Creative Biogene
Zebrafish Platform

Zebrafish

Disease Model Services

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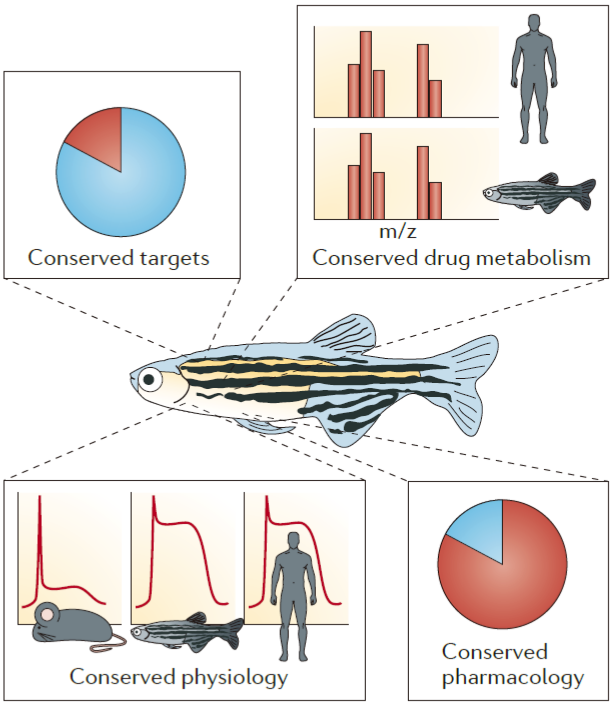
Zebrafish Disease Models

Zebrafish (Danio rerio) have been widely used as animal models in various fields of biomedical research. The transparency of the zebrafish embryo enables real-time visualization of the development and morphogenesis of almost all its tissues and organs. Zebrafish are amenable to genetic manipulation, for which advanced genetic and molecular techniques are constantly being introduced. Meanwhile, a pair of adult zebrafish can produce up to hundreds of eggs in a single clutch, and the resulting embryos and larvae are sufficiently small that they can be housed in 96-well or 384-well plates, allowing phenotypic analysis by high-throughput microscopy and behavioral analytical systems.



Zebrafish are suitable for drug discovery[1]

Zebrafish has many physiological and genetic similarities with humans, including the brain, digestive tract, vasculature, musculature, and innate immune system. The cloning of mutated genes screened for specific phenotypes in zebrafish has similarities in humans and thus serves as a model for human disease and investigation of underlying mechanisms. Multiple mutants that show phenotypic similarities to human disease have been screened and identified. These include neurological disorders, cardiovascular diseases, hematological disorder, muscle disease, cancers, anxiety, Parkinson's disease, and posttraumatic stress disorder.

As a global provider, Creative Biogene provides drug screening, efficacy evaluation, and safety testing services utilizing the zebrafish platform for researchers worldwide.

[1]: MacRae, C., Peterson, R. Zebrafish as tools for drug discovery. Nat Rev Drug Discov 14, 721–731 (2015).

Drug Efficacy Evaluation

Oncology Models	
Models / Assays	**Main Indicators**
Zebrafish anti-angiogenesis model	Number of intact intersegmental vessels
	Area of sub-intestinal vessels
Xenotransplantation cancer model	Fluorescence intensity of apoptotic cells
	Tumor size
	Metastatic distance of tumor cells
	Antitumor drug distribution and excretion
	Number of tumor neovascularization
	Tumor cell proliferation
	Number of tumor-associated lymphatics
	Tumor histopathology
P-glycoprotein (P-gp) inhibitor screening	P-glycoprotein function inhibition
Targeted cancer drug screening	Wnt pathway: body axis length
	Wnt pathway: regeneration length of broken tail
	Number of iris pigmented cells
Apoptosis model	Fluorescent signal intensity of Caspase substrate metabolites

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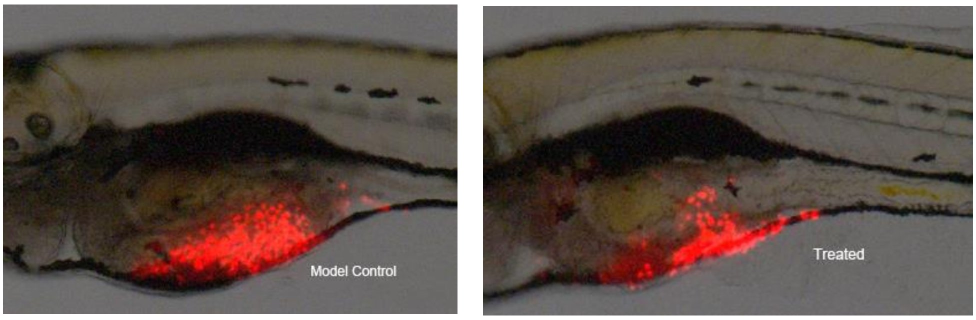
Oncology Model Examples

Xenotransplantation cancer model

Zebrafish only have non-specific immunity in the early stage, and do not have perfect specific immunity, so there is no rejection of human tumor cells. Therefore, zebrafish can be directly inoculated with human tumor cells to screen broad-spectrum antitumor drugs. In addition, zebrafish have similar tumorigenesis and development-related signaling pathways to humans and thus can be used to screen targeted anti-tumor drugs.

