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Short Communication

A shortcut in phage screening technique

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Abstract

A simple modification of the traditional Benton & Davis technique for phage screening is presented that avoids the tedious sample dilutions of putative spots/phages towards the second screening. With the use of a sole agar plate and nylon filter, the modification distinguishes a true positive recombinant from a false positive, with high probability of success.

Key words: phage screening, shortcut for Benton & Davis technique, single agar-plate/nylon filter.

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We have been working with phages for a long time, and a simplified CsCl protocol for lambda DNA purification was recently proposed by us. It avoids the enzymatic step, as well as the need for preliminary phage titration. This modification, requiring less steps and fewer solutions to prepare, produces a DNA suitable for further manipulation like digestion, ligation, labelling, subcloning, etc. (Santelli and Navarro-Cattapan, 2000). Now we are proposing a shortcut for the Benton & Davis technique (Benton and Davis, 1977). The screening of recombinant phages by this traditional technique is extremely efficient, but the confirmation of true positives in the second round frequently results in some false positives. This implies a waste of time and material. To diminish the frequency of these false recombinants, we conceived a modification of the original technique (Sambrook and Russell, 2001). This new approach has allowed us to distinguish, with great accuracy, a true positive spot from a false one, using a single agar-plate/nylon filter for a previously selected collection of potential positive phages (spots).

The technique consist of exposing the nylon filters to a PhosphorImager system (Storm, Molecular Dynamics) after the hybridization of the first screening and printing the results. Then, the image is printed to a transparency sheet. This makes the superposition of the transparency against the filters and agar plates easier, allowing a precise co-localization of the chosen spots on the agar plates. With the help of the wide side (diameter of approximately 4 mm) of a Pasteur pipette, the agar plugs corresponding to each spot are transferred to tubes containing 0.5 mL of lambda buffer (Tris pH 7.5 10 mM - $MgCl_2$ 10 mM) and a drop of chloroform. After approximately 2 h, 5 µL of each suspension are placed onto a fresh agarose upper layer made with competent bacteria (DL 538 *E. coli* strain) as usual. At least eight samples can be positioned on a sole agar plate. The next day, the plate with several individual large lysis plaques is covered with a nylon filter, and DNA transfer is performed according to the traditional procedure, devised by the above cited authors (Benton and Davis, 1977). Finally, the filter is hybridized using the same probe solution used in the original screening.

After many experiments using this technique, we observed that positive phages develop darker spots than the control (a plug removed from another region of the same plate). Experiment duplicates confirmed the reproducibility of the method. The most frequent result that distinguishes a positive recombinant from a false one is the presence of some dark mini-spots over the gray background area, resembling the labeling of an isolated phage plate (see Figure 1).

We have applied this simple modification with great success to the isolation of phages from a genomic library of the insect *Rhynchosciara americana*, as compared to the positivity estimate by the traditional methods (Table 1). This modification is less tedious than the plating of several dilutions of putative samples normally used in the second screening round. Another obvious advantage is that it avoids the waste of several expensive nylon filters.

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Table 1 - Quantification of positive phages obtained in some experiments by using the present technique, compared with the traditional one (Benton and Davis, 1977).

Expt	cDNA probe	N. of plugs tested	Positive phages observed	Positive phages confirmed*		Benton & Davis** %
				N.	(%)	
1	RT	8	4	4	100	50.0
2	gag	8	3	2	100	37.5
3	puff B	4	2	2	100	50.0
4	Ell	4	2	2	100	50.0
5	Hsp83	3	1	1	100	33.0
6	Histone	16	7	2	100	43.7
7	Actin	8	3	2	100	37.5

*Frequency of positive phages confirmed by Southern blot and DNA sequencing.
**The frequency of positive phages by the Benton & Davis technique was estimated from the ratio between the number of positive phages and the total number of selected plugs.

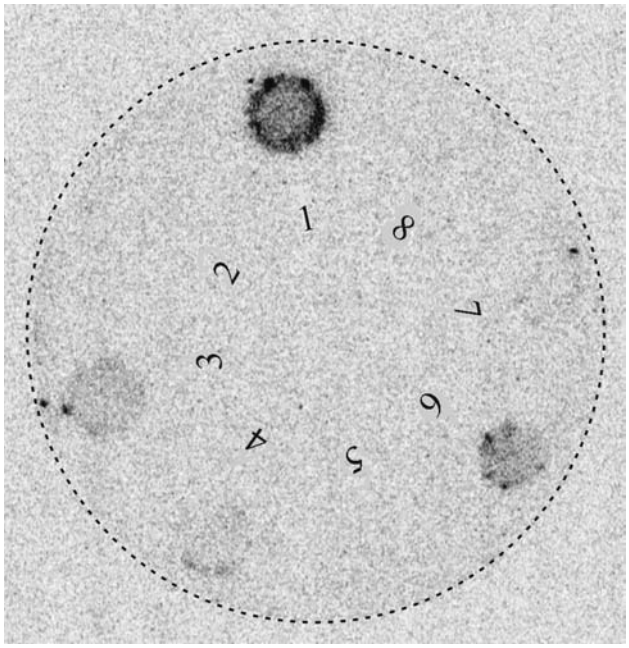

Figure 1 - Detection of true positives 5µL phage suspensions from eight selected agar plugs were placed on an agar plate overlayed with freshly made top agarose plus competent bacteria. Samples 1, 3, 4 and 6 resulted in positive recombinants; the others (2, 5 and 7) were assumed to be false positives by comparison with other several applications of this method. Sample 8 is the control (a plug withdrawn from another place of the same dish).

Acknowledgments

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