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## Proposal for a unified nomenclature for target site mutations associated with resistance to fungicides

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## Abstract

Evolved resistance to fungicides is a major problem limiting our ability to control agricultural, medical and veterinary pathogens and is frequently associated with substitutions in the amino acid sequence of the target protein. The convention for describing amino-acid substitutions is to cite the wild type amino acid, the codon number and the new amino acid, using the one letter amino acid code. It has frequently been observed that orthologous amino acid mutations have been selected in different species by fungicides from the same mode of action class, but the amino acids have different numbers. These differences in numbering arise from the different lengths of the proteins in each species. The purpose of the current paper is to propose a system for unifying the labelling of amino acids in fungicide target proteins. To do this we have produced alignments between fungicide target proteins of relevant species fitted to a well-studied “archetype” species. Orthologous amino acids in all species are then assigned numerical “labels” based on the position of the amino acid in the archetype protein.

## Background

Evolved resistance to fungicides is a major problem limiting our ability to control agricultural, medical and veterinary pathogens<sup>1,2</sup>. Research over the last 30 years has often defined the mechanism conferring reduced sensitivity to the fungicide. Many cases of resistance have been ascribed to the activity of efflux pumps<sup>3</sup>, or to over-expression of target genes<sup>4</sup> but the majority are due, at least partly, to substitutions (or indels) in the amino acid sequence of the target protein.

The convention for describing amino acid substitutions is to cite the wild type amino acid, the codon number and the new amino acid, using the one letter amino acid code (see<sup>5</sup> box 6.1 page 138 for a description of the system). A well-known example is the alanine (A) for glycine (G) substitution in the cytochrome b gene at position 143 conferring resistance to strobilurin fungicides, referred to as G143A<sup>6</sup>. Further alterations can be amino acid deletions designated with a  $\Delta$  and insertions with an “ins”.

Target site amino acid substitutions have been described for seven fungicide groups (named here according to the FRAC convention,<sup>7</sup>) and their target proteins. These are C3 and cytochrome b (Cytb) (Table 1); G1 and two sterol C14-demethylases (paralogs Cyp51A and Cyp51B), (Tables 2 and 3); B1/B2 and *b*-tubulin, (Table 4); C2 and three of the sub-units of the succinate dehydrogenase complex (SdhB, SdhC and SdhD) (Table 5, 6 and 7); H5 and cellulose synthase A3 (CesA3) (Table 8); E3 and the Os1 family (group III) histidine kinase (Os-1, includes Bos1, BcOS1, Daf1, HK1, HIK1 and NIK1<sup>8</sup>)(Table 9), G3 and the 3-keto reductase (Erg27). Where more than one species has been studied, it has frequently been observed that orthologous amino acid mutations have been associated with resistance to fungicides with the same mode of action.

In cases where the proteins are strongly conserved between species, the mutations have identical numbers. For example, the orthologous Cytb G143A mutation has been found in 22

species (Table 1). However in other cases orthologous mutations have different numbers – e.g. Cyp51B amino acid Y137 in *Zymoseptoria tritici* is orthologous to amino acids numbered from 132 to 145 in different species (Table 3). Similarly, SdhB amino acid H277 in *Pyrenophora teres* is orthologous to amino acids numbered from 249 to 278 (Table 5). These differences in numbers creates unnecessary confusion and obscures the relationships between mutations in different species.

Resistance caused by insertions in promoters and in efflux pumps have a much lower level of homology and so are not considered here.

### **The Proposal**

The differences in numbering arise from the different lengths of the fungicide target protein in each species. The purpose of the current paper is to propose a system for unifying the labelling of mutant amino acids in fungicide target proteins. We propose that orthologous amino acids (i.e. ones presumed to be descended from the same amino acid in the common ancestor of these species) are given the same number in all species regardless of the actual position. The advantages of a unified system is that it would be easier to memorise common changes, to determine whether the changes were novel or were repetitions of what has already been seen in other species and to link changes to particular active ingredients. Orthologous mutations would be assigned the same ‘mutation label’.

We distinguish between ‘mutation labels’ which refers to the orthology between proteins from different species, from ‘amino acid numbering’, which remains the order of the amino acids in each protein in each species. To avoid confusion, we propose that mutation labels should be italicised and mutation numbers should use regular lettering.

In several cases, amino acid substitutions have been found in the target protein but have not been definitively associated with any change in sensitivity either *in vitro* or in the field. It may be that the mutation underlying the amino acid substitution is a random event and of no obvious relevance. Definitively linking a mutation to a sensitivity change can be technically very demanding. If resistance to the same class of fungicide is linked to mutations affecting orthologous codons in different species, this is strong, if still circumstantial, evidence of the importance of the mutation. Unifying the mutant labelling system will make it much easier to identify important codon changes. This would assist the prioritisation of research aiming to functionally characterise mutations.

### **Options for producing the alignments**

We have produced a set of draft alignments of each target protein for which resistance to multiple species has been reported (Figures 1 to 9) and tables of putatively orthologous amino acids in other species where fungicide resistance has been reported (Tables 1 to 9). The species included in these tables and alignments have been referred to by their European and Mediterranean Plant Protection Organization (EPPO) codes<sup>9</sup> as listed in Table 10.

The alignments for *b*-tubulin and Cytb are essentially co-linear in fungi studied to date and hence there are no changes to be made on the current nomenclature. For the other genes, we have considered four possible methods to generate the alignment. The alignment could be;

1. Fitted to the longest gene in the gene set.
2. Fitted to a strict consensus alignment.
3. Fitted to the gene from the species that is currently the most researched species for the fungicide resistance concerned.
4. Fitted to the gene from the species that was the first species for the fungicide resistance concerned.

The aim is to create a set of alignments that would be stable into the foreseeable future and would invoke the least relabelling of mutations that have already been described and published. We favour method 3 (basing the alignment on the species with the most currently-described resistance mutations), but also taking into account method 1 (using the longer gene) when alternative species are candidates. We propose that Cyp51A is fitted to ASPEFU (*Aspergillus fumigatus*), Cyp51B and Cytb are fitted to SEPTRI (*Zymoseptoria tritici*), *b*-tubulin to ASPEND (*Aspergillus nidulans*), the SDH proteins to PYRNTE (*Pyrenophora teres*), CesA3 to PHYTIN (*Phytophthora infestans*) and Os-1 to BOTCIN (*Botrytis cinerea*). For Erg27, mutations associated with resistance have currently been described only in BOTCIN and thus we propose this species as the archetype. The alignments have been summarised and fungicide resistance associated mutations are given in the Tables. By way of example, in Cyp51B the mutation Y136F in ERYSGH would be given the label Y137F. In CANDAL (*Candida albicans*) the orthologous amino acid is Y132 and has been mutated to both F and H. The Y132H mutation would therefore be given the label Y137H. V151 in SEPTRI is clearly demonstrated to be orthologous to I145F in PHAKPA (*Phakopsora pachyrhizi*). This mutation would be labelled I151F in PHAKPA and V151F in SEPTRI. The other proposed relabellings are listed in Tables 1 to 9.

By examining the species that have amino acids mutations with common labels, we can infer that positions 137, 148, 461, 467, 483 and 524 in Cyp51B are especially important in conferring resistance to triazole fungicides. This is consistent with numerous functional studies<sup>10,11</sup>. We expect that the alignments should assist the identification of key amino acids in target proteins of newer fungicide classes.

### **The proposal in practice**

The system must also allow for mutations to be discovered in new species. The parameters used to make the alignments are described below and can be applied to an alignment between the new species and the archetype. We envisage regularly updating the alignments based on new published knowledge.

A potential problem with the system we propose might occur if an amino acid in a newly described mutant gene corresponded to a gap in the archetype protein's sequence. In such a case, the mutation could be labelled as X50.2Y if it concerned the second extra amino acid after number 50 in the archetype sequence. To our knowledge, no examples of mutations of

such poorly conserved amino acids causing resistance have been described, but the possibility remains.

We hope that future studies will refer to the archetype by indicating that the mutation X123Y in the target protein associated with resistance corresponds to the archetype X145Y and refer to this paper or a related web page for support.

We suggest that other target genes from medically important fungi (e.g. the FKS1/2 genes that are targets of Echinocandins) and from herbicide- and insecticide- resistant weeds and insects might also benefit from this approach.

We commend this scheme to the community and seek comment and support. And we urge Journal editors to encourage authors to use this new system.

#### Note on the alignments

Amino acid sequences were downloaded from NCBI GenBank and annotated with reported amino acid substitutions<sup>8, 12-15</sup>, using Geneious 6.1.8 software (Biomatters). Alignments of sequences were generated using ClustalW<sup>16</sup> algorithm with Blosum scoring matrix, gap opening penalty 10, gap extension penalty 0.5, free end gaps.

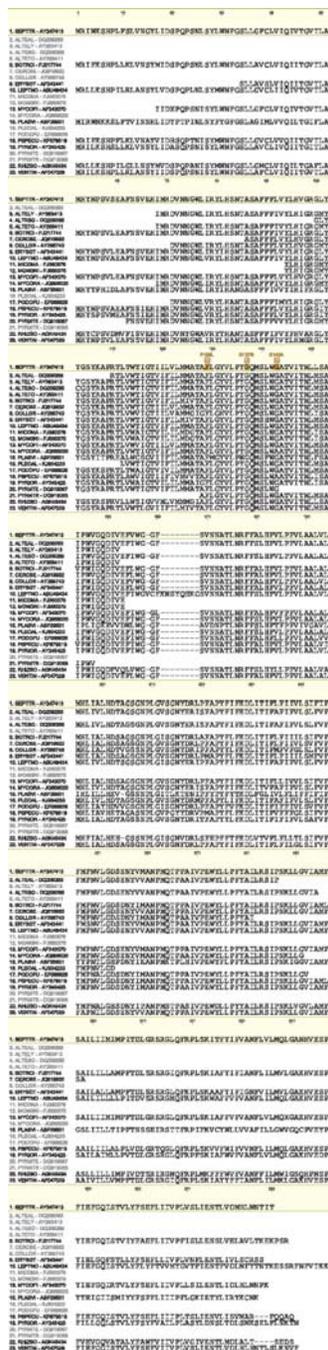
The alignments are available as .doc file and as fasta files as supplementary data,

#### References

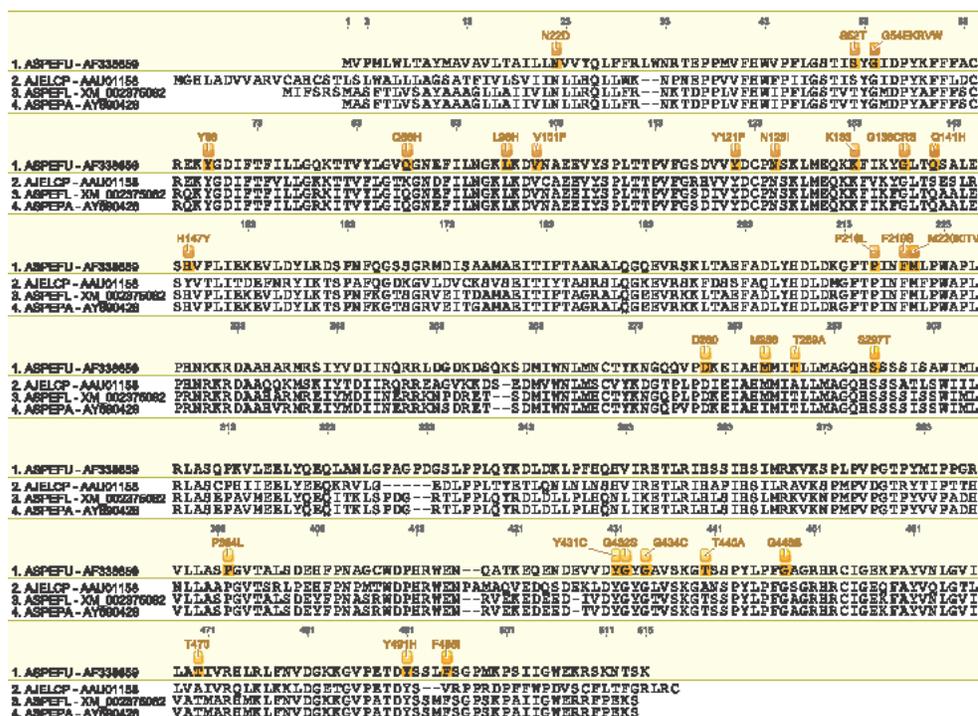
1. Lucas JA, Hawkins NJ and Fraaije BA. The Evolution of Fungicide Resistance. In *Advances in Applied Microbiology*, pp. 29-92 (2015).
2. Grimmer MK, van den Bosch F, Powers SJ and Paveley ND, Fungicide resistance risk assessment based on traits associated with the rate of pathogen evolution. *Pest Management Science*; **71**(2): 207-215 DOI Electronic Resource Number (2015).
3. Del Sorbo G, Schoonbeek H and De Waard MA, Fungal transporters involved in efflux of natural toxic compounds and fungicides. *Fungal Genet Biol*; **30**(1): 1-15 DOI Electronic Resource Number (2000).
4. Cools HJ, Fraaije BA, Bean TP, Antoniw J and Lucas JA, Transcriptome profiling of the response of *Mycosphaerella graminicola* isolates to an azole fungicide using cDNA microarrays. *Molecular Plant Pathology*; **8**(5): 639-651 DOI Electronic Resource Number (2007).
5. Oliver RP and Hewitt HG. *Fungicides in crop protection: Second edition*, (2014).
6. Sierotzki H, Parisi S, Steinfeld U, Tenzer I, Poirey S and Gisi U, Mode of resistance to respiration inhibitors at the cytochrome bc1 enzyme complex of *Mycosphaerella fijiensis* field isolates. *Pest Management Science*; **56**(10): 833-841 DOI Electronic Resource Number (2000).
7. FRAC, FRAC Code List. <http://www.frac.info/docs/default-source/publications/pathogen-risk/pathogen-risk-list.pdf?sfvrsn=8> [accessed January 10th 2016 2016].
8. Ishii H and Hollomon DW. *Fungicide Resistance in Plant Pathogens: Principles and a Guide to Practical Management*. Springer, (2015).
9. EPPO, EPPO Global Database. <https://gd.eppo.int> [accessed January 21st 2016].
10. Kelly HM, Wright DL, Dufault NS and Marois JJ, Decision models for fungicide applications for soybean rust. *Plant Health Progress*; **16**(2): 80-83 DOI Electronic Resource Number (2015).

