MIT CEHS NEWSLETTER

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Massachusetts Institute of Technology
CENTER FOR ENVIRONMENTAL HEALTH SCIENCES

Spring/Summer 2014

DNA Repair Goes Technicolor

Test analyzing cells' ability to fix different kinds of broken DNA could help doctors predict cancer risks

Article by Anne Trafton, MIT News Offices

Our DNA is under constant attack from many sources, including environmental pollutants, ultraviolet light, and radiation. Fortunately, cells have several major DNA repair systems that can fix this damage, which may lead to cancer and other diseases if not mended.

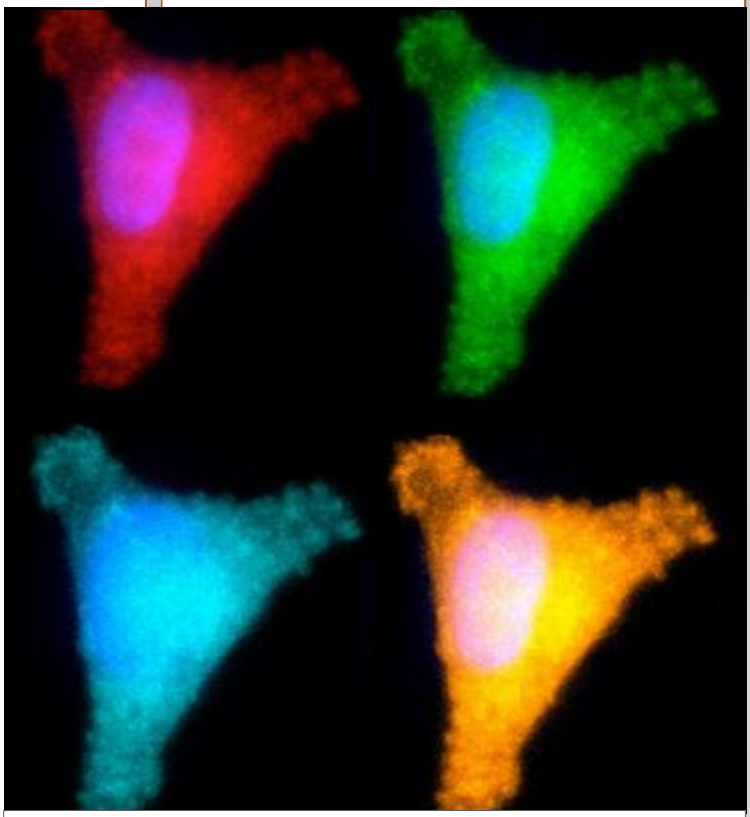
The effectiveness of these repair systems varies greatly from person to person; scientists believe that this variability may explain why some people get cancer while others exposed to similar DNA-damaging agents do not. A team of MIT researchers has now developed a test that can rapidly assess several of these repair systems, which could help determine individuals' risk of developing cancer and help doctors predict how a given patient will respond to chemotherapy drugs.

The new test, described in the *Proceedings of the National Academy of Sciences* the week of April 21, can analyze four types of DNA repair capacity simultaneously, in less than 24 hours. Previous tests have been able to evaluate only one system at a time.

"All of the repair pathways work differently, and the existing technology to measure each of those pathways is very different for each one. It takes expertise, it's time-consuming, and it's labor-intensive," says Zachary Nagel, an MIT postdoc and lead author of the *PNAS* paper. "What we wanted to do was come up with one way of measuring all DNA repair pathways at the same time so you have a single readout that's easy to measure."

The research team, led by professor Leona Samson, used this approach to measure DNA repair in a type of immortalized human blood cells called lymphoblastoid cells, taken from 24 healthy people. They found a huge range of variability, especially in one repair system where some people's cells were more than 10 times more efficient than others.

"None of the cells came out looking the same. They each have their own spectrum of what they can repair well and what they don't repair well. It's like a fingerprint for each person," says Samson, who is the Uncas and Helen Whitaker Professor, an American Cancer Society Professor, and a member of MIT's departments of biological engineering and of biology, Center for Environmental Health Sciences, and Koch Institute for Integrative Cancer Research.



