**CRISPR Guide RNA Tutorial**

The gene products of the nuclear-encoded copies of the gene *rbcS* interact with the chloroplast-encoded copies of the gene *rbcL* to form a heterodimer that functions as a subunit in the Rubisco complex. Hybrid polyploid coffee was originally formed by hybridization between *Coffea eugenioidies* (maternal progenitor) and *Coffea canephora*, but since the hybridization event, the *C. canephora* copy of the *rbcS* gene has been replaced by the *C. eugenioides* copy,such that both copies of *rbcS* now found in coffee resemble the maternally derived gene, and the paternal copy is absent. We have therefore hypothesized that the paternally derived copy of *rbcS* was incompatible with the maternally derived *rbcL* gene encoded in coffee’s chloroplast, resulting in reduced rates of carbon fixation in complexes with the paternal copy of *rbcS* than in complexes with the maternal copy of *rbcS*. To test this, we want to use CRISPR to edit the *rbcS* gene copies to once again resemble the paternally derived copy and test whether the resulting transgenic coffee plants exhibit reduced rates of carbon fixation. To do so, we need to design CRISPR guide RNAs for the following mRNA sequence (the site we want to edit is marked in RED from TTG to AAG):

GGTGAACCACATAATCCAATGGCGGACGATGGTCTAAGATCAGGATGATGGACTTTTGTCCGTTAGATATAGGAGCCATGGAAGAGCAAGTAGTTGCATTATATATAGAAAGGGTTCTGTAGAGCAAAGGCCATATGATTGATTCCCTTGCTATTATATCAGAAGAAAAAGGAAGGGAACGAGCTAGCGAGAATGGCATCCTCAATGATCTCCTCGGCAGCTGTTGCCACCACCACCAGGGCCAGCCCTGCTCAAGCTAGCATGGTTGCACCCTTCACCGGCCTCAAAGCTGCATCTTCTTTCCCCATTTCCAAGAAGTCCGTCGACATTACTTCCCTTGCCACCAACGGTGGAAGGGTCCAATGCATGCAGGTACCATTACCAACCACAAAATACTAGCACTCTCTCTCTCTATATATACATATACATATATATATATATATATATATATATATATATTCAACTCAAGTTTAATTTGAACACACATACATTTAATTTTAGGTGTGGCCACCAACTGGAAAGTTGAAGAACGAGACTTTTTCATATCTTCCAGATCTTACCGACGAGCAATTGCTCAAGGAAATTGATTACCTTATCCGCAATGGATGGATTCCTTGCTTGGAATTCGAGTTGGAGGTAAAAAAAAAAAAATTTGTTACACAGATAAGATGTTTGCATGTACTAACATAGAATTATTTTTCAGTGGCGGAAAGATTTATACAAACAAATAAAAGAAAGTATAGAGACAGGCATTTAATATTTATACTGAAGCTAATACGTTCGTTTGGTTAATGTTAATAGCAGTAGAGTAGAGTAGAGTAGATAGATTAATATGCTGATGCGGGGTTTGTGATTTGGTGGGTTGAACGTGTAGAAAGGACATGTGTACCGTGAATACCACAGGTCACCGGGATACTATGACGGACGCTACTGGACCATGTGGAAGCTGCCTATGTTCGGCTGCACGGACGCAACTCAGGTGCTGAAGGAGGTTCGGGAATGCCTGAAGGAATACCCAAATTGCTGGGTCAGGATCATCGGATTCGACAACGTCCGCCAGGTGCAGTGTATCAGTTTCATTGCCGCCAAGCCAAAGGGTTTTTAAGCCCCTTCTTCACAAATTCGGCCCCGGCCCCGTCCTCTTCCCCTCAAATTTGAGGCTACGTTTCTTGGCAGTTGACAGCTAGTTGTCAATAAAATTGAGAACTGGGGCTGTACTTTCAGGTGTTTTTCTTTTTTATTTGCCTTTCCCGTGGTGGGTCTGGTTTTGCTTCTATTCTTCTCCTTTCTTTTTTTTCCGCTTTGACATTCGGTTTCGGTGTATGTTTCCGGATTTCCAAAGATATGTACGAGACTTTAATCAT

**Procedure:**

1. Open a browser and proceed to <http://chopchop.cbu.uib.no/>
2. For the target, type in: GSCOCT00006888001
3. For the box “IN” type in coffea, and select the Coffea canephora v1.0 genome
4. Select CRISPR/Cas9, and knock-in for the remaining options
5. Press submit. Wait…keep waiting….
6. Once the results pop up, look for guide RNAs that are within 30 nucleotides of the GTT sequence above \*\*\***NOTE that the sequence above is mRNA, not DNA, so it lacks introns\*\*\***
7. Place your guide RNA here: