Vector NTI[™] **Express Quick Reference**

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

This quick reference provides brief descriptions of the Vector NTI[™] Express graphical user interface, including the **Database Explorer** and the **Molecule Viewer**, and step-by-step instructions for using the most common features and functions of the software. 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.

Vector $NTI^{™}$ Express has been developed to provide the comprehensive functionality of Vector $NTI^{™}$ Advance in a single convenient software application.



Open Vector NTI[™] Express software

To launch the software, click **Start ➤ All Programs ➤ Life Technologies ➤ Vector NTI**[™] **Express** or click the Vector NTI[™] Express icon () found on the desktop. The screen opens to provide the toolbar for further uses.

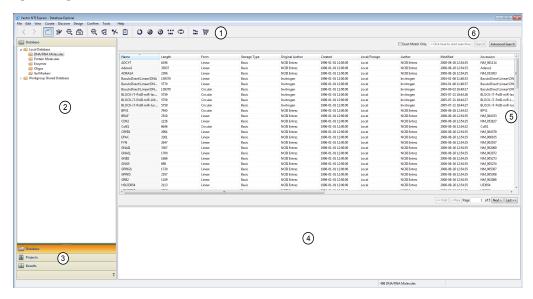


Figure 1 Database Explorer Screen

- 1 Toolbar
- 2 Data Types View
- (3) Data Types Folders

- 4 Property View
- ⑤ Explorer View
- (6) Search Tool

Local database

Database Explorer is the main tool for accessing the information in your local Vector NTI^{TM} Express database. Using the explorer, you can import, open, export, and organize molecules and database items, as well as launch other Vector NTI^{TM} Express modules.

The local database stores records for different types of biological data. Each database record includes all of the information for that object (e.g., a DNA molecule record includes the DNA sequence, defined features of the molecule, and other information).

Biological data are categorized by types: DNA molecules, protein molecules, etc. When first opening the software, DNA/RNA Molecules is the default object type. Click on the folder for the desired molecule type to access the molecules in the database.

Three types of data are stored within the database:

- Sequence records DNA/RNA molecules, Protein molecules, Enzymes, Oligos and Gel Marker Fragment Lists
- Projects Alignment, Contig Assembly, Cloning and Gene Synthesis projects
- Results BLAST and Analysis results

To open an object from the local database, click on Database Explorer and select the object name in the **Records Viewer** pane. 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 Editor**.

Note: When you install Vector NTI[™] Express the default local database is created in a folder called VntiExpress_Database in the root directory of your computer (e.g., C:\VntiExpress_Database).

All Vector NTI^{TM} Express applications can be launched by clicking on the appropriate icon found in the toolbar.



- 1 Previous/Next application
- 2 Database Explorer
- (3) Molecule Editor
- 4 Public Database Search
- (5) Genome Bench
- (6) BLAST Search
- 7 AlignX
- (8) 3D Molecule Viewer
- 9 Analysis Monitor
- (10) Clon2Seq
- (1) GateWay Cloning
- (12) TOPO Cloning
- (13) GeneArt Cloning
- (14) Parts Assembler
- (15) Contig Express
- 16 Ordering

When you open more than one application, the **Previous/Next Application** icons allow navigation between the different application windows.

Backup/Restore the database

Note: It is strongly recommended to routinely back up the local database. You can launch the Database Backup manually or use the Database Backup Reminder to trigger the task automatically as configured.

To manually perform a database backup:

- 1. From the menu bar, select **Tools** Database Utilities.
- 2. In the **Database Utilities** window, click on **Backup Database Now** to launch the backup.

To set up a database backup reminder:

- 1. From the menu bar, select **Tools** Database Utilities Configure Reminder.
- Navigate to and name the folder for the Database Backup. If needed, configure
 the Database Backup Reminder parameters. Review and edit the Set Date, Set
 Number of Days, Set Automatic Backup, and Switch Reminder Off sections as
 needed.
 - In the Backup Reminder Configuration window, you can set up a specific date or interval (e.g., backup every 15 days) to automatically perform backups. If you do not want to receive reminders, you can also choose to turn them off.
- 3. Click **OK** to save your settings.

To restore a backup or to switch to another database, return to the **Database Utilities** window. From here, you can choose to either use a different database or restore a backup of your database.

To manually perform a database change:

- 1. Select Database Utilities ▶ Switch Active Database By ▶ Select Existing VE Database.
- 2. Navigate to and select the folder of your choice for the new database. Click **OK**. The software will need to relaunch to load the new data set.

