Quick Chromatin Digestions

(Modified from Nick Kent, http://www2.bioch.ox.ac.uk/~nakent/method.html)

If you really want to read in depth, see the original protocol noted above.
Yields enough DNA for ~ 4-8 Southerns.

Reagents:

- 1M Sorbitol
- Zymolyase solution (made fresh): Zymolyase 20T in 1M Sorbitol and 5mM b-Mercaptoethanol. (See below for Zymolyase concentrations).
- Spheroplast digestion buffer (SDB): 1M Sorbitol, 50 mM NaCl, 10 mM Tris pH 8, 5 mM MgCl2, 1 mM CaCl2, 1 mM b-Mercaptoethanol, 0.5 mM spermidine, 0.075% NP40. (bME added fresh)
- MNase or DNaseI (see below)
- Buffer G2 (Qiagen): 800 mM guanidine HCl, 30 mM Tris pH 8, 30 mM EDTA, 5% Tween-20, 0.5% Triton X-100.

Protocol:

1) Grow up 100 ml cells to OD660 = 0.7, or close enough.
2) Spin down in 2 x 50 ml Falcon tubes 2′ at 3000 rpm in J-6. Dump media. Vortex pellet briefly to resuspend.
3) While cells are pelleting, label 5 normal microfuge tubes and add either 15 U DNaseI (Roche units) or 75 U MNase (Roche units) to each. (If you want a range of digestions, try 2-3 tubes each, but you will recover only ~2-4 Southerns worth of DNA for each. If this is desirable, I would use 7.5 and 15 U DNaseI and 25 and 75 U MNase, or try a range of concentrations and see what you like.)
4) Transfer resuspended pellet to 2 ml microfuge tube. Spin briefly (let microfuge accelerate to ~10,000 rpm and then stop). Remove Media with P1000.
5) Resuspend pellet in 950 μl Zymolyase solution by pipetting up and down (rapidly, but without spilling it everywhere). If you grow cells in glucose, Zymolyase solution should contain 10 mg/ml Zymo, and you should incubate at room temperature for ~50′ while inverting tube in your hand. If cells grown in raffinose or galactose, up the Zymo to 50 mg/ml (yes 50, don't tell Toshi) and incubate for 2′ at room temp. N. Kent says the spheroplasting times are different for different strains, so you may have to play around with it a bit.
6) Pellet spheroplasts in microfuge (as before with cell pellet) and add 950 μl 1M Sorbitol. Reverse orientation of tube and pellet again. (This will sort of wash spheroplasts as they drift from one side of tube to other.) Repeat (2 washes total).
7) Resuspend pellet in 1.2 ml SDB. Add 200 μl aliquots to each nuclease-laden tube, mixing by pipetting up and down 2-3 times. Work rapidly, so that the first tubes are not digested significantly longer than the last. Quickly transfer to 37 degree incubator. After 1′ incubation, briefly flick tubes to mix and distribute heat.
8) After 4′ total incubation (3′ after flicking), remove tubes and add 800 l G2 Buffer to each and vortex. This stops reaction. Incubate tubes from 20′ to quite-a-while (if you are too busy to process) at 37 degrees to allow complete lysis (or nearly complete) and spin in microfuge tubes 2 at full speed. Pour all 5 identical aliquots into a 14 ml Falcon tube and add RNaseA (~40 mg) and incubate at 42 degrees 0.5-1 hour. Add proteinase K (150 mg) and incubate 3 hours to overnight at 50-55 degrees.

9) Vortex tubes for 10'. Spin tubes at 4000 rpm in J-6 for 10'.
10) While waiting, equilibrate Qiagen Genomic-tip 20/G column with 1 ml QBT and aliquot some buffer QF into a tube and incubate at 50 degrees.
11) Pour from 14 ml tubes directly into equilibrated column. (Only 2.5-3 ml will fit, so do it in 2 steps.)
12) After liquid flows through, add 1 ml QC buffer. I pipet buffer around circumference of column to wash sides. After flow through, repeat wash.
13) Transfer column to 14 ml tube, using column girdles and add 900 μl of 50 degree buffer QF. After flow through, repeat (total 2 elutions).
14) Transfer elutions to two normal microfuge tubes and add 700 μl isopropanol to each. Invert a few times (At this stage, you can put in -20 °C for a while, or not.) and spin at full speed for 15'.
15) Remove all liquid, while not disturbing pellets. Let pellets dry and resuspend in 40 μl total (20 μl in each tube and combine). There may be some insoluble shit in the tube at this point (resin from columns?). Just do a hard spin before each time you remove DNA for southern blotting.

Southern Blotting (Indirect end labeling):

- I like to pick restriction sites that are ~1.7-2.3 kb apart and one is 600-1.1 kb from the region of interest.
- I try to make the probe 180-250 bp in length.
- Digests: I do them in 50 μl, with 2 μl of each enzyme and precipitate/resuspend in 10 μl l TE after. I usually quantitate [DNA] by spectrophotometer and adjust amounts of DNA accordingly. 1.5 to 2 mg DNA is usually sufficient.
- Gel: 1.7% agarose in 1X TBE. Run slowly overnight (esoterica: BufferPuffer requires ~1300 volt-hours (Vh) under these conditions.) Pour sort of thin (6-7mm).
- Denature/neutralize 45 each (or so). Capillary transfer onto uncharged nylon membrane. Cross-link w/Stratalinker.
- Hybridize with probe at 55 degrees (overnight is preferable). Wash 1x 5' in 1X SSC/0.5% SDS; 2x 15' in same buffer (all washes at 55 degrees).
- Exposure depends on signal, but 2 days on MR film not uncommon.

Primer Extension:

i. **I could only get this to work for MNase-digested samples**
ii. **The same amount of MNase for indirect end labeling works for primer extensions**

- Primer: design primer to be at least 30 bp and at least 60^0 Tm and probably about 50 bp from region of interest. I end-label 50 pmol w/PNK in 50ul reaction. Purify on Biogel 6 column (elutes in 30 μl or so). Use 0.3 μl (so ~.5-1 pmol) per extension.
- Extensions:

H20 + DNA (~1ug)	18 μl
Rxn Buffer*	5 μl
10 mM dNTPs	0.5 ul
Taq	1 μl
Labeled primer	0.3 μl

Cycling parameters:

1X	95^0	3 minutes
30X	95^0	30 seconds
	60^0	30 seconds
	72^0	30 seconds
	4^0	hold

- ETOH precipitate samples and resuspend in 2 μl TE and add 8 μl of Stop Solution**
- Boil 2 minutes and add to prerun sequencing gel
- Dry down gel and expose

*Rxn Buffer (5X): 50 mM Tris pH 8.3, 250 mM KCl, 15 mM MgCl2, 0.25% NP-40, 0.25% Tween-20 (from Shimizu et al, 1992)

**Stop Solution: 95% formamide, 20 mM EDTA, 0.05% Bromophenol Blue, 0.05% Xylene Cyanol