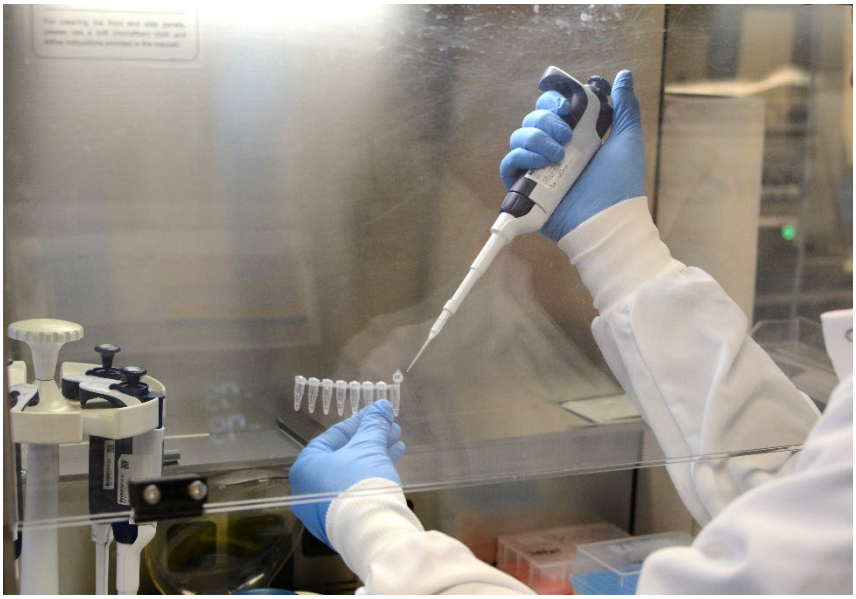
SHERLOCK Genetic Run Assignment of Winter-Run Length-at-Date Chinook Salmon at Salvage Facilities

Water Years 2023 and 2024



State of California
California Natural Resources Agency

Department of Water Resources

Division of Integrated Science and Engineering
Collaborative Science and Innovation Section

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Acronyms and Abbreviations

Term	Abbreviation/Acronym
AIM	ancestry-informative markers
ART	adult return timing
CDFW	California Department of Fish and Wildlife
CFS	Cramer Fish Sciences
CRISPR	clustered regularly interspaced short palindromic repeats
CVP	Central Valley Project
DWR	California Department of Water Resources
ESU	Evolutionarily Significant Unit
GeM	Genetic Monitoring
GSI	genetic stock identification

Term	Abbreviation/Acronym
GT-seq	genotyping-in-thousands
ITP	California Endangered Species Act Incidental Take Permit 2081-2023-054-00, *Long-Term Operation of the State Water Project in the Sacramento-San Joaquin Delta*
LAD	length-at-date
NMFS	National Marine Fisheries Service
SHERLOCK	specific high-sensitivity enzymatic reporter unlocking
SWP	State Water Project
SWP-FF	State Water Project Fish Facilities unit
WR LAD	winter-run length-at-date
WY	Water Year

1 Overview

1.1 Executive Summary

The California Department of Water Resources' (DWR's) Collaborative Science and Innovation Section conducted a pilot study to assess the use of a clustered regularly interspaced short palindromic repeats (CRISPR)-based rapid genetic tool called specific high-sensitivity enzymatic reporter unlocking (SHERLOCK) for Chinook salmon (*Oncorhynchus tshawytscha*) run assignment during Water Years (WYs) 2023 and 2024. SHERLOCK results were obtained from the DWR Genetic Monitoring (GeM) laboratory and were compared to genotyping-by-thousands (GT-seq) results obtained from Cramer Fish Sciences' (CFS') Clemento panel (Clemento et al. 2014) and, to a limited extent, the National Marine Fisheries Service (NMFS) Chinook full GT-seq panel adopted for salvaged Chinook in 2025 (Anderson panel) (Anderson et al. 2025). All genetic results were contextualized with run assignments of salvaged Chinook salmon generated by the size-based and error-prone length-at-date (LAD) model. Recommendations and next steps are outlined here for further consideration of adopting the SHERLOCK assay at salvage facilities.

1.2 Numbers at a Glance

- Across WY 2023 and WY 2024, DWR screened 1,654 DNA samples of Chinook salmon from salvage facilities using SHERLOCK. State Water Project (SWP) and Central Valley Project (CVP) staff collected 17 and 624 winter-run LAD (WR LAD) Chinook salmon at the salvage facilities in WY 2023 and WY 2024, respectively.
- Winter-run identification: SHERLOCK had a 100% concordance rate with GT-seq for homozygous winter-run individuals.
- Individuals heterozygous at SHERLOCK assay loci cannot be resolved to a run type. Other genetic tools were used to assign run types for these fish (10.3% of total samples). These individuals then undergo additional genetic screening (e.g., neutral panels using GT-seq).
- For calling adult return timing (ART) genotypes using Ots28 loci, SHERLOCK had a 98% concordance rate with CFS GT-seq.

2 Background

Chinook salmon entering the SWP and CVP salvage facilities in the Sacramento-San Joaquin Delta (Delta) are assigned to a run type for incidental take reporting and loss threshold triggers. Since 1997, the Delta model LAD criteria have been used to determine run type at salvage. Inaccurate assumptions underlying the model and overlapping juvenile length distributions across runs have prompted increased use of genetic approaches for run assignment. Currently, most genetic approaches use many neutral markers to assign run type based on population structure (e.g., Clemento et al. 2011, Clemento et al. 2014, and Meek et al. 2016).

An alternative approach is to use a small number of near-diagnostic markers, such as a region associated with ART (Thompson et al. 2020), in combination with SHERLOCK, a CRISPR-based detection technology (Baerwald et al. 2023 and Kellner et al. 2019). An adult migration-associated marker/SHERLOCK approach has situational advantages over the current GT-seq method, including the potential to obtain results in under two hours after a fish is collected at salvage facilities and for under $20, in contrast to GT-seq, which costs hundreds of dollars in materials alone and takes at least 12 hours under ideal conditions.

