

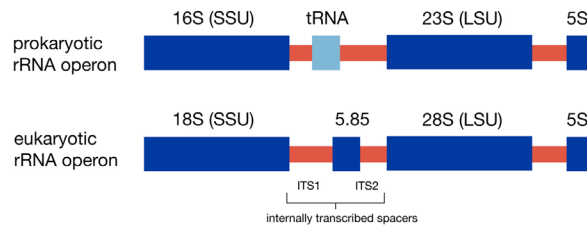
# Microbial Identification

## By rRNA Gene Analysis

### Introduction

The identification of microorganisms has traditionally relied on phenotypic methods. With advancements in genetic methods, various classification methodologies based on nucleotide sequences are increasingly popular due to their specificity and speed. Gene sequencing-based techniques can serve to detect, identify, and monitor microbes, even poorly known or uncultured microorganisms. Comparative gene sequence analysis of ribosomal DNA (rDNA) has been shown to have the highest accuracy of microbial identification system technologies and has been considered the gold standard for microbial identification for over a decade.

Poochon's Rapid Microbial Identification System uses a highly accurate phylogenetic approach for microbial identification based on the sequencing of the 16S rRNA gene for bacteria or the internal transcribed spacer (ITS) region and 28S rRNA gene for fungi (Figure 1). The resulting sequences of microbial samples are compared to sequences in the National Center for Biotechnology Information's database.



**Figure 1:**  
*Illustration  
of ribosomal  
RNA operon*

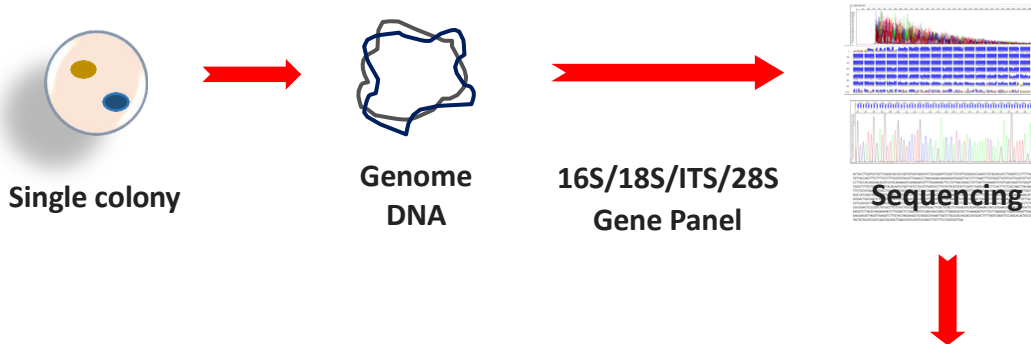
### Specifications

- **Method** – 16S/18S/28S/ITS Gene Panel and Applied Biosystems™ automated DNA sequencing
- **Key Instruments** – Veriti™ 96-Well Fast Thermal Cycler, Applied Biosystems™ 3730xl DNA Analyzer
- **Sensitivity** – Highly sensitive, 100% for single colony
- **Specificity** – Highly specific to detect 16S/18S/28S/ITS rRNA genes
- **Acceptable Samples** – Single colony on plates or in culture media
- **Turnaround Time** – Reports are typically ready within 3 business days of specimen receipt

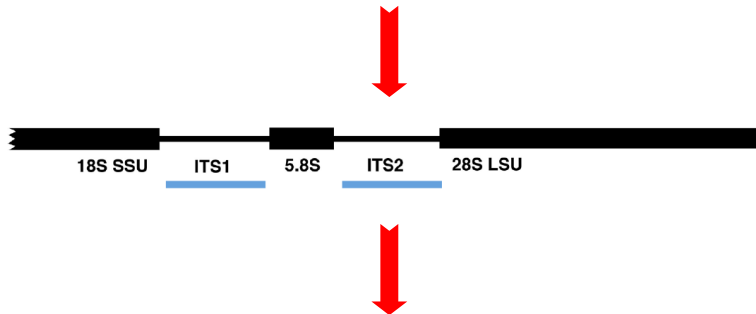
# Microbial Identification

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## 16S/18S/ITS/28S rRNA Gene Sequencing



