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Quick and efficient method for recovering of bacteriophages from soft agar after their propagation by the plate lysate technique

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Bacteriophages, in addition to their role as a research model, are commonly used tools in different fields of molecular genetics and biotechnology. For example, bacteriophage λ was first used as a cloning vehicle [1 - 3] and many versatile and sophisticated λ vectors are currently available which are designed for cloning large fragments of DNA, construction of genomic libraries, expression of cDNA as a β-galactosidase fusion protein, and immunological screening [4]. Single stranded, filamentous bacteriophage vectors such as derivatives of M13 are commonly used as a source of recombinant DNA templates for DNA sequencing by the dideoxy chain-termination method, generating DNA probes for hybridization and for site directed mutagenesis [4]. P1 phage is the most common tool for construction of recombinant *Escherichia coli* strains by general transduction procedures.

Several methods for propagation of bacteriophages have been described (see [4] and [5] for details) and one of the most commonly used is the plate lysate technique in which the bacteriophages are propagated in bacteria grown in soft agar. The last step of this technique is the isolation of phage particles from agar following lysis of bacterial cells. Usually the soft agar layer is scraped off into a sterile tube. To liberate phages the agar gel, after addition of chloroform and buffer, is disrupted by extensive pipetting or vortexing for several minutes or by rotation or shaking for a longer period. Alternatively, a few milliliters of buffer can be added onto the plate and such plate is stored at 4°C for several hours (usually overnight) with gentle

Table 1
Efficiency of recovering of phages from agar using different methods

Phage	p.f.u./ml of lysate obtained using:		
	pipetting	vortexing	mixer
λcIb2	4.7×10^{10}	8.5×10^{10}	6.0×10^{11}
P1vir	1.5×10^{9}	1.2×10^{9}	4.0×10^{9}
M13	2.4×10^{11}	6.0×10^{11}	1.7×10^{12}

Bacterial strains MC1061 [6] or MC1061/F^+ (in propagation of M13 phage) were used. For propagation and titration of bacteriophages we used TAd plates [1% Trypton (Difco), 1% NaCl, 1.5% Bacto-agar (Difco)] and TAg soft agar [1% Trypton, 0.5% NaCl, 0.7% Bacto-agar]. For propagation and titration of P1 phage, $CaCl_2$ was added to TAg soft agar to a final concentration of 5 mM. For propagation of bacteriophages, 0.3 ml samples of the overnight bacterial culture in the LB medium (or LB containing 5 mM $CaCl_2$) was mixed with the bacteriophage (10^6 p.f.u). The mixture was incubated for 15 min at room temperature and then 3 ml of molten TAg (or TAg containing 5 mM $CaCl_2$) soft agar (45 - 50°C) was added. The sample was mixed and immediately poured onto a plate containing TAd agar. The plate was incubated for 8 h at 37°C. Then the soft agar was scraped off into a sterile centrifuge tube with a sterile bent glass rod. One ml of TM buffer (10 mM Tris/HCl, pH, 7.2, 10 mM $MgSO_4$) and 1 ml of chloroform were added. The agar gel was disrupted alternatively by extensive pipetting, vortexing or by using the mixer until a uniform emulsion was obtained. The sample was centrifuged at 4000 × *g* for 15 min at 4°C and the supernatant containing phage particles was titrated with the appropriate bacterial strain

shaking to allow phage particles to diffuse from agar into the buffer. Although the last mentioned method is simple, it is however, time consuming. On the other hand, rapid "gel-disrupting" techniques are often inconvenient. We propose another method for disrupting agar gel, which is simple, rapid and efficient, and takes less than one minute. We have constructed a simple mixer (Fig. 1) which contains a 12 V motor and can be used with standard laboratory power supply. We recommend to use voltage of about 6 - 10 V. A long metallic arm of the mixer with a loop on its end can be easily sterilized by simply dipping it into a beaker containing 95% ethanol and then holding it in the flame of a Bunsen burner to ignite alcohol. We have tested the mixer for efficiency of recovering bacteriophages from soft agar in comparison with two common methods, i.e. by pipetting and vortexing. As shown in Table 1, the use of the mixer is even more efficient than other techniques in the case of all tested bacteriophages.

Moreover, it takes only one minute to get an emulsion, suitable for efficient recovery of phages while pipetting or vortexing takes several minutes. Besides, the use of the mixer allows to avoid splitting of the mixture.

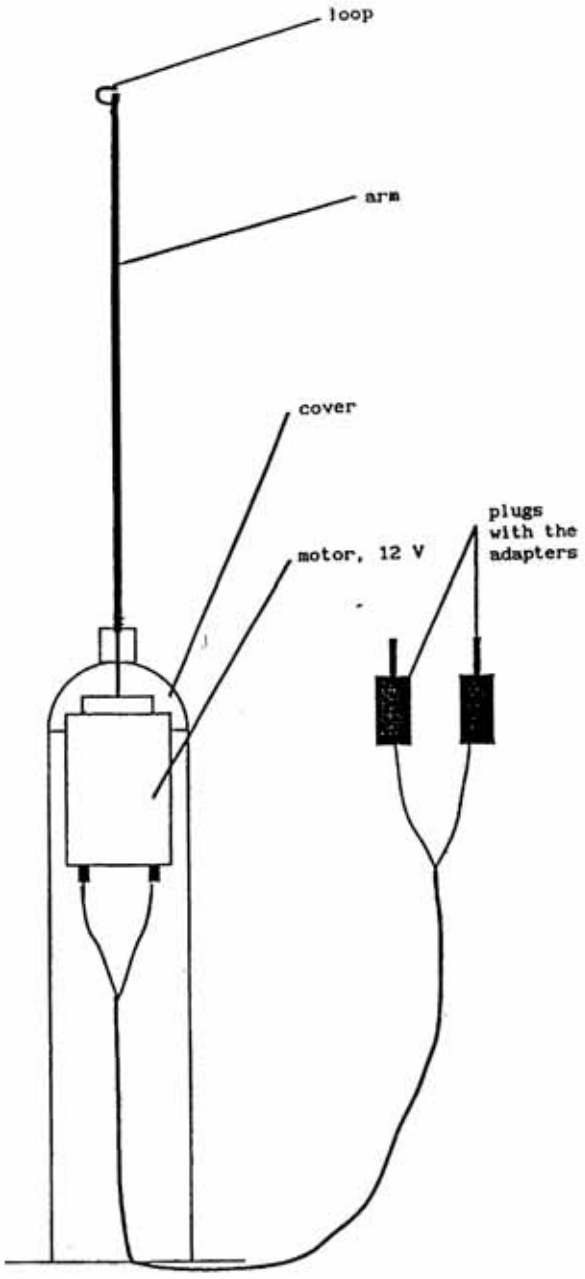

Fig. 1. The scheme of the mixer

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