ⓘ Population Structure Versus Diagnostic Markers

Population structure assignment matches up the broader genetic background of unknown fish with those of known reference populations. In contrast, ART markers target a genome region strongly associated with Chinook salmon run types since runs are based on ART. SHERLOCK uses four assays to identify runs: two to distinguish early-returning fish (spring or winter) from late-returning fish (fall- and late-fall) and two to distinguish between spring- and winter-run within the early-returning classification.

2.1 Current Regulations and GT-seq

The 2024 version of DWR's California Endangered Species Act Incidental Take Permit 2081-2023-054-00, *Long-Term Operation of the State Water Project in the Sacramento-San Joaquin Delta* (ITP) (California Department of Water Resources 2025) triggers reductions in water exports upon exceedance of annual and weekly loss thresholds for natural-origin winter-run Chinook per ITP Conditions of Approval 8.2.1, 8.4.3, and 8.4.4. These thresholds are based on data from the *Winter-Run Chinook Juvenile Production Estimate* (California Department of Water Resources 2024), an annual forecast of winter-run Chinook salmon young expected to reach the Delta. WR LAD Chinook salmon taken at the fish salvage facilities are presumed

to be true winter-run until they were screened with GT-seq or SHERLOCK to determine if they are genetically winter-run. Genetic screening may be expedited to avoid hitting thresholds or to inform adaptive management. While SHERLOCK is being piloted, it can also be used in a regulatory context. However, currently, if it disagrees with GT-seq results, then the GT-seq results will be used for regulatory decisions.

2.2 SHERLOCK Pilot Study

The pilot study began in January 2023 and is currently underway at SWP and CVP salvage facilities. Concordance was determined by comparing run type results between SHERLOCK and existing genetic methods (e.g., GT-seq with the Clemento panel). Full-scale adoption of SHERLOCK by both SWP and CVP depends on this pilot study's results and approval of use for regulatory decisions by the California Department of Fish and Wildlife (CDFW) and NMFS. Planning for the 2023 pilot study began in November 2022. Planning activities included developing fish sampling and laboratory protocols, purchasing equipment and supplies, training the SWP Fish Facilities (SWP-FF) unit on laboratory procedures, training SWP and CVP operators on mucus collection protocols, and coordinating with managers. Mucus swab samples were collected briefly in 2023 in addition to fin clip DNA samples. Samples were transported from the facilities by SWP-FF in 2023 and by the Central Valley Tissue Archive in 2024.

3 Results

Key Findings Summary

- WY 2023—Perfect concordance between SHERLOCK and GT-seq for winter-run identification and ART.
- WY 2024—Larger sample size (624 WR LAD fish) with 100% winter-run identification for homozygous fish and 98.6% adjusted concordance rate across all LAD categories with GT-seq. Over 80% of all Chinook sampled were fall- or late-fall-run with late ART genotypes.

The primary regulatory-related product for WR LAD individuals is a classification as either a winter-run or a non-winter-run. All genetic results can identify the three Chinook salmon Evolutionarily Significant Units (ESUs) found in the Central Valley (i.e., winter-run, spring-run, and fall-/late-fall-run), so those results are included below. Assays compared in this report do not reliably distinguish fall-run from late-fall-run salmon, and SHERLOCK assays do not identify tributaries of origin. The NMFS Anderson panel is reported to distinguish between fall- and late-fall-runs.

3.1 Water Year 2023

3.1.1 Winter and Non-Winter Genetic Assignments

SHERLOCK and GT-seq results (i.e., a Clemento panel completed and analyzed by CFS) were compared after samples (mucus and/or fin clips) were taken from the WR LAD samples collected during the sampling period. Three mucus samples did not amplify for SHERLOCK assays. Aside from these three non-amplifications from mucus samples, all other individuals (n=14) show 100% concordance across sample types when comparing SHERLOCK and GT-seq results. For simplicity, this report primarily discusses SHERLOCK results from fin clips. While mucus samples were moderately successful, DNA from fin clips are known to perform consistently well for genotyping fish samples, and comparisons between SHERLOCK and GT-seq were confounded when comparing results across different sample types (i.e., mucus swab versus fin clip). Both SHERLOCK and Clemento panel GT-seq identified one winter-run individual and 16 non-winter samples (Table 1).

Table 1. Genetic Assignments for 17 Winter-Run Length-at-Date Chinook Salmon Collected at Salvage, Water Year 2023

Method	Winter	Non-Winter
GT-Seq (Clemento et al. 2014)	1	16
SHERLOCK (Baerwald et al. 2023)	1	16

3.1.2 Adult Return Timing (Ots28) Genotypes

Both GT-seq and SHERLOCK Chinook assays target the same regions on the Chinook salmon chromosome 28 (Ots28) associated with ART. The two genetic methods had 100% concordance for Ots28 when fin clip DNA was used for both.

3.1.3 Run Type Genetic Assignment

Run Type Concordance

Different GT-seq panels produced different results due to the inclusion of Feather River spring individuals in the spring-run baseline by CFS when using the Clemento panel. However, SHERLOCK and GT-seq were 100% concordant after the Anderson panel (run by a CDFW laboratory) was used to resolve discrepancies.

