4th INTERNATONAL CONFERENCE ON GMP, GCP & QUALIITY CONTROL

ORAL PRESENTATON

ON

'ZEBRAFISH AS A MODEL SYSTEM FOR DRUG TARGET SCREENING AND VALIDATION'

PRESENTATION BY:

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Content

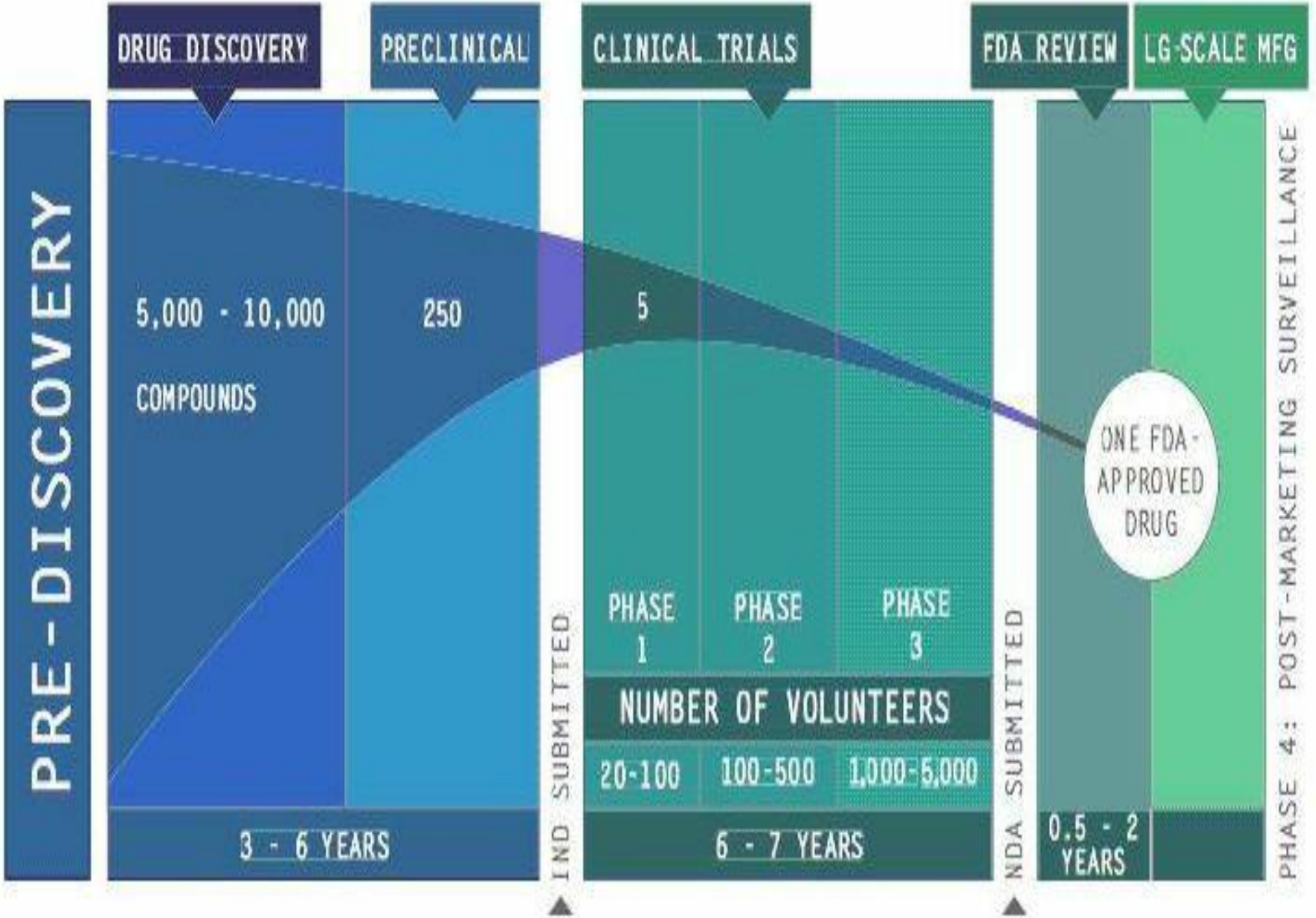
- Introduction
- Drug Discovery Process
- Reasons of Drug Failure
- Why Zebrafish as Model?
- Current Trends/Requirement
- Advantages/Benefits of Model
- Conclusion
- References

INTRODUCTON

- **The Pharmaceutical industry is short of new drugs. In the 2nd part of the 20th century, about 50-60 new drugs (NCEs) were approved by the FDA every year.**
- **Conversely, research costs for a new drug are estimated to be in the $1-1.5 Bi. range. Considering all high-profile failures in recent drug discovery, this figure is unlikely to drop substantially.**
- **There is also increasing pressure to limit animal use to situations in which they are absolutely necessary, such as in preclinical toxicity and safety assessment.**
- **Mammalian models of absorption, distribution, metabolism, excretion and efficacy are expensive, laborious and consume large quantities of precious compounds.**
- **Lengthy process: takes 10-15 years to develop**
- **Also, results are unguaranteed.**
- **The current processes by which drugs are discovered are long and expensive.**
- **Many compounds still fall out of the discovery pipeline due to lack of efficacy and mechanism-based toxicity.**
- **Central to these reasons is a failure to understand properly all of the biological roles of potential drug targets in normal and disease processes.**
- **This knowledge failure results in ignorance of the many potential unpleasant consequences that could be rendered by compound modulation of the target's activity *in vivo*.**

Current New Drug Discovery

- Expensive, time consuming and difficult process
- Result unguaranteed
- Costly: A single new drug can cost 1.2billion euros
- Long: take 10- 15 years to develop


PRE-DISCOVERY
DRUG DISCOVERY
PRECLINICAL
CLINICAL TRIALS
FDA REVIEW
LG-SCALE MFG
5,000 - 10,000
COMPOUNDS
250
5
ONE FDA-APPROVED DRUG
PHASE 1
PHASE 2
PHASE 3
NUMBER OF VOLUNTEERS
20-100
100-500
1,000-5,000
3 - 6 YEARS
6 - 7 YEARS
0.5 - 2 YEARS
IND SUBMITTED
NDA SUBMITTED
PHASE 4: POST-MARKETING SURVEILLANCE

