

Office of Biological and Environmental Research Biological Systems Science Division Update

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## **Update on Programmatic Activities**

## Completed Reviews/PI Meetings

- ✓ GSP/BSP PI Meetings (This week!)
- ✓ eBERLight review at the Advanced Photon Source (APS)
- ✓ LANL Microbiome SFA
- FOA and Lab Funding Opportunities
  - Early Career Program (FOA and LAB) posted 11/16/22
  - Integrative Computational Tools for Systems Biology Research (FOA) posted 12/1/22
  - Funding for Accelerated, Inclusive Research (FAIR) (FOA) posted 12/15/22
  - Reaching a New Energy Sciences Workforce (RENEW) FOA posted 1/9/23
  - Energy Earthshot Research Centers (LAB) posted 1/18/23
  - Biopreparedness Research Virtual Environment (BRaVE) (LAB) posted 1/24/23
  - Accelerate Innovations in Emerging Technologies (LAB) posted 2/16/23
  - Science Foundations for Energy Earthshots (FOA) posted 3/21/23

## **External Activities**

- ✓ E.O. on Biotechnology and Biomanufacturing Sec 3 reports
- Measuring the Bioeconomy (ongoing)
- Data for the Bioeconomy (ongoing)



#### Workshop/Reports Updates

## Artificial Intelligence/Machine Learning (AI/ML) for BioEnergy Research (AMBER)



Report is complete BERAC Update from Huimin Zhao – workshop chair See QR code in handout

#### Sept 12, 2022 Executive Order Reporting

Bold-Goals-for-U.S.-Biotechnology-and-Biomanufacturing-Harnessing-Research-and-Development-To-Further-Societal-Goals-FINAL.pdf





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## Genomes to Structure and Function Workshop Report

Report is complete and available at : <u>BER Structural Biology Portal</u> **New** Overcoming Barriers in Plant Transformation: A Focus on Bioenergy Crops

Planned for mid-Sept 2023 Chair: Wayne Parrott (UGA)

Apr 2023 BERAC Meeting

**BSSD Updates** 



## E.O. Reports: A Compilation of Five Reports Addressing:

Climate Change Solutions Food and Agricultural Innovation Supply Chain Resilience Human Health Cross-Cutting Advances







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BSSD Updates

## Ten Bold Goals for Climate Change Solutions

## **Transportation and Stationary Fuels**

**Goal 1.1: Expand Feedstock Availability** – In 20 years, collect and process 1.2 billion metric tons of conversion-ready, purpose-grown plants and waste-derived feedstocks and utilize >60 million metric tons of exhaust gas CO2 suitable for con version to fuels and products, while minimizing emissions, water use, habitat conversion and other sustainability challenges.

**Goal 1.2:** Produce Sustainable Aviation Fuel (SAF) – In 7 years, produce 3 billion gallons of SAF with at least 50% (stretch 70%) reduction in GHG lifecycle emissions relative to conventional aviation fuels, with production rising to 35 billion gallons in 2050.

**Goal 1.3: Develop Other Strategic Fuels** – In 20 years, develop technologies to replace 50% (>15 billion gallons) of maritime fuel, off-road vehicle fuel, and rail fuel with low net GHG emission fuels.

## **Chemicals and Materials**

**Goal 2.1: Develop Low-Carbon-Intensity Chemicals and Materials** — In 5 years, produce >20 commercially viable bioproducts with >70% reduced lifecycle GHG emissions over current production practices.

**Goal 2.2: Spur a Circular Economy for Materials** – In 20 years, demonstrate and deploy costeffective and sustainable routes to convert bio-based feedstocks into recyclable-by-design polymers that can displace >90% of today's plastics and other commercial polymers at scale.











## Ten Bold Goals for Climate Change Solutions Cont'd

## **Climate-Focused Agricultural Systems and Plants**

**Goal 3.1: Develop Measurement Tools for Robust Feedstock Production Systems** – In 5 years, develop new tools for measurement of carbon and nutrient fluxes in agricultural and bioeconomy feedstock systems that contribute to a national framework.

**Goal 3.2: Engineer Better Feedstock Plants** – In 5 years, engineer plants and manipulate plant microbiomes to produce drought tolerant feedstocks capable of growing on underutilized land with >20% improvement in nitrogen and phosphorus use efficiency.

**Goal 3.3: Engineer Circular Food Protein Production Systems** – In 5 years, demonstrate viable pathways to produce protein for food consumption including from biomass, waste and CO2 that achieve >50% lifecycle GHG emissions reduction and cost parity relative to current production methods.

## Carbon Dioxide (CO2) Removal

**Goal 4.1: Develop Landscape-Scale Biotechnology Solutions** – In 10 years, develop technologies to expand implementation of landscape-scale soil carbon sequestration and management techniques on tens of millions of acres, increasing soil health and drought resilience and supporting U.S. climate targets.

**Goal 4.2: Enable Biomass with Carbon Removal and Storage (BiCRS)** – In 9 years, demonstrate durable, scalable biomass CO2 removal for <\$100/net metric ton, on a path to enabling gigaton-scale removal.





#### March 28-29, 2022





**DOE Energy Earthshots** 

## **DOE Energy Earthshots Initiative**

**Energy-Earthshots-Initiative** 

Hydrogen Shot<sup>™</sup> Long Duration Storage Shot<sup>™</sup> Carbon Negative Shot<sup>™</sup> Enhanced Geothermal Shot<sup>™</sup> Floating Offshore Wind Shot<sup>™</sup> Industrial Heat Shot<sup>™</sup>



RD&D to remove CO2 from the atmosphere

- biological sequestration
- abiotic sequestration
- fundamental reactions/kinetics
- measurement/validation

*Efforts at DOE Labs - Energy Earthshot Research Centers and within the Academic communities via a separate FOA*  RD&D to reduce carbon intensity of Industrial processes

- reduce carbon footprint of heating
- alternative to thermal heating
- heat recovery and use



## Bioenergy Crops Reduce Carbon Emissions – If Grown on the Right Land



#### Background/Objective

• Estimate the global warming intensity (GWI) and greenhouse gas mitigation potential of biofuel produced from switchgrass grown on marginal land

#### Approach

 Modeled crop yields and soil organic carbon levels resulting from planting switchgrass on recently-tilled abandoned farm fields and other marginal lands in Michigan grown with and without fertilizer.

#### Results

A field of switchgrass at Michigan State University's Kellogg Biological Station.

• These lands could produce more than 150 million gallons of biofuels from nitrogen-fertilized switchgrass, of which 96% meet the Renewable Fuel Standard and 73%–75% are carbon negative. However, carbon storage decreases in carbon-rich soils like wetlands, resulting in biofuel with higher GWI than gasoline.

#### Significance/Impacts

• This research highlights the strong climate mitigation potential of biofuel derived from switchgrass grown on marginal lands as well as the need to avoid planting in carbon-rich soils.

Kim, S., *et al.* Global warming intensity of biofuel derived from switchgrass grown on marginal land in Michigan. *GCB Bioenergy* (2023). [DOI: 10.1111/gcbb.13024]



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**BSSD Updates** 

## Transformation and Gene Editing in the Bioenergy Grass Miscanthus

#### Background

This report demonstrates, for the first time, that the genomes of three *Miscanthus* species can be edited via the CRISPR/Cas9 system.

#### Approach

Using sequence information from both *Miscanthus* and sorghum, orthologs of maize *lw1* were identified. Embryogenic calli of *M. sacchariflorus*, *M. sinensis* and *M. x giganteus* were transformed via particle bombardment (biolistics) or *Agrobacterium tumefaciens* introducing the *Cas9* gene and three gRNAs to edit *lw1*.

#### Results

Gene editing procedures for *Miscanthus* were developed using CRISPR/Cas9 enabling the mutation of a specific (targeted) endogenous gene to knock out its function. Leaves on edited *Miscanthus* plants displayed the same phenotypes noted in *lw1*-edited maize (leaves were chlorotic and/or striped), and Sanger sequencing confirmed editing.

