### Chapter 3: Nascent-seq

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#### Abstract

#### Introduction

#### Materials & Methods

#### Results

*Total mRNA levels correlate well with nascent mRNA levels at all timepoints*

As embryos at the stages utilized for transcriptome measurements are highly dynamic systems, with rapidly fluctuating levels of transcripts, we used Nascent-seq to confirm that the transcriptome measurements were indicative of actual transcription rates, and not overwhelmed by the various contributions of maternal mRNA contribution or differential rates of mRNA maturation and degradation. Sequencing of nascent RNA has been utilized to monitor fluctuating mRNA levels, for example following induction of an immune response in cell culture{Bhatt, 2012 #2995}. In *Drosophila*, nascent-seq has been used to monitor cotranscriptional splicing in adult flies{Khodor, 2011 #2081}, as well as circadian transcript cycling{Rodriguez, 2013 #1782}, in which the authors saw significant differences in total mRNA and nascent mRNA levels over ninety minute collections.

Embryos were collected at each timepoint and fractionated to isolate chromatin-associated RNA, which is enriched for nascent transcripts. Efficient fractionation was confirmed by immunobloting for cytoplasmic and nuclear components (*Supplemental figure*).

#### Discussion

#### References

#### Figures and Tables