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Researching Potential Options for an On-Vessel Portable Genotyping Laboratory for the Port Moller Salmon Test Fishery

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Alaska Department of Fish and Game **Division of Commercial Fisheries**

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Weights and measures (metric)

centimeter	cm
deciliter	dL
gram	g
hectare	ha
kilogram	kg
kilometer	km
liter	L
meter	m
milliliter	mL
millimeter	mm

Weights and measures (English)

cubic feet per second	ft^3/s
foot	ft
gallon	gal
inch	in
mile	mi
nautical mile	nmi
ounce	oz
pound	lb
quart	qt
yard	yd

Time and temperature

day	d
degrees Celsius	°C
degrees Fahrenheit	°F
degrees kelvin	K
hour	h
minute	min
second	s

Physics and chemistry

all atomic symbols	
alternating current	AC
ampere	A
calorie	cal
direct current	DC
hertz	Hz
horsepower	hp
hydrogen ion activity (negative log of)	pH
parts per million	ppm
parts per thousand	ppt, ‰
volts	V
watts	W

General

Alaska Administrative Code	AAC
all commonly accepted abbreviations	e.g., Mr., Mrs., AM, PM, etc.
all commonly accepted professional titles	e.g., Dr., Ph.D., R.N., etc.
at	@
compass directions:	
east	E
north	N
south	S
west	W
copyright	©
corporate suffixes:	
Company	Co.
Corporation	Corp.
Incorporated	Inc.
Limited	Ltd.
District of Columbia	D.C.
et alii (and others)	et al.
et cetera (and so forth)	etc.
exempli gratia (for example)	e.g.
Federal Information Code	FIC
id est (that is)	i.e.
latitude or longitude	lat or long
monetary symbols (U.S.)	$, ¢
months (tables and figures): first three letters	Jan,...,Dec
registered trademark	®
trademark	™
United States (adjective)	U.S.
United States of America (noun)	USA
U.S.C.	United States Code
U.S. state	use two-letter abbreviations (e.g., AK, WA)

Mathematics, statistics

all standard mathematical signs, symbols and abbreviations	
alternate hypothesis	H_A
base of natural logarithm	*e*
catch per unit effort	CPUE
coefficient of variation	CV
common test statistics	(F, t, χ^2, etc.)
confidence interval	CI
correlation coefficient (multiple)	R
correlation coefficient (simple)	r
covariance	cov
degree (angular)	°
degrees of freedom	df
expected value	*E*
greater than	>
greater than or equal to	≥
harvest per unit effort	HPUE
less than	<
less than or equal to	≤
logarithm (natural)	ln
logarithm (base 10)	log
logarithm (specify base)	$\log_2$, etc.
minute (angular)	'
not significant	NS
null hypothesis	H_O
percent	%
probability	P
probability of a type I error (rejection of the null hypothesis when true)	α
probability of a type II error (acceptance of the null hypothesis when false)	β
second (angular)	"
standard deviation	SD
standard error	SE
variance	
population	Var
sample	var

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RESEARCHING POTENTIAL OPTIONS FOR AN ON-VESSEL PORTABLE GENOTYPING LABORATORY FOR THE PORT MOLLER SALMON TEST FISHERY

by

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ABSTRACT

The Port Moller Test Fishery inseason estimates of stock- and age-specific run strengths for sockeye salmon (*Oncorhynchus nerka*) returning to Bristol Bay, Alaska, are used by the fleet and industry for planning and by the Alaska Department of Fish and Game (ADF&G) for management. To produce stock-specific run strengths, samples are shipped to the ADF&G Gene Conservation Laboratory (GCL) in Anchorage for genetic analysis. Shipping the samples requires a vessel to deliver the samples to Port Moller and limits the ability to sample the entire transect. An on-vessel genotyping lab would eliminate the delivery time and potentially alleviate the need for a second vessel, saving the program funds. We investigated the feasibility of an on-vessel genotyping lab by assessing 5 DNA extraction methods and 3 genotyping methods we identified as likely candidates after searching the literature and evaluating the experience of other researchers. Among the variables we assessed were cost, space needs, skills needed, sensitivity to movement, method accuracy, and data compatibility with the current methods. Based on these assessments, we identified the Macherey-Nagel extraction and Fluidigm genotyping methods as the most promising. These could be housed in a modified shipping container with equipment placed on pneumatic antivibration base(s). Raw genotyping data would be sent via satellite internet to the GCL for scoring, mixed stock analysis, and reporting. Personnel selected to work in the on-vessel lab would need to be adequately trained and able to work on a vessel. We recommend testing these methods on a vessel prior to investing in a complete lab. We estimate that the base price for a mobile genotyping lab for the top-ranked methods would cost about $280,000. This price would be higher if redundant equipment or additional stabilization methods are required. This price does not include annual training, personnel, or supply costs.

Keywords: Port Moller, Bristol Bay, sockeye salmon, Oncorhynchus nerka, genetic stock identification, test fishery, single-nucleotide polymorphism, SNP, DNA extraction, genotyping, portable laboratory

INTRODUCTION

The Port Moller Test Fishery (PMTF) has been conducted since 1967 using drift gillnets set at fixed stations ranging from 30 to 150 nautical miles offshore from Port Moller, Alaska (Raborn and Link 2020). The primary goal of the test fishery is to predict the run strength of sockeye salmon (*Oncorhynchus nerka*) 1 week before they enter fishing districts in Bristol Bay. The results from the PMTF are important to Bristol Bay fish processors, fishery participants, and the to Alaska Department of Fish and Game (ADF&G) because they allow time for ADF&G to respond to deviations from the preseason run forecast.

In 2006, inseason genetic mixed stock analysis (MSA) was added to the PMTF, allowing for estimates of stock-specific run strengths. Samples are collected from fish at the end of each drift gillnet set and preserved. At the end of each 2-day sampling trip, the samples are offloaded in Port Moller and shipped to the ADF&G Gene Conservation Laboratory (GCL) in Anchorage for genetic analysis. Stock composition estimates from the MSA are generally made available 3 or 4 days after sample collection (Dann et al. 2013). The results are valuable to ADF&G managers (managing for sustainable fisheries), fishery participants (determining which district to fish), and fish processors (distribution of their resources).

Although there are 26 test fish stations in the PMTF transect, until recently only stations 2, 4, 6, 8, 10, 12, and 14 (35–95 miles from Port Moller) have been fished (Raborn and Link 2020). A second test fish vessel was added in 2018 as a pilot study to fish stations 16, 18, 20, 22, and 24 (Raborn and Link 2018). The results from that study observed a large portion of the run migrating farther offshore from station 12 and different contributions of Bristol Bay stocks at the outer stations. Additional funding was added to the project to run 2 test fish vessels in 2019 and 2020, 1 for inner stations and 1 for outer stations. Currently, genetic samples from the outer vessel are transferred to the inner vessel at sea for transport to Port Moller.

Using a test fish vessel to deliver samples to Port Moller reduces the number of stations fished by 1 vessel each day by up to 40% on a given 2-day trip, making the task of delivering samples to shore costly. Samples are typically delivered to shore every other day. Travel time between the innermost stations (stations 2 and 4) and Port Moller typically takes 3.5–4.5 hours 1 way for a total of 7–9 hours round-trip travel time over the 2 days, which is equivalent to the time it takes to fish 4–6 stations. Logistics at and around the dock in Port Moller often adds more time; the vessel may have to wait a few hours for high tide (for sufficient water depth) to access the Peter Pan Seafoods dock, and there are sometimes delays getting up to the dock because of other vessel traffic. With combined travel and logistic time, the vessel can forego the equivalent of up to 1 day of fishing time per trip to shore to deliver samples. Finally, once in port, the vessel is a long way from the test fishing stations, and short windows of suitable fishing weather on a given day are sometimes missed because the vessel is unable to reach the test fishing stations before the weather is expected to worsen. When this happens, the vessel stays in port to wait for a large window of good weather.

After the samples are delivered to Port Moller, they need to be transported to Anchorage, adding logistical challenges and uncertainty to the project. Transit time from Port Moller to the GCL in Anchorage can take over 3 hours if there is a direct flight, or up to several days if the shipment goes through multiple airports. Weather delays can also increase the sample transport time to the GCL, especially in June when dense fog can persist for multiple days. Additionally, the Peter Pan Seafoods facility was sold in 2020 and its future is uncertain. Without this facility, the logistics and cost of the PMTF project would increase substantially.

Under current conditions, delivering samples to Port Moller mandates that the project use 2 fishing vessels to cover the entire transect. It is impossible for a single vessel to fish sufficient stations to adequately sample the run and deliver samples to Port Moller. Genotyping the tissue samples on a test fish vessel would greatly reduce the logistical challenges and cost of the PMTF project. Most significantly, an at-sea lab might also allow a single vessel to cover a sufficient portion of the transect on a daily basis, reducing the cost to the project of chartering fishing vessels by close to 50% (about $150,000 annually).

The current lab equipment and methods employed by the GCL to genotype tissue samples from the PMTF are not compatible for use on a moving vessel without modifications. Currently, DNA is extracted from tissues using NucleoSpin 96 Tissue Kits (Macherey-Nagel) and the genotyping of 24 single nucleotide polymorphism (SNP) markers is conducted on a Fluidigm integrated fluidic circuit. During the extraction process, a centrifuge is used at high speed to filter out the DNA from the digested tissues. Running a centrifuge at high speed on a moving vessel could be hazardous because of the conservation of angular momentum. Additionally, the Fluidigm instruments used in the genotyping process are sensitive to movement and would need to be stabilized in some way to work on a moving vessel.

