Tutorial

Folding RNA Molecules

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Sample to Insight

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Introduction

The purpose of this tutorial is to illustrate how to use *CLC Main Workbench* or *CLC Genomics Workbench* for predicting the secondary structure of an RNA molecule. We focus on the following:

- Download the RNA sequence.
- Predict the secondary structure.
- View and interact with the graphical displays of the secondary structure.

Data used in this tutorial

This tutorial uses AB009835, a mitochondrial tRNA molecule from *Drosophila melanogaster*. tRNA molecules have a distinctive secondary structure with four main arms: an acceptor stem and three hairpin loops that form the shape of a three-leafed clover (figure 1).

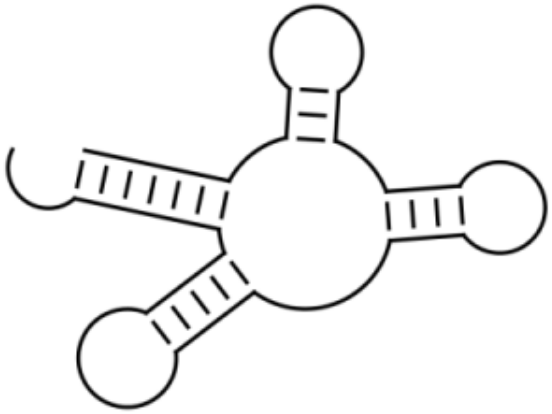

Figure 1: *The secondary structure of a tRNA molecule.*

Prerequisites

For this tutorial, you must be working with *CLC Main Workbench* or *CLC Genomics Workbench* 25.0 or higher. Note that versions higher than 25.0 may produce slightly different results than those shown here.

General tips

- Throughout this tutorial, we provide links to relevant manual pages, which we recommend exploring for additional details.
- Tools can be found in the Toolbox, but it is often easier to launch them using Quick Launch (), found in the top toolbar (shortcut Ctrl+Shift+T or ⌘ +Shift+T on Mac). Quick Launch displays the full Toolbox path, making it easy to identify the location of the tool or workflow if needed.
- The in-built manual can be accessed by clicking the **Help** button on wizards or by selecting the **Help** option under the **Help** menu.
- Within wizards, the **Reset** button can be used to change settings to their default values.
- Many data elements produced by *CLC Main Workbench* tools have multiple views, indicated as icons in the lower left corner of elements opened in the View Area. Clicking on one of the view icons while pressing the Ctrl (⌘ on Mac) key will open in split view such that both views are visible at the same time. Often, if viewing a table and a graphical representation in split view, selecting entries in the table will highlight them in the graphical representation. The order of the views can be changed using drag and drop, see Arrange views in View Area.

Download the sequence

We start by downloading the AB009835 sequence from GenBank using Search for Sequences at NCBI.

1. Start *CLC Main Workbench* or *CLC Genomics Workbench*.
2. Launch **Search for Sequences at NCBI** () using Quick Launch ().
3. Type AB009835 in the search text field and click on the **Start search** () button (figure 2).

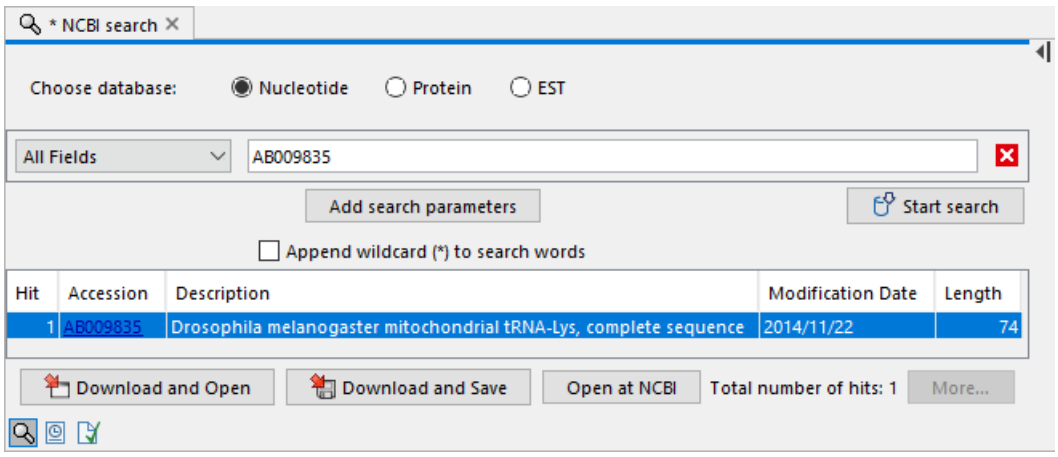

Figure 2: *Search for AB009835 at NCBI. When a hit is selected, the sequence can be downloaded locally.*

One hit is found with accession AB009835.

4. Select the hit and click on the **Download and Save** ( ) button (figure 2).

   Select a suitable location in the `Navigation Area` to save the downloaded sequence and click on **Ok**.

Once the download is completed, "AB009835" ( ) is visible in the Navigation Area.

Predict the secondary structure

We will now predict the RNA secondary structure using `Predict Secondary Structure`.

1. Launch **Predict Secondary Structure** ( ) using Quick Launch ( ).
2. In the next step, "Select nucleotide sequences", select "AB009835" as input.
3. In the next step, "Set parameters", check **Compute sample of suboptimal structures** and keep the remaining default settings (figure 3).

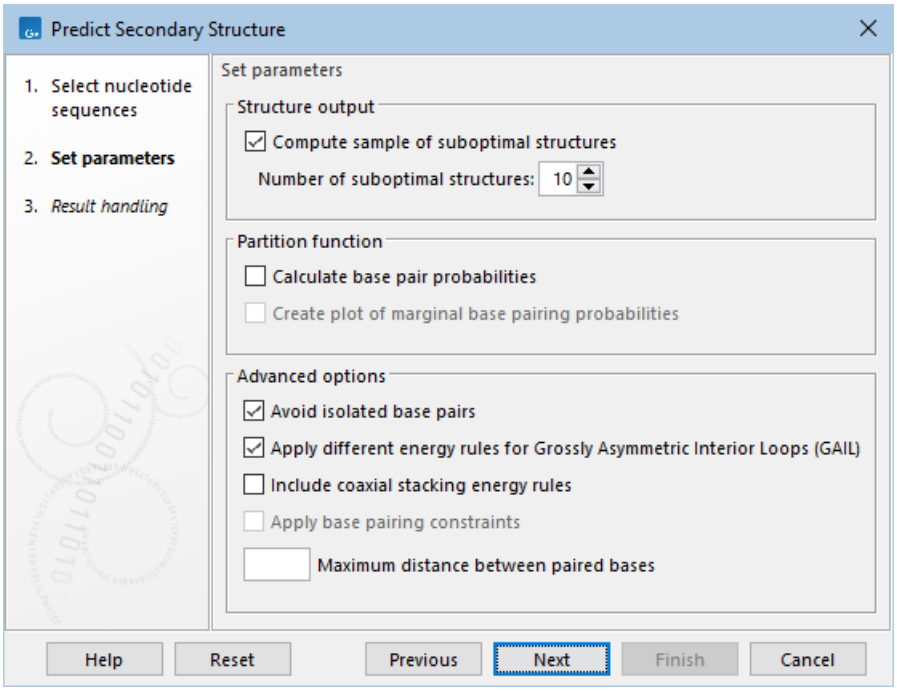

Figure 3: *Predict Secondary Structure configured to compute suboptimal structures.*

4. In the final step, keep the default settings and click on **Finish**.

Once the prediction is complete, the linear sequence view opens (figure 4). The components of the best structure are shown as annotations above the sequence, while a textual representation of the ten suboptimal structures appears below the sequence.

To view the best predicted structure in 2D, click on the Secondary Structure 2D icon ( ) at the bottom of the view (figure 5). This structure differs from the expected one shown in figure 1.