To manually perform a database restore:

- 1. Select Database Utilities ▶ Switch Active Database By ▶ Restore Earlier Database Backup.
- 2. Navigate to and select the correct backup folder.
- 3. Click **OK** to start restoring the data. The software will relaunch automatically to load the backup dataset.

Molecule Editor

Molecule Editor * displays information about DNA, RNA, and protein molecules.

To open molecule records:

- Stored in your local database In Database Explorer, double click on the
 desired molecule record row in the viewing panel (molecule records are divided
 into folders of DNA/RNA Molecules, Protein Molecules, Enzymes, Oligos, and
 Gel Markers). This launches the editor with the associated record.
- Stored on your desktop Click File > Open and choose a molecule record type (DNA, Protein, Gel Marker, etc.) from the menu. Navigate to and select a molecule record. Click File > Save to store the record to your local database.

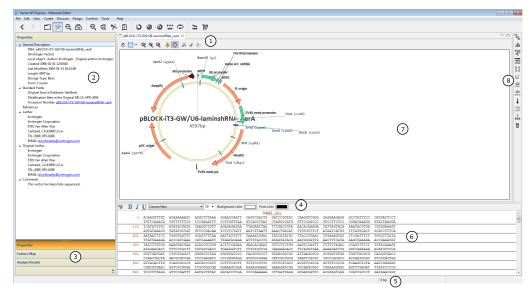
To import molecule records:

• Click **File** • **Import** and choose a molecule record type (DNA, Protein, Gel Marker, etc.) from the menu.

To add a new molecule:

- Click **File** ▶ **New** and choose a molecule record type (DNA, Protein, Gel Marker, etc.) from the menu.
- Provide a name and sequence for the record. For DNA/RNA molecules, choose **linear** or **circular**.
- (Optional) Click **Edit Properties** to edit further details in the record.

The **Molecule Editor** screen contains tools to modify chosen molecules.



- 1 Display Tools
- 2 Properties Pane
- ③ Properties, Feature Map, and Analysis Results Tabs
- 4 Sequence Formatting Tools
- (5) Sequence Selection Tool
- 6 Sequence Pane
- (7) Graphics Pane
- 8 Molecule Analysis Tools
- **Properties** tab displays general information about the molecule, including the sequence length, author information, and accession number.
- Feature Map tab lists all of the annotated features on the molecule.
- Analysis Results tab displays information about analyses performed on the molecule using the Molecule Analysis Tools.

To modify within the **Graphics Display**:

- Modify the **Graphic Pane** by clicking **Edit Graphic**. Alternatively, from the **View** menu, click **View Edit Graphic**.
- Edit text or graphics by selecting, then right clicking to select **Properties** or going to **View Properties**.
- To capture the final image, right click on the image and select the **Camera** tool.
- (Optional) Restore the graphics display by clicking **Standard Arrangement** or selecting **View Standard Arrangement**.

Molecule Editor Toolbar

Analysis Tools for manipulation and analysis of the molecule records in the database are found using the tool ribbons on the right hand side of the **Molecule Editor** window. These are typically referred to as the **Analysis Toolbars** in this manual. Sets of tools are presented according to the type of molecule record currently open. DNA-specific tools are open for DNA or RNA records; protein-specific tools for protein records.

Figure 2 Function Icons in DNA Analysis Toolbar

- 1 ORF Finder
- 2 Translation
- (3) Restriction Analysis
- 4 Motif Finder
- (5) Primer Design
- (6) BioAnnotator
- (7) SIM4
- 8 Spidey
- Thermodynamics
- (10) Oligo Duplex Analysis
- (1) Silent Mutation Analysis
- 12 GeneArt Gene Synthesis



Figure 3 Function Icons in Protein Analysis Toolbar

1 Motif Finder

4 Regenerator

2 InterProScan

5 Protein Domain

(3) Back Translation

Active icons are highlighted while the tool is in use. The results from many tools are written to the **Analysis Results** tab.

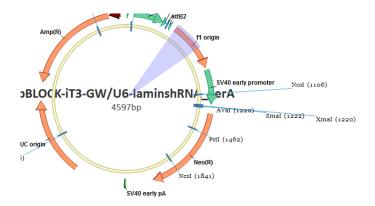
Select and edit molecule sequences

Molecule Editor allows you to select part of a molecule sequence using different methods:

 Hold down the mouse button and drag the cursor across the sequence in the Sequence Pane (Fig. 4) or Graphics Pane (Fig. 5).



