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Preparing Mouse Genomic DNA using the EPICENTRE MasterPure complete DNA purification kit

This protocol is taken from the publication: "Genomic DNA as a general cohybridization standard for ratiometric microarrays", Brian A. Williams, Richele M. Gwirtz and Barbara J. Wold. Methods in Enzymology, (2006) DNA microarrays Part A: Array platforms and wet-bench protocols, volume 410, chapter 14.

Label incorporation and yield of target are strongly influenced by the source of genomic DNA used as template. In our hands, genomic DNA from several commercial suppliers gave inefficient labeling compared with companion reactions using DNA prepared directly from mouse tissue. This method allows specification of the strain, genotype and sex of the genomic DNA standard, thereby allowing the evaluation of qualitative differences in Y-linked genes, and quantitative differences in X-linked genes.

We chose the EpiCentre MasterPure DNA purification kit for in-lab production of mouse genomic DNA, and supplemented it with 3 additional RNAses available from EpiCentre. Mouse kidney was prepared for DNA extraction by freezing immediately after dissection in liquid nitrogen, grinding in a mortar and pestle under liquid nitrogen, and aliquoting in cryovials stored at -80°C. Using this protocol, careful attention should be paid to the appropriate volume of lysis buffer relative to the amount of tissue being extracted, using the manufacturer's directions for guidance. A small aliquot of the crude preparation from the kit was set aside for agarose gel electrophoresis, to visualize the intact genomic DNA and any residual RNA remaining after the RNAse A treatment in the kit (Figure 1). We then used EpiCentre's Riboshredder, as a potent, broad spectrum RNAse to target any residual RNA, which, if later labeled, would introduce prep to prep variation. Next, we added RNAse I, to degrade any remaining single stranded RNAs, and then RNAse H, to remove any remaining RNA in heteroduplex form with the genomic DNA.

Size fractionation of genomic DNA was accomplished by random shearing with a sonicator to an average size of 1-2 kb with a maximum of approximately 4 kb (Figure 1). Our labeling and yield measurements were consistently higher for this size fragment when compared to shorter sheared products or DNA digested with DpnII. Likewise, the signal intensity on array features after hybridization was consistently greater for longer sheared fragments than for shorter DNA, just as one would predict. Published results have suggested that shorter fragments might increase specific signal strength by increasing target mobility (Dai et al., 2002), but over the size ranges we surveyed, longer DNA gave a stronger signal.

The following protocol produces ~90 µgs of DNA from 70 mgs of mouse kidney tissue, which is enough for 36, 2.5 µg aliquots or 12 microarray experiments. Keep in mind that if only male mouse kidney is chosen, X-linked genes will be represented in the standard at half the abundance of autosomal genes.

Equipment and Reagents

- Liquid nitrogen
- Mortar and pestle for grinding
- Oven or heating block set at 65°C that can accommodate a 14 ml centrifuge tube.
- Eppendorf 5417C microcentrifuge with variable RPM/RCF adjustment at room temperature (any good variable speed lab microcentrifuge should work, make sure that it is capable of speeds greater than 10,000 g).
- Microcentrifuge with variable RPM/RCF adjustment at 4°C.
- Laboratory balance
- UV spectrophotometer
- Microson XL 2007 sonicator with small tip to fit inside microcentrifuge tubes. (Note: any good sonicator with a microtip will work. You will have to experiment with the power and duty settings to

achieve fragmentation of the DNA to achieve a smear topping out at about 3-4 kb, with average size about 2 kb, as seen on a 1% agarose gel. See Figure 1 for an example.

- Zymo 25 μg capacity DNA Clean and Concentrator columns (catalog # D4005)
- Distilled H_2O adjusted to pH 8.0 with NaOH
- Equipment and reagents for standard agarose gel electrophoresis.
- Microcentrifuge tubes, gloves, aerosol barrier pipette tips, dry ice, wet ice, ethanol, isopropanol.
- 1M NaCl solution
- 100 mM $MgCl_2$ solution
- 1 M Tris-HCl pH 7.5 solution
- EPICENTRE MasterPure complete DNA purification kit (catalog # MCD85201)
- EPICENTRE Riboshredder (catalog # RS12100)
- EPICENTRE RNAse I (catalog # N6901K)
- EPICENTRE RNAse H (catalog # R0601K)
- ISC BioExpress GeneMate 2 ml microcentrifuge tubes (catalog # C-3219-1)

Freezing and pulverization

1. Mouse kidneys are quickly dissected and flash frozen in liquid nitrogen.
2. It will be important to get a reasonably accurate weight of the tissue aliquots. Before preparing to grind the kidneys under liquid nitrogen, prepare about 30 tubes to hold tissue aliquots. Weigh the tubes prior to filling, and mark the empty tube weight on each tube. Place the marked tubes in ground dry ice to cool.
3. Kidneys are ground in liquid nitrogen in a mortar and pestle which has been pre-chilled using liquid nitrogen.
4. Aliquot ground tissue at approximately 20 mg per tube. Keep on dry ice. (CAUTION: do not close tube immediately, pressure can build up due to gas release from liquid nitrogen and cause tube to pop).
5. Re-weigh the tubes cold to get an accurate estimate of tissue weight, but don't bother trying to readjust individual tubes by redistributing ground tissue. Keep the dry ice near when weighing, to avoid freeze/thaw. Mark the filled tube weight on each tube.
6. Store aliquots separately at -80°C.

Tissue lysis

It is important to maintain the correct proportions of T&C lysis buffer and Proteinase K (MasterPure kit) to the estimated weight of your tissue sample. If this is not done, you will have difficulty achieving complete lysis, which will affect your yield of genomic DNA.

