SUPPORTING INFORMATION

Cell-free transcription in *Xenopus* egg extract

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Figure S1

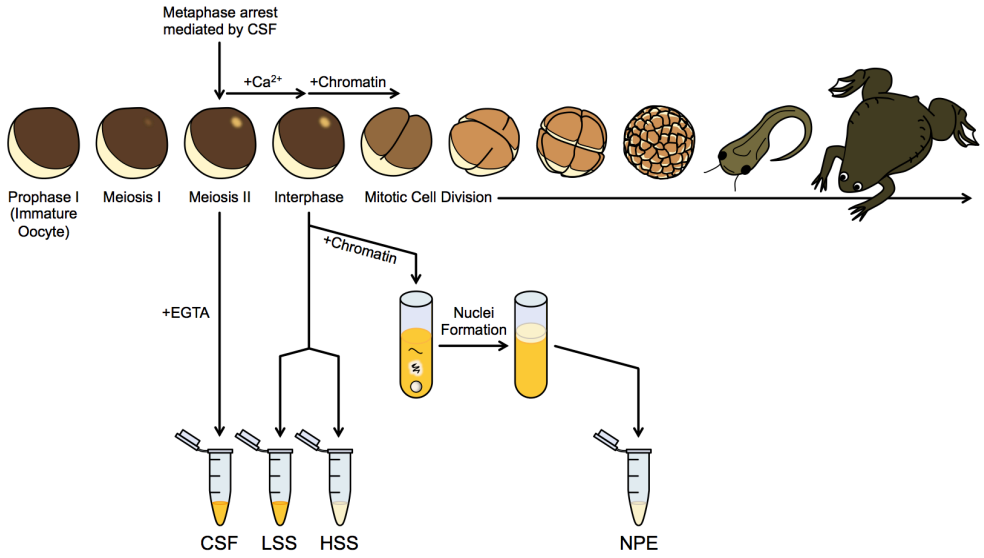

Figure S1. Schematic showing *Xenopus laevis* egg extracts prepared at different developmental stages. "Traditional" extracts are produced with mature, unfertilized *Xenopus* eggs. These eggs can be driven to interphase by the addition of exogenous calcium (Ca^{2+}), which mimics the release of calcium generated during fertilization (1). Some extracts derived from interphase-arrested eggs can promote chromatin decondensation and nuclear formation (2). Although nuclear formation assays support RNA polymerase III activity (involved in tRNA synthesis) (3), they do not promote mRNA transcription by RNA polymerase II (4). Unfertilized eggs can also be lysed in the presence of a calcium chelator (EGTA) to induce a mitotic state (5). Extracts made from mitotic eggs can be incubated with chromatin to promote spindle assembly (6). Spindle assembly is dependent on RNAPII-dependent centromeric transcription, but does not involve widespread transcription of the genome (7,8). Extracts have also been produced with immature oocytes isolated from frog ovaries (arrested in prophase I). Although these extracts retain some level of transcriptional activity, they lack robust chromatin assembly that is required for regulated transcription and pre-mRNA processing (9,10). CSF (cytostatic factor-arrested extract), LSS (low-speed supernatant of interphase eggs), HSS (high-speed supernatant of interphase eggs), NPE (nucleoplasmic extract).

