Update on the Tilapia Rickettsia Like Organism (TRLO) Infecting Tilapia On Oahu.

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CTAHR/HAAA Workshop

Windward Community College

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REGIONAL BIOSECURITY: OPERATIONAL BIOSECURITY AND DIAGNOSTIC SURVEILLANCE – Year 2

- Grant #: 2008-202(b)
- Reporting Period: April 30, 2011 – May 1, 2012.
- Funding Level: $50,000
- Participants:
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  - Leina'ala Bright
  - Stan Kodama
  - Ron Koza

College of Tropical Agriculture and Human Resources

Aquaculture & Livestock Support Services
DEPARTMENT OF AGRICULTURE

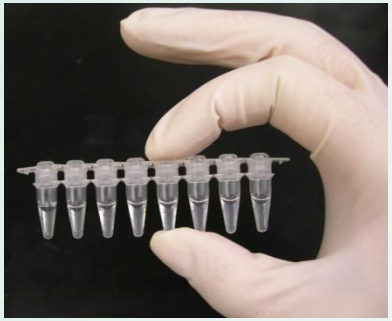
MOANA Technologies Inc.

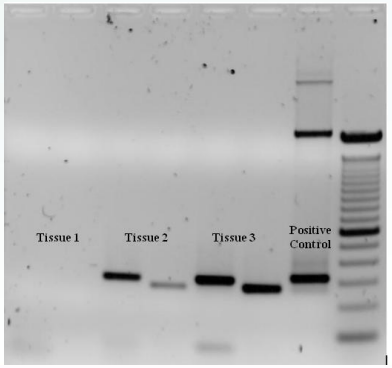
Rationale for Project

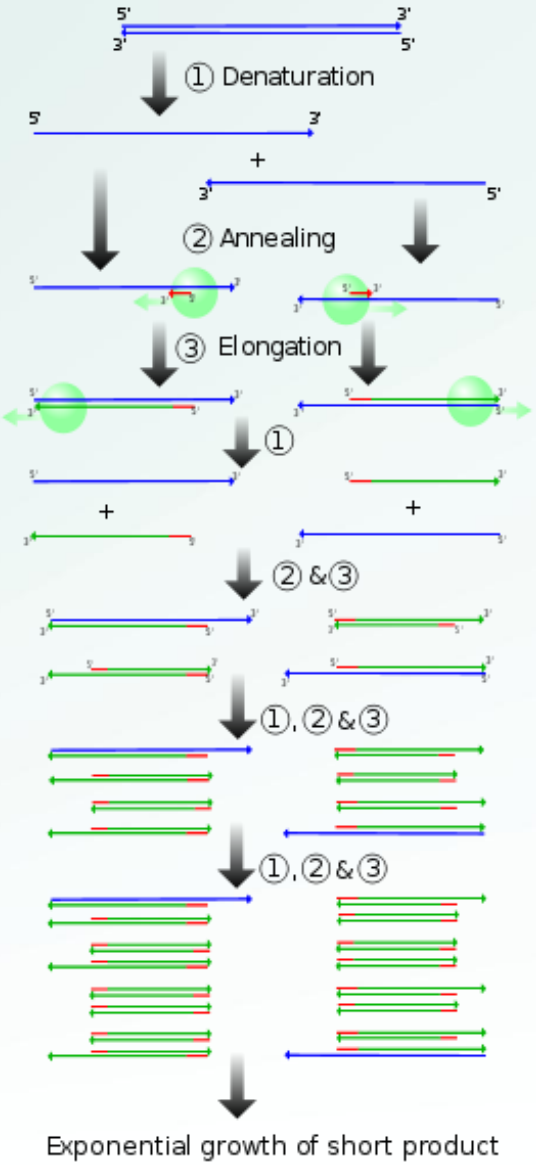
- In 1994, wild and farm-raised populations of tilapia on Oahu began to die of an unknown disease.
- Only tilapia were affected and mortalities occurred only during the cooler months (October to April) and were restricted to Oahu.
- Department of Agriculture issues PQ Policy 98-09, Section 150A-8, HRS effective November 5, 1998: Oahu shipments presented to PQB should not be certified for movement to other islands.
- Development and widespread availability of molecular detection techniques provides an opportunity to address the challenges posed by this particular pathogen.

Polymerase Chain Reaction (PCR)

The polymerase chain reaction (PCR) is a technique in molecular biology that amplifies a piece of DNA by generating millions of copies of a particular DNA sequence.







Primer's for TRLO/FLB

FLB16S180f: 5'-GCG-GAT-TAA- AGG-TGG-CCT-TTG-3' (forward primer)

