Effect of days after irradiation on lethal rate of F3 progeny in the fruit fly mutation isolation system

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Heavy-ion beam mutagenesis is generally recognized as an effective method for mutation breeding[1, 2]. Although this method was greatly successful with a plant, its application is still limited for animals. We therefore plan to acquire more basic data to set up optimal conditions for the irradiation system by heavy-ion beam using *Drosophila melanogaster* (fruit fly) as a useful model.

In a previous study, we have developed and improved a stable mutant isolation system using fruit flies by employing carbon-ion beam irradiation. In the system, we prepared a useful genetic tool, called balancer chromosome, to prevent genomic recombination. We also prepared small commercial cuvettes with a plane surface to apply heavy-ion beam equally to overcome unstable results[3]. Then, we could observe a linear correlation between a number of F1 progeny and an irradiation dose when the males were irradiated with a carbon-ion beam[3].

In this report, we introduced a new machine to improve the mutant isolation system [Fig. 1a]. If fruit flies are put in empty cuvettes for irradiation, they die because of dryness. So, it is necessary to put fly foods in the cuvettes to avoid dryness. However, it was difficult to prepare many samples without polluting the irradiated surface because the food was stuffed manually. The machine made it possible to dispense fly foods to many cuvettes without polluting the side surface of the cuvettes.

We prepared powdered fly foods for the machine, because it is easy to use and is commercially available. We considered using a needle tip for dispensing viscous fly foods to many cuvettes continuously [Fig. 1b]. Then we optimized the water-to- powder ratio for injection. Next, we set parameters for the dispensing machine using a program, such that the surface of fly foods was slanted in a cuvette [Fig. 1c]. As a result of these examinations, we were able to prepare more than 300 cuvettes in 2 hours.

To optimize the conditions for the mutant isolation system, we estimated the suitable state of F1 progeny that includes a possibility of chromosome damages. Various developmental cells are intermingled in testis of irradiated fruit fly. The repair mechanism to DNA damages and the sensitivity to irradiation are different in each cell[4]. So, it is important to know when flies with high probability of DNA damage are born. Because DNA damages to important genes for survival can be judged by the homozygotes born in F3 progeny, we measured a frequency of lethal rate of F3 progeny [Fig. 2]. The progeny being born 4 days after irradiation at 10 Gy dose levels recorded the maximum frequency of lethal rate [Fig. 2]. These data will be helpful for optimizing the irradiation system in the future.

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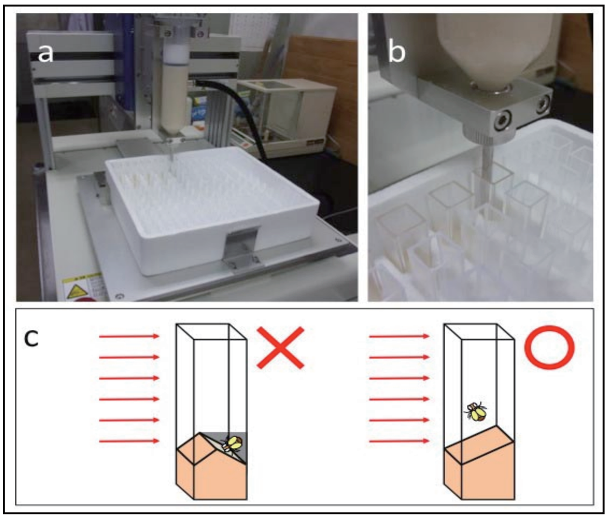

Fig. 1. a) A photograph of a micro dispensing machine (Shopmaster 300DS made by Musashi engineering, Inc.). b) A photograph of magnified view of dispensing fly foods into cuvettes. c) A diagram of a comparison of a surface of fly foods in the cuvettes. A dispensing program has been tuned so that a shadow does not form in the cuvettes by fly foods. The left figure shows a failure caused by fly food shadow. The right figure shows appropriate placement of fly food. Heavy-ion beams are indicated as red arrows.

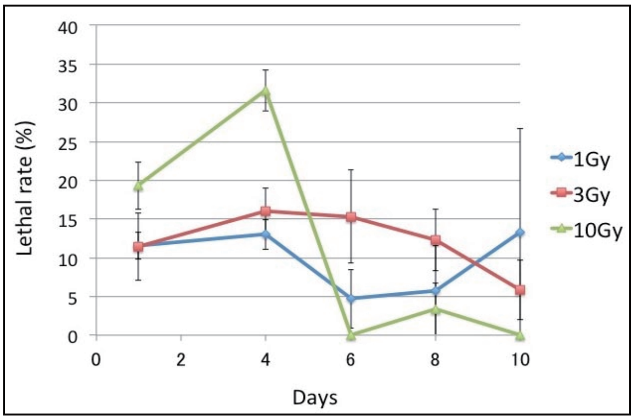

Fig. 2. A change in frequency of lethal rate of F3 progeny. Parental male flies were irradiated with a carbon-ion beam with linear energy transfer values of 80keV/μm. F: filial generation.

References

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