Hera Services & Technology

Hera BioLabs

About Hera & Project Execution
Oncology Model Examples
Gene Editing Tools & Capabilities

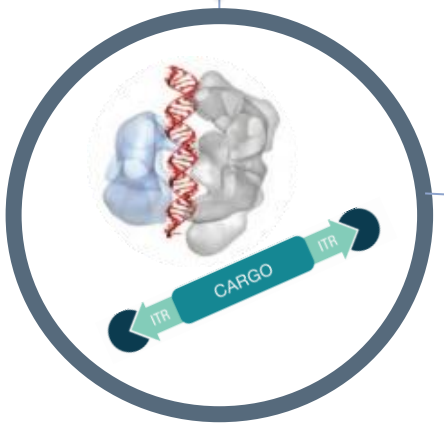
Chris Brenzel
Sr. Director, Business Development
cbrenzel@herabiolabs.com
859-967-9672

About Hera BioLabs

Proprietary
Gene Editing Technologies

piggyBac® **Cas-CLOVER™** **TAL-CLOVER™**

Research Field of Use
Reagents & Technology Licensing



Custom Genome Engineering

- Cell-line gene editing
- Disease and animal models

Contract Research Services

- Oncology studies
- Model characterization and validation

Proprietary Genetically Engineered Rodent Models

- SRG rat (***OncoRat***®)
- Humanization of immune system and liver

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Our Facility and Vivarium

- AAALAC accredited animal welfare program
- Barrier facility with Innovive IVC caging system
- Digital data acquisition via StudyLog®systems software
- Surgical suite & gowning room
- Molecular and cell culture facility
- Highly trained and certified staff

aalac INTERNATIONAL
PROUDLY, AN AAALAC ACCREDITED PROGRAM

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Rodent Toxicology

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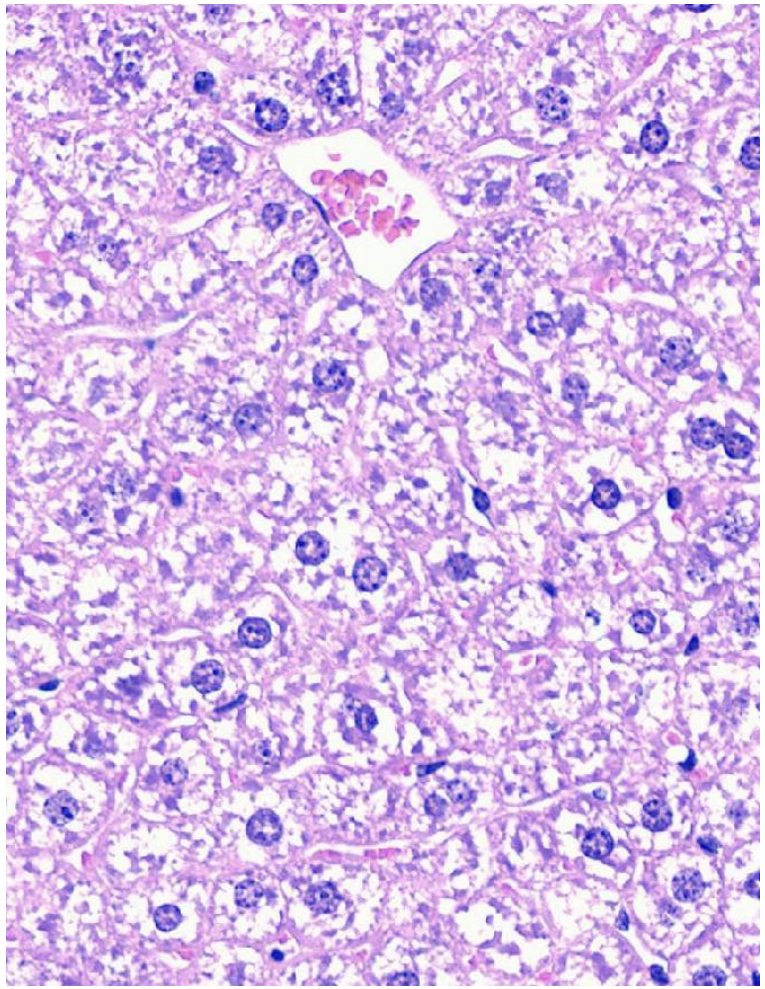
Toxicology Studies in mice and rats



Services

Max tolerable dose
Pharmacokinetics
Pharmacodynamics
Clinical examination
CBC & toxicology
Urinalysis
Other clinical chemistries

Necropsy:

Organ weight
Body composition
FFPE, OCT, flash freeze

Study designs:

Acute
Subchronic
Chronic

Routes:

Oral
Subcutaneous
Infusion
Intravenous
Intraperitoneal
Rectal

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In vivo Oncology Models

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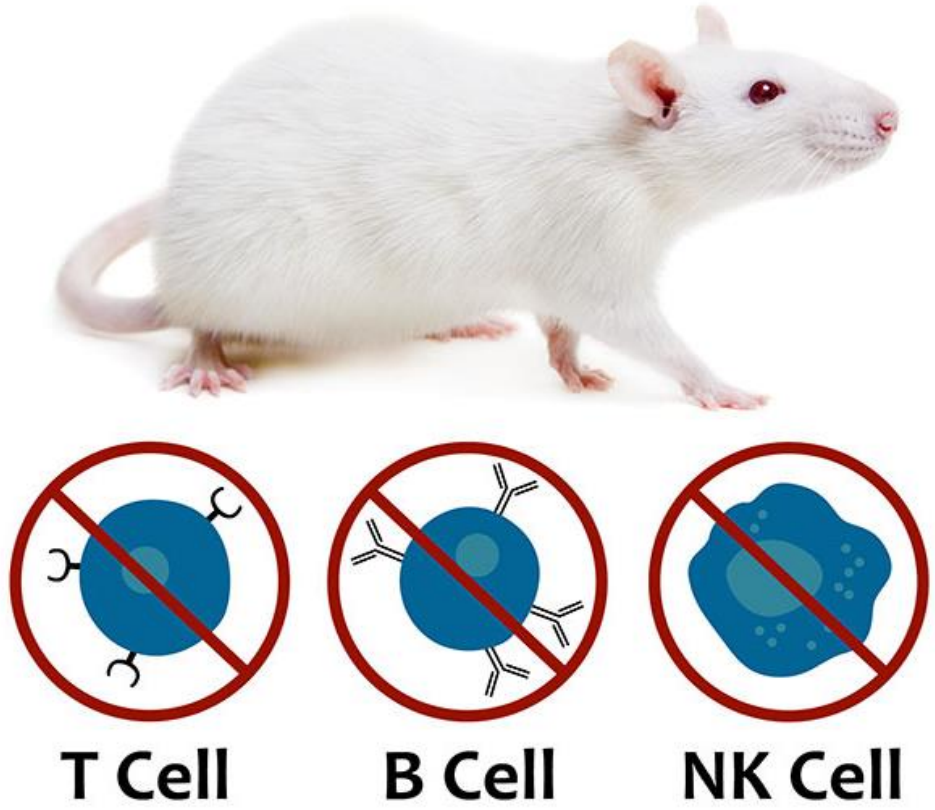
The *SRG OncoRat*®



SRG OncoRat: Building a Better Trap for Cancer

Developed using Hera Biolabs' advanced gene editing technology, is a **SCID** rat on the **Sprague-Dawley** background that harbors a **double knockout for the Rag2 and Il2Rγ genes**.

- Enhanced immunodeficiency: lacks B-cells, T-cells, and NK-cells.
- Enhanced engraftment rates
- Improved tumor growth for both cell-line tumor models and patient-derived xenografts (PDXs)
- Perform serial tumor biopsies
- Combine efficacy, pharmacokinetic (PK), biomarker, and toxicology studies

Noto et al. PLoS One. 2020 Oct 7;15(10):e0240169.

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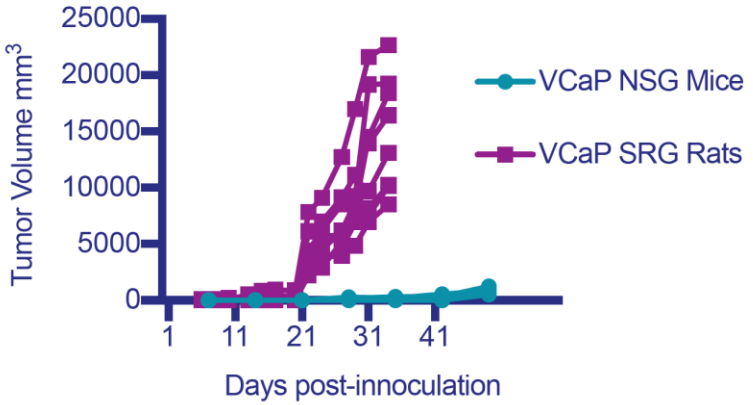
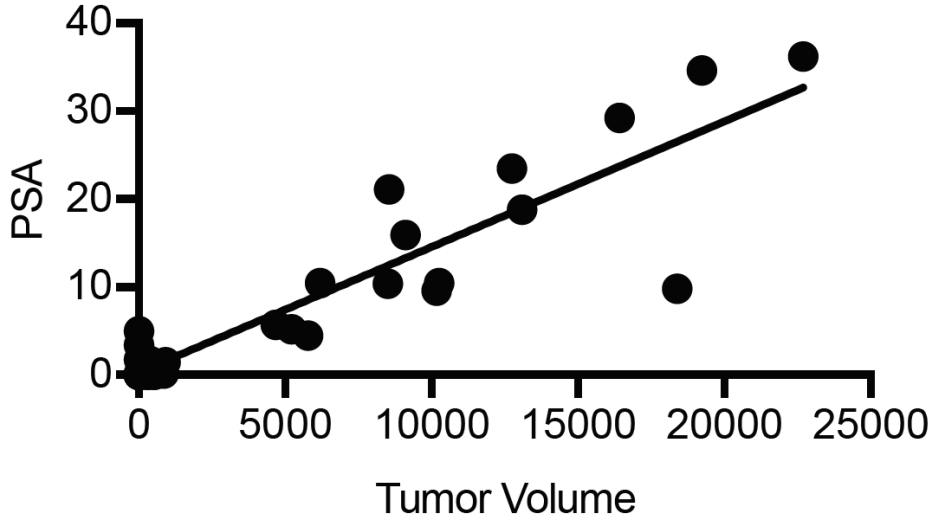
Cancer Xenograft Studies: Human Prostate Example