1. When tissue samples are brought from the freezer, appropriate amounts of T&C lysis buffer are quickly added to each tube (300 μL of T&C lysis buffer per 5 mg tissue, plus 1μL of Proteinase K per 5 mg tissue). We process 14-15, 5 mg samples at a time; therefore select aliquot tubes from the freezer that will total ~70 to 75 mg of tissue mass.
2. The samples are incubated separately for 5 minutes at 65°C, and are then combined in a single 14 ml centrifuge tube.
3. The incubation is then continued for another 10 minutes at 65°C, vortexing at 5 minutes and again at 10 minutes. If lysis is not complete at 15 minutes, you can continue incubation for an additional 5 minutes with an additional vortexing.
4. Incubate at 37°C for 5 min.
5. 1 μL of RNAse A (MasterPure kit) is added per 5 mg of tissue.
6. Incubate at 37°C for 30 minutes, vortexing once at 15 minutes, and more importantly at the end of the 30 minute incubation (prior to splitting). While waiting, prepare 14 separate fresh tubes and place in a 4°C ice bath.

Protein precipitation

7. Split sample into the 14 chilled tubes (~300 µL per tube). Chill on wet ice for 4 minutes.
8. Add 150 µL MPC protein precipitation reagent (MasterPure kit) to each tube.
9. Vortex 10 seconds.
10. Spin at 10,000g for 10 minutes, room temperature.

Genomic DNA precipitation and resuspension

11. Remove the supernatant to 14 fresh tubes.
12. Add 500 µL of isopropanol (room temperature) to each tube.
13. Invert tubes 40 times, DO NOT VORTEX.
14. Spin at 12,000 g for 10 minutes at 4°C.
15. Aspirate and discard supernatant.
16. Rinse pellets twice with about 300 µL 70% ethanol (briefly vortex and spin down each time).
17. Remove trace remaining ethanol with a fine tip and let the samples air dry on the benchtop for about 30 minutes (may take longer).
18. Resuspend pellet in each tube in 35 µL of TE (MasterPure kit).
19. Incubate at 37°C for 20 minutes.
20. Resuspend each sample by trituration with pipette tip, then combine all 14 samples into a single tube and count 2 µL on the spectrophotometer. Typical estimated yield at this point is 20-25 µgs per 5 mg sample. The 260/280 ratio will be about 1.4-1.6.

If you "park" the preparation here, approximately 4.5 hours have elapsed since beginning the extraction. The sample volume is now 490 µL. The preparation can be stored at -20°C overnight, or at 4°C if you want to avoid freeze/thaw.

RNAse digestion

1. Add 8 µL of RiboShredder to the sample.
2. Incubate at 37°C for 30 minutes.
3. Add (4.3 µL per sample) X (14 samples) = 60.2 µL (~60 µL) of 1M NaCl
4. Add (2 µL per sample) X (14 samples) = 28 µL of 1:10 dilution of RNAse I in dilution buffer (for 1:10 dilution, use 3 µL stock to 27 µL dilution buffer)
5. Incubate at 37°C for 30 minutes.
6. Add (5 µL per sample) X (14 samples) = 70 µL 100 mM $MgCl_2$ (10 mM final)
7. Add (2.5 µL per sample) X (14 samples) = 35 µL 1M Tris HCl pH 7.5 (40 mM final).
8. Add 8 µL of RNAse H
9. Incubate at 37°C for 30 minutes.
10. Reserve 5 µL for electrophoresis here.
11. Final volume is now ~700 µL.

Sonication

12. Split the preparation into 3 aliquots of 233 µL (~230 µL) each into 2 ml microcentrifuge tubes (ISC BioExpress).
13. Place each 2 ml tube in -20°C ethanol while sonicating at setting 18 on a Microson XL 2007 sonicator for 50 seconds.
14. Recombine the sonicated aliquots (~700µL total) in a 4 ml Falcon Tube (#35 2063).

Column cleanup

15. The entire preparation will be cleaned up over 4 Zymo columns (25 µg capacity for each column).
16. Add 2 volumes (1400 µL) of Zymo DNA binding buffer.
17. Vortex lightly and spin down briefly to bring liquid down from sides of tube.
18. Split the preparation into 4 samples of 525 µL each.

19. The entire volume (525 µL) of each sample is passed over each column twice, in order to bind as much DNA as possible to the column. However, the column load volume is only 400 µL, so this will require 4 spins.
20. Add the first volume of each sample to the Zymo column.
21. Set the centrifuge speed to maximum, and watch the RCF counter. When it hits 10,000g, begin timing for 10 seconds. Then stop the centrifuge.
22. Repeat with same volume (the "flowthrough") and then discard.
23. Bind the second volume (the remainder of each sample) with 2 spins as above.
24. Wash with 200 µL of wash buffer. Time the spin as above.
25. Repeat the wash, but this time spin for 30 seconds after 10,000g is reached, then stop the centrifuge.

To elute the DNA:

26. Add 35 µL ddH_2O that has been adjusted to pH 8.0 (*important step*).
27. Incubate for 5 minutes at room temperature.
28. Spin for 30 seconds total elapsed time with the centrifuge set at maximal speed. Do not wait until the centrifuge hits maximal speed and then start timing.
29. Repeat elution as above (step 26).
30. Count 3 µL in the UV spec. Typical yield is 1.3 µgs genomic DNA per mg tissue input. 260/280 should be 1.8 – 1.9.
31. A 2 µg aliquot run on a 1% agarose gel should show a smear topping out at about 3-4 kb, with average size about 2 kb (see Figure 1).
32. Store in 2.5 µg aliquots at -80°C.