MIT biological engineers have developed a way to test several different DNA repair pathways on a cell by cell basis, millions per minute. In each of these images, the cell is producing a different colored fluorescent protein where each color indicates whether or not a specific DNA lesion has been repaired.

Image: Aprotim Mazumder

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UPCOMING EVENTS

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CEHS RETREAT TO BE HELD ON AUGUST 27, 2014

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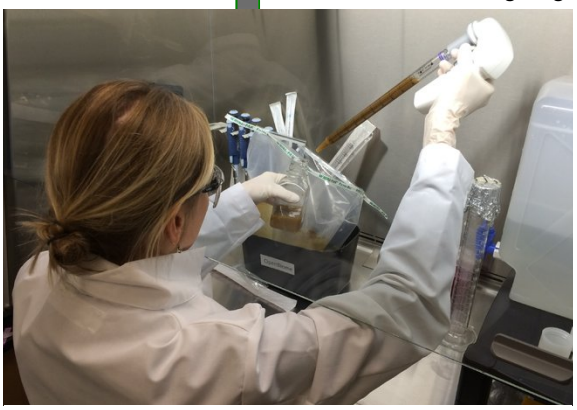
A New Kind of Transplant Bank

Article published by Peter Andrey Smith at The New York Times on February 17, 2014
http://www.nytimes.com/2014/02/18/health/a-new-kind-of-transplant-bank.html?_r=0

CAMBRIDGE, MASS. — Around noon on a recent Friday, Donor Five, a healthy 31-year-old, walked across M.I.T.'s frigid, wind-swept campus to a third-floor restroom to make a contribution to public health.

Less than two hours later, a technician blended the donor's stool into preparations that looked like chocolate milk. The material was separated and stored in freezers at an M.I.T. microbiology lab, awaiting shipment to hospitals around the country. Each container was carefully labeled: Fecal Microbiota Preparation.



Eliska Didyk stores a human stool sample in OpenBiome's laboratory facilities at MIT. **Image**: Carolyn Edelstein

Nearly a year ago, Mark Smith, a 27-year-old doctoral candidate, and three colleagues launched OpenBiome, the nation's first human stool bank. Its mission: to provide doctors with safe, inexpensive fecal material from screened donors to treat patients with Clostridium difficile, a gastrointestinal infection that kills at least 14,000 Americans a year.

"People are dying, and it's crazy because we know what the solution is," Mr. Smith said. "People are doing fecal transplants in their basements and may not be doing any of the right screening or sterile preparation. We need an intermediate solution until there are commercial products on the market."

C. diff, as it's known, resides among trillions of other bacteria in normal, healthy humans. When antibiotics wipe out the competition, the bacteria can produce toxins, causing persistent diarrhea. The bacteria are increasingly resistant to conventional treatments. But researchers have discovered an alternative: A donor's stool can be transplanted in the intestine or colon of a sick patient via an enema, colonoscopy or nasogastric tube. The healthy bacteria fight off C. diff and re-establish a normal community in the gut.

A study published last year in The New England Journal of Medicine found that fecal transplants were nearly twice as effective as antibiotics in treating patients with recurring C. difficile.

But where to get healthy donor stool? For doctors, it's a tedious, time-consuming process, and some patients turn awkwardly to relatives or friends. Since September, OpenBiome has delivered more than 135 frozen, ready-to-use preparations to 13 hospitals. The nonprofit project fields dozens of requests from doctors, hospitals and patients every week. (The preparations are not sent directly to patients.)

Carol Capps, 75, a retired nurse in Clemmons, N.C., had been in and out of hospitals for months with a C. diff infection that was not going away despite multiple courses of antibiotics. After a recurrence, her doctor suggested OpenBiome, and she received a fecal transplant. By that afternoon, Ms. Capps said, she felt like a new person and has been healthy since. "I am just thankful for whoever donates," she said. "It's such a miracle."

