Indian Journal of Geo Marine Sciences
Vol. 47 (08), August 2018, pp. 1695-1698

Studies on the genomic DNA integrity of marine fishes through chip-based gel electrophoresis

T. Shanmuga Priya[1], V. Balasubramanian[1], T. Marudhupandi[2], C. Kumar[2] & D. Inbakandan[2]*

[1]Centre for Ocean Research, Academy of Maritime Education and Training (AMET), Chennai, Tamil Nadu 603 112, India

[2]Centre for Ocean Research, Sathyabama Institute of Science and Technology, Chennai 600 119, India

[E.Mail: inbakandan@gmail.com]

Received 30 January 2017; revised 30 March 2017

Present study consists the extraction of gDNA from marine fish gut samples that belong to the Chaetodontidae family. Eppendorf BioSpectrometer kinetic, based on UV spectroscopy, and Qubit 2.0, was employed for this purpose, with ladder. Thus on-chip, gel electrophoresis was performed for the analysis of gDNA quantification and sizing in the NGS sample preparation workflow. Quantification of 2 different gDNAs from gut samples of Pakistan butterfly (*Chaetodon collare*) and Indian vagabond (*Chaetodon decussates*) was made with a standard DNA.

[Keywords: Genomic DNA, Bio-Spectrometer kinetic, UV spectroscopy, Qubit fluorometer, Gel electrophoresis, Marine butterfly fish].

Introduction

Deoxyribonucleic acid is a genetic material that enables the storage of information from one generation to another. Various factors such as source, age and size of the sample plays a major role in the isolation process. Knowing the purity and accuracy of DNA concentration is very important in various stages of molecular cloning[1,2]. There are a number of methods available to quantify DNA. Assessment of the quantity and integrity of gDNA of marine fish sample is an important step in the preparation of NGS libraries. Analysis of these samples prior to downstream NGS applications can prevent the time and marine fish resources that are wasted. A method for DNA quantification employing UV spectroscopy is that of UV-induced fluorescence using intercalating dyes is the Qubit fluorometer that gives the total concentration of DNA in the samples. The Qubit 2.0® measures DNA sample concentrations as low as 10pg/µl, which is perfect when measuring immunoprecipitated DNA that is outside the UV spectrometer range. Commercially available chip-based nucleic acid analysis system is used to plot the electropherogram of the gDNA samples. Agilent DNA 1000 Bioanalyser, microfluidic technology was used here to generate the electropherogram plot with fluorescence intensity versus migration time that will be displayed as a “virtual gel” using a densitometry plot[3].

The marine ornamental butterfly fishes (Chaetodontidae) are common, vividly colored inhabitants of coral reef communities across the world. Because they are conspicuous and abundant, species within this group have been the subject of a wealth of ecological, behavioral, systematic, and biogeographic research[4-7.] Despite this attention, the process of diversification in this group is poorly understood. Reef fishes in the coral reef habitat of Gulf of Mannar, India were studied during the period from July 2007 to 2008. Overall 117 species under 79 genera, 42 families were recorded by the visual census method. Among them 29 species were noticed in all study stations. The abundance of in the study area was estimated for fishes Lethrinidae (11 and 10.6 %), Chaetodontidae (9.9 and 11.1 %), and Pomacentridae (8 and 9.2 %) family was dominated in all the study stations followed by Labridae (8.4 and 6.9 %), Serranidae (8.7 and 6.4 %), Apogonidae (6.6 and 5.8 %), respectively. Based on these data, we have targeted Family: Chaetodontidae, and selected 2 different marine ornamental butterfly fish species like, Pakistan butterfly (*Chaetodon collare*) and Indian vagabond (*Chaetodon decussates*), respectively for this experimental study.

Materials and Methods

Marine butterfly fishes, *C. collare* (TL = 6 ± 0.5 cm, n = 4) and *C. decussates* (TL = 6 ± 0.5 cm, n = 4)

were procured from ornamental fish traders in Chennai. These were transported to the laboratory and maintained in seawater aquarium equipped with biofilters, canisters and protein skimmers. Shrimp, boiled oyster and clam meat were used to feed the fish for three times a day (08:00, 12:00 and 16:00 hrs). Excretory material and remnant food particles were siphoned out an hour after the feeding. The tanks were illuminated with a 40 W fluorescent tube suspended 45 cm above the water surface. Water quality parameters in the tanks were maintained as temperature 28 ± 1 °C, salinity 32 ± 1 ppt, pH 8.1 ± 0.2 and dissolved oxygen 6.5 ± 0.3 mg l^{-1}. Light intensity of 800 lux was maintained for 12 h (07:00–19:00 hrs). Once a week the tanks were given 50% water change. Thus the fishes were maintained for 30 days in a controlled condition prior the experiment.

A modified protocol was followed based on the work reported by Wu et al., 2016.[10] Total community genomic DNA extraction of the marine butterfly fish gut samples were performed using a QIAamp DNA Stool Mini Kit (Qiagen, Germany), following the manufacturer's instructions. We measured the concentration of the DNA using a UV-vis spectrophotometer (Eppendorf BioSpectrometer kinetic) to ensure that adequate amounts of high-quality genomic DNA had been extracted (>300 µg/µl).

