

SEQUENCHER™

Tutorial for Windows and Macintosh

Assembly Strategies

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T C A G E N E
A G T C O D E S

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Assembly Strategies

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Assembly Strategies Tutorial

Sequencher offers a wide variety of assembly options. The most frequently used options, **Assemble Automatically**, **Assemble Interactively**, and **Assemble to Reference** are available as prominent buttons in the Project window. However, there are numerous other options under the **Contig** menu that may be more suitable for a given project.

In addition to the assembly options described here, investigate the tutorials for Reference Sequence and for Assemble by Name. The Reference Sequence has a number of special properties that affect the assembly process. Assemble by Name allows you to further automate the assembly process, providing greater quality control while saving you time.

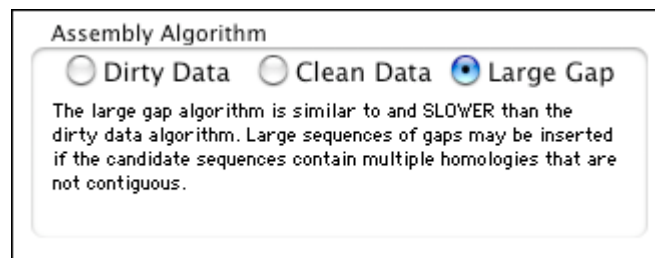
THE LARGE GAP ASSEMBLY ALGORITHM

The **Large Gap** algorithm allows you to assemble sequences that are expected to contain insertions and deletions greater than 10 bases long. Typical examples of assemblies that require the **Large Gap** algorithm include the assembly of a cDNA sequence to a genomic sequence and the assembly of related genes with alternative splicing.

- **Launch Sequencher.**
- **Go to the File menu, select Import > Sequencher Project..., and double-click on Large Gap.SPF.**

This file contains two sequences, the p53 cDNA and the p53 genomic sequences.

- **Click on the Assembly Parameters button.**
- **Click on the Large Gap radio button, then click OK.**

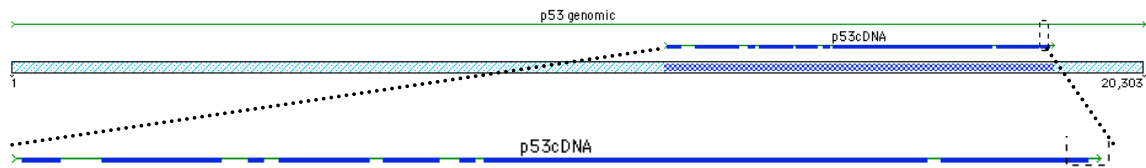


- **Select both the cDNA and genomic sequences if they are not already selected.**
- **Click on the Assemble Automatically button.**

Sequencher will assemble the cDNA to the genomic sequence in less than one second.

- **Close out of the Assembly Completed dialog.**
- **Double-click on the Contig[0001] icon to open the Contig Editor Overview.**
- **Make sure that the Display Motifs and Colors As Backgrounds menu items on the View menu are checked.**
- **To see a graphic representation of the gap alignment, from the Window menu, click on Motif Definitions....**
- **Type a ":" as a Motif and select the "Blue" Display Style option from the drop down menu.**
- **Close the Motifs window.**

Now the location of each gap will be highlighted in both the **Overview** and in the text displays of the sequences.



- Double click on the name of the P53cDNA sequence to open its Fragment Editor.

Gaps have been placed in the cDNA in order to assemble the sequence to the genomic.

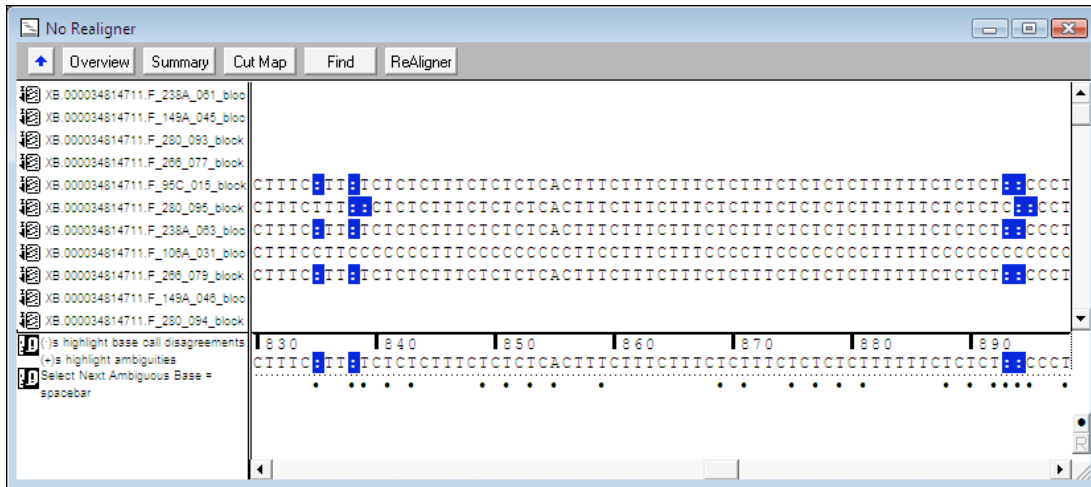
- Close the Fragment Editor by clicking on the close box.
- Click on the **Bases** button to switch from the Overview to the Bases view.
- Place your cursor on the first base in the consensus.
- Select **Select > Next Ambiguous Base**.

