PHYLONIX

COMPANY BACKGROUND

Phylonix is a Contract Research Organization developing and marketing *in vivo* zebrafish based assays for therapeutic drug screening for research and preclinical studies. The Company has established a state of the art aquaculture facility in Cambridge, MA with the capacity to produce thousands of embryos per week. Resources include automated sample handling devices, microplate readers, microscopes and flow cytometers for performing high throughput drug screens.

A New Era in Animal Testing

Zebrafish develop rapidly. Three days after fertilization, the embryo is essentially complete, with a functioning heart, circulatory and nervous system. This rapid development is comparable to three months of human development. In addition, zebrafish have a relatively short generation time (2-3 months) and produce large clutches of embryos (100-200) per mating.

By day four, the zebrafish embryo has hatched and can eat and swim. Intestinal epithelial cells are polarized and express digestive enzymes. Hepatocytes secrete bile. Pancreatic islets and acini produce insulin and carboxypeptidase. Since zebrafish embryos are transparent, every event in early develoment can be observed visually. Quantitative endpoint assays can also be performed using microplate readers, similar to cell based formats.

Advantages of Zebrafish

Zebrafish currently rival mice and rats as a popular laboratory animal model. Interest in zebrafish for drug screening is rapidly increasing due to several inherent advantages, including:

- *Access to All Developmental Stages*
- *Easy Manipulation for Automated or Visual Screens*
- *Drug Administration Directly to Fish Water or by Microinjection*
- *Rapid Vertebrate Organogenesis*
- *Statistically Significant Number of Animals per Test*
- *Small Amount of Drug Required*
- *Low Cost*

PHYLONIX
100 Inman Street, Suite 300 - Cambridge, Massachusetts 02139
Tel: 617-441-6700 - Fax: 617-441-6766
www.phylonix.com

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TOXICITY SERVICES

Phylonix is a leader in providing zebrafish screening services for drug toxicity profiling.

General Toxicity

We have evaluated general toxicity including: LD50, teratogenic effects, organ and developmental toxicity for numerous clinically available drugs. Zebrafish exhibits mammalian-comparable toxic responses. We have also demonstrated that zebrafish embryos provide a unique opportunity for investigating developmental drug effects. Phylonix has established standard procedures to efficiently and accurately evaluate general toxicity in various drug regimens. We can rapidly profile compound toxicity prior to expensive and time-consuming mammalian animal testing. Drugs are delivered either by addition to the growth medium or by microinjection. General toxicity studies include:

- *Acute Single Dose*
- *Sub-Chronic Multiple Dose*
- *Chronic Dosing*

Specific Toxicity

We have developed *in vivo* vital dye staining protocols, organ-specific antibodies, and flow cytometry methods for performing toxicity studies on organs and tissues. The morphology of each organ can be assessed by visual assays and early disease status can be determined by fluorescent staining and flow cytometry analysis.

Specific assays are available for the following:

- *Angiogenesis*
- *Cardiovascular Development*
- *Neurotoxicity*
- *Liver Toxicity*
- *Cartilage Development*
- *Carcinogenicity*
- *Gastrointestinal Toxicity*
- *Cell Proliferation*
- *Gene Expression*
- *Reproductive Toxicology*
- *Developmental Toxicity*

Genetic Toxicology

- *DNA Fragmentation*

To investigate drug induced genotoxicity, we offer *in vivo* testing for DNA fragmentation using electrophoresis, vital dye staining and flow cytometry.

Pathology

We provide three types of pathology studies:

- *In situ Hybridization*
- *Necropsy*
- *Apoptosis*

Expression patterns of specific genes at different developmental stages can be determined by *in situ* hybridization. Pathology studies using *in vivo* fluorescent staining can be performed to assess the sites of necrosis and apoptosis. Cellular events of interest can be quantitated by flow cytometry.

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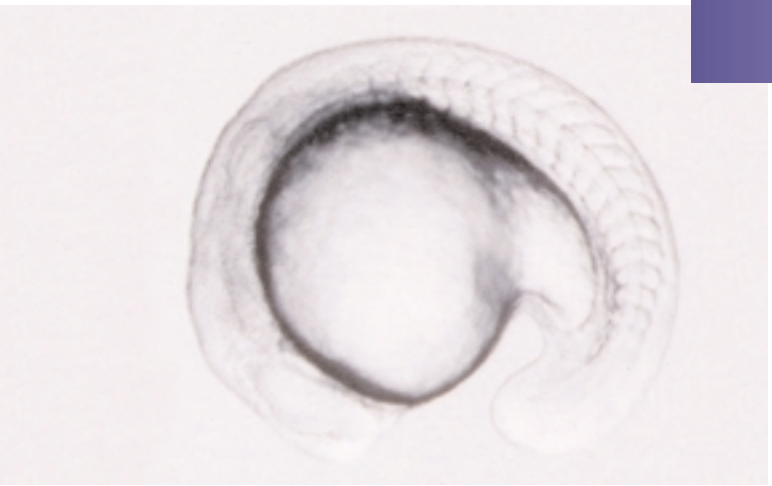


CUSTOM DESIGNED STUDIES

Our team of Developmental Biologists, Biochemists, Toxicologists, Pharmocologists, Cell Biologists, Geneticists, Molecular Biologists, and Aquaculturists work closely with Customers under the direction of a Project Leader to provide the integrated services necessary to reduce the time and expense of research and pre-clinical studies. Services include protocol design and specialized assay development tailored to Customer specifications.

