

Sequencing project steps:

Use this to remind yourself of the steps we have already done and what we have yet to do.

Question: what plant species are in my herbal supplement?

- 1) Select supplement(s) (you can choose two different herbal supplements)
- 2) Extract DNA
- 3) PCR1: PCR amplify target gene(s) (ITS2 for plants)
- 4) Run agarose gel to see if PCR worked
- 5) Clean PCR reaction with magnetic beads
- 6) PCR2: Add indexes and sequencing primers by running a second PCR reaction using previous PCR product as a template
- 7) Run agarose gel to see if PCR2 worked
- 8) Clean PCR reaction with magnetic beads (we'll do this for you)
- 9) Bridge PCR (the UAF DNA core lab will do this)
- 10) Sequencing by synthesis on Illumina miSeq DNA sequencer (core lab)
- 11) Analyze data (Dr. Naoki Takebayashi will do this for us)
 - a. Trim primer/adaptor sequences
 - b. Overlap forward and reverse reads from the same cluster
 - c. Assemble similar sequences to create a Consensus sequence
 - d.
 - e. Use RDP classifier to search a curated database and statistically estimate the confidence in species assignments
 - f. Visualize species assignments with Krona
- 12) You will BLAST your consensus sequences to identify species with similar DNA sequences in the NCBI nucleotide database (Genbank)