

Genetic Population Structure of the Blue Starfish (*Linckia laevigata*) in the Indo-Malay Archipelago Based on Microsatellites

Introduction

- Population genetics is the study of naturally occurring genetic differences or polymorphism among organisms (Hartl, 2000). It can be approached by several genetic markers, such as allozymes, mitochondrial DNA (mtDNA) and microsatellites.
- Microsatellites are tandem repeats of one to six nucleotides sequence found in the nuclear genomes (Selkoe & Toonen, 2006).
- The blue starfish (*Linckia laevigata*) is a common benthic animal associated with coral reefs in the Indo-West Pacific (Fig. 1).
- A previous study on the population genetic structure of *L. laevigata* in the Indo-Malay Archipelago based on mitochondrial cytochrome oxidase subunit I gene (COI) revealed a genetic break between the Indian and Pacific Oceans, but panmixing on most parts of archipelago (Kochzius et al. 2009).

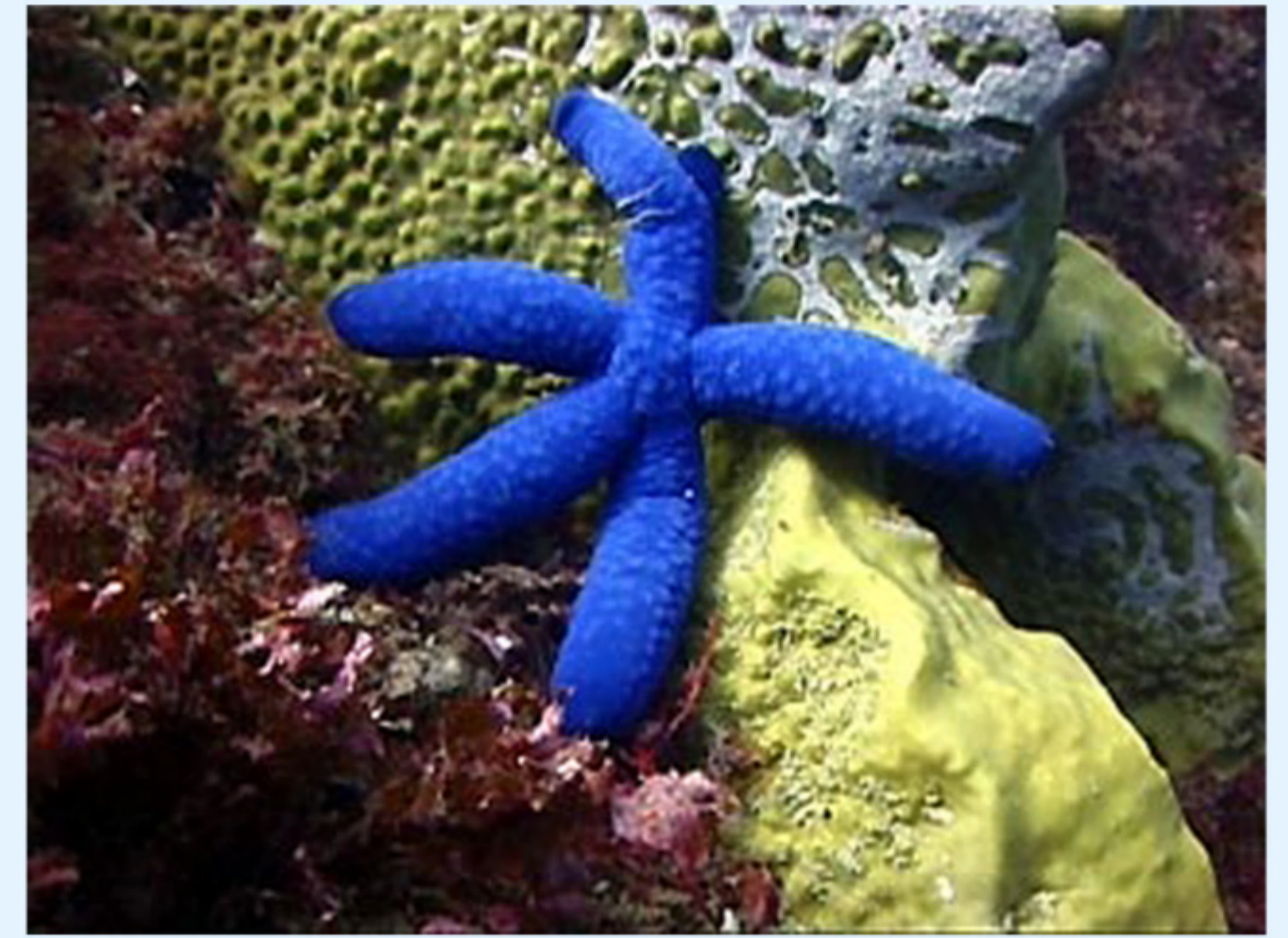


Figure 1. The blue starfish (*Linckia laevigata*)
Source: http://www.plankton.com.au/dorey/wsp/hal2004/large/linckia_laevigata.jpg

Objectives

- Characterizing the genetic variability based on microsatellites,
- Assessing the genetic population structure of *L. laevigata* in the Indo-Malay Archipelago (Fig.2), and
- Comparing the results to the previous study based on COI.

