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Analysis of Environmental Samples with Yeast-Based Bioluminescent Bioreporters

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1. Introduction

Extensive research over the past decade has found the widespread presence of organic wastewater contaminants (OWC) in surface waters around the globe including the United States, (Alvarez et al., 2009; Focazio et al., 2008; Kolpin et al., 2002; Owens et al., 2007; Zheng et al., 2008), Asia (Ma et al., 2007), Europe (Cargouet et al., 2007; Cespedes et al., 2005; Gros et al., 2009; Reemtsma et al., 2006) and South America (Bergamasco et al., submitted; Jardim et al., 2011; Kuster et al., 2009). These OWC include pesticides, plasticizers, pharmaceuticals, and natural and synthetic hormones as well as pollutants from chemical spills into the environment. These compounds may be introduced into surface waters by runoff from land application of biosolids, through leaking sewer lines and septic systems, or by incomplete removal from wastewater treatment systems. Further, a wide variety of these chemicals have been implicated in endocrine disruption in invertebrates and vertebrates (Cooper & Kavlock, 1997; Fang et al., 2000; Folmar et al., 2002; Fossi & Marsili, 2003; Guillette et al., 1999; Hayes et al. 2010; Kavlock et al., 1996; Kidd et al. 2007; Ropstad et al., 2006; Sonne et al., 2006; Tyler et al., 1998).

An endocrine disruptor is an exogenous substance that causes adverse health effects in an organism or its offspring by way of alteration in the function of the endocrine system. As such endocrine disruption is a mechanism leading to a variety of adverse health effects, most of which are considered as reproductive or developmental toxicities (OECD, 2002). The complex nature of reproductive and developmental effects suggests that *in vivo* tests are necessary to detect endocrine disruption. Several i*n vivo* mammalian assays (e.g. O'Connor et al., 2002) and *in vitro* assays (e.g. Fang et al., 2000; Zacharewski, 1997) exist for measuring estrogenic effects in various biological systems. However, these are not suitable for rapid, high-throughput screening of chemicals or necessarily screening of environmental samples. Yeast-based *in vitro* estrogen and androgen screens have been firmly established as a means for rapidly identifying chemicals with potential endocrine disrupting activity. This chapter will review the development and use of yeast-based bacterial bioluminescent bioreporters for the detection of endocrine disruption compounds.

1.1 Bioreporters

Reporter gene fusions have been widely used for the detection and quantification of chemical, biological, and physical agents (Daunert et al., 2000). The principle is to fuse a specific genetic promoter or response element with a reporter gene. Induction by a specific target chemical initiates transcription/translation of the bioreporter molecule, which generates a measurable signal. There are three widely-used classes of bioreporters: colorimetric (e.g. *lacZ, cat*), fluorescent (e.g. *gfp*), and bioluminescent (e.g. *luc, lux*). One example of a colorimetric-based bioreporter is the *lacZ* gene which encodes the β-galactosidase enzyme. β-Galactosidase mediates the breakdown of lactose to glucose + galactose. As a bioreporter, β-galactosidase is widely used in molecular biology in the blue-white screening assay. The chromophore X-gal (bromo-chloro-indolyl-galactopyranoside) is cleaved into galactose and an indole moiety that turns the medium blue. For chemical detection, *lacZ* is fused to a chemical-responsive promoter and when the cells are exposed to chromophores, such as chlorophenol red-β-D-galactopyranoside (CPRG), the assay medium changes from yellow to red. This type of colorimetric bioreporter is inexpensive and can be used in a qualitative or quantitative type of assay. Color density can be measured on a standard spectrophotometer.

Fluorescent assays take advantage of the green fluorescent protein (GFP). GFP was originally isolated from the jellyfish *Aequorea victoria* (Johnson et al., 1962; Shimomura et al., 1962). GFP is widely used as a bioreporter in eukaryotic systems for its simplicity to clone and no requirement for an organic substrate other than excitation with either UV or blue light. Quantification of the signal is by a fluorescent spectrophotometer or plate reader. There are different versions of *gfp* including blue-, red-, and yellow-shifted variants each requiring different excitation wavelengths and each of which fluoresce at different wavelengths (Hein & Tsien, 1996; Kendall & Badminton, 1998). In some cases this may be advantageous, especially when multiple bioreporters will be used simultaneously. These genes have been used extensively since they were first employed as gene expression biomarkers (Chalfie et al., 1994).

Firefly luciferase is another well-used bioreporter in eukaryotic systems. The luciferase, encoded by the *luc* gene (*lucFF*), was originally isolated from *Photinus pyralis* (firefly) and generates luciferase by a two-step conversion of D-luciferin to oxyluciferin (de Wet et al., 1985). This reaction generates light at 560 nm. However, the gene does not encode for the D-luciferin substrate and therefore substrate addition in any assay is required, which adds processing time and expense to the assay. Luc-based assays may also be constrained by the requirement for a cell lysis step followed by addition of the D-luciferin, adding both time and expense to the assay.

Bacterial bioluminescence has been widely used as a bioreporter in prokaryotic systems. The *lux* operon (*lux*CDABE) was originally isolated from *Vibrio fischeri* (Engebrecht et al., 1983), *Vibrio harveyi* (Cohn et al., 1983), and *Photorhabdus luminescens* (Szittner & Meighen, 1990). The *lux* operon encodes for the luciferase enzyme (*luxAB*) and the long-chain aldehyde substrate (*luxCDE*) for that reaction. An assay employing bacterial bioluminescence does not require an external organic substrate; the only requirement is for oxygen (O_2). A long chain aldehyde and a reduced flavin mononucleotide ($FMNH_2$) are converted by luciferase (LuxAB) to a long chain carboxylic acid and FMN, producing light at 490 nm wavelength (Meighen & Dunlap, 1993). The *luxAB* (without *luxCDE*) can also be used as a bioreporter and while these strains also produce light at 490 nm, they are less suited for high

throughput analysis due to additional handling steps (costly substrate addition) and additional cost.

The *luc* genes have been reported to be more sensitive than *lux*-based systems, however in a recent comparison of *luc*- and *lux*-based hormone-sensing bioreporters, Svobodova and Cajthaml (2010) determined that some *lux*-based bioreporters (BLYES/BLYAS bioassays, discussed below) are of comparable sensitivity and in some cases much more sensitive than *luc*-based bioreporters.

Several reviews are available on the properties and use of *luc*, *luxAB, luxCDABE, gfp,* and *gfp*-derived reporter genes in environmental systems (Hakkila et al., 2002; Keane et al., 2002; Ripp et al., 2010). Each of these reporter technologies has advantages and disadvantages depending on the application. For high throughput analysis of samples, bioreporters with the *luxCDABE* genes expressed are particularly well-suited for screening large numbers of samples. For both *luxAB*- and *lucFF*-based bioreporters, costly substrates must be continually added to the cells for visualization of the reaction. This increases not only handling difficulty but also costs to perform the assay. For GFP-based bioreporters, no exogenous substrates are necessary but fluorescent molecules must be excited by a light source to fluoresce. Each of these types of bioreporters produces signals for different lengths of time and has different light emission maxima and optimum temperatures. For example, while the *Photorhabdus luminescens* luciferase (Lux) is stable up to 42^{o}C, firefly luciferase (Luc) has a temperature optimum at 25^{o}C and is thermally inactivated above 30^{o}C (Keane et al., 2002). Bioreporter fusions incorporating the full *lux* cassette are advantageous in that they do not require exogenous substrates, cell lysis is not required, the signal is quantitative and reproducible (King et al., 1990). Further, continuous on-line monitoring is possible (e.g. DiGrazia et al., 1991; Heitzer et al., 1994; Heitzer et al., 1992; King et al., 1990).

1.2 Bacterial *lux* expression in *Saccharomyces cerevisiae*

Prior to 2003, the *lux* genetic system was previously limited only to expression in prokaryotic systems. However, Gupta et al. (2003) were successful in expressing the *P. luminescens lux* cassette in the yeast *S. cerevisiae*. Specifically, the *luxA, -B, -C, -D*, and *-E* genes from *P. luminescens* and the *frp* gene from *Vibrio harveyi* were re-engineered for expression in *Saccharomyces cerevisiae*. The *lux* operon was engineered using two pBEVY yeast expression vectors (Miller et al., 1998), which allowed bidirectional, constitutive expression of the individual *luxA, -B, -C, -D*, and *-E* genes. The *luxA* and *luxB* genes were independently expressed from divergent yeast constitutive promoters GPD and ADH1 on pBEVY-U (Figure 1). The *luxCD* and *luxE-frp* genes were independently expressed from a second plasmid (pBEVY-L), also using the GPD and ADH1 promoters. An internal ribosome entry site (IRES) was inserted between the *luxC* and *luxD* genes and the *luxE* and *frp* genes. The IRES allows translation of multiple genes from a single promoter in eukaryotes (Hellen & Sarnow, 2001).