Sequencer will bring you to the beginning and end of each large gap in the assembly with each selection of **Next Ambiguous Base**.

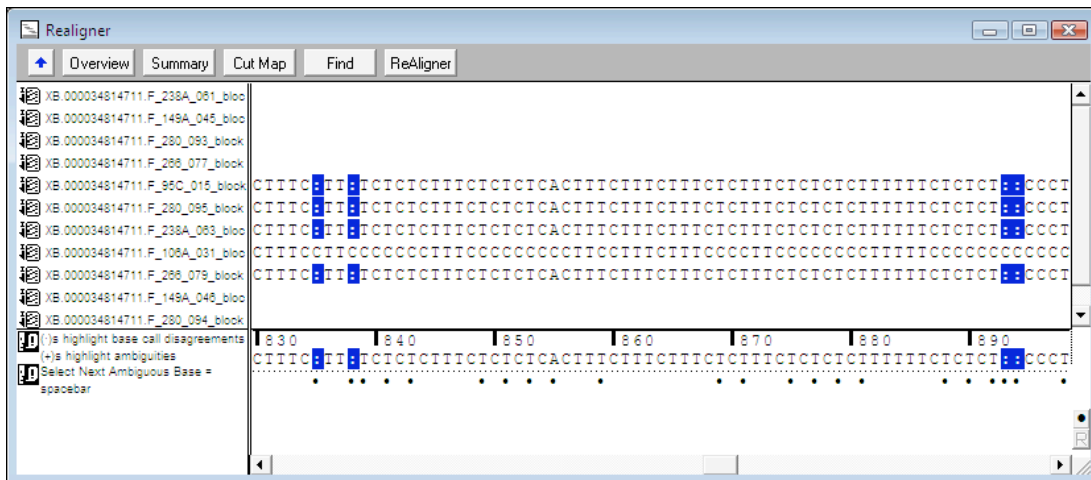
- From the **File** menu, select **Close Project** without saving.

ASSEMBLY WITH REALIGNER

Realigner is an optional step that can augment the standard assembly algorithm. The **ReAligner** function evaluates the placement of gaps within a contig and optimizes their placement. **ReAligner** facilitates editing in the consensus sequence and more clearly displays the effect of insertions and deletions.



The sequences in the assemblies above and below are the same. The top contig was assembled without **ReAligner**. The bottom contig was assembled with the **ReAligner** option. Note that the location of the inserted bases is much clearer in the assembly below than it is in the one above.



To test the **ReAligner** option, use the following steps to open the Sequencer Realigner project.

- Select **File > New Project**.
- Go to the **File** menu, select **Import > Sequencer Project...** and then double-click on **Realigner.SPF**.

The project contains the two contigs, **Realigner** and **No Realigner**.

- Select the contig **Realigner** and dissolve the assembly by choosing **Dissolve Contig** from the **Contig** menu.
- Dismiss the warning dialog by clicking on the **Dissolve Contig** button.

The contig will revert to its original 11 fragments.

- To reassemble the contig with the ReAligner option, click on the **Assembly Parameters** button.
- The check boxes to **Use ReAligner** and **Prefer 3' Gap Placement** should be on by default. If not, select them.
- Click **OK**.
- Ensure that all the fragments from the dissolved contig are still selected.
- Click on the **Assemble Automatically** button.

You will assemble all of the 11 sequences and run the **ReAligner** in less than one second.

Note: Sequencher assembles and dissolves contigs so quickly that it is easy to optimize assembly parameters and options.

- Select the **Close** button to close the **Assembly Completed** dialog.
- From the **File** menu, select **Close Project** without saving.