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AAATCCTGAAATTCAGGTTGACTCTGAAAGAGGAAAGCGTAAATATACGCCACCTCGGCACAGTGAAGAGCCCGCTCGCAACTGCCTTTAAC
AATTTATCAGACAATCTGTGTGGGCACTCGAAGATACGGATTCTTAAAGTTCGCAAGACGAAAAATGAATACCAAGTCTCAAGAGTGAACACGTAAT
CATTACGAAGTTTAAATCTTTGAGCATCAAACCTTTAAATTGAAAGCTTGGTTGATGGCTCAGTTGAACCGCTGGCGGACGGCCTAACACATGCAAG
TCGAACGGTAACAGGAAACAGCTTGCTGTTTCGCTGACGAGTGGCGGACGGGTGAGTAATGCTGGGAAACTGCCTGATGGAGGGGGATAACTACTG
GAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTCGGGCCTTTGCCATCGGATGTGCCAGATGGGATTAGCTTGTGG
TGGGGTAAACGGCTCACCAAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACCTGAGACACGGTCCAGACTCCACGGGAGCC
AGCAGTGGGGAATATGCACAATGGGCGCAAGCCTGATGCAGCCATGCCCGGTGATGAAGAAGCCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAG
GAAGGGAGTAAAGTAAATACCTTTGCTCATTGACGTTACCCGAGAAGAAGCACCAGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCA
AGCCTTAAATCGGAATTAAGCGGTAAGCGCACGCGAGGCGGTTTGTAAAGTCAGATGTGAAATCCCGGGCTCAACCTGGGAACTGCATCTGATAC
TGCAAGCTTGAGTCTCGTAGAGGGGGTAGAATCCAGGTGTAGCGGTGAATCCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGGCCCT
GGACGAAGACTGACGCTCAGGTGCGAAAGCGTGGGAGCAAACAGGATTAGATACCTGGTAGTCCAGCCGTAAACGATGTGCGACTTGGAGGTTGT
GCCCTTGAGCGTGGCTTCCGGAGCTAACCGCTTAAAGTCGACCGCCTGGGAGTACGGCCGCAAGGTTAAACTCAAATGAAATGACGGGGGGCCGC
ACAAGCGTGGAGCATGTGGTTTAAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACATCCAGGAAGTTTTCAGAGATGAGAATGTGCCTTC
GGGAACCGTGAGACAGGTGCTGCATGGCTGCTGTCAGCTCGTGTGTAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCCTTATCCTTTGTTGC
CAGCGGTCGGCCGGGAACCTCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGTGGGGATGACGTCAGTCAAGTCAATCATGGCCCTTACGACCAGGGCTA
CACACGTGCTACAATGGCGCATACAAAGAGAAGCGACCTCCGCGAGAGCAAGCGGACCTCATAAAGTGCCTCGTAGTCCGGATTGGAGTCTGCAACTC
GACTCCATGAAGTCGGAATCGCTAGTAATCGTGGATCAGAATGCCAGGTGAATACGTTCCCGGGCCTGTACACACCCCGCTCACACCATGGGAG
TGGTTGCCAAAAGAGTAGTAGCTTAACCTTCGGGAGGGCGCTTACCACCTTGTGATTTCATGACTGGGGTGAAGCTGCTACAGGTAAGCTGAGG
GAACCTCGGTTGGATCACCTCCTTACCTTAAAGAAGCTACTTTGCAGTGCTCACACAGATTGTCTGATGAAATGAGCAGTAAACCTCTACAGG
CTTGTAGCTCAGTGGTTAGAGCGCACCCCTGATAAGGGTGAGGTCGGTGTTCAGTCCACTCAGGCCCTACCAAAATTTGCACGGGCAAAATTTGAAGA
GGTTTTAACTACATGTTATGGGGCTATAGCTCAGCTGGGAGAGCGCCTGCTTTCACGCAGGAG
```



### Report

Species and Strain-level Identity

5350 Partners Court, Suite C, Frederick, MD 21703

Email: [support@poochonscientific.com](mailto:support@poochonscientific.com)

Tel: 301-761-4835

[www.poochonscientific.com](http://www.poochonscientific.com)



# Microbial Identification

By rRNA Gene Analysis

## Report Example

### CERTIFICATE OF ANALYSIS

#### General Information

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**Sample Name:** No.1059BSC10102NOV202101

**Description:** One plate growing a single fungus colony

#### Identification Results

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**Fungus:** *Aspergillus sydowii*

*Aspergillus sydowii* determined by the internal transcribed spacer (ITS) region and 28S rRNA gene sequencing of the colony