Method

- Inject fluorescently labeled human tumor cells into zebrafish to build tumor models.
- Administrate test articles to zebrafish after injecting tumor cells.
- After a period of time, record and collect images of zebrafish to observe the growth and metastasis of tumor cells to measure apoptosis, inflammatory responses, and morphological changes and calculate survival periods.



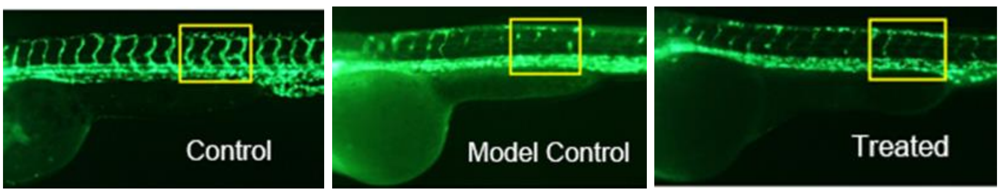
Tumor growth phenotype

Anti-angiogenesis model

Anti-angiogenesis drugs exert anti-angiogenic effects mainly through mechanisms such as affecting the expression of vascular growth factors, blocking the activity of angiogenic factor receptors, inhibiting endothelial cell proliferation, and limiting the activity of endothelial cell-specific integrins.

Method

- Endothelial fluorescent transgenic zebrafish are used to manifest and track angiogenesis.
- Zebrafish ingesting angiogenesis inhibitors consist of a significantly smaller portion of sub-intestinal vessels than normal zebrafish, while intersegmental vessels can break off to lose integrity.



Inhibition of intersegmental angiogenesis by the test article

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Drug Efficacy Evaluation

Metabolic Disease Models / Assays	
Models / Assays	**Main Indicators**
High-sugar and high-fat diet-induced model (e.g., diabetes and hyperlipidemia)	Glucose level
	Triglyceride level
	Cholesterol level
	Venous blood flow velocity
	Fluorescent signal intensity of perivenous macrophages
	Peripheral motor nerve fluorescent signal intensity
	Peripheral motor nerve neutrophil count
	Retinal lesions (pathological sections)
Hypolipidemic drugs screening model	Triglyceride level
	Cholesterol level
Fat metabolism model	Intestinal and vascular fat content
	Yolk sac fluorescent signal intensity
	Pancreatic lipase optical density value
Energy metabolism regulator screening model	Fluorescent signal intensity of energy metabolite detection reagents
Gastrointestinal motility inhibitor screening model	Fluorescent signal intensity of intestinal contents
Antidote screening model	Behavioral testing
	Cardiac output
	Blood flow velocity

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Metabolic Disease Model Examples

Hyperlipidemia model

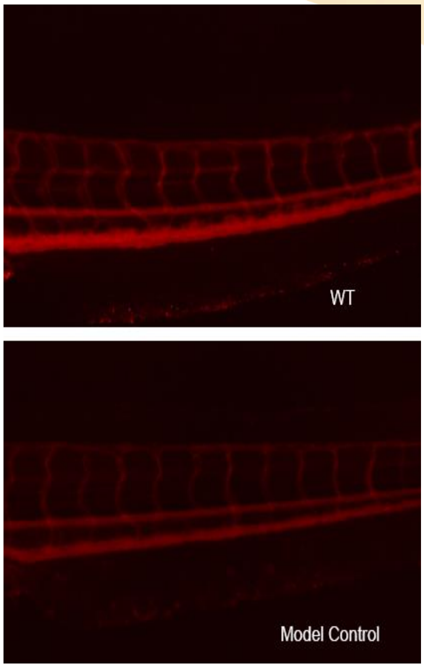
Egg yolk is made up of 60% lipids. Hence, feeding zebrafish with egg yolk powder can rapidly enhance the fat content of blood within zebrafish, thereby establishing a zebrafish model of hyperlipidemia.

Method

Feed zebrafish egg yolk powder and fluorescently labeled cholesterol to establish a hyperlipidemia model, and then administrate the zebrafish with the test article.

Evaluate the efficacy of test articles on zebrafish using 4 indicators:

1. zebrafish lipid phenotype map;
2. zebrafish total triglyceride content;
3. zebrafish cholesterol phenotype map;
4. zebrafish total cholesterol content.



Cholesterol phenotype map

Type 2 diabetes model

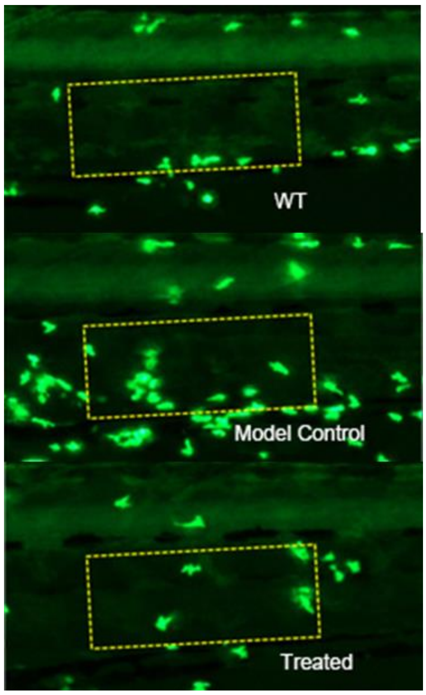
Zebrafish exhibit persistent hyperglycemia in a sugar-loaded state, which can be induced with high-glucose and high-fat diets, thus mimicking neurological complications of type 2 diabetes in humans.

