Josh - DNA Barcode Testing 11/12/11

Purpose:
Determine ideal sample prep method, test barcoding primers

Use DNA extract from previous week, samples:

Sample	Source	Preparation
1	Fresh fish	Lacerate tissue with sterile nail
2	Fresh fish	5 square millimeter fin webbing cut
3	Fresh fish	5mm x 2mm x 2mm meat chunk
4	Fresh fish	Wire stab
5	Cooked fish	5mm x 2mm x 2mm meat, chopped
6	Fresh crab	5 square millimeter chunk
7	Fresh fish skin scraping	5 square mm
8	Fresh fish	20 uL meat, chopped in tube

PCR Test #1 - FISHCO1L/HBC 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
35 cycles:
    Denature: 30 secs @ 95 C
    Anneal: 1 min @ 56 C
    Extend: 1 min @ 72 C
Final Extension: 5 min @ 72 C

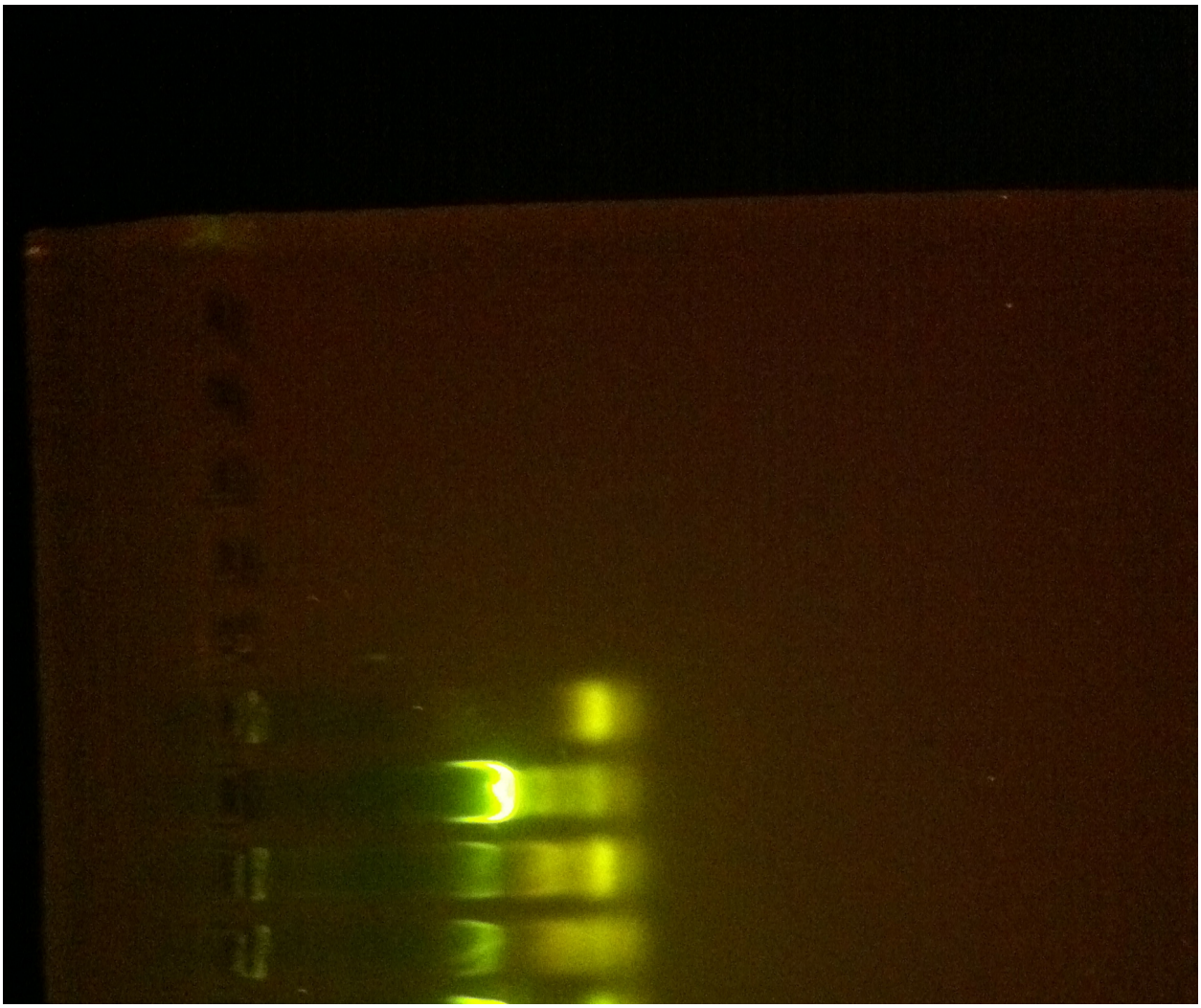
Gel: 1.5% agarose TAE, 100 bp ladder

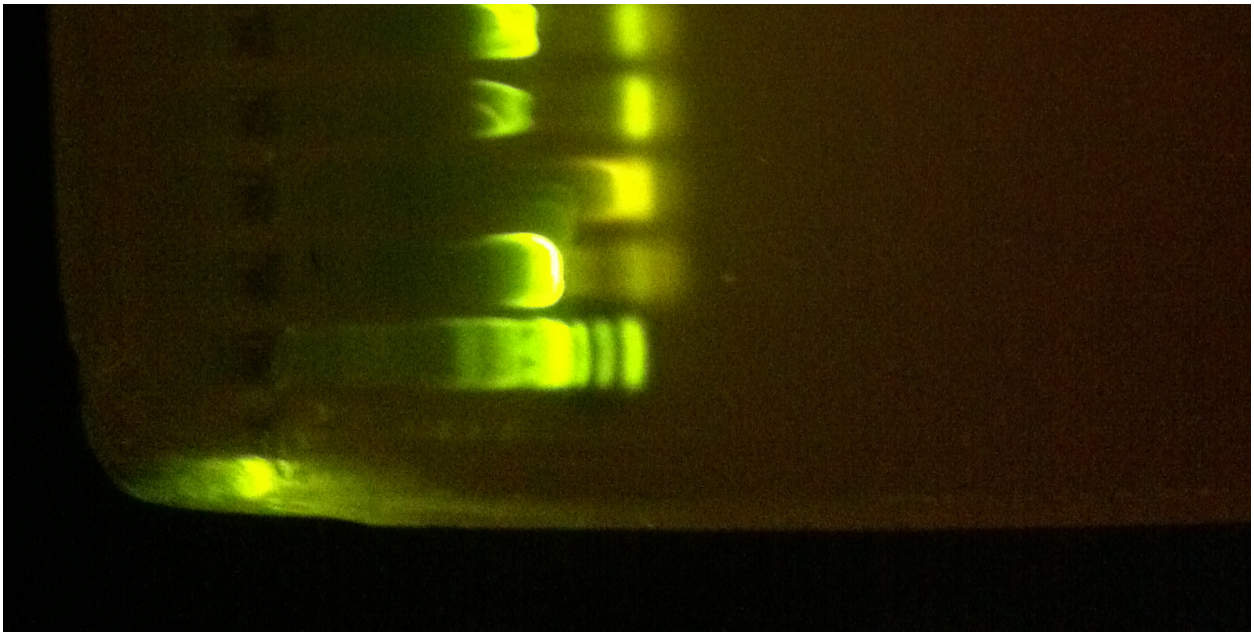
Sequencing:
Primer: FishCO1LBC (TCAACYAATCAYAAAGATATYGGCA)
Amplicon length: 704

Identification: BOLD species level

Gel/Sequencing Results:

