Alaska Hatchery Research Program **Technical Document**:[1] 3

1
2 **Title**: Evaluation of tissue quality for pedigree samples collected in 2013 **Version:** 1.0
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4 **Date:** August 13, 2014

5 ## Abstract

6 The ADF&G Gene Conservation Lab conducted a pilot study to determine the feasibility of
7 obtaining high-quality genetic samples for parentage analyses using tissue taken from
8 moribund/dead chum salmon from Southeast Alaska. Genotyping success was compared
9 between heart tissues (*bulbus arteriosus*) from moribund/dead fish sampled from four pedigree
10 streams and standard axillary process tissue taken from live fish collected in Fish Creek. Overall
11 genotyping success rate (percent of scorable loci) for heart tissues was 92% compared to 96% for
12 axillary process tissues, irrespective of the collection state observed in the field. When collection
13 state was taken into account, the genotyping success rate for heart tissues was ≥98% for all non-
14 rotten fish sampled. Overall, across tissues 82% of individuals were successfully genotyped for
15 at least 95% of markers and 92% of individuals were successfully genotyped for at least 80% of
16 markers. The lowest average success rate was for Sawmill Creek fish (79%), which were
17 sampled late in the season with a higher proportion of rotten fish than other pedigree streams.
18 There was no difference in success rates among non-rotten collection states. These results
19 suggest that using heart tissues should work for parentage analyses, provided tissues are
20 collected before or shortly after death.

21 ## Background of AHRP

22 Extensive ocean-ranching salmon aquaculture is practiced in Alaska by private non-profit
23 corporations (PNP) to enhance common property fisheries. Most of the approximately 1.7B
24 juvenile salmon that PNP hatcheries release annually are pink salmon in Prince William Sound
25 (PWS) and chum salmon in Southeast Alaska (SEAK; Vercessi 2013). The large scale of these
26 hatchery programs has raised concerns among some that hatchery fish may have a detrimental
27 impact on the productivity and sustainability of natural stocks. Others maintain that the potential
28 for positive effects exists. ADF&G convened a Science Panel for the Alaska Hatchery Research
29 Program (AHRP) whose members have broad experience in salmon enhancement, management,

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30 and natural and hatchery fish interactions. The AHRP was tasked with answering three priority
31 questions:

32 I. *What is the genetic stock structure of pink and chum salmon in each region (PWS and*
33 *SEAK)*?
34 II. *What is the extent and annual variability in straying of hatchery pink salmon in PWS and*
35 *chum salmon in PWS and SEAK*?
36 III. *What is the impact on fitness (productivity) of natural pink and chum salmon stocks due*
37 *to straying of hatchery pink and chum salmon*?

38 ## Introduction

39 ### *Measuring the Impact on Fitness*

40 To answer the third question, we need to know the origin and pedigree of each fish captured in
41 select streams across multiple generations. **Origin** refers to the type of early life-history habitat
42 (hatchery or natural) that a fish experienced. **Pedigree** refers to the family relationship among
43 parents and offspring. **'Ancestral origin'** refers to the origin of an individual's ancestors (e.g.,
44 two parents of a single origin [hatchery/hatchery or natural/natural] or two parents of mixed
45 origin [hatchery/natural]). These ancestral origins can be determined by combining information
46 from three sources: identification of hatchery origin from otolith marks, pedigree from genetic
47 data, and age from scales (for chum salmon from SEAK). By pairing these data within fish and
48 across generations, we can estimate **reproductive success** (**RS**) among cross types (i.e. hatchery-
49 hatchery, hatchery-natural, and natural-natural origin crosses). The AHRP is using the **relative**
50 **reproductive success** (**RRS**) *of hatchery-origin fish to natural-origin fish as the measure of*
51 *fitness in this study* (Tech Doc 1 – Shedd et al. 2014).

52 ### *Problem: Will Tissue from Dead Fish be of Sufficient Quality for Pedigree Analysis?*

53 Estimating RRS with high precision requires high proportions of fish with known pedigree. The
54 proportion of fish for which pedigree can be identified by parentage analysis is a function of the
55 proportion of fish sampled (both parent and offspring), allelic frequencies of genetic markers,
56 and proportion of fish accurately genotyped for current and previous generations. In this study,
57 tissues were sampled for genetic analysis from fish that were either moribund or dead to reduce
58 the chance that sampled fish represented fish that were destined to spawn in other creeks (i.e.
59 nosing-in fish) and to pair origin (otoliths) with tissue without impacting reproductive success.

60 DNA decay in dead fish is affected by time, temperature, chemical environment, and solar
61 radiation (Cadet et al. 1997). Previous studies have documented how DNA from poor quality
62 tissues can produce unreliable data and questionable estimates of stock composition in mixed
63 stock analyses (Paetkau 2003; ADF&G unpublished data). As a result, the Gene Conservation
64 Laboratory (GCL) generally implements an "80% Rule", whereby individuals missing genotypes
65 for 20% or more of screened markers are removed from further analysis (Dann et al. 2009).

66 While this genotypic data quality criterion improves the accuracy and precision of mixed stock
67 analyses, it is even more important for parentage analyses due to the large influence that missing
68 or incorrect genotypes can have on parentage assignments relative to stock of origin assignments.
69 We therefore chose to sample heart tissue (*bulbus arteriosus*) because (1) it is one of the last
70 tissues to die, (2) it is protected from the solar radiation that can damage DNA, and (3) previous
71 tests of this tissue type from live salmon indicated we could genotype single nucleotide markers
72 (SNPs) with high success. SNPs were chosen because they lend themselves to high-throughput
73 genotyping and have been successfully used for parentage analysis in salmonids (Anderson and
74 Garza 2006; Hauser et al. 2011). However, we do not know how long DNA remains viable for
75 SNP genotyping after death.

76 An alternative to acquiring tissues from carcasses for DNA analysis but still only analyzing
77 tissues from fish that died in the stream is to double sample fish. This method would entail two
78 sampling events: 1) capturing live fish in the stream, sampling axillary fins, tagging the fish with
79 a uniquely-numbered floy tag, and then releasing the fish back into the stream and 2) collecting
80 dead fish later in the season, sampling the otolith, and taking the floy tag. The otolith and
81 genetic samples would be matched using the floy tag number.

82 *Goals of Technical Document*

83 In this technical document we investigate the success rates of genotyping moribund or dead
84 chum salmon that were sampled for the fitness aspect of this study. Three goals of this technical
85 document are to:

86 1) Describe the methodology we used to evaluate DNA quality of tissues collected in the
87 field for 2013 pedigree samples;
88 2) Evaluate genotyping success for heart and axillary tissue samples collected from chum
89 salmon in 2013; and
90 3) Determine the relationship between field-evaluated tissue quality (collection state) and
91 genotyping success and accuracy.

