

斑马鱼药物筛选研究简介

国家水生生物种质资源库 (NABRC)

国家斑马鱼资源中心 (CZRC)

潘鲁媛

www.zfish.cn 国家水生生物种质资源库

国家斑马鱼资源中心

luyuanpan@ihb.ac.cn

• 斑马鱼药物筛选概况

- 药物筛选实验概述
- 斑马鱼的生物学特性与化合物筛选
- 斑马鱼药物筛选的基本流程与系统

• 斑马鱼药物筛选实例解析

- 糖尿病药物筛选实例
- 癌症药物筛选实例
- 中药相关筛选实例
- 进入临床的筛选实例

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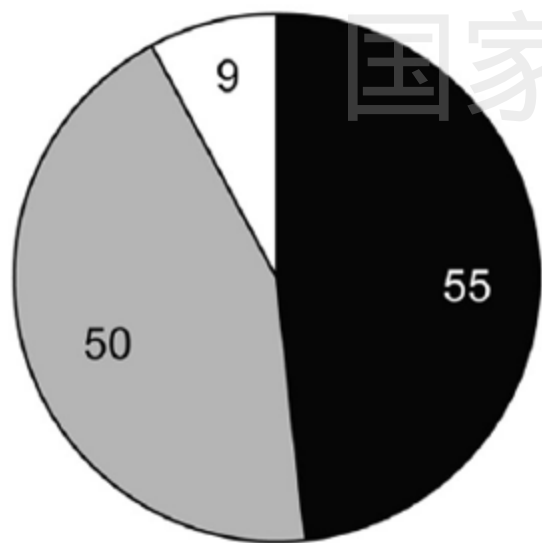
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应用斑马鱼进行化合物筛选

- 始于2000年
- 到2017年，共有114篇发表的工作



Rennekamp&Peterson, 2015



- Angiogenesis
- Bone
- Central nervous system
- Fin
- Gut
- Hair cells/lateral line
- Heart
- Hematopoiesis
- Kidney
- Liver
- Lymphangiogenesis
- Muscle
- Melanocytes/iridophores

Zhang & Peterson, 2021

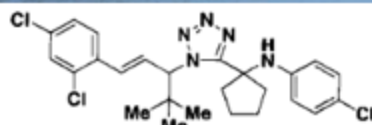
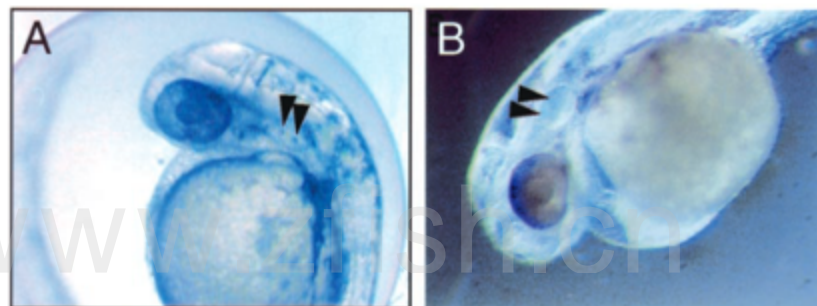
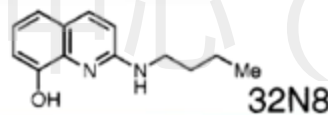
化合物筛选的目的

- 发现新药
- 发现已有药物的替代药物（强效、副作用少、特异性强）
- 发现已有药物的新作用
- 了解/缓解药物的毒副作用
- 发现影响生物学过程的新化合物
- 了解已有药物的作用机理，探索疾病成因
- 探索化合物之间的相互作用

斑马鱼中的首次化合物筛选

First screen: Harvard University, Department of Molecular and Cellular Biology, 2000

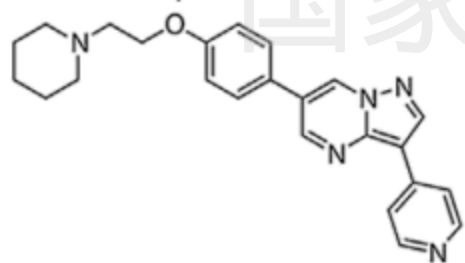
- ✓ Small molecules
- ✓ 1100 synthetic small molecules
- ✓ early development
- ✓ fertilized zebrafish eggs
- ✓ Wild type
- ✓ 96-well plates
- ✓ 3 embryos per well (per compound)
- ✓ Molecules were added to water



- ✓ Screen what?
- ✓ Size of the library?
- ✓ What phenotype?
- ✓ Animal age?
- ✓ Animal genotypes?
- ✓ Size of screening system?
- ✓ Sample size of animal?
- ✓ How to apply chemicals?

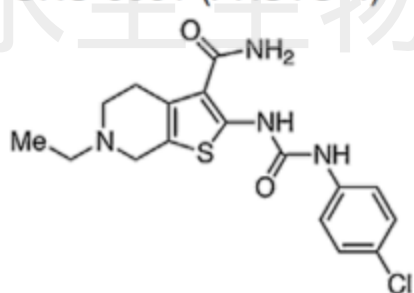
斑马鱼的药物筛选成功进入临床

Dorsomorphin



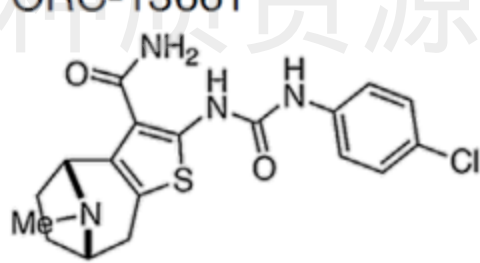
进行性骨化性
纤维发育不良

ORC-0001 (PROTO-1)

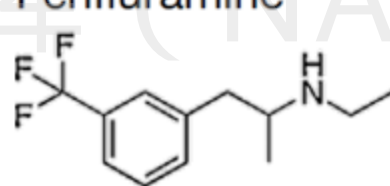


氨基糖苷类抗生素引起的毛细胞死亡

ORC-13661

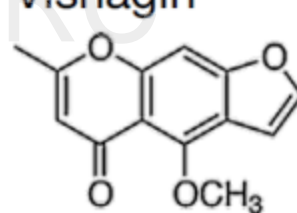


Fenfluramine



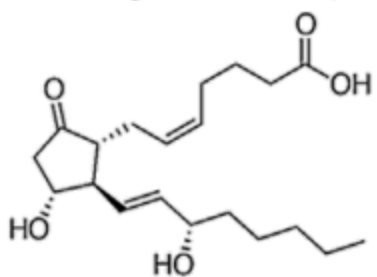
Dravet
Syndrome

Visnagin

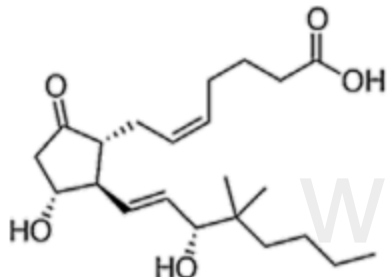


多柔比星引发心肌病

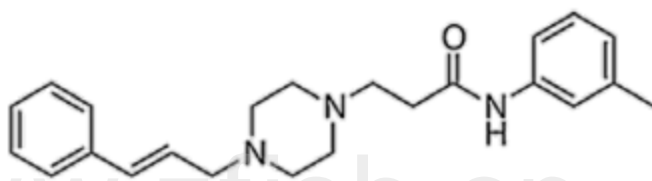
Prostaglandin E2 (PGE2) dmPGE2



脐带血干细胞移植

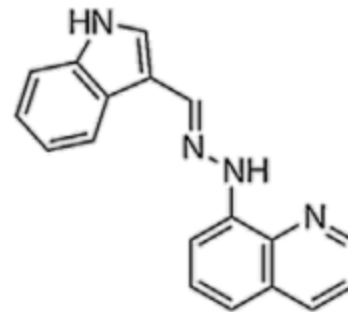


Finazine



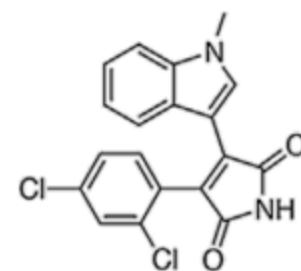
精神病

Lenaldekar



白血病

SB216763



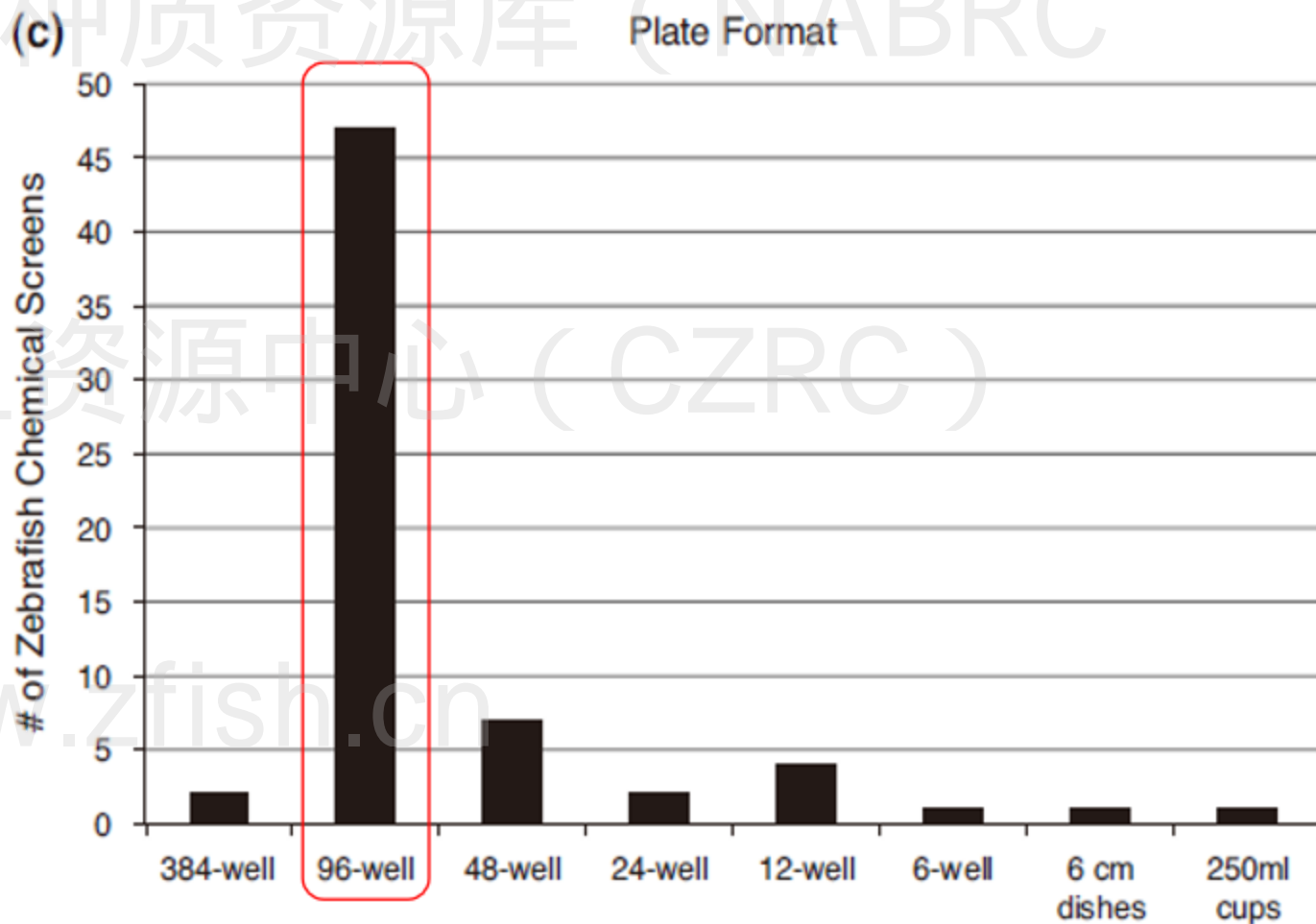
心律失常性心肌病

斑马鱼的生物学特性及优势

- 体型小 (成鱼2-5厘米、养殖密度10条/L、占用空间小)
- 易于养殖 (低成本、低硬件门槛、高样品数)
- 发育快 (24hpf完成早期发育、3个月可性成熟)
- 四季产卵、产卵量大 (>200枚/对, 每周可交配产卵, 实验方便)
- 体外受精、体外发育 (易于实现多样的实验操作)
- 胚胎透明 (易于操作, 易于进行活体观察)
- 与人类基因组同源性高 (利于建立各类人类疾病模型)

