Plant Biotechnology 25, 119–122 (2008)

Technical Note

Heavy-ion beam irradiation facility for biological samples in RIKEN

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Received September 21, 2007; accepted December 20, 2007 (Edited by K. Yazaki)

Abstract Biological samples are irradiated with heavy-ion beams at a heavy-ion beam irradiation facility in RIKEN to perform mutation breeding. The energies of the heavy-ion beams are sufficiently high to irradiate biological samples of macroscopic thickness in air. A uniform dose distribution is a key to a systematic study of the effect of the heavy-ion beams, and thus to the improvement of mutation efficiency. We selected a sufficiently high beam energy to avoid the Bragg peak to realize a uniform dose distribution along the beam path. The outline of the beam line is presented. The linear energy transfers (LETs) of the heavy-ion beams are selected using a range shifter and an energy adjuster to investigate the LET dependence of the irradiation effect. More than five hundred samples are automatically sent to the beam position using an automatic sample changer, which put the heavy-ion beam induced mutation breeding to practical use. The structure and function of the automatic irradiation system are also presented.

Key words: Heavy-ion beam, mutagenesis, plant breeding, linear energy transfer.

A heavy-ion beam irradiation facility is in operation at the RIKEN RI-beam factory (RIBF). The RIBF is one of the heavy-ion accelerator complexes that have the capacity to accelerate heavy-ion beams to sufficiently high energies to irradiate biological samples of macroscopic thickness in air. At the RIBF, ions from hydrogen to uranium can be accelerated. The RIBF consists of four ring cyclotrons together with two injectors (Yano 2007). Three of the four ring cyclotrons, namely, a fixed-frequency ring cyclotron, an intermediate-stage ring cyclotron, and a superconducting ring cyclotron, were newly constructed for nuclear physics experiments requiring intense high-energy heavy-ion beams. The typical heavy-ion beam used for such purposes is a uranium beam at 82 GeV. The acceleration of heavy-ion beams for the irradiation of biological samples is performed using the remaining ring cyclotron, the RIKEN ring cyclotron (RRC), together with one of the two injectors, that is, another cyclotron.

The capacity of heavy-ion beams for mutagenesis in animal cells has been demonstrated since the construction of the beam line for irradiation of biological samples (Watanabe et al. 1988). Plant scientists started to apply heavy-ion beams for plant breeding in 1993. Soon, high efficiency of ion beam became clear in inducing mutation in tobacco embryos during fertilization without damaging other plant tissue (Abe et al. 1995). Many types of tobacco mutants were isolated including albino, periclinal chimera, sectorial chimera, and herbicide-tolerant and salt-tolerant phenotypes (Abe et al. 2000). Six new flower cultivars were put on the market in Japan, USA, Canada, and EU since 2002 (Kanaya et al. in this issue, Miyazaki et al. 2006). The development period of these new cultivars was only three years. The ion beam irradiation technique realizes a high mutation rate without severe growth inhibition at relatively low doses. However, to reach the stage of practical application, it was crucially important to have a method to irradiate numerous samples. Therefore, we developed an automatic irradiation system which drastically increases the number of samples that can be irradiated in a limited beam time. Some works (Ichida et al. 2007 and Kazama et al. in this issue) was achieved by using the automatic irradiation system.

Beam line for irradiation of biological samples

Heavy-ion beams accelerated using the RRC are transported through a vacuum duct to the E5B beam line

