Supplementary Figure Legends

Supplementary Figure 1. No detectable DNA is pulled down from an untagged control N2 worm strain. Biotinylated chromatin isolation and affinity purification using monomeric avidin were performed on an untagged N2 worm strain and the Biotag::H3.3/BIRA worm strain. Using the same pull-down conditions, we were able to detect DNA in the pull-down lane from Biotag::H3.3/BIRA, but not from the untagged N2 worm strain. Lanes 1 and 2: pull-down and unbound fractions from the untagged N2 control worm strain, respectively; lanes 5 and 6: pull-down and unbound fractions from the Biotag::H3.3/BIRA worm strain, respectively; lanes 3 and 4, DNA molecular weight standard.

Supplementary Figure 2. Biotinylated histones are not detectable in *C. elegans* chromatin. Core histones from an untagged worm strain eluted from chromatin with 2.5 M NaCl were electrophoresed and stained with Coomassie blue (left panel) or analyzed on a western blot probed with streptavidin-HRP (middle panel) or an antibody against histone H3 dimethylated at lysine 4 (right panel). This sample contains a high concentration of core histones based on Coomassie blue staining and histone H3 antibody staining. However, no biotinylated histones were detectable in the 2.5 M NaCl chromatin eluate, suggesting that endogenous worm histones are not biotinylated in chromatin.

Supplementary Figure 3. In vivo biotinyation of BioTag2::H3.3 in *C. elegans*. Western blot analysis using streptavidin-HRP for mixed stage worm strains expressing BioTag**2**::H3.3 (lanes 2 and 5) or BioTag::H3.3 (lanes 3 and 6) in combination with BIRA. Twice as much worm lysate was used in lanes 4 to 6. Biotinylation of BioTag**2**::H3.3 (~18.8 kDa) could be detected in a worm strain expressing BioTag**2**::H3.3/BIRA, and the

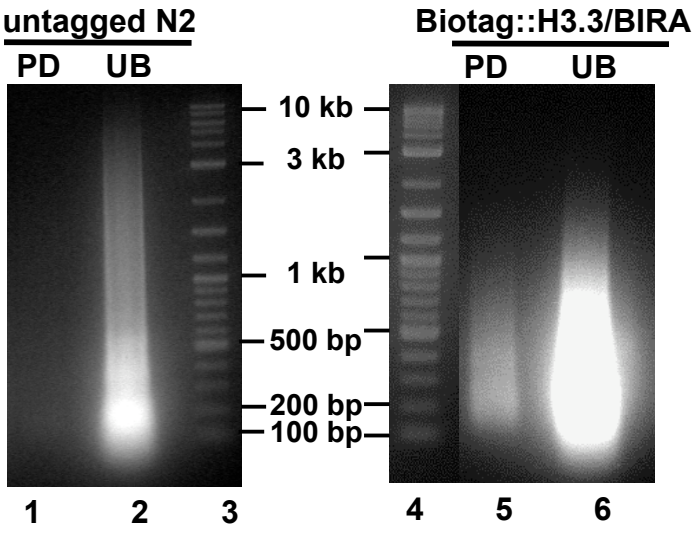
intensity of the signal suggests that the biotinylation of BioTag**2**::H3.3 is just as efficient as BioTag::H3.3 (~21.4 kDa). The band residing between 16 and 25 kDa in the NA22 bacteria (used to feed the worms) lane is an endogenous biotinylated protein in bacteria.

Supplementary Figure 4. Average ends analysis profiles of operons. The 1151 operons annotated in WormBase were aligned at their 5' and 3' ends and averaged for each of the datasets displayed in Figures 5 and 7 (brown curves). These are shown in comparison to corresponding profiles for all 8244 annotated loci used in the gene analyses (blue curves). (A-C) Salt fraction profiles; (D-E) H3.3 profiles.

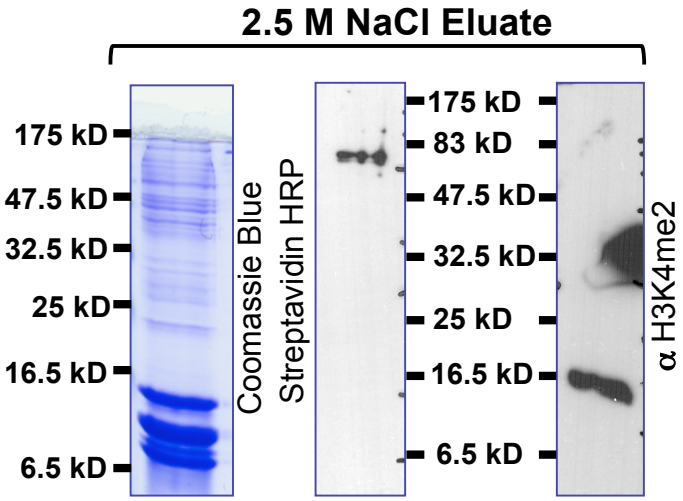
Supplementary Figure 5. Chromatin isolation and salt fractionation using mixed-stage adult worms. Nuclei isolated from N2 mixed-stage adult worms were resuspended in buffer A containing ~17 mM salt for MNase digestion, and the digestion was stopped with EGTA. Next, centrifugation was performed to separate the supernatant containing MNase from the nuclear pellet. The nuclei were then resuspended in 80 mM salt, extracted, centrifuged, and the nuclear pellet was re-extracted with 600 mM salt. DNA recovered from different chromatin fragments was resolved on a 1.5% agarose gel and stained with ethidium bromide. MNase-digested chromatin (lanes 3 and 4) produced a typical nucleosomal ladder. Under these conditions, no mononucleosomes leak into the supernatant during the centrifugation step performed after MNase digestion (lane 5). The 80 mM salt soluble fraction consists of only mononucleosomes (lane 6), while the 600 mM salt soluble fraction shows a typical nucleosomal ladder (lane 7). A small amount of DNA remains in the pellet (lane 9). Lane 1, DNA molecular standard; lane 2, nuclei; lanes 3 and 4, samples treated by MNase for 4 and 10 minutes, respectively; lane 5, supernatant; lane 6, 80 mM salt-soluble fraction; lane 7, 600 mM fraction; lane 8, 600 mM insoluble pellet fraction.

