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A simple strategy for identifying
a single complementing clone in a
96-well microtiter dish

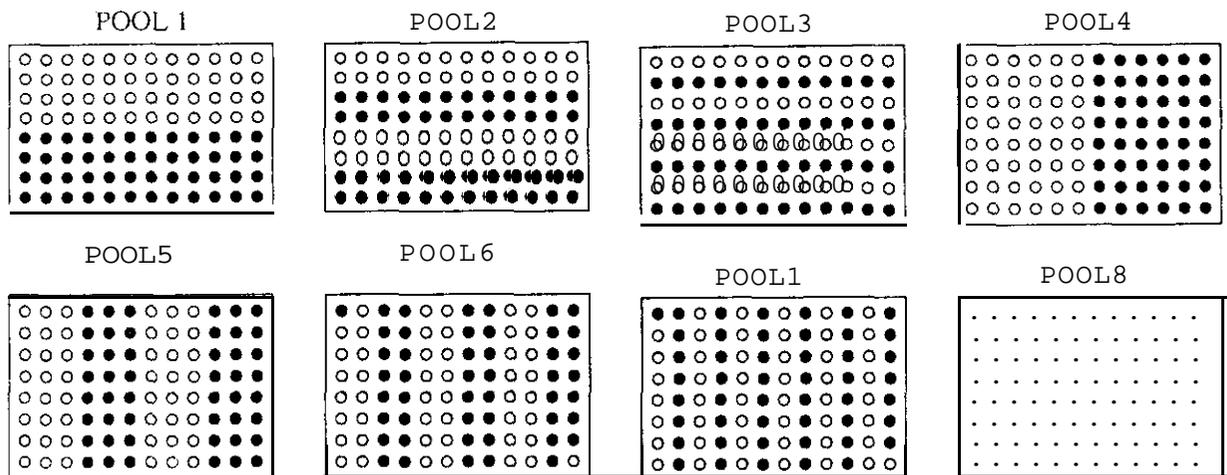
microtiter plates of 96 wells each, with each well containing an E. coli strain carrying a single cosmid bearing a fragment of Neurospora DNA. By replicating the clones from each plate to growth medium, one can prepare a quantity of pooled DNA from the clones on each plate and identify the plate or plates which contain a complementing clone. The problem then becomes one of minimizing the labor of identifying which of 96 clones is responsible for the complementation.

Complementation of a Neurospora mutant host by plasmids or cosmids carrying fragments of wild type Neurospora DNA has been a successful approach to cloning the wild type gene corresponding to a particular mutation (Akins and Lambowitz 1985 Mol. Cell. Biol. 5:2272-2278); Vollmer and Yanofsky 1986 Proc. Nat. Acad. Sci. U.S. 83:4869-4873).

The Vollmer-Yanofsky cosmid library consists of 32

Obviously, it is not necessary to prepare 96 different DNA samples and test them each for transforming ability. One could prepare DNA from clones of each of the eight rows, A through H, and from clones of each of the 12 files, 1 through 12, and by testing each of the 20 preparations for ability to transform, one could identify the well with the transforming clone. However, it is almost as obvious that even this strategy is far from optimal. The number of binary choices needed to specify one among 96 candidates is $\log_2 96$, or 6.58. Therefore, seven properly chosen pools of clones must be sufficient to identify the unique transforming clone. In practice, we will suggest that an eighth, mathematically redundant pool be prepared, since in any case, it is convenient to do centrifugations of even numbers of samples. This "pool 8" is the complete set, i.e., simply a repeat of the whole plate, and acts as a positive control for the rest.

The patterns in which colonies are grown up on plates to make the seven incomplete pools and the eighth, complete pool are shown below. The simple binary information obtained from the first five pools is self-explanatory, but the sixth and seventh pools have small, deliberately-introduced irregularities: wells 1A and 12H represent a break in the pattern in each of these pools of information inherent in there being seven pools rather than the physically meaningless minimum of 6.58 pools. The presence of clone 1A in both pools 6 and 7 insures that at least one of the seven incomplete pools will contain the desired clone. This provides a second positive control along with pool 8. The absence of clone 12H from both pools 6 and 7 guarantees that at least one of the pools will NOT contain the clone. Along with a "no DNA control" this acts as a second negative control for the possibility of reversion of the host or other unexpected events. The identification of the desired clone from the pattern of "yes" and "no" answers on transforming ability by pools 1-7 is self-explanatory.



Inoculating cultures one at a time onto seven petri plates to make the incomplete pools would be tedious, and it is simple to cut masks that allow the use of a 96-pin replicator, with the mask preventing contact between the end of the pin and the plate at positions where no inoculum is desired. Translucent orange "Bio-Check" Biohazard Bags (distributed by American Scientific Products, Cat. #A9500-11) are made of thin, autoclavable plastic, and have excellent dimensional stability and flatness. The masks need not have individual holes cut in them; blocks of holes can be cut out with an exacto knife. The masks are autoclaved between paper sheets and then arranged on oversized plates or cafeteria trays of medium and the microtiter cultures are printed out with the replicator.

The strategy we have described for identifying the complementing clone within a microtiter plate could, in principle, be applied at an earlier stage as well-- identifying which microtiter plate among the 32 plates contains a complementing clone. This extension of the approach would probably be ill-advised. Correct identification of a candidate clone from binary (yes/no) results on transforming activity of a series of pools requires that the collection under examination contain only one complementing clone. This condition will almost always be met when the collection being examined is one 96-well microtiter plate, but will often not be met in a collection of 32 such plates. The seemingly more laborious approach of preparing 32 pools of clones for the first screening is probably to be recommended. Supported by NIH Grant GM 08995 to RLM and by a grant from the Lucille P. Markey Charitable Trust, Miami FL, to SK. - - - Dept. of Physiological Chemistry, University of Wisconsin, Madison, WI 53706