



# Development and Application of a Bioluminescent Yeast-Reporter System for Screening Chemicals for Estrogenic and Androgenic Effects.

M.L. Eldridge, J. Sanseverino, A.C. Layton, J. Easter, T.W. Schultz, G.S. Saylor



Project ID: RD-831302

## Science Question

Two widely used receptor/reporter assays for detecting estrogenic and androgenic compounds are the Yeast Estrogen Screen (YES) (Routledge and Sumpter, 1996) and the Yeast Androgen Screen (YAS) (Purvis et al., 1991). These *Saccharomyces cerevisiae* (*S. cerevisiae*) strains contain the human estrogen or androgen receptor and a plasmid-based response element-lacZ reporter fusion. For example, when an estrogen-like compound binds to the estrogen receptor protein, this protein-compound complex binds to the estrogen response element (ERE), inducing transcription of lacZ. This assay has been used extensively to measure estrogenic and androgenic responses to various organic pollutants. Although proven effective for the *in vitro* determination of estrogenic and androgenic activity, the assays' incubation time of 3-5 days is impractical when considering the 87,000 chemicals requiring Tier 1 screening. To overcome this issue, bioluminescent versions of these reporters have been constructed.

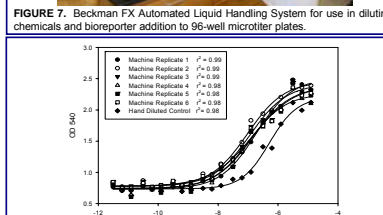
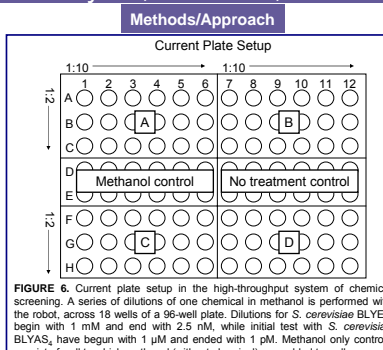
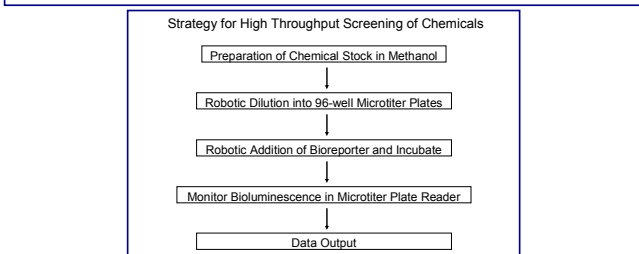
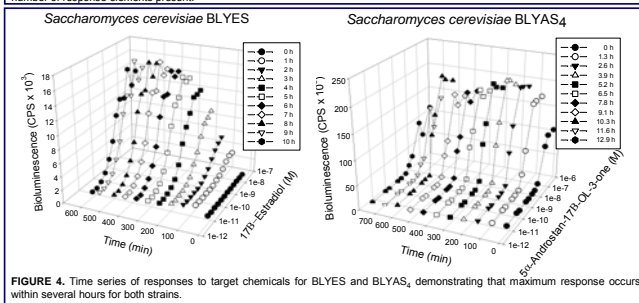
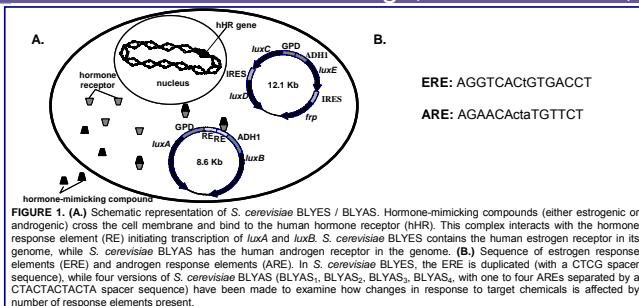
Gupta et al. (2003) functionally expressed the *luxA*, *B*, *C*, *D*, and *E* genes from *Photobacterium luminescens* and the *trp* gene from *Vibrio harveyi* in *S. cerevisiae*. The present work extends this system by developing estrogen- and androgen-responsive yeast-based bioluminescent bioreporters and demonstrating its usefulness against known endocrine disrupting compounds.

## Research Goals

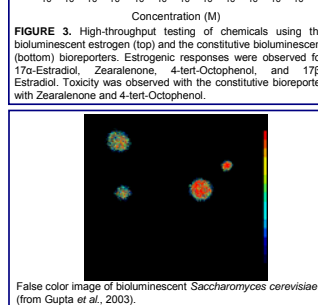
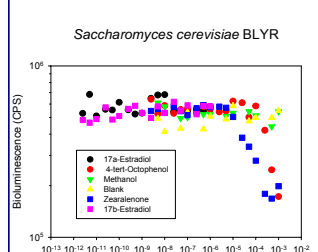
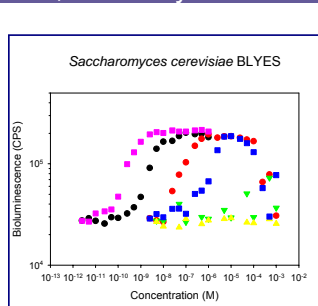
The purpose of Tier 1 screening is to identify substances that have the potential to interact with the endocrine system (O'Connor et al., 2002). The colorimetric-based YES has been widely used in the literature and this laboratory and is a very useful tool for assessing estrogenicity of a compound or environmental sample. Development of the bioluminescent version of the YES system (*S. cerevisiae* BLYES) has the potential to enhance the utility of this system. The primary objective of this research is to (i) validate the BLYES system and develop a standard operating procedure for routine chemical analysis; and (ii) develop an androgen bioluminescent reporter system analogous to the BLYES system.

The specific tasks of this research are:

1. Test the *S. cerevisiae* BLYES using the proposed 78 substances (see ICCVAM, 2002) listed for validation of estrogen receptors and correlate to the colorimetric *S. cerevisiae* YES assay.
2. Develop the *S. cerevisiae* BLYES into a standard assay suitable for high-throughput screening of chemicals.
3. Modify plasmid pEREAB construct for improved sensitivity.
4. Develop a yeast-based reporter for detection of androgens.



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
COMPUTATIONAL TOXICOLOGY



## Results/Conclusions

Two bioluminescent bioreporter strains responsive to estrogens and androgens have been developed and preliminary testing has been completed. Response times for each strain ranged from 2 - 6 hours compared (Fig. 2) to 3 - 5 days for the colorimetric version. Assay development and validity testing using the recommendations outlined in the ICCVAM report (ICCVAM, 2002) is underway. Chemicals are being tested for endocrine activity as well as for toxicity (Fig. 3). As a demonstration of the usefulness of these assays, a high-throughput testing strategy is being developed using a Beckman FX Automated Liquid Handling System (ALH). The ALH can dilute and distribute chemicals to 96-well microtiter plates as well as distribute reporter cells to the test chemicals. Further, the ALH can be linked potentially to an incubator and a microtiter plate reader to record bioluminescence.

## Impact and Outcomes

This research has standardized two bioluminescent yeast assays for screening estrogen- and androgen-mimicking compounds. Autonomously produced bioluminescence as a reporter has several advantages over colorimetric and *luc*-based reporters including:

- A reagent-less reporter system eliminating the extra manipulation and cost of adding exogenous reagents (such as luciferin).
  - Speed; a response is observed in as little as two hours and a maximum response in six hours.
  - A range of equipment can be used to detect bioluminescence.
  - Chemical dilution and dispensing of culture medium and cells into microtiter plates can be automated.
  - Cells for the assay can be prepared fresh and used or stored at -80°C.
  - Data collection and interpretation can be automated.
  - No animals are used in this assay.
  - Reduction in the use of labor-intensive cell culture assays.
- Further, *S. cerevisiae* BLYES and BLYAS when combined with appropriate photodetection technology can be used for remote, near real-time monitoring of our nation's waterways for endocrine disrupting activity (Bolton et al., 2001; Nivens et al., 2004).

## Future Directions

At the conclusion of this project, a standard operating procedure will be written for the use of each strain in testing unknown chemicals. Long-term uses of these strains include direct monitoring of, for example, wastewater treatment effluent, confined animal feeding operation runoffs, and groundwater monitoring.

## References

\*Bolton, EK, GS Saylor, DE Nivens, JM Rochelle, S Ripp, ML Simpson (2002) Integrated CMOS photodetectors and signal processing for very low-level chemical sensing with the bioluminescent bioreporter integrated circuit. *Sens Actuators B* 95: 179-185.  
\*EDSTAC (1998) Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) Final Report. EPA/743/R-98/003.  
\*Owata, EK, SS Patterson, S Ripp, GS Saylor (2003) Expression of the *Photobacterium luminescens lux* genes (*luxA*, *B*, *C*, *D*, and *E*) in *Saccharomyces cerevisiae*. *FEMS Yeast Res* 4: 305-313.  
\*Horie-Inoue, K, N Bono, Y Ozaki, S Ito (2004) Identification and functional analysis of consensus estrogen response elements in human prostate cancer cells. *Biochem Biophys Res Com* 325: 1312-1317.  
\*Nivens, DE, TE McKnight, SA Moser, SJ Osbourn, ML Simpson, GS Saylor (2004) Bioluminescent bioreporter integrated circuit: potentially small, rugged and inexpensive whole-cell biosensors for remote environmental monitoring. *J Appl Microbiol* 96: 33-46.  
\*O'Connor, JC, JC Cook, MD Murray, LG Davis, AM Kaplan, EW Carney, (2002) Evaluation of tier 1 screening approaches for detecting endocrine-active compounds (EACs). *Crit Rev Toxicol* 32: 521-549.  
\*Purvis, U, D Chohli, SW Dykes, DS Lubahn, FS French, EM Wilson, AN Hodson (1991) An androgen-inducible expression system for *Saccharomyces cerevisiae*. *Gene* 100: 35-42.  
\*Routledge, EJ, JP Sumpter (1996) Estrogenic activity of surfactants and some of their degradation products assessed using a recombinant yeast system. *Environ Toxicol Chem* 15: 241-248.

## Acknowledgements

We express appreciation for technical support provided by Victoria Garrett and Leslie Sadtak. We express appreciation to E.A. Meghen for the *P. luminescens luc*COMBET operon, and J.P. Sumpter for the Glauco Welcome YES and YAS strains. This research was supported by the U.S. Environmental Protection Agency's STAR program through grant RD-831302.

This poster does not necessarily reflect EPA policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.