

Review of Satterthwaite et al. 2015: *Multidisciplinary Evaluation of the Feasibility of Parentage-Based Genetic Tagging (PBT) for Management of Pacific Salmon*

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Background on Issuance of RFP, Report Development and Report Review

In October of 2013, the Committee on Scientific Cooperation (CSC) submitted a proposal, titled *Assessment of emerging technologies with potential to enhance and/or replace the current CWT system*, jointly to the Northern and Southern Funds for their consideration. This original proposal called for issuance of two RFPs for the following tasks: (a) a feasibility study of Parentage-Based Tagging (PBT) as a possible replacement for or complement to the existing Coded Wire Tag (CWT) system, and (b) an assessment of the current status and cost of miniaturized Radio Frequency Identification (RFID) tags that might be suitable for tagging juvenile Pacific salmon as a potential "second generation" alternative to CWTs. The Southern Fund granted funding for the PBT feasibility study. A total of \$83,000 was awarded: \$60,000 for development of a report consistent with the PBT RFP, with remaining funds to support travel to allow an Oversight Committee to develop the RFP, select an appropriate contractor, and review the delivered product. The Oversight Committee consisted of all four members of the Committee on Scientific Cooperation as well as the following individuals: Terry Beacham, Gayle Brown and Arlene Tompkins (DFO), John Carlile and Bill Templin (ADFG), Andy Gray (NMFS), and Marianna Alexandersdottir (NWIFC). A copy of the RFP issued by the PSC is attached as Appendix I.

The RFP was timely because a 2005 Expert Panel Report on the Future of the CWT System had concluded that "There is no obvious viable short-term alternative to the CWT system that could

provide the data required for cohort analysis and implementation of PST management regimes for Chinook and coho salmon. Therefore, agencies must continue to rely upon CWTs for several years (at least 5+ years), even if agencies make decisions for development and future implementation of alternative technologies." The Expert Panel had also noted the development of a new technology, now termed Parentage-Based Genetic Tagging (PBT), that might, if feasible and cost-effective, replace or complement the CWT system in the future.

Since the 2005 report, there has been increasing application of PBT, primarily in the Columbia River system and in California's Central Valley system, as well as technological advancements in genomic technology. The CSC therefore believed it appropriate to revisit the potential management applications of this newly developing technology. Challenges facing the existing CWT program and identified in 2005 have persisted and in some cases increased. These challenges include: escalating complexity of salmon fishery management, increasing demand for finer scale management, more widespread implementation of Mass Marking and Mark-Selective Fisheries (MM&MSF), and incomplete sampling for Double Index Tags (DIT) in ocean and freshwater fisheries. All of these challenges have placed increased demands on the existing CWT system, but fiscal support for the CWT system has generally diminished. (Subsequent to the 2005 Expert Panel Report, the governments of the United States and Canada provided \$7.5 million each to improve the performance of the existing CWT system, but those funds have since been exhausted.)

The issued RFP called for a detailed assessment of the feasibility of PBT to deliver estimates of parameters (age- and fishery-specific exploitation rates in ocean and freshwater fisheries, and survival rates from release to specified ocean age) that currently are used by the Pacific Salmon Commission (PSC) and the Pacific Fishery Management Council (PFMC) to assess performance of fisheries, ensure that stocks of concern are not overfished, and guide development of fishing regulations. Estimates of these parameters are currently based on cohort reconstruction methods applied to recoveries (expanded by sampling fractions) of fish from CWT release groups (of both hatchery- and natural-origin) generated from sampling of ocean and freshwater fisheries and natural spawning escapements, and enumeration of returns to hatcheries. In addition, the RFP called for a comparison of the probable cost of a coast-wide system based on PBT with that of the existing CWT system. The RFP specifically noted that a multidisciplinary team, including fisheries modelers with expertise in salmon management as well as geneticists with expertise in development or application of the PBT concept, would be needed to develop an adequate response to the RFP.

In August of 2014, the Oversight Committee awarded the RFP to a highly qualified team with direct knowledge of contemporary management of Pacific salmon and of PBT development and application. Team membership included five fisheries scientists from the National Marine

Fisheries Service Ecology Lab in Santa Cruz, CA: Will Satterthwaite and Michael Mohr (fisheries modeling and salmon management), Carlos Garza and Eric Anderson (genetics and PBT) and Cameron Spier (economics); and two fisheries geneticists with expertise using PBT in the Columbia River system: Shawn Narum (Columbia River Inter-Tribal Fish Commission) and Matt Campbell (Idaho Department of Fish and Game). This team submitted a draft report to the Oversight Committee in early January of 2015; two members of the team (Satterthwaite and Spier) gave a preliminary oral report of progress at the February 2015 PSC meeting; and the final report was submitted on 01 April 2015, and posted at the PSC website on 28 April 2015. Members of the Oversight Committee reviewed and discussed the submitted final report, with the CSC responsible for development of this written assessment of the report's implications by July-August 2015.

Summary of CSC Findings and Recommendations

In a PBT-based system, as the authors envisage, all hatchery broodstock would be genotyped, thereby resulting in genetic tagging of all hatchery fish. All progeny produced from specific sets of genotyped parents would be reared and released in an identical fashion, thereby generating "PBT release groups" directly analogous to current "CWT release groups". Sampling programs for ocean and freshwater fisheries, spawning escapements, and hatcheries would produce recoveries of fish from PBT release groups via statistical matching of genotypes of sampled individuals to two genotyped hatchery parents (mother and father - offspring *trios*) or to single parents (parent-offspring *pairs*). All parent genotypes would be stored in a coast-wide database of hatchery parent genotypes, and a Regional Mark Information System (RMIS)-like database would be used to store observed and expanded PBT recoveries.

The ability to efficiently and cost-effectively tag the entire production of a hatchery by genotyping a relatively small number of parents has considerable conceptual appeal, and recent preliminary applications of PBT suggest that this approach could feasibly generate recovery data required for current management of Chinook and coho salmon by the Pacific Salmon Commission. Whether or not the PBT approach could (or should) replace or be used to complement the existing CWT system, however, depends on the relative operating costs of the two systems (or of hybrid systems), and on the quality and quantity of information generated by the two systems. It would only make sense to switch to PBT-based or PBT-augmented systems if the operating costs of these systems were less than those of the current CWT system and the information generated were at the very least equivalent, *or* if the costs of a PBT-based system were only modestly greater but the quality and quantity of generated information were considerably greater than for CWT. Assessing the relative costs of competing schemes is, in principle, relatively straightforward. Costs of applying and recovering CWTs are well-defined and well documented. Costs of genotyping required to implement PBT have fluctuated

considerably across laboratories and technologies, but in principle meaningful costs per fish of genotyping can be based on current experience and technology or on anticipated near-term changes in technologies. In their calculations of the relative costs of a number of alternative PBT-based systems (a synopsis of report methodologies and findings is presented as Appendix II), the authors have assumed two possible costs per fish of genotyping (192 SNPs):

1. \$7/fish for *genotyping by sequencing*, GBS (a low cost anticipated to be achievable by a new developing technology, but at an unknown time in the future); and,
2. \$22.50/fish for genotyping by *exonuclease-based sequencing*, ExN (approximate current cost for an existing proven technology).

