

OVERVIEW SHEET

Cardiovascular Disease Applications

Bioluminescent imaging offers the advantage of non-invasively tracking the progression of disease in animal models (McCaffrey et al., 2003). Therefore, the same animal can be followed over time and the response to treatment can be assessed without the need for measuring circulating markers, or terminal histological assessments. Several applications coupling luciferase reporters with a sensitive imaging system (IVIS® Imaging System) can be used to study cardiovascular diseases including cardiac ischemia, hypertrophy, stroke, and potentially the inflammation component of arteriosclerosis. A variety of transgenic mice have been developed that use the promoters from specific genes to drive luciferase expression. These bioluminescent animals (LPTA® animal models) produced by Xenogen, and others created in academic laboratories, could be useful for non-invasively monitoring cardiovascular disease. For data sheets and/or publications describing the LPTA® animal models created by Xenogen, visit the Xenogen website at <http://www.xenogen.com>. For the models created by academic laboratories we have cited the appropriate references.

Ischemic Heart Disease

A transgenic mouse model has been developed that fuses the human promoter for the brain (B-Type) natriuretic protein gene to firefly luciferase (*hBNP-luc*; He et al., 2001). The BNP gene is significantly induced by ventricular tissue damage and circulating levels of BNP are a good predictor of left ventricular dysfunction. BNP is secreted into the circulation and functions as a natriuretic, diuretic and vasorelaxant. Therefore, BNP gene induction also may be predictive of the extent of tissue damage due to a myocardial infarct. A standard model of

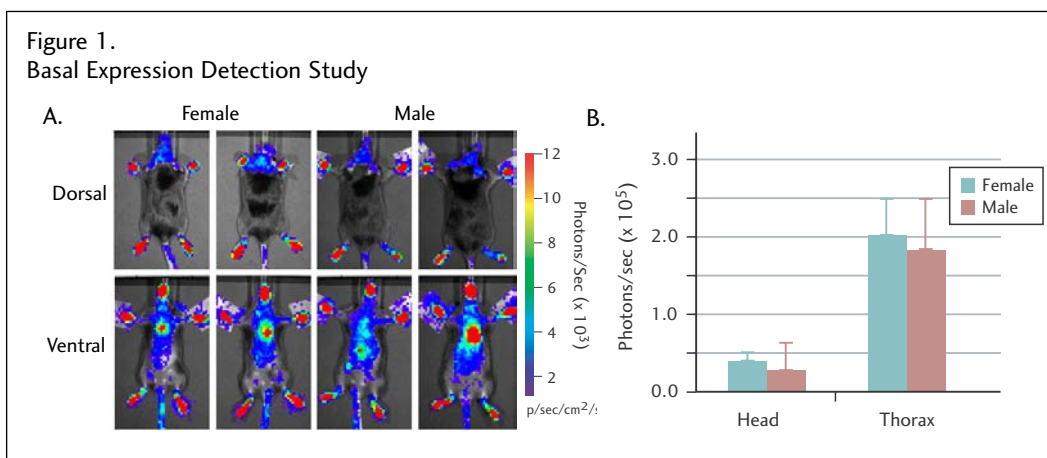


Figure 1. Male and female *hBNP-luc* mice were anesthetized, injected with luciferin (150 mg/kg), and imaged with an IVIS® Imaging System 200 Series. Images were collected for two minutes from 2 male and 2 female mice from the dorsal and ventral sides. Basal expression was detected in the head and the thoracic region confirming the results of He et al., (2001) who showed that the highest basal expression was in the heart and brain.

cardiac ischemia is ligation of the left anterior descending coronary artery (e.g., Yang et al., 1997). Ligation of this artery results in significant hypoxia and tissue damage to coronary muscle. A baseline luciferase signal is detectable in ventricle tissue extracts of the *hBNP-luc* mice. Coronary artery ligation results in approximately a 5-fold induction of the luciferase reporter signal measured in ventricle tissue homogenates 48 hours after infarct. Recently, we have taken the *hBNP-luc* mice described by He et al., (2001) and have determined that a baseline luciferase signal is detectable by imaging (Figure 1, page 1). Therefore, it may be possible to use this transgenic model for non-invasively following the consequences of infarct over time after coronary artery ligation.

Heme-oxygenase-1 is a ubiquitously expressed "heat shock" protein (HSP-32) that is induced by toxins as well as ischemia. This enzyme catalyzes the first and rate-limiting step in heme degradation and also has other cellular functions. Over-expression of the HO1 protein in the heart has been shown to protect cardiac muscle from ischemic damage (Pachori et al., 2004). Furthermore, HO1 protein levels are elevated in LDLR (low density lipoprotein receptor) $-/-$ transgenic mice with arteriosclerosis, perhaps due to the activation of the prostacyclin PGI₂ (Egan et al., 2004). A transgenic mouse has been constructed by Xenogen that fuses the promoter of the murine HO1 gene to firefly luciferase (*mHo1-luc*; Zhang et al., 2002). This model has been used to monitor toxicity due to a variety of agents including heavy metals and drugs causing known liver or kidney toxicity (Malstrom et al., 2004). Figure 2 below shows the induction of this reporter in *mHo1-luc* transgenic mouse liver following treatment with doxorubicin. The HO1 gene is known to be induced in a variety of tissues due to ischemia including brain (Imaizumi et al., 2003) and heart (Masini et al., 2003). Therefore, the *mHo1-luc* transgenic mouse could be used as an *in vivo* sensor to assess both the extent of tissue ischemia as well as induction of this gene by potential therapeutic compounds. Xenogen has also developed other transgenic mice that express luciferase under the control of the mSOD1 promoter, and the my-GCS promoter. Both of these genes are induced by