This report summarizes results from our investigation into the feasibility of on-vessel genotyping as an alternative to inseason genotyping at the GCL for the Port Moller sockeye salmon project. Available technologies were investigated, with a review of the constraints, potential limitations, and estimated costs.

METHODS

Investigations on available methodologies were conducted by the following:

1. searching the scientific literature for genetic studies that have been conducted on vessels;
2. contacting researchers who might have information or experience with DNA extraction and genotyping methods on vessels; and
3. contacting representatives from companies that produce DNA extraction supplies, produce instruments capable of genotyping SNPs, convert shipping containers into labs, and produce stabilization equipment for information on products that may be compatible for use on moving vessels.

Through this research, we identified DNA extraction and genotyping methods that have potential to be used for on-vessel analyses and we provide the following information to assess suitability.

The information for extraction methods included the following:

1. the amount of time it takes to extract 190 samples (divided into total time from start to finish and amount of heads-down personnel time);
2. the cost of equipment and supply cost for extracting 190 samples;
3. the lab equipment and supplies required for each method;
4. the power requirements for equipment;
5. lab bench space needed for cutting tissues, pipetting, and for equipment;
6. the personnel training time needed;
7. whether the method is sensitive to movement; and
8. potential modifications that would allow the method to work on a moving vessel.

The information for genotyping methods included the following:

1. the amount of time it takes to genotype 190 samples for 24 SNPs (divided into total time from start to finish and amount of heads-down personnel time for producing raw genotype output files, scoring time at the GCL, and overall time to produce genotypes for MSA);
2. the cost of equipment and supply cost for genotyping 190 samples for 24 SNPs;
3. the lab equipment and supplies required for each method;
4. the power requirements for equipment;
5. lab bench space needed for pipetting and for equipment;
6. the personnel training time needed;
7. whether the method is sensitive to movement;
8. potential modifications that would allow a method to work on a moving vessel;
9. genotyping accuracy;
10. whether the baseline would need to be updated with new genetic markers;
11. the file size of the raw output from each method that would be electronically transferred to the GCL for scoring and MSA; and
12. which of the potential DNA extractions methods could be used without modification and potential modifications that would allow an extraction method to be used.

We then indicated how certain we are in each item of information using a 3-level rating system indicated by 1–3 asterisks: very confident (***), somewhat confident (**), and not very confident (*).

RESULTS

REVIEW OF SCIENTIFIC LITERATURE FOR ON-VESSEL GENOTYPING

Most genetic analyses that have been performed on research vessels to date have sequenced DNA rather than target specific SNP genotypes (Lim et al. 2014; Moroz 2015; Ducluzeau et al. 2019). Although these studies do not provide insight into methods for genotyping SNP markers on a PMTF vessel, they demonstrate that conducting genetic analyses on a moving research vessel is possible.

The first documented use of a next-generation sequencer on a research vessel in the field was on a 2013 expedition in the South Pacific Ocean (Lim et al. 2014). During the expedition, they extracted and purified the DNA from bacteria using Nucleospin Tissue Kits (Macherey-Nagel; see section below for descriptions for all potentially applicable methods for on-vessel genotyping) and sequencing was performed using an Ion Torrent Personal Genome Machine. Although the expedition was successful, Lim et al. (2014) noted some challenges they experienced while sequencing on a vessel. To reduce the risk of using a high-speed centrifuge on a moving vessel, they had to place it in the lowest part of the ship, and they eliminated it from their protocol when possible. In those cases, they used a mini-centrifuge for DNA extraction and vacuum-based purification protocols. Unexpected equipment failures were also challenging because they had no access to technical support or replacements. During data transfer from the Personal Genome Machine hard drive to their data storage server, they experienced problems with corrupted files, which they believe were caused by the movement of the ship affecting the server hard drive or uneven power on the ship. The final challenge was finding enough people with the expertise to analyze the sequence data in a timely manner, which they said could be solved with a shipwide Wi-Fi system allowing all members of their team to access the data, or by sending the data off-ship for analysis.

In 2014, a pilot study was conducted to test the feasibility of sequencing tornaria larvae and ctenophores while in remote locations in the Caribbean Sea (Moroz 2015). For the study, a shipping container was converted into a mobile lab and placed on the deck of a 141-foot yacht. The lab was equipped with all the instrumentation needed to perform dissections, isolation of DNA and RNA, construction of next-generation sequencing libraries, and sequencing and bioinformatic analysis on site and in real-time. Sequencing was performed on an Ion Torrent semiconductor sequencer because it was able to handle the ship's movement and changes in humidity and temperature. The study successfully performed 22 sequencing cycles while on the vessel. The article about this study (Moroz 2015) was written for a symposium and did not include detailed methods. Online searches for an article containing detailed methods were not successful and there was no response from the author when contacted by email.

In 2018, the University of Alaska Fairbanks ran a sequencing workshop for undergraduate students on the R/V *Sikuliaq* while in transit from Nome to Seward, Alaska (Ducluzeau et al. 2019). Although they experienced 3 days of stormy weather and rough seas, they successfully extracted DNA from microbes in water samples using single-tube Qiagen DNeasy PowerWater Kits and sequenced the DNA using Oxford Nanopore Technology (ONT) MinION sequencers. They chose to use MinION because they are portable, do not require a traditional lab setup to implement, can be powered and controlled via a laptop computer USB, are relatively inexpensive, and have user-friendly hardware and software. They chose a single-tube extraction method because it allowed them to use a small benchtop centrifuge during the

extraction process instead of a high-speed centrifuge. In a telephone conversation with the lead author on December 12, 2020, Anne-Lise Ducluzeau provided additional information on their lab setup and working conditions they experienced. In the ship lab, all equipment and materials were securred to working surfaces using bolts or bungee cords, and benchtops were covered with rubber mats to reduce vibrations. When they experienced rough seas, they found it necessary to have 2 people doing the lab work; 1 person did the majority of the work and the second person held the person pipetting to keep them from falling over, fetched supplies, and took over pipetting when the first person was too tired or seasick to continue.

Only 1 study could be found where SNP genotyping was performed on a research vessel. In 2019, researchers with the Department of Fisheries and Oceans Canada used the MinION on a research vessel in the Gulf of Alaska to produce SNP genotypes from coho salmon (*O. kisutch*) samples for stock identification using genotyping-by-sequencing (GBS) methods (C. Deeg, Postdoctoral Fellow, Department of Forest and Conservation Sciences, University of British Columbia, personal communication, April 8, 2021). In this study, DNA was extracted from 80 coho salmon tissue samples using QuickExtract DNA Extraction Solution (Lucigen) and then sequenced to produce genotypes for 296 SNPs. When comparing the SNP calls from the MinION analysis using R9 flow cells to those produced for the same fish using an Ion Torrent sequencer, they found 83% matching calls.

Although the Department of Fisheries and Oceans Canada study has not been published yet, 2 land-based studies have used the MinION for genotyping SNPs (Cornelis et al. 2017; Malmberg et al. 2019). Cornelis et al. (2017) investigated the use of the MinION for forensic GBS of a female single-contributor control DNA sample for 52 SNPs. The study compared the MinION genotypes to those produced using an Illumina sequencer and found corresponding genotypes for 51 SNP loci and 1 SNP that could not be called correctly due to allelic imbalance in the mapped subreads. Another SNP was not considered successfully genotyped, even though it had corresponding genotypes for both sequencers, because it had severe allelic imbalance. Both SNPs with allelic imbalance were located between or inside homopolymer stretches.

Malmberg et al. (2019) examined whether the MinION could produce accurate SNP genotypes when analyzing samples from a species with highly duplicated genomes. The study extracted DNA from 9 canola (*Brassica napus)* leaf samples with the DNeasy 96 Plant Kit (Qiagen) and used low coverage long read sequencing on the MinION to examine genotypes at 4 million pre-validated SNP positions. They filtered their data for SNPs with read depths between 2 and 5 and for SNPs with genotypes in at least 7 of the 9 samples. They assessed genotyping accuracy for the filtered set of SNPs by comparing the genotypes with those produced for the same SNPs using an Illumina sequencer. They found that 75% of SNPs were accurately genotyped for all samples. However, when heterozygous MinION genotype calls were removed, they found that 84.2% of SNPs were accurate for all samples. They noted that, due to improved alignment of long reads, the MinION data was likely correct for genotypes that were heterozygous in the Illumina data but homozygous in the MinION data. After adjusting for these genotypes, they found that 97.2% of SNPs had accurate genotypes for all samples using the MinION.

POTENTIAL DNA EXTRACTION METHODS FOR ON-VESSEL USE

To conduct inseason genotyping of sockeye salmon tissue samples on a PMTF vessel, it is important to select a simple, quick, and high-yield DNA extraction method that can be used on a vessel. Through our literature search, discussions with researchers and technicians who work on

vessels, and discussions with companies creating DNA extraction kits, we identified the following 5 available extraction methods that meet these criteria and were evaluated for use on a research vessel: Macherey-Nagel NucleoSpin Tissue kit, Macherey-Nagel NucleoSpin 96 Tissue kit, Qiagen QIAcube Connect, QuickExtract DNA Extraction Solution, and Chelex 100. Below is a summary of each method and an analysis of suitability for on-vessel use with the PMTF. Methods are presented in order from most to least suitable according to our assessment. See Table 1 to compare select characteristics of potential extraction methods.

Macherey-Nagel NucleoSpin Tissue Kit and Macherey-Nagel NucleoSpin 96 Tissue Kit

Note: Both methods have been used by the GCL to extract DNA from fish axillary processes and fin tissues.