Figure S2

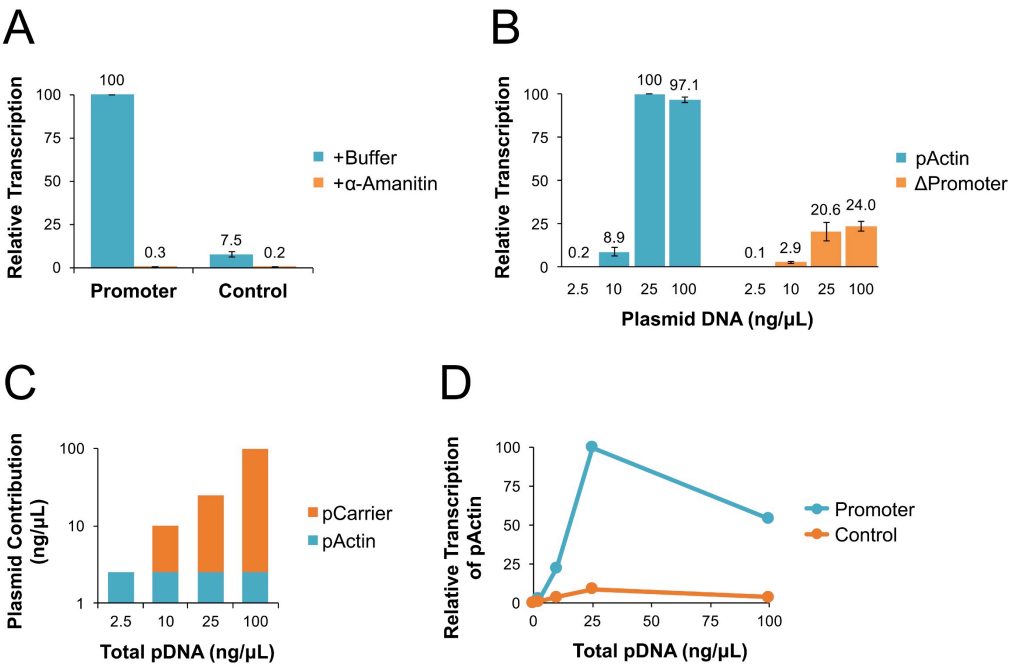

Figure S2. (**A**) Experimental replicates of Figure 2B are shown for transcription of pActin in NPE using Promoter and Control primers at 60 minutes (n=4). (**B**) Experimental replicates of Figure 2C (n=3). Data labels show average relative transcription and error bars represent +/- one standard deviation. (**C**) Schematic graph showing the amount of pActin and pCarrier included in each reaction depicted in *(D)*. (**D**) NPE was incubated with a fixed 2.5 ng/μL of pActin and increasing amounts of pCarrier for 120 minutes. RNA was then isolated and quantified by RT-qPCR using the Promoter and Control primers.

Figure S3

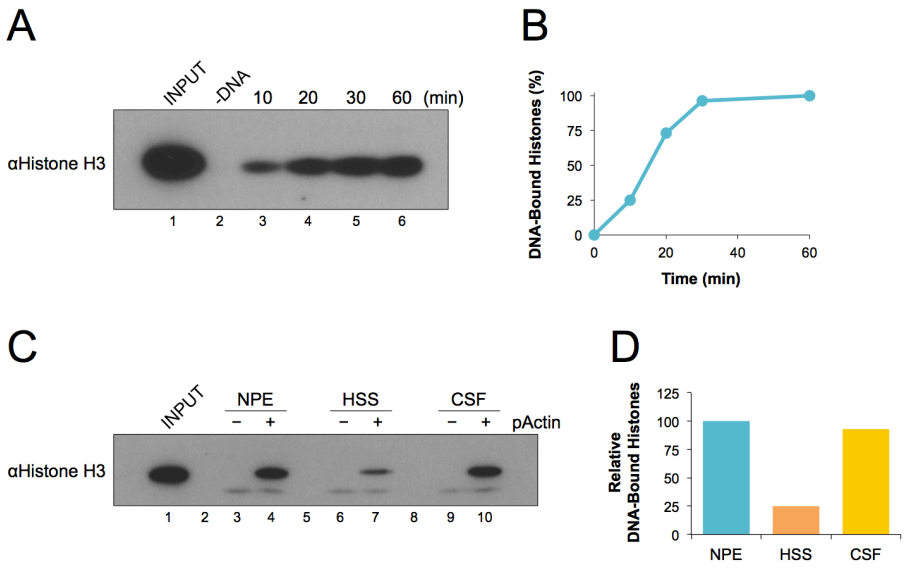

Figure S3. Chromatinization of plasmid DNA in NPE. (**A**) pActin was incubated at 10 ng/µL in NPE. At the indicated time, DNA-bound proteins were isolated by plasmid pull-down and analyzed by Western blot with histone H3 antibodies. (**B**) DNA-bound histone H3 from *(A)* was quantified and graphed relative to peak intensity. (**C**) pActin was incubated at 10 ng/µL in NPE, HSS, or CSF for 60 minutes and DNA-bound histone H3 was visualized as described in *(A)*. (**D**) DNA-bound histone H3 from *(C)* was quantified and graphed relative to binding in NPE.

