Clone Manager 11

NEBuilder® HiFi DNA Assembly Cloning Method

NEBuilder HiFi DNA Assembly is a New England Biolabs method for assembling DNA fragments. New England Biolabs promotes the method as allowing seamless assembly of multiple DNA fragments, regardless of fragment length or end compatibility. The reaction uses a mix of enzymes working in the same reaction buffer. More information on the method can be found in the references.

The NEBuilder HiFi DNA Assembly Master Mix consists of several enzymes: a 5' exonuclease creates 3' overhangs that allow annealing between adjacent fragments; a DNA polymerase fills in the gaps; a DNA ligase seals the junction.

While the NEBuilder method is similar to the Gibson and In-Fusion assembly cloning methods, NEBuilder is more flexible when dealing with 3' and 5' end mismatches.

The DNA polymerase used has 3' end proof reading capabilities. This allows for cutting the vector at a restriction site but trimming a few bases at the 3' end to allow manipulations such as preserving a reading frame.

NEBuilder wizard

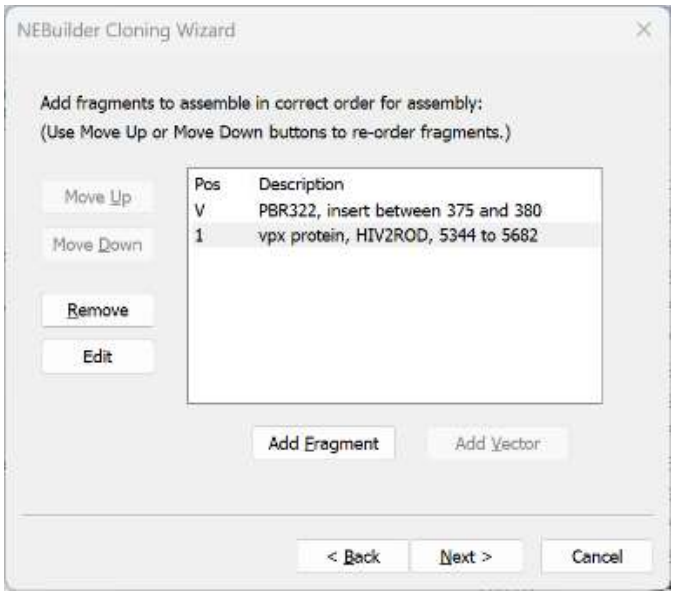
The NEBuilder wizard is designed to assist in selection and preparation of fragments to be assembled. Start the wizard using the NEBuilder option on the main Clone menu.

The wizard is very similar to the Gibson and In-Fusion assembly cloning wizards: Assembly design settings can be changed; Vector and insert fragments can be added with customization of how the fragments should be prepared; PCR can be used to amplify a part of a larger molecule and introduce the necessary overlaps for assembling onto the adjacent fragments.

The first page of the wizard is a simple introduction of the method. The next page allows you to control the design conditions and will be described in more detail later.

The main page of the wizard is the fragments list that shows the fragments selected for assembly.

