

SUPPLEMENTAL FIGURE LEGENDS**Supplemental Figure 1**

(A) For each preparation, MNase digestion is analyzed by agarose gel electrophoresis. The arrows indicate the mononucleosomal DNA. The samples used to isolate mononucleosomes for sequencing are shown with an asterisk. **(B)** For each replicate, histograms show the distribution of the sequenced fragment lengths, which are the distance between the outer edges of read pairs. **(C)** The distribution of sequenced fragment length between X and autosomes for the first replicate of data from embryos. The results were similar for all the other datasets. **(D)** We used only those sequenced fragments longer than 146 to calculate nucleosome occupancy across the genome. Normalized embryo nucleosome occupancy per base pair was averaged across all transcripts, and plotted across the transcript starts of autosomes and the X chromosome.

Supplemental Figure 2

UCSC Genome Browser was used to illustrate nucleosome occupancy at a ~20 kb region on chromosome II **(A)** and ~15 kb region on the X chromosome **(B)**. Averaged and normalized nucleosome coverage from embryos and adults are plotted. For each replicate, raw nucleosome coverage of each base pair is also shown. Lower panels zoom into smaller regions. Wormbase genes transcribed from the top and bottom strand are shown on top and bottom, respectively.

Supplemental Figure 3

Nucleosome occupancy from germlineless adults **(A)**, germline containing adults **(B)**, and mixed stage worms (Valouev *et al.* 2008) **(C)** is plotted. For each, normalized occupancy at each coordinate is averaged across transcripts and plotted across the transcript start sites. **(D)** Same as A-C, but genes are grouped by the distance of their transcript starts to nearby genes. **(E)** Average nucleosome occupancy from embryos (y axis) is plotted against GC content (x axis). Averages were determined from 300 bp upstream to 200 bp downstream of the transcript

start sites. **(F)** Pearson correlation coefficient of two datasets was calculated from base by base correlation. The scale is shown to the right.

Supplemental Figure 4

(A) For embryo dataset, 147 bp sequenced nucleosomal fragments were aligned at the dyad, and the fraction of AA/AT/TT/TA and CC/CG/GC/GG dinucleotides (3-bp moving average) at each position of the alignment is plotted. For each fragment, the complementary sequence is also taken; therefore the graphs are symmetric at the center. Data from X (right panel) and autosomal (left panel) nucleosomal fragments are shown. The number of fragments that fulfill the criteria are for autosomes:~1,214.4, and chrX:~318.5 thousand. **(B)** Same as in A, but data from naked DNA digested with MNase were used. The number of fragments that fulfill the criteria are for autosomes:~208.2, and chrX:~53.8 thousand. **(C)** Same as in A, but only those nucleosomal fragments that are within the -300 to +200 bp of the transcript start sites were taken. The number of fragments that fulfill the criteria are for chrI:~15.4, chrII:~19.2, chrIII:~14.9, chrIV:~17.1, chrV:~26.1, and chrX:~21.2 thousand. 20-30 bp from the nucleosome dyad is indicated with a bracket.

Supplemental Figure 5

(A,B) For each coordinate, the fraction of mappable bases is plotted across the transcript start **(A)**, and end **(B)** site. **(C)** Average nucleosome occupancy in 5 kb contiguous windows was calculated for embryos and adults. The distribution of values in these windows is plotted as a histogram. **(D)** Embryo nucleosome occupancy per base was averaged across exon and intron start coordinates and plotted for each chromosome. Only those exons that are longer than 150 bp were used.

SUPPLEMENTAL METHODS

Following protocols were used in this study. Additional protocols are available through www.modENCODE.org, or upon request.

Worm embryo growth and harvest

Adapted from a protocol by Christina Whittle.

(1) Grow gravid N2 hermaphrodites at 20°C on 12 10 cm NGM plates. Pick 10 gravid hermaphrodites onto each 10cm NGM plate seeded with OP50 and grow 2 generations until the plate is full of gravid hermaphrodites. During the 2nd generation, add a few drops of concentrated food to keep the worms from starving until they reach adulthood and are gravid.