#### Significance/Impacts

**CABBI developed procedures for gene editing via CRISPR/Cas9 in** *Miscanthus* **for the first time.** This included five genotypes representing three *Miscanthus* species. The designed gRNAs targeted all copies of *lw1* (homeologous copies and their alleles); results also confirmed *lw1* made a good editing target in species other than *Z. mays*. The ability to target specific loci to enable endogenous gene editing presents a new avenue for genetic improvement of this important biomass crop.

Trieu, A., Belaffif, M., Hirannaiah, P., Manjunatha, S., Wood, R., Bathula, Y., Billingsley, R.L., Arpan, A., Sacks, E.J., Clemente, T.E., Moose, S.P., Reichert, N.A., Swaminathan, K. Dec 28, 2022. "Transformation and Gene Editing in the Bioenergy Grass Miscanthus." *Biotechnology for Biofuels and Bioproducts* 15 (148). <u>https://doi.org/10.1186/s13068-022-02241-8</u>.





Leaf phenotypes in five *Miscanthus* genotypes transformed with gene editing vector pHA194.



## Advanced One-pot Deconstruction and Valorization of Lignocellulosic Biomass into Triacetic Acid Lactone Using Rhodosporidium toruloides

#### Background

- Lignocellulosic biofuels can be more profitable if produced along with valuable bioproducts such as triacetic acid lactone (TAL)
- TAL is capable of undergoing chemical conversion to sorbic acid, fungicides, and valuable chemicals such as resorcinol, phloroglucinol, and 1,3,5-trihydroxybenezene. Other applications include polymers, plasticizers, organic synthesis, adhesives and emulsifiers.

#### Approach

The yeast *Rhodosporidium toruloides* was engineered to produce TAL from lignocellulose. Both field dried and ensiled sorghum were tested as feedstocks.

#### Results

We found the *R. toruoides* can produce TAL and that it was able to make more TAL (almost 4 g/L) from hydrolysates of ensiled sorghum relatives to the dried sorghum.

#### Significance/Impacts

We demonstrate that *R. toruloides* is an excellent host for production of a range of biofuels and bioproducts, and that list now included TAL. This works moves us closer to the realization of economical production of biofuels and bioproducts.









## Engineering Auxotrophic Agrobacterium Improves **Plant Transformation**

#### **Objective**

• Develop more effective ways to control growth of *Agrobacterium* growth during plant transformation.

#### Approach

- Agrobacterium tumefaciens strains auxotrophic for methionine (EHA105<sub>met</sub> and LBA4404<sub>met</sub>) were engineered via homologous recombination as an alternative counter-selective agent to cure plants of the bacterium.
- Switchgrass transformation was chosen to assess the performance of the engineered auxotrophic Agrobacterium.

#### Results

- •The auxotrophic strains minimized the use of antibiotics and exhibited the same transformation frequency as the parental strains.
- •Auxotrophy has no effect on the number of T-DNA copies delivered by any of the Agrobacterium strains evaluated.

#### Significance/Impacts

• Engineering auxotrophic strains eliminates the use of antibiotics while preventing bacterial overgrowth on plants. Furthermore, use of the engineered strains permits for the biological containment of bacteria carrying transgenes.

Prías-Blanco M et al. Transgenic Research. 2022.; doi.org/10.1007/s11248-022-00328-4





Agrobacterium growth in co-cultivation with switchgrass tissue.

EHA105	EHA105 <sub>met</sub>	LBA4404	LBA4404 <sub>met</sub>
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GUS staining in a switchgrass Performer 7 callus including all Agrobacterium strains used in this study at five days of co-cultivation.



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**BSSD Updates** 

#### **ORNL Plant Microbe Interfaces**

## Salicin Attracts Multiple Bacteria to the Poplar Microbiome

#### **Objective**

 Populus produces large quantities of phenolic glycosides (PGs), such as salicin. There is limited data on the function of PGs in shaping the microbiome. The PMI team designed experiments to understand the molecular mechanism of salicin degradation and its role(s) in the polar microbiome.

#### Approach

- Genomics, proteomics, and metabolomics elucidated the relevant degradation pathways in the two organisms.
- Team identified Rahnella can grow by utilizing the glucosyl moiety of salicin, and the secreted salicyl alcohol can be subsequently utilized by a Pseudomonas strain.
- Metabolic use of salicin between *Rahnella* and *Pseudomonas* results in a unidirectional cross-feeding relationship.

#### Result/Impacts

 These results suggest the potential for multi-trophic interactions occurring between rhizosphere microbiota and that these interactions may be important for recruitment and structuring of the *Populus* microbiome.



Proposed mechanism of salicin metabolism by a bacterial co-culture. In the Populus-Rahnella-Pseudomonas model, Rahnella can utilize salicin produced by Populus and releases the salicyl alcohol as a by-product. Salicyl alcohol is then utilized by Pseudomonas.

Dahal S., et al., **Metabolites.** (2023). [DOI: 10.3390/metabo13020140]



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#### Environmental Microbiome

### Temperature and Nutrient Availability Impact Microbial Food Webs





Objective

- Understand the combined effect of changes in nutrients and temperatures on microbial food webs
- Model the phenotypic changes that occur across trophic levels

#### Approach

- Set up artificial microbial food web composed of complex bacterial communities, a bacterivorous protist (*Tetrahymena pyriformis*), and an omnivorous top predator (*Euplotes sp.*)
- Conducted time series studies by varying temperature and nutrients
- Used statistical modeling to characterize ecological dynamics

#### Result/Impacts

- Interacting effects of temperature and nutrients on food webs are more common at higher trophic levels
- Temperature and nutrients interact to shift the relative strength of top-down versus bottom-up control
- Rapid phenotypic change mediates responses to changes in temperature and nutrients.
- The work will lead to better prediction of trophic level interactions. This will improve ecosystem model at all levels.

Han Z-Y, Wieczynski DJ, Yammine A, Gibert JP. 2023 Temperature and nutrients drive eco-phenotypic dynamics in a microbial food web. **Proc. R. Soc. B** 290: 20222263. https://doi.org/10.1098/rspb.2022.2263

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nutrient

#### Secure Bioystems Design

## Engineering a bacteriophage for precision editing of its hosts within a mixed community

#### Objective

Develop a phage-based DNA delivery technology for specific and efficient microbial gene editing within the context of a microbial community *in situ*.

#### Approach

A single-step engineering approach using a modified CRSIPR-Cas system and a DNA repair template was developed to introduce a base editor into the lambda phage. The engineered phage would deliver the machinery for precise C-to-T base conversions within the host genome in the context of a microbial community.

#### *Result/Impacts*

- The phage-based delivery system inactivated multiple chromosome- and plasmidencoded target genes within the host by introducing stop codons through base editing.
- The research demonstrated the potential of phages for precise genome editing within a microbial community context in a fabricated ecosystem (EcoFAB) device that recapitulates a soil ecosystem.





Nethery et al., PNAS 119, e2206744119 (2022)



<u>Sustainability</u> <u>Research</u>

## QTL and Candidate Genes Controlling Oilseed Traits are Identified in Camelia

#### Objective

The Chaofu Lu lab has designed experiments to better understand oilseed yield and nitrogen use efficiency in camelina.

#### Approach

- A collection of 222 *Camelina sativa* accessions were resequenced for their genomes in collaboration with the DOE Genome Joint Institute. These populations were grown in field for two consecutive years under two nitrogen levels. The population structure of the camelina accessions was determined using 161,301 SNPs.
- Genome wide association studies complemented with linkage mapping using a recombinant inbred line population have identified quantitative trait loci (QTLs) and candidate genes controlling important oilseed traits such as seed size, fatty acid composition, and flowering time.
- Soil samples were collected from 33 camelina growing sites to analyze microbiomes. Screening and testing for key plant growth microbes are underway. Soil microbes are an important factor influencing the absorbance and utilization of nitrogen.

#### *Result/Impacts*

Results from this project will provide tools for the development of high-yielding camelina that requires minimum input of fertilizers.

Li et al. (2021). The Plant Genome DOI: 10.1002/tpg2.20110





Camelina seed and oil yields are determined by traits such as plant biomass, seeds per plant, seed size and oil content, which are genetically controlled and vary in natural populations. Soil nutrients and microbes may also affect plant growth and yield.



