SP8 DNA damage assay development: Bevin P. Engelward, Biological Engineering, MIT
Funding Source and Period: NCI P01-CA026731 2014 – 2019 (Tannenbaum, PI; Engelward, Project 2 co-lead). **Associated With:** TRD1, (4)

Significance: Dr Engelward, MIT, is a leader in understanding on how DNA damages lead to carcinogenesis and other diseases. More specifically, her work often focuses on homologous recombination leading to the creation many novel technologies for detecting these rare sequence changes in vivo and to measure genomic damages in vitro(1-33). Engelward laboratory created the first transgenic model to study homologous recombination (HR) using fluorescent protein reporters (7, 26, 30, 33). This technology can be used to detect rare HR events in vivo in disease models. In the in vitro front, her group recently modernized a traditional DNA repair assay called the 'comet assay' to increase its repeatability and throughput (5, 12, 34). Furthermore, Dr. Engelward's group is working on improving the throughput of few other common in-vitro toxicology assays including the γ-H2AX assay. γ-H2AX assay is an important and commonly used technique to quantify DNA double strain breaks (DSBs; and other DNA lesions) based on an established method for labeling repair proteins. Recently, Dr. Engelward's team has also turned their attention to the relation of DNA damage and infections. Results from her new studies suggest that, following infection, DNA damage could affect the disease progression.

Approach: LBRC has long worked with Dr. Engelward on developing in-vivo and in-vitro assays for DNA damage. In the current cycle, Dr. Engelward has developed a new animal model to study rare homologous recombination (HR) in-vivo. These HR events are very rare (on the order of 1 in 10^5 or 10^6) and flow cytometry has proven effective to detect the frequency of these rare cells. However, the frequency of fluorescent cells is not just a reflection of an HR event, but depends also on the probability of clonal expansion. Therefore, the Engelward laboratory uses imaging based assays to quantify the number of HR events by identifying cell clusters that formed based on the same HR event. Freshly excised tissue is compressed between two coverslips and imaged, after which the fluorescent foci (which reflects single cells and cell clusters), are identified and counted to quantify the number of HR events. It has been identified that manual analysis of this kind can give rise to inter and/or intra rater variability. Hence, to eliminate human error, LBRC has provided Engelward laboratory automated image analysis algorithm. This approach eliminates any human bias and allow the inclusion of subtle image parameters that cannot be estimated by eye (7). Dr. Engelward's group is also developing high-throughput DNA damage quantification assays based on γ-H2AX immunolabeling. Ionizing radiation causes various types of DNA damages including DSBs. DSBs are often recognized by DNA repair protein ATM which forms γ-H2AX foci at the site of the DSBs that can be visualized using immunohistochemistry (2). Most of such experiments are of low throughput in terms of imaging and image analysis. Imaging is usually done in 2D because of speed limitation. As a result, the number of cells imaged is limited to a couple hundred. Furthermore, most laboratories use labor-intensive, imprecise, manual counting or classification and hence limited to quantifying foci only in cells with few of them (< 5); for cells with more foci, they are often grouped together only as one class. In order to overcome these limitations, Engelward laboratory utilizes LBRC technology to develop a high-throughput protocol that uses a high-speed 3D imaging and image processing pipeline. A large population of cells with highly clustered foci inside nuclei are imaged in 3D with submicron resolution (Fig. 1) using an existing LBRC high throughput 3D image cytometer based on structured light illumination. This LBRC in-house service instrument features 3D cell imaging throughput approach 1000 cells/second that is almost an order of magnitude faster than any commercial 3D image cytometer(35, 36). LBRC further provides 3D image analysis algorithms to automate counting of the number of foci per cell nucleus. Initial results suggest that while most of the other 2D imaging and manual quantification studies can count only up to about 5 foci per nucleus, LBRC approach has enable accurate counting of foci per nucleus beyond 100 at much higher throughput.

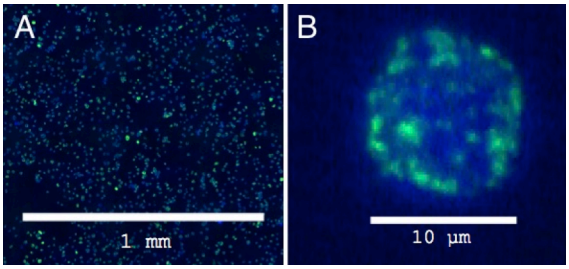

Fig. 1: (A) A depth resolved image of a cell population with g-H2AX foci. (B) An image of a representative nucleus in 'A'. Shown in green are g-H2AX foci inside the nucleus.

Center Offering: First, LBRC provides access to a high-throughput 3D imaging cytometer to image large population of cells for the next generation γ-H2AX toxicology assay. With tissue optical clearing, these approach may also work for quantifying HR in the tissue of Dr. Engelward's novel mouse models. Second, LBRC provides computational support for fully automated data analysis microscope images; these user friendly image analysis software enables LBRC service users, like Dr. Engelward and her colleagues, to quickly quantify their image data with unbiased statistics.

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