Bleach to get embryos

- Collect each plate by washing off worms with M9 and combine samples in 50ml conical tubes.
- Pellet worms by gravity or centrifugation at 3000 g for 3 minutes. Remove supernatant and wash once with M9. **If the worms are dirty (excess bacteria or agar pieces) do additional washes with M9.
- Add bleaching solution to each tube (no more than 5mL packed worms per 50mL tube).
- Rock for ~5-10 minutes until free-floating embryos and broken worms are seen. A small sample can be taken to check under the microscope.
- Spin down 3000 g for 3 minutes and pipet off supernatant. Immediately wash with M9. Pellet can be pipetted up and down to break it up.
- Spin down 3000 g for 3 minutes. Remove supernatant. Wash 2 more times with M9.

(2) Starving for L1s

Resuspend pellet in 5-10mL M9 and incubate while gently rocking at 20°C overnight to hatch and starve. The next day, filter starved L1s into a new 50mL conical tube over 20uM nylon mesh. L1s will pass through the mesh into the new tube. Wash and bring the volume of the conical tube up to 50mL with M9. Spin down 3000 g for 3 minutes to collect L1s at the bottom of the tube. Remove supernatant. Transfer L1s into prepared liquid culture flask.

(3) Liquid Culture

Prepare liquid culture media (S-medium): To 475 mL S-Basal add:

5mL 1M Potassium Citrate pH 6.0
5mL 100X Trace Metals Solution
10mL 50:50 Nystatin:100X PSN
1.5mL 1M CaCl2
1.5mL 1M MgSO4
3ml concentrated E.coli

Add ~200ul packed L1s to each prepared flask

Incubate the cultures shaking at 230 rpm at 25°C, checking each day for stage and how much food is left, and add food (HB101 E.Coli 1V:V in M9+5% glycerol) if necessary.

(4) Collecting Liquid Cultures (embryos)

- Pour the culture of gravid hermaphrodites over a 35 μ M Nitex membrane lined funnel. This will collect the gravid hermaphrodites. Wash with M9.
- Collect the worms by washing the membrane with M9 into a 50mL conical tube. Spin at 3000 g for 3 min to collect. Remove supernatant.
- Split the pellet into multiple tubes, so there is no more than ~5ml packed worms per tube. Wash with M9, and spin down. Add bleaching solution to each tube (no more than 5mL packed worms per 50mL tube). Perform bleaching as described above.
- Resuspend embryos to 45mL M9. Add 2.8 mL 37% w/v CHO (formaldehyde) and incubate with rocking for 15 minutes.
- Add 2.5mL 2.5M glycine and incubate 5 minutes with rocking.
- Spin down 3000 g for 3 min to collect.
- Wash twice with M9, 1 times with FA (50 mM HEPES/KOH pH 7.5, 1 mM EDTA, 1% Triton X-100, 0.1 % sodium deoxycholate; 150 mM NaCl) buffer. Remove supernatant. Quick-freeze tubes in liquid nitrogen.

Reagents

Medium (1 L) (autoclave)

0.1 M NaCl (20 ml of 5M NaCl)

0.05 M Potassium phosphate pH 6 (50 ml of 1M Potassium phosphate pH 6)

1ml cholesterol (5mg/ml in EtOH)

Trace Metals Solution (1 L) (autoclave)

5 mM Disodium EDTA

2.5 mM FeSO₄.7H₂O

1 mM MnCl₂.4H₂O

1 mM ZnSO₄.7H₂O

0.1 mM CuSO₄.5H₂O

M9 buffer (1 L) (autoclave)

3 g KH₂PO₄

6 g Na₂HPO₄

5 g NaCl

Add 1 ml 1M MgSO₄ after the buffer cools down.

Bleaching solution: Prepare fresh before use

0.25 M NaOH

1.25% NaOCl (check bleach bottle, typically 5-6%)

Worm germlineless adult soma glp-1 (q224) growth and harvest

Kindly provided by Christina Whittle.

Starting liquid culture is same as embryos except the worms (JK1107) were grown at 15°C. Collection culture is grown at 25°C. Do not allow starvation or crowding at 25°C. Collect worms once they reach adulthood. Because they have no germline, appearance of eggs will not identify them as adults and they will be skinnier. Look for the white patch on the belly with the vulva.

