

1. Identify and sequence zebrafish orthologue
2. Design morpholino (MO/gene); at least one splice blocker MO
3. Assess maternal transcript expression

Test efficacy of splice-blocking MO

1. Inject 12 ng MO
2. RT-PCR at 24 hpf to test for altered splicing

No

Redesign MO

Altered
splicing?

Yes

Titrate MO dose (1-12 ng) injected at 1-2 cell stage; starting at 12ng and titrate down as needed

MO-knockdown analysis (>60 embryos)

1. 24 hpf: general morphology, viability, non-specific toxicity
2. 48 hpf: Laterality defects (*cardiac looping, curly phenotype, Otolith development, hydrocephalus*).
Heart rate, contractility, rhythm, chamber size
1. 72 hpf: Blood circulation and embryo movement
2. 7 day: Kidney cysts, embryo morphology, survival rate

Output

1. Create datafile (gene architecture, efficacy of splice-blocking MO, Phenotype Table).
2. Upload datafile to GNomEx; associate with topic/subtopic
3. Update cumulative log of results (gene name, cardiovascular phenotype, human correlate, link to GNomEx. Send to NERI for public website
4. Consultation w/referring center.