To support a collaboration between Dr. Cat Lutz of The Jackson Laboratory and the Friedrich’s Ataxia Research Alliance, our Model Generation Services (MGS) team undertook a project to create a conditional knockout allele of the Frataxin (Fxn) gene using the Cre-loxP Recombination System. Friedrich’s Ataxia is a progressive degenerative disease marked by neurodegeneration and cardiomyopathy. Genetically, it is conferred by an autosomal recessive Fxn allele. In order to learn more about the biology of this disease, a useful mouse model was desired. Fxn-null mice are embryonic lethal (Cossee et al., 2000), so a different strategy was needed. Thus, in order to allow for normal development of the mice but be able to study the resulting biology of Fxn knockout in a specific tissue or at a later developmental stage, Dr. Lutz’s team wanted to create a conditional knockout allele of Fxn. The Cre-Lox system is commonly used for this purpose. In this strategy, recombinase loxP sites are engineered into a gene sequence of interest. These sites are placed into the genome such that expression of the targeted gene is not affected. In the presence of Cre, the region spanned by the loxP sites is excised, resulting in the “knockout” of the gene of interest at the time and in the cells and/or tissues in which Cre Recombinase is expressed.

The challenge with creating a conditional allele is that the template provided to repair the Cas9-excised locus often requires a relatively large sequence. If the loxP sites flank an exon, for instance, the template for repair might span multiple kb. The longer the sequence to be inserted (also, the larger the template that must be provided), the lower the efficiency of the properly integrated insertion. The strategy our MGS team devised to create the conditional allele of Fxn was to flank exon 2 of the gene with loxP sites, as shown in Figure 1. In this strategy, the length of the “floxed” or loxP-flanked sequence was 300 bp. However, in order to maximize the efficiency of the homology-directed repair, long homology arms flanking this region were incorporated into the donor plasmid. The left homology arm was approximately 2.1 kb while the right was 2.8 kb. In sum, the repair template was 5.2 kb.
Figure 1. Strategy to introduce loxP sites flanking exon 2 of the Fxn gene. Long left and right homology arms (HA) allow for high fidelity repair of Cas9-induced DNA break, as well as multiple short and long-range PCR options using primers at the regions indicated with blue arrows.

Using oligo sequences as diagrammed in the figure, both long-range and short-range PCR analyses were utilized to determine whether the potential founder mice carried the properly inserted loxP sites. Founder mice were produced and our genetic validation strategies were able to efficiently identify founders resulting in the delivery of N1 animals within our expected timeline of 33-37 weeks.