetulus* spp. gi 19115*M. ochrogastra* gi 52030804*M. musculus* gi 6755046*M. musculus* gi 387427 PDB C3GSUB*H. sapiens* gi 42741659 PDB 6COV*G. gorilla* gi 428356791*P. troglodytes* gi 114614226*P. abelli* gi 395738781*C. aethiops* gi 00326712*M. mulatta* gi 74136329*M. fascicularis* gi 544421742*C. jacchus* gi 296209780*O. garnetti* gi 395818915*F. catcus* gi 32806558*C. lupus familiaris* gi 67462127*A. melanoleuca* gi 170576332*O. divergens* gi 472383372*E. callubus* gi 545214020*S. scrofa* gi 335295539*O. orca* gi 465971828*A. aries* gi 125629444*B. taurus* gi 528930244*E. telfairi* gi 50764369*C. porcellus* gi 523580052*H. glaber* gi 513001951*O. degus* gi 507639834*C. lanigera* gi 533185054*M. domestica* gi 334388453*X. tropicalis* gi 148234563*D. rerio* gi 326674984*P. panicus* gi 39709337*M. mulatta* gi 355606707*C. elegans* gi 17541710 PDB 4F4C

...TMD 6

NBD 1...

Walker A

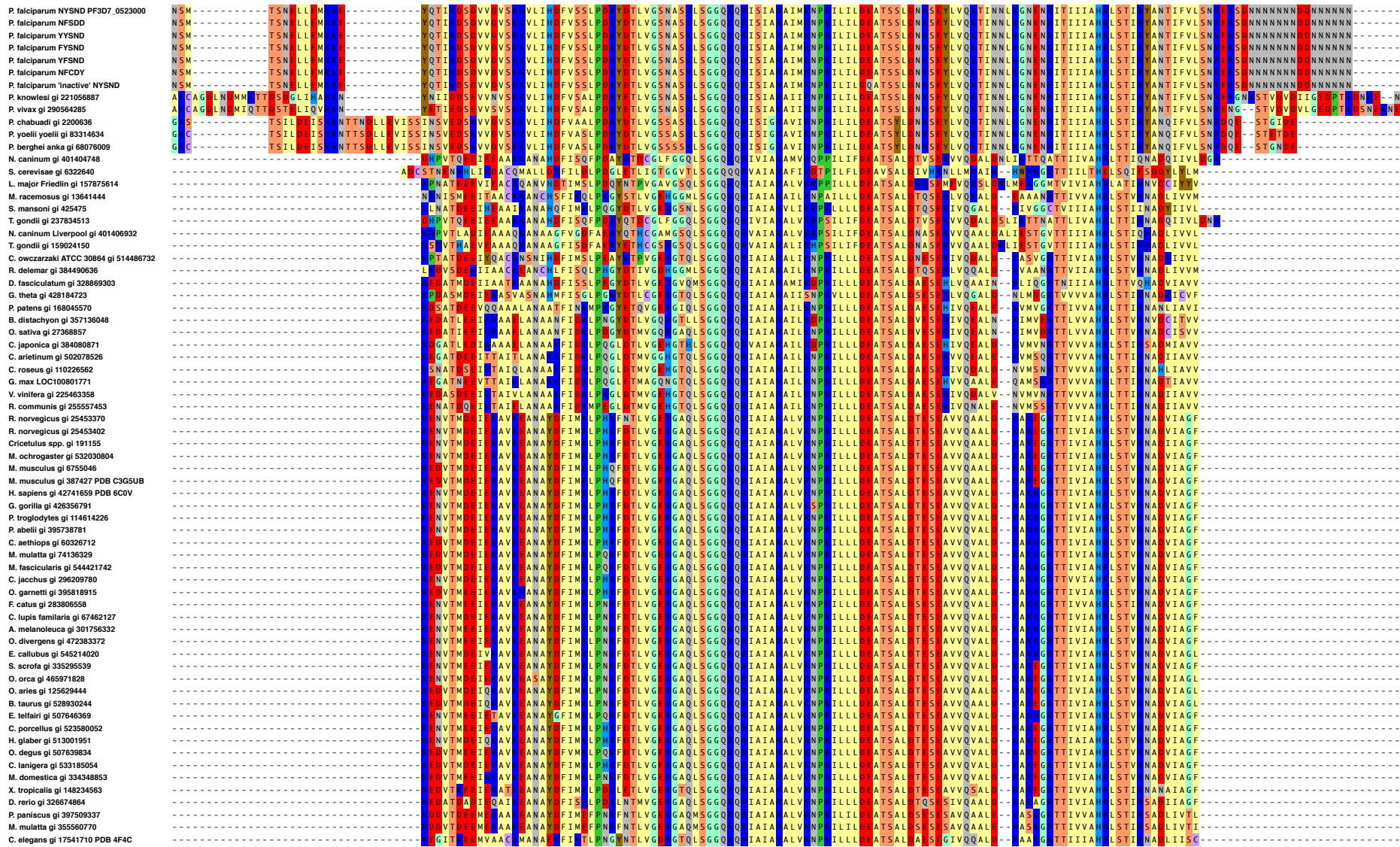
Q-loop



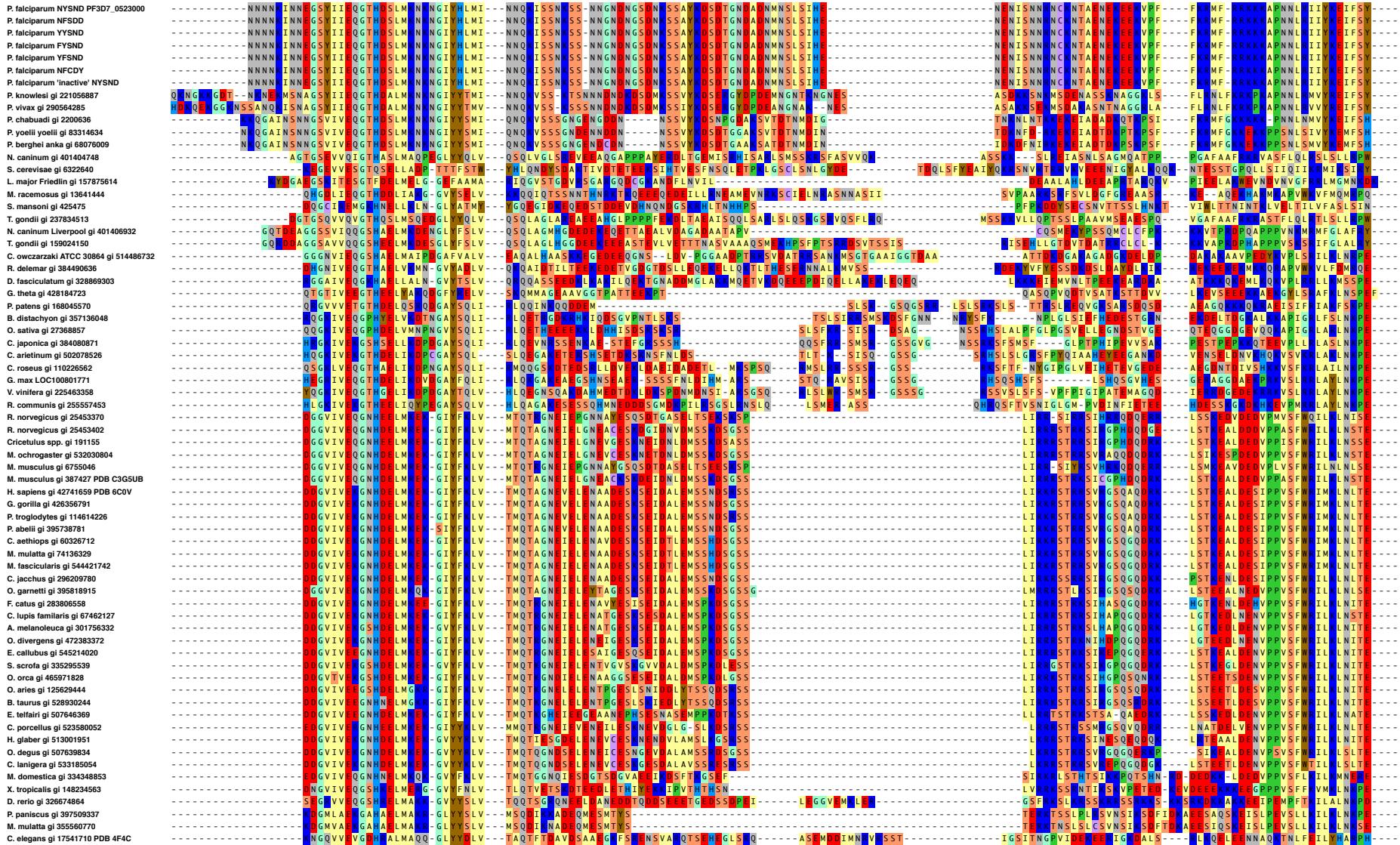
# ...NBD 1...