Supplementary Figure 6. Similarities between embryo and adult mononucleosome landscapes. MNase digests from embryos (A, C) and adults (B, C) were resolved on agarose gels and the mononucleosome-sized DNA was excised. See Figure 4 legend for details. Averages from two independent experiments are shown.

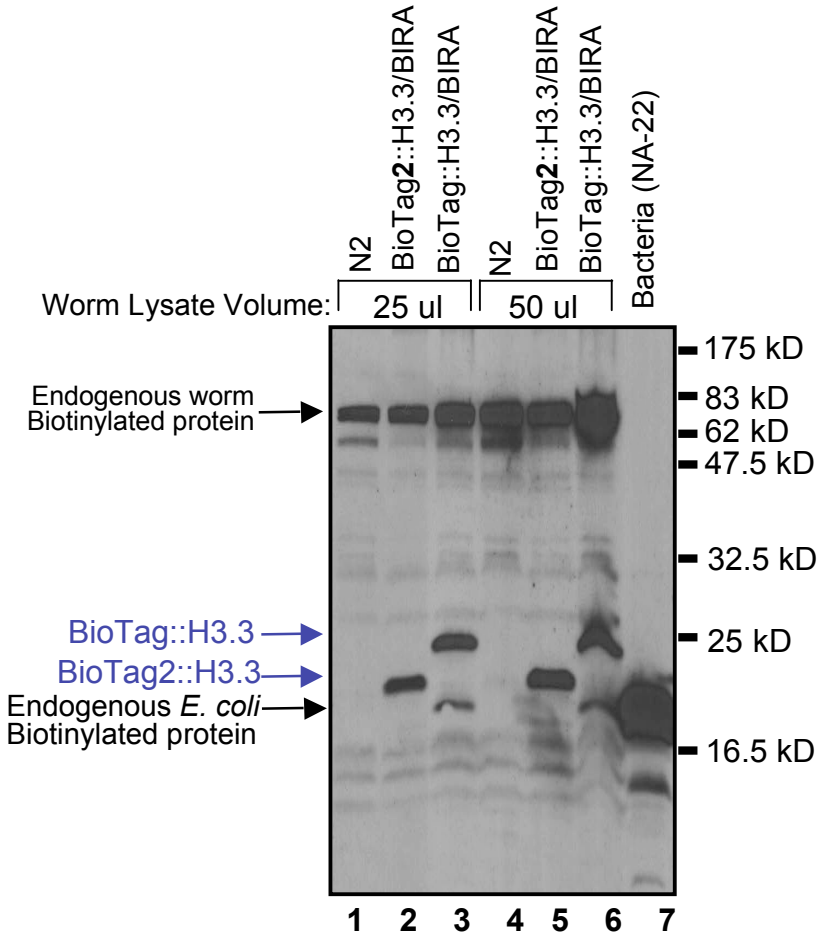
Supplementary Figure 7. Biotinylated BioTag::H3.3 is present in the nuclei of embryos and the somatic cells of adult animals. (A) Adult hermaphrodite and (B) embryos fixed and stained for Streptavidin-FITC and DNA. Biotinylated BioTag::H3.3 is present in the nuclei of embryos, and also in the somatic cells (e.g. neuronal and pharyngeal nuclei), but not the germline of adult hermaphrodites. Scale bar, 50 μm.



