RNase Treatment (Post-Extraction) Protocol

Note: DNA is less soluble in isopropanol, so it will fall out of solution faster and at a lower concentration, but the downside is that the salt will too. It is *okay* to chill an isopropanol precipitated sample if you are sure that it is not excessively salty. You may choose to wash the pellet several times with 70% ethanol after pelleting, to reduce the amount of salt you carry over.

1. Nanodrop your sample and record the values. You may choose to remove 4-8 ul of untreated xDNA and set aside for comparison later on your gel.
2. Measure and record your starting volume.
3. Add 1/100 volume of RNase One (Promega) to your sample (e.g. for 100ul of xDNA, ad 1ul of RNase). Mix by back pipetting.
4. Incubate at 37°C for 30 min.
5. Add 1/10 volume of 3M sodium acetate.
6. Add 1 volume of room temperature isopropanol. Mix by back pipetting.
7. Incubate at one ice for 10 minutes
8. Centrifuge at max speed for 20 minutes 4°C. Be sure to note the orientation of the tubes so that you know where to expect a pellet to form.
9. Check for pellet formation. The isopropanol pellet is sometimes more difficult to see compared to the ethanol pellet and can look clear and glassy.
10. Carefully decant off the isopropanol and add ice cold 70% ethanol (~120 ul).
11. While keeping track of the pellet, centrifuge again for 15 minutes at 4°C. Decant of the 70% ethanol wash. You may choose to do multiple washes (2 washes works well).
12. Allow the tube to drain upside down for a few minutes and then air dry (in incubator at 56 °C or speed vac dry). Be careful not to over-dry. 5-10 minutes of drying time is usually fine.
13. Incubate the AE elution buffer to 50-60°C. Add the appropriate volume of AE buffer and allow the pellet to dissolve. After dissolving pellet, you may choose to continue incubating your sample for about 5 minutes to allow the pellet to completely dissolve.
14. Nanodrop again and run the treated (and untreated sample, if you wish) sample out on a gel (1.5-2% agarose gel at 120V for 90 minutes)