Persistent high blood glucose levels led to diabetic complications, with hyperglycemia involving central and peripheral neuropathy and neuroinflammation.

Method

Build the neutrophil and motor nerve hybrid fluorescence strain zebrafish into type 2 diabetes models using the high-glucose and high-fat diet.

Then administrate zebrafish with hypoglycemic drugs; The number of neutrophils in zebrafish peripheral motor nerves is observed under the microscope.



Peripheral motor nerve neutrophil count

Drug Efficacy Evaluation

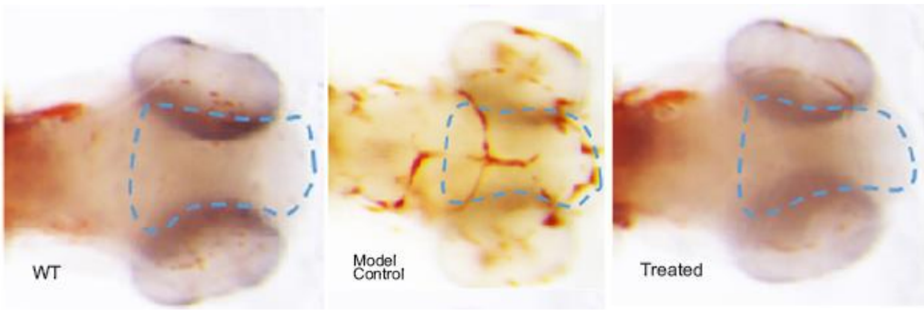
Cardiovascular & Cerebrovascular Models / Assays	
Models / Assays	**Main Indicators**
Pro-angiogenesis model	Sub-intestinal sprouting angiogenesis
	Area of sub-intestinal vessels
Vascular endothelial injury protection evaluation	Vessel diameter
	Thrombus formation
	Number of cardiac red blood cells
	Blood flow velocity
Platelet aggregation thrombosis	Thrombus formation
	Number of cardiac red blood cells
	Blood flow velocity
Erythrocytic thrombosis	Thrombus formation
	Number of cardiac red blood cells
	Blood flow velocity
Drug screening model for heart failure treatment	Pericardial edema area
	Venous stasis area
	Cardiac output
	Blood flow velocity
Myocardial injury protective agent screening model	Fluorescent signal intensity of myocardial apoptotic cells
	Cardiac output
	Blood flow velocity
Cardiac hemorrhage model	Incidence of cardiac hemorrhage
	Exercise improvement (Behavioral testing)
	Blood flow improvement
Cerebral hemorrhage model	Incidence of cerebral hemorrhage
	Cardiac output
	Exercise improvement (Behavioral testing)
	Blood flow improvement
Cerebral ischemia model (cerebral thrombosis)	Incidence of cerebral thrombosis
	Exercise improvement (Behavioral testing)
	Blood flow improvement

Thrombosis model

After ponatinib enters the human body, it causes thrombosis, inhibits the proliferation, migration, and angiogenesis of vascular endothelial cells, and affects nitric oxide synthesis. As a result, ponatinib leads to vascular endothelial injury and cerebral thrombosis. The zebrafish brain reserves typical morphological characteristics of the vertebrate brain as well as coagulation factors and platelet receptors, which can induce cerebral thrombosis after ingesting a large amount of ponatinib.

Method

Build the zebrafish thrombus model using ponatinib. Administrate zebrafish in the treatment group with both ponatinib and antithrombotic drugs. After a period of time, observe the formation of the thrombus in the head by erythrocyte-specific staining on the whole zebrafish.



Cerebral thrombosis phenotype

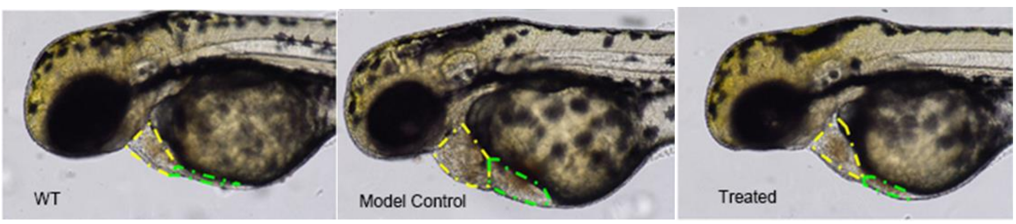
Heart failure model

Zebrafish and mammalian hearts reserve similar functions, including blood flow direction, a high-pressure system driven by specialized endocardial muscle tissue, a heart rhythm regulated by an electrical system, and a heartbeat associated with pacing activity. The pharmacological response of zebrafish to typical cardiotoxins is similar to that of humans.

Verapamil is a phenylalanine calcium antagonist which can induce zebrafish heart failure. Compared with normal zebrafish, zebrafish with heart failure manifest pericardial edema, venous stasis, reduced cardiac output and slowed blood flow.

