TARDIGARD

protocol

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Protocol for Radiation Resistance Test

In order to evaluate the effect of the protein Dsup on *E. coli*, we designed this protocol. Dsup is a self-expressed protein that can protect the bacteria from radiation by binding with DNA[3]. It is expected that the LD_{50} of UV can be obtained by setting up a dose gradient.

Equipment and materials:

Ultraviolet source, microscope, hemocytometer[1], trypan blue stain (0.8mM in polybutylene succinate, PBS)[1].

1. Radiation disposal[4]

- We expect to use a wide spectrum of UV radiations with a peak of emission at 312nm (UV-B), which is considered the most cytotoxic and mutagenic wavelength among types of solar radiation[5].
- Set up a gradient of UV doses, e.g. $10kJm^{-2}$ - $100kJm^{-2}$.
- Apply newly cultured bacteria in LB broth respectively on small culture dishes.
- Put the dishes under the UV lamp without cover. The UV lamp was positioned 30 cm above the samples. Expose the sample for a period of 180 min.
- The temperature should be kept constant during the whole process of irradiation, and the surroundings should be kept dark.
- For un-irradiated control, another dish is placed in the same temperature without direct UV radiation.

2. Survival rate assay[1]

- Mix irradiated cells 1:1 with trypan blue solution (for no more than 30 min because of its toxicity).
- Count the number of blue (dead) and unstained (living) cells respectively. For usage of hemocytometer, see https://en.wikipedia.org/wiki/Hemocytometer[2].
- Calculate the proportion of two types of cells.

References:

1. Sandy Westerheide, Northwestern University, *Protocol for use of tripan blue dye.*

2. Hemocytometer - Wikipedia -.

3. Takuma Hashimoto *et al. Extremotolerant tardigrade genome and improved radiotolerance of human cultured cells by tardigrade-unique protein.*

4. Tiziana Altiero *et al. Ultraviolet radiation tolerance in hydrated and desiccated eutardigrades.*

5. Connelly SJ. *et al. Temperature effects on survival and DNA repair in four freshwater cladoceran Daphnia species exposed to UV radiation.*