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Development of optimal EST-SSR marker creation method in rice

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[Introduction]

As the DNA marker technology has been developed, the rapid generation of numerous genetic and physical maps was promoted and genetic map comparisons of different species, regardless of their genetic distance could be possible. As the interest of these application of molecular markers is increasing, molecular marker has been improved, and EST-SSR (expressed sequence tag - single sequence repeat) molecular marker have especially benefits for potential of candidate gene mapping and potential for studying adaptive genetic variation.

[Materials and Methods]

RNA was extracted from Oryza sativa L. cv. Nagdong and populations for construction of genetic map were Cheongcheong, Nagdong, and CNDH (Cheongcheong, Nagdong) lines. Rneasy plant Mini kit (QIAGEN, Germany) was used to extract RNA. cDNA library construction was performed by using cDNA PCR library kit (TAKARA, Japan, code 6119). pGEM-T Easy Vector System I kit (Promega, USA, cod A1360) and E. coli strain DH5á were used to cDNA transformation

[Results and Discussions]

Plasmid DNA extraction was performed by using QIAPREP Spin Miniprep (QIAGEN, Germany). For confirm of inserted cDNA fragments, cDNA was enzyme digested by EcoRI (TAKARA, Japan) and band profiles of recombinant plasmid was performed after digestion with EcoRI in 0.8% agarose gel. Sequence was analysed by SolGent Co, Korea and cDNA region was identified by using http://blast.ncbi.nlm.nih.gov/Blast.cgi. EST-SSR marker was designed by http://www.wsmartins.net/websat/ and sequence was analysed by BioEdit Sequence Alignment Editor. The amplification reaction was conducted by using GeneAmp PCR System 2700(Applied Biosystems, USA). For the condition of PCR, initial denaturation was 96 ℃ for 5 min. Followed by 35 cycles of 96 ℃ denaturation for 1 min, annealing temperature according to the primer used for 30 sec, and 72 ℃ for 5 min. Amplified band by EST-SSR markers was analysed using QIAxcel (QIAGEN, Germany).

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