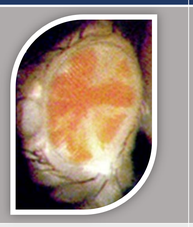
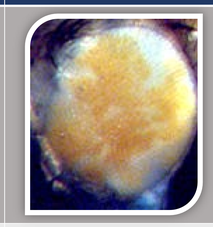
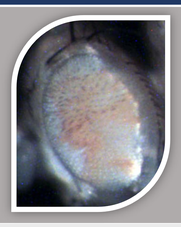
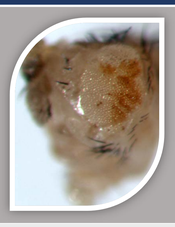
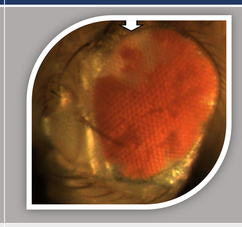
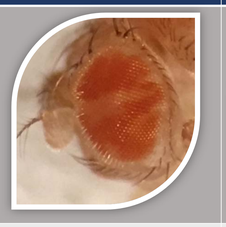
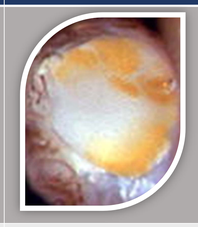
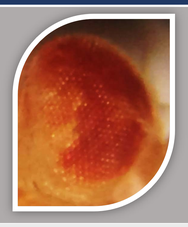
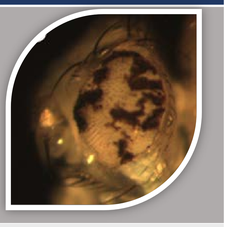
WGS and bioinformatics analysis combined with genetic mapping of EMS mutants in *Drosophila melanogaster* with balancer chromosomes

Kayla Bieser[1], Richard Tillett[2], and Jacob Kagey[3]

[1]Nevada State College, Henderson, NV kayla.bieser@nsc.edu; [2]University of Nevada Reno, Reno, NV rltillett@unr.edu; [3]University of Detroit Mercy, Detroit, MI kageyja@udmercy.edu

Table 1: Genetic Screen, Complementation Mapping, and Whole-Genome Sequencing candidate genes for EMS *Drosophila melanogaster* mutants

Mutant	*Dark* [82] Control	*Cos2*	*L.3.1*	*H.2.2*	*A.4.4*	*B.2.16*	*B.2.13*	*N.1.4*	*L.3.2*	*N.1.1*
Mosaic Eye										
Failure to Complement 2R(Df)kit (bp)	NA	2R:7,382,176..7,387,115	2R:21,522,420..21,559,977	2R:17,716,263..17,748,161	2R:22,592,996..22,661,827	None	2R:8,655,629..8,724,129	None	2R:11,823,771..11,892,100	2R:21,056,798..21,088,247
Alleles Failed to Complement	NA	*Cos2*	*Egfr* 10.17912/micropub.biology.000098	None	None https://doi.org/10.17912/MICROPUB.BIOLOGY.000069	None	*Ptc* https://doi.org/10.1016/j.mod.2012.05.007	None	None	*Cpa* 10.4236/abb.2016.710036
Unique SNPs from WGS	NA	*72*	*49*	627	113	33	*37*	39	38	*29*
Gene candidate from WGS	NA	*Cos2*	*Egfr*	Hyccin	3	1 possibly linked to *Dark*^{82}	*ptc*	39 possible SNPs	none within FTC region	*cpa*
Effect	NA	Leu951 → Gln	Cys620 → Ser	Stop gained (Trp160 → stop)	3 missense within FTC	Unknown	Trp173 → Arg	Unknown	Unknown	Splice donor variant & intron variant

Fly-CURE Analysis

***w;FRT42D,Dark^{82}/CyO* treated with EMS**

Screen for mutants:

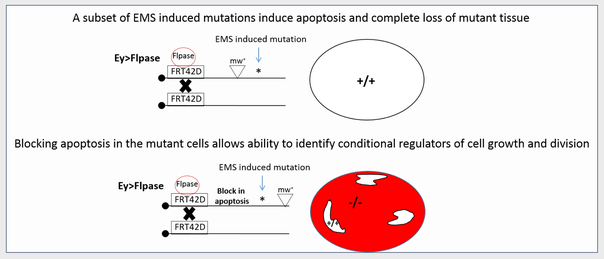


Fig 1: *Ey>Flp/FRT* system to screen for cell overgrowth utilizing *Dark*^{82} to block apoptosis in EMS mutant cells.

Complementation Mapping: Virgin ♀ *w;FRT42D,Dark^{82},EMS/CyO X Df(2R)/balancer*

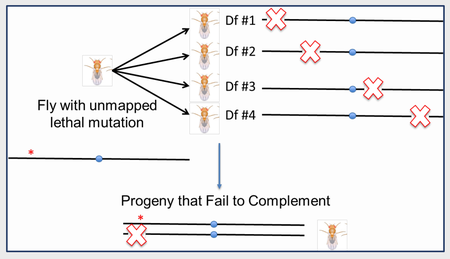


Fig 2: Model of complementation mapping to the BDSC 2R deficiency kit. Locations that failed to complement were further assessed by additional mapping and whole genome sequencing.

Additional complementation mapping to predicted alleles

Whole-genome sequencing (see Genomics Pipeline)

Verification of mutation utilizing Sanger Sequencing (Fig. 3)

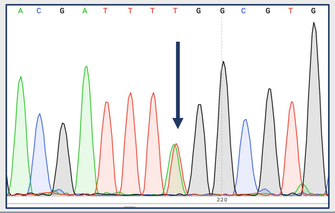


Fig 3: Sanger sequencing chromatogram of the heterozygous *cos2* mutation as predicted from genomic sequencing analysis. Arrow indicates the double peak.

Genomics Pipeline

Sequence 9 mutant lines + *Dark^{82}* control
(2 x Illumina NextSeq 500)

Align to *D. melanogaster* genome
(BWA vs. BDGP6)

Call variants, Filter INDELs & MNPs, Genotype
(bcftools pileup, bcftools call)

Predict SNP effects (missense, nonsense, splicing impacts)
(SnpEff, SnpSift)

Filter for SNP *exclusivity* to single mutant
(dplyr joins of all sites)

Discussion

- To our knowledge, this is the first time WGS methodology has been utilized in EMS mutated *Drosophila* with balancer chromosomes.
- WGS successfully identified SNPs in 5 of the 9 mutants and identified possible genes in the remaining 4 mutants for which future methods such as Crispr could be utilized.
- This is the first allele identified in the *Hyccin* gene.
- The combination of complementation mapping and WGS provides powerful methodology for the identification of EMS generated mutants.

Funding:
The project described was supported by a grant from the National Institute of General Medical Sciences (GM103440), ReBUILDetroit, NIH: UL1GM118982, TL4M118983, and RL5GM118981, and NIGMS IPERT grant 1R25GM125674-01.