Allele ladder-based standardization of existing coho salmon microsatellite data and implementation in the GAPS database

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Report to the Pacific Salmon Commission Southern Boundary Restoration & Enhancement Fund;

Project SF-2008-I-4

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Summary

This project used a demonstrated method to simplify microsatellite DNA standardization and to consolidate existing data for coho salmon microsatellite loci currently in common use among multiple laboratories. By analyzing an allele ladder sample for eight microsatellite markers (loci), five different laboratories converted existing data to a common allele naming system. Genotypes from 8,879 coho salmon collected from 110 locations were uploaded into the Genetic Analysis of Pacific Salmonids (GAPS) database. Forty two of the locations were represented by samples collected in multiple years. These data represent the first coho salmon data to be included in the GAPS database. Analyses showed that these data can be used for genetic stock identification (GSI) of coho mixed-fisheries, as well as for population structure analyses to identify genetically significant stock groups.

Introduction

Microsatellite DNA data has proven to be useful for mixture analyses and delineating genetic population structure of coho salmon (Beacham et al 2001; Ford et al. 2004; Van Doornik et al. 2007; Johnson and Banks 2008). With multiple laboratories collecting such data, having the ability to pool data together greatly increases the usefulness of these types of datasets, compared to the efforts of a single laboratory working alone. Combining data collected by different laboratories using different genotyping platforms has been difficult for a number of reasons (LaHood et al. 2002), but the recent successes of creating standardized baselines of genetic data for Chinook salmon (Seeb et al. 2007) and steelhead (Stephenson et al. In press) have proven that these types of standardization efforts are worth the effort. The development of allele ladders has overcome many of the challenges of standardizing microsatellite data, and has proven to be a highly efficient and accurate way to transform inter-laboratory microsatellite data to a common allele naming system (LaHood et al. 2002).

This project was designed to extend the power and utility of the current Genetic Analysis of Pacific Salmonids (GAPS) database to include coho salmon, and would make those data readily available to harvest managers interested in Genetic Stock Identification (GSI) applications, and to others interested in a wide range of ecological studies such as coho salmon juvenile migration, habitat use, and population structure. Facilitating access to these types of data in an interactive GSI environment will support (and be supported by) a wide range of ecological genetic studies and fishery management objectives.

This report describes the efforts undertaken to achieve the project’s three main objectives:

1) Collect reference DNA samples and create and distribute allele ladders for at least eight microsatellite loci among five laboratories - Northwest Fisheries Science Center (NWFSC), Oregon State University (OSU), Southwest Fisheries Science Center (SWFSC), Washington Department of Fish and Wildlife (WDFW), U.S. Fish & Wildlife Service – Alaska (USFWS);

2) Standardize and upload existing microsatellite data from contributing laboratories, as well as any available single nucleotide polymorphisms (SNP) data, into the GAPS web accessible database already in place and maintained by the NWFSC;

3) Collect user input on the coho database from GAPS collaborators and interested PSC parties for improvements in this and future performance periods.

Methods

Locus selection/Ladder construction

We identified 4 microsatellite loci (Ocl8, Oki1, Ots103, P53; Table 1) for which data were or had been collected for coho salmon by the 4 laboratories participating in this study within the Southern Fund's region (NWFSC, OSU, SWFSC, WDFW). An additional 4 loci were selected (Omy1011, One13, OtsG422, Ots213), based upon which ones were already in use by the greatest number of laboratories, which had the most existing data, and which could be reliably amplified by all of the participating laboratories. Samples were then selected from archived samples at the NWFSC for each locus that would represent the entire known allelic range for each locus. Allele ladders were then constructed using these samples following the procedures described by LaHood et al. (2002).

Data Standardization

Allele ladders for the eight chosen loci and 96 reference samples were distributed to each of the participating laboratories. Information regarding the name given to each allele in the ladders, and the genotypes of the samples on the reference plate (as determined by the project lead) were also distributed. Each laboratory used their own genotyping methods to analyze the ladders and reference samples and compare them to coho salmon samples they had previously analyzed. They then compared their allele names to those designated by the ladders and made the necessary conversions to their data.

Data Submission

Laboratories with previously existing data or who were currently collecting data, converted their data so that it was standardized to the allele ladders, and submitted the standardized data to the project lead. Each laboratory was responsible for implementing their own quality control to assure that the data submitted was free of errors, however, the project lead did check all submitted data for conformance to the proper data format, and examined it for any missing or inconsistent data. The data were then uploaded into the GAPS database by IT personnel at the NWFSC.

Data Analyses

A few basic analyses were conducted by the project leader to examine the capabilities of the newly compiled database. Only samples with data for all eight loci were used. Conformance of the observed allele frequencies to expected Hardy-Weinberg proportions was tested with a Fisher's exact test (Guo and Thompson 1992) in the computer program GENEPOP (Raymond and Rousset 1995). The sequential Bonferroni method (Rice 1989) was then used to adjust the critical significant level for multiple tests. Observed and expected heterozygosity were determined using the program GDA (Lewis and Zaykin 2002). Allelic richness values for each locus were determined using the program FSTAT (Goudet 1995).

The genetic population structure of the populations was examined by calculating the amount of gene diversity (F_{st}) among populations using FSTAT (Goudet 1995). For these analyses, and all subsequent ones, temporal replicates collected from the same population were pooled together. Genetic distances among populations were visualized by calculating Corvalli-Sforza and Edwards (1967) chord distances over 1000 bootstrap replicates with the program PHYLIP (Felenstein 2005), and creating a consensus neighbor-joining tree.

In order to test the baseline's ability to estimate stock of origin for mixture samples we used the program ONCOR (Kalinowski 2007) to make proportional stock estimates and individual assignments of a mixture of coho salmon of known origins. This sample consisted of 197 fish collected as juveniles off the coasts of Washington and Oregon that had been implanted with a coded wire tag (CWT). Thus, their region of origin was known. Estimates were made to nine reporting groups. Some of these groups consisted of multiple regions combined (ex. Hood Canal was combined with Puget Sound; Strait of Juan de Fuca was combined with north and south Washington Coast). Only fish with four or more loci genotyped were used in the baseline created to make these estimates. The baseline and mixture sample were bootstrapped 1000 times when estimating mixture proportions to generate 95% confidence intervals. Individual assignment results included not only the estimated reporting group of origin, but also the probability (P) that the individual originated from that region compared to all other reporting groups.

Results

Standardization

All participating laboratories successfully used the allele ladders and reference plate to standardize their coho salmon microsatellite genotyping (Appendix A). There were a small number of discrepancies noted while genotyping the reference samples. These were most likely due to the low DNA concentration of some of the reference plate samples. Overall, all of the participating laboratories expressed confidence in their ability to produce standardized data for the eight microsatellite loci.

Database Creation

Genotypes from 8,879 coho salmon were compiled and added to the current GAPS database. These represent 175 samples collected from 110 populations (42 populations were sampled in 2 or more years).

Of these, 154 had genotypes for all 8 loci. Sample locations ranged from Big Creek Hatchery on Scott Creek in Central California to the Kuskokwim River in Alaska, however the majority of samples were concentrated in Washington and Oregon (Figure 1). We grouped the populations into 20 geographic regions (Table 2). The boundaries of these regions were based upon geography and previous studies of coho salmon population structure (Weitkamp et al. 1995; Beacham et al. 2001; Ford et al. 2004; Van Doornik et al. 2007).

In addition to genotypes for eight loci, the database includes other important information for each individual fish. When available and appropriate, this information includes:

- Run timing
- Origin (hatchery vs. wild)
- Life stage collected (parr, smolt, adult)
- Collecting agency
- Collecting method
- Collection year
- Brood year
- Genotyping agency
- Latitude and longitude of sample location
- Other notes on collection, sampling or genotyping

The database also has an interactive map feature that allows the user to quickly visualize the geographic coverage of available data, and to easily choose samples of interest for download (Figure 1). The database can output data in GENEPOP format, which can then be easily converted for use with other programs. The database resides on computer servers at the NWFSC and can be accessed via the internet at http://webapps.nwfsc.noaa.gov/gaps. Access to the database is controlled through the use of user names and passwords. A user name and password can be obtained by contacting the NWFSC's Scientific Data Management group at nwfsc.sdm@noaa.gov.

Data Analyses

A total of 298 alleles were observed in the eight loci. The number of alleles per locus ranged from 16 for Omy1011 to 71 for OtsG422 (Table 1). We found that 8.1% of the tests for conformance to expected Hardy-Weinberg proportions were significant at $P < 0.05$. Over half of the significant tests (52%) occurred at Ots103, a locus known to have null alleles (Beacham et al. 2001). Over all eight loci, we observed a heterozygosity value of 0.811. Expected heterozygosity was slightly higher at 0.885. Allelic richness values ranged from 6.8 for Omy1011 to 13.8 for Ots103. F_{st} among all populations was 0.062 (95% confidence interval = 0.047 – 0.074).

A dendrogram of genetic distances showed that samples from within the same geographic area tended to cluster together (Figure 2). However, there were several exceptions, including the odd clustering of Yakoun R. from Queen Charlotte Island, Grizzly Cr. from Puget Sound, Hoko R. from the Washington Coast, and Rockybrook Cr. from Hood Canal.

Proportional stock estimates of a mixture of known origins are shown in Table 3. Estimates were fairly accurate. With the exception of the North/Central Oregon Coast, whose proportion was significantly overestimated, the true proportion was within the 95% confidence interval for each reporting group. Attempting to assign each individual fish to a reporting group yielded an accuracy rate of 75.1%. If only assignments where $P > 0.95$ are considered, the accuracy improves to 87.4%, with 104 of 119 individuals correctly identified to its group of origin. Improved mixture estimates and individual assignments, as well as having the ability to use smaller reporting units, may be possible pending further analyses and the addition of more data to the database.

Discussion

This project has completed its three main objectives. First, we successfully created allele ladders for eight microsatellite loci to be used for coho salmon. These allele ladders contain alleles that span most of the known range of each locus. In addition to distributing the allele ladders to each of the participating laboratories, several aliquots of each allele ladder are being stored at the NWFSC and will be available for distribution to any other laboratory who wants to become standardized for these loci.

