

ADDITIONAL UV KILLING EXPERIMENTS

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See previous protocol on *Ultraviolet Killing of Bacteria* for preparation of the plates used here.

PHOTO-REACTIVATION DEMONSTRATION:

Ultraviolet damage can be corrected by several mechanisms in most organisms, including bacteria. One of these repair mechanisms is photoreactivation in which pyrimidine dimers are snipped apart *in situ* by an enzyme which uses visible light as the source of energy. This process may be demonstrated by modifying the UV killing assay as follows:

1. **Prepare a seeded plate** by pouring 2 mL melted and cooled top agar which has been inoculated with 0.1 mL ON culture of bacteria to be tested. (see *Agar Overlay Technique*) Mark with a 16 square grid as on page one of this protocol.
2. **Label the four crossing lanes** with the exposure times to UV: 3, 1, 0.3 and 0 minutes.
3. **Label the four vertical lanes** with the exposure times to visible light: 27, 9, 3 and 0 minutes.
4. **Expose the plate to UV** according to the labeled times as directed on page 1.
5. **Rotate the plate 90°, expose to visible light**, covering all but the 27 minute band with opaque cardboard, place under a bright visible light (glass-filtered sunlight, or 100 watt bulb at 25 cm) for 18 minutes.
6. **Move the cardboard** to expose the 27 and 9 minutes bands for 6 more minutes.
7. **Move the cardboard** to expose the 27, 9 and 3 minute bands for 3 more minutes.
8. **Incubate at 37°C in the dark** for 24 to 48 hours, note any difference in populations in the agar overlay. Propose a mechanism for any differences observed.

SUNTAN LOTION PROTECTION?

Paint bands of several suntan lotions of given blocking powers across the top of a plastic petri dish. Pour a top agar layer of *E. coli* on top of nutrient agar plate. Expose the plate as above to 0, 20 seconds, 1 and 3 minutes as in the protocol for *Ultraviolet Killing of Bacteria*. Incubate and note any protection which may be observed.

TEST THE BACTERIOCIDAL EFFECTS OF TANNING BEDS?

Shortly before going to a tanning saloon, prepare a nutrient agar plate with a fresh lawn of *E. coli* in top agar (step 1 above). At the tanning bed, cover the open plate with blocking cardboard, expose for 9, 3, 1 and 0 minutes as directed on page 1. Incubate ON and read bacterial density of exposed areas. Discuss the implication for your exposed skin for these times. *Extra credit will be given for anyone performing this experiment!*