Identify Disease (loci of interest) in humans and the candidate protein/isoforms of the protein implicated in the disease.

Search for homologous loci in fish genome. Look for variants, duplicate genes, to determine the exact identity (based on the sequence in the database) of the gene (locus) to be modulated by Talens.

Submit the Gene ID to Mojo Hand.

Pick an exon with a suitable Talen (preferably 2nd or 3rd exon in the gene). A suitable Talen ideally, would be one that (i) does not have certain RVDs repeated multiple times in the Talen sequence (based on our lab experience, pHD2), (ii) has a unique restriction site preferably in the center of the spacer region, (iii) the restriction enzyme preferably digests completely in PCR buffer and it **does not require** methylation activity to digest since PCR products are not methylated.

Search for suitable primers (not AT or GC rich), likely to work easily for subsequent genomic PCR screen, within ~500 bp of the TALEN arms

Amplify PCR products using the genomic screen PCR primers and genomic DNA from ‘candidate’ wild-type fish by mating several ‘Wild Type’ fish and collecting 2-3 embryos/clutch.

Sequence PCR products from embryos from different clutches and confirm that there are no polymorphisms in the Talen binding region or in the restriction enzyme site being used to screen for Talen activity. The PCR products may need to be cloned if sequence is unclear due to polymorphisms or differences in alleles.
8. If these sites are altered due to polymorphisms, submit the manually sequenced region in FASTA format to Mojo Hand and redesign your Talens. If it is difficult to design optimal talens in that stretch of sequence, choose another region or exon, repeat steps 6 and 7 until you find a region where there are no polymorphisms in the Talen binding sites or in the restriction enzyme screening site.

9. Once a pair of Talens has been chosen, amplify the region for screening Talen activity and test the screening primers on Wild Type fish embryos. Digest with the screening enzyme and confirm complete digestion.


11. Synthesize RNA from Talens cloned in Goldy Tal. Check for RNA integrity on an agarose gel. Aliquot RNA samples and freeze at -80C.

12. Inject embryos with varying amounts of RNA to determine an optimal dose for injecting Talen RNA. Could start with 50 pg and check for toxicity.

13. Lyse injected sibling embryos (8-10) and screen for Talen activity via PCR and restriction enzyme digest. Clone and sequence the ‘mutated’ PCR products from 4-5 embryos and 2-3 colonies per embryo.

14. Raise sibling embryos. When fish are mature check for somatic Talen activity by lysing fin-clips from 8-10 fish, followed by PCR, and restriction enzyme digest of PCR products.
15. Separate fish that test positive for Talen activity.

16. Outcross fish from step 13 with wild–type fish.

17. Collect F1 embryos and test for germline embryos as in step 12.

18. Clone and sequence germline mutations and raise germline fish.