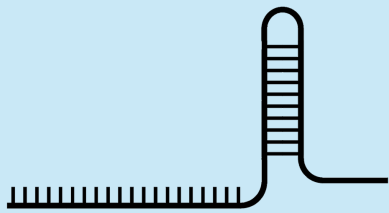


# SUCCESSFUL ZEBRAFISH KNOCK-INS



The 6 Essential Steps to a Successful Knock-in Project



## 1 Know Your Gene

Good knowledge of your gene, alternate isoforms expressed and related orthologs is required for designing an efficient experimental strategy.



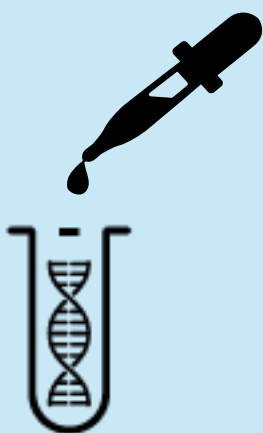
## 2 Choose Good sgRNAs

To ensure you use an sgRNA that guides efficient cutting, 2-3 sgRNAs with different recognition and PAM sites should be designed for each knock-in experiment



## 3 Validate sgRNAs

All sgRNAs should be validated in vivo to ensure that they are not toxic and that they efficiently guide Cas9 cutting of the DNA. [Click here for our article on validation.](#)



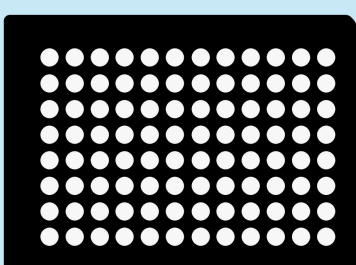
## 4 Create the Right Donor DNA Template

The size of your knock-in insert determines the type of template (double-stranded vs. single-stranded) and the size of the homology arms used.



## 5 Design and Test Primers

Developing a robust assay to detect your edit is critical when you begin screening the embryos produced by the F0 founders.



## 6 Screen, Screen, Screen

Knock-ins are notoriously low efficiency, and on top of that, the F0 germline is mosaic and can transmit multiple edits. The rate of transmission to your F1 generation will vary depending on when the F0 germline edit was made in development. Ranges from 3% to 50% have been observed. Be sure to grow enough F1 animals that you will be able to find your F1 heterozygotes.