11. Cools HJ, Parker JE, Kelly DE, Lucas JA, Fraaije BA and Kelly SL, Heterologous expression of mutated eburicol 14 $\alpha$ -demethylase (CYP51) proteins of *Mycosphaerella graminicola* to assess effects on azole fungicide sensitivity and intrinsic protein function. *Applied and Environmental Microbiology*; **76**(9): 2866-2872 DOI Electronic Resource Number (2010).
12. Becher R and Wirsel SG, Fungal cytochrome P450 sterol 14 $\alpha$ -demethylase (CYP51) and azole resistance in plant and human pathogens. *Applied microbiology and biotechnology*; **95**(4): 825-840 DOI Electronic Resource Number (2012).
13. Morio F, Loge C, Besse B, Hennequin C and Le Pape P, Screening for amino acid substitutions in the *Candida albicans* Erg11 protein of azole-susceptible and azole-resistant clinical isolates: new substitutions and a review of the literature. *Diagnostic microbiology and infectious disease*; **66**(4): 373-384 DOI Electronic Resource Number (2010).
14. Howard SJ and Arendrup MC, Acquired antifungal drug resistance in *Aspergillus fumigatus*: epidemiology and detection. *Medical mycology*; **49**(sup1): S90-S95 DOI Electronic Resource Number (2011).
15. Sagatova AA, Keniya MV, Wilson RK, Monk BC and Tyndall JD, Structural insights into binding of the antifungal drug fluconazole to *Saccharomyces cerevisiae* lanosterol 14 $\alpha$ -demethylase. *Antimicrobial agents and chemotherapy*; **59**(8): 4982-4989 DOI Electronic Resource Number (2015).
16. Thompson JD, Higgins DG and Gibson TJ, CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic acids research*; **22**(22): 4673-4680 DOI Electronic Resource Number (1994).

Figure Legends



**Figure 1.** Amino acid sequence alignment of the Cytb family. Sequences are named by species EPPO code and NCBI gene accession number. Residues highlighted in yellow in the archetype sequence from *Zymoseptoria tritici* denote amino acid substitutions associated with fungicide resistance at an orthologous position in any of the sequences. Numerical mutation labels shown above the alignment are based on the position number of the amino acid in the archetype protein.



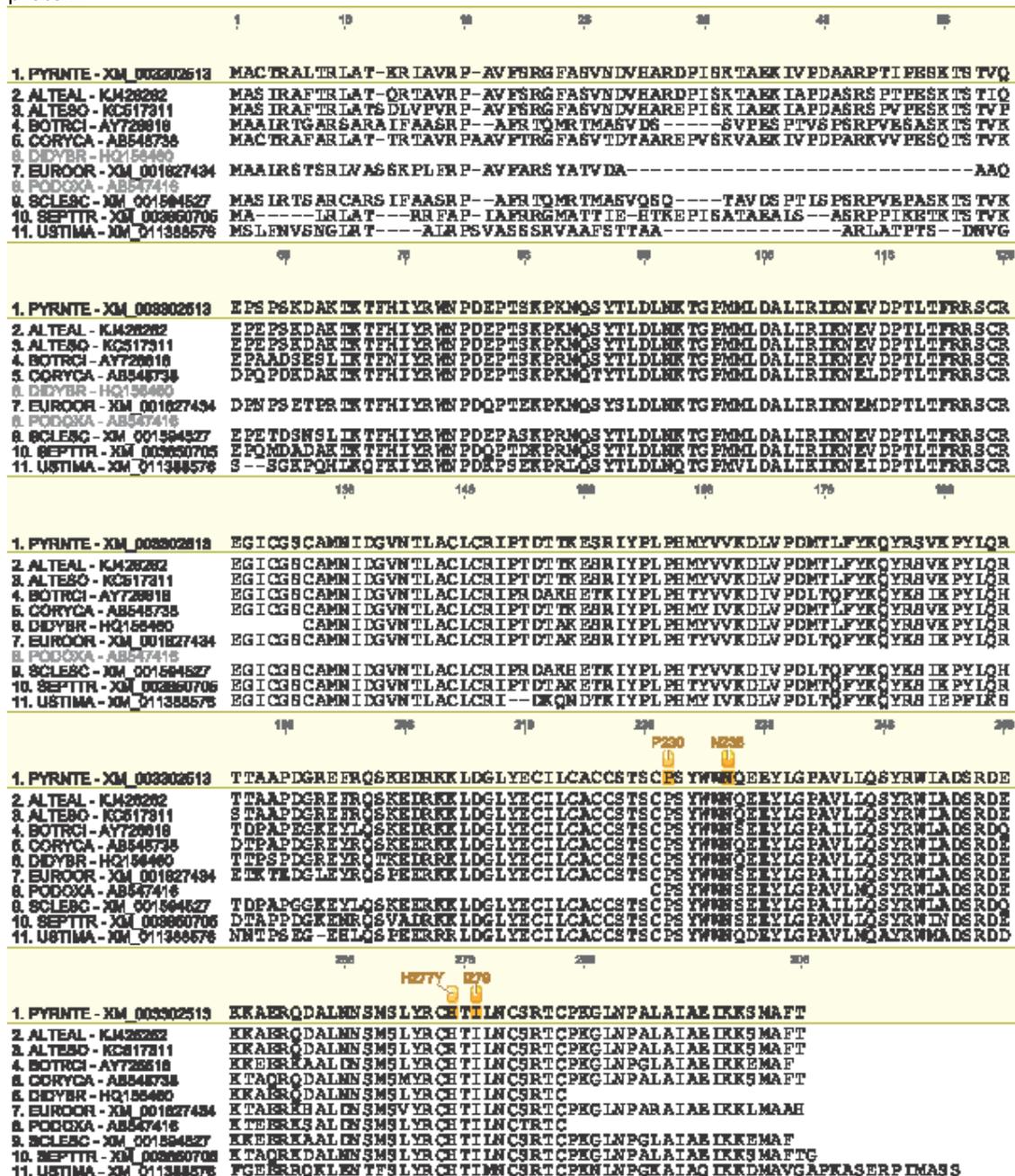
**Figure 2.** Amino acid sequence alignment of the Cyp51A family. Sequences are named by species EPO code and NCBI gene accession number. Residues highlighted in yellow in the archetype sequence from *Aspergillus fumigatus* (Cyp51A) denote amino acid substitutions associated with fungicide resistance at an orthologous position in any of the sequences. Numerical mutation labels shown above the alignment are based on the position number of the amino acid in the archetype protein.

