

HepG2-CYP™ cell panel

New tools to assist in identifying human routes of metabolism, CYP phenotyping and drug/drug interactions

Hera BioLabs

services@herabiolabs.com

The central graphic is a composite image. On the left, a word cloud contains terms such as 'Neurotoxicity Screening', 'ADME', 'Translational', 'Predictive', 'Hepatotoxicity', 'Drug safety', 'Inhibition', 'Cellular', 'Disease models', 'Patient specific', 'Cardio-tox', 'Toxicology', 'PKPD', 'ADME', 'Patient specific', 'Pharmacokinetic', 'Induction', 'DILI', 'Induction', and 'Xenografts'. In the center, there is a stylized illustration of a liver with various colored regions. On the right, a diagram shows a large white mouse with a red mouse and four smaller white mice, with arrows indicating a flow or relationship between them. The background is a light blue gradient.



Precision Toxicology™ & Efficacy
CRO Services

- 🧬 HepG2 is one of the most commonly used cell systems for *in vitro* screening, but HepG2 cells have low expression and activity of CYP enzymes which leads to missed or underestimated cytotoxicity of test compounds and/or metabolites
- 🧬 Hera Biolabs has assembled a panel of HepG2 transgenic cell lines that each stably express one of the major human CYPs
- 🧬 Hera has also created a CYP reductase (POR) knockout HepG2 cell line
- 🧬 Used in appropriate assay panels, these cells can address a variety of questions pertaining to CYP mediated metabolism and drug/drug interaction
 - CYP metabolism phenotyping can be accomplished directly with minimal or no use of inhibitors
 - Direct or indirect determination of CYP inhibition
 - CYP specific cytotoxicity

HepG2-CYP Cell Panel	Additional Cell Lines
HepG2 CYP reductase KO	HepG2-CYP1A1
HepG2-CYP1A2	HepG2-CYP2A6
HepG2-CYP2B6	HepG2-CYP2E1
HepG2-CYP2C9	HepG2-CYP2D6
HepG2-CYP3A4	HepG2-CYP2C19
	HepG2-CYP2C8

The HepG2-CYP cell panel has been fully validated in-house at Hera and published in our SOT 2017 poster *Effect of Transfecting HepG2 with Human CYP Enzymes on Chemical Toxicity*

Additional Cell Lines were created by & validated at Takeda Pharmaceutical Company Limited (Osaka, Japan)

