Natural variation in R-loop formation in *Drosophila melanogaster*

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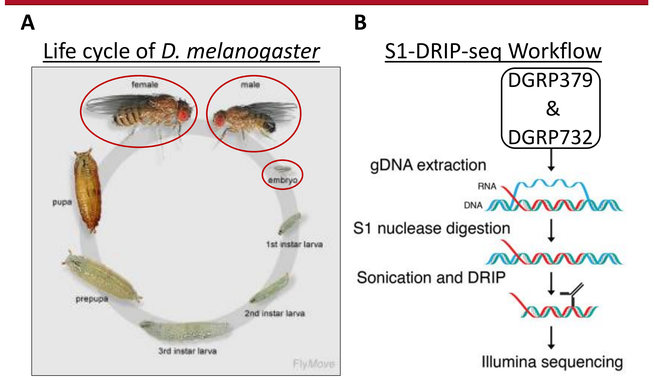
Introduction

R-loops are three-stranded nucleotide structures consisting of a DNA:RNA hybrid and a displaced ssDNA non-template strand. Originally viewed as a byproduct of transcription, R-loops are now recognized as important regulators of gene expression and genomic stability. R-loops are primarily associated with active Pol II transcription but have also been found at tRNA genes, mitochondrial genes, and transposable elements. Persistent dysregulation of R-loop maintenance can result in replication stress, DNA double-strand breaks, and chromosomal rearrangements that contribute to diseases such as neurological disorders and cancer. Although R-loops are conserved across cell types in mammals, little is known about natural variation in R-loop formation between individuals and throughout development.

Using DNA:RNA immunoprecipitation followed by high-throughput sequencing (DRIP-seq), we have mapped the R-loop profiles of two *D. melanogaster* individuals from the Drosophila Genetic Reference Panel (DGRP) at both embryonic and adult stages. Our current work assesses *in vivo* the underlying sequence and architectural determinants of R-loop formation and variation with respect to development and sex.

Methods and Materials



A) Embryos, adult females, and adult males were collected and used for subsequent DRIP-seq. B) Two wild strains of *D. melanogaster* DGRP379 and DGRP732 were processed for DRIP-seq from the stages indicated in *A*. S1 nuclease digestion preserves R-loop structures during sonication, allowing for immunoprecipitation of R-loops from highly repetitive regions of the genome.

DRIP-seq peak-calling and shared peaks overlap

Table 1. DRIP-seq peak-calling and shared peaks across conditions

	Embryos		Females		Males	
	DGRP-379	DGRP-732	DGRP-379	DGRP-732	DGRP-379	DGRP-732
Rep1	8316	8644	20783	19438	14287	15952
Rep2	10467	6916	18194	19340	13636	15429
Shared	*4797*	*3718*	*14056*	*14668*	*9933*	*10943*

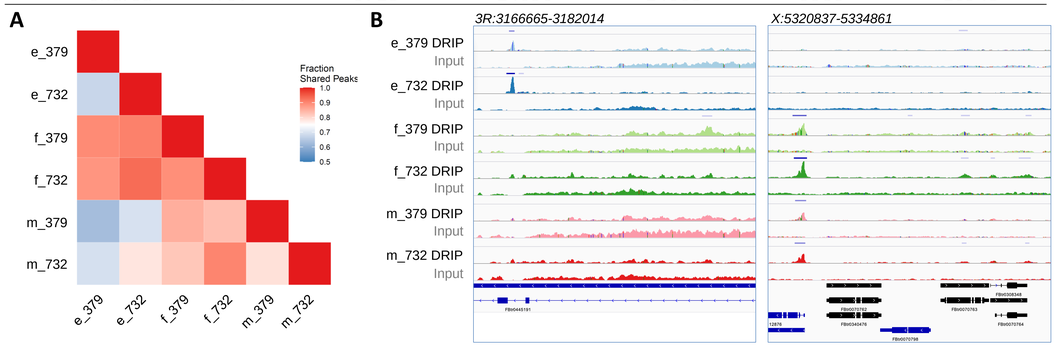


Table 1. Aligned DRIP-seq reads were used to call peaks with MACS2 (p-value cutoff = $1x10^{-3}$). Shared peaks between replicates were determined using *bedtools intersect*. A) Cross-conditional peak analysis plotted as fraction of shared peaks relative to the mate with fewer peaks called. B) DRIP-seq peaks unique to embryos (left) or adults (right).

