Localized Codon-Optimization (LCO) algorithm

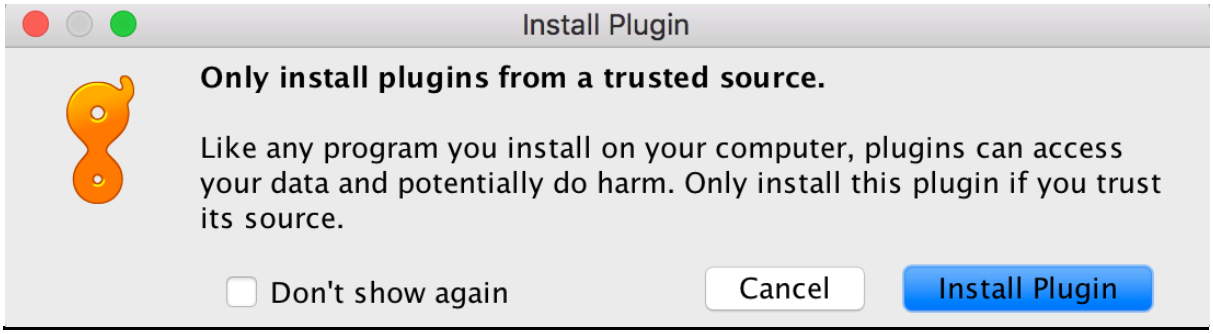
Background:

For Background information refer to Materials and Methods section of the manuscript:

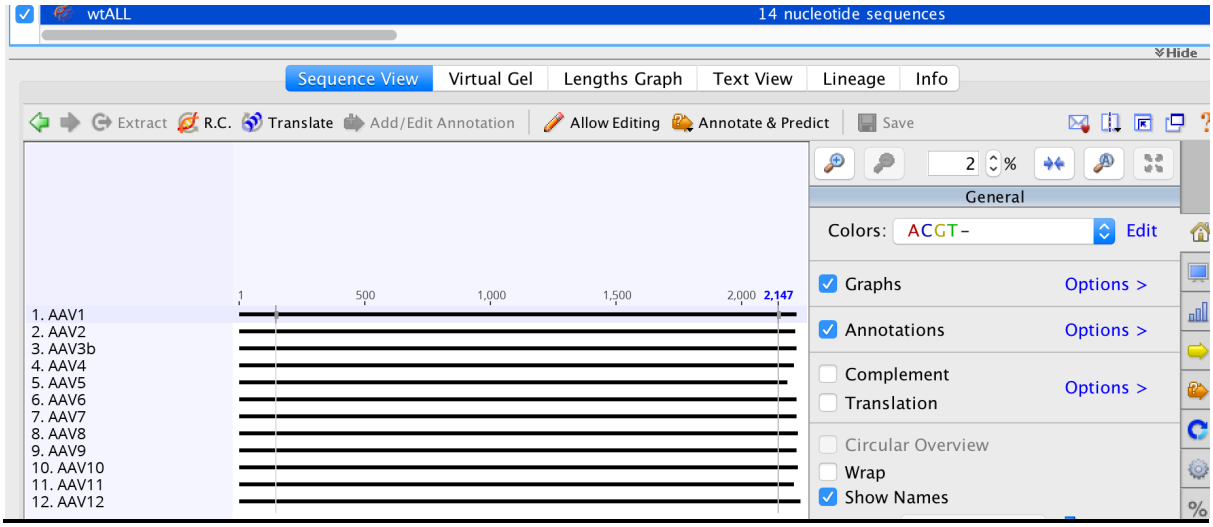
Cabanes-Creus M et al, Codon-Optimization of Wild-Type Adeno-Associated Virus Capsid Sequences Enhances DNA Family Shuffling while Conserving Functionality. Mol Ther Methods Clin Dev. 2018 Nov 1;12:71-84.

Step by step guide:

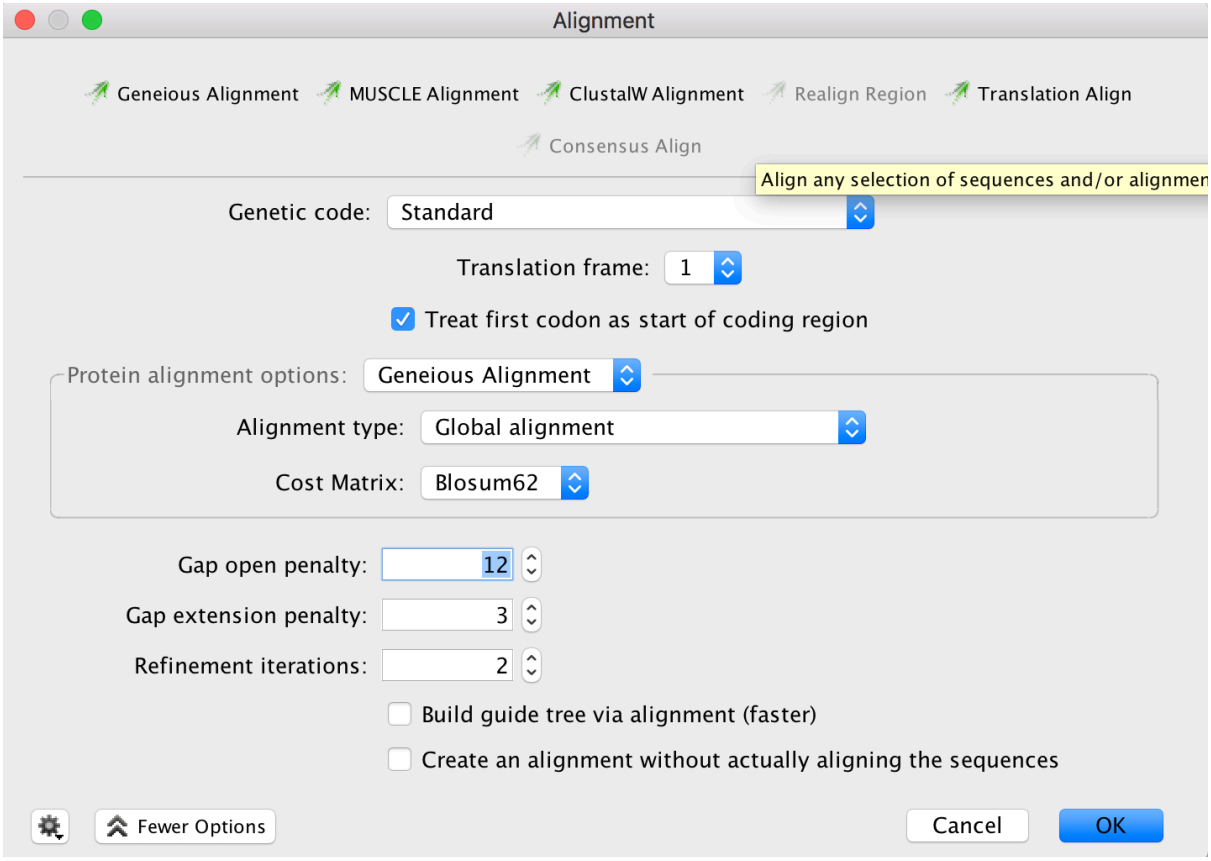
1. Download the "Localized Codon-Optimization.gplugin" from https://github.com/CMRI-TVG/AAVcodons
2. Install plugin on Geneious.



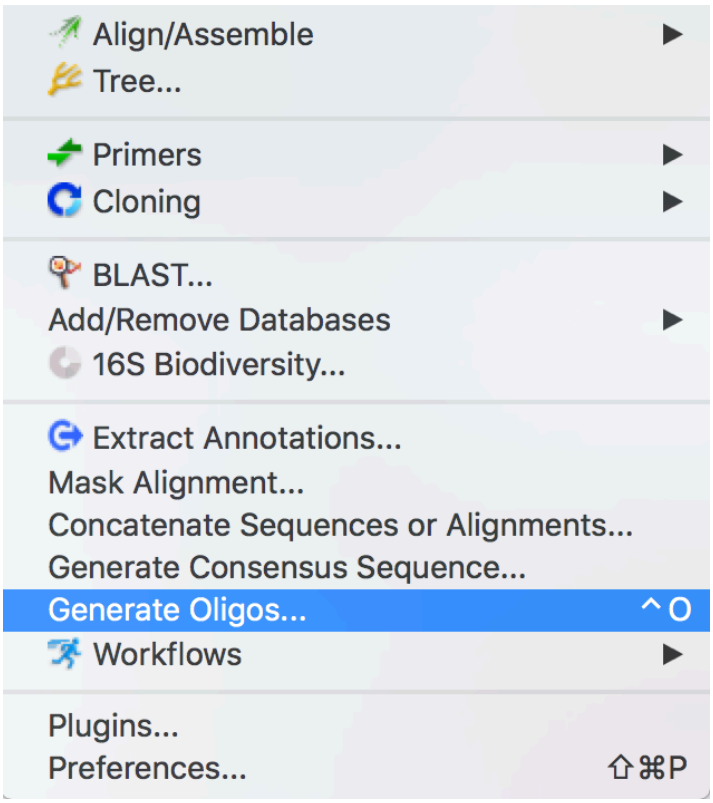
3. In order to use the plugin, first generate a Geneious FASTA file with all the parental AAV serotypes/ genes of interest that need to be local codon optimized.



4. Perform a Translation based Multiple sequence alignment by clicking on Align/Assemble in the main menu -> Multiple Align… -> Translation align. Use the following settings and click OK.