Secondly, the allele ladders that were constructed allowed all participating laboratories to standardize their data. The reference samples that were distributed also proved to be valuable part of the standardization process. The two combined gave each laboratory multiple examples of most of the alleles being standardized. By comparing the genotyping results of these samples to their own samples, each laboratory could determine the proper standardized name to give to each allele observed in their samples.

A sizeable amount of coho salmon genetic data has now been loaded into the existing GAPS database as a result of this project, where it will complement the Chinook salmon data already present. Although we expect this database to grow in the number of samples and loci it contains, our preliminary analyses showed that as it currently exists, it is capable of providing information about coho salmon population structure, and can be used to make accurate proportional stock estimates of coho salmon mixtures in the southern part of their range. We expect that as more data is added to the database, and as more people use the data, its full capabilities and limitations will become better known. Although the samples in the baseline represent a large geographic range, the best coverage is in the southern regions. Samples are few and far between from central British Columbia northward. Hopefully, future sample collecting and genotyping by the participating laboratories will add data for these areas. While no SNP data for coho salmon were available to upload, the database is easily capable of adding such data in the future when it becomes available.

Finally, the goal of collecting user input on the coho database from GAPS collaborators has been accomplished. Success of this project is largely due to the fact that all of the participating laboratories in this project have experience standardizing microsatellite data for other salmonid species, and are accustomed to collaborating with each other. All participants provided valuable insight, ideas and data throughout the course of this project. This expertise and cooperation has helped create the first database of standardized genetic data for coho salmon, which will be the foundation of a coastwide database that will be useful for numerous management applications and ecological studies. We expect that further input from interested parties will occur as additional people make use of the database.

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Table 1. Microsatellite loci added to the GAPS database for 110 populations of coho salmon. For each locus, the number of alleles, expected heterozygosity (H_e), observed heterozygosity (H_o) and allelic richness (A_r) was calculated.

Locus	N alleles	H_e	H_o	A_r	Primer reference
Ocl8	30	0.912	0.836	10.1	Condrey and Bentzen, 1998
Oki1	23	0.864	0.784	8.3	Smith et al. 1998
Omy1011	16	0.816	0.762	6.8	Spies et al. 2005
One13	26	0.915	0.834	9.8	Scribner et al. 1996
Ots103	65	0.967	0.803	13.8	Small et al. 1998
Ots213	42	0.785	0.739	7.3	Greig et al. 2003
OtsG422	71	0.965	0.932	13.7	Williamson et al. 2002
P53	25	0.856	0.800	7.5	de Fromentel et al. 1992
Overall	298	0.885	0.811		

Table 2. List of coho salmon samples that have been genotyped at eight microsatellite loci and added to the GAPS database. Letter abbreviations are as follows: Cr = creek, E = early, H = hatchery, L= late, Lk = lake, R = river, W = Wild.

Region / Population	Collection Year	N Fish Genotyped
Kuskokwim		
Arolik R.	1997	88
Big R., Kuskokwim	2008	23
Highpower Cr.	2004	29
	2005	21
Kisaralik R.	1997	82
Middle Fork	2008	21
Salmon R., Kuskkokwim	2007	197
South Fork	2008	65
Stony R.	2008	27
Tin Cr.	2008	120
Windy Fork R.	2008	27
Kuskokwim Total		700
North British Columbia Coast		
Babine R.	1996	22
Cedar R.	1995	40
Clearwater Cr.	1995	58
Zolzap Cr., Nass R.	1996	38
North British Columbia Coast Total		158
Queen Charlotte Is.		
Yakoun R.	1995	79
Queen Charlotte Is. Total		79
West Vancouver Island		
Nitinat R. H.	1996	24
	1997	23
	1998	24
	2000	24
Tranquil Cr. H.	1998	32
	1999	32
	2001	31
Up. Kennedy R. H.	1996	24
	1999	23
	2000	25
West Vancouver Island Total		262
East Vancouver Island		
Cowichan H.	1998	45

	1999	30
	2000	14
Goldstream H.	1998	96
Nanaimo R. H.	1996	24
	1997	24
	1998	24
	1999	24
East Vancouver Island Total		281
Lower Fraser R.		
Chehalis H.	1996	39
	1997	22
	1999	32
Chilliwack H.	1997	22
	1998	23
	2000	46
Inch Cr. H.	1996	31
	1998	29
	2000	32
Lower Fraser R. Total		276
Mid Fraser R. / Thompson R.		
Dunn Cr.	1997	24
	1998	23
	1999	23
	2000	22
Bridge Cr.	1996	30
	1998	49
	1999	15
Bessette Cr.	1996	4
	1997	45
	1998	13
	1999	6
	2000	1
	2001	1
	2002	11
Mid Fraser R. / Thompson R. Total		267
South British Columbia Coast		
Capilano H.	1997	31
	1998	31
	2000	30
Homathko R.	1998	36
	2002	40
Tenderfoot H.	1998	48
	1999	48
South British Columbia Coast Total		264

Puget Sound		
Ennis Cr.	1997	12
	1999	42
Grizzly Cr.	1999	48
Minter Cr. H.	1995	40
	2002	48
Nooksack H.	2003	96
Skagit H.	2003	96
Soos Cr. H.	1997	48
	1998	47
Puget Sound Total		477
Hood Canal		
Big Beef Cr.	2003	77
Dewatto R.	1997	44
	1998	37
Rockybrook Cr., Dosewallips R.	2003	34
George Adams H.	1999	94
Quilcene H.	2000	48
	2001	48
	2002	48
Grizzly Cr., Skokomish R.	2003	96
Hood Canal Total		526
Strait of Juan de Fuca		
Dungeness H.	2003	48
Elwha R. H.	2005	96
Hoko R.	2002	78
Snow Cr.	2002	47
	2003	48
	2004	48
Strait of Juan de Fuca Total		365
North Washington Coast		
Makah H.	2001	48
	2002	47
	2003	47
Queets R.	2002	92
Quinault H.	2002	48
SolDuc H. Fall	2003	95
SolDuc H. Summer	2003	96
SolDuc R. Summer	1995	101
North Washington Coast Total		574
South Washington Coast		
Bingham Cr. H.	1995	47
Hope Cr.	1999	47
Naselle H.	2003	96

Nemah H.	2003	48
South Washington Coast Total		238
Columbia R.		
Big Cr. H.	2002	96
Bonneville H.	2002	96
Clackamas R. E.	1998	61
Clackamas R. L.	1998	31
Cowlitz H.	2002	95
	2003	48
Eagle Cr. H.	2001	96
Elochoman H.	2003	94
Fallert H.	2003	96
Kalama Falls H.	2003	90
Lewis H. E.	2003	47
Lewis H. L.	2003	48
Sandy H.	2002	95
Columbia R. Total		993
North Oregon Coast		
Alsea R. W.	2002	102
Beaver Cr. W.	2002	33
Devils Cr. W.	2002	38
Necanicum R. W.	2002	31
Nehalem H.	2002	96
Nehalem R. W.	2002	140
Nestucca R. W.	2002	54
Salmon R. W., OR	2002	36
Trask H.	2002	96
North Oregon Coast Total		626
Mid Oregon Coast		
Coos R. H.	2004	96
Coos R. W	2004	78
Coquille R. H.	2004	49
Coquille R. W.	2002	40
	2004	47
Devil's Lk.	2002	39
Siletz R.	2000	66
	2001	11
Siuslaw R.	2000	76
Yachats R. W.	2003	27
	2004	26
Yaquina R.	2000	43
	2001	30
Mid Oregon Coast Total		628
Oregon Lakes Complex		

Mercer Lk.	2003	28
Siltcoos Lk.	2000	31
	2001	27
Siltcoos Lk.	2002	24
Sutton Cr.	2002	50
Sutton Lk.	2002	35
Tahkenitch Lk.	2000	32
	2001	26
	2002	39
	2004	48
Tenmile Lk.	2000	35
	2001	30
	2002	99
Oregon Lakes Complex Total		504
Umpqua R.		
Calapooya Cr.	2000	22
	2001	17
	2006	85
	2007	112
	2008	96
Elk Cr.	2000	24
	2001	10
South Fork Umpqua R.	2000	39
	2001	29
Smith R.	1997	33
	2000	38
	2001	37
Umpqua R. W.	2002	277
	2004	48
Umpqua R. Total		867
South Oregon Coast / North California		
Iron Gate H.	2002	48
Klamath R.	2003	47
Redwood Cr.	2002	48
Rogue R. W.	2002	125
South Fork Eel R.	2003	96
Sharber & Dutch Cr., Trinity R.	2003	96
South Oregon Coast / North California Total		460

Central California		
Big R.	2003	48
Lagunitas Cr.	2001	94
Scott, Big Cr. H.	2006	96
South Fork Noyo R.	2001	96
Central California Total		334
Grand Total		8,879

Table 3. Estimated stock proportions of a mixture of 197 coded-wire tagged coho salmon that were caught off the coasts of Washington and Oregon. Estimates were made using the 8 microsatellite locus GAPS coho salmon database.

Reporting group	Estimated proportion	95% Confidence interval	True proportion
Kuskokwim	0.000	0.000 - 0.011	0.000
North British Columbia	0.000	0.000 - 0.012	0.000
South British Columbia	0.022	0.006 - 0.077	0.010
Puget Sound	0.049	0.023 – 0.130	0.025
Washington Coast	0.183	0.097 – 0.250	0.244
Columbia R.	0.645	0.521 – 0.692	0.675
North/Mid Oregon Coast	0.099	0.048 – 0.166	0.046
South Oregon/North California Coast	0.002	0.000 – 0.0224	0.000
Central California	0.000	0.000 – 0.000	0.000

Figure 1. A screen capture from the GAPS database showing the locations of the 110 coho salmon populations currently in the database.

