



国家水生生物种质资源库 (NABRRC)

斑马鱼中基于PGC移植的突变体构建技术

国家斑马鱼资源中心 (CZRC)

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20231130 武汉

主要内容

国家水生生物种质资源库（NABRC）

- 原始生殖细胞简介
- 原始生殖细胞移植及其应用
- 原始生殖细胞移植操作流程
- 诱导型原始生殖细胞简介
- 诱导型原始生殖细胞移植及其应用

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国家斑马鱼资源中心（CZRC）

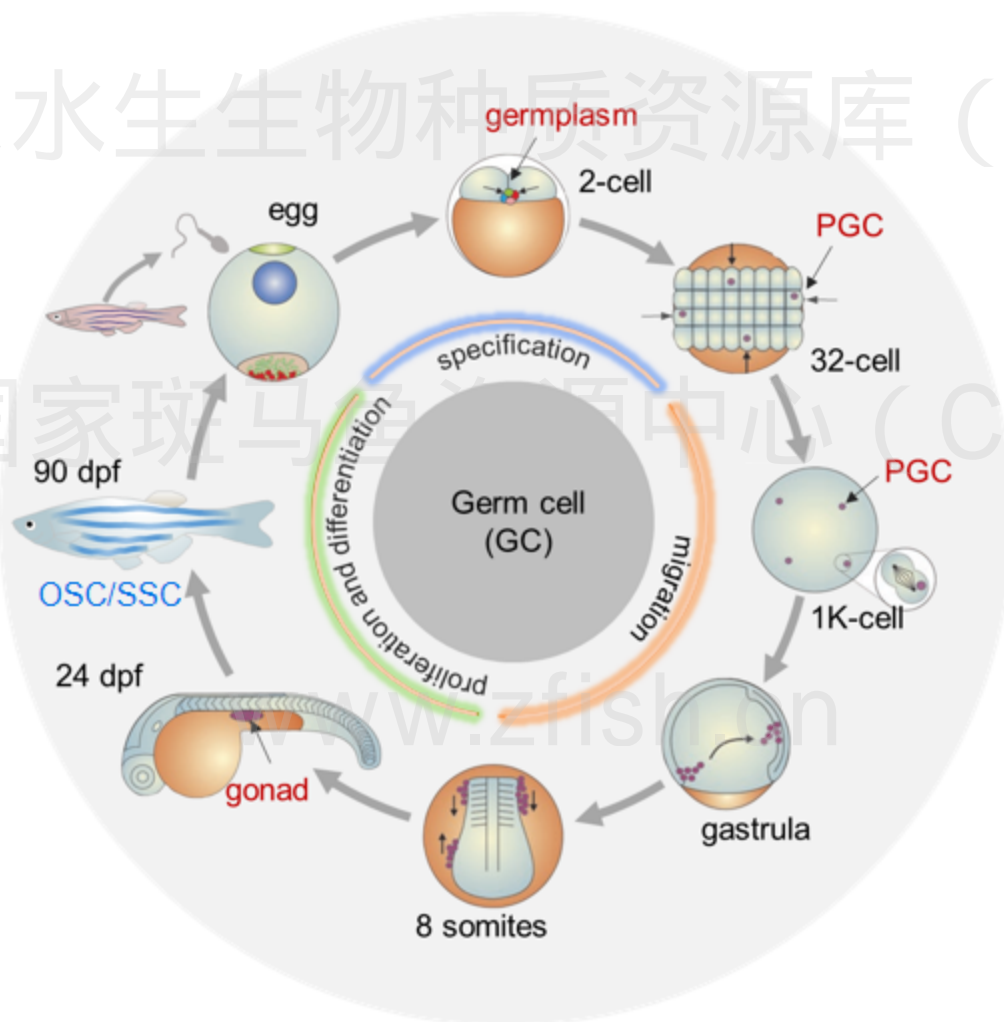
- 原始生殖细胞移植操作流程

- 诱导型原始生殖细胞简介

- 诱导型原始生殖细胞移植及其应用

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生殖细胞是遗传物质传递的载体



Germplasm: 生殖质

PGC: Primordial Germ Cell (原始生殖细胞)

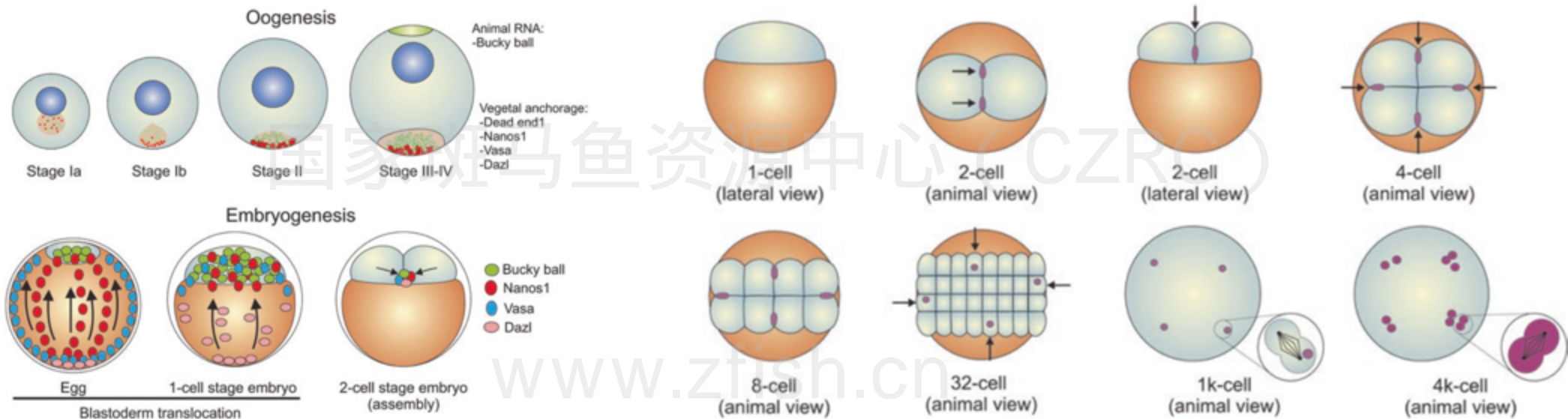
GSPC: germline stem and progenitor cell (生殖干祖细胞)

OSC: oogonia stem cells (卵原干细胞)

SSC: spermatogonia stem cell (精原干细胞)

PGC的产生

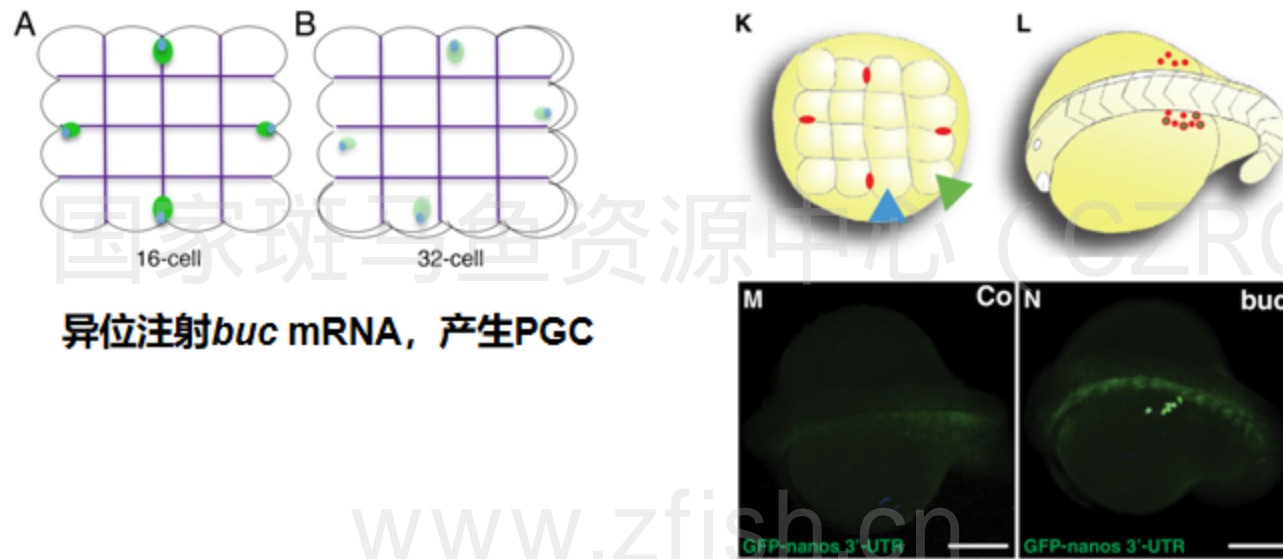
PGC: Primordial Germ Cell (原始生殖细胞)



pPGC: PGC precursor

异位诱导产生PGC

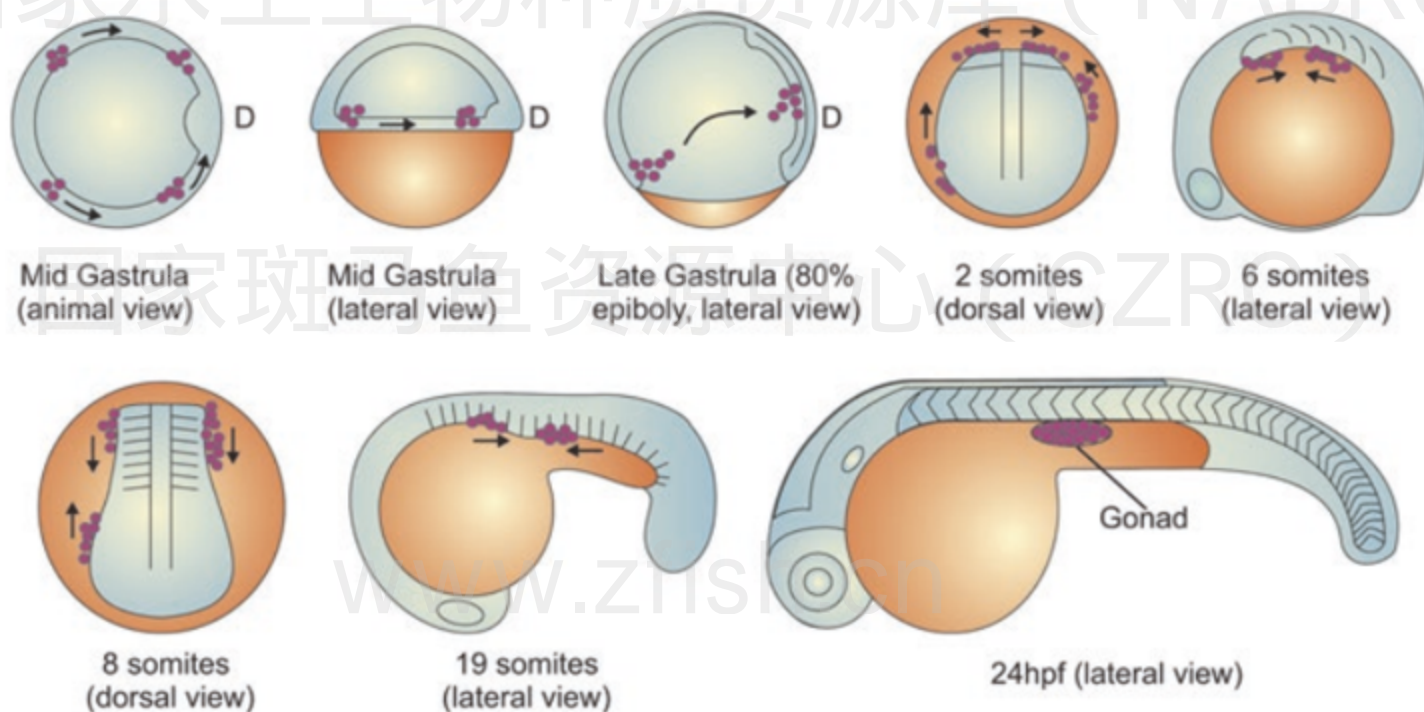
国家水生生物种质资源库 (NABRC)



斑马鱼PGC是由生殖质因子决定的

PGC的迁移

国家水生生物种质资源库 (NABRC)



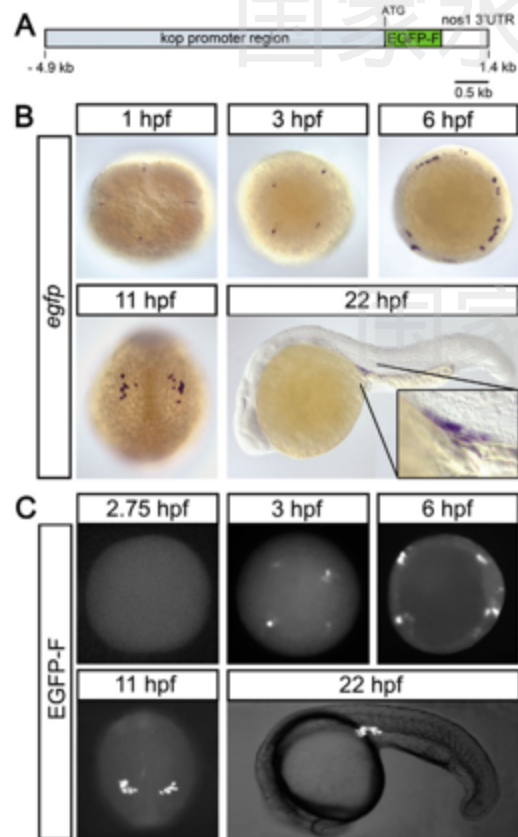
斑马鱼生殖细胞发育相关基因

Germ name	Protein	Function
Nanos1	RNA binding zinc-finger proteins	Acts as a translational repressor that regulates translation of specific mRNAs by forming a complex with Pumilio that associates with the 3'-UTR of mRNA targets
Dazl	RNA binding proteins	DAZL activates the translation efficiency of the target mRNAs through direct binding to cis-elements in their 3' UTRs. Interacts with poly(A)-binding proteins, which are critical for the initiation of translation. Protect mRNAs against miRNA repression in germ cells
Dead end 1 (dnd1)	RNA binding proteins	Protect mRNAs against miRNA repression in germ cells
Vasa	A DEAD-box RNA helicase	Crucial role during germ-line specification, migration, survival, and maintenance. Involved in female meiosis, differentiation, and maintenance of germ-line stem cells
Cxcr4b	Seven transmembrane G-protein coupled receptor	Cxcr4b receptor for the chemokine Sdf1a. Critical role in PGC migration, required specifically for their chemotaxis
Sdf-1a (stromal cell-derived factor-1)	Chemokine	Sdf1a guides migrating PGCs towards their intermediate and final targets
Staufen1/2	RNA binding proteins	Stau1 and Stau2 proteins are involved in survival and maintenance of germ cells. PGC migration is aberrant, and the mis-migrating PGCs do not survive in Stau-compromised embryos

Germ name	Protein	Function
Ziwi/Zili	RNA binding proteins	Ziwi proteins associate with a class of germ-cell-specific small RNA molecules named Piwi-Interacting RNAs (piRNAs). Have a role in germ cell maintenance and transposon silencing
Buc (Buckyball)	Novel protein (unrelated to any known family protein)	Germ plasm defect in bucky ball mutants precedes the loss of polarity, indicating that Buc primarily controls Balbiani body formation
Tdrd7 (Tudor domain containing protein-7)	RNA binding proteins	Tdrd7 has a critical role for structural integrity of granules in PGCs
Mgn (Magellan microtubule actin crosslinking factor 1 [macf1] gene)	Spectraplakins family of cytoskeletal linker proteins	In mgn mutants, the oocyte nucleus is mislocalized; and the Balbiani body, localized mRNAs, and organelles are absent from the periphery of the oocyte, consistent with a function for macf1 in nuclear anchoring and cortical localization
Pum 1/2 (Pumilio2)	Puf family of RNA-binding proteins	Translational repression of target mRNA. Pumilio proteins regulate the translation of specific proteins required for germ cell development and morphogenesis
Puf-A	Puf family of RNA-binding proteins	<i>puf-A</i> plays an important role not only in eye development, but also in PGC migration and the specification of germ cell lineage
Brul (Bruno-like)	Elav-type RNA-binding protein that belongs to the Bruno-like family	Brul genes may have a conserved function in axis specification (Dm analogy) and germ cells maintenance

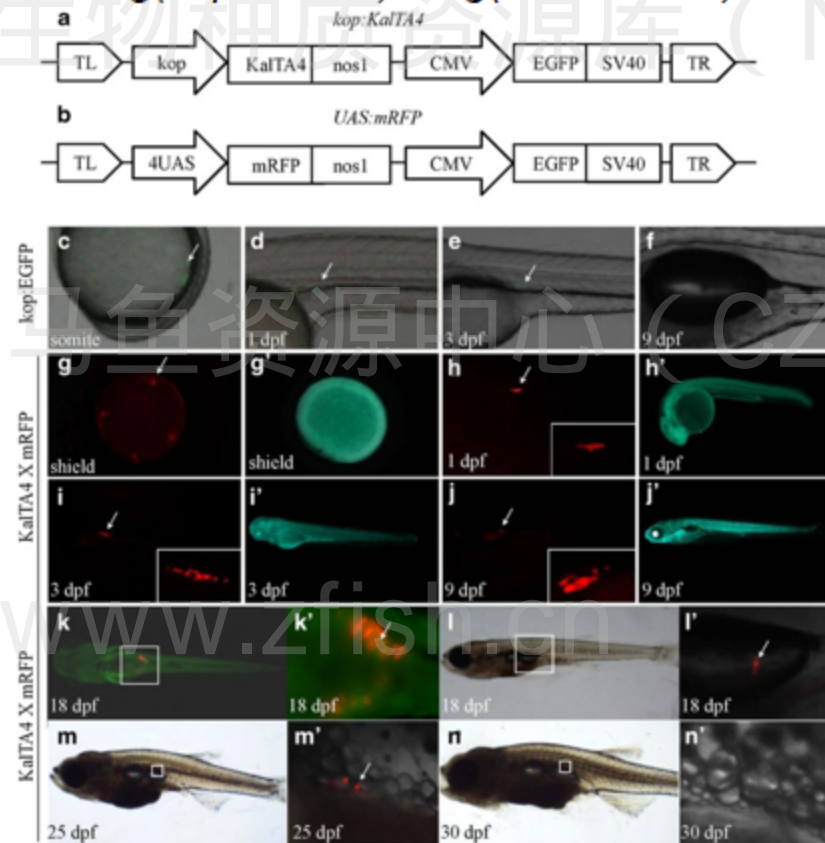
斑马鱼生殖细胞的标记

Tg(kop:EGFP-UTRnanos3)

