Making “sticky-ends” on your PCR product (insert-to-be) and vector

1) After PCR, your DNA fragment of interest is “blunt-ended” and needs to be digested with the appropriate restriction enzymes to make it “sticky-ended”.
2) Your vector also needs to be digested with the appropriate enzymes in preparation for ligation with your PCR product (insert-to-be)
3) Use the enzymes whose recognition sites you added to your DNA sequence when you designed your PCR primers. Usually EcoRI/BamHI for pGEX vectors.
4) Use PCR purification kit to remove PCR buffer. Digest with the first enzyme in appropriate buffer. Use PCR purification kit to remove first restriction enzyme's buffer. Then digest with the second enzyme in appropriate buffer.
    - If your enzyme is EcoRI or BamHI, incubate 2-4 hours at 37°C per digestion. Run PCR purification kit before you leave for the night, don't leave the DNA sitting with EcoRI or BamHI.
    - If your enzyme is NdeI, incubate >6 hours or overnight at 37°C, PCR purification kit next morning.
5) Example restriction digest 80 0 5011040 0 50 1 “

- BamHI enzyme 2.5 µl

TOTAL **60 µl**

7) After last restriction digest, the vector (just the vector, not the PCR insert!) needs to be treated with shrimp alkaline phosphatase (SIP) to remove phosphates and prevent intramolecular ligation. After the incubation period, add 1 µl of SIP to last restriction digest. SIP will be active in the restriction enzyme buffer. Incubate an additional 30 minutes at 37°C. Then, heat-inactivate 22 0 Tm (t) Tj 50 044 0