Constitutive expression of the *luxCDABEfrp* genes in *S. cerevisiae* W303a generated approximately 9,000,000 photons per second per unit optical density (Gupta et al., 2003). This is comparable to similar expression in prokaryotic systems. This was a significant milestone in expression of bacterial operons in lower eukaryotic systems and created possibilities for screening organic wastewater contaminants with mammalian health significance.

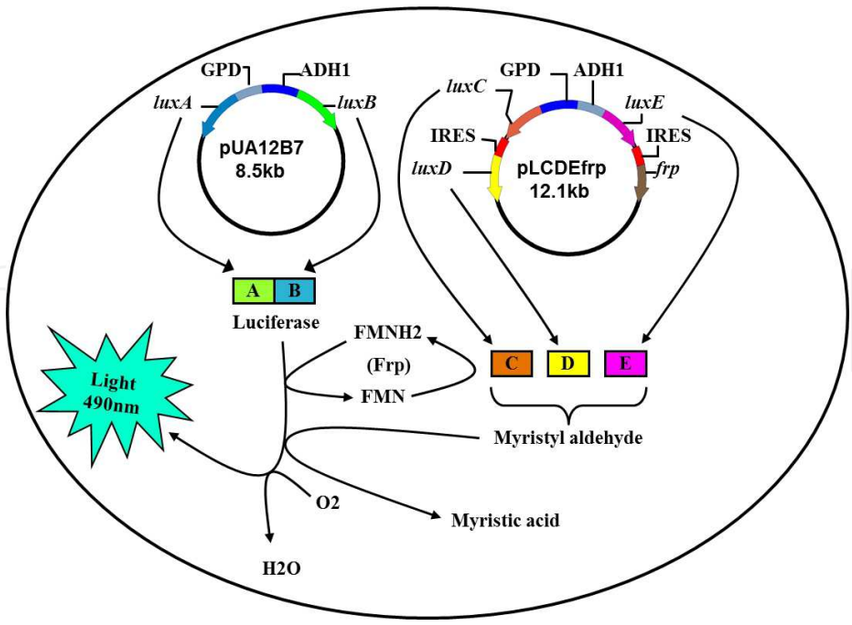


Fig. 1. Schematic representation of *S. cerevisiae* BLYEV (currently known as BLYR). This strain produces light continuously by constitutive expression of the *luxCDABE* genes from *Photorhabdus luminescens* and the *frp* gene from *Vibrio harveyi*.

2. Chemical detection using *S. cerevisiae*-based bioluminescent bioreporters

Yeast-based bioassays containing human receptors for estrogens and androgens fall into the recombinant receptor/reporter gene assay category. Estrogen or androgen response elements linked to a bioreporter molecule offer a low-cost method for screening samples rapidly for determining the presence of possible endocrine disruptors. Two widely used receptor/reporter assays for detecting estrogenic and androgenic compounds are the Yeast Estrogen Screen (YES) (Routledge & Sumpter, 1996) and the Yeast Androgen Screen (YAS) (Purvis et al., 1991). The *S. cerevisiae* YES and YAS bioreporters are colorimetric *lacZ*-based estrogen and androgen-sensing strains, respectively. The *S. cerevisiae* host strain for YES and YAS, contains the human estrogen receptor (hER-α) and human androgen receptor, respectively (Purvis et al., 1991; Routledge & Sumpter, 1996). Further, each host strain contains a series of either human estrogen response elements (EREs) or human androgen response elements (AREs) fused to the *lacZ* gene. The *lacZ* gene product, β-galactosidase, transforms the chromogenic substrate CPRG to a red product, measured by absorbance at 540 nm. These were the first widely used assays for yeast-based detection of estrogenic compounds.

The YES and YAS assays have been used extensively to measure endocrine responses to specific chemicals including polychlorinated biphenyls (PCBs) and hydroxylated derivatives (Layton et al., 2000; Schultz, 2002; Schultz et al., 1998), polynuclear aromatic hydrocarbons (PAH) (Schultz & Sinks, 2002), pesticides (Sohoni et al., 2001) and other compounds (Schultz et al., 2002). These assays have been adapted to environmental matrices including environmental waterways (Thomas et al., 2002), aquifers (Conroy et al., 2005), wastewater treatment systems (Layton et al., 2000) and dairy manure (Raman et al., 2004). Additional yeast-based bioreporters have been developed using either a colorimetric detection (Bovee et al., 2004; Gaido et al., 1997; Le Guevel & Pakdel, 2001; Rehmann et al., 1999), green

fluorescent protein (Bovee et al., 2007; Bovee et al., 2004) or the firefly luciferase bioreporter (Bovee et al., 2004; Leskinen et al., 2005; Michelini et al., 2005).

While the YES and YAS assays were highly specific for their target compounds, the colorimetric assays have disadvantages including addition of the chromophore for color development and a 3-5 day reaction time. This latter requirement hindered their ability for high-throughput analysis. Further, after 3 -5 days of incubation, it was unknown if any oxidation reactions were occurring that may activate the target compound. Some newer colorimetric assays have dramatically shortened the time required for color development (4-6 h) through the use of alternative substrates but have the disadvantage of requiring cell lysis steps (Jaio et al., 2008).

To overcome these limitations, bioluminescent version of the YES and YAS reporters were developed by modifying the plasmid constructs of Gupta et al. (2003). Triple repeats of the human ERE were inserted in between the GPD and ADH1 constitutive promoters regulating the *luxA* and *luxB* genes, respectively (Figure 2) generating strain BLYES (Sanseverino et al., 2005). A similar strategy was used for strain BLYAS (Eldridge et al., 2007), which functions in the same way except that it contains the human androgen receptor gene on its genome and *luxAB* are under control of four androgen response elements (AREs), while the constitutive strain (BLYR) has both the *luxAB* and *luxCDEfrp* genes constitutively produced therefore it makes light constantly. The BLYR strain is used to determine whether samples or chemicals are toxic to the yeast, preventing false negatives. If a chemical is highly toxic, killing or inhibiting the cells, no light will be produced and it would be easy to mistake toxicity for no estrogenic response. However, if bioluminescence of the BLYR strain is reduced, since it produces light constitutively, it is obvious that toxicity exists in the sample.

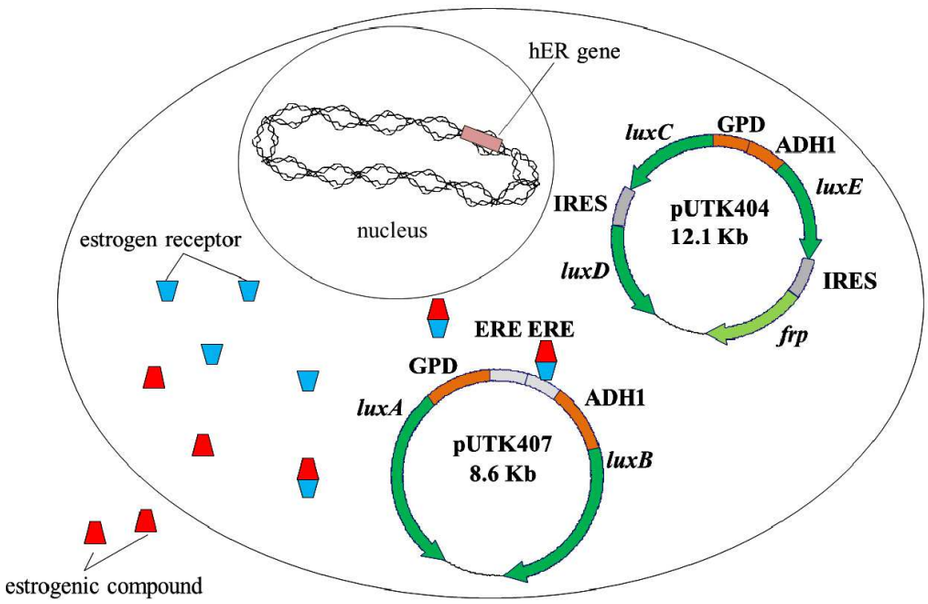
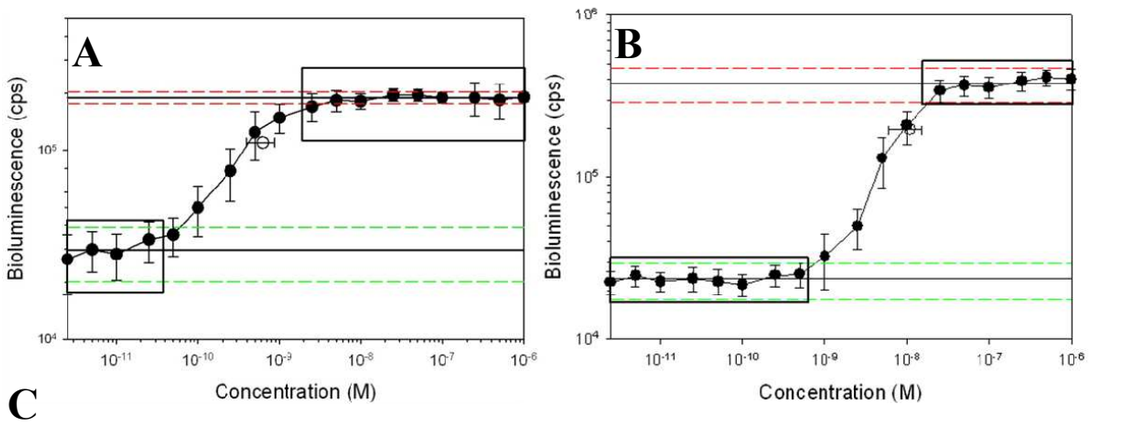


Fig. 2. Schematic representation of *S. cerevisiae* BLYES. Estrogenic compounds cross the cell membrane and bind to the human estrogen receptor (hER). This complex interacts with estrogen response elements (RE) initiating transcription of *luxA* and *luxB*. *S. cerevisiae* BLYES contains the human estrogen receptor in its genome, while *S. cerevisiae* BLYAS has the human androgen receptor in the genome.

