PACIFIC SALMON FOUNDATION

APPLICATION OF 'OMICS' TECHNOLOGIES IN THE SALMONID ENHANCEMENT PROGRAM

Wendy Vandersteen, PhD

Pacific Salmon Foundation
1682 W 7th Ave, Vancouver, BC, V6J 4S6

2022

www.psf.ca

TABLE OF CONTENTS

Citation for this publication:

Vandersteen, W. 2022. Application of 'Omics' technologies in the Salmonid Enhancement Program. 36p. Pacific Salmon Foundation, Vancouver, BC. doi.org/10.48689/3f201316-5f3f-4f8a-ac79-3a58339c33d5

Cover photos by: top and left: Nicole Christiansen, centre: Colin Middleton and right: Sam James

INTRODUCTION

Hatcheries managed by the Salmonid Enhancement Program (SEP) have been an integral part of the history of salmon management in British Columbia. Releases of billions of juvenile salmon into BC waters have provided fishing opportunities, preserved endangered populations, and enhanced awareness of salmon conservation. These activities have provided ecosystem and societal values, but also involve trade-offs between these benefits and potential impacts/risks on wild salmon populations. These effects can be complex and have proven difficult to quantify. But recently developed "omics" technologies can assist with understanding and estimation of risks, and finding a balance between successful production of hatchery-reared salmon and minimizing impacts on wild salmon.

Pacific Salmon Foundation (Houde 2021) recently reviewed the genomic tools available for research that may help advance hatchery management and salmon production. The review provided technical details on the use of the various tools with examples of their relevance to hatchery deliverables. The intent of this document is to complement Houde's review by providing a user-focused manual that highlights the genomic applications in terms of how they could fit in current hatchery operations and learning.

This document first provides a summary of the relevant regulatory components of the SEP management framework. After a summary of each regulatory component in the SEP management framework is an overview of the broad genomics approach that may be relevant to that management structure. Details on the genomics approach are included at the end of the document with information on the types of questions that can be addressed, considerations that must be made when sampling and storing tissues, and comments on the level of readiness of each technique for implementation.

Photo by: Sam James

GENETICS VERSUS GENOMICS

Genetics refers to the study of the function and composition of single genes. The scope of genetics is the heredity of traits of an organism and the variation of that trait in a population. In contrast, genomics refers to approaches that consider the complete genome of an organism (Fig. 1). The complete genome is the entire collection of coding (i.e. genes) as well as non-coding DNA sequences in an organism. It relies on a combination of techniques to sequence, assemble, and analyze the structure and function of genomes. It differs from classical genetics in that it considers an organism's full complement of hereditary material, rather than one gene or one gene product at a time. Genomics focuses on interactions between loci and alleles within the genome and other interactions such as epistasis, pleitropy, and heterosis. Genomics also considers the impact of environment and epigenetic modifications on gene expression and ultimately expressed phenotype.

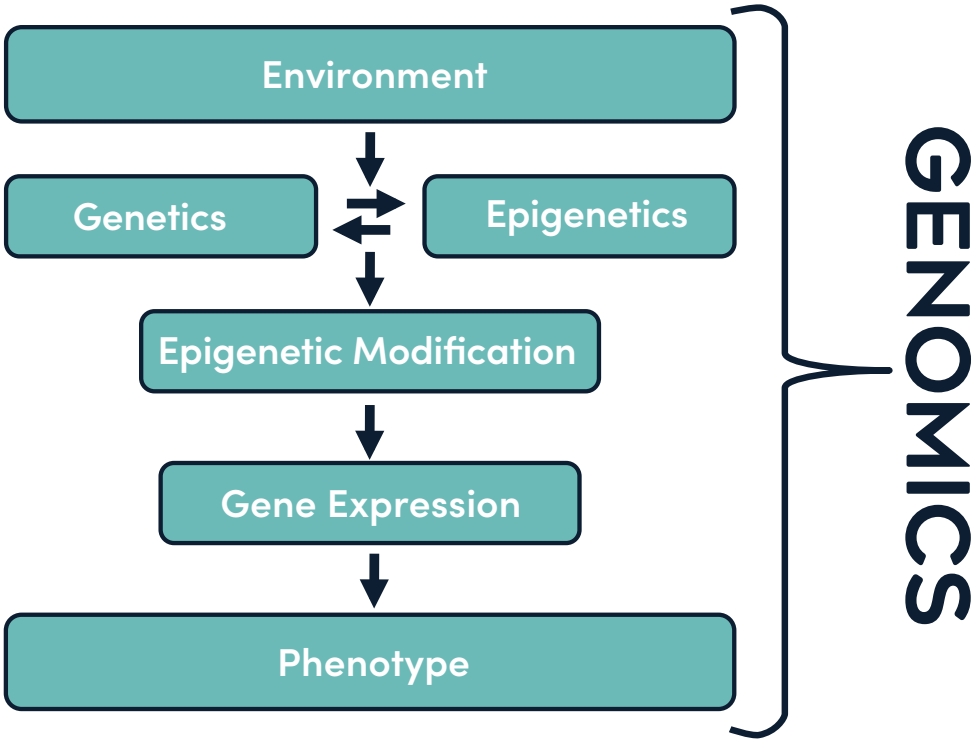


Figure 1. Genomics incorporates genetic control of phenotype in addition to the influence of environment and epigenetic modification on gene expression and subsequent phenotype.

> WHY WOULD YOU WANT TO EXPAND BEYOND GENETICS AND ADD MORE COMPLEXITY WITH GENOMICS?

Genomics approaches can help to provide better resolution to important questions such as determining the genetic basis of phenotypic expression or assessing family-based marine survival, and can be used more broadly as mixed-stock analysis, an adaptive basis for defining management units, quantification of adaptive divergence between populations, or understanding connectivity between populations. Genomics is also useful to help define a smaller subset of genetic markers that can be used to address a specific use, such as development of pathogen screening arrays or smoltification readiness assays. Genomics can be used for exploratory work to understand how populations adapt to climate change or to monitor variation in the environment.

Some genomic tools are currently in use, such as single nucleotide polymorphisms (SNPs) for parental-based tagging (PBT) of Chinook and coho salmon. This enables much greater accuracy in stock identification, but within hatcheries the information could also be used to identify genetic-based differences in family survival rates and marine survival; and/or examine the genetic basis of quantitative genetic traits. The full potential of genomic tools to assist in intensive culture has yet to be appreciated.

Some of the most relevant hatchery questions that genomics could advance would include:

1. PBT and study of family-based survival during hatchery and marine life-stages.
2. Assessment of genotype x environment interactions through assessment of family variation in epigenetic modification to better reduce domestication and improve fitness in the wild.
3. Quantify genetic interactions between hatchery and native populations to directly assess impacts.
4. Develop of assays for pathogen screening and smolt status and assess impact of factors associated with climate change.
5. Refine understanding of the genetic basis of quantitative traits such as age at maturity and develop techniques to measure rate of loss of genetic diversity over generation.

Photo by: Colin Middleton

HOW DO YOU CHOOSE WHERE TO START?

Given the unlimited potential of genomic applications it can be confusing to consider where to start. The flow-diagrams below (Fig. 2a, b) can provide some guidance.

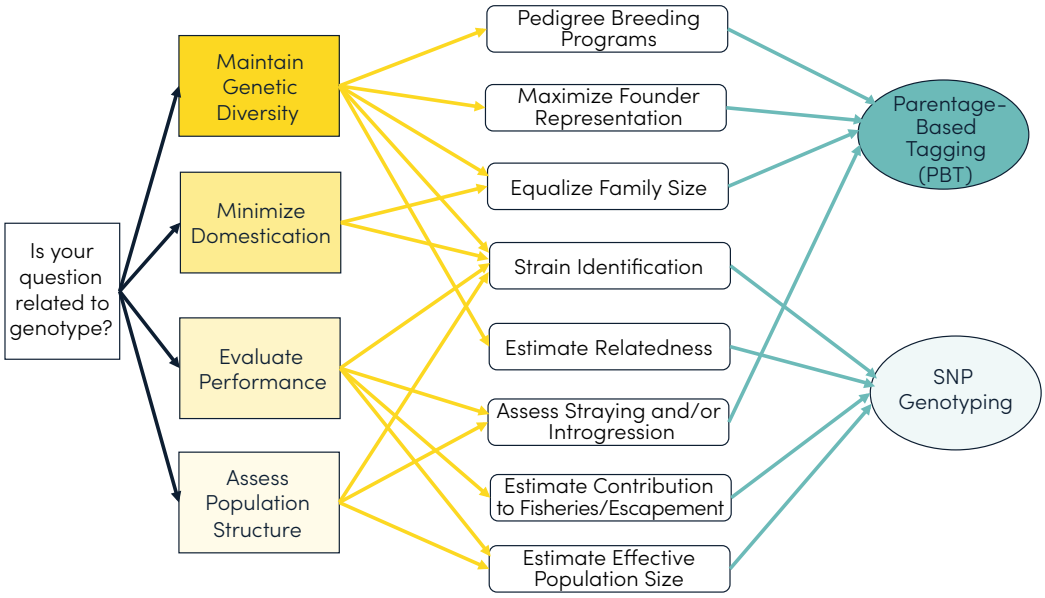


Figure 2a. Questions related to genotype may include a broad number of questions related to how conservation breeding programs are managed. This flow diagram can help to identify some specific objectives that can help meet the overall goal, and defines if this is best approached using specific Parentage-Based Tagging programs or a more general assessment of SNPs.

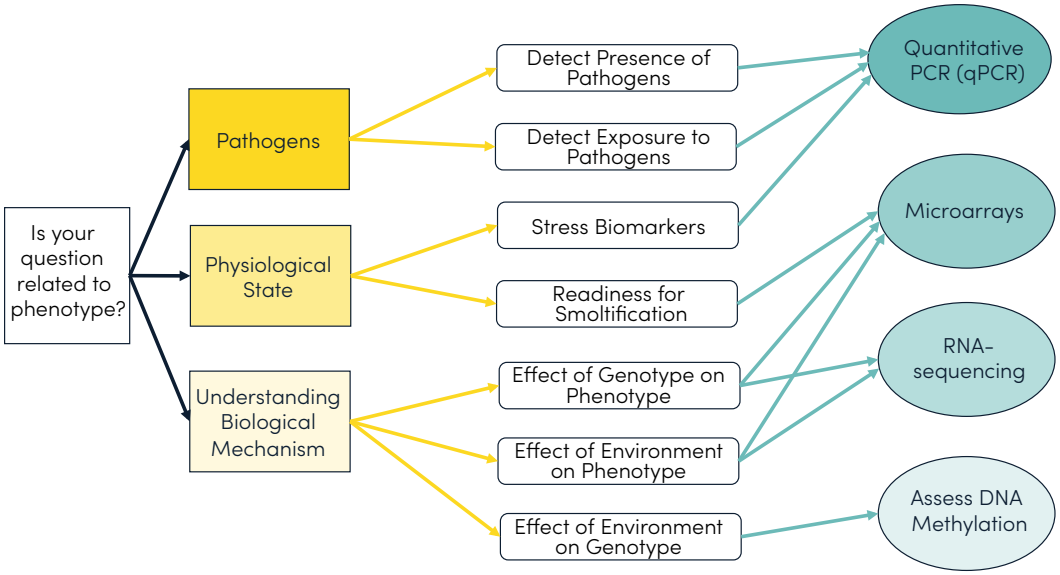


Figure 2b. Goals may not be directly associated with management of breeding programs but may relate more to disease, physiology, or other visible phenotypic states. This flow diagram helps to assign specific objectives to these questions and outlines the best genomic approach to address the desired objective.

