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A rapid and efficient replica plating technique

BY THOMAS H. WOOD AND SURESH K. MAHAJAN*
Department of Physics, University of Pennsylvania, Philadelphia Pa. 19104

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SUMMARY

A new type of apparatus for replica plating is described, which reduces operator error and fatigue. Several replica plates can be made immediately, thus eliminating the need for a master plate in many cases.

In microbial genetics it is often necessary to grow colonies in an ordered array on a Petri dish (the master plate) in order to facilitate further tests (selection of mutants, determination of the genetic constitution, specification of mating type, etc.) (Jacob & Wollman, 1961). Normally inocula from colonies to be tested are transferred to the surface of the master plate by sterile needles or toothpicks and after proper incubation, the individual colonies are simultaneously tested for the desired properties using the velveteen pad replica plating technique of Lederberg & Lederberg (1952). We describe here a new method which greatly facilitates the transfer of cells to form the master plate and in most cases allows the subsequent tests to be carried out directly without the production of a master plate.

In this technique sterile straight pins whose points have been touched one by one to colonies to be analysed are individually placed, and supported points downward, in holes drilled in a metal plate (the grid plate, Fig. 1). This plate is held in a simple mechanical printer which can be lowered until the pin points just touch the agar surface of a Petri dish containing a particular assay medium, thereby transferring cells which may grow to produce colonies. This process can be repeated 10–12 times with little loss of ability to transfer cells (Table 1).

The grid plates (Fig. 1) are made of aluminium plate (0·25 cm thick × 7 cm × 7 cm). 100 holes slightly larger than the pin diameters are drilled in a 10 × 10 grid, 5 cm on the side. A grid plate is held in a horizontal plane by the support plate (aluminium: 9 cm × 12 cm × 0·6 cm thick); the centre portion of this plate (6·5 cm × 6·5 cm) is cut away so that the pins held by the grid plate may hang freely. The grid plate is accurately positioned by means of dowel pins mounted on the surface of the support plate.

Four round steel rods (0·6 cm diameter, 7 cm long) are mounted vertically on the surface of the base plate (1 cm thick × 10 cm × 16 cm, any suitable material) such that they pass through four holes drilled in the corners of the support plate. Easily compressible springs are positioned on the support rods and keep the base plate

* Permanent address: Bhabha Atomic Research Centre, Biology Division, Trombay, Bombay (India).

near the top of the rods. Two dowel pins are located on the surface of the base plate so as to accurately position a Petri dish directly under the hanging pins. Adjustable screw stops are attached to the support plate and are set to allow the points of the hanging pins to come to a predetermined distance from the base plate when the support plate is pushed downwards.

There is only one critical aspect in the construction of the apparatus. The pin holes must be large enough to allow the pins to ride smoothly in them but not large

Table 1. *Replica plating efficiencies*

Conditions*	Number colonies tested	Colonies missing on successive replicas (%)									
		1	0	0	4	5	6	7	8	9	10
Surface colonies (dia. ~ 1 mm)	850	0	0	0	0	0	0	0	0	0·1	0·1
Surface colonies (dia. ~ 0·4 mm)	150	0	0	0	1·3	2·7	2·7	4·0	7·3	0·3	8·7
Colonies under agar (48 h growth)	1400	0	0	0	0	0·1	0·2	0·7	1·2	1·6	1·9

* Stainless steel pins (0·050 cm dia.) with acid-etched points and top-loading of 200 mg/pin.