Comparison of the BLYES and BLYAS strains to their colorimetric counterparts and proof-of-concept as to their utility has been established (Eldridge et al., 2007; Sanseverino et al., 2005). The BLYES and BLYAS assays are consistent with previously published yeast-based reporter assays (Sanseverino et al., 2009). The 40 - 50% variability of the EC_{50} values shown in Figure 3 reaffirms the suggestion that no single assay should be used to determine an absolute EC_{50} value but rather as a first step in estimating the hormonal activity of a chemical (Beresford et al., 2000).



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Assay	Chemical Standard	EC_{50} (M)	Upper Limit of Detection (M)	Lower Limit of Detection (M)
BLYES	17β-Estradiol	$6.3 \pm 2.4 \times 10^{-10}$	5.0×10^{-9}	2.5×10^{-11}
BLYAS	5α-Dihydrotestosterone	$1.1 \pm 4.6 \times 10^{-8}$	5.0×10^{-8}	1.0×10^{-10}

Fig. 3. **A.** *S. cerevisiae* BLYES standard curve (n = 13) using 17β-estradiol. **B.** *S. cerevisiae* BLYAS standard curve (n = 13) using dihydrotestosterone as a standard. Open circle: calculated EC_{50} values with error bars. A 50% effective concentration (EC_{50}) value was determined from the midpoint of the linear portion of the sigmoidal dose response curve. The mean and standard deviation values were calculated from replicate EC_{50} values for each standard to determine the variability between assays. **C.** Summary of EC_{50} values for BLYES and BLYAS strain with upper and lower limits of detection.

S. cerevisiae BLYES, *S. cerevisiae* BLYAS, *S. cerevisiae* BLYR, were used to assess their reproducibility and utility in screening 69, 68, and 71 chemicals for estrogenic, androgenic, and toxic effects, respectively (Sanseverino et al., 2009). This screening was part of an assessment of the United States Environmental Protection Agency's Tiered screening of chemicals for endocrine-disrupting ability. The 3-tier system includes (i) priority setting, (ii) Tier 1 screening, and (iii) Tier 2 screening. Priority setting focuses on identifying chemicals that require further testing; i.e., excluding chemicals with little or no known hormonal activity and that are generally regarded as safe. The intent of Tier I screening is to rapidly identify chemicals that interact with the estrogen, androgen, and thyroid systems while Tier 2 screenings provide a more in-depth study of how each chemical interacts with each endocrine system. In this study, EC_{50} values were $6.3 \pm 2.4 \times 10^{-10}$ M (n = 18) and $1.1 \pm 0.5 \times 10^{-8}$ M (n = 13) for BLYES and BLYAS, using 17β-estradiol and 5α-dihydrotestosterone

(DHT) over concentration ranges of 2.5 x 10^{-12} thru 1.0 x 10^{-6} M, respectively. Based on analysis of replicate standard curves, comparison to background controls, and screening a variety of chemicals, a set of quantitative rules was formulated to interpret data and determine if a chemical is potentially hormonally active, toxic, both, or neither (Sanseverino et al., 2009). The results demonstrated that these assays were applicable for Tier I chemical screening in EPA's Endocrine Disruptor Screening and Testing Program as well as for monitoring endocrine disrupting activity of unknown chemicals in water.

Additional *S. cerevisiae* bioluminescent bioreporters for estrogens and androgens have been developed using the firefly luciferase as the reporter molecule. The bioreporters of Leskinen et al., (2005) each contain the firefly luciferase gene (*lucFF*) under control of hormone-responsive promoters. The four strains, designated BMAEREluc/ERα, BMAEREluc/ERβ, BMAEREluc/AR, and BMA64/luc were used to detect estrogens (two versions), androgens, and toxicity, respectively. This bioassay is unique in that it uses two estrogen-sensing bioreporters; one contains the alpha form of the estrogen receptor and one contains the beta form (ERα, ERβ). These bioreporters were used by Svobodova et al. (2009) to test commercially available PCB mixtures and triclosan for estrogenic and androgenic activity but did not detect any activity with these samples (estrogenic or androgenic). This lack of estrogenic response in the bioluminescent assays may be due to the different mode of action of chemicals like triclosan (Stoker et al., 2010). In a study that examined the effects of triclosan exposure on female Wistar rats, triclosan advanced the onset of puberty symptoms. Also, a combination of ethinyl estradiol (EE2) and triclosan increased uterine weight significantly more than EE2 alone while triclosan alone had no effect. Therefore the mode of action of triclosan appears to have a synergistic effect on EE2 activity in Wistar rats. This effect appears to be independent of estrogen receptor binding given that bioluminescent yeast bioassays (Svobodova et al. 2009, Eldridge et al. unpublished data), which measure binding to the hER and then EREs, did not respond to triclosan.

In addition to hormone-mimicking chemicals, several other types of contaminants are also detectable with *S. cerevisiae*-based bioluminescent bioreporters. For example, the aryl hydrocarbon-sensing strain of Leskinen et al. (2008) contains genomically integrated human aryl hydrocarbon receptor and human aryl hydrocarbon nuclear translocator genes. In addition, it carries a plasmid-encoded copy of the firefly luciferase gene (*lucFF*) that is regulated by a series of aryl hydrocarbon receptor complex (AHRC) response elements (also called dioxin response elements or xenobiotic response elements, AhREs/DREs/XREs). Aryl hydrocarbon receptor proteins interact with both their AH ligand and the nuclear translocator protein then bind to the AhRE region of the *luc*-containing plasmid, activating transcription of luciferase, similarly to the receptor-response element system present in the BLYES bioassay. Since this bioassay is *luc*-based, D-luciferin must be added.

Another *S. cerevisiae*-based bioreporter has been created to measure arsenate and also UV damage (Bakhrat et al., 2011). This strain is based on the BLYES strain of Sanseverino et al. (2005), containing a constitutive *luxCDEfrp* plasmid and a *luxAB* plasmid that has been re-engineered to be under control of the UFO1 promoter, which specifically responds to DNA damage by UV light and also arsenate. The strain is able to detect very low concentrations of arsenate (1x10^{-12} to 1x10^{-6} M), which makes them useful for environmental monitoring. It was also used to evaluate the level of UV protection in commercial sunscreens. When films of Saran wrap were placed between the cells with SPF100 or SPF15 sunscreen on them, the sunscreen provided 100% and 90% protection, respectively, in comparison to a control in

which samples were shielded with only Saran wrap. Studies of this type demonstrate this bioassay's usefulness on complex samples.

3. Analysis of aqueous environmental samples

For use on environmental samples, the BLYES/BLYAS/BLYR bioreporter suite is particularly well-suited. They require no substrate addition or illumination source, are inexpensive to use, and are optimized for 96-well plate formats. For water samples where OWC are typically found in the ppb range, a concentration step is necessary. Figure 4 outlines the procedures for analysis of aqueous samples. For wastewater effluents and source drinking water samples, solid phase extraction is performed to isolate and concentrate any chemical contaminants.