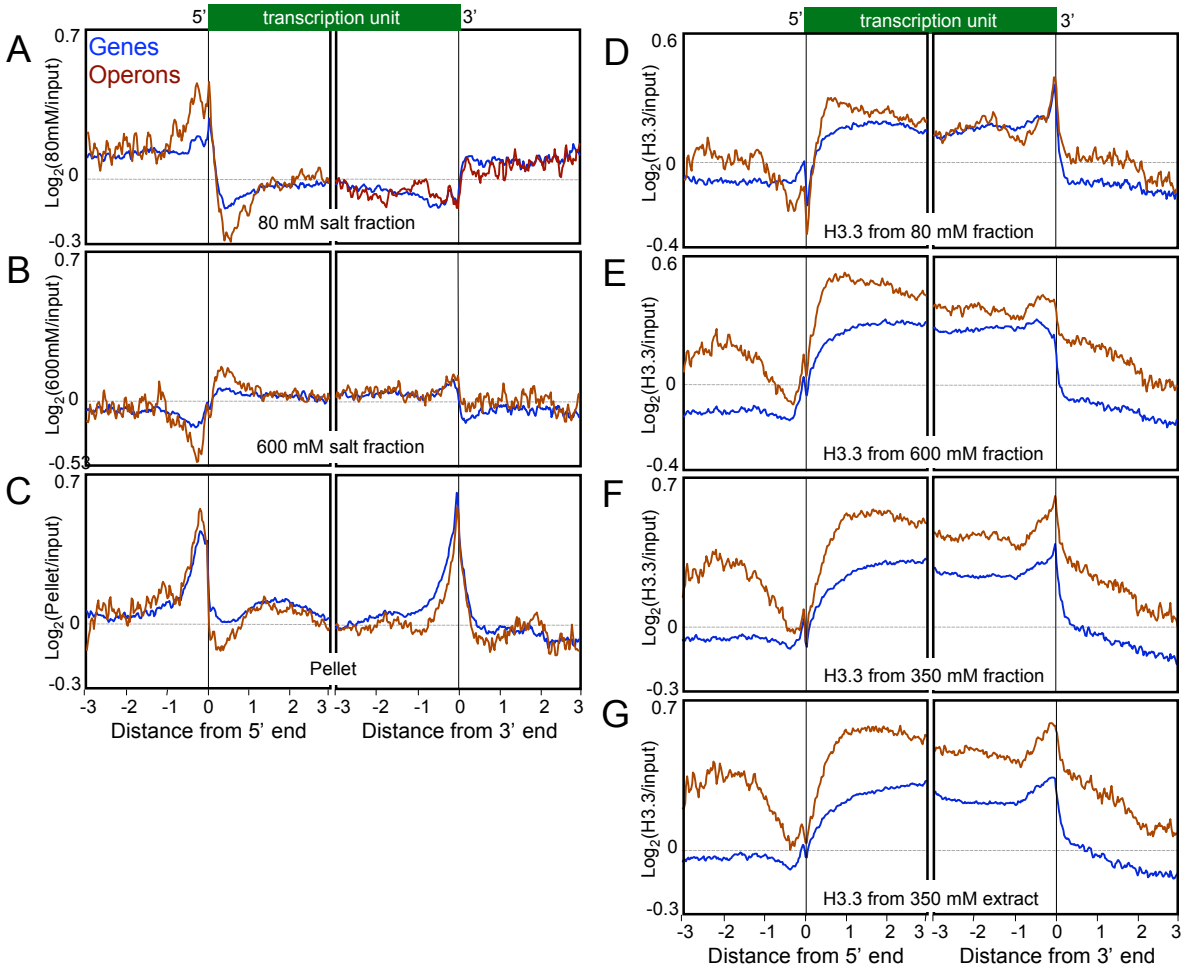
Supp. Fig. 1 Ooi et al.



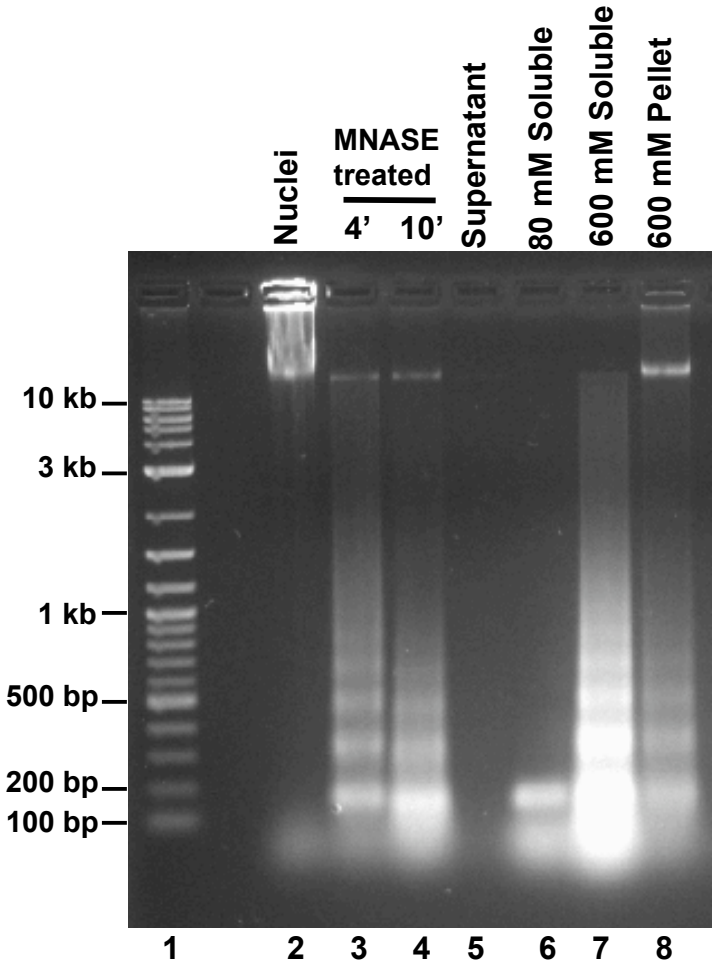
Supp. Fig. 2 Ooi et al.



