DNA Sequencing in 10 Easy Steps

- 1. Separate and individually label samples for reference.
- 2. Perform DNA Extraction, labeling extractions to match above. Save in the refrigerator.
- 3. Individually amplify 1-2uL of each sample via PCR, labeling amplifications to match above.
- 4. Electrophores 3uL of each PCR productand DNA ladder on an agarose gel. Save remaing PCR product at room temp.
- 5. Stain and photodocument the gel. Don't throw your gel away until you have a good picture.
- 6. "Clean" 5uL of sucessful PCR products by digesting with SAP/EXO. Save extra PCR product at room temp.
- 7. Use 1-2uL of your cleaned PCR product in DNA sequencing reaction. You will perform one reaction separately for each primer for each sample.
- 8. Precipitate DNA sequening reactions by adding 40uL of 70% isopropyl alcohol. Wait 1 hour, centrifuge 1 hour, remove supernate and dry the DNA pellet.
- 9. Resuspend DNA pellet in DNA sequencing loading buffer and load onto sequencer for analysis.
- 10. Edit resulting sequences in Sequencher and compile for data analysis.