Figure 4 Selecting DNA sequence by dragging in Sequence Pane



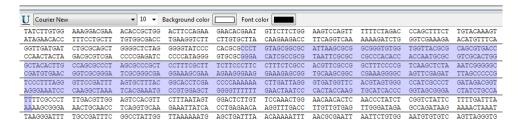


Figure 5 Selecting DNA sequence by dragging in Graphics Pane

- Go to the **Sequence Selection Tool** at the bottom of the **Molecule Editor** window, select **Range** and enter the sequence base-pair range in the dialog box.
- Click on a defined feature in the Feature Map pane.

To copy a molecule sequence:

- 1. Select a sequence using one of the methods above.
- 2. Right click and Copy or type CTRL + C on the keyboard to copy to the WindowsTM clipboard.

To paste a molecule sequence:

- 1. With the sequence in text format on the Windows[™] clipboard, click on the point in the **Sequence Pane** to add the insert.
- 2. Right click and **Paste** or type CTRL + V on the keyboard. The Insert Sequence dialog box will open displaying the sequence to be inserted.
- 3. Click **OK** to complete the insertion.

To delete a molecule sequence:

- 1. Select a sequence using one of the methods above.
- 2. Right click and **Delete** or type the Delete key on the keyboard.

Design PCR primers from a sequence

Vector NTI^{TM} Express software can analyze a selected sequence and design PCR primers for it based on parameters including melting temperature (Tm), GC content, and amplicon length.

With a DNA or RNA molecule open in Molecule Editor:

- 1. Select the sequence section to use for designing primers.
- 2. Select the Primer Design tool on the Analysis toolbar.
- **3.** Select the **Find PCR Primers Inside Selection** option to find primers within the sequence.

Alternatively, in the **Graphics Pane**, select a segment of DNA or a feature and right click to display the **Graphics Options** menu. In the Options menu, navigate down to **Primer Design** ➤ **Find PCR Primers Inside Selection** (other amplification sections are available; see the Vector NTI[™] Express user's guide for more information.

- **4.** In the dialog box, select the primer-design parameters. Note that most of the parameters have default values based on typical PCR primers.
- **5.** Click **Run**. The results will appear under the Analysis Results tab.

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

- Right-click on the PCR Analysis folder in the Text Pane.
- Select Save as file or Save all analysis results to Database.
- Right-click on the primer sequence to add the primer to the Oligos list or Oligos database or write to a file for external ordering.
- Right-click to add the primer to the Oligo database as an oligo record.

Sequencing primer design and view customization

The software can analyze a selected sequence and design sequencing primers for it based on parameters such as desired melting temperature (T_m) , GC content, and length.

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

- 1. Select the part of the sequence for which you want design primers.
- 2. Click **Primer Design** on the **Analysis** toolbar.
- 3. Click Sequencing Primers to find primers within the sequence. Alternatively, right-click on a sequence in the graphic, then navigate down to Primer Design ➤ Sequencing Primers. Other amplification selections are available. See the Vector NTI™ Express User Manual for more information.

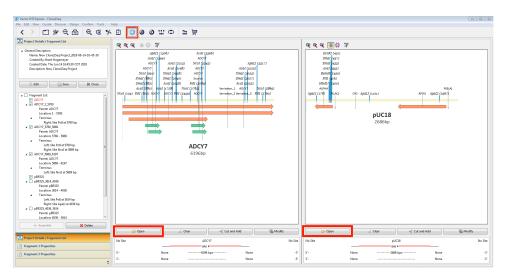
- **4.** In the **Primer Design Sequencing Primers** pane, select the desired primerdesign parameters. Note that most of these parameters have default values based on typical PCR primers.
- 5. Click **Run**. Results appear under **PCR Analysis** in the **Text** pane.
- **6.** In the **Analysis Results** pane, select only the Sequencing Primers results.

Clone 2 fragments with Clone2Seq[™]

Clone2Seq $^{\text{\tiny TM}}$ offers a method for cloning 2 fragments.

- In Database Explorer or Molecule Viewer, click Design ➤ Clone2Seq[™] or click the Clone2Seq[™] icon .
- **2.** To load an insert and/or vector into the left and right panes, click **Open** at the bottom of each pane.

In this example, click **Open** in the lefthand pane and navigate to the ADCY7 record in the data from the local database. Select the record and click **OK**. In the righthand molecule pane, go through a similar method and identify a vector to clone the ADCY7 molecule into.



3. Select a fragment by restriction site by clicking on Site #1 and shift-clicking on Site #2 on each molecule.

Note: 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.

