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Evaluation of DNA extraction protocols for low coverage whole genome sequencing (lcWGS) of the jumbo flying squid (*Dosidicus gigas*)

Chile

Evaluation of DNA extraction protocols for low coverage whole genome sequencing (lcWGS) of the jumbo flying squid (*Dosidicus gigas*)

Valentina Córdova-Alarcón[1,3], Carlos I. Vargas[1,2], Cristian Araneda[1,2], María Angélica Larraín[1,3]

(1) Universidad de Chile, Food Quality Research Center, Santiago, Chile.

(2) Universidad de Chile, Departamento de Producción Animal, Facultad de Ciencias Agronómicas, Santiago, Chile.

(3) Universidad de Chile, Departamento de Ciencia de los Alimentos y Tecnología Química, Facultad de Ciencias Químicas y Farmacéuticas, Santiago, Chile.

1. Background

During the 12th meeting of the SPRFMO Scientific Committee (SC12), the delegations of Peru, Chile, China, and Ecuador agreed to advance a coordinated population genomics study for the jumbo flying squid. This agreement arose precisely from the need to overcome the comparability limitations of previous studies and to establish a common design, from sampling to bioinformatic analysis. The squid Genetics and connectivity group defined its objective as evaluating the population genomics of the three phenotypic size groups described for the species (small, medium, and large) across its distribution range in the Southeast Pacific.

The agreement incorporated spatial and temporal sampling criteria, the collection of females in gonadal maturity stages III and IV, and a sample size of 30 individuals per phenotypic group and area. Furthermore, it was established that muscle tissue or DNA samples would be sent to a single sequencing facility to generate data using low-coverage Whole Genome Sequencing (lcWGS), ensuring comparable results across countries. Additionally, it was agreed to hold virtual meetings throughout 2025 to build consensus on protocols for sampling, DNA extraction, quality control, sequencing, and population genomic analysis.

Within this framework, Chile's specific commitment entails collecting *D. gigas* samples in zones E3 and E4, both of which are associated with the large phenotypic size group. Consequently, the objective of the present report is to detail the progress achieved by Chile's delegation in the collection and preparation of *Dosidicus gigas* samples for lcWGS, in accordance with the commitments assumed by Chile within the SPRFMO jumbo flying squid Genetics and Connectivity group. Specifically, this report emphasizes the efforts in zones E3 and E4, the standardization of sampling procedures, and the preliminary evaluation of DNA extraction protocols intended for comparative genomic analyses.

2. Methods

2.1 Sample collection and tissue preservation

Sample collection was conducted in accordance with the SPRFMO jumbo flying squid Genetics and connectivity group agreements, applying standardized tissue collection, biological recording, and preservation protocols previously established at SC13-SQ05. Sampling of targeted EEZ national fishing grounds corresponding to zones E3 and E4 was carried out in collaboration with local artisanal fishers during routine operations. Mantle muscle tissue was collected exclusively from the large phenotypic group, specifically targeting females exceeding 70 cm in total mantle length at gonadal maturity stage III. For each specimen, recorded metadata included the sampling date, fishing zone, WGS84 geographic coordinates (when available), and a unique identification code. Biological parameters, including total mantle length (cm) and total weight (kg), were documented, and sex was determined via macroscopic gonadal inspection. Finally, to validate the macroscopic maturity assessments, histological sections were performed on the gonads of a sample of individuals.

Briefly, mantle tissue fragments (approximately 1 × 1 cm) were excised from each specimen using sanitized forceps and scissors. To minimize DNA degradation, tissues were immediately immersed in 96% ethanol in pre-labeled 15 mL Falcon tubes, maintaining a minimum ethanol-to-tissue volumetric ratio of 5:1. Samples were transported to the laboratory under refrigerated conditions. Upon arrival, the ethanol was replaced, and the tissues were further sectioned to enhance penetration of the preservative. Strict anti-contamination and preservation protocols, including instrument sterilization between specimens and continuous cold-chain management, were implemented to ensure sample viability for downstream genomic sequencing. Finally, the samples were stored at −20 °C at the Laboratory of Genetics and Biotechnology in Aquaculture (Faculty of Agronomic Sciences, University of Chile).

