CONSTRUCTION OF EST DATABASE FROM OVARIES OF WILD MATURING EELS

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Introduction:

Since a research project for artificial production of the Japanese eel, *Anguilla japonica*, was commenced in the late 1960s, a continuing process of trial and error has finally achieved the production of second-generation larvae in 2010. However, quality of eggs obtained through controlled maturation is still highly variable, and the early survival rates of the larvae are usually extremely low. The low egg quality may be caused by abnormal oocyte development induced artificially by salmon pituitary injections. To address this issue, we began seeking wild maturing eels in their spawning area.

Methods:

On receiving a success in capturing post-spawning Japanese eels with their gonads appeared to be degenerated in 2008 [1, 2], a large-scale trawling survey was conducted by four research cruises in the following spring to summer, at the southern part of the West Mariana Ridge. During a 2009 trawling survey, four males and four females of the Japanese eel in spawning condition and two mature males and one female of the giant mottled eel (*Anguilla marmorata*) were caught [3]. Trawling surveys were also carried out in 2010, and one female of *A. marmorata* in maturing condition was captured. Total RNA was extracted from the ovaries of *A. japonica* and *A. marmorata*. Oligo-dT primed cDNA libraries were used for sequencing. Sequencing was performed using Genetic Analyzer II (GAII, Illumina) and 454 FLX Titanium (454FLX, Roche). Sequence reads obtained by GAII were assembled by Velvet assembler, then the GSII contigs and sequence reads obtained by 454FLX were assembled together into contigs by using TGICL clustering software.

Results and discussion:

Two of three female *A. japonica,*t captured in the new moon night, were in a condition just after spawning. The ovaries possessed clear post-ovulated follicles and oocytes in early to mid-vitellogenic stage. This ovary in the early to mid-vitellogenic stage was used for sequencing. The third female had still ovulated oocytes (eggs before spawning). The eggs seemed to be over-ripened judging from the over-conflated oil droplet. The electrophoresis profiles of the RNA extracted from the eggs seemed to occur its fragmentation. *A. marmorata* captured in 2010 had ovary in late-vitellogenic stage with oocytes that occur transparent in their peripheral region. The late-vitellogenic ovary was not obtained in the wild *A. japonica*, therefore, *A. marmorata* ovary was used for sequencing as a reference. From *A. japonica* ovary in the early to mid-vitellogenic stage, GAII single sequencing run produced 14,372,520 reads, with average length of 43 nucleotides. These reads were assembled into 63,622 contigs, with average 214 bases. A half-plate run of 454FLX sequencing produced 320,429 reads, with average length of 293 nucleotides. These reads were assembled into 98,310 contigs including 85,673 singlets, with average length of 447 bases. The contigs constructed from GAII sequences and sequence reads from 454FLX were assembled together by TGICL clustering software. This assemble produced 128,840 contigs, with average length of 341 nucleotides (Table 1). From *A. marmorata* ovary in the late-vitellogenic stage, GAII single sequencing run produced 34,612,320 reads, with average length of 76 nucleotides. A half-plate run of 454FLX sequencing produced 615,163 reads, with average length of 414 nucleotides. These reads were assembled into 91,691 contigs including 65,455 singlets (Table 2). An attempt for cross mapping between *A. japonica* and *A. marmorata* short sequence reads produced by GAII

Table 1 Sequence reads and contigs from *A. japonica* ovary

	Number of reads	Average read length	Number of contigs	Average contig length
GAII	14,372,520	43	63,622	214
454FLX	320,429	293	98,310	447
GAII + 454FLX	-	-	128,840	341

Table 2 Sequence reads and contigs from *A. marmorata* ovary

	Number of reads	Average read length	Number of contigs	Average contig length
GAII	34,612,329	76	not yet	not yet
454FLX	615,163	414	91,691	not yet

resulted in 71 % and 62 % of GAII reads of *A japonica* and *A. marmorata* could be mapped onto contigs of *A. japonica*, respectively.

Conclusion:

EST database was constructed from wild matured *A. japonica* and maturing *A. marmorata* ovaries by the aid of two types of next generation sequencing. GAII produced higher number of short sequence, in contrast, 454FLX produced longer sequences but in lower number. Clustering of these sequence reads together appeared to accelerate formation of contigs. The result from that almost same percentage of GAII sequence reads from *A. japonica* and *A. marmorata* could mapped onto *A. japonica* contigs may indicates that *A. marmorata* sequence reads can be used for transcriptome analyses in *A. japonica* ovaries.

References:

[1]CHOW, S., KUROGI, H., MOCHIOKA, N., KAJI, S., OKAZAKI, M., TSUKAMOTO K. 2009. Discovery of mature freshwater eels in the open ocean. Fish. Sci., 75: 257-259.

[2]KUROGI, H., OKAZAKI, M., MOCHIOKA, N., JINBO, T., HASHIMOTO, H., TAKAHASHI, M., TAWA, A., AOYAMA, J., SHINODA, A., TSUKAMOTO, K., TANAKA, H., GEN, K., KAZETO, Y., CHOW, S. 2011. First capture of post-spawning female of the Japanese eel *Anguilla japonica* at the southern West Mariana Ridge. Fish. Sci., 77: 199-205.

[3]TSUKAMOTO, K., CHOW, S., OTAKE, T., KUROGI, H., MOCHIOKA, N., MILLER, J.M., AOYAMA, J., KIMURA, S., WATANABE, S., YOSHINAGA, T., SHINODA, A., KUROKI, M., OYA, M., WATANABE ,T., HATA, K., IJIRI, S., KAZETO, Y., NOMURA, K.,TANAKA, H. 2011. Oceanic spawning ecology of freshwater eels in the western North Pacific. Nature Communications 2: Article 179.