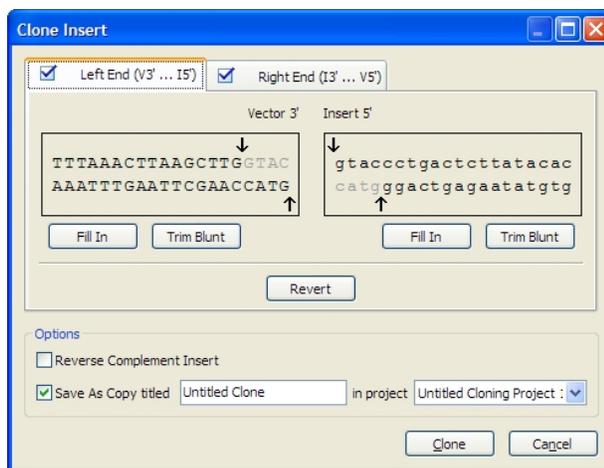## Creating a Restriction Clone

Restriction cloning is a general cloning method that uses restriction enzymes to cut insert and vector sequences at specific locations. Once the vector and insert sequences are properly prepared, a ligation reaction is typically used to create a recombinant clone.

1. Select **File > New Cloning Project**. The *Untitled Cloning Project* window is displayed.

2. Select the *Inserts* folder.

3. Select **File > Import** and locate the *Tn5wPCR.sbd* sequence in the *C:\Program Files\DNASTAR\Lasergene\Demo Data* folder.

4. Double-click the file to add it to the *Inserts* folder.

5. Select **File > Open**, locate the *CloningVectors.sbp* file in the *C:\Program Files\DNASTAR\Lasergene* folder, and double-click to open it.

6. Locate the pcDNA5/FRT/TO/CAT$^{©}$ vector in the *Restriction Vectors* folder.

7. Right-click on the vector, and select **Copy**, then close the Cloning Vectors catalog.

8. Return to the *Untitled Cloning Project* window, highlight the *Vectors* folder and use **Edit > Paste** to copy the vector into your project.

9. Open the *Inserts* folder and double-click the TN5wPCR insert sequence to open it.

10. Display and choose your enzymes by clicking on an enzyme label and highlighting the nucleotides between the *Acc* 65I and *Apa* I sites.

11. Select **Cloning > Copy Restriction Insert**. The insert is copied to the clipboard.

12. Return to the *Untitled Cloning Project* window (minimize the *Inserts* window).

13. Double-click the pcDNA5/FRT/TO/CAT© vector sequence to open it.

14. *Shift+click* on the sites for *Acc* 65I and *Apa* I.

15. Select **Cloning > Clone Restriction Insert**. The *Clone Insert* dialog opens, displaying the ends of the vector and insert sequence for compatibility. If the ends are not compatible, the

checkboxes in the *Left End* and *Right End* tabs at the top will be unchecked.

16. Modify the ends, if needed, by sliding the positional arrows or by clicking *Fill in* or *Trim Blunt*. When both ends are compatible and both *Left End* and *Right End* checkboxes are checked, the *Clone* button will be active.

17. Enter a name for the new clone (e.g., `Test Clone`) in the *Save as Copy Titled* box on this screen, and save to any project listed in the *In Project* box.

18. Click *Clone*. The new clone is displayed in a new window, and it is stored in the *Clones* folder of the previously selected project.

19. Save the project and the information in its subfolders by selecting **File > Save Project As**.

Creating a TA Clone

TA Cloning is a cloning method that takes advantage of the terminal transferase activity of some DNA polymerases that add a 3′-dA overhang to each end of a PCR product. A linearized vector with prepared 3′-T overhangs facilitates ligation of the PCR product.

1. Select **File > New Cloning Project**. The *Untitled Cloning Project* window is displayed.

2. Select the *Inserts* folder.

3. Select **File > Import** and locate the *Tn5wPCR.sbd* sequence in the *C:\Program Files\DNASTAR\Lasergene\Demo Data* folder.