Fragments list

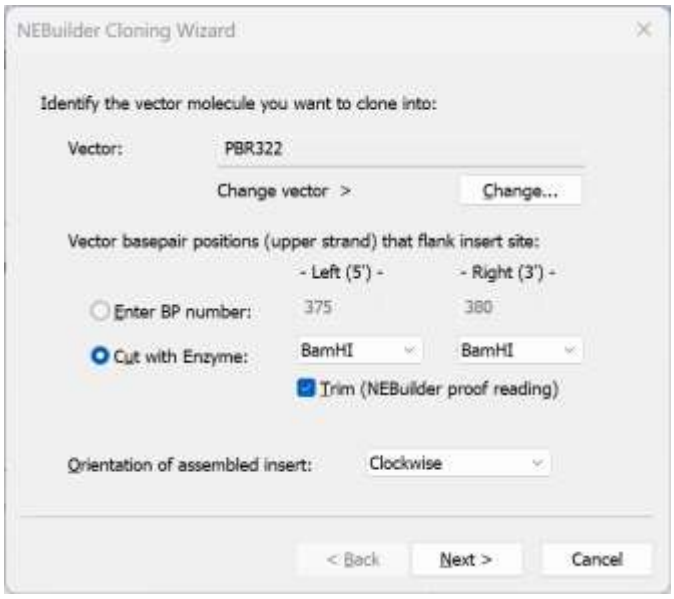


The main page of the wizard allows you to enter, and manage, the fragments that are to be assembled. Typically, one or more fragments will be assembled into a vector and the result circularized.

The wizard page shows a list of the fragments entered for assembly. Buttons under the list allow for adding one or more fragments and adding the vector. In the sample, a vector and one insert have been added to the llist.

Fragments should be entered in the order that they will be assembled. Use the 'Move' up and down buttons on the left to reorder the fragments if needed. The Edit button allows for customizing the selected fragment.

Vector



Select the vector to be used using the 'Add Vector' button under the list of fragments and then using the 'Change' button to select from the Molecule List.

You can enter the base pair positions that flank the insertion site or you can cut with one, or two, restriction enzymes.

In the example we have selected to cut at a single BamHI site.

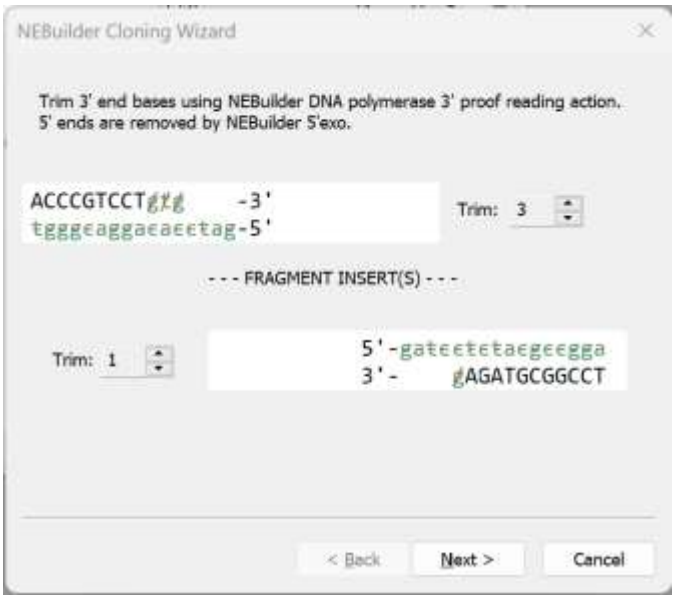
The optional 'Trim' check box allows you to specify how many bases at the 3' end of the cut vector should be removed by the 3' end proof reading property of the DNA polymerase.

You can ignore the Trim function if you will be assembly fragments that have already been PCR amplified to create the necessary overlap. In this case the overlap will automatically be detected.

In this example we have checked the 'Trim' box so that we will later be able to design the correct PCR primers for amplifying the insert fragment.

If you want to insert your fragments into the vector in the inverse direction, you can change the orientation of the assembled insert.

Clicking the wizard Next button will show the page displaying the ends of the cut vector and allowing trimming of 3’ end bases.



The bases at the 5’ ends of the cut vector are removed by the 5’ exonuclease contained in the Master enzyme mix. These bases are marked with dashes to indicate that they do not participate in the assembly reaction.

Use the appropriate spinner control to change the number of bases to remove from the 3’ end of the cut vector. Trimmed bases are shown in lower case and with a slash mark.