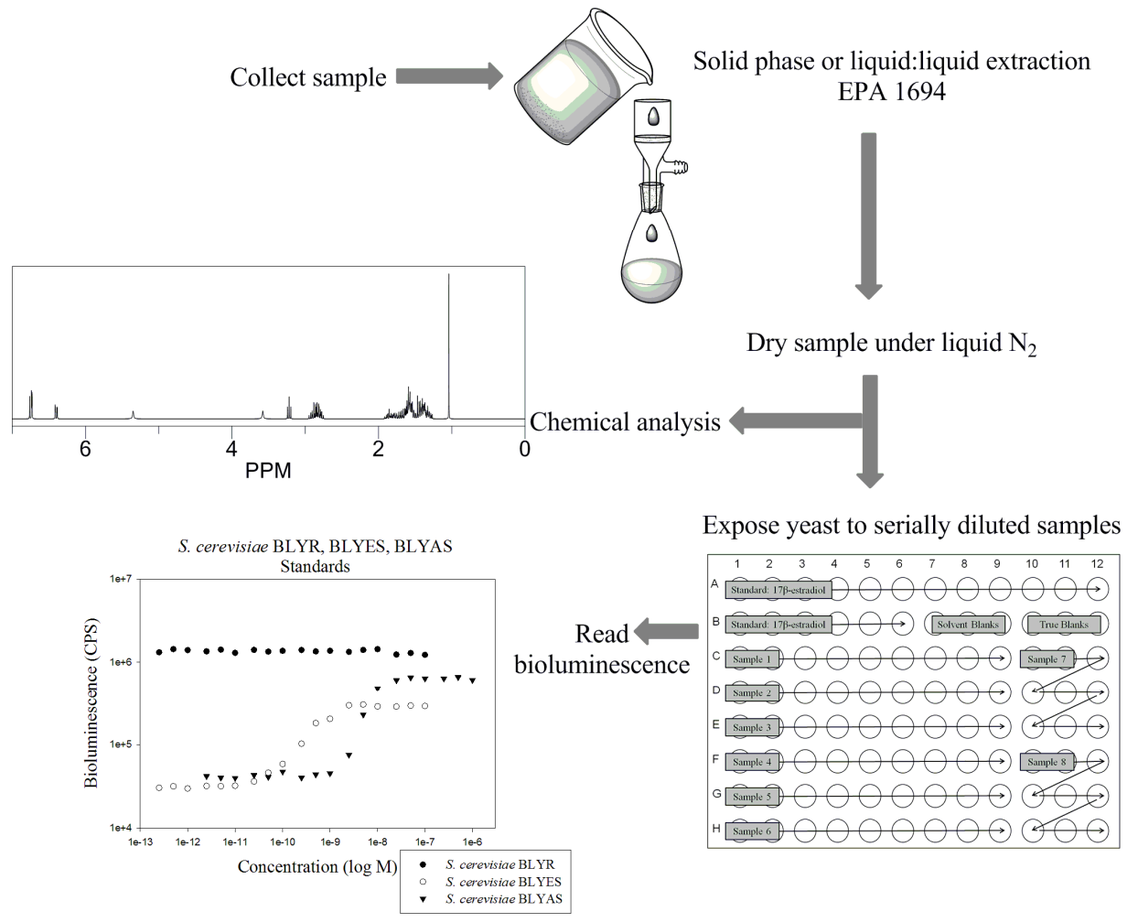

Fig. 4. Schematic of sample preparation and analysis. Typically, 1 L samples are collected aseptically and passed through a solid phase extraction unit. After elution by an appropriate solvent, concentrating the sample 1,000-fold, the sample is analyzed by the bioassays and/or with chemical analysis such as GC/MS or LC/MS. Typically, eight samples are analyzed on a 96-well plate, including standards and control wells (both solvent control and no treatment controls). By combining multiple plates in one assay run, numerous samples are processed at one time. Bioluminescence is monitored and recorded over time using a photon-counting system.

Numerous methods for solid phase extraction exist but commonly a modification of United States EPA 1694 (2007) is used. Briefly, Oasis filters (Waters, Inc.) or cartridges are conditioned with methanol and water, then the sample (typically ~1 L) is passed though the membrane slowly under a small amount of pressure. Chemicals are eluted in a solvent, either singly or in combinations, such as methanol or a methanol:acetone mixture. The solvents are evaporated to dryness and may be used immediately or stored at -20°C for future use. For the BLYES/BLYAS/BLYR assays, samples are resuspended in methanol (or DMSO) such that they are 1000x concentrated compared to the original sample, e.g. 1 L of sample is concentrated, dried, and resuspended in 1 mL of solvent, yielding an effective concentration factor of 1000x. This may then be split for chemical analysis and bioassays.

In the bioassays, samples are serially diluted in methanol to achieve a range of concentrations (1000x-2.5x). In addition, standard chemicals (17β-estradiol (E2) for BLYES/BLYR and 5α-dihydrotestosterone (DHT) for BLYAS) are suspended in methanol at 0.01 M and then serially diluted 18 times to generate a concentration range of $4x10^{-7}$ M to $1x10^{-12}$ M for E2 and $4x10^{-6}$ M to $1x10^{-11}$ M for DHT. Samples and standards (50 µL) are then spotted into the wells of 96-well plates (Figure 4). Triplicate plates are made (one for each of three strains) and then methanol is evaporated at room temperature.

For preparation of the bioassay, each yeast strain is grown overnight at 28°C with shaking (150 rpm) in Yeast Minimal Media (YMM) without leucine or uracil (Routledge & Sumpter, 1996) to an OD_{600} of 1.0. Yeast strains (200 µL) are spotted into the wells of 96-well plates containing dry samples and standards, beginning the exposure. This generates a concentration range of 250x-0.625x for environmental samples, $1x10^{-7}$ M to $2.5x10^{-13}$ M for E2, and $1x10^{-6}$ M to $2.5x10^{-12}$ M for DHT. Negative controls included wells with (i) medium + cells and (ii) medium + cells + evaporated methanol, to monitor whether estrogenic or androgenic substances are present in the solvent. Plates are then placed into a plate reader (such as Perkin-Elmer Victor2 Multilabel Counter) with an integration time of 1 s/well. Bioluminescence is measured every 30-60 min for four hours. Relative light unit data (as counts per second) is plotted versus the log of concentration in SigmaPlot (or similar statistical software) (Figure 5).

For each chemical, the log of bioluminescence (counts per second) versus the log of chemical concentration (M) is plotted, generating a sigmoidal curve for hormonally active compounds. A 50% effective concentration (EC_{50}) value is determined from the midpoint of the linear portion of the sigmoidal curve. The mean and standard deviation values are calculated from replicate EC_{50} values for standards to determine the variability between assays. Detection limits are determined by calculating the concentration of chemical at background bioluminescence plus three standards deviations. Toxicity is calculated as the concentration of sample that reduces the signal from the constitutively bioluminescent strain (BLYR) by 20% (IC_{20}). For environmental samples, the concentration factor that yields 50% maximal response is considered the EC_{50} and when this value is divided by the EC_{50} for that assay's standard, estrogenic or androgenic equivalents are calculated (in terms of E2 or DHT, respectively); this determines the amount of potentially estrogenic substances that are present in a sample relative to the standard.

For samples in which DMSO is the preferred solvent, a 4% solution of DMSO is used for the serial dilutions of environmental samples and standards (by incubating the sample in a small volume of 100% DMSO for 15 minutes then adding ultra-pure water to achieve a final DMSO concentration of 4%). Next, 100 µL of sample or standard are spotted into 96-well plates along with 100 µL of yeast cells (without drying the samples/standards), yielding a

final DMSO concentration of 2% in all wells. Negative controls should consist of wells with (i) medium + cells and (ii) medium + cells + DMSO to monitor whether DMSO is toxic to yeast cells and whether the solvent contains potentially estrogenic substances.

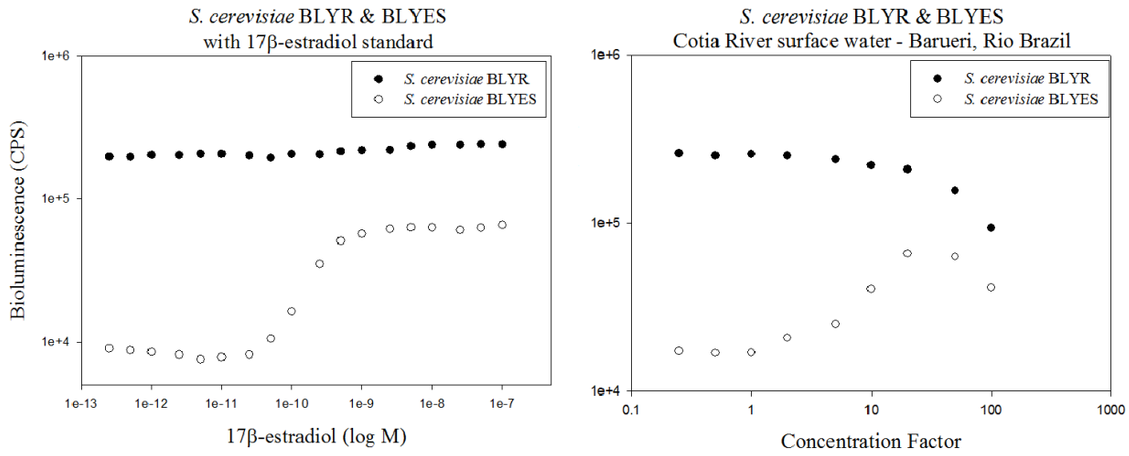

Fig. 5. Yeast assay data using environmental samples. The graphs show the responses of the yeast strains *S. cerevisiae* BLYR and BLYES in response to the 17β-estradiol standard and serial dilutions of a solid phase extracted sample. The sample consisted of surface water from the Cotia River in Brazil, which has a high concentration of estrogenic substances present (1.2 ng E2 equivalents/L) and exhibits marked toxicity. Analysis of surface and groundwater are of particular interest to regulatory agencies (and the public) because they are source waters for drinking water treatment plants.

Using this bioassay, surface water samples were surveyed from the U.S. and Brazil (Eldridge-Umbuzeiro, unpublished data, Figures 5 and 6), with both studies determining that the estrogen-sensing strain detects more estrogen-like activity than predicted through chemical analysis alone. This is expected however, given that chemical analysis targets certain contaminants and cannot be expected to screen samples for all known estrogens. In addition, it is relatively unknown if/how chemicals act synergistically to promote estrogen- or androgen-like activity. The assay can provide a clear evaluation on the levels of potential estrogenicity in monitoring studies of surface water samples as can be seen from Figure 6 (unpublished data). The levels varied from 0.01 to 19.3 ng/L of E2 equivalents per liter of water. In this particular case, the river water that was monitored was from Brazil, with the highest levels of pollution expected to occur in the dry season (corresponding to June-October).

In Jardim et al. (2011), surface water samples from Brazil were also examined. The samples were collected from sites classified by the São Paulo State Environmental Agency (CETESB) as excellent, good, medium, fair, and poor. Both bioassays and chemical analysis were performed on samples following solid phase extraction. The authors targeted estrone (E1), 17β-estradiol (E2), ethinyl estradiol (EE2), estriol (E3), bisphenol A (BPA), 4-n-octophenol (OP), and 4-n-nonylphenol (NP) in their chemical analysis and used the estrogen-sensing BLYES as the bioassay. From this data, the authors determined that the bioassay data is not fully explained by the amount and strength of the detected estrogens. For example, the highest estrogenic response determined by the yeast bioassay (BLYES) was also determined to have the highest concentrations of estrogen by chemical analysis. Also, in drinking water

samples in which targeted estrogens were not detected by chemical analysis, the yeast bioassay also did not detect any estrogenic activity. However, in some samples from different surface water intake points, often yeast bioassays detected estrogenic activity at levels that chemical analysis data did not predict. For example, BPA detected at 3.53 ng/L (according to chemical analysis) is not a sufficient concentration to elicit an estrogenic response. However, the same sample elicited a response equivalent to 0.7 ng/L of E2 equivalents (according to the yeast bioassay). This suggests that *S. cerevisiae* BLYES was responding to a) something that was not recognized by chemical analysis, b) by-products of the targeted chemicals, or c) that a mixture effect is causing a synergistic estrogen response. The reader is cautioned that these assays should be a first determination of estrogenic activity. *S. cerevisiae* does not have an endocrine system and cannot explicitly identify endocrine disruptors. Advanced testing with alternate assays (i.e. mammalian-based assays) should be used for confirmation of endocrine-disrupting activity.

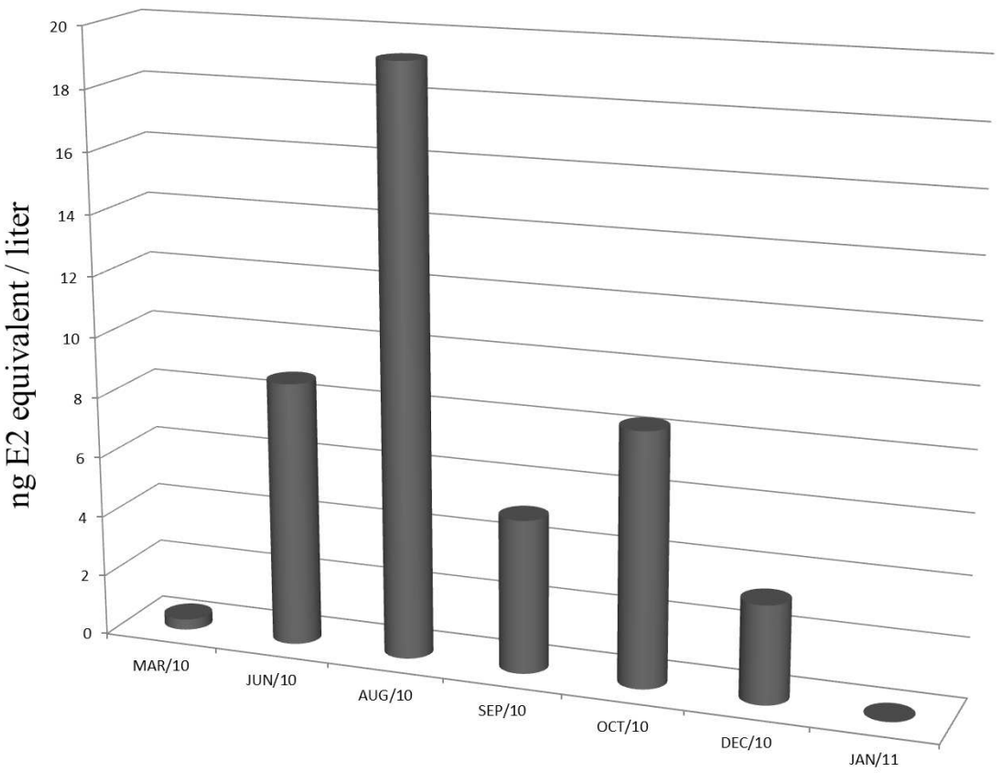

Fig. 6. Surface water samples from Brazil assessed with the BLYES bioassay. Surface water samples were solid phase extracted and then processed with the *S. cerevisiae* BLYR and BLYES.

Alvarez et al. (2009) used BLYES for the analysis of Potomac River water samples in a study on the reproductive health of bass. The authors criticized the collection of single grab samples, in favor of using passive samplers to concentrate contaminants. They examined extracts from passive samplers that had been deployed for 31 days in the Potomac River and its tributaries, which receive significant amounts of flow from WWTP effluents. Samples

were collected once yearly, for two years (2005-2006), both upstream and downstream of known WWTP discharge sites. They also performed both chemical analysis (targeting E1, E2, EE2, and E3) and bioassays (using BLYES and BLYR). They were able to detect potentially estrogenic compounds at levels statistically different than the field blanks. Levels of E2 equivalents were detected in the nanomolar range. The authors were able to measure estrogenic responses with BLYES but they were not able to detect a seasonal difference in estrogenicity (some chemicals were detected seasonally via chemical analysis but others were not) though it is unclear whether there was no seasonal effect or whether the estrogens were detected at such a low concentration that a conclusion cannot be drawn.

In both studies (Alvarez et al., 2009; Jardim et al., 2011), the expected response with bioassays was lower than the actual response determined with the bioassay. Expected responses are calculated by multiplying a chemical's concentration (determined through chemical analysis) by its potency relative to a reference estrogen, such as E2. It is expected that if all contaminating estrogenic molecules are detected by chemical analysis then the expected responses should match actual bioassay responses. However, it is difficult to anticipate (and therefore target) all possible endocrine-active contaminants that are present in environmental samples. In addition, prediction of the effects of mixtures of chemicals, especially at low concentrations, has proven to be problematic. Moreover, bioassays are likely to detect metabolites of estrogenic chemicals, as long these molecules continue to interact with the human hormone receptor/response element-sensing systems. Given these reasons, it is natural to expect that chemical analysis is unlikely to ever fully predict actual bioassay responses.

The androgen-sensing strain of Leskinen et al. (2005) has been used to monitor wastewater before and after treatment in wastewater treatment plants in several cities in Italy (Michelini et al. 2005). It was determined that both samples (pre- and post-treatment) contained chemicals with androgenic activity, however treatment decreased this activity. They determined that approximately 30% of androgenic activity was typically removed but occasionally activity was reduced by 90%. They attributed the decreased activity to the presence of carbon-based filters, which should bind chemicals, thereby removing them from wastewater. This study illustrates the effectiveness of yeast-based bioreporters for the rapid analysis of samples before and after water treatment. It also demonstrates that wastewater treatment does not necessarily remove chemicals associated with potential endocrine disrupting activity.

In addition, the strains of Leskinen et al. (2005) (BMAEREluc/ERα, BMAEREluc/ERβ, BMAEREluc/AR, and BMA64/luc) were used to test several lotion samples, as a simulation of using the strains on complex sample matrices. Five of the seven lotion samples demonstrated estrogenic activity, even at dilutions as low as 1:175. The authors attributed this activity to parabens present in the lotions, given that samples with no parabens were not estrogenic but samples with mixtures of parabens were. The authors state that parabens are present in many cosmetic products and are generally considered safe (Soni et al., 2002), despite having been demonstrated to produce an estrogenic response (Routledge et al., 1998) and being present in breast cancer tumors (Darbre et al., 2004). No androgenic activity was found for any of the samples.