Collecting Adults

- Pour the culture of germlineless adults over a 20 μ M Nitex membrane lined funnel. This will collect the germlineless adults from the culture media. Wash well with M9.
- Collect the worms by washing the membrane with M9 into a 50mL conical tube. Spin at 3000 g for 3 min to collect. Remove supernatant. Repeat wash.
- Wash with PBS and spin down. Remove Supernatant.
- Resuspend the worm pellet in an equal volume of PBS + protease inhibitors (Calbiochem).
- Making popcorn: Pour some liquid nitrogen into the labeled 50mL conical tube. Add the worm-PBS slurry in droplets to the liquid nitrogen until it is all added. Allow the remaining liquid nitrogen to evaporate off (but do not let thaw!), put the cap on (closed, but not airtight), and store at -80°C.

Worm germ line containing adult fem-2 (b245ts) soma growth and harvest

Kindly provided by Hoang Pham.

Preparation of frozen concentrated *C. elegans* food (HB101)

1. Inoculate 120 L of LB with fresh HB101 and grow to saturation
2. Harvest by centrifugation, weigh pellet, and resuspend at 1:1 with potassium phosphate buffer, pH 7.0.
3. Aliquot into 40 mL volumes and flash freeze in liquid nitrogen. Store at -80°C for future use.

Growing worms on plates for liquid culture

1. Chunk starved worms onto ~7-8 plates (60 mm) for each 400 mL culture approximately one and a half weeks before starting asynchronous culture.
2. Leave plates at the proper temperature (15°C) until just before worms are starved.

Asynchronous Culture

1. Make 400 mL of S complete in a 2 L wide-mouth flask for each set of starved plates.

2. Thaw HB101, and add 10 mL into 400 mL S complete.
3. Wash worms off plate with S basal and inoculate into S complete.
4. Place the culture at 15°C in shaker at 160 rpm (or whatever speed is appropriate to minimize foaming).
5. Leave culture for one to two generations until majority are gravid adults. Check culture every few days for overcrowding and food concentration.

Starving Plates for next Maintenance Culture (optional)

1. If growing worms for another round of harvesting, chunk starved worms onto ~7-8 plates (60 mm) for each 400 mL culture for next maintenance culture.
2. Leave plates at the proper temperature (15°C) until just before worms are starved.

Bleaching worms

1. Place M9 on ice.
2. Place asynchronous culture on ice for ~20 minutes to allow worms to settle.
3. Pour off all but ~40 mL of media, and transfer worms to 50 mL conicals.
4. Pellet worms at 3000 g for 3 minutes, pour off supernatant, wash twice with cold M9, and dividing worms as necessary to ensure that no more than 5 mL of worms are in each 50 mL conical.
5. Add bleaching solution to each 50 mL conical, vortex well and place on rocker for no more than 5 minutes (or until ¾ of worms are broken, and you can see free floating embryos when you examine a 20 µL aliquot using a dissecting microscope).
6. Centrifuge at 3000 g for 2 minutes, and pour off supernatant. Wash twice with cold M9 ensuring pellets are broken up.
 - a. (Optional) If few worms were broken, follow up with a second bleach for 1 minute. Centrifuge again at 3000 g for 2 minutes.
7. Resuspend embryos in 50-100 mL S complete without HB101.
8. Place the culture in 15°C shaker at 160 rpm (or whatever speed is appropriate to minimize foaming). Embryos will hatch and arrest at L1 stage overnight without food.

Synchronous culture

1. At least 24 hours after bleaching, but no more than 72 hours, take 5-10 µL from starved culture to examine under microscope. Dilute starved culture by S complete (for total volume of 400 mL) until worm concentration is 5000 worms per mL.
2. Add 3 mL HB101 into each 400 mL culture in a 2 L wide mouth flask.
3. Place the culture at 23°C in shaker at 160 rpm (or whatever speed is appropriate to minimize foaming).
4. Add 4 mL food for two subsequent days.

5. Take a small aliquot of culture, and check stage of worms under a dissecting microscope. When adults with mature oocytes are present, harvest worms.