ABC signature

Walker B



## ...NBD 1





*P. falciparum* NYSND PF3D7\_0523000

*P. falciparum* NFSDD

*P. falciparum* YFSND

*P. falciparum* YFSND

*P. falciparum* NFCDY

*P. falciparum* 'inactive' NYSND

P. knowlesi gi 221058887

P. vivax gi 290564285

P. chabaudi gi 2200635

P. yoelii yoelii gi 83314634

P. berghei anka gi 68076009

N. caninum gi 401404748

S. cerevisea gi 6322640

L. major Friedlin gi 157875614

M. recusosum gi 13641444

S. mansoni gi 425475

T. gondii gi 23783453

N. caninum Liverpool gi 401406932

T. gondii gi 50204150

C. owczarski ATCC 30864 gi 514486732

R. delemer gi 384490636

D. fasciculatum gi 328869303

G. theta gi 242184723

P. patens gi 168045570

B. distachyon gi 571365048

O. sativa gi 27368857

C. japonica gi 384080781

C. arrietinum gi 502078526

C. roseus gi 110226562

G. max LOC100801771

V. vinifera gi 328663538

R. communis gi 255557453

R. norvegicus gi 25453370

R. norvegicus gi 25453402

Cricetulus spp. gi 191155

M. ochrogaster gi 532030804

M. musculus gi 6755046

M. musculus gi 387427 PDB C3G5UB

H. sapiens gi 42741659 PDB 6C0V

G. gorilla gi 426356791

P. troglodytes gi 11614226

P. abelli gi 395738781

C. ethiopius gi 60326712

M. mulatta gi 74136329

M. fascicularis gi 544421742

C. jacchus gi 996209780

O. garnetti gi 395818915

F. cati gi 28306558

C. lupus familiaris gi 67462127

A. melanoleuca gi 371056332

O. divergens gi 472383372

E. calabus gi 545214020

S. scrofa gi 335295539

O. orca gi 465971928

O. aries gi 125629444

B. taurus gi 528930244

E. telfairi gi 507646369

C. porcellus gi 523500052

H. glaber gi 513001951

O. degeuri gi 507639834

C. lanigera gi 533185054

M. domestica gi 334348853

X. tropicalis gi 148234563

D. rerio gi 326674864

P. panicus gi 397509337

M. mulatta gi 355560770

C. elegans gi 1754110 PDB 4F4C

TMD 10

TMD 11

*P. falciparum* NYSND PF3D7\_0523000*P. falciparum* NFSDD*P. falciparum* YVSND*P. falciparum* YFSND*P. falciparum* NFCDY*P. falciparum* 'inactive' NYSND