- **4.** Add selected fragments by clicking on **Cut and Add** in both the left and right panes.
- **5.** Click **Assemble** in the **Project Details** pane.

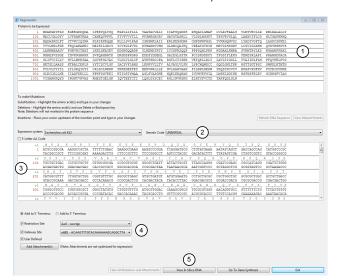
In-Silico Gene Synthesis with ReGENerator $^{^{\mathsf{TM}}}$

ReGENerator offers the fastest way to build your desired DNA from a protein sequence, while also optimizing for expression, adding amino acid mutations, and including flanking sequences needed for expression, purification, or detection. When completed, you can then easily send your DNA sequence to the GeneArt secure server for rapid synthesis.

To design the DNA from an amino acid sequence:

- With the source protein molecule loaded into the Molecule Viewer, click ReGENerator[™]

 on the toolbar.
- Mutate the protein molecule to insert, delete, or replace single or mutiple amino acids by highlighting the amino acid residues to be changed or placing the cursor at the insertion/deletion site in either the **Protein to be Expressed** or **In Silico DNA Sequence** fields. To make the change, type the desired amino acid at the cursor.
- 3. Select the desired Codon Usage Table for the organism under In Silico DNA Sequence > Expression System.
- 4. Select the desired Genetic Code under In Silico DNA Sequence > Genetic Code .
 - **Note:** You may also add attachment sequences to the terminus of the DNA. Choose a terminus (3' or 5') and choose an **Attachment Type** of either gateway or custom sequences.
- 5. If desired, add attachment sequences to the terminus of the DNA. Choose a terminus (3' or 5') and choose an **Attachment Type** of either gateway or custom sequences.



6. To view the back-translated DNA, click View In Silico DNA.

Figure 6 ReGENerator Screen

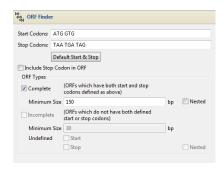
- 1 Original Protein Sequence
- (2) Host and Codon Table Selections
- 3 Sequence Editing Tool
- 4 Attachment Selector Tool
- (5) View DNA Sequence
- 6 Submit for Synthesis

Identify Open Reading Frames (ORFs)

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

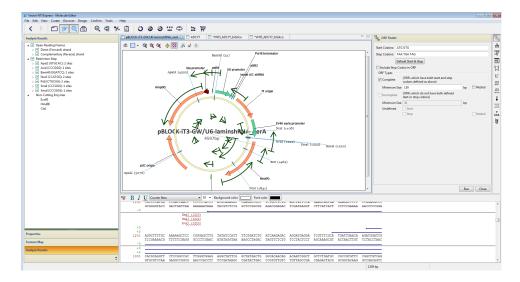
With a DNA or RNA molecule open in the **Molecule Editor**:

- 1. In the Analysis toolbar, click on **ORF Finder**
- **2.** In the dialog box, select the parameters for identifying and marking ORFs in the molecule.



After clicking **Run**, 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 **Analysis Results** pane.

- **3.** Click on a directional ORF arrow in the **Graphics Pane** to highlight its sequence in the **Sequence Pane**.
 - Alternatively, open a folder under **Open Reading Frames** in the **Analysis Results Pane**, identify the box corresponding to the ORF name, and select or deselect to highlight it in the **Graphics** and **Sequence Panes**.
- **4.** 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 **Create feature from selection**.



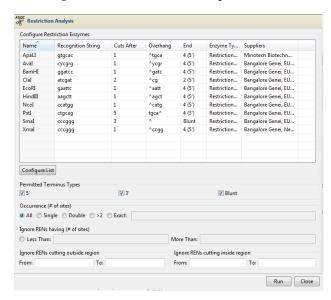
- **5.** Select a**Feature Type** and name the feature using the **Feature Name** field. If needed, add any **Annotations**.
- 6. Click **OK** to display the feature in the **Graphics Pane**.

Create a restriction map

Using a comprehensive library of restriction enzymes, Vector NTI[™] Express can analyze a DNA/RNA molecule and identify the restriction sites.

With a DNA or RNA molecule open in **Molecule Editor**:

- 1. From the Analysis toolbar, click **Restriction Analysis** \(\frac{\pi}{\pi}\).
- 2. In the Restriction Map Setup dialog box, review the list of restriction enzymes in the **Configure Restriction Enzymes** field. These are the enzymes that will be used to generate the restriction map.



a. Click on **Configure List** to add and remove enzymes from the list. Confirm your selection by selecting **OK**.

