MiraiBio

DNASIS® MAX

Version 2.5

Contig Manager
User's Manual

For Research Use Only
Part no. C-51125-10202

Preface

Thank you for purchasing DNASIS® MAX from MiraiBio. DNASIS® MAX incorporates an excellent, user-friendly graphical user interface (GUI) and Contig Manager database for taking full control of fragments and contigs. And by installing the Contig Manager Version 2.0, users can take advantage of sequence assembly at even higher precision and higher-speed. Read this manual thoroughly to ensure correct usage.

Note:

- The copyrights for this software and user manual belong to Hitachi Software Engineering Co., Ltd.
- It is prohibited to copy or reproduce the contents of this manual in part or in whole.
- This manual is subject to change without prior notice.
- Hitachi Software Engineering Co., Ltd. is not responsible for any erroneous or incorrect descriptions in this manual.
- The use of this software and user manual is based upon agreement to the stipulations laid out in the Product License Agreement.

First Edition July 2002 (invalid)
Second Edition February 2003 (invalid)
Third Edition April 2004

© 2002, 2004 Hitachi Software Engineering Co., Ltd. All rights reserved.

DNASIS is a registered trademark of Hitachi Software Engineering Co., Ltd.

DNASpace is a registered trademark of Hitachi Software Engineering Co., Ltd.

Windows is a registered trademark of Microsoft Corporation.

All other company and product names mentioned in this manual are trademarks or registered trademarks of their owners.

Under the approval of UK Medical Research Council, TraceViewer program uses the io_lib library developed by Staden Package of the UK.

Phred/Phrap is licensed from the University of Washington. The Phred/Phrap option for DNASIS is a product of MiraiBio (USA).

Phred/Phrap Option Installation and Default Settings

Operating Platform

DNASIS MAX Phred/Phrap Option Ver2.0 operates in the following environment.

Hardware

CPU Pentium® or higher (Pentium® 4, 1.0 GHz or higher recommended)

RAM 128 MB or more (1 GB or more recommended)

Hard Disk 150 MB or more (additional capacity will be required for data)

CD-ROM Drive (Required for installation)

Video card & Display 1024 × 768 dots, 256 colors or more

Operating Systems

Windows 2000

Windows XP

*This program will not function on Windows 95/98/Me/NT.

Note

DNASIS MAX V2.5 must be installed on the computer before DNASIS MAX Contig Manager Ver2.0 can be installed and used. The key codes specific to DNASIS MAX Contig Manager Ver2.0 are also required.

Installation

This section explains how to install DNASIS MAX Phred/Phrap Option Ver2.0. The user must logon to the system with administrator rights in order to install the DNASIS MAX Phred/Phrap Option Ver2.0. DNASIS MAX needs DNASIS MAX V2.5, MSDE2000 for DNASIS and Contig Manager to be installed before you attempt to install the Phred/Phrap Option Ver2.0. Please refer to the Installation Guide that came with your DNASIS MAX software to install DNASIS MAX V2.5, MSDE2000 for DNASIS and Contig Manager.

To install Phred/Phrap Option V2.0, insert the DNASIS MAX Phred/Phrap Option Ver2.0 CD-ROM in the computer, and follow the instructions to proceed with installation.

If an installation dialog does not appear when you insert the CD-ROM, open the CD-ROM with Explorer or another file manager, and then double click the Setup.exe file (fig. 2). (Depending on the Explorer settings, the .exe extension may not be displayed.)

fig. 1 Setup program icon

Key Code Input

The DNASIS Key Code Manager shown in fig. 2 will start up once installation has completed.

fig. 2 DNASIS Key Code Manager

Select Phred/Phrap Option V2.0, click Unlock… to display the Unlock Product dialog shown in fig. 4, and then enter the key code issued by your Regional Support Center (see Appendix A: User Support Contact Information) in the Key Code field. Refer to the section on Key Code Issuance Procedures for details on acquiring the key code.

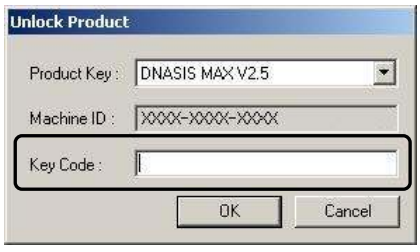


fig. 3 Unlock Product dialog

If the DNASIS Key Code Manager does not start up, select Programs > DNASIS MAX > Key Code Manager from the Start menu.

Key Code Issuance Procedures

Fill in the required details on the Key Code Issuance & User Forum Registration Form included in the package, and either fax or mail it (for email, see below) to your Regional Support Center (see Appendix A). The Key Code Issuance Notification and User Forum Registration Certificate will be sent back to you by fax and mail.

For e-mail requests, fill in the details for Key Code Issuance and send it to your Regional Support Center by e-mail. (Be sure to include the Validation Code that came with your software.)

The Machine ID of the computer on which DNASIS MAX Contig Manager Ver2.0 will be installed is also required for key code issuance. The Machine ID is displayed in the Machine ID field of the Unlock Product dialog when Unlock on the Key Code Manager is clicked. Be sure to include this machine ID number in your request.

fig. 4 Unlock Product dialog

Tutorial Data

Select Programs > DNASIS MAX > Tutorial Data from the Windows Start menu to open the folder that contains the tutorial data. The data used by the ContigManager tutorial is stored in the ContigManager folder. Refer to Chapter 1 Contig Manager Tutorial for details on how to use the tutorial data.

Start up the Contig Manager

Select Programs > DNASIS MAX > Contig Manager from the Start menu. The window shown in fig. 5 will appear.

fig. 5 Initial window

Chapter 1 Contig Manager Tutorial

This chapter explains the basic operations of the Contig Manager, which enables the sample data to be used through a series of simple operations.

1.1 Foreword

1.1.1 Contig Manager

The Contig Manager is a user interface that enables sequencing to be carried out with DNASIS MAX Contig Manager both simply and graphically.

1.1.2 Contigs and Fragments

A contig is a consensus sequence constructed by assembling sequences. A fragment is sequence segment used for constructing contigs.

1.1.3 Data Management by Project

The Contig Manager enables the entered fragment types, sequences, trimming results, assembled sequence results, quality values and other data to be managed under single analysis units known as projects.

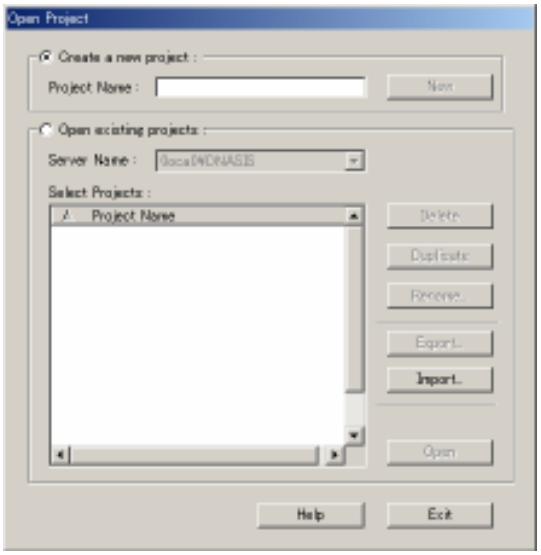
Multiple projects can be created to allow data management to be performed independently for each user or for each sequence analysis.

1.1.4 Start up the Contig Manager

Select Programs > DNASIS MAX > Contig Manager from the Start menu to start up the Contig Manager.

1.1.5 Create Projects

The dialog shown below will display when the Contig Manager starts normally.



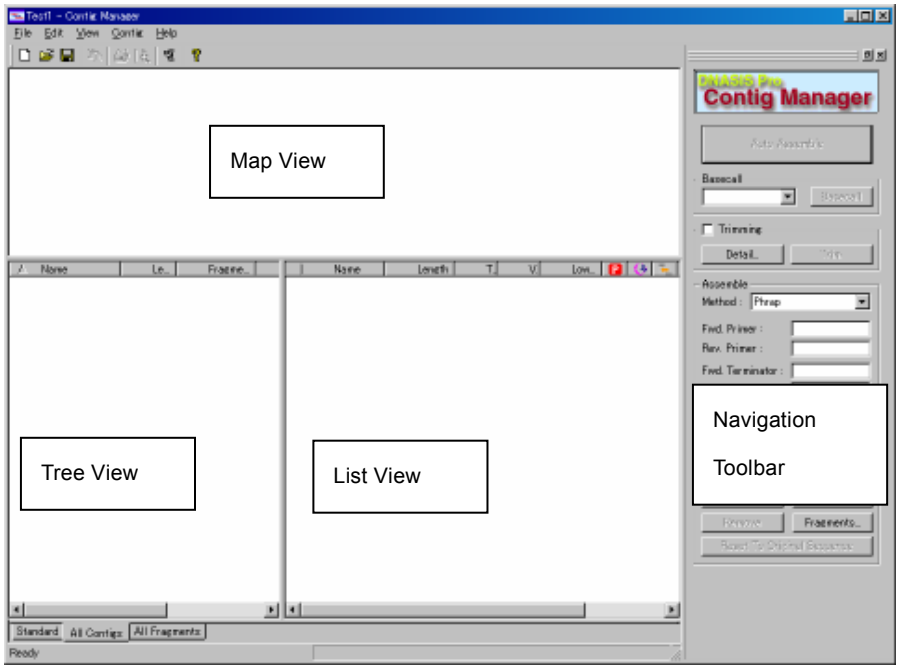
Open Project dialog

Before using the Contig Manager for analysis, select whether to create a new project or open an existing project (a list is displayed in the lower part of the dialog) with this dialog. For this tutorial a new project is created.

Select the “Create a new project:” radio button and enter “Sample” in the Project Name text box, and then click New.

1.1.6 Part Names and Descriptions

The main Contig Manager window shown below will appear when a new project is created.



Part Names of Main Contig Manager Window

Item	Description
Map View	Graphically displays a list of contigs that reside in the folder selected in Tree View.
Tree View	Displays the folder hierarchy to manage the sequence data, and a list of contigs.
List View	Displays the contigs and fragments that reside in the folder selected in Tree View, or a list of the fragments that make up the contig selected in Tree View.
Navigation Toolbar	A group of tools for operating the Contig Manager.

1.1.7 Close the Contig Manager

Select File > Exit from the menu to exit the Contig Manager.

1.1.8 Data Used in the Tutorial

The data used in the tutorial is installed together with the Contig Manager. Select Programs > DNASIS MAX > Tutorial Data from the Start menu to open the folder that contains the DNASIS MAX tutorial data.

The tutorial use the data contained in the ContigManager folder.

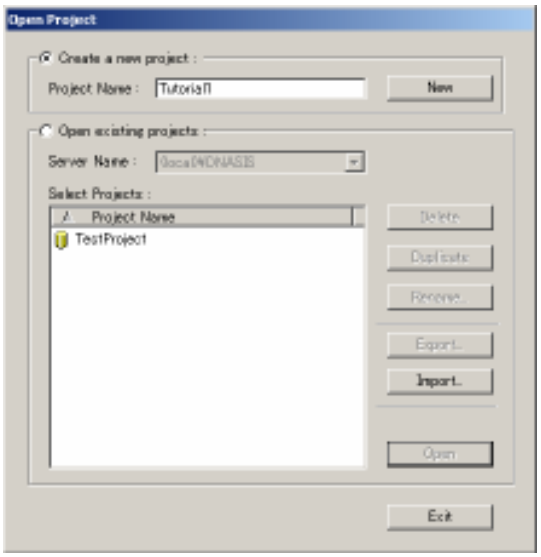
To use this tutorial, you need to install the DNASIS MAX Contig Manager Version 2.0.

1.2 Analysis Example 1 - Assembling Trace Data

This section explains how to use the Contig Manager to load trace data output from sequences, to perform Phred basecalling, vector trimming and Phrap assembly.

1.2.1 Start up the Contig Manager

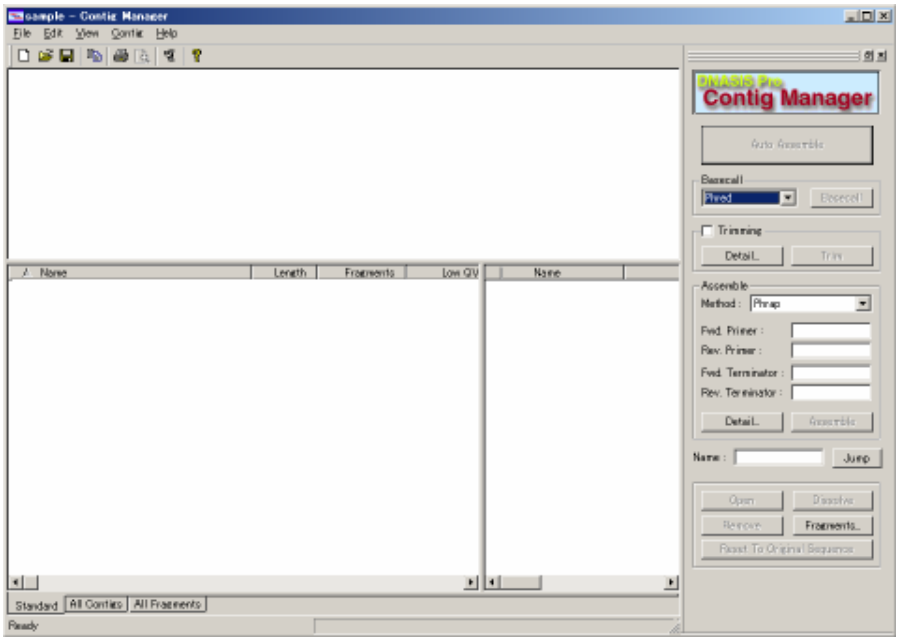
Select Programs > DNASIS MAX > Contig Manager from the Start menu to display the Open Project dialog shown below.



Open Project dialog

1.2.2 Create a Project

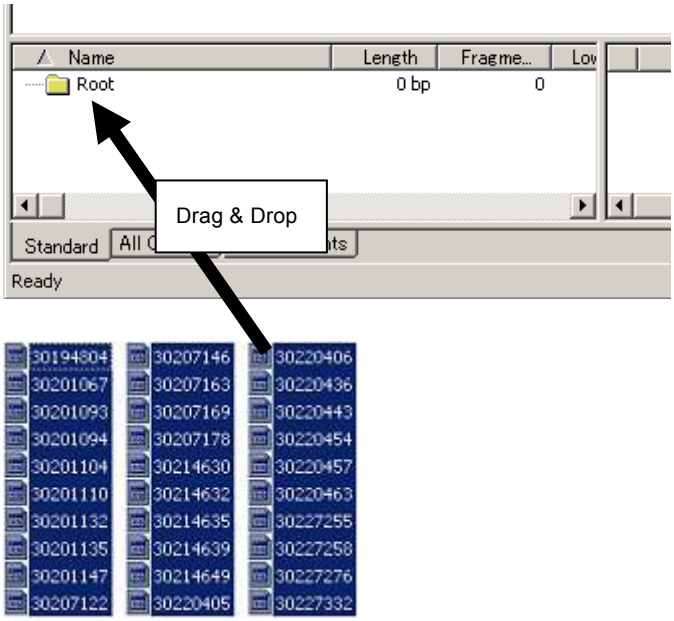
1. Select the “Create a new project:” radio button on the Open Project dialog.
2. Enter “Tutorial1” in the Project Name text box, and then click New.
3. The main Contig Manager window appears.



Main Contig Manager window

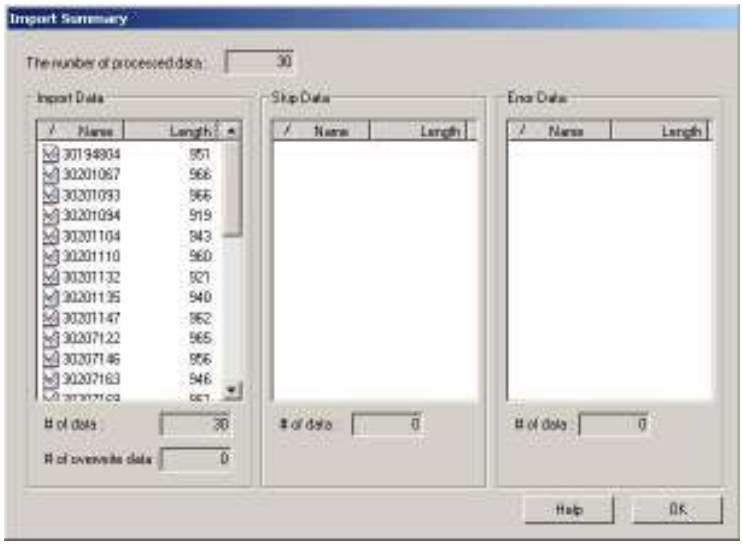
1.2.3 Enter Trace Data

1. Select Programs > DNASIS MAX > Contig Manager Tutorial Data from the Start menu to open the TutorialData folder.
2. Then open ContigManager > TutorialData1 > TraceData1_1.
3. Select all of the files contained in the TraceData1_1 folder, and then drag and drop them into the Root folder in Tree View of the Contig Manager or into the List View.
4. When dropping the files into Tree View, be sure that the data overlaps the destination folder (the Root folder in this case).



Enter Sequence Data by Drag & Drop

5. File reading will complete after a few moments. The Import Summary dialog will show the total number of data, the number and names of imported data, the number and names of skipped data, and the number and names of error data.

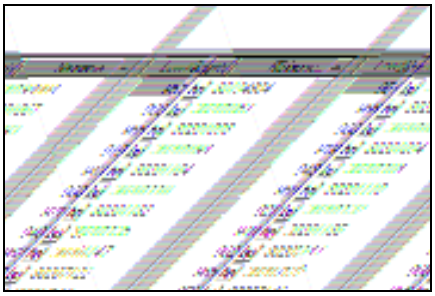


Import Summary dialog

6. Click OK to close the dialog after first checking the imported fragment contents.

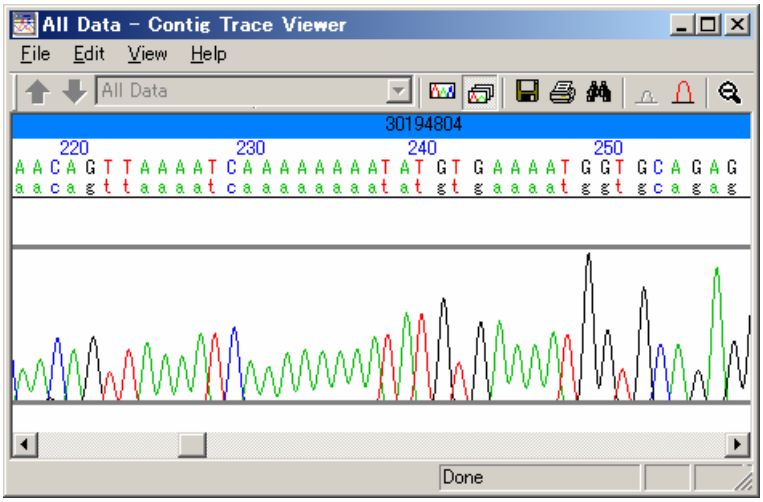
1.2.4 Display Trace Data

1. A list of the fragments imported in the previous step will appear in List View on the main Contig Manager window.



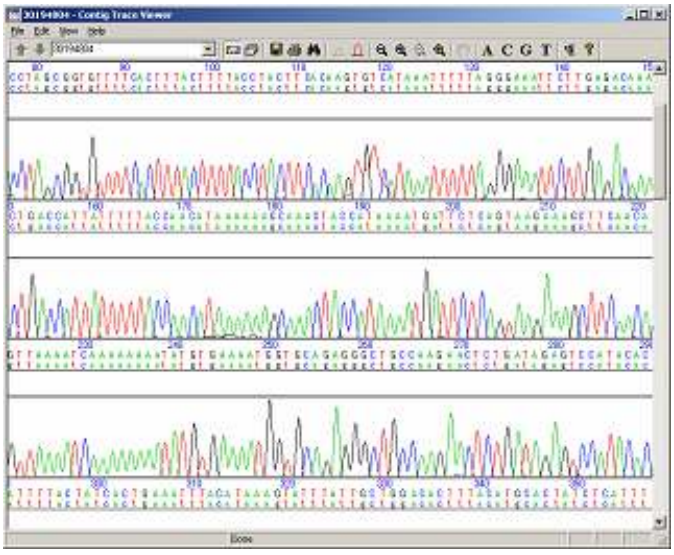
Fragments in List View

2. Double click on any item in the list to display the fragment trace data.



Trace Display using the Trace Viewer

3. Select View > Show Single Data from the Trace Viewer menu or click on Single Data ( ) located on the Toolbar to display the trace data in multiple stages so that it can be viewed over as wide a range as possible.



Display Single Trace Data over Multiple Stages

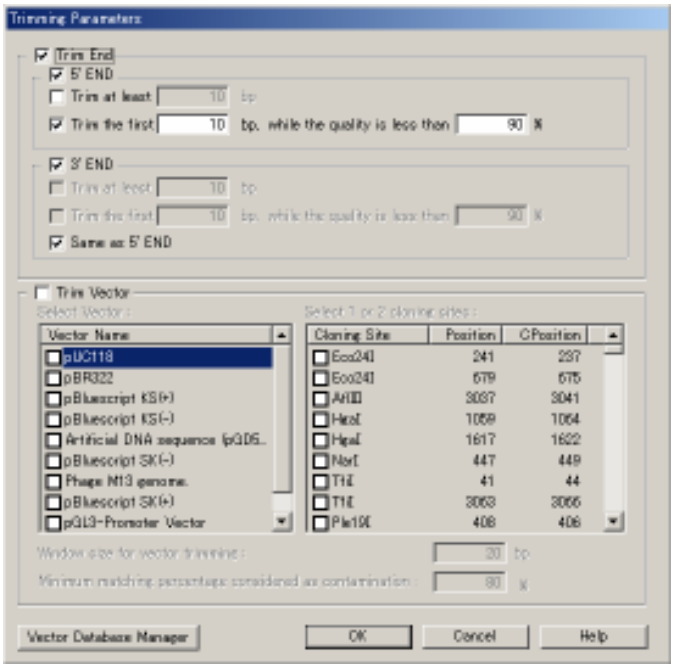
4. Select multiple fragments from List View in Contig Manager, and then click Open on the Navigation Toolbar to sort and display multiple trace data.

Display Multiple Trace Data with the Trace Viewer

1.2.5 Trimming Parameter Setup 1

This procedure sets the parameters for erasing vector sequences, sequencing primer sequences and other elements before the fragments are assembled. Removing these sequences prior to assembly enables the sequences to be assembled with even higher levels of precision.

Click Detail... under Trimming group on the Navigation Toolbar to display the Trimming Parameters dialog.



Trimming Parameters dialog

This dialog is used to set up the parameters for trimming. Below are parameter descriptions:

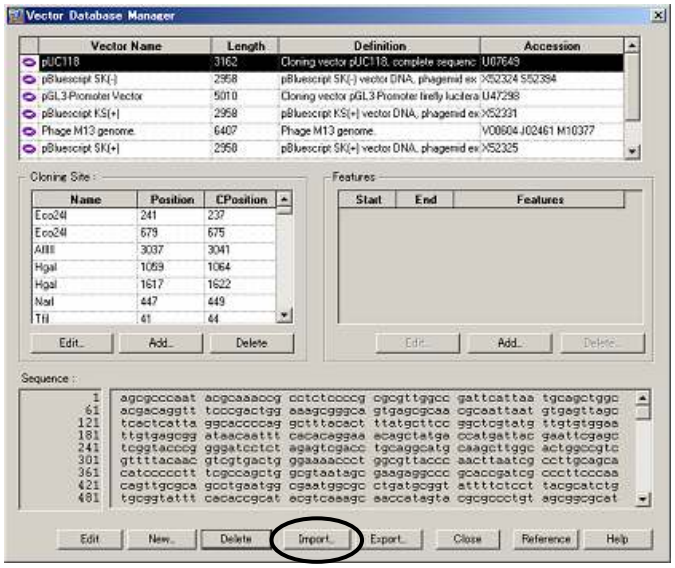
Item	Description
Trim End	The parameter for trimming the end of fragments.
Trim at least	The base value for unconditional 5' and 3' ends trimming.
Trim the first XX bp, while the quality is less than XX %.	The base value for average movement (trim the first XX bp) with the unstable base (N) percentage for the fragment's end (quality is less than XX%.)
Trim Vector	The parameter for removing vector sequences included in fragments.
Select Vector	The check boxes for selecting vectors to be trimmed.
Select 1 or 2 cloning sites	The check boxes for the cloning sites to be trimmed.
Window size for vector trimming	The parameter for setting the base length (window size) to be extracted from the vector cloning site sequence that is used for determining the vector sequence area. Set with integers of 15 or higher.
Minimum matching percentage considered as contamination	The parameter for setting the minimum percentage for matching the vector cloning site sequence with the sequence targeted for trimming. Vector sequences that are larger than the percentage set here will be ignored.

Trimming is performed on 5' ends and 3' ends with the conditions set for "Trim the first 10bp, while the quality is less than 90%." for explanatory purposes in this tutorial.

Note that trimming will not be carried out for registered vector sequences with the sample fragments provided by the tutorial. Proceed onto the next section on Add Vector Data.

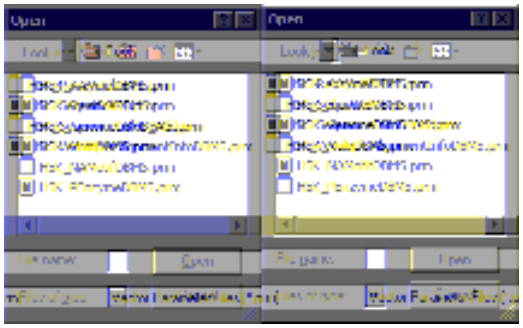
1.2.6 Add Vector Data

1. Click Vector Database Manager on the Trimming Parameters dialog.
2. The Vector Database Manager for managing registered vector data will start up.



Vector Database Manager

3. Click Import... to open the dialog for importing vectors.



Dialog to Import Vector Data

4. Select ContigManager > TutorialData 1 > TutorialVector from the tutorial data (default: C:\HSK_DB\TutorialData), select the TutorialVectorA.prm file, and then click Open.
5. The window will return to the Vector Database Manager, and TutorialVectorA will be added to the vector list.
6. Perform the same procedure for TutorialVectorB.prm and TutotialVectorC.prm to add a total of three vectors.

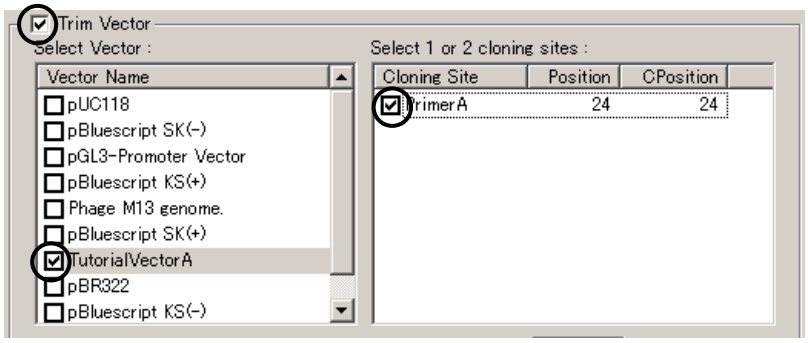
- When registering the vectors to be trimmed with actual analysis data, add any of the vectors from the VectorData folder (default: C:\HSK_DB\VectorData) that is specified beneath the Database directory when DNASIS MAX is installed. (If the location cannot be found, select Programs > DNASIS MAX > Tutorial Data from the Start menu. The required folder is in the first hierarchy of folders in the database.)
- This folder contains more than 900 different types of vector data. Refer to the VectorTable.txt file located in the VectorData folder for details. This file can be easily viewed with Microsoft Excel or another spreadsheet application.

7. Register the vector, and then click Close to end the Vector Database Manager.

1.2.7 Trimming Parameter Setup 2

This procedure sets the parameters required trimming using the vectors added in the previous section on Add Vector Data.

1. Select the Trim Vector check box on the Trimming Parameters dialog.
2. Select the TutorialVectorA check box on the Select Vector list.
3. Select the PrimerA check box on the Cloning Sites list.



Select the vector and cloning site check boxes

4. Repeat the same procedure to select the TutorialVectorB and PrimerB check boxes, and the TutorialVectorC and PrimerC check boxes.

- Check the imported vectors and corresponding cloning sites when performing actual analysis.

5. Set a value that is lower than the default value for the “Minimum matching percentage considered as contamination:” parameter with the tutorial data. Amend the default value to 85%.

6. Click OK to close the Trimming Parameters dialog once this setting has been made.
7. Return to the main window, and select the Trimming check box on the Navigation Toolbar.

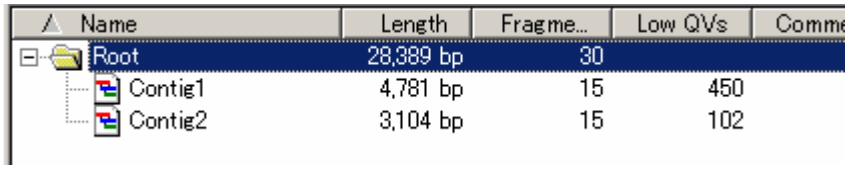
1.2.8 Perform Auto Assemble

This section explains how to perform basecalling, trimming and assembly of trace data.

1. Select all of the fragments displayed in List View on the main Contig Manager window (an easy way to select all data is to click on any data item in List View then press Ctrl + A or select Edit > Select All from the menu).
2. Confirm that all fragments are highlighted then click Auto Assemble on the Navigation Toolbar.

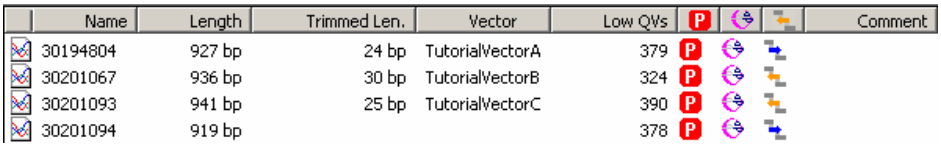
- If the Use Phred check box was selected under the Basecall group, Phred basecalling will be performed with assembly taking into account the quality values (QVs). If the check box is not selected, assembly will be performed based on the trace’s internal basecall data, without regard to the QVs.

3. The contig sequence information is added to Tree View once analysis is completed.



New contigs

4. Trimming results and direction information are added to each fragment in List View.



The List View with the new information added

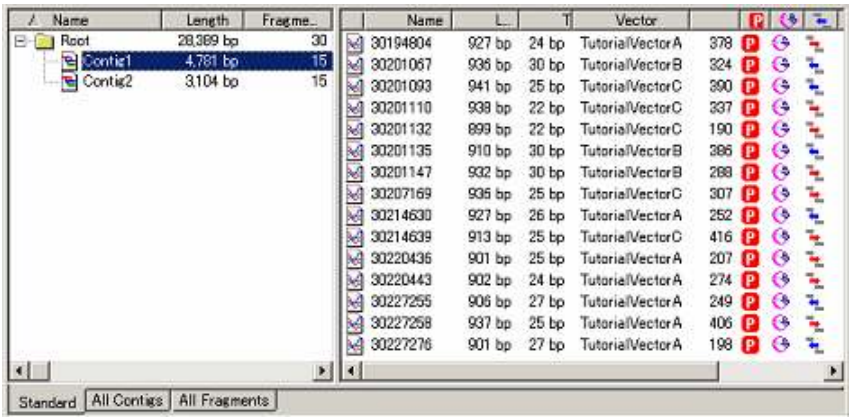
In the example above, the length of fragment 30194804 is 927bp after trimming, the trimmed bp count is 24bp, the name of the vector used for trimming is TutorialVectorA, the number of base pairs within the range set as Low QV with the QV as 379bp, the basecall procedure was performed with Phred, trimming has been completed, and assembly was carried out with the normal strand.

The details for each column are listed below:

Item	Description
Icons by data type	Indicates the data type.
	The icon attached to fragments that possess trace data.
	The icon attached to fragments that possess only sequence data.
	The icon attached to fragments that have been entered as trace data, but for which the trace data has been moved or deleted.
	The icon attached to contigs.
Name	Displays the name of the corresponding data.
Length	Displays the sequence length of the corresponding data.
Trimmed Len.	Displays the total bp count of the trimmed area when trimming has been performed. This column remains blank if trimming is not performed or no fragments were trimmed.
Vector	Displays the name of the vector for which trimming was performed.
Low QVs	When using Phred basecalling, displays the number of bases in bp units that are within the range of Low QVs set in View > Preferences from the menu.
Phred icon 	Displays if Phred basecalling is performed on the fragment.
Trimming icon 	Displays if the fragment has been trimmed. It will also display even if trimming was not performed.
Assembly Direction Icon	Indicates the direction in which the fragment was assembled. Data that does not display this icon has not been assembled.
	This icon is displayed when assembly has been performed with the same strand as the entered sequence.
	This icon is displayed when assembly has been performed with complementary strands of the entered sequence.
Comment	Displays a character string when a comment has been entered for the corresponding data.

1.2.9 Display Contig Results

1. Select any of the contigs displayed in Tree View on the main window to display the fragment information of the new contig.
2. A list of the fragments forming the contig selected from the List View together with each assembly direction will display.



List of Fragments Forming the Contig

3. Double click Contig1 on the Tree View.
4. The Contig Viewer will start up, and the status of the Contig1 fragment assembly will be displayed graphically.

Graphical Display of the Composition

The Contig Viewer is divided into top and bottom panes. The top pane graphically displays the entire subject (Map View) and the bottom (Sequence View) displays the contig sequence (top stage) and each fragment sequence.

1.2.10 Sort and Display Trace Data

Actual trace data can be sorted and displayed to evaluate assembly results.

1. Click the base value for the trace that is to be sorted and displayed on the contig sequence, and then either select View > Chromatograms from the menu, or click Show Chromatograms ( ) with the base value highlighted.

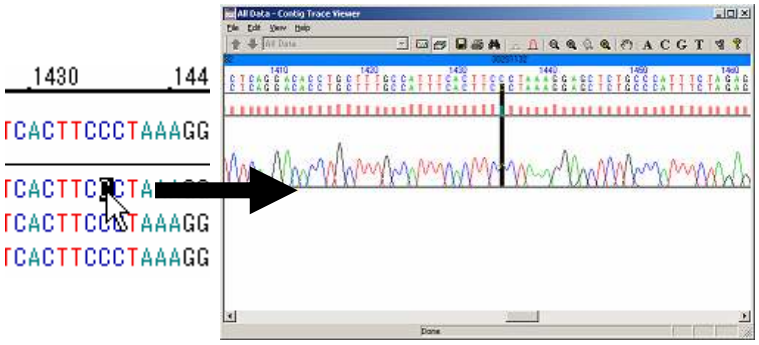
Highlighted base values

2. The trace data will be aligned and displayed based on the selected base value.

Trace data sorted and displayed

3. The status of the trace data display is linked into the selected position of the sequence in the Contig Viewer and can be changed accordingly. Click the contig sequence in the Contig Viewer or the base value on the fragment sequence to re-sort and display the trace data based on the selected base value.

The linked display of the trace data when the base value on the contig is clicked



The linked display of the trace data when the base value on the fragment is clicked

1.2.11 Reassembly

This section explains how to add a new sequence and perform reassembly with the added fragments included.

1. Select Programs > DNASIS MAX > Tutorial Data from the Start menu to open the TutorialData folder.
2. Then open ContigManager > TutorialData1 > TraceData1_2.
3. Drag and drop the data contained in the folder to the Root folder in Tree View.
4. The name of the sequence and the number involved entered on the Import Summary dialog will be displayed. Click OK to close the dialog.
5. Select the Root folder in Tree View and confirm that the corresponding fragments have been added to the List View.
6. Select the required fragment and click Basecall on the Navigation Toolbar to perform Phred basecalling and calculate the quality value.
7. Select the corresponding fragment and click Trim on the Navigation Toolbar to perform the vector trimming.
8. Select all of the fragments in the List View and press Assemble. A message stating, “Some of the selected fragments are connected. Are you sure to dissolve the connection and continue operation?” will appear. Click Yes to continue with the process.

9. The assembly process will be performed including the added fragments, and a new contig sequence will be created.
10. These will be assembled into a single contig if the tutorial data is used.

1.2.12 Exit the Contig Manager

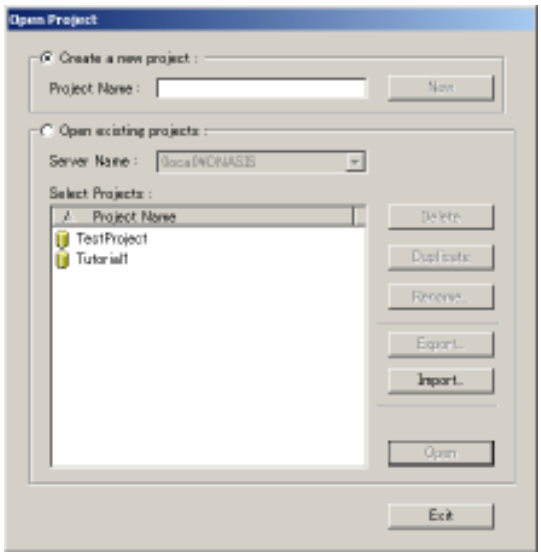
Select File > Exit from the Contig Manager's menu if analysis has completed.

1.3 Analysis Example 2 - Sequence Clustering and DNASIS MAX Links

This section explains how to perform clustering on the EST sequences registered in Genbank using the Contig Manager. It also explains how to perform simple analysis of new contig sequences with the use of DNASIS MAX.