More recently, Svobodova et al. (2009) examined the endocrine disrupting potential of a commercial PCB mixture (Delor 103) and a series of potential PCB degradation metabolites (chlorobenzoic acids and cholorophenols). The authors did not detect any estrogenic activity with any of the chemicals or mixtures tested using bioluminescent yeast, except that 5 mg/L

chlorophenol caused a response. This is in contrast to the results obtained with the Yeast Estrogen Screen (YES) colorimetric bioreporter, which detected estrogenic activity with all the tested chemicals except chlorophenols. One reason for this difference may be the different length of exposure time between the two bioassays. The YES was incubated with chemicals for three days whereas the Leskinen strains were only incubated with the chemicals for 2.5 h prior to sample processing. It is possible that over three days' time, the PCBs may have oxidized (yeast are incubated in aerobic conditions) to forms that are more likely estrogenic. Indeed, hydroxylated PCBs have been demonstrated to harbor estrogenic activity (Korach et al., 1988; Schultz, 2002; Schultz et al., 1998). Interestingly, using the bioluminescent androgen-sensing bioreporter (BMAEREluc/AR), androgenic activity was detected with the commercial PCB mixture, but not with chlorobenzoic acids, chlorophenols, or triclosan. Triclosan has been demonstrated to have no activity with the BLYES and BLYAS bioassays as well (data not shown).

4. Future applications

Saccharomyces cerevisiae-based bioluminescent bioreporters offer excellent opportunities beyond bacterial bioreporters for rapid analysis of chemicals with human and environmental significance. Expression of the bacterial *lux* cassette in a lower eukaryote offers many opportunities not only for high-throughput screening systems but also bioprocess monitoring, diagnostic applications, fungal gene expression analysis, and *in vivo* sensing of fungal infections (Gupta et al., 2003). Expression in *S. cerevisiae* has led to advances in transferring this system to mammalian cell lines (Close et al., 2010; Patterson et al., 2005).

The advantages for detection of endocrine-disrupting chemicals in water by *S. cerevisiae lux*-based bioreporters are numerous including accuracy, ease of use, not expensive, and amenable to automation in performing and collection of data. In addition to screening aqueous samples, BLYES, BLYAS, and BLYR, and other variants described in the literature are useful for Tier I screening as proposed by the EPA, analysis of wastewater influent and effluent, chemical leaching from manufactured products, for example. In fact, the State Environmental Agency of São Paulo (CETESB) in Brazil is considering using the *S. cerevisiae* BLYES bioassay for routine monitoring of surface and ground water samples for the presence of potentially estrogenic substances. Two of the authors (M.E. and G.S.) have begun routine monitoring of wastewater treatment plant effluents from a treatment facility in TN as well as screening 250 water samples across the state of Tennessee in a broad survey.

Ideally, detection of potential endocrine disruptors (or any other chemical of interest) by bioluminescent bioreporter strains would be coupled to remote detection systems for continuous real-time monitoring. Bioluminescent bioreporter integrated circuits fuse reporter cells to an integrated circuit containing a photodetector (e.g. Sayler et al., 2001; Nivens et al., 2004; Sayler et al., 2004). These devices could be distributed in networks and coupled with wireless communications would send signals indicating the presence/absence of chemical contaminants. Roda et al. (2011) have developed a device that couples estrogen- or androgen-sensing *S. cerevisiae* expressing firefly bioluminescence to fiber optics with detection by a CCD sensor, yielding a fully functional biosensor. While this device resulted in strains whose detection limit was approximately 10-fold higher than bioassays performed in the lab and was larger than previously reported remote detection systems, it does

demonstrate this device's usefulness in future environmental monitoring. Remotely deployed devices may allow long integration times to account for chronic exposure to low-levels (ppb) of a potential endocrine disrupter that may not be captured in a single grab sample.

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6. References

Alvarez, D.A., Cranor, W.L., Perkins, S.D., Schroeder, V.L., Iwanowicz, L.R., Clark, R.C., Guy, C.P., Pinkney, A.E., Blazer, V.S. & Mullican, J.E. (2009). Reproductive health of bass in the Potomac, USA, drainage: part 2. Seasonal occurrence of persistent and emerging organic contaminants. *Environmental Toxicology and Chemistry* 28: 1084-1095.

Bakhrat, A., Eltzov, E., Finkelstein, Y., Marks, R.S. & Raveh, D. (2011). UV and arsenate toxicity: a specific and sensitive yeast bioluminescence assay. *Cell Biology and Toxicology* 27: 227-236.

Beresford, N., Routledge, E.J., Harris, C.A. & Sumpter, J.P. (2000). Issues arising when interpreting results from an in vitro assay for estrogenic activity. *Toxicology and Applied Pharmacology* 162: 22-33.

Bergamasco, A.M., Eldridge, M.L., Sanseverino, J., Sodré, F.F., Montagner, C.C., Pescara, I.C., Jardim, W.D. & Umbuzeiro, G.D. Bioluminescent yeast estrogen assay (BLYES) as a promising tool to monitor surface and drinking water for estrogenicity. *Journal of Environmental Monitoring* (in press).

Bovee, T.F.H., Helsdingen, R.J.R., Hamers, A.R.M., van Duursen, M.B.M., Nielen, M.W.F. & Hoogenboom, R. (2007). A new highly specific and robust yeast androgen bioassay for the detection of agonists and antagonists. *Analytical and Bioanalytical Chemistry* 389: 1549-1558.

Bovee, T.F.H., Helsdingen, R.J.R., Koks, P.D., Kuiper, H.A., Hoogenboom, R. & Keijer, J. (2004). Development of a rapid yeast estrogen bioassay, based on the expression of green fluorescent protein. *Gene* 325: 187-200.

Cargouet, M., Perdiz, D. & Levi, Y. (2007). Evaluation of the estrogenic potential of river and treated waters in the Paris area (France) using in vivo and in vitro assays. *Ecotoxicology and Environmental Safety* 67(1): 149-156.

Cespedes, R., Lacorte, S., Raldua, D., Ginebreda, A., Barcelo, D. & Pina, B. (2005). Distribution of endocrine disruptors in the Llobregat River basin (Catalonia, NE Spain). *Chemosphere* 61: 1710-1719.

Chalfie, M., Tu, Y., Euskirchen, G., Ward, W.W. & Prasher, D.C. (1994). Green fluorescent protein as a marker for gene-expression. *Science* 263: 802-805.

Close, D.M., Patterson, S.S., Ripp, S., Baek, S.J., Sanseverino, J. & Sayler, G.S. (2010). Autonomous bioluminescent expression of the bacterial luciferase gene cassette (*lux*) in a mammalian cell line. *Plos One* 5(8).

Cohn, D.H., Ogden, R.C., Abelson, J.N., Baldwin, T.O., Nealson, K.H., Simon, M.I. & Mileham, A.J. (1983). Cloning of the *Vibrio harveyi* luciferase genes - use of a

synthetic oligonucleotide probe. *Proceedings of the National Academy of Sciences of the United States of America-Biological Sciences* 80: 120-123.

Conroy, O., Quanrud, D.M., Ela, W.P., Wicke, D., Lansey, K.E. & Arnold, R.G. (2005). Fate of wastewater effluent hER-agonists and hER-antagonists during soil aquifer treatment. *Environmental Science & Technology* 39: 2287-2293.

Cooper, R.L. & Kavlock, R.J. (1997). Endocrine disruptors and reproductive development: a weight-of-evidence overview. *Journal of Endocrinology* 152: 159-166.

Darbre, P.D., Aljarrah, A., Miller, W.R., Coldham, N.G., Sauer, M.J. & Pope, G.S. (2004). Concentrations of parabens in human breast tumours. *Journal of Applied Toxicology* 24: 5-13.

Daunert, S., Barrett, G., Feliciano, J.S., Shetty, R.S., Shrestha, S. & Smith-Spencer, W. (2000). Genetically engineered whole-cell sensing systems: coupling biological recognition with reporter genes. *Chemical Reviews* 100: 2705-2738.

de Wet, J.R., Wood, K.V., Helinski, D.R. & Deluca, M. (1985). Cloning of firefly luciferase cDNA and the expression of active luciferase in *Escherichia coli*. *Proceedings of the National Academy of Sciences of the United States of America* 82: 7870-7873.

DiGrazia, P., King, J., Blackburn, J., Applegate, B., Bienkowski, P., Hilton, B. & Sayler, G. (1991). Dynamic response of naphthalene biodegradation in a continuous flow soil slurry reactor. *Biodegradation* 2: 81-91.

Eldridge, M.L., Sanseverino, J., Layton, A.C., Easter, J.P., Schultz, T.W. & Sayler, G.S. (2007). *Saccharomyces cerevisiae* BLYAS, a new bioluminescent bioreporter for detection of androgenic compounds. *Applied and Environmental Microbiology* 73: 6012-6018.

Engebrecht, J., Nealson, K. & Silverman, M. (1983). Bacterial bioluminescence - isolation and genetic-analysis of functions from *Vibrio fischeri*. *Cell* 32: 773-781.

