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## A SIMPLIFIED METHOD OF COLONY HYBRIDIZATION USING RADIOLABELED PROBES IN SEALED PETRI DISHES

### ABSTRACT

*A simplified colony hybridization procedure was developed using sealed disposable plastic petri dishes in place of sealed plastic bags. Prior to hybridization, the dishes were sealed with successive layers of parafilm, plastic wrap and aluminum foil to prevent evaporation of the solution. This self-contained procedure eliminates some of the technical problems such as spilling of radioactive materials, leakage of solution, sealing of plastic bags and the formation of air bubbles. Therefore, this method allows for safer and easier handling of radioactive materials during hybridization procedures.*

Screening bacterial colonies by hybridization in plastic bags with gene probes usually requires incubation of nitrocellulose or nylon membranes at 37C, 42C in solutions containing formamide or at 68C when using aqueous solutions (Montenegro *et al.* 1984; Perbal 1988; Sambrook *et al.* 1989). Hybridization at 68C in aqueous solutions offers the advantage of a two- to threefold faster rate of hybridization than in solutions containing formamide (Sambrook *et al.* 1989) among other advantages of avoiding the use of formamide. Hybridization at high temperatures in disposable petri dishes has not been used due to the problem of evaporation of the hybridization solution during incubation. Successful performance of colony hybridization requires efficient handling of radioactive probes and minimization of technical problems associated with the use of plastic bags.

Therefore, a simplified colony hybridization procedure was developed using sealed disposable plastic petri dishes (90 × 15 mm) to replace sealed plastic bags.

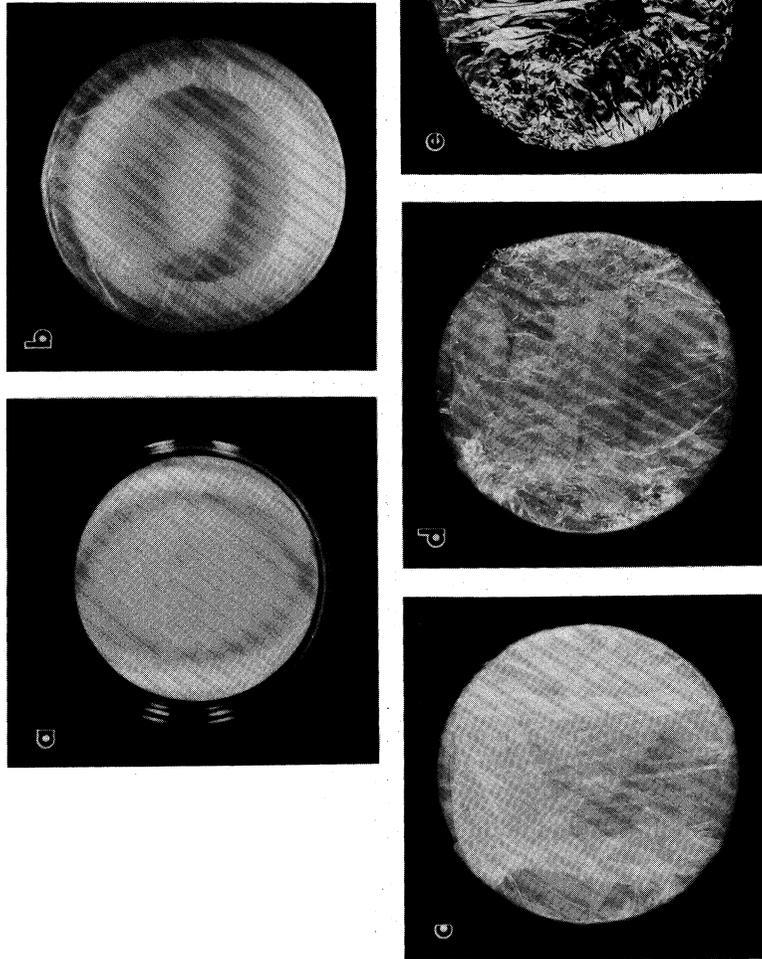
The mobilization of DNA and the hybridization conditions used in this method were according to Sambrook *et al.* (1989). DNA immobilized on nitrocellulose/nylon membranes (diameter 82 mm) was prehybridized for 2 h in petri dishes in an oven set at 68C, the prehybridization solution removed, and hybridization solution added (Fig. 1a). The dishes were sealed as follows:

1. An initial tight layer of parafilm was placed around the rim (Fig. 1b).
2. To further ensure complete sealing, the petri dishes were wrapped around the entire surface with:
  - a. A complete tight layer of parafilm (Fig. 1c).
  - b. A layer of Saran™ wrap (Fig. 1d).
  - c. A layer of aluminum foil (Fig. 1e).

