

OligoAnalyzer Guide

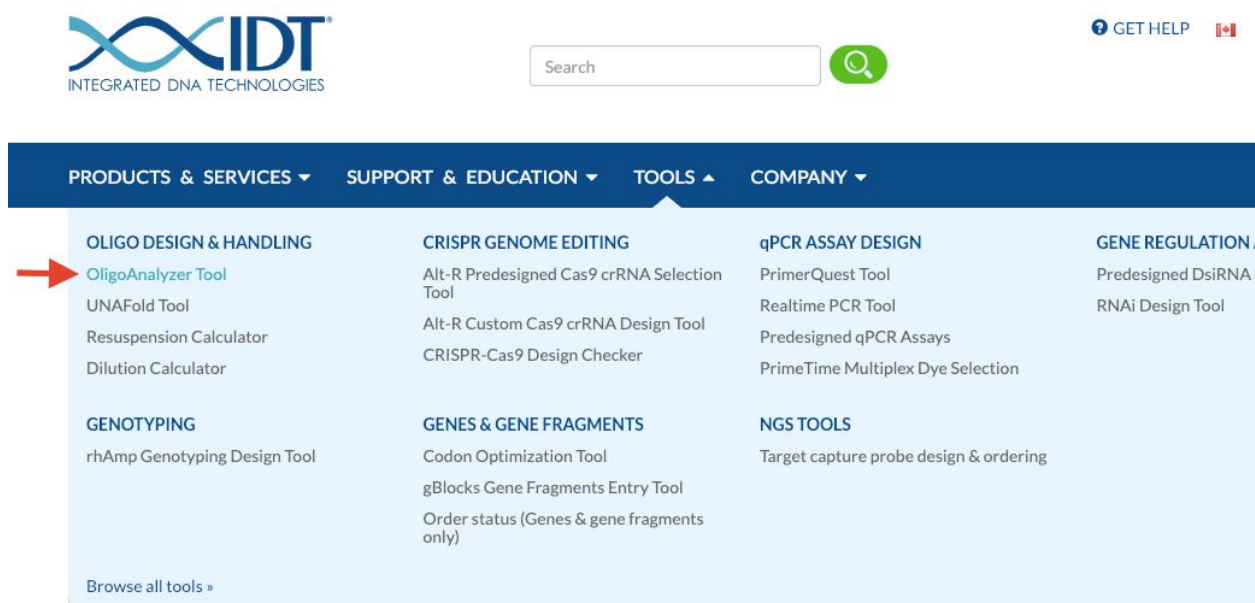
IDT's OligoAnalyzer tool is useful for analyzing single stranded oligonucleotide sequences (such as RNA or primers) and visualizing potential secondary structure or dimerization that may interfere with function. It should be used to confirm and supplement the primer analysis functions present in Benchling.

Access the OligoAnalyzer tool at

<https://www.idtdna.com/calc/analzyer>

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:

OligoAnalyzer

Sequence
5' MOD
INTERNAL
3' MOD
MIXED BASES

Bases 0

CLEAR SEQUENCE

Try the new batch mode here

Parameter sets
SpecSheet (Default)

Target type
DNA

Oligo Conc
0.25
μM

Na⁺ Conc
50
mM

Mg⁺⁺ Conc
0
mM

dNTPs Conc
0
mM

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ANALYZE

HAIRPIN

SELF-DIMER

HETERO-DIMER

NCBI BLAST

TM MISMATCH

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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:

TGA TGA ACT TCG AGG ACG G

Bases 19

CLEAR SEQUENCE

Try the new batch mode here

Parameter sets
SpecSheet (Default)

Target type
DNA

Oligo Conc
0.25
μM

Na⁺ Conc
50
mM

Mg⁺⁺ Conc
0
mM

dNTPs Conc
0
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Results
RESUSPENSION
DILUTION

SEQUENCE	5'- TGA TGA ACT TCG AGG ACG G -3'
COMPLEMENT	5'- CCG TCC TCG AAG TTC ATC A -3'
LENGTH	19
GC CONTENT	52.6 %
MELT TEMP	54.3 °C
MOLECULAR WEIGHT	5892.9 g/mole
EXTINCTION COEFFICIENT	189500 L/(mole-cm)
nmole/OD ₂₆₀	5.28
μg/OD ₂₆₀	31.1

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:

General Information

Image Batch date: 1/30/2019 5:37 PM

Sequence: TGATGAACCTTCGAGGACGG

Nucleotide type: DNA

Na Concentration: 50 mM

Mg Concentration: 0 mM

Suboptimality: 50 %

Sequence type: Linear

Temperature: 25 °C

Max Foldings: 20

Start Position: 0

Stop Position: 0

UPDATE ADD TO ORDER

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NCBI BLAST

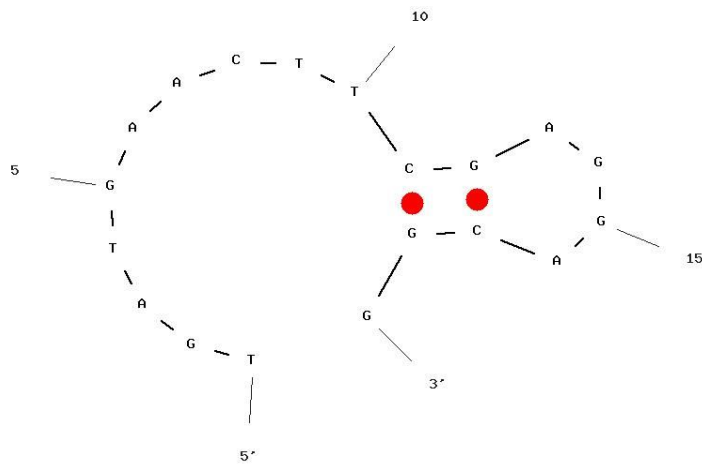
TM MISMATCH

ADD TO ORDER

Structures

structure	Image	ΔG (kcal.mole ⁻¹)	T_m (°C)	ΔH (kcal.mole ⁻¹)	ΔS (cal.K ⁻¹ mole ⁻¹)	Output
1		-0.95	37.1	-24.5	-78.97	Ct Det

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:



dG = -0.954 91ef987e-b28e-48ef-8c66-8a06005f4b38

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:

Homo-Dimer Analysis

The delta G is calculated by taking into account the longest stretch of complementary bases. These pairs of complementary bases are represented by a solid line. Dotted lines represent additional complementary bases for that dimer structure, but their presence does not impact calculated delta G values. Actual delta G values may vary based on presence of additional complementary bases. The Maximum Delta G value refers to the free energy of the oligo sequence binding to its perfect complement.

Dimer Sequence:

5'- TGATGAACTTCGAGGACGG -3'

Maximum Delta G: -36.39 kcal/mole

Delta G: -6.76 kcal/mole Base Pairs: 4

```
5' TGATGAACTTCGAGGACGG
   : ||| :
3'   GGCAGGAGCTTCAAGTAGT
```

Delta G: -3.61 kcal/mole Base Pairs: 2

```
5' TGATGAACTTCGAGGACGG
   ||  ::
3'   GGCAGGAGCTTCAAGTAGT
```

Delta G: -3.61 kcal/mole Base Pairs: 2

5' TGATGAACTTCGAGGACGG

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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:

Delta G: -17.11 kcal/mole Base Pairs: 6

```
5'  TGATGAACTTCGCGCGCAATTGGGCCC
      :  :  :  |||||  :  :  :
3'  CCCGGGTTAACGCGCGCTTCAAGTAGT
```

Delta G: -16.64 kcal/mole Base Pairs: 6

```
5'  TGATGAACTTCGCGCGCAATTGGGCCC
      :      :  |||||  :      :
3'  CCCGGGTTAACGCGCGCTTCAAGTAGT
```

Delta G: -15.42 kcal/mole Base Pairs: 6

```
5'  TGATGAACTTCGCGCGCAATTGGGCCC
                        |||||
3'                               CCCGGGTTAACGCGCGCTTCAAGTAGT
```

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:

OligoAnalyzer

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Sequence

5' MOD INTERNAL 3' MOD MIXED BASES

TGA TGA ACT TCG AGG ACG G

Bases 19

CLEAR SEQUENCE

Try the new batch mode here

Parameter sets

SpecSheet (Default)

Target type DNA

Oligo Conc 0.25 μ M

Na⁺ Conc 50 mM

Mg⁺⁺ Conc 0 mM

dNTPs Conc 0 mM

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ADD TO ORDER

Hetero-Dimer Analysis

Primary Sequence:

5'- TGA TGA ACT TCG AGG ACG G -3'

Secondary Sequence:

5'- -3'

CREATE COMPLEMENT CALCULATE

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.

Hetero-Dimer Analysis

The delta G is calculated by taking into account the longest stretch of complementary bases. These pairs of complementary bases are represented by a solid line. Dotted lines represent additional complementary bases for that dimer structure, but their presence does not impact calculated delta G values. Actual delta G values may vary based on presence of additional complementary bases. The Maximum Delta G value refers to the free energy of the oligo sequence binding to its perfect complement.

Primary Sequence: 5'-TGATGAACTTCGAGGACGG-3'
Secondary Sequence: 5'-AGGAAAGGACAGTGGGAGTGG-3'
Maximum Delta G: -40.24 kcal/mole

Delta G: -3.54 kcal/mole **Base Pairs:** 3

```
5'      TGATGAACTTCGAGGACGG
      ::  |||
3' GGTGAGGGTGACAGGAAAGGA
```

Delta G: -3.52 kcal/mole **Base Pairs:** 3

```
5'      TGATGAACTTCGAGGACGG
      |||
3' GGTGAGGGTGACAGGAAAGGA
```

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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!