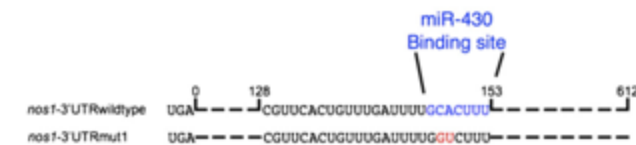
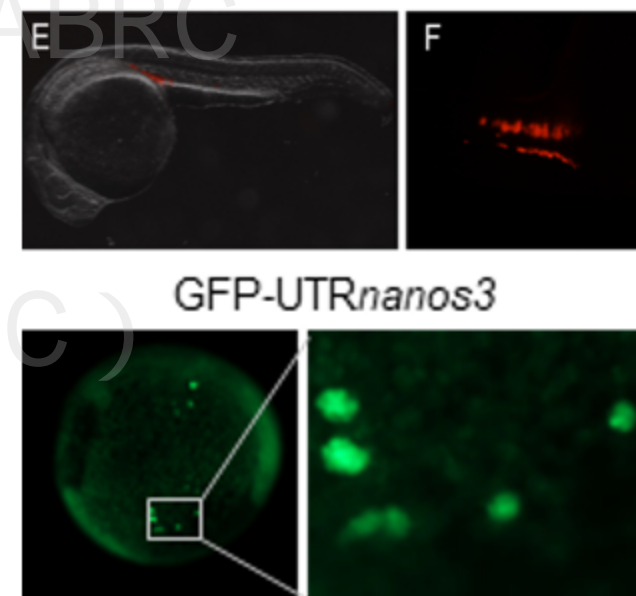


标记迁移的PGC

Tg(kop:KalTA4) x Tg(UAS:mRFP)



Tg(nanos3:mCherry-UTRnanos3)

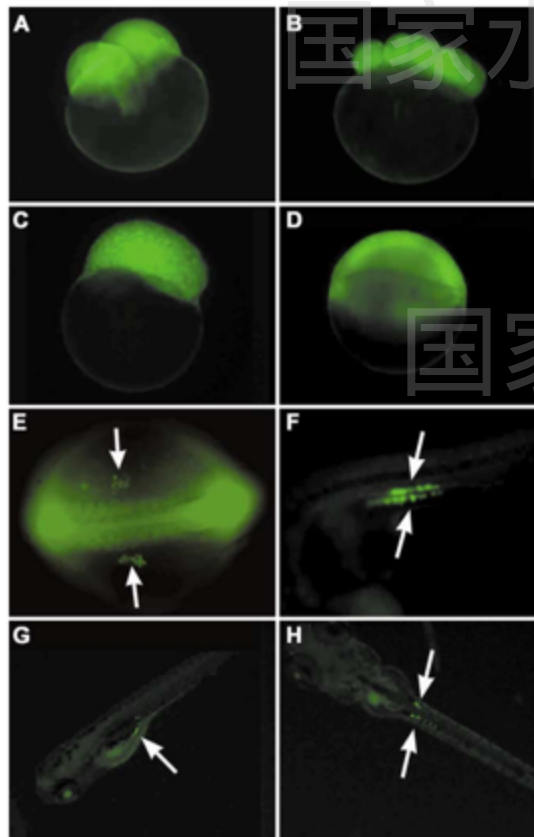


标记早期生殖细胞

Blaser et al., 2005
Xiong et al., 2013
Zhou et al., 2018
Wang et al., 2023

斑马鱼生殖细胞的标记

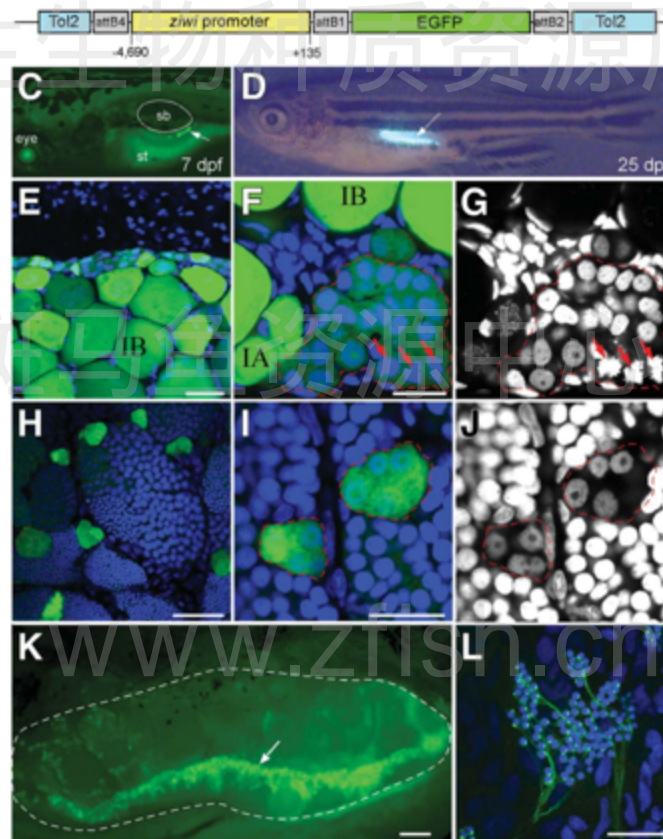
Tg(ddx4:EGFP)



优点：标记精原/精母细胞

缺点：不能标记1dpf前PGC

Tg(ziwil1:EGFP)



优点：标记精原/精母细胞，卵原/卵母细胞

缺点：不能标记7dpf前的生殖细胞

Tg(zpc:EGFP)

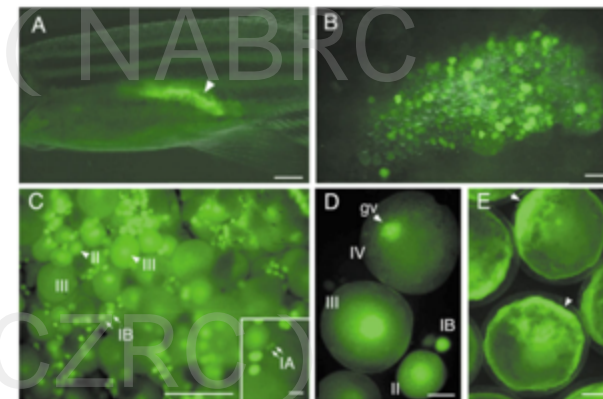
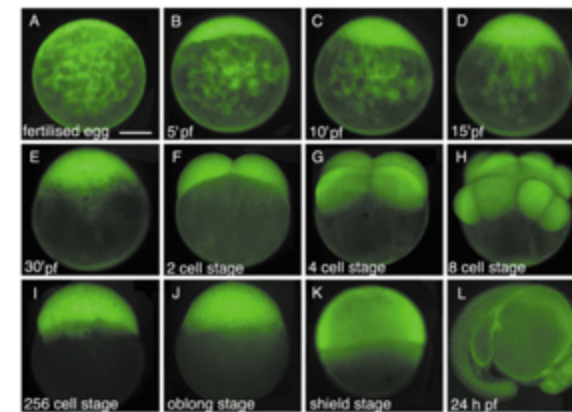


Fig. 2.

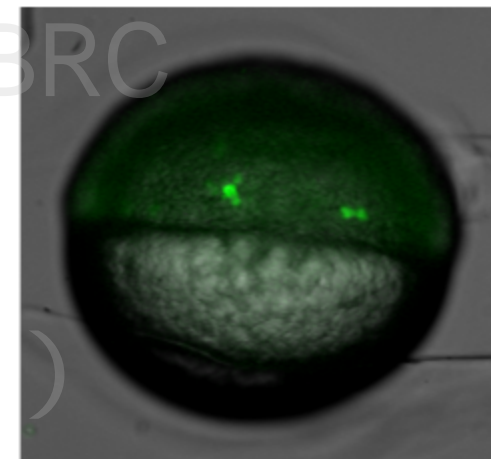
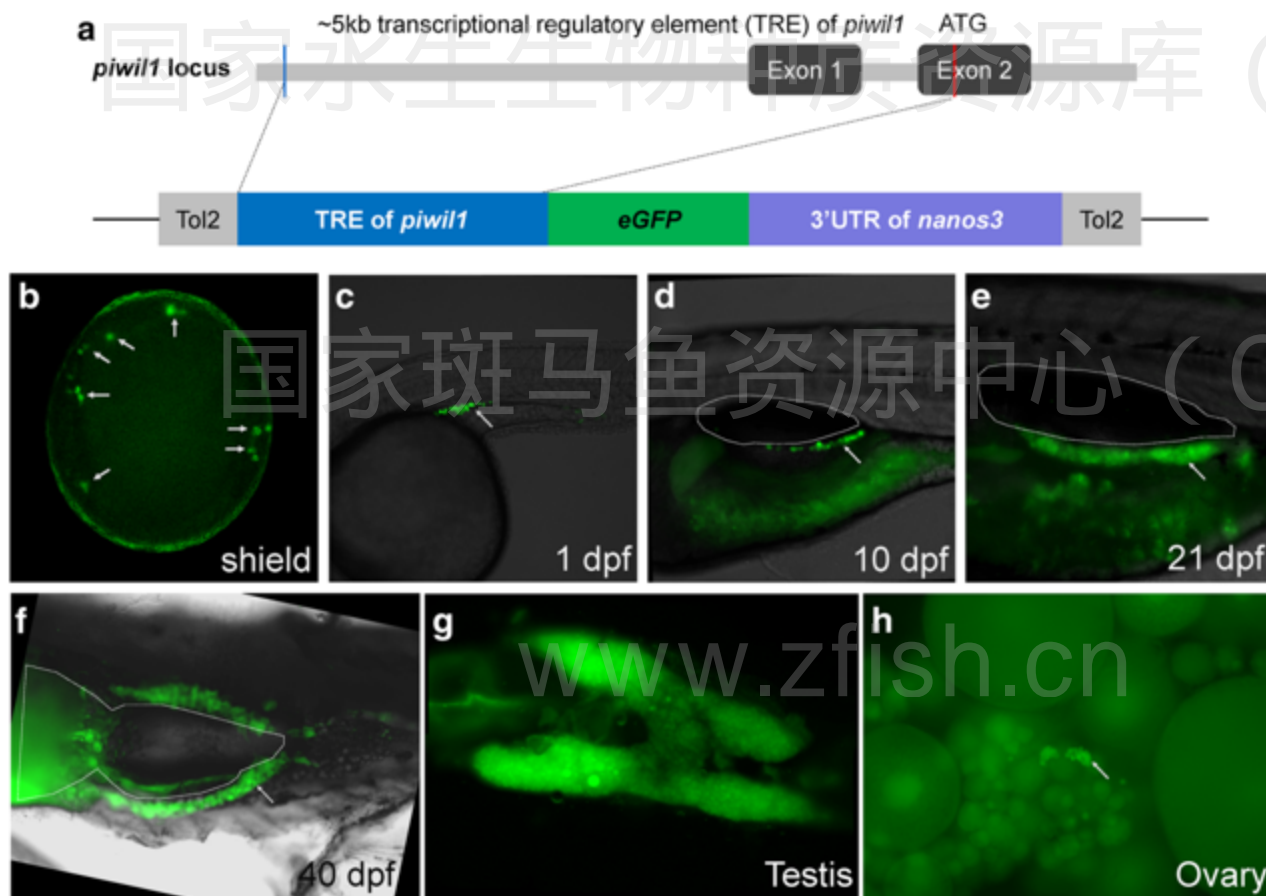


优点：标记所有期卵母细胞

缺点：不能标记早期生殖细胞

Krovel et al., 2002
David et al., 2005
Onichtchouk et al., 2003

全时相标记斑马鱼生殖细胞

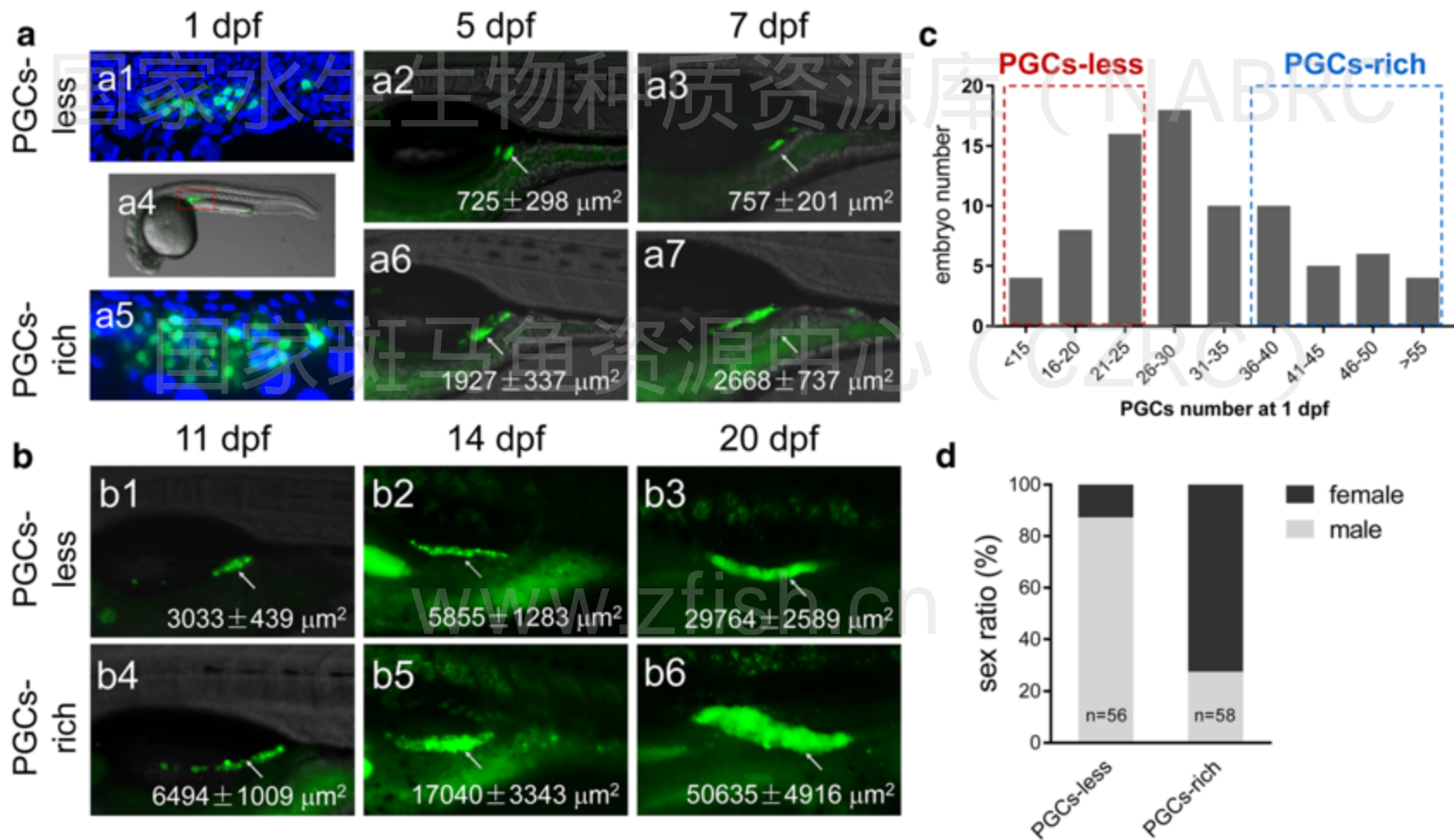


Tg(piwil1:egfp-UTRnanos3)

Nature of the transgene

The *ihb327Tg* allele was generated by random integration of *Tg(piwil1:EGFP-UTRnanos3)* construct.