|                          |  |
|--------------------------|--|
| 1. SEPTTR - AY253234     | MGLLQEVLAQFDQAQFG-----QTHLNKLVGLGFLAFSTLAILLNVLSQLLE   |
| 2. ASPEFL - K0C13903     | MGILAVILDSVRCERCS-----GSSLNMLSTVALLSILVSVVINVLRQLLE  |
| 3. CANDAL - AF163960     | MAIVETVID-----GINYFLSLSVTQOISILLGVPVYVNLVWQVLY   |
| 4. CERCBE - HM778021     | MGLLQDVAVAFDNRFG-----QTATWKLVLVLAFTFFLLSVVNVLRQVLE   |
| 5. ERYSGH - AJ578781     | MGLISLSEFMFYLQPLI-----QLGFGIALASGILSILLDLTFFINVLKQLLE  |
| 6. ERYSGH - AJ578781     | MKGPFSEFMSFYLOPLI-----QGFSGIALASGILSILLDLTFFINVLKQLLE  |
| 7. FILBNF - AF228914     | MSAITIQVQQLLQVQAQFPPWFALLESIKVALAVVGTIPALITIGINVFQGLCL   |
| 8. MONIFC - AF470821     | MGVLETTIAGFDAQEIS-----QRSIGTITLAAVVAEAVVDAVVINVLQVLE   |
| 9. MYCOPI - XP_007928762 | MGLLDLDALEDAQVFG-----QTATWKLVLVLAFTFFLLSVVNVLRQVLE   |
| 10. PHAKPA - K0741476    | MSSSV---LIDQVLY-----SFTSLLITSELSLTIIVLNWQLLE   |
| 11. PUCCRT - FJ978683    | MSSVSEVLELEPIG-----SFTSFTNOVLLIYLVAVVSIISINIFDQLAI   |
| 12. SACCCE - AAB88433    | MSATKIVGSALEYVNI-----GLSHFLALEFLAQRISLIIIPFTYINLVWQLLY   |
| 13. UNCNE - AAC49812     | MYIADILSLLTQQT-----RYGWIWMVTSIAPSILLAVGLNVLSQLLE   |
| 1. SEPTTR - AY253234     | RGRSSDPPLVFWVPPFISG <sup>106</sup> ITTYGIDPYKFFFS <sup>280</sup> CREKYGDVFTFILLGKKTIVCLGT  |
| 2. ASPEFL - K0C13903     | KNY--KEPPLVFWVPPFISGTTISYGMDDPYRFFFNCREKYGDVFTFVLLGKKTIVYLG  |
| 3. CANDAL - AF163960     | SIRKDRAPLWFWYIWFVFGSAAISYGOQPYEFPFESCRQKYGDVFTFILLGKIMTVYLG  |
| 4. CERCBE - HM778021     | RQPKMKEPPLVFWVPPFISGTTISYGLDPYKFFFS <sup>280</sup> CRQKYGDVFTFILLGKKTIVCLGT  |
| 5. ERYSGH - AJ578781     | KNP--MEPPIVFWVPPFISGTTISYGMNPKYFPHESQAKYGNFTFILLGKKTIVYLG  |
| 6. ERYSGH - AJ578781     | KNP--MEPPIVFWVPPFISGTTISYGMNPKYFPHESQAKYGNFTFILLGKKTIVYLG  |
| 7. FILBNF - AF228914     | PRKDLPPVFWVPPFISGTTISYGLDPYKFLFECRDKYGDVFTFILLGRRITVVALG   |
| 8. MONIFC - AF470821     | ANP--MEPPIVFWVPPFISGTTISYGLDPYRFFFDCKRKYGDVFTFILLGKKTIVYLG   |
| 9. MYCOPI - XP_007928762 | RNP--MEPPIVFWVPPFISGTTISYGLDPYKFFACRQKYGDVFTFILLGKKTIVYLG  |
| 10. PHAKPA - K0741476    | --KDRMTPPLVFWVPPFISGTTISYGMDDPYQFEDCKRKHGNVFTFVLLNKKVTVVALG  |
| 11. PUCCRT - FJ978683    | PKDPTAPPVFWVPPFISGTTISYGLDPYAFLES <sup>280</sup> CRQKYGNVFTFVLLNKKVTVVALG  |
| 12. SACCCE - AAB88433    | SIRKDRAPLWFWYIWFVFGSAVVYGMKPYEFPFESCRQKYGDVFTFVLLGRVMTVYLG   |
| 13. UNCNE - AAC49812     | RRP--YEPVFWVPPFISGTTISYGLDPYKFFYFDCRQKYGDVFTFILLGKKTIVYLG  |
| 1. SEPTTR - AY253234     | KGN <sup>D107V</sup> DFILNGKLRDVNABEYIS <sup>119</sup> PLTTFVFGK <sup>L120</sup> DVVYDC <sup>D134Q</sup> PNSK <sup>V138AG</sup> LMEQ <sup>V157F</sup> KK <sup>M14E</sup> EVRYGLT <sup>K149</sup> T <sup>V151</sup> HAL   |
| 2. ASPEFL - K0C13903     | KGNDFILNGKLRDVNABEYISPLTTFVFGKDVVYDCPNASKLMEQKKEVRYGLTSHAL   |
| 3. CANDAL - AF163960     | KGEFEVFNAKLSDVSAEDAYKHLDTFVFGKGVYDCPNASKLMEQKKFAKALTTDSF   |
| 4. CERCBE - HM778021     | KGNDFILNGKLRDVNABEYISPLTTFVFGKDVVYDCPNASKLMEQKKFVKGLTSSAAL   |
| 5. ERYSGH - AJ578781     | QGNDFILNGKLRDVNABEYITVLTTFVFGTDVVYDCPNASKLMEQKKFMKAALTTFAE   |
| 6. ERYSGH - AJ578781     | QGNDFILNGKLRDVNABEYISVLTTFVFGTDVVYDCPNASKLMEQKKFMKAALTTFAE   |
| 7. FILBNF - AF228914     | KGNDLISLGGKISQVSAEAYTHLDTFVFGKGVYDCPNEMLMQOKKFKISGLTSESL   |
| 8. MONIFC - AF470821     | KGNDFILNGKLRDVNABEYITVLTTFVFGKDVVYDCPNASKLMEQKKFMKIGLSTFAE   |
| 9. MYCOPI - XP_007928762 | KGNDFILNGKLRDVNABEYISPLTTFVFGTDVVYDCPNASKLMEQKKFVKRYGLTSSAL  |
| 10. PHAKPA - K0741476    | EGNTLVNLGKILSEVNABEAYTHLDTFVFGKDVVYDVPNSILMQOKKFKAGLTKTEK  |
| 11. PUCCRT - FJ978683    | EGNALVNLGKILSQVNABEAYTALDTFVFGTDVVYDVPNSAILMQOKKFKVSGLTNENF  |
| 12. SACCCE - AAB88433    | KGEFEVFNAKLADVSAARAAYALHDTFVFGKGVYDCPNASKLMEQKKFVKGLTKEAF  |
| 13. UNCNE - AAC49812     | QGNDFILNGKLRDVNABEYITNLTTFVFGKDVVYDCPNASKLMEQKKFMKALTTEAF  |
| 1. SEPTTR - AY253234     | QSYVTLIAAETRQ <sup>D176</sup> FD <sup>N178S</sup> DRNNPEK <sup>220T</sup> FAS <sup>220T</sup> TSGTIDLEPPALAE <sup>220T</sup> LIYTASRSIQKEVREG  |
| 2. ASPEFL - K0C13903     | RSYVRLITEVEVDFQKSS---ALQFNGVFDVCKTIAEITTYTASRSIQKEVRSR   |
| 3. CANDAL - AF163960     | KRYVPKIREYILNYFTLDESFKLMS <sup>220T</sup> THGVANV <sup>220T</sup> KEPITTYTASRSIQKEVRSR   |
| 4. CERCBE - HM778021     | QSYVTLITEVTRQFSPKNNPEK <sup>220T</sup> FAS <sup>220T</sup> TSGTIDLEPPALAE <sup>220T</sup> LIYTASRSIQKEVRSR   |
| 5. ERYSGH - AJ578781     | RSYVPIIQHEVKSFT <sup>220T</sup> EKKCD--DFRSEGININIAVMAEITTYTASRSIQKEVRSR   |
| 6. ERYSGH - AJ578781     | RSYVPIIQHEVKSFT <sup>220T</sup> EKKCD--DFRSEGININIAVMAEITTYTASRSIQKEVRSR   |
| 7. FILBNF - AF228914     | QSYVPMITSECEDFPKV <sup>220T</sup> EVG <sup>220T</sup> I--SQE <sup>220T</sup> PSATL <sup>220T</sup> DLKANS <sup>220T</sup> ELI <sup>220T</sup> LTASRTIQKEVRSR   |
| 8. MONIFC - AF470821     | RSYVPIIQHEVFNPKRSS---AFRQGEKGTANIPMAEITTYTASRSIQKEVRSR   |
| 9. MYCOPI - XP_007928762 | RSYVLI <sup>220T</sup> IK <sup>220T</sup> ET <sup>220T</sup> DF <sup>220T</sup> SK <sup>220T</sup> DN <sup>220T</sup> ES <sup>220T</sup> KK <sup>220T</sup> FAS <sup>220T</sup> THG <sup>220T</sup> TV <sup>220T</sup> DL <sup>220T</sup> PPMAE <sup>220T</sup> LIYTASRSIQKEVRSR   |
| 10. PHAKPA - K0741476    | RKYVGIIVREATSYLEDDH <sup>220T</sup> LF <sup>220T</sup> C--S <sup>220T</sup> PV <sup>220T</sup> KS <sup>220T</sup> V <sup>220T</sup> TK <sup>220T</sup> DV <sup>220T</sup> HD <sup>220T</sup> IT <sup>220T</sup> CTAAT <sup>220T</sup> LIQKEVREG  |
| 11. PUCCRT - FJ978683    | RKYVSLIAEETISYLEDDH <sup>220T</sup> VF <sup>220T</sup> E--N <sup>220T</sup> PK <sup>220T</sup> TQ <sup>220T</sup> TK <sup>220T</sup> RD <sup>220T</sup> FK <sup>220T</sup> VASE <sup>220T</sup> ITCTAS <sup>220T</sup> ALQKEVREA   |
| 12. SACCCE - AAB88433    | KRYVPLIAEVEYK <sup>220T</sup> YFR <sup>220T</sup> DS <sup>220T</sup> KN <sup>220T</sup> FL <sup>220T</sup> N <sup>220T</sup> ER <sup>220T</sup> IT <sup>220T</sup> GT <sup>220T</sup> ID <sup>220T</sup> VM <sup>220T</sup> TP <sup>220T</sup> EM <sup>220T</sup> IT <sup>220T</sup> TASRS <sup>220T</sup> LLQKEVREK   |
| 13. UNCNE - AAC49812     | RSYVPIIQHEVEAYINNCV---S <sup>220T</sup> FQ <sup>220T</sup> ES <sup>220T</sup> GT <sup>220T</sup> VN <sup>220T</sup> ISK <sup>220T</sup> VMAEITTYTASRSALQKEVREN   |
| 1. SEPTTR - AY253234     | FDS <sup>270</sup> SPADLY <sup>270</sup> LDL <sup>270</sup> MG <sup>270</sup> FT <sup>270</sup> FIN <sup>270</sup> FML <sup>270</sup> WAP <sup>270</sup> L <sup>270</sup> PQ <sup>270</sup> NRR <sup>270</sup> DY <sup>270</sup> AQ <sup>270</sup> KK <sup>270</sup> MS <sup>270</sup> ET <sup>270</sup> YMS <sup>270</sup> I <sup>270</sup> IQ <sup>270</sup> RR <sup>270</sup> ES--  |
| 2. ASPEFL - K0C13903     | FDS <sup>270</sup> TEA <sup>270</sup> LY <sup>270</sup> LDL <sup>270</sup> MG <sup>270</sup> FAP <sup>270</sup> IN <sup>270</sup> FML <sup>270</sup> WAP <sup>270</sup> L <sup>270</sup> PN <sup>270</sup> RKR <sup>270</sup> DA <sup>270</sup> AQ <sup>270</sup> KK <sup>270</sup> MT <sup>270</sup> ET <sup>270</sup> YMS <sup>270</sup> I <sup>270</sup> IK <sup>270</sup> RR <sup>270</sup> EAG--  |
| 3. CANDAL - AF163960     | FDS <sup>270</sup> TEA <sup>270</sup> LY <sup>270</sup> LDL <sup>270</sup> MG <sup>270</sup> F <sup>270</sup> TP <sup>270</sup> IN <sup>270</sup> F <sup>270</sup> PN <sup>270</sup> L <sup>270</sup> PL <sup>270</sup> PH <sup>270</sup> Y <sup>270</sup> WR <sup>270</sup> DA <sup>270</sup> AQ <sup>270</sup> KK <sup>270</sup> IS <sup>270</sup> AT <sup>270</sup> Y <sup>270</sup> M <sup>270</sup> KE <sup>270</sup> I <sup>270</sup> IK <sup>270</sup> RR <sup>270</sup> ERAG--   |
| 4. CERCBE - HM778021     | FDS <sup>270</sup> TEA <sup>270</sup> LY <sup>270</sup> LDL <sup>270</sup> MG <sup>270</sup> F <sup>270</sup> TP <sup>270</sup> IN <sup>270</sup> F <sup>270</sup> ML <sup>270</sup> WAP <sup>270</sup> L <sup>270</sup> PN <sup>270</sup> RR <sup>270</sup> DY <sup>270</sup> AQ <sup>270</sup> KK <sup>270</sup> MT <sup>270</sup> ET <sup>270</sup> YMS <sup>270</sup> I <sup>270</sup> IK <sup>270</sup> RR <sup>270</sup> EAGD--  |
| 5. ERYSGH - AJ578781     | FDS <sup>270</sup> TEA <sup>270</sup> LY <sup>270</sup> LDL <sup>270</sup> MG <sup>270</sup> F <sup>270</sup> TP <sup>270</sup> IN <sup>270</sup> F <sup>270</sup> ML <sup>270</sup> WAP <sup>270</sup> L <sup>270</sup> PN <sup>270</sup> RR <sup>270</sup> DHA <sup>270</sup> Q <sup>270</sup> RT <sup>270</sup> VAK <sup>270</sup> IY <sup>270</sup> ME <sup>270</sup> I <sup>270</sup> IN <sup>270</sup> SR <sup>270</sup> RT <sup>270</sup> QK--  |
| 6. ERYSGH - AJ578781     | FDS <sup>270</sup> TEA <sup>270</sup> LY <sup>270</sup> LDL <sup>270</sup> MG <sup>270</sup> F <sup>270</sup> TP <sup>270</sup> IN <sup>270</sup> F <sup>270</sup> ML <sup>270</sup> WAP <sup>270</sup> L <sup>270</sup> PN <sup>270</sup> RR <sup>270</sup> DHA <sup>270</sup> Q <sup>270</sup> RT <sup>270</sup> VAK <sup>270</sup> IY <sup>270</sup> ME <sup>270</sup> I <sup>270</sup> IN <sup>270</sup> SR <sup>270</sup> RT <sup>270</sup> QK--  |
| 7. FILBNF - AF228914     | LNG <sup>270</sup> Q <sup>270</sup> FA <sup>270</sup> K <sup>270</sup> Y <sup>270</sup> EDL <sup>270</sup> MG <sup>270</sup> F <sup>270</sup> TP <sup>270</sup> IN <sup>270</sup> F <sup>270</sup> ML <sup>270</sup> WAP <sup>270</sup> L <sup>270</sup> PL <sup>270</sup> PS <sup>270</sup> Y <sup>270</sup> RR <sup>270</sup> DA <sup>270</sup> AQ <sup>270</sup> KK <sup>270</sup> MS <sup>270</sup> DF <sup>270</sup> Y <sup>270</sup> L <sup>270</sup> IM <sup>270</sup> EN <sup>270</sup> RR <sup>270</sup> KEG--                            |
| 8. MONIFC - AF470821     | FDS <sup>270</sup> TEA <sup>270</sup> LY <sup>270</sup> LDL <sup>270</sup> MG <sup>270</sup> F <sup>270</sup> TP <sup>270</sup> IN <sup>270</sup> F <sup>270</sup> ML <sup>270</sup> WAP <sup>270</sup> L <sup>270</sup> PN <sup>270</sup> RR <sup>270</sup> DHA <sup>270</sup> Q <sup>270</sup> RT <sup>270</sup> VAK <sup>270</sup> IY <sup>270</sup> ME <sup>270</sup> I <sup>270</sup> IN <sup>270</sup> SR <sup>270</sup> RT <sup>270</sup> QK--  |
| 9. MYCOPI - XP_007928762 | FDS <sup>270</sup> TEA <sup>270</sup> LY <sup>270</sup> LDL <sup>270</sup> MG <sup>270</sup> F <sup>270</sup> TP <sup>270</sup> IN <sup>270</sup> F <sup>270</sup> ML <sup>270</sup> WAP <sup>270</sup> L <sup>270</sup> PN <sup>270</sup> RR <sup>270</sup> DA <sup>270</sup> AQ <sup>270</sup> KK <sup>270</sup> MA <sup>270</sup> EV <sup>270</sup> Y <sup>270</sup> TA <sup>270</sup> I <sup>270</sup> IK <sup>270</sup> RR <sup>270</sup> KEG--   |
