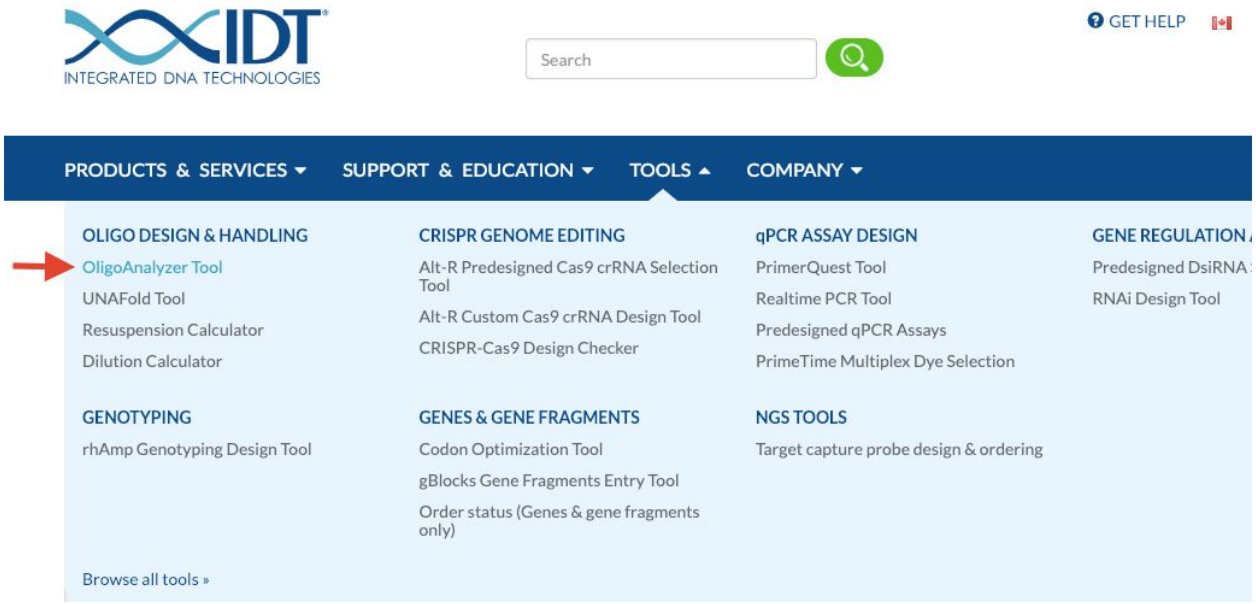
OligoAnalyzer Guide

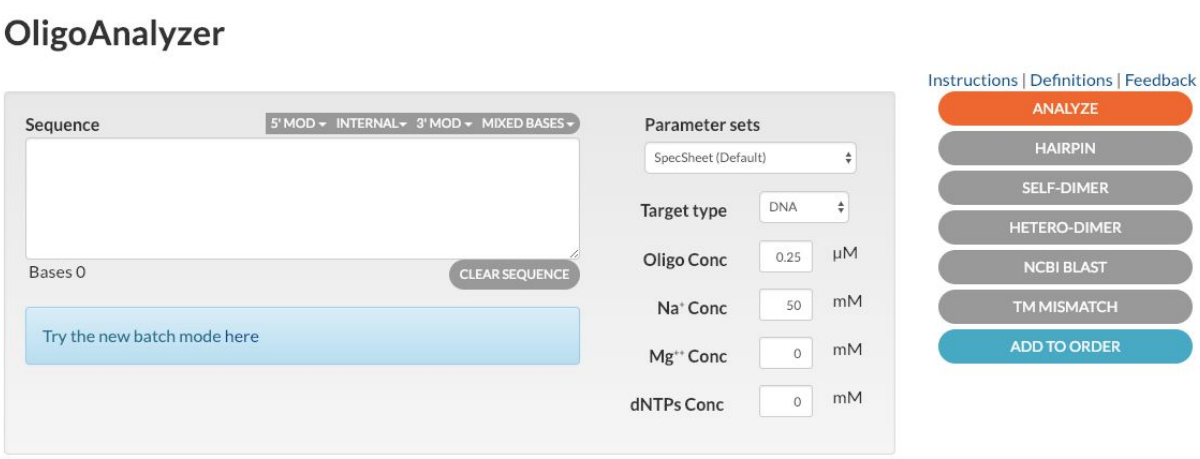
IDT's OligoAnalyzer tool is useful for analyzing single stranded oligonucleotide sequences (such as RNA or primers) and visualizing potential secondary structure or dimerization that may interfere with function. It should be used to confirm and supplement the primer analysis functions present in Benchling.

