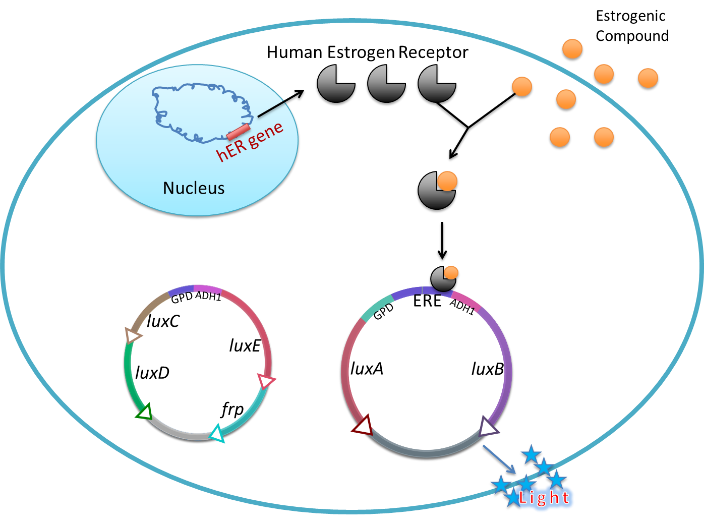
The Human estrogen receptor alpha (hERα) is a key protein responsible for sensing estrogenic ligands and regulating genes and pathways that maintain the normal growth, reproduction and functioning of various tissues and organs ([Delfosse, Grimaldi et al. 2012](#_ENREF_1)). Malfunction of the hERα gene and its corresponding transcriptional pathways has been found to contribute to many critical human health issues such as breast cancer, osteoporosis, delayed development, cardiovascular disease, obesity etc. ([Huang, Chandra et al. 2010](#_ENREF_2)). In human cells, hERα becomes activated through the binding of 17β-estradiol (E2) to its binding site, which can then interact with estrogen response elements (ERE) on upper streams of regulated genes and initiate down-stream gene expression. As our understanding of hERα deepens, many chemical ligands originated from both natural and synthetic sources were found capable of interfering the function of hERα through the same ligand binding mechanism. These chemicals are often referred to as endocrine disrupting chemicals (EDCs). EDCs can be found in a variety of common substances humans use every day such as pharmaceuticals, personal care products, pesticides and even food packaging. Unfortunately, because of harmful human activities such as chemical spills and inappropriate disposal of pharmaceuticals, EDCs now have a rather significant presence in the environment. There have been reports of many severe problems in wild animals caused by the exposure to environmental EDCs such as reversed sexuality, reduced ability to reproduce etc. ([Lathers 2002](#_ENREF_3)). The health effects of EDCs to human have also been a great public concern due to increased EDC exposure. Therefore, the scientific community has been focusing on identifying potential EDCs as well as determining the health effects of known EDCs. The Environmental Protection Agency also initiated an ambitious Endocrine Disruptor Screening program in order to identify hormonally active compounds from a list of 8,700 existing chemicals ([Sanseverino, Eldridge et al. 2009](#_ENREF_5)).

**Figure 2:** The structures shown above represent various estrogenic compounds

**Figure 1:** Human Estrogen Receptor α Ligand Binding Domain

Human exposure to EDCs have several varation factors. First, humans are exposed to a complex mixture of EDCs rather than single ligands alone. Second, different populations could be exposed to different EDCs, and lastly, not all humans carry the same genetic background. It has been observed that genetic variations called single nucleotide polymorphism (SNP) are fairly prevalent in human([Sachidanandam, Weissman et al. 2001](#_ENREF_4)). Regarding EDC exposure, an important question being asked today is how would people with different genetic variations respond to different EDCs.

**Figure 3:** A representational illustration of the pathway estrogenic compounds follow by binding to hERα and initiating transcription of the lux gene cassette, which produces light.

Previously, PhD student Jun Wang at the Center for Environmental Biotechnology has investigated the potential effects of six point mutations close to hERα binding pockets using computational methods, which include Molecular Dynamics Simulation and Ensemble Docking. The primary focus of his project is to understand how SNPs could alter the binding affinity of different EDCs and whether these changes would alter transcriptional activity regulated by hERα. As part of this project, I will focus on validating his computational results using the yeast-based bioluminescent reporter system previously developed in CEB([Sanseverino, Eldridge et al. 2009](#_ENREF_5)). ***My hypothesis is that mutations close to the ligand-binding site of hERα will significantly change its affinity to various ligands, which will directly impact the transcriptional activation function of hERα.***

Specifically, I will be targeting the effects of three specific mutations of hER that are occurring in humans: Met396Val, Asn519Ala and Glu353Val. To validate the effects these mutations, I will use recombinant *Saccharomyces cerevisiae* as a model by incorporating a natural or mutated hERα gene as well as the plasmids containing the *lux* gene cassette (Figure 3). First, a wild type and mutated hERα gene will be incorporated into the genome of *Saccharomyces cerevisiae* SEY6210 by homologous recombination. Through the use of polymerase chain reaction (PCR) we can verify that the target genes have been successfully integrated and are being expressed correctly. Two bacterial plasmids (pUTK404 and pUTK407), which carry the bacterial luciferase gene cassette (*lux* CDABE) and estrogen reporter elements (EREs), will be transformed into the recombinant yeast. I will then use these strains’ response to serial diluted E2 as a measurement for more selection in order to obtain a stable cell line. Once this is accomplished, I will begin chemical testing the wild type and mutant yeasts by measuring their responses to selected EDCs through bioluminescent reporters.

The benefit of this research experience towards my education and career goals would serve as an excellent bridge for me into the scientific community at the University of Tennessee Knoxville, as well as the greater scientific world. As a microbiology and biochemistry, cellular and molecular biology major with intentions of pursuing doctoral level education in the biomedical sciences, gaining laboratory experience and a thorough understanding of the research process are necessary components I need to foster growth so that I may become a better student, scientist and researcher. Memorizing information, processes, and structures allows only for one-dimensional comprehension and lower level thinking, but with appropriate application, a much higher level of understanding is attainable. After participating in research in the Center for Environmental Biotechnology (CEB) in previous semesters (Fall 2013/Spring 2014), I have been exposed to a number of the procedures and material that will be related to this specific project such as plasmid cloning, genetic engineering, and various uses of bioreporters. I will be able to begin working on this project in the spring of 2014 with Jun Wang and should be able to finish by near the end of the summer. Receiving this stipend would be a critical step for me in furthering my abilities and desire to work in biomedical research, would instill a greater appreciation and understanding of the research process, and provide me with a much better understanding of the relationship between the environment and human health systems, which are both exciting and relevant to me and my future field of study.

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**I certify that this is my own work and has been reviewed and approved by my research mentor(s).**

Signature/e-Signature:\_\_\_\_Peter Hjorth\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_