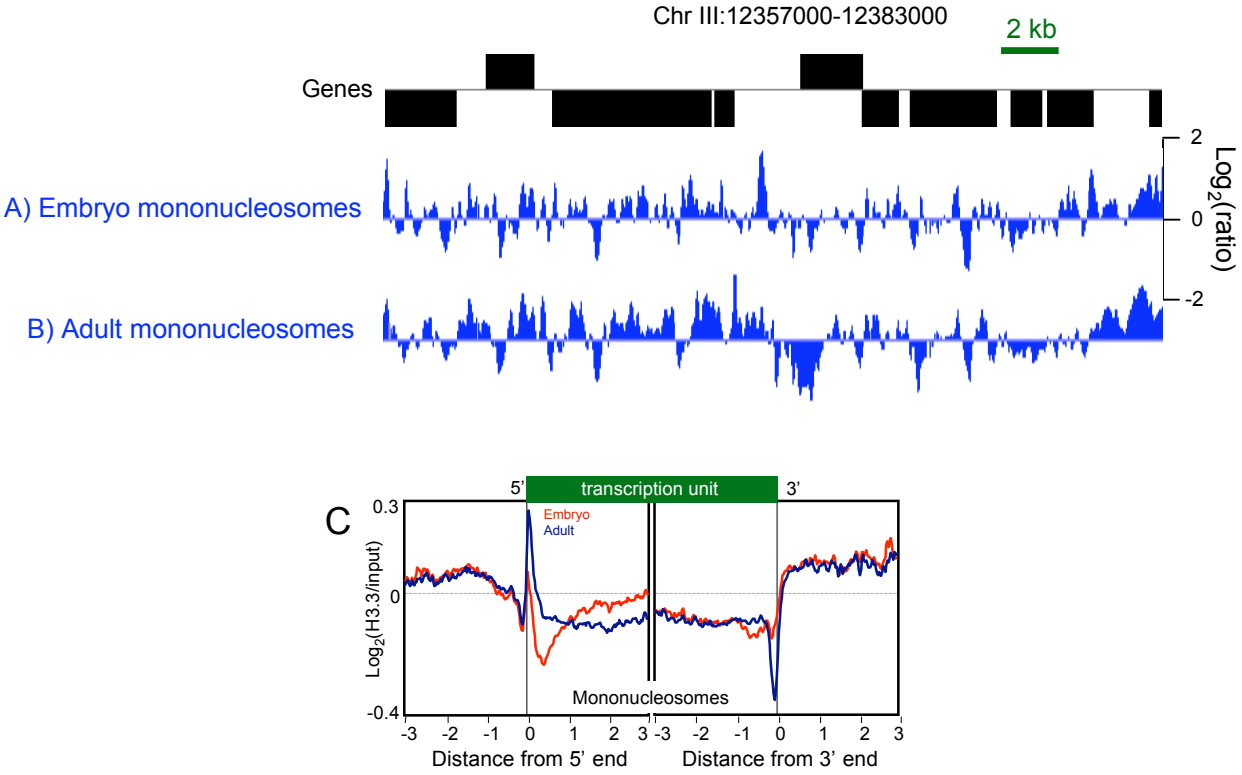
Supp. Fig. 3 Ooi et al.



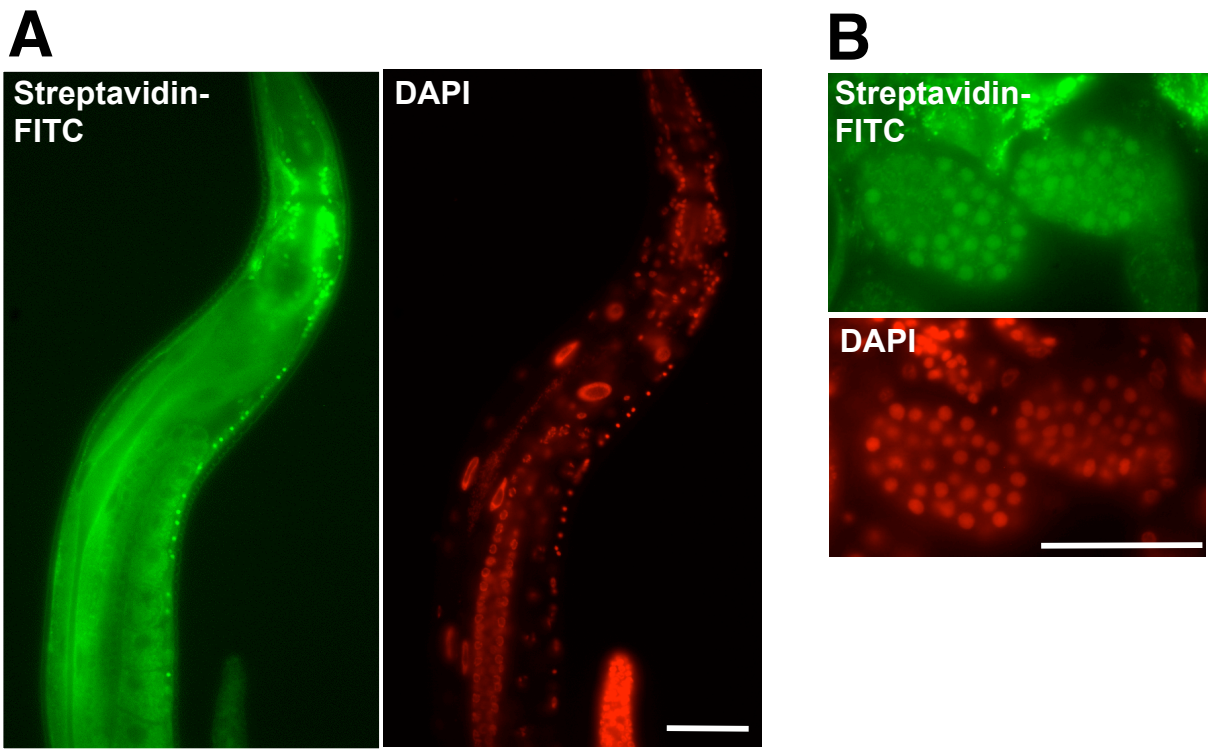
Supp. Fig. 4 Ooi et al.