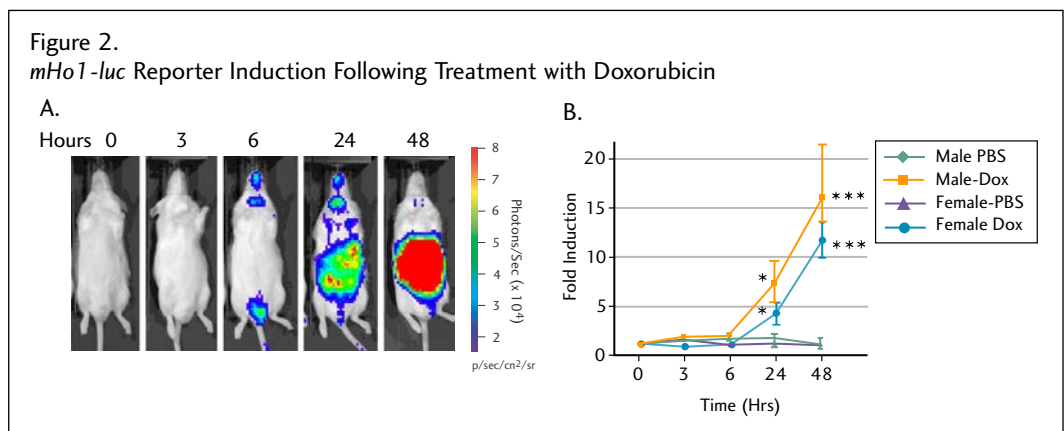


Figure 2. The left panel presents ventral images of a representative male *mHo1-luc* mouse treated with 15mg/kg of doxorubicin (i.p.) and imaged at baseline and 3, 6, 24 and 48 hours after injection. The chart panel presents the fold induction of the luciferase signal, relative to baseline, for the liver region of interest for male and female, control and doxorubicin-treated, *mHo1-luc* mice. After dox injection, induction of the luciferase reporter was observed by 24 hours after injection and a 13–16 fold induction was observed 48 hours after injection.

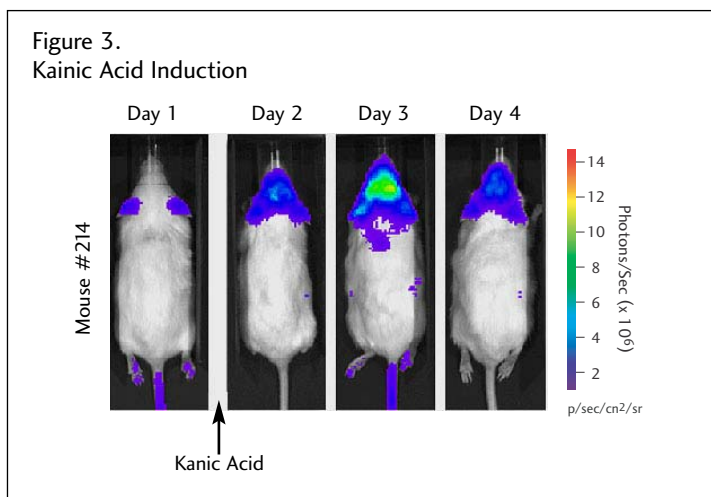


Figure 3. Male and female *mGfap-luc* mice were injected with kainic acid (intraperitoneal; 30mg/kg) and imaged on the day of injection and daily thereafter for 4 days using the IVIS® Imaging System 100 Series. A representative mouse is shown in this figure. Peak induction of the luciferase reporter occurred approximately 48 hrs after kainic acid and this correlated with both endogenous Gfap mRNA levels and neuronal damage in the hippocampus.

oxidative stress and the transgenic mice could be useful reporters of cardiac ischemia.

Another approach to study cardiac ischemia by bioluminescent imaging is to utilize adeno-associated viral vectors to deliver reporter genes to the heart that are driven by cardiac specific promoters. These vectors can be delivered by either i.v. injection or by injecting them directly into cardiac muscle. For example, one rAAV vector was constructed which consisted of hypoxia response elements (HREs), a heart specific promoter (mLC2v), a therapeutic gene, and a green fluorescent protein (Phillips et al., 2002; also see Tang et al., 2005). Following i.v. injection, green fluorescent protein mRNA was detectable only in cardiac tissue, and it was induced in a mouse model of myocardial infarct. Such an approach could be used with a luciferase reporter to track non-invasively the physiological changes induced by ischemia and reperfusion injury.