Table 2. Genotyping Method Run Type Concordance for 16 Winter-Run Length-at-Date Chinook Salmon, Water Year 2023

Fin Clip SHERLOCK Compared to …	Concordance (All Run Types)
Clemento panel GT-seq population structure	56% (9/16 samples)
Anderson panel GT-seq population structure	100% (7/7 samples)

Comparison of run types (including winter-run versus non-winter) requires other genome regions outside of Ots28. For SHERLOCK, after Ots28 genotyping has identified an individual as having an early adult migration genotype (i.e., spring-run or winter-run ESUs), additional assays target another region of the genome to distinguish spring from winter. While GT-seq does produce an ART call, GT-seq run type assignment did not use ART-associated loci on Ots28 as part of its run identification or population assignment before WY 2025. One sample was identified as heterozygous by both Ots28 methods (by DWR GeM and CFS), and this sample was removed from comparisons as SHERLOCK does not produce final run type calls for heterozygous individuals. Individuals genotyped as heterozygous via SHERLOCK were flagged for assignment using a population structure approach such as GT-seq. SHERLOCK and Clemento panel GT-seq had 56% (9/16) concordance when fin

clipping was used for both (Table 2). The CDFW Fisheries Genetics laboratory analyzed the seven individuals with discrepant Clemento panel assignments using the Anderson panel. These individuals were all assigned to fall- or late-fall-run population assignments, concordant with SHERLOCK.

ⓘ GT-Seq Panel Differences

The GT-seq panels discussed in this report are the Clemento panel and the newer Anderson panel. The Clemento panel was developed to determine coastwide population structure. In contrast, the Anderson panel and reference baseline were developed specifically for Chinook salmon genetic stock identification (GSI) within the complex population structure of the California Central Valley.

The Anderson panel was used to characterize individuals with late Ots28 genotypes assigned to spring population structure IDs by the Clemento panel. Data generated from the Anderson panel and presented here used a developmental version of the panel. In late 2024, genetic labs, including CFS, began using a finalized, full version of the Anderson panel with several additional genetic loci.

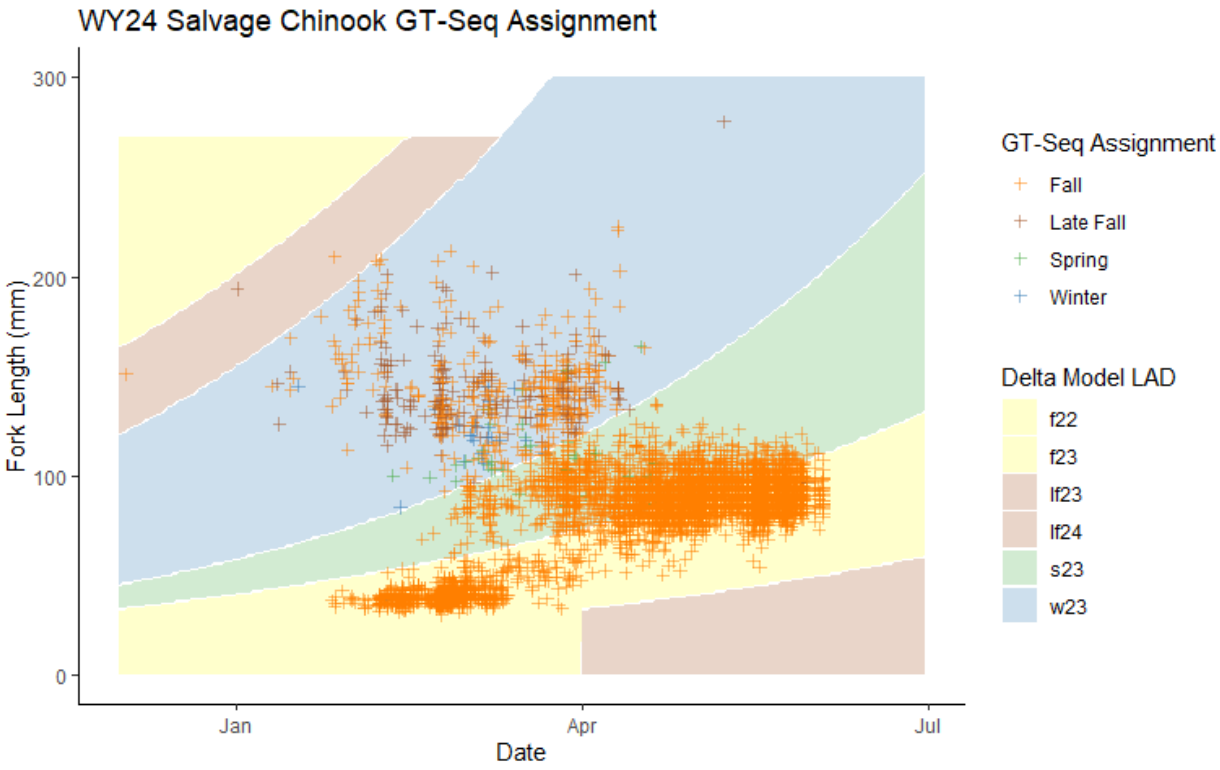
The discrepancies discussed here are due to CFS' decision to include Feather River spring individuals in their reference baseline as part of their spring reporting group. As a result of historical mixing in the Feather River, fall- and spring-run Chinook salmon in the Feather River often have genomic backgrounds resembling either fall or spring populations while retaining the adult migration timing of the other (Meek et al. 2020). The common and current consensus among California salmon geneticists is that Feather River spring individuals should be part of the fall reporting group when using a GSI approach, as their broader genomic background resembles fall fish, and including them in the spring group can lead to misclassification. This is the approach taken by the Anderson panel baseline, which has and will continue to produce more accurate run type assignments for Feather River fish. SHERLOCK was 100% concordant with GT-seq after the Anderson panel (analyzed by CDFW) resolved discrepancies.

3.2 Water Year 2024

WY 2024 saw a noted increase in unmarked juvenile Chinook collected at SWP and CVP salvage facilities compared to WY 2023, with 624 WR LAD individuals (Figure 1). Where run type discrepancies or ambiguities were observed in any genetic method, up to two additional screening methods were used for further investigation: ancestry-informative markers (AIM) (Meek et al. 2016) and the

Anderson panel. Fin clip DNA was used for all genetic screening methods. The DWR GeM laboratory also developed additional process improvements for SHERLOCK and the salvage genetic identification process. These includes piloting pre-prepared, frozen SHERLOCK assays to reduce turnaround times and a Shiny app for customizable visualization of genetic results over time.