WHAT ARE THE OBJECTIVES OF THE SALMON ENHANCEMENT PROGRAM?

There are five objectives outlined within the SEP Production Planning Framework (DFO 2018). Every group of fish at a hatchery fits within at least one of these objectives, and a single group may be assigned more than one objective. Understanding this framework used by SEP is important because the assigned objectives help to determine the guidelines to be followed for fish health management, brood collection, and spawning strategies (within the logistical and resource limitations of the individual hatchery programs). Therefore, these objectives are the basis of activities at SEP and implementation of any genomics technologies will need to be integrated within these biological objectives (these objectives are described in DFO 2018).

1. Harvest

Enhancement for harvest opportunities for First Nations, recreational, or commercial fisheries. Production targets are based on natural spawning estimates and harvest requirements. The focus of this objective is to increase contribution to fisheries.

2. Assessment

Fish are marked and used to obtain stock assessment information to address specific Pacific Region assessment priorities, assess hatchery production, or other defined assessment priorities.

3. Conservation

Enhancement of a stock identified as 'at risk' of or 'highly vulnerable' to extirpation or extinction (DFO 2018). This objective may include re-establishing or rebuilding extinct or extirpated conservation units. Production targets aim for levels required to rebuild the naturally spawning population while maintaining the genetic diversity without changing the genetic variation within the population. Standard guidelines are that salmon returns of hatchery origin should be less than 50% of the target escapement for the population, and brood collection should be less than 30% of total escapement. However, different approaches may be established for specific recovery plans.

4. Rebuilding

Enhancement of a stock that is below apparent carrying capacity. These populations may need to be enhanced due to depletion or to mitigate against habitat loss. Production targets and guidelines may be similar to conservation objectives.

5. Stewardship and Education

These are typically small numbers of fish that are produced for stewardship or educational opportunities.

Photo by: Ryan Miller

As new technologies are developed, and new knowledge becomes available, further improvements and refinements to practices can be made. The figure below (Figure 1, from DFO 2018) provides a visual overview of how the different areas of SEP work together to inform production planning (essentially, how many fish are to be produced from each facility in SEP). The SEP Risk Management Framework outlines an approach to identify, minimize, and manage impacts of hatchery salmon on wild salmon. The SEP Biological Assessment Framework describes methods for quantifying production of hatchery salmon and the effects of hatchery salmon on wild salmon. These frameworks inform the SEP Production Planning Framework that is used to define hatchery production and specifies the targets for SEP Hatchery Production. Genomics technologies can be used at each of these levels to assist in meeting the objectives outlined in the framework.

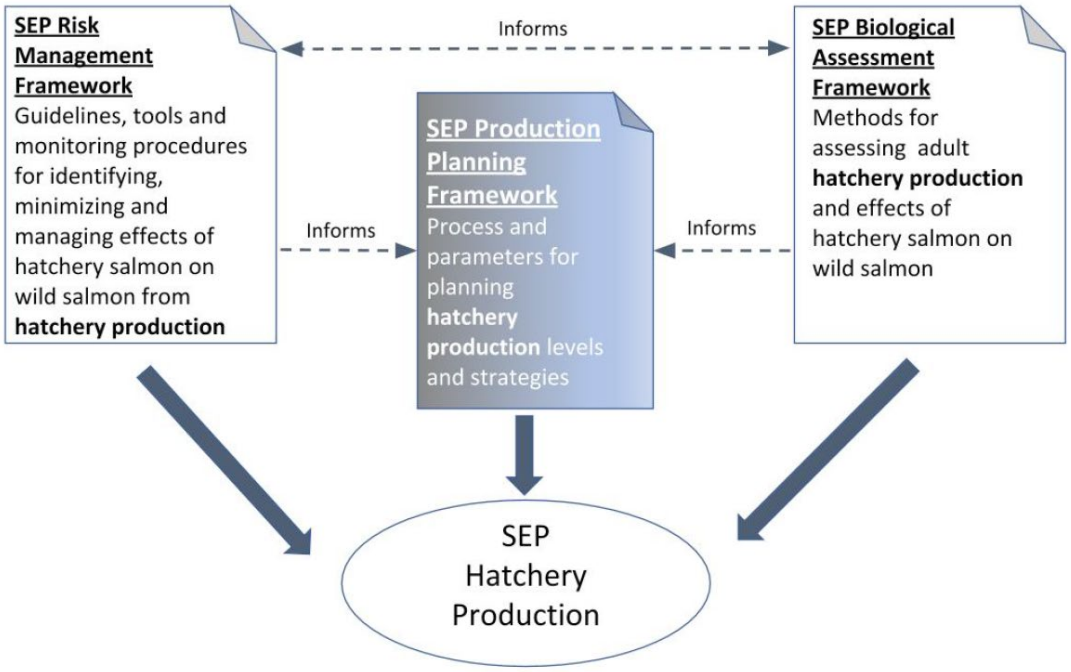


Figure 3. Overview of the SEP Integrated Framework (from DFO 2018).

SEP BIOLOGICAL RISK MANAGEMENT FRAMEWORK

> OVERVIEW OF THE SEP BIOLOGICAL RISK MANAGEMENT FRAMEWORK (DFO 2013)

This SEP framework (DFO 2013) serves to assess and manage the biological risks to wild salmon populations from hatchery production. It provides details on the program components used to assess and manage risks at the level of the production facilities. These assessments also provide input into decision making processes for SEP production planning.

Risk management within this framework requires identification or description of risk. Once identified and defined, risks are assessed for severity and likelihood of occurrence, and then mitigation measures are determined. Risk assessment involves review of all production components according to the following approach (described in DFO 2013):

1. Assess need for enhancement with consideration of benefits and risks.

2. Implement risk management and mitigation measures for any enhancement project, and apply any existing regulations, policies, and operation guidelines.

3. Identify further gaps and uncertainties and develop mechanisms for risk management.

Within the risk management framework eight operational activities have been identified: adult collection, holding, and sorting; spawning practices; adult carcass management; incubation; rearing; release location; release time, size, and condition; and assessment. There is also risk assessment for spawning channel activities. These specific operational activities will be discussed further below in consideration with how they link to daily activities at the hatchery level and the potential for support from genomic technologies.

> HOW CAN GENOMICS HELP WITH BIOLOGICAL RISK MANAGEMENT?

Development of conservation breeding programs could be aided by integration of available genomic tools. These tools can help to estimate the benefits and risks of enhancement, develop risk management and mitigation guidelines, and investigate any uncertainties in developing the conservation strategy.

Every individual hatchery program must balance competing interests between achieving a successful enhancement program versus risks to wild populations that can be categorized as either genetic, disease, or ecological risks. Genetic risks include the potential for intentional or unintentional changes to the genetic makeup of native populations (and includes domestication). Disease risks are just that, unintentional exposure of populations to higher pathogen loads due to increased rearing densities or exposure to novel pathogens within hatchery environments. Ecological risks include factors such as alterations to environmental carrying capacity (such as the availability of limited food resources), increased competition, increased predation, altered behavior, and/or impacts of increased harvest on non-targeted stocks.

It is important to define the level of biological risk that is being assessed and managed, as this will guide which genomic resources may be most applicable. The SEP Risk Management framework assesses risk for each production line, and at each stage of the enhancement process. Risk to wild salmon is defined at the level of the population.

Photo by: Nicole Christiansen

SEP BIOLOGICAL RISK ASSESSMENT FRAMEWORK

> OVERVIEW OF THE SEP BIOLOGICAL RISK ASSESSMENT FRAMEWORK (DFO 2019)

The objectives for the SEP Biological Assessment Framework include (DFO 2019):

1. Program performance measurement – these include assessment requirements for national and public reporting.
2. Program efficiency and optimization – these assessments help to prioritize resources within SEP.
3. Effects of hatchery salmon on wild salmon populations.

To meet these objectives, each enhancement project is assessed on total adult production, survival rate, exploitation rate, biological metrics, and effects of hatchery fish on wild. Assessments may be direct assessments based on mark, release, and recapture; some directly assessed stocks are considered indicator stocks to be representative of other stocks in the area. Assessments may also be indirect and based on bio-standards from indicator stocks (note that juvenile production is directly assessed in both cases).

> HOW CAN GENOMICS HELP WITH BIOLOGICAL RISK ASSESSMENT?

Based on the assessments outlined in the biological assessment framework, metrics are currently estimated for:

1. Total adult production — the number of hatchery fish that survive to adult stage, including fish that contribute to harvest opportunities and escapement.
2. Survival rate — rate of juveniles released relative to total production (defined above).
3. Exploitation rate — ratio of total catch to total production.
4. Index of gene flow — such as Proportionate Natural Influence (PNI) — between natural and hatchery environments; used to assess genetic risk.

The ability to measure and track these indices can be strengthened with the use of available genomics resources. Genomic tools will advance understanding of effects within facilities and can open opportunities to mitigate these effects once understood.

Photo by: Nicole Christiansen

SEP PRODUCTION PLANNING FRAMEWORK

> OVERVIEW OF THE SEP PRODUCTION PLANNING FRAMEWORK (DFO 2018)

SEP production planning is based on identification of priorities that are established based on risks identified by SEP. Current priorities to be addressed by SEP include provision of harvest opportunities, existing Pacific Salmon Treaty commitments, Marine Stewardship Council conditions, and recovery of vulnerable populations. These priorities may fluctuate in ranking over time and by facility due to events such as recruitment failure, habitat loss, or climate change, but the overarching objectives are expected to remain the same.

> HOW CAN GENOMICS HELPS WITH SEP PRODUCTION PLANNING?

Establishing priorities is a dynamic process not only due to factors identified above, but also due to accumulation of new data that may have influence on the decision-making process. Some of the challenges and/or uncertainties include:

1. Incorporation of scientific understanding of salmon population structure and the effects of introgression (gene flow) between hatchery and wild fish.

In particular, SEP priorities must adhere to the Wild Salmon Policy principle that "conservation of wild Pacific salmon and their habitats is the highest priority in resource management decision making" (DFO 2005). Therefore, knowledge is required to understand the relationship between impacts of hatchery rearing on hatchery-influenced and surrounding wild populations. One recently adopted approach is the use of PNI — proportionate natural influence — as an index of gene flow between the natural and hatchery environment. The PNI is a value between 0 and 1 that is calculated from the estimated proportion of wild-origin fish in the hatchery brood and hatchery-origin fish on the spawning grounds. PNI metrics can be used to assign biological designations for populations (wild, wild-stray influenced, integrated-wild, integrated transition, integrated -hatchery) and the biological designation will in turn modify the operation guidelines for brood collection and spawning practices (Withler *et al.* 2018). Based on the concept of PNI, genetic risks can be minimized through management practices that minimize size of the hatchery program, manipulate the composition of the brood-stock (i.e. increase use of natural-origin spawners), or preventing hatchery-origin fish from spawning in the river. As indicated in the introduction, there are trade-offs between genetic risks and socio-economic benefits, and being able to accurately estimate PNI can help to manage these trade-offs. This requires a mechanism to accurately identify wild- and hatchery-origin adults prior to spawning. Use of PBT would provide a direct measure of this uncertainty.