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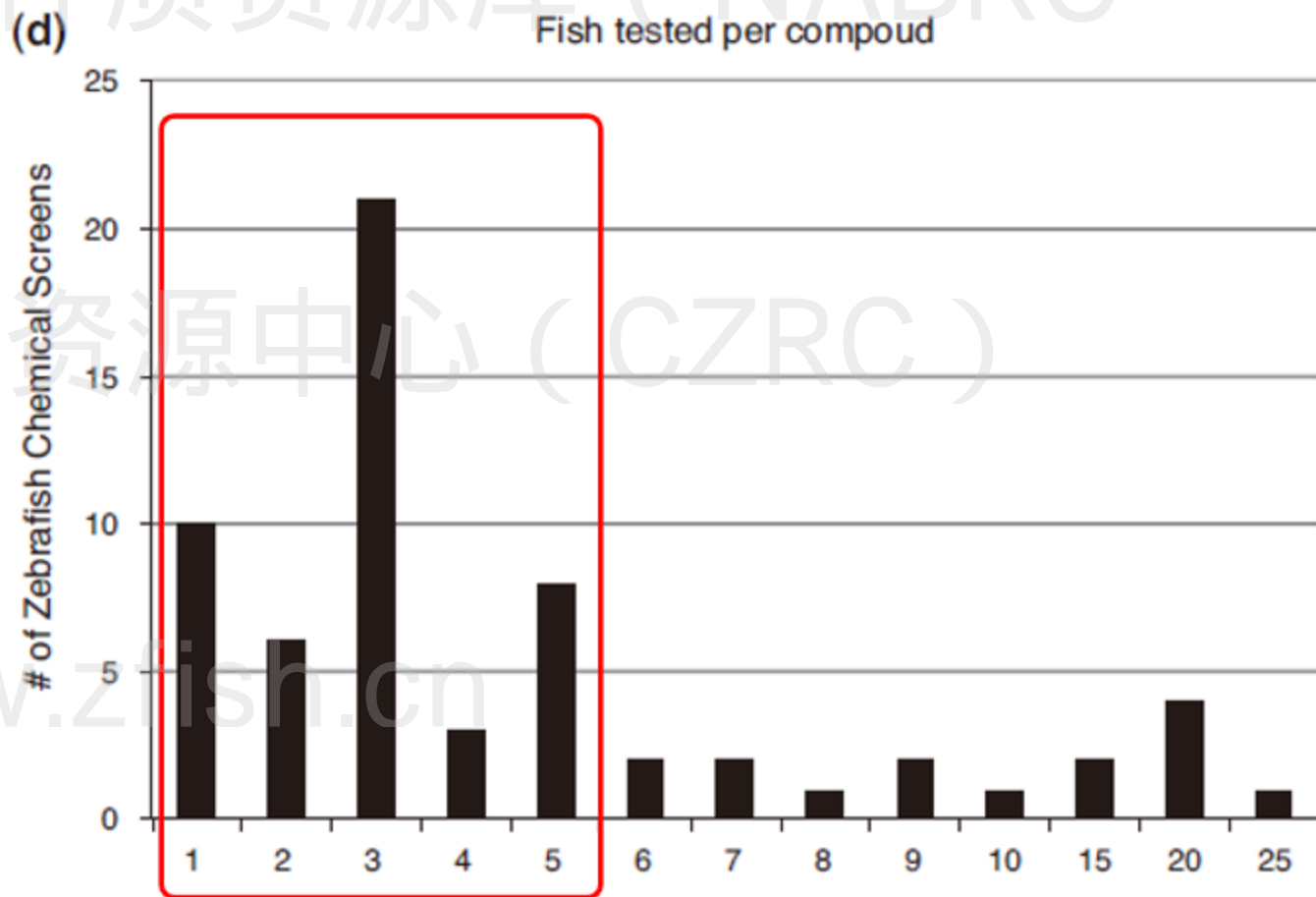
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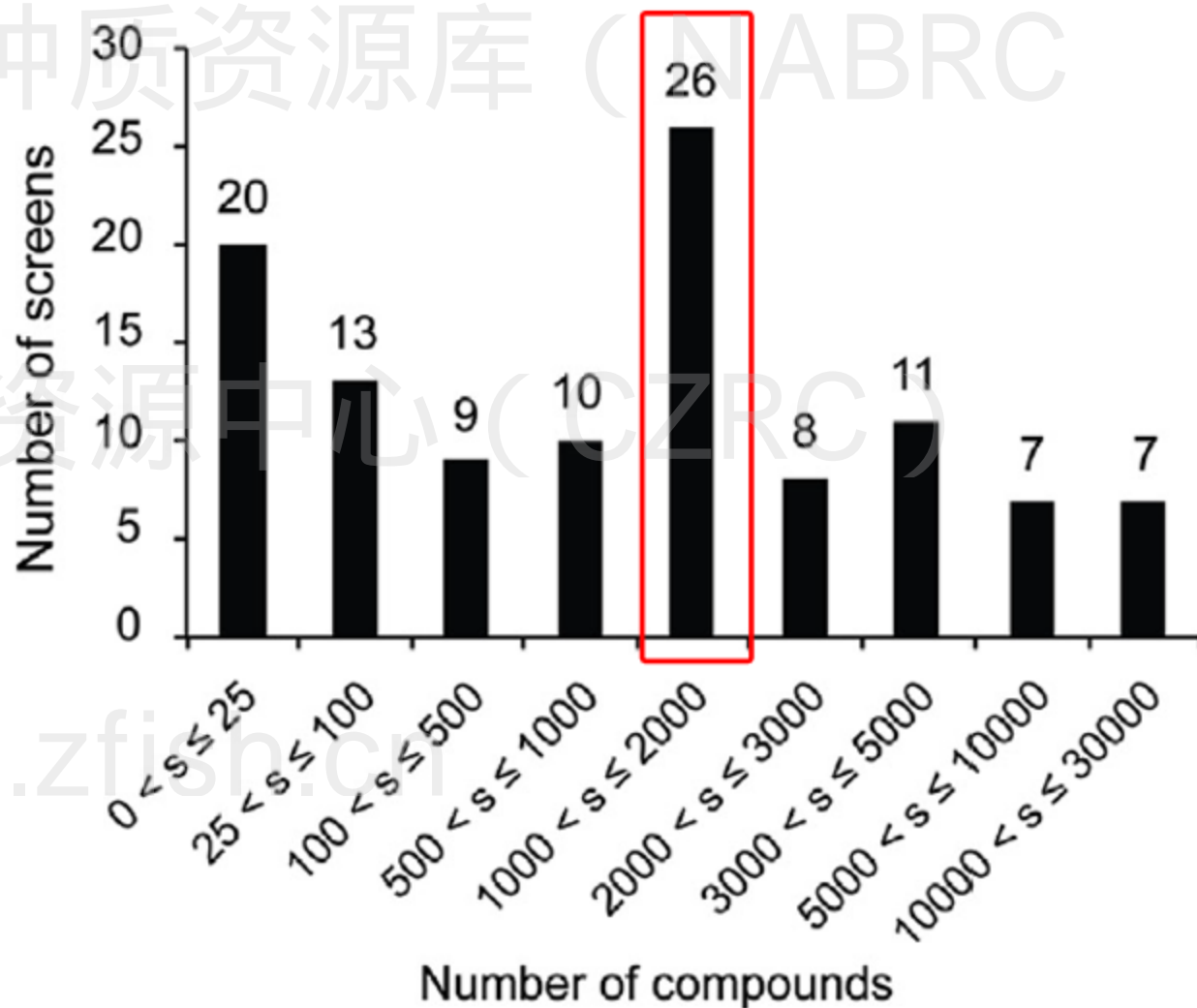
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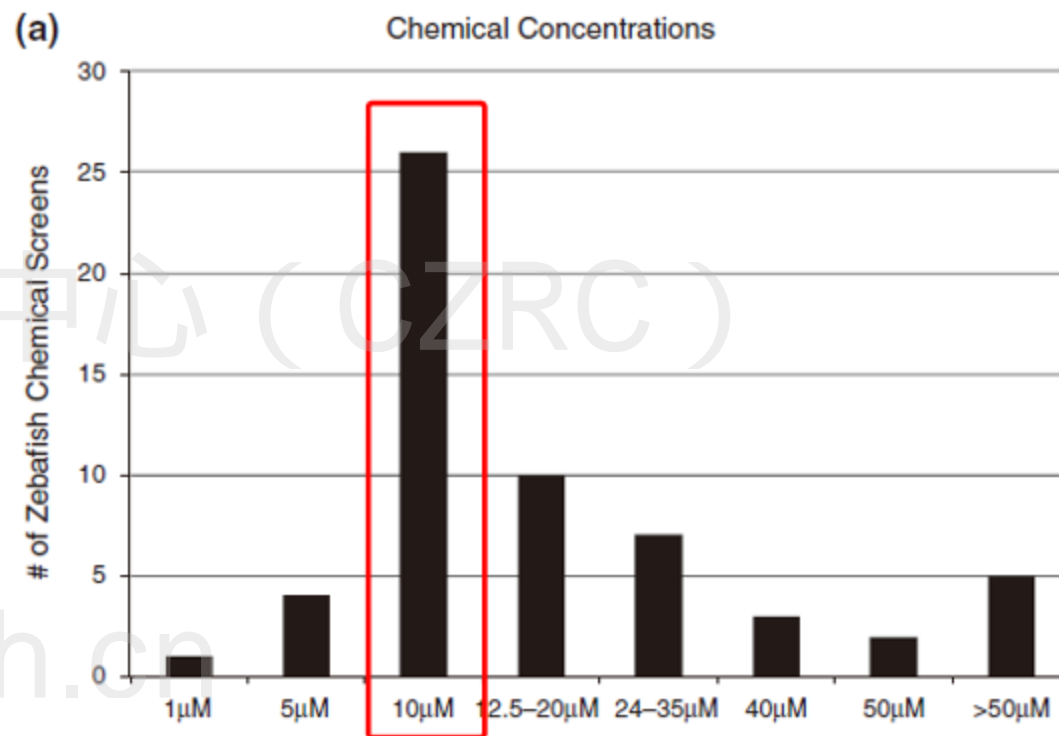
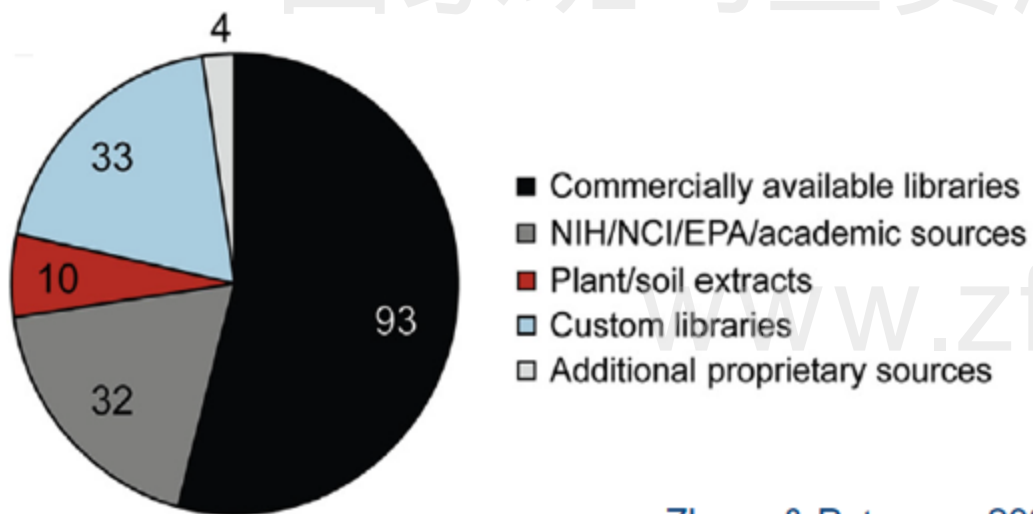
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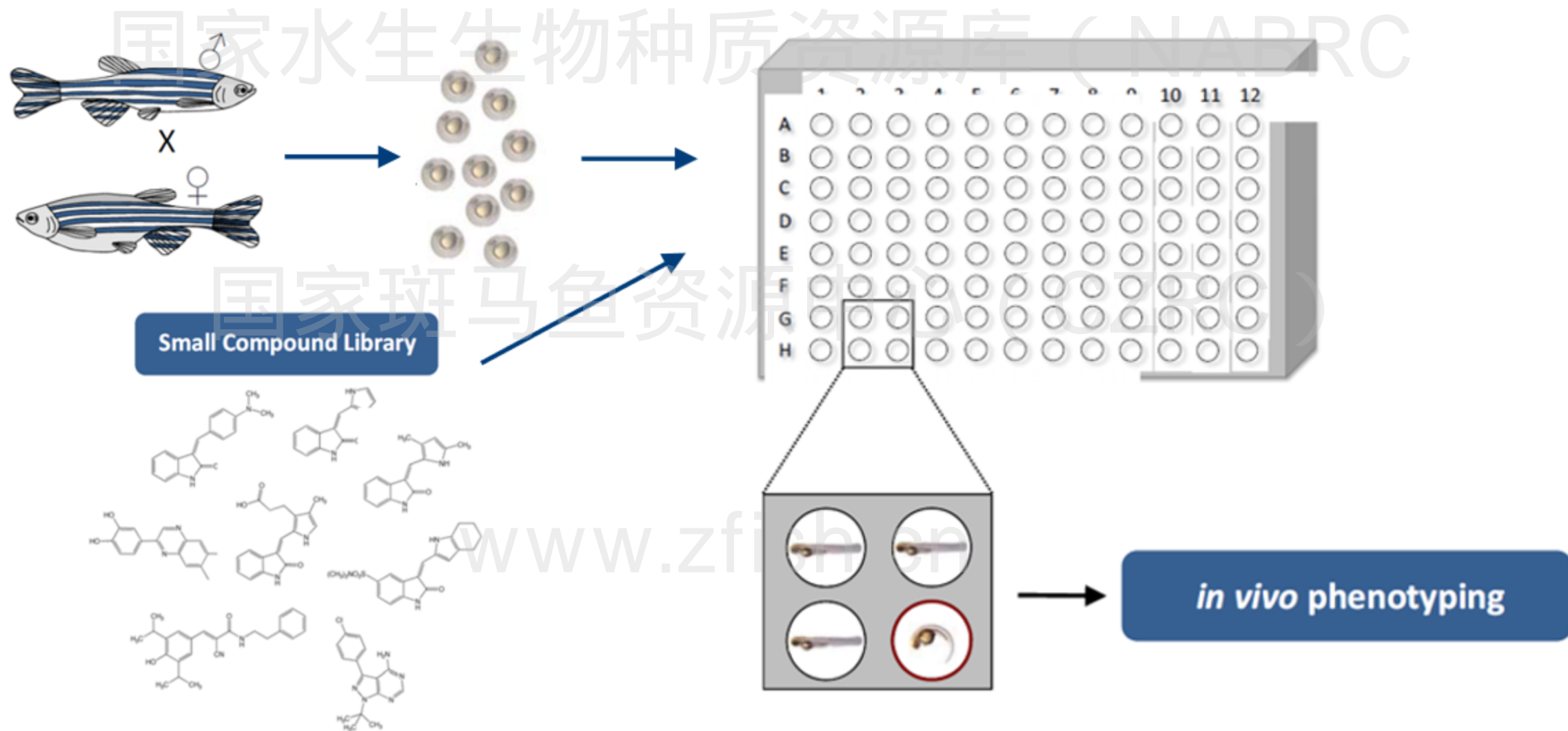