It is much more difficult to place a monetary value on information generated from tag recovery data, even though modern salmon management is heavily reliant on such data. The authors of the report made no attempt to quantify the relative value of information generated from PBT-based and CWT-based systems, but instead restricted their analyses to the more limited comparison of total operating costs of three of their five proposed alternative PBT-based systems with those of the existing CWT system. (Note: "total operating costs" excluded costs of sampling required to collected heads (CWT) or genetic material (PBT), per the RFP instructions. Sampling costs were assumed to be identical for PBT or CWT systems.)

Using the two alternative genotyping costs per fish and existing data on costs per fish of applying adipose fin clips (ADC), inserting CWT or blank agency wire, etc., and data summarizing hatchery releases and recent ocean fishery sampling statistics, the authors found that the total costs (tagging plus recovery) of all three of the alternative PBT-based systems (and variants of these systems) exceeded those of the existing CWT system for the current ExN genotyping method, but that the total costs of a "hybrid" system (two variants) were similar to or slightly less than total costs of the existing CWT system for the low-cost GBS genotyping method. (In hybrid systems, PBT would be used to tag all hatchery fish, but CWT would be used to tag all natural-origin fish.) The high estimated costs of non-hybrid PBT-based systems relative to the existing CWT system were primarily due to two factors: (a) inability to directly apply the PBT concept to natural-origin smolts which cannot be tagged via genotyping of parents, but must instead be genotyped individually and recovered using DNA fingerprinting; and (b) difficulties in design of a recovery system which can cost-effectively reduce the number of ADC and unmarked fish which would need to be genotyped in ocean and freshwater sampling programs. (The second of these factors is a direct consequence of mass marking in which large numbers of hatchery fish are now released with ADC but without CWT, associated mark-selective fisheries, and double-index tagged (DIT) releases of unmarked fish with CWTs.)

As noted above, for the hybrid systems *which assume low GBS genotyping costs*, hatchery fish would be all tagged with PBT but natural-origin fish (and possibly also some very small hatchery

populations and special unplanned releases) would be tagged with ADC+CWT as at present. Thus, the hybrid schemes would require simultaneous operation of two coordinated coast-wide systems: one for PBT releases and recoveries, and one for CWT releases and recoveries. For one of the hybrid systems considered, Alternative 5, the calculated total operating cost was just slightly higher (\$19.02 million) than the equivalent cost of the existing CWT system (\$18.87 million). A second hybrid system, Alternative 5a, assumed that some secondary mutilation mark (e.g., left ventral fin clip) could be used to allow visual identification of ADC fish that belonged to PBT release groups (or non-ADC fish that belonged to DIT groups), thereby greatly reducing genotyping sample sizes. Calculated total cost for Alternative 5a (\$16.46 million) was less than that for the existing CWT system. Calculated *break even* costs per genotype (cost per fish which make a proposed alternative hybrid systems equal in cost to the existing CWT system) were \$6.84/fish and \$12.97/fish for Alternatives 5 and 5a, respectively.

Based on our review of the authors' report, members of the CSC conclude the following:

- 1. An exclusively PBT-based system, though intrinsically feasible, is not cost-effective at this time when compared to the existing CWT system.**
- 2. Cost-effectiveness of the proposed hybrid systems is unclear at present and appears to depend on whether or not the proposed approximately \$7/fish genotyping cost of a GBS system is a realistic value for genotyping in the near future.**
- 3. If a \$7/fish cost per fish for GBS could be achieved in the future, additional factors should be considered in assessing the relative costs of PBT- and CWT-based system. These factors were outside the scope of the report and include the following:**
 - **Assessment of infrastructure costs.** In their calculations of the relative costs of the proposed hybrid systems, the authors did not consider costs associated with the coast-wide infrastructure and databases that would be needed to maintain two distinct systems: a PBT system and a CWT system. A comprehensive comparison with the existing CWT system warrants consideration of such costs.
 - **Assessment of the probability that a hybrid system would be "sustainable"** in the long term for both hatchery and wild stock tagging. In the proposed hybrid systems, PBT is used to tag close to 100% of all hatchery releases. Coast-wide support for the CWT system might wane if it were used only to generate recoveries of natural-origin fish. CWT tagging of natural-origin fish is currently of relatively small magnitude when compared to overall levels of CWT tagging of hatchery fish and also varies regionally in importance. We note, for example, that natural origin CWT tagging is clearly of great importance for naturally spawning populations of Chinook salmon in southeast Alaska

(including the transboundary Taku and Stikine rivers) and in the Lewis River and Hanford Reach of the Columbia River, but natural origin Chinook salmon are not marked in British Columbia. Wild stocks of coho salmon are marked with CWT in both British Columbia and Washington for use in the Coho Technical Committee management model. Total CWT recoveries from natural-origin fish are very small relative to those from all CWT'd hatchery populations combined.

- **Assessment of the feasibility of a secondary external mutilation mark.** The CSC considers it highly unlikely that a suitable secondary external mutilation mark will become available to support implementation of the more economically attractive hybrid system, Alternative 5a. Indeed, the desirability of identifying such a secondary mutilation mark was noted in the 2005 Expert Panel Report on the Future of the CWT Program (in the context of sampling problems associated with mass marking and large scale release of ADC fish without CWT). No suitable mark has yet been identified/introduced due to concerns over mark-induced mortality (especially for Chinook populations with subyearling smolts), suspected large errors of mark recognition at recovery, and suspected difficulties of developing auto-tagging procedures for asymmetric mark types. Instead, electronic tag detection (ETD) was introduced to identify presence of CWT among ADC fish (or among unmarked fish with CWT that belong to DIT groups).
- **Assessment of capital costs.** The Report did not consider in detail capital costs that would be required to establish a new coast-wide PBT-based tag recovery system, yet such costs need to be appropriately assessed to support a more rigorous comparison of system costs.