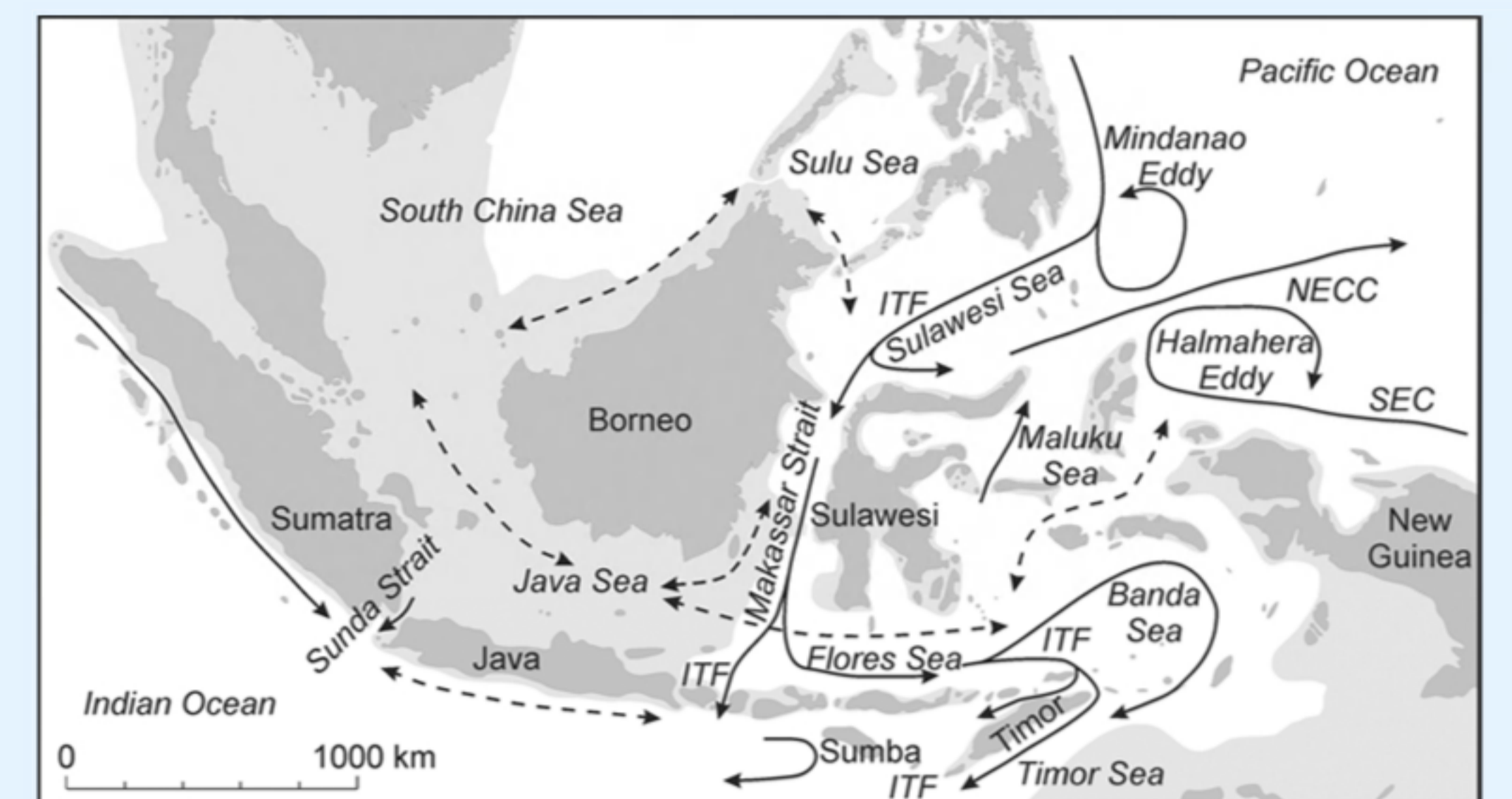


Figure 2. Map of Indo-Malay Archipelago

Materials and Methods

Sampling

Tissue samples of *L. laevigata* were collected at 30 sites (Fig.3) across the Indo-Malay Archipelago in 2004-2007.

A fragment, approximately 1 cm length was cut from blue starfish arms, then separately preserved in 96% ethanol and stored in 4°C.



Figure 3. Sampling sites in Indo-Malay Archipelago

DNA Extraction

DNA extraction was carried out using the Chelex method.