Chemical Libraries

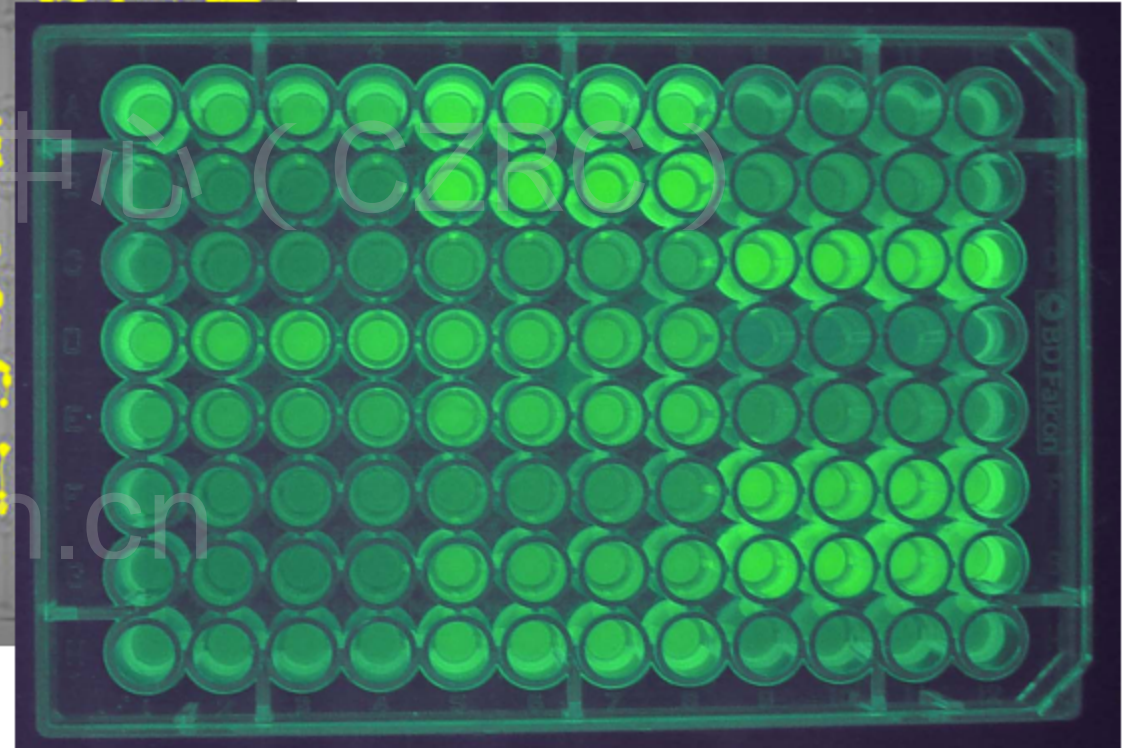
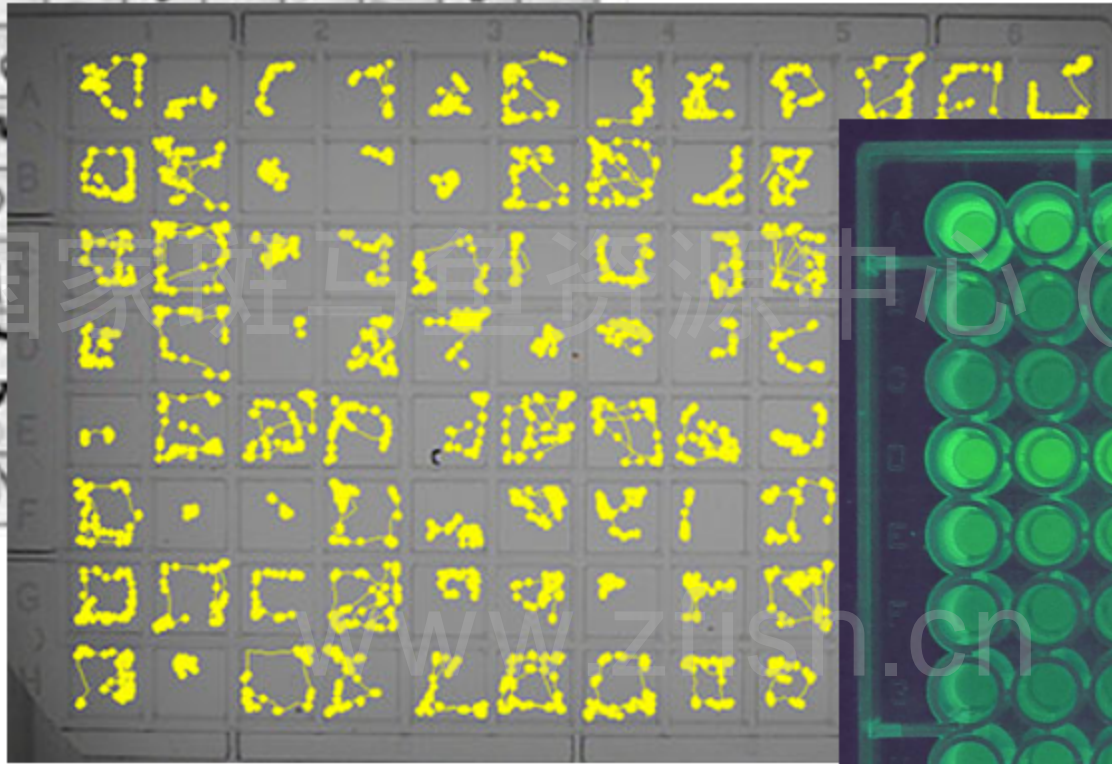
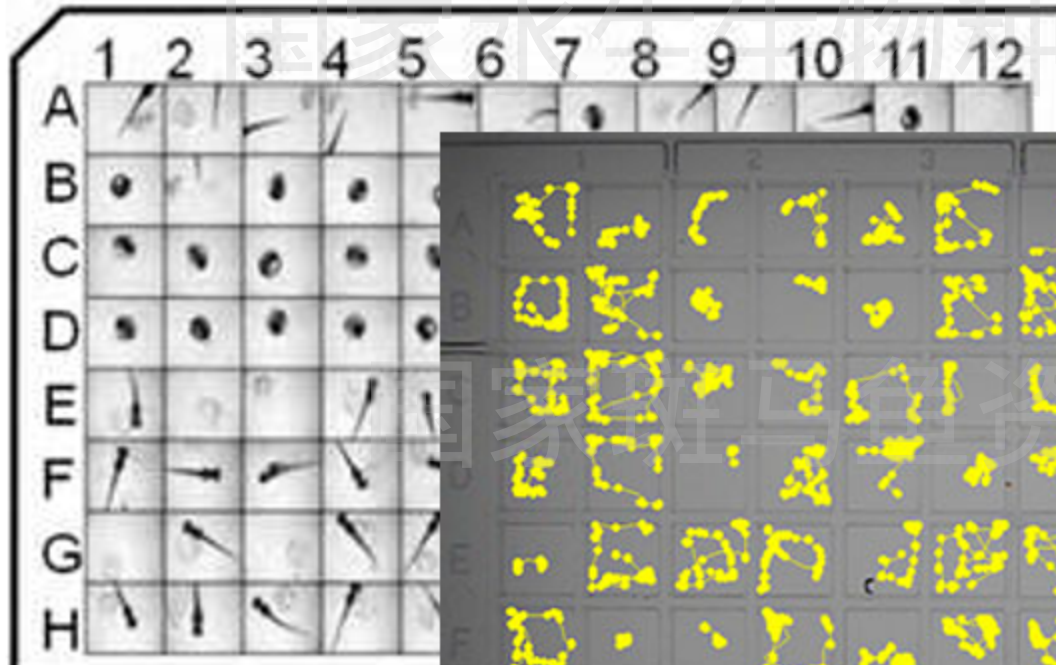
Library name	Number of screens
Prestwick Chemical Library (Prestwick Chemical) [®]	16
The Spectrum Collection (MicroSource Discovery Systems)	15
LOPAC ^{®1280} (Millipore Sigma)	11
SCREEN-WELL [®] ICCB Known Bioactives library (Enzo)	9
NINDS Custom Collection 2	8
DIVERSet [®] Libraries (ChemBridge)	8
SCREEN-WELL [®] FDA Approved Drug Library (Enzo)	6
NIH Clinical Collection (1 and/or 2)	4
EPA ToxCast [™] Phase-I Library	3
SCREEN-WELL [®] Nuclear Receptor ligand library (Enzo)	3



斑马鱼高通量化合物筛选的基本流程

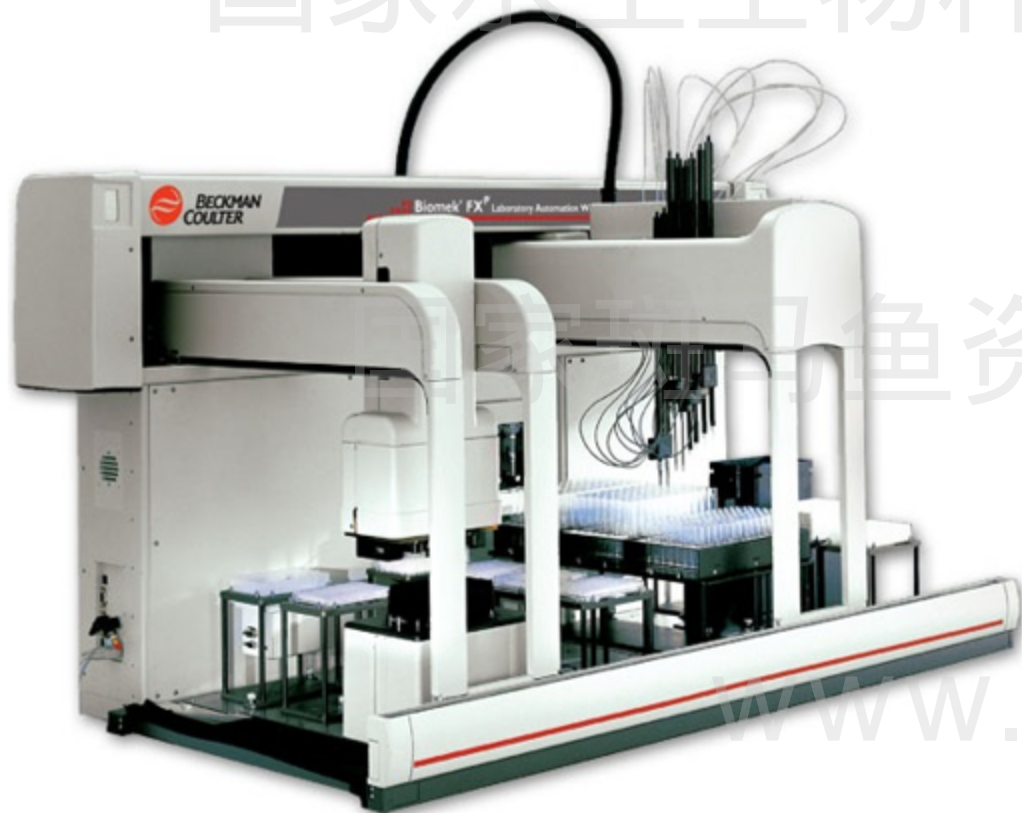


筛选表型



设备系统

国家水生生物种质资源库 (NABRC)



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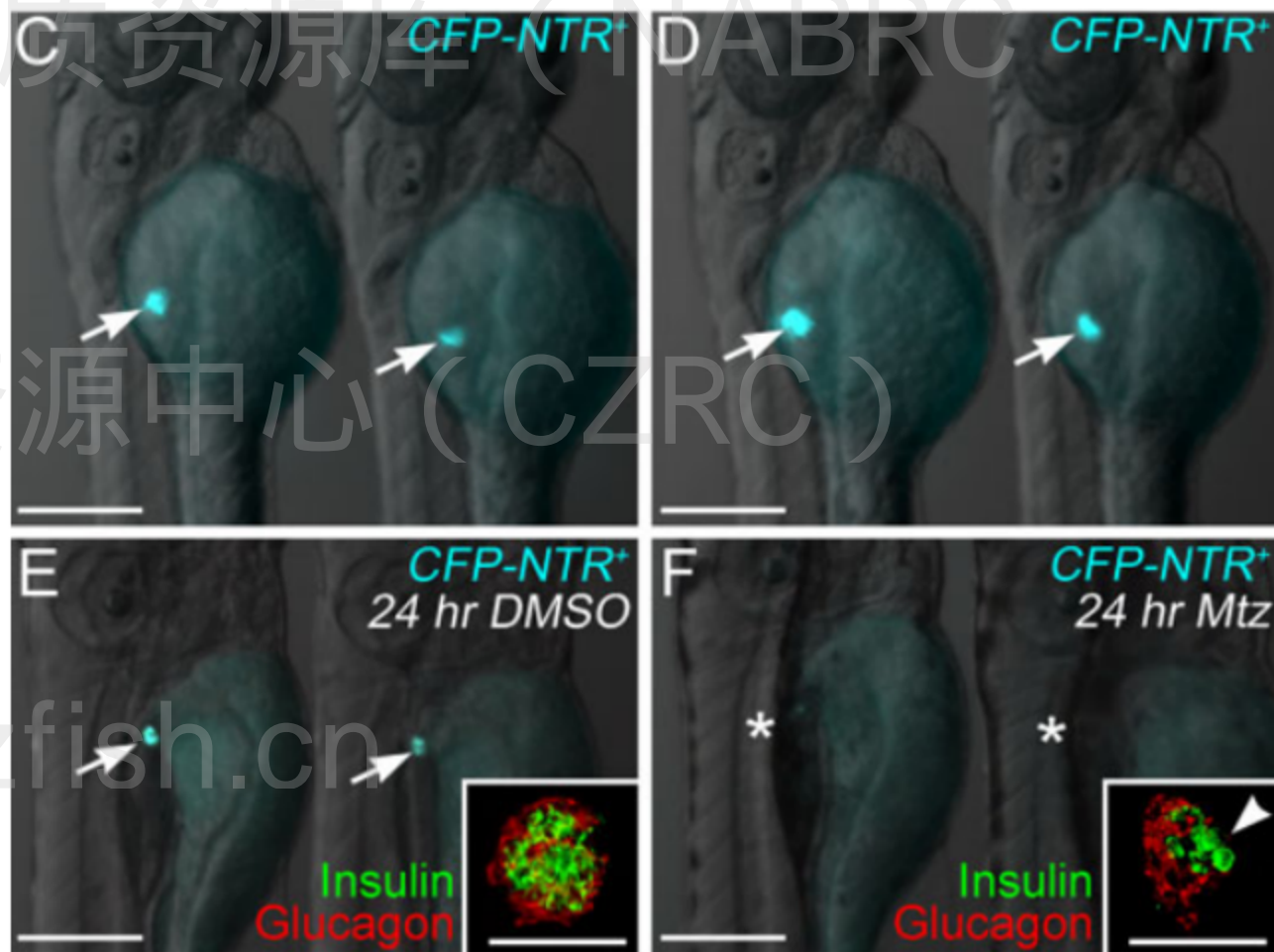
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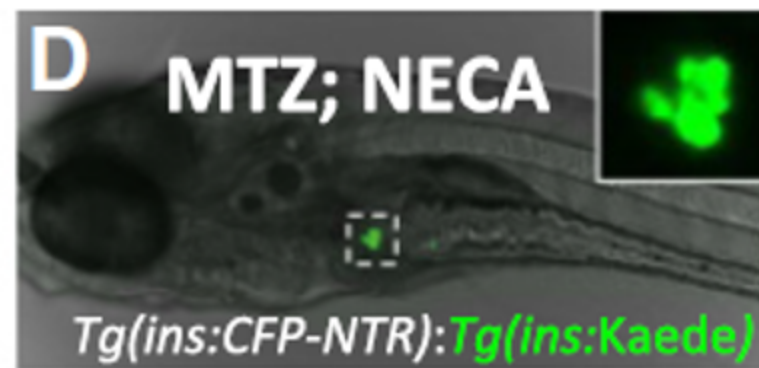
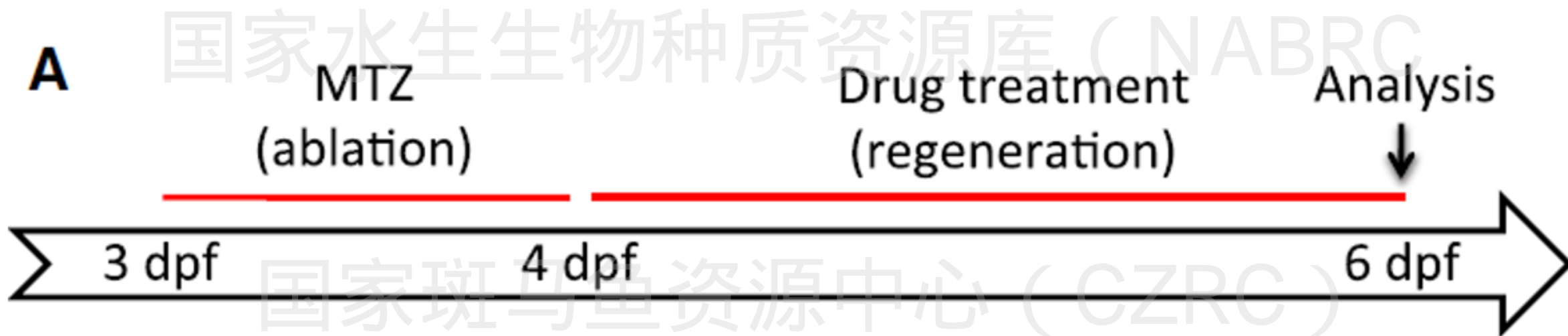
- 糖尿病药物筛选实例
(转基因致病工具模型)
- 癌症药物筛选实例
(人源化模型)
- 中药相关筛选实例
(外部干预+转基因标记模型)
- 进入临床的筛选实例
(内源突变+行为学检测模型)