4. The report makes clear that a number of problematic issues with the existing CWT-based system that were identified in the 2005 Expert Panel Report also pose problems for development of a PBT-based system. One such issue is mass-marking for mark-selective fisheries. For either CWT or PBT, sampling complexity and cost are greatly increased due to mass-marking, the resolution of data is sometimes decreased, and the DIT approach (to assess fishery impacts on unmarked populations) has yet to be fully implemented. (Ocean and freshwater fisheries seem generally not sampled for catches of DIT fish: see the most recent Selective Fishery Evaluation Committee report). When stocks are exposed to MSF, the fishery exploitation rates experienced by AD+CWT hatchery fish can no longer be assumed the same as those for unmarked natural origin populations of conservation concern.

Although the report indicates that a PBT-based system is theoretically feasible and could generate the same information as currently generated by the CWT program, the CSC concludes that the existing CWT system remains a more cost-effective system for providing the information required for PSC management models. Further study of the issues we have identified above is recommended for a more comprehensive comparison of the costs of CWT- and PBT-Based systems. The CSC also recognizes the substantial value of data that may be generated by genotyping parents and their returning progeny at fish hatcheries. Such data, if augmented by estimated age-specific mortality rates based on CWT recovery data, could be used to establish inheritance of traits such as age at maturity, fecundity or growth rates, to assess variation in family size, and for many other purposes that could enhance our scientific understanding of Pacific salmon and steelhead and strengthen fishery management practices and hatchery operations. In light of this, and considering the pace of development and downward evolution of costs in the PBT-field, the CSCS recommends that a reassessment of the relative costs and merits of the PBT-based or hybrid PBT/CWT systems should be undertaken again in five years or possibly sooner if technological changes or significant reductions in cost warrant it.

The CSC believes that the authors of the report have provided an outstanding and objective assessment of the feasibility and costs of implementing a PBT-based system for management of Pacific salmon. We thank them for their willingness to take on a very difficult task with very modest funding, and we commend them for their efforts. We also thank the members of our Oversight Committee for their time and effort supporting the CSC members in their preparation of this review.

Finally, we note that the CSC had also originally proposed issuance of an RFP for an assessment of the current status and cost of miniaturized RFID devices that might alternatively replace the CWT in a system that would otherwise be essentially unchanged. In principle, miniaturized RFID tags would allow real-time (and possibly repeated) non-lethal recoveries and elimination of costs associated with extraction and reading of CWTs. Although this proposal was not supported, the CSC continues to recognize the merits of such a study and advocates for its initiation in the immediate future. In the meantime, the CSCS strongly encourages the PSC and its cooperating agency partners to fully support the existing coordinated coast-wide CWT system.

APPENDIX I. Posted RFP.

REQUEST FOR PROPOSALS:

EVALUATION OF THE FEASIBILITY AND COST-EFFECTIVENESS OF DEVELOPING A COORDINATED COAST-WIDE TAG RECOVERY SYSTEM USING PARENTAL BASED TAGGING (PBT)

BACKGROUND STATEMENT

The coast-wide Coded Wire Tag (CWT) Recovery System was developed in the early 1970s and for the past 40 years has generated critical information that supports the management of Chinook and coho salmon fisheries along the Pacific Coast of North America, from central California to southeast Alaska. The importance of CWT recovery data for salmon management is exemplified in the 1985 Pacific Salmon Treaty (Memorandum of Understanding, August 13, 1985, Section B: Data Sharing, 1985 PST Agreement) that obligates the US and Canada to maintain the CWT system to support management of PSC salmon fisheries.

The level of coast-wide coordination that has been accomplished with the CWT recovery system represents an unprecedented achievement in collaborative and cooperative management of salmon fisheries. Recent issues raised by mass marking and mark-selective fisheries, as well as reduced or insecure funding for certain aspects of the CWT system, have generated recent consideration of the future of the CWT system. A 2005 Expert Panel report prepared by the Pacific Salmon Commission concluded that the CWT system was clearly the only viable means to generate critical information required for salmon management “for at least the next five to ten years”. The time therefore appears ripe to reevaluate this assessment.

Since 2005, a new genetic approach, termed Parental Based Tagging (PBT), has emerged as a potential alternative to the CWT system and substantial experience has been gained in application of this approach on local scales (i.e., within watersheds). Although some proponents of PBT have argued or implied that PBT should quickly replace the CWT system on a coast-wide basis, many fishery scientists who have for many years used CWT recovery data for fishery management are highly skeptical that an effective and highly coordinated coast-wide PBT system could be developed and provide the same type and level of information as the CWT system for all stocks of interest. There is also skepticism that it could be operated in a cost-competitive fashion when compared with the existing CWT system.

No transition from the coast-wide CWT system to any alternative approach, such as PBT or perhaps new RFID tags, would make sense unless it met the following criteria:

1. The alternative system would need to have long-term annual operating costs that would be no more than or, ideally, substantially less than that of the existing CWT system.
2. The alternative system would need to generate at least the information that is currently generated from the CWT system via run reconstruction (cohort) analyses of estimated recoveries from individual CWT release groups.

Assuming that transition from the existing CWT system to a coast-wide system based on PBT might be feasible and cost-effective, it is important to recognize that it would be highly desirable to maintain (and secure funding for) both systems for at least one full Chinook salmon brood cycle (5 years) so that direct empirical comparisons could be made concerning the performance of the two systems and comparability of information generated from the two systems. Therefore, unless the cost of a coast-wide PBT system were *substantially* less than that of the existing CWT system, a transition from the existing CWT system to PBT or some alternative system would not make sense unless:

3. The alternative system delivers additional or novel information, not provided by the existing CWT system, that would inform management of fisheries for coho and Chinook salmon.

REQUEST FOR PROPOSALS

We seek proposals for development of a report that would achieve the following overarching objective:

Evaluate the feasibility and cost of developing a coordinated coast-wide tag recovery program that would be based on the PBT concept.

Requested Report Structure

The developed report would have the following required structure and content and should, wherever judged appropriate, distinguish between issues raised for Chinook as compared to coho salmon:

Part I. Current Status of the CWT System and of the PBT Concept and Applications.

- A. Update on the current status , operation, and concerns with the existing CWT system based on reports and experiences since publication of the 2005 Expert Panel Report on the Future of the Coded Wire Tag Recovery Program for Pacific Salmon. This update should focus on the following specific issues:
 1. Progress and concerns identified by the Coded Wire Tag Improvement Team (CWTIT) since 2005;
 2. Current status of mass marking (100% AD-clip), mark-selective fisheries (MSF: coast-wide extent and locations of implementation) and assessments of MSF impacts for coho and Chinook salmon, and of Pacific coast hatchery marking programs generally (including California);
- B. Overview of the PBT concept and a review of recent applications of this concept, including both published applications and on-going implementations that have not yet generated published reports.