Microsatellite loci will be amplified through Multiplex PCR based on 23 microsatellite primers described by Wainwright et al. (2012).

Temperature profile for PCR will be denaturation at 95°C for 10 minutes, follow by 38 cycles of 30s at 94°C, 45s at 55°C and 45s at 72°C; and final extension 30 minutes at 72°C.

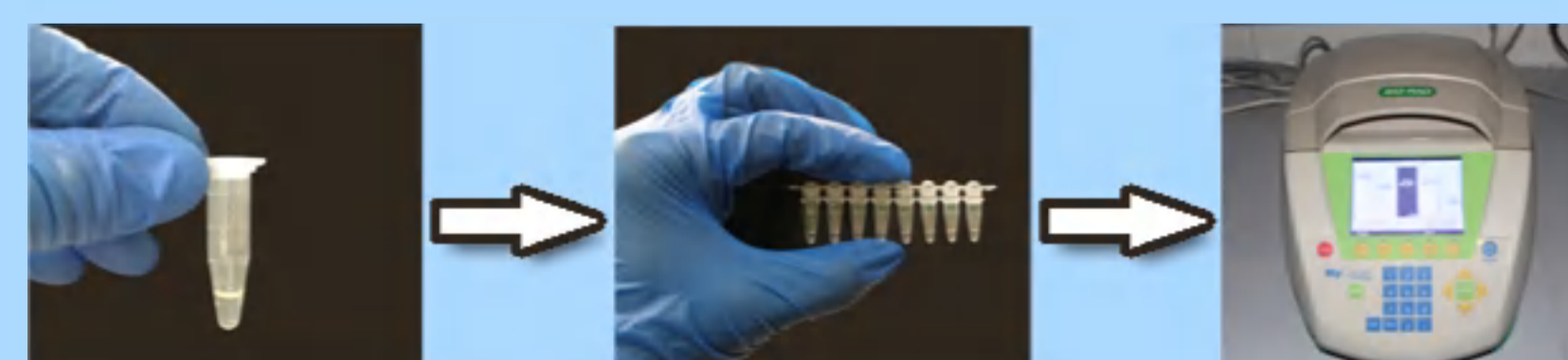


Figure 4. Scheme of DNA extraction and PCR

Gel Electrophoresis

PCR products will be visualized on a gel imaging system.

Gel are made of 2% Agarose in TBE 1X, and it will be run on 80 Volt, 400 mA for 90 minutes.