Despite the promise, the Food and Drug Administration has grappled with the regulation of fecal transplants. In early 2013, the agency announced that it would treat them as biologic drugs requiring an Investigational New Drug application, which typically precedes a clinical trial.

Following outcry from patients and doctors, the F.D.A. announced it would "exercise enforcement discretion" — in effect, saying that it would not go after physicians performing fecal transplants for C. diff. Jennifer Rodriguez, an F.D.A. spokeswoman, said the agency planned to issue industry guidelines later this year.

Because of the legal ambiguity, some researchers are not preparing fecal microbiota for sale (usually at cost) — including Dr. Alexander Khoruts, a gastroenterologist at the University Minnesota, who first published the methodology for doing so.

Dr. Khoruts worries that approval of OpenBiome's efforts will slow the development of next-generation therapeutics beyond the crude preparations available today. "We desperately need some clarity," Dr. Khoruts said. "We're going to be stuck with the OpenBiome model, and nothing better's going to come along."

At the same time, Mr. Smith and Eric J. Alm, an M.I.T. microbiologist and adviser to OpenBiome, said the F.D.A.'s classification of fecal transplants as drugs hinders research into their possible uses to treat inflammatory bowel diseases and obesity.

"It's going to give a monopoly to whatever company gets the drug approved," Mr. Smith said.

"We think it should be regulated, but unlike most products the F.D.A. oversees, there's a real risk of the black market," he said. "If you restrict access, there's going to be lots of people doing it underground."

NEWS ARTICLE

Seeing is Believing: Detection of Rare DNA Sequence Changes by Fluorescence

Homologous recombination is a critical DNA repair pathway for protecting against DNA damage-induced cytotoxicity. Although generally accurate, homologous recombination carries with it a risk for associated mutations. For example, recombination between repeat sequences can cause insertions and deletions, and recombination between homologous chromosomes can cause loss of heterozygosity (LOH). In cells that harbor one mutant and one wild type tumor suppress gene, homologous recombination is the predominant cause of LOH. Taken together, virtually all cancers harbor the finger print of one or more homologous recombination events.

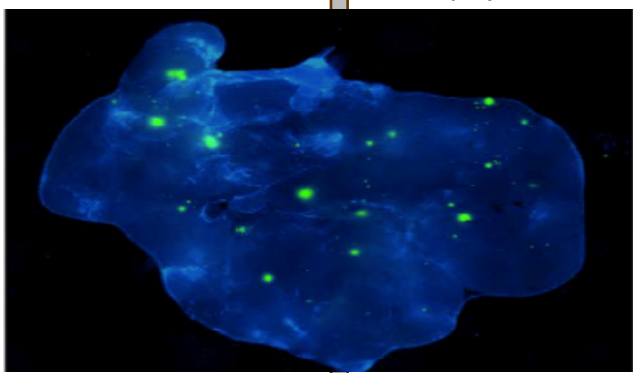


Figure 1. Fluorescent recombinant cells (some of which have undergone clonal expansion) appear as bright foci against a background of DAPI stained blue nuclei.

Given the importance of homologous recombination in driving mutations that cause cancer, the Engelward laboratory set out to develop tools to study the impact of genetic and environmental factors on homologous recombination in a mouse model. The Engelward laboratory recently developed a 'second generation recombomouse', called the Rosa26 Direct Repeat (RaDR) mouse model. The RaDR mice harbor a direct repeat wherein homologous recombination gives off a fluorescent signal, making it possible to literally see recombinant cells within intact tissues (Figure 1). Importantly, the reporter sequences were targeted to the Rosa26 locus, which has nearly ubiquitous expression, making it possible to study recombination in virtually any tissue. Michelle Sukup-Jackson, Orsolya Kiraly, Jennifer Kay and Li Na worked together to demonstrate the efficacy of the model for studying various tissues. Jennifer Kay worked on the effect of aging on recombination and Li Na developed single-cell PCR conditions that can be used to get insights into the underlying molecular mechanism of homologous recombination events. This was also a productive project for three undergraduates who are coauthors: Daniel Chow, Elizabeth Rowland, and Kelly Winther.

