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Client: Tiffany Craig, WKRG

Date: May 9, 2005

Species: red snapper

Project # 20097

Sample type: frozen tissue

Received: April 12, 2005

Number received: 9

Test(s) performed: DNA sequencing test for species identification

Samples: Commercial samples: Atlantis Fish Market
Landry's
Zeke's Landing
Foley Fish Company
King Neptune Restaurant
Joe Patti's Seafood
J.D. Seafood
City Seafood
The Pillar's Restaurant

Enclosures: Summary report
Invoice

THERION INTERNATIONAL
REVISED FINAL REPORT
Project # 20097

Conclusions:

Fish filet samples were submitted from nine commercial sources and DNA sequencing analysis was performed to determine the likely species of origin. DNA from the samples was first sequenced using primer pairs to the mitochondrial DNA control region of red snapper. For those samples with ambiguous results, additional sequencing was performed with universal primers to the mitochondrial cytochrome *b* gene. The sequences obtained from the samples were compared to those in the sequence database at the National Center for Biotechnology Information (NCBI) of the National Institutes of Health (NIH). The sequences were also compared to those obtained from two previously analyzed samples from Caribbean red snapper and lane snapper.

Samples from Atlantis Fish Market, King Neptune Restaurant, Joe Patti's Seafood, J. D. Seafood and City Seafood were determined to be red snapper. The samples from Landry's and Foley Fish Company were determined to be lane snapper. The sample from The Pillar's was not snapper. Grouper was the species with the highest Blast call but perch and sculpin also generated similar E values. The sample from Zeke's was determined to be emperor red snapper.

Methodology:

DNA was extracted from the tissue samples using the Wizard SV Genomic DNA Isolation Kit from Promega. An aliquot of the DNA was amplified by the polymerase chain reaction (PCR™) using primer pairs to the mitochondrial DNA control region of red snapper (Garber, et al., 2004, Mar. Biotechnol. 6, 175-185). Samples that gave ambiguous results were also amplified using universal primer pairs mcb398 and mcb869 to the mitochondrial cytochrome *b* gene (Verma & Singh, 2003, Molecular Ecology Notes, 3, 28-31).

The amplified DNA was purified (High Pure PCR Product Purification Kit, Roche), quantified and run through the Big Dye Cycle Sequencing kit (version 3.1, Applied Biosystems, Inc.). The sequenced samples were ethanol precipitated and run on an ABI® Prism 3100-Avant Genetic Analyzer™. The results were analyzed using ABI® DNA Sequencing Analysis™ software version 5.1. The sequences obtained from the samples were subjected to a BLAST search of the database at the National Center for Biotechnology Information (NCBI) of the National Institutes of Health (NIH) to determine likely species of origin. Sequences were also compared directly to those produced from the Caribbean red snapper (*Lutjanus purpureus*) and lane snapper (*Lutjanus synagris*) controls to further clarify species assignment.

Results:

Control samples (control gene) - The NCIB database at NIH did not contain any sequences from the mitochondrial DNA control region for *Lutjanus purpureus* (Caribbean red snapper) and *Lutjanus synagris* (lane snapper). Therefore the closest match in the database for these samples was *Lutjanus campechanus* (northern red snapper).

Fish filet samples (control gene) - The sequences obtained from the fish filet samples were subjected to a BLAST search of the NIH database. Some of the sequences were also directly compared to the DNA sequence obtained from the Caribbean red snapper and lane snapper control samples. The results are shown below in Table 1. The Expect value (E) and the percent homology to the red snapper (Rs) and lane snapper (Ls) controls are also indicated. The E value is a measure of the random background noise that exists for matches between sequences. The closer the E value is to “0” the more “significant” the match is. [For clarification an entry of 3.0E-22 means 3.0×10^{-22} .] The E value also takes into account the length of the query sequence (~285 bases) and the size of the database used for the comparison (<2.8 million sequences).

Table 1. DNA sequencing results from red snapper primers

SITE	Blast call	E value	% to Rs	E value	% to Ls	E value	Species call
Atlantis Fish Market	<i>L. campechanus</i>	1.0E-109					red snapper
Landry’s	<i>Lutjanus sp.</i>	1.0E-115	82%	4.0E-53	91%	1.0E-125	lane snapper
Zeke’s Landing	<i>L. campechanus</i>	2.0e-9	20%	2.0E-10	10%		snapper?
Foley Fish Company	<i>L. campechanus</i>	1.0 ^E -78	22%	3.0 ^E -24	85%	1.0 ^E -89	lane snapper
King Neptune Rest.	<i>L. campechanus</i>	1.0E-51					red snapper
Joe Patti’s Seafood	<i>L. campechanus</i>	3.0E-95					red snapper
J.D. Seafood	<i>L. campechanus</i>	2.0E-71					red snapper
City Seafood	<i>L. campechanus</i>	1.0E-124					red snapper
The Pillar’s Rest.	ambiguous result						see below

Rs is the red snapper control, Ls is the lane snapper control.
Lutjanus campechanus is the northern red snapper.

Samples from Atlantis Fish Market, King Neptune Restaurant, Joe Patti’s Seafood, J. D. Seafood and City Seafood were determined to be red snapper. The results from Landry’s and Foley Fish Company suggested that they might be lane snapper. The samples from Zeke’s Landing and The Pillar’s gave ambiguous results. Therefore, these last four samples were sequenced further using universal primer pairs mcb398 and mcb869 to the mitochondrial cytochrome *b* gene. The results are shown below in Table 2.

Table 2. DNA sequencing results from cytochrome *b* primers

SITE	Blast call	E value	Species call
Landry's	<i>Lutjanus synagris</i>	4.0E-95	lane snapper
Zeke's Landing	<i>Lutjanus sebae</i>	1.0E-159	emperor red snapper
Foley's Fish Company	<i>Lutjanus synagris</i>	6.0E-91	lane snapper
The Pillar's Restaurant	<i>Epinephelus sp.</i>	4.0E-87	grouper **

Epinephelus species is grouper.

**Two other species came up with similar E-values:

M. wakiyae (silverbelly sea perch) and *L. microptera* (sculpin)

Fish filet samples (cytochrome *b* gene) - The samples from Landry's and Foley's Fish Company produced E values of 4.0E-95 and 6.0E-91 in the BLAST search match to *Lutjanus synagris* (lane snapper) corroborating the results reported above. The sample from Zeke's showed a match in the Blast search with a very high E value to *Lutjanus sebae*. This is saltwater fish from the western Pacific Ocean. The sample from The Pillar's Restaurant showed a match in the BLAST search to *Epinephelus species*, which is grouper. Similar E values were also obtained for *Malakichthys wakiyae* (silverbelly sea perch) and *Lepidotrigla microptera* (sculpin).

Summary information for all species calls is presented below in Table 3.

Table 3. Cumulative DNA sequencing results

SITE	Species call
Atlantis Fish Market	red snapper
Landry's	lane snapper
Zeke's Landing	emperor red snapper
Foley Fish Company	lane snapper
King Neptune Rest.	red snapper
Joe Patti's Seafood	red snapper
J.D. Seafood	red snapper
City Seafood	red snapper
The Pillar's Rest.	grouper