Identification and Comparison of Indigenous Yeast on Grapes Located within the Lake Erie Appellation by Sequencing PCR Amplified rDNA

By

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Submitted in Partial Fulfillment of the Requirements
for the Degree of
Master of Science
in the
Biological Sciences
Program

Youngstown State University
December, 2002

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Abstract

Aureobasidium pullulans and *Rhodotorula glutinis* were identified as the two most common indigenous yeast species identified in the Lake Erie Appellation, using Polymerase Chain Reaction (PCR) and DNA sequence analysis (Beckman CEQ 2000XL) of the ITS (Internal Transcribed Spacer) regions. Field and grape type did not affect variation in distribution of indigenous yeast species present within one vineyard. In addition, field and grape type showed little sequence alignment variation within the same indigenous yeast species. There were, however, outliers identified within the *R. glutinis* species, but they were not associated with any particular field or grape type. One vineyard from each of the three subdistricts in the Lake Erie Quality Wine Alliance was studied and the dominant species present showed no regional differences in rDNA sequence. *Sporobolomyces marcillae* was only found in the vineyard located in the Western subdistrict, and was not identified in the eastern and central subdistricts. Year to year variation was seen in indigenous yeast species in that one new isolate was identified (*Sporidiobolus pararoseus*), but not in rDNA sequence alignment.

Acknowledgements

I would like to thank my advisor, Dr. Carl Johnston, for all his help, patience, and devotion to this study.

I would like to thank my committee member Dr. Heather Lorimer for her help, support, and guidance throughout my studies.

I would like to thank Roland Riesen and my committee member Dr. John Usis, for without him this study would never have happened.

I would like to thank Dr. Bruce Levison, Diana Arnette, and Ray Cummings for their constant help and unwavering patience with DNA sequencing and computer problems.

I would like to thank the Biology Department for all their kindness and help through all the day to day dilemmas of graduate life.

I would like to extend a very special thank you to the vineyard owners: Arnie Esterer, Lee Klingshirn, and Doug Moorehead. Without your kindness, help, and welcoming spirits this study would have never succeeded.

I would like to thank the Ohio Plant Biotech Consortium for funding my thesis research.

I would like to especially thank my family for their patience and support along the way. You have always been a strength in my life.

Finally, I would like to send a heartfelt thank you to my friends who have helped with this project and who have been there to support and encourage me whenever I needed them: Becky Liptak, Allison Jakubec, Rebecca Rupert, Dan Drum, Katie Bolan, Bruce Miller, Jen Humphries, Terri Zolka, and Ed Ickert.

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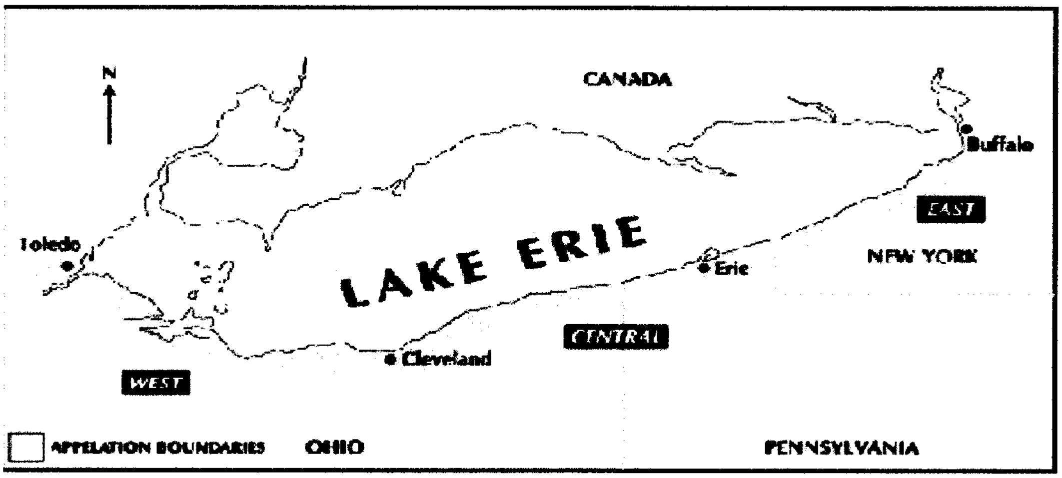
Chapter I: Introduction

I. Background

Ohio was once the largest wine producing state in the United States and its wineries produced more than one third of the U.S. total wine production (22). Over time wine grapes had to withstand cold temperatures, disease, wars, industrialization, and the Prohibition act. It was virtually impossible to grow the ever-popular European wine grapes in such a difficult environment, so with such odds stacked against it Ohio fell from prominence by going from fifty-two wineries to thirty-nine in the late 1800s with numbers continuously dwindling (21), until recently.

In the late forties Dr. Konstantin Frank successfully introduced and grew *Vitis vinifera* within the Finger Lakes region. Soon after, in the sixties, Arnie Esterer and his partner Tim Hubbard, successfully grew Chardonnay and Riesling grapes within the Lake Erie Appellation of Northern Ohio (Fig. 1). As of 1990, his success helped the number of wineries in Ohio grow to 49 and ranked Ohio tenth in the nation (22). As of 1983, the belt has been recognized as an American Viticultural Area. It spans Ohio, Pennsylvania, and New York (Fig. 1). It is the first multi-state Viticultural Area in the United States and is represented by the Lake Erie Quality Wine Alliance (LEQWA, 24). The regional and year-to-year differences in "terroir," or more simply environmental elements, provide an interesting setting to seek out and study yeast species variations between vineyards. This newfound interest in Ohio wine has led to the study and identification of the yeast species found in the northeastern Ohio region (Fig. 2). Since indigenous yeast species have been found to aid in taste, spontaneous fermentation, and biological control (10),

Figure 1: The three sub-districts of the LEQWA: West, East, and Central sub-districts.


N
CANADA
Buffalo
Toledo
LAKE ERIE
EAST
NEW YORK
Erie
CENTRAL
Cleveland
WEST
APPELATION BOUNDARIES
OHIO
PENNSYLVANIA

the main goal was to identify the indigenous yeast species present on wine grape surfaces within the Markko, Presque Isle, and Klingshirn vineyards. There was also interest in the distribution of indigenous yeast strains between each of the aforementioned vineyards as well as year to year variation, from the 2000 and 2001 season, within the Markko vineyard.

II. Native Yeasts

The most frequently isolated native indigenous yeast species are the "apiculate yeasts" (cell with a short projection, apiculum, at one or both ends) like *Kloeckera apiculata*, or more likely its sexual counterpart *Hanseniaspora uvarum*. Regularly encountered, but less prevalent yeast are *Metschnikowia pulcherima*, *Hansenula anomala*, *Pichia membranaefaciens*, and the weakly fermentative pink/red yeast *Rhodotorula minuta* (7). *Aureobasidium pullulans* is occasionally isolated. This "black yeast" is in fact not yeast; it is considered a taxonomically separate fungus and is termed "yeast-like."

Hanseniaspora uvarum is a member of the *Saccharomyces* clade (group) and is an anamorph (very similar) of *Kloeckera apiculata*. It reproduces asexually by bipolar budding; the cells are apiculate, ovoidal, or elongate. *H. uvarum* cells may or may not possess pseudohyphae and asci do arise without conjugation. Also, glucose is fermented (14) as *H. uvarum* can be seen in the early stages of spontaneous fermentation. From the order Hyphomycetales and the family Moniliaceae (12), *Aureobasidium pullulans* (Fig. 3a) is highly tolerant to irradiation and dehydration (23). This genus is a member of the "black yeasts" along with *Hormonema*, and probably *Hortaea* (14). When the colonies are young the unicellular budding yeast cells are the only structures visible

Figure 2: The Lake Erie Appellation of Northern Ohio. Each vineyard is indicated by arrows.

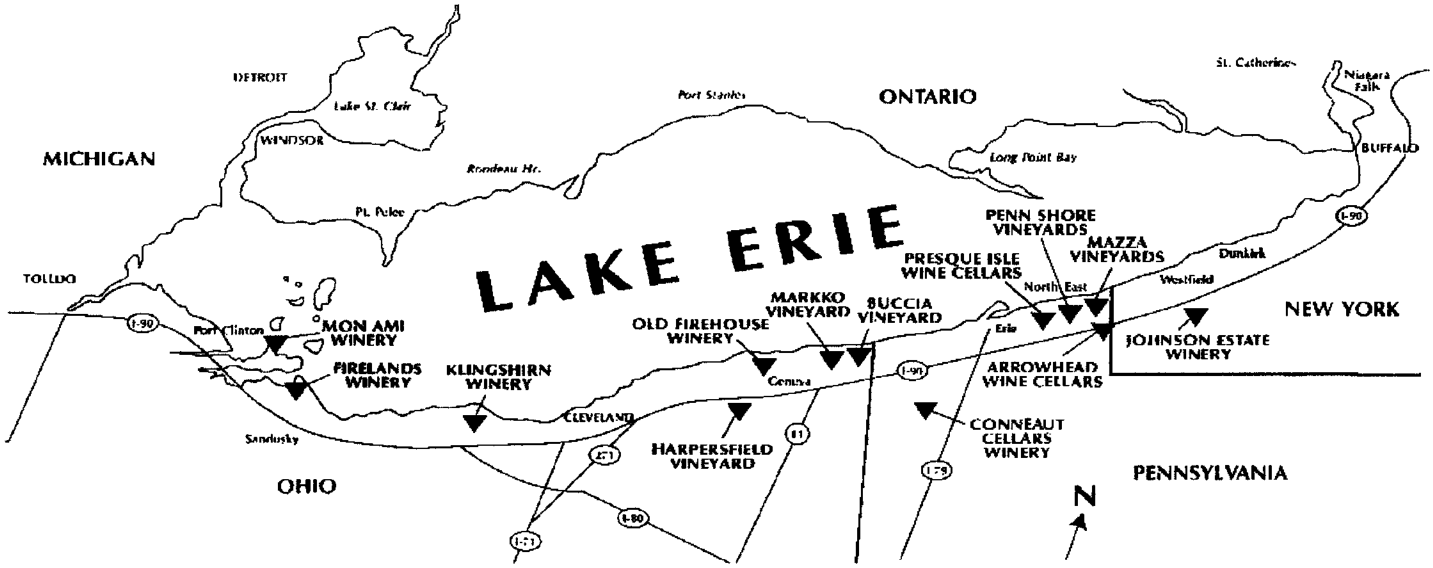
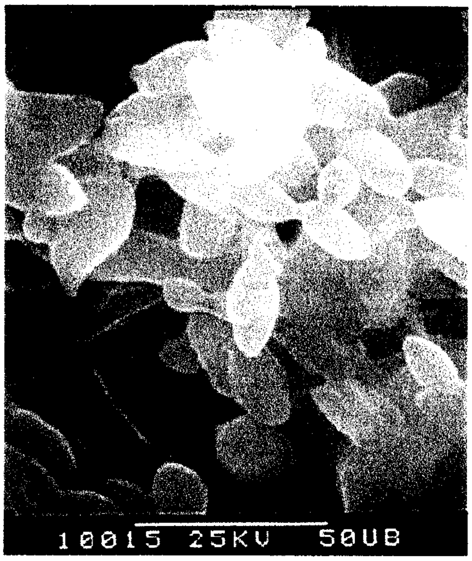
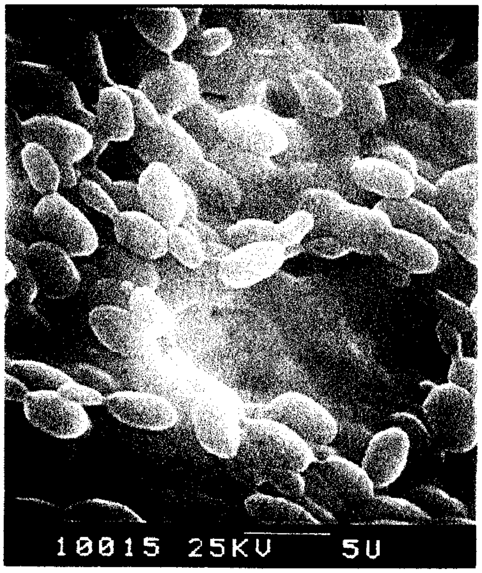

DETROIT
Lake St. Clair
WINDSOR
MICHIGAN
Port Stanley
ONTARIO
St. Catherines
Niagara Falls
Long Point Bay
BUFFALO
Rondeau Hr.
Pt. Pelee
LAKE ERIE
TOLEDO
I-90
Port Clinton
MON AMI WINERY
FIRELANDS WINERY
KLINGSHIRN WINERY
Sandusky
CLEVELAND
OHIO
I-271
I-71
I-80
HARPERSFIELD VINEYARD
11
OLD FIREHOUSE WINERY
Geneva
MARKKO VINEYARD
BUCCIA VINEYARD
I-90
PRESQUE ISLE WINE CELLARS
PENN SHORE VINEYARDS
MAZZA VINEYARDS
North East
Erie
Dunkirk
Westfield
I-90
NEW YORK
JOHNSON ESTATE WINERY
ARROWHEAD WINE CELLARS
CONNEAUT CELLARS WINERY
179
PENNSYLVANIA
N

Figure 3: a) *Aureobasidium pullulans*. Magnification: 4.0K, Aperture: 3, Tilt 0°. b) *Rhodotorula glutinis*. Magnification: 3.5K, Aperture: 3, Tilt: 65°.

a.



b.



microscopically. The colony surfaces are white or pale pink and become brown to black with age as the hyphae become more visible (25). *A. pullulans* is an antagonist of post-harvest fungal pathogens. It prevents fungal disease in field conditions due to such factors as competition for space and nutrients (5).

From the family Cryptococcaceae, *Rhodotorula glutinis* (Fig. 3b) is an anamorph of Basidiomycetes and forms rapid growing, salmon pink to coral red colonies (12). Yeast cells are globose to subglobose, ellipsoidal, and ovoidal to elongate (14). This nonfermenting yeast reproduces by multilateral or polar budding and along with *R. mucilaginosa* is one of the most abundant species identified in food (13). It is believed that *R. glutinis*, along with *A. pullulans*, adheres to plant surfaces by way of the presence of glycoproteins and mannose residues present at the site of bud development. *R. glutinis* is a common phylloplane epiphyte that shows biological control potential against species like *Botrytis cinerea*, a phytopathogenic fungus with a host range of more than 235 identified plant species including that of grapes (1, 3, 16). *B. cinerea* growth occurs on immature or damaged grapes, entering directly or through microscopic lesions, causing "slipskin" and the disease known as "grey mold" (1, 7). It can also be considered a more sensitive yeast than other competing "killer" yeast species. Meaning the killer yeast can release a toxin that will later rid the sensitive yeast strain from the population (10). Usually seen late in the growing season is the yeast species *Saccharomyces cerevisiae*, which likely reflects its preference for a high sugar environment of grape juice and fermentation. This explains why population numbers increase, as grape sugars become more available (7).

III. Research Strategy

In 2000, samples were collected from select fields (Brother, Marie, Bottom, Dr. Frank and Midhill) in the Markko vineyard containing Chardonnay, Riesling, Merlot, Cabernet Sauvignon, and Pinot Noir grapes (Fig. 4). In 2001, samples of Riesling grapes were collected from Markko, Klingshirn, and Presque Isle (Fig.2). This study set out to identify and compare the indigenous yeast present from one year to another by culturing on SAB plates, isolating distinct colonies, growing pure cultures, and using DNA extraction and polymerase chain reaction of the ITS rDNA regions, ITS4 and ITS5 (Fig. 4).

Molecular techniques have recently been used as an alternative for the morphological and physiological characterization traditionally used to identify the species and strains of *Saccharomyces*, one of the main yeasts present during fermentation. In this study molecular techniques such as polymerase chain reaction (DNA amplification) and DNA sequence analysis were used for the identification of indigenous yeast present on grape surfaces prior to harvest. Forward and reverse internal transcribed spacer (ITS5 and ITS4, respectively) primers were used to amplify a region of the rDNA gene repeat unit that includes two functionally important non-coding regions, between the 18S and 28S rDNA genes, and the 5.8S rDNA gene. Also, the PCR products can show a high length (base pair) variation in the ITS1 and ITS2 regions (Fig. 5) for the various yeast species found (9). We chose the ITS rDNA regions due to the fact that rDNA genes have a high copy number as well as interspersed conserved and variable (ITS1 and ITS2 regions) rDNA sequences, potentially making them ideal targets for yeast species and strain identification (3). The advantages of using the ITS regions are that they are becoming very dependable in species differentiation among distantly as well as

Figure 4: Map of Markko vineyard. Grapes were sampled from each of the fields in 2001 and from Dr. Frank in 2002.

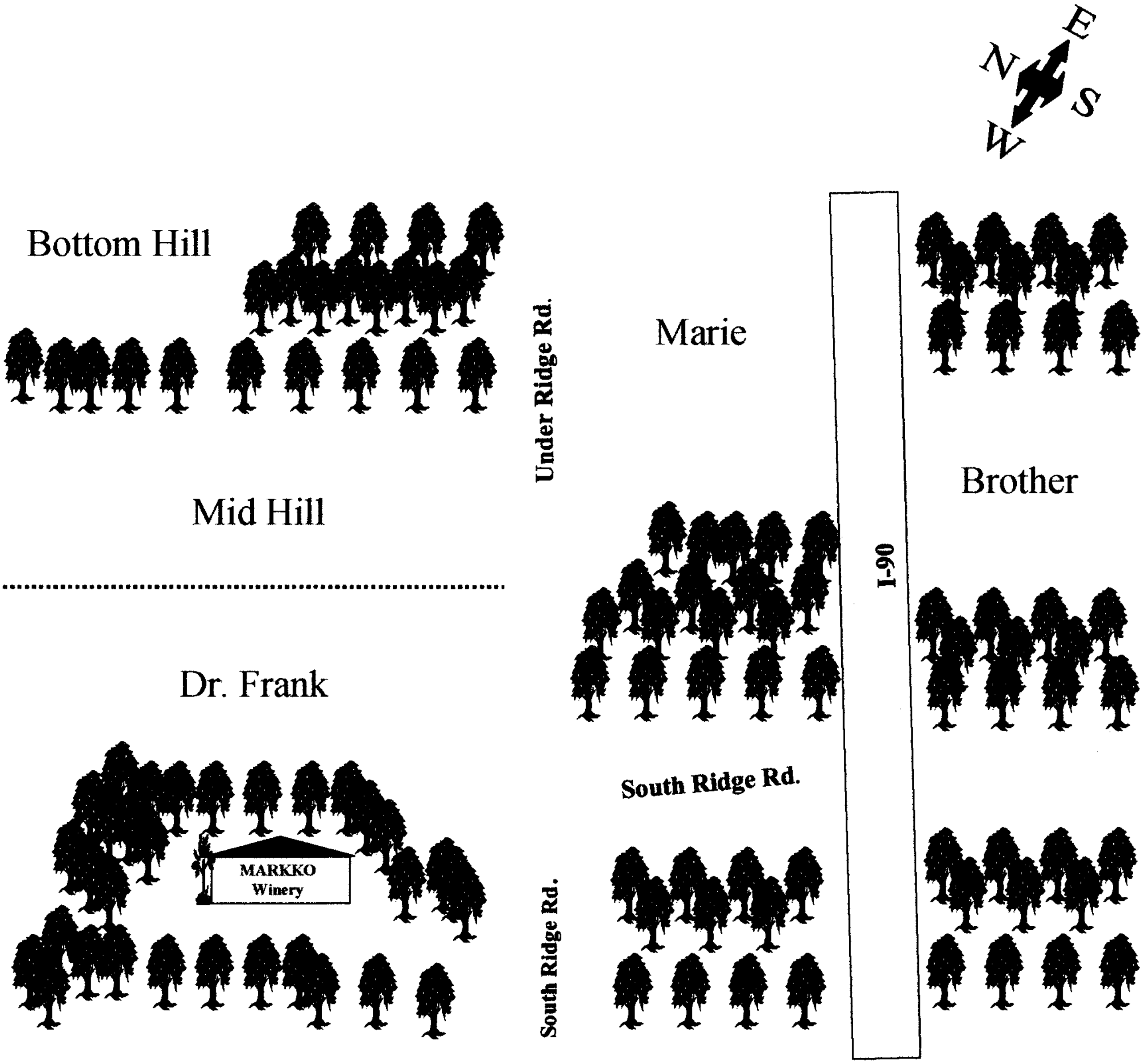
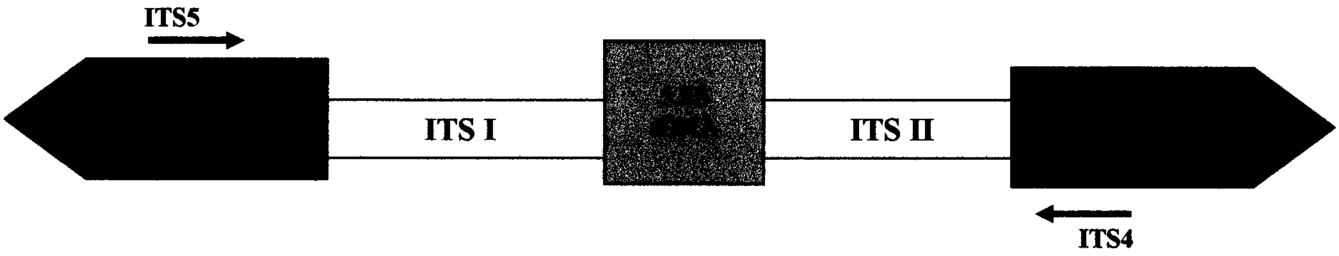

E
N
S
W
Bottom Hill
Under Ridge Rd.
Marie
I-90
Brother
Mid Hill
Dr. Frank
South Ridge Rd.
MARKKO
Winery
South Ridge Rd.

Figure 5: Nuclear Ribosomal RNA gene Repeat Unit showing internal transcribed spacers (ITS) region and location of the ITS4 and ITS5 primers. The amplified PCR products using primers ITS4 and ITS5 are approximately 600 – 800 bp in length.


ITS5
ITS I
ITS II
ITS4

very closely related species of fungi. DNA sequences from this region have been used to distinguish between closely related species once thought to be taxonomically the same (6, 11, 13).

Following identification of indigenous yeast present within Markko vineyard, it was desired to determine whether there was variation in yeast species and rDNA sequence as a result of grape type and field (Fig. 4) during the 2000-growing season. Then the next step was to determine whether there was regional variation in the distribution of yeast species or in their rDNA sequence within the Lake Erie Appellation (Fig. 2) by sampling only Riesling grapes from 2001-growing season. Lastly, we wished to determine whether there was variation in the distribution of yeast species and rDNA sequence from one year (2000) to the next (2001) within Markko vineyard.

IV. Goals

1. Identify indigenous yeast species present on wine grapes prior to harvest within the Lake Erie Quality Wine Alliance.
2. a) Determine the extent of variation of indigenous yeast species found in each field and on each type of grape within one vineyard.

   b) Determine the extent of variation of rDNA sequences within species present in each field and on each type of grape within one vineyard.
3. a) Determine the extent of variation in indigenous yeast species found on Riesling grapes between the three subdistricts of the Lake Eire Quality Wine Alliance.

b) Determine the extent of variation in rDNA sequence of indigenous yeast species identified on Riesling grapes between the three subdistricts of the Lake Erie Quality Wine Alliance.

4. a) Determine the extent of variation in indigenous yeast species found from year to year within one vineyard.

   b) Determine the extent of variation in rDNA sequence within indigenous yeast species found from year to year within one vineyard.

Chapter II: Materials & Methods

Vineyard grape samples:

In 2000, the yeast samples were obtained from the surfaces of grapes collected from the Markko vineyard (Fig. 6) in Conneaut, Ohio on July 27, 2000, August 21, 2000, and September 21, 2000.

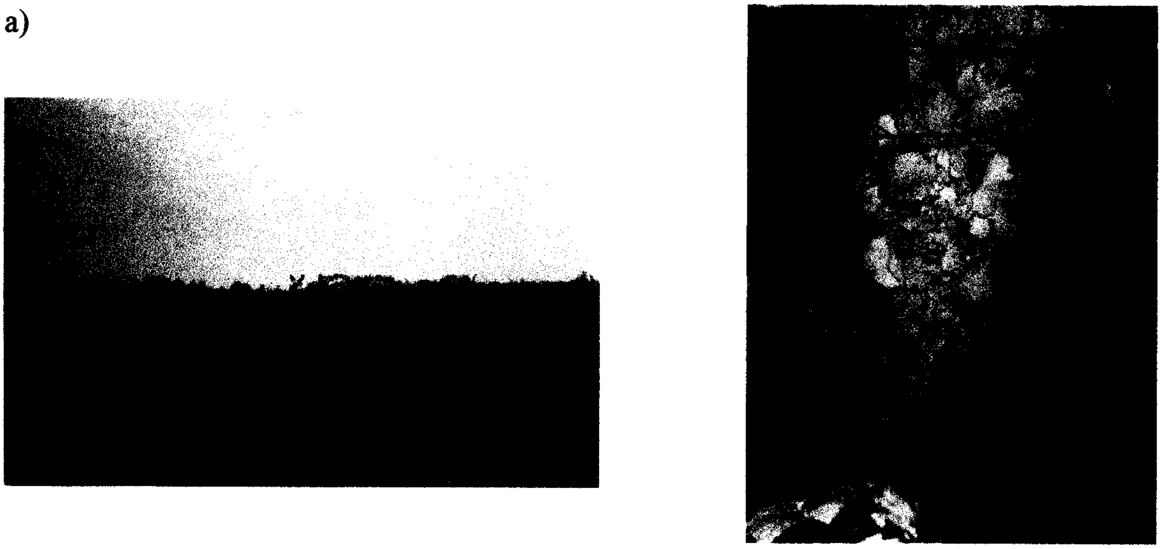
In 2001, yeast samples were collected using a randomized block design, from the surface of grapes collected from three different vineyards of the Lake Erie Quality Wine Alliance (LEQWA). One of each of the three sub-districts was represented [Markko (Fig. 6a), Klingshirn (Fig. 6b), Presque Isle (Fig. 6c)]. Klingshirn samples were collected on August 30, 2001, September 23, 2001, and October 5, 2001. Markko samples were collected on August 24, 2001, September 22, 2001, and October 14, 2001. Presque Isle samples were collected on August 24, 2001, September 22, 2001, and October 7, 2001.

Plating:

Yeast isolates, along with yeast colony forming units (CFU's), were obtained by adding 200 g of grapes were 100 ml of autoclaved 10 Mega Ohm (MΩ) water and blended in a Waring Blender. 1 ml of resultant grape juice was added to 9 ml of autoclaved 10 MΩ water to yield a 10^1 dilution. Serial dilutions were repeated until a 10^4 dilution was obtained. One hundred microliters from the 10^2 through 10^4 dilutions were spread plated in duplicate on to SAAB nutrient media, rich in glucose, becoming 10^3

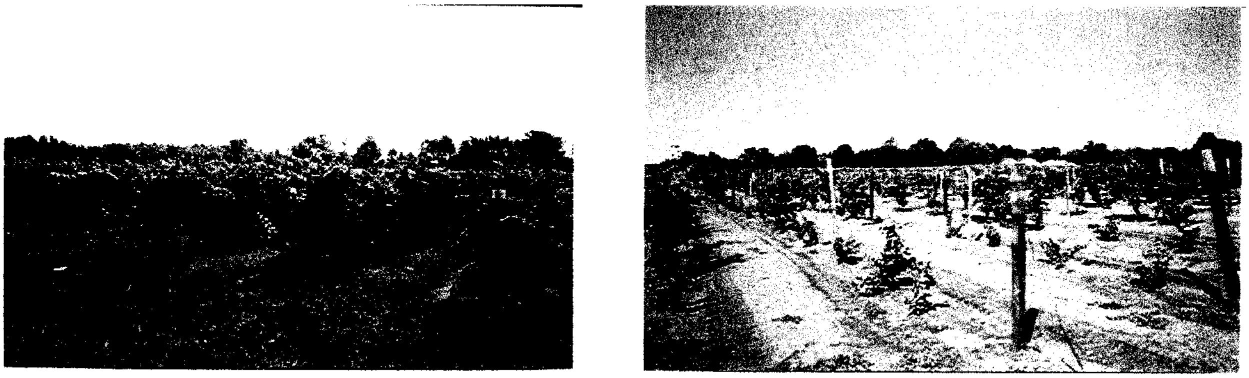
Figure 6: a) Markko vineyard. b) Klingshirn vineyard. c) Presque Isle vineyard.

a)



b)

c)



through 10^5 dilution plates. The final set of grapes collected in 2002 were diluted and spread plated in the same manner as described above as well as being spread plated onto Difco's Wallerstein (WL) Nutrient media containing Bacto Yeast Extract, Bacto Casitone, Bacto Dextrose, Monopotassium Phosphate, Potassium Chloride, Calcium Chloride and Magnesium Sulfate, Ferric Chloride and Manganese Sulfate, Bacto Agar, and Brom Cresol Green. WLN media excludes such predominantly fermentative yeast as *Saccharomyces cerevisiae* and preferentially selects for wild yeast.

Broth cultures:

Yeast colonies from plates were streaked until isolated colonies were obtained. Isolates were designated by colony color (morphology). The colonies were designated Peach and White in 2000, and following closer attention in 2001 were designated Peach, Pink, and White. Only isolated colonies were chosen as samples for further analysis.

Then, YEP broth was made containing yeast extract, Bacto Peptone, TAPd, and water. To create YEPD broth from YEP broth, a 20% glucose solution was made first by adding 20 g of dextrose to 80 ml of 10 MΩ milli-Q water. The solution was heated, stirred, and autoclaved for 15 min. at 121°C. 12 ml of 20% glucose were added to 100 ml of YEP media yielding a 2% [glucose] YEPD broth. The broth (3 ml) was inoculated using a sterile loop containing isolated yeast colonies. The individual yeast samples were then incubated at 25°C in YEPD broth on a rotation wheel. After 2 – 3 days of incubation, tubes were centrifuged at ~10,000 rpm for 10 s. Supernatant was separated from pellets and stored at about -20°C.

DNA extraction:

Two methods were used. A method adapted from Hoffman and Winston (13b) was used for DNA extractions from the 2000 harvest. Acid washed glass beads, 0.3 g, were added to cell pellets along with 200 µl Lysis buffer and 200 µl phenol, chloroform, and isoamyl alcohol at a ratio of 25:24:1 (stored at 4°C). Each sample was placed on ice for 5 min and vortexed for 2 min until the cell pellet was broken up. 200 µl of TE was added and centrifuged for 5 min; the aqueous layer was removed and placed in a sterile Eppendorf tube containing the amount of phenol chloroform equal to the amount of aqueous layer removed. The aqueous layer was then removed and added to 750 µl of 100% EtOH (stored at -20°C) followed by the addition of 50 µl NaOAc. Then the samples were mixed and placed in a freezer at -20°C for 5 – 10 min, centrifuged at ~10,000 rpm for 10 min, followed by careful removal of the supernatant. The pellet was gently washed with 70% EtOH (stored at -20°C), the EtOH was carefully removed and the pellet dried overnight.

The MasterPure™ Yeast DNA Purification Kit technique, obtained from Epicentre Technologies™, was used in the 2001 harvest. 300 µl of Yeast cell Lysis solution was added to each yeast cell pellet and vortexed, to suspend the sample, followed by incubation at 65°C for 15 min and an ice bath for 5 min. 150 µl of MPC Protein Precipitation Reagent was added and vortexed for 10 s and centrifuged for 10 min at ~10,000 rpm to pellet any cellular debris. The addition of 500 µl isopropanol followed and the mixture was subjected to centrifugation for 10 min at ~10,000 rpm to pellet the DNA. The supernatant was removed and the pellet was washed with 0.5 ml of 75%

ethanol. The ethanol was removed and the DNA was suspended in 50 µl autoclaved 10 MΩ milli-Q water at -20°C.

Polymerase Chain Reaction:

PCRs were performed using a PTC-200 Peltier thermal cycler. The TAQurate master mix (Epicentre Technologies™) was placed into the PCR tube by the addition of: 34 µl autoclaved 10 MΩ water, 10 µl 10X TAQurate PCR buffer, 16µl 2mM $MgCl_2$, 30 µl MasterAmp 10X PCR Enhancer, 2 µl 10µM dNTPs, 1 µl 20 pmol ITS4, 1 µl 20 pmol ITS5, 1 µl of 2.5 U/µl MasterAmp TAQurate DNA polymerase mix, and 5 µl extracted DNA for a total volume of 100 µl. To denature the template DNA, the reaction mixtures were initially heated for 3 min at 92°C with all components followed by 30 cycles of denaturation (92.0°C for 15 s), annealing (51.7°C for 30 s), and a final extension (72°C for 30 s).

Primers ITS4 and ITS5 that are complementary to conserved regions of fungal 18S and 28S rRNA sequences were used (Fig. 5). The primer sequence for ITS4 is TCC TCC GCT TAT TGA TAT GC and for ITS5 is GGA AGT AAA AGT CGT AAC AAG G.

Gel Electrophoresis:

To determine rDNA recovery and quality from the DNA extraction, 5 µl rDNA or 5 µl (50 ng) Lambda Weight ladder (Sigma) were added to 1 µl BioMarker™Low tracking dye and placed in 8 mm wells and electrophoresed at 50 V to 60 V for 1 h. Agarose gels (0.8%) were made by adding 0.4 g of agarose powder to 50 ml of 1X TAE buffer. Gels were stained with Ethidium Bromide (EtBr) in an EtBr bath (EtBr + 1X TAE buffer) for 30 min and documented using KODAK DC290 digital camera and KODAK 1D™ imaging software.

To test for the presence of PCR amplified rDNA, 1% agarose gels were made by adding 0.5 g of Fisher high melt agarose powder to 50 ml of 1X TAE (Tris-acetate, 2 mM $Na_2EDTA \cdot H_2O$) buffer. Then 5 µl PCR reaction mixture or 5 µl (50 ng) BioMarker™Low Weight ladder (BioVentures, Incorporated) were added to 1 µl BioMarker™Low tracking dye and placed in 8 mm wells and electrophoresed at 50 V to 60 V for 1 h. Gels were stained and documented as above.

Purification of PCR amplified DNA:

Following PCR, the QIAquick PCR Purification Kit Protocol from QIAGEN using a microcentrifuge was used according to the manufacturers recommended protocol. Five volumes of Buffer PB were added to 1 volume PCR reaction mixture in a QIAquick column and centrifuged for 60 s at ~10,000 rpm to bind the DNA. Flow through was discarded and 0.75 ml of Buffer PE was added to QIAquick column and centrifuged for 60s at ~10,000 rpm to wash. Flow through was discarded and QIAquick column was placed in a sterile 1.5 ml Eppendorf tube and 30 µl Buffer EB (10 mM Tris-Cl, pH 8.5) was then added, allowed to stand for 1 min and centrifuged at ~10,000 rpm for 60 s to elute the DNA. The PCR amplified rDNA solution was stored at -20°C until used.

DNA sequencing:

Sequencing was done using Beckman Coulter CEQ2000XL Dye Terminator Cycle Sequencer. Purified DNA samples were subjected to the Beckman Coulter sequencing protocol. PCR dye labeling was done as follows: 45 cycles of denaturation (96°C for 20 s), annealing (50°C for 20 s), and extension (60°C for 4 min). Sequences were identified using EBI/GenBank FASTA program for fungal DNA analysis (www.embl.org/genbank or www.ebi.ac.uk/fasta).

Alignment:

Sequences were aligned using Clustal W version 1.8 found on the Baylor College of Medicine (BCM) website (www.searchlauncher.bcm.tmc.edu) under the heading "multiple sequence alignments." Reverse compliments of reverse primer sequences were done also on the aforementioned website, but under the heading "sequencing tools." Clustal W version 1.82 alignments were done on the EBI-EMBL international website, www.ebi.ac.uk/clustalw.com.

Chapter III: Results and Discussion

Most studies focus on the yeast species present during the fermentation process following a sulfur treatment that kills indigenous yeasts. However, some indigenous yeast present in the fields prior to harvest have been found to be the initiators in spontaneous fermentations of alcohol, that progress without sulfur treatment (3, 5). This, perhaps, aids in the natural flavor that distinguishes one vineyard's wine from another like France, for instance, which uses spontaneous fermentation methods that result in expensive, high quality wines.

Surprisingly, in this study the indigenous yeast species identified were different than the yeast species expected. Some authors state that *Hanseniaspora, Metschnikowia, Candida, Pichia, Hansenula,* and *Zygosaccharomyces* are found more frequently on wine grapes (8, 12) around the world, yet the dominant indigenous yeast species identified in this study were *Rhodotorula* sp. and *A. pullulans*, followed far behind by *Hanseniaspora uvarum*, *Sporidiobolus pararoseus*, and *Metschnikowia bicuspidata*. Also, the identification of *Sporobolomyces marcillae* only within the Klingshirn vineyard was another unexpected outcome. This difference may be due to changes in the micro environments of the vineyards studied.

All vineyards showed 10^4 colony forming units/milliliter (CFUs/ml) in August, 2001. By early October, the amount of CFUs in Markko vineyard remained relatively constant while CFUs in Presque Isle increased to 10^6 and CFUs in Klingshirn increased to 10^7. These data indicate that there were regional differences in the numbers of CFUs during this time interval just prior to harvest. Difco's Wallerstein Nutrient media (WLN) was only used during early October, just prior to harvest. Similar results were obtained from the comparison of CFUs grown on WLN media to those grown on SAAB nutrient media: Markko vineyard at 10^4 CFUs/ml, Presque Isle at 10^6 CFUs/ml, and Klingshirn vineyard at 10^7 CFUs/ml.

Within the Markko vineyard in 2000 there were two dominant indigenous yeasts: *Aureobasidium pullulans* (Fig. 3a) and *Rhodotorula glutinis* (Fig. 3b). These species were identified from isolated colonies using DNA extraction, Polymerase Chain Reaction, and DNA sequencing of ITS rDNA. As expected, PCR products ranged from about 500 to 750 bp in size. Of the dominant indigenous yeast species found, PCR products from *A. pullulans* and *R. glutinis* were about 600 bp in size, and PCR product from *H. uvarum* was about 740 bp in size. The DNA sequences were entered into the EBI-EMBL FASTA databank of nucleotide fungal species, and the percent match comparing the entered sequence to the FASTA matches within the databank were given. Peach colonies were identified as *Rhodotorula glutinis* with an average percent match of 96.5% and a standard deviation of 4.26 (n = 17; Table 2). The white colonies were identified as *Auriobasidium pullulans*, with an average percent match of 96.9% and a standard deviation of 1.53 (n = 23), and *Hanseniaspora uvarum* (90.6% match), using ITS rDNA (Table 1).

There were only two isolates from Markko vineyard that were not identified as *A. pullulans* and *R. glutinis*. One isolate was identified as *H. uvarum* just prior to harvest, and one isolate was tentatively identified as *Saccharomyces cerevisiae* (data not shown). This was likely another unknown yeast species due to such a poor match (69%). Since each of the two dominant species were found throughout the vineyard, it was concluded

Table 1: Identification of 23 isolates of *Aureobasidium* pullulans collected from Markko vineyard during the 2000-growing season. The percent match indicates the percent similarity of the sample to the EMBL species. The nt overlap indicates the number of nucleotides that overlapped from the two sequences (sample sequence vs. EMBL sequence). a) Arranged by field.
b) Arranged by grape.

Key For Naming Isolates

First number	**1 – 13**	were collected on 7/27/00
	21 – 33	were collected on 8/20/00
	41 – 53	were collected on 9/20/00
First letter	**W**	white colonies
Date	**xx/xx/xx**	date collected
Second letter	**M**	Markko vineyard
Last letter	**C**	Chardonnay
	P	Pinot Gris
	R	Riesling
	M	Merlot
	N	Pinot Noir
	B	Cabernet Sauvignon.

TOTAL
(*A. pullulans*)

n=23

avg=96.91

stdev=1.53

a) Arranged by field.

Isolate	Field	%Match	nt overlap	
10W 7/27/00-MR	Bottom	98.772	570 nt	n=4 avg=98.6 stdev=1.4
11W 7/27/00-MC	Bottom	95.652	368 nt	
30W 8/20/00-MR	Bottom	97.582	579 nt	
31W 8/20/00-MC	Bottom	98.435	575 nt	
1W 7/27/00-MB	Brother	98.763	485 nt	n=12 avg=96.4 stdev=1.58
3W 7/27/00-MC	Brother	97.628	548 nt	
4W 7/27/00-MN	Brother	94.585	591 nt	
5W 7/27/00-MP	Brother	94.949	495 nt	
21W 8/20/00-MC	Brother	95.229	503 nt	
22W 8/20/00-MM	Brother	98.014	554 nt	
23W 8/20/00-MC	Brother	97.707	567 nt	
24W 8/20/00-MN	Brother	96.87	575 nt	
25W 8/20/00-MP	Brother	95.563	586 nt	
43W 9/20/00-MC	Brother	95.637	573 nt	
44W 9/20/00-MN	Brother	98.084	574 nt	
45W 9/20/00-MP	Brother	94.14	529 nt	
12W 7/27/00-MC	Dr. Frank	94.605	519 nt	n=4 avg=97.4 stdev=1.85
13W 7/27/00-MR	Dr. Frank	98.101	474 nt	
32W 8/20/00-MC	Dr. Frank	98.609	575 nt	
53W 9/20/00-MR	Dr. Frank	98.087	575 nt	
6W 7/27/00-MC	Marie	96.701	576 nt	n=3 avg=97.3 stdev=0.93
7W 7/27/00-MC	Marie	96.875	576 nt	
8W 7/27/00-MC	Marie	98.393	560 nt	

b) Arranged by grape.

Isolate	Field	%Match	nt overlap	
1W 7/27/00-MB	Brother	98.763	485 nt	n=2 avg=97 stdev=2.499
21W 8/20/00-MC	Brother	95.229	503 nt	
11W 7/27/00-MC	Bottom	95.652	368 nt	n=10 avg=97.0 stdev=1.37
31W 8/20/00-MC	Bottom	98.435	575 nt	
3W 7/27/00-MC	Brother	97.628	548 nt	
23W 8/20/00-MC	Brother	97.707	567 nt	
43W 9/20/00-MC	Brother	95.637	573 nt	
12W 7/27/00-MC	Dr. Frank	94.605	519 nt	
32W 8/20/00-MC	Dr. Frank	98.609	575 nt	
6W 7/27/00-MC	Marie	96.701	576 nt	
7W 7/27/00-MC	Marie	96.875	576 nt	
8W 7/27/00-MC	Marie	98.393	560 nt	
22W 8/20/00-MM	Brother	98.014	554 nt	n=1
5W 7/27/00-MP	Brother	94.949	495 nt	n=3 avg=94.9 stdev=0.71
25W 8/20/00-MP	Brother	95.563	586 nt	
45W 9/20/00-MP	Brother	94.14	529 nt	
4W 7/27/00-MN	Brother	94.585	591 nt	n=3 avg=96.5 stdev=1.78
24W 8/20/00-MN	Brother	96.87	575 nt	
44W 9/20/00-MN	Brother	98.084	574 nt	
10W 7/27/00-MR	Bottom	98.772	570 nt	n=4 avg=98.1 stdev=0.49
30W 8/20/00-MR	Bottom	97.582	579 nt	
13W 7/27/00-MR	Dr. Frank	98.101	474 nt	
53W 9/20/00-MR	Dr. Frank	98.087	575 nt	

Table 2: Identification of 17 isolates of *Rhodotorula glutinis* collected from Markko vineyard during the 2000-growing season. The percent match indicates the percent similarity of the sample to the EMBL species. The nt overlap indicates the number of nucleotides that overlapped from the two sequences (sample sequence vs. EMBL sequence). a) Arranged by field. b) Arranged by grape.

Key For Naming Isolates

First number	**1 – 13**	were collected on 7/27/00
	21 – 33	were collected on 8/20/00
	41 – 53	were collected on 9/20/00
First letter	**P**	peach colonies
Date	**xx/xx/xx**	date collected
Second letter	**M**	Markko vineyard
Last letter	**C**	Chardonnay
	P	Pinot Gris
	R	Riesling
	M	Merlot
	N	Pinot Noir
	B	Cabernet Sauvignon.

TOTAL
(*R. glutinis*)

n=17
avg=96.52
stdev=4.26

a) Arranged by field.

Isolate	Field	%Match	nt overlap	
10P 7/27/00-MOR	Bottom	99.29	563 nt	n=2 avg=90.5 stdev=12.4
51P 9/21/00-MOC	Bottom	81.7	541 nt	
3P 7/27/00-MBC	Brother	98.58	563 nt	n=5 avg=98.36 stdev=0.723
5P 7/27/00-MBP	Brother	98.58	599 nt	
22P 8/20/00-MBM	Brother	98.01	502 nt	
42P 9/21/00-MBM	Brother	97.35	567 nt	
44P 9/21/00-MPB	Brother	99.29	562 nt	
12P 7/27/00-MFC	Dr. Frank	97.36	606 nt	n=3 avg=98.1 stdev=0.795
52P 9/21/00-MFC	Dr. Frank	98.94	565 nt	
53P 9/21/00-MFR	Dr. Frank	97.99	596 nt	
8P 7/27/00-MMC	Marie	98.97	585 nt	n=4 avg=96.34 stdev=2.831
28P 8/20/00-MMC	Marie	97.14	595 nt	
46P 9/21/00-MMC	Marie	96.91	420 nt	
47P 9/21/00-MMC	Marie	92.32	495 nt	
9P 7/27/00-MHR	Mid Hill	96.08	586 nt	n=4 avg=96.69 stdev=2.185
29P 8/20/00-MHR	Mid Hill	98.41	565 nt	
49P 9/21/00-MHR	Mid Hill	93.85	553 nt	

b) Arranged by grape.