The first method uses single NucleoSpin columns (single-tube extraction) and the second method uses 96-well NucleoSpin binding plates (96-well extraction). Both methods use proteinase K/SDS solution for sample lysis (i.e., to breakdown the cell membrane). After DNA is released from the cells, it is bound to a silica membrane in a NucleoSpin Tissue column (single-tube) or binding plate (96-well). Two different wash buffers are used to purify DNA on the silica membrane. Finally, the purified DNA is eluted with a slightly alkaline elution buffer. A high-speed centrifuge is currently used by the GCL for this method; however, GCL staff have experimented with using a 96-well vacuum manifold instead of a centrifuge (Appendix A1). In the experiment they found that vacuum manifold extraction time was similar to centrifuge extraction time; however, there was some concern about potential for contamination using a vacuum manifold. Because of the dangers of using a high-speed centrifuge on a moving vessel, we only report the results for this method using a vacuum manifold.

Characteristics

1. Extraction time for 190 samples[1] **
    a. Single tube: 6.5 hours (5 hours personnel time).
    b. 96-well: 5 hours (3.5 hours personnel time).
2. Cost ***
    a. Single tube
        i. Equipment: $6,213 (Table 2).
        ii. Supplies for 190 samples: $640.
    b. 96-well
        i. Equipment: $13,591 (Table 3).
        ii. Supplies for 190 samples: $349.
3. Equipment **
    a. Single tube: NucleoVac 24 tube vacuum manifold (Macherey-Nagel), NucleoVac Valves, lab vacuum pump, 1.5 mL tube racks, and vortex mixer.
    b. 96-well: NucleoVac 96 Vacuum Manifold (Macherey-Nagel), NucleoVac 96 Vacuum Regulator, lab vacuum pump, multichannel pipettors (0.5–1,200 microliters (µL)), and an electronic multichannel pipettor (15–1,250 µL).
    c. Both methods: Small incubator, single channel pipettors (0.5–1,000 µL), and a small refrigerator for storing chemicals and extracted DNA.

[1] This time will be influenced by environmental conditions.

4. Supplies ***
    a. Single tube: Macherey-Nagel NucleoSpin Tissue kit.
    b. 96-well: Macherey-Nagel NucleoSpin® 96 Tissue kit and reagent reservoirs.
    c. Both methods: Pipette tips.
5. Power ***
    a. All equipment - 120V AC.
6. Space **
    a. 2×4 ft bench to pipette and cut tissues, 2×4 ft bench for equipment.
7. Personnel ***
    a. Training time: This method would require about 1 month of training at GCL for an untrained person and about 1 week for a trained staff member.
8. Sensitivity to vessel movement *
    a. Vessel movement may cause contamination between samples using the 96-well manifold, but this needs to be tested to confirm.
9. Potential mitigation for vessel movement **
    a. Using 24-tube vacuum manifold with 1-way valves could prevent cross-contamination due to vessel movement.

QIAcube HT System

This is a QIAGEN product and is designed to extract DNA automatically. The preparation steps are similar to the Macherey-Nagel NucleoSpin Tissue kit, which include sample lysis, DNA binding to a silica membrane, DNA purification, and DNA elution. The instrument can be operated via a computer, which can display the time remaining for each step during DNA extraction process. The instrument used for this automated method is equipped with a vacuum driven system.

Characteristics

1. Extraction time for set of 190 samples[2] **
    a. 13.75 hours (3.75 hours personnel time).
2. Cost **
    a. Equipment: $65,524 (Table 4).
    b. Supplies for 190 samples: $431.
3. Equipment **
    a. QIAcube HT system with laptop computer, uninterruptible power supply, low-speed plate microcentrifuge with swing-out rotor, small incubator, single channel pipettors (0.5–1,000 µL), multichannel pipettors (0.5–1,200 µL), and a small refrigerator for storing chemicals and extracted DNA.
4. Supplies **
    a. QIAamp 96 DNA QIAcube HT Kit, pipette tips, 96-well plates.
5. Power ***
    a. All equipment - 120V AC, QIAcube HT system should be connected to an uninterruptible power supply (i.e., battery backup).

[2] This time will be influenced by environmental conditions.

6. Space **
    a. 2×4 ft bench to pipette and cut tissues, 2×6 ft bench for equipment.
7. Personnel ***
    a. Training time: This method would require about 1 month of training at GCL for an untrained person and about 2 weeks for a trained staff member.
8. Sensitivity to vessel movement **
    a. Movement is not likely to be an issue, but testing needs to be done to confirm this.
9. Potential mitigation for vessel movement
    a. N/A

QuickExtract DNA Extraction Solution

This is a fast and simple method to extract DNA from tissue. Heat treatment is the most important step in the process. No toxic chemicals or spin columns are used in the preparation. In an incubator, the tissue is lysed, DNA is released, and compounds that inhibit amplification are degraded. The DNA from this method can be used for all kinds of polymerase chain reaction (PCR)-based analyses. This method can be used to easily process many samples in 96-well plates without the use of a high-speed centrifuge and has the option of using a robotic automation system to reduce the overall time for the extraction process. The DNA from QuickExtract extractions would require filtering or a 2-hour preamplification step for some genotyping platforms.

Characteristics

1. Extraction time for set of 190 samples[3] **
    a. 5 hours (5 hours personnel time).
2. Cost ***
    a. Equipment: $15,049 (Table 5).
    b. Supplies for 190 samples: $705.
3. Equipment ***
    a. 24-tube microcentrifuge, low-speed plate microcentrifuge with swing-out rotor, small incubator, tube racks, 96-well plate racks, single channel pipettors (0.5–1,000 µL), multichannel pipettors (0.5–1,200 µL), vortex mixer, and a small refrigerator for storing extracted DNA.
4. Supplies **
    a. QuickExtract solution, 2 mL tubes, 96-well plates, and pipette tips.
5. Power ***
    a. All equipment - 120V AC.
6. Space ***
    a. 2×4 ft bench to pipette and cut tissues, 2×4 ft bench for equipment.
7. Personnel ***
    a. Training time: This method would require about 1 month of training at GCL for an untrained person and about 2 weeks for a trained staff member.
8. Sensitivity to vessel movement **
    a. Movement is not likely to be an issue, but testing needs to be done to confirm this.

[3] This time will be influenced by environmental conditions.

9. Potential mitigation for vessel movement
    a. N/A

Chelex 100 Chelating Resin (BioRad)

Note: This method has been used in the past by the GCL to extract DNA from fish tissue samples.

The resin is composed of styrene divinylbenzene copolymers containing iminodiacetic ions, which function as chelating groups (Walsh et al. 1991). For this method, Chelex 100 is added to water to form a solution. The solution is then added to the either a single tube or a 96-well plate containing tissue samples. The samples in the Chelex solution go through 2 heating steps to lyse the cells and release DNA, which can be done using either 2 dry block incubators or with a thermocycler. Finally, the samples in the Chelex solution are vortexed and then spun down. This extraction method is simple, relatively inexpensive, and does not require a high-speed centrifuge, organic solvents or transferring the solution from tube to tube. The DNA from Chelex 100 extractions would require purification or a 2-hour preamplification step for some genotyping platforms.

Characteristics

1. Extraction time for set of 190 samples[4] **
    a. 7.75 hours (3.75 hours personnel time).
2. Cost **
    a. Equipment: $13,526 (Table 6).
    b. Supplies for 190 samples: $139.
3. Equipment ***
    a. 250 mL beaker, lab scale, hotplate with magnetic stirrer, 2 dry block incubators, 96-well plate racks, single channel pipettors (0.5–1,000 µL), multichannel pipettors (0.5–1,200 µL), vortex mixer, and a small refrigerator for storing extracted DNA.
4. Supplies ***
    a. Chelex 100 chelating resin, 2 mL tubes, 96-well plates, and pipette tips.
5. Power ***
    a. All equipment - 120V AC.
6. Space ***
    a. 2×4 ft bench to pipette and cut tissues, 2×4 ft bench for equipment.
7. Personnel ***
    a. Training time: This method would require about 1 month of training at GCL for an untrained person and about 2 weeks for a trained staff member.
8. Sensitivity to vessel movement **
    a. Movement and vibration will be a concern when weighing with lab scale.
9. Potential mitigation for vessel movement ***
    a. Weigh out multiple batches Chelex 100 on land before the season begins.

[4] This time will be influenced by environmental conditions.

POTENTIAL GENOTYPING METHODS FOR ON-VESSEL USE WITH THE PORT MOLLER TEST FISHERY

SNP assays and GBS are currently the most used SNP genotyping methods. After reviewing the literature and talking with researchers who have genotyped on vessels in the field, we selected 3 methods that were most promising for genotyping in this application. These included 2 potential SNP assay methods and 1 potential GBS method. For the SNP assay methods, we selected the QuantStudio 5 System (ThermoFisher Scientific) and Fluidigm SNP Genotyping Technology. There are many sequencers that can be used for the GBS method, including Ion Torrent next-generation sequencing systems (ThermoFisher Scientific), NextSeq 500 System (Illumina), and MinION (ONT). However, for this study we selected only the MinION (Oxford Nanopore) as a potential method because it has properties that make it uniquely suited for field work and it is currently the least expensive sequencer available. Below is a summary of each method and characteristics for on-vessel use. Methods are in order from most to least suitable according to our assessment. See Table 7 to compare select characteristics of potential genotyping methods.

Fluidigm SNP Genotyping Technology

Note: This genotyping method is one of 2 genotyping systems used by the GCL and is currently used to genotype samples from the PMTF in season.

This technology is efficient at high-throughput SNP genotyping, provides an easy workflow by using microfluidic technology, and supports both TaqMan and SNP Type assays. As such, GCL personnel are extremely familiar with the process and we place more confidence in our assessment of the suitability of this method to meet the needs of at-sea processing tissue samples.