92 **Methods**

93 *Selection of Tissues*

94 We received 1,947 heart tissue samples of chum salmon sampled from four pedigree streams in
95 Southeast Alaska (

Collection State	Tissue	n	SNP genotypes	Failures	Success rate
Alive	Ax	95	9,025	395	95.6%
	BA	132	12,540	176	98.6%
Pink Gill	BA	37	3,515	33	99.0%
Grey Gill	BA	63	5,985	137	97.7%
Rotting	Ax	1	95	80	15.8%
	BA	51	4,845	1,699	64.9%

	Overall	379	36,005	2,520	93.0%

96

97 Table 5.– Results from Tukey's HSD pairwise comparisons of mean number of loci genotyped
98 per individual between collection states and tissue types.

Category	Pairwise comparison	Diff. in mean number loci genotyped	2.5% CI	97.5% CI	*P*
Collection state	Rotting-Pink Gill	-33.32	-41.11	-25.53	0.00
	Rotting-Grey Gill	-32.04	-38.82	-25.25	0.00
	Rotting-Alive	-31.70	-37.26	-26.13	0.00
	Pink Gill-Grey	1.28	-6.22	8.78	0.97
	Pink Gill-Alive	1.62	-4.80	8.04	0.91
	Grey Gill-Alive	0.34	-4.82	5.50	1.00
Tissue type	Heart-Axillary	2.82	-0.44	6.07	0.09

99 **Figures**

100 Figure 1) by the Sitka Sound Science Center (SSSC) after they were separated from their paired
101 otoliths by the ADF&G Mark, Tag, and Age Laboratory in Juneau, Alaska. At the time of tissue
102 receipt, we lacked associated data including sampling location (pedigree stream) and collection
103 state (i.e. tissue condition). In addition to the heart tissues, GCL staff sampled axillary processes
104 from 241 live chum salmon from Fish Creek on July 18-20, 2013. We randomly sampled 380
105 individuals from these two types of collections for extraction and genotyping, 284 from the heart
106 samples and 96 from the axillary process samples, regardless of collection state or location.

107 *Extraction and Genotyping*

108 Genomic DNA was isolated from tissue samples using a DNeasy® 96 Tissue Kit by QIAGEN®
109 (Valencia, CA). Genotyping was first accomplished using Applied Biosystems' SNP TaqMan
110 assay analysis methods. The following five genetic markers were used to evaluate tissue quality:
111 *Oke_ccd16-77*, *Oke_CKS-389*, *Oke_GPH-105*, *Oke_U1018-50*, and *Oke_u217-172*. These
112 markers were selected from past GCL projects specifically to differentiate high quality from low
113 quality DNA. Each reaction on this platform was performed in 384-well reaction plates in a 5μL
114 volume consisting of 5-40 ng/μl of template DNA, 1x TaqMan® Universal PCR Master Mix
115 (Applied Biosystems), and 1x TaqMan® SNP Genotyping Assay (Applied Biosystems).
116 Thermal cycling was performed on a Dual 384-Well GeneAmp® PCR System 9700 (Applied
117 Biosystems) as follows: an initial denaturation of 10 min at 95°C followed by 50 cycles of 92°C
118 for 1 s and annealing/extension temperature for 1 min. The plates were scanned on an Applied
119 Biosystems (ABI) Prism 7900HT Sequence Detection System after amplification and scored
120 using ABI Sequence Detection Software version 2.2.

121 Once initial results were obtained, the tissue quality for all samples was determined to be high
122 enough to screen the samples using 96 SNPs on the Fluidigm® 96.96 Dynamic Arrays
123 (http://www.fluidigm.com) protocol. These 96 SNPs were chosen for the Western Alaska
124 Salmon Stock Identification Program (WASSIP; DeCovich et al. 2012; Tables

125 Table 1) and are representative of our normal genotyping process. The Fluidigm® 96.96
126 Dynamic Array contains a matrix of integrated channels and valves housed in an input frame.
127 On one side of the frame are 96 inlets to accept the sample DNA from individual fish and on the
128 other are 96 inlets to accept the assays for 96 SNP markers. Once in the wells, the components
129 are pressurized into the chip using the IFC Controller HX (Fluidigm). The 96 samples and 96
130 assays are then systematically combined into 9,216 parallel reactions. Each reaction is a mixture
131 of 4 μl of assay mix (1x DA Assay Loading Buffer (Fluidigm), 10x TaqMan® SNP Genotyping
132 Assay (ABI), and 2.5x ROX (ABI)) and 5 μl of sample mix (1x TaqMan® Universal Buffer
133 (ABI), 0.05x AmpliTaq® Gold DNA Polymerase (ABI), 1x GT Sample Loading Reagent
134 (Fluidigm) and 60-400 ng/μl DNA) combined in a 7.2 nL chamber. Thermal cycling was
135 performed on an Eppendorf IFC Thermal Cycler as follows: 70°C for 30 min for "Hot-Mix"
136 step, initial denaturation of 10 min at 96°C followed by 40 cycles of 96° for 15 s and 60° for 1

137 min. The Dynamic Arrays were read on a Fluidigm® EP1™ System after amplification and
138 scored using Fluidigm® SNP Genotyping Analysis software.

139 *Summarizing of Data*

140 We read genotypes directly from Biomark files into *R* version 2.14.1 for further analysis (*R*
141 Development Core Team 2011). We summarized observed heterozygosity for each SNP as well
142 as over all SNPs. We calculated success rate as the number of SNPs that produced scorable
143 genotypes divided by the total number of SNP assays that were successful for at least one
144 sample. These success rates were summarized by tissue type, collection state, and stream.
145 While paired collection state information was not available when tissues were selected for
146 genotyping, paired sample data later became available for analyses (with the exception of the one
147 axillary process from Admiralty Creek that did not have data for collection state). Collection
148 state was visually determined in the field by the SSSC as one of four factor levels: alive, pink
149 gill, grey gill, and rotten, corresponding to the progression of senescence after spawning. We
150 used an analysis of variance (ANOVA) to test for differences in genotyping success rate between
151 tissue types and collection states. Tukey's Honestly Significant Difference (Tukey's HSD) *post*
152 *hoc* tests were used to examine pairwise relationships.

153 We also summarized individuals by the number of SNPs for which they were missing genotypes.
154 This gave us an indication of how many individuals would be removed from future parentage
155 analyses based upon different criteria for allowing missing genotypes. For example, we could
156 investigate how many individuals would be removed from parentage analysis using our standard
157 '80% Rule' compared to more restrictive rules allowing for only individuals with 90% or 95%
158 genotyping success Many parentage programs, such as CERVUS (Kalinowski et al. 2007),
159 include this criterion as an analysis parameter, and we wished to know how many individuals
160 would be excluded from future parentage analysis at different parameter levels.