Method

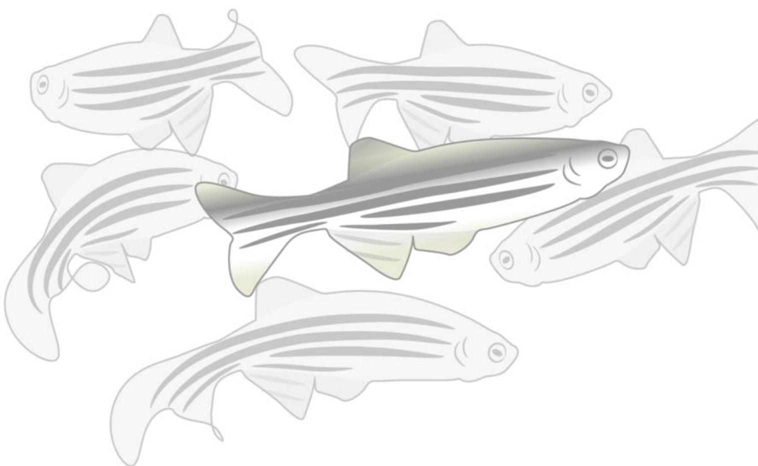
A zebrafish heart failure model is constructed using verapamil. Zebrafish in the drug intervention group ingest both verapamil and anti-heart failure drugs. After a period of time, the formation of pericardial edema and venous stasis is observed in zebrafish; heartbeat output and blood flow velocity are measured with the heartbeat flow analysis system.



Heart failure phenotype

Drug Efficacy Evaluation

Liver & Kidney Disease Models / Assays	
Models / Assays	**Main Indicators**
Hepatoprotective drug screening model	Liver size
	Degree of liver degeneration
	Hepatic histopathological testing
	Yolk sac size
Alcoholic fatty liver model	Degree of hepatic steatosis
	Liver tissue section (H&E staining)
Alcoholic liver injury model	Liver size
	Degree of liver degeneration
	Liver tissue section (H&E staining)
	Yolk sac size
Non-alcoholic fatty liver model	Degree of hepatic steatosis
	Liver tissue section (H&E staining)
Kidney protectant screening model	Renal edema
	Glomerular filtration rate
Cytochrome P450 analysis	CYP3A4 activity
	CYP2D6 activity



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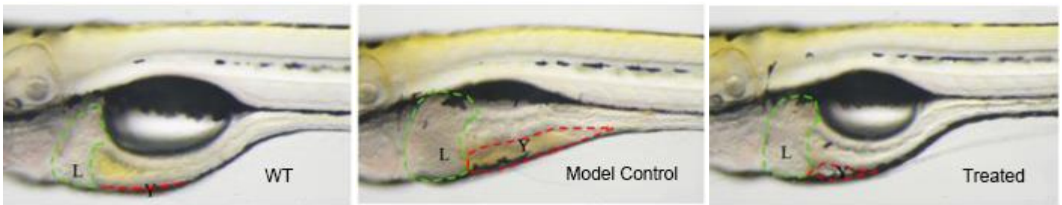
Liver & Kidney Disease Model Examples

Alcoholic liver injury model

Zebrafish genes have shown 87% similarity to human genes. Moreover, zebrafish liver contains various lipid metabolic enzymes homologous to mammals. These enzymes can defend against foreign chemicals, including enzymatic induction and oxidative stress. Moreover, the larvae of zebrafish are transparent throughout. Hence, toxic phenotypes can be easily observed.

Method

Build the zebrafish alcoholic liver injury model using ethanol. Administrate zebrafish in the treatment group with both ethanol and hepatoprotective agents. After a period of time, observe the liver and yolk sac phenotype of zebrafish using the microscope, and obtain pathological sections for the evaluation of pathological structural changes in the liver.



Liver (L) and yolk sac (Y) phenotype

Kidney protectant screening

The zebrafish kidney shares many similarities with mammalian in the development and onset of kidney diseases, involving many highly conserved genes. Therefore, zebrafish is suitable for the evaluation of renal protective efficacy.

In both humans and zebrafish, aristolochic acid can induce nephrogenic edema and abnormal kidney morphology, causing irreversible kidney damage and death.

Method

Build the zebrafish renal injury model using aristolochic acid. Administrate zebrafish in the treatment group with both aristolochic acid and neuroprotectants. After taking neuroprotectants for a period of time,

1. calculate the incidence rate of renal edema in zebrafish,
2. record and collect images of zebrafish with the microscope and analyze the glomerular filtration rate through the whole-body fluorescence intensity of zebrafish.

Renal edema phenotype

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Drug Efficacy Evaluation

Neurological Diseases Models / Assays	
Models / Assays	**Main Indicators**
Anti-epileptic drug screening model	Rapid movement distance
	Gabra1 gene expression level
	C-FOS gene expression level
Multiple sclerosis drug screening model	Fluorescent signal intensity of myelin staining
Alzheimer's disease drug screening model	Responsiveness to stimulation
	Expression levels of related genes
	Acetylcholinesterase activity
	Recovery rate of intersegmental vascular injury
	Monoamine oxidase inhibition
	Recovery evaluation of movement disorder
	Specific phenotype (e.g., no brain and no eyes)
Peripheral nerve injury model	Peripheral motor nerve length
Axis regeneration model	Axonal fluorescence intensity
CNS injury model	Fluorescent signal intensity of apoptotic cells in the central nervous system
Anti-depression model	Monamidase (MAO) activity
Parkinson's disease model	Antidote screening model
	Dopamine neuron area
Hair cell protective agent screening model	Hair cell protection (%) - Hair cell fluorescence intensity
Bipolar psychiatric disorder model	Behavioral testing
	Gene expression level of gamma-aminobutyric acid type A receptor (GABA-A)
	Gene expression level of melatonin receptor
Insomnia model	Behavioral testing
	Gene expression level of gamma-aminobutyric acid type A receptor (GABA-A)
	Gene expression level of melatonin receptor
	Monamidase (MAO) activity
Anxiety model	Behavioral testing
	Monamidase (MAO) activity

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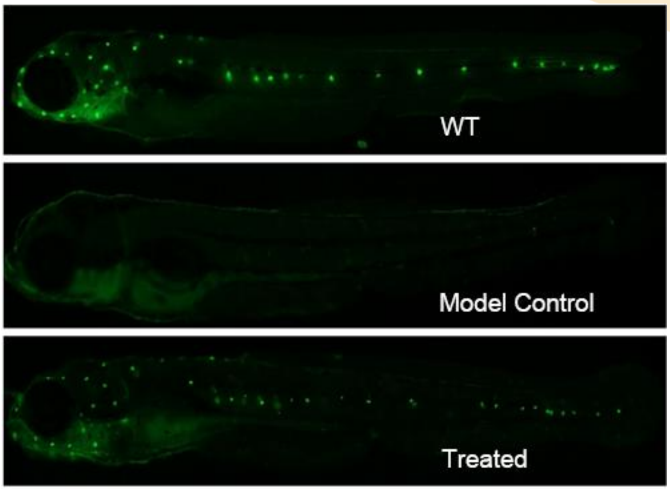
CNS Disease Model Examples

Demyelination model

The structural features of the myelin sheath and the differentiation process of oligodendrocytes in zebrafish are highly consistent with those of mammals, which can reflect changes caused by the dysfunction of the nervous system under the action of foreign compounds macroscopically. Zebrafish axonal damage can be induced by ethanol to mimic multiple sclerosis in humans.



Axonal phenotype

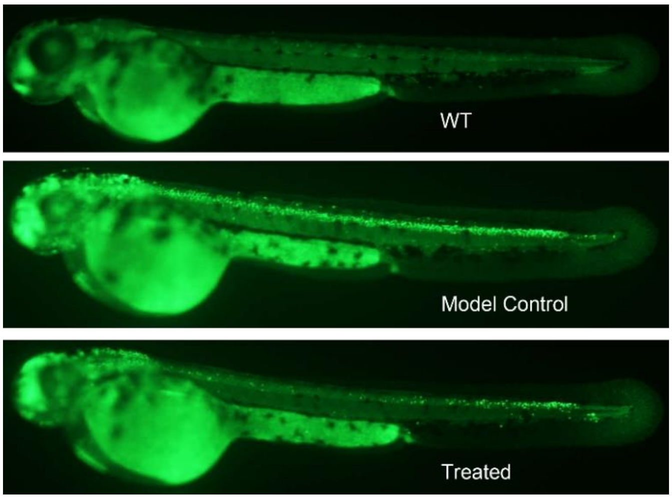
Method

Build the zebrafish demyelination model on the fluorescently-label axon zebrafish using ethanol. Administrate zebrafish in the treatment group with both ethanol and drugs that promote axonal regeneration. After a period of time, analyze the fluorescence intensity of zebrafish axonal under the fluorescence microscope.

Nerve regeneration model

Zebrafish is a vertebrate with high homology with humans, and its brain has typical vertebrate brain morphological features. The myelin structural features and oligodendrocyte differentiation process of zebrafish are highly consistent with those of mammals. Moreover, zebrafish has a short growth and development cycle and a simple nervous system, which make it conducive to the study of central neuroprotective agents.

Mycophenolate mofetil is a commonly used chemical drug for inducing zebrafish nerve injury, as it can inhibit the growth of neuronal axons and interfere with nerve and cell migration, leading to neuronal cell apoptosis.



Central nervous phenotype

Method

Build the zebrafish nerve injury model using mycophenolate mofetil. Administrate the zebrafish in the treatment group with both mycophenolate mofetil and central neuroprotectors. After a period of time, perform specific fluorescent staining on zebrafish to observe the apoptosis of central nervous cells.

Bone & Muscle Disease Models / Assays	
Models / Assays	**Main Indicators**
Analgesic drug screening	Total movement distance
Tissue regeneration/wound healing	Melanin content in damaged areas of torso skin
	Cell proliferative capacity at the wound site
	Apoptosis at the wound site
	Number of necrotic cells at the wound site
	Number of leukocyte at the wound site
	Regeneration length of adult caudal fin
	Regeneration area of adult caudal fin
Skeletal development promoter screening model	Number of vertebrae
Cartilage injury model	Chondrofluorescence intensity
	Meckel's cartilage length and angle
	Ceratohyal bone length and angle
Osteoporosis therapeutic agent screening model	Expression level of bone morphogenetic protein (BMP)
	Bone density

Bone & Muscle Disease Model Examples

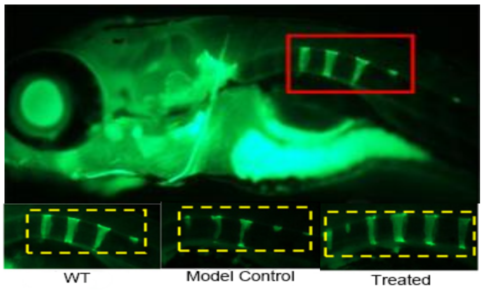
Osteoporosis model

Prednisone is a commonly used agent of glucocorticoids. Corticosteroid treatment causes a change in the ratio between osteoprotegerin (OPG) and nuclear factor KB receptor activator ligand (RANKL), leading to increased bone resorption during the first 3 to 6 months, making it more likely to develop osteoporosis. Therefore, prednisone is an ideal chemical drug to induce zebrafish osteoporosis.

