

# **Bio Detectors for Radiation Detection: Diversifying the System**

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# EFFECTS OF IONIZING RADIATION ON LIVE ORGANISMS

- Ionizing radiation affects live organisms by causing damage of their DNA.
- Radiation also causes damage of other (macro)molecules including proteins and pigment complexes.

# HOW MICROORGANISMS SURVIVE THE DAMAGE?

- **Microorganisms switch on a “SOS system” for damage repair**
- **SOS system is a set of genes which are induced by stress**
- **Microorganisms have SOS subsystems, which are induced by different stress factors, including radiation**

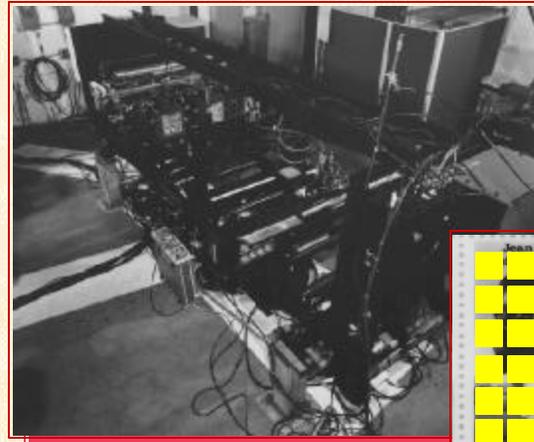
# HOW DOES MICROBIAL RAD SOS SYSTEM WORK?

- **Ionizing radiation causes DNA and protein damage, which cannot be repaired by regular systems**
- **Such damage triggers induction of the genes that encode proteins that repair DNA (*recA*), or respond to protein damage (*grpE*), or to the oxidative stress (*katG*)**
- **A *umuCD* gene family is switched on in response to environmentally-induced DNA damage**

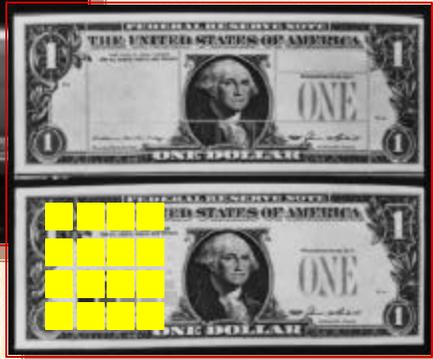
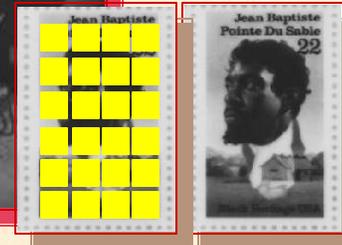
# Stable Fluorescent Bioreporter Products Provide Historical Record of Exposure



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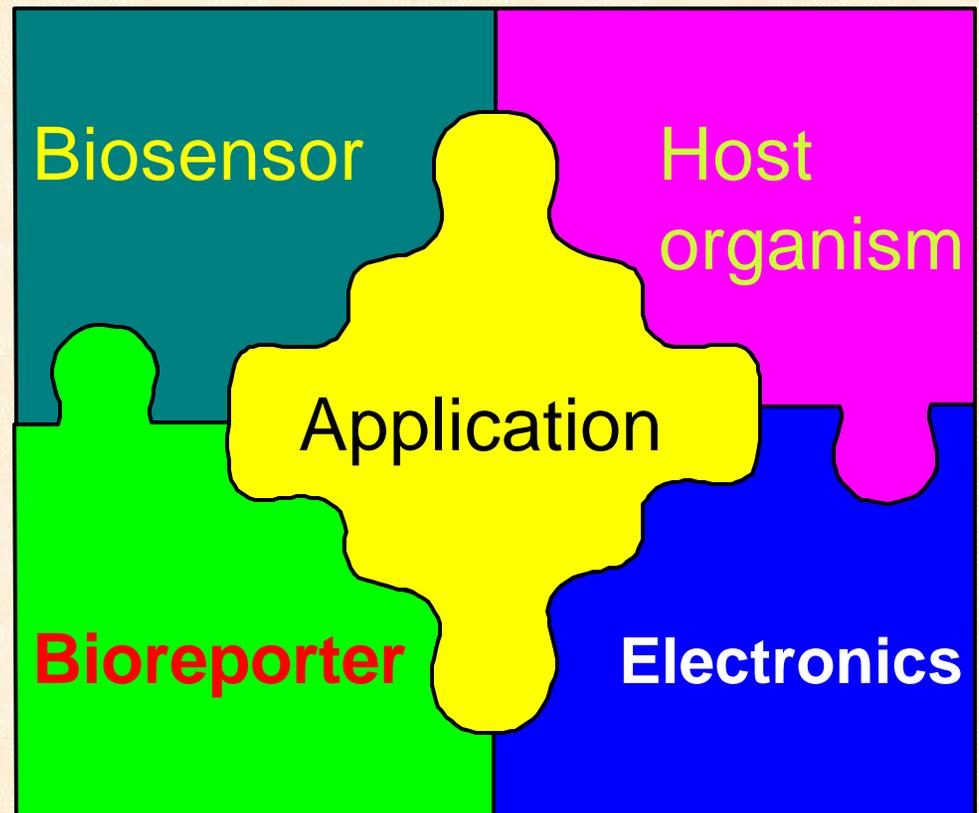
*postage stamp inspection*