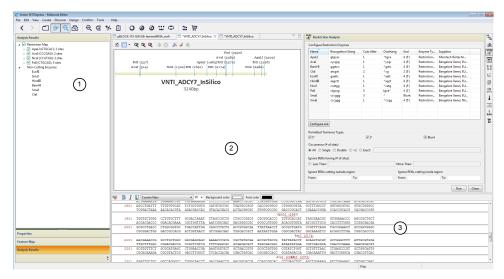
Note: If you click on **Configure List**, the **Select Restriction Enzymes** dialog will open, listing all the enzymes in the database. Select enzymes in the list by clicking on each one needed from the **Available Enzymes** list, then add them to the **Selected Enzymes** list by clicking the right arrow > found between the lists, or remove them from the list of **Selected Enzymes** by clicking on each one to remove and then clicking on the left arrow <.

b. Filter the enzymes in the Ignore RENs having more than 1 site.

Note: Filtering aids in identifying single/unique cutters that can be used for cloning or for restriction analysis of plasmids.

3. Click **Run** in the **Restriction Analysis** dialog box.

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 Map** in the **Analysis Results Pane**.



- 1 Analysis Results Pane showing Restriction Map
- 2 Graphics Pane
- 3 Sequence Pane

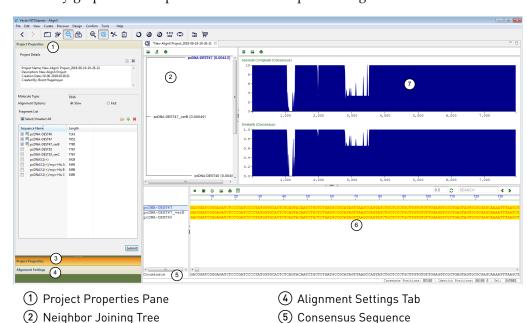
Align molecules

Two or more sequences of DNA/RNA molecules can be aligned using the AlignX function.

To align sequences:

- 1. Click 🔁 AlignX in the toolbar.
- 2. Click on an **Alignment Option** of **Slow** or **Fast**.
- 3. Click to add sequences to the **Fragment List**.
- 4. Click **Submit** to begin aligning.

Note: Alignment can take several minutes depending on the length and number of molecules selected.



Results display in the **AlignX Pane** as shown below. This pane shows different similarity graphs and the points at which the sequences align.

The project can be saved to the local database. Saved projects can be found under the **Alignment Projects** folder of the **Projects** tab in **Database Explorer**. The consensus sequence can be saved to the local database as a molecule record by right clicking **Consensus Sequence** and selecting **Save As Molecule**.

6 Sequence Alignment

Contig assembly

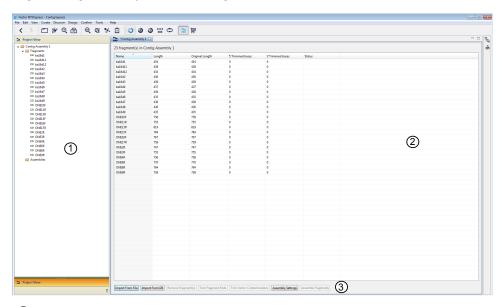
The **ContigExpress** function can assemble DNA fragments, both text sequences and chromatograms from automated sequencers, into longer contiguous sequences or "contigs".

In Database Explorer, click ContigExpress **:::**:

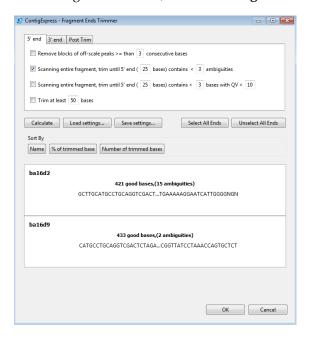
(3) Project Properties Tab

- 1. In the **Contig Assembly Project** dialog, enter a **Name** and **Description** as needed. Click **New**.
- 2. Click **Import From File** or **Import From DB** in the **Assembly** toolbar at the bottom of the window.
- For importing from a file, identify the type of file to import and navigate to the directory containing your fragment sequence files. Select the files and click Open. Alternatively, to import from Database Explorer, choose fragments and click OK.