Part II. Structure, Feasibility and Cost of a Coordinated Coast-Wide PBT Tag Recovery System.

- A. Detailed description of the structure of and requirements for a coordinated coast-wide PBT tag recovery system that could allow the same kind of tag group-specific run reconstruction analyses that are currently performed based on recoveries of CWTs. The description must include locations and requirements for tagging and sampling for tag recoveries; address the timeliness of sample analysis for both in-season and post-season applications; quantify the required laboratory capacities (throughput, precision/accuracy of genotyping and assignments, and resolution); identify the computing resources required to perform and store data related to parental assignments; and address coastwide coordination, data sharing, and analytical verification of parental assignments and QA/QC. Requirements should be given separately for a system that would generate information from unmarked (adipose fin intact) fish belonging to paired groups designed to assess impacts of mark-selective fisheries, and for a system that does not attempt to generate this information.
- B. Description of the requirements for hatchery programs to implement a parental-based tagging program, to maintain tagged groups without mixing between different tagged groups, and to accurately assess the number of tagged individuals per tagged group at the time of release. This section would also determine the degree to which substantial hatchery infrastructure changes would be needed to implement PBT.
- C. Assessment of the degree to which this system could or could not deliver estimates of the key life history and fishery parameters that are currently delivered from the CWT program and do so with similar or better accuracy (i.e., consider errors of estimation). Identify areas or issues where implementation of PBT on a coast-wide basis seems most problematic.
- D. Identification of additional information that could be generated from a coast-wide PBT system, over and above the kind of information that is currently generated from CWTs.
- E. Identification of any qualitative benefits that might be realized if PBT were adopted (e.g., no need to remove heads on fish destined for “whole fish” market; no issues re cooperation of fishermen with recovery of heads).
- F. Assessment of whether or not the PBT concept could be applied to tagging of wild stocks, specifically when access to parent spawners is impossible or impractical.
- G. Assess more limited and targeted applications of the PBT technology that could cost-effectively supplement or replace “parts” of the existing CWT system.

- H. Assess the degree to which additional specific issues (see Appendix A) might rule out feasible or cost-effective application of PBT (for fisheries management purposes) on a coast-wide basis.
- I. Quantify the probable range of costs for implementation of a coast-wide tag recovery system based on PBT and compare the cost of this system against the costs of supporting the existing CWT tag recovery system. (See Appendix B for further details.)
- J. If judged meaningful, determine the “break-even” cost-per-fish of genotyping that would generate approximately equal costs for support of CWT-based and PBT-based systems.

Proposal Due Date, Available Funding, and Time Frame for Report Development

Proposals are due no later than July 15, 2014.

Funds available to support preparation of the requested report are \$60,000, to cover all expenses (including indirect costs, if any), awarded on a not-to-exceed basis.

We anticipate the following dates for achieving key milestones in development of the requested report:

mid-August 2014:	Bid proposals reviewed by Oversight Committee and selection made. Initial teleconference of contractor (lead party) with Oversight Committee.
early November 2014	Preliminary Report of Progress. Teleconference with members of the Oversight Committee, if judged necessary.
15 December 2014:	Draft report from contractors due for Oversight Committee Review and payment of \$30,000 to the contractor.
15 January 2015:	Comments on Draft report due back to contractor.
11 February 2015:	Presentation of draft findings by RFP contractor to the Pacific Salmon Commission’s science community, including preliminary response to comments.
01 April 2015	Submission of Final Report to the PSC and payment of remaining \$30,000 to contractor.

Requested Respondent Proposal Packages:

Respondents must submit the following:

1. A plan (including timeline and budget costs) for development of the requested report;
2. A listing of individuals proposed to participate in development of the requested report, including identification of their specific areas of expertise, and brief (2 page) CVs for each participating individual;
3. Names and contact information for references who could be contacted concerning prior success in developing reports in response to RFPs;

Selection of Awardee:

The selected awardee must show expertise and understanding regarding the following areas:

1. Salmon fisheries management in the PST and PFMC jurisdictions.
2. Current methods used by PSC technical teams for analysis of CWT data.
3. Procedures for insertion of, sampling for, and detection of CWTs.
4. Theory and application of PBT in salmon research and management in the PST and PFMC areas.

Respondent proposal packages will be evaluated on the basis of their proposed plan as well as the level and relevance of experience possessed by individuals participating in report preparation.

Recommended References for Preparation of Proposals (posted at the PSC web site).

Anderson, E. C. 2012. Large-scale parentage inference with SNPs: an efficient algorithm for statistical confidence of parent pair allocations. *Statistical applications in genetics and molecular biology* 11: article 12. <http://www.psc.org/pubs/csc/Anderson2012.pdf>

Anderson, E.C., and J.C. Garza. 2006. The power of single-nucleotide polymorphisms for large-scale parentage inference. *Genetics* 172: 2567-2582.
<http://www.psc.org/pubs/csc/AndersonAndGarza2006.pdf>

Beacham, T. 2014. Genetic Stock Identification/Parental Based Tagging for Pacific Salmon. Powerpoint presentation given at Strategy Session, February 2014, Seattle.
<ftp://ftp.psc.org/pub/tcchinook/PBT/>

Coded Wire Tag Improvement Team (CWTIT) Annual Reports. Available in annual reports of the Chinook Technical Team (2006-present) :
http://www.psc.org/publications_tech_techcommitteereport.htm#TCCHINOOK

Morishima, G., and M. Alexandersdottir. 2013. Q&A About Parental Based Tagging (PBT). Report prepared for NWITFC.
[http://www.psc.org/pubs/csc/MorishimaAndAlexandersdottir2013ParentalBasedTagging10-17-2013\(1\).pdf](http://www.psc.org/pubs/csc/MorishimaAndAlexandersdottir2013ParentalBasedTagging10-17-2013(1).pdf)

Northwest Power Planning Council Memos and Reports: <http://www.nwcouncil.org/fw/tag/home/> :
specifically see ☐ [FTF Decision memo](#) ([April 30, 2013]), ☐ [IEAB FTF Report](#)

Pacific Salmon Commission. 2005. Report of the expert panel on the future of the coded wire tag recovery program for Pacific salmon. available at
http://www.psc.org/publications_tech_psctechreport.htm See, in particular: Part I (BACKGROUND INFORMATION p. 1-21); Part II (Issues Raised by Mass Marking & Mark-Selective Fisheries; Existing and Future Technologies that Might Complement or Replace the CWT System p. 23-27); p. 79-90 (summary of full parental genotyping); APPENDIX A. Proposed Scheme for Estimation of Total Age-Specific Non-Catch Mortalities to Unmarked Chinook Salmon Subject to a Mixture of Non-Selective and Mark-Selective Fisheries; APPENDIX F. Alternative Schemes for Estimating Total Age-Specific Non-landed Mortalities to Unmarked Salmon Subject to a Mixture of Non-Selective and Mark-Selective Fisheries (166-); Appendix H. Comparison of Sampling Requirements for CWT and Genetic Based Methods(198-207).