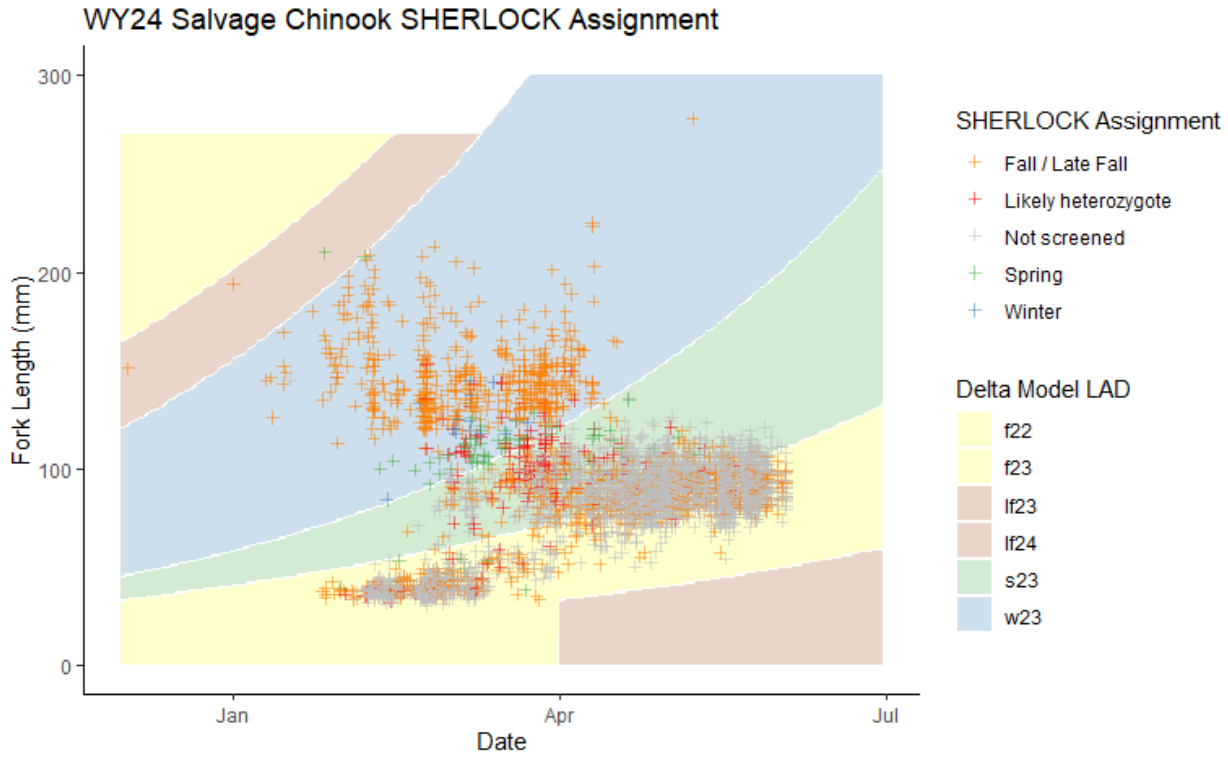
Figure 1. Water Year 2024 Genetic Assignments, All Length-at-Date Groups GT-Seq Assignment of Water Year 2024 Salvage Chinook Salmon



Two steelhead trout were misidentified as WR LAD Chinook at the facilities. CFS identified these individuals as steelhead through their normal GT-seq process. The SHERLOCK Chinook assays showed no signal on these samples, further distinguishing them from actual Chinook salmon results.

In addition to WR LAD fish, the DWR GeM laboratory screened a subsample of 1,023 non-WR LAD Chinook with the early and late SHERLOCK assays to examine concordance in ART genotypes (Figure 2). CFS re-screened individuals of extra concern to CDFW and DWR management (i.e., late ART genotypes with assignment to spring-run using the Clemento panel) using the Anderson panel in early 2025. Where CFS provided Anderson panel run identifications, those results were used for comparison with SHERLOCK in place of Clemento panel results.

Figure 2. SHERLOCK Assignment of Water Year 2024 Salvage Chinook Salmon



3.2.1 Winter and Non-Winter Genetic Assignments

Winter-Run Identification

SHERLOCK and GT-seq were 100% concordant for winter-run identification of homozygous fish. 43% of genetic winter-run fish from the season were heterozygous or ambiguous at the SHERLOCK loci and determined to be winter-run by other genetic methods.

CFS identified 23 genetic winter-run individuals out of the 624 WR LAD individuals screened (3.7%). Of the 23, SHERLOCK unambiguously identified 13 as winter-run. The remaining 10 of 23 individuals (43%) returned heterozygous or ambiguous SHERLOCK assay results, which were confirmed by the DWR GeM laboratory to be winter-run using another genetic method. SHERLOCK identified one fish, C240552SWP, as a winter-run individual, which was categorized as fall-run by CFS, spring-run with AIM, and winter-run by CDFW on the Anderson panel, likely reflecting a fish of ambiguous or mixed ancestry. There were no other winter-/non-winter assignment discrepancies between SHERLOCK and the CFS Clemento panel data.

3.2.2 Adult Return Timing (Ots28) Genotypes

Across the 624 WR LAD and 1,023 non-WR LAD samples screened with the SHERLOCK Ots28 assays, 1,423 individuals were recorded as homozygous by GT-seq and SHERLOCK. SHERLOCK provided ART genotype calls concordant with GT-seq ART results in 98% of these homozygous individuals. Since GT-seq panels and the SHERLOCK assay use different loci for calling ART genotypes, discordance is expected in a fraction of cases and likely generally represents organisms with mixed genotypes rather than misidentification by either method. For ART comparisons, only homozygous genotypes were compared between the two methods.

Fall- and late-fall-run salmon dominated salvage across all LAD categories, with more than 80% of all Chinook salmon collected genetically assigned as fall-run or late-fall-run with late ART genotypes (Table 3).

