

Beatrice and Grant Duckett
Duckett Truffieres
400 Lowry's Rd.
Parksville, BC V9P 2B5

September 09, 2009

Fragment Analysis and DNA Sequencing Services
University of British Columbia Okanagan
3333 University Way
Kelowna, BC V1V1V7

Dear Beatrice and Grant Duckett,

I have completed the molecular prep and DNA sequencing for the six fungal samples received on August 27th, 2009. DNA from all six samples was successfully extracted and amplified. Subsequent DNA sequencing was successful on three of the six samples, providing a high degree of confidence in species identification. A second attempt to sequence the three unsuccessful samples using different primer sets for amplification and subsequent sequencing were also unsuccessful. It was observed that the successful samples were those from the fruiting bodies whereas the samples which were not successful appeared to be soil samples. The following is a brief summary of the results. Please see the attached document for more details.

Sample #1-2008- *Tuber melanosporum*; 100% identity over 571bp

Sample #2-2009- *Tuber melanosporum*; 100% identity over 551bp

Sample #3-2009- *Tuber melanosporum*; 100% identity over 240bp

Sample #4-2009- Uncultured fungus genomic DNA sequence, 99% identity over 521bp

Sample #5-2009- unable to sequence

Sample #6-2009- Uncultured mycorrhizal fungus genes, 95% identity over 693bp

Sample 5 showed multiple bands in sequencing which makes it hard to sequence it with regular sequencing methods.

Please do not hesitate to contact me if you have any questions or concerns.

Sincerely,

Mahsa Amirabbasi
FADSS Technician
(250) 807-9610
mahsa.amirabbasi@ubc.ca

Results Summary

- Sample #1-2008- *Tuber melanosporum*; 100% identity over 571bp
- Sample #2-2009- *Tuber melanosporum*; 100% identity over 551bp
- Sample #3-2009- *Tuber melanosporum*; 100% identity over 240bp
- Sample #4-2009- Uncultured fungus genomic DNA sequence, 99% identity over 521bp
- Sample #5-2009- unable to sequence
- Sample #6-2009- Uncultured mycorrhizal fungus genes, 95% identity over 693bp

Sample #1-2008

Contig sequence derived from ITS1 and LR21 primers (891bp)


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TTGTGTATTCCCGAACACAAACCTCTCTGCGTATCACTCCATGTTGCTTCCACA
GGTTAAGTGACCATGCTGGCACCTGTGGGAGATCTCTATGTTAACGGAGTATTT
GATTGTGCTGTCTGAGCTGGCCATGTGTCAGATTTAGTAAAGTAAAACTTTCA
ACAACGGATCTCTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAG
GTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCG
CCCTTTGGTATTCCTTAGGGCATGCCTGTTGAGCGTCACTACACACCTTATCA
CAAAGTTTGTGGTCTTGGCAGGAGTGAATTGCTAGTCTATCAAATGTTCCAG
CTGTACACTCTGCTAAAATTATGAGAAGGTTACCAGGCATGAACGACGGACT
TTATAAACGGTTATAAGACCTGGATCAGTCACAAGTCTTGTCTGGTCCTTACCT
TAAGGACCCCATCCTAGATGAACCTATGGGTTGACCTCGAATCAGGGAGGGAT
ACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAAAAGAAACCAACAGGGA
TTGCCCTAGTAACGGCGAGTGAAGCGGCAAAGCTCAAATTTGAAATCTGGC
ACCTTTGGTGTCCGAATTGTAATTTGGAGAGGCAACTTCGGGTAGGACCCAGT
CTATGTTCCCTTGGAACAGGACGTCATAGAGGGTGAGAATCCCGTTCATGACTG
GATGTTCCCTACTAGTACGTAGTGCCTTCTACGAGTCGAGTTGTTTGGGAATGCA
GCTCAAATGGGTGGTAAATTCATCTAAAGCTAAATATTGGCGAGAGACCGA
TAGCGCACAAAGTAGAGTGATCGAAAGATGAAAAGCACT

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Blastn top result for percent identity using the Nucleotide Collection (nr/nt) database optimized for highly similar sequences (megablast)

