MIAMI BIO/TECHNOLOGY WINTER SYMPOSIUM

BETWEEN THE MAP AND THE TERRITORY

MIAMI, Fla.—*In vitro* nucleic acid hybridization is as important to molecular biologists as *in vivo* hybridization is to molecular biology.

In the context of the human genome initiative, it is the only way currently available to answer questions about the expression of cloned segments of DNA. Such assays are, however, tedious and expensive. For these reasons, the idea of designing hybridization assays based on their *in vivo* equivalent, homologous recombination, has been attractive since it was first proposed by Brian Seed (*Nuc. Acids Res.* 11:2427) in 1983. As participants at this year's Miami Bio/Technology Winter Symposium heard from David Kurnit (Howard Hughes Medical Institute, Ann Arbor, MI), reliable recombination-based assays may at last be at hand.

In a recombination-based assay, DNA homologies are detected by plaques of lambda phage on a lawn of bacteria instead of bands or spots on a piece of nitrocellulose. Since it is possible to examine millions of possible homologies simultaneously because of the powerful selection systems that can be designed, such assays are, in principle, efficient and elegant. This selectivity, however, has also plagued their practical application since even small amounts of unwanted homologies are enough to give false positive plaques. With their newest vectors and strains, Kurnit and his colleagues have, they hope, eliminated the sources of such contamination.

To illustrate the problems and their proposed solutions, Kurnit described how a recombination-based assay could be used to determine the extent to which a piece of genomic DNA is represented in a lambda cDNA library by using only two petrie dishes, and in the case of a relatively rare sequence, to isolate its cDNA using one more. To begin, the DNA of interest is cloned into a plasmid that carries both an antibiotic resistance gene (to select transformants) and a bacterial amber suppressor gene (*supF*), to select the desired recombinants. The plasmid is then used to transform a recombination proficient *Escherichia coli* host, which is infected with one to ten million phage from the expression library. If the infecting phage contains at least a 25-base-pair exact homology with the genomic sequence on the plasmid, a chimera containing both the phage and plasmid sequences will be generated by recombination. These are recovered from the lysate by plating onto a second strain that selects for the presence of the *supF* gene.

This special property is conferred by an ingenious balance of two DNA replication genes—an amber mutation of the *E. coli dnaB* gene whose otherwise lethal phenotype is compensated by a P1 phage gene called *ban* (*dnaB* analog). Although the host can utilize the P1 gene to replicate its DNA, lambda cannot and therefore its replication in such bacteria is dependent on suppressing the *dnaB* amber mutation by expressing *supF*.

The number of plaques formed on this host is therefore a direct measure of the frequency with which the plasmid-borne sequence is represented in the cDNA library.

Because the copy number of *ban* must be kept low for the selection to work, Kurnit had previously used a single-copy lambda lysogen to introduce the gene. However, the low level of recombination between incoming *supF* phage and the lambda lysogen prevented the assay being useful for preparative purposes. To overcome this, the researchers have now constructed a selector strain that utilizes the property of P1 to lysogenize *E. coli* as a single-copy plasmid. This new strain expresses the *ban* gene even in the presence of the P1 repressor and carries other mutations that inactivate the P1 restriction system thereby allowing lambda to form plaques efficiently.

Another source of unanticipated recombination can arise because lambda cDNA libraries are often passed through strains carrying ColE1-like plasmids with which they share homology. Thus if the cloning vector is derived from ColE1, even very low levels of recombination between the incoming phage and the plasmid will result in unacceptable ambiguities in the assay. To avoid this problem, Kurnit has now introduced the *supF* gene into an R6K replicon, which does not share any homology with ColE1-based plasmids. These R6K replicons can be used to screen cDNA libraries constructed in a variety of lambda vectors independent of the presence of ColE1 sequences.

Finally, in order to ensure that a given plaque is the result of the desired recombination (and at the same time recover the cDNA of interest) it is necessary to be able to counterselect the chimeric phage for loss of the *supF* gene. This has now been achieved in a simple manner by constructing an *E. coli* strain which carries an amber mutation in a gene of phage P2 called *old*. Lambda is unable to replicate in the presence of an active Old protein and thus *supF*-containing phage are prevented from forming plaques on this host.

If the new vector and the two new selector strains function as advertised, recombination-based assays should now begin to fulfill their many potential applications, and in the process demonstrate that classical molecular biology based on *Escherichia coli* and its phages still has an important role, even in these days of "reverse" genetics.

—Harvey Bialy

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