

**FY10 INVITATION
PROPOSAL SUMMARY PAGE**

Project Title: Pilot Sequence: High Density DNA Sequencing to Detect Population Structure of Pacific Herring (Project Number: 10100165)

Project Period: October 1, 2009 – February 1, 2010

Primary Investigator(s): J Seeb (jseeb@u.washington.edu), L Hauser (lhauser@u.washington.edu), L Seeb (lseeb@u.washington.edu) (U. of Washington) & W Templin (bill.templin@alaska.gov)(ADFG)

Study Location: Prince William Sound. Key words: next generation sequencing, transcriptome, SNP.

Abstract: This is a demonstration project to document the value and low risk of the high density sequencing approach for population genetics study. We propose to sequence the transcribed genome of a single reference individual, report the sequence that will include SNPs in many thousands of genes, and annotate those genes that belong to gene families known to respond to oil exposure and disease.

Abstract of Parent Project: Pacific herring in Prince William Sound is one of only two resources still classified as ‘not recovered’. The recovery of PWS herring depends crucially on levels of connectivity within PWS and with the Alaskan coast, determining the relative importance of immigration and recruitment in the recovery process. We propose to investigate the genetic structure of Pacific herring by analyzing discrete spawning waves and age classes in three spatially and temporally isolated locations in PWS, within the context of Kodiak, Togiak, and SE Alaska outgroups. In contrast to previous studies, we will use high-density sequencing to discover many 10,000s of single nucleotide polymorphisms (SNPs) in the expressed genome of Pacific herring. We will identify discriminatory SNPs using a 1536-SNP array that will specifically incorporate genes known to be under natural selection. Highly discriminatory SNPs will be assembled in an information-rich 96-SNP array for subsequent genetic monitoring of recovery efforts.

Estimated Budget:
EVOS Funding Requested (*must include 9% GA*)

FY10	FY11	FY12	FY13	Total
\$71.3				\$71.3

Non-EVOS Funds to be used:

FY10	FY11	FY12	FY13	Total

PROJECT PLAN

I. NEED FOR THE PROJECT

This is a demonstration project to document the value and low risk of the high density sequencing approach for population genetics study.

The parent project addresses numerous issues that are fundamentally important to understanding and managing herring restoration in PWS. Science Panel reviews were favorable with a recommended "fund reduced."

Science Panel Comments:

This proposal focuses on developing single nucleotide polymorphism (SNP) technology for determining population structure of Pacific herring along the Pacific coast. This state-of-the-art technology would provide the strongest evidence regarding stock structure of herring not only within PWS but also in the Pacific. Furthermore, since this approach identifies highly evolving proteins common to reproductive, immune, and physiologically adaptive processes, it also holds promise in identifying genes involved in disease susceptibility/resistance as well as "health" of the PWS population. This is a very expensive, high-risk proposal with potential for high pay off in several areas beyond population structure. It is recommended that sampling frequency be cut significantly with an appropriate budget reduction.

The Science Coordinator recommended "do not fund" based upon the perceived high risk and high cost.

We suggest revising the submission for FY 2011 to reduce the broad geographic scope and cost while retaining the core objectives identified above that are key to identifying population structure and promoting sound restoration activities. Additionally, the risk identified by the Science Panel is greatly reduced based upon the recent success that our group has had applying this SNP discovery strategy. The strategy was novel when we drafted the original herring proposal, but we now routinely assemble and align high-density sequence in Pacific salmon for similar projects. In doing so we are discovering thousands of SNPs for use in population structure studies. Many of these SNPs have been found in genes involved in immune response and tumor suppression--genes that are key to the scan for adaptively important genes that is proposed in the parent project.

In this pilot project we propose to sequence the transcribed genome of a single reference individual, report the sequence that will include SNPs in many thousands of genes, and annotate those genes that belong to gene families known to respond to natural selection.

II. PROJECT DESIGN

A. Objective

The specific objective for the pilot study:

Demonstrate SNP discovery. Use the latest techniques of ultra high-density sequencing to sequence and identify 1,000s of SNPs from one Pacific herring.

This is the reference sequence described in the parent proposal. Assembling this sequence makes the sequencing of additional individuals much easier and less risky.

B. Procedural and Scientific Methods

We will use high-resolution techniques that were not available until recent technical advances allowed the collection and assembly of large-scale DNA sequence data. In this pilot project we will sequence the entire transcribed genome from one Pacific herring from Prince William Sound to be used for a reference sequence for further SNP discovery. DNA will be obtained from four tissues: gill, heart, liver, testes.

