

DNAMAN

Sequence Analysis Software

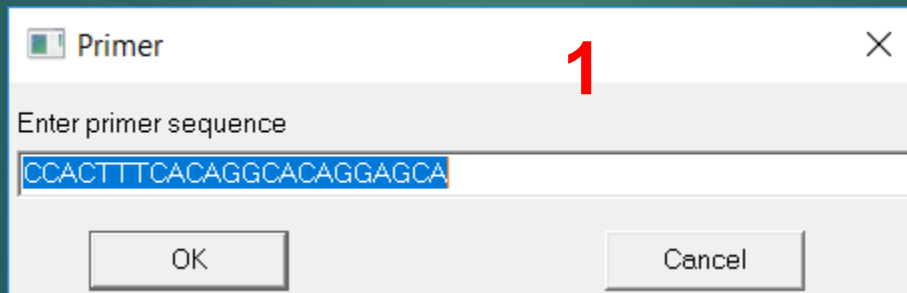
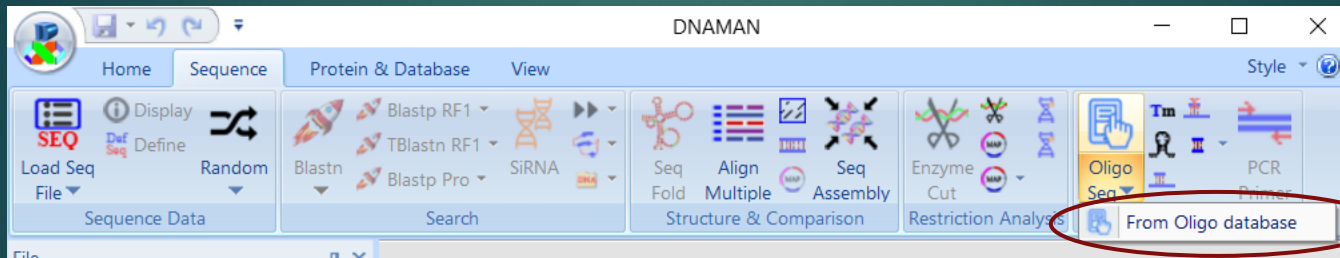
*Oligo Sequences  
Analysis and PCR  
Primers*

BIOINFORMATICS PLATFORM

# Oligo Sequences and PCR Primers

- ▶ Oligo Sequence Analysis
- ▶ Oligo Secondary Structure
- ▶ PCR Primers

# Enter Oligo Sequence

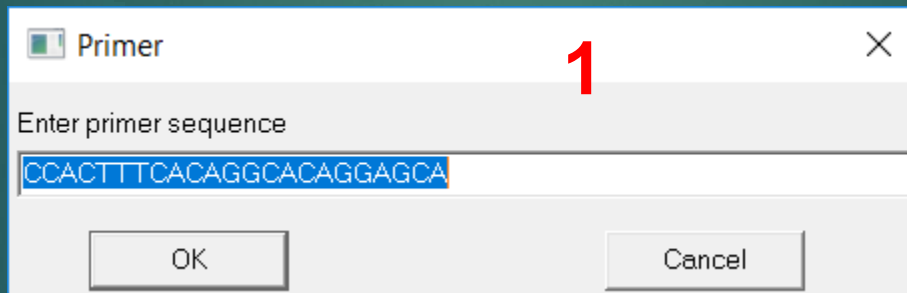
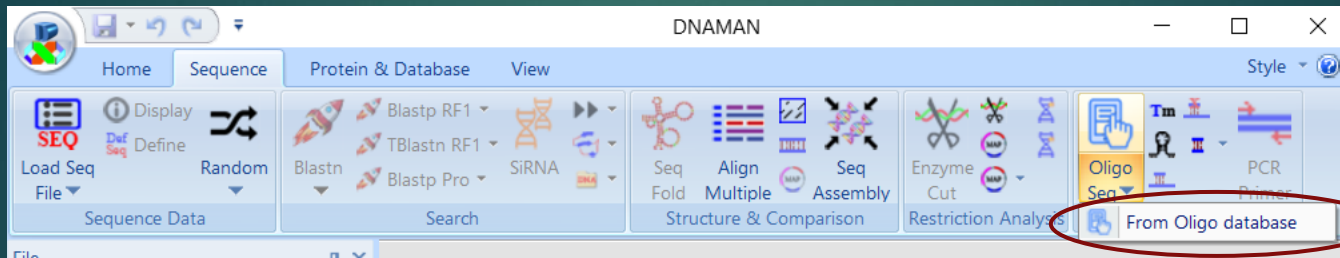