DNA samples and assays for each SNP are loaded into integrated fluidic circuits (IFCs), using Fluidigm IFC Controllers. The IFC is then placed on an Fluidigm IFC thermal cycler to amplify the DNA segments. After DNA amplification, the IFC is read on the Fluidigm EP1 System to collect data from the amplification product. The data from the chip is then scored (i.e., assigned genotypes for each SNP) using Fluidigm SNP Genotyping Analysis software.

Characteristics

1. Genotyping time for a set of 190 samples and 24 SNPs ***
    a. Producing raw data on vessel: 2 hours (1 hour personnel time).[5]
    b. Scoring time at GCL: 30 minutes.[6]
    c. Total time: 2.5 hours.
2. Cost ***
    a. Equipment: $193,881 (Table 8).
    b. Supplies for 190 samples and 24 SNPs: $640.
3. Equipment ***
    a. Fluidigm IFC controller (RX), FC1 thermocycler, and EP1 reader, uninterruptible power supplies, low-speed plate microcentrifuge with swing-out rotor, single channel pipettors (0.5–1,000 µL), multichannel pipettors (0.5–1,200 µL), vortex mixer, and a small refrigerator for storing PCR reagents.

[5] This time will be influenced by environmental conditions.

[6] The raw data can be sent, via satellite internet connection, to the GCL for scoring.

4. Supplies ***
    a. 192.24 IFC from Fluidigm, PCR reagents, 96-well plastic plates, 15 mL plastic tubes, pipette tips, and reagent reservoirs.
5. Power ***
    a. All equipment - 120V AC; IFC controller (RX), FC1 thermocycler, and EP1 reader should be connected to an uninterruptible power supply.
6. Space ***
    a. 2×4 ft bench to prepare plates for PCR, 3×8 ft bench for equipment.
7. Personnel ***
    a. Training time: This method would require about 1 month of training at GCL for an untrained person and about 1 week for a trained staff member.
8. Sensitivity to vessel movement **
    a. Fluidigm engineers say that vibrations would be a concern for the EP1 reader.
9. Potential mitigation for vessel movement **
    a. Place EP1 reader on top of a pneumatic self-leveling vibration-isolation base (Appendix C1) and secure to workbench.
10. SNP genotyping accuracy ***
    a. Fluidigm claims 99.75% genotyping accuracy for 192.24 IFCs (Appendix B1), which is in line with reproducibility estimates from GCL projects.
11. Baseline update ***
    a. No update needed; current genetic markers work with this method.
12. Electronic transfer file size of raw data for 190 samples and 24 SNPs **
    a. About 40 megabytes.
13. Extraction method ***
    a. Macherey-Nagel and QIAcube extractions will work without modification. The DNA from QuickExtract and Chelex 100 extractions are not filtered and would require filtering or a 2-hour preamplification step before loading on an IFC.

MinION Genotyping by Sequencing

The first step of GBS on the MinION is referred to as library prep. During library prep, PCR is performed on the DNA of each sample, which can be done in a single multiplex reaction for each sample in a 96-well plate. Unique barcodes are then added to the PCR products for each sample using the ONT barcoding ligation kit. The PCR products are then pooled and ligate reverse adaptors are added. The pooled DNA goes through an additional PCR step to concatenate the DNA from the samples. After concatenation, ONT ligate sequencing adapter are added to the libraries and then loaded on the MinION for sequencing. After sequencing, base calling is performed using a program called Guppy (ONT) which contains basecalling algorithms. The output files from Guppy (FASTQ) are read into another program (e.g., Porechop; https://github.com/rrwick/Porechop) to deconcatenate the loci and bin the sequencing reads by barcode and produce separate FASTA files for each sample. Sequence alignment is performed for each sample using another program such as the Burrows-Wheeler alignment tool (Li and Durbin 2010) or MiniMap2 (Li 2018). Finally, scoring is performed using a custom script. This summary is just one approach for GBS with the MinION; some steps during library prep could be performed in a different order and sequence alignment could be done using a custom script.

Characteristics

1. Genotyping time for a set of 190 samples and 24 SNPs *
    a. Producing raw sequence data on vessel: 21 hours (8 hours personnel time).[7]
    b. Scoring at GCL: 4–8 hours.[8]
    c. Total time: 25–29 hours.
2. Cost *
    a. Equipment: $35,261 (Table 9).
    b. Supplies for 190 samples and 24 SNPs: $978.[9]
3. Equipment **
    a. MinION sequencer and a laptop computer, uninterruptible power supply, low-speed plate microcentrifuge with swing-out rotor, 96-well plate racks, single channel pipettors (0.5–1,000 µL), multichannel pipettors (0.5–1,200 µL), vortex mixer, and a small refrigerator for storing chemicals.
4. Supplies **
    a. Chemicals, pipette tips, 96-well plastic plates.
5. Power ***
    a. All equipment – 120V AC; the laptop for the MinION should be connected to an uninterruptible power supply[10] (i.e., battery backup).
6. Space ***
    a. 2×4 ft bench for library prep work, 2×4 ft bench for equipment.
7. Personnel **
    a. Training time: This method would require a person with previous GBS lab experience in addition to about 2 months of training at GCL for an untrained person and about 1 month for a trained staff member.
8. Sensitivity to vessel movement **
    a. Sensitivity to movement is likely not an issue—this instrument was designed for field use.
9. Potential mitigation for vessel movement
    a. N/A
10. SNP genotyping accuracy *
    a. Malmberg et al. (2019) found 97.2% genotyping accuracy when comparing to genotypes produced by Illumina sequencer. This estimate is biased high (error rate is likely higher) because they filtered out SNPs with low read depth.
11. Baseline updating *
    a. Yes, a new set of genetic markers will likely be needed for this method. This would increase the initial cost significantly.
12. Electronic transfer file size of raw data for 190 samples and 24 SNPs *
    a. About 10 gigabytes.

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[7] This time will be influenced by environmental conditions.

[8] The raw sequence data can be sent, via a satellite internet connection, to the GCL for the final steps needed to produce SNP genotypes.

[9] The number of sequencing reads needed for GBS on the MinION is uncertain until laboratory tests are conducted, making it difficult to estimate the cost of genotyping supplies. This estimate is based the cost of supplies for analyzing 190 samples for 24 SNPs using an Illumina NextSeq sequencer at the GCL.

[10] Although the MinION can run off a laptop's battery, a battery backup should be connected to the laptop in case the ships power is off for an extended period.

13. Extraction method **
    a. All proposed extraction methods should work without modification.

QuantStudio 5 System

Note: This is designed for a real-time PCR using TaqMan assays and is comparable to what is used by the GCL.

During real-time PCR, the instrument collects the fluorescence intensity related to PCR product concentration from each amplification step. This system can also be used as an endpoint scanner/reader to collect SNP genotype information from PCR products in multiwell plates, which is the current GCL workflow. Prior to endpoint reading on the QuantSudio, the ProFlex Dual 384-Well PCR System, a thermocycler, is used to amplify DNA in separate PCR reaction mixtures for each SNP in 384-well plates. The thermocycler can be preprogrammed to raise and lower the temperature to make copies of target nucleic acid segment. A low-speed centrifuge is used to spin down the plates prior to DNA amplification.

Characteristics

1. Genotyping time for a set of 190 samples and 24 SNPs ***
    a. Producing raw data on vessel: 7.5 hours (7.5 hours personnel time).[11]
    b. Scoring at GCL: 1 hour.[12]
    c. Total time: 8.5 hours.
2. Cost ***
    a. Equipment: $132,933 (Table 10).
    b. Supplies for 190 samples and 24 SNPs: $1,470.
3. Equipment ***
    a. 3 ProFlex Dual 384-Well PCR System thermocyclers, QuantStudio 5 system, uninterruptible power supply, low-speed plate microcentrifuge with swing-out rotor, electronic multichannel pipettor (0.5–12.5 µL), single channel pipettors (0.5–1,000 µL), multichannel pipettors (0.5–1,200 µL), vortex mixer, and a small refrigerator for storing PCR chemicals.
4. Supplies ***
    a. PCR chemicals, pipette tips, 96-well plastic plates, 384-well plastic plates, 15 mL plastic tubes, reagent reservoirs, and PCR plate adhesive sealing film.
5. Power ***
    a. All equipment - 120V AC; the ProFlex Dual 384-Well PCR System thermocyclers and QuantStudio 5 system should be connected to uninterruptible power supplies (i.e., battery backups).
6. Space **
    a. 2×4 ft bench to prepare plates for PCR, 3×8 ft bench for equipment.
7. Personnel ***
    a. Training time: This method would require about 1 month of training at GCL for an untrained person and about 1 week for a trained staff member.

[11] Time estimates are based on the how long it takes 1 person to genotype in a land-based laboratory. Genotyping time on a PMTF vessel may take longer due to variable environmental conditions.

[12] The raw data can be sent, via satellite internet connection, to the GCL for scoring.

8. Sensitivity to vessel movement *
    a. This information is unknown, but movement and vibrations may be a concern for the ProFlex Dual 384-Well PCR and QuantStudio 5 systems.
9. Potential mitigation for vessel movement *
    a. Place the ProFlex Dual 384-Well PCR and QuantStudio 5 systems and on top of pneumatic self-leveling vibration-isolation bases.
10. SNP genotyping accuracy **
    a. ThermoFisher Scientific does not have this information; however, it is likely that this method is as accurate or more accurate than the Fluidigm method because it uses a higher volume PCR reaction. In a quality control analysis, the GCL compared 7,672 genotypes produced using the QuantStudio and Fluidigm methods and found a 99.8% genotyping concordance, which is in line with the reproducibility rates for Fluidigm.
11. Baseline updating ***
    a. No update needed; current genetic markers work with this method.
12. Electronic transfer file size of raw data for 190 samples and 24 SNPs **
    a. About 17 megabytes.
13. Extraction method **
    a. All proposed extraction methods should work without modification.