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>  gb|U89359.1|TMU89359 Tuber melanosporum internal transcribed spacer
sequence
Length=620

Score = 1055 bits (571), Expect = 0.0
Identities = 571/571 (100%), Gaps = 0/571 (0%)
Strand=Plus/Plus

Query 1 TTGTGTATTCCCGAACACAAACCTCTCTGCGTATCACTCCATGTTGCTTCCACAGGTTAA 60
      |||
Sbjct 50 TTGTGTATTCCCGAACACAAACCTCTCTGCGTATCACTCCATGTTGCTTCCACAGGTTAA 109

Query 61 GTGACCATGCTGGCACCTGTGGGAGATCTCTATGTTAACGGAGTATTTGATTGTGCTGTC 120
      |||
Sbjct 110 GTGACCATGCTGGCACCTGTGGGAGATCTCTATGTTAACGGAGTATTTGATTGTGCTGTC 169

Query 121 TGAGCTGGCCATGTGTCAGATTTAGTAAAGTAAAACTTTCAACAACGGATCTCTTGGCT 180

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Sbjct	170	 TGAGCTGGCCATGTGTCAGATTTAGTAAAGTAAAACTTTCAACAACGGATCTCTTGGCT	229
Query	181	CTCGCATCGATGAAGAACGCAGCGAAATGCGATAGGTAATGTGAATTGCAGAATTCAGTG	240
Sbjct	230	 CTCGCATCGATGAAGAACGCAGCGAAATGCGATAGGTAATGTGAATTGCAGAATTCAGTG	289
Query	241	AATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCTTAGGGCATGCCTGTTC	300
Sbjct	290	 AATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCTTAGGGCATGCCTGTTC	349
Query	301	GAGCGTCACTACACACCTTATCACAAAGTTTGTGGTCTTGGCAGGAGTGAATTGCTAGTC	360
Sbjct	350	 GAGCGTCACTACACACCTTATCACAAAGTTTGTGGTCTTGGCAGGAGTGAATTGCTAGTC	409
Query	361	TATCAAAATGTTCCAGCTGTACACTCTGCTAAAAATTATGAGAAGGTTACCAGGCATGAA	420
Sbjct	410	 TATCAAAATGTTCCAGCTGTACACTCTGCTAAAAATTATGAGAAGGTTACCAGGCATGAA	469
Query	421	CGACGGACTTTATAAACGGTTATAAGACCTGGATCAGTCACAAGTCTTGTCTGGTCCTTA	480
Sbjct	470	 CGACGGACTTTATAAACGGTTATAAGACCTGGATCAGTCACAAGTCTTGTCTGGTCCTTA	529
Query	481	CCTTAAGGACCCCATCCTAGATGAACTATGGGTTGACCTCGAATCAGGGAGGGATACCC	540
Sbjct	530	 CCTTAAGGACCCCATCCTAGATGAACTATGGGTTGACCTCGAATCAGGGAGGGATACCC	589
Query	541	GCTGAACTTAAGCATATCAATAAGCGGAGGA	571
Sbjct	590	 GCTGAACTTAAGCATATCAATAAGCGGAGGA	620

Sample #2-2009

Contig sequence derived from ITS1 and LR21 primers (869bp)