#### Summary

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The single colony from the plate (A.1059BSC1.01.02NOV2021.01) was processed for genomic DNA isolation followed by PCR of the ITS region and 28S rRNA gene. The amplified ITS region and 28S rRNA gene product was further analyzed by automated DNA sequencing using Applied Biosystems™ 3730xl DNA Analyzer. The results indicated that the microbe is Fungus *Aspergillus sydowii*.

# Microbial Identification

## By rRNA Gene Analysis

### Report Example Continued

## CERTIFICATE OF ANALYSIS

### ITS Sequencing Results

**Sample:** No.1059BSC10102NOV202101

**F1\_P2\_ITS1\_2021-11-23\_B07**

GGGGGCCCCGCTGCGGGCTGCCTCCGGGCGCCACCTCCCACCCGTGAATACCTAACACTGTTGCTTCGGCGGGGAGCTCCCTCGGGGG  
CGAGCCGCGGGGACTACTGAACCTCATGCCTGAGAGTGATGCAGTCTGAGTCTGAATATAAAATCAGTCAAACCTTTCAACAATGGATCT  
CTTGTTCCGGCATCGATGAAGAACGAGCGAACTGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGAACGCACA  
TTGGCCCCCTGGCATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCATCAAGCCCCGGTGTGTGTTGGGTCGTCTCCCCCCC  
GGGGGACGGGCCGAAAGGCAGCGGGCCGACCGTGTCCGGTCTCGAGCGTATGGGGCTTTATCACCCGCTCGACTAGGGCCGGCCGG  
CGCCAGCCGACGTCTCAACCATTTTTCTCAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACCTAAGCATATCAATAAGCGGAGG  
AACACACTCAGATTGATTGACTGGCACCCACACAACCTTTGAGTATGTGAATTAATTGAAGAAAACATCCTTACCTTACCAAATCTTTGAC  
ATCCTTTGAACATTCTAGAGATCCTTTTTTCCCTTCATACGGAAAAGTGACGGGTGGGTTCTGGTCTGTCTGTCTGTCTGTCTGTGAGAT  
GATGGCCTAAATCCCAACAACCGTAATCCTTATGTTACATGTCATTAATAAATAGAAAACCTAATGTGACTGACAATGACAAAACAGG  
AGGAAAAATGCCGAATAATCTCCATTACCTGTCCCTTTATGATTATGGGCCTCGCACATGCTTACGATGCACAATACTTAGGATCCCGGA  
AGCCGACAGAGAAATACTCAAATCCCATATGTTGGTCTCCATTACGGATTGCATTCCGGCCACTCGAACTACCTGAAATGTGTAATCGCC  
TCGCAATCCGAAAACAGTCATGGATACCGTAAATACTTCTCGGCGTCTTGTCCATCCGCCCTCAACACCACTAGAATCTGTAATCCTCC  
CGAAGCCACTGCACGCATCCATTCTGCACCTAATGCCGATCAGTAATGTTGGGAAAAGATTGGGGTGCCA

**F1\_P2\_ITS3\_2021-11-23\_E07**

TCCCCGCGCCCCATGTTGTGATTGCAGATTCAGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGCATTCCGGGGGGCATGCC  
TGTCCGAGCGTCATTGCTGCCATCAAGCCCGCTTGTGTGTTGGGTCGTCTCCCCCGGGGGACGGGCCGAAAGGCAGCGGGCGGCA  
CGGTGTCCGGTCTCGAGCGTATGGGGCTTTATCACCCGCTCGACTAGGGCCGGCCGGGCGCCAGCCGACGTCTCAACCATTTTTCTTCA  
GGTTGACTCGGATCAGGTAGGGATACCCGCTGAACCTAAGCATATCAATAAGCGGAGGAATGATATTTACAACAAGGTCCTCCCCCTGCC  
TCACTCCTCCGCTTAATCTTGTCCCTACGAGTCTCGAGACTATTCCTGAATAGTGTTCCTGATACTACCCGCATAACCAACCCATTT  
CTAAGATTGACCTGTTTTACGCTTCTACCCGATGAACCTAAGCTTATCATCTCCGCTGGGACTACCGTCAACTGTAAGTATAAGA  
ATTGACTGCACCCGCACAACAACCGGAGTATGTGTTTTATTCAAACCAACGAAAACAACCTACCATGTAATGACTCTATCTGACAAAAACA  
CATAAGTACCTTTCTCGTACATTACAGACTGGTATGCATGGTGTCTTCTCTGTGTGAGATGTTAGTCTGTCACCCCAAGCC  
ACACCTTGATCATC

**Matched to:**

>KY828886.1 *Aspergillus sydowii* isolate FBKL3.0133 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence

CATTACTGAGTGCGGGCTGCCTCCGGGCGCCAACTCCCACCCGTGAATACCTAACACTGTTGCTTCGGCGGGGAGCTCCCTCGGGGGCG  
AGCCGCGGGGACTACTGAACCTCATGCCTGAGAGTGATGCAGTCTGAGTCTGAATATAAAATCAGTCAAACCTTTCAACAATGGATCTCT  
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GCGCCCCCTGGCATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCATCAAGCCCCGGTGTGTGTTGGGTCGTCTCCCCCGG  
GGGACGGGCCGAAAGGCAGCGGGCCGACCGTGTCCGGTCTCGAGCGTATGGGGCTTTATCACCCGCTCGACTAGGGCCGGCCGGGCG  
CCAGCCGACGTCTCAACCATTTTTCTCAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACCTAAGCATATCAATAAGCGGAGGAA