1.3.1 Start up the Contig Manager

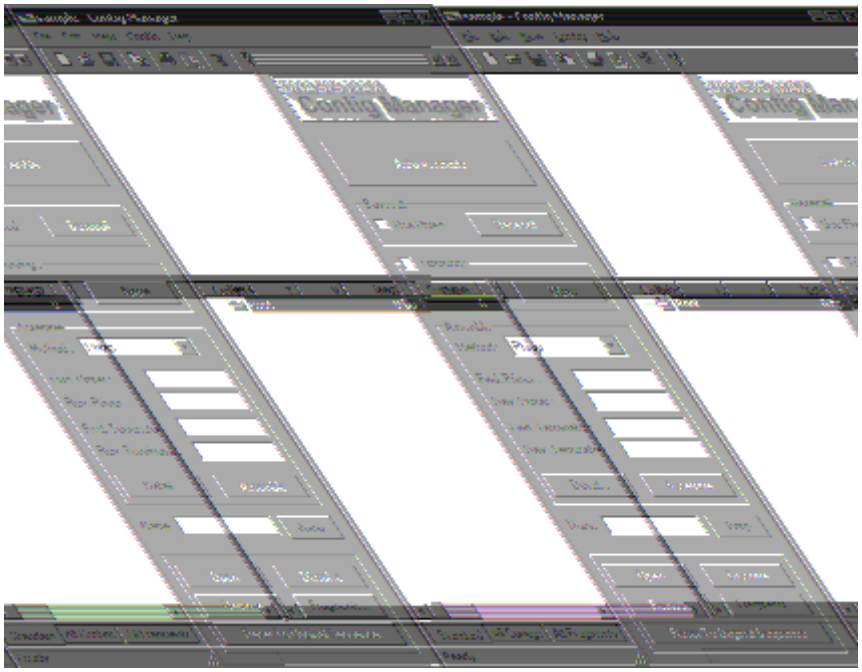
Select Programs > DNASIS MAX > Contig Manager from the Start menu to display the Open Project dialog shown below.



Open Project dialog

1.3.2 Create a Project

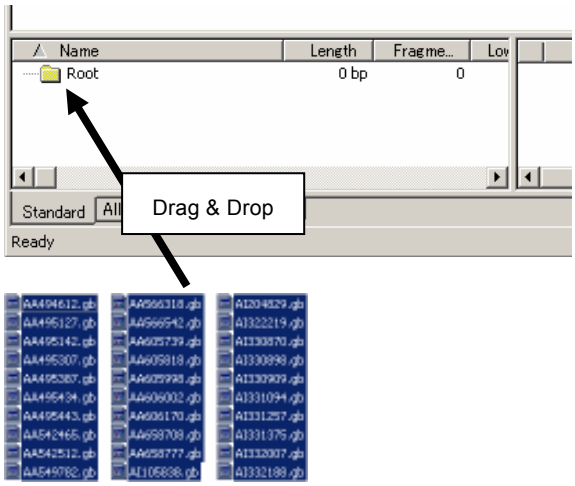
1. Select the “Create a new project:” radio button on the Open Project dialog.
2. Enter “Tutorial2” in the Project Name text box, and then click New to open the main Contig Manager window.



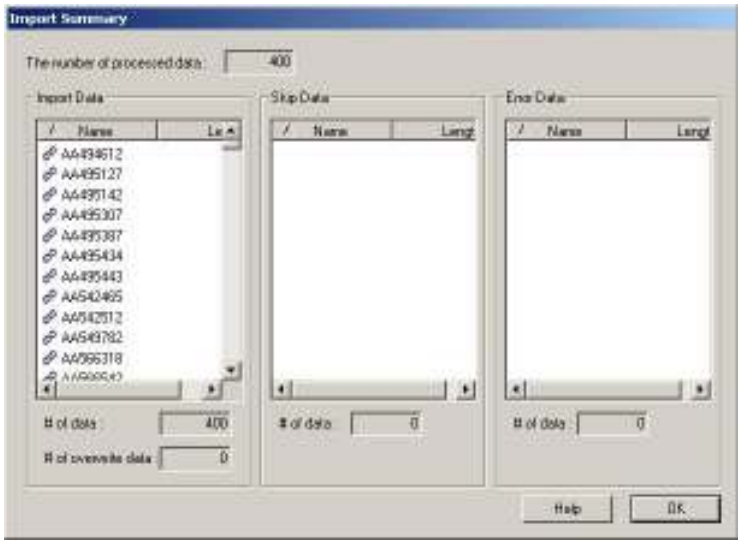
Main Contig Manager Window

1.3.3 Enter Trace Data

1. Select Programs > DNASIS MAX > Tutorial Data from the Start menu to open the TutorialData folder.
2. Open ContigManager > TutorialData2 > TraceData2_1.
3. Select all of the files contained in the TraceData2_1 folder, and then drag and drop them into the Root folder on the Tree View of the Contig Manager or into the List View.



4. File reading will complete after a few moments. The Import Summary dialog will show the total number of data, the number and names of imported data, the number and names of skipped data, and the number and names of error data.



Import Summary dialog

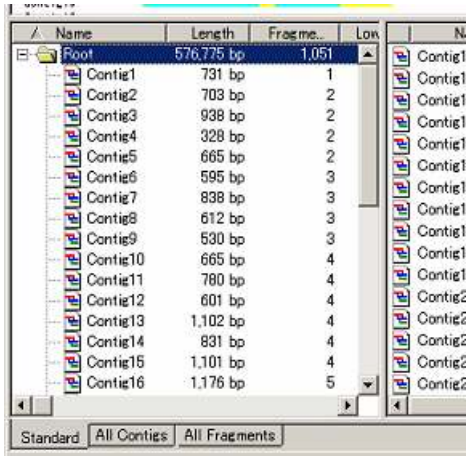
5. Click OK to close the dialog.

1.3.4 Sequence Assembly

1. Select all fragments added to the List View on the main Contig Manager window.

- Select any data item in List View, and then press Ctrl + A, or select Edit > Select All from the menu to select all fragments.

2. Click Assemble on the Navigation Toolbar.
3. The assembled contigs will appear in Tree View.



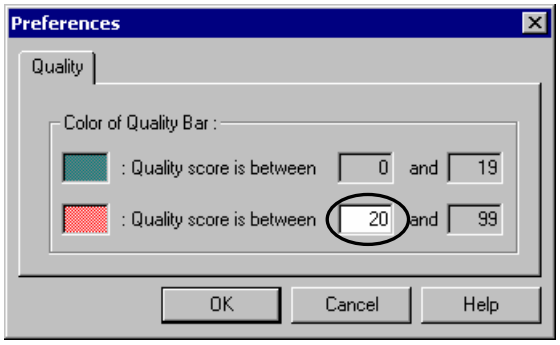
Composition Results

4. 36 contigs will be created if analysis is performed with the default values. This may be interpreted as the entered fragment groups forming 36 clusters.

1.3.5 Adjust the QV Threshold

It is possible to specify different colors for the Map View and set up high/low threshold values as the parameters for the Low QVs count in Tree View and List View. In this tutorial, low QV can be changed from 1 to 29 and high QV from 30 to 99.

1. Select View > Preferences… from the menu, or click Preferences ( ) on the Toolbar to display the Preferences dialog.

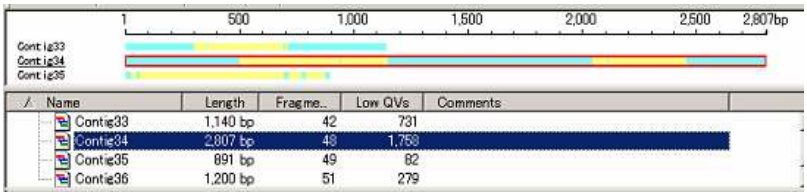


Preferences dialog

2. Change the value circled in the dialog above between 20 and 30 then click OK to close the dialog.
3. The Map View will be redrawn, and the values in Tree View and List View will be re-calculated.

1.3.6 Examine Assembly Results

Contig34 created through analysis with the default values is used in this tutorial.

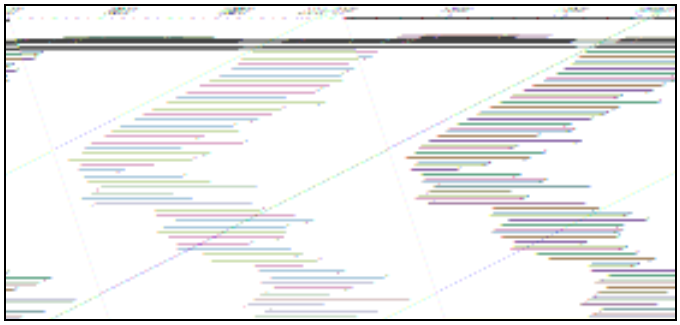


Contig34

The view above shows a Contig34 length of 2807bp, consisting of 48 fragments, and a base value of 1758bp for the QV (set at 30 or less for this example.) It is also clear that the low quality areas are situated on both ends and in the center of the contig.

1.3.7 Display Assembly Status Details

1. Double click on Contig34 to view actual assembly details in List View.



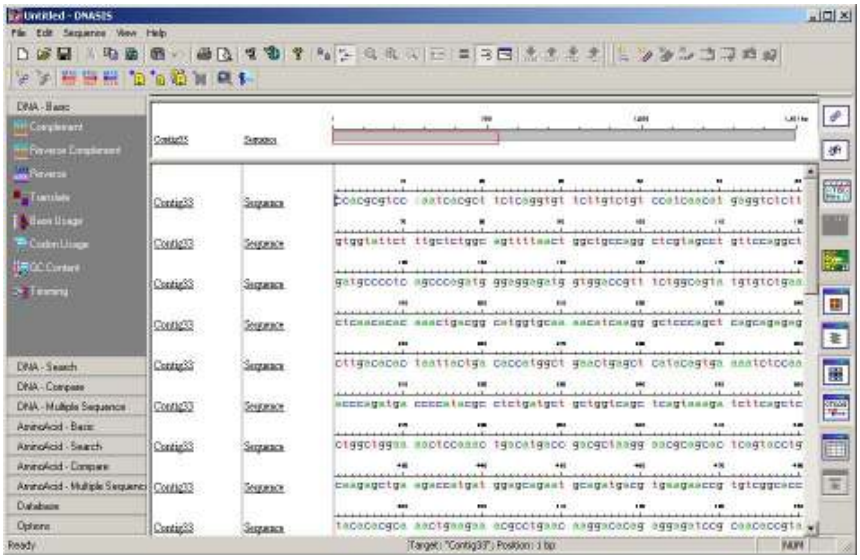
Map View

2. The layout of fragments in Map View of the Contig Viewer indicates that both ends of the contig have been read comparatively well, whereas the central area has not.
3. Viewing the sequence enables us to estimate that a Poly-A region exists near the 3' end of the contig sequence.

Poly-A Region

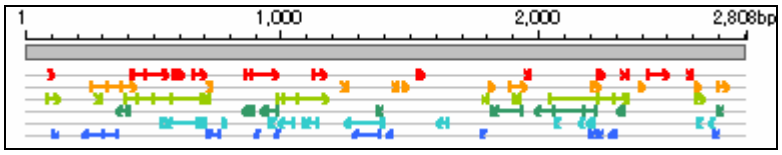
1.3.8 Link with DNASIS MAX

1. Select File > Export Contig to DNASIS MAX from the Contig Viewer menu, or click Export Contig to DNASIS MAX () on the Toolbar to analyze the new contig sequence with DNASIS MAX.
2. DNASIS MAX will start up when the contig sequence is read.



DNASIS Start Up window

3. Try an ORF search at this point by clicking ORF in the DNA-Search group. And click Execute in the Analysis dialog. The analysis will be performed and results similar to those shown below will appear.



ORF search results

4. These results show that errors still exist in the contig sequence.
5. As shown by the contig's quality value the sequence accuracy between values close to 1150bp to values close to 2050bp are relatively low. The next step is to design a primer for increasing sequence accuracy by performing sequencing with the use of the primer working method from areas with high levels of sequence precision.
6. Select 1050bp to 1100bp from the DNASIS MAX contig sequence, and then press Primer Design in the DNA-Search group. A primer that increases the width of the relevant area will be designed when the sequence is selected.

Result of Primer Design

7. By performing analyses and linking with DNASIS MAX and by repeating the primer design, sequencing and re-assembly in this way, it is possible to increase the accuracy of the contig sequence.

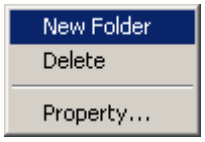
1.3.9 Manage Sequence Data by Folder

The most convenient method to manage a large volume of sequence data is to create hierarchical folders in Tree View and manage the sequence data by folder.

Managing sequence data by folder without dividing the projects enables work to be efficiently and simply carried out for such tasks as sequence clustering with contig creation, adding sequences and performing re-assembly after contigs have been created.

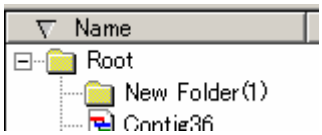
1.3.10 Create a New Folder

1. Right click the Root folder and select New Folder from the popup menu that appears.



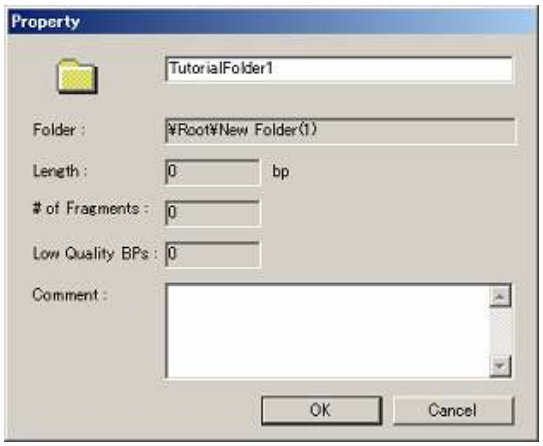
Popup menu

2. A folder named New Folder(1) is created under the Root folder.



New Folder

3. Right click the new folder and select Property... from the popup menu that appears. The Property dialog appears.
4. The first text box of the dialog is for the folder name, which can be changed.
5. For this Tutorial use TutorialFolder1.

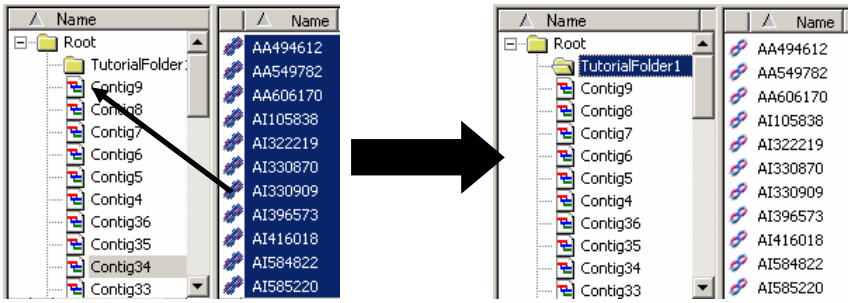


Property dialog

1.3.11 Move Fragments

Fragments and contigs are moved to other folders by dragging and dropping. For example, the following explains how to move all the fragments that make up Contig34 to TutorialFolder1.

1. Select Contig34 from the Tree View to display a list of fragments that make up Contig34 on the List View.
2. Select all the fragments in ListView. Drag and drop all fragments into the TutorialFolder1.
3. The selected fragments move to TutorialFolder1.



1.3.12 Add Sequence Data

This section explains how to add sequence data from sequencing using a combination of DNASIS MAX features like primer design and others.

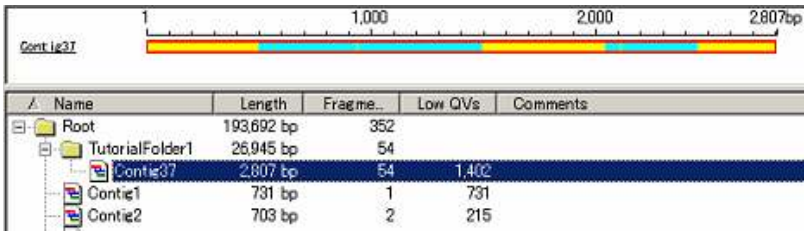
1. Select Programs > DNASIS MAX > Tutorial Data from the Start menu to open the TutorialData folder.
2. Then open ContigManager > TutorialData2.
3. Select TutorialData2_2.txt from the TutorialData2 folder, and then drag and drop it into the TutorialFolder1 folder on the Contig Manager's Tree View.
4. File reading will complete after a few moments. The Import Summary dialog will show the total number of data, the number and names of imported data, the number and names of skipped data, and the number and names of error data.

Dropping this file into TutorialFolder1 enables the fragments contained in the folder to be read directly.

1.3.13 Sequence Assembly With Only the Sequence Data Contained in the Folder

1. Click TutorialFolder1 in Tree View to display a list of the contigs and fragments contained in the folder in List View.
2. Select all of the fragments in List View and click Assemble on the Navigation Toolbar.
3. A message stating, "Some of the selected fragments are connected. Are you sure to dissolve the connection and continue operation?" will appear. Click Yes to continue with the process.

4. The assembly process will be performed, and Contig37 will be created immediately beneath TutorialFolder1.



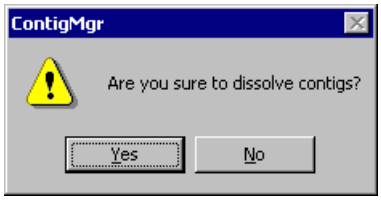
Contig37

5. Clearly, adding fragments and re-assembling the data has reduced the low region quality value from 1758bp to 1402bp, and that the accuracy of the contig sequence has been improved.

1.3.14 Dissolve Assembled Sequences

To dissolve assembled sequences, select the contig assembly to dissolve from Tree View or List View, and then click Dissolve on the Navigation Toolbar. In this example, Contig36 will be dissolved. A new folder will also be created for easy confirmation of dissolving results. All fragments will be moved to this folder to perform dissolving.

1. Right click on the Root folder, and then select New Folder from the popup menu.
2. Right click on the New Folder (1) created in the previous step, and then select Property... from the popup menu.
3. The Property dialog for the folder will appear. Amend the folder name from New Folder (1) to TutorialFolder2.
4. Select Contig36 from Tree View to display a list of fragments that make up this contig in List View.
5. Click on any item of data displayed in List View, and then press Ctrl + A, or select Edit > Select All from the menu.
6. Drag and drop the fragments selected in List View and move them to TutorialFolder2.
7. Select Contig36 from the List View, and then click Dissolve on the Navigation Toolbar.
8. A confirmation dialog with a message, “Are you sure to dissolve contigs?” will display. Click Yes to continue with the dissolving process.

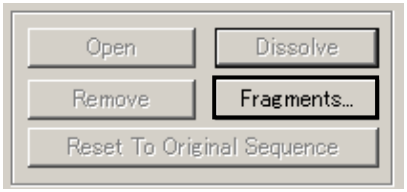


9. Click TutorialFolder2 on the Tree View to display a list of all fragments contained in that folder (the fragments that make up Contig36). The absence of the icons that indicate the assembled status confirms that assembled sequence has been dissolved.

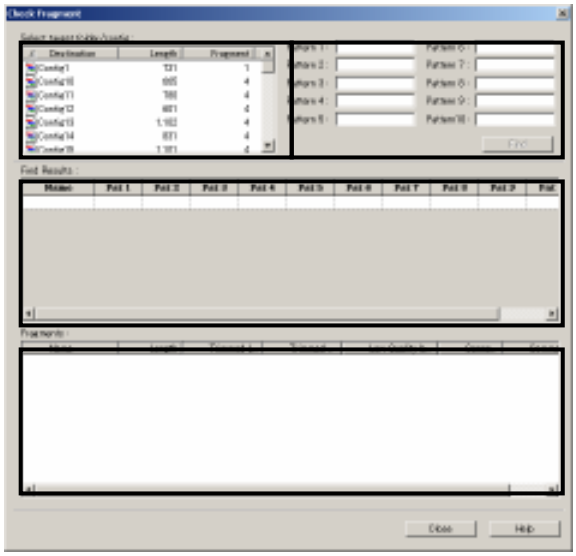
1.3.15 Search for Fragments

Fragment names can be specified to run searches on which folder or contig they belong to. Wildcards can be used as search strings to enable fragment names that share specific patterns to be retrieved as efficiently as possible.

1. Click Fragments on the Navigation Toolbar.



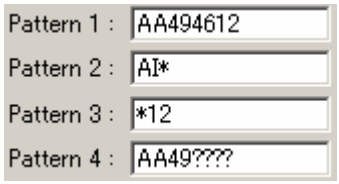
2. Check Fragment dialog appears.



Check Fragment dialog

Navigation Toolbar	Description
1. Select target folder / contig	Select the folder or contig for the search target.
2. Pattern1 - Pattern10	Enter the name of the fragments for which the search is to be run. Searches can be run with a maximum of ten fragments. The following two methods are available for specifying the wildcard: *: Random character strings ?: Single random character Names that represent perfect matches and names that include the wildcard can be entered.
3. Find Results	Displays a list of the fragments found that match the search strings.
4. Fragments	Displays a list of the fragments found during the search.

3. Enter AA494612 in Pattern 1, AI* in Pattern 2, *12 in Pattern 3, and AA49???? in Pattern 4.



Search Pattern Entry

• A494612: Searches for data with a sequence name of AA494612.

• AI*: Searches for data for which the sequence name starts with AI.

• *12: Searches for data for which the sequence name ends with 12.

• AA49????: Searches for data for which the sequence name starts with AA49 and contains four other random characters consecutively.

4. Select all of the contigs and folders on the Select target folder/contig list, and then click Find.

- When selecting multiple elements from the list, click the mouse button while pressing the Ctrl button to add required selections, or click the mouse button while pressing the Shift button to sequentially select all of the data between the first click and the second click.

Search target

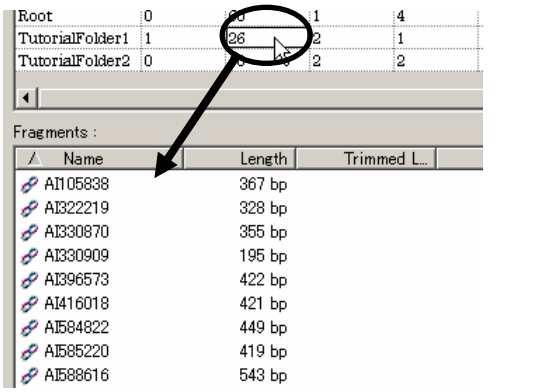
Find Results :

Name	Pat 1	Pat 2	Pat 3	Pat 4
Contig5	0	0	0	1
Contig6	0	3	0	0
Contig7	0	0	0	0
Contig8	0	0	0	0
Contig9	0	1	0	0
Root	0	60	1	4
TutorialFolder1	1	26	2	1
TutorialFolder2	0	10	2	2

Number of search results for each pattern.

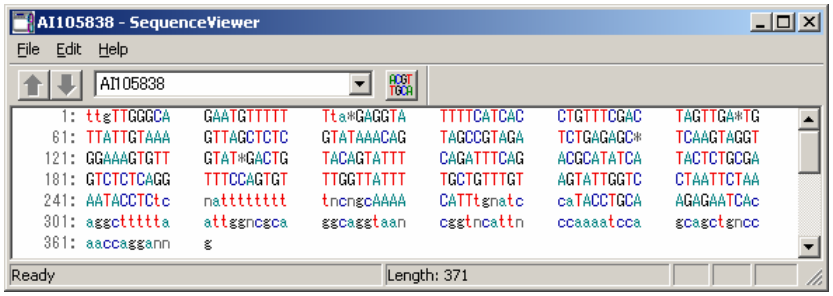
Search result

5. The Find Results shows that the AA494612 fragment entered in Pattern 1 resides in TutorialFolder1, and those three fragments that start with the AI entered in Pattern 2 reside in Contig6, 1 in Contig9, 60 in the Root folder, 26 in TutorialFolder1, and 10 in TutorialFolder2.
6. Click on the cell for search results to display a list of the fragments that match the search conditions in the Fragments list.

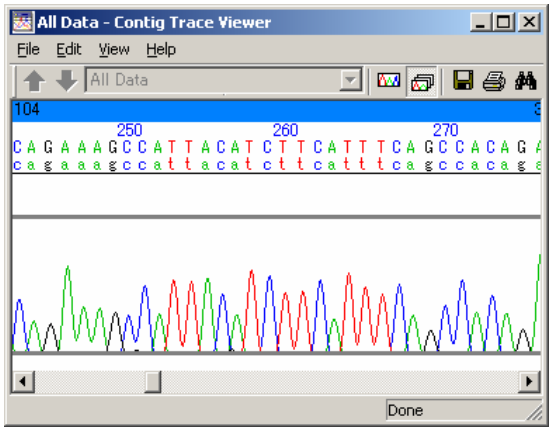


Fragment search result list

7. Double click on any of the fragments displayed on the Fragments list to display the sequence for that specific fragment.
8. Trace data will be displayed together with the sequence if fragments that possess trace data are found.



Fragment sequence display



Fragment trace display

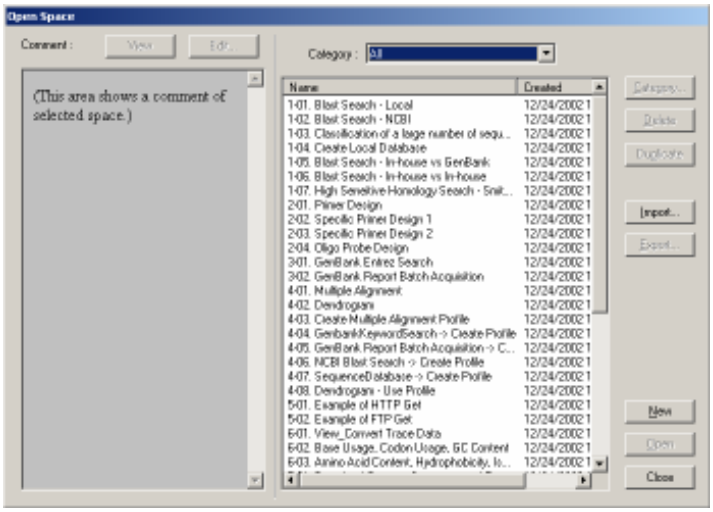
1.4 Analysis Example 3 - Analyses Using Phred Quality Values

This section explains how to run homology searches using sequence selection and only high quality regions based on Phred quality values.

***The DNASIS MAX DNASpace option is required for this tutorial.**

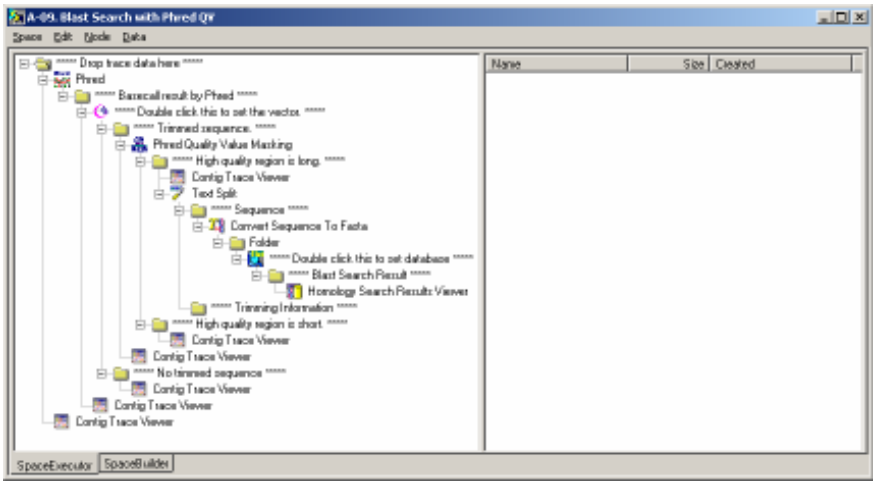
1.4.1 Start up DNASpace and Opening Space

1. Select Programs > DNASIS MAX > DNASpace from the Start menu.
2. The Open Space dialog shown below will appear.



Open Space dialog

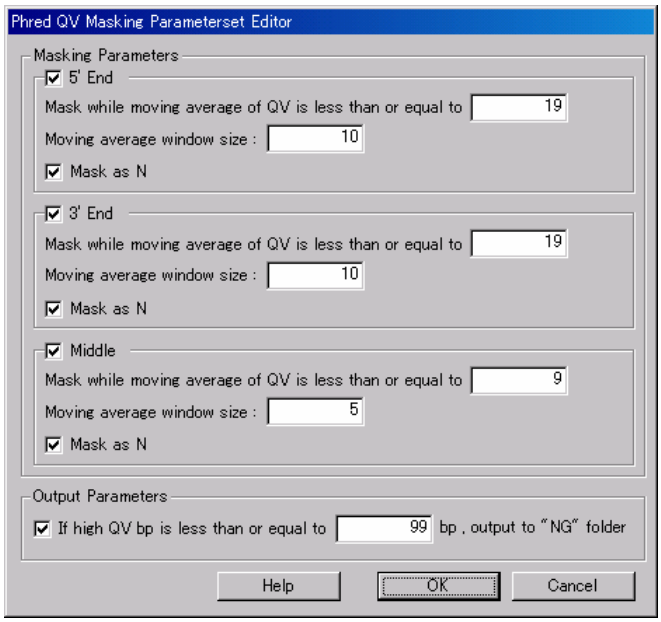
3. Select “A-09. Blast Search with Phred QV” from the list. Click Open and the following space appears.



A-09. Blast Search with Phred QV

1.4.2 Set Sequence Masks and Selection Parameters of Quality Values

1. Double click on Phred Quality Value Masking on the Space dialog to open the Parameter Set Editor.



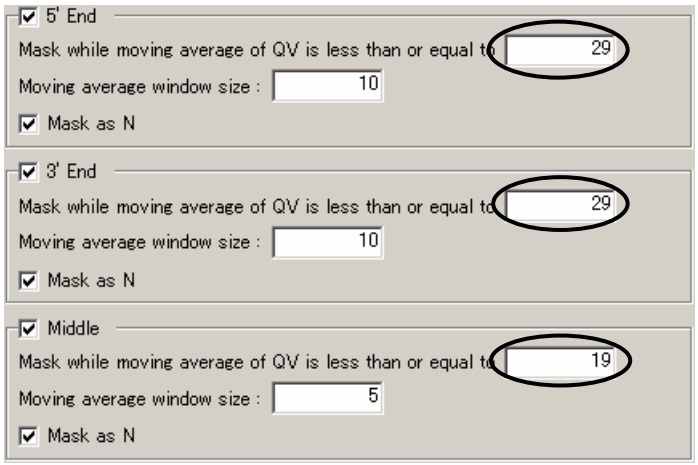
Phred QV Masking Parameterset Editor

Item	Description
Masking Parameters	Sets the masking parameters based on the input sequence quality value.
5' End	Sets whether or not to perform masking with the QV set for the 5' end sequence. Select this check box to perform masking.
Mask while moving average of QV is less than or equal to XX	Obtains the moving average of the QV from the 5' end and performs masking while the average value is XX or less. Masking ends when the average value exceeds the value specified here.
Moving average window size	Specifies the window size for obtaining the moving average when masking with 5' end sequences.
Mask as N	Select this check box if the base value is to be replaced with N for the relevant region when the moving average of the 5' end sequence's QV is the same or less than the threshold value.
3' End	Sets whether or not to perform masking with the QV set for the 3' end sequence. Select this check box to perform masking.
Mask while moving average of QV is less than or equal to XX	Obtains the moving average of the QV from the 3' end and performs masking while the average value is XX or less. Masking ends when the average value exceeds the value specified here.
Moving average window size	Specifies the window size for obtaining the moving average when masking with 3' end sequences.
Mask as N	Select this check box if the base value is to be replaced with N for the relevant area when the moving average of the 3' end sequence's QV is the same or less than the threshold value.
Middle	Sets whether or not to perform masking with the QV set for the entire sequence. Select this check box if masking is to be performed.
Mask while moving average of QV is less than or equal to XX	Obtains the moving average of the QV from the closest 5' end to the closest 3' end of non-masked regions, and performs masking while the average value is XX or less.
Moving average window size	Specifies the window size for obtaining the moving average when masking with the entire sequence.
Mask as N	Select this check box if the base value is to be replaced with N for the relevant region

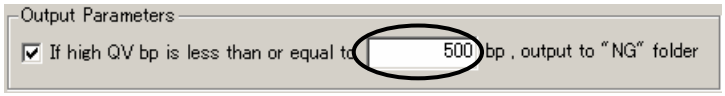
Item	Description
	when moving average of the entire sequence's QV is the same or less than the threshold value.
Output Parameters	Sets the parameters for the folder where masking result data is output.
If high QV bp is less than or equal to XX bp, output to "NG" folder	The results are output to the "NG" folder if the length of unmasked region is XXbp or less when this check box is selected.

2. In this tutorial, the threshold of the QVs for both ends are set to 29, the threshold of the QVs for the entire sequence are set to 19, and the bp count of the high quality regions to be output to the NG folder is set at 500bp and lower.

Masking Parameters



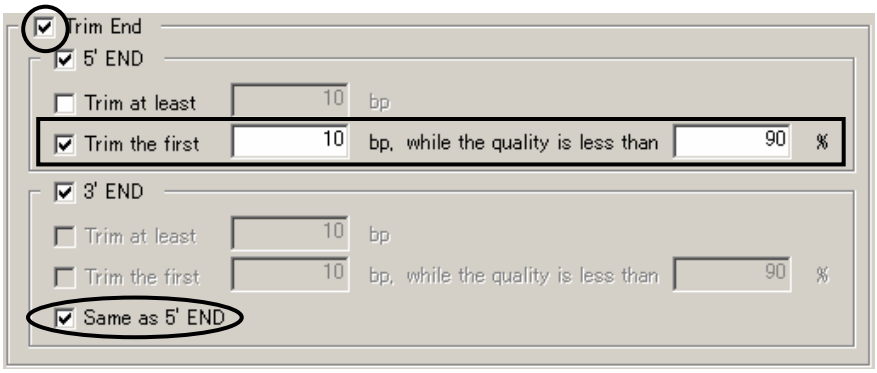
Output Parameters



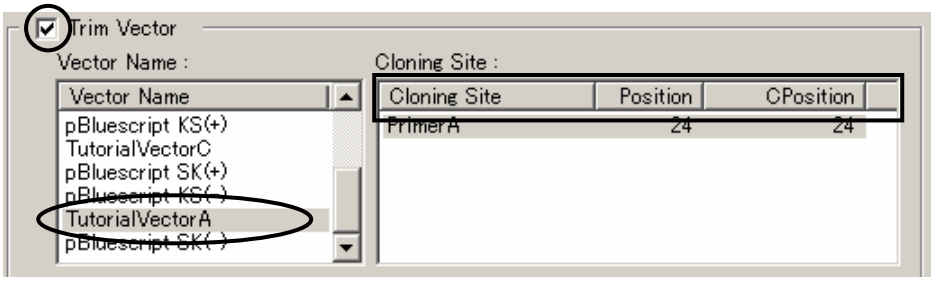
1.4.3 Trimming Setup

This tutorial uses the vector registered in section 1.2. If the relevant vector has not been registered, refer to 1.2.6 "Add Vector Data" to register the vector before continuing with this tutorial.

1. Double click ***** Double click this to set the vector. ***** to open the trimming settings dialog.
2. Select the Trim End check box at the top, and then set 5' End to "Trim the first 10bp, while the quality is less than 90%", and 3' End to Same as 5' End.



3. Select the Trim Vector check box, select TutorialVectorA from the Vector Name: list, and then select PrimerA from the Cloning Site: list.

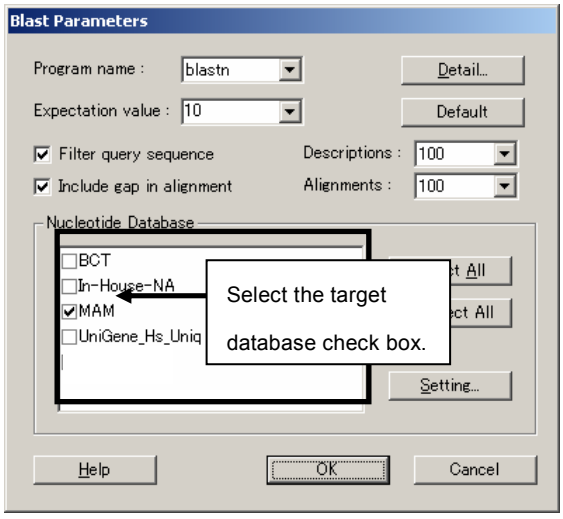


Trimming setup

4. Close the dialog with OK once the settings have been made.

1.4.4 Blast Search Setup

Double click ***** Double click this to set database ***** to open the parameter setup dialog.

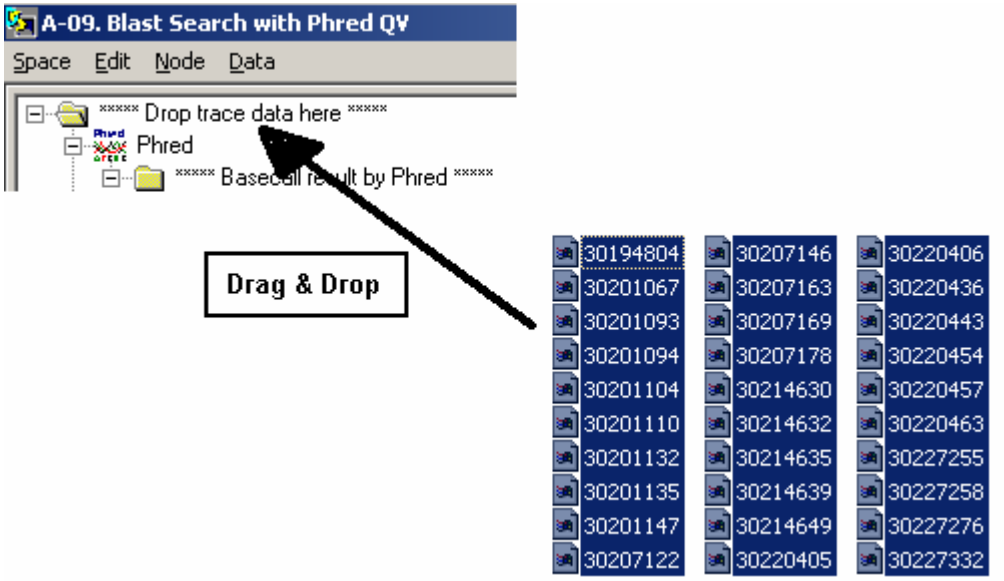


Blast Parameters dialog

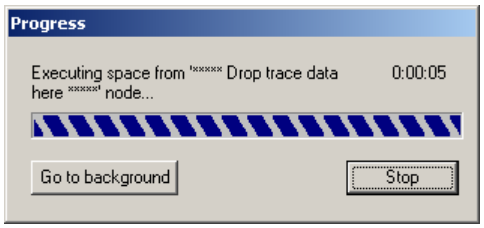
It is not necessary to modify the parameters for use with this tutorial. When performing actual analyses, set the target search database and other details in this dialog.