ACCTCTCTGCGTATCACTCCATGTTGCTTCCACAGGTTAAGTGACCATGCTGGC
ACCTGTGGGAGATCTCTATGTTAACGGAGTATTTGATTGTGCTGTCTGAGCTGG
CCATGTGTCAGATTTAGTAAAGTAAAACTTTCAACAACGGATCTCTTGGCTCT
CGCATCGATGAAGAACGCAGCGAAATGCGATAGGTAATGTGAATTGCAGAATT
CAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATTCCTTAGGG
CATGCCTGTTTCGAGCGTCACTACACACCTTATCACAAAGTTTGTGGTCTTGGC
AGGAGTGAATTGCTAGTCTATCAAAATGTTCCAGCTGTACACTCTGCTAAAAAT
TATGAGAAGGTTACCAGGCATGAACGACGGACTTTATAAACGGTTATAAGACC
TGGATCAGTCACAAGTCTTGTCTGGTCCTTACCTTAAGGACCCCATCCTAGAT
GAACTATGGGTTGACCTCGAATCAGGGAGGGATACCCGCTGAACTTAAGCATA
TCAATAAGCGGAGGAAAAGAAACCAACAGGGATTGCCCTAGTAACGGCGAGT
GAAGCGGCAAAAGCTCAAATTTGAAATCTGGCACCTTTGGTGTCCGAATTGTA
ATTTGGAGAGGCAACTTCGGGTAGGACCCAGTCTATGTTCCCTTGGAACAGGAC
GTCATAGAGGGTGAGAATCCCGTTCATGACTGGATGTTCCCTACTAGTACGTAGT
GCCTTCTACGAGTCGAGTTGTTTGGGAATGCAGCTCAAAATGGGTGGTAAATT
TCATCTAAAGCTAAATATTGGCGAGAGACCGATAGCGCACAAGTAGAGTGATC
GAAAGATGAAAAGCA

Blastn top result for percent identity using the Nucleotide Collection (nr/nt) database optimized for highly similar sequences (megablast)

```
> gb|U89359.1|TMU89359 Tuber melanosporum internal transcribed spacer
sequence
Length=620

Score = 1018 bits (551), Expect = 0.0
Identities = 551/551 (100%), Gaps = 0/551 (0%)
Strand=Plus/Plus

Query 1 ACCTCTCTGCGTATCACTCCATGTTGCTTCCACAGGTTAAGTGACCATGCTGGCACCTGT 60
      |||
Sbjct 70 ACCTCTCTGCGTATCACTCCATGTTGCTTCCACAGGTTAAGTGACCATGCTGGCACCTGT 129

Query 61 GGGAGATCTCTATGTTAACGGAGTATTTGATTGTGCTGTCTGAGCTGGCCATGTGTCAGA 120
      |||
Sbjct 130 GGGAGATCTCTATGTTAACGGAGTATTTGATTGTGCTGTCTGAGCTGGCCATGTGTCAGA 189

Query 121 TTTAGTAAAGTAAAACTTTCAACAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGC 180
      |||
Sbjct 190 TTTAGTAAAGTAAAACTTTCAACAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGC 249

Query 181 AGCGAAATGCGATAGGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACG 240
      |||
Sbjct 250 AGCGAAATGCGATAGGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACG 309

Query 241 CACATTGCGCCCTTTGGTATTCCTTAGGGCATGCCTGTTTCGAGCGTCACTACACACCTTA 300
      |||
Sbjct 310 CACATTGCGCCCTTTGGTATTCCTTAGGGCATGCCTGTTTCGAGCGTCACTACACACCTTA 369

Query 301 TCACAAAGTTTGTGGTCTTGGCAGGAGTGAATTGCTAGTCTATCAAAATGTTCCAGCTGT 360
      |||
Sbjct 370 TCACAAAGTTTGTGGTCTTGGCAGGAGTGAATTGCTAGTCTATCAAAATGTTCCAGCTGT 429

Query 361 ACACTCTGCTAAAAATTATGAGAAGGTTACCAGGCATGAACGACGGACTTTATAAACGGT 420
      |||
Sbjct 430 ACACTCTGCTAAAAATTATGAGAAGGTTACCAGGCATGAACGACGGACTTTATAAACGGT 489

Query 421 TATAAGACCTGGATCAGTCACAAGTCTTGTCTGGTCCTTACCTTAAGGACCCCCATCCTA 480
      |||
Sbjct 490 TATAAGACCTGGATCAGTCACAAGTCTTGTCTGGTCCTTACCTTAAGGACCCCCATCCTA 549

Query 481 GATGAACTATGGGTTGACCTCGAATCAGGGAGGGATACCCGCTGAACTTAAGCATATCAA 540
      |||
Sbjct 550 GATGAACTATGGGTTGACCTCGAATCAGGGAGGGATACCCGCTGAACTTAAGCATATCAA 609

Query 541 TAAGCGGAGGA 551
      |||
Sbjct 610 TAAGCGGAGGA 620
```

