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SHORT NOTES

A quick and efficient method for interruption of bacterial conjugation*

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Jacob & Wollman (1961) produced interruption of chromosome transfer during conjugation in *E. coli* K-12 by exposing conjugating pairs to high shear forces created by a propeller-type mixer (a Waring blendor). In our laboratory, efforts to use this method were not completely successful because of the relatively long times required to 'blend' each sample and the large variations from sample to sample in the efficiency of separating mating couples ('blending efficiency'). The variations in blending efficiency were thought to be due to lack of homogeneity of mixing in the blendor chamber. We have obtained much more satisfactory results by using a vibratory mixer which shakes the entire sample chamber and thoroughly agitates all the cells. Furthermore this vibratory blendor is much easier to use and permits taking at least twice as many samples per unit time, as compared to the usual blendor method.

BLENDOR APPARATUS

The new blendor apparatus consists of a holder for securing a small culture tube which contains the mating cells to be separated. An electric motor drive is required for violently shaking the culture tube holder. Figure 1 is a diagram of the holder with a culture tube held in place for shaking. The holder consists of two aluminium cups which are aligned by two long bolts and held together by two springs as shown. The spring tension holds the culture tube in place and layers of rubber in each cup cushion the tube in order to prevent breakage. A small piece of Parafilm‡ is wrapped over the top of the culture tube before insertion into the blendor, in order to prevent leakage and contamination.

A carpenter's oscillating sabre saw with the saw blade removed serves as an adequate and inexpensive motor drive for the blendor. The motor housing is inverted and mounted securely with the drive shaft in an upright position. The holder assembly is mounted on the drive shaft as shown in the figure. A typical sabre saw motor produces approximately fifty $\frac{3}{4}$ in. oscillations per second. For convenience the motor power cord can be plugged into an interval timer actuated by a push-button.

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‡ Parafilm 'M' obtainable from Marathon Division of American Can Co., Neenah, Wisconsin, U.S.A.

TECHNIQUES AND DISCUSSION

Blending of the mating pairs is accomplished by adding an aliquot of the mating suspension to a 10 ml. culture tube which contains 3 ml. of the desired blending liquid. This tube is covered with a small piece of Parafilm, inserted into the blendor holder and agitated (normally for 5 sec.). The blended suspension may now be diluted and plated onto recombinant-selective plates.

We have found it convenient to use soft agar (0·75% agar, kept in the 10 ml. culture tubes at 45°C. before use) for the blending liquid. In this case the proper aliquot of the mating suspension is added directly to the soft agar, agitated, and poured directly onto

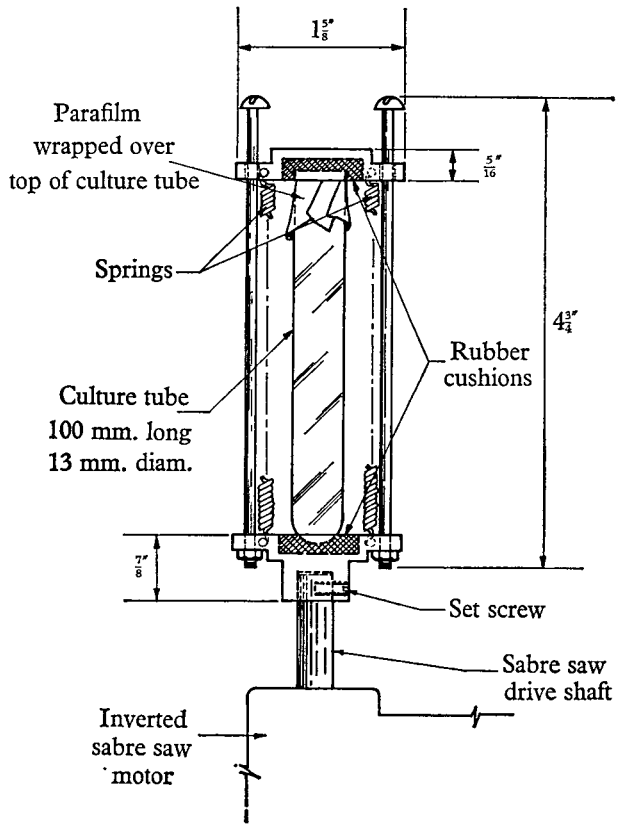

Fig. 1. Diagram of blendor showing essential parts and typical dimensions.

the proper assay plate. Alternatively, one may use broth or buffer for the blending liquid; after agitation aliquots may be spread onto various selective plates. If matings are carried out on cellulose acetate filters, mating pairs may be removed from the filter and separated in one operation by this apparatus.