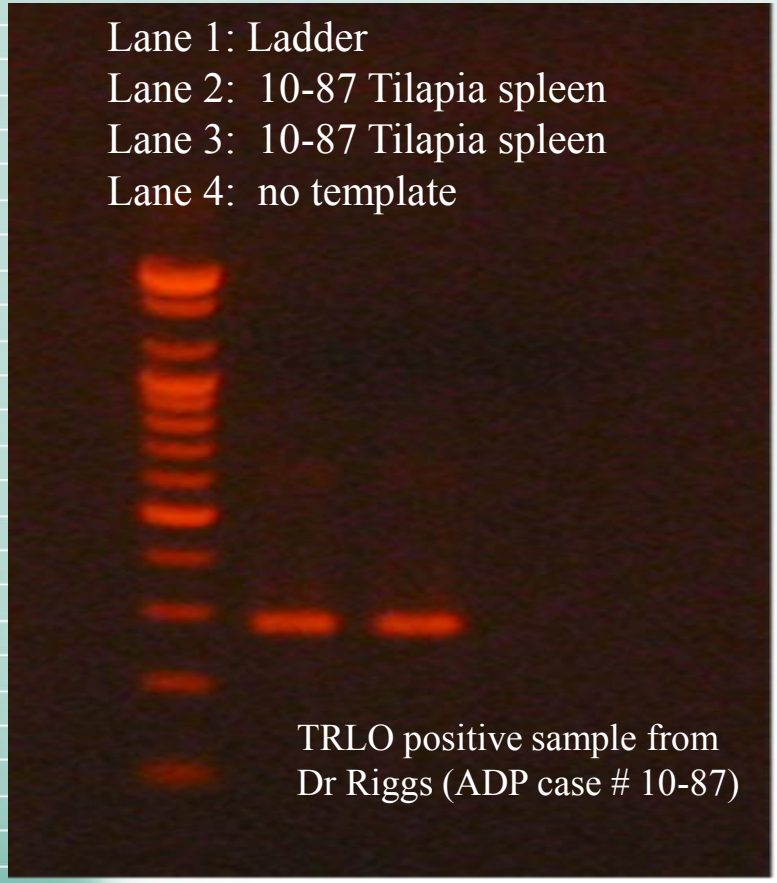
FLB16S465r: 5'-CCT-GCA-AGC-TAT-TAA-CTC-ACA- 3' (reverse primer).

Hsieh et.al., 2007. PCR and in situ hybridization for the detection and localization of a new pathogen *Francisella*-like bacterium (FLB) in ornamental cichlids. *Diseases of Aquatic Organisms* **75**, 29-36.

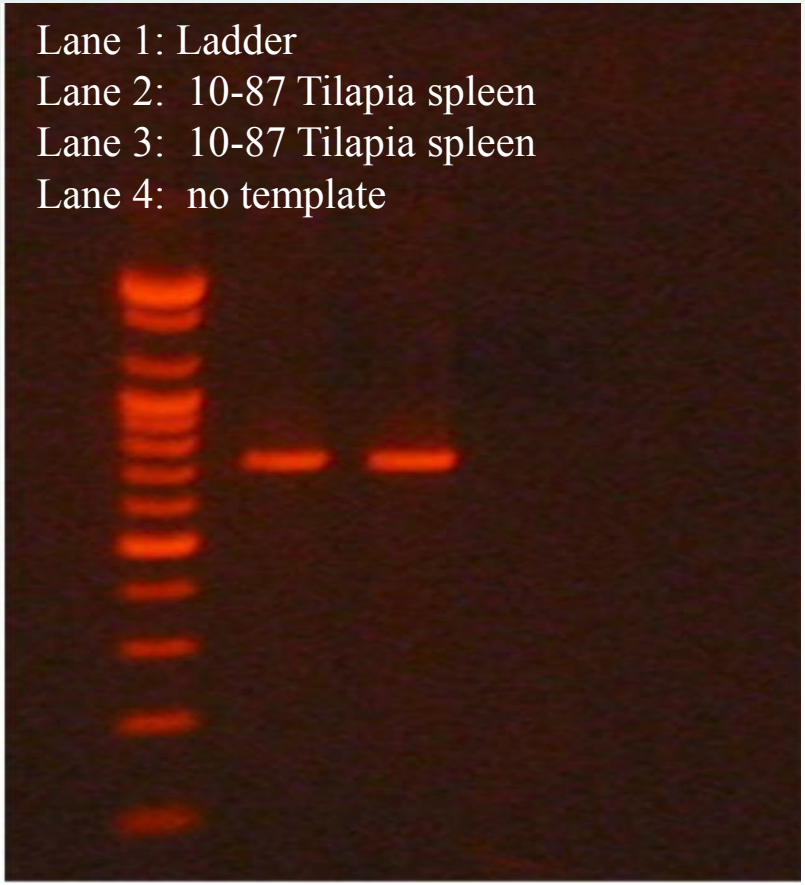
Misumi, et al., 2010 Identification and isolation of *Francisella*-like bacteria (FLB) from tilapia (*Oreochromis mossambicus*) for the first time in Hawaii. Submitted to Fisheries Science.

Validation of PCR Test for TRLO/FLB

Primers: TRLO F/R



Primers: CO-I (Fish DNA)