I型糖尿病药物筛选

- I型糖尿病：胰腺 β 细胞自身免疫性破坏
- 斑马鱼在发育的第一周，只有一颗胰岛
- 双重转基因品系：特异性标记胰岛细胞，和特异性在胰岛中产生硝基还原酶 (NTR)
- 以甲硝哒唑 (Mtz) 处理特异性杀灭表达NTR的细胞
- 胰岛标记：*Tg(ins:Kaede)*
- NTR表达：*Tg(ins:CFP-NTR)*



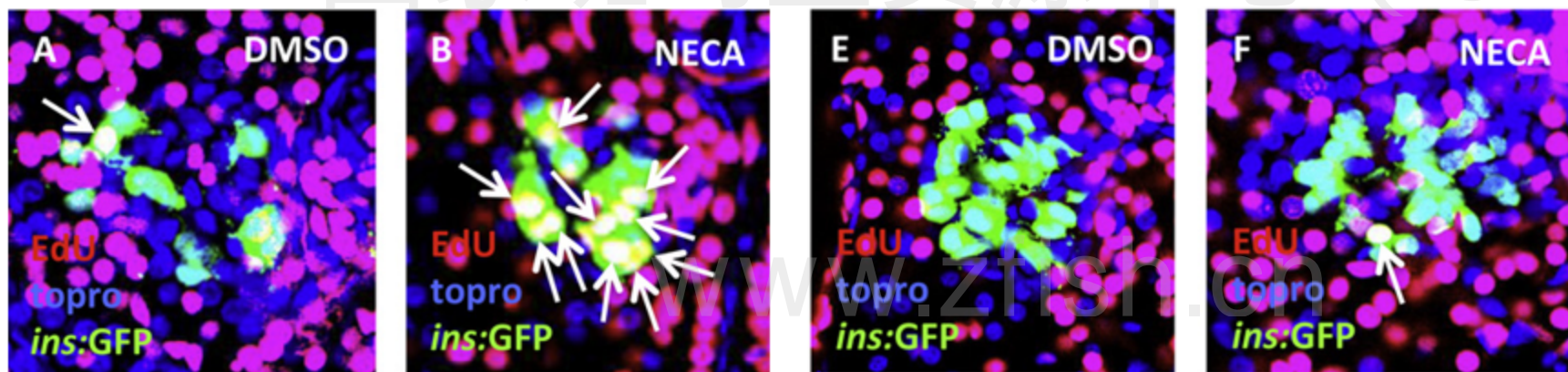
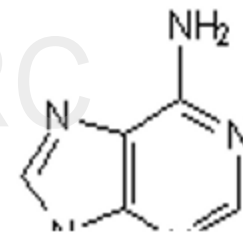
I 型糖尿病药物筛选



筛选过程和筛选结果

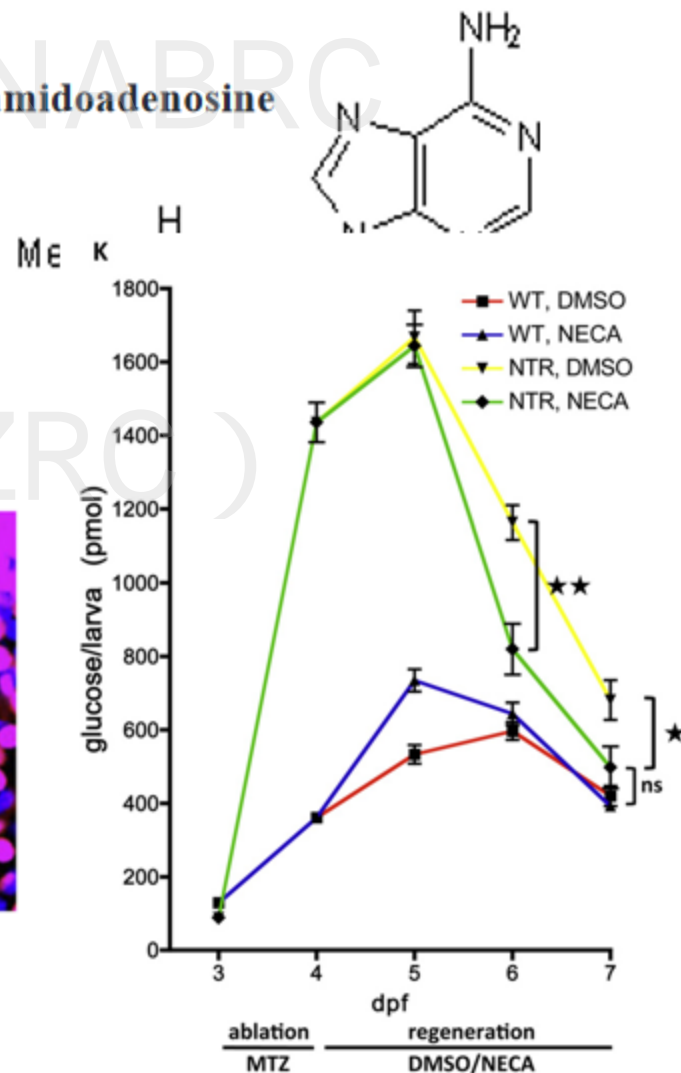
- 7,186 compounds
- ~ 100,000 larvae
- 5 compounds doubled the number of β cells
- 4 of 5 converge on the adenosine signaling pathway

50-N-ethylcarboxamidoadenosine (NECA)

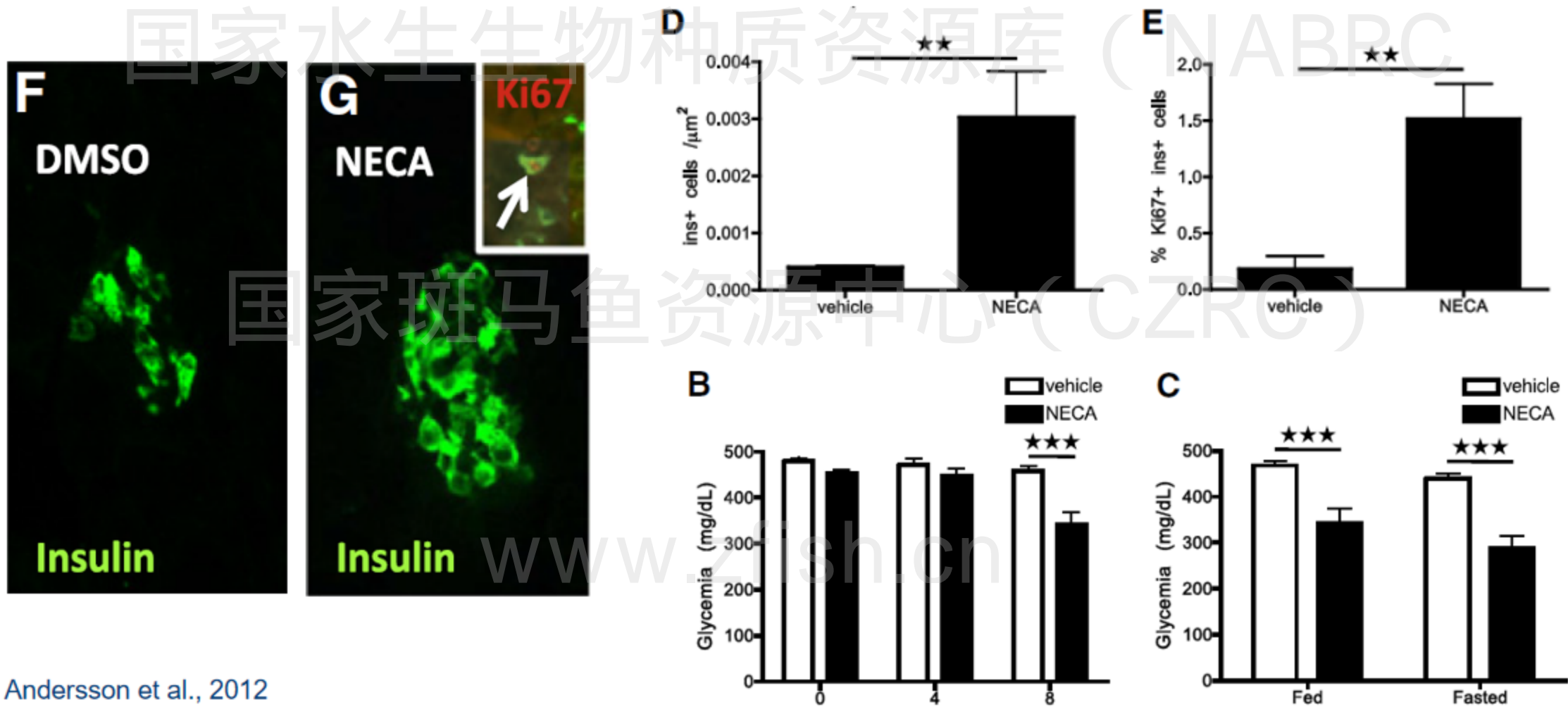


Ablated cells

Normal cells

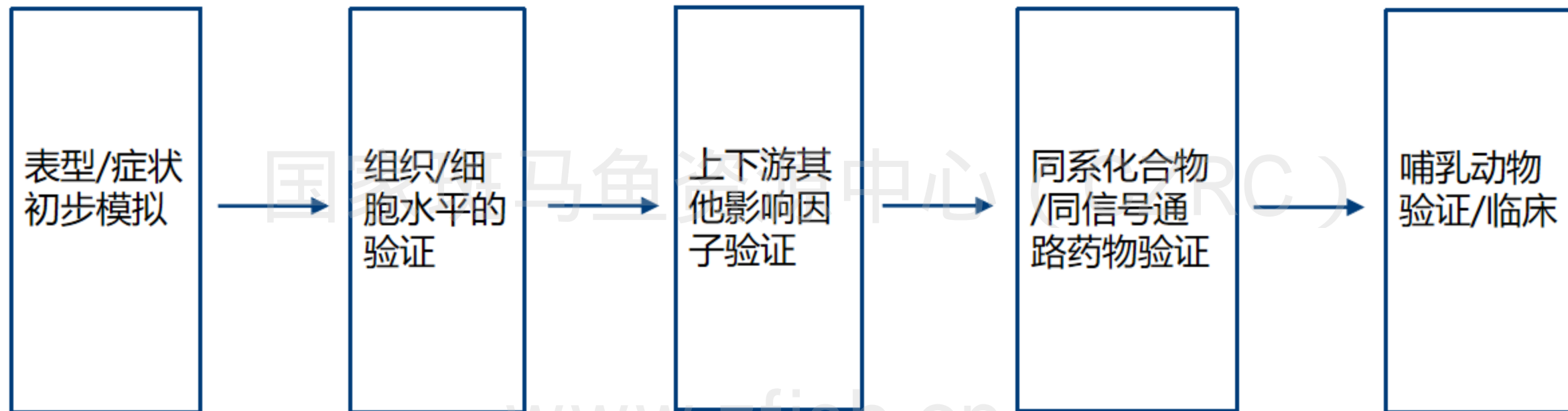


NECA的进一步验证 (小鼠)



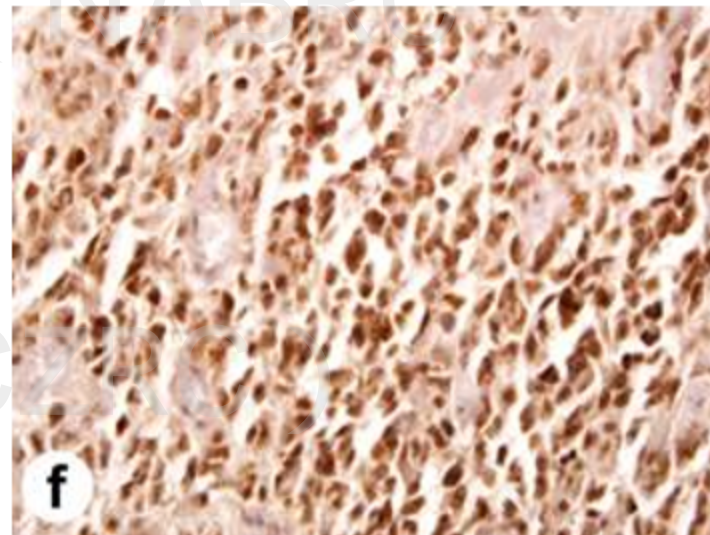
疾病模型用于化合物筛选的基本路径

国家水生生物种质资源库 (NABRC)



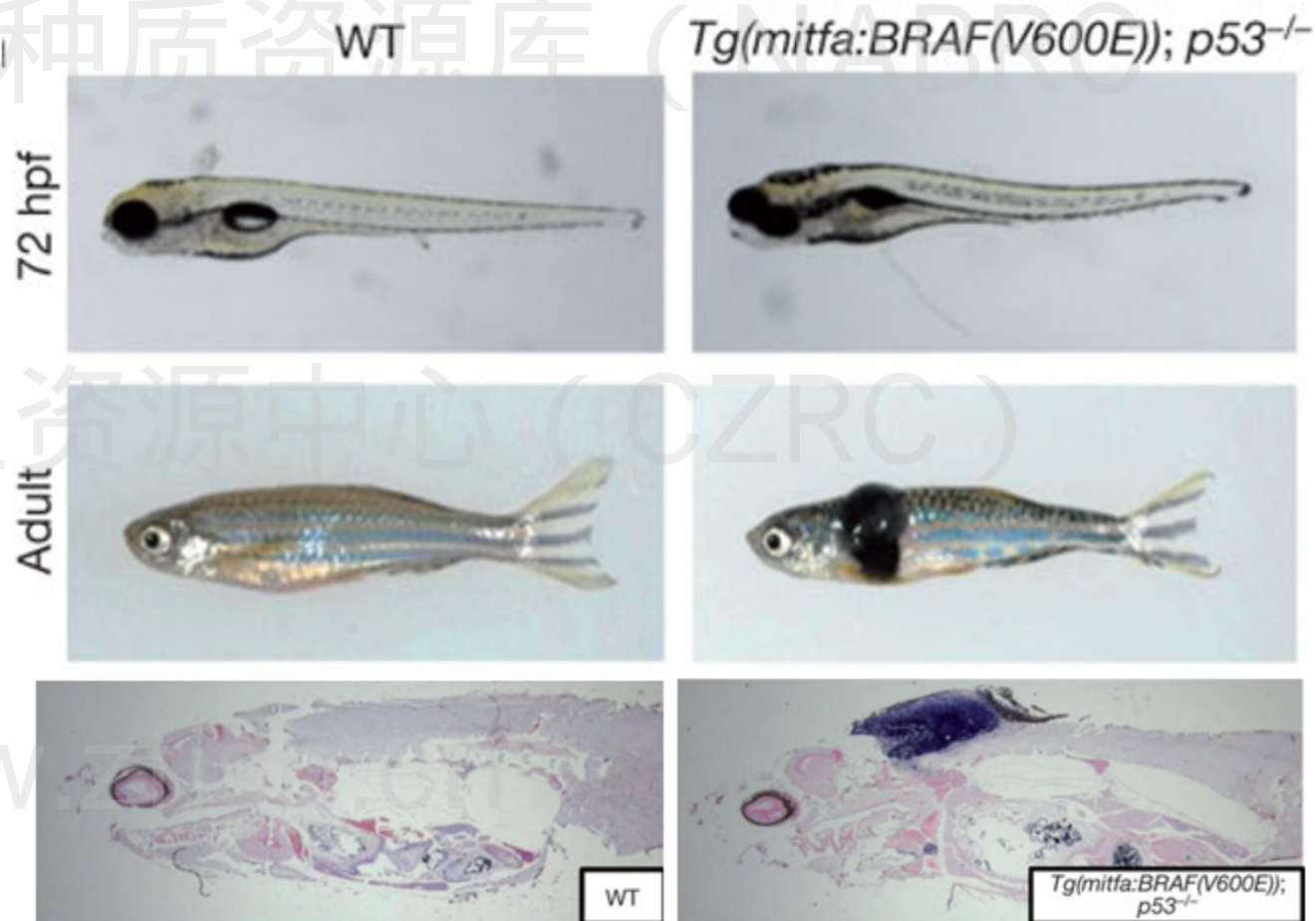
人源化模型: $BRAF^{V600E}$

- 黑色素瘤: 黑色素细胞恶变, 可发于皮肤、粘膜、内脏
- 已知oncogene, BRAF, 是人类黑色素瘤中最常见突变
- 最常见的致癌/抑癌基因, 如: *p53*, *apc*, *pten*, *BRAF*, 在斑马鱼中都有同源基因
- 在斑马鱼中引入人类 $Tg(mitfa:BRAF^{V600E})$ 突变



