



## Development of CRISPR/Cas9 mutagenesis and transgenics in the fire ant

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To better understand gene function in fire ants, it is necessary to develop techniques for genetic manipulation. CRISPR/Cas9 mediated mutagenesis has revolutionized the testing of gene function in both model and non-model organisms, including two basal ant species. We have developed a fire ant embryo microinjection protocol for CRISPR/Cas9 mutagenesis. As proof-of-principle we generated individuals mosaic for the knockout of *spitz*. In *Drosophila*, *spitz* has multiple functions, including oenocyte formation. Consistent with conserved function, mutating *spitz* results in larvae with reduced or eliminated oenocytes. We verified that injected individuals have high rates of mutations, with some having >90% mutation rate per individual. Not all individuals mutated for *spitz* based on PCR assays show an oenocyte phenotype; a possible explanation is genetic redundancy. To complement gene mutation, we are developing transgenesis. We have used the *piggyBac* transposon to generate mosaic individuals expressing GFP under the fire ant actin promoter. Our success indicates that both mutagenesis and transgenesis can be used to study gene function in the fire ant at the individual, and possibly the social, level.