Complex disease targets

Cost

Adverse reactions

Poor absorption

Low levels in body

Not effective enough

Intellectual Property

Not sufficiently selective

Side effects

Unsafe

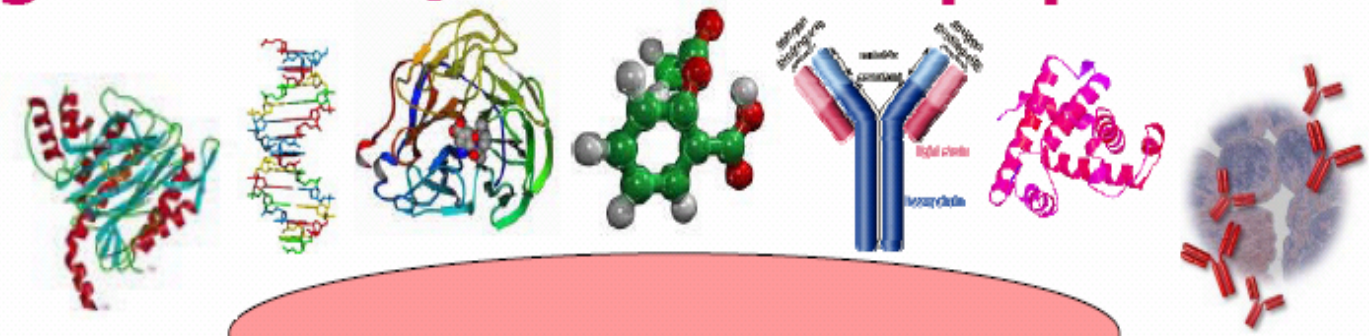
Unstable

Competition

Impractical to make

Confidence in Rationale

Drug discovery and develop. process



Target Identification

Target Validation

In Silico Modeling

DISCOVERY PLATFORMS

Compound Screening

Lead Identification

Lead Optimization

Toxicology

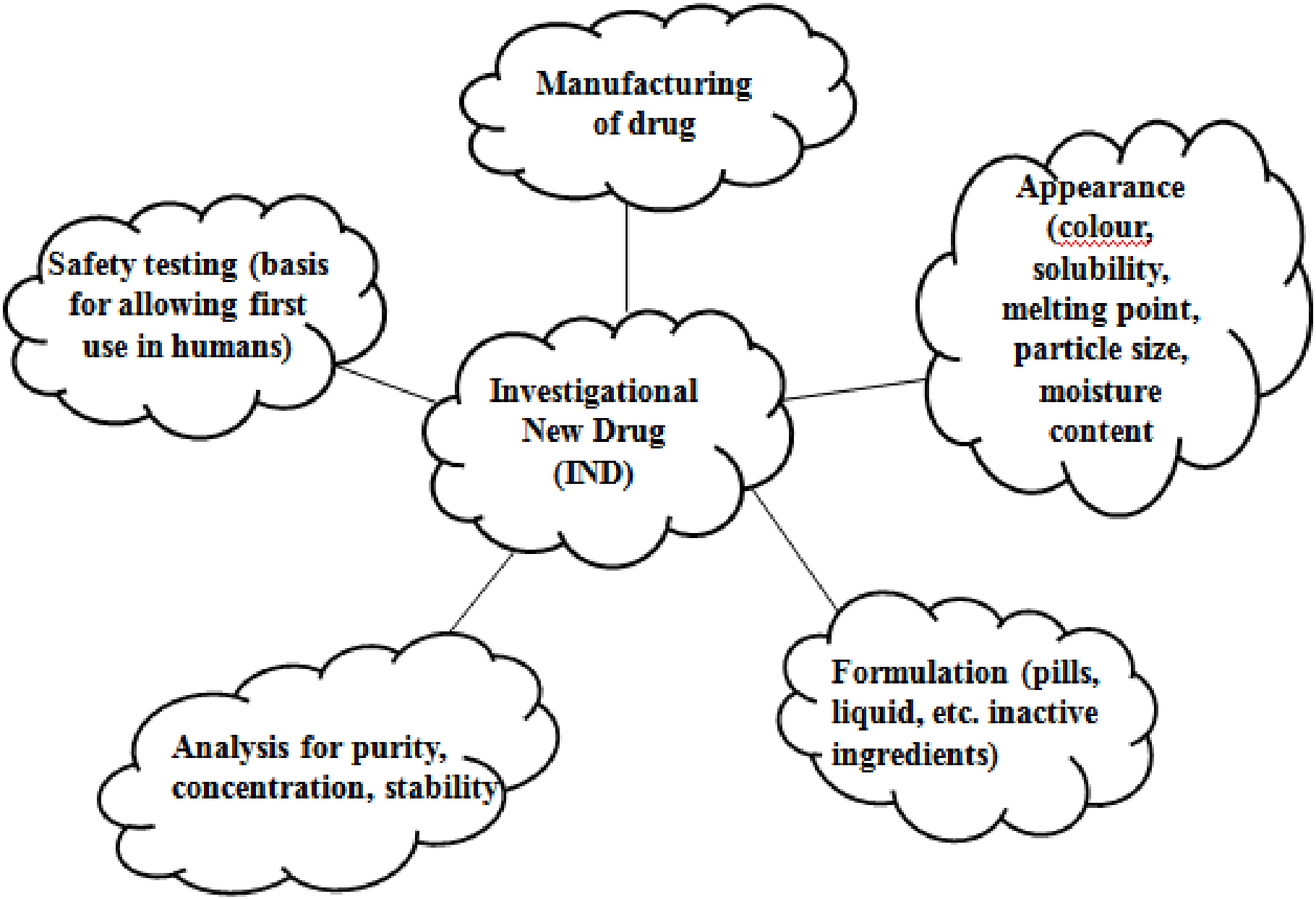
Animal Studies

PRODUCT DEVELOPMENT

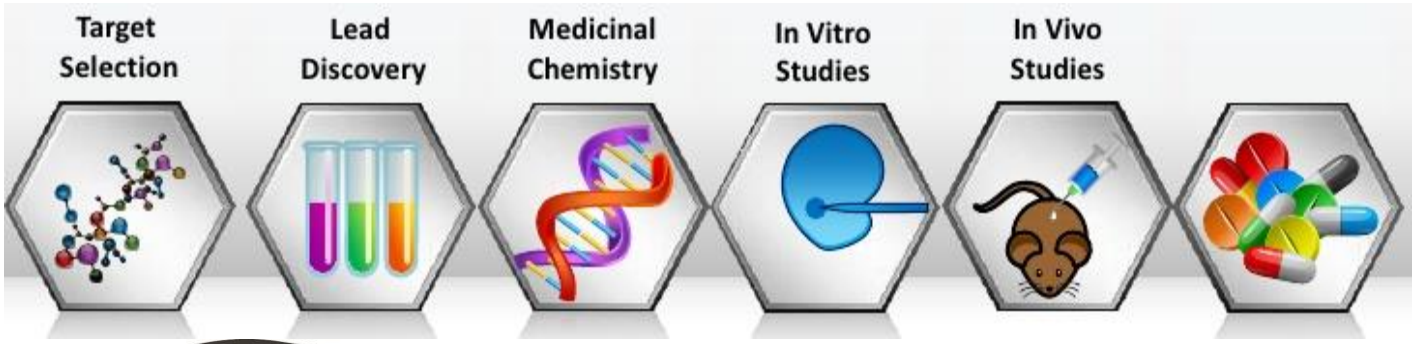
Pre-Clinical Development

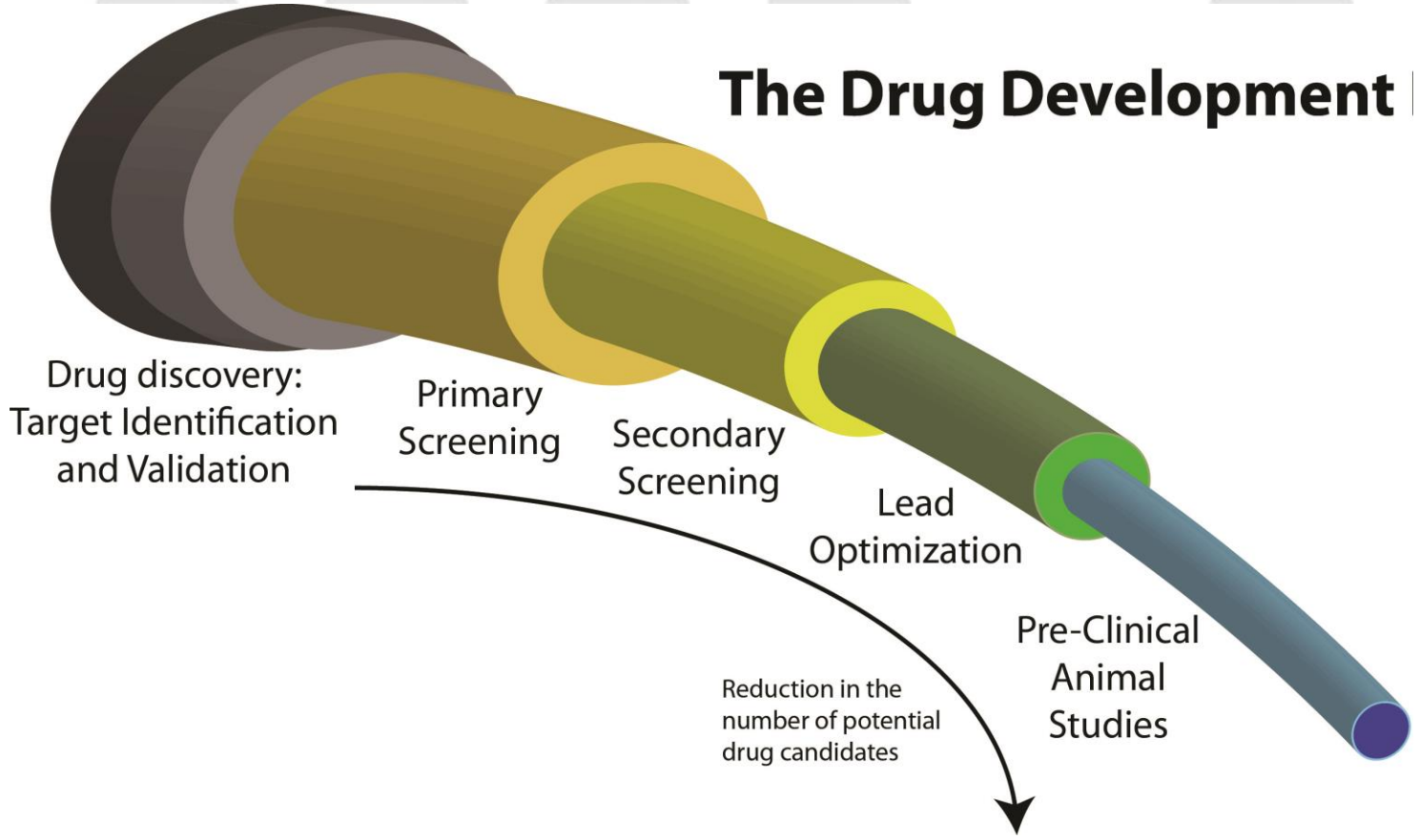
Human Trials

Drug characteristics shown by IND

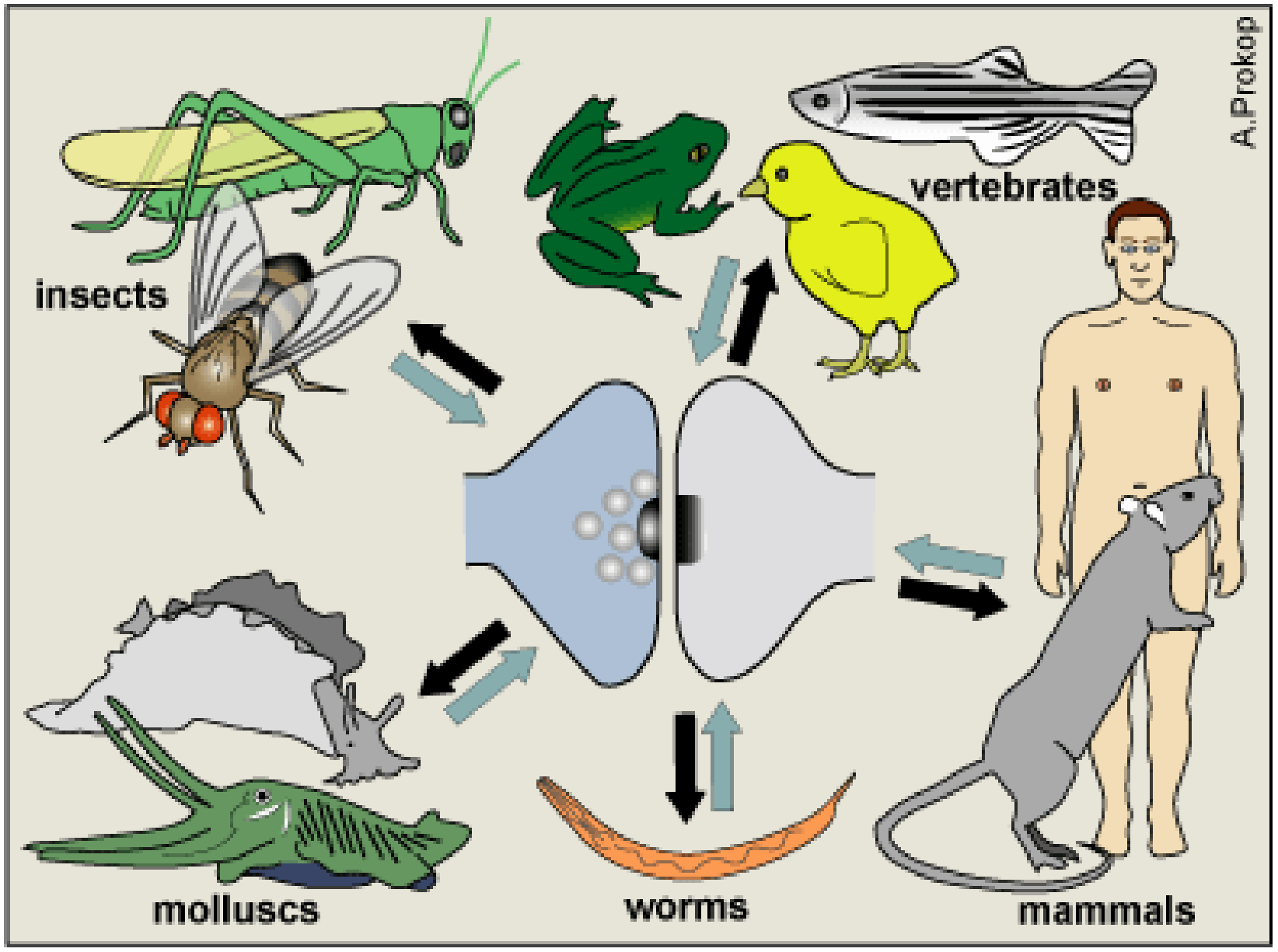


TARGET VALIDATION: A DOOR TO DRUG DISCOVERY

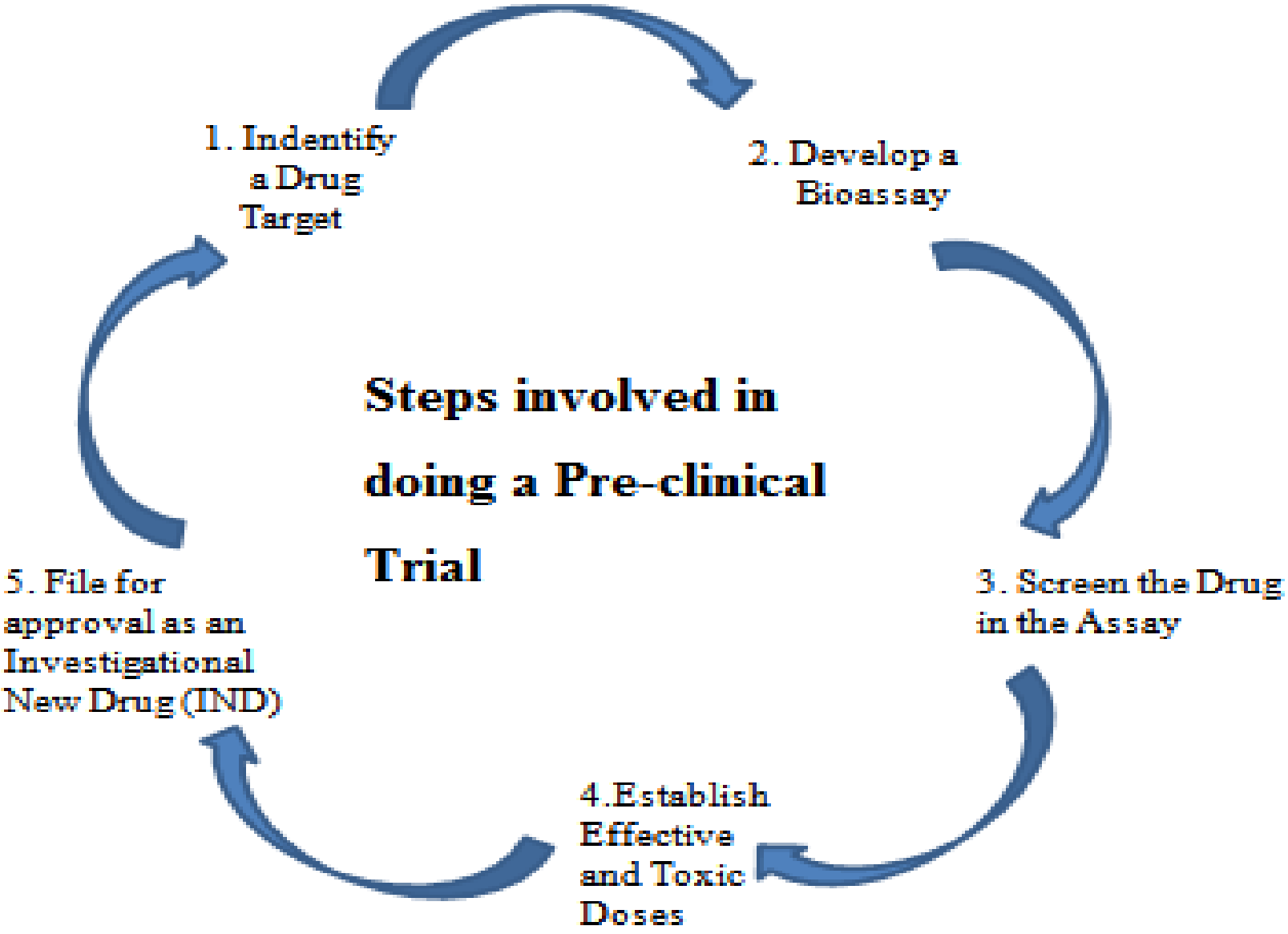


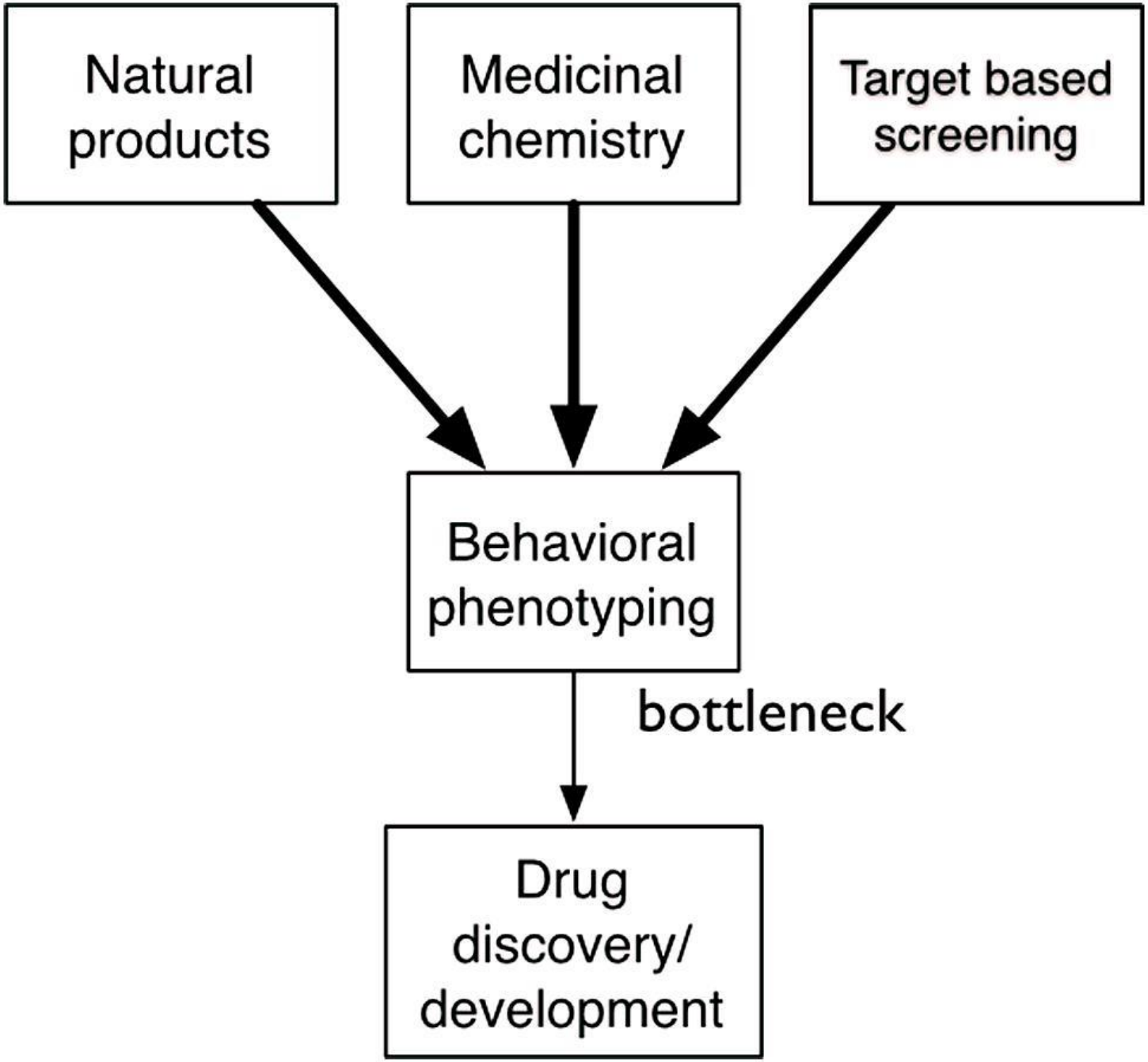


TARGET VALIDATION: A DOOR TO DRUG DISCOVERY

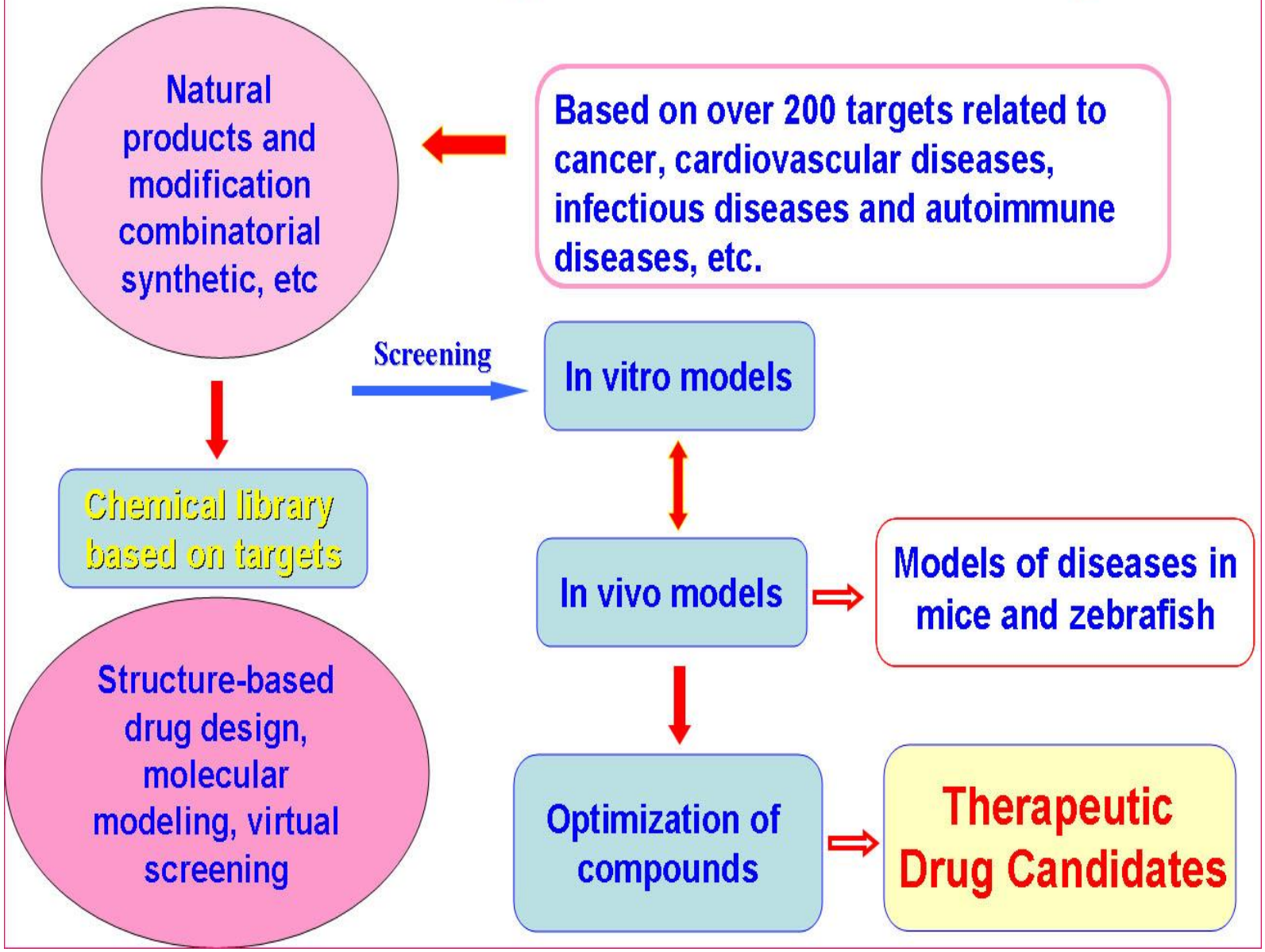

insects
vertebrates
molluscs
worms
mammals
A.Prokop

Steps involved in doing a Pre-clinical Trial



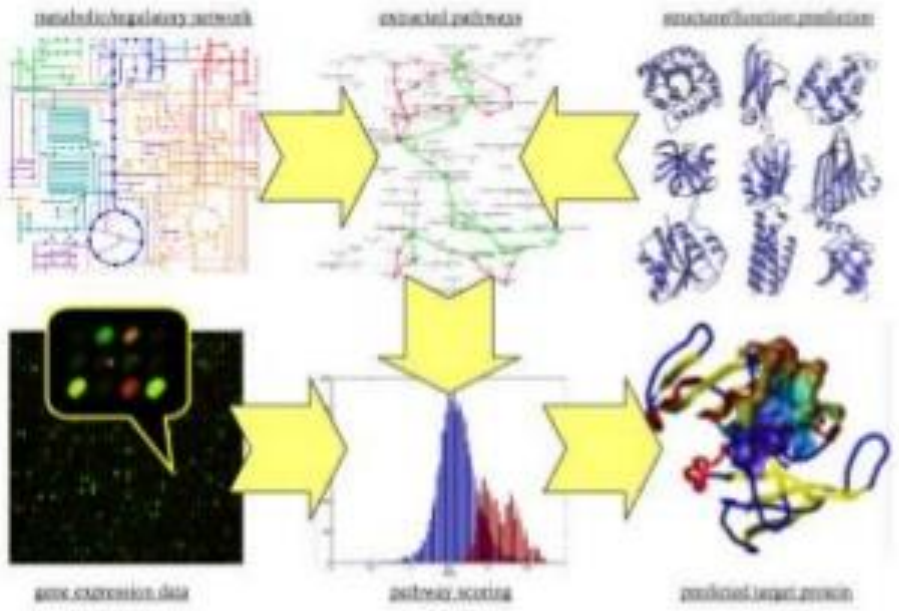

Natural products
Medicinal chemistry
Target based screening
Behavioral phenotyping
bottleneck
Drug discovery/ development

