Progress Report to Southern Fund for Phase I of Coho Salmon GSI: SNP baseline development
Year 1 of 4
Contract: SF-2019-SP-7A

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Background

We proposed the development of a bilateral CAN-US Coho Salmon single nucleotide polymorphism (SNP) genetic baseline as the foundation for identifying Coho Salmon stocks in mixed stock fisheries. In Phase I of the project, DFO and WDFW will screen the 484 SNP loci in use at the DFO lab in a common set of 190 tissue samples to identify loci that work on the WDFW SNP genotyping platform and to confirm that the genotyping platforms in the two labs produce identical genotypes. These loci will constitute the West Coast Coho SNP panel. Both labs use amplicon sequencing methods (WDFW uses GTseq and DFO uses Ion Torrent technology), and while the data produced by both platforms are compatible, the technologies differ, and locus combinations must be worked out independently. In Phase II, tissue samples will be collected from 60 representative stocks of Coho Salmon from southern British Columbia (SBC, N=30) and Washington State (WA, N=30) and genotyped with the West Coast Coho SNP panel. This will establish the core of the West Coast Coho Salmon bilateral SNP baseline. Because of advances in sequencing technology and the economy of SNP genotyping, DFO and WDFW labs have shifted infrastructure to SNP genotyping and the existing Coho microsatellite baseline is obsolete. Further, no comprehensive Coho Salmon baseline exists for US stocks. DFO and WDFW, along with supportive tribal, academic, and federal partners, seek to generate Coho Salmon SNP data that can be shared directly and develop a collaborative coastwide Coho Salmon genetic baseline. The primary application of the SNP baseline is to identify the origins and contributions of Coho Salmon populations in Southern Boundary Region mixed fisheries to support international and local Coho Salmon fisheries management and conservation. The same SNP panel would have many other uses, for example, for parent-based tagging to identify hatchery and wild interactions, and as a conservation tool.

In Phase I of this project, WDFW was tasked with converting DFO's Coho Salmon SNP panel from Ion Torrent sequencing technology (Beacham et al. 2017) to GTseq genotyping which employs Illumina as the sequencing platform (Campbell et al. 2015). GTseq is widely used by government and academic laboratories in the Pacific Northwest. WDFW currently uses a Coho Salmon GTseq panel that was designed by Columbia River Inter-Tribal Fish Commission (CRITFC) and ascertained with only lower Columbia River Coho Salmon populations. DFO's Coho Salmon SNP panel includes some SNPs from

CRITFC's panel, plus additional SNPs ascertained using collections from a broad spatial range, including populations from Washington State and southern BC, and thus may provide better information on population structure and improved genetic stock identification.

Phase I was broken down into four main steps and the completion of each step is reported on below:

Step 1: Purchase primers for DFOs panel:

After submission of the original proposal, DFO dropped a locus from their panel, thus all work in Phase I was based on the DFO panel of 483 SNP loci. The complete list of loci in the DFO panel and respective SNPs are listed in Table 1. In order to maximize efficiency and assess how all loci in the panel would amplify in a pool, the entire panel of 483 loci was screened through BatchPrimer3 (You et al. 2008). This program takes sequence data and corresponding SNP positions and designs PCR primers with similar sizes and melting temperatures, producing similar amplicon sizes while minimizing deleterious interactions between primers such as primer dimers and mispriming. Of DFO's original 483 loci, 454 loci were identified by BatchPrimer3 as candidates for pooled amplification. We added the necessary small RNA subunit adapter to the forward and reverse primer sequences for GTseq sequencing capabilities and ordered primers for 454 loci.

Step 2: Develop *in silico* probes for use in the GTseq genotyping protocol:

WDFW used the sequence data provided by DFO to create *in silico* probes that surround the SNP by 7 base pairs on either side for each locus. These *in silico* probes are used post-sequencing to obtain genotypes from amplicon sequencing data. A series of perl scripts are then used to identify and count each probe, and by proxy each SNP, as part of the GTseq genotyping pipeline (https://github.com/GTseq/GTseq-Pipeline).

Step 3: Choose collections to use in concordance testing between DFO and WDFW labs (i.e. one 96-well plate from each lab for a total of 190 indviduals):

WDFW sent DFO a 96-well plate of samples to be run on their Ion Torrent 483 SNP coho panel. This plate consisted of 48 Coho Salmon samples taken from Coho Salmon in the Upper Skagit River basin in Washington State, USA (WDFW collection code 17PT) and 48 samples taken from Coho Salmon in the Stillaguamish River basin in Washington State, USA (WDFW collection code 17LB). DFO sent WDFW a 96-well plate of samples from Robertson Creek Hatchery in Port Alberni, BC (WDFW collection code 15UF) (Table 2). These collections are from population aggregates used in Coho Salmon management.

Step 4: Run test genotyping with general PCR and sequencing parameters. Eliminate SNP loci that amplify or otherwise perform poorly. Re-genotype test plates with reduced SNP panel. Repeat until only SNP loci that amplify or otherwise perform satisfactorily remain in the panel. Additional steps may be taken to ensure that the species ID marker and sex ID marker remain in the panel:

WDFW ran three rounds of sequencing optimization to create the final GTseq panel. In the first round of optimization, loci were eliminated from the panel if they had two or more occurrences of the following scenarios: outliers in on-target ratios of the probe/forward primer reads, poor on-target priming of the forward primer, double-complement primer-artifacts, and forward and reverse primer mis-primes. Based on these criteria, 44 loci were dropped from further consideration and the remaining 410 loci were pooled

for round two of optimization. In the second round of optimization, potential paralogs and loci with poor separation of genotypes by read count were removed. This was done by plotting the allele 1 vs allele 2 count distribution relative to the allele ratio thresholds used to call genotypes a homozygote, heterozygote, or no call. If the ratio of allele counts is largely, but not completely, skewed to one homozygote genotype, this may be an indication of a paralogous locus. Loci with allele count ratios that fall outside of ranges expected for homozygotes or heterozygotes may be difficult to score accurately even if they are not paralogous. In addition, read depth outliers (e.g., loci with extremely high read counts) and loci with low on-target amplification were also dropped from further consideration. In total, an additional 40 loci were removed during round two of optimization leaving 370 loci.

The locus OkiOts_120255_113 (Table 1) is used by DFO for species identification by evaluating 15 different variable sites within the amplicon rather than a single SNP. Because the original amplicon length (212 bp) exceeds the capabilities of Illumina technology for efficient GTseq genotyping this locus was dropped from further consideration. Testing of WDFW's Chinook Salmon GTseq panel on non-Chinook salmonid species revealed a high rate of amplification failure and very high homozygosity in loci that did amplify thus allowing identification of non-Chinook. A similar test will be undertaken with the Coho Salmon GTseq panel to discover if non-Coho can be identified the same way.

