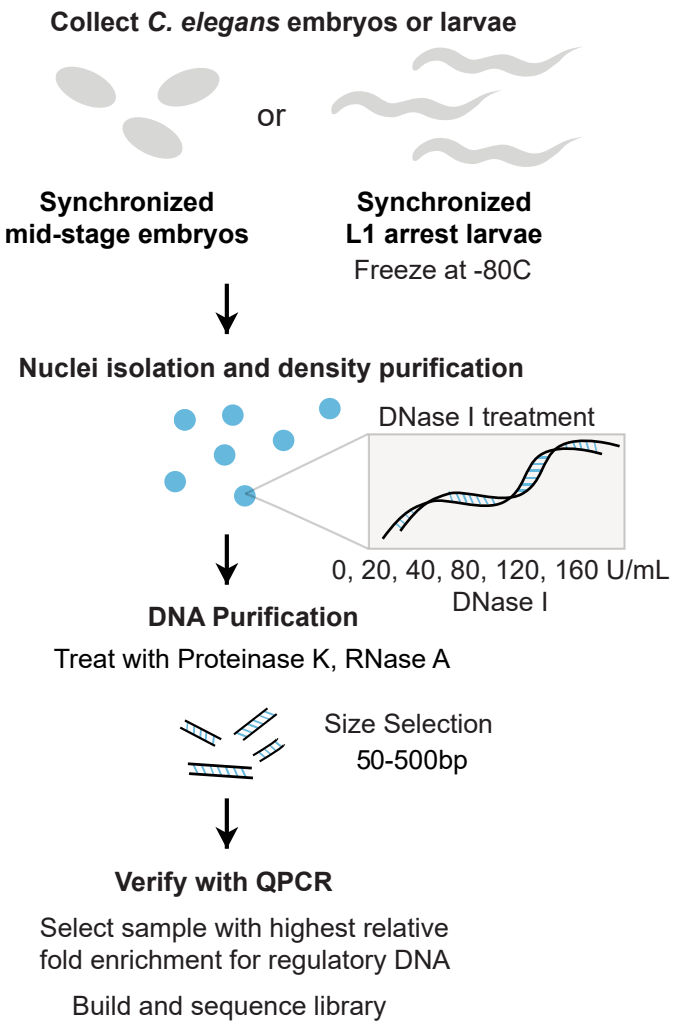
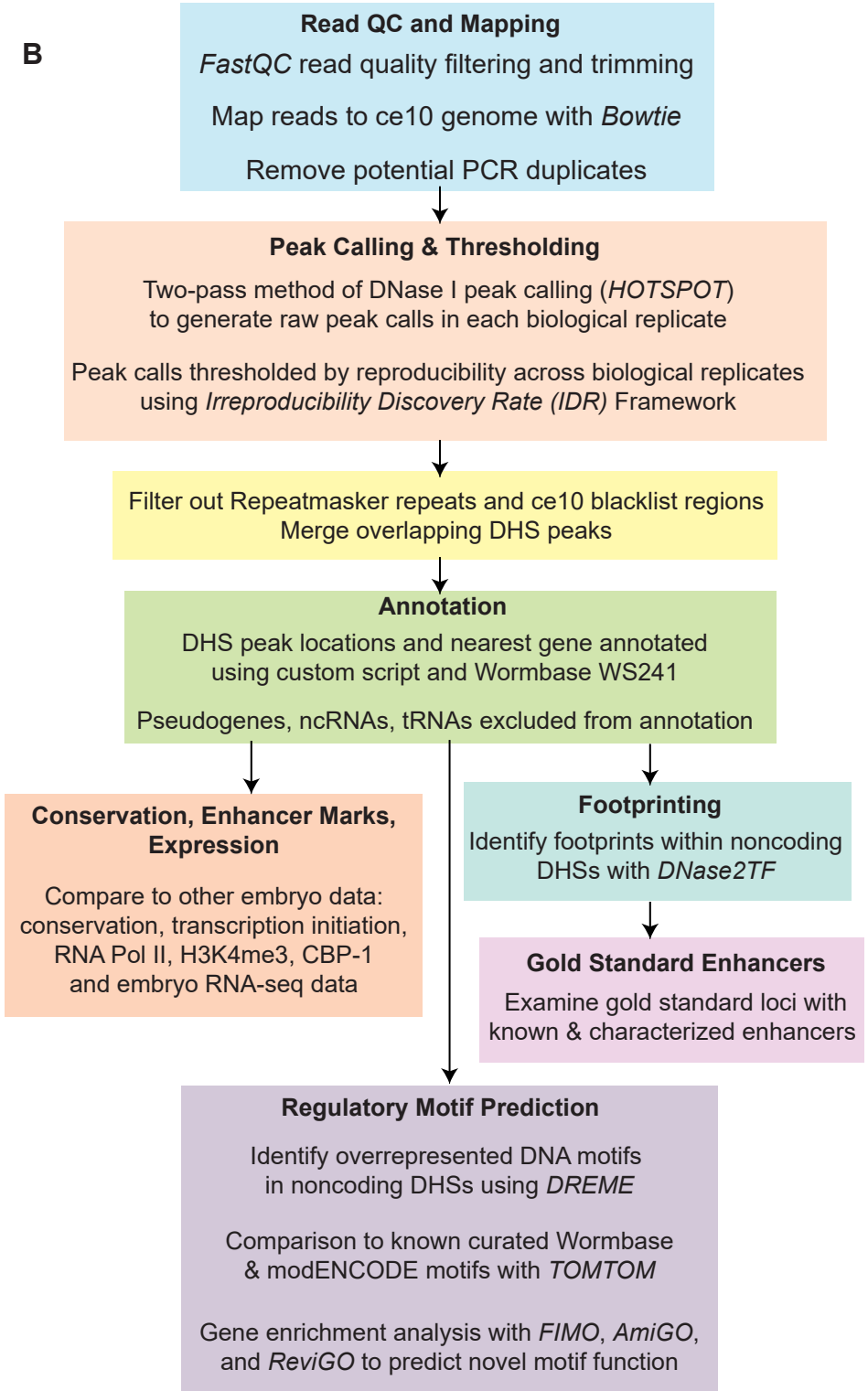
**Figure S1**