2

Oligo sequence can be entered:

1. Directly from Enter box
2. From Oligo database

# Enter Oligo Sequence

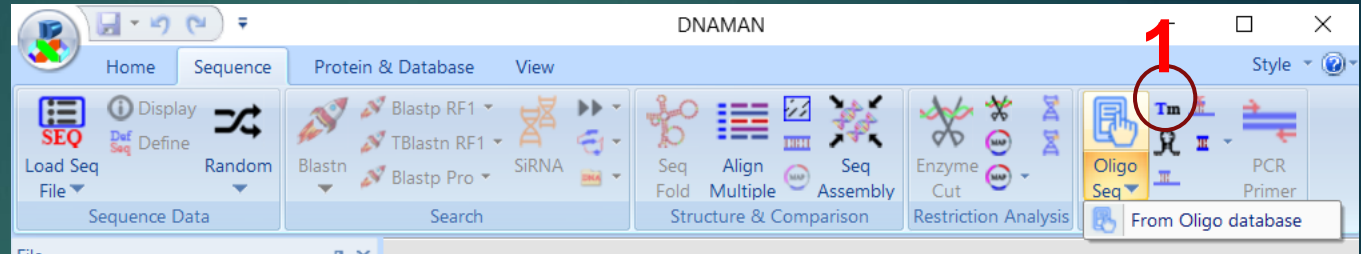


2

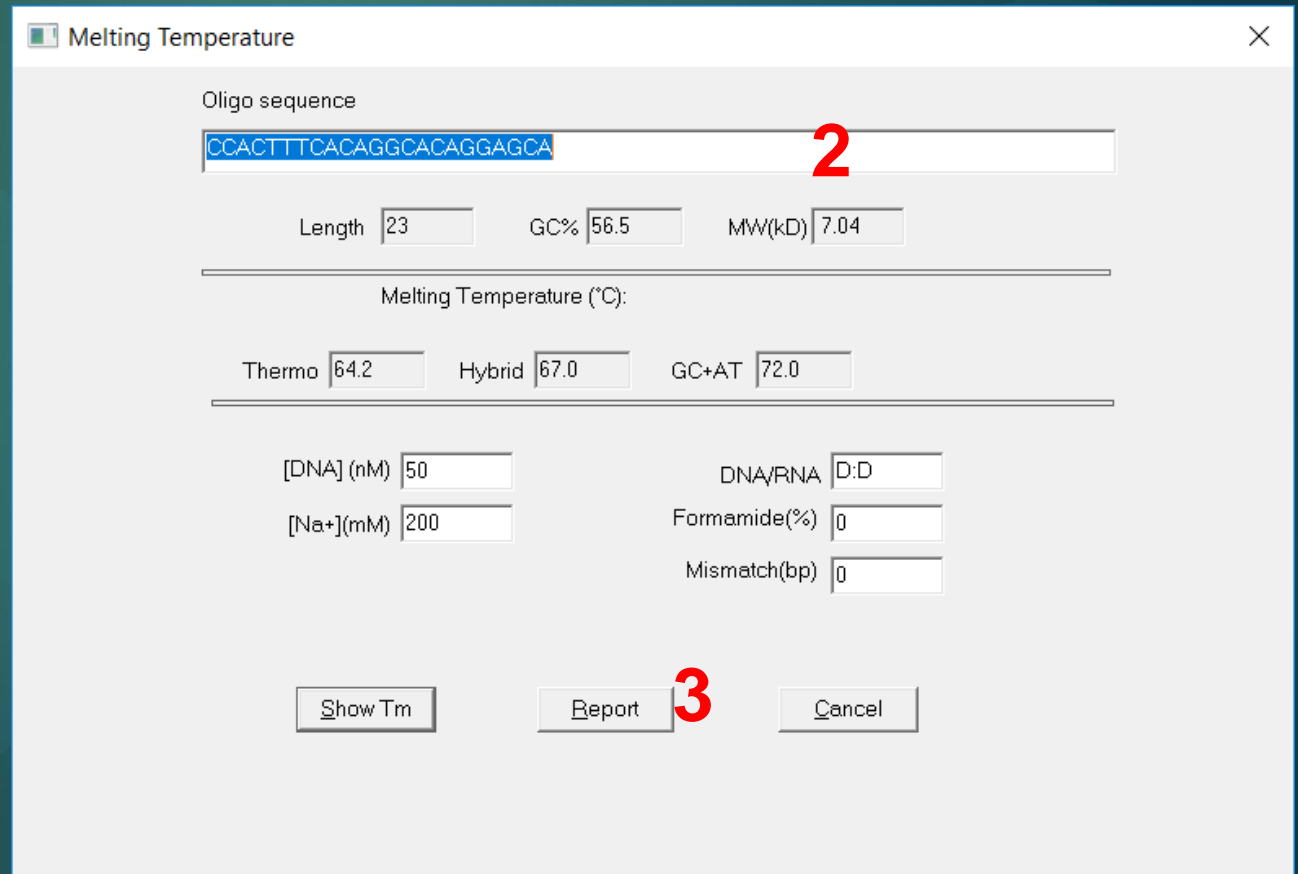
Oligo sequence can be entered:

1. Directly from Enter box
2. From Oligo database

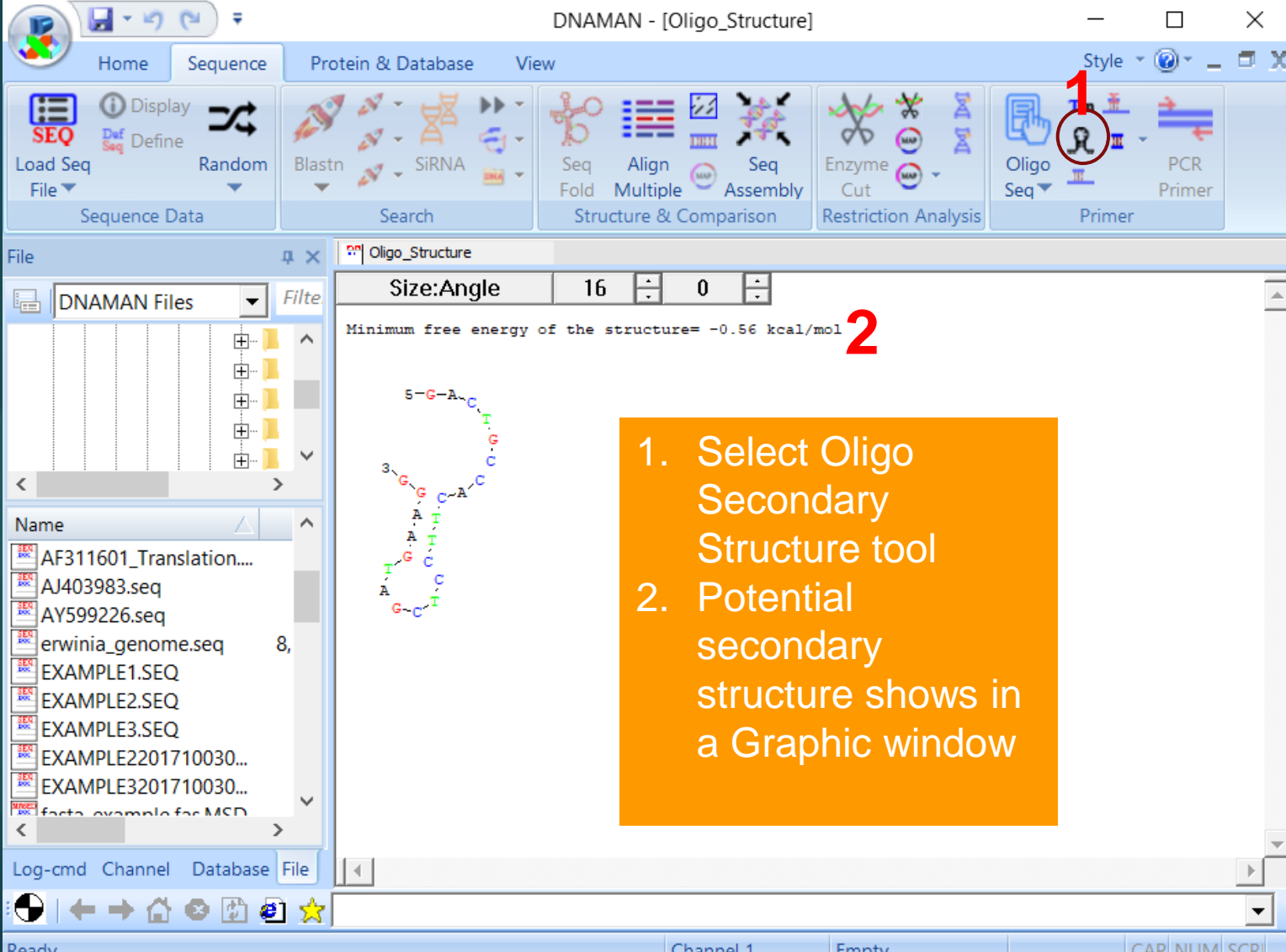
# Oligo Properties



1. Select Oligo Properties tool
2. Properties shown in a dialog box. Oligo sequence can be modified.
3. Press Report button to show properties in Text window



# Secondary Structure



The screenshot shows the DNAMAN software interface. The ribbon at the top includes tabs for Home, Sequence, Protein & Database, and View. The Sequence tab is active, and the Oligo Seq tool is highlighted with a red circle and the number 1. The main window displays the Oligo Structure tool, showing a secondary structure diagram of a sequence. The diagram is a circular structure with a minimum free energy of -0.56 kcal/mol, indicated by a red number 2. The sequence is 5'-G-A-C-T-G-C-A-C-A-T-T-T-C-C-C-G-A-3'. The status bar at the bottom shows 'Ready', 'Channel 1', 'Empty', and 'CAP NUM SCR'.

1. Select Oligo Secondary Structure tool

2. Potential secondary structure shows in a Graphic window

# PCR Primers

The screenshot shows the DNAMAN software interface with the following components:

- File List:** A list of sequence files is shown on the left. The file **EXAMPLE1.SEQ** is circled in red, with a red number '1' next to it.
- Sequence View:** The main window displays the sequence for **EXAMPLE1.SEQ** (886 bp). It includes sequence statistics (Composition: 256 A, 159 C, 195 G, 276 T, 0 OTH; Percentage: 28.9% A, 17.9% C, 22.0% G, 31.1% OTH), COLOURS (sequence = 1, features = 0), FEATURES (intron at 31..161, cds at 163..601), and ORIGIN (nucleotide sequence from position 1 to 661).
- PCR Primers Tool:** A tooltip for the PCR Primers tool is visible, containing the text: "Search PCR primer for current sequence". A red number '2' is placed next to this tooltip.
- Toolbar:** The top toolbar contains various tools. The PCR Primers tool, represented by a blue and red double-headed arrow icon, is circled in red.