Supp. Fig. 5 Ooi et al.



Supp. Fig. 6 Ooi et al.



Supp. Fig. 7 Ooi et al.

Supplemental Material 1 Ooi et al.

Biotinylated chromatin purification with Streptavidin-Sepharose from *C. elegans* embryo

Worm growth

1. Day 1. Patch three plates (100 x 15 mM NGM plate seeded with OP50) of unsynchronized worm strain expressing biotinylated BioTag::H3.3 onto five (150 x15 mM, refered to as "big" plate hereafter) peptone rich plates containing NA22 bacteria (PR-NA22 plates). *Worms were grown on PR-NA22 plates, because we could detect reproducible biotinylation of BioTag::H3.3.*
2. Incubate at 22˚C and let the worms grow until adult stage.
3. Day 4. Bleach worms in 10% Bleach/0.5 N KOH/M9, and expand it to seven big PR-NA22 plates.
4. Incubate at 22˚C and let the worms grow until adult stage.
5. Day 7, Bleach worms in 10% Bleach/0.5N KOH/M9, and expand it to 30 PR-NA22 plates at a concentration of 1 million embryos per plate. *As a rough estimate, this translates to ~30 million embryos in 30 plates total.*
6. Incubate at 22˚C and let the worms grow until adult stage. *Assuming that each embryo becomes an adult worm containing about 10 embryos, our growth procedure yields about 10 million embryos per plate or 300 million embryos in total.*

Worm embryo and blastomere preparation

7. Collect worms from the 30 big plates into two 500 ml bottles
8. Spin worm down at 380 g (1500 rpm in a Sorvall GS3 rotor) for 4 min
9. Bleach worms in 800 ml of M9 containing 10% Bleach and 0.5 N KOH in two 500-ml bottles

	Volume per bottle	Total Volume	Final [ ]
Adult Worm in M9	340 ml	680 ml	
5 N KOH	20 ml	40 ml	0.5 N
Bleach Solution	40 ml	80 ml	10%
Total	400 ml	800 ml	

10. Spin down at 380 g (1500 rpm in a Sorvall GS3 rotor) for 4 min
11. Wash 1 time in ~400 ml of M9
12. Filter the sample through two layers of Miracloth (475855, Calbiochem) set as a V-shaped cone on a 300 ml-beaker.
13. Pellet embryo at 380 g (1500 rpm in a Sorvall GS3 rotor) for 4 min
14. Add 1 U/ml chitinase (from *Streptomyces griseus*, Sigma C6137, resuspended in M9 as 1 unit/ml stock) to the embryo pellet (to a final concentration of ~ 0.2 U/ml)

	Total Rx Volume (20ml)	Final [ ]
Embryo in M9	16 ml	
1 U/ml chitinase	4 ml	0.2 U/ml
Total	20 ml	

15. Rock gently at RT for 30 min
16. Bring volume up to 50 ml with buffer A.

Nuclei preparation using NP-40 substitute and glass homogenizer

17. Wash blastomeres 2X in buffer A by pelleting at 2000g (4100 rpm in F15S Fiberlite rotor) for 10 minutes.
18. Rinse in 4 volumes of buffer A.
19. Let cells swell on ice for 15 min.
20. Pellet cells at 2000g (4100 rpm in F15S Fiberlite rotor) for 10 minutes.
21. Resuspend in 7 ml of buffer A containing 0.25% NP-40 (Fluka 74385) and 0.1% Triton X-100.
22. Dounce 15 strokes with loose (A) followed by tight (B) pestle.
23. Spin at 100 g (900 rpm in F15S Fiberlite rotor) for 5 min to remove junk. Nuclei are in the supernatant, aspirate supernatant (Nuclei 1) to a new 50 ml conical tube and set on ice. *The pellet is rather soft, so it is not possible to perfectly separate the supernatant from the pellet.*
24. Resuspend the pellet with 7 ml of buffer A containing 0.25% NP-40 and 0.1% Triton X-100 again.
25. Dounce 15 strokes with loose (A) followed by tight (B) pestle.
26. Spin at 100 g (900 rpm in F15S Fiberlite rotor) for 5 min to remove junk. Nuclei (Nuclei 2) are in the supernatant, aspirate supernatant (Nuclei 2) to a new 50 ml conical tube and set on ice. At this step, the pellet should be rather small, and it may be impossible to separate the pellet from the supernatant, which is fine.
27. Pellet nuclei 1 and 2 at 4000 g (5900 rpm in F15S Fiberlite rotor) for 10 min. *The supernatant is dilute cytoplasm, while the nuclei is in the pellet.*
28. Aspirate the supernatant off, and pool nuclei 1 and 2 by transferring them into two eppendorf tubes with some residual buffer A.
29. Spin at 800 g (3 krpm in table top Eppendorf 5425R) for 5 min to pellet nuclei
30. Aspirate the supernatant off, resuspend nuclei in 2 ml (1 ml per eppendorf tube) of TM2 (10 mM Tris.Cl, pH 7.5, 2 mM $MgCl_2$, .5 mM PMSF) buffer. *Starting from this step (until step xx, after nuclei has extracted in salt), nuclei will be handled in smaller volume (1 µl to 1 ml) using 20-µl to 1 ml Pipetman pipettes. It is important to note that starting from this point, pipeting of the nuclei should be performed using bored pipette tips by cutting off the pipette tip end with a clean scissor or razor blade. The nuclei volume should be around 200-400 µl. High quality nuclei are creamy or white in appearance, easy to resuspend, and goes into solution easily when you flick the tube.*