Harvesting worms

1. Place flask on ice, and allow worms to settle for 20-30 minutes. Pour off supernatant after allowing worms to settle.
 - a. (Optional) Place a 35 uM Nitex filter into a Buchner funnel set in a filter flask. Pour synchronous culture into Buchner funnel to collect adults from culture media.
 - b. Wash worms with several hundred mLs of cold M9. Turn off vacuum, form the Nitex into a "basket", add some M9 and collect into 50 mL conicals.
2. Centrifuge at 3000 g to pellet worms and pour off supernatant.
3. Wash with cold M9, centrifuge at 3000 g and pour off supernatant. Divide worms into separate 50 mL conicals so that pellet volume is no greater than 5 mL.
4. Fill up to 25 mL cold 0.1M NaCl and 25 mL cold 60% sucrose. Invert a few times to mix. Centrifuge at 1500 g for 5 minutes.
5. HB101 and various debris should pellet at bottom, while adult worms should float near surface. Collect top 25 mL into new 50 mL conicals and wash twice with cold M9.
6. Wash with 50 mL EBS + PA and PMSF.
7. Centrifuge at 3000 g, and pour off supernatant until 2X pellet volume. Add 1:100 volume of Halt Protease and Phosphatase Inhibitor.

Place sieve into an ice bucket and fill with liquid nitrogen. Pipet 1 mL culture and dispense into liquid nitrogen to make worm popcorn. After all worms are frozen, pour worm popcorn into 50 mL conical filled halfway with liquid nitrogen. Store popcorn at -80°C freezer.

Solutions

1M KPO4 pH 7.0

Make 1M solution of KH2PO4 Potassium Phosphate Monobasic

Make 1M solution of K2HPO4 Potassium Phosphate Dibasic

pH the 1M solution of KH2PO4 to 7.0 using the 1M solution of K2HPO4.

For 4.5 L of PO4 buffer (300 ml x 15) 175.5 ml KH2PO4 274.5 ml K2HPO4 pH to 7 add 450 mls 100% glycerol and bring up to 4.5 L

10X EB (500 mL)

250 mM HEPES (pH 7.5)	1 M stock 125 mL
1180 mM NaCl	5 M stock 118 mL
480 mM KCl	2 M stock 120 mL
20 mM EDTA	0.5 M stock 20 mL
5 mM EGTA	0.5 M stock 5 mL

EBS + PA and PMSF (50 mL)
10X EB 5 mL
Sucrose (final 340 mM) 4.28 g
0.15 M Spermidine (-20°C aliq.) 67 uL
0.5 M Spermine (-20°C aliq.) 50 uL
0.1 M PMSF (4°C stock) 500 uL

Bleaching solution
0.25 M NaOH
1.25% NaOCl (check bleach bottle, typically 5-6%)

Worm XO hermaphrodite growth and harvest

Growth was done as in embryos, except worms were collected by settling when they reach L3 stage (~22-26 hours).

Worm adult nucleosome preparation

Start with worm “pop corns” 1V worms+1V buffer. In this protocol volume refers to volume of the worms.

Worm powdering:

1. Chill biopulverizer (<http://www.biospec.com/BioPulverizer.htm>) and mortar and pestle (<http://www.biospec.com/Cryo-cup%20Grinder.htm>) with liquid nitrogen. At these steps make sure the samples do not thaw. Transfer the popcorn into the biopulverizer and squeeze spring-loaded hammer 5 times. Collect powder in the middle and break another 5 times for 10 times more. In between cool samples with liquid nitrogen but be careful not to pour a lot and splash the sample out. Transfer sample to the chilled mortar and turn it into a fine white powder. This takes 5-10 minutes. Make sure the whole worms are broken into more than 5 pieces.
2. Add the powder to 10 volumes of PBS+1mM PMSF +protease inhibitors+1% formaldehyde. Incubate 1 minute at room temperature while dissolving the powder by inverting the tube. Add 125 mM Glycine and incubate 1 minute.

Chromatin digestion:

From here all steps are at 4°C. The buffers should contain protease inhibitors and be chilled at 4°C.