| 10. PHAKPA - K0741476    | DN <sup>270</sup> KS <sup>270</sup> FA <sup>270</sup> QL <sup>270</sup> LDL <sup>270</sup> MG <sup>270</sup> F <sup>270</sup> TP <sup>270</sup> IN <sup>270</sup> F <sup>270</sup> ML <sup>270</sup> WAP <sup>270</sup> L <sup>270</sup> PL <sup>270</sup> PS <sup>270</sup> Y <sup>270</sup> RR <sup>270</sup> DA <sup>270</sup> AQ <sup>270</sup> KK <sup>270</sup> MS <sup>270</sup> DF <sup>270</sup> Y <sup>270</sup> L <sup>270</sup> IM <sup>270</sup> EN <sup>270</sup> RR <sup>270</sup> KEG--  |
| 11. PUCCRT - FJ978683    | DN <sup>270</sup> KS <sup>270</sup> FA <sup>270</sup> QL <sup>270</sup> LDL <sup>270</sup> MG <sup>270</sup> F <sup>270</sup> TP <sup>270</sup> IN <sup>270</sup> F <sup>270</sup> ML <sup>270</sup> WAP <sup>270</sup> L <sup>270</sup> PL <sup>270</sup> PS <sup>270</sup> Y <sup>270</sup> RR <sup>270</sup> DA <sup>270</sup> AQ <sup>270</sup> KK <sup>270</sup> MS <sup>270</sup> DF <sup>270</sup> Y <sup>270</sup> L <sup>270</sup> IM <sup>270</sup> EN <sup>270</sup> RR <sup>270</sup> KEG--  |
| 12. SACCCE - AAB88433    | DD <sup>270</sup> TD <sup>270</sup> FA <sup>270</sup> LY <sup>270</sup> LDL <sup>270</sup> MG <sup>270</sup> F <sup>270</sup> TP <sup>270</sup> IN <sup>270</sup> F <sup>270</sup> ML <sup>270</sup> WAP <sup>270</sup> L <sup>270</sup> PN <sup>270</sup> RR <sup>270</sup> DHA <sup>270</sup> Q <sup>270</sup> RT <sup>270</sup> VAK <sup>270</sup> IY <sup>270</sup> ME <sup>270</sup> I <sup>270</sup> IN <sup>270</sup> SR <sup>270</sup> RT <sup>270</sup> QK--  |
| 13. UNCNE - AAC49812     | FDS <sup>270</sup> TEA <sup>270</sup> LY <sup>270</sup> LDL <sup>270</sup> MG <sup>270</sup> F <sup>270</sup> TP <sup>270</sup> IN <sup>270</sup> F <sup>270</sup> TY <sup>270</sup> WAP <sup>270</sup> L <sup>270</sup> PN <sup>270</sup> RR <sup>270</sup> DHA <sup>270</sup> Q <sup>270</sup> RT <sup>270</sup> VAK <sup>270</sup> IY <sup>270</sup> ME <sup>270</sup> I <sup>270</sup> IN <sup>270</sup> SR <sup>270</sup> RT <sup>270</sup> QK--  |
| 1. SEPTTR - AY253234     | --KTGEBEEDMIENLMQ <sup>N294H</sup> --CKYKDGNAIPDKETAENMIALLMAG <sup>E300</sup> HSS <sup>H302Y</sup> SATESWITLRL <sup>A311Q</sup>   |
| 2. ASPEFL - K0C13903     | --SKRDS <sup>E300</sup> EDM <sup>H302Y</sup> VN <sup>A311Q</sup> ML <sup>G312A</sup> MS--CMYKDGTPV <sup>E300</sup> PDE <sup>H302Y</sup> ETAE <sup>A311Q</sup> MMIALLMAG <sup>G312A</sup> HSS <sup>E300</sup> SS <sup>H302Y</sup> TAAN <sup>A311Q</sup> VIL <sup>G312A</sup> LEL  |
| 3. CANDAL - AF163960     | --DIDFNRDLIDSLLEH <sup>E300</sup> STYK <sup>H302Y</sup> DG <sup>A311Q</sup> VR <sup>G312A</sup> MTDQ <sup>E300</sup> EIAN <sup>H302Y</sup> LLIG <sup>A311Q</sup> ILM <sup>G312A</sup> GGCH <sup>E300</sup> TS <sup>H302Y</sup> AS <sup>A311Q</sup> TS <sup>G312A</sup> AW <sup>E300</sup> FL <sup>G312A</sup> LEL  |
| 4. CERCBE - HM778021     | KTS <sup>E300</sup> ENG <sup>H302Y</sup> EDMT <sup>A311Q</sup> KN <sup>G312A</sup> LMS--CTYKDGTPV <sup>E300</sup> PDR <sup>H302Y</sup> EVARE <sup>A311Q</sup> MMIALLMAG <sup>G312A</sup> HSS <sup>E300</sup> SS <sup>H302Y</sup> TS <sup>A311Q</sup> SW <sup>G312A</sup> FL <sup>E300</sup> LEL  |
| 5. ERYSGH - AJ578781     | --ETDSDMLD <sup>E300</sup> MM <sup>H302Y</sup> QL <sup>A311Q</sup> ML <sup>G312A</sup> MR--SS <sup>E300</sup> YK <sup>H302Y</sup> DGT <sup>A311Q</sup> PV <sup>G312A</sup> PK <sup>E300</sup> ETA <sup>H302Y</sup> ENMIALLMAG <sup>G312A</sup> HSS <sup>E300</sup> SS <sup>H302Y</sup> TS <sup>A311Q</sup> W <sup>G312A</sup> IM <sup>E300</sup> WL <sup>E300</sup> LEL  |
| 6. ERYSGH - AJ578781     | --ETDSDMLD <sup>E300</sup> MM <sup>H302Y</sup> QL <sup>A311Q</sup> ML <sup>G312A</sup> MR--SS <sup>E300</sup> YK <sup>H302Y</sup> DGT <sup>A311Q</sup> PV <sup>G312A</sup> PK <sup>E300</sup> ETA <sup>H302Y</sup> ENMIALLMAG <sup>G312A</sup> HSS <sup>E300</sup> SS <sup>H302Y</sup> TS <sup>A311Q</sup> W <sup>G312A</sup> IM <sup>E300</sup> WL <sup>E300</sup> LEL  |
| 7. FILBNF - AF228914     | --SDEE--DM <sup>E300</sup> EN <sup>H302Y</sup> LQ <sup>A311Q</sup> S--CK <sup>E300</sup> YR <sup>H302Y</sup> NG <sup>A311Q</sup> V <sup>G312A</sup> PL <sup>E300</sup> SD <sup>H302Y</sup> RD <sup>A311Q</sup> ETAE <sup>E300</sup> MMIALLMAG <sup>G312A</sup> HSS <sup>E300</sup> SS <sup>H302Y</sup> TS <sup>A311Q</sup> W <sup>G312A</sup> IM <sup>E300</sup> WL <sup>E300</sup> LEL  |
| 8. MONIFC - AF470821     | --TEAE <sup>E300</sup> PK <sup>H302Y</sup> S <sup>A311Q</sup> DM <sup>G312A</sup> EN <sup>LQ</sup> S--CK <sup>E300</sup> YR <sup>H302Y</sup> NG <sup>A311Q</sup> V <sup>G312A</sup> PL <sup>E300</sup> SD <sup>H302Y</sup> RD <sup>A311Q</sup> ETAE <sup>E300</sup> MMIALLMAG <sup>G312A</sup> HSS <sup>E300</sup> SS <sup>H302Y</sup> TS <sup>A311Q</sup> W <sup>G312A</sup> IM <sup>E300</sup> WL <sup>E300</sup> LEL  |
| 9. MYCOPI - XP_007928762 | PTSG <sup>E300</sup> KK <sup>H302Y</sup> EQ <sup>A311Q</sup> DM <sup>G312A</sup> EN <sup>LQ</sup> S--CK <sup>E300</sup> YR <sup>H302Y</sup> NG <sup>A311Q</sup> V <sup>G312A</sup> PL <sup>E300</sup> SD <sup>H302Y</sup> RD <sup>A311Q</sup> ETAE <sup>E300</sup> MMIALLMAG <sup>G312A</sup> HSS <sup>E300</sup> SS <sup>H302Y</sup> TS <sup>A311Q</sup> W <sup>G312A</sup> IM <sup>E300</sup> WL <sup>E300</sup> LEL   |
| 10. PHAKPA - K0741476    | --RQ <sup>E300</sup> DF <sup>H302Y</sup> EN <sup>A311Q</sup> DM <sup>G312A</sup> EN <sup>LQ</sup> S--CK <sup>E300</sup> YR <sup>H302Y</sup> NG <sup>A311Q</sup> V <sup>G312A</sup> PL <sup>E300</sup> SD <sup>H302Y</sup> RD <sup>A311Q</sup> ETAE <sup>E300</sup> MMIALLMAG <sup>G312A</sup> HSS <sup>E300</sup> SS <sup>H302Y</sup> TS <sup>A311Q</sup> W <sup>G312A</sup> IM <sup>E300</sup> WL <sup>E300</sup> LEL   |
| 11. PUCCRT - FJ978683    | --RQ <sup>E300</sup> DF <sup>H302Y</sup> EN <sup>A311Q</sup> DM <sup>G312A</sup> EN <sup>LQ</sup> S--CK <sup>E300</sup> YR <sup>H302Y</sup> NG <sup>A311Q</sup> V <sup>G312A</sup> PL <sup>E300</sup> SD <sup>H302Y</sup> RD <sup>A311Q</sup> ETAE <sup>E300</sup> MMIALLMAG <sup>G312A</sup> HSS <sup>E300</sup> SS <sup>H302Y</sup> TS <sup>A311Q</sup> W <sup>G312A</sup> IM <sup>E300</sup> WL <sup>E300</sup> LEL   |
| 12. SACCCE - AAB88433    | --DIQ--DR <sup>E300</sup> DL <sup>H302Y</sup> IDS <sup>A311Q</sup> LQ <sup>G</sup> S--CTYK <sup>E300</sup> DGR <sup>H302Y</sup> PL <sup>A311Q</sup> MD <sup>G312A</sup> ETAE <sup>E300</sup> MMIALLMAG <sup>G312A</sup> HSS <sup>E300</sup> SS <sup>H302Y</sup> TS <sup>A311Q</sup> W <sup>G312A</sup> IM <sup>E300</sup> WL <sup>E300</sup> LEL   |
| 13. UNCNE - AAC49812     | --RSG <sup>E300</sup> EN <sup>H302Y</sup> KN <sup>A311Q</sup> DM <sup>G312A</sup> EN <sup>LQ</sup> S--CTYK <sup>E300</sup> DGR <sup>H302Y</sup> PL <sup>A311Q</sup> MD <sup>G312A</sup> ETAE <sup>E300</sup> MMIALLMAG <sup>G312A</sup> HSS <sup>E300</sup> SS <sup>H302Y</sup> TS <sup>A311Q</sup> W <sup>G312A</sup> IM <sup>E300</sup> WL <sup>E300</sup> LEL   |
| 1. SEPTTR - AY253234     | AAR <sup>I333</sup> PD <sup>I333</sup> IQ <sup>I333</sup> DEL <sup>I333</sup> LQ <sup>I333</sup> EQ <sup>I333</sup> R <sup>I333</sup> Q <sup>I333</sup> DK <sup>I333</sup> ML <sup>I333</sup> GV <sup>I333</sup> NADGS--IKELTYANLSK <sup>I333</sup> LT <sup>I333</sup> LN <sup>I333</sup> Q <sup>I333</sup> V <sup>I333</sup> Y <sup>I333</sup> RE <sup>I333</sup> TL <sup>I333</sup> R <sup>I333</sup> IA <sup>I333</sup> PA <sup>I333</sup> V <sup>I333</sup>  |
| 2. ASPEFL - K0C13903     | AAS <sup>I333</sup> PE <sup>I333</sup> ITE <sup>I333</sup> ELY <sup>I333</sup> Q <sup>I333</sup> EQ <sup>I333</sup> LR <sup>I333</sup> IL <sup>I333</sup> GH <sup>I333</sup> D----MP <sup>I333</sup> EL <sup>I333</sup> TY <sup>I333</sup> EN <sup>I333</sup> LQ <sup>I333</sup> RL <sup>I333</sup> DL <sup>I333</sup> MA <sup>I333</sup> KY <sup>I333</sup> IK <sup>I333</sup> ET <sup>I333</sup> LR <sup>I333</sup> IA <sup>I333</sup> PA <sup>I333</sup> V <sup>I333</sup>  |
| 3. CANDAL - AF163960     | GEK <sup>I333</sup> PH <sup>I333</sup> LD <sup>I333</sup> Q <sup>I333</sup> V <sup>I333</sup> I <sup>I333</sup> Y <sup>I333</sup> Q <sup>I333</sup> EV <sup>I333</sup> VL <sup>I333</sup> LL <sup>I333</sup> KE <sup>I333</sup> G----GD <sup>I333</sup> LN <sup>I333</sup> DL <sup>I333</sup> TY <sup>I333</sup> ED <sup>I333</sup> LQ <sup>I333</sup> KL <sup>I333</sup> PS <sup>I333</sup> V <sup>I333</sup> NT <sup>I333</sup> IK <sup>I333</sup> ET <sup>I333</sup> LR <sup>I333</sup> IA <sup>I333</sup> PA <sup>I333</sup> V <sup>I333</sup> |
| 4. CERCBE - HM778021     | ATRR <sup>I333</sup> DI <sup>I333</sup> Q <sup>I333</sup> DEL <sup>I333</sup> V <sup>I333</sup> Q <sup>I333</sup> EQ <sup>I333</sup> DL <sup>I333</sup> GV <sup>I333</sup> NEDGS--IKELTYANLSK <sup>I333</sup> LT <sup>I333</sup> LN <sup>I333</sup> Q <sup>I333</sup> V <sup>I333</sup> Y <sup>I333</sup> RE <sup>I333</sup> TL <sup>I333</sup> R <sup>I333</sup> IA <sup>I333</sup> PA <sup>I333</sup> V <sup>I333</sup>  |
| 5. ERYSGH - AJ578781     | AAR <sup>I333</sup> PD <sup>I333</sup> ITE <sup>I333</sup> ELY <sup>I333</sup> Q <sup>I333</sup> EQ <sup>I333</sup> LE <sup>I333</sup> LG <sup>I333</sup> SE----LP <sup>I333</sup> PK <sup>I333</sup> Y <sup>I333</sup> ED <sup>I333</sup> LS <sup>I333</sup> KL <sup>I333</sup> DS <sup>I333</sup> L <sup>I333</sup> Q <sup>I333</sup> NV <sup>I333</sup> L <sup>I333</sup> KE <sup>I333</sup> VL <sup>I333</sup> RL <sup>I333</sup> IA <sup>I333</sup> PA <sup>I333</sup> V <sup>I333</sup>  |
| 6. ERYSGH - AJ578781     | AAR <sup>I333</sup> PD <sup>I333</sup> ITE <sup>I333</sup> ELY <sup>I333</sup> Q <sup>I333</sup> EQ <sup>I333</sup> LE <sup>I333</sup> LG <sup>I333</sup> SE----LP <sup>I333</sup> PK <sup>I333</sup> Y <sup>I333</sup> ED <sup>I333</sup> LS <sup>I333</sup> KL <sup>I333</sup> DS <sup>I333</sup> L <sup>I333</sup> Q <sup>I333</sup> NV <sup>I333</sup> L <sup>I333</sup> KE <sup>I333</sup> VL <sup>I333</sup> RL <sup>I333</sup> IA <sup>I333</sup> PA <sup>I333</sup> V <sup>I333</sup>  |
| 7. FILBNF - AF228914     | AAR <sup>I333</sup> PD <sup>I333</sup> ITE <sup>I333</sup> ELY <sup>I333</sup> Q <sup>I333</sup> EQ <sup>I333</sup> LE <sup>I333</sup> LG <sup>I333</sup> SE----LP <sup>I333</sup> PK <sup>I333</sup> Y <sup>I333</sup> ED <sup>I333</sup> LS <sup>I333</sup> KL <sup>I333</sup> DS <sup>I333</sup> L <sup>I333</sup> Q <sup>I333</sup> NV <sup>I333</sup> L <sup>I333</sup> KE <sup>I333</sup> VL <sup>I333</sup> RL <sup>I333</sup> IA <sup>I333</sup> PA <sup>I333</sup> V <sup>I333</sup>  |
| 8. MONIFC - AF470821     | ADR <sup>I333</sup> P <sup>I333</sup> D <sup>I333</sup> ITE <sup>I333</sup> ELY <sup>I333</sup> Q <sup>I333</sup> EQ <sup>I333</sup> LE <sup>I333</sup> LG <sup>I333</sup> SE----LP <sup>I333</sup> PK <sup>I333</sup> Y <sup>I333</sup> ED <sup>I333</sup> LS <sup>I333</sup> KL <sup>I333</sup> DS <sup>I333</sup> L <sup>I333</sup> Q <sup>I333</sup> NV <sup>I333</sup>  |