We evaluate osteoporosis with two indicators: 1. bone morphogenetic protein (BMP) expression level; 2. bone mineral density (fluorescent signal intensity).

Method

Build the zebrafish alcoholic liver injury model using ethanol. Administrate zebrafish in the treatment group with both ethanol and hepatoprotective agents. After a period of time, observe the liver and yolk sac phenotype of zebrafish using the microscope, and obtain pathological sections for the evaluation of pathological structural changes in the liver.



Vertebral bone fluorescence phenotype

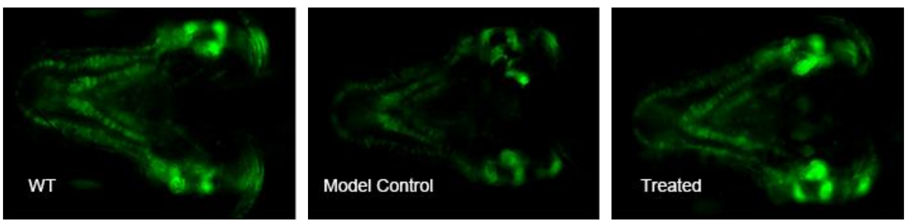
Wound healing and regeneration model

The skeletal development of zebrafish reserved relatively high similarity to that of other vertebrates and, therefore, can be used to evaluate cartilage repair efficacy.

The cartilage of zebrafish is mainly distributed in the head, including seven pairs of pharyngeal cranial cartilage arches and cerebro cranial cartilage. Based on the characteristics of cartilaginous transgenic fluorescent zebrafish, the cartilage fluorescence intensity of zebrafish suffering from cartilage damage is significantly dimmer than that of normal zebrafish.

Method

Build the zebrafish cartilage injury model using dexamethasone. Administrate the zebrafish in the treatment group with both dexamethasone mofetil and test articles. After a period of time, observe changes in cartilage fluorescence.



Cartilage phenotype

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Drug Efficacy Evaluation

Inflammation & Immune Disease Models / Assays	
Models / Assays	**Main Indicators**
Mechanical injury-induced inflammation model (Tail docking)	Neutrophil count
	Inflammatory cytokine expression levels
Bacterial infection-induced inflammation model	Neutrophil count
	Inflammatory cytokine expression levels
Neuroinflammation model (copper sulfate induced)	Neutrophil count
	Inflammatory cytokine expression levels
Colitis model	Intestinal mucosal thickness
	Intestinal lumen diameter
	Intestinal lumen area
	Distribution of neutrophils in colitis tissue
	Intestinal histopathology
Arthritis model	COX-2 activity
Immunomodulator screening model	Neutrophil count
	Macrophage count
	Macrophage phagocytic capacity
	T cell count
	Inflammatory cytokine changes
Antibacterial drug screening model	Bacterial content (fluorescent)
Anti-pneumonia efficacy evaluation (bacterial)	Neutrophil count in swim bladder
	Macrophage count in swim bladder
	TNF-α and IL-1β expression
	Pathological detection of swim bladder
	Video recording of macrophages and neutrophils spilling out of blood vessels
Anti-pneumonia efficacy evaluation (viral)	Neutrophil count in swim bladder
	Macrophage count in swim bladder
	TNF-α and IL-1β expression
	Pathological detection of swim bladder
	Video recording of macrophages and neutrophils spilling out of blood vessels

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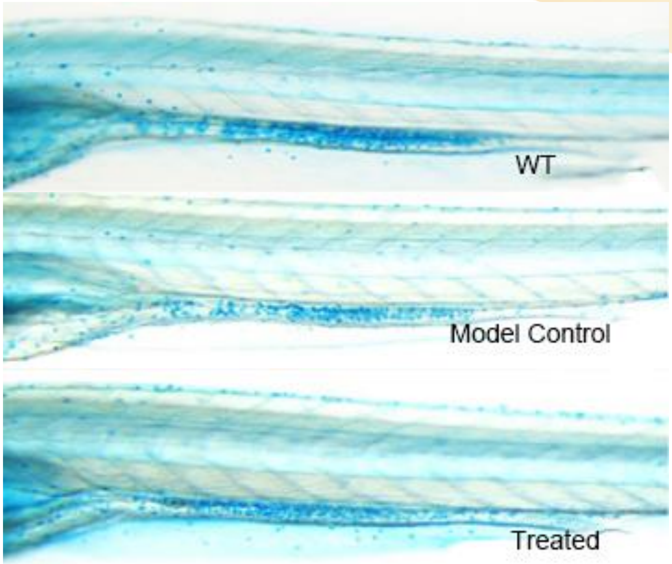
Colitis model

The anatomy and cytoarchitecture of the zebrafish digestive tract are similar to that of humans, involving concentric layers of inner epithelium, connective tissue, circular muscle and outer longitudinal muscle layer.