Cardiac Hypertrophy

A transcription factor called NFAT (nuclear factor of activated T cells) is important for both immune function and mediating cardiac hypertrophy in heart failure (Ritter et al., 2002). This transcription factor is activated via dephosphorylation by calcineurin. Transgenic mice have been created by Wilkins et al., (2004) using a transgene constructed by fusing 9 NFAT binding sites to the minimal promoter of the α -myosin heavy chain gene and the coding sequence for firefly luciferase. Ligation of the left anterior descending coronary artery induced luciferase activity of this reporter approximately 5-fold in the failing hearts of mice 21 days after ligation. In addition, constriction of the transverse aortic arch causing a pressure overload was used to induce cardiac hypertrophy in the *NFAT-RE-luc* mice. Aortic constriction more than doubled the luciferase signal in heart tissue extracts two days after placing the ligature. The elevated luciferase signal gradually increased over time and was 3 fold above sham-operated levels by day 56 post ligation. Therefore, this model could be a useful model for tracking heart failure in mice non-invasively by bioluminescent imaging. However, these mice have not yet been imaged to determine the feasibility of using this model for *in vivo* studies.

Stroke

The glial fibrillary acidic protein (GFAP) gene is expressed exclusively in the central nervous system in astrocytes and the gene is induced following stroke (Clark et al., 1993), as well as Alzheimer's disease, and during inflammation after a closed-head injury. A variety of experimental procedures and genetic manipulations in the mouse have been developed to produce

neuronal cell death, including blunt head trauma, cerebral artery occlusion, and neurotoxin treatment. Xenogen has developed an LPTA® animal model that expresses luciferase under the control of the murine GFAP promoter (*mGfap-luc*; Zhu et al., 2004). Following peripheral intravenous injection of the neurotoxin, kainic acid, there is a clear induction of the transgene in *Gfap-luc* mice by 48 hours following injection (see Figure 3, page 3). The luciferase signal is correlated with expression of the endogenous gene and with neuronal damage in the hippocampus. Therefore, the *mGfap-luc* model may be a useful tool for non-invasively tracking the effects of CNS ischemia and resulting neuronal death in the mouse. Other LPTA® animal models that sense hypoxia (e.g., *mH₁-luc*) may also be of use to study CNS recovery from stroke; however, these other models have not yet been tested for that application.

Inflammation and Arteriosclerosis

Xenogen has developed a set of LPTA® animal models using the promoters from genes that participate in various inflammatory responses and pathways. Promoters from murine iNOS (inducible nitric oxide synthase; see Zhang et al., 2003), iκB (inhibitor of NFκB; see Zhang et al., 2005), IL2 (interleukin-2), and TNFα (tumor necrosis factor alpha) have been used to create transgenic mice expressing luciferase. In addition, the response elements for NFκB fused to the SV40 promoter has been used to drive luciferase expression in a transgenic mouse (Carlsen et al., 2002). Several of these genes and pathways are induced in the endothelial and/or smooth muscle cells at the site of arteriosclerotic plaque formation. For example, the NFκB cascade is activated in the vascular wall of the major arteries in arteriosclerosis (for review see Monaco et al., 2004) and macrophages expressing iNOS are abundant in atherosclerotic plaques (Boyle, 2005).

Since there is a significant inflammatory response that contributes to plaque formation in arteriosclerosis, the Xenogen inflammation LPTA® mice could be used to study the inflammation component of plaque formation. A number of transgenic mouse models are available in which arteriosclerotic plaques develop when the animals are placed on a high cholesterol diet. Transgenic mice coexpressing the CETP and ApoB100 genes (Grass et al., 1995), and the LDLR knockout mice and other transgenic models (Tangirala RK et al., 1995), develop complex plaques in the aorta. These models of arteriosclerosis could be bred to one of the Xenogen LPTA® animal models that express luciferase under the control of the iNOS, NFκB response elements, or the IL2 promoter. Alternatively, lesions on the intima develop following ligation/irritation of the carotid artery (Harmon et al., 2000) and this surgical procedure, applied to the *miNOS-luc* or the *mNFκB-RE-luc* models, would be expected to produce a local inflammatory response that could be used to track and monitor the extent of plaque formation and response to treatment.

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Note: For LPTA[®] animal model lines CYP3a11, CYP3A4 rat, Epx, Vegfr2 and Vegf: these product lines and their use are claimed by pending U.S. and foreign patent applications owned by Xenogen Corporation.

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In vivo imaging in mammals is covered by one or more U.S. and foreign patents controlled by Xenogen Corporation, including the following: U.S. patent numbers 6,649,143, 6,217,847, and 5,650,135 and European Union patent number 0861093. A license from Xenogen Corporation is required to practice under these patents.

Xenogen Corporation, 860 Atlantic Avenue, Alameda, CA 94501, USA Toll Free 877.936.6436
Phone 510.291.6100 Fax 510.291.6196 E-mail: imaging@xenogen.com www.xenogen.com