Figure S4

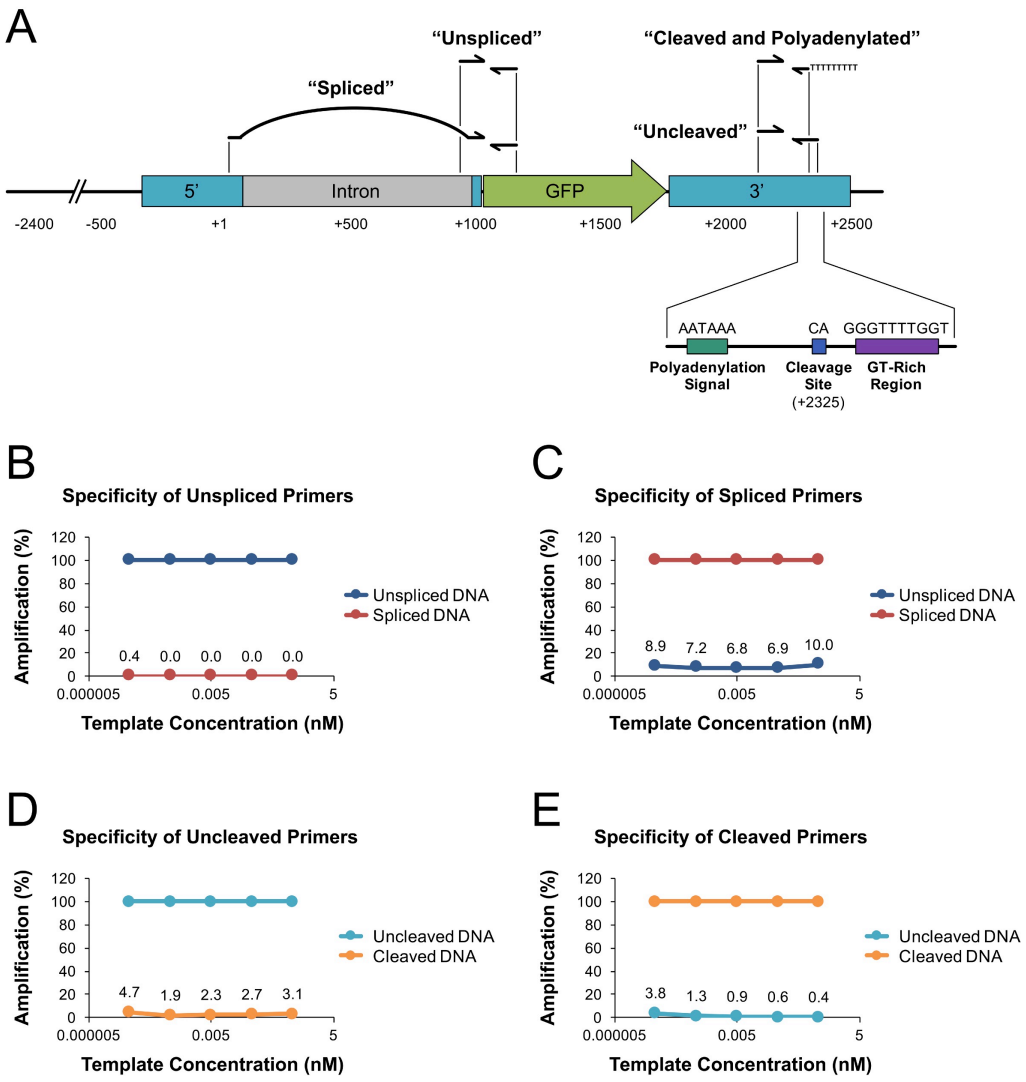

Figure S4. Specificity of pre-mRNA processing primers. (**A**) pActin schematic showing the relative positions of Unspliced, Spliced, Uncleaved, and Cleaved and Polyadenylated primer pairs. (**B-E**) The specificity of each primer pair was analyzed by qPCR with the corresponding unprocessed and processed DNA templates. "Background" amplification of the incorrect DNA template was graphed as a percentage of total amplification measured with the correct DNA template.

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