Sample	Operon Tube	Gel Result	Sequencing Result, bp, ID %
1	AF9914	Ok - Band w/ smaller products, strong signal	Good, 684, 100%
2	AF9913	Bad - No amplification	Bad, no match
3	AF9912	Good	Poor, no match
4	AF9911	Good	Ok, 686, 100%
5	AF9852	Poor - Band w/ smaller products, weak signal	Poor, 681, 97.47%
6	AF9848	Poor - Band w/ smaller products	Poor, 678, 99.66%
7	AF9910	Ok - Band w/ smaller products, strong signal	Good, 680, 100%
8	AF9847	Bad - No amplification	Bad, no match





PCR Test #2 - 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
35 cycles:
    Denature: 30 secs @ 95 C
    Anneal: 1 min @ 66 C
    Extend: 1 min @ 72 C
Final Extension: 5 min @ 72 C

Gel: 1.5% agarose TAE, 100 bp ladder

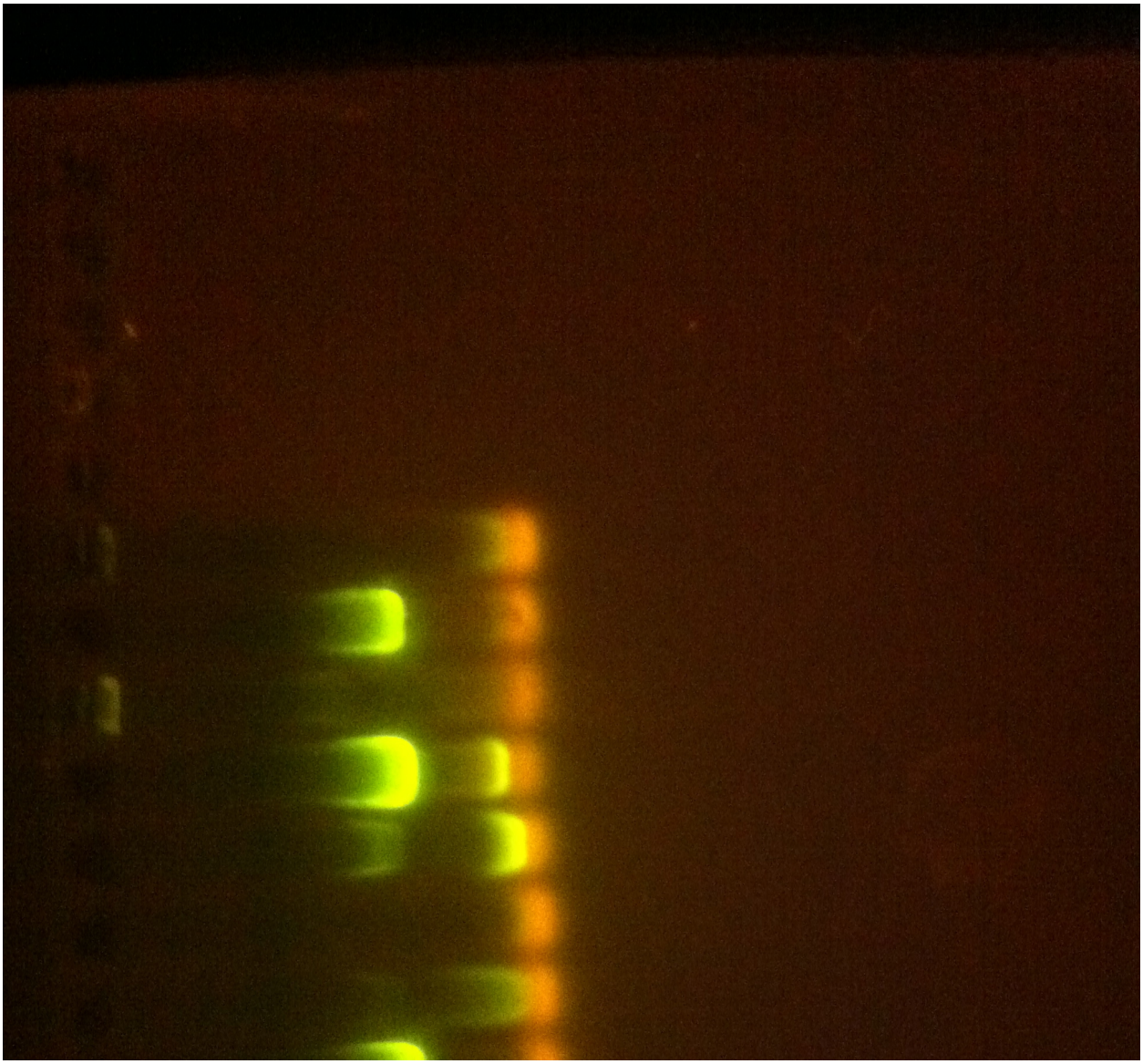
Sequencing:
Primer: M13F (CACGACGTTGTAAAACGAC)
Amplicon length: 743