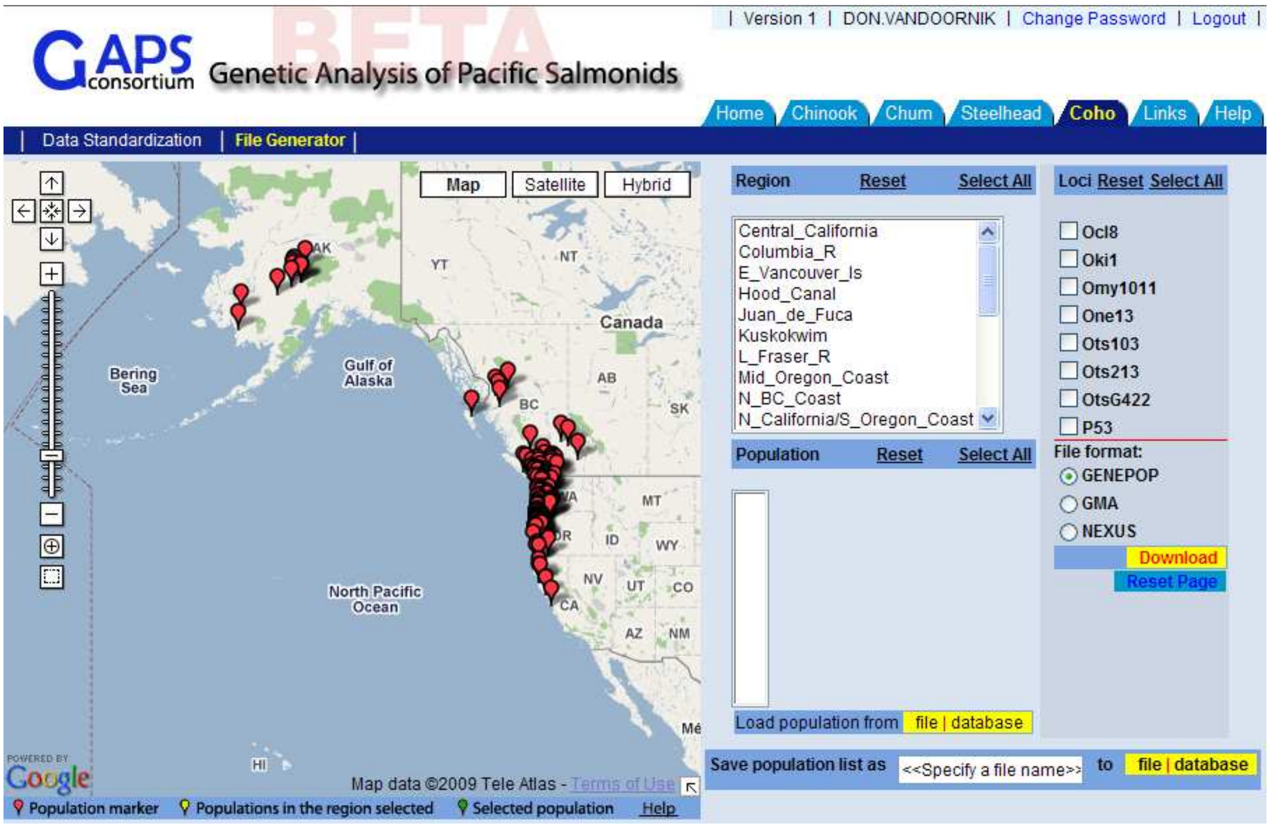
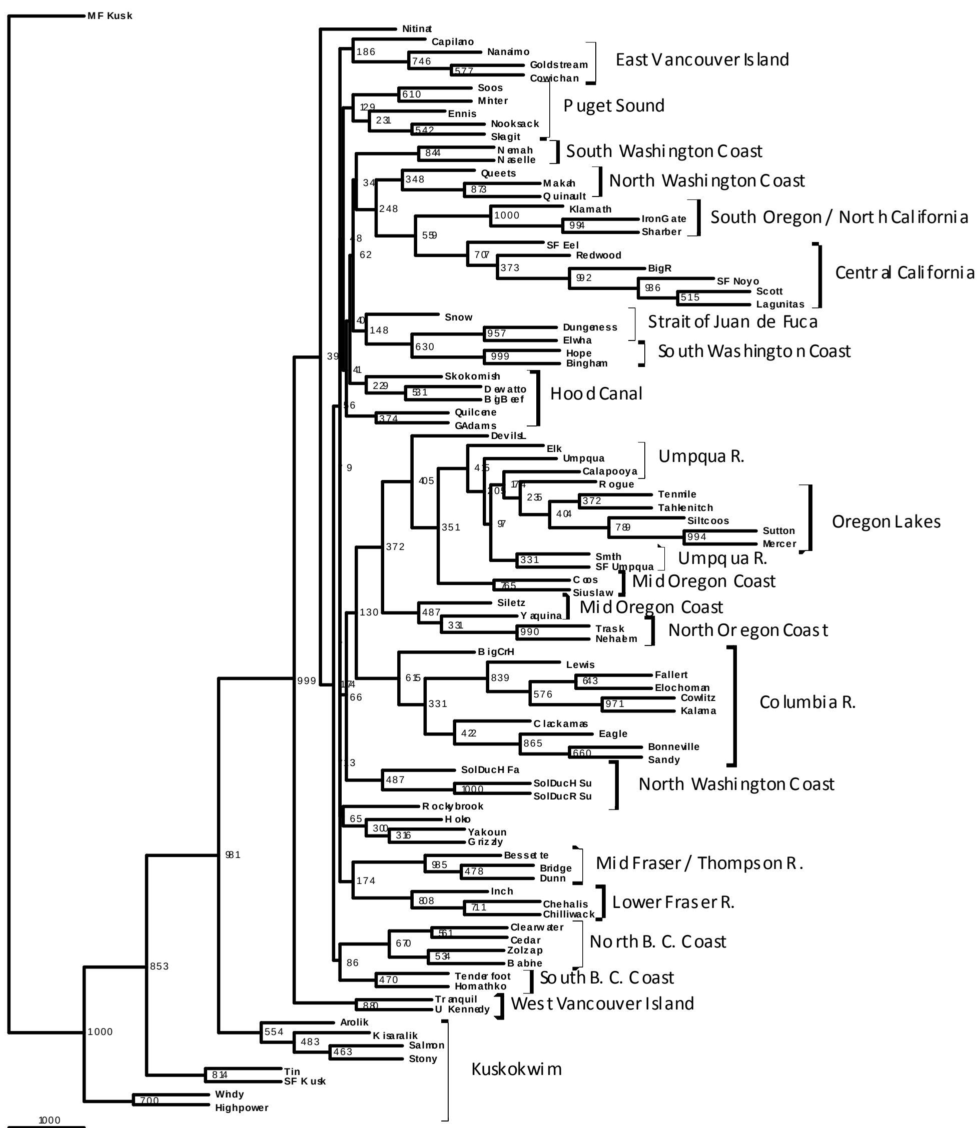


Figure 2. Neighbor-joining dendrogram of Corvalli-Sforza and Edwards (1967) chord distances for 92 coho salmon populations, with bootstrap values given for each branch (out of 1,000 bootstraps). Clusters of two or more populations from the same region are identified.



Appendix A: Reports received from participating laboratories

Southwest Fisheries Science Center, NOAA Fisheries

This report summarizes the work conducted at the Southwest Fisheries Science Center, Fisheries Ecology Division for the process of standardization of eight microsatellite markers in coho salmon.

A list of 8 microsatellite loci to be standardized among participating laboratories was determined with a survey of participating laboratories conducted by project leader Don VanDoornik, NWFSC. Using the provided size range information for each of the selected loci, along with fluorescent dye labeled primers, two panels of markers were constructed such that loci could be multiplexed on two ABI377 automated sequencer gel runs: one panel with 5 loci and the other with 3 loci (Table 1). Of the 8 selected loci, prior to this standardization process, 6 had been amplified successfully and extensively used to genotype coho salmon in this lab, one gave marginal success but was not widely used (Omy1011) and the other had not been previously assessed by us in coho salmon (Ots213).

Upon receipt of the reference DNA plate and 8 allelic ladders, a dilution tray was made, containing 1:10 dilutions (in 5mM Tris) of reference samples and a 1:5 dilution of each allelic ladder. In the interest of efficiency and per the project leader's suggestion, in order to run both reference samples and ladders on the same gel, one column (4a-4h) of reference sample DNA was omitted from the dilution tray and replaced with the 8 allelic ladders; hence, a total of 88 reference samples and 8 ladders were analyzed.

Loci were amplified according to PCR recipes and protocols (available upon request) already proven successful in this lab, using 35-cycle thermal-cycling profiles with annealing at 53/55˚C (Omy1011, Ots213, OtsG422, P53, Ots103) or 55/57˚C (Oki1, Ocl8, One13). For each panel of markers, PCR products were pooled at the post-PCR stage and electrophoresed on an ABI377 automated sequencer. Gels were tracked using GeneScan 3.0 and allele calls made in Genotyper 2.1 software (Applied Biosystems, Inc.). All loci but one (OtsG422) successfully amplified in most individuals on the first attempt. Some loci had somewhat high dropout rates and/or low overall signal, and these were re-amplified and re-run to obtain more complete data coverage: Ocl8, Ots103 and Omy1011. The FAM-labeled version of OtsG422 F, on Panel A, was found to not amplify in any individuals on two separate PCR attempts using two separate batches of primer mix, so it was concluded that there may be an error in the sequence of the F primer. An existing TET-labeled version of OtsG422 F, redesigned and widely used in coho salmon in our lab, called OtsG422b F, was used instead for this standardization process.

For each marker, raw decimal data from scored peaks was used to construct categories in Genotyper 2.1, such that the category (allele) names matched the GAPS allele names provided in the reference plate genotype data. As amplification of or distinction between the allelic ladder peaks was inconsistent between markers, only some of the ladders proved useful in ground-truthing the allele names. Once the GAPS categories had been created, decimal allele calls were changed to reflect the GAPS names. As is customary in this lab, allele calls were made independently by two people, and all discrepancies were resolved by reviewing the raw data in Genotyper 2.1.