Typical results for an *E. coli* Hfr × F^- interrupted mating are shown in Fig. 2. In this case samples were blended and plated alternately on four types of recombinant-selective plates, so that the entry times for four male markers were determined in one experiment.

As indicated above, 5 sec. of shaking in the blendor is an adequate time interval for disrupting essentially all of the mating pairs in the culture tube. Longer periods of blending neither increase the blending efficiency nor injure the cells as determined by

plate counts. The short blending time required (5 sec.) enables one experimenter to take samples at intervals of less than 1 min. With two or more experimenters collaborating, samples can be blended two or three times as often.

An indication of the blending efficiency is obtained by comparing the number of recombinants of a particular type appearing after the plateau is reached (see Fig. 2) with

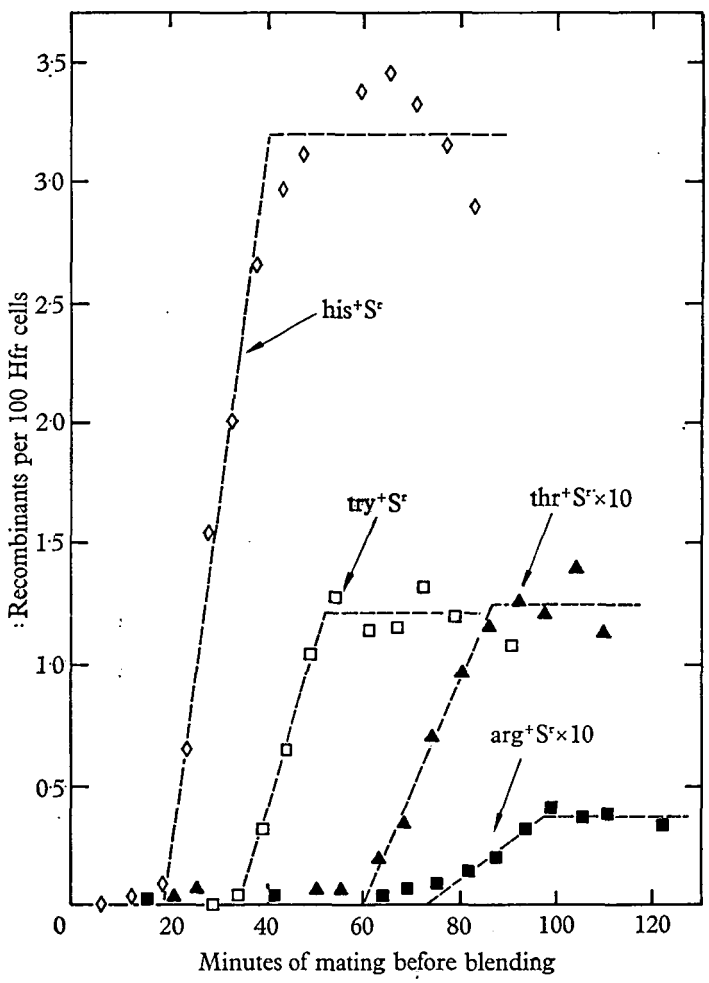

Fig. 2. Results of an interrupted mating experiment using *E. coli* Ra-1 Hfr (prototrophic and streptomycin-sensitive, S^s) and PA309 F^- (auxotrophic for histidine (his), tryptophan (try), threonine (thr) and arginine (arg), and streptomycin-resistant, S^r) cells (Joset *et al.*, 1964). Exponentially growing male and female cultures were mixed in a 1:100 ratio and shaken gently at 37°C. Samples for blending were taken at approximately 1 min. intervals, and the number of recombinants of the various types were determined as a function of the time between the start of mating and blending. These numbers were all normalized to the female titre at the time of blending.

the number obtained before the marker entry time. (Here the marker entry time is taken as the time when the straight portion of the blendor curve extrapolates to zero.) The pre-entry time level is usually less than 0·1% of the plateau level. The pre-entry time

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plate counts may be due to mating pairs formed in the small interval between blending and solidification of the agar after pouring onto the plate.

REFERENCES

JACOB, F. & WOLLMAN, E. L. (1961). *Sexuality and the Genetics of Bacteria*, pp. 130–131. Academic Press, New York.

JOSET, F., LOW, B. & KRISCH, R. E. (1964). *Biochem. Biophys. Res. Commun.* **17**, 742.

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