161 **Results**

162 *Selection of Tissues*

163 Of the 1,947 tissue samples collected by the SSSC, we randomly sampled 284 heart tissues and 1
164 axillary process for DNA extraction and genotyping. Of the 241 axillary process samples
165 collected by the ADF&G, we randomly sampled 95 for DNA extraction and genotyping. The
166 total number of fish genotyped was 380.

167 *Extraction and Genotyping*

168 Selected tissues were successfully extracted and genotyped on both genotyping systems for all
169 but one SNP. *Oke_U1015-255* failed to load on the Biomark system and did not produce
170 genotypes for these samples (Figure 2C). This failure to load was a result of the Biomark loader
171 and was not influenced by the tissues being analyzed.

172 *Summarizing of Data*

173 Observed heterozygosity's ranged from 0.00-0.55 and overall observed heterozygosity was 0.32
174 (Tables

175 Table 1; Figure 3). Overall genotyping success rate across all samples and markers was 93%.
176 The success rate of axillary processes collected from Fish Creek was 96%, and the one axillary
177 process sampled from Admiralty Creek failed for 80 markers. This compares to a 99% success
178 rate for heart samples collected from Fish Creek and a 92% success rate for heart samples from
179 all pedigree streams that were sampled by the SSSC (range of 79-99%; Table 2). Heart samples
180 from Sawmill Creek had a markedly lower success rate (79%) than heart samples from other
181 creeks. Success rates by marker were fairly consistent (Figure 4), but varied considerably among
182 streams and tissue types (Figure 5).

183 Success rates by individual were significantly different between collection states, but not tissue
184 types ($F_{3,374}$=77.67, p<0.001; and $F_{1,374}$=3.68, p=0.056, respectively). Tukey's HSD showed that
185 although there are no pairwise differences between alive, pink gill, and grey gill collection states,
186 all three of these had significantly higher success rates than rotten fish (Table 4 & 5, Figure 6).
187 While there was no significant difference in success rate between axillary tissue (standard for
188 GCL collections) and heart tissue for an α=0.05, heart tissues did have a higher average success
189 rate than axillaries, when controlling for differences in tissue quality (Table 4, Figure 6).

190 Disregarding the SNP assay that failed to load, a majority of individuals had either complete 95
191 SNP genotypes (173 or 46% of total) or were only missing genotypes for a single SNP and (76 or
192 20% of total; Table 3). Results varied considerably among streams and tissue types, with heart
193 samples from Fish Creek having the greatest number of individuals with complete genotypic data
194 (58%), and Sawmill Creek having the lowest (32%, excluding the single axillary sample from
195 Admiralty which failed at 80 markers). Overall, 82% of individuals had 90+ SNP genotypes.

196 Discussion

197 We randomly sampled chum salmon tissues collected from Southeast Alaska pedigree streams in
198 2013 to evaluate what level of genotyping success we can expect for parentage analysis in this
199 project. Genotyping success was generally high, although one stream had markedly lower
200 success rates than the others. Sawmill Creek was sampled late in the season and had a higher
201 proportion of rotten fish (32 out of 50 genotyped) than others. These results stress the
202 importance of appropriate timing of sampling efforts in order to obtain tissues as soon after death
203 as possible.

204 Other parentage analysis studies have used cut offs for genotyping success rates that range from
205 75% (Hauser et al. 2011, Araki et al. 2007) to 93% (Hess et al 2012) depending on whether
206 individuals are assigned to single parents or to parent pairs. Overall, 81% (230/284) of heart
207 samples met the most stringent level of 95% individual genotyping success rate (genotypes for

90+ SNPs), with 91 % (259/284) of heart samples meeting the GCL's standard "80% Rule" for individual genotyping success rate (genotypes for 76+ SNPs). These results suggest that using heart tissue should work for parentage analyses, provided tissues are collected before or shortly after death.

The results presented here represent the success rates we can expect for tissues of this quality and type under our standard operating procedures in the laboratory. It may be possible to improve genotyping success in the laboratory using additional methods. For example, pre-amplification of DNA prior to normal genotyping can improve success rates when the quantity of DNA available from a sample is low (but not when the quality is poor). However, our results for these SNP markers show that we can obtain a high success rate with heart tissue, without the need for pre-amplification.

Questions for the AHRP

1. Is it reasonable to use heart tissue sampled from moribund/dead individuals for parentage analysis?
2. Is it worth performing a cost-benefit analysis for using the double sampling methods that use floy tags?

AHRP Review and Comments

This technical document was discussed at the December 12, 2014 meeting of the AHRG. In addition it was reviewed by email exchange prior to the meeting.

Use of heart tissue is recommended for all parentage analysis. The additional sampling in conjunction with floy tags is considered unnecessary.

This document is acceptable to the AHRG.

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277 **Tables**

278 Table 1.–Observed heterozygosity (H_O) and source for 96 chum salmon SNPs used to evaluate
279 tissue quality.

Assay	H_O	Source[a]	Assay	H_O	Source[a]
Oke_ACOT-100	0.467	A	*Oke_MLRN-63*	0.516	A
Oke_AhR1-78	0.486	B	*Oke_Moesin-160*	0.269	C
Oke_arf-319	0.474	C	*Oke_nc2b-148*	0.470	A
Oke_ATP5L-105	0.388	A	*Oke_ND3-69*[b]	0.000	A
Oke_azin1-90	0.506	A	*Oke_NUPR1-70*	0.284	A
Oke_brd2-118	0.175	A	*Oke_pgap-111*	0.482	A
Oke_brp16-65	0.338	A	*Oke_pgap-92*	0.396	A
Oke_CATB-60	0.054	A	*Oke_PPA2-635*	0.162	B
Oke_ccd16-77	0.503	A	*Oke_psmd9-57*	0.226	A
Oke_CD81-108	0.044	A	*Oke_rab5a-117*	0.499	A
Oke_CD81-173	0.314	A	*Oke_ras1-249*	0.490	B
Oke_CKS1-94	0.388	A	*Oke_RFC2-618*	0.022	C
Oke_CKS-389	0.454	D	*Oke_RH1op-245*	0.340	C
Oke_Cr30[b]	0.000	A	*Oke_RS27-81*	0.281	A
Oke_Cr386[b]	0.000	A	*Oke_RSPRY1-106*	0.256	A
Oke_ctgf-105	0.166	B	*Oke_serpin-140*	0.475	C
Oke_DCXR-87	0.125	A	*Oke_slc1a3a-86*	0.419	A
Oke_e2ig5-50	0.421	A	*Oke_sylc-90*	0.364	A
Oke_eif4g1-43	0.386	A	*Oke_TCP1-78*	0.060	B
Oke_f5-71	0.369	A	*Oke_Tf-278*	0.486	B
Oke_FANK1-166	0.436	A	*Oke_thic-84*	0.357	A
Oke_FBXL5-61	0.249	A	*Oke_U1002-262*	0.493	A
Oke_gdh1-191	0.346	A	*Oke_U1008-83*	0.282	A
Oke_gdh1-62	0.468	A	*Oke_U1010-251*	0.471	A
Oke_GHII-3129	0.025	B	*Oke_U1012-241*	0.457	A
Oke_glrx1-78	0.473	A	*Oke_U1015-255*[c]	N/A	A
Oke_GPDH-191	0.334	C	*Oke_U1016-154*	0.469	A
Oke_GPH-105	0.423	B	*Oke_U1017-52*	0.260	A
Oke_HP-182	0.311	B	*Oke_U1018-50*	0.014	A
Oke_il-1racp-67	0.256	C	*Oke_U1021-102*	0.425	A
Oke_IL8r2-406	0.264	A	*Oke_U1022-139*	0.221	A
Oke_KPNA2-87	0.062	B	*Oke_U1023-147*	0.433	A
Oke_LAMP2-186	0.493	A	*Oke_U1024-113*	0.073	A
Oke_mgll-49	0.432	A	*Oke_U1025-135*	0.011	A