2. Identify strategies to produce the number of adults desired to meet objectives.

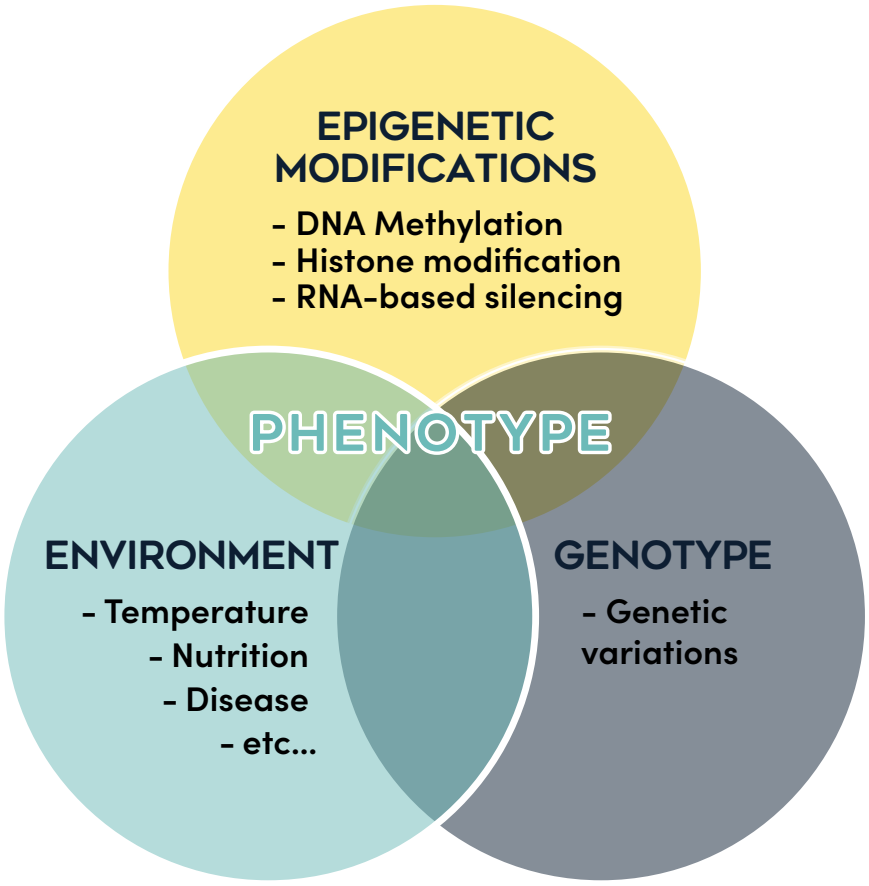
Production planning is a complex process that aims to establish release targets and must consider species interactions, predict effects on existing stocks, harvest, habitat capacity, project capacity, and overall objectives. The production plan must specify donor stocks, egg-take and juvenile release targets, release sites, and stages at release. This challenging task can be directly aided by strategically applied genomics resources.

3. Minimizing epigenetic effects caused by hatchery rearing.

It is known that environmental differences during rearing can cause differences in DNA methylation patterns that subsequently lead to differences in gene expression. It is possible that if these epigenetic changes occur in germ cells, the impacts of the environment could be passed to future generations. It has become evident that the hatchery environment is capable of causing epigenetic impacts on salmon that may reduce their ability to survive in the wild (Granada *et al.* 2017, Roy *et al.* 2021). However, research is required to better understand the major drivers of the hatchery environment causing these changes and develop methods to reduce the potential for epigenetic change.

WHAT EXACTLY ARE EPIGENETIC EFFECTS?

Epigenetic effects are changes in gene activity, or expression, that can arise during development. It is basically the process by which organisms access and use the information stored in their DNA. If access to this information is altered, then you can see different phenotypes arising from the same genotype. Epigenetic effects arise from molecular mechanisms that influence how, where, and when genes are expressed. Gene expression is regulated by the interaction between DNA sequence, histones, and non-histone proteins (such as transcription factors). Chromatin and nucleosomes (you can think of these as DNA scaffolds) are also key players in epigenetic effects because they can alter the folding of DNA and make it more or less exposed to subsequent expression. For a great in-depth review of epigenetics in fish, refer to Best *et al.* 2018.



GENOMIC APPLICATIONS CAN SUPPORT OBJECTIVES OF THE SEP FRAMEWORK

The previous section has provided a general overview of the operational framework of the SEP and provides an indication of how program objectives are prioritized and managed. Now we must direct our focus to the daily operational activities of the SEP at the hatchery level. This is where the raw data are collected, where program activities are conducted, and where genomic tools can be integrated.

For each operational activity, there is a teal box containing suggested genomics approaches that could be useful. More detailed information on the genomics tools is found in the appendices. Additional information on these techniques, including examples of applications in fisheries management, can be found in the report: 'Genetics and 'Omics' Technologies Review for Salmon Hatcheries.

> OVERVIEW OF THE SEP OPERATIONAL ACTIVITIES

At the operation level, hatchery managers need to structure site activities according to the following objectives:

1. Maximize survival of hatchery fish to ensure that programs are being operated as efficiently as possible in terms of hatchery resources and the number of broodstock required to meet production targets.

2. Meet management objectives as determined by SEP Production Planning with input from SEP Risk Management and SEP Biological Assessment.

3. Minimize risk to wild populations while still meeting the objectives of the specific program.

> INTEGRATION OF GENOMICS INTO THE SEP OPERATIONAL ACTIVITIES

These daily operational objectives are impacted by all operational activities that occur at the SEP hatcheries, and for each activity there are examples where the SEP managers would benefit from information that could be provided through application of genomic technologies. The following section discusses the major operational activities identified within the SEP Risk Management Framework (DFO 2013) and provides examples of where genomics tools could assist with management decisions. Note that the suggested genomics tools are highlighted in the box. Click on the teal box to see the table of associated information.

1. Adult Collection, Holding, and Sorting

Hatchery brood should represent the genetic diversity in the original wild population. Removal of fish for hatchery brood should not negatively impact abundance and diversity of wild spawning fish. Managers are instructed to follow the Genetic Management Guidelines for broodstock collection protocols and spawning protocols.

For broodstock collection, guidelines indicate that managers must collect sufficient numbers for the duration of the return migration to ensure genetic diversity is captured. Collection protocols must be appropriate for the population size and enhancement objective, and wild salmon are to be included as part of the broodstock pool. Several challenges are posed here and some available genomics resources may be helpful. These include knowledge on how to sample a broodstock pool that is representative of the original population and tools to help identify wild from hatchery fish.

Genotyping

2. Spawning Practices

Mating design and spawning practice guidelines are based on the same principles as broodstock collection — to ensure that the genetic diversity is represented in the offspring. These guidelines can benefit from use of genetic markers to establish a fully pedigreed program, or as needed to monitor and manage genetic diversity. Below are some critical guidelines with examples of how genomic technologies may assist with meeting these guidelines.

- Minimize removal of brood, especially wild brood, from the natural environment.
- Avoid inbreeding, the mating of genetically related. Inbreeding may lead to a reduction in fitness due to increased expression of deleterious recessive alleles, or through loss of heterozygosity and any associated heterozygosity advantage.
- Avoid outbreeding (of strays).

Genotyping

3. Adult Carcass Management

Salmon carcasses provide abundant nutritional resources to river habitats, and guidelines have been developed for placement of carcasses into these habitats after spawning at hatcheries. There is a minimal risk of transfer of pathogens from carcasses disposed in natal streams for nutrient enrichment. Recommendations are to only use local carcasses to ensure no introduction of novel pathogens to the river system. However, in some cases, carcasses may be placed in nearby streams within the same 'Transfer Zone'. In this case, disease screening is required to ensure that there are not high levels of endemic pathogens or presence of pathogens unique to the watershed. Standard approaches test for specific pathogens of concern using developed assays. More exploratory approaches could also be possible using genomics techniques if new or novel pathogens are suspected.

Pathogen Screening

4. Incubation

Appropriate incubation protocols have been developed for salmon species reared in hatchery environments. Attempts have been made to optimize these protocols to maximize hatch success, and for the most part would match temperatures experienced by eggs developing in the wild. Genetic risks could arise if there is differential mortality of a specific batch of eggs (i.e. due to a disruption in water flow or an equipment malfunction) but these are risks that cannot be mitigated with genomic technologies. There is a chance that differences in incubation environment between the hatchery and natural environment could lead to epigenetic differences between the hatchery and naturally spawned fish. In this case, genomics tools discussed in the "rearing" section below could be applied here. Surface disinfection of eggs prior to incubation mitigates much of the disease risk at this stage.

Photo by: Eiko Jones

5. Rearing

Rearing of salmonids encompasses many variables that can be controlled such as feeding regimes, water temperature, water flow rate, rearing densities, light regimes, etc. These factors may all have some influence on the survival of fish once released from the hatchery. Incorporating techniques associated with genotyping could enable hatchery managers to develop experimental trials that determine the rearing variables associated with better survival at release.

Genotyping

It is possible that altered rearing environments may cause unintentional genetic changes due to domestication. Recent evidence suggests that epigenetic effects could be an even greater concern; if epigenetic changes occur in gametes, these could be passed to future generations.

Detection of Epigenetic Effects

During disease outbreaks effluent may pose risk of transfer of pathogens to wild fish. Disease screening could be useful here.

Pathogen Screening

6. Release Location

Choice of release location has been identified as a possible factor in the risk of outbreeding depression due to the potential for straying of hatchery fish into other non-natal populations. Guidelines recommend releasing fish in locations that maximize the likelihood of return to the release site without straying into other systems.

Genotyping

Choice of release location may also be influenced by the habitat carrying capacity (due to presence of resident fish or habitat quality). These considerations may be important for prioritizing limited hatchery resources in terms of number and/or location of release.