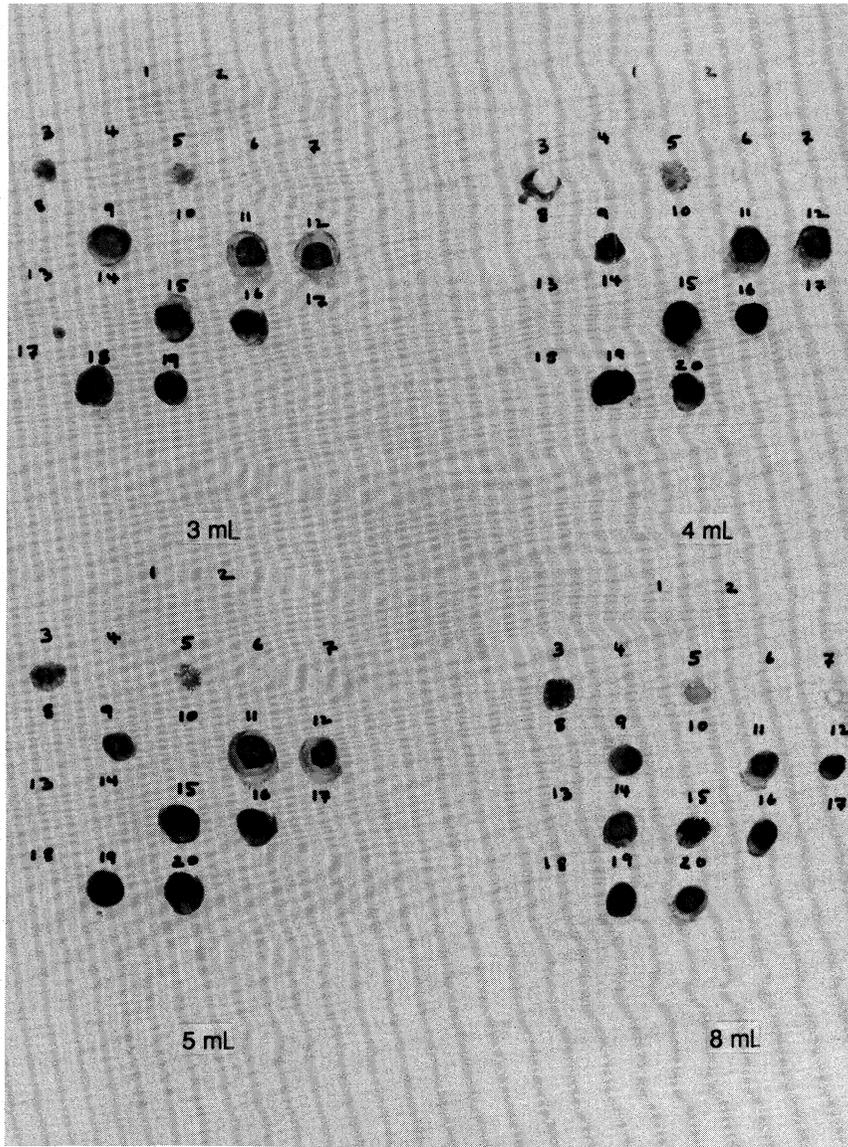
A tight seal with these layers of wrapping is essential to prevent excessive evaporation of the hybridization solution at 68C for 18 h. The bottom of the petri dish must be kept flat to maintain an even contact between the membrane and the hybridization solution. The parafilm wrap melted during incubation at 68C and sealed the dish, but this did not interfere with subsequent steps. Omission of any of these successive layers in any combination allowed loss of hybridization solution which ranged from a small amount to complete evaporation. The minimum amount of hybridization solution to be used during hybridization at 68C was determined. The autoradiograph of membranes incubated with either 3, 4, 5 or 8 mL of hybridization solution is shown in Fig. 2. The results showed that a minimum volume of 3.0 mL could be used for hybridization with negligible loss of hybridization solution (Fig. 2). This small loss probably resulted from adherence of solution to the surface of the membrane and to the petri dish, but this did not affect the hybridization. In case of minimal availability of probe, two membranes can be placed in the dish (Fig. 3). It should be possible to hybridize several membranes at once using a deeper petri dish; however, the volume of hybridization solution must be increased to cover them completely.