Our target was the V3–V4 hyper-variable region of the bacterial 16S rRNA gene of the marine butterfly fish gut samples. PCR was started immediately after the DNA was extracted. The 16S rRNA V3–V4 amplicon was amplified using KAPA HiFi Hot Start Ready Mix (2×) (TaKaRa Bio Inc., Japan). Two universal bacterial 16S rRNA gene amplicon PCR primers (PAGE purified) were used: the amplicon PCR forward primer (CTACGGGNGGCWGCAG) and amplicon PCR reverse primer (GACTACHVGGGTATCTAATCC). The reaction was set up as follows: microbial DNA (5 ng/µl) 2.5 µl; amplicon PCR forward primer (1 µM) 5 µl; amplicon PCR reverse primer (1 µM) 5 µl; 2× KAPA HiFi Hot Start Ready Mix 12.5 µl (total 25 µl). The plate was sealed and PCR performed in a thermal instrument (Applied Biosystems 9700, USA) using the following program: 1 cycle of denaturing at 95 °C for 3 min, followed by 25 cycles of denaturing at 95 °C for 30 s, annealing at 55 °C for 30 s, elongation at 72 °C for 30 s, and a final extension at 72 °C for 5 min. The PCR products were checked using electrophoresis in 1 % (w/v) agarose gels in TBE buffer (Tris, boric acid, EDTA) stained with ethidium bromide (EB) and visualized under UV light. A bioanalyzer (Agilent 2100, USA) with DNA 1000 chip was used to verify the size of the PCR product of the marine butterfly fish gut samples.

We used AMPure XP beads to purify the free primers and primer dimer species in the amplicon product. To sequence our amplicon, we attached dual indices and Illumina sequencing adapters using the Nextera XT Index Kit and purified the amplicon again using AMPure XP beads[11]. The DNA concentration of each PCR product of the marine butterfly fish gut sample was determined using a Qubit® 2.0 Green double-stranded DNA assay and it was quality controlled using a bioanalyzer (Agilent 2100, USA).

Results and Discussion

The concentration of DNA was accurately measured by the UV absorbance spectrophotometer. Amount of UV radiations absorbed by solution of DNA is directly proportional to the amount of DNA in the sample. Usually, the absorbance is measured at 260 nm at which wavelength of absorbance A 260 of 1.0 corresponds to 50 µg. The purity of DNA is determined with the pure sample of DNA where the ratio of the absorbance at 260 nm and 280 nm (A260/A280) was 1.8. The ratio less than 1.8 indicates the presence of contaminants such as proteins or with phenol. Similarly the purity of DNA is determined with the pure sample of DNA where the ratio of the absorbance at 260 nm and 230 nm (A260/A230) was 2.2. The standard of known DNA concentration was prepared and the readings were measured by UV spectrophotometer at 260 nm. As the DNA concentration increased the OD value also increased. OD is directly proportional to DNA concentration (Psifidi, 2010).

The Agilent DNA Chip software offers a significantly improved smear analysis with an easy to use integrator. The improved smear identification provided sliders within the electropherogram that allow size adjustments of the smear region. One adjustable smear region was predefined in the Agilent DNA Chip software, but additional regions could be added. The software automatically determined the average size (bp), size distribution in CV (%), concentration (pg/µL), % of total, and molarity (pmol/L) for each defined smear region. The region table displayed underneath the electropherogram summarizes the smear results of all regions per

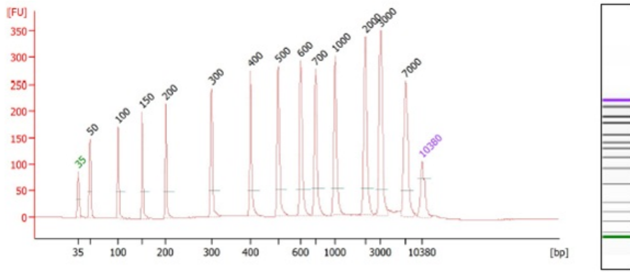

Fig. 1A — Eletropherogram peaks and gel images for standard DNA ladder

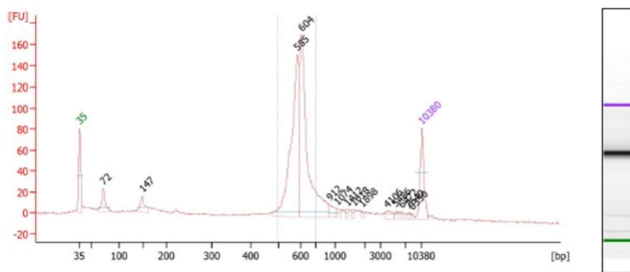

Fig. 1B — Eletropherogram peaks and gel images for gDNA isolated from the gut of Pakistan butterfly (*Chaetodon collare*)