Table 3. Late and Total Adult Return Timing Genotype Counts of Salvaged Chinook Salmon, Water Year 2024 (GT-Seq)

LAD	Total	Late	Percentage Late	LAD-Genetic Match Rate
Fall	3,957	3,784	95.6%	100%
Spring	2,368	1,995	84.2%	0.2%
Winter	622	518	83.3%	3.8%
Late-fall	3	2	66.7%	66.7%

3.2.3 Run Type Genetic Assignment

SHERLOCK and CFS GT-seq were 94% concordant across all LAD classes in 1,335 out of 1,420 individuals.

SHERLOCK relies solely on its Ots28 assay to sort fish into fall- and late-fall-run versus spring- and winter-run categories. Starting in WY 2025, CFS will use a similar assignment logic agreed upon by NMFS, CDFW, U.S. Fish and Wildlife Service, U.S. Bureau of Reclamation, and DWR geneticists, using ART genotypes to inform ESU assignment. This will likely result in fewer discrepancies between SHERLOCK and GT-seq in the future. Of the discordant individuals, 65 (76.5%) would have been concordant with the new assignment logic. After adjusting for the new assignment logic, SHERLOCK's expected concordance rate with GT-seq using the Anderson panel is estimated to be roughly 98.6% for homozygous fish, which represent 89.7% of the study totals for WYs 2023 and 2024.

3.3 State Water Project and Central Valley Project Salvage Facilities

Facilities Overview

While SWP and CVP have historically similar total Chinook salvage numbers, SWP salvages significantly more WR LAD Chinook (approximately 76% of WR LAD salvaged in WY 2024). SHERLOCK performed consistently at both facilities with similar concordance rates when compared to GT-seq.

SWP and CVP operations in the Delta are jointly operated and historically have similar take numbers (Figure 3). However, the SWP has historically salvaged more winter-run LAD Chinook salmon than the CVP facility (Figure 4). This was also true in WY 2024, with SWP salvaging 76% of the total WR LAD Chinook salmon. SHERLOCK results did not meaningfully vary in concordance rates between the two facilities (Table 4).

Figure 3. Unmarked Chinook Salmon at State Water Project and Central Valley Project Salvage Facilities

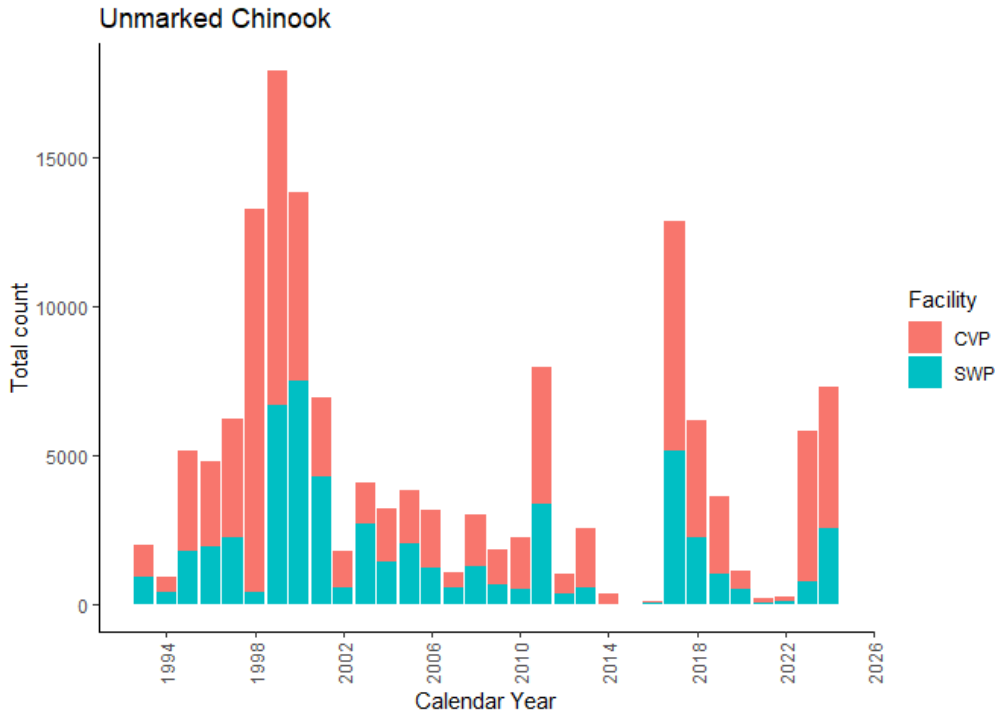
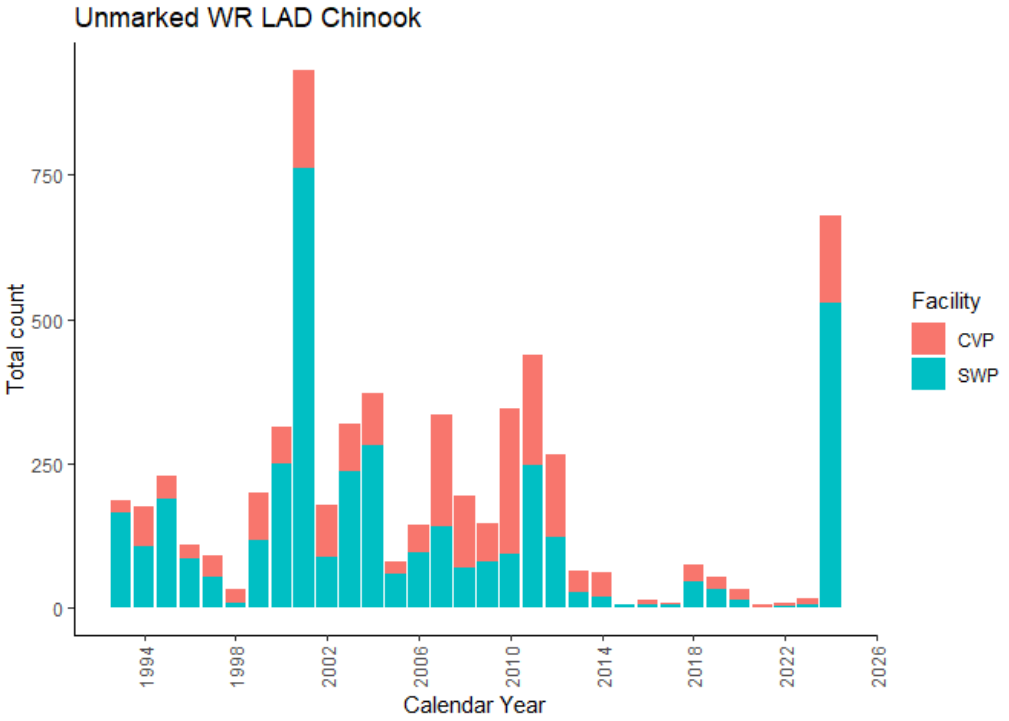