After genotype data had been error-checked, our allele calls were compared to the reference genotype data in a similar manner. The majority of the discrepancies at this stage were call/no-call, and these were left in the dataset. An additional 15 discrepancies involved actual allele call discrepancies. Of these, 8 appear to be true discrepancies involving an allele that amplified in this lab but not in the lab where the reference data was generated, or vice versa. These 8 discrepancies, involving 1 or 2 individuals each for Omy1011, Ots213, One13, Ots103 and P53, were also left in the dataset, each with a comment explaining the rationale for the discrepancy. There were no instances of GAPS category or allele name discrepancies in any of the 8 loci. Sample DNA quality was highly variable, with 48 of the 88 samples assayed providing full 8 locus genotypes, but 5 of these samples failed at 5 loci or more. The overall missing data rate was ~13%, or a mean of approximately 2 allele calls (of 16) per sample.

Conservation Genetics Laboratory, U. S. Fish and Wildlife Service

This report summarizes the work conducted at U.S. Fish and Wildlife Alaska Region Conservation Genetics Laboratory (CGL) as directed by the principal investigator, Don VanDoornik, NWFSC.

Eight microsatellite loci were chosen for use on an Applied Biosystem Incorporated (ABI) 3730 DNA Analyzer. The NWFSC distributed recommendations for amplification and scoring of these loci, allelic ladders and a 96-sample DNA reference plate to participating laboratories.

Four of these loci had been used previously in the CGL for coho salmon using Li-Cor IR$^{2®}$ DNA scanner: Ocl8, Oki1, Ots213, and Omy1011. Conversion factors for these loci were determined by comparing previously scored populations from the Li-Cor platform to new data for the same populations generated with the ABI platform.

It is important to note that there are multiple primer sequences for Ocl8 in circulation (Table 1). For the purpose of this study, we used the sequences provided by NWFSC. However, both the forward and reverse sequences they provided differ from the original sequences published in: Condrey and Benzen (1998) Characterization of coastal cutthroat trout (*Oncorhychus clarki clarki*) microsatellites and their conservation in other salmonids, Molecular Ecology, 7, 783-792.

The allelic ladders distributed by NWFSC were amplified to create scoring bins with the ABI GeneMapper v4.0 software. The ladders all amplified successfully and we assigned bin names based on those provided by NWFSC.

After creating the bins, the reference DNA plate was analyzed. Initial amplification yielded lower than normal success rates. We believe this was due, primarily to the low concentration of the DNA in the reference plate. The CGL typically standardizes all DNA to 30 ng/µl for PCR amplification. Quantification of the DNA in the reference plate revealed DNA concentrations several orders of magnitude lower (≤0.01 ng/µl). After adjusting PCR conditions by increasing TAQ, primer concentrations, and DNA, results were improved. However, the overall missing data rate remained abnormally high (~20%), and near 40% for OtsG422 and Ots213. For the 96 reference samples, complete genotypes for all 8 loci were obtained for only 40 samples, and 12 samples failed at 5 or more loci. Given the extremely low quantity of DNA provided, we determined that additional rounds of amplification would not be cost effective.

The CGL scores were compared to those provided for the reference plate samples. There were 1209 data points that allowed for direct comparisons between the CGL and NWFSC scores and only five discrepancies (0.4%). In all five cases, the discrepancies were scored as heterozygotes by the CGL, but as homozygotes by NWFSC (Table 2).

Table 1. Ocl8 Primer Sequences.

Ocl8 Reverse Sequences	Source
CCC TGT CCC TTC CAT CTC T	NWFSC
CAC CTT CCA TCT CTC ATT CCA C	Condrey and Benzen 1998
Ocl8 Forward Sequences	
TAg TgT TTC gTg TTC gCC Tg	NWFSC
TAg TgT TCC gTg TTC gCC Tg	Condrey and Benzen 1998

Table 2. Scoring Discrepancies

Locus	Well Location	CGL Score	Reference Score
Ocl8	F7	98/104	98/98
Omy1011	E2	186/206	186/186
Ots213	A5	155/271	155/155
P53	F7	161/171	171/171
P53	H7	161/177	177/177

State of Washington
Department of Fish and Wildlife
Fish Management Program - Science Division
Conservation Biology - Molecular Genetics Laboratory

March 3, 2009

To: Don Van Doornik

From: Todd W. Kassler

Subject: WDFW coho standardization

A plate of genomic DNA sent to WDFW was used to compare allele sizes of a standard plate of individuals to allele sizes generated at WDFW using existing equipment and conditions. After analyzing the standard plate a conversion table was generated to align allele names from WDFW to a standardized naming that can be used by all participating labs for eight microsatellite loci. Aligning data among the labs has allowed sharing of datasets to expand baselines for data analysis of coho populations.

Descriptions of the eight loci and the PCR conditions used by WDFW are given in Table 1. Microsatellite alleles were sized using an internal size standard. GENEMAPPER (Version 3.7) software (Applied Biosystems) was used to collect and analyze the microsatellite data. Allele binning and naming were accomplished using MicrosatelliteBinner-v1h (Young, WDFW available from the author). MicrosatelliteBinner creates groups (bins) of alleles with similar mobilities (alleles with the same number of repeat units). The upper and lower bounds of the bins are determined by identifying clusters of alleles separated by gaps (nominally 0.4 base pairs in size) in the distribution of allele sizes. The bins are then named as the mean allele size for the cluster rounded to an integer.

Allele sizes for the eight loci analyzed by WDFW were aligned to the allele sizes of the standardized plate of individual coho (Table 2). The size adjustment between WDFW and the standardized data are shown in Table 3. This conversion table can be used for future conversion of coho data to the standardized format.

Literature Cited

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Table 1. Microsatellite loci used for coho standardization including dye, number alleles, and allele size range for each locus.

Locus	Annealing temp C°	dye	WDFW Allelic Range	Number of alleles per locus	Citation
Oki-1	54	hex	91 - 162	16	Smith et al. 1998
Ocl-8	55	hex	98 - 135	17	Condrey and Bentzen 1998
p53	46	hex	163 - 197	13	Baker et al. 2002
One-13M	56	hex	193 - 226	16	Scribner et al. 1996
Omy-1011	54	ned	179 - 211	8	Spies et al. 2005
Ots-103 [b]	56	hex	85 - 291	42	Small et al. 1998
Ots-213	54	pet	183 - 316	21	Greig et al. 2003
Ots-G422	54	6fam	228 - 418	27	Williamson et al. 2002

Table 2. Comparison of allele sizes between WDFW and the standardized plate for eight microsatellite loci.

Sample#	Plate Well	WDFW Ocl-8a	WDFW Ocl-8b	Ocl8_1	Ocl8_2	Diff	Diff
90210-13228	A01			110	110		
90210-13229	B01			102	108		
90210-13231	C01			126	126		
90210-13232	D01			102	124		
90210-13233	E01			122	126		
90210-13234	F01			098	098		
90210-13235	G01			098	102		
90210-13239	H01			128	128		
90210-13241	A02	125	127	124	126	001	001
90210-13294	B02	106	110	106	110	000	000
90210-13295	C02	102	106	102	106	000	000
90210-13300	D02	110	123	110	122	000	001
90210-13348	E02	106	127	106	126	000	001
90210-13349	F02			110	116		
90210-13350	G02	108	117	108	116	000	001
90210-13351	H02	106	129	106	128	000	001
90210-13352	A03	119	127	118	126	001	001
90210-13353	B03	102	110	102	110	000	000
90210-13358	C03			098	126		
90210-13371	D03			122	122		
90210-13373	E03	106	123	106	122	000	001
90210-13374	F03	117	125	116	124	001	001
90210-13375	G03	121	121	120	120	001	001
90210-13377	H03	104	112	104	112	000	000
90210-13378	A04	123	133	122	132	001	001
90210-13379	B04	104	127	104	126	000	001
90210-13380	C04			110	122		
90210-13381	D04	102	127	102	126	000	001
90210-13382	E04	123	127	122	126	001	001
90210-13383	F04	104	106	104	106	000	000
90210-13384	G04			114	126		
90210-13385	H04	102	127	102	126	000	001
90210-13386	A05	98	127	098	126	000	001
90210-13387	B05	98	110	098	110	000	000
90210-13388	C05	108	123	108	122	000	001
90210-13389	D05	98	123	098	122	000	001
90210-13390	E05			106	122		
90210-13391	F05	98	119	098	118	000	001
90210-13392	G05			110	122		
90210-13393	H05	127	133	126	132	001	001
90210-13394	A06	106	123	106	122	000	001
90210-13395	B06	127	133	126	132	001	001
90210-13396	C06	98	115	098	114	000	001
90210-13398	D06	98	106	098	106	000	000
90210-13399	E06	127	127	126	126	001	001
90210-13400	F06	110	110	110	110	000	000
90210-13401	G06	104	115	104	114	000	001
90210-13402	H06	121	127	120	126	001	001

Table 2 continued.

Sample#	Plate Well	WDFW Ocl-8a	WDFW Ocl-8b	Ocl8_1	Ocl8_2	Diff	Diff
90210-13407	A07	102	123	102	122	000	001
90215-13962	B07	115	117	114	116	001	001
90215-13963	C07	117	119	116	118	001	001
90215-14031	D07	110	127	110	126	000	001
90215-14032	E07	98	123	098	122	000	001
90215-14034	F07	98	98	098	098	000	000
90215-14035	G07	102	115	102	114	000	001
90215-14036	H07	104	117	104	116	000	001
90215-14037	A08	98	127	098	126	000	001
90215-14113	B08	98	123	098	122	000	001
90215-14114	C08	110	121	110	120	000	001
90215-14115	D08	98	121	098	120	000	001
90215-14116	E08	98	127	098	126	000	001
90215-14117	F08	110	127	110	126	000	001
90215-14174	G08	119	127	118	126	001	001
90215-14175	H08	123	127	122	126	001	001
90215-14176	A09	110	127	110	126	000	001
90215-14177	B09	98	108	098	108	000	000
90215-14178	C09	106	106	106	106	000	000
90215-14179	D09			110	138		
90215-14181	E09	104	112	104	112	000	000
90215-14182	F09	98	117	098	116	000	001
90215-14183	G09	108	115	108	114	000	001
90215-14457	H09	108	135	108	134	000	001
90215-14505	A10	106	129	106	128	000	001
90215-14618	B10	123	127	122	126	001	001
90215-14625	C10	123	127	122	126	001	001
90215-14639	D10	104	104	104	104	000	000
90215-14640	E10	106	123	106	122	000	001
90215-14661	F10	110	127	110	126	000	001
90253-17700	G10	108	108	108	108	000	000
90253-17703	H10	102	102	102	102	000	000
90253-17704	A11	106	108	106	108	000	000
90253-17712	B11	108	108	108	108	000	000
90253-17713	C11	102	121	102	120	000	001
90253-17714	D11	108	108	108	108	000	000
90253-17715	E11	104	108	104	108	000	000
90253-17836	F11	110	127	110	126	000	001
90253-17837	G11	115	127	114	126	001	001
90253-17838	H11	110	127	110	126	000	001
90253-17866	A12	98	98	098	098	000	000
90253-17867	B12	123	123	122	122	001	001
90253-17868	C12	123	127	122	126	001	001
90253-17869	D12			122	126		
90253-17871	E12	98	112	098	112	000	000
90253-17872	F12			098	102		
90253-17893	G12	108	119	108	118	000	001
90253-17995	H12	106	121	106	120	000	001

Table 2 continued.