P. knowlesi gi 22105887

P. vivax gi 290564285

P. chabaudi gi 220636

P. yoelii yoelii gi 83314634

P. berghhei anka gi 68076009

N. caninum gi 40140748

S. cerevisiae gi 6322640

L. major Friedlin gi 157875614

M. musculus gi 13641444

S. mansoni gi 425475

T. gondii gi 237834513

N. caninum Liverpool gi 401406932

T. gondii gi 159024150

C. oswcarzaki ATCC 30864 gi 514486732

R. dellemar gi 384490536

D. fasciculatum gi 328869303

G. theta gi 428184723

F. patens gi 16804557

B. distachyon gi 357136048

O. sativa gi 27368857

C. japonica gi 384080871

C. arietinum gi 502078526

C. roseus gi 110226562

G. max LOC GI00800177

V. vinifera gi 225453358

R. communis gi 255557453

R. norvegicus gi 25453370

R. norvegicus gi 25453402

Cricetus spp. gi 191155

M. ochroaster gi 532030804

M. musculus gi 6755046

M. musculus gi 387427 PDB C3G5UB

H. sapiens gi 27741659 PDB 6C0V

G. gorilla gi 246356791

P. troglodytes gi 114614226

P. abelli gi 395738781

C. aethiops gi 60326712

M. mulatta gi 74136329

M. fascicularis gi 544421742

C. jacchus gi 296209780

O. garnetti gi 305918915

F. catus gi 283806558

C. lupus familiaris gi 67462127

A. melanoleuca gi 301756332

O. divergens gi 472383372

E. callibus gi 545214020

S. scrofa gi 33529539

O. orca gi 465971828

O. aries gi 125629444

B. taurus gi 528930244

E. telfairi gi 507646369

C. porcellus gi 523580052

H. glaber gi 513001951

O. degus gi 507639834

C. lanigera gi 533185054

M. domestica gi 334348853

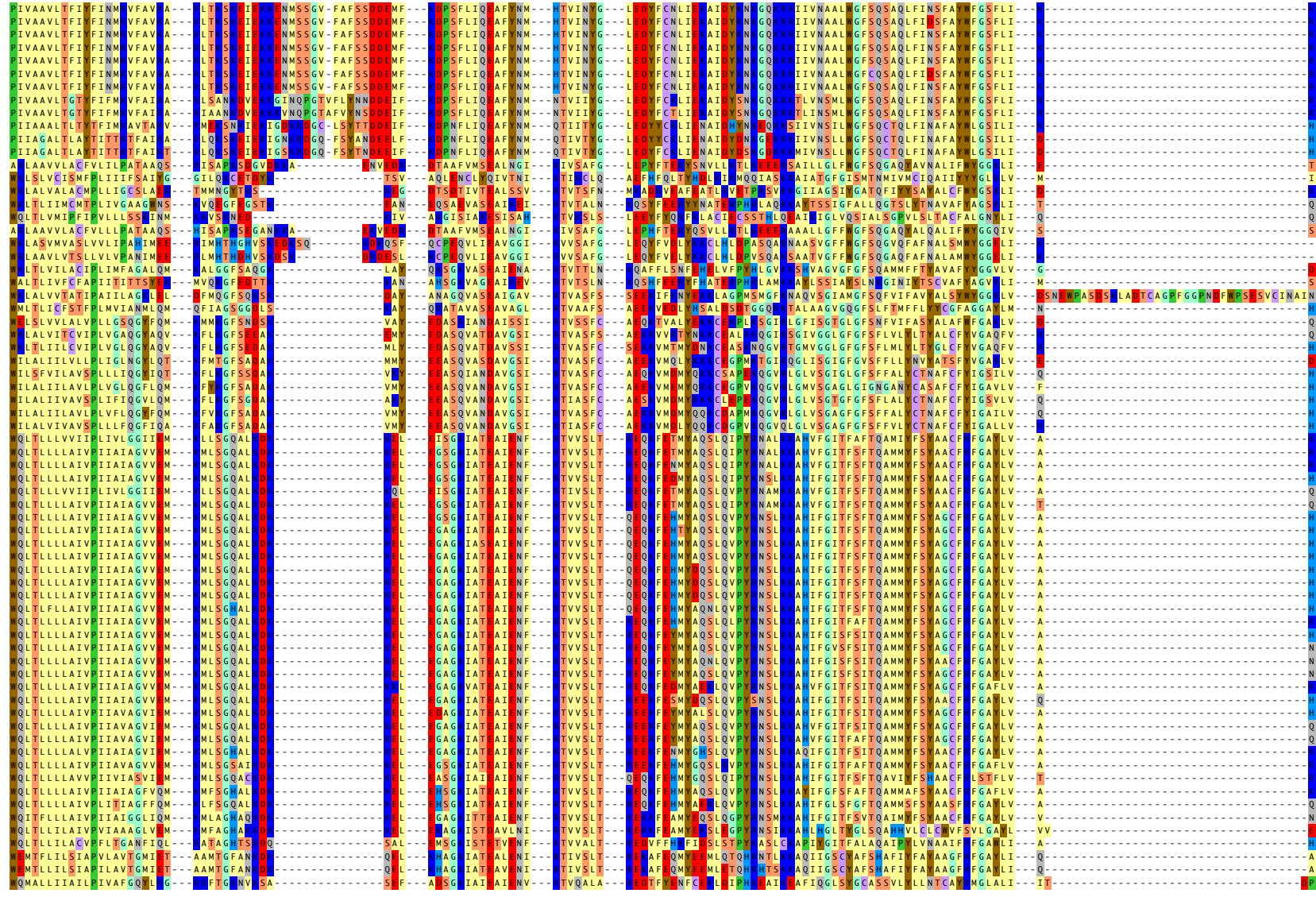
X. tropicalis gi 148234563

D. rerio gi 326674864

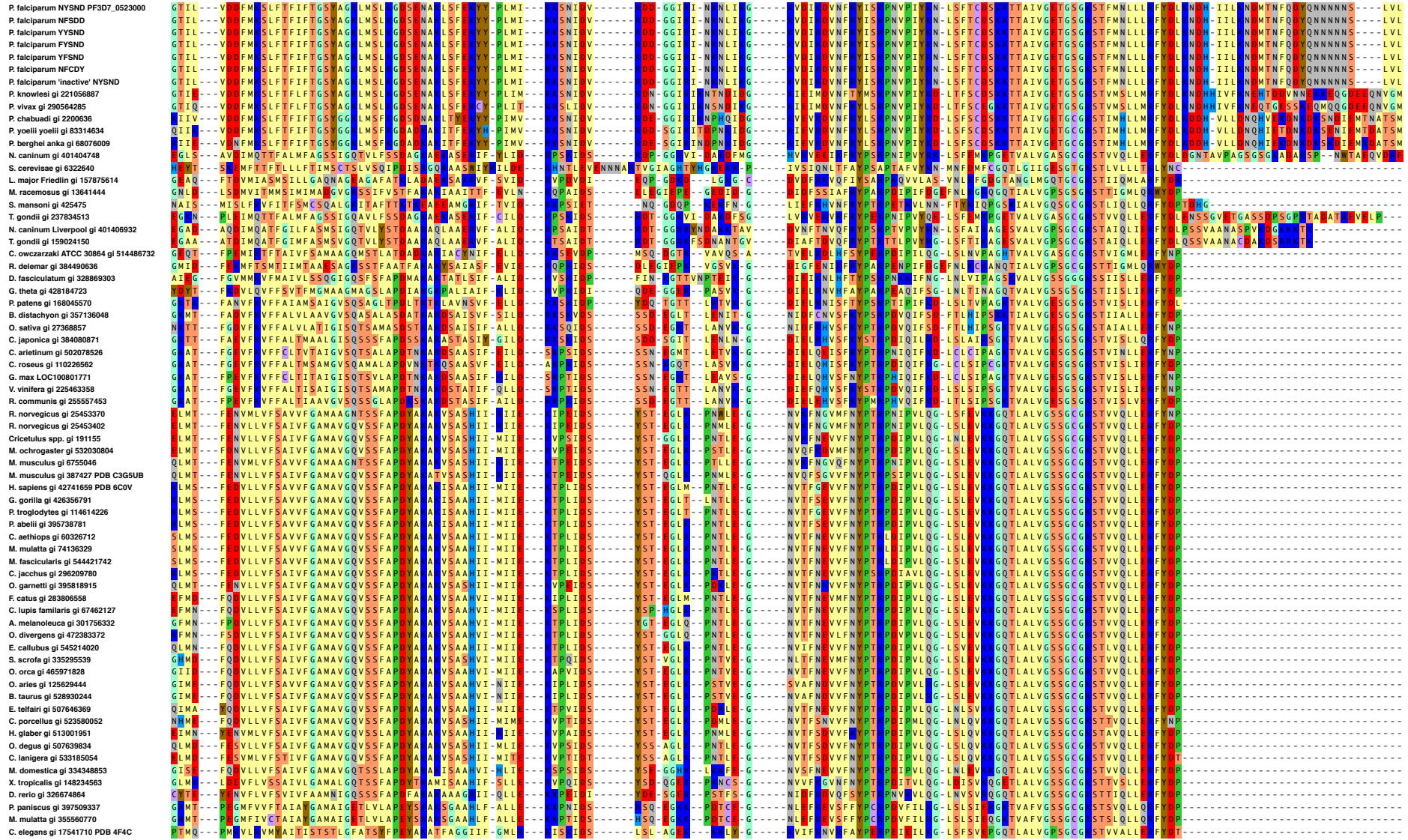
P. panicus gi 397509337

M. mulatta gi 355560770

C. elegans gi 17541710 PDB 4FC4C



## TMD 12

NBD 2...  
Walker A

...NBD 2

## Q-loop

ABC signature

Walker B