Figure 4. Unmarked Winter-Run Length-at-Date Chinook Salmon at State Water Project and Central Valley Project Salvage Facilities



Note: Where genetic data are available, most of these fish are not genetically winter-run.

Table 4. Concordance Rates Between SHERLOCK and GT-seq for Winter-Run, Run Type, and Adult Return Timing for Comparison Between State Water Project and Central Valley Project Salvage Facilities, Water Year 2024

Facility	Winter-Run Concordance Rate	Run Type Concordance Rate	ART Concordance Rate
CVP	100%	95%	95%
SWP	100%	93%	97%

4 Recommendations

4.1 Adopting SHERLOCK at Salvage Facilities

Recommendation 1

Implement SHERLOCK rapid screening to distinguish early versus late ART genotypes. This can be performed either directly at salvage facilities (for less than nine individuals at a time) or at a dedicated genetic laboratory (for larger sample sizes).

Based on pilot results, SHERLOCK accurately distinguishes early versus late ART genotypes. Adult Chinook salmon with late ART genotypes typically return later in the year and are part of the fall- and late-fall-run ESU. Over 80% of Chinook sampled at salvage facilities were part of the fall- and late-fall-run ESU (Table 3), so most fish must only be screened with the early and late ART SHERLOCK assays to determine their run identity. This will reduce processing resource needs since SHERLOCK processing of a single sample on-site will take about two hours to complete and cost about $30 in supplies. In comparison, processing a single sample at a genetic laboratory in Sacramento using GT-seq takes 20–44 hours (including travel and waiting times) and costs about $400 in materials. As sample numbers increase, GT-seq costs per sample considerably reduce, but the processing time remains the same.

Recommendation 2

For Chinook salmon with early ART genotypes identified by SHERLOCK at salvage facilities, immediately initiate the GT-seq process parallel to SHERLOCK. Both SHERLOCK and GT-seq can be used to distinguish spring- versus winter-run, but GT-seq can also be used to identify tributary of origin for spring-run Chinook salmon populations, enabling the identification of salmon from tributaries of concern.

To determine their run identity, salvaged Chinook salmon with homozygous early ART SHERLOCK genotypes (8.2% in WY 2024) must be screened with spring/winter SHERLOCK assays or GT-seq. While SHERLOCK can identify the majority of early ART fish down to the run-level, immediately escalating all fish with early/early (homozygous early) SHERLOCK results to rapid GT-seq is recommended due to management's desire to have tributary-level source information to identify Core 1 spring-run Chinook salmon. The Anderson panel is a good choice for this purpose,

as it was developed for Central Valley Chinook salmon GSI, and initial data suggest that the identifications are reliable.

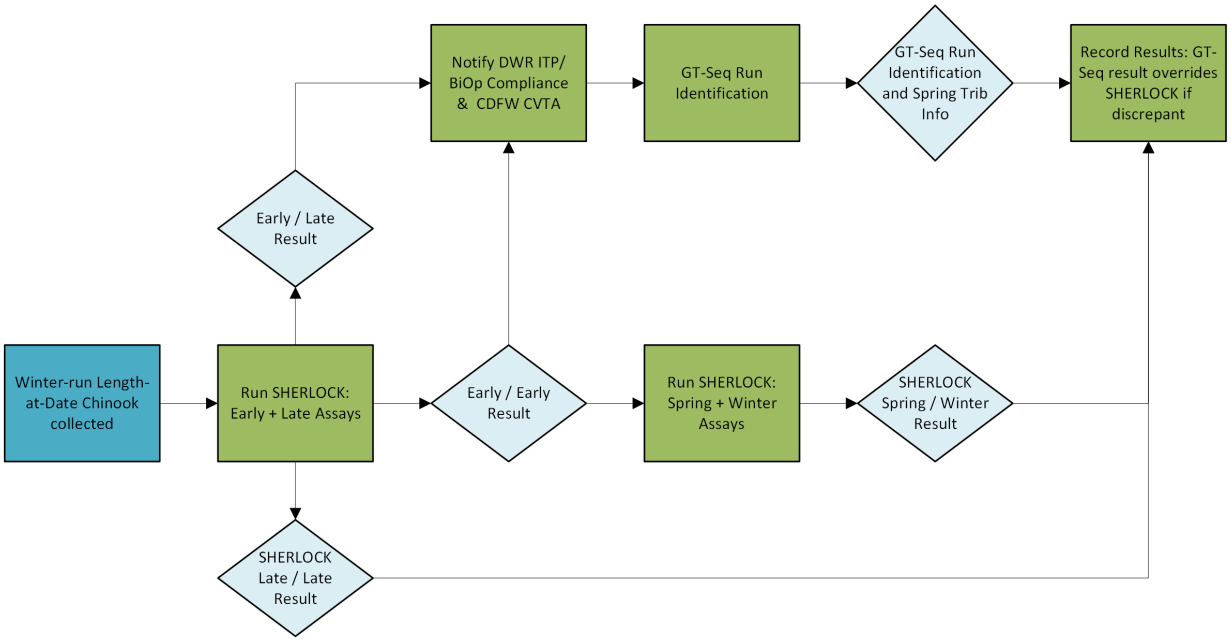
Upon receiving an early/early positive SHERLOCK result, initiating coordination among DWR, CDFW, and DWR's genetic contractor is recommended to obtain GT-seq results. Simultaneously, spring and winter SHERLOCK assays can be used to identify the sample run type with SHERLOCK. Per the ITP, the SHERLOCK assay result may be used for regulatory calls. If the GT-seq run identification results disagree with SHERLOCK results, the GT-seq results will overrule SHERLOCK results for compliance purposes.

