RUA # 1384

UNIVERSITY OF CALIFORNIA, DAVIS
ENVIRONMENTAL HEALTH & SAFETY, HEALTH PHYSICS

Radiation Use Authorization

Safety Protocol

Provide a copy of this protocol to each co-worker participating in the experiment.

Title of Project: Initiation of development in *Myxococcus xanthus*
Purpose To understand how changes in gene expression lead to changes in behavior and morphology.

Radioisotopes ^{35}S, ^{32}P, and ^{33}P
Chemical Form: ^{35}S amino acids and dNTPs
^{33}P dNTP
^{32}P dNTP and NTPs

A. What personal protection methods will be used to prevent contamination and internal exposures to radiation?
Disposable gloves __X__ Disposable booties_____ Lab coat __X__ Glove box_____
Fume hood __X__ (Flow rate: _____ FPM) Absorbent paper __X__ Respirator _____
Other: Lucite shields and boxes will be provide for storage and work shielding. All areas and equipment used for radioactive work will be clearly marked and shielded. A designated "hot bench" will be used for most radioactive work, this bench will contain a microcentrifuge, heating block, water bath and aspirator dedicated for radioactive work. No eating or drinking will be permitted near radioactive work areas.

B. How will you detect radioactive contamination and/or radiation fields?
Wipes and liquid scintillation counting
G.M. Survey Meter Model: Not yet purchased

C. At what frequency will you check your laboratory for contamination?
After each experiment __X__ Biweekly __X__

D. Explain your method for decontamination of nondisposable objects contaminated with radioactivity:
Non-disposable items will be cleaned with "count-off" and water. Rinses will be placed in to the appropriate liquid radioactive waste and paper towel into dry waste containers.

E. What personal protection methods will be used to prevent external exposures.
Shielding __X__ (see below) Devices __X__ (see below)

Isotopes will be stored behind Lucite shields and all physical manipulations will be performed behind Lucite shielding. Gloves, lab coats and eye shields will be worn at all times. Pipetting devices and disposable tips will be used.

F. List the types of radioactive waste you will have and where you will store each form of waste until EH&S picks it up Estimate the volume per month:

		Storage Location	**Quantity/Month**
Dry	X	270 Briggs Hall	1-2 cubic feet
Liquid	X	270 Briggs Hall	1-5 gallons
Biological	none	270 Briggs Hall	
Scintillation vials	X	270 Briggs Hall	1-5 flats (100 vials/flat)

Liquid Chemical form (*i.e.*, H_2O, ethanol, etc.)	H_2O
	Ethanol
	Acetone
	Trichloroacetic acid (neutralized)

G. Protocols

I. In vivo protein labeling and Immunoprecipitations. This protocol is to be performed at the designated hot bench. All radioactive waste containers (liquid and dry), dedicated vacuum aspirator, microcentrifuge, water-bath and heating-block used exclusively for radioactive work are located at the hot bench. All equipment and radioactive waste containers are appropriately shielded and marked. All work is done behind Lucite shields and over absorbent paper.

Radioactive amino acids (Tans ^{35}S-label, 25 μCi/pulse reaction) will be added to growing cell culture for 2-10 minutes to pulse-label bulk proteins, then chased with 40μg/ml of cold methionine for 2 minutes. The reaction is then stopped by the addition of 1 ml of 5% ice cold Trichloroacetic acid (TCA).

Labeling reactions are prepared a head of time by mixing label with 100μl of medium. Label is stored behind Lucite shields until needed and is immediately returned to storage after the appropriate amount has been removed and all log sheets are completed. ^{35}S-trans label is opened in the hood after venting into a syringe with a charcoal trap (trap is disposed into dry waste); ^{35}S-trans label (25 μCi/pulse) is added to 100 μl of sterile culture medium using a mechanical pipetting device and disposable tips (tips are disposed into dry waste). Enough labeling mix is made for each pulse in the experiment (total volume of labeling mix = number of pulses x 100 μl medium, total amount of label required = number of pulses x 25 μCi), 100 μl of the labeling mix is then transferred to reaction tubes (disposable tubes) and placed on ice until required. Any remaining labeling mix is removed and discarded into liquid radioactive waste (the pipette tip and labeling mix tube is discarded into dry waste). At the appropriate time interval 100 μl of the growing culture is added to the pulse tube, which has been placed in a water bath 2 minutes prior to use to warm the reaction mix to the appropriate temperature. The culture is allowed to incubate with the labeling mix for 2-5 minutes at the appropriate temperature (volume is 200 μl) at which point the reaction is chased with 40μg/ml of cold methionine for 2 minutes. The reaction is stopped by the addition of 1 ml of ice cold 5% TCA to precipitate the macromolecules and is then placed on ice behind Lucite shielding until all samples have been taken. This is repeated for each time point. The precipitated proteins are recovered by centrifugation, the TCA is removed by vacuum aspiration into a side arm flask (flask contains a secondary trap to prevent vacuum contamination). The pellet is washed 3 times with ice cold 80% v/v of acetone, the acetone is removed by vacuum aspiration. Samples are dried and resuspended in IP (immunoprecipitation) buffer. The Acetone-TCA wash is first neutralized with sodium hydroxide and then transferred to the appropriate liquid waste container.

