Optimised Workflow for HMW DNA Extraction, Sample Preparation and Sequencing for Marine Vertebrates on the PacBio Revio System

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Background

- Ocean Genomes is an initiative enabled by Minderoo Foundation OceanOmics Division and delivered in collaboration with the University of Western Australia.
- We are focused on creating openly accessible, high-quality reference genomes for marine vertebrates. This initiative aligns with the Earth BioGenome Project.
- Our goal is to develop robust, scalable workflows that accelerate the production of reference-quality genomes.

Workflow

Here we describe our ocean genome laboratory workflow developed for processing marine vertebrates for HiFi sequencing on the PacBio Revio System. We outline methods optimised for High Molecular Weight (HMW) DNA extraction using the Nanobind PanDNA kit from various tissue types, our strategy for size selection, and the implementation of automated library preparation method using the Beckman Biomek i7 system.
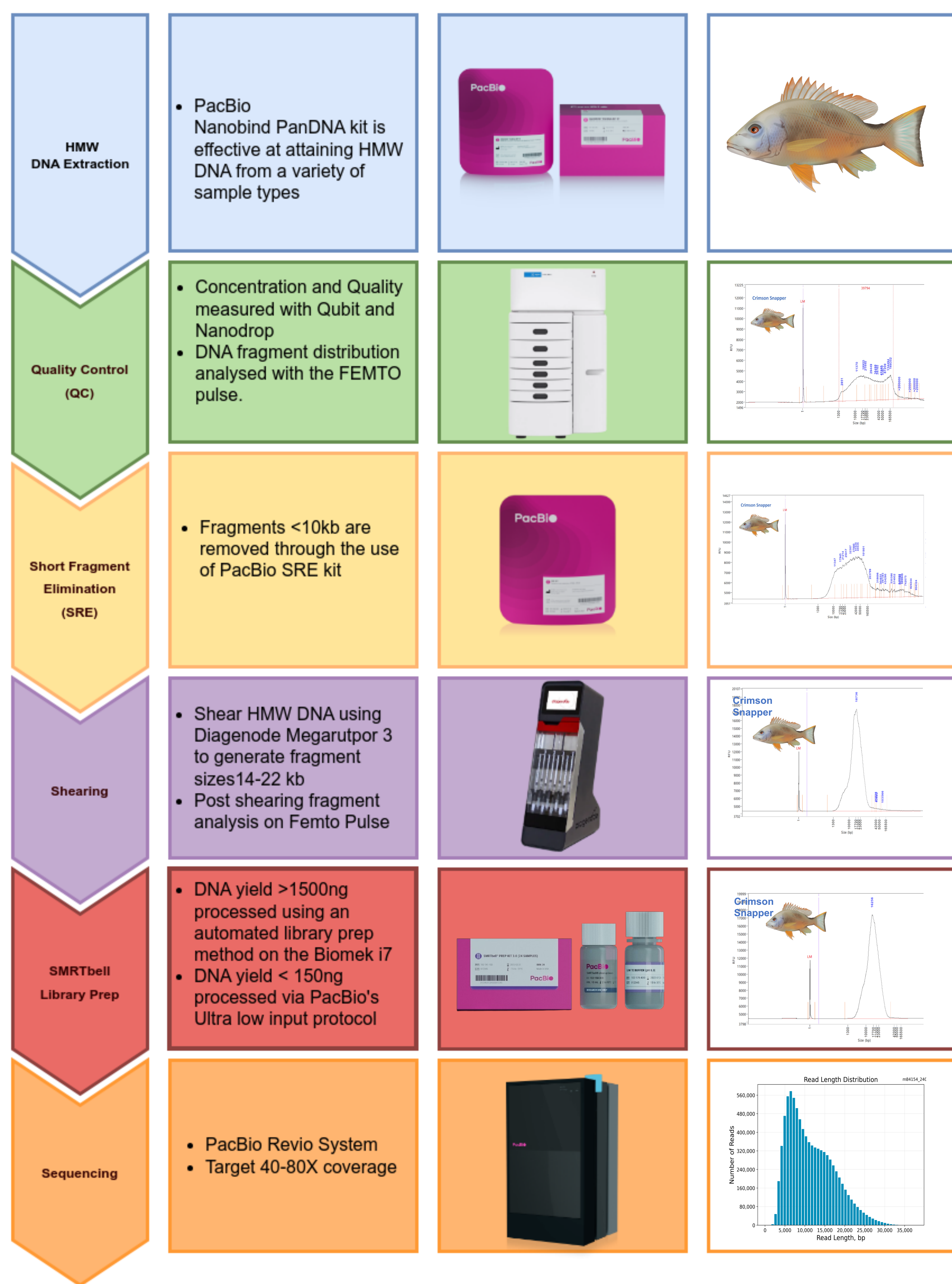
HMW DNA extraction and sequencing result