2.2 DNA extraction protocols

To establish a reproducible protocol for yielding sequencing-grade genomic DNA, three extraction methods were comparatively evaluated for yield, purity, and molecular integrity in three *D. gigas* tissue samples: (i) a modified phenol-chloroform protocol (Larraín et al., 2012), (ii) the E.Z.N.A.® Tissue DNA Kit (Omega Bio-tek), and (iii) the GeneJET Genomic DNA Purification Kit (Thermo Scientific, Cat. No. K0722). DNA extractions were performed using 30 mg of minced dry tissue following the manufacturer's instructions. DNA integrity and potential degradation were assessed via 0.8% agarose gel electrophoresis. To determine the size of the DNA obtained, the Lambda/*Hind*III size ladder (Lambda DNA HindIII Digest, New England Biolabs) and a 1 kb ladder (ZR 1 kb DNA Marker, Zymo Research) were used. Sample purity (A260/280 and A260/230 ratios) was evaluated using a NanoDrop spectrophotometer, while final DNA concentration was quantified using a Qubit fluorometer equipped with the dsDNA High Sensitivity Kit (Thermo Scientific). The phenol-chloroform protocol modified by

Larraín et al. (2012) was selected for its widespread use in extracting high-molecular-weight DNA and its potential to yield intact DNA from preserved tissues. The commercial kits, E.Z.N.A. (Omega Bio-tek) and GeneJET (Thermo Scientific), were included because of their higher operational standardization, reduced inter-operator variability, and minimized exposure to hazardous reagents.

3. Results

3.1 Sample collection and tissue preservation

A total of 60 female specimens representing the large phenotypic group (>70 cm mantle length) were successfully collected from zones E3 and E4 on the coast of Chile. Strictly adhering to the SPRFMO Genetics and Connectivity group protocols, 30 individuals were sampled from the Northern Macrozone E3 (Coquimbo Region), and 30 individuals from the Central Macrozone E4 (Maule Region). Complete metadata—including biometric parameters, capture dates, geographic coordinates, and sex—were successfully recorded for all 60 individuals.

3.2 DNA extraction protocols

The three evaluated extraction protocols, phenol-chloroform, E.Z.N.A. (Omega Bio-tek), and GeneJET (Thermo Scientific), successfully yielded high-molecular-weight genomic DNA from three *Dosidicus gigas* muscle tissue samples. Agarose gel electrophoresis revealed minor to moderate smearing across all treatments, indicating low levels of DNA degradation (Figure 1). Spectrophotometric quality metrics, including DNA concentration and purity ratios, are detailed for each sample and extraction method in Table 1. The highest media DNA concentrations were obtained with the phenol-chloroform method (Nannodrop: 2,368.7 ng/ul; Qubit: 75.83 ng/ul), followed by the EZNA kit (Nannodrop: 425.02 ng/ul; Qubit: 35.13 ng/ul) and the GeneJET kit (Nannodrop: 34.47 ng/ul; Qubit: 13.31 ng/ul). The greatest DNA degradation was observed with the phenol-chloroform method, followed by the EZNA and GeneJET kits. Regarding quality, the A260/A280 absorbance ratio for all samples was close to 1.8, which is considered acceptable. The A260/A230 ratio was lower for the GeneJET kit than for the other two methods. Overall, comparative analysis demonstrated that the GeneJET commercial kit exhibited the best performance among the evaluated methods, yielding superior DNA quality and molecular integrity.

