Improved Phenol:Chloroform:Isoamyl alcohol (25:24:1) extraction of DNA - Rahul Patharkar

Reagents:
Phenol chloroform:isoamyl alcohol 25:24:1 (PCI) – Fisher
3.0 M Sodium Acetate p 5.2 (http://cshprotocols.cshlp.org/content/2015/6/pdb.rec085761.full?rss=1)
20 µg/µL Linear polyacrylamide (LPA) – (can be purchase from Fisher Scientific or made yourself)
Isopropanol
70% Ethanol

Before starting note: use the smallest tubes you can handle. You should have at least 20 µL starting volume for 0.2 mL tubes; 30 µL starting volume for 0.5 mL tubes; 50 µL starting volume for 1.5 mL tubes. If you have less than the indicated volume at the start of this procedure, add water to the indicated minimum volume.

1. Add Sodium Acetate to 0.3M final concentration (divide starting volume by 9) to your DNA. Add 1 µl of LPA.
2. Add 1/10th volume of PCI to your sample (i.e. if the step 1 total volume is 20 µL, add 2 µL PCI).
3. Shake by hand vigorously for 5-10 seconds.
4. Spin at max speed (>= 10,000xg) for 30 seconds - 1 minute.
5. Carefully transfer the upper phase to a new tube without taking any of the interphase.
6. Add 0.6 volumes of isopropanol and mix well (i.e. if you got 15 µL of upper phase, add 9 µL isopropanol).
7. Spin at max speed (>= 10,000xg) for 5 minutes.
8. Remove the supernatant by pipetting (get as much supernatant out as possible without disturbing the pellet).
9. Wash the pellet with 70% ethanol (fill the tube half way to ¾ the way full). Mix by gently inverting the tube. Spin for 5 seconds. Pipet the supernatant away.
10. After getting rid of the 70% ethanol, spin the residual supernatant down and pipet it out. Dry the pellet by placing the tube on its side.
11. Resuspend the pellet in the desired buffer (like TE) and volume.

For a video protocol see: https://rahulpatharkar.000webhostapp.com/?p=291