DNA was extracted from different tissues or blood of multiple marine vertebrates. DNA yield is between 6 to 79 ug and mode size from 22 to 202 kb. Longer DNA size was observed from nucleated blood samples. HiFi sequencing generated from 44X to 108X coverage with mean HiFi read length between 11 to 15 kb.

Species	Common name	Illustration	Sample Type Used	Input material	DNA yield	DNA Fragment size	SMRT Cell used	HiFi yield	Mean HiFi RL	Medium QV	Coverage
Lutjanus erythropterus	Crimson snapper		Gill	20 mg	**19.5 µg**	45 kb	1	**102 Gb**	11.6 kb	Q38	**108x**
Galeocerdo cuvier	Tiger shark		Nucleated blood	10 mg	**11.9 µg**	124 kb	3	**234 Gb**	14.7 kb	Q34	**72x**
Aprion virescens	Green Jobfish		Heart	20 mg	**79.5 µg**	23 kb	1	**93 Gb**	11.0 kb	Q38	**79x**
Aluterus scriptus	Scrawled leatherjacket		Gill	20 mg	**15 µg**	202 kb	0.5	**38 Gb**	14.2 kb	Q35	**83x**
Urogymnus asperrimus	Porcupine Ray		Nucleated blood	10 mg	**6 µg**	193 kb	2	**156 Gb**	15.4 kb	Q35	**44x**

SMRTbell library prep automation on the Beckman Coulter Biomek i7

The Biomek i7 automated liquid handling workstation is designed to deliver advanced pipetting technologies with a flexible, modular deck layout tailored for diverse laboratory workflows.

Key features include:
- Multiple dual-arm configurations with multichannel heads (96/384) and/or Span-8 pipetting capabilities
- Volume handling range from 0.5 µL to 1 mL, ensuring high accuracy and precision
- 45 available deck positions, providing exceptional workflow flexibility and scalability
- Optional on-deck modules, including a thermal cycler, orbital shaker, and Peltier for temperature control

Workflow	Hands-on time	Total processing Time
8 x Manual Library prep	155 minutes	240 minutes
8x Automated Library prep	30 minutes	220 minutes
24xManual Library prep	315 minutes	400 minutes
24x Automated Library prep	30 minutes	240 minutes
48x Automated Library prep	30 minutes	260 minutes