Detection of Epigenetic Effects

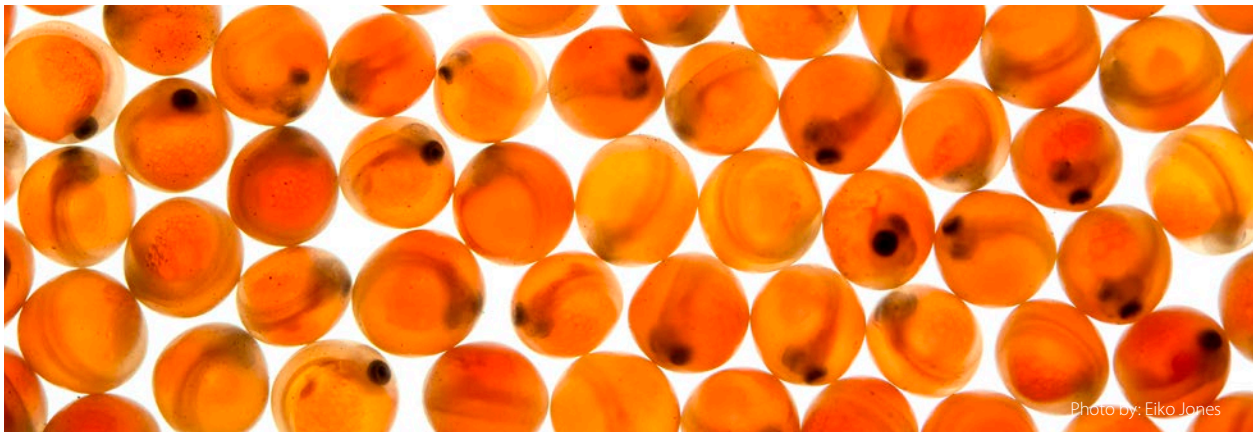


Photo by: Eiko Jones

7. Release Time, Size, Condition

Many factors can influence the survival of juveniles once they are released from the hatchery into the wild. These include the physiological status and health of the fish, as well as ecological factors of the release environment.

The physiological status of the fish can be a significant factor in determining successful transition to the natural environmental and survival to maturity. The level of smoltification can have a strong influence on whether the fish are ready for transfer to seawater. Disease screening prior to release will help to ensure that hatchery fish are not carrying pathogen loads that could potentially impact wild populations or limit their ability to transition to the natural environment.

Gene Expression Biomarkers

Presence of other conspecifics in the environment, especially if resources such as food are limited, could make it challenging for the fish to locate and capture enough food to successfully grow and survive.

Presence of predators in the freshwater environment can have significant impacts on survival.

Environment DNA (eDNA) Analysis

8. Assessment

It is important that assessment activities do not restrict movement of non-target populations (such as may happen with the use of fences to collect and count returning broodstock).

Genotyping

Environment DNA (eDNA) Analysis

9. Spawning Channels

Inadvertent loss of genetic variation could occur if the entire run does not have equal access to the spawning channel.

Genotyping

Environment DNA (eDNA) Analysis

Elevated levels of pathogens could occur due to high loading densities and elevated water temperatures.

Pathogen Screening

> DAILY MANAGEMENT OF HATCHERY PRODUCTION

Within the management framework described above, hatchery managers are tasked with meeting specific production targets, and must prioritize daily activities to best meet those targets. This can be a difficult task and it is important to obtain a frontline perspective on these daily operational challenges. Prioritizing genomic applications that address both daily challenges experienced by hatchery managers and challenges and objectives outlined in the SEP Framework would likely have the greatest impact on improving SEP effectiveness and would have the greatest likelihood for success.

Through personal discussion and a review of interviews conducted with hatchery managers there were several areas of concern that were consistent across different hatcheries and programs. These areas of concern identified by individual hatchery managers fit well with the challenges and objectives outlined in the SEP Management Framework, and provide additional detail based on hands-on perspectives that make it possible to formulate hypotheses that can then be tested through incorporation of genomics techniques. These questions are summarized below along with some examples of how genomics could be applied.

1. What are the epigenetic effects of hatchery rearing?

The prevalence of this concerns indicates that awareness of the potential for epigenetic effects due to hatchery environments is common knowledge with hatchery staff. Within this general concern, there was a specific interest in the epigenetic effects associated with differential immune response as well as better understanding of the long-lasting effects of hatchery rearing (in other words, the potential for heritable epigenetic effects from hatchery environments). Hatchery environment variables of most interest included the effect of rearing container and environmental enrichment. There is also interest in understanding the effect of different rearing strategies (i.e. spawning channels, fed fry, 2-year smolt program) and whether this information could be subsequently used to support program planning and allow for weighting of impacts of hatchery/wild interactions based on the origin of the hatchery progeny.

Whole Genome Bisulfite Sequencing can be used to assess epigenetic variation across the entire genome. This approach could be used to examine the impact of specific components of the hatchery environment, such as rearing density or water temperature, on DNA methylation patterns and assess if these changes are associated with differential fitness due to disease susceptibility or other factors.

2. How do we better optimize release timing?

Managers have realized that establishing the timing of release is a complicated question that is impacted by many factors including the physiological state of the fish, presence of predators (i.e. Sherker *et al.* 2021 and references therein) or competitors in the receiving environment, availability of food, and many other identified and unknown factors. In particular, managers wanted to know if it was possible to link release timing with marine productivity to ensure better survival of hatchery fish and less impact on wild populations. There was also a specific interest in incorporating measures of fish physiology as a guide to optimize release timing decisions as well as the feed regime of the fish prior to release.

Genomic resources could be applied to test many interesting hypotheses associated with this topic. Hypotheses should be developed based on the specific relevant environmental characteristics of the hatchery initiating the study. Some examples include:

- Use of Parentage-based Tagging to track differential success of groups exposed to different variables in their rearing environment to determine which conditions were associated with better survival after release;
- Use of gene expression arrays (i.e. Fit-Chips) to assess the physiological state of smolts to determine the gene expression profile that is most strongly associated with better survival after release; and
- Development of environmental DNA analysis to assess trends in marine productivity and/or presence of predators to better time releases to maximize survival.

3. How can we adapt to climate change?

Managers, especially those with a long history withing the SEP, have intimate understanding of the variation and change in environmental conditions over time and the impact it could have on the operations and the fish stocks.

One important piece of the response to climate change is ensuring that populations retain the genetic potential for adaptation to climate change. This means that diversity within and among populations should be prioritized instead of focusing solely on preservation of the species as a whole. This concept is also referred to as the "portfolio effect" and highlights the importance of having sufficient genetic diversity within a population to adapt to future (and presumably greater) environmental variation (see Price *et al.* 2021 and references therein).

Understanding the genetic basis of traits such as adaptation to changing water temperatures, adaptation to new food sources, or changes in run timing will provide some understanding of the ability of a population to respond to these changes in their environment. Applying genomics techniques with an experimental approach can help to assess the level of inter- and intra-population variation, develop understanding of the importance of variation in specific traits, and provide an ability to assess evolutionary potential in a population. This would require incorporation of both genotyping and gene expression approaches, and could be developed into a comprehensive research program.

Pathogen screening will also be an important part of the approach to climate change adaptation. Changes in environmental conditions may lead to differences in pathogen prevalence, and/or new pathogens, due to: altered migrations routes of salmon populations, shifts in species distribution, and increased stress increasing susceptibility to pathogens.

Photo by: Nicole Christiansen

CHALLENGES AND LIMITATIONS

From the examples listed above, it is clear that use of molecular resources can provide tools that help to support the objectives of the SEP. There is strong support for the use of genomics to reduce inbreeding depression, ensure use of local populations, and help to monitor the status of wild populations in enhanced systems. Genomic tools help to support adaptive management approaches to salmon conservation and can be used to refine optimal release strategies to ensure efficient use of hatchery resources. Despite these valid benefits, the challenges associated with using large-scale genomics data to refine management practices are well acknowledged (and these include both challenges for adoption of the techniques as well as challenges of the techniques themselves). Hatchery managers intimately understand the complexity of this research and the impact that variable environments across years and habitats have on the ability to use these techniques in a manner that provides reliable and useful information. However, we hope that the material presented here shows how application of genomics techniques can actually help to better understand variation due to environment and is worth the time and money.

One technical challenge is the use of evidence of local adaptation to define conservation units. As genomes become analysed in more depth it becomes easier to detect loci associated with local adaptation. However, increasing the number of conservation units to maintain these local adaptations may not necessarily be the best option for an effective conservation strategy (Waples *et al.* 2020), and genomic data must be considered in context with other influencing factors.

Use of more novel and innovative genomic technologies such as eDNA assessment (Baillie *et al.* 2019) require rigorous positive and negative controls to ensure reliability of results. For the eDNA example, these data can be strongly influenced by contamination and environmental conditions (such as water temperature). Verification and development of rigorous guidelines for sampling, data analysis, and interpretation is recommended prior to using these data for management decisions. When developing any new tools, not just eDNA assessments, it is important to budget adequate time and money for preliminary verification and development of the approach to address specific questions.

The science underlying these "omics" tools, and the approaches required for data handling and analysis, can be complex, and represents a major technical challenge to uptake of these techniques. A lack of understanding of the potential value of genetic information has been identified as one of the impediments of uptake of this technology to fisheries management (Bernatchez *et al.* 2017). Hatchery managers, program managers, and biologists may require strong scientific support to identify areas where genomic tools could be beneficial, and to help develop a specific approach to ensure reliable and meaningful data are generated. This gap between integration of genomics resources with conservation strategies can be mitigated with improved communication, availability of training material that will increase confidence in being able to discuss potential genomic applications, and availability of funding for genomics approaches to conservation challenges (Taylor *et al.* 2017). These can be achieved by ensuring communications are done using plain language and facilitating collaborative opportunities between hatchery managers and geneticists.

There may also be challenges for adoption that are due to non-technical factors. There must be appreciation for the fact that often other management decisions within the hatchery program may appear to outweigh the effort and cost of incorporating genomic approaches. With this limitation in mind, geneticists should ensure that proposed techniques and approaches are reliable and robust, with consideration made for the relative cost of different approaches. For example, McKinney *et al.* 2020 found that SNP discovery based on RADseq followed by GT-seq panel development was a cost-effective approach that was successfully used to discriminate population structure in stocks that have historically been difficult to assess. Studies such as these reflect the importance of realizing that a reduced set of focused markers are easier and more cost-effective to assay, increasing likelihood of uptake into fisheries management. With more in-depth understanding of the benefits of using genomic approaches it should become apparent that the costs of not incorporating these approaches may be higher than the cost of incorporating these technologies into SEP management. For example, a comprehensive PBT program would increase efficiencies overall in addition to providing direct measurement of data important for SEP management decisions.

One approach to developing capacity for the implementation of genomics techniques within the SEP is to develop some hatcheries as research facilities. Personnel and resources could be made available to these research hatcheries to assess the value of incorporating these approaches into the SEP. They could undergo research on improving hatchery effectiveness and ability to respond to climate change and thereby act as a genomic resource for other hatcheries. This would facilitate development and retention of the required skillset to understand and implement these approaches.

