Application note

The BioArk Fish collection kit: simplicity with efficiency

Introduction

Global wild fish stocks are exploited to such an extent that, without urgent measures, we could be the last generation to catch food from the oceans[1]. The application of genomics in the aquaculture industry is one of the key options that could help turn the tide. The arrival of next generation sequencing (NGS) technologies and ultra-high throughput platforms provide the means to introgress complex traits quickly through approaches such as genomic selection (GS). One of the current caveats for the application of NGS to aquaculture includes the requirement for intact, high molecular weight DNA. This is only achievable through high-quality DNA extractions from reliable starting material.

Current protocols for fish sample collection are rife with inefficiencies and waste. These include large volume storage issues, the need for additional labour to identify samples and the transfer of samples into extraction-capable plates for screening, as well as inconsistent fin sizes that can lead to variability in DNA concentrations. In addition, protocols consume and waste vast quantities of plastics and ethanol, generate energy costs from freezing of samples, risk sample compromise during shipping or storage, and are subject to enhanced shipping costs for flammable or frozen materials. These challenging realities of securing consistent and well-preserved samples for DNA extraction have meant that applying genomics in aquaculture often starts with a disadvantage.

In contrast, the BioArk Fish collection kit (LGC, Biosearch Technologies) does not require ethanol or freezing to stabilise sample

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material: consistently sized samples are taken, deposited and stabilised directly into a compact 96-well format. Stabilised samples can then be shipped back to Biosearch Technologies for high-throughput processing, or used as starting material for in-house, automated or manual DNA isolation protocols. This application note demonstrates the effectiveness of the BioArk Fish collection kit, and its suitability for aquaculture at all scales, as it does not depend on access to electricity, ethanol storage, or large containers of samples.

Materials and methods

Biosearch Technologies worked together with an industry partner to address current sampling issues and subsequently identify the most effective and relevant resolutions to these. Throughout development, we focused on keeping the solution simple and sufficiently inexpensive for the global aquaculture community.

Current traditional protocols require sampling of variably sized fish fins into individual tubes containing 5 mL 70% ethanol. For an average GS experiment (3,000 samples), this equates to around 15 L of 70% ethanol, which is costly, difficult to store, and expensive to ship.

The BioArk Fish collection kit solution is composed of a 96-well plate, pierced strip cap tubes, desiccant, a plastic bag for the completed plate, a fish punch, and a mat. The kit allows collection of punched fin samples directly into a 96-well format, which are then immediately stabilised using the desiccant included in the kit via the perforated strip cap lids. The unique desiccant is key in maintaining sample integrity and enables the isolation of high-quality, intact DNA. The use of a punch to sample the fish fins ensure that samples are of a consistent size, which subsequently improves the consistency of the DNA that is obtained (Figure 1). The punch tool also facilitates direct transfer of the sample into a well, without needing to use forceps or scissors.

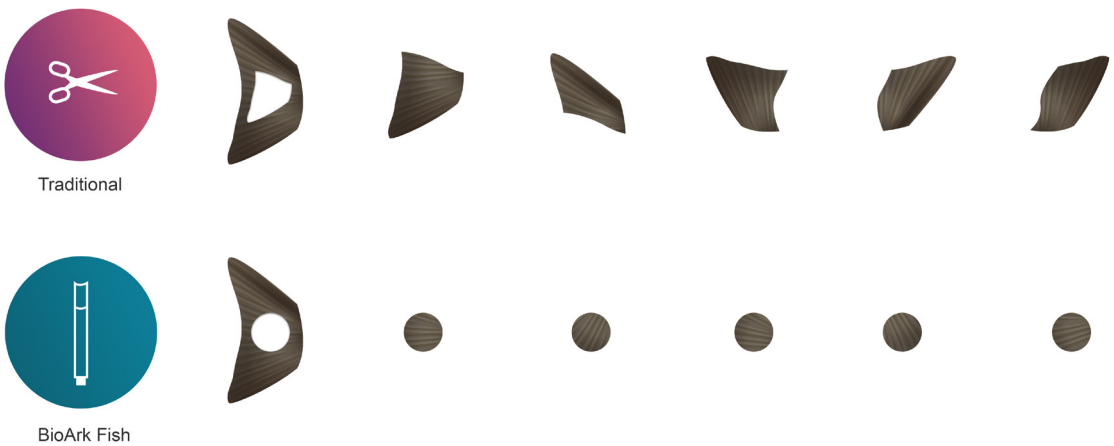

Figure 1. Illustrating the difference between fin samples obtained through traditional and BioArk Fish sampling processes. Samples collected using the BioArk Fish collection kit and round fin punch are of a consistent size (5 mm diameter), whereas traditionally sampled fins are more variable in shape and size.