A sex ID marker was identified in the DFO panel, Ots_SEXY3_1 (Table 1). This marker was originally ascertained in Chinook Salmon and did not amplify as part of the Coho Salmon GTseq panel. However, CRITFC recently designed two sex ID loci specific to Coho Salmon. These loci were designed from whole genome sequencing and genome-wide association tests that identified a region on Coho Salmon Chromosome 30 as significantly correlated with sex. Primers were designed for the two loci, Oki30_5473492 and Oki30_5551409, where heterozygotes are males and homozygotes are females for both loci (Horn et al. in review). These two loci were pooled with the rest of the new GTseq panel and amplified well. The final GTseq Coho Salmon panel (Oki.372) thus consists of 372 loci, 370 of which are in the DFO 483 SNP panel and the remaining two are sex ID loci (Table 1).

To date, WDFW has genotyped both plates of DNA (Table 2) at the Oki.372 GTseq panel and has received genotypes from DFO for the Robertson Creek Canadian samples. WDFW has not yet received genotypes for the Coho Salmon from the Skagit and Stillaguamish rivers sent to DFO. The delay in concordance genotyping is due to laboratory closure in response to the SARS CoV-2 coronavirus pandemic. In an effort to avoid further delays, we proceeded by comparing genotypes of the 96 Robertson Creek Hatchery Coho Salmon samples that have been genotyped by both labs.

Concordance was checked using two algorithms. The first algorithm is built into the GTseq genotyping pipeline and checks for duplicates and concordance between a subset of samples genotyped twice, first as a regular member of a project and second processed separately a second time as positive controls. The second algorithm calculates an allele sharing distance (ASD) using a script developed in-house at WDFW. ASD is essentially an allele sharing metric produced by making pairwise allele comparisons among all individuals of a batch of samples. Based on experiments in the WDFW lab, genotype concordance of the same individual genotyped twice at any of our GTseq SNP panels should be 97% or greater, with a few mismatching loci typically due to differences in minimum coverage thresholds across loci and individuals from sequencing run-to-run. Both the GTseq and ASD algorithms showed less than 100% concordance. The GTseq duplicate check provides a percent alike value for each individual; the

same samples genotyped at the DFO and WDFW panel had a percent alike value between 72% and 90%. The ASD between the same individuals (DFO panel compared to WDFW panel) were much lower (average ASD = 0.028) than the distance between two different individuals (average ASD = 0.208), but still greater than expected between the same individuals (same individual genotyped twice at the WDFW panel; average ASD = 0.005). Investigation by both labs revealed that a high number of genotype mismatches were a result of incorrect heterozygote calls made by the default method used in the Ion Torrent Variant Caller pipeline. When DFO generated genotypes using the same allele ratio methods as GTseq there was demonstrated improvement in concordance. GTseq uses a more stringent filtering method to exclude intermediary genotypes where poor separation between a homozygote and heterozygote genotype occurs. As a result, the majority of original mismatches between WDFW and DFO shift to no genotype called for both pipelines (Table 3).

There are 11 loci where a relatively high number of mismatches remain (Table 3), even after parameter adjustment in the Ion Torrent genotype pipeline. These are loci that amplify poorly in both panels (Oki_RAD42356_106) or still have discrepancies in heterozygote vs. homozygote calls between the two labs (10 loci). In order to resolve these mismatches, several actions will be taken. First, the flanking sequence surrounding these loci, from both labs, will be confirmed and compared to identify any discordance. In addition, we will compare major to minor allele ratios for mismatched genotypes in an attempt to identify the presence of null alleles. If discrepancies cannot be resolved, these markers will be dropped from the WDFW GTseq SNP panel.

For the majority of the 370 shared loci there is reliable concordance between genotypes generated from the DFO 483 SNP Ion Torrent panel and the WDFW Oki.372 SNP GTseq panel. After additional analysis for the 11 remaining unresolved mismatches we will be in a good position to begin Year 1 of Phase II. We anticipate further analysis to be completed in a timely fashion. In the meantime, discussions regarding selection of 60 representative stocks of Coho Salmon from southern British Columbia and Washington State can be initiated.

Acknowledgements

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References cited

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Table 1. Loci and their respective SNPs in DFO and WDFW coho panels.

DFO 483 SNP Ion Torrent Panel	Present in WDFW Oki.372 SNP GTseq Panel	SNP	Comment
Oki100771_83	Yes	T/C	
Oki100884_210	Yes	A/C	
Oki100974_293	Yes	G/T	
Oki101419_103	Yes	A/C	
Oki101770_525	Yes	A/C	
Oki102267_166	Yes	A/T	
Oki102414_499	Yes	A/T	
Oki102801_511	Yes	T/C	
Oki102867_667	Yes	A/C	
Oki103271_161	Yes	G/A	
Oki103577_70	Yes	A/T	
Oki103713_182	Yes	G/A	
Oki104515_99	Yes	G/T	
Oki104519_45	Yes	T/C	
Oki104569_261	Yes	G/T	
Oki105105_245	Yes	G/T	
Oki105115_49	Yes	G/T	
Oki1051321_169	Yes	T/C	
Oki105235_460	Yes	G/T	
Oki106172_60	Yes	T/C	
Oki106313_353	Yes	G/A	
Oki106419_292	Yes	G/T	
Oki106479_278	Yes	G/A	
Oki107336_45	Yes	A/C	
Oki107607_213	Yes	G/A	
Oki107974_46	Yes	G/A	
Oki109525_359	Yes	G/A	
Oki109651_152	Yes	A/T	
Oki109874_122	Yes	A/C	
Oki109894_418	Yes	A/C	
Oki110078_191	Yes	G/T	
Oki110381_77	Yes	G/A	
Oki111681_407	Yes	A/T	
Oki113457_324	Yes	G/A	
Oki114315_360	Yes	G/A	

DFO 483 SNP Ion Torrent Panel	Present in WDFW Oki.372 SNP GTseq Panel	SNP	Comment
Oki114448_101	Yes	T/C	
Oki114587_309	Yes	G/T	
Oki116362_411	Yes	A/T	
Oki116865_244	Yes	G/A	
Oki117043_374	Yes	T/C	
Oki117144_64	Yes	T/C	
Oki117815_369	Yes	G/T	
Oki118152_314	Yes	G/A	
Oki118654_330	Yes	T/C	
Oki120024_226	Yes	G/T	
Oki122593_430	Yes	G/A	
Oki123205_88	Yes	G/A	
Oki123470_92	Yes	G/A	
Oki123921_90	Yes	G/A	
Oki125998_324	Yes	G/T	
Oki126160_142	Yes	CT/TG	
Oki127760_301	Yes	G/A	
Oki128302_547	Yes	G/A	
Oki128851_185	Yes	G/A	
Oki129870_552	Yes	G/A	
Oki130295_48	Yes	G/A	
Oki130524_184	Yes	T/C	
Oki131460_243	Yes	A/C	
Oki131802_368	Yes	G/A	
Oki94903_192	Yes	A/T	
Oki95318_100	Yes	T/C	
Oki96158_278	Yes	T/C	
Oki96222_70	Yes	G/T	
Oki97954_228	Yes	A/C	
Okiafp410_66	Yes	T/C	
Okiarp_105	Yes	T/C	
OkiaspAT_273	Yes	T/C	
OkibcAKal_274	Yes	A/T	
Okicarban_107	Yes	A/T	
oki_epic4_001	Yes	A/G	
oki_epic4_002	Yes	A/G	
oki_epic4_003	Yes	G/A	
oki_epic4_004	Yes	G/A	
oki_epic4_005	Yes	A/T	
oki_epic4_006	Yes	G/T	
oki_epic4_008	Yes	T/G	