Access the OligoAnalyzer tool at
https://www.idtdna.com/calc/analyzer
Note: you will need to make a free IDT account in order to access this tool. You should use your UCalgary email address to do so.

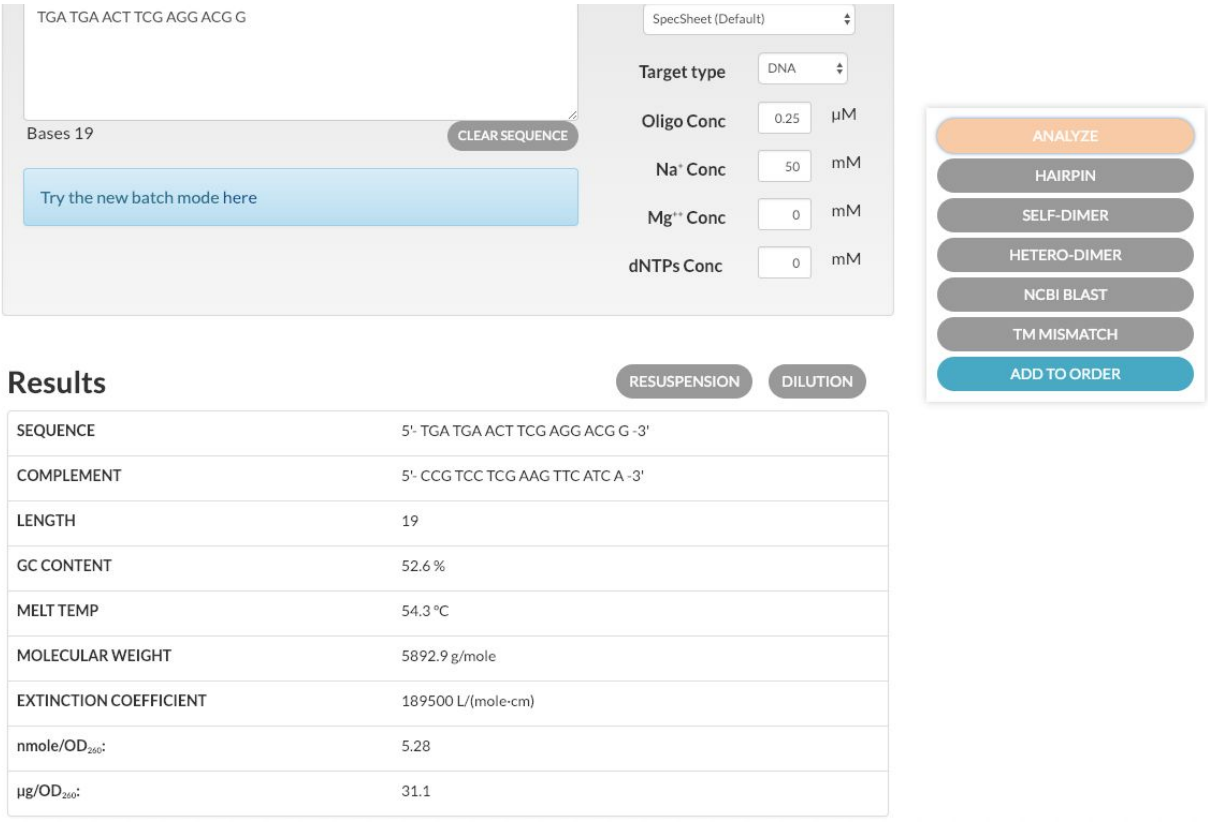
You can also access this tool from anywhere on the IDT website using the drop-down menu. Click 'Tools' and 'OligoAnalyzer Tool'.