**Figure 3.** Amino acid sequence alignment of the Cyp51B family. Sequences are named by species EPPO code and NCBI gene accession number. Residues highlighted in yellow in the archetype sequence from *Zymoseptoria tritici* denote amino acid substitutions associated with fungicide resistance at an orthologous position in any of the sequences. Numerical mutation labels shown above the alignment are based on the position number of the amino acid in the archetype protein.

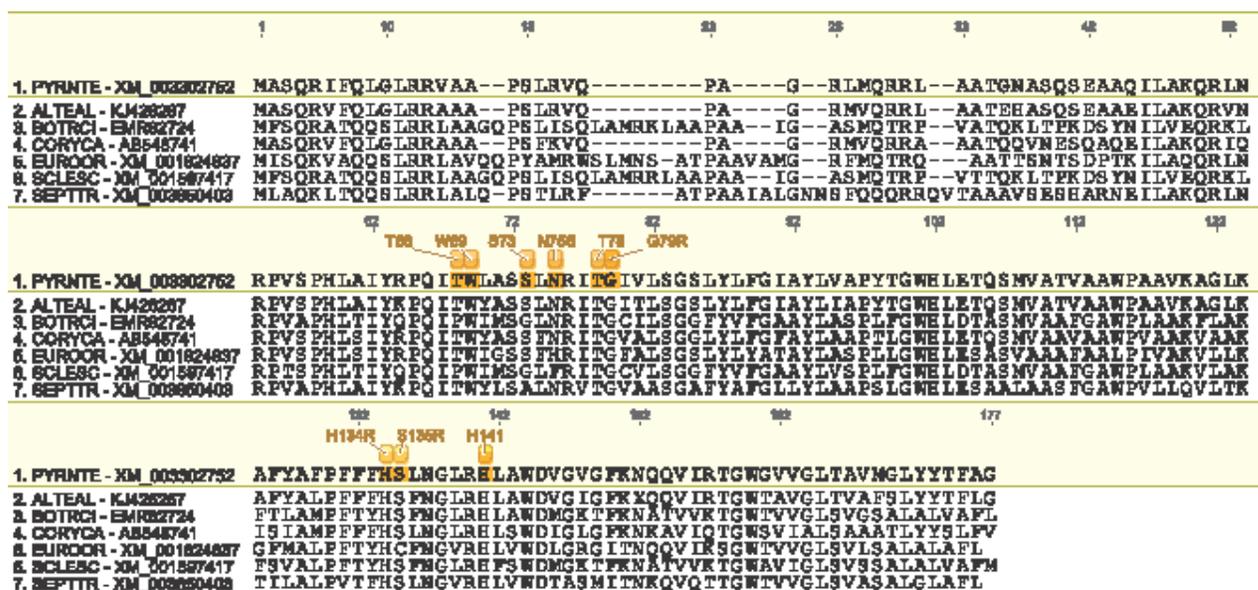
|                                      |  |               |              |              |             |     |              |
|--------------------------------------|--|---------------|--------------|--------------|-------------|-----|--------------|
|                                      | 1  | 10            | 20           | 30           | 40          | 50  | 60           |
|                                      |  | <b>HILY</b>   |              |              |             |     | <b>YSGNS</b> |
| 1. ASPEND berA beta-tubulin - M17519 | MREIVHLQGGCGNQIGAAFWQITISGHEGLDGGGVYNGTSDLCLERMNVITFNASGDKYVPRAV   |               |              |              |             |     |              |
| 2. BOTRC1 - Z96243                   | MREIVHLQGGCGNQIGAAFWQITISGHEGLDGGGVYNGTSDLCLERMNVITFNASGDKYVPRAV   |               |              |              |             |     |              |
| 3. CERC8E - AY555373                 | MREIVHLQGGCGNQIGAAFWQITISGHEGLDGGGVYNGTSDLCLERMNVITFNASGDKYVPRAV   |               |              |              |             |     |              |
| 4. COCHHE - AB000971                 | MREIVHLQGGCGNQIGAAFWQITISGHEGLDGGGVYNGTSDLCLERMNVITFNASGDKYVPRAV   |               |              |              |             |     |              |
| 5. GIBBFU beta 1-tubulin - U27303    | MREIVHLQGGCGNQIGAAFWQITISGHEGLDGGGVYNGTSDLCLERMNVITFNASGDKYVPRAV   |               |              |              |             |     |              |
| 6. GIBBFU beta 2-tubulin - KF771181  | MREIVHVFQGGCGNQVSSFWITVSKHEGIDGGGVYNGTSDLCLERMNVITFNASGDKYVPRAV    |               |              |              |             |     |              |
| 7. GIBBZE beta 2-tubulin - FJ214683  | MREIVHVFQGGCGNQVSSFWITVSKHEGIDGGGVYNGTSDLCLERMNVITFNASGDKYVPRAV    |               |              |              |             |     |              |
| 8. HELMSO - Y10870                   | MREIVHLQGGCGNQIGAAFWQITISGHEGLDGGGVYNGTSDLCLERMNVITFNASGDKYVPRAV   |               |              |              |             |     |              |
| 9. HYFMOD - Y12256                   | MREIVHLQGGCGNQIGAAFWQITISGHEGLDGGGVYNGTSDLCLERMNVITFNASGDKYVPRAV   |               |              |              |             |     |              |
| 10. LEPTMO - S68822                  | MREIVHLQGGCGNQIGAAFWQITISGHEGLDGGGVYNGTSDLCLERMNVITFNASGDKYVPRAV   |               |              |              |             |     |              |
| 11. MONFC - AY253676                 | MREIVHLQGGCGNQIGAAFWQITISGHEGLDGGGVYNGTSDLCLERMNVITFNASGDKYVPRAV   |               |              |              |             |     |              |
| 12. MONLA - AY349149                 | MREIVHLQGGCGNQIGAAFWQITISGHEGLDGGGVYNGTSDLCLERMNVITFNASGDKYVPRAV   |               |              |              |             |     |              |
| 13. NEUSCR - M13530                  | MREIVHLQGGCGNQIGAAFWQITISGHEGLDGGGVYNGTSDLCLERMNVITFNASGDKYVPRAV   |               |              |              |             |     |              |
| 14. PENIAU - JN112033                | MREIVHLQGGCGNQIGAAFWQITISGHEGLDGGGVYNGTSDLCLERMNVITFNASGDKYVPRAV   |               |              |              |             |     |              |
| 15. PENEX - KQ042690                 | MREIVHLQGGCGNQIGAAFWQITISGHEGLDGGGVYNGTSDLCLERMNVITFNASGDKYVPRAV   |               |              |              |             |     |              |
| 16. PENIT - KQ072770                 | MREIVHLQGGCGNQIGAAFWQITISGHEGLDGGGVYNGTSDLCLERMNVITFNASGDKYVPRAV   |               |              |              |             |     |              |
| 17. PYRPBR - KC342227                | MREIVHLQGGCGNQIGAAFWQITISGHEGLDGGGVYNGTSDLCLERMNVITFNASGDKYVPRAV   |               |              |              |             |     |              |
| 18. RHYNSE - X81046                  | MREIVHLQGGCGNQIGAAFWQITISGHEGLDGGGVYNGTSDLCLERMNVITFNASGDKYVPRAV   |               |              |              |             |     |              |
| 19. SCLEHO - KF765483                | MREIVHLQGGCGNQIGAAFWQITISGHEGLDGGGVYNGTSDLCLERMNVITFNASGDKYVPRAV   |               |              |              |             |     |              |
| 20. SCLESC - XM 001584784            | MREIVHLQGGCGNQIGAAFWQITISGHEGLDGGGVYNGTSDLCLERMNVITFNASGDKYVPRAV   |               |              |              |             |     |              |
| 21. VENTIN - M87951                  | MREIVHLQGGCGNQIGAAFWQITISGHEGLDGGGVYNGTSDLCLERMNVITFNASGDKYVPRAV   |               |              |              |             |     |              |
|                                      | 70   | 80            | 90           | 100          | 110         | 120 |              |
|                                      |  | <b>M73</b>    |              |              |             |     |              |
| 1. ASPEND berA beta-tubulin - M17519 | LVLDLEPGTMDAVRAGPEGQLERPDNPFVFGQSGAGNNWAKGNYTEGAEIWDQVLDVVRREAECCD |               |              |              |             |     |              |
| 2. BOTRC1 - Z96243                   | LVLDLEPGTMDAVRAGPEGQLERPDNPFVFGQSGAGNNWAKGNYTEGAEIWDQVLDVVRREAECCD |               |              |              |             |     |              |
| 3. CERC8E - AY555373                 | LVLDLEPGTMDAVRAGPEGQLERPDNPFVFGQSGAGNNWAKGNYTEGAEIWDQVLDVVRREAECCD |               |              |              |             |     |              |
| 4. COCHHE - AB000971                 | LVLDLEPGTMDAVRAGPEGQLERPDNPFVFGQSGAGNNWAKGNYTEGAEIWDQVLDVVRREAECCD |               |              |              |             |     |              |
| 5. GIBBFU beta 1-tubulin - U27303    | LVLDLEPGTMDAVRAGPEGQLERPDNPFVFGQSGAGNNWAKGNYTEGAEIWDQVLDVVRREAECCD |               |              |              |             |     |              |
| 6. GIBBFU beta 2-tubulin - KF771181  | LVLDLEPGTMDAVRAGPEGQLERPDNPFVFGQSGAGNNWAKGNYTEGAEIWDQVLDVVRREAECCD |               |              |              |             |     |              |
| 7. GIBBZE beta 2-tubulin - FJ214683  | LVLDLEPGTMDAVRAGPEGQLERPDNPFVFGQSGAGNNWAKGNYTEGAEIWDQVLDVVRREAECCD |               |              |              |             |     |              |
| 8. HELMSO - Y10870                   | LVLDLEPGTMDAVRAGPEGQLERPDNPFVFGQSGAGNNWAKGNYTEGAEIWDQVLDVVRREAECCD |               |              |              |             |     |              |
| 9. HYFMOD - Y12256                   | LVLDLEPGTMDAVRAGPEGQLERPDNPFVFGQSGAGNNWAKGNYTEGAEIWDQVLDVVRREAECCD |               |              |              |             |     |              |
| 10. LEPTMO - S68822                  | LVLDLEPGTMDAVRAGPEGQLERPDNPFVFGQSGAGNNWAKGNYTEGAEIWDQVLDVVRREAECCD |               |              |              |             |     |              |
| 11. MONFC - AY253676                 | LVLDLEPGTMDAVRAGPEGQLERPDNPFVFGQSGAGNNWAKGNYTEGAEIWDQVLDVVRREAECCD |               |              |              |             |     |              |
| 12. MONLA - AY349149                 | LVLDLEPGTMDAVRAGPEGQLERPDNPFVFGQSGAGNNWAKGNYTEGAEIWDQVLDVVRREAECCD |               |              |              |             |     |              |
| 13. NEUSCR - M13530                  | LVLDLEPGTMDAVRAGPEGQLERPDNPFVFGQSGAGNNWAKGNYTEGAEIWDQVLDVVRREAECCD |               |              |              |             |     |              |
| 14. PENIAU - JN112033                | LVLDLEPGTMDAVRAGPEGQLERPDNPFVFGQSGAGNNWAKGNYTEGAEIWDQVLDVVRREAECCD |               |              |              |             |     |              |
| 15. PENEX - KQ042690                 | LVLDLEPGTMDAVRAGPEGQLERPDNPFVFGQSGAGNNWAKGNYTEGAEIWDQVLDVVRREAECCD |               |              |              |             |     |              |
| 16. PENIT - KQ072770                 | LVLDLEPGTMDAVRAGPEGQLERPDNPFVFGQSGAGNNWAKGNYTEGAEIWDQVLDVVRREAECCD |               |              |              |             |     |              |
| 17. PYRPBR - KC342227                | LVLDLEPGTMDAVRAGPEGQLERPDNPFVFGQSGAGNNWAKGNYTEGAEIWDQVLDVVRREAECCD |               |              |              |             |     |              |
| 18. RHYNSE - X81046                  | LVLDLEPGTMDAVRAGPEGQLERPDNPFVFGQSGAGNNWAKGNYTEGAEIWDQVLDVVRREAECCD |               |              |              |             |     |              |
| 19. SCLEHO - KF765483                | LVLDLEPGTMDAVRAGPEGQLERPDNPFVFGQSGAGNNWAKGNYTEGAEIWDQVLDVVRREAECCD |               |              |              |             |     |              |
| 20. SCLESC - XM 001584784            | LVLDLEPGTMDAVRAGPEGQLERPDNPFVFGQSGAGNNWAKGNYTEGAEIWDQVLDVVRREAECCD |               |              |              |             |     |              |
| 21. VENTIN - M87951                  | LVLDLEPGTMDAVRAGPEGQLERPDNPFVFGQSGAGNNWAKGNYTEGAEIWDQVLDVVRREAECCD |               |              |              |             |     |              |
|                                      | 130  | 140           | 150          | 160          | 170         | 180 | 190          |
|                                      |  | <b>Q134K</b>  |              | <b>A185V</b> | <b>F167</b> |     |              |
| 1. ASPEND berA beta-tubulin - M17519 | CLQGGQITHEBLGGGTGAGNSILLISKREELPFORMATISVVVSPKVSVDITVVFYPMATLSVBQL |               |              |              |             |     |              |