Screening of Small Molecule Drugs

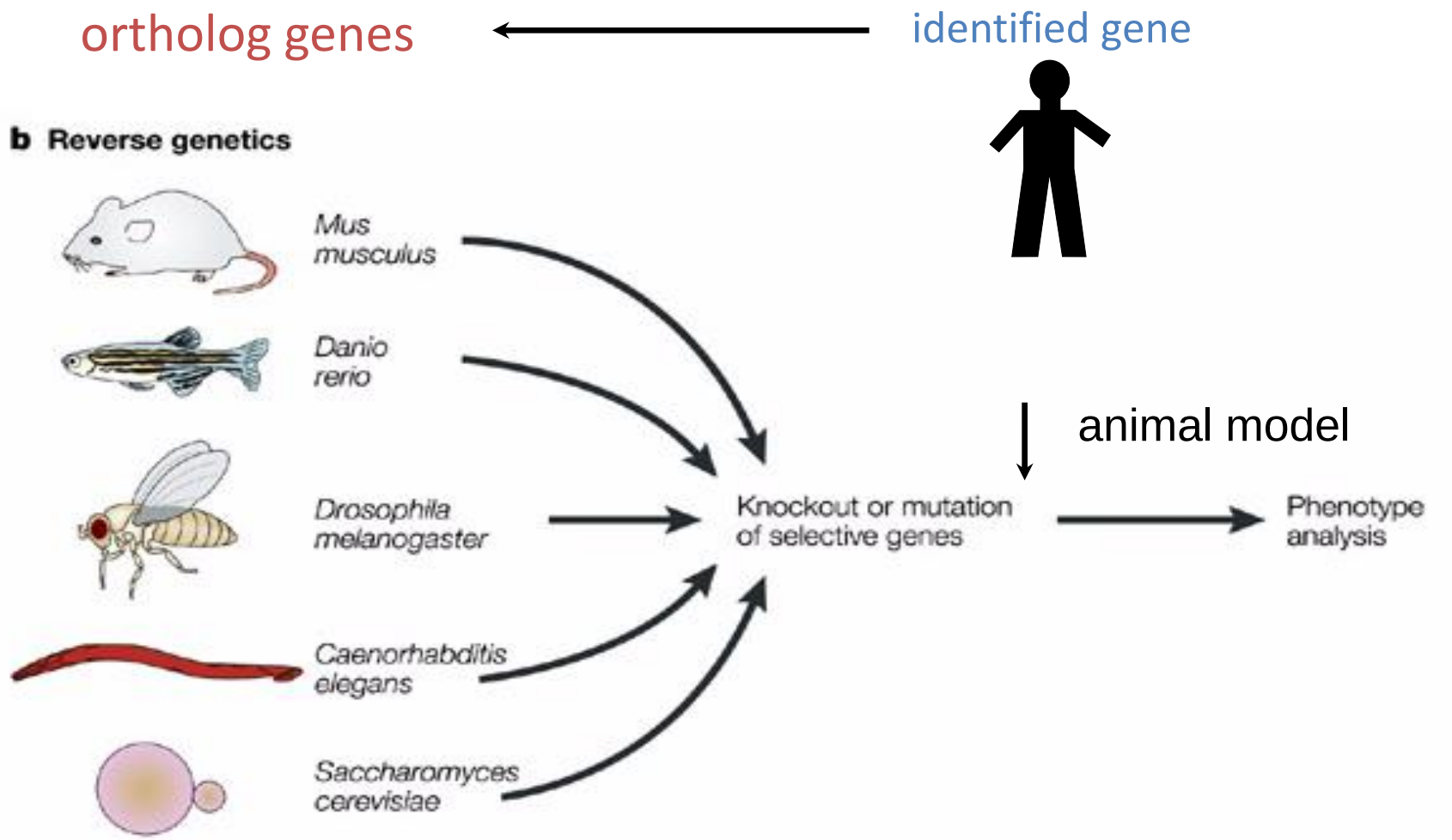


Discovery processes: Target identification strategies

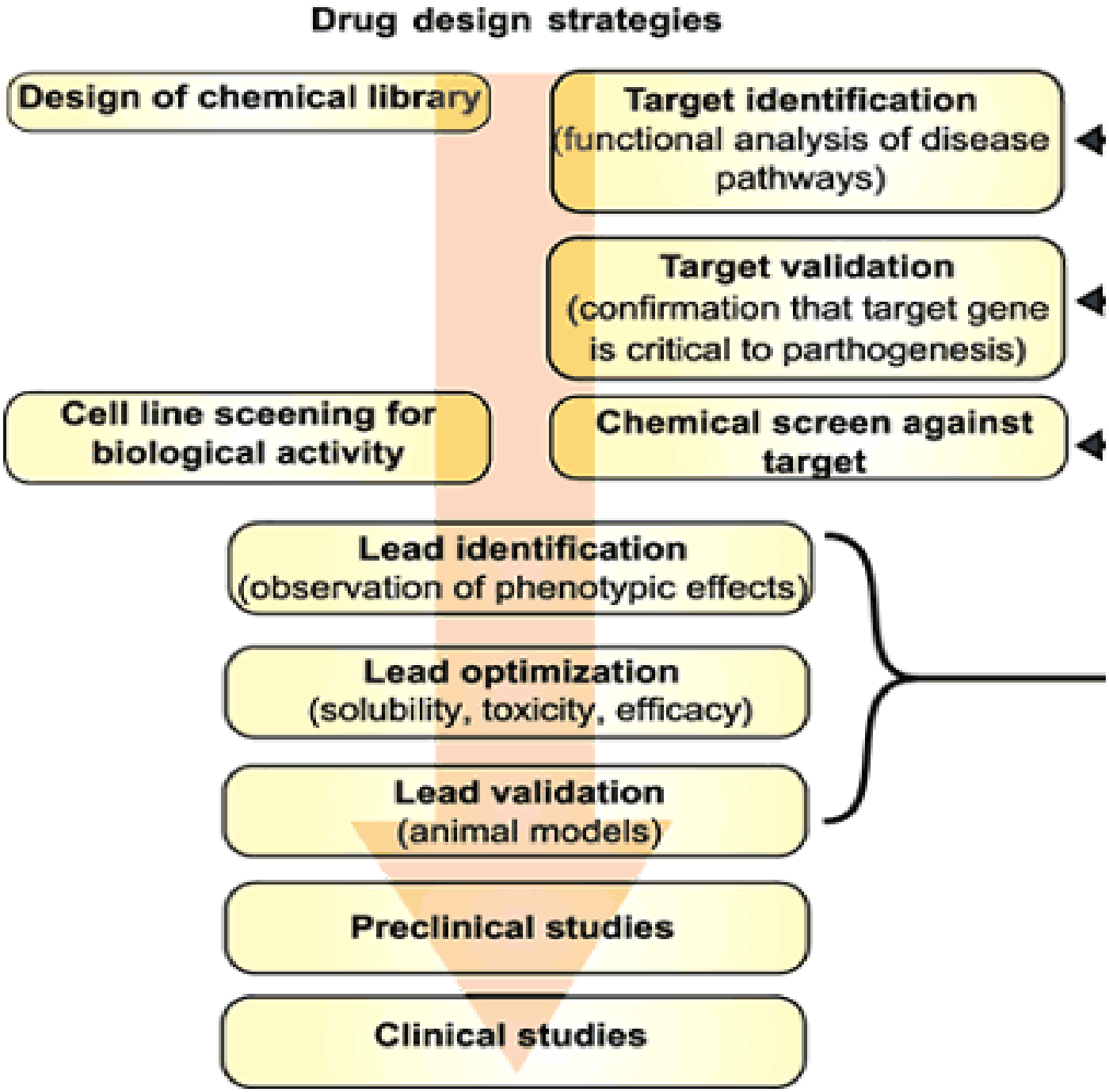
- Gene expression profiling
- Focused proteomics, e.g. activity-based protein profiling
- Pathway analysis – pathway databases, e.g. GeneGo Metacore & Ariadne
- Phenotype analysis – phenomic database
- Functional screening (siRNAs, shRNAs)
- Genetic association
- Scientific Literature

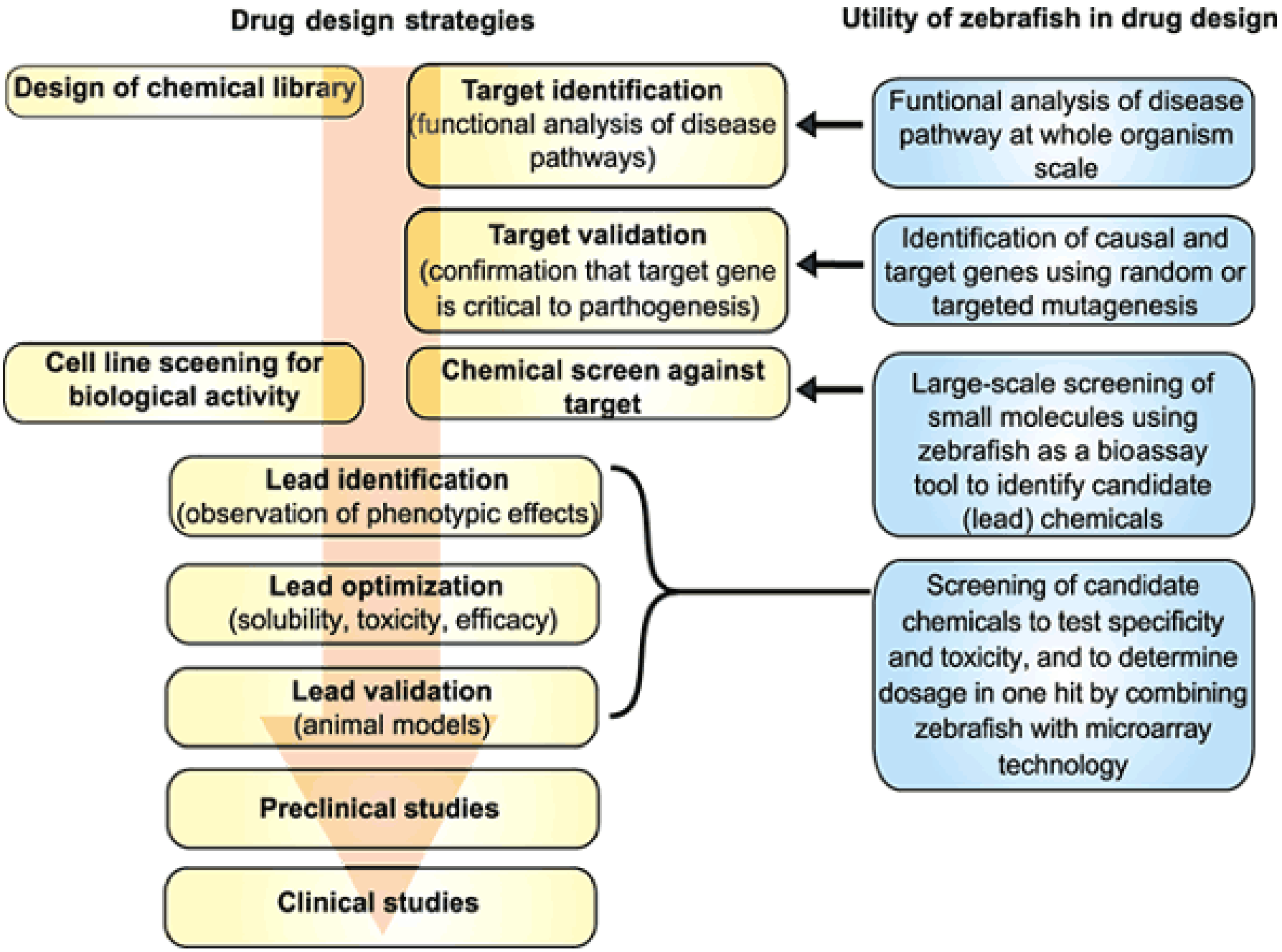


Towards the target



Reverse genetics: Modifications of the genotype by directed mutations


Drug design strategies
Design of chemical library
Target identification
(functional analysis of disease pathways)
Target validation
(confirmation that target gene is critical to parthogenesis)
Cell line sceening for biological activity
Chemical screen against target
Lead identification
(observation of phenotypic effects)
Lead optimization
(solubility, toxicity, efficacy)
Lead validation
(animal models)
Preclinical studies
Clinical studies


Drug design strategies
Utility of zebrafish in drug design
Design of chemical library
Target identification (functional analysis of disease pathways)
Funtional analysis of disease pathway at whole organism scale
Target validation (confirmation that target gene is critical to parthogenesis)
Identification of causal and target genes using random or targeted mutagenesis
Cell line sceening for biological activity
Chemical screen against target
Large-scale screening of small molecules using zebrafish as a bioassay tool to identify candidate (lead) chemicals
Lead identification (observation of phenotypic effects)
Lead optimization (solubility, toxicity, efficacy)
Lead validation (animal models)
Screening of candidate chemicals to test specificity and toxicity, and to determine dosage in one hit by combining zebrafish with microarray technology
Preclinical studies
Clinical studies

Why should use Zebrafish

- Being vertebrates, they share most major organ systems with humans.
- Zebrafish are robust, small, and reproduce quickly.
- Moreover, their eggs are transparent and develop outside of the body of the mother.
- This allows researchers to observe the development of organs or even individual cells in the embryo as well as in the larva, which is also transparent, without harming adult animals.
- The fish are ideally suited to studying the causes of cancer, heart disease, and behavioral disorders and to evaluating potential drugs.
- This makes them ideal model organisms to study the causes

The zebrafish (Danio rerio),

a new model system for proof of concept studies

Meet the Zebrafish

- **Small, fresh water aquatic vertebrate**
- **Lifespan 1-2 years**
- **Independently swimming by day of life 3**

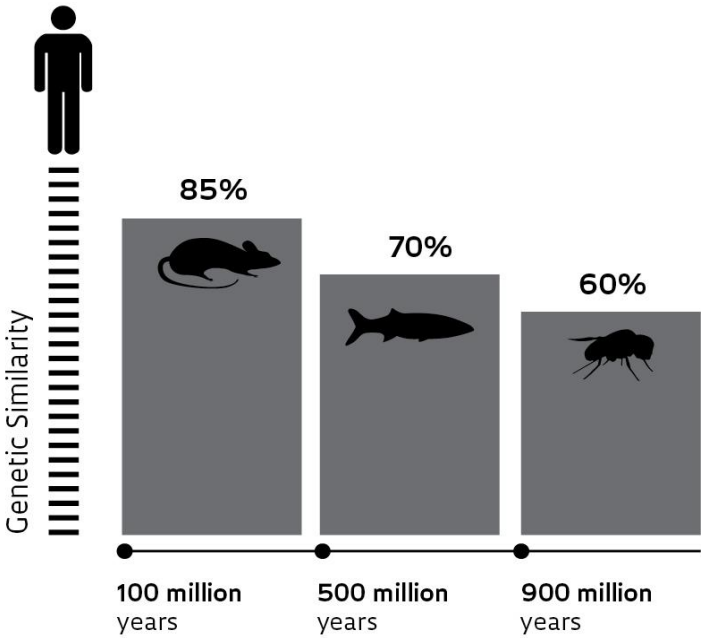
The models and their advantages

High reproduction rate, low maintenance cost and embryo development outside the mother's body are some of the zebrafish's attractions

	FRUIT FLY	*ZEBRAFISH*	MOUSE
FERTILIZATION	Internal	External	Internal
EMBRYO DEVELOPMENT	External	External	Internal
EMBRYO	Not transparent	Transparent	Not transparent
PRODUCTION OF OFFSPRING	100 eggs/day	100 eggs/day	10 babies/2 months
TIME TO REPRODUCTIVE AGE	20 days	60 to 90 days	85 days
DAILY MAINTENANCE	-	R$ 0.60	R$ 8.00
BODY TYPE	Invertebrate, 6 legs and wings	Vertebrate, no legs	Vertebrate, 4 paws

