Quantitate your isolated genomic DNA on the NanoDrop.

Set up PCR rxn in PCR tube:

10 µl 5X *Taq* Master Mix

2.5 µl Forward primer

2.5 µl Reverse primer

_____ 100 - 250 ng Template DNA

_____ DDW

50 µl Total volume

Program Thermocycler and run PCR reaction O/N

1X	94°C	4 min
30X	94°C	30 sec
	56°C	60 sec
	72°C	90 sec
1X	72°C	10 min
1X	4°C	∞

Electrophorese 5-20 µl of PCR reaction on **Invitrogen E-Gel**

While your gel is electrophoresing:

Clean up PCR rxn with **Qiagen MinElute PCR Purification Kit**

1. Add 5 volumes of Buffer PB to 1 volume of the PCR reaction and mix.
2. Check that the color of the mixture is yellow. If the color of the mixture is orange or violet, add 10 µl of 3 M sodium acetate, pH 5.0 and mix.
3.
4. Place a MinElute column in a provided 2 ml collection tube in a suitable rack.
5. To bind DNA, apply the sample to the MinElute column and centrifuge for 1 min. For maximum recovery, transfer all traces of sample to the column.
6. Discard flow-through.
7. Place the MinElute column back into the same tube.
8. Add 750 µl Buffer PE to the MinElute column and centrifuge for 1 min. This wash step removes any nonspecific binding on nucleotides and small primers.
9. Discard flow-through and place the MinElute column back in the same tube.
10. Centrifuge the column for an additional 1 min at maximum speed. IMPORTANT: Residual ethanol from Buffer PE will not be completely removed unless the flow-through is discarded before this additional centrifugation
11. Place the MinElute column in a clean 1.5 ml microcentrifuge tube.
12. To elute DNA, add 10 µl Buffer EB (10 mM Tris·Cl, pH 8.5) or water to the center of the membrane, let the column stand for 1 min, and then centrifuge for 1 min. IMPORTANT: Ensure that the elution buffer is dispensed directly onto the center of the membrane for complete elution of bound DNA. The average eluate volume is 9 µl from 10 µl elution buffer volume. Elution efficiency is dependent on pH. The maximum elution efficiency is achieved between pH 7.0 and 8.5. When using water, make sure that the pH value is within this range, and store DNA at -30°C to -15°C as DNA may degrade in the absence of a buffering agent.

Quantitate your purified PCR product on the NanoDrop.

Assemble your sequencing reactions:
Dilute forward primer to 5 µM (pmol/µl)
Dilute reverse primer to 5 µM (pmol/µl)
Need 5 µl for each sequencing rxn.

Dilute your purified PCR product to 4 ng/µl
Need 10 µl per sequencing reaction

Place in a 0.5 ml microfuge tube:
10 µl of purified PCR product
5 µl of primer
You will need two tubes one with forward primer and one with reverse primer.
We will be sequencing from both directions but in separate reactions.
Label your tube