斑马鱼PGC数目与性别分化密切相关



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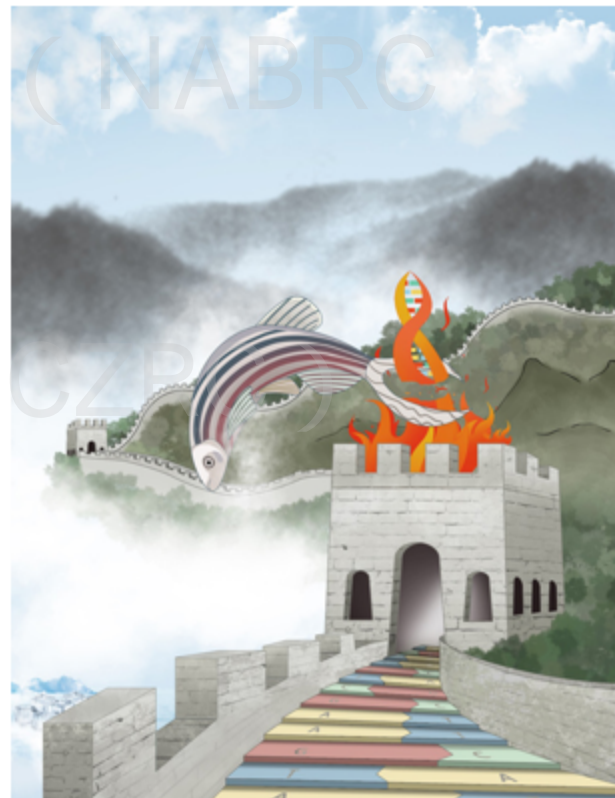
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基于CRISPR/Cas9构建斑马鱼突变体库

Systematic genome editing of the genes on zebrafish Chromosome 1 by CRISPR/Cas9

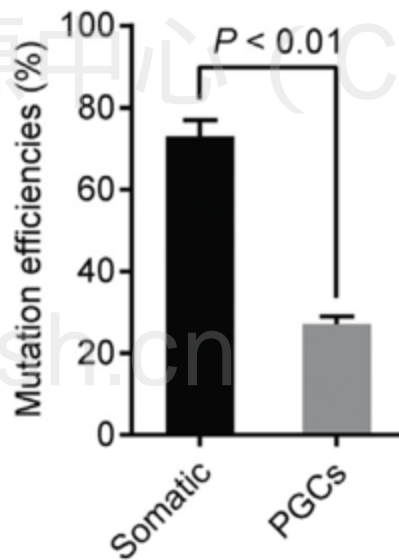
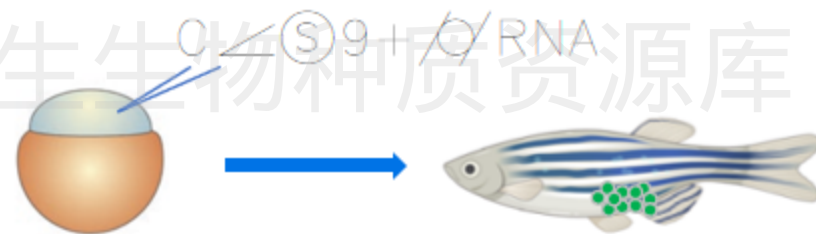
Yonghua Sun,¹ Bo Zhang,² Lingfei Luo,³ De-Li Shi,⁴ Han Wang,⁵ Zongbin Cui,¹ Honghui Huang,³ Ying Cao,⁶ Xiaodong Shu,⁷ Wenqing Zhang,⁸ Jianfeng Zhou,⁹ Yun Li,⁹ Jiulin Du,¹⁰ Qingshun Zhao,¹¹ Jun Chen,¹² Hanbing Zhong,¹³ Tao P. Zhong,¹⁴ Li Li,³ Jing-Wei Xiong,¹⁵ Jinrong Peng,¹² Wuhan Xiao,¹ Jian Zhang,¹⁶ Jihua Yao,¹⁷ Zhan Yin,¹ Xianming Mo,¹⁸ Gang Peng,¹⁹ Jun Zhu,²⁰ Yan Chen,²¹ Yong Zhou,²² Dong Liu,¹³ Weijun Pan,²² Yiyue Zhang,⁸ Hua Ruan,³ Feng Liu,²³ Zuoyan Zhu,¹ Anming Meng,²⁴ and The ZAKOC Consortium²⁵

“斑马鱼1号染色体全基因敲除计划”
“从基因型到表型” 的斑马鱼反向遗传学研究



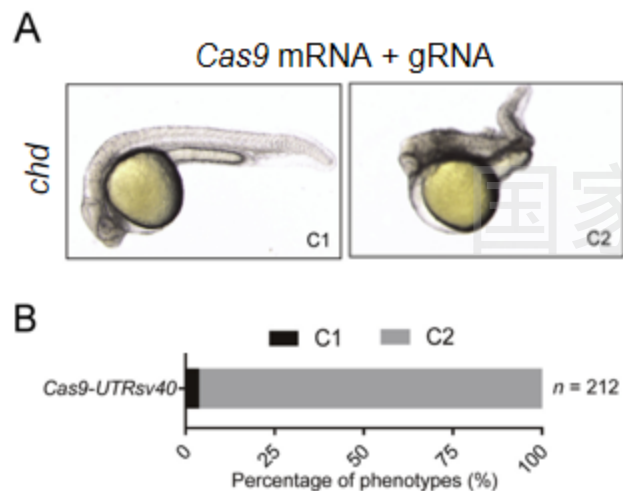
Sun et al., 2019

体细胞的基因突变效率高于生殖细胞突变效率



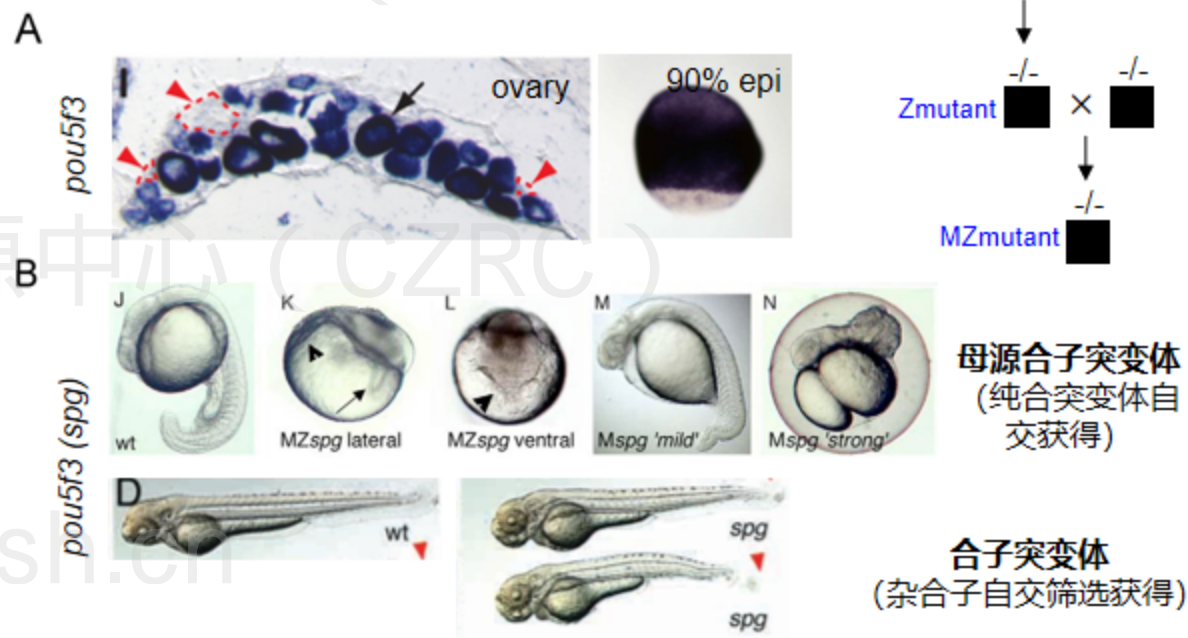
突变体构建中面临的难题

1. 致死基因的合子突变体 (*chd*, *bmp2b*, *bmp7a* ...)



- 胚胎高突变率伴随着高致死率
- 突变配子传递效率低

2. 致死基因的母源合子突变体 (*pou5f3*, *nanog* ...)



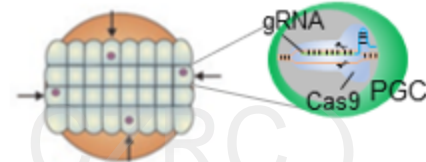
- 难以获得母源合子突变体

PGC是遗传操作的理想靶细胞

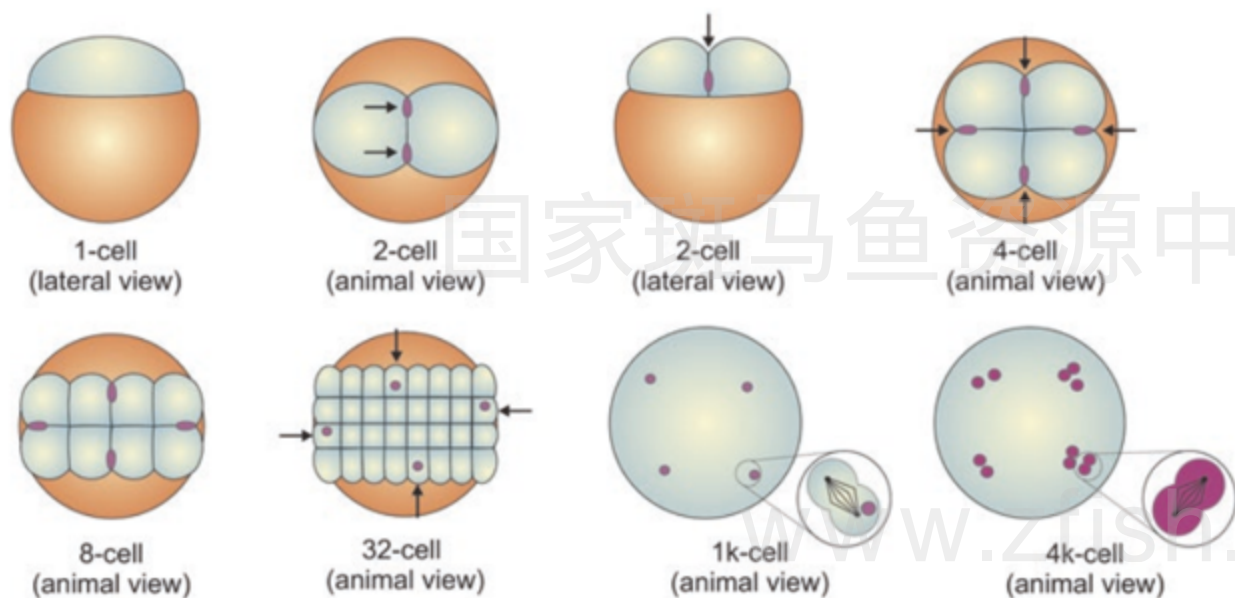
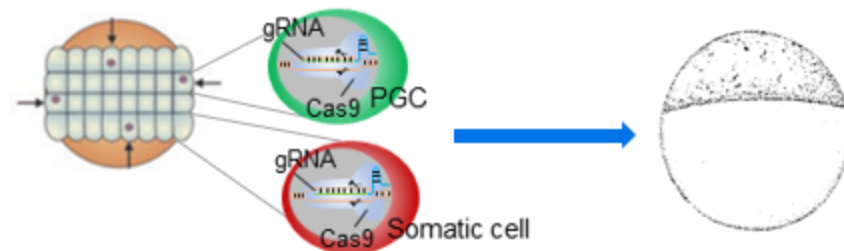
国家水生生物种质资源库 (NABRC)

构建生殖细胞突变策略

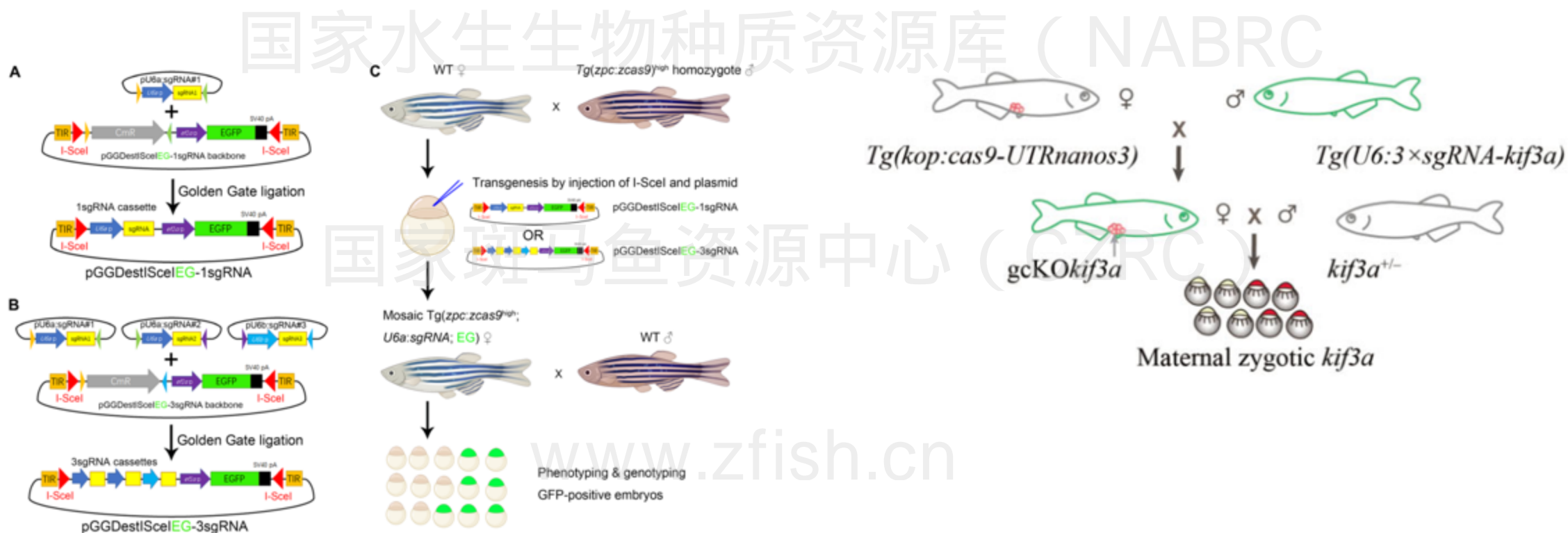
(1) 生殖细胞特异表达cas9/gRNA



(2) PGC移植借腹生殖



基于生殖细胞表达Cas9的转基因品系构建突变体



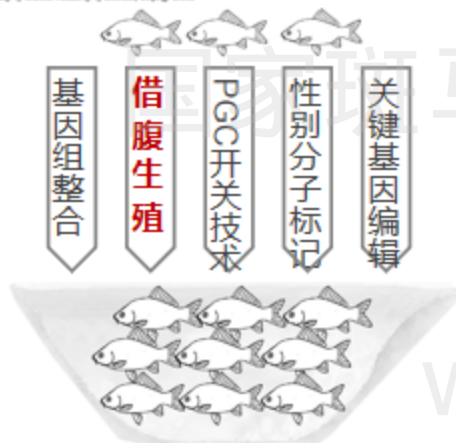
借腹生殖



Rethinking fish biology and biotechnologies in the challenge era for burgeoning genome resources and strengthening food security

Jian-Fang Gui^{a,b,*}, Li Zhou^{a,b}, Xi-Yin Li^{a,b}

^a State Key Laboratory of Freshwater Ecology and Biotechnology, Hubei Hongshan Laboratory, The Innovation Academy of Seed Design, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China
^b University of Chinese Academy of Sciences, Beijing, China



桂建芳院士
中科院水生所



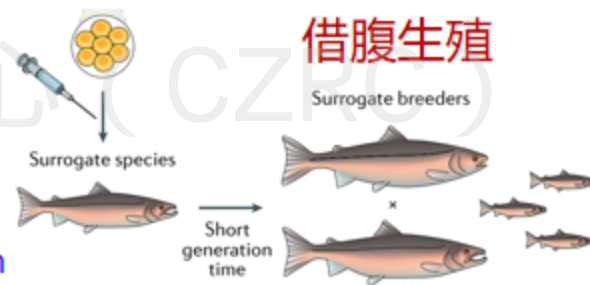
Prof. Houston
英国罗斯林研究所



Ross D. Houston[✉], Tim P. Bean, Daniel J. Macqueen, Manu Kumar Gundappa, Ye Hwa Jin, Tom L. Jenkins, Sarah Louise C. Selly, Samuel A. M. Martin, Jamie R. Stevens, Eduarda M. Santos, Andrew Davie & Diego Robledo

Nature Reviews Genetics 21, 389–409(2020) | Cite this article

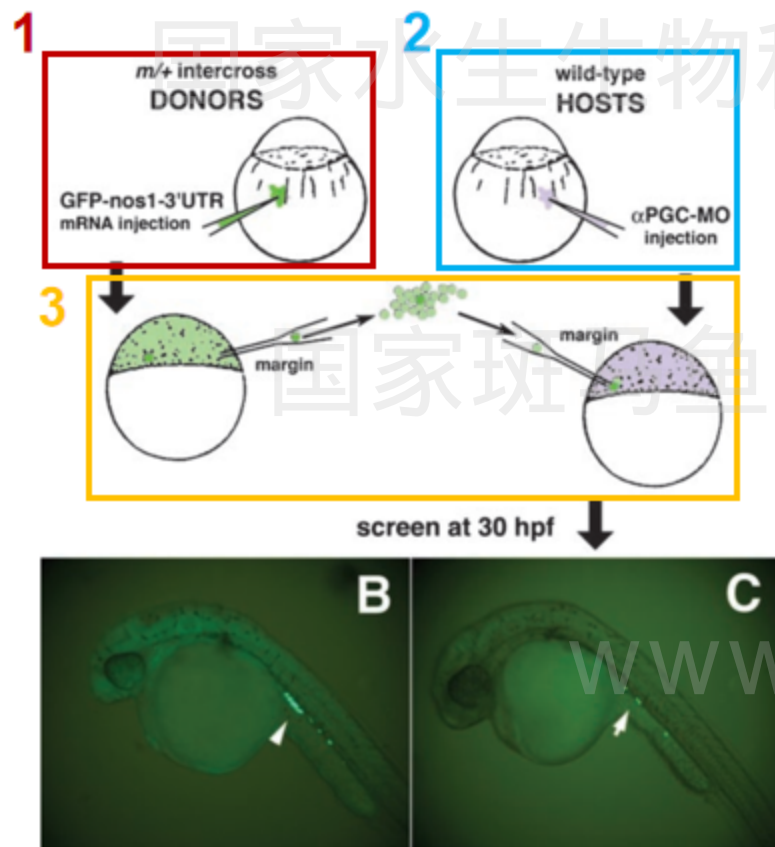
4916 Accesses | 2 Citations | 105 Altmetric | Metrics



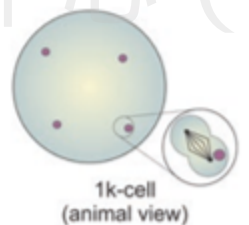
将创新性的生物技术——如“借腹生殖”等应用于养殖鱼类遗传育种，将大大加速鱼类品种培育的进程。

