Identification and genotyping of zebrafish with *lmna* KO

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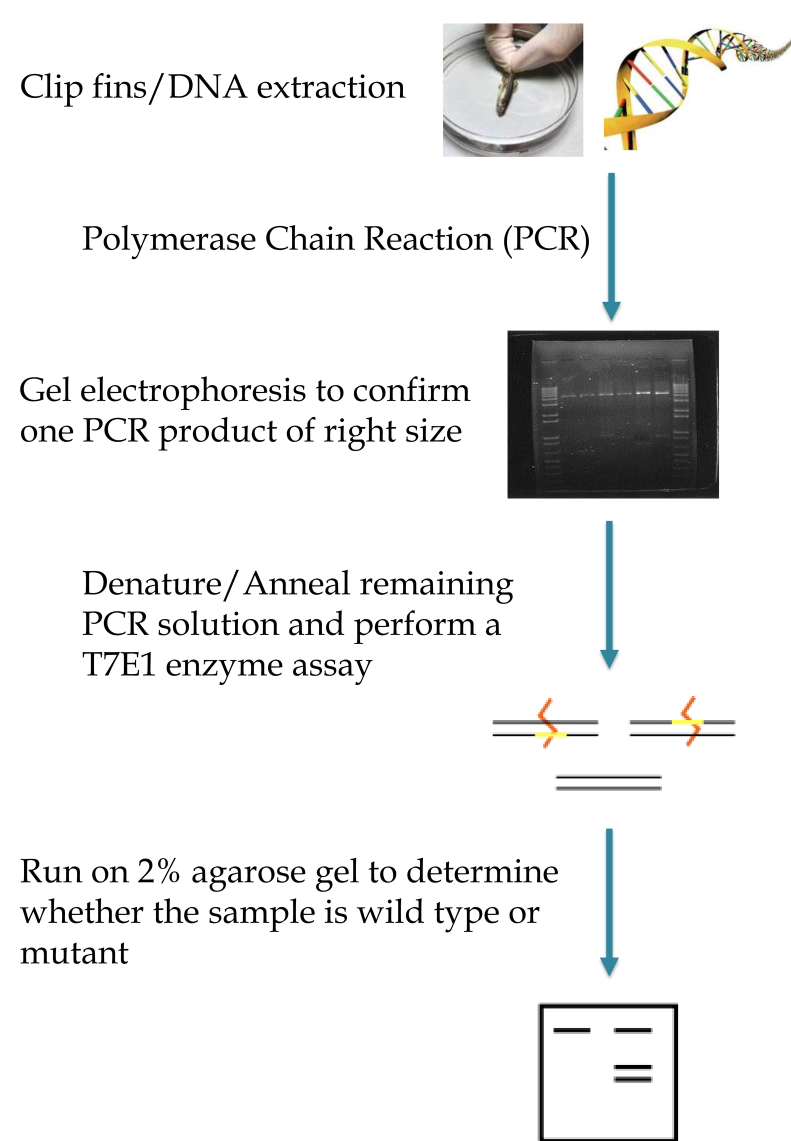
Background

The lamin A/C protein coded by the *LMNA* gene is involved in many nuclear processes such as maintaining nuclear stability. Dilated cardiomyopathy (DCM), atrial fibrillation (AF) and Emery-Dreifuss Muscular Dystrophy (EDMD) are diseases associated with mutations in *LMNA*[2,5]. The CRISPR/Cas9 technology was used to knockout (KO) *lmna* in zebrafish. This technology is a system for targeted genomic cleavage. Guide RNA (gRNA) directs the Cas9 enzyme to the target site where Cas9 induces a double stranded break (DSB). The repair of the DSB may result in insertion/deletion which interrupts the gene potentially leading to gene KO [6]. One cell zebrafish embryos were micro-injected with the Cas9 enzyme mRNA and the gRNA that targets *lmna*.

Research Objective

To determine which of the (now adult) micro-injected fish carry the *lmna* interruption.

Methodology



Results/Discussion

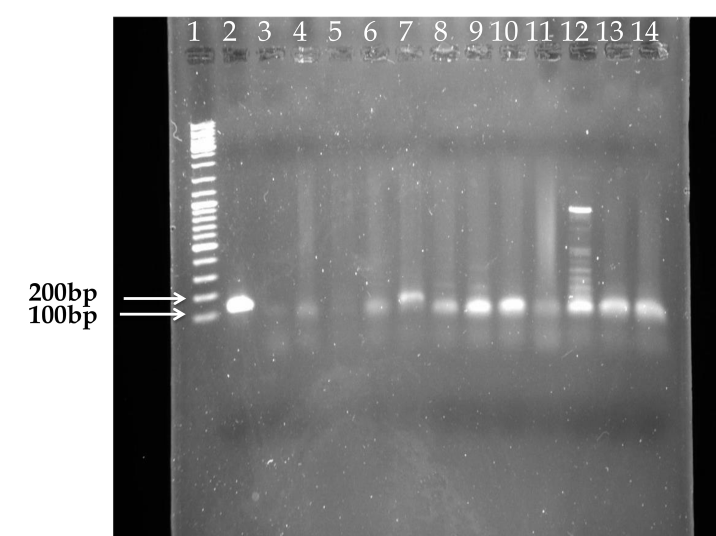


Figure 1: PCR was run on fin clips and a 5 uL aliquot was loaded on a 1% agarose gel to verify the amplicon. The gel was run for 40 minutes at 120V. Lane 1: GeneRuler – DNA Ladder Mix, lane 2: positive control (β-actin), lane 3: negative control (no DNA), lane 4 and 6: wt control for fish micro-injected with gRNA targeting *lmna* exon 7, lane 5: wt control for fish micro-injected with gRNA targeting *lmna* exon 2, lane 7: DNA sample from a fin clip of a fish that has been injected with Cas9 mRNA and *lmna* gRNA targeting exon 2, lanes 8– 14: DNA samples from fin clips of different fish that have been injected with Cas9 mRNA and *lmna* gRNA targeting exon 7.

