Purification Protocol (Fermentas)

All centrifugation are at 13,500 rpm.

1. Weigh a 2 ml tube.
2. Excise DNA from TAE gel. Cut as close to DNA as possible. Use glass plate under gel to minimize damaging the UV transluminator light box.
3. Place gel into tube and weigh the tube again.
4. Add 1X volume of binding buffer to gel.
5. Incubate at 50-60° C for 10 min or until gel is completely dissolved. Mix periodically by inverting. Ensure gel is completely dissolved.
6. Check the pH of DNA gel solution by color. (should be yellow)
7. Add 1X volume of isopropanol to sample & mix by inverting.
8. Place gel solution in GeneJET purification column, centrifuge for 1 min.
9. Discard flow through.
10. Add 700 µl wash buffer to purification column, centrifuge for 1 min.
11. Discard flow through.
12. Centrifuge again for 1 min.
13. Place purification column in 1.5 ml tube.
14. Add 20-50 µl elution buffer to center of purification column, centrifuge for 1 min. (normally 50 µl, use less for less DNA)
15. Discard purification column and store DNA at -20°C

Q-PCR

1:10 dilutions

A → B → C → D → E → F → G