SOURCE JOSÉ XAVIER NETO / LNBIO, MONICA RYFF VIANNA / PUC-RS E DENIS ROSEMBERG / UNCHAPECÓ

Comparison with human beings



CNS

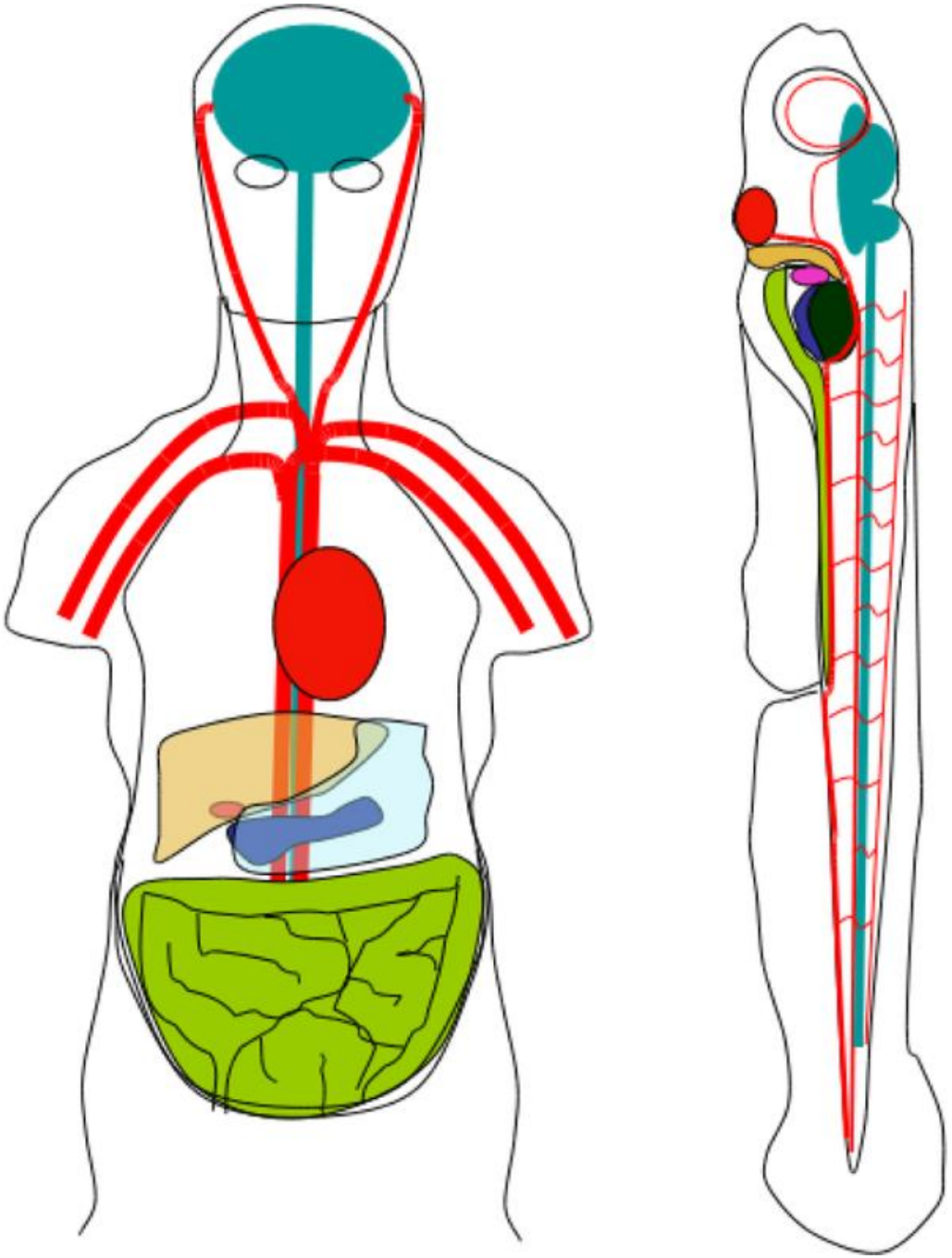
Cardiovascular system

Liver

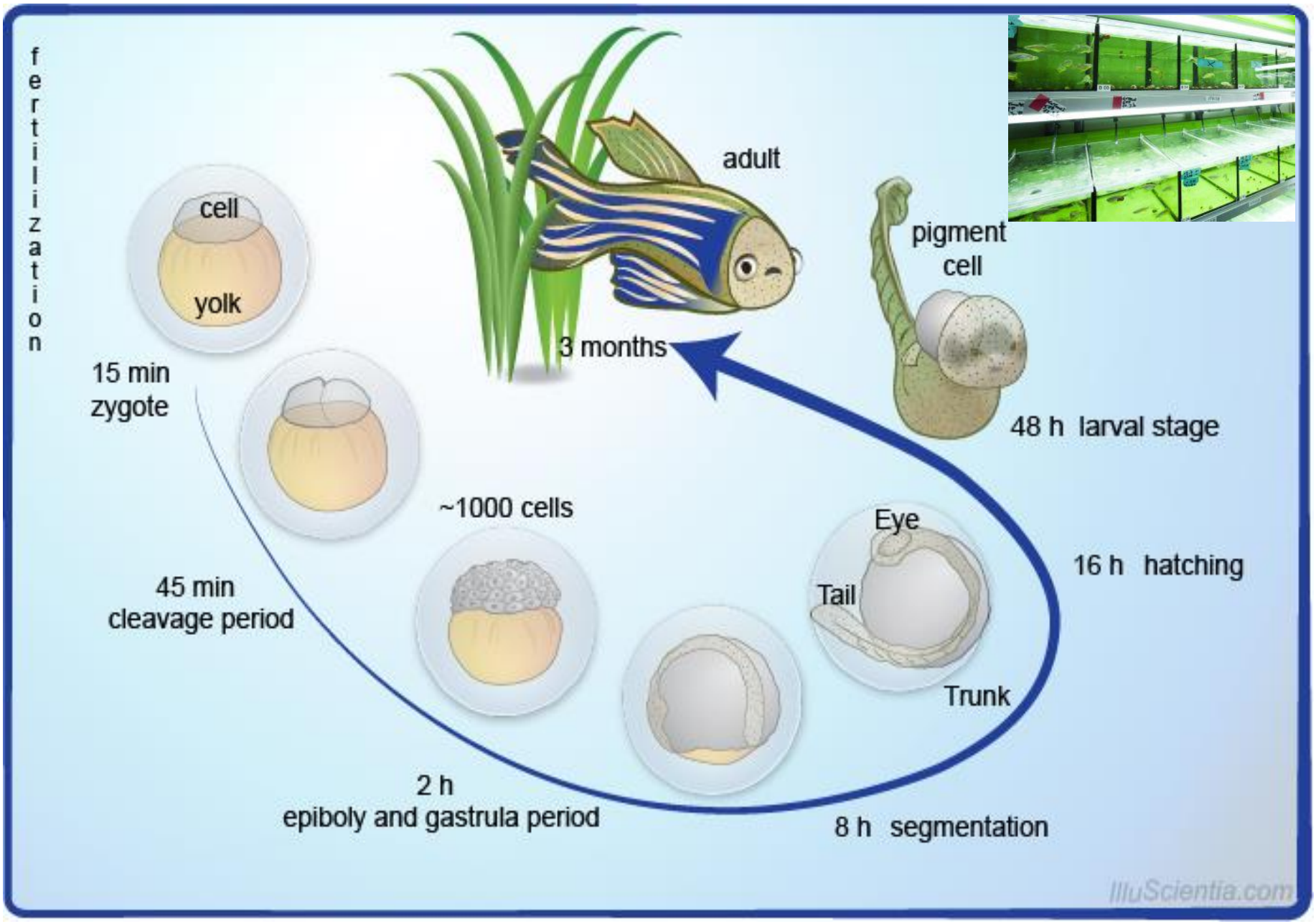
Pancreas

Gall bladder

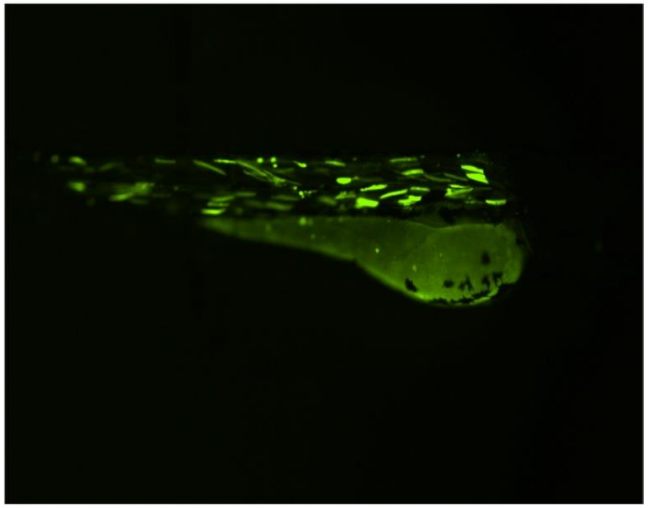
Intestine

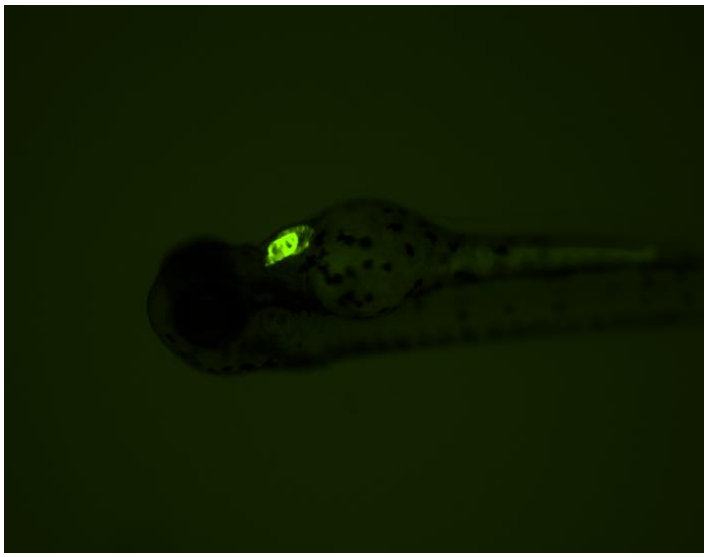


Life cycle of a zebra fish from fertilization to an adult fish



Why zebrafish???





- Crystal clarity!
  - Zebrafish are optically translucent allowing for live imaging of muscle and heart

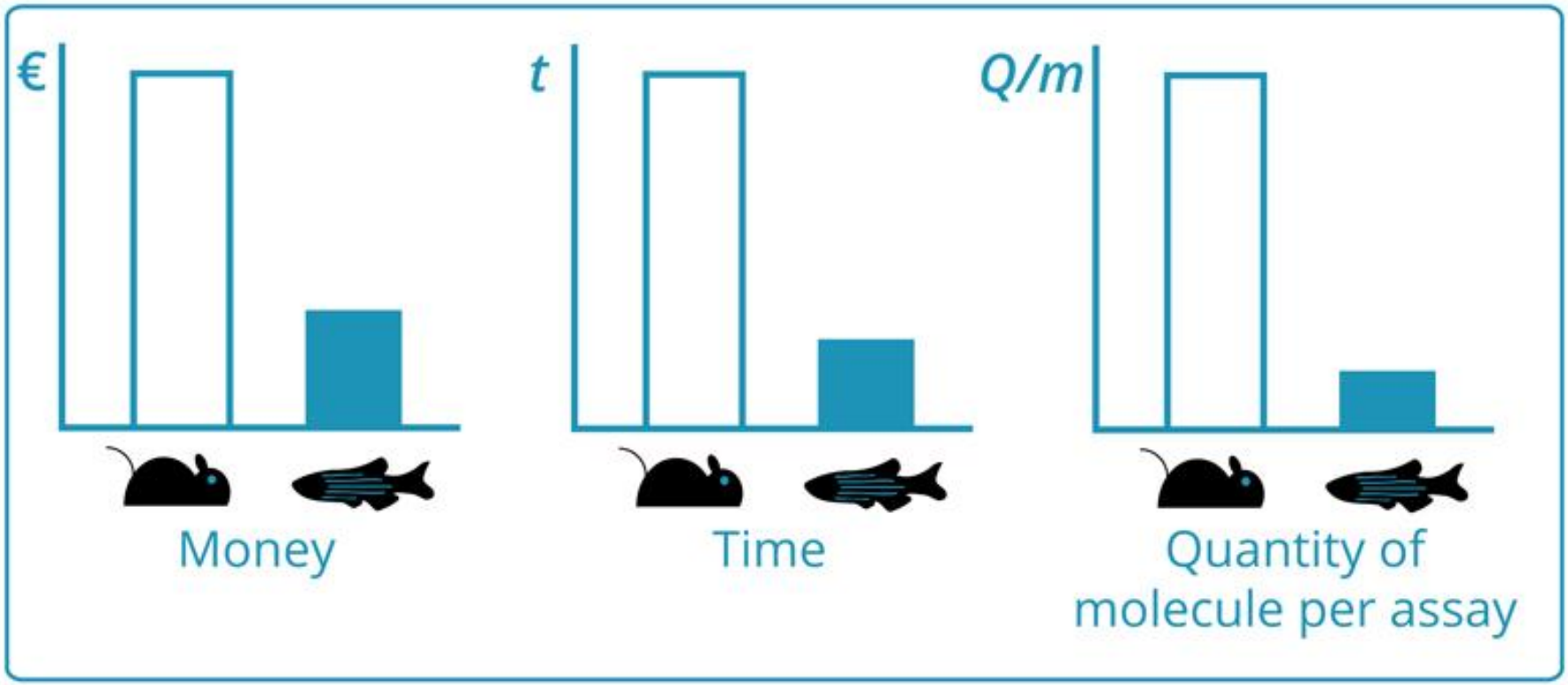
Why Zebrafish???

- Invertebrate style genetics
  - -Large number of offspring
  - -Can easily introduce DNA/RNA
  - -Can do saturating mutagenesis screening
- Vertebrate style genome
  - -Genome at least as complex as ours
  - -Genome sequenced as part of the NIH genome project
  - -All known muscular dystrophy genes are found in the zebrafish

Advantages

- They can be kept at fairly high densities in a small tank
- They lay large numbers of easily collectible eggs
- The eggs are clear and easily observed and manipulated
- They develop fast
- Their generation time (egg to adult) is short
- They are vertebrates
- Expense. Fish are cheaper to maintain than mice, but more expensive than flies--another powerful model organism

Feature	Benefit
Easy maintainence	Low housing costs
Year round spawning	Research can run continuously
High fecundity(300-600 by single female at one time)	Low cost per assay
Optical transparency of early stages	Real time (live) imaging of developmental processes Easy selection of precise developmental stages(in contrast to mammals)
Swimming begins at hatching(48-72 hpf) and more complex behaviuor (food seeking) at 5 dpf	Behavioural studies can be made on very early stages
Very rapid development	Large number of experiments possible in short time period
Fertilization is external	Embryos accessible non- invasively, can be continuously imaged there is no placental barrier or maternal compartment to influence drug experiments
Minimal parental care	Reduced epigenetic parental influence on experimental outcome
Mutants available, genome sequenced , morpholino knockdowns possible	Genetic basis of teratogenesis can be investigated
Animal protection laws often less stringent for Zebrafish embryos than for mammals	Fewer legal restrictions on research
Eggs develop in non- sterile, simple buffers	Easy to raise and maintain embryos
Genome has important similarities to human(e.g. nearly all mammalian genes have Zebrafish counterpart l; high conservation of key developmental genes with human)	Common molecular pathways can be studied
Very small size of early embryos(0.8-1.2 mm diameter with chorion)	Only very low quantities of expensive test drugs and staining reagents needed Suitable for high throughput screening in 96 and 384 multi-well plates
Small egg size and external fertilization	Very precise control of drug delivery and dosage
Early embryo is permeable to many compounds	Suitable for drug testing

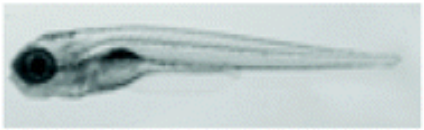


ZeClinics

Advantages of using zebrafish *vs* rodents

• Developmental toxicity
: phenotype-based chemical screening

• Organ-specific toxicity
: cardiotoxicity, nephrotoxicity, hepatoxicity, otoxicity, neurotoxicity (behavioral toxicity)



• Small size
• High fecundity
• Rapid embryogenesis
• Transparency
• Genetic manipulations

• High-througput toxicity testing
: automated analysis

• Toxicogenomics
: integrating genomics and toxicology

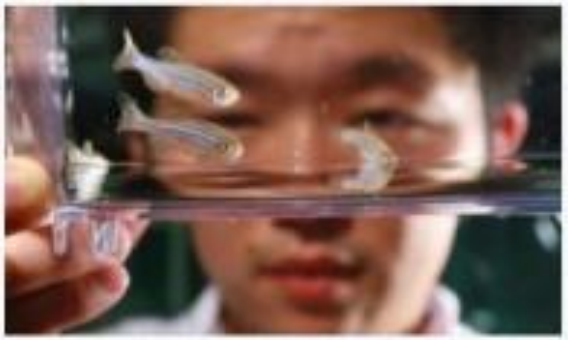
• Omics
: transcriptomics, proteomics, metabolomics

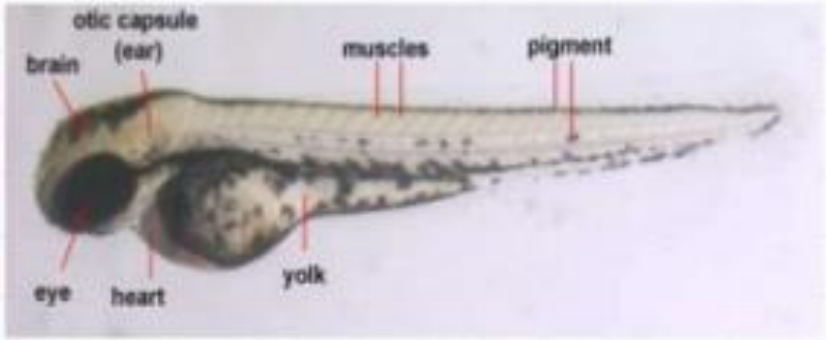
• Alternative toxicity testing to mammalian models

• Ecotoxicology
: fish embryos toxicity test (FET), alternative to fish acute toxicity test

Zebrafish a model system

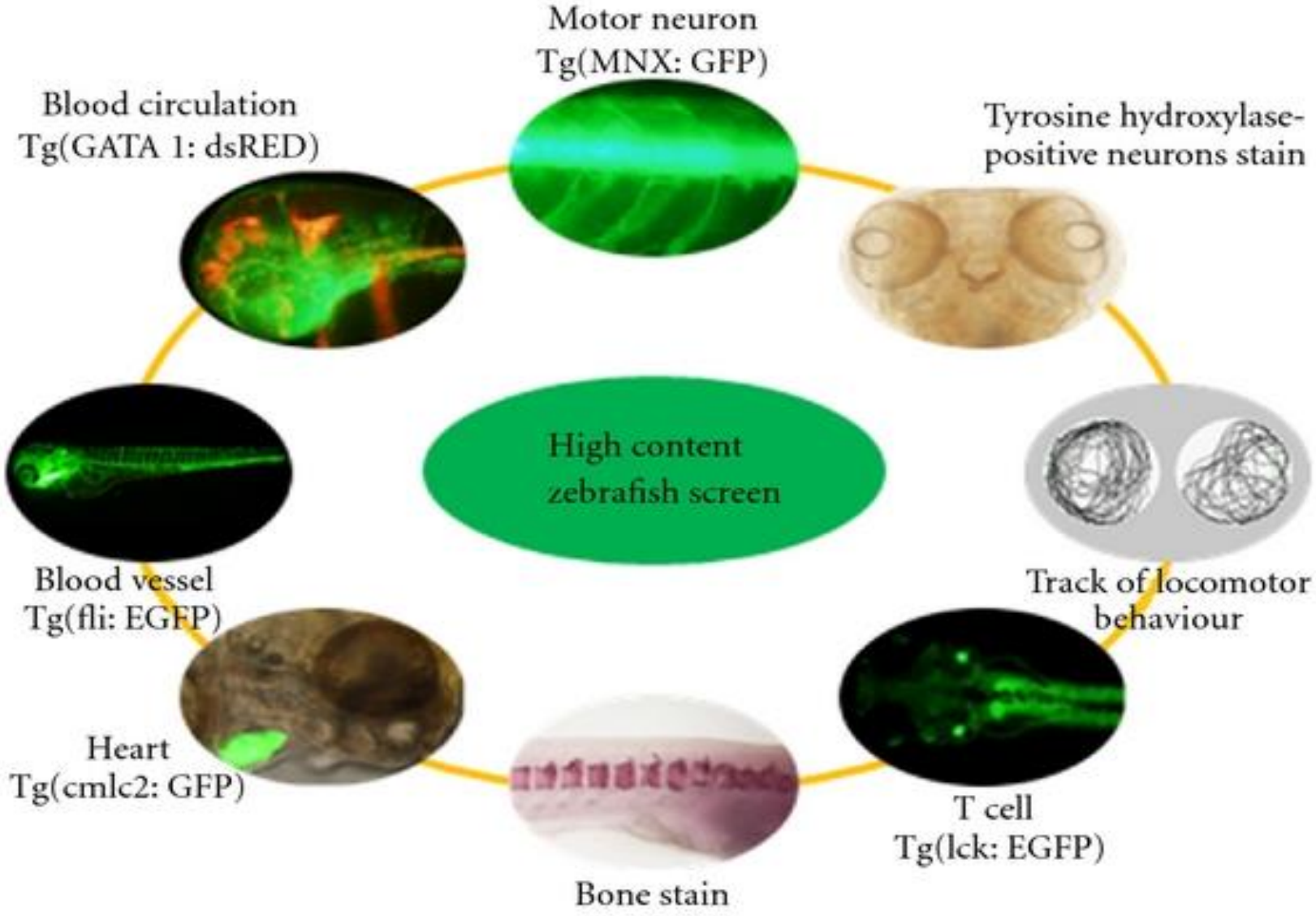
- Small size
- Short life cycle & generation time
- Good reproduction captivity
- External fertilization
- **Optically transparent embryo**
- Rapid embryonic development