Pacific Salmon Commission. 2014. *2013 Exploitation Rate Analysis and Model Calibration. Volume One.* February 2014. Joint Chinook Technical Committee. Available at
http://www.psc.org/publications_tech_techcommitteereport.htm#TCCHINOOK

Steele, C.A., E. C. Anderson, M. W. Ackerman, M. A. Hess, N. R. Campbell, S. R. Narum, and M.R. Campbell. 2013. A validation of parentage-based tagging using hatchery steelhead in the Snake River basin. *Can. J. Fish. Aquat. Sci.* **70**: 1046–1054
http://www.psc.org/pubs/csc/Steele_et_al2013.pdf

Appendix A. ADDITIONAL SPECIFICATIONS FOR REPORT CONTENTS: Assessment of the degree to which the following issues might rule out feasible or cost-effective application of PBT (for fisheries management purposes) on a coast-wide basis. (See Part II. E.)

1. Is there any way to efficiently apply the PBT concept to wild stocks? For example, some wild AK populations that have no hatchery indicators are currently CWT'd (wild smolts), but access to adults for PBT is essentially impossible;
2. How could PBT be used for mark-selective fisheries evaluation? Is there any possible DIT analogue for PBT and, if so, what would the sampling requirements be to achieve the equivalent of DIT groups?
3. Coast-wide coordination of PBT databases and analyses would be required to implement a useful scheme. What genetic data would be reported and to whom? (e.g., just summaries of assignments of sampled fish to PBT parental groups, or genotypes for individual sampled fish)?
4. Achieving the equivalent of CWT release groups (where hatchery fish are released at different times/location/sizes/methods) using PBT would appear to require that all progeny from a particular set of genotyped and spawned parents are held separately from others throughout their rearing prior to release. Would significant new hatchery infrastructure be needed to support such separation of progeny from different sets of genotyped parents? Also important is ensuring that tagged fish are "representative" of all hatchery releases of the same type/time of release. How could this be accomplished? Finally, how could PBT be used to achieve the equivalent of "unanticipated" CWT groups that might need to be released in response to events (e.g., drought or unusually low flows) that could not have been foreseen at the time when parents were spawned?
5. How feasible would it be to develop a consistent and effective coast-wide set of SNPs that could be used at all laboratories, along with a consistent and mutually agreed upon procedures for tissue handling, genotyping, QA/QC, data management, and algorithms for generating assignments to PBT groups?
6. Would detection of PBT- tagged groups occurring at very low proportions in fisheries be a more serious problem for PBT than for CWT?
7. The ability to use electronic detection to locate fish (heads) with CWTs provides an efficient way to screen out 'untagged' fish from fishery or escapement samples. This reduces costs associated with shipping, storing and dissection. Could there be a PBT analogue for this capability?

8. The California Hatchery Scientific Review Group has recently recommended that all hatchery Chinook salmon should be released from CA hatcheries with CWT, but that only a fraction (about 25%) should also be released with externally visible adipose fin clips. Would this marking scheme pose special problems for implementation of PBT?

Appendix B. ADDITIONAL SPECIFICATIONS FOR REPORT CONTENTS: Quantify the probable range of costs for implementation of a coast-wide tag recovery system based on PBT and compare the cost of this system against the costs of supporting the existing CWT tag recovery system. (See Part II.G.)

The contractor shall provide information to compare costs of marking, mark recovery sampling, and mark detection between CWT and PBT. The cost estimates should assume that adipose fin clips will in most areas continue to be used as an indicator of tag presence for either mark type (CWT or PBT) and for mass-marking of hatchery fish released to support mark-selective fisheries. Costs should be calculated for two alternative PBT-based systems: (a) a system that would generate information from unmarked (adipose fin intact) fish belonging to paired groups designed to assess impacts of mark-selective fisheries, and (b) for a system that does not attempt to generate this information. Cost comparisons between PBT-based and CWT-based systems should assume existing levels of CWT tagging for hatchery and wild stocks used for the CTC exploitation rate analysis (Table 2.1, TCCHINOOK (14)-1.v1).

1. Marking Costs

- a. Hatchery Releases. Assume equivalent costs of adipose fin-clipping for both CWT and PBT.
 - i. Estimate the current range of CWT/fish costs for marking hatchery populations and releasing current numbers of fish released with CWT.
 - ii. Estimate the current cost per fish of parental genotyping for marking hatchery population.
 - iii. Apply and compare costs to the average annual releases and average number of parents at hatcheries used for the CTC exploitation rate analysis (Table 2.1, TCCHINOOK (14)-1.v1). (Note: Table of CWT releases will be provided.) Include costs of DIT tagging where indicated.
- b. Wild stock releases. Assume equivalent costs to capture and adipose fin-clip fish.
 - i. Estimate the range of CWT/fish costs for wild populations.
 - ii. Estimate the range of costs per fish to genotype either fin tissue from marked juveniles or from adults to characterize wild population.
 - iii. Apply costs to the average annual tags and escapement levels of the five wild stocks used for the CTC exploitation rate analysis. (Table 2.1, TCCHINOOK (14)-1.v1).

- 2. Fisheries Sampling (Recovery sampling). Assume equivalent costs to screen the same proportion of the catch (typically about 20%) for adipose fin clips and to remove heads or take genetic samples as needed.