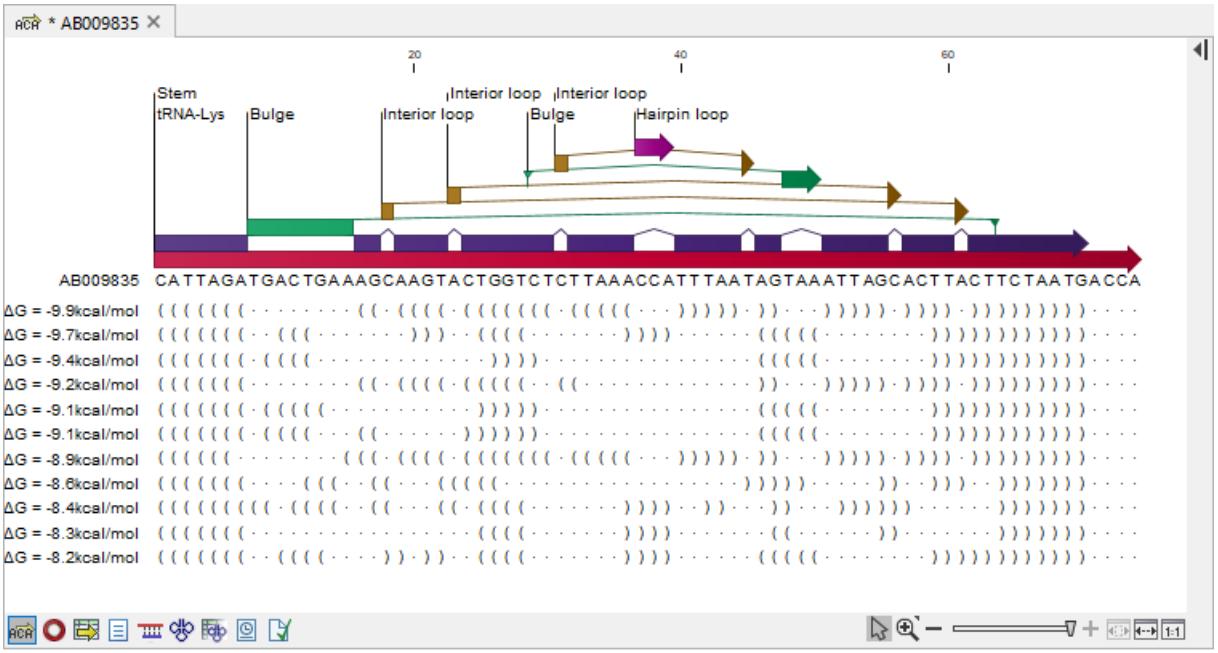

Figure 4: *Linear sequence view of the predicted secondary structures.*

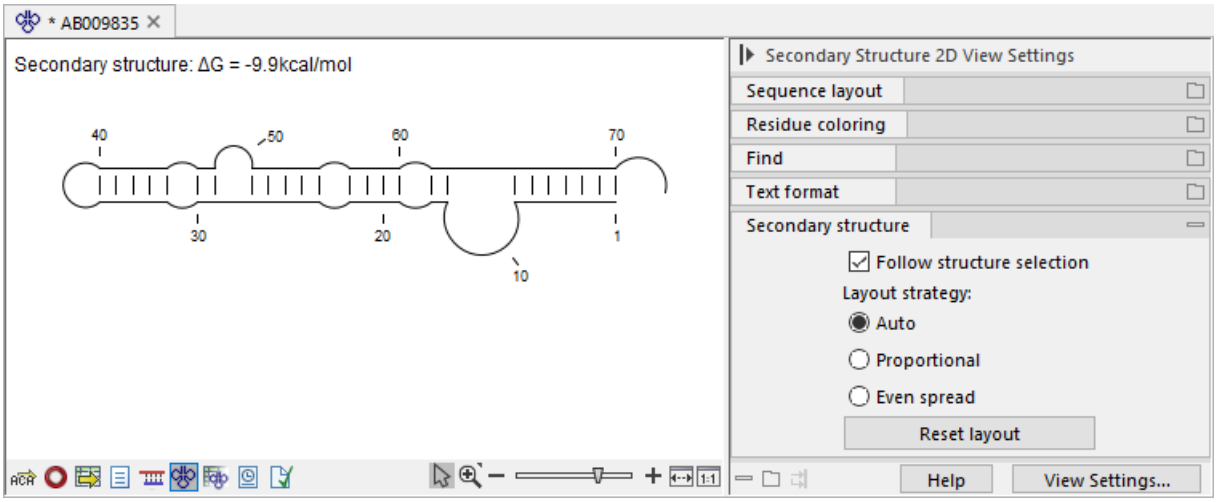

Figure 5: *2D view of the best predicted secondary structure.*

To investigate the suboptimal structures, click on the Secondary Structure Table icon ( ) at the bottom of the view while pressing Ctrl (⌘ on Mac). This opens a table with the best predicted structure and the ten suboptimal ones. Click on the second row and the 2D view at the top will show the selected structure (figure 6).