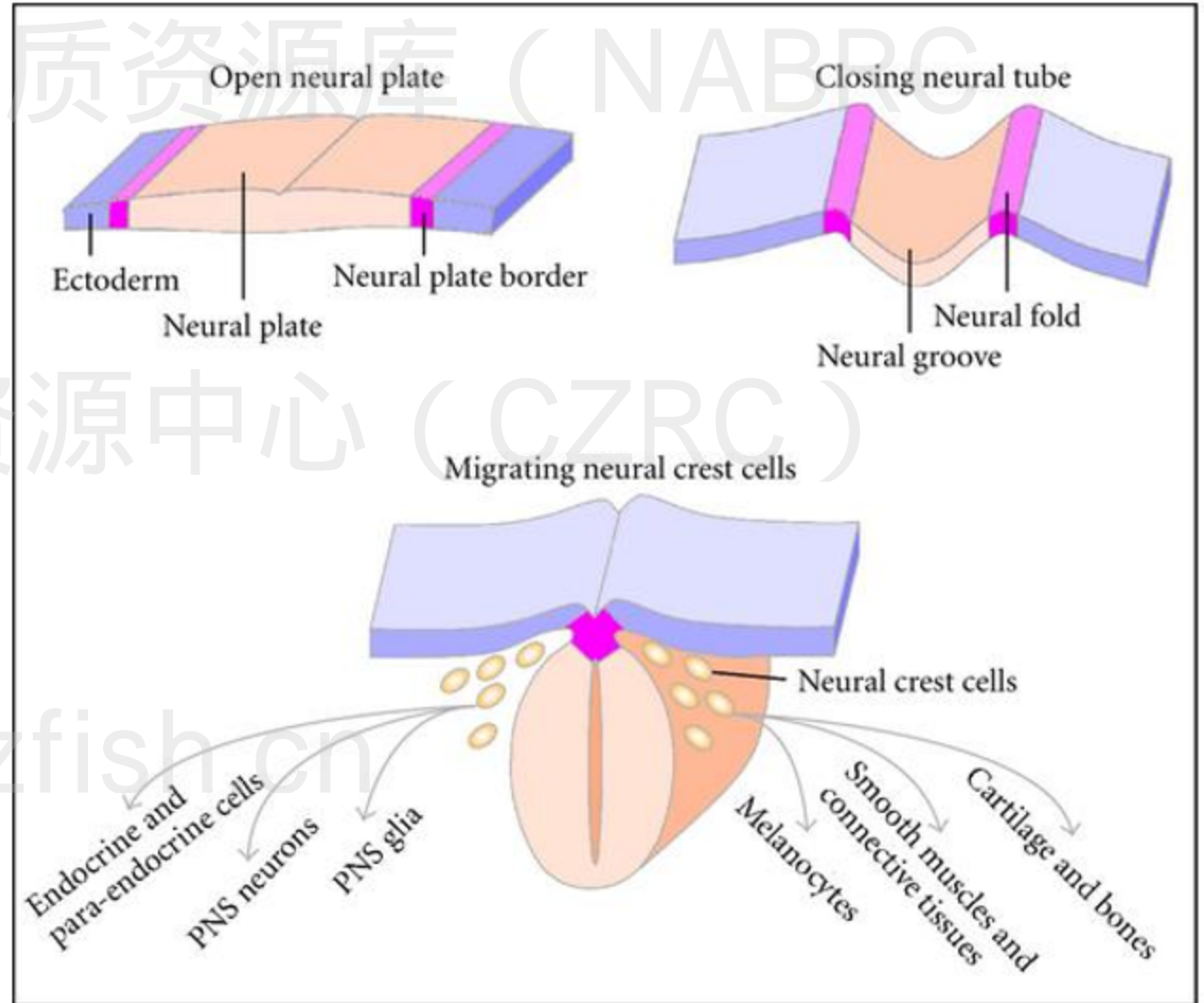
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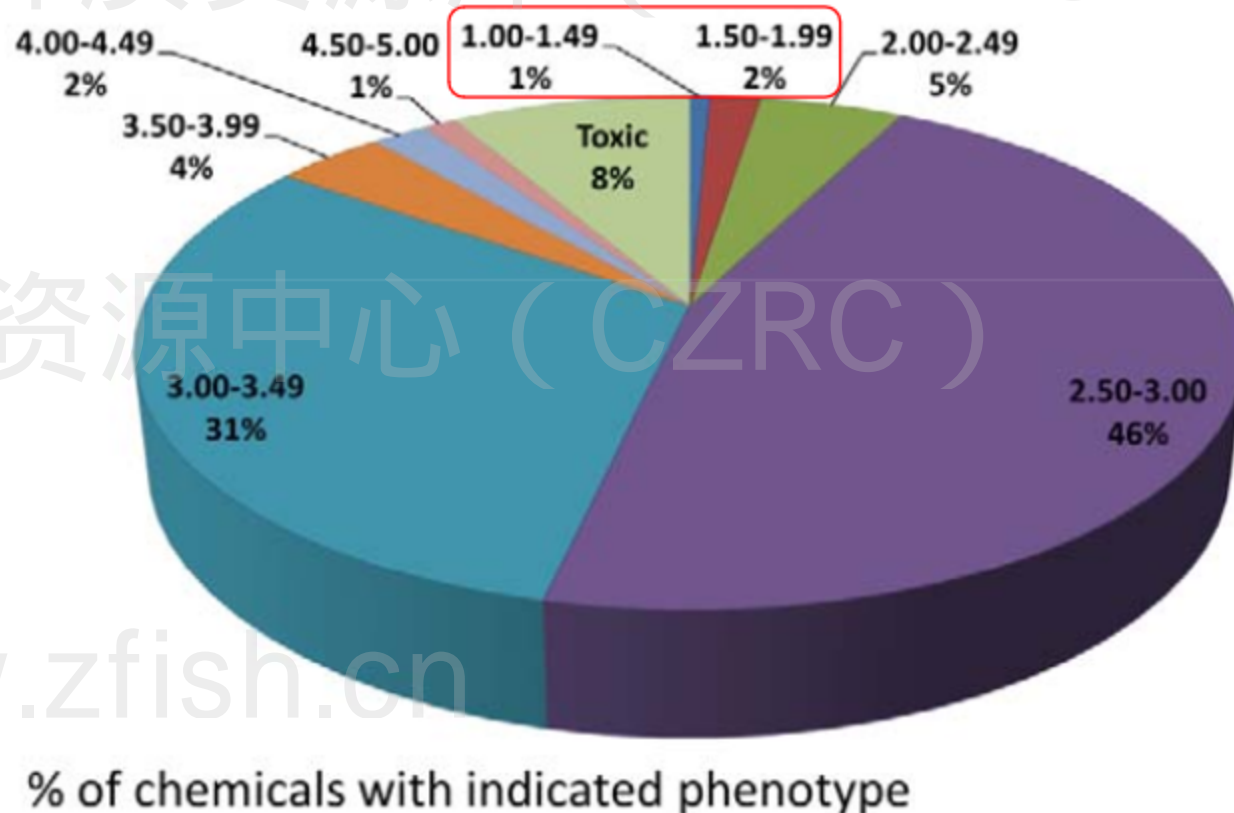
药物筛选策略

- 黑色素细胞, 最早是由embryonic neural crest发育而来
- 已知某些抑制neural crest发育的因素, 也可以抑制黑色素瘤的生长
- 检测neural crest cell的标记基因表达
- 筛选>2000个化合物
- 药物直接加入水中

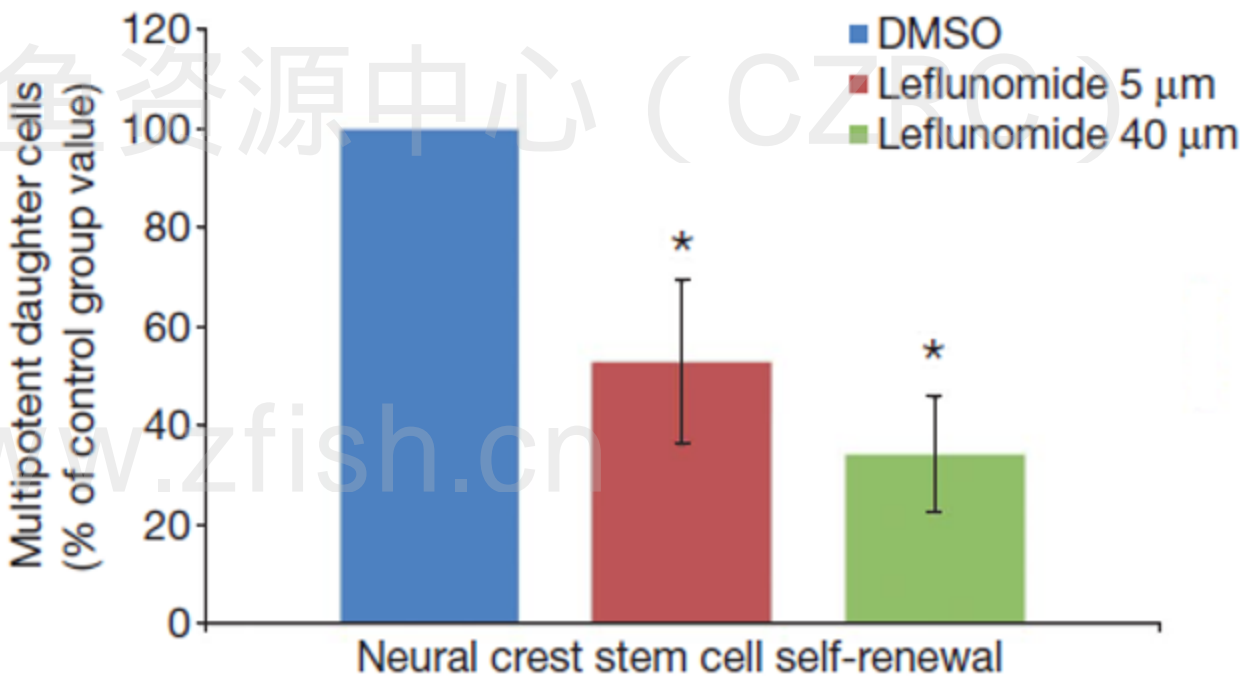
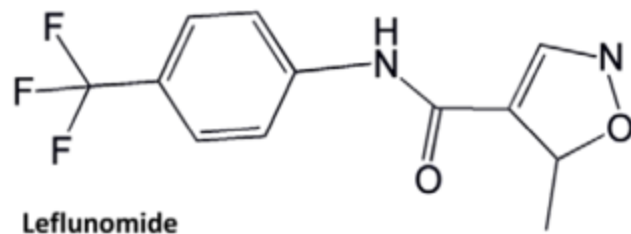
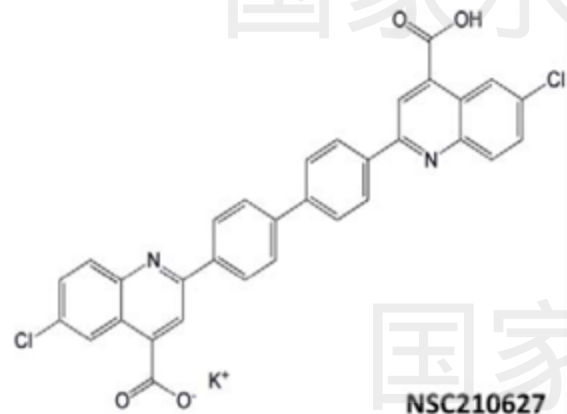


筛选结果

- 检测neural crest cell的标记基因表达量
- 药物处理后，表达量1-5分级定量
- 3是表达量没有变化，1是表达消失，5是表达增加
- 定量 <2 ，被认为有效药物，占整个化合物库的3%



筛选结果验证



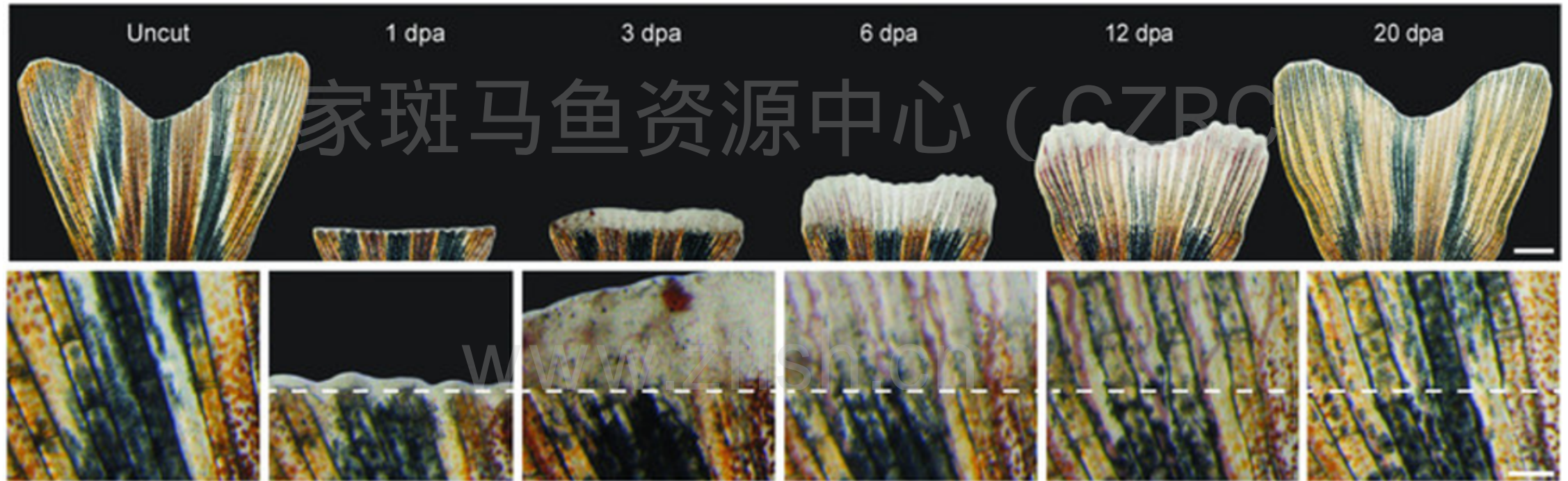
Leflunomide的进一步测试

- Microarray (for gene expression)
- Relative chemicals
- Upstream & downstream factors
- Function on cultured melanoma cells
- Dosage effect
- Test on cancer cell transplanted mice