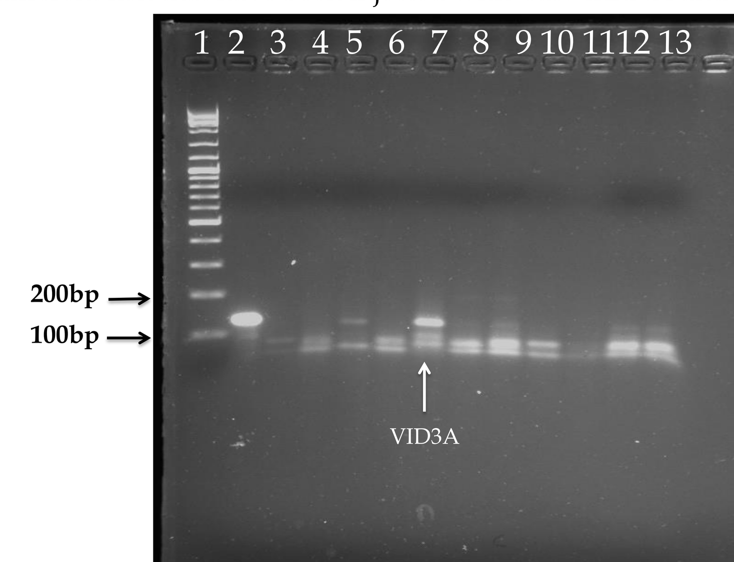


Figure 2: PCR samples digested by the T7E1 enzyme was run on a 2% agarose gel for 50 minutes at 120V. The following are run on each lane - 1: GeneRuler – DNA Ladder Mix, 2: positive control (β-actin), 3: negative control (no enzyme), lane 4 and 6: wt control for fish micro-injected with gRNA targeting *lmna* exon 7, lane 5: wt control for fish micro-injected with gRNA targeting *lmna* exon 2, lane 7: DNA sample from a fin clips of a fish that has been injected with Cas9 mRNA and *lmna* gRNA targeting exon, lanes 8-13: DNA samples from fin clips of different fish that have been injected with Cas9 mRNA and *lmna* gRNA targeting exon 7.

Lane #	Zebrafish	Targeted site	T7E1 assay	# of bands	Genotype
4,6	IE3A	ctrl – WT exon 7	✓	2	WT
5	IE3A	crtl – WT exon 2	✓	2	WT
7	VID3A	*lmna* 2	✓	3	Mutated
8	ID8A	*lmna* 7	✓	2	WT
9	ID10E	*lmna* 7	✓	2	WT
10	IE1A	*lmna* 7	✓	2	WT
11	ID10A	*lmna* 7	✓	2	WT (?)
12	ID10C	*lmna* 7	✓	2	WT
13	ID10D	*lmna* 7	✓	2	WT

Table 1: Summary of results for identifying the genotype of the DNA samples from fin clips of fish that have been injected with Cas9 mRNA and *lmna* gRNA targeting either exon 2 or 7. The lane number corresponds to Figure 2. Zebrafish were coded by rack and tank number. Each fish had a determined targeted region, either exon 2 or 7. The genotype is identified by the number of bands and the size of the bands compared to the controls.

The first gel (figure 1) is ran to ensure that only one product of appropriate size is successfully amplified. Figure 1 shows one band in each lane (except for lane 12) which corresponds to the expected band size which is between 100-200bp. Since the sample in lane 12 had multiple bands, it was not included in the next step.

The second gel (Figure 2) is ran after the T7E1 enzyme digest to check the digestion profile of the samples compared to the wild type. Lane 7 which has the sample from a fish that was micro-injected with the gRNA targeting *lmna* exon 2 presents with three bands as opposed to the two found in the wild type (lane 5). This suggests that the sample in lane 7 is from a mutant fish. Three bands are due to the endonuclease I enzyme cutting heteroduplex DNA. For lanes 8 to 13, a wild type genotype was determined because the number and the size of the bands are the same as the WT controls (lanes 4 and 6).

Conclusion

Our results suggest that at least one of our fish (*lmna* exon 2 targeted) has the *lmna* interruption. In order to determine whether an insertion or deletion occurred at the targeted site, sequencing can be performed on this fish' DNA sample or on its progenies. Sequencing will also indicate if the fish is homozygous or heterozygous regarding the *lmna* disruption.

Future Work

LMNA caused DCM and EDMD tend to be severe [2]. There is currently no cure for DCM or EDMD and only treatments are available to alleviate symptoms [3]. Thus, the development of zebrafish *lmna* KO model can be used to study the development of cardiac and skeletal muscle laminopathies and unravel the molecular mechanisms involved in the pathogenesis of laminopathies and hopefully help in developing diagnostic and/or therapeutic tools for patients with these diseases.

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