Gel extraction of PCR product

1) After your last restriction digest of the PCR reactions and SIP treatment of the vector, use gel purification to remove PCR template and uncut vector.
2) Prepare 500 mL of 1 x TAE (10 mL of 50x TAE to 500 mL MilliQ water to cylinder in hood).
3) Make a 1% agarose gel, 0.5g per 50 mL 1x TAE in 250 mL Ehrlenmeyer. Heat to near boiling, check & swirl, repeat until no floating agarose particles are visible to eye (~2x1 minute in our microwave). Use folded paper towel to hold onto neck of flask. Cool in running tap water stream with cooling to ~ 50ºC. Add 3 uL of Ethidium Bromide. Dispose of EtBr tips in can in hood. Pour gel into mold and insert the large tooth combs.
4) Add the 1/6th volume of loading dye to the restriction digest (probably 10 uL to 60 uL digest).
5) Load the mixture into 2 wells; also load ~3uL of DNA marker alongside and run the gel at ~90 volts for ~30 minutes.
6) Photograph gel. Then cut out the bands and place in a 2mL eppendorf. To cut, use the UV lamp to locate your bands (if they're difficult to see by eye). Wear eye protection. Press clean razor vertically down around the bands, minimizing unnecessary agarose.
7) The gel slices need to be weighed immediately after excision. Zero the small balance with empty 2mL eppendorf. Weigh the tubes containing the gel slices and write the amount on the eppendorf. You can now wait overnight or a few days before finishing the subcloning, if you need to.
8) Use the Qiagen Gel extraction protocol to extract your DNA from the gel. If the gel weight is more than 400mg, perform the protocol in duplicate by splitting the gel in two and putting it in 2 eppendorf tubes. The Qiaquick columns are limited to 400mg gel.