A Bioluminescent Yeast-Reporter System for Screening Chemicals for Estrogenic Effects

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Abstract

The Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) created by the Environmental Protection Agency was mandated with developing methods to screen approximately 87,000 chemicals for biological effects on estrogen, androgen and thyroid hormone systems. As part of this mandate, EDSTAC proposed that EPA develop rapid, high throughput screening systems to assess a compound's effects on hormonal systems. The Center for Environmental Biotechnology at the University of Tennessee has re-engineered the *Saccharomyces cerevisiae* YES colorimetric estrogen reporter system (Routledge and Sumpter, 1996) to produce bioluminescence in response to estrogen or environmental estrogens (*S. cerevisiae* BLYES; Figure 1; Gupta *et al.*, 2003). Bioluminescence is a reagentless system eliminating the need for expensive chromophores. Light detection is more sensitive than absorbance detection thus shortening the development time of the assay.

In previous work, strain BLYES was exposed to the estrogenic compounds 17β-estradiol, 17α-estradiol, 17α-ethynyl estradiol, estrone, and 3,4′,5-trichloro-4-biphenylol and compared to the YES assay (Fig. 2). The EC_{50} values correlated linearly (R^2=0.97) between the 2 assays. Sensitivities of both assays decreased in the order 17β-estradiol > 17α-ethynyl - estradiol > estrone > 17α-estradiol, with no significant response generated from 3,4′,5-trichloro-4-biphenylol where the hydroxyl group is the sterically hindered by the paired ortho chlorines. The BLYES screen consistently detected estrogenic potencies at 5- to 10-fold lower levels than those attained in the YES assay. Moreover, bioluminescence was detectable in less than 4 hours as compared to 3 days for the colorimetric YES strain.

The primary objective of this research is to validate the BLYES system and develop a standard operating procedure for routine chemical analysis. Work is in progress to test the *S. cerevisiae* BLYES using the proposed 78 substances (ICCVAM, 2002) listed for validation of estrogen receptors and correlate to the colorimetric *S. cerevisiae* YES assay. Parallel research includes developing the *S. cerevisiae* BLYES into a standard assay suitable for HTS of chemicals and to modify the *lux* genes for optimum transcription/translation in *S. cerevisiae* thus increasing sensitivity of the assay.

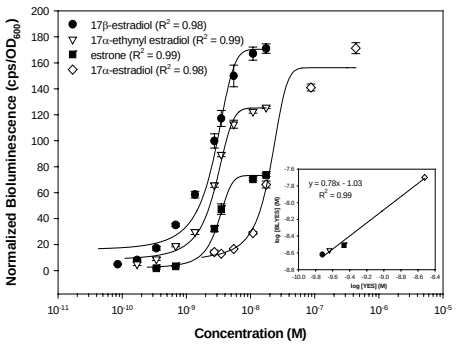
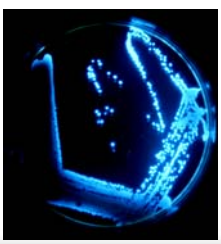


Figure 2. EC_{50} dose response profiles of the *S. cerevisiae* BLYES bioreporter to the estrogenic compounds 17β-estradiol (●), 17α-ethynyl estradiol (▽), estrone (■), and 17α-estradiol (◇); (n = 4). Inset: EC_{50} dose response correlations between the *lacZ* based YES and *lux* based BLYES estrogenic assays.

Impact

This EPA-sponsored research seeks to standardize a bioluminescent yeast assay for screening substances that potentially interact with the human estrogen receptor. The bioluminescent reporter system has several advantages including:

- A low-cost, reagentless reporter system that eliminates the extra manipulation and cost of adding exogenous reagents.
- Speed; preliminary data using 17β-estradiol indicated a bioluminescent response in 2-4 hours and a maximum response in 15 hours (Fig. 3).
- Amenable to automated, high throughput analysis, data collection and interpretation.
- Cells for the assay can be prepared fresh or stored at –80ºC.
- No animals are used in this assay.
- Elimination of labor-intensive cell culture assays.



Acknowledgements

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Saccharomyces cerevisiae BLYES

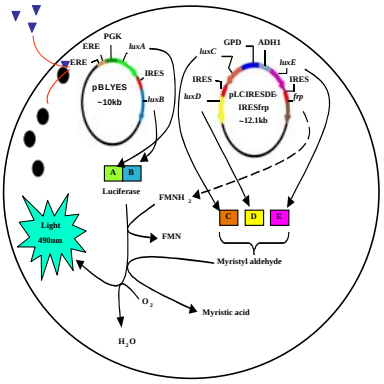

Figure. 1. Construction of the estrogen inducible bioreporter *S. cerevisiae* BLYES. Synthesis of *luxA* is regulated on plasmid pEREAB by upstream incorporation of two sequential estrogen response elements (ERE) coupled to a human phosphoglycerate kinase (PGK) promoter. The *luxB* component of the luciferase is supplied via independent expression from a fused IRES. The *luxC*, *luxD*, *luxE* and *frp* genes are under control of constitutive promoters on the plasmid pLCDEfrp. The human estrogen receptor (hER-α) is inserted in the *S. cerevisiae* chromosome.

Goals

The purpose of Tier 1 screening is to identify substances that have the potential to interact with the endocrine system. The colorimetric-based YES assay 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 HTS of chemicals.
3. Modify plasmid pEREAB construct for improved sensitivity.
4. Develop a yeast-based reporter for the detection of androgens.

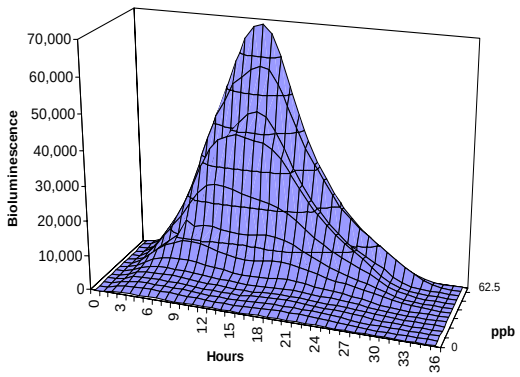

Figure 3. 3-Dimensional plot of bioluminescence vs. time for 0-62.5 ppb 17β-estradiol. Initial bioluminescence is observed in as little as 2 hours and peaks at approximately 15 h.

Literature Cited

Gupta, R.K., S.S. Patterson, S. Ripp, M.L. Simpson, and G.S. Sayler. 2003. Expression of the *Photorhabdus luminescens lux* genes (*luxA, B, C, D,* and *E*) in *Saccharomyces cerevisiae.* FEMS Yeast Research. 4:305-313.

Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). 2002. Expert Panel Evaluation on the Validation Status of In Vitro Test Methods for Detecting Endocrine Disruptors: Estrogen Receptor and Androgen Receptor Binding and Transcription Activation Assays. Expert Panel Final Report.

Routledge, E.J., and J.P. Sumpter. 1996. Estrogenic activity of surfactants and some of their degradation products assessed using a recombinant yeast screen. Environ. Toxic. Chem. 15(3):241-248.