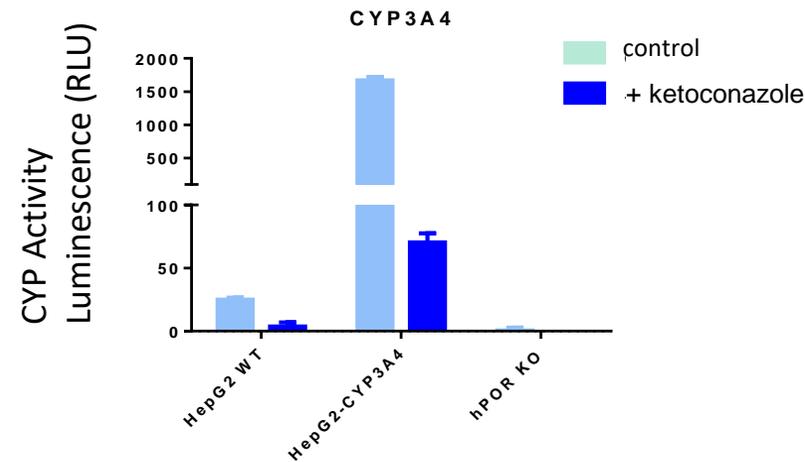
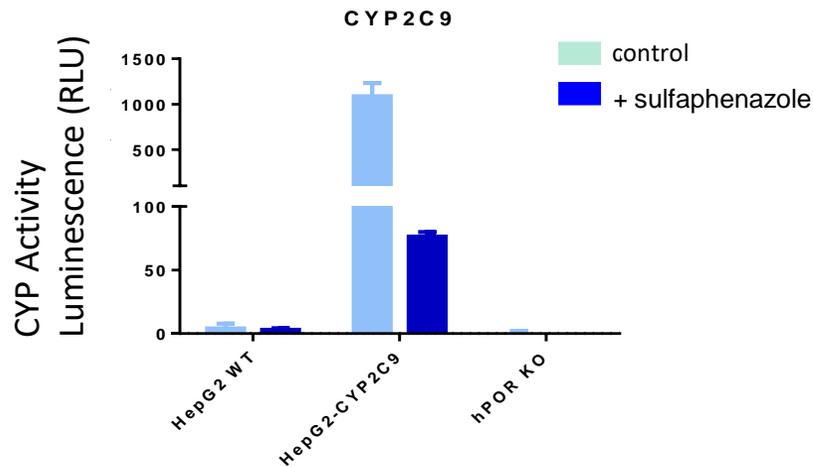
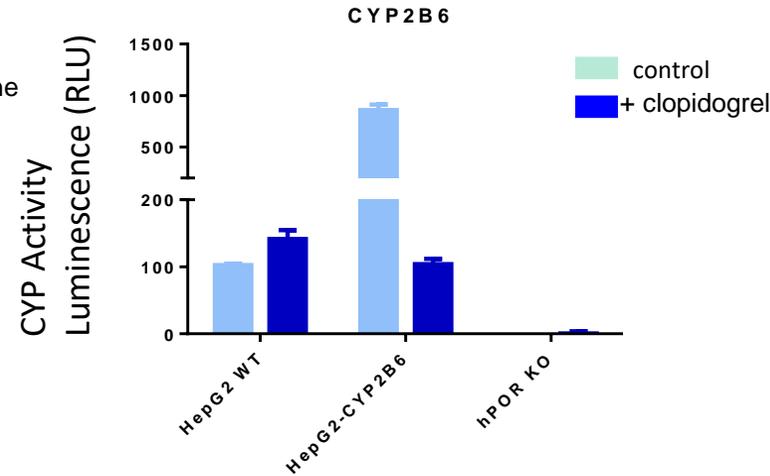
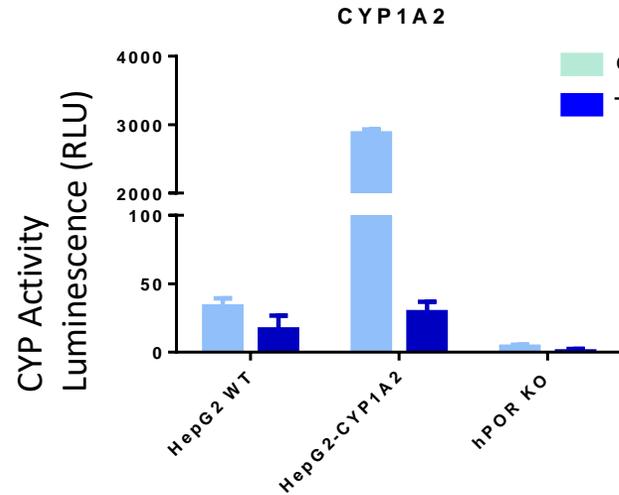
The CYP reductase (POR) knockout was created using gene editing technology at Hera

The transgenic CYP HepG2 cell lines were created and published by Takeda Pharmaceutical Company Limited (Osaka, Japan)

Takeda transgenic CYP publications

- *Establishment of the transformants expressing human cytochrome P450 subtypes in HepG2, and their applications on drug metabolism and toxicology* (Hashizume et al, 2010)
- *Advantages of Human Hepatocyte-Derived Transformants Expressing a Series of Human Cytochrome P450 Isoforms for Genotoxicity Examination* (Yoshitomi et al, 2001)
- *In vitro micronucleus test in HepG2 transformants expressing a series of human cytochrome P450 isoforms with chemicals requiring metabolic activation* (Hashizume et al, 2009)

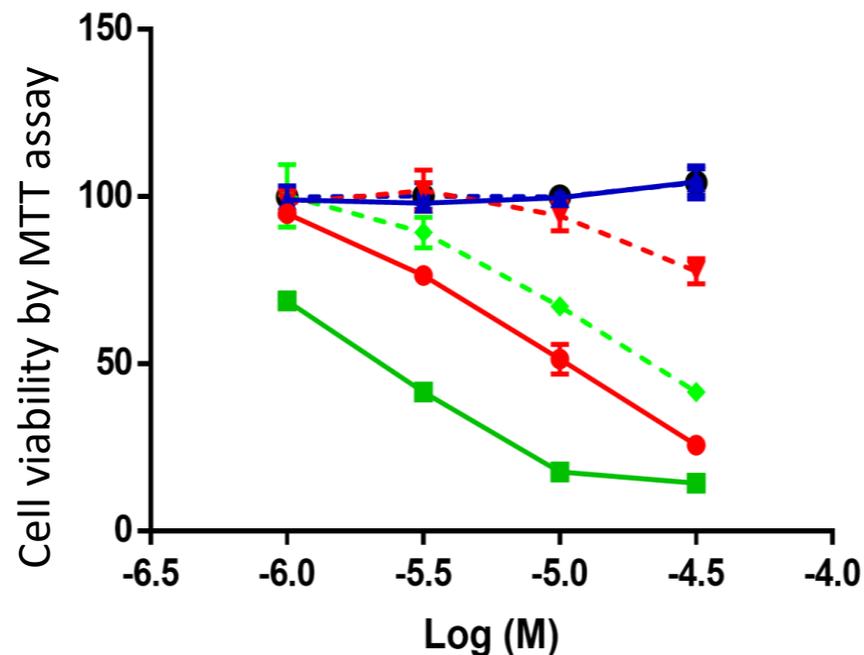
HepG2-CYP™ cell restore while reductase KO abolishes CYP activity