280

281 Table 1.–page 2 of 2.

Assay	H_O	Source[a]
Oke_u200-385	0.520	C
Oke_U2006-109	0.471	A
Oke_U2007-190	0.465	A
Oke_U2011-107	0.221	A
Oke_U2015-151	0.063	A
Oke_U2025-86	0.550	A
Oke_U2029-79	0.501	A
Oke_U2031-37	0.046	A
Oke_U2032-74	0.151	A
Oke_U2034-55	0.497	A
Oke_U2035-54	0.017	A
Oke_U2037-76	0.097	A
Oke_U2041-84	0.461	A
Oke_U2043-51	0.104	A
Oke_U2048-91	0.438	A
Oke_U2050-101	0.231	A
Oke_U2053-60	0.442	A
Oke_U2054-58	0.113	A
Oke_U2056-90	0.455	A
Oke_U2057-80	0.478	A
Oke_U212-87	0.049	C
Oke_u217-172	0.424	C
Oke_U302-195	0.040	B
Oke_U502-241	0.091	B
Oke_U504-228	0.300	B
Oke_U506-110	0.479	B
Oke_U507-286	0.428	B
Oke_U509-219	0.486	B
Overall	0.323	

282 a A=International Program for Salmon Ecological Genetics at the University of Washington (Petrou et al. 2013);
283 B=Elfstrom et al. 2007; C=Smith et al. 2005b; and D=Smith et al. 2005a.

284 b These are mitochondrial SNPs so there are no heterozygotes.

285 c This assay failed to load in the laboratory and is not indicative of tissue quality.

286 Table 2.–Stream, tissue type (Ax=axillary process; BA=*bulbus arteriosus*), sample size (n), number of potential SNP genotypes,
287 number of failed genotypes, and success rate of SNP assays for chum salmon tissues collected from pedigree streams in Southeast
288 Alaska as part of the AHRP.

Stream	Tissue	n	SNP genotypes	Failures	Success rate
Admiralty	Ax	1	95	80	15.8%
	BA	51	4,845	330	93.2%
Fish Creek	Ax	95	9,025	395	95.6%
	BA	112	10,640	151	98.6%
Prospect	BA	71	6,745	551	91.8%
Sawmill Creek	BA	50	4,750	1,013	78.7%
	Overall	380	36,100	2,520	93.0%

289

290 Table 3.–Number of individuals missing genotypes for a given number of SNPs by stream and tissue type (Ax=axillary process;
291 BA=*bulbus arteriosus*). Individuals missing genotypes for greater than 5 SNPs are pooled in the "> 5" category, and percentages (in
292 parentheses) denote the cumulative proportion of individuals from a given stream and tissue type missing a given number or fewer
293 SNPs (e.g. 75% of BA tissues from individuals from Admiralty were missing genotypes for 2 or fewer SNPs).

		Individuals missing genotypes for a given # of SNPs (and cumulative %)							
Stream	Tissue	0	1	2	3	4	5	> 5	Total
Admiralty	Ax	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (100%)	1
	BA	21 (41%)	15 (71%)	2 (75%)	2 (78%)	0 (78%)	1 (80%)	10 (100%)	51
Fish Creek	Ax	33 (35%)	19 (55%)	19 (75%)	6 (81%)	1 (82%)	2 (84%)	15 (100%)	95
	BA	65 (58%)	23 (79%)	8 (86%)	4 (89%)	1 (90%)	1 (91%)	10 (100%)	112
Prospect	BA	38 (54%)	14 (73%)	4 (79%)	1 (80%)	1 (82%)	2 (85%)	11 (100%)	71
Sawmill Creek	BA	16 (32%)	5 (42%)	4 (50%)	1 (52%)	0 (52%)	1 (54%)	23 (100%)	50
	Overall	173 (46%)	76 (66%)	37 (75%)	14 (79%)	3 (80%)	7 (82%)	70 (100%)	380

294 Table 4.– Collection state (alive, pink gill, grey gill, and rotting), tissue type (Ax=axillary
295 process; BA=*bulbus arteriosus*), sample size (n), number of potential SNP genotypes, number of
296 failed genotypes, and success rate of SNP assays for chum salmon tissues collected from
297 pedigree streams in Southeast Alaska as part of the AHRP. Note that the overall sample size is
298 379 due to one tissue that did not have data for collection state.

Collection State	Tissue	n	SNP genotypes	Failures	Success rate
Alive	Ax	95	9,025	395	95.6%
	BA	132	12,540	176	98.6%
Pink Gill	BA	37	3,515	33	99.0%
Grey Gill	BA	63	5,985	137	97.7%
Rotting	Ax	1	95	80	15.8%
	BA	51	4,845	1,699	64.9%
	Overall	379	36,005	2,520	93.0%