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**BSSD Updates** 

## Quantum Sensing and Imaging

*Quantum Sensing: High throughput single-molecule detection of protein or viral RNA in crude bio-samples* 

# Flow chamber

Nanodiamond Quantum Sensors

- Nitrogen-Valency quantum sensor in nanodiamonds
- Reduced costs, No purification
- Insensitive to non-specific binding

DOE Quantum Information Science Center, Q-NEXT Peter Maurer, David Awschalom, Argonne Labs and U. Chicago Commercial collaboration with SomaLogic, Stoll & Jayich Lab

#### Q-NEXT is Co-Funded with Basic Energy Sciences and Advanced Scientific Computing Research

#### Quantum Imaging: Million-fold lower illumination is sufficient, providing damage free images

#### Cells imaged with entangled photons or classical photons.



Entangled Photons - a low-light beam detects sample while second, correlated beam is measured by camera

#### BER Bioimaging Award to T. Goodson III, U. of Michigan





## Busting the Unbreakable Lignin



Removing lignin to produce fuels from the cellulose and hemicellulose in plant cell walls is a resource-intensive process. Lignin breakdown by anaerobic fungi was thought to be impossible, yet chemical clues had researchers wondering if that was true. They tested rumen fungi that can efficiently produce enzymes that get through lignin to use the other two components.

#### Approach

A UC Santa Barbara team worked with researchers at the DOE Joint Genome Institute, the Joint BioEnergy Institute and the Great Lakes Bioenergy Research Center. Anaerobic fungi were cultivated on feedstock crop samples in an oxygen-free environment. The JGI conducted RNA sequencing and alignment to the reference fungal genome. JGI data portals and pipelines also helped identify potential genes responsible for lignin deconstruction. NMR data was generated at JBEI and analyzed by the GLBRC.

#### *Result/Impacts*

- First real irrefutable proof that lignin can be deconstructed by fungi in the absence of oxygen.
- Using NMR and other advanced imaging techniques, the team identified specific lignin bond breakages caused by the anaerobic fungi.





**Top:** Scanning electron micrograph of the fungus *Neocallimastix californiae* (Chuck Smallwood/PNNL.) **Bottom**: Art by Bianca Susara/Berkeley Lab



Publication: Lankiewicz TS. *et al.* "Lignin deconstruction by anaerobic fungi." Nature Microbiology (2023). doi: 10.1038/s41564-023-01336-8





## 98 JGI Publications October 1, 2022-March 24, 2023

#### Selected Highlights

- Genome of *Paspalum vaginatum* and the role of trehalose mediated autophagy in increasing maize biomass. *Nature Communications*
- Hyperactive nanobacteria with host-dependent traits pervade Omnitrophota. *Nature Microbiology*
- Multiomics in the central Arctic Ocean for benchmarking biodiversity change. PLOS Biology
- Substrate availability and not thermal acclimation controls microbial temperature sensitivity response to long-term warming. *Global Change Biology*
- Lignin deconstruction by anaerobic fungi. *Nature Microbiology*
- Mining metatranscriptomes reveals a vast world of viroid-like circular RNAs. *Cell*
- The IMG/M data management and analysis system v.7: content updates and new features. *Nucleic Acids Research*
- Twenty-five years of Genomes OnLine Database (GOLD): data updates and new features in v.9. *Nucleic Acids Research*

#### JGI News Releases and Highlights:

jgi.doe.gov/news-publications

Office of

Science

jgi.doe.gov/category/science-highlights



IMG/M い・い

TEGRATED MICROBIAL GENOMES & MICROBIOME







## Honoring the Structural Biology Center (SBC) and Andrzej Joachimiak

- Dr. Andrzej Joachimiak has led SBC since September 1997 26 years!
- SBC is a macromolecular crystallography sector at the Advanced Photon Source (APS) with 2 beamlines (19-ID and 19-BM); and advanced protein characterization facility.
- 19-ID is among the most productive macromolecular crystallography beamlines in the world.
- SBC has served the entire biological community with astounding productivity:
  - 2,782 unique users
  - 3,256 projects
  - 6,381 structures deposited in PDB
  - 2,805 publications
  - H-index 217

# SBC will collect its last data set on April 17 as the APS goes "dark" for a year-long shut-down necessary for the facility upgrade







## Thank you

https://science.osti.gov/ber

https://www.energy.gov/science/ber/biological-andenvironmental-research



## DOE Systems Biology Knowledgebase

**Objective:** Integrate and share metagenome analysis tools, data types, and execution capabilities into KBase to accelerate the discovery of microbial genomes and uncover the genetic potential of microbial communities.