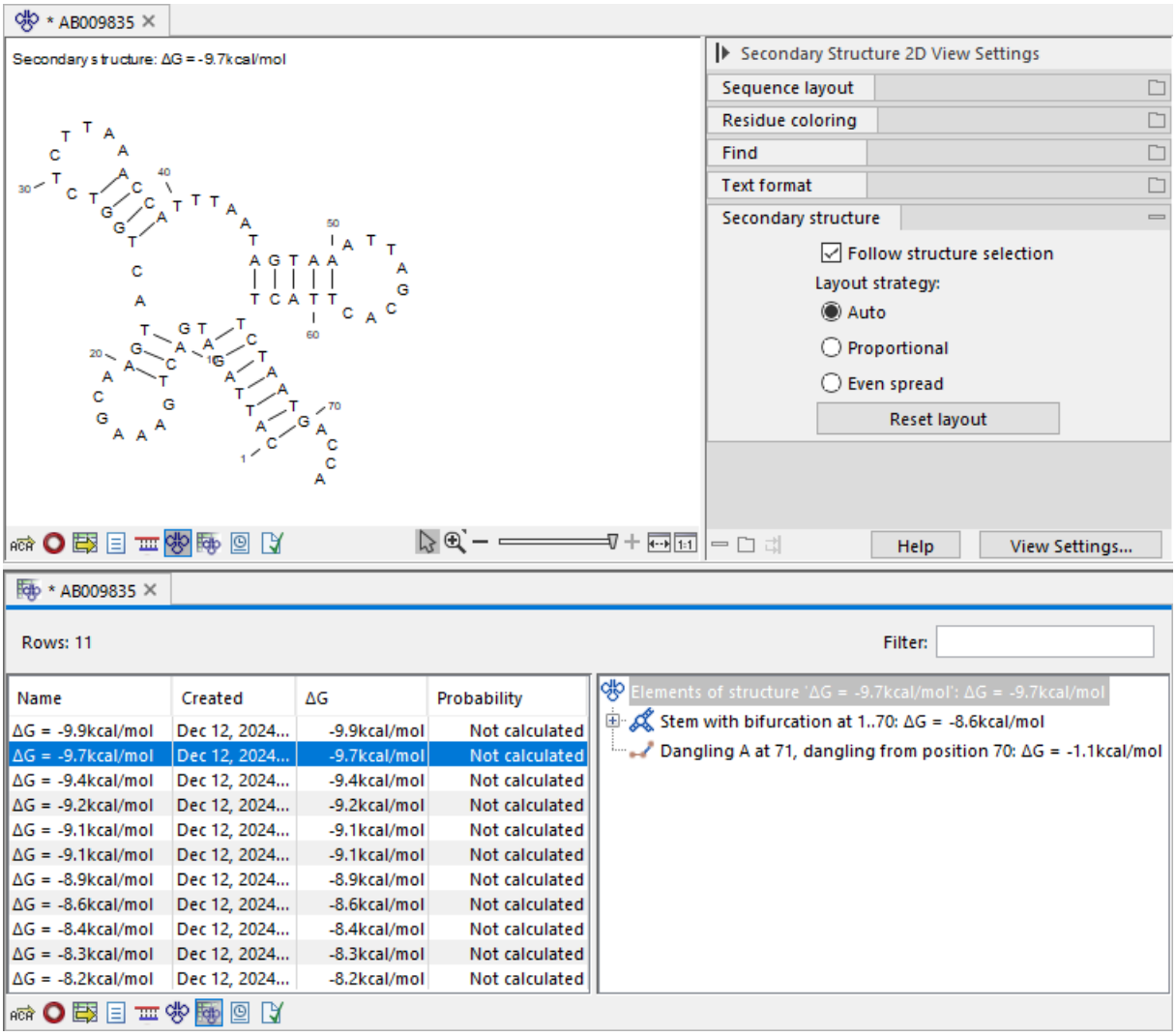

Figure 6: *A split view showing a table of all predicted structures at the bottom and the 2D representation of the structure selected from the table at the top.*

This structure resembles the expected one shown in figure 1. To improve the layout (figure 7):

1. In the "Secondary structure" palette of the `Side Panel`, check **Proportional**.
2. Right-click on the magnifier ( ) and click on the hand icon ( ) to select the Pan mode.
