Purification of Amplified DNA

Kit: QIAquick PCR Purification Kit

1. Thaw the samples if stored at -20 degrees Celsius on ice and spin down the samples in the picocentrifuge.
2. In a 1.5ml eppendorf tube, add 458.5 ul of Buffer PB for each sample. Add the amplified product to the buffer PB and mix by pipetting.
3. Place a QIAquick column in a 2 ml collection tube and pipette the entire sample into the column and centrifuge (17,9000 x g or 13,000 rpm) for 60 seconds. Discard the flow through.
4. To wash, add 750 ul of Buffer PE to the column and repeat centrifugation for 60 seconds. Discard the flow through.
5. Centrifuge the column once more in a 2 ml collection tube for 1 minute to remove residual wash. Discard the flow through and collection tube.
6. Place the column in a 1.5 ml eppendorf tube and add 50 ul of H_2O to the center of the column. Let the column stand for 1 minute and then spin in the centrifuge for 1 minute at 17,900 x g or 13,000 rpm.
7. Once spun, take the concentration and OD on the nanodrop at 260 nm.

Run a 2.5% gel in the same manner as done after extraction. Save the picture. Load 5 ul of sample with 1 ul of DNA 6X loading dye to observe if amplification is sufficient and to validate the nanodrop readings. Once satisfied, store the samples at -20 degrees Celsius.