Day 3, PCR Session: Designing PCR primers for Bar Code in Castenea rbcL region

Lab: I want to design primers to pick up the ~600nt region used to bar code the rbcL gene region in Castenea mollissima (Chinese Chestnut) chloroplasts.

Step 1: collect the necessary bar code sequence.

I looked for a publication from the Barcode Consortium, and found one describing the bar code on Arabidopsis thaliana (a small flowering plant) – it describes using the first 599nt of the gene sequence, so now I know what part was chosen.

How do I find the rbcL sequence from C. mollissima?

1. Go to the main ncbi Home page.
2. To select the database in the first drop-down box, select Gene
3. Enter Castenea mollissima AND rbcL
4. You will see some graphics that you can mouse over to explore
5. Continue down to the section RefSeq, it shows one sub-heading Genomic, gives a range (which means the start and stop positions for this gene on the chloroplast genome
   1. Write down the start and stop positions (60590-62017) – note that this is longer than the bar code region, and longer than an ideal amplicon for sequencing (you want it to be 600-700 nt long)
   2. Download the sequence as FASTA, copy and save to a Word document on your desktop.
6. You want to include the full 600bp bar code region in the amplicon. For the reverse primer you are fine – the gene is longer than the bar code region. But for the forward primer you need to be back in the chloroplast sequence – capture about 100 nt upstream of the start of the gene.
7. Ir you retrieve the full chloroplast sequence, then select a beginning that is at 60490 and an end that is 600nt of bar code +100 nt for primer design = 700 nt from the start of the gene = 61290, this will give you a region to design your primers in with Primer3 Plus.
8. Find the Castenea mollissima chloroplast genome again
   1. Ncbi Genomes, Organelles tab, page 14 🡪 castenea mollissima
9. Request the subset of sequence as indicated above, download as FASTA, copy and paste to Word, save to desktop as text.
10. Copy the sequence into the Primer3Plus target box.
11. Make sure you include the 600 nt in the middle and use the 100nt at each end for primer design.

Save your best primer pair and we will compare them. See the back for another project – aimed at the summer science camp.

**Extra challenge:**

I want to amplify the entire Chloroplast genome as 15,000nt chunks, covering the entire 150,000bp in about 11-12 pieces.

1. I want the pieces to overlap by about 200nt, so the second chunk starts about 200nt before the end of the first amplicon.
2. There are a couple of inverted repeat regions (so primers may bind in 2 different places, which is not allowed) so you may have to do some fiddling around with amplicon sizes – they can be as small as 10,000nt, but no larger than 16,000nt.
3. Don’t forget that this is a circular genome, so the numbers on your last amplicon are going to be confusing, it will look like the end comes before the beginning.
4. If you get this far and provide and entire set I will order them and we will try them out this summer.