Sample#	Plate Well	WDFW Oki-1a	WDFW Oki-1b	Oki1_1	Oki1_2	Diff	Diff
90210-13228	A01			106	142		
90210-13229	B01			098	134		
90210-13231	C01			094	094		
90210-13232	D01			098	110		
90210-13233	E01			098	118		
90210-13234	F01			098	110		
90210-13235	G01			106	110		
90210-13239	H01			094	114		
90210-13241	A02	103	107	102	106	001	001
90210-13294	B02	107	127	106	126	001	001
90210-13295	C02	099	127	098	126	001	001
90210-13300	D02	095	095	094	094	001	001
90210-13348	E02	095	099	094	098	001	001
90210-13349	F02			090	130		
90210-13350	G02	099	107	098	106	001	001
90210-13351	H02	095	107	094	106	001	001
90210-13352	A03	107	123	106	122	001	001
90210-13353	B03	099	158	098	154	001	004
90210-13358	C03			114	118		
90210-13371	D03			098	130		
90210-13373	E03	095	115	094	114	001	001
90210-13374	F03			098	118		
90210-13375	G03			094	118		
90210-13377	H03			094	098		
90210-13378	A04	115	119	114	118	001	001
90210-13379	B04	099	111	098	110	001	001
90210-13380	C04						
90210-13381	D04	107	140	106	138	001	002
90210-13382	E04	107	136	106	134	001	002
90210-13383	F04	095	115	094	114	001	001
90210-13384	G04			098	106		
90210-13385	H04	107	127	106	126	001	001
90210-13386	A05	095	107	094	106	001	001
90210-13387	B05	099	115	098	114	001	001
90210-13388	C05	107	115	106	114	001	001
90210-13389	D05	115	145	114	142	001	003
90210-13390	E05	127	140	126	138	001	002
90210-13391	F05	095	136	094	134	001	002
90210-13392	G05			098	098		
90210-13393	H05	099	127	098	126	001	001
90210-13394	A06	095	123	094	122	001	001
90210-13395	B06	095	127	094	126	001	001
90210-13396	C06	099	111	098	110	001	001
90210-13398	D06	099	107	098	106	001	001
90210-13399	E06	099	103	098	102	001	001
90210-13400	F06			098	134		
90210-13401	G06	107	111	106	110	001	001
90210-13402	H06	099	115	098	114	001	001

Table 2 continued.

Sample#	Plate Well	WDFW Oki-1a	WDFW Oki-1b	Oki1_1	Oki1_2	Diff	Diff
90210-13407	A07	099	099	098	098	001	001
90215-13962	B07	095	136	094	134	001	002
90215-13963	C07	095	099	094	098	001	001
90215-14031	D07	099	103	098	102	001	001
90215-14032	E07	099	119	098	118	001	001
90215-14034	F07	095	095	094	094	001	001
90215-14035	G07	099	119	098	118	001	001
90215-14036	H07	099	099	098	098	001	001
90215-14037	A08	103	107	102	106	001	001
90215-14113	B08	103	140	102	138	001	002
90215-14114	C08	099	111	098	110	001	001
90215-14115	D08	095	099	094	098	001	001
90215-14116	E08	103	119	102	118	001	001
90215-14117	F08			094	098		
90215-14174	G08	099	111	098	110	001	001
90215-14175	H08	119	136	118	134	001	002
90215-14176	A09	095	099	094	098	001	001
90215-14177	B09	099	103	098	102	001	001
90215-14178	C09	103	132	102	130	001	002
90215-14179	D09	095	099	094	098	001	001
90215-14181	E09	107	119	106	118	001	001
90215-14182	F09	103	162	102	158	001	004
90215-14183	G09	095	099	094	098	001	001
90215-14457	H09			098	098		
90215-14505	A10	099	099	098	098	001	001
90215-14618	B10	099	103	098	102	001	001
90215-14625	C10	095	107	094	106	001	001
90215-14639	D10	099	127	098	126	001	001
90215-14640	E10	095	115	094	114	001	001
90215-14661	F10			098	138		
90253-17700	G10	099	132	098	130	001	002
90253-17703	H10	099	099	098	098	001	001
90253-17704	A11	099	107	098	106	001	001
90253-17712	B11	111	111	110	110	001	001
90253-17713	C11	099	111	098	110	001	001
90253-17714	D11	095	099	094	098	001	001
90253-17715	E11	099	127	098	126	001	001
90253-17836	F11	095	123	094	122	001	001
90253-17837	G11	103	107	102	106	001	001
90253-17838	H11	107	136	106	134	001	002
90253-17866	A12	091	107	090	106	001	001
90253-17867	B12	095	140	094	138	001	002
90253-17868	C12	095	136	094	134	001	002
90253-17869	D12	115	140	114	138	001	002
90253-17871	E12	099	127	098	126	001	001
90253-17872	F12	095	115	094	114	001	001
90253-17893	G12	099	127	098	126	001	001
90253-17995	H12	111	145	110	142	001	003

Table 2 continued.

Sample#	Plate Well	WDFW Omy-1011a	WDFW Omy-1011b	Omy1011_1	Omy1011_2	Diff	Diff
90210-13228	A01			186	190		
90210-13229	B01			182	198		
90210-13231	C01			178	182		
90210-13232	D01			182	190		
90210-13233	E01			182	190		
90210-13234	F01			178	210		
90210-13235	G01			186	190		
90210-13239	H01			190	210		
90210-13241	A02	183	187	182	186	001	001
90210-13294	B02	179	183	178	182	001	001
90210-13295	C02	179	183	178	182	001	001
90210-13300	D02	183	211	182	210	001	001
90210-13348	E02	187	187	186	186	001	001
90210-13349	F02						
90210-13350	G02	183	191	182	190	001	001
90210-13351	H02			194	194		
90210-13352	A03	183	211	182	210	001	001
90210-13353	B03	179	183	178	182	001	001
90210-13358	C03			186	210		
90210-13371	D03			178	182		
90210-13373	E03	183	199	182	198	001	001
90210-13374	F03			182	182		
90210-13375	G03			182	186		
90210-13377	H03			194	194		
90210-13378	A04	179	187	178	186	001	001
90210-13379	B04			186	210		
90210-13380	C04						
90210-13381	D04			178	210		
90210-13382	E04			186	186		
90210-13383	F04	191	211	190	210	001	001
90210-13384	G04						
90210-13385	H04			206	206		
90210-13386	A05	187	191	186	190	001	001
90210-13387	B05	183	187	182	186	001	001
90210-13388	C05			178	198		
90210-13389	D05	183	187	182	186	001	001
90210-13390	E05	183	187	182	186	001	001
90210-13391	F05	183	187	182	186	001	001
90210-13392	G05			186	186		
90210-13393	H05	191	211	190	210	001	001
90210-13394	A06			178	190		
90210-13395	B06			186	198		
90210-13396	C06			206	218		
90210-13398	D06			182	194		
90210-13399	E06			182	186		
90210-13400	F06			186	190		
90210-13401	G06			178	194		
90210-13402	H06	187	187	186	186	001	001

8

Table 2 continued.

Sample#	Plate Well	WDFW Omy-1011a	WDFW Omy-1011b	Omy1011_1	Omy1011_2	Diff	Diff
90210-13407	A07	187	191	186	190	001	001
90215-13962	B07	187	187	186	186	001	001
90215-13963	C07	187	187	186	186	001	001
90215-14031	D07	183	199	182	198	001	001
90215-14032	E07			186	194		
90215-14034	F07	179	187	178	186	001	001
90215-14035	G07			186	190		
90215-14036	H07			182	186		
90215-14037	A08	183	183	182	182	001	001
90215-14113	B08	183	187	182	186	001	001
90215-14114	C08			186	198		
90215-14115	D08	183	187	182	186	001	001
90215-14116	E08	211	211	210	210	001	001
90215-14117	F08	191	195	190	194	001	001
90215-14174	G08	187	191	186	190	001	001
90215-14175	H08	187	187	186	186	001	001
90215-14176	A09	179	187	178	186	001	001
90215-14177	B09	187	187	186	186	001	001
90215-14178	C09			178	190		
90215-14179	D09	179	183	178	182	001	001
90215-14181	E09			186	210		
90215-14182	F09	179	187	178	186	001	001
90215-14183	G09	187	187	186	186	001	001
90215-14457	H09						
90215-14505	A10	183	187	182	186	001	001
90215-14618	B10	187	191	186	190	001	001
90215-14625	C10	183	191	182	190	001	001
90215-14639	D10	179	187	178	186	001	001
90215-14640	E10	187	187	186	186	001	001
90215-14661	F10			182	190		
90253-17700	G10	183	191	182	190	001	001
90253-17703	H10	187	191	186	190	001	001
90253-17704	A11	183	183	182	182	001	001
90253-17712	B11	183	187	182	186	001	001
90253-17713	C11	183	187	182	186	001	001
90253-17714	D11	179	183	178	182	001	001
90253-17715	E11	183	187	182	186	001	001
90253-17836	F11	187	191	186	190	001	001
90253-17837	G11	187	187	186	214	001	-027
90253-17838	H11	179	183	178	182	001	001
90253-17866	A12			198	202		
90253-17867	B12	187	199	186	198	001	001
90253-17868	C12			178	206		
90253-17869	D12	187	187	186	186	001	001
90253-17871	E12	179	203	178	202	001	001
90253-17872	F12	187	187	186	186	001	001
90253-17893	G12	187	191	186	190	001	001
90253-17995	H12	187	199	186	198	001	001

Table 2 continued.