The efficiency of hybridization with the petri dish method as demonstrated by autoradiography was identical to that found using plastic bags (Fig. 4). This self-contained procedure eliminates the need for plastic bags which have had the following problems:

- (1) Spilling of radioactive liquid during its addition or subsequent sealing of the bag.
- (2) Formation of air bubbles.
- (3) Leakage of hybridization solution into the water bath or in the incubator.
- (4) Spilling of radioactive liquid from the sealed plastic bags when punctured or cut open for changing buffers or removing the membranes from the bags.



**FIG. 1. PHOTOGRAPHS OF SEQUENCE OF SEALING OF PETRI DISH BY WRAPPING**  
A detailed description of sealing of the petri dish is described in the text. (a) Nitrocellulose membrane with hybridization solution in the petri dish; (b) Initial wrapping of parafilm around the rim of the petri dish; (c) Layer of parafilm completely enclosing the petri dish; (d) Layer of saran enclosing the petri dish; (e) Complete covering with aluminum foil.



**FIG. 2. EVALUATION OF THE ADDITION OF DIFFERENT VOLUMES OF HYBRIDIZATION MIXTURE ON THE EFFICIENCY OF HYBRIDIZATION IN SEALED PETRI DISHES**  
 Hybridization was done at 68C using volumes of 3, 4, 5, and 8 mL as shown in the figure.  
 The petri dishes were sealed as described in Fig. 1.