Powerful model organism

- Genetics
- Developmental biology
- **Toxicology**
- Pharmacology
- DNA repair
- Cancer


Motor neuron
Tg(MNX: GFP)
Blood circulation
Tg(GATA 1: dsRED)
Tyrosine hydroxylase-
positive neurons stain
High content
zebrafish screen
Blood vessel
Tg(fli: EGFP)
Track of locomotor
behaviour
Heart
Tg(cmlc2: GFP)
T cell
Tg(lck: EGFP)
Bone stain

Zebrafish use in ecotoxicology

- Eco-environmental monitoring
- Sensitivity to different contaminants
- Toxic heavy metals, endocrine disruptors & organic pollutants
- Changes in morphology, gene expression, behavior or physiology

(Dai *et al.*, 2014)

Organ-specific toxicity

Preparation	Concentration	Observed effect/toxicity
Doxorubicin	30 mg/l	Teratogenicity, nephrotoxicity, Hepatotoxicity, cardiotoxicity
Dexamethasone	324 mg/l	Nephrotoxicity, hepatotoxicity, GIT lesion
Methotrexate	454 mg/l	Teratogenicity, nephrotoxicity, Hepatotoxicity, cardiotoxicity, GIT lesion
Fluorouracil	3.3 mg/l	Nephrotoxicity, hepatotoxicity
Cyclosporin A	69 mg/l	Teratogenicity, nephrotoxicity, Hepatotoxicity, cardiotoxicity
Caffeine	108 mg/l	Change of locomotor activity, Muscular spasticity

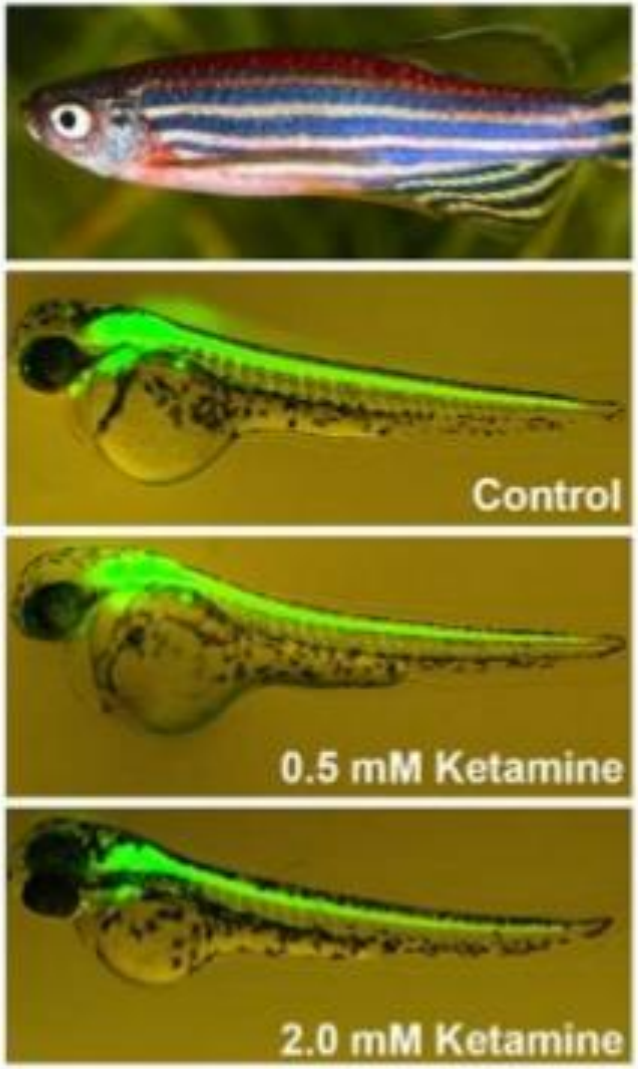
Cardiotoxicity

- Zebrafish larvae (3 dpf)
- Exposure period- 3 h
- ventricular & auricular heart rate, cardiac output & stroke volume
- Cardiac morphology

Wu *et al.*, 2013

Toxicology

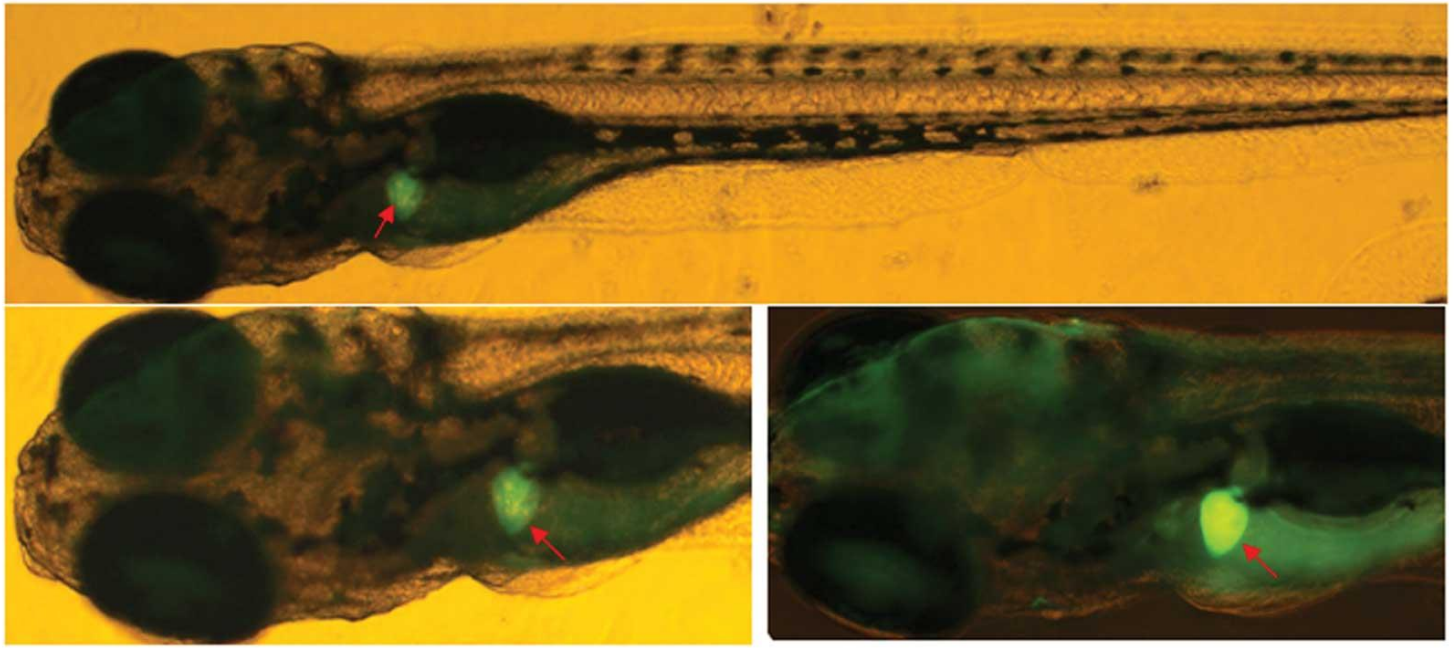
Zebrafish Embryo Toxicity Test (ZEFT) : Robust and sensitive model



Why using Zebrafish?
-Very small size - High fertility - High predictivity for Human - Embryo transparency and fast development

A

B



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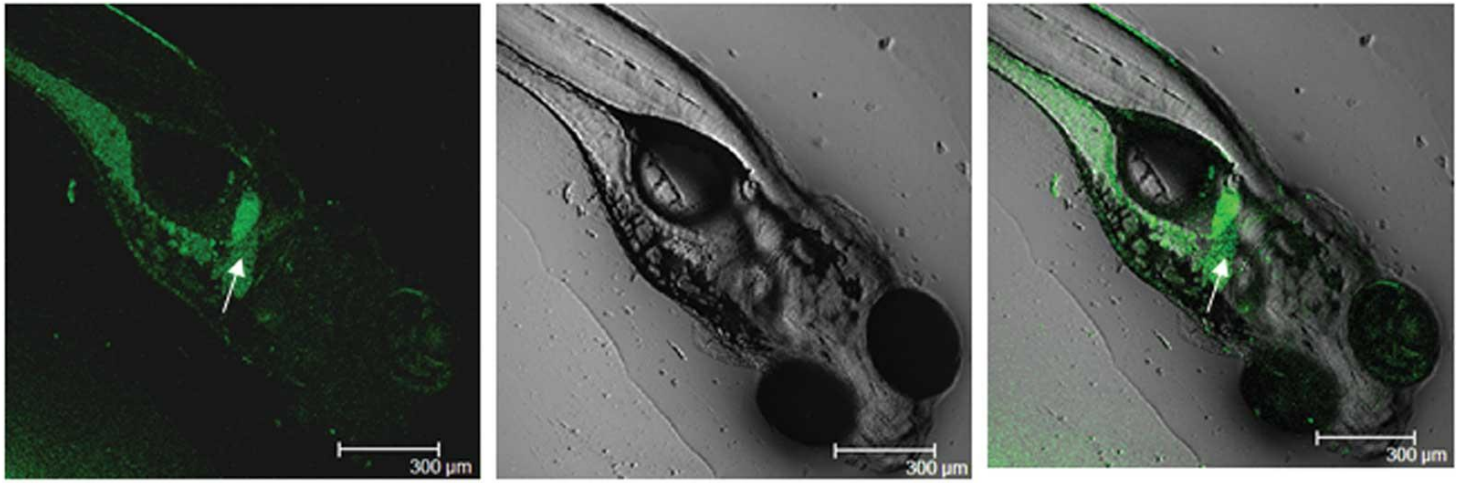


larvae at 10 dpf

MODEL / FEATURE	FRUIT FLY	ZEBRAFISH	MOUSE
			
FERTILIZATION	Internal	External	Internal
EMBRYO DEVELOPMENT	external	external	internal
EMBRYO	Not transparent	transparent	Not transparent
PRODUCTION OF OFFSPRING	100 eggs/day	100 eggs/day	10 babies/ 2 months
TIME TO REPRODUCTIVE AGE	20 days	60 to 90 days	85 days
DAILY MAINTENANCE	-	R$0.60	R$8.00
BODY TYPE	Invertebrate, 6 legs and wings	Vertebrate, No legs	Vertebrate, 4 paws

Comparative Zebrafish Biology For Modeling Human Disease

Characteristics	Key similarities to humans	Key differences and unknowns
General biology		
Genome structure	Diploid: essentially contains the full vertebarate repertoire of genes	Gene duplication resulting from ancestral whole-genome duplication, resulting in subfunctionalisation and neofunctionalisation
Anatomy	Vertebrate body plan	Aquatic adaptations include streamlined body plan and different locomotar strategies
Diet and metabolism	Omnivorous	Poikilothermic, grows optimally at 28.5°C
Growth	Growth is determinate (proceeds to a limited maximum adult size)	Significant capacity for regeneration of many tissues and organs for example, heart, fin retina
Lifespan	Juvenile and adult phases of growth around the point of reproductive maturity	Lifespan 3-5 years; generation time of 3 months.

Drugs and Chemicals Used and Assessed Toxicity in Zebrafish

Drugs	Drug type	Type of toxicity study
Retinoic acid	Acidified form of vitamin A	Abnormal pectoral fin bud morphology Abnormal development of the caudal midbrain and anterior hindbrain RA- mediated gene expression in transgenic reporter zebrafish
Cyclopamine	Treatment agent in basal cell carcinoma, medulloblastoma and rhabdomyosarcoma	Elimination of primary motoneurons Role of shh in the induction and patterning of the pituitary Inhibition of fin outgrowth Role of hedghog signalling in eye development
17-beta estradiol	Attenuated acetylcholine-induced coronary arterial constriction in women but not men with coronary heart disease	Effects on mortality and hatching, consequences for CNS Vitellogenin as an estrogenic biomarker
17alpha ethinylestradiol	Synthetic steroid	Effects on sex ratio and breeding success
Neomycin	An aminoglycoside antibiotic	Bioassay for assessing toxicity

Table No. : Selected Zebrafish Models of Human Diseases and Syndromes

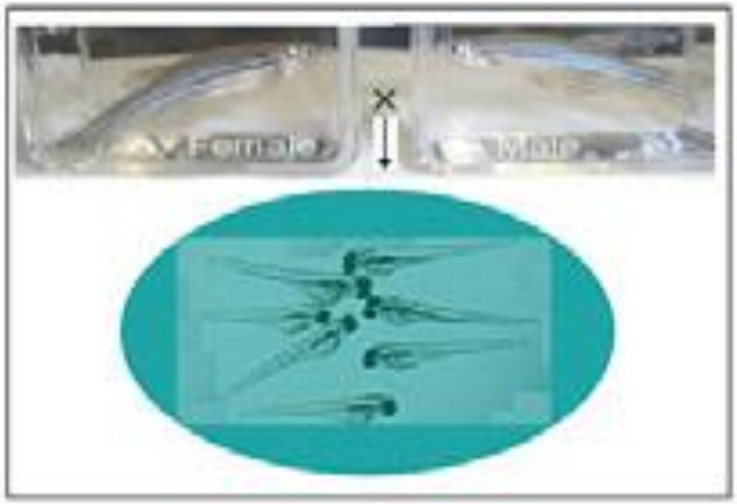
Human Condition	Zebrafish model	Zebrafish genes
Cardiac arrhythmia: short QT syndrome	Reggae mutant (reg)	zERG
Cardiac arrhythmia: QT prolongation	Rate of atrial and ventricular rates	-
Parkinson's disease	Oxidative stress, dopamine neuronal loss	DAT,TH and Dj-1
Inflammatory bowel disease	Gut morphology, peristalsis	
Cerebral cavernous malformations	Ccm1mutant	Ccm1
Polycystic kidney disease	Bicaudal C and Polycystic kidney disease mutant (Bicc1, pkd2)	Bicc1, pkd2
Polycythemia vera	Janus kinase 2 mutant disease	$Jak2^{V518F}$
Waardenburg syndrome type IV	Sex determining region Y mutant (sox 10)	Fgf8,sox9a,sox9b, sox 10
Variegate porphyria (porphyrias)	Montalcino mutant	ppox

Zebrafish Husbandry

- 70% of all human disease genes
- High fecundity
- Embryos are transparent
- Develop outside the body
- Development is rapid
- Easy and inexpensive

Forward Genetics

- Chemical Mutagenesis
- Insertional Mutagenesis



Reverse Genetics

- Gene knockdown
- Targeting Induced Local Lesions in Genomes
- Zinc finger Endonucleases
- Transcription Activator-Like Effector Nucleases
- Transgenesis

Vertebrate Development

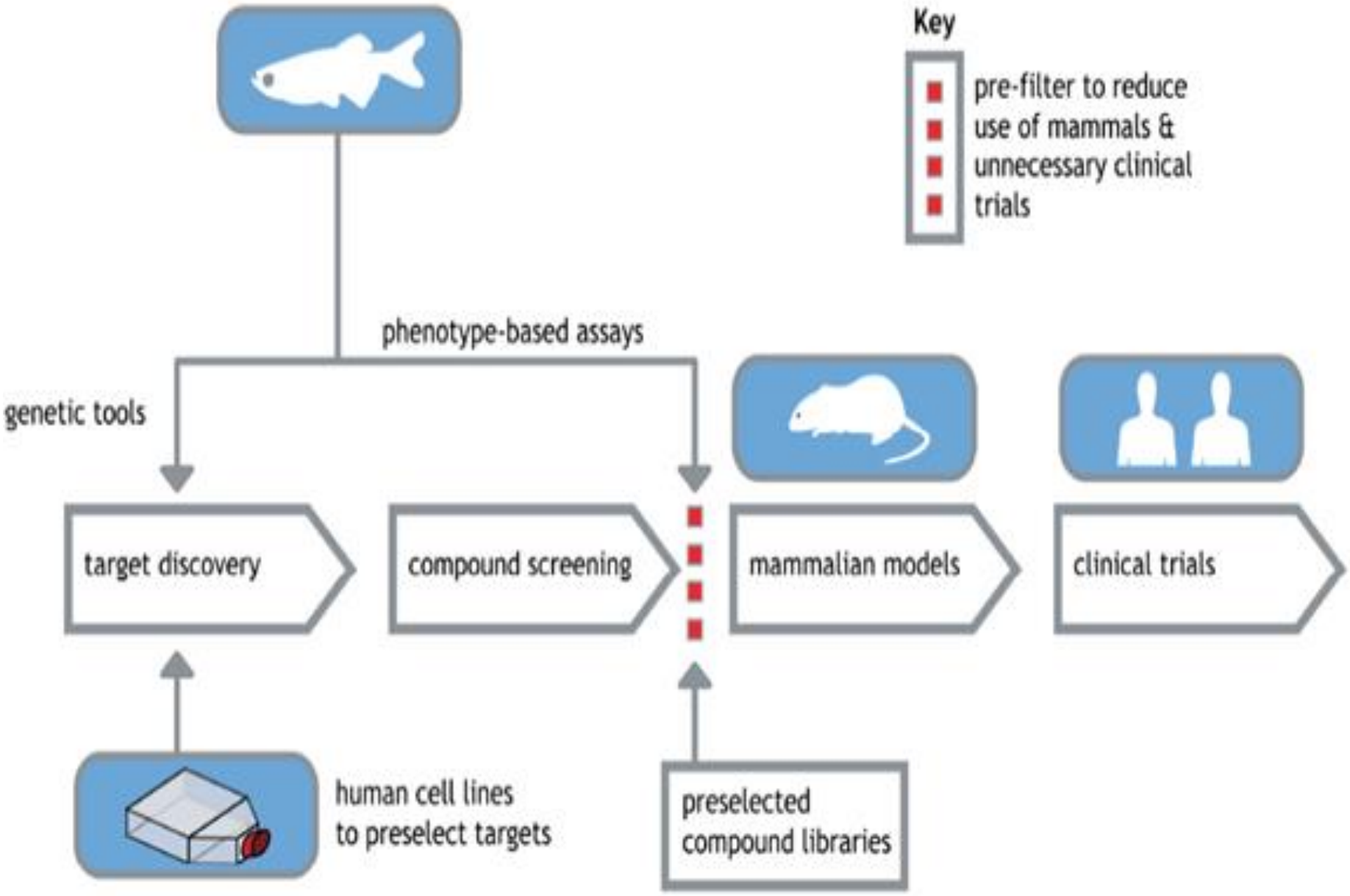
- Heart development
- Endoderm development
- Motor neuron development
- Craniofacial development

