

TARGET VALIDATION

■ Introduction

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. So far, many *in silico* and *in vitro* techniques have failed due to the pharmacological promiscuity of the compounds as well as the lack of complete information about protein interaction and compensation.

Zebrafish shows a great potential to be used in early stages of discovery, thanks to its properties that include transparency, easiness to treat with compounds, high conservation with other vertebrates, cost-effectiveness and possibility to generate transgenic lines targeting specific organs and pathways. Zebrafish has also been proposed as a good model to validate a target function due to its capacity to assess a specific process combined with a general toxicity assessment or drug screening. It also allows combining different morpholinos (MOs) at the same time to unravel possible synergistic effects among different genes.

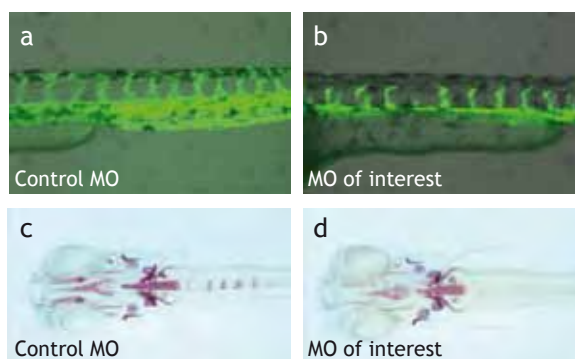
Different strategies and tools can be used to unravel the function of a specific gene. Among them, the use of Morpholinos (MO) - a phosphorodiamidate morpholino oligo- is a common one [1,2,3]. The Morpholino is a short sequence of antisense oligonucleotides used to inhibit the translation of a specific gene. There are two main mechanisms to knock genes down with MO: targeting to the ATG site or to the splicing site of a pre-mRNA. Morpholinos do not act as the siRNA degrading the target molecules, but binding to the specific sequence and impeding the translation of the mRNA.

■ Methods

Two case-examples are presented regarding genes regulating either angiogenesis or bone calcification. 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 order to visualize the organ or pathway of interest, the analysis was carried out using a transgenic line or a specific histological staining (Figure 1).

The assay is under the scope of the Good Laboratory Practice (GLP) certification obtained by Biobide.

Transgenic line



■ 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 days old embryo with calcified vertebrae. (d) Injection of a MO shows the involvement of the gene in the process of calcification.

■ Conclusions

- Morpholinos can be used in zebrafish as a tool to unravel the function of a specific gene helping in the target validation process.
- Zebrafish is a good model to find genes involved in specific processes and/or check activity of a desired gene in a specific process. It is also possible to combine target validation with a general toxicity assessment or drug screening.
- Target Validation is a fast, statistically significant, and highly repeatable assay.

■ Bibliography

- [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] Nasevicius A, Ekker SC. Effective targeted gene 'knockdown' in zebrafish. *Nat Genet*. 2000 Oct;26(2):216-20.