LAB PERSONNEL SKILLSET

The extraction and genotyping methods above all require lab personnel with the same base skillset. The person chosen to work in the on-vessel lab should have a high attention to detail and the ability to remain focused for long periods of time, follow detailed protocols, and manage multiple tasks with varying timelines. They would also need to be self-sufficient and have a proven track record for working on a moving vessel. However, because of the many technical steps required for genotyping with the MinION, that method would also require a person that has previous GBS lab experience.

POTENTIAL CONTAINER FOR LABORATORY

At the beginning of this study, the Bristol Bay Science and Research Institute requested that our top-ranked methods include the cost of setting up a mobile lab that is easily deployable and can be removed from the vessel at the end of the season. Shipping containers are designed to be moved and secured and are durable, waterproof, and can handle extreme environmental conditions, making them a good container for a mobile lab on a vessel (e.g., Moroz 2015). A 20-foot shipping container would have plenty of space to set up a mobile genotyping lab and would be small enough to fit on the deck of a PMTF vessel. Container Specialties of Alaska is a company that converts shipping containers in Anchorage, Alaska. We called the company to inquire about the cost of converting a 20-foot container. They said that lab conversions, including the cost of the shipping container,[13] can range from $20,000 to $80,000 depending on the specifications and setup required (e.g., insulation, doors, windows, electrical, plumbing, etc.). Custom nonshipping containers that are designed to fit a specific place on a vessel may provide a

[13] The current cost of a new 20-foot shipping container is around $6,000.

lighter and smaller container that still satisfies the specifications (space, power, configuration) for the lab. We did not explore the costs or designs of any custom structures.

INSTRUMENT STABILIZATION

During our investigations, we explored 2 different methods for stabilizing lab equipment to counter the effects of vessel movement and vibration. We explored stabilization of the most sensitive piece of equipment, the Fluidigm EP1, on the bench with a hexapod (a.k.a. Stewart Platform) and a desktop vibration isolation base. Stabilization of the EP1 using a hexapod would cost about $90,000 and would require sufficient space around it for movement. Stabilizing the EP1 using a desktop vibration isolation base would cost about $3,000 and would be limited to isolation from vibration and self-leveling. After discussing these 2 options with representatives from Fluidigm, the second method appeared to be adequate for stabilizing the EP1 because it is mainly affected by vibrations, not large movements. Fluidigm engineers are in the process of running simulations using the vibration isolation platform under the EP1 to determine if it will be effective or not.

DISCUSSION

GENETIC BASELINE CONSIDERATIONS: EXISTING VERSUS NEW

MSA requires that the mixture samples be analyzed for the same set (or a subset) of markers that are in the baseline. Baselines contain genetic data from populations that represent all potential stocks that may be present in an MSA mixture sample. The genetic baseline currently used in the MSA of the PMTF samples contains data for 24 SNP markers and 19,614 fish obtained from 146 locations in Bristol Bay and other parts of Western Alaska (Dann *In prep*).

The Fluidigm and QuantStudio genotyping methods both support the use of TaqMan assays and would be able to produce genotypes for all baseline markers; however, the MinION GBS method may not be able to produce genotypes for an adequate set of markers if assays for this method cannot be developed for some current markers using this technology. If not enough of the current markers can be assayed with the MinION, then the baseline would need to be updated with high-resolution markers that can be screened by the MinION.

Updating the genetic baseline for a new set of SNP markers would require regenotyping all baseline samples, and a statistical analysis that would include tests to evaluate MSA reporting group performance. A baseline update would take at least 1 year to produce genotypes for statistical analysis and would cost roughly $750,000. Validation of the new baseline would require an assessment of simulated MSA results to determine accuracy and precision and a reanalysis of 20 mixtures of 190 fish from past PMTF samples for concordance with existing methods. These validation steps would add an additional $120,000 to the cost of developing a new baseline; however, it is uncertain whether the updated baseline would perform as well as or better than the current baseline for PMTF. In any event, these tasks to develop a new baseline would require about $1 million and a couple of calendar years to produce a fully validated baseline for MSA.

ON-VESSEL LABORATORY CONSIDERATIONS

Top-Ranked Methods

We used the information we gathered to assess the suitability of several DNA extraction and genotyping methods for use on a PMTF vessel as a basis for our rankings. Because choosing an appropriate extraction method depends on which genotyping method is used, we begin with our ranking for a genotyping method.

Genotyping

To select the genotyping method for an on-vessel lab, we considered the time it takes to produce genotypes, genotyping accuracy, whether it will work with our current baseline SNPs, equipment and supply costs, the file size of the raw output, the skillset and training required, and whether it is sensitive to movement. Based on these considerations, we believe that the Fluidigm method is most suitable for use in an on-vessel lab because it takes the least amount of personnel time to produce genotypes, has very high genotyping accuracy, would work with the current baseline, has the lowest supply costs, and the file size of its raw output is relatively small for electronic transfer to the GCL. However, this method has the highest equipment cost of the 3 methods we considered and because the antivibration base has not been tested yet with the EP1, there is some uncertainty of whether this method will work on a moving vessel. If the antivibration base testing is unsuccessful with the Fluidigm system, it is also likely to be unsuccessful with the QuantStudio system. Our second ranked method would be the MinION, because it is not sensitive to movement; however, this method has some drawbacks. The biggest drawback of the MinION is that the current set of baseline SNP markers would probably not work, and the baseline would have to be updated and evaluated for MSA, which would be expensive and time-consuming. Other drawbacks of the MinION are that it has highly technical steps requiring personnel with more training, takes about a day to produce genotypes, has the lowest genotyping accuracy of the 3 methods we investigated, and has a relatively high supply cost. We ranked the QuantStudio method third only because it ranked below the Fluidigm system and is also likely to be affected by vessel movement. The QuantStudio has high genotyping accuracy, would also work with the current baseline, and has the smallest raw data file size.

Extraction

To select the appropriate DNA extraction method for an on-vessel lab, we considered which method would work with our top-ranked genotyping methods, the extraction time, the steps involved, supply and equipment costs, and whether the method is sensitive to movement. Because all extraction methods we investigated require about the same amount of training and skillset, we did not consider those items in our selection. Based on these considerations, we believe that the Macherey-Nagel 96-well extraction method using the vacuum manifold is the most suitable extraction method for use with the Fluidigm system. We chose this extraction because it produces high-quality DNA that can be used without an additional preamplification step, it has less steps and takes less time than the single-tube Macherey-Nagel method, extraction time is not too long, equipment and supply costs are relatively low, and it will most likely work on a moving vessel. However, this method has not been tested on a moving vessel where cross contamination may be an issue. If vessel movement is an issue, our second ranked extraction method for the Fluidigm system would be the Macherey-Nagel single-tube extraction method. The QIAcube extraction method could also be used with the Fluidigm system without a preamplification step and has a similar extraction time and supply costs to the Macherey-Nagel

method; however, equipment costs for this method are higher and it is uncertain whether this method is affected by movement. For the MinION, the Chelex 100 method would be the top-ranked method to extract DNA from tissues. We ranked this method at the top because it can be used for sequencing without any additional steps, it has the second lowest extraction time, the second lowest equipment costs, and the lowest supply costs of all the methods we investigated, and it is not sensitive to vessel movement.

Field Testing of Laboratory Equipment

Although our top-ranked methods may work on a moving vessel, tests should be performed in a similar environment to that on the PMTF vessel before purchasing expensive equipment and setting up a mobile lab. Ideally, lab equipment testing would be performed on the same PMTF vessel where the mobile lab would be placed; however, testing the lab equipment on a vessel in Cook Inlet would be less expensive than shipping it to Port Moller for testing. The Cook Inlet offshore test fish vessel fishes a transect with multiple fishing stations from July 1 to July 30 each year, and experiences similar environmental conditions as the PMTF vessels. The Cook Inlet test fishery also samples their sockeye salmon catch for genetics using similar methods to those used by the PMTF. The current Cook Inlet test fish vessel is the R/V *Solstice*, and it has space on board to set up a temporary lab, where test analyses could be performed on samples collected by the test fish crew. We recommend borrowing or leasing the Fluidigm IFC controller, FC1 thermocycler, EP1 reader, and vibration isolation base from Fluidigm and running several mixture analyses of 190 fish on the R/V *Solstice* while it is at sea. These tests would assess whether the Fluidigm equipment will work on a moving vessel and the levels of contamination with the vacuum extraction method. These tests would also highlight any unforeseen problems with genotyping on a moving vessel, so mitigation steps can be taken before designing the lab, ordering the equipment, and genotyping on a PMTF vessel.

Laboratory Cost

We roughly estimate that setting up a mobile lab for our top-ranked extraction and genotyping methods would cost about $280,000 (Table 11). This cost includes laboratory and stabilization equipment and the high-end cost of modifying a shipping container. However, this setup cost does not include the cost for testing the equipment on a vessel in Cook Inlet, training personnel, shipping the mobile lab to Port Moller, or mounting the lab on the deck of a PMTF vessel.

Although we provide cost estimates for extracting and genotyping 190 samples, they do not include personnel costs. Each season, lab personnel would work for about 1 month on the test fish vessel and about 2 weeks' time for traveling to and from Port Moller, preparing the lab before the season, and securing the lab for storage postseason (i.e., about 1.5 months). Either a Fishery Biologist (FB) I or II would likely possess the skills and experience needed for working in the on-vessel lab. At the Port Moller pay scale for 1.5 months, salary and benefits would cost about $18,000 for an FB I and $20,000 for an F BII. Training costs will be higher to pay for training time if staff without GCL training are hired to conduct on-vessel genotyping.