The ethanol solution of trinitrobenzenesulfonic acid (TNBS) can dissolve the mucus on the surface of the intestinal mucosa and temporarily destroy the intestinal mucosal barrier. Hence, TNBS can combine with intestinal tissue proteins to form a complete antigen, resulting in a delayed response of the intestinal mucosal immune system to the antigen and damaging the intestinal mucosa.

Method

Build the zebrafish colitis model using TNBS. Administrate the zebrafish in the treatment group with both TNBS and test articles. After a period of time, observe changes in the intestinal lumen area and measure the number of goblet cells in zebrafish.



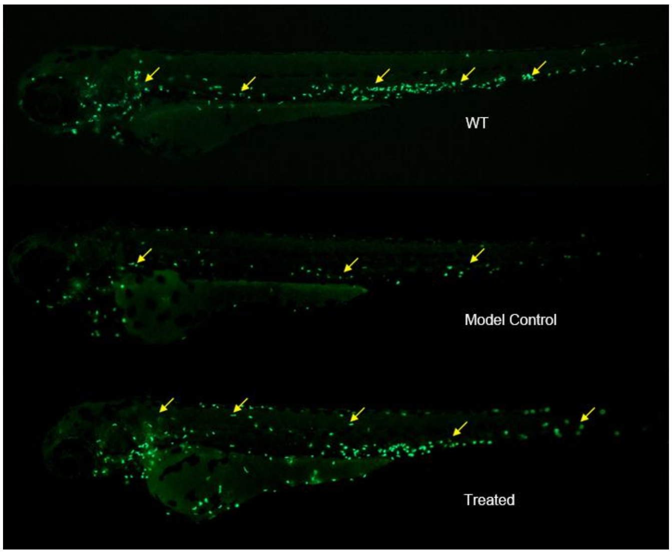
Intestinal goblet cell phenotype

Immunocompromised model

A zebrafish immunocompromised model can be constructed by intravenous administration of vincristine. The high dose of vincristine results in significant myelosuppression, leading to a decrease in platelets, erythrocytes and leukocyte counts (neutrophils, macrophages, T cells, etc.). Human and zebrafish are very similar in cellular composition of the immune system. The adult zebrafish are the smallest of all individual animals with both specific and non-specific immunity, which makes them suitable for high-throughput evaluation of regulatory immune efficacy.

Method

Build the immunocompromised zebrafish model by intravenous administration of vincristine on transgenic zebrafish with neutrophil expressing green fluorescent. Administrate zebrafish in the treatment group with both vincristine and immunomodulator. After a period of time, observe changes in the intensity of green fluorescence.



Neutrophil phenotype

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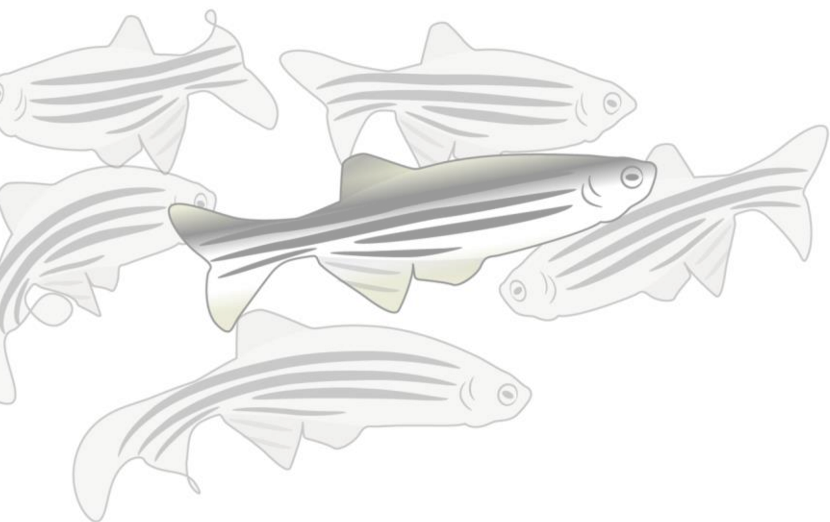
Drug Efficacy Evaluation

Hematological Disease Models / Assays	
Models / Assays	**Main Indicators**
Anemia model	Erythrocyte content (chemotherapy drug-induced cancer anemia model)
	Erythrocyte signal intensity (hemolytic anemia model)
Neutropenia model	Neutrophil count

Visual Function Disease Models / Assays	
Models / Assays	**Main Indicators**
Ophthalmic protective agent screening model	Apoptosis of ocular cells
	Ocular histopathology
Ocular vascular hyperplasia model	Ocular vascular area
	Ocular histopathology



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Hematological Ddisease Model Examples

Anemia model

The transcription factors and signal transduction pathways associated with the formation of the zebrafish hematopoietic system have a high degree of homology with humans, and these features make zebrafish widely used in the study of the human hematopoietic system and blood disorders.

Phenylhydrazine is a suitable inducer for the zebrafish anemia model. Phenylhydrazine can induce red blood cell eversion and oxidative stress in humans and zebrafish. Meanwhile, red blood cell eversion can cause blood cell aggregation, and oxidative stress leads to endothelial cell damage, which in turn causes platelet aggregation, fibrinogen formation, ultimately, thrombosis.