Isolate	Field	%Match	nt overlap	
51P 9/21/00-MOC	Bottom	81.7	541 nt	n=8 avg=95.24 stdev=5.873
3P 7/27/00-MBC	Brother	98.58	563 nt	
12P 7/27/00-MFC	Dr. Frank	97.36	606 nt	
52P 9/21/00-MFC	Dr. Frank	98.94	565 nt	
8P 7/27/00-MMC	Marie	98.97	585 nt	
28P 8/20/00-MMC	Marie	97.14	595 nt	
46P 9/21/00-MMC	Marie	96.91	420 nt	
47P 9/21/00-MMC	Marie	92.32	495 nt	
22P 8/20/00-MBM	Brother	98.01	502 nt	n=2 avg=97.68 stdev=0.462
42P 9/21/00-MBM	Brother	97.35	567 nt	
5P 7/27/00-MBP	Brother	98.58	599 nt	n=1
44P 9/21/00-MPB	Brother	99.29	562 nt	n=1
10P 7/27/00-MOR	Bottom	99.29	563 nt	n=6 avg=97.34 stdev=2.013
53P 9/21/00-MFR	Dr. Frank	97.99	596 nt	
9P 7/27/00-MHR	Mid Hill	96.08	586 nt	
29P 8/20/00-MHR	Mid Hill	98.41	565 nt	
49P 9/21/00-MHR	Mid Hill	93.85	553 nt	

that there was no variation in indigenous yeast species was seen between each field and each grape type within the vineyard.

Following PCR, rDNA purification, DNA sequence analysis, using the ITS4 primer, *Aureobasidium pullulans* strains, showed relatively more sequence variability whereas *Rhodotorula glutinis* showed a relatively constant ClustalW alignment pattern throughout. However, when trying to indicate the 28S ribosomal region it was found to be in between the 18S and ITS1 region instead of following the ITS2 region (App. 4). This alignment was checked with both ClustalW 1.8 and ClustalW 1.82, so it may be due to some variation between the rDNA sequence of the sample and the EMBL match. This anomaly was seen in three sequences but not seen with all sequences tested. The *A. pullulans* strains, sequenced using the ITS5 primer, showed a more variable alignment pattern throughout as did *R. glutinis*, using ITS5 (App. 5). More sequence variability was shown when using the ITS5; therefore only sequence alignments for ITS4 are shown.

Neither type of grape, field, nor sample date appeared to be selective for sequence variation in *R. glutinis* and *A. pullulans*. There was significant gapping within the *A. pullulans* sequence alignment, but the sequences between gaps align consistently throughout each field and grape type (App. 4). When seven sequence outliers identified as *R. glutinis* from the EMBL nucleotide databank were removed (alignment not shown), better alignment was allowed in the remaining sequences. The field and grape type did not appear to affect distribution of indigenous yeast strains since these alignment outliers were identified throughout the same fields and grape types as the other sequences of *R. glutinis* that aligned consistently.

Regional distribution of species showed little variation in the dominant indigenous yeast species detected. However, there was variability in the less dominant indigenous yeast species detected across the appellation. The dominant indigenous yeast species found on Riesling grapes from the Markko, Presque Isle, and Klingshirn were once again identified as *Aureobasidium pullulans* (n = 29; avg.= 97.7%; s.d.= 1.37) and *Rhodotorula* sp. (n = 9; avg.= 97.9%; s.d.= 3.18). A few *Rhodotorula* sp. could not be specifically identified due to difficulty in determining between two closely related species, so they became identified as *Rhodotorula* sp., their top match on the EMBL website search engine (Table 3).

Hanseniaspora uvarum and *Sporidiobolus pararoseus* showed dominance as well (Table 3). Both species showed little variation in alignment (App. 8 and 9). *A. pullulans*, *Rhodotorula* sp., *H. uvarum*, and *S. pararoseus* have been identified within all three vineyards, but additional indigenous yeast species (*Rhodotorula graminis, Coniophora marmorata, Metschnikowia bicuspidata, Filobasidium floriforme, Cryptococcus wieringa, Cryptococcus victoriae*) have been identified within Presque Isle and Klingshirn (Table 3) and one species, *Sporobolomyces marcillae*, was only seen within the Klingshirn vineyard showing variability in yeast species throughout the Lake Erie Quality Wine Alliance.

Generally, little year to year variation was seen in indigenous yeast species. *A. pullulans* and *Rhodotorula* sp. were dominant in both 2000 and 2001, and some variations in minor species were found. *H. uvarum* was identified once in 2000 and twice in 2001. *Sporidiobolus pararoseus* was only identified once in 2001 and not in 2000. Similarly, there was little year to year variation seen in rDNA sequence. Using ClustalW

1.8 and 1.82, alignments of rDNA sequences identified within the Markko vineyard have shown that *Aureobasidium pullulans* and *Rhodotorula* sp. have individually shown consistent alignment patterns from 2000 to 2001 (Apps. 11 and 12), although when comparing *A. pullulans* alignments there was consistency, but frequent gaps.

When *Sporidiobolus pararoseus* was aligned with the other *S. pararoseus* species found within the Markko, Klingshirn and Presque Isle vineyards little variation in rDNA sequence was seen (App. 8). There was not much variability when aligning rDNA sequences from the dominant indigenous yeast species identified in the 2000 harvest to those species identified in 2001, but indigenous yeast species identification varied in that less frequent species *Hanseniaspora uvarum*, from 2000, was seen slightly more in 2001 (Tables 1,3).

Comparison of rDNA sequences within a species in one year showed little sequence variation, the same was expected in 2001. Alignments of rDNA sequences of indigenous yeast identified from the 2001 harvest showed little variation, as expected, when compared to other sequences in the same species. rDNA sequences, using ITS4 primer, of *A. pullulans* showed regular consistency among sequences (App. 6) whereas sequences using the ITS5 primer showed very poor consistency (data not shown). *A. pullulans* showed a more consistent alignment in 2001 than in 2000 (App. 4, App. 6), yet when comparing year to year they align consistently. *R. glutinis* showed consistency when sequenced using either primer, but sequences using the ITS5 primer were still less reliable (data not shown). The rDNA sequence alignment outliers seen in 2001 remained outliers in the year to year comparison. These sequences may indicate different strains of *R. glutinis* or a new species closely related to *R. glutinis* like that of *R. graminis* (14).

Table 3: Identification of yeast isolates collected from Riesling grapes within the Markko (**M**), Presque Isle (**P**), and the Klingshirn (**K**) vineyards during the 2001 season. a) *A. pullulans* b) Other dominant indigenous yeast species. c) Minor indigenous yeast species.

Key For Naming Isolates

First number	**1 – 12**	were collected on 8/24/01
	13 – 18	were collected on 8/30/01
	21 – 32	were collected on 9/22/01
	33 – 38	were collected on 9/23/01
	41 – 46	were collected on 10/14/01
	47 – 52	were collected on 10/5/01
	53 – 58	were collected on 10/7/01
First letter	**W**	white colonies
	WF	white feather colonies
	P	peach colonies
	PK	pink colonies
Date	**xx/xx/xx**	date collected
Second letter	**M**	Markko vineyard
	P	Presque Isle vineyard
	K	Klingshirn vineyard

3a.

	EBI	**EBI**
Isolate	**% Match**	**nt overlap**
3W 8/24/01-M	98.604	573
6W 8/24/01-M	95.39	564
10W 8/24/01-P	94.703	472
17W 8/30/01-K	97.736	265
26W 9/22/01-M	97.163	423
27W 9/22/01-P	97.762	581
41WF 10/14/01-M	98.255	573
44WF 10/14/01-M	95.979	572
46WF 10/14/01-M	96.853	572
50WF 10/7/01-P	94.464	578
53WF 10/7/01-K	95.965	570
57WF 10/5/01-K	97.642	424
58WF 10/5/01-K	95.66	530
7W 8/24/01-P	98.62	580
58WF 10/5/01-K	98.969	582
4W 8/24/01-M	97.793	589
3W 8/24/01-M	99.12	568
27P 9/22/01-P	98.964	579
2W 8/24/01-M	99.129	574
22W 9/22/01-M	98.01	584
12W 8/24/01-P	98.282	582
8W 8/24/01-P	98.955	574
57WF 10/5/01-K	98.454	582
5W 8/24/01-M	98.451	581
58WF 10/5/01-K	98.623	581
53WF 10/7/01-K	98.637	587
53P 10/7/01-K	97.942	583
50WF 10/7/01-P	98.462	585
14W 8/30/01-K	98.936	564
n=29 avg=97.7 stdev=1.366		

3b.

		EBI	**EBI**
Isolate	**Yeast species**	**% Match**	**nt overlap**
3P 8/24/01-M	*Rhodotorula glutinis*	98.003	601
33P 9/23/01-K	*Rhodotorula glutinis*	97.815	595
44P 10/14/01-M	*Rhodotorula glutinis*	91.385	592
46PKS 10/14/01-M	*Rhodotorula glutinis*	96.321	598
46PKW 10/14/01-M	*Rhodotorula graminis*	100	569
53P 10/5/01-K	*Rhodotorula sp. KB 651 18S*	99.833	600
53P2 10/5/01-K	*Rhodotorula sp. KB 651 18S*	99.315	584
58P10/5/01-K	*Rhodotorula sp. KB 651 18S*	99.486	584
51PK 10/7/01-P	*Rhodotorula sp. KB 651 18S*	99.341	607
	n=9 avg=97.94 stdev=2.731		

		EBI	**EBI**
Isolate	**Yeast species**	**% Match**	**nt overlap**
58W 10/5/01-K	*Hanseniaspora uvarum*	94.82	695
43W 10/14/01-M	*Hanseniaspora uvarum*	89.474	646
35W-4 9/23/01-K	*Hanseniaspora uvarum*	96.991	698
58WS 10/5/01-K	*Hanseniaspora uvarum*	97.626	716
50W 10/7/01-P	*Hanseniaspora uvarum*	97.765	716
50W 10/7/01-P	*Hanseniaspora uvarum*	98.825	681
44PK 10/14/01-M	*Hanseniaspora uvarum*	92.988	656
	n=7 avg=95.498 stdev=3.317		

		EBI	**EBI**
Isolate	**Yeast species**	**% Match**	**nt overlap**
29P-4 9/22/01-P	*Sporidiobolus pararoseus*	90.4	250
22P 9/22/01-M	*Sporidiobolus pararoseus*	98.621	580
17P 8/30/01-K	*Sporidiobolus pararoseus*	98.331	599
35P 9/23/01-K	*Sporidiobolus pararoseus*	98.319	595
31P 9/23/01-P	*Sporidiobolus pararoseus*	97.4	577
14P2 8/30/01-K	*Sporidiobolus pararoseus*	98.013	604
	n=6 avg=96.847 stdev=3.186		

3c.

		EBI	EBI
Isolate	**Yeast species**	**% Match**	**nt overlap**
27W 9/23/01-P	*Coniophora marmorata*	99.603	504
41P 10/14/01-M	*Coniophora marmorata*	97.89	474
n=2; avg=98.75; stdev=1.211			
56PK 10/5/01-K	*Cryptococcus wieringa*	99.659	587
33PMK 9/23/01-K	*Cryptococcus victoriae*	99.598	497
n=2; avg=99.63; stdev=0.043			
15W 8/30/01-K	*Filobasidium floriforme strain*	98.892	632
n=1; avg=98.9; stdev=0			
53PK 10/7/01-K	*Metschnikowia bicuspidata*	79.545	308
n=1; avg=79.5; stdev=0			
13P 8/30/01-K	*Sporobolomyces marcillae*	92.816	515
15P 8/30/01-K	*Sporobolomyces marcillae*	94.981	259
17P 8/30/01-K	*Sporobolomyces marcillae*	93.458	535
38P 9/23/01-K	*Sporobolomyces marcillae*	91.349	578
n=4; avg=94.15; stdev=1.51			

The variation that did exist between vineyards along the Lake Erie Quality Wine Alliance (Tables 1, 2, 3) was in the less prevalent indigenous yeast species identified in 2001. However there were smaller numbers of isolates of those species so the sequence alignments were not done because only one or two sequences were identified.

Surprisingly, in this study the indigenous yeast species identified were different than the yeast species expected. Some authors state that *Hanseniaspora, Metschnikowia, Candida, Pichia, Hansenula,* and *Zygosaccharomyces* are found more frequently on wine grapes (8, 12) around the world, yet the dominant indigenous yeast species identified in this study were *Rhodotorula* sp. and *A. pullulans*, followed far behind by *Hanseniaspora uvarum*, *Sporidiobolus pararoseus*, and *Metschnikowia bicuspidata*. Also, the identification of *Sporobolomyces marcillae* only within the Klingshirn vineyard was another unexpected outcome. This difference may be due to changes in the micro environments of the vineyards studied.

The rDNA sequences of the indigenous yeast identified have been found to have matching strains when identified through EBI-EMBL and GenBank, but it appears that using the ITS primers to sequence over the conserved regions 18S, 28S, and 5.8S posed a problem when trying to achieve a specific accession number from the EMBL databank. Although other studies have found that the EBI-EMBL nucleotide sequence database is highly effective (2, 10, 26), it was difficult to get a specific species accession number due to the numerous sequences for each species and closely related species, so the top match listed was chosen as the identified indigenous yeast species. This appears to be the problem for identifying *Rhodotorula* sp. beyond the species level for four isolates (Table 3). However, once sequences were aligned with the EBI-EMBL sequences identified for

Rhodotorula glutinis there was not much variability in sequence strain probably due to the presence of the highly conserved regions of rDNA.

Although all the regions showed consistencies in alignment, the 5.8S region showed the most consistent alignment throughout due to it being a very highly conserved region among yeast species, followed closely by the ITS2 variable region. The 18S, 28(6)S, 5.8S, ITS1, and ITS2 regions of those indigenous yeast strain samples with the highest percent match (within each species) from EBI/FASTA sequence identification were compared to those of the yeast species designated by the given EBI accession number from FASTA sequence comparison. For example, sample 2W was identified as *A. pullulans* with a sequence accession number of AF013229. The sequence given by EBI was divided into each of the above regions, if the regions were designated on the EBI website, and compared to the entire sequence of 2W 8/24/01-M, sequenced using primer ITS4, and 2W 8/24/01-M, sequenced using primer ITS5, (App. 3) to determine where each region was located and to better understand where the variability, if any, in the alignments lies. To find the conserved and variable regions, one isolate each for *A. pullulans*, *Rhodotorula sp.*, and *H. uvarum*, were divided up by region (not shown).

Two potential drawbacks in our study were our limited sample size and plate collection methods. Firstly, with more rDNA sequences, more specific alignment patterns could have been seen. Also, closer alignments were found within one year than from year to year possibly due to climate changes and/or different vineyard protection measures such as fungicide application. Secondly, isolation using the agar plate collection methods is highly selective and only permits the growth of a small portion of microbes. The high selectivity likely led to an underestimate of the indigenous species found.

Restriction enzyme analysis and denaturing gradient gel electrophoresis (DGGE) have been found to be very fast and reliable in yeast species identification (5, 17, 18). On the other hand, if the previously mentioned obstacles are overcome, further studies can benefit from sequence analysis of indigenous yeast species as a more thorough and definitive method for identification than previously used protocols for restriction enzyme analysis and DGGE which depend on pattern and/or location for identification.

Further studies could be improved by using larger sample sizes and more encompassing collection methods. In additon, a study could be done to determine how climatic changes (terroir) may affect indigenous yeast species and rDNA sequence variation. Also, a study that focuses on whether or not harmful yeast are identified within a vineyard could be helpful to winemakers. This can determine whether preventative measures against wine grape diseases are dependable inhibitors. Finally, long term studies that take into account the many factors, in addition to those mentioned above, that could help determine the exact role that indigenous yeast play in winery success.

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APPENDEX 1:

DNA Sequencing Protocol and CEQ 2000XL Setup

DNA Sequencing protocol and setup (following purification of DNA after PCR)

First make Stop Solution for Step 3 (stop polymerases):

3M NaOAc	40□l per row
0.5M EDTA	8□l per row
H_2O	32□l per row
Glycogen	20□l per row

Note: On original protocol amounts used differ.
Use 5□l per reaction

1. Pipette 5□l of Stop Solution into each tube.
2. Add DNA from PCR to Stop Solution (20□l solutions)
3. Add 60□l of cold (-20□C freezer) 95% Ethanol/dH20
4. Mix thoroughly
5. Immediately centrifuge 15min for precipitate

Machine Setup:

While centrifuging:

1. Choose rows
2. File/Save (All droplets on screen must turn blue)
3. Make sure setting at bottom right is DTCS-3
4. Save
5. Click on "Running Guy"
6. Direct Control
7. Go to *Unload plate*
8. Click on *unload*
9. Pull out Wittig Tray and fill with sterile water
   note: be careful of time limit

DNA Sequencing (cont.)

6. Remove from centrifuge and pipette out supernatant
7. Add 200□l 70% ethanol to pellets <u>without</u> vortexing
8. Repeat Step 7 twice and centrifuge 3min each

Note: If pellet comes loose then centrifuge again for about 5min so pellet can stick again

9. Speed vac (dry) tubes for 40min
10. Take formamamide out to thaw

Machine setup (cont.)

11. Click on *load/unload* and select *unload*
12. Load buffer
13. Select *load*
14. Click on capillary picture which tells you to load
    a) Remove capillary holder
    b) Remove manifold plug (pull red lever) and pull out
    c) Load capillary
15. Click *Done*
16. Click on *Uninstall Gel*
    a) pull out yellow plug and load acrylomide gel
17. Click on *Direct Control*
18. *Gel capillary fill* will appear and select *Fill*

DNA Sequencing (cont.)

10. Resuspend in 40□l of formamamide
11. Vortex tubes until pellet dissolve and let sit for 5min
12. Pipette out solution and load into tray
13. Add 1 drop of oil over top
14. Check for bubbles on top
15. Select *load/unload*
    note: be careful of time limit
16. Click on *unload*
17. Load tray
18. Click on *Setup Plate*
    a) check
19. Run sample plate
    a) all whites: will run; black: no run
20. Click on *Start*
21. Select DATA Monitor
22. Pick 4 data letters
    a) A is pUC and **it must work** because it is the standard. If pUC has no read than sequencing won't work.
    b) Watch voltage: usually around 10kV

Note: usually takes about 30min to see any readings

Calculations:

$$V = \frac{\text{fmol (DNA bp) (649) } (10^{-6})}{\text{ng DNA}}$$

Sample chart for Sequencing setup using Beckman Coulter Protocol:

Row		**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
		pUC (control)	Sample	Sample	Sample	Sample	Sample	Sample	Sample
1	DNA	0.5							
	Primer	2							
	Premix	12							
	H_2O	5.5							
	Total	20							
2		Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
	DNA								
	Primer								
	Premix								
	H_2O								
	Total								

APPENDIX 2:

ClustalW 1.8 and 1.82 Alignment Procedures

DNA Sequence Search

1. www.ebi.ac.uk/fasta33
2. Paste sequence.
3. Select "Nucleic Acid" DATABASE.
4. Select "FUNGI."
5. Make other selections if needed.
6. Click "Run Fasta3."
7. Matches page should automatically appear.

ITS4 Primer Sequence - Reverse Compliment

1. www.searchlauncher.bcm.tmc.edu
2. Select "Sequence Utilities."
3. Select "Reverse Compliment."
4. Paste Sequence.
5. Click "Submit."

Multiple Sequence Alignments

- Baylor College of Medicine

1. www.searchlauncher.bcm.tmc.edu
2. Select "Multiple Sequence Alignments."
3. Select "Clustal 1.8."
4. Paste Multiple Sequences.
    a) Example:
    >Sequence 1
    AAGGTTAACCG
    >Sequence 2
    AAGGTTATACC
5. Click "Submit."
6. Copy Alignment Data in Fasta Format (Green Box).
7. Click BOXSHADE Link at bottom of page.
8. Paste sequence into BOXSHADE area.
9. Select RTF_new Output format.
10. Select Consensus Line with Symbols.
11. Select "other" for Input Sequence Format.
12. Click "Run BOXSHADE."
13. Click Link to Word file.
14. BOXSHADE alignments should appear on screen.

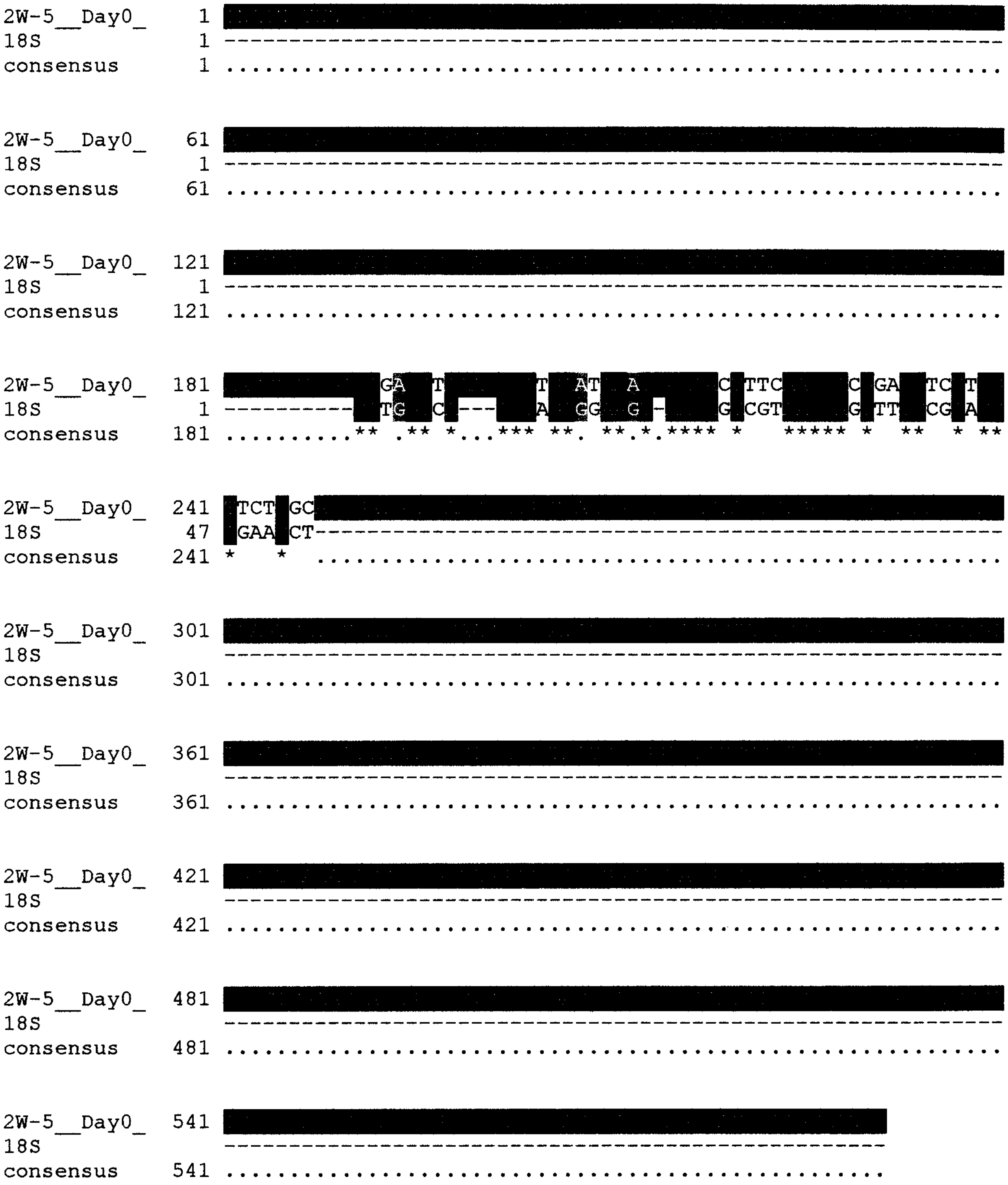
- EMBL-EBI Databank

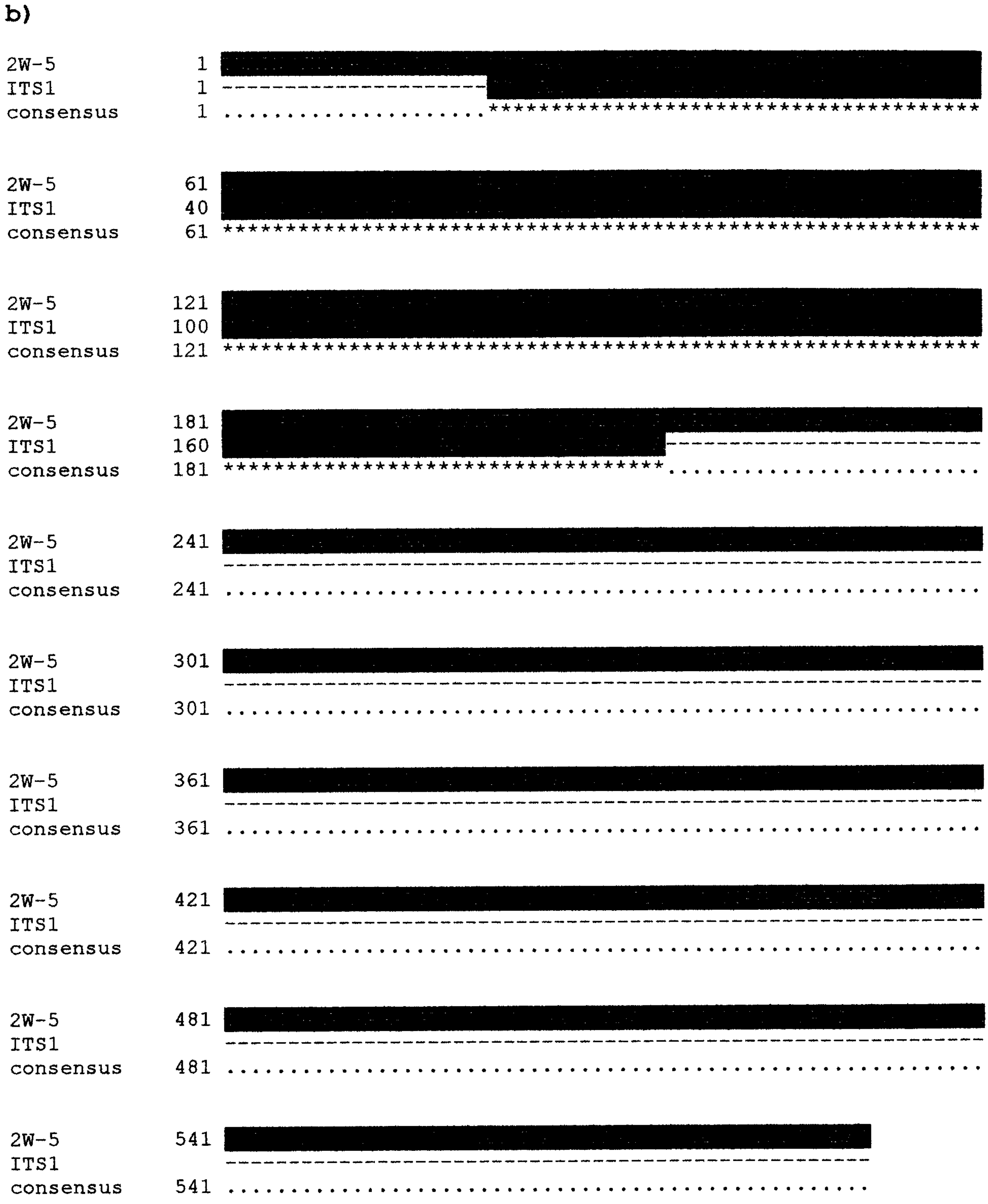
1. www.ebi.ac.uk/clustalw
2. Paste sequence and choose settings.
3. Click "Run."
4. Alignment information page will open automatically.

Appendix 3:

Alignments showing conserved and ITS rDNA regional alignments for *Aureobasidium pullulans*, for isolate 2W_8/24/01-M. Sequencing reactions were with primer ITS5.
a) 18S b) ITS1 c) 5.8S d) ITS2 e) 28S

a)


2W-5__Day0_ 1
18S 1
consensus 1
2W-5__Day0_ 61
18S 1
consensus 61
2W-5__Day0_ 121
18S 1
consensus 121
2W-5__Day0_ 181 GA T T AT A C TTC C GA TC T
18S 1 TG C --- A GG G - G CGT G TT CG A
consensus 181 ....** .** *...*** **. **.*.**** * ***** * ** * **
2W-5__Day0_ 241 TCT GC
18S 47 GAA CT
consensus 241 * *
2W-5__Day0_ 301
18S
consensus 301
2W-5__Day0_ 361
18S
consensus 361
2W-5__Day0_ 421
18S
consensus 421
2W-5__Day0_ 481
18S
consensus 481
2W-5__Day0_ 541
18S
consensus 541


b)
2W-5 1
ITS1 1
consensus 1
2W-5 61
ITS1 40
consensus 61
2W-5 121
ITS1 100
consensus 121
2W-5 181
ITS1 160
consensus 181
2W-5 241
ITS1
consensus 241
2W-5 301
ITS1
consensus 301
2W-5 361
ITS1
consensus 361
2W-5 421
ITS1
consensus 421
2W-5 481
ITS1
consensus 481
2W-5 541
ITS1
consensus 541

c)

```
2W-5          1 [redacted]
5.8S          1 ------------------------------------------------------------
consensus     1 ............................................................

2W-5         61 [redacted]
5.8S          1 ------------------------------------------------------------
consensus    61 ............................................................

2W-5        121 [redacted]
5.8S          1 ------------------------------------------------------------
consensus   121 ............................................................

2W-5        181 [redacted]
5.8S          1 -----------------------------------[redacted]
consensus   181 ...................................*************************

2W-5        241 [redacted]
5.8S         26 [redacted]
consensus   241 ************************************************************

2W-5        301 [redacted]
5.8S         86 [redacted]
consensus   301 ************************************************************

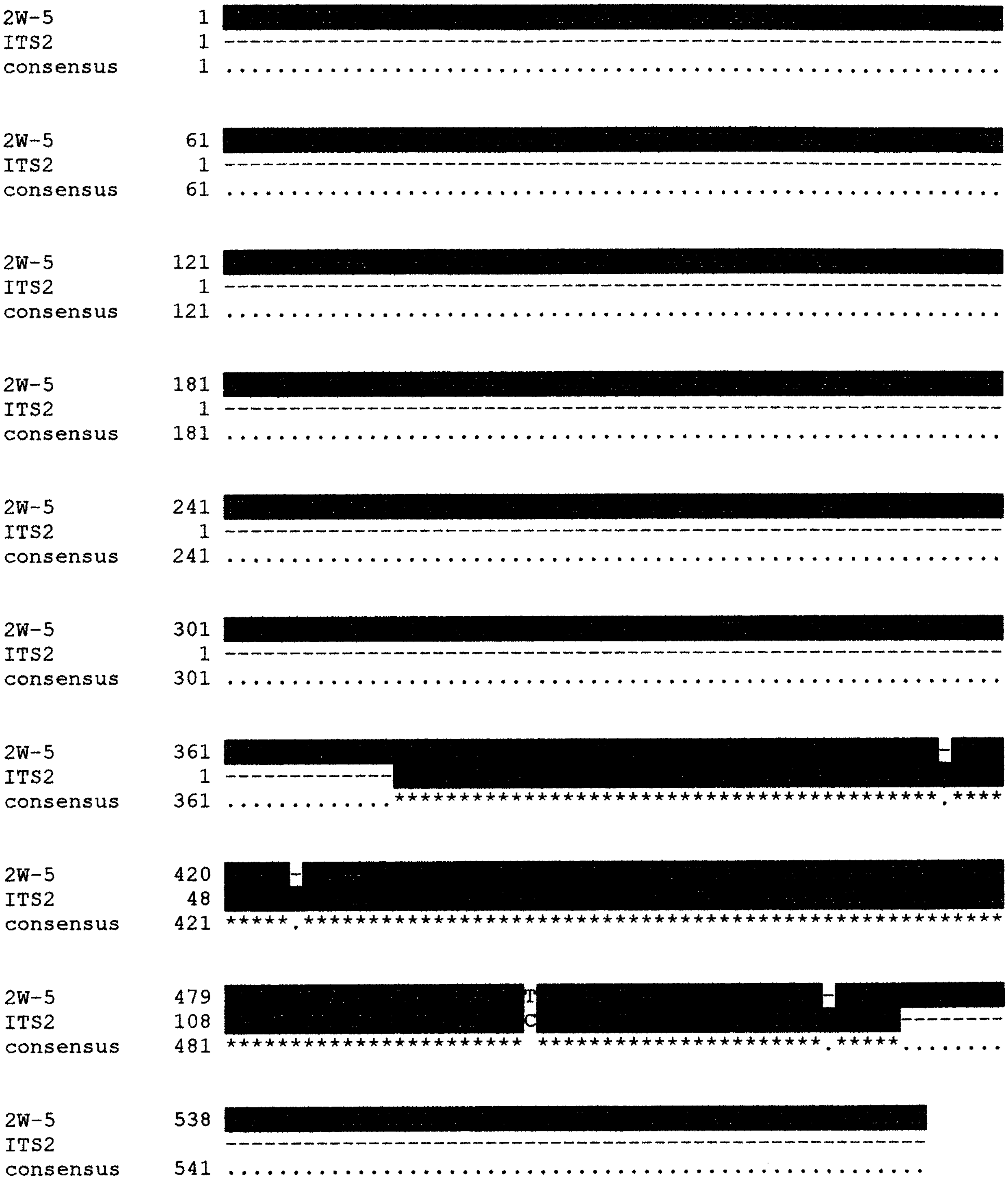
2W-5        361 [redacted]-[redacted]
5.8S        146 [redacted]----------------------------------------------
consensus   361 *.************..............................................

2W-5        420 [redacted]
5.8S            ------------------------------------------------------------
consensus   421 ............................................................

2W-5        480 [redacted]
5.8S            ------------------------------------------------------------
consensus   481 ............................................................

2W-5        540 [redacted]
5.8S            ----------------------------------------------------
consensus   541 ....................................................
```

d)


2W-5 1
ITS2 1
consensus 1
2W-5 61
ITS2 1
consensus 61
2W-5 121
ITS2 1
consensus 121
2W-5 181
ITS2 1
consensus 181
2W-5 241
ITS2 1
consensus 241
2W-5 301
ITS2 1
consensus 301
2W-5 361
ITS2 1
consensus 361
2W-5 420
ITS2 48
consensus 421
2W-5 479
ITS2 108
consensus 481
T
C
2W-5 538
ITS2
consensus 541

e)

```
2W-5      1 [illegible]
28S       1 ------------------------------------------------------------
consensus 1 ............................................................

2W-5      61 [illegible]
28S       1 ------------------------------------------------------------
consensus 61 ............................................................

2W-5      121 [illegible]
28S         1 ------------------------------------------------------------
consensus 121 ............................................................

2W-5      181 [illegible]
28S         1 ------------------------------------------------------------
consensus 181 ............................................................

2W-5      241 [illegible]
28S         1 ------------------------------------------------------------
consensus 241 ............................................................

2W-5      301 [illegible]
28S         1 ------------------------------------------------------------
consensus 301 ............................................................

2W-5      361 [illegible]
28S         1 ------------------------------------------------------------
consensus 361 ............................................................

2W-5      421 [illegible]
28S         1 ------------------------------------------------------------
consensus 421 ............................................................

2W-5      481 [illegible]
28S         1 -------------------------------------------------[illegible]
consensus 481 .................................................***********

2W-5      541 [illegible]AN[illegible]
28S        12 [illegible]---[illegible]GG[illegible]-
consensus 541 ******************************************...**. *.
```

Appendix 4:

Sequence alignment of *Aureobasidium pullulans* strains sampled from Markko vineyard in the 2000 season. Sequencing reactions were done using reverse primer ITS4. DNA sequence alignments using the EMBL ClustalW 1.8 Multiple Sequence Alignment website. Conserved and ITS regions labeled. Dashes represent gaps. Blue circle indicates where ITS1 and 5.8S overlap. Region 28S is not labeled due to a discrepancy in region location.

```
                                                                       18S
                                                  |-------------------------------------------
44W-4__9/20/2000-MN       ----TATC---CTAC--CTG-ATC-C-GAGG--TCT-------A-CCTAG----AAAAA 33
45W-4__9/20/2000-MP       ----TATC---CTAC--CTG-ATC-C-GAGG--TNT-------A-CCTAG----AAAAA 33
24W-4__8/20/2000-MN       ----TATC---CTAC--CTG-ATC-C-GAGG--TCT-------A-CCTAG----AAAAA 33
1W-4_7/27/2000-MB         -----------CTACTTCTG-GGG-C-GAGGTTCAGGTGTGTCATCCTATC---AAAAA 43
53W-4__9/20/2000-MR       -----TATC--CTAC--CTG-ATC-C-GAGG--TCA-------A-CCTAG----AAAAA 33
43W-4__9/20/2000-MC       -------TC--CTAC--CTG-ATC-C-GAGG--TCT-------AACCTAG----AAAAA 32
8W-4__7/27/2000-MC        -----------------CTG-ATC-C-GAGG--TCA-------A-CCTAG----AAAAA 25
23W-4__8/20/2000-MC       -------TG--CTAC--CTGTATCGCTGAGG--TCAG------A-CCTAGAGAGAATAA 39
7W-4__7/27/2000-MC        -TGGTAGTCG-CTAC--CTG-ATCGC-GAGG--TCA-------A-CCTAGT---AAAAA 40
10W-4__7/27/2000-MR       --GGTATCCG-CTAC--CTG-ATCGC-GAGG--TCA-------A-CCTAG----AAAAA 38
4W-4__7/27/2000-MN        ---GTAGTCCGCTAC--CTG-ATCGC-GAGG--TCA-------A-CCTAGT---AAAAA 39
25W-4__8/20/2000-MP       ----TATC---CTAC--CTG-ATC-C-GAGG--TCT-------A-CCTAG----AAAAA 33
32W-4__8/20/2000-MC       ----AGTCG--CTAC--CTG-ATCGC-GAGG--TCA-------A-CCTAGA---GAAAA 36
6W-4__7/27/2000-MP        -TGGTATGC--CTAC--CTG-ATC-C-GAGG--TCT-------AACCTAGA---GAAAA 39
31W-4__8/20/2000-MC       ----TATC---CTAC--CTG-ATC-C-GAGG--TCT-------A-CCTAG----AAAAA 33
30W-4__8/20/2000-MR       ----TATCC--CTAC--CTG-ATC-C-GAGG--TCT-------A-CCTAG----AAAAA 34
22W-4__8/20/2000-MM       ---GTATCC--CTAC--CTG-ATC-C-GAGG--TCT-------A-CCTAG----AAAAA 35
3W-4__7/27/2000-MC        ----TATCC--CTAC--CTG-ATC-C-GAGG--TCT-------A-CCTAG----AAAAA 34
                                           ***     * ****  *         * ****      * **

                                         18S                         ||     ITS1
                          -----------------------------------------------||--------------
44W-4__9/20/2000-MN       TAAA-GGTT-TCA-GT--CGGC-AG-AGT-TCCTCTC-CTTT--GAC----A-GACGT-T 76
45W-4__9/20/2000-MP       TAAA-GGTT-TCA-GT--CGGC-AG-AGT-TCCTCTC-CTTT--GAC----A-GACGT-T 76
24W-4__8/20/2000-MN       TAAA-GGTT-TCA-GT--CGGC-AG-AGT-TCCTCTC-CTTT--GAC----A-GACGT-T 76
1W-4_7/27/2000-MB         TAAAGGTT-TCA-GT--CGGC-AG-A-T-TCCTCTC-CTTT--GAC----A-GACGT-T 86
53W-4__9/20/2000-MR       TAAA-GGTT-TCA-GT--CGGC-AG-AGT-TCCTCTC-CTTT--GAC----A-GACGT-T 76
43W-4__9/20/2000-MC       TAAA-GGTT-TCA-GT--CGGC-AG-AGT-TCCTCTC-CTTT--GAC----A-GACGT-T 75
8W-4__7/27/2000-MC        TAAA-GGTT-TCA-GT--CGGC-AG-AGT-TCCTCTC-CTTT--GAC----A-GACGT-T 68
23W-4__8/20/2000-MC       TAAAGGGTATTCA-GTGGTGCC-AGCAGTCTCCTCTC-CTTCTCGAC----ACGACGTGT 92
7W-4__7/27/2000-MC        TAAA-GGTG-TCA-GT--CGGC-AG-AGT-TCCTCTC-CTTT--GAC----A-GACGTGT 84
10W-4__7/27/2000-MR       TAAA-GGTG-TCA-GT--CGGC-AG-AGT-TCCTCTC-CTTT--GAC----A-GACGTGT 82
4W-4__7/27/2000-MN        TAAA-GGTG-TCA-GT--CGGC-AG-AGT-TCCTCTC-CTTT--GAC----A-GACGTGT 83
25W-4__8/20/2000-MP       TAAA-GGTT-TCA-GT--CGGC-AG-AGT-TCCTCTC-CTTT--GAC----A-GACGTGT 77
32W-4__8/20/2000-MC       TAAA-GGTGATCA-GTG-CGGC-AG-AGT-TCCTCTC-CTTT--GAC----A-GACGTGT 82
6W-4__7/27/2000-MP        TAAA-GGTA-TCAAGTG-CGGC-AGCAGT-TCCTCTC-CTTT--GAC----A-GACGTGT 86
31W-4__8/20/2000-MC       TAAA-GGTT-TCA-GT--CGGC-AG-AGT-TCCTCTC-CTTT--GAC----A-GACGT-T 76
30W-4__8/20/2000-MR       TAAA-GGTT-TCA-GT--CGGC-AG-AGT-TCCTCTC-CTTT--GAC----A-GACGT-T 77
22W-4__8/20/2000-MM       TAAA-GGTT-TCA-GT--CGGC-AG-AGT-TCCTCTC-CTTT--GAC----A-GACGT-T 78
3W-4__7/27/2000-MC        TAAA-GGTT-TCA-GT--CGGC-AG-AGT-TCCTCTC-CTTT--GAC----A-GACGT-T 77
                          **** ***  *** **   * * ** * * ******* ***   ***    * ***** *

                                                   ITS1
                          ------------------------------------------------------------
44W-4__9/20/2000-MN       CGAAT-AAAT-----TCTACTACAGCCTAAAGCCGGAG-TGGCCTCGTCCGAGGTCTTTA 129
45W-4__9/20/2000-MP       CGAAT-AAAT-----TCTACTACNGCCTAAAGCCGGAG-TGGCCTCGTCCGAGGTCTTTA 129
24W-4__8/20/2000-MN       CGAAT-AAAT-----TCTACTAC-GCCTAAAGCCGGAG-TGGCCTCGTCCGAGGTCTTTA 128
1W-4_7/27/2000-MB         CGAAT-AAAT-----TCTACTAC-GCCTAAAGCCGGAG-TGGCCTCG-CCGAGGTCTTTA 137
53W-4__9/20/2000-MR       CGAAT-AAAT-----TCTACTAC-GCCTAAAGCCGGAG-TGGCCTCG-CCGAGGTCTTTA 127
43W-4__9/20/2000-MC       CGAAT-AAAT-----TCTACTAC-GCCTAAAGCCGGAG-TGGCCTCG-CCGAGGTCTTTA 126
8W-4__7/27/2000-MC        CGAAT-AAAT-----TCTACTAC-GCCTAAAGCCGGAG-TGGCCTCG-CCGAGGTCTTTA 119
23W-4__8/20/2000-MC       CGAAT-AAATC----TCTACTAC-GCCTAAAGCCGGAGATGGCCTCG-CCGAGGTCTTTA 145
7W-4__7/27/2000-MC        CGAATGAAATG----TCTACTAC-GCCTAAAGCCGGAG-TGGCCTCG-CCGAGGTCTTTA 137
10W-4__7/27/2000-MR       CGAAT-AAAT-----TCTACTAC-GCCTAAAGCCGGAG-TGGCCTCG-CCGAGGTCTTTA 133
4W-4__7/27/2000-MN        CGAATGAAATG----TCTACTAC-GCCTAAAGCCGGAG-TGGCCTCG-CCGAGGTCTTTA 136
25W-4__8/20/2000-MP       CGAAT-AAAT-----TCTACTAC-GCCTAAAGCCGGAG-TGGCCTCG-CCGAGGTCTTTA 128
32W-4__8/20/2000-MC       CGAAT-AAAT-----TCTACTAC-GCCTAAAGCCGGAG-TGGCCTCG-CCGAGGTCTTTA 133
6W-4__7/27/2000-MP        CGAAT-AAAT-----TCTACTAC-GCCTAAAGCCGGAG-TGGCCTCGGCCGAGGTCTTTA 138
31W-4__8/20/2000-MC       CGAAT-AAAT-----TCTACTAC-GCCTAAAGCCGGAG-TGGCCTCG-CCGAGGTCTTTA 127
30W-4__8/20/2000-MR       CGAAT-AAAT-----TCTACTAC-GCCTAAAGCCGGAG-TGGCCTCG-CCGAGGTCTTTA 128
22W-4__8/20/2000-MM       CGAAT-AAAT-----TCTACTAC-GCCTAAAGCCGGAG-TGGCCTCG-CCGAGGTCTTTA 129
3W-4__7/27/2000-MC        CGAAT-AAAT-----TCTACTAC-GCCTAAAGCCGGAG-TGGCCTCG-CCGAGGTCTTTA 128
                          ***** ****     ******** ************** ******** ************
```