Data Analysis and Reporting

To reduce the workload and the expertise required for the on-vessel lab, the genotype scoring, data archiving, MSA of the genotype data, and reporting of the results would be conducted by staff at the GCL in Anchorage using established methods. The raw data produced by the on-vessel lab would be sent to the GCL via a satellite internet connection. At that point, scoring

would be performed by experienced lab staff and the resulting genotypes would be uploaded to the GCL database, *Loki*. The GCL's PMTF project leader would then conduct the MSA and disseminate the results to the Bristol Bay Science and Research Institute, ADF&G, and the public. Adequate bandwidth in the satellite internet connection on the PMTF vessel would be required for this approach.

Training Personnel

The personnel selected to work in the on-vessel lab will need sufficient lab training and experience to complete highly detailed tasks without supervision and to troubleshoot minor equipment issues. Inseason genotyping for the PMTF project is currently conducted at the GCL by multiple experienced lab personnel in a stable environment. During the extraction process, lab personnel check each other's work for mistakes. If problems occur during extraction or genotyping, there is generally someone available that can help resolve the issue. However, the personnel selected for genotyping on a PMTF vessel will not have this support. Moreover, the changing environmental conditions experienced on a moving vessel would make genotyping even more challenging—especially while pipetting—so it is important that personnel have enough experience that genotyping tasks are almost second nature. Therefore, we recommend that untrained personnel have at least 1 month of experience extracting DNA and at least 1 month of experience genotyping before they work in the on-vessel lab. Personnel selected for the on-vessel genotyping lab should also have a proven and successful record of working on the same or similar vessels to that used for the PMTF.

ADDITIONAL RECOMMENDATIONS AND CONSIDERATIONS

Besides our top-ranked methods and recommendations above, we have some additional recommendations and considerations for the on-vessel lab. We recommend that all equipment be placed on elastomeric/silicone mats to help absorb vibrations from the vessel and then secured with bolts or straps. The lab work benches should also have some means of securing plate and tube racks during pipetting steps. To increase reliability, consider purchasing and storing redundant equipment on the test fish vessel, so that if equipment fails, it can be replaced. Depending on what level of redundancy is considered appropriate, this may add substantially to the capital costs. Although 1 person could extract and genotype 190 samples in 6–9.5 hours over a 2-day period, 2 people might be better to add reliability to the program. An additional person could help prepare tissues for extraction, take over if the person pipetting gets too seasick or tired to continue, fetch lab supplies, help troubleshoot problems, and double-check the other person's work for mistakes. This would reduce the overall burden on each person and the potential for human error during the genotyping process. Vessel movement may also increase the potential for pipetting errors during the extraction and genotyping processes. To reduce the possibility of pipetting errors, consider purchasing a 96-channel semi-automated electronic pipette (e.g., Eppendorf epMotion 96; cost: ~$25,000). This piece of lab equipment would allow lab personnel to transfer fluids from one 96-well plate to another in a single motion instead of 12 motions with an 8-channel pipette. Lastly, we recommend using GCL staff that are normally assigned to analyze the PMTF samples to run the on-vessel lab because they are highly skilled and would require little or no training. However, we did not canvass or propose to existing lab personnel to see if any were suitably experienced and willing and able to run the on-vessel lab.

If an on-vessel genotyping lab is implemented by the PMTF project, considerations also need to be made for the inseason age composition analysis. For this analysis, scale samples collected

from sockeye salmon on the PMTF vessel are offloaded in Port Moller and sent to ADF&G personnel in King Salmon for aging. Because an on-vessel genotyping lab would eliminate the need for the PMTF vessel to return to port to offload samples, acetate impressions of scales may need to be taken at sea and aging could be done on the vessel or digital images of the scale impressions could be electronically transferred to ADF&G personnel for aging.

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TABLES

Table 1.–Characteristics of potential extraction methods: personnel and total extraction time for 190 samples, total equipment cost and supply cost for extracting 190 samples, training time needed for trained and untrained staff, movement sensitivity and potential mitigation, and whether a filtering/preamplification step would be needed for some genotyping methods.

	Extraction time (hours)		Cost		Training time		Movement		
Method	Pers.	Total	Equip.	Supp.	Trained	Untrained	Sensitivity	Potential mitigation	Filter/ Preamp
Macherey-Nagel (single tube)	**	**	***	***	***	***	*		***
	5.00	6.50	$6,213	$640	1 week	1 month	No	N/A	No
Macherey-Nagel (96-well)	**	**	***	***	***	***	*	**	***
	3.50	5.00	$13,591	$349	1 week	1 month	Maybe	Yes	No
QIAcube HT	**	**	**	**	***	***	**		***
	3.75	13.75	$65,524	$431	2 weeks	1 month	Not likely	N/A	No
QuickExtract	**	**	***	***	***	***	**		***
	5	5	$15,049	$750	2 weeks	1 month	Not likely	N/A	Yes
Chelex 100	**	**	**	**	***	***	**	***	***
	3.75	7.75	$13,526	$139	2 weeks	1 month	Yes	Yes	Yes

Note: Asterisks indicate the level of certainty for each item: very confident (***), somewhat confident (**), and not very confident (*)

Table 2.–Estimated equipment costs for extracting DNA into single tubes using the Macherey-Nagel NucleoSpin Tissue kit with a 24-tube vacuum manifold.

Item	Brand	Cost
single channel pipettors (0.1–1,000 µL)	Rainin	$3,276
lab vacuum pump	Gardner Denver	$1,172
vortex mixer	Corning	$595
small incubator	Quincy Lab	$540
NucleoVac 24 tube vacuum manifold	Macherey-Nagel	$301
tube racks	VWR	$219
small refrigerator	Frigidaire	$110
	Total cost	$6,213

Table 3.–Estimated equipment costs for extracting DNA into 96-well plates using the Macherey-Nagel NucleoSpin 96 Tissue kit with a 96-well vacuum manifold.

Item	Brand	Cost
multichannel pipettors (0.5–1,200 µL)	Rainin	$5,604
single channel pipettors (0.1–1,000 µL)	Rainin	$3,276
electronic multichannel pipettor (15–1,250 µL)	ThermoFisher Scientific	$1,973
lab vacuum pump	Gardner Denver	$1,172
NucleoVac 96 Vacuum Manifold	Macherey-Nagel	$708
small incubator	Quincy Lab	$540
NucleoVac 96 Vacuum Regulator	Macherey-Nagel	$208
small refrigerator	Frigidaire	$110
	Total cost	$13,591

Table 4.–Estimated equipment costs for extracting DNA into 96-well plates using the QIAcube HT system.

Item	Brand	Cost
QIAcube HT system	Qiagen	$51,664
multichannel pipettors (0.5–1,200 µL)	Rainin	$5,604
uninterruptible power supply (Smart-UPS 3000VA)	APC	$3,570
single channel pipettors (0.1–1,000 µL)	Rainin	$3,276
plate microcentrifuge with swing-out rotor	Benchmark Scientific	$760
small incubator	Quincy Lab	$540
small refrigerator	Frigidaire	$110
	Total cost	$65,524

Table 5.–Estimated equipment costs for extracting DNA using QuickExtract DNA Extraction Solution.

Item	Brand	Cost
multichannel pipettors (0.5–1,200 µL)	Rainin	$5,604
24-tube microcentrifuge	Eppendorf	$3,870
single channel pipettors (0.1–1,000 µL)	Rainin	$3,276
plate microcentrifuge with swing-out rotor	Benchmark Scientific	$760
vortex mixer	Corning	$595
small incubator	Quincy Lab	$540
tube racks	VWR	$219
small refrigerator	Frigidaire	$110
96-well plate racks	MTC Bio	$75
	Total cost	$15,049

Table 6.–Estimated equipment costs for extracting DNA using Chelex 100 chelating resin.

Item	Brand	Cost
multichannel pipettors (0.5–1,200 µL)	Rainin	$5,604
single channel pipettors (0.1–1,000 µL)	Rainin	$3,276
lab scale	Mettler Toledo	$1,344
dry block incubators	ThermoFisher Scientific	$900
plate microcentrifuge with swing-out rotor	Benchmark Scientific	$760
vortex mixer	Corning	$595
hotplate with magnetic stirrer	ThermoFisher Scientific	$516
tube racks	VWR	$219
250 mL beaker	Heidolph	$127
small refrigerator	Frigidaire	$110
96-well plate racks	MTC Bio	$75
	Total cost	$13,526

Table 7.–Characteristics of potential genotyping methods: personnel and total time to produce raw data for 190 samples, total equipment cost and supply cost for genotyping 190 samples, training time needed for trained and untrained staff, movement sensitivity and potential mitigation, SNP genotyping accuracy, whether a baseline update would be required, the raw data file size for electronic transfer to the Gene Conservation Laboratory for scoring, and which extraction methods would work without a filtering/preamplification step.

	Raw data time (hours)		Cost		Training time		Movement					
Method	Pers.	Total	Equip.	Supp.	Trained	Untrained	Sens.	Mitig.	Acc.	Base. update	File size[a]	Extraction methods[b]
Fluidigm	***	***	***	***	***	***	**	**	***	***	**	***
	1.00	2.00	$193,881	$640	1 week	1 months	yes	yes	99.75%	No	40 MB	MN, QC
MinION GBS[c]	*	*	*	*	**	**	**	**	*	*	*	**
	8.00	21.00	$35,261	$978	1 month	2 months	no	N/A	<97.20%	Yes	10 GB	MN, QC, QE, C
QuantStudio	***	***	***	***	***	***	*	*	**	***		**
	7.50	7.50	$132,933	$1,470	1 week	1 months	maybe	yes	>99.80%	No	17 MB	MN, QC, QE, C

Note: Asterisks indicate the level of certainty for each item: very confident (***), somewhat confident (**), and not very confident (*).

