

TARGET VALIDATION & DISEASE MODELS

Biobide is a biotechnology company offering drug discovery services to Pharma, Biotech, Chemical, Cosmetic and Nutraceutical companies. Our services are based on the **zebrafish model** and the capacity to offer highly efficient **tailor made assays**.

1 TARGET VALIDATION WITH MORPHOLINOS

Target identification and validation is gaining relevance in early phases of Drug Discovery. This process allows characterizing the role of a protein or pathway of interest and provides selection arguments to define the required properties of the compounds to be screened.

Zebrafish has been proposed as a good model to find genes involved in specific processes and/or check activity of a desired gene in a specific process. Different strategies and tools can be used to unravel the function of a specific gene. Among them, the transient inhibition with Morpholinos (MO) is a common one [1-2]. Furthermore, the zebrafish animal model allows combining different MOs at the same time to unravel possible synergistic effects between different genes.

Case-example: inhibition of genes regulating angiogenesis/bone calcification with MO

A set of 100 embryos at one-cell stage are injected at three different concentrations with a micro-injector. The assay also includes a control MO injection. Once injected, embryos are scored for a specific phenotype at the defined stage.

In this case-example the analysis is carried out using a transgenic line or a specific histological staining (Figure 1) to visualize the organ or pathway of interest.

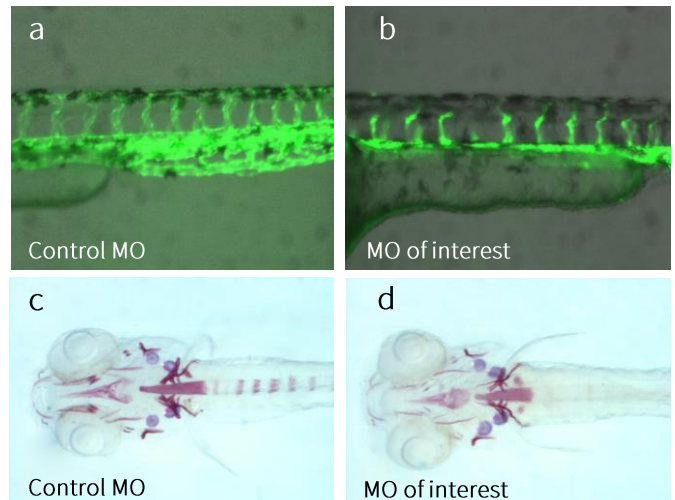


Figure 1. (a) Zebrafish embryo with a normal developed vasculature. (b) Zebrafish embryo showing an inhibition of angiogenesis as a consequence of the injection with a MO. (c) 9-day-old embryo with calcified vertebrae. (d) Injection of a specific MO shows the involvement of the gene in the process of calcification.

2 DISEASE MODELS GENERATION

Biobide is specialized developing zebrafish disease models to be used in preclinical stages for drug discovery and/or target identification.

Thanks to its wide experience and know-how, Biobide has the ability to generate transgenic or mutant zebrafish models of diseases through different technologies/techniques (Table 1).

Genetic mutations

- ENU induced mutations
- Viral induced mutations
- Zinc Finger mutations

Transgenic induced gene alterations

- CRISPR/Cas and TALENS
- Protein overexpression
- Tissue specific toxicity

Drug induced metabolism alterations

- Hormone
- Cytotoxic
- Others



Table 1. General technologies/techniques used by Biobide to generate transgenic or mutant zebrafish models.

Biobide also has the ability to develop zebrafish xenograft models [3], interesting for analyzing the effects produced, such as tumor generation, proliferation, migration and metastasis.

General toxicity (acute toxicity, teratotoxicity) or organ-specific toxicity (cardiotoxicity, hepatotoxicity, neurotoxicity, ototoxicity..) assays, could be also evaluated together with the efficacy of the treatment(s).

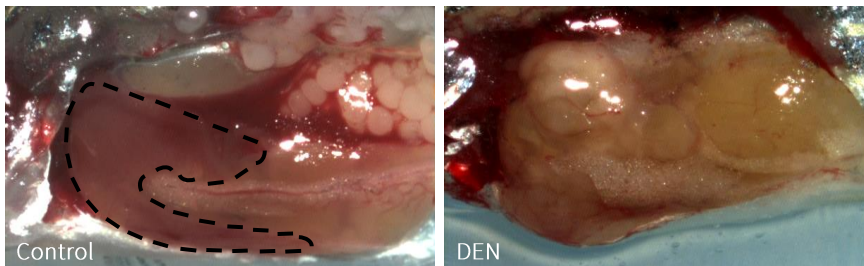


Figure 2. Hepatocarcinoma by chemical induction with diethyl nitrosamine (DEN). Immersion in DEN solution (100 ppm) of 2-month-old wild type zebrafish during 8 weeks. Tumors are first detected 1 month after DEN treatment (mainly in females).

- Morpholinos can be used in zebrafish as a tool to unravel the function of a specific gene helping in the target validation process.
- Target validation is a fast, statistically significant and highly repeatable assay, as well as the zebrafish disease model generation.
- Technological innovation has helped the zebrafish embryo gain ground as a disease model and to be an assay system for drug screening.

[1] Brent LJ, Drapeau P. Targeted "knockdown" of channel expression in vivo with an antisense morpholino oligonucleotide. *Neuroscience*. 2002;114(2):275-8.

[2] Deiters A, Yoder JA. Conditional transgene and gene targeting methodologies in zebrafish. *Zebrafish*. 2006;3(4):415-29.

[3] Eguiara A, Holgado O, Beloqui I, Abalde L, Sanchez Y, Callol C, Martin AG. Xenografts in zebrafish embryos as a rapid functional assay for breast cancer stem-like cell identification. *Cell Cycle*. 2011 Nov 1;10(21):3751-7.