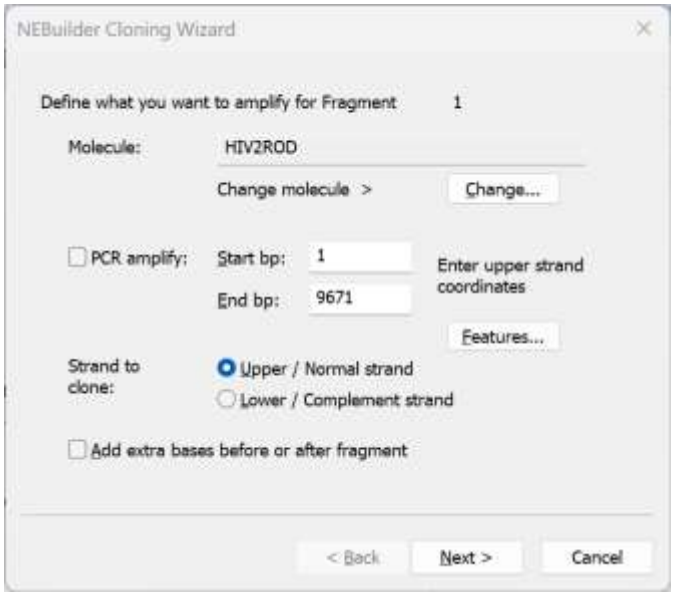
In this example, we have trimmed 3 bases from the vector end preceding the inserted fragments and 1 base following. Up to 10 bases can be trimmed in this operation.

Clicking the wizard Next button will return to the main fragment list page and show the vector in the first position (marked with ‘V’ symbol).

Use the ‘Edit’ button to review, or make changes to, the settings for the vector fragment.

Selecting a vector for the cloning is optional. If no vector is selected, then the fragments will be assembled in order as a linear recombinant molecule. If a vector is selected, then the resultant recombinant will be circularized.

Insert(s)



Add one, or more, fragments to assemble using the ‘Add Insert’ button under the list of fragments and then using the ‘Change’ button to select from the Molecule List.

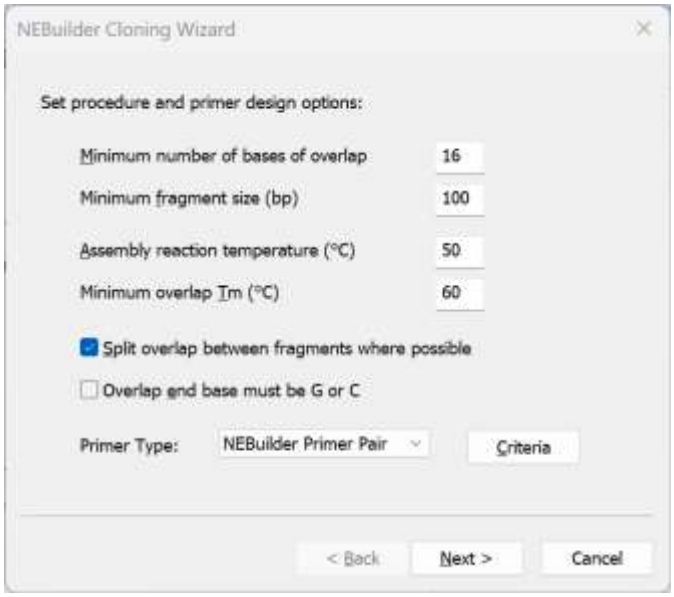
The wizard page allows you to select a part of the molecule to clone using PCR. Either enter the start and end base positions of the region or click the ‘Features’ button to select a gene or region. The PCR amplify check box will automatically be checked when you click the Next button.

You can also select the strand to clone using the radio buttons. Selecting the Lower, or Complementary, strand will invert the fragment when assembling the fragments.

Check the ‘Add extra bases’ box to use PCR to add extra bases around the region selected for insertion into the final recombinant.

If your selected insert molecule has already been amplified by PCR, or is a synthesized oligo, then simply accept the full-length fragment coordinates. The wizard will automatically determine the overlap with adjacent fragments.

Design Conditions



This wizard page allows control of the conditions used to design cloning using the NEBuilder assembly method.

Minimum number of bases of overlap: sets the minimum length of the overlap that will be generated when designing PCR primers. If inserts have already been amplified, or synthesized, then this is the minimum length of overlap that will be accepted.

