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 of PCR (always add the enzyme buffer second to last and the enzyme last):

• PCR (*always measure*; *adjust water accordingly*)	46 µl
• sdH20	5.0 µl
• 100x BSA (special for BamHI only)	0.6 µl
• 10x BamHI buffer	6 µl
• BamHI enzyme	2.5 µl
TOTAL	**60 µl**

6) Example restriction digest of vector. Will need at least two of these digests to have enough vector for ligations, so digest in duplicate.

• Vector miniprep	20 µl
• sdH20	31 µl
• 100x BSA (special for BamHI only)	0.6 µl
• 10x BamHI buffer	6 µl

• 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 SIP by transferring to 60°C heat block for an additional 10 minutes.
8) After the second restriction digest (and SIP for vector), the DNAs are ready for gel purification.