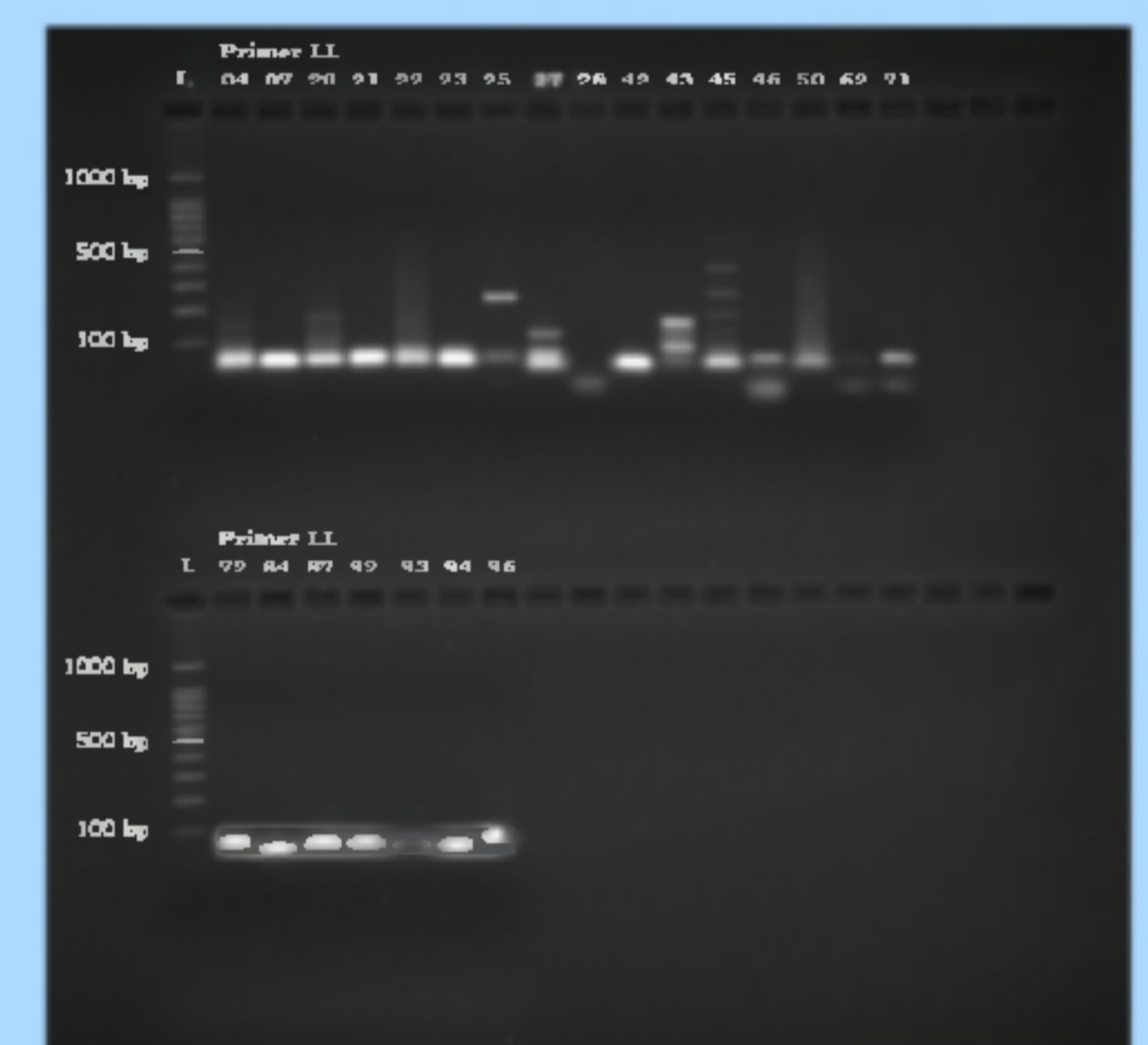


Figure 5. Visualization of 23 primers on gel.

References

- Hartl DL (2000) A Primer of Population Genetics. Sinauer Associates, Inc., Sunderland, Massachusetts.
- Kochzius M, Seidel C, Hauschild J, Kirchhoff S, Mester P, Meyer-Wachsmuth I, Nuryanto A, Timm J (2009) Genetic population structures of the blue starfish *Linckia laevigata* and its gastropod ectoparasite *Thyca crystallina*. Marine Ecology Progress Series 396: 211-219.
- Selkoe KA, Toonen RJ (2006) Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. Ecology Letters 9: 615-629.
- Wainwright BJ, Arlyza IS, Karl SA (2012) Isolation and characterization of twenty-three polymorphic microsatellite loci for *Linckia laevigata*. Conservation Genet Resour: 1-3.