Preparation of PRO-cap libraries: Cells were permeabilized and run-on reactions with two biotin NTPs were carried out with the appropriate number of cells per reaction as indicated in each experiment (method described previously and adapted from Mahat et al., 2016 with modifications). Drosophila S2 cells were spiked into the run-on reaction whenever necessary. RNA was isolated using standard TRIzol/chloroform method. Next, biotin-labeled RNA is enriched by affinity purification using streptavidin-coated magnetic beads. Two sequencing adapter ligations were performed. Between adapter ligations, cap state selection reactions were performed. Lastly, the RNA was reverse transcribed, PCR amplified, purified, and quantified before submission for sequencing.