ITS1

```
44W-4__9/20/2000-MN AGGCGC--GCCCAA-CTAAG-GACGA--CGCCC----AAT--AC-C--AAGC-AT----A 169
45W-4__9/20/2000-MP AGGCGC--GCCCAA-CTAAG-GACGA--CGCCC----AAT--AC-C--AAGC-AT----A 169
24W-4__8/20/2000-MN AGGCGC--GCCCAA-CTAAG-GACGA--CGCCC----AAT--AC-C--AAGC-AT----A 168
1W-4_7/27/2000-MB   AGGCGC--GCCCAA-CTAAG-GACGA--CGCCC----AAT--AC-C--AAGC-AT----A 177
53W-4__9/20/2000-MR AGGCGC--GCCCAA-CTAAG-GACGA--CGCCC----AAT--AC-C--AAGC-AT----A 167
43W-4__9/20/2000-MC AGGCGC--GCCCAA-CTAAG-GACGA--CGCCC----AAT--AC-C--AAGC-AT----A 166
8W-4__7/27/2000-MC  AGGCGC--GCCCAA-CTAAG-GACGA--CGCCC----AAT--AC-C--AAGC-AT----A 159
23W-4__8/20/2000-MC AGGCGACTGCCCAA-CTAAGCGACGCACCGCCC----AAT--AA-CCTAAGC-AT----A 192
7W-4__7/27/2000-MC  AGGCGC--GCCCAA-CTAAG-GACGA--CGCCC----AAT--AC-C--AAGC-AT----A 177
10W-4__7/27/2000-MR AGGCGC--GCCCAA-CTAAG-GACGA--CGCCC----AAT--AC-C--AAGC-AT----A 173
4W-4__7/27/2000-MN  AGGCGC--GCCCAA-CTAAG-GACGA--CGCCC----AAT--AC-C--AAGC-ATG---A 177
25W-4__8/20/2000-MP AGGCGC--GCCCAA-CTAAG-GACGA--CGCCC----AAT--AC-C--AAGC-AT----A 168
32W-4__8/20/2000-MC AGGCGC--GCCCAA-CTAAG-GACGA--CGCCC----AAT--AC-C--AAGC-AT----A 173
6W-4__7/27/2000-MP  AGGCGC--GCCCAA-CTAAG-GACGA--CGCCC----AAT--AC-C--AAGC-AT----A 178
31W-4__8/20/2000-MC AGGCGC--GCCCAA-CTAAG-GACGA--CGCCC----AAT--AC-C--AAGC-AT----A 167
30W-4__8/20/2000-MR AGGCGC--GCCCAA-CTAAG-GACGA--CGCCC----AAT--AC-C--AAGC-AT----A 168
22W-4__8/20/2000-MM AGGCGC--GCCCAA-CTAAG-GACGA--CGCCC----AAT--AC-C--AAGC-AT----A 169
3W-4__7/27/2000-MC  AGGCGC--GCCCAA-CTAAG-GACGA--CGCCC----AAT--AC-C--AAGC-AT----A 168
                    *****   ****** ***** ****   *****    ***  *  *  **** **    *
```

ITS1

```
44W-4__9/20/2000-MN GCT-TG-A-GT-GGTGT---A-AT-G-ACGC-TCGAA--CAGG--CATG----CCCCTCG 210
45W-4__9/20/2000-MP GCT-TG-A-GT-GGTGT---A-AT-G-ACGC-TCGAA--CAGG--CATG----CCCCTCG 210
24W-4__8/20/2000-MN GCT-TG-A-GT-GGTGT---A-AT-G-ACGC-TCGAA--CAGG--CATG----CCCCTCG 209
1W-4_7/27/2000-MB   GCT-TG-A-GT-GGTGT---A-AT-G-ACGC-TCGAA--CAGG--CATG----CCCCTCG 218
53W-4__9/20/2000-MR GCT-TG-A-GT-GGTGT---A-AT-G-ACGC-TCGAA--CAGG--CATG----CCCCTCG 208
43W-4__9/20/2000-MC GCT-TG-A-GT-GGTGT---A-AT-G-ACGC-TCGAA--CAGG--CATG----CCCCTCG 207
8W-4__7/27/2000-MC  GCT-TG-A-GT-GGTGT---A-AT-G-ACGC-TCGAA--CAGG--CATG----CCCCTCG 200
23W-4__8/20/2000-MC GCTCTGCA-GT-GGTGT---A-AT-G-ACGC-TCGTAACAGG--CATG----CCCCTCG 237
7W-4__7/27/2000-MC  GCT-TG-A-GT-GGTGT---A-AT-G-ACGC-TCGAA--CAGG--CATG----CCCCTCG 218
10W-4__7/27/2000-MR GCT-TG-A-GT-GGTGT---A-AT-G-ACGC-TCGAA--CAGG--CATG----CCCCTCG 214
4W-4__7/27/2000-MN  GCT-TG-A-GT-GGTGT---A-AT-G-ACGC-TCGAA--CAGG--CATG----CCCCTCG 218
25W-4__8/20/2000-MP GCT-TG-A-GT-GGTGT---A-AT-G-ACGC-TCGAA--CAGG--CATG----CCCCTCG 209
32W-4__8/20/2000-MC GCT-TG-A-GT-GGTGT---A-AT-G-ACGC-TCGAA--CAGG--CATG----CCCCTCG 214
6W-4__7/27/2000-MP  GCT-TG-A-GT-GGTGT---A-AT-G-ACGC-TCGAA--CAGG--CATG----CCCCTCG 219
31W-4__8/20/2000-MC GCT-TG-A-GT-GGTGT---A-AT-G-ACGC-TCGAA--CAGG--CATG----CCCCTCG 208
30W-4__8/20/2000-MR GCT-TG-A-GT-GGTGT---A-AT-G-ACGC-TCGAA--CAGG--CATG----CCCCTCG 209
22W-4__8/20/2000-MM GCT-TG-A-GT-GGTGT---A-AT-G-ACGC-TCGAA--CAGG--CATG----CCCCTCG 210
3W-4__7/27/2000-MC  GCT-TG-A-GT-GGTGT---A-AT-G-ACGC-TCGAA--CAGG--CATG----CCCCTCG 209
                    *** ** * ** *****   * ** * **** *** *   ***  ****    *******
```