4. Double-click the file to add it to the *Inserts* folder.

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5. Open the *Inserts* folder and double-click the TN5wPCR insert sequence to open it, then select the sequence range that has been PCR-amplified (in this case, the neomycin/kanamycin resistance sequence).
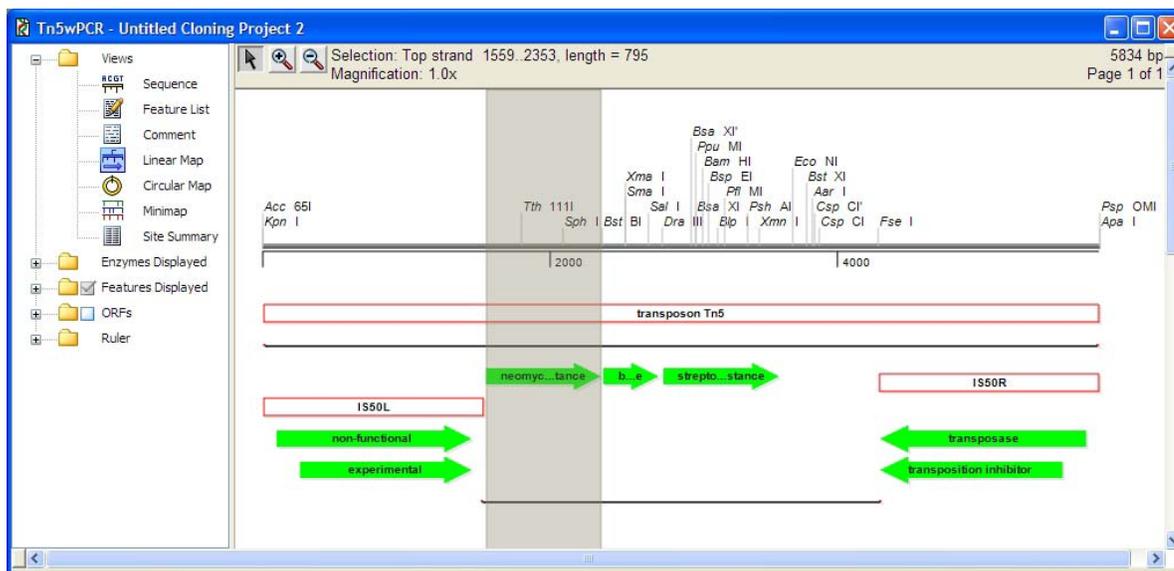
6. Select **Cloning > Create TA Cloning Insert**. The PCR-amplified insert is placed in the *Inserts* folder, and the *TA Cloning Advisor* dialog is displayed.
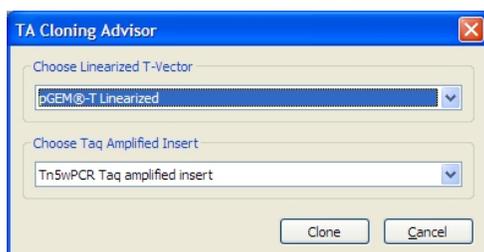
7. Verify your selected insert, or select a new insert, then select a linearized T-vector such as *pGEM®-T Linearized*. Click *Clone*. The clones, both forward and reverse orientations, are displayed in the *Clones* folder in the *Untitled Cloning Project* window.

8. Go to the *Clones* folder in the *Untitled Cloning Project* window, right-click on each *Untitled TA Clone*, and rename the clones.

9. In the open project window, double-click one of the clones just created (either forward or reverse) to open it. Then click *Circular Map* in the *Views* pane, and click the *Feature Display* checkbox within the curtain to view the results.

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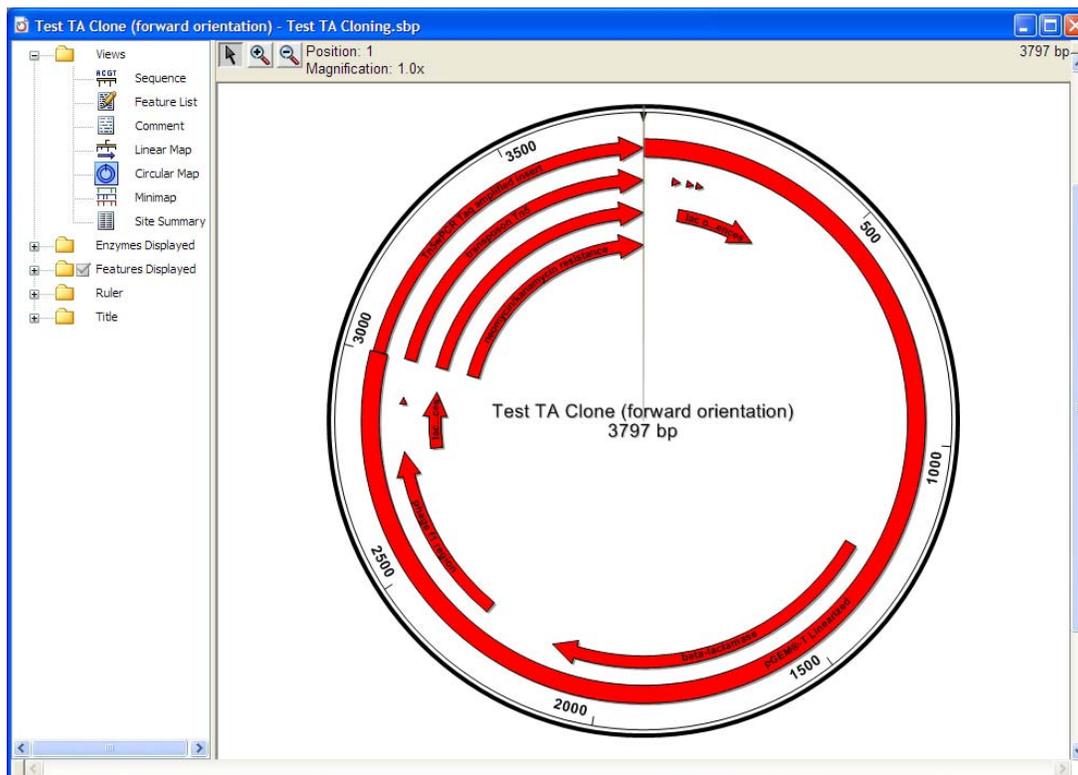
10. Save the project and the information in its subfolders by selecting **File > Save Project As**.

Creating a Gateway® Clone

Gateway® Cloning is a widely used molecular technique that allows the transfer of DNA inserts between different cloning vectors while maintaining the reading frame and orientation of the insert. This technology eliminates the need for restriction enzyme digestion, ligation, and colony screening for recombinants and is based on the site-specific recombination system of phage I.

1. Select **File > New Cloning Project**. The *Untitled Cloning Project* window appears.

2. Select the *Inserts* folder.

3. Select **File > Import** and locate the *Tn5wPCR.sbd* sequence in the *C:\Program Files\DNASTAR\Lasergene\Demo Data* folder.

4. Double-click the file to add it to the *Inserts* folder.

5. Open the *Inserts* folder and double-click the TN5wPCR insert sequence to open it, then select the neomycin/kanamycin resistance sequence.

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