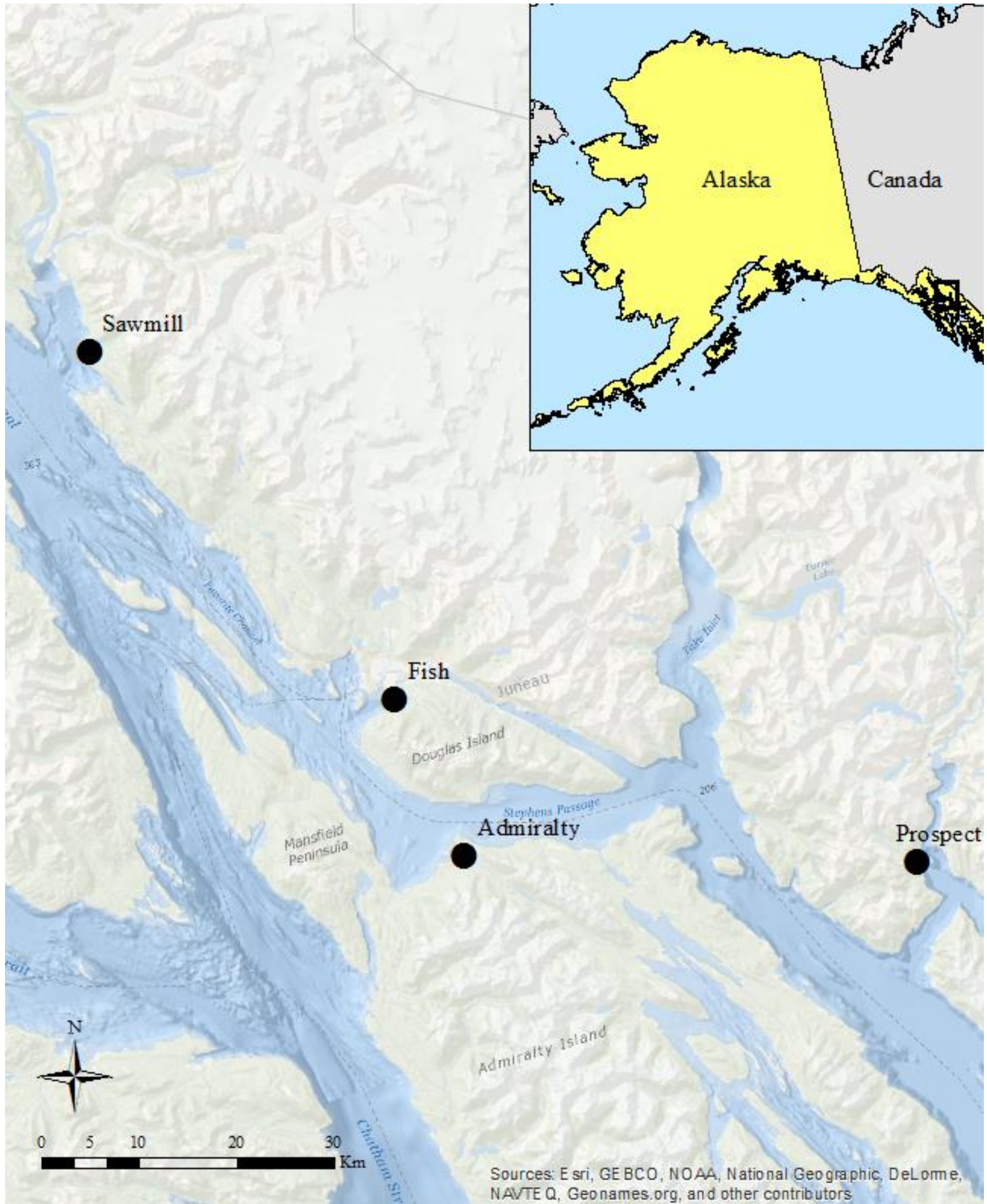
299

300 Table 5.– Results from Tukey's HSD pairwise comparisons of mean number of loci genotyped
301 per individual between collection states and tissue types.

Category	Pairwise comparison	Diff. in mean number loci genotyped	2.5% CI	97.5% CI	*P*
Collection state	Rotting-Pink Gill	-33.32	-41.11	-25.53	0.00
	Rotting-Grey Gill	-32.04	-38.82	-25.25	0.00
	Rotting-Alive	-31.70	-37.26	-26.13	0.00
	Pink Gill-Grey	1.28	-6.22	8.78	0.97
	Pink Gill-Alive	1.62	-4.80	8.04	0.91
	Grey Gill-Alive	0.34	-4.82	5.50	1.00
Tissue type	Heart-Axillary	2.82	-0.44	6.07	0.09

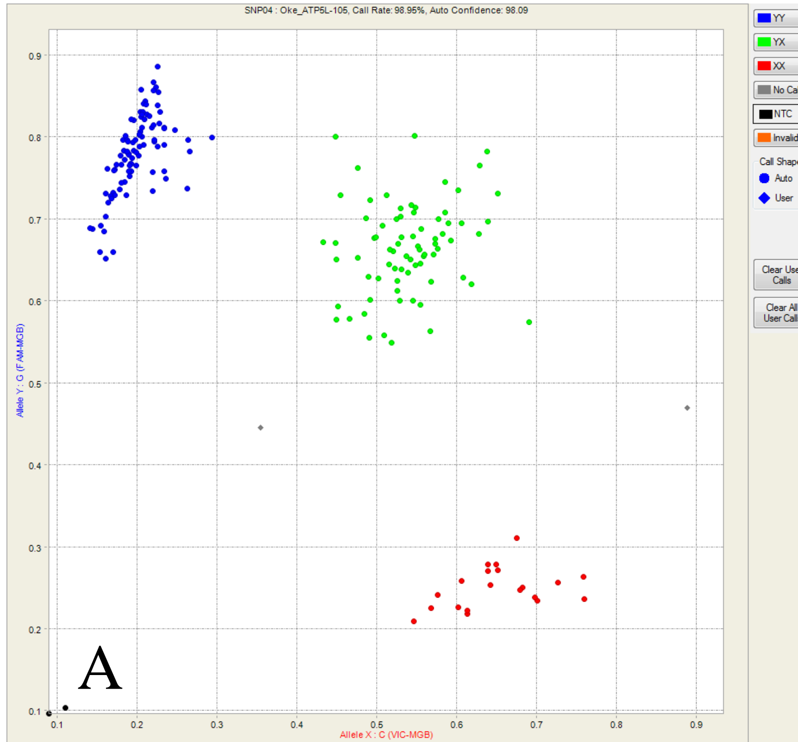
Figures

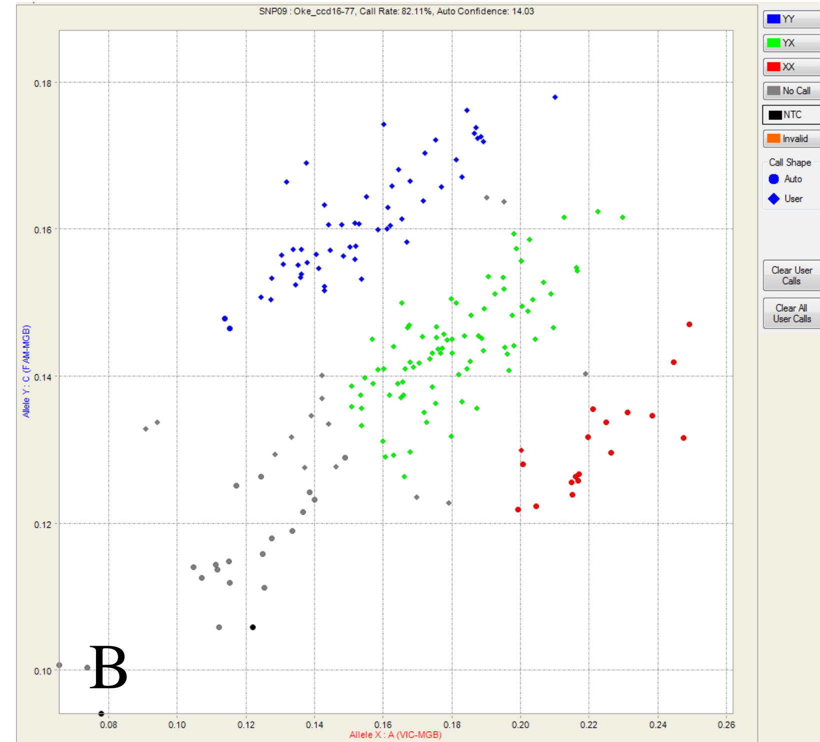
Figure 1.–Map of four pedigree streams in SEAK where chum salmon samples were collected in 2013 as part of the AHRP.



