VectorNTI - Workflow

1. **Main setup**
    a. Download VectorNTI from http://www.thermofisher.com/us/en/home/life-science/cloning/vector-nti-software/vector-nti-advance-software/vector-nti-advance-downloads.html (press Download (.exe))
    b. Install database
    c. Start program: Start, All programs, Invitrogen, Vector NTI Advance 11, Vector NTI
    d. Examine the 3 main areas: text area: general text description of sequence, Graphical area: manipulation of sequence, Sequence area
    e. If you get stuck use the user manual: https://tools.thermofisher.com/content/sfs/manuals/VectorNTI_UserManual.pdf
    f. To enter your license key -- Start, All programs, Invitrogen, License Manager, Applications Tab, Trial, Paste your key, Activate

        Under Download (.exe) press “Get my trial license key”, fill out the information and get the key
2. **Local database**
    a. Press the Local database button to open up the list of DNA/RNA molecules, protein molecules, enzymes, oligos, blast results, analysis results in VectorNTI
    b. Minimize the local database screen, select any DNA/RNA or protein molecule and drag it into the graphics pane to view. Use] or [to zoom in or out. Use Window option to switch between open windows
        i. DNA molecule – ADCY7
        ii. DNA Phage BaculoDirect Linear DNA
        iii. Protein 1B14_HUMAN
        iv. pBR322 vector
    c. Molecule subset: select some molecules and click on this icon to make new subset of molecules
3. **Edit graphic**
    a. Right click on a feature on the graphic (of pBR322)
        i. Look at feature properties (feature map, restriction map, etc…)
        ii. Change styles, colors
        iii. Translate sequences to protein (Right click, Translate, feature)
4. **Add protein feature**
    a. Click File menu point, create [using sequence editor Protein]
    b. Enter name and sequence [pasting is not allowed in the demo version]
    c. Enter protein sequence, Click Ok, ok, ok
    d. Open up Analysis, review parameters such as length, weight, amino acids, etc.

    e. Analyses menu POINT, Back translate [useful if you want to synthesize a gene which produces this protein]
        i. Test by sliding bar from least to most degenerate, see how the nt bases change to IUPAC symbols
        ii. Change genetic code

5. **Search protein sequence against PFAM database**
    a. Open 41_HUMAN from local Database Proteins
    b. Select "Dom Misc 1" from the protein sequence, then do: Analyses menu point, Bioannotator, PFAM search
    c. Keep the default parameters and press "Submit"
    d. Wait for the PFAM search to complete, the Object URL tells you the name of the search
    e. Click on results at bottom to show hits with PFAM database
        i. Left text pane shows the results under External Analyses, PFAM Analysis
        ii. Clicking on + shows hit details
        iii. In graphics pane we can see the hits with dotted lines

6. **Find ORFs**
    a. Open "BaculoDirect Linear DNA Cloning Fragment" from local database
    b. Right click in graphic view, Display setup , uncheck everything
    c. ORF button
    d. Minimum size 300 bp
    e. In the text pane see how many ORFs you got and their coordinates on both strands and in all three frames
    f. Either highlight sequence or select an ORF
    g. Then either translate direct or complementary strand

7. **Design primers**
    a. Open ColE1 DNA molecule
    b. Set selected region (Ctrl+G or Edit, set selection range) to 5200-6400
    c. Primer design menu, find primers in selected region
    d. Set GC%, amplicon region, max repeats and max palindrome under Amplicon and Structure tabs
    e. Press Okay, and inspect the kinds of primers that you have made
        i. Right click, then select thermodynamic properties
            1. Press Analyze to see what kinds of repeats it has
            2. Then press dimers and loops to see if you have these structures as well

ii. Right click, Save to database (if you check, it will be in local database)

8. View/Design oligos

a. List menu point, Oligo list
b. Load “M13 Forward (-20)”
c. Press Analyze button, calculates GC%, molecular weight, etc.
    i. Press Dimers and hairpin loops to show if these structures are present
d. Press Duplexes button, Analyze button: calculates possible duplexes
e. Can also edit (Edit button, “Oligo” tab to change sequence) or save to database (Save button)

9. AlignX

a. Align menu point, Open New Alignment Window
b. Click on the load from database icon (4th from left, 2nd row)
c. Click on the load proteins button at the bottom
d. Grab the 41_BB molecule and drag it into the AlignX pane
    i. Make the local database window a bit smaller
    ii. Open it up and review its features (feature map, analysis)
e. Grab the 5H1A proteins and drag them to the AlignX pane (all 3 at once)
f. Highlight all three 5H1A sequences using Shift key, then press the Align button
    i. Review alignment, consensus sequence, complexity panels
    ii. Highlight portions of the alignment
g. You can use alignment setup to change alignment parameters
h. At present only the complexity and the similarity profiles are visible in the graphics panel
i. View dot plot matrix
    i. Try 2 5H1A molecules then 1 5H1A and 41_BB
j. Select 41_BB and add it to alignment
    i. Plots automatically recalculate themselves