PCR Validation: DNA Sequence

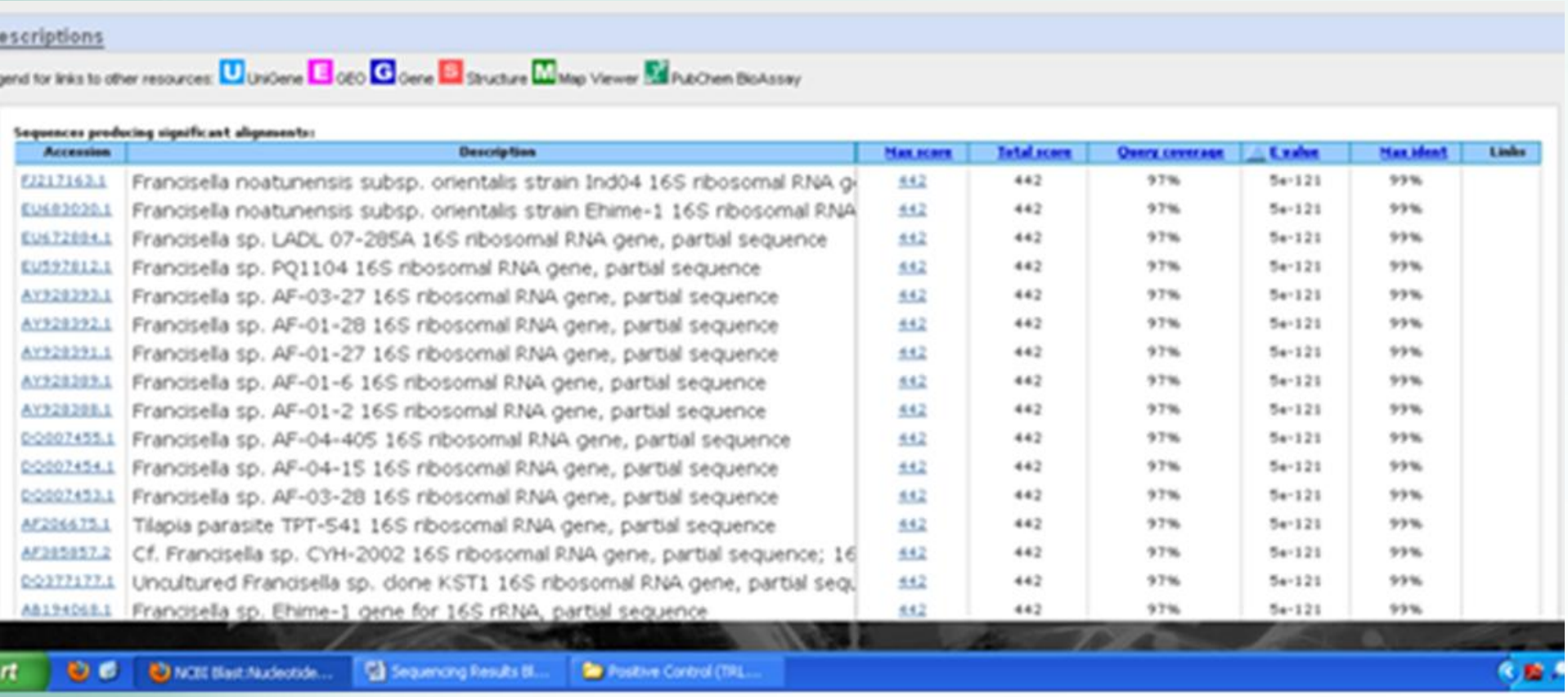
10-87 (+TRLO)

GGATCTACTGCGTTGGATAGCTAGTTGGTGGGGTAAGGGCCTACCAAGGCTACGATCCATA
GCTGATTTGAGAGGATGATCAGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGG
AGGCAGCAGTGGGGAATATTGGACAATGGGGGAAACCCTGATCCAGCAATGCCATGTGTG
TGAAGAAGGCTCTAGGGTTGTAAAGCACTTTAGTTGGGGAGGAAAGCCTGTGAGTTATAG
CTTGCAGGAA

MTI-10-22C1 (+CO-I fish DNA)

ACGATCTGCATTGGTGCTTGAGCCGGATAGTAGGACTGCACTAAGTCTCCTTATTCGGGCA
GAACTAAGCCAGCCCGGCTCTCTCCTCGGAGACGACCAGATTTATAATGTAATTGTTACAG
CACATGCTTTCGTAATAATTTTCTTTATAGTAATACCAATTATAATTGGAGGGTTTGGAAACT
GACTGGTGCCACTTATGATTGGAGCACCAGACATGGCCTTCCCTCGAATAAATAACATGAG
TTTTTGACTCCTTCCCCCCTCATTTCTCCTTCTCCTCGCCTCATCCGGAGTCGAAGCAGGA
GCCGGTACAGGATGAACTGTTTATCCTCCCCTCGCAGGCAATCTCGCCCACGCTGGACCTT
CTGTTGACTTGACCATCTTTTCCCTCCACTTGGCCGGGGTGTCATCTATTTTAGGCGCAATT
AATTTTATCACAACCATTATTAACATAAAACCCCCTGCCATCTCTCAATATCAAACACCCCT
CTTTGTGTGATCCGTTCTAATTACCGCAGTATTACTCCTACTATCCCTGCCCGTTCTTGCCGC
CGGCATCACAATACATCTAACAGATCGAAACCTGGACGCAAGCTTCTCTCACCCTGCCAG
AAAAAGAGACTC

PCR Validation: TRLO = *Francisella* like bacteria (FLB) DNA Sequence



escriptions

gend for links to other resources: UniGene GEO Gene Structure Map Viewer PubChem BioAssay

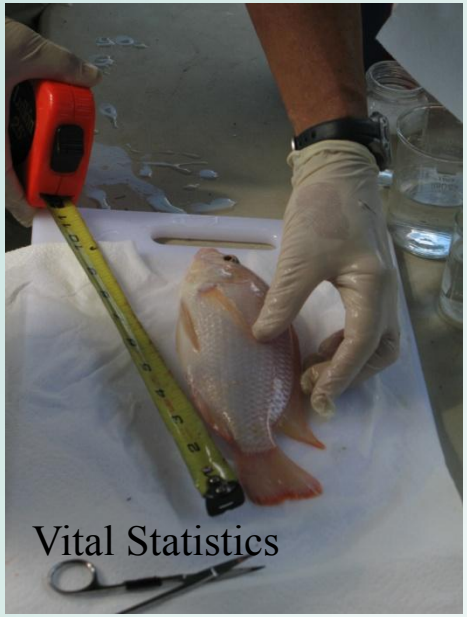
Sequences producing significant alignments:

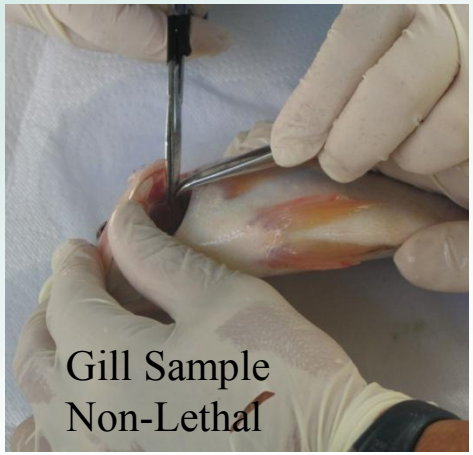
Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
FJ217163.1	Francisella noatunensis subsp. orientalis strain Ind04 16S ribosomal RNA g	442	442	97%	5e-121	99%	
EU683030.1	Francisella noatunensis subsp. orientalis strain Ehime-1 16S ribosomal RNA	442	442	97%	5e-121	99%	
EU672694.1	Francisella sp. LADL 07-285A 16S ribosomal RNA gene, partial sequence	442	442	97%	5e-121	99%	
EU597812.1	Francisella sp. PQ1104 16S ribosomal RNA gene, partial sequence	442	442	97%	5e-121	99%	
AY928393.1	Francisella sp. AF-03-27 16S ribosomal RNA gene, partial sequence	442	442	97%	5e-121	99%	
AY928392.1	Francisella sp. AF-01-28 16S ribosomal RNA gene, partial sequence	442	442	97%	5e-121	99%	
AY928391.1	Francisella sp. AF-01-27 16S ribosomal RNA gene, partial sequence	442	442	97%	5e-121	99%	
AY928389.1	Francisella sp. AF-01-6 16S ribosomal RNA gene, partial sequence	442	442	97%	5e-121	99%	
AY928388.1	Francisella sp. AF-01-2 16S ribosomal RNA gene, partial sequence	442	442	97%	5e-121	99%	
DQ007455.1	Francisella sp. AF-04-405 16S ribosomal RNA gene, partial sequence	442	442	97%	5e-121	99%	
DQ007454.1	Francisella sp. AF-04-15 16S ribosomal RNA gene, partial sequence	442	442	97%	5e-121	99%	
DQ007453.1	Francisella sp. AF-03-28 16S ribosomal RNA gene, partial sequence	442	442	97%	5e-121	99%	
AF206675.1	Tilapia parasite TPT-541 16S ribosomal RNA gene, partial sequence	442	442	97%	5e-121	99%	
AF385857.2	Cf. Francisella sp. CYH-2002 16S ribosomal RNA gene, partial sequence; 16	442	442	97%	5e-121	99%	
DQ377177.1	Uncultured Francisella sp. clone KST1 16S ribosomal RNA gene, partial seq	442	442	97%	5e-121	99%	
AB194068.1	Francisella sp. Ehime-1 gene for 16S rRNA, partial sequence	442	442	97%	5e-121	99%	

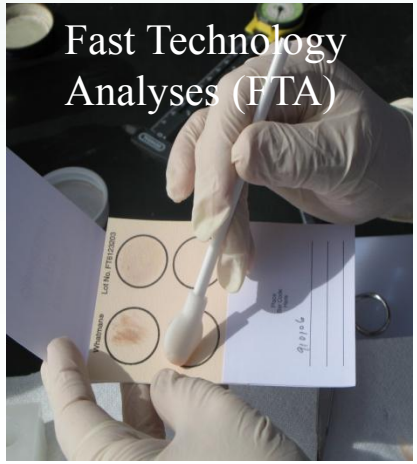
NCBI Blast:Nucleotide... Sequencing Results Bl... Positive Control (TRL...

Basic Local Alignment Search Tool (BLAST)