DFO 483 SNP Ion Torrent Panel	Present in WDFW Oki.372 SNP GTseq Panel	SNP	Comment
oki_epic4_009	Yes	A/G	
oki_epic4_010	Yes	C/T	
oki_epic4_011	Yes	C/T	
oki_epic4_014	Yes	C/T	
oki_epic4_019	Yes	G/A	
oki_epic4_020	Yes	A/T	
oki_epic4_021	Yes	G/A	
oki_epic4_024	Yes	T/C	
oki_epic4_026	Yes	T/G	
oki_epic4_028	Yes	C/T	
oki_epic4_029	Yes	C/G	
oki_epic4_030	Yes	G/A	
oki_epic4_031	Yes	A/G	
oki_epic4_032	Yes	G/A	
oki_epic4_033	Yes	A/G	
oki_epic4_036	Yes	A/G	
oki_epic4_037	Yes	C/G	
oki_epic4_038	Yes	A/G	
oki_epic4_041	Yes	G/A	
oki_epic4_042	Yes	T/A	
oki_epic4_043	Yes	T/A	
oki_epic4_044	Yes	A/C	
oki_epic4_046	Yes	T/C	
oki_epic4_047	Yes	A/C	
oki_epic4_048	Yes	G/T	
oki_epic4_049	Yes	C/T	
oki_epic4_050	Yes	C/G	
oki_epic4_051	Yes	A/G	
oki_epic4_052	Yes	C/T	
oki_epic4_053	Yes	A/C	
oki_epic4_054	Yes	G/C	
oki_epic4_056	Yes	T/C	
oki_epic4_057	Yes	C/T	
oki_epic4_058	Yes	T/G	
oki_epic4_059	Yes	T/A	
oki_epic4_061	Yes	A/T	
oki_epic4_063	Yes	G/A	
oki_epic4_066	Yes	T/C	
oki_epic4_068	Yes	T/A	
oki_epic4_070	Yes	A/G	
oki_epic4_072	Yes	T/C	

DFO 483 SNP Ion Torrent Panel	Present in WDFW Oki.372 SNP GTseq Panel	SNP	Comment
oki_epic4_073	Yes	A/G	
oki_epic4_074	Yes	C/T	
oki_epic4_075	Yes	A/G	
oki_epic4_076	Yes	G/A	
oki_epic4_082	Yes	G/A	
oki_epic4_083	Yes	A/C	
oki_epic4_084	Yes	G/T	
oki_epic4_085	Yes	T/C	
oki_epic4_086	Yes	G/T	
oki_epic4_087	Yes	T/A	
oki_epic4_088	Yes	C/A	
oki_epic4_090	Yes	A/G	
oki_epic4_091	Yes	A/T	
oki_epic4_094	Yes	C/A	
oki_epic4_095	Yes	A/T	
oki_epic4_096	Yes	G/A	
oki_epic4_097	Yes	C/T	
oki_epic4_098	Yes	T/G	
oki_epic4_101	Yes	T/A	
oki_epic4_103	Yes	T/C	
oki_epic4_105	Yes	C/T	
oki_epic4_107	Yes	C/T	
oki_epic4_109	Yes	C/T	
oki_epic4_110	Yes	T/C	
oki_epic4_111	Yes	C/A	
oki_epic4_112	Yes	G/A	
oki_epic4_116	Yes	T/G	
oki_epic4_118	Yes	C/T	
oki_epic4_119	Yes	G/A	
oki_epic4_120	Yes	A/C	
oki_epic4_121	Yes	A/G	
oki_epic4_122	Yes	G/A	
oki_epic4_123	Yes	G/A	
oki_epic4_124	Yes	G/A	
oki_epic4_127	Yes	T/C	
oki_epic4_128	Yes	C/T	
oki_epic4_132	Yes	C/T	
oki_epic4_133	Yes	G/A	
oki_epic4_134	Yes	A/G	
oki_epic4_135	Yes	T/A	
oki_epic4_136	Yes	A/G	

DFO 483 SNP Ion Torrent Panel	Present in WDFW Oki.372 SNP GTseq Panel	SNP	Comment
oki_epic4_139	Yes	G/T	
oki_epic4_143	Yes	T/G	
oki_epic4_144	Yes	T/A	
oki_epic4_145	Yes	T/G	
oki_epic4_146	Yes	T/A	
oki_epic4_148	Yes	T/G	
oki_epic4_149	Yes	A/T	
oki_epic4_154	Yes	G/A	
oki_epic4_155	Yes	C/G	
oki_epic4_157	Yes	G/T	
oki_epic4_159	Yes	A/G	
oki_epic4_160	Yes	G/T	
oki_epic4_163	Yes	C/T	
oki_epic4_165	Yes	C/T	
oki_epic4_166	Yes	T/G	
oki_epic4_169	Yes	A/C	
oki_epic4_170	Yes	C/T	
oki_epic4_172	Yes	C/T	
oki_epic4_174	Yes	T/G	
oki_epic4_176	Yes	A/T	
oki_epic4_178	Yes	C/T	
oki_epic4_179	Yes	A/T	
oki_epic4_180	Yes	C/T	
oki_epic4_181	Yes	T/G	
oki_epic4_182	Yes	G/A	
oki_epic4_183	Yes	T/C	
oki_epic4_189	Yes	C/T	
oki_epic4_190	Yes	A/T	
oki_epic4_193	Yes	A/C	
oki_epic4_195	Yes	C/T	
oki_epic4_196	Yes	G/T	
oki_epic4_206	Yes	G/A	
oki_epic4_208	Yes	A/C	
oki_epic4_209	Yes	G/A	
oki_epic4_210	Yes	C/T	
oki_epic4_214	Yes	T/C	
oki_epic4_216	Yes	A/G	
oki_epic4_218	Yes	C/T	
oki_epic4_219	Yes	C/T	
oki_epic4_220	Yes	C/T	
oki_epic4_221	Yes	T/C	