Fang, H., Tong, W.D., Perkins, R., Soto, A.M., Prechtl, N.V. & Sheehan, D.M. (2000). Quantitative comparisons of *in vitro* assays for estrogenic activities. *Environmental Health Perspectives* 108: 723-729.

Focazio, M.J., Kolpin, D.W., Barnes, K.K., Furlong, E.T., Meyer, M.T., Zaugg, S.D., Barber, L.B. & Thurman, M.E. (2008). A national reconnaissance for pharmaceuticals and other organic wastewater contaminants in the United States - II) Untreated drinking water sources. *Science of the Total Environment* 402: 201-216.

Folmar, L.C., Hemmer, M.J., Denslow, N.D., Kroll, K., Chen, J., Cheek, A., Richman, H., Meredith, H. & Grau, E.G. (2002). A comparison of the estrogenic potencies of estradiol, ethynylestradiol, diethylstilbestrol, nonylphenol and methoxychlor in vivo and in vitro. *Aquatic Toxicology* 60: 101-110.

Fossi, M.C. & Marsili, L. (2003). Effects of endocrine disruptors in aquatic mammals. *Pure and Applied Chemistry* 75: 2235-2247.

Gaido, K.W., Leonard, L.S., Lovell, S., Gould, J.C., Babai, D., Portier, C.J. & McDonnell, D.P. (1997). Evaluation of chemicals with endocrine modulating activity in a yeast-based steroid hormone receptor gene transcription assay. *Toxicology and Applied Pharmacology* 143: 205-212.

Gros, M., Petrovic, M. & Barcelo, D. (2009). Tracing pharmaceutical residues of different therapeutic classes in environmental waters by using liquid chromatography/quadrupole-linear ion trap mass spectrometry and automated library searching. *Analytical Chemistry* 81: 898-912.

Guillette, L.J., Brock, J.W., Rooney, A.A. & Woodward, A.R. (1999). Serum concentrations of various environmental contaminants and their relationship to sex steroid concentrations and phallus size in juvenile American alligators. *Archives of Environmental Contamination and Toxicology* 36: 447-455.

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

Hakkila, K., Maksimow, M., Karp, M. & Virta, M. (2002). Reporter genes *lucFF*, *luxCDABE*, *gfp*, and *dsred* have different characteristics in whole-cell bacterial sensors. *Analytical Biochemistry* 301: 235-242.

Hayes, T.B., V. Khoury, A. Narayan, M. Nazir, A. Park, t. Brown, L. Adame, E. Chan, D. Bucholz, T. Stueve, & S. Gallipeau. (2010). Atrazine induces complete feminization and chemical castration in make African clawed frogs (*Xenopus laevis*). *Proceeding of the National Academy of Science*, USA, 107:4612-4617.

Hein, R. & Tsien, R.Y. (1996). Engineering green fluorescent protein for improved brightness, longer wavelengths and fluorescence resonance energy transfer. *Current Biology* 6: 178-182.

Heitzer, A., Malachowsky, K., Thonnard, J.E., Bienkowski, P.R., White, D.C. & Sayler, G.S. (1994). Optical biosensor for environmental online monitoring of naphthalene and salicylate bioavailability with an immobilized bioluminescent catabolic reporter bacterium. *Applied and Environmental Microbiology* 60: 1487-1494.

Heitzer, A., Webb, O.F., Thonnard, J.E. & Sayler, G.S. (1992). Specific and quantitative assessment of naphthalene and salicylate bioavailability by using a bioluminescent catabolic reporter bacterium. *Applied and Environmental Microbiology* 58: 1839-1846.

Hellen, C.U.T. & Sarnow, P. (2001). Internal ribosome entry sites in eukaryotic mRNA molecules. *Genes & Development* 15: 1593-1612.

Jaio, B.W., Yeung, E.K.C., Chan, C.B. & Cheng, C.H.K. (2008). Establishment of a transgenic yeast screening system for estrogenicity and identification of the anti-estrogenic activity of malachite green. *Journal of Cellular Biochemistry* 105: 1399-1409.

Jardim, W., Montagner, C., Pescara, I., Umbuzeiro, G., Bergamasco, A., Eldridge, M. & Sodre, F. (2011). An intergrated approach to evaluate emerging contaminants in drinking water. *Separation and Purification Technology*. *In press.*

Johnson, F.H., Gershman, L.C., Waters, J.R., Reynolds, G.T., Saiga, Y. & Shimomura, O. (1962). Quantum efficiency of *Cypridina* luminescence, with a note on that of *Aequorea*. *Journal of Cellular and Comparative Physiology* 60: 85-103.

Kavlock, R.J., Daston, G.P., DeRosa, C., FennerCrisp, P., Gray, L.E., Kaattari, S., Lucier, G., Luster, M., Mac, M.J., Maczka, C., Miller, R., Moore, J., Rolland, R., Scott, G., Sheehan, D.M., Sinks, T. & Tilson, H.A. (1996). Research needs for the risk assessment of health and environmental effects of endocrine disruptors: a report of the US EPA-sponsored workshop. *Environmental Health Perspectives* 104: 715-740.

Keane, A., Phoenix, P., Ghoshal, S. & Lau, P.C.K. (2002). Exposing culprit organic pollutants: a review. *Journal of Microbiological Methods* 49: 103-119.

Kendall, J.M. & Badminton, M.N. (1998). *Aequorea victoria* bioluminescence moves into an exciting new era. *Trends in Biotechnology* 16: 216-224.

Kidd, K. A., Blanchfield, P. J., Mills, K. H., Palace, V. P., Evans, R. E., Lazorchak, J. M., & Flick, R. W. (2007). Collapse of a fish population after exposure to a synthetic estrogen. *Proceeding of the National Academy of Science*, USA, 104:8897–8901.

King, J.M.H., Digrazia, P.M., Applegate, B., Burlage, R., Sanseverino, J., Dunbar, P., Larimer, F. & Sayler, G.S. (1990). Rapid, sensitive bioluminescent reporter technology for naphthalene exposure and biodegradation. *Science* 249: 778-781.

Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B. & Buxton, H.T. (2002). Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999-2000: A national reconnaissance. *Environmental Science & Technology* 36: 1202-1211.

Korach, K.S., Sarver, P., Chae, K., McLachlan, J.A. & McKinney, J.D. (1988). Estrogen receptor-binding activity of polychlorinated hydroxybiphenyls - conformationally restricted structural probes. *Molecular Pharmacology* 33: 120-126.

Kuster, M., Azevedo, D.A., de Alda, M.J.L., Neto, F.R.A. & Barcelo, D. (2009). Analysis of phytoestrogens, progestogens and estrogens in environmental waters from Rio de Janeiro (Brazil). *Environment International* 35: 997-1003.

Layton, A.C., Gregory, B.W., Seward, J.R., Schultz, T.W. & Sayler, G.S. (2000). Mineralization of steroidal hormones by biosolids in wastewater treatment systems in Tennessee USA. *Environmental Science & Technology* 34: 3925-3931.

Le Guevel, R. & Pakdel, F. (2001). Streamlined beta-galactosidase assay for analysis of recombinant yeast response to estrogens. *Biotechniques* 30: 1000-1004.

Leskinen, P., Hilscherova, K., Sidlova, T., Kiviranta, H., Pessala, P., Salo, S., Verta, M. & Virta, M. (2008). Detecting AhR ligands in sediments using bioluminescent reporter yeast. *Biosensors & Bioelectronics* 23: 1850-1855.

Leskinen, P., Michelini, E., Picard, D., Karp, M. & Virta, M. (2005). Bioluminescent yeast assays for detecting estrogenic and androgenic activity in different matrices. *Chemosphere* 61: 259-266.

Ma, M., Rao, K.F. & Wang, Z.J. (2007). Occurrence of estrogenic effects in sewage and industrial wastewaters in Beijing, China. *Environmental Pollution* 147: 331-336.

Meighen, E.A. & Dunlap, P.V. (1993). Physiological, biochemical and genetic-control of bacterial bioluminescence. In *Advances in Microbial Physiology, Vol 34*. London, Academic Press Ltd. 34: 1-67.

Michelini, E., Leskinen, P., Virta, M., Karp, M. & Roda, A. (2005). A new recombinant cell-based bioluminescent assay for sensitive androgen-like compound detection. *Biosensors & Bioelectronics* 20: 2261-2267.

Miller, C.A., Martinat, M.A. & Hyman, L.E. (1998). Assessment of aryl hydrocarbon receptor complex interactions using pBEVY plasmids: expression vectors with bi-directional promoters for use in *Saccharomyces cerevisiae*. *Nucleic Acids Research* 26: 3577-3583.

Nivens, D.E., McKnight, T.E., Moser, S.A., Osbourn, S.J., Simpson, M.L., & Sayler, G.S. (2004). Bioluminescent bioreporter integrated circuits: potentially small, rugged and inexpensive whole-cell biosensors for remote environmental monitoring. *Journal of Applied Microbiology* 96:33-46.