This reference transcriptome sequence for Pacific herring will be assembled using paired-end reads from mRNA-seq libraries. Briefly, a standard mRNA-seq library is constructed according to the manufacturer's protocol (Illumina) with minor modification. First, rRNA depleted RNA is fragmented and reverse-transcribed to generate cDNA. Fragment ends of double-stranded cDNA are polished and non-templated 'A' added, prior to ligation with paired-end adapters. Following ligation the library is enriched by amplification. Libraries are size fractionated, recovering fragments of approximately 300bp. Paired, 100bp reads will be generated from the mRNA-seq libraries using standard, sequencing-by-synthesis chemistry on an Illumina Genome Analyzer (GAII). The reference sequence will be assembled from these 100bp reads using the Genomics Workbench (CLC-bio) software package or other short-read assembler.

C. Coordination and Collaboration with other Efforts

All of the DNA instrumentation used at the University of Washington was purchased by State of Washington or Foundation funds. No equipment will be purchased funds from this project. We operate an extensive genotyping laboratory equipped with state-of-art instrumentation capable of the uniplex and multiplex qPCR. All of the high density DNA sequencing will be done by the UW High-Throughput Genomics Unit. Dr. Michael Dorschner, Unit Director, is currently conducting related DNA sequencing projects for us on Chinook, chum, and sockeye salmon. All of the complex bioinformatics for DNA sequence and assembly will be provided by Dr. Steven Roberts at the University of Washington (<http://fish.washington.edu/research/genefish/robertslab/Home.html>). Dr. Roberts has extensive experience in this area and is currently assembling next-generation sequences for salmon and shellfish.

III. SCHEDULE

A. Project Milestones

DNA sequence completed	December 1, 2009
Assembly and annotation	January 1, 2010
SNP discovery	January 15, 2010
Final Report	February 1, 2010

Budget Justification

Personnel

S. Roberts is responsible for alignment and assembly of the herring transcriptomes.

Commodities

Here we include the costs for RNA extraction, cDNA preparation, and high density sequencing of one individual at the UW core facility.

Indirect

This work will be done on campus at the federally audited rate of 56%.

**EXXON VALDEZ OIL SPILL TRUSTEE COUNCIL
DETAILED BUDGET FORM FY 10- FY 12**

Budget Category:	Proposed FY 10	Proposed FY 11	Proposed FY 12	Proposed FY 13	TOTAL PROPOSED
Personnel	\$9.9			\$0.0	\$9.9
Travel				\$0.0	\$0.0
Contractual				\$0.0	\$0.0
Commodities	\$32.0			\$0.0	\$32.0
Equipment	\$0.0			\$0.0	\$0.0
Indirect (<i>will vary by proposer</i>)	\$ 23.5				\$23.5
SUBTOTAL	\$65.4			\$0.0	\$65.4
General Administration (9% of subtotal)	\$5.9			\$0.0	\$5.9
PROJECT TOTAL	\$71.3			\$0.0	\$71.3
Other Resources (Cost Share Funds)	\$0.0	\$0.0	\$0.0	\$0.0	\$0.0

COMMENTS: In this box, identify non-EVOS funds or in-kind contributions used as cost-share for the work in this proposal. List the amount of funds, the source of funds, and the purpose for which the funds will be used. Do not include funds that are not directly and specifically related to the work being proposed in this proposal.

FY10 - 13

Project Title: High Density DNA Sequencing to Detect Population Structure of Pacific Herring
Lead PI: J. Seeb

**FORM 4A
NON-TRUSTEE
AGENCY SUMMARY**

**EXXON VALDEZ OIL SPILL TRUSTEE COUNCIL
DETAILED BUDGET FORM FY 10- FY 12**

New Equipment Purchases: Description	Number of Units	Unit Price	Equipment Sum
			0.0
none			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
New Equipment Total			\$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency
ABI 7900 qpcr	1	UW
LightCycler 480 qpcr	1	UW
BioMark qpcr	1	UW
Janus Robot	1	UW

FY10

**Project Title: High Density DNA Sequencing to
Detect Population Structure of Pacific Herring
Lead PI: J. Seeb**

**FORM 4B
EQUIPMENT
DETAIL**

**EXXON VALDEZ OIL SPILL TRUSTEE COUNCIL
DETAILED BUDGET FORM FY 10- FY 12**

Personnel Costs:		GS/Range/ Step	Months Budgeted	Monthly Costs	Benefits	Personnel Sum
Name	Project Title					
						0.0
Seeb J						
Hauser L						
Seeb L						
Roberts S						
Research Sci IV Technician						
						0.0
						0.0
						0.0
						0.0
						0.0
Subtotal			0.0	0.0	0.0	
Personnel Total						\$0.0