| 2. BOTRC1 - Z96243                   | CLQGGQITHEBLGGGTGAGNSILLISKREELPFORMATISVVVSPKVSVDITVVFYPMATLSVBQL |               |              |              |             |     |              |
| 3. CERC8E - AY555373                 | CLQGGQITHEBLGGGTGAGNSILLISKREELPFORMATISVVVSPKVSVDITVVFYPMATLSVBQL |               |              |              |             |     |              |
| 4. COCHHE - AB000971                 | CLQGGQITHEBLGGGTGAGNSILLISKREELPFORMATISVVVSPKVSVDITVVFYPMATLSVBQL |               |              |              |             |     |              |
| 5. GIBBFU beta 1-tubulin - U27303    | CLQGGQITHEBLGGGTGAGNSILLISKREELPFORMATISVVVSPKVSVDITVVFYPMATLSVBQL |               |              |              |             |     |              |
| 6. GIBBFU beta 2-tubulin - KF771181  | CLQGGQITHEBLGGGTGAGNSILLISKREELPFORMATISVVVSPKVSVDITVVFYPMATLSVBQL |               |              |              |             |     |              |
| 7. GIBBZE beta 2-tubulin - FJ214683  | CLQGGQITHEBLGGGTGAGNSILLISKREELPFORMATISVVVSPKVSVDITVVFYPMATLSVBQL |               |              |              |             |     |              |
| 8. HELMSO - Y10870                   | CLQGGQITHEBLGGGTGAGNSILLISKREELPFORMATISVVVSPKVSVDITVVFYPMATLSVBQL |               |              |              |             |     |              |
| 9. HYFMOD - Y12256                   | CLQGGQITHEBLGGGTGAGNSILLISKREELPFORMATISVVVSPKVSVDITVVFYPMATLSVBQL |               |              |              |             |     |              |
| 10. LEPTMO - S68822                  | CLQGGQITHEBLGGGTGAGNSILLISKREELPFORMATISVVVSPKVSVDITVVFYPMATLSVBQL |               |              |              |             |     |              |
| 11. MONFC - AY253676                 | CLQGGQITHEBLGGGTGAGNSILLISKREELPFORMATISVVVSPKVSVDITVVFYPMATLSVBQL |               |              |              |             |     |              |
| 12. MONLA - AY349149                 | CLQGGQITHEBLGGGTGAGNSILLISKREELPFORMATISVVVSPKVSVDITVVFYPMATLSVBQL |               |              |              |             |     |              |
| 13. NEUSCR - M13530                  | CLQGGQITHEBLGGGTGAGNSILLISKREELPFORMATISVVVSPKVSVDITVVFYPMATLSVBQL |               |              |              |             |     |              |
| 14. PENIAU - JN112033                | CLQGGQITHEBLGGGTGAGNSILLISKREELPFORMATISVVVSPKVSVDITVVFYPMATLSVBQL |               |              |              |             |     |              |
| 15. PENEX - KQ042690                 | CLQGGQITHEBLGGGTGAGNSILLISKREELPFORMATISVVVSPKVSVDITVVFYPMATLSVBQL |               |              |              |             |     |              |
| 16. PENIT - KQ072770                 | CLQGGQITHEBLGGGTGAGNSILLISKREELPFORMATISVVVSPKVSVDITVVFYPMATLSVBQL |               |              |              |             |     |              |
| 17. PYRPBR - KC342227                | CLQGGQITHEBLGGGTGAGNSILLISKREELPFORMATISVVVSPKVSVDITVVFYPMATLSVBQL |               |              |              |             |     |              |
| 18. RHYNSE - X81046                  | CLQGGQITHEBLGGGTGAGNSILLISKREELPFORMATISVVVSPKVSVDITVVFYPMATLSVBQL |               |              |              |             |     |              |
| 19. SCLEHO - KF765483                | CLQGGQITHEBLGGGTGAGNSILLISKREELPFORMATISVVVSPKVSVDITVVFYPMATLSVBQL |               |              |              |             |     |              |
| 20. SCLESC - XM 001584784            | CLQGGQITHEBLGGGTGAGNSILLISKREELPFORMATISVVVSPKVSVDITVVFYPMATLSVBQL |               |              |              |             |     |              |
| 21. VENTIN - M87951                  | CLQGGQITHEBLGGGTGAGNSILLISKREELPFORMATISVVVSPKVSVDITVVFYPMATLSVBQL |               |              |              |             |     |              |
|                                      | 200  | 210           | 220          | 230          | 240         | 250 |              |
|                                      |  | <b>E190DQ</b> | <b>F207Y</b> |              | <b>L240</b> |     |              |
| 1. ASPEND berA beta-tubulin - M17519 | VENSDETFICIDNEALYDIDCMRPLKLSNPSYGLNRLVSAVMSGVTTCLRAFPGQLNSDLKRLAVN |               |              |              |             |     |              |
| 2. BOTRC1 - Z96243                   | VENSDETFICIDNEALYDIDCMRPLKLSNPSYGLNRLVSAVMSGVTTCLRAFPGQLNSDLKRLAVN |               |              |              |             |     |              |
| 3. CERC8E - AY555373                 | VENSDETFICIDNEALYDIDCMRPLKLSNPSYGLNRLVSAVMSGVTTCLRAFPGQLNSDLKRLAVN |               |              |              |             |     |              |
| 4. COCHHE - AB000971                 | VENSDETFICIDNEALYDIDCMRPLKLSNPSYGLNRLVSAVMSGVTTCLRAFPGQLNSDLKRLAVN |               |              |              |             |     |              |
| 5. GIBBFU beta 1-tubulin - U27303    | VENSDETFICIDNEALYDIDCMRPLKLSNPSYGLNRLVSAVMSGVTTCLRAFPGQLNSDLKRLAVN |               |              |              |             |     |              |
| 6. GIBBFU beta 2-tubulin - KF771181  | VENSDETFICIDNEALYDIDCMRPLKLSNPSYGLNRLVSAVMSGVTTCLRAFPGQLNSDLKRLAVN |               |              |              |             |     |              |
| 7. GIBBZE beta 2-tubulin - FJ214683  | VENSDETFICIDNEALYDIDCMRPLKLSNPSYGLNRLVSAVMSGVTTCLRAFPGQLNSDLKRLAVN |               |              |              |             |     |              |
| 8. HELMSO - Y10870                   | VENSDETFICIDNEALYDIDCMRPLKLSNPSYGLNRLVSAVMSGVTTCLRAFPGQLNSDLKRLAVN |               |              |              |             |     |              |
| 9. HYFMOD - Y12256                   | VENSDETFICIDNEALYDIDCMRPLKLSNPSYGLNRLVSAVMSGVTTCLRAFPGQLNSDLKRLAVN |               |              |              |             |     |              |
| 10. LEPTMO - S68822                  | VENSDETFICIDNEALYDIDCMRPLKLSNPSYGLNRLVSAVMSGVTTCLRAFPGQLNSDLKRLAVN |               |              |              |             |     |              |
| 11. MONFC - AY253676                 | VENSDETFICIDNEALYDIDCMRPLKLSNPSYGLNRLVSAVMSGVTTCLRAFPGQLNSDLKRLAVN |               |              |              |             |     |              |
| 12. MONLA - AY349149                 | VENSDETFICIDNEALYDIDCMRPLKLSNPSYGLNRLVSAVMSGVTTCLRAFPGQLNSDLKRLAVN |               |              |              |             |     |              |
| 13. NEUSCR - M13530                  | VENSDETFICIDNEALYDIDCMRPLKLSNPSYGLNRLVSAVMSGVTTCLRAFPGQLNSDLKRLAVN |               |              |              |             |     |              |
| 14. PENIAU - JN112033                | VENSDETFICIDNEALYDIDCMRPLKLSNPSYGLNRLVSAVMSGVTTCLRAFPGQLNSDLKRLAVN |               |              |              |             |     |              |
| 15. PENEX - KQ042690                 | VENSDETFICIDNEALYDIDCMRPLKLSNPSYGLNRLVSAVMSGVTTCLRAFPGQLNSDLKRLAVN |               |              |              |             |     |              |
| 16. PENIT - KQ072770                 | VENSDETFICIDNEALYDIDCMRPLKLSNPSYGLNRLVSAVMSGVTTCLRAFPGQLNSDLKRLAVN |               |              |              |             |     |              |
| 17. PYRPBR - KC342227                | VENSDETFICIDNEALYDIDCMRPLKLSNPSYGLNRLVSAVMSGVTTCLRAFPGQLNSDLKRLAVN |               |              |              |             |     |              |
| 18. RHYNSE - X81046                  | VENSDETFICIDNEALYDIDCMRPLKLSNPSYGLNRLVSAVMSGVTTCLRAFPGQLNSDLKRLAVN |               |              |              |             |     |              |
| 19. SCLEHO - KF765483                | VENSDETFICIDNEALYDIDCMRPLKLSNPSYGLNRLVSAVMSGVTTCLRAFPGQLNSDLKRLAVN |               |              |              |             |     |              |
| 20. SCLESC - XM 001584784            | VENSDETFICIDNEALYDIDCMRPLKLSNPSYGLNRLVSAVMSGVTTCLRAFPGQLNSDLKRLAVN |               |              |              |             |     |              |
| 21. VENTIN - M87951                  | VENSDETFICIDNEALYDIDCMRPLKLSNPSYGLNRLVSAVMSGVTTCLRAFPGQLNSDLKRLAVN |               |              |              |             |     |              |
|                                      | 260  | 270           | 280          | 290          | 300         | 310 | 320          |
|                                      |  | <b>M87L</b>   |              |              |             |     |              |
| 1. ASPEND berA beta-tubulin - M17519 | MVFFERLEITWVGFAPLTSRGAESFRVAVVPELTOQMDENKMAASDFRNGRYLTCBALVAGE     |               |              |              |             |     |              |
| 2. BOTRC1 - Z96243                   | MVFFERLEITWVGFAPLTSRGAESFRVAVVPELTOQMDENKMAASDFRNGRYLTCBALVAGE     |               |              |              |             |     |              |
| 3. CERC8E - AY555373                 | MVFFERLEITWVGFAPLTSRGAESFRVAVVPELTOQMDENKMAASDFRNGRYLTCBALVAGE     |               |              |              |             |     |              |
| 4. COCHHE - AB000971                 | MVFFERLEITWVGFAPLTSRGAESFRVAVVPELTOQMDENKMAASDFRNGRYLTCBALVAGE     |               |              |              |             |     |              |
| 5. GIBBFU beta 1-tubulin - U27303    | MVFFERLEITWVGFAPLTSRGAESFRVAVVPELTOQMDENKMAASDFRNGRYLTCBALVAGE     |               |              |              |             |     |              |
| 6. GIBBFU beta 2-tubulin - KF771181  | MVFFERLEITWVGFAPLTSRGAESFRVAVVPELTOQMDENKMAASDFRNGRYLTCBALVAGE     |               |              |              |             |     |              |
| 7. GIBBZE beta 2-tubulin - FJ214683  | MVFFERLEITWVGFAPLTSRGAESFRVAVVPELTOQMDENKMAASDFRNGRYLTCBALVAGE     |               |              |              |             |     |              |
| 8. HELMSO - Y10870                   | MVFFERLEITWVGFAPLTSRGAESFRVAVVPELTOQMDENKMAASDFRNGRYLTCBALVAGE     |               |              |              |             |     |              |