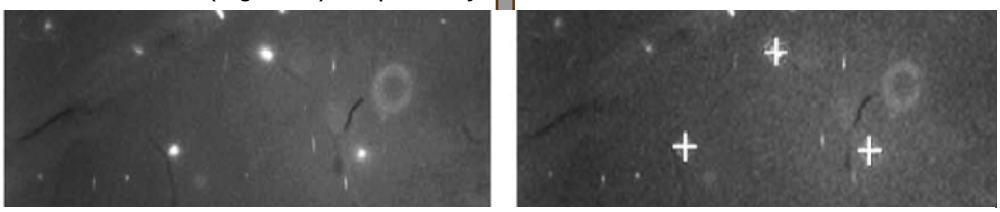


Figure 2. Software developed in the laboratories of Peter So and Jagath Rajapakse can specifically identify recombination events that occur in somatic stem cells by their size and shape following clonal expansion.

One of the major goals is to study homologous recombination in animals following exposures or as they age. It turned out that there were so many recombination events in animals exposed to a DNA damaging agent that quantification by eye became impractical. To overcome this limitation, the Engelward laboratory collaborated with Peter So's research team (Prof. So is in the Department of Mechanical and Biological Engineering at MIT, and he has a research group in Singapore as part of the MIT-Singapore Alliance for Research and Technology). Vijay Singh and Dushan Wadduwage teamed up to write software to automatically identify and quantify recombination events that are visible as fluorescent foci.

The So laboratory also worked with Jagath Rajapakse from Singapore to develop software that makes it possible to quantify homologous recombination events within somatic stem cells for first time (Figure 2). Mutations in somatic stem cells are thought to drive cancer, making this a powerful new tool for studies of cancer etiology.

This new mouse model results from fruitful collaborations among many scientists and promises to open doors to an integrated understanding of homologous recombination throughout the body and will provide a valuable tool for studies of exposure biology. Current projects include collaborations with Susan Erdman, Steve Tannenbaum, Jerry Wogan and Jim Fox to study infection/inflammation-induced sequence rearrangements in vivo.

This publication can be viewed here http://www.plosgenetics.org/article/info%3Adoi%2F10.1371%2Fjournal.pgen.1004299

CEHS News

Welcome a New Faculty Center Member

We are pleased to announce a new Center Faculty Member.

Edward Boyden, Associate Professor of Biological Engineering and Brain and Cognitive Sciences, who's research interest is in developing new technologies to enable the systematic and integrative analysis of how the environment shapes physiological processes throughout entire complex biological systems, such as the brain, or even the entire body. Please visit his website for further information of his research and publications http://syntheticneurobiology.org

MIT IS&T Announcement: Backup Services now *FREE*

Beginning July 1, 2014, MIT IS&T will roll out a new, cloud-based backup service offering: CrashPlan. Between July 2014 and June 2015, all existing TSM desktop / laptop users (almost 6000 devices!) will be migrated to the new service. As of July 1, 2014, backup services will be made available to the entire MIT community without charge as part of our ongoing effort to eliminate chargebacks and to enhance our services for the MIT community.

MIT IS&T will begin contacting existing TSM users in the month of July to coordinate their migration to the new service. New user registrations for CrashPlan will be available in a similar timeframe. Please refer to http://ist.mit.edu/backup for additional information.

It's our hope that CrashPlan will provide the community with an improved user experience for backup services and make it easier to protect your critical data. Further information on this free service can be found here http://adminconnect.mit.edu/news/ist-roll-out-new-cloud-based-backup-service-crashplan.

2014-2015 Pilot Project Call issued

Announcement of award recipients to be released in mid-July 2014.