1.4.5 Enter Trace Data

1. Select Programs > DNASIS MAX > Contig Manager Tutorial Data from the Start menu, and then open the TutorialData folder.
2. Open the ContigManager > TutorialData1 > TraceData1_1 folder.
3. Select all of the files contained in the TraceData1_1 folder, and drag and drop them into the ***** Drop trace data here ***** folder in the Space dialog.



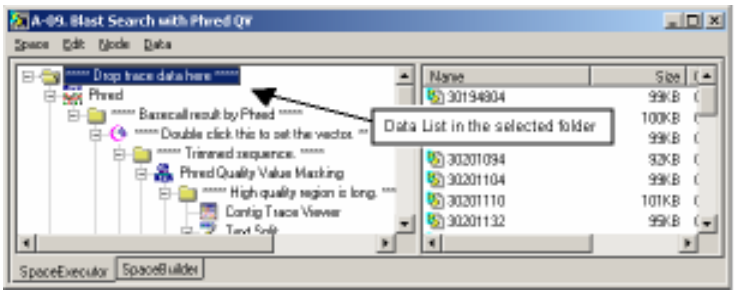
4. The progress dialog will appear, and then close when processing has completed.



Progress dialog

1.4.6 Results

1. Data will accumulate in the folders located in the Space window when the processing has completed. Double click ***** Drop trace data here ***** and confirm that the entered trace data has been saved.

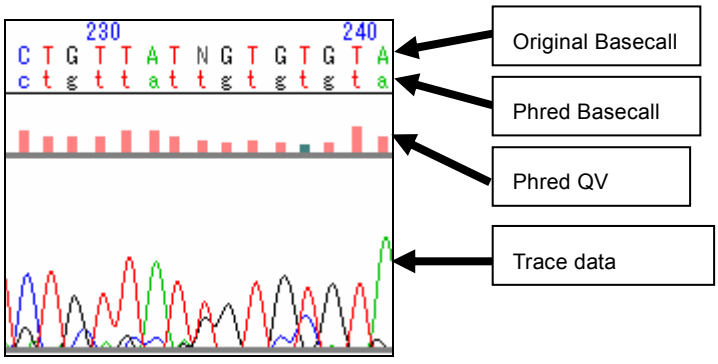


Data confirmation in the Space window

2. Double click on any item listed in the selected folder to display data details.

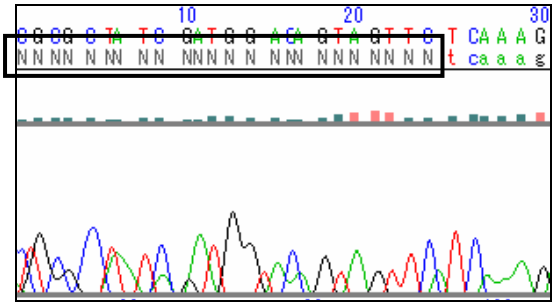
Trace data

3. The Phred basecall data is saved in the ***** Basecall result by Phred ***** folder. Double click this data to display the trace data. The original basecall, the Phred basecall, the Phred QV, and the trace data can be viewed.



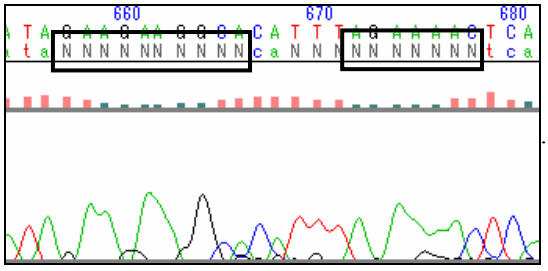
Results of Phred

4. The vector sequences deleted with trimming are output to the ***** Trimmed sequence. ***** folder. Of the 30 items of data input with this tutorial, 17 have been vector trimmed. This shows the vectors regions replaced with N.



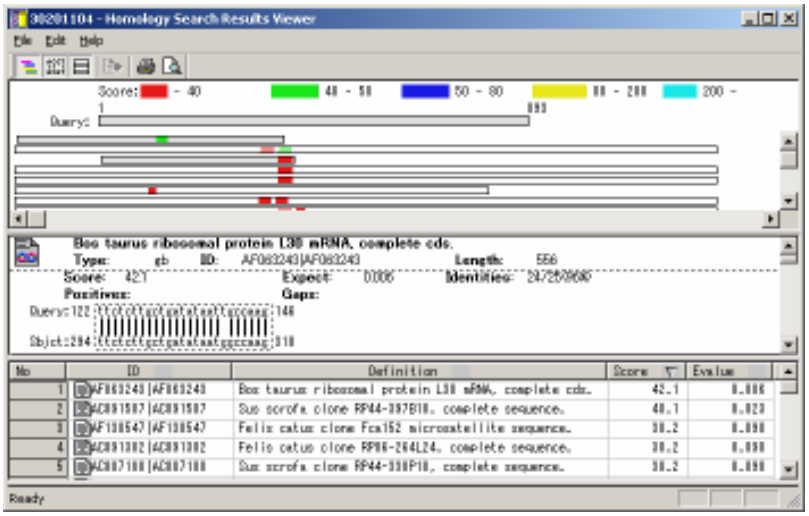
Trimming Results

5. The vector sequences that could not be deleted are output to the ***** No trimmed sequence ***** folder. Of the 30 items of data input with this tutorial, 13 have been separated as data that could not be vector trimmed.
6. The results of Phred QV masking and the data for which the high quality region bp count is longer than the threshold value (500bp with the tutorial) are output to the ***** High quality region is short. ***** folder. Of the 17 items of trimmed data input with this tutorial, 12 items of data are judged to have long high quality regions. Double click on the data to display the results and show the low quality regions masked by N.



Masking results with Phred QV

7. The data for which the high quality region bp count is shorter than the threshold value is output to the ***** High quality region is short. ***** folder. Of the 17 items of trimmed data input with this tutorial, 5 are judged to have short high quality regions.
8. The Blast search results that have vector sequences were deleted, high quality regions longer than 500bp and the low quality regions have been masked by N are output to the ***** Blast Search Result ***** folder. Double click on the data to display the search results graphically.



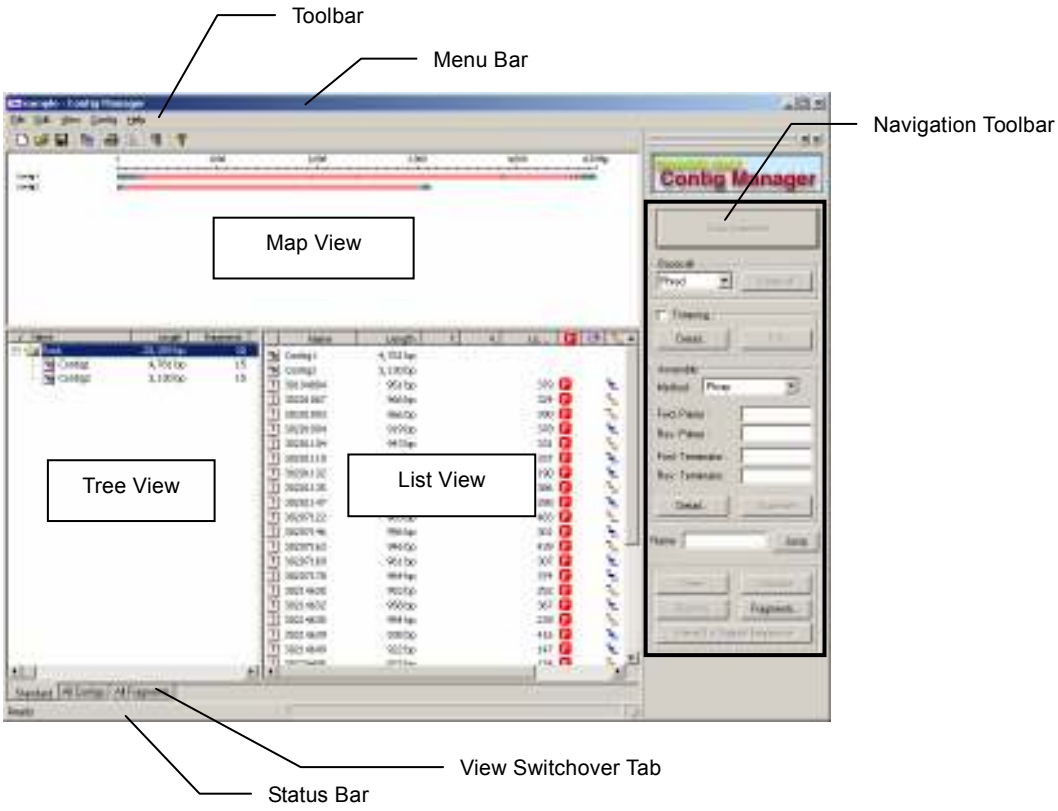
Blast search results

Chapter 2 Window Descriptions

2.1 Online Help

Online Help is available in Contig Manager. Select Help > Contents from the menu or click on the Toolbar. Also, by clicking Help of each dialog, Help for the dialog will appear.

2.2 Project Window



2.2.1 Menu

File menu	Description
New	Opens a new project in a new window. Displays Open Project dialog.
Open	Opens a specified project file. It is also possible to specify multiple files to open.
Revert	Returns the project to the pre-edit status. If the project was not saved, returns the project to the status when opened.
Save Project	Stores a project by overwriting it.
Print Setup...	Displays the Set Printing Information dialog and gives the setting of the paper size and printer information.
Print Preview	Displays a print image.
Print...	Carries out printing.
Import	Obtains a specified project or sequence.
Import Project…	Imports a project. Merges the data of other project with the currently opened project.
Import Sequence…	Reads the sequence from the specified file.
Export Sequence	Stores a sequence by giving it a name.
Export Original Sequence...	Stores a sequence by giving it a name before trimming or assembly.
Export Sequence...	Stores a specified sequence in the List View by giving it a name.
Exit	Terminates the Contig Manager.

Edit menu	Description
Copy	The active view decides the target of copy. When Map View is active: The shapes drawn by Map View are copied to Clipboard. When Tree View is active: The data is copied as text data to Clipboard, with all the data hierarchies of the Tree View open. The depth of each hierarchy is expressed with the number of tabs at the line head, so when pasted to applications such as Microsoft Excel, browsing becomes easy. When List View is active: The information highlighted on List View is copied to Clipboard as tab delimited text.
Delete	Deletes a selected folder, fragment, or contig.
Select All	When Map View is active, every contig displayed in Map View is highlighted. When List View is active, every fragment and contig displayed in List View is highlighted.
Deselect All	Every selection made in the active view is canceled.

View menu	Description
Map View	Views/hides the Map View.
Check Fragments Composition...	Displays the Check Fragments dialog. For details refer to 2.2.11 “Check Fragments dialog”.
Open Data...	Opens the highlighted data in the active view.
Summary...	Displays the Import Summary dialog. For details refer to 2.2.10 “Import Summary dialog”.
Navigation Tool bar	Views/hides the Navigation Toolbar.
Standard Toolbar	Views/hides the Standard Toolbar.
Status Bar	Views/hides the Status Bar.
Preferences...	Displays the Preferences dialog. For details refer to 2.2.8 “Preferences dialog”.

Contig menu	Description
New Folder	Creates a new folder. This feature is enabled when Tree View is active and a folder is selected. The new folder is created under the selected folder.
Assemble	Assembles what is selected in List View such as fragments.
Basecall	Performs basecall assembly of the trace data selected in List View from the Basecall setting on the Navigation Toolbar.
Trimming	Removes terminal regions with numerous N's and vector sequences from fragments selected in List View. Refer to 2.2.15 "Trimming Parameter dialog" for setting details.
Phrap Assemble	Assembles fragments selected in List View, utilizing Phrap. Refer to 2.2.13 "Phrap Parameter dialog" for setting details. To use Phrap, you need to install the Contig Manager.
DNASIS Assemble	Automatically searches and assembles the fragments selected in List View. Refer to 2.2.12 "DNASIS Assemble Parameter dialog" for setting details.
Auto Assemble	Based on the Navigation Toolbar settings, performs basecalling, trimming and assembly of fragments selected in List View.
Dissolve Contig	Removes the contig alignment selected in the currently active view.
Remove Selected Sequence	Removes contig fragments selected in List View. Reassembles the contig without the fragments selected for removal.
Copy Contig As Fragment	Copies the selected contig as a fragment. When reassembling the contig, use the copied fragment.
Relink Trace Files...	Resets the links to trace files. Refer to 2.2.16 "Relink Trace Files dialog" for setting details.
Parameters...	Displays the parameter setting window.
Trimming...	Sets the trimming parameters. Refer to 2.2.14 "Trimming Parameter dialog" for setting details.
Vector DB Manager...	Sets the Vector Database Manager. Refer to 2.2.15 "Vector Database Manager dialog" for setting details.
Phrap...	Sets the Phrap parameters. Refer to 2.2.13 "Phrap Parameter dialog" for setting details.
DNASIS Assemble	Set the parameters for DNASIS Assemble. Refer to 2.2.12 "DNASIS Assemble Parameter dialog" for further details.
Property...	Displays the folder, contig and fragment information selected in Tree View or List View. The names and comments can be edited.

Help menu	Description
Contents	Displays online help.
User Forum Web Page	Displays the User Forum website of DNASIS MAX. Requires connection to the Internet.
About Contig Manager...	Displays the version information.

2.2.2 Toolbar

Icon	Description
	Opens a new project in a new window. Displays Open Project dialog. The same as selecting File > New from the menu.
	Opens a specified project file. It is also possible to specify multiple files to open. The same as selecting File > Open… from the menu.
	Stores a project by overwriting it. The same as selecting File > Save Project from the menu.
	Not supported in the current version.

Icon	Description
	Copies data selected in Tree View or List View to the clipboard. The same as selecting Edit > Copy from the menu.
	Not supported in the current version.
	Carries out printing. The same as selecting File > Print from the menu.
	Displays a print image. The same as selecting File > Print Preview from the menu.
	Displays the Preferences dialog. For details refer to 2.2.8 “Preferences dialog”. The same as selecting View > Preference… from the menu.
	Displays online help. The same as selecting Help > Contents from the menu.

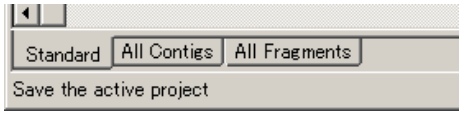
2.2.3 Navigation Toolbar

Item	Description
Auto Assemble	Based on the Navigation Toolbar Settings, performs basecalling, trimming and assembly.
Basecall	Sets trace basecall parameters.
Use Phred	Selects the parameter to use for basecalling. Original: Basecalling using the sequence information stored in the trace file. Phred: Basecalling using Phred. To use Phred, you need to install the Contig Manager.
Basecall	Perform a basecall using the trace selected in List View.
Trimming	Performs trimming if Auto Assemble is checked.
Detail...	Displays the Trimming Parameter dialog. Refer to 2.2.14 “Trimming Parameter dialog” for setting details.
Trim	Trims the fragments selected in List View based on the settings in Detail....
Assemble	Sets the assembly parameters.
Method	Selects the algorithm to use for assembling. DNASIS Assemble: Aligns using DNASIS Assemble. Phrap:

Item	Description
	Aligns using Phrap. When Phrap is selected, the following parameters will be displayed. To use Phred, you need to install the Contig Manager.
Fwd. Primer	Trace data is forward read using the dye primer method and when distinct name patterns are identified for a fragment they are input. Forward linking is given priority if the direction cannot be determined.
Rev. Primer	Trace data is reverse read using the dye primer method and when distinct name patterns are identified for a fragment they are input. Reverse linking is given priority if the direction cannot be determined.
Fwd. Terminator	Trace data is forward read using the terminator method and when distinct name patterns are identified for a fragment they are input. Forward linking is given priority if the direction cannot be determined.
Rev. Terminator	Trace data is reverse read using the terminator method and when distinct name patterns are identified for a fragment they are input. Reverse linking is given priority if the direction cannot be determined.
Detail...	When selecting DNASIS Assemble in Method: Displays the DNASIS Assemble Parameter dialog. Refer to 2.2.12 “DNASIS Assemble Parameter dialog” for details. When selecting Phrap in Method: Displays the Phrap Parameter dialog. Refer to 2.2.13 “Phrap Parameter dialog” for details.
Assemble	Assembles the fragments selected in List View based on the settings.
Jump	Searches for fragments. Enter the name of the fragment you want to search in the box and click Jump. Fragments with identical names will be selected when List View is active.
Open	Opens the selected fragment or contig in an active view.
Dissolve	Removes the contig alignment selected in the currently active view.
Remove	Removes contig fragments selected in List View. Reassembles the contig without the fragments selected for removal.
Fragments…	Displays Check Fragment dialog. Refer to 2.2.11 “Check Fragment dialog” for setting details.
Reset To Original Sequence	Returns the selected fragments to the sequence at the time of input. When the fragments are linked, the links are removed.

2.2.4 Status Bar

The Status Bar displays the names of each menu button of menu and Toolbar at the mouse cursor, and each application on the application palette.



2.2.5 Map View

Map View graphically displays the project contigs with quality values. Refer to 3.11.1 “Map View” for the details.

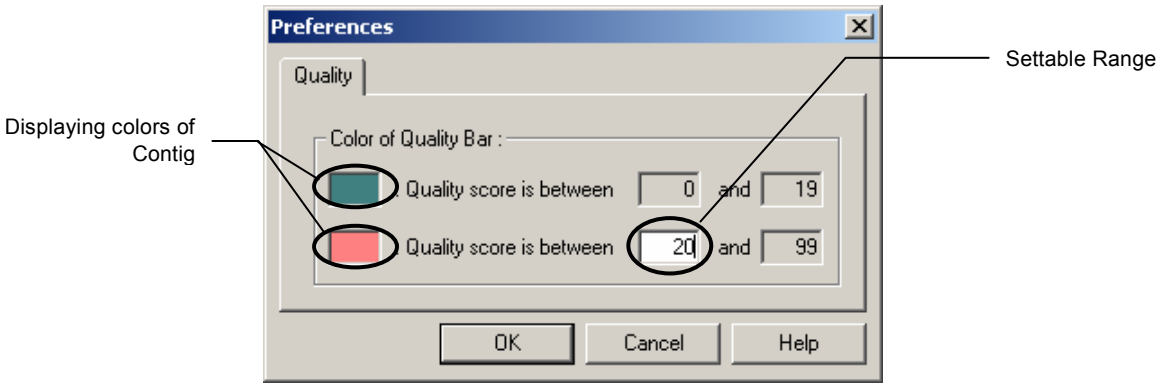
2.2.6 Tree View

Tree View displays the project structure, with folders, contigs, and fragments that compose it. Refer to 3.11.2 “Tree View” for the details.

2.2.7 List View

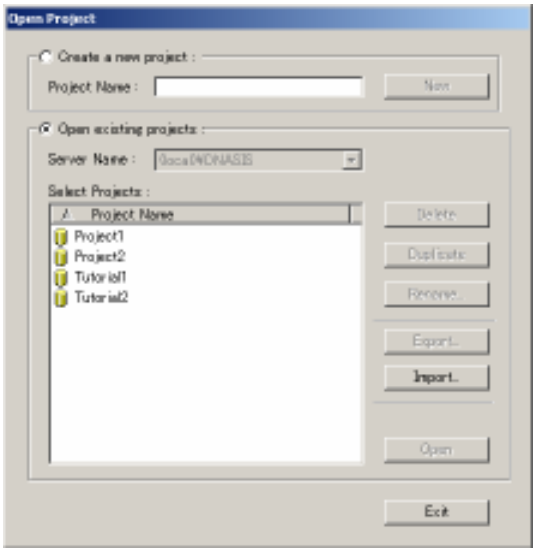
List View displays the contents of the folder selected in Tree View. Refer to 3.11.3 “List View” for the details.

2.2.8 Preferences dialog



Item	Description
Quality score is between X and Y	Sets the boundaries when coloring the contigs in Map View based quality values. Boundary values can range is from 1 to 99.
Displaying colors of Contig	Sets the display color when quality value is within the set range. When double clicking the display color with the mouse the color setting dialog will display.

2.2.9 Open Project dialog

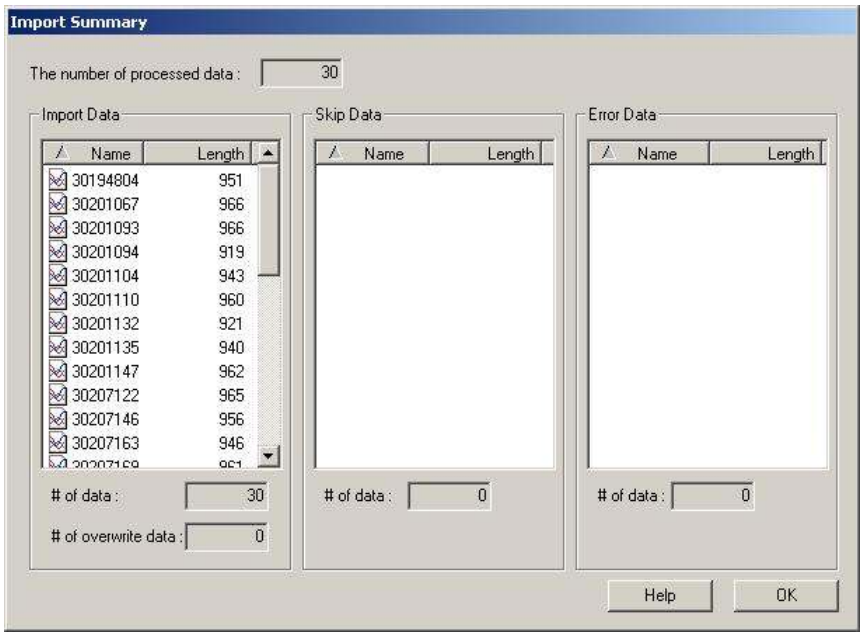


Item	Description
Create a new project	Creates a new project.
Project Name	Enter a project name. Up to 120 characters can be used. The following characters cannot be used in the project name: ( ) * = | ! [ ] @ { } ; ? \ , /
New	Creates a new project with the name input in the Project Name textbox.
Open existing project	Opens an existing project.
Server Name	Specifies the server to store data. In the current version, the server cannot be changed.
Select Projects	Displays a list of existing projects.

Item	Description
Delete	Deletes the project selected in the Select Projects list.
Duplicate	Copies the project selected in the Select Projects list. After pushing the button you will be prompted to enter a new name, which is required.
Rename	Renames the project selected in the Select Projects list.
Export…	Exports the project selected in the Select Projects list. The whole project can be stored in a file.
Import…	Imports the project exported in Export…. Project names can be specified for import.
Open	Opens the project selected in the Select Projects list.
Exit	Closes the Open Project dialog.

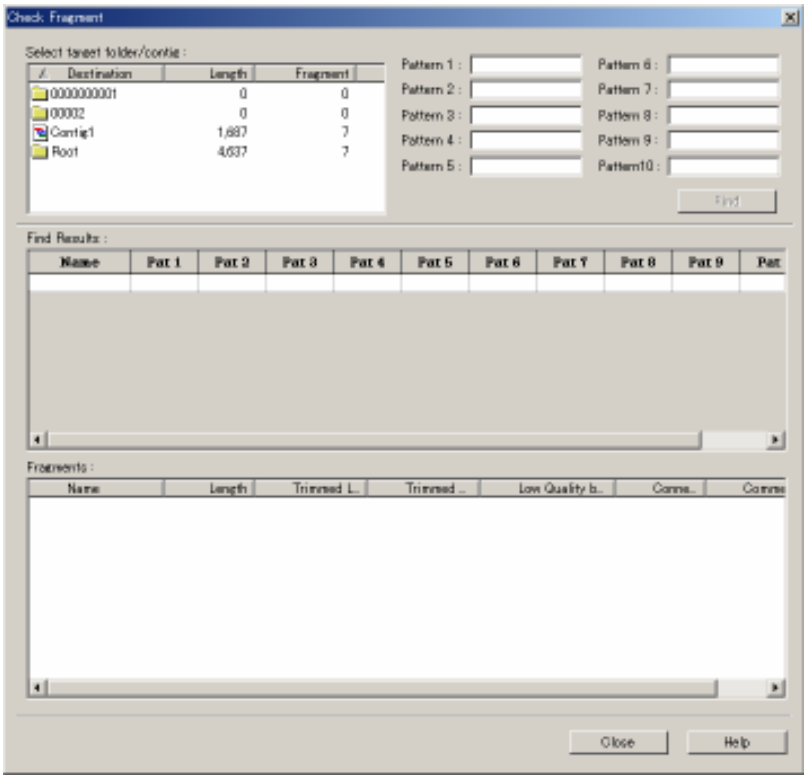
2.2.10 Import Summary dialog

When the imported data is not an ACE file, the dialog displays the contents of the imported data.



Item	Description
The number of processed data	Displays the number of data processed for import.
Import Data	Displays a list of the imported data. Also displays the names and sequence length. The data can be sorted by clicking each column header.
Skip Data	Specifies files to skip when trying to import data with the same name as data already present. The data can be sorted by clicking each column header.
Error Data	Displays data failed to import. The data can be sorted by clicking each column header.
# of data	Displays the number of imported data.
# of overwrite data	Displays the number of data overwritten on importing.
OK	Closes the dialog.

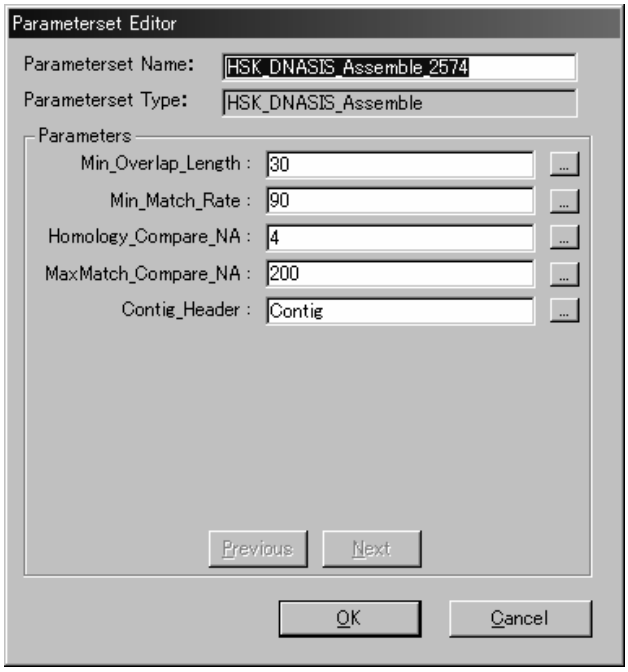
2.2.11 Check Fragment dialog



Item	Description
Select target folder/contig	Selects the search target folder or contig.
Pattern	Input the fragment name for search. Up to ten fragments can be searched. * and ? are available as wild cards. *: any string ?: any one character
Find Results	Lists the number of fragments found with the search string.
Fragments	Displays a list of fragments found with the search string.
Name	Displays the name of the fragment.
Length	Displays the length of the fragment.
Trimmed Length	Displays the length of the region masked by trimming.
Trimmed Vector	If masked by a vector sequence in trimming, displays the name of the vector.
Low Quality bps	For fragments resulting from Phred basecalling of a trace, the total bp number in the region where the Phred QV value of the fragment is low will display.
Connected	Displays the process done to the corresponding fragment. Phred: Phred basecalled fragments Trimmed: Trimmed fragments Assemble(N): Fragments linked with a Normal strand Assemble(C): Fragments linked with a complementary strand
Comment	Displays the comment of the corresponding fragment.

2.2.12 DNASIS Assemble Parameter dialog

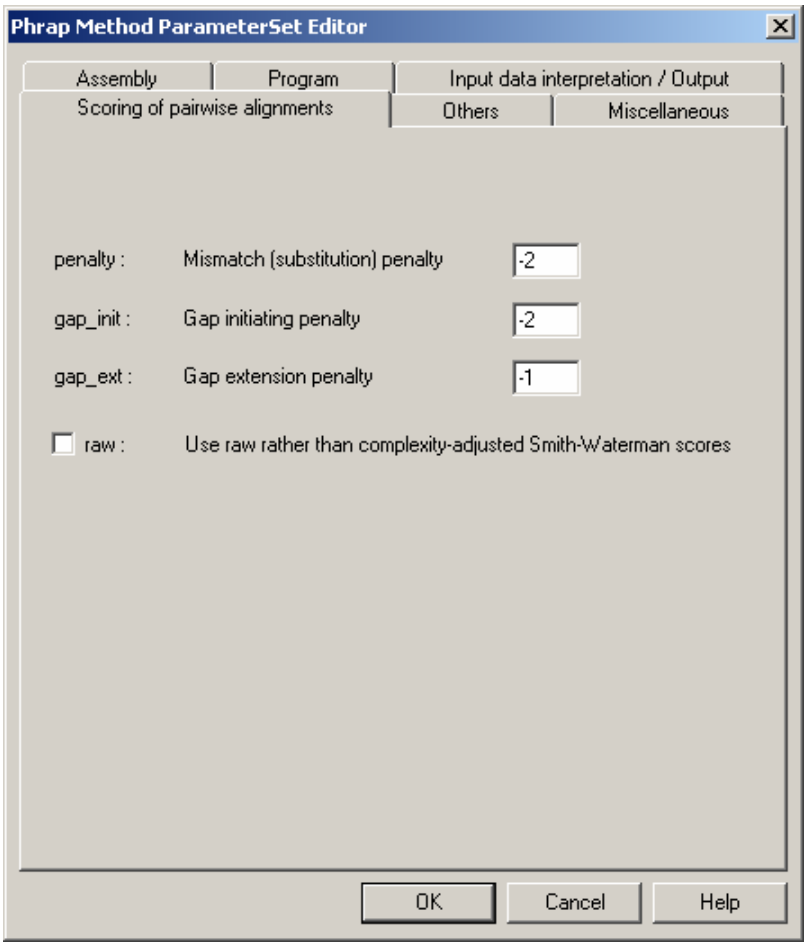
Parameterset Editor



Item	Description (Initial setting)
Min_Overlap_Length	Sets the minimum overlap of the fragments necessary to determine whether the pieces of the fragments can be linked. The setting can range from 1 to 100, and it cannot be smaller than the value set in Homology_Compare_NA. (30)
Min_Match_Rate	Sets the minimum percentage of the matching fragments to determine whether the pieces of the fragments can be linked. The setting can range from 1 to 100. (90)
Homology_Compare_NA	Sets the number of bases (bp) to compare when searching for regions of fragment homology. The starting and ending points for searches will be perfectly identical points, more than the number of bases set here. The setting can range from 1 to 6. (4)
MaxMatch_Compare_NA	Sets the number of bases (bp) to compare in the maximum matching of fragments. When the part to compare is greater than the number of bases set here, the ends of the part to compare will be repeatedly compared by half the value set here. The setting can range from 200 to 500. (200)
Contig_Header	To embed MIME information, sets the header name for contig information (the actual name of a contig will be “the value set here + the order of creation”). Value up to 64 letter characters, both capitals and lower cases, and the underbar. (_)

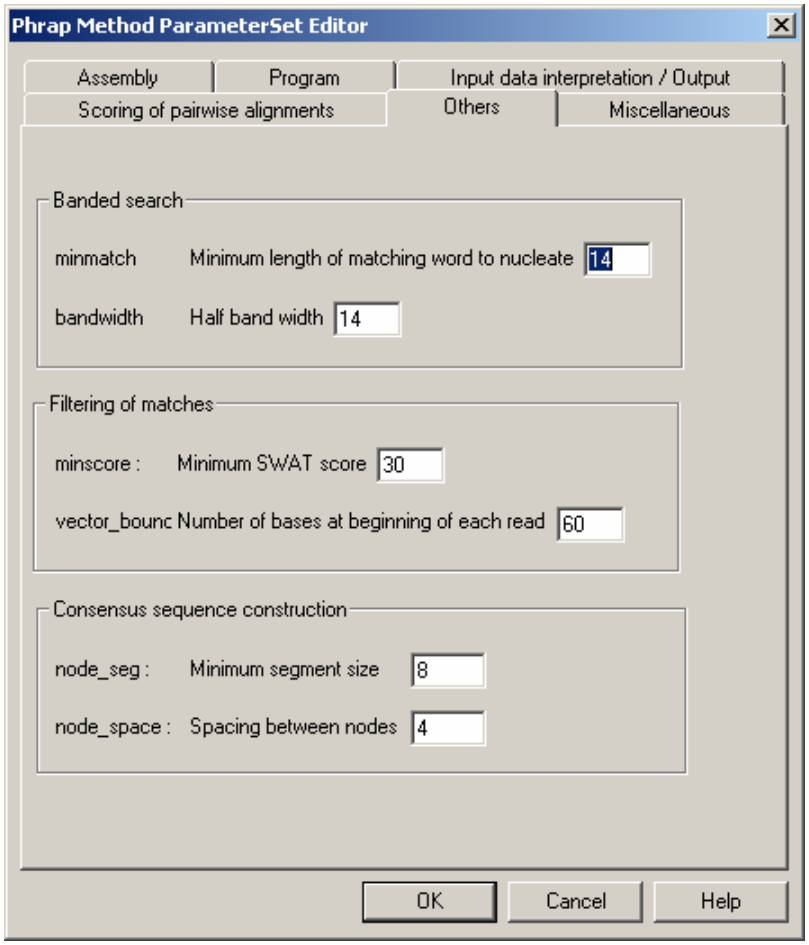
2.2.13 Phrap Parameter dialog

Scoring of pairwise alignments



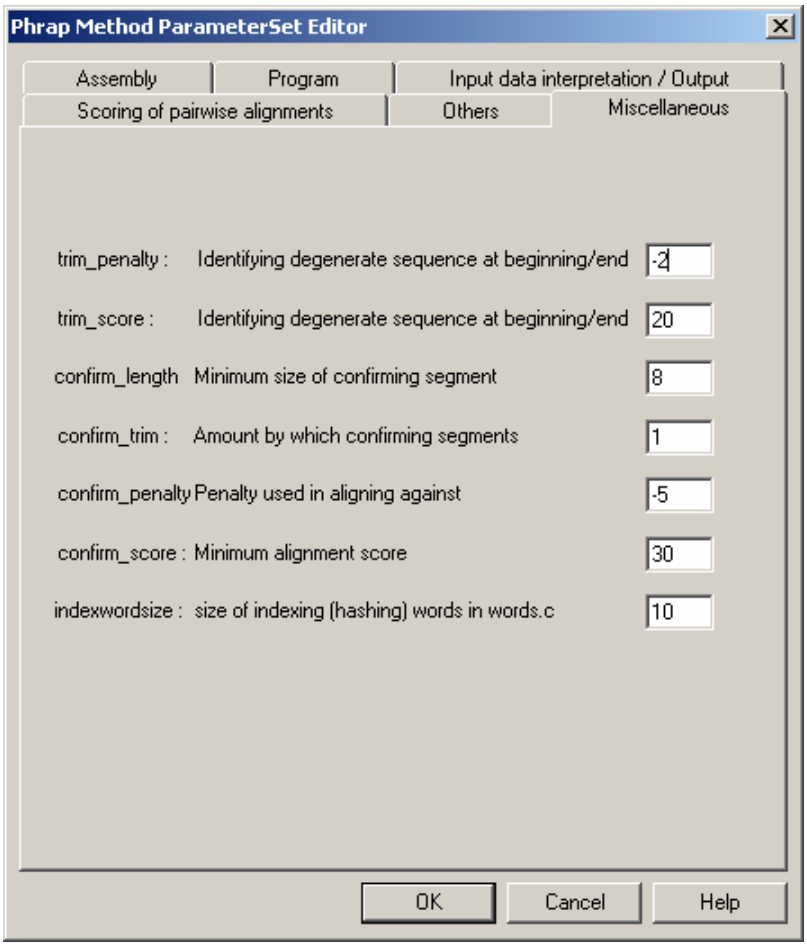
Item	Description (Initial setting)
penalty	Sets the mismatch penalty for SWAT comparisons. (-2)
gap_init	Sets the gap initiating penalty for SWAT comparisons. (-2)
gap_ext	Sets the gap extension penalty for SWAT comparisons. (-1)
raw	Uses raw rather than the existing Smith-Waterman scores. (□)

Others



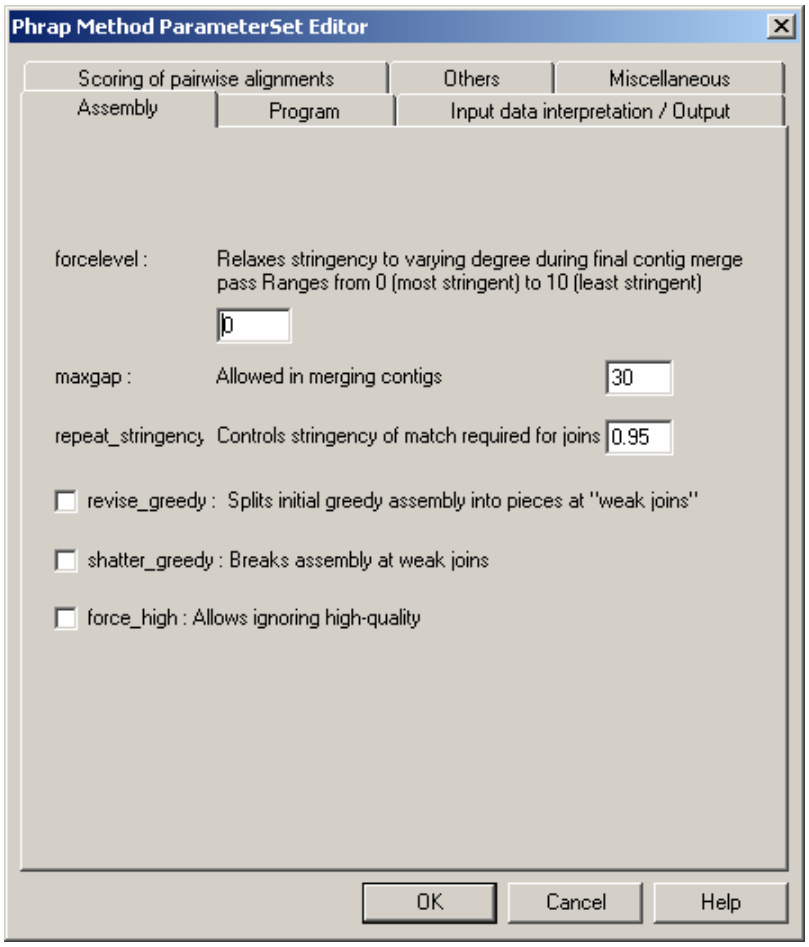
Item	Description (Initial setting)
minmatch	Sets the minimum length of matching word to nucleate SWAT. If the value is set to 0, the matching process will terminate. (14)
bandwidth	Sets the region value at half band width for banded SWAT search. (Full width is 2n+1.) (14)
minscore	Sets the minimum SWAT score. (30)
vector_bound	Sets the number of bases at the beginning of each read, matches within which are assumed to be a vector. (60)
node_seg	Sets the minimum segment size. (8)
node_space	Sets the spacing width between nodes. (4)

Miscellaneous



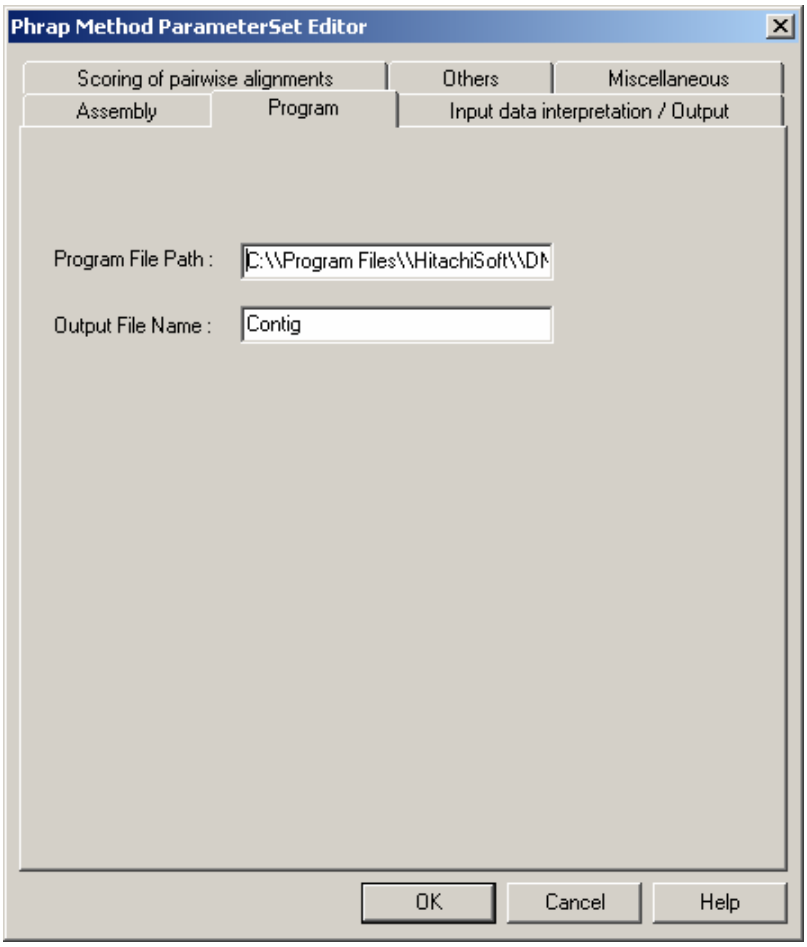
Item	Description (Initial setting)
trim_penalty	Sets the penalty for identifying a degenerate sequence at beginning/end of a read. (-2)
trim_score	Sets the minimum score for identifying a degenerate sequence at beginning/end of a read. (20)
confirm_length	Sets the minimum size of confirming segments. (8)
confirm_trim	Sets the amount by which confirming segments are trimmed at the edges. (1)
confirm_penalty	Sets the penalty used in aligning against “confirming” reads. (-5)
confirm_score	Sets the minimum alignment score for a read to be allowed to “confirm” part of another read. (30)
indexwordsize	Sets the size of an indexing (hashing) word in words.c. This parameter has a small effect on run time and memory usage. (10)

Assembly



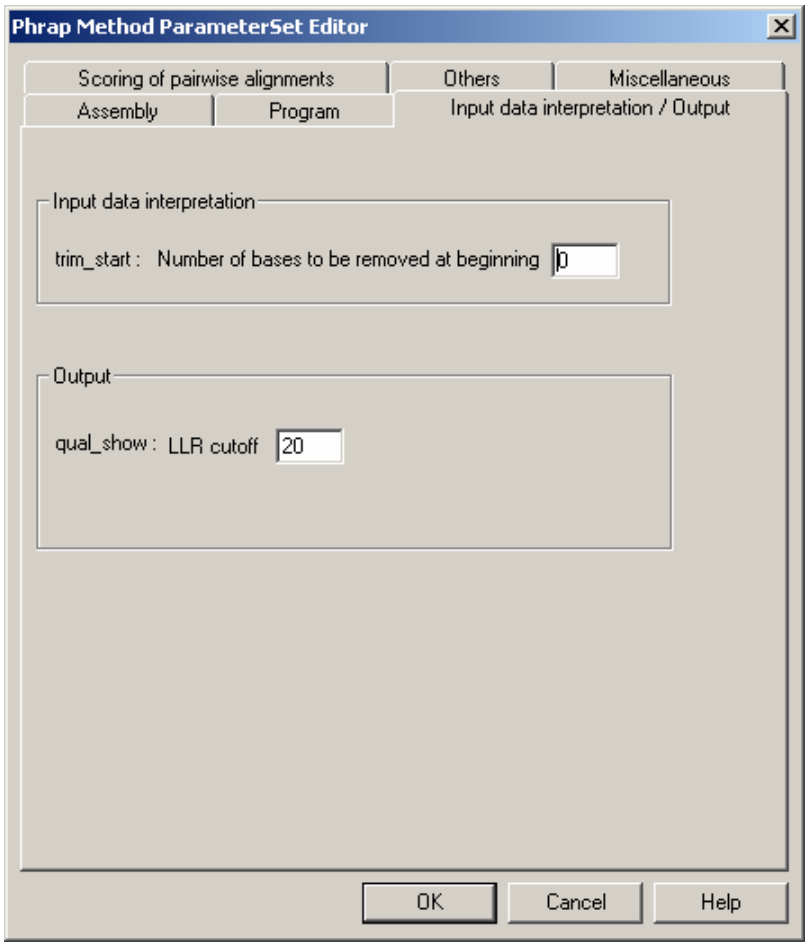
Item	Description (Initial setting)
forcelevel	Sets the stringency during final contig merge pass. Values can range from 0 (most stringent) to 10 (least stringent). (0)
maxgap	Sets the maximum permitted size of an unmatched region in merging contigs, during first (most stringent) merging pass. (30)
repeat_stringency	Sets the stringency of match required for joins. (0.95)
revise_greedy	Splits initial greedy assembly into pieces at “weak joins”, and then tries to reattach them to give a higher overall score. (□)
shatter_greedy	Breaks the assembly at weak joins, as with revise_greedy, but does not try to reattach pieces. (□)
force_high	Causes edited high-quality discrepancies to be ignored during final contig merge pass. (□)

Program



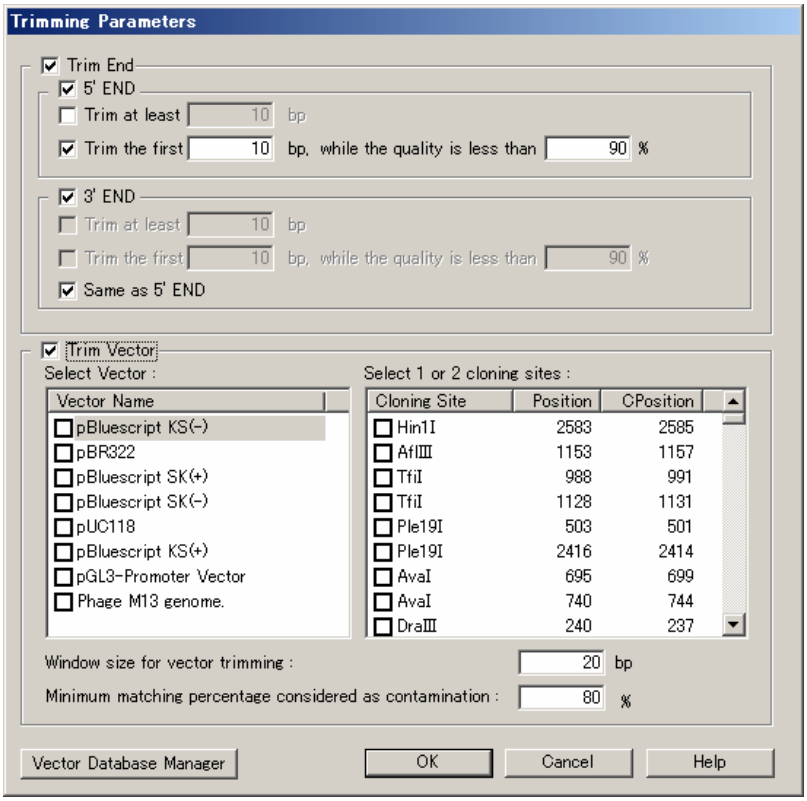
Item	Description (Initial setting)
Program File Path	Sets the drive path for storing phrap.exe. Automatically set at installation, and usually it is not necessary to change.
Output File Name	Sets the name of output files in MIME types files. Set without using an extension. (Contig)

Input data interpretation / Output



Item	Description (Initial setting)
trim_start	Sets the number of bases to be removed at beginning of each read. (0)
qual_show	Sets the LLR score. The LLR score is a measure of overlap length and quality. High quality discrepancies that might indicate different copies of a repeat lead to low LLR scores. (20)

2.2.14 Trimming Parameters dialog

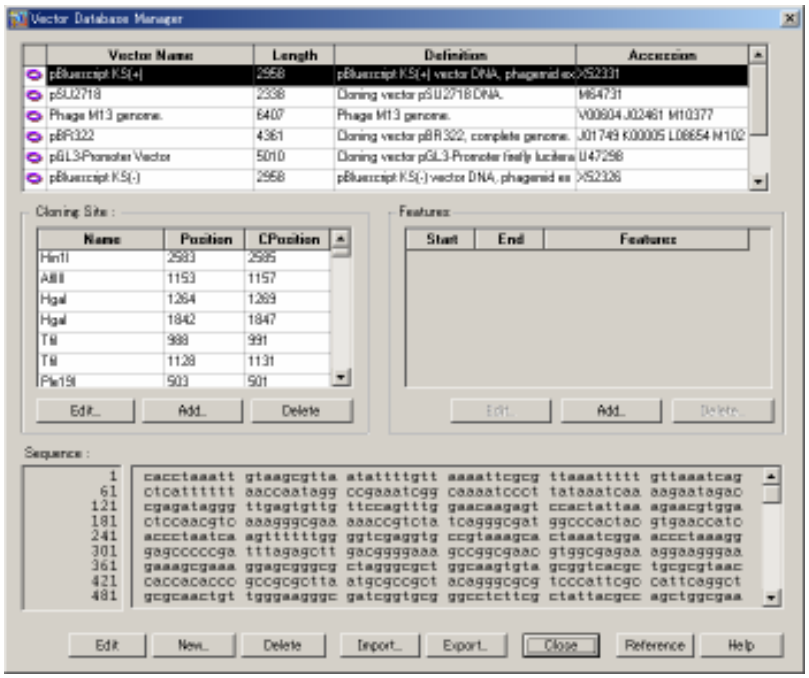


Item	Description
Trim End	Sets whether to trim off any base pairs at the ends of reads.
5'End	When checked, trims from the 5' end. When both "Trim at least" and "Trim the first" are checked, first run "Trim at least" followed by "Trim the first".
Trim at least	Unconditionally trims the length of sequence specified from the 5' end. Trims when checked. Sequence lengths must be integers greater than 0.
Trim the first	When checked, trims where quality is low from the 5' end. When determining the quality, set integers greater than 0 for window lengths and quality thresholds. Below is the trimming procedure: 1. Calculate the quality of the sequence length (called a window) set from the 5' end. 2. When the quality is lower than the threshold, move the window toward the 3' end for one base, and repeat 1. 3. When the above produces a quality higher than the threshold, trimming will be done from the 5' end to N closest to the 3' end in the current window.
3'End	When checked, trims the 3' end. When both "Trim at least" and "Trim the first" are checked, first run "Trim at least", followed by "Trim the first".
Trim at least	Unconditionally trims the length of sequence specified from the 3' end. Trims when checked. For sequence lengths must be integers greater than 0.
Trim the first	When checked, trims where quality is low from the 3' end. When determining the quality, set integers greater than 0 for window lengths and quality thresholds. Below is the trimming procedure: 1. Calculate the quality of the sequence length (called a window) set from the 3' end. 2. When the quality is lower than the threshold, move the window toward the 5' end for one base, and repeat 1. 3. When the above produces a quality higher than the threshold, trimming will be done from the 3' end to N closest to the 5' end in the current window.

Item	Description
Same as 5'End	Sets whether to make the trimming conditions from the 3' end the same as those from the 5' end. Check to make them the same.
Trim Vector	Sets whether to remove a vector sequence. Check when trimming. Up to six vectors can be checked simultaneously.
Vector Name	Select the vectors to be removed from a list. Trimming can be done with up to six vectors simultaneously.
Cloning Site	Sets a cloning site from a list for each vector to be trimmed. Up to two cloning sites can be selected for each vector. Check the cloning sites to use for trimming.
Window size for vector trimming	Sets the base length (window size) to extract from the vector cloning site sequence to use for determining the portion of vector sequence. It is also used to correct the matching percentage. Sets 15 or more integers.
Minimum matching percentage considered as contamination	Sets the minimum matching percentage between the alignment of a vector cloning site and the alignment to be trimmed. When the matching percentage is larger than the set value, it is considered to be a vector alignment.
Vector Database Manager	Displays the Vector Database Manager. Refer to 2.2.15 "Vector Database Manager dialog" for the details of the Vector Database Manager.
OK	Closes the Dialog, enabling what is set.
Cancel	Closes the Dialog, disabling all the changes.
Help	Displays online help.

2.2.15 Vector Database Manager dialog

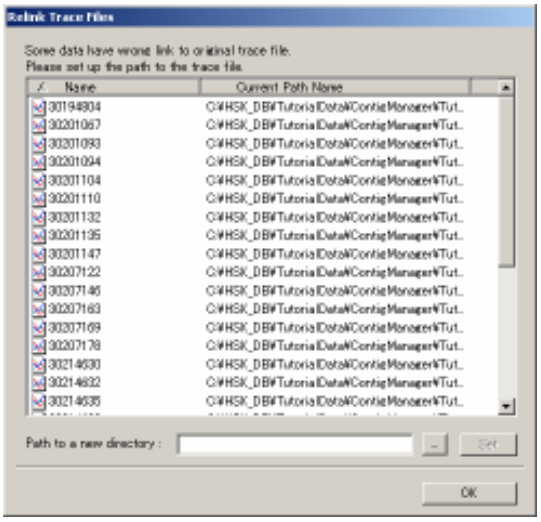
The Vector Database Manager dialog displays a list of vectors registered in the database. It also creates, modifies, and deletes vectors.



Item	Description
Vector Database Manager	Displays a list of registered vectors.
Vector Name	Displays vector names.
Length	Displays vector lengths.
Definition	Displays vector definitions.

Item	Description
Accession	Displays accession numbers of vectors.
Cloning Site	Edits vector cloning sites.
Cloning Site	Displays a list of cloning sites.
Name	Displays cloning site names.
Position	Displays the cloning site positions of Normal strands.
CPosition	Displays the cloning site positions of complementary strands.
Edit…	Displays the update window to update the cloning site selected in the cloning site list.
Add…	Displays the registration window to add a new cloning site.
Delete	Deletes the cloning sites selected in the list.
Features	Sets the vector features.
Features	Displays a list of features.
Start	Displays the starting position of the corresponding feature.
End	Displays the ending position of the corresponding feature.
Features	Displays the features.
Edit…	Displays the update window to update the features selected in the list.
Add	Displays the registration window to add a new feature.
Delete	Deletes the features selected in the list.
Edit	Changes vector contents selected in the vector list.
New ...	Adds a new vector.
Delete	Deletes the vectors selected in the vector list.
Import...	Imports vectors by specifying vector data files.
Export...	Outputs the vector information selected in the vector list into a vector data file, so that the file can be exported to DNASIS on other PCs.
Close	Exits the Vector Database Manager.
Reference	Displays the reference information of the vectors selected in the vector list.
Help	Displays online help.

2.2.16 Relink Trace Files dialog



Item	Description
Relink Trace Files	Displays a list of fragments whose links have been disconnected.
Name	Displays the data names. The data can be sorted by clicking column headers.
Current Path Name	Displays the path to the currently linked trace file.
Path to a new directory:	When setting where to link, designates the path to the directory where trace files are stored.
…	Click this button to a path to a new directory.
Set	When selecting data from the list to link to a new directory and then pushing this button, if data with the same name as the selected data is already present, a new link is created to the file.

2.3 Contig Window

2.3.1 Menu

File menu	Description
Export...	Stores sequences in the selected fragment range in Fasta format. When multiple fragments are selected and stored, they are stored in multi-Fasta format.
Export Contig…	Stores a contig sequence in a file.
Export Contig to DNASIS MAX	Outputs the currently displayed contig sequence into DNASIS MAX. The data will be displayed in Sequence View of DNASIS MAX. This is the same as on the Toolbar.
Revert...	Refreshes the Viewer by rereading the stored data. When the data has not been saved, the data at the creation of the contig will be displayed.
Save	Overwrites the sequence currently displayed in Sequence View to the original file. The quality value in editing the sequence can be set with the Quality Tab in the dialog, which is displayed when selecting View > Preferences ... from the menu.
Print...	Prints the currently active view. Only the displayed part of the page in each view will be printed. Refer to 10.1.2 “Print from the Contig Viewer” for printing.
Print Preview	Displays the print image of the currently active view. Print preview shows the area displayed in each view.

File menu	Description
Print Setup	Displays the standard print setup dialog for setting the paper size, printing direction.
Exit	Closes the Contig Viewer.

Edit menu	Description
Cut	Not supported in the current version.
Copy	When clicking the contig sequence, copies the names of fragments and bases to the clipboard as tab delimited text for all aligned fragments.
Copy Image	Copies the contents of the active view to the clipboard in meta-file format.
Paste	Not supported in the current version.
Select All	Selects all the bases in all fragment sequences.
Deselect All	Cancels the selection of all the bases in all fragment sequences.
Find…	Displays the Search dialog and searches designated sequence in the contig. Valid when a contig sequence is selected in Sequence View.
Jump to Next Marker	Jumps to the mark to the right of the cursor in the Sequence View.
Jump to Previous Marker	Jumps to the mark to the left of the cursor in the Sequence View.

View menu	Description
Toolbar	Displays/hides the Toolbar.
Status Bar	Displays/hides the Status Bar.
Display Type	Sets the display style in Sequence View.
Sequence	Displays only the sequence. The same as clicking  on the Toolbar.
Quality Map	Displays a diagram of the sequence and quality value. The same as clicking  on the Toolbar.
Quality Numeric	Displays the sequence and quality value. The same as clicking  on the Toolbar.
Chromatograms	Displays the trace data of the sequence selected in Sequence View. The same as clicking  on the Toolbar.
Quality Lists	Displays the list of the consensus and the high quality region of a fragment in a dialog. The same as clicking  on the Toolbar. The criteria for determining the high quality region can be set with the Quality Tab in the dialog, which is displayed when selecting View > Preferences ... from the menu. Additionally, selecting a high quality region from the list in the dialog and clicking OK will underline the corresponding sequence and region in Sequence View.
Auto Scroll	Sets whether to automatically compensate the display position of the Sequence View.
Preferences...	Displays the parameter setting window. The same as clicking  on the Toolbar.

Help menu	Description
Contents	Displays the Help for Contig Manager Contig View.
About Contig Viewer...	Displays the contig version information.

2.3.2 Toolbar

Button	Description
	Prints the contents of the currently active view. What is displayed will be printed. The function is the same as selecting File > Print from the menu.
	Searches sequences. The function is the same as selecting Edit > Find… from the menu.
	Outputs the currently open contig to DNASIS MAX. The data is displayed in the Sequence View of DNASIS MAX. The function is the same as selecting File > Export Contig to DNASIS from the menu.
	Expands the Map View.
	Shrinks the Map View.
	Displays the Map View at 100% size.
	Jumps to the mark to the right of the cursor in the Sequence View.
	Jumps to the mark to the left of the cursor in the Sequence View.
	Displays the Sequence View in sequence. The function is the same as selecting View > Display Type > Sequence from the menu.
	Displays a sequence diagram and quality value in Sequence View. The function is the same as selecting View > Display Type > Quality Map from the menu.
	Displays a sequence and quality value in Sequence View. The function is the same as selecting View > Display Type > Quality Numeric from the menu.
	Displays sequence chromatograms selected in Sequence View. If the selected sequence does not have trace information, Chromatograms will not display. Same as selecting View > Chromatograms from the menu.
	Displays the list of the selected consensus, and the quality information of the sequence used to link the consensus. Same as selecting View > Quality Lists from the menu.
	Displays the Parameter Editor. Same as selecting View > Preferences from the menu.
	Displays online help. Same as selecting Help > Contents from the menu.

2.3.3 Status Bar

The Status Bar displays a description of each button on the menu and Toolbar at the mouse cursor.

Displays the location of the mouse cursor on the contig in Sequence View.

2.3.4 Map View

The Map View graphically displays contigs and the fragments composing the contigs.
Refer to 8.2 “Contig Map View” for the details.

2.3.5 Sequence View

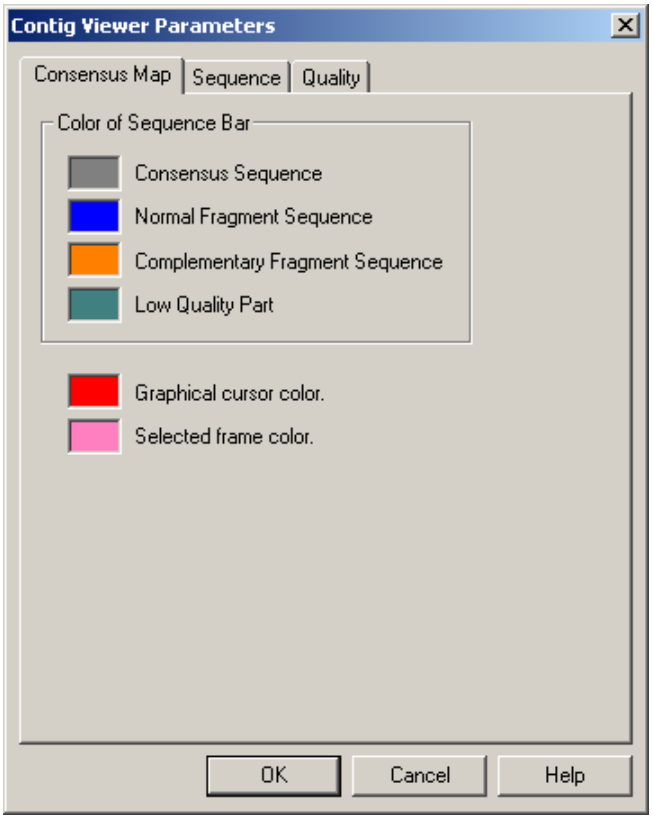
Sequence View displays contigs and fragment sequences.

Displays the sequences using the color parameters set for Base A, Base G, Base C, Base T, and others.

Refer to 8.3 “Sequence View” for the details.

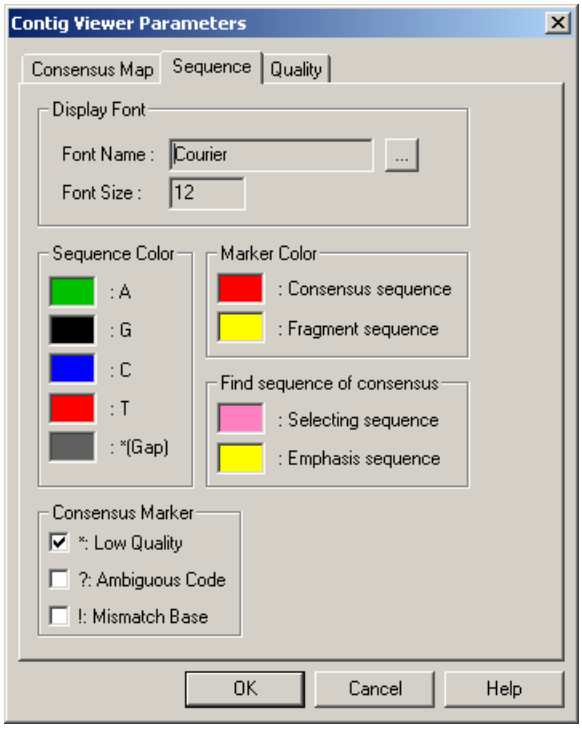
2.3.6 Contig Viewer Parameters

Consensus Map



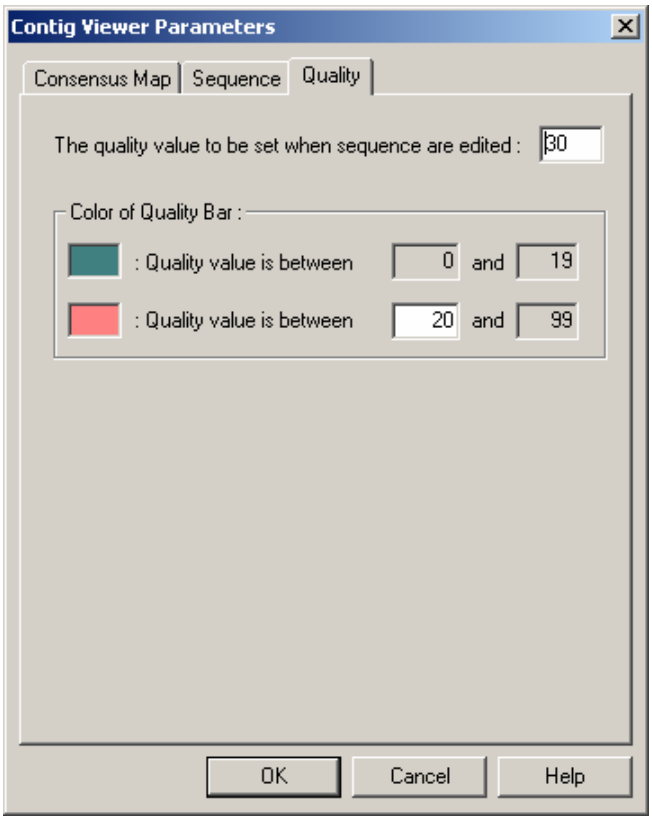
Item	Description
Color of Sequence Bar	Sets the display color of the contigs and fragments in Map View. When double clicking on the displayed colors with the mouse cursor, a color setting dialog will display.
Consensus Sequence	Sets the display color of contigs.
Normal Fragment Sequence	Sets the display color of fragments linked with the strand as the input data.
Complementary Fragment Sequence	Sets the display color of fragments linked with complementary strands of the input data.
Low Quality Part	Sets the display color of the lower quality parts than the set value.
Graphical cursor color.	Sets the color of the graphical cursor.
Selected frame color.	Sets the color of the frame.

Sequence



Item	Description
Display Font	Sets the display font of sequences.
Font Name	Sets the font type. Click "…", and set the display font in the font setting dialog.
Font Size	Sets the size of font. Click "…", and set the display font size in the font setting dialog.
Sequence Color	Sets the sequence color. Double clicking each color with mouse will display the color setting dialog.
A	Sets the color of Base A.
G	Sets the color of Base G.
C	Sets the color of Base C.
T	Sets the color of Base T.
*(Gap)	Sets the color of elements other than Bases □, G, C, T.
Marker Color	Sets the color of the markers.
Consensus Sequence	Sets the color of the contig sequence markers. When set to display Low Quality, Ambiguous Code, and Mismatch Base, the respective marker will be the color specified here.
Fragment Sequence	Sets the marker color of the fragment sequences. The color will be the background color of the fragments bases, which do not match the contig.
Find sequence of consensus	Sets the background color of the region where searching strings are found in the alignment search.
Selecting sequence	Sets the background color of the last searched region.
Emphasis sequence	Sets the previously searched region when performing consecutive searches.
Consensus Marker	Displays a marker on a consensus sequence. When a check box is selected that item will display with a marker.
*:Low Quality	Attaches an asterisk (*) to low quality sequences. The quality threshold can be changed with the parameters in the Quality page.
?:Ambiguous Code	Attaches a question mark (?) to a contig sequence when the code is ambiguous.
!:Mismatch Base	Attaches an exclamation mark (!) to mismatched bases in fragments.

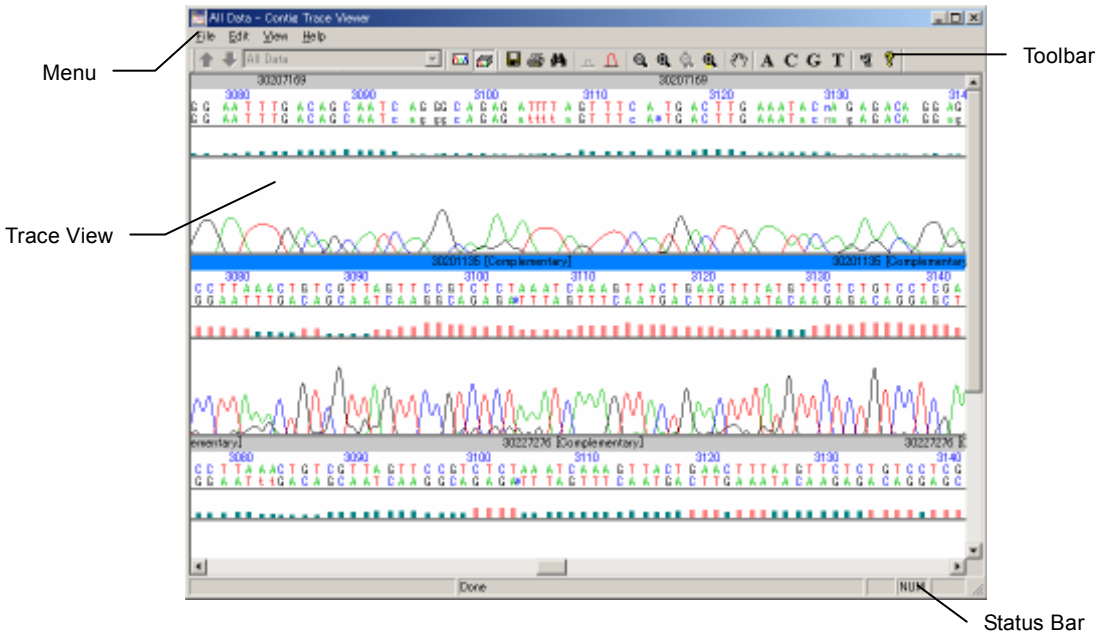
Quality



Item	Description
The quality value to be set when sequence are edited:xx	When sequences are edited, the quality value of the corresponding bases will be the value set here. The valid range is 0 to 99.
Color of Quality Bar:	Sets the color of the Quality Bar.
Quality value is between xx and yy	When the quality value is within the set range, it will display in the specified color. Only the lower limit of the high quality value can be changed.

2.4 Trace Window

The Trace Window displays trace data in ABI and SCF formats as trace data. Features include copying and searching bases.



2.4.1 Menu

File menu	Description
Export	Stores the original basecall (the upper sequence) of the active data in a file. The file can be saved in Fasta or SCF format. When a range is selected, only the sequence in the selected region will be stored in Fasta format.
Export Phred…	Stores the Phred basecall (the lower sequence) of the active data in a file. The storing format can be chosen from either Fasta format or SCF format. When a range is selected, only the sequence in the selected region will be stored in Fasta format.
Print Setup…	Sets the printer.
Print Preview	Displays the printing image. To exit the print preview mode and return to the previous window, click Close.
Print…	Performs printing.
Exit	Closes the window.

Edit menu	Description
Copy	Copies the selected sequence or the trace value of the selected part to the clipboard. Refer to 9.6.2 “Copying Bases” and 9.6.3 “Copying Traces” for details of operation.
Select All	Selects the whole original sequence of the selected data.
Find	Searches a particular base from the displayed data. Refer to 9.5 “Searching a Sequence” for the details of operation.

View menu	Description
Toolbar	Toggles the Toolbar to display/hide it.
Status Bar	Toggles the status bar to display/hide it.
Show Single Data	Switches to the Single Data mode.
Show All Data	Switches to the Parallel Data mode.
Preference…	Displays the parameter dialog of the view. Refer to 2.4.5 “Trace Viewer Parameter” for the details.

Help menu	Description
Contents	Displays the help for Contig Trace Viewer.
About Contig Trace Viewer…	Displays the version information of Contig Trace Viewer.

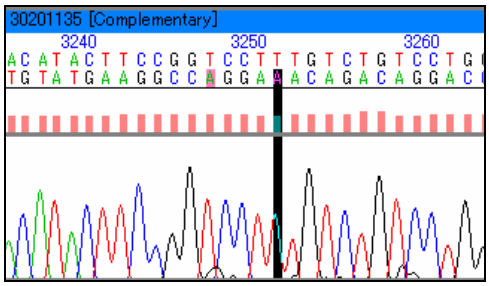
2.4.2 Toolbar

Button	Description
	Displays the previous data in the Single Data mode.
	Displays the next data in the Single Data mode.
	Displays the data list in the Single Data mode.
	Switches to the Single Data mode.
	Switches to the Parallel Data mode.
	Stores the original sequence in a file. The same as selecting File > Export... from the menu.
	Prints the window. The same as selecting File > Print… from the menu.
	Displays the Search dialog for bases. The same as selecting Edit > Find… from the menu.
	Decreases the vertical width of a trace.
	Increases the vertical width of a trace.
	Decreases the horizontal width of a view.
	Increases the horizontal width of a view.
	Decreases the vertical width of a view.
	Increases the vertical width of a view.
	Turns ON/OFF the hand tool (for scrolling through individual items of data) in the parallel data mode.
	Displays/hides a trace of lane A.
	Displays/hides a trace of lane C.
	Displays/hides a trace of lane G.
	Displays/hides a trace of lane T.
	Displays the dialog to set view parameters.
	Displays online help.

2.4.3 Status Bar

The Status Bar displays the names of each menu and Toolbar button at the mouse cursor, and each application on the application palette.

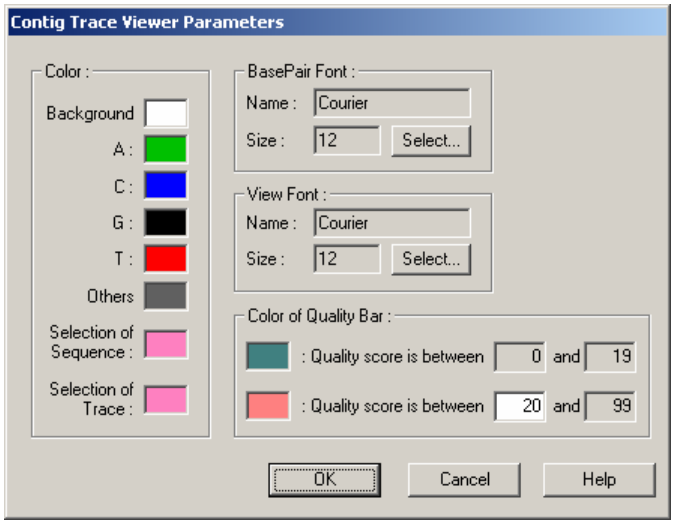
2.4.4 Trace View



The Trace View displays four big rows, which are the Sequence Name, Base Display, Quality Bar, and Trace Data, from top to bottom.

Refer to 8.5.2 “How to Read the Trace Display” for details.

2.4.5 Trace Viewer Parameter



Item	Description
Color	Sets the colors of the Trace View.
Background	Sets the background color of the Contig Trace View.
A	Sets the color for Base A and a trace of lane A.
C	Sets the color for Base C and a trace of lane C.
G	Sets the color for Base G and a trace of lane G.
T	Sets the color for Base T and a trace of lane T.
N	Sets the color for Base N and a trace of lane N.
Selection of Sequence	Sets the color for the selection of sequence. Double click the color box to display the color setting dialog.

Item	Description
Selection of Trace	Sets the color for the selection of trace. Double click the color box to display the color setting dialog.
BasePair Font	Sets the display font for the number of bases (bp). Click Select… to display the font dialog.
Name	Displays the currently set font type.
Size	Displays the currently set font size. When changed, the View Font will automatically be set to the same size.
View Font	Sets the display font for sequences. Click Select… to display the font dialog.
Name	Displays the currently set font type.
Size	Displays the currently set font size. When changed, the BasePair Font will automatically be set to the same size.
Color of Quality Bar	Sets the color of the Quality Bar.
Quality score is between xx and yy	When the quality value is within the set range, it will display in the specified color. To change the high quality range, adjust the lower limit.

Chapter 3 Project Window

3.1 What is a Project?

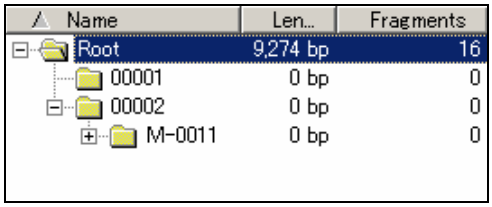
In the Contig Manager a project is an analytical unit in which operations are performed. A project contains information on sequences, how they are linked, how contigs are formed and user defined parameters.

3.2 Components of a Project

A project is composed of a folder(s), contig(s), and fragment(s).