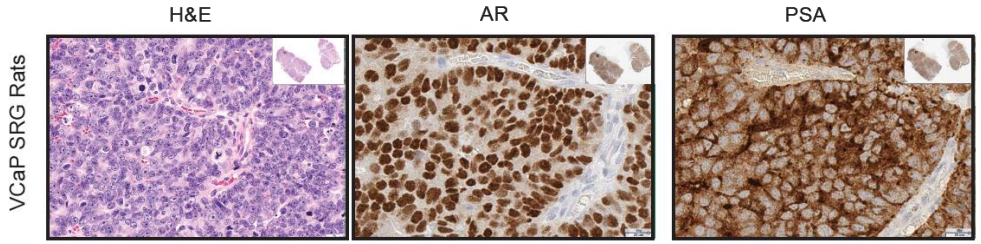
VCaP tumor kinetics rat vs. mice

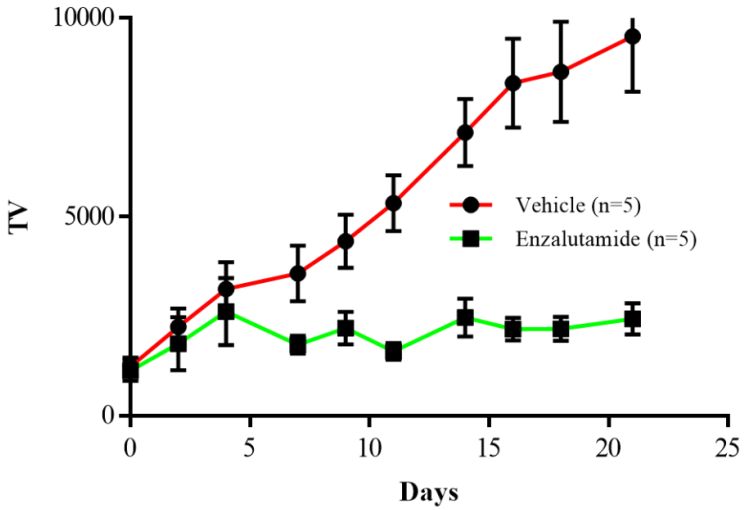




VCaP tumor serum PSA

IHC of VCaP tumors confirms AR and PSA





VCaP is responsive to Positive Control

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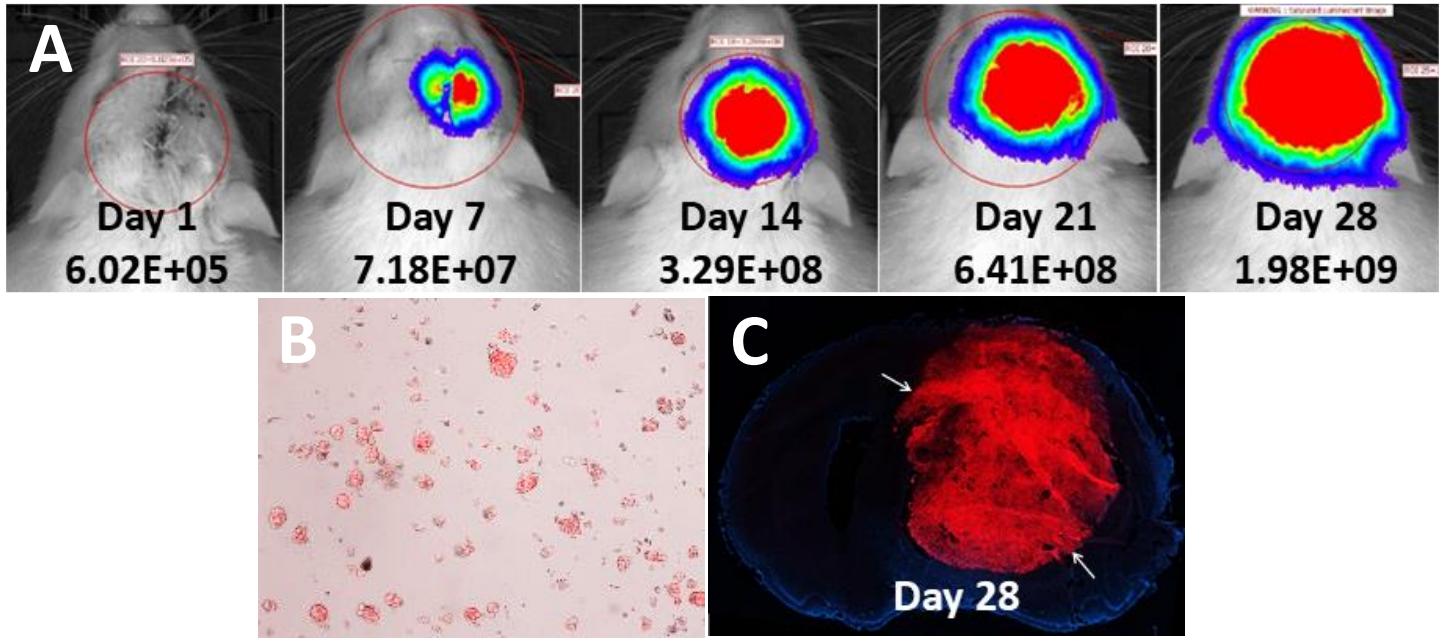
SRG rat brain size permits translational interventions and imaging

In vivo bioluminescent imaging to track tumor growth



A) signal expansion over 4 weeks. The tumors become symptomatic after ~4 weeks of growth.

B) Patient GBM stem-like cells (4x magnification) grown in spheres and transduced to express luciferase and tdTomato.

C) Day 28 GBM PDX histology showing the tdTomato against DAPI.

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List of cell-derived tumor models validated in the SRG rat

Model	Cancer Type	Notes
MOLM-13	Adult acute myeloid leukemia	Mutation in FLT3; Unexplicit; Internal tandem duplication
MDA-MB-231	Breast adenocarcinoma	Triple negative breast cancer; Mutations in CDKN2A, CDKN2B, BRAF, KRAS, TERT, TP53
MX-1	Breast adenocarcinoma	Mutation in TP53
HCC1954	Breast ductal carcinoma	HER2+ studies, ER- studies, AR inhibitors; Gene fusion CLTC-VMP1; Mutations in PIK3CA, TP53
MCF7	Invasive breast carcinoma	Derived from pleural effusion metastasis; Mutations in GATA3, PIK3CA, TP53
HCT-116	Colon carcinoma	Tumorigenesis, colorectal cancer metastasis, CDK inhibition, TGFβ+; Mutations in ACVR2A, BRCA2, CDKN2A, CTNNB1, EP300, KRAS, PIK3CA, TGFBR2
HT-1080	Fibrosarcoma	Cachexia studies; Gene deletion CDKN2A; Mutations in IDH1, NRAS
SNU-5	Gastric Carcinoma	Mutations in CDKN2A, TP53
U118-MG	Glioblastoma/Astrocytoma	Mutations in PTEN, TP53
U87-MG	Glioblastoma	Historic data comparisons (some model controversy); Mutations in NF1, PTEN, TERT
Hep3B	Hepatocellular carcinoma, pediatric	a.k.a Hep 3B2.1-7; Mutations in AXIN1, RB1
OCI-AML2	Leukemia, adult acute myeloid	NOS and VEGF signaling, angiogenesis inhibitors, DNA methylation; Gene fusion KMT2A-AFDN
K-562	Leukemia, blast chronic myelogenous	Cachexia studies; BCR-ABL1 positive. Gene fusion BCR-ABL1; Mutation in TP53
A-549	Lung adenocarcinoma	Mutations in KRAS, STK11, TP53
NCI-H1975	Lung adenocarcinoma	Mutations in EGFR, PIK3CA, TP53
NCI-H226	Lung, pleural epithelioid mesothelioma	Gene deletion CDKN2A
NCI-H322	Lung adenocarcinoma, minimally invasive	Mutation in TP53

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List of cell-derived tumor models validated in the SRG rat