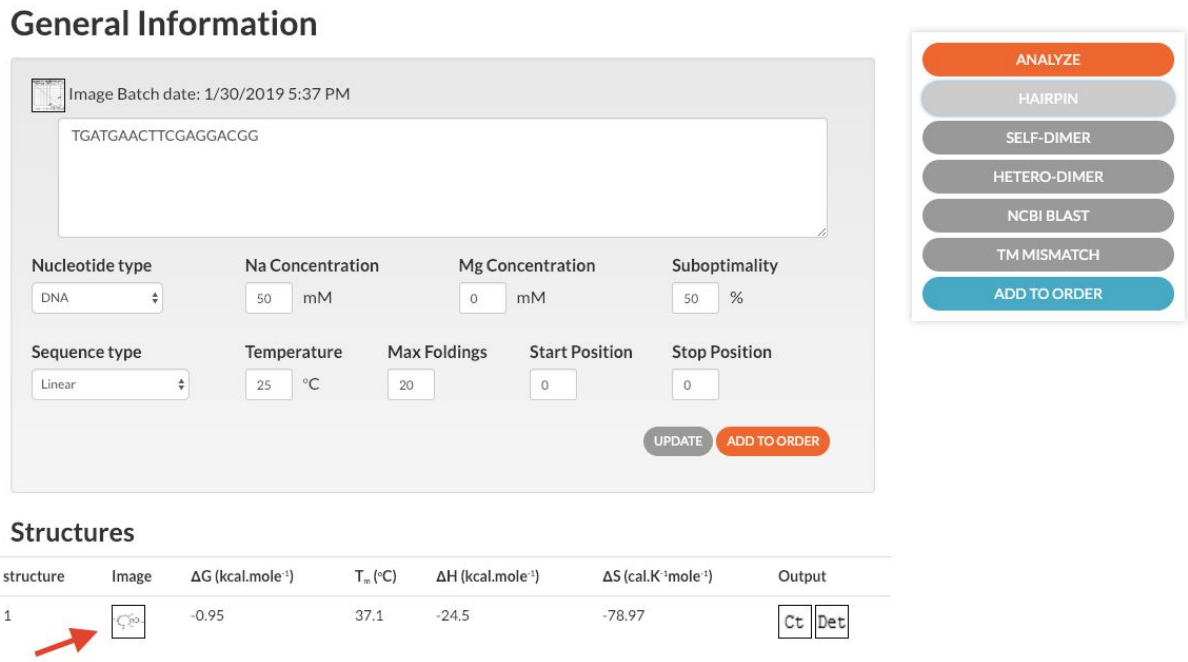
When you have made your account, clicking through the link should bring you to this interface:



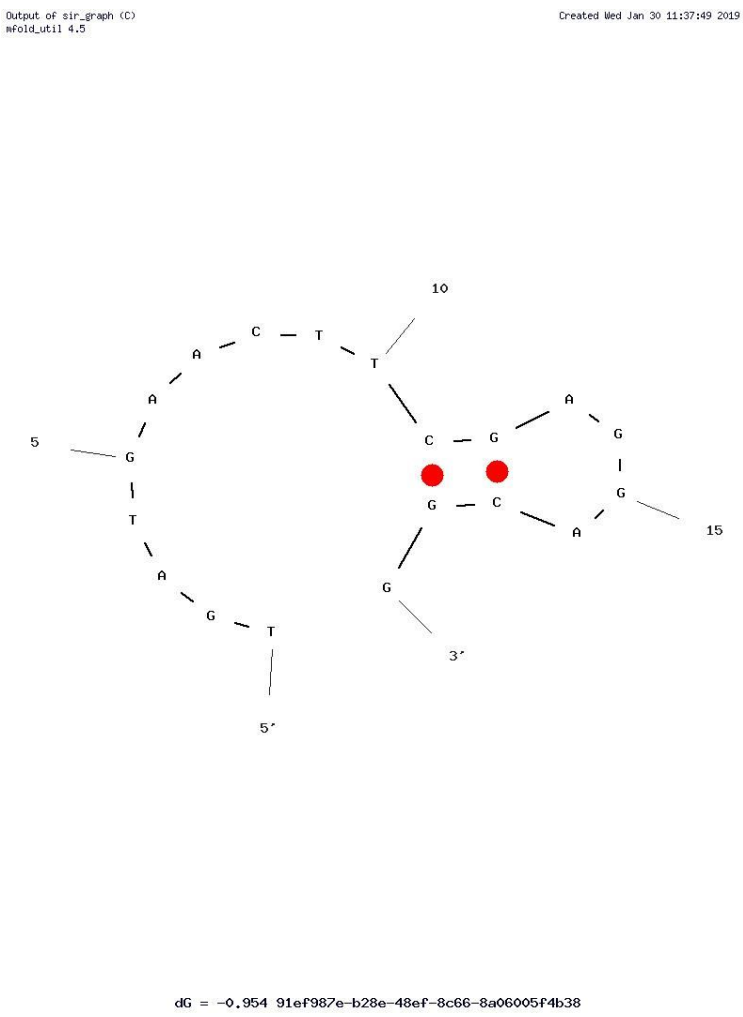
To get started, paste in your oligonucleotide sequence. For our uses, this will most likely be a primer sequence. Be sure that you are pasting the sequence in the 5'-3' direction, but you don't have to format it specially in any way (it will automatically re-format). Hit 'Analyze' on the right when complete. It will bring you to a page that looks like this:



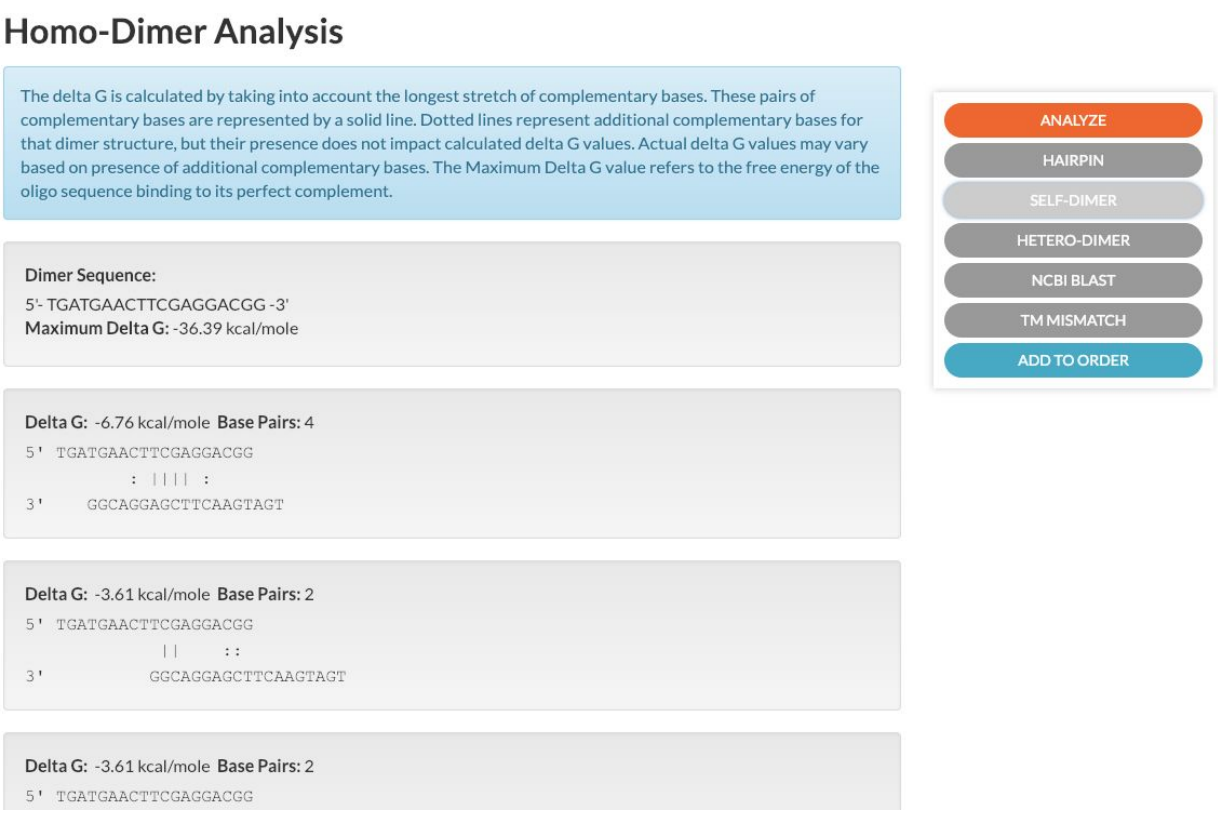
This is not overly helpful as Benchling already has most of the information here that is pertinent. Instead, you should click on the 'Hairpin' button in the right-hand menu. This feature is very good at analyzing secondary structure that may form. This is what you will see:



You can change the parameters depending on your reaction solution. Generally, we want the ΔG to be greater than -2 kcal/mol for all suggested structures. For primers, the melting temperature (T_m) is also a useful piece of information. If you click on the image (indicated by the red arrow), a representation of the secondary structure will open:

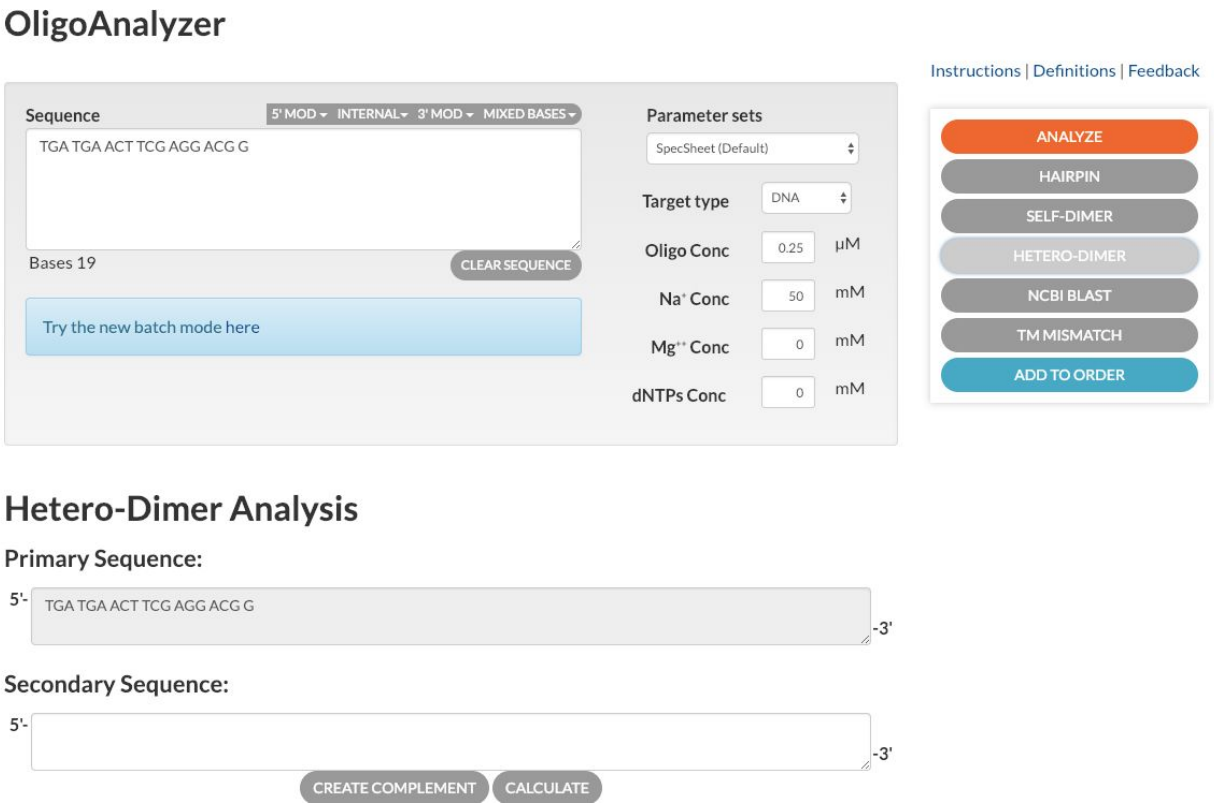


The next feature that we want to analyze is the formation of self-dimers. Click on the ‘Self-dimer’ button on the right-hand side menu. This is what you will see:

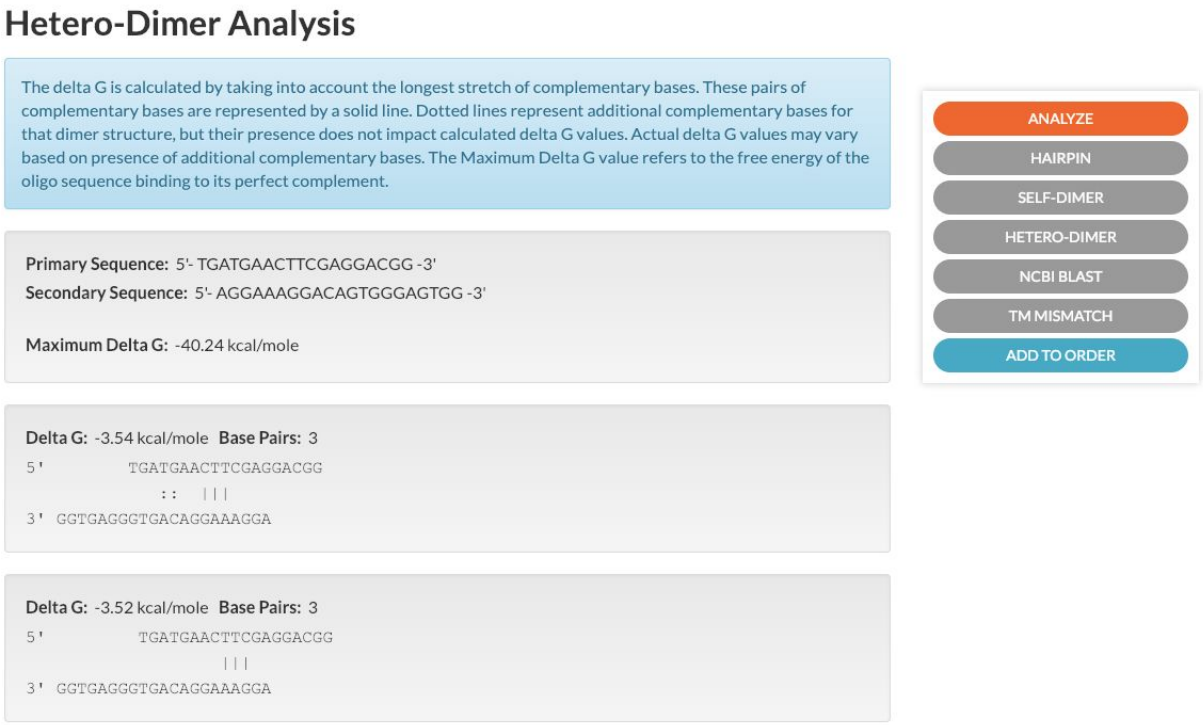


What is shown here are representations of the possible primer-dimers formed between two molecules of your primer. As a general rule, any structures with ΔG greater than -5 kcal/mol are inconsequential and can be ignored, but sometimes you may expand this range to -10 kcal/mol. Any primer dimers with ΔG less than -5 kcal/mol run the risk of forming primer-dimers that block primer function. This is especially problematic when the dimerization occurs primarily at the 3’ ends of the primer, because this is the region that must be available to bind the DNA and allow polymerization. Below is an example of a poor primer that would need to be redesigned:

A very similar process is done for heterodimer analysis (dimerization between your forward and reverse primers). Click on the 'Hetero-dimer' button. This is the screen that you will see:



Enter your second primer sequence in the ‘secondary sequence’ box. Be sure that the sequence is in the 5’-3’ direction! To analyze, hit the ‘Calculate’ button. You will see a very similar screen as the self-dimer analysis. The same general rules apply.



The other features present in this tool are not particularly useful for us. If you use the OligoAnalyzer tool in conjunction with the Benchling tools, you’ll be more likely to have successful PCR reactions!