A fast and convenient way to produce single stranded DNA from a phagemid

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Despite the increased number of methods using double stranded DNA for sequencing, site directed mutagenesis, etc..., it is often more convenient to use vectors able to produce single stranded DNA for such usages. The phagemids are more convenient for many purposes in molecular biology than M13 phage because they are smaller and can grow on bacteria as colonies instead of plaques, However the obtention of single stranded DNA from phagemids is longer than from M13 since it needs an infection step with a helper phage. A convenient protocol for single stranded DNA production from phagemids is proposed here. Used with pEMBL8$^+$ and derived vectors, this method provides similar yields than other described methods (1−3). It avoids absorbance monitored culture, saves up to one day and is far less consuming in helper phage stock.

A colony of male *E. coli* strain harboring the phagemid of interest, taken from a fresh selective medium plate (less than one week-old), is suspended in 10−50 μl of 2YT medium. The bacterial suspension is then infected by addition of 1 μl of M13 KO7 helper phage stock (more than 10^{10} pfu/ml) and incubated 15 min at RT. 500 μl of 2YT with antibiotic for phagemid selection are added to the infected cells which are then incubated at 37°C for one hour to let helper phage express antibiotic resistance. 100 to 200 μl of incubated cells are added to 3−4 ml of 2YT medium with appropriate antibiotics (we used 150 mg/l ampicillin and 75 mg/l kanamycin), The culture is proceeded for 18−20 hours at 37°C. The culture should be lead to saturation for good single strand production. The virion particles are isolated and single stranded DNA is prepared as in (3). 1.5 ml culture leading to 30 μl of preparation should provide concentrations suitable for most applications (see figure).

A convenient way to pick up the colony is to use a blue sterile cone placed on a Pipetman. The Pipetman is set around 150 μl, the push button is depressed and the cone applied on the colony with light pressure avoiding damage to the agarose surface. The colony is pumped and resuspended in the 10−50 μl of medium by rotating the adjustment ring back and forth.

The cultures have been performed on volumes up to 50 ml, times longer than 24 hours and temperatures down to 29°C with none of the problems which can occur in case of M13 production. This method was used with bacterial strain such as RZ1032 to obtain uracil-containing template DNA for mutagenesis with Kunkel's method (4).

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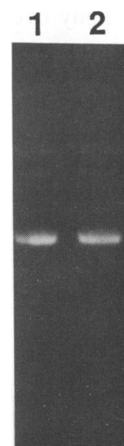

1% agarose gel electrophoresis of single stranded DNA prepared from *E. coli* TG2 cells harboring pEMBL8$^+$ and superinfected with phage M13K07. 3 μl were layed from a 30 μl preparation. Lane 1: Culture done as in ref 3. Lane 2: Culture done as described.