- a. Estimate range of costs/head for processing by recovery agencies listed in Table 1, Morishima and Alexanderdottir (2013).
 - b. Estimate range of current genotyping costs/fish for fish required to be processed for PBTs.
 - c. Apply and compare costs to average annual sample statistics detailed in Table 1, Morishima and Alexanderdottir (2013) assuming either that (a) the PBT-based system would generate information from unmarked (adipose fin intact) fish belonging to paired (DIT) groups designed to assess impacts of mark-selective fisheries, or (b) the PBT-based system does not attempt to generate this information.
3. Escapement sampling.
 - a. Hatcheries. Calculate cost of screening fish for presence of CWT and for recovery of CWTs. (Note: PBT costs for escapement sampling have already been accounted for under Marking as all returning individuals must be genotyped.) Apply costs to hatcheries used for CTC exploitation rate analysis as per 1.a.iii.
 - b. Spawning grounds. Assume costs of obtaining escapement samples (carcass or live sampling) are equivalent.
 - i. Apply head processing costs identified in 2.a. to expected number of heads collected on natural spawning grounds.
 - ii. Apply genotypic costs identified in 2.b. to expected number of fish that would need to be genotyped given sampling rates on natural spawning grounds currently used to estimate number of CWTd fish that fail to enter hatcheries.
 - c. Wild stocks. Assume costs of obtaining escapement samples (carcass or live sampling) are equivalent.
 - i. Apply head processing costs identified in 2.a. to expected number of heads from five wild stocks used as CTC exploitation rate indicators.
 - ii. Apply genotyping costs identified in 2.b. to expected number of samples from five wild stocks used as CTC exploitation rate indicators.
4. Discuss comparative costs for coastwide information systems and data management required for the two mark types.
5. If judged meaningful, calculate the genotyping cost per fish that would be allowable if a PBT-based system were to be cost-equivalent to the current CWT program. under two scenarios: 1) PBT is used to develop the equivalent of DIT to assess impacts of mark-selective fisheries; and 2) PBT is not used to develop DIT equivalents

Appendix II. Abbreviated Synopsis of Report Structure, Methods and Contents

The final Report (135 pages in length) directly addressed all of the specific issues identified in the issued RFP (see Appendix I) and was organized around these specific issues. In part as a consequence, the merits of the analysis methods and the implications of the report calculations are difficult to fully grasp from just a single reading. Therefore, in this section we provide an abbreviated synopsis of the Report's content, methods, and findings.

Part I of the Report reviews the current status, operation and concerns regarding the CWT system since the 2005 Expert Panel Report, and also summarizes the parentage-based tagged (PBT) concept and its applications/implementations since 2005. Part II of the Report proposes the structure of five alternative approaches that might be taken to develop exclusively PBT-based systems or to develop hybrid PBT-CWT systems; addresses a number of practical hatchery management issues that need to be addressed to successfully implement PBT (with illustrative examples taken from large-scale application of PBT for hatchery steelhead within the Columbia River system); and presents cost analyses designed to allow comparison of the total annual operating costs of three of the five alternatives (and numerous variants) with total operating costs of the existing CWT system.

Two Report appendices include a review of the Snake River experience in transition to a PBT-based system for steelhead and Chinook salmon, and an assessment of the statistical errors in estimation of PBT recoveries in ocean fisheries (as compared to errors of estimation of CWT recoveries) that might emerge as a consequence of uncertainty in PBT tagging rate. The first of these appendices demonstrates that it is indeed feasible to use the PBT approach on a large scale. The second appendix responds to concerns raised by the OC concerning effects of uncertainty of PBT tagging rates and shows that errors of estimation of PBT recoveries in ocean fisheries would likely be no larger than those for the existing CWT system.

Below we provide brief descriptions of the five alternative systems that were proposed, a brief

review of the methods that were used to compare operating costs of the alternative systems, and we reproduce a number of Report tables that seem most pertinent for summarizing the authors' findings. We also provide an abbreviated glossary of acronyms used in the Report and in this synopsis.

Abbreviated List of Acronyms and Abbreviations

ADC	adipose fin clip
AWT	agency wire tag
CWT	coded wire tag
DIT	double index tagging (paired groups released with ADC+CWT, and CWT only), used to assess fishery impacts for unmarked fish subjected to mark-selective fisheries
ETD	electronic tag detection (use of wands to detect presence of CWT or AWT)
ExN	exonuclease-based sequencing
GBS	genotyping by sequencing
GSI	genetic stock identification
MM	mass marking (marking of all hatchery releases with ADC)
MSF	mark-selective fishery (or fisheries)
PBT	parentage-based tag (or tagging)
RFP	request for proposals
SNP	single nucleotide polymorphism

Five Alternative Approaches for Using PBT to Generate Data Now Generated by CWT System

The existing CWT system is referred to as System 0. Five alternative systems (Systems 1-5) were described in detail and were qualitatively assessed regarding various issues that were raised by specific systems. For each approach, it was assumed that a (highly successful) attempt would be made to genotype 100% of hatchery broodstock at each hatchery (i.e., full parental genotyping, with the exception of rare genotyping failures or hatchery logistics errors). The authors assumed that *all* progeny from specific sets of genotyped parents could be held and

reared separately from other juveniles and released at a common date and location as part of a (relatively small) number of *PBT release groups*. Some of these PBT release groups would be directly analogous to current *CWT release groups* (groups of hatchery fish sharing group-specific common CWT codes, with all members of specific CWT groups released at approximately the same date, size and location, often at sizes, dates and locations that are intended to represent natural populations for which a particular hatchery population serves as an indicator). Whether or not PBT could be fully implemented in this fashion at most hatcheries is unclear, but even if it could not be fully implemented that would not materially alter the issues identified in the report because the numbers of fish released with CWT are usually small (no more than 25% of total releases) compared to the total number of releases made at most hatcheries. We believe that it would generally be logistically feasible at most hatcheries to rear and release all progeny from *some* specific sets of genotyped parents so as to generate the equivalent of existing CWT release groups.

The five alternative systems are as follows:

- **System 1. Replicate Existing CWT system.**

This system uses a combination of PBT, ADC, AWT and ETD to essentially replicate the structure of the existing CWT system, but using PBT instead of CWT as the tag, using ADC as an external mark (to identify hatchery fish as a MM and to support MSF (OR, WA, BC), or as an indicator for the presence of a CWT (CA)), and using AWT to allow identification (via electronic detection, ETD) of members of an ADC+PBT release group (or unmarked PBT DIT group). Natural-origin smolts would be genotyped and ADC to allow later identification via DNA fingerprinting. Ocean and freshwater sampling would rely on presence of ADC to identify hatchery fish, ETD to identify that fish belonged to an ADC PBT release group (or an unmarked PBT DIT group), and genotyping used to establish PBT group membership.

- **System 2. PBT Only.**

For this system, distinct sets of genotyped parents would be used to generate PBT hatchery release groups and associated DIT groups. ADC would be used to identify hatchery fish and marked natural-origin fish as for current MM or constant fractional marking regimes.