ITS1

```
44W-4__9/20/2000-MN G---AATA--C-CAAGGGG---CGCAAT-GTGCG----T-TCAAAG-ATAC-GATGA-TT 252
45W-4__9/20/2000-MP G---AATA--C-CAAGGGG---CGCAAT-GTGCG----T-TCAAAG-ATAC-GATGA-TT 252
24W-4__8/20/2000-MN G---AATA--C-CAAGGGG---CGCAAT-GTGCG----T-TCAAAG-ATAC-GATGA-TT 251
1W-4_7/27/2000-MB   G---AATA--C-CAAGGGG---CGCAAT-GTGCG----T-TCAAAG-ATTC-GATGA-TT 260
53W-4__9/20/2000-MR G---AATA--C-CAAGGGG---CGCAAT-GTGCG----T-TCAAAG-ATTC-GATGA-TT 250
43W-4__9/20/2000-MC G---AATA--C-CAAGGGG---CGCAAT-GTGCG----T-TCAAAG-ATTC-GATGA-TT 249
8W-4__7/27/2000-MC  G---AATA--C-CAAGGGG---CGCAAT-GTGCG----T-TCAAAG-ATTC-GATGA-TT 242
23W-4__8/20/2000-MC A---GATATACTCAAGGGG---CGCAAT-GTGCG----TATCAAAG-ATTC-GATGATTT 284
7W-4__7/27/2000-MC  G---AATA--C-CAAGGGG---CGCAAT-GTGCG----T-TCAAAG-ATTC-GATGA-TT 260
10W-4__7/27/2000-MR G---AATA--C-CAAGGGG---CGCAAT-GTGCG----T-TCAAAG-ATTC-GATGA-TT 256
4W-4__7/27/2000-MN  G---AATA--C-CAAGGGG---CGCAAT-GTGCG----T-TCAAAG-ATTC-GATGA-TT 260
25W-4__8/20/2000-MP G---AATA--C-CAAGGGG---CGCAAT-GTGCG----T-ACAAAG-ATTC-GATGA-TT 251
32W-4__8/20/2000-MC G---AATA--C-CAAGGGG---CGCAAT-GTGCG----TATCAAAG-ATTC-GATGA-TT 257
6W-4__7/27/2000-MP  G---AATA--C-CAAGGGG---CGCAAT-GTGCG----T-ACAAAG-ATTC-GATGA-TT 261
31W-4__8/20/2000-MC G---AATA--C-CAAGGGG---CGCAAT-GTGCG----T-TCAAAG-ATTC-GATGA-TT 250
30W-4__8/20/2000-MR G---AATA--C-CAAGGGG---CGCAAT-GTGCG----T-TCAAAG-ATTC-GATGA-TT 251
22W-4__8/20/2000-MM G---AATA--C-CAAGGGG---CGCAAT-GTGCG----T-TCAAAG-ATTC-GATGA-TT 252
3W-4__7/27/2000-MC  G---AATA--C-CAAGGGG---CGCAAT-GTGCG----T-TCAAAG-ATTC-GATGA-TT 251
                         ***  * *******   ****** *****    *  ***** ** * ***** **
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                        ITS1
                                       5.8S
44W-4__9/20/2000-MN   C-ACTG-AATTCT--GCA-ATTC--ACATT-----ACTTATCG--CATTT----CGCTG- 293
45W-4__9/20/2000-MP   C-ACTG-AATTCT--GCA-ATTC--ACATT-----ACTTATCG--CATTT----CGCTG- 293
24W-4__8/20/2000-MN   C-ACTG-AATTCT--GCA-ATGC--ACATT-----ACTTATCG--CATTT----CGCTG- 292
1W-4_7/27/2000-MB     C-ACTG-AATTCT--GCA-ATTC--ACATT-----ACTTATCG--CATTT----CGCTG- 301
53W-4__9/20/2000-MR   C-ACTG-AATTCT--GCA-ATTC--ACATT-----ACTTATCG--CATTT----CGCTG- 291
43W-4__9/20/2000-MC   C-ACTG-AATTCT--GCA-ATTC--ACATT-----ACTTATCG--CATTT----CGCTG- 290
8W-4__7/27/2000-MC    C-ACTG-AATTCT--GCA-ATTC--ACATT-----ACTTATCG--CATTT----CGCTG- 283
23W-4__8/20/2000-MC   C-ACTG-AATTCT--GCC-ATTC--ACATT-----ACTTATCG--CATTT----CGCTG- 325
7W-4__7/27/2000-MC    C-ACTG-AATTCT--GCA-ATTC--ACATT-----ACTTATCG--CATTT----CGCTG- 301
10W-4__7/27/2000-MR   C-ACTG-AATTCT--GCA-ATTC--ACATT-----ACTTATCG--CATTT----CGCTG- 297
4W-4__7/27/2000-MN    C-ACTG-AATTCT--GCA-ATTC--ACATT-----ACTTATCG--CATTT----CGCTG- 301
25W-4__8/20/2000-MP   C-ACTG-AATTCT--GCA-ATTC--ACATT-----ACTTATCG--CATTT----CGCTG- 292
32W-4__8/20/2000-MC   C-ACTG-AATTCT--GCA-ATTC--ACATT-----ACTTATCG--CATTT----CGCTG- 298
6W-4__7/27/2000-MP    C-ACTG-AATTCT--GCA-ATTC--ACATT-----ACTTATCG--CATTT----CGCTG- 302
31W-4__8/20/2000-MC   C-ACTG-AATTCT--GCA-ATTC--ACATT-----ACTTATCG--CATTT----CGCTG- 291
30W-4__8/20/2000-MR   C-ACTG-AATTCT--GCA-ATTC--ACATT-----ACTTATCG--CATTT----CGCTG- 292
22W-4__8/20/2000-MM   C-ACTG-AATTCT--GCA-ATTC--ACATT-----ACTTATCG--CATTT----CGCTG- 293
3W-4__7/27/2000-MC    C-ACTG-AATTCT--GCA-ATTC--ACATT-----ACTTATCG--CATTT----CGCTG- 292
                      * **** ******  **  ** *  *****     ********  *****    *****

                                       5.8S
44W-4__9/20/2000-MN   --CGTT----CTT-CAT---CGA-----TGCGA----GAACC-------AAGAGATCCG- 326
45W-4__9/20/2000-MP   --CGTT----CTT-CAT---CGA-----TGCGA----GAACC-------AAGAGATCCG- 326
24W-4__8/20/2000-MN   --CGTAA---CTT-CAT---CGA-----TGCGA----GAACC-------AAGAGATCCG- 326
1W-4_7/27/2000-MB     --CGTT----CTT-CAT---CGA-----TGCGA----GAACC-------AAGAGATCCG- 334
53W-4__9/20/2000-MR   --CGTT----CTT-CAT---CGA-----TGCGA----GAACC-------AAGAGATCCG- 324
43W-4__9/20/2000-MC   --CGTT----CTT-CAT---CGA-----TGCGA----GAACC-------AAGAGATCCG- 323
8W-4__7/27/2000-MC    --CGTT----CTT-CAT---CGA-----TGCGA----GAACC-------AAGAGATCCG- 316
23W-4__8/20/2000-MC   --CGTTA---CTT-CAT---CGA-----TGCGAT---GAACC-------TAGAGATCCG- 360
7W-4__7/27/2000-MC    --CGTT----CTT-CAT---CGA-----TGCGA----GAACC-------AAGAGATCCG- 334
10W-4__7/27/2000-MR   --CGTT----CTT-CAT---CGA-----TGCGA----GAACT-------A-GAGATCCG- 329
4W-4__7/27/2000-MN    --CGTT----CTT-CAT---CGA-----TGCGA----GAACC-------AAGAGATCCG- 334
25W-4__8/20/2000-MP   --CGTT----CTT-CAT---CGA-----TGCGA----GAACC-------AAGAGATCCG- 325
32W-4__8/20/2000-MC   --CGTT----CTT-CAT---CGA-----TGCGA----GAACC-------AAGAGATCCG- 331
6W-4__7/27/2000-MP    --CGTT----CTT-CAT---CGA-----TGCGA----GAACC-------AAGAGATCCG- 335
31W-4__8/20/2000-MC   --CGTT----CTT-CAT---CGA-----TGCGA----GAACC-------AAGAGATCCG- 324
30W-4__8/20/2000-MR   --CGTT----CTT-CAT---CGA-----TGCGA----GAACC-------AAGAGATCCG- 325
22W-4__8/20/2000-MM   --CGTT----CTT-CAT---CGA-----TGCGA----GAACC-------AAGAGATCCG- 326
3W-4__7/27/2000-MC    --CGTT----CTT-CAT---CGA-----TGCGA----GAACC-------AAGAGATCCG- 325
                        ***     *** ***   ***     *****    ****          ********

                                       5.8S
44W-4__9/20/2000-MN   -TT--GTTGATAA-GT-GTTG-ATTT-ATTC--AAAATT-T-TA--ACTC----AGACGA 369
45W-4__9/20/2000-MP   -TT--GTTGANAA-GT-GTTG-ATTT-ATTC--AAAATT-T-TA--ACTC----AGACGA 369
24W-4__8/20/2000-MN   -TT--GTCGA-AA-GT-CTTG-ATTTTATTC--AAAATT-T-TA--ACTC----AGACGA 369
1W-4_7/27/2000-MB     -TT--GTTGA-AA-GT-TTTG-ATTT-ATTC--AAAATT-T-TA--ACTC----AGACGA 376
53W-4__9/20/2000-MR   -TT--GTTGA-AA-GT-TTTG-ATTT-ATTC--AAAATT-T-TA--ACTC----AGACGA 366
43W-4__9/20/2000-MC   -TT--GTTGA-AA-GT-TTTG-ATTT-ATTC--AAAATT-T-TA--ACTC----AGACGA 365
8W-4__7/27/2000-MC    -TT--GTTGA-AA-GT-TTTG-ATTT-ATTC--AAAATT-T-TA--ACTC----AGACGA 358
23W-4__8/20/2000-MC   -CTTCGTTGAAAC-GT-CTTTGATTTATTTC--AAAATGGTCTAA-CCTC----AGACGA 410
7W-4__7/27/2000-MC    -TT--GTTGA-AA-GT-TTTG-ATTT-ATTC--AAAATT-T-TA--ACTC----AGACGA 376
10W-4__7/27/2000-MR   -TT--GTTGA-AA-GT-CTTG-ATTA---TC--AAAATG-T--A--ACTC----AGACGA 368
4W-4__7/27/2000-MN    -TT--GTTGA-AA-GT-TTTG-ATTT-ATTC--AAAATT-T-TA--ACTC----AGACGA 376
25W-4__8/20/2000-MP   -TT--GTTGA-AA-GT-TTTG-ATTT-ATTC--AAAATT-T-TA--ACTC----AGACGA 367
32W-4__8/20/2000-MC   -TT--GTTGAAAC-GT-TTTG-ATTT-ATTC--AAAATT-TCTAT-ACTC----AGACGA 376
6W-4__7/27/2000-MP    -TT--GTTGAAAC-GT-TTTG-ATTT-ATTC--AAAATT-TCTAT-ACTC----AGACGA 380
31W-4__8/20/2000-MC   -TT--GTTGA-AA-GT-GTTG-ATTT-ATTC--AAAATT-T-TA--ACTC----AGACGA 366
30W-4__8/20/2000-MR   -TT--GTTGA-AA-GT-CTTG-ATTT-ATTC--AAAATT-T-TA--ACTC----AGACGA 367
22W-4__8/20/2000-MM   -TT--GTTGA-GA-GT-GTTG-ATTT-ATTC--AAAATT-T-TA--ACTC----AGACGA 368
3W-4__7/27/2000-MC    -TT--GTTGA-AA-GT-TTTG-ATTT-ATTC--AAAATT-T-TA--ACTC----AGACGA 367
                        *  ** **    **  **  ***    **  *****  *  *   ***    ******
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                                        5.8S                 |         ITS2
44W-4__9/20/2000-MN  CCGG--TTAAA--TAGCAA-GAGTTT----GGTT-TAACTCTG-GCGGGCGCTCGCC-TG 417
45W-4__9/20/2000-MP  CCGG--TTAAA--TANCAA-GAGTTT----GGTT-TAACTCTG-GCGGGCGCTCGCC-TG 417
24W-4__8/20/2000-MN  CCGG--TTAAA--TAACAT-GAGTCT----GGTT-TAACTCTG-GCGGGCGCTCGCC-TG 417
1W-4_7/27/2000-MB    CCGG--TTAAA--TAACAA-GAGTTT----GGTT-TAACTCTG-GCGGGCGCTCGCC-TG 424
53W-4__9/20/2000-MR  CCGG--TTAAA--TAACAA-GAGTTT----GGTT-TAACTCTG-GCGGGCGCTCGCC-TG 414
43W-4__9/20/2000-MC  CCGG--TTAAA--TAACAA-GAGTTT----GGTT-TAACTCTG-GCGGGCGCTCGCC-TG 413
8W-4__7/27/2000-MC   CCGG--TTAAA--TAACAA-GAGTTT----GGTT-TAACTCTG-GCGGGCGCTCGCC-TG 406
23W-4__8/20/2000-MC  CCGG--TTAAA--TAACAA-GAGTTCT---GGTTCTAACTCTGTGCGGGCTCTCGCC-TG 461
7W-4__7/27/2000-MC   CCGG--TTAAA--TAACAA-GAGTTT----GGTT-TAACTCTG-GCGGGCGCTCGCC-TG 424
10W-4__7/27/2000-MR  CCGG--TTAA---TAACA--GAGTTC----GGTC--AGCTCTG-GCGG-CGCT-GCC-TG 411
4W-4__7/27/2000-MN   CCGG--TTAAA--TAACAA-GAGTTT----GGTT-TAACTCTG-GCGGGCGCTCGCC-TG 424
25W-4__8/20/2000-MP  CCGG--TTAAA--TAACAA-GAGTTT----GGTT-TAACTCTG-GCGGGCGCTCGCC-TG 415
32W-4__8/20/2000-MC  CCGG--TTAAA--TAACAA-GAGTTTC---GGTT-TAACTCTG-GCGGGCGCTCGCC-TG 425
6W-4__7/27/2000-MP   CCGG--TTAAA--TAACAA-GAGTGT----GGTT-TAACTCTG-GCGGGCGCTCGCC-TG 428
31W-4__8/20/2000-MC  CCGG--TTAAA--TAACAA-GAGTTT----GGTT-TAACTCTG-GCGGGCGCTCGCC-TG 414
30W-4__8/20/2000-MR  CCGG--TTAAA--TAACAA-GAGTCT----GGTT-TAACTCTG-GCGGGCGCTCGCC-TG 415
22W-4__8/20/2000-MM  CCGG--TTAAA--TAACAA-GAGTTT----GGTT--AGCTCTG-GCGGGCGCTCGCC-TG 415
3W-4__7/27/2000-MC   CCGG--TTAAA--TAACAA-GAGTTT----GGTT-TAACTCTG-GCGGGCGCTCGCC-GG 415
                     ****  ****   ** **  ****      ***   * ***** **** * ** ***  *

                                                  ITS2
44W-4__9/20/2000-MN  GGACGAATCCCCAGCGGC-TCGAGACCGAGCGGTTCCGCCAAAGCAACATG-GTAGTATT 475
45W-4__9/20/2000-MP  GGACGAATCCCCAGCGGC-TCGAGACCGAGCGGTNCCGCCAAAGCANCATN-GTAGTATT 475
24W-4__8/20/2000-MN  GGACGAATCCCCAGCCGC-TCGAGACCGAGCGGTCCCGCCAAAGC-ACACG-GTAGTATT 474
1W-4_7/27/2000-MB    GGACGAATCCCCAGCGGC-TCGAGACCGAGCGGTCCCGCCAAAGCAACAAG-GTAGTTTT 482
53W-4__9/20/2000-MR  GGACGAATCCCCAGCGGC-TCGAGACCGAGCGGTCCCGCCCAAGCCACAAG-GTAGTTTT 472
43W-4__9/20/2000-MC  GGACGAATCCCCAGCGGC-TCGAGACCGAGCGGTCCCGCCAAAGCAACAAG-GTAGTTTT 471
8W-4__7/27/2000-MC   GGACGAATCCCCAGCGGC-TCGAGACCGAGCGGTCCCGCCAAAGCAACAAT-GTAGTTTT 464
23W-4__8/20/2000-MC  GGACGAATCCCCAGCGGCATCGAGACCGAGCGGTCCCGCCAAAGCAGCAAG-GTAG-ATT 519
7W-4__7/27/2000-MC   GGACGAATCCCCAGCGGC-TCGAGACCGAGCGGTCCCGCCAAAGCAACAAT-GTAGTATT 482
10W-4__7/27/2000-MR  G-ACGACTCCC-AGC-GC-T-GAGAC-GAGCG-T-CCGCC-AAGC-ACAT--GTAG-AT- 457
4W-4__7/27/2000-MN   GGACGAATCCCCAGCGGC-TCGAGACCGAGCGGTCCCGCCAAAGCAACAAT-GTAGTATT 482
25W-4__8/20/2000-MP  GGACGAATCCCCAGCGGC-TCGAGACCGAGCGGTCCCGCCAAAGCAACAAT-GTAGTATT 473
32W-4__8/20/2000-MC  GGACGACTCCCCAGCGGC-TCGAGACCGAGCGGTCCCGCCAAAGCAACAATGGTAGTAAT 484
6W-4__7/27/2000-MP   GGACGACTCCCCAGCGGC-TCGAGACCGAGCGGTCCCGCCAAAGCAACAATGGTAGTATT 487
31W-4__8/20/2000-MC  GGACGAATCCCCAGCGGC-TCGAGACCGAGCGGTCCCGCCAAAGCAACAAG-GTAGTATT 472
30W-4__8/20/2000-MR  GGACGAATCCCCAGCGGC-TCGAGACCGAGCGGTCCCGCCAAAGCAACAAG-GTAGTTTT 473
22W-4__8/20/2000-MM  GGACGAATCCC-AGCGGC-TCGAGACCGAGCGGT-CCGCCAAAGC-ACAG--GTAGTAT- 468
3W-4__7/27/2000-MC   GGACGA-TCCCCAGCGGC-T-GAGAC-GAGCG--TCCGCC-AAGC-ACAG-----GTGTT 462
                     * **** **** *** ** * ***** *****   ***** ****  **      *

                                                  ITS2
44W-4__9/20/2000-MN  -AACAACGA-----GGTAGG-AC-GTCGGG-CGCTGAGCACCG-TTACTCATTTAATGAT 525
45W-4__9/20/2000-MP  -AACAACGA-----NNNAGG-AN-GTCNGG-CGCTGAGCACCN-TTACTCNTTTAATGAT 525
24W-4__8/20/2000-MN  -AGC-ACAGA---GGGTAGG-AG-GTCTGG-CGCTGAGCACCT-TTACGC-TGGAATGAT 524
1W-4_7/27/2000-MB    -AACAACAAA---GGGTTGG-AG-GTCGGG-CGCTGAGCACCC-TTACTC-TTTAATGAT 533
53W-4__9/20/2000-MR  -AGCAACAAA---GGGTTGG-AG-GTCGGG-CGCTGAGCACCC-T-ACTC-TT-AATGAT 521
43W-4__9/20/2000-MC  -AACAACAAA---GGGTTGG-AG-GTCGGG-CGCTGAGCACCC-TTACTC-TTTAATGAT 522
8W-4__7/27/2000-MC   -AACAACAAA---GGGTTGG-AG-GTCGGG-CGCTGAGCACCC-TTACTC-TTTAATGAT 515
23W-4__8/20/2000-MC  TAACAACAAA---GGGTTGG-AG-GTCAGG-CGATGTGCACCC-TTACTCTTTAATTGAT 572
7W-4__7/27/2000-MC   -AACAACAAA---GGGTTGG-AG-GTCGGG-CGCTGAGCACCC-TTACTC-TTTAATGAT 533
10W-4__7/27/2000-MR  -AAC-ACGAC----GGTTG--AG-GTCGG--AGCTGAGCACCC--TACTC-TTTAATGAT 503
4W-4__7/27/2000-MN   -AACAACAAA---GGGTTGG-AG-GTCGGG-CGCTGAGCACCC-TTACTC-TTTAATGAT 533
25W-4__8/20/2000-MP  -AACAACAAA---GGGTTGG-AG-GTCGGG-CGCTGAGCACCC-TTACTC-TTTAATGAT 524
32W-4__8/20/2000-MC  TAACAACAAAC--GGGTTGGGAGGGTCGAGGCGCTGAGCAGCCCTTACTC-TTTAATGAT 541
6W-4__7/27/2000-MP   AAATAACGAAC--GGGTTGG-AG-GTCGAGACGCTGAGCACCCCTTACTC-TTCAATGAT 542
31W-4__8/20/2000-MC  -AACAACAAA---GGGTTGG-AG-GTCGGG-CGCTGAGCACCC-TTACTC-TTTAATGAT 523
30W-4__8/20/2000-MR  -AGCGACAGA---GGGGTGGCAC-GTCGGG-CGCTGAGCACCC-TTACTC-TTTACTGAT 525
22W-4__8/20/2000-MM  -AAC-ACGA-----GGGTGG-AG-GTCGG--CGCTGAGCACCC-T-ACTC--TGAATGAT 513
3W-4__7/27/2000-MC   -TAC-ACAA-----GGTTGA----GTCGCC-GGC--ACCATCT-TATATC-TCCAGTCA- 505
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                                   ITS2
44W-4__9/20/2000-MN    GCATGACGCA-CGGACACCATACGGAACTCTTT-GCTTACGACGTAGTAGCTAGCAA--- 580
45W-4__9/20/2000-MP    GCATGACGCA-CGNACANCNTACNGNANNCNTT-GCTNACGACGTANTANCTNGNAAACC 583
24W-4__8/20/2000-MN    GCTTGCCGCA-GGTACACC-TACGGAATACCCT-GTTTACGACATAT--ACTAGGCA--- 576
1W-4_7/27/2000-MB      CCTT-CCGCA-GGTTCACC-TACGGAA-ACCT--GTTTACGACTTTT--ACTGGCAACAC 585
53W-4__9/20/2000-MR    -CTT-CCGCA-GGTTCACC-TAC-GAA-ACCT---GTTACGACTTA---ACTT------- 562
43W-4__9/20/2000-MC    CCTT-CCGCA-GGTTCACC-TACGGAA-ACCT---GTTACGACTTT---ACGAAAAAA-- 570
8W-4__7/27/2000-MC     CCTT-CCGCA-GGTTCACC-TACGGGA-ACCCT-GGTTACGACTTT---ATTAGCAA-AT 566
23W-4__8/20/2000-MC    CCTT-CCGCA-TGTTCACC-TACGGAA-ACCTT-GATTACGAACTTTTACTTAGTCA-AT 626
7W-4__7/27/2000-MC     CCTT-CCGCA-GGTTCACC-TACTGAA-CCCT---GTTACGACTTC------TTGTGGAA 580
10W-4__7/27/2000-MR    TCCT-CCGCAGGGTTCACC-TACGGAAGACCT--GGTTACGACTTTT----ACTGTACAA 555
4W-4__7/27/2000-MN     CCTT-CCGCA-TGTACACC-TACTGAG-ACCT---GTTACGACTAT------ATGTA-AA 579
25W-4__8/20/2000-MP    CCTT-CCGCA-TGTACACC-TACTGAG-ACCT---GTTACGACTGT----ATTTACATAT 573
32W-4__8/20/2000-MC    TCTT-CCGCA-TGTACACC-TACTGAG-ACCT---GATACGACTGT----ACTGTCATAT 590
6W-4__7/27/2000-MP     TCTTCCCGCA-TGTACACC-TACTGAAGTCCT--GGTTACGACTTTT---ACTTGCATAT 595
31W-4__8/20/2000-MC    GCCT-CCGCA-GGTTCACC-TACGGAA-ACCT---GTTACGACTAT------ATTGACAA 570
30W-4__8/20/2000-MR    -CTT-CCGCA-GGTACACC-TACTGAA-CCAT---GTTACGACATTT---ACTTGAAAAA 574
22W-4__8/20/2000-MM     CCT--CCGCA-GGTTCACC-TACGGGAACCCT---GGTACGACTTTT--ACTTGGCAAAA
3W-4__7/27/2000-MC     -C---ACGAACTGTAGACT-TATGCAACAAATGGGGGCCCGGCCGGGGGACGGGGCAGAC 560
                        *    ** *  *   *   **                 **


44W-4__9/20/2000-MN    -----------------------------------------------
45W-4__9/20/2000-MP    CCCTTTGCGGGGNNC-------------------------------- 598
24W-4__8/20/2000-MN    -----------------------------------------------
1W-4_7/27/2000-MB      TAGCCTGCCGGCC---------------------------------- 598
53W-4__9/20/2000-MR    -----------------------------------------------
43W-4__9/20/2000-MC    -----------------------------------------------
8W-4__7/27/2000-MC     --TTCTAT-ATGCGG-------------------------------- 578
23W-4__8/20/2000-MC    CATTCTATTACGCAGGGAAAGAGAAAAAAAAAAAAAATTAAAAAAA--- 672
7W-4__7/27/2000-MC     ATTTA-TAAAGCGAG-------------------------------- 594
10W-4__7/27/2000-MR    TTTAA-GTAAGCGAGGA------------------------------ 571
4W-4__7/27/2000-MN     TTTTATGTATGCGGAAAA----------------------------- 597
25W-4__8/20/2000-MP    CAATA-GCGAAGGAAA------------------------------- 588
32W-4__8/20/2000-MC    CATGA-GCGAGGAAAAA------------------------------ 606
6W-4__7/27/2000-MP     CACTATGCGGAGAAA-------------------------------- 610
31W-4__8/20/2000-MC    -----------------------------------------------
30W-4__8/20/2000-MR    -----------------------------------------------
22W-4__8/20/2000-MM    -----------------------------------------------
3W-4__7/27/2000-MC     CAAGGGGACAGGGGGGACCAAGCCGGGCAACAGGGACG--------- 598
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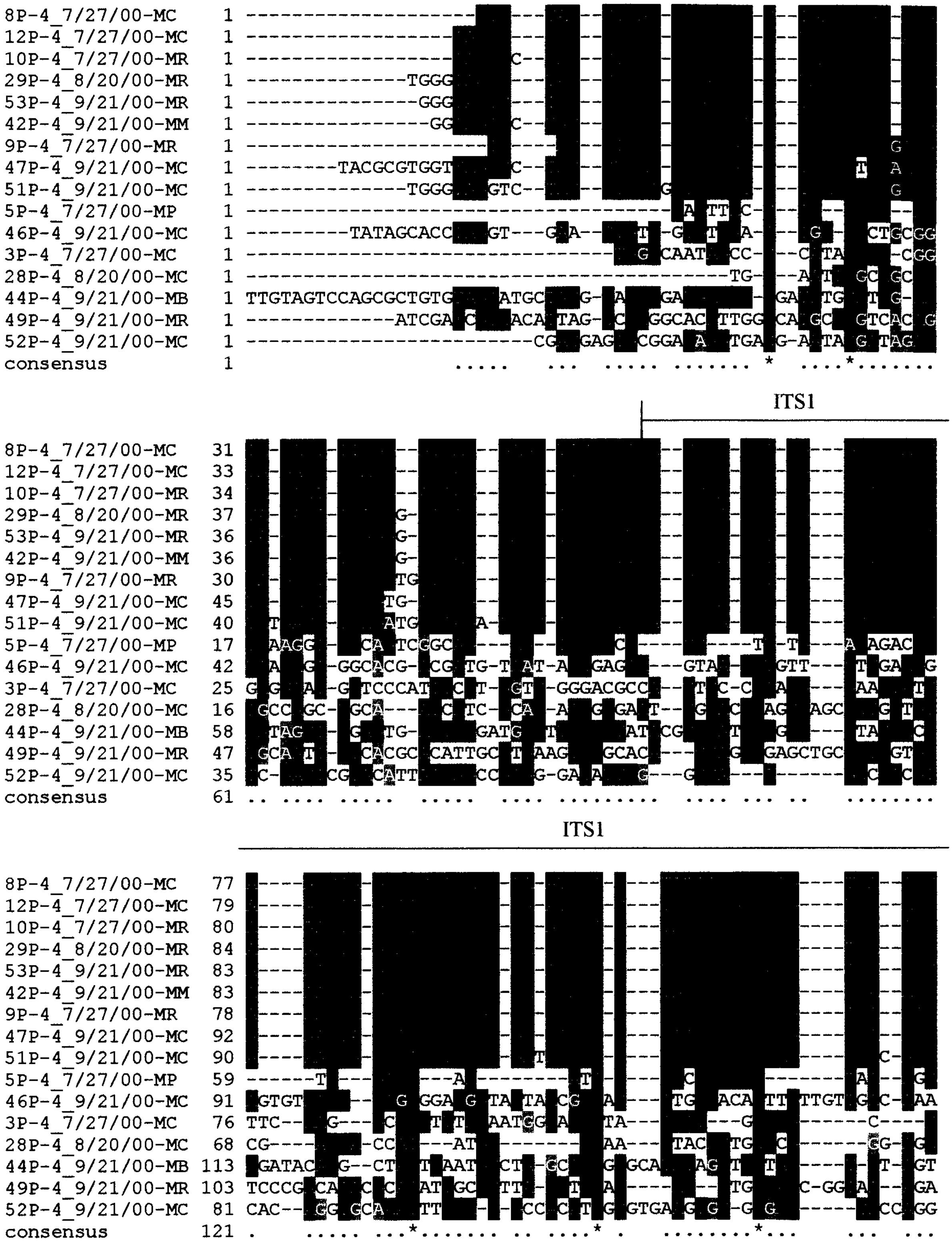
Appendix 5:

Sequence alignment of *Rhodotorula glutinis* strains sampled from Markko vineyard in the 2000 season. Sequencing reactions were done using reverse primer ITS4. DNA sequence alignments done using the Baylor College of Medicine Clustal 1.8 Multiple Sequence Alignment website. Conserved and ITS regions labeled. Dashes represent gaps. (18S and 26S are not represented due to error in alignment.)


ITS1
ITS1
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12P-4_7/27/00-MC
10P-4_7/27/00-MR
29P-4_8/20/00-MR
53P-4_9/21/00-MR
42P-4_9/21/00-MM
9P-4_7/27/00-MR
47P-4_9/21/00-MC
51P-4_9/21/00-MC
5P-4_7/27/00-MP
46P-4_9/21/00-MC
3P-4_7/27/00-MC
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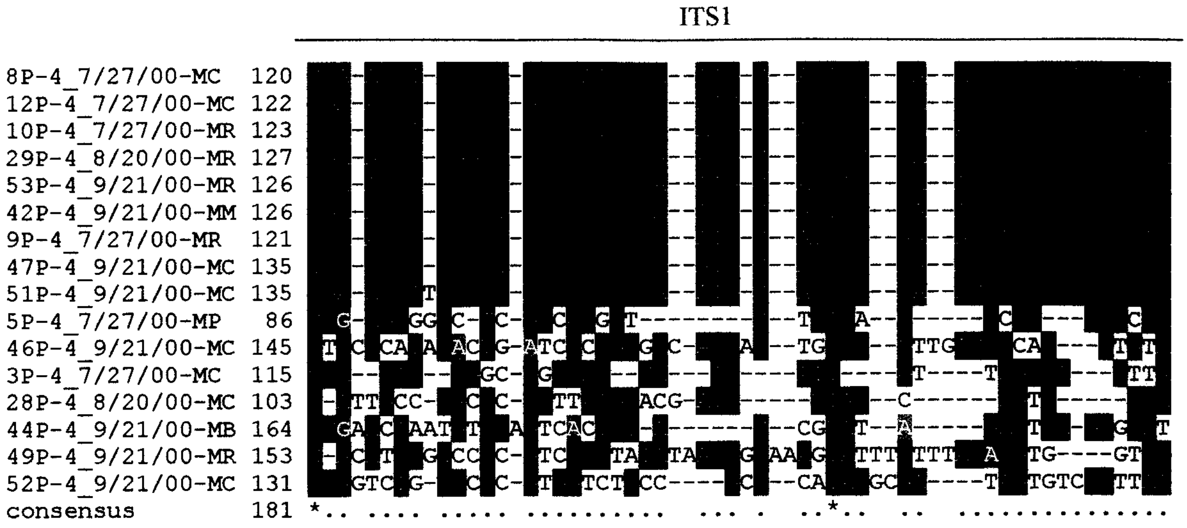
52P-4_9/21/00-MC
consensus


ITS1
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29P-4_8/20/00-MR 127
53P-4_9/21/00-MR 126
42P-4_9/21/00-MM 126
9P-4_7/27/00-MR 121
47P-4_9/21/00-MC 135
51P-4_9/21/00-MC 135
5P-4_7/27/00-MP 86
46P-4_9/21/00-MC 145
3P-4_7/27/00-MC 115
28P-4_8/20/00-MC 103
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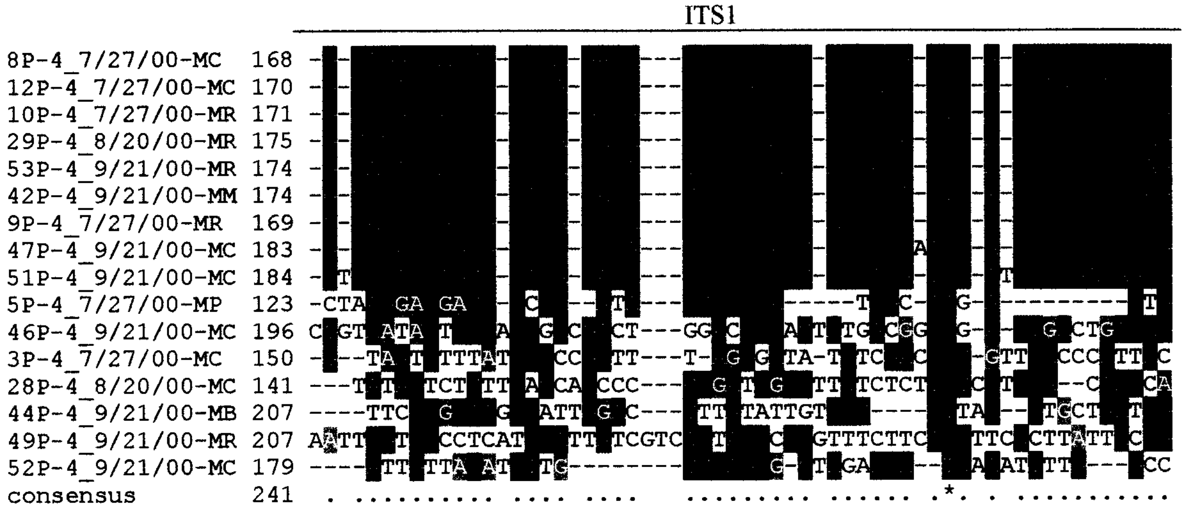
consensus 181


ITS1
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10P-4_7/27/00-MR 171
29P-4_8/20/00-MR 175
53P-4_9/21/00-MR 174
42P-4_9/21/00-MM 174
9P-4_7/27/00-MR 169
47P-4_9/21/00-MC 183
51P-4_9/21/00-MC 184
5P-4_7/27/00-MP 123
46P-4_9/21/00-MC 196
3P-4_7/27/00-MC 150
28P-4_8/20/00-MC 141
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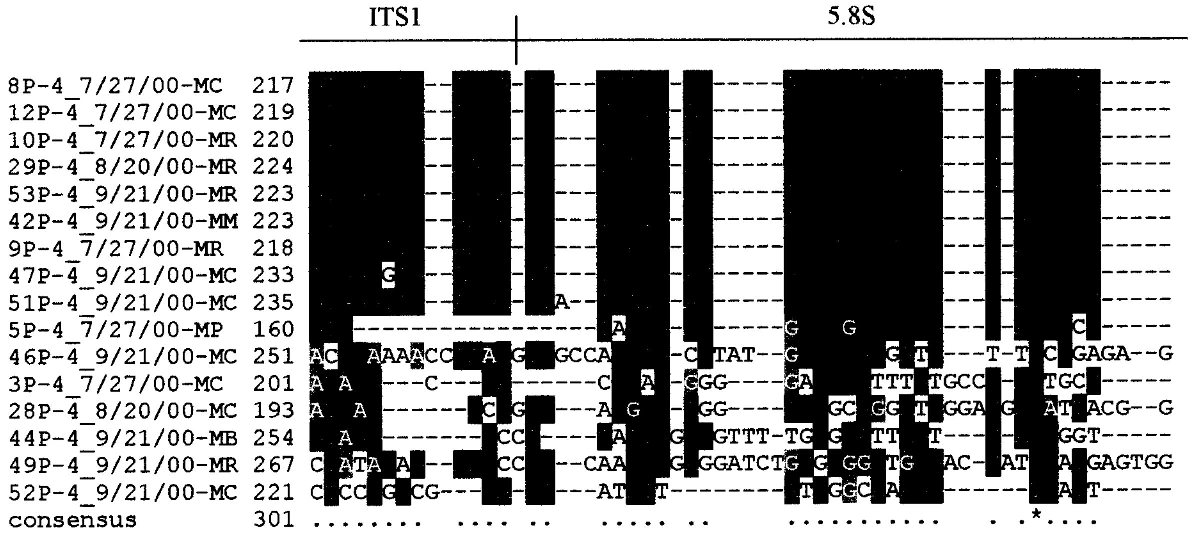
consensus 241


ITS1
5.8S
8P-4_7/27/00-MC 217
12P-4_7/27/00-MC 219
10P-4_7/27/00-MR 220
29P-4_8/20/00-MR 224
53P-4_9/21/00-MR 223
42P-4_9/21/00-MM 223
9P-4_7/27/00-MR 218
47P-4_9/21/00-MC 233
51P-4_9/21/00-MC 235
5P-4_7/27/00-MP 160
46P-4_9/21/00-MC 251
3P-4_7/27/00-MC 201
28P-4_8/20/00-MC 193
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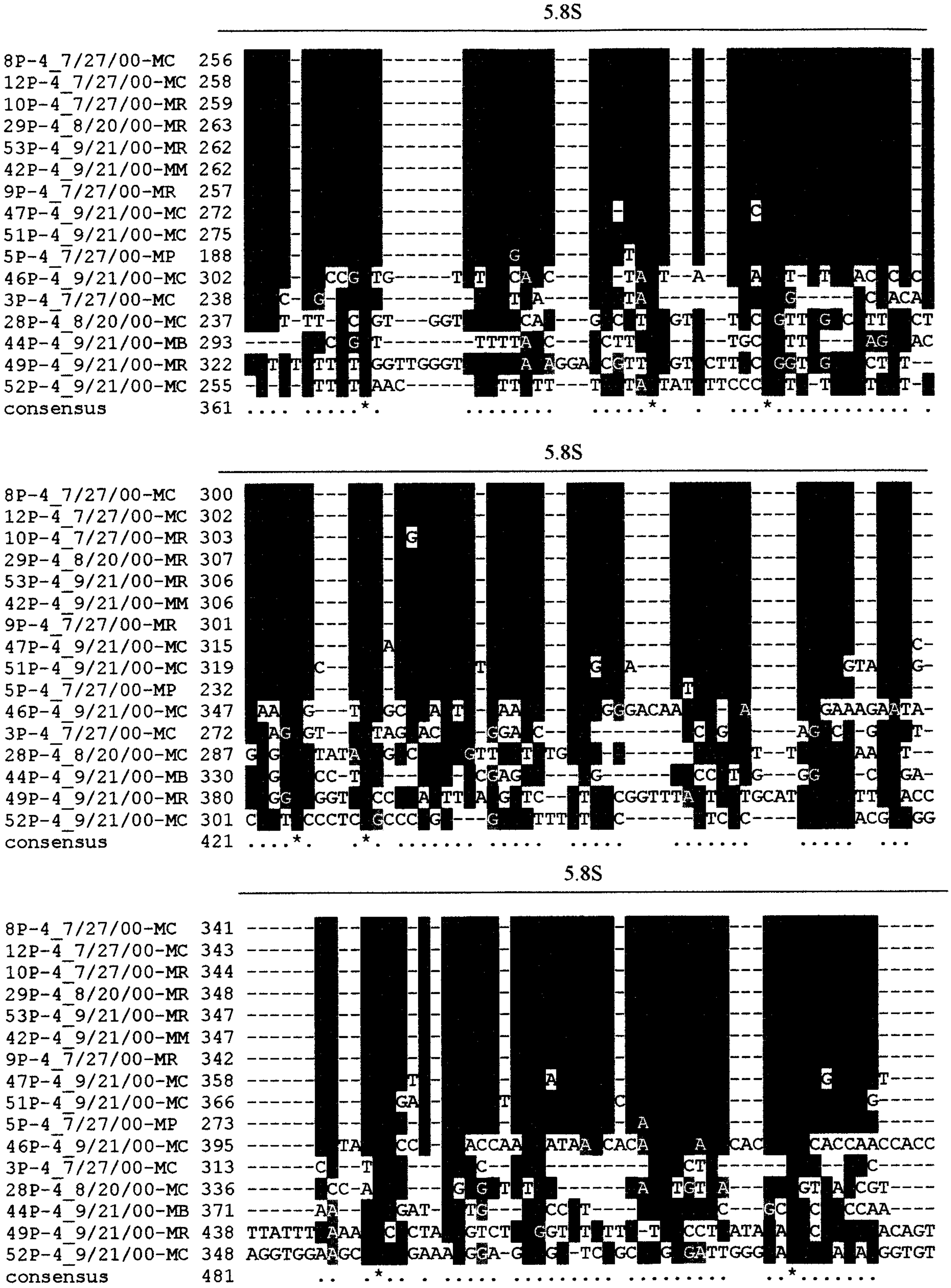
consensus 301


5.8S
8P-4_7/27/00-MC 256
12P-4_7/27/00-MC 258
10P-4_7/27/00-MR 259
29P-4_8/20/00-MR 263
53P-4_9/21/00-MR 262
42P-4_9/21/00-MM 262
9P-4_7/27/00-MR 257
47P-4_9/21/00-MC 272
51P-4_9/21/00-MC 275
5P-4_7/27/00-MP 188
46P-4_9/21/00-MC 302
3P-4_7/27/00-MC 238
28P-4_8/20/00-MC 237
44P-4_9/21/00-MB 293
49P-4_9/21/00-MR 322
52P-4_9/21/00-MC 255
consensus 361
5.8S
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12P-4_7/27/00-MC 302
10P-4_7/27/00-MR 303
29P-4_8/20/00-MR 307
53P-4_9/21/00-MR 306
42P-4_9/21/00-MM 306
9P-4_7/27/00-MR 301
47P-4_9/21/00-MC 315
51P-4_9/21/00-MC 319
5P-4_7/27/00-MP 232
46P-4_9/21/00-MC 347
3P-4_7/27/00-MC 272
28P-4_8/20/00-MC 287
44P-4_9/21/00-MB 330
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52P-4_9/21/00-MC 301
consensus 421
5.8S
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12P-4_7/27/00-MC 343
10P-4_7/27/00-MR 344
29P-4_8/20/00-MR 348
53P-4_9/21/00-MR 347
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9P-4_7/27/00-MR 342
47P-4_9/21/00-MC 358
51P-4_9/21/00-MC 366
5P-4_7/27/00-MP 273
46P-4_9/21/00-MC 395
3P-4_7/27/00-MC 313
28P-4_8/20/00-MC 336
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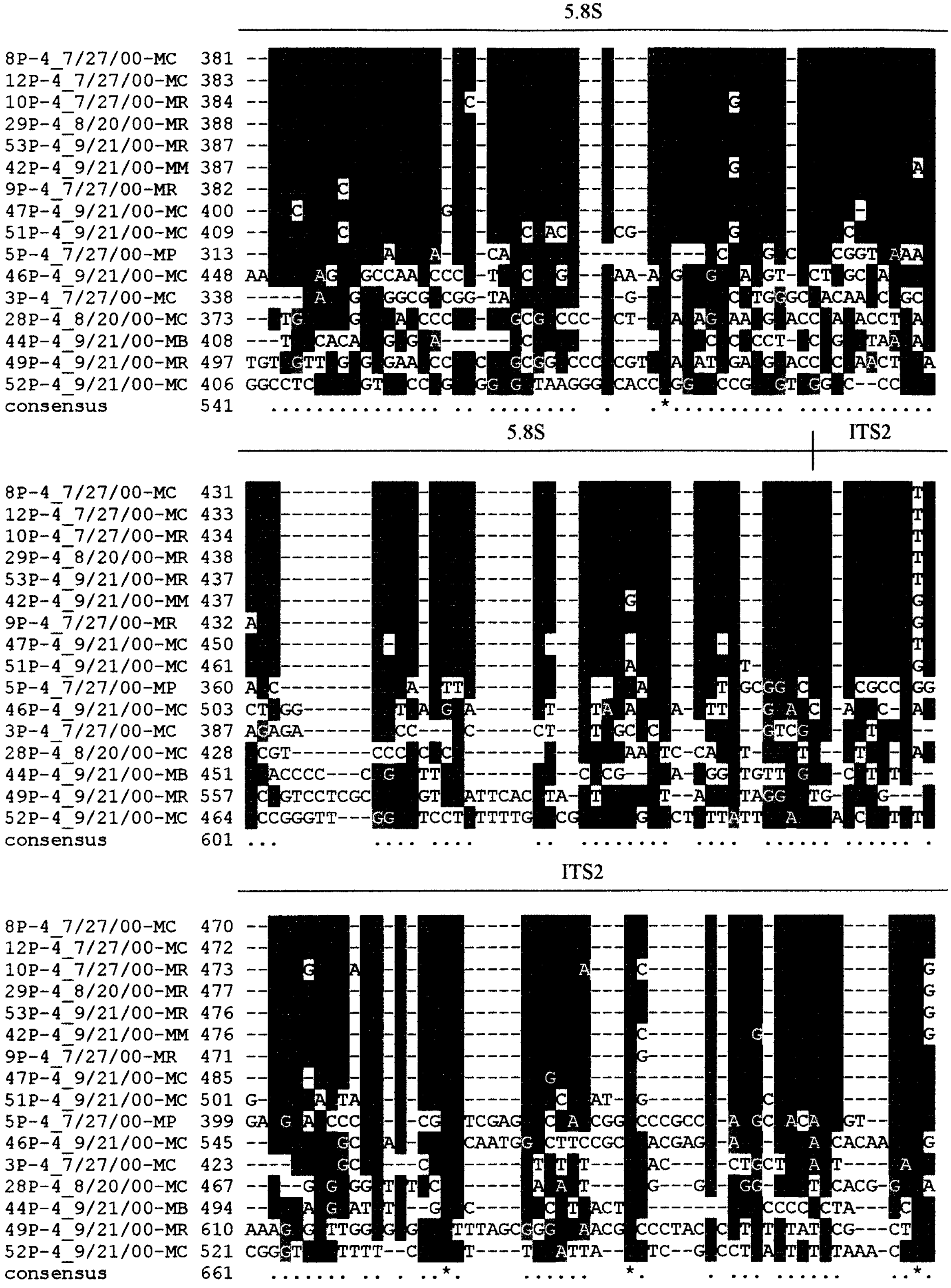
49P-4_9/21/00-MR 438
52P-4_9/21/00-MC 348
consensus 481


5.8S
5.8S
ITS2
ITS2
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29P-4_8/20/00-MR
53P-4_9/21/00-MR
42P-4_9/21/00-MM
9P-4_7/27/00-MR
47P-4_9/21/00-MC
51P-4_9/21/00-MC
5P-4_7/27/00-MP
46P-4_9/21/00-MC
3P-4_7/27/00-MC
28P-4_8/20/00-MC
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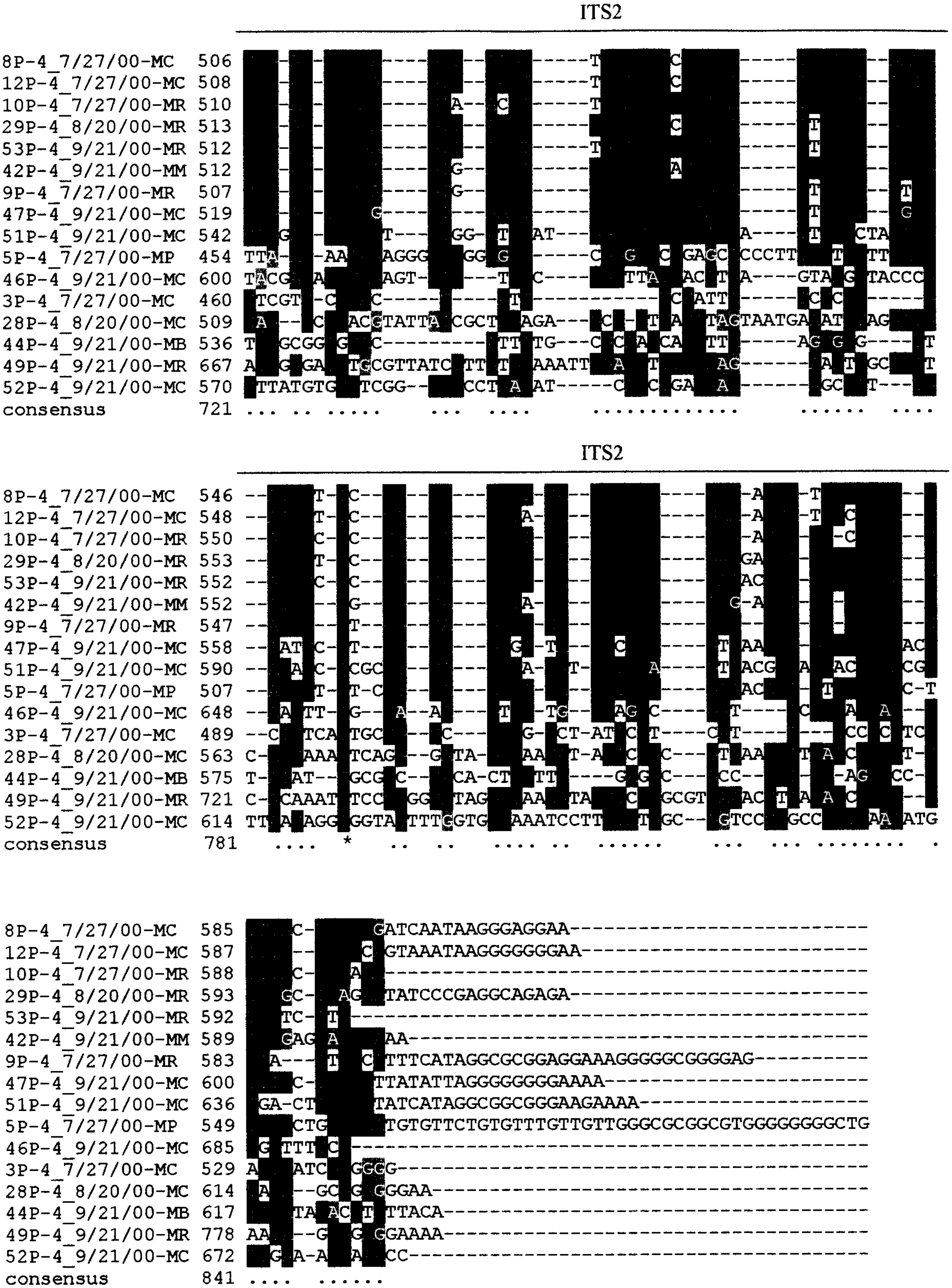
49P-4_9/21/00-MR
52P-4_9/21/00-MC
consensus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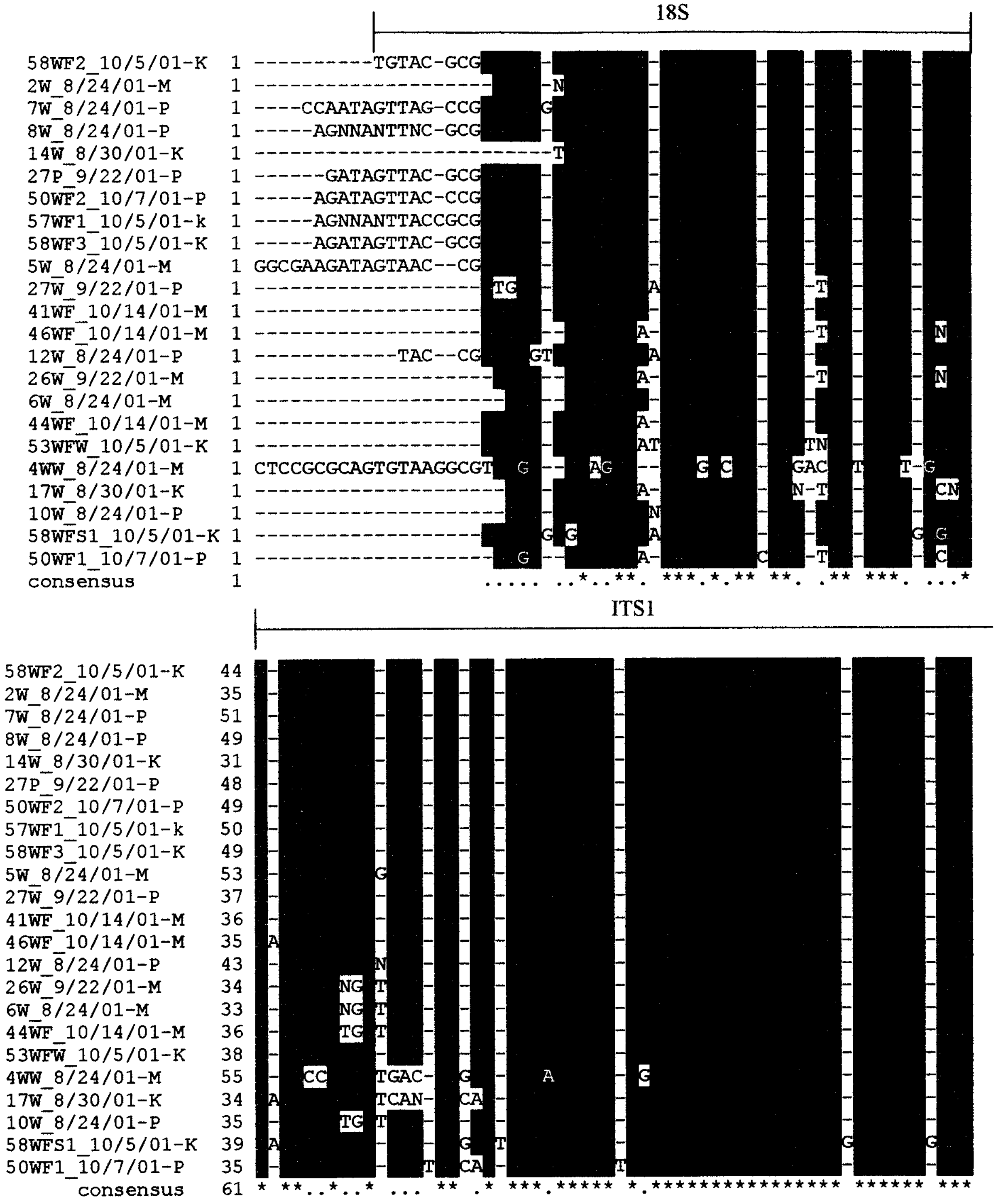
29P-4_8/20/00-MR 513
53P-4_9/21/00-MR 512
42P-4_9/21/00-MM 512
9P-4_7/27/00-MR 507
47P-4_9/21/00-MC 519
51P-4_9/21/00-MC 542
5P-4_7/27/00-MP 454
46P-4_9/21/00-MC 600
3P-4_7/27/00-MC 460
28P-4_8/20/00-MC 509
44P-4_9/21/00-MB 536
49P-4_9/21/00-MR 667
52P-4_9/21/00-MC 570
consensus 721
ITS2
8P-4_7/27/00-MC 546
12P-4_7/27/00-MC 548
10P-4_7/27/00-MR 550
29P-4_8/20/00-MR 553
53P-4_9/21/00-MR 552
42P-4_9/21/00-MM 552
9P-4_7/27/00-MR 547
47P-4_9/21/00-MC 558
51P-4_9/21/00-MC 590
5P-4_7/27/00-MP 507
46P-4_9/21/00-MC 648
3P-4_7/27/00-MC 489
28P-4_8/20/00-MC 563
44P-4_9/21/00-MB 575
49P-4_9/21/00-MR 721
52P-4_9/21/00-MC 614
consensus 781
8P-4_7/27/00-MC 585
12P-4_7/27/00-MC 587
10P-4_7/27/00-MR 588
29P-4_8/20/00-MR 593
53P-4_9/21/00-MR 592
42P-4_9/21/00-MM 589
9P-4_7/27/00-MR 583
47P-4_9/21/00-MC 600
51P-4_9/21/00-MC 636
5P-4_7/27/00-MP 549
46P-4_9/21/00-MC 685
3P-4_7/27/00-MC 529
28P-4_8/20/00-MC 614
44P-4_9/21/00-MB 617
49P-4_9/21/00-MR 778
52P-4_9/21/00-MC 672
consensus 841

Appendix 6:

Sequence alignment of *Aureobasidium pullulans* strains sampled from Markko, Presque Isle, and Klingshirn vineyards in the 2001 season. Sequencing reactions were done using reverse primer ITS4. DNA sequence alignments done using the Baylor College of Medicine ClustalW 1.8 Multiple Sequence Alignment website Box Shade View. Dashes represent gaps. Conserved and ITS regions labeled.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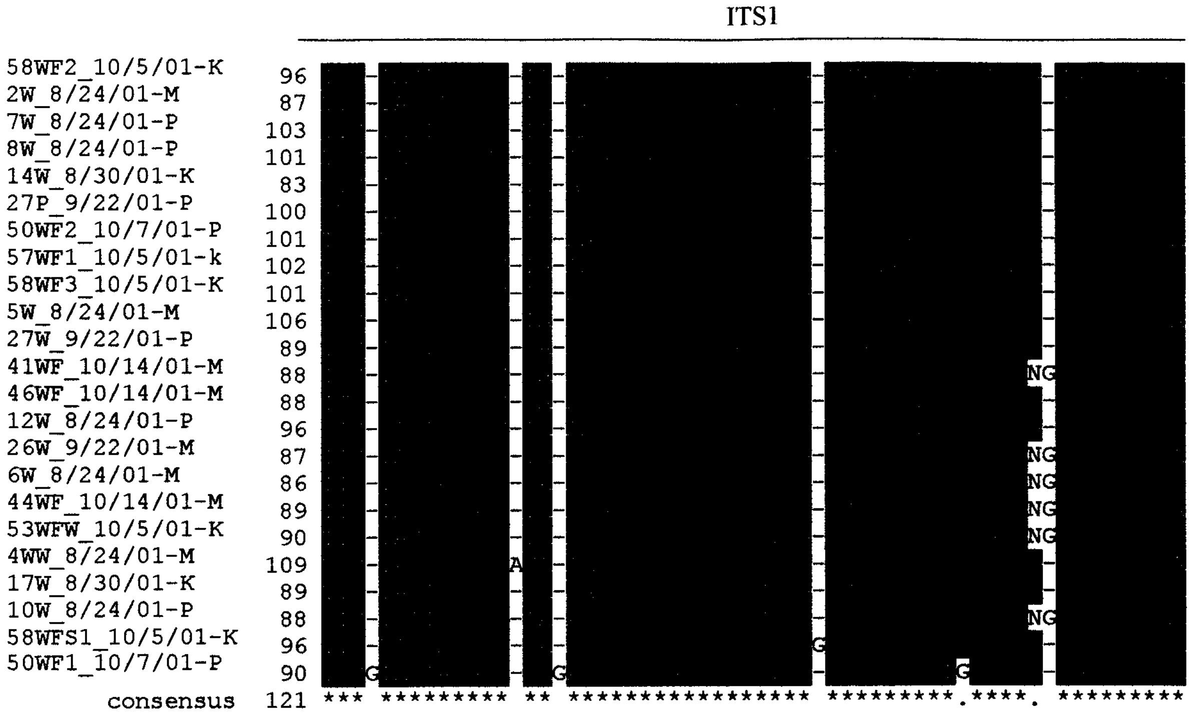
2W_8/24/01-M 1
7W_8/24/01-P 1 ----CCAATAGTTAG-CCG
8W_8/24/01-P 1 -----AGNNANTTNC-GCG
14W_8/30/01-K 1
27P_9/22/01-P 1 ------GATAGTTAC-GCG
50WF2_10/7/01-P 1 -----AGATAGTTAC-CCG
57WF1_10/5/01-k 1 -----AGNNANTTACCGCG
58WF3_10/5/01-K 1 -----AGATAGTTAC-GCG
5W_8/24/01-M 1 GGCGAAGATAGTAAC--CG
27W_9/22/01-P 1
41WF_10/14/01-M 1
46WF_10/14/01-M 1
12W_8/24/01-P 1 ------------TAC--CG
26W_9/22/01-M 1
6W_8/24/01-M 1
44WF_10/14/01-M 1
53WFW_10/5/01-K 1
4WW_8/24/01-M 1 CTCCGCGCAGTGTAAGGCGT
17W_8/30/01-K 1
10W_8/24/01-P 1
58WFS1_10/5/01-K 1
50WF1_10/7/01-P 1
consensus 1
ITS1
58WF2_10/5/01-K 44
2W_8/24/01-M 35
7W_8/24/01-P 51
8W_8/24/01-P 49
14W_8/30/01-K 31
27P_9/22/01-P 48
50WF2_10/7/01-P 49
57WF1_10/5/01-k 50
58WF3_10/5/01-K 49
5W_8/24/01-M 53
27W_9/22/01-P 37
41WF_10/14/01-M 36
46WF_10/14/01-M 35
12W_8/24/01-P 43
26W_9/22/01-M 34
6W_8/24/01-M 33
44WF_10/14/01-M 36
53WFW_10/5/01-K 38
4WW_8/24/01-M 55
17W_8/30/01-K 34
10W_8/24/01-P 35
58WFS1_10/5/01-K 39
50WF1_10/7/01-P 35
consensus 61


ITS1
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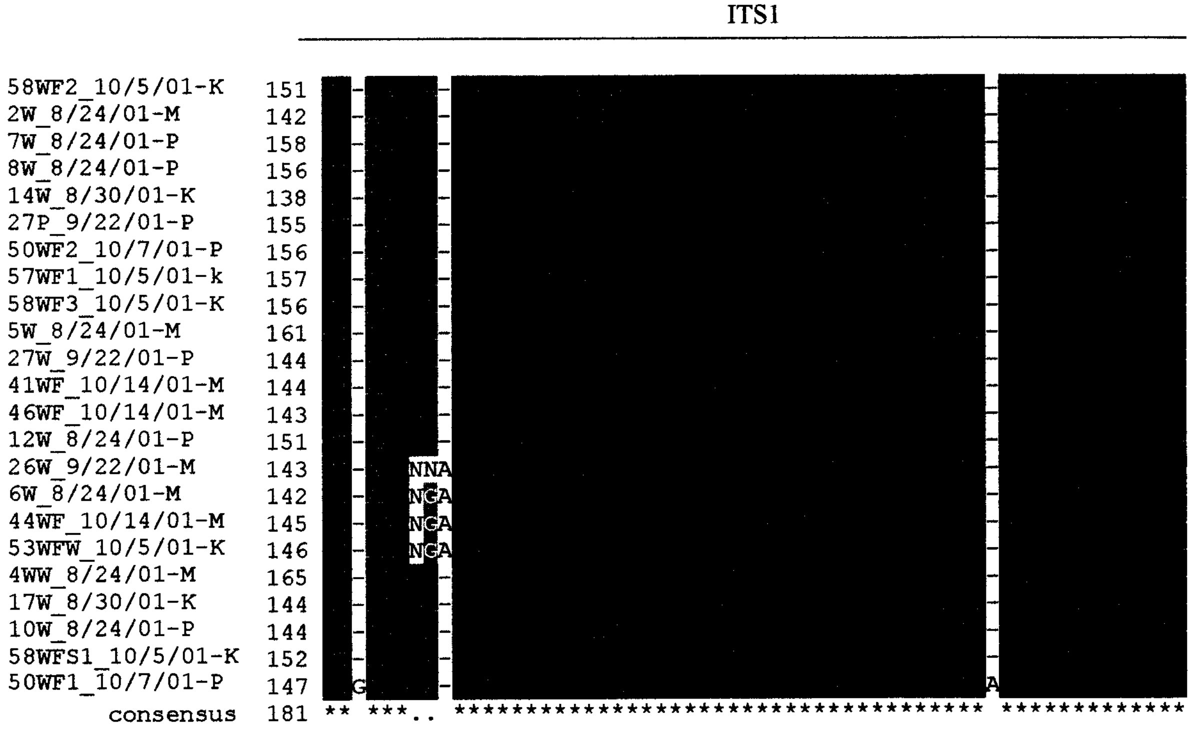
2W_8/24/01-M 87
7W_8/24/01-P 103
8W_8/24/01-P 101
14W_8/30/01-K 83
27P_9/22/01-P 100
50WF2_10/7/01-P 101
57WF1_10/5/01-k 102
58WF3_10/5/01-K 101
5W_8/24/01-M 106
27W_9/22/01-P 89
41WF_10/14/01-M 88
46WF_10/14/01-M 88
12W_8/24/01-P 96
26W_9/22/01-M 87
6W_8/24/01-M 86
44WF_10/14/01-M 89
53WFW_10/5/01-K 90
4WW_8/24/01-M 109
17W_8/30/01-K 89
10W_8/24/01-P 88
58WFS1_10/5/01-K 96
50WF1_10/7/01-P 90
consensus 121
NG
NG
NG
NG
NG
NG
A
G
G
G
G
*** ********* ** ***************** *********.****. *********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8W_8/24/01-P 156
14W_8/30/01-K 138
27P_9/22/01-P 155
50WF2_10/7/01-P 156
57WF1_10/5/01-k 157
58WF3_10/5/01-K 156
5W_8/24/01-M 161
27W_9/22/01-P 144
41WF_10/14/01-M 144
46WF_10/14/01-M 143
12W_8/24/01-P 151
26W_9/22/01-M 143
6W_8/24/01-M 142
44WF_10/14/01-M 145
53WFW_10/5/01-K 146
4WW_8/24/01-M 165
17W_8/30/01-K 144
10W_8/24/01-P 144
58WFS1_10/5/01-K 152
50WF1_10/7/01-P 147
consensus 181
NNA
NGA
NGA
NGA
G
A
** ***.. ************************************* *************

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                                  ITS1                          |                5.8S
                         ----------------------------------------------------------------------
58WF2_10/5/01-K      208  [illegible]
2W_8/24/01-M         199  [illegible]
7W_8/24/01-P         215  [illegible]
8W_8/24/01-P         213  [illegible]
14W_8/30/01-K        195  [illegible]
27P_9/22/01-P        212  [illegible]
50WF2_10/7/01-P      213  [illegible]
57WF1_10/5/01-k      214  [illegible]
58WF3_10/5/01-K      213  [illegible]
5W_8/24/01-M         218  [illegible]
27W_9/22/01-P        201  [illegible]
41WF_10/14/01-M      201  [illegible]
46WF_10/14/01-M      200  [illegible]
12W_8/24/01-P        208  [illegible]
26W_9/22/01-M        201  [illegible]
6W_8/24/01-M         200  [illegible]
44WF_10/14/01-M      203  [illegible]
53WFW_10/5/01-K      204  [illegible]
4WW_8/24/01-M        222  [illegible]
17W_8/30/01-K        201  [illegible]
10W_8/24/01-P        201  [illegible]
58WFS1_10/5/01-K     209  [illegible]
50WF1_10/7/01-P      206  [illegible]
          consensus  241  ************************************************************
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                                               5.8S
                         ----------------------------------------------------------------------

58WF2_10/5/01-K      268  [illegible]
2W_8/24/01-M         259  [illegible]
7W_8/24/01-P         275  [illegible]
8W_8/24/01-P         273  [illegible]
14W_8/30/01-K        255  [illegible]
27P_9/22/01-P        272  [illegible]
50WF2_10/7/01-P      273  [illegible]
57WF1_10/5/01-k      274  [illegible]
58WF3_10/5/01-K      273  [illegible]
5W_8/24/01-M         278  [illegible]
27W_9/22/01-P        261  [illegible]
41WF_10/14/01-M      261  [illegible]
46WF_10/14/01-M      260  [illegible]
12W_8/24/01-P        268  [illegible]
26W_9/22/01-M        261  [illegible]
6W_8/24/01-M         260  [illegible]
44WF_10/14/01-M      263  [illegible]
53WFW_10/5/01-K      264  [illegible]
4WW_8/24/01-M        282  [illegible]
17W_8/30/01-K        261  [illegible]
10W_8/24/01-P        261  [illegible]
58WFS1_10/5/01-K     269  [illegible]
50WF1_10/7/01-P      266  [illegible]
          consensus  301  ************************************************************
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                                                   5.8S
                            ____________________________________________________________

58WF2_10/5/01-K    328  [illegible]
2W_8/24/01-M       319  [illegible]
7W_8/24/01-P       335  [illegible]
8W_8/24/01-P       333  [illegible]
14W_8/30/01-K      315  [illegible]
27P_9/22/01-P      332  [illegible]
50WF2_10/7/01-P    333  [illegible]
57WF1_10/5/01-K    334  [illegible]
58WF3_10/5/01-K    333  [illegible]
5W_8/24/01-M       338  [illegible]
27W_9/22/01-P      321  [illegible]
41WF_10/14/01-M    321  [illegible]
46WF_10/14/01-M    320  [illegible]
12W_8/24/01-P      328  [illegible]
26W_9/22/01-M      321  [illegible]
6W_8/24/01-M       320  [illegible]
44WF_10/14/01-M    323  [illegible]
53WFW_10/5/01-K    324  [illegible]
4WW_8/24/01-M      342  [illegible]
17W_8/30/01-K      321  [illegible]
10W_8/24/01-P      321  [illegible]
58WFS1_10/5/01-K   329  [illegible]
50WF1_10/7/01-P    326  [illegible]
       consensus   361  ************************************************************

                         5.8S   |                 ITS2
                        ________|___________________________________________________
                                |

58WF2_10/5/01-K    388  [illegible]
2W_8/24/01-M       379  [illegible]
7W_8/24/01-P       395  [illegible]
8W_8/24/01-P       393  [illegible]
14W_8/30/01-K      375  [illegible]
27P_9/22/01-P      392  [illegible]
50WF2_10/7/01-P    393  [illegible]
57WF1_10/5/01-K    394  [illegible]
58WF3_10/5/01-K    393  [illegible]
5W_8/24/01-M       398  [illegible]
27W_9/22/01-P      381  [illegible]
41WF_10/14/01-M    381  [illegible]
46WF_10/14/01-M    380  [illegible]
12W_8/24/01-P      388  [illegible]
26W_9/22/01-M      381  [illegible]
6W_8/24/01-M       380  [illegible]
44WF_10/14/01-M    383  [illegible]
53WFW_10/5/01-K    384  [illegible]
4WW_8/24/01-M      402  [illegible]
17W_8/30/01-K      381  [illegible]
10W_8/24/01-P      381  [illegible] N [illegible]
58WFS1_10/5/01-K   389  [illegible] C [illegible]
50WF1_10/7/01-P    386  [illegible]
       consensus   421  ********************************************.*.*************
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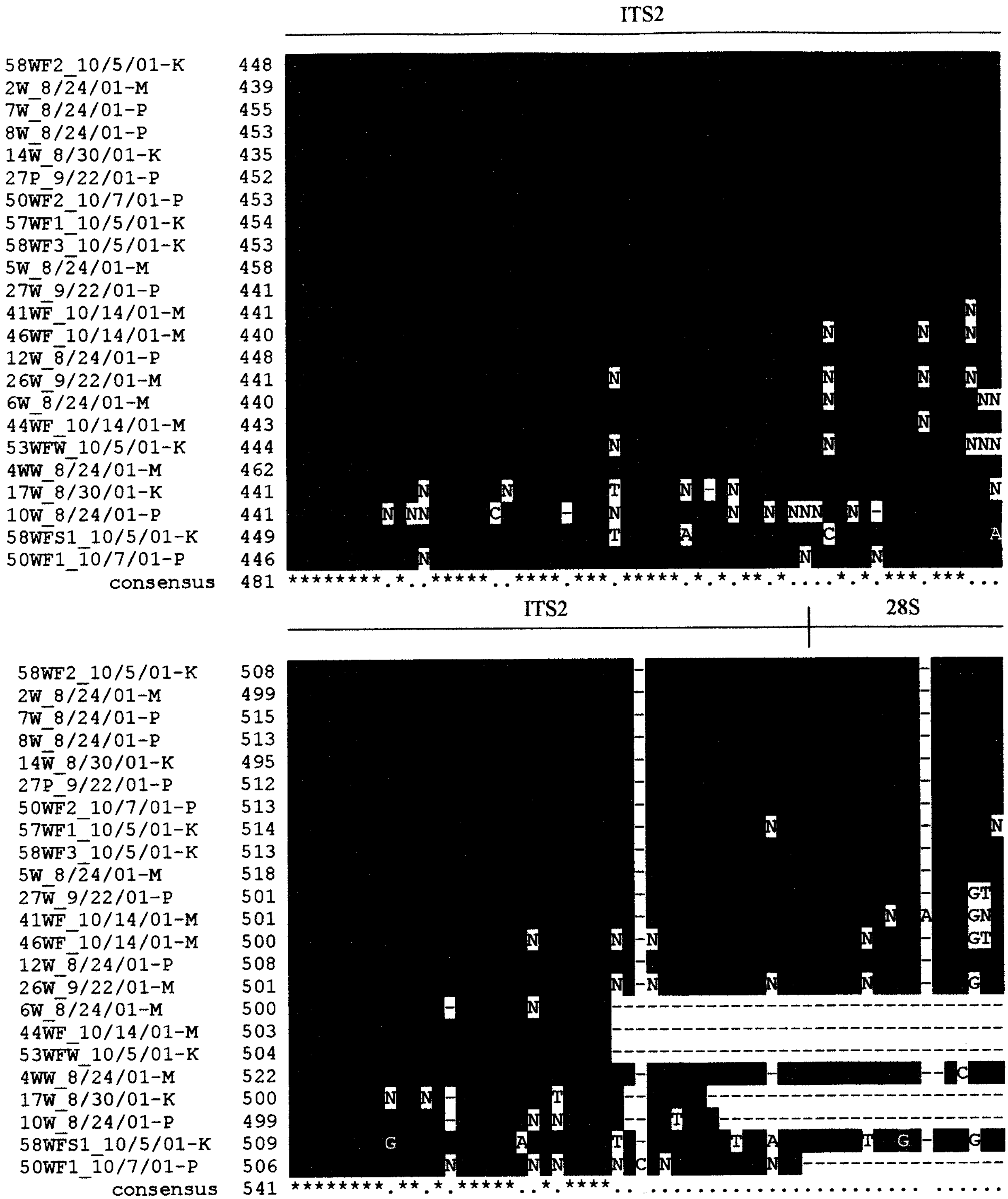
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8W_8/24/01-P 453
14W_8/30/01-K 435
27P_9/22/01-P 452
50WF2_10/7/01-P 453
57WF1_10/5/01-K 454
58WF3_10/5/01-K 453
5W_8/24/01-M 458
27W_9/22/01-P 441
41WF_10/14/01-M 441
46WF_10/14/01-M 440
12W_8/24/01-P 448
26W_9/22/01-M 441
6W_8/24/01-M 440
44WF_10/14/01-M 443
53WFW_10/5/01-K 444
4WW_8/24/01-M 462
17W_8/30/01-K 441
10W_8/24/01-P 441
58WFS1_10/5/01-K 449
50WF1_10/7/01-P 446
consensus 481
ITS2
28S
58WF2_10/5/01-K 508
2W_8/24/01-M 499
7W_8/24/01-P 515
8W_8/24/01-P 513
14W_8/30/01-K 495
27P_9/22/01-P 512
50WF2_10/7/01-P 513
57WF1_10/5/01-K 514
58WF3_10/5/01-K 513
5W_8/24/01-M 518
27W_9/22/01-P 501
41WF_10/14/01-M 501
46WF_10/14/01-M 500
12W_8/24/01-P 508
26W_9/22/01-M 501
6W_8/24/01-M 500
44WF_10/14/01-M 503
53WFW_10/5/01-K 504
4WW_8/24/01-M 522
17W_8/30/01-K 500
10W_8/24/01-P 499
58WFS1_10/5/01-K 509
50WF1_10/7/01-P 506
consensus 541

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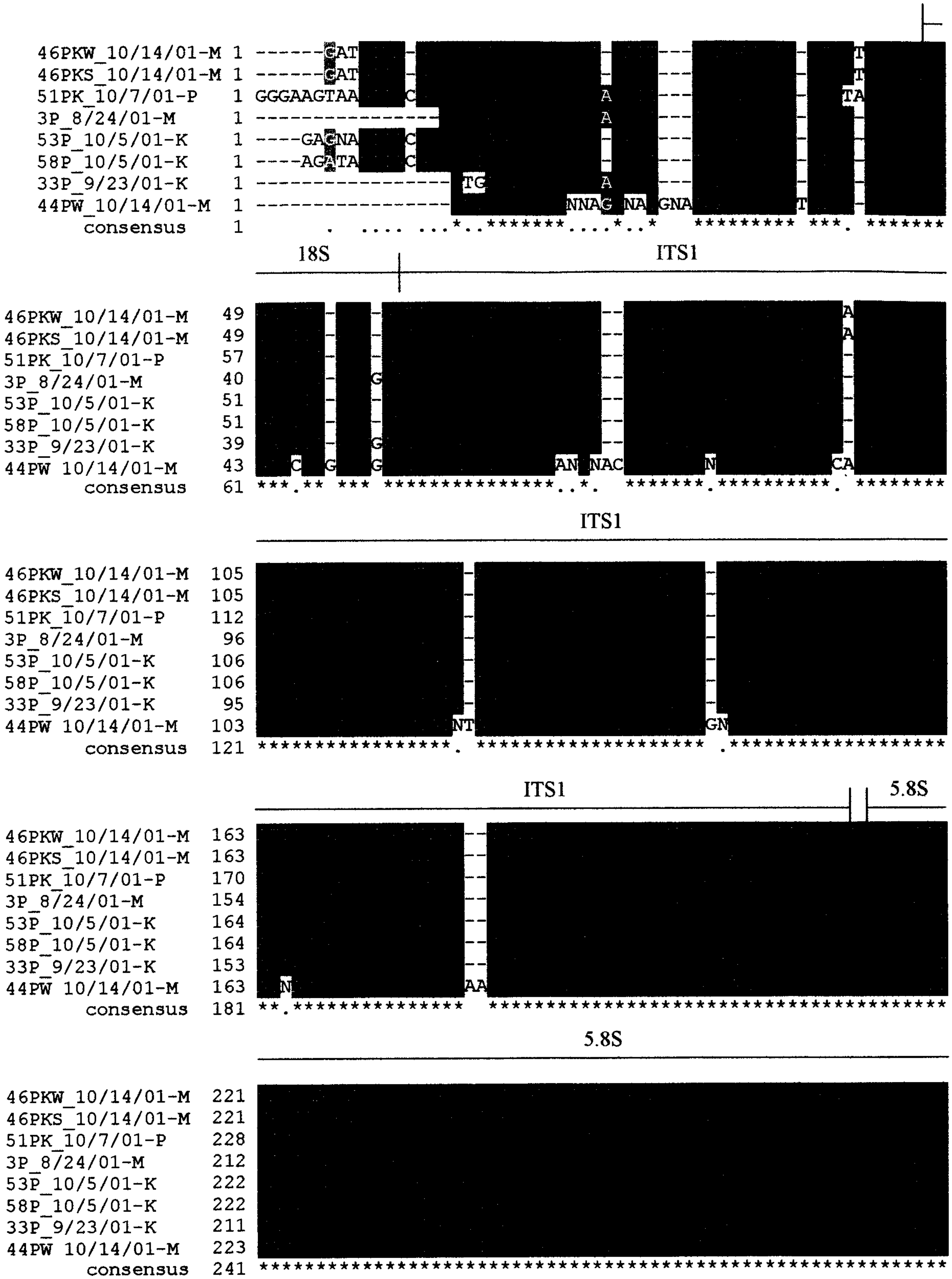
58WF2_10/5/01-K    566 [illegible]A-CTTGGGAAAAA-
2W_8/24/01-M       557 N[illegible]N-CTA---------
7W_8/24/01-P       573 [illegible]T-CTTTGGCAAA--
8W_8/24/01-P       571 N[illegible]T-AC----------
14W_8/30/01-K      553 [illegible]T-ATTTGGGAAAAA
27P_9/22/01-P      570 [illegible]T-ACTTGGGAAAAA
50WF2_10/7/01-P    571 [illegible]T-ACTTGGGCAAA-
57WF1_10/5/01-K    572 N[illegible]A-TTT---------
58WF3_10/5/01-K    571 [illegible]--CTAGGGCAAAAA
5W_8/24/01-M       576 [illegible]TTACTGGGCAAA--
27W_9/22/01-P      559 [illegible]----------------
41WF_10/14/01-M    560 [illegible]---------------
46WF_10/14/01-M    558 [illegible]-------------------
12W_8/24/01-P      566 [illegible]TTACTTNGACA---
26W_9/22/01-M      559 [illegible]-----------------
6W_8/24/01-M           ----------------------
44WF_10/14/01-M        ----------------------
53WFW_10/5/01-K        ----------------------
4WW_8/24/01-M      578 [illegible]T-ACTAGCAA----
17W_8/30/01-K          ----------------------
10W_8/24/01-P          ----------------------
58WFS1_10/5/01-K   567 [illegible]TTACTCGC------
50WF1_10/7/01-P        ----------------------
        consensus  601 ........
```

Appendix 7:

Sequence alignment of *Rhodotorula* sp. strains sampled from Markko, Presque Isle, and Klingshirn vineyards in the 2001 season. Sequencing reactions were done using reverse primer ITS4. DNA sequence alignments done using the Baylor College of Medicine ClustalW 1.8 Multiple Sequence Alignment website Box Shade view. Conserved and ITS regions labeled. Dashes represent gaps and blue circle indicates area where regions overlap.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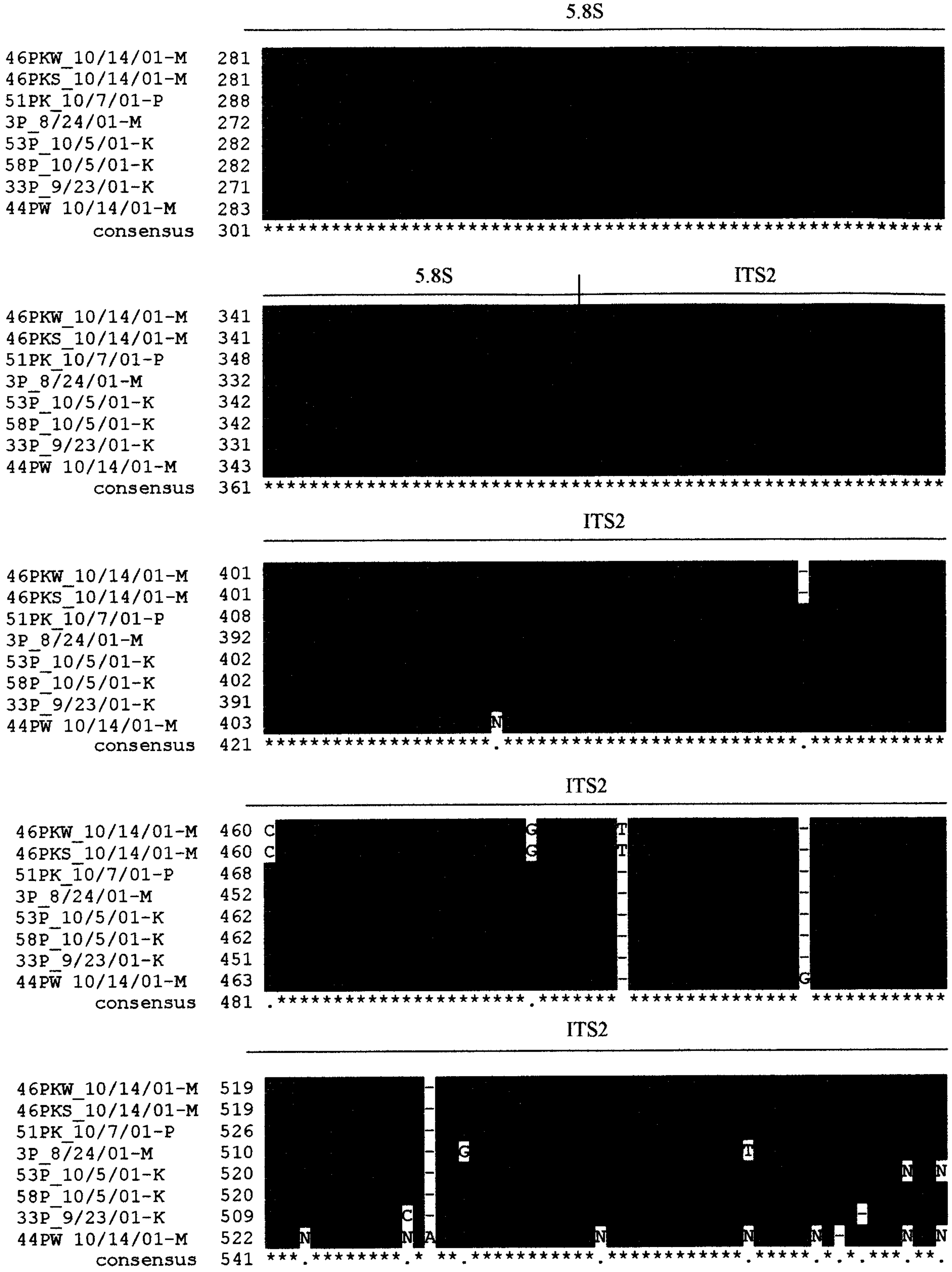
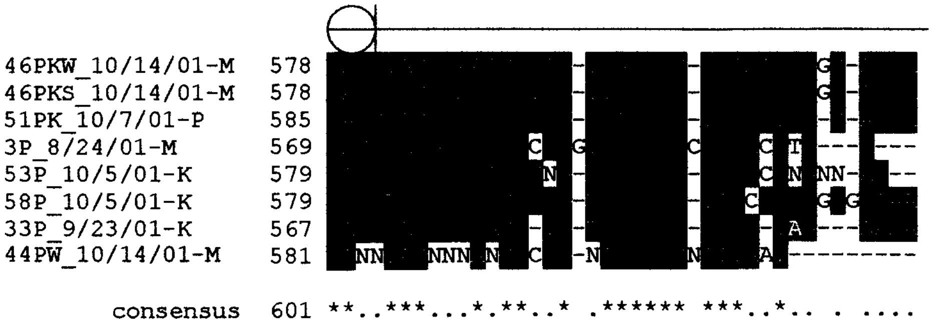
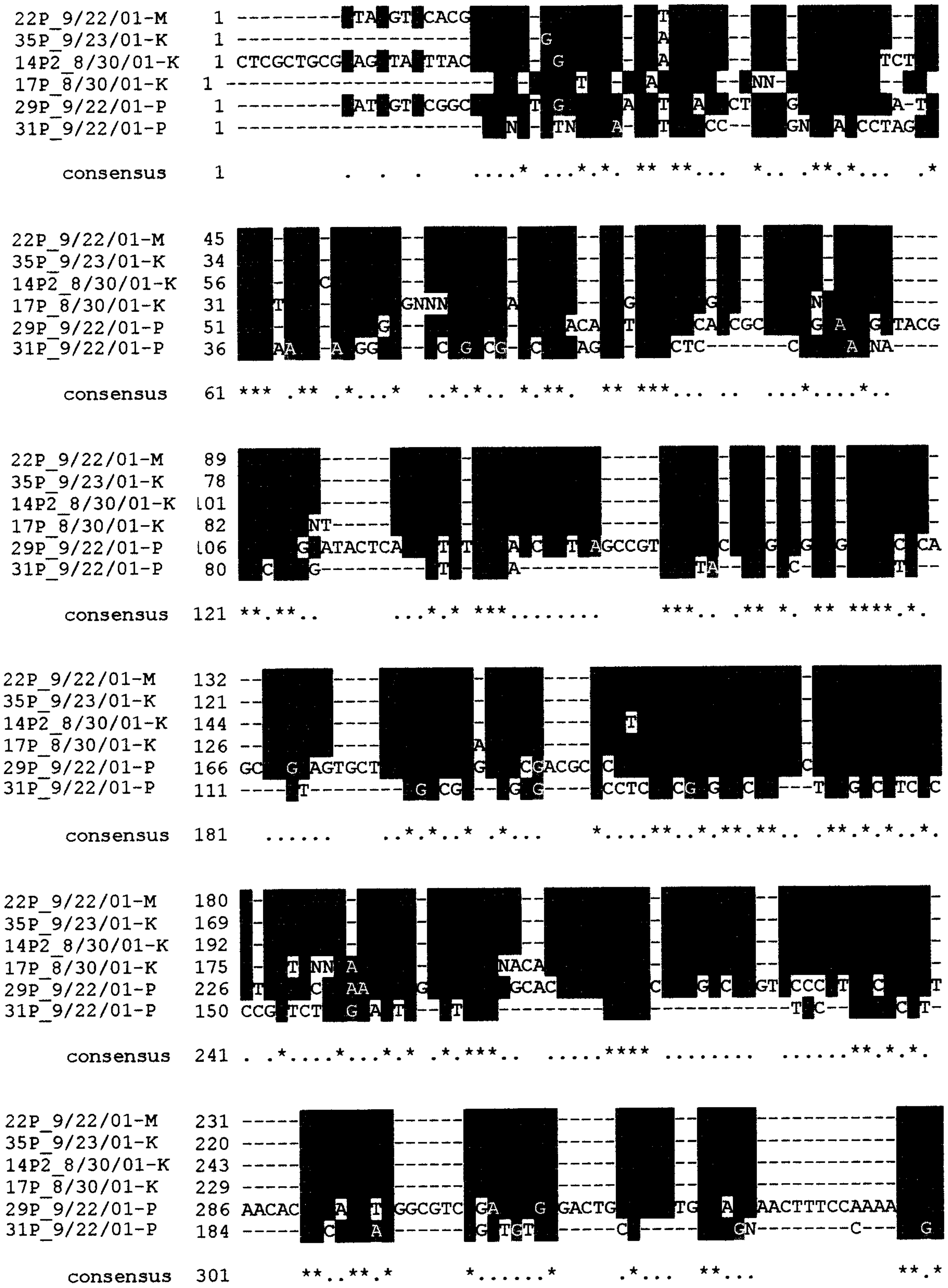
3P_8/24/01-M 1 A
53P_10/5/01-K 1 ----GAGNA C
58P_10/5/01-K 1 ----AGATA C
33P_9/23/01-K 1 TG A
44PW_10/14/01-M 1 NNAG NA GNA T
consensus 1
18S
ITS1
46PKW_10/14/01-M 49
46PKS_10/14/01-M 49
51PK_10/7/01-P 57
3P_8/24/01-M 40
53P_10/5/01-K 51
58P_10/5/01-K 51
33P_9/23/01-K 39
44PW 10/14/01-M 43
consensus 61
ITS1
46PKW_10/14/01-M 105
46PKS_10/14/01-M 105
51PK_10/7/01-P 112
3P_8/24/01-M 96
53P_10/5/01-K 106
58P_10/5/01-K 106
33P_9/23/01-K 95
44PW 10/14/01-M 103
consensus 121
ITS1
5.8S
46PKW_10/14/01-M 163
46PKS_10/14/01-M 163
51PK_10/7/01-P 170
3P_8/24/01-M 154
53P_10/5/01-K 164
58P_10/5/01-K 164
33P_9/23/01-K 153
44PW 10/14/01-M 163
consensus 181
5.8S
46PKW_10/14/01-M 221
46PKS_10/14/01-M 221
51PK_10/7/01-P 228
3P_8/24/01-M 212
53P_10/5/01-K 222
58P_10/5/01-K 222
33P_9/23/01-K 211
44PW 10/14/01-M 223
consensus 241

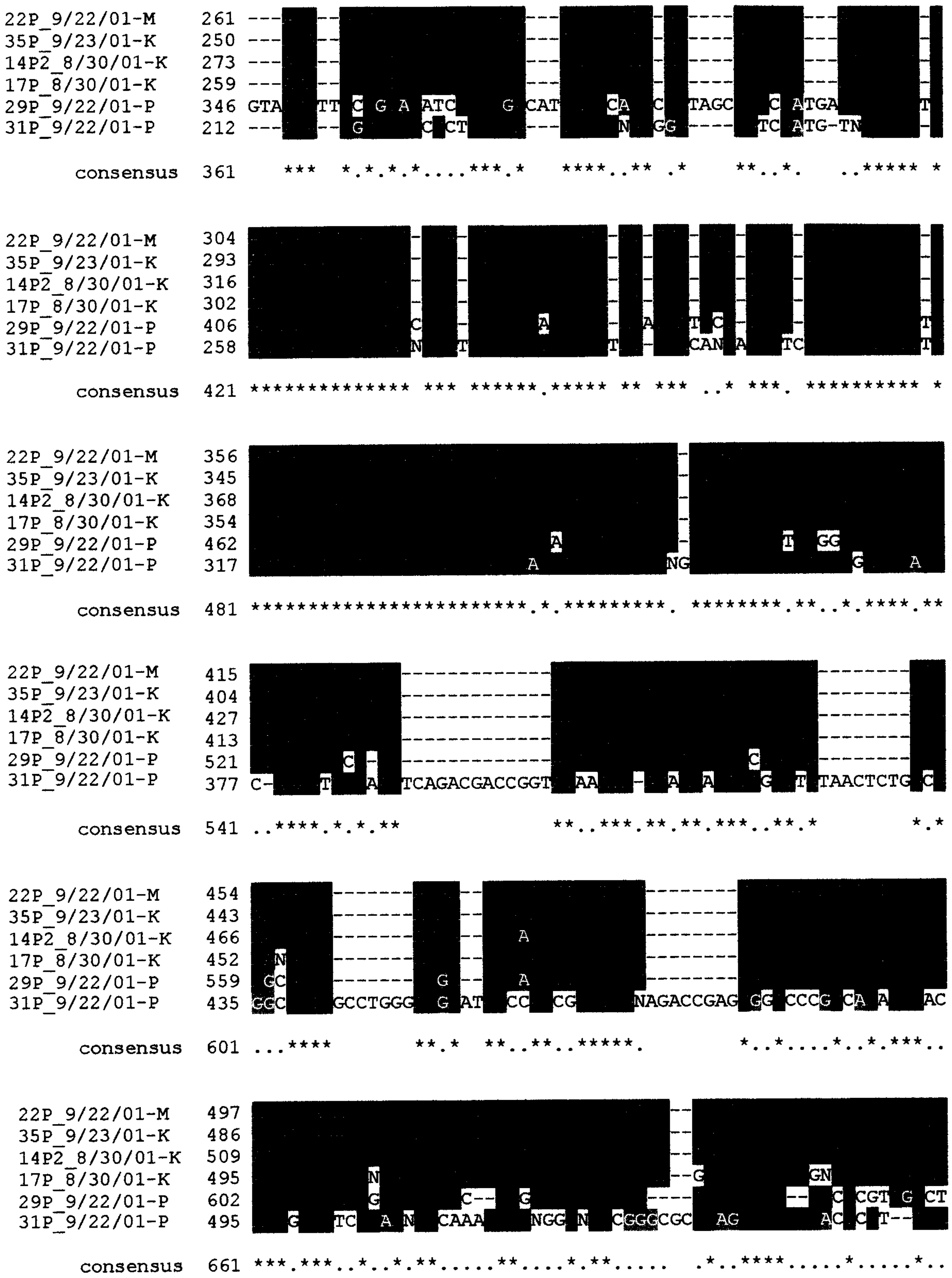

5.8S
46PKW_10/14/01-M 281
46PKS_10/14/01-M 281
51PK_10/7/01-P 288
3P_8/24/01-M 272
53P_10/5/01-K 282
58P_10/5/01-K 282
33P_9/23/01-K 271
44PW 10/14/01-M 283
consensus 301
5.8S
ITS2
46PKW_10/14/01-M 341
46PKS_10/14/01-M 341
51PK_10/7/01-P 348
3P_8/24/01-M 332
53P_10/5/01-K 342
58P_10/5/01-K 342
33P_9/23/01-K 331
44PW 10/14/01-M 343
consensus 361
ITS2
46PKW_10/14/01-M 401
46PKS_10/14/01-M 401
51PK_10/7/01-P 408
3P_8/24/01-M 392
53P_10/5/01-K 402
58P_10/5/01-K 402
33P_9/23/01-K 391
44PW 10/14/01-M 403 N
consensus 421
ITS2
46PKW_10/14/01-M 460 C G T
46PKS_10/14/01-M 460 C G T
51PK_10/7/01-P 468
3P_8/24/01-M 452
53P_10/5/01-K 462
58P_10/5/01-K 462
33P_9/23/01-K 451
44PW 10/14/01-M 463 G
consensus 481
ITS2
46PKW_10/14/01-M 519
46PKS_10/14/01-M 519
51PK_10/7/01-P 526
3P_8/24/01-M 510 G T
53P_10/5/01-K 520 N N
58P_10/5/01-K 520
33P_9/23/01-K 509 C
44PW 10/14/01-M 522 N N A N N N N N
consensus 541

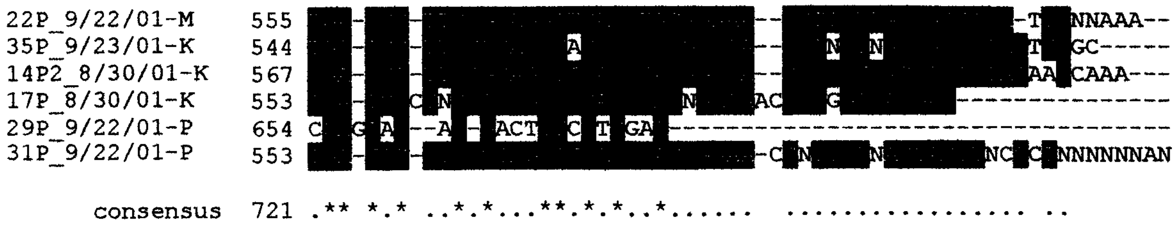

46PKW_10/14/01-M 578
46PKS_10/14/01-M 578
51PK_10/7/01-P 585
3P_8/24/01-M 569
53P_10/5/01-K 579
58P_10/5/01-K 579
33P_9/23/01-K 567
44PW_10/14/01-M 581
consensus 601 **..***...*.**..* .****** ***..*.. . ....

Appendix 8:

Sequence alignment of *Sporidiobolus pararoseus* strains sampled from Markko, Presque Isle, and Klingshirn vineyards in the 2001 season. Sequencing reactions were done using reverse primer ITS4. DNA sequence alignments done using the Baylor College of Medicine ClustalW 1.8 Multiple Sequence Alignment website Box Shade view. Dashes represent gaps.

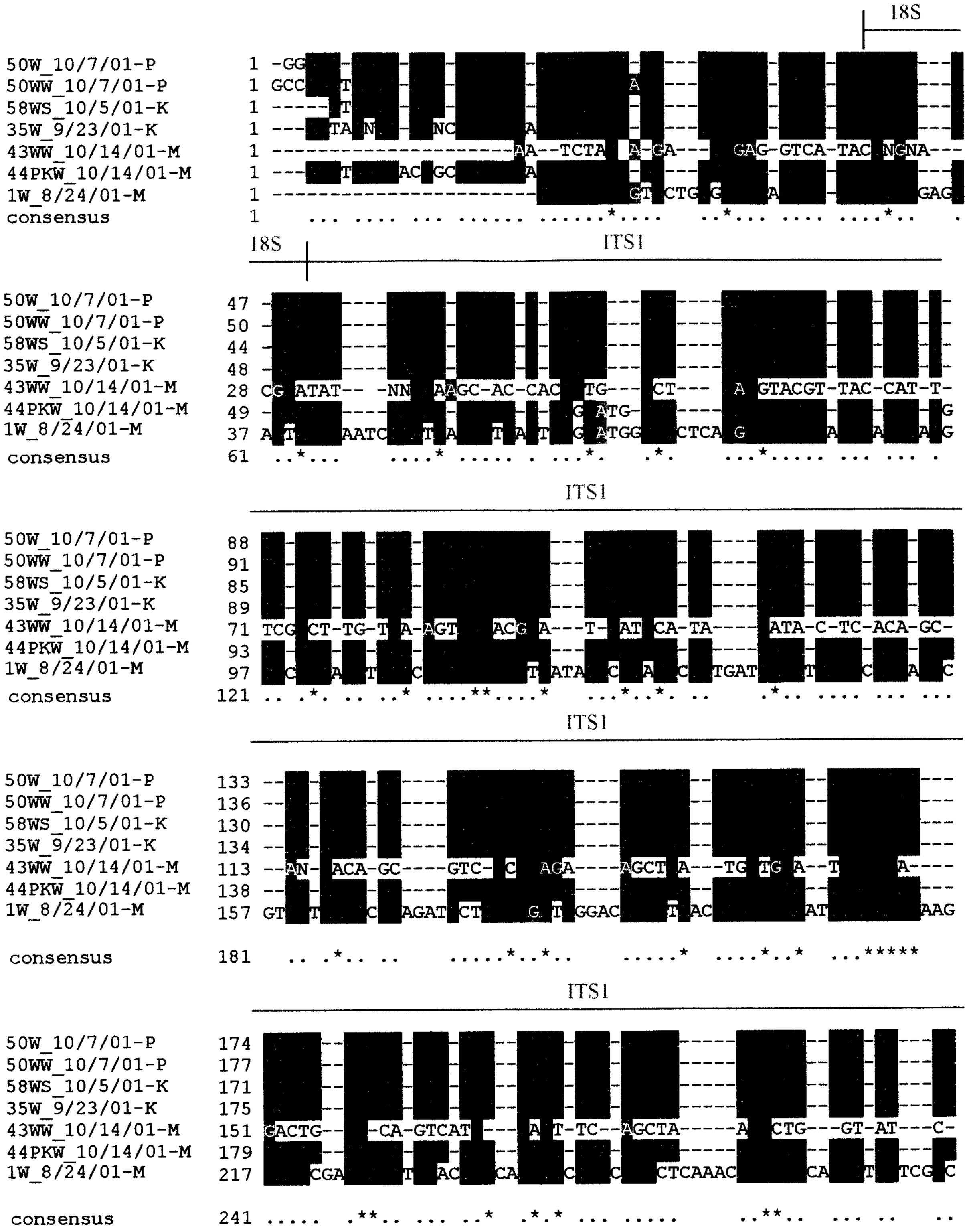

22P_9/22/01-M 1
35P_9/23/01-K 1
14P2_8/30/01-K 1
17P_8/30/01-K 1
29P_9/22/01-P 1
31P_9/22/01-P 1
consensus 1
22P_9/22/01-M 45
35P_9/23/01-K 34
14P2_8/30/01-K 56
17P_8/30/01-K 31
29P_9/22/01-P 51
31P_9/22/01-P 36
consensus 61
22P_9/22/01-M 89
35P_9/23/01-K 78
14P2_8/30/01-K 101
17P_8/30/01-K 82
29P_9/22/01-P 106
31P_9/22/01-P 80
consensus 121
22P_9/22/01-M 132
35P_9/23/01-K 121
14P2_8/30/01-K 144
17P_8/30/01-K 126
29P_9/22/01-P 166
31P_9/22/01-P 111
consensus 181
22P_9/22/01-M 180
35P_9/23/01-K 169
14P2_8/30/01-K 192
17P_8/30/01-K 175
29P_9/22/01-P 226
31P_9/22/01-P 150
consensus 241
22P_9/22/01-M 231
35P_9/23/01-K 220
14P2_8/30/01-K 243
17P_8/30/01-K 229
29P_9/22/01-P 286
31P_9/22/01-P 184
consensus 301

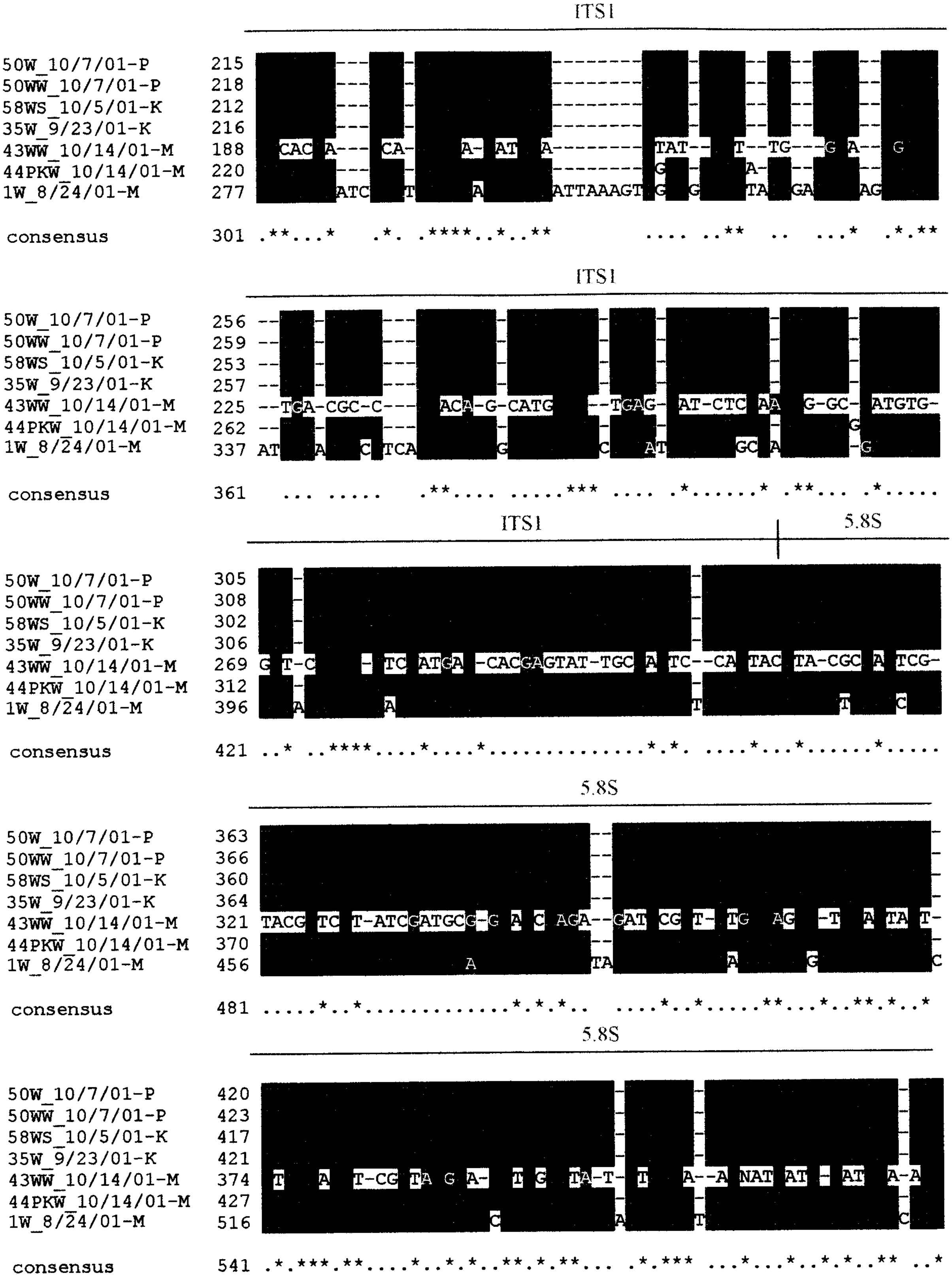

22P_9/22/01-M 261
35P_9/23/01-K 250
14P2_8/30/01-K 273
17P_8/30/01-K 259
29P_9/22/01-P 346
31P_9/22/01-P 212
consensus 361
22P_9/22/01-M 304
35P_9/23/01-K 293
14P2_8/30/01-K 316
17P_8/30/01-K 302
29P_9/22/01-P 406
31P_9/22/01-P 258
consensus 421
22P_9/22/01-M 356
35P_9/23/01-K 345
14P2_8/30/01-K 368
17P_8/30/01-K 354
29P_9/22/01-P 462
31P_9/22/01-P 317
consensus 481
22P_9/22/01-M 415
35P_9/23/01-K 404
14P2_8/30/01-K 427
17P_8/30/01-K 413
29P_9/22/01-P 521
31P_9/22/01-P 377
consensus 541
22P_9/22/01-M 454
35P_9/23/01-K 443
14P2_8/30/01-K 466
17P_8/30/01-K 452
29P_9/22/01-P 559
31P_9/22/01-P 435
consensus 601
22P_9/22/01-M 497
35P_9/23/01-K 486
14P2_8/30/01-K 509
17P_8/30/01-K 495
29P_9/22/01-P 602
31P_9/22/01-P 495
consensus 661


22P_9/22/01-M 555
35P_9/23/01-K 544
14P2_8/30/01-K 567
17P_8/30/01-K 553
29P_9/22/01-P 654
31P_9/22/01-P 553
consensus 721
-T NNAAA--
A N N T GC-----
AA CAAA---
C N N AC G
C G A --A - ACT C T GA
-C N N NC C NNNNNNNAN
.** *.* ..*.*...**.*.*..*...... ....

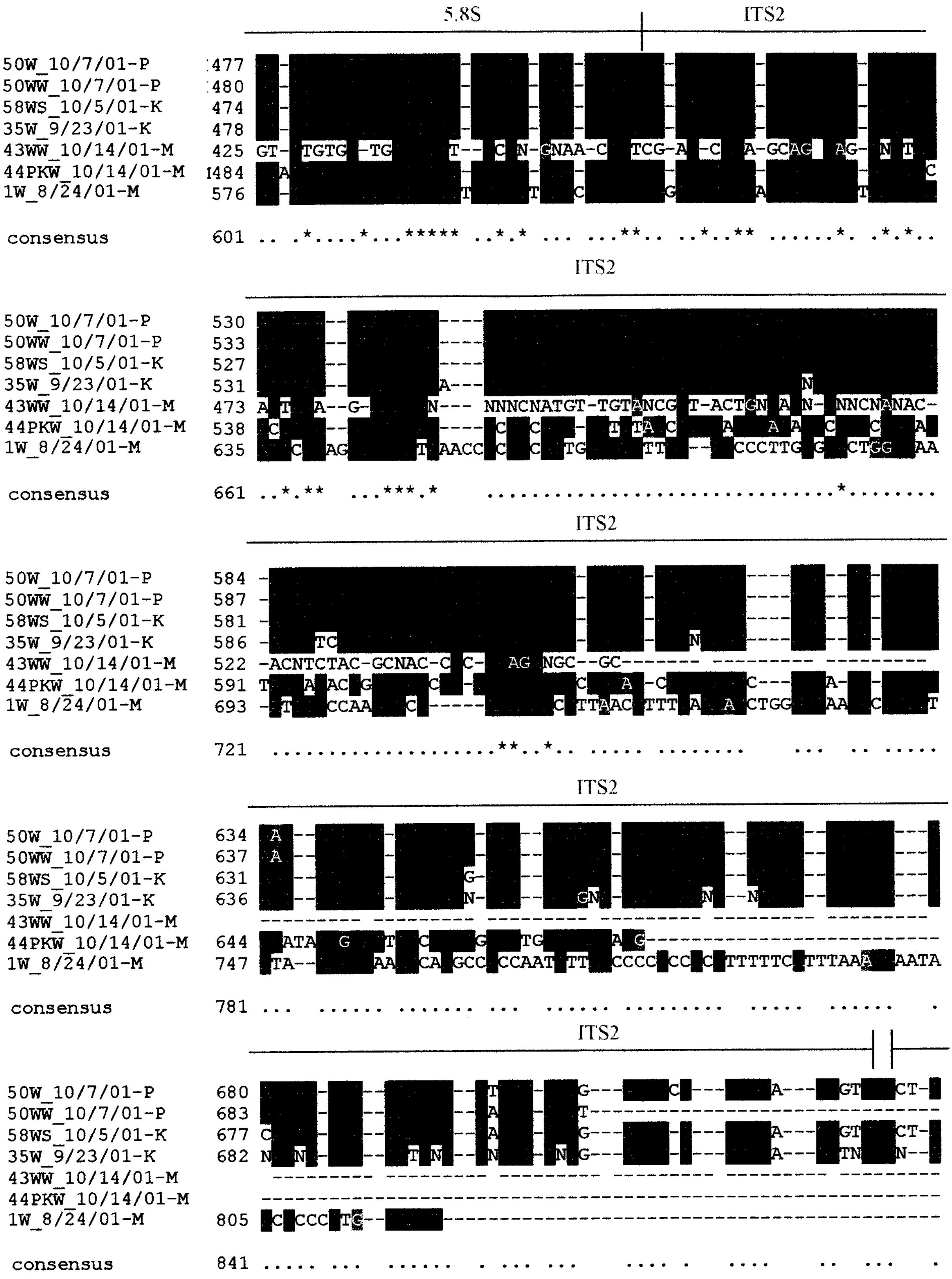
Appendix 9:

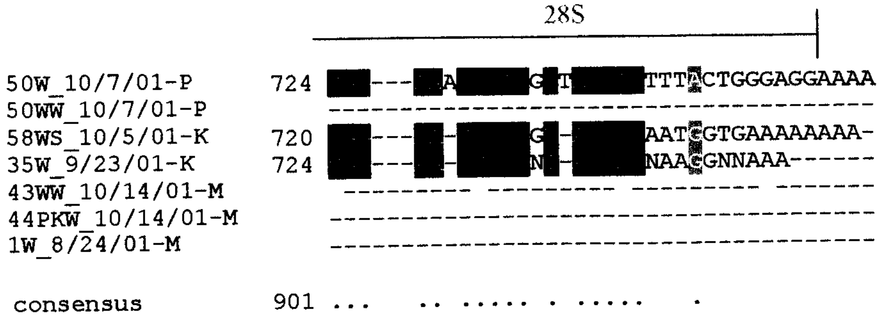
Sequence alignment of *Hanseniaspora uvarum* strains sampled from Markko, Presque Isle, and Klingshirn vineyards in the 2001 season. Sequencing reactions were done using reverse primer ITS4. DNA sequence alignments done using the Baylor College of Medicine ClustalW 1.8 Multiple Sequence Alignment website Box Shade view. Conserved and ITS regions labeled. Dashes represent gaps. (Note: 43WW_10/14/01-M is not boxshaded, but does align with other sequences. Boxshade error.)


18S
ITS1
50W_10/7/01-P
50WW_10/7/01-P
58WS_10/5/01-K
35W_9/23/01-K
43WW_10/14/01-M
44PKW_10/14/01-M
1W_8/24/01-M
consensus



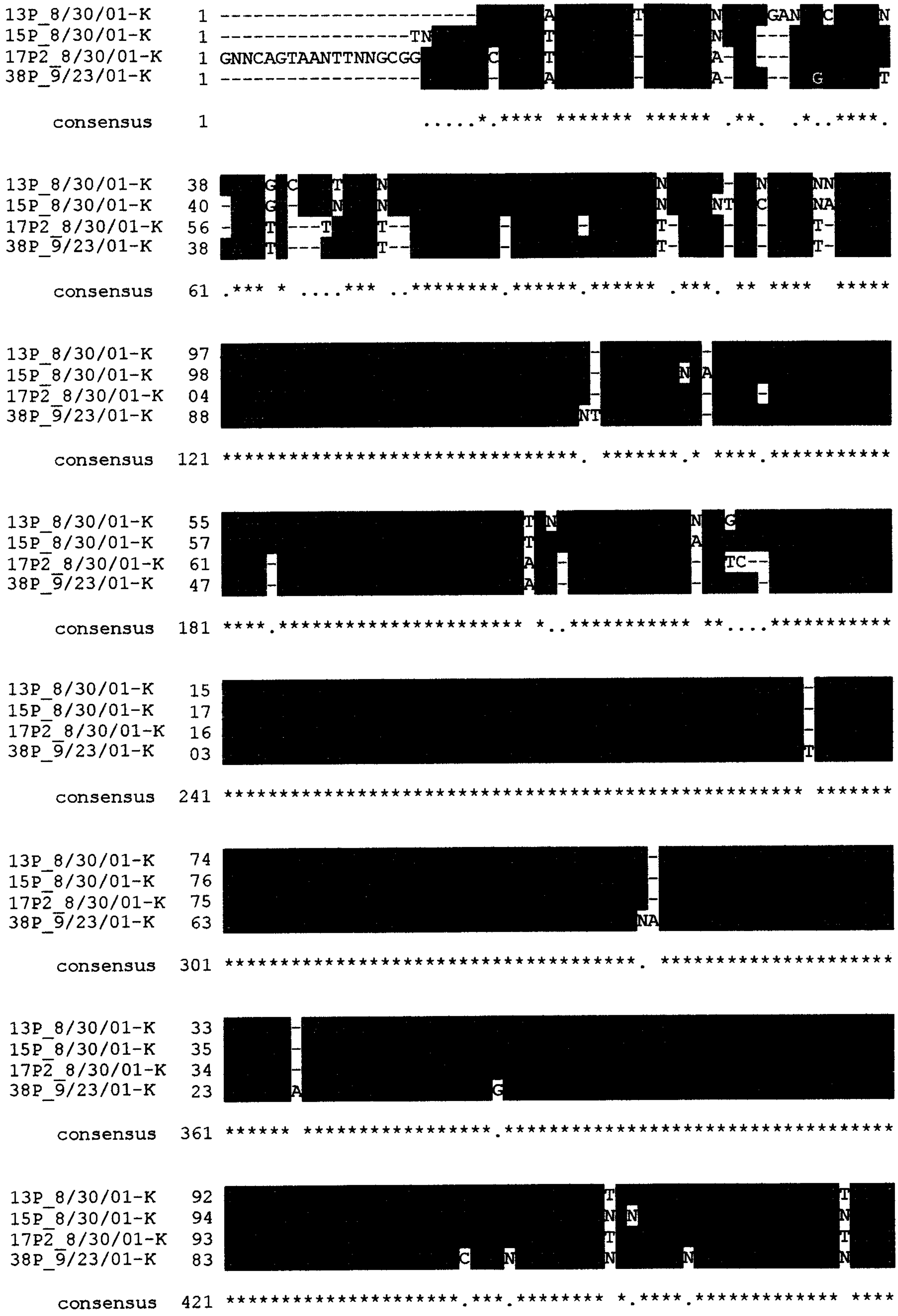
ITS1
50W_10/7/01-P 215
50WW_10/7/01-P 218
58WS_10/5/01-K 212
35W_9/23/01-K 216
43WW_10/14/01-M 188
44PKW_10/14/01-M 220
1W_8/24/01-M 277
consensus 301
ITS1
50W_10/7/01-P 256
50WW_10/7/01-P 259
58WS_10/5/01-K 253
35W_9/23/01-K 257
43WW_10/14/01-M 225
44PKW_10/14/01-M 262
1W_8/24/01-M 337
consensus 361
ITS1
5.8S
50W_10/7/01-P 305
50WW_10/7/01-P 308
58WS_10/5/01-K 302
35W_9/23/01-K 306
43WW_10/14/01-M 269
44PKW_10/14/01-M 312
1W_8/24/01-M 396
consensus 421
5.8S
50W_10/7/01-P 363
50WW_10/7/01-P 366
58WS_10/5/01-K 360
35W_9/23/01-K 364
43WW_10/14/01-M 321
44PKW_10/14/01-M 370
1W_8/24/01-M 456
consensus 481
5.8S
50W_10/7/01-P 420
50WW_10/7/01-P 423
58WS_10/5/01-K 417
35W_9/23/01-K 421
43WW_10/14/01-M 374
44PKW_10/14/01-M 427
1W_8/24/01-M 516
consensus 541

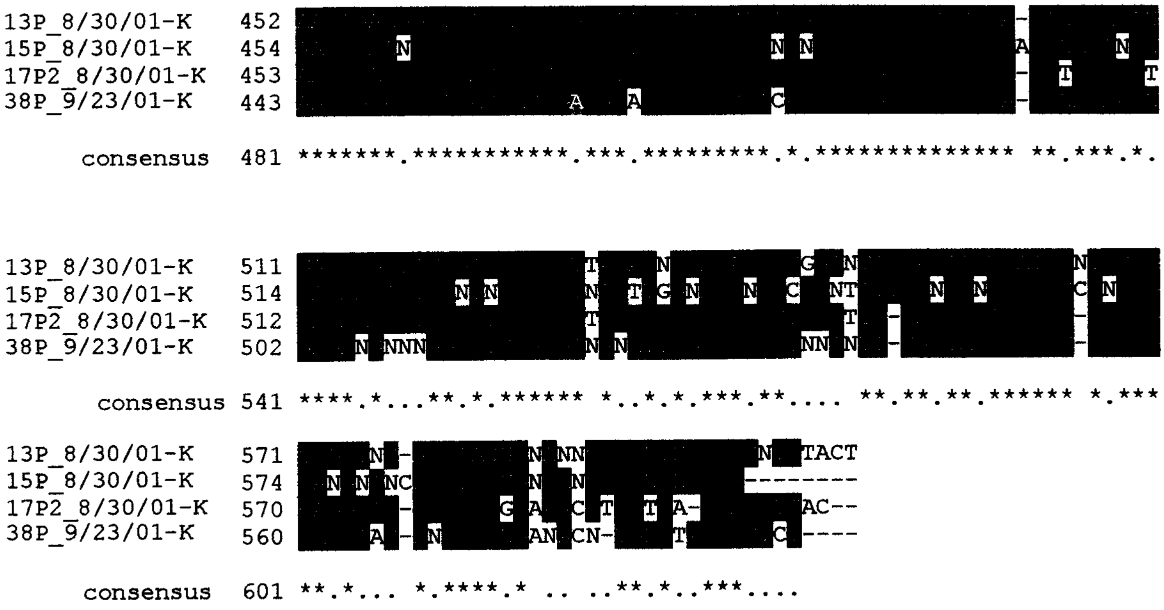

5.8S
ITS2
50W_10/7/01-P 477
50WW_10/7/01-P 480
58WS_10/5/01-K 474
35W_9/23/01-K 478
43WW_10/14/01-M 425
44PKW_10/14/01-M 1484
1W_8/24/01-M 576
consensus 601
ITS2
50W_10/7/01-P 530
50WW_10/7/01-P 533
58WS_10/5/01-K 527
35W_9/23/01-K 531
43WW_10/14/01-M 473
44PKW_10/14/01-M 538
1W_8/24/01-M 635
consensus 661
ITS2
50W_10/7/01-P 584
50WW_10/7/01-P 587
58WS_10/5/01-K 581
35W_9/23/01-K 586
43WW_10/14/01-M 522
44PKW_10/14/01-M 591
1W_8/24/01-M 693
consensus 721
ITS2
50W_10/7/01-P 634
50WW_10/7/01-P 637
58WS_10/5/01-K 631
35W_9/23/01-K 636
43WW_10/14/01-M
44PKW_10/14/01-M 644
1W_8/24/01-M 747
consensus 781
ITS2
50W_10/7/01-P 680
50WW_10/7/01-P 683
58WS_10/5/01-K 677
35W_9/23/01-K 682
43WW_10/14/01-M
44PKW_10/14/01-M
1W_8/24/01-M 805
consensus 841


28S
50W_10/7/01-P 724 ---A G T TTTACTGGGAGGAAAA
50WW_10/7/01-P
58WS_10/5/01-K 720 --- G - AATCGTGAAAAAAAA-
35W_9/23/01-K 724 --- N - NAACGNNAAA------
43WW_10/14/01-M
44PKW_10/14/01-M
1W_8/24/01-M
consensus 901 ... .

Appendix 10:

Sequence alignment of *Sporobolomyces marcillae* strains sampled from Markko, Presque Isle, and Klingshirn vineyards in the 2001 season. Sequencing reactions were done using reverse primer ITS4. DNA sequence alignments done using the Baylor College of Medicine ClustalW 1.8 Multiple Sequence Alignment website Box Shade view. Dashes represent gaps.


13P_8/30/01-K
15P_8/30/01-K
17P2_8/30/01-K
38P_9/23/01-K
GNNCAGTAANTTNNGCGG
consensus
1
61
121
181
241
301
361
421


13P_8/30/01-K 452
15P_8/30/01-K 454
17P2_8/30/01-K 453
38P_9/23/01-K 443
consensus 481 *******.***********.***.*********.*.************** **.***.*.
13P_8/30/01-K 511
15P_8/30/01-K 514
17P2_8/30/01-K 512
38P_9/23/01-K 502
consensus 541 ****.*...**.*.****** *..*.*.***.**.... **.**.**.****** *.***
13P_8/30/01-K 571
15P_8/30/01-K 574
17P2_8/30/01-K 570
38P_9/23/01-K 560
consensus 601 **.*... *.****.* .. ..**.*..***....

