Sample preparation for ion-beam irradiation for biological experiments

Y. Hayashi*1 and T. Abe*1

RIKEN has developed a unique technology for mutation induction by using heavy-ion beams from particle accelerators at the RI Beam Factory (RIBF). At relatively low doses, ion beams induced mutations at a high rate without severely inhibiting growth. The irradiation treatment given to the various plant materials is quick, lasting between a few seconds and a few minutes, but is sufficient to induce mutation. By using this method, RIKEN has already put 39 new varieties on the market in Japan, the United States, and the European Union since 2001.[1,2] Furthermore, high-yielding rice, good growth wakame (*Undaria pinnatifida*), lettuce with low browning, microalgae with high oil content, and larger rotifer were identified as beneficial mutants.

We have developed an automatic irradiation system consisting of a range shifter and an automatic sample changer to cope with numerous requests with various biological samples such as dry seeds, wet seeds, callus, cultured tissue materials, pollens, seedings, cuttings *etc.*[3] Biological samples are stored in sample containers. Typical sample containers include plant boxes for regenerated plants and seedlings, 35-, 60-, and 90 mm-diameter plastic petri dishes for cultured tissues and seeds, and square plastic boxes for cuttings. The sample containers containing biological samples are attached to unique frames that support each sample containers.[4] Irregularly shaped samples placed in zipper bags or hybri-bags are fixed directly to the frame in line with the irradiation position. For example, Arabidopsis seeds were placed in a hybri-bag and arranged in a single layer by deaeration, then we found the most effective LET for mutation induction.[5] A uniform dose distribution is important for reproducible treatment on biological materials using ions with stable LET. The thickness of the sample is limited by the irradiation conditions. Therefore, it is necessary to place the sample in the most suitable container and attach the container to the frame.

We built a new beam line, 'WACAME,' to increase available nuclides with higher LETs and longer ranges. The Ar-ion beams are accelerated by RRC and IRC to 160 MeV/nucleon and sent to the E5 beam lines. Ar-ion irradiation is now available in tubes, increasing the number of microbes and microalgae users. We have created two types of cassettes for liquid cultures of microbes and microalgae (Fig. 1). One is for 15 mL centrifuge tube, and the other is for 1.5 mL micro and 200 μL PCR tubes. The first one is also available for cuttings that do not fit into square plastic boxes. The second one is rubber lined to prevent slipping down of tubes. Samples are fixed by springs to adapt various size of samples in both cassettes. Holes were drilled in the frame to check the sample's center. Each cassette also has a hole, and by aligning it with the hole in the frame, accurate placement in the irradiation position was made possible. In addition, yellow stickers were placed on the plates so that the irradiation field could be seen. We will continue to develop new frames and cassettes that promote efficiency in irradiation experiments as a tailor-made mutagen treatment technology to meet user requirements.

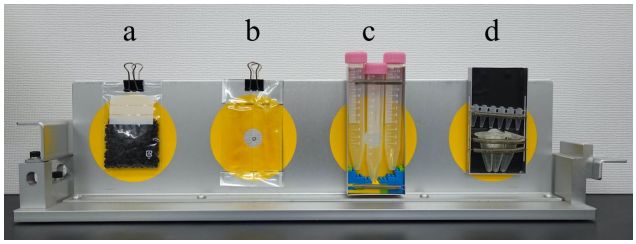

Fig. 1. Various samples set in the frame of automatic sample changer.
a: seed in the zipper bag, b: liquid sample in the hybri-bag, c: centrifuge tube fixed in the cassette, d: micro tube and PCR tube fixed in the cassette.

Table 1. Sample containers available in the frames of automatic sample changer.

Samples	Containers
Cultured tissues	Petri dish, Plant box
Seeds	Petri dish, Zipper bag, Square plastic box, Hybri-bag
Cuttings	Zipper bag, Square plastic box
Liquid	Centrifuge tube (15 ml), Micro tube (1.5-2 ml), 8 strips PCR tube*1, Hybri-bag*2

*1: Use 6 strips in the middle and cut off the both ends.
*2: The thickness of sample for Fe-ion irradiation must be less than 2 mm.

*1 RIKEN Nishina Center

References
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