azti tecnalia

GECKA

Genetic close-kin analysis on white anglerfish (*Lophius piscatorius*) for abundance estimates in support of deep sea fisheries management under the Common Fisheries Policy

M2.1_DNA EXTRACTION PERFORMED AND RAD-SEQ LIBRARIES PREPARED

PROJECT TITLE: Genetic close-kin analysis on white anglerfish (*Lophius piscatorius*) for abundance estimates in support of deep sea fisheries management under the common fisheries policy

ACRONYM: GECKA

FUNDING BODY: JOINT RESEARCH CENTER, EUROPEAN COMMISION

WEBSITE: http://www.azti.es/gecka

CONTACT: nrodriguez@azti.es

Index

1. SAMPLE SELECTION

324 individuals maximizing area and season coverage were selected for DNA extraction (Table 1).

	WINTER	SPRING	SUMMER	AUTUMN
A	6	0	25	17
B	0	20	0	22
C	24	1	0	22
D	5	19	0	42
E	6	16	0	21
F1	2	11	11	22
F2	0	0	0	15
G	0	0	4	3
MED	0	0	10	0

Table 1: Number of samples per area and season selected for DNA extraction.

2. DNA EXTRACTION AND QUALITY CHECKING

Genomic DNA from 324 individuals was extracted from about 20 mg of muscle tissue using the Wizard® Genomic DNA Purification kit (Promega, WI, USA) following manufacturer's instructions for "Isolating Genomic DNA from Tissue Culture Cells and Animal Tissue". Extracted DNA was suspended in Milli-Q water and concentration was determined with the Quant-iT dsDNA HS assay kit using a Qubit® 2.0 Fluorometer (Life Technologies). DNA integrity was assessed by electrophoresis, migrating about 100 ng of GelRed™-stained DNA on an agarose 1.0% gel and assigning values 1, 2 o 3 depending if they are bad, medium or high quality. 288 samples with quality score 2 or 3 and sufficient DNA quantity were selected for RAD-sequencing (Annex I).

3. RAD-SEQ LIBRARY PREPARATION AND SEQUENCING

Restriction-site-associated DNA libraries were prepared following the methods of Etter et al. (2011). Briefly, starting DNA (ranging from 250 to 600ng, depending on integrity) was digested with the SbfI restriction enzyme and ligated to modified Illumina P1 adapters containing 5bp unique barcodes. Pools of 32 individuals (Table 2) were sheared using the Covaris® M220 Focused-ultrasonicator™ Instrument (Life Technologies) and size selected to 300-500 bp by cutting agarose migrated DNA (Figure 1). After Illumina P2 adaptor

ligation, each library was amplified using 14 PCR cycles. Each pool was sent for paired-end sequenced (100 bp) on an Illumina HiSeq2000.

POOL	n	A	B	C	D	E	F1	F2	G	MED	total
GECKA-01	32	8	2	6	10	1	4	1	0	0	32
GECKA-02	32	7	2	6	6	0	11	0	0	0	32
GECKA-03	32	3	0	6	8	0	15	0	0	0	32
GECKA-04	32	2	8	4	4	4	0	4	5	1	32
GECKA-05	32	8	3	5	1	8	4	0	1	2	32
GECKA-06	32	1	12	4	1	5	0	7	1	1	32
GECKA-07	32	5	4	5	4	9	0	3	0	2	32
GECKA-08	32	5	6	5	4	12	0	0	0	0	32
GECKA-09	32	3	5	1	4	3	12	0	0	4	32
Total	288	42	42	42	42	42	46	15	7	10	288

Table 2: Number of individuals per area included in each of the 9 RAD-seq pools.