Cellular Activity

We have developed biochemical methods to detect reactive oxygen species and nitric oxide, as well as fluorescence microplate assays for caspase activity. Our flow cytometry service conducts cell cycle analysis on cells from whole embryos or specific cell types after drug treatment. Analysis includes:

- *Reactive Oxygen Species*
- *Caspase Activity*
- *Cell Cycle Profiling*

Behavior

Zebrafish exhibit simple behavior making it an ideal model for studying neuronal defects. Assessments include:

- *Touch Response*
- *Motor Activity*
- *Auditory Startle*
- *Chemical Sensitivity*

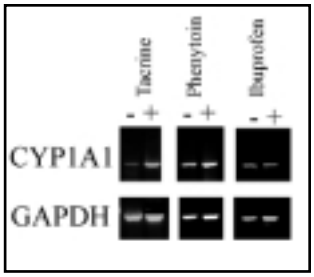
Target Validation

We use gene specific anti-sense probes for drug target validation.

Gene Profiling

We are using zebrafish arrays to examine gene expression patterns after drug treatment. We have correlated results with effects on neurotoxicity, gastrointestinal toxicity and developmental defects observed in mammals. Our data base can be used to predict gene response in the following categories:

- *Apoptosis*
- *Xenobiotic Enzymes*
- *Inflammatory Response*
- *Stress Response*
- *Drug Resistance*
- *Proliferation*
- *Cell Cycle*



Cell Cycle

Our flow cytometry service also performs cell cycle analysis after drug treatment to investigate and validate the effects of cell cycle blockers on whole embryos or specific tissues or organs.

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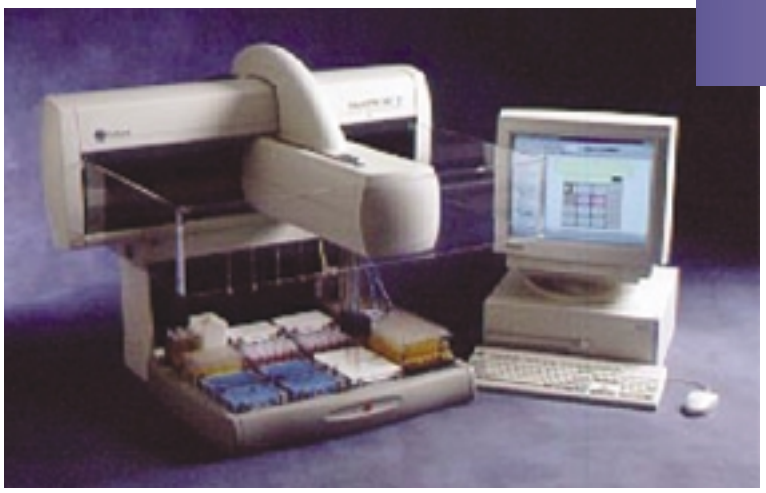

HTS OF CHEMICAL LIBRARIES USING ZEBRAFISH EMBRYOS

We offer quantitative microplate based whole animal High Throughput Screening Services for evaluating drug effects on Angiogenesis and Apoptosis. We have combined liquid sample handling devices and microplate readers to analyze large chemical libraries. Small molecules are delivered directly in the fish water and proteins are injected. Additional effects of "hits" can be assessed visually.

Advantages of Zebrafish for HTS

Delivery of small molecules by direct addition to the embryo in a microwell is an advantage for use with automated sample handling instrumentation. The ability to screen large libraries and rapidly assess statistically significant numbers of animals are important advantages of our approach. High throughput screening of whole animals after drug treatment will complement testing in cell-based assays. Assessment of drug effects in a convenient vertebrate model, prior to proceeding to evaluation in mice, will streamline drug development and dramatically reduce costs.

Advantages include:

- *Short Assay Time (3-5 Days)*
- *Single Dosing*
- *Small Drug Amount*
- *Statistically Significant Numbers of Animals*
- *Quantitative Endpoint Assays*
- *Easy Manipulation*
- *Rapid Assessment of Large Chemical Libraries*

Angiogenesis

We use a rapid, enzyme specific primary drug screen and a vessel specific antibody based secondary screen. We analyze whole embryos in a quantitative 96-microwell format or in a multiparameter visual screen. An *in vivo* microangiography assay is used for validation and our array analysis can detect vessel-specific gene expression.

Apoptosis

We use fluorescent staining to assess the sites of apoptosis *in vivo*. The cell type and the degree of apoptosis is assessed by flow cytometry or fluorescence microplate assays. We can precisely detect apoptotic activity in whole embryos and rank potential apoptotic activators and inhibitors.

Additional High Throughput Screens Are Under Development.

Please Inquire.

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