借腹生殖——利用代孕受体生产供体来源配子的技术，主要包括**PGC移植借腹生殖**和**精原/卵原细胞移植借腹生殖**。是国内外公认的前瞻性鱼类育种技术之一，用于快速获得繁殖周期长、养殖和繁育技术复杂鱼类的功能性配子。

PGC移植借腹生殖



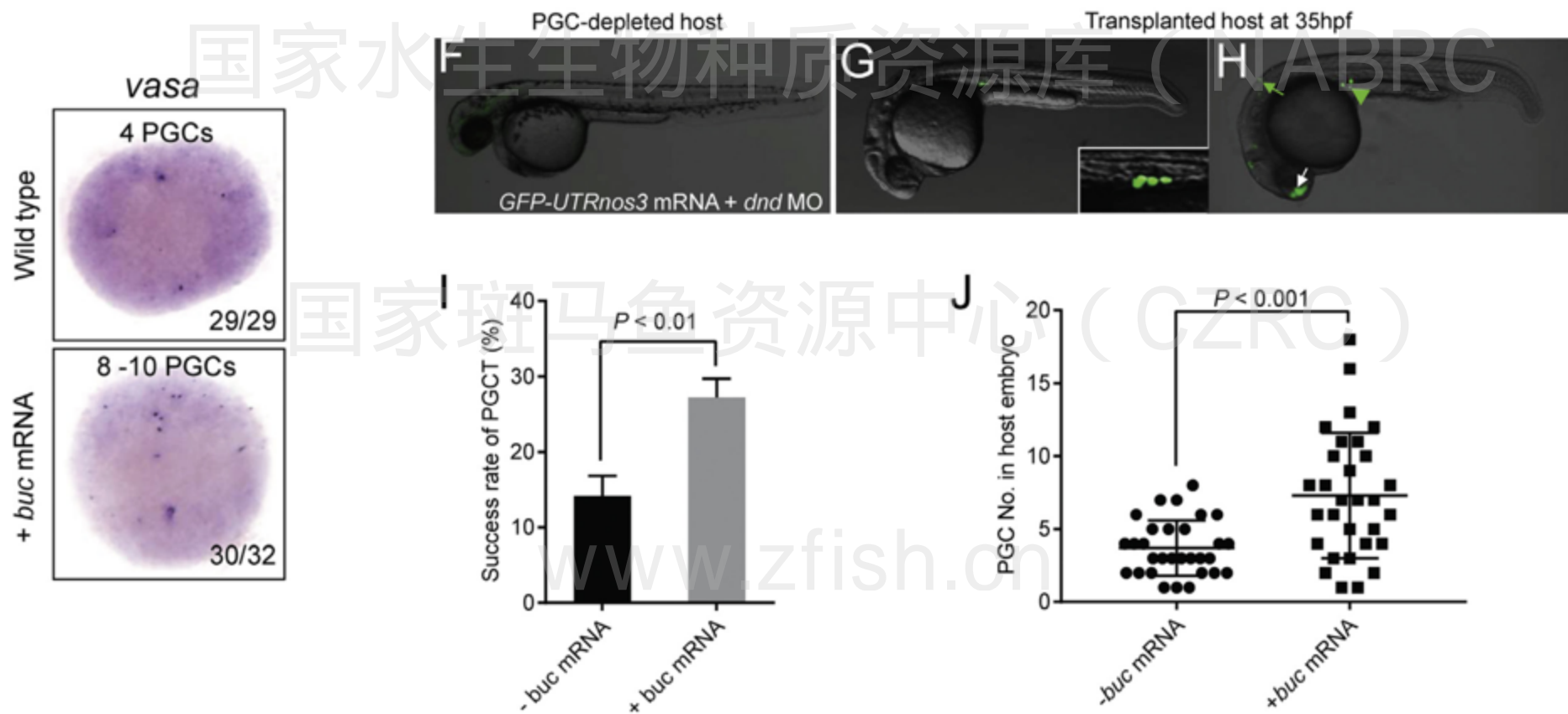
需先获得杂合突变体成鱼
(费时费力)



供体PGC数目少 (1K时期只有4颗PGC)
(限制PGC移植成功率)

PGCT success rate: 11.8%
MZmutant success rate更低

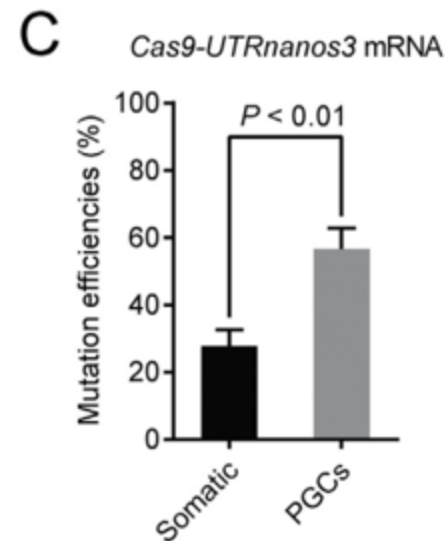
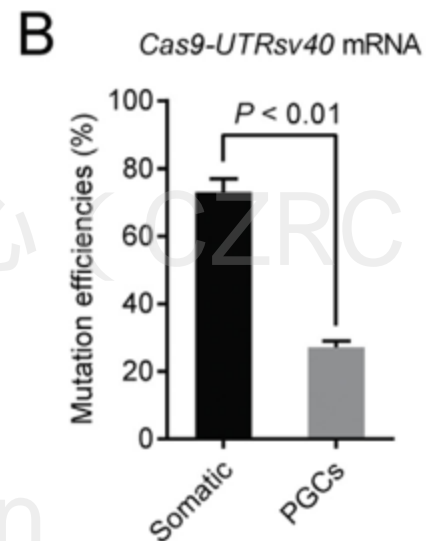
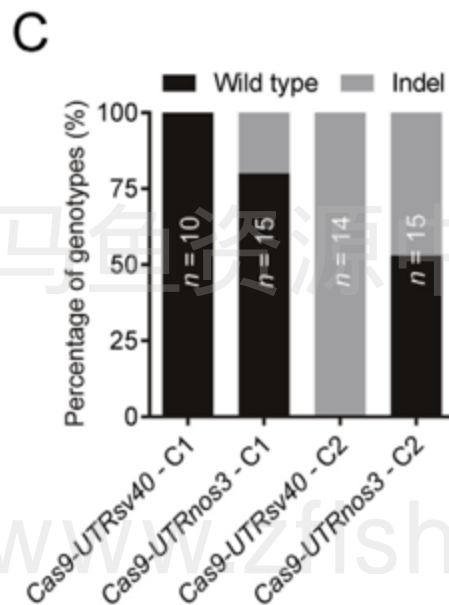
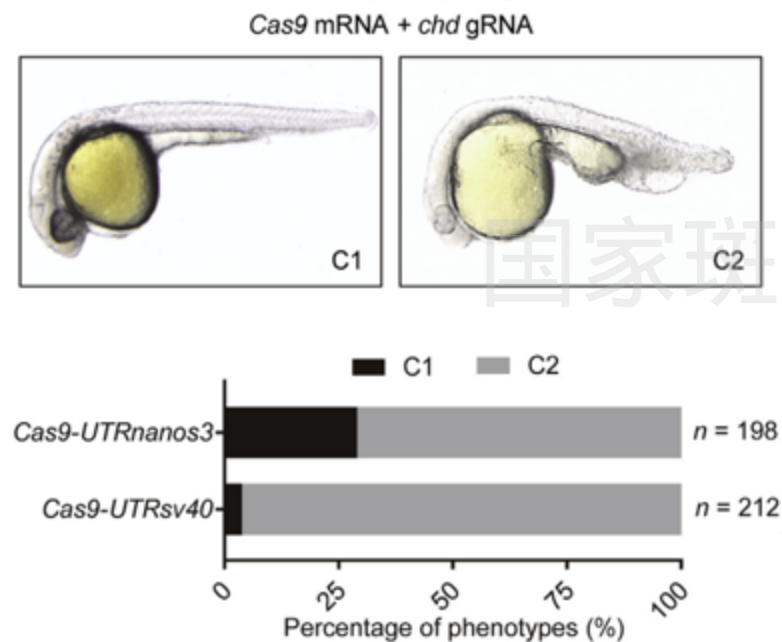
PGC数目少是制约PGCT成功率的关键因素之一



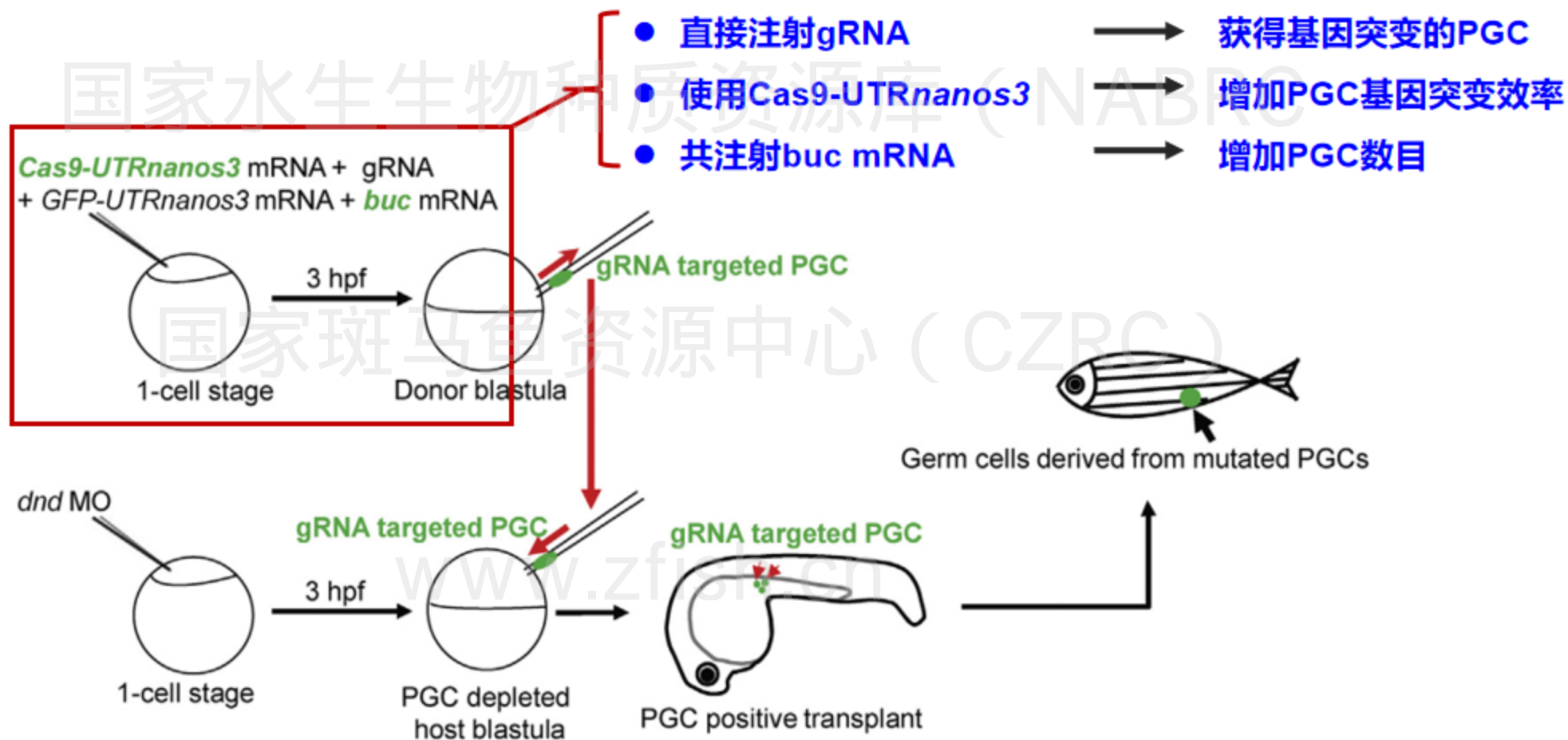
PGCT success rate: 14.2% to 27.2%

生殖细胞

国家水生生物种质资源库 (NABRC)



基于PGC移植快速构建斑马鱼突变体



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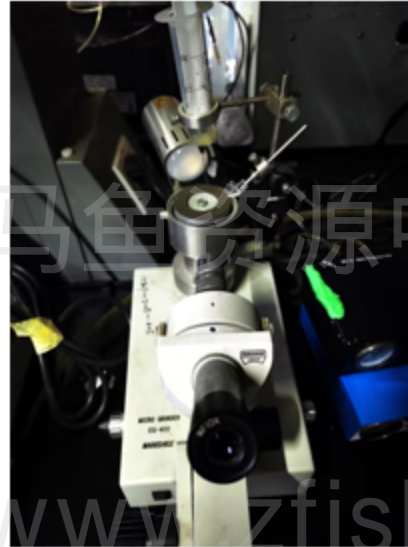
www.zfish.cn

1. 移植针

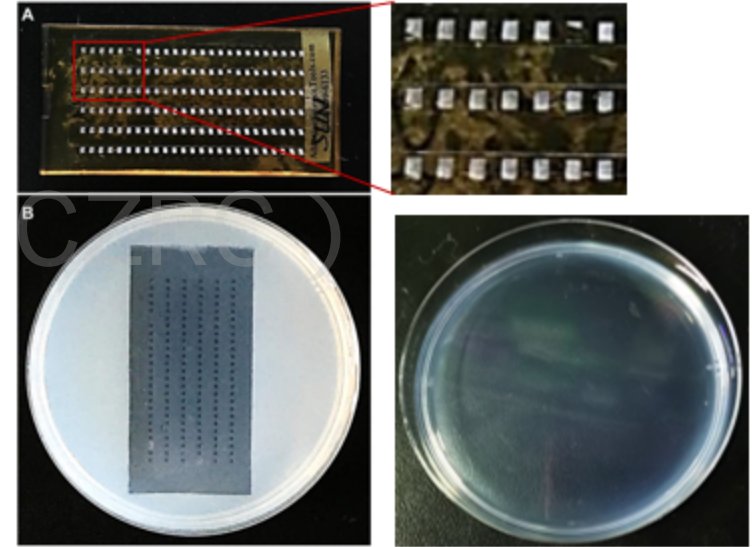


无内芯毛细吸管
内径：0.75 mm
外径：1.0 mm
端口直径：50-60 µm
角度：45°

2. 磨针仪



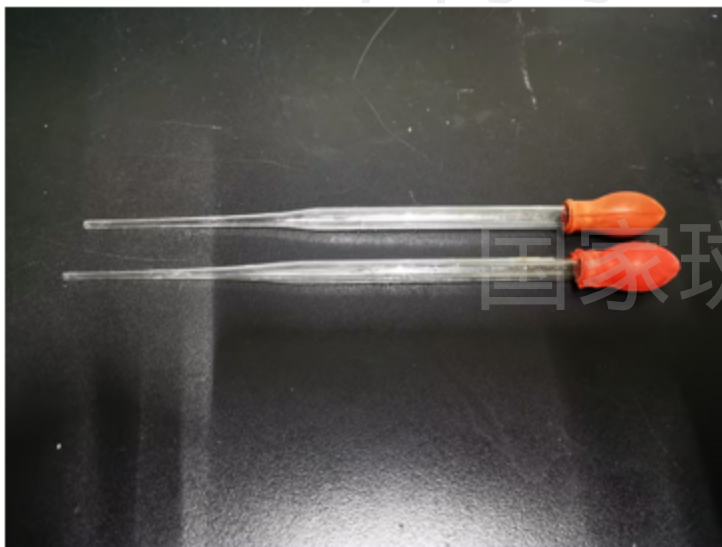
3. 移植模具及平皿



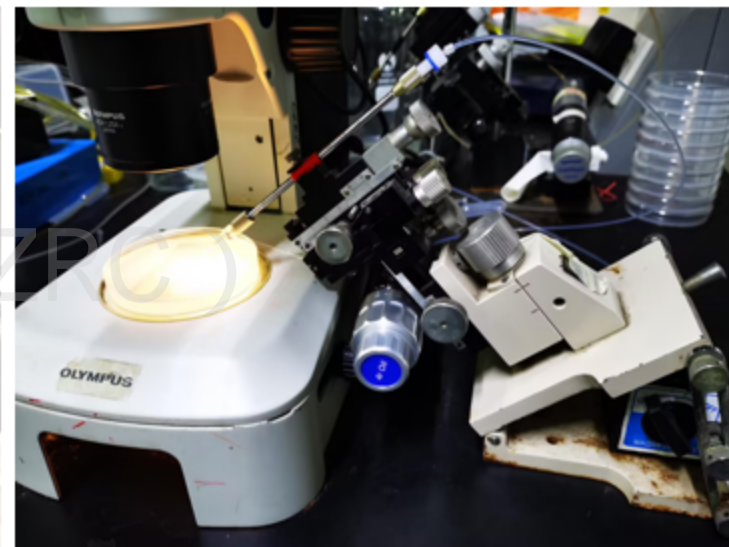
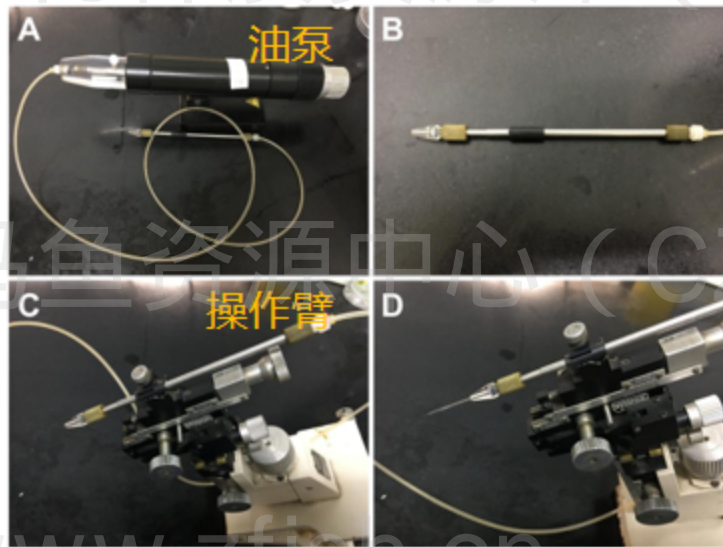
2% 琼脂