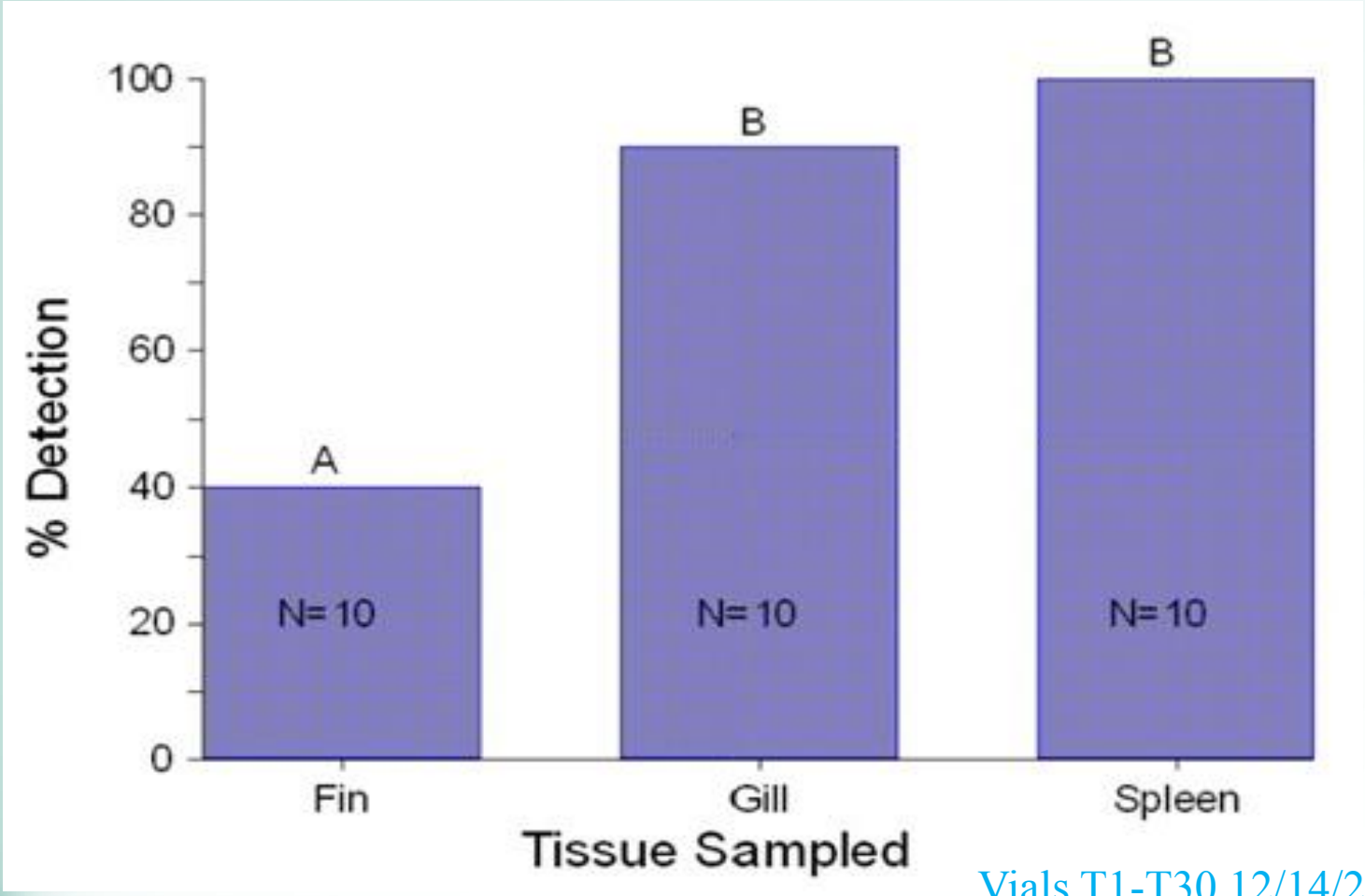
Processing a specimen for PCR testing





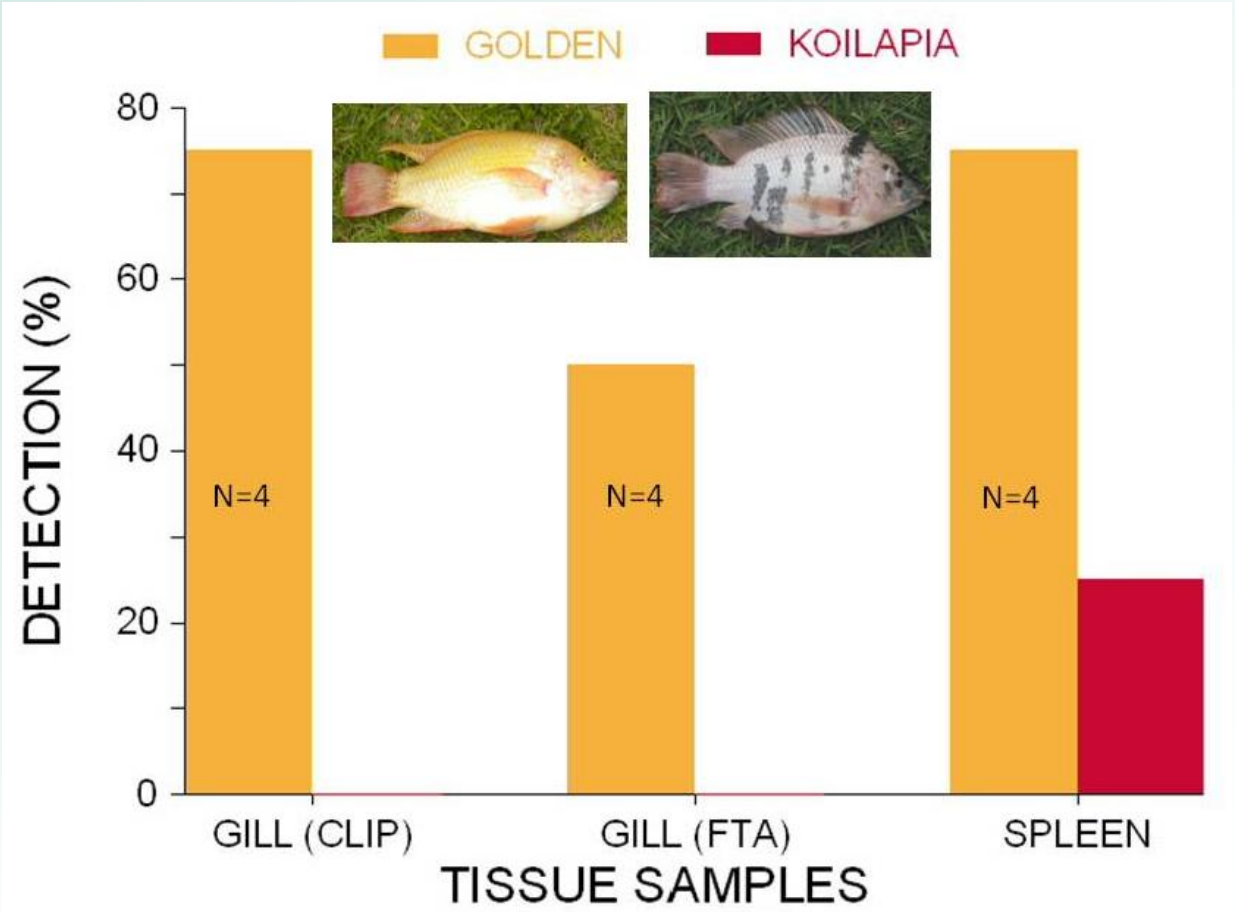


Tissue distribution during an active infection



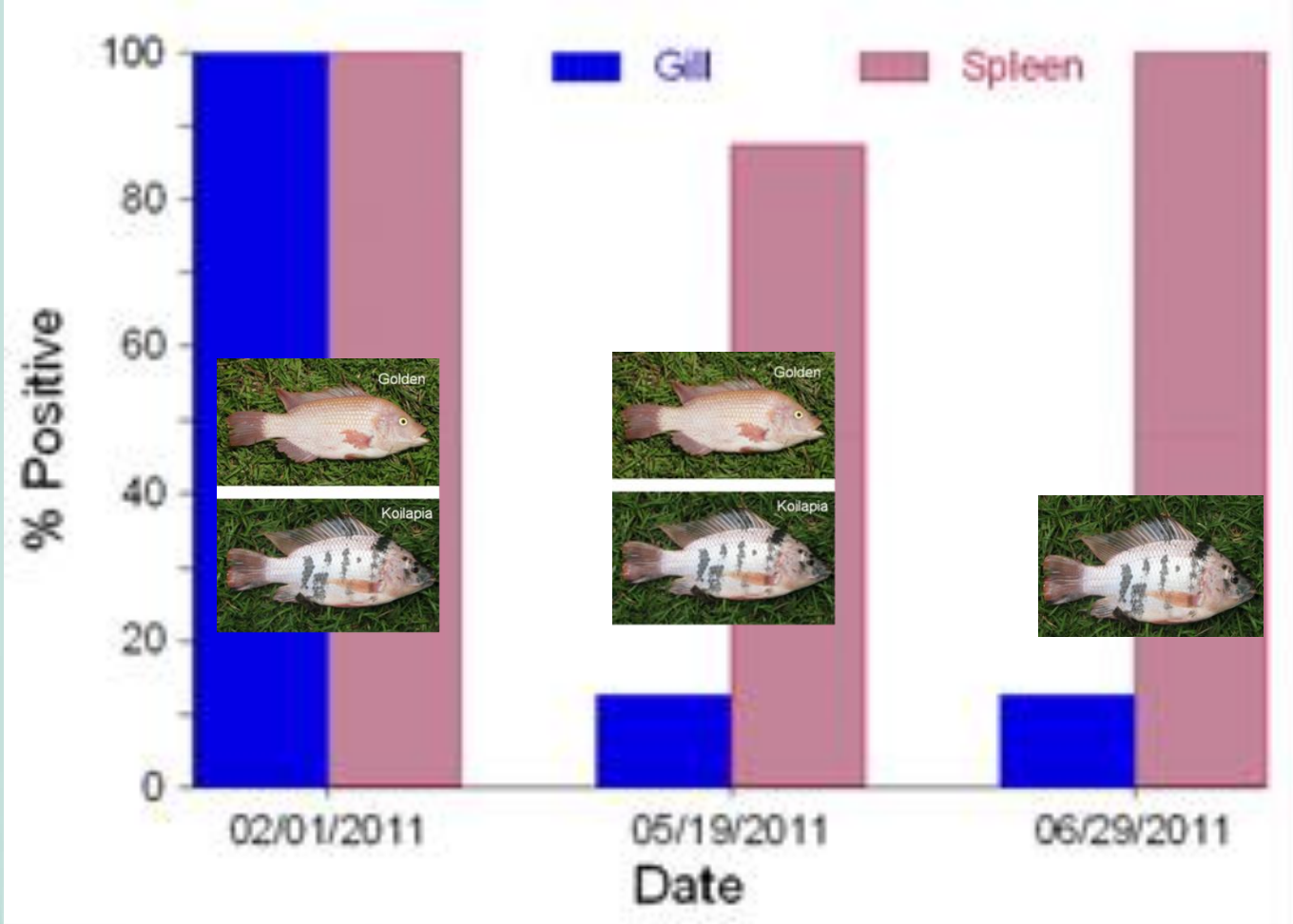
Vials T1-T30 12/14/2010

Species and tissue distribution of TRLO/FLB during an active infection



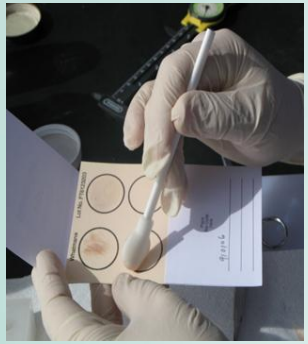
Vials T79 – T120 03/02/2011

Change in tissue distribution of TRLO/FLB during active infection that becomes an asymptomatic population