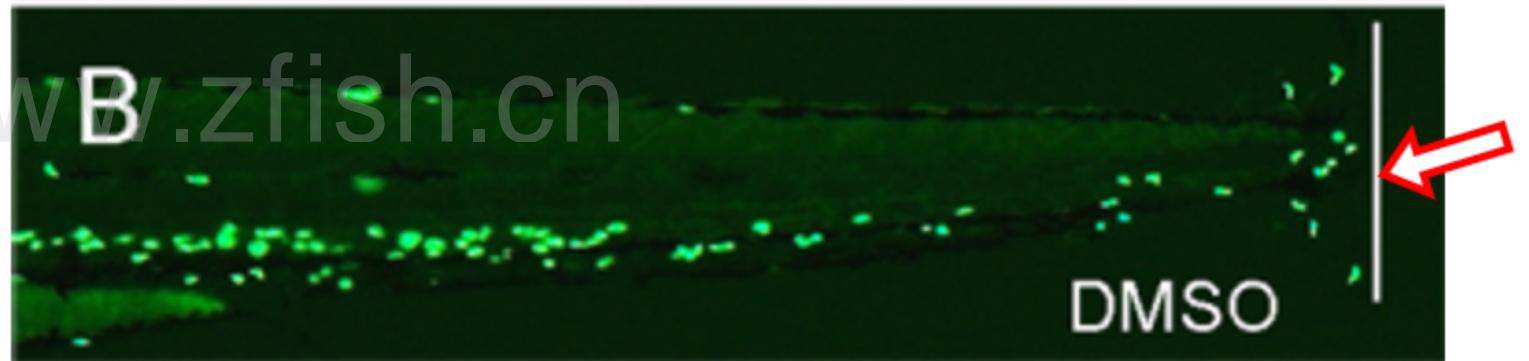
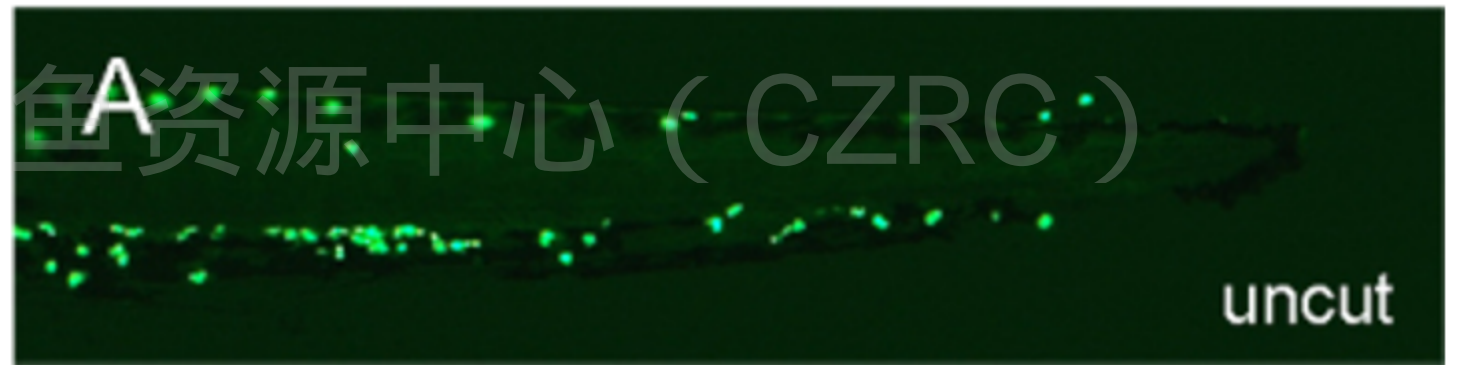
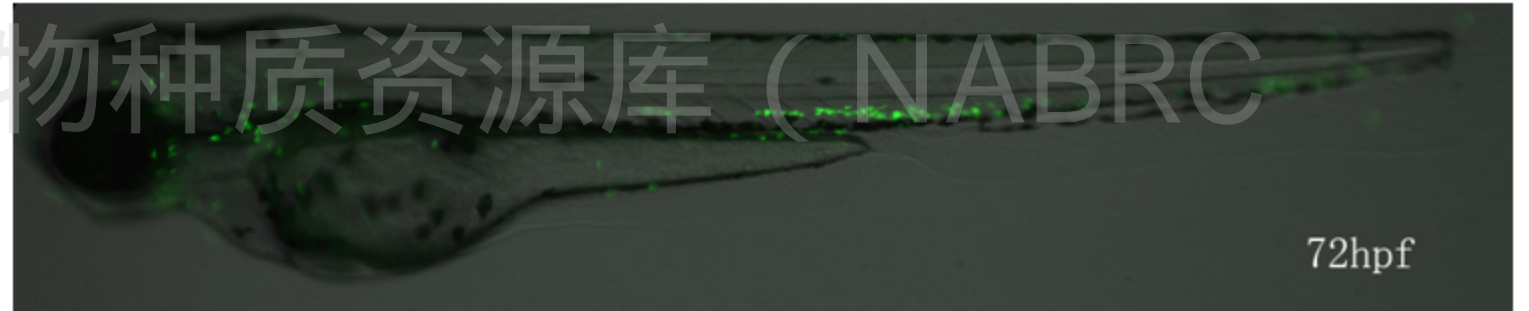
炎症与损伤修复药物筛选

- 斑马鱼是研究损伤修复、组织再生的理想模型 (NABRC)
- 斑马鱼可以快速修复尾鳍、视网膜、肝脏、纤毛细胞、心脏、神经系统

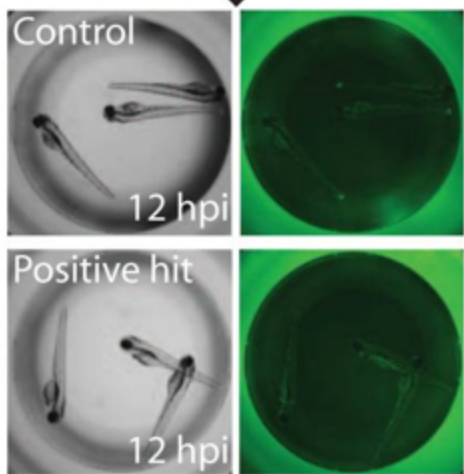
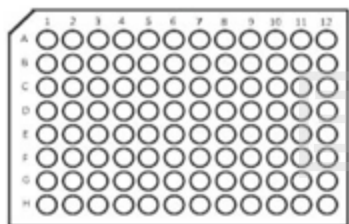
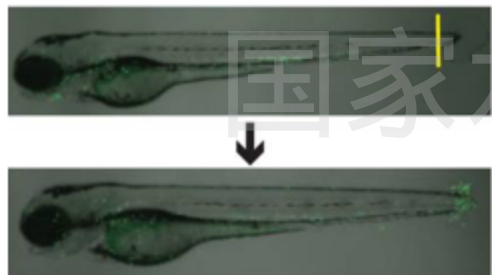


尾鳍损伤后中性粒细胞在伤口聚集

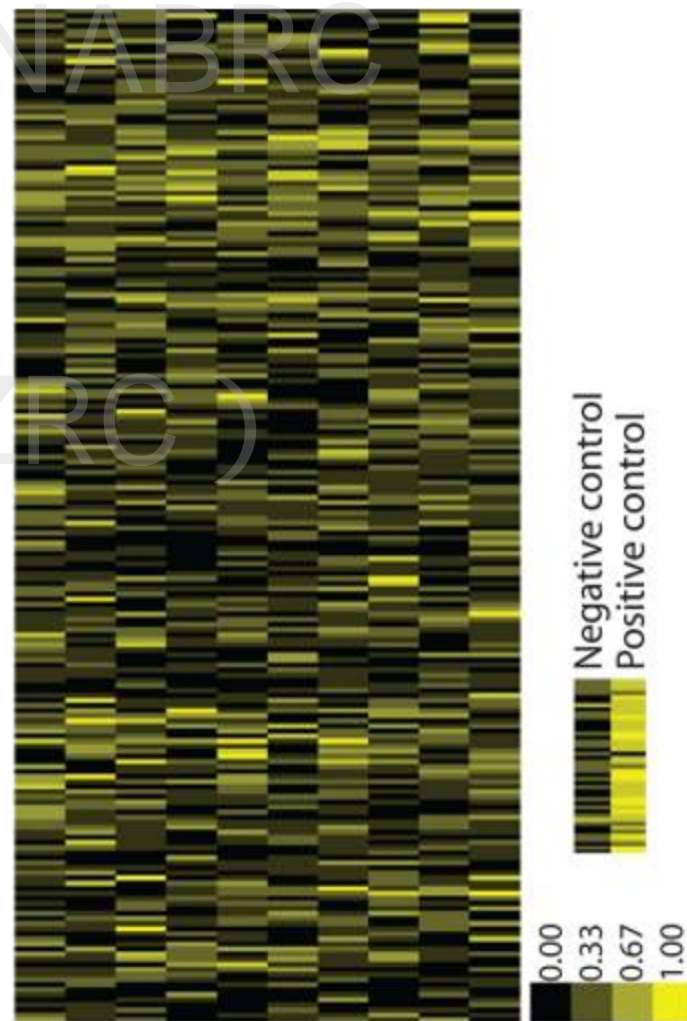
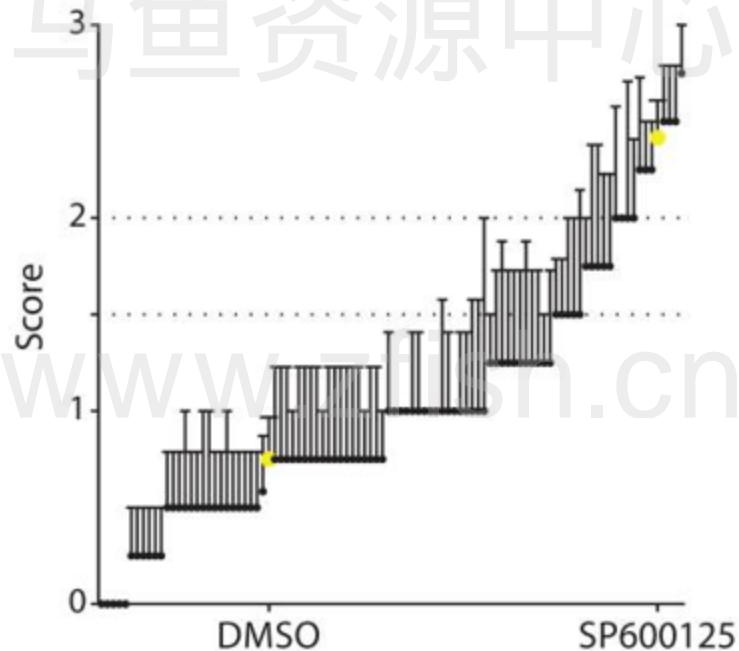
- Neutrophils are highly evolved at injury site for host defense
- Neutrophils must be removed from the site by either apoptosis or reverse migration
- Disable to do neutrophil clearance may rise persistent inflammation
- *Tg(mpx:GFP)*



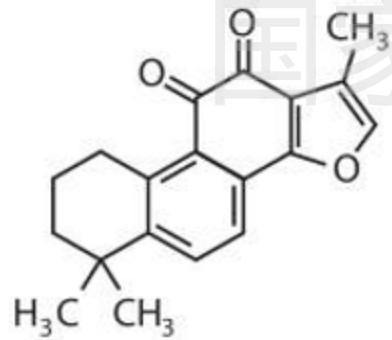
损伤修复促进药物筛选



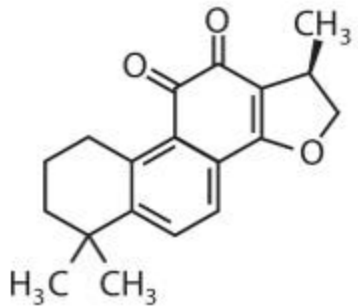
- 4hpa开始用药物处理, 处理8小时
- 2000 compounds
- 21 positive hits
- 2 shared close structural similarity