Drug Screen

- Identification of Disease phenotypes
- Suppression by lead components
- Validation by tissue specific transgenic lines

Human Disease

- Hematologic diseases
- Cardiovascular Diseases
- Cancer
- Muscle disorders
- Neurologic disorders



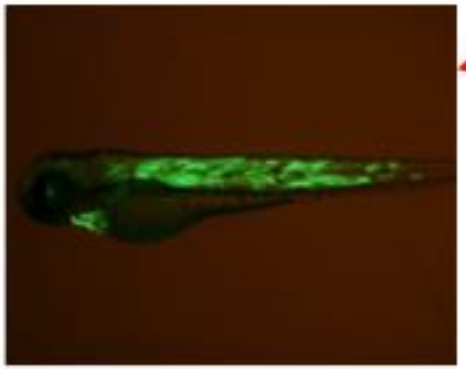
Drug discovery pipeline involving novel Zebrafish models

cDNA
mRNA
siRNA/miRNA
Peptides
Chemicals

Combinatorial Library

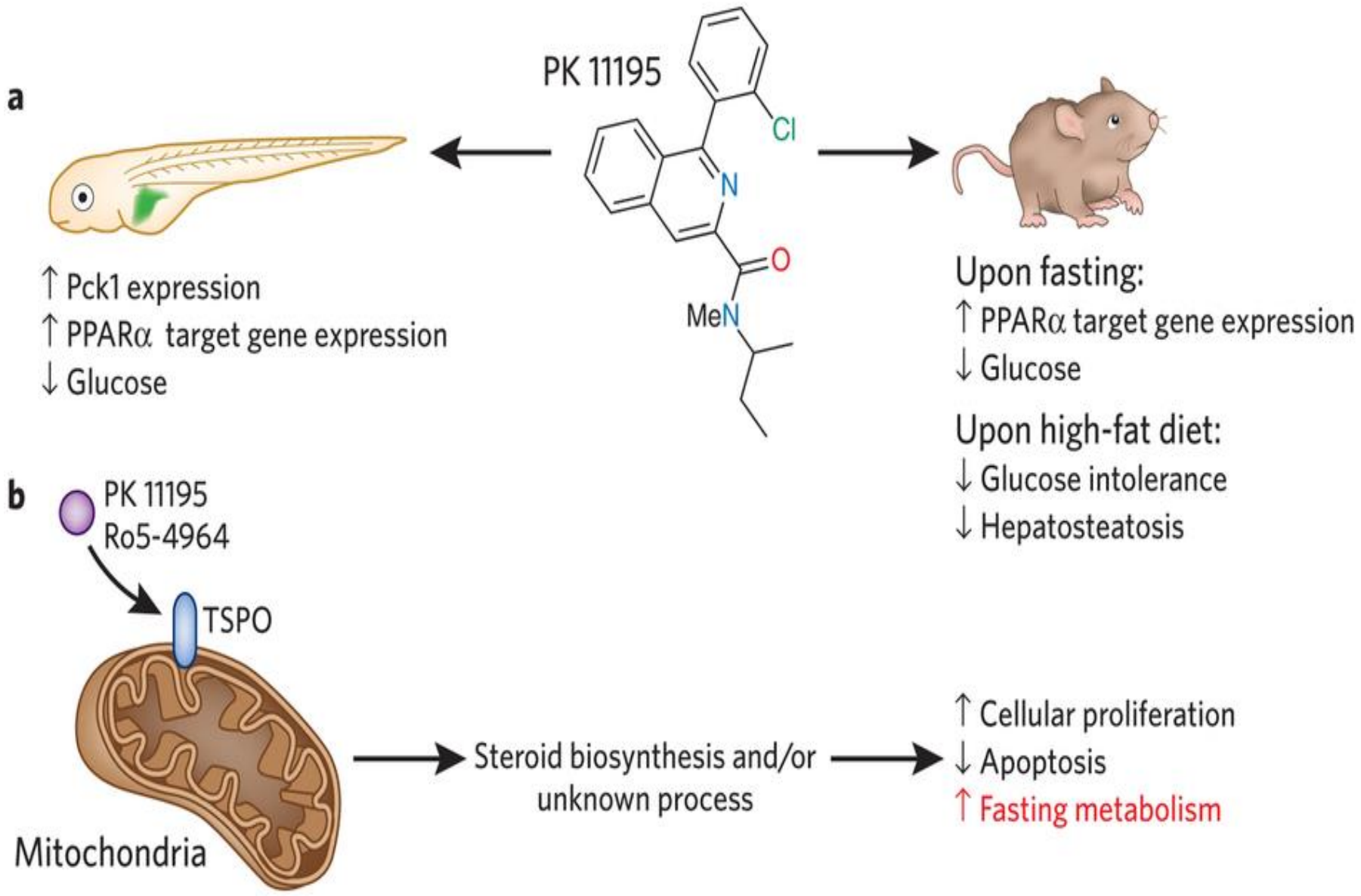
HTP Microinjection

Detection of target genes by WISH



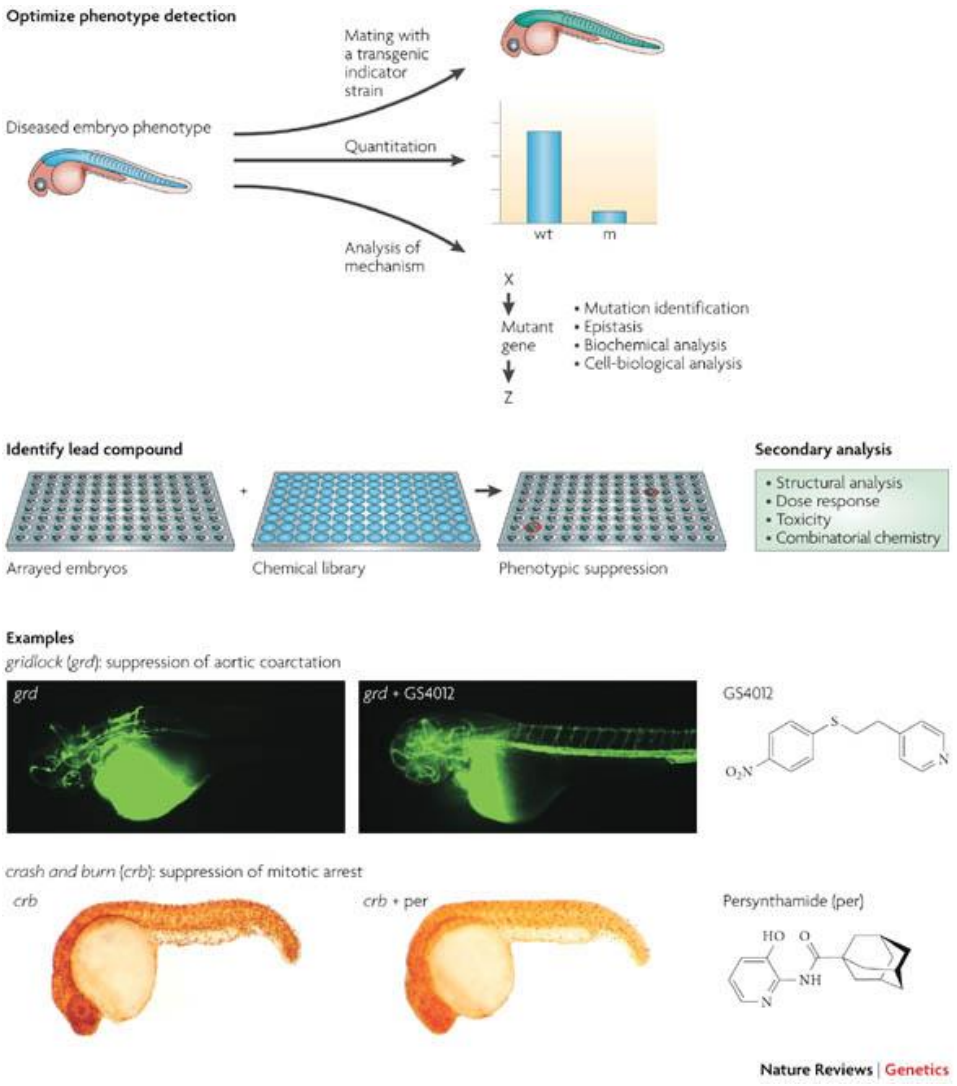
Detection of target gene products by GFP

Network analysis of target genes/ products


a
PK 11195
Cl
N
O
MeN
↑ Pck1 expression
↑ PPARα target gene expression
↓ Glucose
Upon fasting:
↑ PPARα target gene expression
↓ Glucose
Upon high-fat diet:
↓ Glucose intolerance
↓ Hepatosteatosis
b
PK 11195
Ro5-4964
TSPO
Mitochondria
Steroid biosynthesis and/or
unknown process
↑ Cellular proliferation
↓ Apoptosis
↑ Fasting metabolism

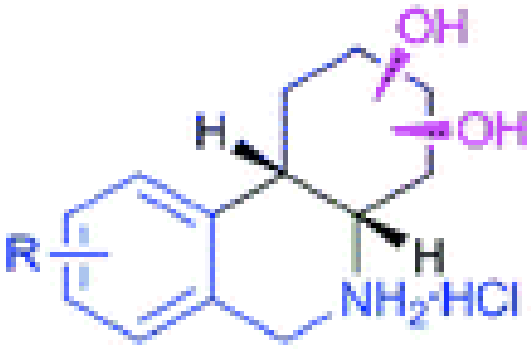
Drug screening in the zebrafish

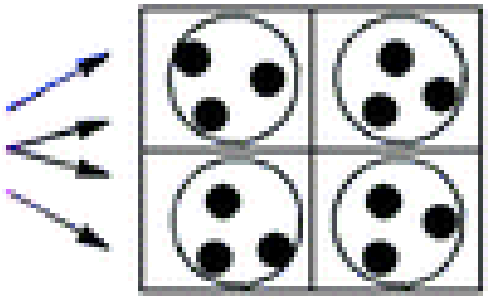
- **ZF and drug screening**
  - **Large number of offspring**
  - **Frequent mating**
  - **Easily absorb drugs in media**
  - **Translucent body plan plus many GFP markers**
- **Muscle specific phenotypes for drug screens**
  - **Birefringence**
  - **Motor function**
  - **Other targets? (for example, cardiac phenotypes)**



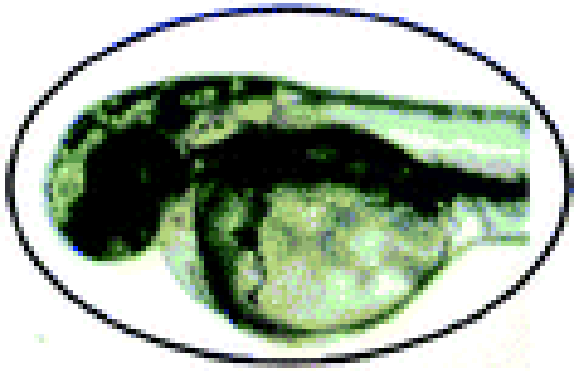
Rapid library synthesis

Phenotypic evaluation

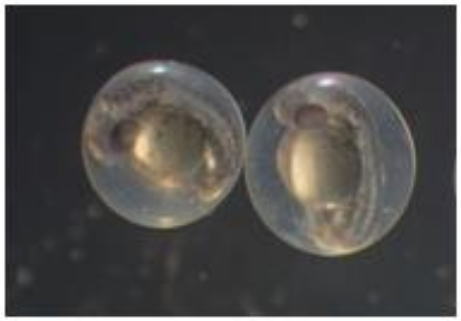


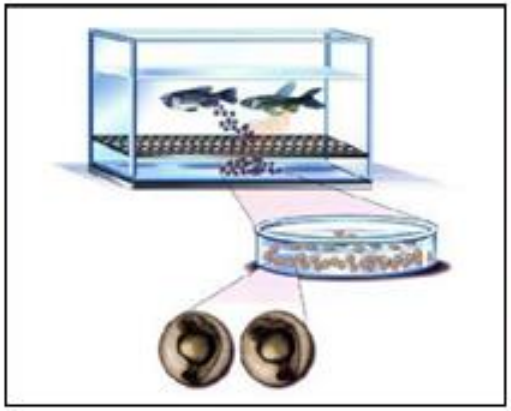


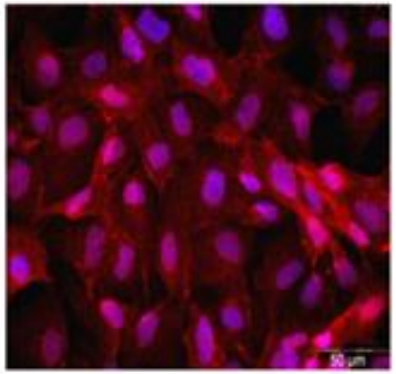
3-5 days

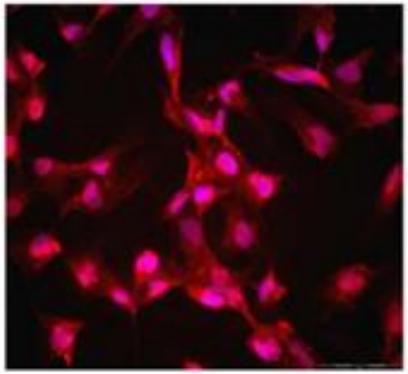


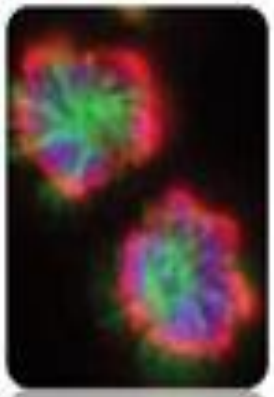
R = H, Me, F, OMe,
-OCH_2O- etc.





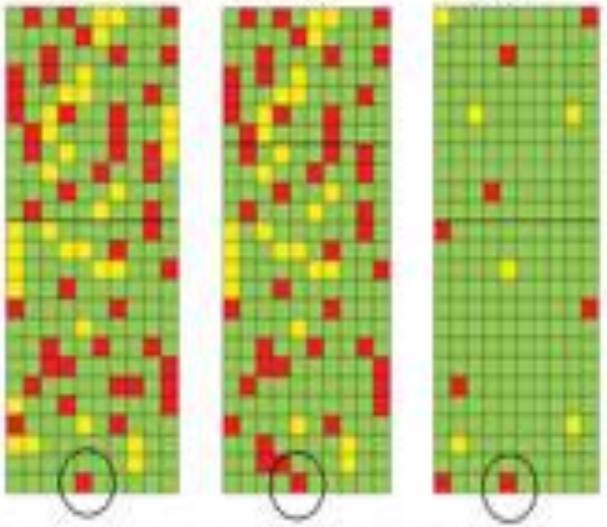






Phenotypic Cell Assay

Phenotypic Screen Array's Kinase Med Chem Collection



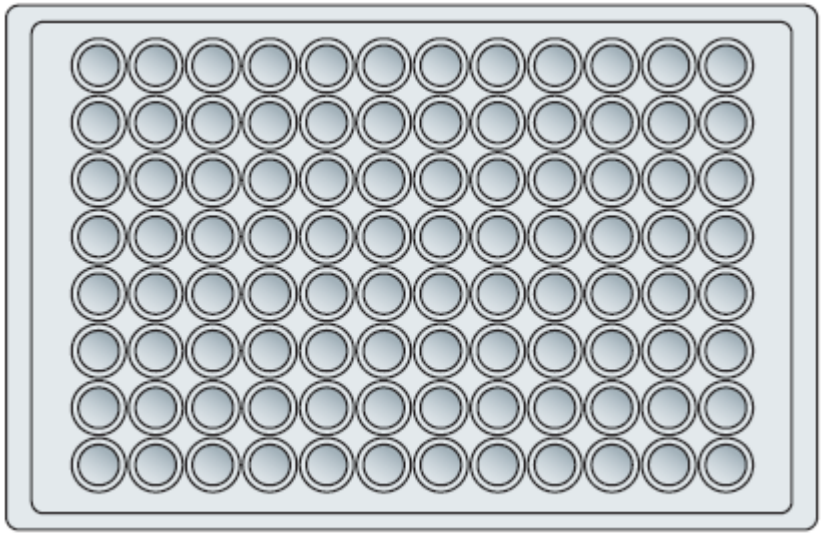
Kinase selectivity profiling of hits

Array Bioinformatics to De-convolute

Target Validation & Lead Generation

Lead optimization on Novel target

Throughput



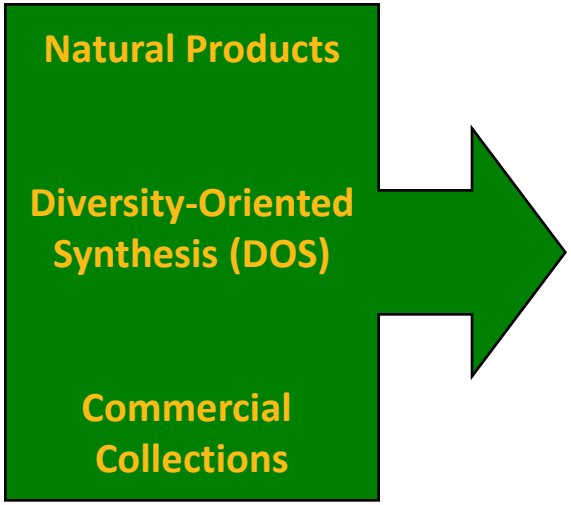
96-well plate, 384-well plate

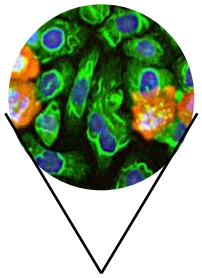
HTS uses some well designed models or assays to screen large quantity of compounds in relative short time