Model	Cancer Type	Notes
HCC-95	Lung, squamous cell carcinoma	Derived from metastatic site: Pleural effusion
NCI-H2170	Lung squamous cell carcinoma	Mutations in RHOA and TP53
NCI-H1734	NSCLC, Lung adenocarcinoma	Mutations in ATM, KRAS, RB1, TP53
NCI-H2122	NSCLC, Lung adenocarcinoma	Mutations in KRAS, TP53
NCI-H358	NSCLC, Lung adenocarcinoma	KRAS & EGFR signaling, metastasis to lungs; Gene deletion TP53; Mutation in KRAS
NCI-H441	NSCLC, papillary adenocarcinoma	Mutations in KRAS, TP53
Daudi	Lymphoma, EBV-related Burkitt	Gene fusion MYC-IGH; Mutation B2M, CTNNB1, TP53
OV81*	Ovarian, serous	Mutation in BRCA2, *Developed by Hera BioLabs
Capan-2	Pancreatic ductal adenocarcinoma	Mutations in CDKN2A, KRAS, TP53
MIA PaCa-2	Pancreatic ductal adenocarcinoma	Epithelial-to-mesenchymal transition (EMT); Gene deletion CDKN2A. Mutations in KRAS, TP53
22Rv1	Prostate carcinoma	Mutations in KMT2D, PIK3CA, TP53
LNCaP	Prostate carcinoma	AR inhibtors, castration resistance studies; Mutations in AR, MEN1, PIK3R1, PTEN, TP53
NCI-H660	Prostate small cell carcinoma	Gene fusion TMPRSS2-ERG
PC-3	Prostate carcinoma	Derived from metastatic site: Bone. Mutation in TP53
VCaP-EnzR	Prostate carcinoma	Enzalutime drug resistance studies, castration resistant studies
VCaP	Prostate Carcinoma	AR resistance, castration resistant studies; Gene fusion TMPRSS2-ERG; Mutation MLH1; TP53;
786-O	Renal Cell Carcinoma	VEGF inhibition studies, bone metastasis development, hypoxia and tumor progression; Mutations in PTEN, TERT, TP53, VHL
SCC-090	Tongue squamous cell carcinoma	Cells are positive for Human Papilloma Virus (HPV)

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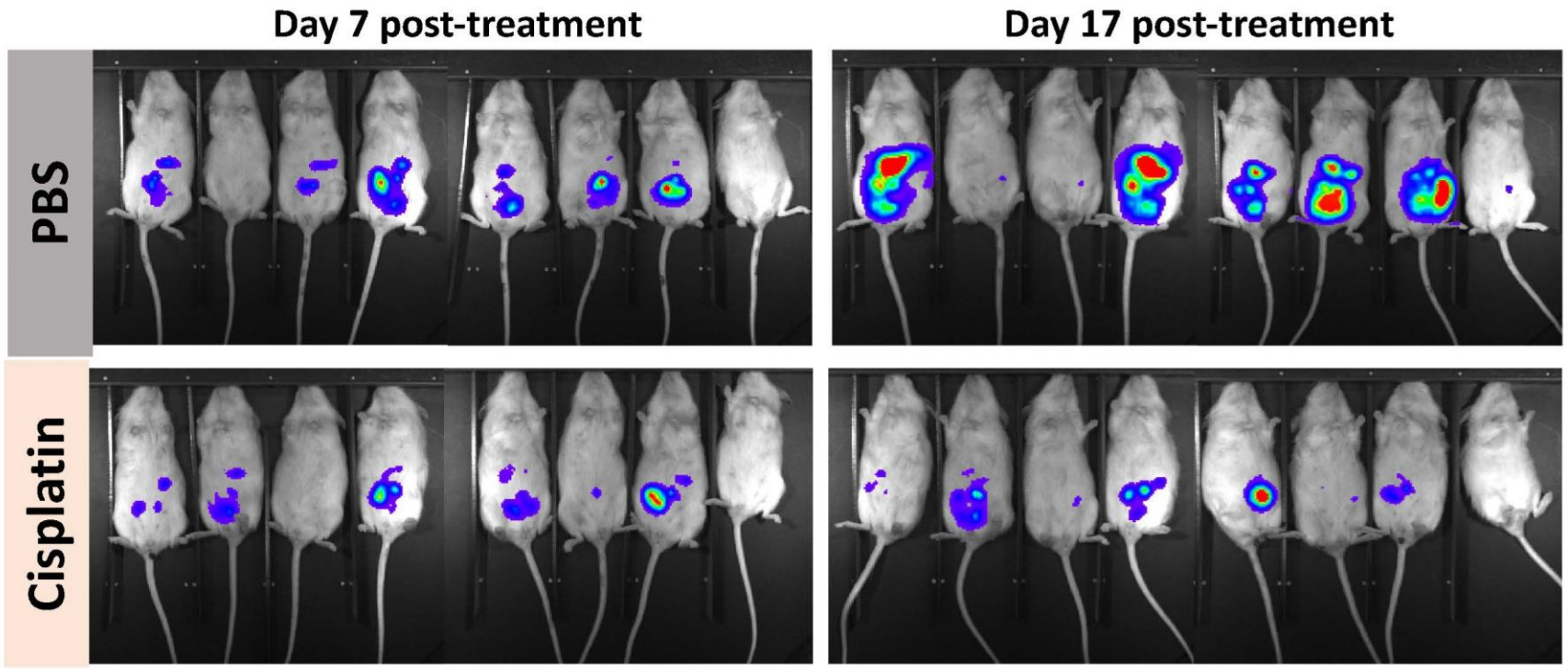
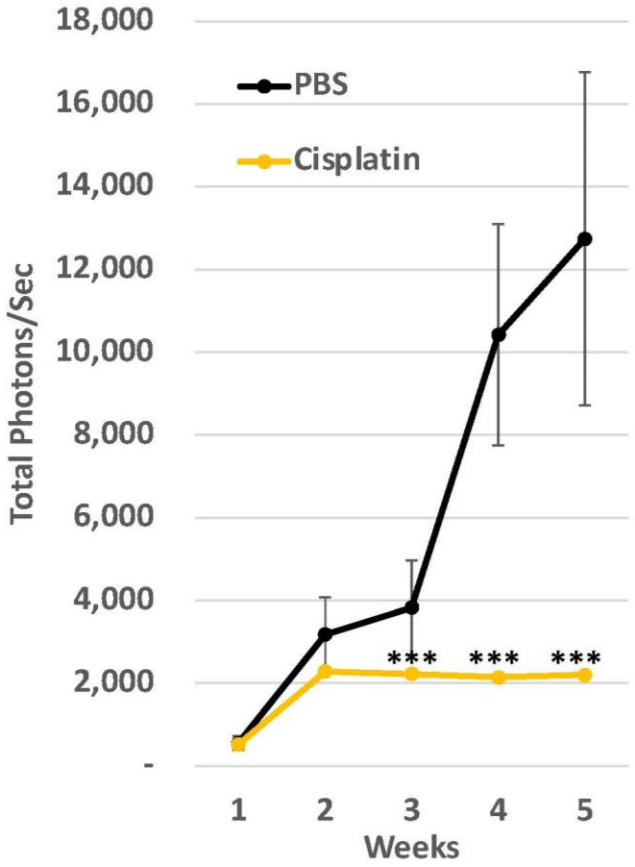
NSG mice: human ovarian cancer xenograft example

OV81.2-luciferase platinum study: NSG mice inoculated with 1 x 10^6 OV81.2-luc cells
Fluorescent reporters can be imaged in vivo with our ***Ami HT*** from ***Spectral Instruments Imaging***





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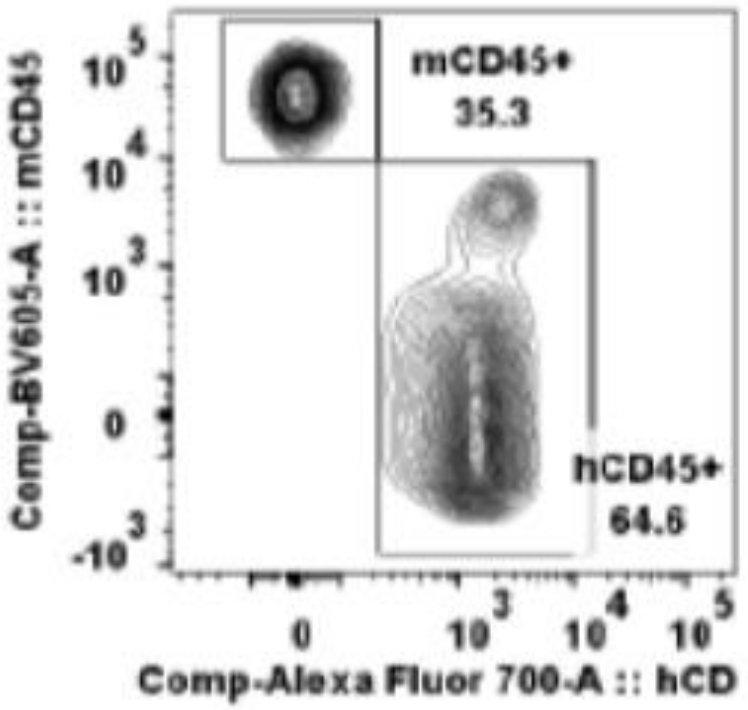
Immuno-Oncology Services

Humanized immune mouse models

- Immuno-oncology: mice engrafted with CD34+ hematopoietic stem/progenitor cells (HSPCs) for development of multi-lineage human immune cells.
- Humanized mouse models or your specific donor, whether HLA matching or engineered cells for your preclinical studies.

