**CRISPR/Cas9 *Drosophila* pooled knockout library**

**Library description**: The library consists of on average 3 unique sgRNAs targeting common coding exons shared between the maximum number of transcript isoforms for each *Drosophila* genes. The total library size is 40,279 guides targeting 13,501 genes, with 8,989 genes covered by 3 or more sgRNAs.



A tab delimited text file (compatible with most spreadsheets) describing the genes, sgRNA sequences and counts in the original library, and a BED file (that can be uploaded to genome browsers such as UCSC) describing the locations of the sgRNAs are available for download. Original data is provided at the NCBI Gene Expression Omnibus (GEO) under the accession number GSE67339.

The vector used carries both the sgRNA and Cas9 components, and is outlined below (Addgene #49330). It expresses the sgRNA from a U6 promoter, and Cas9 and the puromycin resistance gene from an Actin 5c promoter.



**Screening:** After amplification of the library DNA according to the provided protocol, the library needs to be diluted in inert carrier DNA to at least a 1:100 dilution, with the aim of obtaining approximately 1 sgRNA per cell upon transfection. Cells are transfected with the diluted library, and selected in puromycin (5 ug/ml), beginning 2 days after transfection to obtain cells that have integrated the sgRNA constructs. Cells are then grown for a further 3-10 days to allow mutations to be produced, and proteins to be depleted. Screening can then be performed (e.g. for drug resistance), and sgRNA populations between control and treated populations compared by high throughput sequencing.

It is important to maintain coverage of the library throughout the screening process, and ideally 500-1000 cells should be screened per sgRNA (20-40 million cells) in order that it is possible to perform statistical analysis on the resulting population. This represents the population after transfection and selection for integration of the sgRNA/Cas9 plasmid that would be screened after selection, so the original number of cells may be significantly higher. However, the exact number of cells necessary depends on the effect size of the treatment applied, with smaller effects requiring larger numbers of cells.

When analyzing the sgRNA populations after treatment, it is equally important to amplify gDNA from an equivalent number of cells, and PCRs therefore need to be performed from a substantial amount of starting material, and high throughput sequencing performed to at least this number of reads.