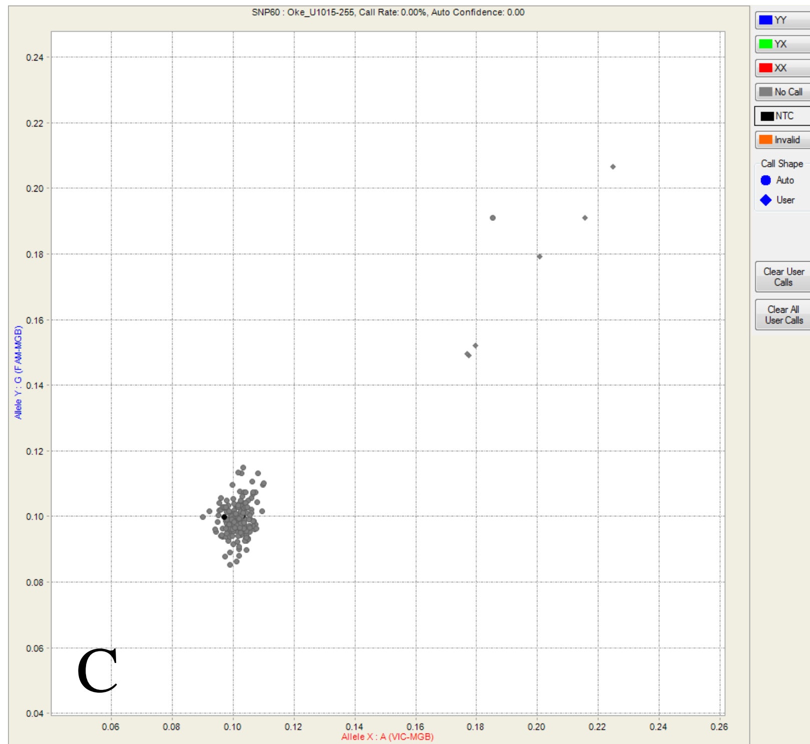
306 Figure 2.–Genotype plots showing example PCR assays with A) good separation of genotypes (88% of markers), and B) fairly poor
307 separation (11% of markers). Blue and red data points indicate homozygous genotypes, green indicates heterozygous genotypes, and
308 gray indicates inconclusive result.

309



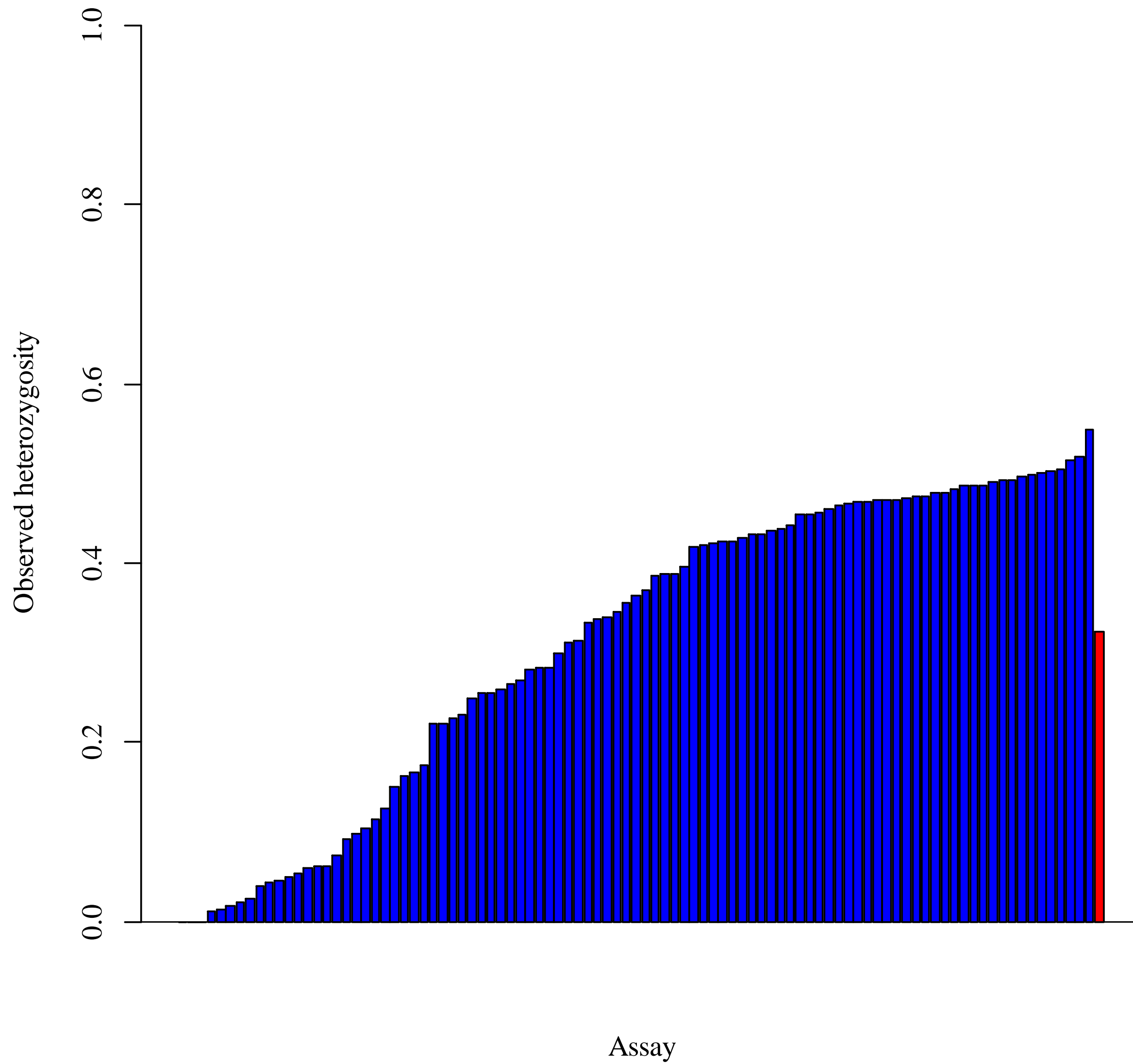


310 Figure 2.–Genotype plots showing example PCR assays with C) no separation for the one PCR assay that failed to load (*Oke_U1015-*
311 *255*). Blue and red data points indicate homozygous genotypes, green indicates heterozygous genotypes, and gray indicates
312 inconclusive result.