Appendix 11:

Markko vineyard 2000 to 2001 sample comparison of *A. pullulans.* Sequencing reactions were done using reverse primer ITS4. DNA sequence alignments done using the EMBL ClustalW 1.8 Multiple Sequence Alignment website. Conserved and ITS regions labeled. Dashes represent gaps. M, P, K – 2001.

```
                                                        18S
                                |-------------------------------------------------
5W-4_7/27/2000-M_     ----------ATAAAGTGGTGTATCTGAAGAGTGCGGTGGCGAT-GCTAAG--TTCGTGC 47
13W-4_7/27/2000-M_    ------------------GTGTATTCAAGGTGC---GTGGCCAT-GTCAGT--TCTGCCT 36
3W-4_7/27/2000-M_     ---------------------TATCCCTAC-CT-GATC--CGAG-GTCTA---CCTAGAA 31
12W-4_7/27/2000-M_    --------------------GTATCC-TAC-CT-GATC--CGAG-GT-TA---CCTAGAA 30
4WW-4_8/24/2001       ------CTCCGCGCAGTGTAAGGCGTGTGT-CCAGCCGA-TGCCAGGTGACACTCTATGA 52
43W-4_9/20/2000-M_    ----------------------------TC-CTACCTGA-TCCGAGGTC-TAACCTAGAA 29
1W-4_7/27/2000-M_     -----------CTACTTCTGGGGCGAGGGT-TCAGGTG--TGTCATCCT-----ATCAAA 41
31W-4_8/20/2000-M_    ----------------TATCCTACCTGATC-CGAGGTC--TACC-----------TAGAA 30
53W-4_9/20/2000-M_    ----------------TATCCTACCTGATC-CGAGGTC--AACC-----------TAGAA 30
17W-4_8/30/2001-K     ---------------------TATCCCTAC-CA-GATC--CGAG-GTNTA--CCTAGACN 32
50WF-S-410/7/2001-P   --------------------GTGTCC-TAC-CA-GATC--CGAGCGTCTA--CCTAGACA 33
58WFS1-4_10/5/2001-K  ------------------GGTATGCGCTAC-CTAGATC--CGAG-GTCAAC-CTAGGAGA 37
6W-4_7/27/2000-M_     -----------------TGGTATGC-CTAC-CT-GATC--CGAG-GTCTAA-CCTAGAGA 36
25W-4_8/20/2000-M_    ---------------------TATC-CTAC-CT-GATC--CGAG-GTCTA--CCTAGA-A 30
32W-4_8/20/2000-M_    ---------------------AGTCGCTAC-CT-GATCG-CGAG-GTCAA--CCTAGAGA 33
4W-4_7/27/2000-M_     ------------------GTAGTCCGCTAC-CT-GATCG-CGAG-GTCAA--CCTAGTAA 36
8W-4_7/27/2000-M_     -------------------------------CT-GATC--CGAG-GTCAA--CCTAG-AA 22
7W-4_7/27/2000-M_     -----------------TGGTAGTCGCTAC-CT-GATCG-CGAG-GTCAA--CCTAGTAA 37
30W-4_8/20/2000-M_    ---------------------TATCCCTAC-CT-GATC--CGAG-GTCTA--CCTAGA-A 31
12W-4_8/24/2001-P     -------------TACCGGGTAGTCCCTAC-CTAGATC--CGAG-GTCAA---CCTAGAA 40
27W-4_9/22/2001-P     -------------------GTGATCCCTAC-CTAGATC--CGAG-GTCTA---CCTAGAA 34
2W-4_8/24/2001-M      -------------------GTAT-NCCTAC-CT-GATC--CGAG-GTCAA---CCTAGAA 32
57WF-S-4_10/5/2001-K  ----AGNNANTTACCGCGGGTAT-CCCTAC-CT-GATC--CGAG-GTCAA---CCTAGAA 47
8W-4_8/24/2001-P      -----AGNNANTTNCGCGGGTAT-CCCTAC-CT-GATC--CGAG-GTCAA---CCTAGAA 46
5W-4_8/24/2001-M      GGCGAAGATAGTAAC-CGGGTAT-CCCTAC-CT-GATC--CGAG-GTCAA---CCTAGAA 50
58WFS-4_10/5/2001-K   -----AGATAGTTACGCGGGTAT-CCCTAC-CT-GATC--CGAG-GTCAA---CCTAGAA 46
14W-4_8/30/2001-K     ------------------------TCCTAC-CT-GATC--CGAG-GTCAA---CCTAGAA 28
27P-4_9/22/2001-P     ------GATAGTTACGCGGGTAT-CCCTAC-CT-GATC--CGAG-GTCAA---CCTAGAA 45
58WFS2-4_10/5/2001-K  ----------TGTACGCGGGTAT-CCCTAC-CT-GATC--CGAG-GTCAA---CCTAGAA 41
50WFS3-4_10/7/2001-P  -----AGATAGTTACCCGGGTAT-CCCTAC-CT-GATC--CGAG-GTCAA---CCTAGAA 46
7W-4_8/24/2001-P      ----CCAATAGTTAGCCGGGTATGCCCTAC-CT-GATC--CGAG-GTCAA---CCTAGAA 48
41WFW-4_10/14/2001-M  ------------------GGTAT-CCCTAC-CT-GATC--CGAG-GTCAA---CCTAGAA 33
44WFW-4_10/14/2001-M  -------------------GGTATCCCTAC-CA-GATC--CGAG-GTCAA---CCTAGAA 33
26W-4_9/22/2001-M     --------------------GTATCC-TAC-CA-GATC--CGAG-GTCTA--CCTAGANA 32
46WFW-4_10/14/2001-M  -------------------GGTATCC-TAC-CA-GATC--CGAG-GTCTA--CCTAGANA 33
53WFW-4_10/7/2001-K   -------------------GGTATCCCTAC-CATGATC--CGAG-GTCTN--ACCTAGAA 35
6W-4_8/24/2001-M      ----------------------TATCCTAC-CT-GATC--CGAG-GTCAA---CCTAGAA 30
22W-4_8/20/2000-M     --------------------GTATCCCTAC-CT-GATC--CGAG-GTCTA---CCTAGAA 32
24W-4_8/20/2000-M     ---------------------TATCC-TAC-CT-GATC--CGAG-GTCTA---CCTAGAA 30
44W-4_9/20/2000-M_    ---------------------TATCC-TAC-CT-GATC--CGAG-GTCTA---CCTAGAA 30
45W-4_9/20/2000-M_    ---------------------TATCC-TAC-CT-GATC--CGAG-GTNTA---CCTAGAA 30
10W-4_8/24/2001-P     ---------------------TATCCCTAC-CTNGATC--CGAG-GTCAA---CCTAGAA 32
23W-4_8/20/2000-M_    ------------------------TGCTAC-CTGTATCG-CTGAGGTCAGA-CCTAGAGA 33
21W-4_8/20/2000-M_    ----------------TAGGTCGCGCTTACACTGGATCGCTGAGCGCTCGAACGCTAGAG 44
11W-4_7/27/2000-M_    ------------------------------------------------------------
```

```
                                                      ITS1
                          |--------------------------------------------------------
5W-4_7/27/2000-M_         ACGTGCGTGCA--CCCTGTCTCGATCACACGCTACCTAGTGTTCTGTGATAT---GAAAA 102
13W-4_7/27/2000-M_        GCGT---------TCCTTTCTCGAT-ACACGCTACT--GTGTTCTG-GATAT---GAAAA 80
3W-4_7/27/2000-M_         AAATAA----A-----GGTT-TCAGT-CG-GC-A-G-AGTTC-CTCTCCTTT---GACA- 71
12W-4_7/27/2000-M_        AAATAA----A-----GGTT-TCAGT-CG-GC-A-G-AGTTC-CTCTCCTTT---GACA- 70
4WW-4_8/24/2001           AAATAC----C-----GGTTTGACGTGCG-GC-A-A-AGTTC-CGCTCCTTT---GACA- 94
43W-4_9/20/2000-M_        AAATAA----A-----GGTTT--CAGTCG-GC-A-G-AGTTC-CTCTCCTTT---GACA- 69
1W-4_7/27/2000-M_         AATAAA----A-----GGTTT--CAGTCG-GC-A-G-A-TTC-CTCTCCTTT---GACA- 80
31W-4_8/20/2000-M_        AAATAA----A-----GGTTT--CAGTCG-GC-A-G-AGTTC-CTCTCCTTT---GACA- 70
53W-4_9/20/2000-M_        AAATAA----A-----GGTTT--CAGTCG-GC-A-G-AGTTC-CTCTCCTTT---GACA- 70
17W-4_8/30/2001-K         AAATAA----A-----GGTTTCANGTCAG-GC-A-G-AGTTC-CTCTCCTTT---GACA- 74
50WF-S-410/7/2001-P       AA-TAA----A-----GGTTTCATGTCAG-GC-A-G-AGTTCTCTCTCCTTT---GACA- 75
58WFS1-4_10/5/2001-K      AAATAA----A-----GGTTTCA-GTGCG-TGCA-G-AGTTC-CTCTCCTTT---GACA- 79
6W-4_7/27/2000-M_         AAATAA----A-----GGTATCAAGTGCG-GCAG-C-AGTTC-CTCTCCTTT---GACA- 79
25W-4_8/20/2000-M_        AAATAA----A-----GGTT-TCAGT-CG-GC-A-G-AGTTC-CTCTCCTTT---GACA- 70
32W-4_8/20/2000-M_        AAATAA----A-----GGTGATCAGTGCG-GC-A-G-AGTTC-CTCTCCTTT---GACA- 75
4W-4_7/27/2000-M_         AAATAA----A-----GGTGTCA-GT-CG-GC-A-G-AGTTC-CTCTCCTTT---GACA- 76
8W-4_7/27/2000-M_         AAATAA----A-----GGTTTCA-GT-CG-GC-A-G-AGTTC-CTCTCCTTT---GACA- 62
7W-4_7/27/2000-M_         AAATAA----A-----GGTGTCA-GT-CG-GC-A-G-AGTTC-CTCTCCTTT---GACA- 77
30W-4_8/20/2000-M_        AAATAA----A-----GGTTTCA-GT-CG-GC-A-G-AGTTC-CTCTCCTTT---GACA- 71
12W-4_8/24/2001-P         AAATAA----A-----GGTTNTCAGT-CG-GC-A-G-AGTTC-CTCTCCTTT---GACA- 81
27W-4_9/22/2001-P         AAATAA----A-----GGTTT-CAGT-CG-GC-A-G-AGTTC-CTCTCCTTT---GACA- 74
2W-4_8/24/2001-M          AAATAA----A-----GGTT-TCAGT-CG-GC-A-G-AGTTC-CTCTCCTTT---GACA- 72
57WF-S-4_10/5/2001-K      AAATAA----A-----GGTT-TCAGT-CG-GC-A-G-AGTTC-CTCTCCTTT---GACA- 87
8W-4_8/24/2001-P          AAATAA----A-----GGTT-TCAGT-CG-GC-A-G-AGTTC-CTCTCCTTT---GACA- 86
5W-4_8/24/2001-M          AAATAA----A-----GGTTGTCAGT-CG-GC-A-G-AGTTC-CTCTCCTTT---GACA- 91
58WFS-4_10/5/2001-K       AAATAA----A-----GGTT-TCAGT-CG-GC-A-G-AGTTC-CTCTCCTTT---GACA- 86
14W-4_8/30/2001-K         AAATAA----A-----GGTT-TCAGT-CG-GC-A-G-AGTTC-CTCTCCTTT---GACA- 68
27P-4_9/22/2001-P         AAATAA----A-----GGTT-TCAGT-CG-GC-A-G-AGTTC-CTCTCCTTT---GACA- 85
58WFS2-4_10/5/2001-K      AAATAA----A-----GGTT-TCAGT-CG-GC-A-G-AGTTC-CTCTCCTTT---GACA- 81
50WFS3-4_10/7/2001-P      AAATAA----A-----GGTT-TCAGT-CG-GC-A-G-AGTTC-CTCTCCTTT---GACA- 86
7W-4_8/24/2001-P          AAATAA----A-----GGTT-TCAGT-CG-GC-A-G-AGTTC-CTCTCCTTT---GACA- 88
41WFW-4_10/14/2001-M      AAATAA----A-----GGTT-TCAGT-CG-GC-A-G-AGTTC-CTCTCCTTT---GACA- 73
44WFW-4_10/14/2001-M      AAATAA----A-----GTGTTTCAGT-CG-GC-A-G-AGTTC-CTCTCCTTT---GACA- 74
26W-4_9/22/2001-M         AATAAA----G-----NGTT-TCAGT-CG-GC-A-G-AGTTC-CTCTCCTTT---GACA- 72
46WFW-4_10/14/2001-M      AAATAA----A-----GGTT-TCAGT-CG-GC-A-G-AGTTC-CTCTCCTTT---GACA- 73
53WFW-4_10/7/2001-K       AAATAA----A-----GGTT-TCAGT-CG-GC-A-G-AGTTC-CTCTCCTTT---GACA- 75
6W-4_8/24/2001-M          AAATAA----A-----GNGTTTCAGT-CG-GC-A-G-AGTTC-CTCTCCTTT---GACA- 71
22W-4_8/20/2000-M         AAATAA----A-----GGTT-TCAGT-CG-GC-A-G-AGTTC-CTCTCCTTT---GACA- 72
24W-4_8/20/2000-M         AAATAA----A-----GGTT-TCAGT-CG-GC-A-G-AGTTC-CTCTCCTTT---GACA- 70
44W-4_9/20/2000-M_        AAATAA----A-----GGTT-TCAGT-CG-GC-A-G-AGTTC-CTCTCCTTT---GACA- 70
45W-4_9/20/2000-M_        AAATAA----A-----GGTT-TCAGT-CG-GC-A-G-AGTTC-CTCTCCTTT---GACA- 70
10W-4_8/24/2001-P         AAATAA----A-----GTGTTTCAGT-CG-GC-A-G-AGTTC-CTCTCCTTT---GACA- 73
23W-4_8/20/2000-M_        GAATAATAAAG-----GGTATTCAGTGGT-GCCA-GCAGTCTCCTCTCCTTCTC-GACAC 85
21W-4_8/20/2000-M_        AGATAATTATAAGAGTGATTCAAAGTGCGTGCCATGCAGTCTCCTCTCCTTCTCGAACAC 104
11W-4_7/27/2000-M_        ------------------------------------------------------------
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ITS1

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5W-4_7/27/2000-M_          ATGCTTC---ATCACTTGTATC-TGTACCGCTC-----GTATATAGGGACGACGGTGGTA 153
13W-4_7/27/2000-M_         ---TCTT---CTCACTTAC------TGTCGCT-------TATATAGCGCCG--GGTAG-- 117
3W-4_7/27/2000-M_          G-ACGT----TCGA-ATAAATT--CTACTAC-------GCCTA-A-AGCCGGAG--TG-- 110
12W-4_7/27/2000-M_         G-ACGT----TCGA-ATAAATT--CTACTAC-------GCCTA-A-AGCCGGAG--TG-- 109
4WW-4_8/24/2001            G-ACGT----TCGA-ATAAATT--CTACTAC-------GCCATAA-AGCCGGAG--TG-- 134
43W-4_9/20/2000-M_         G-ACGT----TCGA-ATAAATT--CTACTAC-------GCC-TAA-AGCCGGAG--TG-- 108
1W-4_7/27/2000-M_          G-ACGT----TCGA-ATAAATT--CTACTAC-------GCC-TAA-AGCCGGAG--TG-- 119
31W-4_8/20/2000-M_         G-ACGT----TCGA-ATAAATT--CTACTAC-------GCC-TAA-AGCCGGAG--TG-- 109
53W-4_9/20/2000-M_         G-ACGT----TCGA-ATAAATT--CTACTAC-------GCC-TAA-AGCCGGAG--TG-- 109
17W-4_8/30/2001-K_         G-ACGT----TCGA-ATAAATT-C-TACTAC-------GCCTA-A-AGCCGGAG--TG-- 113
50WF-S-410/7/2001-P        G-ACGT----TCGA-ATAAATT-CGTACTAC-------GCCTAGA-AGCCGGAG--TG-- 116
58WFS1-4_10/5/2001-K       G-ACGTG---TCGA-ATGAAAT-TCTACTAC-------GCCTA-A-AGCCGGAG--TG-- 120
6W-4_7/27/2000-M_          G-ACGTG---TCGA-AT-AAAT-TCTACTAC-------GCCTA-A-AGCCGGAG--TG-- 119
25W-4_8/20/2000-M_         G-ACGTG---TCGA-AT-AAAT-TCTACTAC-------GCCTA-A-AGCCGGAG--TG-- 110
32W-4_8/20/2000-M_         G-ACGTG---TCGA-AT-AAAT-TCTACTAC-------GCCTA-A-AGCCGGAG--TG-- 115
4W-4_7/27/2000-M_          G-ACGTG---TCGA-ATGAAATGTCTACTAC-------GCCTA-A-AGCCGGAG--TG-- 118
8W-4_7/27/2000-M_          G-ACGT----TCGA-ATAAAT--TCTACTAC-------GCCTA-A-AGCCGGAG--TG-- 101
7W-4_7/27/2000-M_          G-ACGTG---TCGA-ATGAAATGTCTACTAC-------GCCTA-A-AGCCGGAG--TG-- 119
30W-4_8/20/2000-M_         G-ACGT----TCGA-ATAAATT--CTACTAC-------GCCTA-A-AGCCGGAG--TG-- 110
12W-4_8/24/2001-P_         G-ACGT----TCGA-ATAAATT--CTACTAC-------GCCTA-A-AGCCGGAG--TG-- 120
27W-4_9/22/2001-P          G-ACGT----TCGA-ATAAATT--CTACTAC-------GCCTA-A-AGCCGGAG--TG-- 113
2W-4_8/24/2001-M           G-ACGT----TCGA-ATAAATT--CTACTAC-------GCCTA-A-AGCCGGAG--TG-- 111
57WF-S-4_10/5/2001-K       G-ACGT----TCGA-ATAAATT--CTACTAC-------GCCTA-A-AGCCGGAG--TG-- 126
8W-4_8/24/2001-P           G-ACGT----TCGA-ATAAATT--CTACTAC-------GCCTA-A-AGCCGGAG--TG-- 125
5W-4_8/24/2001-M           G-ACGT----TCGA-ATAAATT--CTACTAC-------GCCTA-A-AGCCGGAG--TG-- 130
58WFS-4_10/5/2001-K        G-ACGT----TCGA-ATAAATT--CTACTAC-------GCCTA-A-AGCCGGAG--TG-- 125
14W-4_8/30/2001-K          G-ACGT----TCGA-ATAAATT--CTACTAC-------GCCTA-A-AGCCGGAG--TG-- 107
27P-4_9/22/2001-P          G-ACGT----TCGA-ATAAATT--CTACTAC-------GCCTA-A-AGCCGGAG--TG-- 124
58WFS2-4_10/5/2001-K       G-ACGT----TCGA-ATAAATT--CTACTAC-------GCCTA-A-AGCCGGAG--TG-- 120
50WFS3-4_10/7/2001-P       G-ACGT----TCGA-ATAAATT--CTACTAC-------GCCTA-A-AGCCGGAG--TG-- 125
7W-4_8/24/2001-P           G-ACGT----TCGA-ATAAATT--CTACTAC-------GCCTA-A-AGCCGGAG--TG-- 127
41WFW-4_10/14/2001-M       G-ACGT----TCGA-ATAAATT--CTACTAC-------GCCTA-A-AGCCGGAG--TG-- 112
44WFW-4_10/14/2001-M       G-ACGT----TCGA-ATAAATT--CTACTAC-------GCCTA-A-AGCCGGAG--TG-- 113
26W-4_9/22/2001-M          G-ACGT----TCGA-ATAAATT--CTACTAC-------GCCTA-A-AGCCGGAG--TG-- 111
46WFW-4_10/14/2001-M       G-ACGT----TCGA-ATAAATT--CTACTAC-------GCCTA-A-AGCCGGAG--TG-- 112
53WFW-4_10/7/2001-K        G-ACGT----TCGA-ATAAATT--CTACTAC-------GCCTA-A-AGCCGGAG--TG-- 114
6W-4_8/24/2001-M           G-ACGT----TCGA-ATAAATT--CTACTAC-------GCCTA-A-AGCCGGAG--TG-- 110
22W-4_8/20/2000-M          G-ACGT----TCGA-ATAAATT--CTACTAC-------GCCTA-A-AGCCGGAG--TG-- 111
24W-4_8/20/2000-M          G-ACGT----TCGA-ATAAATT--CTACTAC-------GCCTA-A-AGCCGGAG--TG-- 109
44W-4_9/20/2000-M_         G-ACGT----TCGA-ATAAATT--CTACTACA------GCCTA-A-AGCCGGAG--TG-- 110
45W-4_9/20/2000-M_         G-ACGT----TCGA-ATAAATT--CTACTACN------GCCTA-A-AGCCGGAG--TG-- 110
10W-4_8/24/2001-P_         G-ACGT----TCGA-ATAAATT--CTACTAC-------GCCTA-A-AGCCGGAG--TG-- 112
23W-4_8/20/2000-M_         G-ACGTG---TCGA-ATAAATC-TCTACTAC-------GCCTA-A-AGCCGGAGA-TG-- 127
21W-4_8/20/2000-M_         GCACGTGTTCTGGATATGAAATGCTTCTCACTTACTGTCCTTATATAGCCGGAGATCG-- 162
11W-4_7/27/2000-M_         ------------------------------------------------------------
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                                                   ITS1
                        ------------------------------------------------------------
5W-4_7/27/2000-M_       CGATCGTCGTGCACTCTCGCGGTCCTCCCGTACAGATGATGCCTCTGTCAGAGGGTGGCG 213
13W-4_7/27/2000-M_      -----ATGGGGCTCTCCGGC-------TCGTA--AGTGAT-TCTCTGTCATAGG--GGCG 160
3W-4_7/27/2000-M_       -----GCCTC-GCC-GAGG------TCTT-TAAGG-CGC--GCCCAA-CTAAGG---ACG 149
12W-4_7/27/2000-M_      -----GCCTC-GCCCGAGG------TCTT-TAAGG-CGC--GCCCAAACTAAGG---ACG 150
4WW-4_8/24/2001         -----GCCTC-G-CCGAGG------TCTT-TAAGG-CGC--GCCCAACT-AAGG---ACG 173
43W-4_9/20/2000-M_      -----GCCTC-G-CCGAGG------TCTT-TAAGG-CGC--GCCCAACT-AAGG---ACG 147
1W-4_7/27/2000-M_       -----GCCTC-G-CCGAGG------TCTT-TAAGG-CGC--GCCCAACT-AAGG---ACG 158
31W-4_8/20/2000-M_      -----GCCTC-G-CCGAGG------TCTT-TAAGG-CGC--GCCCAACT-AAGG---ACG 148
53W-4_9/20/2000-M_      -----GCCTC-G-CCGAGG------TCTT-TAAGG-CGC--GCCCAACT-AAGG---ACG 148
17W-4_8/30/2001-K       -----GCCTC-G-CCGAGG------TCTT-TAAGG-CGC--GCCCAACT-AAGG---ACG 152
50WF-S-410/7/2001-P     -----GCCTC-G-CCGAGG------TCTG-TAAGG-CGC--GCCCAACTGAAGG---ACG 156
58WFS1-4_10/5/2001-K    -----GCCTC-GGCCGAGG------TCTT-TAAGG-CGC--GCCCAACT-AAGG---ACG 160
6W-4_7/27/2000-M_       -----GCCTC-GGCCGAGG------TCTT-TAAGG-CGC--GCCCAACT-AAGG---ACG 159
25W-4_8/20/2000-M_      -----GCCTC-G-CCGAGG------TCTT-TAAGG-CGC--GCCCAACT-AAGG---ACG 149
32W-4_8/20/2000-M_      -----GCCTC-G-CCGAGG------TCTT-TAAGG-CGC--GCCCAACT-AAGG---ACG 154
4W-4_7/27/2000-M_       -----GCCTC-G-CCGAGG------TCTT-TAAGG-CGC--GCCCAACT-AAGG---ACG 157
8W-4_7/27/2000-M_       -----GCCTC-G-CCGAGG------TCTT-TAAGG-CGC--GCCCAACT-AAGG---ACG 140
7W-4_7/27/2000-M_       -----GCCTC-G-CCGAGG------TCTT-TAAGG-CGC--GCCCAACT-AAGG---ACG 158
30W-4_8/20/2000-M_      -----GCCTC-G-CCGAGG------TCTT-TAAGG-CGC--GCCCAACT-AAGG---ACG 149
12W-4_8/24/2001-P       -----GCCTC-G-CCGAGG------TCTT-TAAGG-CGC--GCCCAACT-AAGG---ACG 159
27W-4_9/22/2001-P       -----GCCTC-G-CCGAGG------TCTT-TAAGG-CGC--GCCCAACT-AAGG---ACG 152
2W-4_8/24/2001-M        -----GCCTC-G-CCGAGG------TCTT-TAAGG-CGC--GCCCAACT-AAGG---ACG 150
57WF-S-4_10/5/2001-K    -----GCCTC-G-CCGAGG------TCTT-TAAGG-CGC--GCCCAACT-AAGG---ACG 165
8W-4_8/24/2001-P        -----GCCTC-G-CCGAGG------TCTT-TAAGG-CGC--GCCCAACT-AAGG---ACG 164
5W-4_8/24/2001-M        -----GCCTC-G-CCGAGG------TCTT-TAAGG-CGC--GCCCAACT-AAGG---ACG 169
58WFS-4_10/5/2001-K     -----GCCTC-G-CCGAGG------TCTT-TAAGG-CGC--GCCCAACT-AAGG---ACG 164
14W-4_8/30/2001-K       -----GCCTC-G-CCGAGG------TCTT-TAAGG-CGC--GCCCAACT-AAGG---ACG 146
27P-4_9/22/2001-P       -----GCCTC-G-CCGAGG------TCTT-TAAGG-CGC--GCCCAACT-AAGG---ACG 163
58WFS2-4_10/5/2001-K    -----GCCTC-G-CCGAGG------TCTT-TAAGG-CGC--GCCCAACT-AAGG---ACG 159
50WFS3-4_10/7/2001-P    -----GCCTC-G-CCGAGG------TCTT-TAAGG-CGC--GCCCAACT-AAGG---ACG 164
7W-4_8/24/2001-P        -----GCCTC-G-CCGAGG------TCTT-TAAGG-CGC--GCCCAACT-AAGG---ACG 166
41WFW-4_10/14/2001-M    -----GCCTC-G-CCGAGG------TCTT-TAAGNGCGC--GCCCAACT-AAGG---ACG 152
44WFW-4_10/14/2001-M    -----GCCTC-G-CCGAGG------TCTT-TAAGNGCGC--GCCCAACTAAGNG---ACG 154
26W-4_9/22/2001-M       -----GCCTC-G-CCGAGG------TCTT-TAAGNGCGC--GCCCAACTAAGNN---ACG 152
46WFW-4_10/14/2001-M    -----GCCTC-G-CCGAGG------TCTT-TAAGG-CGC--GCCCAACTAAGGA----CG 151
53WFW-4_10/7/2001-K     -----GCCTC-G-CCGAGG------TCTT-TAAGNGCGC--GCCCAACTAAGNG---ACG 155
6W-4_8/24/2001-M        -----GCCTC-G-CCGAGG------TCTT-TAAGNGCGC--GCCCAACTAAGNG---ACG 151
22W-4_8/20/2000-M       -----GCCTC-G-CCGAGG------TCTT-TAAGG-CGC--GCCCAACT-AAGG---ACG 150
24W-4_8/20/2000-M       -----GCCTC-GTCCGAGG------TCTT-TAAGG-CGC--GCCCAACT-AAGG---ACG 149
44W-4_9/20/2000-M_      -----GCCTC-GTCCGAGG------TCTT-TAAGG-CGC--GCCCAACT-AAGG---ACG 150
45W-4_9/20/2000-M_      -----GCCTC-GTCCGAGG------TCTT-TAAGG-CGC--GCCCAACT-AAGG---ACG 150
10W-4_8/24/2001-P       -----GCCTC-G-CCGAGG------TCTT-TAAGNGCGC--GCCCAACT-AAGG---ACG 152
23W-4_8/20/2000-M_      -----GCCTC-G-CCGAGG------TCTT-TAAGGCGACT-GCCCAACTAAGCG---ACG 169
21W-4_8/20/2000-M_      -----GCCTCCGGCCGTAGGA-TGCTCTTATAGGGCGACT-GCCCTAACTAAGGC-GACG 214
11W-4_7/27/2000-M_      ------------------------------------------------------------
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                                                    ITS1
                          ------------------------------------------------------------
5W-4_7/27/2000-M_         GACTGTACCGCTACACCCTGAGAGGCGAAACCGCAGCCGCACCGCCACATTGAACGCTAT 273
13W-4_7/27/2000-M_        GACTGTCCCGCTACACC-TAAGAGGCGA---CGCA-CCGCCCCGC-ACAT-GAACGCTAA 213
3W-4_7/27/2000-M_         A--CGCCC-AATA--CC-----AAGC-ATAGCTTGAG--TGGTGT---AATG-ACGCTC- 191
12W-4_7/27/2000-M_        A--CGCCCCAATA--CC-----AAGCCATAGCCTGGAGTTGGTGTTAAAATGGACGCCTC 201
4WW-4_8/24/2001           A--CGCCC-AATA--CC-----AAGCAT-AGCTTG-AG-TGGTGT---AAT-GACGCTC- 215
43W-4_9/20/2000-M_        A--CGCCC-AATA--CC-----AAGCAT-AGCTTG-AG-TGGTGT---AAT-GACGCTC- 189
1W-4_7/27/2000-M_         A--CGCCC-AATA--CC-----AAGCAT-AGCTTG-AG-TGGTGT---AAT-GACGCTC- 200
31W-4_8/20/2000-M_        A--CGCCC-AATA--CC-----AAGCAT-AGCTTG-AG-TGGTGT---AAT-GACGCTC- 190
53W-4_9/20/2000-M_        A--CGCCC-AATA--CC-----AAGCAT-AGCTTG-AG-TGGTGT---AAT-GACGCTC- 190
17W-4_8/30/2001-K         A--CGCCC-AATA--CC-----AAGCAT-AGCTTG-AG-TGGTGT---AAT-GACGCTC- 194
50WF-S-410/7/2001-P       A--CGCCC-AATA--CC-----AAGCAT-AGCTTG-AG-TGGTGT---AATAGACGCTC- 199
58WFS1-4_10/5/2001-K      A--CGCCC-AATA--CC-----AAGCAT-AGCTTG-AG-TGGTGT---AAT-GACGCTC- 202
6W-4_7/27/2000-M_         A--CGCCC-AATA--CC-----AAGCAT-AGCTTG-AG-TGGTGT---AAT-GACGCTC- 201
25W-4_8/20/2000-M_        A--CGCCC-AATA--CC-----AAGCAT-AGCTTG-AG-TGGTGT---AAT-GACGCTC- 191
32W-4_8/20/2000-M_        A--CGCCC-AATA--CC-----AAGCAT-AGCTTG-AG-TGGTGT---AAT-GACGCTC- 196
4W-4_7/27/2000-M_         A--CGCCC-AATA--CC-----AAGCATGAGCTTG-AG-TGGTGT---AAT-GACGCTC- 200
8W-4_7/27/2000-M_         A--CGCCC-AATA--CC-----AAGCAT-AGCTTG-AG-TGGTGT---AAT-GACGCTC- 182
7W-4_7/27/2000-M_         A--CGCCC-AATA--CC-----AAGCAT-AGCTTG-AG-TGGTGT---AAT-GACGCTC- 200
30W-4_8/20/2000-M_        A--CGCCC-AATA--CC-----AAGCAT-AGCTTG-AG-TGGTGT---AAT-GACGCTC- 191
12W-4_8/24/2001-P         A--CGCCC-AATA--CC-----AAGCAT-AGCTTG-AG-TGGTGT---AAT-GACGCTC- 201
27W-4_9/22/2001-P         A--CGCCC-AATA--CC-----AAGCAT-AGCTTG-AG-TGGTGT---AAT-GACGCTC- 194
2W-4_8/24/2001-M          A--CGCCC-AATA--CC-----AAGCAT-AGCTTG-AG-TGGTGT---AAT-GACGCTC- 192
57WF-S-4_10/5/2001-K      A--CGCCC-AATA--CC-----AAGCAT-AGCTTG-AG-TGGTGT---AAT-GACGCTC- 207
8W-4_8/24/2001-P          A--CGCCC-AATA--CC-----AAGCAT-AGCTTG-AG-TGGTGT---AAT-GACGCTC- 206
5W-4_8/24/2001-M          A--CGCCC-AATA--CC-----AAGCAT-AGCTTG-AG-TGGTGT---AAT-GACGCTC- 211
58WFS-4_10/5/2001-K       A--CGCCC-AATA--CC-----AAGCAT-AGCTTG-AG-TGGTGT---AAT-GACGCTC- 206
14W-4_8/30/2001-K         A--CGCCC-AATA--CC-----AAGCAT-AGCTTG-AG-TGGTGT---AAT-GACGCTC- 188
27P-4_9/22/2001-P         A--CGCCC-AATA--CC-----AAGCAT-AGCTTG-AG-TGGTGT---AAT-GACGCTC- 205
58WFS2-4_10/5/2001-K      A--CGCCC-AATA--CC-----AAGCAT-AGCTTG-AG-TGGTGT---AAT-GACGCTC- 201
50WFS3-4_10/7/2001-P      A--CGCCC-AATA--CC-----AAGCAT-AGCTTG-AG-TGGTGT---AAT-GACGCTC- 206
7W-4_8/24/2001-P          A--CGCCC-AATA--CC-----AAGCAT-AGCTTG-AG-TGGTGT---AAT-GACGCTC- 208
41WFW-4_10/14/2001-M      A--CGCCC-AATA--CC-----AAGCAT-AGCTTG-AG-TGGTGT---AAT-GACGCTC- 194
44WFW-4_10/14/2001-M      A--CGCCC-AATA--CC-----AAGCAT-AGCTTG-AG-TGGTGT---AAT-GACGCTC- 196
26W-4_9/22/2001-M         A--CGCCC-AATA--CC-----AAGCAT-AGCTTG-AG-TGGTGT---AAT-GACGCTC- 194
46WFW-4_10/14/2001-M      A--CGCCC-AATA--CC-----AAGCAT-AGCTTG-AG-TGGTGT---AAT-GACGCTC- 193
53WFW-4_10/7/2001-K       A--CGCCC-AATA--CC-----AAGCAT-AGCTTG-AG-TGGTGT---AAT-GACGCTC- 197
6W-4_8/24/2001-M          A--CGCCC-AATA--CC-----AAGCAT-AGCTTG-AG-TGGTGT---AAT-GACGCTC- 193
22W-4_8/20/2000-M         A--CGCCC-AATA--CC-----AAGCAT-AGCTTG-AG-TGGTGT---AAT-GACGCTC- 192