CEHS

Submission Deadline:
May 16, 2014

Anticipated Start Date:
July 1, 2014

Questions regarding the application process should be directed to:

Professor John M. Essigmann, Director (jessig@mit.edu) or Professor Bevin P. Engelward, Deputy Director (bevin@mit.edu)

Questions of an administrative or financial nature should be directed to:

Amanda Tat
Administrative Officer
(atat@mit.edu).

Check our website for further details:
http://cehs.mit.edu/pilot.html

THE M.I.T. CENTER FOR ENVIRONMENTAL HEALTH SCIENCES

Call for Pilot Project Proposals in Basic Research &Translational Research

The MIT Center for Environmental Health Sciences (CEHS), funded by the National Institute of Environmental Health Sciences (NIEHS), invites MIT faculty and research staff with PI privileges to submit applications for funding of Pilot Projects related to environmental health, to support either basic or translational research. **The Center anticipates funding six (6) pilot projects at $25,000 (direct costs) for each project.** Basic Research is self-explanatory. Translational Pilot Projects should be designed to move basic/fundamental research closer to its application to prevent or treat human diseases caused by environmental exposures. This definition spans a range of research activities, from applying a method or device to cell- or animal-based models of human disease, to initiating a study involving human tissue samples. We encourage proposals involving collaborations with clinicians or other interdisciplinary collaborations, for example between engineers and animal model experts, that move environmental health science closer to human applications. In all cases, the trajectory to human application must be clear and feasible.

The Pilot Program seeks to:

- Provide initial support for new investigators to establish research in the area of environmental health.
- Allow established investigators to explore innovative new directions in environmental health research representing a significant departure from ongoing funded research.
- Stimulate investigators from diverse fields of endeavor to apply their expertise to environmental health research.
- Encourage and foster multi-disciplinary research collaborations.
- Provide an opportunity for investigators to move their basic research to the translational level.

Proposal Guidelines:

Applicants should submit a four-page proposal that outlines the Specific Aims and Research Strategy (Significance, Innovation, and Approach). Please distinguish in the project title whether your pilot project application is "Basic Research" or "Translational Research".

Applications should include a detailed budget (Form Page 4) and budget justification along with an NIH biographical sketch, using the PHS398 forms.
(http://grants.nih.gov/grants/funding/phs398/phs398.html)

Completed applications should be submitted to: Ms. Amanda Tat, Administrative Officer of the CEHS via email (atat@mit.edu).

CEHS 2014 Poster Session Winners

The Center for Environmental Health Sciences (CEHS) at MIT held its annual poster session on May 9, 2014 at the Morss Hall, Walker Memorial Building (50-140). The session highlighted the work of the environmental health research communities of MIT and some of our sister institutions. Sixty posters were presented from the science and engineering laboratories affiliated with the Center. We would like to thank all of the poster presenters for participating in this event.

The CEHS has an overall mission to study the biological effects of exposure to environmental agents in order to understand, and predict, how such exposures affect human health. Moreover, by uncovering the chemical, biochemical and genetic bases for environmental disease, sometimes we are able to leverage that understanding to delay or even prevent the development of disease in human populations. To that end, the center brings together 35 MIT faculty members from a total of nine MIT departments (in both the School of Science and the School of Engineering) plus two Harvard faculty members; from the Harvard School of Public Health (HSPH) and the Harvard Medical School affiliated hospital (Massachusetts General Hospital).

This year's CEHS cash prizes were increased significant which are awarded in two categories, graduate students and postdoctoral scholars. For each category, the prize for first-place was $1,000, second-place prize was $500, and the third-place prize was both $200 and CEHS memorabilia. The cash prizes were made possible by the Myriam Marcelle Znaty Research Fund, which was established nearly 30 years ago to support the research of young scientists at MIT.

Graduate Students and Postdoctoral Scholars presented the results of their research at MIT's Morss Hall. The CEHS 2014 Poster Winners are listed below.

Eric Ma of Professor Jonathan Runstadler's lab won first place in the graduate student category. Eric presented his work on the, "Development of Network-Based Methods for Identifying Reassortant Influenza Viruses." In second place was Christina Birch of Professor Jacquin Niles' lab, who presented her work on, "RNA Aptamers for Studying Pregnancy-Associated Malaria." Finally, in third place was Jing Ge of Professor Bevin P. Engelward's lab of who presented work on, "CometChip: Enabling Translation of DNA Damage and Repair Assays."

In the postdoctoral scholar category, first place went to Ravindra Kodihalli of Professor Steven R. Tannenbaum's lab who presented his work on, "Functional Proteomics of Matrix Metalloproteases in a Model of Osteoarthritis." Second place went to Christopher Corzett of Professor Martin F. Polz's lab who presented his work on, "The Ecology of Algal Polysaccharide Degradation: Characterizing Novel Fucoidan-Degrading Bacteria." And lastly, Simone Moser from Elizabeth M. Nolan's lab took third place after presenting her work on, "Probing the Effects of Human Defensin 5 on *E. Coli* Using Microscopy and a Genetic Screening Approach."

Pictures of the winners:

Graduate Student winners (from left to right): Jing Ge (3rd place), Christina Birch (2nd place), and Eric Ma (1st place).

Postdoctoral Scholar winners (from left to right): Simone Moser (3rd place), Ravindra Kodihalli (1st place), and Christopher Corzett (2nd place)

CEHS COE^{2}C HIGHLIGHTS

Five Snapshots of Recent Bidirectional Successes from CEHS COE^{2}C

By Dr. Kathleen Vandiver, CEHS COE^{2}C Director

MIT COE^{2}C is continually engaged in discussion with members of our community who are concerned about environmental health. Through an open dialog, five requests for COE^{2}C support have recently emerged. These requests were for a summer environmental health camp program for middle schoolers, a workshop on molecular genetics at a local nursing school, a PEPH webinar about climate change and air pollution for teacher professional development, an emissions briefing about Boston Logan International Airport for the NE American Lung Association, and an advisory meeting with Friends of the Malden River.

The following results demonstrate the strength of our collaborative efforts:

1) A five day camp program was developed around environmental health concepts. The program will be piloted as one of many course offerings at i2 Camp, a STEM camp that will be hosted at 19 sites across the US this summer (beginning June 16, 2014).

2) COE^{2}C delivered a hands-on molecular biology workshop at Boston College's Connor School of Nursing (September 27, 2013) and as a result, the dean of the school is coauthoring an article with us that will be submitted to the Journal of Nursing Education.

3) PEPH' s request for a teacher professional development webinar (delivered August 9, 2013) for teaching materials about climate and air pollution has led to the posting of the PEPH webinar on YouTube, helping to further disseminate NIEHS teaching tools. Furthermore, NIEHS reports that the Louisiana State University Superfund Research Translation Core is now using these materials in their local outreach programming to teach about air pollution and health.

4) COE^{2}C also facilitated two briefing sessions on the MIT campus between CEHS members and the American Lung Association of the North East (March, 2014). The discussions focused on MIT's computer models that have produced some eye-opening information about global mercury transport and early deaths attributable to airport proximity.

Lastly, 5) COE^{2}C facilitated a meeting (February 20, 2014) between the Friends of the Malden River and CEHS toxicologists. Here the discussion focused on finding the most cost effective way to obtain a new human health risk assessment for the river. This report aims to improve the public's access to the Malden River for healthy, recreational activities such as boating and nature walks in this highly urbanized area.

Resources:

i2 Camp: http://i2camp.org/

Boston College Nursing School: http://www.bc.edu/schools/son/

Youtube for the PEPH Webinar: http://www.youtube.com/watch?v=2nfzRp30dBM

Louisiana State University Superfund Research Program uses the "Understanding Air" teacher workshop materials developed by MIT CEHS: http://lsusrp.wordpress.com/2014/02/28/lsu-srp-researchtranslation-core-and-trainees-attend-the-2014-louisiana-environmental-education-symposium/

Friends of the Malden River: http://maldenriver.wordpress.com/

American Lung Association of the North East: http://www.lung.org/associations/charters/northeast/

CEHS Featured Article Continued

Continued from page 1

Measuring repair

With the new test, the MIT team can measure how well cells repair the most common DNA lesions, including single-strand breaks, double-strand breaks, mismatches, and the introduction of alkyl groups caused by pollutants such as fuel exhaust and tobacco smoke.