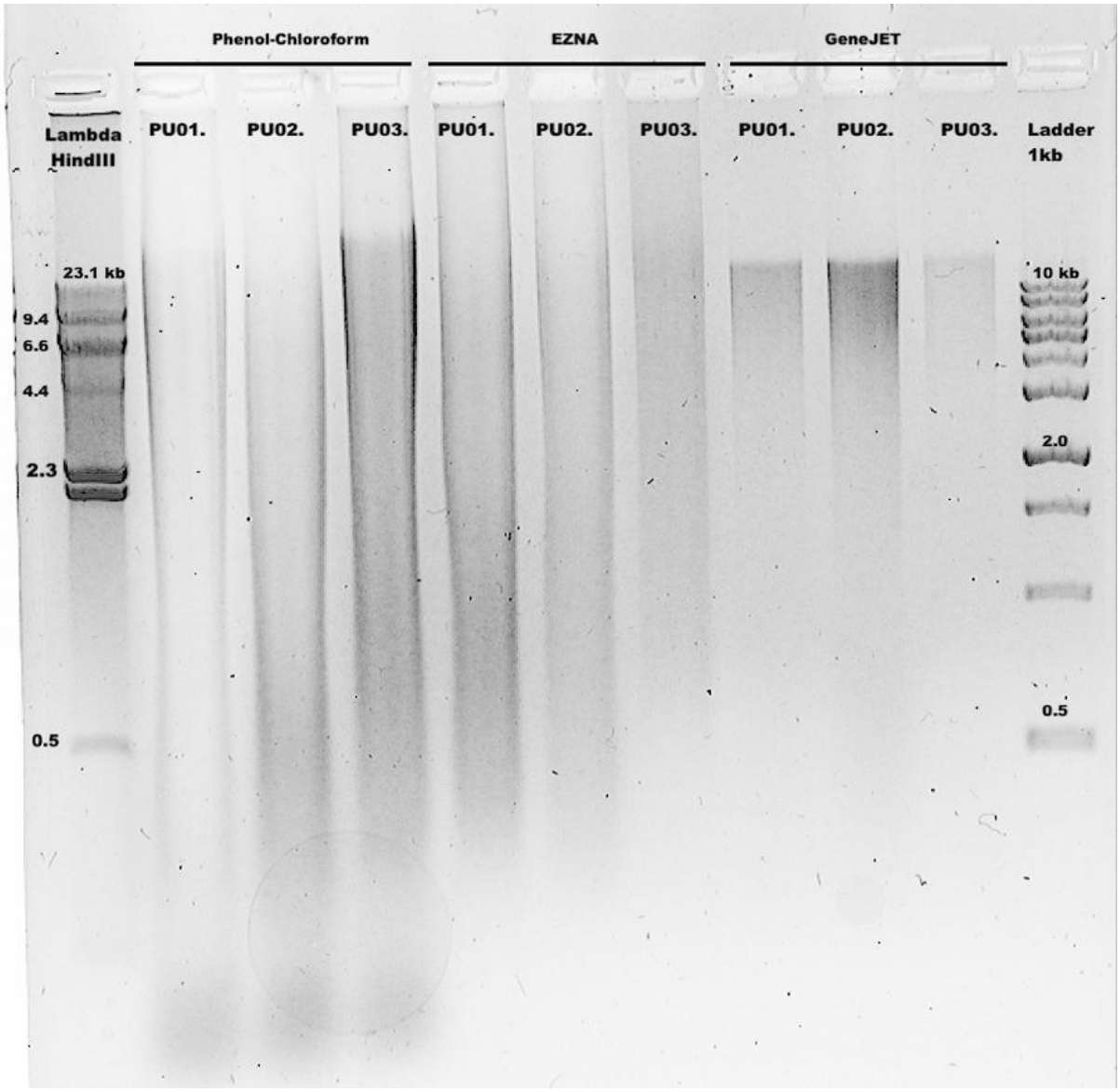

Figure 1. DNA Integrity in a 0.8% agarose gel for each DNA extraction protocol tested.

Table 1. Spectrophotometric quality metrics for extracted DNA from the 3 tested protocols.