丹参酮促进损伤修复

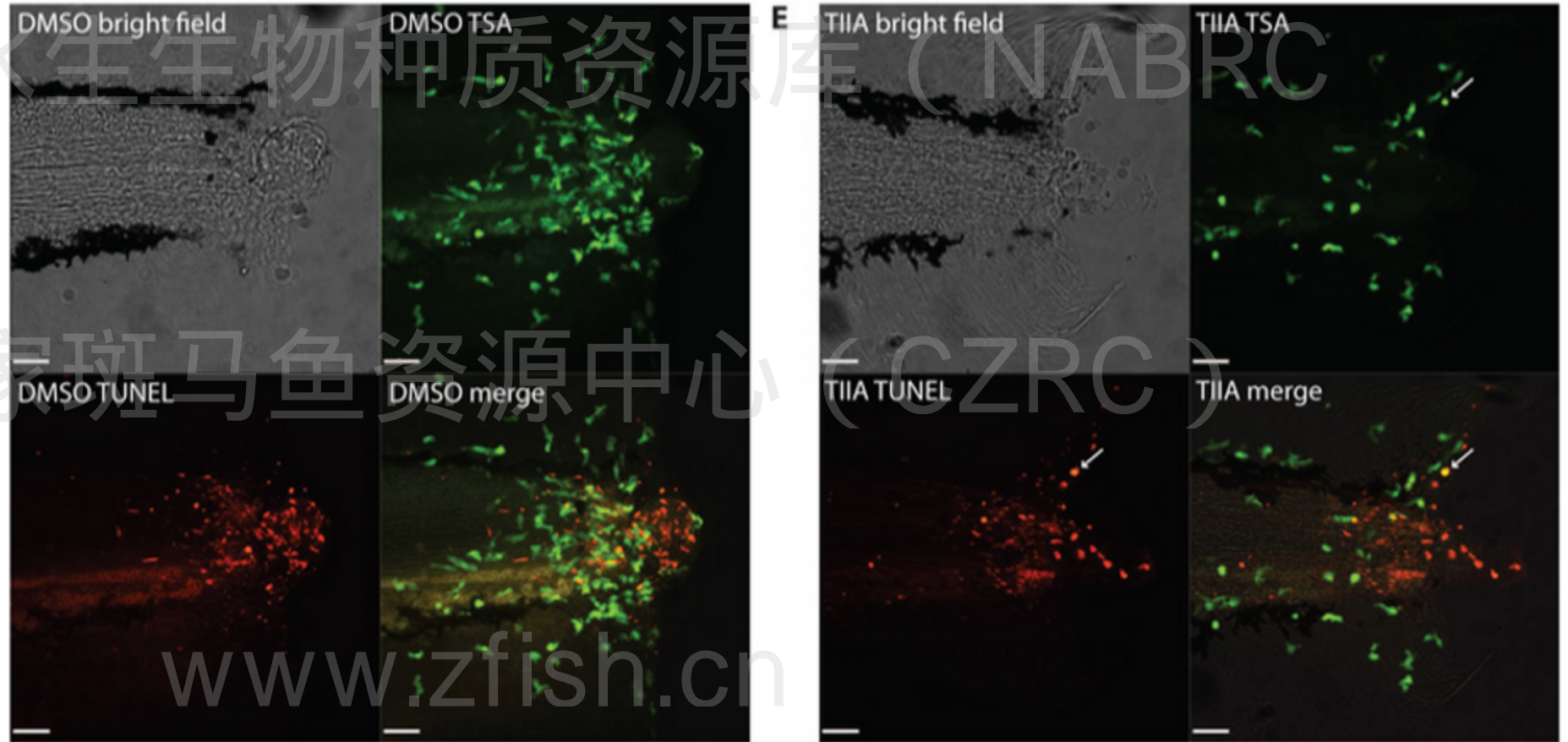


Tanshinone IIA



Cryptotanshinone

Robertson et al., 2014



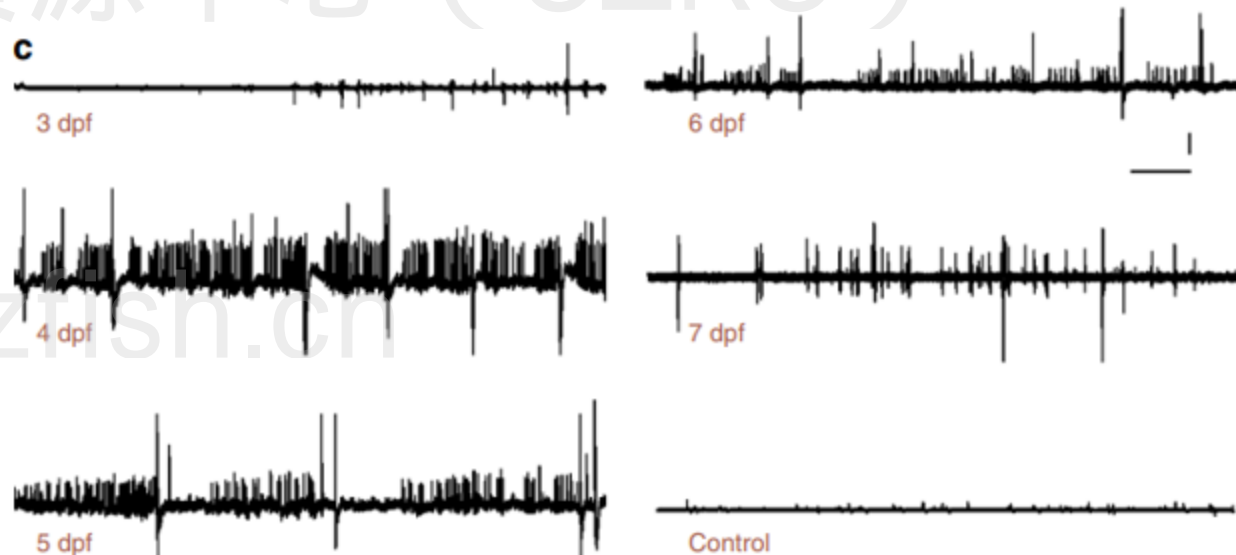
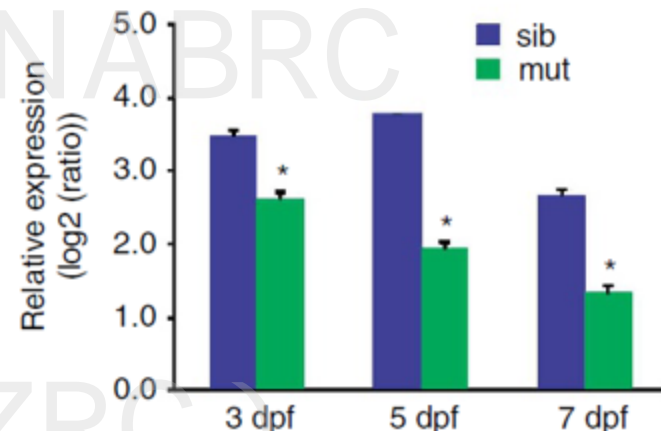
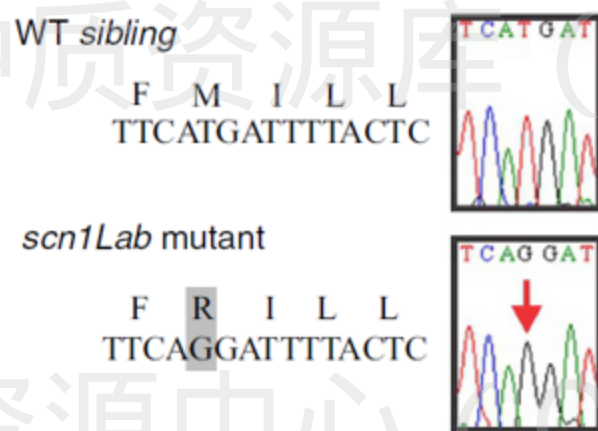
丹参酮IIA促进中性粒细胞的凋亡和反向转移

丹参酮IIA不影响中性粒细胞在损伤部位的聚集

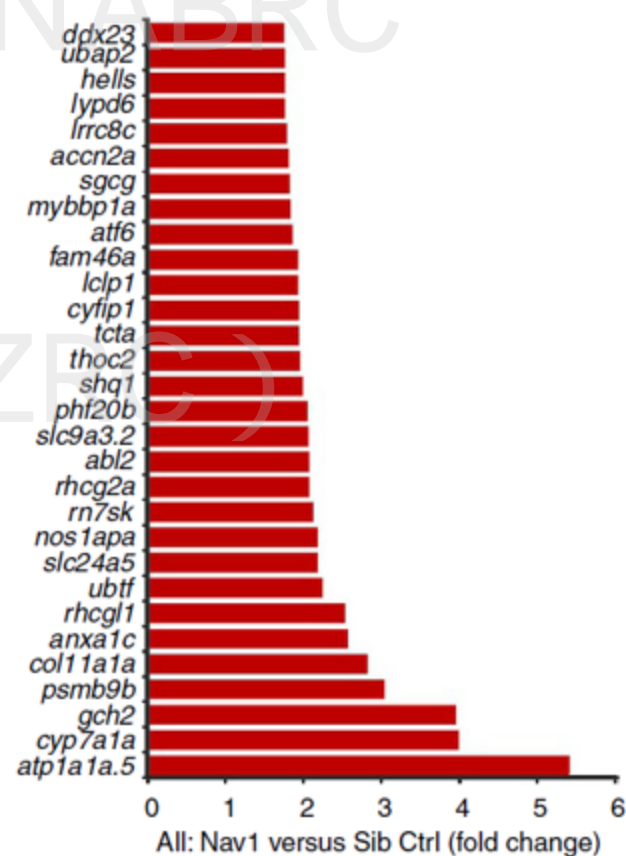
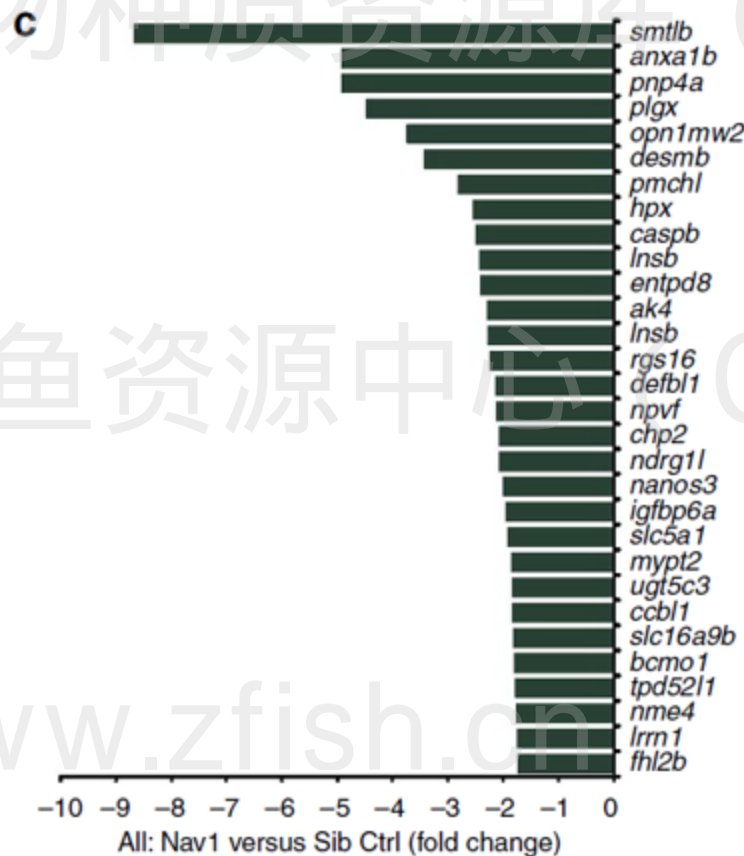
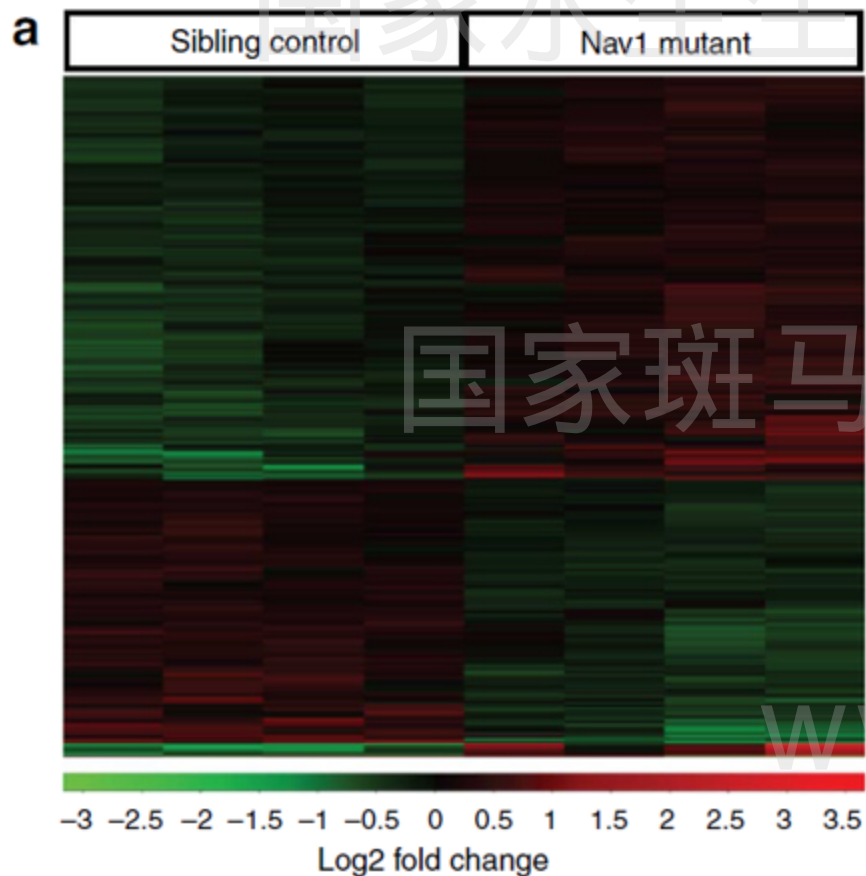
www.zfish.cn

行为学特性筛选: Dravet syndrome

- Dravet syndrome: autosomal dominant genetic disorder, prolonged seizure, delayed language/motor development, sleeping difficulties, cognitive deficit and ASD
- linked to >600 de novo mutations in SCN1A, nearly 85% of patients
- Zebrafish homolog: *scn1lab*

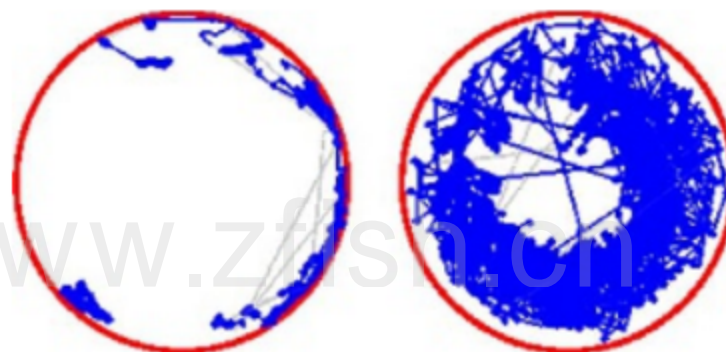
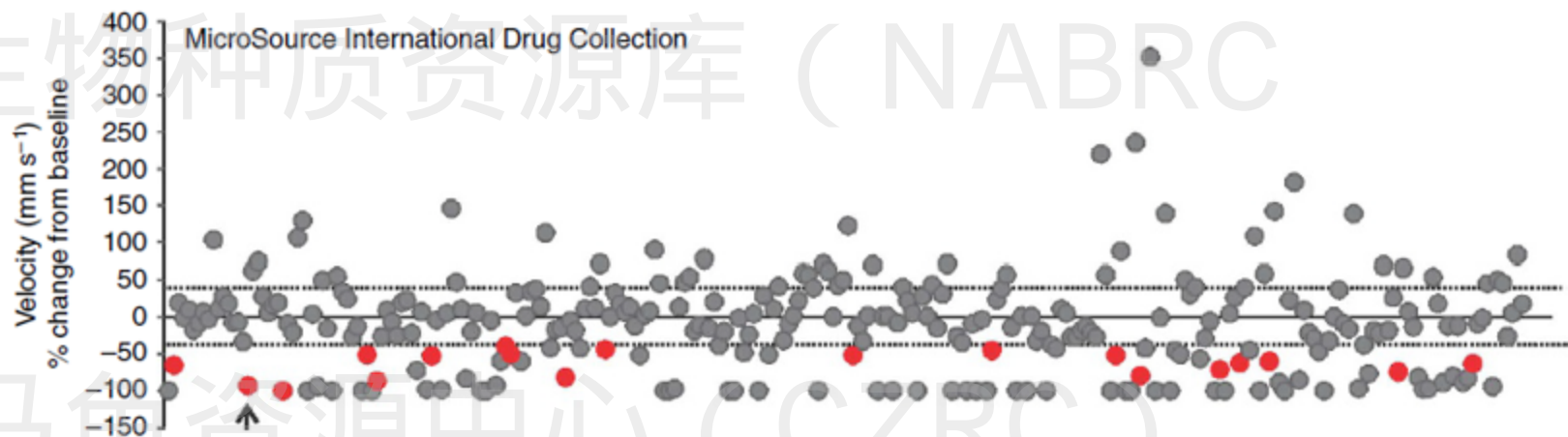


行为学特性筛选: Dravet syndrome



行为学特性筛选: Dravet syndrome

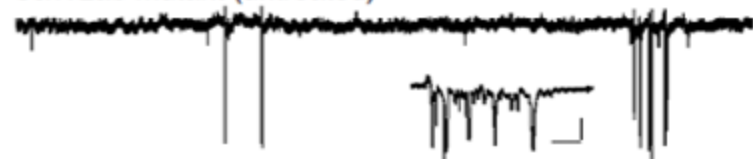
- swim activity
- 320 compounds
- 5dpf larvae
- 6-12 larvae/compound
- Tested at 667 μ M
- 18 significantly inhibit spontaneous seizures
- Retested at 667, 67, 6.7 μ M
- 4 left for 3rd round test
- only **clemizole** picked