## Alignment of MDR1 proteins used to generate the PfMDR1 homology model.

A selection of MDR1 proteins from different organisms, including the *H. sapiens*, *M. musculus*, and *C. elegans* homologues that have had their structures determined [1, 2, 3, 4], were aligned using ClustalW and the resulting alignment was manually edited in MacVector 12.7.5. The gene identifier (NCBI accession number — PF3D7, gi, or LOC) of each protein is given. The sequence of PfMDR1<sup>NYSND</sup> is at the top of the alignment followed by the sequences of the other PfMDR1 isoforms included for study. The regions predicted to span the transmembrane domains (TMDs), the nucleotide-binding domains (NBDs), and the N- and C-termini are marked with black bars. Residues are shaded as follows: positively charged (arginine and lysine), dark blue; negatively charged (aspartic acid and glutamic acid), red; serine and threonine, orange; aromatic (tyrosine and tryptophan), brown; asparagine and glutamine, gray; cysteine, purple; proline, green; histidine, blue; glycine, turquoise; non-polar (methionine, alanine, isoleucine, leucine, valine, and phenylalanine), yellow. Pink triangles denote the globally prevalent polymorphisms found at positions 86, 184, 1034, 1042, and 1246 in PfMDR1. Blue triangles denote the mutations made within the Walker B motif of each NBD to generate a catalytically-inactive version of PfMDR1<sup>NYSND</sup>. Consensus sequences within the NBDs are annotated with gray boxes to indicate the positions of the Walker A and B motifs, the ABC signature sequences, and the Q-loops.

1. Jin MS, Oldham ML, Zhang Q, Chen J. Crystal structure of the multidrug transporter P-glycoprotein from *Caenorhabditis elegans*. *Nature*. 2012;490: 566-569.
2. Kim Y, Chen J. Molecular structure of human P-glycoprotein in the ATP-bound, outward-facing conformation. *Science (New York, NY)*. 2018;359: 915-919.
3. Li J, Jaimes KF, Aller SG. Refined structures of mouse P-glycoprotein. *Protein science: a publication of the Protein Society*. 2014;23: 34-46.
4. Ward AB, Szewczyk P, Grimard V, Lee C, Martinez L, Doshi R, et al. Structures of P-glycoprotein reveal its conformational flexibility and an epitope on the nucleotide-binding domain. *Proc Natl Acad Sci USA*. 2013;110: 13386-13391.