**A**


Collect C. elegans embryos or larvae
or
Synchronized mid-stage embryos
Synchronized L1 arrest larvae
Freeze at -80C
Nuclei isolation and density purification
DNase I treatment
0, 20, 40, 80, 120, 160 U/mL DNase I
DNA Purification
Treat with Proteinase K, RNase A
Size Selection 50-500bp
Verify with QPCR
Select sample with highest relative fold enrichment for regulatory DNA
Build and sequence library

**B**


Read QC and Mapping
FastQC read quality filtering and trimming
Map reads to ce10 genome with Bowtie
Remove potential PCR duplicates
Peak Calling & Thresholding
Two-pass method of DNase I peak calling (HOTSPOT) to generate raw peak calls in each biological replicate
Peak calls thresholded by reproducibility across biological replicates using Irreproducibility Discovery Rate (IDR) Framework
Filter out Repeatmasker repeats and ce10 blacklist regions
Merge overlapping DHS peaks
Annotation
DHS peak locations and nearest gene annotated using custom script and Wormbase WS241
Pseudogenes, ncRNAs, tRNAs excluded from annotation
Conservation, Enhancer Marks, Expression
Compare to other embryo data: conservation, transcription initiation, RNA Pol II, H3K4me3, CBP-1 and embryo RNA-seq data
Footprinting
Identify footprints within noncoding DHSs with DNase2TF
Gold Standard Enhancers
Examine gold standard loci with known & characterized enhancers
Regulatory Motif Prediction
Identify overrepresented DNA motifs in noncoding DHSs using DREME
Comparison to known curated Wormbase & modENCODE motifs with TOMTOM
Gene enrichment analysis with FIMO, AmiGO, and ReviGO to predict novel motif function