To achieve this, the researchers created five different circular pieces of DNA, four of which carry a specific type of DNA damage, also called DNA lesions. Each of these circular DNA strands, or plasmids, also carries a gene for a different colored fluorescent protein. In some cases, the DNA lesions prevent those genes from being expressed, so when the DNA is successfully repaired, the cell begins to produce the fluorescent protein. In others, repairing the DNA lesion turns the fluorescent gene off.

By introducing these plasmids into cells and reading the fluorescent output, scientists can determine how efficiently each kind of lesion has been repaired. In theory, more than five plasmids could go into each cell, but the researchers limited each experiment to five reporter plasmids to avoid potential overlap among colors. To overcome that limitation, the researchers are also developing an alternative tactic that involves sequencing the messenger RNA produced by cells when they copy the plasmid genes, instead of measuring fluorescence.

In this paper, the researchers tested the sequencing approach with just one type of DNA repair, but it could allow for unlimited tests at one time, and the researchers could customize the target DNA sequence to reveal information about which type of lesion the plasmid carries, as well as information about which patient's cells are being tested. This would provide the ability for many different patient samples to be tested in the same batch, making the test more cost-effective.

Making predictions

Previous studies have found that many different types of DNA repair capacity can vary greatly among apparently healthy individuals. Some of these differences have been linked with cancer vulnerability; for example, a genetic defect in a type of DNA repair called nucleotide excision repair often leads to a condition called xeroderma pigmentosum, in which DNA damage caused by ultraviolet light goes unrepaired and leads to skin cancer.

Scientists have also identified links between DNA repair and neurological, developmental, and immunological disorders, but useful predictive DNA-repair-based tests have not been developed, largely because it has been impossible to rapidly analyze several different types of DNA repair capacity at once.

Samson's lab is now working on adapting the new test so it can be used with blood samples taken from patients, allowing researchers to identify people who are at higher risk and potentially enabling prevention or earlier diagnosis of diseases linked to DNA repair. Such a test could also be used to predict patients' response to chemotherapy drugs, which often work by damaging cancer cells' DNA, or to determine how much radiation treatment a patient can tolerate.

The researchers also believe this test could be exploited to screen for new drugs that inhibit or enhance DNA repair. Inhibitors could be targeted to tumors to make them more susceptible to chemotherapy, while enhancers could help protect people who have been accidentally exposed to DNA-damaging agents, such as radiation.

Another important application for this test could be studying fundamental biological processes such as how cells recruit backup repair systems to fill in when another pathway is overwhelmed, says Samuel Wilson, a principal investigator at the National Institute of Environmental Health Sciences (NIEHS), part of the National Institutes of Health (NIH).

"There's an opportunity to use these multiplexed plasmids in biological assays where several repair pathways can be probed at the same time, offering a very advanced tool to allow us to make much better interpretations about the repair status of a cell," says Wilson, who was not part of the research team.

Graduate students Carrie Margulies and Isaac Chaim; technical assistants Siobhan McRee and Patrizia Mazzucato; and research scientists Vincent Butty, Anwaar Ahmad, Ryan Abo, and Anthony Forget also contributed to the research, which was funded by the NIH and NIEHS.

This article was also highlighted on the National Institute of Environmental Health Sciences Environmental Factor June 2014 Newsletter, which can be found via this link below: http://www.niehs.nih.gov/news/newsletter/2014/6/science-dna_repair/

This research was also highlighted on Boston Fox WFXT. That interview can e viewed here http://s3.amazonaws.com/TVEyesMediaCenter/UserContent/201799/3333182.9609/WFXT_05-08-2014_17.27.15.mp4