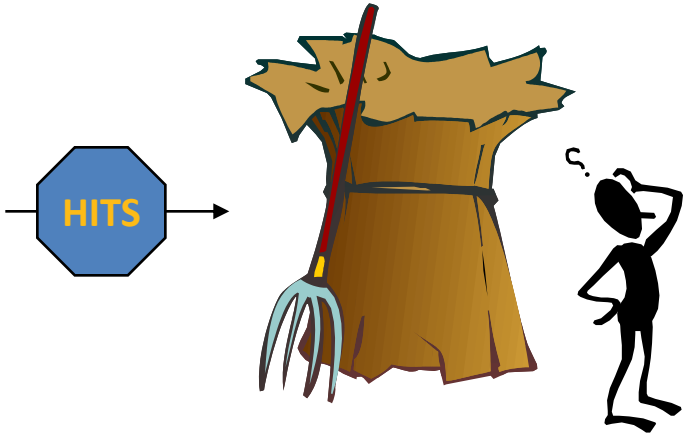
In assays, the activities of compounds are visualized: images (in Forward CG) or fluorescent signals (in Reverse CG)

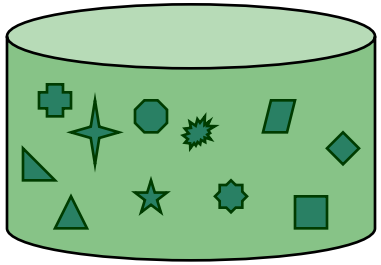
Forward chemical genetics involves 3 basic stages:

- a library of compounds
- an assay, usually a phenotypic assay
- a strategy to trace an active compound to its biological target

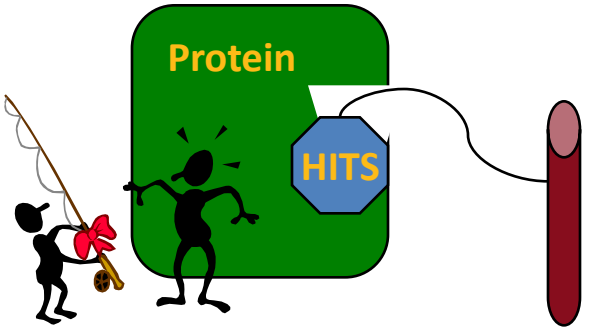








A Phenotypic Screen

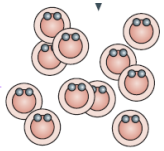


How to Find New Target Protein and New Leading Compound

Mammalian cells

Homology to humans

Produce eggs

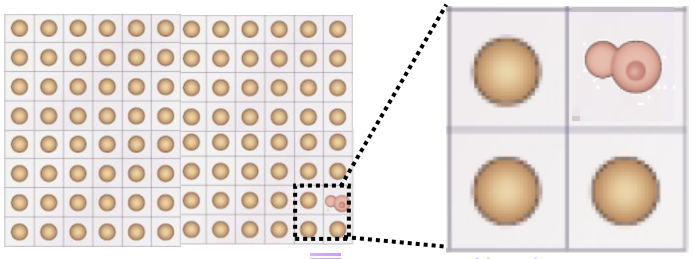


organisms

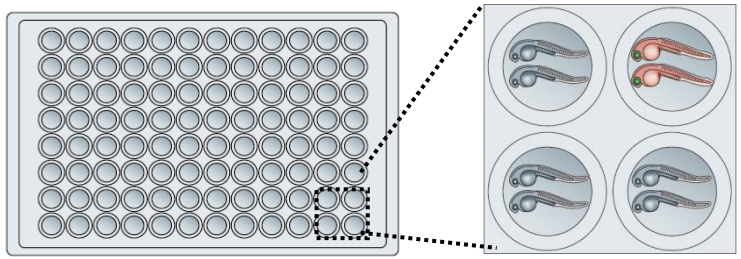
Physiological context

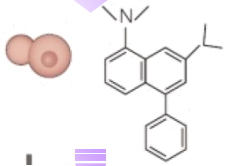
Diverse Library

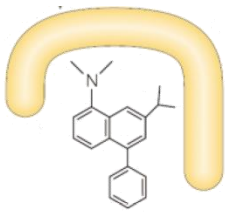
???

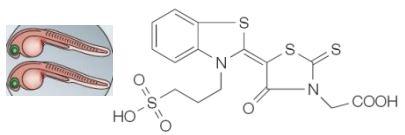


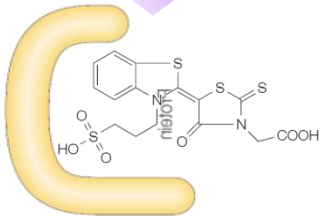
96-well plate, one well one compound







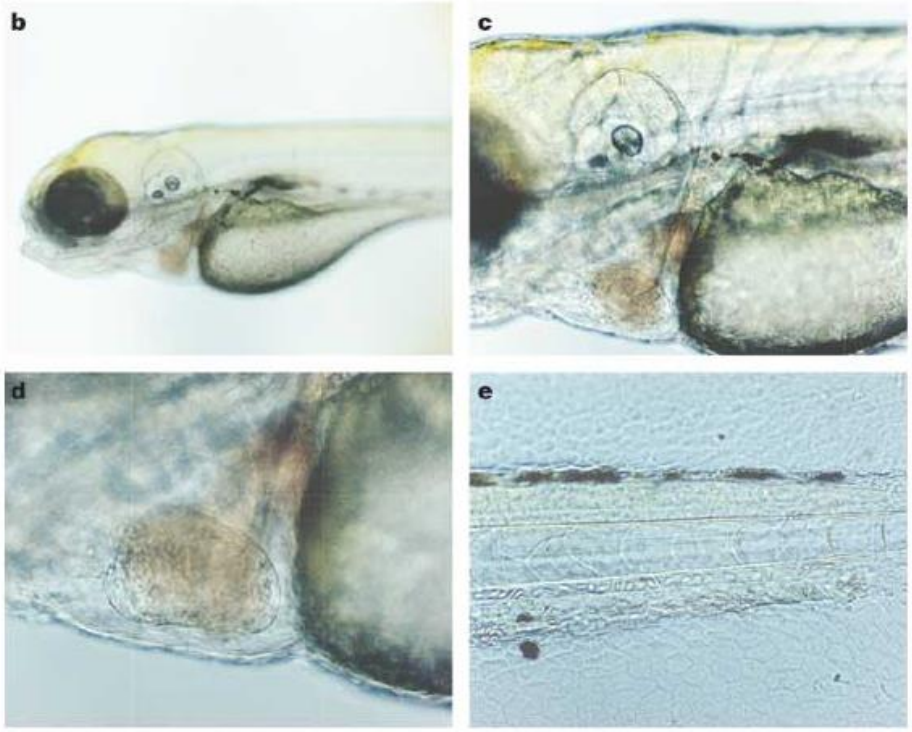




Zebra fish as animal model

Due to their size, zebra fish (*Danio rerio*) are easy to handle. Moreover, during their embryonal and larva stadium they are translucent, which facilitates the analysis of *in vivo* studies.

Thus High Throuput Screening regarding the consequences on the phenotype is possible.

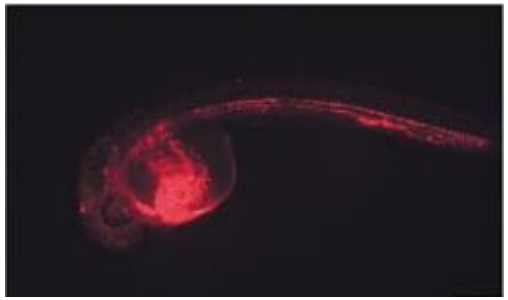


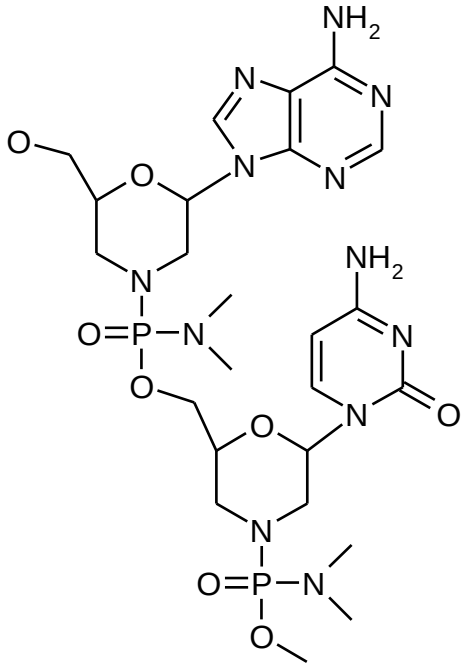
Zebra fish as animal model

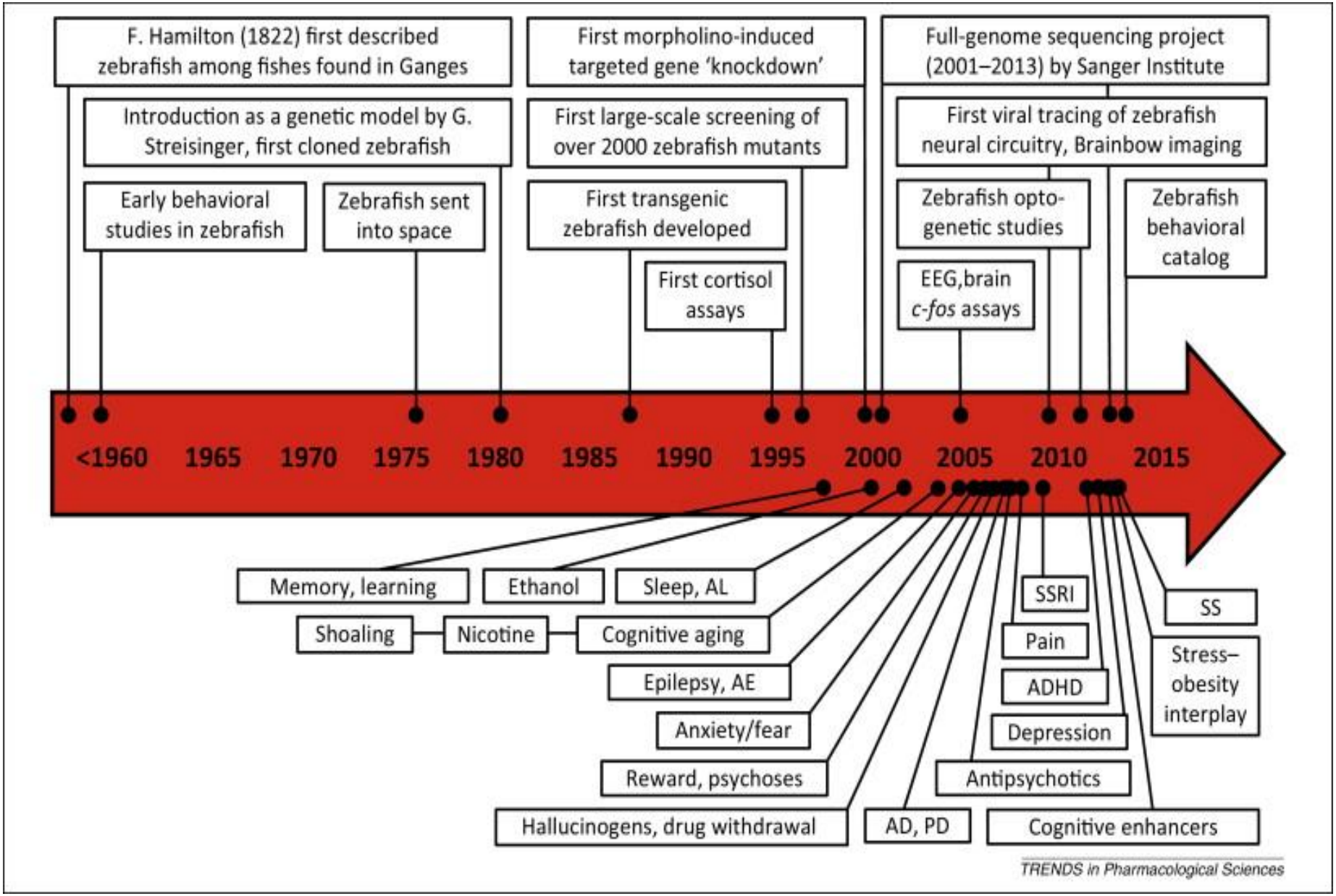
Furthermore there are a number of standard tools for genetic manipulations, e.g.

Knock down using morpholino oligonucleotides (cf. siRNA)

As well as the usual transgenic methods





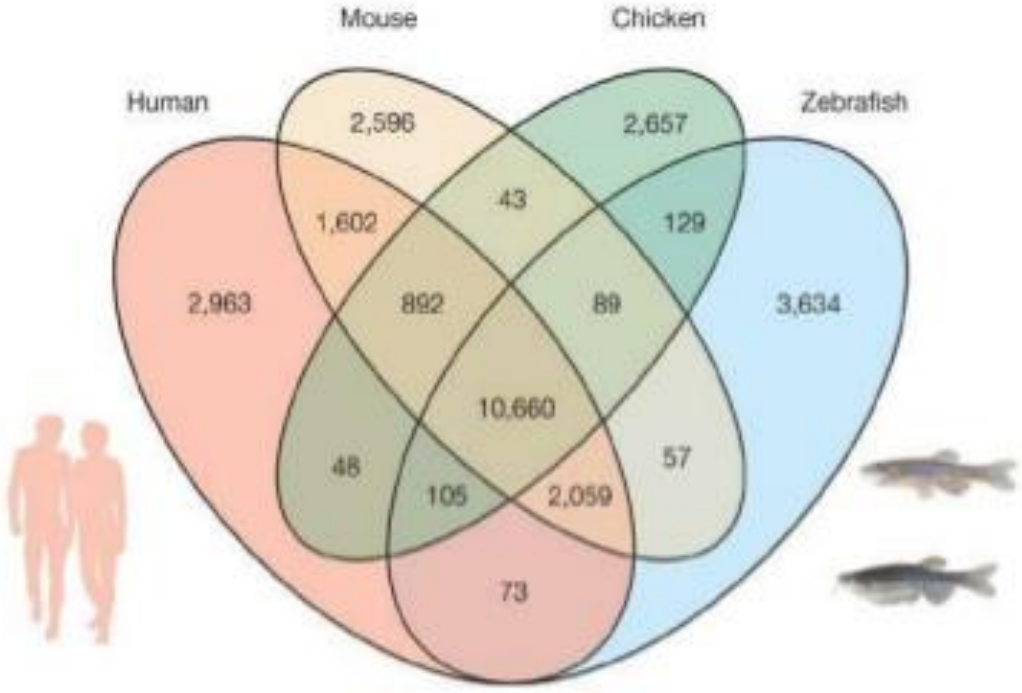

F. Hamilton (1822) first described zebrafish among fishes found in Ganges
First morpholino-induced targeted gene 'knockdown'
Full-genome sequencing project (2001–2013) by Sanger Institute
Introduction as a genetic model by G. Streisinger, first cloned zebrafish
First large-scale screening of over 2000 zebrafish mutants
First viral tracing of zebrafish neural circuitry, Brainbow imaging
Early behavioral studies in zebrafish
Zebrafish sent into space
First transgenic zebrafish developed
Zebrafish opto-genetic studies
Zebrafish behavioral catalog
First cortisol assays
EEG,brain c-fos assays
<1960
1965
1970
1975
1980
1985
1990
1995
2000
2005
2010
2015
Memory, learning
Ethanol
Sleep, AL
Shoaling
Nicotine
Cognitive aging
Epilepsy, AE
Anxiety/fear
Reward, psychoses
Hallucinogens, drug withdrawal
SSRI
Pain
ADHD
Depression
Antipsychotics
AD, PD
SS
Stress–obesity interplay
Cognitive enhancers
TRENDS in Pharmacological Sciences

Selected zebrafish models of human diseases and syndromes.

Human condition	zebrafish model	Zebrafish genes
cardiac arrhythmia: short QT syndrome	reggae mutant (reg)	*zERG*
cardiac arrhythmia: QT prolongation	rate of atrial and ventricular rates	-
Parkinson's disease	oxidative stress, dopamine neuronal loss	*DAT*, *TH* and *Dj-1*
Inflammatory bowel disease	Gut morphology, peristalsis	-
Epilepsy	Startle response	-
Cerebral cavernous malformations	Ccm1 mutant	*Ccm1*
Polycystic kidney disease	bicaudal C and Polycystic kidney disease mutant (Bicc1, Pkd2)	*Bicc1*, *Pkd2*,
Ullrich congenital muscular dystrophy	collagen VI mutant (Col6a1)	*Col6a1*
Polycythemia vera	Janus kinase 2 mutant (jak2a)	$jak2a^{V581F}$
Waardenburg syndrome type IV	sex determining region Y mutant (sox10)	*fgf8*, *sox9a*, *sox9b* and *sox10*
Variegate porphyria (porphyrias)	Montalcino mutant	*ppox*
cancer	Transplantations of cancer cell lines (WM-266-4, SW620, FG CAS/Crk, CCD-1092Sk). Quantification of cancer cells in zebrafish	-

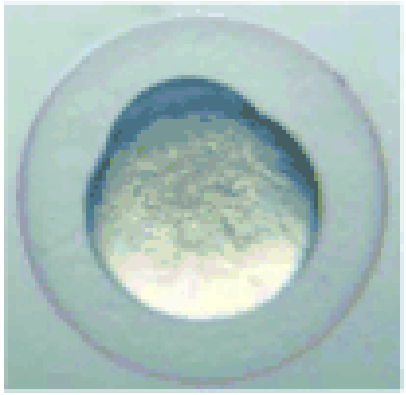
Genetics

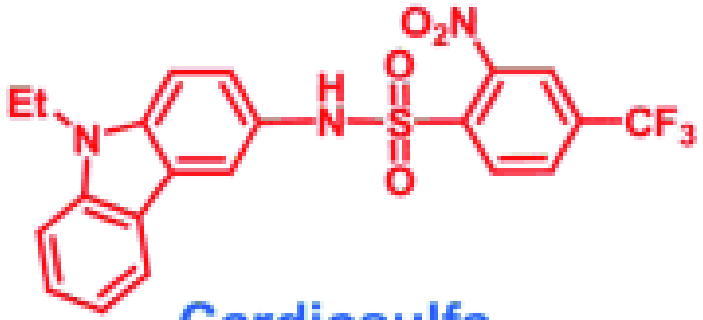
- According to a paper published in *Nature*, 70 per cent of protein-coding human genes are related to genes found in the zebrafish (*Danio rerio*), and 84 per cent of genes known to be associated with human disease have a zebrafish counterpart.



