基因编辑斑马鱼

南模生物斑马鱼技术服务平台提供斑马鱼基因编辑服务，并可通过分析基因敲除、敲低、过表达斑马鱼模型的表型改变研究基因、蛋白在体内的功能以及相关信号通路，筛选致病基因、探索基因功能。

南模生物斑马鱼技术服务平台拥有受过严格训练的技术与分析人员，可提供高质量的斑马鱼基因编辑服务，并可通过分析基因敲除、敲低、过表达斑马鱼模型的表型改变研究基因、蛋白在体内的功能以及相关信号通路，筛选致病基因、探索基因功能。

我们提供以下服务：

- **基因Knock-down及表型分析（Morpholino knock-down）**

- **基因Over-expression及表型分析**

- **基因敲除斑马鱼定制（Cas9-KO）**

- **转基因斑马鱼定制**

联系我们了解更多服务详情，欢迎来电：400-728-0660，或来函 info@modelorg.com。

基因Knock-down及表型分析（Morpholino knock-down）
Morpholino knock-down技术原理

图1. Morpholino 技术原理。（a）Morpholino与RNA结合。（b）Morpholino 通过阻断翻译过程而发挥作用。（c-e）Morpholino阻断RNA的正常剪接。

Morpholino Knockdown应用案例

图2. Morpholino Knockdown 靶点位置示意图。The zebrafish gene-X was targeted by three specific morpholino antisense strategies to prevent either the translation of the zebrafish gene (ATG-MO) or proper splicing of exon 3 (E3I3-MO).
Morpholino Knockdown 表型分析

图3. Panels A through H show lateral views of control MO injected zebrafish embryos (Panel A and Panel E) and embryos injected with gene-X morpholino oligonucleotides (MO) (Panel B through C, Panel F through G), gene-X-e3i3-MO plus nonmutant zebrafish gene-X (Panel D and Panel H). Coinjection of nonmutant zebrafish gene-X mRNA rescued U-shaped somites (red arrow) and curved body axis (blue dotted line) in gene-X morphants at 52 hpf. The bar graph in Panel I shows the percentage of embryos with development defects. Panels J Effectiveness of gene-X knockdown was confirmed by RT-PCR and sanger sequencing. hpf, hours post fertilization.

Morpholino Knockdown 信号通路机制分析

图4. Endogenous shha, ptch1, ptch2, sufu, gli1, gli2a, gli2b, and gli3 in wild-type control and geneX morphants assessed by qRT-PCR (n = 100 individual embryos). geneX fine-tunes Hedgehog patterning activity maybe through a novel regulatory feedback loop.
基因 Knockdown 后抑制斑马鱼血管生成

图5. GeneX knock down inhibits the trunk angiogenesis in zebrafish. (A-D) Representative fluorescent images of zebrafish embryos at 32h post-fertilization (hpf). (C-E) Compared with wild-type control, embryos injected with geneX-MO present a lower number of incomplete ISVs and only occasional sprouts (asterisk) of dorsal aorta. The boxed regions are shown at higher magnification in the right panels. DLAV, dorsal longitudinal anastomotic vessels; ISV, intersegmental vessels; DA, dorsal aorta; PCV, posterior cardinal vein.

基因 Knockdown 后导致中枢神经系统特异性细胞凋亡

图6. Morpholino knock down of geneX induces potent CNS-specific apoptosis. Wild-type control embryos and embryos injected with geneX-MO were stained with acridine orange (AO) at 32hpf. Apoptotic cells are visible as black spots, and less bright homogenous black staining is unspecific background staining. (A-B) Uninjected wild-type control zebrafish exhibited few or no apoptotic cells in CNS (central nervous system). In contrast, significantly increased staining was observed throughout the CNS in zebrafish injected with geneX-MO (C-D). The blue boxed regions are shown at higher magnification in the right panels. A-D: lateral view, anterior, left.

基因 Knockdown 后具有潜在听毒性
图7. GeneX knock down induces potent ototoxicity in zebrafish. Wild-type control embryos and embryos injected with geneX-MO were stained with the mitochondrial potentiometric dye DASPEI at 6-dpf. Hair cells stereotypically located on the lateral line were stained as green dots (white arrow). Uninjected wild-type control zebrafish exhibited normal hair cell number. In contrast, significantly decreased hair cell staining was observed in zebrafish injected with geneX-MO. Fluorescent DASPEI images were inverted for particle analysis. The fluorescence particle signal was quantified using morphometric analysis. dpf, days post fertilization.

文献发表

图8. Nonmutant bmp10 overexpression causes development defects in zebrafish. (A–C) Gross morphology at 32hpf. Compared with uninjected wild-type control embryos, nonmutant zebrafish bmp10 overexpression causes decreased body length (black dotted line) and curved body axis (blue dotted line) in zebrafish. The bar graph in Panel D through E show the percentage and body length of embryos with development defects. hpf, hours post fertilization.
图9. Nonmutant bmp10 overexpression inhibits the trunk angiogenesis in zebrafish. (A-F) Representative bright field and fluorescent images of zebrafish embryos at 32h post-fertilization (hpf). Red arrow indicates haemorrhage in the tail (B). (C-H) Compared with wild-type control, nonmutant zebrafish bmp10 mRNA (200pg) injection present a lower number of incomplete ISVs and only occasional sprouts (asterisk) of dorsal aorta. The red boxed regions are shown at higher magnification in the right panels. DLAV, dorsal longitudinal anastomotic vessels; ISV, intersegmental vessels; DA, dorsal aorta; PCV, posterior cardinal vein.

文献发表


基因敲除斑马鱼定制（Cas9-KO）

利用CRISPR/Cas9技术，针对靶基因序列设计sgRNA，指导Cas9蛋白在特定基因位点引起DNA双链断裂，在非同源性末端接合修复断裂DNA的过程中，靶基因碱基突变或缺失被引入到斑马鱼基因组中，最终导致靶基因无法正常转录翻译，达到基因敲除的目的。目前我们利用CRISPR-Cas9技术，提供AB品系的基因敲除斑马鱼制备，AB系是最为普遍使用的标准纯遗传背景品系之一。同时也可根据委托方的需求，提供TU、Casper以及其它遗传背景的基因敲除斑马鱼服务。
基因敲除斑马鱼应用案例

南模生物自主研发构建tyr基因敲除斑马鱼。tyr（酪氨酸酶基因）是黑色素合成关键酶，该基因敲除后胚胎色素形成受到干扰。
图10. tyr Cas9-KO induces pigmentation defects.

转基因斑马鱼定制

利用Tol2转座子系统构建肝脏特异性EGFP绿色荧光蛋白转基因TG(fabp10a:EGFP)斑马鱼模型，以及绿色荧光蛋白标记巨噬细胞转基因TG(zlyz:EGFP)斑马鱼模型。
图11. Establishment of TG(fabp10a:EGFP) and TG(zlyz:EGFP) transgenic zebrafish.