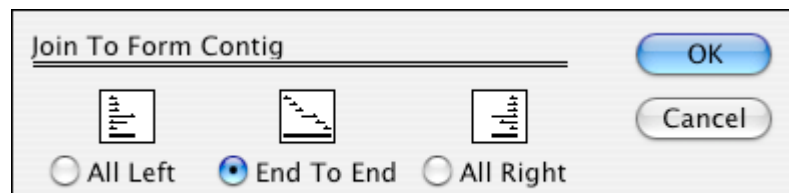
MINDLESSLY JOIN

Mindlessly Join is the kind of tool that every assembly program ought to have, but very few do. It is the kind of tool that allows you to really tinker with sequences outside of the conventional sets of algorithms.

- From the **File** menu, select **New Project**.
- Go to the **File** menu, select **Import > Sequencher Project...**, and then double-click on **Mindlessly Join.SPF**.

The Project window now contains the sequences for each of the eleven exons of p53. You can use the **Mindlessly Join** command to perform a virtual ligation and create a cDNA sequence from these exons by following the steps below.

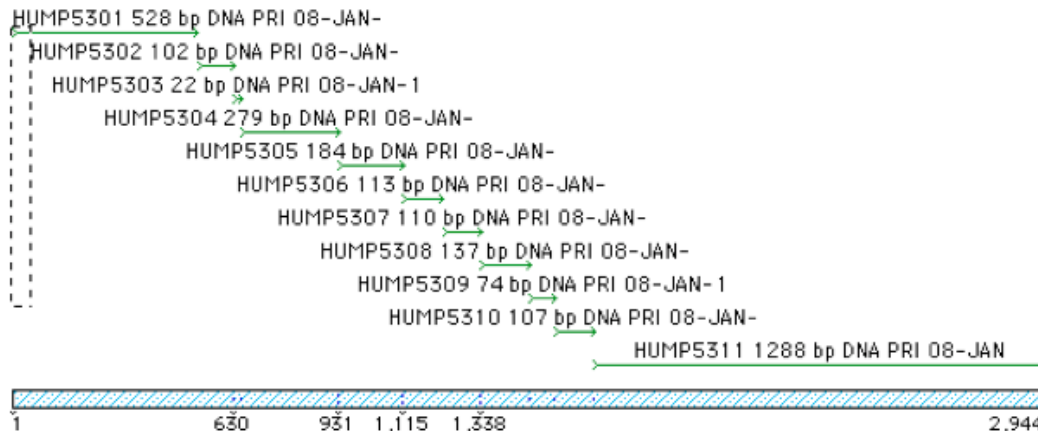
- Use your mouse to drag a selection box around the exons in the order in which you wish them to assemble.
- Select **Contig > Assemble Contigs > Mindlessly Join...**



- Select **End to End**, then click **OK**.

Sequencher will build the new contig instantly.

- Double click on the new Contig[0001] icon to open the Overview.



Note that there is no overlap between the sequences. The other options, **All Left** and **All Right**, are valuable for aligning sequences that do not assemble based on standard assembly criteria.

- From the **File** menu, select **Close Project** and don't save changes when prompted.

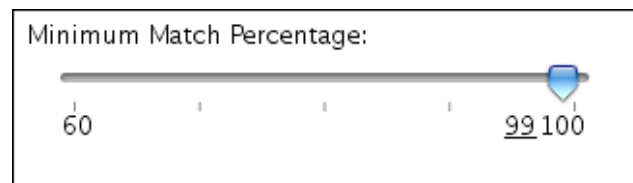
ADD SELECTED ITEMS TO OTHERS

Add Selected Items To Others is the favorite algorithm of those working in large sequencing projects. Assemblies of tens of thousands of fragments are greatly expedited by using this command in lieu of the standard, **Assemble Automatically** command. **Add Selected Items To Others** not only saves you time, but it also gives you more control over the direction of your assemblies. Uses for this function include genomic sequencing and clustering ESTs.

- From the **File** menu, select **New Project**.
- Go to the **File** menu, select **Import > Sequencer Project...**, and select **Add Selected Items.SPF**.
- Click **Open**.

The project contains 83 sequences, all related to each other, and all about 1100 bases in length. Our objective is to identify which, if any, of the sequences in the project are most closely related to the first sequence in the project—HLA:HLA00001.

- To do this, modify the **Assembly Parameters** to require a **Minimum Match Percentage** of 99%. Click on the **Assembly Parameters** button and slide the **Minimum Match Percentage** control to 99%.