DFO 483 SNP Ion Torrent Panel	Present in WDFW Oki.372 SNP GTseq Panel	SNP	Comment
oki_epic4_222	Yes	G/T	
oki_epic4_225	Yes	A/T	
oki_epic4_226	Yes	A/C	
oki_epic4_227	Yes	C/A	
oki_epic4_229	Yes	C/T	
oki_epic4_230	Yes	T/G	
oki_epic4_232	Yes	T/A	
oki_epic4_233	Yes	T/C	
oki_epic4_235	Yes	C/T	
oki_epic4_236	Yes	C/G	
oki_epic4_237	Yes	G/A	
oki_epic4_239	Yes	C/T	
oki_epic4_240	Yes	T/C	
oki_epic4_244	Yes	T/C	
oki_epic4_247	Yes	A/C	
oki_epic4_248	Yes	T/C	
oki_epic4_249	Yes	T/C	
oki_epic4_250	Yes	C/T	
oki_epic4_255	Yes	T/C	
oki_epic4_258	Yes	A/G	
oki_epic4_259	Yes	G/T	
oki_epic4_260	Yes	C/T	
Okigdh189_132	Yes	A/T	
Okigh183_160	Yes	G/A	
Okigshpx152_132	Yes	A/C	
Okihsc71p313_136	Yes	G/T	
Okihsf1b_85	Yes	G/C	
Okihsp90B_83	Yes	A/T	
Okiitpa_85	Yes	G/T	
OkimapK3p_93	Yes	G/A	
Okinips_100	Yes	G/T	
Okipigh33_33	Yes	A/T	
Okipoop5265_175	Yes	G/T	
Oki_RAD100331_48	Yes	G/A	
Oki_RAD100507_58	Yes	T/G	
Oki_RAD101032_66	Yes	C/T	
Oki_RAD101136_60	Yes	A/G	
Oki_RAD101377_37	Yes	C/G	
Oki_RAD101607_49	Yes	C/T	
Oki_RAD102175_55	Yes	A/T	
Oki_RAD104180_61	Yes	G/T	

DFO 483 SNP Ion Torrent Panel	Present in WDFW Oki.372 SNP GTseq Panel	SNP	Comment
Oki_RAD104335_44	Yes	T/C	
Oki_RAD105692_35	Yes	T/G	
Oki_RAD106749_40	Yes	T/C	
Oki_RAD109706_63	Yes	C/T	
Oki_RAD111748_36	Yes	A/T	
Oki_RAD112734_32	Yes	T/C	
Oki_RAD115799_69	Yes	C/G	
Oki_RAD14549_57	Yes	G/A	
Oki_RAD15228_35	Yes	G/C	
Oki_RAD17843_62	Yes	G/T	
Oki_RAD23085_53	Yes	G/T	
Oki_RAD27801_45	Yes	A/T	
Oki_RAD345_59	Yes	A/G	
Oki_RAD35869_61	Yes	T/G	
Oki_RAD37037_59	Yes	T/G	
Oki_RAD37278_54	Yes	C/T	
Oki_RAD37493_51	Yes	A/G	
Oki_RAD37537_45	Yes	T/C	
Oki_RAD37979_59	Yes	G/T	
Oki_RAD39712_67	Yes	G/A	
Oki_RAD40740_40	Yes	T/C	
Oki_RAD41030_31	Yes	C/T	
Oki_RAD42227_43	Yes	T/G	
Oki_RAD42296_68	Yes	G/A	
Oki_RAD42356_106	Yes	T/G	
Oki_RAD42662_33	Yes	A/G	
Oki_RAD43051_33	Yes	G/A	
Oki_RAD44444_52	Yes	G/A	
Oki_RAD45691_45	Yes	G/T	
Oki_RAD45746_54	Yes	T/C	
Oki_RAD45971_66	Yes	A/G	
Oki_RAD46141_169	Yes	C/A	
Oki_RAD46160_48	Yes	C/G	
Oki_RAD46744_47	Yes	T/C	
Oki_RAD4695_38	Yes	A/G	
Oki_RAD47313_50	Yes	A/G	
Oki_RAD49257_48	Yes	C/G	
Oki_RAD49488_47	Yes	C/A	
Oki_RAD51585_47	Yes	T/G	
Oki_RAD51713_42	Yes	C/A	
Oki_RAD51912_66	Yes	A/T	

DFO 483 SNP Ion Torrent Panel	Present in WDFW Oki.372 SNP GTseq Panel	SNP	Comment
Oki_RAD53121_66	Yes	C/T	
Oki_RAD53155_32	Yes	A/C	
Oki_RAD53655_42	Yes	T/C	
Oki_RAD55090_49	Yes	A/C	
Oki_RAD55249_49	Yes	T/A	
Oki_RAD55327_41	Yes	A/T	
Oki_RAD55690_46	Yes	A/C	
Oki_RAD56094_43	Yes	G/T	
Oki_RAD58946_67	Yes	A/G	
Oki_RAD59556_32	Yes	T/C	
Oki_RAD62768_58	Yes	G/A	
Oki_RAD63267_39	Yes	A/G	
Oki_RAD65902_30	Yes	C/T	
Oki_RAD66265_54	Yes	T/C	
Oki_RAD66397_30	Yes	T/G	
Oki_RAD66663_68	Yes	A/G	
Oki_RAD66976_58	Yes	A/C	
Oki_RAD66994_58	Yes	G/A	
Oki_RAD68033_63	Yes	G/A	
Oki_RAD68125_68	Yes	G/C	
Oki_RAD68190_55	Yes	A/G	
Oki_RAD69161_64	Yes	T/G	
Oki_RAD70496_44	Yes	G/A	
Oki_RAD70600_60	Yes	A/G	
Oki_RAD70812_52	Yes	T/A	
Oki_RAD71346_48	Yes	G/A	
Oki_RAD71442_69	Yes	A/G	
Oki_RAD72460_47	Yes	G/A	
Oki_RAD72503_39	Yes	A/C	
Oki_RAD72979_40	Yes	G/A	
Oki_RAD73094_68	Yes	G/A	
Oki_RAD73130_59	Yes	C/T	
Oki_RAD73526_35	Yes	A/G	
Oki_RAD75608_42	Yes	G/T	
Oki_RAD75909_38	Yes	T/C	
Oki_RAD77210_64	Yes	A/T	
Oki_RAD77342_49	Yes	T/C	
Oki_RAD77803_60	Yes	G/A	
Oki_RAD78773_67	Yes	G/T	
Oki_RAD81387_37	Yes	G/A	
Oki_RAD85448_97	Yes	T/G	