Because neither AWT nor ETD would be relied upon, all ADC fish encountered during recovery sampling would need to be genotyped, thereby generating additional information (via GSI) on untagged fish (ADC or unmarked), but also greatly increasing the number of fish that would need to be genotyped. In areas where unmarked members of DIT groups are expected to be present (e.g., in fishery areas where ADC and unclipped fish are both allowed to be captured), some fraction of unclipped fish would need to be genotyped. (Note that the size of PBT DIT groups could be greatly expanded at little cost to allow reduction in ocean fishery sampling rates needed to generate equivalent DIT recoveries but at greatly reduced genotyping expense.)

- **System 3. PBT but with AWT as a secondary mark.**

This system builds on System 2 by using AWT as a “secondary mark” indicating ADC fish that do *not* belong to a PBT release group, thereby reducing genotyping requirements. AWT would be applied to the equivalent of current levels of fish released with ADC but without CWTs. Natural-origin smolts would be genotyped to allow later identification via DNA fingerprinting, and would typically be ADC (unless subject to MSF). ETD (negative detection) would be used to identify ADC-only fish that belonged to PBT release groups or natural-origin ADC tag groups. (Note that the Report authors did not attempt to calculate the annual operating costs of this alternative.)

- **System 4. Combine PBT, ADC, AWT and ETD along with a new at-sea sampling program.**

This complicated system would provide improved information on impacts of MSFs and natural-origin stocks and full details are presented in the Report. Sampling would require observers on fishing vessels in MSF marine and freshwater fisheries. DIT would not be needed for this system. (Note that the Report authors did not attempt to calculate the annual operating costs of this alternative.)

- **System 5. Hybrid PBT/CWT System.**

This system would rely almost exclusively upon PBT to tag hatchery fish but would use ADC+CWT for natural-origin tagging. At hatcheries, genotype all parents for PBT release groups and ADC all members of PBT release groups as for existing levels of ADC. For natural-origin stocks, apply CWT to natural-origin smolts and ADC (unless subject to MSF). Fish in unmarked components of DIT groups would receive CWT but not ADC. ADC + CWT might also be used for low production hatcheries or small and/or unplanned release groups. Ocean and freshwater sampling would screen ADC fish for CWT using ETD and heads would be extracted; sampled ADC fish without CWT would be genotyped.

Relative Costs of PBT-based Alternative Tag Recovery Systems

Operating costs of three of the above alternative PBT schemes (systems 1, 2 and 5 and variants of each) were calculated and compared with the equivalent operating costs of the existing CWT system. Costs include those associated with tagging and marking at release and (some of) those costs associated with recovery. Recovery costs were limited to those associated with extracting/decoding CWTs (for existing CWT system) or genotyping (for PBT systems). Costs for sampling of ocean and freshwater fisheries and spawning escapements to obtain recoveries were assumed to be the same for all systems (including CWT), as requested in the RFP, and were therefore not included in cost calculations. Thus, calculated operating costs for the existing CWT system and the proposed alternative PBT-based systems are less than true total operating costs, as

they exclude costs of sampling required to collected heads (for CWTs) or tissues (for PBT) in sampling of fish in fisheries, spawning escapements and at hatcheries, but they should nevertheless be directly comparable.

Based on an assumption that the mean number of fish released per female Chinook and coho salmon are 3,800 and 1,800, respectively, the authors calculated (from RMIS release statistics) that their recommended full (100%) parental genotyping at hatcheries would require 135,709 Chinook broodstock genotypes and 87,489 coho salmon broodstock genotypes per year. Full parental genotyping would mean that all hatchery releases would be tagged via genotyping of their parents.

Total costs of tag or mark application for ADC, CWT and AWT are based on average cost per fish data provided by various agencies multiplied by the numbers of juvenile fish that would need to be tagged at hatcheries or in natural areas (wild stock tagging). Numbers of fish that are currently released and tagged with and without ADC and CWT are based on an average for 2010-2012, as reported by the RMIS data system, and were summarized in their Table II.I.5 (reproduced below).

For natural populations, the authors estimate that approximately 900,000 fish are currently tagged with CWT and released annually on a coast-wide basis from natural populations, most of which are also ADC. Assumed costs per fish of tagging and marking in different contexts were reported in their Table II.I.8 (reproduced below).

Table II.I.5 Annual average (2010-2012) of total juvenile Chinook and coho salmon released, marked, and tagged by state or province. Source: RMIS database query

State/Province	Total Releases (A)	ADC+CWT (B)	ADC only (C)	CWT only (D)
<i>Chinook</i>				
Alaska	8,733,799	954,632	173,282	0
British Columbia	40,956,206	4,720,789	95,251	121,505
Washington	115,752,460	13,964,150	85,967,225	6,624,108
Idaho	14,916,114	2,001,648	9,274,477	2,586,519
Oregon	31,449,600	6,492,490	21,083,507	1,000,907
California	46,038,662	14,824,064	92,388	42,803
Coastwide Total – Chinook	257,846,840	42,957,774	116,686,130	10,375,841
<i>Coho</i>				
Alaska	27,175,400	923,726	24,324	411
British Columbia	12,264,848	759,501	5,554,653	146,457
Washington	31,763,644	3,006,772	23,250,324	2,469,814
Idaho	384,940	0	0	71,438
Oregon	6,361,877	447,986	5,690,097	152,478
California	788,427	117,391	2,348	66,441
Coastwide Total - Coho	78,739,136	5,255,375	34,521,746	2,907,040

“Decoding” costs per fish were assumed to be \$5/fish for CWT (extraction and reading of a CWT) and were assumed to be either \$7/fish for GBS (genotyping-by-sequencing, a developing technology) or \$22.50/fish for ExN (exonuclease genotyping, an existing technology). These costs per fish were applied to the estimated number of fish for which heads would be needed to be decoded (CWT) or for which genotypes would need to be taken based on Morishima and Alexandersdottirs (2013) coast-wide summaries of recent recovery sampling effort and tag/mark presence for Chinook and coho salmon sampled from primarily ocean fisheries along the Pacific Coast (the authors’ Table II.I.7, reproduced below).