Sample#	Plate Well	WDFW One-13Ma	WDFW One-13Mb	One13_1	One13_2	Diff	Diff
90210-13228	A01			161	171		
90210-13229	B01			161	173		
90210-13231	C01			173	173		
90210-13232	D01			161	185		
90210-13233	E01			151	179		
90210-13234	F01			159	173		
90210-13235	G01			159	179		
90210-13239	H01			151	185		
90210-13241	A02	201	203	159	161	042	042
90210-13294	B02	203	223	161	181	042	042
90210-13295	C02	203	209	161	167	042	042
90210-13300	D02	193	201	151	159	042	042
90210-13348	E02			151	159		
90210-13349	F02			161	173		
90210-13350	G02	201	221	159	179	042	042
90210-13351	H02			179	185		
90210-13352	A03	193	203	151	161	042	042
90210-13353	B03	217	226	175	185	042	041
90210-13358	C03			159	161		
90210-13371	D03			151	179		
90210-13373	E03	203	215	161	173	042	042
90210-13374	F03			163	185		
90210-13375	G03			161	161		
90210-13377	H03			159	185		
90210-13378	A04			151	179		
90210-13379	B04			179	179		
90210-13380	C04			000	000		
90210-13381	D04	193	209	151	167	042	042
90210-13382	E04	193	203	151	161	042	042
90210-13383	F04			173	179		
90210-13384	G04			151	151		
90210-13385	H04	217	226	175	185	042	041
90210-13386	A05	215	221	173	179	042	042
90210-13387	B05	209	217	167	175	042	042
90210-13388	C05	209	217	167	175	042	042
90210-13389	D05	193	201	151	159	042	042
90210-13390	E05	193	201	151	159	042	042
90210-13391	F05	193	193	151	151	042	042
90210-13392	G05			161	161		
90210-13393	H05	193	193	151	179	042	014
90210-13394	A06	193	193	151	151	042	042
90210-13395	B06			151	161		
90210-13396	C06			151	159		
90210-13398	D06	193	218	151	177	042	041
90210-13399	E06	203	218	161	177	042	041
90210-13400	F06			151	159		
90210-13401	G06			159	161		
90210-13402	H06	193	193	151	151	042	042

10

Table 2 continued.

Sample#	Plate Well	WDFW One-13Ma	WDFW One-13Mb	One13_1	One13_2	Diff	Diff
90210-13407	A07	193	193	151	151	042	042
90215-13962	B07			161	175		
90215-13963	C07	193	221	151	179	042	042
90215-14031	D07	201	218	159	177	042	041
90215-14032	E07			155	159		
90215-14034	F07			159	171		
90215-14035	G07			173	181		
90215-14036	H07	215	215	173	173	042	042
90215-14037	A08	203	215	161	173	042	042
90215-14113	B08			151	179		
90215-14114	C08			173	181		
90215-14115	D08	215	221	173	179	042	042
90215-14116	E08	201	207	159	165	042	042
90215-14117	F08			151	171		
90215-14174	G08	223	223	181	181	042	042
90215-14175	H08	201	221	159	179	042	042
90215-14176	A09	209	221	167	179	042	042
90215-14177	B09	205	209	163	167	042	042
90215-14178	C09	198	215	157	173	041	042
90215-14179	D09	215	221	173	179	042	042
90215-14181	E09	193	218	151	177	042	041
90215-14182	F09	193	203	151	161	042	042
90215-14183	G09	193	226	151	185	042	041
90215-14457	H09			167	185		
90215-14505	A10	218	226	177	185	041	041
90215-14618	B10	193	203	151	161	042	042
90215-14625	C10	201	203	159	161	042	042
90215-14639	D10	215	217	173	175	042	042
90215-14640	E10	193	193	151	151	042	042
90215-14661	F10			151	173		
90253-17700	G10			173	179		
90253-17703	H10	221	224	179	183	042	041
90253-17704	A11	215	221	173	179	042	042
90253-17712	B11	221	221	179	179	042	042
90253-17713	C11	201	221	159	179	042	042
90253-17714	D11	226	226	185	185	041	041
90253-17715	E11	193	203	151	161	042	042
90253-17836	F11	213	218	171	177	042	041
90253-17837	G11	193	213	151	171	042	042
90253-17838	H11	193	203	151	161	042	042
90253-17866	A12	195	203	153	161	042	042
90253-17867	B12	221	221	179	179	042	042
90253-17868	C12	193	217	151	175	042	042
90253-17869	D12			159	161		
90253-17871	E12	203	221	161	179	042	042
90253-17872	F12			151	179		
90253-17893	G12	193	207	151	165	042	042
90253-17995	H12	203	221	161	179	042	042

Table 2 continued.

Sample#	Plate Well	WDFW Ots-103a	WDFW Ots-103b	Ots103_1	Ots103_2	Diff	Diff
90210-13228	A01			077	149		
90210-13229	B01			093	093		
90210-13231	C01			093	161		
90210-13232	D01			149	149		
90210-13233	E01			153	153		
90210-13234	F01			069	117		
90210-13235	G01			085	145		
90210-13239	H01			085	085		
90210-13241	A02	154	225	145	217	009	008
90210-13294	B02	093	125	085	117	008	008
90210-13295	C02			073	117		
90210-13300	D02	093	197	085	189	008	008
90210-13348	E02	154	248	145	241	009	007
90210-13349	F02			069	101		
90210-13350	G02	125	174	117	165	008	009
90210-13351	H02	133	133	125	125	008	008
90210-13352	A03	113	170	105	161	008	009
90210-13353	B03	109	125	101	117	008	008
90210-13358	C03			165	197		
90210-13371	D03			125	205		
90210-13373	E03	125	190	117	181	008	009
90210-13374	F03			077	077		
90210-13375	G03			125	269		
90210-13377	H03			085	085		
90210-13378	A04	125	158	117	149	008	009
90210-13379	B04	150	158	141	149	009	009
90210-13380	C04			105	125		
90210-13381	D04	170	240	161	233	009	007
90210-13382	E04	085	193	077	185	008	008
90210-13383	F04	137	158	129	149	008	009
90210-13384	G04			161	165		
90210-13385	H04	105	117	097	109	008	008
90210-13386	A05	101	137	093	129	008	008
90210-13387	B05	154	213	145	205	009	008
90210-13388	C05			173	193		
90210-13389	D05	197	197	189	189	008	008
90210-13390	E05	154	197	145	189	009	008
90210-13391	F05	209	237	201	229	008	008
90210-13392	G05			177	177		
90210-13393	H05	162	186	153	177	009	009
90210-13394	A06	154	154	145	145	009	009
90210-13395	B06	150	186	141	177	009	009
90210-13396	C06	125	125	117	117	008	008
90210-13398	D06	113	145	105	137	008	008
90210-13399	E06	105	150	097	141	008	009
90210-13400	F06			145	153		
90210-13401	G06	117	125	109	117	008	008
90210-13402	H06	120	182	113	173	007	009

Table 2 continued.

Sample#	Plate Well	WDFW Ots-103a	WDFW Ots-103b	Ots103_1	Ots103_2	Diff	Diff
90210-13407	A07	170	288	161	281	009	007
90215-13962	B07	170	291	161	285	009	006
90215-13963	C07	120	158	113	149	007	009
90215-14031	D07	120	178	113	169	007	009
90215-14032	E07	125	252	117	245	008	007
90215-14034	F07	097	097	089	089	008	008
90215-14035	G07	174	201	165	193	009	008
90215-14036	H07			073	085		
90215-14037	A08	089	133	081	125	008	008
90215-14113	B08	145	174	137	165	008	009
90215-14114	C08	128	162	121	153	007	009
90215-14115	D08	105	217	097	209	008	008
90215-14116	E08	105	120	097	113	008	007
90215-14117	F08			073	121		
90215-14174	G08	174	186	165	177	009	009
90215-14175	H08	125	233	117	225	008	008
90215-14176	A09			073	077		
90215-14177	B09	209	209	201	201	008	008
90215-14178	C09	158	186	149	177	009	009
90215-14179	D09	205	205	197	197	008	008
90215-14181	E09	141	209	133	201	008	008
90215-14182	F09	145	145	137	137	008	008
90215-14183	G09	097	113	089	105	008	008
90215-14457	H09	170	217	161	209	009	008
90215-14505	A10	217	217	209	209	008	008
90215-14618	B10	158	162	149	153	009	009
90215-14625	C10	166	174	157	165	009	009
90215-14639	D10	101	209	093	201	008	008
90215-14640	E10	162	162	153	153	009	009
90215-14661	F10			165	221		
90253-17700	G10	186	190	177	181	009	009
90253-17703	H10	109	182	101	173	008	009
90253-17704	A11			077	109		
90253-17712	B11	113	174	105	165	008	009
90253-17713	C11						
90253-17714	D11	128	137	121	129	007	008
90253-17715	E11	158	166	149	157	009	009
90253-17836	F11	154	190	145	181	009	009
90253-17837	G11	105	178	097	169	008	009
90253-17838	H11	125	170	117	161	008	009
90253-17866	A12	089	105	081	097	008	008
90253-17867	B12	237	237	229	229	008	008
90253-17868	C12	128	178	121	169	007	009
90253-17869	D12	178	197	169	189	009	008
90253-17871	E12	113	154	105	145	008	009
90253-17872	F12			117	117		
90253-17893	G12	105	137	097	129	008	008
90253-17995	H12						

Table 2 continued.