Sample #3-2009

Contig sequence derived from ITS1F and ITS2 primers (268bp)

TAGAGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGA
TCATTATGAATGCTTTGGGATACCGTTGTGTATTCCCGAACACAAACCTCTCTG
CGTATCACTCCATGTTGCTTCCACAGGTTAAGTGACCATGCTGGCACCTGTGG
GAGATCTCTATGTTAACGGAGTATTTGATTGTGCTGTCTGAGCTGGCCATGTGT

CAGATTTAGTAAAGTAAAACTTTCAACAACGGATCTCTTGGCTCTCGCATCG
AT

Blastn top result for percent identity using the Nucleotide Collection (nr/nt) database
optimized for highly similar sequences (megablast)

[gb|DQ388877.1|](#) Tuber melanosporum isolate Tmel-eu08 18S ribosomal RNA
gene,
partial sequence; internal transcribed spacer 1 and 5.8S ribosomal
RNA gene, complete sequence; and internal transcribed
spacer 2, partial sequence
Length=548

Score = 444 bits (240), Expect = 1e-121
Identities = 240/240 (100%), Gaps = 0/240 (0%)
Strand=Plus/Plus

```
Query 29 TCCGTAGGTGAACCTGCGGAAGGATCATTATGAATGCTTTGGGATACCGTTGTGTATTCC 88
          |||
Sbjct 1 TCCGTAGGTGAACCTGCGGAAGGATCATTATGAATGCTTTGGGATACCGTTGTGTATTCC 60

Query 89 CGAACACAAACCTCTCTGCGTATCACTCCATGTTGCTTCCACAGGTTAAGTGACCATGCT 148
          |||
Sbjct 61 CGAACACAAACCTCTCTGCGTATCACTCCATGTTGCTTCCACAGGTTAAGTGACCATGCT 120

Query 149 GGCACCTGTGGGAGATCTCTATGTTAACGGAGTATTTGATTGTGCTGTCTGAGCTGGCCA 208
          |||
Sbjct 121 GGCACCTGTGGGAGATCTCTATGTTAACGGAGTATTTGATTGTGCTGTCTGAGCTGGCCA 180

Query 209 TGTGTCAGATTTAGTAAAGTAAAACTTTCAACAACGGATCTCTTGGCTCTCGCATCGAT 268
          |||
Sbjct 181 TGTGTCAGATTTAGTAAAGTAAAACTTTCAACAACGGATCTCTTGGCTCTCGCATCGAT 240
```

Sample#4-2009

GAAGTAAAAGTCGTAACAAGGTCTCCGTTGGTGAACCAGCGGAGGGATCATT
ACCGAGTTTACAACCTCCCAAACCCCTGTGAACATACCTTAATGTTGCCTCGGC
GGATCAGCCCGCGCCCCGTAAAACGGGACGGCCCGCCAGAGGACCCAAACTC
TAATGTTTCTTATTGTAACCTTCTGAGTAAAACAAACAATAAATCAAACTTTC
AACAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCAAATGCGATA
AGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGC
GCCCCGCTGGTATTCCGGCGGGCATGCCTGTTTCGAGCGTCATTTCAACCCTCAA
GCCNCGGGTTTGGTGTTGGGGATCGGCTCTGCCTTCTGGCGGTGCCGCCCCC
GAAATACATTGGCGGTCTCGCTGCAGCCTCCATTGCGTAGTAGCTAACACCTC
GCAACTGGAACGCGGCGCGGCCATGCCGTAACCCCAACTTCTGAATGTTG
ACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATA

Blast top result for percent identity using the Nucleotide Collection (nr/nt) database
optimized for highly similar sequences (megablast)

[emb|FN397346.1|](#) Uncultured fungus genomic DNA sequence containing 18S rRNA
gene,
ITS1, 5.8S rRNA gene, ITS2 and 28S rRNA gene, clone N14_P_4_E7
Length=598