Flow cytometry of peripheral blood for human CD45+ 8 weeks after BMT



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Other Disease Models

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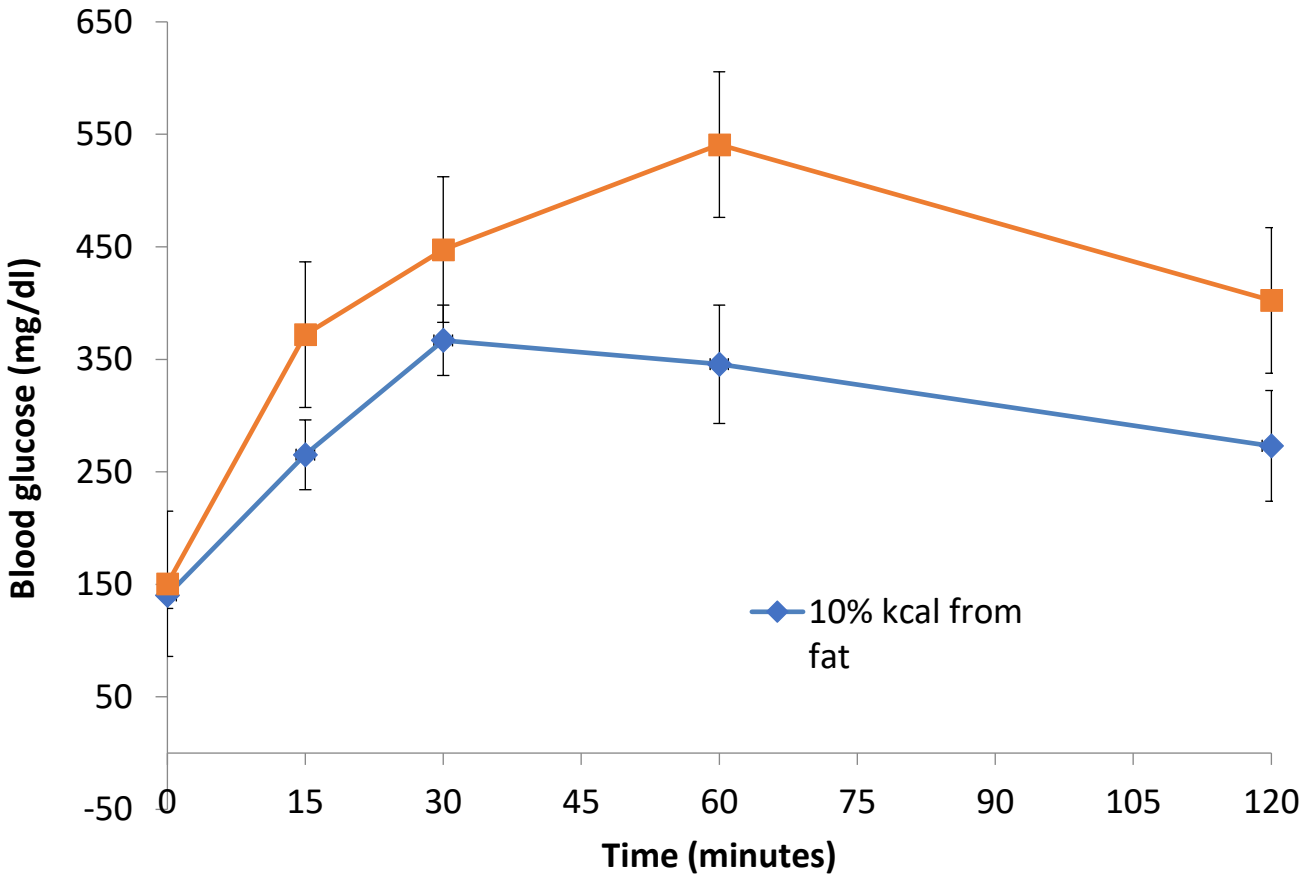
Diabetes and metabolism

The SRG rat develops impaired glucose tolerance after 10 weeks of high fat diet.



- Mice & Rats
- Diet-induced obesity
- Streptozotocin
- Glucose tolerance testing
- Insulin tolerance testing
- Ex vivo body composition

The SRG Rat can be used to test cell-based therapies for Type 1 and Type 2 Diabetes.

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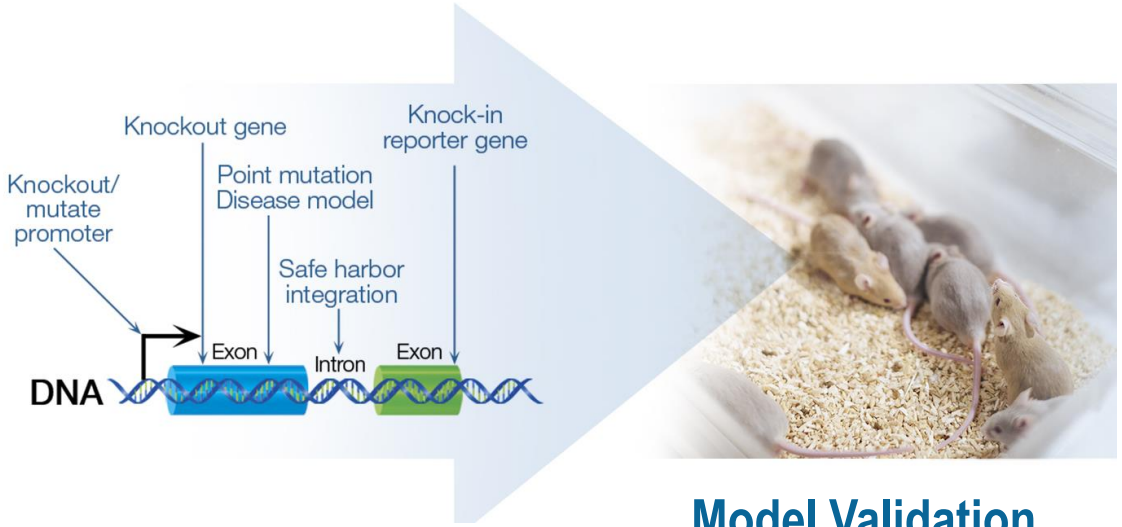
Animal model creation



Research Model Creation

Model Validation
***In vivo* Services**

- Custom rat and mouse models
  - Knockout, knock-in, transgenic, BAC transgenic
- Embryo electroporation or PNI
- Breeding and colony maintenance, genotyping, and phenotyping
- Drug efficacy studies

Hera's full-service capabilities allow for projects to move directly from model creation, to validation, to *in vivo* drug efficacy studies saving time and resources.

