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Application Bulletin

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USES OF THE CL-1000 UV CROSSLINKER IN THE LABORATORY

The CL-1000 UV Crosslinker is a multi-purpose ultraviolet exposure instrument for use in the laboratory. Utilizing a 254nm shortwave ultraviolet (UV) radiation, the Crosslinker has the ability to perform a wide variety of applications.

CL-1000 CROSSLINKER

INTRODUCTION

Ultraviolet radiation is a fast, easy, and effective method to fix nucleic acids to nitrocellulose, nylon, and nylon-reinforced membranes after Northern, Southern, slot or dot blotting. Ultraviolet radiation catalyzes the covalent attachment of nucleic acids to these membranes by activating interactions between thymines or uracils with the amine groups on the membrane matrix [1]. The result is higher resolution and sensitivity of subsequent hybridization analysis. The entire fixing process performed on the Crosslinker is around two to ten minutes, compared to the two hour period required for fixing by the baking method.

The Crosslinker is not limited to just fixing nucleic acid. The versatile wavelength is effective in many applications, compatible with most molecular biology experiments. CL-1000 UV Crosslinker can be used to nick ethidium bromide stained DNA in agarose gels, a step in alternating contour-clamped homogeneous electric field gel electrophoresis (CHEF) [2]. CHEF is a type of pulsed field gel electrophoresis, when used with the CL-1000 UV Crosslinker, can resolve DNA fragments greater than five megabases with clarity and ease.

UV irradiation of DNA provides an easy way to control the extent of restriction endonuclease digestion due to the fact that UV radiation dimerizes neighboring thymidines (TT, TTT, etc.). The restriction enzymes can not recognize and cleave the DNA if the thymidines within their restriction sites were dimerized [3]. The Crosslinker allows a greater control over partial digestions.

The Crosslinker offers a simple method to test for *recA* mutations. *RecA+* strains repair UV-induced damages and grow normally whereas mutations in *recA* prevent the cells to grow because of inability to repair damages [4]. By irradiating a strain with an unknown genotype, a mutation can be easily detect due to the properties mentioned above.

Ultraviolet radiation is often used in sterilization. The Crosslinker is effective in killing bacteria cultures, viruses, bacteriophages, and small organisms on surfaces [5]. It is an efficient alternative to the traditional heat germicide.

UV radiation can solve the problem of PCR (polymerase chain reaction) contamination. UV irradiated fragments form pyrimidine dimers which function as termination sites [6]. The formation of these termination sites eliminates most contamination caused by the reagents from the previous amplified material.

MATERIALS AND METHODS

A. FIXING NUCLEIC ACIDS ON TO MEMBRANES

Run agarose gel electrophoresis [7]. Transfer nucleic acids on to the membrane by capillary or other transfer methods [8]. Lay the membrane, nucleic acid side up, on a piece of tin foil. Do not cover. Turn the Crosslinker on. Press one of the

auto settings: UV energy exposure or time. Remove the membrane when the Crosslinker beeps five times. Continue with hybridization [9, 10].

B. NICKING ETHIDIUM BROMIDE STAINED DNA IN AGAROSE GELS

Prepare DNA samples. Run CHEF gel electrophoresis using a hexagonal array of electrodes. After electrophoresis, the gel is stained with ethidium bromide (0.5 μg/ml) and irradiated by the Crosslinker for about one minute [2]. The gel is then subjected to hybridization [10].

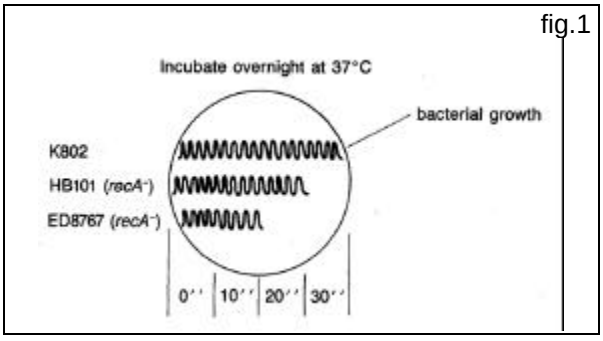
C. GENE MAPPING FOR CREATING CLEAVAGE-INHIBITING THYMINE DIMERS

Prepare DNA samples. Add 0.1-1 μg of DNA and 20 μl of restriction buffer. The restriction buffer must contain MgCl2. In the experiment conducted by Whittaker it was added into the mixture when the appropriate oligo was end-labeled. This mixture is irradiated for up to 60 minutes [3]. Then the mixture can be analyzed by agarose gel electrophoresis [7]. Longer irradiation time equals less cleavage by the restriction enzymes.

D. TESTING recA FUNCTION

Streak the strain being tested along with *recA+* strain on a petri dish. Us a piece of cardboard to cover about three quarters of each streak, expose the remaining portion to the Crosslinker for about ten seconds. Move the cardboard so half of the dish is covered. Expose the dish to the Crosslinker for ten seconds. Then move the cardboard so three forth of the petri dish is exposed, place it in the Crosslinker for ten seconds. Incubate the dish at 37°C overnight [4]. The *recA-* strain should be shriveled and shorter than the wild type strain (fig. 1).

fig.1



E. ULTRAVIOLET STERILIZATION

Place the surfaces to be sterilized in to the Crosslinker for a set amount of time. Consult the Bacteria Destruction Chart on the UVP Internet home page for suggested time. Note: the Crosslinker cannot sterilize liquid nor solids.

F. ELIMINATION OF PCR CONTAMINATION

Irradiate the target DNA with the Crosslinker, five minutes is sufficient. For best results, the photoinduced defects should be in the sequence region bounded by the 3' ends of the PCR primers [11]. Add the PCR reaction components. Perform amplification as practiced [12].

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