Samples are then mixed with appropriate anti-body and buffer and incubated 1 hr to over night at 4˚C. After incubation 50 μl of Staph-A cells (or Staph-A agarose beads) are added and incubated on ice for 30 minutes. The samples are centrifuged in a microcentrifuge for 1-2 minutes to precipitate the Staph A-Antibody-Antigen complex. The supernatant is removed by vacuum aspiration, and the pellet washed 4 times with buffer, aspirating the wash off each time. The pellet is resuspended in 20-25 μl of SDS sample buffer + bromphenol blue dye, incubate at 95˚C for 5 minutes and centrifuged to remove the cell debris. The supernatant is transferred to a new tube by mechanical pipetting devise (pipette tip, tube and pellet are discarded into dry radioactive waste). The radioactive TCA and acetone washes are neutralized with sodium hydroxide and transferred to the radioactive liquid waste container. The sample is then analyzed by

polyacrylamide gel electrophoresis. Once the gel has run the appropriate length of time, the gel is removed dried and subjected to autoradiography. Running buffers are typically non-radioactive since the amount of radioactivity at this point is extremely low, since we are working with immunoprecipitates. Once the gel is no longer needed it is disposed into dry radioactive waste. Work areas are monitored by direct surface assay (wipe tests) as soon as they are completed and any contaminated areas are cleaned.

II. ^{32}P labeled DNA Probes: This protocol is to be performed at the designated hot bench. All radioactive waste containers (liquid and dry), dedicated vacuum aspirator, microcentrifuge, water-bath and heating-block used exclusively for radioactive work are located at the hot bench. All equipment and radioactive waste containers are appropriately shielded and marked. All work is done behind Lucite shields and over absorbent paper. These protocols require the experimenter to wear lab coat, safety glasses, and gloves.

For Northern, Southern, and S1 protocols ^{32}P-labeled DNA probes are used. Probes are made by one of two protocols: Kinase reactions or Random Priming reactions using DNA polymerase. In both protocols ^{32}P nucleotide triphosphates are mixed with DNA, buffer and enzymes to label the DNA fragment. Label is removed from storage and the appropriate log forms completed. For kinase reactions 50-100 μCi of ATP/reaction is used and for Random Priming 25-50 μCi of dCTP (or dATP)/reaction is used. Label is mixed with appropriate buffers, DNA and enzyme in a 1.5 ml screw cap microfuge tube and incubated. All manipulations and incubations are performed behind Lucite shields and monitored with a Geiger counter. Reactions are stopped, and the DNA precipitated and washed with 95% ethanol. All radioactive supernatants (washes) are removed with a mechanical pipetting device with disposable tips (washes are discarded into ^{32}P-liquid waste and the tips into ^{32}P-dry waste). The precipitated DNA is then resuspended into the appropriate buffer and used as required (see below).

III. Northern and Southern analysis. RNA (Northern) and/or DNA (Southern) blots are incubated with ^{32}P-labeled probe (see above). Incubation is performed either in a sealable plastic bag or a sealed glass hybridization tube. The sealed tubes are preferential to sealed bags (due to ease of manipulations), but both containment vessels minimize exposure to radioactive liquids. The sealed vessel is placed into a hybridization oven or water-bath and incubated over night. The probe is then removed and the blot washed several times. The radioactive washes and used probe are transferred to the ^{32}P-liquid waste. The blot is then wrapped in saran wrap and exposed to autoradioagraphy. Once the blot is no longer needed, they are discarded into the ^{32}P-dry waste.

IV. S1 analysis. ^{32}P-labeled probe is mixed with RNA and buffer and incubated at 65˚C behind a Lucite shield for 12-24 hours. Enzyme is added and the reaction incubated at 37˚C, stopped and the nucleotides precipitated. The radioactive supernatant is discarded using a mechanical pipetting device and disposable tips (supernatant to the liquid waste, the tip to the dry waste). The nucleic acids are resuspended and analyzed by polyacrylamide gel-electrophoresis. The gel is dried and exposed to autoradiography. The used gel is finally discarded into dry waste. The gel buffer is typically non-radioactive and simply discarded. For all of these procedures, all manipulations will be done behind Lucite shields and the work areas monitored during the experiment with a Geiger counter. Water-baths, heating blocks, microcentrifuges and all other equipment will be marked appropriately and shielded. All contaminated areas will be decontaminated immediately.

V. DNA sequence analysis. Radioactive nucleotides (^{35}S-dATP or ^{33}P-dNTP) will be used to label DNA in vitro using for DNA sequence analysis. In a typical sequencing reaction 10 μCi of labeled nucleotide (^{35}S-dATP or ^{33}P-dNTP) is placed in a microcentrifuge tube along with DNA, enzymes, and reaction buffer. The reactions are allowed to incubate and then spun in a microcentrifuge. The samples are then subjected to polyacrylamide gel electrophoresis followed by auto radiography. All manipulations will be done behind Lucite shields and the work areas monitored during the experiment with a Geiger counter. Water baths, heating blocks and all other equipment will be marked appropriately and shielded. In addition, the experimenter will wear a lab coat, safety glasses and gloves during the procedure. After each

experiment the work areas will be cleaned, monitored and any radioactive waste will be stored in the appropriately labeled Lucite waste containers.