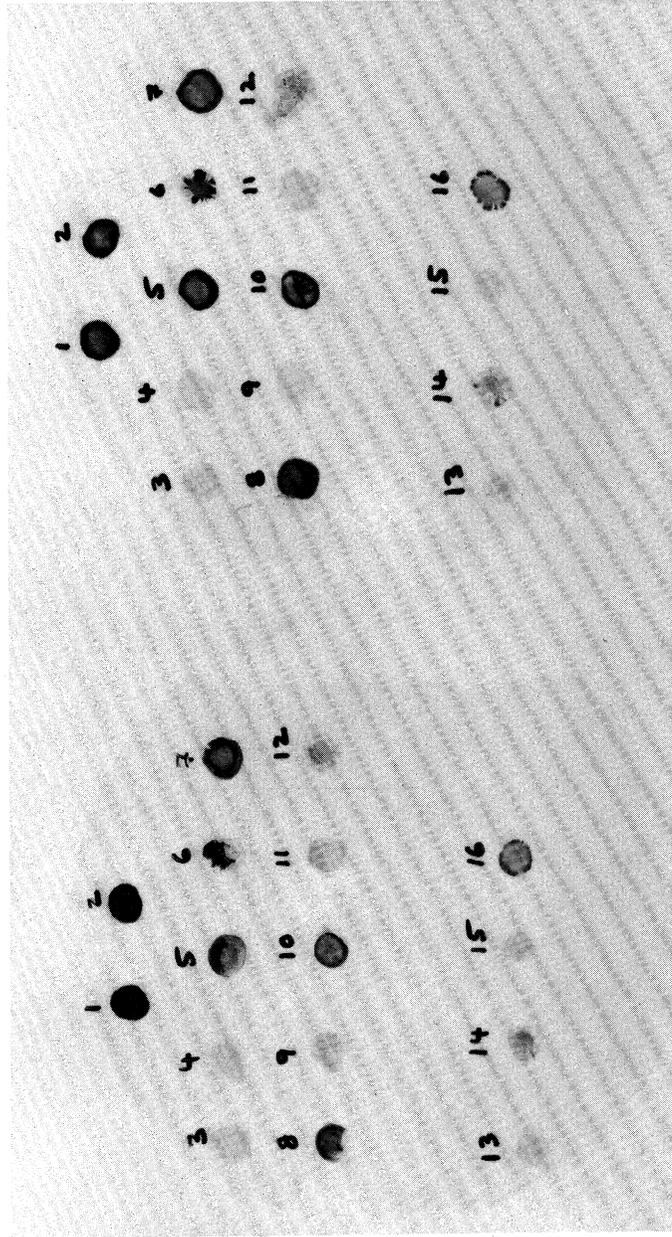
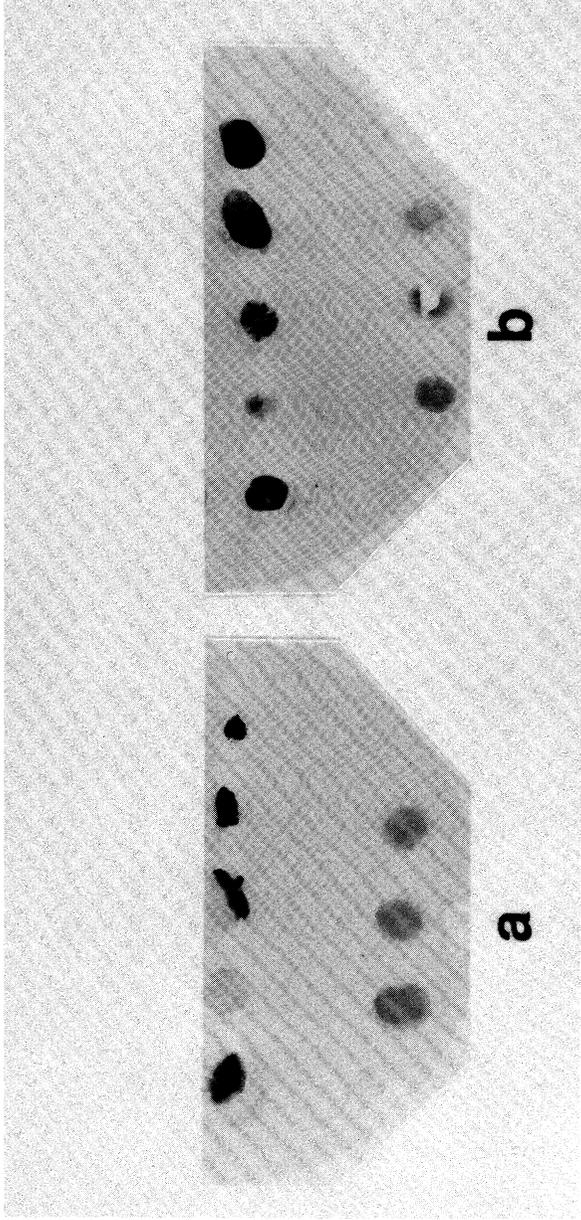


FIG. 3. EFFICIENCY OF HYBRIDIZATION WITH TWO MEMBRANES IN ONE SINGLE PETRI DISH  
 Hybridization was done with 3 mL solution in a petri dish at 68C sealed as described in Fig. 1.



**FIG. 4. COMPARISON OF EFFICIENCY OF HYBRIDIZATION IN SEALED PETRI DISH AND PLASTIC BAG**  
Hybridization was done at 68C in a sealed petri dish with 3 mL solution (a). Hybridization was done in a sealed plastic bag with 5 mL solution in a waterbath at 68C as described by Sambrook *et al.* (b)

In this method there is no direct contact of radioactive liquid with the hands; this could occur from accidental leakage from the bags or breakage of protective plastic gloves while handling the radioactive solutions. Exposure to radioactivity is minimal due to reduced risk of mishandling of radioactive materials. Rapid and efficient processing of a large number of hybridizations can now be possible using petri dishes. In the case of nonradioactive probes, similar problems are encountered using plastic bags and these can be eliminated using the sealed petri dish method. This procedure is also applicable to Southern, Northern and dot hybridization techniques.

#### REFERENCES

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