Table II.1.8 Estimated Unit Costs -Marking and Tagging

Step	Estimated Unit Cost (2014 US Dollars)
<i>CWT-based alternative systems</i>	
ADC+CWT • Auto-tagging trailer	0.154
ADC+CWT • Hand tag	0.236
CWT only • Auto-tagging trailer	0.154
CWT only • Hand tag	0.236
ADC only • Auto-tagging trailer	\$ 0.048
ADC only • Hand tag	\$ 0.1095
<i>PBT-based alternative systems</i>	
ADC+AWT • Auto-tagging trailer	\$ 0.104
ADC+AWT • Hand tag	\$ 0.186
ADC only • Auto-tagging trailer	\$ 0.048
ADC only • Hand tag	\$ 0.1095
AWT only • Auto-tagging trailer	\$ 0.104
AWT only • Hand tag	\$ 0.186
ADC+ alternative mark • Auto-tagging trailer	\$ 0.064
ADC+ alternative mark • Hand tag	\$ 0.146
Alternative mark only • Auto-tagging trailer	\$ 0.064
Alternative mark only • Hand tag	\$ 0.146
<i>Natural-origin Stock Tagging</i>	
ADC+CWT • Hand tag	\$ 0.236
ADC only (with tissue sample) • Hand tag	\$ 0.146
ADC+ alternative mark • Hand tag	\$ 0.146
ADC+AWT • Hand tag	\$ 0.186
AWT only • Hand tag	\$ 0.186

Table II.I.7 Annual average (2008-2011) of total sampled adult Chinook and coho salmon sampled and recovered by state or province of sampling. Source for Columns A-D: Table 1, Morishima and Alexandersdottir (2013). U.S. Fed includes fish sampled by USFWS, NMFS, and NMFSNWR. Source for Columns E-F: Authors' calculations as described in section above.

State or Province	Sampled (A)	Processed (B)	Tags Decoded (C)	Ad Clipped, current mark rate (D)	Estimated Ad Clipped 100% mark with DIT (E)	Estimated Ad Clipped 100% mark without DIT (F)
<i>Chinook</i>						
AK	116,369	10,198	6,040	10,198	83,545	85,253
BC	44,049	7,282	2,958	7,785	11,436	12,165
WA	270,612	34,529	30,623	169,366	191,208	197,843
ID	0	0	0	0	0	0
OR	90,415	13,484	12,093	27,088	35,731	36,685
CA	52,161	15,362	14,590	15,360	28,215	28,215
U.S. Fed.	81,852	15,971	14,115	52,540	59,316	61,374
Coastwide Chinook	655,458	96,826	80,419	282,337	409,451	421,535
<i>Coho</i>						
AK	635,861	10,329	7,789	10,291	54,781	55,889
BC	42,119	588	221	636	1,253	1,351
WA	438,469	62,144	32,107	339,317	388,546	403,371
ID	0	0	0	0	0	0
OR	67,406	6,989	6,366	31,168	36,220	37,671
CA	16	8	1	8	10	9
U.S. Fed.	18,695	4,324	4,033	15,199	17,404	18,068
Coastwide Coho	1,202,566	84,382	50,517	396,619	498,214	516,359

Using the information contained in Tables II.I.5, II.I.8, and II.I.7, the authors calculated the annual operating costs of three of the alternative PBT-based systems (and several variants of each) and the existing CWT system for the GBS genotyping scheme (at genotyping cost of \$7/fish and for the ExN genotyping scheme (at genotyping cost of \$22.50/fish). GBS costs were reported in their Table II.I.3 (reproduced below). ExN costs were reported in their Table II.I.4 (reproduced below)

Table II.I.3 Detailed summary of costs by step assuming genotypes performed using GBS (\$7 per genotype). At 100% PBT tag rate, Chinook hatchery broodstock = 135,709 and coho hatchery broodstock = 87,489. All values are millions of U.S. dollars.

System	Mark and Tag	Genotype Parents	Handle Natural-origin Fish	Genotype Natural-origin Fish	Decode Tag	Total
0 CWT	17.71	-	0.25	-	0.91	18.87
1	14.64	1.56	0.20	7.54	1.27	25.20
1a	22.39	1.56	0.20	7.54	6.35	38.04
1b	14.64	0.26	0.20	7.54	1.27	23.90
1c	12.18	1.56	0.16	7.54	1.27	22.70
2	20.98	1.56	0.16	7.54	7.90	38.14
2a	20.98	1.56	0.16	7.54	13.01	43.24
2b	21.64	1.56	0.16	7.54	6.57	37.46
2c	10.38	1.56	0.16	7.54	4.75	24.39
2d	22.78	1.56	0.16	7.54	1.27	33.30
5	12.45	1.56	0.25	-	4.75	19.02
5a	13.37	1.56	0.25	-	1.27	16.46

Table II.I.4 Detailed summary of costs by step assuming genotypes performed using ExN (\$22.50 per genotype). At 100% PBT tag rate, Chinook hatchery broodstock = 135,709 and coho hatchery broodstock = 87,489. All values are millions of U.S. dollars.

System	Mark and Tag	Genotype Parents	Handle Natural-origin Fish	Genotype Natural-origin Fish	Decode Tag	Total
0	17.71	-	0.25	-	0.91	18.87
1	14.64	5.02	0.20	24.22	4.08	48.16
1a	22.39	5.02	0.20	24.22	20.42	72.25
1b	14.64	0.84	0.20	24.22	4.08	43.97
1c	12.18	5.02	0.16	24.22	4.08	45.66
2	20.98	5.02	0.16	24.22	25.40	75.79
2a	20.98	5.02	0.16	24.22	41.80	92.19
2b	21.64	5.02	0.16	24.22	21.10	72.14
2c	10.38	5.02	0.16	24.22	15.28	55.06
2d	22.78	5.02	0.16	24.22	4.08	56.25
5	12.45	5.02	0.25	-	15.28	33.00
5a	13.37	5.02	0.25	-	4.08	22.73

A number of features of the calculated operating costs for the three PBT-based alternative systems compared to the existing CWT system are worth noting:

- Total estimated costs for the ExN genotyping system exceed those of the existing CWT system (\$18.87 million) for all explored alternatives, often by very large

amounts.

- High costs of exclusively PBT-based systems (i.e., non-hybrid systems) are the consequence of high recovery expenses (“Decode Tag”) and high costs of genotyping natural-origin smolts, not of high costs of parental genotyping (tagging).
- The cost of genotyping natural-origin smolts for exclusively PBT-based alternatives is extremely high: \$7.54 million for GBS and \$24.22 million for ExN.
- Estimated total operating costs for the GBS genotyping system are comparable to or lower than the operating costs of the existing CWT system only for the hybrid schemes (Alternatives 5 and 5a). For the hybrid alternatives, all natural-origin smolts would be tagged with CWT.