Combined with rapid analysis tools, such as those developed by ORNL for the U.S. Postal Service and Bureau of Engraving and Printing, bioreporter signature arrays can provide a powerful tool for identifying and locating chemical residues indicative of weapons manufacturing.

# APPLICATION VERSATILITY CAN BE ACHIEVED THROUGH MIX-AND-MATCH STRATEGY

- Biosensing damage repair systems (DNA, protein)
- Potential host organisms (*E.coli*, other bacteria, yeast, algae)
- Bioreporting systems (*lux*, *gfp*, *lacZ*, other)
- We have a variety of electronic signal reporting systems

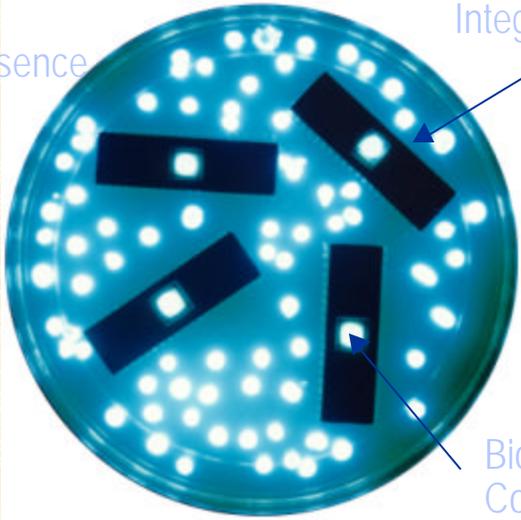


# GENES INVOLVED IN BACTERIAL RESPONSE TO RADIATION ARE KNOWN

- **These genes have been isolated and sequenced**
- **Sequences of the genes are available, promoters are known**
- **Prior research showed that the *recA*-based systems can provide linear response to gamma-ray doses of 1 - 50 Gy**

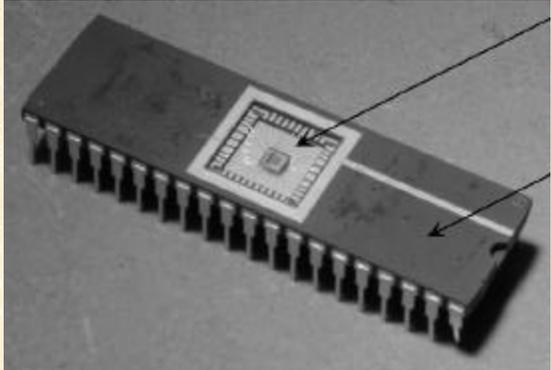
# Bioluminescent Bioreporter Integrated Circuits - complete sensing technology in a compact, low power package.

TVA8 Strain indicating presence of toluene.



Integrated Circuit

Bioreporting Colony



2.2 mm square

mW power consumption

Bacteria are genetically modified to produce light (or fluorophores) in response to the amount of exposure (chemical or radiological) received in the environment.

We have developed CMOS-based integrated circuits that transduce bioluminescence to provide direct measurement of contaminant or radiological exposure.

