Thermal tolerance is a quantitative trait influenced by a combination of genetic and environmental factors. It is an essential survival skill in many species, particularly *Drosophila melanogaster* (fruit flies), whose body temperature changes with the ambient temperature. Several studies have characterized thermal tolerance in fruit flies, however, we still know little about the genetic basis of this complex trait. Regulatory changes that alter gene expression are one of the potential genomic changes that could influence an individual's thermal tolerance. As a mode of gene expression modification, alternative splicing is instrumental in diversifying protein expression and forming different isoforms within the genome. We used the Drosophila Synthetic Population Resource (DSPR), a large, multi-parental population of Recombinant Inbred Lines (RILs) consisting of approximately 1,600 RILs, to investigate the role of alternative splicing in thermal tolerance. A previous study in our lab identified 8 QTL influencing thermal tolerance by assaying a total of 741 RILs (~40,000 individual) of flies at 41°C. We first validated these thermal tolerance measurements by assaying a subset of high and middle thermal tolerance RILs at a higher temperature of 43°C to determine the upper thermal limits of the DSPR RILs and whether the assay was replicable. Next, we hypothesized that where alternative splicing occurs within the genome, flies with high thermal tolerance will have similar isoform formation and gene expression in comparison to the low thermal tolerance population. We performed pooled RNAseq on the 7 high thermal tolerance and 7 low thermal tolerance RILs and followed with the "New Tuxedo Package" RNAseq analyzing tool to identify splice variants and isoforms that differ between the high and low thermal tolerance pools. We then linked these results to our QTL mapping results by identifying splice variants that occur within our QTL intervals of interest to identify potential candidate genes. By identifying splice variants within the DSPR, we aim to identify which genes are causal in thermal tolerance and how the diversification of gene expression through alternative splicing affects phenotypic expression.