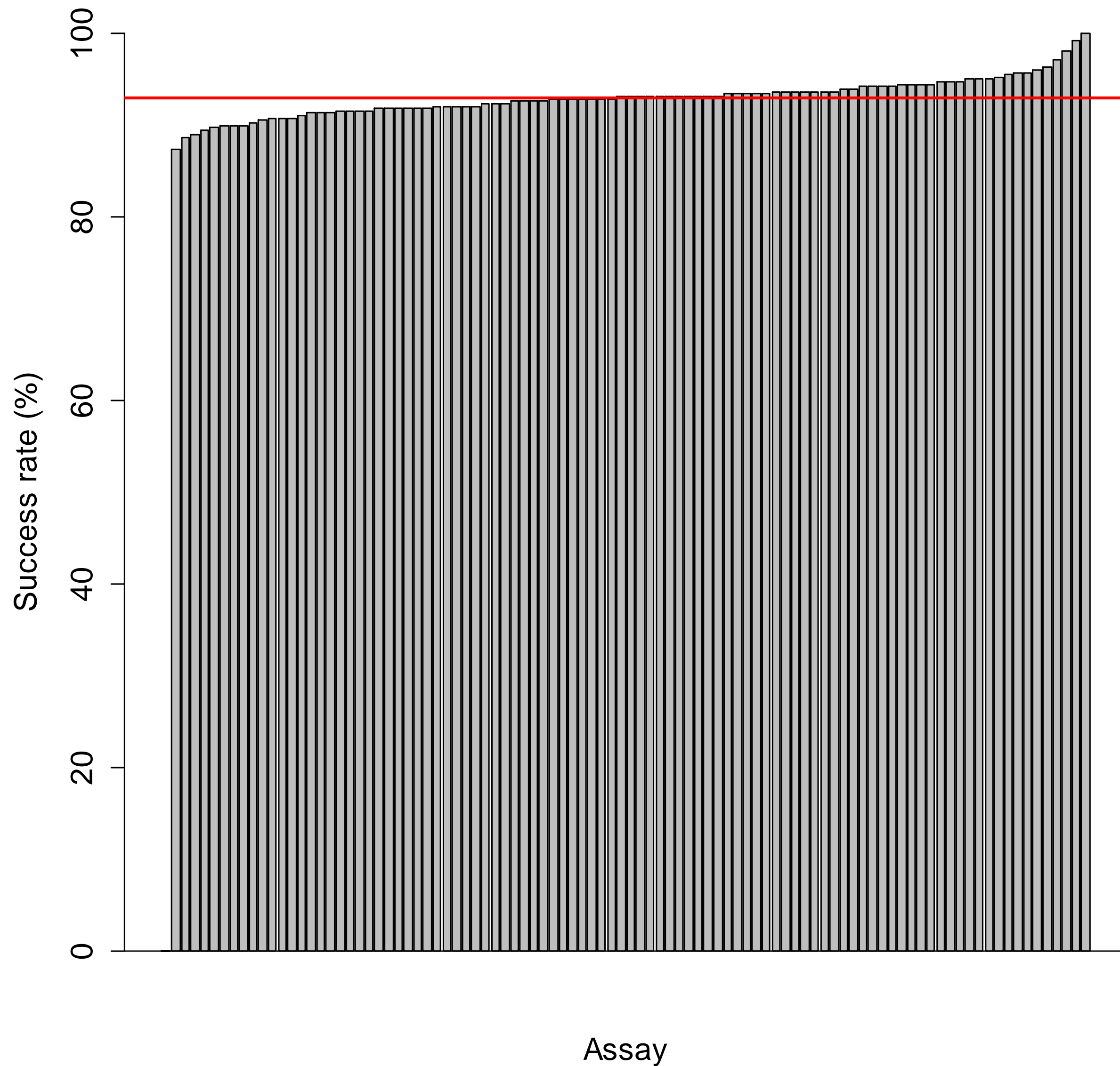
313

314 Figure 3.–Observed heterozygosity (H_O) for 93 nuclear SNP assays used to evaluate the quality
315 of chum salmon tissues collected from 4 pedigree streams in SEAK as part of the AHRP.
316 Individual marker (SNP assay) values are blue bars and the overall heterozygosity across
317 markers is indicated with the red bar.