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To illustrate the effectiveness of the BioArk Fish collection kit, 5 mm punches were collected from *Salmo salar* (salmon) fins using the kit, extracted using our sbeadex™ chemistry, and eluted in 50 µL of elution buffer. Figure 2 illustrates the sampling process using the fin punch. Resultant DNA was visualised using agarose gel electrophoresis (0.7% gel, 12 samples), and all 96 samples were quantified using the PicoGreen™ dsDNA quantitation assay (Thermo Fisher Scientific).

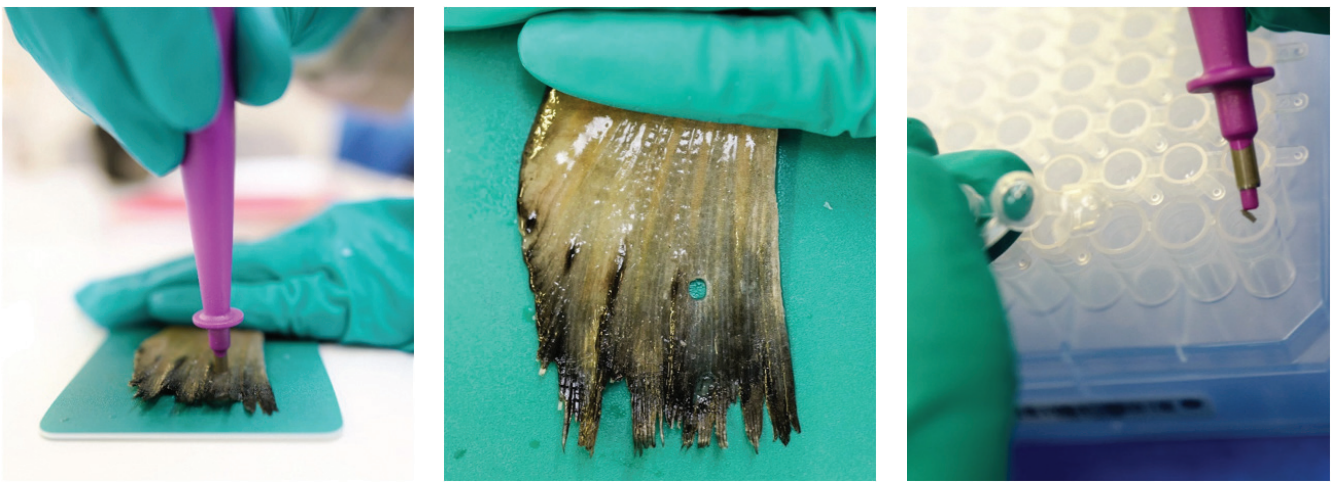

Figure 2. Fish fin sampling using the BioArk Fish collection kit. This demonstrates how a punch is taken, an example of the piece excised, and the sample transfer from the fin punch into a well of the provided 96-well plate.

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Results

The fish fin sampling protocol developed for the BioArk Fish collection kit is significantly less time consuming than traditional sampling methods. Figure 3 compares a typical timeline for traditional sampling with the timeline for the BioArk Fish collection kit. When the BioArk Fish collection kit is used, the total time required to generate extraction-ready samples is reduced by approximately 1.5 days.

