

1. Multiple sequence alignment.

- A. Get the three CR elements from. Go to <http://www.plantbio.uga.edu/~kelly/courseInfo/3210/GENE3210.html> and copy/save 'get sequence of CRs' to the hard drive.
- B. Go to <http://www2.ebi.ac.uk/clustalw/>
- C. Paste your three sequences in the box.
- D. Click "Run."
- E. You will get this type of display

CLUSTAL W (1.81) multiple sequence alignment

```
rice_CR          AGTGGTAAGGCGAAGGTAACAAAACGGTGCACATTAATTTTGCAATTGGAAATTACCAT 3830
maize_CR         AGTGGTAAGGTC AAGGTAACCAAGCTGGTACGAATTAATTTTGCTATTGGTTCATATCGT 3611
barley_CR        AGTGGCAAGGTTAAGGTAACACGTACTGTTTCGTGTGCATTTTAGCATTGCTACATATTCT 3815

rice_CR          GATGTTGTTGAATGTGATGTTGTGCCCATGCAAGCATGTAATATTCTGCTAGGTAGACCA 3890
maize_CR         GATGTTGTTGACTGTGATGTTGTGCCTATGGATGCTTGTAAATATTCTGCTAGGTAGACCA 3671
barley_CR        GATTTTGTGATTGTGATGTGGTACCCATGCAAGCATGCTCCGTTTTACTTGGTAGACCA 3875

rice_CR          TGGCAATTTGATAGGGATTCTATGCATCATGGTAGGTCCAACCAGTACTCTTTTCTGTAC 3950
maize_CR         TGGCAATTTGATT CAGATTGTATGCATCATGGTAGATCAAATCAATATTCTCTCATAACAC 3731
barley_CR        TGGCAATTTGATAAAAATTCTGTACACCATGGTAGAACAAATCAGTATACTCTTGTTCAT 3935
```

2. Primer design. At this point you want to scan the sequence for two good primers. You want to choose a region with no more than two degenerate positions. We want primers that are 21bp long and have between 45-55% GC content. The amplicon should be between 500 and 1000 bp. You can do this by eye fairly easily.