仪器设备

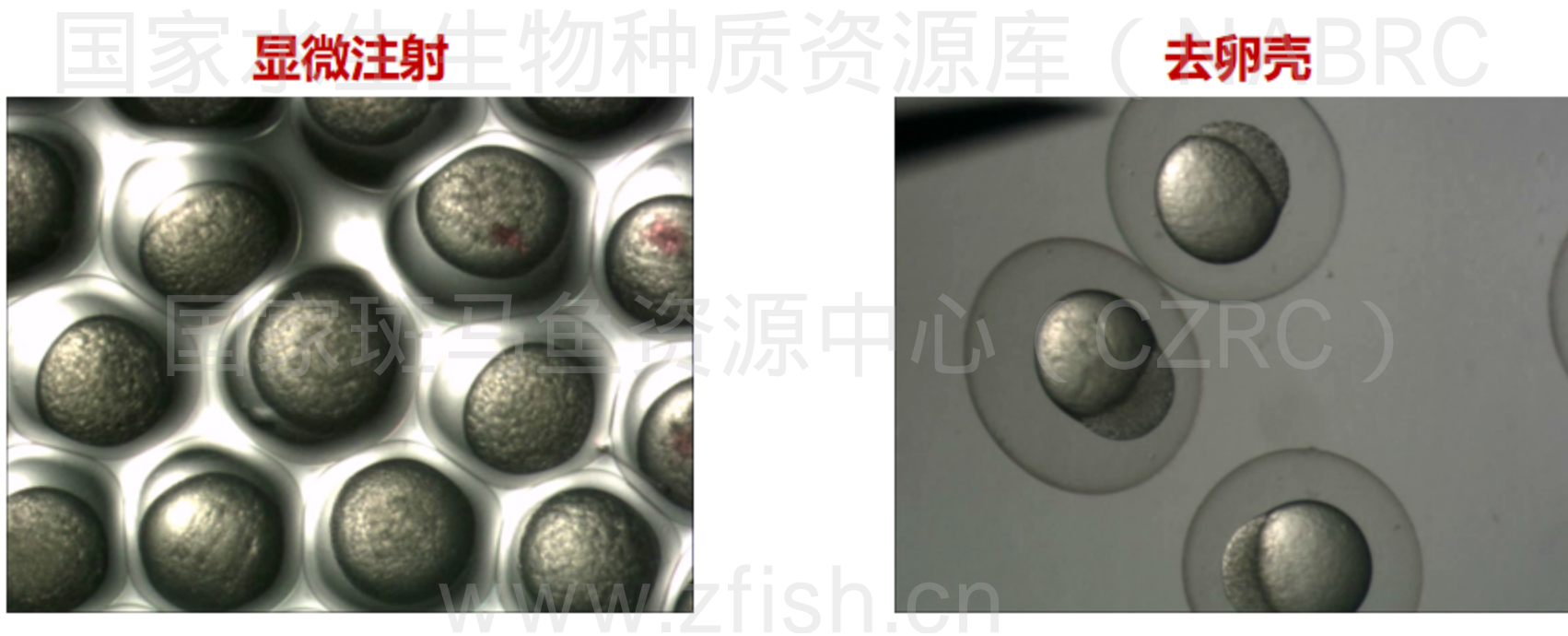
4. 玻璃吸管



5. 移植仪器



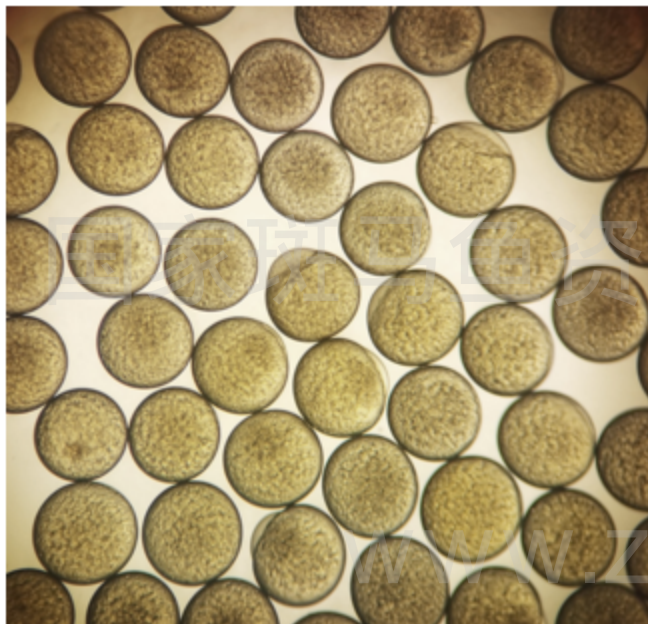
供/受体的制备



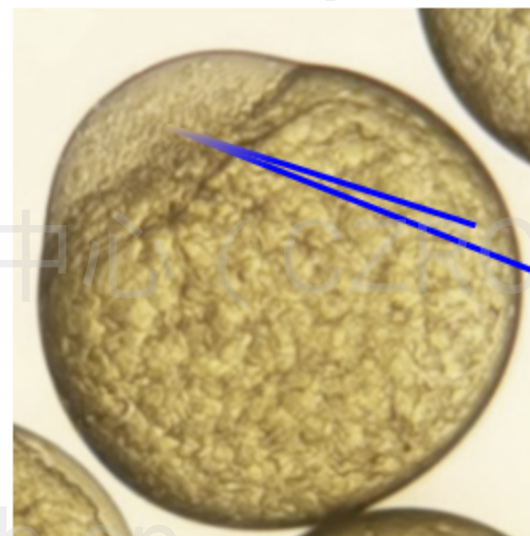
受体: *dnd1* MO

供/受体的制备

国家水生生物种质资源库 (NABRC)



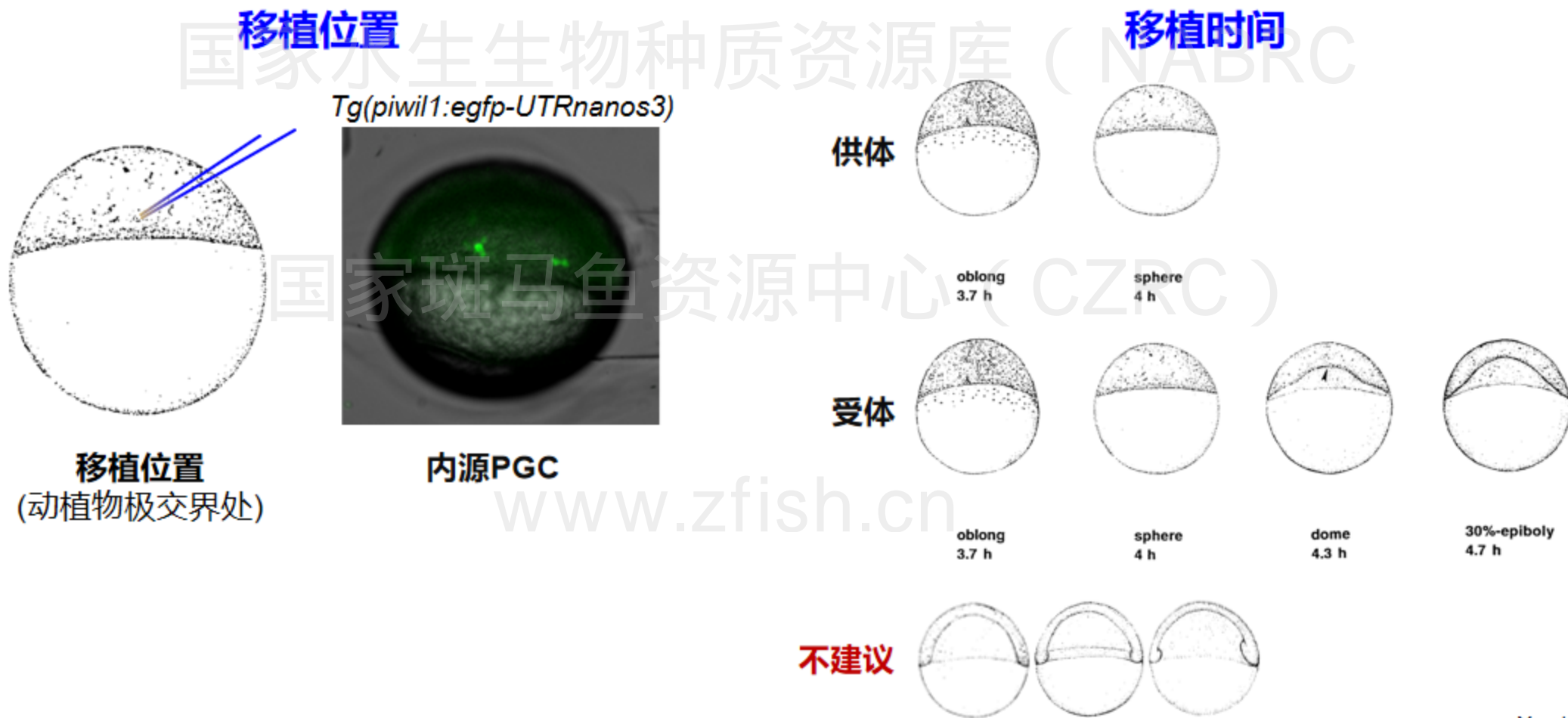
酶处理去除卵壳: 0.25%
胰蛋白酶处理1-cell胚胎



注射动物极
(从植物极到动物极)

供体: GFP-UTRnanos3
+cas9+gRNA+
buc mRNA

PGC移植位置和移植时间



PGC移植操作

国家水生生物种质资源库（NABRC）

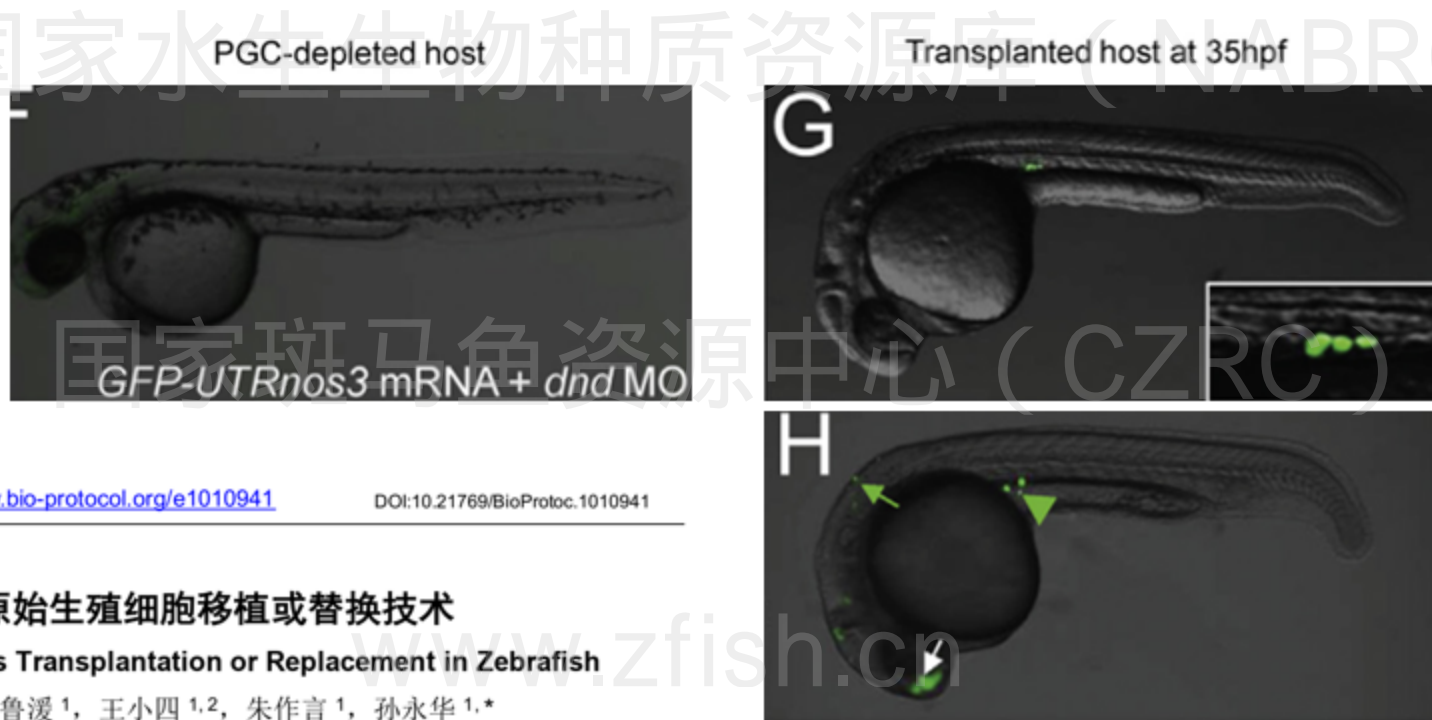
国家斑马鱼资源中心（CZRC）



www.zfish.cn

PGCT

PGC移植成功金标准——PGC迁移到生殖嵴



bio-101

www.bio-protocol.org/e1010941

DOI:10.21769/BioProtoc.1010941

斑马鱼原始生殖细胞移植或替换技术

Primordial Germ Cells Transplantation or Replacement in Zebrafish

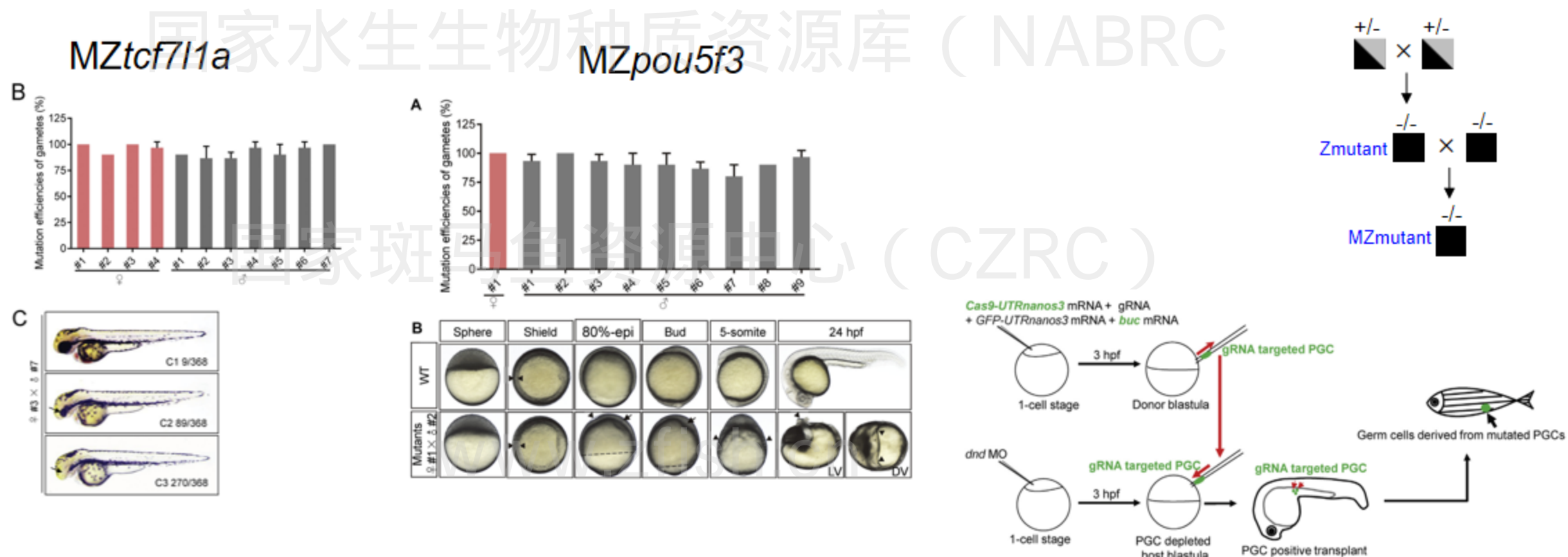
张峰华^{1,2}, 潘鲁漫¹, 王小四^{1,2}, 朱作言¹, 孙永华^{1,*}

¹ 国家斑马鱼资源中心, 国家水生生物种质资源库, 中国科学院水生生物研究所, 武汉, 湖北

² 生命科学学院, 中国科学院大学, 北京, 中国

*通讯作者邮箱: yhsun@ihb.ac.cn

快速获得母源合子突变体



仅需一代时间即可获得致死基因的母源合子突变体

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诱导型原始生殖细胞 (iPGC)

nature communications



Article

<https://doi.org/10.1038/s41467-023-43587-3>

Induced formation of primordial germ cells from zebrafish blastomeres by germplasm factors

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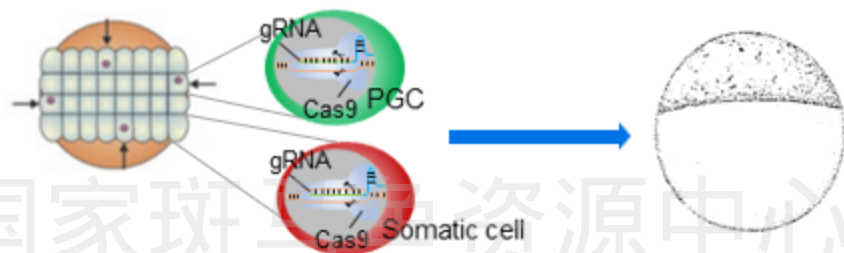
Xiaosi Wang^{1,2,3} , Junwen Zhu^{1,2}, Houpeng Wang^{1,3}, Wenqi Deng^{1,2}, Shengbo Jiao^{1,2}, Yaqing Wang^{1,2,3}, Mudan He^{1,2,3}, Fenghua Zhang^{1,2}, Tao Liu^{1,2}, Yongkang Hao^{1,2}, Ding Ye^{1,2,3} & Yonghua Sun^{1,2,3}