GENOTYPING TECHNIQUES

TECHNIQUE	SCALE	SAMPLE	STORAGE REQUIREMENTS	INFORMATION CAN BE USED TO...	WHY?	LEVEL OF READINESS	LIMITATIONS / ACCESSIBILITY
Parentage-Based Tagging (PBT) using SNP Genotyping (Steele *et al.* 2019)	Set of identified genetic markers	Fin clips, tissue, or blood	Frozen at -20C or in 95% ethanol or dry storage on absorptive paper	Develop pedigreed breeding programs	Minimize inbreeding	Emerging, and in use	Requires database of parental genotypes
				Estimate contribution of hatchery fish to fisheries and escapment	Improve estimates of Total Adult Production and Survival Rate, and provide a direct measure of accuracy of Coded Wire Tag data	Emerging, and in use	Requires database of genotypes; see Beacham *et al.* 2020 for an example of use.
				Evaluate group performance	Determine environmental factors associated with better survival	Emerging, and in use	Requires an appropriate experimental design and collection of parental and offspring genotypes
				Quantify or detect straying	Optimize choice of release location to minimize straying and subsequent outbreeding depression; ensure maximum returns to hatchery	Emerging, and in use	Requires database linking genotypes to parents or strains
				Age or sex determination		Emerging, and in use	Age identification would require reference to database of parents over all possible generations
				Equalize family sizes at fertilization or release	Limit loss of genetic diversity and genetic adaptation to the hatchery	Emerging	Requires reference to PBT program

TECHNIQUE	SCALE	SAMPLE	STORAGE REQUIREMENTS	INFORMATION CAN BE USED TO...	WHY?	LEVEL OF READINESS	LIMITATIONS / ACCESSIBILITY
SNP Genotyping	Whole genome	Fin clips, tissue, or blood	Frozen at -20C or in 95% ethanol	Obtain estimates of linkage disequilibrium to estimate effective population size	Assessing trends in effective population size can provide some indication if a program is increasing or decreasing a population	Emerging	Maintaining effective population size also ensure adaptive potential (i.e. Franklin and Frankham 1998).
				Differentiate composition of mixed fisheries	Ensure stocks of concern are not being targeted	Some development required	Diagnostic SNP panels could be developed to identify specific strains
				Determine level of genetic introgression	Assess gene flow between hatchery and wild populations	In use for research	
				Estimate relatedness and inbreeding coefficients	Identify inbreeding depression and management practices associated with better preservation of genetic integrity	In use for research	
				Define population structure in combination with PBT	Determine genetic differences between hatchery and wild populations; identify genetic markers associated with local adaptation	Emerging	
				Species / strain identification	Prevent inadvertent capture of non-target species / strains	Some development required	Diagnostic SNP panels could be developed to identify specific strains
				Identify hatchery versus wild fish	Limit adaptation to the hatchery environment by selecting wild fish for spawning, if possible, or ensuring wild brood remain in the native environment to spawn	Some development required	Diagnostic SNP panels could be developed to identify specific strains

GENE EXPRESSION TECHNIQUES

TECHNIQUE	SCALE	SAMPLE	STORAGE REQUIREMENTS	INFORMATION CAN BE USED TO...	WHY?	LEVEL OF READINESS	LIMITATIONS / ACCESSIBILITY
Quantitative PCR (qPCR)	Specific gene targets	High quality tissue samples	Frozen at -80C or stored in RNALater	Compare relative or absolute quantification of gene transcripts from RNA	Detect exposure to pathogens	In use	Many pathogen specific qPCR tests have been developed
					Measure stress biomarkers	In use	Many stress biomarkers have been identified and can be analysed with qPCR
Microarrays	Known set of ESTs (expressed sequence tags) bound to a microarray slide	High quality tissue samples	Frozen at -80C or stored in RNALater	Assess physiological status of fish	Assess readiness for smoltification; assess disease states	In use	Well-defined analysis protocols are in place; microarray slides are available for salmonids
					Detect exposure to pathogens	In use	Well-defined analysis protocols are in place; microarray slides are available for salmonids
RNA-sequencing	Identifies a larger number of differentially expressed genes	High quality tissue samples	Frozen at -80C or stored in RNALater	Provide more insight into biological mechanism	Understand effect of environment on phenotype; assess appropriateness of transplants; predict ability to respond to habitat change	Ready to use	Requires appropriated experimental design; more complex and extensive bioinformatic analysis

PATHOGEN SCREENING

TECHNIQUE	SCALE	SAMPLE	STORAGE REQUIREMENTS	INFORMATION CAN BE USED TO...	WHY?	LEVEL OF READINESS	LIMITATIONS / ACCESSIBILITY
DNA Sequencing	Whole Genome	Fin clips, tissue, or blood	Frozen at -20C or in 95% ethanol	Detect and identify pathogens	Assess health status of brood; test for presence of pathogens in water	In use for research	Requires a high-level of bioinformatics ability
Gene Expression Approaches	Known set of ESTs (expressed sequence tags) bound to a microarray slide	High quality tissue samples	Frozen at -80C or stored in RNALater	Assess physiological status of fish	Detect exposure to pathogens	In use	Well-defined analysis proto-cols are in place; microarray slides are available for salmonids
Protein biomarkers	Specific	High quality tissue samples	Frozen at -80C	Detect presence of proteins associated with disease of pathogens	Detect exposure to pathogens and help with vaccine development	In use for research	Biomarkers have been identified for some diseases and/or pathogen antigens

ENVIRONMENTAL DNA (eDNA) SCREENING

TECHNIQUE	SCALE	SAMPLE	STORAGE REQUIREMENTS	INFORMATION CAN BE USED TO...	WHY?	LIMITATIONS / ACCESSIBILITY / LEVEL OF READINESS
Sequence-based metabar-coding	Genome	Water must be filtered	Preserved with alcohol precipitation, filtration, and/or chemical preservation	Assess genetic variation in specific habitats or watersheds	Understand genetic variation within and among populations	Experimental development required; Method of sampling and storage can have strong impact on results
				Species presence / absence	Estimate habitat carrying capacity; assess level of competitors and/ or predators in the environment	Experimental development required; Method of sampling and storage can have strong impact on results
				Species abundance	Estimate habitat carrying capacity; assess level of competitors and/ or predators in the environment	Experimental development required; Method of sampling and storage can have strong impact on results

DETECTION OF EPIGENETIC EFFECTS

TECHNIQUE	SCALE	SAMPLE	STORAGE REQUIREMENTS	INFORMATION CAN BE USED TO...	WHY?	LIMITATIONS / ACCESSIBILITY / LEVEL OF READINESS
Bisulfite Sequencing	Whole genome or specific genes	High quality tissue samples	Frozen at -80C	Measure DNA methylation	Detect epigenetic effects from different environmental conditions	If testing specific genes, these may first need to identified using gene expression approaches (i.e. Beemelmanns *et al.* 2021)
Chromatin Immuno-precipitation (ChIP)	Specific protein-DNA interactions or whole genome	High quality tissue samples	Frozen at -80C	Track changes in DNA methylation and chromatin structure	Detect epigenetic effects from different environmental conditions	

RESOURCES

Baillie SM, McGowan C, May-McNally S, Leggatt R, Sutherland BJG, Robinson S. 2019. Environmental DNA and its applications to Fisheries and Oceans Canada: National needs and priorities. Can. Tech. Rep. Fish. Aquat. Sci. 3329: xiv + 84p.

Beemelmanns A, Ribas L, Anastasiadi D, Moraleda-Prados J, Zanuzzo FS, Rise ML, Gamperl AK. 2021. DNA methylation dynamics in Atlantic salmon (Salmo salar) challenged with high temperature and moderate hypoxia. Frontiers in Marine Science 14:604878.

Bernatchez L. *et al.* 2017. Harnessing the power of genomics to secure the future of seafood. Trends in Ecology and Evolution 32:665-680.

Best C, Ikert H, Kostyniuk DJ, Craig PM, Navarro-Martin L, Marandel L, Mennigen JA. 2018. Epigenetics in teleost fish: from molecular mechanisms to physiological phenotypes. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology. 224:210-244.

Beacham TD, Jonsen K, McIntosh B, Sutherland BJG, Willis D, Lynch C, Wallace C. 2020. Large-scale parentage-based tagging and genetic stock identification applied in assessing mixed-stock fisheries and hatchery brood stocks for coho salmon in British Columbia, Canada. Canadian Journal of Fisheries and Aquatic Sciences 77:1505-1517.

DFO. 2019. SEP Biological Assessment Framework. Salmonid Enhancement Program, Fisheries and Oceans Canada, Pacific Region.

DFO. 2018. SEP Production planning: a framework. Salmonid Enhancement Program, Fisheries and Oceans Canada, Pacific Region.

DFO. 2016. A compilation of operation and planning guidelines for the Salmonid Enhancement Program. Salmonid Enhancement Program, Fisheries and Oceans Canada, Pacific Region.

DFO. 2013. A biological risk management framework for enhancing salmon in the Pacific Region. Salmonid Enhancement Program, Fisheries and Oceans Canada, Pacific Region.

DFO. 2005. Canada's policy for conservation of wild Pacific salmon. Fisheries and Oceans Canada, Pacific Region.

Franklin IR and Frankham R. 1998. How large must populations be to retain evolutionary potential? Animal Conservation 1:69-73.

Granada L, Lemos MFL, Cabral HN, Bossier P, Novais SC. 2017. Epigenetics in aquaculture – the last frontier. Reviews in Aquaculture 10: 994-1013.

Leitwein M, Laporte M, Le Luyer J, Mohns K, Normandeau E, Withler R, Bernatchez L. 2021. Epigenomic modifications induced by hatchery rearing persist in germ line cells of adult salmon after their oceanic migration. Evolutionary Applications 14: 2402– 2413.

McKinney GJ, Pascal CE, Templin WD, Gilk-Baumer SE, Dann TH, Seeb LW, Seeb JE. 2020. Dense SNP panels resolve closely related Chinook salmon populations. Canadian Journal of Fisheries and Aquatic Sciences 77:451-461.

Price MHH, Moore JW, Connors BM, Wilson KL, Reynolds JD. 2021. Porfolio simplification arising from a century of change in salmon population diversity and artificial production. Journal of Applied Ecology 58: 1477– 1486.

Roy S, Kumar V, Behera BK, Das BK. 2021. Epigenetics: Perspectives and potential in aquaculture. In: Pandey PK, Parhi J (Eds) Advances in Fisheries Biotechnology. Springer, Singapore. https://doi.org/10.1007/978-981-16-3215-0_9

Sherker ZT, Pellett K, Atkinson J, Damborg J, Trites AW. 2021. Pacific Great Blue Herons (*Ardea herodias fannini*) consume thousands of juvenile salmon (*Oncorhynchus* spp). Canadian Journal of Zoology 99:349-361.

Steele CA, Hess M, Narum S, Campbell M. 2019. Parentage-based tagging: reviewing the implementation of a new tool for an old problem. Fisheries 44:412-422.

Taylor HR, Dussex N, van Heezik Y. 2017. Bridging the conservation genetics gap by identifying barriers to implementation for conservation practioners. Global Ecology and Conservation 10:231-242.

Waples RS, Naish KA, Primmer CR. 2020. Conservation and management of salmon in the age of genomics. Annual Review of Animal Biosciences 8:117-143.

Waters CD, Hard JJ, Fast DE, Knudsen CM, Bosch WJ, Naish KA. 2020. Genomic and phenotypic effects of inbreeding across two different hatchery management regimes in Chinook salmon. Molecular Ecology 29:658-672.

Withler RE, Bradford MJ, Willis DM, Holt C. 2018. Genetically based targets for enhanced contributions to Canadian Pacific Chinook salmon populations. DFO Can. Sci. Advis. Sec. Res. Doc. 2018/019. Xii + 88 p.

