OBTAINING TALENS FROM THE MUTATION SERVICE CORE
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I. Choosing a target sequence

A. Client chooses a target gene and submits to the Core a *TALEN Request Form* that includes: Client's name and email, PI name, account to be charged, gene name, and sequences of two 'potential target regions' of the gene.

Criteria for 'potential target regions':

- Preferred minimum size of region: 200bp
- Often the two 'potential target regions' would be two different exons. One large exon can be submitted instead of two separate regions. To create a null allele it is best to target an exon that is as 5' in the gene as possible, and certainly that is 5' of a sequence encoding a conserved portion of the protein (for example upstream of a DNA binding domain).
- If only small exons are available, please submit the exon along with 20bp of upstream and downstream intron sequence
- We normally avoid exon 1 to avoid possible alternative transcription start sites, if the client can rule these out it is ok to target exon 1
- Beware of alternatively spliced exons

B. Core identifies 2-3 best candidate TALEN target sequences and sends this information to Client. A typical TALEN target will be 15-21bp binding site – 14-18bp spacer – 15-21bp binding site

C. Client chooses one of the candidate target sequences and **verifies** that their model organism to be used has the exact TALEN-binding sequence without polymorphisms.

One approach to doing this in Zebrafish is:

- Fin clip and prepare genomic DNA from a series of adults. Amplify from one gDNA sample 300 – 600 bp of genomic sequence that includes the target site and sequence the amplicon. If the sequence matches the candidate target, analyze more adults. If the sequence does not match the candidate target, try a different WT strain.
- Genotype additional adult fish (4 - 8 from each sex from the same strain). This can be accomplished by either of two strategies: 1) sequence additional adults; 2) use High Resolution Melt Analysis to determine if the additional adults have any signs of polymorphisms in the region. We highly recommend preparing HRMA primers, as they will likely be used to detect mutations. It is advisable to design the sequencing primers above so that one of the two sequencing primers can also be used for HRMA assays (HRMA primers should generate a 75 – 120bp amplicon that includes the entire target sequence). Once you find adults that carry the target sequence, the target sequence is considered **verified**.
- Once the target site is **verified**, contact the Core to request TALEN gene construction against the target. The Core will not begin construction until the target is verified.

II. TALEN gene construction

Typically the Core will generate two plasmids, encoding Left and Right Talens, in a pCS2-TAL3 plasmid backbone. Constructions are sequence-verified and are completed in less than two weeks.

III. Optimizing HRMA conditions to detect polymorphisms

The Client needs to prepare primers for HRMA and be able to detect newly induced polymorphisms at the target site. Please the Core (mutrus@genetics.utah.edu) for any help with the design or implementation of HRMA detection of polymorphisms.

IV. TALEN-induced mutagenesis in Zebrafish

Client prepares capped in vitro synthesized mRNA and injects 1-cell embryos. Typically we start by co-injecting 50pg of each mRNA (left and right TALEN) and upwards of 300pg of each mRNA. Typically we analyze for induced mutations in 24 injected 1 dpf embryos and 4 control un-injected embryos. Extract DNA and analyze by HRMA. We often obtain 100% injected embryos with evidence of mutations. If this is the case, assume that you will recover germ line mutations from about 90% of the injected fish that grow to adulthood. We suggest you raise 50 – 75 to adulthood.

V. Feedback to the Core

We need to monitor how well our methods are working. This is a new technology, and it is still being optimized! For example, we want to collect information as to what is the optimal spacer size. We rely on your help! Upon testing the induction of mutations, please contact the Core and report to us:

- Gene name and TALEN name, # injected embryos with evidence of new mutations / # injected embryos assayed. Please ask for advice and give us feedback. We are here to be helpful.