Faster & cleaner transporter assays with cPgp/cBcrp double knockout, and “humanized” MDCKII cell lines stably expressing human ABCB1 (MDR1) gene encoding P-glycoprotein and ABCG2 gene encoding BCRP.
About Hera BioLabs

**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

<table>
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<th>2015</th>
<th>2016</th>
<th>2017</th>
<th>2017/18 plan</th>
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<td>Hera spun-out of Transposagen &amp; licenses IP for gene editing technology; development of SCID rats begins; awarded phase II SBIR grant</td>
<td>Completion of a 10,000 ft² facility; Scientific team assembled with <em>in vitro</em> &amp; <em>in vivo</em> efficacy &amp; toxicity capabilities</td>
<td>Introduction of SDR™ &amp; SRG™ SCID rats and efficacy services; Engineered HepG2 and MDCK cells; <em>in vivo</em> toxicity studies and humanized liver mice; custom gene editing, breeding and screening services in mouse and rat</td>
<td>Humanization of the liver &amp; immune system of SRG™ rats for toxicity and immuno-oncology services</td>
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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

Hera has freedom to operate through multiple licenses to issued and pending patents

- Gene knock-in & knockout
- Stable, transgenic integrations
- Genetic screens
- Better project design
- Off-target analysis
- Footprint-free™ gene editing
- Gene editing enhancements
MDCK canine cells are desired for easy handling and a high proliferation rate. MDCKII cells form monolayers for transporter assays in 3 days vs Human Caco-2 cells take 21-days. However, endogenous canine transporters interfere in transfected MDCKII cells.

Hera Biolabs has generated cPgp/cBcrp double knockout MDCKII cell line (MDCKII-BP-null™), and “humanized” cell lines stably expressing ABCB1 (MDR1) gene encoding hPgp and ABCG2 gene encoding hBCRP on the MDCKII-BP-null cell background (hMDR1-MDCKII-BP-null and hBCRP-MDCKII-BP-null).

Application: (1) MDR1 or BCRP substrate assessment; (2) MDR1 and BCRP inhibitor assessment.
Making Humanized MDCKII Cells

NextGEN CRISPR/Cas9 transfection into MDCKII + dual reporter plasmid with targeting sites

Sort RFP⁺/GFP⁺ cells
(highly concentrate cells in which CRISPR/Cas9 cut properly)

Single cell clones isolation

Genomic verification (PCR, sequencing)
Functional verification (transporter assay)

Check cutting efficiency
(Cell I assay)

MDCKII-BP-null cell line

MDCKII-BP-null cell line

PB-hMDR1/PB-hBCRP + PiggyBac transposase transfection

G418 selection

Single cell clones isolation

Genomic verification (PCR, sequencing)
Gene expression (RT-PCR, IF staining)
Functional verification (transporter assay)

hMDR1-MDCKII-BP-null cell line
hBCRP-MDCKII-BP-null cell line
Frameshift mutation on cPgp and cBcrp gene lead to premature stop codons downstream in early exons.

Transport activity of cPgp and cBcrp was abolished in MDCKII-BP-null cells. The Pgp and Bcrp activity to transport substrate DiOC₂(3) were detected by Multidrug Resistance Direct Dye Efflux Assay (ECM910, Millipore).
hMDR1/hBCRP-MDCKII-BP-null cell pool characterization

**BCRP: Hoechst Efflux 33342 assay**

MDCKII WT, MDCKII-BP-null (KO) and hBCRP-MDCKII-BP-null (KO-BCRP) pool cells were incubated with 5 μM Hoechst for 1 hour at 37°C (before effluxing). Then cells were switched to Hoechst-free medium with/without BCRP inhibitor Fumitremorgin C for 1, 2 and 4 hours. Hoechst remaining in the cells was measured in a fluorescence spectrophotometer (Ex/Em=350nm/460nm), and was expressed as percentage of the value determined after 1 hours of incubation. hBCRP-MDCKII-BP-null cells pool show BCRP-mediated efflux as BCRP specific inhibitor Fumitremorgin C inhibits the efflux.

**MDR1 Efflux: Calcein – AM assay**

MDCKII WT, MDCKII-BP-null (KO) and hMDR1-MDCKII-BP-null (KO+ PB-MDR1 pool cells were incubated with 5 μM Calcein AM for 1 hour with/without MDR1 inhibitor vinblastine. Calcein-AM accumulating in the cells was measured in a fluorescence spectrophotometer (Ex/Em=485nm/520nm). Compared to MDCKII-BP-null cells, hMDR1-MDCKII-BP-null cells show lower Calcein-AM accumulation, indicating possible human MDR1 activity.
MDCK II WT, MDCKII-BP-null (KO), hBCRP-MDCKII-BP-null pool (KO-BCRP) and individual clones (A1, B2, B3, C4, D2, D3) cells were incubated with 5 μM Hoechst for 1 hour at 37°C (before effluxing). Then cells were switched to Hoechst-free medium with/without Bcrp inhibitor Fumitremorgin C for 1 hours. Hoechst remaining in the cells was measured in a fluorescence spectrophotometer (Ex/Em=350nm/460nm), and was expressed as percentage of the value determined after 1 hours of incubation.

Cell imagines of MDCKII cells in Hoechst efflux assay. Hoechst dye binds to the cell nucleus (blue). MDCKII WT cells (WT) efflux Hoechst dye (blue intensity decrease) through transporters other than canine Bcrp, because Bcrp inhibitor Fumitremorgin C did not inhibit the efflux. MDCKII-BP-null (KO) cells show much lower efflux activity. hBCRP-MDCKII-BP-null (KO-BCRP B2) cells show obvious dye efflux which is inhibited by BCRP inhibitor, indicating the human BCRP contributes the efflux.
Hera’s products & services

Cancer Xenografts

- Xenograft/PDX Efficacy studies
- Off-the-shelf SCID rats models

In Vivo & In Vitro
Lead Optimization, Toxicity and Metabolism

- HepG2-CYP™ metabolism and toxicity cell panel
- hu-MDCK™ humanized transporter cells
- Humanized liver rodent models
- *In vivo* early discovery services

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
Hera BioLabs Leadership

Jack Crawford, M.S.
**CEO**

Formerly directed the Sales, Marketing, and Business Development Divisions at Transposagen. Experience in product development, licensing, technology and patent evaluation, and fundraising.

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.

Chris Chengelis, Ph.D., DABT
**Senior Scientific Advisor**

Former CSO at WIL Research. 35 years+ experience in the preclinical toxicology industry, facility design, study design and execution.

Fallon Noto, Ph.D.
**Senior Scientist**

10+ years working with mice and rats, expertise in rodent humanization, cell and tissue transplantation, microsurgery, and ethical animal care.

Kamesh Ravi, Ph.D.
**Senior Scientist**

10+ years of experience in preclinical oncology, cancer xenograft models, tumor efficacy studies and onco-nephrology.

Goutham Narla, M.D., Ph.D.
**SAB Member & Consultant**

The Pardee Gerstacker Professor of Cancer Research and a Medical geneticist at Case Western Reserve University. CSO and Scientific Founder of Dual Therapeutics, Inc. Expertise in cancer genetics and xenograft and transgenic models of cancer with over 58 publications in the field.

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