O'Connor, J.C., Cook, J.C., Marty, M.S., Davis, L.G., Kaplan, A.M. & Carney, E.W. (2002). Evaluation of Tier I screening approaches for detecting endocrine-active compounds (EACs). *Critical Reviews in Toxicology* 32: 521-549.

OECD (2002). Appraisal of test methods for sex-hormone disrupting chemicals. Detailed Review Paper. Series on testing andassessments. No. 21. Paris, France, Organization for Economic Co-operation and Development (OECD).

Owens, C.V., Lambright, C., Bobseine, K., Ryan, B., Gray, L.E., Gullett, B.K. & Wilson, V.S. (2007). Identification of estrogenic compounds emitted from the combustion of computer printed circuit boards in electronic waste. *Environmental Science & Technology* 41: 8506-8511.

Patterson, S.S., Dionisi, H.M., Gupta, R.K. & Sayler, G.S. (2005). Codon optimization of bacterial luciferase (*lux*) for expression in mammalian cells. *Journal of Industrial Microbiology & Biotechnology* 32: 115-123.

Purvis, I.J., Chotai, D., Dykes, C.W., Lubahn, D.B., French, F.S., Wilson, E.M. & Hobden, A.N. (1991). An androgen-inducible expression system for *Saccharomyces cerevisiae*. *Gene* 106(1): 35-42.

Raman, D.R., Williams, E.L., Layton, A.C., Burns, R.T., Easter, J.P., Daugherty, A.S., Mullen, M.D. & Sayler, G.S. (2004). Estrogen content of dairy and swine wastes. *Environmental Science & Technology* 38: 3567-3573.

Reemtsma, T., Weiss, S., Mueller, J., Petrovic, M., Gonzalez, S., Barcelo, D., Ventura, F. & Knepper, T.P. (2006). Polar pollutants entry into the water cycle by municipal wastewater: a European perspective. *Environmental Science & Technology* 40: 5451-5458.

Rehmann, K., Schramm, K.W. & Kettrup, A.A. (1999). Applicability of a yeast oestrogen screen for the detection of oestrogen-like activities in environmental samples. *Chemosphere* 38: 3303-3312.

Ripp, S., DiClaudio-Eldridge, M.L. & Sayler, G.S. (2010). Biosensors as environmental monitors. In *Environmental Microbiology, 2nd Edition*. R. Mitchell and J. D. Gu. Hoboken, NJ, Wiley-Blackwell: 213-233.

Roda, A., Cevenini, L., Michelini, E. & Branchini, B.R. (2011). A portable bioluminescence engineered cell-based biosensor for on-site applications. *Biosensors & Bioelectronics* 26: 3647-3653.

Ropstad, E., Oskam, I.C., Lyche, J.L., Larsen, H.J., Lie, E., Haave, M., Dahl, E., Wiger, R. & Skaare, J.U. (2006). Endocrine disruption induced by organochlorines (OCs): field studies and experimental models. *Journal of Toxicology and Environmental Health-Part a-Current Issues* 69: 53-76.

Routledge, E.J., Parker, J., Odum, J., Ashby, J. & Sumpter, J.P. (1998). Some alkyl hydroxy benzoate preservatives (parabens) are estrogenic. *Toxicology and Applied Pharmacology* 153: 12-19.

Routledge, E.J. & Sumpter, J.P. (1996). Estrogenic activity of surfactants and some of their degradation products assessed using a recombinant yeast screen. *Environmental Toxicology and Chemistry* 15: 241-248.

Sanseverino, J., Eldridge, M.L., Layton, A.C., Easter, J.P., Yarbrough, J., Schultz, T.W. & Sayler, G.S. (2009). Screening of potentially hormonally active chemicals using bioluminescent yeast bioreporters. *Toxicological Sciences* 107: 122-134.

Sanseverino, J., Gupta, R.K., Layton, A.C., Patterson, S.S., Ripp, S.A., Saidak, L., Simpson, M.L., Schultz, T.W. & Sayler, G.S. (2005). Use of *Saccharomyces cerevisiae* BLYES expressing bacterial bioluminescence for rapid, sensitive detection of estrogenic compounds. *Applied and Environmental Microbiology* 71: 4455-4460.

Sayler, G.S., Ripp, S., Nivens, D., & Simpson, M. (2001). Bioluminescent Bioreporter Integrated Circuits: Sensing Analytes and Organisms with Living Microorganisms. *Journal of Environmental Biotechnology* 1: 33-39.

Sayler, G.S., Simpson, M.L., & Cox, C.D. (2004). Emerging foundations: nano-engineering and bio-microelectronics for environmental biotechnology. *Current Opinion in Microbiology* 7:267-273.

Schultz, T.W. (2002). Estrogenicity of biphenylols: activity in the yeast gene activation assay. *Bulletin of Environmental Contamination and Toxicology* 68: 332-338.

Schultz, T.W., Kraut, D.H., Sayler, G.S. & Layton, A.C. (1998). Estrogenicity of selected biphenyls evaluated using a recombinant yeast assay. *Environmental Toxicology and Chemistry* 17: 1727-1729.

Schultz, T.W. & Sinks, G.D. (2002). Xenoestrogenic gene exression: structural features of active polycyclic aromatic hydrocarbons. *Environmental Toxicology and Chemistry* 21: 783-786.

Schultz, T.W., Sinks, G.D. & Cronin, M.T.D. (2002). Structure-activity relationships for gene activation oestrogenicity: Evaluation of a diverse set of aromatic chemicals. *Environmental Toxicology* 17: 14-23.

Shimomura, O., Johnson, F.H. & Saiga, Y. (1962). Extraction, purification and properties of aequorin, a bioluminescent protein from luminous hydromedusan, *Aequorea*. *Journal of Cellular and Comparative Physiology* 59: 223-239.

Sohoni, P., Lefevre, P.A., Ashby, J. & Sumpter, J.P. (2001). Possible androgenic/anti-androgenic activity of the insecticide fenitrothion. *Journal of Applied Toxicology* 21: 173-178.

Soni, M.G., Taylor, S.L., Greenberg, N.A. & Burdock, G.A. (2002). Evaluation of the health aspects of methyl paraben: a review of the published literature. *Food and Chemical Toxicology* 40: 1335-1373.

Sonne, C., Leifsson, P.S., Dietz, R., Born, E.W., Letcher, R.J., Hyldstrup, L., Riget, F.F., Kirkegaard, M. & Muir, D.C.G. (2006). Xenoendocrine pollutants may reduce size of sexual organs in East Greenland polar bears (*Ursus maritimus*). *Environmental Science & Technology* 40: 5668-5674.

Stoker, T.E., Gibson, E.K. & Zorrilla, L.M. (2010). Triclosan exposure modulates estrogen-dependent responses in the female Wistar rat. *Toxicological Sciences* 117: 45-53.

Svobodova, K. & Cajthaml, T. (2010). New in vitro reporter gene bioassays for screening of hormonal active compounds in the environment. *Applied Microbiology and Biotechnology* 88: 839-847.

Svobodova, K., Plackova, M., Novotna, V. & Cajthaml, T. (2009). Estrogenic and androgenic activity of PCBs, their chlorinated metabolites and other endocrine disruptors estimated with two in vitro yeast assays. *Science of the Total Environment* 407: 5921-5925.

Szittner, R. & Meighen, E. (1990). Nucleotide-sequence, expression, and properties of luciferase coded by *lux* genes from a terrestrial bacterium. *Journal of Biological Chemistry* 265: 16581-16587.

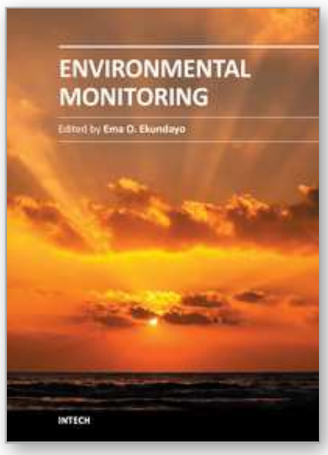
Thomas, K.V., Hurst, M.R., Matthiessen, P., McHugh, M., Smith, A. & Waldock, M.J. (2002). An assessment of in vitro androgenic activity and the identification of environmental androgens in United Kingdom estuaries. *Environmental Toxicology and Chemistry* 21: 1456-1461.

Tyler, C.R., Jobling, S. & Sumpter, J.P. (1998). Endocrine disruption in wildlife: a critical review of the evidence. *Critical Reviews in Toxicology* 28: 319-361.

United States-EPA (2007). Pharmaceuticals and personal care products in water, soil, sediment, and biosolids by HPLC/MS/MS. *Method 1694*.

Zacharewski, T. (1997). *In vitro* bioassays for assessing estrogenic substances. *Environmental Science & Technology* 31: 613-623.

Zheng, W., Yates, S. & Bradford, S. (2008). Analysis of steroid hormones in a typical dairy waste disposal system. *Environmental Science & Technology* 42: 530-535.



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