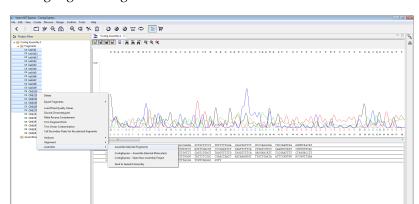
Fragments will load into **ContigExpress** in the Fragments Folder. To view a fragment, click on it in the fragment list to load it in the **Fragment Viewer**. To begin contig assembly, select the fragments to be assembled.



- 1 Project View and Fragment Folder
- ② Fragment Explorer
- 3 Assembly Toolbar
- 4. With a fragment selected, click Trim Fragment Ends. Click Calculate. Click OK.



Fragments are analyzed and trimmed with results listing in the **Fragment Explorer** window along with results for each fragment.



5. Select the fragments to assemble in the Project View window, then right click on the highlighted fragments and click **Assemble** • **Assemble Selected Fragments**.

The contig assembly is analyzed and saved under the **Assemblies** folder under **Project View**. Right click the contig box to rename the contig assembly.

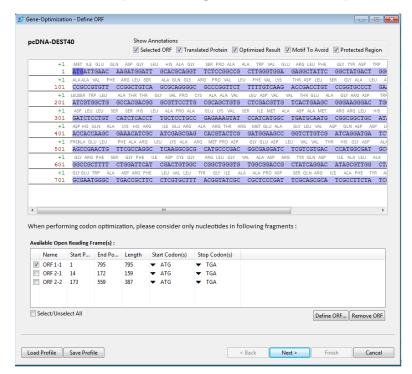
- **6.** If needed, to edit the contig, open the **Contig** folder and select the assembled contig. This launches the **Contig Assembly Viewer**.
 - In the contig alignment window, the individual fragment assemblies and the consensus sequence can be examined and edited. Right click on the fragment name and choose **Show/Hide Chromatogram** to show the electropherogram data. Right click on the fragment name and choose **Set as reference** to set a fragment as the reference sequence for the region.
 - The consensus sequence shows SNP data as black circles. Gaps or poor coverage calls are highlighted with + signs. Select a consensus residue to delete or change the residue call.
- 7. After editing the consensus sequence, right-click the Contig project to export the consensus sequence to the Database Explorer as a molecule record. Click File > Save As to save the contig assembly to the Contig Assembly Projects portion of the Projects tab in Database Explorer.

Codon optimization

The software can perform codon optimization on a DNA sequence and format the result into a gene synthesis order or save it to the database for subsequent usage in projects.

With a DNA or RNA molecule open in Molecule Editor:

- 1. Select the part of the sequence for codon optimization.
- 2. Click **GeneArt Portal o**n the **Analysis** toolbar.



3. Select the Gene-Optimization option to launch Gene Optimization.

- 4. Select the desired **Open Reading Frame** from the list, or define the **Available Open Reading Frame(s)** manually. Click **Next**.
- 5. If necessary, select any **Restriction Sites To Avoid** and add any **Custom Motif(s) To Avoid**. Click **Next**.
- 6. Select Optimization Parameters and click Optimize.
- 7. Click Finish.

Gene Synthesis ordering

Gene Synthesis works on a DNA sequence to format a result for a gene synthesis order.

With a DNA or RNA molecule open in the Molecule Editor:

- 1. Select a part of the sequence to synthesize. Start with anything from a sequence region or an entire constructed clone.
- 2. Click **GeneArt Portal** on the **Analysis** toolbar.
- 3. Select the **Gene-Synthesis** option to launch the **Gene Synthesis** workflow.

Note: If you previously performed **Gene Optimization**, selecting the **Finish** button takes you to this tool.

- Select the **Biosafety classification**.
- If a specific antibiotic resistance marker is required to be used or avoided in the cloning vector, choose the Vector resistance option. Alternatively, the GeneArt Portal will select a primary cloning vector based upon your criteria and provide a list of supported secondary cloning vectors for sub-cloning.
- Synthesis projects can be stored as simple sequence records or as projects.
- Right click on a project to send it to the GeneArt Portal or to go to a previously uploaded project.

Additional information

Free technical support for Vector NTI^{TM} Express is available exclusively online in the Software Support section of the Vector NTI^{TM} Express webpages at **thermofisher.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 Thermo Fisher Scientific at bioinfosales@thermofisher.com.

To receive technical support:

United States

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

Email: bioinfosupport@thermofisher.com

Europe, Middle East, Africa, Asian Pacific

Phone: +44 781 696 2707

Email: bioinfosupport@thermofisher.com

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