

## **Pipeline for generating stable large genomic deletions in zebrafish, from small domains to whole gene excisions.**

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### **Supplemental material**

#### **Figure S01.**

Tyrosinase (*tyr*) cDNA sequence presenting gene ORF as well as sgRNA target sites. Snapgene file extension.

#### **Figure S02.**

Example of 2dpf wild type embryos injected with 400pg of Cas9 protein complexed with either (A-B) a sgRNA-free solution or (C-D) a 400pg four-guides anti-*tyr* cocktail. The absence of pigment evidence bi-allelic knockout of the *tyr* gene. Scale bar 200µm.

#### **Figure S03**

DNA/oligo template to be used for designing optimised top and bottom oligos, easing the generation of reproducible high yield of sgRNAs. The 20-bases feature highlighted in red and annotated “Genomic target from CRISPRscan/CHOPCHOP to include” should be replaced by the guide of interest. As described in the method section, both Top and bottom (complementary sequence/oligo) have to be ordered and annealed prior to RNA synthesis, thereby abolishing the need to perform an elongation or amplification step (which seems to often result in inconsistent yield). Snapgene file extension.

#### **Figure S04**

Annotated neurexin sequences (snapgene file extension) as well as sequencing data and alignment analysis (from bulk PCR or gel extracted products).

#### **Figure S05**

Detailed step-by-step protocol.

### Figure S06

Schematic workflow describing how to manually read a sequencing chromatogram presenting two distinct alleles, and how to identify the associated mutation/deletion (figure made based on the chromatogram file *IA\_TMR\_8-6R\_180.ab1* presented in supplemental zip file S04). For complex cases, such as in the presence of multiple deletions, we recommend following up with a gel extraction to confirm the presence and the nature of the mutation(s).

### Figure S07

Sequencing results associated with **figure 03**.

### Table S01

DNA oligos and methods used in this study for generating our sgRNAs.

### Table S02

DNA primers used in this study.

### Table S03

Template/guide for easing the preparation of Cas9/multiple-sgRNA cocktails.

### Table S04

Table summarising the different neurexin mutations selected during our study as well as their incidence and the Cas9/sgRNA mix ratio initially used. \*Sequence addition impacting the transmembrane domain region. “Full” for whole gene deletion, “Partial” for removal of the  $\alpha$ -isoform and “tmr” for the transmembrane domain region.