Weitemier K, Penaluna BE, Hauck LL, Longway LJ, Garcia T, Cross R. 2021. Estimating the genetic diversity of Pacific salmon and trout using multigene eDNA metabarcoding. Molecular Ecology 30: 4970-4990. DOI: 10.1111/mec.15811

GLOSSARY

Biomarker – a measurable product in an organism indicative of a specific characteristic such as exposure to pathogen or toxin, disease, or physiological state.

DNA barcoding – use of a specific set of genetic markers to identify an organism.

DNA methylation – addition of a methyl group to DNA that can modify gene expression; an epigenetic modification.

DNA sequencing – determining the order of nucleotides in a sequence of DNA.

Environmental DNA (eDNA) – DNA extracted from environmental samples, such as water or soil.

Epigenetic effects – changes to the physical structure of DNA from external factors; these changes in structure include DNA methylation and histone modification, and lead to changes in gene expression; if the changes occur in DNA contained within gametes (eggs or sperm) then the epigenetic effects could become hereditary.

Genetic markers – identifiable sequences of DNA that are transmitted from one generation to the next; these markers arise through mutation and can be used to identify species, population, strain, family, or individual, depending on the type of marker.

Genotyping – detection of genetic markers.

High-throughput sequencing – also known as next-generation sequencing, these are technologies designed to sequence DNA or RNA rapidly and cost-effectively.

Inbreeding coefficients – a measure of how closely related two individuals are; specifically, it is the probability that two genes selected at random are identical by descent.

Inbreeding depression – the reduced fitness of a population due to inbreeding.

Linkage disequilibrium – non-random association of alleles from different loci, or locations on the DNA; this information can be used, for example, to understand evolutionary history, to map genes associated with specific traits, or to understand evolution of linked sets of genes.

Metabarcoding – this is a technique that combines DNA barcoding with high-throughput sequencing; essentially, this technique can identify multiple organisms within a sample without the need for isolation.

Microsatellites – these are a type of genetic marker composed of non-coding repetitive DNA sequences usually several base pairs in length.

Parentage Based Tagging (PBT) – this technique involves sampling and genotyping parents of offspring; these offspring are then genetically tagged because they can be assigned to their respective parents through genotyping.

Single Nucleotide Polymorphism – genetic variation caused by a single point mutation, or single base pair change.

A DEEPER DIVE INTO GENOTYPING

> WHAT IS GENOTYPING?

Genotyping techniques are essentially methods to detect genetic variation. Over time, mutations in DNA lead to genetic variation, which leads to differences among individuals, strains, populations, species, etc... These genetic differences are essentially "genetic markers". Many different types of mutations can occur to cause mutations, and all of these types of mutations are common in an organism's genome. These include:

- SNPs (single nucleotide polymorphisms)
- Indels – insertions or deletions
- Inversions
- Rearrangements of DNA around a locus

> WHAT MAKES A USEFUL GENETIC MARKER?

Useful genetic markers are those that are heritable, detectable (either as a visible phenotype or as a genetic difference that can be distinguished using molecular techniques), and polymorphic. For hatchery purposes, we have focused on SNPs, but below is a summary of other genetic markers for reference.

GENETIC MARKER	WHAT ARE THEY?	WHAT ARE THEY USED FOR?	POSITIVE ATTRIBUTES?	LIMITATIONS?
Allozymes	Variants of proteins caused by different alleles at a single locus; one of the earliest markers used in aquaculture genetics	Tracking inbreeding; stock identification; parentage analysis; linkage mapping	Codominant markers; easy to use; low cost	Limited number; heterozygote deficiencies due to null alleles; large amount of good quality tissue required; some genetic changes are masked at the protein level (i.e. silent and synonymous mutations)
Mitochondrial DNA (mtDNA) Markers	Sequency polymorphisms in the mtDNA; technically, mtDNA markers are RFLP markers but the target is mtDNA instead of nuclear DNA	Stock structure; broodstock identification; population differentiation	High level of diversity therefore they can clarify relationships among closely related species; evolves faster than nuclear DNA; multiple copies in each cell	Only transferred through maternal lineage therefore any gender-based selection or introgression may not be reflected; back mutation (mutations that revert back to original sequence); parallel substitution (same mutation in different populations); mutation hotspots (unusual high areas of mutation)

Restriction Fragment Length Polymorphism (RFLP)	Digestion of DNA with restriction enzymes produces fragments that will vary in size due to mutations among individuals, populations, and species	Differentiate species, strains, or populations	Codominant markers; easy to score because size differences are typically large	Low level of diversity; sequence information or probes required so could be difficult to develop markers in species lacking molecular information
Random Amplified Polymorphic DNA (RAPD)	Unknown segments of nuclear DNA obtained by polymerase chain reaction (PCR); presumed to be selectively neutral; dominant inheritance	Species identification; analysis of population structure; analysis of genetic impact of environmental stressors; assessment of genetic diversity	Commercially available primers; no prior knowledge of target DNA required; can easily screen a large number of individuals	Inability to distinguish between homozygotes and heterozygotes; low reproducibility due to low annealing temperature during PCR amplification
Amplified Fragment Length Polymorphism (AFLP)	PCR-based technique combining some aspects of RFLP and RAPD; included mutations at restriction sites and mutations at PCR primer sites; using known DNA adaptors to select a subset of fragments for separation (by electrophoresis)	Generate markers that can identify strains or populations, including hybrids; analysis of gynogens and androgens; generation of high-resolution linkage maps	Large numbers of polymorphisms; high reproducibility; relatively affordable; does not require any prior molecular information	Codominant scoring possible for well-characterized families but can difficult for populations; requires specialized equipment to do efficiently (automated gene sequencers)
Microsatellites	Multiple copies of simple sequence repeats ranging from 1 to 6 base pairs; polymorphism is reflected as size differences due to varying numbers of repeats for different alleles at a given locus	Genome mapping; parentage; kinship analysis; stock structure	Codominant inheritance; highly abundant; even genomic distribution; high polymorphism	Each locus needs to be identified and its flanking region sequenced; efficient marker development requires genomic DNA libraries
Single Nucleotide Polymorphisms (SNP)	Polymorphisms caused by point mutations (single base pair changes)	Broodstock identification; identification of quantitative trait loci (QTL); traceability; food safety; genomic selection	The most abundant polymorphism; can be automated; reveals hidden polymorphism not detected with other methods	Large-scale SNP analysis requires expensive equipment

> HOW TO ASSAY SNPS IN A POPULATION

SNPs can be assayed on chips that have already been developed for many species of salmonids or custom chips can be developed for specific populations. Some resources available include:

- SNP chip for Coho salmon Parentage Based Tagging through the EPIC4 research program at University of Victoria, Simon Fraser University, and University Laval
- Commercially available resources available through Center for Aquaculture Technologies Genotyping - CAT-Center for Aquaculture Technologies (aquatechcenter.com) for different salmonid species
- Sockeye and Chinook salmon sequencing and SNP panel development by scientists at the Molecular Genetics Lab of DFO and through Simon Fraser University
- It is strongly recommended that you form a collaborative relationship with a researcher to develop an assay that will meet your objectives

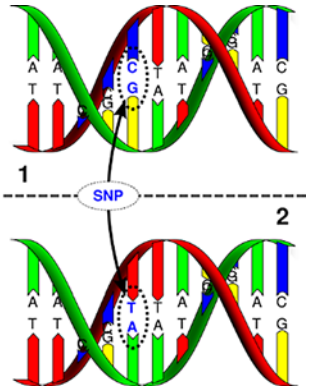



Photo by: Nicole Christiansen

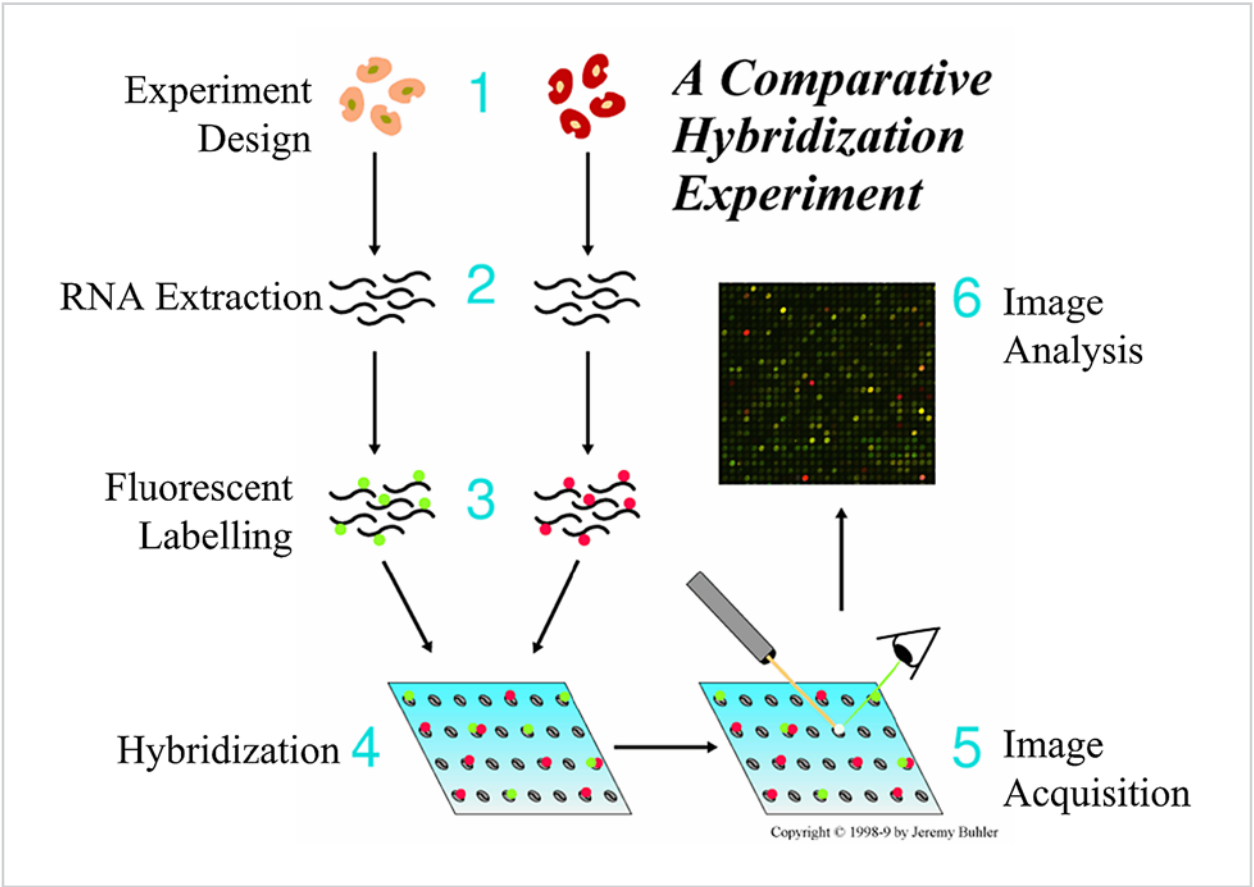
A DEEPER DIVE INTO GENE EXPRESSION TECHNIQUES

> WHAT CAN GENE EXPRESSION TELL US?

Having the ability to measure expression of genes can provide insight into how genetics and environment are working together to control phenotype. This technology can help to address questions related to the effect of rearing environment on survival. Use of microarrays that can detect 1000s of genes at once can be used to select a specific panel of genes related to disease states, stress state, smolt readiness, or maturation, for example.

> MICROARRAYS

Below is an overview of the flow for a microarray experiment (in this case, it is a comparative hybridization experiment which means that two samples are hybridized together on the same microarray slide). The first and most important consideration is the experiment design and sample collection. The reason that this is so important is that you must take care to ensure all environmental conditions are as identical as possible except for the factor(s) being tested. Once the samples are collected, they must be carefully preserved to ensure that the RNA is not degraded. The RNA is then extracted and labelled with fluorescent dye (each sample is labelled a different colour, in this case red or green). The RNA is hybridized to the slide that is spotted with 1000s of cDNA sequences that will bind to RNA in the samples. After washing off any unbound RNA, the image is captured and then analysed. In essence, the level of RNA will be reflected by the colour intensity of the fluorescent label (which can be quantified).



> HOW CAN THE DATA BE USED?

Below is an example of a heat map representing data from a microarray experiment after quantification and normalization. These heat maps are useful because they can be analyzed to show clusters of samples and/or genes with similar expression patterns. This can help to identify specific genes that may be useful for assessing physiological states.

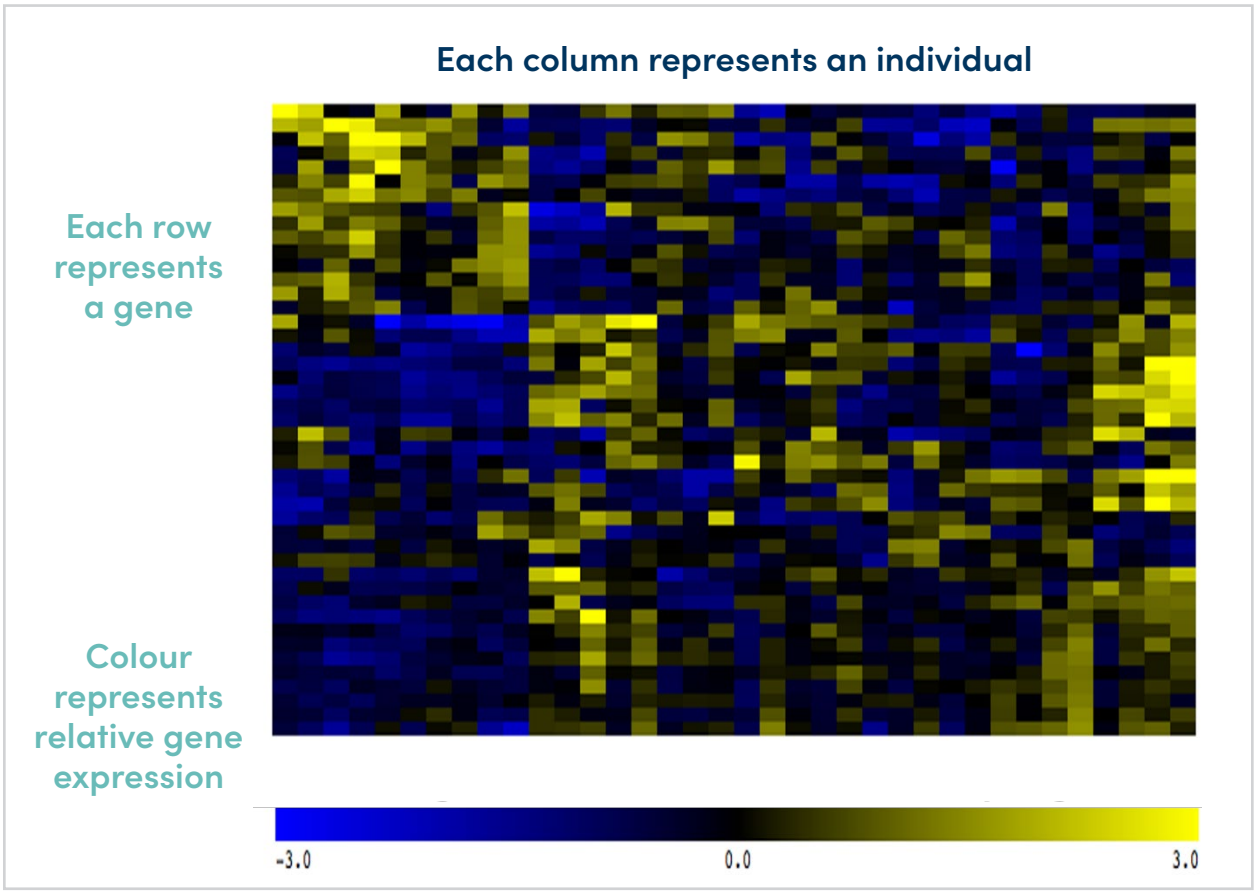



Photo by: Sam James

A DEEPER DIVE INTO EPIGENETICS

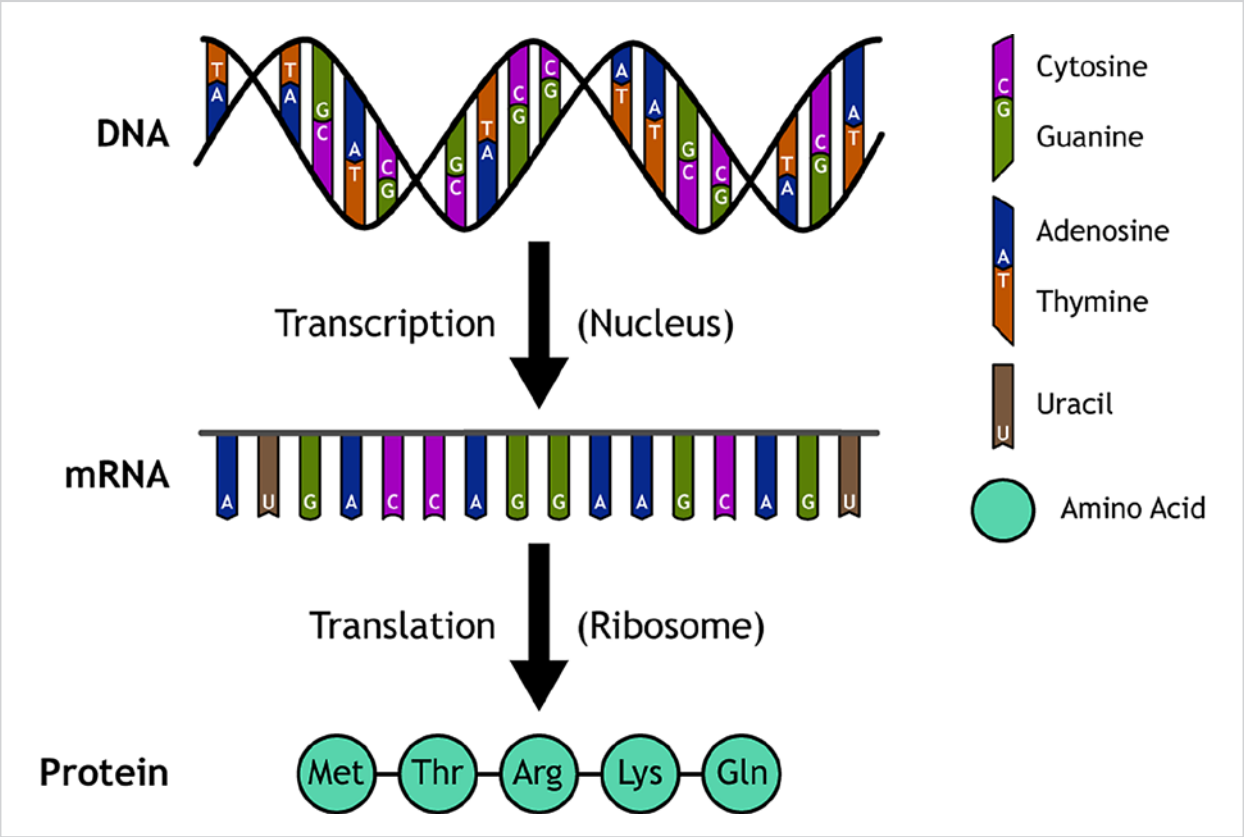
> WHAT CAN GENE EXPRESSION TELL US?

Epigenetics is the study of how environment can cause changes in phenotype without causing changes in DNA sequence (in other words, the genetics remains the same).

> HOW CAN PHENOTYPE BE ALTERED WITHOUT CHANGING GENOTYPE?

First, remember the Central Dogma of genetics: DNA → RNA → Protein. This is how genotype (DNA) gets translated into phenotype. However, environment can have a significant effect on phenotype through genomic modifications that do not alter the DNA sequence.

DNA in a cell is tightly packed into chromosomes. Formation of chromosomes is facilitated by the winding of DNA around proteins called histones. Before DNA can be transcribed into RNA, it must unwind from these histones. Epigenetic modifications can include the attachment of methyl groups to the DNA or histones, thereby affecting the ability of the DNA to unwind for transcription. There are other types of epigenetic modifications, but attachment of methyl groups (or methylation) is currently the most common modification analysed.



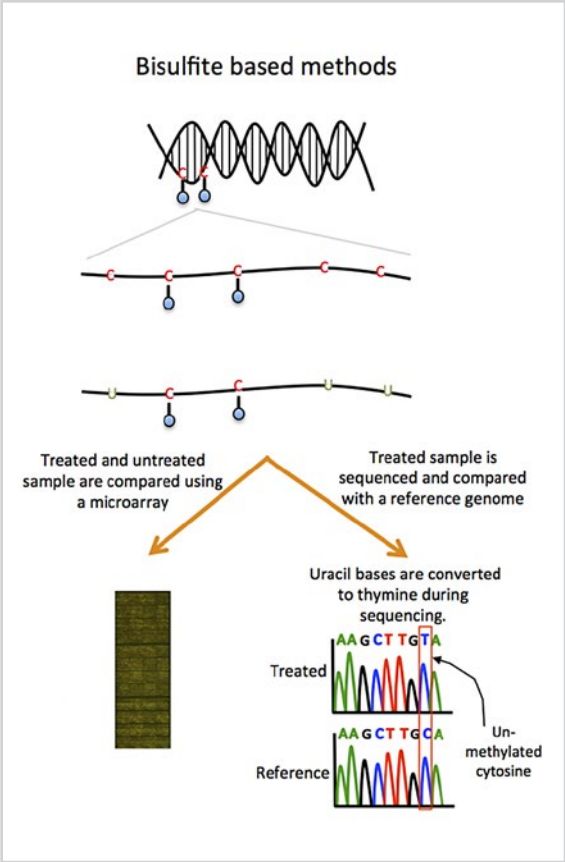
> HOW CAN METHYLATION BE DETECTED?