Sample#	Plate Well	WDFW Ots-213a	WDFW Ots-213a	Ots213_1	Ots213_2	Diff	Diff
90210-13228	A01			155	155		
90210-13229	B01			155	155		
90210-13231	C01			155	155		
90210-13232	D01			155	159		
90210-13233	E01			155	159		
90210-13234	F01			159	159		
90210-13235	G01			155	195		
90210-13239	H01			155	275		
90210-13241	A02	183	183	155	155	028	028
90210-13294	B02	183	187	155	159	028	028
90210-13295	C02			159	243		
90210-13300	D02	183	183	155	155	028	028
90210-13348	E02	183	187	155	159	028	028
90210-13349	F02			159	163		
90210-13350	G02			163	247		
90210-13351	H02			155	155		
90210-13352	A03	187	264	159	239	028	025
90210-13353	B03			159	159		
90210-13358	C03			155	195		
90210-13371	D03			155	159		
90210-13373	E03	183	187	155	159	028	028
90210-13374	F03			227	275		
90210-13375	G03			155	155		
90210-13377	H03	187	264	159	239	028	025
90210-13378	A04	187	249	159	223	028	026
90210-13379	B04			155	155		
90210-13380	C04			159	235		
90210-13381	D04	187	187	159	159	028	028
90210-13382	E04	183	183	155	155	028	028
90210-13383	F04			155	195		
90210-13384	G04			155	195		
90210-13385	H04	183	187	155	159	028	028
90210-13386	A05	183	183	155	155	028	028
90210-13387	B05	183	183	155	155	028	028
90210-13388	C05	183	190	155	163	028	027
90210-13389	D05	183	256	155	231	028	025
90210-13390	E05			155	155		
90210-13391	F05			155	243		
90210-13392	G05	183	183	155	155	028	028
90210-13393	H05	183	183	155	155	028	028
90210-13394	A06			155	159		
90210-13395	B06	183	183	155	155	028	028
90210-13396	C06	183	308	155	283	028	025
90210-13398	D06	272	276	247	251	025	025
90210-13399	E06	183	187	155	159	028	028
90210-13400	F06	183	183	155	155	028	028
90210-13401	G06	187	268	159	243	028	025
90210-13402	H06	183	187	155	159	028	028

Table 2 continued.

Sample#	Plate Well	WDFW Ots-213a	WDFW Ots-213a	Ots213_1	Ots213_2	Diff	Diff
90210-13407	A07	183	316	155	291	028	025
90215-13962	B07	183	249	155	223	028	026
90215-13963	C07	183	187	155	159	028	028
90215-14031	D07	183	183	155	155	028	028
90215-14032	E07	183	183	155	155	028	028
90215-14034	F07	187	190	159	163	028	027
90215-14035	G07			155	155		
90215-14036	H07			163	243		
90215-14037	A08	183	187	155	159	028	028
90215-14113	B08	183	183	155	155	028	028
90215-14114	C08			159	171		
90215-14115	D08	268	288	243	263	025	025
90215-14116	E08	187	190	159	163	028	027
90215-14117	F08	183	187	155	159	028	028
90215-14174	G08	183	187	155	159	028	028
90215-14175	H08	183	187	155	159	028	028
90215-14176	A09	183	300	155	275	028	025
90215-14177	B09	183	296	155	271	028	025
90215-14178	C09	183	183	155	155	028	028
90215-14179	D09			159	251		
90215-14181	E09	183	183	155	155	028	028
90215-14182	F09	183	183	155	155	028	028
90215-14183	G09	183	190	155	163	028	027
90215-14457	H09			171	171		
90215-14505	A10	187	198	159	171	028	027
90215-14618	B10	183	260	155	235	028	025
90215-14625	C10	183	190	155	163	028	027
90215-14639	D10			155	159		
90215-14640	E10	183	183	155	155	028	028
90215-14661	F10			155	155		
90253-17700	G10			159	271		
90253-17703	H10	187	190	159	163	028	027
90253-17704	A11	183	187	155	159	028	028
90253-17712	B11	190	209	163	183	027	026
90253-17713	C11	187	312	159	287	028	025
90253-17714	D11			159	231		
90253-17715	E11			159	235		
90253-17836	F11			155	155		
90253-17837	G11	187	312	159	287	028	025
90253-17838	H11	183	187	155	159	028	028
90253-17866	A12			159	259		
90253-17867	B12	183	183	155	155	028	028
90253-17868	C12	183	183	155	155	028	028
90253-17869	D12	183	312	155	287	028	025
90253-17871	E12	183	190	155	163	028	027
90253-17872	F12			155	223		
90253-17893	G12	183	183	155	155	028	028
90253-17995	H12	183	187	155	159	028	028

Table 2 continued.

Sample#	Plate Well	WDFW Ots-G422a	WDFW Ots-G422b	OtsG422_1	OtsG422_2	Diff	Diff
90210-13228	A01			308	316		
90210-13229	B01			272	336		
90210-13231	C01			292	352		
90210-13232	D01			296	304		
90210-13233	E01			300	332		
90210-13234	F01			280	328		
90210-13235	G01			312	324		
90210-13239	H01			304	328		
90210-13241	A02	305	374	304	372	001	002
90210-13294	B02			292	308		
90210-13295	C02	268	322	268	320	000	002
90210-13300	D02	309	414	308	412	001	002
90210-13348	E02			348	368		
90210-13349	F02			328	336		
90210-13350	G02	293	313	292	312	001	001
90210-13351	H02			288	312		
90210-13352	A03			324	364		
90210-13353	B03	281	313	280	312	001	001
90210-13358	C03			364	372		
90210-13371	D03			304	320		
90210-13373	E03			312	312		
90210-13374	F03	272	301	272	300	000	001
90210-13375	G03			328	352		
90210-13377	H03			280	328		
90210-13378	A04			300	328		
90210-13379	B04			324	404		
90210-13380	C04			320	320		
90210-13381	D04	330	334	328	332	002	002
90210-13382	E04			268	344		
90210-13383	F04	281	289	280	288	001	001
90210-13384	G04			316	348		
90210-13385	H04			288	332		
90210-13386	A05	289	354	288	352	001	002
90210-13387	B05	305	334	304	332	001	002
90210-13388	C05			280	296		
90210-13389	D05			312	364		
90210-13390	E05			368	396		
90210-13391	F05			300	300		
90210-13392	G05			304	352		
90210-13393	H05			276	332		
90210-13394	A06	272	366	272	364	000	002
90210-13395	B06			284	296		
90210-13396	C06	272	330	272	328	000	002
90210-13398	D06			260	280		
90210-13399	E06			308	316		
90210-13400	F06			328	348		
90210-13401	G06			272	392		
90210-13402	H06			296	372		

Table 2 continued.

Sample#	Plate Well	WDFW Ots-G422a	WDFW Ots-G422b	OtsG422_1	OtsG422_2	Diff	Diff
90210-13407	A07	289	346	288	344	001	002
90215-13962	B07			328	372		
90215-13963	C07			224	300		
90215-14031	D07			280	328		
90215-14032	E07			332	356		
90215-14034	F07			300	352		
90215-14035	G07			268	352		
90215-14036	H07			252	340		
90215-14037	A08	285	293	284	292	001	001
90215-14113	B08			268	308		
90215-14114	C08			276	316		
90215-14115	D08			296	320		
90215-14116	E08			304	352		
90215-14117	F08			268	356		
90215-14174	G08			276	336		
90215-14175	H08			300	368		
90215-14176	A09	276	285	276	284	000	001
90215-14177	B09			320	328		
90215-14178	C09			296	304		
90215-14179	D09			332	348		
90215-14181	E09			268	348		
90215-14182	F09			308	308		
90215-14183	G09	228	330	228	328	000	002
90215-14457	H09			328	344		
90215-14505	A10	281	346	280	344	001	002
90215-14618	B10			292	356		
90215-14625	C10			272	332		
90215-14639	D10			328	332		
90215-14640	E10			292	320		
90215-14661	F10			356	364		
90253-17700	G10			252	300		
90253-17703	H10			304	356		
90253-17704	A11			296	300		
90253-17712	B11	289	390	288	388	001	002
90253-17713	C11	342	354	340	352	002	002
90253-17714	D11	285	322	284	320	001	002
90253-17715	E11	276	318	276	316	000	002
90253-17836	F11	334	338	332	336	002	002
90253-17837	G11	285	313	284	312	001	001
90253-17838	H11			268	304		
90253-17866	A12			280	300		
90253-17867	B12	301	301	300	300	001	001
90253-17868	C12	289	297	288	296	001	001
90253-17869	D12			340	356		
90253-17871	E12	313	313	312	312	001	001
90253-17872	F12	330	418	328	416	002	002
90253-17893	G12	276	305	276	304	000	001
90253-17995	H12	326	342	324	340	002	002

Table 2 continued.

Sample#	Plate Well	WDFW p53a	WDFW p53b	P53_1	P53_2	Diff	Diff
90210-13228	A01			179	181		
90210-13229	B01			177	181		
90210-13231	C01			163	177		
90210-13232	D01			163	169		
90210-13233	E01			169	177		
90210-13234	F01			163	171		
90210-13235	G01			175	177		
90210-13239	H01			177	181		
90210-13241	A02	178	182	177	181	001	001
90210-13294	B02	178	182	177	181	001	001
90210-13295	C02	182	184	181	183	001	001
90210-13300	D02	172	178	171	177	001	001
90210-13348	E02			165	185		
90210-13349	F02			165	181		
90210-13350	G02	178	182	177	181	001	001
90210-13351	H02			169	177		
90210-13352	A03	163	195	163	193	000	002
90210-13353	B03	176	178	175	177	001	001
90210-13358	C03			181	183		
90210-13371	D03			169	171		
90210-13373	E03	172	178	171	177	001	001
90210-13374	F03			163	177		
90210-13375	G03			177	181		
90210-13377	H03			163	181		
90210-13378	A04	178	184	177	183	001	001
90210-13379	B04	172	172	171	171	001	001
90210-13380	C04			177	181		
90210-13381	D04	163	182	163	181	000	001
90210-13382	E04			169	171		
90210-13383	F04	172	178	171	177	001	001
90210-13384	G04			169	183		
90210-13385	H04	178	182	177	181	001	001
90210-13386	A05	180	182	179	181	001	001
90210-13387	B05	182	197	181	195	001	002
90210-13388	C05			163	185		
90210-13389	D05	172	182	171	181	001	001
90210-13390	E05	170	182	169	181	001	001
90210-13391	F05	178	180	177	179	001	001
90210-13392	G05			177	181		
90210-13393	H05	182	184	181	183	001	001
90210-13394	A06	178	182	177	181	001	001
90210-13395	B06			163	169		
90210-13396	C06	178	182	177	181	001	001
90210-13398	D06	170	178	169	177	001	001
90210-13399	E06	184	184	183	183	001	001
90210-13400	F06			169	177		
90210-13401	G06			181	181		
90210-13402	H06	163	172	163	171	000	001

Table 2 continued.