1. Double click a sequence file to open
2. Press PCR Primer tool

# PCR Primers

## Step 1: Primer Filters

Step 1 of 3: Primer filtration

**Primer locations on target sequence** **1**

Product size (bp) from  to

Sense primer from  to

Antisense primer from  to

**Primer properties** **2**

Shortest primers  G/C at 3' end

Length (base)  to

T<sub>m</sub> (°C)  to

GC (%)  to

**Reject primers** **3**

3' Dimers (bp) >  Hairpin Stem (bp) >

PloyN (base) >  3' Unique (base) <

Primer-Primer: Continuous(bp)>  All matches(%)>

**Concentrations for T<sub>m</sub> calculation** **2**

Primer (nM)

Salt (mM)

**4**

Product for hybridization

Product T<sub>m</sub>(°C) range from  to

Product GC content (%) from  to

Hybridization [Salt] (mM)

Exclude product with polyN (bp) >

1. Select primer locations on template DNA
2. Set primer properties with component concentrations
3. Set Rejection metrics to filter low quality primers
4. Set PCR product properties if the product will be used for hybridization



# PCR Primers

## Step 2: Primer Filters

Step 2 of 3: Refinement and pair selection

13 Sense primers Export List **3** 8 Antisense primers

|     |                         |        |     |                       |        |
|-----|-------------------------|--------|-----|-----------------------|--------|
| 29  | TTACTGCCAAGGACATICTGG   | 56.1°C | 497 | GCCTCTGGAATCCGTTGAAAG | 57.5°C |
| 32  | CTGCCAAGGACATICTGGAC    | 56.4°C | 526 | GCACAGGAGCATCGCATAATG | 58.3°C |
| 33  | TGCCAAGGACATICTGGACC    | 57.8°C | 533 | TTACAGGGCACAGGAGCATC  | 58.2°C |
| 94  | TICTATGTGGCAGACCTGGG    | 56.9°C | 536 | ACTTTCACAGGCACAGGAGC  | 58.4°C |
| 189 | TGATAGCAAAGCCAAAGACGAAG | 57.6°C | 540 | TTCCACTTTCACAGGCACAG  | 56.2°C |
| 193 | AGCAAAGCCAAAGACGAAGAC   | 56.7°C | 874 | CCCATTCTGCCATACACAAGC | 57.8°C |
| 196 | AAAGCCAAAGACGAAGACGAG   | 56.1°C | 878 | TTAGCCCATCTGCCATACAC  | 56.3°C |
| 198 | AGCCAAAGACGAAGACGAGAG   | 57.4°C | 880 | GCTTAGCCCATCTGCCATAC  | 57.3°C |
| 320 | CCTAAACCAGATGACGGCTG    | 56.2°C |     |                       |        |
| 321 | CTAAACCAGATGACGGCTGC    | 56.9°C |     |                       |        |
| 323 | AAACCAGATGACGGCTGCTAC   | 58.9°C |     |                       |        |
| 476 | ACTTTCACAGGATTCAGAGG    | 56.2°C |     |                       |        |
| 478 | TITCAACGGATTCAGAGGC     | 56.0°C |     |                       |        |

Reject primer pairs **1** Primer Tm difference (°C) >   
 Mispriming analysis on target sequence Cut off% >=

No restriction analysis **2**  Keep primers with restriction site  
 Keep primers without restriction site

- Sense and anti-sense primers are selected. Set parameters to select pairs.
1. Set parameters to reject pairs with different Tm or mispriming target DNA
  2. Set restriction site requirement to allow or reject primers
  3. All primers can be exported to a Text window for records