Save 60µl out of 2 ml (3%) as Nuclei DNA Sample #1 and 20 µl out of 2 ml (1%) as Nuclei Protein Sample #1.

Micrococcal nuclease (MNase) digestion

31. Pre-warm nuclei at 37˚C for 5 min
32. Perform MNAse treatment in a total of 2 ml. Add 2.16 µl of 1 M CaCl2, followed by 8 µl of 1U/5µl MNase (Sigma N3755)

	Volume/tube	Total Rx Volume	Final [ ]
Nuclei in TM2	1 ml	2 ml	
1 M CaCl2	2.1 µl	4.2 µl	2 mM
MNase (1U/5µl)	8 µl	16 µl	.8 unit / 1.011 ml
Total Volume/tube	1.011 ml	2.022 ml	

33. Incubate the reaction at 37˚C for 10 min
34. Stop the reaction by adding 10.2 µl 0.2 M EGTA to each tube.

0.2 M EGTA	10.2 µl	20.4 µl	2 mM
Total	1.021 ml	2.042 ml	

Save 200 µl out of 2 ml (as MNase Total DNA sample #2, 10%) and 20 µl out of 2 ml (as MNase Total Protein Sample #2, 1%).

35. Collect nuclei by spinning at 400 g (2000 rpm on tabletop eppendorf centrifuge) for 10 min at 4˚C.
36. Aspirate supernatant to a new tube. *The supernatant is SUP1. The goal is to have all nucleosomes remain in the pellet and not leak into the supernatant.*

Save 300 µl out of 2 ml (as SUP1 DNA sample #3, 15%) and 20 µl out of 2 ml (as SUP1 protein sample #3, 1%).

80 mM and 0.6 M Salt Extraction

37. Resuspend pellet in 2 ml (1 ml/tube) total of prechilled 80 mM salt extraction buffer (70 mM NaCl/10 mm Tris.Cl, pH 7.4/ 2 mm $MgCl_2$/2 mM EGTA/0.5 mM PMSF/0.1% Triton X-100), and rotate at 4˚C for 2 hours to obtain 80 mM soluble chromatin fraction.
38. Collect nuclei by spinning at 400 g (2000 rpm on tabletop eppendorf centrifuge) for 10 min at 4˚C.
39. Aspirate supernatant to two new tubes. The supernatant will be used to prepare the future 80 mM INPUT (Sample #4). Set it at 4˚C for processing tomorrow.
40. Resuspend pellet in 2 ml (1 ml/tube) total of prechilled 0.6 M salt extraction buffer (585 mM NaCl/10 mm Tris.Cl, pH 7.4/ 2 mm $MgCl_2$, /2 mM EGTA/0.5 mM PMSF/0.1% Triton X-100), and rotate at 4˚C for overnight.
41. Collect nuclei by spinning at 400 g (2000 rpm on tabletop eppendorf centrifuge) for 10 min at 4˚C.
42. Aspirate supernatant to two new tubes. The supernatant will be used to prepare the future .6 M INPUT (Sample #7). Set it at 4˚C for further processing.
43. Resuspend the pellet in 2 ml of 0.6 M Triton buffer.

Save 300 µl out of 2 ml (as .6 M PELLET DNA Sample #10, 15%) and 20 µl out of 2 ml (as .6 M PELLET protein sample #10, 1%).

80 mM and 0.6 M Input Processing for Pull-Down Experiments

44. Spin the future 80 mM INPUT and 0.6 M INPUT samples at 5900 g (8000 rpm on tabletop Eppendorf centrifuge) for 5 min at 4˚C. *This step is very important in order to separate nucleosomes from undigested HMW DNA.*
45. Aspirate supernatants to new tubes.

For each salt concentration, save 400 µl out of 2 ml (as DNA sample, 20%) and 20 µl out of 2 ml (as protein sample, 1%) as 80 mM Salt Soluble Input (Sample #4) and .6 M Salt Soluble Input (Sample #7), respectively.

Streptavidin Pull Down of Biotinylated Nucleosomes

Streptavidin Beads Preparation

46. Use nutator to resuspend Streptavidin Sepharose (Streptavidin Sepharose High Performance, GE Healthcare, 17-5113-01). *Each 1 ml reaction requires 100 µl of streptavidin beads. We need 400 µl of beads (2 ml of 80 mM INPUT and 2 ml of 0.6 M INPUT).*
47. Aliquot ~220 µl (440 µl total) of Streptavidin Sepharose into two eppendorf tubes.