The combination of genome editing and primordial germ cell (PGC) transplantation has enormous significance in the study of developmental biology and genetic breeding, despite its low efficiency due to limited number of donor PGCs. Here, we employ a combination of germplasm factors to convert blastoderm cells into induced PGCs (iPGCs) in zebrafish and obtain functional gametes either through iPGC transplantation or via the single blastomere

PGC数目少是限制PGCT成功率的关键因素之一

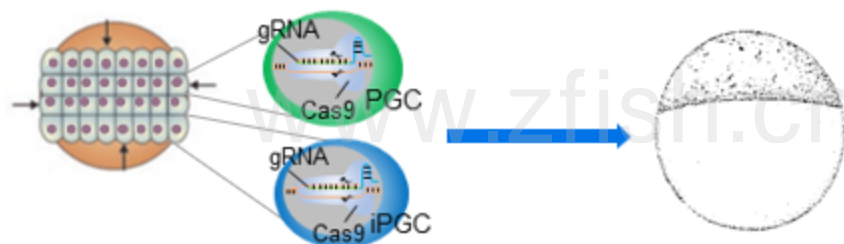
国家水生生物种质资源库 (NABRC)

(1) PGC移植借腹生殖



PGC数目少

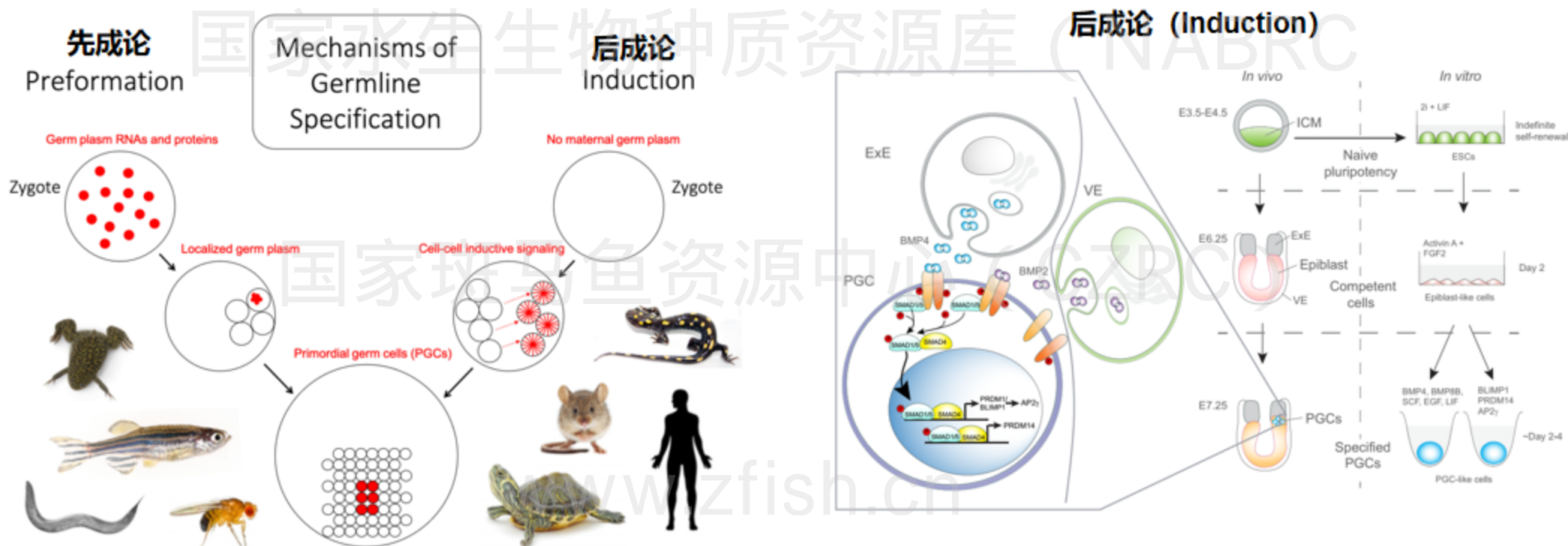
(2) iPGC移植借腹生殖



PGC获取自由

如何高效产生原始生殖细胞?

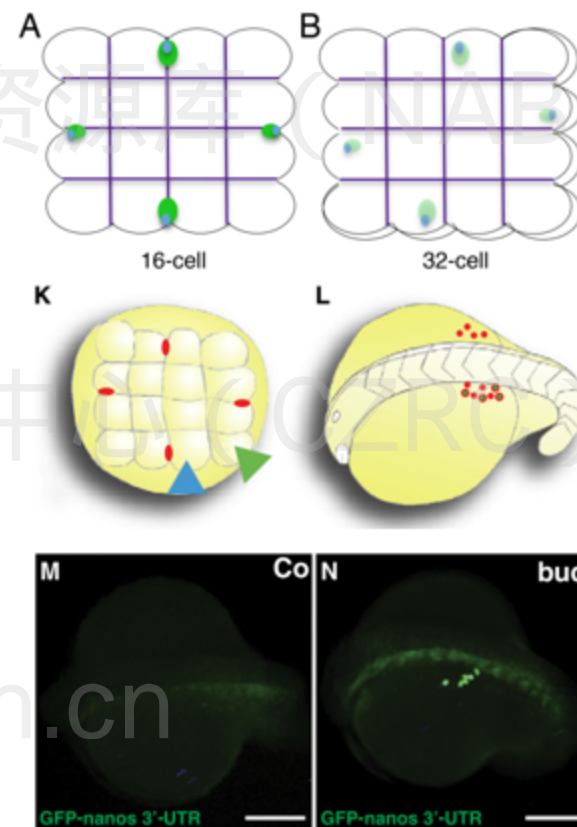
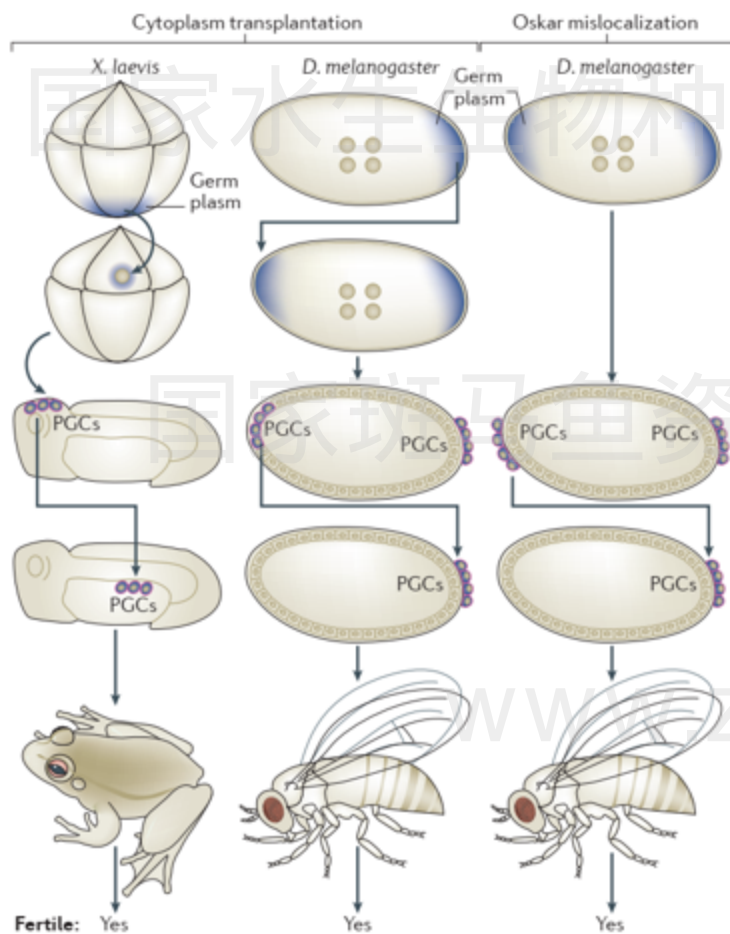
PGC形成模式——“先成论”和“后成论”



Hansen et al., 2021
Magnúsdóttir et al., 2014

如何在“先成论”物种中产生原始生殖细胞？

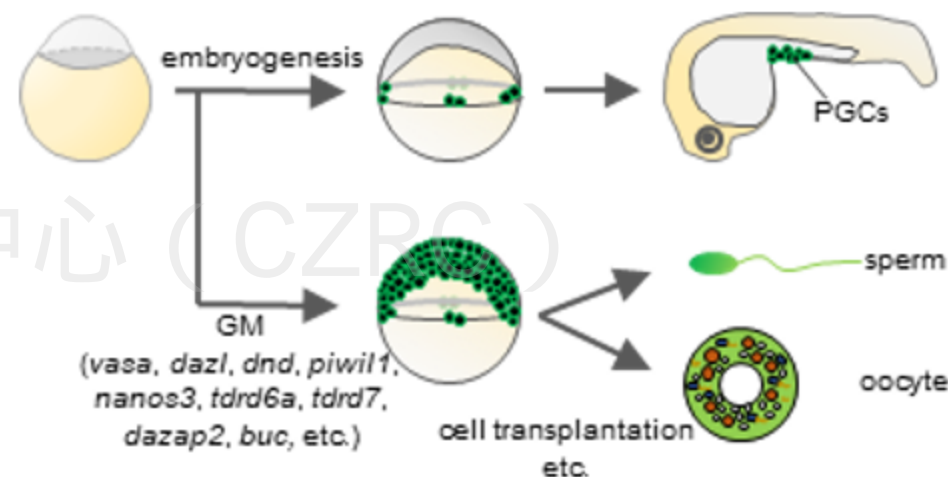
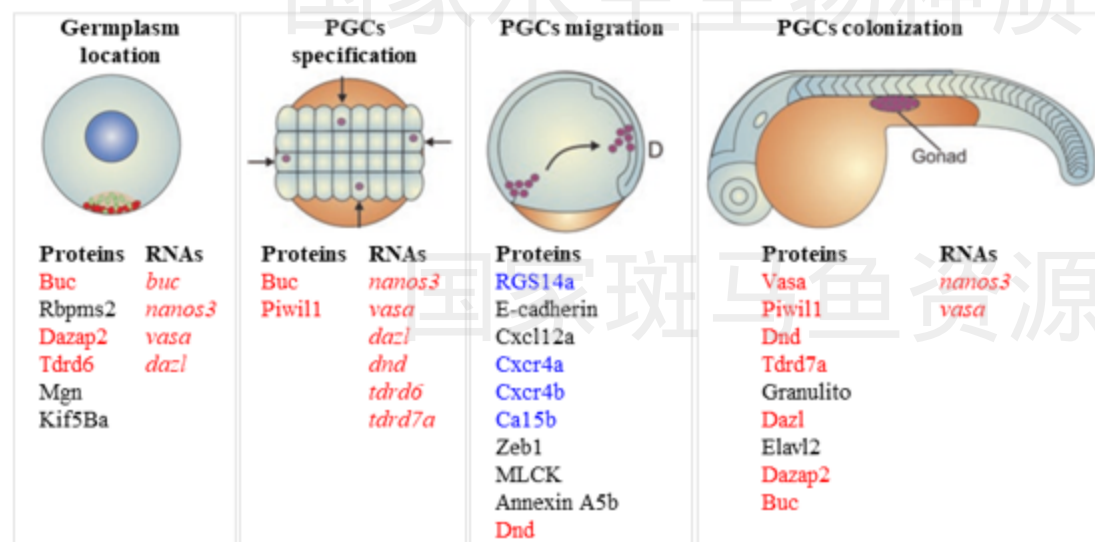
“先成论” 动物中PGC的诱导策略



PGC诱导的关键因子是什么？

Strome et al., 2015
Bontems et al., 2009

PGC发育相关的生殖质因子

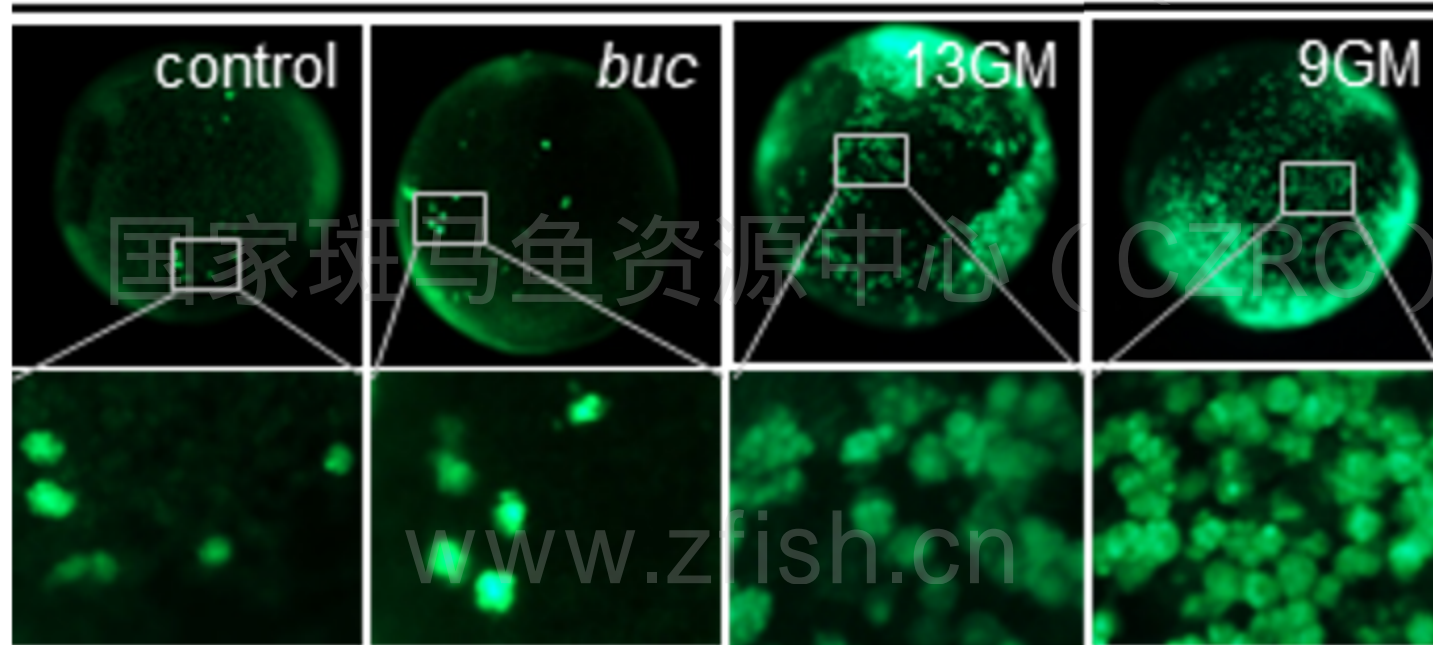


13GM = 9GM (specification related factors) + 4GM (migration related factors)

高效产生斑马鱼诱导型PGC (iPGC)