- Click **OK** to dismiss the dialog.
- Select only the sequence HLA:HLA00001 icon in the Project window.
- Under the **Contig** menu, select **Assemble Contigs > Add Selected Items To Others**.

Sequencher will only assemble the selected sequence with the sequences that match the original selected sequence with a 99% identity.

This assembly option is also useful when you have a growing data set. For instance, if you generate 96 new EST sequences per day, you may wish to assemble the newly generated sequences to the previously assembled sequences. Newly imported data is automatically selected in Sequencher. By using the **Add Selected Items to Others** assembly command, you direct Sequencher to perform all of the possible comparisons with the new data without repeating the comparisons that you have already performed with the old data.

- **Close out of the Assembly Completed dialog.**
- **From the File menu, select Close Project without saving.**

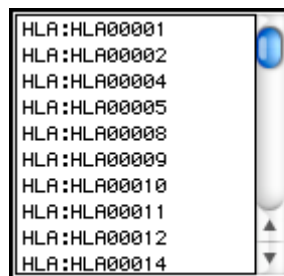
ASSEMBLE INTERACTIVELY

The **Assemble Interactively** option can be used whenever you want the ultimate control in the assembly of a batch of sequences. The **Assemble Interactively** window provides you with detailed information regarding overlap, mismatch, and gapping for any given sequence and a set of candidate sequences. The candidate assemblies are driven by your user-defined **Assembly Parameter** settings.

- **Go to the File menu and select New Project.**
- **Then, from the File menu again, select Import > Sequencher Project... and then select Assemble Interactively.SPF.**
- **Click Open.**

You will open a project that contains the same 83 sequences that we used in the Add Selected Items project.

- Click on the **Assembly Parameters** button.
- Set the following parameters:
 - Dirty Data
 - ReAligner: Off Prefer 3'Gap Placement: Off
 - Minimum Match Percentage: 90%
 - Minimum Overlap: 20 bases
 - Assemble by Name: not Enabled
- Click **OK**.
- With all of the sequences in the project still selected, click on the **Assemble Interactively** button.



- Select the first sequence in the **Candidates** list.

The **Matches** window displays a list of all of the selected sequences that would assemble to the candidate sequence given the current assembly parameters. The list also displays the %Match, the length of the overlap with the candidate sequence, the number of mismatches, the number of gaps, and the length of the new contig. The list is sorted by %Match.

Matches	%Match	Overlap	Mismatch	Gaps	New Contig Len
HLA:HLA01496	≈99	≈1,098	≈1	≈0	≈1,098
HLA:HLA00004	≈99	≈1,099	≈0	≈1	≈1,099
HLA:HLA00110	≈99	≈1,098	≈4	≈0	≈1,098
HLA:HLA00002	≈99	≈1,098	≈5	≈0	≈1,098
HLA:HLA00043	≈98	≈1,098	≈12	≈0	≈1,098
HLA:HLA01297	≈98	≈1,098	≈13	≈0	≈1,098
HLA:HLA00047	≈98	≈1,098	≈14	≈0	≈1,098
HLA:HLA00046	≈98	≈1,098	≈14	≈0	≈1,098
HLA:HLA00040	≈98	≈1,098	≈18	≈0	≈1,098
HLA:HLA00037	≈98	≈1,098	≈19	≈0	≈1,098

- Select the sequence with only one mismatch, **HLA:HLA01496** in the **Matches** list.

Sequencher automatically displays the alignment of the selected sequence with the candidate sequence, and provides the following description of the putative alignment.

ATGGCCGTCATGGCGCCCCGAAACCCTCCTCCTGCTACTCTCGGGGGCCCTGGCCCTGACCCAGACCTGGGCGGGCTCC
ATGGCCGTCATGGCGCCCCGAAACCCTCCTCCTGCTACTCTCGGGGGCCCTGGCCCTGACCCAGACCTGGGCGGGCTCC
1 10 20 30 40 50 60 70
ATGGCCGTCATGGCGCCCCGAAACCCTCCTCCTGCTACTCTCGGGGGCCCTGGCCCTGACCCAGACCTGGGCGGGCTCC

The view at the bottom of the window displays and scrolls through the new assembly and the new consensus.

- If you choose to create this assembly, click on the **Assemble** button.
- Name the new contig and click **OK**.

The new contig will appear in the Candidates window and in your Project window when you close the **Assemble Interactively** dialog.

- Close the **Assemble Interactively** window by clicking the **Done** button.
- Close the Project without saving and exit Sequencher if desired.