Dna Extraction Protocol	Sample code	Nucleic Acid (ng/uL)	A260/A280	A260/A230	Qubit (ng/ul)
Phenol-Chloroform	PU-01	3498.34	1.94	1.49	87.2
	PU-02	2090.65	1.97	1.85	62.1
	PU-03	1515.81	1.96	2.09	78.2
E.Z.N.A. kit Omega Bio-tek	PU-01	638.73	1.95	1.86	49.4
	PU-02	520.28	1.95	1.39	38.9
	PU-03	116.06	1.91	2.17	17.1
GeneJET kit Thermo Scientific	PU-01	44.62	1.71	0.70	17.2
	PU-02	88.28	1.74	0.76	33.3
	PU-03	20.24	1.65	0.44	6.9

4. Discussion

Establishing a unified DNA extraction protocol across all participating nations is imperative to ensure high-quality sequencing and reliable data for the SPRFMO international genomic study on *Dosidicus gigas*. Securing high-quality, high-molecular-weight (HMW) DNA is a fundamental prerequisite for successful sequencing operations, particularly when samples collected from distinct geographical regions must be integrated and compared within a shared genomic strategy. Consequently, this document promotes the adoption of a standardized extraction methodology to prevent methodological biases and guarantee the integrity of the cooperative genetic assessment of the jumbo flying squid.

The successful implementation of low-coverage Whole Genome Sequencing (lcWGS) demands strict quality standards and highly intact DNA to ensure uniform library insert sizes and even genomic coverage. Highly degraded DNA or the presence of copurified inhibitors can lead to sequencing bias, uneven coverage, and ultimately, elevated rates of missing data during variant calling. Consequently, the selected extraction protocol must prioritize not only DNA yield but also molecular integrity, purity, cross-laboratory reproducibility, and strict compatibility with lcWGS library construction requirements.

In this context, our comparative evaluation of extraction methodologies revealed significant chemical and operational differences. The traditional phenol-chloroform method, which relies on organic phase separation, effectively isolates HMW DNA but is highly labor-intensive, heavily operator-dependent, and poses a significant risk of phenol carryover. Such chemical contamination strongly inhibits downstream enzymatic reactions required for library preparation. Conversely, silica-membrane-based commercial kits (such as the E.Z.N.A. and GeneJET kits) utilize chaotropic salts and spin-column washing steps. While commercial kits present a higher initial cost per sample regarding consumables, they drastically reduce hands-on labor time, minimize inter-operator variability, and eliminate the need for hazardous waste disposal. Our preliminary results indicate that the GeneJET kit (Thermo Scientific) exhibited the best overall performance, providing an optimal balance of HMW integrity, high purity, and operational efficiency. This finding should guide a unified technical decision within the SPRFMO, preventing divergent extraction protocols from introducing batch effects or quality biases among samples collected by different national delegations.

Furthermore, we strongly recommend convening a dedicated technical working group among researchers from Peru, Ecuador, China, Chile, and the SPRFMO. This meeting should focus exclusively on standardizing DNA extraction protocols, quality control (QC) parameters, minimum acceptance criteria, and bioinformatic workflows. Verifiable operational agreements must be established, encompassing minimum thresholds for DNA concentration and purity (e.g., A260/280 and A260/230 ratios), metadata recording formats, sample exclusion criteria, effective sequencing depth, alignment parameters, and variant calling and filtering criteria.

5. Conclusions

1. The phenol-chloroform protocol yielded the highest DNA yield, averaging 2368 ng/uL across samples.
2. All DNA extraction protocols consistently achieved optimal protein purity (A260/A280), indicating minimal to no protein contamination.
3. Phenol-Chloroform and E.Z.N.A. kit resulted in high DNA degradation.
4. The GeneJet kit exhibited the best overall performance, providing an optimal balance of high molecular-weight DNA integrity, high purity, and operational efficiency for low-coverage Whole Genome Sequencing of the jumbo flying squid.

6. Reference

Larraín, M., Díaz, F., Lamas, C., Vargas, C. & Araneda, C. 2012. Genetic composition of Mytilus species in mussel populations from southern Chile. Lat. Am. J. Aquat. Res., 40(4): 1077-1084, 2012. http://dx.doi.org/10.3856/vol40-issue4-fulltext-23