Disease	Genes and their products
Alzheimer's disease	Presenilin-1 Presenilin-2 Acetylcholine esterase Amyloid precursor protein *anoE*
Amyotrophic lateral sclerosis	*Sod-1*
Muscular dystrophy	Dystroglycan Dystrophin *Dp71*
Leukemia	*Runx1* *Cbfb*
Thrombosis	Factor VII *COX-1* *COX-2* (*COX*—cyclooxygenase)
Cardiomyopathy	Cardiac troponin T Titin (TTN)
Diabetes mellitus	Insulin IA-2 autoantigen IA-2β autoantigen (IA—islet antigen)

Zebrafish embryo

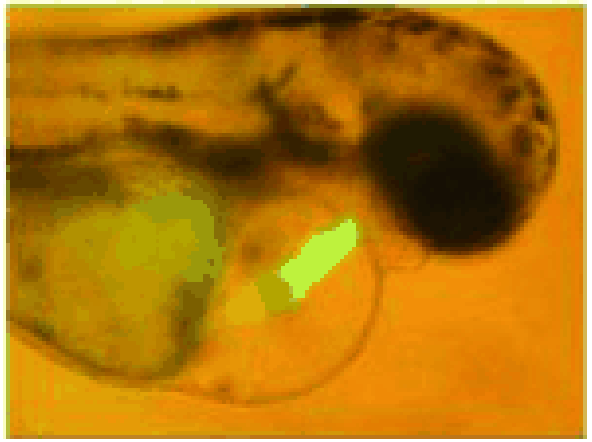




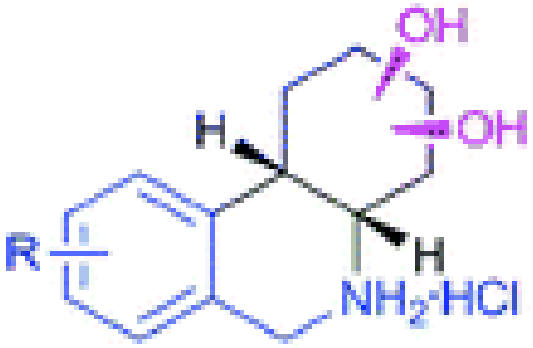
Cardiosulfa

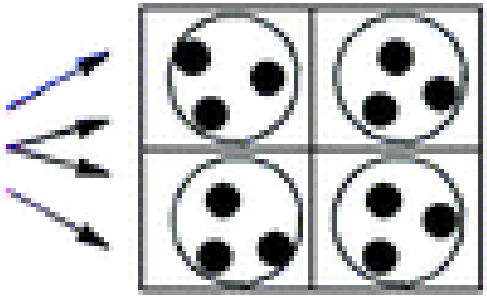
Activation of the
AhR signaling pathway

Heart deformation



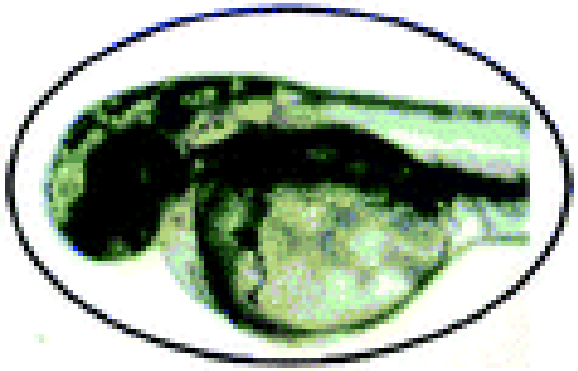
Rapid library synthesis





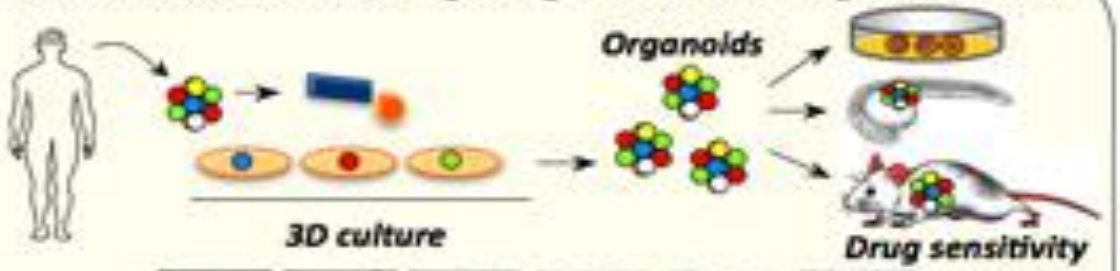
3-5 days

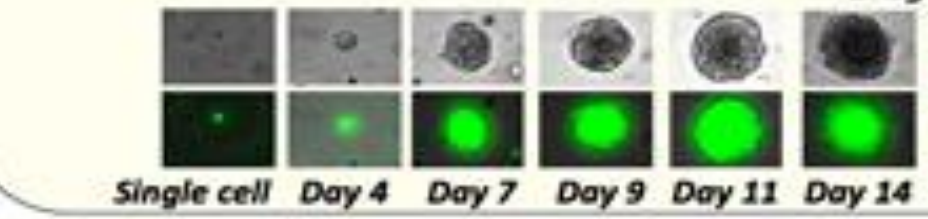
Phenotypic evaluation

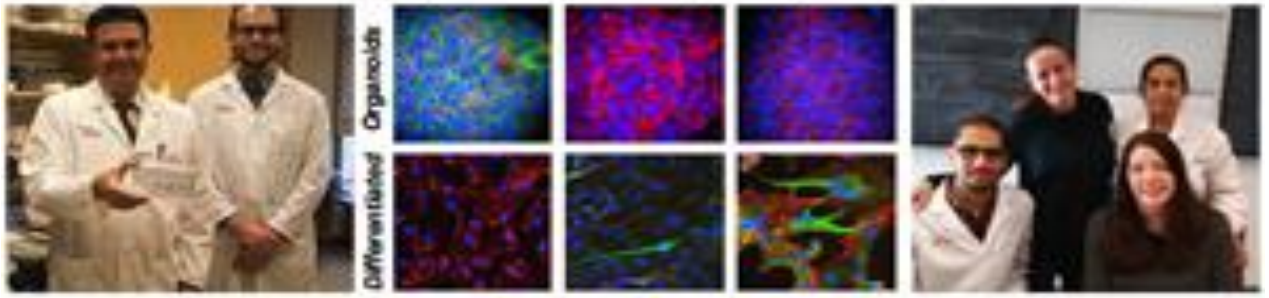


R = H, Me, F, OMe,
-OCH_2O- etc.

Stem cells, Cancer, Organogenesis and Regeneration

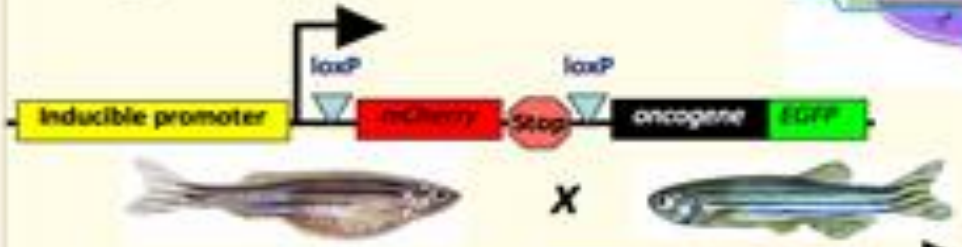




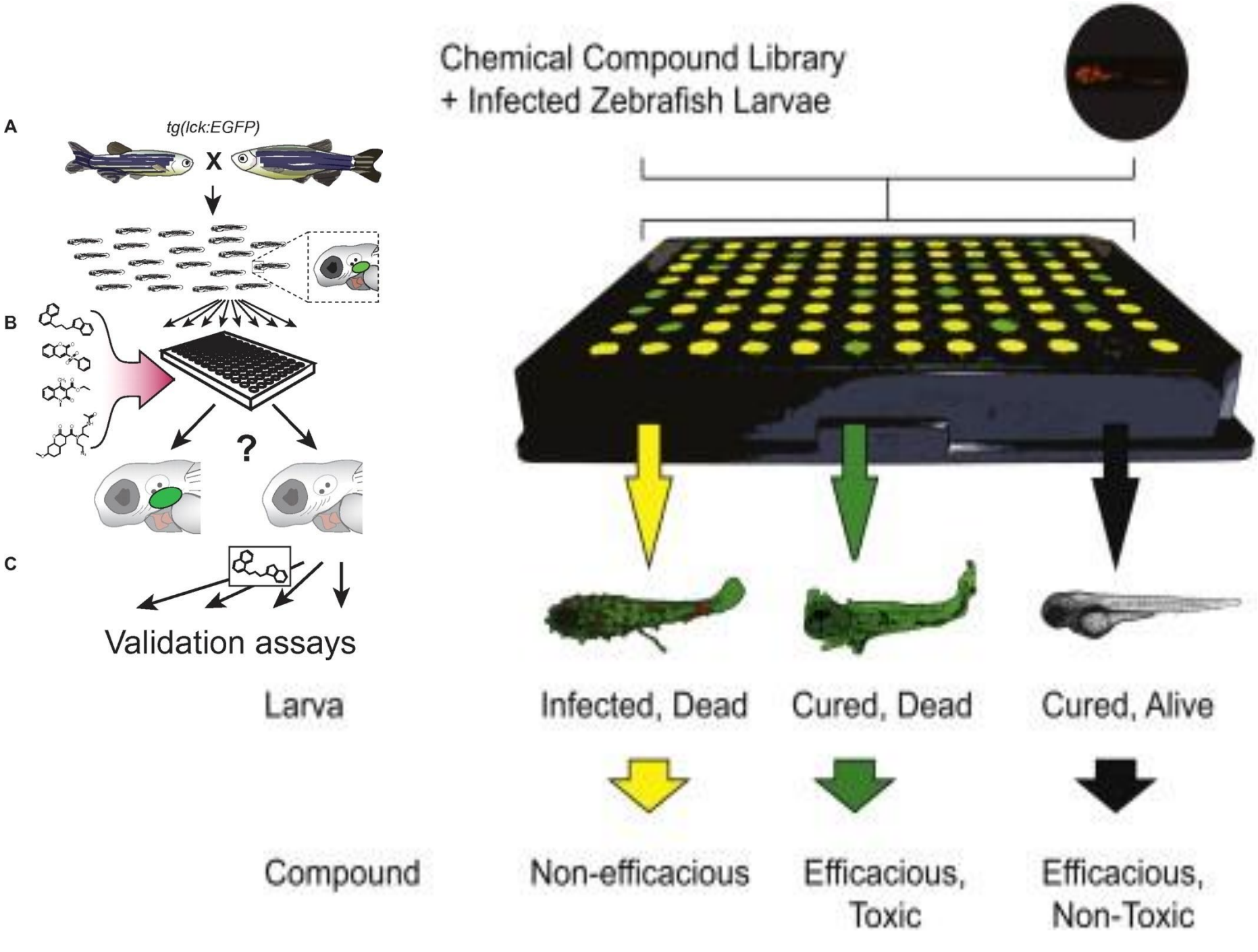


Zebrafish stem cell and oncogenic reporters

CRISPR-mediated genome editing



Cancer drug discovery

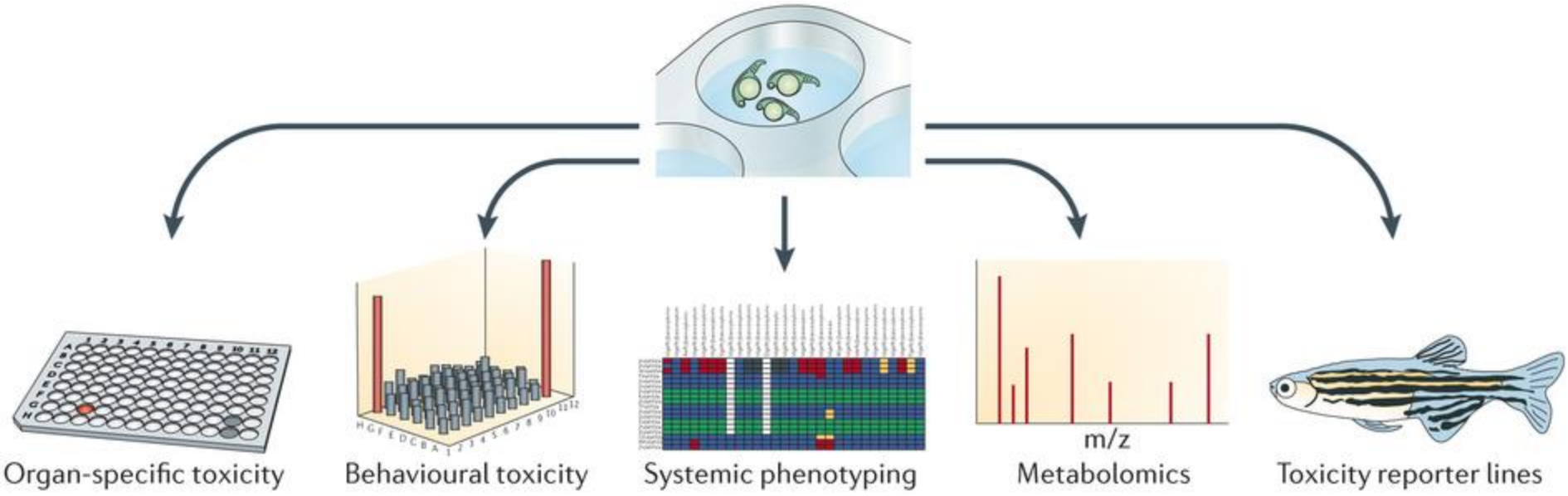

A
tg(lck:EGFP)
X
B
?
C
Validation assays
Chemical Compound Library
+ Infected Zebrafish Larvae
Larva
Infected, Dead
Cured, Dead
Cured, Alive
Compound
Non-efficacious
Efficacious, Toxic
Efficacious, Non-Toxic

Zebrafish Phenotypic assay

Natural Products

Developmental defects associated with perturbed signaling pathways closely shared between zebrafish and human

Opportunity for the development of targeted cancer therapies


Organ-specific toxicity
Behavioural toxicity
Systemic phenotyping
m/z
Metabolomics
Toxicity reporter lines

Limitation

- They require water systems to maintain them
- Reverse genetics has not been worked out for zebrafish as it has in the mouse.
- No way of targeting mutations. In mice, for example, you can "knock out" a gene if you have the sequence, and ask why it is needed. In zebrafish, we create random mutations and look for specific defects. Then we have to go and find what sequence is responsible for the defect.

Conclusion

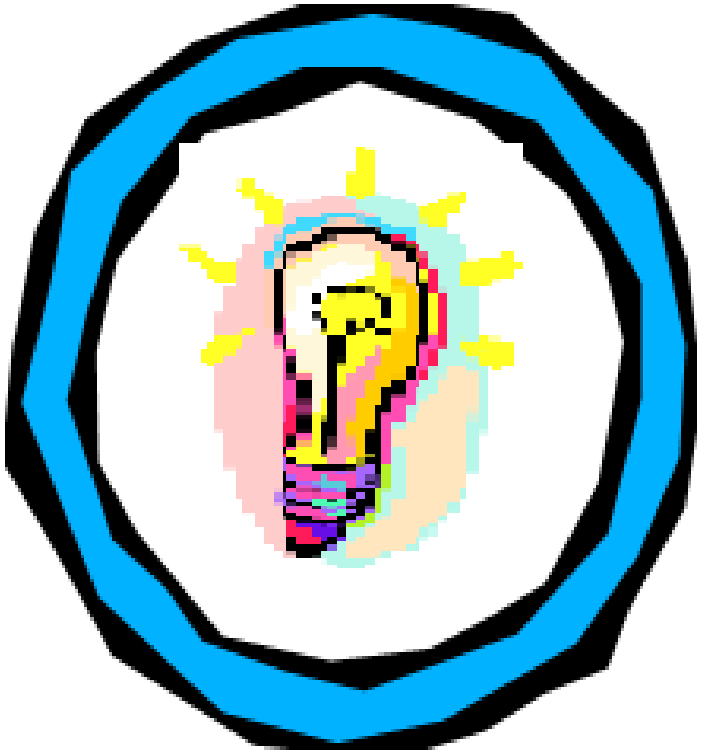
- The use of zebrafish in pharmaceutical research and discovery and drug development is mainly screening of lead compounds, target identification, target validation, morpholino oligonucleotide screens, assay development for drug discovery, physiology based drug discovery, quantitative structure-activity relationship (QSAR) and structure -activity relationships (SAR) study and drug toxicity study.
- Current drug discovery strategies include both molecular and empirical approaches by using Zebrafish Model.
- Possible to obtain scientific input on issues related to sensitivity, specificity, reproducibility and quality control of genotypic and phenotypic assays.
- Phenotypic approaches to be the more successful strategy.
- Approaches for categorizing mutational patterns for assessing their prognostic value on treatment outcome.
- Organism-based process has given way in recent decades to systematic, high-throughput assays using purified proteins, cells, or cell extracts.
- Zebrafish, make it possible to combine the advantages of organism-based small molecule discovery with the technologies and throughput of modern screening.
- Presently, the research using zebrafish is expanding into other areas such as pharmacology, clinical research as a diseases model and interestingly in drug discovery.

REFERENCES:

1) Target validation: A door to drug discovery, Xiu-Ping Chen,Guan-Hua Du*, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China., Drug Discov Ther 2007;1(1):23-29
2) Zebrafish: A Complete Animal Model for In Vivo Drug Discovery and Development Chiranjib Chakraborty,*Govindasamy Agoramoorthy et.al., Department of Marine Biotechnology and Resources, College of Marine Science and Division of Marine Biotechnology, Asia-Pacific Ocean Research Center, National Sun Yat-sen University, Kaohisung, Taiwan; Department of Pharmacy, Tajen University, Yanpu, Pingtung 907, Taiwan,Current Drug Metabolism, 2009, 10, 116-124
3) Zebrafish: From disease modeling to drug discovery, Amy L Rubinstein, Current Opinion in Drug Discovery & Development 2003 6(2):218-223


Thank U...

THANK YOU



ANY QUESTIONS