DNA Extraction of 2 x 96 samples using a modified Qiagen kit

Written by Brook Moyers (following Horne *et al*. 2004) on 18 August 2013

Before starting:

* **Day before**: autoclave a set of 2 racks of 1000/1200μl (depending on your pipette choice) pipette tips with the last ~3-5 mm of the tip cut off (consistent length is best). This prevents much of the mechanical shearing that is usually observed with the Qiagen columns.
* Heat ~105 ml of AP1 to 65 C in a new container (for working lysis buffer)
* Defrost aliquot of 1M DTT (~1 ml) on ice
* Chill 31 ml of buffer P3 in –20 C
1. Disrupt your tissue in the provided collection microtubes with one 3.2 mm chrome bead. Less is better; I use one 7mm diameter lyophilized leaf disc per extraction. Use no more than 10 mg dry or 50 mg fresh tissue. I usually run four rounds of 45 s. disruptions at slightly below max speed (25 Hz), switching the plates between sides of the disruptor and grinding in two orientations (‘A’ row out / ‘H’ row out) on each side. Tap the plates against a bench to knock as much of the ground tissue off the caps as you can.
2. While your tissue is being disrupted, prepare the working lysis buffer:
	1. 105 ml pre-warmed AP1
	2. 260 μl RNase A
	3. 260 μl Reagent DX (prevents foaming)
	4. ~1 ml 1M DTT (final concentration ~10 mM)
3. Carefully remove caps and add 500 μl of the working lysis buffer to each tube. If you’re careful to keep them properly oriented, you can replace the same caps; otherwise seal with new caps, cover with the clear plastic rack tops, and shake each rack for several seconds.
4. Incubate racks in 65 C (I use the drying oven) for 45 minutes. Check and shake every 10 minutes or so. It is a good idea to put something heavy on top of the rack covers, to keep the tubes sealed.
5. Pulse spin for 15 s at 1500 rcf; remove and discard caps. Add 150 μl of chilled buffer P3 to each tube. Cover with new caps and the rack cover and shake vigorously with both hands for a full 15 s. Pulse spin for 15 s at 1500 rcf to collect fluid from caps.
6. Incubate at –20 C for 10 minutes. You can prolong this step if you need to.
7. Centrifuge at max (~6000 rcf) for 7 minutes.
8. Carefully transfer 400–450 μl of the supernatant by using the cut pipette tips (try not to contaminate with the pelleted materials; I use the manual 1200 μl multi-channel pipette but a single 1000 μl would also work) to a new racked collection microtube. Make sure to label and orient them correctly before transfer.
9. Add 1.5 volumes (600–675 μl) of buffer AW1 to each tube. Cap carefully (if you used larger volumes they may be full) and add the clear rack tops, each with a clean folded paper towel inserted. Shake vigorously with both hands for a full 15 s. Pulse spin for 15 s at 1500 rcf to collect fluid from caps.
10. Remove and discard caps. Place DNeasy 96 plate on top of an S-block (both labeled and in the same orientation). Carefully transfer 1 ml of your sample to the DNeasy plate columns. Seal with tape sheet.
11. Spin for 4 minutes at max (~6000 rcf). If you are working with larger volumes, repeat steps 10 and 11 with the remainder of your samples, after emptying the flow through from the S-blocks. You may reuse the tape sheet. If some of your sample remains on top of the columns after one spin, spin for another 4 minutes at max.
12. Remove the tape sheet and add 800 μl of buffer AW2 to each well. Reseal with a new tape sheet and centrifuge at max (~6000 rcf) for 5 minutes. Discard flow through.
13. Add 800 μl of 100% ethanol and spin for 15 minutes at max speed, without the tape sheet, to dry the membrane. Discard the flow through.
14. Place the DNeasy 96 plate on a rack of collection microtubules (labeled and in correct orientation). Add 50–100 μl of buffer AE (or whatever you want to elute into). Carefully tap the plates against the bench to ensure that all elution buffer is on the membrane and seal with a new tape sheet. Incubate for 3 min at 65 C (I use the oven). Centrifuge at max for 2 minutes.
15. Repeat step 15 with an additional 50–100 μl of elution buffer.
16. Cap with the provided rubber strips and store at –20 C. If your DNA quality decays with time and it will not interfere with downstream applications, add DTT to a final concentration of 10 mM before storing.