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論文審査結果の報告（甲）

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論文題名　Development of rapid and versatile genome editing technologies toward creation of genome diversity in yeast
（ゲノムの多様性創出を目指した出芽酵母ゲノム工学技術の開発）

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論文審査結果の要旨

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Development of rapid and versatile genome editing technologies toward creation of genome diversity in yeast
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論文の要旨

This Ph. D thesis by Farhana Easmin, a MEXT supported International Ph. D student, deals with the development of three novel genome engineering technologies in yeast. It consists of four chapters. In Chapter 1 as general introduction she reviewed the development of a variety of genome engineering technologies, i.e., PCS (PCR-mediated chromosome splitting), PCD (PCR-mediated chromosome segmental deletion), PCRep (PCR-mediated chromosomal segmental replacement) and PCDup (PCR-mediated chromosome segmental duplication) technologies, developed by the laboratory to which she belongs and also recently developed CRISPR/Cas9 genome editing technology. Through reviewing those technologies, she made an assertion that

integrating and combining those technologies is indispensable for further development of genome engineering field. She, especially, argued that expression and delivering system of guide RNA (gRNA) is one of the key technologies which should be simplified because previously developed gRNA expression systems are all plasmid-based and require cloning of respective target sequence and therefore laborious and time-consuming.

In Chapter 2, to overcome the drawback, she developed a system called gRNA Transient Expression System (gRNA-TES) for producing gRNA in a short period of time. Her basic idea was to exploit PCR system, one of simplest techniques in molecular biology field, to prepare PCR fragment which expresses gRNA in yeast host. Using gRNA-TES system, she established the system which enables preparation of the PCR fragment and yeast transformation step within only one day whereas plasmid-based system takes at least 3 to 4 days to construct and verify the gRNA-expression plasmids. She claimed that gRNA-TES is a very useful tool for genome engineering to which both basic biologists and biotechnologists can easily access without any difficulty.

In Chapter 3, she developed additional two genome engineering technologies, called CRISPR-PCD and CRISPR-PCRep by integrating conventional PCD and PCRep technologies with CRISPR/Cas9 system. She revealed that CRISPR-PCD and CRISPR-PCRep technologies are effective and speed up simultaneous deletion of two and three regions by one-step transformation when gRNA-TES was exploited. She also found that the frequency of getting transformants by CRISPR-PCD and CRISPR-PCRep technologies was much higher compared with conventional PCD and PCRep and even 500 kb large chromosomal region was deleted through replacement with marker.

In Chapter 4, she summarized all the observations concerning three newly developed genome engineering technologies that she developed in this study and discussed the importance and significance of her study in genome engineering and related fields. Finally, she concluded that these newly developed genome engineering technologies offer a more rapid means for manipulating multiple chromosomal regions toward creation of genome diversity both for breeding and understanding genome function in yeast. She also emphasized that these powerful technologies are applicable to not only yeasts but also a broad range of organisms.

最終試験結果の要旨

After the defense, a variety of questions, comments and even criticism were given to her. She answered those questions very well and also responded to comments and criticisms in appropriate way. Her achievements surely contribute to significant progress of genome engineering and related fields. Judging from these facts, this dissertation deserves the degree of Doctor of Engineering.