Recommendation 3

For Chinook salmon with heterozygous ART assay results, escalate to GT-seq immediately. SHERLOCK cannot produce run identifications for fish with heterozygous genotypes.

SHERLOCK cannot resolve fish that are heterozygous at the loci currently used (approximately 10% of fish at salvage during WYs 2023 and 2024). These fish must be rapidly screened with GT-seq to determine their run identity. Thus, DWR's ITP/BiOp Implementation Section should be immediately notified to coordinate GT-seq of the sample whenever a heterozygous SHERLOCK result is received. Figure 5 is a flowchart of the total recommended process.

Figure 5. Salvage SHERLOCK-GTSeq Hybrid Process Flowchart



For homozygous fish, adopting SHERLOCK at-facility genotyping can produce results as quickly as within two hours of fish collection and fin clip preparation, allowing for rapid decision-making at the facilities. For heterozygous or otherwise ambiguous SHERLOCK assay results, waiting for SHERLOCK results before escalation to GT-seq may delay Central Valley Tissue Archive notification for daily sample transport in occasional cases. Immediate or early morning processing of collected Chinook fin clips would prevent delays and allow rapid GT-seq to continue on the current schedule without disruption. Given the majority of late ART Chinook that would no longer need to undergo the resource-intensive GT-seq process, the overall time savings may compensate for this trade-off even if SHERLOCK cannot be started before regular working hours.

5 Next Steps

5.1 In-Facility Protocol Development

DWR's GeM laboratory began training SWP-FF scientists on in-facility SHERLOCK protocols and data management in early 2025. SWP-FF scientists will collaborate with DWR's GeM laboratory to develop, test, and refine facilities-based workflows, data, and sample management practices. It is possible that, in the future, DWR's GeM laboratory and SWP-FF scientists can also train CVP staff on SHERLOCK protocols and data management practices, if desired. DWR's GeM laboratory will provide ongoing support, including premade SHERLOCK assay mixes, to SWP-FF as needed.

5.2 Personnel

For the long-term adoption of SHERLOCK at the fish facilities, additional staff support may be needed to ensure that SHERLOCK is run promptly and does not impact the current GT-seq workflow. Unmarked WR LAD Chinook salmon are a small fraction of the total fish taken at the facilities, but they arrive at all times of the day and night. SWP-FF does not have 24/7 on-site staff resources and thus cannot process salvage samples in real-time. Furthermore, SWP-FF staff are primarily based in Sacramento and only travel to facilities for research and monitoring activities as needed.

Facility operators have expressed concern that the extra workload of SHERLOCK may impact their other responsibilities or result in out-of-class work. In its current form, SHERLOCK requires careful work, extra training, operating scientific equipment, and adherence to good scientific practices. However, the DWR GeM laboratory and SWP-FF scientists are working together to make protocol improvements to simplify procedures and maximize ease and efficiency.

A human resources solution, which is beyond the scope of this report, will be required to address staffing issues before SHERLOCK can be fully implemented at facilities.

6 Methods

6.1 Winter-Run Length-at-Date Collection

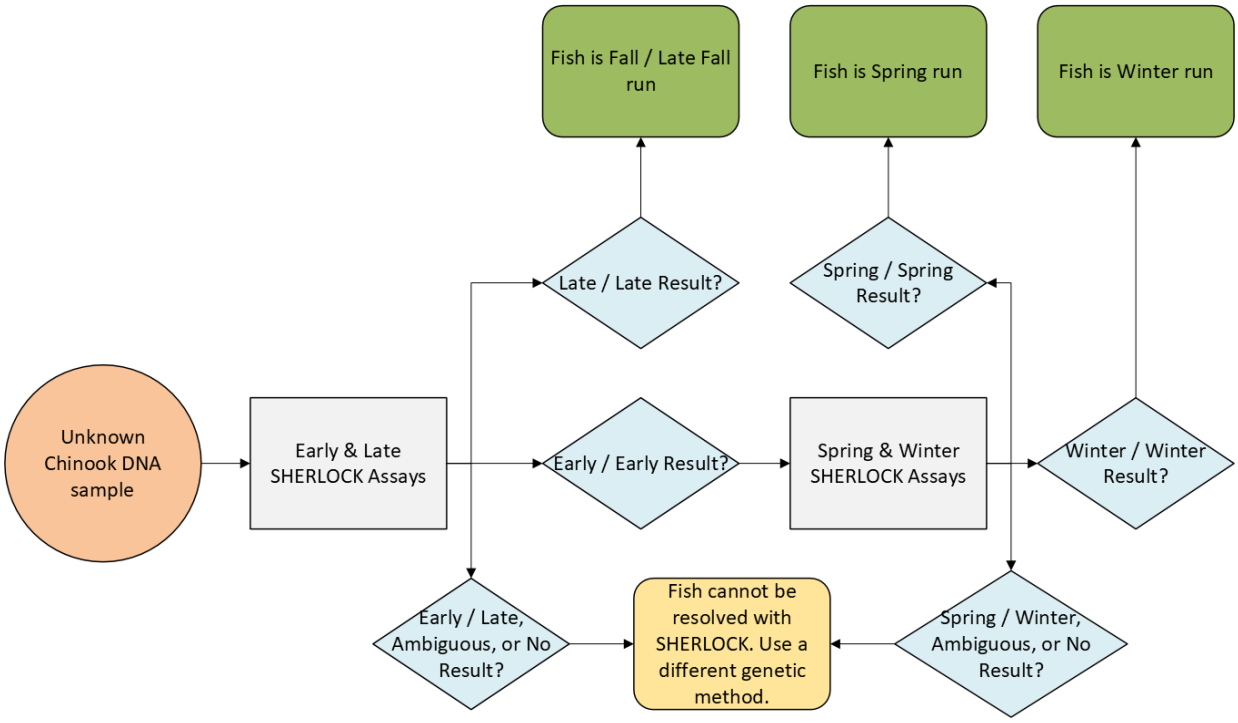
Mucus collections were piloted to increase the speed of results since a DNA extraction step is not always necessary (Baerwald et al. 2023). All WR LAD samples from WY 2023 had both mucus and a fin clip collected. Samples from WY 2024 had only fin clips collected and extracted for DNA. CFS performed fin clip DNA extractions using a Qiagen DNeasy Blood and Tissue Kit, and then aliquots were transferred to the DWR GeM laboratory.