**Figure 4.** Amino acid sequence alignment of the *b*-tubulin family. Sequences are named by species EPP0 code and NCBI gene accession number. Residues highlighted in yellow in the archetype sequence from *Aspergillus nidulans* (benA) denote amino acid substitutions associated with fungicide resistance at an orthologous position in any of the sequences. Numerical mutation labels shown above the alignment are based on the position number of the amino acid in the archetype protein.

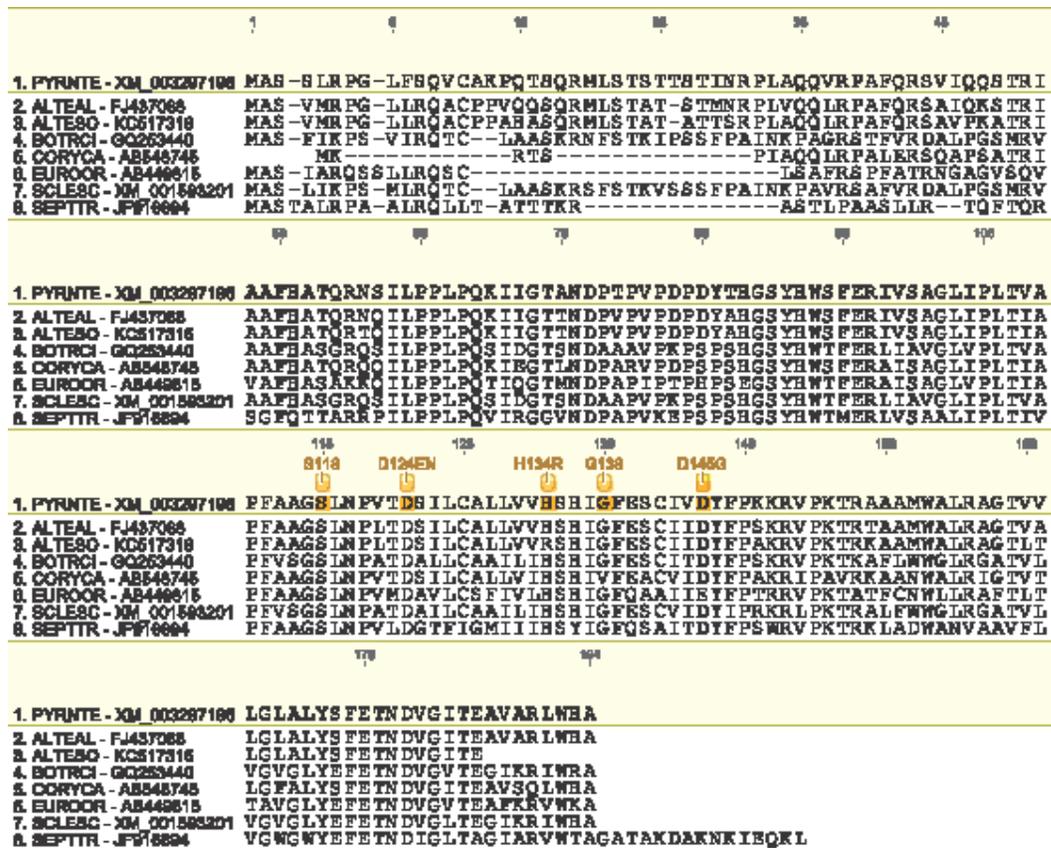


**Figure 5.** Amino acid sequence alignment of the SdhB family. Sequences are named by species EPP0 code and NCBI gene accession number. Residues highlighted in yellow in the archetype sequence from *Pyrenophora teres* f. sp. *teres* denote amino acid substitutions associated with fungicide

resistance at an orthologous position in any of the sequences. Numerical mutation labels shown above the alignment are based on the position number of the amino acid in the archetype protein.



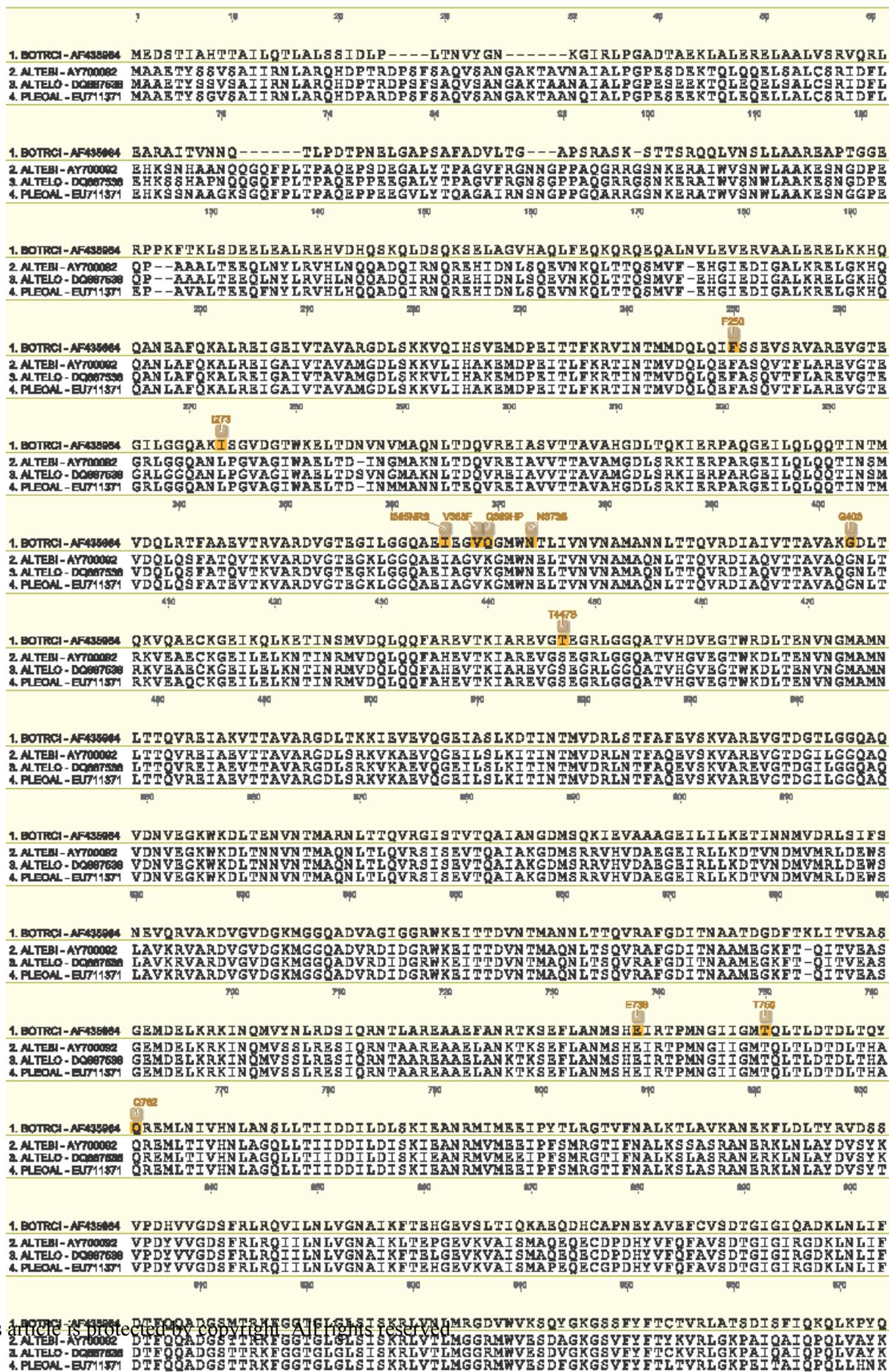
**Figure 6.** Amino acid sequence alignment of the SdhC family. Sequences are named by species EPPD code and NCBI gene accession number. Residues highlighted in yellow in the archetype sequence from *Pyrenophora teres* f. sp. *teres* denote amino acid substitutions associated with fungicide resistance at an orthologous position in any of the sequences. Numerical mutation labels shown above the alignment are based on the position number of the amino acid in the archetype protein.



**Figure 7.** Amino acid sequence alignment of the SdhD family. Sequences are named by species EPPO code and NCBI gene accession number. Residues highlighted in yellow in the archetype sequence from *Pyrenophora teres* f. sp. *teres* denote amino acid substitutions associated with fungicide resistance at an orthologous position in any of the sequences. Numerical mutation labels shown above the alignment are based on the position number of the amino acid in the archetype protein.



**Figure 8.** Amino acid sequence alignment of the CesA3 family. Sequences are named by species EPPO code and NCBI gene accession number. Residues highlighted in yellow in the archetype sequence from *Phytophthora infestans* denote amino acid substitutions associated with fungicide resistance at an orthologous position in any of the sequences. Numerical mutation labels shown above the alignment are based on the position number of the amino acid in the archetype protein



**Figure 9.** Amino acid sequence alignment of the OS-1 family. Sequences are named by species Eppo code and NCBI gene accession number. Residues highlighted in yellow in the archetype sequence from *Botrytis cinerea* denote amino acid substitutions associated with fungicide resistance at an orthologous position in any of the sequences. Numerical mutation labels shown above the alignment are based on the position number of the amino acid in the archetype protein.

**Table 1 CytB**

Position number based on alignment to reference sequence from *Zymoseptoria tritici* (NCBI gene accession number AY247413).

| Amino acid substitution(s) in archetype | Homologous position in other species |
|---|--------------------------------------|
| <b>F129L</b>                            | F129L in PHAKPA                      |
|   | F129L in PLASVI                      |
|   | F129L in PYRIOR                      |
|   | F129L in PYRNTE                      |
|   | F129L in PYRNTR                      |
|   | F129L in RHIZSO                      |
| <b>G137</b>                             | G137R in PYRNTR                      |
| <b>G143A</b>                            | G143A in ALTEAL                      |
|   | G143A in ALTELY                      |
|   | G143A in ALTESO                      |
|   | G143A in ALTETO                      |
|   | G143A in BOTRCI                      |
|   | G143A in CERCBE                      |
|   | G143A in COLLGR                      |
|   | G143A in ERYSGT                      |
|   | G143A in LEPTNO                      |
|   | G143A in MICDMA                      |
|   | G143A in MONGNI                      |
|   | G143A in MYCOFI                      |
|   | G143A in MYCORA                      |
|   | G143A in PLASVI                      |
|   | G143A in PLEOAL                      |
|   | G143A in PODOFU                      |
|   | G143A in PSPECU                      |
|   | G143A in PYRIOR                      |
|   | G143A in PYRNTR                      |
|   | G143A in RHIZSO                      |
| G143A in VENTIN                         |                                      |

**Table 2 Cyp51A**

Position number based on alignment to reference sequence from *Aspergillus fumigatus* (Cyp51A) (NCBI gene accession number AF338659).

| <b>Amino acid substitution(s) in archetype</b> | <b>Homologous position in other species</b> |
|--|---|
| N22D   | NA  |
| S52T   | NA  |
| G54E/K/R/V/W                                   | G54W in ASPEPA                              |
| Y68  | Y132N in ASPEFL                             |
| Q88H   | NA  |
| L98H   | NA  |
| V101F  | NA  |
| Y121F  | Y136F in AJELCP                             |
| N125I  | NA  |
| K133   | K197N in ASPEFL                             |
| G138C/R/S                                      | NA  |
| Q141H  | NA  |
| H147Y  | NA  |
| P216L  | NA  |
| F219S  | NA  |
| M220K/I/T/V                                    | NA  |
| D280   | D282E in ASPEFL                             |
| M286   | M288L in ASPEFL                             |
| T289A  | NA  |
| S297T  | NA  |
| P394L  | NA  |
| Y431C  | NA  |
| G432S  | NA  |
| G434C  | NA  |
| T440A  | NA  |
| G448S  | NA  |
| T470   | T469S in ASPEFL                             |
| Y491H  | NA  |
| F495I  | NA  |

**Table 3 Cyp51B**

Position number based on alignment to reference sequence from *Zymoseptoria tritici* (NCBI gene accession number AY253234).