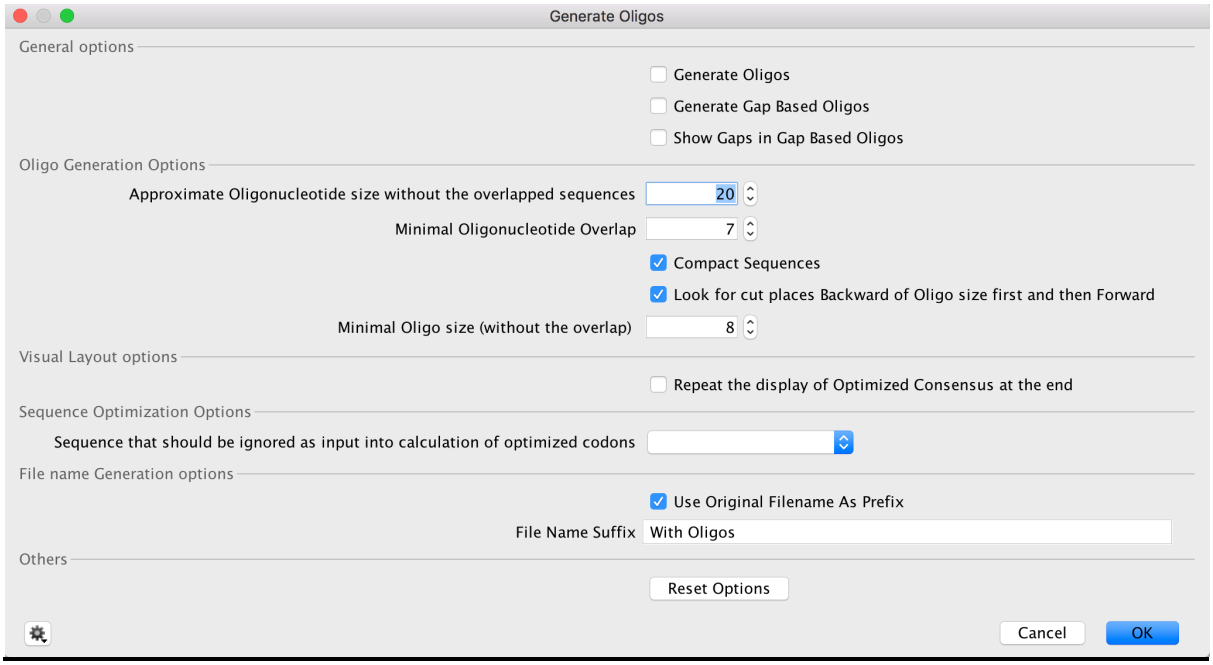


5. On the tools section on main menu, click on ‘Generate Oligos…’

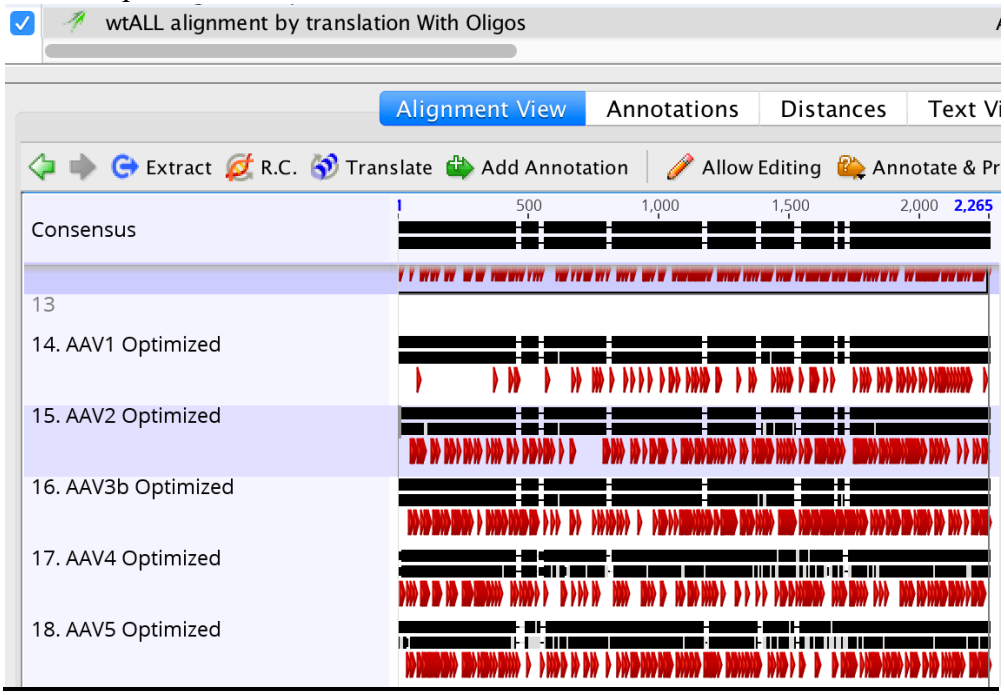


6. Mark/Unmark options for optimization:
    a. The plugin allows the generation of forward/reverse oligonucleotides that will cover all the *cap* region after optimization, for library building. Unmark 'Generate Oligos' if only local codon optimization is required.
    b. Optional: at the Sequence Optimization Option, select a variant that will be excluded into the calculation of optimized codons.

    Click OK.



7. A new file will be generated with the parental sequences followed by the Codon-Optimized variants.



8. Details on Codon Optimization can be found on the annotations (Red) displayed under the Sequence.