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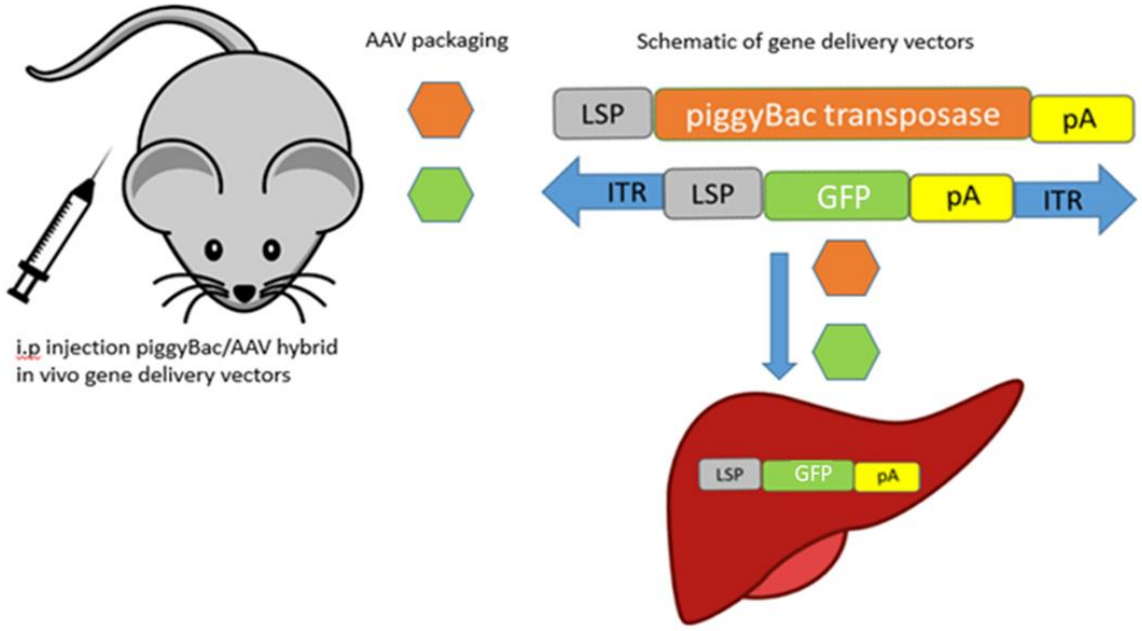
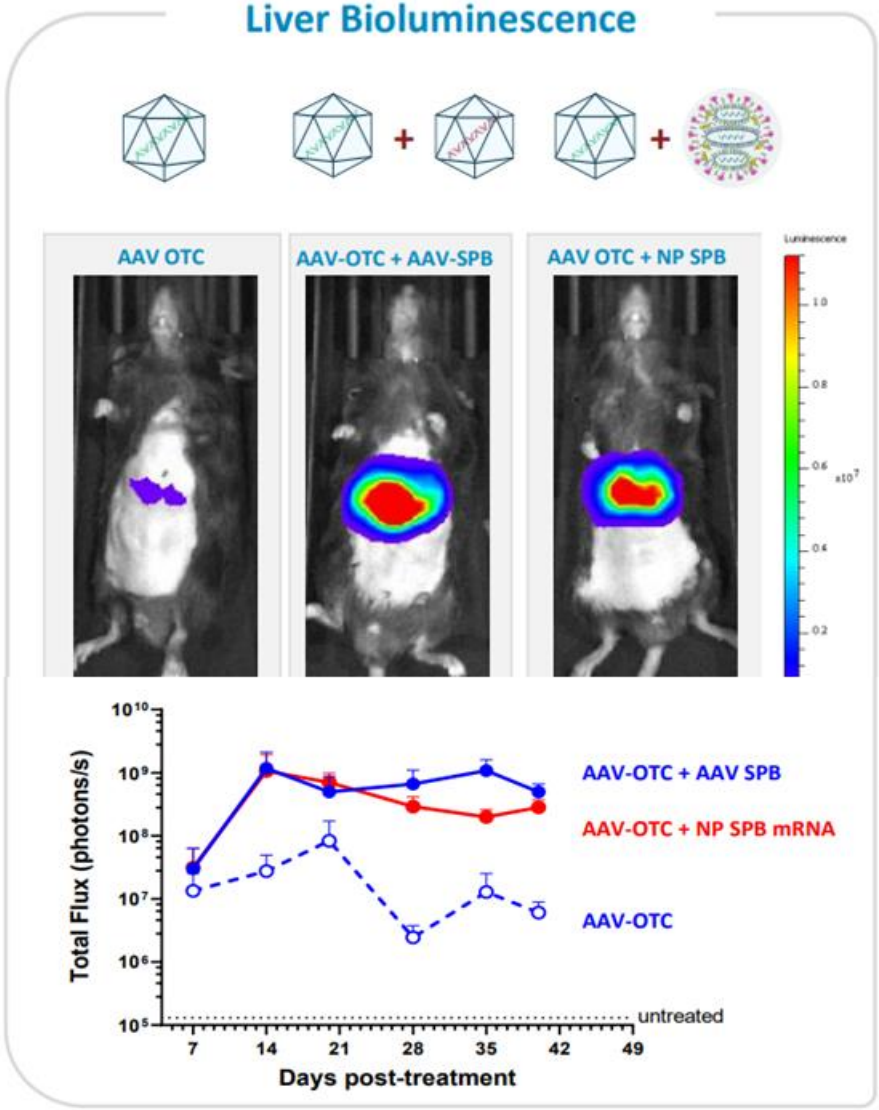
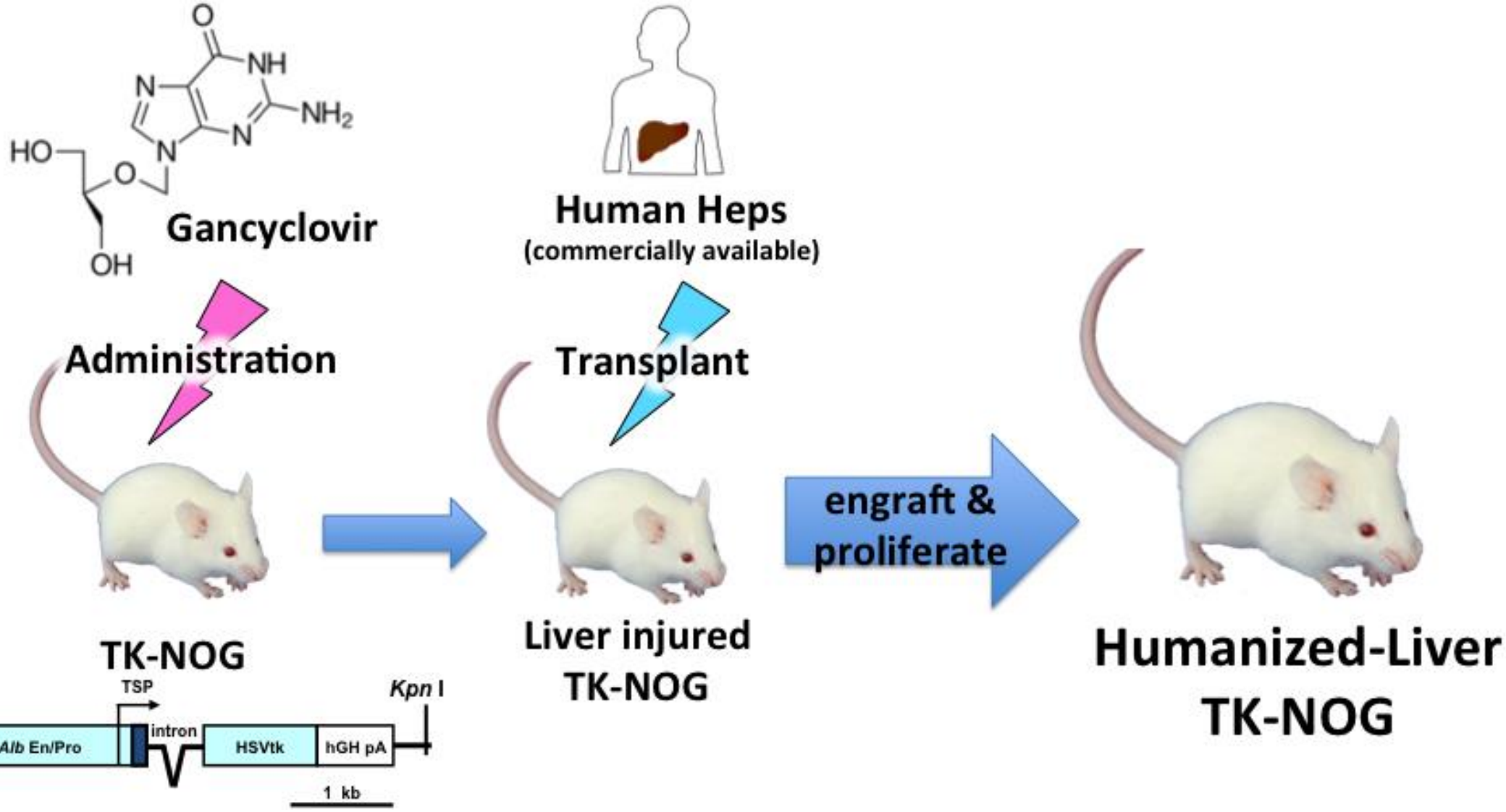
In vivo gene delivery

- piggyBac is highly efficient at delivering transgenes in-vivo eliminating the need to create transgenic animal models.