CYP activity in HepG2 WT, HepG2-CYP transgenic and HepG2-CYP reductase KO (POR) cells

CYP activity was measured with P450 –Glo™ assay kits. Specific CYP activity in transgenic cells was confirmed with CYP-selective competitive inhibitors, namely 5 μM α-naphthoflavone (CYP1A2), 1 μM clopidogrel (CYP2B6), 2 μM sulfaphenazole (CYP2C9), and 1 μM ketoconazole (CYP3A4).

HepG2-CYP panel correctly identifies CYP3A4 role in Aflatoxin B1 cytotoxicity



- HepG2
- HepG2-CYP3A4
- ▲ hPOR KO
- ▼ HepG2 + ketoconazole
- ◆ HepG2-CYP3A4 + ketoconazole
- hPOR KO + ketoconazole

🧬 Cytotoxicity is absent in CYP reductase (POR) KO indicating **CYP mediated toxicity**

🧬 CYP3A4 specific cells demonstrates increased cytotoxicity **suggesting 3A4 involvement**

🧬 CYP3A4 specific **inhibitor** partially prevents cytotoxicity in WT and CYP3A4 transgenic **confirming 3A4 involvement**

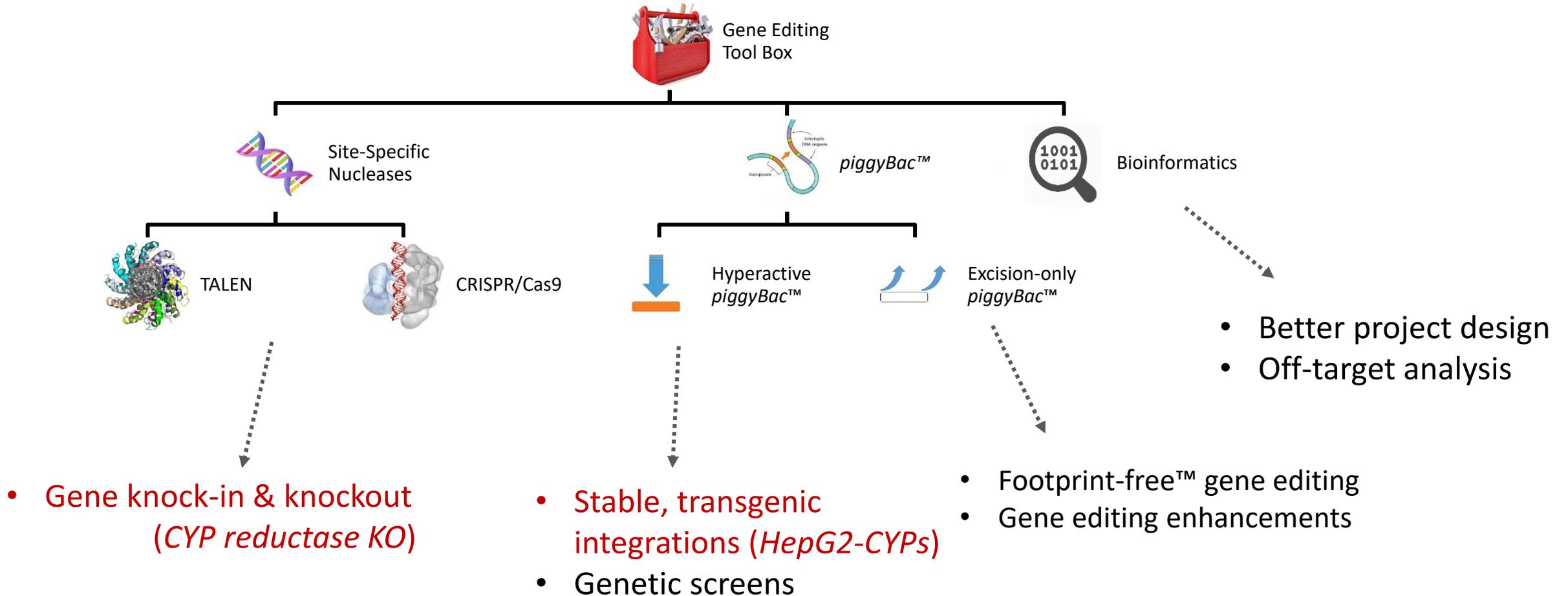
Cells were treated with varied concentrations of aflatoxin B1 with or without 1 μ M ketoconazole (CYP3A4 inhibitor). In cells treated with aflatoxin B1, concentration-dependent cytotoxicity (based on loss of cell viability) was enhanced by the expression of CYP3A4 and suppressed by CYP reductase knockout. In HepG2 WT cells and HepG2-CYP3A4 cells, co-incubation with 1 μ M ketoconazole partially protected against the toxic effect of aflatoxin B1.