iPGC: induced PGC

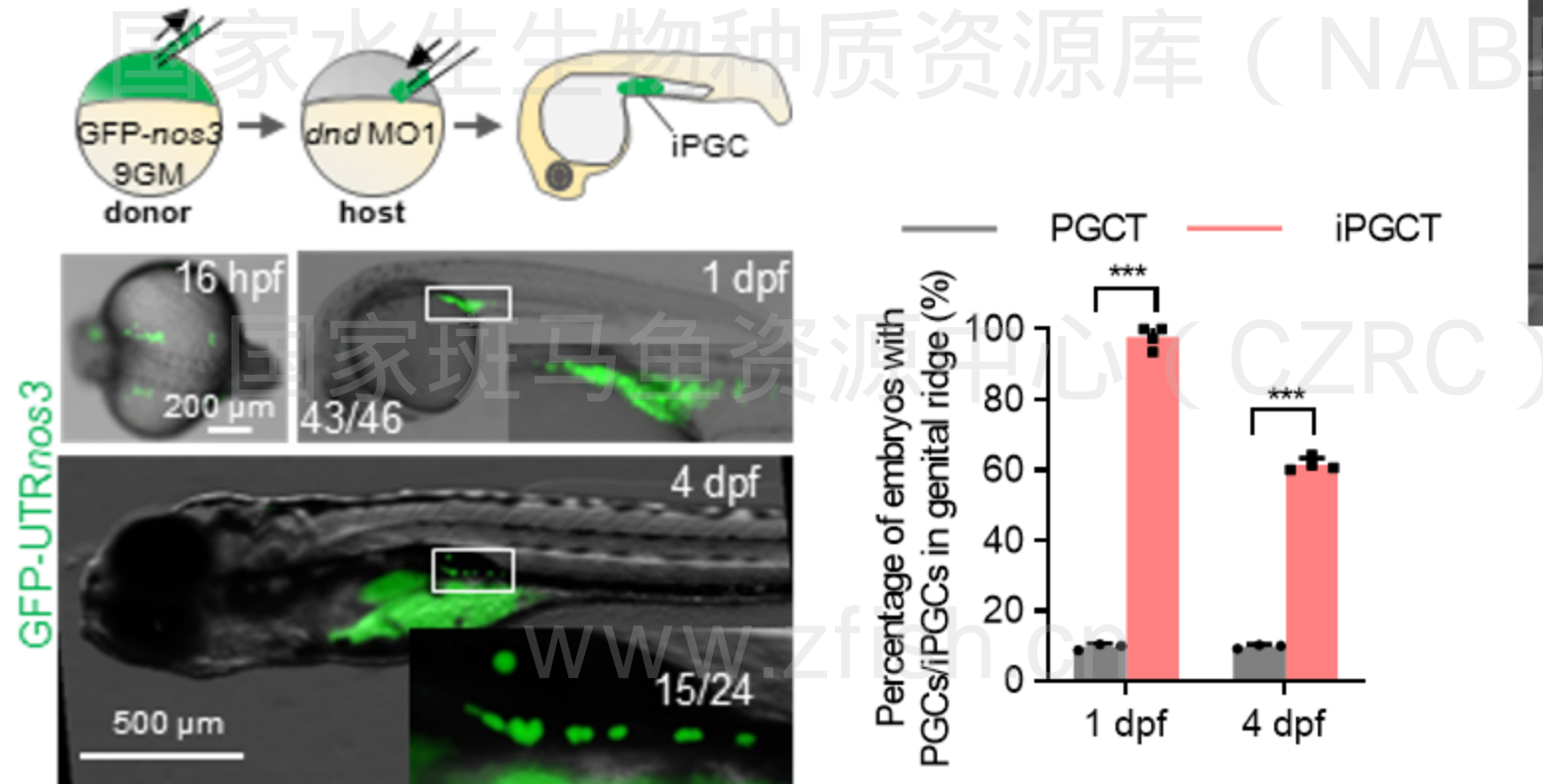
GFP-UTR*nanos3*



9GM几乎将整个胚盘细胞诱导成GFP-UTR*nanos3*阳性细胞

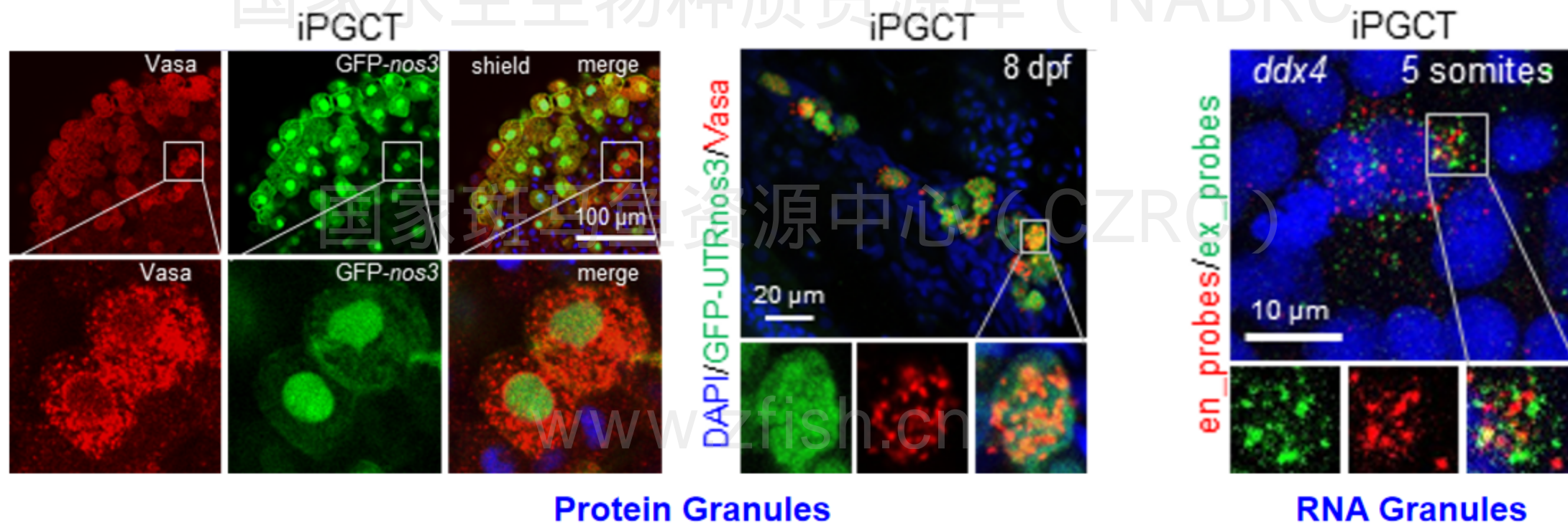
iPGC具有PGC典型特征

1. 迁移到生殖嵴



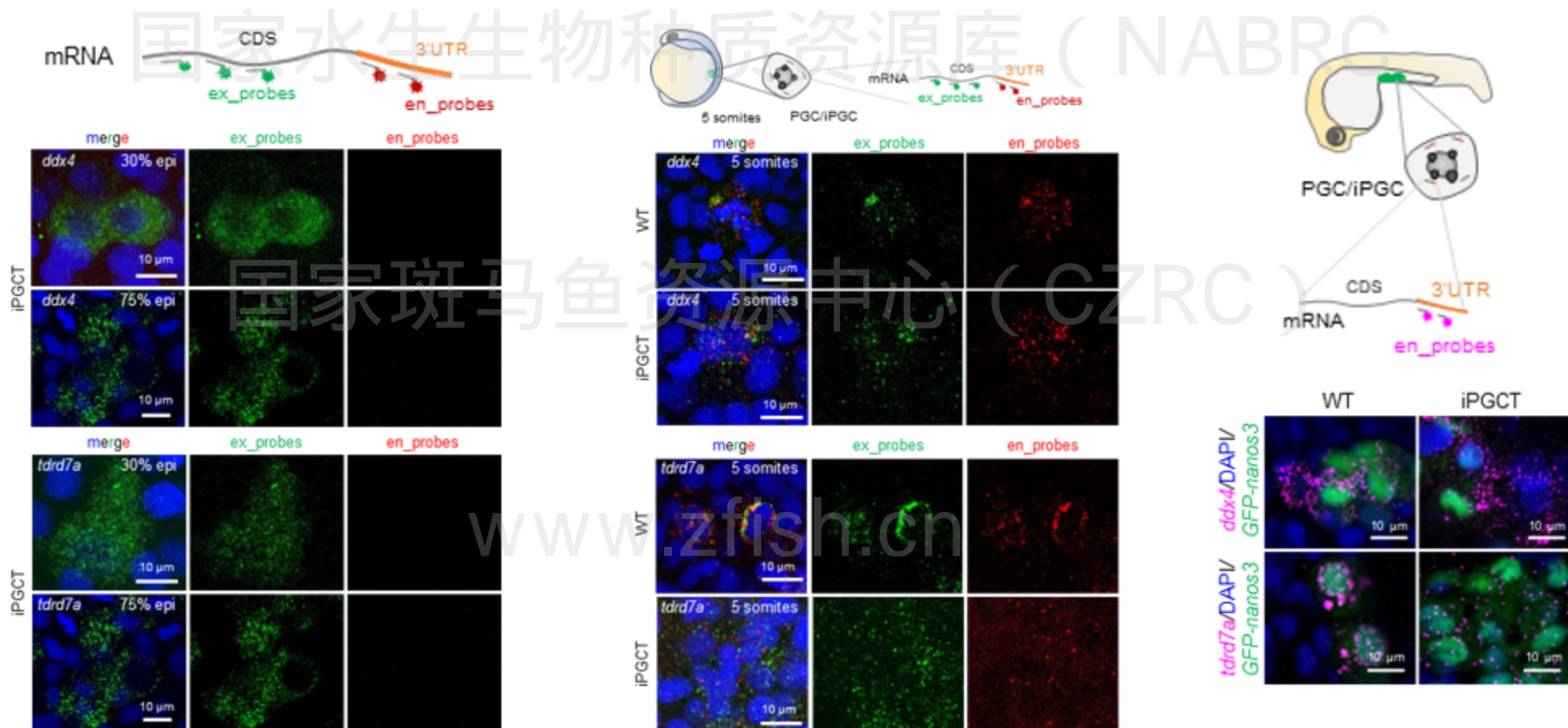
iPGC具有PGC典型特征

2. 生殖颗粒



iPGC具有PGC典型特征

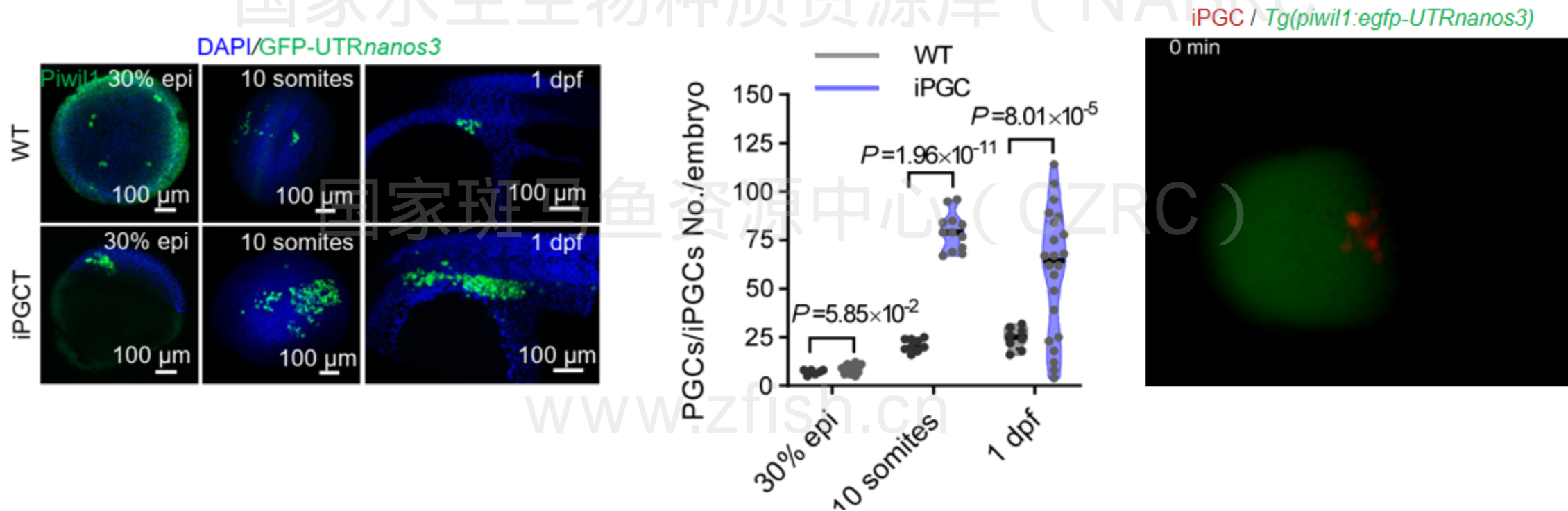
3. 激活生殖质基因表达



Endogenous germplasm mRNAs were initiated at 5-somites stage

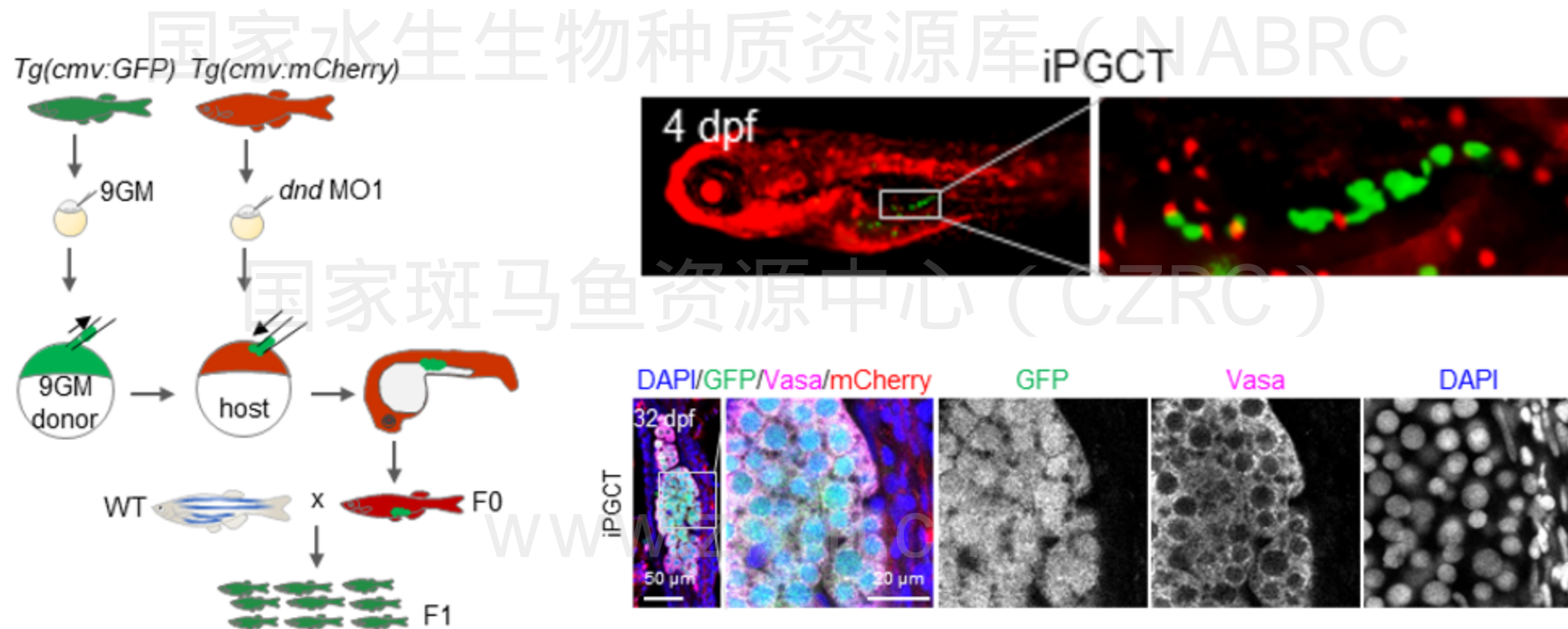
Wang et al., Nature Communications, 2023