DFO 483 SNP Ion Torrent Panel	Present in WDFW Oki.372 SNP GTseq Panel	SNP	Comment
Oki_RAD85949_47	Yes	G/A	
Oki_RAD86669_50	Yes	A/G	
Oki_RAD87446_62	Yes	A/G	
Oki_RAD87621_67	Yes	G/T	
Oki_RAD88551_51	Yes	A/G	
Oki_RAD88786_44	Yes	C/G	
Oki_RAD90772_47	Yes	T/C	
Oki_RAD91362_68	Yes	A/C	
Oki_RAD91470_66	Yes	C/A	
Oki_RAD91750_33	Yes	C/T	
Oki_RAD92243_63	Yes	A/G	
Oki_RAD92616_68	Yes	G/A	
Oki_RAD92875_31	Yes	C/T	
Oki_RAD92978_42	Yes	T/C	
Oki_RAD93028_59	Yes	G/A	
Oki_RAD94260_66	Yes	G/T	
Oki_RAD95780_30	Yes	G/A	
Oki_RAD96072_42	Yes	A/G	
Oki_RAD97168_52	Yes	A/T	
Oki_RAD97325_35	Yes	T/A	
Oki_RAD97903_41	Yes	T/G	
Oki_RAD98485_66	Yes	A/G	
Oki_RAD99298_47	Yes	T/A	
Oki_RAD99659_52	Yes	G/T	
Okirpo2j235_212	Yes	A/C	
OkiSClkF2R2_67	Yes	G/T	
Okiserpin328_119	Yes	TT/GA	
Okispf30119_107	Yes	G/A	
OkiSWS1op38_141	Yes	G/T	
Okiu6257_187	Yes	A/T	
Ots_106747_266	Yes	T/G	
Ots_108390_387	Yes	T/G	
Ots_130720_411	Yes	C/T	
Ots_CCR7_182	Yes	C/T	
Ots_crRAD20376_46	Yes	G/A	
Ots_crRAD21752_87	Yes	C/A	
Ots_crRAD27247_97	Yes	T/C	
Ots_crRAD30341_147	Yes	C/A	
Ots_crRAD32203_124	Yes	C/G	
Ots_crRAD33491_135	Yes	C/T	
Ots_crRAD53756_119	Yes	C/T	

DFO 483 SNP Ion Torrent Panel	Present in WDFW Oki.372 SNP GTseq Panel	SNP	Comment
Ots_crRAD57366_94	Yes	G/T	
Ots_crRAD73823_115	Yes	T/G	
Ots_FARSLA_224	Yes	T/C	
Ots_hnRNPL_58	Yes	A/T	
Ots_il_1racp_174	Yes	G/T	
Ots_nkef_148	Yes	G/C	
Ots_u202_249	Yes	A/T	
OkiOts_120255_113	No	A/G	species ID in DFO panel
Oki_RAD57956_47	No	G/C	
Oki_RAD60246_68	No	A/C	
Oki101554_359	No	G/A	
Oki108505_331	No	T/C	
Oki109243_480	No	G/A	
Oki117286_291	No	A/C	
Oki96127_66	No	G/T	
Okianp_168	No	T/C	
Oki_RAD102786_61	No	C/T	
Oki_RAD104779_63	No	C/G	
Oki_RAD107732_42	No	C/T	
Oki_RAD112339_67	No	T/G	
Oki_RAD20524_31	No	A/C	
Oki_RAD23108_39	No	A/G	
Oki_RAD27348_46	No	T/C	
Oki_RAD3702_58	No	T/C	
Oki_RAD37101_64	No	T/G	
Oki_RAD42611_52	No	C/T	
Oki_RAD43472_33	No	C/G	
Oki_RAD49978_70	No	G/T	
Oki_RAD49991_69	No	T/C	
Oki_RAD53705_68	No	A/T	
Oki_RAD62917_33	No	A/G	
Oki_RAD63468_42	No	C/A	
Oki_RAD65345_34	No	T/C	
Oki_RAD65466_31	No	C/T	
Oki_RAD66324_35	No	T/C	
Oki_RAD66922_64	No	C/A	
Oki_RAD68238_42	No	A/G	
Oki_RAD69779_33	No	C/T	
Oki_RAD76045_68	No	C/T	
Oki_RAD78988_32	No	T/C	
Oki_RAD8763_51	No	A/C	

DFO 483 SNP Ion Torrent Panel	Present in WDFW Oki.372 SNP GTseq Panel	SNP	Comment
Oki_RAD90555_50	No	G/A	
Oki_RAD90652_58	No	T/C	
Oki_RAD95534_53	No	C/T	
Oki_RAD9927_35	No	G/A	
Oki_RAD99813_32	No	C/A	
oki_epic4_007	No	G/T	
oki_epic4_012	No	A/C	
oki_epic4_013	No	T/C	
oki_epic4_015	No	G/A	
oki_epic4_016	No	C/T	
oki_epic4_017	No	A/G	
oki_epic4_022	No	C/T	
oki_epic4_023	No	T/G	
oki_epic4_034	No	T/A	
oki_epic4_039	No	T/C	
oki_epic4_060	No	A/G	
oki_epic4_062	No	C/T	
oki_epic4_065	No	C/A	
oki_epic4_067	No	C/A	
oki_epic4_071	No	C/T	
oki_epic4_078	No	A/G	
oki_epic4_079	No	C/T	
oki_epic4_089	No	C/T	
oki_epic4_092	No	G/A	
oki_epic4_093	No	T/A	
oki_epic4_114	No	A/G	
oki_epic4_115	No	T/A	
oki_epic4_117	No	C/T	
oki_epic4_131	No	G/T	
oki_epic4_140	No	C/T	
oki_epic4_141	No	T/C	
oki_epic4_147	No	C/G	
oki_epic4_161	No	A/T	
oki_epic4_162	No	C/T	
oki_epic4_167	No	T/C	
oki_epic4_171	No	G/A	
oki_epic4_173	No	T/A	
oki_epic4_175	No	G/A	
oki_epic4_184	No	G/T	
oki_epic4_185	No	T/C	
oki_epic4_187	No	G/T	

DFO 483 SNP Ion Torrent Panel	Present in WDFW Oki.372 SNP GTseq Panel	SNP	Comment
oki_epic4_188	No	A/G	
oki_epic4_191	No	T/C	
oki_epic4_192	No	G/T	
oki_epic4_194	No	A/C	
oki_epic4_197	No	C/G	
oki_epic4_198	No	A/G	
oki_epic4_203	No	T/C	
oki_epic4_215	No	C/G	
oki_epic4_223	No	G/A	
oki_epic4_224	No	A/G	
oki_epic4_228	No	A/C	
oki_epic4_231	No	C/T	
oki_epic4_234	No	A/C	
oki_epic4_241	No	C/G	
oki_epic4_242	No	A/C	
oki_epic4_243	No	G/A	
oki_epic4_246	No	C/T	
oki_epic4_251	No	T/A	
oki_epic4_256	No	G/A	
oki_epic4_257	No	T/C	
oki_epic4_261	No	G/C	
Ots_105401_242	No	G/C	
Ots_107806_821	No	A/T	
Ots_110201_250	No	C/A	
Ots_128693_382	No	C/T	
Ots_apoc1_832	No	A/G	
Ots_Chin30up_495	No	T/G	
Ots_crRAD17324_192	No	C/A	
Ots_crRAD17527_50	No	-/T	
Ots_crRAD18336_135	No	C/A	
Ots_crRAD44588_193	No	-/G	
Ots_crRAD46081_199	No	GGA/-	
Ots_crRAD57520_52	No	A/C	
Ots_mapKpr_377	No	A/C	
Ots_RAD3470_45	No	G/T	
Ots_SEXY3_1	No	TCAGCGAAGTGGAGAT/-	sex ID in DFO panel
Ots_TAPBP_579	No	A/C	
Ots_U5121_459	No	C/T	
NA	Oki30_5473492	C/T	sex ID in WDFW panel
NA	Oki30_5551409	T/C	sex ID in WDFW panel

Table 2. Collections and number of individuals genotyped as part of the concordance genotype testing between the DFO and WDFW coho panels.