| <b>Amino acid substitution(s)<br/>in archetype</b> | <b>Homologous position in<br/>other species</b> |
|--|---|
| <b>T66</b>   | A61V in CANDAL                                  |
| <b>C80</b>   | S79T in ERYSGT                                  |
| <b>D107V</b>                                       | NA  |
| <b>L126</b>  | F120L in PHAKPA                                 |
| <b>D134G</b>                                       | NA  |
| <b>V136A/C/G</b>                                   | NA  |
| <b>Y137F</b>                                       | Y132F/H in CANDAL                               |
|  | Y132F/H in PHAKPA                               |
|  | Y134F in PUCCRT                                 |
|  | Y136F in ERYSGH                                 |
|  | Y136F in ERYSGT                                 |
|  | Y136F in MONIFC                                 |
|  | Y136F in MYCOFI                                 |
|  | Y136F in UNCINE                                 |
|  | Y140F/H in SACCCE                               |
| Y145F in FILBNF                                    |   |
| <b>M145L</b>                                       | NA  |
| <b>K148</b>  | K142R in PHAKPA                                 |
|  | K143E in CANDAL                                 |
|  | K147Q in ERYSGH                                 |
| <b>V151</b>  | I145F in PHAKPA                                 |
| <b>D176</b>  | K175N in ERYSGT                                 |
| <b>N178S</b>                                       | NA  |
| <b>S208T</b>                                       | NA  |
| <b>N284H</b>                                       | NA  |
| <b>E300</b>  | E297K in CERCBE                                 |
| <b>H303Y</b>                                       | NA  |
| <b>A311G</b>                                       | A313G in MYCOFI                                 |
| <b>G312A</b>                                       | NA  |
| <b>I333</b>  | I330T in CERCBE                                 |
| <b>A379G</b>                                       | A381G in MYCOFI                                 |
| <b>I381V</b>                                       | NA  |
| <b>P391</b>  | P394S in CERCBE                                 |
| <b>A410T</b>                                       | S405F in CANDAL                                 |
| <b>G412A</b>                                       | NA  |
| <b>H430</b>  | H399P in ASPEFL                                 |
| <b>A453</b>  | D411N in ASPEFL                                 |
| <b>Y459C/D/N/S/P/Δ</b>                             | Y461D in MYCOFI                                 |
| <b>G460D/Δ</b>                                     | G462A in MYCOFI                                 |
| <b>Y461D/H/S</b>                                   | F449S in CANDAL                                 |
|  | Y463D/H/N in MYCOFI                             |
| <b>G476</b>  | G464S in CANDAL                                 |
|  | G484S in FILBNF                                 |

|              |                 |
|--------------|-----------------|
| <b>R479</b>  | R467K in CANDAL |
| <b>I483</b>  | I471T in CANDAL |
|              | I475T in PHAKPA |
| <b>V490L</b> | NA              |
| <b>T496</b>  | T454P in ASPEFL |
| <b>G510C</b> | NA              |
| <b>S524T</b> | S508T in PYRPBR |
|              | S509T in ERYSGH |

**Table 4 *b*-Tubulin**

Position number based on alignment to reference sequence from *Aspergillus nidulans* (benA) (NCBI gene accession number M17519).

| <b>Amino acid substitution(s) in archetype</b> | <b>Homologous position in other species</b> |
|--|---|
| <b>H6L/Y</b>                                   | H6Y in LEPTNO                               |
|  | H6Y in MONIFC                               |
| <b>Y50N/S</b>                                  | Y50N in GIBBFU $\beta_1$ -tubulin           |
|  | Y50C in GIBBZE $\beta_2$ -tubulin           |
|  | Y50C in HYPMOD                              |
| <b>M73</b>                                     | Q73R in GIBBZE $\beta_2$ -tubulin           |
| <b>Q134K</b>                                   | NA  |
| <b>A165V</b>                                   | NA  |
| <b>F167</b>                                    | F167Y in CERCBE                             |
|  | F167Y in COCHHE                             |
|  | F167Y in GIBBZE $\beta_2$ -tubulin          |
|  | F167Y in NEUSCR                             |
|  | F167Y in PENIEX                             |
| <b>E198D/K/Q</b>                               | E198A/G/K/V in BOTRCI                       |
|  | E198A in CERCBE                             |
|  | E198V in GIBBFU $\beta_2$ -tubulin          |
|  | E198K/L/Q in GIBBZE $\beta_2$ -tubulin      |
|  | E198A/Q in HELMSO                           |
|  | E198A/K in MONIFC                           |
|  | E198G in NEUSCR                             |
|  | E198A/K in PENIAU                           |
|  | E198A/K/V in PENIEX                         |
|  | E198K in PENIIT                             |
|  | E198A/G in PYRPBR                           |
|  | E198G/K in RHYNSE                           |
|  | E198A/K in SCLEHO                           |
|  | E198A in SCLESC                             |
|  | E198A/K in VENTIN                           |
| <b>F200Y</b>                                   | F200Y in BOTRCI                             |
|  | F200Y in GIBBFU $\beta_2$ -tubulin          |
|  | F200Y in GIBBZE $\beta_2$ -tubulin          |
|  | F200Y in PENIAU                             |
|  | F200Y in PENIIT                             |
|  | F200Y in RHYNSE                             |
|  | F200Y in VENTIN                             |
| <b>L240</b>                                    | L240F in MONILA                             |
|  | L240F in PYRPBR                             |
|  | L240F in VENTIN                             |
| <b>M257L</b>                                   | NA  |

**Table 5 SdhB**

Position number based on alignment to reference sequence from *Pyrenophora teres* f. sp. *teres* (NCBI gene accession number XM\_003302513).

| Amino acid substitution(s) in archetype | Homologous position in other species |
|---|--------------------------------------|
| <b>P230</b>                             | P225F/L/T in BOTRCI                  |
| <b>N235</b>                             | N225I/T in SEPTTR                    |
|   | N230I in BOTRCI                      |
| <b>H277Y</b>                            | H249L/N/Y in EUROOR                  |
|   | H257L in USTIMA                      |
|   | H267L/R/Y in SEPTTR                  |
|   | H273Y in SCLESC                      |
|   | H272L/R/V/Y in BOTRCI                |
|   | H277R/Y in ALTEAL                    |
|   | H277R/Y in ALTESO                    |
|   | H277R/Y in DIDYBR                    |
|   | H278R/Y in CORYCA                    |
| H->Y in PODOXA <sup>a</sup>             |                                      |
| <b>I279</b>                             | I269V in SEPTTR                      |

<sup>a</sup>Amino acid position number for this substitution is unknown as only 176-bp fragment of SdhB gene has been sequenced in both sensitive and resistant isolates

**Table 6 SdhC**

Position number based on alignment to reference sequence from *Pyrenophora teres* f. sp. *teres* (NCBI gene accession number XM\_003302752).

| Amino acid substitution(s) in archetype | Homologous position in other species |
|---|--------------------------------------|
| <b>T68</b>                              | T79I/N in SEPTTR                     |
| <b>W69</b>                              | W80S in SEPTTR                       |
| <b>S73</b>                              | S73P in CORYCA                       |
|   | A84V in SEPTTR                       |
|   | A85V in BOTRCI                       |
| <b>N75S</b>                             | N86K/S in SEPTTR                     |
| <b>T78</b>                              | T90I in EUROOR                       |
| <b>G79R</b>                             | G90R in SEPTTR                       |
| <b>H134R</b>                            | H134R in ALTEAL                      |
|   | H146R in SCLESC                      |
| <b>S135R</b>                            | NA                                   |
| <b>H141</b>                             | H152R in SEPTTR                      |

**Table 7 SdhD**

Position number based on alignment to reference sequence from *Pyrenophora teres* f. sp. *teres* (NCBI gene accession number XM\_003297196).

| Amino acid substitution(s) in archetype | Homologous position in other species |
|---|--------------------------------------|
| <b>S118</b>                             | S89P in CORYCA                       |
| <b>D124E/N</b>                          | NA                                   |
| <b>H134R</b>                            | H132R in BOTRCI                      |
|   | H132R in SCLESC                      |
|   | H133R in ALTEAL                      |
|   | H133R in ALTESO                      |
| <b>G138</b>                             | G109V in CORYCA                      |
| <b>D145G</b>                            | D124E in EUROOR                      |
|   | D129E in SEPTTR                      |

**Table 8 CesA3**

Position number based on alignment to reference sequence from *Phytophthora infestans* (NCBI gene accession number EF563995).

| Amino acid substitution(s) in archetype | Homologous position in other species |
|---|--------------------------------------|
| <b>Q1077</b>                            | Q1077K in PHYTCP                     |
| <b>G1105A/V</b>                         | G1105S/V in PLASVI                   |
|   | G1105V/W in PSPECU                   |
| <b>V1109L</b>                           | V1109L/M in PHYTCP                   |
|   | V1109L in PHYTDR                     |

**Table 9 OS-1**

Position number based on alignment to reference sequence from *Botrytis cinerea* (Bos1) (NCBI gene accession number AF435964).

| Amino acid substitution(s) in archetype | Homologous position in other species |
|---|--------------------------------------|
| <b>F250</b>                             | F267L in PLEOAL                      |
| <b>I273</b>                             | L290S in PLEOAL                      |
| <b>I365N/R/S</b>                        | NA                                   |
| <b>V368F</b>                            | NA                                   |
| <b>Q369H/P</b>                          | NA                                   |
| <b>N373S</b>                            | NA                                   |
| <b>G403</b>                             | G420D in ALTELO                      |
| <b>T447S</b>                            | NA                                   |
| <b>E738</b>                             | E753K in ALTEBI                      |
| <b>T750</b>                             | T765R in PLEOAL                      |
| <b>Q762</b>                             | Q777R in PLEOAL                      |

**Table 10 Abbreviations of Species Names**

| <b>Abbreviation (EPPO code)</b> | <b>Name of pathogen</b>                        |
|---------------------------------|--|
| AJELCP                          | <i>Ajellomyces capsulatus</i>                  |
| ALTEAL                          | <i>Alternaria alternata</i>                    |
| ALTEBI                          | <i>Alternaria brassicicola</i>                 |
| ALTELO                          | <i>Alternaria longipes</i>                     |
| ALTELY                          | <i>Alternaria arborescens</i>                  |
| ALTESO                          | <i>Alternaria solani</i>                       |
| ALTETO                          | <i>Alternaria tomato</i>                       |
| ASPEFL                          | <i>Aspergillus flavus</i>                      |
| ASPEFU                          | <i>Aspergillus fumigatus</i>                   |
| ASPEND                          | <i>Emericella nidulans</i>                     |
| ASPEPA                          | <i>Aspergillus parasiticus</i>                 |
| BOTRCI                          | <i>Botryotinia fuckeliana</i>                  |
| CANDAL                          | <i>Candida albicans</i>                        |
| COCHHE                          | <i>Cochliobolus heterostrophus</i>             |
| COLLGR                          | <i>Glomerella graminicola</i>                  |
| CORYCA                          | <i>Corynespora cassiicola</i>                  |
| CERCBE                          | <i>Cercospora beticola</i>                     |
| DIDYBR                          | <i>Stagonosporopsis cucurbitacearum</i>        |
| ERYSGH                          | <i>Blumeria graminis</i> f. sp. <i>hordei</i>  |
| ERYSGT                          | <i>Blumeria graminis</i> f. sp. <i>tritici</i> |
| EUROOR                          | <i>Eurotium oryzae</i>                         |
| FILBNF                          | <i>Filobasidiella neoformans</i>               |
| GIBBFU                          | <i>Gibberella fujikuroi</i>                    |
| GIBBZE                          | <i>Gibberella zeae</i>                         |
| HELMSO                          | <i>Helminthosporium solani</i>                 |
| HYPMOD                          | <i>Hypomyces odoratus</i>                      |
| LEPTNO                          | <i>Parastagonospora nodorum</i>                |
| MONGNI                          | <i>Monographella nivalis</i>                   |
| MONIFC                          | <i>Monilinia fructicola</i>                    |
| MONILA                          | <i>Monilinia laxa</i>                          |
| MICDMA                          | <i>Microdochium majus</i>                      |
| MYCOFI                          | <i>Mycosphaerella fijiensis</i>                |
| MYCORA                          | <i>Didymella rabiei</i>                        |
| NEUSCR                          | <i>Neurospora crassa</i>                       |
| PENIAU                          | <i>Penicillium aurantiogriseum</i>             |
| PENIEX                          | <i>Penicillium expansum</i>                    |
| PENIIT                          | <i>Penicillium italicum</i>                    |
| PHAKPA                          | <i>Phakopsora pachyrhizi</i>                   |
| PHYTCP                          | <i>Phytophthora capsici</i>                    |
| PHYTDR                          | <i>Phytophthora drechsleri</i>                 |
| PHYTIN                          | <i>Phytophthora infestans</i>                  |
| PLASVI                          | <i>Plasmopara viticola</i>                     |
| PLEOAL                          | <i>Pleospora allii</i>                         |
| PODOFU                          | <i>Podosphaera fusca</i>                       |
| PODOXA                          | <i>Podosphaera xanthii</i>                     |
| PSPECU                          | <i>Pseudoperonospora cubensis</i>              |
| PUCCRT                          | <i>Puccinia triticina</i>                      |
| PYRIOR                          | <i>Magnaporthe oryzae</i>                      |

|        |                                     |
|--------|-------------------------------------|
| PYRNTE | <i>Pyrenophora teres</i>            |
| PYRNTR | <i>Pyrenophora tritici-repentis</i> |
| PYRPBR | <i>Pyrenopeziza brassicae</i>       |
| RHIZSO | <i>Thanatephorus cucumeris</i>      |
| RHYNSE | <i>Rhynchosporium secalis</i>       |
| SACCCE | <i>Saccharomyces cerevisiae</i>     |
| SCLEHO | <i>Sclerotinia homoeocarpa</i>      |
| SCLESC | <i>Sclerotinia sclerotiorum</i>     |
| SEPTTR | <i>Zymoseptoria tritici</i>         |
| UNCINE | <i>Erysiphe necator</i>             |
| USTIMA | <i>Ustilago maydis</i>              |
| VENTIN | <i>Venturia inaequalis</i>          |