3. To `rotate parts of the structure`:
   (a) Place the cursor on the opening of a stem. A visual indication of an anchor point used for rotating the stem is shown.
   (b) Click-and-drag to rotate the stem.
   (c) Repeat for all four stems until the desired layout is achieved.

The 2D structure can be `printed` or `exported to a graphics format file`.

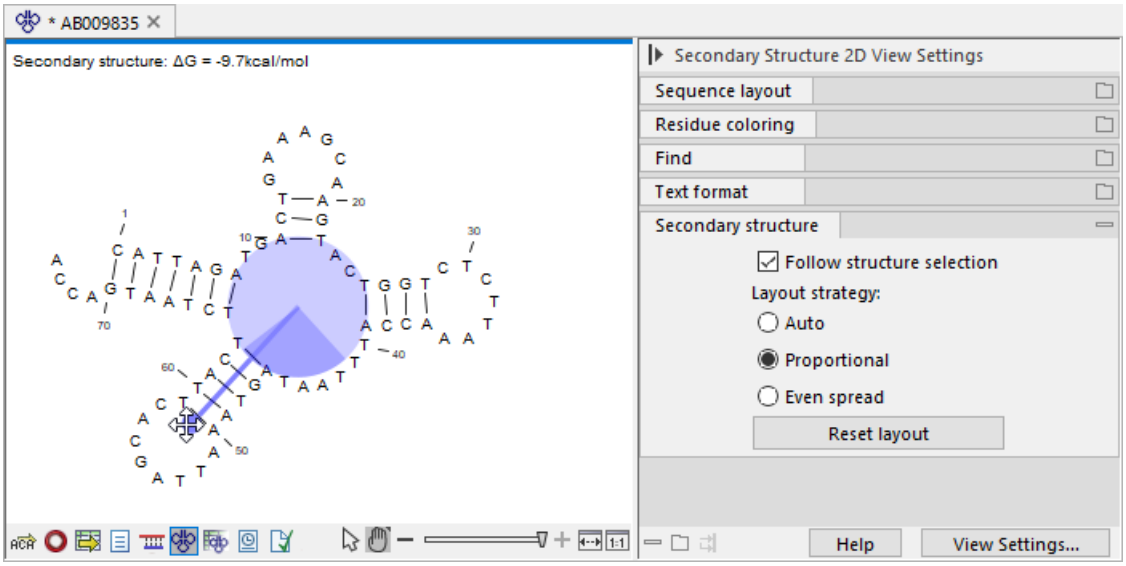

Figure 7: *The layout of the 2D secondary structure is updated by rotating stems in the Pan mode.*