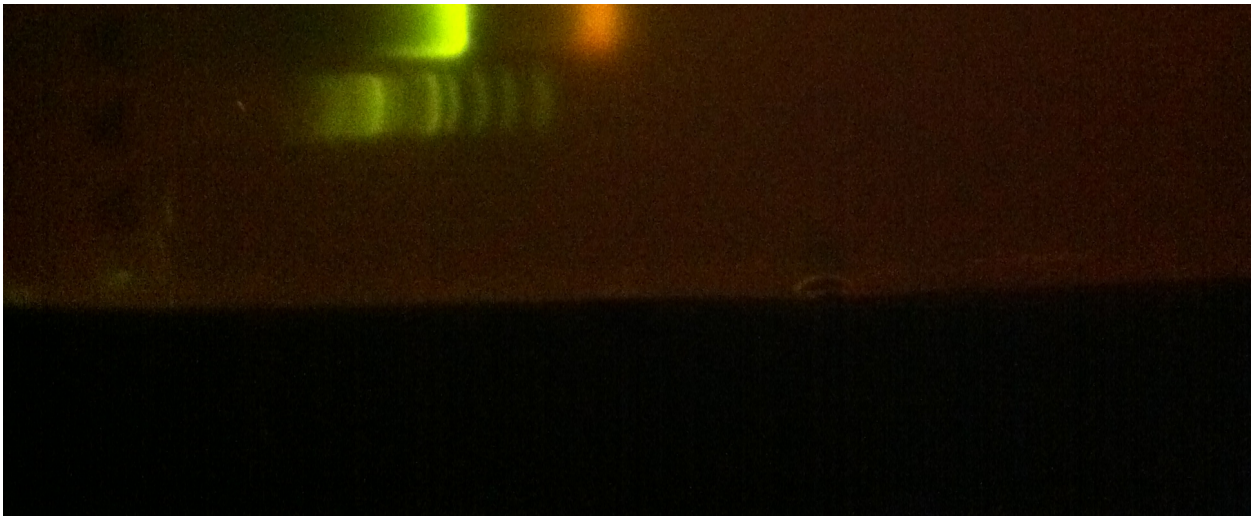
Identification: BOLD species level

Gel/Sequencing Results:

Sample	Operon Tube	Gel Result	Sequencing Result, bp, ID %

1	AF9853	Good	Good, some background, 716, 99.85%
2	AF9854	Bad - minute amplification w/ smaller smear	Bad, no match
3	AF9855	Bad - no amplification	Bad, no match
4	AF9856	Ok - weak amplification	Poor, 712, 100%
5	AF9858	Good	Good, 714, 99.85%
6	AF9857	Bad - smear	Bad, no match
7	AF9959	Good	Poor, high background, 100%
8	AR8588	Bad - no amplification	Bad, no match





Analysis
Based on gels, lacerating tissue with sterile nail and skin scraping provided the best overall results, but other methods may be made to work better with different template amount. Awaiting sequencing results.