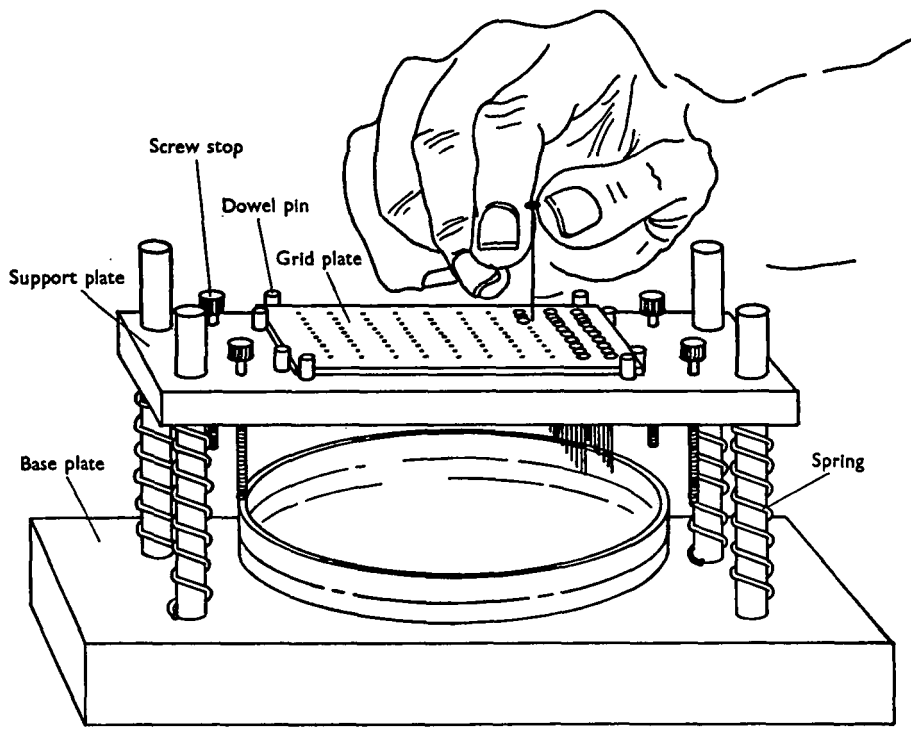

Fig. 1. Diagram of replica printing device showing essential parts, relative dimensions, and use. Individual sterile pins whose points have been touched to colonies to be analysed are placed points downward in the holes of the grid plate. When the grid plate is filled with pins, the support plate is pressed downwards until the pin points touch the surface of the medium of a Petri dish immediately below and the pin heads ride 1 or 2 mm above the grid plate. The printing process may be repeated 10–12 times on additional Petri dishes with little loss of transfer efficiency (Table 1).

enough to allow the points of the pins to come into contact with each other if they are accidentally jarred; we routinely use stainless steel pins that are 0·050 cm in diameter and 0·061 cm holes (no. 73 drill).

For most uniform colony size and most rapid growth, the pins should not dig deeply into the agar surface but should be light enough in weight so that when their points touch the agar their heads are lifted by 1 or 2 mm from the grid plate; for larger pins this may be facilitated by slightly blunting their points. However, if many plates are to be printed, a higher efficiency of transfer is obtained if the pins dig slightly into the agar ($\frac{1}{2}$ to 1 mm); this can be accomplished by placing a second empty grid plate on top of the pin heads (top-loading of 200 mg/pin). Efficiency of multiple printing can also be increased by slightly blunting or acid-etching (5% per chloric acid for 2–4 h) the points of the pins. The grid plates may be sterilized by flaming or by other means; the inoculating pins are dry-heat sterilized and may be discarded or resterilized after use.

If very small colonies are to be analysed, the inocula carried on the pins may be insufficient to give 100% efficiency of transfer after several prints have been made (Table 1). In such cases a single print may be made by the pins on a plate of the selective medium (or a complete medium); after incubation replica prints are made from this master plate using the modified velveteen printing technique described by Curtiss & Stallions (1969).

We have found that this technique has several important advantages over other methods. Operator fatigue associated with the formation of the master plate is minimized because the positions of the pins in the grid plate automatically inform the operator of the next position to be filled. The fixed grid also completely eliminates the possibility of spotting inocula from two colonies at the same position. The uniform colony size produced (which is independent of the 'touch' of the operator) and regularity of the grid lead to ease and precision of scoring. In our experience all of these factors lead to a considerable saving of operator time. Since it is not necessary usually to make a master plate from which replicas can be printed, results may be obtained one day earlier. The extreme regularity of the grid also allows 100 (or more) colonies to be analysed per plate with consequent economy in the consumption of plates.

It should be noted that this technique is basically different from others utilizing fixed pins or 'flower frogs' which are often used for the isolation of auxotrophs (Braendle & Szybalski, 1957; Roberts, 1959). With fixed-pin devices it is necessary to work from Petri dishes containing almost confluent surface colonies if a reasonable fraction of the inoculating needles is to carry cells; under such conditions a large fraction of the needles transferring cells would be multiply infected with cells from two or more colonies. (For overall transfer efficiencies of 10, 25, 50, 75 and 90% the corresponding percentages of multiple infection would be 6, 14, 31, 54 and 75, respectively, assuming Poisson statistics.) For a proper genetic analysis it is imperative that there be no multiple infection of this type and thus it is necessary to sample at very low efficiency (below 10%) or to sample each colony to be tested on an individual basis.

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