Implementation of automated library preparation enable larger series of sample and robust workflow. It's now possible to prepare 48 libraries in 4 hours and 30 minutes with only 30 minutes hands on time.
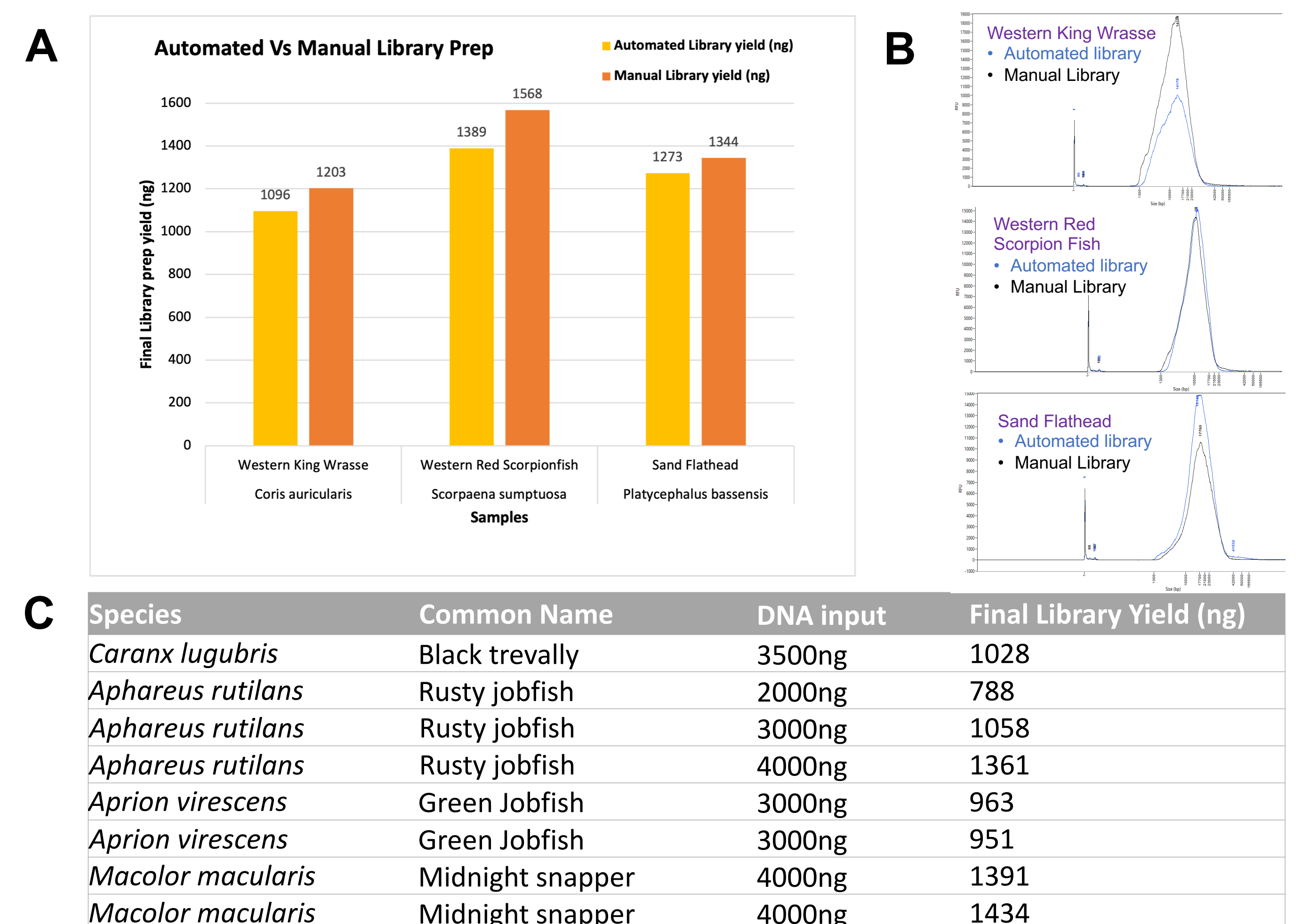

Species	Common Name	DNA input	Final Library Yield (ng)
Caranx lugubris	Black trevally	3500ng	1028
Aphareus rutilans	Rusty jobfish	2000ng	788
Aphareus rutilans	Rusty jobfish	3000ng	1058
Aphareus rutilans	Rusty jobfish	4000ng	1361
Aprion virescens	Green Jobfish	3000ng	963
Aprion virescens	Green Jobfish	3000ng	951
Macolor macularis	Midnight snapper	4000ng	1391
Macolor macularis	Midnight snapper	4000ng	1434

Figure 1. Automation validation of PacBio SMRTbell library prep on the Biomek i7. (A) Manual vs automated final library yield compared. **(B)** Similar FEMTO pulse traces of the manual and automated library prep shows that the automated method does not cause undesirable fragmentation to occur. Table **(C)** Validation results from testing automated method across a range of DNA inputs, demonstrating its consistency and capability to process various levels of DNA input.