Travel Costs:	Ticket Price	Round Trips	Total Days	Daily Per Diem	Travel Sum
Description					
					0.0
Seattle-Anchorage					
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
Travel Total					

FY11

**Project Title: High Density DNA Sequencing to
Detect Population Structure of Pacific Herring
Lead PI: J. Seeb**

**FORM 4B
PERSONNEL &
TRAVEL DETAIL**

**EXXON VALDEZ OIL SPILL TRUSTEE COUNCIL
DETAILED BUDGET FORM FY 10- FY 12**

Contractual Costs: Description	Contract Sum
If a component of the project will be performed under contract, the 4A and 4B forms are required.	Contractual Total
	\$0.0

Commodities Costs: Description	Commodities Sum
DNA chemistry and TaqMan reagents for genotyping	
misc dna lab chems and supplies: dna resequencing and high resolution melt	
	Commodities Total
	\$0.0

FY11

**Project Title: High Density DNA Sequencing to
Detect Population Structure of Pacific Herring
Lead PI: J.Seeb**

**FORM 4B
CONTRACTUAL &
COMMODITIES
DETAIL**

**EXXON VALDEZ OIL SPILL TRUSTEE COUNCIL
DETAILED BUDGET FORM FY 10- FY 12**

New Equipment Purchases: Description	Number of Units	Unit Price	Equipment Sum
			0.0
none			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
New Equipment Total			\$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency
ABI 7900 qpcr	1	UW
LightCycler 480 qpcr	1	UW
BioMark qpcr	1	UW
Janus Robot	1	UW

FY11

**Project Title: High Density DNA Sequencing to
Detect Population Structure of Pacific Herring
Lead PI: J.Seeb**

**FORM 4B
EQUIPMENT
DETAIL**

**EXXON VALDEZ OIL SPILL TRUSTEE COUNCIL
DETAILED BUDGET FORM FY 10- FY 12**

Personnel Costs:		GS/Range/ Step	Months Budgeted	Monthly Costs	Benefit	Personnel Sum
Name	Project Title					
						0.0
Seeb J						
Hauser L						
Seeb L						
Roberts S						
Research Sci IV Technician						
						0.0
						0.0
						0.0
						0.0
						0.0
Subtotal			0.0	0.0	0.0	
Personnel Total						\$0.0

Travel Costs:	Ticket Price	Round Trips	Total Days	Daily Per Diem	Travel Sum
Description					
					0.0
Seattle Anchorage					
Conference travel (2 trips)					
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
Travel Total					\$0.0

FY12

**Project Title: High Density DNA Sequencing to
Detect Population Structure of Pacific Herring
Lead PI: J.Seeb**

**FORM 4B
PERSONNEL &
TRAVEL DETAIL**

**EXXON VALDEZ OIL SPILL TRUSTEE COUNCIL
DETAILED BUDGET FORM FY 10- FY 12**

New Equipment Purchases: Description	Number of Units	Unit Price	Equipment Sum
			0.0
none			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
			0.0
New Equipment Total			\$0.0

Existing Equipment Usage: Description	Number of Units	Inventory Agency
ABI 7900 qpcr	1	UW
LightCycler 480 qpcr	1	UW
BioMark qpcr	1	UW
Janus Robot	1	UW

FY12

**Project Title: High Density DNA Sequencing to
Detect Population Structure of Pacific Herring
Lead PI: J.Seeb**

**FORM 4B
EQUIPMENT
DETAIL**

**EXXON VALDEZ OIL SPILL TRUSTEE COUNCIL
DETAILED BUDGET FORM FY 10- FY 12**

Personnel Costs:		GS/Range/ Step	Months Budgeted	Monthly Costs	Overtime	Personnel Sum
Name	Project Title					
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
						0.0
Subtotal			0.0	0.0	0.0	
Personnel Total						\$0.0

Travel Costs:	Ticket Price	Round Trips	Total Days	Daily Per Diem	Travel Sum
Description					
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
					0.0
Travel Total					\$0.0

FY13

Project Title:
Lead PI:

**FORM 4B
PERSONNEL &
TRAVEL DETAIL**

**EXXON VALDEZ OIL SPILL TRUSTEE COUNCIL
DETAILED BUDGET FORM FY 10- FY 12**

Contractual Costs: Description	Contract Sum
If a component of the project will be performed under contract, the 4A and 4B forms are required.	Contractual Total
	\$0.0

Commodities Costs: Description	Commodities Sum
	Commodities Total
	\$0.0

FY13

Project Title:
Lead PI:

**FORM 4B
CONTRACTUAL &
COMMODITIES
DETAIL**