1. Spin down the sample at 4000 g for 5 minutes.
2. Wash in 2V dounce buffer.
3. Resuspend in 2V dounce buffer and incubate on ice for 10 minutes. Dounce on ice 20 strokes with loose (A) pestle then 20 strokes with tight (B) pestle.
4. Spin down 100 g for 2 minutes.

5. Take out SNT, which contains the nuclei. Resuspend the pellet in 2V dounce buffer 2 and dounce with type B pestle 20 strokes. Spin down to 100 g for 2 minutes. Take out SNT.
6. Repeat step 6.
7. Combine the SNTs and the last pellet (to have everything in the reaction) and spin 4000 g for 10 minutes.
8. Wash with 1V MNase digestion buffer.
9. Resuspend in 1.5V of MNase digestion buffer. Divide into 600 ul aliquots and digest with increasing amounts of Mnase.
10. After nuclei is divided into 600 ul aliquots, put each tube at 25C for 5 minutes. Add MNase to desired concentration. This is empirically determined and may change with differing protocols, try 0, 50, 250, 750, 1000 units/600 ul when started with 2 grams of adults. Mix the enzyme by flicking the tube or briefly vortexing and incubate at 25°C for 10 minutes. It is important to watch the clock during the reaction and control each parameter of digestion as well as possible.
11. Add 30 ul 0.5 M EDTA to stop the reaction, mix and place on ice. Continue with DNA preparation.

DNA purification:

1. To 600 ul samples: Add 65 ul 10% SDS, 12 ul 5 M NaCl, and 2 ul 10 mg/ml Proteinase K.
2. Incubate at 55°C for 2 hours and reverse Crosslink overnight at 65°C.
3. Phenol/Chloroform extract once. Add 4 ul 10 mg/ml RnaseA and incubate at 37 °C for 2 hours.
4. Phenol/Chloroform extract. Repeat extraction.
5. Add 10% volume of 3 M NaAcetate pH 5.2 and 2 volumes of cold EtOH.
6. Incubate at -20°C or -80 °C for 30 min- indefinitely.
7. Pellet DNA by centrifuging 15 minutes at 13,000 g at cold room.
8. Wash with 500 ul of cold 70% EtOH.
9. Dry DNA pellet and resuspend in 50 ul 0.1X TE.
10. Purify desired nucleosomal DNA fragment by Qiagen Gel extraction kit.

Reagents:

Chitinase (sigma #C7809): 2 unit/ml made up in Embryo Buffer

Digitonin (sigma #D5628): 20mg/ml made up in DMSO

MNase (Worthington LS004797)

Make Worthington MNase stocks in water at 50 units/ul concentration and save 20 ul aliquots at -80. When an aliquot is taken out, do not put the left over back because the potency of the enzyme changes and you need a rough idea of how the enzyme would cut to make predictable enzyme dilutions.

Dounce Buffer: 0.35 M Sucrose 15 mM HEPES-KOH, pH 7.5 0.5 mM EGTA 5 mM MgCl₂ 10 mM KCl 0.1 mM EDTA 1 mM DTT 0.5% TritonX-100 0.25% NP-40

Mnase Digestion Buffer: 110 mM NaCl 40 mM KCl 2 mM MgCl₂ 1 mM CaCl₂
50 mM Hepes/KOH pH 7.5

Worm embryo nucleosome preparation

Embryo chitinase digestion

1. Thaw frozen embryos on ice. Wash embryos twice with 1 ml of Embryo Buffer (Pellet at 2000 g for 5 minutes). Note the volume of the embryo pellet (V) and use this for future steps.
2. Pellet at 2000 g for 5 minutes and add 2V of Embryo Buffer and 500 ul of chitinase/ml of embryo pellet.
3. Rock gently at RT for 20-30min. Pipet up and down with a blue tip several times, this presumably helps break up the cells. Check embryos under the scope, the folded ones release from the eggshell, others change shape.

Chromatin digestion:

From here all steps are at 4°C. The buffers should contain protease inhibitors and be chilled at 4°C.