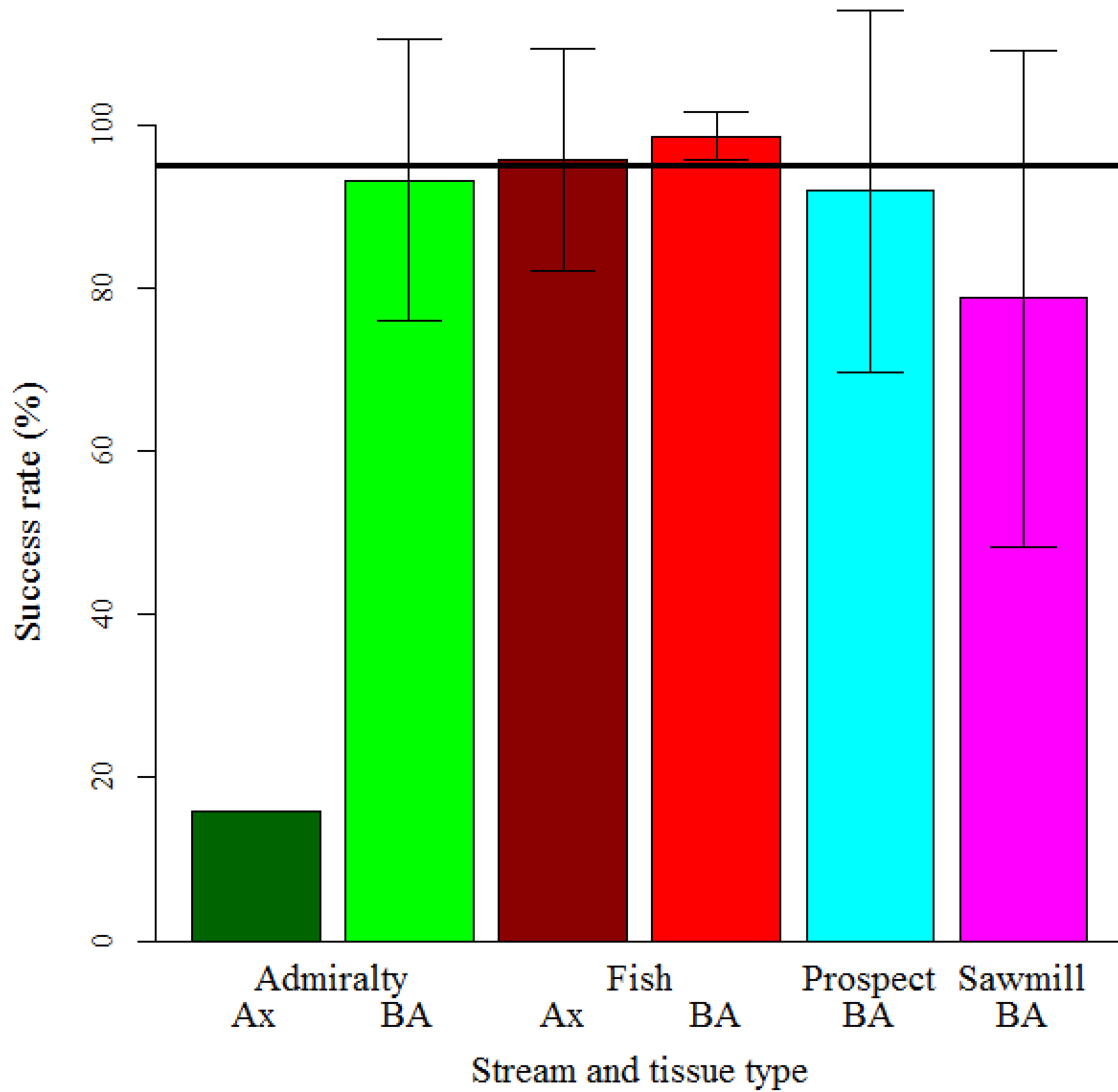
318

319 Figure 4.–Ordered values of genotyping success rate for 96 SNP assays (percent of fish samples,
320 regardless of tissue type, that produced a scorable genotype for a given locus) used to evaluate
321 the quality of chum salmon tissues collected from 4 pedigree streams in SEAK as part of the
322 AHRP. The horizontal red line depicts average success rate of the 95 assays that produced
323 genotypes (this does not include the 1 assay that failed to load).



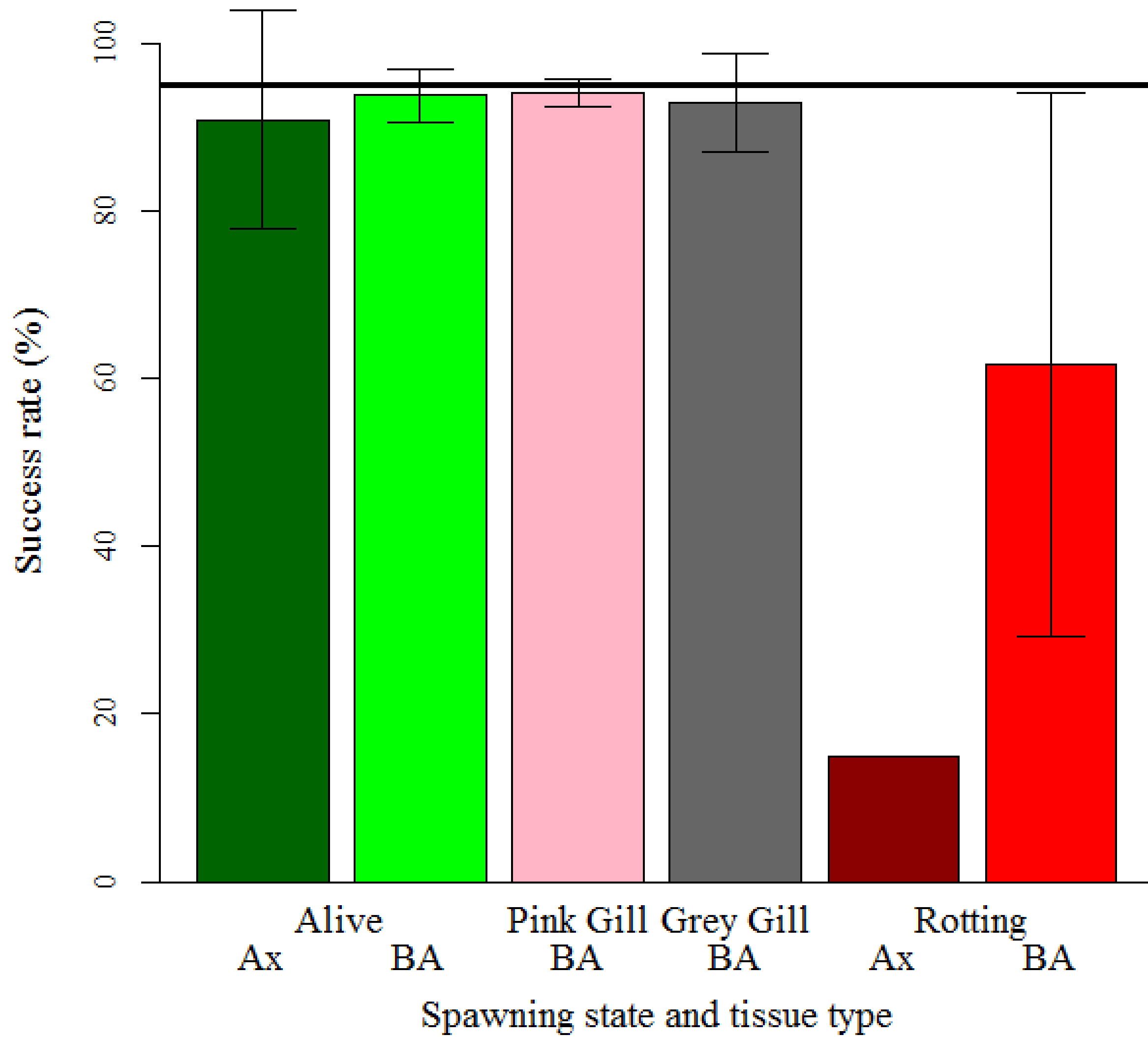
324

325 Figure 5.–Genotyping success rates (mean ± SD) by stream and tissue type (Ax=axillary process;
326 BA=*bulbus arteriosus*) for the 95 SNPs that produced genotypes for chum salmon tissues
327 collected from pedigree streams in SEAK as part of the AHRP. Horizontal line indicates 95%
328 success rate.



329

330 Figure 6.– Genotyping success rates (mean ± SD) by collection state (alive, pink gill, grey gill,
331 and rotting) and tissue type (Ax=axillary process; BA=*bulbus arteriosus*) for the 95 SNPs that
332 produced genotypes for chum salmon tissues collected from pedigree streams in SEAK as part of
333 the AHRP. Horizontal line indicates 95% success rate.



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