WDFW collection code	Region	N
15UF	Robertson Creek, BC	96
17LB	Stillaguamish River, WA	31
17PT	Skagit River, WA	64

Table 3. A summary of genotype concordance between the DFO and WDFW coho panels.

	Original Parameters			**Updated DFO Parameters**		
Locus	Match	Mismatch	NA	Match	Mismatch	NA
oki_epic4_196	96	0	0	96	0	0
oki_epic4_010	96	0	0	96	0	0
oki_epic4_047	96	0	0	96	0	0
oki_epic4_008	96	0	0	96	0	0
oki_epic4_050	96	0	0	96	0	0
oki_epic4_038	96	0	0	96	0	0
oki_epic4_139	96	0	0	96	0	0
oki_epic4_132	96	0	0	96	0	0
oki_epic4_110	96	0	0	96	0	0
oki_epic4_219	95	1	0	96	0	0
oki_epic4_086	96	0	0	96	0	0
oki_epic4_095	96	0	0	96	0	0
oki_epic4_041	96	0	0	96	0	0
oki_epic4_239	96	0	0	96	0	0
oki_epic4_044	96	0	0	96	0	0
oki_epic4_120	96	0	0	96	0	0
oki_epic4_145	96	0	0	96	0	0
oki_epic4_085	96	0	0	96	0	0
oki_epic4_176	96	0	0	96	0	0
oki_epic4_004	96	0	0	96	0	0
oki_epic4_107	96	0	0	96	0	0
oki_epic4_109	96	0	0	96	0	0
oki_epic4_258	96	0	0	96	0	0
oki_epic4_222	96	0	0	96	0	0
oki_epic4_059	96	0	0	96	0	0
oki_epic4_237	96	0	0	96	0	0
oki_epic4_135	96	0	0	96	0	0
oki_epic4_143	95	1	0	96	0	0

Oki100771_83	96	0	0	96	0	0
Oki111681_407	96	0	0	96	0	0
Oki102867_667	96	0	0	96	0	0
Okihsp90B_83	96	0	0	96	0	0
Oki109894_418	96	0	0	96	0	0
Oki114315_360	96	0	0	96	0	0
Oki102267_166	96	0	0	96	0	0
Oki_RAD106749_40	96	0	0	96	0	0
Oki_RAD44444_52	96	0	0	96	0	0
Oki_RAD56094_43	96	0	0	96	0	0
oki_epic4_121	96	0	0	95	0	1
oki_epic4_221	95	0	1	95	0	1
oki_epic4_155	95	0	1	95	0	1
oki_epic4_144	95	0	1	95	0	1
oki_epic4_235	95	0	1	95	0	1
oki_epic4_068	95	0	1	95	0	1
oki_epic4_240	95	0	1	95	0	1
oki_epic4_159	96	0	0	95	0	1
oki_epic4_082	95	0	1	95	0	1
oki_epic4_019	95	1	0	95	0	1
oki_epic4_105	95	0	1	95	0	1
oki_epic4_112	95	0	1	95	0	1
oki_epic4_033	95	1	0	95	0	1
oki_epic4_157	96	0	0	95	0	1
oki_epic4_227	95	0	1	95	0	1
oki_epic4_179	95	0	1	95	0	1
Oki104519_45	95	0	1	95	0	1
Okipoop5265_175	95	0	1	95	0	1
Oki117043_374	95	0	1	95	0	1
Oki128302_547	95	0	1	95	0	1
Oki_RAD4695_38	93	2	1	95	0	1
Oki_RAD55249_49	96	0	0	95	0	1
Oki_RAD58946_67	96	0	0	95	0	1
Oki_RAD72503_39	95	0	1	95	0	1
Oki_RAD71346_48	95	0	1	95	0	1
Oki_RAD97325_35	96	0	0	96	0	0
Ots_130720_411	96	0	0	96	0	0
Ots_nkef_148	96	0	0	96	0	0
Ots_crRAD30341_147	96	0	0	96	0	0
Ots_crRAD33491_135	96	0	0	96	0	0
Ots_crRAD53756_119	96	0	0	96	0	0
oki_epic4_030	94	0	2	94	0	2
oki_epic4_247	94	0	2	94	0	2

oki_epic4_028	94	0	2	94	0	2
oki_epic4_021	95	0	1	94	0	2
oki_epic4_076	94	0	2	94	0	2
oki_epic4_048	94	1	1	94	0	2
oki_epic4_119	94	0	2	94	0	2
oki_epic4_098	95	0	1	94	0	2
oki_epic4_181	96	0	0	94	0	2
Oki107607_213	95	0	1	94	0	2
OkiSWS1op38_141	94	1	1	94	0	2
Oki100974_293	94	0	2	94	0	2
Oki100884_210	95	0	1	94	0	2
OkibcAKal_274	95	0	1	94	0	2
Oki113457_324	94	1	1	94	0	2
Oki123921_90	95	0	1	94	0	2
Okihsc71p313_136	94	1	1	94	0	2
Oki_RAD73130_59	94	1	1	94	0	2
Ots_108390_387	95	0	1	95	0	1
Ots_crRAD27247_97	95	0	1	95	0	1
oki_epic4_229	94	0	2	93	0	3
oki_epic4_006	94	1	1	93	0	3
oki_epic4_208	94	2	0	93	0	3
oki_epic4_087	95	0	1	93	0	3
oki_epic4_118	93	0	3	93	0	3
oki_epic4_091	93	0	3	93	0	3
oki_epic4_009	93	3	0	93	0	3
oki_epic4_084	94	1	1	93	0	3
oki_epic4_183	95	0	1	93	0	3
Oki109874_122	93	0	3	93	0	3
Oki105235_460	93	1	2	93	0	3
Oki_RAD35869_61	93	0	3	93	0	3
Oki_RAD37493_51	93	1	2	93	0	3
Oki_RAD40740_40	93	1	2	93	0	3
Oki_RAD63267_39	91	2	3	92	0	4
Oki_RAD86669_50	94	1	1	93	0	3
oki_epic4_037	93	0	3	93	0	3
Oki_RAD99298_47	93	1	2	94	0	2
Oki_RAD99659_52	94	0	2	94	0	2
Ots_106747_266	94	0	2	94	0	2
Ots_FARSLA_224	94	1	1	94	0	2
oki_epic4_214	92	1	3	92	0	4
oki_epic4_002	94	0	2	92	0	4
oki_epic4_073	92	2	2	92	0	4
oki_epic4_001	93	2	1	92	0	4