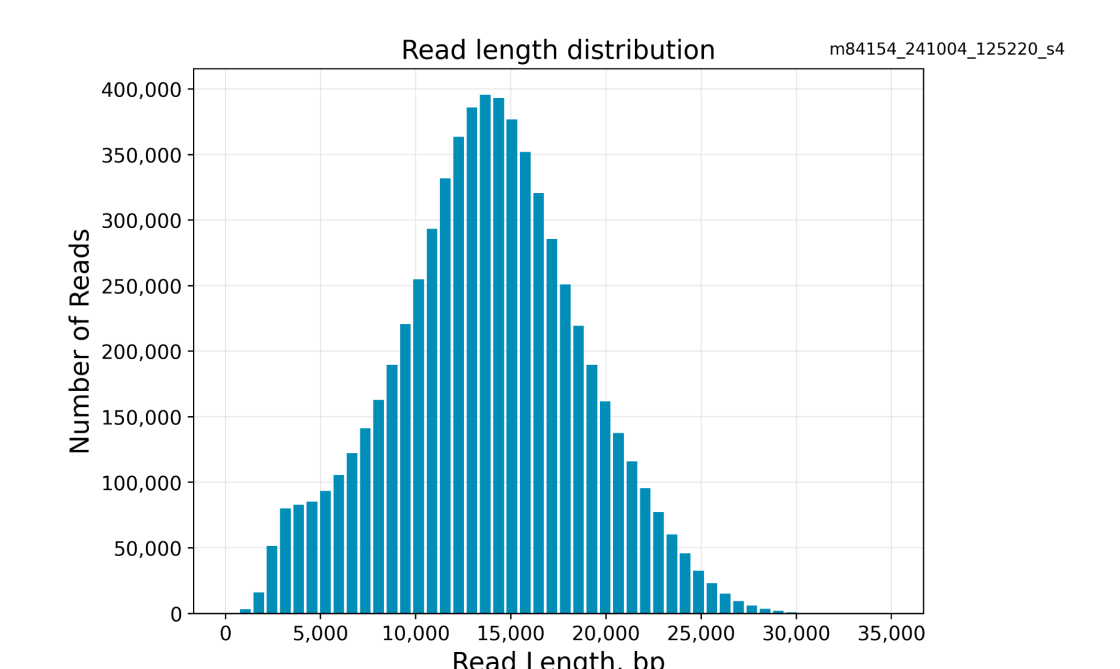| Metric	Value
Hifi reads	6.6 M
Hifi reads yield	90.0 Gb
HiFi read length N50	13.7 kb
HiFi read quality (median)	Q36
Base quality ≥Q30 (%)	94%

Figure 2. Revio sequencing results from two pooled automated libraries of the following specimens; Coral Rockcod (*Cephalopholis miniata)* and Mangrove Jack (*Lutjanus argentimaculatus).* These two libraries were prepared from the automated method on the Biomek i7. Sequenced on a single Revio SMRT Cell using a 24-hour movie time.

Conclusion:

- The Ocean Genomes initiative has established an optimised, high-throughput workflow for HMW DNA extraction, automated library preparation, and HiFi sequencing on the PacBio Revio system.
- Automation through the Biomek i7 has increased efficiency, reduced hands-on time, and enhanced sample consistency across diverse DNA inputs.
- This workflow has led to the generation of HiFi data for over 130 marine vertebrates, providing invaluable data for conservation and research efforts.