1. Spin down the blastomeres at 2000 g for 10 minutes.
2. Wash in 4V dounce buffer 1 twice. Spin down the sample at 4000 g for 5 minutes.
3. Resuspend in 4V dounce buffer 1 and incubate on ice for 10 minutes. Dounce on ice 20 strokes with loose (A) pestle then 20 strokes with tight (B) pestle.
4. Spin down 100 g for 2 minutes.
5. Take out SNT, which contains the nuclei. Resuspend the pellet in 4V dounce buffer 2 and dounce with type B pestle 20 strokes. Spin down to 100 g for 2 minutes. Take out SNT.
6. Repeat step 5.
7. Combine the SNTs and the pellet and spin 4000 g for 10 minutes. Wash with 8V MNase digestion buffer, spin 4000 g for 10 minutes.
8. Resuspend in 6V of MNase digestion buffer. Divide into 600 ul aliquots and digest with increasing amounts of Mnase.
9. After nuclei is divided into 600 ul aliquots, put each tube at 25°C for 5 minutes. Add MNase to desired concentration. This is empirically determined and may change with differing protocols, if using 500 mg of embryos, divide to 5 tubes and digest with add 0, 50, 200, 500, 1000 units of Mnase. Mix the enzyme by flicking the tube or briefly vortexing and incubate at 25°C for 10 minutes. It is important to watch the clock during the reaction and control each parameter of digestion as well as possible.
10. Add 30 ul 0.5 M EDTA to stop the reaction, mix and place on ice. Continue with DNA preparation as described above.

Embryo Buffer 110 mM NaCl 40 mM KCl 2 mM CaCl₂ 2 mM MgCl₂ 25 mM HEPES-KOH pH 7.5

Solexa library preparation

Blunting:

DNA+H2O 36 uL (50 ng DNA)
10x NEB ligation Buffer 5 uL
10 mM dNTP mix 2 uL
NEB PNK 3 uL
NEB Klenow 5U/uL diluted 1:5 1 uL
NEB T4 DNA polymerase 3 uL
Total 50 uL

Incubate 30 minutes at 20°C in PCR machine or Thermo-block.

Cleanup using Qiagen PCR Purification Column:

Use 250 uL PB buffer Spin at 13,000 g Elute with 42 uL EB

Adding A overhang:

DNA 40 uL
10x NEB Buffer 2 5 uL
10 mM dATP 1 uL
Exo (-) Klenow (5 U/uL) 3 uL
Total 50 uL

Incubate 1 Hour at 37°C

Cleanup using Qiagen Mini-Elute PCR Purification Column:

Use 250 uL PB buffer

Spin at 13,000 g

Elute with 13 uL EB

Ligating adaptors:

DNA 12.5 uL
2X NEB Quick Ligation buffer 15 uL
Solexa pair end adapter (1:10 dilution) 1 uL
NEB Quick ligation ligase 1.5 uL
Total 30 uL

Incubate 20 minutes at 23°C in PCR machine or Thermo-block.

Cleanup using Qiagen PCR Purification Column:

Use 150 uL PB buffer

Spin at 13,000 g

Elute with 36 uL EB

PCR amplification:

DNA 36 uL
50 uM Solexa pair end primer mix 2 uL
10X PfuUltra II Reaction Buffer 10 uL
2.5 mM dNTP 10 uL
Phusion polymerase 1 uL
H2O 41 uL
Total 100 uL

PCR cycle
98°C 30 sec
Repeat 18 times {98°C 20 sec, 65°C 30 sec, 72°C 30 sec}
72°C 5 min
4°C

Cleanup using Qiagen Mini-Elute Column
Use 500 uL PB buffer+10 uL of 3 M NaOAc
Spin at 13,000 g
Elute with 11 uL EB

Size select mononucleosomal fragment:

1. Add 3 uL Loading Buffer per 10 uL sample
(loading buffer: 50 mM Tris pH 8.0, 40mM EDTA, 40% (w/v) sucrose).
2. Run sample on 1.5% agarose gel for ~1 hr.
3. Excise the desired DNA range.
4. Purify using Qiagen Gel Extraction Column
Use 6x QG Buffer and dissolve at room temperature.
Use 2x Isopropanol
Spin at 13,000 g
Elute with 30 uL EB
5. Measure DNA concentration using Qubit fluorometer (Invitrogen)