oki_epic4_236	96	0	0	92	0	4
oki_epic4_096	94	2	0	92	0	4
oki_epic4_094	93	1	2	92	0	4
oki_epic4_154	93	0	3	92	0	4
Oki114587_309	93	1	2	92	0	4
Oki101419_103	91	2	3	92	0	4
Okiitpa_85	93	0	3	92	0	4
OkiaspAT_273	92	1	3	92	0	4
Oki106479_278	93	1	2	92	0	4
Oki118654_330	92	2	2	92	0	4
Oki127760_301	93	0	3	92	0	4
Oki_RAD102175_55	95	1	0	92	0	4
Oki_RAD111748_36	92	2	2	92	0	4
Oki_RAD41030_31	92	3	1	92	0	4
Oki_RAD42296_68	92	0	4	92	0	4
Oki_RAD53655_42	92	0	4	92	0	4
Oki_RAD55327_41	92	2	2	92	0	4
Oki_RAD68033_63	92	1	3	92	0	4
Oki_RAD68125_68	94	1	1	92	0	4
Oki_RAD72460_47	93	1	2	92	0	4
Oki_RAD72979_40	93	1	2	92	0	4
Okiserpin328_119	92	0	4	92	0	4
oki_epic4_072	90	3	3	91	0	5
oki_epic4_075	93	1	2	91	0	5
oki_epic4_195	91	1	4	90	0	6
oki_epic4_128	91	0	5	91	0	5
oki_epic4_005	93	0	3	91	0	5
oki_epic4_070	91	0	5	91	0	5
oki_epic4_088	96	0	0	91	0	5
oki_epic4_074	92	2	2	91	0	5
oki_epic4_233	91	0	5	91	0	5
Oki105105_245	91	2	3	91	0	5
Oki114448_101	91	2	3	91	0	5
OkimapK3p_93	91	3	2	91	0	5
Oki123470_92	90	0	6	90	0	6
Okihsf1b_85	92	2	2	91	0	5
Oki95318_100	94	0	2	91	0	5
Oki103713_182	93	1	2	91	0	5
Oki_RAD112734_32	93	1	2	91	0	5
Oki_RAD345_59	92	1	3	91	0	5
Oki_RAD46744_47	94	0	2	91	0	5
Oki_RAD70600_60	92	3	1	91	0	5
Oki_RAD70812_52	92	1	3	91	0	5

Oki_RAD75909_38	91	2	3	91	0	5
Oki103271_161	92	1	3	91	0	5
Oki_RAD92616_68	95	1	0	92	0	4
oki_epic4_058	74	20	2	90	0	6
oki_epic4_165	91	2	3	90	0	6
oki_epic4_122	91	1	4	90	0	6
oki_epic4_180	91	1	4	90	0	6
oki_epic4_259	92	0	4	90	0	6
oki_epic4_225	92	0	4	90	0	6
oki_epic4_029	90	1	5	90	0	6
oki_epic4_169	92	1	3	90	0	6
oki_epic4_146	92	2	2	90	0	6
Oki131802_368	92	1	3	90	0	6
Oki102801_511	95	1	0	90	0	6
Oki118152_314	91	2	3	90	0	6
Oki131460_243	94	0	2	90	0	6
Oki107974_46	91	3	2	90	0	6
Oki_RAD17843_62	92	2	2	90	0	6
Oki_RAD27801_45	91	0	5	90	0	6
Oki_RAD46160_48	91	1	4	90	0	6
Oki_RAD65902_30	90	0	6	90	0	6
Okispf30119_107	88	5	3	90	0	6
oki_epic4_052	90	1	5	89	0	7
oki_epic4_031	91	0	5	89	0	7
oki_epic4_020	91	2	3	89	0	7
oki_epic4_193	90	1	5	89	0	7
Oki1051321_169	93	1	2	89	0	7
Oki97954_228	90	1	5	89	0	7
Oki117144_64	92	1	3	89	0	7
Okirpo2j235_212	90	1	5	89	0	7
Oki123205_88	90	1	5	89	0	7
Oki129870_552	89	3	4	89	0	7
Okinips_100	90	4	2	89	0	7
Oki_RAD100507_58	91	4	1	89	0	7
Oki_RAD115799_69	89	1	6	89	0	7
Oki_RAD49257_48	93	0	3	89	0	7
Oki_RAD51585_47	91	3	2	89	0	7
oki_epic4_090	90	2	4	88	0	8
oki_epic4_248	88	3	5	88	0	8
oki_epic4_124	88	1	7	88	0	8
oki_epic4_042	94	0	2	88	0	8
oki_epic4_046	89	3	4	88	0	8
Oki94903_192	90	2	4	88	0	8

Oki96222_70	89	0	7	88	0	8
Oki_RAD101377_37	90	4	2	88	0	8
Oki_RAD104180_61	88	0	8	88	0	8
Oki_RAD104335_44	89	4	3	88	0	8
Oki_RAD37278_54	91	4	1	88	0	8
Oki_RAD77803_60	92	1	3	88	0	8
Oki_RAD81387_37	88	5	3	87	0	9
Oki_RAD88551_51	89	2	5	89	0	7
Oki_RAD97903_41	91	2	3	89	0	7
Oki_RAD98485_66	89	1	6	89	0	7
Ots_crRAD73823_115	90	0	6	89	0	7
oki_epic4_160	87	0	9	87	0	9
Oki110078_191	91	1	4	87	0	9
OkiSClkF2R2_67	88	1	7	87	0	9
Oki110381_77	88	4	4	86	0	10
Oki109525_359	91	2	3	87	0	9
Okiafp410_66	90	2	4	86	0	10
Oki102414_499	89	1	6	87	0	9
Oki122593_430	87	1	8	87	0	9
Oki128851_185	87	8	1	87	0	9
Oki_RAD101136_60	88	4	4	87	0	9
Oki_RAD105692_35	89	1	6	87	0	9
Oki_RAD15228_35	89	3	4	87	0	9
Oki_RAD42227_43	88	2	6	87	0	9
Oki_RAD66994_58	88	4	4	87	0	9
Oki_RAD73094_68	88	4	4	87	0	9
Oki130295_48	91	2	3	87	0	9
oki_epic4_220	87	4	5	86	0	10
oki_epic4_127	88	2	6	86	0	10
oki_epic4_244	89	1	6	86	0	10
oki_epic4_136	89	2	5	86	0	10
Okiu6257_187	91	2	3	86	0	10
Oki101770_525	89	5	2	86	0	10
Oki116362_411	88	5	3	86	0	10
Oki_RAD100331_48	92	3	1	86	0	10
Oki_RAD109706_63	87	4	5	86	0	10
Oki_RAD14549_57	86	4	6	86	0	10
Oki_RAD42662_33	88	2	6	86	0	10
Oki_RAD45971_66	81	8	7	86	0	10
Oki_RAD51713_42	89	3	4	86	0	10
Oki_RAD75608_42	89	1	6	86	0	10
Oki_RAD45746_54	86	2	8	85	0	11
Oki_RAD96072_42	89	2	5	87	0	9