48. Fill one tube up to 1 ml with 80 mM salt extraction buffer without Triton X-100 (70 mM NaCl/10 mm Tris.Cl, pH 7.4/ 2 mm $MgCl_2$/2 mM EGTA/0.5 mM PMSF), and fill the second tube up to 1 ml with .6 M salt extraction buffer without Triton X-100 (585 mM NaCl/10 mm Tris.Cl, pH 7.4/ 2 mm $MgCl_2$ /2 mM EGTA/0.5 mM PMSF).
49. Spin at 2300 g (5000 rpm on tabletop Eppendorf Centrifuge) for 2 min at 4˚C.
50. Aspirate supernatant off
51. Resuspend the two tubes each in 500 µl of 80 mM or .6M salt buffer without Triton, respectively
52. Spin at 2300 g (5000 rpm on tabletop Eppendorf Centrifuge) for 2 min at 4˚C.
53. Aspirate supernatant off
54. Repeat the wash step (step 4-6) one more time
55. Resuspend the two tubes of Streptavidin Sepharose in 220 µl of 80 mM or .6 M salt buffer without Triton X-100, respectively

Biotinylated Nucleosome Pull Down

56. Aliquot 100 µl of equilibrated Streptavidin Sepharose beads resuspended in the appropriate salt buffer to the two tubes of 80 mM INPUT and 0.6 M INPUT respectively. *At this point, there should be about 1.6 ml (0.8 ml in each eppendorf tube) of 80 mM INPUT and 0.6 M INPUT left respectively.*
57. Incubate 1 hour at 4˚C with rotation.
58. Spin at 2300 g (5000 rpm on tabletop Eppendorf Centrifuge) for 2 minutes at 4˚C.
59. Aspirate the supernatant, which is the unbound fraction, to new tubes. For each salt concentration, save 300 µl out of 1.8 ml (as DNA sample, 17%) and 20 µl out of 1.8 ml (as protein sample, 1%) as 80 mM Salt Unbound Fraction (Sample #5) and .6 M Salt Unbound Fraction (Sample #8).
60. Resuspend beads in 1 ml (per Eppendorf tube) of either 80 mM or 0.6 M Salt extraction buffer (without Triton X-100), respectively. Invert the tubes 2-3 times.
61. Spin at 2300 g (5000 rpm on tabletop Eppendorf Centrifuge) for 2 minutes at 4˚C.
62. Repeat the wash one more time.
63. Resuspend Streptavidin Sepharose Pulldown of each salt concentration in 300 µl of 0.2 M NaCl/PBS/2 mM EDTA/0.5 mM PMSF (150 µl in each eppendorf tube). For each salt concentration, save 280 µl out of 300 µl (as DNA sample, 93%) and 20 µl out of 300 µl (as protein sample, 7%) as 80 mM Salt Pulldown (Sample #6) and .6 M Salt Pulldown fraction (Sample #10), respectively.

DNA purification

64. Adjust all samples to a final volume of 300 µl in 0.2 M Salt using TNE buffer (0.2 M NaCl/10 mM Tris.Cl, pH 7.5/1 mM EDTA). *This step could be done by using 5 M NaCl to adjust for the salt concentration. Refer to Supplemental Table 1. Note that the final volume of the input is 400 µl and the appropriate volume of reagent to be added to the inputs is marked in blue color.*
65. Add .5 µg/ µl of RNase A (5 µl /6.25 µl) to each sample.
66. Incubate at room temperature for 30 min.
67. Add 20% SDS to final concentration of 0.5% (7.5 / 9.4 µl).
68. Add 20 mg/ml Proteinase K (3.5 / 4.375 µl) to a final concentration of .2 µg/µl.
69. Incubate at 55˚C for 30 min.

	Rx Vol	Rx Vol	Final [ ]
Samples in .2 M salt	300 µl	400 µl	.2 M NaCl
RNase A (.5 µg/µl)	5 µl	6.25	2.5 µg in 300 µl
Incubate at RT for 30 min			
20 % SDS	7.5 µl	9.375	0.5 %
20 mg/ml Proteinase K	3.5 µl	4.375	200 µg/ml =0.2 µg/µl
Total Volume	300	400	
Incubate at 55˚C for 30 min			

70. For samples containing Streptavidin Sepharose, spin at 2300 g (5000 rpm on tabletop Eppendorf Centrifuge) for 2 minutes at 4˚C. *The purpose of this step is to separate the DNA from Streptavidin Sepharose.*
71. Remove supernatant into a new tube.
72. Add 300 µl (400 µl) of phenol/chloroform for extraction.
73. Spin at 16100 g (13200 rpm on tabletop Eppendorf Centrifuge) at room temperature for 5 min.
74. Add 300 µl (400 µl) of chloroform to each tube, perform the extraction again
75. Add 750 µl (1 ml) of 100% EtOH.
76. Set on ice or at -20˚C for half an hour.
77. Spin at 16100 g (13200 rpm on tabletop Eppendorf Centrifuge) 4˚C for 15 min
78. Wash in 1 ml of 70% EtOH.
79. Resuspend in 25-50 µl of TE buffer.

Protein Analysis Preparation

80. To prepare for protein western blot analysis, for each 20 µl of protein sample, add 10 µl of 3X SDS buffer.
81. To prepare pull down samples for western blot analysis, add 10 µl of 8 M Urea to 20 µl of streptavidin sepharose pull-down, followed by 15 µl of 3X SDS buffer with β-mercaptoethanol. *Streptavidin Sepharose pull-down sample requires denaturation step by urea because of the irreversible interaction between biotin and streptavidin. However, even using condition described above, it is still difficult to recover all biotinylated protein for visualization on a western blot.*
82. Boil at 100˚C for 10 min
83. Store at -20˚C until use.