Score = 1048 bits (567), Expect = 0.0
Identities = 568/569 (99%), Gaps = 0/569 (0%)

Strand=Plus/Minus

```
Query 1 GAAGTAAAAGTCGTAACAAGGTCTCCGTTGGTGAACCAGCGGAGGGATCATTACCGAGTT 60
|||||
Sbjct 584 GAAGTAAAAGTCGTAACAAGGTCTCCGTTGGTGAACCAGCGGAGGGATCATTACCGAGTT 525

Query 61 TACAACCTCCCAAACCCCTGTGAACATACCTTAATGTTGCCTCGGCGGATCAGCCCGCGCC 120
|||||
Sbjct 524 TACAACCTCCCAAACCCCTGTGAACATACCTTAATGTTGCCTCGGCGGATCAGCCCGCGCC 465

Query 121 CCGTAAAACGGGACGGCCCGCCAGAGGACCCAAACTCTAATGTTTCTTATTGTAACCTTCT 180
|||||
Sbjct 464 CCGTAAAACGGGACGGCCCGCCAGAGGACCCAAACTCTAATGTTTCTTATTGTAACCTTCT 405

Query 181 GAGTAAAACAAACAAATAAATCAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGA 240
|||||
Sbjct 404 GAGTAAAACAAACAAATAAATCAAACTTTCAACAACGGATCTCTTGGTTCTGGCATCGA 345

Query 241 TGAAGAACGCAGCAAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAA 300
|||||
Sbjct 344 TGAAGAACGCAGCAAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAA 285

Query 301 TCTTTGAACGCACATTGCGCCCGCTGGTATTCCGGCGGGCATGCCTGTTTCGAGCGTCATT 360
|||||
Sbjct 284 TCTTTGAACGCACATTGCGCCCGCTGGTATTCCGGCGGGCATGCCTGTTTCGAGCGTCATT 225

Query 361 TCAACCCCTCAAGCCNCGGGTTTGGTGTGGGGATCGGCTCTGCCTTCTGGCGGTGCCGC 420
|||||
Sbjct 224 TCAACCCCTCAAGCCCTCGGGTTTGGTGTGGGGATCGGCTCTGCCTTCTGGCGGTGCCGC 165

Query 421 CCCCAGAAATACATTGGCGGTCTCGCTGCAGCCTCCATTGCGTAGTAGCTAACACCTCGCA 480
|||||
Sbjct 164 CCCCAGAAATACATTGGCGGTCTCGCTGCAGCCTCCATTGCGTAGTAGCTAACACCTCGCA 105

Query 481 ACTGGAACGCGGCGCGGCCATGCCGTAAAACCCCAACTTCTGAATGTTGACCTCGGATCA 540
|||||
Sbjct 104 ACTGGAACGCGGCGCGGCCATGCCGTAAAACCCCAACTTCTGAATGTTGACCTCGGATCA 45

Query 541 GGTAGGAATACCCGCTGAACTTAAGCATA 569
|||||
Sbjct 44 GGTAGGAATACCCGCTGAACTTAAGCATA 16
```

Sample#5-2009

Unable to obtain sequencing results.

Sample#6-2009

```
AAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTATTGAAT
ACGATTGGTACTGATGCTGGCTCTTCACTGAGCATGTGCTCGTCCATCTATTTAT
CTTCTCTTGTGCACATCTTGTAGTCTTGAATTGAAACCCCTCGCAGTCAAATGC
GGTTTGGGAGGTTGGGCGCGAGCCCTCCTTCTGCTTCTTCAAGGCTATGTTTTC
ATATACTATAAAGTTACAGAATGTCTTTAACGATTGTGCTAGTCGCAGTCAT
TAAACCTATACAACTTTCAGCAACGGATCTCTTGGCTCTCCTATCGATGAAGAA
CGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAA
TCTTTGAACGCACCTTGCGCCCTTTGGTATTCCGAAGGGCATGCCTGTTTGAGT
GTCATTAAATTATCAACCTTGCTCTCGCTTTTACCGGCTTGAGTGAGGCTTGA
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Sbjct 643 | | | |
TAAG 646