P. falciparum NYSND PF3D7\_0523000  
P. falciparum NFSDD  
P. falciparum YYNSND  
P. falciparum FYSND  
P. falciparum YFSND  
P. falciparum NFCDV  
P. falciparum 'inactive' NYSND  
P. knowlesi gi 22105887  
P. vivax gi 290564285  
P. chabaudi gi 2200623  
P. yoelii yoelii gi 33314634  
P. berghei anka gi 68076009  
*N. caninum* gi 401407478  
*S. cerevisiae* gi 6332240  
*L. major* Friedlin gi 157875614  
*M. musculus* gi 13641444  
*S. mansoni* gi 425475  
*T. gondii* gi 237834513  
*N. caninum* Liverpool gi 401406932  
*T. gondii* gi 159024150  
*C. owczarszaki* ATCC 30864 gi 514486732  
*R. delemer* gi 384490636  
*D. fasciculatum* gi 3288699303  
*G. thetai* gi 428184723  
*P. patens* gi 168045570  
*B. distachyon* gi 357136048  
*O. sativa* gi 27368657  
*C. japonica* gi 384080871  
*C. arietinum* gi 502087526  
*C. roseus* gi 110226562  
*G. max* LOC100801771  
*V. vinifera* gi 225463358  
*R. communis* gi 255557453  
*R. norvegicus* gi 25453370  
*R. norvegicus* gi 25453402  
*Cricetulus* spp. gi 191155  
*M. ochrogaster* gi 5320320804  
*M. musculus* gi 6750546  
*M. musculus* gi 387427 PDB C3G5UB  
*H. sapiens* gi 42741569 PDB 6C0V  
*G. gorilla* gi 243567691  
*P. troglodytes* gi 114614226  
*P. abelli* gi 395738781  
*C. ethiopae* gi 60326712  
*M. mulatta* gi 7413392  
*M. fascicularis* gi 54421742  
*C. jacchus* gi 296209780  
*O. garnetti* gi 395818915  
*F. catus* gi 283806558  
*C. lupis familiaris* gi 67462127  
*A. melanoleuca* gi 301756332  
*O. divergens* gi 47233372  
*E. callubius* gi 545214020  
*S. scrofa* gi 33295539  
*O. orca* gi 465971828  
*O. aries* gi 125629444  
*B. taurus* gi 528930244  
*E. telfairi* gi 507846360  
*C. porcellus* gi 523580052  
*H. glaber* gi 513001951  
*O. degus* gi 507639834  
*C. lanigera* gi 533185054  
*M. domestica* gi 334348853  
*X. tropicalis* gi 148234563  
*D. rerio* gi 326674864  
*P. panicus* gi 397509337  
*M. mulatta* gi 355560770  
*C. elegans* gi 17541710 PDB 4F4C