Ots_hnRNPL_58	92	1	3	86	0	10
oki_epic4_206	92	0	4	86	0	10
oki_epic4_149	85	0	11	85	0	11
oki_epic4_209	85	4	7	85	0	11
oki_epic4_057	85	0	11	85	0	11
Oki107336_45	87	5	4	85	0	11
Okigshpx152_132	90	0	6	85	0	11
Oki_RAD101607_49	87	4	5	85	0	11
Oki_RAD37537_45	88	3	5	85	0	11
Oki_RAD53155_32	87	5	4	85	0	11
Oki_RAD66976_58	85	5	6	84	0	12
Oki_RAD69161_64	86	3	7	84	0	12
Oki_RAD62768_58	88	0	8	85	0	11
Oki_RAD92875_31	88	4	4	86	0	10
Ots_il_1racp_174	87	1	8	86	0	10
oki_epic4_014	83	3	10	83	0	13
oki_epic4_036	87	3	6	84	0	12
Oki_RAD55090_49	89	2	5	84	0	12
Oki_RAD66397_30	84	0	12	84	0	12
oki_epic4_226	89	3	4	83	0	13
Oki116865_244	84	0	12	83	0	13
Oki105115_49	88	3	5	83	0	13
Oki_RAD37037_59	87	5	4	83	0	13
Oki_RAD49488_47	86	3	7	83	0	13
Oki_RAD66663_68	88	3	5	83	0	13
Oki_RAD85448_97	83	0	13	83	0	13
Ots_u202_249	83	0	13	83	0	13
Oki106172_60	86	5	5	82	0	14
Oki_RAD46141_169	83	2	11	82	0	14
Oki_RAD68190_55	88	6	2	82	0	14
Oki_RAD77210_64	84	6	6	82	0	14
Oki_RAD78773_67	87	3	6	82	0	14
Oki106313_353	84	6	6	82	0	14
Oki_RAD88786_44	85	1	10	83	0	13
Oki_RAD91470_66	87	3	6	83	0	13
oki_epic4_210	86	4	6	81	0	15
oki_epic4_250	88	4	4	81	0	15
oki_epic4_051	81	0	15	81	0	15
oki_epic4_003	84	0	12	80	0	16
Oki103577_70	81	1	14	81	0	15
oki_epic4_056	81	4	11	80	0	16
Oki104515_99	90	4	2	80	0	16
Oki_RAD23085_53	80	8	8	80	0	16

Oki_RAD66265_54	81	8	7	80	0	16
Oki_RAD70496_44	82	7	7	80	0	16
Oki_RAD85949_47	82	6	8	80	0	16
Oki_RAD87446_62	87	4	5	81	0	15
Ots_crRAD32203_124	81	0	15	81	0	15
Okipigh33_33	88	2	6	80	0	16
oki_epic4_043	79	1	16	79	0	17
Oki_RAD55690_46	87	7	2	79	0	17
oki_epic4_163	79	5	12	78	0	18
Oki_RAD71442_69	78	2	16	78	0	18
oki_epic4_232	77	2	17	77	0	19
Ots_crRAD57366_94	81	6	9	77	0	19
Oki_RAD94260_66	79	3	14	75	0	21
Oki_RAD39712_67	74	0	22	74	0	22
Ots_CCR7_182	79	5	12	75	0	21
Oki130524_184	78	1	17	73	0	23
Oki_RAD37979_59	74	1	21	73	0	23
Oki_RAD47313_50	73	4	19	73	0	23
Oki_RAD77342_49	72	7	17	72	0	24
oki_epic4_024	65	3	28	66	0	30
oki_epic4_134	52	0	44	52	0	44
oki_epic4_097	49	0	47	49	0	47
Oki104569_261	30	0	66	30	0	66
oki_epic4_216	0	0	96	0	0	96
oki_epic4_249	0	0	96	0	0	96
oki_epic4_255	0	0	96	0	0	96
oki_epic4_011	0	0	96	0	0	96
oki_epic4_026	0	0	96	0	0	96
Oki117815_369	0	0	96	0	0	96
Oki126160_142	0	0	96	0	0	96
Oki96158_278	0	0	96	0	0	96
oki_epic4_101	94	1	1	94	1	1
oki_epic4_174	95	1	0	94	1	1
oki_epic4_148	93	2	1	93	1	2
oki_epic4_116	93	2	1	92	1	3
oki_epic4_133	93	1	2	92	1	3
Oki_RAD91750_33	88	4	4	88	1	7
oki_epic4_103	90	2	4	86	1	9
oki_epic4_049	86	4	6	86	1	9
oki_epic4_083	87	2	7	85	1	10
Okicarban_107	87	6	3	85	1	10
oki_epic4_218	86	3	7	84	1	11
oki_epic4_182	88	2	6	83	1	12

Oki120024_226	85	1	10	83	1	12
Oki_RAD101032_66	89	2	5	82	1	13
Oki_RAD97168_52	74	2	20	69	1	26
Oki_RAD91362_68	79	9	8	68	1	27
Oki_RAD43051_33	63	3	30	64	1	31
Oki_RAD92978_42	81	11	4	65	1	30
oki_epic4_063	65	4	27	63	1	32
Oki_RAD59556_32	80	6	10	55	1	40
oki_epic4_066	45	1	50	45	1	50
Oki_RAD95780_30	79	7	10	43	1	52
oki_epic4_170	37	1	58	37	1	58
Ots_crRAD21752_87	0	1	95	0	1	95
Oki_RAD53121_66	87	5	4	86	2	8
Oki_RAD73526_35	83	4	9	82	2	12
Okigh183_160	80	6	10	74	2	20
Oki_RAD87621_67	73	15	8	36	2	58
oki_epic4_190	82	4	10	82	3	11
Oki_RAD92243_63	81	9	6	77	3	16
Okigdh189_132	66	25	5	72	3	21
Oki125998_324	92	4	0	92	4	0
oki_epic4_230	91	4	1	91	4	1
Oki_RAD90772_47	73	6	17	72	4	20
oki_epic4_032	88	7	1	88	6	2
Oki_RAD45691_45	84	9	3	79	6	11
Okiarp_105	80	9	7	78	6	12
oki_epic4_260	76	9	11	76	6	14
Oki_RAD42356_106	65	10	21	30	6	60
oki_epic4_189	79	10	7	79	7	10
oki_epic4_061	84	10	2	84	9	3
oki_epic4_054	84	9	3	83	9	4
Oki106419_292	71	10	15	68	10	18
Oki109651_152	79	13	4	75	11	10
Ots_crRAD20376_46	61	19	16	62	11	23
oki_epic4_178	50	15	31	34	13	49
Oki_RAD51912_66	48	21	27	48	16	32
oki_epic4_111	33	31	32	21	22	53
oki_epic4_172	52	39	5	52	39	5
oki_epic4_123	7	39	50	7	39	50
oki_epic4_053	41	44	11	41	42	13
Oki_RAD93028_59	42	48	6	39	44	13
oki_epic4_166	5	74	17	5	71	20