24W-4_8/20/2000-M         A--CGCCC-AATA--CC-----AAGCAT-AGCTTG-AG-TGGTGT---AAT-GACGCTC- 191
44W-4_9/20/2000-M_        A--CGCCC-AATA--CC-----AAGCAT-AGCTTG-AG-TGGTGT---AAT-GACGCTC- 192
45W-4_9/20/2000-M_        A--CGCCC-AATA--CC-----AAGCAT-AGCTTG-AG-TGGTGT---AAT-GACGCTC- 192
10W-4_8/24/2001-P         A--CGCCC-AATA--CC-----AAGCAT-AGCTTG-AG-TGGTGT---AAT-GACGCTC- 194
23W-4_8/20/2000-M_        CACCGCCC-AATAACCT-----AAGCATAGCTCTGCAG-TGGTGT---AAT-GACGCTC- 217
21W-4_8/20/2000-M_        CACCGCCCACATAACCT-----AAGCAT-AGCTCTGCATGTGTGTAGTAAT-GACGCTC- 266
11W-4_7/27/2000-M         ------------------------------------------------------------
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                                                           ITS1
                            --------------------------------------------------------|-----
5W-4_7/27/2000-M_           AAGGACCATTGCAAGA--ACATCTGGGCATGGTGTGTGGTTAGGTTGT-----AATATTG 326
13W-4_7/27/2000-M_          AGACATCAAGACATCT--GCATGTTG-TGTAGTATATAGCACGGCTCG-----TACAC-A 264
3W-4_7/27/2000-M_           -GAAC---AGGCATG----CCCCTCGGAATA-----CCAAGGGG-CGC-------AATGT 230
12W-4_7/27/2000-M_          GGAACCAGGGGCATGGCCCCCCCTCGGGAAAATAACCCAAGGGG-GGCCGCCAAGGGTGG 260
4WW-4_8/24/2001             G-AACA---GGCATG----CCCCTCGGAATAC-----CAAGGGG-CGC-------AATGT 254
43W-4_9/20/2000-M_          G-AACA---GGCATG----CCCCTCGGAATAC-----CAAGGGG-CGC-------AATGT 228
1W-4_7/27/2000-M_           G-AACA---GGCATG----CCCCTCGGAATAC-----CAAGGGG-CGC-------AATGT 239
31W-4_8/20/2000-M_          G-AACA---GGCATG----CCCCTCGGAATAC-----CAAGGGG-CGC-------AATGT 229
53W-4_9/20/2000-M_          G-AACA---GGCATG----CCCCTCGGAATAC-----CAAGGGG-CGC-------AATGT 229
17W-4_8/30/2001-K           G-AACA---GGCATG----CCCCTCGGAATAC-----CAAGGGG-CGC-------AATGT 233
50WF-S-410/7/2001-P         G-AACA---GGCATG----CCCCTCGGAATAC-----CAAGGGG-CGC-------AATGT 238
58WFS1-4_10/5/2001-K        G-AACA---GGCATG----CCCCTCGGAATAC-----CAAGGGG-CGC-------AATGT 241
6W-4_7/27/2000-M_           G-AACA---GGCATG----CCCCTCGGAATAC-----CAAGGGG-CGC-------AATGT 240
25W-4_8/20/2000-M_          G-AACA---GGCATG----CCCCTCGGAATAC-----CAAGGGG-CGC-------AATGT 230
32W-4_8/20/2000-M_          G-AACA---GGCATG----CCCCTCGGAATAC-----CAAGGGG-CGC-------AATGT 235
4W-4_7/27/2000-M_           G-AACA---GGCATG----CCCCTCGGAATAC-----CAAGGGG-CGC-------AATGT 239
8W-4_7/27/2000-M_           G-AACA---GGCATG----CCCCTCGGAATAC-----CAAGGGG-CGC-------AATGT 221
7W-4_7/27/2000-M_           G-AACA---GGCATG----CCCCTCGGAATAC-----CAAGGGG-CGC-------AATGT 239
30W-4_8/20/2000-M_          G-AACA---GGCATG----CCCCTCGGAATAC-----CAAGGGG-CGC-------AATGT 230
12W-4_8/24/2001-P           G-AACA---GGCATG----CCCCTCGGAATAC-----CAAGGGG-CGC-------AATGT 240
27W-4_9/22/2001-P           G-AACA---GGCATG----CCCCTCGGAATAC-----CAAGGGG-CGC-------AATGT 233
2W-4_8/24/2001-M            G-AACA---GGCATG----CCCCTCGGAATAC-----CAAGGGG-CGC-------AATGT 231
57WF-S-4_10/5/2001-K        G-AACA---GGCATG----CCCCTCGGAATAC-----CAAGGGG-CGC-------AATGT 246
8W-4_8/24/2001-P            G-AACA---GGCATG----CCCCTCGGAATAC-----CAAGGGG-CGC-------AATGT 245
5W-4_8/24/2001-M            G-AACA---GGCATG----CCCCTCGGAATAC-----CAAGGGG-CGC-------AATGT 250
58WFS-4_10/5/2001-K         G-AACA---GGCATG----CCCCTCGGAATAC-----CAAGGGG-CGC-------AATGT 245
14W-4_8/30/2001-K           G-AACA---GGCATG----CCCCTCGGAATAC-----CAAGGGG-CGC-------AATGT 227
27P-4_9/22/2001-P           G-AACA---GGCATG----CCCCTCGGAATAC-----CAAGGGG-CGC-------AATGT 244
58WFS2-4_10/5/2001-K        G-AACA---GGCATG----CCCCTCGGAATAC-----CAAGGGG-CGC-------AATGT 240
50WFS3-4_10/7/2001-P        G-AACA---GGCATG----CCCCTCGGAATAC-----CAAGGGG-CGC-------AATGT 245
7W-4_8/24/2001-P            G-AACA---GGCATG----CCCCTCGGAATAC-----CAAGGGG-CGC-------AATGT 247
41WFW-4_10/14/2001-M        G-AACA---GGCATG----CCCCTCGGAATAC-----CAAGGGG-CGC-------AATGT 233
44WFW-4_10/14/2001-M        G-AACA---GGCATG----CCCCTCGGAATAC-----CAAGGGG-CGC-------AATGT 235
26W-4_9/22/2001-M           G-AACA---GGCATG----CCCCTCGGAATAC-----CAAGGGG-CGC-------AATGT 233
46WFW-4_10/14/2001-M        G-AACA---GGCATG----CCCCTCGGAATAC-----CAAGGGG-CGC-------AATGT 232
53WFW-4_10/7/2001-K         G-AACA---GGCATG----CCCCTCGGAATAC-----CAAGGGG-CGC-------AATGT 236
6W-4_8/24/2001-M            G-AACA---GGCATG----CCCCTCGGAATAC-----CAAGGGG-CGC-------AATGT 232
22W-4_8/20/2000-M           G-AACA---GGCATG----CCCCTCGGAATAC-----CAAGGGG-CGC-------AATGT 231
24W-4_8/20/2000-M           G-AACA---GGCATG----CCCCTCGGAATAC-----CAAGGGG-CGC-------AATGT 230
44W-4_9/20/2000-M_          G-AACA---GGCATG----CCCCTCGGAATAC-----CAAGGGG-CGC-------AATGT 231
45W-4_9/20/2000-M_          G-AACA---GGCATG----CCCCTCGGAATAC-----CAAGGGG-CGC-------AATGT 231
10W-4_8/24/2001-P           G-AACA---GGCATG----CCCCTCGGAATAC-----CAAGGGG-CGC-------AATGT 233
23W-4_8/20/2000-M_          GTAACAA--GGCATG----CCCCTCGAGATATA--CTCAAGGGG-CGC-------AATGT 261
21W-4_8/20/2000-M_          GTAACA---GGCATG---CCTCCTCGGAATATA--CTCAAGGGGTCGC-------AATGT 311
11W-4_7/27/2000-M           ----------------------------GTTT-----GGAAGAGCCAT-----CAGTCAT 22
                                                                       *
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                                                         5.8S
5W-4_7/27/2000-M_           AGGCAACCGGGCCTTCCCGGTAACATCCAAAGGGGTCCAT-TGTGGCCCACTCTCT-TTC 384
13W-4_7/27/2000-M_          AGGCAT----GCCTCCTCGAGAATATACTCAAGGG--CGT-CGCAATC----GTGC-GTA 312
3W-4_7/27/2000-M_           GCGTTC--AAAGATTC--GATGA--TTCACTG-AATTC---TGCAATTCACATTAC-TTA 279
12W-4_7/27/2000-M_          CGGTTCCAAAGGATTC--GATGAC-TTCACCGGAATTC---TGCA-TTCACATTAC-TTA 312
4WW-4_8/24/2001             GCGTT--CAAAGATTC--GATGAT-T-CACTG-AATTC---TGCAATTCACATTAC-TTA 303
43W-4_9/20/2000-M_          GCGTT--CAAAGATTC--GATGAT-T-CACTG-AATTC---TGCAATTCACATTAC-TTA 277
1W-4_7/27/2000-M_           GCGTT--CAAAGATTC--GATGAT-T-CACTG-AATTC---TGCAATTCACATTAC-TTA 288
31W-4_8/20/2000-M_          GCGTT--CAAAGATTC--GATGAT-T-CACTG-AATTC---TGCAATTCACATTAC-TTA 278
53W-4_9/20/2000-M_          GCGTT--CAAAGATTC--GATGAT-T-CACTG-AATTC---TGCAATTCACATTAC-TTA 278
17W-4_8/30/2001-K           GCGTT--CAAAGATTC--GATGAT-T-CACTG-AATTC---TGCAATTCACATTAC-TTA 282
50WF-S-410/7/2001-P         GCGTT--CAAAGATTC--GATGAT-T-CACTG-AATTC---TGCAATTCACATTAC-TTA 287
58WFS1-4_10/5/2001-K        GCGTT--CAAAGATTC--GATGAT-T-CACTG-AATTC---TGCAATTCACATTAC-TTA 290
6W-4_7/27/2000-M_           GCGTA--CAAAGATTC--GATGAT-T-CACTG-AATTC---TGCAATTCACATTAC-TTA 289
25W-4_8/20/2000-M_          GCGTA--CAAAGATTC--GATGAT-T-CACTG-AATTC---TGCAATTCACATTAC-TTA 279
32W-4_8/20/2000-M_          GCGTAT-CAAAGATTC--GATGAT-T-CACTG-AATTC---TGCAATTCACATTAC-TTA 285
4W-4_7/27/2000-M_           GCGTT--CAAAGATTC--GATGAT-T-CACTG-AATTC---TGCAATTCACATTAC-TTA 288
8W-4_7/27/2000-M_           GCGTT--CAAAGATTC--GATGAT-T-CACTG-AATTC---TGCAATTCACATTAC-TTA 270
7W-4_7/27/2000-M_           GCGTT--CAAAGATTC--GATGAT-T-CACTG-AATTC---TGCAATTCACATTAC-TTA 288
30W-4_8/20/2000-M_          GCGTT--CAAAGATTC--GATGAT-T-CACTG-AATTC---TGCAATTCACATTAC-TTA 279
12W-4_8/24/2001-P           GCGTT--CAAAGATTC--GATGAT-T-CACTG-AATTC---TGCAATTCACATTAC-TTA 289
27W-4_9/22/2001-P           GCGTT--CAAAGATTC--GATGAT-T-CACTG-AATTC---TGCAATTCACATTAC-TTA 282
2W-4_8/24/2001-M            GCGTT--CAAAGATTC--GATGAT-T-CACTG-AATTC---TGCAATTCACATTAC-TTA 280
57WF-S-4_10/5/2001-K        GCGTT--CAAAGATTC--GATGAT-T-CACTG-AATTC---TGCAATTCACATTAC-TTA 295
8W-4_8/24/2001-P            GCGTT--CAAAGATTC--GATGAT-T-CACTG-AATTC---TGCAATTCACATTAC-TTA 294
5W-4_8/24/2001-M            GCGTT--CAAAGATTC--GATGAT-T-CACTG-AATTC---TGCAATTCACATTAC-TTA 299
58WFS-4_10/5/2001-K         GCGTT--CAAAGATTC--GATGAT-T-CACTG-AATTC---TGCAATTCACATTAC-TTA 294
14W-4_8/30/2001-K           GCGTT--CAAAGATTC--GATGAT-T-CACTG-AATTC---TGCAATTCACATTAC-TTA 276
27P-4_9/22/2001-P           GCGTT--CAAAGATTC--GATGAT-T-CACTG-AATTC---TGCAATTCACATTAC-TTA 293
58WFS2-4_10/5/2001-K        GCGTT--CAAAGATTC--GATGAT-T-CACTG-AATTC---TGCAATTCACATTAC-TTA 289
50WFS3-4_10/7/2001-P        GCGTT--CAAAGATTC--GATGAT-T-CACTG-AATTC---TGCAATTCACATTAC-TTA 294
7W-4_8/24/2001-P            GCGTT--CAAAGATTC--GATGAT-T-CACTG-AATTC---TGCAATTCACATTAC-TTA 296
41WFW-4_10/14/2001-M        GCGTT--CAAAGATTC--GATGAT-T-CACTG-AATTC---TGCAATTCACATTAC-TTA 282
44WFW-4_10/14/2001-M        GCGTT--CAAAGATTC--GATGAT-T-CACTG-AATTC---TGCAATTCACATTAC-TTA 284
26W-4_9/22/2001-M           GCGTT--CAAAGATTC--GATGAT-T-CACTG-AATTC---TGCAATTCACATTAC-TTA 282
46WFW-4_10/14/2001-M        GCGTT--CAAAGATTC--GATGAT-T-CACTG-AATTC---TGCAATTCACATTAC-TTA 281
53WFW-4_10/7/2001-K         GCGTT--CAAAGATTC--GATGAT-T-CACTG-AATTC---TGCAATTCACATTAC-TTA 285
6W-4_8/24/2001-M            GCGTT--CAAAGATTC--GATGAT-T-CACTG-AATTC---TGCAATTCACATTAC-TTA 281
22W-4_8/20/2000-M           GCGTT--CAAAGATTC--GATGAT-T-CACTG-AATTC---TGCAATTCACATTAC-TTA 280
24W-4_8/20/2000-M           GCGTT--CAAAGATAC--GATGAT-T-CACTG-AATTC---TGCAATGCACATTAC-TTA 279
44W-4_9/20/2000-M_          GCGTT--CAAAGATAC--GATGAT-T-CACTG-AATTC---TGCAATTCACATTAC-TTA 280
45W-4_9/20/2000-M_          GCGTT--CAAAGATAC--GATGAT-T-CACTG-AATTC---TGCAATTCACATTAC-TTA 280
10W-4_8/24/2001-P           GCGTT--CAAAGATTC--GATGAT-T-CACTG-AATTC---TGCAATTCACATTAC-TTA 282
23W-4_8/20/2000-M_          GCGTAT-CAAAGATTC--GATGAT-TTCACTG-AATTC---TGCCATTCACATTAC-TTA 312
21W-4_8/20/2000-M_          GCGTAT-CAAAGATGCT-GATGAT-T-TACTG-AATTCATGCACATTCCACATTGCCTTA 366
11W-4_7/27/2000-M_          GCGTATTTAAATATTG--AGTCGT-TTTGGTGCGACTTCGTTTTAATTTAT-TTATCTTT 78
                              *          *           *                           *    *
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                                                 5.8S
                          -----------------------------------------------------------
5W-4_7/27/2000-M_         CCGGAGGAAA-TATGTAACCTCCAAAAGGGGGGGCGGTCTGTACAAA-ATTC-G---ATA 438
13W-4_7/27/2000-M_        TCAAAGATTG-CTGATGATCTCTACTAGAATTCATGGCACATTCCAACATTT-GCCTATA 370
3W-4_7/27/2000-M_         TCGCATTTCGCTGCGTT-CTTCATCGATGCGA-G-AACCAAGAGATCCGTT--GTTGAAA 334
12W-4_7/27/2000-M_        TCCCATT-CGCCGGGGT-TCTCATCGAGGCAA-A-ACCCAAAAACTCGGTT--GTTGGAA 366
4WW-4_8/24/2001           TCGCATTTCGCTGCGTT-CTTCATCGATGCGA-G-AACCAAGAGATCCGTT--GTTGAAA 358
43W-4_9/20/2000-M_        TCGCATTTCGCTGCGTT-CTTCATCGATGCGA-G-AACCAAGAGATCCGTT--GTTGAAA 332
1W-4_7/27/2000-M_         TCGCATTTCGCTGCGTT-CTTCATCGATGCGA-G-AACCAAGAGATCCGTT--GTTGAAA 343
31W-4_8/20/2000-M_        TCGCATTTCGCTGCGTT-CTTCATCGATGCGA-G-AACCAAGAGATCCGTT--GTTGAAA 333
53W-4_9/20/2000-M_        TCGCATTTCGCTGCGTT-CTTCATCGATGCGA-G-AACCAAGAGATCCGTT--GTTGAAA 333
17W-4_8/30/2001-K         TCGCATTTCGCTGCGTT-CTTCATCGATGCGA-G-AACCAAGAGATCCGTT--GTTGAAA 337
50WF-S-410/7/2001-P       TCGCATTTCGCTGCGTT-CTTCATCGATGCGA-G-AACCAAGAGATCCGTT--GTTGAAA 342
58WFS1-4_10/5/2001-K      TCGCATTTCGCTGCGTT-CTTCATCGATGCGA-G-AACCAAGAGATCCGTT--GTTGAAA 345
6W-4_7/27/2000-M_         TCGCATTTCGCTGCGTT-CTTCATCGATGCGA-G-AACCAAGAGATCCGTT--GTTGAAA 344
25W-4_8/20/2000-M_        TCGCATTTCGCTGCGTT-CTTCATCGATGCGA-G-AACCAAGAGATCCGTT--GTTGAAA 334
32W-4_8/20/2000-M_        TCGCATTTCGCTGCGTT-CTTCATCGATGCGA-G-AACCAAGAGATCCGTT--GTTGAAA 340
4W-4_7/27/2000-M_         TCGCATTTCGCTGCGTT-CTTCATCGATGCGA-G-AACCAAGAGATCCGTT--GTTGAAA 343
8W-4_7/27/2000-M_         TCGCATTTCGCTGCGTT-CTTCATCGATGCGA-G-AACCAAGAGATCCGTT--GTTGAAA 325
7W-4_7/27/2000-M_         TCGCATTTCGCTGCGTT-CTTCATCGATGCGA-G-AACCAAGAGATCCGTT--GTTGAAA 343
30W-4_8/20/2000-M_        TCGCATTTCGCTGCGTT-CTTCATCGATGCGA-G-AACCAAGAGATCCGTT--GTTGAAA 334
12W-4_8/24/2001-P         TCGCATTTCGCTGCGTT-CTTCATCGATGCGA-G-AACCAAGAGATCCGTT--GTTGAAA 344
27W-4_9/22/2001-P         TCGCATTTCGCTGCGTT-CTTCATCGATGCGA-G-AACCAAGAGATCCGTT--GTTGAAA 337
2W-4_8/24/2001-M          TCGCATTTCGCTGCGTT-CTTCATCGATGCGA-G-AACCAAGAGATCCGTT--GTTGAAA 335
57WF-S-4_10/5/2001-K      TCGCATTTCGCTGCGTT-CTTCATCGATGCGA-G-AACCAAGAGATCCGTT--GTTGAAA 350
8W-4_8/24/2001-P          TCGCATTTCGCTGCGTT-CTTCATCGATGCGA-G-AACCAAGAGATCCGTT--GTTGAAA 349
5W-4_8/24/2001-M          TCGCATTTCGCTGCGTT-CTTCATCGATGCGA-G-AACCAAGAGATCCGTT--GTTGAAA 354
58WFS-4_10/5/2001-K       TCGCATTTCGCTGCGTT-CTTCATCGATGCGA-G-AACCAAGAGATCCGTT--GTTGAAA 349
14W-4_8/30/2001-K         TCGCATTTCGCTGCGTT-CTTCATCGATGCGA-G-AACCAAGAGATCCGTT--GTTGAAA 331
27P-4_9/22/2001-P         TCGCATTTCGCTGCGTT-CTTCATCGATGCGA-G-AACCAAGAGATCCGTT--GTTGAAA 348
58WFS2-4_10/5/2001-K      TCGCATTTCGCTGCGTT-CTTCATCGATGCGA-G-AACCAAGAGATCCGTT--GTTGAAA 344
50WFS3-4_10/7/2001-P      TCGCATTTCGCTGCGTT-CTTCATCGATGCGA-G-AACCAAGAGATCCGTT--GTTGAAA 349
7W-4_8/24/2001-P          TCGCATTTCGCTGCGTT-CTTCATCGATGCGA-G-AACCAAGAGATCCGTT--GTTGAAA 351
41WFW-4_10/14/2001-M      TCGCATTTCGCTGCGTT-CTTCATCGATGCGA-G-AACCAAGAGATCCGTT--GTTGAAA 337
44WFW-4_10/14/2001-M      TCGCATTTCGCTGCGTT-CTTCATCGATGCGA-G-AACCAAGAGATCCGTT--GTTGAAA 339
26W-4_9/22/2001-M         TCGCATTTCGCTGCGTT-CTTCATCGATGCGA-G-AACCAAGAGATCCGTT--GTTGAAA 337
46WFW-4_10/14/2001-M      TCGCATTTCGCTGCGTT-CTTCATCGATGCGA-G-AACCAAGAGATCCGTT--GTTGAAA 336
53WFW-4_10/7/2001-K       TCGCATTTCGCTGCGTT-CTTCATCGATGCGA-G-AACCAAGAGATCCGTT--GTTGAAA 340
6W-4_8/24/2001-M          TCGCATTTCGCTGCGTT-CTTCATCGATGCGA-G-AACCAAGAGATCCGTT--GTTGAAA 336
22W-4_8/20/2000-M         TCGCATTTCGCTGCGTT-CTTCATCGATGCGA-G-AACCAAGAGATCCGTT--GTTGA-G 334
24W-4_8/20/2000-M         TCGCATTTCGCTGCGTAACTTCATCGATGCGA-G-AACCAAGAGATCCGTT--GTCGA-A 334
44W-4_9/20/2000-M_        TCGCATTTCGCTGCGTT-CTTCATCGATGCGA-G-AACCAAGAGATCCGTT--GTTGATA 335
45W-4_9/20/2000-M_        TCGCATTTCGCTGCGTT-CTTCATCGATGCGA-G-AACCAAGAGATCCGTT--GTTGANA 335
10W-4_8/24/2001-P         TCGCATTTCGCTGCGTT-CTTCATCGATGCGA-G-AACCAAGAGATCCGTT--GTTGAAA 337
23W-4_8/20/2000-M_        TCGCATTTCGCTGCGTTACTTCATCGATGCGATG-AACCTAGAGATCCGCTTCGTTGAAA 371
21W-4_8/20/2000-M_        TCGCATGTCGCTGCGTGACTTCATCGATGCGATGCAACCAAGAGATCCGTTCGTTTGACA 426
11W-4_7/27/2000-M         TACGTGGTAATAACGTGGGTTTGTGTTGGCCC---AGCCTAGAGCTTCATC---TTAAGG 132
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                                                    5.8S
                      ____________________________________________________________
5W-4_7/27/2000-M_     GCCCGGGTATTCAAA-AATGAAGTTGTTTTAC-TTTCAA-TCATTCGACCGTAGTAGAAT 495
13W-4_7/27/2000-M_    TTTCGTCCATTTGTTCGGCTTTGCCGTTTTACCTTTCAT-TTCCGAAATTGTCCGAATGC 429
3W-4_7/27/2000-M_     GTTTTG--ATTTA----TTCAAAAT--TTTAA--CTCAG-ACGACCGGTT-AAATAACAA 382
12W-4_7/27/2000-M_    AGTTTGGGATTTAA--TTCCAAAAA--TTT-----TTAA-ACCCCCGA---ACGCAACCG 413
4WW-4_8/24/2001       -GT-TTTGATTT----ATTCAAAAT--TTTAA--CTCAG-ACGACCGGTT-AAATAACAA 406
43W-4_9/20/2000-M_    -GT-TTTGATTT----ATTCAAAAT--TTTAA--CTCAG-ACGACCGGTT-AAATAACAA 380
1W-4_7/27/2000-M_     -GT-TTTGATTT----ATTCAAAAT--TTTAA--CTCAG-ACGACCGGTT-AAATAACAA 391
31W-4_8/20/2000-M_    -GT-GTTGATTT----ATTCAAAAT--TTTAA--CTCAG-ACGACCGGTT-AAATAACAA 381
53W-4_9/20/2000-M_    -GT-TTTGATTT----ATTCAAAAT--TTTAA--CTCAG-ACGACCGGTT-AAATAACAA 381
17W-4_8/30/2001-K     -GT-TTTGATTT----ATTCAAAAT--TTTAA--CTCAG-ACGACCGGTT-AAATAACAA 385
50WF-S-410/7/2001-P   -GT-TTTGATTT----ATTCAAAAT--TTTAA--CTCAG-ACGACCGGTT-AAATAACAA 390
58WFS1-4_10/5/2001-K  -GT-TTTGATTT----ATTCAAAAT--TTTAA--CTCAG-ACGACCGGTT-AAATAACAA 393
6W-4_7/27/2000-M_     CGT-TTTGATTT----ATTCAAAAT--TTCTATACTCAG-ACGACCGGTT-AAATAACAA 395
25W-4_8/20/2000-M_    -GT-TTTGATTT----ATTCAAAAT--TTTAA--CTCAG-ACGACCGGTT-AAATAACAA 382
32W-4_8/20/2000-M_    CGT-TTTGATTT----ATTCAAAAT--TTCTATACTCAG-ACGACCGGTT-AAATAACAA 391
4W-4_7/27/2000-M_     -GT-TTTGATTT----ATTCAAAAT--TTTAA--CTCAG-ACGACCGGTT-AAATAACAA 391
8W-4_7/27/2000-M_     -GT-TTTGATTT----ATTCAAAAT--TTTAA--CTCAG-ACGACCGGTT-AAATAACAA 373
7W-4_7/27/2000-M_     -GT-TTTGATTT----ATTCAAAAT--TTTAA--CTCAG-ACGACCGGTT-AAATAACAA 391
30W-4_8/20/2000-M_    -GT-CTTGATTT----ATTCAAAAT--TTTAA--CTCAG-ACGACCGGTT-AAATAACAA 382
12W-4_8/24/2001-P     -GT-TTTGATTT----ATTCAAAAT--TTTAA--CTCAG-ACGACCGGTT-AAATAACAA 392
27W-4_9/22/2001-P     -GT-TTTGATTT----ATTCAAAAT--TTTAA--CTCAG-ACGACCGGTT-AAATAACAA 385
2W-4_8/24/2001-M      -GT-TTTGATTT----ATTCAAAAT--TTTAA--CTCAG-ACGACCGGTT-AAATAACAA 383
57WF-S-4_10/5/2001-K  -GT-TTTGATTT----ATTCAAAAT--TTTAA--CTCAG-ACGACCGGTT-AAATAACAA 398
8W-4_8/24/2001-P      -GT-TTTGATTT----ATTCAAAAT--TTTAA--CTCAG-ACGACCGGTT-AAATAACAA 397
5W-4_8/24/2001-M      -GT-TTTGATTT----ATTCAAAAT--TTTAA--CTCAG-ACGACCGGTT-AAATAACAA 402
58WFS-4_10/5/2001-K   -GT-TTTGATTT----ATTCAAAAT--TTTAA--CTCAG-ACGACCGGTT-AAATAACAA 397
14W-4_8/30/2001-K     -GT-TTTGATTT----ATTCAAAAT--TTTAA--CTCAG-ACGACCGGTT-AAATAACAA 379
27P-4_9/22/2001-P     -GT-TTTGATTT----ATTCAAAAT--TTTAA--CTCAG-ACGACCGGTT-AAATAACAA 396
58WFS2-4_10/5/2001-K  -GT-TTTGATTT----ATTCAAAAT--TTTAA--CTCAG-ACGACCGGTT-AAATAACAA 392
50WFS3-4_10/7/2001-P  -GT-TTTGATTT----ATTCAAAAT--TTTAA--CTCAG-ACGACCGGTT-AAATAACAA 397
7W-4_8/24/2001-P      -GT-TTTGATTT----ATTCAAAAT--TTTAA--CTCAG-ACGACCGGTT-AAATAACAA 399
41WFW-4_10/14/2001-M  -GT-TTTGATTT----ATTCAAAAT--TTTAA--CTCAG-ACGACCGGTT-AAATAACAA 385
44WFW-4_10/14/2001-M  -GT-TTTGATTT----ATTCAAAAT--TTTAA--CTCAG-ACGACCGGTT-AAATAACAA 387
26W-4_9/22/2001-M     -GT-TTTGATTT----ATTCAAAAT--TTTAA--CTCAG-ACGACCGGTT-AAATAACAA 385
46WFW-4_10/14/2001-M  -GT-TTTGATTT----ATTCAAAAT--TTTAA--CTCAG-ACGACCGGTT-AAATAACAA 384
53WFW-4_10/7/2001-K   -GT-TTTGATTT----ATTCAAAAT--TTTAA--CTCAG-ACGACCGGTT-AAATAACAA 388
6W-4_8/24/2001-M      -GT-TTTGATTT----ATTCAAAAT--TTTAA--CTCAG-ACGACCGGTT-AAATAACAA 384
22W-4_8/20/2000-M     AGT-GTTGATTT----ATTCAAAAT--TTTAA--CTCAG-ACGACCGGTT-AAATAACAA 383
24W-4_8/20/2000-M     AGT-CTTGATTTT---ATTCAAAAT--TTTAA--CTCAG-ACGACCGGTT-AAATAACAT 384
44W-4_9/20/2000-M_    AGT-GTTGATTT----ATTCAAAAT--TTTAA--CTCAG-ACGACCGGTT-AAATAGCAA 384
45W-4_9/20/2000-M_    AGT-GTTGATTT----ATTCAAAAT--TTTAA--CTCAG-ACGACCGGTT-AAATANCAA 384
10W-4_8/24/2001-P     -GT-TTTGATTT----ATTCAAAAT--TTTAA--CTCAG-ACGACCGGTT-AAATAACAA 385
23W-4_8/20/2000-M_    CGTCTTTGATTTA---TTTCAAAATGGTCTAAC-CTCAG-ACGACCGGTT-AAATAACAA 425
21W-4_8/20/2000-M_    -GTCTTGGATTT----ATCCCAAAATGTTTA---CTCAGTACGACCGGTTTAAATAACAA 478
11W-4_7/27/2000-M     TTTCCTGGTCATA---GTTCTTCTC--TGTGGGGTAAAAAGCGCATGGGT----TTGCGA 183
                                                 *         *
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                           5.8S |                    ITS2
5W-4_7/27/2000-M_          TATGCATAGCC-CGACAATT-----GTTCCGA--AACAA-ATTTTTGAACCCGTTAATTA 546
13W-4_7/27/2000-M_         AAACCCTAAAG-AAAGAATT--CCCGCTTCATTTGACAACGTCTTTGGAATTTTT--TCA 484
3W-4_7/27/2000-M_          GAGTTTGGTTT--AACTCTG-GCGGGCGCTCGCCGGGGACGA-TCCCCAGCGGCTGAGAC 438
12W-4_7/27/2000-M_         GGTTTAAAATT--ACC------CAGGAGGTTTTGGGGTTTTA------------------ 447
4WW-4_8/24/2001            GAGTTT-GGTT-TAACTCTG-GCGGGCGCTCGCCTGGGACGAATCCCCAGCGGC-TCGAG 462
43W-4_9/20/2000-M_         GAGTTT-GGTT-TAACTCTG-GCGGGCGCTCGCCTGGGACGAATCCCCAGCGGC-TCGAG 436
1W-4_7/27/2000-M_          GAGTTT-GGTT-TAACTCTG-GCGGGCGCTCGCCTGGGACGAATCCCCAGCGGC-TCGAG 447
31W-4_8/20/2000-M_         GAGTTT-GGTT-TAACTCTG-GCGGGCGCTCGCCTGGGACGAATCCCCAGCGGC-TCGAG 437
53W-4_9/20/2000-M_         GAGTTT-GGTT-TAACTCTG-GCGGGCGCTCGCCTGGGACGAATCCCCAGCGGC-TCGAG 437
17W-4_8/30/2001-K          GAGTTT-GGTT-TAACTCTG-GCGGGCGCTCGCCTGGGACGAATCCCCAGCGGC-TCGAG 441
50WF-S-410/7/2001-P        GAGTTT-GGTT-TAACTCTG-GCGGGCGCTCGCCTGGGACGAATCCCCAGCGGC-TCGAG 446
58WFS1-4_10/5/2001-K       GAGTTT-GGTT-TAACTCTG-GCGGGCGCTCGCCTGGGACGACTCCCCAGCGGC-TCGAG 449
6W-4_7/27/2000-M_          GAGTGT-GGTT-TAACTCTG-GCGGGCGCTCGCCTGGGACGACTCCCCAGCGGC-TCGAG 451
25W-4_8/20/2000-M_         GAGTTT-GGTT-TAACTCTG-GCGGGCGCTCGCCTGGGACGAATCCCCAGCGGC-TCGAG 438
32W-4_8/20/2000-M_         GAGTTTCGGTT-TAACTCTG-GCGGGCGCTCGCCTGGGACGACTCCCCAGCGGC-TCGAG 448
4W-4_7/27/2000-M_          GAGTTT-GGTT-TAACTCTG-GCGGGCGCTCGCCTGGGACGAATCCCCAGCGGC-TCGAG 447
8W-4_7/27/2000-M_          GAGTTT-GGTT-TAACTCTG-GCGGGCGCTCGCCTGGGACGAATCCCCAGCGGC-TCGAG 429
7W-4_7/27/2000-M_          GAGTTT-GGTT-TAACTCTG-GCGGGCGCTCGCCTGGGACGAATCCCCAGCGGC-TCGAG 447
30W-4_8/20/2000-M_         GAGTCT-GGTT-TAACTCTG-GCGGGCGCTCGCCTGGGACGAATCCCCAGCGGC-TCGAG 438
12W-4_8/24/2001-P          GAGTTT-GGTT-TAACTCTG-GCGGGCGCTCGCCTGGGACGAATCCCCAGCGGC-TCGAG 448
27W-4_9/22/2001-P          GAGTTT-GGTT-TAACTCTG-GCGGGCGCTCGCCTGGGACGAATCCCCAGCGGC-TCGAG 441
2W-4_8/24/2001-M           GAGTTT-GGTT-TAACTCTG-GCGGGCGCTCGCCTGGGACGAATCCCCAGCGGC-TCGAG 439
57WF-S-4_10/5/2001-K       GAGTTT-GGTT-TAACTCTG-GCGGGCGCTCGCCTGGGACGAATCCCCAGCGGC-TCGAG 454
8W-4_8/24/2001-P           GAGTTT-GGTT-TAACTCTG-GCGGGCGCTCGCCTGGGACGAATCCCCAGCGGC-TCGAG 453
5W-4_8/24/2001-M           GAGTTT-GGTT-TAACTCTG-GCGGGCGCTCGCCTGGGACGAATCCCCAGCGGC-TCGAG 458
58WFS-4_10/5/2001-K        GAGTTT-GGTT-TAACTCTG-GCGGGCGCTCGCCTGGGACGAATCCCCAGCGGC-TCGAG 453
14W-4_8/30/2001-K          GAGTTT-GGTT-TAACTCTG-GCGGGCGCTCGCCTGGGACGAATCCCCAGCGGC-TCGAG 435
27P-4_9/22/2001-P          GAGTTT-GGTT-TAACTCTG-GCGGGCGCTCGCCTGGGACGAATCCCCAGCGGC-TCGAG 452
