Josh - 12/8/11 Fish Extraction Testing

Purpose:
Test fish extraction methods

Sample	Source	Preparation
1	Smoked Salmon	5 ul slice, lacerated with pipette tip
2	Smoked Salmon	Toothpick stab
3	Smoked Salmon w/ Ketchup	5 ul slice, lacerated with pipette tip, some ketchup included
4	Smoked Salmon w/ Ketchup	Toothpick stab, stabbed through ketchup
5	Stuffed Scallops	5 ul slice, lacerated with pipette tip
6	Stuffed Scallops	Toothpick stab
7	Shrimp	5 ul slice, lacerated with pipette tip
8	Shrimp	Toothpick stab
9	Negative control	Toothpick stab
10	Smoked Salmon	Toothpick stab 5 times

DNA Extraction
Use 75 ul Epicentre BuccalAmp buffer in PCR tube, no vortexing, incubate 65 C 6 minutes, heat inactivate 98 C 2 minutes.
For toothpick stab samples, swirl toothpick end in 75 ul buffer a few times. Toothpick samples stabbed once unless noted. Toothpick was cylindrical with 1 cm cone on one end.

PCR Test - FISHCO1L/HBCm13 Primers

Reaction:
1 uL template
2 uL 10 uM primer mix
17 uL ddH20
20 uL Bioneer tube

PCR Program:
Initial Denaturing: 5 min @ 95 C
30 cycles:
    Denature: 30 secs @ 95 C
    Anneal: 1 min @ 65 C
    Extend: 1 min @ 72 C
Final Extension: 5 min @ 72 C

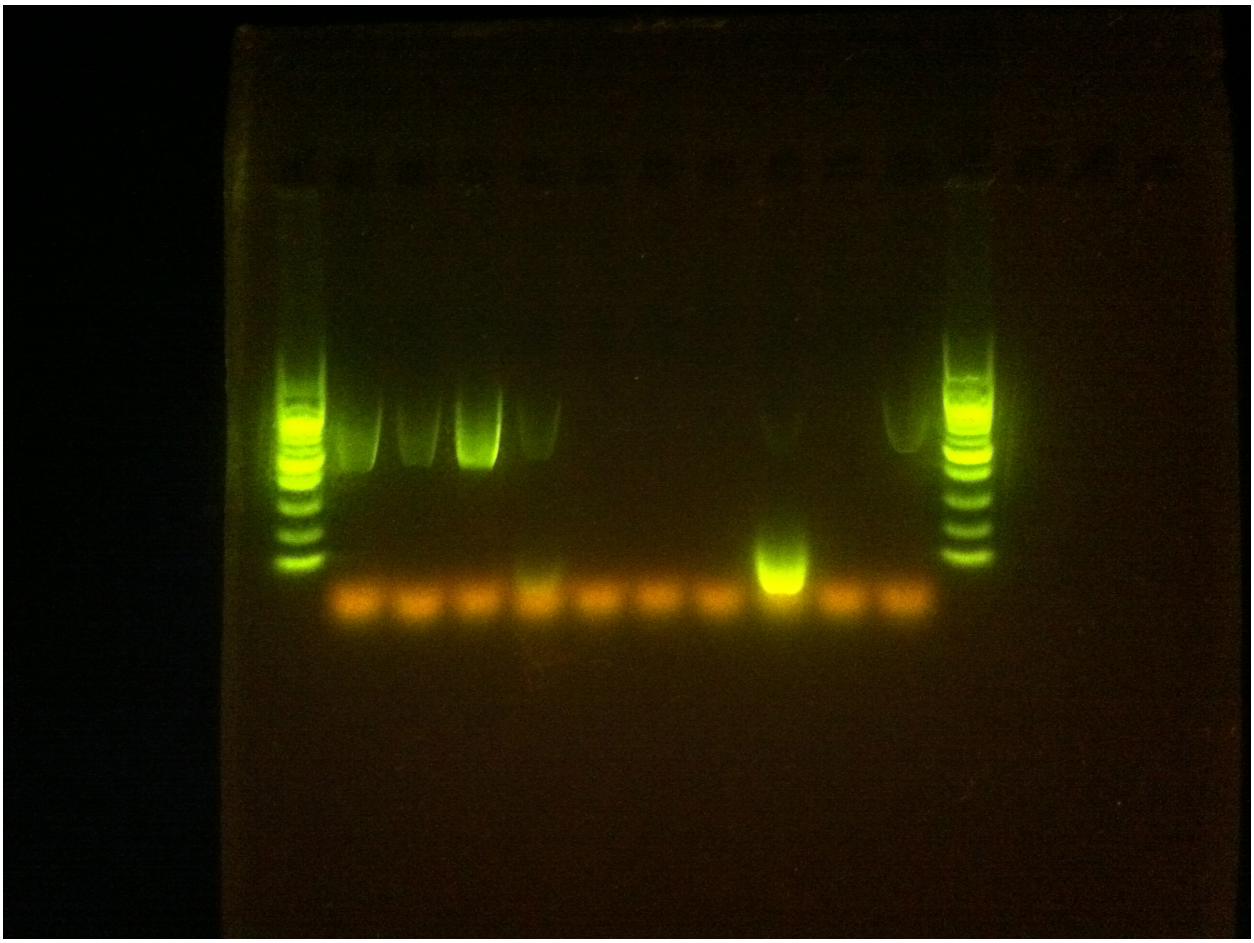
Gel: 1.5% agarose TAE, 100 bp ladder

Sequencing:
Primer: M13F (CACGACGTTGTAAAACGAC)
Amplicon length: 743

Identification: BOLD species level

Gel/Sequencing Results:

Sample	Gel Result	Operon Tube	Sequencing Result	Species ID
1	Good			
2	Ok, weaker amplification			
3	Good			
4	Ok, weaker amplification			
5	No Amplification			
6	No Amplification			
7	No Amplification			
8	Bad, Tiny Amplification, tons of primer dimers			
9	No Amplification			
10	Ok			



Follow-up
Ran 10 more cycles on sample #10, re-ran on same gel (lane 13) to see if sample intensity improved: