

Vector NTI Advance™ 11 Quick Start Guide

Catalog no. 12605050, 12605099, 12605103

Version 11.0
December 15, 2008
12605022

Published by:
Invitrogen Corporation
5791 Van Allen Way
Carlsbad, CA 92008
U.S.A.

www.invitrogen.com

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Introduction

This Quick Start Guide is designed to get you started using **Vector NTI Advance™ 11**. It provides brief descriptions of the **Vector NTI Advance™ 11** graphical user interface, including **Vector NTI Explorer** and the **Molecule Viewer**, and step-by-step instructions for using the most common features and functions of the software. The topics covered include locating the desired tools, displaying molecules, designing PCR primers, cloning two fragments, gene synthesis, aligning molecules, performing a restriction analysis, and assembling contigs.

This guide assumes that you have a working knowledge of basic Microsoft® Windows® and Mac OS® features and functions (how to open and save files, how to use your mouse, and so on) and that **Vector NTI Advance™ 11** is installed on your computer.

Opening Vector NTI Advance™ 11

The **QuickStart Page** is a single page that consolidates most commonly used modules, tools, and utilities that Vector NTI Advance provides.

To launch the QuickStart Page, select **Start > All Programs > Invitrogen > Vector NTI Advance 11 > Quick Start**.



Figure 1. QuickStart Page


You can configure the software to open both the **Molecule Viewer** and **Vector NTI Explorer** when you select **Vector NTI** from the **Start** menu.

1. In the **Molecule Viewer** window, go to the **Edit** menu and select **Options**.
2. In the **General** tab of the dialog, select the **Open Local Explorer At Startup** checkbox.
3. Click **OK** to make the change.

Local Database

Vector NTI Explorer is the main tool for accessing the information in your local **Vector NTI Advance™** database. Using the **Explorer**, you can import, open, export, and organize molecules and other database items, and launch other **Vector NTI Advance™** modules (Figure 2).

To launch **Vector NTI Explorer**:

- On **QuickStart Page**, click on **Local Database**.
- In the **Molecule Viewer**, click on the **Local Database icon** ().
- From the Windows® Start menu, select Programs | Invitrogen | **Vector NTI Advance 11** | Vector NTI Explorer.
- The local database in **Vector NTI Advance™** contains records for different types of molecular biology objects. Each database record includes all the information for that object (e.g., a DNA molecule record includes the DNA sequence, defined features of the molecule, and other information). Objects in the database can include molecules, analysis results, BLAST search results, citations, and other types of information.

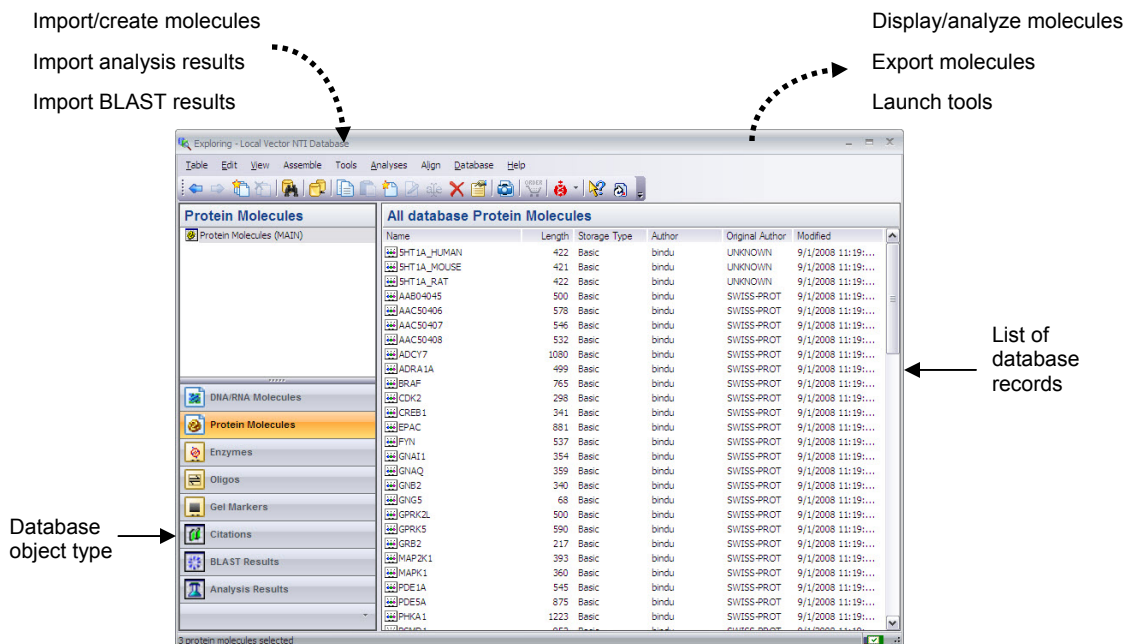


Figure 2. Vector NTI Explorer (Local Database) window.

Database objects in **Vector NTI Advance™** are categorized by type (DNA molecules, protein molecules, and so on). Some molecules are installed with the software. When you first open the software, **DNA/RNA Molecules** is the selected object type. Click on the tab in the lower left corner of the **Vector NTI Explorer** to select from the other available database objects.

To open an object from the local database, double-click on the object name in the right-hand pane of the **Explorer**. Depending on the object type, information about that object may be displayed in a dialog box, or the object may be loaded into a viewer. For example, DNA, RNA, and protein molecules are displayed in the **Molecule Viewer**.



When you install **Vector NTI Advance™**, the default local database is created in a folder called **VNTI Database** in the root directory of your computer (e.g., C:\VNTI Database).

Database Backup/Restore

It is strongly recommended that the local database be backed up routinely. You may launch the Database Backup manually, or use the Database Backup Reminder to trigger the task automatically as configured (Figure 3).

To manually perform Database Backup:

- From the **Vector NTI Explorer** menu, select Database | **Backup Database Now**.

To configure the **Database Backup Reminder**:

- From the **Vector NTI Explorer** menu, select Database | **Database Backup Reminder**.
- To set a specific date or interval (e.g., backup every 15 days), click on **Set Reminder**.

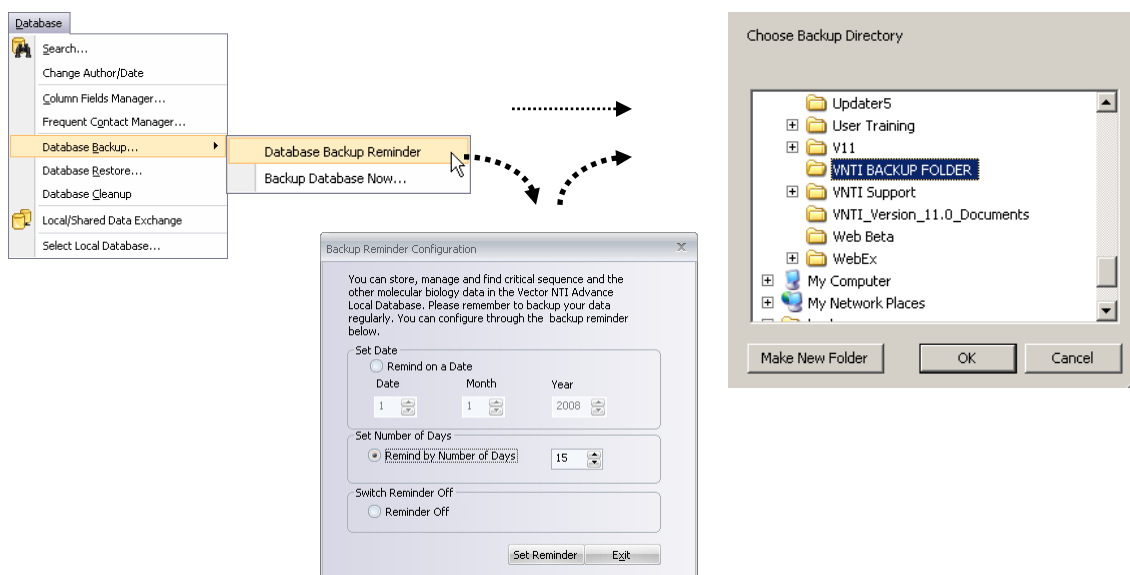


Figure 3. Database Backup

To restore a database:

- From **Vector NTI Explorer** menu, select Database | **Database Restore**.

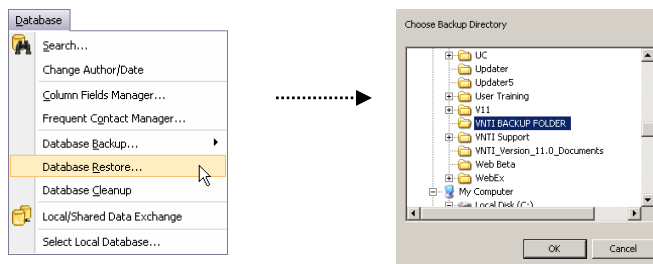


Figure 4. Database Restore

Molecule Viewer

The **Molecule Viewer** displays information about DNA, RNA, and protein molecules. To launch the **Molecule Viewer**:

- Click on **Molecule Viewer** on the **QuickStart page**, or
- From the Windows® Start menu, select Programs | Invitrogen | Vector NTI Advance 11 | **Vector NTI**, or
- Double-click on a molecule name in the **Vector NTI Explorer**.

To open a molecule from within the **Molecule Viewer**, click on the **Open button** () on the main toolbar and select the molecule name from the dialog box.

The molecule will be loaded into the **Molecule Viewer**.

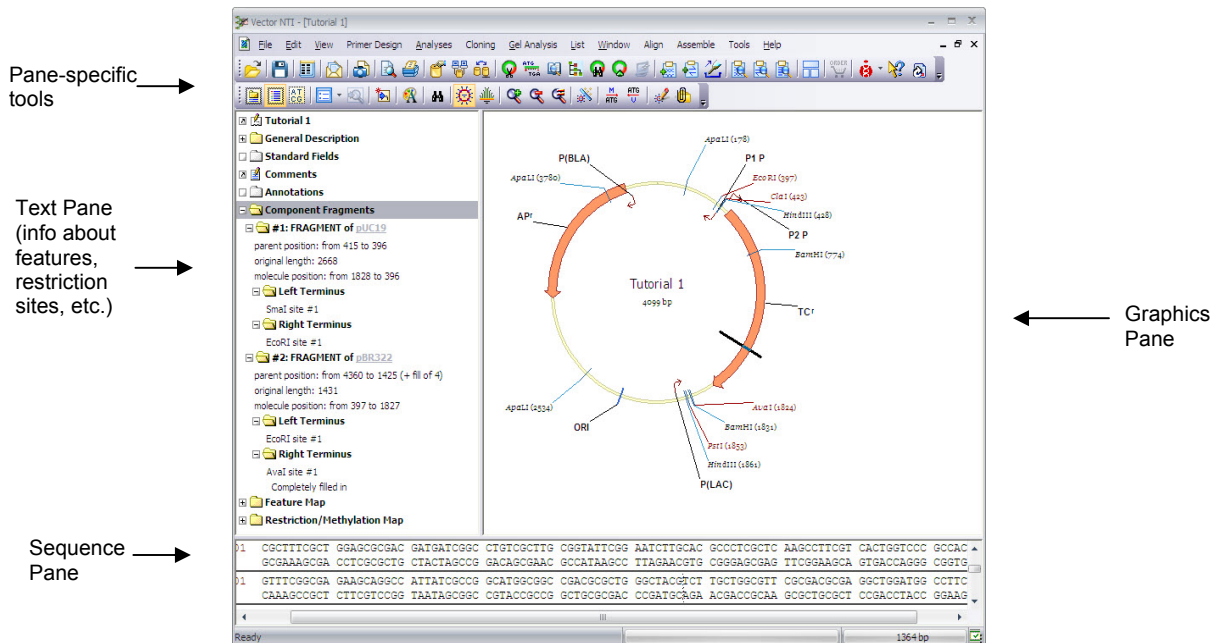


Figure 5. Molecule Viewer window for a DNA molecule.


The **Molecule Viewer** window has different panes for displaying different types of information about the molecule, as shown in Figure 5. Click inside a pane to make it the active pane. The available tools and right-click menu options will change depending on which pane is active.

Use tools on the dropdown menus and toolbars to add information about the molecule and perform various analysis functions, as described in the step-by-step instructions on the following pages.

Multiple molecules can be displayed in separate windows of the **Molecule Viewer**.

Selecting and Editing Molecule Sequences

In the **Molecule Viewer**, you can select part of a molecule sequence in several different ways:

- Hold down the mouse button and drag the cursor across the sequence in the Sequence Pane or Graphics Pane (Figure 6).
- Go to the *Edit menu*, select **Set Selection**, and enter the sequence base-pair range in the dialog box.
- Click on a defined feature in the Graphics Pane.
- Click on a defined feature in the Text Pane, and click on **Find** () on the main toolbar.

The selected sequence will appear highlighted in both the Graphics Pane and the Sequence Pane.

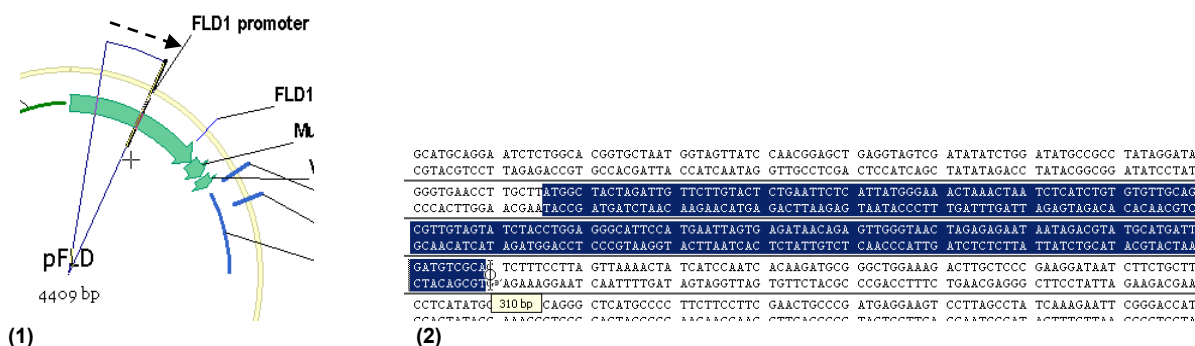


Figure 6. Selecting a DNA sequence by (1) dragging in Graphics Pane or (2) dragging in Sequence Pane.

To **copy** a molecule sequence:

1. Select it as described above.
2. To copy it to the Windows[®] clipboard, use the CTRL + C keyboard command, or

To copy the sequence as a text file, go to the *Edit menu* and select **Copy to > File**. You will be prompted to select a format and enter a name for the file.

To **delete** a molecule sequence:

1. Select it as described above.
2. Click on the DELETE key on your keyboard.

To **paste** a molecule sequence:

1. With the sequence in text format on the Windows[®] clipboard, click on the point in the Sequence Pane where you want to add the insert.
2. Click on CTRL + V on your keyboard.
3. The **Insert Sequence dialog** will open, displaying the sequence to be inserted.
4. Click on **OK** to complete the insertion.

Designing PCR Primers from a Sequence

Vector NTI Advance™ 11 can analyze a selected sequence and design PCR primers for it, based on parameters such as desired melting temperature (T_m), GC content, and amplicon length.

With a DNA or RNA molecule open in the **Molecule Viewer**:

1. Select the part of the sequence for which you want to design primers, as described on the previous page.
2. Go to the **Primer Design** menu.
3. Select **Find PCR Primers** to find primers within the sequence (Figure 7), or

Select **Amplify Selection** to find primers in the regions before and after the sequence (other amplification selections are available; see the **Vector NTI Advance™ 11** User's Manual for more information).

4. In the dialog box, select the desired primer-design parameters. Note that most of these parameters have default values based on typical PCR primers.
5. Click on **OK**. The results will appear under **PCR Analysis** in the Text Pane.

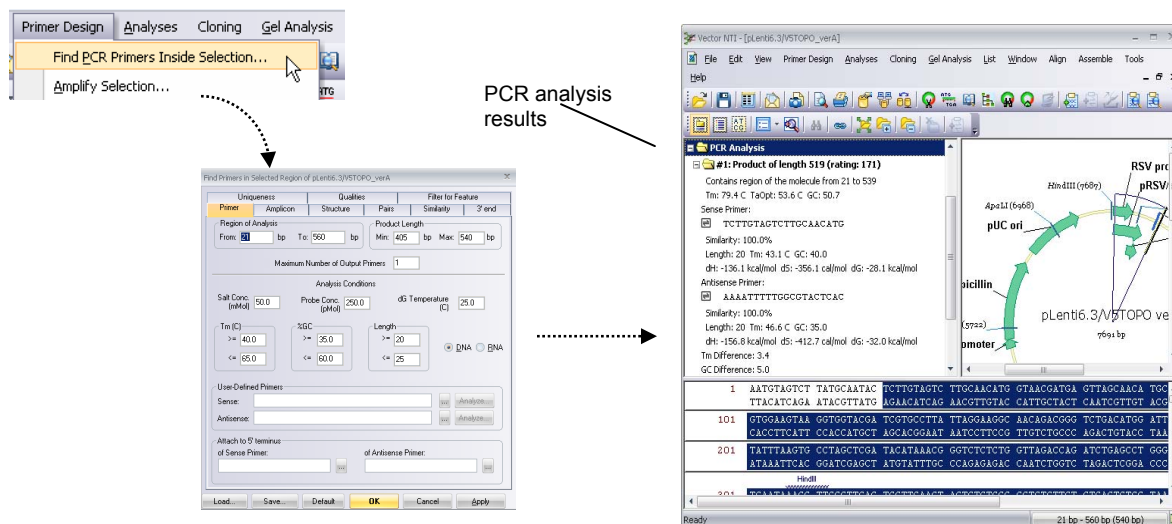


Figure 7. Designing PCR primers within a selected region.

To save the PCR analysis results as a separate object in the database:

1. Right-click on the **PCR Analysis** folder in the Text Pane.
2. Select **Save as Analysis Result**.

The saved results will be listed under the Analysis Results object type in the **Vector NTI Explorer** (Figure 8).

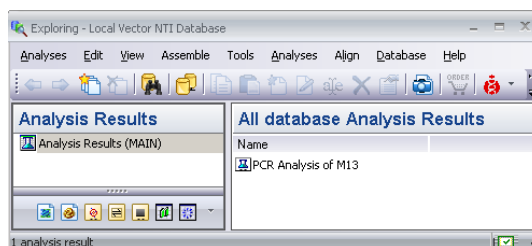


Figure 8. PCR analysis results listed in Vector NTI Explorer.

Clone Two Fragments with Clone2Seq

Clone2Seq offers the easiest way to clone two fragments. To use Clone2Seq:


1. In the Molecule Viewer, go to Cloning | **Clone2Seq**, or click on the **Clone2Seq** button () on the main toolbar.
2. To load Insert and/or Vector, click on **Load Molecule**.
3. Select fragment by Restriction Site by clicking on Site #1, shift-clicking on Site #2 on each molecule.
4. Make sure the left terminus of the first fragment is compatible with the right terminus of the second fragment, and the right terminus of the first fragment is compatible with the left terminus of the second fragment.
5. Add selected fragments by clicking on **Add Fragment**.
6. Click on **Clone**.




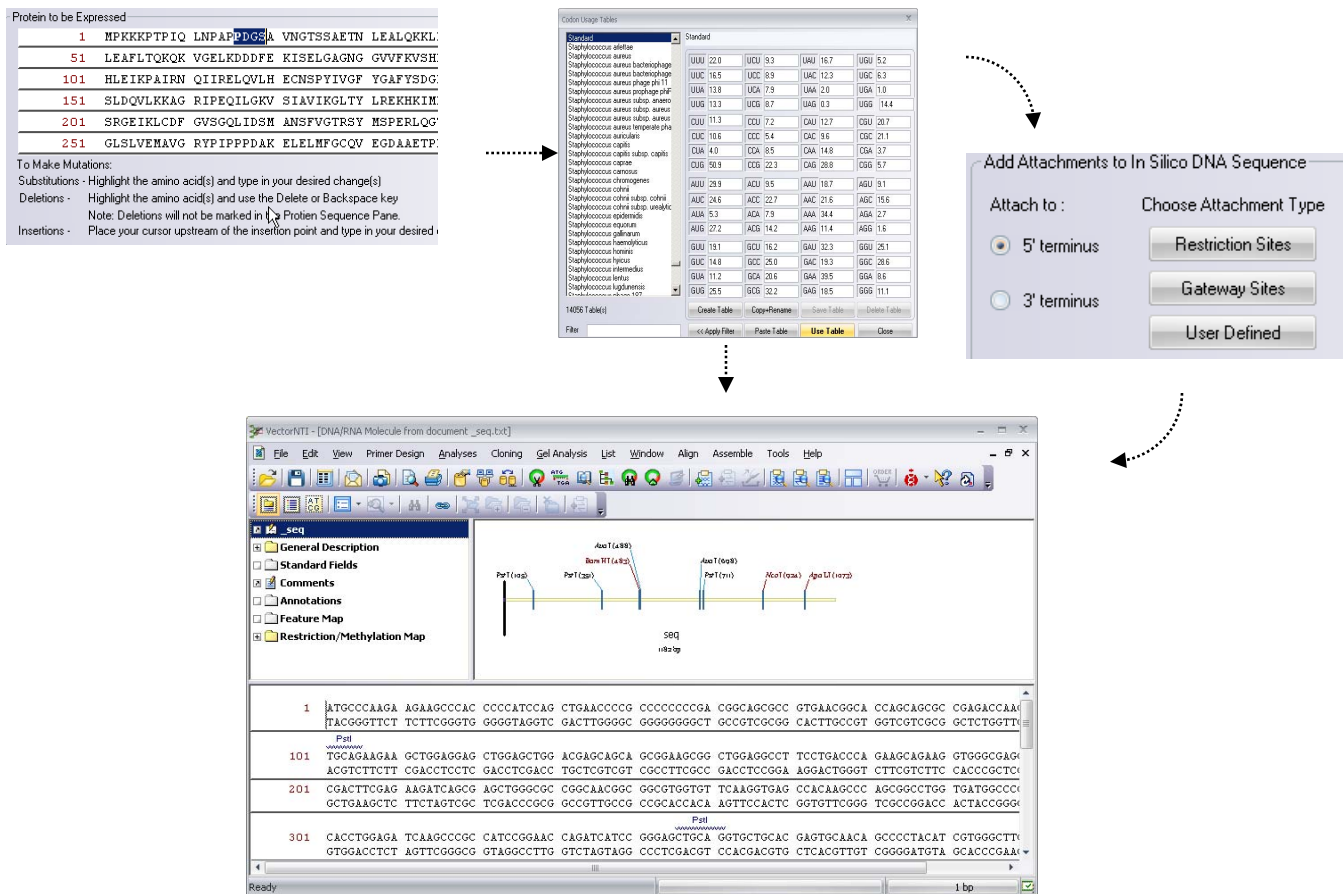
Figure 9. Clone 2 fragments with Clone2Seq.

In-Silico Gene Synthesis with ReGENERator

ReGENERator offers the fastest way to build your desired DNA from the ground up, optimized for expression, with any amino acid mutation you want, and with the flanking sequences you need for expression, purification, or detection. Then, right from your desktop, send your DNA sequence to our partner Blue Heron® Bio's secure server for rapid synthesis.

To design the DNA from an amino acid sequence:

1. With the source protein molecule loaded in the Molecule Viewer, select Cloning | ReGENERator or click on the  icon on the main toolbar.
2. Mutate the protein molecule by inserting, deleting, or replacing single or multiple amino acids.
3. Select the desired Codon Usage Table for the organism.
4. Select the desired Genetic Code.
5. You may also add attachment sequences to the terminus of the DNA:
 - Choose terminus (5' or 3')
 - Choose Attachment Type
6. To view the back-translated DNA, click **View Molecule**.
7. To send the DNA sequence for synthesis at Blue Heron®, click **Send for Synthesis**.



The figure illustrates the workflow for in-silico gene synthesis using ReGENERator. It consists of several interconnected windows:

- Protein to be Expressed:** A window showing a protein sequence with a mutation highlighted in red. The sequence is:


```

            1  MPKKKPTPIQ LNFAPDGGSA VNGTSSAETN LEALQKKL
            51  LEAFLTQKQK VGLKDDDFE KISELGAGWG GVVFVKVSH
            101 HLEIKPAIRN QIIRELQVLH ECNSPYIVGF YGAFYSDG
            151 SLDQVLKKG RIPEQLKGV SIAVIKGLTY LREKHKIM
            201 SRGEIKLCDF GVSQQLDSM ANSFVGTIRSY HSPERLQG
            251 GLSLVEMAVG RYPIPPDAK ELELMFGQCV EGDAAETP
            
```
- To Make Mutations:** A panel with instructions for substitutions, deletions, and insertions.
- Codon Usage Tables:** A window displaying a list of organisms and their corresponding codon usage tables. The 'Standard' table is selected.
- Add Attachments to In Silico DNA Sequence:** A dialog box where the user can choose to attach sequences to the 5' or 3' terminus using Restriction Sites, Gateway Sites, or User Defined sequences.
- VectorNTI:** The main software window showing the back-translated DNA sequence with various restriction sites (NotI, BamHI, XbaI, SpeI, EcoRI) and a primer map overlaid on the sequence. The DNA sequence is:


```

            1  ATGCCCAAGA AGAAGCCAC CCCCATCCAG CTGAACCCCG CCCCCCCGGA CGGCAGCGCC GTGAACGGCA CCAGCAGCGC CGAGACCAA
            101 TACGGGTTC TCTTCGGGTG GGGGTAGGTC GACTTGGGGC GGGGGGGGGT GCGCTCGCGC CACTTGCCTG GTCGTCGCG GCTCTGGTT
            201 TGCAGAAGAA GCTGGAGGAG CTGGAGCTGG ACGAGCAGCA GCGGAAGCGG CTGGAGGCC TCTGACCCA GAAGCAGAAG GTGGGCGAG
            301 CGACTTCGAG AAGATCAGCG AGCTGGGCGC CGGCAACGGC GCGGTGGTGT TCAAGGTGAG CCACAGGCC AGGGCGCTGG TGAATGCCG
            401 GCTGAAGCTC TTATGATCGC TCGACCCCGC GCCGTTGCC CCGCACCA CA AGTTGCACCT GGTGTTGGGG TCGCCGGACC ACTACCGGG
            501 CACCTGGAGA TCAAGCCCGC CATCCGGAA CAGATCATCC GGGAGCTGCA GGTGCTGCAC GAGTGCAGA GCCCCTACAT CGTGGGCTT
            601 GTGGACCTCT AGTTCGGCGC GTAGGCCCTG GTCTAGTAG CCCTCGACGT CCACGACGCG CTCACGTTGT CGGGATGTA GCACCCGAA
            
```

Figure 10. In-Silico Gene Synthesis with ReGENERator.

Identifying Open Reading Frames (ORFs)

Vector NTI Advance™ 11 can analyze a DNA/RNA molecule and identify the open reading frames (ORFs) in it, based on start and stop codons within the molecule.

With a DNA or RNA molecule open in the **Molecule Viewer**:

1. Go to the *Analyses menu* and select **ORF** (Figure 11).
2. In the dialog box, select the parameters for identifying and marking ORFs in the molecule.
3. When you click on **OK**, the sequences identified as ORFs will be marked with directional arrows in the Graphics Pane and Sequence Pane, and the ORFs will be listed in the Text Pane.
4. To identify an ORF in the different panes:
 - Click on a directional ORF arrow in the Graphics Pane to highlight its sequence in the Sequence Pane, or
 - Open a folder under **Open Reading Frames** in the Text Pane, right-click on the ORF name, and select **Find ORF** to highlight it in the Graphics and Sequence Panes.
5. To save an ORF to the feature map of the molecule, right-click on the ORF arrow in the Graphics Pane or the ORF folder in the Text Pane, and select **Add ORF to FMap**.

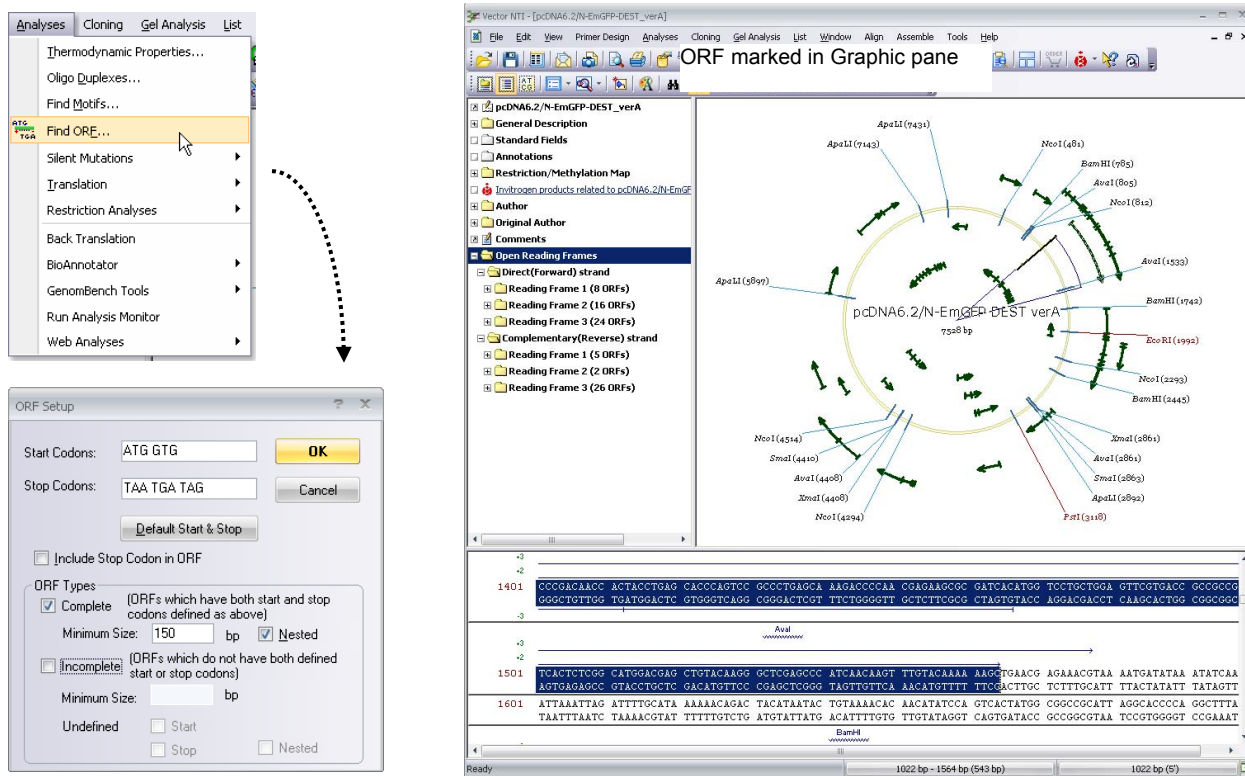


Figure 11. Identifying ORFs

Creating a Restriction Map

Vector NTI Advance™ 11 can analyze a DNA/RNA molecule and identify the restriction sites in it, using the software's comprehensive library of restriction enzymes.

With a DNA or RNA molecule open in the **Molecule Viewer**:

1. Go to the *Analyses menu* and select **Restriction Analyses > Restriction Sites** (Figure 12).
2. In the **Restriction Map Setup dialog**, review the list of restriction enzymes in the *Use Enzymes:* field. These are the enzymes that will be used to identify the restriction sites. Click on the **< Add**, **> Remove**, and **>> Remove All** buttons to add and remove enzymes from the list.

Note: If you click on **< Add**, the **Choose Database Enzymes dialog** will open, listing all the enzymes in the database. Select enzymes in the list by clicking on them or click on the **Select All** button, and then click on the **OK** button to add them to the **Restriction Map Setup dialog**.

3. Click on **OK** in the **Restriction Map Setup dialog**. The restriction enzymes and their binding sites will be shown in the Graphics Pane and Sequence Pane. The specific cut site of each enzyme will be listed under **Restriction/Methylation Map** in the Text Pane.

The figure illustrates the workflow for creating a restriction map in Vector NTI. It shows the following steps and components:

- Analyses Menu:** The 'Restriction Sites...' option is selected under the 'Restriction Analyses' sub-menu.
- Restriction Map Setup Dialog:** This dialog allows users to configure the analysis. It includes fields for 'Use Enzymes:' (with Add, Remove, and Remove All buttons), 'Sort Sites in the Text Pane by' (Enzyme Name or Number of Restriction Sites), and options to ignore restriction enzymes cutting outside or inside a specific region.
- Choose Database Enzymes Dialog:** This dialog provides a list of enzymes from a database. The table below shows a portion of this list:

Name	Recognition String	Terminus Type
AarI	cacctgc	5' Overhang
AacII	gacgctc	3' Overhang
Acc65I	ggtacc	5' Overhang
AccI	gtnkac	5' Overhang
AceIII	caagctc	5' Overhang
AccI	ccgc	5' Overhang
AccI	aacggt	5' Overhang
AccI	ctgaag	3' Overhang
AfeI	agcgct	Blunt
AflIII	cttaag	5' Overhang
AflIII	acrygt	5' Overhang
AgeI	accggt	5' Overhang


- Main Software Window:** The 'Restriction/Methylation Map' pane shows a circular map of the pTarget-DEST plasmid (5589 bp) with various restriction sites marked. The 'Sequence Pane' shows the corresponding DNA sequence with the BamHI site (GGATCC) circled.

Figure 12. Creating a Restriction Map.

Aligning Molecules

Vector NTI Advance™ 11 can align the sequences of two or more DNA/RNA molecules. The tool for doing this is called **AlignX**. This tool can be launched from either the **Molecule Viewer** or **Vector NTI Explorer**.

To align sequences using **Vector NTI Explorer**:

1. In the **Explorer**, select the molecules that you want to align using **SHIFT + CLICK** or **CTRL + CLICK** key commands (Figure 13).
2. Go the **Align menu** and select **AlignX—Align Selected Molecules**. The **AlignX Window** will open, with the molecules you selected listed in the upper left Text Pane.
3. In the **AlignX Window**, use **SHIFT + CLICK** or **CTRL + CLICK** key commands to select two or more molecules in the Text Pane list to align.
4. To begin the alignment, click on the **Align button** () on the toolbar. The alignment may take several minutes, depending on the length and number of the molecules selected.
5. When the alignment is complete, the results are displayed in the **AlignX Window**, as shown in Figure 13. The **AlignX Window** has panes showing different similarity graphs and the points at which the sequences align.

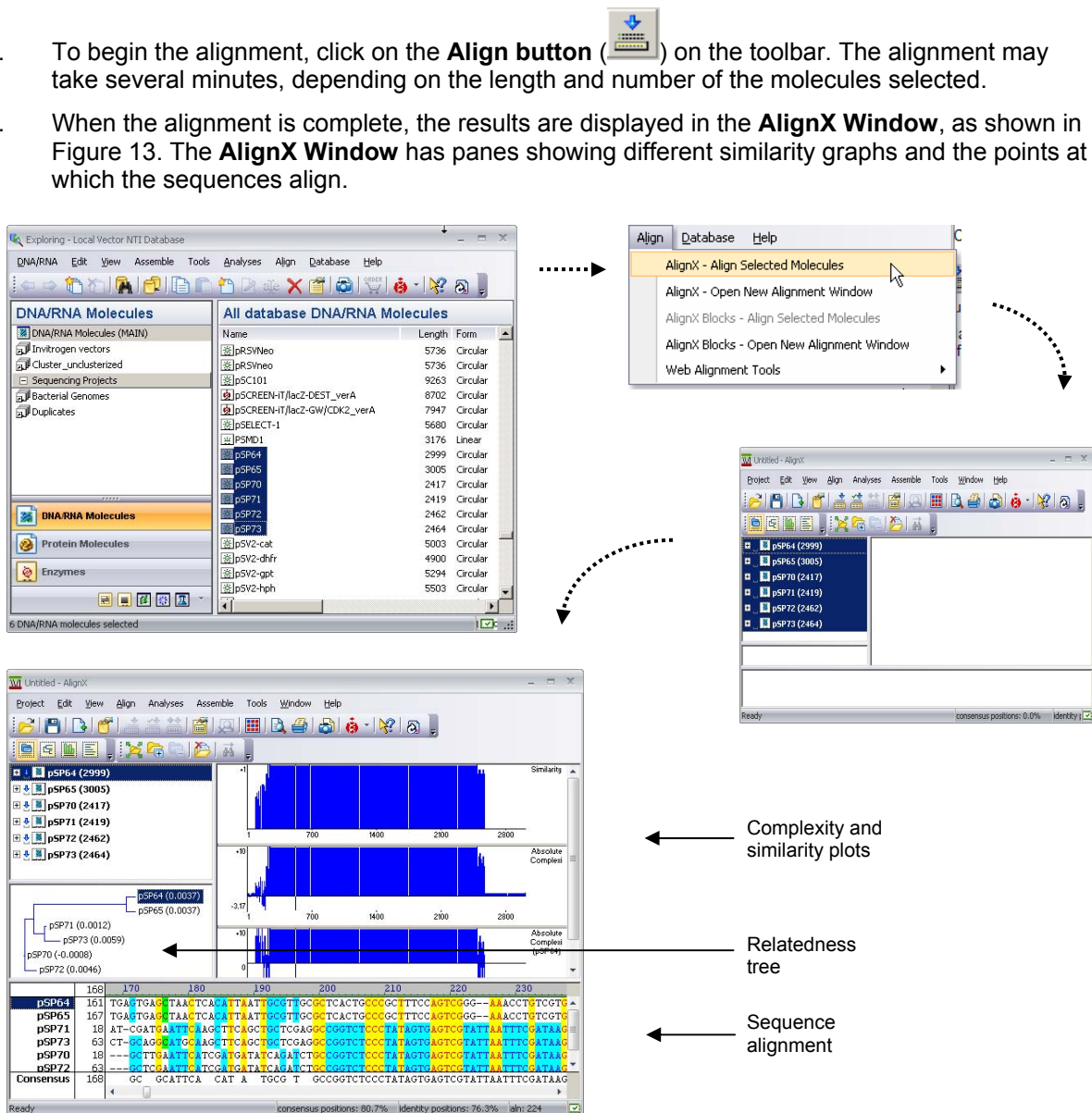



Figure 13. Aligning molecules.

Contig Assembly

Vector NTI Advance™ 11 can be used to assemble DNA fragments—both text sequences and chromatograms from automated sequencers—into longer contiguous sequences or “contigs.” The tool for doing this is called **ContigExpress**.

In **Vector NTI Explorer** or the **Molecule Viewer**:

1. Go to the *Assemble menu* and select **ContigExpress—Open New Assembly Project** (Figure 144).
2. In the **ContigExpress Project Explorer**, go to the *Project menu* and select **Add Fragments >**. Select your fragment file type from the submenu list. The **Import Sequence dialog** will open.
3. In the **Import Sequence dialog**, navigate to the directory containing your fragment sequence files. Select the files and click on **Open**.
4. Depending on the file type, you may be prompted to list the fragments by their Windows® file names or by their internal fragment names. Select the desired option. The fragments will be loaded in the **ContigExpress Project Explorer**.
5. To view a particular fragment, double-click on it in the **Project Explorer** list. It will be loaded into the **Fragment Viewer**.
6. When you are ready to perform contig assembly, select the fragments in the **ContigExpress Project Explorer**.
7. Click the **Assemble Selected Fragments icon** () on the main toolbar. Fragments will be analyzed and assembled into one or more contigs, which will be listed in the **Project Viewer** along with the fragments in each contig.
8. Double-click on a contig in the list. It will be displayed in the **Contig Viewer**. The Sequence Pane at the bottom shows the sequence of the assembly. The Graphics Pane on the right shows the orientations of the fragments in the assembly. The Text Pane on the left lists the fragments in the assembly.
9. If you wish to edit the contig, enable the Enhanced Edit Mode by clicking the icon **Use Enhanced Edit Mode** (far left on the toolbar in the Contig window) before making any reasonable changes.
10. There are three trimming options in **ContigExpress**. Fragments can be trimmed for ambiguities, Phred quality scores, and vector contamination. Refer to the **Vector NTI Advance™ 11 User's Manual** for details.

Continued on the following page

Contig Assembly, continued

The process begins with the 'Add Fragments' menu, where 'From ABI file...' is selected. This leads to a file selection dialog showing a list of ABI files in the 'Demo Projects' directory. The selected files are then loaded into the 'ContigExpress Project' window, which displays a 'History View' showing 12 fragments in the project.

The 'Fragment Viewer' displays the following table of fragments:

Name	Length	Original length	5' Trimmed bases
ONE17KANR	759	759	0
ONE2KANR	747	747	0
ONE3KANR	755	755	0
ONE4KANR	756	756	0
ONE6KANR	770	770	0
ONE8KANR	764	764	0
ONE9KANR	758	758	0
ONE10KANF	758	758	0
ONE11KANR	755	755	0
ONE13KANR	819	819	0
ONE15KANF	784	784	0
ONE16KANF	767	767	0

The 'Assembly View' shows the following table of items:

Name	Length	Original length	5' Trimmed bases
Contig 1	3675		
ONE17KANR	737	759	0
ONE17KANR	723	819	96
ONE15KANF	567	784	76
ONE10KANF	688	758	70
ONE11KANR	698	755	38
ONE16KANF	599	767	90
ONE9KANR	719	758	38
ONE6KANR	714	770	39
ONE3KANR	704	755	38
ONE2KANR	705	747	42
Contig 2	764		
ONE8KANR	762	764	0
ONE4KANR	719	756	37

Figure 14. Assembling a Contig

Additional Information

Invitrogen's free technical support for **Vector NTI Advance 11** is available exclusively through the web. For more information, check out the Software Support section of the Vector NTI website at <http://www.invitrogen.com/VectorNTI>.

To obtain personalized technical support by telephone or email, you must have an annual support contract. You may purchase an Advanced Support Contract by contacting Invitrogen at bioinfosales@invitrogen.com.

To receive technical support, use the following contacts:

United States

Phone: 800-955-6288 x 67990 (Toll-free, U.S.)

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Europe, Middle East, Africa, Asian Pacific

Phone: +44 781 696 2707

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