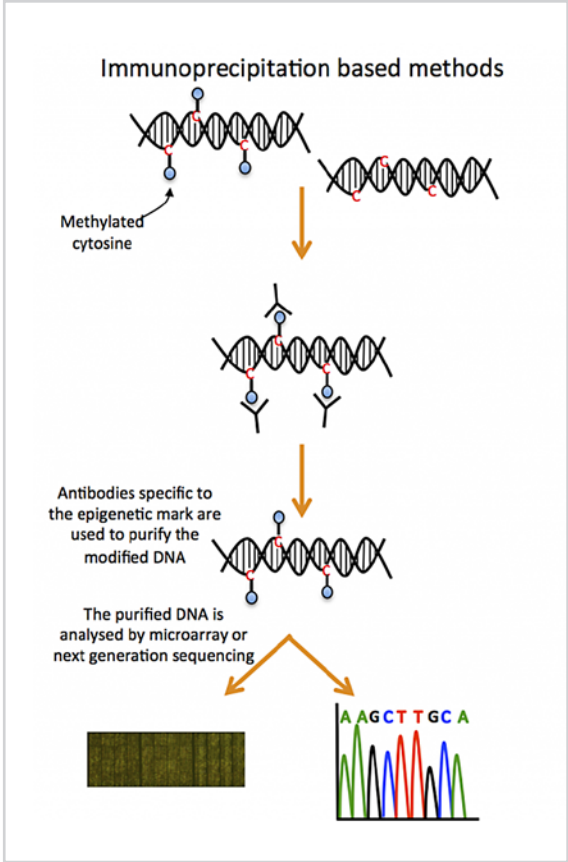
Methylation of DNA can be measured using bisulfite-based methods or chromatin immunoprecipitation (ChIP). Whole-genome bisulfite sequencing was used in Wellband *et al.* 2021 to determine for the first-time epigenetic changes in sperm of cultured salmon. Dr. Wellband is now a scientist at PSEC in West Vancouver and could be a great option for collaborative work on epigenetic questions related to SEP.

Bisulfite-based methods involve treatment of the DNA to convert unmethylated cytosine bases to a uracil base, while leaving methylated residues as a cytosine. The treated DNA is then analysed by microarray or sequencing. Microarray analysis can compare the level of methylation (or epigenetic modification) between the bisulfite-treated sample and untreated samples. The signal intensity ratio between the samples is used to infer the level of methylation in specific regions of the genome. The treated and untreated samples may also be compared by sequencing to identify specific methylation sites within the genome. This approach requires a well annotated genome.

Chromatin immunoprecipitation (ChIP)-based methods use antibodies specific to methylation to purify methylated regions of the genome. This is followed by microarray or sequencing to identify the specific regions (just as with bisulfide-based approaches).

Either of these approaches could also be used to specifically target histone modifications (as opposed to DNA modifications described above).



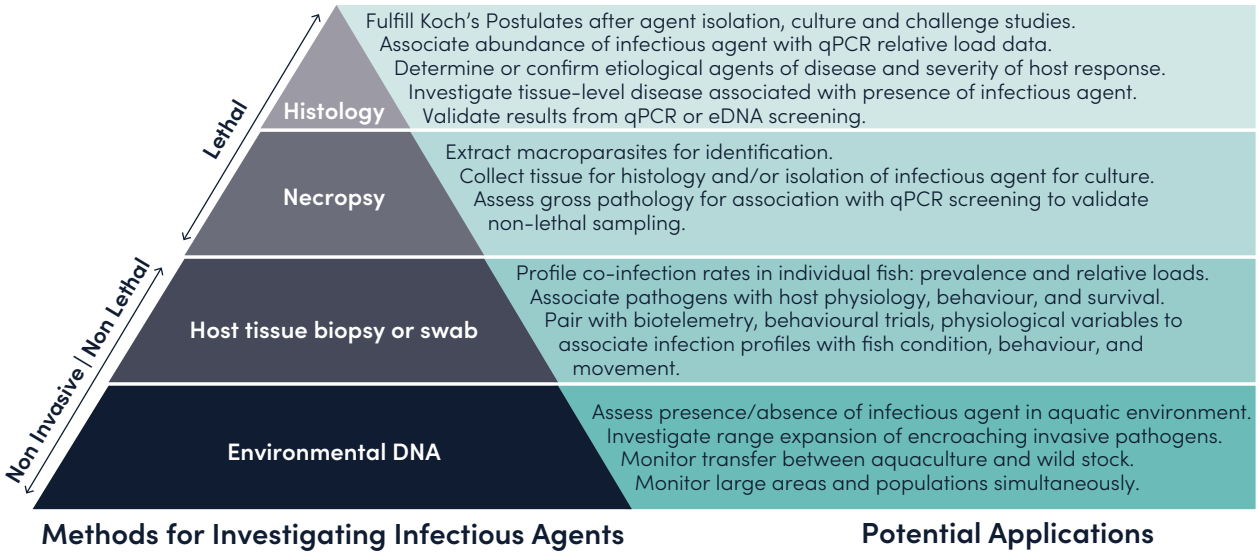


> REFERENCE

Wellband K, Roth D, Linnansaari T, Curry RA, Bernatchez L. 2021. Environment-driven reprogramming of gamete DNA methylation occurs during maturation and is transmitted intergenerationally in Atlantic salmon. G3 GENES|GENOMES|GENETICS 11:JKAB353.

A DEEPER DIVE INTO PATHOGEN SCREENING

Much work has been done over the past several years to develop methods for non-lethal detection of disease agents in salmonid populations. For a more complete overview of these methods and their potential application you are encouraged to read Chapman *et al.* 2021.



The figure shown above (from Chapman *et al.* 2021) shows that pathogen screening can range from non-invasive methods, to more invasive but non-lethal methods, to lethal methods. Development of pathogen screening assays have a goal for rapid non-invasive or non-lethal methods, but lethal methods of necropsy and histology are required at least for validation of the use of other methods.

> PATHOGEN DETECTION USING SEQUENCING

It is possible to obtain non-lethal tissue samples or swabs from fish (such as a small gill tissue punch). The DNA can be extracted from these samples and sequenced. Extensive and growing sequence databases can be used to identify any pathogens present in the sample. This approach is useful if the pathogens are not known and can be used to develop more specific quantitative PCR assays (described below).

> QUANTITATIVE PCR TECHNIQUES

These techniques may also use non-lethal sample collection. Quantitative PCR (detection of specific target genetic sequences) can be done in a highly multiplex format such that a number of genes and samples can be assayed at one time. There have been specific salmon disease screening assays developed that can be applied immediately, or work could be done to develop custom assays.

This technique has also been done to detect the physiological response of fish to presence of pathogens (in this case, the expression of specific genes is being measured). When fish are exposed to pathogens, certain genes will respond by increasing or decreasing expression. These patterns are detectable and may provide some understanding of the presence of pathogens and how they are affecting the fish.

> USE OF ENVIRONMENTAL DNA FOR PATHOGEN SCREENING

See the appendix below (A DEEPER DIVE INTO ENVIRONMENTAL DNA ANALYSIS) for additional details on how these assays are done. Basically, a water sample can be obtained from an area of interest and the DNA contained within this sample can be replicated, sequenced, and identified to test for presence of pathogens of interest.

The following website may be helpful for further understanding of potential applications and for developing collaborative projects: BAIEA – Broughton Area Integrated Ecosystem Assessment

> REFERENCE

Chapman JM, Kelly LA, Teffer AK, Miller KM, Cooke SJ. 2021. Disease ecology of wild fish: opportunities and challenges for linking infection metrics with behaviour, condition, and survival. Can. J. Fish. Aquat. Sci. 78: 995–1007.

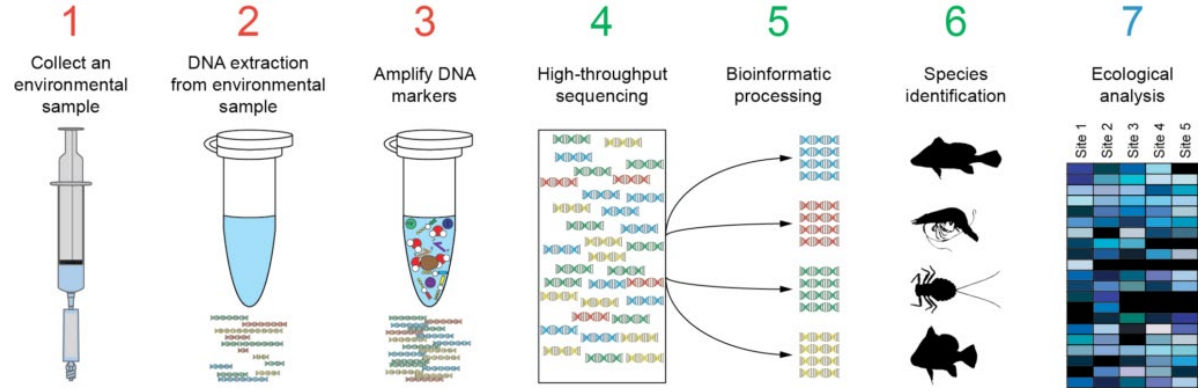
A DEEPER DIVE INTO ENVIRONMENTAL DNA ANALYSIS

Below is a summary of some of the published applications of environmental DNA (eDNA) analysis. Uses applicable to SEP range from pathogen screening at sites, assessing food availability for determining optimal release timing, assessing diversity of fish in an area, or monitoring for invasive species. Fisheries and Oceans Canada has acknowledged that eDNA analysis is likely being underutilized and holds a lot of potential for addressing questions related to aquaculture and fisheries management (Baillie *et al.* 2019).

> HOW DO YOU DO THIS ANALYSIS?

A Fisheries and Oceans Canada document prepared by Baillie *et al.* 2019 provides a comprehensive overview of applying eDNA analysis to questions highly relevant for SEP and may provide some contacts for further collaborative work and development within the department.

Below is a high-level overview of the process of eDNA analysis with some consideration of the current limitations of the technology. The first important point to keep in mind is that DNA can be present in the water or sediment from a number of sources: free DNA, feces, organism, etc. This means that factors such as disturbing settled layers may cause detection of DNA from organisms that are no longer present or relevant to the area. There can also be a high opportunity for contamination of DNA from other sources. How samples are collected may also lead to biases in the results, and this includes consideration of how to filter, preserve, store, and transport the samples. Cutting edge technology includes hand-held devices that can provide analysis at time of collection, however these devices currently cannot handle detection of multiple targets. Once the samples are collected and brought to the lab, the DNA is amplified and then sequenced. Bioinformatic processing is used to identify the species present in the sample.



> REFERENCE

Baillie SM, McGowan C, May-McNally S, Leggatt R, Sutherland BJG, and Robinson S. 2019. Environmental DNA and its applications to Fisheries and Oceans Canada: National needs and priorities. Can. Tech. Rep. Fish. Aquat. Sci. 3329: xiv + 84 p.

Photo by: D. Swainson

PACIFIC SALMON FOUNDATION

1682 West 7th Ave,
Vancouver, BC, V6J 4S6
Tel: 604-664-7664
Email: salmon@psf.ca

Fisheries and Oceans Canada
Pêches et Océans Canada

BRITISH COLUMBIA

Funding for the project is provided by the BC Salmon Restoration and Innovation Fund, a contribution program funded jointly between Fisheries and Oceans Canada and the Province of BC.