6.2 SHERLOCK

DNA aliquots were diluted in nuclease-free water. Through experimentation, the DWR GeM laboratory has found that dilution up to 100 times still produces satisfactory SHERLOCK results. The SHERLOCK assay was constructed as in Baerwald et al. 2023 with the modification of 2 microliters of template DNA added to each reaction. Samples (e.g., salvaged WR LAD) were screened in triplicate. Depending on the number of samples processed concurrently, reactions were processed with either 384-well assay plates (10 microliter volumes) or 8-strip tubes (20 microliter volumes). Assays were incubated at 37 degrees Celsius for a minimum of one hour. Each run contained at least one positive control, one negative control, and one no-template control per assay. Samples that amplified above a background fluorescence threshold for only the late assay were classified as fall- or late-fall-run. In contrast, samples that amplified only for early were moved onto spring and winter assays (Figure 6).

Samples that amplified for both early and late assays or both spring and winter assays were considered to be heterozygotes. These heterozygous individuals are not resolvable with SHERLOCK; thus, these individuals were additionally screened by DWR with AIM markers (Meek et al. 2016), which is a population structure method. In cases where additional genetic information was helpful for comparison, the CDFW Fishery Genetics laboratory provided GSI with a pre-publication version of the Anderson panel.

Figure 6. Chinook SHERLOCK Flowchart



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8 References

Anderson EC, Clemento AJ, Campell MA, Pearse DE, Beulke AK, Columbus C, Campbell E, Thompson NF, Garza JC. 2025. "A Multipurpose Microhaplotype Panel for Genetic Analysis of California Chinook Salmon." *Evolutionary Applications*. May. https://doi.org/10.1111/eva.70110

Baerwald MR, Funk EC, Goodbla AM, Campbell MA, Thompson T, Meek MH, Schreier, AD. 2023. "Rapid CRISPR-Cas13a genetic identification enables new opportunities for listed Chinook salmon management." *Molecular Ecology Resources* 25(5) https://onlinelibrary.wiley.com/doi/10.1111/1755-0998.13777.

California Department of Water Resources. 2024. *Long-Term Operations of the State Water Project Final Environmental Impact Report*. Prepared by ICF. October. https://water.ca.gov/News/Public-Notices/2024/Oct-24/FEIR-LTO-SWP-2024

California Department of Water Resources. 2025. California Endangered Species Act Incidental Take Permit 2081-2023-054-00, *Long-Term Operation of the State Water Project in the Sacramento-San Joaquin Delta*. Prepared for the California Department of Fish and Wildlife. January. https://cawaterlibrary.net/document/california-endangered-species-act-incidental-take-permit-no-2081-2023-054-00/

Clemento AJ, Abadía-Cardoso A, Starks HA, Garza JC. 2011. "Discovery and characterization of single nucleotide polymorphisms in Chinook salmon, *Oncorhynchus tshawytscha*." *Molecular Ecology Resources*, 11(1):50–66. https://onlinelibrary.wiley.com/doi/10.1111/j.1755-0998.2010.02972.x

Clemento AJ, Crandall ED, Garza JC, Anderson EC. 2014. "Evaluation of a single nucleotide polymorphism baseline for genetic stock identification of Chinook Salmon (Oncorhynchus tshawytscha) in the California Current large marine ecosystem." *Fishery Bulletin* 112(2–3):112–130. https://spo.nmfs.noaa.gov/content/evaluation-single-nucleotide-polymorphism-baseline-genetic-stock-identification-chinook-0

Kellner MJ, Koob JG, Gootenberg JS, Abudayyeh OO, Zhang F. 2019. "SHERLOCK: Nucleic acid detection with CRISPR nucleases." *Nature Protocols*, 14:2986–3012. https://doi.org/10.1038/s41596-019-0210-2

Meek MH, Baerwald MR, Stephens MR, Goodbla A, Miller MR, Tomalty KMH, May B. 2016. "Sequencing improves our ability to study threatened migratory species: Genetic population assignment in California's Central Valley Chinook salmon." *Ecology and Evolution* 6:7706–7716. https://onlinelibrary.wiley.com/doi/10.1002/ece3.2493

Meek MH, Stephens MR, Goodbla A, May B, Baerwald MR. 2020. "Identifying hidden biocomplexity and genomic diversity in Chinook salmon, an imperiled species

with a history of anthropogenic influence." *Canadian Journal of Fisheries and Aquatic Sciences* 77:534–547. https://doi.org/10.1139/cjfas-2019-0171

Thompson NF, Anderson EC, Clemento AJ, Campbell MA, Pearse DE, Hearsey JW, Kinziger AP, Garza JC. 2020. "A complex phenotype in salmon controlled by a simple change in migratory timing." *Science* 370:609–613. https://www.science.org/doi/10.1126/science.aba9059