3.2.1 Folders

A folder is a place to store data. A folder can contain folders, contigs, and fragments, and manage a hierarchical structure of data. The folder at the top hierarchy is called a root folder. Folders are displayed with icons as in the following figure.



Users can give a folder any non-duplicate name. However, the root folder cannot be renamed.

3.2.2 Contigs

A sequence made by linking fragments is called a contig, and displayed using the icon.

3.2.3 Fragments

Fragments are classified into trace data and sequence data with only base alignments.

Trace Data

The data output from a sequencer in ABI Format and SCF Format can be imported as trace data. On the Contig Manager, trace data is displayed using the icon.

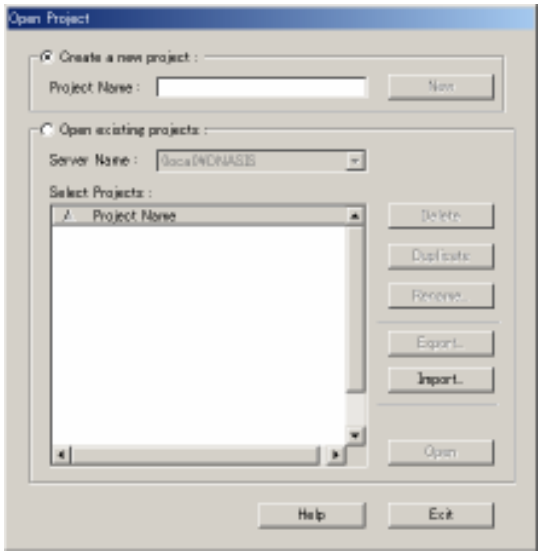
Sequence Data

The kinds of data that can be imported as sequence data are Fasta Format, Multi-Fasta Format, Genbank Format, Multi-Genbank Format, EMBL Format, Multi-EMBL Format, DNASIS for Windows Format, and Simple Text Format. In a view, sequence data is displayed using the icon.

3.3 Create a New Project

At the start of the Contig Manager, the Open Project dialog is displayed. A new project can be created following the procedure below.

1. Select Program > DNASIS MAX > Contig Manager from the Start menu. The Contig Manager will start, and the Open Project dialog will appear.



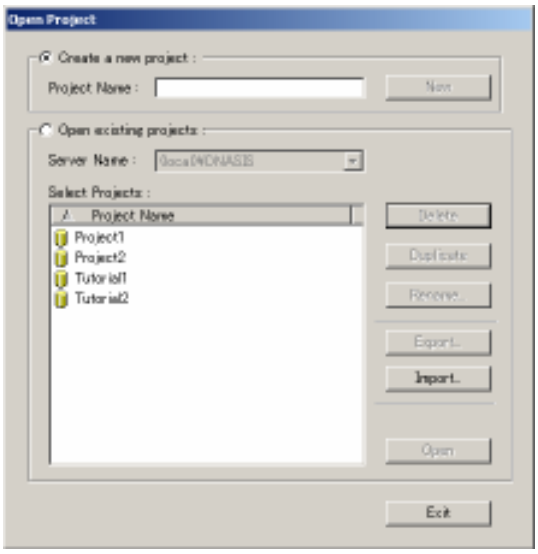
2. Check that the “Create a new project:” radio button is selected and type any project name in the Project Name box.
3. Click New to create a new project with the typed in name.

- A new project can be created during the operation of the Contig Manager. Select File > New from the menu or click on the Toolbar to open the Open Project dialog.

3.4 Open Existing Projects

Follow the procedure below to open an existing project.

1. Select Program > DNASIS MAX > Contig Manger from the Start menu. The Contig Manger will start and the Open Project dialog will appear.



2. Select a project in the Select Projects list.
3. Click Open to open the selected project.

3.5 Delete Projects

1. Select Program > DNASIS MAX > Contig Manager from the Start menu. The Contig Manager will start and the Open Project dialog will appear.
2. Select a project to delete in the Select Projects list.
3. Click Delete to delete the selected project.

3.6 Copy Projects

1. Select Program > DNASIS MAX > Contig Manager from the Start menu. The Contig Manager will start and the Open Project dialog will appear.
2. Select a project to copy in the Select Projects list.
3. Click Duplicate to display a dialog for entering a new project name. The selected project will be copied.

3.7 Save Projects

To save a project, select Save Project from the File menu, or click  on the Menu Bar. The whole project's current condition will be saved.

When any of the following operations are performed, a project is automatically saved. Therefore, performing Revert will not restore the project.

- AutoAssemble, Basecall, Trim, Assemble
- When opening ContigViewer, ContigTraceViewer or SequenceViewer.
- When importing data into the project.
- When opening another project.
- When terminating a project.

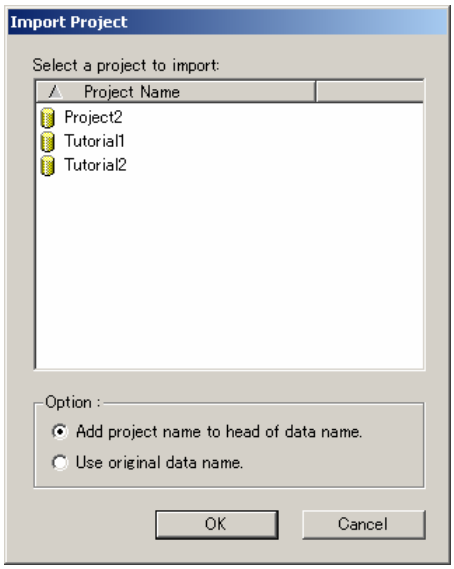
3.8 Revert Projects

To revert a project, select File > Revert from the menu. The project will revert to the last saved status.

3.9 Merge Projects

The currently open project can be merged with another project.

1. Select Import > Import Project… from the Contig Manager. The Import Project dialog will appear.



Item	Description
Select a project to import	Displays a list of existing projects.
Option	Sets options when merging projects.
Add project name to head of data name.	When merging into an opened project, the corresponding project name is added to the heads of the contigs and fragments of the project to be merged.
Use original data name.	When merging into an opened project, the names of the contigs and fragments of the project to be merged are added as they are. If the currently opened project has contigs and fragments with the same name, merging is not possible.

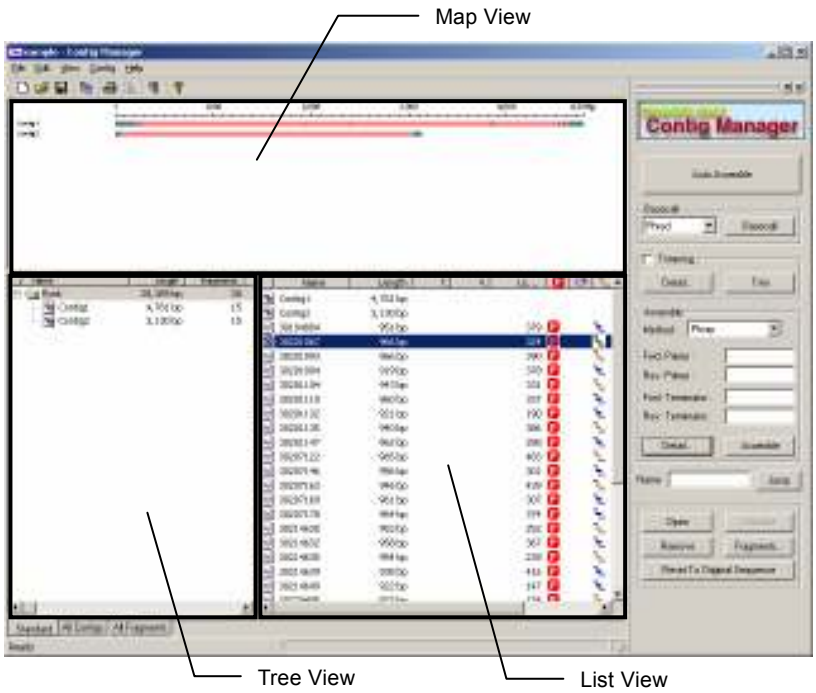
2. Specify a project to merge from the projects displayed in the list, and select the Option.
3. Click OK to merge the selected project.

3.10 Close Projects

Select Exit from the File menu to close the Contig Manager. The operation status will automatically be saved.

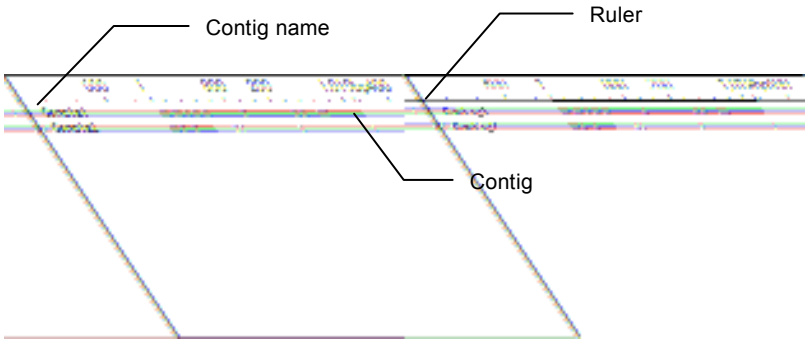
3.11 Structure of the Main Window

The Main Window of the Contig Manager is explained below. The Main Window consists of Map View, Tree View, and List View.

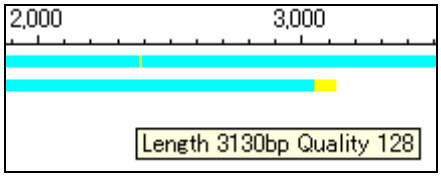


3.11.1 Map View

Map View colors the contigs in a project according to their quality values and displays them graphically. The contig names are displayed at the left side of the view, and the contigs are displayed under the ruler, colored according to their quality values.



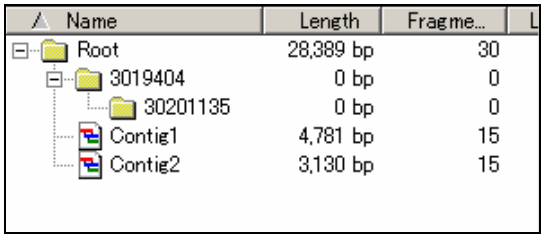
When the mouse pointer is moved over a contig, the bp number and the quality value of the contig will display.



3.11.2 Tree View

The Tree View displays folders and contigs in a project in a hierarchical structure.

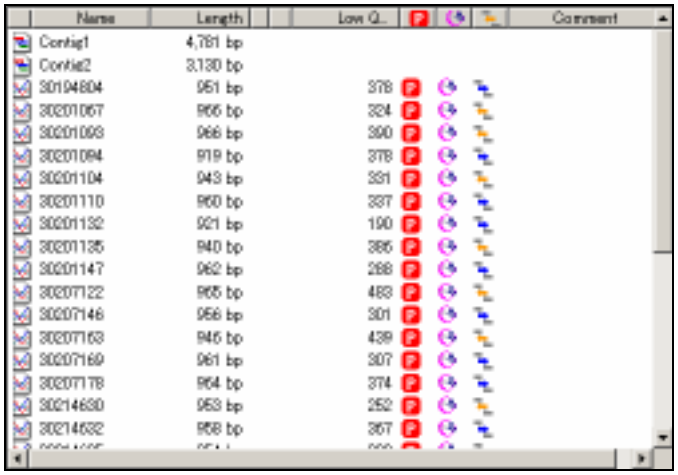
The hierarchical structure can be changed by using the mouse. Refer to 3.14 “Operations in Tree View” for details.



Column header item	Description
Name	Displays the names of folders and contigs. Each time the column header is clicked, the names are sorted in ascending and descending orders.
Length	Displays the total bp length of fragments in a folder, and the bp length of each contig. Each time the column header is clicked, the names are sorted in ascending and descending orders.
Fragments	Displays the number of fragments in a folder and the number of fragments composing a contig. Each time the column header is clicked, the names are sorted in ascending and descending orders.
Low QVs	Displays the number of base pairs in a contig whose quality is less than the threshold quality. The threshold of quality can be set in 2.2.8 “Preferences dialog”. Each time the column header is clicked, the names are sorted in ascending and descending orders.
Comment	If comments are attached to folders or fragments, they are displayed here. Each time the column header is clicked, the names are sorted in ascending and descending orders.

3.11.3 List View

The List View displays the contents of the folder selected in Tree View.



Column header item	Description
Name	Displays the names of the contig and fragment. Each time the column header is clicked, the names are sorted in ascending and descending orders.
Length	Displays the bp length of contigs and fragments. Each time the column header is clicked, the names are sorted in ascending and descending orders.
Trimmed Len.	Displays the number of base pairs trimmed by performing Trimming.
Vector	Displays the corresponding vector name when a fragment is trimmed by a vector.
Low QVs	Displays the number of low quality base pair regions in the corresponding fragment. The threshold of quality can be set in 2.2.8 “Preferences dialog”.
P	Indicates fragments for which Phred was performed.
	Indicates trimmed fragments.
	Indicates the link direction for fragments that are linked.
Comment	If comments are attached to folders or fragments, they are displayed here. Each time the column header is clicked, the names are sorted in ascending and descending orders.

3.12 Three Display Modes

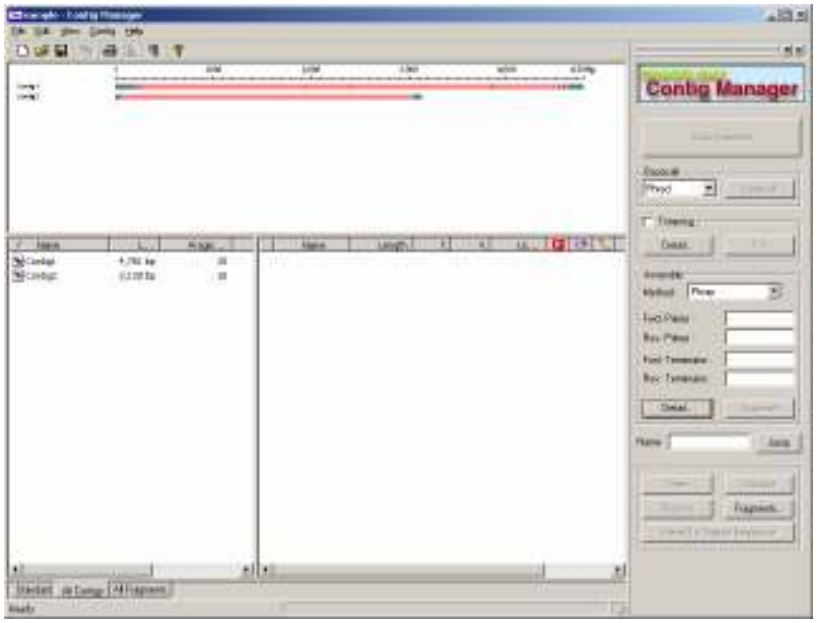
The Main Window of Contig Manager has three display modes: Standard, All Contigs, and All Fragments. By clicking the tabs in the Main Window, the modes can be changed.

3.12.1 Standard Display Mode

Click the Standard Tab to display this mode. It is the standard display. The Map View, Tree View, and List View are shown and folders, contigs, and fragments are displayed.

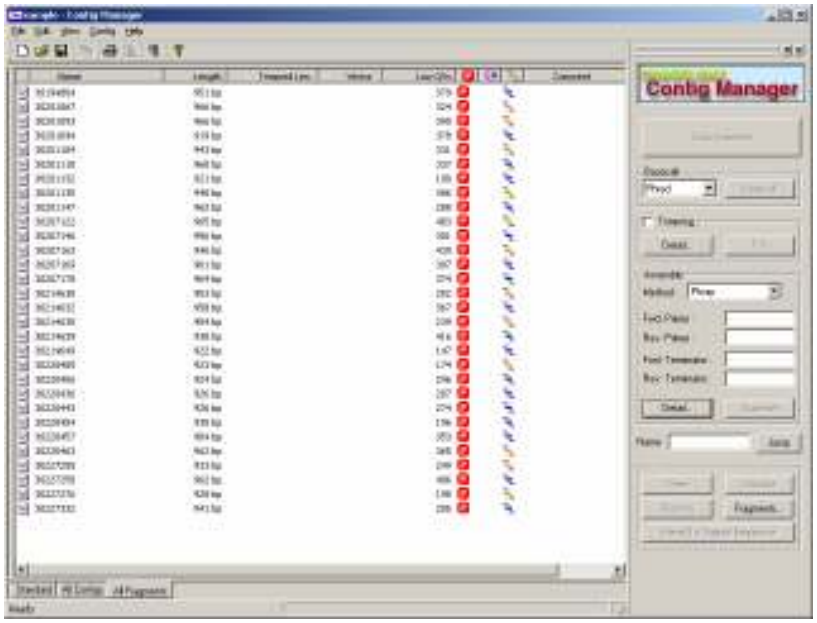
3.12.2 All Contigs Display Mode

Click the All Contigs Tab to display this mode. In Map View and Tree View, all the contigs included in a project are display. In List View, the fragments composing the contigs selected in Tree View are displayed.



3.12.3 All Fragments Display Mode

Click the All Fragments Tab to display this mode. This mode displays all the fragments included in a project.



3.13 Operations in Map View

In the Map View, the following operations can be performed.

Displaying Contigs

3.13.1 Display Contigs from Map View

When double clicking the contig displayed in Map View, the Contig Viewer will start and the linking status of fragments in the corresponding contig will graphically display.

The window is divided into Map View and Sequence View. Map View gives an overall view of the fragments and contigs along with the quality value graph. In Sequence View, the contig alignments and each fragment alignment are displayed.

Refer to 2.3 “Contig Window” for the details of the Contig Window.

3.14 Operations in Tree View

In Tree View, the following operations can be performed.

Operations for folders

Operations for contigs

Operations for fragments

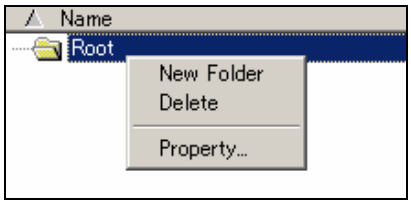
Sorting data

Operating trees

3.14.1 Operations for Folders

Create Folders

1. In Tree View, select the directory to create a new folder.
2. Select Contig > New Folder from the menu, or right click the mouse on the folder and select New Folder from the popup menu (see below).
3. A new folder will be created under the selected or right clicked folder.

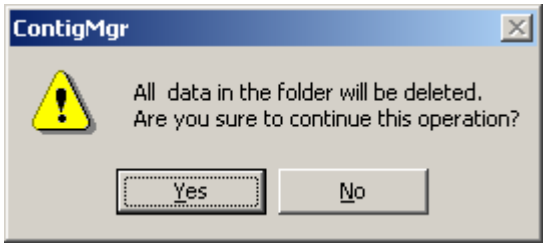


Move Folders

Select a folder and drag-and-drop it to the destination. This operation is typical of Windows-based programs such as the Windows Explorer.

Delete Folders

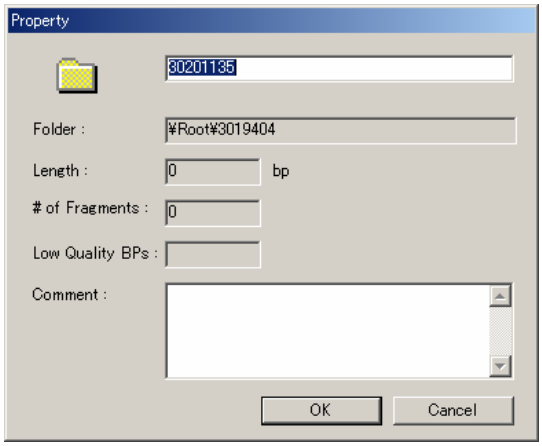
1. Select a folder to delete in Tree View, and press the Delete key on the keyboard, or right click the folder and select Delete. A delete confirmation message will appear.



2. Click Yes. The folder and all the folders, contigs, and fragments within will be deleted.

Display Folder Properties

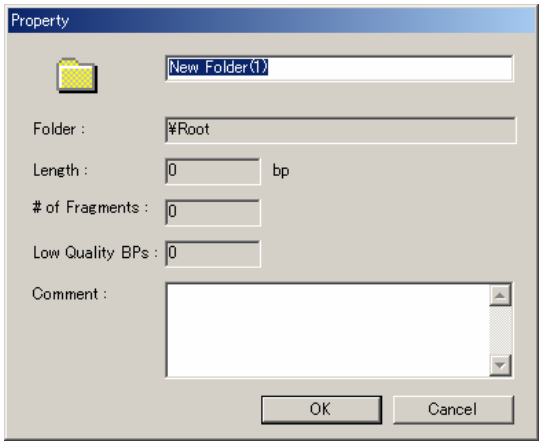
1. Right click the folder to display the property in List View.
2. Select Property… from the popup menu.
3. The Property dialog will appear.



Item	Description
	Displays the folder name. Can be renamed. Refer to “Rename Folders” for the details.
Folder	Shows the location of the corresponding folder with the path from the root folder.
Length	Displays the total number of base pairs in the fragments of a folder.
# of Fragments	Displays the number of fragments in a folder.
Low Quality BPs	Displays the number of base pairs in low quality regions in the fragments of the corresponding folder.
Comment	Displays folder comments. They are editable by the user.

Rename Folders

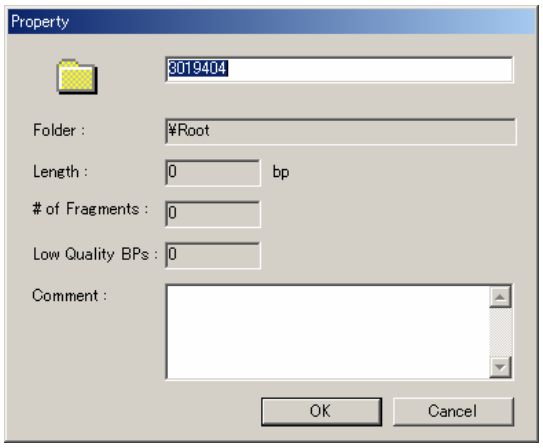
1. Right click the folder to rename.
2. Select Property… from the popup menu.
3. The Property dialog will appear.



4. Type the new name for the folder in the textbox of the icon.
5. Press the Enter key or click OK. The folder will be renamed.

Attach Comments to Folders

1. Right click the folder to attach a comment.
2. Select Property… from the popup menu displayed. The Property dialog will appear.

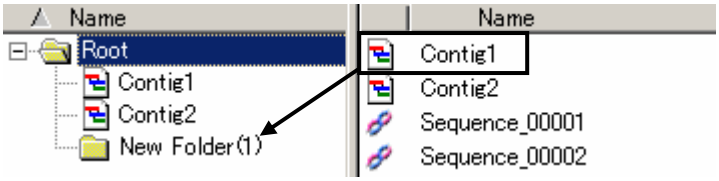


3. Type a comment in the Comment box. Up to 255 characters can be used.
4. Press the Enter key or click OK.

3.14.2 Operations for Contigs

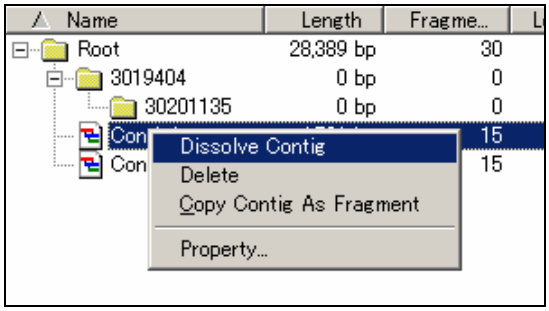
Move Contigs

Select a contig to move in Tree View, and drag-and-drop it to the destination. This operation is typical of Windows-based programs such as the Windows Explorer.

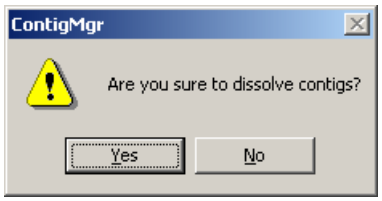


Dissolve Contigs

1. Right click on the contig to dissolve.
2. Select Dissolve Contig from the popup menu.

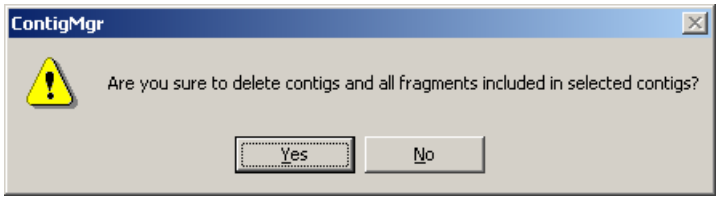


3. A confirmation message will appear. Click Yes to dissolve the link with the corresponding contig.



Delete Contigs

1. Select a contig to delete, and press the Delete key on the keyboard. A confirmation message will appear.



2. Click Yes and delete the corresponding contig and the fragments that composed the contig in the project.

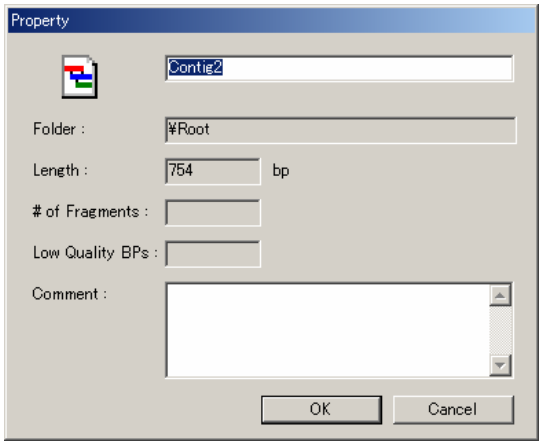
Display Contigs from Tree View

1. Double click the contig to display. Or select the contig, and click Open on the Navigation Toolbar.

2. The Contig Viewer will start, and the corresponding contig will appear.

Display Contig Properties

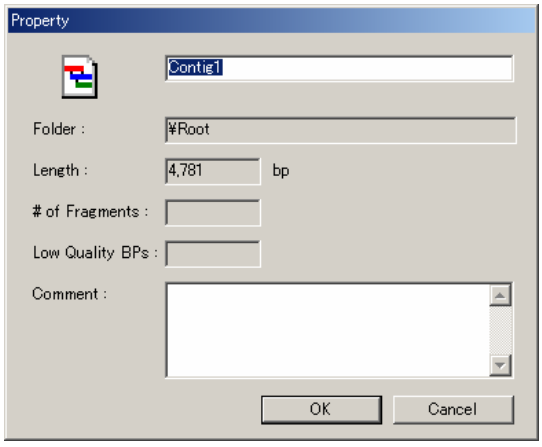
1. Right click the contig to display the property in Tree View.
2. Select Property… from the popup menu displayed.
3. The Property dialog will appear.



Item	Description
	Displays the name of a contig. Can be renamed.
Folder	Shows the location of the contig with the path from the root folder.
Length	Displays the number of base pairs in the contigs.
# of Fragments	Displays the number of fragments composing the contigs.
Low Quality BPs	Displays the number of base pairs in low quality region in the contigs.
Comment	Displays comments. They are editable by the user.

Renaming Contigs

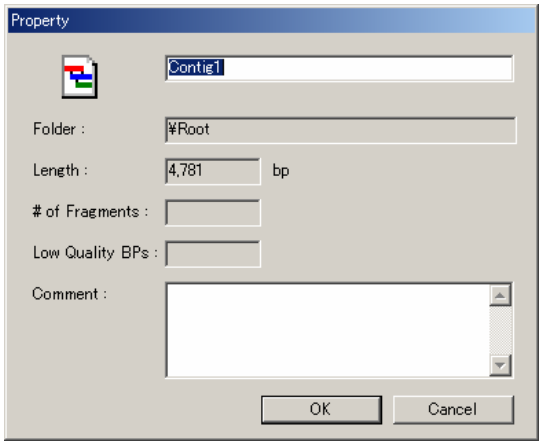
1. Right click the contig to rename.
2. Select Property… from the popup menu displayed.
3. The Property dialog will appear.



4. Type the new name for the contig in the textbox to the right of the icon.
5. Press the Enter key or click OK. The contig will be renamed.

Attach Comments to Contigs

1. Right click the contig to attach a comment.
2. Select Property… from the popup menu. The Property dialog will appear.



3. Type a comment in the Comment box. Up to 255 characters can be used.
4. Press the Enter key or click OK.

3.14.3 Operations for Fragments

Import Fragments

It is possible to import fragment data from a window to a given folder in Contig Manager.

1. Select a fragment file from the window to import. More than one file can be imported simultaneously.
2. Drag-and-drop the file(s) to the destination folder in Contig Manager. Be sure to drop when the mouse pointer is over the destination folder.

3. The Import Summary dialog will display a list of imported data, skipped data, and import-error data.
4. Check the contents of the dialog, and click OK.

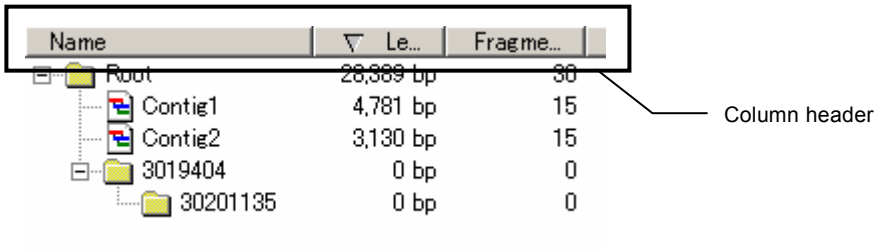
Move Fragments

Select a fragment displayed in List View and drop it in the destination folder in Tree View. This will move the dropped fragment to the corresponding folder. This operation is typical of Windows-based programs such as the Windows Explorer.

3.14.4 Other Operations

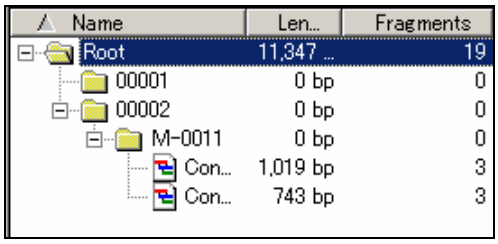
Sort Display Items

It is possible to sort by clicking on the column header of each item. ▽ means the items are in descending order, and △ ascending order.

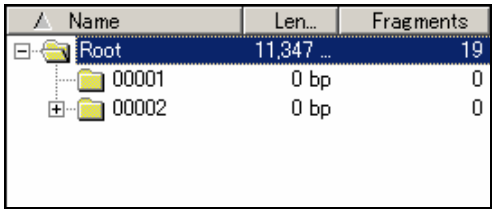


Expand/Shrink Trees

The folders with ⊞ on the left mean they contain folders or contigs.



To display the contents of a folder, click ⊞, or double click the folder. This is called “expanding” a folder.



In reverse, collapsing a tree so that the low-order part of the tree will not be seen is called “shrinking” a folder. Click ⊟ to the left of the folder, and the folder will shrink.

3.15 Operations in List View

In List View, the following operations are possible.

Operations for contigs

Operations for fragments

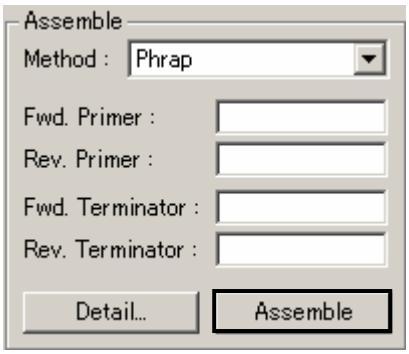
Display data

Sort data

3.15.1 Operations for Contigs

Create Contigs

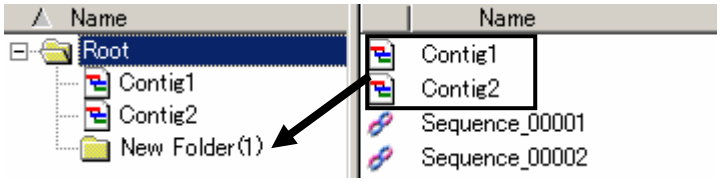
1. Select a fragment to create a contig.
2. Select Contig > Assemble > Phrap Assemble from the menu, or click Assemble on the Navigation Toolbar.



3. In a few moments analysis will complete. When a contig is created its data is added.

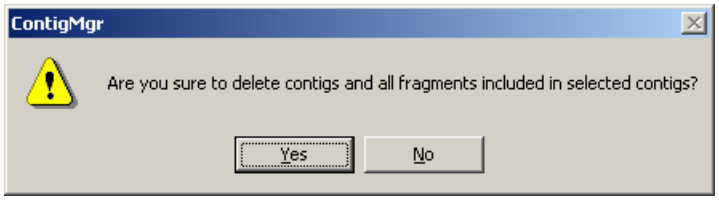
Move Contigs

Select a contig to move in Tree View, and drag-and-drop it to the destination. This operation is typical of Windows-based programs such as the Windows Explorer.



Delete Contigs

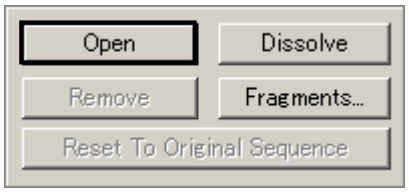
1. Select a contig to delete and press the Delete key on the keyboard. A confirmation message will appear.



2. Click Yes. The corresponding contig and the fragments composing the contig from the project will be deleted.

Display Contigs from Tree View

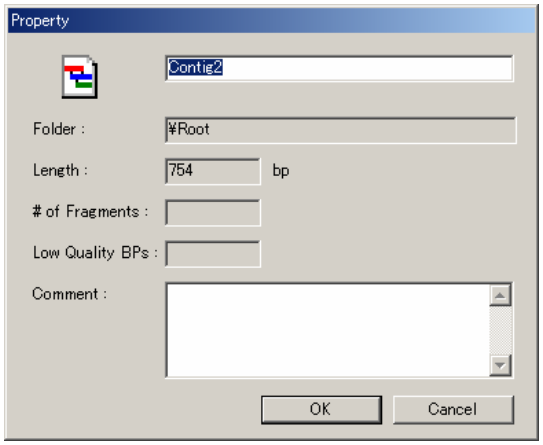
1. Double click the contig to display. Or select the contig and click Open on the Navigation Toolbar.



2. The Contig Viewer will start, and the corresponding contig will appear.

Display Contig Properties

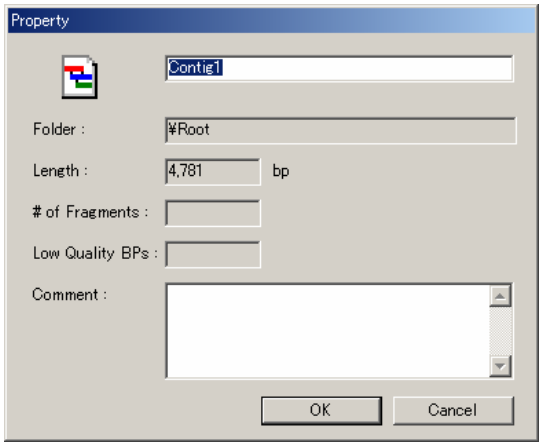
1. Right click the contig to display its properties in Tree View.
2. Select Property… from the popup menu.
3. The Property dialog will appear.



Item	Description
	Displays the name of the contig. Can be renamed.
Folder	Shows the location of the contig with the path from the root folder.
Length	Displays the number of base pairs in the contig.
# of Fragments	Displays the number of fragments composing the contig.
Low Quality BPs	Displays the number of base pairs in low quality regions in the contig.
Comment	Displays comments. They are editable by the user.

Rename Contigs

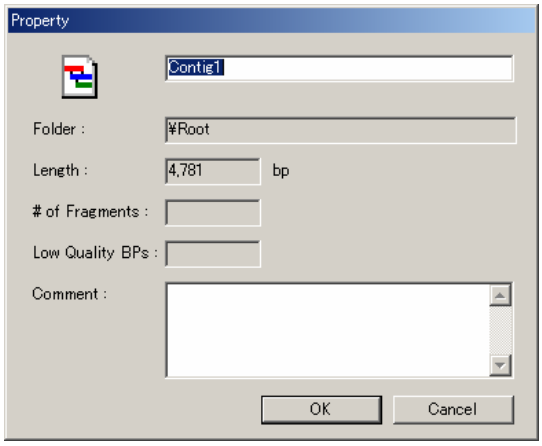
1. Right click the contig to rename.
2. Select Property… from the popup menu.
3. The Property dialog will appear.



4. Type the new name for the contig in the textbox to the right of the icon.
5. Press the Enter key or click OK. The contig will be renamed.

Attaching Comments to Contigs

1. Right click the contig to attach a comment.
2. Select Property… from the popup menu. The Property dialog will appear.



3. Type a comment in the Comment box. Up to 255 characters can be used.
4. Press the Enter key or click OK.

3.15.2 Operations for Fragments

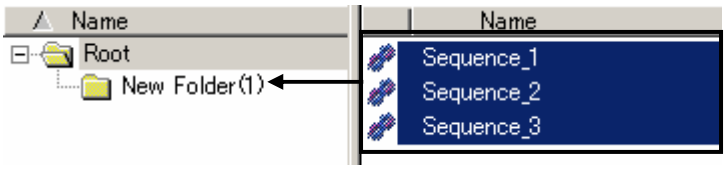
Import Fragments

It is possible to import fragment data from a window to a given folder in Contig Manager.

1. Click the destination folder in Tree View of Contig Manager to display the contents of the corresponding folder in List View.
2. Select a fragment file from the window to import. More than one file can be imported simultaneously.
3. Drag-and-drop the file(s) in List View of Contig Manager. The fragment data will be imported to the folder selected in Tree View.
4. The Import Summary dialog will display a list of imported data, skipped data, and import-error data.
5. Check the contents of the dialog, and click OK.

Move Fragments

Select a fragment displayed in List View and drop it in the destination folder in Tree View. This will move the dropped fragment to the corresponding folder. This operation is typical of Windows-based programs such as the Windows Explorer.



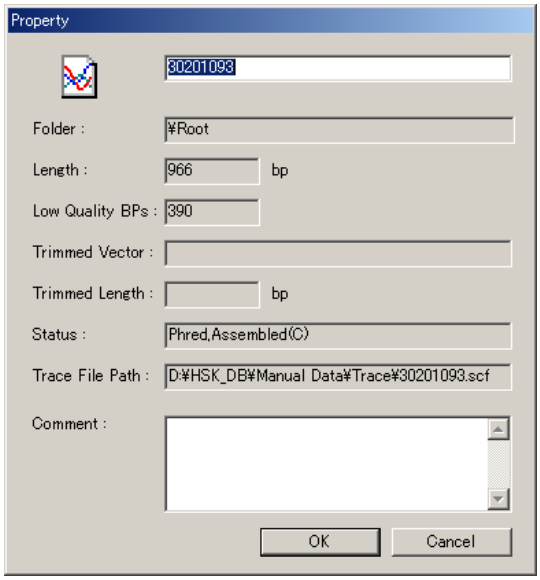
Delete Fragments

1. Select a fragment to delete in List View, and press the Delete key on the keyboard. A confirmation message will appear.

2. Click Yes to delete the corresponding fragment from the project.

Display Fragment Properties

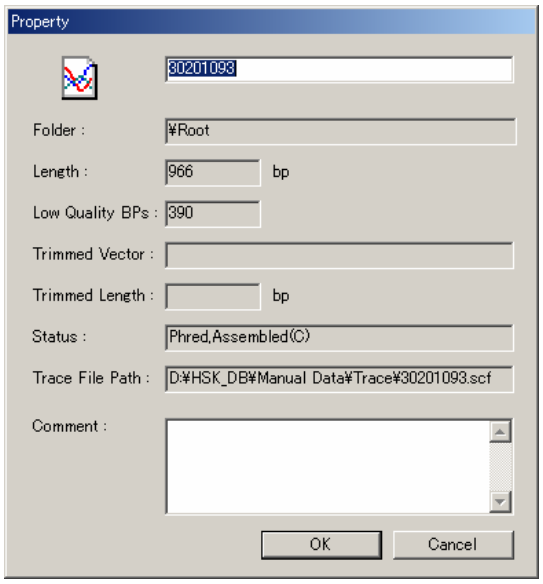
1. Right click the fragment to display its properties in List View.
2. Select Property… from the popup menu.
3. The Property dialog will appear.



Item	Description
	Displays the name of the fragment. Can be renamed. When the fragment is trace data, the left icon is used, and when it is sequence data, the right icon.
Folder	Shows the location of the fragment with the path from the root folder.
Length	Displays the number of base pairs in the fragment.
Low Quality BPs	Displays the number of base pairs in low quality regions in the fragment.
Trimmed Vector	Displays the vector name when a fragment is trimmed with by a vector.
Trimmed Length	Displays the number of trimmed base pairs.
Status	Displays the status (listed below) of the corresponding fragment. Trimmed: trimmed fragments Phred: Phred basecalled fragments Assembled(N): fragments linked with normal strand Assembled(C): fragments linked with complementary strand
Trace File Path	For trace data, displays the path to the linked trace file.
Comment	Displays comments. They are editable by the user.

Rename Fragments

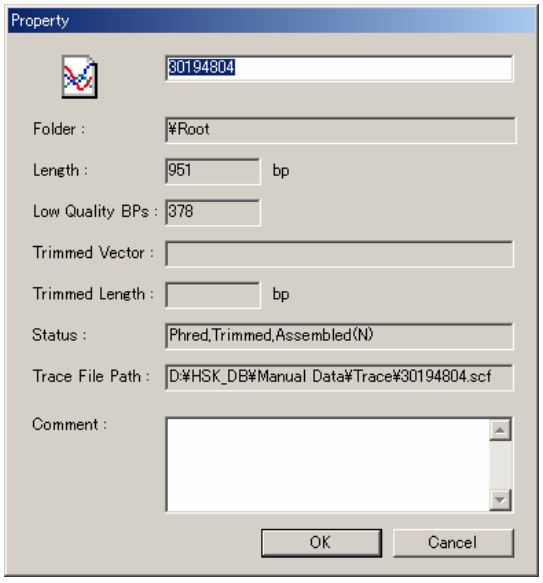
1. The Property dialog will appear.
2. Right click the fragment to rename.
3. Select Property… from the popup menu.
4. The Property dialog will appear.



5. Type the new name for the fragment in the textbox to the right of the icon (in case of sequence data, the icon).
6. Press the Enter key or click OK. The fragment will be renamed.

Attach Comments to Fragments

1. Right click the fragment to attach a comment.
2. Select Property… from the popup menu. The Property dialog will appear.

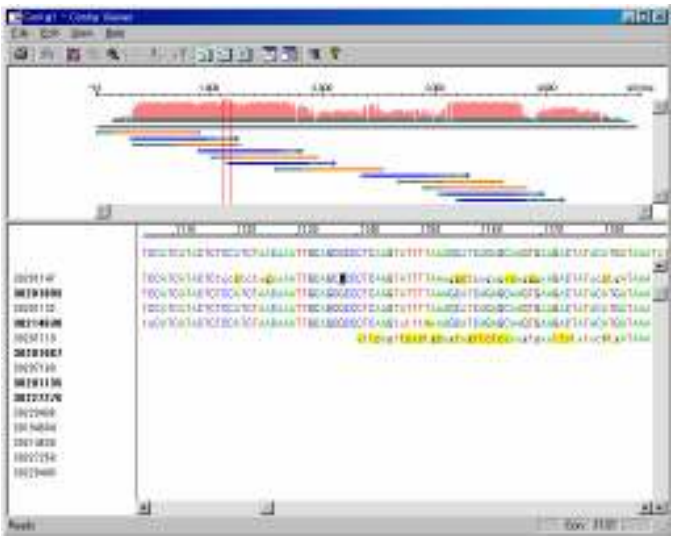


3. Type a comment in the Comment box. Up to 255 characters can be used.
4. Enter the new name and click OK. The fragment will be renamed.

3.15.3 Display Data

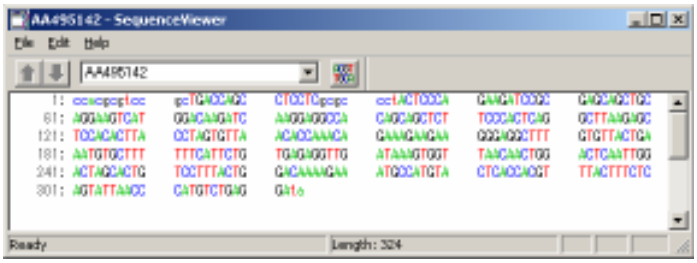
Display Contig Data

Double click a contig on the List View. The Contig Viewer will start, and the corresponding contig will display.



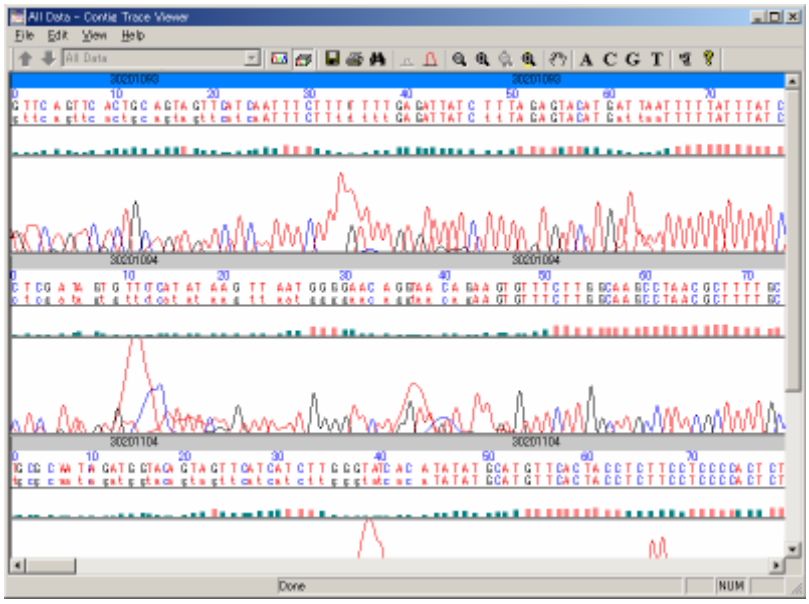
Display Sequence Data

Double click sequence data in List View. The Sequence Viewer will start, and the corresponding sequence data will display.



Display Trace Data

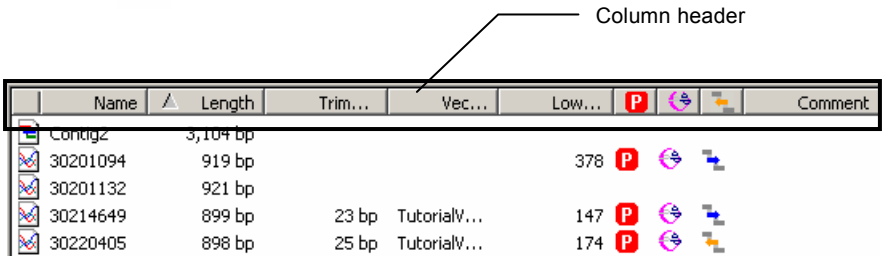
The specified file can be displayed in trace from Tree View. Double click the file to display. More than one file can be selected simultaneously.



3.15.4 Other Operations

Sort Displayed Items

It is possible to sort by clicking on the column header of each item. means the items are in descending order, and ascending order.



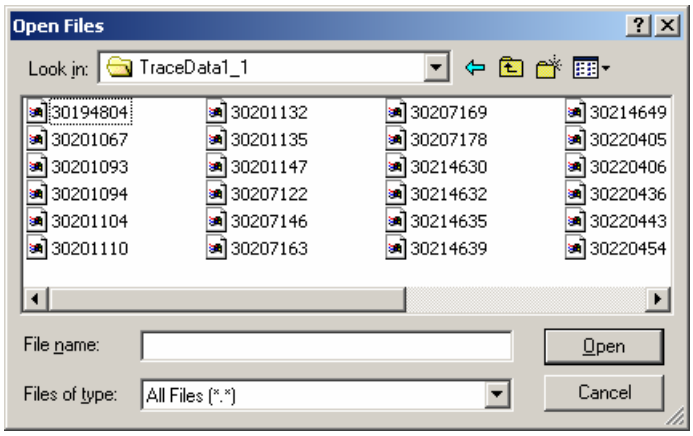
In List View, it is possible to sort Name, Length, with/without Phred, with/without Trimming, linking direction, and comments.

Chapter 4 Import and Export

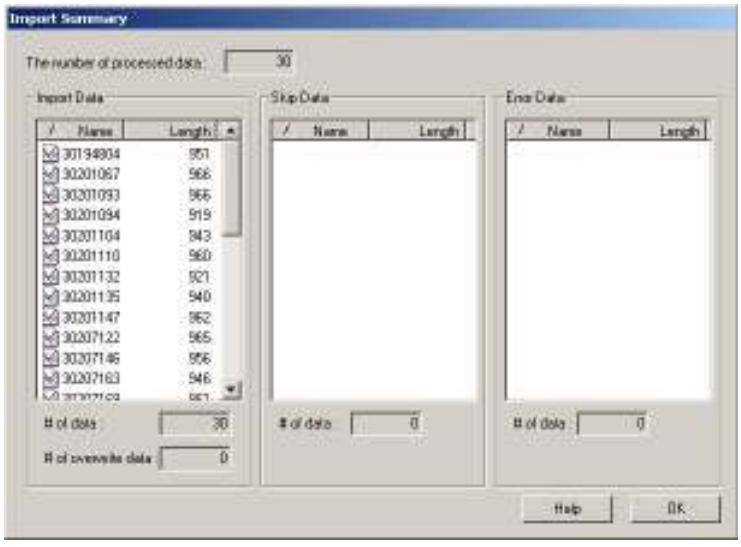
4.1 Import Trace Files

It is possible to directly import trace files output from DNA Auto sequencer. The formats of trace in ABI and SCF formats can be imported.

1. Select File > Import > Import sequence… from the menu. The Open Files dialog will display.



2. Select a file to import and click Open. More than one file can be selected at the same time.
3. The data is imported and the Import Summary dialog will display. Check the contents and click OK.

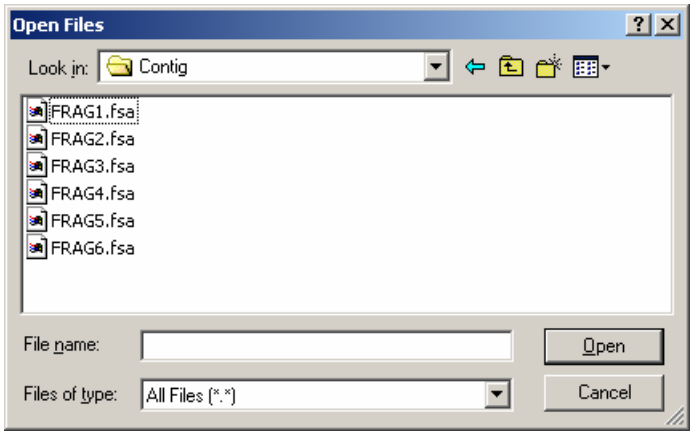


4. The selected trace is imported, and will display as trace data in List View. Trace data is displayed using the  icon.

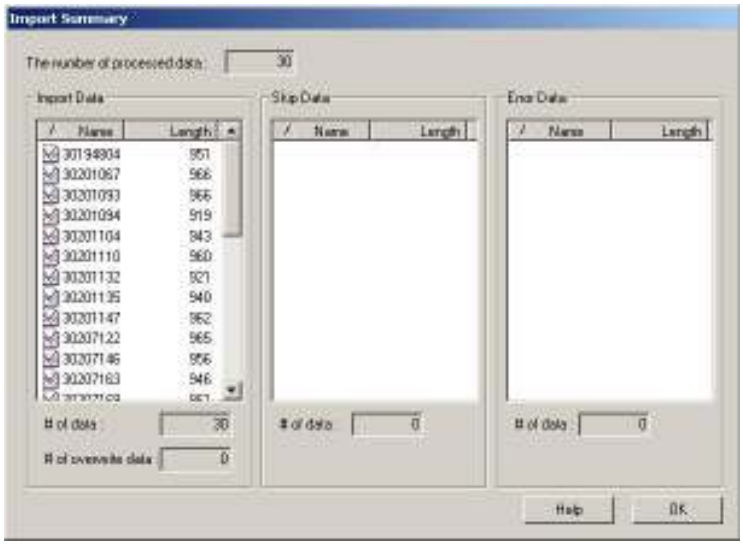
4.2 Import Sequence Files

It is possible to import sequence files that don't have trace data. The formats that can be imported as sequence files are text file in which only sequences are written, Fasta Format, Multi-Fasta Format, Genbank Flat File Format, Multi-Genbank Flat File Format, EMBL Format, Multi-EMBL Format, and DNASIS for windows Format.

1. Select File > Import > Import Sequence… from the menu. The Open Files dialog will display.



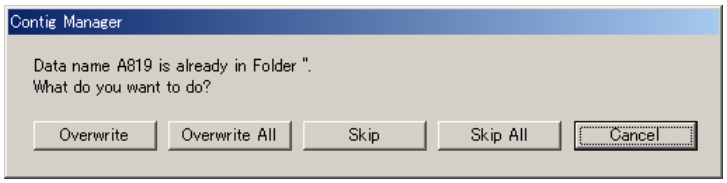
2. Select a file to import and click Open. More than one file can be selected at the same time.
3. The data is imported and the Import Summary dialog will display. Check the contents and click OK.



4. The sequence stored in the selected file is imported and will display as sequence data in List View.

   Sequence data is displayed using the icon.

- When a file is imported and has the same data name as the one already existing in the project, the following dialog will display.

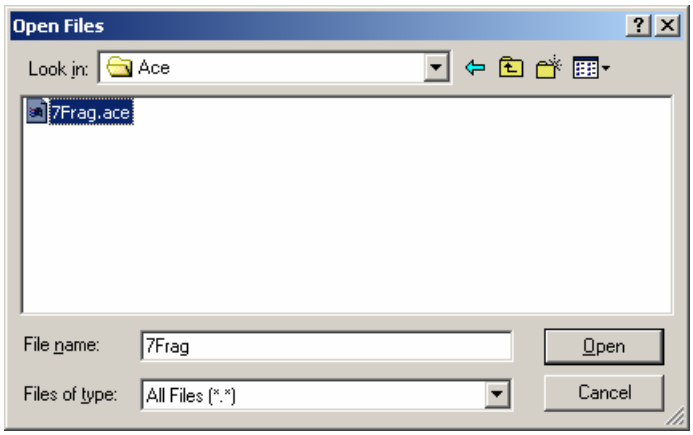


Button	Description
Overwrite	Overwrites the data.
Overwrite All	Overwrites all the data that have the duplicate names. Used when multiple data files are specified.
Skip	Skips data. The skipped data will not be imported.
Skip All	Skips all the data that have the same names. Only data that has a different name will be imported.
Cancel	Cancels file import process. No data will be imported.

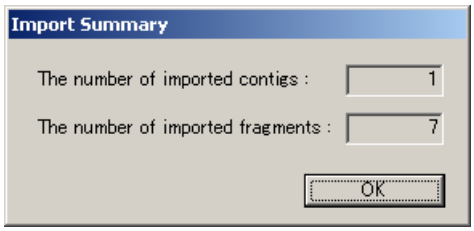
4.3 Import ACE Files

ACE files can be imported when no data exist in a project.

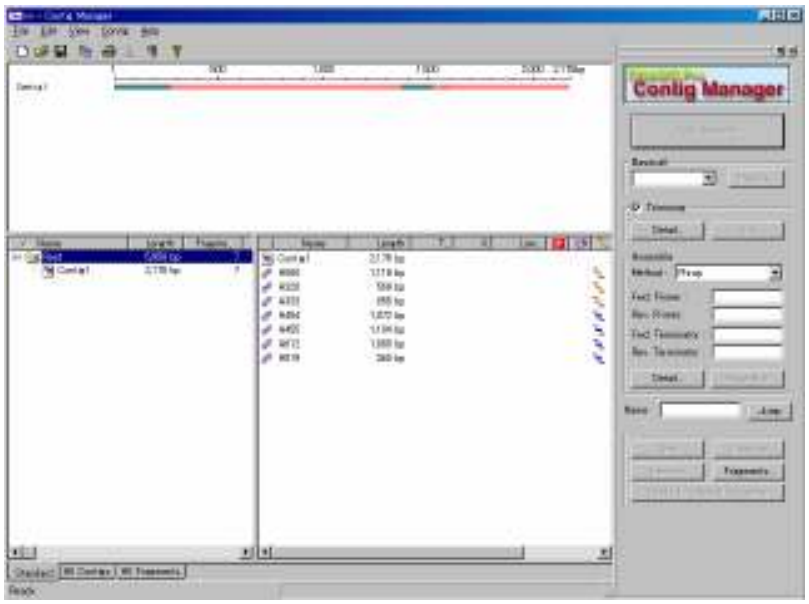
1. Select File > Import > Import Sequence… from the menu. The Open Files dialog will display.



2. Select ACE files to import, and click Open. The files are imported, and the Import Summary dialog will display.



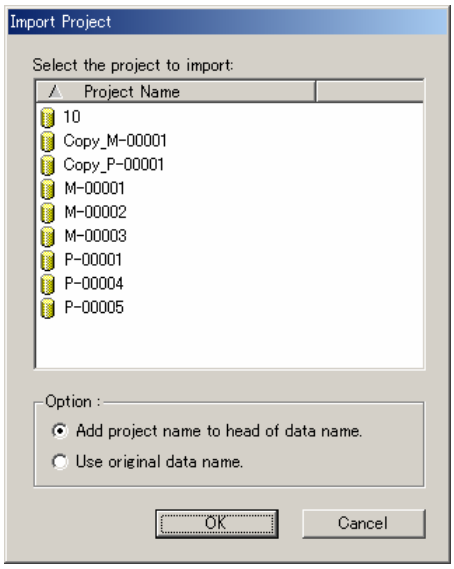
3. Check the contents and click OK. When importing is completed, the fragments and contigs imported to the Contig Manager will display.



4.4 Import Projects

Loading data of another project into the currently opened project is called “import.”

1. Select File > Import > Import Project… from the menu. The Import Project dialog will display.



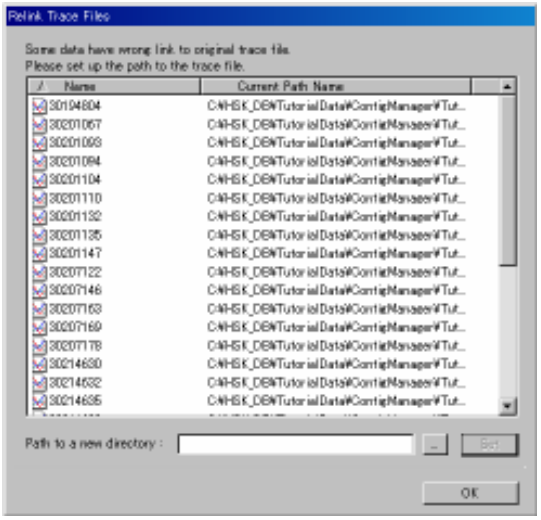
2. The existing project will display in the window. Select a project to import.
3. Check the Option box, and click OK.

Add project name to head of data name.	Project names are added to the front of the names of each fragment and contig included in the imported projects.
Use original data name	Adds data to the currently opened project, leaving the names of each fragment and contig included in the imported projects as they are. If importing would create duplicate fragment or contig names, the data cannot be imported.

4.5 Relink of Trace data

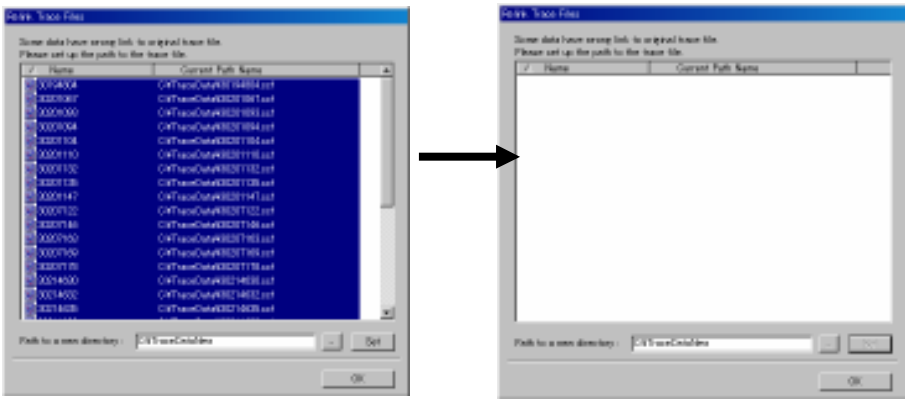
The trace data imported into a project don’t actually contain trace information, they contain path information to the trace files. Therefore, if the trace data are moved or deleted after the import process, the path information of the trace files will not be correct and a  icon will display in the Contig Manager.

Relocating the trace data files in Contig Manager is called relinking. Select Contig > Relink Trace Files… from the menu to display the Relink Trace File dialog.



Item	Description
List	Displays the trace data information of broken links.
Name	Displays the trace data names of broken links. The data can be sorted in ascending or descending order by clicking the column headers.
Current Path Name	Displays the current path name of the trace data with broken link. The data can be sorted in ascending or descending order by clicking the column headers.
Path to a new directory	Designate the directory to relink.
…	Displays the Browse dialog to designate a directory to relink.
Set	When pushed, the trace data selected in the list and the files in the directory designated for relinking are compared, and if the data names and file names match, relinking process will be performed.
OK	Closes the Relink Trace File dialog.

1. In the dialog, the trace data with broken links to trace files are displayed. Select data to relink.
2. Type the correct path in the “Path to a new directory:” text box.
3. To browse to the path, click “…”, select the folder to link, and click OK. The selected folder will display in the “Path to a new directory:” text box.
4. Check the path and click Set.



5. Click OK to close the dialog.

4.6 Export Sequences

4.6.1 Export Sequences of Fragments

To export fragment sequences, use the methods below. Exporting by these methods, will output sequences reflecting Phred basecall, trimming, and Phrap respectively. Note that the data linked in reverse by Phrap will be output as complementary strands.

Method to Export from Contig Manager

1. Select the fragment sequence to export in List View of Contig Manager.
2. Select File > Export Sequence > Export Sequence… from the menu.
3. When the “Save As ...” dialog opens, designate the location and the file name to save, and click Save.
4. When performed in List View with more than one data item selected, the sequence will be exported in multi-Fasta Format.

Method to Export from Contig Viewer

1. Select the fragment sequence region to export in Sequence View of Contig Viewer.
2. Select File > Export ... from the menu.
3. When the “Save As ...” dialog opens, designate the location and the file name to save, and click Save.
4. When exporting the whole fragment length, by clicking the arrow of the corresponding fragment in Map View, the entire fragment can be selected.
5. When performed with more than one fragment selected, the sequence will be exported in multi-Fasta Format.

Method to Export from Trace Viewer

It is possible to export from Trace Viewer only with trace data.

1. Click the fragments to export in Sequence View of Contig Viewer to make them active.
2. Select View > Chromatograms from the menu of Contig Viewer.
3. The trace data of the corresponding fragment will display in the Contig Trace Viewer.
4. Select File > Export Phred… from the menu of the Contig Trace Viewer.
5. When the “Save As ...” dialog opens, ensure that the file type is FASTA, assign an appropriate name, and click Save.
6. If a part of Phred Basecall (the lower row of the sequence data) is selected and exported, only that selected region will be exported.

4.6.2 Export the Original Fragment Sequences

To export fragment sequences, use the methods below. Exporting by these methods, sequences will be exported as they were at the time of input, no matter what condition the sequences are in.

Method to Export from Contig Manager

1. Select the fragment sequence to export in List View of Contig Manager.
2. Select File > Export Sequence > Export Original Sequence ... from the menu.
3. When the “Save As ...” dialog opens, designate the location and the file name to save, and click Save.
4. When performed in List View with more than one data item selected, the sequence will be exported in multi-Fasta Format.

Method to Export from Trace Viewer

1. Double click the trace data fragment to export in List View of Contig Manager.
2. The trace data of the corresponding fragment will display in the Contig Trace Viewer.
3. Select File > Export… from the menu of the Contig Trace Viewer.
4. When the “Save As ...” dialog opens, ensure that the file type is FASTA, assign an appropriate name, and click Save.
5. If selecting a part of the original basecall (the upper row of the sequence data) to export, only the selected sequence region will be exported.

Method to Export from Sequence Viewer

1. Double click the sequence data fragment to export in List View of the Contig Manager.
2. The sequence data of the corresponding fragment will display in the Sequence Viewer.
3. Select File > Export… from the menu of the Sequence Viewer.
4. When the “Save As ...” dialog opens, assign an appropriate name, and click Save.

4.6.3 Export Contig Sequences

To export contig sequences, use the following methods.

Method to Export from Contig Manager

1. Select the contig data to export from List View, Tree View, or Map View of Contig Manager.
2. Select File > Export Sequence > Export Sequence… or File > Export Sequence > Export Original Sequence… from the menu.
3. When the “Save As ...” dialog opens, assign an appropriate name, and click Save.
4. When performed in List View with more than one data item selected, the sequence will be exported in multi-Fasta Format.

Method to Export from Contig Viewer

1. Double click the contig data to export from List View, Tree View, or Map View of Contig Manager.
2. The corresponding contig will display in the Contig Viewer.
3. Select File > Export Consensus… from the Contig Viewer menu.

4. When the “Save As ...” dialog opens, assign an appropriate name, and click Save.

4.6.4 Export Contig Sequence to DNASIS MAX

Follow the procedure below to export contig sequences directly to DNASIS MAX.

1. Double click the contig data to export from List View, Tree View, or Map View of Contig Manager.
2. The corresponding contig will display in the Contig Viewer.
3. Select File > Export Contig to DNASIS MAX from the Contig Viewer menu, or click  on the Toolbar.
4. The contig sequence will display in DNASIS MAX.

4.7 Export Trace Files

It is possible to export trace files not only by original basecall but also by Phred basecall.

4.7.1 Export Original Basecall as Trace Data

1. Double click the trace data in List View of Contig Manager.
2. The corresponding data will display in the Contig Trace Viewer.
3. Select File > Export… from the menu of the Contig Trace Viewer.
4. When the “Save As ...” dialog opens, change the file type to SCF (*.scf).
5. Assign an appropriate name, and click Save.

4.7.2 Export Phred Basecall as Trace Data

1. Double click the Phred Basecalled trace data in List View of Contig Manager.
2. The corresponding data will display in the Contig Trace Viewer.
3. Select File > Export Phred… from the menu of the Contig Trace Viewer.
4. When the “Save As ...” dialog opens, change the file type to SCF (*.scf).
5. Assign an appropriate name, and click Save.

Chapter 5 Phred Basecall and Quality Evaluation

Contents in this section assume that the Phred/Phrap Option is installed.

5.1 Phred Basecall and Quality

This section explains the basics of Phred.

5.1.1 Phred Basecall

Basecalling converts trace data output from a sequencer into ACGT strings. Usually, the sequencer that outputs the trace data file has its own algorithm for basecalling.

Phred is a program that independently analyzes trace data and basecalls them, without using the algorithm that the sequencer has.

5.1.2 Quality Value

As the basecall information written in trace data files output from the sequencer contains only ACGT characters, regions that are read clearly and not clearly are treated as equivalent information.

On the other hand, Phred calculates the accuracy of called bases, attaching a numerical Quality Value (QV) to the results.

QV is defined in the equation below.

$$Q = -10 \text{ X } \log_{10} P$$

Q: Quality Value representing the accuracy of a called base.

P: Estimated error probability of a called base.

The relationship between basecall QV and accuracy is shown in below.

QV *Q*	**Error Probability *P***	**Accuracy (%)**
10	0.1	90
20	0.01	99
30	0.001	99.9
40	0.0001	99.99

5.2 Display Basecall Results and Quality Values

Basecalls and quality values can be displayed following the procedure below.

5.2.1 Prepare Trace Data

Start the Contig Manager and create a new project.

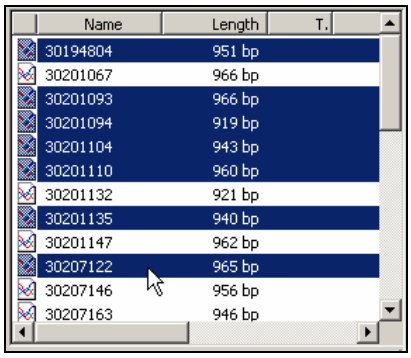
Refer to 3.3 “Create a New Project” for creating new projects.

Import trace data into the project. Phred basecalling can only calculate the quality values (QV) for trace files. QV cannot be calculated from a basecalled sequence that doesn’t have trace data.

Refer to 4.1 “Import trace files” for importing trace data.

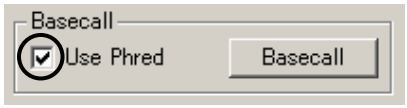
5.2.2 Phred Basecalling

1. Select data to Phred basecall on the List View.

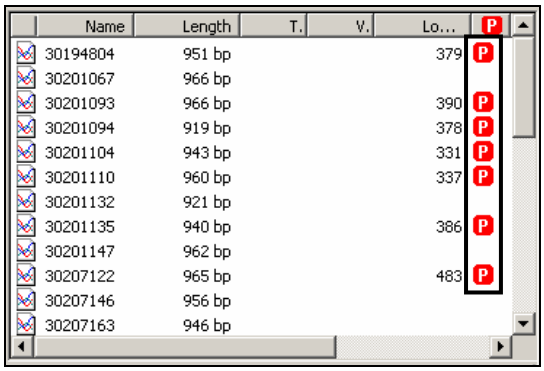


Selecting Data to Phred Basecall

2. Ensure that Use Phred is checked in Basecall group on the Navigation Toolbar, click Basecall.



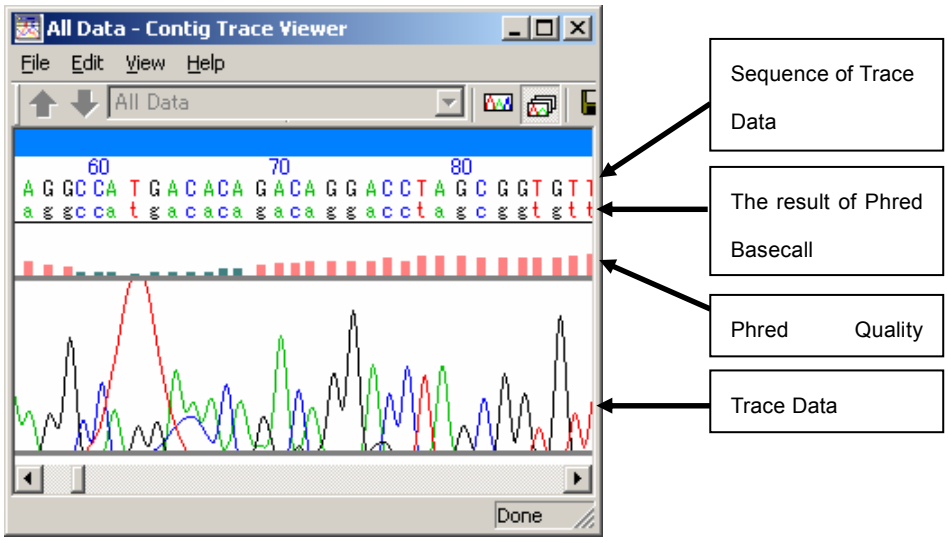
3. After a while performance of Phred ends, and Phred completed icons will be displayed on the List View.



Phred basecalled data

5.2.3 Display Basecall Results

By double clicking the data with Phred completed icons in List View, Phred basecall and QV bar are displayed in the Contig Trace Viewer, along with trace data.

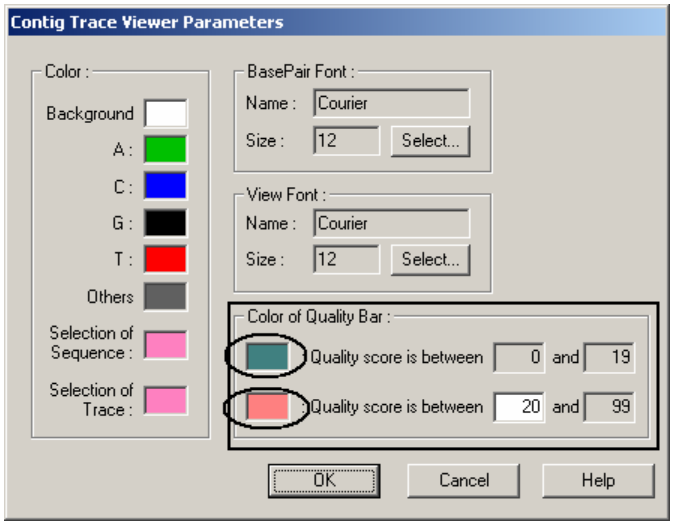


Basecall results in the Contig Trace Viewer

5.3 Search Low Quality Value Regions

5.3.1 Change Colors of Quality Value Bars

Select View > Preference… from the Contig Trace Viewer menu, or click Preference ( ) on the Toolbar, to open the Contig Trace Viewer Parameter dialog.



Contig Trace Viewer Parameter dialog

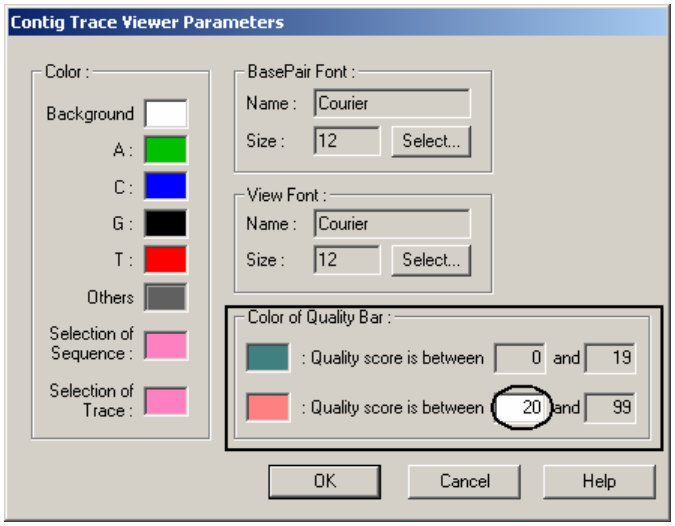
The respective colors (inside the ellipses in the figure above) in the Color of Quality Bar group will display in the quality value graph for bases whose QV are within the specified range (in the example above, 0 to 19 and 20 to 99). By double clicking inside these ellipses, the dialog will appear. Select any color and click OK to change the graph color.

Color palette

5.3.2 Set Quality Value Thresholds

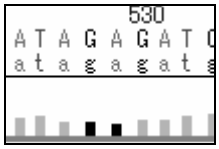
It is possible to change threshold colors of the quality value graph.

Select View > Preference… from the Contig Trace Viewer menu, or click Preference ( ) on the Toolbar to open the Contig Trace Viewer Parameters dialog.



Contig Trace Viewer Parameter dialog

The number in the ellipsis above is the lower threshold for coloring the quality value graph. By changing this value along with colors for threshold values above and below, low quality regions can be easily found.

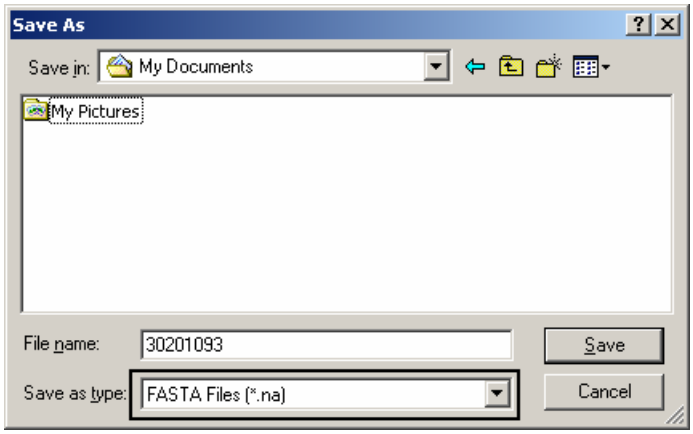


Low quality region emphasis

5.4 Export Phred Results

5.4.1 Export Phred Basecall Results as Strings

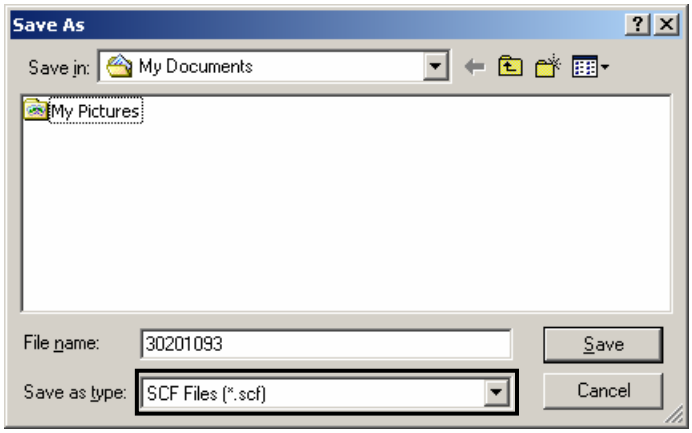
1. When File > Export Phred… is selected from the Contig Trace Viewer menu, the Save As dialog will appear.



2. Check that File Type is FASTA Files (*.na), and click Save.

5.4.2 Export Phred Basecall Results in SCF Format

1. When File > Export Phred… is selected from the Contig Trace Viewer menu, the Save As dialog will appear.

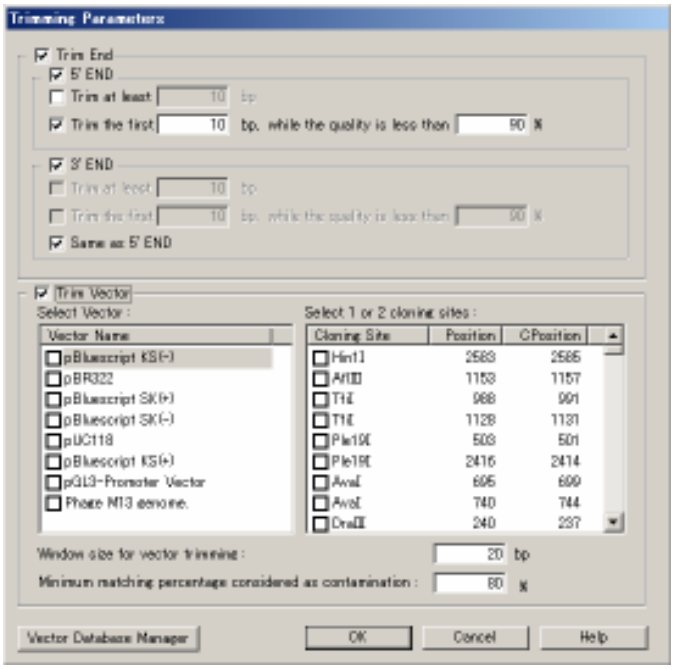


2. Check that File Type is SCF Files (*.scf), and click Save.

Chapter 6 Vector Trimming

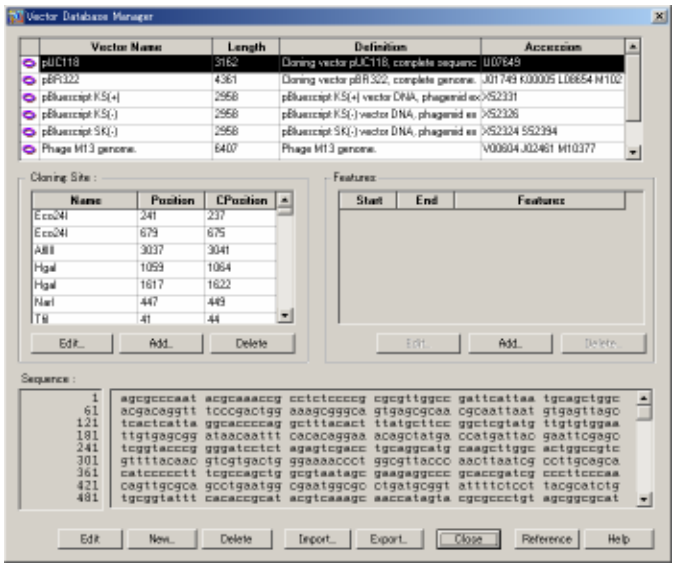
6.1 Set up Vectors

Click Detail... under Trimming on the Navigation Toolbar to display the Trimming Parameters dialog. All of the parameters required for vector trimming are located here. Refer to 2.2.15 “Vector Database Manager dialog” for details.

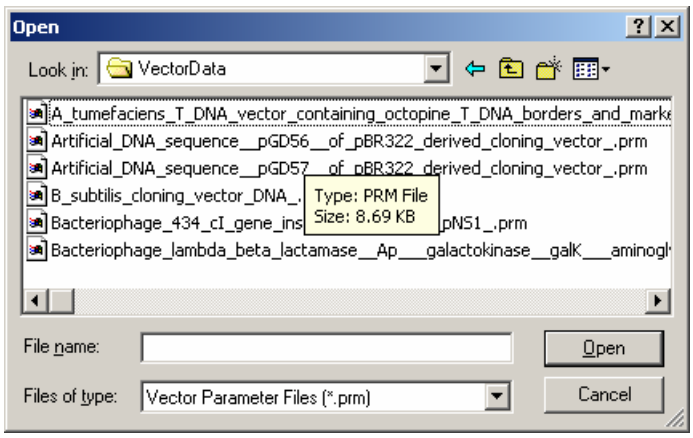


6.2 Register Vectors

1. Click Vector Database Manager on the Trimming Parameters dialog to display the Vector Database Manager.



2. Click Import.... The dialog below appears.



3. Select the vector data that is to be imported, and then click Open. The Vector Database Manager dialog appears again, and the new vector will be added to the vector list.

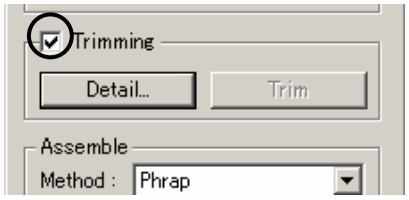
- The vectors are registered in the VectorData folder. This folder is located inside the Database directory created when installing DNASIS MAX. The default path is C:\HSK_DB\VectorData.
- More than 900 vector databases reside in the VectorData folder. The contents of these vectors can be viewed with the VectorTable.txt file located in the VectorData folder. The most convenient way of viewing this data is to use MS-Excel or a similar spreadsheet application.

---

4. Once the required vector data has been registered, click Close to exit the Vector Database Manager.

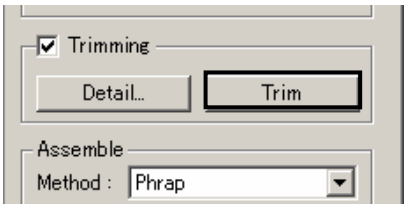
6.3 Trimming

1. This procedure sets up the trimming parameters. Refer to 6.1 “Set up Vectors” for details.
2. Place a check in the Trimming check box on the Navigation Toolbar.

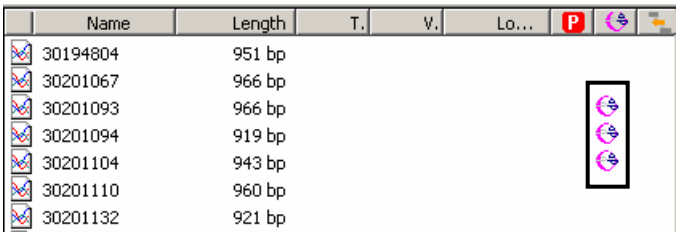


3. Select the fragments to be trimmed from the List View.

4. Click Trim under Trimming on the Navigation Toolbar.



5. The Progress dialog will appear, and analysis will run. A symbol will be displayed in the column beside the fragments that have been trimmed once the process completes.



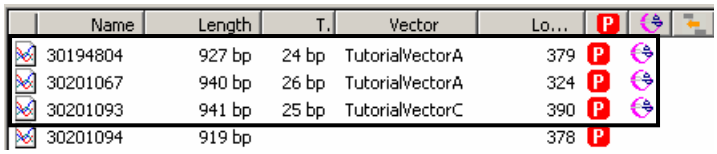
6.4 Display Trimming Results

Displaying trimming results is different for trace data and sequence data.

6.4.1 Trace Trimming Results

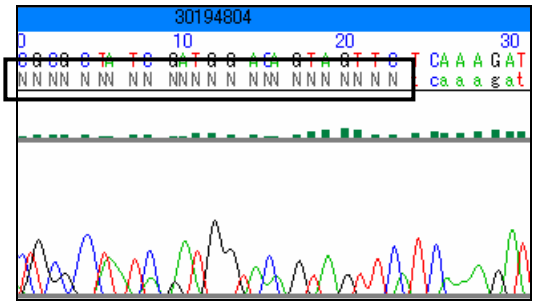
The trace trimming results are sorted sequentially and displayed.

Double click on the trimmed trace data.



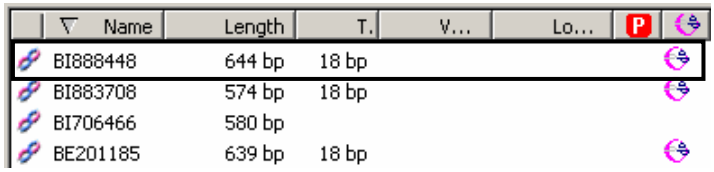
Trimmed Trace Data

The Contig Trace Viewer will start up and display the sequence data and trace data. The series of N's are the areas that have been trimmed.



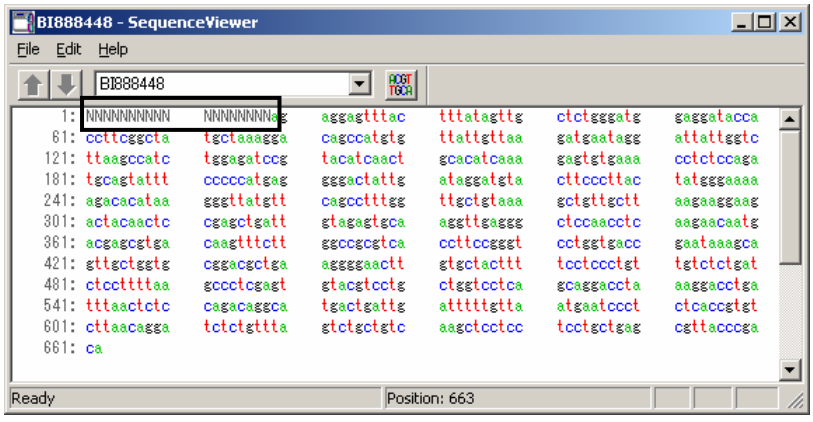
6.4.2 Trimming Results of Sequence Data

Double click on the trimmed sequence data.



Trimmed Sequence Data

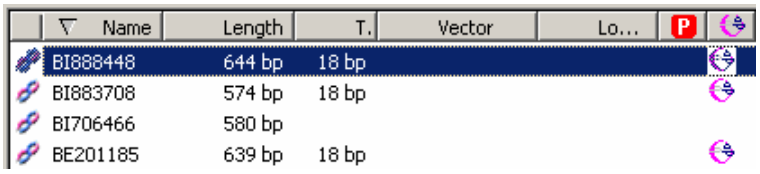
The Sequence Viewer will start up and display the trimmed sequence data. The series of N's are the areas that have been trimmed.



6.5 Reset Test Data

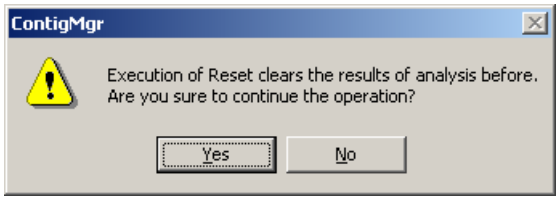
It is possible to restore the trimming to the original input sequence. However, note that this process does not only clear the results of trimming, but also the Phred and Phrap results for the corresponding data.

1. Select the trimming results to reset to the original sequence data from the List View.

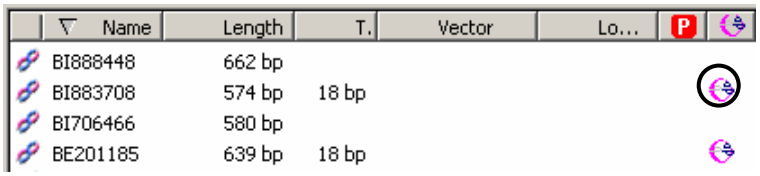


2. Click Reset To Original Sequence on the Navigation Toolbar.

3. A confirmation dialog will appear. Click Yes to go ahead and clear the Phred, trimming and Phrap results for the corresponding data.



4. The trimming results will be cleared, and the sequence will return to its previous status. The corresponding trimming icon will disappear, indicating that the trimming results have been cleared.



Chapter 7 Phrap Assembly

Contents in this section assume that the Phred/Phrap Option is installed.

7.1 Assembly

The procedure for assembling a sequence is as follows.

1. In List View, select the data to be assembled. (To select all items listed, press Ctrl+A or select Edit > Select All from the menu.)
2. Click Assemble in the Assemble group.
3. After a few moments the assembly is complete and the resulting contig displays in List View, with each fragment directional marker.

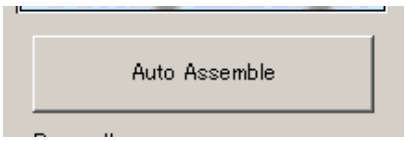
7.2 Fully Automatic Assembly

It is possible to perform automated processing for basecalling, trimming, and assembly, from settings in the Navigation Toolbar.

1. To use a trace data basecall as the original basecall (the sequence data described in the trace data file itself), select Original on the Navigation Toolbar. To use the Phred basecall, select Phred. This parameter has no effect if the input data is not trace.

2. To perform trimming, select the Trimming check box on the Navigation Toolbar. If no trimming is to be performed, clear the Trimming check box. See 6.1 “Vector Settings”, for detailed information on trimming settings.

3. Specify the assembly parameters. See 2.2.13 “Phrap Parameter dialog”, for detailed information on parameter settings.
4. In List View, select the data to be assembled. (To select all items listed, press Ctrl+A or select Edit > Select All from the menu.)
5. Click Auto Assemble on the Navigation Toolbar.



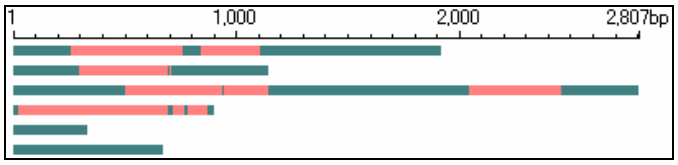
6. After a few moments the operation is complete and List View displays analysis results based on the settings.

7.3 Display Assembly Results

The items displayed for assembly results and generated contigs differ depending on the view.

7.3.1 Map View

In this view each contig is displayed as a single bar, with the quality value equal to or greater than the threshold value and the quality value lower than the threshold value shown in the specified colors settings.

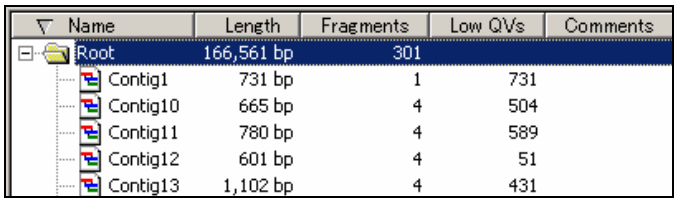


When a contig is selected in Map View, a list of the fragments composing it is displayed in List View.

7.3.2 Tree View

This view displays the contigs branching down from the folder containing them.

For each contig the contig name, contig length, number of fragments, number of low-QV bases, and comments are displayed.

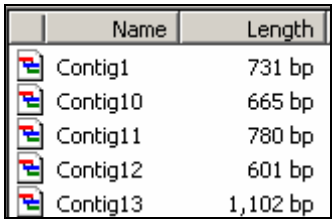


When a contig is selected in Tree View, a list of the fragments composing it is displayed in List View.

7.3.3 List View

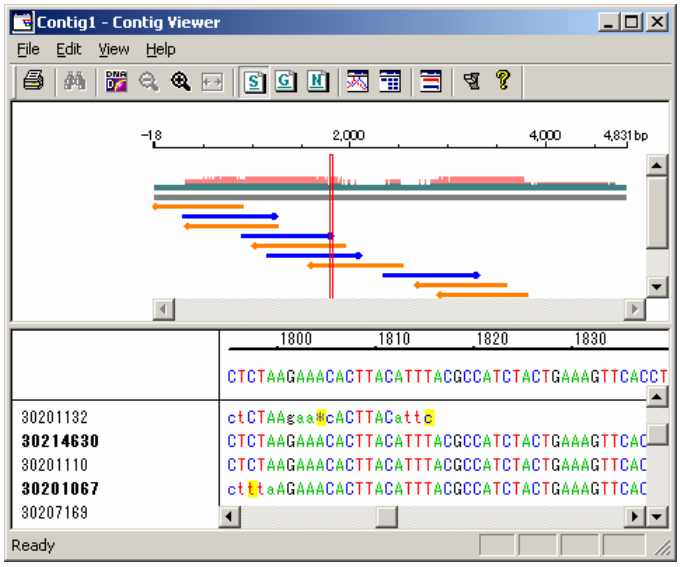
This view displays the contigs contained in the folder selected in Tree View.

For each contig the contig name and contig length are displayed.



7.4 Contig Detail View

In Map View, Tree View, or List View, you can start the Contig Viewer and view contig, fragment sequence and other detailed information, either by double clicking a contig or clicking Open on the Navigation Toolbar.

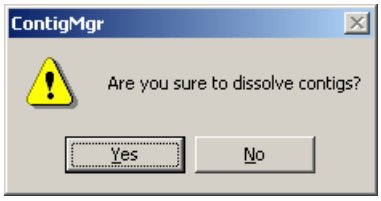


Refer to 8.1 “Contig Viewer Display”, for more information on the display items.

7.5 Dissolve Contigs

To dissolve contigs, select the contigs to be removed in Map View, Tree View, or List View and click Dissolve on the Navigation Toolbar.

A confirmation dialog with the message “Are you sure to dissolve contigs?” is displayed. Click Yes to dissolve.



The selected contigs are removed from the view and each fragment directional marker composing the contig disappear.

7.6 Reassemble Contig Sequences

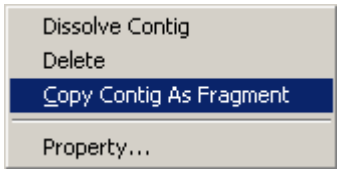
There are two methods for assembling contig sequences. Each method may produce different results, so it is important to select the method that is appropriate for the application.

7.6.1 Assemble a Contig as a Single Fragment

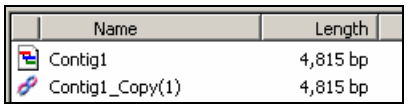
It is possible to assemble a contig as a single fragment.

This reduces the amount of computing time required if the number of fragments contained in the contig is large, but it may produce results that differ from those that would have been produced if data were added to the individual fragments before assembly.

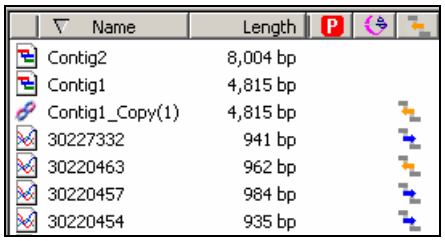
1. Right click a contig in the Tree View or List View.
2. Select Copy Contig As Fragment from the popup menu.



3. The contig sequence is copied as a fragment. The fragment is copied to the folder in which the contig resides.



4. The copied contig is treated exactly the same as other fragments. It can be used to assemble new contigs in the normal manner.

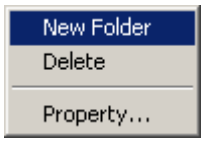


7.6.2 Assemble a Contig Using Constituent Fragments

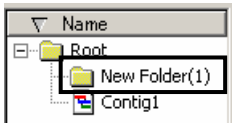
It is possible to add more fragments to the fragments composing a contig before performing assembly.

This requires more time than assembling a contig as a fragment because it is necessary to first dissolve the contig and then realign it, but the result reflects the data from each fragment more accurately.

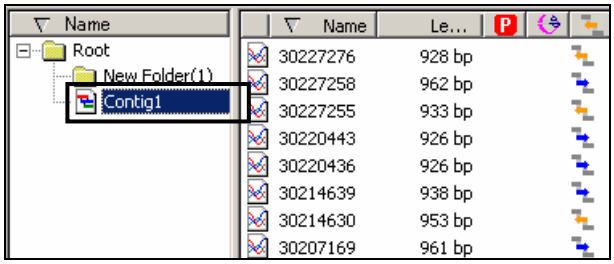
1. Create a new folder for storing all of the fragments composing the contig. Right click the Root folder in Tree View.
2. Select New Folder from the popup menu.



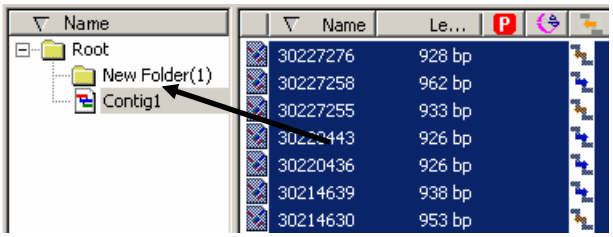
3. A new folder is created in Tree View.



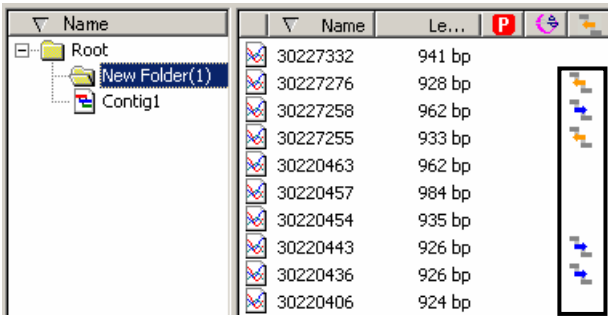
4. Select the contig to be assembled in Tree View. A list of the fragments composing the contig is displayed in List View.



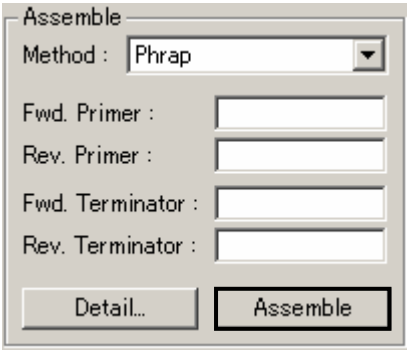
5. Select all of the fragments shown in List View and drag and drop them to the new folder. (To select all of the fragments, click in the List View display area and then press Ctrl+A or select Edit > Select All from the menu.)



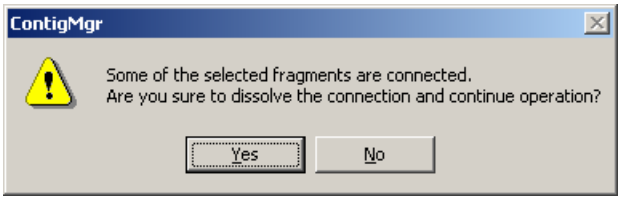
6. All of the fragments composing the contig are moved to the new folder. Next, add any other fragments to be assembled to the folder.



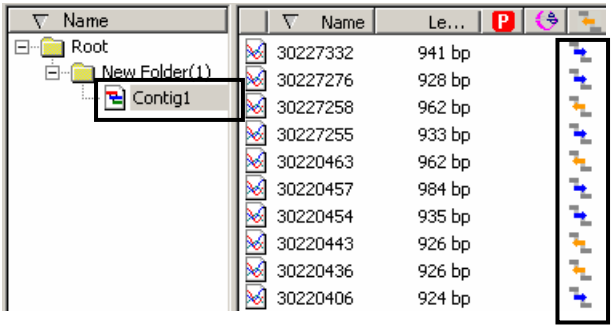
7. Select the new folder and select all of the fragments in the folder.
8. Click Assemble on the Navigation Toolbar.



9. The program begins to reassemble the fragments composing the contig, and displays a confirmation dialog with the message “Some of the selected fragments are connected. Are you sure to dissolve the connection and continue operation?” To dissolve the contig and realign it, with the added fragments included, click Yes.

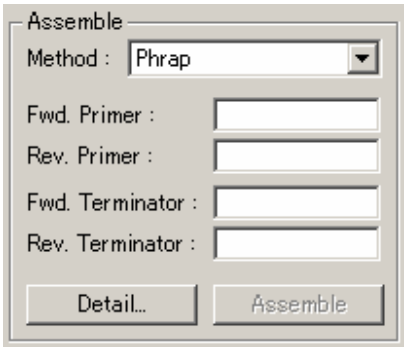


10. After a few moments the operation completes and the new contig is created.



7.7 Parameters

The assembly parameters on the Navigation Toolbar are listed below.



Navigation Toolbar Assembly Parameters

Item	Description
Method	Selects the algorithm used for assembly.
DNASIS Assemble	Performs assembly using the DNASIS Assemble algorithm.
Phrap	Performs assembly using the Phrap algorithm. When Phrap is selected, the following parameters are displayed. To use Phrap, you need to install the Contig Manager.
Fwd. Primer	Trace data is forward read using the dye primer method and when distinct name patterns are identified they are input. Forward linking is given priority if the direction cannot be determined. Example: "_FP" is input for C0001_FP, C0002_FP.
Rev. Primer	Trace data is reverse read using the dye primer method and when distinct name patterns are identified they are input. Reverse linking is given priority if the direction cannot be determined. Example: "_RP" is input for C0001_RP, C0002_RP.
Fwd. Terminator	Trace data is forward read using the terminator method and when distinct name patterns are identified they are input. Forward linking is given priority if the direction cannot be determined. Example: "_FT" is input for C0001_FT, C0002_FT.
Rev. Terminator	Trace data is reverse read using the terminator method and when distinct name patterns are identified they are input. Reverse linking is given priority if the direction cannot be determined. Example: "_RT" is input for C0001_RT, C0002_RT.

7.8 Advanced Parameters

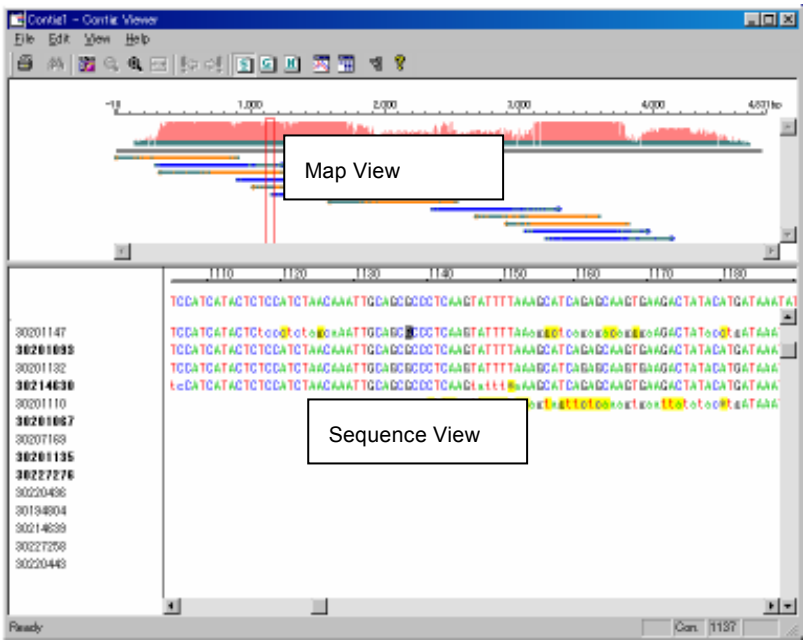
It is possible to set other parameters by clicking Detail... in the Assemble group on the Navigation Toolbar.

Refer to 2.2.12 "DNASIS Assemble Parameter dialog", and 2.2.13 "Phrap Parameter dialog", for an explanation of advanced parameters.

Chapter 8 Contig Editing

8.1 Contig Viewer

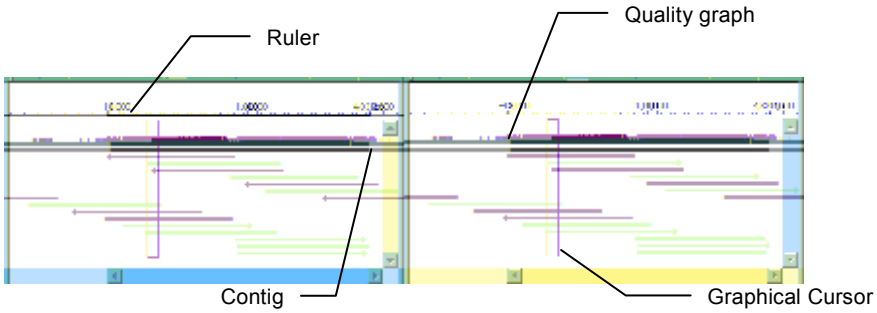
It is possible to view contig details in Contig Viewer by clicking on it in Contig Manager. The Contig Viewer window appears as shown below. The top pane is the Map View and the bottom is the Sequence View.



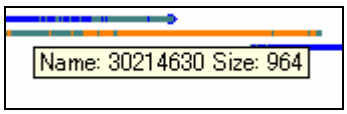
8.2 Contig Map View

This view displays the contig and the fragments that compose it in graphical form. The upper gray bar is the contig. Above that is the quality value (QV) bar graph quality value with color coded threshold values. The threshold value and the colors can be changed* by the user. Below the contig bar are fragment bars that compose the contig. A blue right-facing arrow indicates a fragment linked in the normal direction, and an orange left-facing arrow one that is linked by a complementary strand. The bar lengths and positions indicate the fragment positions and lengths within the contig sequence. In addition, the dark colored parts of fragment bars are of lower quality than the threshold value.

* Refer to 2.3.6 “Contig Viewer Parameters” for details.



Move the mouse pointer over a contig or fragment bar to display the name, size, and number of sequences for the corresponding contig or fragment.

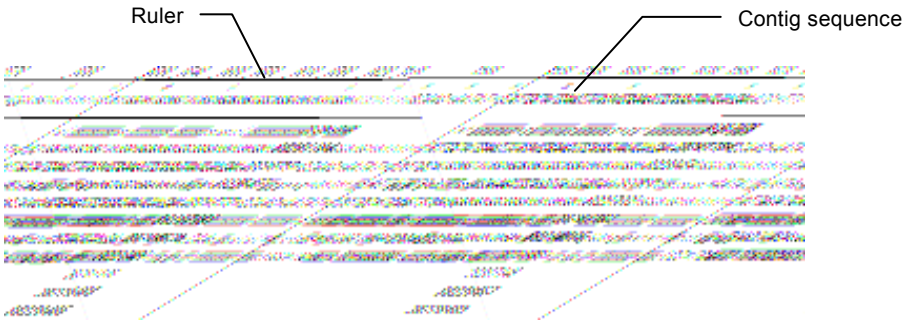


Map View can be resized horizontally. Click the icon on the Toolbar to expand the view one step and the icon to reduce the view one step. Click the icon to restore the 100% display.

The red rectangular box is the cursor. The portion inside the box is displayed in expanded form in the Sequence View. When the red box is dragged with the mouse to a different location the content shown in the Sequence View changes to match.

8.3 Sequence View

This view displays the alignment of contig and fragment sequences.



Alignment

The sequence shown at the very top is the contig (consensus) sequence, and the lines displayed below it each correspond to fragment sequences. The names of the fragment sequences are displayed at the left edge of the window. The names of fragments linked by complementary strands are displayed in bold type. The colors and fonts used to display different bases can be changed from the Preferences dialog. Refer to 2.3.6 “Contig Viewer Parameter Sequence Tab” for details.

Ruler

A ruler is displayed above the contig sequence. Since the 5' end of a contig sequence is always 1bp, the ruler may begin with a negative bp value if there is a portion of a fragment sequence that does not compose part of the contig. In addition, the ruler is calculated with gaps included.

Mismatch Display

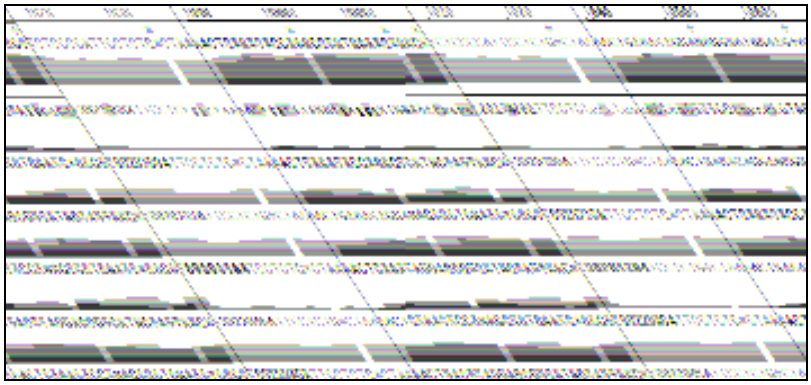
Bases that do not match the contig sequence are highlighted in yellow. Gaps are indicated by an asterisk (*). The display colors can be changed from the Preferences dialog. Refer to 2.3.6 “Contig Viewer Parameter Sequence Tab” for details about this dialog.

Scrolling

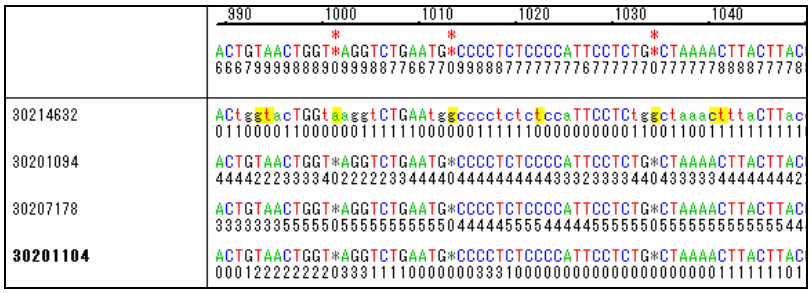
The alignment sequence can be scrolled to the left or right using the horizontal scroll bar at the bottom of the Sequence View pane or by moving the cursor in Map View. It is possible to have the display automatically scroll vertically so that the bases are always displayed at the top of the Sequence View. To select this option, clear the View > Auto Scroll check.

Quality Value Display

It is possible to display quality values below the base values of contig and fragment sequences. To select this option, select View > Display Type > Quality Map from the menu or click  on the Toolbar. The quality values of the bases are indicated by bar graphs.



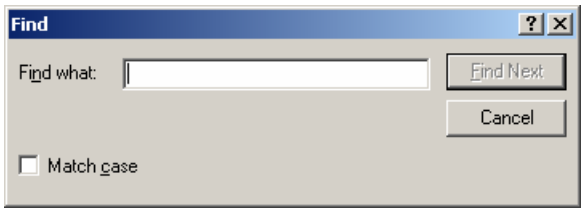
Alternately, select View > Display Type > Quality Numeric from the menu or click  on the Toolbar to display the second digit of the quality values in numeric form.



8.4 Search a Consensus Sequence

It is possible to search for data within a consensus sequence.

1. In Sequence View, click a consensus sequence. The base clicked on is selected and highlighted.
2. The search menu Toolbar becomes active. Next, select Edit > Find from the menu or click  on the Toolbar.
3. A dialog like the one shown below is displayed. Input the sequence of bases to be searched for in the text box then click Find Next.



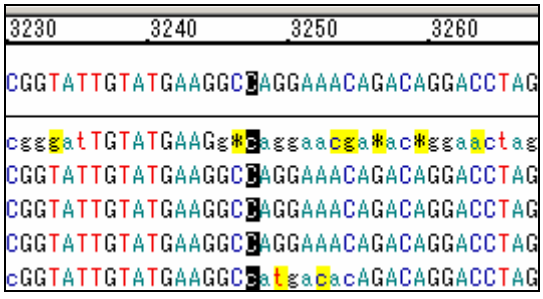
4. The search begins from the selected base and proceeds toward the 3' end. Matching portions of the sequence are displayed in pink and highlighted. Clicking Find Next again causes the program to search for the next match. The matches found thus far remain displayed in yellow.
5. Clicking Cancel clears all highlighted items and closes the search dialog.

8.5 Trace Display

It is possible line up the actual trace data in order to evaluate the assembly results.

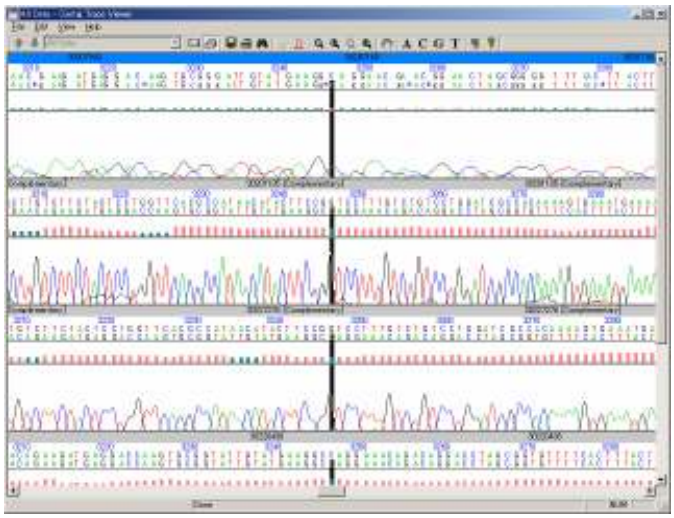
8.5.1 Trace Display Methods

1. Click the base in the contig sequence you want to display as trace data to select it. Clicking as base in the contig sequence causes the corresponding bases in the fragment sequences to be selected as well. Clicking a base in a fragment sequence will only select that base.

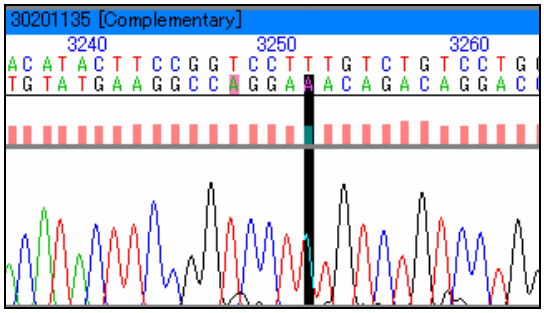


2. Select View > Chromatograms from the menu or click  on the Toolbar. The relevant trace data is displayed, with the selected base in the center. This step can be omitted if trace data is already being displayed.