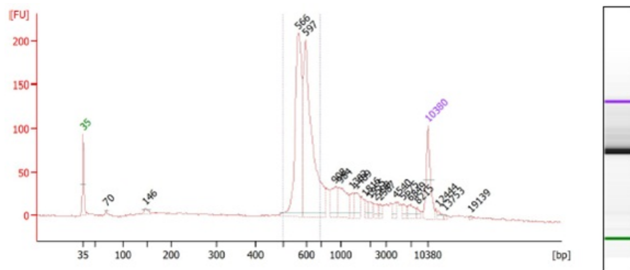

Fig. 1C — Eletropherogram peaks and gel images for gDNA isolated from the gut of Indian vagabond (*Chaetodon decussates*)

sample (Agilent High Sensitivity DNA Kit Guide, 2009). The Agilent DNA HS kit was developed to resolve different DNA size ranges, 50-7000bp and 100-12000bp respectively. Each kit exhibits the optimal separation performance for the targeted DNA fragment range and provided less separation in other size ranges, by utilizing different gel matrices for the separation of the DNA fragments. Libraries were validated using bioanalyser by loading 1µl of to 1 well on Agilent DNA HS Chip for the separation, sizing and quantification of dsDNA fragments from 35 to 10380 bp for the of the marine butterfly fish gut samples.

The Agilent analysis software displayed both an Electropherogram view and a Region view. The Region view was designed for analyzing samples that appeared as a smear (for example, NGS libraries) and gave slightly different information to that displayed in the electropherogram view. Electropherogram view was designed with discrete peaks, and the default size reported was that of the highest point of the peak. Region view calculated data over a whole region and reported the distribution of sizes within that marine butterfly fish gut samples. Fig: 1-A shown the standard eletropherogram with various ladder peaks from 35bp as a lower marker and 10380bp as an upper marker. Fig: 1-B and 1-C revealed the eletropherogram view and the region view for the marine butterfly fish gut samples.

Conclusions

The outcome of Next Generation Sequencing (NGS) and other molecular screening assays often rely on the overall quality of the genomic DNA (gDNA) starting material. It is therefore widely recommended to quantify and assess the integrity of the gDNA, especially for costly workflow such as NGS to prevent wasted time and resources. Traditionally, agarose gel electrophoresis is used for gDNA analysis. This method only offers limited data though, as a second fluorometric or spectrophotometric method is required for gDNA quantification. In addition to being labor intensive and a manual process, it also does not provide a numerical assessment of the gDNA integrity. To address these limitations, chip-based gel electrophoresis method was utilized through Agilent bioanalyzer (Agilent 2100, USA system), for measuring the quantity and integrity of the gDNA starting material. Thus eletropherogram peak and gel images for gDNA isolated from the gut of Indian vagabond (*Chaetodon decussates*) and Pakistan butterfly (*Chaetodon collare*) were quantified compared with standard DNA.

Acknowledgement

Authors thank Chancellor and Management of Sathyabama University, Chennai for their kind support.

References

1. Saenger, W., Principles of Nucleic Acid Structure, *Springer-Verlag: New York*, (1984) 90-123.
2. Bruce, A., Johnson, A., Lewis, J., Raff, M., Roberts, K., Walters, P., *Molecular Biology of the Cell, 4th Edition*, (Garland Science: New York) 2002
3. Robertson, C., *Spectrophotometry's next generation technology*, (American Biotechnology Laboratory) 2003.
4. Helfman, G., Collette B., Facey, D.E., Bowen, B.W., *The Diversity of Fishes: Biology, Evalution and Ecology, 2nd Edition,* (Malden: Blackwell) (1972) pp. 176.

5. Paxton, J., Eschmeyer, W., *Enclyclopedia of fishes- second edition*. (San Diego, CA: Academic press) 1998.
6. Kuiter, R, H., *Coastal fishes of south-Easterrn Australia*, (Honolulu, HI: University of Hawaii press) 1993.
7. Nelson, J., *Fishes of the world- third edition. New York*. (John Wiley and sons) 1994.
8. Schander, C., Kenneth, H.M., DNA, PCR and formalinized animal tissue - a short review and protocols. *Organ. Divers. Evol.*, 3 (2003) 195-205.
9. Psifidi A, Dovas CI, Banos G. A comparison of six methods for genomic DNA extraction suitable for PCR-based genotyping applications using ovine milk samples. *Mol. Cell. Probes.,* 24 (2010) 93-8.
10. Wu, X., Zhang, H., Chen, J., Shang, S., Wei, Q., Yan, J., Tu, X. Comparison of the fecal microbiota of dholes high-throughput Illumina sequencing of the V3–V4 region of the 16S rRNA gene. *Appl. Microbiol. Biotechnol.,* 100 (2016) 3577-86.
11. Kozich,J.J., Westcott, S.L., Baxter,N.T., Highlander, S.K., Schloss, P.D., Development of a Dual-Index Sequencing Strategy and Curation Pipeline for Analyzing Amplicon Sequence Data on the MiSeq Illumina Sequencing Platform, *Appl. Environ. Microbiol.,* 79(2013) 5112–5120.