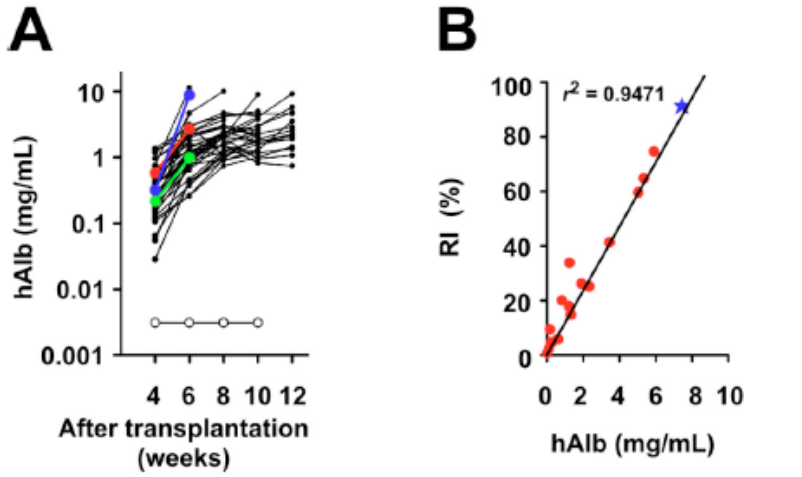




Liver humanization in the TK-NOG mouse

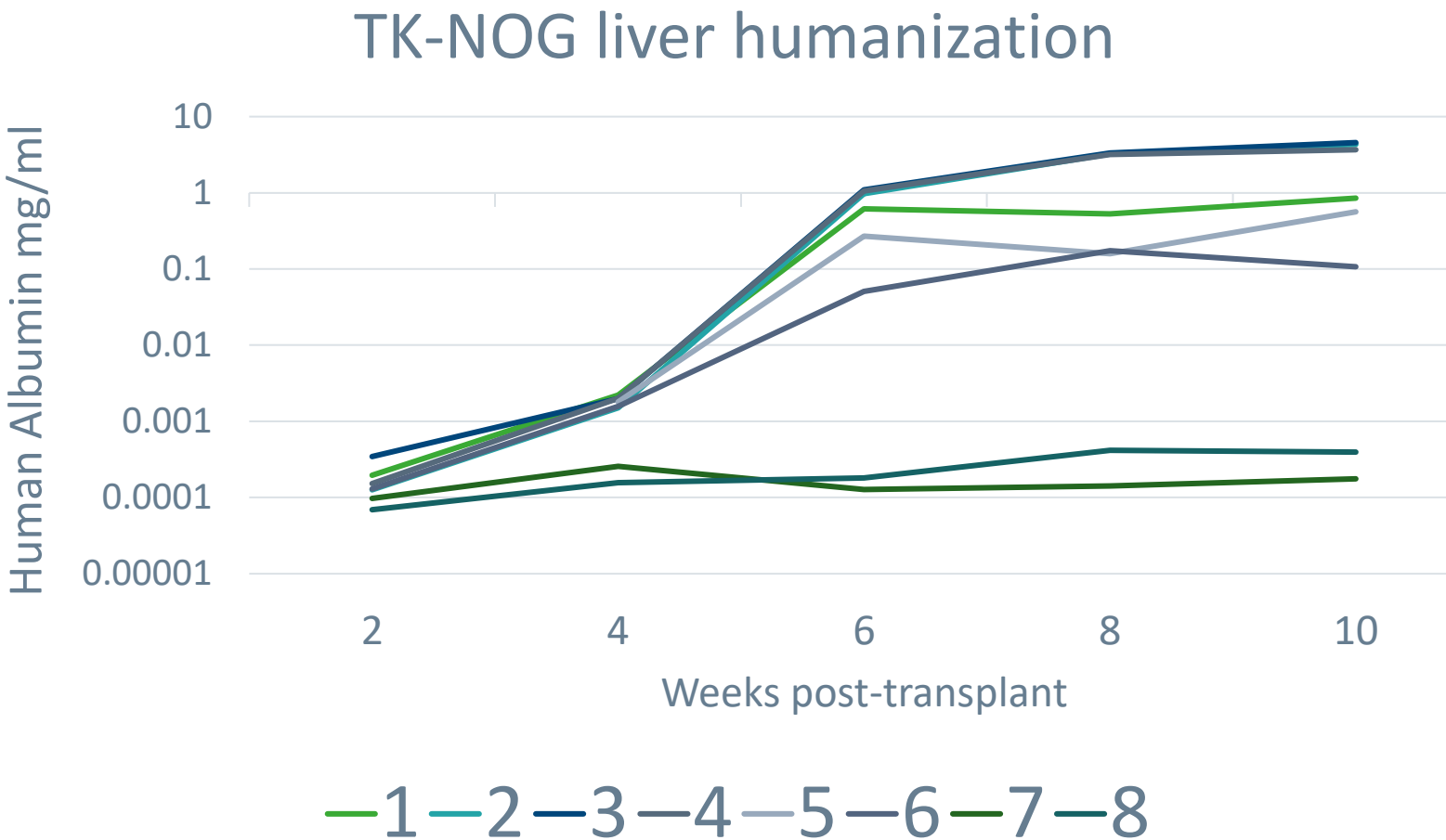


TK-NOG mouse human albumin levels



Humanized

Very low humanization



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Molecular Biology & Gene Editing Tools

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Molecular biology

At Hera

Genotyping and Gene expression

PCR and qPCR

(Applied Biosystems StepOnePlus)

ELISA & enzymatic assays

colorimetric, fluorescent, chemilum

In coordination with subcontractors

Flow cytometry & FACS

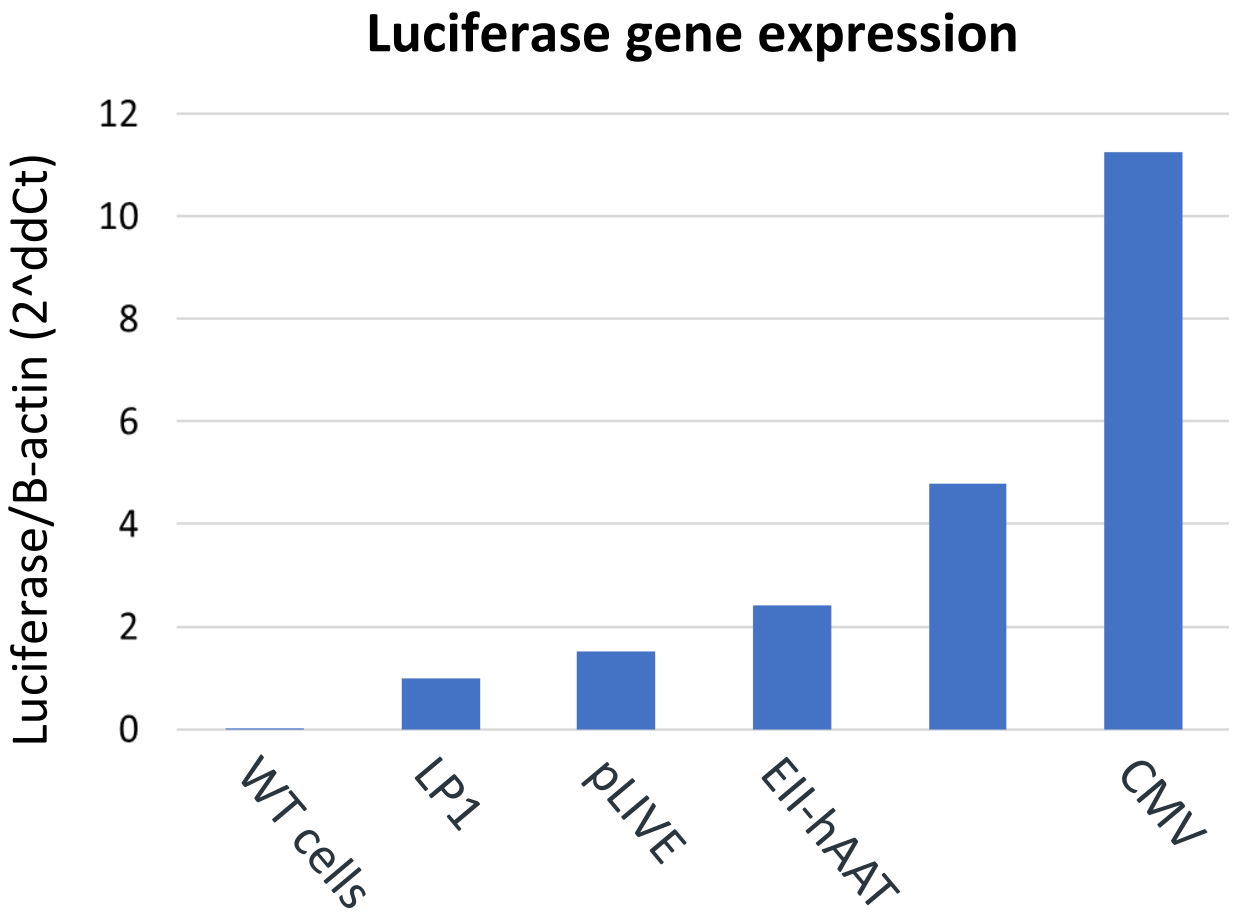
Multi-plex assays (MSD or Luminex)