**May be coupled with integrated-circuit-based telemetry to provide wireless wide area and peripheral monitoring for chemical, biological, and radiological agents.**

# COMPARISON OF RADIATION-RESPONSIVE GENETIC SYSTEMS

## **DNA repair (*recA*, *uvrB*,**

Induced by radiation  
of different types

Found in all  
organisms

## **DNA and protein repair (*kat*, *umu* etc.)**

Induced by different  
agents, incl. chemical

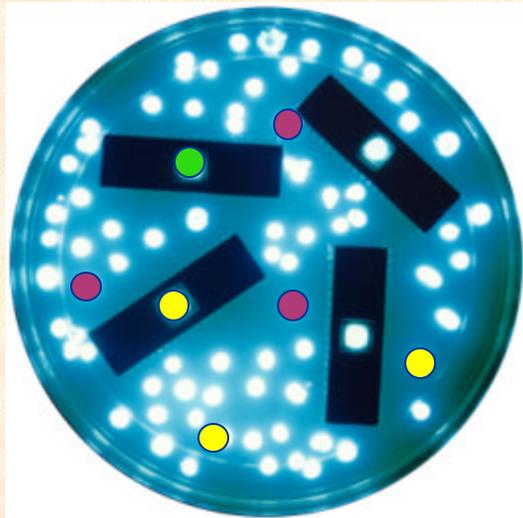
Found in all  
organisms

## **Photosynthetic complex repair**

Induced by ionizing  
radiation, not UV-A

Found only in  
microalgae

Continued development of Bioreporting Integrated Circuits Systems, either with discrete excitation/filtering or integrated optical advances, will provide low power, spectrally responsive systems - ideal for transduction of fluorescent protein reporting methods.



#### Advantages:

Low Cost (bulk fabrication of CMOS can be very inexpensive, i.e., consumer electronics)

Low Power (operation, including RF spread spectrum transmission mwatts)

Compact, rugged packaging.

Parallel expandability, providing bioreporting arrays for chemical/exposure signature analysis.

# MAIN FEATURES OF THE DESIGN OF OUR BIOSCINTILLATOR

- We will use promoters of two genes: *recA* and *umuC*
- We will fuse a reporter gene *gfp* (Green Fluorescent Protein) to work from under these promoters
- We will use several bacterial hosts for the construct
- We will design electronic detector system in parallel with microorganisms in order to provide the best integration of the systems

# WHY USE SEVERAL MICROBIAL HOSTS?

- Commonly used strains of *Escherichia coli* are unstable and their viability is affected by doses over 50 Gy. They are also prone to desiccation.
- We will use Gram(+) organisms (food-grade *Staphylococci*) yeast, and radioresistant organisms which are robust
- We will also use algae, which are capable of withstanding high levels of radiation.

# ADVANTAGES OF OUR BIOSCINTILLATOR

- **Design that involves different hosts will allow robustness and use of the bioscintillator under different environmental conditions**
- **Pigments present in algae will serve additional indicators of radiation at higher doses**
- **Co-design of “bioware” and hardware will provide ideal integration of the systems**

# OUR PRIOR EXPERIENCE

- **First-hand knowledge and experience with expression of different genetic reporter systems**
- **Extensive experience in development and use of genetic manipulation systems in Gram(-), Gram(+) microorganisms, prokaryotic and eukaryotic algae, yeast, and mammalian cells**
- **Extensive experience in design of electronic systems for amplification and monitoring of bioluminescence**

# RESEARCH TASKS

- Engineer plasmids with *gfp* reporter downstream from *recA* and *umuC* promoters.
- Transform plasmids into *Staphylococcus*, *Anabaena* sp. (cyanobacterium), *Chlamydomonas* (green alga), and yeast
- Analyze response and survivability of the recombinant organisms to ionizing radiation between 0.1 - 1,000 Gy
- Analyze contribution of oxidative damage response by comparing responses to radiation under aerobic and anaerobic conditions
- Design the system for amplification and monitoring of the signal
- Test the prototype

# BUDGET AND TIMEFRAME

- **Total request, depending on the combination of the tasks, will be \$1.6 - 2.1M**
- **We will be able to complete this research and demonstrate the prototype in 24 - 27 months**
- **Collaborating scientists: Drs. Eli Greenbaum (CTD), Tim McKnight (I&C), Gary Saylor (UT), Thomas Seed (AFRRI)**