Sample#	Plate Well	WDFW p53a	WDFW p53b	P53_1	P53_2	Diff	Diff
90210-13407	A07	178	182	177	181	001	001
90215-13962	B07			161	167		
90215-13963	C07	182	195	181	193	001	002
90215-14031	D07	178	187	177	185	001	002
90215-14032	E07	178	184	177	183	001	001
90215-14034	F07	172	172	171	171	001	001
90215-14035	G07			169	181		
90215-14036	H07			177	177		
90215-14037	A08	174	174	173	173	001	001
90215-14113	B08			177	193		
90215-14114	C08	178	180	177	179	001	001
90215-14115	D08	163	178	163	177	000	001
90215-14116	E08	182	184	181	183	001	001
90215-14117	F08	170	182	169	181	001	001
90215-14174	G08	178	182	177	181	001	001
90215-14175	H08	182	184	181	183	001	001
90215-14176	A09	182	182	181	181	001	001
90215-14177	B09	163	178	163	177	000	001
90215-14178	C09	174	178	173	177	001	001
90215-14179	D09	178	180	177	179	001	001
90215-14181	E09	170	178	169	177	001	001
90215-14182	F09	170	178	169	177	001	001
90215-14183	G09	163	172	163	171	000	001
90215-14457	H09			177	179		
90215-14505	A10	172	178	171	177	001	001
90215-14618	B10	180	184	179	183	001	001
90215-14625	C10	178	187	177	185	001	002
90215-14639	D10			169	177		
90215-14640	E10	172	182	171	181	001	001
90215-14661	F10			171	183		
90253-17700	G10	182	182	181	181	001	001
90253-17703	H10	178	182	177	181	001	001
90253-17704	A11	178	184	177	183	001	001
90253-17712	B11	172	178	171	177	001	001
90253-17713	C11	178	182	177	181	001	001
90253-17714	D11	172	184	171	183	001	001
90253-17715	E11	163	178	163	177	000	001
90253-17836	F11	170	172	169	171	001	001
90253-17837	G11	178	182	177	181	001	001
90253-17838	H11			175	177		
90253-17866	A12	172	187	171	185	001	002
90253-17867	B12	163	182	163	181	000	001
90253-17868	C12	184	195	183	193	001	002
90253-17869	D12			169	179		
90253-17871	E12	170	191	169	189	001	002
90253-17872	F12			169	177		
90253-17893	G12			163	169		
90253-17995	H12	182	195	181	193	001	002

Table 3. Lookup table for converting coho data from WDFW allele naming to standardized naming.

Lower Bounds	Upper Bounds	WDFW Ocl-8	Ocl-8
97.72	98.27	98	98
101.83	102.77	102	102
104.02	104.49	104	104
106.19	106.8	106	106
108.19	108.91	108	108
110.07	110.61	110	110
112.28	112.71	112	112
114.55	114.81	115	114
116.41	116.63	117	116
118.44	119.06	119	118
120.76	120.93	121	120
122.25	123.23	123	122
124.81	124.94	125	124
126.51	127.04	127	126
129.14	129.4	129	128
133.19	133.34	133	132
135.45	135.46	135	134

Lower Bounds	Upper Bounds	WDFW Oki-1	Oki-1
90.7	90.71	91	90
94.4	94.78	95	94
98.59	98.99	99	98
102.62	102.95	103	102
106.71	107.19	107	106
110.69	111.06	111	110
114.88	115.27	115	114
119.08	119.22	119	118
123.16	123.29	123	122
127.31	127.55	127	126
131.54	131.58	132	130
135.83	136.09	136	134
140.16	140.5	140	138
144.82	145.1	145	142
157.84	157.85	158	154
161.83	161.84	162	158

Table 3 continued.

Lower Bounds	Upper Bounds	WDFW Omy-1011	Omy-1011
179.36	179.64	179	178
183.16	183.65	183	182
187.05	187.57	187	186
191.13	191.47	191	190
195.3	195.31	195	194
199.03	199.2	199	198
202.77	202.78	203	202
210.46	210.58	211	210

Lower Bounds	Upper Bounds	WDFW One-13M	One-13
192.39	193.1	193	151
194.92	194.93	195	153
198.35	198.36	198	157
200.34	200.94	201	159
202.47	202.92	203	161
204.89	204.9	205	163
206.33	206.76	207	165
208.5	208.84	209	167
212.46	212.59	213	171
214.16	214.83	215	173
216.42	216.8	217	175
218.39	218.65	218	177
220.24	220.71	221	179
222.59	222.74	223	181
224.47	224.48	224	183
226.31	226.7	226	185

Table 3 continued.

Lower Bounds	Upper Bounds	WDFW Ots-103	Ots-103
84.83	84.84	85	77
88.77	88.96	89	81
92.75	92.86	93	85
96.74	96.9	97	89
100.82	101.01	101	93
104.6	104.99	105	97
108.68	108.74	109	101
112.41	112.66	113	105
116.57	116.64	117	109
120.37	120.56	120	113
124.41	124.79	125	117
128.37	128.55	128	121
132.6	132.77	133	125
136.62	136.91	137	129
141.08	141.09	141	133
145.38	145.51	145	137
149.82	150.01	150	141
153.9	154.18	154	145
157.88	158.1	158	149
161.8	162	162	153
165.84	165.92	166	157
169.67	169.93	170	161
173.74	173.95	174	165
177.69	177.88	178	169
181.69	181.79	182	173
185.56	185.74	186	177
189.53	189.65	190	181
193.37	193.38	193	185
197.37	197.51	197	189
201.25	201.26	201	193
205.28	205.29	205	197
208.97	209.15	209	201
213.1	213.11	213	205
216.84	217.04	217	209
224.78	224.79	225	217
232.54	232.55	233	225
236.33	236.61	237	229
240.39	240.4	240	233
248.13	248.14	248	241
252.06	252.07	252	245
287.52	287.53	288	281
291.33	291.34	291	285

Lower Bounds	Upper Bounds	WDFW Ots-213	Ots-213
182.33	183.28	183	155
186.42	186.88	187	159
190.29	190.56	190	163
197.73	197.8	198	171
209.33	209.34	209	183
226.37	226.54	226	155
230.34	230.35	230	159
241.43	241.44	241	171
248.59	248.85	249	223
256.31	256.32	256	231
260.48	260.49	260	235
264.42	264.56	264	239
268.01	268.09	268	159
271.86	271.87	272	247
275.82	275.83	276	251
287.7	287.71	288	263
295.57	295.58	296	271
299.52	299.53	300	275
307.61	307.62	308	283
311.77	312.8	312	243
315.65	315.66	316	291

Table 3 continued.

Lower Bounds	Upper Bounds	WDFW Ots-G422	Ots-G422
228.03	228.04	228	228
268.40	268.41	268	268
272.29	272.54	272	272
276.36	276.57	276	276
280.52	280.59	281	280
284.46	284.75	285	284
288.44	288.70	289	288
292.70	292.80	293	292
296.79	296.80	297	296
300.82	301.10	301	300
304.83	305.10	305	304
309.15	309.16	309	308
313.26	313.42	313	312
317.50	317.51	318	316
321.47	321.68	322	320
325.89	325.90	326	324
329.72	329.92	330	328
333.85	334.18	334	332
338.29	338.30	338	336
342.32	342.37	342	340
346.30	346.40	346	344
354.20	354.27	354	352
365.95	365.96	366	364
373.99	374.00	374	372
389.60	389.61	390	388
413.95	413.96	414	412
418.10	418.11	418	416

Lower Bounds	Upper Bounds	WDFW p53	P53
163.37	163.75	163	163
169.58	169.9	170	169
171.69	172.22	172	171
173.75	173.93	174	173
175.94	175.95	176	175
177.94	178.34	178	177
180.01	180.35	180	179
182.12	182.56	182	181
184.28	184.69	184	183
186.33	186.81	187	185
190.77	190.78	191	189
194.74	195.06	195	193
196.85	196.86	197	195

Appendix B: Financial Statement of Expenditures

The following table lists the amount of funds spent and the budget that was originally submitted with the proposal for this project. Five thousand dollars that had originally been budgeted for additional Information Technology Specialist labor was instead used to purchase software licensing to maintain the database which maintains the data created by this project. Also, $1,000 had originally been budgeted for travel for the project lead, but no travel was needed to complete the project. Those funds were instead applied to expenses incurred for supplies.

PSC Southern Boundary Restoration & Enhancement Fund

Contract #: SF-2008-I-4 20-May-2009

Title: Allele Ladder Based Standardization of existing coho salmon microsatellite data and implications in GAPS

PI: Don Van Doornik

Item	Description	Amount spent	Budgeted amount
Labor	Technical level Molecular Geneticist - 240 hours to conduct lab work Technical level Molecular Geneticist - 240 hours to conduct lab work and coordinate project Information Technology Specialist - 240 hours to carry out database work	$33,700	$39,876
Contractual Services	To OSU for allele ladder standardization	$15,000	$15,000
	To WDFW for allele ladder standardization	$15,000	$15,000
	To IT Department for Oracle database maintenance	$5,000	$0
Interagency Agreement	To USFWS for allele ladder standardization	$15,000	$15,000
Interagency Transfer	To SWFSC for allele ladder standardization	$15,000	$15,000

Supplies & Materials	To cover "in house" lab costs	$15,255	$12,924
Travel	To cover travel for project lead	$0	$1,000
Overhead (indirect costs)	NOAA, NMFS, NWFSC, GSA rent	$18,045	$18,200
TOTAL		$132,000	$132,000

I certify, to the best of my knowledge, that the above expenditures accurately reflect how the funds in question were expended.

Signature: Paul Aebersold Title: Division Coordinator Date: 6/7/2009