Histochemistry, immunohistochemistry

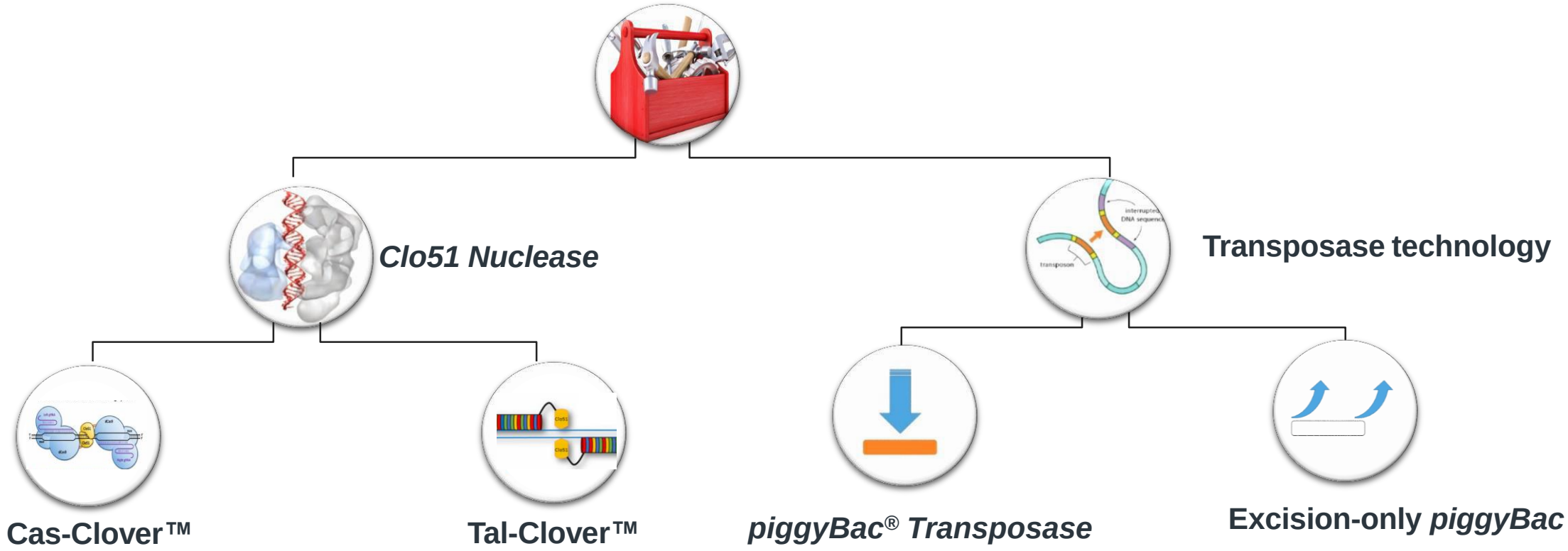
Cell physiology

Kill curves & viability

Other custom services



Proprietary Genetic Engineering Technologies



"Molecular Scissors" introduce targeted double strand breaks in genomic DNA

- Knockouts, knock-ins, base-pair edits
- **No IP entanglements**

Introduce small to very large gene cargo

Scarless removal of gene cargo

- Transgenics & stable cell lines
- *Footprint-Free* gene editing

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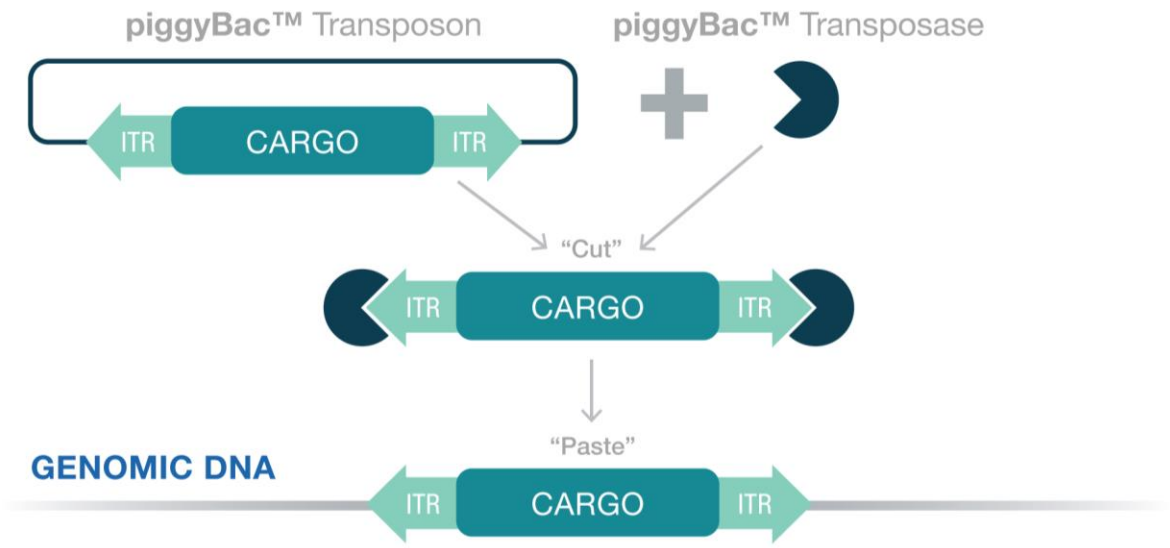
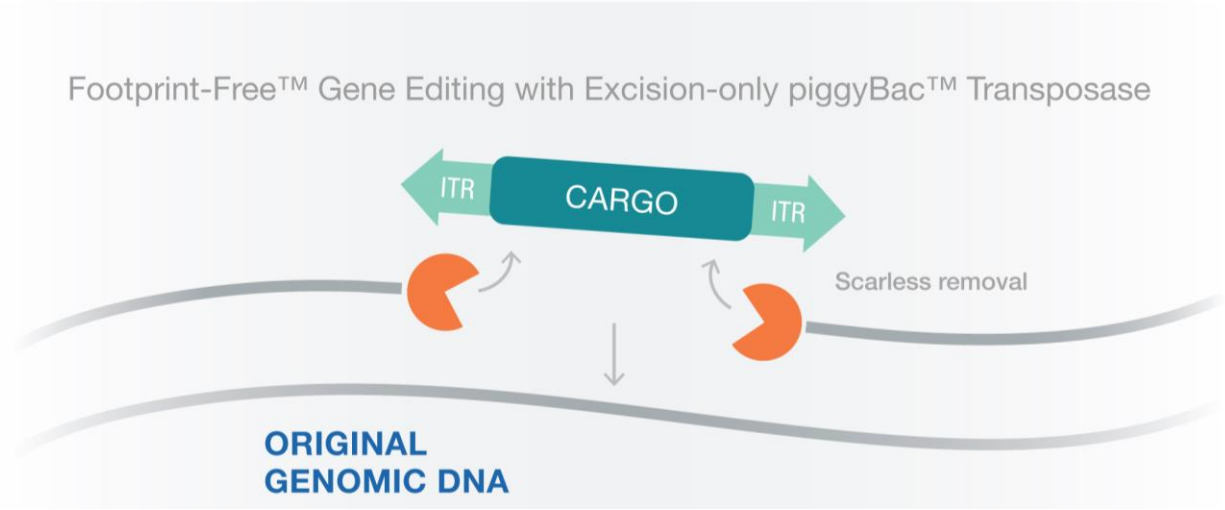
Gene Editing Tools: piggyBac transposase





Features

- ✓ Small to enormous gene integration (200kb+)
- ✓ Very efficient
- ✓ High expression
- ✓ Stable
- ✓ Scarless removal with *Footprint-Free*™ gene editing

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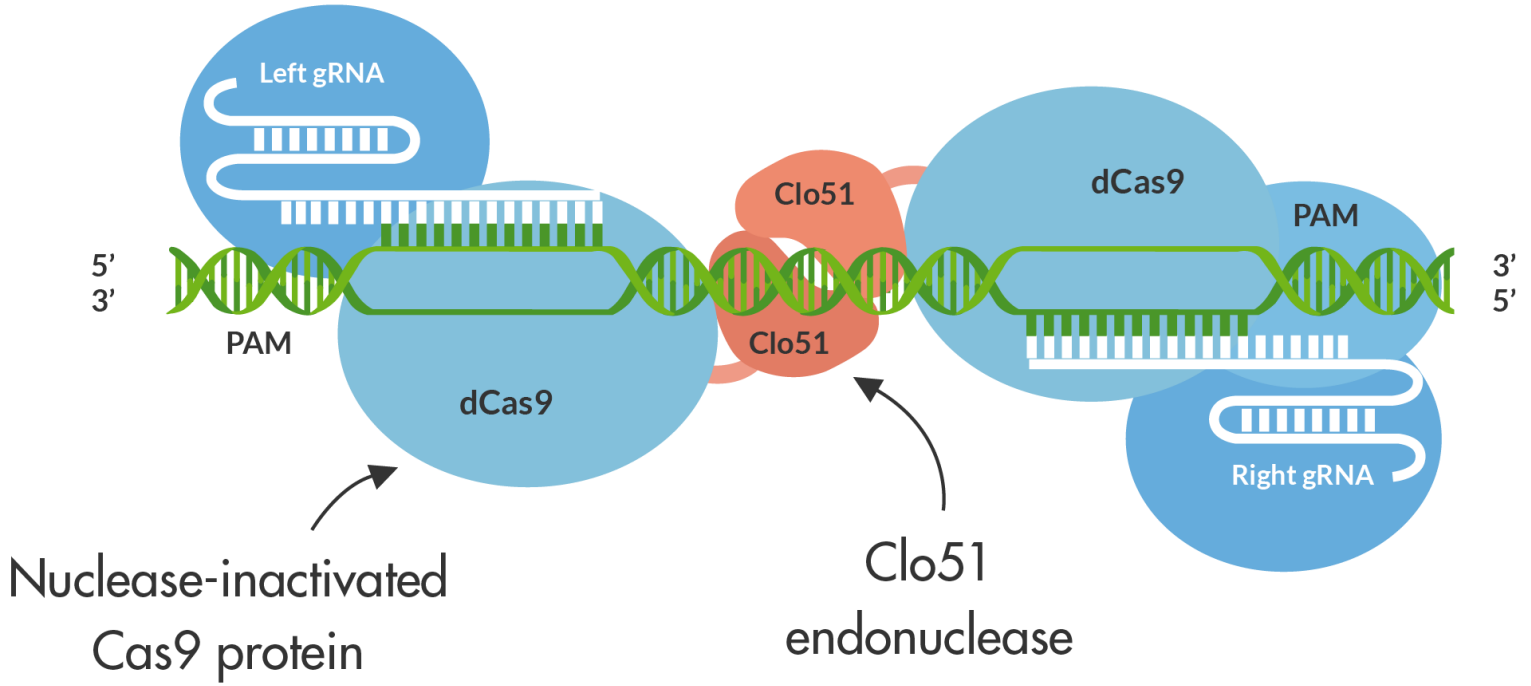
The Cas-CLOVER™ System

Benefits

- Ease of use: double gRNA guided
- High efficiency: dCas9-guided RNA recognition
- High fidelity: cuts only when Clo51 nuclease dimerizes



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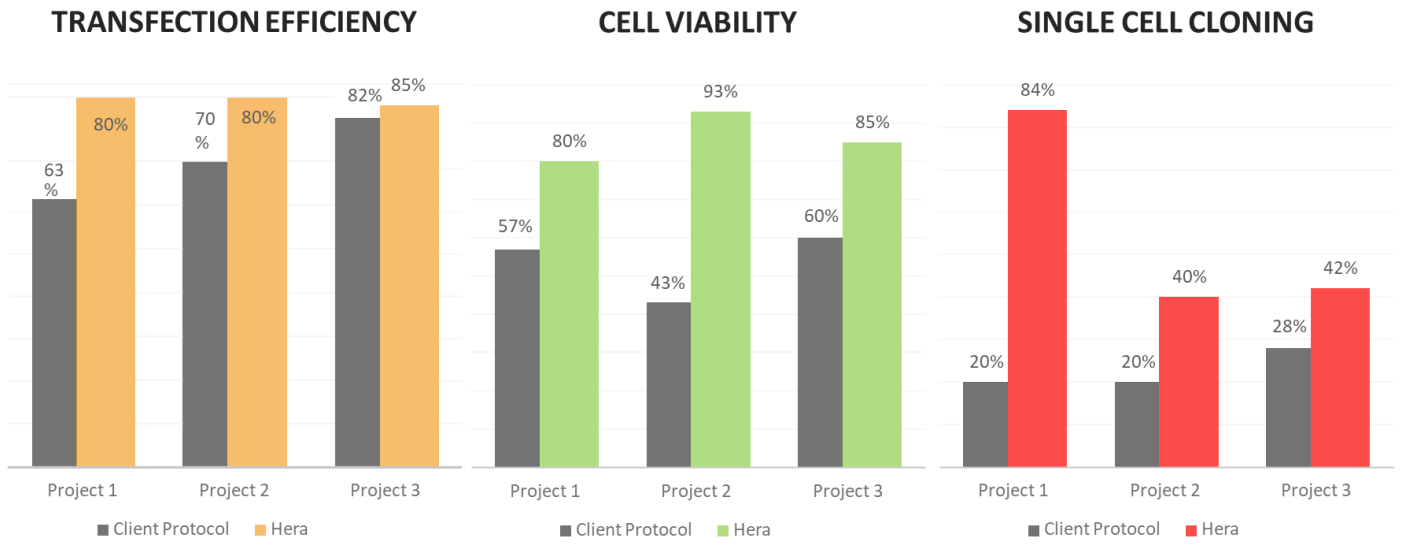
Custom Cell Line Engineering/Gene Editing Services