-  The HepG2-CYP cell lines are available as a panel or can be purchased individually

-  Hera provides *in vitro* screening services using the HepG2-CYP panel as well other cell lines including: primary hepatocytes, HepaRG, Upcyte hepatocytes, Corning HepatoCells
 - Services include:
 - Cytotoxicity - cell viability (MTT & ATP)
 - Metabolic stability & metabolite profiling
 - CYP phenotyping and drug-drug interaction studies

-  Custom gene editing services (knockout, knock-in and transgenic) to improve metabolic capability of any cell line as well as further modifications to any of the cell lines from the HepG2-CYP panel

Hera's gene editing technology & capabilities



Hera's gene editing tool box for product development

Custom gene editing, phenotyping and screening services in cells, rats & mice available

Cancer Xenografts



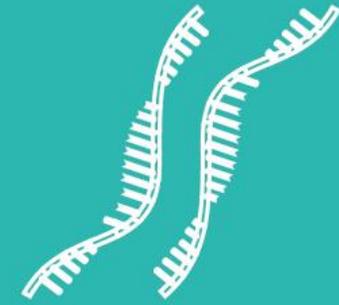
- [Xenograft/PDX Efficacy studies](#)
- [Off-the-shelf SCID rats models](#)

In Vivo & In Vitro Toxicity and Metabolism



- [HepG2-CYP™ metabolism and toxicity cell panel](#)
- [hu-MDCK™ humanized BCRP transporter cells](#)
- Humanized liver rodent models
- Hepatotoxicity studies in rats

Disease Modeling



- [In vivo liver gene delivery for disease model creation and gene therapy efficacy](#)
- Custom genome engineering in rat and mouse
- [Colony management and phenotyping](#)

Links for specific product and service information above

Precision Toxicology™ & Efficacy: utilizing precisely gene-edited models such as SCID rats, humanized rodents and engineered cell lines for producing more rapid, consistent and clinically-relevant data

2015

Hera spun-out of Transposagen & licenses IP for gene editing technology; development of SCID rats begins; awarded phase II SBIR grant

2016

Completion of a 10,000 ft² facility; Scientific team assembled with *in vitro* & *in vivo* efficacy & toxicity capabilities

2017

Introduction of SDR™ & SRG™ SCID rats and efficacy services; Engineered HepG2 and MDCK cells; *in vivo* toxicity studies and humanized liver mice; custom gene editing, breeding and screening services in mouse and rat

2017/18 plan

Humanization of the liver & immune system of SRG™ rats for toxicity and immuno-oncology services

Tseten Yeshi, Ph.D.

VP, R & D

Former Director of R&D at Transposagen. An expert in genome editing with well-developed scientific program management skills and experience. A molecular biologist with full range experience in performing complex gene editing in a broad range of cell types including rodent embryonic stem cells and spermatogonial stem cells. Hands on start to finish experience in targeted nuclease design, their on-target and off-target cutting analysis, creation of custom cell lines, performing wide-range of molecular and cellular assays for their characterization

Wei Zhang, Ph.D.

Senior Scientist

Background of Toxicology and Cell Biology. Experience in genetically engineered cell line (knock-in, knockout and reporter cell lines) development, mammalian cell culture and manipulation, bioactivity screening and in vitro toxicity testing. Has worked extensively with cell based assays measuring cell viability, cell apoptosis, cellular stress, drug metabolism enzymes activity, transporter activity, gene expression, etc.

Chris Chengelis, Ph.D., DABT

Senior Scientific Advisor

Former CSO at WIL Research. 35 years+ experience in the CRO industry, facility design, study design and execution. Considerable hands on experience, with publications in toxicology and ADME/PK

Fallon Noto, Ph.D.

Senior Scientist

Proficient in cell culture techniques of mammalian cells, primary cells, and pluripotent stem cells, including differentiation assays and viral transduction. Skilled in molecular techniques, including tissue histology, PCR, and protein expression analysis.

Contact Us: services@herabiolabs.com 859-414-0648
2277 Thunderstick Dr. #500 Lexington, KY 40505