Sib ctrl

Nav1.1 mut

scn1Lab mutant (untreated)



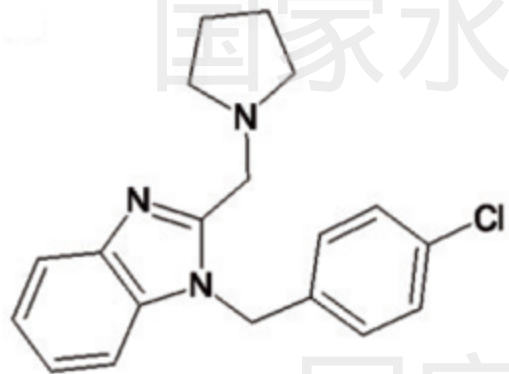
scn1Lab mutant (Clem)



scn1Lab mutant (Zox)

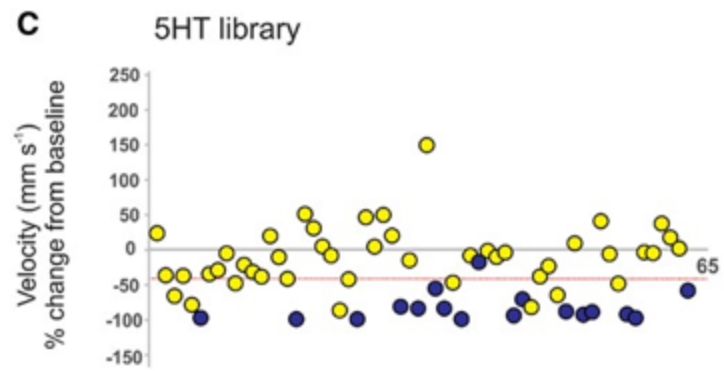
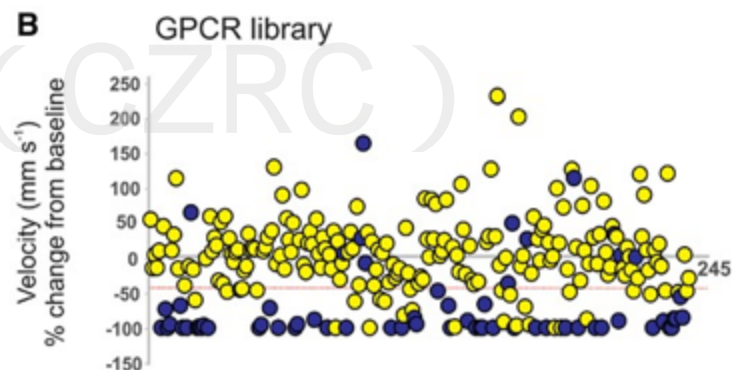
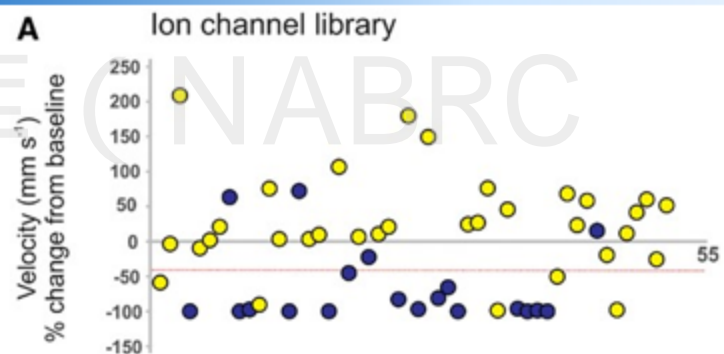
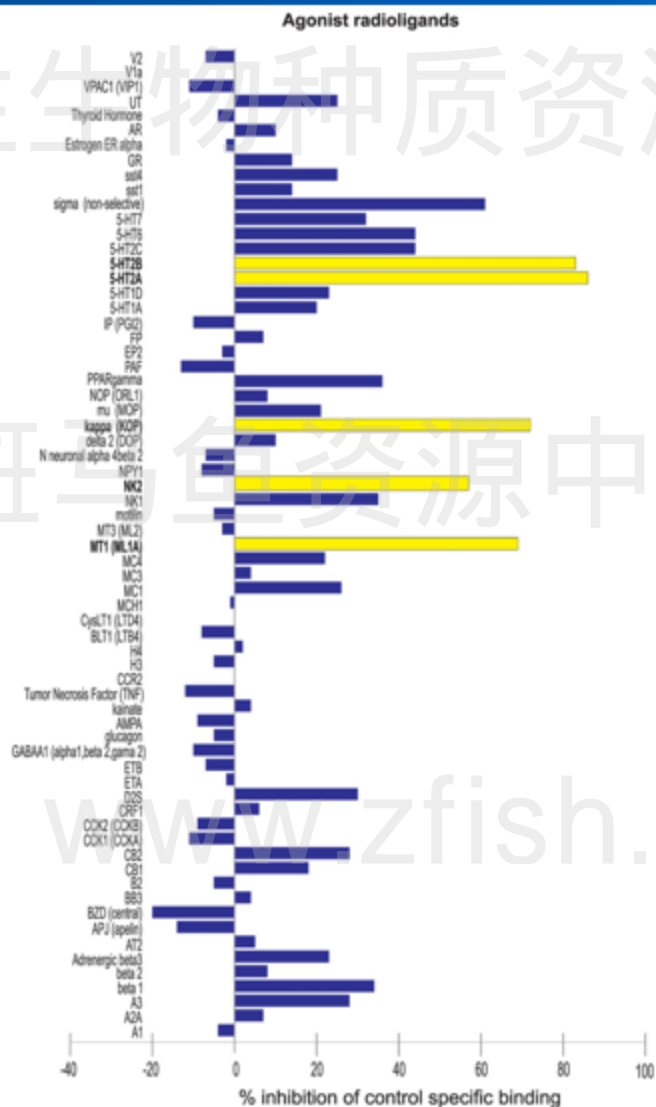


行为学特性筛选: Dravet syndrome

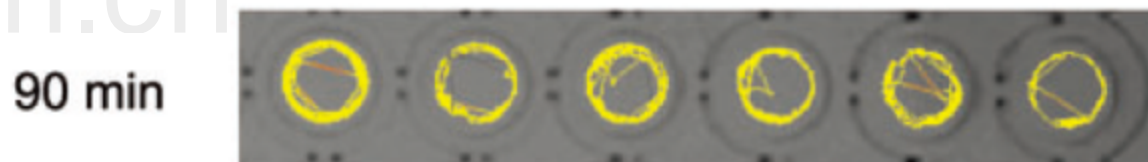
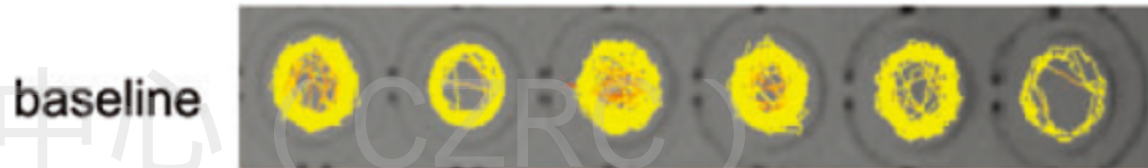
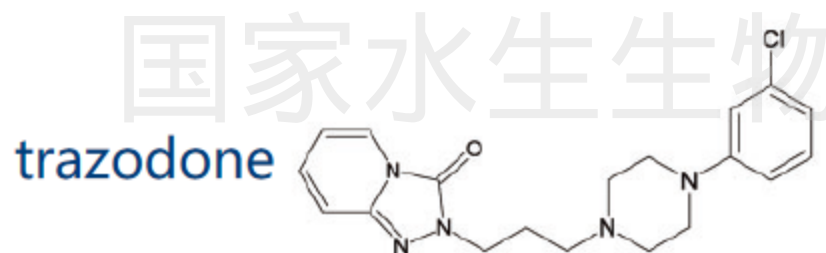


- compound libraries modulating ion channels
- 368 compounds
- trazodone and lorcaserin

Griffin et al., 2017



行为学特性筛选: Dravet syndrome

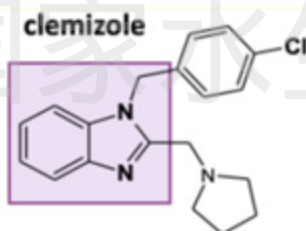


行为学特性筛选: Draw

- Belviiq® (lorcaserin)
- Children's Hospital Colorado (Aurora, CO)
- 5 children (7-18 year old)
- All exhibited a reduction in the total number of seizures
 - 3: 1-2 seizure-free days per week
 - 1: 3 seizure-free week
 - 1: 2 seizure-free week
- common side effect was decreased appetite

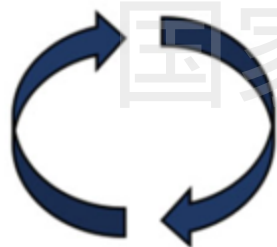
Patient	1	2	3	4	5
Age (years)	10	18	10	7	14
Weight (kg)	28	46	23	24	35
Dose (mg/kg/day)	0.25	0.27	0.19	0.32	0.31
Prior treatments	CLZ CZP KD LMT LVT PRM OXC RUF TPX VPA	CBZ CBD CLZ CLB CZP FBM LMT LVT PRM PHB TPM VPA CC KD VNS	ESM FBM LMT LVT MSM VPA VMP ZNM KD	CZP ESM LVT LZP STP TPM ZNM KD	CBZ FBM GBP LCM LMT LVT OXC PHB PRED RUF STP VNS VPM ZNM KD CLB TPX VPA
Concurrent AEDs	CLB STP VPA	CZP STP ZNM	KD TPM VPA	BRO CBD CLB VPA	CLB TPX VPA
Prior seizure frequency	FS: 50/day GTC dusters: 1/ month	MS: numerous daily FS + GTC: 10/ month (requires rescue medications)	MS: daily GTC seizures: 100/ month (clusters 7-10)	AS: 12/h FS: 3-5 / week NCS: 1/ month 1-2 seizure free days/ week	MS: constant throughout the day GTC seizures: 1-2/ week MS: initially reduced in the morning then increases to constant throughout the late afternoon GTC: 1-2/ week
Seizure frequency after treatment: first 3 months	Seizure free initial 3 weeks, cluster of seizures then again seizure free for 2 weeks	Seizure free for 2 weeks	GTC seizures: 46/ month (GTC clusters of 1-3 seizures)	AS or FS: 3/ month GTC: 1-2 /day	MS: initially reduced in the morning then increases to constant throughout the late afternoon GTC: 1-2/ week
Seizure frequency after treatment: following first 3 months	Gradual increase in seizures with return to baseline frequency	MS: occasional FS + GTC: 1/month (no rescue medications)	MS: daily Seizures gradually decreased to 16/ month with some seizure free nights then seizures increased to baseline	NCS: 1/ month Gradual increase in seizures, seizure free days stopped 9 months after treatment	Unchanged, Belviiq tapered off with no change in seizure frequency
Duration treatment (months)	12 months, still taking	MS: clusters 1-2/ week FS + GTC: 1-2/ month and (no rescue medications required)	12 months, still taking	14 months Restarted due to increased seizures treated for 2 months, stopped to participate in other drug study	13 months No increase in seizure when medication stopped
Side effects	none	none	14 months Restarted due to increased seizures treated for 2 months, stopped to participate in other drug study	13 months No increase in seizure when medication stopped	9 months Decreased appetite
			Vomiting and decreased appetite	Decreased appetite	Decreased appetite

行为学特性筛选: Dravet syndrome



Clemizole analog library

Receptor identification



Antiseizure screening

Electrophysiology

Fenfluramine

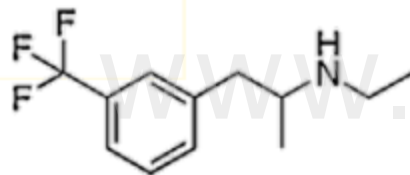
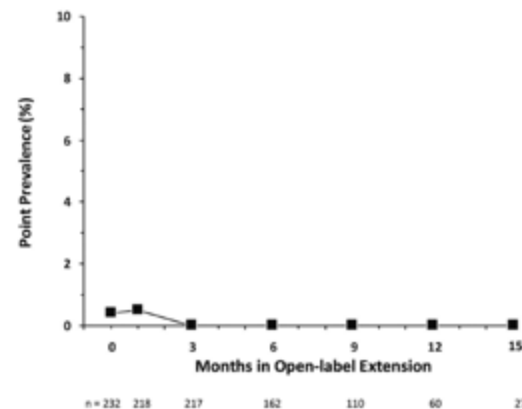
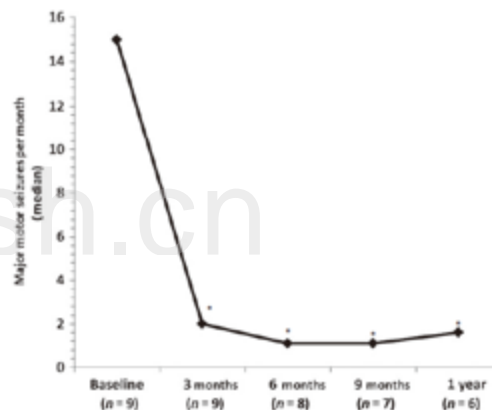


Table 2 Individual patient therapeutic information

Patient	Initial FFA dose		Most recent FFA dose		Treatment duration (years)	Major motor seizures/month ^a		
	mg/day	mg/kg/day	mg/day	mg/kg/day		3-month baseline period	FFA treatment ^b	Percent reduction ^c
1	10	0.29	20	0.44	5.06	15.0 ^d	4.5 ^d	-70%
2	5	0.50	12.5	0.69	4.70	2.5 ^d	0.4 ^d	-84%
3	5	0.29	10	0.62	0.78	0.4 ^d	0 ^d	-100%
4	10	0.25	15	0.36	1.50	39.7 ^d	7.3 ^d	-82%
5	5	0.10	15	0.25	1.64	2.0 ^d	0.7 ^d	-68%
6	10	0.21	10	0.19	1.57	2.3 ^d	1.5 ^d	-37%
7	10	0.17	15	0.27	1.02	18.3	13.2	-28%
8	5	0.21	5	0.17	0.63	20.4	0.8	-96%
9	10	0.16	10	0.16	0.30	23.8	6.0	-75%
Mean	7.8	0.24	12.5	0.35	1.9	13.8	3.8	-71%
Median	10	0.23	12.5	0.29	1.5	15.0	1.5	-75%



• 斑马鱼药物筛选概况

- 药物筛选实验概述
- 斑马鱼的生物学特性与化合物筛选
- 斑马鱼药物筛选的基本流程与系统

• 斑马鱼药物筛选实例解析

- 糖尿病药物筛选实例
(转基因致病工具模型)
- 癌症药物筛选实例
(人源化模型)
- 中药相关筛选实例
(外部干预+转基因标记模型)
- 进入临床的筛选实例
(内源突变+行为学检测模型)

国家水生生物种质资源库 (NABRC)

本讲内容完毕

欢迎交流

国家斑马鱼资源中心 (CZRC)



中国斑马鱼信息中心