- Experience with >70 different cell types
- Knockout, knock in, transgenic, and humanization
- ***Footprint-Free*** enables success where others have failed
  - Difficult edits or cell lines (iPSCs)
- All projects include cell line optimization step



Example List – Cell Projects

HEK293
A549
293T
H358
Hs578T
Keratinocytes/neonatal foreskin
Spermatogonial Stem cell
hIPSC
THP-1
U-937
HT-29
A375
HCC-4006
MV-4-11
MOLM-13
FreeStyle 293F
KHYG-1
TF-1
Jurkat-E6-1
K562
CHO-K1
CHO-ZN

DLD-1
Feline ES cells
TALL-104
HUH-7
LNCap
H596
CCD841
MCF10A
NCM356
NCM-460
HEK293F
HeLa S3
HeLa
C1498
EL-4
E.G7 OVA
MC38
MOPC-315
B16 F10
MPC-11
A-20
NK-92

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Gene Editing Reagents

Cas-CLOVER™

- Custom designed nucleases
- Ready to use DNA or RNA reagents

piggyBac®

- Transposon vector catalogue (>20)
  - Specific promoters/selection markers for various projects
- Transposases
  - GMP & research grade mammalian codon optimized mRNA
  - Excision-only transposase DNA and mRNA

Gene Editing Kits

- Cas-CLOVER + DNA templates
  - Knock-in projects paring Cas-CLOVER w/ DNA templates
  - In combination w/ piggyBac (Footprint-Free® Gene Editing)
- Gene Editing Kit Vectors

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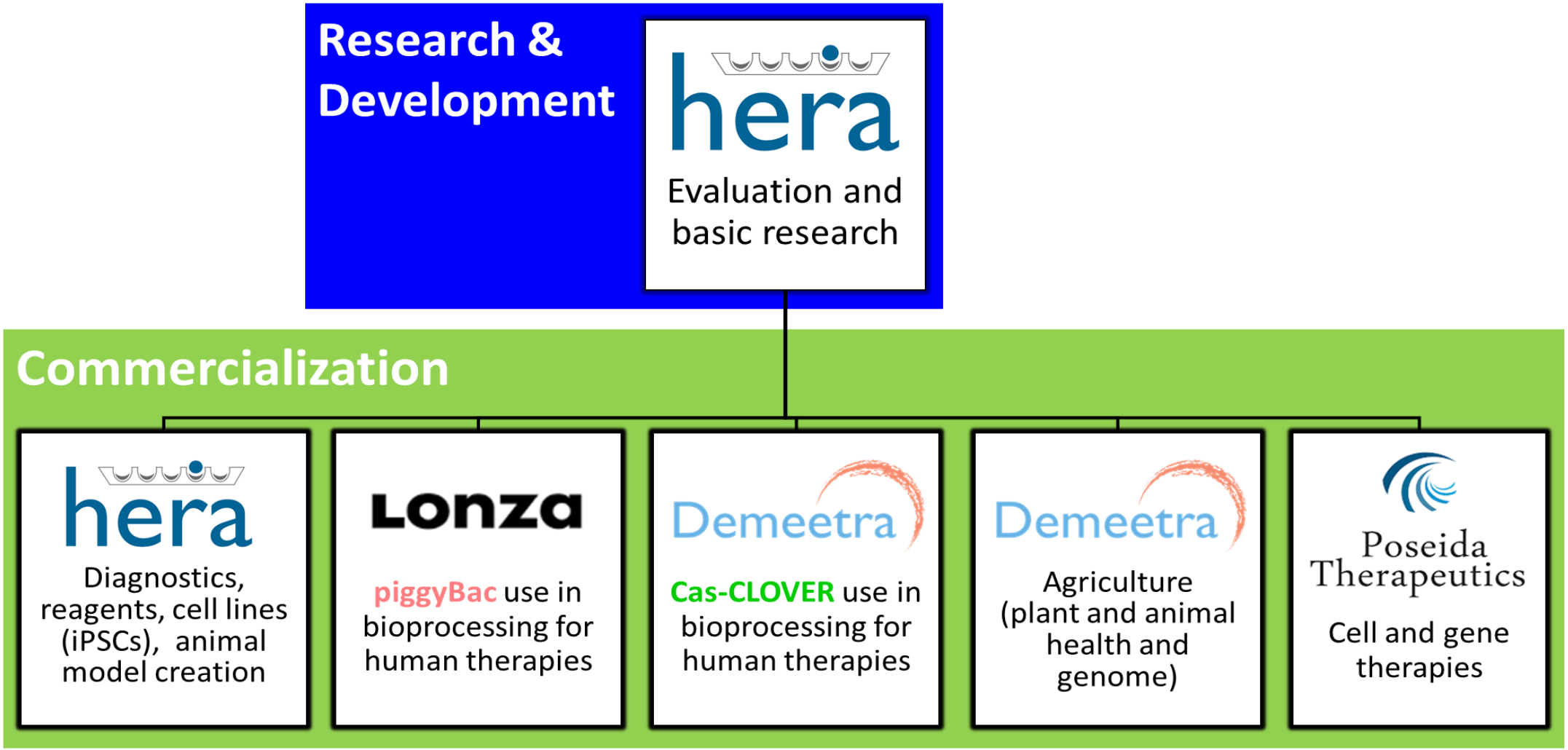
Cas-CLOVER and piggyBac licensing



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The Hera Team

Michael Schlosser, Ph.D., DABT
Chief Executive Officer
Mike joined Hera BioLabs in 2019 and has over 25 years of pharma/biotech industry experience with startups and small/large pharma. He founded Midwest BioResearch (MBR) in 2003, a nonclinical research service lab acquired in 2009 by WIL Research (now Charles River Labs). Prior to starting MBR, he was head of nonclinical safety at Pharmacia/Pfizer (Skokie) and held previous director level positions at Searle and Astra Pharmaceuticals. Mike has a PhD in pharmacology/toxicology from University of Mississippi (Ole Miss) and is a Diplomate of the American Board of Toxicology.

Fallon Noto, Ph.D.
Executive Director, R&D/In Vivo Services
Dr. Noto has 15+ years working with mice and rats, expertise in rodent humanization, cell and tissue transplantation, microsurgery, and ethical animal care with 10+ publications in the field. She is proficient in cell culture techniques of mammalian cells, primary cells, and pluripotent stem cells, including differentiation assays and viral transduction. Dr. Noto is skilled in molecular techniques, including tissue histology, PCR, and protein expression analysis.

Chris Brenzel
Director, Scientific Business Development
Chris has been with Hera BioLabs since its inception in 2015. He has extensive experience in scientific business development, market research, and licensing for startups and fast-growing technology-based contract research organizations. He grew the gene-editing and animal models development businesses at Transposagen Biopharmaceuticals, a company acquired by Hera BioLabs in 2018. Contact Chris at cbrenzel@herabiolabs.com or 859-414-0648.

Diane Begemann, Ph.D.
Study Director, In Vivo Services
Dr. Diane Begemann earned her PhD in Toxicology & Cancer Biology from the University of Kentucky in 2020. Dr. Begemann did her dissertation work in the lab of Dr. Natasha Kyprianou, focusing on mechanisms of chemotherapeutic resistance in models of prostate cancer. After moving with her mentor, her dissertation work was completed at the Tisch Cancer Institute at Mount Sinai Hospital.

Dr. Valeriya Steffey
Director, In Vitro Services
Dr. Valeriya Steffey worked for Transposagen Biopharmaceuticals Inc. for over ten years, prior to joining Hera, where she distinguished herself as an innovative leader in both technology and business development. Dr. Steffey was the Director of Cell Line Engineering at Transposagen. Under her management, her team had a proven track record of successful completion of cell line engineering, engineered animal models and R&D projects.

R. Grace Walton, Ph.D.
Scientist, Business Development
Dr. Walton earned her Ph.D. at the University of Alabama at Birmingham, where she studied type 2 diabetes. She moved to the University of Kentucky for a post-doctoral fellowship with Philip Kern M.D. and Charlotte Peterson Ph.D., where she studied molecular mechanisms of exercise physiology. She remained in Dr. Peterson's lab as scientist and then as Research Assistant Professor.

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