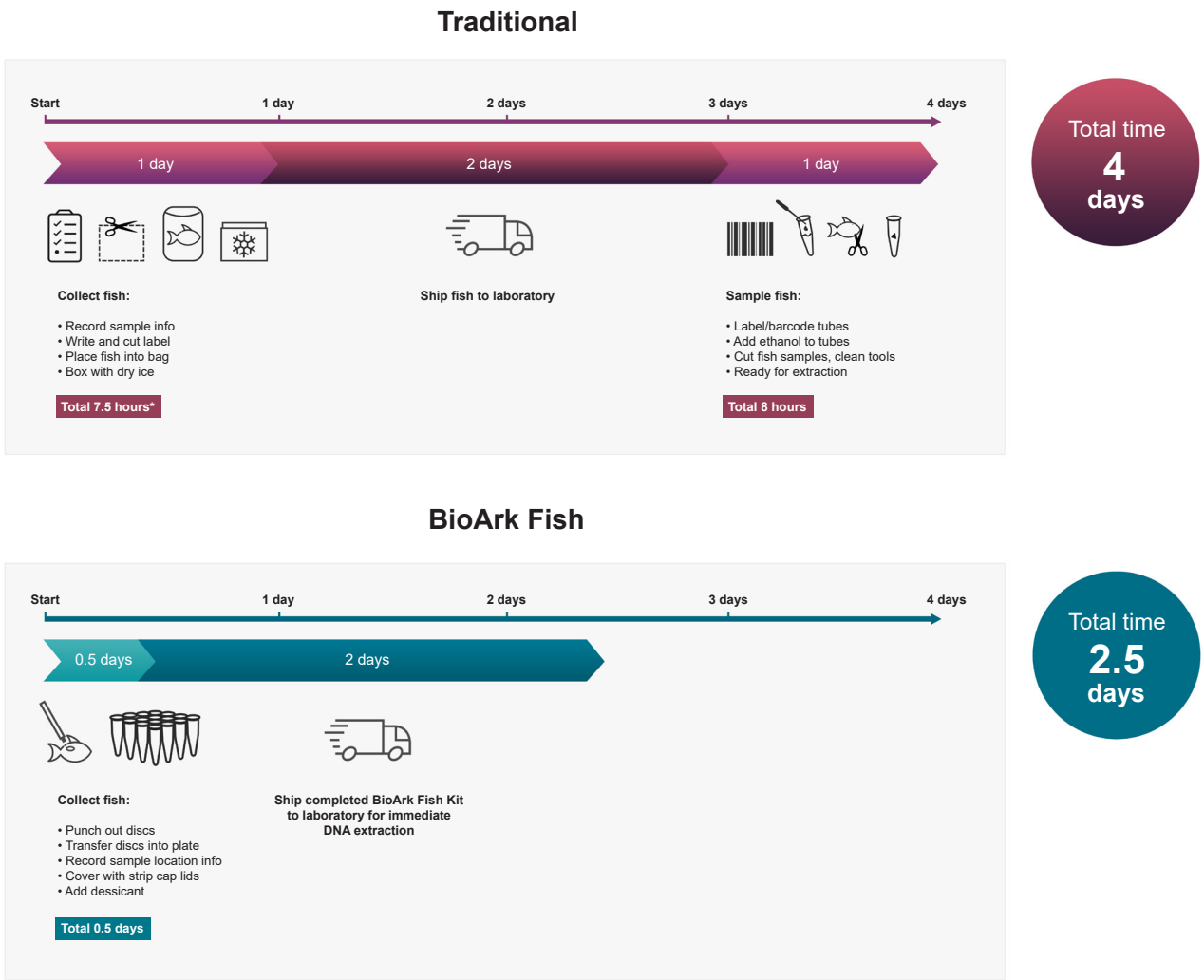

Figure 3. Timeline comparison for traditional and BioArk Fish collection kit sampling procedures. The total time required to complete sampling using the BioArk Fish collection kit is 1.5 days shorter than traditional methods. *Time required for fish collection varies depending on the total number of fish to be sampled; this estimate is based on 96 fish.

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The DNA purified from fish fins collected using the BioArk Fish collection kit was of high molecular weight, as illustrated by the heavy bands toward the top of the gel image in Figure 4. Average DNA concentration (n=96) was 110 ng/µL ± 31.2 ng/µL, and concentrations for all samples are shown in Figure 5. In addition, we have observed up to 8-week stability of fins that have been stabilised in the BioArk Fish collection kit; high-quality DNA was purified from these fins (data not shown). We would also expect samples to remain stable beyond this time period (data not yet available).

