5. B. J. Bachmann, *Bacteriol. Rev.* **36**, 525 (1972).
6. L. W. Enquist and A. Skalka, *J. Mol. Biol.* **75**, 185 (1973).
7. D. Uyemura and I. R. Lehman, *J. Biol. Chem.* **251**, 4078 (1976).
8. K. Struhl, in preparation.
9. E. M. Southern, *J. Mol. Biol.* **98**, 503 (1975).
10. M. Thomas and R. Davis, unpublished data.
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Use of Isolators in Recombinant DNA Research

The use of negative pressure rooms has been recommended as a means of physical containment for recombinant DNA experiments. The major drawback of such rooms is that the experimenter, because he is in contact with the potentially hazardous material, may contaminate himself and serve as a dissemination vector. By using an isolator, the experimenter can avoid any direct contact with the hazardous materials, and he needs no special safety training. Isolators in positive or negative pressure have been used for many years for the manipulation of radioactive compounds and toxic chemicals and bacteria, and they have proved efficient in preventing outside contamination.

The aim of this report is to emphasize the advantages of using isolators in recombinant DNA research. To be really safe, an isolator must be equipped with a transfer system which allows the experimenter to take material in and out without ever coming into contact with it. The Elster isolator (*1*) (Fig. 1) which we started using recently possesses a particularly elaborate transfer system (*2*, *3*): material can be taken in or out in a tightly closed container, and this container can be transferred to another isolator or directly autoclaved. The isolator is in negative pressure, with air filtrated through Hepa filters, and its internal temperatures can be regulated from 20° to 40°C so that it can be used as an incubator for plates and cultures.

Isolators of this type but in positive pressure have been used for surgical operations (*3*), the protection of immunodeficient patients (*2*, *4*), and for the

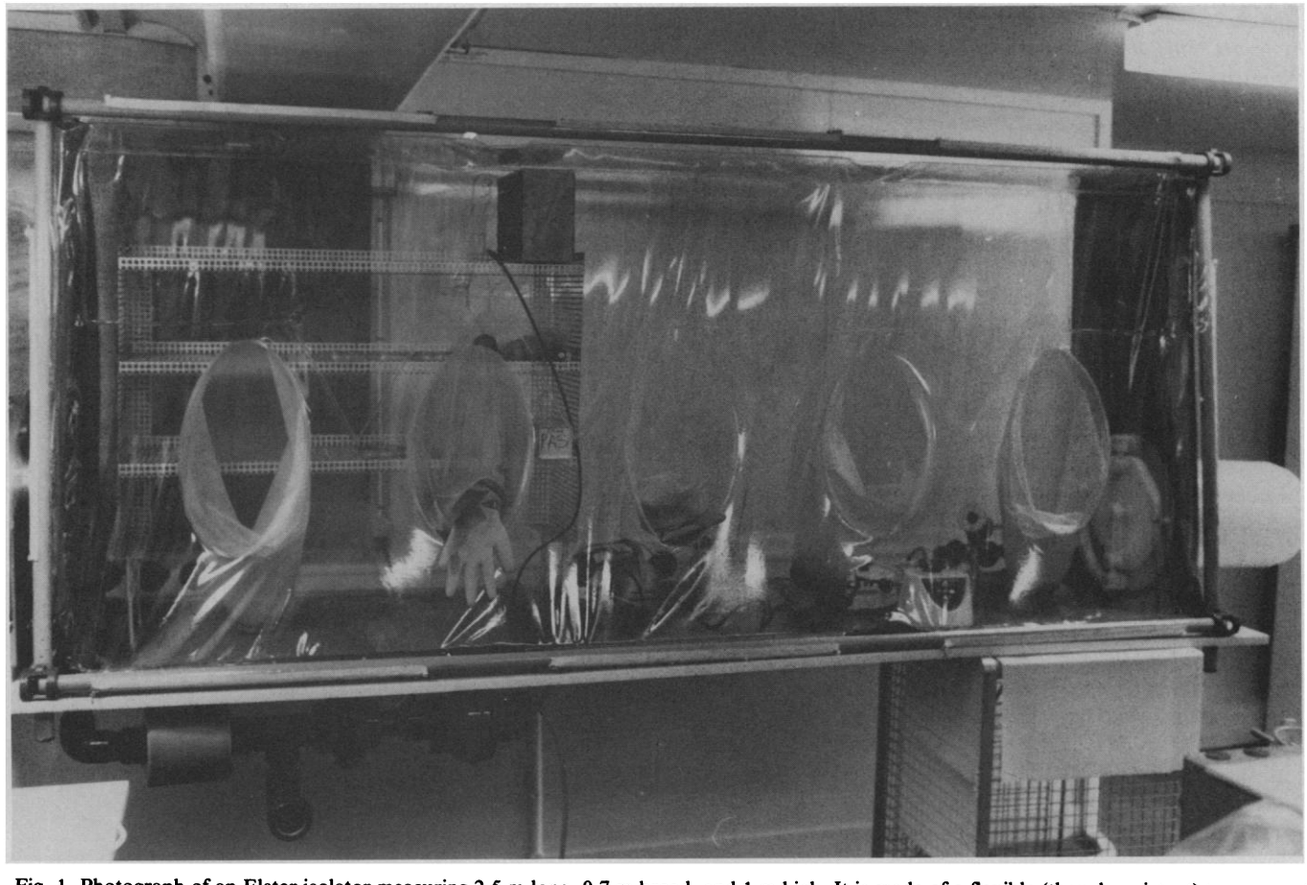

Fig. 1. Photograph of an Elster isolator measuring 2.5 m long, 0.7 m broad, and 1 m high. It is made of a flexible (though resistant) transparent plastic sheet held by a tubular frame, set up on a table with filters and machinery attached underneath. There are five regularly spaced gloves along the front, which permit manipulation in all parts of the internal volume. The isolator is maintained under negative pressure (which can be varied from 0 to −7 mm of water). The incoming and extracted air is passed through Hepa filters. Because of the limited air flow, these filters will last for many years. There are two circular openings, one on each side. The biggest one, on the left, is for taking in or out large sized equipment. The other, which is the transfer system door, shown here with a container plugged in, is used to take materials in or out during experiments. When the container with its lid is applied onto the transfer door and rotated, it triggers the isolator's door which can now be opened from inside. The lid of the container is stuck to the door in such a way that the external faces of both the lid and the door are protected from contamination. The tightly closed container can be transferred to another isolator or autoclaved (with metal containers not shown). The inlet for formol or peracetic acid is at the bottom left. The internal volume is closed and remains safe even if air extraction stops. Since plates may have to be incubated or cultures grown at temperatures ranging from 30° to 40°C, the entire isolator is thermally regulated, and the internal temperature can be varied at will. Plates are incubated on the bench, and cultures are grown on a shaking platform. A magnetic stirrer is available for cultures of 1 to 2 liters, and a small electric pump can be used to bubble air through the culture. Samples could be subjected to ultracentrifugation by sealing the opening of the ultracentrifuge in the same or another isolator while the centrifuge itself remains on the outside.

breeding of axenic animals (5). For this reason, a large body of data on the efficiency of the decontamination and air filtration systems is available. The data show excellent efficiency, at least as far as bacterial contamination is concerned (6).

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References and Notes

1. Elster Company, 9 avenue Fremiet, 75016 Paris.
2. B. Saint-Martin, R. Ducluzeau, J. C. Ghnassia, C. Griscelli, B. Lauvergeon, *Rev. Fr. Gynécol. Obstet.* **70**, 585 (1975).
3. J. Lannelongue, P. Burdin, J. Pelicot, J. Cas taing, *Rev. Chirurg. Orthopéd.* **62**, 389 (1976).
4. J. C. Ghnassia and C. Griscelli, *Nouv. Presse Méd.* **3**, 1429 (1974).
5. P. Raibaud, R. Ducluzeau, M. C. Muller, *Ann. Microbiol. (Paris)* **126 B**, 357 (1975).
6. J. Pelicot, *Aérobiocontamination en Milieu Hospitalier* (Thèse de Médecine, Tours, 1974).

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Degradation of DNA by Nucleases in Intestinal Tract of Rats

Abstract. *Strains of* Escherichia coli *K12 have been constructed as safer hosts for use in recombinant DNA research. These strains are unable to survive passage through the intestinal tracts of rats because of a constellation of mutations conferring bile sensitivity and requirements for diaminopimelic acid and thymine. Since death caused by diaminopimelic acid deprivation could release recombinant DNA before DNA is degraded because of thymine starvation, it is important to determine the "survival potential" of the released DNA's. Bacterial and plasmid DNA's extracted from bacterial cells are rapidly degraded when added to low dilutions of rat intestinal contents. This observation, coupled with the stringent requirements necessary for in vitro transformation or transfection, make in vivo transmission of naked recombinant DNA in the rat intestinal tract highly improbable.*

The design, construction, and testing of strains of *Escherichia coli* K12 that are more useful and safer for recombinant DNA molecule research is one of the goals of our laboratory (*1*). The first of such strains, χ1776, is unable to survive passage through the intestinal tracts of rats at frequencies of less than 10^{-11} bacteria per milliliter because of a constellation of mutations that cause sensitivity to bile and requirements for diaminopimelic acid (DAP) and thymine (*1*). Although DNA degradation accompanies thymine-less death in in vitro experiments and is an all-or-none response for any given cell (*1*), it is likely that lysis associated with DAP-less death may liberate DNA prior to its degradation. Since undegraded recombinant DNA might be taken up by robust microorganisms encountered in natural environments by transformation or as a consequence of concomitant phage infection (*2*), it is important to determine the "survival potential" of such DNA in nonlaboratory controlled environments. We determined the rate of degradation of *E. coli* chromosomal and plasmid DNA in extracts of the intestine contents of rats. We were unable to locate any literature reports indicating the fate of "naked" DNA's in the intestines of warm-blooded animals and believed that a likely route of escape of chimeric microorganisms from the laboratory is ingestion by laboratory workers.

The small and large intestines were aseptically removed from asphyxiated rats (Charles River) (*3*), and after longitudinal incisions were made the contents were removed by washing with 5.0 ml of buffered saline with gelatin (BSG) (*4*) containing 10 m*M* $MgSO_4$. Each extract had a volume of approximately 6.0 ml and thus was a 1 : 6 dilution of the original intestine contents. The data in Fig. 1 show that there is an initial rapid degradation of the DNA in the 1 : 6, 1 : 12, and 1 : 60 dilutions of the small intestine extracts while no detectable degradation is observable in the large intestine extracts. After 4 hours, the level of DNA degradation in the diluted large intestine contents is comparable to the initial level of degradation in the small intestine contents, while complete degradation occurs in the 1 : 6, 1 : 12, and 1 : 60 dilutions of the small intestine contents. There was no DNA degradation during the experiment in the boiled extracts and controls containing BSG and 10 m*M* $MgSO_4$. In order to ascertain if the immediate reduction in acid-insoluble radioactivity in the diluted small intestine contents was due to quenching, the total soluble and insoluble radioactivity was monitored throughout the experiment and found to be equal to that in the input DNA. Results similar to those in Fig. 1 were obtained in another experiment in which the intestine contents were diluted in BSG without added $MgSO_4$. Thus a suitable ionic environment exists within the intestine for nuclease activity and our results are not enhanced by the addition of Mg^{2+} to the buffer used for suspending and diluting intestine contents. Comparable but somewhat more extensive DNA degradation occurs in the intestine contents from gnotobiotic rats (Fig. 2). These results rule out bacterial nucleases as being the sole cause for DNA degradation in the intestine contents from conventional rats. It therefore appears that the intestine contents of rats contain nucleases that rapidly degrade free bacterial DNA and are probably secreted into the lumen of the intestine by cells in the intestinal wall (*5*) or from the pancreas (*6*).

Data on the rate of bacterial DNA deg-

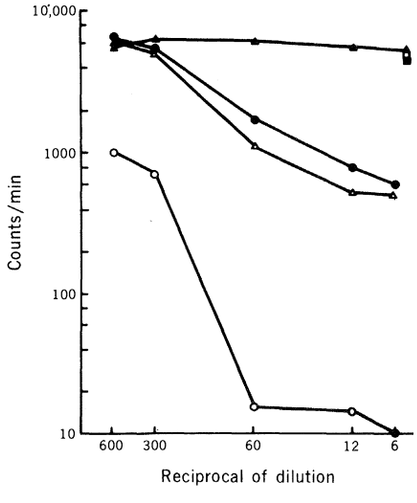

Fig. 1. Degradation of bacterial DNA by intestine contents from conventional rats. DNA labeled with tritiated thymidine was isolated from *E. coli* χ1776 (*1*). Lysozyme-generated spheroplasts were made in buffer containing 50 m*M* tris, 50 m*M* EDTA, 50 m*M* NaCl, and 10 percent sucrose (*p*H 8.0) and were lysed by adding 0.2 ml of spheroplasts to 1.0 ml of the intestine contents that were diluted to the extent indicated in BSG plus 10 m*M* $MgSO_4$. This method was believed to most closely mimic the in vivo entry of an enfeebled host into the lumen of the intestine. All incubations were at 37°C. Samples of 25 μl were taken immediately after mixing (0 hours) and at 4 hours and spotted on Whatman No. 3 filters. The filters were immersed sequentially in 10 and 5 percent TCA and 95 percent ethanol by standard methods and dried. The acid-insoluble counts were determined in a Beckman scintillation counter with 2,5-bis-2(5-*tert*-butylbenzoxazolyl)thiophene (BBOT) in toluene as a scintillation fluid. Data are shown for small intestine contents at 0 hours (●) and after 4 hours (○); large intestine contents at 0 hours (▲) and after 4 hours (△); small intestine contents boiled for 10 minutes before adding DNA at both 0 and 4 hours (■); and large intestine contents boiled for 10 minutes before adding DNA at both 0 and 4 hours (□). The control was DNA diluted into BSG plus 10 m*M* $MgSO_4$; its radioactivity remained at approximately 6200 count/min at 0 and 4 hours.