Minimum fragment size: this provides a warning when short insert fragments are selected. Since the NEBuilder master mix includes a 5’ exonuclease, short fragments will need a shorter reaction time and may need to be provided at a higher concentration. This warning can be ignored if the short fragment is a single strand oligonucleotide since the 5’ exonuclease will not affect it.

Assembly and overlap temperatures: control the recognition of overlap regions and design of PCR primers.

Split overlap: when designing PCR primers, the overlap will be split between the 2 fragments.

Overlap end base must be G or C: enables you to specify that PCR primers should end in a G or C base.

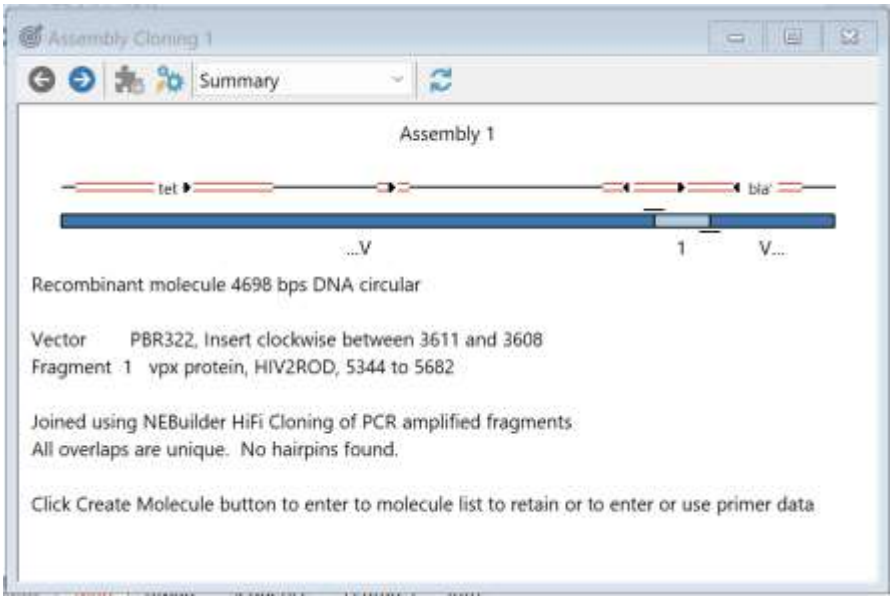
Primer Type and Criteria: allows customization of the PCR design conditions.

Wizard Finish

The final page of the wizard shows a brief outline of the goal of the cloning experiment and the size of the expected recombinant. Click the ‘Finish’ button to complete the design and show the results.

When viewing the results, you can return to the wizard to make changes by clicking the Redefine toolbar button.

View Results



A sample results view is shown on the right. It consists of an action toolbar at the top, an iconic map of the recombinant and a description of the cloning process.

Toolbar:

Hovering your mouse cursor over a toolbar button will show a tooltip describing the function. The left and right arrows allow you to toggle the main view between the summary and primer design views. This can also be changed using the drop down combo-box.

Create the recombinant molecule by clicking the toolbar button. This button will also allow you to create and export any primers used to PCR amplify any fragment(s).

You can change the wizard design parameters using the redefine button at the right end of the toolbar. This returns you to the design wizard where you can change any setting.

Iconic Map:

This shows the placement of the assembled fragments and distinguishes vector (…V and V…) from the insert(s). In this example, insert fragment 1 was amplified by PCR and the primers are shown as short lines overlapping the junction with the vector.

Cloning description:

Shows the size of the recombinant molecule and the fragments that were used for the cloning. Also shown is the method and any warnings of factors that might affect the assembly process.

Wizard results output

The results view can be copied to the clipboard or a file using the menu View, Send view options. Results view can be printed using the menu File, Print.

References:

Application Overview: https://www.neb.com/applications/cloning-and-synthetic-biology/dna-assembly-and-cloning/nebuilder-hifi-dna-assembly