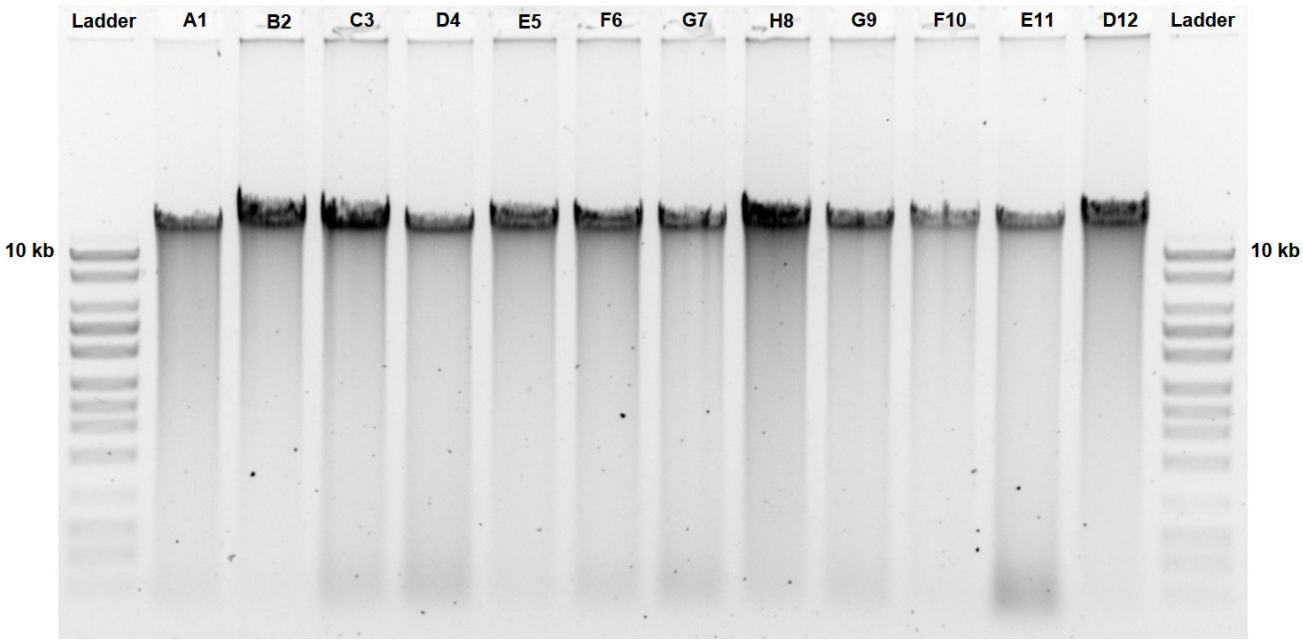

Figure 4. Visualisation of DNA obtained using the BioArk Fish Collection kit. A 0.7% gel was prepared, and 12 DNA samples (after sbeadex extraction) from across the 96-well plate were run alongside a 10 kb ladder. Clear bands illustrate that all samples contain intact, high molecular weight DNA.

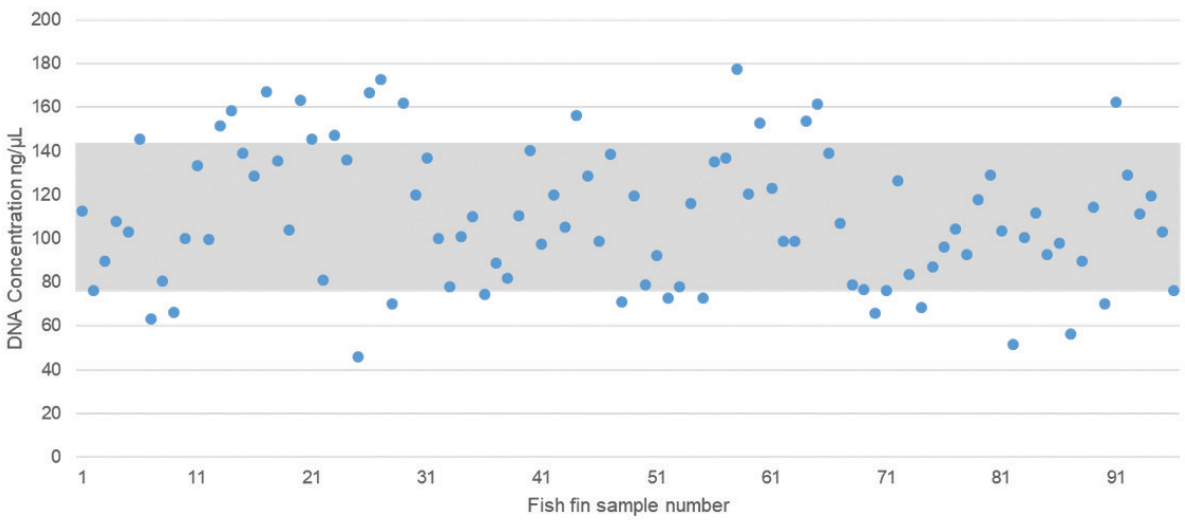

Figure 5. DNA concentration of samples obtained using the BioArk Fish collection kit. The mean DNA concentration (n=96) obtained from a 5 mm fin punch eluted in 50 µL elution buffer was 110 ng/µL ± 31.2 ng/µL.

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Conclusions

Biosearch Technologies have developed the BioArk Fish collection kit for the *in situ* stabilisation of samples intended for DNA extraction, reducing sample handling whilst maintaining the integrity and quality of the starting material. We demonstrate that intact high molecular weight DNA can be purified from fin samples collected using this kit, with sufficient DNA for many downstream applications. This simple-to-use and inexpensive solution provides a pragmatic update to process flows in aquaculture farms and research centres, no matter how remote or wild the location may be. The kit does not depend on access to electricity or require any ethanol, and results in savings of vast quantities of time, money, and consumables with a strong positive environmental impact. Our motto of 'Science for a Safer World' is driving development of solutions for the aquaculture industry, through improvement of sampling methods, elimination of waste, and streamlined access to new technologies.

References

1. How the world's oceans could be running out of fish. Gaia Vince, 21st September 2012, BBC Future, https://www.bbc.com/future/article/20120920-are-we-running-out-of-fish

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