BENJAMIN DUNCAN and JAMES WALTERS, Dept of Applied Science, Bluefield State College, Bluefield, WV 24701. Constructing a NPC1L1 Knockout Line in Zebrafish using CRISPR Cas9.

How the intestinal absorption of lipids impact dyslipidemias such as obesity and diabetes is currently under debate. NPC1L1 is a critical transport protein in intestinal enterocyte cholesterol absorption. To understand the mechanism of *NPC1L1* uptake within the enterocyte, we are creating a *npc1l1 -/-* (knockout) line in zebrafish. We hypothesize that the *npc1l1 -/-* larval zebrafish will show reduced intestinal cholesterol absorption when challenged to a high fat diet compared to wild type larvae. To do this, we have used a gene editing tool known as CRISPR to create genomic mutations within the *npc1l1* sequence. The CRISPR complex will create double stranded breaks that the embryo will try to mitigate by using non-homologous end joining (NHEJ) repair. We hypothesize some of the mutations will result in premature stop codons disrupting the translation of the protein. The crRNA:tracrRNA guide complex was assembled and Cas9 mRNA was amplified, the crRNA:tracrRNA complex and the Cas9 mRNA were injected into embryos where the complex and Cas9 combine to form the CRISPR duplex. Two of four guide complexes were injected showing survival rates of 16.7% (complex 329) and 8.7% (complex 291). After raising injected embryos, we will screen for frame shifts by sequencing to identify mutations where we will then raise a *npc1l1 -/-* line. To date, no frameshift mutation has been observed, however; we hypothesize that newly placed PCR primers will validate our hypothesis.

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