#### Results/Impacts

- Chivian, D. *et al.* Metagenome-assembled genome extraction and analysis from microbiomes using KBase. *Nature Protocols* 18 (2022). DOI:10.1038/s41596-022-00747-x
- Integrated microbiome tools CheckM, DRAM, CONCOCT, and GTDB-tk into KBase, alongside DOE developed tools like MetaBAT and GOTTCHA2
- New pipeline integrated within a broader set of KBase tools designed to facilitate mechanistic understanding of complete microbiomes, including: metabolic modeling, phenotype prediction, multi-omics integration, and cheminformatics

#### **KBase User Publications**

- Heinken A, et al. Genome-scale metabolic reconstruction of 7,302 human microorganisms for personalized medicine. Nature Biotechnology. 2023; 1–12. doi:10.1038/s41587-022-01628-0
- Rosconi F, et al. A bacterial pan-genome makes gene essentiality strain-dependent and evolvable. Nature Microbiology. 2022;7: 1580–1592. doi:10.1038/s41564-022-01208-7
- Keller-Costa T, et al. Metagenomics-resolved genomics provides novel insights into chitin turnover, metabolic specialization, and niche partitioning in the octocoral microbiome. Microbiome. 2022;10: 151. doi:<u>10.1186/s40168-022-01343-7</u> DOE Funded Research
- AminiTabrizi R, et al. Microbial sensitivity to temperature and sulfate deposition modulates greenhouse gas emissions from peat soils. Global Change Biology. 2023;29: 1951–1970. doi:10.1111/gcb.16614 DOE Funded Research







**Enabling Capabilities** 

## National Microbiome Data Collaborative (NMDC)

**Objective:** Connect data, people, and ideas to advance microbiome innovation and discovery.

Submission Portal Data Portal

NMDC EDGE

**Approach:** Support a FAIR microbiome data sharing network, through <u>infrastructure</u>, <u>data standards</u>, and <u>community building</u>, that addresses pressing challenges in environmental sciences.

#### Result & Impacts:

- Increased data in the Data Portal
- New workflows available in NMDC EDGE: metaproteomics and plasmid/virus detection
- New features in the Submission Portal & support for JGI and EMSL user projects
- Onboarded new Ambassador cohort, SAB, and support 50 Champions
- New publication! Cohort-based learning for microbiome research community standards, 2023 Nature Microbiology (accepted)







Apr 2023 BERAC Meeting

**BSSD** Updates

## **Background on DOE's Previous Low Dose Radiation Research Program**

- ➢ Managed in DOE-BER for 18 years (1998 − 2016)
- > Extension of DOE's historical radiobiology efforts extending back decades
  - Focused on doses below 100mSv
- Research performed in university and DOE laboratory-led projects
- A focus of technology development (ex. microbeam technology)
- Some fundamental discoveries:
  - New insights to the "hit model", bystander effect, Reactive Oxygen Species(ROS) impacts, adaptive response
- > Ended in 2016 as the DOE-BER portfolio shifted towards bioenergy and environment
  - Some good insights into the effects of low dose radiation on cellular function
  - No impact on radiation protection regulations or ameliorating public fear of radiation/nuclear power



## Congressional Interest in Restoring a Low Dose Research Program in BER

- There has been a concerted lobbying effort in Congress to restore low dose radiation research in BER since 2016.
- > Congress has continued to press DOE to reinstate a low dose radiation research program
- > Congress directed BER to fund a NASEM Strategic Plan that was completed in June 2022
- Congress has appropriated funds in FY20-23 (5-8M each year). These funds have been used to supplement an existing DOE-NCI computational effort to adapt AI/ML techniques for advancing cancer research, to the challenge of low dose radiation exposure.

The FY24 President's Budget includes \$10M for low dose research. This is first time since 2016 that funds for low dose research have been requested by DOE.