[a] MB = megabytes, GB = gigabytes.

[b] MN = Macherey-Nagel, QC = QIAcube HT, QE = QuickExtract, and C = Chelex 100.

[c] Genotyping-by-sequencing.

Table 8.–Estimated equipment costs for genotyping with the Fluidigm system.

Item	Brand	Cost
EP1 reader	Fluidigm	$124,296
IFC controller (RX)	Fluidigm	$26,057
FC1 thermocycler	Fluidigm	$19,132
Fluidigm System Basic Install Application Training	Fluidigm	$7,247
multichannel pipettors (0.5–1,200 µL)	Rainin	$5,604
uninterruptible power supply (Smart-UPS 3000VA)	APC	$3,570
single channel pipettors (0.1–1,000 µL)	Rainin	$3,276
Fluidigm System Install	Fluidigm	$3,234
plate microcentrifuge with swing-out rotor	Benchmark Scientific	$760
vortex mixer	Corning	$595
small refrigerator	Frigidaire	$110
	Total cost	$193,881

Table 9.–Estimated equipment costs for genotyping-by-sequencing with the MinION.

Item	Brand	Cost
ProFlex Dual 96-Well PCR System thermocycler	ThermoFisher Scientific	$18,336
multichannel pipettors (0.5–1,200 µL)	Rainin	$5,604
single channel pipettors (0.1–1,000 µL)	Rainin	$3,276
Vostro 15 7500 laptop	Dell	$2,654
electronic multichannel pipettor (0.5–12.5)	ThermoFisher Scientific	$2,387
MinION Sequencer	Oxford Nanopore Technologies	$1,200
plate microcentrifuge with swing-out rotor	Benchmark Scientific	$760
vortex mixer	Corning	$595
uninterruptible power supply (Back-UPS Pro 1500)	APC	$264
small refrigerator	Frigidaire	$110
96-well plate racks	MTC Bio	$75
	Total cost	$35,261

Table 10.–Estimated equipment costs for genotyping with the QuantStudio 5 System.

Item	Brand	Cost
QuantStudio 5 Real-Time PCR System	ThermoFisher Scientific	$62,640
3 ProFlex Dual 384-Well PCR System thermocyclers	ThermoFisher Scientific	$52,416
multichannel pipettors (0.5–1,200 µL)	Rainin	$5,604
uninterruptible power supply (Smart-UPS 3000VA)	APC	$3,570
single channel pipettors (0.1–1,000 µL)	Rainin	$3,276
electronic multichannel pipettor (0.5–12.5)	ThermoFisher Scientific	$2,387
uninterruptible power supply (Smart-UPS SRT 1500VA)	APC	$1,500
plate microcentrifuge with swing-out rotor	Benchmark Scientific	$760
vortex mixer	Corning	$595
small refrigerator	Frigidaire	$110
96-well plate racks	MTC Bio	$75
	Total cost	$132,933

Table 11.–Estimated capital costs for setting up a mobile lab to extract DNA with Macherey-Nagel NucleoSpin 96 Tissue kit with a 96 well vacuum manifold and genotype with the Fluidigm system.

Item	Brand/Company	Cost
Extraction Equipment		
electronic multichannel pipettor (15–1,250 µL)	ThermoFisher Scientific	$1,973
lab vacuum pump	Gardner Denver	$1,172
NucleoVac 96 Vacuum Manifold	Macherey-Nagel	$708
small incubator	Quincy Lab	$540
NucleoVac 96 Vacuum Regulator	Macherey-Nagel	$208
Genotyping Equipment		
EP1 reader	Fluidigm	$124,296
IFC controller (RX)	Fluidigm	$26,057
FC1 thermocycler	Fluidigm	$19,132
Fluidigm System Basic Install Application Training	Fluidigm	$7,247
uninterruptible power supply (Smart-UPS 3000VA)	APC	$3,570
Fluidigm System Install	Fluidigm	$3,234
vibration isolation base for EP1	Chuo Precision Industrial	$3,000
vortex mixer	Corning	$595
Shared Equipment		
multichannel pipettors (0.5–1,200 µL)	Rainin	$5,604
single channel pipettors (0.1–1,000 µL)	Rainin	$3,276
plate microcentrifuge with swing-out rotor	Benchmark Scientific	$760
small refrigerator	Frigidaire	$110
Mobile Laboratory		
modified 20-foot shipping container (range: $20,000–80,000)	Container Specialties of Alaska	$80,000
	Total cost	$281,482

Note: Costs will increase if additional stabilization is required (up to another estimated $90K). Also not included are transportation costs, modifications to the vessel, if needed, and cost for testing the equipment. These are the costs for the bare minimum equipment; redundant equipment for reliability will increase costs. These costs also do not include training personnel to conduct analyses on a vessel.

APPENDIX

Appendix A.–Lab report for tests that compared extracting DNA with the Macherey-Nagel NucleoSpin 96 Tissue kit using vacuum manifold to extracting with the same kit using a centrifuge.

T019 Vacuum vs. Centrifugation NucleoSpin Methods

Authors: Zach Pechacek & Heather Hoyt

Report finalized 07/13/2017

Purpose

The purpose of this experiment was to test the vacuum protocol for the NucleoSpin extraction kits, and to determine how well it compares to the centrifugation protocol in regards to the time it takes to isolate the DNA in elution, the general usability of the protocol, and the quality of data produced using both methods. This experiment consisted of cutting two duplicate plates and extracting DNA using both NucleoSpin protocols. Duplicate plates were made of samples collected in bulk bottles as well as those collected on Whatman cards.

Methods

Duplicate plates were extracted using the NucleoSpin extraction method and the NucleoSpin vacuum manifold. The vacuum manifold was attached to a Welch brand air pump during the test. The vacuum manifold was setup with a waste tray during the wash steps, and then the elution plate for the final step.

A sample was prepared for extraction by cutting a small piece and putting it into the corresponding well on the rack of tube strips. 180 ul of Buffer T1 and 25 ul of ProK were added to each well, and the rack of tube strips as put in the 56°C incubator overnight for lysis. The next day, 400 ul of Buffer BQ1 was added to each well and everything was transferred into the binding plate that was setup inside of the vacuum manifold.

The lysate was transferred into the binding plate. The official NucleoSpin protocol for vacuum processing called for the vacuum to be adjusted to -0.2 bar for five minutes. The vacuum could not be adjusted below -0.6 bar for this step, so that amount of pressure was used instead.

The wash steps called for 600 ul of Buffer BW, 900 ul of Buffer B5, and an addition 900 ul of Buffer B5 with the vacuum turned to -0.2 bar for five minutes in-between each wash. In order to maintain a constant -0.2 bar on the vacuum, the wells of the binding plate had to be covered with paper.

The waste tray of the vacuum manifold was dumped out and replaced before the drying the binding plate. To dry the membrane of the binding plate, the vacuum was adjusted to -0.6 bar for ten minutes. To maintain -0.6 bar, the wells of the binding plate were covered with a sheet of paper.

The waste tray was removed from the manifold and the elution plate was inserted in its place. The binding plate was transferred on top of the elution plate. 200 ul of Buffer Be was added to each well of the binding plate. The setup was left to incubate at room temperature for twenty minutes. The vacuum was applied for two minutes at -0.4 bar. The final elution was capped and put in the refrigerator for storage.

T019 Vacuum vs. Centrifugation NucleoSpin Methods

Results

The extraction using the vacuum protocol was performed by Zach Pechacek. Because there was only one vacuum manifold in-house, plates had to be extracted one at a time after the transfer of fluid to the binding plate. The procedure was completed in a time comparable to that of the centrifugation protocol. The breakdown of the time it took to complete DNA extraction using the vacuum can be seen in Table 1.

	Step	Start Time	End Time
Duplicate of 59045	digest	5/2/2017 12:40	5/3/2017 10:20
	transfer	10:40	
	wash (x3)	10:50	11:12
	room temp incubation	11:26	11:46
	completed		11:50
	total time	1.5 hours	
Duplicate of 59073	digest	5/2/2017 12:40	5/3/2017 10:20
	transfer	11:55	
	wash (x3)	12:05	12:24
	room temp incubation	12:38	12:58
	completed	13:05	
	total time	1.5 hours	

Table 1: Time table for vacuum protocol of duplicate plates

The vacuum pump used in the test could not be adjusted easily. The vacuum pressure was much higher when fluid was present in the binding plate. The vacuum did work with the binding plate; however, the increased pressure caused bubbling and a build-up of foam in the waste block. Figures 1 and 2 below show how the fluid was observed using the vacuum manifold.

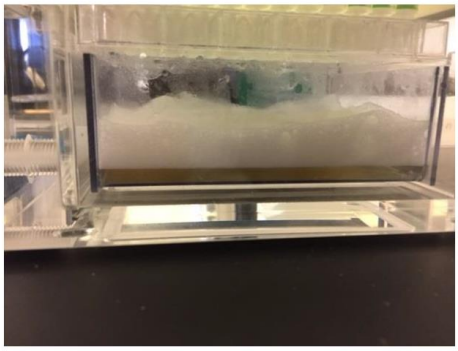


Figure 1: waste block during post-transfer vacuum

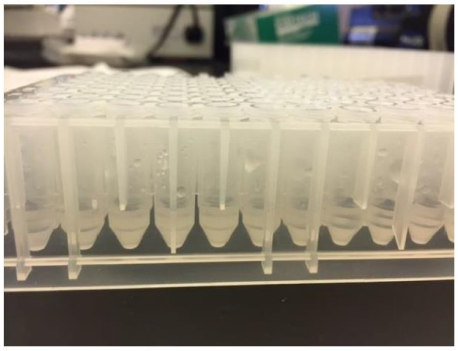


Figure 2: Final elution plate after vacuum

T019 Vacuum vs. Centrifugation NucleoSpin Methods

Summary
The results of Project T019 showed little difference in the time to isolate the DNA between the centrifugation and vacuum protocol for the NucleoSpin extraction kits. The difference was not significant enough to suggest that the vacuum procedure was the better method.