58WFS2-4_10/5/2001-K       GAGTTT-GGTT-TAACTCTG-GCGGGCGCTCGCCTGGGACGAATCCCCAGCGGC-TCGAG 448
50WFS3-4_10/7/2001-P       GAGTTT-GGTT-TAACTCTG-GCGGGCGCTCGCCTGGGACGAATCCCCAGCGGC-TCGAG 453
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41WFW-4_10/14/2001-M       GAGTTT-GGTT-TAACTCTG-GCGGGCGCTCGCCTGGGACGAATCCCCAGCGGC-TCGAG 441
44WFW-4_10/14/2001-M       GAGTTT-GGTT-TAACTCTG-GCGGGCGCTCGCCTGGGACGAATCCCCAGCGGC-TCGAG 443
26W-4_9/22/2001-M          GAGTTT-GGTT-TAACTCTG-GCGGGCGCTCGCCTGGGACGAATCCCCAGCGGC-TCGAG 441
46WFW-4_10/14/2001-M       GAGTTT-GGTT-TAACTCTG-GCGGGCGCTCGCCTGGGACGAATCCCCAGCGGC-TCGAG 440
53WFW-4_10/7/2001-K        GAGTTT-GGTT-TAACTCTG-GCGGGCGCTCGCCTGGGACGAATCCCCAGCGGC-TCGAG 444
6W-4_8/24/2001-M           GAGTTT-GGTT-TAACTCTG-GCGGGCGCTCGCCTGGGACGAATCCCCAGCGGC-TCGAG 440
22W-4_8/20/2000-M          GAGTTT-GGTT--AGCTCTG-GCGGGCGCTCGCCTGGGACGAATCCC-AGCGGC-TCGAG 437
24W-4_8/20/2000-M          GAGTCT-GGTT-TAACTCTG-GCGGGCGCTCGCCTGGGACGAATCCCCAGCCGC-TCGAG 440
44W-4_9/20/2000-M_         GAGTTT-GGTT-TAACTCTG-GCGGGCGCTCGCCTGGGACGAATCCCCAGCGGC-TCGAG 440
45W-4_9/20/2000-M_         GAGTTT-GGTT-TAACTCTG-GCGGGCGCTCGCCTGGGACGAATCCCCAGCGGC-TCGAG 440
10W-4_8/24/2001-P          GAGTTT-GGTT-TAACTCTG-GCGGGCGCTCGCCTGGGACGAATNCCCAGCGGC-TCGAG 441
23W-4_8/20/2000-M_         GAGTTCTGGTTCTAACTCTGTGCGGGCTCTCGCCTGGGACGAATCCCCAGCGGCATCGAG 485
21W-4_8/20/2000-M_         GAGTGC-GGTG-CAACTCTT-GCGGGCTCTCGCCTGGTACGACTCCCCAGCAGGCACGAG 535
11W-4_7/27/2000-M_         AGTTGTTTGCG-GGACATCC--CAACTGTCAGTTGAACTCGCTTATTTGGAGCG---TTG 237
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13W-4_7/27/2000-M_        AAAGTGTGTCTTGCTCG---GACGACCGGTTTTAAATAACAAGAGTGTCGGTTCAACTCT 541
3W-4_7/27/2000-M_         GAGCGTCCGCCAAGCAC---AGGTGTTTACAC-AAGGTTGAGTCGCC--GGCACCATC-- 490
12W-4_7/27/2000-M_        -------------------------------------------------------------
4WW-4_8/24/2001           ACCGAGCGGTCCCGCCA---AAGCAACAAGG--TAGTT-TTAACAAC--AAA-GGGTT-- 511
43W-4_9/20/2000-M_        ACCGAGCGGTCCCGCCA---AAGCAACAAGG--TAGTT-TTAACAAC--AAA-GGGTT-- 485
1W-4_7/27/2000-M_         ACCGAGCGGTCCCGCCA---AAGCAACAAGG--TAGTT-TTAACAAC--AAA-GGGTT-- 496
31W-4_8/20/2000-M_        ACCGAGCGGTCCCGCCA---AAGCAACAAGG--TAGTA-TTAACAAC--AAA-GGGTT-- 486
53W-4_9/20/2000-M_        ACCGAGCGGTCCCGCCC---AAGCCACAAGG--TAGTT-TTAGCAAC--AAA-GGGTT-- 486
17W-4_8/30/2001-K         ACCGAGCGGTNCCGCCA---NAGCAACAATG--TAGTN-T-ANCAAC--AAA-GGGTT-- 489
50WF-S-410/7/2001-P       ACCGAGCGGTNCCGCCA---AAGCAACAAGG--TAGTT-TTAACAAC--ANA-GGGTN-- 495
58WFS1-4_10/5/2001-K      ACCGAGCGGTCCCGCCA---AAGCAACAATG--TAGTATTAA-CAAC--AAACGG-TT-- 498
6W-4_7/27/2000-M_         ACCGAGCGGTCCCGCCA---AAGCAACAATGG-TAGTATTAAATAAC--GAACGGGTT-- 503
25W-4_8/20/2000-M_        ACCGAGCGGTCCCGCCA---AAGCAACAATG--TAGTA-TTAACAAC--AAA-GGGTT-- 487
32W-4_8/20/2000-M_        ACCGAGCGGTCCCGCCA---AAGCAACAATGG-TAGTAATTAACAAC--AAACGGGTT-- 500
4W-4_7/27/2000-M_         ACCGAGCGGTCCCGCCA---AAGCAACAATG--TAGTA-TTAACAAC--AAA-GGGTT-- 496
8W-4_7/27/2000-M_         ACCGAGCGGTCCCGCCA---AAGCAACAATG--TAGTT-TTAACAAC--AAA-GGGTT-- 478
7W-4_7/27/2000-M_         ACCGAGCGGTCCCGCCA---AAGCAACAATG--TAGTA-TTAACAAC--AAA-GGGTT-- 496
30W-4_8/20/2000-M_        ACCGAGCGGTCCCGCCA---AAGCAACAAGG--TAGTT-TTAGCGAC--AGAGGGGTG-- 488
12W-4_8/24/2001-P         ACCGAGCGGTCCCGCCA---AAGCAACAAGG--TAGTT-TTAACAAC--AAA-GGGTT-- 497
27W-4_9/22/2001-P         ACCGAGCGGTCCCGCCA---AAGCAACAAGG--TAGTT-TTAACAAC--AAA-GGGTT-- 490
2W-4_8/24/2001-M          ACCGAGCGGTCCCGCCA---AAGCAACAAGG--TAGTT-TTAACAAC--AAA-GGGTT-- 488
57WF-S-4_10/5/2001-K      ACCGAGCGGTCCCGCCA---AAGCAACAAGG--TAGTT-TTAACAAC--AAA-GGGTT-- 503
8W-4_8/24/2001-P          ACCGAGCGGTCCCGCCA---AAGCAACAAGG--TAGTT-TTAACAAC--AAA-GGGTT-- 502
5W-4_8/24/2001-M          ACCGAGCGGTCCCGCCA---AAGCAACAAGG--TAGTT-TTAACAAC--AAA-GGGTT-- 507
58WFS-4_10/5/2001-K       ACCGAGCGGTCCCGCCA---AAGCAACAAGG--TAGTT-TTAACAAC--AAA-GGGTT-- 502
14W-4_8/30/2001-K         ACCGAGCGGTCCCGCCA---AAGCAACAAGG--TAGTT-TTAACAAC--AAA-GGGTT-- 484
27P-4_9/22/2001-P         ACCGAGCGGTCCCGCCA---AAGCAACAAGG--TAGTT-TTAACAAC--AAA-GGGTT-- 501
58WFS2-4_10/5/2001-K      ACCGAGCGGTCCCGCCA---AAGCAACAAGG--TAGTT-TTAACAAC--AAA-GGGTT-- 497
50WFS3-4_10/7/2001-P      ACCGAGCGGTCCCGCCA---AAGCAACAAGG--TAGTT-TTAACAAC--AAA-GGGTT-- 502
7W-4_8/24/2001-P          ACCGAGCGGTCCCGCCA---AAGCAACAAGG--TAGTT-TTAACAAC--AAA-GGGTT-- 504
41WFW-4_10/14/2001-M      ACCGAGCGGTCCCGCCA---AAGCAACAAGG--TAGTT-TTAACAAC--AAA-GGGTT-- 490
44WFW-4_10/14/2001-M      ACCGAGCGGTCCCGCCA---AAGCAACAAGG--TAGTT-TTAACAAC--AAA-GGGTT-- 492
26W-4_9/22/2001-M         ACCGAGCGGTCCCGCCA---AAGCAACAANG--TAGTT-TTAACAAC--AAA-NGGTT-- 490
46WFW-4_10/14/2001-M      ACCGAGCGGTCCCGCCA---AAGCAACAAGG--TAGTT-TTAACAAC--AAA-NGGTT-- 489
53WFW-4_10/7/2001-K       ACCGAGCGGTCCCGCCA---AAGCAACAANG--TAGTT-TTAACAAC--AAA-NGGTT-- 493
6W-4_8/24/2001-M          ACCGAGCGGTCCCGCCA---AAGCAACAAGG--TAGTT-TTAACAAC--AAA-NGGTT-- 489
22W-4_8/20/2000-M         ACCGAGCGGTCC-GCCA---AAGCA-CA-GG--TAGTA-T-AACAC---GAG--GGT--- 479
24W-4_8/20/2000-M         ACCGAGCGGTCCCGCCA---AAGCA-CACGG--TAGTA-TTAGCACA--GAG--GGTA-- 487
44W-4_9/20/2000-M_        ACCGAGCGGTTCCGCCA---AAGCAACATGG--TAGTA-TTAACAAC--GAG--G-TA-- 487
45W-4_9/20/2000-M_        ACCGAGCGGTNCCGCCA---AAGCANCATNG--TAGTA-TTAACAAC--GAN--N-NA-- 487
10W-4_8/24/2001-P         ACCGAGCNGNNCCGCCC---AAGCA-CAANG--TAGTT-TTANCANC--NNN--GGNT-- 488
23W-4_8/20/2000-M_        ACCGAGCGGTCCCGCCA---AAGCAGCAAGG--TAGAT-TTAACAAC--AAA-GGGTT-- 534
21W-4_8/20/2000-M_        ACCGAGCGGTCTCGCCGCATAGCCAGCGATGGTAAGTATTTAACTACCGAAACGGGTTTG 595
11W-4_7/27/2000-M_        GAGGAGTACTTCTAGGGTAGGTTTTATAATG--CGATAATCAAAACTGGGGTGATATTGG 295
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                                                         ITS2
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3W-4_7/27/2000-M_          TTATATCTCC-AGTCACACGAACTGTAGACTTATGCAACAAATGGGGGCCCGGCCGGGGG 549
12W-4_7/27/2000-M_         ------------------------------------------------------------
4WW-4_8/24/2001            GGA--GGTCG-GGCGCTGAGCA-CCCTTACTC-TTTAATGATCCTTCC-GCAGGT-CACC 564
43W-4_9/20/2000-M_         GGA--GGTCG-GGCGCTGAGCA-CCCTTACTC-TTTAATGATCCTTCC-GCAGGTTCACC 539
1W-4_7/27/2000-M_          GGA--GGTCG-GGCGCTGAGCA-CCCTTACTC-TTTAATGATCCTTCC-GCAGGTTCACC 550
31W-4_8/20/2000-M_         GGA--GGTCG-GGCGCTGAGCA-CCCTTACTC-TTTAATGATGCCTCC-GCAGGTTCACC 540
53W-4_9/20/2000-M_         GGA--GGTCG-GGCGCTGAGCA-CCCT-ACTC-TT-AATGAT-CTTCC-GCAGGTTCACC 537
17W-4_8/30/2001-K          GGA--GGTCG-GNCGCTGAGCN-CCNTA-CTC-TTTATTGATC--TTCCG---------- 531
50WF-S-410/7/2001-P        GGA--GGTCG-GGCGCTGAGCA-CCCTNACTC-TTNANTGATNCCTNCCGCAGGTNCA-- 548
58WFS1-4_10/5/2001-K       GGA--GGTCG-GACGCTGAGCGCCC-TTACTC-TATAATGATTCTTCC-GCATGTACACC 552
6W-4_7/27/2000-M_          GGA--GGTCGAGACGCTGAGCACCCCTTACTC-TTCAATGATTCTTCCCGCATGTACACC 560
25W-4_8/20/2000-M_         GGA--GGTCG-GGCGCTGAGCA-CCCTTACTC-TTTAATGATCCTTCC-GCATGTACACC 541
32W-4_8/20/2000-M_         GGGAGGGTCGAGGCGCTGAGCAGCCCTTACTC-TTTAATGATTCTTCC-GCATGTACACC 558
4W-4_7/27/2000-M_          GGA--GGTCG-GGCGCTGAGCA-CCCTTACTC-TTTAATGATCCTTCC-GCATGTACACC 550
8W-4_7/27/2000-M_          GGA--GGTCG-GGCGCTGAGCA-CCCTTACTC-TTTAATGATCCTTCC-GCAGGTTCACC 532
7W-4_7/27/2000-M_          GGA--GGTCG-GGCGCTGAGCA-CCCTTACTC-TTTAATGATCCTTCC-GCAGGTTCACC 550
30W-4_8/20/2000-M_         GCA--CGTCG-GGCGCTGAGCA-CCCTTACTC-TTTACTGATC--TTCCGCAGGTACACC 541
12W-4_8/24/2001-P          GGA--GGTCG-GGCGCTGAGCA-CCCTTACTC-TTTAATGATCCTTCC-GCAGGTTCACC 551
27W-4_9/22/2001-P          GGA--GGTCG-GGCGCTGAGCA-CCCTTACTC-TTTAATGATCCTTCC-GCAGGTTCACC 544
2W-4_8/24/2001-M           GGA--GGTCG-GGCGCTGAGCA-CCCTTACTC-TTTAATGATCCTTCC-GCAGGTTCACC 542
57WF-S-4_10/5/2001-K       GGA--GGTCG-GGCGCTGAGCA-CCCTTACTC-TTTAATGATCCTTCC-GCAGGTNCACC 557
8W-4_8/24/2001-P           GGA--GGTCG-GGCGCTGAGCA-CCCTTACTC-TTTAATGATCCTTCC-GCAGGTTCACC 556
5W-4_8/24/2001-M           GGA--GGTCG-GGCGCTGAGCA-CCCTTACTC-TTTAATGATCCTTCC-GCAGGTTCACC 561
58WFS-4_10/5/2001-K        GGA--GGTCG-GGCGCTGAGCA-CCCTTACTC-TTTAATGATCCTTCC-GCAGGTTCACC 556
14W-4_8/30/2001-K          GGA--GGTCG-GGCGCTGAGCA-CCCTTACTC-TTTAATGATCCTTCC-GCAGGTTCACC 538
27P-4_9/22/2001-P          GGA--GGTCG-GGCGCTGAGCA-CCCTTACTC-TTTAATGATCCTTCC-GCAGGTTCACC 555
58WFS2-4_10/5/2001-K       GGA--GGTCG-GGCGCTGAGCA-CCCTTACTC-TTTAATGATCCTTCC-GCAGGTTCACC 551
50WFS3-4_10/7/2001-P       GGA--GGTCG-GGCGCTGAGCA-CCCTTACTC-TTTAATGATCCTTCC-GCAGGTTCACC 556
7W-4_8/24/2001-P           GGA--GGTCG-GGCGCTGAGCA-CCCTTACTC-TTTAATGATCCTTCC-GCAGGTTCACC 558
41WFW-4_10/14/2001-M       GGA--GGTCN-GGCGCTGAGCA-CCCTTACTC-TTTAATGATCCTTCC-GCAGGTTCACC 544
44WFW-4_10/14/2001-M       GGA--NGTCG-GGCGCTGAGCA-CCCTTACTC-TTTAATGAT------------------ 529
26W-4_9/22/2001-M          GGA--NGTCN-GGCGCTGAGCA-CCCTTACTC-TTTAATGATNCNTCC-GCAGGTNCACC 544
46WFW-4_10/14/2001-M       GGA--NGTCN-GGCGCTGAGCA-CCCTTACTC-TTNAATGATNCNTCC-GCAGGTTCACC 543
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6W-4_8/24/2001-M           GGA--GGTCG-NNCGCTGAGCA--CCCTACTC-TTNAATGAT------------------ 525
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10W-4_8/24/2001-P          GGA--GGTCG-GGCGCTGAGCA--CCCTACTC-TTNANTGATCTTTCGC----------- 531
23W-4_8/20/2000-M_         GGA--GGTCA-GGCGATGTGCA-CCCTTACTCTTTAATTGATCCTTCC-GCATGTTCACC 589
21W-4_8/20/2000-M_         TGAAGGGTCGGAGGACCGCATGAAGCCAGCCCCTTAAACTTCTTATCAAATTTGATTTCC 655
11W-4_7/27/2000-M_         TCATACAGAAAGAGGTTTAACAGATGTTGATT--TGAACGGGAATTGAGGGGTATTAGTC 353
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3W-4_7/27/2000-M_        ACGGGGCAGACCAAGGGGACAGGGGGGACCAAGCCGGGCAACAGGGACG----------- 598
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43W-4_9/20/2000-M_       -TACGGAA-A-CCTGTT-ACGACTT--T-ACGAAAAAA---------------------- 570
1W-4_7/27/2000-M_        -TACGGAA-A-CCTGTTTACGACTT--TTACTGGCAACACTAGCCTGCCGGCC------- 598
31W-4_8/20/2000-M_       -TACGGAA-A-CCTGTT-ACGACTA--T-ATTGACAA----------------------- 570
53W-4_9/20/2000-M_       -TACG-AA-A-CCTGTT-ACGACTT--AACTT---------------------------- 562
17W-4_8/30/2001-K        ------------------------------------------------------------
50WF-S-410/7/2001-P      ------------------------------------------------------------
58WFS1-4_10/5/2001-K     -TACTGAG-A-CCTGGTTACGACTT--TTACTCGC------------------------- 582
6W-4_7/27/2000-M_        -TACTGAA-GTCCTGGTTACGACTT--TTACTTGCATATCACTATGCGGAGAAA------ 610
25W-4_8/20/2000-M_       -TACTGAG-A--CCTGTTACGACTG--T-ATTTACATATCAATAGCGAAGGAAA------ 588
32W-4_8/20/2000-M_       -TACTGAG-A--CCTGATACGACTG--T-ACTGTCATATCATGAGCGAGGAAAAA----- 606
4W-4_7/27/2000-M_        -TACTGAG-ACCT--GTTACGACTA--TAT--GTAAATTTTATGTATGCGGAAAA----- 597
8W-4_7/27/2000-M_        -TACGGGA-ACCCTGGTTACGACTT--TATTAGCAAATTTC-TATATGCGG--------- 578
7W-4_7/27/2000-M_        -TACTGAA-CCCT--GTTACGACTT--CTTGTGGAAATTTATAAAGCGAG---------- 594
30W-4_8/20/2000-M_       -TACTGAA-C--CATGTTACGACAT--TTACTTGAAAAA--------------------- 574
12W-4_8/24/2001-P        -TACGGAA-A-CCTTGTTACGACTT--TTACTTNGACA---------------------- 584
27W-4_9/22/2001-P        -TACGGAA-A-CCTGTTTACGAC------------------------------------- 564
2W-4_8/24/2001-M         -TACGGAA-A-CCTTGTNACGACTT--NCTA----------------------------- 568
57WF-S-4_10/5/2001-K     -TACGGAA-A-CCTTGNNACGACTT--ATTT----------------------------- 583
8W-4_8/24/2001-P         -TACGGAA-A-CCTTGTNACGACTT--TAC------------------------------ 581
5W-4_8/24/2001-M         -TACGGAA-A-CCTTGTTACGACTT--TTACTGGGCAAA--------------------- 595
58WFS-4_10/5/2001-K      -TACGGAA-A-CCTTGTTACGACTT--CTA--GGGCAAAAA------------------- 590
14W-4_8/30/2001-K        -TACGGAA-A-CCTTGTTACGACTT--TATTTGGGAAAAA-------------------- 573
27P-4_9/22/2001-P        -TACGGAA-A-CCTTGTTACGACTT--TACTTGGGAAAAA-------------------- 590
58WFS2-4_10/5/2001-K     -TACGGAA-A-CCTTGTTACGACTT---ACTTGGGAAAAA-------------------- 585
50WFS3-4_10/7/2001-P     -TACGGAA-A-CCTTGTTACGACTT--TACTTGGGCAAA--------------------- 590
7W-4_8/24/2001-P         -TACGGAA-A-CCTTGTTACGACTT--TCTTTGGCAAA---------------------- 591
41WFW-4_10/14/2001-M     -TACGGNA-AACCTGNTTACGACT------------------------------------ 566
44WFW-4_10/14/2001-M     ------------------------------------------------------------
26W-4_9/22/2001-M        -TACNGAA-A-CCTGGTTACGA-------------------------------------- 563
46WFW-4_10/14/2001-M     -TACNGAA-A-CCTGTTTAC---------------------------------------- 560
53WFW-4_10/7/2001-K      ------------------------------------------------------------
6W-4_8/24/2001-M         ------------------------------------------------------------
22W-4_8/20/2000-M        -TACGGGA-ACCCTGGT-ACGACTT-TTACTTGGCAAAA--------------------- 564
24W-4_8/20/2000-M        -TACGGAATACCCTGTTTACGACAT-ATACTAGGCA------------------------ 576
44W-4_9/20/2000-M_       ATACGGAACTCTTTGCTTACGACGTAGTAGCTAGCAA----------------------- 580
45W-4_9/20/2000-M_       NTACNGNANNCNTTGCTNACGACGTANTANCTNGNAAACCCCCTTTGCGGGGNNC----- 598
10W-4_8/24/2001-P        ------------------------------------------------------------
23W-4_8/20/2000-M_       -TACGGAA-ACCTTGATTACGAACTTTTACTTAGTCAATCATTCTATTACGCAGGGAAAG 647
21W-4_8/20/2000-M_       TTTCCCAG--GCAGTGGTACCACCC-CTGACTGCAAAGCCCTTGGATTACGACTTGTACA 712
11W-4_7/27/2000-M        AGCTAATTTAATGGTAACTTAACTAAGAAATTGTGGGAGCGTTT---------------- 397
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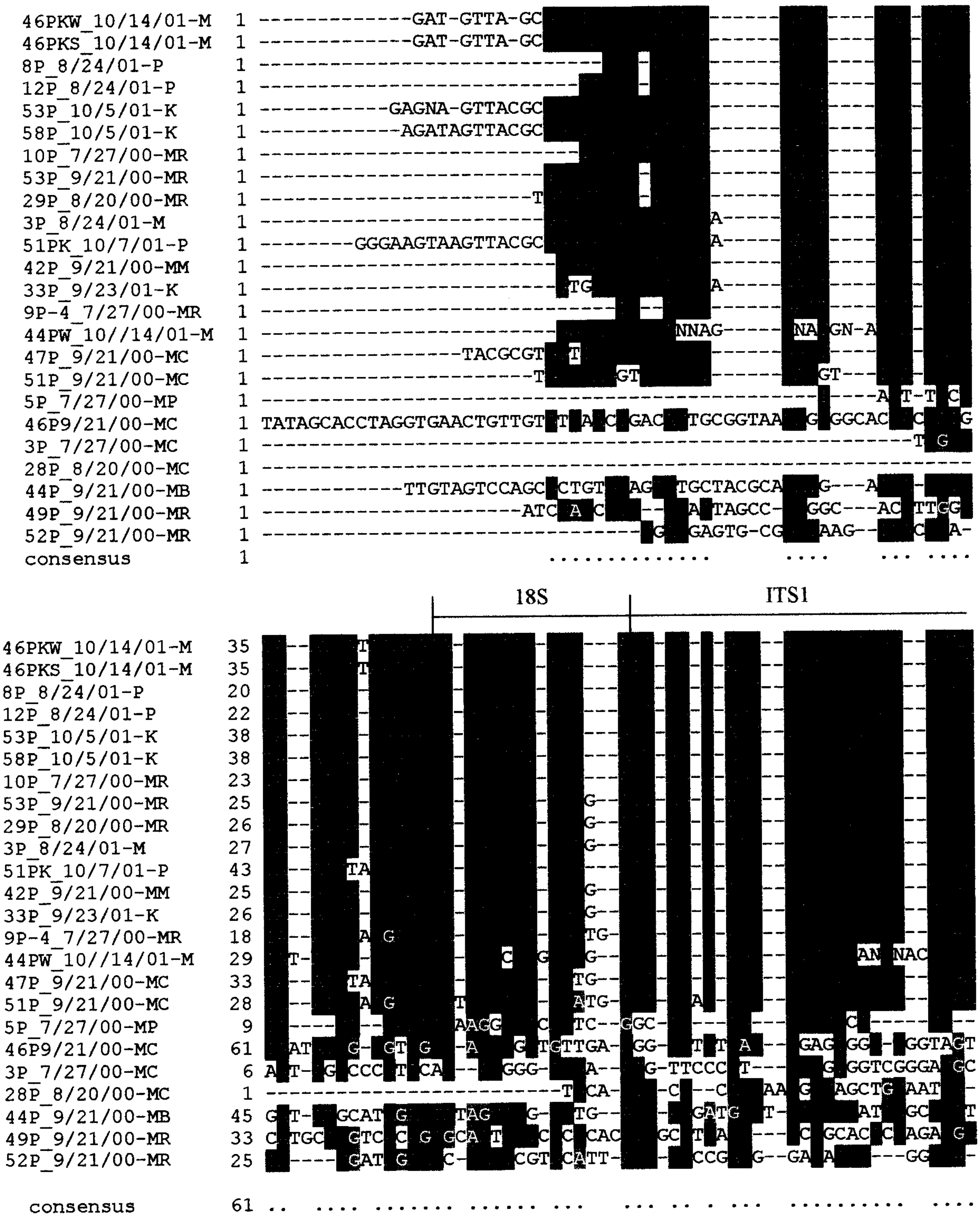
Appendix 12:

Markko vineyard 2000 to 2001 sample comparison of *Rhodotorula* sp. Sequencing reactions were done using reverse primer ITS4. DNA sequence alignments done using the Baylor College of Medicine ClustalW 1.8 Multiple Sequence Alignment website Box Shade view. Conserved and ITS regions labeled. Dashes represent gaps. M, P, K – 2001.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29P_8/20/00-MR
3P_8/24/01-M
51PK_10/7/01-P
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33P_9/23/01-K
9P-4_7/27/00-MR
44PW_10//14/01-M
47P_9/21/00-MC
51P_9/21/00-MC
5P_7/27/00-MP
46P9/21/00-MC
3P_7/27/00-MC
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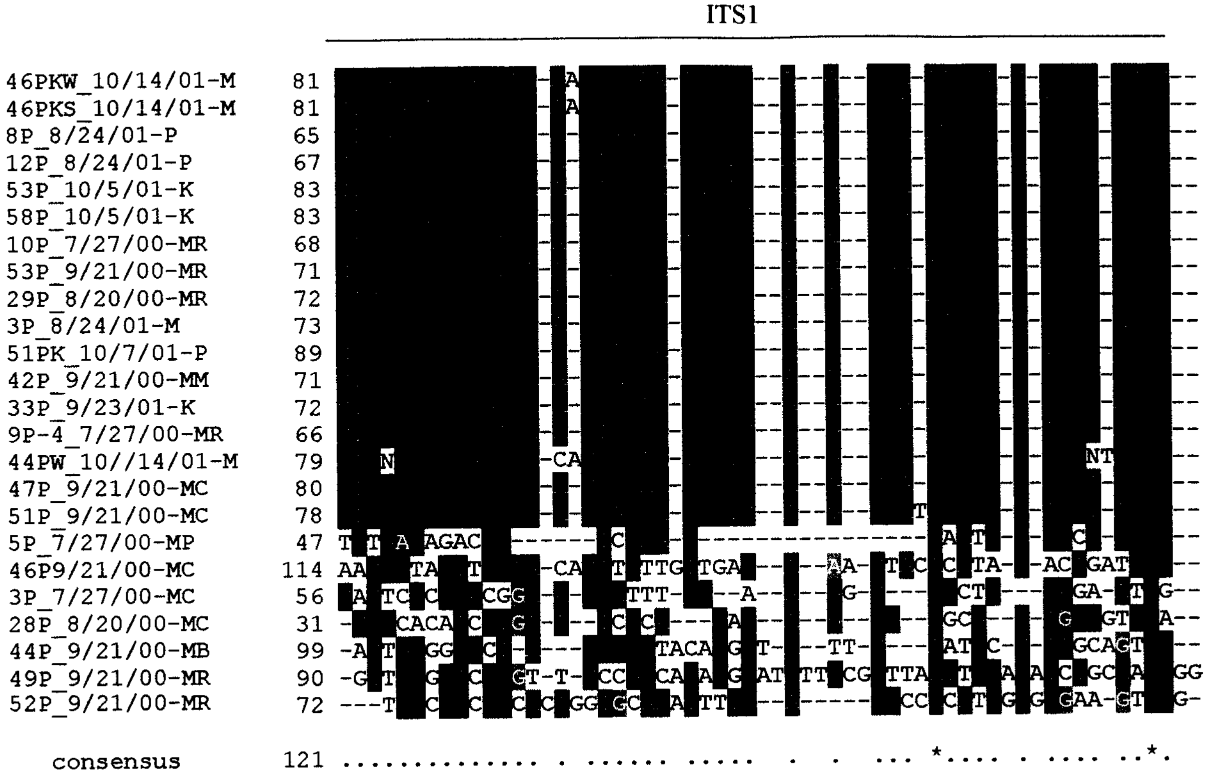
consensus
18S
ITS1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53P_10/5/01-K 83
58P_10/5/01-K 83
10P_7/27/00-MR 68
53P_9/21/00-MR 71
29P_8/20/00-MR 72
3P_8/24/01-M 73
51PK_10/7/01-P 89
42P_9/21/00-MM 71
33P_9/23/01-K 72
9P-4_7/27/00-MR 66
44PW_10//14/01-M 79
47P_9/21/00-MC 80
51P_9/21/00-MC 78
5P_7/27/00-MP 47
46P9/21/00-MC 114
3P_7/27/00-MC 56
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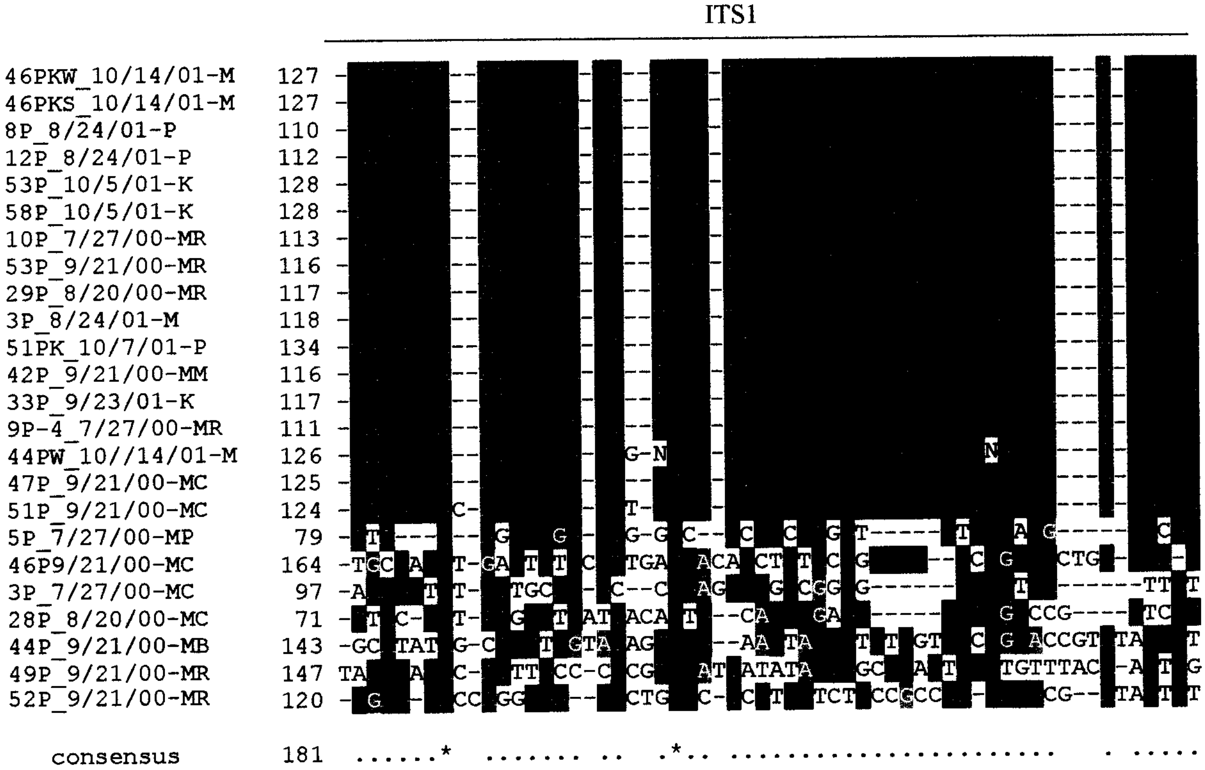
52P_9/21/00-MR 72
consensus 121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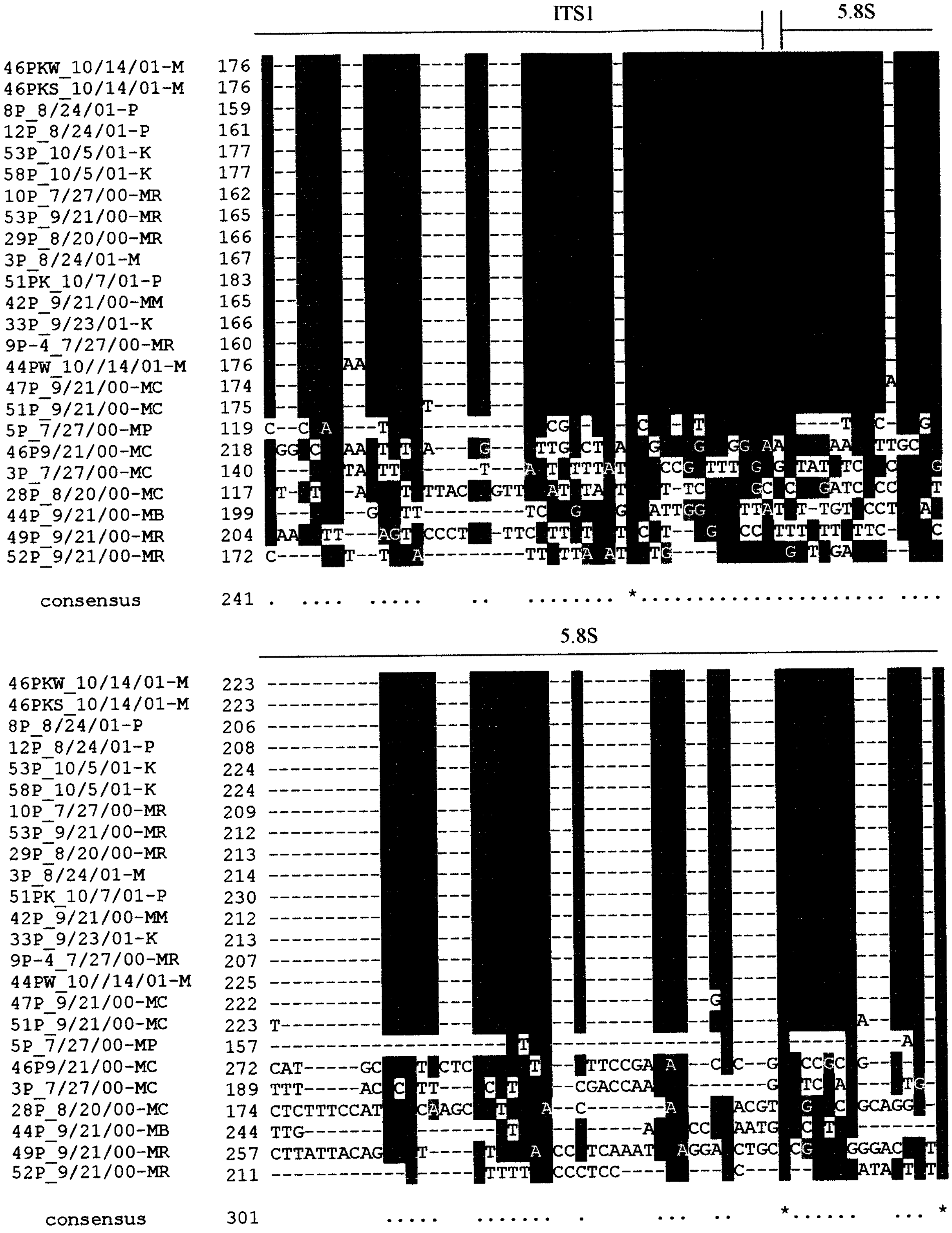
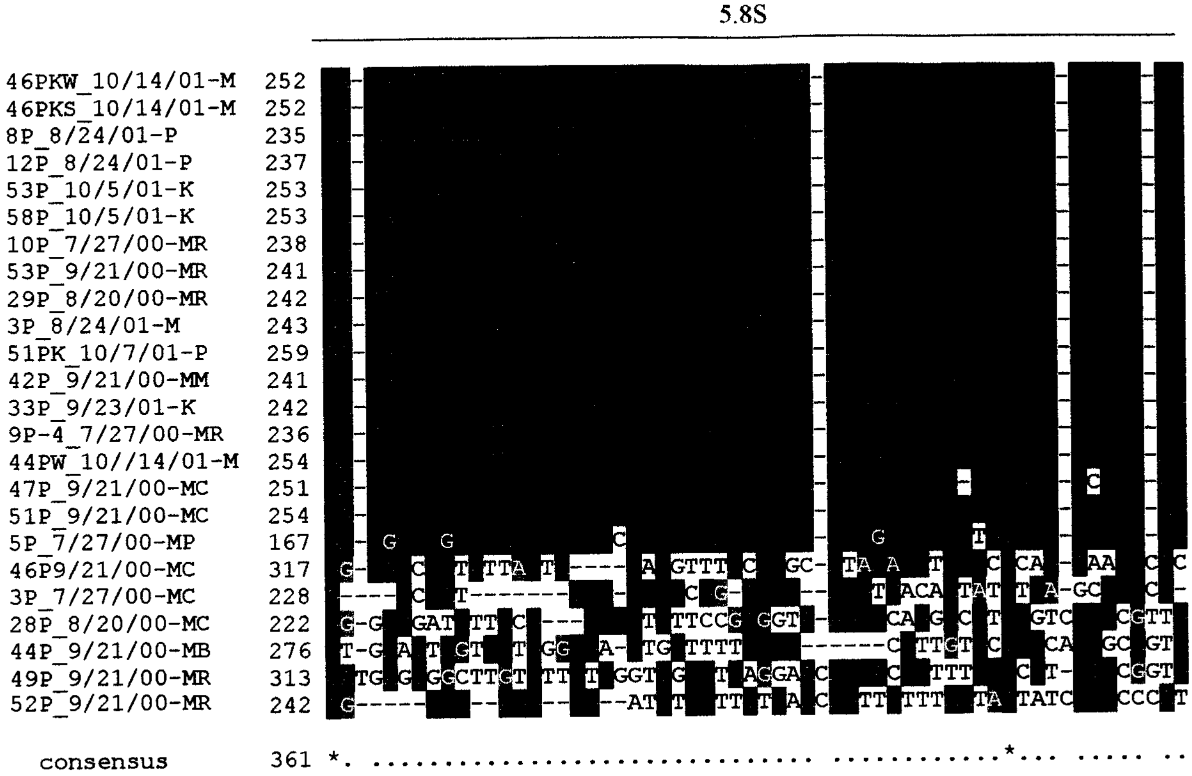
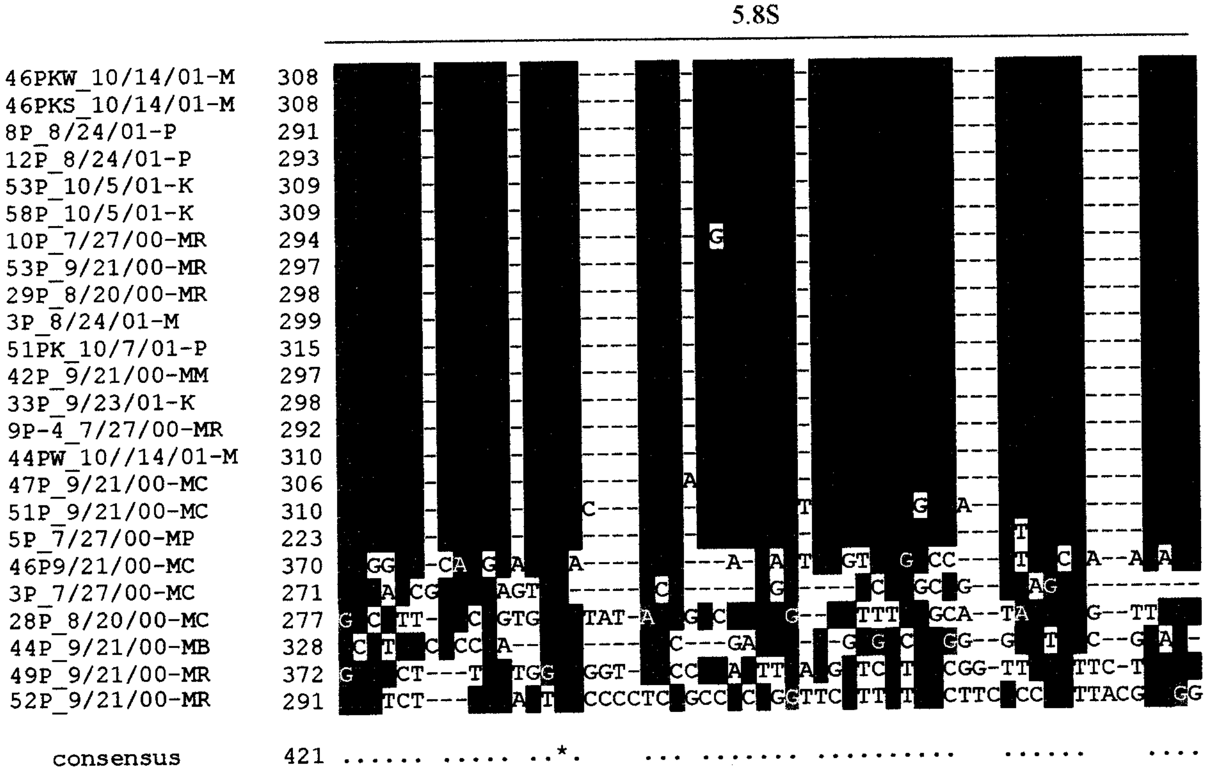
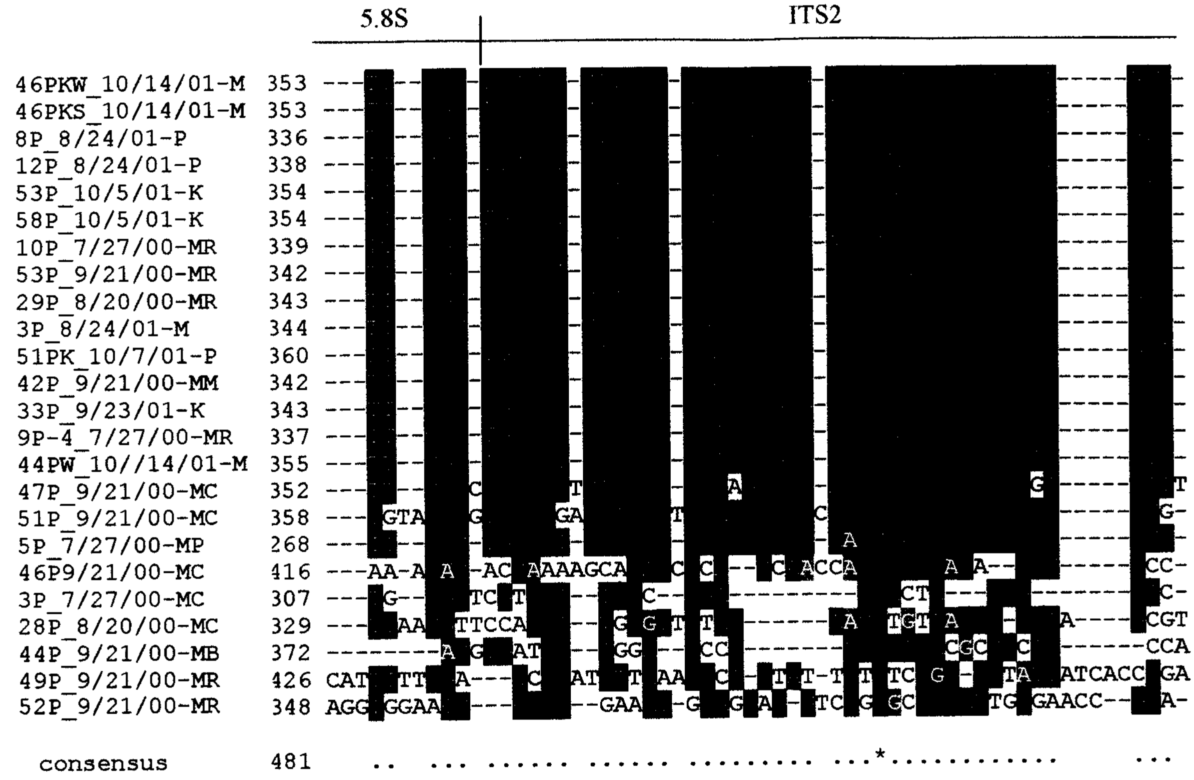
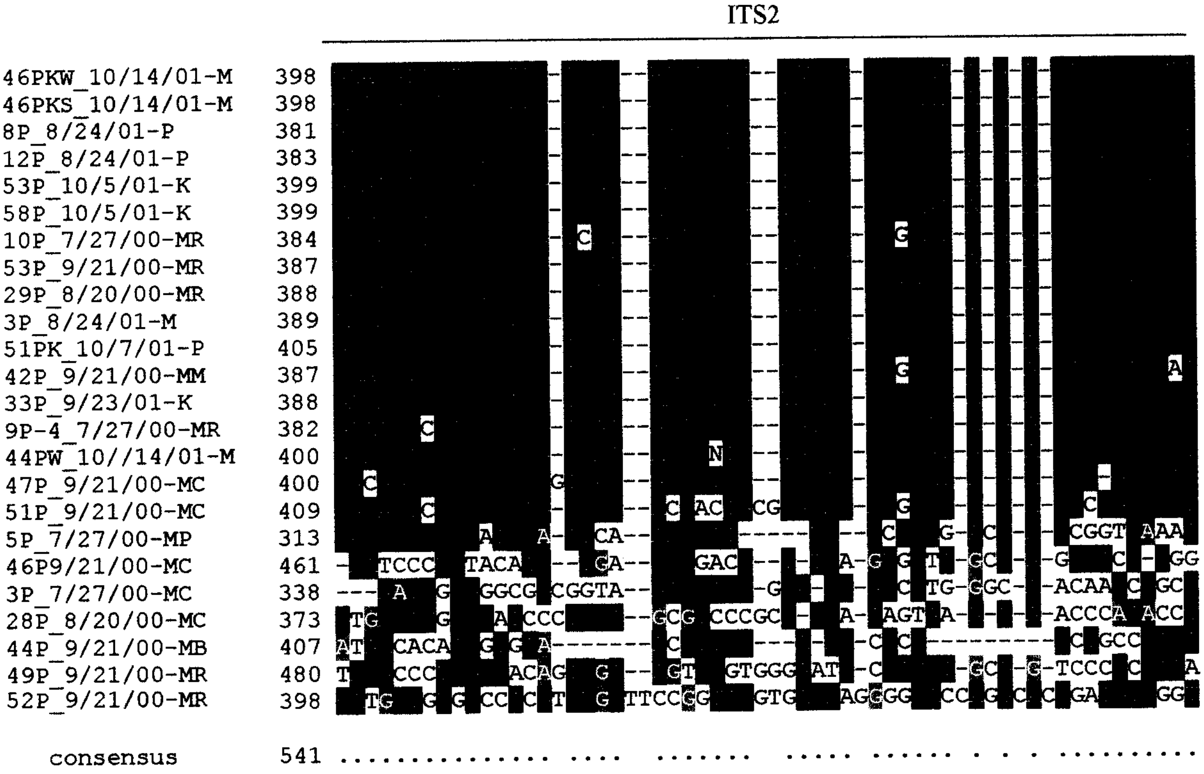
12P_8/24/01-P 112
53P_10/5/01-K 128
58P_10/5/01-K 128
10P_7/27/00-MR 113
53P_9/21/00-MR 116
29P_8/20/00-MR 117
3P_8/24/01-M 118
51PK_10/7/01-P 134
42P_9/21/00-MM 116
33P_9/23/01-K 117
9P-4_7/27/00-MR 111
44PW_10//14/01-M 126
47P_9/21/00-MC 125
51P_9/21/00-MC 124
5P_7/27/00-MP 79
46P9/21/00-MC 164
3P_7/27/00-MC 97
28P_8/20/00-MC 71
44P_9/21/00-MB 143
49P_9/21/00-MR 147
52P_9/21/00-MR 120
consensus 181

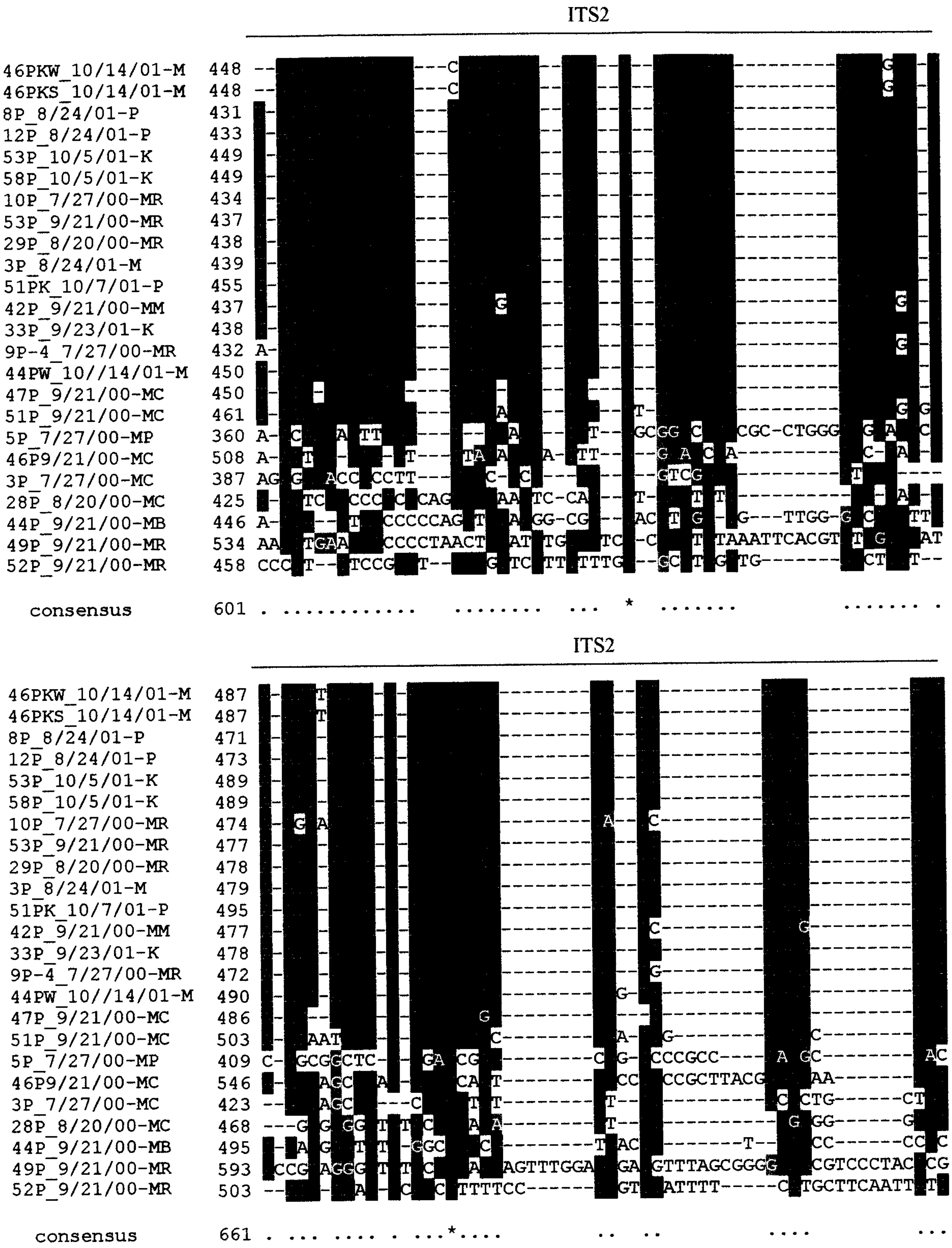

ITS1
5.8S
46PKW_10/14/01-M 176
46PKS_10/14/01-M 176
8P_8/24/01-P 159
12P_8/24/01-P 161
53P_10/5/01-K 177
58P_10/5/01-K 177
10P_7/27/00-MR 162
53P_9/21/00-MR 165
29P_8/20/00-MR 166
3P_8/24/01-M 167
51PK_10/7/01-P 183
42P_9/21/00-MM 165
33P_9/23/01-K 166
9P-4_7/27/00-MR 160
44PW_10//14/01-M 176
47P_9/21/00-MC 174
51P_9/21/00-MC 175
5P_7/27/00-MP 119
46P9/21/00-MC 218
3P_7/27/00-MC 140
28P_8/20/00-MC 117
44P_9/21/00-MB 199
49P_9/21/00-MR 204
52P_9/21/00-MR 172
consensus 241
5.8S
46PKW_10/14/01-M 223
46PKS_10/14/01-M 223
8P_8/24/01-P 206
12P_8/24/01-P 208
53P_10/5/01-K 224
58P_10/5/01-K 224
10P_7/27/00-MR 209
53P_9/21/00-MR 212
29P_8/20/00-MR 213
3P_8/24/01-M 214
51PK_10/7/01-P 230
42P_9/21/00-MM 212
33P_9/23/01-K 213
9P-4_7/27/00-MR 207
44PW_10//14/01-M 225
47P_9/21/00-MC 222
51P_9/21/00-MC 223
5P_7/27/00-MP 157
46P9/21/00-MC 272
3P_7/27/00-MC 189
28P_8/20/00-MC 174
44P_9/21/00-MB 244
49P_9/21/00-MR 257
52P_9/21/00-MR 211
consensus 301

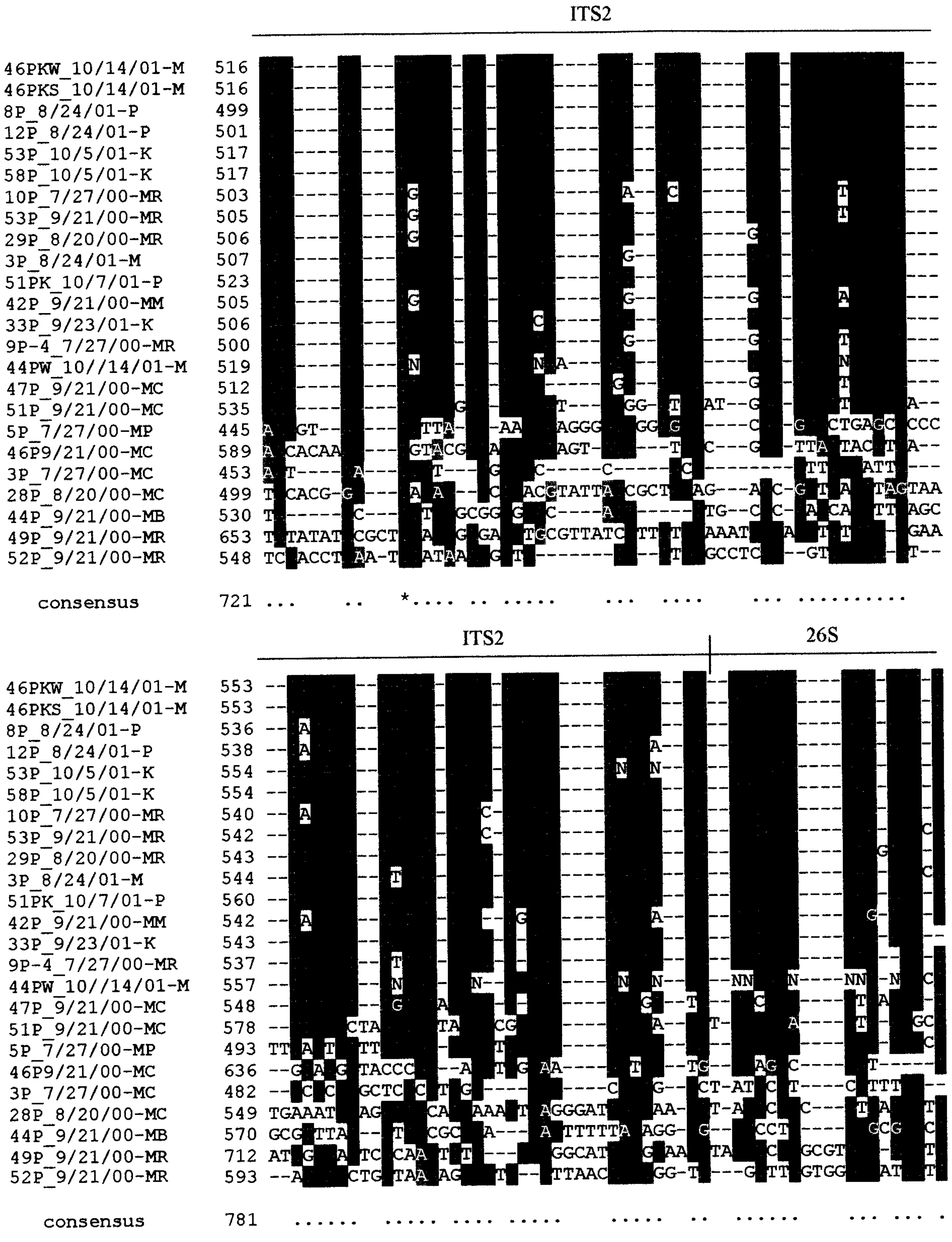

5.8S
46PKW_10/14/01-M 252
46PKS_10/14/01-M 252
8P_8/24/01-P 235
12P_8/24/01-P 237
53P_10/5/01-K 253
58P_10/5/01-K 253
10P_7/27/00-MR 238
53P_9/21/00-MR 241
29P_8/20/00-MR 242
3P_8/24/01-M 243
51PK_10/7/01-P 259
42P_9/21/00-MM 241
33P_9/23/01-K 242
9P-4_7/27/00-MR 236
44PW_10//14/01-M 254
47P_9/21/00-MC 251
51P_9/21/00-MC 254
5P_7/27/00-MP 167
46P9/21/00-MC 317
3P_7/27/00-MC 228
28P_8/20/00-MC 222
44P_9/21/00-MB 276
49P_9/21/00-MR 313
52P_9/21/00-MR 242
consensus 361

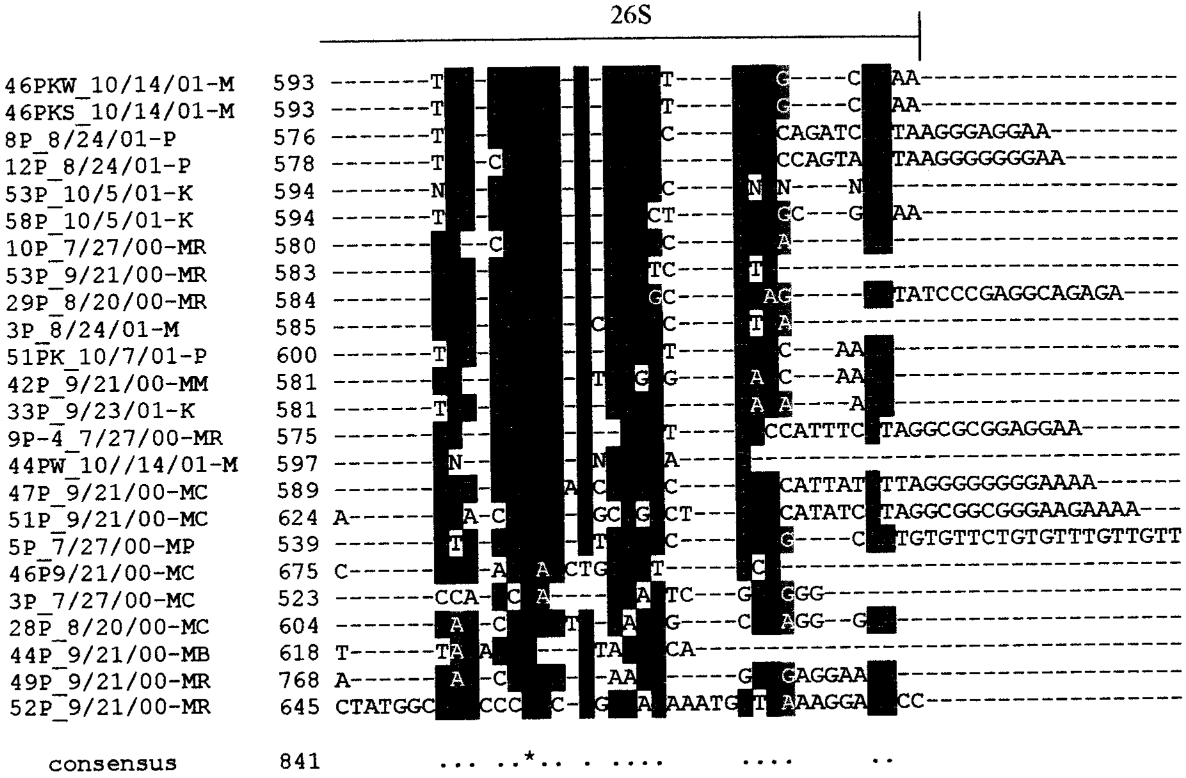

5.8S
46PKW_10/14/01-M 308
46PKS_10/14/01-M 308
8P_8/24/01-P 291
12P_8/24/01-P 293
53P_10/5/01-K 309
58P_10/5/01-K 309
10P_7/27/00-MR 294
53P_9/21/00-MR 297
29P_8/20/00-MR 298
3P_8/24/01-M 299
51PK_10/7/01-P 315
42P_9/21/00-MM 297
33P_9/23/01-K 298
9P-4_7/27/00-MR 292
44PW_10//14/01-M 310
47P_9/21/00-MC 306
51P_9/21/00-MC 310
5P_7/27/00-MP 223
46P9/21/00-MC 370
3P_7/27/00-MC 271
28P_8/20/00-MC 277
44P_9/21/00-MB 328
49P_9/21/00-MR 372
52P_9/21/00-MR 291
consensus 421


5.8S
ITS2
46PKW_10/14/01-M 353
46PKS_10/14/01-M 353
8P_8/24/01-P 336
12P_8/24/01-P 338
53P_10/5/01-K 354
58P_10/5/01-K 354
10P_7/27/00-MR 339
53P_9/21/00-MR 342
29P_8/20/00-MR 343
3P_8/24/01-M 344
51PK_10/7/01-P 360
42P_9/21/00-MM 342
33P_9/23/01-K 343
9P-4_7/27/00-MR 337
44PW_10//14/01-M 355
47P_9/21/00-MC 352
51P_9/21/00-MC 358
5P_7/27/00-MP 268
46P9/21/00-MC 416
3P_7/27/00-MC 307
28P_8/20/00-MC 329
44P_9/21/00-MB 372
49P_9/21/00-MR 426
52P_9/21/00-MR 348
consensus 481


ITS2
46PKW_10/14/01-M 398
46PKS_10/14/01-M 398
8P_8/24/01-P 381
12P_8/24/01-P 383
53P_10/5/01-K 399
58P_10/5/01-K 399
10P_7/27/00-MR 384
53P_9/21/00-MR 387
29P_8/20/00-MR 388
3P_8/24/01-M 389
51PK_10/7/01-P 405
42P_9/21/00-MM 387
33P_9/23/01-K 388
9P-4_7/27/00-MR 382
44PW_10//14/01-M 400
47P_9/21/00-MC 400
51P_9/21/00-MC 409
5P_7/27/00-MP 313
46P9/21/00-MC 461
3P_7/27/00-MC 338
28P_8/20/00-MC 373
44P_9/21/00-MB 407
49P_9/21/00-MR 480
52P_9/21/00-MR 398
consensus 541


ITS2
46PKW_10/14/01-M 448
46PKS_10/14/01-M 448
8P_8/24/01-P 431
12P_8/24/01-P 433
53P_10/5/01-K 449
58P_10/5/01-K 449
10P_7/27/00-MR 434
53P_9/21/00-MR 437
29P_8/20/00-MR 438
3P_8/24/01-M 439
51PK_10/7/01-P 455
42P_9/21/00-MM 437
33P_9/23/01-K 438
9P-4_7/27/00-MR 432
44PW_10//14/01-M 450
47P_9/21/00-MC 450
51P_9/21/00-MC 461
5P_7/27/00-MP 360
46P9/21/00-MC 508
3P_7/27/00-MC 387
28P_8/20/00-MC 425
44P_9/21/00-MB 446
49P_9/21/00-MR 534
52P_9/21/00-MR 458
consensus 601
ITS2
46PKW_10/14/01-M 487
46PKS_10/14/01-M 487
8P_8/24/01-P 471
12P_8/24/01-P 473
53P_10/5/01-K 489
58P_10/5/01-K 489
10P_7/27/00-MR 474
53P_9/21/00-MR 477
29P_8/20/00-MR 478
3P_8/24/01-M 479
51PK_10/7/01-P 495
42P_9/21/00-MM 477
33P_9/23/01-K 478
9P-4_7/27/00-MR 472
44PW_10//14/01-M 490
47P_9/21/00-MC 486
51P_9/21/00-MC 503
5P_7/27/00-MP 409
46P9/21/00-MC 546
3P_7/27/00-MC 423
28P_8/20/00-MC 468
44P_9/21/00-MB 495
49P_9/21/00-MR 593
52P_9/21/00-MR 503
consensus 661


ITS2
46PKW_10/14/01-M 516
46PKS_10/14/01-M 516
8P_8/24/01-P 499
12P_8/24/01-P 501
53P_10/5/01-K 517
58P_10/5/01-K 517
10P_7/27/00-MR 503
53P_9/21/00-MR 505
29P_8/20/00-MR 506
3P_8/24/01-M 507
51PK_10/7/01-P 523
42P_9/21/00-MM 505
33P_9/23/01-K 506
9P-4_7/27/00-MR 500
44PW_10//14/01-M 519
47P_9/21/00-MC 512
51P_9/21/00-MC 535
5P_7/27/00-MP 445
46P9/21/00-MC 589
3P_7/27/00-MC 453
28P_8/20/00-MC 499
44P_9/21/00-MB 530
49P_9/21/00-MR 653
52P_9/21/00-MR 548
consensus 721
ITS2
26S
46PKW_10/14/01-M 553
46PKS_10/14/01-M 553
8P_8/24/01-P 536
12P_8/24/01-P 538
53P_10/5/01-K 554
58P_10/5/01-K 554
10P_7/27/00-MR 540
53P_9/21/00-MR 542
29P_8/20/00-MR 543
3P_8/24/01-M 544
51PK_10/7/01-P 560
42P_9/21/00-MM 542
33P_9/23/01-K 543
9P-4_7/27/00-MR 537
44PW_10//14/01-M 557
47P_9/21/00-MC 548
51P_9/21/00-MC 578
5P_7/27/00-MP 493
46P9/21/00-MC 636
3P_7/27/00-MC 482
28P_8/20/00-MC 549
44P_9/21/00-MB 570
49P_9/21/00-MR 712
52P_9/21/00-MR 593
consensus 781


26S
46PKW_10/14/01-M 593
46PKS_10/14/01-M 593
8P_8/24/01-P 576
12P_8/24/01-P 578
53P_10/5/01-K 594
58P_10/5/01-K 594
10P_7/27/00-MR 580
53P_9/21/00-MR 583
29P_8/20/00-MR 584
3P_8/24/01-M 585
51PK_10/7/01-P 600
42P_9/21/00-MM 581
33P_9/23/01-K 581
9P-4_7/27/00-MR 575
44PW_10//14/01-M 597
47P_9/21/00-MC 589
51P_9/21/00-MC 624
5P_7/27/00-MP 539
46P9/21/00-MC 675
3P_7/27/00-MC 523
28P_8/20/00-MC 604
44P_9/21/00-MB 618
49P_9/21/00-MR 768
52P_9/21/00-MR 645
consensus 841
CAGATC TAAGGGAGGAA
CCAGTA TAAGGGGGGGAA
TATCCCGAGGCAGAGA
CCATTTC TAGGCGCGGAGGAA
CATTAT TTAGGGGGGGAAAA
CATATC TAGGCGGCGGGAAGAAAA
TGTGTTCTGTGTTTGTTGTT
CTATGGC
AAATG
AAGGA