Protocol for cleaning of PCR product and DNA quantification

Before products from reactions can contribute to downstream reactions, they must be cleaned by removing unincorporated primers, dNTPs and dyes. This is done easily using ethanol precipitation and centrifugation. Equipment, reagents and protocols are listed below.

Reagents:
Distilled de-ionized H_2O (ddH_2O)
100% Ethanol (ETOH)
70% ETOH

Equipment and supplies:
Eppendorf tubes (eppi tubes)
Micropipettor (10-100µl, and 100-1000µl)
Centrifuge

PCR Clean Protocol:

1) **Add** 25µL of **100% ETOH** to each sample with PCR product.
2) **Transfer** everything to 1.5 mL eppi tube. Mix by pipette once before transferring.
3) **Vortex** and let it sit for at least 1 minute at room temperature.
4) **Centrifuge** at maximum speed for **5 minutes**. (Make sure the hinges of the tube are facing up!)
5) CAREFULLY **aspirate** the ETOH without disturbing the pellet. (Remove using a micropipettor, or aspirator on the faucet to the sink opposite the refrigerated centrifuge.)
    a. The pellet will not be visible. Be aware that if you placed the tubes in the centrifuge with the hinges facing up, that the pellet will be located on the side of the tube with the hinge, at the base. In such cases it is best to hold the tube at an angle, with the hinge and lid facing down. Then remove the ETOH by carefully running the tip of the pipette along the inside of the tube facing up (your PCR product will be on the bottom) at the same time you are drawing up the ETOH. With practice, this process will become easier and happen more quickly.
6) **Wash** the pellet by adding 250-500µL of **70% ETOH**.
7) **Vortex** or finger flick to rinse the sides of the eppi tubes with ETOH.
8) **Centrifuge** at maximum speed for 3 minutes.
9) **Aspirate ETOH** (carefully!) as thoroughly as possible using same instructions as indicated in STEP 5 above.
10) **Evaporate** remaining ETOH by placing the tubes on a 65-90°C heat block or letting it sit t room temp for 20-30 minutes.
11) **Re-suspend** the PCR product (pellet) by adding ~20µL of **ddH_2O**.
12) **Label** your tubes! You need to indicate:
    a. Specimen ID# **AND**…
    b. Name of DNA region that was amplified using PCR (eg "ITS" or "RPB2")

DNA Quantification with Qubit (Qubit 4):

1) Set up 0.5 mL tubes (use only tubes by Qubit) for your samples plus two additional tubes for standards
2) *SKIP if working solution is already prepared*. Make Qubit working solution—you will need ~200µL per sample. Your master mix should be:

	per reaction
ds DNA BR reagent	1µL
ds DNA BR buffer	199µL

3) Make two standards (standard #1 and standard #2).
    a. For each, add 190µL working solution and 10µL of standard.
    b. Vortex briefly (avoid adding bubbles when you vortex).
4) Dilute DNA samples into working solution. Add 1-20µL of DNA, then add working solution to make a final volume of 200µL.
    a. Vortex samples briefly.
5) Let sit at room temp for 2 minutes.
6) On the home screen of the Qubit, press DNA, then pick dsDNA Broad range or dsDNA high sensitivity for the assay type.
7) On standards screen, select yes to run a new calibration or no to use the last calibration.
   NOTE: it is probably a good idea to do a calibration for each set of samples you are measuring.

8) Read standards. Insert standard 1 and press read. When the Qubit prompts you for the next sample, insert standard #2 and press read.
9) Read DNA samples.
    a. Indicate how many µL (1-20) of original DNA sample has been added to each tube.
    b. Insert (next) sample.
    c. Enter read and record results.
    d. Remove sample and return to 9b.