10. 3D protein structure viewing [separate program involved]

a. Start 3D molecular viewer: Start, Program, Invitrogen, 3D Molecule Viewer
b. Open the downloaded 1GX9 (Protein Data Bank, PDF format) from the course web page
    i. 1Gx9 is a bovine beta-lactoglobulin with retinoic acid
c. Measure distance mode – click two points to measure distance between them
d. Enter measure angle mode
    i. Click three atoms to get the angle between them

e. Torsion marks , click four atoms here
f. removes all measurement marks
g. Press structure button
h. Go through different structural elements and mark/unmark them

11. ReGENerator for generating protein via DNA sequence

a. Open BRAF protein
b. Open ReGENerator program or Cloning, ReGENerator
c. In top view you can insert amino acids or delete them, or overwrite them (substitution)
d. Insert restriction sites, gateway or user-defined sequences at 5' or 3' end [these do not affect the protein sequence]
e. Press in silico DNA to view DNA sequence

12. Gel analysis

a. Press gel icon for gel setup [watch for time increment]
b. In Electrophoresis profile select Agarose gel, then press Run, run screen comes up
c. Create sample [4th button from left on the bottom]
d. Add gene ADCY7, restriction enzymes AarI produces 5 fragments
e. Give a sample name and add to gel, then Close
f. Add marker lane [e.g. 1 kb extension ladder]
g. Click on left pane under General Description to see what kind of fragments there are
h. Press Graphics page, [2nd from left, bottom row of buttons]
i. Press forward button to see how gel is simulated
j. Select fragments with cursor, calculate time to separation
k. Press fast forward button to watch simulation of fragments in the gel

13. BioAnnotator

a. Analyses menu point, BioAnnotator, Open New Analyzer Window
b. Open the pSV2-dhfr molecule from DNA/RNA Molecules in separate VectorNTI window
c. Find ORFs, like how we did in practice #8
d. Click on the DFHR gene
e. File, Create new sequence, Using translation procedure, direct strand
f. Rename DFHR
g. Select molecule, Anayses, BioAnnotator, Analyze Selected Molecule

h. The hydropathicity/hydrophobicity profiles are showed only

i. Analyses list

14. ContigExpress - Assembly

a. Start menu, Programs, Invitrogen, Vector NTI Advance, ContigExpress

b. Project, Open Project, Demo Projects folder, DemoProject.cep

c. Project, Add Fragments, From ABI file, select Rb.abi and Sample1303.abi and Open (Choose Yes to keep the internal fragment names)

d. Select all fragments by Shift+Click on all except xb-control

e. Assemble, Assembly Setup, browse the default values and Cancel

f. After Assemble, Assemble 1 includes assembled Contig 1 and 2

g. For a child of Assembly 1, Shift click on ONE17K and the following six fragments and Assemble Selected

h. View, Options, Details View, select Non-Ambiguous bases and press >>, Displayed Fields, select Non-Ambiguous Bases, Move Up to the top, OK

i. Different views with list display buttons (Show Contigs, Expand Contigs, Show Unassembled Fragments, Show Other Fragments)

j. Content View

15. ContigExpress – Fragment Viewer

a. Double-click the fragment ONE8R

b. Click on Sequence Pane button or tab, then Show Two Strands button

c. At the end of the sequence, notice the last 2 bases dropped down and replaced with arrows. This is the result of clipping during the assembly process.

d. Viewing Options, Blocks per Line = 15, OK and change it back from View, Groupings, Set Number of Blocks = 10

e. Click Chromatogram Pane button or tab, try clicking on Show Trace for A, C, G or T (Alternatively, View, Show Traces)

f. Click on Position Box at the bottom right on the status bar of the ContigExpress

window to change the current caret position

g. Click on Selection Box for range selection, e.g. from 10 bp to 30 bp

h. Show ORF (or View Options, ORF)

i. Select the region from127 to 138 bp in Sequence Pane and click Translate Complementary button

j. View, Show 1-letter AA codes toggles between 1 and 3 letter codes

k. View, Clear All Translation clears all translations

l. View, Show ORFs removes the displayed ORFs

16. ContigExpress – Contig Viewer

a. Double-click Contig 1 of Assembly 1 in ContigExpress Project window

b. Info Pane shows contig details

c. Graph Pane shows overlapping contigs and the assembled larger contig. Zoom In and Out to fit the view. View, Show ORFs or click on the button. Green bars indicate individual ORFs. ORF options can be changed from View, View Options, then ORF.

d. Alignment Pane displays the sequences. Identify fragment names, fragment position, scale, fragment sequences, and consensus sequences (nucleotide or symbol format)

    i. Dot is an IUPAC ambiguity symbol

    ii. Plus symbol (+) is an ambiguous position containing specific base disagreements and/or gaps

e. Click on the name ONE6R in the Alignment Pane. Right-click anywhere in the Alignment Pane and select Show Chromatogram for ONE6R. Again right-click, then select Show All Chromatograms. Hide All Chromatograms clears them.

f. View, Consensus Translation > 1st, 2nd, or 3rd frame or click on the button (Note: Gaps are ignored and do not introduce frameshift)

g. Show 1-letter AA codes button toggles the translation display