Irreproducible Discovery Rate (IDR) Analysis

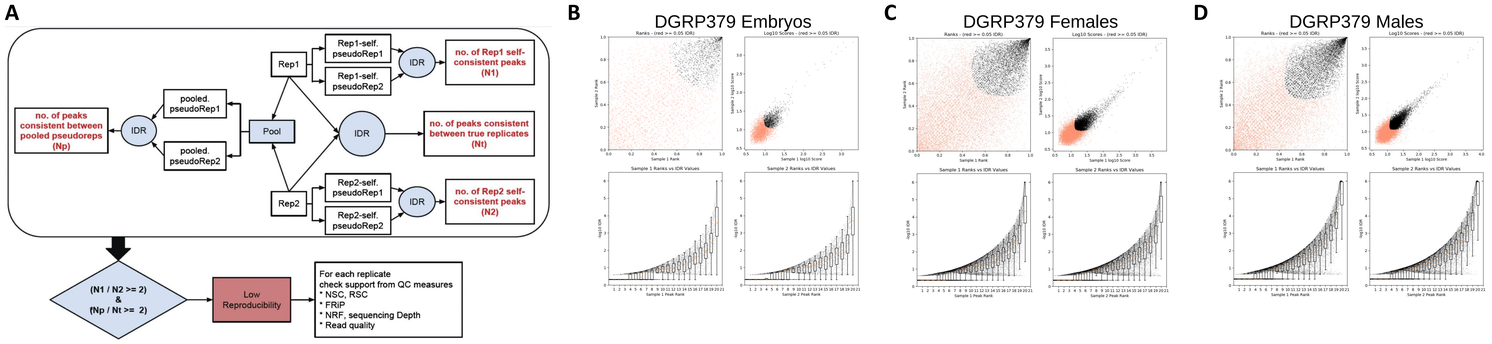
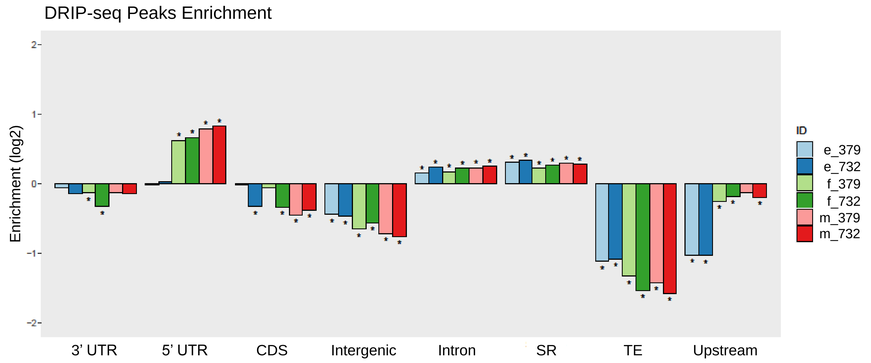


Table 2. IDR analysis determines the rate of reproducibility between replicates.

	Embryos		Females		Males	
	DGRP-379	DGRP-732	DGRP-379	DGRP-732	DGRP-379	DGRP-732
Nt	1352	936	5156	6170	3763	4227
Np	2778	1982	8073	8091	5836	6927
Np/Nt	*2.05*	*2.12*	*1.57*	*1.31*	*1.55*	*1.64*
N1	619	648	3824	3374	2639	3051
N2	793	389	2460	3094	1880	3175
N1/N2	*1.28*	*1.03*	*1.22*	*1.03*	*1.13*	*1.04*

A) IDR workflow from *ENCODE ChIP-seq Guidelines*. B-D) IDR Analysis of DGRP379 for embryos (B), females (C), and males (D). For each plot: *Upper Left* = Rep1 vs Rep2 peak ranks; peaks with IDR < 0.05 are colored red. *Upper right* = Rep1 vs Rep2 log10 peak scores; peaks with IDR < 0.05 are colored red. *Bo bottom row* = peak ranks vs IDR score for each true replicate. Table 2) Total peaks with IDR < 0.05 between true replicates (Nt) and pooled pseudo-replicates (Np) as well as between self-pseudoreplicates (N1 and N2) below a factor of 2 indicate good reproducibility.

R-loops are enriched/depleted at specific genetic elements



DRIP-seq peak enrichment (observed/expected) at specific genetic features was determined using *bedtools intersect*: 5' UTR, 3' UTR, CDS, Intergenic, Introns, short repeats, transposable elements (TE) and upstream regions. * indicates $p < 0.05$.

Acknowledgments and References

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1. Wahba L, Costantino L, Tan FJ, Zimmer A, Koshland D. S1-DRIP-seq identifies high expression and polyA tracts as major contributors to R-loop formation. *Genes Dev*. 2016;30(11):1327–1338. doi:10.1101/gad.280834.116
2. Landt SG, Marinov GK, Kundaje A, et al. ChIP-seq guidelines and practices of the ENCODE and modENCODE consortia. *Genome Res.* 2012;22(9):1813–1831. doi:10.1101/gr.136184.111