Buffer A : 15 mM Tris.Cl, pH 7.5/2 mM MgCl2/.34 M sucrose/.15 mM spermine/.5 mM spermidine/1 mM DTT/0.5 mM PMSF

		Volume (µl) required for	
	Stock [M]	50 ml	500 ml
15 mM Tris.Cl, pH 7.5	1	750	7.5 ml
2 mM MgCl2	1	100	1 ml
.34 M sucrose	1	17000	170 ml
.15 mM spermine	0.25	30	300 µl
.5 mM spermidine	2	12.5	125 µl

To be added just before use	1 mM DTT	1	50
To be added just before use	0.5 mM PMSF	100 mM	250

Buffer A with NP40 and Triton X-100: 15 mM Tris.Cl, pH 7.5/2 mM MgCl2/.34 M sucrose/.15 mM spermine/.5 mM spermidine/1 mM DTT/0.5 mM PMSF/.25 % NP-40 substitute/.1% Triton X-100

		Volume (µl) required for	
	Stock [ ]	50 ml	500 ml
15 mM Tris.Cl, pH 7.5	1	750	7.5 ml
2 mM MgCl2	1	100	1 ml
.34 M sucrose	1	17000	170 ml
.15 mM spermine	0.25	30	300 µl
.5 mM spermidine	2	12.5	125 µl

To be added just before use	1 mM DTT	1	50	
To be added just before use	0.5 mM PMSF	100 mM	250	
To be added just before use	.25 % NP-40 substitute	100%	125	
To be added just before use	.1% Triton X-100	20%	250	

TM2 Buffer: 10 mM Tris.Cl/ 2 mM MgCl2/0.1% Triton X-100
Buffer to resuspend nuclei for Mnase treatment

	Final [ ]	Stock [ ]	10 ml	50 ml
	10 mM Tris.Cl, pH 7.5	1 M	100 µl	500 µl
	2 mM MgCl2	1 M	20 µl	100 µl
To be added just before use	0.5 mM PMSF	100 mM	50	250

80 mM Salt Extraction Buffer : 10 mM Tris.Cl, PH 7.5/2 mM MgCl2/70 mM NaCl/2 mM EGTA/0.5 mM PMSF/0.1% Triton X-100

	Final [ ]	Stock [ ]	10 ml	50 ml
	10 mM Tris.Cl, pH 7.5	1 M	100	500
	2 mM MgCl2	1 M	20	100
	70 mM NaCl	5 M	140	700
	2 mM EGTA	.2 M	100	500
To be added just before use	0.5 mM PMSF	100 mM	50	250
To be added just before use	0.1% Triton X-100.	20%	50	250

600 mM Salt Extraction Buffer : 10 mM Tris.Cl, PH 7.5/2 mM MgCl2/585 mM NaCl/2 mM EGTA/0.5 mM PMSF/0.1% Triton X-100

	Final [ ]	Stock [ ]	10 ml	50 ml
	10 mM Tris.Cl, pH 7.5	1 M	100	500
	2 mM MgCl2	1 M	20	100
	585 mM NaCl	5 M	1.17 ml	5.85 ml
	2 mM EGTA	.2 M	100	500
To be added just before use	0.5 mM PMSF	100 mM	50	250
To be added just before use	0.1% Triton X-100.	20%	50	250

TNE Buffer : 10 mM Tris.Cl, PH 7.5/ .2 M NaCl/ 1 mM EDTA

Final [ ]	Stock [ ]	10 ml	50 ml
10 mM Tris.Cl, pH 7.5	1 M	100	500 µl
.2 M NaCl	5 M	400	2 ml
1 mM EDTA	0.5 M	20	100

Recipe for Peptone Rich plate

	Gram	Final concentration
Agar	25	2.50%
Peptone (Fisher)	20	
Nacl (Sigma)	1.2	
water	to 975 ml	
Autoclave, cool to 55°C, add remaining ingredients		
1M PPB (Potassium Phosphate Buffer)	25 ml	25 mM
1M MgCl2	1 ml	1 mM
5 mg/ml cholesterol in ethanol	1 ml	5 µg/ml

Pour ~100 ml onto each 150 x15 mM plate
When the plate is dry, seed each plate with NA22 bacteria

Supplemental Table 1.

				80 mM			.6 M			
	Nuclei	MNase Total	SUP1	Input	Unbound Fraction	Pulldown	Input	Unbound Fraction	Pulldown	.6M Pellet
Sample Number	1	2	3	4	5	6	7	8	9	10
DNA Sample Volume (µl)	60	200	300	400	300	280	400	300	280	300
Out of a total volume of (in ml)	2	2	2	2	1.8	0.3	2	1.8	0.3	2
% of sample used for DNA Prep	3%	10%	15%	20%	17%	93%	20%	17%	90%	15%
Existing Salt concentration (mM)	12 mM	12 mM	12 mM	80 mM	80 mM					
Volume (µl) of 5 M NaCl to add	2	8	12	15	12					
Volume (µl) of .2 M TNE to add	240	100	--	--	--					
Protein Sample Volume	20	20	20	20	20	20	20	20	20	20
% of sample used for Protein Analysis	1%	1%	1%	1%	1%	7%	1%	1%	7%	1%