# PCR Primers

## Step 3: Primer Pairs

Step 3 of 3: Final

Product=679bp. 5'primer 20 bases GC%=50 Tm=56.1. 3'primer 21 bases GC%=52 Tm=57.8

|     |                       |        |     |     |                       |        |
|-----|-----------------------|--------|-----|-----|-----------------------|--------|
| 189 | TGATAGCAAAGCCAAGACGAG | 57.6°C | and | 874 | CCCATTCTGCCATACACAAGC | 57.8°C |
| 189 | TGATAGCAAAGCCAAGACGAG | 57.6°C | and | 878 | TTAGCCCATCTGCCATACAC  | 56.3°C |
| 193 | AGCAAAGCCAAGACGAGAC   | 56.7°C | and | 874 | CCCATTCTGCCATACACAAGC | 57.8°C |
| 193 | AGCAAAGCCAAGACGAGAC   | 56.7°C | and | 878 | TTAGCCCATCTGCCATACAC  | 56.3°C |
| 196 | AAAGCCAAGACGAGACGAG   | 56.1°C | and | 874 | CCCATTCTGCCATACACAAGC | 57.8°C |
| 196 | AAAGCCAAGACGAGACGAG   | 56.1°C | and | 878 | TTAGCCCATCTGCCATACAC  | 56.3°C |
| 198 | AGCCAAGACGAGACGAGAG   | 57.4°C | and | 874 | CCCATTCTGCCATACACAAGC | 57.8°C |
| 198 | AGCCAAGACGAGACGAGAG   | 57.4°C | and | 878 | TTAGCCCATCTGCCATACAC  | 56.3°C |
| 198 | AGCCAAGACGAGACGAGAG   | 57.4°C | and | 880 | GCTTAGCCCATCTGCCATAC  | 57.3°C |

9 pairs   Order    Position    Product size    Tm1+Tm2    delta Tm    Mispriming   Zoom In   Zoom out   Off

88   176   264   352   440   528   616   704   792   880

1. Select a pair of primers visualize the properties
2. Primer pair locations shown in the target DNA diagram
3. Product length and primer information
4. Primer quality can be sorted in Position/Product Size/Tm

# PCR Primers

## Final: Primer Pair List

The screenshot shows the DNAMAN software interface. The main window displays a primer list for a sequence named 'example1'. The primer list is as follows:

```
Primer list for sequence: example1
189 TGATAGCAAAGCCAAGACGAAG 57.6°C and 874 CCCATTCTGCCATACACAAGC 57.8°C
189 TGATAGCAAAGCCAAGACGAAG 57.6°C and 878 TTAGCCCATCTGCCATACAC 56.3°C
193 AGCAAAGCCAAGACGAAGAC 56.7°C and 874 CCCATTCTGCCATACACAAGC 57.8°C
193 AGCAAAGCCAAGACGAAGAC 56.7°C and 878 TTAGCCCATCTGCCATACAC 56.3°C
196 AAAGCCAAGACGAAGACGAG 56.1°C and 874 CCCATTCTGCCATACACAAGC 57.8°C
196 AAAGCCAAGACGAAGACGAG 56.1°C and 878 TTAGCCCATCTGCCATACAC 56.3°C
198 AGCCAAGACGAAGACGAGAG 57.4°C and 874 CCCATTCTGCCATACACAAGC 57.8°C
198 AGCCAAGACGAAGACGAGAG 57.4°C and 878 TTAGCCCATCTGCCATACAC 56.3°C
198 AGCCAAGACGAAGACGAGAG 57.4°C and 880 GCTTAGCCCATCTGCCATAC 57.3°C
```

The interface includes a menu bar with 'Home', 'Sequence', 'Protein & Database', and 'View'. The 'Sequence' menu is open, showing options like 'Load Seq File', 'Display Def Seq', 'Random', 'Blastn', 'SiRNA', 'Seq Fold', 'Align Multiple', 'Seq Assembly', 'Enzyme Cut', 'Restriction Analysis', 'Oligo Seq', and 'PCR Primer'. The 'PCR Primer' option is highlighted. The 'File' menu is also open, showing a list of files including 'DNAMAN Files', 'EXAMPLE1.SEQ', 'EXAMPLE2.SEQ', 'EXAMPLE3.SEQ', and 'facta\_example\_fac MSD'.