iPGC能够快速增殖

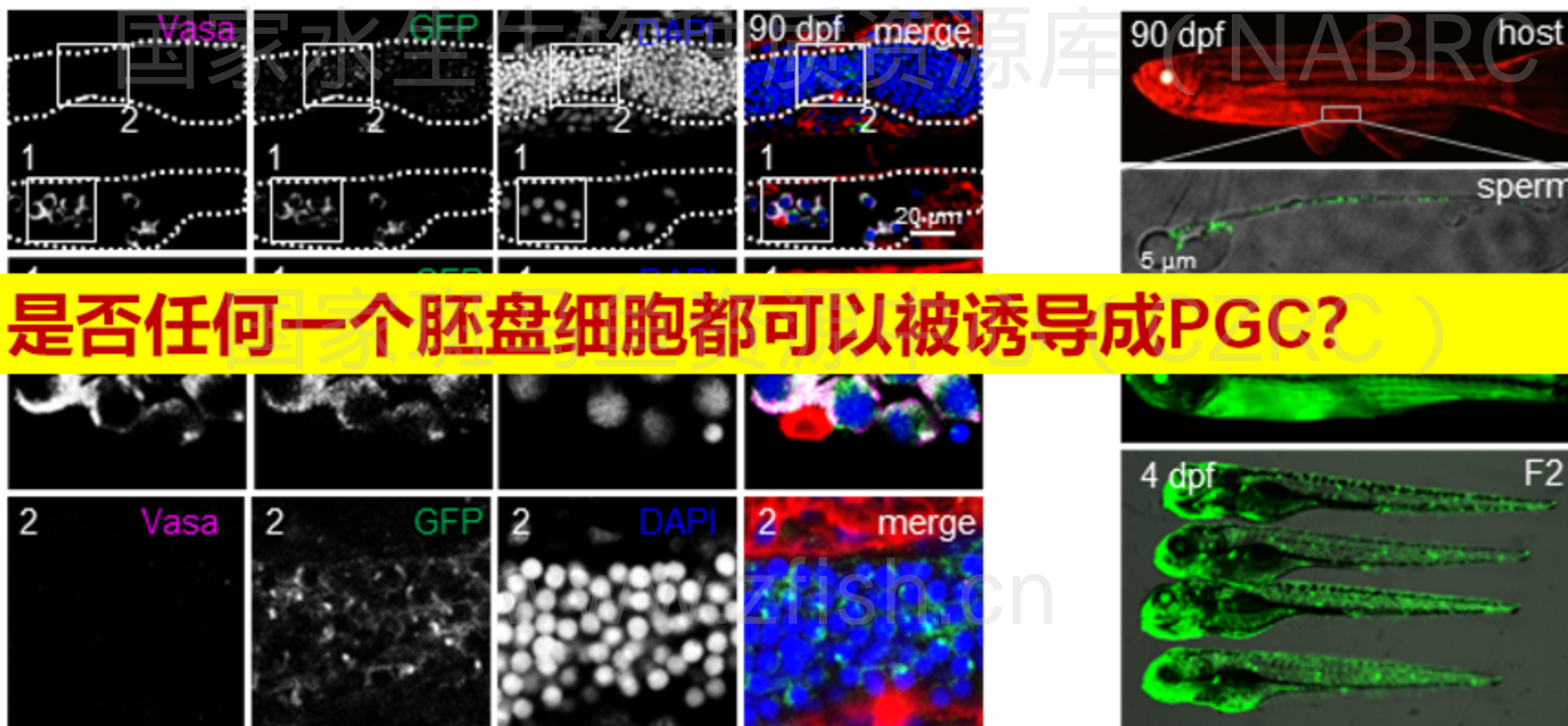


早期的iPGC具有生殖细胞特征同时又具有体细胞快速增殖特征

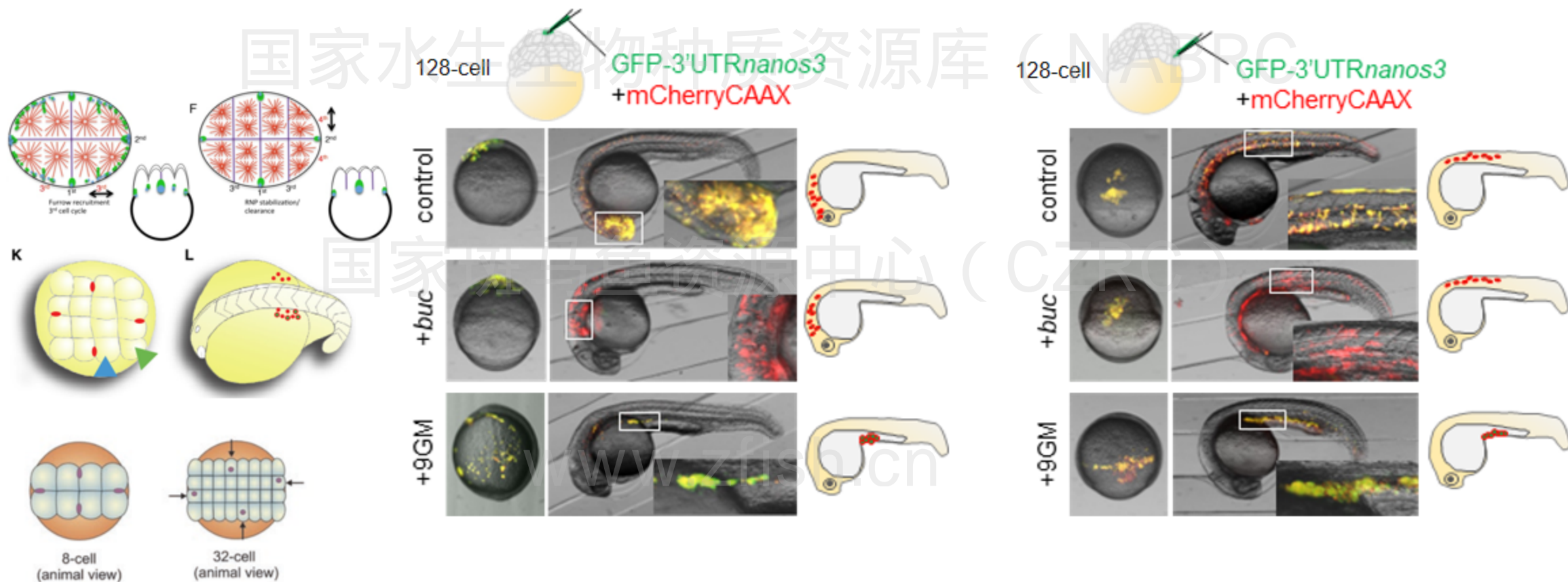
iPGC移植



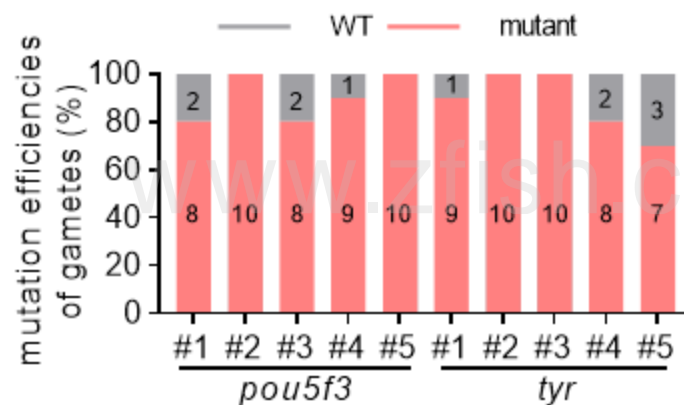
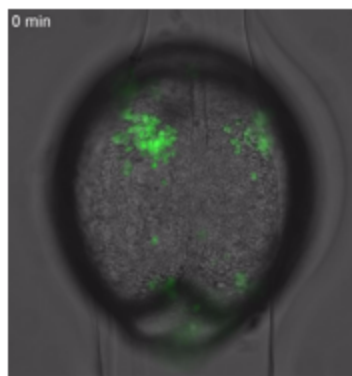
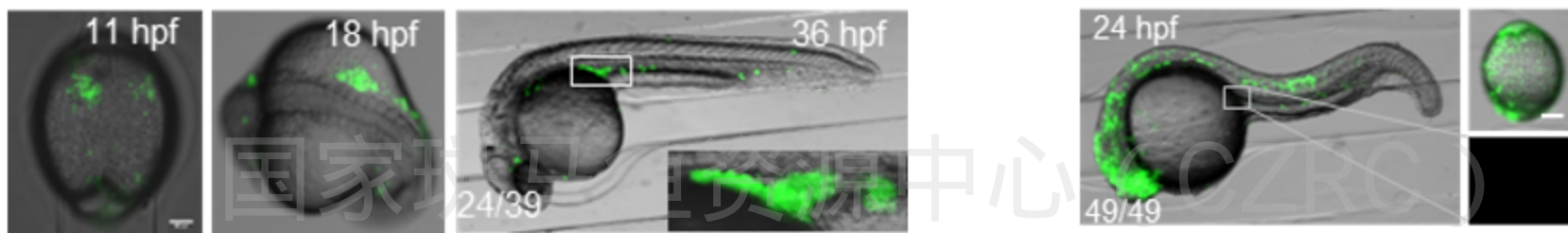
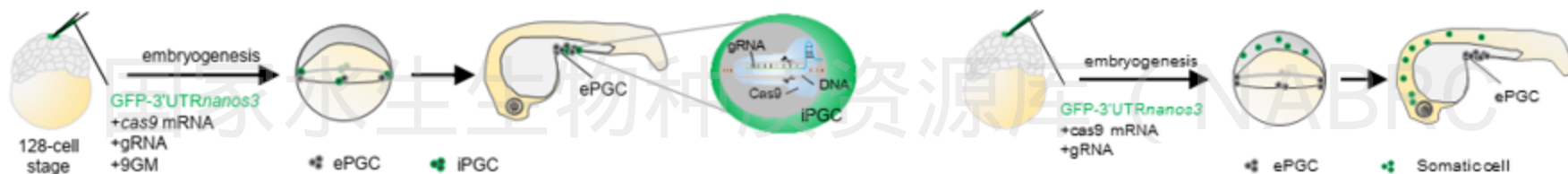
iPGCT高效产出供体来源的功能性配子



任何一个胚盘细胞均可以被诱导成为iPGC



快速高效获得基因编辑配子



主要内容

国家水生生物种质资源库（NABRC）

➤ 原始生殖细胞简介

➤ 原始生殖细胞移植及其应用

国家斑马鱼资源中心（CZRC）

➤ 原始生殖细胞移植操作流程

➤ 诱导型原始生殖细胞简介

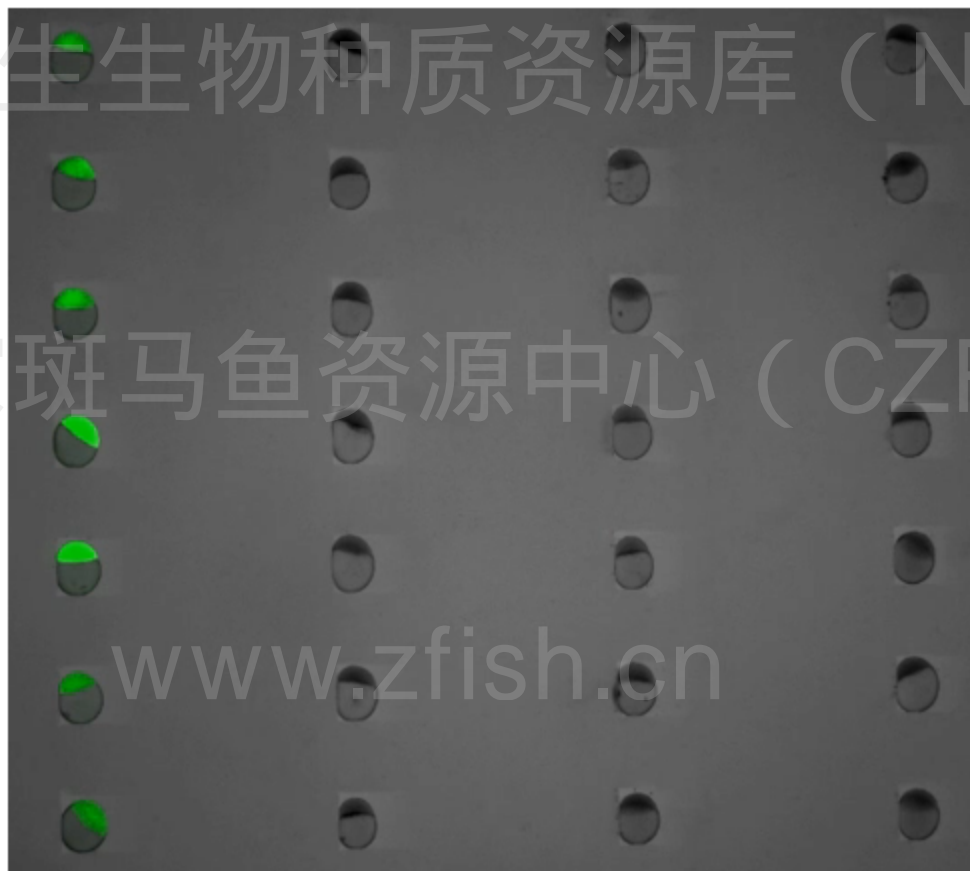
➤ 诱导型原始生殖细胞移植及其应用

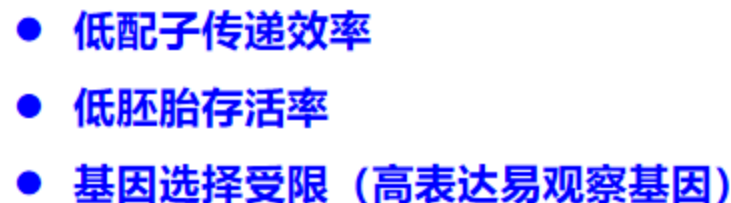
iPGC移植

国家水生生物种质资源库 (NABRC)

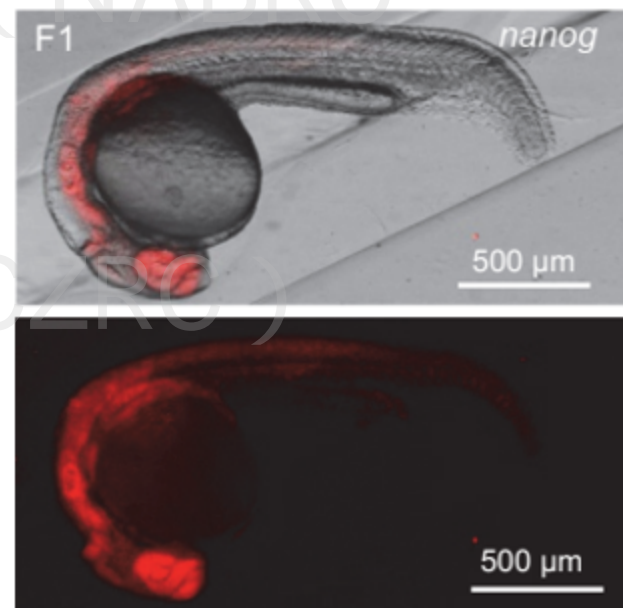
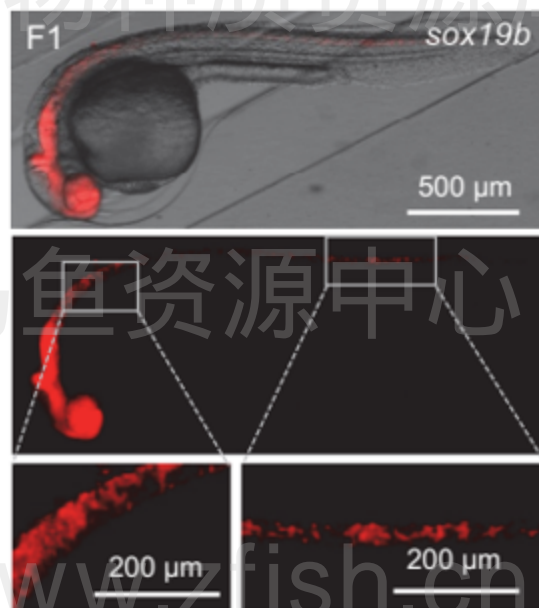
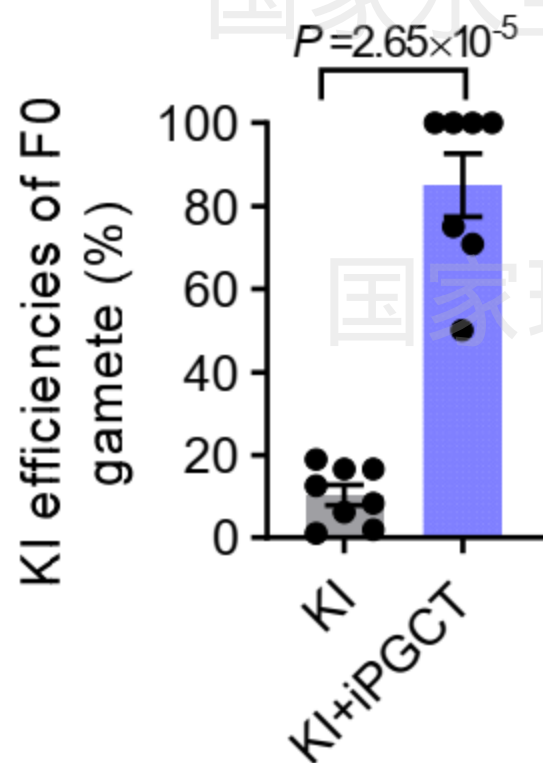
国家斑马鱼资源中心 (CZRC)

www.zfish.cn



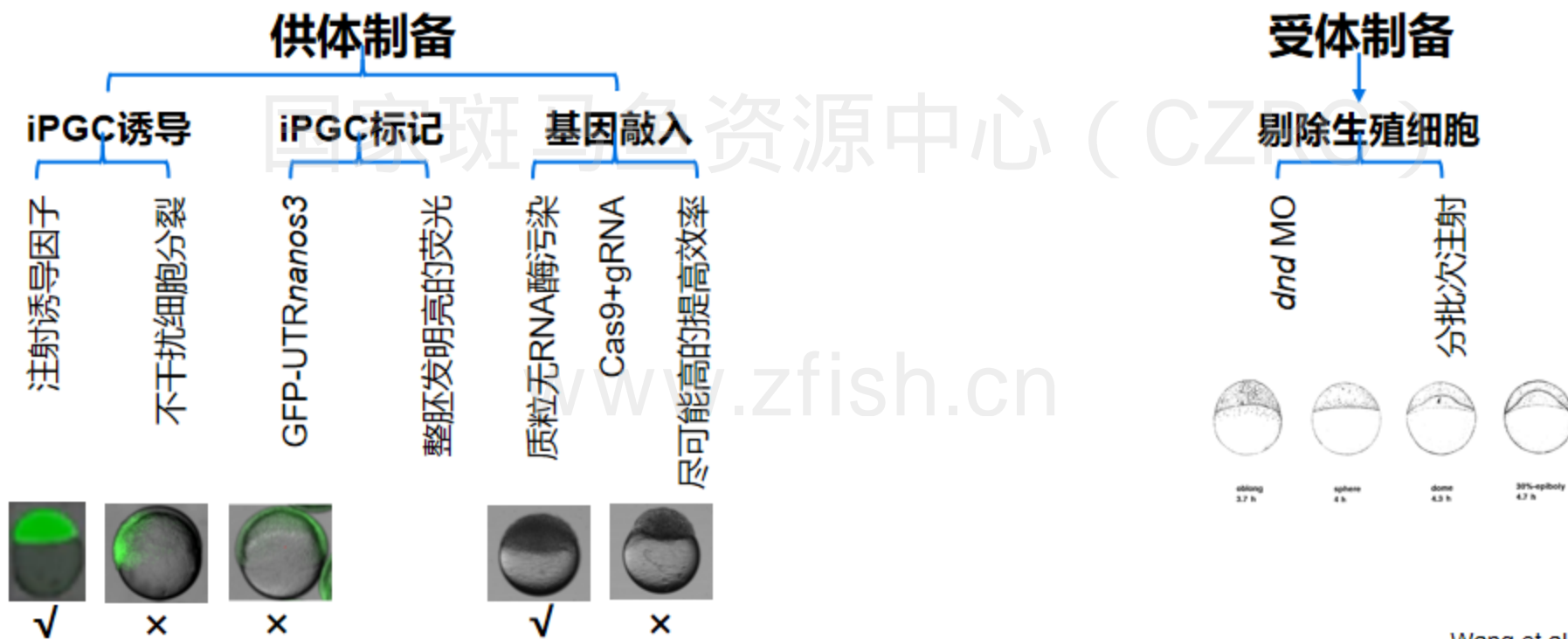


基于iPGCT快速构建基因敲入品系



iPGC移植

国家水生生物种质资源库 (NABRC)



注意事项及解决办法

注意事项

- ① 选择内源PGC所在位置进行细胞的吸取和移入(PGC移植成功的关键)
- ② 移植针开口直径适当 (成功吸取和注射细胞的关键)
- ③ 移植的细胞数量不易过多 (移植胚胎成活的关键)
- ④ 受体胚胎勿移入过多的溶液 (移植胚胎成活的关键)
- ⑤ 选取高质量的卵子作为受体

解决方法

- ① 贴近胚盘边缘进行吸取和移入细胞, 勿碰到卵黄
- ② 移植针开口直径50-60 μm ; 清理磨完针后的碎玻璃渣
- ③ 对于PGC移植: 供体细胞控制在60-100个; 对于iPGC移植: 供体细胞控制在10-20个
- ④ 吸取细胞是控制细胞吸入的速度, 避免吸入过多溶液

iPGCT

- ① 诱导因子必须精准注射到1细胞中 (高诱导效率的关键)
- ② 供体细胞可以来源于胚胎任意位置的细胞 (PGC获取自由)

总结

国家水生生物种质资源库 (NABRC)

基于PGC移植借腹生殖的突变体快速构建技术

PGC体内高效诱导技术

基于iPGC移植借腹生殖的突变体和基因敲入品系快速构建技术

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