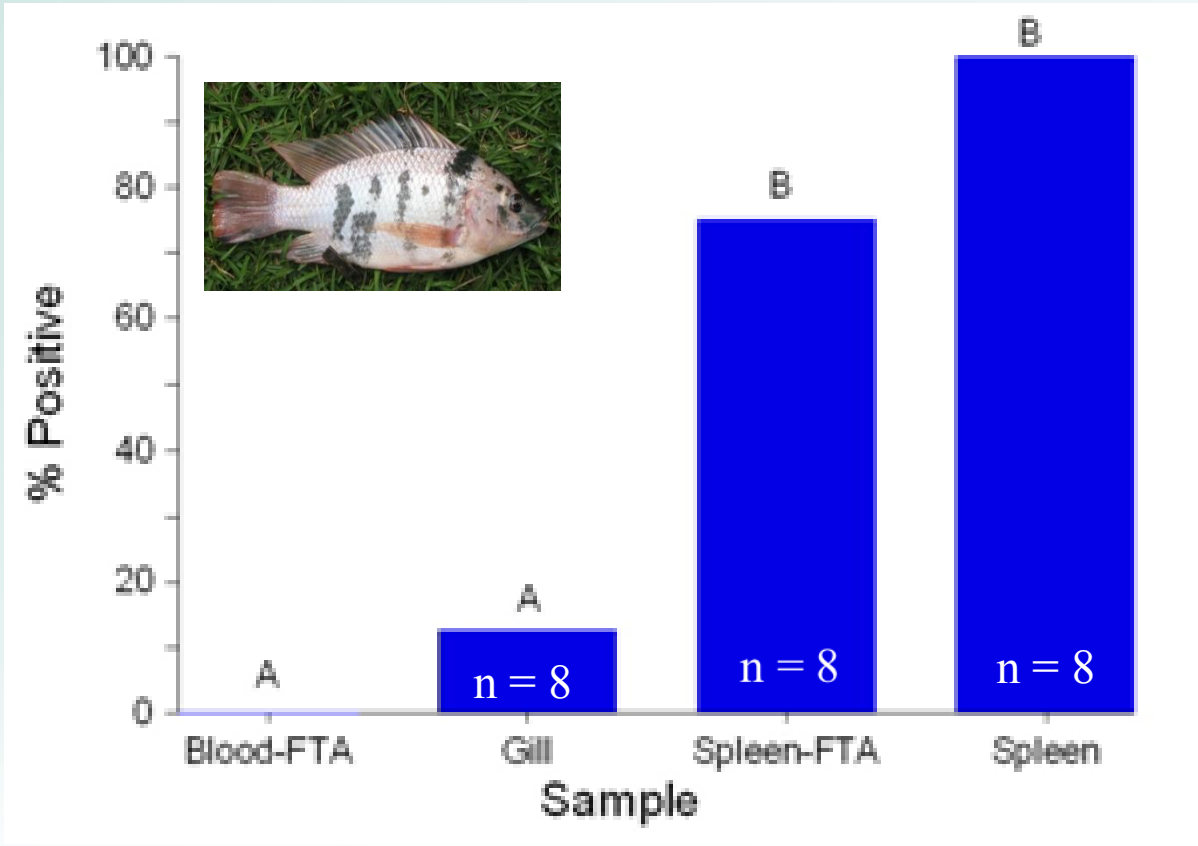
Tissue distribution of TRLO/FLB in an asymptomatic population

