

How to use UV for mutagenesis

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Background: One step in the discovery of genes and gene products involved in a biochemical function or a developmental process is to identify mutations that change a function or process. Ultraviolet light (UV) is a strong mutagen (in the wavelength that DNA absorbs, roughly 225-300 nm) and primarily generates dimers between adjacent pyrimidine bases on the same DNA strand. These dimers cause the strand to buckle, disrupting normal base pairing. This prevents proper replication and can result in point mutations, deletions, and chromosomal rearrangements. The effects of mutagenesis can be reversed by photoreactivation, a light-dependent DNA repair mechanism.

Prototcol: A conidial suspension from 7 day old conidia is prepared in water and filtered through cotton or glass wool to remove mycelial fragments. The conidial concentration of the suspension is determined using a hemocytometer (e.g. Reichert Brightline; Warner-Lambert Technologies Inc., Buffalo, N.Y.). Subsequently the suspension is calibrated so that the suspension contains 5×10^{-6} conidial/ml. Ten ml. of this suspension is dispensed into a 150X15 mm Petri dish and placed on a magnetic stirrer at 25°C to keep the conidia in suspension. A 15-Watt General Electric germicidal lamp with approximately 90% of its ultraviolet output at 2537 Å was used for irradiation. Remove the lid of the Petri dish before irradiation. Also, keep the plates in the dark during incubation to prevent photoreactivation. However, photoreactivation usually requires a high light intensity.

In general a dosage of ca $6,000 \text{ ergs/mm}^2$ as measured with a General Electric photoelectric ultraviolet meter is used. The distance from the UV light determines the dosage. If you do not have access to a meter, determine the survival of the conidia at a given distance of 10 cm to 15 cm at different time intervals ranging from 0.5 to 6 minutes. To determine survival rate, samples of the irradiated suspension are plated to determine survival at 10^{-5} and 10^{-6} (see protocol how to plate conidia and ascospores). The survival rate = number of colonies on irradiated plate / number of colonies on unirradiated plates at the same culture dilution factor. A 20% to 50% survival was determined as the optimal dosage for induction of reversions and mutations.

References:

Davis, R.H. and de Serres, F.J. (1970) Genetic and microbiological research techniques for *Neurospora crassa*. *Methods in Enzymology* 17: 79-143.

Giles, NH (1951) Studies on the mechanism of reversion in biochemical mutants of *Neurospora crassa*. *Cold Spring Harbor Symposium on Quantative Biology*, 16: 283-313.