The usability of the vacuum pump proved to be more of a challenge. The pressure of the vacuum could not be adjusted to the required bars when liquid was present in the binding plate. Because of this, there was foam produced in the waste block and a spattering of liquid in the final elution plate. This is seen as an increased likelihood of contamination between wells, and the method does not provide the same confidence in quality as the centrifugation protocol.

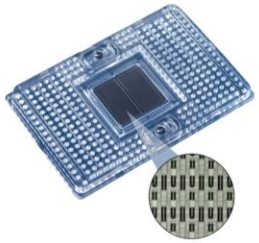
Further testing to genotype these samples was not completed. There is not enough cost or time savings in this methodology to compensate for the challenges in implementation or the potential lack of quality product, so a decision was made to end the experiment at this point. The DNA from the duplicate plates was discarded.

Further considerations
The vacuum procedure with NucleoSpin is not recommended based on the observations of Project T019. It is possible that the technique used in the test was somehow different than that used by NucleoSpin technicians, and may be worthwhile bringing in a Macherey-Nagel representative to observe the procedure.

Appendix B.–SNP genotyping data sheet for Fluidigm 192.24 Integrated Fluidic Circuits.

Fluidigm

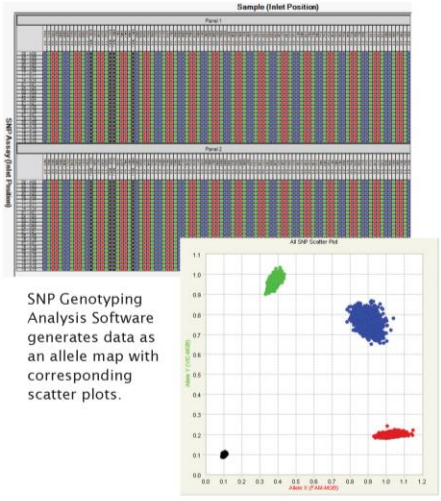
SNP GENOTYPING | DATA SHEET



192.24 Dynamic Array™ IFC

KEY BENEFITS

- Outstanding Quality—99% call rate and 99.75% accuracy
- Accelerated Workflow—delivers 4,608 SNPs in one hour
- Low cost per data point—uses fewer SNP assays against high number of samples



SNP Genotyping Analysis Software generates data as an allele map with corresponding scatter plots.

The Fluidigm 192.24 Dynamic Array Integrated Fluidic Circuit (IFC) provides the ideal solution for high sample throughput SNP genotyping. It is designed to genotype 192 samples against 24 SNP assays in a single run and incorporates innovative design features to greatly increase sample throughput, provide outstanding data quality, and an accelerated workflow.

Outstanding Data Quality

Fluidigm Dynamic Array IFCs have a proven history of providing outstanding data quality and the 192.24 Dynamic Array IFC continues that tradition. Call rates of 99 percent or higher and accuracy of 99.75 percent or higher can be routinely achieved. Additionally, existing SNP genotyping assays can be used.

Accelerated Workflow

The 192.24 Dynamic Array IFC's new flow-through design allows for ultra-fast loading and thermal cycling, resulting in a greatly accelerated workflow. Genotyping results can be obtained in about **one hour*** and five times more IFCs can be run in a single day. Up to sixteen 192.24 chips can be run in one day enabling users to process over **3,000 samples**.

Lower Genotyping Costs

The 192.24 Dynamic Array IFC requires minimal reagents and lowers running costs because each reaction volume is only eight nanoliters. It is also designed to automatically assemble reactions to minimize the number of pipetting steps required **by up to 100-fold**.

* The FC1™ Cycler or the BioMark™ HD System is required to attain fast thermal cycling rates.

SNP GENOTYPING | DATA SHEET

FAST AND EASY WORKFLOW

1. **Transfer** – Easily transfer samples and assays into the IFC using standard SBS+ formats.
2. **Load** – Place the IFC on the IFC Controller RX to automatically set up reaction chambers.
3. **Thermal Cycle** – Place the IFC onto the FC1 Cycler and start the PCR protocol.
4. **Read** – Place the IFC on the EP1 Reader or BioMark HD Reader for fluorescence detection.
5. **Analyze** – Use analysis software to view and interpret results.

SPECIFICATIONS

Parameter	
Number of uses per IFC	1
Call rate	99.00% or greater
Call accuracy	99.75% or greater
Chip footprint	128 mm x 85 mm x 14 mm
Inlet spacing on input frame	4.5 mm pitch
Assay inlets	24
Sample inlets	192
Reaction chambers	4,608
Instrument compatibility	EP1 Reader, BioMark HD System, IFC Controller RX, FC1 Cycler

ORDERING INFORMATION

Product	P/N
192.24 Dynamic Array IFC for SNP Genotyping	BMK-M-192.24GT
IFC Controller RX (192.24)	IFC-RX
192.24 GT + Sample/Loading Kit – 10 Chip Package	BMK-M10-192.24GT
GT 192.24 Dynamic Array Sample & Assay Loading Reagent Kit – 10 Chips	100-3459

FLUIDIGM SOLUTIONS FOR GENETIC ANALYSIS

- **BioMark™ HD System** – Integrated hardware/software for gene expression, genotyping and digital PCR using IFCs
- **EP1™ Reader** – Integrated hardware/software for genotyping and digital PCR using IFCs
- **IFC Controller RX** – Integrated hardware/software for loading IFCs
- **FC1™ Cycler** – Integrated hardware/software for thermal cycling of IFCs
- **Reusable Dynamic Array™ IFCs** – Reusable IFCs for high-throughput SNP genotyping
- **Dynamic Array™ IFCs** – Consumable IFCs for high-throughput gene expression analysis and SNP genotyping
- **Digital Array™ IFCs** – Consumable IFCs for digital PCR
- **Software Suite** – Analysis software for gene expression analysis, SNP genotyping, and digital PCR
- **Service Plans** – Hardware service and maintenance plans

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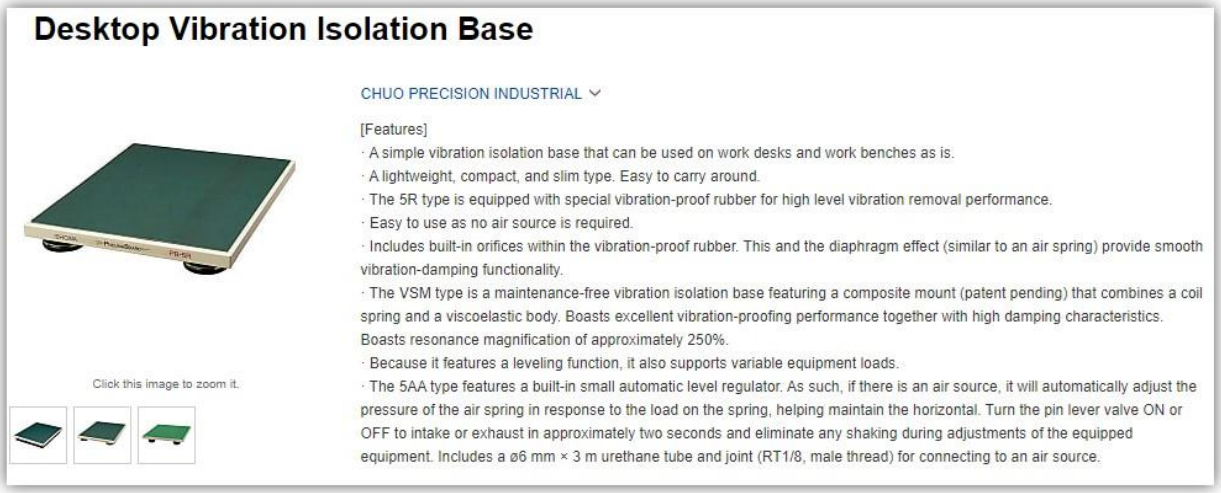
Appendix C.–Description of vibration isolation base that would be used to stabilize the Fluidigm EP1 reader.



Desktop Vibration Isolation Base

CHUO PRECISION INDUSTRIAL

[Features]
· A simple vibration isolation base that can be used on work desks and work benches as is.
· A lightweight, compact, and slim type. Easy to carry around.
· The 5R type is equipped with special vibration-proof rubber for high level vibration removal performance.
· Easy to use as no air source is required.
· Includes built-in orifices within the vibration-proof rubber. This and the diaphragm effect (similar to an air spring) provide smooth vibration-damping functionality.
· The VSM type is a maintenance-free vibration isolation base featuring a composite mount (patent pending) that combines a coil spring and a viscoelastic body. Boasts excellent vibration-proofing performance together with high damping characteristics. Boasts resonance magnification of approximately 250%.
· Because it features a leveling function, it also supports variable equipment loads.
· The 5AA type features a built-in small automatic level regulator. As such, if there is an air source, it will automatically adjust the pressure of the air spring in response to the load on the spring, helping maintain the horizontal. Turn the pin lever valve ON or OFF to intake or exhaust in approximately two seconds and eliminate any shaking during adjustments of the equipped equipment. Includes a ø6 mm × 3 m urethane tube and joint (RT1/8, male thread) for connecting to an air source.

Click this image to zoom it.