FTA Card



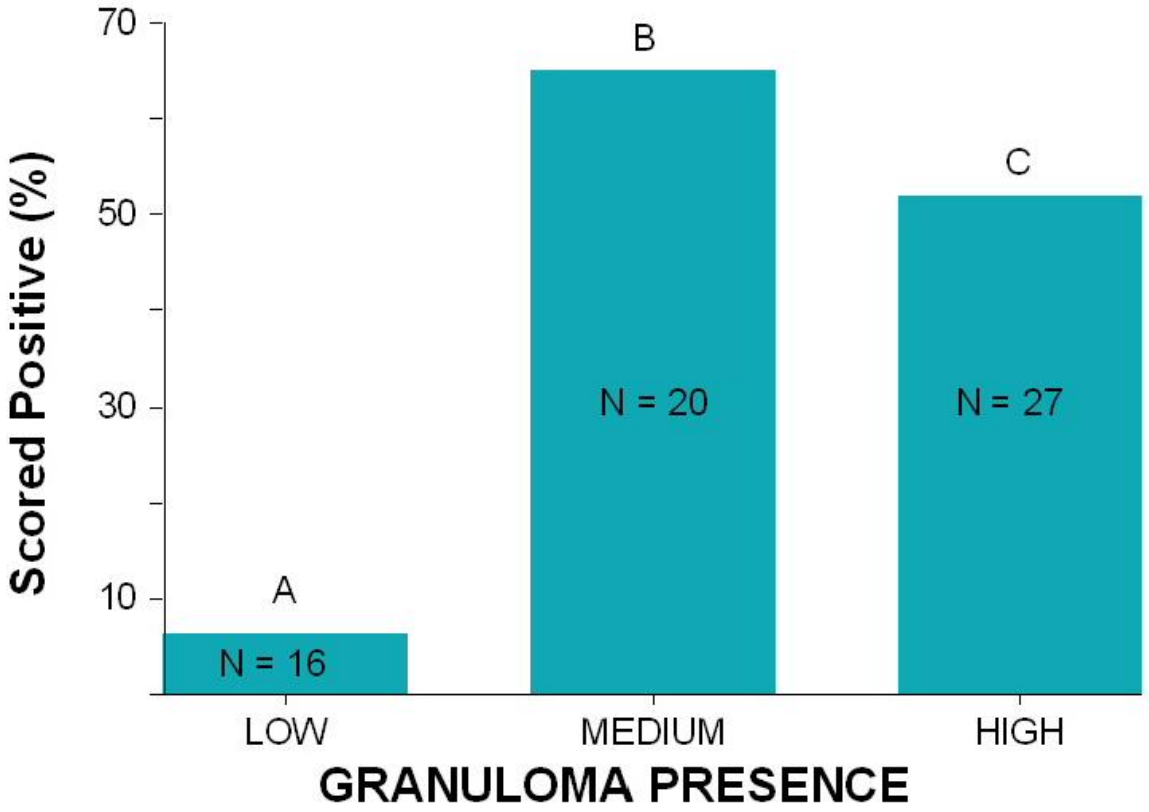
Gill Non-lethal

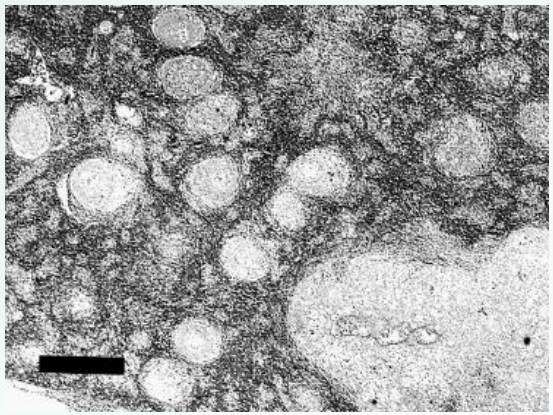
Spleen-lethal



Vials T-143 - T174 06/29/2011

Use of granulomas as an indicator of TRLO/FLB



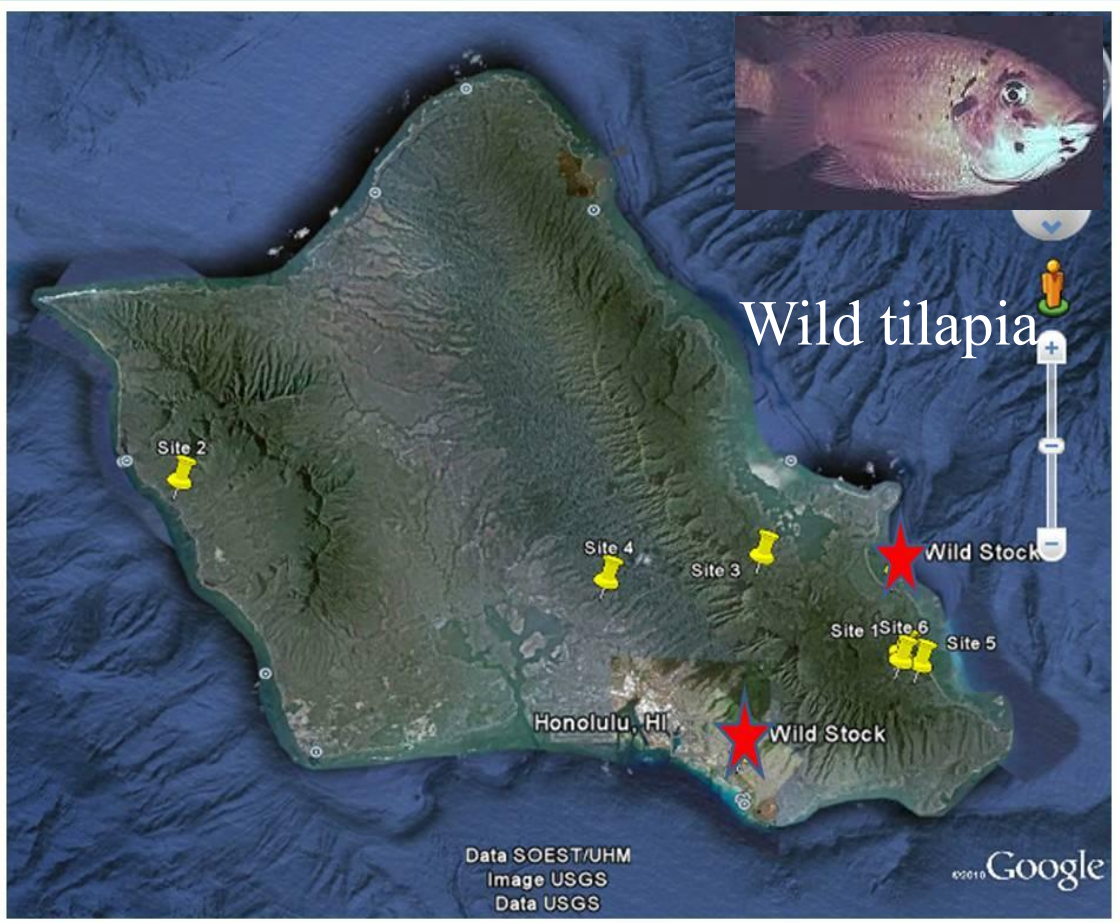


H&E stained spleen showing multiple granulomas. Scale bar = 50 µm.

From Mauel, et al., 2005. J. Vet. Diag. Invest. 17:601-605.

Distribution of positive cases on Oahu Dec 2010 – Current

- Six cases from cultured stock
- Two cases from wild stocks
- More being tested



Summary and Conclusions

- Capacity for PCR testing of FLB (previously known as TRLO) established, validated and operational
- Several tissues can harbor FLB during an active infection
- Only spleen and lethal sampling protocol useful for identifying asymptomatic carriers
- Some tilapia strains may be resistant to FLB

MAHALO and Thanks for Listening