8.5.2 How to Read the Trace Display



The display consists of four sections. They show, starting from the top, the sequence name, the bases, a graph indicating quality, and the traces. Since data for multiple sequences is displayed, arranged from top to bottom, the data displayed on one line does not wrap to the next line. To see more of a particular sequence, use the horizontal scroll bar to scroll the display.



Sequence Name

A sequence name with a blue background indicates the target of menu and toolbar operations. To change the target, click a different area of the trace data. In addition, if “Complementary” appears after the sequence name, the corresponding sequence is linked by complementary strands in the contig. In this case the trace and original base sequence are displayed with left and right reversed.

Base Display

The sequences displayed below show the original data read from the trace files. The sequence displayed at the top shows original sequence stored in the trace file. The sequences displayed below are the assembled sequences (that were linked together). That is, the sequences that were basecalled using Phred. They may have been edited by the user, have had gaps inserted by the assembly process, or have become linked by complementary strands.

Quality Graph

Base quality in graphical format. The display color differs depending on whether the quality value is higher or lower than the threshold value set by the user. The user can set the display color and threshold value.

Trace Display

The trace data is displayed based on the ABI and SCF formats.

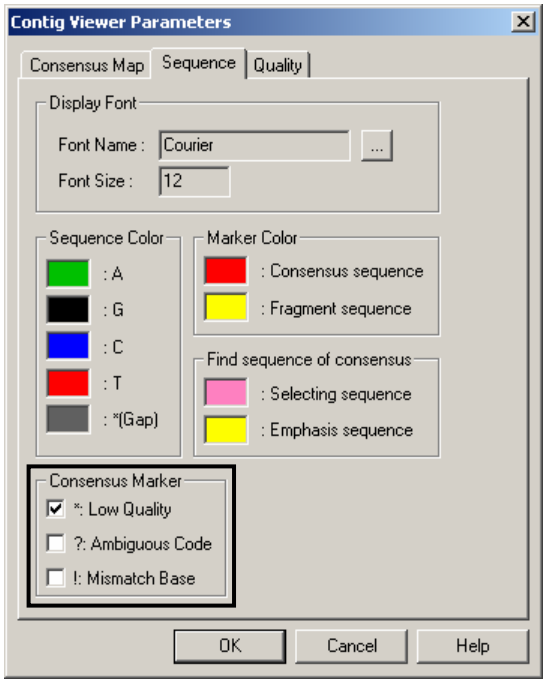
8.6 Markers

It is possible to display marks on the contig sequence.

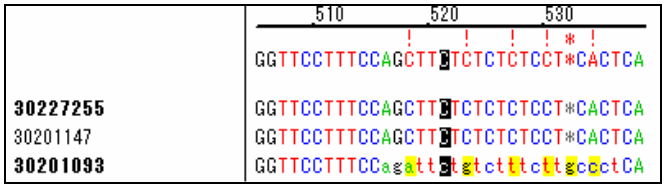
8.6.1 Marker Settings

1. Select View > Preferences... from the menu or click on the Toolbar.
2. Select the Sequence tab from the dialog that is displayed.
3. Under the Consensus Marker item, select the check boxes for the items you wish to display as marks.

   Refer to 2.3.6 “Contig Viewer Parameter” for a description of the Preferences dialog.



4. Click OK. The markers are displayed above the contig sequence.



8.6.2 Jumping to Markers on the Contig Sequence

After specifying marker types to display, it is possible to jump to marked bases in the contig sequence. Click the contig series in Sequence View. Now, clicking (or) causes the focus to jump from the current cursor position to the next marker to the right (or to the left). It is possible to continue jumping until you reach the marker closest to the end of the contig sequence.

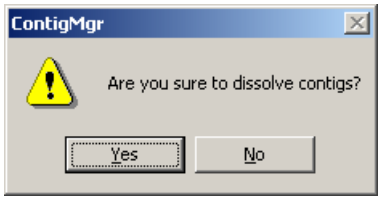
8.6.3 Jumping to Markers on Fragment Sequences

It is possible to jump to positions where the fragments and contig sequence do not match. Click on a fragment sequence in Sequence View. Now, clicking  (or ) causes the focus to jump from the current cursor position to the next place to the right (or to the left) where there is a mismatch between the fragment and the contig sequence. It is possible to continue jumping until you reach the marker closest to the end of the fragment sequence.

8.7 Dissolve a Contig

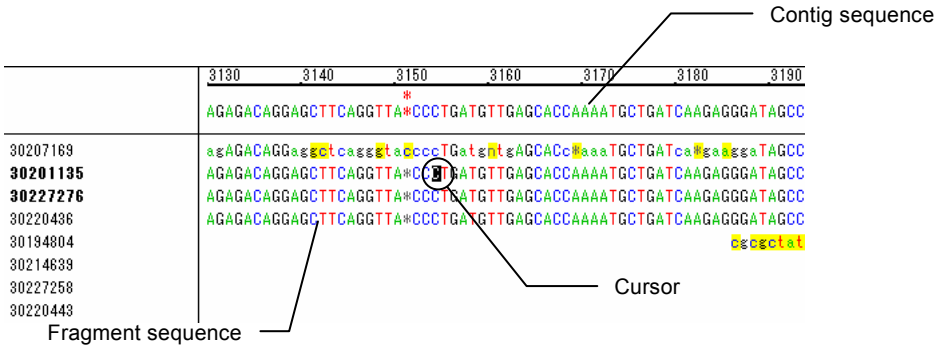
It is possible to dissolve a contig created previously. This is performed from the project window.

1. In Map View, Tree View, or List View, select the contig you wish to dissolve. Then click Dissolve on the Navigation Toolbar. Alternately, right click on the contig and select Dissolve Contig from the popup menu, or select Contig > Dissolve Contig from the menu.
2. A confirmation dialog with the message “Are you sure to dissolve contigs?” is displayed. Click Yes to dissolve the contig.



8.8 Sequence Editing

It is possible to edit the base sequence of a contig, or the base sequences of the fragments that compose the contig. The editor supports replace, delete, and backspace operations. The editing unit is one base only. It is not possible to define a range of bases and then apply the replace, delete, or backspace operations.



8.8.1 Contig Sequence Editing

1. Click a contig base sequence.
2. The cursor moves to the base sequence you clicked. (The base sequences of the contig on which the cursor is positioned and the fragments composing it are highlighted.)
3. Move the cursor to the place in the base sequence you wish to edit.

4. To move the cursor, either use the ← and → keys or click the base sequence you wish to edit directly with the mouse.

Replace	Move the cursor to the replace position in the base sequence. Input the replacement using the A, G, C, or T key. When the replacement is made in the contig base sequence, the corresponding location in the consensus sequence will also be replaced with the input base value.

8.8.2 Fragment Sequence Editing

1. Click a fragment base sequence.
2. The cursor moves to the base sequence you clicked. (The base sequence on which the cursor is positioned is highlighted.)
3. Move the cursor to the place in the base sequence you wish to edit.
4. To move the cursor, either use the ↑, ↓, ↑, and → keys or click the base sequence you wish to edit directly with the mouse.

Replace	Move the cursor to the place in the base sequence where the replacement is to take place. Input the replacement using the A, G, C, or T key. When the replacement is made in the fragment base sequence, the corresponding location in the contig sequence is replaced with an ambiguous code.
Backspace	Move the cursor to the place in the base sequence where the backspace is to take place and press the Backspace key. The base at the cursor position is moved backwards one space, replacing the base that was to its left. If there is a consensus at the same position as the cursor position, the base in the consensus base sequence is replaced with an ambiguous code. In addition, the portion of the consensus extending to the right from the cursor position to the data end is all replaced with an ambiguous code.
Delete	Move the cursor to the place in the base sequence where the deletion is to take place and press the Delete key. The base at the cursor position is deleted. If there is a consensus at the same position as the cursor position, the base in the consensus base sequence is replaced with an ambiguous code. In addition, the portion of the consensus extending to the left from the cursor position to the data end is all replaced with ambiguous codes.

Note: The backspace or delete function can only be performed once.

8.9 Reassembly after Removing Sequences

It is possible to select some of the fragments composing a contig for removal and then reassemble the contig.

In the project window, select the fragments you wish to remove. It is possible to select multiple fragments at once.

Click Remove on the Navigation Toolbar. Alternately, select Contig > Remove Selected Sequences from the menu bar or right click on one of the selected fragments and select Remove selected sequences from the popup menu.

A confirmation dialog with the message “This may dissolve the contig. Do you wish to continue?” is displayed. Click Yes to proceed with reassembly of the contig.

8.10 Display Color and Font Settings

It is possible to change the colors used to display traces and bases.

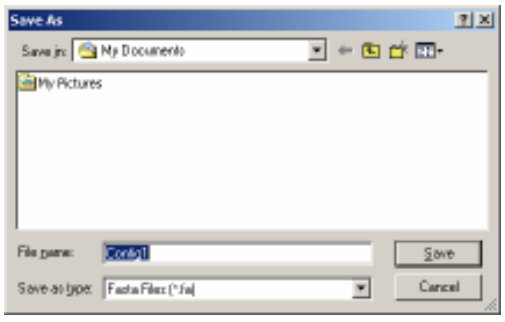
1. Select View > Preference from the menu or click on the Toolbar. The Preferences dialog is displayed.

2. Enter the desired settings and click OK. Refer to 2.3.6 “Contig Viewer Parameters” for a description of the Preferences dialog.

8.11 Export Contig Sequences

It is possible to export contig sequences. The output files use the Fasta format. If multiple contigs are selected for export, they are saved in the Multi-Fasta format.

1. In the project window, select the data you wish to export.
2. In Contig Viewer, the displayed contig sequence is exported.
3. Select File > Export Sequence > Export Sequence... from the project window menu. The Save As dialog is displayed.
4. In Contig Viewer, select File > Export Contig... from the menu.

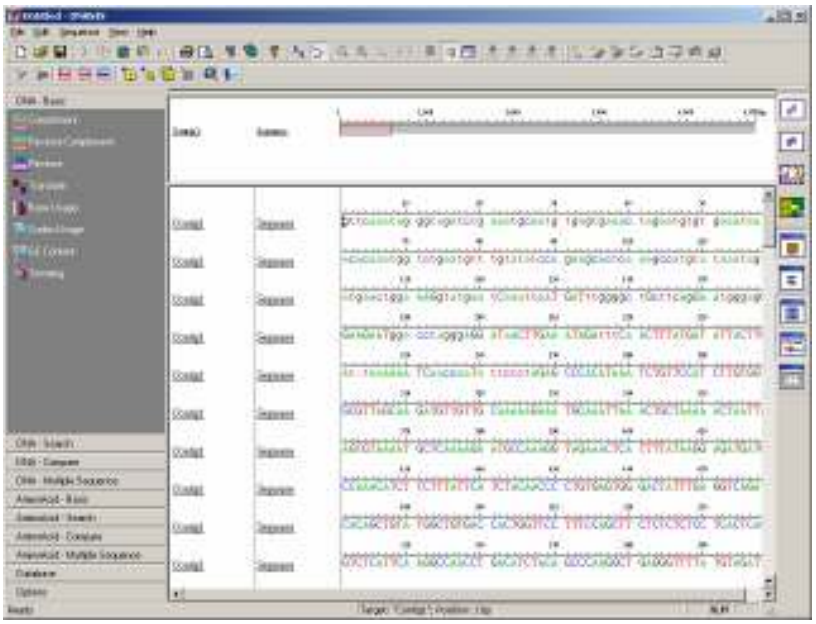


5. Specify the file name and the location then click Save.

8.12 Use DNASIS MAX to Analyze a Consensus Sequence

It is possible to use DNASIS MAX to analyze the contigs you create.

1. In the project window Map View, click the contig you wish to analyze to display it in Contig View.
2. Select File > Export Contig to DNASIS MAX, or click  on the Toolbar. DNASIS MAX starts and the sequence is displayed.

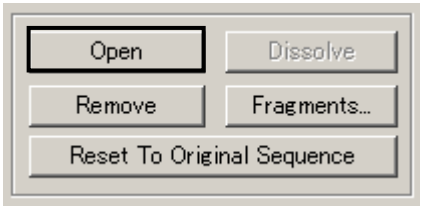


- Refer to the DNASIS MAX user's manual for details on DNASIS MAX analysis procedures

Chapter 9 Trace Display

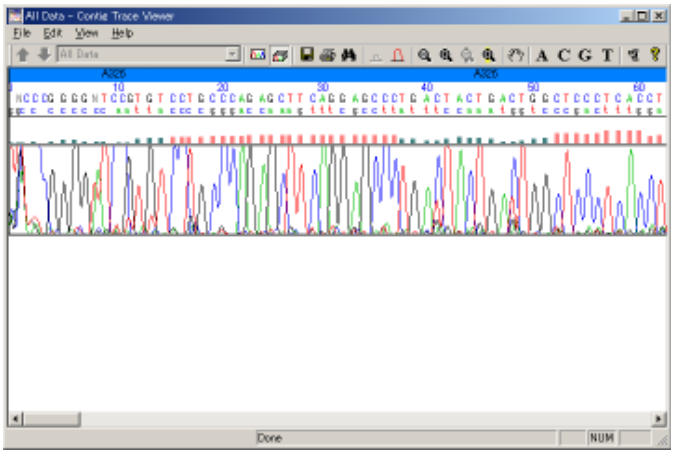
9.1 Open Trace File from the Project Window

It is possible to display a trace graphically by double clicking on a trace data fragment in Contig Manager. Alternately, you can select a trace data fragment and click Open on the Navigation Toolbar. The Open button is located in the lower part of the Navigation Toolbar (see figure below).



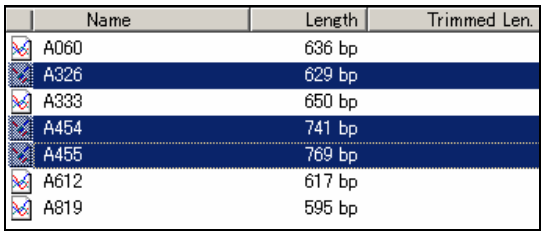
Performing one of the above operations causes the Contig Trace Viewer to open with the trace of the specified trace data fragment displayed.

For details, refer to 8.5.2 “How to Read the Trace Display”.



It is also possible to display multiple trace data fragments at the same time.

Select multiple trace data fragments in Contig Manager (see figure below). With the fragments still selected, click Open on the Navigation Toolbar.



Name	Length	Trimmed Len.
A060	636 bp	
A326	629 bp	
A333	650 bp	
A454	741 bp	
A455	769 bp	
A612	617 bp	
A819	595 bp	

Performing the above operation displays the trace data fragments from the top downwards. The display order matches the selection order of the trace data fragments in Contig Manager.

9.2 Open Trace Files from the Contig Trace Viewer

It is possible to display the traces of trace data fragments linked to a contig displayed in the Contig Trace Viewer window.

Select a contig in Contig Manager. Then double click it or click Open on the Navigation Toolbar to open the Contig Viewer.

Continue with the procedure below.

1. Click the base in the contig sequence you wish to display. This will select it. The base in the selected contig sequence and the bases in the corresponding positions in the fragments composing the contig sequence are highlighted.

	1090 1100 1110 1120 1130
	GAGATTAAGCCATAAAGGTGGAGTCCATGTTCGTGGGCTT*AGGACCT
30214632	GAGATTAAgccctaAAGGTGGAGTCCATGTTCGTGGGCTtaaggac*t
30201094	GAGATTAAGCCATAAAGGTGGAGTCCATGTTCGTGGGCTt*aaggccg
30207178	GAGATTAAGCCATAAAGGTGGAGTCCATGTTCGTGGGCTT*AGGACCT
30201104	GAGATTAAGCCATAAAGGTGGAGTCCATGTTCGTGGGCTT*AGGACCT
30220454	GAGATTAAGCCATAAAGGTGGAGTCCATGTTCGTGGGCTT*AGGACCT
30220457	GAGATTAAGCCATAAAGGTGGAGTCCATGTTCGTGGGCTT*AGGACCT
30227332	GAGATTAAGCCATAAAGGTGGAGTCCATGTTCGTGGGCTT*AGGACCT

2. Select View > Chromatograms from the menu, or click  on the Toolbar. The contig sequence and the fragment sequences are displayed with the position of the selected base displayed in black and aligned vertically, as shown in the figure below.

9.3 Export Trace Files

Trace fragment data can be stored as a FASTA file or as an SCF file. You can select them as sequence to be saved as “the sequence originally basecalled by the sequencer” or “the sequence basecalled by Phred.”

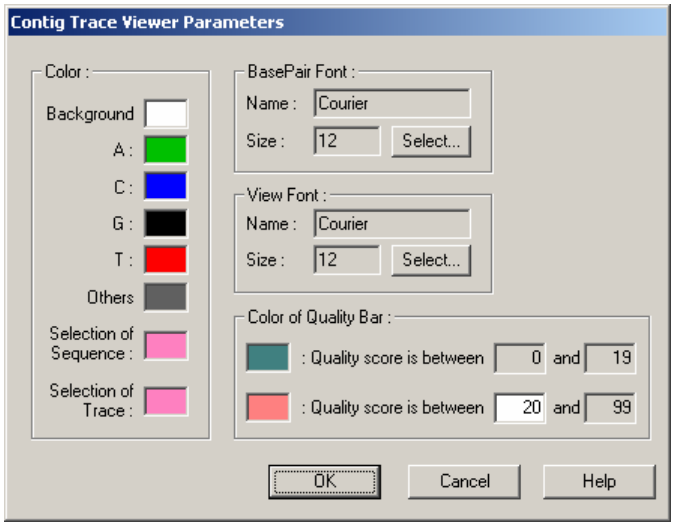
1. To store the sequence basecalled by the sequencer, select File > Export... from the menu. To store the sequence basecalled by Phred, select File > Export Phred... from the menu.

   In either case, the Save As dialog opens.
2. Specify the output destination, the filename, and the file type, then click the Save button. The available formats for saving files are FASTA (*.na) and SCF (*.scf).

9.4 Display Color Settings

It is possible to change the display colors for the background, traces, bases, quality values, and also the font used to display bases.

1. Select View > Preferences from the menu, or click  on the Toolbar. A dialog displays like the one shown below.



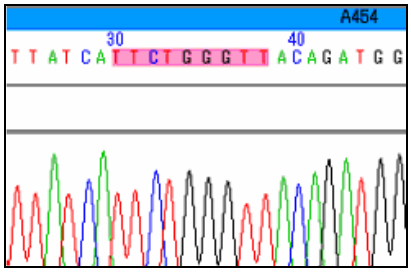
2. Enter the desired settings and click OK. Refer to 2.4.5 “Trace Viewer Parameters”, for a description of the Preferences dialog.

9.5 Search a Sequence

It is possible to search for a specific sequence within a contig sequence or fragment sequence.

1. Select Edit > Find from the menu or click  on the Toolbar. The Search dialog is displayed.
2. Input the search string of letters (bases) in the text box.

3. Click Find Next.
4. If a matching string is found, that string is selected.

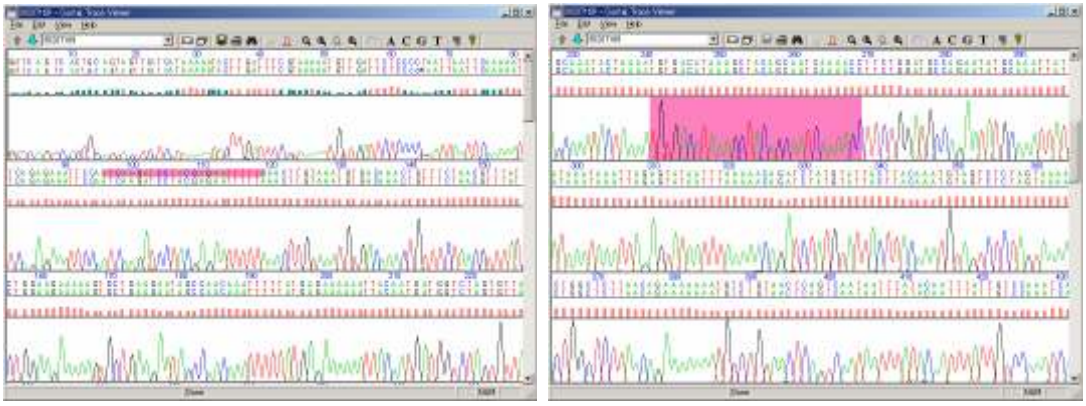


- You can click Find Next to continue searching as long as there are matching base sequences left. To quit the search, click Cancel. In the parallel data display mode the search covers all the data that is being displayed. If you select a sequence of bases in the data that you wish to search and then open the Find dialog, that string of bases is entered in the text box for you.
- It is only possible to search for base sequences within the original basecall base sequences output by the sequencer.

9.6 Operations within Trace Viewer

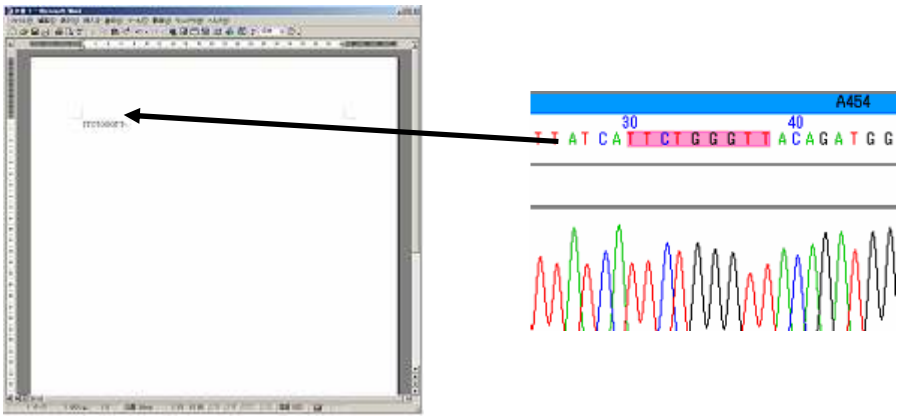
9.6.1 Select Bases and Traces

Drag the cursor along the portion of the base sequence or the trace you wish to select. The selected portion of the base sequence or trace changes color. To cancel the selection click outside of the selected area.



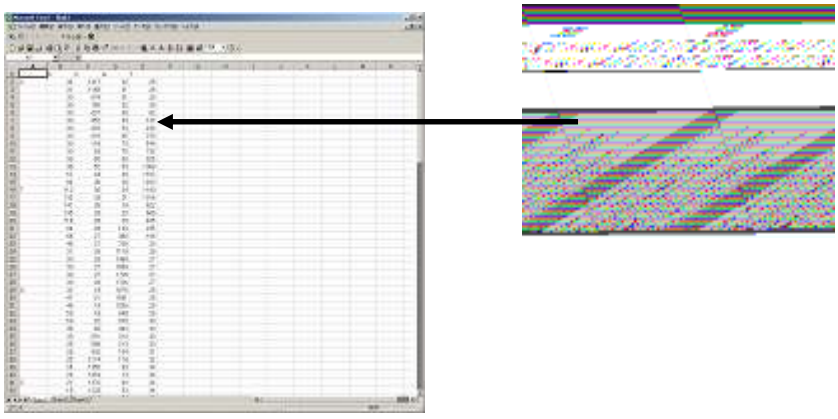
9.6.2 Copy Bases

Select a portion of a base sequence and select Edit > Copy from the menu. The selected base sequence is copied to the clipboard and can be copied into another application, such as Microsoft Word, for editing. It is also possible to drag and drop sequences to paste them directly to a new location.



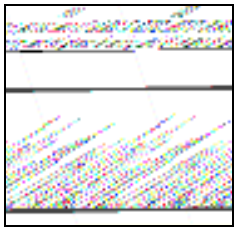
9.6.3 Copy Traces

Select a portion of a trace and select Edit > Copy from the menu. The base traces for the selected portion of the trace are copied to the clipboard in tabular format and can be copied into another application, such as Microsoft Excel, for editing. It is also possible to drag and drop portions of traces to paste them directly to a new location. The selected portion of a trace cannot be pasted as an image.



9.6.4 Hide Specific Traces

To hide traces for A, C, G, or T (one or more), click the corresponding lane button (or buttons). The specified traces disappear from the display and the associated bases are displayed in an italic font. For example, click A to hide A traces. The display then appears as shown below.



Chapter 10 Output

This chapter explains how to print and how to copy windows. For information on project and sequence export functions please refer to chapter 4, Import and Export.

10.1 Printing

It is possible to print the various views from Contig Manager. The procedure and the print format are described in this section.

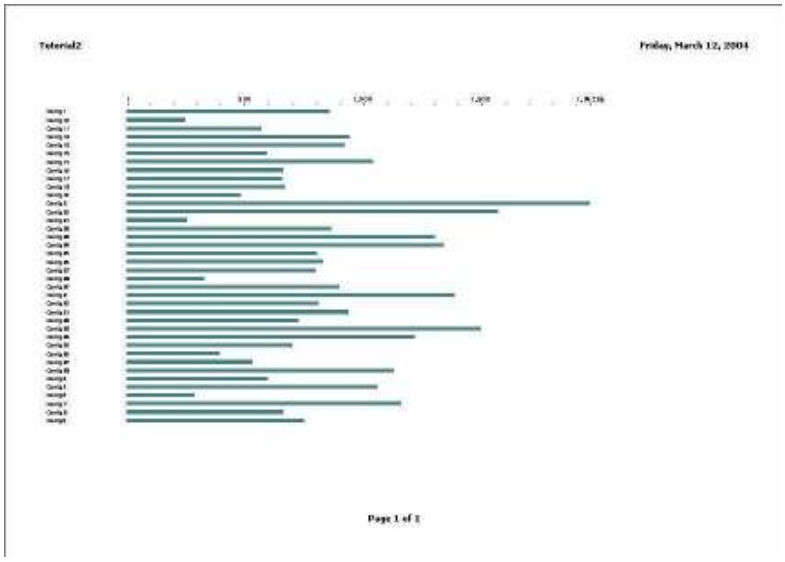
10.1.1 Print from the Project Window

From the project window you can print the Map View, Tree View, and List View.

Print the Map View

Click in the Map View pane. Then select File > Print... from the menu or click  on the Toolbar. The data print range is what currently displays in Map View.

You can display a preview showing what the printout will look like by selecting File > Print Preview from the menu.



- The page orientation setting should be set to landscape before printing. Printing with the orientation set to portrait can result in output that is partially cut off. Use the Print Setup dialog to set the paper orientation. To display this dialog, select File > Print Setup... from the menu.

Print the Tree View

Click in the Tree View pane. Then select File > Print... from the menu or click  on the Toolbar.

Print the List View

Click in the List View pane. Then select File > Print... from the menu or click  on the Toolbar.

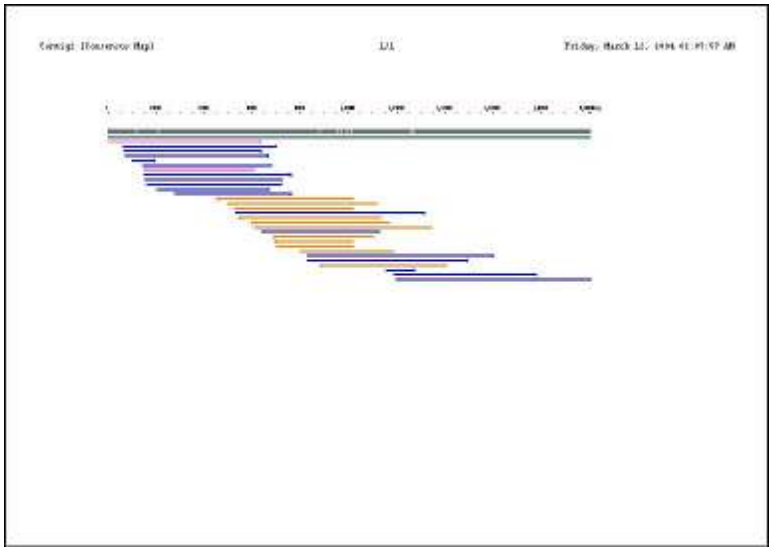
10.1.2 Print from the Contig Viewer

From the Contig Viewer you can print the Map View and Sequence View.

Print the Map View

Click in the Map View pane. Then select File > Print... from the menu or click  on the Toolbar. The data print range is what currently displays in Map View.

You can display a preview showing what the printout will look like by selecting File > Print Preview from the menu.

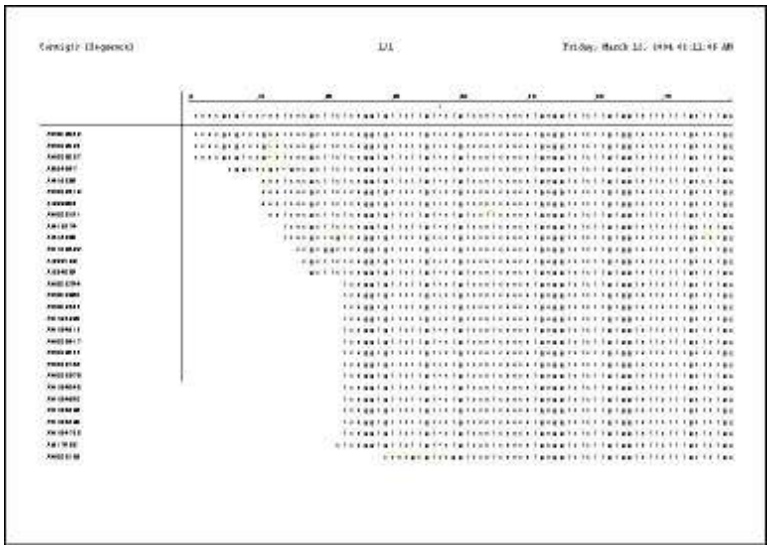


- The page orientation setting should be set to landscape before printing. Printing with the orientation set to portrait can result in output that is partially cut off. Use the Print Setup dialog to set the paper orientation. To display this dialog, select File > Print Setup... from the menu.

Print the Sequence View

Click in the Sequence View pane. Then select File > Print... from the menu or click  on the Toolbar. The data print range is what currently displays in Sequence View.

You can display a preview showing what the printout will look like by selecting File > Print Preview from the menu.

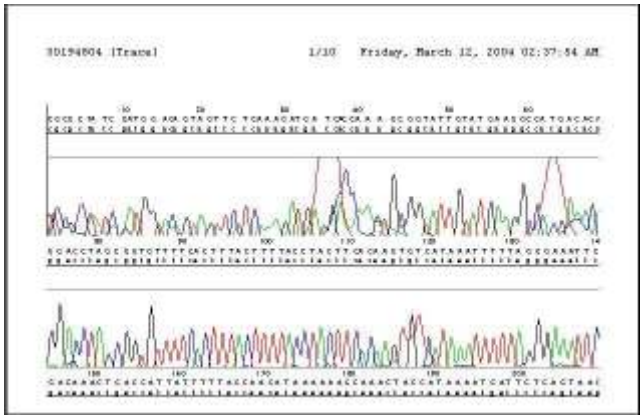


- The page orientation setting should be set to landscape before printing. Printing with the orientation set to portrait can result in output that is partially cut off. Use the Print Setup dialog to set the paper orientation. To display this dialog, select File > Print Setup... from the menu.

10.1.3 Print the Trace View

From the Trace View window, select File > Print... from the menu or click  on the Toolbar. The printed output appears as shown below. All of the data is printed.

You can display a preview showing what the printout will look like by selecting File > Print Preview from the menu.



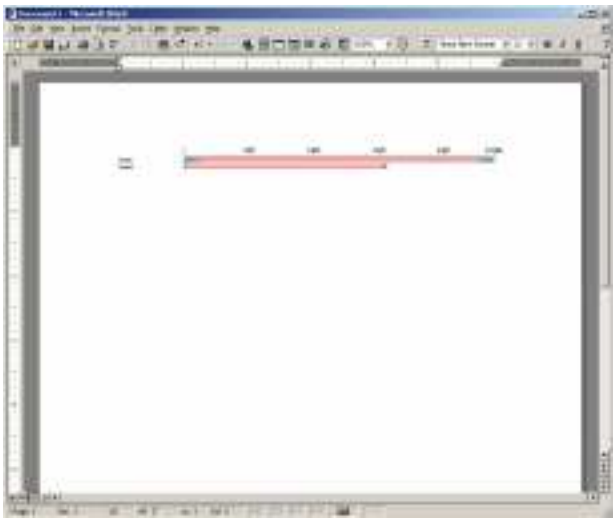
- The page orientation setting should be set to landscape before printing. Printing with the orientation set to portrait can result in output that is partially cut off. Use the Print Setup dialog to set the paper orientation. To display this dialog, select File > Print Setup... from the menu.

10.2 Copying Images

10.2.1 Copy from the Project Window

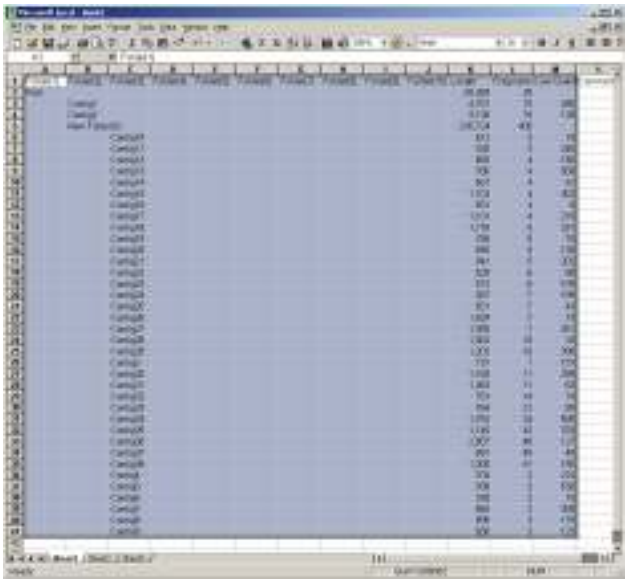
Map View

Click in the Map View pane. Then select Edit > Copy from the menu or click  on the Toolbar. The Map View data is copied to the clipboard. The graphics are copied as vector data, so they can be copied into other applications, such as Microsoft Word, and printed out in high quality.



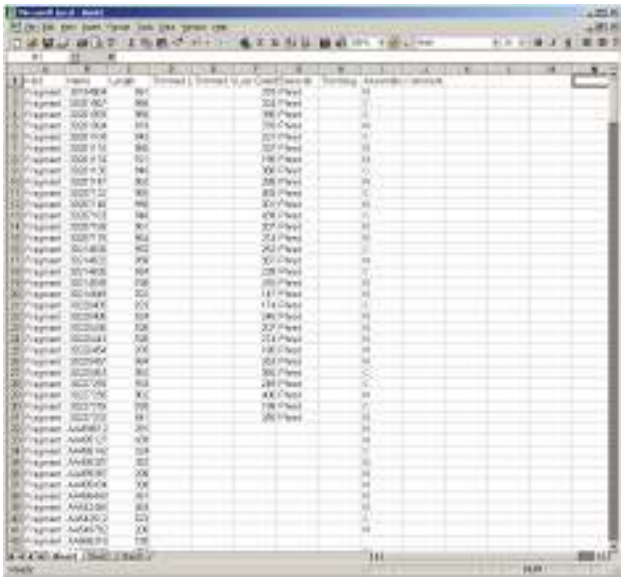
Tree View

Click in the Tree View pane. Then select Edit > Copy from the menu. All the data in the view is copied to the clipboard as tab-delimited text. It can then be copied into other applications, such as Microsoft Excel, and edited as needed.



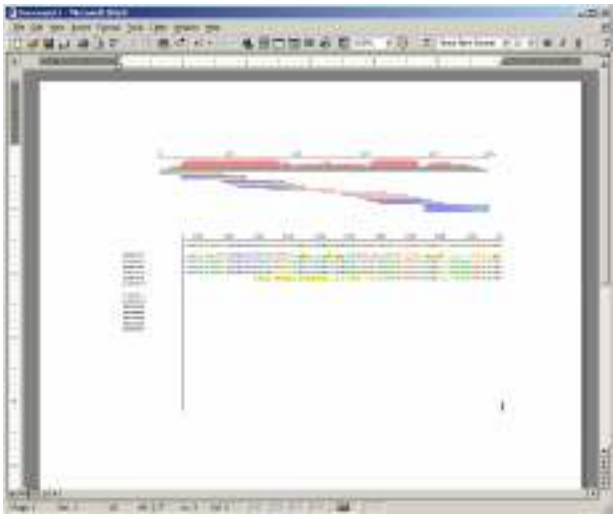
List View

Click in the List View pane. Then select Edit > Copy from the menu. All the data in the view is copied to the clipboard as tab-delimited text. It can then be copied into other applications, such as Microsoft Excel, and edited as needed.



10.2.2 Copy from the Contig Viewer

Click in the pane of the View you wish to copy from. Then select Edit > Copy from the menu. In Map View and Sequence View the range of data copied to the clipboard is what currently displays in the View. The graphics are copied as vector data, so they can be copied into other applications, such as Microsoft Word, and printed out in high quality.



10.2.3 Copy from the Trace Viewer

Select the part of the sequence you wish to copy. Then select Edit > Copy from the menu. The selected part is copied to the clipboard as text data. In parallel display mode the sequence to copy from should first be made active.

Index

T

V

Appendix A: User Support Contact Information:

Please use the following information to contact your regional support center with questions on using or purchasing DNASIS® MAX.

MiraiBio, Inc.: (The Americas, Asia, and Pacific Regions)
1201 Harbor Bay Parkway Ste. 150
Alameda, CA 94502
U.S.A
Web: www.miraibio.com
Tel: +1-510-337-2000
Fax: +1-510-337-2099
E-mail: dnasiskey@miraibio.com (For obtaining key codes)
gene@miraibio.com (For other information or inquiries).

Hitachi Software Engineering Europe AG: (Europe, Middle East, and Africa Regions)
Neues Kranzler Eck
Kurfürstendamm 22
10719 Berlin
Germany
Web: www.hitachisoft-bio.com
Tel: 49-30-8877-2600
Fax: 49-30-8877-2610
E-mail: dnasis@hitachisoft.de (For obtaining key codes)
info-bio@hitachisoft.de (For other information or inquiries).