DNA purification using <u>Wizard® DNA Clean-Up System</u>

(1) For **ground water** *(or samples with very low DNA concentrations)* purify approximately half the sample.

(2) Bring volume of the sample up to 50-100 µL with nuclease-free H_2O

(3) Add 100 µL Direct Purification Buffer (50 mM KCl, 10 mM Tris-HCl (pH 8.8), 1.5 mM $MgCl_2$, 0.1% Triton® X-100)

<u>Direct Purification Buffer</u> (100 mL)
2.5 mL 2 M KCl
1 mL 1 M Tris-HCl
150 µL 1 M $MgCl_2$
100 µL Triton X-100

(4) Mix 1 mL resin with DNA by inversion, incubate at room temperature 30 min, mixing every 10 min. *Make sure the resin is well-mixed prior to use.*

(5) Attach the minicolumn to the syringe barrel (both provided with the kit). Add DNA/resin mix to the barrel. Apply a vacuum to draw the sample through the minicolumn. Once all the samples have passed through the column, release the vacuum.

(6) Transfer 2 mL 80 % isopropanol to the column and apply a vacuum. Continue to apply a vacuum for 30 s after the wash solution has been pulled through the column (*no more than 30 s*). Remove the syringe barrel and place minicolumn into a microcentrifuge tube.

(4) Centrifuge the minicolumn for 2 min 10,000 x *g* to remove excess isopropanol.

(5) Transfer the minicolumn to a new microcentrifuge tube and place 100 µL pre-warmed (80 °C) nuclease-free water to the minicolumn. Incubate at 80 °C for 10 min (*you can keep the microcentrifuge and minicolumn in a hotplate set at 80 °C).*

(6) Centrifuge the minicolumn for 20 s, 10,000 x *g* to elute the DNA

(7) Repeat steps 5 and 6 with an additional 100 µL of water

(7) Check the concentration and purity with nanodrop.
If the 260/280 ratio is too low (<1.8), repeat purification process.
If the color of the sample is too dark, repeat the purification – *the DNA extracts should be colorless.*
If the 260/230 ratio is low (<1.7), proceed with the desalting protocol.

(8) Store DNA at -20 ℃ or -80 ℃

Desalting Protocol

If the 260/230 ratio is low after purification (<1.7), the desalting protocol can be used to remove contaminants (most likely guanidine from the resin in the purification kit).
If you are concerned about the quantity of DNA you have, please discuss with Liyou prior to desalting. Another option is to desalt only half of your DNA and see how much DNA is recovered.

(1) Precipitate the Wizard purified product with 2.5 vol of 100 % ice cold ethanol and 1:10 volume of 3 M NaOAc (pH 5.2). Incubate the samples overnight at -20 ℃. *If you can see the DNA precipitating you do not need to incubate overnight.*

For 100 μL purification product add 250 μL ethanol and 10 μL NaOAc

(2) Centrifuge 30 min at 13,000 x *g* to pellet the DNA

(3) Decant the supernatant and wash the pellet with 1 mL 70 % ethanol (cold). *Keep the supernatant until you are sure that DNA has been recovered.*

(4) Centrifuge the pellet 10 min at 13,000 x *g*

(5) Decant the supernatant

(6) Dry the pellet for ~30 min (may take longer). *Make sure to not over dry the sample.*

(7) Resuspend the pellet in nuclease free water

(8) Some impurities may still be present, which will not dissolve – possibly from the purification filter. Centrifuge the samples after dissolving and transfer the supernatant to a fresh tube.

(9) Check DNA using nanodrop
260/230 ratio should be $\geq$ 1.7

(10) Store DNA at -20 ℃ or -80 ℃

At this point the optimum numbers are:
260/280 -- 1.8
260/230 – $\geq$1.7