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Abbreviations: LET, linear energy transfer; RIBF, RI-Beam Factory; RRC, RIKEN Ring Cyclotron
This article can be found at http://www.jspcmb.jp/

placed at the northwestern end of the RIBF. The E5B beam line was originally developed for the irradiation of biological samples in 1991 (Kanai et al. 1991). The heavy-ion beams transported from the RRC are spread laterally using a pair of wobbler magnets and a scatterer foil (Renner and Chu 1987). Figure 1 shows a schematic view of the E5B beam line. The beam is wobbled around the beam axis to draw a circle at the position of the samples using a pair of wobbler magnets. The diameters of the circles drawn by carbon, nitrogen, and neon beams are approximately 57 mm, and those drawn by argon and iron beams are approximately 58 mm. Two sheets of gold foil are placed behind the wobbler magnets; foil thicknesses are approximately 0.3 and 0.2 mm. The 0.3-mm-thick foil is used to spread the carbon, nitrogen, and neon beams by multiple Coulomb scattering, and the 0.2-mm-thick foil is used to spread the argon and iron beams. After passing through the gold foil, heavy ions move in a straight direction, forming a uniform radiation field at the position of the samples. The beam is extracted into air from the vacuum duct through 50-μm-thick aluminum foil. An ionization chamber is placed immediately downstream of the aluminum foil separating the air and vacuum. The ionization chamber is used to measure the integrated beam current. Behind the beam monitor, biological samples are irradiated with heavy-ion beams. The samples are automatically sent to the beam position and irradiated with the control of the linear energy transfer (LET) and dose using an automatic irradiation system.

Automatic irradiation system

The automatic irradiation system consists of an automatic sample changer and a range shifter. Figure 2 shows a photograph of the automatic irradiation system. Sample containers are filled with biological samples. Typical sample containers are a 50-mm-wide 75-mm-high 18-mm-thick rectangular plastic box (Figure 3D), 35-, 60-, and 90-mm-diameter plastic Petri dishes (Figure 3C), and a 60-mm-wide 80- or 100-mm-high 60-mm-thick plant box (Figure 3A). The sample containers with biological samples are attached to frames supporting the sample containers. Table 1 shows a list of the frames. Four to seven sample containers can be attached to one frame, depending on the type of sample container. The frames are numbered, and the sample containers are distinguished by the frame number and their attachment position on the frame. Dose and LET are indicated for each sample container by specifying the frame number and the attachment position of the sample container on the frame. The frames are placed on the stages of the automatic sample changer. The automatic sample changer automatically carries the frames to the topmost stage using lifts and air cylinders. The frame number is read using devices at the upstream end of the stage, and the frame is carried onto a movable table by a transfer arm. The movable table shifts a sample container on the frame to the beam position. An ionization chamber for calibration is placed on the movable table. A ZnS(Ag)

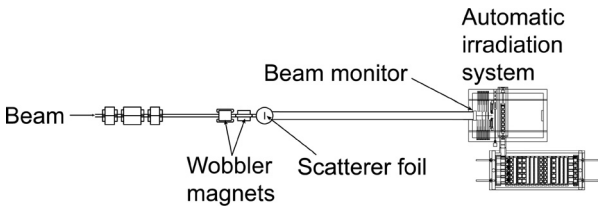

Figure 1. Schematic view of the E5B beam line.

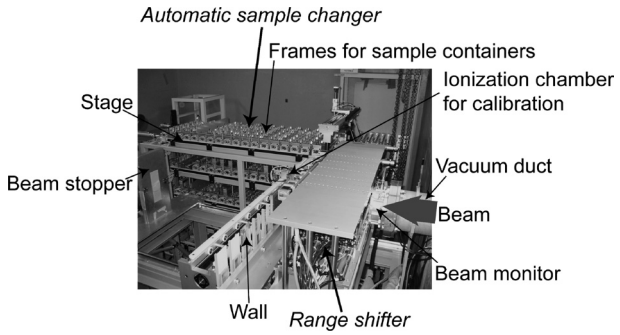

Figure 2. Photograph of the automatic irradiation system.

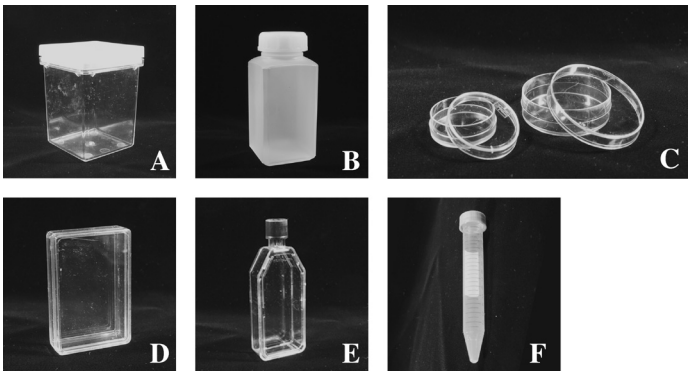

Figure 3. Photographs of sample containers for automatic sample changer.
(A) Plant box. (B) Polypropylene bottle. (C) Petri dish. (D) Plastic rectangular box. (E) Cell culture flask. (F) Centrifuge tube.

Table 1. List of frames of automatic sample changer

Frame No.	No. of samples on each frame	Sample container				
		Type	Size	Maker	Catalog No.	Photo
1xx	5	Plant box	60×60×100 mm	IWAKI CUL-JAR300	41-012-002	A
2xx	6	Polypropylene bottle	55×55×126 mm	SANPLATEC	2172	B
3xx–4xx	4	ϕ90 mm petri dish	ϕ90mm			C
5xx	6	ϕ60 mm petri dish	ϕ60mm			C
6xx	7	ϕ35 mm petri dish	ϕ35mm			
7xx	6	Plastic rectangular box	75×50×18 mm	AS ONE	1-4698-3	D
8xx–9xx	7	Cell culture flask	Culture area 24 cm^2	NUNC	152094	E
10xx	7	Micro tube, Plastic bag, etc.	90×60 mm (max.)			
11xx	4		90×90 mm (max.)			
12xx	7	Centrifuge tube	15 ml	IWAKI, CORNING etc.		F

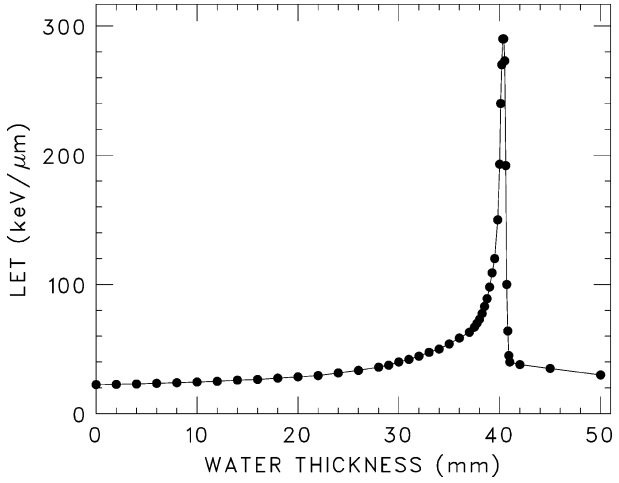

Figure 4. LET of the carbon ion passing through water (Kanai et al. 1933). The horizontal and vertical axes indicate the water thickness and LET, respectively.

phosphor screen and "walls" are placed on another movable table. The walls are used during calibration to reproduce the effect of sample containers. The width of a pair of slits is automatically adjusted to that of the sample container. The LET of the beam in the biological samples is selected using the range shifter. The range shifter consists of twelve aluminum disks attached to air cylinders. The thicknesses of the aluminum disks range from 24 to 20 mm. The thickness of the aluminum disk through which a beam passes can be changed from 0 to 41 mm by combining all the aluminum disks. The beam loses kinetic energy as it passes through the aluminum disk. Figure 4 shows the LET of carbon ions (Kanai et al. 1993). The horizontal axis indicates the water equivalent thickness at which carbon ions penetrate, and the vertical axis indicates the LET of the carbon ions. The LET gradually increases as the energy of the carbon ions decreases; thus, we can control the LET by changing the kinetic energy of a heavy-ion beam. By decreasing the kinetic energy of the heavy-ion beam, the range of the ions in the sample and LET are shifted.

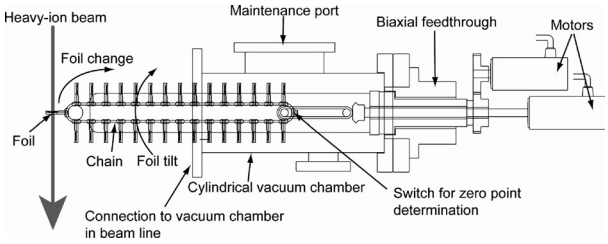

Figure 5. Schematic view of the energy adjuster.

Energy adjuster

The range shifter can be used to select only some discrete LETs because the total aluminum thickness is the sum of the aluminum thicknesses selected from the limited number of aluminum disks. The smallest interval is determined by the thickness of the thinnest aluminum disk (24 μm), and it is difficult to stably move a very thin foil of large area in air. Thus, an energy adjuster was constructed to enable a finer selection of LET (Ryuto et al. 2007). Figure 5 shows the schematic view of the energy adjuster. Thirty foils are attached to chain loops stretched in a cylindrical vacuum chamber. A beam viewer is also attached to the chain loops. An opening is left to allow beams to pass through without hitting any foil, and this part is also used to determine the zero point of the loops using switches. Two motors are placed at one end of the cylindrical vacuum chamber. The other end is connected to a common vacuum chamber in the beam transport line. One of the motors is used to change the foil at the beam position by rotating the chain loops through a biaxial feed through. The other motor is used to tilt the foil. The foil can be tilted from 0 to 60°; thus, the effective thickness of the foil can be continuously increased up to two times the initial foil thickness.

Summary of beams

At present, biological samples are irradiated with ^{12}C, ^{14}N, ^{20}Ne, ^{40}Ar, and ^{56}Fe beams. Table 2 shows a summary of the parameters of these ion beams. Energies and LETs at the surfaces of the samples were estimated using calculation codes of energy loss (Bazin et al. 2002, Tarasov and Bazin 2004, Ziegler et al. 1985). The

Table 2. Summary of heavy-ion beams used to irradiate biological samples showing energies on the surfaces of the samples, LETs at the energies shown in the table, and range of ions in water

Ion	$^{12}C^{6+}$	$^{14}N^{7+}$	$^{20}Ne^{10+}$	$^{40}Ar^{17+}$	$^{56}Fe^{24+}$
E (GeV)	1.52	1.76	2.46	3.18	3.77
LET (keV/μm)	22	30	63	293	651
Range in water (mm)	40	33	22	6	3

energies are lower than the extraction energy of the RRC because of the energy loss caused by the scatterer foil, the foil separating air and vacuum, the beam monitor, and air. Generally, an ion deposits its kinetic energy on a sample concentratedly at the Bragg peak considerably changing the LET before it stops in the sample. However, a uniform dose distribution is a key to the systematic study (Kazama et al. in this issue, Ichida et al. 2007), so the sufficiently high energies that the ions pass through the sample without serious variation of the LET are usually used. The LETs of ^{12}C, ^{14}N, and ^{20}Ne ions after passing through 10-mm-thick water are calculated to be 25, 35, and 81 keV μm^{-1}, respectively. The distribution of the beam intensity is symmetrical around the beam axis. Within an 8-cm-diameter circular area, the beam intensity is uniform within a level of 5%.

The RI-Beam Factory is open to the researchers in the field of nuclear physics, and material and life science. Please refer our web page

(RIKEN Nishina Center, http://www.nishina.riken.jp/Eng/index.html) and make contact with the User Liaison and Support Office (mailto:UserSupportOffice-M@ribf.riken.jp).

Acknowledgements

This work was supported in part by the Ministry of Agriculture, Forestry and Fisheries of Japan through a fund for research projects utilizing advanced technologies in agriculture, forestry and fisheries.

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