Method

Build the zebrafish anemia model using phenylhydrazine. Administrate the treatment group zebrafish with phenylhydrazine after ingesting anti-anemia drugs. Then analyze the erythrocyte signal intensity of zebrafish using the erythrocyte-specific staining on zebrafish.

Anemia phenotype

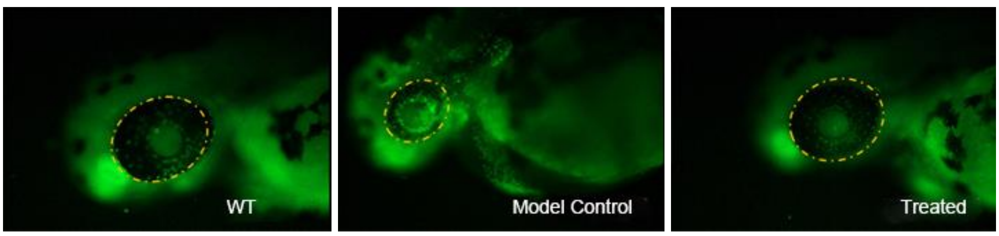
Visual Function Disease Model Examples

Eye protectant screening model

The ocular vascular and retinal structures of zebrafish are very similar to those of humans, and mycophenolate mofetil can induce apoptosis of zebrafish eye cells.

Method

Build the zebrafish eye injury model using mycophenolate mofetil. Administrate the treatment group zebrafish with both mycophenolate mofetil and eye protectants. After a period of time, analyze apoptotic cells by staining zebrafish fluorescently and evaluate the pathological structural changes of zebrafish eyes through pathological sections.



Ocular apoptosis phenotype

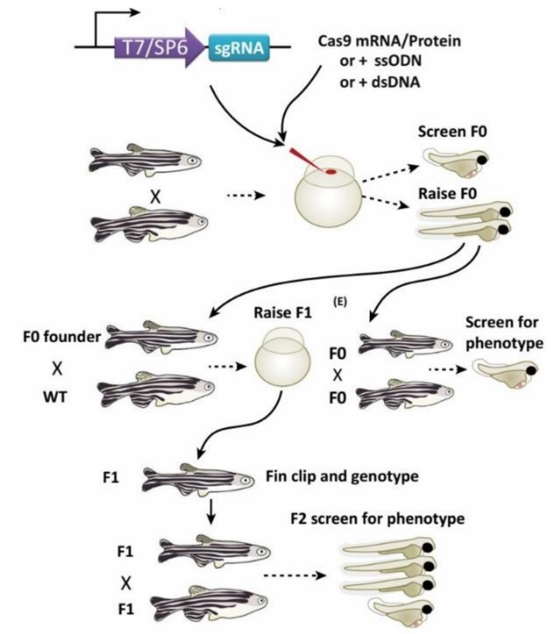
Gene-edited Models

As a model organism, zebrafish reserved advantages of small size, short generation time, easy mass culture, high spawning capacity, transparent embryos, and high genomic similarity with human genomes.

Gene editing is an important tool in zebrafish research, which are mainly used in the following aspects:
1. Gene identification and functional study by site-specific knockout technology;
2. Using reporter genes to study the function of receptors and other proteins;
3. Various zebrafish mutants were constructed to study human diseases and screen drugs through zebrafish disease models;
4. Construction of biosensors containing specific promoters for environmental monitoring.

Creative Biogene provides gene editing services, including knockout, knock-in, and targeted mutation in zebrafish by the Tol2 transposon system, as well as CRISPR-Cas9 and Morpholino technologies.

We are well experienced in the service of gene editing. Please do not hesitate to contact us anytime at your convenience if you have any specific requirements regarding genes and loci, and a quick and sufficient solution will be provided around the corner.



Strategy for zebrafish genome engineering with CRISPR/Cas91

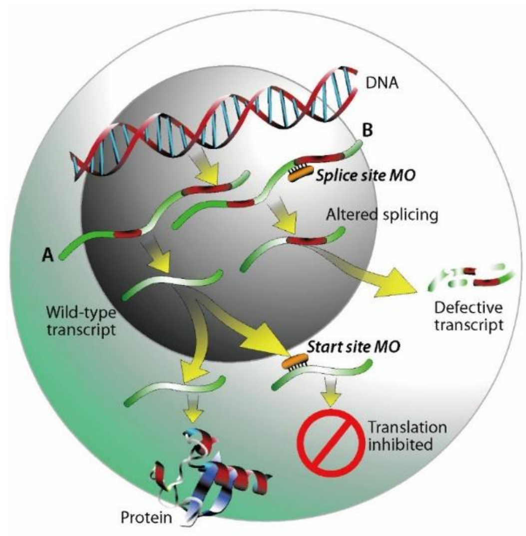


Illustration of how the start site (A) and splice site (B) morpholinos work2

[1]: Li M, et al. Zebrafish genome engineering using the CRISPR–Cas9 system. Trends in Genetics, 2016, 32(12): 815-827.
[2]: Timme-LaragyA R, et al. Gene knockdown by morpholino-modified oligonucleotides in the zebrafish (Danio rerio) model: applications for developmental toxicology. Developmental Toxicology. Humana Press, Totowa, NJ, 2012: 51-71.

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